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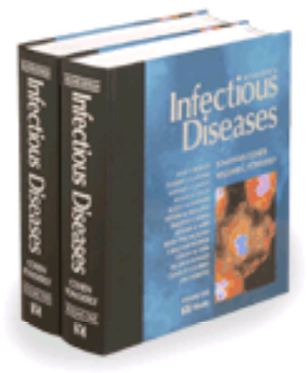

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Infectious Diseases

SECOND EDITION

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Preface

When we sat down five years ago to plan the first edition of *Infectious Diseases* we were determined to make it make it innovative, comprehensive and accessible. It is very gratifying to record that the responses we have had from colleagues all over the world suggest that, in large part, we succeeded in those aims. Many of the ideas that we introduced have proved popular with our readers, in particular the use of full-color illustrations, the down-loadable slide picture library, the Practice Points feature of common but difficult problems, and the international scope of both the content and the authorship. It was this success, as well as the breathtaking speed with which new developments in infectious diseases were occurring, that persuaded us that a second edition could no longer be delayed.

In putting together this second edition we have looked rigorously at all parts of the book, re-structuring where necessary, adding and updating material and inviting new editors to strengthen the team. The emergence of unsuspected clinical syndromes (West Nile fever in the USA is just one example), and unwanted challenges for infectious diseases physicians (sadly, the spectre of bioterrorism has found its way on to these pages) has resulted in new chapters and new authors. But we were also keen to continue the innovative approach that we took for the first edition, and have added a substantial new element to the book, the *Infectious Diseases* website. Although this existed in a rudimentary form before, for this second edition Steven Opal has taken the lead in creating an extraordinary resource of material that will complement the book.

No project of this size and complexity can be undertaken without the help of a very large number of people. Section editors and authors have worked against a very challenging timetable to ensure that the book is as up-to-date as possible. We are also indebted to the publishers, and in particular to Shuet-Kei Cheung, who was in at the beginning of the project and worked tirelessly to see it through to production, and also to Deborah Russell and Jill Day for their contributions.

A final word of thanks must go to the Section Editors who worked on the first edition: Claude Carbon, David Durack, Don Louria, Bruce Polsky and Paul Quie, and to Donald Armstrong, whose vision contributed so much. Without their input we would never have had the opportunity to work on this second edition. We are greatly indebted to them.

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Section 1 - INTRODUCTION TO INFECTIOUS DISEASES

Claus O Solberg

Chapter 1 - Nature and Pathogenicity of Micro-organisms

Menno Kok
Jean-Claude Pechère

In our daily life we are surrounded by a wealth of micro-organisms, the majority of which are inoffensive. Human existence would be impossible without these micro-organisms, as they play critical roles in processes as diverse as photosynthesis, nitrogen fixation, production of vitamins in the human intestine and decomposition of organic matter. They are the sole, true 'recyclers' of our planet. Micro-organisms are also the major driving force behind the evolution of life. They evolved photosynthesis and respiration, which have since been acquired by present-day eukaryotes, and they mediate genome rearrangements in infected host cells.

In a rather simplified view, micro-organisms may be considered to be no more than 'little machines that multiply'. In fact, this is what they do best. We are starting to understand some of the strategies micro-organisms have developed to stay alive, grow and reproduce. The lifestyle of a micro-organism is intimately related to its environment, whether that environment is the human body or a polluted riverbed. Some highly specialized micro-organisms have adapted to the harsh conditions of hot ocean vents, oil tanks or nuclear reactors; others prosper on waste dumps. Still others have been tempted by the abundant resources provided by higher organisms, such as plant root-colonizing bacteria and our own intestinal flora.

In this chapter we shall examine the lifestyle of pathogenic micro-organisms and how they infect us, reproduce and cause disease. We shall use the word 'pathogenicity' to indicate the capacity to cause disease (or damage). Although the word 'virulence' is often used in the same sense, it refers more specifically to transmissibility or infectiousness of micro-organisms.

The world of pathogenic microbiology is immensely diverse, ranging from prion proteins to worms. A better understanding of the behavior of these infectious agents will help us to design strategies for disease prevention and treatment.

DEFINITION AND COMPARISON OF INFECTIOUS AGENTS

The definition of an 'infectious agent' was proposed by J Henle in 1840 and put to the test by the German physician Robert Koch. In 1876, Koch reported experiments on mice with *Bacillus anthracis* showing that:

- a single micro-organism could be isolated from all animals suffering from anthrax;
- the disease could be reproduced in an experimental host by infection with a pure culture of this bacterium; and
- the same micro-organism could subsequently be reisolated from the experimental host.

These three criteria define an infectious agent.

Even though a clear oversimplification, we will divide infectious agents into four groups, presented here in the order of increasing complexity.

Prions

Prions are the simplest infectious agents. They consist of a single protein molecule denoted PrP (Prion Protein). The infectious particle, known as PrP^{Sc} (Sc denotes scrapie), is identical to the ubiquitous cellular protein PrP, except that it is folded in an abnormal conformation. The PrP^{Sc} particle has the following characteristics:

- the prion disease spongiform encephalopathy is transmissible between different species such as sheep and goats (see Chapter 26);
- even with the most advanced analytic techniques, no nucleic acid could be detected in infective prion particles; therefore, prions do not carry the genetic code for their own de novo synthesis;
- the PrP^{Sc} protein catalyzes the conformational change of PrP into PrP^{Sc} . The latter is remarkably resistant to heat and chemical agents, making it difficult to disinfect contaminated material; and
- as prions induce the conversion of an endogenous protein, their ability to produce disease is host dependent and subject to genetic variation.

For a detailed description of the biology of prions see Chapter 223 .

Viruses

Viruses contain at least two types of macromolecules: nucleic acid and protein. All viruses share the following characteristics:

- they are small — the largest known virions are produced by poxvirus (approximately 230 × 270nm) and most viruses of medical importance are smaller than 200nm in diameter;
- they contain only one species of nucleic acid, either DNA or RNA, whereas bacteria always have both species;
- they attach to their host cell with a specific receptor-binding protein;
- they cannot replicate autonomously; in order to reproduce its genomic information a virus requires the assistance of a living eukaryotic or prokaryotic cell; and
- when a virus infects a cell, information contained in the viral genome is used to divert the cellular machinery towards the production of new viral particles.

'Defective' and 'simplified' viruses

Some infective agents share many features with viruses but seem to be even more primitive. Some very small viruses require the assistance of another virus in the same host cell for their replication. The nonpathogenic dependoviruses owe their name to their dependence on an adenovirus or, occasionally, a herpesvirus to assist in their replication. The delta agent, also referred to as hepatitis D virus, is too small to code for even a single capsid protein and needs help from hepatitis B virus for transmission. Hepatitis B and D are often co-transmitted. Viroids are ssRNA molecules that do not code for any protein species. As the delta agent, they are likely to replicate by using the cellular RNA polymerase.

Bacteria and archaea

Bacteria (eubacteria) and archaea (archaeabacteria) have long been united under the name 'prokaryotes'. Today, this terminology ('everything that is not eukaryote') is considered inadequate.[1] The main characteristics of prokaryotes, compared with eukaryotes, are given in Table 1.1 . Bacteria and archaea invariably have a DNA genome which, unlike the eukaryotic genome, is not physically

TABLE 1-1 -- Comparison of prokaryotes and eukaryotes.

COMPARISON OF PROKARYOTES AND EUKARYOTES		
Feature	**Prokaryotes**	**Eukaryotes**
Chromosome	Single, circular	Multiple
Gene organization	Operon-polycistronic mRNA	Single genes and block of genes
Nucleosomes	No	Yes
Nuclear membrane	No	Yes
Mitosis	No	Yes
Introns in genes	No	Yes
Transcription	Coupled with translation	Separate from translation
mRNA	No terminal polyadenylation (except archaeabacteria); polygenic	Terminal polyadenylation; usually monogenic
First amino acid	Unstable formylmethionine (except archaeabacteria)	Methionine
Ribosome	70S (30S + 50S)	80S (40S + 60S)
Cell wall	Presence of muramic acid, D-amino acids, peptidoglycan (except archaeabacteria and mycoplasma)	No muramic acid, D-amino acids, or peptidoglycan
Membrane	No sterols or phosphatidyl-choline (except mycoplasma)	Sterols and phosphotidyl-choline
Endoplasmic reticulum	No	Yes
Mitochondria	No	Yes
Lysosomes and peroxysomes	No	Yes
Movement	By flagella, composed of a single fiber	Ameboid, by cilia or cilia-like flagella

separated from the rest of the cell contents by a membrane. Neither size (mycoplasmas are as small as viruses, about 200nm in diameter) nor obligatory reproduction

in eukaryotic cells supplies definitive criteria to distinguish bacteria and archaea from viruses. However, in contrast to viruses, prokaryotes always contain both DNA and RNA. Even obligate intracellular bacteria, like *Chlamydia* spp. and *Rickettsia* spp., which appear to have adopted a virus-like lifestyle, remain enclosed within their own cell envelope throughout their life cycle and provide their own nucleic acid and protein reproduction machineries.

Based on extensive nucleotide sequence data, it has been suggested that archaea are more closely related to eukaryotes than to bacteria. Indeed, these micro-organisms, which seem to have a particular preference for hostile environments seeded with toxic

TABLE 1-2 -- **Comparison of bacteria and fungi.**
Adapted from Kobayashi.[2]

COMPARISON OF BACTERIA AND FUNGI		
Characteristics	**Bacteria**	**Fungi**
Cell volume (μm^3)	0.6–5.0	Yeast: 20–50; molds; greater than yeast
Nucleus	No membrane	Membrane
Mitochondria	No	Yes
Endoplasmic reticulum	No	Yes
Sterol in cytoplasmic membrane	No (except for mycoplasma grown on sterols)	Yes
Cell wall components	Muramic acids and teichoic acids; no chitin, glucans or mannans	Chitin, glucans and mannans; no muramic acids or teichoic acids
Metabolism	Autotropic or heterotropic	Heterotropic
Sensitivity to polyenes	No	Yes

chemicals, deep-sea hydrothermal vents and oil deposits, share with eukaryotes at least one feature that is absent from bacteria — their genes frequently bear introns. Archaea do not play any role in human medicine. In contrast, eubacteria include the hundreds of bacterial species that are commensal or pathogenic for humans. Bacteria come in various shapes and sizes (typically in the range of 1–2µm diameter). With the exception of *Mycoplasma* spp., bacteria characteristically have a rigid cell wall.

Eukaryotes

Eukaryotes have subcellular compartimentalization. DNA transcription, photosynthesis, respiration and protein modification are physically restricted to specific organelles: the nucleus, chloroplasts, mitochondria and the Golgi system.

TABLE 1-3 -- The four major classes of fungi.

THE FOUR MAJOR CLASSES OF FUNGI	
Class	**Representative genera**
Phycomycetes	*Rhizopus, Mucor*
Ascomycetes	*Neurospora, Penicillinium, Aspergillus*
Basidiomycetes	Mushrooms, rusts, smuts
Denteromycetes (or fungi imperfecti)	Most human pathogens

TABLE 1-4 -- Proptozoa that are important in humans.

IMPORTANT PROTOZOA IN HUMANS			
Category	**Species**	**Disease**	**Estimated worldwide prevalence of human infections**
Protozoa	*Toxoplasma gondii*	Toxoplasmosis	1–2 billion
	Entamoeba histolytica	Amebiasis	200–400 million
	Trichomonas vaginalis	Trichomoniasis	15% of women
	Plasmodium spp.	Malaria	200–300 million
	Giardia lamblia	Giardiasis	200 million
	Trypanosoma cruzi, T. brucei	Chagas' disease: African sleeping sickness	15–20 million
	Leishmania donovani, L. tropica	Leishmaniasis	1–2 million
Helminths	*Ascaris lumbricoides*	Ascariasis	1 billion
	Necator americanus	Hookworm disease	800–900 million
	Schistosoma mansoni	Schistosomiasis	200–300 million
	Wuchereria bancrofti	Lymphatic filariasis	200 million
	Enterobius vermicularis	Pinworm infection	60–100 million
	Strongyloides stercoralis	Strongyloidiasis	50–80 million
	Onchocerca volvulus	Onchocerciasis	50 million

Fungi

Fungi and bacteria play similar roles in the biosphere, share the capacity to produce infectious disease and both have rigid cell walls, but their cellular architecture is completely different (Table 1.2). Pathogenic fungi occur in two forms: the filamentous molds and the unicellular yeasts. There are four major classes of fungi (Table 1.3). *Pneumocystis carinii*, which causes severe pneumonia in immunosuppressed hosts, was long considered to be a protozoan. However, its ribosomal RNA, which was recently sequenced, showed greater similarity to that of fungi than to protozoa.[3]

Protozoa

Protozoa (Table 1.4) are unicellular eukaryotes. In contrast to fungi, they have a flexible cell membrane. Their movements can be ameboid or directed by cilia or cilia-like flagella. Pathogenic protozoa often have complex life cycles with both intrahuman and extrahuman stages. The sources of parasites in the environment are called reservoirs, which include other animals or free forms of the parasite found in the external environment (e.g. food contaminated with *Toxoplasma gondii* oocysts from cat feces).

Some protozoa that are pathogenic for humans, such as the malaria parasite, trypanosomes, *Leishmania* spp. and *Toxoplasma* spp., invade deep tissues and reside inside host cells, at least during part of their life cycle. These protozoa do not survive for long in the external environment and they are often transmitted by living vectors such as flies and mosquitoes. Other protozoa are extracellular (e.g. the agents of amebiasis and giardiasis) and possess vegetative and resistant forms. The

trophozoite produces the active disease and allows vegetative growth and multiplication, whereas the highly resistant cyst form, which is able to survive in hostile environments, assures transmission between hosts.

Parasitic worms

Helminths are the largest parasites that infect humans. They are multicellular organisms ranging from 1cm to 10m in size. They usually are encased by an outer membrane or cuticle that protects internal differentiated organ systems. Helminths (or worms) are classified into three groups, generally distinguishable by their shape:

- nematodes (or roundworms),
- cestodes (or tapeworms), and
- trematodes (or flukes).

Some helminths have complex life cycles that may include successive animal reservoirs and insect vectors.

GENERAL PROPERTIES AND CLASSIFICATION OF VIRUSES

Structure of viruses

The whole virus particle, the virion, is designed to protect the viral genome and to mediate the migration of the virus and the invasion of the target host cell. The viral genome can be packaged in a nucleocapsid, which in some virus families contains a number of enzymes required for the early stages of virus multiplication. The capsid may in turn be surrounded by an outer membrane.

The genome

The genome is made up of either DNA or RNA, associated with proteins or polyamines. The size of nucleic acid per virion ranges from 3 to 300kb. There may be only one gene in the smallest virions, whereas the largest genomes, such as vaccinia, may encode hundreds of proteins. The Parvoviridae, which include the virus that causes erythema infectiosum, have a 5.5kb DNA genome, which codes for only three polypeptides. This may explain why these viruses need help from a larger virus, i.e. adenovirus, for their replication.

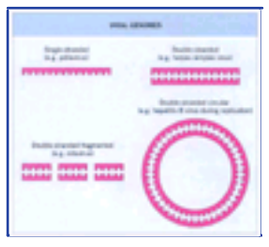

Figure 1-1 Viral genomes.

The nucleic acid may be either double stranded or single stranded (Fig. 1.1). The nucleic acid of all DNA viruses except parvoviruses is double stranded. In contrast, the nucleic acid of all RNA viruses except the reoviruses (e.g. influenza, bunya and arenaviruses) is single stranded. The genome may be linear or circular, and non-segmented or segmented. Genome segmentation, a general feature of reoviruses, favors gene exchange between co-infecting virions. RNA virus genomes change at high frequency through point mutations, which helps viral evasion of the human immune response.

Some viral DNA molecules may contain alternative nucleotides, which inhibit host cell nucleases and thus protect the viral genome. Linear DNA genomes may contain terminal redundancies, allowing incomplete replication products to recombine, or they may carry proteins at both ends, that play a role in priming of DNA replication. Some viral DNA is flanked by repeat sequences, which suggests relatedness with transposable elements.

Most human RNA viruses have single-stranded genomes. This RNA molecule may have either of two possible polarities. The positive-strand RNAs can act directly as messengers for protein synthesis; they resemble eukaryotic RNAs with a cap at the 5' end and a poly-A chain at the 3' end. The negative-strand RNAs need to be transcribed by a viral RNA transcriptase into mRNA. Negative-strand RNA genomes have neither a cap structure nor a poly-A tail. Retroviruses first synthesize a DNA copy of the positive-strand RNA genome, which integrates into the cellular DNA and may subsequently serve as a template for mRNA synthesis.

The capsid

The viral genome is protected by a protein coat, the capsid or nucleocapsid. The capsid is made of knob-like structures known as capsomeres, which consist entirely of proteins coded by the viral genome. The capsid accounts for a large portion of the viral mass. Papilloma virus produces only two capsid proteins and poliovirus four, but more complex viruses may encode a much larger variety of capsid proteins. Different nucleocapsid morphologies have been observed by electron microscopy (Fig. 1.2).

Picornaviruses, adenoviruses and papovaviruses have a nucleocapsid structure with icosahedral symmetry. The capsid consists of 20 triangular facets and 12 corners or apices. Influenza, measles and rabies virus form capsids with helical symmetry. The central core is formed by the nucleic acid genome, around which the nucleocapsid proteins are arranged like the steps of a spiral staircase, forming long cylinders.

More complex virion morphologies also exist. Bacteriophages, which use bacteria as hosts, have additional attachment structures fixed to the capsid. The nucleocapsid of orthopoxviruses, such as variola and vaccinia virus, consists of a network of tubules, sometimes surrounded by an envelope, forming a brick-shaped virion.

The envelope

In some viruses the nucleocapsid is surrounded by an outer envelope. Enveloped viruses contain nucleocapsids of either icosahedral (e.g. herpesviruses and togavirus, which causes rubella) or helical symmetry (e.g. influenza virus). The outer envelope consists of a lipid bilayer, derived from the host cell membrane, in which the viral glycoproteins are embedded. The viral matrix proteins (M proteins) are firmly associated with the envelope. Matrix proteins play an important role in the structural organization of the virion and are thought to connect the capsid to the viral glycoprotein inserted in the lipid bilayer. Besides oligosaccharide residues, the glycoproteins contain a membrane anchor and, in many cases, one or two molecules of fatty acid. Glycoproteins play a key role in the attachment of virions to the cell surface and penetration into the cell. Some viruses, such as the influenza virus, have glycoproteins with neuraminidase activity; this promotes the release of newly formed viral particles from the host cell membrane. Once released from the host cell, virions are metabolically inert. The virus only comes 'alive' after entry into a suitable host cell and activation of its genome.

Classification of viruses

Viruses are classified into families, subfamilies and genera. The most important families are summarized in Table 1.5 . Classification criteria include the nucleic acid species, the number and polarity of the nucleic acid strands, the presence or absence of a lipid envelope and the symmetry (icosahedral, helical or complex) of the nucleocapsid.

Viral gene expression strategies

Viral infection can be separated into two phases. In the early phase of infection, the virus establishes the proper cell environment required for viral genome replication and the viral DNA or RNA polymerase is produced. In the late phase, the viral genome is amplified and the structural components of the virion accumulate. At this point a considerable part of the cellular metabolism is committed to viral reproduction. Eventually the virion is assembled from its components and leaves the cell. Four viral replication strategies can be distinguished (Fig. 1.3).

Positive-strand RNA viruses

In positive-strand RNA viruses, the viral genome has the right polarity to serve immediately as a messenger RNA. The first step in viral infection consists of a complete translation of the genome to produce a polyprotein, which is sequentially processed into smaller polypeptides. Enzymatic cleavage of the composite protein is at least partially autocatalytic. In the early phase, processing preferentially produces proteases and the RNA-dependent polymerase. In the late phase of infection, processing is reoriented towards the production of the structural proteins. This scheme applies to picornaviruses, flaviviruses and hepatitis C viruses.

For other virus families (e.g. togaviruses, coronaviruses, caliciviruses and hepatitis E viruses), the incoming genome is only partially translated to produce the proteases and the RNA polymerase. The portion of the genetic information that encodes the structural proteins is expressed from a transcript derived from the RNA intermediate in genome replication.

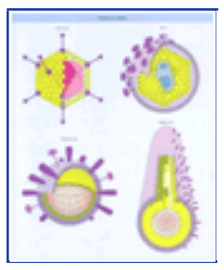

Figure 1-2 Examples of virions. Adenovirus is an icosahedral DNA virus without an envelope; fibers extend from the 12 points of the icosahedral coat; DNA forms a ribbon-like molecule. Approximate size 80nm. HIV-1; glycoprotein (GP) molecules protrude through the lipid membrane; the icosahedral capsid encloses a vase-shaped nucleocapsid, in which the diploid RNA is enclosed. Approximate size 100nm. Influenza virus is an enveloped RNA virus, containing nucleocapsid of helical symmetry; spikes of hemagglutinin and neuraminidase protrude from the lipid bilayer. Approximate size 100–200nm. Rabies virus is a helical RNA nucleocapsid with a bullet-shaped lipoprotein envelope, in which approximately 200 GPs are embedded. Approximate size 150nm. (The diagram is not to relative scale.) *Adapted from Collier and Oxford[4] by permission of Oxford University Press.*

TABLE 1-5 -- Classification of viruses.

CLASSIFICATION OF VIRUSES				
Family name	**Example**	**Genome size (kb) and polarity (+ or -)**	**Morphology**	**Envelope**
DNA viruses				
Single-stranded				
Parvoviridae	Human parvovirus B19	5 (±)	Icosahedral	No
Mixed-stranded				
Hepadnaviridae	Hepatitis B	3 (±)	Icosahedral	Yes
Double-stranded				
Papovaviridae	Wart virus	8 (±)	Icosahedral	No
Adenoviridae	Adenovirus	36–38 (±)	Icosahedral	No
Herpesviridae	Herpes simplex	120–220 (±)	Icosahedral	Yes
Poxviridae	Vaccinia	120–280 (±)	Complex	Yes
RNA viruses				
Single-stranded				
Picornaviridae	Poliovirus	7.2–8.4 (+)	Icosahedral	No
Togaviridae	Rubella	12 (+)	Icosahedral	Yes
Flaviviridae	Yellow fever	10 (+)	Icosahedral	Yes
Coronaviridae	Infectious bronchitis	16–21 (+)	Helical	Yes
Rhabdoviridae	Rabies	13–16 (-)	Helical	Yes
Paramyxoviridae	Measles	16–20 (-)	Helical	Yes
Ozthomyxoviridae	Influenza	14 (-)	Helical	Yes
Bunyaviridae	California encephalitis	13–21 (-)	Helical	Yes
Arenaviridae	Lassa fever	10–14 (-)	Helical	Yes
Retroviridae	HIV-1	3–9 (+)	Icosahedral	Yes
Filoviridae	Marburg, Ebola	19 (-)	Helical	Yes
Double-stranded				
Reoviridae	Rotavirus	16–27 (±)	Icosahedral	No

Negative-strand RNA viruses

The early phase is characterized by 'primary transcription' of the infecting genome by an RNA-dependent RNA polymerase. Primary transcription generates a positive-strand RNA species, which can act as a mRNA for viral protein synthesis. In the late phase of genome replication, transcription and viral protein synthesis are simultaneously amplified.

In the case of the ambisense viruses, such as arenaviruses and some members of the bunyaviruses, the situation is somewhat more complex. The intermediate in genome-replication, positive-strand RNA species also acts as a template for mRNA synthesis. This transcription strategy does not, however, result in the synthesis of complementary mRNA, because half of the genome is transcribed in one polarity and the other half in the opposite polarity.

DNA viruses

In the early phase, the virus 'takes the decision' whether or not to pursue exponential replication of its genome. If the cellular physiology favors virus amplification, the early phase is used to create the conditions that allow efficient DNA synthesis and the cellular S phase is induced. Alternatively, viral gene expression is confined to the functions that prevent the efficient genome synthesis and the virus remains in a latent form.

In the case of ssDNA viruses (e.g. parvoviruses), the incoming genome is first used to express proteins that permit the synthesis of the complementary DNA strand.

Double-stranded DNA is an obligatory replication intermediate.

The late phase is devoted to the accumulation of the structural components of the virion.

Viruses using reverse transcription

In the case of the retroviruses, genome synthesis takes place in two distinct steps:

- In a preliminary step, the viral RNA genome enters the cell together with the viral reverse transcriptase, which was synthesized and incorporated into the virion during the previous infection cycle.
- The RNA genome is converted to a dsDNA copy by reverse transcriptase and integrates into the cellular chromosome as a proviral genome. This remains in the 'dormant state' as long as the cells are quiescent, which represent conditions that do not favor virus multiplication.

In the case of the more complex retroviruses (e.g. spumaviruses, lentiviruses), transcription by the cellular RNA polymerase II of the integrated viral DNA first produces multispliced mRNAs, which direct the synthesis of regulatory proteins. As a consequence of the accumulation of certain regulatory proteins, the processing of the viral transcripts changes and becomes oriented towards the production of unspliced or simply spliced mRNAs representing the viral genomes and serving the synthesis of the structural proteins.

GENERAL PROPERTIES AND CLASSIFICATION OF BACTERIA

Bacteria are small (0.6–4.0μm) unicellular organisms; 3×10^{12} bacteria weigh in the order of 1g. Under optimal physiologic conditions, a bacterium may divide between two and three times per hour. This means that theoretically in one day nearly 300g of bacterial mass can be produced from a single bacterial cell. Such small organisms profit from a favorable cell surface-to-volume ratio, which allows metabolic fluxes largely superior to those attained by the larger eukaryotic cells. Bacteria react very quickly to environmental changes, using regulation at the level of gene transcription to adapt their physiology.

Bacteria were probably the first cells to appear on earth more than 3.5 billion years ago. They have since developed into an overwhelming

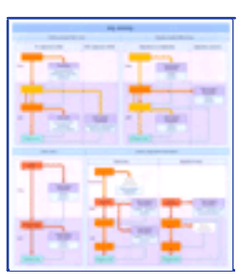

Figure 1-3 Viral 'lifestyles'.

diversity representing the bulk of the world's biomass today. Although evolution has not lead to bacteria associating into multicellular organisms, they are capable of cell-to-cell communication.[5] By using low molecular weight compounds, bacteria have found a way to 'see' how dense their local population is and decide whether or not to activate developmental programs such as plasmid conjugation, light production (in association with deep-sea fish) or virulence gene expression.

Different cell morphologies can be observed with light microscopy (e.g. spherical cocci, rod-shaped bacilli, curved vibrios). Electron

Figure 1-4 Bacterial cell walls. (a) *Mycoplasma pneumoniae* has a single membrane, made up of phospholipids and membrane proteins. (b) In Gram-positive organisms the cytoplasmic membrane is covered with a thick layer of peptidoglycan; chains of lipoteichoic acid protrude outside. (c) The cell wall of a Gram-negative rod is more complex. The layers are: the cytoplasmic membrane; the periplasmic space; a layer of peptidoglycan, which is thinner than that in Gram-positive bacteria; and an asymmetric outer membrane. The inner leaflet of the outer membrane is made of phospholipids. The outer leaflet has lipopolysaccharides as its principal lipids; porins, which are channel-forming proteins often organized as trimers, allow the penetration of hydrophilic molecules through the outer membrane. (d) The peptidoglycan of *Staphylococcus aureus* has polysaccharide chains ('backbone') that are alternating residues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). Tetrapeptides are attached to MurNAc and are linked together by pentaglycines bridging the L-lysine of each tetrapeptide chain to the D-alanine of the neighboring one.

microscopy unveils a distinctive cell wall, a simple nuclear body without a nuclear membrane and the presence in the cytoplasm of ribosomes and mesosomes, sometimes granules of reserve material, but no endoplasmic reticulum and no organelles such as mitochondria or chloroplasts.

Bacterial dichotomy revealed by a simple staining technique

In 1884, the Danish bacteriologist Hans-Christian Gram developed a simple staining technique that distinguishes two types of bacteria: the Gram-positive and the Gram-negative bacteria. The distinction is based on the ability of one group of bacteria, the Gram positives, to retain a crystal-violet-iodine dye in the presence of alcohol or acetone. Gram-negatives lose the dye and can be counterstained with other dyes such as fuchsin. This simple observation turned out to reflect distinctive structures. Gram-positive bacteria characteristically have a thick wall made up mainly of a vast molecule of peptidoglycan, with protruding chains of teichoic acids. Gram-negative bacteria have an additional membrane (the outer membrane) surrounding the peptidoglycan skeleton in the periplasm (Fig. 1.4). *Escherichia coli* is an example of a Gram-negative bacterium; it is rod shaped and growing cells are between 2 and 4μm long.

The rigid cell wall determines the shape of bacteria and allows them to resist the osmotic pressure caused by the large difference in solute concentration between the cytoplasm and the environment. *Mycoplasma* spp. lack peptidoglycan and thus have neither a rigid wall nor a defined shape.

Organization of the bacterial cell

The bacterial cytoplasm does not contain physically separated compartments. Thus DNA replication, transcription, protein synthesis, central metabolism and respiration all take place in the same environment. Complex biochemical processes may nonetheless be spatially organized in the cell. Transcription of DNA into mRNA and translation of the mRNA into protein are coupled processes. This means that polysomes are linked to the DNA, via the enzyme RNA polymerase (Fig. 1.5). The cytoplasmic membrane not only contains numerous metabolite transport systems, but it is the site of intense enzymatic activity as well. Like eukaryotic cells, bacteria possess efflux systems that allow them to expel unwanted substances from the cytoplasm into the environment.

The genetic information is usually stored in a single chromosome. Bacterial chromosomes vary considerably in size. The *Haemophilus influenzae* chromosome, the first completely sequenced genome of a cellular life form, is 1.83 million base pairs (Mbp) long and encodes 1703 putative proteins. [7] The chromosome of laboratory strains of *Escherichia coli* K12 is approximately 2.5 times bigger (5Mbp), though still rather small if compared with the 30Mbp *Bacillus megaterium* genome, and is more than 500 times the length of the cell (Fig. 1.6).

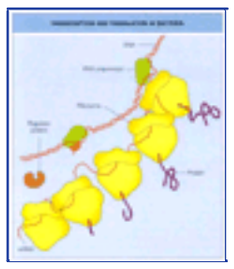

Figure 1-5 Transcription and translation in bacteria (*Escherichia coli*).

The bacterial chromosome codes for polypeptides and stable RNA molecules such as transfer RNA and ribosomal RNA molecules. *E. coli* probably contains well over 1500 different polypeptides with a variety of functions, such as maintenance of membrane structure; transport; respiration; degradation of nutrients; synthesis of amino acids, sugars, nucleotides, lipids and vitamins; and production of polymers such as DNA, RNA, proteins and polysaccharides. Mobile genetic elements, such as plasmids, bacteriophages and transposable elements, are important sources of genetic variation. They supply genes that are not essential for bacterial growth but may offer a selective advantage under specific conditions. Virulence factors and antibiotic resistance elements are frequently associated with these

Figure 1-6 Genetic information in bacteria. This example is *Escherichia coli*. Additional genetic information may be supplied by extrachromosomal elements such as plasmids or bacteriophages. Bacteria may carry a variety of these 'mobile genetic elements', which may transfer readily from one cell to another. The electron micrograph shows a 8.65kb *E. coli* plasmid that confers sulfonamide and streptomycin resistance (left) and a single-stranded derivative of the plasmid (right).

mobile DNA structures. Comparison of the genome sequences of the harmless laboratory strain *E. coli* K12 and the human pathogen *E. coli* 0157:H7, first isolated and identified following a 1982 outbreak of hemorrhagic colitis caused by contaminated hamburgers, revealed impressive differences between the two strains.[8] No less than 1387 new genes were identified in the latter, of which at least some will be involved in virulence.

Transcription and translation in bacteria

Gene expression is usually regulated at the level of transcription initiation by regulator proteins and occasionally by small RNA molecules, which interact with the 'promoter DNA' and with the enzyme RNA polymerase (see Fig. 1.5). The promoter is the site where RNA polymerase opens ('melts') the dsDNA to synthesize an RNA copy of one of the two DNA strands. A sigma factor transiently interacts with the polymerase when it binds the promoter DNA and determines the nucleotide sequence specificity of the enzyme. Bacterial cells produce multiple sigma factors, each controlling the expression of a set of genes. Three types of RNA are produced: regulatory RNA, 'stable' RNA and messenger RNA (mRNA). Stable RNAs include the transfer RNA molecules, which position the amino acids on the ribosomes during protein synthesis and are important structural components of the ribosomes. Messenger RNA molecules are generally quite unstable but are protected from premature degradation by ribosomes, the protein synthesis machines.[6] Transcription and translation are coupled in bacteria; ribosomes bind the mRNA as soon as it 'leaves' RNA polymerase and start protein synthesis by coupling the initiator amino acid (formyl-methionine) to the second amino acid in the coding sequence and uncoupling it from the tRNA molecule. As mRNA elongation proceeds, more ribosomes bind to the messenger RNA to form a 'polysome'. The polypeptides that are produced by the ribosomes fold either spontaneously or with the help of molecular chaperones into their native structures. Bacterial mRNAs generally encode more than one protein. The bacterial protein synthesis machinery is an important target for antibiotics.

Motility

Many bacterial species are equipped with a sophisticated detection system — 'chemotaxis' — which allows them to detect very small variations in concentrations of either valuable or harmful substances in the surrounding environment. [9] Flagella are the effectors of chemotaxis (Fig. 1.7). By changing the direction of flagellar rotation, micro-organisms swim towards sites favorable to survival and growth. Amino acids and sugars are powerful chemoattractants.

Figure 1-7 Flagella and motility in bacteria.

TABLE 1-6 -- Important steps in microbial pathogenesis.

IMPORTANT STEPS IN MICROBIAL PATHOGENESIS
• Encounter
• Attachment to host cells
• Local or general spread in the body (invasion)
• Cell and tissue damage
• Evasion of host defenses
• Shedding from the body

Although many pathogenic species are flagellated, a role for motility in virulence has not been established in all cases.

PATHOGENESIS OF INFECTIOUS DISEASE

The key microbial factors involved in the onset and spread of microbial infection can be identified by carefully analyzing the interaction of the micro-organism with its host (Table 1.6). Molecular techniques have contributed considerably to our present understanding of microbial pathogenesis. Insight into the intimate relationship between host and pathogen will help us find the answers to the all-important questions: how can we eliminate the cause of disease and how can we reduce its harmful effects on the human body?

Each pathogen has its own infection strategy, resulting in the development of a disease pattern with distinct symptoms. In the following sections we shall examine the lifestyles of some pathogenic species.

Contamination

In the developed areas of the world, the majority of human infections are caused by pathogens belonging to the normal microflora of the host (so-called endogenous infections), whereas those caused by exogenous micro-organisms have steadily declined over the past century. In contrast, exogenous infections are still prevalent in poorer areas.[10]

Endogenous infections and normal microbial flora of the human host

The fetus *in utero* is normally sterile but right after birth it starts building up its indigenous microflora, which will quickly outnumber its own cell content; a normal adult carries more than 10 [14] bacteria, which represents roughly 10 bacteria for each eukaryotic cell. In addition to bacteria, we may provide hospitality to an estimated 150 viral species, to fungi, protozoa and worms. The indigenous flora, or 'normal flora', is found in any part of the body exposed to the outside environment — the mouth, nose and the oropharynx, the anterior part of the urethra and vagina and other moist areas of the skin (Fig. 1.8). The human microbial population is especially dense in the large intestine; it has been estimated that each gram of stool specimen contains about 10[12] bacteria. The normal flora is well adapted to its niche and may multiply rapidly under favorable nutritional conditions such as those found in the colon. Although the host's age and physical condition, and especially antibiotic treatment, may induce more or less important variations, the microbial population of the gastrointestinal tract seems to be stable, consisting of more than 99% of obligate anaerobic species. Facultative anaerobes such as *E. coli*, which are frequently used as markers for environmental pollution with human feces, represent less than 1% of the normal flora.

Figure 1-8 Contamination of humans by micro-organisms. Many parts of the body are colonized by normal flora, which can be the source of endogenous infection. Large numbers of micro-organisms are found in moist areas of the skin (e.g. the groin, between the toes), the upper respiratory tract, the digestive tract (e.g. the mouth, the nasopharynx), the ileum and large intestine, the anterior parts of the urethra and the vagina. Other routes are interhuman transmission of infections and exposure to exogenous contamination.

Transient micro-organisms, ingested with food or water, will normally pass through the high flow rate, central region of the gastrointestinal tract without being able to penetrate the mucous gel that overlays the intestinal epithelium or to reach the epithelial surface, which is densely populated by the indigenous flora. The top two-thirds of the ileum are less densely populated by the normal flora, probably owing to a combination of high motility and the acidity of the stomach contents. Population levels of the different areas of the gastrointestinal tract are controlled mainly at the level of metabolic competition, the normal flora being well adapted to the low oxidation reduction potentials and tightly adherent to the mucosal epithelium. Pathogens that use the gastrointestinal tract as a portal of entry must find ways of dealing with the fierce microbial competition.

The skin is much less densely populated by the indigenous flora. In comparison with the gastrointestinal tract, it supplies a considerably less stable microenvironment and one that is often devoid of water. Although impermeable to bacteria, a number of parasites, among them *Schistosoma mansoni* which poses a major health threat in developing countries, can penetrate the intact human skin. Moreover, skin disruptions due to lacerations or insect bites may allow entry of pathogenic microbes into the body.

The large majority of micro-organisms that belongs to the human flora reside on the body surface without creating any damage. This peaceful co-habitation can be called symbiotic if in a 'both sides win' relationship; it is beneficial for both the host and the microbes. Some bacteria find shelter and food in the intestine and, in turn, supply vitamins or digest cellulose. However, symbiotic relationships are rather uncommon. More frequently, the micro-organisms, rather than the host, derive benefit from the association. These inhabitants of our body are called commensals. True commensals do not invade the host and, therefore, do not elicit an immune response. Parasitism constitutes a third category where the micro-organisms, after invading the host, cause an infection.

The separation between parasitism, commensalism and symbiosis is not always clearly defined and the condition of the host may make a big difference. Some micro-organisms, referred to as opportunistic pathogens, are commensals in the majority of people but cause disease in an immunocompromised host. With the progress of medicine, more and more highly immunocompromised hosts can be saved from a premature death, creating at the same time a growing human reservoir for opportunistic pathogens.

The host and its indigenous microflora maintain a delicately balanced relationship that, when disrupted, may lead to the development of infectious disease.

An inevitable consequence of antibiotic treatment is the (local) elimination of susceptible bacteria which, owing to fierce competition, are quickly replaced by antibiotic-resistant species. This phenomenon can cause diseases such as candidiasis, pseudomembranous colitis or severe enterococcal superinfection.

Any rupture of the body surface may favor the development of an infection. *Staphylococcus aureus* on our hands will become an invader and cause an infection as soon as we neglect a local wound. Dirty wounds containing soil particles are readily infected. Organisms with less pronounced pathogenic potential, such as *Staphylococcus epidermidis*, may also be involved.

The case of opportunistic infections in the immunocompromised host, already discussed, is another example of endogenous infection

promoted by a rupture of the balance between the host and its microflora.

Probiotics (live micro-organisms) may help to restore the natural flora. For example, *Saccharomyces boulardii* may be used to treat colitis associated with *Clostridium difficile*.

Exogenous infections

Exogenous infections occur after a direct contamination from microbial populations in the environment:

- in air, soil and water,
- in live animals,
- in other people with infections, and
- in healthy people who are carriers.

Humans are continuously in intimate contact with the large exogenous microbial populations in the air, soil and water, which all harbor highly pathogenic bacteria such as *Clostridium tetani* and *Bacillus anthracis*. Important pathogenic species, such as *Staphylococcus aureus, Clostridium perfringens* and *Clostridium botulinum*, may be present in our food and cause food poisoning.

Live animals represent another important source of exogenous micro-organisms. Infectious diseases of animals that may be transmitted to humans — the so-called zoonoses — include brucellosis, tularemia, plague, toxoplasmosis and rabies. In addition, microbial pathogens can be transmitted from animals to humans by insect vectors like flies, mosquitoes and ticks.

The most important sources of exogenous infections are probably humans themselves (see Fig. 1.8). Well-known examples of human-to-human transmission include the common cold, AIDS, other sexually transmitted diseases, measles, diphtheria, tuberculosis and typhoid fever. Cross-infection in hospitals poses enormous problems, especially in intensive care units.

Several regions of the body may be exposed to exogenous contamination (see Fig. 1.8). Healthy people may be carriers if they harbor and excrete potentially disease-producing micro-organisms. For instance, people recovering from typhoid fever may retain *Salmonella typhi* in the gallbladder and continue to excrete the pathogen in the feces long after recovery from the disease. These people are chronic carriers, even though they usually have mounted a protective immune response against the bacterium.

Exogenous infections, predominant in the past, have dramatically declined in the developed world thanks to improved hygiene, vaccination programs and infection control programs. They are, however, still prevalent in areas with limited resources. Community-acquired pneumonia, diarrheal diseases, malaria, AIDS and

tuberculosis are the main causes of over-mortality in developing countries.

The infection process

Three stages in the infection process may be functionally distinguished:

- attachment of the micro-organism to the target cell(s) and, for intracellular pathogens, entry into the host cell;
- development of the infection, local multiplication of the pathogen and spread of the micro-organism to distant sites; and
- shedding of the organism and transfer to a new host.

Attachment to host cells

Only a few pathogens have the capacity to penetrate our body directly through the skin. Examples include the cercariae of various schistosome species, which can invade the skin with the help of their glandular secretions, and pathogens that enter the body after a bite (e.g. *Simulium* blackfly bite for *Onchocercus volvulus*, anopheles mosquito bite for malaria), intramuscular or intravenous injection, blood transfusion or after injury of the body surface.

Although 'free' micro-organisms exist (for instance, in the lumen of the intestine or in the saliva) most members of the human flora need to be attached to a cellular surface to avoid being swept away by the biologic fluxes such as urine or the passage of the alimentary bolus. For many microbial pathogens, adherence to the epithelial surface of the respiratory, digestive or reproductive mucosa is a compulsory step in pathogenesis.

Adherence

The approach of micro-organisms to an epithelial surface is guided by a balance between attractive and repulsive forces. Eventually, multiple high-affinity contacts between the microbe and the cellular surface may establish a virtually irreversible association between the two. Such contacts may involve nonspecific interactions, such as those between exposed hydrophobic structures on the microbial cell envelope and lipophilic areas on the cell membrane. Glycocalyx, made essentially of a mixture of polysaccharides, and 'slime', produced in particular by *Staphylococcus epidermidis*, may mediate nonspecific adherence between prokaryotic and eukaryotic cells.

Specific adherence involves microbial adhesins on the one side and host cell receptors on the other. Although the interaction between adhesins and cell receptors may be highly specific, this is not always the case. The specificity can be tested by artificially blocking adherence with an excess of purified adhesin or receptor or with antibodies directed against one of these two. The specificity accounts for the early observation that many pathogens distinctively infect certain areas or organs of the body and not others. For instance, *Streptococcus pneumoniae* causes pneumonia but not urethritis, whereas *Neisseria gonorrhoeae* exhibits the opposite pattern of specificity. The receptors for poliovirus, rhinovirus and HIV are expressed only by specific cell types, restricting virus infection accordingly. These and many other examples support the notion that adhesins determine the tropism of microbial pathogens. On the other hand, cell receptors for many organisms are ubiquitous and these organisms (e.g. influenza virus) have no tissue restriction.

Ubiquitous receptors

Fibrinogen, fibronectin, collagen and heparin-related polysaccharides are major components of the extracellular matrix (ECM), which coats the mucosal surface of epithelial cells. Members of the integrin family are involved in the interaction between the ECM and the underlying epithelium. A number of components of the ECM are used as receptors for microbial adhesins. Fibronectin specifically binds fibronectin-binding factors on the cell envelopes of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Treponema pallidum* and *Mycobacterium* spp.; fibrinogen binds groups A, C and G streptococci and a member of the integrin family binds the major invasion factor of *Yersinia pseudotuberculosis*. Their abundance and structural conservation among mammalian species make ECM components ideal targets for bacterial adhesins.

Bacterial adhesins

Close contact between micro-organism and host cell represents an essential step in pathogenesis. It optimizes the interaction of microbial virulence factors with the target cell to allow the pathogen to penetrate or cause local cell damage, or both. Other possible functions of adhesins include modulation of the inflammatory response, adhesin-directed degranulation from mast cells and adhesin-mediated bacterial phagocytosis by neutrophils. Bacteria use two general strategies to attach themselves to host cells: fimbrial and afimbrial adhesion (Fig. 1.9).[11]

Pili and fibrillae

Attachment of bacteria to the plasma membrane can be mediated by filamentous structures protruding from the bacterial surface,

Figure 1-9 Bacterial adherence.

called fimbriae or fibrillae. The classification of these colonization factors is based on morphologic criteria. Fimbriae (or common pili) are rigid hair-like structures with a regular diameter, whereas fibrillae are flexible and have an irregular diameter. These structures are distinct from flagella, which are responsible for bacterial motility (see Fig. 1.7), and sex pili, which are associated with bacterial conjugation.

Twenty different colonization factors have been described for *E. coli*.[12] One of these, the so-called P-pili expressed by uropathogenic *E. coli* strains, mediates adherence of the bacterium to the urinary mucosa to avoid elimination by the urinary flux. P-pili consist of a long and rigid base section attached to an outer membrane scaffold, and a short flexible tip (Fig. 1.10).[13] The rigid section measures about 7nm in diameter, with a central channel approximately 1.5nm wide, and is 1–2µm long. It is composed of hundreds of pyelonephritis-associated (PapA) pilin subunits arranged in a right-handed helix. The pilus tip is 2nm in diameter with a 15nm pitch composed of PapE monomers. The PapG monomer is located at the end of the tip and is the actual adhesin. It recognizes the glycolipid receptor globobiose (a-1–4 linked di-galactose) on the host cell surface. During pilus formation, the tip is assembled and exported first, followed by the

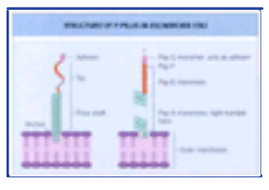

Figure 1-10 Structure of P-pilus in *Escherichia coli.*

addition of the pilin subunits forming the shaft. The assembly of pili requires periplasmic chaperones, which assist in protein folding and assembly but never become part of the pili structure.

Afimbrial adhesins

Afimbrial adhesins, such as lectins (carbohydrate-binding proteins), also mediate tight binding between the bacteria and the host cell but, unlike pili, they do not form supramolecular structures. Similar adhesins exist in viruses, fungi and protozoa. Afimbrial binding has been extensively studied in *Streptococcus pyogenes* (Fig. 1.11). Two surface components are believed to be critical in the colonization of an epithelial surface: lipoteichoic acid and fibronectin-binding protein.

Purified lipoteichoic acid binds to fibronectin and inhibits the binding of *S. pyogenes* to oral epithelial cells. The binding properties are confined to the lipid moiety of lipotechoic acid. Similarly, artificially added fibronectin-binding protein inhibits adhesion of *Strep. pyogenes* to epithelial cells even after the streptococci have been

depleted of lipoteichoic acid.

The complex surface of this micro-organism also includes the M protein.[15] This protein is a major virulence factor but it does not seem to be involved in adherence to epithelial cells, as was previously assumed. However, the M protein binds fibrinogen in a stoichiometric fashion and exerts an antiphagocytic effect, which may partially explain its role in virulence.

Viral adhesion

Viral adhesion and invasion are generally mediated by the same viral proteins and may be considered as a single continuous event. Initial attachment represents the first in a series of steps that ultimately leads to the delivery of the viral genome to its site of replication. Nonenveloped viruses appear to pass or slide through the plasma membrane directly. Enveloped viruses, such as measles and mumps virus, enter the cell after fusion with the plasma membrane.[16] These virions have a fusion protein that initiates the contact between the two membranes. Virus internalization may be mediated by a protein called clathrin, which forms membrane invaginations containing the virion. Once in the cytoplasm, the virus escapes from the clathrin-coated pits to reach the cytosol. The low pH inside the vacuole triggers escape of the virion.

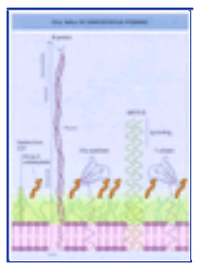

Figure 1-11 Cell wall of *Streptococcus pyogenes.* The proposed model of the M protein is based on current sequence and structural data. ARP, immunoglobulin A receptor protein; FcR, receptor for the Fc portions of immunoglobulin. *Adapted from Kehoe.*[14]

Receptor availability on the cell determines whether a virus particle will bind to its surface. Cell specificity may thus be rather relaxed for viruses that use ubiquitous receptors and, on the other hand, be strongly restricted for viruses that require two or more cellular receptors simultaneously. An example of the latter is HIV; it requires co-expression of CD4 and chemokine receptors for efficient adhesion and invasion of the target cell. Herpes simplex virus 1 has an enveloped icosahedral capsid. The envelope contains at least 12 glycoproteins, of which two (glycoproteins B and C) interact with heparin sulfate on the plasma membrane. A second, specific interaction is probably required between glycoprotein D and an as yet unidentified cellular receptor to trigger fusion of the viral envelope and the plasma membrane. Following fusion, the capsid is released in the cytosol and is transported to the nuclear membrane. Herpes simplex virus first infects epithelial cells of the skin and mucosal surfaces, where the initial replication cycles take place, to pass into axon termini of neurons, the ultimate infection site.

Viral adherence and invasion can be blocked by neutralizing antibodies, which specifically bind the active site(s) of the adhesin(s). However, many viruses have hidden this region in a protein pocket (or 'canyon'), making it physically inaccessible to potentially neutralizing antibodies, thus escaping humoral immunity.

INVASION

Invasive and noninvasive micro-organisms

Many micro-organisms, including those of the natural flora, remain at the epithelial surface without invading the underlying tissue (Table 1.7). This type of colonization is usually harmless although it may, in some cases, induce damage to adjacent cells through the production of toxins or elicit a local inflammatory or allergic response. Nonpenetrating micro-organisms include *Streptococcus pyogenes* and *Corynebacterium diphtheriae*, which cause pharyngitis, *Mycoplasma pneumoniae*, which causes atypical pneumonia, and *Trichomonas vaginalis*, a cause of vaginitis.

Other micro-organisms gain access to deeper tissues only after a physical or chemical injury of the epithelial barrier. *Staphylococcus aureus*, a harmless microbe when on the skin, may become a dangerous toxin-producing pathogen once it penetrates the body.

Invasive micro-organisms exhibit the capacity to penetrate the target tissue to which they adhere without the need for local disruption of the protective epithelium. Invasive bacteria have developed the capacity to enter host cells, which are not naturally phagocytic. Penetration into these 'nonprofessional' phagocytes is achieved by:

- specific attachment to the host cell; and
- induction of local rearrangements of the cytoskeleton, through polymerization and depolymerization of actin.

This results in the formation of pseudopod-like structures, which eventually engulf the pathogen into the host cell (Fig. 1.12). In order to induce ingestion by the host cell, the pathogens may produce surface proteins called invasins.

In some cases infection remains confined to the epithelial surface (see Table 1.7), but in others the micro-organism may be transported across the superficial epithelium to be released into subepithelial space. This process is called transcytosis and involves the host cell actin network (see below). After transcytosis, the underlying tissues may be invaded and infected and the infection may eventually spread all over the body (e.g. *Neisseria meningitidis* may get across the pharyngeal epithelium and cause meningitis, and *Salmonella typhi* may cross the intestinal epithelium and cause typhoid fever). For a more detailed analysis of the mechanisms of invasion, we shall use the example of enteroinvasive pathogens.

Enteroinvasive pathogens and the membranous cell gateway

Acute infectious diarrhea may cause the clinical spectrum of dysentery and bloody diarrhea. It occurs when the pathogen invades the intestinal mucosa and causes structural damage to the intestine. The immunologic protection of the intestine is performed by the gut-associated lymphoid tissues, which are separated from the intestinal lumen by a specialized follicle-associated epithelium. In the follicle-associated epithelium, membranous cells (M cells) play a prominent role because they are specialized in the transport of antigens. Enteroinvasive viruses, protozoa and bacteria exploit the transport facilities provided by M cells to invade the host. Entry (and passage) of M cells by these pathogens is preceded by adherence, in the case of reovirus type 1 through the specific adhesins s1 or µ1 of the outer capsid. Enteroinvasive bacteria such as *Salmonella*, *Shigella* and *Yersinia* spp. appear to distinguish between different subsets of M cells. Membranous cells produce glycocalyx, which contains a distinctive profile of lectin-binding sites. Diversity in lectin-binding sites between different locations of the gut may account for the tropism of enteric pathogens, such as the preferential colonization of colonic mucosa by *Shigella* spp. rather than *Salmonella* spp., which are more commonly found at the end of the ileum. Following adherence, the interactions with the M cells vary according to the pathogen (Fig. 1.13). Enteroadherent *E. coli* is not internalized and

TABLE 1-7 -- Interaction of micro-organisms with epithelial cells.

INTERACTION OF MICRO-ORGANISMS WITH EPITHELIAL CELLS			
	Order	**Micro-organism**	**Disease**

Generally confined to epithelial surfaces	Bacteria	*Bordetella pertussis*	Pertussis
		Chlamydia trachomatis	Trachoma, urethritis
		Corynebacterium diphtheriae	Diphtheria
		Streptococcus pyogenes	Uncomplicated pharyngitis
	Viruses	Coronaviruses	Common cold
		Rhinoviruses	Common cold
		Rotaviruses	Diarrhea
	Fungi	*Candida albicans*	Thrush
		Trichophyton spp.	Athlete's foot
	Protozoa	*Giardia lamblia*	Diarrhea
		Trichomonas vaginalis	Vaginitis
Enter through the epithelium	Bacteria	*Mycobacterium tuberculosis*	Tuberculosis
		Brucella melitensis	Brucellosis
		Neisseria meningitidis	Meningitis
		Salmonella typhi	Typhoid fever
		Treponema pallidum	Syphilis
		Yersinia pestis	Plague
	Viruses	Measles virus	Measles
		Rubella virus	Rubella
		Varicella	Chickenpox
		Poliovirus	Poliomyelitis
	Fungi	*Cryptococcus* spp.	Cryptococcosis
		Histoplasma spp.	Histoplasmosis
	Protozoa	*Toxoplasma gondii*	Toxoplasmosis
		Entamoeba histolytica	Liver abscess

hence is not invasive. *Vibrio cholerae* is taken up and transported by the M cells but rapidly killed thereafter. It is considered to be invasive at the cellular level but not at the clinical level.

Detailed molecular analyses of virulence factors produced by enteroinvasive *Shigella* spp. have revealed that all virulent species harbor a 220kb plasmid, of which a 31kb fragment, encoding 32 genes, is both necessary and sufficient for invasion of epithelial cells.[18] The four invasion plasmid antigens (IpaA, B, C and D) encoded by this fragment are key players in the invasion process. Secretion of the 'Ipa complex' is induced by contact with the target cells and is accomplished by a specialized entry-associated secretion apparatus encoded by a set of genes (*mxi*, *spa*) located in the same region of the virulence plasmid. The *Salmonella* spp. entry functions are clustered in a 35–40kb region of the chromosome at centisome 63.[19] Such clustering of virulence genes is a typical example of a genetic 'pathogenicity island'.[20] These pathogenicity islands are often

Figure 1-12 Opsonization and phagocytosis of bacteria. Bacteria are covered with IgG, specific for surface antigens. Bound IgG interacts with the phagocyte Fc?-receptor and pseudopods are formed, engulfing the bacterium into the host cell.

transmissible from one microbial species to another as a single DNA fragment by way of mobile genetic elements such as plasmids, transposons or bacteriophages. In *Vibrio cholerae* the A- and B-subunits of cholera toxin are encoded by a bacteriophage that integrates into the bacterial genome and may, in concert with yet another bacteriophage, facilitate horizontal spread of the toxin genes.[21]

The *Salmonella* and *Shigella* spp. genes involved in invasion of the eukaryotic host cell are homologous and have been remarkably well conserved with respect to both the individual coding sequences and their genetic organization (Fig. 1.14).[22] Using a needle-like complex, [23] the bacteria translocate a number of effector proteins into the cytosol and the plasma membrane of the target cell.[24] Some of these effector proteins specifically modify the activities of cellular GTPases, inducing the alterations of the cytoskeleton required for bacterial internalization. [25] The interplay between target cell and bacteria during the invasion processes of

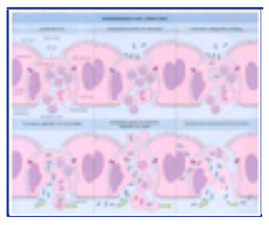

Figure 1-13 Enteropathogen-M cell interactions. (a) An uninfected M cell, enclosed between two adjacent enterocytes. The basolateral side forms a pocket where lymphocytes and macrophages are located. (b) Enteroadherent *Escherichia coli* forms microcolonies at the M cell surface, but is not internalized. (c) *Vibrio cholerae* undergoes transcytosis but is efficiently phagocytosed in the submucosa. (d) *Campylobacter jejuni* and *Yersinia* spp. undergo transcytosis, replicate in the submucosa and disseminate. (e) *Salmonella* spp. are transported across M cells, leading to destruction of the M cell. (f) *Shigella flexneri* is endocytosed by M cells, escapes into the cytoplasm, replicates, is propelled by actin tails and spreads to adjacent enterocytes. *Adapted from Siebers and Finlay.*[17]

these two genera is different, however. Another important difference between the pathogenic lifestyles of these two bacterial species involves the intracellular fate of the bacteria. Once internalized, the bacteria find themselves enclosed by a host cell membrane in an endocytic vesicle, deprived of nutrients. In professional phagocytes, such as macrophages and dendritic cells, these endosomes are programmed to fuse to a prelysosome, releasing the hydrolytic enzymes required for the destruction of the bacterial cell. Soon after entry into the cell, *Shigella* spp. escape from the endosome into the nutritious cytoplasm, but *Salmonella* spp. have adopted an entirely different strategy. Salmonellae modify the endocytic pathway of the host cell by means of virulence factors encoded largely, but not exclusively, by a second pathogenicity island,[26] thus avoiding exposure to bactericidal mechanisms of the cell. Although only some of the cellular targets of the translocated bacterial virulence proteins have been identified to date, it is clear that the physiology of the infected cell is profoundly modified to suit bacterial growth and maintenance.

Actin-based intracellular motility of microbial pathogens

Enteroinvasive micro-organisms use passive actin modification to invade nonprofessional phagocytes such as epithelial cells. Local modification of the cytoskeleton is induced by the pathogen by diverting the signaling pathways of the host cells. In addition, some bacteria use active actin modification to move in the cytoplasm. They induce the formation of actin cross-linked filaments, which assemble in characteristic 'comet-like tails' (Fig. 1.15).[27] [28] Elongation of the actin filaments generates sufficient force to move the micro-organisms through the cytoplasm at rates of 2–100µm/min. Pathogens

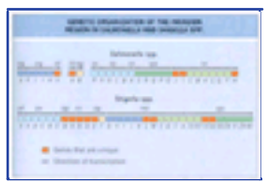

Figure 1-14 Genetic organization of the invasion region in *Salmonella* and *Shigella* spp. Identical patterns indicate topologically conserved blocks of genes. Each genus has genes that are unique. Despite remarkable genetic similarities, the invasion strategies of the two bacteria are quite different (see Fig. 1.13). *Adapted from Galan.*[19]

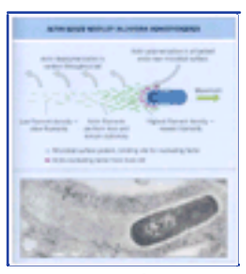

Figure 1-15 Actin-based motility in *Listeria monocytogenes.* The bacterium moves forwards at the rate of actin-filament growth behind the pathogen. *Adapted from Sanders and Theriot.*[27] The EM shows a section of a CaCo-2 cell infected with *Listeria monocytogenes*; the bacterium protrudes into the cytoplasm of an adjacent cell; protrusion is limited by a double membrane (arrowheads).

that use the actin skeleton for intracellular spread include bacteria (e.g. *Listeria monocytogenes*, *Shigella* spp., *Rickettsia* spp.) and viruses (e.g. vaccinia, measles, rabies).

The intracellular life cycle of *L. monocytogenes* illustrates this strategy (Fig. 1.16). [29] [30] Under natural conditions, *Listeria* first penetrates enterocytes and probably M cells and subsequently spreads through the body to infect a variety of host cells, including endothelial cells, Kupffer cells, hepatocytes and phagocytes. Entry is facilitated

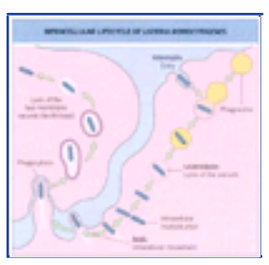

Figure 1-16 Intracellular life cycle of *Listeria monocytogenes.*

by the products encoded by the internalin (*inl*) family of genes, which seem to confer tropism for different cell types. Once inside the cell, *L. monocytogenes* remains confined to the phagosome for only a short time. Following lysis of the endosomal membrane, it escapes into the cytosol. Membrane lysis is achieved by a production of listeriolysin-O, which attains maximum activity under the acidic conditions of the intravacuolar environment. Once in the cytosol, the bacteria multiply and migrate towards the plasma membrane by using the actin-based mechanism as described above. Actin polymerization is mediated by the *L. monocytogenes* protein ActA, localized at one end of the bacterium. For spread to neighboring cells, *L. monocytogenes* requires bacterial lecithinase and phospholipase C, which stimulate lysis of the two membranes that separate the bacterium from the cytoplasm of the newly infected cell. Interestingly, most of the virulence genes associated with this process are clustered in a single region of the *L. monocytogenes* chromosome.

Subepithelial invasion and spread through the body

Invasion from the site of infection can only be achieved by micro-organisms that effectively resist the host defense mechanisms in the subepithelial space, most prominently phagocytosis.

The lymphatic network is often used as a means of transport and successful micro-organisms may rapidly reach the nearest local lymph nodes, which have an important filtering function. In the lymph nodes, resident macrophages and polymorphonuclear cells actively fight the invaders. As a result the first line of lymph nodes are often inflamed. If the invading micro-organism is sufficiently virulent or present in sufficiently large numbers, it may pass into efferent lymphatic vessels to be conducted to the bloodstream. The result is primary bacteremia or viremia. Some microbes can enter directly into the blood vessels via an injury. A typical example is provided by viridians streptococci, which enter the bloodstream during dental extraction, enabling them to infect a cardiac valve and produce endocarditis. Insect bites (malaria and arthropod-borne viruses) or damage to the blood vessel wall inflicted by hemorrhagenic viruses are alternative ways to circumvent the body's first line of defense: the mucosal immune system.

Once in the bloodstream, the micro-organisms may circulate as either an extracellular or an intracellular species. Pathogens have been found in polymorphonuclear cells (staphylococci), lymphocytes (HIV), macrophages (*Mycobacterium tuberculosis*) and even in red blood cells (*Plasmodium* spp.), which provide protection against potent humoral factors in the serum, such as complement.

Infection of distant target organs

Transported by the bloodstream, the invasive micro-organisms can reach distant target organs and create infective metastases throughout the body. Almost any tissue can be reached, but the organs containing abundant capillary and sinusoid networks (e.g. lungs, liver, kidneys) are especially exposed, because blood flows slowly at these sites and transported micro-organisms get the opportunity to adhere and establish an infection. From the target organs, the invaders may produce a secondary bacteremia or viremia, in which microbial counts in the blood are generally higher than during primary infections.

The example of measles virus

Measles virus adheres to the CD46 receptor.[31] Infection of a nonimmune host proceeds by invasion of the epithelial surface of the respiratory mucosa, where the virus undergoes limited replication. The virus subsequently migrates to the regional lymph nodes and those micro-organisms that survive local macrophage attack will enter the bloodstream, causing a primary viremia. When the infection becomes generalized, several target areas are affected, including the lungs, the skin and the central nervous system. At this stage, the virus undergoes further replication in the leukocytes (causing leukopenia) as well as in the lymphoid tissues and in the target organs. The result is secondary viremia and fever. Rash appears later and is due to the destruction of infected cells by cytotoxic T cells rather than to a direct cytopathic effect of the virus on skin cells.

These pathogenic steps correspond to different clinical periods. During the initial encounter with the virus, the viral spread to target organs and the primary viremia, there are no clinical symptoms; this corresponds to the incubation period, which lasts for about 10 days. After this, disease develops and about 4 days later (i.e. typically 14 days after contamination) the skin rash occurs, corresponding to local inflammation and cellular immunity.

Serum resistance in Neisseria gonorrhoeae *and* Salmonella *spp.*

Immunocompetent hosts contracting gonorrhea usually do not develop a systemic disease. Human serum contains a variety of humoral factors, including the complement system, which provide effective protection against *N. gonorrhoeae*.

Complement is both a major effector of specific humoral immunity and a participant in natural (innate) immunity. The complement system involves some 30 serum proteins and is aimed at extracellular pathogens. It may be activated by one of two convergent pathways. The 'classic pathway' is activated by specific binding of either two IgG or one IgM molecule to a circulating target. The first component of the complement system (C1) binds in a co-operative fashion to two adjacent Fc fragments of the immunoglobulin attached to the target and initiates a proteolytic activation cascade, culminating in the erection of a multiprotein complex that strongly stimulates phagocytosis. The complement system may directly induce cytolytic activity through the membrane attack complex anchored on the microbial envelope. The

'alternative pathway' does not depend on the availability of specific immune globulins and is initiated directly by fixation of complement proteins to specifically recognized structures on the target surface. The outcome of both pathways is in many respects similar.

The ability to resist complement killing contributes to the virulence of *Salmonella* spp. Complement resistance can be provided by very long O-side chains of *Salmonella* lipopolysaccharide (LPS). In addition, the outer membrane protein Rck (resistance to complement killing) may provide protection of the bacterium against complement-mediated killing. The last step in the assembly of the membrane attack complex onto the bacterial membrane (insertion of polymerized C9 into the outer membrane) is prevented by Rck. The *rck* gene is located on the large virulence plasmids of *Salmonella dublin* and *Salmonella typhimurium*.

Some strains of *N. gonorrhoeae* are serum resistant and do cause disseminated infection in normal hosts. These strains are protected against complement by changes in the carbohydrate portion of their lipo-oligosaccharide. In some of the resistant strains, a galactose residue of LPS covalently binds an activated form of sialic acid from human blood, thus abolishing complement activation.[32] In addition, individuals with genetic defects in the terminal complement components (C6–C9) are unable to assemble the membrane attack complex. They are more susceptible to disseminated neisserial infections although there is a lower case mortality.

Cell and tissue damage induced by micro-organisms

Infectious disease is often characterized by cell and tissue damage. Paralysis in poliomyelitis, exanthem in varicella, gastroduodenal ulcers in *Helicobacter pylori* infections and bloody diarrhea in shigellosis all result from damage caused directly or indirectly by micro-organisms. Cell damage can be generated by a variety of different mechanisms (Table 1.8).

Bacterial toxins

Bacteria produce a large diversity of toxins, which have been classified according to their mode of action (Table 1.9 , Fig. 1.17). Traditionally, exotoxins (or excreted toxins) are distinguished from endotoxin (equivalent to the lipopolysaccharide of the outer membrane of Gram-negative bacteria). However, some of the so-called exotoxins are actually intracellular and are released into the environment only after cell lysis. The pneumolysin of *Streptococcus pneumoniae*, for example, is cytoplasmic, the adenylate cyclase of *Bordetella pertussis* is associated with the cytoplasmic membrane and the heat-labile toxin I (LT-1) from *E. coli* is periplasmic. The genetic information that encodes bacterial toxins is frequently carried on mobile DNA elements, which may readily pass from one microbial host to another. The toxins associated with diphtheria, botulism and scarlet fever, as well as Shiga-like toxin in *E. coli*, are encoded by temperate bacteriophages. Genes for LT-I and methanol-susceptible heat-stable toxin (Sta) of *E. coli* are carried on plasmids.

Toxins deregulate the physiology of the host cell before or during bacterial adhesion and invasion. The bacteria may profit from the induced damage, which compromises the cellular defense against the intruder and release of nutrients from the cytosol.

The diphtheria toxin as example of an A–B toxin

Diphtheria toxin belongs to the so-called A–B toxins (Fig. 1.18). These toxins are bifunctional molecules. Portion A mediates the enzymatic activity responsible for the toxicity after internalization into the target cell, but cannot penetrate by itself. Portion B is not toxic but binds to a cell receptor localized on the cell surface and mediates the translocation of the A chain into the cytosol. Portion B accounts for the cell specificity of the A–B toxins. The receptor recognized by the B chain of diphtheria toxin is a heparin-binding precursor of epidermal growth factor. Epidermal growth factor is an important hormone for growth and differentiation of many different cell types.

Uptake of diphtheria toxin proceeds via receptor-mediated endocytosis. Acidification of the endocytic vesicle induces a confirmational

TABLE 1-8 -- Mechanisms of cell and tissue damage produced by micro-organisms.

MECHANISMS OF CELL AND TISSUE DAMAGE PRODUCED BY MICRO-ORGANISMS		
	Mechanism	**Examples**
Direct damage by micro-organisms	Production of toxins	See Table 1.9
	Production of enzymes	Proteases, coagulase, DNAses produced by *Staphylococcus aureus*
	Apoptosis	HIV ($CD4^+$ T cells); *Shigella flexneri* (macrophages)
	Virus-induced cytopathic effects:	
	Cell lysis	Cytomegalovirus
	Formation of syncytium	Respiratory syncytial virus
	Inclusion bodies	
	Intracytoplasmic	Rabies
	Nuclear	Herpesviruses
	Transformation	Human papillomaviruses type 16
Damage via the host immune response	Cytotoxic T cells and natural killer lymphocytes	Production of the measles rash
	Autoimmunity	Acute rheumatic fever
	Immediate hypersensitivity	Rashes associated with helminthic infections
	Cytotoxic hypersensitivity	Cell necrosis induced by hepatitis B
	Immune complexes	Glomerulonephritis in malaria
	Delayed type hypersensitivity	Tuberculous granuloma

TABLE 1-9 -- Examples of bacterial toxins.

EXAMPLES OF BACTERIAL TOXINS					
Toxin type	**Example of sources**	**Toxin**	**Targets**	**Mechanisms**	**Effects**
Endotoxin (LPS, lipid A)	Gram-negative bacteria	Endotoxin	Macrophages, neutrophils, lymphocytes, plasma components	Activation of target cells, complement; release of IL-1, TNF, kinins	Septic shock
Membrane-disrupting toxins	*Staphylococcus aureus*	a-Toxin	Many cell types	Formation of pores	Tissue necrosis
	Listeria monocytogenes	Listeriolysin	Many cell types	Formation of pores at acidic pH	Escape from the phagosome
	Clostridium perfringens	Perfringolysin-O	Many cell types	Phospholipase (removes polar head groups from phospholipids)	Gas gangrene

A–B-type toxins	*Clostridium tetani*	Tetanospasmin	Synaptic transmission	Inhibits release of inhibitory neurotransmitters	Spastic paralysis
	Clostridium diphtheriae	Diphtheria toxin	Many cell types	ADP ribosylation of EF-2	Paralysis
	Vibrio cholerae	Cholera toxin	Intestinal cells	ADP ribosylation of adenylate cyclase, leading to rise in cyclic AMP	Profuse watery diarrhea
Superantigen	*Streptococcus pyogenes*	Streptococcal pyogenic exotoxin	T cells, macrophages	T cell stimulation, release of IL-1, IL-2, TNF; possible enhancement of LPS activities	Fever, eruption, toxic shock-like syndrome
	Staphylococcus aureus	Toxic shock toxin	T cells, macrophages	Same as streptococcal pyrogenic toxin	Toxic shock syndrome

change in the enclosed holotoxin, enabling the A subunit to traverse the membrane and reach its cytoplasmic target. The A subunit of diphtheria toxin catalyzes ADP-ribosylation of the elongation factor-2 (EF-2). After attachment of the ADP-ribosyl group, EF-2 becomes inactive, causing the death of the target cell.

Only *Corynebacterium* lysogenic for temperate bacteriophage carrying the *tox* gene produce the toxin. The *tox* gene is under the control of the repressor protein DtxR, which forms a complex with iron, DtxR-Fe (Fig. 1.19), binds DNA and represses *tox* expression. Thus diphtheria toxin is only synthesized under low iron conditions, suggesting that it may be produced to stimulate iron release from target cells. Interestingly, the *Pseudomonas aeruginosa* exotoxin A has a very similar structure, but uses a different cell receptor: the a-2 macroglobin low-density lipoprotein receptor. Like diphtheria toxin, exotoxin enters the cell via receptor-mediated endocytosis but the toxin is released only after passage through the Golgi system.

Hydrolyzing enzymes

Microbial pathogens often secrete hydrolyzing enzymes, such as proteases, hyaluronidases, coagulases and nucleases. As such, these enzymes cannot harm the host cells and they are therefore not considered to be toxins. However, in the context of an ongoing infection

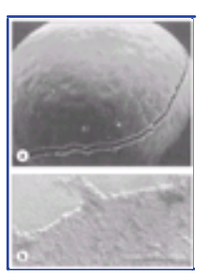

Figure 1-17 Action of bacterial toxins. (a) *Xenopus* oocyte treated with the cytolytic delta toxin (perfringolysin) of *Clostridium perfringens*. (b) Rabbit erythrocyte exposed to a very small quantity of streptolysin-O, produced by *Streptococcus* A,C,G. Hemoglobin escapes from sites of membrane rupture. *Courtesy of Dr J Alouf.*

they are assumed to facilitate colonization of host tissues by a variety of mechanisms, such as proteolysis of IgA; fluidification of pus; induction of plasma clotting, which may hinder the influx of phagocytes into the focus of infection; and a general disorganization of the host tissue structure. The release of hydrolytic enzymes by phagocytes damaged by a bacterial toxin may have similar effects.

Apoptosis

Apoptosis is a process in which the cell activates an intrinsic suicide program. It plays a key role in processes like organ development, tissue repair and maintenance of the dynamic equilibrium of the immune system. These processes critically depend on the generation of self-limiting organized structures through addition of new cells and elimination of 'old' cells. The morphologic changes associated with apoptotic death are a reduction of the volume of the cytosol and nuclear condensation (Fig. 1.20). The genome is fractionated by an endonuclease activity that cuts the DNA into multiples of 180–200bp. [33] Finally, the remains of the cell are removed by phagocytosis.

Apoptosis is distinct from necrosis. In necrosis, which may, for example, be induced by a bacterial toxin, the cell does not participate actively in its own death. Another important difference between apoptosis and necrosis is that the former does not usually induce proinflammatory responses that usually accompany the latter.

Many viruses trigger apoptotic death of the infected host cell. For instance, apoptosis seems to contribute to the depletion of $CD4^+$ T cells, both in cell culture and in HIV-infected people.[34] Apoptotic cells have also been observed in infections caused by Epstein-Barr virus and adenoviruses. Bacteria can also induce apoptosis. *Bordetella pertussis*, the agent of whooping cough, triggers macrophage apoptosis by interfering with cellular regulation at the level of the cytoplasmic second messenger cyclic AMP (cAMP).[35] The bacterium induces high levels of cytoplasmic cAMP, favoring the induction of apoptosis. *Shigella flexneri*, the etiologic agent of dysentery, can kill macrophages by apoptosis. Cell death is induced by invasion plasmid antigen B (IpaB) encoded by the *Shigella* virulence plasmid (see Fig. 1.14). [36] The *Shigella* IpaB protein binds to the host cytoplasmic enzyme interleukin-1ß converting enzyme (caspase-1) and activates it.[37] Caspase-1 activates the proinflammatory cytokines IL-1 and IL-18 by proteolytic cleavage and initiates one of the proapoptotic pathways. In *Salmonella* infection of macrophages and dendritic cells, the IpaB homolog SipB similarly activates caspase-1 to stimulate secretion of the proinflamatory

Figure 1-18 Diphtheria toxin synthesis and mode of action. (Top) The 25-residue leader sequence is cleaved off by the bacterial leader peptidase; the A and B subunits are generated from the precursor protein by a 'trypsin-like enzyme'. Once in the cytoplasm of a targeted eukaryotic cell, the A chain, responsible for ADP-ribosyl transfer, is disconnected from the B chain, responsible for receptor binding and membrane insertion. (Bottom) The B chain binds to a specific receptor on the eukaryotic cell. After endocytosis, acidification in the endosome induces insertion of the B chain into the endosomal membrane and translocation of subunit A into the cytosol, where it catalyzes the ADP ribosylation of EF-2. As a result, protein synthesis is inhibited and the targeted cell dies.

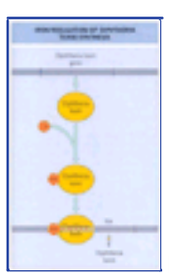

Figure 1-19 Iron regulation of diphtheria toxin synthesis. High iron concentrations in the environment repress the synthesis of diphtheria toxin: when bound to iron, DtxR-Fe acts as a transcriptional repressor of the *tox* gene.

cytokine IL-18 and induce apoptosis.[38] Timely induction of apoptosis in dendritic cells may well allow Salmonellae to exploit the mobility of these host cells to migrate away from the intestinal mucosa and establish systemic infection.

Virus-induced cytopathic effect

Most viruses severely damage the cells they infect, sometimes inducing distinctive cytopathic effects that may be useful in diagnosis (Fig. 1.21). A large variety of mechanisms may be involved in these cytopathic effects, some being direct consequences of the presence of the virus, others resulting from the host immune response.

Virus infection may result in the intracellular accumulation or release of a number of small molecules, including reactive oxygen and nitrogen intermediates, which may play an important role in certain types of cell destruction, particularly in macrophages. Rotavirus, cytomegalovirus and HIV can produce significant increases in intracellular calcium, which seems to be a common pathway for the development of irreversible cell injury.

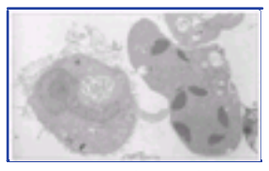

Figure 1-20 Apoptosis induced by Sendai virus. Morphologic changes in the apoptotic Sendai infected cell (right) include the typical condensation of chromosomal DNA. *Courtesy of Dr Dick Compans and Dr Kiyoshi Tanebayashi.*

In addition to cell lysis, other cytopathic effects exist. Paramyxoviruses such as respiratory syncytial virus, parainfluenza viruses, and measles virus, as well as herpesvirus and some retroviruses, cause the formation of multinucleated giant cells. The formation of these giant cells (syncytia) is mediated by virus-encoded fusion proteins. Viral infection can also produce eosinophilic or basophilic inclusion bodies, which appear in the cytoplasm or the nucleus. Inclusion bodies may represent aggregations of mature virions, areas of altered staining at sites of viral growth or simply degenerative changes.

Host cell transformation by viruses results in increased cellular multiplication rates and disorderly growth. It may be caused by DNA viruses (for instance, Burkitt's lymphoma associated with Epstein-Barr virus) or retroviruses (adult T-cell leukemia caused by human T-cell lymphotropic virus type 1). Malignancy is induced by the expression of viral oncogenes. High-risk human papilloma viruses (such as HPV16) usually only transiently infect the basal cells of the cervical mucosa, to be cleared by the host immune response. However, in a minority of women persistent infections with high levels of viral DNA may develop. These persistent infections may eventually progress to invasive carcinoma.[39] Two viral proteins, E6 and E7, play co-operative roles throughout viral multiplication, pathogenesis and malignant transformation, which correlates with integration of viral DNA into the genome.[40] By specifically interacting with an impressive variety of cellular targets these proteins remodel the cell cycle and modify cell differentiation of the basal cells of the cervical epithelium, creating an environment

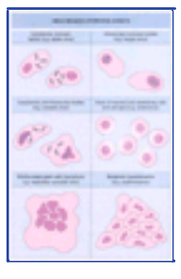

Figure 1-21 Virus-induced cytopathic effects.

supportive of viral replication. Due to the inactivation of tumor suppressor proteins p53 and Rb by E6 and E7, chromosomal destabilization, enhancement of foreign DNA integration and mutagenicity, the infected cells may transform to a malignant phenotype.

Damage resulting from cytotoxic lymphocytes

The most effective host defense mechanism against most viral infections is mediated by the $CD8^+$ cytotoxic T lymphocytes (CTLs). The CTLs recognize, attack and lyse virus-infected cells that present viral antigens on their surface in the context of MHC class I molecules. In addition to CTLs, natural killer lymphocytes similarly kill virus-infected cells. The cytotoxic reaction contributes to the pathologic and clinical picture of many viral diseases. The characteristic measles rash is produced after the attack of CTLs on skin cells infected by the measles virus. This explains why children with defects in cell-mediated immunity do not develop a rash during measles infection. In this disease, rash indeed represents a good immune response by the host, whereas its absence may signal uncontrolled viral growth. It is also believed that lymphocyte-induced cytotoxicity contributes to the pathology associated with persistent virus infections, such as the subacute sclerosing panencephalitis caused by a defective measles virus.

Harmful immune responses

The destructive potential of the immune system is considerable. It can damage the host in a variety of ways.

Autoimmunity

Autoimmune reactions break the rules of the 'self versus nonself' dichotomy. Autoimmune reactions, directed against 'self-proteins', may result from partial identity of antigenic determinants of the host and an infective agent or from alterations of self-components caused by infection. Acute rheumatic fever occurring after group A streptococcal pharyngitis has been associated with antigens found in the cell wall of the streptococcus. These antigens cross-react with components of the endocardium and the joint synovial membrane molecules and thus induce an autoimmune response. Heat shock proteins, which are omnipresent and remarkably conserved proteins in nature, are often associated with autoimmunity. Mycobacterial infection may give rise to antibodies and T cells that are reactive to both the microbial (nonself) and the host (self) heat shock proteins.

Autoimmune reactions that follow an infection may also result from the release of self-components that are normally sequestered in a compartment that is relatively inaccessible to the immune system. Multiple sclerosis might provide an example of this type of autoimmune disease, although this is hypothetical because correlation of the disease with a microbial infection has yet to be established.

Hypersensitivity reactions

Hypersensitivity reactions occur if the host immune system seemingly overreacts to microbial infection. Hypersensitivity reactions have been classified by Gell and Coombs into four types.

Type I or immediate hypersensitivity

Type I hypersensitivity occurs within minutes of antigen exposure. It results from antigen binding to mast cell-associated IgE. Vasoactive amines are released and anaphylactic reactions may develop. Certain forms of rash after helminth infections seem to be due to this type of hypersensitivity.

Type II or cytotoxic hypersensitivity

Type II hypersensitivity is a consequence of the binding of specific antibodies to cell surface-associated antigens. Antibody binding mediates cytoxicity via complement activation or natural killer cells. Thus cells bearing microbial antigens may be lyzed via an antibody-dependent mechanism. Such a mechanism has been suggested to account for liver cell necrosis during hepatitis B infection.

Type III or immune complex-mediated hypersensitivity

Type III hypersensitivity is induced by classic complement activation, caused by extracellular antibody-antigen complexes. This causes inflammation and changes in

vascular permeability and it attracts neutrophils to tissues where the immune complexes are deposited, including the kidneys, joints and small vessels of the skin. Glomerulonephritis in malaria is probably due to this mechanism.

Type IV or delayed-type hypersensitivity

Type IV hypersensitivity typically occurs at least 48 hours after exposure to an antigen. It involves activated T cells, which release cytokines, macrophages attracted by these cytokines, and cytotoxic $CD8^+$ T cells.

Prolonged antigen exposure

During prolonged antigen exposure, such as in chronic infections, granuloma can be formed. Delayed-type hypersensitivity and granuloma play a major role in tissue damage observed during infections with slow-growing intracellular organisms, such as *Mycobacterium tuberculosis* (tuberculosis) and *Mycobacterium leprae* (leprosy), and histoplasmosis. Many of the clinical manifestations of chlamydial disease, in particular trachoma, seem to result from a delayed-type hypersensitivity triggered by chlamydial heat shock proteins. In spite of the involvement of bacterial heat shock proteins, this is not an autoimmune phenomenon, because the unique rather than the conserved portions of these proteins seem to be implicated here.

Superantigens and bacterial components associated with toxic and septic shock

Toxic shock and septic shock are exceptionally impressive syndromes associated with a variety of infectious diseases. Severe hypotension, multiple organ failure and intravascular disseminated coagulopathy occur in the most severe cases. Pathogenesis of these syndromes is complex. Various bacterial components, including lipopolysaccharides, peptidoglycan, lipoteichoic acid and (in some cases) toxins acting as superantigens (see Table 1.9) trigger an intense, potentially lethal host response. In the cascade of events leading to this condition, some cells (e.g. macrophages, neutrophils and/or T cells) play important roles (see Chapter 9 and Chapter 56) as well as releasing high levels of inflammatory response mediators, notably tumor necrosis factor and interleukin-1.

How micro-organisms escape host defense

In spite of the efficacy of host defense mechanisms, microbial pathogens can still infect humans and cause disease. This is in part due to the very potent weapons micro-organisms have (a single gram of crystalline botulinum toxin could potentially kill more than 1 million people) but it is also due to the intricate strategies that micro-organisms use to evade host defenses (Table 1.10).

Surviving the phagocyte and complement attack

Immediately after passage of the epithelial surface, the invading micro-organism encounters the most powerful actors of host defense: phagocytes. Two main types of phagocytes are involved, the polymorphonuclear neutrophils (PMNs) and the macrophages. 'Professional' phagocytes can bind micro-organisms with a variety of receptors, some of which specifically interact with bacterial lipopolysaccharide or with antibodies bound to the microbial surface (opsonized micro-organisms). The micro-organisms usually pass into the cell via phagocytosis or pinocytosis, although some (especially viruses) may enter the cytosol directly.

TABLE 1-10 -- Evasion of host defenses.

EVASION OF HOST DEFENSES	
Mechanism	**Examples**
Surviving the phagocyte and complement attack	
Inhibition of chemotaxis	C5a Peptidase by *Streptococcus pyogenes*
Killing the phagocyte before ingestion	a-Toxin and leukocidin by *Staphylococcus aureus*
Avoiding ingestion	Bacterial capsules (e.g. *Streptococcus pneumoniae*)
	LPS O antigen in Gram-negative rods
	Coating with IgA antibodies (*Neisseria meningitidis*)
	M protein (*Streptococcus pyogenes*)
Surviving within phagocytes	Inhibition of phagolysosome fusion (*Chlamydia trachomatis*)
	Escape from phagolysosome (*Listeria monocytogenes*)
	Resistance to lysosomal products (*Salmonella typhimurium*)
	Inhibition of early host gene expression (*Mycobacterium tuberculosis*)
Antigenic variations	Shift and drift in influenza A virus
Tolerance	Prenatal infections
Immunosuppression	
Destroying lymphocytes	Depletion of $CD4^+$ cells by HIV
Proteolysis of antibodies	IgA protease by *Haemophilus influenzae*
Presence in inaccessible sites	Latent infection in dorsal root ganglia (herpes simplex virus)

Bacteria invariably go through an endosomal stage, in which they will be exposed to a multitude of phagocyte defense mechanisms such as acidification, exposure to reactive oxygen species, bacteriocidic peptides and hydrolytic enzymes released after phagosome-lysosome fusion. In addition, in the endosomal pathway, micro-organisms are deprived of the nutritional wealth of the cytosol. Finally, the pathogens are killed and degraded and the microbial antigens may be presented to lymphocytes. However, micro-organisms have developed strategies to avoid, mislead, deregulate or even profit from phagocytes.[41]

Inhibition of the mobilization of phagocytes

Extracellular micro-organisms can avoid phagocytes by inhibiting chemotaxis or complement activation (see below). A bacterial enzyme that degrades complement protein C5a, a main chemoattractant for phagocytes, has been discovered recently in *Streptococcus pyogenes*. Pertussis toxin catalyzes ADP-ribolysation in neutrophils, which causes a rise in intracellular cAMP levels and ultimately impairs chemotaxis. Other examples of toxins that are directed against phagocytes include a-toxins produced by *Staphylococcus aureus*, streptolysins produced by *Streptococcus pyogenes* and the ?-toxin of *Clostridium perfringens*.

Killing the phagocytes before being ingested

Many soluble products excreted by bacteria are potentially toxic for phagocytes entering the foci of infection. Streptolysin O binds to cholesterol in cell membranes, which results in rapid lysis of PMNs. In the process, the lysosomes are also disrupted and release their toxic contents, which may have deleterious effects on the neighboring cells. *Staphylococcus aureus* produces a, ß and ? toxins, as well as leucocidin, which can kill and lyze the PMNs. Several toxins from *Clostridium perfringens* produce similar effects. Indeed, pus sampled from gas gangrene may contain numerous Gram-positive rods without any visible PMNs.

'Professional' phagocytes as vectors

Legionella pneumophila provokes entry in mononuclear phagocytes by accumulating complement factor C3bi on the envelope of the organism. This complement factor is a ligand for the phagocyte receptor CR3, and enhances phagocytosis. Following uptake, *Legionella* remains in the phagosomes, which do not fuse with lysosomes and thus provide protection. Alveolar macrophages are host cells for *Mycobacterium tuberculosis*. [42] Like *Legionella*, phagocytosed *Mycobacterium* prevents fusion with the lysosome and assumes a latent lifestyle. Many years after initial infection, resident *Mycobacterium* may be reactivated and cause acute disease.

Avoiding ingestion

The surface of numerous pathogenic bacteria is covered with a loose network of polymers, which constitutes the bacterial capsule.[43] Capsular material may be very thin, visible only be electron microscopy, as is the case with the hyaluronate capsule of *Streptococcus pyogenes*. In some species (*Streptococcus pneumoniae*, *Klebsiella pneumoniae*) capsule material is abundant, easily visible with a light microscope and responsible for a mucoid aspect of the bacterial colonies. Most of the capsules are composed of polysaccharides, others are made of proteins or a combination of carbohydrate and protein. Some capsule contents mimic host polysaccharides and are thus recognized as 'self' by the host immune system. Examples are the capsules of *Neisseria meningitidis*, which contain sialic acid, and *Streptococcus pyogenes*, which contain hyaluronic acid.

Capsules may protect bacteria from complement activation.[32] As a result, capsulated bacteria are not immediately recognized as invaders by the phagocytes. Capsulated *Streptococcus pneumoniae* resist engulfment by macrophages and PMNs and are virulent; however, noncapsulated strains are easily phagocytosed and are avirulent.[44] There are more than 80 distinct capsular serotypes, with different contributions to virulence, ranging from the highly virulent pneumococci of serotype 3 to the low virulent serotype 37.

The outer membrane of Gram-negative bacteria is covered with LPS, which serves as an attachment site for the complement fragments C3b (required for the triggering of the alternative pathway) and C5b. The polysaccharide chain of LPS (the O antigen) may contain sialic acid, which prevents formation of C3 convertase, and very long O antigen chains prevent the bacterial killing by the membrane attack complex (which is made from C5b, C6, C7, C8 and C9, and forms pores in the outer membrane of Gram-negative bacteria).

Meningococci circulating in the blood are coated with IgA, which is not an activator of the complement cascade. *Schistosoma mansoni* incorporates decay accelerating factors in its membrane; these are host plasma proteins that inhibit deposition of C3 onto host cell membranes. Activation of complement in the blood is thus avoided by the parasite.

Matrix proteins, which form fibrillae (see Fig. 1.11), are considered to be the primary virulence determinants of *Streptococcus pyogenes*. Matrix protein renders the bacteria resistant to phagocytosis by human neutrophils. Matrix fibrillae are approximately 50–60nm in length and exhibit a seven-residue periodicity. They exist as stable dimers, arranged in a coiled coil configuration, with the carboxylterminal portion closely associated with the cell wall (see Fig. 1.11). Streptococci that express M proteins on their surface are poorly opsonized by the alternative pathway and resist PMN phagocytosis. In contrast, streptococci that fail to express M protein are readily opsonized and phagocytosed. Resistance to phagocytosis can be

overcome by antibodies directed against type-specific M epitopes. The mechanism of antiphagocytic activity of M proteins is still unclear. According to one hypothesis, fibrinogen, known to bind to M protein, may hinder access to complement-binding sites on the bacterial surface, disguising the pathogen as 'self'. In another hypothesis, a complement control protein (protein H), which also binds M, may be responsible for the observed complement resistance of virulent *Streptococcus pyogenes*.

Survival within phagocytes

Once ingested by the phagocyte, the pathogen may survive and grow using a variety of strategies (Fig. 1.22). Some microbes prevent exposure to hydrolytic enzymes by inhibiting fusion of the phagosome and the lysosome, others survive within the phagolysosome because they resist enzymatic degradation or neutralize toxic products to which they are exposed in this compartment. Some bacterial pathogens (such as Salmonellae discussed above) extensively modify endosomes into customized survival vesicles. Certain types of bacteria rapidly escape from the phagolysosome and propagate in the cytoplasm, as described above for *Listeria monocytogenes*. Recent studies suggest that intracellular pathogens, notably *Mycobacterium tuberculosis*, may inhibit the early host response at the level of host gene expression.

Inhibition of phagolysosomal fusion

Salmonella spp. have developed several strategies to survive and propagate in macrophages; *Salmonella* spp. that lack this capacity to survive in macrophages are avirulent. Several hours after infection *in vitro*, two distinct *Salmonella* populations can be seen in the macrophage. One consists of rapidly dividing bacteria located in

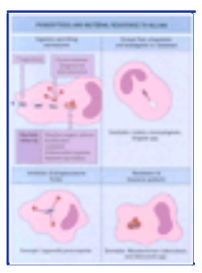

Figure 1-22 Phagocytosis and bacterial resistance to killing.

large unfused phagosomes. This population may rapidly grow and kill the macrophage, leading to the liberation of intracellular bacteria.[45] *In vivo*, this population may be responsible for the acute stage of salmonellosis.

The second population of *Salmonella* consists of nondividing organisms located in phagolysosomes. This population resists the toxic effect of lysosomal products and is believed to account for the prolonged survival of *Salmonella* spp. in the body. Long-living stromal macrophages of the bone marrow may act as long-term *Salmonella* carriers and be responsible for the very late relapses of salmonellosis that are seen in some patients. The dormant phase represents a well-regulated physiologic condition associated with nutrient deprivation *in vitro*.

Inactivation of reactive oxygen species

Reactive oxygen species damage DNA and inhibit the bacterial oxidative phosphorylation. Bacteria may escape from the damaging effect of reactive oxygen species by rapid detoxication of the bactericidal products and by efficient DNA repair. Several bacterial pathogens produce superoxide dismutase (SOD) and catalase, two enzymes that might eliminate the reactive oxygen species and damage to DNA may be efficiently repaired through a RecA-dependent pathway. In Salmonellae the RecA pathway seems to be more important than the production of SOD and catalase because mutants that produce neither SOD nor catalase remain virulent, whereas *rec*A mutants are avirulent. However, the ability of this bacterial species to modify the endocytic pathway of the host cell seems to be the most important mechanism of resistance to reactive oxygen species. In macrophages, virulent Salmonellae localize in phagosomes devoid of NADPH oxidase, the enzyme that drives the respiratory burst. [46]

Resistance to antimicrobial peptides

Several cationic peptides are produced within the lysosomal granules and are believed to kill intracellular pathogens by forming channels in the bacterial cell wall. *Salmonella* spp. resist these antimicrobial peptides by at least two complementary mechanisms, one of which, encoded by the *sap* locus, is characterized in some detail (Fig. 1.23). It seems that the SapA protein forms a complex with the antimicrobial peptides, reducing the deleterious effect on the bacterial membranes.

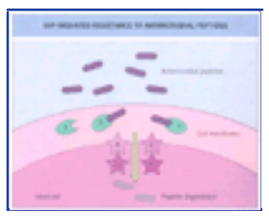

Figure 1-23 Mechanism of resistance to macrophage antimicrobial peptides by *Salmonella* spp. *Salmonella* produces the SapA (A) peptide, which complexes with host cell antimicrobial peptides. Other proteins encoded by the *sap* locus (SapB, SapC and SapD) are required for the transport of the SapA-antimicrobial peptide complex into the cytosol where the antimicrobial peptide is degraded.

TABLE 1-11 -- Examples of antigenic variations.

EXAMPLES OF ANTIGENIC VARIATIONS	
Genetic mechanisms	**Examples**
Recombination between different copies of pilin genes	Pili in *Neisseria gonorrhoeae*
Phase variation — turning expression of an antigen on or off ('flip-flop')	Flagella in *Salmonella*; pili in *Neisseria gonorrhoeae*
Gene reassortment between two strains infecting the same cell	Influenza virus type A
Mutation of surface antigens	Influenza virus type A, B and C
Gene switch leading to surface glycoprotein changes	*Trypanosoma brucei*

Other proteins encoded by the *sap* locus (SapB, SapC and SapD) allow the transport of the SapA-peptide complex into the cytosol. Within the cytosol, peptidases degrade the antimicrobial peptides.

Antigenic and phase variations

A powerful survival strategy for a pathogen would be to mislead the specific host immune response by 'changing appearances'. Three examples of molecular mechanisms used to achieve antigenic variation, one each by a bacterium, a virus and a protozoan, are illustrated below (Table 1.11).

Antigenic variation in Neisseria gonorrhoeae

Neisseria gonorrhoeae varies the composition of at least three major components of its outer membrane: the pili, which mediate the initial attachment to host cells; the membrane protein P.II, responsible for closer attachment resulting in phagocytosis; and LPS, described earlier.

Antigenic variations in the major pilin subunit are essentially due to recombination between different copies of *pil* genes scattered over the chromosome (Fig. 1.24). Only one or two of these are expressed (*pil*E, where E denotes 'expressed') at any point in time, but an array of antigenically distinct pili may be produced in response to an antibody challenge. In addition to this mechanism, pili are subject to phase variation (i.e. switches between *pil*-positive and *pil*-negative variants). Phase variation is controlled at the transcriptional level.

The P.II protein is similarly subject to genetic variation. As a consequence, the specific immune response never quite catches up with genetic variation in the bacterial population. The combination of this mechanism, LPS sialylation (see above) and IgA protease production makes *Neisseria gonorrhoeae* a very recalcitrant pathogen.

Shift and drift in influenza A viruses

Nearly every year, during the recurrent influenza epidemics, vaccination programs are confronted with the problem of antigenic variation. Two different mechanisms account for genetic variation of influenza virus. Antigenic shift results from the infection of a single cell by two different influenza strains. 'New' genomes may be assembled from the available genetic information, leading to gene exchange between the two parent strains. Antigenic shift may result in dramatic changes in the antigenic composition of the surface hemagglutinin (which binds the host cell receptor) or the neuraminidase (which modifies these receptors), and cause devastating epidemics in immunologically unprepared populations. Antigenic drift results from high mutation rates associated with RNA viruses. In influenza viruses A, B and C, mutants with antigenic changes tend to have a selective advantage over the nonmutant viral population. Therefore, new strains are continually being selected, as exemplified by the 1997 outbreak of 'chicken flu' in Hong Kong.

Figure 1-24 Antigenic and phase variations in microbial pathogens. Three mechanisms are shown. (Top) Exchange of DNA between nonexpressed copies of *pil*S and the expressed gene *pil*E in *Neisseria gonorrhoeae* can change the expressed antigen. (Middle) A switch mechanism is responsible for the (mutually exclusive) production of type A and type B flagella in *Salmonella typhimurium.* Phase variation depends on the orientation of a DNA fragment adjacent to the type A flagella gene. When A is expressed (a) from the promoter in the invertable fragment, the repressor for the type B flagella is expressed at the same time. As a consequence the type B flagella gene is repressed. Inversion of the DNA fragment abolishes expression of the A-repressor gene and the B-repressor gene (b). In this situation type B flagella are produced. (Bottom) Antigenic shift by gene reassortment results from infection of a single cell by two different virions.

Antigenic variations in Trypanosoma brucei

African trypanosomes (*Trypanosoma brucei*) are flagellated protozoa, transmitted to humans by several species of *Glossina* (tsetse).

The parasite survives in mammalian body fluids thanks to antigenic variation of the variant surface glycoprotein (VSG), which forms a 15nm thick monolayer covering most of the parasite surface.[47] Within a single generation, most or all of the 10^7 VSG molecules may be replaced by an unrelated species, stemming from a repertoire of an estimated 1000 genomic copies of the gene. The VSG gene is invariably expressed from a polycistronic transcription unit, in the so-called telomeric expression site adjacent to the telomeric repeats. During chronic infection, patients experience successive episodes of parasitemia, each episode coinciding with the expression of a new VSG on the surface of the parasite. With this strategy, trypanosomes avoid complete eradication by the specific immune response, while maintaining the pathogenic burden at sublethal levels. The closely related *T. brucei brucei*, which causes the bovine disease nagana, does not spread to humans because it is sensitive to high-density lipoprotein in human serum.

IMMUNOSUPPRESSION

The most illustrative example of immunosuppression induced by microbial infection is provided by HIV. Human immunodeficiency virus circulating in the bloodstream readily infects $CD4^+$ lymphocytes, macrophages and dendritic cells. The destruction of $CD4^+$ T-helper cells is particularly detrimental to the host and accounts for the emergence of a variety of opportunistic infections as soon as the T-cell counts drops below a critical level.

Other viruses may produce immunosuppression in a more subtle fashion. Measles virus infects both B cells and T cells, interfering with the immunocompetence of the host. As a consequence, in areas with a high prevalence of tuberculosis, measles epidemics may be followed by outbreaks of tuberculosis. Gonococci, meningococci and *Haemophilus influenzae* produce proteases that hydrolyze secretory IgA1 antibodies. Protease-negative mutants of these bacterial strains are less virulent,

suggesting a role for mucosal IgA1 antibodies in host defense against these pathogens.

CONCLUSION

Throughout evolution, humans, like all mammalian species, have maintained an intimate relationship with the microbial world. We have survived thanks to the efficient defense mechanisms we have developed against potentially dangerous micro-organisms. Pathogenic micro-organisms are still here because they have found ways of avoiding elimination by their host or by the microbial competition. 'Successful' pathogens have developed strategies to enter the body and reach and colonize their favorite niche, while defying the powerful human immune system.

In this chapter we have looked into microbial survival strategies. Although some of these have been analyzed in 'molecular detail', a lot remains to be discovered. Future remedies for infectious diseases are likely to be aimed at specific molecular interactions between the pathogenic micro-organism and its host.

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Chapter 2 - Host Responses to Infection

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INTRODUCTION

The need for parasitism, as for evil, has never been satisfactorily explained. In its less severe manifestations — for example, competitive interactions within the food chain — parasitism could have led to the classic 'fight or flight' response because the ability to withstand an 'eat or be eaten' situation would have obvious survival value. However, when competing organisms are placed within a balanced ecosystem they do not require overly aggressive parasitism for survival. The hallmark of such ecosystems is that the individual organisms within it consume no more than they need for survival, for to do so would perturb the whole system.

Recently, it has become increasingly apparent that the effects of interrelated systems permeate our lives, and, as a result, we are all beginning to think ecologically. This readily extends to clinical microbiology and infectious diseases, in which ecologic niches and ecosystems determine the nature and components that make up our microbial flora, as well as their impact on health and disease.

In this sense, 'normal flora' can be considered as an example of 'ecoparasitism', which implies a balanced system that sustains multiple microbial species, each occupying a particular niche. If this is so, then the development of specific host defenses to normal flora might be unnecessary and potentially detrimental if there is a 'cost' resulting from the diversion of metabolic resources from other vital functions to the synthesis of unneeded immune cells and their products. However, host defenses have evolved in a complex and functionally overlapping manner, suggesting that true parasitism has always been a serious threat to survival and that evolution of pathogens and host defenses are linked by selection pressures.

It is important at the outset to understand that host defenses are not limited to the immune response, defined as an induced cellular or humoral defense mechanism that is specific for the challenging agents or their cell-free antigens.[1] The simple presence of an intact integument, effective cough mechanisms, normal gastric acid secretion, peristalsis, or mucin production may exert profound protective effects for the host in the context of host-pathogen interactions. In addition, many metabolic and physiologic events are initiated in the course of infections, and these must be understood as being part of the host response to microbial challenge. The context is even broader, for these events are not restricted to infection but are part of a stereotyped response to other processes that activate the inflammatory response, including trauma and surgery, vasculitis and connective tissue diseases such as rheumatoid arthritis. Nonantigen-specific metabolic events are also triggered by inflammation of any etiology; their relationship to host response is discussed first in this chapter because they are the least appreciated events in host defense, and because they appear to be of survival value and are as dependent on molecular signaling as the antigen-specific responses are.

GENERAL SIGNS, SYMPTOMS AND CONSEQUENCES OF THE HOST RESPONSE TO INFECTION

The classic peripheral signs and symptoms of inflammation (rubor, calor, dolor, tumor and functio laesa — redness, heat, pain, swelling and loss of function) commonly go hand in hand with general systemic signs and symptoms such as fever, chills, myalgias, headache and anorexia (Table 2.1). This relationship between peripheral and systemic responses is one consequence of the common mechanisms that initiate and mediate these events (Fig. 2.1). With progression of the underlying process involved in the activation of inflammation, a clinically recognizable syndrome — the systemic inflammatory response syndrome — may manifest itself. When the systemic inflammatory response syndrome is caused by infection it is called sepsis, which, if uncontrolled, can evolve further into severe sepsis and septic shock, with its life-threatening consequences of refractory hypotension and

TABLE 2-1 -- Consequences of the general symptom responses to infection and inflammation.

CONSEQUENCES OF THE GENERAL CLINICAL RESPONSES TO INFECTION AND INFLAMMATION		
Sign/symptom	**Metabolic effect**	**Benefit for host**
Fever	Increased energy consumption is required to cause and maintain body temperature above normal	Beneficial effect on survival at moderate increases (102–104°F (39–40°C)). May be detrimental with more marked increases (e.g. >107°F (>42°C))
	Enzyme reactions are accelerated	
Anorexia	Decreased nutrient intake requires catabolism of body stores for new protein synthesis	No apparent benefit in infection
	Amino acids are converted to glucose by way of hepatic gluconeogenesis	May permit survival during the healing process after trauma
Lethargy	Decreased voluntary activity reduces energy needs	Benefits of rest documented in some infections (poliovirus, Coxsackie B4 virus) in which exercise increases severity of clinical manifestations
		Allows metabolic support to be directed to host defense responses
Myalgia	Result of muscle activity and muscle catabolism to breakdown muscle protein releases amino acids into the circulation	Generates heat to elevate body temperature
		Provides source of amino acids for increased protein synthesis of host defense molecules and cells

Figure 2-1 Pathways of inflammation induced by microbial components. Components of Gram-negative organisms (LPS) and Gram-positive bacteria (peptidoglycan) can activate similar pathways. C3a, biologically active soluble cleavage product of the activation of complement factor 3; C5a, biologically active soluble cleavage product of the activation of complement factor 5; DIC, disseminated intravascular coagulation; IFN, interferon; IL, interleukin; PMNL, polymorphonuclear leukocyte; TNF, tumor necrosis factor.

multiple-organ dysfunction syndrome (Fig. 2.2 ; see Chapter 56). If these events are viewed in evolutionary terms, there must be a range within which they are beneficial to the host in response to the inflammatory stimulus or for combating an infection, but when they occur in excess and are uncontrolled they may be harmful or even lethal. It is not always clear, however, what the beneficial effect may be or where the threshold for adverse effects begins.

Fever

Fever, the most readily recognized manifestation of inflammation, has no doubt been known since the first parent touched her or his hand to the forehead of a sick

child. The importance of fever as a marker of clinical status and the need for the body

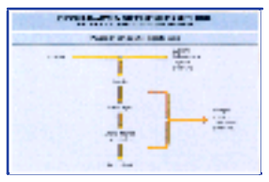

Figure 2-2 Systemic inflammatory response syndrome, septic shock and multiple organ dysfunction syndrome.

to control temperature in response to contagion were also recognized long before the instruments of clinical thermometry were introduced into common use for quantifying body temperature (see Chapter 80). [2]

Fever is now understood as an elevation in body core temperature resulting from a resetting of the thermostatic regulatory system. The core temperature in fever usually follows the same diurnal variation as that exhibited in health but with exaggerations in the slope and height of the peaks, and the temperature often returns to normal levels between fever spikes. Temperature may also be continuously elevated, without returning to a normal baseline. These various fever patterns occur because fever is a regulated process in which alterations in the thermostatic control mechanisms in the hypothalamus

Figure 2-3 Tertian and quartan malarial fever patterns.

of the brain are used to regulate heat production and heat loss in response to the new thermostatic set point to achieve the new temperature. Because there are important individual variations in the fever response to a given stimulus as well as variations in the response to different stimuli and in the effects of light-dark cycles and hormonal controls on body temperature, graphs of fever plotted against time have not proved to have much diagnostic significance (as was once believed when little else of specific diagnostic value was available — for example, tertian and quartan fever patterns were once thought useful for the presumptive etiologic diagnosis of malaria but are rarely observed; Fig. 2.3).

Normal body temperature is classically described as 98.6°F (37°C) ± 1.8°F (1°C) (95% CI); it is lowest in the early morning and highest in the evening (Fig. 2.4). Recent evidence indicates that mean body temperature may actually be closer to 98.2°F (36.8°C) (see Chapter 80 and Chapter 81 for a detailed discussion of thermoregulation and fever). This is one reason why there is no single, uniformly agreed upon definition for the lower limit of fever. Functionally, it has been defined as 99.0°F (37.2°C) in the early morning and 100.0°F (37.8°C) in the evening hours. However, as body temperature cools below 96.8°F (36°C), metabolic processes slow, brain function may become impaired, respiration slows and metabolic needs decline. Controlled hypothermia, as in cardiac surgery, or accidental hypothermia induced by immersion in cold water may, for a while, protect vital organs. However, as the period of hypothermia becomes longer and the temperature continues to drop, metabolism becomes increasingly anaerobic, resulting in acidosis and ultimately in fatal cardiac arrhythmias.

The principal mechanism for generating heat is muscle activity, which burns energy and produces work, with heat as a byproduct. In the development of a febrile response, involuntary muscle activity is controlled by the central nervous system (CNS), resulting in the shivering and rigors that occur as the temperature begins to climb. The slope of the rise and the ultimate peak of the temperature are also regulated by heat-loss mechanisms, and it is the combination of heat production and heat loss that results in the clinical temperature curve (Table 2.2). Heat loss occurs via four principal mechanisms under physiologic control:

- conduction,
- convection,
- radiation, and
- sweating.

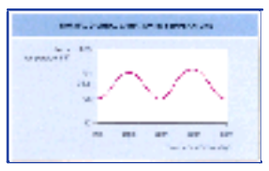

Figure 2-4 Normal diurnal variation in temperature. *These data are from eight healthy volunteers (see Chapter 80).*

TABLE 2-2 -- Physiology and mechanisms of thermoregulation.

PHYSIOLOGY AND MECHANISMS OF THERMOREGULATION			
Process		**Mechanism and clinical manifestations**	**Physiologic regulation**
Heat generation	Metabolic heat production	Involuntary muscle activity generating heat without work; manifested by shivering	Yes, by way of hypothalamic thermoregulatory centers; highly efficient
	Heat retention	Peripheral vasoconstriction lowers skin temperature and reduces heat loss by all mechanisms listed below	Yes, by way of vasomotor responses
Heat loss	Conduction	Heat transfer by direct contact of skin and another surface at a lower temperature; no clinical manifestations	Indirectly, by way of vasomotor responses; inefficient and limited because temperature equilibration rapidly occurs
	Convection	Heat transfer from skin to ambient air, facilitated by moving air; cooling of skin may induce shivering and vasoconstriction	Indirectly, by way of vasomotor responses
	Radiation	Heat transfer to another surface at a lower temperature without direct contact; no clinical manifestations	Indirectly, by way of vasomotor responses
	Sweating	Activation of sweat secretions consumes energy and releases heat through evaporation of sweat	Directly, by control of sweat glands by the hypothalamic thermoregulatory centers Indirectly, by vasomotor responses

Conduction is the direct transfer of heat between two surfaces in direct contact, from the higher to the lower temperature surface, until the temperature at the interface is equalized.

Convection is the removal of heat from a solid body to air at a cooler temperature, and it is facilitated by moving air across the convecting body, as by the use of a fan.

Radiation is the transfer of heat from one surface to another at a lower temperature across a distance. Black surfaces can absorb this radiated heat whereas white surfaces may reflect it; thus, the nature as well as the temperature of the surface receiving radiated heat determine the efficiency of the process. Each of these mechanisms depends on the temperature at the skin surface, and this is physiologically controlled by vasodilatation, which brings warmed blood to the skin and warms the skin surface.

Sweating is an important and effective mechanism of heat loss, as any one who has defervesced after taking aspirin will attest, because profuse sweating accompanies

the rapid decline in body temperature toward the normal range.

The sequential engagement of these mechanisms results in shaking chills as muscle activity and peripheral vasoconstriction are called into play. This is followed by a sensation of fever as vasodilatation occurs to bring heat to the surface for conductive, convective and radiative loss of heat in order to blunt the rise in core temperature. This is followed by profuse sweating as core temperature is rapidly brought to a lower level before the next fever spike initiates the entire sequence again.

Fever also has an upper limit, with temperatures above 106–108°F (41–42°C) rarely if ever noted.[3] At sustained temperatures above this range, as may occur during heat stroke or malignant neuroleptic syndrome, pathologic abnormalities occur; these abnormalities include:

- acid-base changes due to hyperventilation and respiratory alkalosis,
- hypokalemia,
- hypernatremia and other electrolyte abnormalities,
- circulatory failure,
- shock, and
- disseminated intravascular coagulation, with cell swelling and damage in the brain, kidneys and liver, along with widespread hemorrhages.

Structural and metabolic alterations also occur, any one of which carries the potential of death; these changes include:[4]

- hypoxia and mitochondrial damage,
- energy depletion,
- protein denaturation,
- protein phosphorylation,
- ribosomal dysfunction,
- diminished protein synthesis,
- lysosomal enzyme release,
- changes in cytoskeletal and structural proteins,
- altered cell membrane fluidity, owing to altered cholesterol and phospholipid content, and
- degradation or damage of DNA.

Temperature is regulated by warm- and cold-sensitive neurons in the CNS located in the preoptic region, anterior hypothalamus and adjacent septal areas.[5] Direct temperature alterations induced in these regions of the brain lead to all of the behavioral and physiologic events involved in normal thermoregulatory responses. The preoptic region and the anterior hypothalamus are strategically located near the organum vasculosum of the lamina terminalis, the site of transfer of cytokines from blood to brain. This is significant because some cytokines (e.g. interleukin (IL)-1ß and tumor necrosis factor (TNF)-a and, possibly, IL-6 and interferon (IFN)-?) are the principal peripheral signals to the brain to reset the normal temperature set point to cause fever (Fig. 2.5).[6] This signaling is mediated through the production of prostaglandin E_2 and is regulated by a number of possible endogenous antipyretic mediators. These include arginine vasopressin, a-melanocyte-stimulating hormone, catechols, glucocorticoids and their inducers (which block the upregulation of cytokine

Figure 2-5 Mechanisms of fever. Fever may be induced either by exogenous pyrogens, such as microbes or their toxins, or by endogenous pyrogens.

genes), lipocortin (which is a mediator of glucocorticoid function) and natural cytokine inhibitors and soluble cytokine receptors.

Is there any benefit from the fever response? If the results of a set of truly brilliant investigations using the cold-blooded lizard, *Dipsosaurus dorsalis*, can be extrapolated to humans, then fever is a true determinant of the outcome of infection.[7] [8] [9] *Dipsosaurus dorsalis* is a typical poikilotherm and it lacks the physiologic mechanisms for temperature regulation. Nonetheless, in common with other cold-blooded animals, it regulates body temperature as much as possible through the use of behavioral modifications rather than physiologic mechanisms. Thus, it will move between sun and shade as necessary to maintain a constant temperature during the daytime. When placed within an environmental chamber that is able to maintain a gradient of temperature, the animal migrates to the area that allows its core temperature to approximate 101.3°F (38.5°C). However, when the lizards are injected with lipopolysaccharide (LPS) endotoxin or infected with a natural bacterial pathogen, *Aeromonas hydrophila*, the high and low temperature set points triggering migration are both increased (Fig. 2.6) and they migrate in the chamber in order to increase their core temperature by 2–4°F (1–2°C) in the first 24 hours and by another 2–4°F (1–2°C) in the second 24 hours. If the temperature rise is prohibited by keeping the lizards in a chamber with a set temperature or by injecting salicylate, both the incidence of bacteremia and the mortality rate increase (Fig. 2.7). When body temperature is, in a similar manner, fixed at over 107.6°F (42°C), mortality increases further and both infected and uninfected lizards are affected.

These data clearly show that fever is protective and that there is an upper limit beyond which the increased temperature is itself detrimental. There are no clearer demonstrations of the selective advantage of moderate elevations of temperature in host responses than these classic studies of the 1970s. Since then, no convincing mechanism by which this survival advantage is mediated has been demonstrated, in *D. dorsalis* or, if it pertains, in the higher mammals,

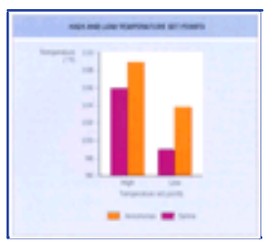

Figure 2-6 High and low temperature set points. The temperature set point is the temperature at which the animal modifies temperature by moving in the temperature gradient chamber. The increased high and low set points in the animals challenged with *Aeromonas hydrophila* result in higher than normal temperature. The data were obtained 3–6 hours after injection with saline or *A. hydrophila*. *Data from Vaughan et al.*[10]

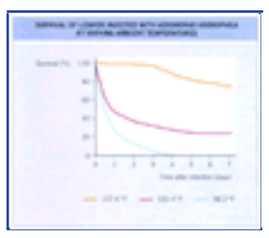

Figure 2-7 Survival of lizards injected with *Aeromonas hydrophila* at varying ambient temperatures. *Data from Kluger et al.*[7]

including humans. However, a murine experimental model has recently been developed to study the impact of alterations of core body temperature by an externally controlled system of temperature regulation. In this model of bacterial peritonitis, a survival advantage was demonstrated in those animals with a febrile core temperature, which demonstrated improved bacterial clearance and enhanced local cytokine generation compared with animals whose core temperatures were maintained in the normothermic range.[11] The question of optimal body temperature for patients during the course of treatment of an ongoing microbial infection has never been satisfactorily answered in a prospective clinical trial. Nonetheless, some investigators have concluded that it is unwise to modify the fever response to infection by the use of antipyretics such as salicylates.[12]

There are consequences of fever other than death that may affect the benefit-risk ratio of this classic host response. One of these is an increase in energy requirements. This increase occurs, in part, because of the biophysical temperature coefficient effect, by which there is an approximately 10% increase in the rate of

enzymatic reactions with an increase in reaction temperature of 1°C (Q_{10} effect). The Q_{10} effect occurs regardless of the cause of the increased temperature; infection triggers this effect, as does artificially increasing the temperature within a heat chamber.

Although this may speed up reactions that yield products of benefit to the host in fighting infection (e.g. the enzymes that result in bactericidal reactive oxygen intermediates such as hydrogen peroxide, superoxide, or hydroxyl radicals), the Q_{10} effect also means that increased energy is required to drive the reactions. This increase in energy needs is not restricted to reactions that benefit the host; all enzyme reactions are affected. Second, the metabolic activity underlying the muscular activity that generates heat in order to raise the core temperature necessitates increased energy expenditures. Third, energy is consumed in the increased metabolic activity needed for the synthesis of the large amounts of new proteins and new cells needed to combat the infection. In fact, energy requirements in humans with sepsis are 35–40% above basal needs.[13]

Anorexia

Loss of appetite is an early manifestation of infection. It is regulated in the CNS, where satiety or hunger is perceived and the appropriate feeding behaviors are triggered. Experiments in which cytokines such as IL-1ß or TNF-a were systemically administered to various species of animals have demonstrated the ability of these cytokines to induce a sharp diminution in food intake.[14] [15] [16] Such experiments suggest that the same cytokines that result in the fever response may also act to alter appetite and food consumption. Principles of 'conservation' of responses may be operative here, for the same response occurs with trauma. Although totally speculative, it is possible that in trauma the injured host may be better served by resting and allowing healing to begin than by foraging for food, and therefore anorexia may make it easier for the injured animal to remain quiescent. In support of this idea, it is striking that IL-1ß induces slow-wave sleep patterns in the brain,[17] which explains why fatigue usually accompanies fever and anorexia in infection and is a part of the teleologic interpretation of these events. Sleep also serves to reduce substrate requirements over and above basal needs, by reducing physical activity and muscle metabolism.

The underlying mechanisms that regulate appetite and food intake are currently being defined, at least in the setting of obesity and hormonal disorders such as diabetes mellitus. Leptin, a recently described cytokine-like peptide mediator produced by adipocytes, is one factor that appears to be involved (Fig. 2.8).[18] Genetically determined obesity disorders in experimental animals have played an essential role in the identification of leptin and the leptin receptor, and it is already clear that defects in either the ligand or the receptor may be responsible for some animal feeding disorders that underlie obesity. Administration of leptin to experimental animals leads to reduced food intake and reduced body weight,[19] and when it is administered directly into the lateral ventricle the effect on food intake occurs within 30 minutes.[20] Leptin appears to act by inhibiting the release of neuropeptide Y in the hypothalamus,[21] which may be the proximate regulator of food-seeking behavior, hyperphagia and energy homeostasis in response to reductions in body energy stores.[22]

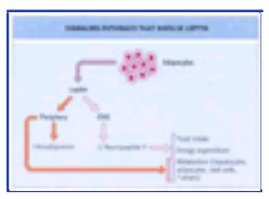

Figure 2-8 Signaling pathways that involve leptin.

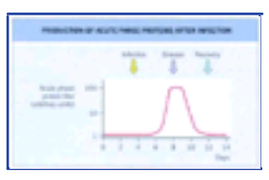

Figure 2-9 Production of acute-phase proteins after infection. Infection causes a rapid increase in the production of these proteins.

TABLE 2-3 -- Acute-phase proteins produced in response to infection in humans.

ACUTE PHASE PROTEINS PRODUCED IN RESPONSE TO INFECTION IN HUMANS		
Acute phase reactant		**Role**
Dramatic increases in concentration	C-reactive protein	Fixes complement, opsonizes
	Mannose-binding protein	Fixes complement, opsonizes
	a_1 -acid glycoprotein	Acts as transport protein
	Serum amyloid A protein	Uncertain
Moderate increases in concentration	a_1 -proteinase inhibitors	Inhibits bacterial proteases
	a_1 -antichymotrypsin	Inhibits bacterial proteases
	C3, C9, factor B	Increase complement function
	Ceruloplasmin	Oxygen scavenger
	Fibrinogen	Coagulation
	Angiotensin	Elevates blood pressure
	Haptoglobin	Binds hemoglobin
	Fibronectin	Cell attachment

Leptin may also be one of a group of proteins whose production is markedly accelerated in the course of an acute inflammatory reaction under the influence of IL-6 (Fig. 2.9). [23] Such proteins are collectively called acute-phase proteins, and it is presumed that they serve some specific role in host response to the acute injury. Some acute-phase proteins have immune regulatory functions (C-reactive protein,[24] IL-11 and IL-6,[25] and G-CSF[26]), others have procoagulant activity (C-reactive protein,[24] reduced synthesis of endogenous anticoagulant,[27] fibrinogen, clotting factors) and yet others stimulate complement (C-reactive protein,[24] synthesis of complement components) or bind to bacterial endotoxin (LPS-binding protein [28] and amyloid proteins[29]). However, as with fever, the extent of the acute-phase response may determine whether the effect is beneficial with respect to the host's response to the initiating stimulus or whether it has harmful consequences (Table 2.3). It is often difficult to sort this out in vivo, because typically there are multiple events occurring at the same time, many of which have overlapping functional impact.

HOST METABOLIC RESPONSES TO INFECTION

Infection results in marked changes in host metabolism, characterized by a dramatic increase in metabolic activity and altered priorities of synthesis. These adaptive responses involve a remarkable array of metabolic activities, including energy, protein, vitamins and minerals.

Energy metabolism

Because of increased metabolic demands in the infected host, it does not make any immediate sense for appetite and food intake to diminish as much as they do in infection; if anything, appetite should be ravenous in order to provide for the increased substrate needs without constricting basal maintenance requirements or resorting to the use of host stores. Yet nature has apparently chosen self-sufficiency as the guiding principle for this situation, and the infected patient reduces voluntary activity and begins to use his or her own sources of energy. Initially the energy store consumed consists primarily of liver glycogen, but within hours this resource is depleted. Because glycogen stores are not sufficient to serve as much more than a buffer for energy needs between feedings it soon becomes necessary to find an alternative source. This is the dilemma of reduced food intake: the energy source must be found within the host. The most abundant energy store in the body is fat; however, during infection and in contrast to starvation, fat utilization is inhibited by the same cytokine mediators that initiate fever and other host responses. This

means that the host must turn to muscle protein as the main reservoir for energy, requiring proteolysis and conversion of amino acids to glucose via gluconeogenesis in the liver. Direct measurements in septic patients have documented increased amino acid turnover in muscle and conversion of amino acids to glucose in the liver.[30] The same events have been shown to occur in patients in whom the inflammatory response is triggered by trauma or surgery.

At the same time, glucose turnover and blood glucose levels are elevated and glucose oxidation is increased; these changes are associated with hyperinsulinemia, hyperglucagonemia and increases in plasma growth hormone levels. In this array of characteristic responses, carbohydrate metabolism in sepsis resembles a state of 'pseudodiabetes'. Peripheral insulin resistance in muscle limits the use of glucose for energy and drives the utilization instead of branched-chain amino acids for energy. Thus, the local proteolytic events in muscle are made more efficient because branched chain amino acids are released and can be used in situ. As the complexities of the changes induced by infection and inflammation become better characterized, the more intricate and coordinated they appear. The response to infection is not a patchwork quilt; rather, it is a work of art.

Vitamin metabolism

Previous studies of vitamin metabolism in infection have not revealed any striking changes that either support the host response or contribute to a metabolic imbalance. Some vitamins are lost in the urine in greater amounts than normal during inflammation, but this has not been seen as clinically important, at least in the short term. Certainly, individual and multiple vitamin deficiencies have been well documented as exerting influences on the host immune system but, with the exception of vitamin A deficiency, the setting of acute infection or inflammation does not itself significantly alter vitamin status. The exception, vitamin A deficiency, has long been known to progress rapidly during infection, and the early changes in the eye that occur in vitamin A deficiency can rapidly progress to blinding keratomalacia following an acute diarrheal infection or measles.

However, a growing body of data indicates that vitamin A is also an acute-phase reactant, being removed from the circulation during infection or acute inflammation. Where the vitamin goes, in what form, how, and what it does there is not known in detail. However, it is reasonable to presume that this, like other acute-phase responses, has evolved through selection and therefore has functional significance in the host response to infection. Vitamin A is commonly known for its role in vision, and for too many students of medicine and biology this is its only recognized role. However, vitamin A and its metabolic products function as transcriptional regulators of many genes, many of which have obvious immunologic functions.[31] [32] Thus, the movement of retinol from plasma to tissue during infection has the potential for transcriptionally activating genes that are critical to host responses. In the mammalian host, this may be an example of a global regulatory pathway that is activated by cytokines released during the response to infection or inflammation.

Vitamin C has received considerable public attention, at least as much for the prominence of some of its proponents as for the evidence that it is an acute-phase reactant with a physiologic role in the host response to infection. Indeed, whether increased vitamin C intake alters the course of infection is still controversial.

Another antioxidant vitamin, a-tocopherol or vitamin E, appears to play a more definitive role in host defense, and a number of vitamin E supplementation trials, especially in older people, have demonstrated that additional vitamin E enhances immune responses and, by this effect, may decrease infectious morbidity.[33] [34] If this is true, it is not yet known whether supplementation is correcting a functional deficiency or not. This is, however, a different issue from that of acute host responses altering vitamin distribution during infection, as occurs with vitamin A.

Minerals

Acute reductions in plasma iron and zinc and an increase in plasma copper have long been known to accompany acute infection and inflammation (Fig. 2.10). The physiologic interpretation of the rapid development of hypoferremia and hypozincemia at the onset of infection has been that of an acute deficiency state. In the case of iron, this has also been interpreted to be of survival value, because the sequestration of iron should reduce the amount available for micro-organisms that require iron for survival, growth and replication. This putative host defense has been termed 'nutritional immunity'. There are many reasons to believe that nutritional immunity may not be physiologically relevant, principally because most bacteria and higher organisms have highly evolved iron acquisition systems that enable the microbes to compete for iron with protein-bound iron in the host. Indeed, many micro-organisms use low concentrations of free iron as a signal to activate genes involved in virulence or resistance to host defenses. Low free iron is also used to signal transcriptional activation of the genes involved in microbial iron binding and

Figure 2-10 Acute cation response in infection and inflammation.

Figure 2-11 Microbial gene regulation via *Fur* gene.

uptake. In one commonly used microbial regulatory gene, ferric uptake regulator (*fur*), the gene product, Fur, is an iron-binding protein that, in the iron-replete form, recognizes a palindromic sequence in the promoter region of iron-regulated genes and blocks transcription (Fig. 2.11). In a low iron environment, Fur does not interact with its DNA binding site and transcription proceeds.[35] [36]

Infection also activates host genes involved in production of the iron-binding protein ferritin, which results in iron uptake into cells, while at the same time reducing synthesis of transferrin, the soluble circulating iron-binding protein that transfers iron from the circulation to an intracellular compartment. [37] [38] Similarly, infection activates the synthesis of metallothienene, the intracellular zinc binding protein, resulting in the sequestration of zinc within cells. These responses are triggered by certain cytokines produced in the inflammatory response (e.g. IL-1ß and TNF-a). In this manner, inflammation, whether due to infection or trauma, induces hypoferremia and hypozincemia. Because IL-1 is so important in the activation of immune host responses, coregulation of the metabolic response and immune activation suggests that the two are, in some way, functionally linked. This view also belies the characterization of infection-mediated hypoferremia and hypozincemia as acute deficiency states.

If the shift of iron and zinc from one host compartment to another is not designed by nature to inhibit microbial growth, then what is its purpose? Both iron and zinc serve as important active centers in metalloenzymes and transcription factors, many of which are involved in DNA synthesis and cell replication such as occurs in response to infectious or inflammatory challenges. In fact, it is not possible for the host to make a response that involves cell division without iron metalloenzymes and zinc-finger transcription factors.

Figure 2-12 Pathologic effects of infection. B, B derived lymphocytes; IFN, interferon; IL, interleukin; PMNL, polymorphonuclear leukocyte; T, T lymphocytes; TNF, tumor necrosis factor.

Because the activation of the humoral and cellular limbs of the immune response is a direct reflection of clonal selection and amplification of lymphoid cells, production of macrophage antigen-presenting cells and increased generation of polymorphonuclear phagocytic cells, the efficient utilization of iron and zinc in support of DNA synthesis and cell division is a prerequisite for the full host response. It appears that a mechanism to promote this has become a part of the acute host response to infection, and the same set of mediators is involved in the catabolic and anabolic events.

ANTIMICROBIAL HOST RESPONSES TO INFECTION

General principles of microbial pathogenesis

If the host response to infection is geared to host defense, then these events should relate to the mechanisms of disease pathogenesis caused by infectious agents. With few exceptions, microbial pathogens act directly on or in the host. The exceptions are limited; they include, for example, micro-organisms that may produce toxic molecules outside the host that can cause disease without infection, such as the *Staphylococcus aureus* enterotoxins involved in food poisoning. However, even these may have profound effects on the immune system in their role as superantigens. Pathogenic organisms typically interact directly with the host, and they are capable of finding a niche in which the organism they can grow, possibly invade across the skin or the mucous membranes and then disseminate, evade host defenses and cause changes in host physiology that translate into symptoms of the illness. Pathogens may produce specific metabolic products that directly damage cellular structure, alter host metabolic processes or produce antigens that elicit potentially injurious immunologic responses (Fig. 2.12). There are at least four separate stages of the host-pathogen interaction that lead to possible pathology or pathophysiologic responses (Table 2.4):

- colonization,
- invasion,
- multiplication, and
- dissemination.

TABLE 2-4 -- Stages in host-pathogen interactions.

STAGES IN HOST-PATHOGEN INTERACTIONS		
Stage	**Mechanism**	**Utility to pathogen**
Colonization	Ligand-specific adherence to host receptors, commonly by way of specific sugar-protein interactions	Provides initial niche for the pathogen to establish and initiate adverse effects on the host
Invasion	Penetration of skin, mucosa or other epithelial membranes to reach the circulation or specific target organ or cell type	Provides entry of pathogen to the host; may also enter immunologic sanctuary, where it is sequestered and protected from host immune responses
Multiplication	Depends on preferred niche of the organism and its growth rate; multiplication may be slow or rapid, intracellular or extracellular	Organism increases in number and may be better able to survive host defenses
Dissemination	Organisms may spread locally or disseminate widely, depending on biologic attributes	Organism infects multiple sites, where it may cause added disease symptoms and survive indefinitely

Colonization

The initial encounter with the host is generally followed by multiplication of the organism. If this occurs on a mucous membrane it is considered colonization. Pathogens must have some means of establishing themselves in their preferred niche. Often these means involve the production of specific colonization factors that allow the organisms both to identify their niche and to attach in a way that allows them to overcome host measures aimed at dislodging them. This is obvious in the gastrointestinal tract (Table 2.5), in which the host produces liters of protease-rich wash fluids in the succus entericus,

TABLE 2-5 -- Microbial attachment in the intestinal tract.

MICROBIAL ATTACHMENT IN THE INTESTINAL TRACT			
Micro-organism	**Disease**	**Attachment site**	**Mechanism**
Vibrio cholerae	Cholera	Intestinal epithelium	Specific bacterial molecule (adhesin) binds to oligosaccharide receptor on cell
Escherichia coli (certain strains)	Diarrhea		
Salmonella typhi	Enteric fever		
Shigella spp.	Dysentery	Colonic epithelium	Bacteria induce epithelial cells to engulf them
Giardia lamblia	Diarrhea	Duodenal, jejunal epithelium	Protozoa bind to mannose-6 phosphate on host cell; also have mechanical sucker
Entamoeba histolytica	Dysentery	Colonic epithelium	Lectin on surface of amebae binds to asialofetuin on host cell
Poliovirus	Poliomyelitis	Intestinal epithelium	Viral capsid protein reacts with specific receptor on cell
Rotavirus	Diarrhea	Intestinal epithelium	Viral outer capsid protein binds to glycolipid receptor on cell

as well as sticky mucins, and then propels these through the gut by means of peristalsis. Without an attachment strategy, potential pathogens may be washed through, explaining why the most efficient pathogens have developed molecular attachment strategies utilizing cell-bound colonization antigens. Typically these are proteins that recognize and bind to specific host glycoconjugates. They are present on the microbial cell surface, often on specialized structures that extend out from the cell surface, such as pili or fimbriae,[39] or sometimes on specialized microbial organelles.[40] This suggests the utility of a host strategy to resist this attachment not only in a non-specific manner but also by developing anti-attachment mechanisms (e.g. by producing specific carbohydrate receptor blocking macromolecules or antibodies).

Figure 2-13 Invasion of micro-organisms across the intestinal mucosa.

Invasion

Attachment may lead to colonization on the mucosal surface but it may also lead to invasion across the epithelial cell layer. Microbial invasion is a complex process resulting from the sequential interaction of microbial products with the host cell.[41] [42] These interactions are often cell-signaling events, and host responses include: [43]

- the activation of protein kinases,
- protein phosphorylations, and
- major rearrangements of the cellular cytoskeleton.

An example is the initial interaction of *Salmonella typhimurium* and the host cell, which results in an active, kinetic 'ruffling' of the cell surface, reminiscent of the effect of growth factors;[44] this is associated with the uptake of the bacteria within host membrane-delimited vesicles. Noninvasive organisms in the vicinity can also be swept up into these vesicles and enter the host cell cytoplasm in a process known as 'passive entry'. [45] The importance of this passive entry depends on the subsequent fate of the ingested organisms, which results from the ability of the organism to resist the microbicidal reactions of the inflammatory response. Recent evidence indicates that gene products from pathogenicity islands of some *Salmonella* spp. actually disrupt oxidative bactericidal mechanisms within phagocytic cells.[46]

Recent studies of the invasion mechanism of *Shigella flexneri* have revealed a different aspect of the role of the host inflammatory response (see Chapter 43 and Chapter 228).[47] First, *Shigella* spp. do not appear to invade across the luminal epithelial cell membrane, as previously thought. Rather, when intestinal epithelial cells are mounted on collagen-coated filters and allowed to form tight junctions, the bacteria are invasive only from the basal surface. Nonetheless, when *S. flexneri* are placed within a loop of intestine in an in-vivo experiment in animals, the organisms invade, cause inflammation and result in fluid production. The interesting aspect of

this is that if the inflammatory response is prevented by the administration of IL-1 antagonists or if the ability of polymorphonuclear leukocytes (PMNLs) to migrate is blocked by the administration of antibodies to PMNL surface antigens involved in locomotion (such as CD18), then no invasion occurs. If neutrophil chemotaxis is blocked, then *Shigella* spp. invasion is markedly reduced and no symptoms of disease ensue.

These findings demonstrate that the initial microbe-host interaction triggers an influx of PMNLs that is necessary for the subsequent, more massive invasion of organisms across the mucosa (Fig. 2.13). These results have changed the paradigm for the sequence of inflammatory responses in shigellosis from that of a reaction to invasion to

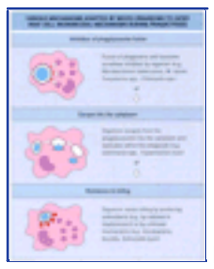

Figure 2-14 Various mechanisms adopted by micro-organisms to avoid phagocytosis.

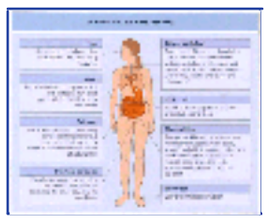

Figure 2-15 Nonimmunologic host defenses.

an integral and essential part of invasion. In other words, infection with *Shigella* spp. is a clear example of host-mediated pathogenesis. This also demonstrates the ingenious nature of microbes in host-pathogen interactions, and the ability of pathogenic micro-organisms to take advantage of and subvert host defense systems for their own welfare. A better-known example of this is the use by HIV of the lymphocyte and macrophage cell surface differentiation antigen CD4, together with host cell chemokine receptors, to enter the target cells.[48] The virus then replicates, thereby initiating the destruction of immunologically active cells in the process and leading, ultimately, to AIDS (see Chapter 120).

Multiplication and dissemination

These examples of colonization and invasion also highlight two other aspects of microbial pathogenesis: multiplication and, in some instances, dissemination. Multiplication may occur on mucosal surfaces, in tissues (e.g. in an abscess) or within cells. Some micro-organisms have become adapted to survival and multiplication within the phagocytic cells, using a number of strategies to evade the host's microbicidal mechanisms (Fig. 2.14). Because of their ability to survive and multiply within these professional host defense cells, such micro-organisms are referred to as 'facultative intracellular pathogens'; they include *Salmonella typhi*, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Leishmania donovani* and *Toxoplasma gondii*. These organisms are able to avoid harmful effects of host defenses by a variety of measures. For example, some prevent the fusion of phagocytic vesicles with lysosomes, some block the acidification response within the vesicle by escaping to the cytoplasm, some interfere with oxidative killing enzymes[46] and others just resist everything the macrophage can throw at them.[49]

Whereas systemic dissemination of micro-organisms is generally associated with systemic manifestations of illness, such manifestations can occur in the absence of dissemination. For example, in

diphtheria, the organisms colonize and multiply on the mucous membranes of the upper airway, but they produce an exotoxin that is transported systemically and leads to clinical illness, owing to its ability to inhibit mammalian cell protein synthesis and cause tissue damage. Systemic invasion may also occur by devious means. For example, the gonococcus can attach to sperm and ride upstream through the female genital tract to the fallopian tubes, where the organism can cause acute salpingitis. [50]

Nonimmunologic host defenses

There are a number of important non-antigen-specific host defenses that are important components of the host barrier to infection (Fig. 2.15). These defenses may be altered by genetics, disease or drugs, and, in the altered state, they can predispose the host to certain groups of infectious agents. It is often possible to improve the resistance of the host by modifying these defenses (if they are recognized and known to the physician and if there are intervention strategies available).

Integument and mucous membranes

The physical barriers to infectious organisms constitute one aspect of host defense; because they are not specific or induced as a response to microbial stimuli, they are not immunologic defenses. For example, the skin and mucous membranes are the first contact between micro-organisms and the host and they are of major importance as barriers. This can be readily appreciated; ask any nail-biter how often he or she develops local cellulitis (paronychia). The most likely answer will be that this is a common occurrence if the integrity of the cuticle is damaged. This provides a portal of entry for *S. aureus*, a colonizer of the skin, which is then able to enter the subcutaneous tissues. The importance of the intact integument has been shown in an experimental reproduction of the nail-biting situation, in which the skin of an experimental animal is painted with viable *S. aureus* and a suture is placed through the skin. Where the suture breaks the intact skin, cellulitis develops; in unbroken skin, nothing happens.

The breached skin barrier is, of course, a hallmark of surgery. A natural example of this also occurs in transmission of the protozoan pathogen *Trypanosoma cruzi*, the cause of South American trypanosomiasis (see Chapter 173). The infectious stage of this parasite develops in the gut of an insect vector from the species of reduviid ('kissing') bugs. When the insect bites a host to take a blood meal, it deposits infectious feces nearby; the parasite can now enter through the broken skin created by the bite, usually because the host rubs the injured site and mechanically brings the organism into the breach in the skin (Fig. 2.16).

It should also be appreciated that many pathogens have developed the means of invading through an intact integument (often via hair follicles) or across mucous membranes after reaching these sites by means of ingestion, inhalation or insertion, as in the gut, respiratory or genital tracts, respectively. The mechanisms employed may differ in detail but are generally similar in principle.[51] To some extent, washing the skin with soap and water can reduce the surface pathogen load; however, excessive washing can remove beneficial lipids from skin that protect against microbial invasion.

Gastric acid

Micro-organisms are vulnerable to extremes of pH, and for many that normally enter the host via the oral route, the low pH that may be achieved in the stomach is sufficient to kill them. In this sense, gastric acid can be considered a non-specific host defense mechanism. For example, *Vibrio cholerae* is very sensitive to acid in vitro, and people who are unable to secrete normal amounts of gastric acid because of gastritis, ulcer surgery or the use of antacids or drugs that block acid secretion are especially susceptible to clinical cholera. This

Figure 2-16 Life cycle of *Trypanosoma cruzi.*

was noted during the seventh pandemic spread of cholera in Europe and Israel in the 1970s, when clinical cases were observed to occur preferentially in hypochlorhydric people. Although acid killing may not be a specific mechanism, certain micro-organisms seem to be able to resist acid-mediated damage by means of genetically controlled properties. Thus, the acid resistance of *Shigella* spp. is associated with the activation of acid-resistance genes, which are growth-cycle regulated as the micro-organisms enter the stationary growth phase. This is a very functional response on the part of the micro-organism because the acid-resistant phenotype is achieved just as the bacterium is excreted into the environment and thus occurs before it infects a new susceptible host (Fig. 2.17). In this manner, *Shigella* spp. are prepared in advance to survive passage through the stomach of the potential new victim. Their ability to resist acid is one reason why so few *Shigella* organisms are needed to cause clinical illness in humans — namely because of their likely safe passage through the stomach (see Chapter 43).

Non-specific defense at the mucosal surface

Mucosal surfaces represent an enormous surface area and carry a huge burden of micro-organisms as normal (or abnormal) flora, especially at the upper and lower ends of the gastrointestinal tract (Fig. 2.18). There, the number of indigenous established micro-organisms, the majority of which are strictly anaerobic bacteria, exceeds the total number of cells that make up the whole host. Whereas many of these organisms are incapable of surviving in the body because they are so susceptible to host defense mechanisms, others become pathogens if they are able to breach the mucosal barrier. Therefore, a healthy mucosal barrier is an important component of host defense. One way of appreciating this is to examine the consequences of altering mucosal cell turnover and replacement by the use of cytotoxic cancer chemotherapeutic agents. These agents often lead to ulcerations of the mucous membranes and increase the risk of systemic invasion by facultative Gram-negative bacilli that normally live in the gastrointestinal tract without causing harm, even in neutropenic patients.

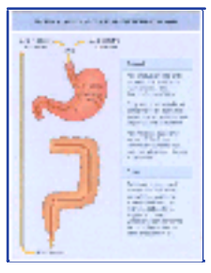

Figure 2-17 Microbial response to acid production by the host.

Mucous membranes use several mechanisms to prevent microbial translocation. Some mucous membranes are the site of secretion of antibacterial proteins such as lysozyme, which hydrolyzes peptidoglycan and leads to the debility and death by lysis of Gram-positive organisms. The functional integrity of ciliated cells and mucus-secreting cells is another general mucosal host defense. Microbes can become embedded in balls of mucus, which are propeled to exit via a stoma by cilia in the upper respiratory tract or by peristalsis in the intestinal tract. Diseases that affect the function of cilia (e.g. viral infections) or the composition of mucus (e.g. cystic fibrosis) diminish the efficiency of this clearance mechanism and predispose to a variety of infections that a normal host would readily resist (Table 2.6).

Mucus itself is more interesting than its appearance as a slick and slippery substance. It is composed of a number of complex glycoconjugates expressing different antigenic epitopes, many of which may mimic receptors or microbial constituents and, by interacting with the host or pathogen surface, block the specific host-pathogen interaction that initiates infection. The mechanism by which breast milk protects the nursing infant from infection may depend on mimicry by the natural glycoconjugates present in colostrum and milk. By the same token, some micro-organisms use these interactions to establish colonization within the slime layers that overlay cells and use this niche to launch a more potent attack on host defenses, leading to systemic infectious diseases.

Initiation of the inflammatory response

Local and systemic manifestations of illness can be initiated by numerous mechanisms (see Chapter 56). For example, the LPS of Gram-negative organisms and the peptidoglycans of Gram-positive

Figure 2-18 Normal flora of the gastrointestinal tract.

organisms can initiate the production and release of proinflammatory cytokines such as IL-1ß and TNF-a. In the case of LPS, the biologic effects are mediated by the lipid A portion of the molecule, a hydrophobic domain in the outer monolayer of the outer microbial cell membrane.[52] Lipopolysaccharide polymers are solublized and converted to LPS monomers via LPS-binding protein (LBP), an acute-phase protein of 456 amino acids that is derived from hepatocytes.[53] It is the LPS-LBP complex that shuttles LPS monomers to CD14-bearing immune effector cells (e.g. neutrophils, monocytes and macrophages; Fig. 2.19).[54]

The identification of infectious agents by means of conserved structural features through pattern-recognition receptors initiates the innate immune response. The conserved components expressed by

TABLE 2-6 -- Interference with ciliary activity in respiratory infections.

INTERFERENCE WITH CILIARY ACTIVITY IN RESPIRATORY INFECTIONS		
Cause	**Mechanisms**	**Importance**
Infecting bacteria (*Bordetella pertussis, Haemophilus influenzae, Pseudomonas aeruginosa, Mycoplasma pneumoniae*)	Production of ciliostatic substance (tracheal cytotoxin from *Bordetella pertussis*, at least two substances from *Haemophilus influenzae*, at least seven substances from *Pseudomonas aeruginosa*)	++
Viral infection	Ciliated cell dysfunction or destruction by influenza viruses or measles virus	+++

Figure 2-19 Lipopolysaccharide activation of macrophages via lipopolysaccharide binding protein (LBP), CD14, Toll-like receptor (TLR) 4, and MD2. IL, interleukin;

TNF, tumor necrosis factor.

TABLE 2-7 -- The Toll-Like receptors (TLRs) and their known microbial ligands.

THE TOLL-LIKE RECEPTORS AND THEIR LIGANDS	
Toll-like receptor	**Microbial ligands**
TLR1	Lipopeptide, lipoteichoic acid (in combination with TLR2)
TLR2	Peptidoglycan, lipopeptide, lipoarabinomannan, fungal cell wall components, lipopolysaccharide of leptospirosis
TLR3	Double-stranded viral RNA
TLR4	Lipopolysaccharide, respiratory syncytial virus proteins
TLR5	Flagellin from Gram-positive or Gram-negative bacteria
TLR6	Zymosan (fungal constituents) along with TLR2
TLR7	Imidazoquinoline antiviral compounds (natural ligand unknown)
TLR8	Imidazoquinoline antiviral compounds (natural ligand unknown)
TLR9	Unmethylated CpG motifs in prokaryotic DNA
TLR10	Natural ligand is currently unknown (may form heterodimers with TLR2 and possibly other TLRs)
Adapted from Opal and Huber[61] and Means et al.[62]	

microbial pathogens that trigger the immune response are termed pathogen-associated molecular patterns (PAMPs).[55] The Toll-like receptors (TLRs) along with CD14 and its accessory molecules are the major pattern recognition receptors that detect these PAMPs.[56] [57] The major microbial elements that are recognized by innate immune cells and their respective pattern recognition receptors are described in Table 2.7 .

Membrane-bound CD14, previously termed 'the endotoxin receptor', is a glycosyl-phosphatidyl-inositol (GPI)-anchored surface protein on myeloid cells. CD14 binds a wide array of microbial constituents in addition to bacterial endotoxin such as peptidoglycan, lipoteichoic acid, and even fungal antigens.[58] [59] [60] CD14 is therefore a prototypical pattern recognition receptor.[58] CD14 is anchored to the cell membrane by a single covalent bound via its GPI-linked tail that lacks a membrane-spanning domain and is incapable of directly transmitting an intracellular signal. The molecular nature of the actual signal transducing surface receptor was not known until its discovery in 1998 as the TLRs.[56]

There are a total of 10 TLRs identified in the human genome. These gene products contain a number of structural and functional similarities. All TLRs express a series of leucine-rich repeats in their ectodomain, a transmembrane domain and an intracellular domain that is homologous to the intracellular domain of the interleukin-1 type 1 receptor. This region of homology is known as the TIR (standing for the Toll-like receptor Interleukin-1 Receptor) domain. When macrophages are activated by LPS, a complex of membrane-bound CD14, an adapter protein known as MD2 and a TLR4 homodimer cluster on the cell surface to initiate an intracellular signal.[61] [62]

The TLRs have greater ligand specificity for the microbial structures than CD14 and they can discriminate between types of bacteria and their products. Gram-positive bacterial components such as peptidoglycan and lipopeptides are recognized by heterodimers consisting of TLR2[63] (in combination with TLR6 (for peptidoglycan) or TLR1 (for bacterial lipopeptides)).[60] [64] Most forms of Gram-negative bacterial LPS are specifically recognized by TLR4.[57] Toll-like receptors may detect additional microbial structures, including bacterial flagellin (TLR5),[63] prokaryotic unmethylated CpG motifs in bacterial DNA (TLR9),[65] mycobacterial lipoarabinomannan (TLR2),[66] fungal constituents (TLR6 and TLR2 heterodimers) [62] and double-stranded viral RNA (TLR3)[61] (Table 2.7).

There is another host protein in the PMNL composed of 456 amino acids, namely bactericidal permeability-increasing protein (BPI), which plays a different role in the host response from that of LPS.[67] The BPI is a very cationic protein, representing approximately 1% of the total protein content of the PMNL. It is produced only by immature PMNLs and it is stored in the primary granules of the cell; it is released when the cells are activated to degranulate, for example by LPS. It acts by forming complexes with LPS in the outer membrane of living Gram-negative organisms, resulting in the very rapid cessation of microbial replication. With time, BPI damages the inner membrane, resulting in a loss of viability of the organism (Fig. 2.20). Extracellular complexing of BPI and LPS inhibits the further cell signaling that is mediated by the LPS.[68] Antibodies to BPI block the microbicidal activity of PMN lysates and inflammatory secretions against Gram-negative bacteria, which suggest that BPI plays a role in PMNL mediated killing of these organisms.

Lipopolysaccaride-binding protein and BPI may compete for LPS binding in tissue sites where Gram-negative infections are present.[69] The opposing effects of BPI (LPS neutralization) and LBP (LPS delivery to monocyte-macrophage or neutrophil membranes) may contribute to the overall host response to LPS release in local infection. The *N*-terminal 199-amino-acid fragment of BPI is fully active in vitro and protects animals against lethal doses of LPS or Gram-negative bacteria. These properties have suggested a potential therapeutic use of this natural antimicrobial agent derived from the human host, and early clinical trials are currently being conducted.[70] Early results with the *N*-terminal portion of BPI in children with

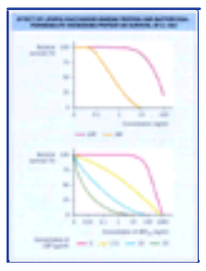

Figure 2-20 Effect of lipopolysaccharide-binding protein (LBP) and bactericidal permeability-increasing protein (BPI) on survival of *Escherichia coli.* (Top) Survival of *E. coli* in the presence of increasing concentrations of LBP or BPI. (Bottom) Synergistic effect on *E. coli* survival of LBP and a recombinant 23kDa *N*-terminal fragment of BPI (rBPI23). *Data from Horwitz et al.*[68]

meningococcemia suggest that this antiendotoxin binding protein may prove to be clinically useful[71] (see Chapter 56). It is interesting to note that the host is prepared to deal with Gram-negative bacteria

TABLE 2-8 -- Specificity and redundancy in host defenses.

SPECIFICITY AND REDUNDANCY IN HOST DEFENSES					
Pathogen		**Type of immune mechanism involved and effect on susceptibility to Infection**			
		Phagocytosis	**T cells**	**Complement**	**Antibody**
Bacteria	*Staphylococcus aureus*	Increased	Not increased	Increased	Increased
	Enterobacteriaceae	Increased	Not increased	Increased	Some increase
	Haemophilus influenzae	Not increased	Not increased	Some increase	Increased
	Mycobacterium tuberculosis	Not increased	Increased	Not increased	Not increased
Viruses	Herpesviruses	Not increased	Increased	Not increased	Not increased
	Enteroviruses	Not increased	Some increase	Not increased	Increased
Fungi	*Candida albicans*	Increased	Increased	Not increased	Not increased
	Aspergillus spp.	Increased	Not increased	Not increased	Not increased
	Cryptococcus spp.	Not increased	Increased	Not increased	Not increased
	Pneumocystis carinii	Not increased	Increased	Not increased	Not increased

Protozoa	*Cryptosporidium* spp.	Not increased	Increased	Not increased	Possibly increased
	Malaria	Not increased	Not increased	Not increased	Possibly increased
	Toxoplasma gondii	Not increased	Increased	Not increased	Not increased
Infectious complications of congenital immunodeficiency syndromes that affect various host defense mechanisms. Genetic analysis has demonstrated that *P. carinii* should be classified with the fungi.					

by way of LBP and BPI, two related, small peptides: LBP binds LPS and allows it to transduce signals to phagocytic cells, resulting in degranulation and release of BPI. The BPI can complex with and neutralize the effects of LPS and bind to and ultimately kill the infecting organisms, thereby releasing the LPS, which again initiates the whole process. This is another example of the exquisite orchestration of host responses to micro-organisms. However, in the cat-and-mouse-like relationship between host defenses and microbial virulence, it is perhaps inevitable that some organisms will have become adept at turning this inflammatory response to their own benefit, as in the case of *Shigella* spp. described above.

Specific elicited (immunologic) responses

Immunologic responses may be defined as those in which microbial antigens elicit a host response that is specific for the structure that initiates the response. The regulation of these responses is rather complex and appears to be ever more complex as more factors involved in regulation are discovered. These factors include:[72]

- cells and secreted products, such as growth factors and signal-transducing mediators;
- soluble immune proteins that may be produced in the course of an immune response, such as antibodies; and
- activation of reaction cascades involving circulating proteins, which result in the formation of protein-protein complexes and the production of hydrolytic products with biologic activity, as occurs during the activation of the complement system.

Cells may interact with cells or proteins, or both, and proteins may interact with other proteins or non-protein constituents such as oligosaccharides or complex carbohydrates. The hallmark of immunologic reactions is specificity for the eliciting antigen of the pathogen, although non-specific or 'bystander' effects can occur and affect organisms other than the pathogen to which the response is directed (see below).

When the antimicrobial functions of these reactions are examined, they are often found to be redundant for certain organisms, to require interactions to be active against other organisms, or to be unique for other organisms. The fascination of this biologic system has never been greater than today, even though we know more about it than ever before, because the answer to one question is the genesis of a whole set of new questions. It is this deepening biologic complexity — along with the ingenuity and genetic plasticity of microbial pathogens, which leads to changes in virulence mechanisms

and new ways of interacting with the host — that makes infectious diseases one of the most vibrant of the disciplines of modern medicine, pediatrics and surgery.

Redundancy of defenses

If there were a single defense mechanism for each class of micro-organism, people with congenital defects in specific limbs of the immune system would be subject to disease with all possible classes of pathogens. This is not the case, and this fact is a major reason to believe that alternative defense mechanisms exist (Table 2.8). For example, if we consider congenital defects in the four major limbs of

Figure 2-21 Pathogenesis of AIDS. The pathogenesis begins with the binding of HIV to CD4 receptors on the regulatory cells of the immune system.

Figure 2-22 The process of phagocytosis.

the immune system (phagocytic cells, T cells, complement and antibody), it is clear that only certain pathogens commonly become problems for the affected host (see Chapter 98):

- defects in phagocytic cells diminish host defenses to certain bacteria and fungi, but not to viruses or protozoa;
- defects in T cells impair cell-mediated immunity to mycobacteria and facultative intracellular bacteria (such as *S. typhi*, *L. monocytogenes* and *L. pneumophila*, certain fungi, certain viruses and some protozoa) but not to pyogenic bacteria such as *S. aureus*;
- defects in complement may impair host defenses to encapsulated micro-organisms but not to fungi or viruses; and
- defects in antibody may impair host responses to encapsulated Gram-negative and Gram-positive bacterial pathogens but do not alter the host response to most viruses and fungi.

At the same time, vigorous immune responses may convey no protection at all and merely be a smoke-screen response elicited by pathogens that are wholly unaffected. Such is the case with malaria or infection with *Leishmania* spp., which elicit a polyclonal antibody response to antigens that do not mediate immune protection; the response consumes energy and amino acid substrates but offers little or no protection. Some micro-organisms, such as *Schistosoma* spp., shed their outer coats as antibodies are produced, thus evading immune recognition. Indeed, it seems that the higher the organism is in the evolutionary tree, the more elaborate its means of avoiding immune destruction. This is not to say that more primitive organisms, despite the relatively limited size of their genome, cannot possess intricate invasive properties. The foremost example of an eloquent evasion of host defences is HIV, with its attack on host immune regulatory cells bearing the CD4 determinant (Fig. 2.21). However, in biologic terms, this is a 'simple' mechanism, although it certainly leads to very complicated results because it disrupts the role of these cells as conductors of the immunologic orchestra (see Chapter 120).

Phagocytosis

Although micro-organisms can invade epithelial cells by inducing a process that appears very similar to phagocytosis, it is the PMNLs and the cells of the monocyte-macrophage line that, by and large, carry out the host function of microbial ingestion and destruction (Fig. 2.22). However, these cells are not very adept at phagocytosis and microbial killing unless they are instructed to do so (Fig. 2.23). This instruction often comes in the form of messages from activated T cells or macrophage antigen-presenting cells, or in the form of soluble proteins derived from complement or immunoglobulin that are able to signal the phagocyte to ingest and kill. Eosinophils are ordinarily considered to be largely restricted to their role in allergy, but they may also ingest very large macroscopic multiple-cell pathogens such as tissue nematodes.[73]

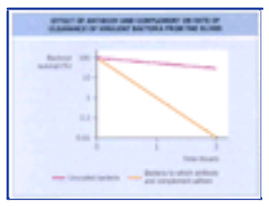

Figure 2-23 Effect of antibody and complement on the rate of clearance of virulent bacteria from the blood. Phagocytosis is greatly potentiated if the microbes are coated with antibody and complement.

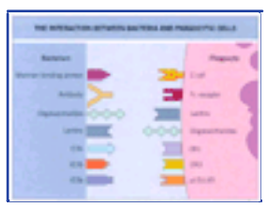

Figure 2-24 The interaction between bacteria and phagocytic cells. This is facilitated by a variety of molecules, the precise nature of which may determine whether uptake occurs and whether killing mechanisms are triggered.

Phagocytosis by PMNLs and the activation of intracellular microbicidal reactions is a multifactorial process that is dependent, in the first instance, on the deposition of activated complement fragments or immunoglobulin on the surface of the organisms, which renders them recognizable by receptors on the surface of the PMNL for C3b and the Fc fragment of immunoglobulin (Fig. 2.24). Subsequent ingestion requires complex signaling via protein phosphorylations that result in the rearrangement of the cytoskeleton, the ingestion of the organism within a vacuole made of host plasma membrane, and the subsequent fusion with primary and secondary granules to form phagolysosomes.[74] These granules contain a number of microbicidal proteins (e.g. BPI) and enzyme systems that are capable of generating reactive oxygen intermediates with microbicidal properties. [75]

To be effective, PMNLs must reach the site of infection. Chemical signals to attract these cells (chemotactic factors or chemoattractants) are produced during the initial host-pathogen interaction (Table 2.9). The ability of host defense cells to reach an infected

TABLE 2-9 -- Chemotactic molecules.

CHEMOTACTIC MOLECULES			
Factor	**Characteristic**	**Source**	**Action on**
C5a	77 amino-acid peptide	*N*-terminus of C5 a chain	Neutrophils, eosinophils, macrophages
F-Met-Leu-Phe	Tripeptide with blocked *N*-terminus	Prokaryotes	
Leukotriene B_4	Arachidonic acid metabolite via lipoxygenase pathway	Mast cells, basophils, macrophages	
Various low-molecular weight chemokines	10kDa proteins	Different leukocyte populations	Selective actions on different leukocyte populations

Figure 2-25 Binding of selectins to carbohydrates expressed on various cells. HEV, high endothelial venules.

area involves the regulated and co-ordinated expression or activation both of leukocyte adhesion molecules and of endothelial cell adhesion molecules, which are called selectins (Fig. 2.25).[76] These adhesins mediate the cell-cell interactions that allow the initial sticking of PMNLs to the capillary endothelium and their subsequent migration across the capillary wall to the site of the infection (Fig. 2.26).[77] The importance of mechanisms of adherence and migration can be appreciated by considering the clinical problems experienced by people with the hyperimmunoglobulin E syndrome (also known as Job's syndrome).[78] Polymorphonuclear leukocytes can accumulate in subcutaneous tissues of these patients in response to microbial challenge, but their further migration is delayed and quantitatively diminished. Although the response is sufficient to prevent systemic spread of *S. aureus* from the skin, it is inadequate to prevent or clear the local subcutaneous lesions. These patients also have a deficiency in IgG and IgA antistaphylococcal antibodies and an excess of IgE, often with demonstrable specificity for *S. aureus* cell walls that, unfortunately, have little clinical importance. It is this

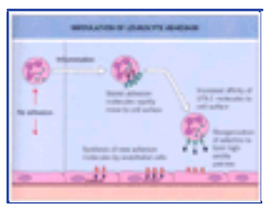

Figure 2-26 Modulation of leukocyte adhesion. There are four ways in which leukocyte binding to endothelium can be enhanced. LFA-1, lymphocyte function associated antigen-1.

finding, probably a reflection of lymphocyte abnormalities in the regulation of IgE production, that gives the syndrome its name.

Several genetic defects in PMNL function (see Chapter 98) confirm the importance of these cells for the defense against infection. These genetic defects include:

- chronic granulomatous disease, a defect in production of microbicidal reactive oxygen intermediates,[79] which renders the host susceptible to infection with *S. aureus* and to a lesser extent with *Aspergillus* spp. and *Candida* spp., *Chromobacterium violaceum*, *Burkholderia cepacia* and various Gram-negative Enterobacteriaceae; and
- Chédiak-Higashi syndrome, a defect in degranulation of lysosomes [80] with abnormal early kinetics for killing of ingested *S. aureus*.

Cyclic neutropenia results in periodic severe neutropenia that exposes the host to the same set of infections that occur in secondary neutropenia as caused, for example, by cancer chemotherapy. The result is increased susceptibility to infection with *S. aureus* and Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*.

Humoral immunity

The best-known component of humoral immunity is antibody, comprising immunoglobulin proteins produced by B lymphocytes and having the property of recognizing unique antigenic molecular structures. Antibodies function in host defense against infection when they are directed to antigenic components of micro-organisms, whether these antigenic components are free in the tissues or present on whole organisms or on the surface of infected cells. For most infectious diseases, the host response depends on the processing of antigens by macrophage antigen-presenting cells, which results in the appearance of antigenic fragments in relation to major histocompatibility complex (MHC) locus determinants; this processing of antigens is regulated by T cells. [81] The repertoire of circulating antibodies generally shifts from an initial IgM to a subsequent IgG predominance (Fig. 2.27), associated with a change from low-affinity antibodies to high-affinity antibodies against specific antigens (Fig. 2.28); this is sometimes referred to as an increase in the 'quality' of the antibody response (or as 'antibody maturation'). The IgM response seems to have its value in being a rapid response to an infectious challenge, although the large size of the IgM molecule largely restricts its activity

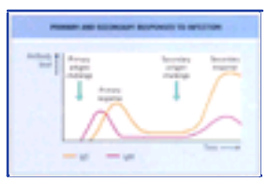

Figure 2-27 Primary and secondary responses to the same antigen during infection.

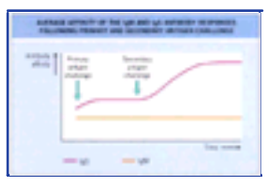

Figure 2-28 Affinity of the antibody responses following primary and secondary antigen challenge.

to the intravascular compartment. Immunoglobulin M is able to activate complement and generate the active byproducts of the classic pathway C3a (which acts as an opsonin) and C5a (which acts as an anaphylatoxin and chemoattractant) in order to facilitate phagocytosis via the C3a receptor of the phagocytic cell and to enhance leukocyte migration to the site of infection. In contrast, the IgG response is more specific and may be more effective as well as wider in distribution, assuming the host has survived the initial challenge. By binding to micro-organisms at the antibody combining site, IgG also acts as an opsonin because the phagocytes have receptors for the Fc portion of the immunoglobulin molecule. To some extent, IgG subclass antibodies act preferentially in certain infections. Thus, congenital IgG_2 subclass deficiency, which is often accompanied by a defect in IgG_4 or a deficiency in IgA, or both, is particularly associated with deficits in antibody to bacterial polysaccharide capsules and recurrent infections with encapsulated organisms.[82]

Recently, a host defense protein known as mannose-binding protein or mannose-binding lectin (MBL) has been characterized with antibody-like and complement-like properties. This lectin binds to mannose-terminated glycoprotein structures and other carbohydrate moieties found on the surface of microbial pathogens such as *N*-acetylglucosamine and fucose.[83] Mannose-binding lectin has a broad range of potential binding sites expressed on the surface of both Gram-positive and Gram-negative bacterial pathogens.[84] It can promote binding and phagocytosis by direct interactions with

pathogens and the CR1 receptor on phagocytic cells.[85] It also activates complement similar to the classical pathway by directly cleaving C3 by its own MBL-associated serine protease in the absence of C1q.[83]

Numerous polymorphisms of exon I of human MBL have been identified, with heterozygotes making up as much as 30% of the population.[86] [87] People who are homozygous or heterozygous for alleles that express low circulating MBL levels have an increased risk of respiratory tract infections,[88] [89] meningococcal disease[90] and infections during chemotherapy for cancer.[91]

In addition to circulating antibodies, the secretory IgA system produces a special dimeric form of IgA, in which the dimers are linked together by a joining peptide, the J piece, with an associated carrier peptide, the secretory component, which facilitates transport of the complete secretory IgA molecule across mucosal surfaces. Secretory IgA is able to act locally to impair microbial colonization and invasion at the mucosal surface, and it probably has many more functions. Although many people with congenital defects in secretory IgA production suffer from repeated infections, others remain clinically healthy, presumably because of the robust redundant response of various alternative defense systems.[62] For example, the introduction of the *Haemophilus influenzae* protein-polysaccharide vaccine to elicit circulating IgG anticapsular antibodies not only protects from invasive *H. influenzae* type b infection but also has shown that IgG can function on a mucosal surface as this has resulted in a marked decrease in upper airway colonization by the organism, which must be related to an effect of the antibody at the nasopharyngeal mucosal surface.[63] Interestingly, protein-energy malnutrition has a marked inhibitory effect on the secretory IgA response, and this is one likely reason why mucosal infections are so prominent in affected children, with respiratory disease predominating among the very young, and diarrheal diseases among those who are older.

Antibodies have highly specific very discrete functions, for example the neutralization of biologic products produced by the microorganism (Fig. 2.29). The oldest and best-known example is the antitoxin response to bacterial toxins, which was used to develop protective bacterial vaccines consisting of biologically inactivated but antigenic toxoids of tetanus and diphtheria toxins. This approach produced spectacular results, even though the natural infection may not lead to significant protection, presumably because so little toxin antigen is produced. It is now known that toxins are often synthesized as heterodimers, with separate A and B subunits mediating, respectively, the enzyme action of the toxin and its binding specificity. Antibody to either the A subunit or the B subunit can be protective, particularly when it is present before the toxin is introduced. Therefore, antitoxin antibody may be useful when it is elicited by a vaccine, although it may not be clinically effective when initiated during infection (as is the case with pertussis), because the pathophysiologic

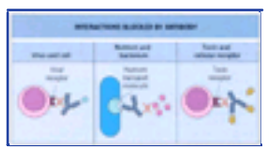

Figure 2-29 Examples of host-pathogen interactions blocked by antibody.

TABLE 2-10 -- Antiviral effects of antibody.

ANTIVIRAL EFFECTS OF ANTIBODY		
Target	**Agent**	**Mechanism**
Free virus	Antibody alone	Blocks binding to cell
		Blocks entry into cell
		Blocks uncoating of virus
	Antibody + complement	Damage to virus envelope
		Blockade of virus receptor
Virus-infected cells	Antibody + complement	Lysis of infected cell
		Opsonization of coated virus or infected cells for phagocytosis
	Antibody bound to infected cells	Antibody-dependent cell-mediated cytotoxicity by natural killer cells, macrophages and neutrophils
Antibody acts to neutralize virus or kill virus infected cells.		

events elicited by the toxin have already occurred by the time the response is mature and antibodies are detectable in situ.

Antibody to viral antigens can also be an effective host defense (Table 2.10). These antibodies may develop during the course of the infection and play a prominent role in the control of infection and clearance of the circulating virus. This occurs in infection with poliovirus and influenza A virus, in both of which virus-neutralizing antibodies develop. Killed parenteral polio vaccine or influenza A vaccine also elicit virus-neutralizing antibodies, which provide significant protection. How these antibodies work is not always clear, and there may be several different mechanisms involved at any one time:

- protection may be related to antibody-induced changes in viral surface charge or shape;
- protection may be due to aggregation and rapid clearance of virus released from cells, preventing viral replication; or
- protection may result in the lysis of infected cells, thus interrupting the life cycle of the virus.

The importance and specificity of antiviral antibody in the host response are shown by the predilection for some viral infections, but not all, in patients with congenital forms of agammaglobulinemia. The infections to which these patients are prone include poliovirus, echovirus and hepatitis B virus infection; the infections to which they are not abnormally prone include measles and rubella. The difference between these groups of viruses may relate to the extent to which cell-mediated immunity contributes to host defense.

Antibody can also function in a co-operative host defense mechanism: antibody-dependent cellular cytotoxicity (ADCC; Fig. 2.30). This is mediated by natural killer lymphocytes (NK cells), which possess high-affinity receptors for the Fc portion of the immunoglobulin molecule but lack markers of either T lymphocytes or B lymphocytes. As a result, they are capable of binding antibody by way of an interaction between the Fc portion of the IgG molecule and the Fc receptor. Antibody-dependent cellular cytotoxicity is distinguished from other cellular cytotoxicity responses by the fact that antigenic specificity is provided by the specificity of the antibody, rather than the cellular effector, and it is not restricted by the MHC locus.[85] In vitro, this mechanism occurs at physiologic concentrations of antibody, and therefore it could be expressed in vivo, especially at sites that are poor in complement activity, such as mucosal surfaces. However, there is as yet no convincing evidence for a role for ADCC in host defense against infection. A variant mechanism of ADCC

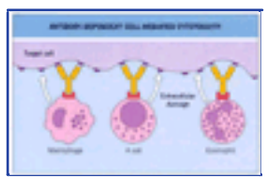

Figure 2-30 Antibody-dependent cell-mediated cytotoxicity. Different effector cells bind to the surface of the target cell via their receptor for antibody.

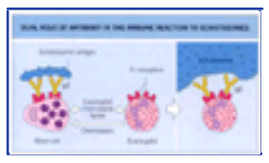

Figure 2-31 Dual role of antibody in the immune reaction to schistosomes. Following contact with the schistosome antigen, mast cells sensitized with anti-schistosome IgE release chemotactic factor, which attracts eosinophils. When the eosinophils arrive they are able to bind to the antibody-coated worm via their Fc receptors and damage the parasite.

involving eosinophils has also been described in vitro as a possible host defense to helminths.[86] These studies demonstrate that eosinophils, which are increased in number by many worm infections, adhere to and damage helminth larvae that have been coated with IgG, resulting in larval death (Fig. 2.31). This form of ADCC has been shown for *Schistosoma mansoni*, *Trichinella spiralis*, *Wuchereria bancrofti* and *Onchocerca volvulus.*

Cell-mediated host immunity

In contrast to humoral immunity resulting from the direct action of soluble host response molecules, such as antibody, cell-mediated immunity requires the action of intact cells of the mononuclear lineage, including macrophages or T cells, or both. This mechanism is dependent on specifically sensitized T cells, which have been activated by antigens of the micro-organism; indeed, in inbred mice, immunity can be adoptively transferred by injecting isolated cells from the spleen into a previously normal, uninfected animal. It is this phenomenon that led to the characterization of the immune response as cell-mediated, in contrast to immunity to pneumococci, which can be transferred by serum antibody alone. Tuberculosis is a particularly well-studied example of cell-mediated immunity (see Chapter 37),[87] in which infection induces clonal expansion of specific mycobacterial antigen-sensitized T cells, which in turn activate the mycobactericidal mechanisms of the macrophage. This event depends on the production

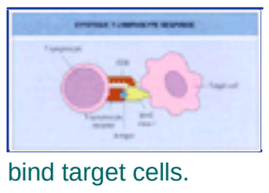

Figure 2-32 Cytotoxic T-lymphocyte response. Cytotoxic T lymphocytes expressing CD8 recognize antigen and major histocompatibility complex (MHC), enabling them to bind target cells.

and release of soluble mediators from the T cells, and it is enhanced by activation of cytokine production from the macrophage, for example IFN-?. Tuberculosis is also a classic example of granuloma formation, a cell-mediated response in which the organism is contained within a collection of macrophages surrounded by activated T cells. The granuloma is a recognizable pathologic unit that is present wherever the organism has spread (e.g. in lung or liver). It is granuloma formation that is initiated by the injection of tuberculin into the skin; indeed, the palpable reaction that characterizes a positive test is a focal granuloma induced by the injection of the antigen, which recruits macrophages and T cells to the site.

Granulomas also characterize the host response in schistosomiasis. It is the granulomatous response that results in the pathology of the infection. Measures that limit granuloma formation protect the host from the damage that results from the production by the granuloma of fibrogenic factors that induce the characteristic scarring of this disease (see Chapter 167).[88]

Another manifestation of cell-mediated immunity is represented by the ability of cytotoxic subpopulations of $CD8^+$ T cells to attack and lyse virus-infected target cells (Fig. 2.32). This cytotoxic lymphocyte response is more firmly established than ADCC and, in contrast to ADCC, it requires no antibody and is genetically restricted by the MHC locus. Congenital defects in this system lead to increased susceptibility to and severity of viral infections (e.g. infections with varicella-zoster virus and other herpesviruses, including cytomegalovirus) and infection with other intracellular pathogens (such as *M. tuberculosis* and *Pneumocystis carinii*). In influenza virus infection, although there is no doubt that vaccine-induced circulating antibody is protective, recovery from disease is best correlated with the appearance of an influenza-specific response by cytotoxic T lymphocytes. Experimental studies involving athymic nude mice and influenza virus have shown that antibody administration blocks shedding of virus for the duration of the treatment, whereas adoptive transfer of virus-specific cytotoxic T lymphocytes clears the virus from the lungs and cures the infection.[89] [90] These findings suggest that the two forms of specific immune response may work together in the response to influenza and presumably to other infections as well.

Natural killer cells

Although they were originally defined as a class of naturally occurring tumoricidal cells, NK cells have since been shown to be involved in the response to infection, especially virus infection. Natural killer cells are distinctive cells that are characterized as relatively large, low-density, granular cells that lack the T-cell receptor and surface immunoglobulin, although they do express a number of cell-surface receptors that are either unique or shared with other

immunologically active cells.[91] Natural killer cells do not require prior activation to function; however, their action is modulated by MHC class I determinants.[92] Cells expressing non-self MHC class I antigens (e.g. transplanted organs) or lacking MHC class I antigens ('loss of self'; e.g. some malignant cells) are subject to attack by NK cells.

The best evidence for the function of NK cells in host defense is in viral infections. Animal models have been extensively used for this purpose, and by depleting and adoptively transferring NK cells, a role in host defense against herpes simplex virus, murine cytomegalovirus and Coxsackie B4 virus infection has been shown, whereas there is no apparent effect of NK cells on lymphocytic choriomeningitis virus. The role of NK cells in humans remains to be clearly shown but a patient has been described with recurrent severe herpes viral infections with deficient NK cell populations.[93]

Interferons

It is now 40 years since the first IFN was described as a host-derived antiviral protein. The IFNs are now known to be a family of proteins, which — rather than being simply antiviral substances — are intimately involved in the regulation of the immune system and play an important role in tumor control. [94] There are, in fact, three classes of IFNs (Table 2.11):

- IFN-a (or leukocyte IFN),
- IFN-ß (or fibroblast IFN), and

¦ IFN-? (or immune IFN).

Interferons a and ß are acid-stable proteins produced in response to viral infections and double-stranded RNA and related polyanions; each of these IFNs is an antiviral protein. In contrast, IFN-?, an acidlabile protein, is made during antigen or mitogen activation of T cells and NK cells, primarily in response to IL-12 released from macrophages (Fig. 2.33). [95] Interferon-?, in turn, feeds back on macrophages to upregulate the expression of TNF-a, which, together with IFN-?, increases the expression of IL-12 and further drives the production of IFN-?. Interleukin-12 together with IFN-? favors the development of the T-helper-1 lymphocyte response, which:[96]

¦ activates macrophages to produce reactive oxygen intermediates such as superoxide and nitric oxide, and to express MHC and type II and Fc receptors;
¦ augments T-cell-mediated cytotoxicity; and
¦ induces NK cells.

TABLE 2-11 -- Human interferons.

HUMAN INTERFERONS			
	Interferon-a	**Interferon-ß**	**Interferon-?**
Alternative name	'Leukocyte' interferon	'Fibroblast' interferon	'Immune' interferon
Principal source	All cells	All cells	T cells
Inducing agent	Viral infection (or double-stranded RNA)	Viral infection (or double-stranded RNA)	Antigen (or mitogen)
Number of species	22	1	1
Chromosomal location of gene(s)	9	9	12
Antiviral activity	+++	+++	+
Immunoregulatory activity:			
Macrophage action	-	-	++
MHC class I upregulation	+	+	+
MHC class II upregulation	-	-	+
MHC, major histocompatibility complex.			

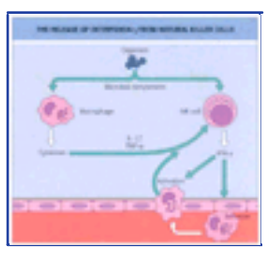

Figure 2-33 The release of interferon (IFN)-? from natural killer (NK) cells. IL, interleukin; TNF, tumor necrosis factor.

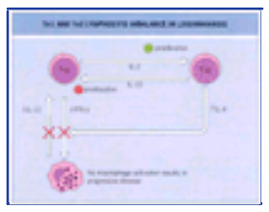

Figure 2-34 T-helper (Th)1 and Th2 lymphocyte imbalance in leishmaniasis. Leishmaniasis is characterized by deficient interferon (IFN)-? production and inhibition of its action. IL, interleukin.

In some intracellular infections of mononuclear cells, IFN-? production is reduced due to increased IL-10 production from activated T-helper-2 lymphocytes (e.g. in tuberculosis, leprosy and leishmaniasis), and this may be a major reason for the progression of these infections (Fig. 2.34). Successful therapy is associated with restoration of IFN-? production. Low levels of IFN-? are due to one of three abnormalities:

¦ abnormal IL-10 and/or IL-12 production,
¦ a deficiency of $CD4^+$ T cells, or
¦ a deficiency of IFN-? receptors.

Deficiency of IFN-? receptors has been identified in a small number of patients, and these patients have a prominent susceptibility to

nontuberculous mycobacterial infection but not to other infections, suggesting a unique function specificity for IFN-? in this infection.

Another cytokine has been identified, namely IL-18 (IFN-? releasing factor), which appears to have an upstream regulatory role in IFN-? regulation. Interleukin-18 is part of the IL-1-like family of cytokines that is activated by caspase 1 and binds to receptor with homology to IL-1 receptor and TLRs. [97] It promotes IFN-? production, induces NK cell cytotoxicity and promotes the T-lymphocyte T-helper-1 phenotype. Its relative role in IFN-? production and other immunologic activities in association with IL-12 are the focus of considerable research interest at the present time.

Interferon-? has been used with success in chronic granulomatous disease, for which it is licensed in several countries, and in the treatment of lepromatous leprosy, visceral and diffuse cutaneous leishmaniasis and disseminated mycobacterial infections. The use of IFN-a is also approved for the treatment of hairy cell leukemia, condyloma acuminatum, Kaposi's sarcoma and hepatitis B and C virus infection. It is likely that the utility of IFN therapy will increase as the number of disease indications in which it has an impact also increases, and the importance of providing the correct co-signal at the right time becomes better known.

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Chapter 3 - Prevention

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Veronique Gibbons

INTRODUCTION

Prevention has been the greatest success in the field of infectious diseases. The largest impact in reducing human premature mortality has occurred through improved standards of living and the impact this has had on infection. However, the emergence of infections such as HIV and Ebola and the re-emergence of infections such as tuberculosis have been a stark reminder of the fact that new infections develop and old ones return if public health interventions lapse, behavior changes, social unrest or changes become prevalent, or various environmental interventions occur. Control of infectious disease is being challenged by development of resistance in bacteria, viruses and parasites such as malaria to both prophylactic and curative drugs. In some countries up to half of all etiologic agents of the most common forms of bacterial meningitis and pneumonia are now resistant to penicillin.[1] Infectious diseases do not respect administrative boundaries and a case of influenza in India does matter directly to someone living in the USA, because with the modern rates of global travel it takes very little time for a microbe to circumnavigate the planet. This was starkly illustrated by the rapid spread of the SARS (severe acute respiratory syndrome) epidemic that emerged in early 2003. Since 11th September 2001 the specter of smallpox is threatening us again after being eradicated from the world. Mass population movements have exacerbated the situation because refugees are vulnerable to infectious diseases and the circumstances in which they are mostly obliged to live are ideal for generating outbreaks.

Although great advances have been made, a considerable burden of morbidity and mortality from infectious diseases remains globally.[2] The majority of the nearly 14 million deaths that occurred in 2000 were preventable through low-cost health interventions that have existed for years to prevent or cure infectious disease[2] [3] (Table 3.1). Pneumonia, tuberculosis, diarrheal diseases, malaria, measles and HIV/AIDS account for half of all premature deaths, killing mostly children and young adults (Table 3.2). Apart from death they also cause long-term disability. Disability adjusted life years (DALYs) were developed to measure lost years of healthy life by a combination of lost years due to premature mortality and lost healthy years through disability.[2] In 2000, 26.8% of the global burden of disease in DALYs was directly attributable to communicable diseases[2] and six out of the leading 20 causes of DALYs were communicable diseases (Table 3.3).

A considerable further burden is from orphan or neglected diseases, which are left out of the priorities of pharmaceutical companies and private-public sector partnerships because there is insufficient market to justify the investment necessary to develop new treatments or vaccines.[4] Infectious diseases are the biggest killer of the young.[3] Measles, diarrhea and pneumonia, often in combination with malnutrition, together claim the lives of more than 5 million children under 5 years of age every year. [5] The impact of such diseases makes people poor and keeps them in poverty.[5] The long-term economic costs of such poverty are projected to be greater than the costs of the solutions. [5] Furthermore, as infectious diseases are

TABLE 3-1 -- Low cost interventions to prevent the global burden of infections.[j]

LOW COST INTERVENTIONS TO PREVENT THE GLOBAL BURDEN OF INFECTIONS	
Intervention	**Impact**
Integrated management of childhood illnesses	Combined therapy for common infectious diseases including oral rehydration and low-cost antibiotics could prevent up to 3 million deaths per year from pneumonia and diarrheal diseases alone
Childhood vaccinations — tuberculosis (TB)	More widespread use of low-cost vaccines could prevent an additional 1.6 million deaths per year
Directly observed therapy, short course (DOTS)	1–2 million TB deaths could be prevented through interventions such as DOTS, supported by the WHO STOP TB initiative
Impregnated bed nets	One in four deaths from malaria could be prevented if children at risk slept under bed nets at night
Availability of essential drugs	More than one-third of the world's population lack regular access to essential life-saving drugs
Prevention of HIV/AIDS	Cheap condoms, safe drug-injecting equipment, safe injection practice, treatment of other sexually transmitted diseases, counseling for HIV-infected mothers, and sex education at school and in the community could effectively reduce HIV transmission
Nutrition — vitamin and mineral supplements	Up to one in four child deaths from infectious diseases could be prevented by vitamin A supplements, and malaria deaths in children can be reduced by the use of iron supplements for anemia
Education	Education of women reduces infant mortality. Health education promotes safe sex, good nutrition and hygiene, immunization, and parents knowing what to do when their child is sick

j* *Adapted from WHO.* [3]

not constrained by administrative boundaries, controlling infections such as multiresistant tuberculosis in the poorest regions of the world where it has emerged has benefits for the whole world. Early intervention to contain epidemics may have dramatic effects and the World Health Organization (WHO) hopes, by taking earlier action, to prevent the HIV epidemic in Asia and Eastern Europe from developing to the same extent as it has in sub-Saharan Africa, where 70% of people with HIV/AIDS currently live.

An additional preventable burden arises from cancer, with up to 16% linked to infections: human papillomavirus infection is linked to cervical cancer; hepatitis B and C virus infection to liver cancer; *Helicobacter pylori* infection to stomach cancer; and schistosomiasis

TABLE 3-2 -- The continuing problem of infectious disease and targets for prevention.[*]

THE CONTINUING PROBLEM OF INFECTIOUS DISEASE AND TARGETS FOR PREVENTION				
Disease	**Deaths per year (millions)**	**New cases per year (millions)**	**% in developing countries**	**UN target**
HIV/AIDS	3	5.3	92	To reduce the number of newly infected young people by 25% by 2010
Tuberculosis	1.9	8.8	84	To halve deaths and prevalence by 2010
Malaria	>1	300	Nearly 100	To reduce disease burden by 50% by 2010
HIV/AIDS, tuberculosis and malaria.				

* *Adapted from WHO.* [5]

to bladder cancer.[6] Hepatitis B virus vaccination programs effectively reduce mortality from hepatocellular carcinoma.[7]

Prevention of infectious diseases can be regarded as a social challenge. Diseases may be indicators of environmental quality as well as patterns of human behavior, and social, environmental or behavioral change may be the most effective intervention. Improvements in the quality of nutrition, the physical environment and provision of safe drinking water in the affluent world led to falls in mortality from practically all infectious diseases before antibiotics or vaccination programs were introduced. Cholera is hardly an infectious disease in a country with good sanitation; imported cases virtually never result in transmission.[8] The incidence of hepatitis A virus infection has fallen in every country with good sanitation and vaccination has played little part in eliminating this virus. The percentage of people with some form of improved water supply and sanitation has risen gradually, but at the beginning of 2000 one-sixth (1.1 billion) of the word's population was without access to improved water supply and two-fifths (2.4 billion) lacked access to improved sanitation.[9] Trends varied by region, with the poorest improved water access in Africa and the poorest sanitation in Asia (Fig. 3.1). Rural areas are generally less well served (Fig. 3.2).

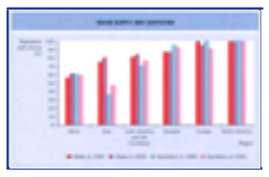


Figure 3-1 Water supply and sanitation. Coverage by region, 1990 and 2000. *Adapted from WHO*[9] .

TABLE 3-3 -- Leading causes of disability adjusted life years.*

LEADING CAUSES OF DALYs		
Rank	**Condition**	**% total DALYs**
1	Lower respiratory tract infections	6.4
2	Perinatal conditions	6.2
3	HIV/AIDS	6.1
4	Unipolar depressive disorders	4.4
5	Diarrheal diseases	4.2
6	Ischemic heart disease	3.8
7	Cerebrovascular disease	3.1
8	Road traffic accidents	2.8
9	Malaria	2.7
10	Tuberculosis	2.4
11	Chronic obstructive pulmonary disease	2.3
12	Congenital abnormalities	2.2
13	Measles	1.9
14	Iron deficiency anemia	1.8
15	Hearing loss, adult onset	1.7
16	Falls	1.3
17	Self-inflicted injuries	1.3
18	Alcohol use disorders	1.3
19	Protein-energy malnutrition	1.1
20	Osteoarthritis	1.1
Both sexes and all ages and percentage of total DALYs, with the total in all WHO regions in 2000 being, 1,472,392 thousand years.		

** With permission from WHO.* [2]

HOST-MICROBE-ENVIRONMENT INTERACTIONS

Infectious diseases are the consequence of interaction of the infectious agent and the host. The environmental setting affects the likelihood that such an interaction will occur and the context in which it takes place (Fig. 3.3).[9] Therefore prevention efforts can be focused on each or any of these components, depending on the disease in question and the availability and acceptability of appropriate interventions.

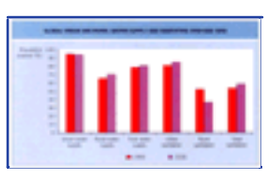


Figure 3-2 Global urban and rural water supply and sanitation, 1990 and 2000. *Adapted from WHO.*[9]

Figure 3-3 The host-agent-environment triad. *Adapted from WHO.*[9]

The specific characteristics of the host that increase the likelihood of exposure to an infectious agent or that predispose to infection, and the severity of the clinical course, are discussed in detail elsewhere in this book. However, there are several general features of the host that, alone or together, can influence the ultimate clinical response and offer the potential for intervention. Similarly, features of the infectious organism can also affect the likelihood that it will establish an infection in humans and subsequently develop into a clinical illness.

Severe malnutrition can precipitate a downward spiral. Weight loss and stunted growth may be the outward manifestations of underlying mucosal damage that exacerbates the state of malnutrition through malabsorption. The weakened mucosal barrier may also increase the success of invading micro-organisms and result in a blunted mucosal immune response. This can result in an increase in the incidence of disease in the population and an increase in the severity of disease in the affected

individual.

In addition to the effects of severe malnutrition, a growing body of evidence suggests that micronutrients such as vitamin A may also have substantial effects on immunity. This effect has been most striking among young children in developing countries when affected with the measles virus. It is estimated that nearly half of the world's children have an inadequate intake of vitamin A, putting them at a 23% higher risk of death from common diseases.[10] This recognition has led to the addition of vitamin A supplementation in infant immunization programs in many developing countries.[11] [12] [13]

There is also evidence that micronutrient supplementation with trace minerals such as zinc and selenium may have a protective effect against infections in malnourished children[14] [15] and institutionalized older people.[16] [17]

Advances in genetics are anticipated to have a great impact in increasing our understanding of the pathogenicity of infectious diseases through better knowledge of both host and pathogen. The human genome project is providing information about the human host, and sequencing of entire bacterial genomes of organisms such as *Mycobacterium tuberculosis*, with the possibility of comparing different related organisms, will provide insight into pathogenicity mechanisms.[18] [19]

The tools and knowledge to ease the burden of HIV/AIDS, tuberculosis and malaria already exist. World leaders have given political commitments and agreed upon United Nations (UN) agency targets for reductions in HIV/AIDS, tuberculosis and malaria (see Table 3.2). Political will at international, national and local levels as well as resources are necessary for success in the control of infectious diseases.[3] [5] Great progress has been made in the control of many diseases and seven have been targeted for eradication or elimination: poliovirus infection, measles, guinea worm disease, tetanus, leprosy, lymphatic filariasis and Chagas' disease.

PREVENTION METHODS

The aim of any control program can be defined by three levels — containment, elimination and eradication. Containment reduces the infection to an acceptable level although the disease remains endemic. Elimination means that there is no endemic transmission

and that imported infection leads only to isolated outbreaks. Eradication removes the disease from the world; smallpox is so far the only example.

Environmental change

Overcrowding in polluted urban environments contributes to the risk of infection. In 1999 more than 1 billion people were estimated to lack access to safe drinking water and around 800 million to lack any access to health services. Changes in land and water use such as deforestation, agricultural development, dams and irrigation schemes can trigger outbreaks of parasitic or other vector-borne diseases. Similar changes may bring people into contact with diseases such as Ebola. An increase in global warming of only 1–2°C over the next 50 years could increase the range of *Anopheles* mosquitoes and extend the range of malaria, dengue and lymphatic filiariasis.[3]

Vector control

By reducing the source of transmission, vector control efforts have had a substantial impact on disease incidence. An estimated 3% of the global burden of disease has been attributed to vector-borne diseases.[20] Malaria is a large component, causing 1.9 million deaths in 2000,[2] but other vector-borne diseases such as dengue fever are out of control in many regions. Roll Back Malaria is a global partnership founded in 1998 by the WHO, the United Nations Development Program (UNDP), the United Nations Children's Fund (UNICEF) and the World Bank. It has the goal of halving the world's malaria burden by 2010 — estimated to be more than 300 million acute illnesses and 1.9 million deaths per year. The Roll Back Malaria partnership includes national governments, civil society and nongovernmental organizations, research institutions, professional associations, UN and development agencies, development banks, the private sector and the media. It aims to expand the use of interventions that are already known to be effective in tackling malaria, including prompt access to effective treatment, promotion of insecticide-treated mosquito nets and improved vector control, prevention and management of malaria in pregnancy and improving the prevention of and response to malaria epidemics and malaria in complex emergencies, as well as supporting research into better drugs and insecticides, malaria vaccines and possibly genetically modified mosquitoes that will not transmit malaria.

Prevention guidelines

Beyond clean water, clean air and available sanitation, our enhanced understanding of microbial pathogenesis, disease transmission and host immunity now provides a number of options for intervention. This has resulted in an increasing number of prevention guidelines, which are updated as new information becomes available. These guidelines represent both primary prevention (a decrease in the likelihood

TABLE 3-4 -- Different categories of guidelines.

DIFFERENT CATEGORIES OF GUIDELINES	
Classification	**Example**
Medical condition	Absent or dysfunctional spleen
Target occupational group	Health care workers
Target professional group	Infection control officers
Organism	Control of hepatitis A virus
Mode of transmission	Sexually transmitted diseases, blood-borne viruses
Intervention	Immunization
Setting	Guidance on infection control in schools, nurseries and day care

of exposure and infection) and secondary prevention (a decrease in the likelihood of disease, given exposure). The details of the many guidelines that are available are far too wide-ranging to include in a single chapter.

Guidelines exist for a variety of purposes and are focused in a variety of ways (Table 3.4). They include pre-exposure and post-exposure protection, and guidance targeted at groups at high risk of exposure as well as groups at high risk from particular infections because of other medical conditions or because they are undergoing procedures (Table 3.5). They have been produced by groups and authorities at local, regional, national and international levels (Table 3.6). Guidelines vary greatly in quality, are different from country to country and vary in the extent to which they incorporate information on implementation or audit and to which they are locally adapted and implemented. They should be produced systematically[21] and undergo regular review to take account of new evidence or interventions and changing epidemiology, for example the emergence of antibiotic resistance or the development of a new antibiotic.

Increasingly, medical practice must be evaluated against standards, and standards should be based upon rigorously evaluated evidence. Evidence-based guidelines set a standard against which practice can be evaluated. As the evidence base for medical practice grows, so the

TABLE 3-5 -- Prevention guidelines — examples of topics.

PREVENTION GUIDELINES — EXAMPLES OF TOPICS	
Category of prevention	**Topic**
Pre-exposure	
At risk from an intervention	Health-care-associated infection — MRSA, nosocomial pneumonia
	Urinary tract infection
	Surgical procedures

Travelers	General advice (Yellow Book — WHO)
	Malaria
	Diarrhea
Underlying conditions at increased risk	Absent or dysfunctional spleen
	Heart disease — prevention of bacterial endocarditis
	Chronic pulmonary disease
	HIV infection — risk of opportunistic infection
	Peritonitis in patients with cirrhosis/ascites
	Sickle cell disease
	Immunosuppression
Postexposure	Hepatitis A
	Rabies
	HIV infection
	Hepatitis B
	Tetanus — wound prophylaxis
	Diphtheria
	Chickenpox
	Sexually transmitted diseases — following sexual assault
Community	Infection control in schools and nurseries
	Meningitis, meningococcal infection, *Haemophilus influenzae* type b infection
	Outbreak investigation and control
Pregnancy and neonates	Group B streptococcal carriage in mother
	RSV immunoglobulin for at-risk infants
	Rash contact (e.g. parvovirus B19 exposure) in pregnancy
	Hepatitis B virus carriage in mother
MRSA, methicillin-resistant *Staphylococcus aureus*; RSV, respiratory syncytial virus.	

TABLE 3-6 -- Sources and scope of guidelines.

SOURCES AND SCOPE OF GUIDELINES		
Scope	**Source and example**	
International	WHO	Tuberculosis and air travel. Guidelines for prevention and control, http://www.who.int/gtb/publications/aircraft/
	European Working Group on *Legionella* Infection	European guidelines for control and prevention of travel-associated Legionnaires' disease, http://www.ewgli.org/public_info/publicinfo_european_guidelines.asp
	Cochrane collaboration	The Cochrane Library UK, http://www.cochrane.org/
National	Australia	Australian Department of Health and Ageing, Population Health Division, http://www.health.gov.au/pubhlth/strateg/communic/index.htm
		Communicable Disease Network Australia, http://www.dhs.vic.gov.au/nphp/cdna/index.htm
		Guidelines for the control of communicable diseases: 'The Blue Book', http://www.dhs.vic.gov.au/phd/hprot/inf_dis/bluebook/
	Canada	Health Canada, http://www.hc-sc.gc.ca/pphb-dgspsp/new_e.html
		Health Care Associated Infection, http://www.hc-sc.gc.ca/pphb-dgspsp/hcai-iamss/index.html
	New Zealand	Ministry of Health — Immunization, http://www.moh.govt.nz/immunisation.html
		The New Zealand Guideline Group, http://www.nzgg.org.nz/index.cfm
	USA	CDC, http://www.cdc.gov/ncidod/hip/Guide/guide.htm
		CDC's Division of Healthcare Quality Promotion, http://www.cdc.gov/ncidod/hip/Guide/guide.htm
Regional	Scotland, UK	Scottish Intercollegiate Guidelines Network, http://www.sign.ac.uk/index.html
	England and Wales	Public Health Laboratory Service, http://www.phls.org.uk/default.htm
Professional bodies	Various countries	Association for Professionals in Infection Control, http://www.apic.org/
		American Academy of Family Physicians, http://www.aafp.org/
		American Heart Association, http://www.americanheart.org/
		American College of Obstetricians and Gynecologists, http://www.acog.org/
		British Society for Antimicrobial Chemotherapy, http://www.bsac.org.uk/
		British Thoracic Society, http://www.brit-thoracic.org.uk/guide/guidelines.html
Examples of a global variety.		

task of producing such guidelines requires a multidisciplinary and dedicated resource. This has been provided by national bodies such as the Centers for Disease Control and Prevention (CDC) in the USA, organizations linked to professional bodies such as the Scottish Intercollegiate Guidelines Network (SIGN)[22] or networks concerned with evidence-based medicine such as the Cochrane collaboration [23] or the National Health Service Centre for Reviews and Dissemination in England, which has been involved in health technology assessment [24] (see Table 3.6). Even so, guidelines produced by such bodies may not always meet published standards.[25]

Surveillance

Early detection of outbreaks is a vital part of their prevention and surveillance is therefore an integral part of infectious disease control. Surveillance has been defined as the ongoing systematic collection, analysis and interpretation of outcome-specific data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease[26] or, more briefly, 'data for action'. Surveillance is carried out with the objectives of identifying changes and patterns to trigger investigation and timely intervention, to identify emerging and re-emerging diseases, and to evaluate, plan and prioritize health services and preventive interventions. To meet these objectives it needs to be simple, timely, accurate and analyzed regularly, and results should be communicated rapidly for appropriate control measures to be taken. Diverse data are used. Statutory notifications, laboratory reports and mortality statistics are most frequently analyzed, followed by

various sentinel systems such as the British Paediatric Surveillance Unit.[27] Reports are made in weekly bulletins in many countries.[28] [29] For vaccination programs, surveillance is also required postlicensure to determine the vaccine efficacy in field conditions, to monitor for changes in vaccine efficacy and to detect rare adverse events as well as vaccination coverage and population susceptibility. Surveillance and ad-hoc reports may lead to outbreak detection, an integral part of the public health role requiring the skills of field epidemiologists trained in programs such as the CDC Epidemic Intelligence Service in the USA[30] or the European Programme for Intervention Epidemiology Training.[31] Public health epidemiology provides the tools to identify potential control measures even before the causative organism is identified or fully characterized, for example in the cases of cholera[32] and HIV infection.[33]

A clear case definition is required for outbreak investigation and other epidemiologic studies and is also an integral part of a good surveillance system.[34] [35] The case definition contributes to the sensitivity and specificity of the surveillance system. When there is an effective intervention such as a vaccination program that has a dramatic impact on the incidence of disease, the positive predictive value of the case definition will change. Case definitions and surveillance systems may have to be adapted to the local epidemiology of the disease and its level of control, [36] and enhanced surveillance systems are often required for rarer diseases and those that are well controlled by vaccination.

In addition to surveillance of known diseases, methods have been developed to detect new and emerging infections and the WHO now has a Department of Communicable Disease Surveillance and Response that acts through partners in a Global Outbreak Alert and Response Network; it has been involved in various outbreaks, such as Ebola.[37] The WHO Influenza Surveillance Network ensures the rapid detection of new influenza viruses and helps to determine the composition of the next season's influenza vaccine, as well as being ready for the detection of pandemic influenza.

Mathematical modeling and health economics

Development of strategies to prevent infection requires a multidisciplinary approach involving clinicians, epidemiologists, public health

Figure 3-4 The basic reproductive number, R_0 — threshold for invasion. The left side shows R_0 = 3 and the right side R_0 = 1. *Modified from Begg and Gay.*[32]

specialists, field workers, mathematicians, modelers and health economists. Infections in populations have certain characteristics that require an understanding beyond the level of infections in individuals. Individual characteristics such as the mode of transmission, reservoirs of infection, the latent period, incubation period and total period of infectivity, the proportion of clinically apparent cases, duration of immunity and the influence of maternal antibody are vital parameters that must be understood for control measures to be appropriate. Infections that lead to life-long immunity, such as measles, tend to occur in regular epidemics, in contrast with diseases in which individuals become susceptible to reinfection on recovery (such as tetanus) or where a carrier state exists (such as hepatitis B virus infection). Other epidemiologic features are specific to particular populations or regions, accounting for different patterns of infection, such as meningococcal infection in Africa.

Modelers have developed approaches to explain the patterns observed to predict the impact of control measures. A fundamental idea is the basic reproduction number (R_0), a summary of these parameters that is specific for each disease. R_0 is defined as the number of secondary (infections) cases produced in a completely susceptible population by a typical infectious individual. The effective reproduction number (R) is the number of secondary cases produced by a typical infectious individual in a particular setting, and so depends upon the proportion of susceptible individuals in the population. Infection only persists in the population if infection is passed

TABLE 3-7 -- Measuring vaccination coverage.

MEASURING VACCINATION COVERAGE				
Type	**Method**	**Advantages**	**Disadvantage**	**Example**
Vaccine usage	Counts doses of vaccine used	Identifies major trends quickly, suitable for centrally distributed vaccine	No information on wasted doses; no information on incomplete courses; no information on vaccine recipients	AFIX, used in the USA for clinic or surgery-based immunization coverage — cannot be extrapolated to provide national coverage data
Sample population assessment	Sampling in different settings; can be done systematically or randomly	Can be a quick and cheap method	Problem with selection bias — may overestimate; pockets of low coverage may be missed	Used in New Zealand, based on immunization benefit claims (moving to total population assessment)
Total population assessment	Denominator: resident children; live births — numerator: completed courses; doses given	Data on primary care doses for age; long-term trends can be observed	Good records needed, migration/poor recording of doses given, may be delay in producing summary statistics	COVER, used in the UK; all infants are entered into the child health register at birth — can be extrapolated to provide national data
Examples of vaccine coverage used in the developing world. COVER, Coverage of Vaccination Evaluated Rapidly.				

to one or more individuals by each infectious person, or in other words if R_0 = 1. For diseases that show natural epidemic cycles, such as measles, pertussis and parvovirus B19, R oscillates around 1 as the proportion of the population that is susceptible oscillates around an equilibrium value.

The concept of reproduction number is directly related to herd immunity, the idea that an infection can be eliminated even if only a proportion of the population is immune, and that nonimmune individuals are thereby protected. A population is said to have herd immunity if on average an infection by a typical primary (infection) case produces less than one secondary infection (R_0 < 1; Fig. 3.4).[32] From such a simple idea, more complex modeling has developed involving both deterministic and stochastic approaches. In combination with health economics, modeling can be used to set health service priorities for preventive programs.[38]

Health economics of infectious disease prevention is a specialized field. For example, there is no standard for evaluating the cost-effectiveness of vaccination programs and many cost-effectiveness analyses are methodologically incorrect because they take no account of indirect protection (herd immunity). The gap between research and practice is large and vaccine efficacy estimated from clinical trials may be quite different from the observed effectiveness of a mass immunization program. Detailed observational studies are impracticable because of the large scale required, making them expensive, and the limitations in finding suitable control populations

Figure 3-5 Global polio incidence. Global and regional summaries of reported cases of poliomyelitis worldwide from 1990–2001. *Modified from WHO.*[43]

for comparison. Modeling allows the impact of mass vaccination to be predicted but still needs to be supported by post-implementation surveillance. Challenges for health economists include the fact that vaccine demand is derived and the 'market' is affected by uncertainty and imperfect information. Parents are generally risk adverse and may find the asymmetry of information between themselves and providers or agents threatening. Vaccination is a public good but the equity issues vary from other fields in that individuals can be protected indirectly through vaccination of others. These and other complex factors need to be incorporated into health economic analyses.

VACCINATION

Immunization has been the most successful medical intervention in reducing the incidence of disease and related deaths worldwide. Guidelines in the form of handbooks, such as the Pink Book in the USA and the Green Book in the UK, and international guidelines from the WHO, have disseminated a greater understanding of the

TABLE 3-8 -- Global measles strategic plan 1999.*

GLOBAL MEASLES STRATEGIC PLAN 1999			
Strategies for achieving sustainable reduction in measles mortality		**Strategies for achieving and maintaining interruption of indigenous measles transmission**	
•	Goal: Reduce the number of annual measles deaths by half by 2005	•	Goal: Achieve and maintain interruption of indigenous measles transmission in large geographic areas
1	Routine immunization — achieve >90% routine vaccination coverage (in each district and nationally) with at least one dose of measles vaccine administered at 9 months of age or shortly thereafter	1	Routine immunization — achieve very high (i.e. >95%) immunization coverage (in each district and nationally) with the first dose of measles vaccine administered through routine services
2	Second opportunity for measles vaccination — for all children through routine or supplemental activities	2	Second opportunity for measles vaccination — to maintain the number of susceptible population below the critical threshold for 'herd' immunity
3	Measles surveillance — establish effective surveillance for measles to report regularly the number, age and vaccination status of children contracting or dying from measles, to conduct outbreak investigations and to monitor immunization coverage	3	Measles surveillance — investigation and laboratory testing of all suspected measles cases (case-based surveillance). Isolation of measles virus should be attempted from all chains of transmission
4	Improve management of complicated cases — including vitamin A supplementation and adequate treatment of complications	4	Improve management of complicated cases — including vitamin A supplementation and adequate treatment of complications
Of all health interventions, measles immunization carries the highest health return for the money spent, saving more lives per unit cost.			

** Modified from WHO, UNICEF.*[44]

epidemiology and prevention of vaccine-preventable diseases to a wider network of professionals.[39] [40]

An essential component of any effective immunization program is a system to measure performance. Vaccine coverage is often used as a surrogate for disease reduction and there are several ways of estimating this (Table 3.7). Assessing vaccine coverage through national, state or local methods is an essential component of any effective immunization program. In the USA, the National Vaccine Advisory Committee strongly encourages the development of community- or state-based immunization registry systems and recommends that vaccination providers participate in these registries whenever possible.[41] The AFIX system (Assessment, Feedback, Incentives and eXchange of information) developed by the CDC is a clinic- or surgery-based vaccine coverage assessment tool that can be used with or without computers. The aim of AFIX is not only to ensure that children are immunized on time but also to provide vaccine providers with feedback about their level of immunization performance in a timely manner. The AFIX system incorporates a mechanism to comment on reasons for low coverage and strategies a vaccine provider might use to improve immunization uptake. A 95% participation

TABLE 3-9 -- Reduction in vaccine-preventable diseases in the USA.*

REDUCTION IN VACCINE-PREVENTABLE DISEASES IN THE USA			
Disease	**Maximum no. reported cases during prevaccine era**	**Reported no. cases during 2001**	**% change in morbidity**
Diphtheria	206,939	2	-99.99
Measles	984,134	116	-99.99
Mumps	152,209	266	-99.83
Pertussis	265,269	7,580	-97.14
Polio (paralytic)	21,269	0	-100.00
Rubella	57,686	23	-99.96
Congenital rubella syndrome	20,000	3	-99.98
Tetanus	1,560	37	-97.63
Haemophilus influenzae type b/unknown (<5 years)	20,000	1,597	-92.01
The figures are reported for 2001 and the maximum number of reported cases during the prevaccine era is estimated for invasive congenital rubella syndrome and polio.			

** Modified from CDC.*[47]

of children aged less than 6 years in fully operational population-based registries is a national health objective for 2010.[42]

The example of smallpox eradication is a model on which other diseases and their eradication programs are drawn. The global smallpox eradication program began in 1967, initially using a 2-fold strategy of mass vaccination campaigns and surveillance systems to detect and contain cases and outbreaks. For example, in isolated pre-20th century populations, R_0 for smallpox was 3.5–6 (R_0 = 1). The persistence of smallpox in the presence of a highly vaccinated population showed that, by isolating people with smallpox and their contacts, outbreaks could be more rapidly contained, even in areas where vaccination coverage was low. This surveillance and containment strategy became a key element in the global eradication program.[39] The world's last indigenous case was in Somalia on 26 October 1977.

The synchronization of immunization among neighboring countries has become a model for eradicating diseases globally. Humans are the only known reservoir for many infections and therefore eradication is a distinct possibility. The success of smallpox eradication demonstrated the power of vaccines when coupled with appropriate strategies. In addition, smallpox had a low R_0 , was distinct and recognizable, and individuals were infectious from the time their earliest lesions were apparent.

In 1988 the World Health Assembly adopted the goal of global eradication of poliovirus by the year 2000. Although this goal has not been met, substantial progress has been made (Fig. 3.5). The Global Polio Eradication Initiative was launched in 1988.[43] Since then, three WHO regions have been certified polio-free: the Americas region (36 countries) in 1994, the Western Pacific region (37 countries, including China) in 2000 and the European region (51 countries) in 2002. Wildly endemic on five continents in 1988, polio is now only found in parts of Africa and South East Asia. The new polio eradication target date is 2005.

Measles is another leading cause of childhood deaths, mainly in developing countries. There were an estimated 30–40 million cases of measles in 2000, causing some 777,000 deaths. Measles accounts for nearly half of the 1.7 million annual deaths due to childhood vaccine-preventable diseases. The WHO and UNICEF have developed the Global Measles Strategic Plan together with the US CDC and numerous experts worldwide (Table 3.8).[44] Measles, unlike smallpox, is not always recognizable and individuals are infectious before the appearance of the erythematous maculopapular rash, leading to 'silent spreaders'. An R_0 of 15–20 means an

individual can infect a large proportion of other susceptible individuals before an index case has been identified. High levels of herd immunity and high vaccination coverage are required to control the infection. Factors in the success of smallpox eradication have not been found

TABLE 3-10 -- Licensed vaccines in the USA, October 1999.[†]

LICENSED VACCINES IN THE USA, OCTOBER 1999
• Adenovirus vaccine, live oral, type 4
• Adenovirus vaccine, live oral, type 7
• Anthrax vaccine, adsorbed
• BCG, live
• Botulinum toxin type A
• Cholera vaccine
• Diphtheria toxoid, adsorbed
• Diphtheria and tetanus toxoids and acellular pertussis vaccine, adsorbed
• Diphtheria and tetanus toxoids and pertussis vaccine, adsorbed and *Haemophilus influenzae* type b, conjugate vaccine
• Diphtheria and tetanus toxoids, adsorbed
• *Haemophilus influenzae* type b, conjugate vaccine
• Hepatitis A vaccine, inactivated
• Hepatitis B vaccine, recombinant
• Influenza virus vaccine, inactivated
• Japanese encephalitis virus vaccine, inactivated
• Lyme vaccine, recombinant vaccine
• Measles, mumps and rubella virus vaccine, live
• Measles and mumps virus vaccine, live
• Measles and rubella virus vaccine, live
• Measles vaccine, live
• Meningococcal polysaccharide vaccine group A
• Meningococcal polysaccharide vaccine group C
• Meningococcal polysaccharide vaccine groups A and C, combined
• Meningococcal polysaccharide vaccine groups A, C, Y and W-135, combined
• Mumps vaccine, live
• Pertussis vaccine, adsorbed
• Plague vaccine
• Pneumococcal vaccine (23-valent)
• Poliovirus vaccine, inactivated
• Poliovirus vaccine, live oral
• Rabies vaccine
• Rotavirus vaccine, live oral reassortant vaccine[*]
• Rubella and mumps vaccine, live
• Rubella vaccine, live
• Tetanus toxoid, adsorbed
• Tetanus and diphtheria toxoids, adsorbed (for adult use)
• Typhoid vaccine, live oral Ty21a
• Typhoid Vi polysaccharide vaccine
• Varicella vaccine, live
• Yellow fever vaccine

† *Modified from the National Institute of Allergy and Infectious Diseases' Jordan Report (National Institutes of Health).*[48]
* On 15 October 1999, Wyeth Lederle Vaccines announced that it had withdrawn RotaShield® from the market.

TABLE 3-11 -- Immune globulins and antitoxins available in the USA and UK.[†]

IMMUNE GLOBULINS AND ANTITOXINS AVAILABLE IN THE USA AND UK				
Immunobiologic	**USA**	**UK**	**Type**	**Indication**
Botulinum antitoxin	?	?	Specific equine antibodies	Treatment of botulism
Cytomegalovirus intravenous immune globulin	?	?	Specific human antibodies	Prophylaxis for bone marrow and kidney transplant recipients
Diphtheria antitoxin	?	?	Specific equine antibodies	Treatment of respiratory diphtheria
Immune globulin	?	?	Pooled human antibodies	Hepatitis A pre-[*] and postexposure prophylaxis; measles postexposure prophylaxis
Intravenous immune globulin	?	?	Pooled human antibodies	Replacement therapy for antibody deficiency disorders; immune thrombocytopenic purpura; hypogammaglobulinemia in chronic lymphocytic leukemia; Kawasaki disease
Hepatitis B immune globulin	?	?	Specific human antibodies	Hepatitis B postexposure prophylaxis
Human rabies immune globulin	?	?	Specific human antibodies	Rabies postexposure management of people not previously immunized with rabies vaccine

Tetanus immune globulin	?	?	Specific human antibodies	Treatment of tetanus; postexposure prophylaxis of people not adequately immunized with tetanus toxoid
Vaccinia immune globulin	?	✗	Specific human antibodies	Treatment of eczema vaccinatum, vaccinia gangrenosa and ocular vaccinia
Varicella-zoster immune globulin	?	?	Specific human antibodies	Postexposure prophylaxis of susceptible immunocompromised people, certain susceptible pregnant women and perinatally exposed newborn infants
The type and indications of use are shown. Immune globulin preparations (called 'immunoglobulins' in the UK) and antitoxins are administered intramuscularly unless otherwise indicated; and human rabies immune globulin is administered around the wounds in addition to intramuscular injection.				

† *Modified from CDC.* [41] [49]
* Not used in the UK for pre-exposure prophylaxis. Hepatitis A vaccine only is offered for pre-exposure up to the day of travel.

to be as effective in the control of measles and no option other than mass immunization has emerged.

The WHO has recommended vaccine coverage to be achieved and maintained at 90% with all childhood vaccines.[45] In general, this target is sufficient to prevent the circulation of vaccine-preventable diseases. Mathematical modeling has shown that the herd immunity threshold, the mechanism by which an infection may be eradicated from a population, does not need to be 100%. The US Healthy People 2000 objective[46] has been consistent with the WHO target level. However, childhood immunization rates are still suboptimal; for example, in 2000 only 82% of children 19–35 months of age had received four doses of DTaP vaccine, down from 84% in 1998.[39] The reduction in vaccine-preventable diseases has had a dramatic effect on morbidity as compared with the prevaccine era in the USA. The most common childhood diseases have all been reduced by over 90% and in the case of paralytic polio by 100% (Table 3.9).[47]

Advances in vaccine technology have enabled development of new vaccines. Novel approaches for vaccine development include DNA vaccines and mucosal vaccines, including the prospect of 'edible' vaccines produced in transgenic plants and vaccines that can be administered by aerosol spray. Although not all vaccines in the process of development will be licensed, the list of vaccines, immune globulins and antitoxins continues to grow (Table 3.10 and Table 3.11). [48] [49] In 1998 the US Food and Drug Administration approved a vaccine using recombinant technology (RotaShield®) that was effective in preventing the serious complications of rotavirus gastroenteritis. In July 1999, the CDC postponed its use and in November 1999 the Advisory Committee on Immunization Practices (ACIP) withdrew its recommendations following reports through the Vaccine Adverse Events Reporting System of an increased risk of intussusception in the first 2 weeks after vaccination. The worldwide burden of rotavirus disease remains substantial and ACIP's decision therefore has implications regarding the risks and benefits of rotavirus vaccination in different settings. [50] At present, new rotavirus vaccines are under development.

The growing number of available vaccines has made the process of immunization increasingly complex. The current childhood schedule in the USA demonstrates the number of vaccines and immunizations required in the first years of life (Fig. 3.6), along with the complexities of catching up those who have uncertain or incomplete immunization status and those who need minimum visits to aid compliance (Table 3.12).[39] [41]

New vaccines for developing countries — the Children's Vaccine Initiative, the Global Alliance for Vaccines and Immunizations, and the Vaccine Fund

In contrast to the vast possibilities that modern technologies offer for vaccine development, the WHO's Expanded Program of Immunizations (EPI) is more limited. This was launched in 1974, following the impressive success of the smallpox eradication program; the WHO looked for other activities that could build on what had already been achieved. The six diseases chosen were tuberculosis, diphtheria, neonatal tetanus, whooping cough, poliomyelitis and measles. During this time, coverage for all EPI target diseases climbed steadily from 5% in developing countries to 80%, although this was not matched by a proportionate decrease in disease incidence. In 1999, serious limitations in global vaccine programs worldwide contributed to a fall in the average vaccination coverage of children under 5 years of age to 74%, and in many countries the infrastructure for vaccine delivery was deteriorating. This means that one child in four in the world remains unimmunized against the six target diseases.[51]

Vaccines available through EPI were further expanded in the 1990s to include yellow fever, hepatitis B and *Haemophilus influenzae* type b (Hib), to countries with high disease burdens from these infections. The aim is for hepatitis B vaccine to be given in all developing countries with adequate delivery systems by 2007 and for Hib

Figure 3-6 Recommended childhood immunization schedule for the USA, 2003. This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of 1 December 2002, for children through age 18 years. DTaP, diphtheria, tetanus, acellular pertussis; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; MMR, measles, mumps, rubella; OPV, oral polio vaccine; PCV, pneumococcal conjugate vaccine; PPV, polysaccharide pneumococcal vaccine; Td, tetanus, low dose diphtheria; Var, varicella. *Redrawn with permission from CDC.*[39]

TABLE 3-12 -- Vaccination of individuals with incomplete immunization status.

VACCINATION OF INDIVIDUALS WITH INCOMPLETE IMMUNIZATION STATUS						
Age	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
<1 year	DTaP1, IPV1, Hib1[* † c], HepB1, PCV1	DTaP2 (4 weeks), IPV2 (4 weeks), Hib2[* †] (4 weeks), HepB2 (4 weeks), PCV2 (4 weeks)	DTaP3 (4 weeks), IPV3 (4 weeks), Hib3[*] (4 weeks), PCV3 (4 weeks)[§]	HepB3[¶] (8 weeks)	DTaP4[£] (6 months), IPV4[¢] (aged 4–6 years)	DTaP5 (6 months)[£]
1–7 years	DTaP1, IPV1, MMR1, Hib1[* † $], HepB1, PCV1[?], PPV23[¥]	DTaP2 (4 weeks), IPV2 (4 weeks), MMR2 (4 weeks), HepB2 (4 weeks)	DTaP3 (4 weeks), IPV3[¢] (4 weeks), PCV2 (8 weeks)[4]	Hib2[* † $] (8 weeks), HepB3[¶]		
7–13 years	Td1, IPV1, MMR1, Var, HepB1	Td2 (4 weeks), IPV2 (4 weeks), MMR2 (4 weeks), HepB2 (4 weeks)	IPV3 (4 weeks)	HepB3[¶] (8 weeks)	Td3[?] (6 months)	Td4[?] (aged 11–12)
13–18	Td1, IPV1, MMR1, Var1, HepB1	Td2 (4 weeks), IPV2 (4 weeks), MMR2 (4 weeks), Var2 (4–8 weeks), HepB2 (4 weeks)	IPV3 (4 weeks)	HepB3[¶] (8 weeks)	Td3 (6 months)[?]	
DO NOT restart series, no matter how long since previous dose. Numbers in brackets indicate minimum recommended gap.						
DTaP, diphtheria, tetanus, acellular pertussis; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; MMR, measles, mumps, rubella; OPV, oral polio vaccine; PCV, pneumococcal conjugate vaccine; PPV, polysaccharide pneumococcal vaccine; Td, tetanus, low dose diphtheria; Var, varicella.						
Modified from CDC.[41]						

* HbOC (HibTITER) and PRP-T (ActHib, OmniHib).
† PRP-OMP (PedvaxHiB).
c‡ If Hib1 given aged 7–11 months, give Hib3 at 12–15 months of age.
§ For infants 7–11 months of age: if unvaccinated, give PCV1 now, PCV2 at 4–8 weeks and PCV3 at 12–15 months.
¶ Overall there must be = 16 weeks between HepB1 and HepB3. Last dose in HepB series should not be given earlier than 6 months of age.
£ If DTaP4 is given before 4th birthday, wait at least 6 months for DTaP5; if DTaP4 is given after 4th birthday, DTaP5 is not needed.
¢ If IPV3 given before 4th birthday, give IPV4 at 4–6 years of age; if IPV3 given after 4th birthday, IPV4 is not needed. If a combination of OPV and IPV have been given, all four doses are needed.
$ For children =15 months and <5 years who have never received Hib vaccine, give only one dose. Not given = 5 years of age.
? For infants 12–23 months: if not previously vaccinated or only one previous dose before 12 months, give PCV1 and PCV2 8 weeks apart. If infant has previously had two doses of PCV, give booster dose 8 weeks or more after previous dose.
¥ If PPV23 is indicated following a course of PCV, allow = 8-week gap.
? Td given aged 11–12 if 5 years has elapsed since last dose. Td given every 10 years, thereafter.

vaccine to be given in 50% of the poorest countries with high disease burdens and adequate delivery systems by 2005. Although no target has been set for yellow fever vaccine, it is estimated that 200,000 yellow fever cases with 30,000 deaths occur each year, mostly in Saharan Africa.

The WHO has overall responsibility for the execution of the EPI program. Recognizing the need for global vaccine deployment and the search for new and improved vaccines, the Children's Vaccine Initiative was founded in 1990 — a collaboration involving UNICEF, UNDP, the World Bank, the Rockefeller Foundation and the WHO.

The impetus for these initiatives stems from the opportunities arising through innovative methods of vaccine production, coupled with an increasing commitment by the international community to prevent disease through vaccination.

To continue and build on the work of the Children's Vaccine Initiative, the Global Alliance for Vaccines and Immunization (GAVI) was launched in 1999. This coalition of international partners includes national governments, international organizations and the private sector. The pharmaceutical industry's participation as a full partner in GAVI is an innovation.

Alongside GAVI is the Global Fund for Children's Vaccines, a financially independent mechanism to raise new resources for immunization, which was launched to address the needs for vaccines and immunizations of the world's 71 poorest countries, those with a per capita GNP of less than US$1000. Funding decisions are based on recommendations of the GAVI board. To date, more than US$600 million of the vaccine fund's resources have been committed to 36 developing country immunization programs.

Vaccines and immunizations: definitions, principles, logistics and use

Vaccines both benefit the individuals who receive the vaccine directly, by preventing them from acquiring and spreading the disease, and in turn indirectly protect those who are unvaccinated or unprotected for any reason, such as age or immune deficiency. Vaccines are biologic agents capable of stimulating immune responses in the host and are called immunobiologics. The vaccines must be immunogenic, thus inducing a strong and measurable immune response in the host. The purpose of this is to provide artificial immunity by priming the recipient's immune system to recognize and attack the disease-causing organism if it is presented again. Immunobiologics are part of a larger grouping that includes antigenic substances (vaccines and toxoids) and antibody-containing products (globulins and antitoxins). Artificially acquired immunity can be either active or passive.

Immunity

A broad definition of immunity is 'a resistance developed in response to stimulus by an antigen and usually characterized by the host'.[52] Active immunity is produced by antibodies that develop in response to antigens (immune response) and can be naturally acquired (where immunity develops after infection or exposure to antigens in the environment) or acquired by active immunization (where immunity develops after administration of an antigen in order to prevent disease). Transfer of antibodies from another person produces passive immunity, and can be achieved either by passive immunization, conferred by administration of antibodies (e.g. immune globulin) to combat infection, or by natural passive immunity, conferred by transfer of maternal antibodies across the placenta.

Vaccine components

In addition to the vaccine antigen that provides a target for the immune response, vaccines may also contain other ingredients, including suspending fluids (e.g. sterile water, saline or fluids derived from the biologic system in which the vaccine is produced); preservatives, stabilizers and antibiotics to prevent bacterial contamination and to protect the antigen from degradation; and adjuvants (substances that enhance the immunologic response). In a patient with known allergies and sensitivities, each of these components should be considered before administration of any vaccine.[39]

Vaccine storage and handling

Each vaccine has its own specific storage and handling requirements. Knowledge of a vaccine's stability can be helpful in determining

Figure 3-7 Vaccine vial monitor showing stages of exposure. The information delivered by the vaccine vial monitor is simple. If the inner square is a lighter color than the outer reference ring than the vaccine can be used. If the inner square is the same color as the outer ring or darker than it, then the vaccine should not be used. *From WHO.*[53]

storage requirements. For example, oral poliovirus vaccine (OPV) and yellow fever vaccine are sensitive to heat and rapidly lose their potency at warm temperatures. In contrast, many vaccines are sensitive to freezing. These include diphtheria and tetanus toxoids, pertussis vaccine, inactivated poliovirus vaccine, Hib conjugate vaccines, hepatitis B vaccine, pneumococcal vaccine and influenza vaccine[53] (see Table 3.13). Maintaining the proper temperature of vaccines from their point of production to the vaccine recipient is referred to as the 'cold chain'. Because it may be difficult to distinguish a potent vaccine from one that has not been strictly maintained at the proper temperature, most vaccines are packed with temperature monitors during shipment. More recently, vaccine vial monitors, which measure exposure to heat, are time- and temperature-sensitive labels attached to vials of vaccine at the time of manufacture (Fig. 3.7).[53] Vaccine vial monitors comply with WHO requirements for heat stability and are being used extensively with the EPI program.

Route of administration

The route of administration is specific to each vaccine based on the process of development and testing for safety and efficacy. Vaccines should only be administered as recommended. In general, it is recommended that all injectable routine childhood vaccines given in the USA be administered by the deep subcutaneous or intramuscular routes.[40] [41] Vaccines containing adjuvants should be injected into muscles; local irritation, induration, skin discoloration, inflammation and granuloma formation are possible adverse events when given subcutaneously or intradermally. One important fact to consider is whether a vaccine is as effective when given at a different site. For example, antibody responses to rabies and hepatitis B vaccines have been shown to be lower when injected in the buttock as compared with intramuscularly into the deltoid. [54] [55] Needle length has been debated in recent studies,[56] [57] but there is insufficient evidence to advise any change in practice.[58] Currently, guidelines are based on age (Table 3.14).[41]

Vaccine schedules

Vaccines may not be effective in very young children with developing immune systems, or they may require several doses to be fully effective. Therefore, the timing sequence of immunizations affects the immune response. The administration of new vaccines is becoming increasingly complex; guidelines have been established for the spacing of vaccines and immune globulin preparations, which may be country- or schedule-specific (Table 3.15 and Table 3.16).[41]

TABLE 3-13 -- Stability of vaccines currently used in national immunization programs.*

STABILITY OF VACCINES CURRENTLY USED IN NATIONAL IMMUNIZATION PROGRAMS				
	Storage temperature (°C)			
Vaccine	**0–8**	**22–25**	**35–37**	**> 37**

Commonly used				
Diphtheria, tetanus and pertussis-containing vaccines (acellular pertussis components)	Will remain stable for 18–24 months in spite of continuous slow decrease in potency of the pertussis component	DT components stable for 4, possibly 6 months; the limiting factor is the pertussis component; some vaccines containing pertussis are stable for only 2 weeks at this temperature	DT components stable for weeks but stability of pertussis component varies with different vaccines; some vaccines containing pertussis lose 50% of potency during storage for 1 week	DT components are: At 45°C: stable for 2 weeks At 53°C: loss of potency after few days At 60–65°C: loss of potency after a few hours Pertussis component: about 10% loss of potency per day at 45°C; rapid loss in potency if stored at 50°C — pertussis component is the limiting factor
Hepatitis B vaccine	Stable for 2–4 years	Stable for 30 days	Stable for 1 week	At 45°C: stable for 3 days
Haemophilus influenzae type b containing vaccines	Stable until use-by date	Stable for at least 24 months when stored at 25°C	Information not available	Information not available
Polio (IPV): DO NOT FREEZE	Stable for up to 2 years	Loss of potency after 20 days	Unstable, discard	Information not available
Measles, mumps and rubella (freeze-dried or unconstituted vaccine) — diluent: DO NOT FREEZE	Stable for 2 years	Retains satisfactory potency for 1 month	Retains satisfactory potency for at least 1 week	50% loss of potency after 2–3 days at 41°C: 80% loss of potency after 1 day at 54°C
Measles, mumps and rubella (reconstituted with diluent): PROTECT FROM LIGHT	Should be used in one vaccination session (8h) if kept cool and protected from sunlight — if not, discard after 1h	Unstable: 50% loss of potency after 1h, 70% loss after 3h	Very unstable: titer may be below acceptable level after 2–7h	Inactivation within 1h
Varicella — Varilrix (freeze dried or unconstituted): MAY BE STORED BELOW 0°C — diluent: DO NOT FREEZE	Stable for 2 years. Protect from light	Information not available	Information not available	Information not available
Varicella — Varilrix (reconstituted with diluent): DO NOT FREEZE	Use promptly and within 90min	Information not available	Information not available	Information not available
Varicella — Varivax: STORE AT -15°C OR BELOW	Administer vaccine immediately after reconstitution; discard if not administered within 30min. Highly unstable — loses potency	Highly unstable — loses potency	Highly unstable — loses potency	Highly unstable — loses potency
Pneumococcal: DO NOT FREEZE	Stable until use by date	Information not available	Information not available	Information not available
Other vaccines				
Polio (OPV) (unopened vials)	Stable for 6–12 months	Unstable: 50% loss of potency after 3 weeks exposure	Very unstable. Loss of satisfactory titre in 1–3 days	Very unstable: At 41°C: 50% loss in 1 day At 50°C: loss of satisfactory titer after 1–3h exposure
BCG (freeze-dried or unconstituted form) — diluent: DO NOT FREEZE	Stable for 1 year. Do not expose to light	Stability varies: 20–30% loss of viability during 2-month exposure. Do not expose to light	Loses potency rapidly. Stability varies. 20% loss of viability during 3–14 days exposure. Do not expose to light	Rapid loss of potency. Up to 73% loss of potency at 3 days. At 70°C: 50% loss during 30min exposure. Do not expose to light
BCG (reconstituted with diluent): DO NOT FREEZE	Very unstable. Protect from light. Keep at 2°C to 8°C when vial is not in use. Discard all unused vaccine at the end of the vaccination session (6 hours)	Very unstable	Very unstable	Very unstable
The vaccines that are most unstable at room temperature are OPV, reconstituted MMR vaccine and reconstituted BCG vaccine. OPV, reconstituted BCG vaccine and reconstituted MMR vaccines must be protected from exposure to light.				

** Modified from WHO.*[53]

TABLE 3-14 -- Injection site and positioning.*

INJECTION SITE AND POSITIONING		
Intramuscular (im)		
Suitable for DTaP, DT, Td, Hib, Hep A, Hep B, influenza and PCV7 vaccine; IPV and PPV23 vaccine can be given im or sc		
Note: insert needle at an 80–90° angle		
Patient's age	**Site**	**Needle size**
Infants (birth to 12 months)	Vastus lateralis muscle in anterolateral aspect of middle upper thigh	7/8" to 1" needle, 23–25 gauge
Young children (12–36 months)	Vastus lateralis muscle preferred until deltoid muscle has developed adequate mass	7/8" to 1" needle, 23–25 gauge
Older children (>36 months) and adults	Thickest portion of deltoid muscle — above level of armpit and below acromion	1" to 2" needle, 23–25 gauge
Subcutaneous (sc)		
Suitable for MMR, varicella, and meningococcal vaccines		
Note: insert needle at 45° angle; make sure you pinch up sc tissue to avoid injecting into muscle		
Patient's age	**Site**	**Needle size**
Infants (birth to 12 months)	Fatty area of thigh	5/8" to 3/4" needle, 23–25 gauge
Young children (12–36 months)	Fatty area of thigh or outer aspect of upper arm	5/8" to 3/4" needle, 23–25 gauge
Older children (>36 months) and adults	Outer aspect of upper arm	5/8" to 3/4" needle, 23–25 gauge

Current guidelines for injection site, needles size and positioning based on age.

** Modified from CDC.[41]*

TABLE 3-15 -- Guidelines for spacing immune globulin preparations and vaccines.‡

GUIDELINES FOR SPACING IMMUNE GLOBULIN PREPARATIONS AND VACCINES		
Simultaneous administration		
Combination		**Minimum interval**
Immunoglobulin and inactivated vaccine		None; can be administered simultaneously at different sites or at any time between doses
Immunoglobulin and live vaccine		Should not be administered simultaneously* ; if unavoidable, administer at different sites and revaccinate or test for seroconversion
Nonsimultaneous administration		
Product administration		
First	**Second**	**Minimum interval**
Immune globulin	Inactivated vaccine	None
Inactivated vaccine	Immune globulin	None
Immune globulin	Live vaccine	Dose related* †
Live vaccine	Immune globulin	2 weeks
Immune globulin preparations include blood products containing large amounts of immune globulin, such as serum immune globulin, specific immune globulins (e.g. tetanus immune globulin and hepatitis B immune globulin), intravenous immune globulin, whole blood, packed red cells, plasma and platelet products.		

‡ *From CDC.[41]*
*Yellow fever and oral Ty21a typhoid vaccine are exceptions to the rule and can be administered at any time before, after or simultaneously with immunoglobulin without decreasing the response.
†The duration of interference of immune globulins on antibody response to measles-containing vaccines and possibly to varicella vaccine, is dose related (see Table 3.16).

Vaccines and schedules are not standardized worldwide and transferring patients on to the national schedule may require knowledge of schedules in other countries. The WHO vaccine preventable diseases monitoring system collects, compiles and disseminates data on immunization coverage and incidence of vaccine preventable diseases, as well as providing nationally recommended immunization schedules[59] (Table 3.17 and Table 3.18).

Vaccine contraindications and precautions

There are several contraindications and precautions to vaccination (Table 3.19).[41] With the exception of moderate or severe underlying illness, contraindications are specific to the vaccine. Among the factors to consider before administering a vaccine are:

- underlying allergies to animal proteins (eggs or chicken embryo), antibiotics (streptomycin or neomycin) or one of the stabilizers;
- immune deficiency; and
- pregnancy.

Some vaccines are prepared in eggs, but persons who are able to eat eggs or egg products without developing allergy should have no difficulty with the receipt of vaccines prepared in eggs or chicken embryos. A review of literature looking at evidence for egg as the agent responsible for allergic reactions to measles, mumps and rubella

TABLE 3-16 -- Suggested intervals between administration of selected immune globulin preparations and measles/varicella vaccine.‡

SUGGESTED INTERVALS BETWEEN ADMINISTRATION OF SELECTED IMMUNE GLOBULIN PREPARATIONS AND MEASLES/VARICELLA VACCINE		
Product indication	**Dose***	**Suggested interval before measles or varicella vaccination (months)**
Tetanus (TIG)	250 units (10mg IgG/kg) im	3
Hepatitis A		
Contact prophylaxis	0.02ml/kg (3.3mg IgG/kg) im	3
International travel*	0.06ml/kg (10mg IgG/kg) im	3
Hepatitis B Ig	0.06ml/kg (10mg IgG/kg) im	3
Rabies Ig	20 IU/kg (22mg IgG/kg) im	4
Varicella zoster Ig (VZIG)	125 units/10kg (20–40mg IgG/kg) im (maximum 625 units)	5
Measles prophylaxis Ig		
Standard	0.25ml/kg (40mg IgG/kg) im	5
Immunocompromised contact	0.50ml/kg (80mg IgG/kg) im	6
IGIV		
Replacement therapy for immune deficiencies†	300–400mg/kg iv	8
Immune thrombocytopenic purpura	400mg/kg iv daily for 5 consecutive days, or	8
Immune thrombocytopenic purpura	1000mg/kg iv daily for one or two consecutive days	10
Kawasaki disease	2g/kg iv	11
This table is not intended to show the correct indications and dosage for using immune globulin products. Recommended intervals are extrapolated from an estimated half-life of 30 days for passively acquired antibody and an observed interference with the immune response to measles vaccine for 5 months after a dose of 80mg IgG/kg. Unvaccinated people may not be fully protected against measles and/or varicella during the entire suggested interval, and additional doses of immune globulin, vaccine or both may be indicated after exposure.		

‡ *Modified from CDC.[41]*
* Recommended for postexposure following travel if hepatitis A vaccine has not been given or if given within 1 month.
† Measles and rubella vaccination is recommended for children with asymptomatic or mildly symptomatic HIV infection but is contraindicated for persons with severe immunosuppression from HIV or

any other immunosuppressive disorder.

TABLE 3-17 -- Immunization schedule for infants recommended by the World Health Organization expanded program on immunization (EPI).[§]

IMMUNIZATION SCHEDULE FOR INFANTS RECOMMENDED BY THE WHO EXPANDED PROGRAM ON IMMUNIZATION			
		Hepatitis B vaccine[*]	
Age	**Vaccine**	**Scheme A**	**Scheme B**
Birth	BCG, OPV0[†]	HB1	
6 weeks	DPT1, OPV1	HB2	HB1
10 weeks	DPT2, OPV2		HB2
14 weeks	DPT3, OPV3	HB3	HB3
9 months	Measles, yellow fever[‡]		
The basis principle guiding the use of EPI vaccine is that protection against the EPI diseases should be achieved prior to the time that infants are at risk from these diseases. Immunization schedules should be designed to provide the first dose of vaccine as early as possible, consistent with the epidemiology of the disease and within the capacity of the vaccine delivery system.			

§ *From WHO.*[60]

* Scheme A is recommended in countries where newborns are at risk of being exposed to hepatitis B through their mothers; scheme B is recommended where this risk does not exist.
† OPV at birth (OPV0) is recommended in countries where poliomyelitis has not been controlled.
‡ Yellow fever vaccine is recommended in countries at risk for yellow fever.

(MMR) or measles vaccine found that MMR is as safe as any other vaccine, and an allergy to eggs should not delay measles vaccination.[61] Recommendations for children to be vaccinated in hospital are those with an allergy to eggs in whom previous exposure led to cardiorespiratory reactions and those with coexisting active, chronic asthma.

Adverse events following vaccination

Like any pharmaceutical product, no vaccine is 100% safe or effective. Almost all vaccines are associated with minor and self-limited

TABLE 3-18 -- Routine immunization schedules in the UK, Canada, Australia and New Zealand, 2002.

ROUTINE IMMUNIZATION SCHEDULES IN THE UK, CANADA, AUSTRALIA AND NEW ZEALAND, 2002	
Country	**Schedule**
UK	2, 3, 4 months: DTP, OPV, Men C, Hib
	12–15 months: MMR
	3.5–5 years: DTaP, MMR, OPV
	13 years: BCG
	15–18 years: Td, OPV
Canada	2, 4, 6 months: DTaP, IPV, Hib, Hep B, PC, Men C
	12 months: MMR, varicella, PC
	18 months: DTaP, IPV, Hib
	4–6 years: DTaP, IPV
	14–16 years: Td or TdaP
Australia	2, 4, 6 months: DTaP, Hib, Hep B, OPV
	12 months: MMR, Hib
	18 months: DTaP
	4 years: DTaP, MMR, OPV
	15–19 years: Td, OPV
New Zealand	6 weeks, 3 months: DTaP, IPV, Hib, Hep B
	5 months: DTaP, IPV, Hep B
	15 months: MMR, DTaP, Hib
	4 years: DTaP, IPV, MMR
	11 years: Td, IPV
Schedules differ from country to country. Awareness of schedules allows individuals to be transferred more easily on to the schedule of the country in which they now reside.	

adverse events, but some vaccines have been associated with very rare, but serious health effects. The paradox of vaccine effectiveness is the growing concern over vaccine safety. As the threat of disease

TABLE 3-19 -- Vaccination contraindications and precautions.[*]

VACCINATION CONTRAINDICATIONS AND PRECAUTIONS	
True contraindications and precautions	**Not contraindications (vaccines may be administered)**
General for all vaccines (DTP, DTaP, OPV, IPV, MMR, Hib, hepatitis B)	

Contraindications	Anaphylactic reaction to a vaccine contraindicates further doses of that vaccine	Mild to moderate local reaction (soreness, redness, swelling) following a dose of an injectable antigen
	Anaphylactic reaction to a vaccine constituent contraindicates the use of vaccines containing that substance	Mild acute illness with or without low-grade fever
	Moderate or severe illnesses with or without fever	Current antimicrobial therapy
		Convalescent phase of illnesses
		Prematurity (same dosage and indications as for normal full-term infants)
		Recent exposure to an infectious disease
		History of penicillin or other non-specific allergies, or family history of such allergies
DTP/DTaP		
Contraindications	Encephalopathy within 7 days of administration of previous dose of DTP	Temperature of <105°F (40.5°C) following previous dose of DTP
		Family history of convulsions
Precautions	Fever of =105°F (40.5°C) within 48h after vaccination with a prior dose of DTP	Family history of sudden infant death syndrome
	Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48h of receiving a prior dose of DTP	Family history of an adverse event following DTP administration
	Seizures within 3 days of receiving a prior dose of DTP	
	Persistent, inconsolable crying lasting =3h within 48h of receiving a prior dose of DTP	
OPV		
Contraindications	Infection with HIV or a household contact with HIV	Breast-feeding
	Known altered immunodeficiency (hematologic and solid tumors; congenital immunodeficiency; long-term immunosuppressive therapy)	Current microbial therapy
	Immunodeficient household contact	Diarrhea
Precautions	Pregnancy	
IPV		
Contraindications	Anaphylactic reaction to neomycin or streptomycin	
Precautions	Pregnancy	
MMR		
Contraindications	Anaphylactic reactions to egg ingestion or to neomycin	Tuberculosis (TB) or positive purified protein derivative skin test
	Pregnancy	Simultaneous TB skin testing
	Known altered immunodeficiency (hematologic and solid tumors; congenital immunodeficiency; long-term immunosuppressive therapy)	Breast-feeding
		Pregnancy of mother of recipient
		Immunodeficient family member or household contact
		Infection with HIV
		Nonanaphylactic reactions to eggs or neomycin
Precautions	Recent immunoglobulin administration	
Hib		
Contraindication	None identified	History of Hib disease
Hepatitis B		
Contraindication	Anaphylactic reaction to common baker's yeast	Pregnancy
Varicella		
Contraindication	Anaphylactic reaction to neomycin	Pregnancy of recipient's mother or other close household contact
	Pregnancy	Immunodeficient family member or household contact
	Known altered immunodeficiency (hematologic and solid tumors; congenital immunodeficiency; long-term immunosuppressive therapy)	Asymptomatic or mildly symptomatic HIV infection
		Humoral immunodeficiency (e.g. agammaglobulinemia)
Precautions	Recent immunoglobulin administration	
	Moderate or severe illness with or without fever	
PCV		
Contraindication	Severe allergic reaction after a previous dose or vaccine component	
Precaution	Moderate or severe acute illness with or without fever	

Note that acetaminophen given before administering DTP and thereafter q4h for 24 hours should be considered for children with a personal or family history of convulsions in siblings or parents. DTP, diphtheria, tetanus, pertussis; DTaP, diphtheria, tetanus, acellular pertussis; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus vaccine; MMR, measles, mumps and rubella; OPV, oral poliovirus vaccine; PCV, pneumococcal conjugate vaccine.

* *From CDC.* [39]

disappears in a community, the risks attributed to vaccines, real and perceived, may achieve increased prominence.

In some countries, particularly in the UK, concern over vaccine safety focused on the whole-cell pertussis vaccine led to erosion of confidence and a decline in the use of vaccines. Without vaccine-induced protective immunity the enlarging pool of susceptible people led to pertussis epidemics.[62]

In the USA, similar concerns led to the creation of the National Childhood Vaccine Injury Act of 1986 and established a compensation program for persons injured by vaccines.[63] This 'no fault' program means that people filing claims are not required to prove negligence on the part of either the health care provider or the manufacturer to receive compensation. The vaccine injury table was created to separate those possibly injured by vaccines from unrelated

claims. The table is adjusted as more research on vaccine side-effects becomes available (Table 3.20).[64]

Adolescent and adult immunization

The focus of reducing the vaccine-preventable diseases of children has been a success around the world, as vaccines have become one of the foundations of routine pediatric care. However, despite the availability of safe and effective vaccines, approximately 50,000–70,000 adults die each year in the USA from complications of infections with *Streptococcus pneumoniae*, influenza virus and hepatitis B virus, infections for which vaccines are routinely recommended for many adults and the elderly. In contrast to the coverage rates of childhood vaccines in the USA (and around the world), current utilization in the USA of these vaccines in target adult populations for which these vaccines are recommended was estimated in 2000 to be 64% for an influenza vaccine in over-65-year-olds and only 53% of seniors had ever received pneumococcal vaccine.

Vaccination of children can have an impact on adolescent and adult populations by altering the epidemiology of disease. Studies have shown that the introduction of varicella vaccine in the USA might result in an increase in zoster cases in the unvaccinated population.[65] [66] Exposure to varicella provides a natural boost to individuals who have previously had chickenpox, thereby delaying or preventing the development of shingles. Reducing the incidence of varicella through vaccination will reduce the number of adults who receive this natural boost.

As new vaccines become available for adolescents and adults, it will be increasingly important to develop delivery systems that can efficiently and effectively administer the vaccines as recommended. It has been suggested that this can be accomplished in part by integrated adolescent and adult vaccinations with other routine preventive services (Table 3.21).[67]

Special populations and circumstances

Although general recommendations can be made for routine vaccination, several groups and circumstances merit additional consideration. This includes an assessment of a person's host factors (e.g. pregnancy or immunocompromising condition) and exposure factors (e.g. known contact with a person with a vaccine-preventable disease, persons living in chronic care facilities and occupations or behaviors that increase the chances of exposure to a vaccine-preventable disease). In general, persons who are immunocompromised should receive special consideration before immunization. [41] [68] Table 3.22 Table 3.23 Table 3.24 summarize the immunization recommendations for immunocompromised persons.

Immunization in pregnancy

Even though all vaccines may be administered safely to children or pregnant women and to breast-feeding mothers, because of the theoretic risk associated with vaccination during pregnancy many health practitioners are understandably cautious when assessing the need for an immunization for pregnant women or women likely to become pregnant during the first 3 months of immunization. The combined tetanus and diphtheria toxoids (Td) are the only vaccines that are routinely recommended for pregnant women, if they have not received a Td booster within the previous 10 years. Before administering hepatitis B vaccine or influenza vaccine, a realistic consideration of the risk of disease often determines the decision to vaccinate. Live virus vaccines are generally not recommended for women who are pregnant, unless the threat of infection and its consequences outweighs concern over a potential adverse event to the developing fetus. Table 3.25 provides a summary of recommendations for vaccination during pregnancy. [41] [69]

Health care workers

Given the potential for health care workers to be exposed to infectious diseases through contact with patients and clinical specimens, and the potential for health care workers to transmit infections to susceptible patients, maintaining immunity is an integral component of prevention and control of infectious diseases in health care settings. Recommendations for the immunization of health care workers have been established; these also take into account the immunization needs of health care workers with special conditions, such as pregnancy, immunosuppression, HIV infection, diabetes, alcoholism and cirrhosis, and renal failure (Table 3.26 and Table 3.27). [70]

TABLE 3-20 -- National vaccine injury compensation program — vaccine injury table.*

NATIONAL VACCINE INJURY COMPENSATION PROGRAM — VACCINE INJURY TABLE		
Vaccine	**Illness, disability, injury or condition covered**	**Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration**
I. Vaccines containing tetanus toxoid (e.g. DTaP, DTP, DT; Td, or TT)	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Brachial neuritis	2–28 days
	C. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
II. Vaccines containing whole-cell pertussis bacteria, extracted or partial cell pertussis bacteria, or specific pertussis antigen(s) (e.g. DTaP, DTP, P, DTP-Hib)	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Encephalopathy (or encephalitis)	72 hours
	C. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
III. Measles, mumps and rubella vaccine or any of its components (e.g. MMR, MR, M, R)	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Encephalopathy (or encephalitis)	5–15 days (not less than 5 days and not more than 15 days) for measles, mumps, rubella or any vaccine containing any of the forgoing as a component
	C. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
IV. Vaccines containing rubella virus (e.g. MMR, MR, R)	A. Chronic arthritis	7–42 days
	B. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
V. Vaccines containing measles virus (e.g. MMR, MR, M)	A. Thrombocytopenic purpura	7–30 days
	B. Vaccine-strain viral infection in an immunodeficient recipient	6 months
	C. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable

VI. Vaccines containing polio live virus (OPV)	A. Paralytic polio	
	• in a non-immunodeficient recipient	30 days
	• in an immunodeficient recipient	6 months
	• in a vaccine-associated community case	Not applicable
	B. Vaccine-strain polio viral infection	
	• in a non-immunodeficient recipient	30 days
	• in an immunodeficient recipient	6 months
	• in a vaccine-associated community case	Not applicable
	C. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
VII. Vaccines containing polio inactivated virus (e.g. IPV)	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
VIII. Hepatitis B vaccines	A. Anaphylaxis or anaphylactic shock	4 hours
	B. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
IX. *Haemophilus influenzae* type b polysaccharide vaccines (unconjugated, PRP vaccines)	A. Early onset Hib disease	7 days
	B. Any acute complication or sequela (including death) of an illness, disability, injury or condition arising within the time period prescribed	Not applicable
X. *Haemophilus influenzae* type b polysaccharide conjugate vaccines	No condition specified	Not applicable
XI. Varicella vaccine	No condition specified	Not applicable
XII. Rotavirus vaccine	No condition specified	Not applicable
XIII. Any new vaccine recommended by the CDC for routine administration to children, after publication by Secretary, HHS of a notice of coverage[†]	A. No condition specified for compensation	
	B. Events described in manufacturer's package insert as contraindications to additional doses of vaccine	Not applicable
Effective date 22 October 1998.		

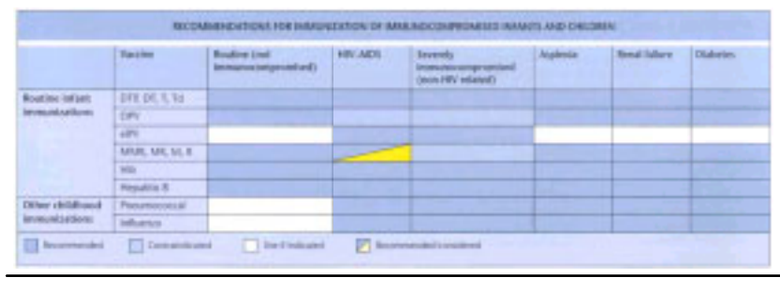
* With permission from US Department of Health and Human Services.[64]
† Pneumococcal conjugate vaccines are included in this category — on 22 May 2001 the secretary published a notice in the Federal Register announcing the addition of pneumococcal conjugate vaccines to the vaccine injury table under category XII.

TABLE 3-21 -- Recommended schedule of vaccinations for adolescents.*

RECOMMENDED SCHEDULE OF VACCINATIONS FOR ADOLESCENTS				
Immunobiologic	**Indications**	**Dose**	**Frequency**	**Route**
Hepatitis A vaccine	Increased risk of hepatitis A infection or its complications	720 EL U/0.5ml (HAVRIX)	A total of two doses at 0 and 6–12 months	im
		25U/0.5ml (VAQTA)	A total of two doses at 0 and 6–18 months	im
Hepatitis B vaccine	Not vaccinated previously for hepatitis B	5µg/0.5ml (Recombivax-HB)	A total of three doses at 0, 1–2 and 4–6 months	im
		10µg/0.5ml (Engerix-B)	A total of three doses at 0, 1–2 and 4–6 months	im
Influenza vaccine	Increased risk for complications caused by influenza or contact with persons at increased risk for these complications	0.5ml	Annually (September–December)	im
Measles, mumps and rubella vaccine	Not vaccinated previously with two doses of measles vaccine at =12 months of age	0.5ml	One dose	sc
Pneumococcal polysaccharide vaccine	Increased risk for pneumococcal disease or its complications	0.5ml	One dose	im or sc
Tetanus and diphtheria toxoids	Not vaccinated within the previous 5 years	0.5ml	Every 10 years	im
Varicella virus vaccine	Not vaccinated previously and no reliable history of chickenpox	0.5ml	One dose	sc
For the upper regimen for hepatitis A vaccine: an alternative dosage and schedule is 360 EL U/0.5ml, and a total of three doses administered at 0, 1 and 6–12 months. 0 Months represents the timing of the initial dose and subsequent numbers represent months after the initial dose. For varicella virus vaccine: adolescents = 13 years of age should be administered a total of two doses (0.5ml/dose) subcutaneously at 0 and 4–8 weeks. These recommendations apply to people aged 11–12 years. EL, enzyme-linked immunosorbent assay (ELISA) units.				

** From CDC.[67]*

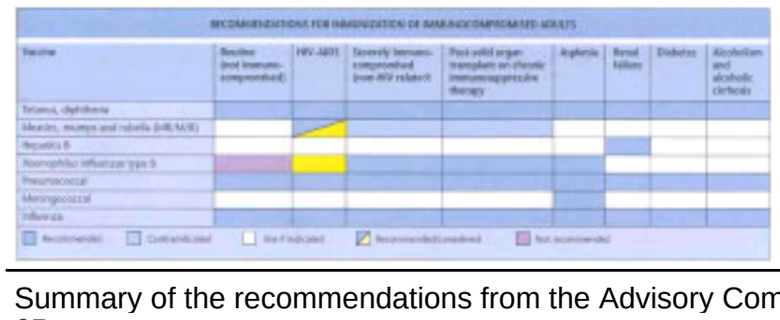
TABLE 3-22 -- Recommendations for immunization of immunocompromised infants and children.*



Summary of the recommendations from the Advisory Committee on Immunization Practices. Pneumococcal vaccine is recommended for those aged 2 years or more. Influenza vaccine is not recommended for children under 6 months of age. D, diphtheria; T, tetanus; P, pertussis; Td, tetanus and diphtheria toxoids; OPV, oral poliovirus vaccine; eIPV, enhanced-potency, inactivated poliovirus vaccine; MMR, measles, mumps and rubella; Hib, *Haemophilus influenzae* type b.

* *From CDC.* [68]

TABLE 3-23 -- Recommendations for immunization of immunocompromised adults.*



Summary of the recommendations from the Advisory Committee on Immunization Practices. Pneumococcal and influenza vaccines are recommended for those aged 65 years or over.

* *From CDC.* [68]

TABLE 3-24 -- Recommendations for the use of immune globulins in immunocompromised persons.*

RECOMMENDATIONS FOR THE USE OF IMMUNE GLOBULINS IN IMMUNOCOMPROMISED PERSONS			
Immune globulin	**Not immunocompromised**	**HIV infected**	**Severely immunocompromised**
Immune globulin	Recommended for infants and adults with contraindication to measles vaccine if exposed to measles	Recommended for symptomatic patients exposed to measles regardless of immunization status	Recommended for patients exposed to measles regardless of immunization status
		Recommended for persons with exposure to hepatitis A virus or who will travel to endemic areas	
Varicella-zoster	Recommended for newborns of mothers who develop chickenpox within 5 days before and 48 hours after delivery	Recommended for susceptible infants and adults after significant exposure to varicella-zoster	Recommended for susceptible infants and adults after significant exposure to varicella-zoster
	Recommended for exposed newborns (=28 weeks gestation) of susceptible mothers		
	Recommended for exposed pre-term (<28 weeks or <1000g body weight)		
	May be used for exposed, susceptible adults, exposed pregnant women, and infants <28 days		
Tetanus	Recommended for those with serious wounds who have received fewer than three doses of tetanus toxoid	Same as for not immunocompromised	Same as for not immunocompromised
Hepatitis B	Recommended for prophylaxis of infants born to hepatitis B surface antigen-positive mothers and susceptible persons with percutaneous, sexual or mucosal exposure to hepatitis B virus	Same as for not immunocompromised	Same as for not immunocompromised
Human rabies	Recommended for postexposure prophylaxis of persons not previously vaccinated against rabies	Same as for not immunocompromised	Same as for not immunocompromised
Summary of the recommendations from the Advisory Committee on Immunization Practices.			

* *From CDC.* [68]

TABLE 3-25 -- Vaccination during pregnancy.*

VACCINATION DURING PREGNANCY			
		Vaccine	**Indications for vaccination during pregnancy**
Live virus vaccines	Measles Mumps Rubella	Live-attenuated	Contraindicated
	Yellow fever	Live-attenuated	Contraindicated except if exposure to yellow fever virus is unavoidable
	Poliomyelitis	Trivalent live-attenuated (oral poliomyelitis vaccine)	Persons at substantial risk of exposure to polio

Inactivated virus vaccines	Hepatitis A	Killed virus	Data on safety in pregnancy are not available. Should weigh the theoretic risk of vaccination against the risk of disease
	Hepatitis B	Recombinant produced, purified hepatitis B surface antigen	Not contraindicated
	Influenza	Inactivated type A and type B virus vaccines	Recommended for women in 2nd and 3rd trimesters of pregnancy during the influenza season
	Japanese encephalitis	Killed virus	Should reflect actual risks of disease and probable benefits of vaccine
	Poliomyelitis	Killed virus (inactivated poliovirus vaccine)	Oral poliovirus vaccine preferred when immediate protection of pregnant females is needed; however, inactivated poliovirus vaccine is an alternative if complete vaccination series can be administered before exposure
	Rabies	Killed virus Rabies immunoglobulin	Should reflect actual risks of disease and probable benefits of vaccine
Live bacterial vaccines	Typhoid (Ty21a)	Live bacterial	Substantial risk of exposure
Inactivated bacterial vaccines	Cholera Typhoid	Killed bacterial	Should reflect actual risks of disease and probable benefits of vaccine
	Plague	Killed bacterial	Selective vaccination of exposed persons
	Meningococcal	Polysaccharide	Only in unusual outbreak situations
	Pneumococcal	Polysaccharide	Only for high-risk persons
	Haemophilus influenzae type b	Polysaccharide-protein conjugate	Only for high-risk persons
Toxoids	Tetanus-diphtheria	Combined tetanus-diphtheria toxoids (adult formulation)	Lack of primary series, or no booster within past 10 years
Immune globulins, pooled or hyperimmune		Immunoglobulin or specific globulin preparations	Exposure or anticipated unavoidable exposure to measles, hepatitis A, hepatitis B, rabies, or tetanus

** From CDC.*[68]

TABLE 3-26 -- Immunizing agents and immunization schedules for health care workers.*

IMMUNIZING AGENTS AND IMMUNIZATION SCHEDULES FOR HEALTH CARE WORKERS				
Generic name	**Primary schedule and booster(s)**	**Indications**	**Major precautions and contraindications**	**Special considerations**
Hepatitis A vaccine	Two doses of vaccine either 6–12 months apart or 6 months apart	Not routinely indicated in USA; recommended in those working with infected primates or with a laboratory researching hepatitis A virus	History of anaphylactic hypersensitivity to alum or 2-phenoxyethanol. The safety of the vaccine in pregnant women has not been determined — the risk associated with vaccination should be weighed against the risk for hepatitis A in women who may be at a high risk of exposure	
Meningococcal polysaccharide vaccine (tetravalent A, C, W135 and Y)	One dose in volume and by route specified by manufacturer; need for boosters unknown	Not routinely indicated in USA	The safety of the vaccine in pregnant women has not been evaluated; it should not be administered during pregnancy unless the risk of infection is high	
Typhoid vaccine, im, sc and po	im: one 0.5ml dose, booster 0.5ml every 2 years; sc: two 0.5ml doses, =4 weeks apart, booster 0.5ml sc or 0.1ml id every 3 years if exposure continues; po: four doses on alternate days The manufacturer recommends revaccination with the entire four-dose series every 5 years	Workers in microbiology laboratories who frequently work with *Salmonella typhi*	Severe local or systemic reactions to a previous dose. Ty21a (oral) vaccine should not be administered to immunocompromised persons or to persons receiving antimicrobial agents	Vaccination should not be considered an alternative to the use of proper procedures when handling specimens and cultures in the laboratory
Vaccinia vaccine (smallpox)	One dose administered with a bifurcated needle; boosters administered every 10 years	Laboratory workers who directly handle cultures with vaccinia, recombinant vaccinia viruses or orthopox viruses that infect humans	Contraindicated in pregnancy, in persons with eczema or a history of eczema, and in immunocompromised persons and their household contacts	Vaccination may be considered for those who have direct contact with contaminated dressings or other infectious material from volunteers in clinical studies involving recombinant vaccinia virus
Other vaccine-preventable diseases				
Tetanus and diphtheria toxoids	Two im doses 4 weeks apart; third dose 6–12 months after second dose; booster every 10 years	All adults	Except in the first trimester, pregnancy is not a precaution. History of a neurologic reaction or immediate hypersensitivity reaction after a previous dose History of severe local (arthus-type) reaction after a previous dose — these people should not receive further routine or emergency doses for 10 years	

Pneumococcal polysaccharide vaccine (23-valent)	One dose, 0.5ml, im or sc; revaccination recommended for those at highest risk 5 or more years after the first dose	Adults who are at increased risk of pneumococcal disease and its complications because of underlying health conditions; older adults, especially those who are aged 65 years or older and who are healthy	The safety of vaccine in pregnant women has not been evaluated; it should not be administered during pregnancy unless the risk of infection is high	Previous recipients of any type of pneumococcal polysaccharide vaccine who are at highest risk of fatal infection or antibody loss may be revaccinated 5 years or more after the first dose
Rubella live-virus vaccine	One dose sc; no booster	Indicated for both men and women with no documentation of prior vaccination on or after their first birthday or laboratory evidence of immunity; people born before 1957, except women who can become pregnant, can be considered immune	Pregnancy Immunocompromised persons Those with a history of anaphylactic reaction after administration of neomycin	The risk for rubella vaccine-associated malformations in the offspring of women pregnant when vaccinated or who become pregnant within 3 months after vaccination is negligible; such women should be counseled about the theoretic basis of concern for the fetus Measles, mumps and rubella vaccine is the vaccine of choice if recipients are likely to be susceptible to measles or mumps as well as to rubella
Varicella-zoster live-virus vaccine	Two 0.5ml doses sc 4–8 weeks apart if 13 years of age or older	Indicated if no reliable history of varicella or no serologic evidence of immunity	Pregnancy Immunocompromised persons History of anaphylactic reaction following receipt of neomycin or gelatin Avoid salicylate use for 6 weeks after vaccination	Vaccine is available from the manufacturer for certain patients with acute lymphocytic leukemia in remission Because 71–93% of persons without a history of varicella are immune, serologic testing before vaccination is likely to be cost-effective
Varicella-zoster immune globulin	If <50kg: 125U/10kg im; if =50kg: 625U	Indicated for those known or likely to be susceptible if at high risk of complications, (e.g. pregnant women) following close and prolonged exposure		Serologic testing may help in assessing whether to administer; if use prevents varicella disease, patient should be vaccinated subsequently
BCG	One percutaneous dose of 0.3ml; no booster dose recommended	Consider only when drug-resistant tuberculosis is prevalent, infection likely and tuberculosis control efforts less than optimal	Should not be administered to immunocompromised persons, pregnant women	In the USA, tuberculosis-control efforts are directed toward early identification, treatment of cases and preventive therapy
Other immunobiologics that are or may be indicated for health care workers				
Immunoglobulin (hepatitis A)	Postexposure: one im dose of 0.02ml/kg given 2 weeks or sooner after exposure	Indicated for those exposed to feces of infectious patients	Contraindicated in persons with IgA deficiency; do not administer within 2 weeks after measles, mumps and rubella vaccine, or 3 weeks after varicella vaccine Delay administration of mumps, measles and rubella vaccine for 3 months or more and varicella vaccine for 5 months or more	Administer in large muscle mass (deltoid, gluteal)
Immunizing agents strongly recommended for health care workers				
Hepatitis B recombinant vaccine	Two doses im 4 weeks apart; third dose 5 months after second; no booster	Those at risk of exposure to blood or body fluids	On the basis of limited data, no risk of adverse effects to developing fetuses is apparent Pregnancy should not be considered a contraindication to vaccination of women Previous anaphylactic reaction to common baker's yeast is a contraindication to vaccination	Prevaccination serologic screening is not indicated for persons being vaccinated because of occupational risk Those who have contact with patients or blood should be tested 1–2 months after vaccination to determine serologic response
Hepatitis B immune globulin	0.06ml/kg im as soon as possible, but no later than 7 days after exposure; a second dose should be administered 1 month later if the hepatitis B vaccine series has not been started	For persons exposed to blood or body fluids containing hepatitis B surface antigen and who are not immune to HBV infection		
Influenza vaccine (inactivated whole-virus and split-virus vaccines)	Annual im vaccination with current vaccine	Those who have contact with patients at high risk for influenza or its complications; those who work in chronic care facilities; those with high-risk medical conditions; those aged 65 years or more	History of anaphylactic hypersensitivity to egg ingestion	No evidence exists of risk to mother or fetus when the vaccine is administered to a pregnant woman with an underlying high-risk condition; influenza vaccination is recommended during second and third trimesters of pregnancy
Measles live-virus vaccine	One dose sc: second dose at least 1 month later	For persons born after 1957 without documentation of physician-diagnosed measles, serologic evidence of immunity or receipt of two doses of live-attenuated vaccine on or after the first birthday. Consider immunization for all health care workers without proof of immunity, even if born before 1957	Pregnancy Immunocompromised persons, including HIV-infected persons who have evidence of severe immunosuppression, anaphylaxis after gelatin ingestion or administration of neomycin Recent administration of immunoglobulin	Measles, mumps and rubella vaccine is the vaccine of choice if recipients are likely to be susceptible to rubella or mumps as well as to measles Persons vaccinated during 1963–1967 with a killed measles vaccine alone, killed vaccine followed by live vaccine, or with a vaccine of unknown type should be revaccinated with two doses of live measles virus vaccine

Mumps live-virus vaccine	One dose sc: no booster	Those believed to be susceptible can be vaccinated; those born before 1957 can be considered immune	Pregnancy Immunocompromised persons History of anaphylactic reaction after gelatin ingestion or administration of neomycin	Measles, mumps and rubella vaccine is the vaccine of choice if recipients are likely to be susceptible to measles and rubella as well as to mumps

** From CDC.*[70]

TABLE 3-27 -- Recommendations for immunization of health care workers with special needs.*

RECOMMENDATIONS FOR IMMUNIZATION OF HEALTH CARE WORKERS WITH SPECIAL NEEDS							
Vaccine	**Pregnancy**	**HIV infection**	**Severe immunosuppression**	**Asplenia**	**Renal failure**	**Diabetes**	**Alcoholism and alcoholic cirrhosis**
BCG	Contraindicated	Contraindicated	Contraindicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Hepatitis A	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Recommended
Hepatitis B	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Influenza	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Measles, mumps and rubella	Contraindicated	Recommended	Contraindicated	Recommended	Recommended	Recommended	Recommended
Meningococcus	Use if indicated	Use if indicated	Use if indicated	Recommended	Use if indicated	Use if indicated	Use if indicated
Poliovirus vaccine (inactivated)	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Poliovirus vaccine (live, oral)	Use if indicated	Contraindicated	Contraindicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Pneumococcus	Use if indicated	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Rabies	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Tetanus and diphtheria	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Typhoid (inactivated and Vi)	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Typhoid (Ty21a)	Use if indicated	Contraindicated	Contraindicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Varicella	Contraindicated	Contraindicated	Contraindicated	Recommended	Recommended	Recommended	Recommended
Vaccinia	Contraindicated	Contraindicated	Contraindicated	Use if indicated	Use if indicated	Use if indicated	Use if indicated
Summary of the recommendations from the Advisory Committee on Immunization Practices. The recommendations for pneumococcus vaccine is based on the person's underlying condition rather than the occupation. Influenza vaccine is recommended for pregnant women who will be in the second or third trimester during the influenza season. Measles, mumps and rubella vaccination is contraindicated in HIV-infected persons who have evidence of severe immunosuppression. Polio vaccination is recommended for unvaccinated health care workers who have close contact with patients who may be excreting wild polioviruses. Primary vaccination with inactivated poliovirus vaccine (IPV) is recommended because the risk for vaccine-associated paralysis after administration of oral live poliovirus vaccine (OPV) is higher among adults than among children. Health care workers who have had a primary series of OPV or IPV and who are directly involved in the provision of care to patients who may be excreting poliovirus may receive another dose of either OPV or IPV. Any suspected case of poliomyelitis should be investigated immediately. If evidence suggests transmission of wild poliovirus, control measures to prevent further transmission should be instituted immediately, including an OPV vaccination campaign.							

** Modified from CDC.*[70]

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Chapter 4 - Emerging and Re-emerging Pathogens and Diseases

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INTRODUCTION

Humans and microbes have been engaged in a complex struggle for survival since human life began. Rapid microbial evolution allows bacteria, viruses and parasites to overcome human defenses (e.g. physiologic mechanisms and manmade drugs) and exploit human behaviors (e.g. sexual practices and methods of food preparation). Moreover, some zoonotic microbes have 'jumped' from animals to humans to evolve into major human pathogens. The ancestors of smallpox virus and malaria parasites, for example, probably became human pathogens 10,000 years ago when humans began to cultivate animals in settlements that were large enough to sustain human-to-human transmission. In recent times the AIDS virus, one of the most destructive pathogens in human history, is thought to have evolved from a virus carried by a nonhuman primate. [1]

Human evolution, though far slower and more difficult to observe, has in turn been influenced by microbes. Some pathogens have apparently been sufficiently virulent and widespread over the course of human history to affect the make-up of the human genome. The best evidence for this concerns malaria parasites, which are less likely to kill individuals who have globin gene alleles that make their red blood cells poor hosts for intracellular parasites (e.g. sickle hemoglobin and a- and ß-thalassemias).[2] Moreover, most or all components of the human immune system probably evolved to prevent

Figure 4-1 Interactions among humans, disease vectors and the environment that contribute to disease emergence. *Source: Institute of Medicine. Emerging infections: microbial threats to health in the United States. Washington DC: National Academy Press; 1992.[3] (In March 2003, the Institute of Medicine published a successor to this report, entitled Microbial threats to health: emergence, detection, and response.)*

disease agents from using human cellular machinery for microbial gene expression and reproduction.

Today, the increasing complexity of human behavior coupled with our ability to change our natural environment has hastened the pace of disease emergence and re-emergence (Fig. 4.1). Many modern human activities facilitate microbial transmission (e.g. air travel and the globalization of the food supply; see Table 4.1),[3] even while others have decreased it (e.g. improvements in sanitation, antimicrobial drug treatments, vaccines and disease eradication programs). Looking ahead, the most significant risk factor for disease emergence in the 21st century is likely to be population growth and urbanization, leading to the creation of megacities (a city with >10 million inhabitants)[4] throughout the developing world. Between 1990 and 2025, the number of people living in urban areas is expected to double to more than 5 billion, with 90% of the increase occurring in the poverty-ridden cities of Asia, Africa and Latin America.[5] By 2007, the number of urban dwellers will equal the number of rural dwellers and by 2015, the number of megacities will have grown from five in 1975 (three in the developing world) to 23 (19 in the developing world).[4] These population increases will be associated with further stresses on natural habitats and resources including clean air, water and food.

TABLE 4-1 -- Factors in the emergence of infectious diseases.

FACTORS IN THE EMERGENCE OF INFECTIOUS DISEASES
Modern demographic and environmental conditions that favor the spread of infectious diseases include:
• global travel;
• globalization of the food supply and centralized processing of food;
• population growth and increased urbanization and crowding;
• population movements due to civil wars, famines and other manmade or natural disasters;
• irrigation, deforestation and reforestation projects that alter the habitats of disease-carrying insects and animals;
• human behaviors, such as intravenous drug use and risky sexual behavior;
• increased use of antimicrobial agents and pesticides, hastening the development of resistance;
• increased human contact with tropical rainforests and other wilderness habitats that are reservoirs for insects and animals that harbor unknown infectious agents;
• deteriorating public health infrastructures in many parts of the world.
Adapted from references[3] [13]

New and re-emerging infectious diseases

Over the past 30 years, more than 40 new pathogens have been identified and characterized, often by using serological and molecular methods (Table 4.2). Some of these microbes cause diseases of global importance (e.g. HIV/AIDS, hepatitis C and rotavirus diarrheal disease). Others appear to be limited (thus far) to particular countries or continents (e.g. Ebola hemorrhagic fever in central Africa (although simian Ebola has also been identified in the Philippines); Argentinian, Bolivian, Venezuelan and Sabia-associated hemorrhagic fevers in South America; hantavirus pulmonary syndrome in the Americas; and new variant Creutzfeldt-Jakob disease in Europe). The most recent example is the outbreak of severe acute respiratory virus syndrome (SARS), almost certainly associated with a novel corona virus. This first emerged in Guangdong province in China but quickly spread through SE Asia. Air travel resulted in its rapid dissemination, most notably to Toronto, Canada. In addition to these newly identified diseases, several 'old' threats have re-emerged in new or drug-resistant forms (Table 4.3). Year-by-year examples of major outbreaks since 1993 are listed in Table 4.4 .

The essential role of the clinician in disease detection and reporting

Physicians are in the best possible position to observe and report unusual illnesses, syndromes and disease risk factors. In 1993, for example, an Indian health service physician reported a cluster of fatal cases of unexplained respiratory disease on Navajo land that proved to be a previously unrecognized disease (hantavirus pulmonary disease). In 1999, the first US outbreak of West Nile encephalitis was identified when an infectious disease physician reported unusual neurologic disease in three elderly people who lived in the same area (Fig. 4.2).[6] West Nile encephalitis-whose causative agent is carried by migratory birds in Asia, Africa and Europe and

transmitted to humans by mosquito bite — had never before been reported in the Western hemisphere. In 2000, physicians in New York, London and Toronto helped identify an international outbreak of leptospirosis among athletes returning home from a competition in Malaysian Borneo. These physicians reported their findings to Geosentinel, the surveillance network of the International Society of Travel Medicine, which alerted travel clinics in 11 countries.[7] An astute clinician was also responsible for reporting the first cases of a multistate outbreak of *Cyclospora* infection that was associated with imported raspberries.[8] Later cases were detected in 20 states, the District of Columbia and two Canadian provinces.

The US anthrax attacks in autumn 2001 also highlighted the crucial role of clinicians in monitoring unusual and dangerous diseases. Six of eight anthrax cases (one inhalational and seven cutaneous) that occurred in New York City were reported to city health authorities by alert physicians. Three were diagnosed by infectious disease physicians (including the inhalational case), one by a dermatologist, one by a public health doctor and one by an emergency room physician. Physicians also reported anthrax cases in Washington DC and New Jersey.

As these examples illustrate, the vigilance of physicians and other health care providers remains the most important factor in infectious disease surveillance and control.

Linkages between animal and human disease surveillance

Most emerging and many re-emerging infectious diseases of human health importance are zoonotic (i.e. animal diseases that can infect humans). The intimate relationship between animal and human health is illustrated by the 1999 outbreak of West Nile encephalitis, which was preceded by die-offs of crows; the 1999 Nipah encephalitis outbreak, which was preceded by illness in pigs; and the 1996 outbreak of new variant Creutzfeldt-Jakob disease, which was preceded by the recognition of an epidemic of bovine spongiform encephalopathy (BSE) among cows.

At the present time, formal mechanisms are being developed to link public health disease monitoring with surveillance for diseases in agricultural animals, migrating birds and wild animals. For example, the Centers for Disease Control and Prevention (CDC) and the US Department of Agriculture (USDA) are working in partnership with zoos across the nation to establish surveillance for West Nile encephalitis among zoo animals. Efforts are also under way to monitor outbreaks of animal influenza (e.g. in chickens and pigs) that might give rise to a new human pandemic strain, as well as to monitor antimicrobial resistance in zoonotic bacteria (e.g. *Salmonella* and *Campylobacter*) that infect agricultural animals. In the future, animal disease surveillance efforts must be expanded to include such potential bioterrorist agents as anthrax, plague, tularemia and Q fever.

In addition to protecting human health, rapid detection and control of animal diseases will benefit agriculture, help preserve wildlife species and avert economic losses due to expensive disease control measures (Table 4.5).

Molecular diagnostics and outbreak detection

To ensure optimal medical care, clinical diagnosis of unusual diseases must be confirmed by laboratory tests. Today's advances in microbial genomics, molecular immunology and bio-informatics are facilitating the development of accurate, rapid and sensitive diagnostics based on detection of microbe-specific or strain-specific nucleic acids or proteins. In the future, advances in human genomics may also allow the identification of individuals with increased genetic susceptibility to certain diseases or to severe manifestations of those diseases (Table 4.6).[9]

Molecular testing has the potential not only to confirm clinical diagnoses but also to facilitate the detection of outbreaks. This function may be especially important when individual cases are not related geographically or by other easily identifiable risk factors. Examples include:

¦ food-borne pathogens transmitted simultaneously to many localities when a contaminated product is shipped to supermarkets or restaurants in several states or countries;

¦ disease agents dispersed by international travelers, like the leptospirosis outbreak in Malaysian Borneo;

¦ an unannounced and undetected (covert) release of a bioterrorist agent in a public place.

TABLE 4-2 -- New microbes

NEW MICROBES			
	Microbe	**Type**	**Disease**
1973	Rotavirus	Virus	Major cause of infantile diarrhea worldwide
1975	Parvovirus B19	Virus	Aplastic crisis in chronic hemolytic anemia
1976	*Cryptosporidium parvum*	Parasite	Acute and chronic diarrhea
1977	Ebola virus	Virus	Ebola hemorrhagic fever
1977	Hantaan virus	Virus	Hemorrhagic fever with renal syndrome (HFRS)
1977	*Legionella pneumophila*	Bacterium	Legionnaires' disease
1977	*Campylobacter jejuni*	Bacterium	Enteric pathogens distributed globally
1980	Human T-lymphotropic virus type I (HTLV-I)	Virus	T-cell lymphoma-leukemia
1981	Toxin-producing strains of *Staphylococcus aureus*	Bacterium	Toxic shock syndrome
1982	*Escherichia coli* 0157:H7	Bacterium	Hemorrhagic colitis; hemolytic uremic syndrome
1982	Human T-lymphotropic virus type II (HTLV-II)	Virus	Hairy cell leukemia
1982	*Borrelia burgdorferi*	Bacterium	Lyme disease
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome (AIDS)
1985	*Helicobacter pylori*	Bacterium	Peptic ulcer disease
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhea
1986	*Cyclospora cayetanensis*	Parasite	Persistent diarrhea
1988	Human herpesvirus 6 (HHV-6)	Virus	Exanthema subitum
1988	Hepatitis E	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chafeensis*	Bacterium	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B hepatitis
1991	Guanarito virus	Virus	Venezuelan hemorrhagic fever
1991	*Encephalitozoon hellem*	Parasite	Conjunctivitis, disseminated disease
1991	New species of *Babesia*	Parasite	Atypical babesiosis
1992	*Bartonella henselae*	Bacterium	Cat-scratch disease; bacillary angiomatosis
1993	Sin Nombre virus	Virus	Hantavirus pulmonary syndrome (HPS)
1993	*Encephalitozoon cuniculi*	Parasite	Disseminated disease
1994	Sabia virus	Virus	Brazilian hemorrhagic fever
1994	Hendra virus	Virus	Encephalitic disease transmitted from horses to humans

1995	Human herpesvirus 8 (HHV-8)	Virus	Associated with Kaposi's sarcoma in AIDS patients
1996	New variant Creutzfeldt-Jakob disease agent	Prion	Progressive degenerative neurologic disease
1997	H5N1 strain of avian influenza	Virus	Influenza transmitted from chickens to humans; often fatal
1999	Nipah virus	Virus	Encephalitic disease transmitted from pigs to humans
2001	Human metapneumovirus	Virus	Acute respiratory infections
2002	Vancomycin-resistant *Staphylococcus aureus*	Bacterium	First vancomycin-resistant *Staph. aureus* strain identified in the United States
Updated from WHO. World Health Report, 1996. Fighting disease: fostering development. Geneva: WHO; 1996.			

Public health tools are already in place in Europe and the United States to identify and trace the source of geographically dispersed food-borne outbreaks by comparing molecular fingerprinting data from clinical and public health laboratories. This is the operating principle behind PulseNET,[10] a molecular subtyping network that has been used to detect several major multistate food-borne outbreaks (Table 4.7). In 1999, for example, PulseNET was used to detect an outbreak of *Salmonella* Newport infections by comparing the molecular fingerprints of 78 *Salmonella* cases from 22 states.

This technology has been shared with Canada, several Pacific Island nations and the European Union. Advances in genome sequencing and bio-informatics will allow this network and molecular epidemiology in general to use increasingly sophisticated typing methods to discover novel disease risk factors and linkages between cases.

In the future, molecular testing may also become a routine part of local efforts to detect intentionally caused outbreaks, for example, by applying environmental sensors that use automated molecular diagnostic tests to detect biologic agents that cause smallpox, anthrax and plague. Once these sensors are in operation, it may become possible to rapidly analyze the genetic make-up of bioterrorist agents (including antibiotic susceptibilities) before symptomatic cases of disease appear.

Being prepared for the unexpected

Infectious pathogens are extraordinarily resilient and have a remarkable ability to evolve, adapt and develop resistance to drugs in an unpredictable and dynamic fashion. Because we do not know what

TABLE 4-3 -- Resurging diseases

RESURGING DISEASES	
Disease or agent	**Factors in re-emergence**
Viral	
Rabies	Breakdown in public health measures; changes in land use; travel
Dengue and dengue hemorrhagic fever	Transportation; travel and migration; urbanization
Yellow fever	Favorable conditions for growth of the mosquito vector
Rift Valley fever	Infected humans, animals or mosquitoes traveled across East Africa to Saudi Arabia and Yemen
Parasitic	
Malaria	Drug and insecticide resistance; civil strife; lack of economic resources
Schistosomiasis	Dam construction, improved irrigation and ecological changes favorable to the snail host
Neurocysticercosis	Immigration
Acanthamebiasis	Introduction of soft contact lenses
Visceral leishmaniasis	War; population displacement; immigrations; habitat changes favorable to the insect vector; and increase in immunocompromised human hosts
Toxoplasmosis	Increase in immunocompromised human hosts
Giardiasis	Increased use of child-care facilities
Echinococcosis	Ecological changes that affect the habitats of the intermediate (animal) hosts
Trypanosomiasis (African sleeping sickness)	Breakdown in public health infrastructure
Bacterial	
Tuberculosis	Human demographics and behavior; industry and technology; international commerce and travel; breakdown of public health measures; microbial adaptation; the HIV/AIDS pandemic
Group A streptococcus	Uncertain
Trench fever	Breakdown of public health measures
Plague	Economic development; land use
Diphtheria	Interruption of immunization programs due to political changes
Pertussis	Refusal to vaccinate in some countries because of belief that pertussis vaccines are not safe
Salmonella	Industry and technology; human demographics and behavior; microbial adaptation; changes in food production
Pneumococcus	Human demographics; microbial adaptation; international travel and commerce; misuse and overuse of antibiotics
Cholera	International travel; introduced to South America from Asia by ship, with spread facilitated by reduced water chlorination and by contaminated food
Adapted from Lorber B. Are all diseases infectious? Ann Intern Med 1996;125;844–51.	

TABLE 4-4 -- Infectious disease challenges, 1993–2003.

INFECTIOUS DISEASE CHALLENGES, 1993–2003	
1993	Hantavirus pulmonary syndrome (United States)
1994	Plague (India)
1995	Ebola fever (Zaire)
1996	New variant Creutzfeldt-Jakob disease (United Kingdom)
1997	H5N1 influenza (Hong Kong); vancomycin-intermediate resistant *Staphylococcus aureus* (Japan, United States)
1998	Nipah virus encephalitis (Malaysia, Singapore)
1999	West Nile encephalitis (Russia, United States)

2000	Rift Valley fever (Kenya, Saudi Arabia, Yemen); Ebola fever (Uganda)
2001	Anthrax (United States); foot and mouth disease (United Kingdom)
2002	Vancomycin-resistant *Staphylococcus aureus* (United States)
2003	Severe acute respiratory syndrome (SARS)

new diseases will arise, we must always be prepared for the unexpected (see Table 4.4). In 1997, for example, an avian strain of influenza that had never before infected humans began to kill previously healthy people (see below). In 2002, the first vancomycin-resistant *Staphylococcus aureus* strain was identified in the United States.[11] Moreover, in 2001, the United States experienced a bioterrorist attack, a multistate outbreak of anthrax that necessitated antibiotic prophylaxis for more than 30,000 people. Rapid medical and public health action limited the outbreak to 11 inhalational cases, 11 cutaneous cases and five deaths.

These examples of unforeseen outbreaks, both naturally occurring and intentional, underscore the need to maintain a strong public health system that is supported by a well-informed and vigilant medical community.

Shifts in view of infectious disease in the past 50 years

Over the past 50 years, there have been significant shifts in how infectious diseases are viewed by the medical and scientific world and by the public. In the years following World War II, it was widely believed that humans were winning the war against infectious microbes. Vaccines and antibiotics, coupled with earlier improvements in urban sanitation and water quality, had dramatically lowered the incidence of infectious diseases. Therefore, it became possible to imagine a world in which infectious pathogens would no longer prey upon humanity. In 1962, the Australian Nobel prize winner Frank Macfarlane Burnet stated:

Figure 4-2 Spread of West Nile virus in the United States. In the 4 years since West Nile virus was first reported in the USA (in New York City), it has been detected in 44 states. *Source: National Center for Infectious Diseases, Centers for Disease Control and Prevention.*

> One can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as an important factor in social life.[12]

Five years later, Surgeon General William H Stewart expressed the views of many US doctors and health experts when he stated that it was time to 'close the book on infectious illnesses', as long as we continued to prevent disease through vaccination. He encouraged the public health community to turn its attention to chronic diseases. Over the following years, biomedical research in the USA became increasingly focused on heart disease, stroke and the 'war on cancer', which was declared by President Richard Nixon in 1971. Local and federal programs aimed at monitoring and studying infectious diseases were greatly reduced or abolished.

New diseases continue to emerge and re-emerge

In spite of optimistic predictions, infectious diseases continued to cause serious problems. As early as the 1950s, certain bacteria, such as *Staphylococcus aureus*, began to develop resistance to penicillin. In 1957 and 1968, new strains of influenza emerged in China and Hong Kong, respectively, and spread rapidly around the globe and in the 1970s there was a resurgence of sexually transmitted diseases in the United States (perhaps due in part to changes in human sexual behaviors and to the importation of antibiotic-resistant strains of gonorrhea by infected soldiers returning from Vietnam). The final blows to our complacent attitude toward infectious diseases came in the 1980s, with the appearance of AIDS and the re-emergence of tuberculosis, including multidrug-resistant strains.

By the early 1990s, many health experts no longer believed that the threat of infectious diseases was receding in the United States and the developed world. Growing concern about the threat of emerging infectious diseases was cogently expressed in a 1992 report issued by the Institute of Medicine (IOM) of the National Academies. The report, *Emerging infections: microbial threats to health in the United States*, emphasized the intimate links between US health and international health.[3] It described the major factors that contribute to disease emergence, including societal changes and microbial evolution (see Table 4.1). The report concluded that emerging infectious diseases are a major threat to US health and it challenged the US government to take action.

In 1994, the CDC answered the challenge by launching a national effort to revitalize the US capacity to protect the public from infectious diseases. This ongoing effort is described in *Preventing emerging infectious diseases: a strategy for the 21st century*.[13]

Looking back to the 1950s and 1960s it is useful to remember that very little was then known about how microbes evolve or develop drug resistance. In the 1970s and 1980s, with the development of molecular biology, biologists learned how resistance genes are carried on plasmids and transmitted from one bacterium to another. They also learned how quickly viruses can evolve by generating mutations with each round of replication, as well as by reassorting gene segments or jumping species barriers. As molecular tools became available, many new viruses (pathogenic and nonpathogenic) were discovered in humans, animals and plants.

Infectious diseases are a national security threat

Before 2001, US concerns about the impact of health issues on national security were focused primarily on events overseas[14] (Table 4.8). During the 1990s, security experts expressed particular concern about the destabilizing effects of HIV/AIDS in poor countries where high death rates among young adults have damaged economic, social, political, military and educational infrastructures and created vast numbers of orphans. In July 2000, the Group of Eight Industrialized Nations pledged to reduce deaths from HIV/AIDS, malaria, tuberculosis and vaccine-preventable diseases by supporting global health initiatives launched by the World Health Organization and other international groups (Table 4.9). These ongoing efforts (including the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM)) reflect a growing consensus that global health security must be a shared responsibility.

Since the autumn of 2001, domestic security concerns have taken center stage. The anthrax attacks illustrated that the United States is vulnerable to bioterrorist weapons. The medical and health care communities must therefore reorient themselves to be prepared for intentional as well as naturally occurring outbreaks.[15]

Physicians are one of the three cornerstones of the 'golden triangle' for bioterrorism preparedness, along with the health care delivery

TABLE 4-5 -- Agricultural costs of controlling diseases carried by food animals.

AGRICULTURAL COSTS OF CONTROLLING DISEASES CARRIED BY FOOD ANIMALS
When a dangerous animal-borne disease threatens human health or food safety, a government may be forced to slaughter large numbers of food animals as a control measure, despite considerable economic costs. Recent examples include two zoonotic diseases (Nipah encephalitis and H5N1 influenza) and two veterinary diseases (foot and mouth disease and bovine spongiform encephalopathy (BSE; also called mad cow disease)). Ingestion of beef containing the causative agent of BSE (a prion) may result in the development of a fatal human neurodegenerative illness (new variant Creutzfeldt-Jakob disease) many years later.
• *Nipah encephalitis.* In 1999 Malaysian health authorities were faced with an outbreak of encephalitis among farm workers, which had a nearly 50% mortality rate. The cause was a previously unknown paramyxovirus called the Nipah virus, which is carried by bats. To control the outbreak, millions of pigs were slaughtered within a few weeks, severely harming the Malaysian meat industry.
• *Avian influenza (strain H5N1).* In 1997, a similar precautionary measure was taken by the government of Hong Kong, which arranged the culling of all 1.6 million chickens on Hong Kong Island and the New Territories to prevent chicken-to-human transmission of a virulent avian form of influenza.

• *Bovine spongiform encephalopathy.* The European Union has temporarily banned the export of live cattle and cattle products (other than milk) from the United Kingdom and trade in these products has been affected on a global basis. Government officials have come under fire and consumers across Europe have changed their eating habits because of concern over the spread of BSE. Control measures, including the slaughter of affected cows, have thus far cost the UK government an estimated £3.5 billion sterling (about US$5 billion).
• *Foot and mouth disease.* The costs of controlling the 2001 outbreak of foot and mouth disease in the United Kingdom and continental Europe dwarfed those of the BSE outbreak and devastated the centuries-old British livestock industry. (Foot and mouth disease does not infect humans but can be spread by travelers who have contaminated soil on their shoes or clothing or who carry contaminated food products.) The St Patrick's Day parade in Ireland was cancelled due to concerns about spreading the virus and the British army was drafted to help bury the carcasses of animals slaughtered because of potential exposure to the disease. Officials credit high-quality animal health surveillance and importation restrictions for the absence of foot and mouth disease in the United States.

TABLE 4-6 -- Examples of genetic factors that influence susceptibility to disease or disease progression.

EXAMPLES OF GENETIC FACTORS THAT INFLUENCE SUSCEPTIBILITY TO DISEASE OR DISEASE PROGRESSION
• Alleles of the chemokine receptor gene CCR5 confer partial protection against HIV infection and the development of AIDS
• Globin gene alleles (e.g. sickle globin and a- and ß-thalassemias) confer partial protection against malaria
• Lack of the Duffy blood group on red cells (due to a mutation in a chemokine receptor gene) confers complete protection against *Plasmodium vivax* malaria
• The tt vitamin D receptor (VDR) genotype may reduce the risk of developing clinical tuberculosis
• Blood group O is associated with severe cholera
• Mutations in the cystic fibrosis gene may confer some protection against cholera
• HLA alleles may influence susceptibility to — or course of infection of — HIV/AIDS, hepatitis B, measles, hantavirus pulmonary syndrome, malaria, tuberculosis, human papillomavirus infection and coccidioidomycosis

system and public health officials.[16] All clinicians, regardless of their specialty, must have basic information about the clinical manifestations of such bioterrorist agents as variola virus (smallpox), *Yersinia pestis* (plague) and *Bacillus anthracis* (anthrax). They must have a high index of suspicion and know how to recognize unusual diseases and report them to local public health and law enforcement officials. The expertise and full engagement of the medical community are essential to the national effort to preserve health security. For further discussion of issues related to bioterrorism, see Chapter 6 .

CATEGORIES OF EMERGING AND RE-EMERGING INFECTIOUS DISEASES

For the purposes of this discussion, emerging and re-emerging threats may be grouped into five (sometimes overlapping) categories:[7]

- drug-resistant diseases;
- food-borne and water-borne diseases;
- zoonotic and vector-borne diseases;
- diseases transmitted through blood transfusions or blood products;
- chronic diseases caused by infectious agents.

Drug-resistant diseases

Drug-resistant pathogens are a growing menace to all people, regardless of age, sex or socio-economic background. They endanger people in affluent, industrial societies like the United States, as well as in less developed nations. Examples of clinically important microbes that are rapidly developing resistance to available antimicrobial agents include bacteria that cause pneumonia, ear infections and meningitis (e.g. *Streptococcus pneumoniae*), skin, bone and bloodstream infections (e.g. *Staphylococcus aureus*), urinary tract infections (e.g. *Escherichia coli*), food-borne infections (e.g. *Salmonella*) and infections transmitted in health care settings (e.g. enterococci) (Table 4.10). Many other pathogens — including the bacteria that cause tuberculosis and gonorrhea; the virus that causes AIDS; the fungi that cause *Candida* infections; and the parasites that cause malaria — are also becoming resistant to standard therapies. If we do not act to address the problem of antimicrobial resistance, it will become impossible to take for granted quick and reliable treatment of infections that have been a manageable problem since the late 1940s. Drug choices for the treatment of common infections will become increasingly limited and expensive and, in some cases, nonexistent.

Reasons for the rapid development of antibiotic resistance include the natural tendency of organisms to mutate and share genetic material. However, this process has been facilitated by:

- inappropriate prescription practices by physicians and veterinarians;
- unrealistic patient expectations that lead to requests for antibiotic treatment of nonbacterial infections;
- overuse of antibiotics by the agricultural industry;
- the economics of pharmaceutical sales;
- increased use of sophisticated medical interventions that require the administration of large quantities of antibiotics (e.g. transplant surgery and immunosuppressive and cytotoxic drug therapy).

Growing antibiotic resistance poses a substantial threat to modern gains in infectious disease control. About 70% of bacteria that cause infections in hospitals in many countries, including the US, are resistant to at least one of the drugs most commonly used to treat infections. The medical community must therefore join with patients and members of the agricultural and pharmaceutical industries in a common effort to promote appropriate use of antibiotics to safeguard their effectiveness for future generations. Steps to be taken by the European Union and the US government to facilitate this process are outlined in the Copenhagen Recommendations[52] and in A Public Health Action Plan to Combat Antimicrobial Resistance respectively. [17]

TABLE 4-7 -- Examples of multistate food-borne outbreaks in the United States, 1994–2002.

EXAMPLES OF MULTISTATE FOOD-BORNE OUTBREAKS IN THE UNITED STATES, 1994–2002			
Year	**Outbreak**	**Number of states**	**Food source**
1994	*Shigella flexneri*	2	Green onions, probably contaminated in Mexico
1994	*Listeria monocytogenes*	3	Milk, contaminated after pasteurization. The milk was consumed in one state, but two of the case patients traveled to other states before becoming ill
1995	*Salmonella enteriditis*	41	Ice-cream premix hauled in trucks that had previously carried raw eggs
1996	*Cyclospora cayetanensis*	20	Raspberries from Guatemala, mode of contamination unclear. Cases were also reported in the District of Columbia and two Canadian provinces
1996	*Escherichia coli* 0157:H7	3	Unpasteurized apple juice, probably contaminated during harvest
1996	Norwalk-like virus	5	Oysters, contaminated before harvest
1997	*Salmonella infantis*	2	Alfalfa sprouts, probably contaminated during sprouting
1997	*Cyclospora cayetanensis*	18	Raspberries imported from Guatemala, mesclun lettuce and products containing basil. Cases were also reported in the District of Columbia and two Canadian provinces
1997	Hepatitis A virus	4	Strawberries from Mexico distributed through the USDA Commodity Program for use in school lunches

1998	*Vibrio parahaemolyticus*	13	Raw oysters, contaminated before harvest
1998	*Shigella sonnei*	4	Imported parsley, probably contaminated during washing after harvest
1999	*Listeria monocytogenes*	22	Hot dogs, probably contaminated at plant after cooking
1999	*Salmonella* Muenchen	20	Unpasteurized orange juice
2000	Hepatitis A virus	2	Green onions or tomatoes served at two outlets of the same fast food chain
2000	*Salmonella* Newport	10	Imported manges, likely contaminated during treatment to kill fruit flies
2000	*Listeria monocytogenes*	8	Deli turkey, contaminated at the plant after cooking
2000	Norwalk-like virus	13	Salads in a 'boxed banquet' sent to several car dealerships in the US
2001	*Salmonella* Poona	16	Imported cantaloupe, probably contaminated in the field or shortly after harvest
2002	*Salmonella* Newport	5	Ground beef from a large commercial grinder
2002	*Escherichia coli* 0157:H7	6	Ground beef from a large commercial grinder
Update from reference[13] .			

TABLE 4-8 -- Biologic national security issues.

BIOLOGIC NATIONAL SECURITY ISSUES
• Diseases that spread across borders, crossing countries and continents (e.g. cholera, bacterial meningitis and measles)
• The emergence of new and antibiotic-resistant diseases that arise in one region and spread throughout the world (e.g. AIDS and drug-resistant TB)
• Environmental issues that may have global effects (e.g. pollution, loss of biodiversity, and global warming, which may affect the growth rate of insect vectors of disease)
• Population overgrowth that may lead to disease, war, famine and political instability
• Bioterrorism: the deliberate release of infectious agents by a terrorist or rogue nation
Adapted from Goldberg J. Our Africa problem. New York Times, March 2, 1997.

Food-borne and water-borne diseases

Twentieth-century improvements in sanitation, food sterilization and processing, and water treatment have greatly reduced the burden of food-borne and water-borne illnesses in developed countries, nearly eliminating many diseases that remain major killers in the developing world (e.g. typhoid fever, cholera and dysentery). Now, in the 21st century, however, there is growing evidence that modern factors such as centralized food processing and the appearance of new types of industrial chemicals may pose challenges to food safety and healthy water.

Food-borne diseases

Food-borne diseases cause an estimated 76 million illnesses, 325,000 hospitalizations and 5000 deaths in the United States each year.[18] As noted above, food-borne pathogens such as *Salmonella, Shigella, Cyclospora, Campylobacter* and *E. coli* 0157:H7 can be transmitted via commercial products that are processed in large quantities and shipped to different states or nations. Although techniques for sterile processing are very advanced, when contamination does occur it can affect many people in many different localities (see Table 4.7). A recent example is an outbreak of *E. coli* 0157:H7 (transmitted via hamburger meat) that affected people in 21 states and led to the recall of 18 tons of beef.[19]

Water-borne diseases

Microbial contamination of water can occur when animal or human sewage contaminates source water that is not adequately disinfected by filtration, chlorination or other methods. In addition, some parasites are resistant to routine water treatment methods. These include *Cryptosporidium*, the causative agent of a major disease outbreak in the Milwaukee drinking water system in 1993 that affected more than 400,000 people.[20] Moreover, some bacteria, viruses and parasites live in 'biofilms' — coatings on pipes and tubing — that may shield them from disinfection.[21] Microbes that live in biofilms in medical devices such as catheters and dialysis machines may be responsible for a significant proportion of health care-associated infections.

Associations between illness and water contaminants are often difficult to evaluate, since most people drink water every day, often from more than one source. Another complicating factor in estimating the national burden of water-related illness is the diversity of US

TABLE 4-9 -- Global health initiatives.

GLOBAL HEALTH INITIATIVES	
Four major global health initiatives were launched between 1998 and 2000.	
• ***Roll Back Malaria***, a global strategy to reduce deaths from malaria by increasing access to prompt and effective treatment (including protective intermittent therapy for pregnant women) and prevention tools (including insecticide-treated bednets); by facilitating rapid response to malaria outbreaks; and by developing new products for the prevention and treatment of malaria.	
• ***Stop TB***, a global strategy to stop the spread of TB around the world. One of its objectives is to promote implementation of the directly observed therapy short-course strategy (DOTS). The effective implementation of DOTS in NYC, in response to the epidemic in the late 1980s and early 1990s, has served as a model in the USA and around the world.	
• ***International Partnership Against AIDS in Africa***, a UNAIDS-led effort to mitigate the effects of the growing HIV/AIDS epidemic. In 1999, as part of this effort, the USA government launched the Leadership and Investment for Fighting an Epidemic (LIFE) Initiative, which provides support to the hardest-hit countries for reducing HIV transmission, improving treatment of HIV/AIDS and opportunistic infections, and strengthening national capacities to collect disease surveillance data and manage national HIV/AIDS programs. The Global AIDS Program is the CDC component of the LIFE Initiative.	
• ***Global Alliance for Vaccines and Immunization (GAVI)***, a global effort to strengthen childhood immunization programs and bring a new generation of recently licensed vaccines into use in developing countries. These include vaccines against hepatitis B, childhood meningitis, yellow fever and respiratory infections, which are the leading cause of death in children under age 5. Substantial resources for this purpose have been pledged by the Bill and Melinda Gates Foundation and the governments of Norway, the Netherlands and the United States. This initiative could save as many as 3 million lives every year.	
Targets for disease reduction	
These targets for disease reduction were endorsed at the Group of Eight Industrialized Nations Summit in Okinawa in July 2000.	
HIV/AIDS:	25% reduction in prevalence in young people by 2010
TB:	50% reduction in deaths by 2010
Malaria:	50% reduction in deaths by 2010

Adapted from reference[40]

source water systems, treatment systems, and distribution systems. Two recent trials [22] [23] found that tap water from selected municipal surface water systems may cause up to 40% of endemic cases of diarrheal illness. Full-scale studies to evaluate the safety of public drinking water systems are underway as part of the Congressionally mandated National Estimate of Waterborne Diease[24] , which will include information on the severity and economic impact of diarrheal illness associated with tap water.

Zoonotic and vector-borne diseases

Zoonotic pathogens

Many of the novel pathogens identified over the past decade are carried by animals. For example, Sin Nombre virus, which is carried by rodents, was identified in 1993 in the USA as the cause of hantavirus pulmonary syndrome. Hendra virus, carried by fruit bats, was identified in Australia in 1994 as a cause of encephalitis in humans and horses.[25] Nipah virus, which is also carried by fruit bats, was identified in 1999 in Malaysia as a cause of encephalitis in humans and swine.[26] Like Sin Nombre virus (a hantavirus), the Nipah and Hendra viruses (paramyxoviruses) are highly dangerous and there are as yet no drugs or vaccines for their treatment or prevention.

TABLE 4-10 -- Examples of emerging resistance in bacterial pathogens.

EXAMPLES OF EMERGING RESISTANCE IN BACTERIAL PATHOGENS
Gram-positive cocci
Methicillin-resistant Staphylococcus aureus
Vancomycin-resistant *Staphylococcus aureus*
Coagulase-negative staphylococci
Penicillin-resistant pneumococci
Macrolide-resistant streptococci
Vancomycin-resistant enterococci
Gram-negative cocci
Penicillin-resistant meningococci
Quinolone-resistant gonococci
Gram-negative bacilli
Enterobacter spp. and other Enterobacteriaceae with chromosomal ß-lactamases
Multidrug-resistant *Pseudomonas aeruginosa*
Stenotrophomonas maltophila
Acinetobacter spp. with novel ß-lactamases, aminoglycoside-modifying enzymes and other resistance mechanisms
Enterobacteriaceae with extended-spectrum ß-lactamases
Multidrug-resistant diarrheal pathogens (*Shigella* spp., *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp.)
Acid-fact bacilli
Multidrug-resistant *Mycobacterium tuberculosis*
Multidrug-resistant *Mycobacterium avium* complex

Zoonotic agents can become established in any geographic area that has a suitable animal reservoir. The arenavirus that causes lymphocytic choriomeningitis (first isolated in 1933) was probably introduced into the New World at the same time as its vector, *Mus musculus*, the common house mouse. Plague (which is both animal-borne and vectorborne) was introduced into the United States in the early 1900s, via infected rats and fleas in ships that arrived at port cities. It quickly became established in the North American prairie ecosystem, infecting a wide range of animals, including native rodents and their fleas, which have been the most frequent sources of human infection. Like plague, newly emergent disease agents that are able to infect many animal species (e.g. Nipah and Hendra viruses) have the potential to spread worldwide.

Zoonotic pathogens are an important public health concern not only because of the illnesses they cause but also because new human diseases can arise from animal reservoirs. Pandemic strains of influenza can emerge from avian and swine reservoirs[27] and many experts believe that Ebola and Marburg hemorrhagic fever viruses are maintained in an animal host. As mentioned above, the AIDS virus probably evolved from a virus carried by a nonhuman primate (e.g. the chimpanzee *Pan troglodytes troglodytes*). [1]

Disease dispersion via insect vectors

Diseases that are carried by insect vectors (i.e. mosquitoes, fleas, ticks and other arthropods) can also spread into new geographical areas and infect new human populations. In 1999, for example, mosquito-borne transmission of three nonendemic diseases — malaria, dengue and West Nile fever — was reported in the United States. West Nile encephalitis may have entered New York City via an imported or migrating bird, an infected person or a mosquito that 'hitch-hiked' on an airplane; dengue fever most likely arrived in Texas via both people and mosquitoes. Asian tiger mosquitoes (*Aedes albopictus*) that can transmit dengue arrived in the United States in Houston in 1987 in imported used-tire casings[28] and more

recently appeared in California, in commercial shipments of a Chinese ornamental indoor plant called 'lucky bamboo'.[29]

While many cases of 'airport malaria' have been reported in Europe and North America (presumably involving small numbers of traveling mosquitoes), locally acquired malaria cases have also been identified that apparently involved at least one cycle of human-to-mosquito transmission. For example, a cluster of non-airport malaria cases was detected in Virginia in 2002, in a community that included a significant number of immigrants from malarious countries who might have asymptomatic malaria infections.[30] Another example of vector-borne disease spread is the recent identification of Rift Valley fever (previously seen only in Africa) in the Middle East.[31] Competent mosquito vectors for Rift Valley fever, a febrile hepatitis associated with encephalitis, retinitis and hemorrhagic fever (among both humans and animals), are present throughout the world.

Without ongoing public health attention, vector-borne diseases may continue to become established (e.g. Japanese encephalitis) or re-established (e.g. malaria or yellow fever) in developed countries in the future.

These concerns are underscored by studies on the effects of global warming in Alaska, which suggest that mosquito populations may grow larger as the area becomes warmer and wetter, increasing the risk of transmission of malaria parasites and encephalitis viruses if these disease agents are introduced into the state.[32]

Diseases transmitted through blood transfusions or blood products

Improvements in donor screening, serologic testing and transfusion practices have made the US blood supply one of the safest in the world, despite its size and complexity. However, because blood is a human tissue, it is a natural vehicle for transmission of infectious agents. During the 1980s, HIV was transmitted through clotting factor and blood transfusions, and during the 1990s, hepatitis C virus was transmitted via intravenous immunoglobulin. More recently, transmissions of Chagas'

disease trypanosomes[33] and West Nile virus[34] have been reported in surgical patients who received organ transplants or blood from infected persons.

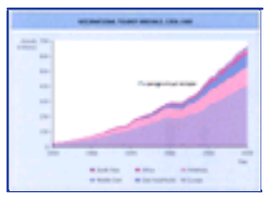

Figure 4-3 International tourist arrivals, 1950–99. International travel has increased by 160 million tourist arrivals since 1990, from 500 million to more than 660 million in 1999. In 1950, 15 countries received nearly 100% of the 25 million international tourist arrivals. In 1999, at least 70 countries and territories each received more than one million international tourist arrivals. The rate of growth in international arrivals in Africa alone was 7.8%, nearly twice the world average. ('Tourism' in these statistics includes business travel.) *Source: World Tourism Organization. WTO tourism highlights 2000, August 2000.*

TABLE 4-11 -- Infectious agents that cause or contribute to neoplastic diseases in humans.

INFECTIOUS AGENTS THAT CAUSE OR CONTRIBUTE TO NEOPLASTIC DISEASES IN HUMANS	
Epstein-Barr virus	Nasopharyngeal carcinoma (undifferentiated)
	Burkitt's lymphoma
	Post-transplant lymphoproliferative disease
	B-cell lymphoma
Hepatitis B and C viruses	Hepatocellular carcinoma
Human immunodeficiency virus	Lymphoma
Human herpesvirus 8	Kaposi's sarcoma
Human T-cell leukemia virus	Adult T-cell leukemia
Human papillomavirus	Cervical carcinoma
Helicobacter pylori	Gastric carcinoma
	Mucosa-associated lymphoid tissue lymphoma
Schistosoma haematobium	Bladder carcinoma
Liver flukes	Cholangiocarcinoma
Adapted from reference[13]	

Infectious diseases also have implications for the availability of blood and blood products, because people who have traveled in countries where they may have been exposed to blood-borne diseases are often excluded as blood donors. Examples include individuals who have recently visited malaria-endemic countries and people who have stayed for 6 months or more in the United Kingdom, where they might have ingested beef from cows with BSE and acquired new variant Creutzfeldt-Jakob disease.

Although research on artificial blood substitutes is under way, it is unlikely that they will be available in the near future. Therefore, continued vigilance is required to ensure the safety of the blood supply. As part of this effort, new blood screening tests must be developed and applied by blood banks as new blood-borne diseases are recognized.

Chronic diseases caused or exacerbated by infectious agents

Several chronic diseases once attributed to lifestyle or environmental factors (such as some forms of cancer, diabetes, heart disease and ulcers) are actually caused by or exacerbated by an infectious agent.[35] [36] Three of the six major causes of cancer death in the world are caused by infectious agents: hepatocellular carcinoma by hepatitis B and C viruses, cervical cancer by human papillomavirus and stomach cancer by *Helicobacter pylori* bacteria (see also Table 4.11). Hepatitis B and C are also major causes of cirrhosis and end-stage liver disease, while *H. pylori* causes peptic ulcer disease in addition to stomach cancer. Moreover, Epstein-Barr virus is associated with nasopharyngeal carinoma, Burkitt's lymphoma, B-cell lymphoma and post-transplant lymphoproliferative disease.

Current research suggests that some chronic cardiovascular, intestinal and pulmonary diseases may also have an infectious etiology. For example, Whipple's disease is caused by a bacterial infection (*Tropheryma whipplei*) [37] and tropical spastic paraparesis is caused by a viral infection (HTLV-I).[38] Potential associations are being investigated for many other illnesses and syndromes, including coronary artery disease (chlamydia infection);[39] Paget's disease (paramyxoviridae infection);[40] and Crohn's disease (mycobacterial infection).[41] There are also preliminary data suggesting that Wegener's granulomatosis responds to antibiotic therapy.[42] These findings raise the possibility that some chronic conditions, including cancer, asthma, arthritis and heart disease, may someday be treated with antimicrobial drugs or prevented by vaccines.

INFECTIOUS DISEASES AND THE GLOBAL VILLAGE

As stressed in the 1992 IOM report, US health and global health are inextricably linked.[3] [43] Modern factors that connect us culturally, commercially and physically such as air travel (Fig. 4.3) and the globalization of the food supply (Table 4.7) put us at risk of exposure to microbes that are endemic in other countries, whether we live in large cities or small rural hamlets. As the HIV/AIDS epidemic has illustrated, a disease that emerges or re-emerges anywhere in the world can spread far and wide.

Several diseases of global public health importance — including epidemic-prone diseases, newly emerging or re-emerging diseases, vaccine-preventable diseases and diseases slated for regional

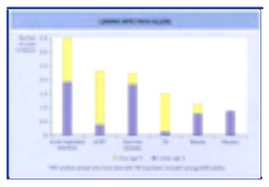

Figure 4-4 Leading infectious killers. *Source: World Health Organization. Overcoming antimicrobial resistance. Geneva: WHO.*

TABLE 4-12 -- Infectious diseases do not recognize borders.

INFECTIOUS DISEASES DO NOT RECOGNIZE BORDERS
From a public health point of view, domestic and international health are inextricably linked. Examples of disease spread from continent to continent include the following:
• *HIV/AIDS.* The AIDS virus apparently emerged in central Africa in the 1950s or earlier[1] and spread through most of Africa, Asia, Europe and the Americas during the 1970s and 1980s.
• *Tuberculosis.* This age-old scourge re-emerged in the 1980s (sometimes in a multidrug-resistant form) in cities around the world, including in the United States. By 2000, approximately 46% of newly identified US TB cases originated in other countries.

• *Malaria.* Although malaria was eliminated in the USA by the 1960s (through swamp-draining and vector control programs), approximately 1500 cases of malaria are reported in the USA each year. One-half occur in US travelers to malaria-endemic countries and the other half occur among foreign nationals who enter the USA already infected.
• *Vibrio cholerae 01, El Tor biotype.* This virulent strain of cholera has caused an ongoing pandemic that has lasted 40 years and affected more than 75 countries. Beginning in 1961, it spread from Indonesia through most of Asia into eastern Europe and Africa. From North Africa it spread to the Iberian peninsula and into Italy in 1973. Small outbreaks occurred in Japan and in the South Pacific in the late 1970s. In January 1991, epidemic cholera appeared in Peru and spread rapidly through most of Latin America, causing more than 1,000,000 cases by 1994. This was the first time in 100 years that a cholera pandemic had reached the New World.
• *Measles.* Fifty-six of the 87 cases of measles identified in the USA in 2000 were traced to importations of the virus from other countries. Twenty-six were direct importations, 18 were secondary cases and eight involved viruses whose DNA sequences suggested a foreign origin.
• *Influenza spread on cruise ships.* A 1997 outbreak of the A/Sydney strain of influenza occurred among people on a cruise that made stops in Canada and New England. The A/Sydney strain had been isolated in Australia too late in the year to be included in the vaccine formulated for the fall/winter flu season in the Northern hemisphere.
• *Polio.* Eliminated from the Western hemisphere since 1991, paralytic polio reappeared in Central America in 2000 (in Haiti and the Dominican Republic) and was attributed to waning immunization coverage rates in those countries.
Adapted from reference[40] .

[1] Zhu T, Korber B, Nahmias AJ, *et al.* An Africa HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature 1998;391:594–7.

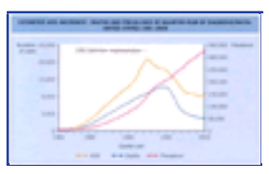

Figure 4-5 Estimated AIDS incidence (*adjusted for reporting delays), deaths and prevalence, by quarter-year of diagnosis/death — United States, 1981–2000. AIDS incidence increased rapidly through the 1980s, peaked in the early 1990s and then declined. The peak of new diagnoses was associated with the expansion of the AIDS surveillance case definition in 1993 (CDC. HIV/AIDS surveillance report, 2000;1 2[1]). As of 1996, sharp declines were reported in AIDS incidence and deaths. From 1998 through June 2000, AIDS incidence and deaths leveled off and AIDS prevalence continued to increase. Throughout the epidemic, approximately 85% of persons diagnosed with AIDS were aged 20–49 years. *Source: Centers for Disease Control and Prevention. HIV and AIDS — United States, 1981–2000. MMWR 2001;50(21);430–4.*

Figure 4-6 Adults and children estimated to be living with HIV/AIDS at the end of 2001. *Source: UNAIDS and World Health Organization, 2001 (http://www.unaids.org/barcelona/presskit/epigraphics/epicore_en4_0602.GIF).*

elimination or worldwide eradication — have resurged in recent years, spreading across countries and (sometimes) continents (Table 4.12). HIV/AIDS, tuberculosis, malaria and measles continue to be major infectious causes of death worldwide, along with acute respiratory infections and diarrheal diseases (Fig. 4.4). Globally important diseases that are of special domestic concern to the United States and other developed countries include HIV/AIDS, tuberculosis and pandemic influenza.

HIV/AIDS

HIV/AIDS incidence in the United States increased rapidly through the 1980s and early 1990s (Fig. 4.5), with the peak of new diagnoses occurring after the expansion of the AIDS surveillance case definition in 1993.[44] After 1996, with the introduction of effective combination antiretroviral therapies, sharp declines were reported in AIDS incidence and deaths, while AIDS prevalence continued to increase. At the present time, US disease prevention efforts are

targeted to young men who have sex with men, the group that currently exhibits the highest incidence nationally.[45]

Worldwide, an estimated 40 million people are living with HIV/AIDS (Fig. 4.6). In 2001 alone, there were 3 million HIV-related deaths and approximately 5 million new infections.

Tuberculosis

Tuberculosis is the attributable cause of one-third of all adult deaths in developing nations.[46] It is also a leading killer of young women. Once thought to be eliminated in Western countries, tuberculosis has reemerged in Europe and the United States, where multidrug-resistant *Mycobacterium tuberculosis* has been reported in 45 of the 50 states.[47] HIV infection confers the greatest known risk for the development of the disease, stimulating both the activation of latent infection and progression to primary disease. UNAIDS estimates that approximately 30% of all AIDS deaths result directly from tuberculosis.[48]

Pandemic influenza

New strains of influenza viruses can emerge unpredictably and spread rapidly and pervasively through susceptible populations, sometimes causing worldwide epidemics. This is due in large part to two features of the influenza virus: its ability to exchange genetic information between strains and its ability to occasionally 'jump' species barriers between mammalian and avian hosts. Experts agree that future pandemics of influenza are inevitable. In the United States alone, preliminary estimates indicate that an influenza pandemic would cause between 88,000 and 227,000 deaths and the economic impact would range from 71 to 166 billion dollars. [49]

The sudden and unpredictable emergence of potential pandemic strains of influenza was illustrated in 1997 by an outbreak of avian influenza A[H5N1] in Hong Kong, which raised the specter of a worldwide epidemic similar to the one that killed 20–50 million people (including 500,000 Americans) in 1918. Epidemiological studies conducted in consultation with the CDC suggested that the H5N1 virus was transmitted from chickens to people and only poorly (if at all) from person to person. Nevertheless, it was feared that the virus might recombine with a human influenza virus during the winter influenza season, creating a virulent strain capable of air-borne human-to-human transmission. To ensure that the H5N1 virus would have no opportunity to evolve, the Hong Kong government authorized the culling of all 1.6 million chickens in Hong Kong (see Table 4.5).

BIOTERRORISM PREPAREDNESS AND RESPONSE

Today, because of recent experiences with bioterrorism, the public health effort to address emerging infections has acquired a new dimension. The medical community must be ready to diagnose and treat not only naturally occurring illnesses but also a wide range of diseases that might be caused by agents engineered for deliberate dissemination.[50]

Instructions for preparing many 'homemade' threat agents are readily available and reports of arsenals of military bioweapons[51] raise the possibility that some terrorists may have access to highly dangerous agents that are engineered for mass dissemination as small-particle aerosols. Some of these agents (like variola virus) are highly contagious and often fatal. There is also concern that bioweapons may be altered physically or genetically to increase their ease of dispersion, change their modes of transmission or render them drug or vaccine resistant.

Responding to outbreaks caused by such agents will require the rapid mobilization of the health care system. There may be a large volume of patients (including both infected people and the 'worried well') and a corresponding need for large quantities of medical supplies, diagnostic tests and hospital beds. Efforts to mitigate illness

and death due to acts of bioterrorism therefore depend on the expertise and full engagement of physicians and other health care providers across the nations. The most likely way in which the next bioterrorist attack will be detected is through the vigilance of an astute clinician who reports something unusual to public health authorities. Rapid treatment of patients and chemotherapeutic prophylaxis or vaccination of exposed persons will also depend on coordinated action by clinicians. Once again, since political and geographic boundaries pose no barrier to the dissemination of these agents, it will be important to think of domestic preparedness in a global context to improve our health security.

Now more than ever, global health and well-being depend on the vigilance of concerned and well-informed clinicians. Each and every clinician plays a critical role in the public health early warning system for unusual infectious diseases, as well as in the public health response to bioterrorism (see Chapter 6).

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Chapter 5 - Diseases of Unknown Etiology: The Role of Infectious Agents

David N Fredricks

INTRODUCTION

'A fool knows everything. A wise man knows his own ignorance.'
(Anon)

We do not understand what causes many diseases. Although risk factors, genetic susceptibility and pathologic manifestations are defined for many diseases, the proximate causes are elusive. Even for a disease such as schizophrenia, with a clear genetic component, environmental factors play a critical role as evidenced by concordance rates of only 30% for monozygotic twins. What are the environmental triggers in these diseases? Among the numerous exogenous factors, infectious agents have several characteristics that make them good candidates for producing occult disease. Microbes can replicate, induce direct tissue damage, promote inflammation and be transmitted to new susceptible hosts. Many microbes are difficult to detect and propagate in the laboratory, so the failure to isolate a pathogen from diseased tissue does not exclude an infectious cause.

Why look for infectious triggers in unexplained diseases? Identification of infectious agents that cause or contribute to disease would create new opportunities for better diagnosis, prevention and treatment.[1] Recent history is replete with instances where novel pathogens have been shown to cause diseases that were previously considered idiopathic. Table 5.1 lists some microbes detected since 1980 and the diseases they cause. These successes provide the impetus to search for occult infectious causes of other unexplained diseases.

The list of diseases with uncertain causation is long and includes many common chronic diseases. Chronic diseases account for 70% of all deaths and 400 billion dollars in annual health care costs in the USA. These chronic diseases include atherosclerosis, cancer and diabetes, all of which have been linked to infectious agents. Table 5.2 provides a partial list of idiopathic diseases for which an infectious cause has been proposed.

NEW MICROBE-DISEASE ASSOCIATIONS: LESSONS LEARNED

The discovery of Lyme arthritis as an infectious disease illustrates the impediments to forming microbe-disease links. In 1975 there was a cluster of cases of oligoarticular arthritis in children from Lyme, Connecticut, that was at first attributed to juvenile rheumatoid arthritis.[2] However, the high attack rate in the community was not consistent with the diagnosis of juvenile rheumatoid arthritis. After prompting by concerned mothers, public health authorities initiated an investigation that eventually resulted in the description of a new disease syndrome, Lyme arthritis. Although evidence suggested that ticks transmit Lyme disease and antibacterial therapy is curative, identification of the causative bacterium was delayed by several years. *Borrelia burgdorferi* was isolated from *Ixodes* ticks in 1982 and identified as the cause of erythema migrans rash, arthritis and other manifestations of Lyme disease.[3] Thus, even with strong evidence implicating a bacterial infection in Lyme disease, identification of the infectious agent was difficult.

Was Lyme disease a new disease? *Borrelia burgdorferi* was detected in a mouse specimen from Massachusetts collected in 1894[4] and ticks from Long Island collected in the 1940s,[5] suggesting that the agent of Lyme disease has been present in the environment for many years. Illness compatible with Lyme disease was described in Europe in the early 1900s. Lyme disease is currently the most common tick-borne illness in the USA. It is hard to believe that Lyme disease was not present in our more bucolic ancestors. It is likely that *Borrelia burgdorferi* has been producing human disease for many decades, if not centuries.

The failure to identify the infectious nature of Lyme disease prior to 1975 reflects at least two problems. The first is inadequate disease surveillance. If the rates of arthritis, pneumonia or encephalitis are not tracked in a community, then we will miss spikes in disease that may represent outbreaks of infectious disease and therefore we will miss the opportunity to identify novel infectious agents. The second problem is our failure to consider the hypothesis of infection. If clinical patterns do not fit our preconceived notions of an infectious disease, we tend to dismiss them as noninfectious and a search for infectious agents is not pursued.

Hantavirus pulmonary syndrome is another disease that has been linked to a recently described infectious agent. A cluster of unexplained respiratory deaths in previously healthy adults was noted in the Four Corners area of the western USA in 1993.[6] Since these patients had a febrile prodrome, an infectious disease was suspected. The US Centers for Disease Control and Prevention initiated an investigation after being alerted by physicians in the Indian health service. Sera from affected patients were screened to detect antibodies to known respiratory pathogens, but no seroreactivity was noted. However, patients' sera did have antibodies that targeted hantavirus antigens. Reverse transcriptase polymerase chain reaction (PCR) using primers complementary to conserved hantavirus RNA sequences identified a novel hantavirus, later named Sin Nombre virus (SNV), as the cause of this respiratory syndrome. A climate-related expansion in the deer mouse population that harbors SNV is believed to have increased contact between mice and humans, resulting in the outbreak. Subsequent investigations have revealed that hantaviruses capable of producing hantavirus pulmonary syndrome are found throughout the Americas.

The etiologic agents of Lyme disease and hantavirus pulmonary syndrome were identified after disease outbreaks with unique clinical syndromes in small, well-circumscribed communities. It is clear that these microbes produced human disease long before their identification as pathogens. Why were these microbes undiscovered for so long? If a novel microbe causes sporadic disease, disease with unremarkable clinical features or only affects a few individuals in a large population, then there may be insufficient evidence to prompt a search for an infectious cause. If the deer mouse population of the Four Corners area of the USA had not increased in 1993, would we know about hantavirus pulmonary syndrome today? What other infectious agents lurk beneath our radar screen for detection?

TABLE 5-1 -- Infectious agents identified since 1980 and the diseases they cause.

INFECTIOUS AGENTS IDENTIFIED SINCE 1980 AND THE DISEASES THEY CAUSE	
Microbe	**Disease**
Helicobacter pylori	Peptic ulcer disease, gastric adenocarcinoma
Borrelia burgdorferi	Lyme disease
Tropheryma whipplei	Whipple's disease
Campylobacter cinaedi	Proctitis
Streptococcus iniae	Cellulitis
Bartonella henselae	Cat scratch disease, bacillary angiomatosis
Escherichia coli 0157:H7	Hemolytic-uremic syndrome
Campylobacter	Guillain-Barré syndrome
Anaplasma phagocytophaga	Human granulocytic 'ehrlichiosis'
Ehrlichia chaffeensis	Human monocytic ehrlichiosis

Rickettsia japonica	Spotted fever
Mycoplasma fermentans	Arthritis
Mycobacterium genavense	Disseminated mycobacteriosis in AIDS
HIV	AIDS
Human herpesvirus 6	Roseola infantum
Human herpesvirus 7	Unknown
Human herpesvirus 8	Kaposi's sarcoma
Human metapneumovirus	Respiratory tract infection
Nipah virus	Encephalitis
Hendra virus	Encephalitis
Hepatitis C virus	Hepatitis, hepatocellular carcinoma
Hepatitis E virus	Hepatitis
Cyclospora cayetanensis	Diarrhea
SARS coronavirus	Severe acute respiratory syndrome (SARS)

TABLE 5-2 -- Diseases of unknown etiology suspected to be caused by infectious agents.

DISEASES OF UNKNOWN ETIOLOGY SUSPECTED TO BE CAUSED BY INFECTIOUS AGENTS
Crohn's disease
Ulcerative colitis
Sprue
Necrotizing enterocolitis of newborns
Sclerosing cholangitis
Primary biliary cirrhosis
Cholelithiasis
Brainerd diarrhea
Scleroderma
Ankylosing spondylitis
Seal finger
Polymyositis
Polyarteritis nodosa
Rheumatoid arthritis
Systemic lupus erythematosus
Wegener's granulomatosus
Behçet's syndrome
Goodpasture's syndrome
Takayasu's arteritis
Eosinophilic pustular folliculitis
Sweet's syndrome
Psoriasis
Kawasaki's disease
Sarcoidosis
Kikuchi's disease
Multiple sclerosis
Schizophrenia
Obsessive-compulsive personality disorder
Diabetes mellitus
Cancer
Chronic fatigue syndrome
Atherosclerosis
Idiopathic pulmonary fibrosis
Bronchiolitis obliterans with organizing pneumonia
Idiopathic pneumonia syndrome
Still's disease
Malakoplakia
Bacterial vaginosis
Nephrolithiasis
Chronic culture-negative prostatitis

A novel paramyxovirus, human metapneumovirus, has been linked to respiratory tract infections in children.[7] The illness resembles respiratory syncytial virus infection and is caused by a phylogenetically closely related virus. Serologic evidence suggests that most people are infected with this virus at an early age. Thus, even though human metapneumovirus infection appears to be ubiquitous, the virus was only recently identified. Another novel paramyxovirus, Nipah virus, was recently linked to encephalitis and respiratory tract infections in pig farmers in Malaysia and Singapore.[8] Japanese encephalitis virus was initially suspected in the outbreak, but this hypothesis proved wrong. A syncytium-forming virus was eventually isolated from cell cultures of cerebrospinal fluid and the infection was identified as a porcine zoonosis. If novel microbes produce disease that mimics other well-known and endemic infectious agents, then their discovery may be impeded. When we encounter a patient with pneumonia or encephalitis who has a negative evaluation for infection, we virtually never push to find novel infectious agents but rather label the illness as idiopathic and move to our next case.

Helicobacter pylori and peptic ulcer disease: a new microbe for an old disease

Another barrier to the identification of microbial triggers of disease is the intellectual inertia that derives from entrenched theories of pathophysiology. Until the 1980s, peptic ulcer disease (PUD) was thought to be caused primarily by excessive gastric acid secretion. Interventions were thus aimed at reducing acid secretion by pharmacologic or surgical means. Although these interventions were usually successful, there was a high rate of disease relapse. In 1984 Marshall and Warren reported finding a unique spiral bacterium in a high percentage of patients with PUD.[9] The association of a novel bacterium with PUD was initially met with great skepticism. Although *H. pylori* was detected in most patients with PUD, it was also detected in patients without PUD, raising questions about the specificity of the association between bacterium and disease. The most compelling argument that *H. pylori* causes PUD comes from antibiotic treatment trials showing that eradication of *H. pylori* eliminates PUD, while bacterial persistence is associated with disease relapse. The shift in therapeutic focus from antiacid to antibacterial interventions has radically changed the diagnosis and management of PUD. Peptic ulcer disease is now curable with antibiotic therapy, and elective surgeries such as vagotomy, antrectomy and gastrectomy for PUD are now virtually obsolete.

Why was the association between *H. pylori* and PUD missed for so many years? *Helicobacter pylori* infection does not behave like other infectious diseases that are more familiar. Patients with *H. pylori* infection lack a prominent acute inflammatory response and the infection typically has a chronic course. If patients with *H. pylori* infection developed high fever, intense leukocytosis and abscess formation, then the infectious nature of PUD would have been more apparent. The fact that a bacterium can produce a chronic, subtle, stable infection emphasizes the need to expand our definition of what syndromes may constitute infectious diseases. *Helicobacter pylori* can be easily visualized in stomach tissue with light microscopy and conventional tissue stains and cultivated in the laboratory using conventional culture media in a micro-aerophilic environment. As noted by Martin Blaser, 'pathologists who now easily identify these organisms using such stains did not previously (before 1983) report and probably did not even see these organisms in histologic sections. Dogma dulls the senses ...'.[10] If a microbe such as *H. pylori* can escape our detection, then it may not be surprising that other more stealthy microbes associated with idiopathic diseases may also escape detection.

Elusive associations and hidden pathogens

If microbes cause some idiopathic diseases, why do they remain concealed? There are several reasons why microbe-disease associations may be missed. The first reason is the failure to consider the

hypothesis of infection. If a disease has metabolic or autoimmune features, then an infectious trigger may not be sought. As evidenced by *H. pylori* and PUD, we need to at least consider the possibility that diseases have an infectious cause, even when we think we understand the pathophysiology.

Second, microbes may initiate an inflammatory cascade but be absent when disease is manifest — a 'hit and run' model of disease causation. Rheumatic heart disease arising from group A streptococcal pharyngitis is an example. The hypothesis of molecular mimicry poses that antibodies directed against bacterial antigens also bind to tissue in the myocardium and heart valves, resulting in myocarditis or valvular heart disease. Disease may occur after *Streptococcus pyogenes* is cleared from the pharynx and is thought to be mediated by ongoing antigenic stimulation from shared epitopes. Trying to detect *S. pyogenes* bacteria in patients with old rheumatic heart disease would thus not be a successful strategy. When microbes have fled the scene of the crime, an immunologic 'fingerprint' may be the only evidence establishing a connection between microbe and disease.

Third, epidemiologic features of disease may mask associations with infectious agents. It is easiest to make an association between an uncommon microbe and a rare but unique clinical syndrome that occurs in all infected patients, such as seen with rabies virus infection and rabies encephalitis. In this setting, all patients who are infected develop disease (high infection 'penetrance'), and all patients who lack infection are free of disease. In contrast, when common microbes cause uncommon diseases, the association between microbe and disease may be obscured. For instance, about 90% of adults are seropositive for Epstein-Barr virus (EBV), but only a small fraction of patients with EBV infection develop EBV-associated malignancies such as nasopharyngeal carcinoma, Burkitt's lymphoma or Hodgkin's malignancy. Being seropositive for EBV does not substantially increase a person's risk for malignancy over that found in the general population because the background seroprevalence is so high. The rarity of these tumors suggests that genetic or additional environmental factors are also required for carcinogenesis. Infection with EBV may be necessary but not sufficient for tumor formation. Identifying rare outcomes associated with common infections thus presents a challenge.

In a similar fashion, the causal relationship between a common infection and a common disease may also be obscured. How would we determine whether human herpesvirus (HHV)-6 causes dementia — an entirely hypothetical proposition? It would be difficult to determine the causal relationship between this common, latent viral infection and a common neurodegenerative condition of the elderly.

Fourth, diseases may result from pathogenic microbial communities. Failure to identify all co-pathogens in the community will lead to spurious conclusions about disease causation. We are familiar with the paradigm in which each disease is caused by a single microbe. What if disease required the interaction of several microbes? In this setting, identification of a single pathogen may not reliably predict disease. The synergistic interaction between hepatitis D virus and hepatitis B virus is an example of this interaction. Hepatitis D virus is a defective virus that requires hepatitis B surface antigen for infectivity. Patients who have chronic stable hepatitis B infection can develop fulminant hepatitis after co-infection with hepatitis D. Bacterial communities may also cause disease. Periodontitis is a disease that has been linked to particular assemblages of pathogenic bacteria at the tooth surface. Some gastrointestinal diseases may be caused by aberrant microbial communities, including ulcerative colitis and Crohn's disease.

Fifth, conventional microbial detection technologies may miss unconventional microbes. Microbes are most likely to be associated with disease when they are present in high numbers, are visible with conventional stains, are large enough to be seen with light microscopy or are cultivated using conventional media. Novel microbes associated with still unexplained diseases likely do not possess these characteristics; the low-hanging fruit has been picked and the remaining fruit will require more work and some technical innovation to grasp a more elusive harvest. When colony-forming units of bacteria are compared with directly observed bacterial counts in environmental niches, only about 1% of bacteria are amenable to laboratory cultivation.[11] When the cultivated bacteria present in human associated niches such as the gut and mouth are compared with the representation of bacteria detected by analysis of bacterial nucleic acid sequences, a large percentage of uncultivated and previously undescribed bacteria are detected with sequence-based methods.[12] [13] These findings underscore the limitations of conventional methods of microbial detection and point the way toward finding novel microbes.

METHODS FOR NOVEL PATHOGEN DISCOVERY

For most idiopathic diseases, the application of conventional microbial detection methods such as cultivation, serology and microscopy has failed to reveal a microbial culprit. If microbes cause some idiopathic diseases, then their discovery will likely necessitate the use of unconventional methods, such as those relying on the detection of microbial nucleic acid sequences.[14] There are several advantages to the nucleic acid approach. All known infectious agents contain nucleic acids (with the possible exception of prions) and thus it should be possible to detect all pathogens. Each unique microbe has a unique complement of nucleic acid so it is possible to distinguish between microbes on the basis of these distinctive sequences and to identify novel microbes based on phylogenetic relationships inferred from shared nucleic acid sequences. Amplification methods, such as PCR, can be used to detect extremely small quantities of microbial nucleic acid, resulting in highly sensitive assays.

Several sequence-based methods have successfully been used to discover novel pathogens. Consensus sequence PCR was used to identify the cultivation-resistant bacterium that causes Whipple's disease (see below). Representational difference analysis was used to identify Kaposi's sarcoma-associated herpesvirus or HHV-8, the cause of Kaposi's sarcoma.[15] This method employs subtractive hybridization and PCR amplification to isolate nucleic acid sequences that are preferentially found in diseased tissue as compared with normal tissue. Microbial nucleic acid sequences can be enriched with this process, although the resulting nucleic acid sequences may not be phylogenetically informative or even derive from a microbe. Hepatitis C virus was discovered by screening cDNA expression libraries with serum from an affected patient.[16] Viral particles were concentrated from the blood of a chimpanzee experimentally infected with the non-A non-B hepatitis agent. Nucleic acid was extracted, cloned into an expression library and then probed with immune serum. From one million clones screened, a single reactive clone was identified and the sequence of this clone was used to identify other overlapping clones and to assemble the genome of hepatitis C, a member of the Flaviviridae.

Other methods for pathogen discovery will likely emerge based on the differential expression of microbial or host nucleic acid sequences in diseased tissue, or on the simultaneous detection of thousands of signature microbial nucleic acid sequences using microarrays (hybridization to high-density nucleic acids anchored to solid supports) or Luminex beads (hybridization to nucleic acids attached to coded beads in solution).

Pitfalls of pathogen discovery

Nucleic acid amplification methods can detect a single molecule of nucleic acid in a reaction. This strength is also a weakness. If billions

of molecules are created in a PCR reaction and a single molecule can produce a positive result, then there is great potential for contamination unless rigorous laboratory procedures are employed to prevent PCR product carry over. Laboratory contamination can also arise from other tissue samples, reagents or equipment. Although reagents and solutions may be sterile, these same solutions frequently contain microbial nucleic acid sequences. Even blood culture bottles contain bacterial and fungal nucleic acids that are likely derived from the media components.[17] Thus, laboratories employing methods for the detection of microbial nucleic acid sequences must adopt stringent measures to control contamination and should confirm results to avoid erroneous conclusions.

The challenge of making new microbe-disease links is illustrated by the association between HHV-8 and sarcoidosis. Sarcoidosis is a systemic inflammatory disease of unknown etiology that is suspected to have an infectious cause. Human herpesvirus-8 is a recently discovered human herpesvirus that causes Kaposi's sarcoma, but may cause other diseases. In 1997 a research team reported finding evidence of HHV-8 DNA in 97% of tissue samples from patients with sarcoidosis, but only 6% of tissue samples from subjects without sarcoidosis.[18] This association was highly statistically significant, with a p value of <0.0001. A nested PCR assay was used to detect HHV-8, a protocol that is prone to contamination from previously amplified PCR products. At first glance, this study gives compelling evidence that HHV-8 is associated with sarcoidosis. Many control samples were processed and found to be negative by HHV-8 PCR and results of the first PCR assay were mostly confirmed in a second PCR assay employing a different HHV-8 gene target. Nevertheless, at least seven laboratories from different countries were not able to confirm the association between HHV-8 and sarcoidosis.[19] Multiple PCR and serologic assays were used to assess HHV-8 infection in sarcoid tissues/sera and the results were strikingly negative. The association between HHV-8 infection and sarcoidosis thus appears to be spurious. This saga demonstrates the potential for rounding up the wrong suspects in our search for etiologic agents of disease. To build a strong case for causation, data should be collected using Hill's criteria for causal association as a guide and results should be reproducible in other laboratories.[14]

MULTIFACTORIAL CAUSATION AND INFECTIOUS DISEASES

For many diseases, our understanding of the causal factors is incomplete. In order to investigate the elements that may contribute to disease production, various measurable risk factors are analyzed to determine whether there is a relationship between exposure to the risk factor and disease. When a risk factor is identified, intervention measures are proposed to reduce its impact. Unfortunately, risk factors may have little or nothing to do with causation. In the case of infectious diseases, easily measured risk factors may overlook occult infectious agents. For example, several studies showed that use of the recreational drug amyl nitrate (poppers) was a risk factor for the development of AIDS. Does amyl nitrate cause AIDS? No, but amyl nitrate use may facilitate risk-taking behaviors that lead to transmission of HIV, which causes AIDS. If intervention studies rely only on risk factor analysis and do not attempt to interrupt the proximate causes of disease, then our efforts at prevention and treatment will be hobbled.

The same caveat is true for atherosclerotic coronary artery disease (CAD). Conventional risk factors associated with CAD include hypertension, smoking, diabetes, hyperlipidemia and family history, but do these factors cause CAD or are they like amyl nitrate and fail to identify the proximate cause of disease? Infectious agents such as *Chlamydia pneumoniae* and cytomegalovirus have been proposed to cause or contribute to CAD (see below). The presence of conventional risk factors for CAD in no way undercuts the validity of the infectious hypothesis.

Many infectious diseases have a swift onset, a short course and a single microbial cause. In contrast, we tend to think of chronic diseases as having a slow onset, a prolonged course and multifactorial causation. These differences are reflected in the different approaches to the epidemiology of infectious versus chronic diseases. For many people, the fact that a disease has multifactorial causation implies that a disease is not infectious. Lewis Kuller wrote, 'The concept of multifactorial etiology of many chronic diseases may be a measure of our ignorance of causality rather than a biological principle'.[20] In fact, all infectious diseases have multifactorial causation. Infectious diseases require a pathogenic microbe, a susceptible host, a suitable environment for transmission and for some diseases a vector. If any of these elements are lacking, disease will not occur.

Whipple's disease: knowing the unknown

The scientific evolution of Whipple's disease from a metabolic disorder to an infectious disease is informative. Whipple's disease is named after the pathologist George Whipple who described a peculiar wasting illness in 1907. [21] The patient was a 36-year-old physician who presented to Johns Hopkins Hospital with complaints of weight loss, abdominal pain, bloating, diarrhea, cough and arthritis. The illness started 5 years prior to presentation with arthritis that affected large joints, causing pain, redness and swelling. These attacks were transient (<24 hours), recurrent and affected almost every joint in succession. In hospital, the patient was noted to have a low-grade fever, abdominal swelling, arthritis and steatorrhea. Tuberculosis or cancer was suspected. He underwent an exploratory laparotomy that revealed mesenteric adenopathy and he died without a diagnosis 2 days later. At autopsy, Whipple noted foamy histiocytes and fat deposits in tissue sections, concluded that the illness results from a derangement in fat metabolism and named it intestinal lipodystrophy. However, upon examination of a silver-stained section of lymph node, Whipple also noted 'great numbers of a rod-shaped organism (?) which, with this stain, is about the diameter of the spirochete of syphilis ...'.[21] Attempts to culture the bacterium failed and the observation of these bacillary structures was ignored for almost 50 years.

Steroid treatment was noted to provide some relief from symptoms of Whipple's disease, but this treatment did not appear to change the ultimate fatal outcome. In 1952, Paulley published a report describing the successful treatment of intestinal lipodystrophy with chloramphenicol.[22] The introduction of antibiotic therapy turned Whipple's disease from an invariably fatal metabolic illness into a frequently curable infectious disease. In the 1960s, electron microscopic studies of Whipple's disease tissues demonstrated small monomorphic bacterial structures inside phagocytes and free in the extracellular spaces. Attempts to culture this bacterium failed.

How can one identify a bacterium when it resists attempts at laboratory propagation? The bacillus of Whipple's disease was identified in the 1990s by molecular phylogenetic analysis of its DNA.[23] Oligonucleotide primers that anneal to highly conserved sequence targets in the bacterial 16S rRNA gene were used in a PCR to amplify bacterial rDNA from infected tissue. The amplified rDNA sequence included regions that are highly polymorphic among the bacteria and allow one to infer evolutionary relationships. Whipple's disease tissues contained a unique 16S rDNA sequence from a novel bacterium and phylogenetic analysis grouped this organism with the actinobacteria. The Whipple bacillus has been named *Tropheryma whipplei*. Fluorescence in-situ hybridization has been used to show that a probe targeting the Whipple bacillus rRNA localizes to bacterial structures in affected tissue and most bacteria are found in the

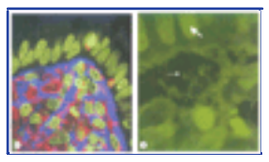
Figure 5-1 Fluorescence micrographs of a formalin fixed intestinal biopsy from a patient with Whipple's disease. (a) Confocal micrograph demonstrating hybridization of a fluorescent *Tropheryma whipplei* rDNA probe (blue) to bacterial rRNA in a small intestine biopsy from a patient with Whipple's disease. Yo-pro dye (green) highlights cell nuclei and an anti-vimentin antibody (red) labels the cytoskeletal protein of human mesenchymal cells. Bacteria localize to the lamina propria and appear abundant in the extracellular spaces. Macrophages and polymorphonuclear leukocytes infiltrate the lamina propria of this enlarged villus. (b) Confocal micrograph of Whipple's disease intestine showing clumps of small bacillary bodies in the lamina propria stained with Yo-pro nucleic acid dye in green (small arrow), and a human epithelial cell nucleus (large arrow). The extremely small size of the Whipple bacillus makes detection difficult using standard methods.

extracellular spaces of the intestinal lamina propria (Fig. 5.1).[24] Over 90 years after George Whipple's description of bacteria in tissue, *T. whipplei* was successfully propagated in the laboratory by co-cultivation with human cells. The remarkably slow doubling time of this bacterium makes routine cultivation impractical and growth on axenic media has not been reported.[25]

Whipple's disease has much to teach us about the infectious causes of idiopathic diseases. The first manifestation of Whipple's disease is frequently a migratory polyarthritis that waxes and wanes. These arthritic symptoms may precede the other manifestations of Whipple's disease by decades. Some infectious diseases may have a prolonged disease course. Whipple's disease can affect any organ and produces a multiplicity of presentations, from isolated gastrointestinal symptoms to isolated neuropsychiatric symptoms or multisystem disease. Some infectious diseases are protean. Although patients with Whipple's disease may improve with steroid treatment, cure is not achieved. A response to steroid medications does not mean a disease is noninfectious. Patients with Whipple's disease usually require a year of antibiotic treatment and relapse is common. Conventional antibiotic courses do not easily eradicate some slow-growing microbes.

EVIDENCE OF MICROBIAL CULPABILITY IN UNEXPLAINED DISEASE: ATHEROSCLEROSIS

Atherosclerosis is the leading cause of mortality in developed countries. Conventional risk factors such as hyperlipidemia and smoking only account for about 50% of atherosclerosis cases. Infectious agents have been proposed as factors that may account for the remaining cases of disease. Recent studies have demonstrated that patients with CAD have circulating markers of inflammation, including elevated levels of C-reactive protein, interleukin-6, tumor necrosis factor a and other cytokines and chemokines.[26] [27] It is believed that the anti-inflammatory properties of aspirin may be responsible for some of its benefits in CAD treatment. If CAD is an inflammatory disease, what is causing this inflammation?

The role of infectious agents in atherosclerosis and the attendant vascular inflammation is a controversial and active area of investigation. Several microbes have been implicated in atherogenesis, including *Chlamydia pneumoniae*, cytomegalovirus and *Helicobacter pylori*, among others. Evidence that *C. pneumoniae* plays a role in atherosclerosis is the most compelling. The first evidence that CAD was linked to *C. pneumoniae* infection came from studies showing that patients with CAD were more likely to have serologic evidence of infection than controls without disease.[28] *Chlamydia pneumoniae* has been detected in atherosclerotic plaque by immunohistochemistry, electron microscopy, PCR, in-situ hybridization and culture. Unfortunately, there is great variability in the reported success rates for detecting *C. pneumoniae* in diseased vascular tissue samples, with rates between 0% and 100% noted. A study of PCR for the detection of *C. pneumoniae* found discrepant results from different laboratories when the same samples were tested. [29] Thus, results are difficult to interpret and compare without standardized diagnostic methods.[30]

In-vitro studies have shown that *C. pneumoniae* can promote adherence of monocytes to vascular endothelium, increase the expression of inflammatory cytokines/chemokines, induce foam cell formation and promote low-density lipoprotein oxidation — all features of atherosclerosis. Animal models of atherosclerosis have yielded additional data supporting a causal role for *C. pneumoniae*. For instance, in a rabbit model, *C. pneumoniae* infected vascular endothelial cells and fostered the release of platelet-derived growth factor.[31] These events were associated with vascular smooth muscle cell proliferation and intimal thickening — histologic hallmarks of atherosclerosis. Early treatment of rabbits with antimicrobial agents active against *C. pneumoniae* can reduce the incidence and extent of atherosclerosis.

The best evidence that *C. pneumoniae* causes atherosclerosis would come from intervention trials showing that the prevention or treatment of *C. pneumoniae* infection reduces the incidence and severity of atherosclerosis in humans. Several small trials have demonstrated some moderate benefit from antibiotic treatment. For instance, a trial comparing 28 days of treatment with roxithromycin versus placebo in men seropositive for *C. pneumoniae* who had peripheral arterial occlusive disease found that antibiotic-treated patients required fewer revascularization procedures, had greater exercise tolerance and experienced reductions in the size of carotid plaques.[32] Some small studies of macrolide therapy in patients with CAD have demonstrated reductions in coronary events for patients treated with antibiotics, but other studies have not been as convincing. Larger trials such as the WIZARD and ACES studies are

underway and may provide the statistical power to determine whether antibiotic therapy is useful in CAD.[33]

There are several caveats to the interpretation of antibiotic intervention trials for atherosclerosis. It may be unreasonable to assume that antibiotic treatment will reduce vascular events in patients with long-standing and advanced disease. If *C. pneumoniae* plays a role in atherogenesis, then eradication of viable bacteria at this stage of disease may not reverse years of smooth muscle proliferation, intimal thickening, calcification and vessel obstruction. The greatest effect from antibiotics is likely to be realized when administered to patients with evidence of early *C. pneumoniae* infection and multiple risk factors for atherosclerosis.

In addition, atherosclerosis is a disease with some autoimmune features. One hypothesis suggests that the immune response to *C. pneumoniae* heat shock protein 60 may induce cross-reactive immune responses to human heat shock proteins in vascular endothelium. Indeed, a study found that antibodies to both *C. pneumoniae* and heat shock protein 60 conferred a relative risk of 82 for the development of CAD.[34] In contrast, individual conventional risk factors for CAD confer relative risks for disease that are generally below 10. Thus, elimination of bacteria may not be sufficient to quench inflammation if human epitopes continue to fan the flames (microbial 'hit and run' — see above).

Finally, conventional courses of antibiotic therapy may not be optimal for eradication of *C. pneumoniae* in atherosclerotic lesions. For instance, an in-vitro study showed that a 30-day treatment course with azithromycin, clarithromycin or levofloxacin did not eliminate bacteria from *C. pneumoniae*-infected cells.[35] Accordingly, one should not be surprised if antibiotic treatment regimens employing short courses of antibiotics fail to impact on atherogenesis. Antibiotic treatment courses designed for acute infections may not be successful in the setting of chronic infections. We need to study which antibiotics (or combinations of antibiotics), doses, intervals and durations are most effective in eradicating chronic *C. pneumoniae* infection before we can conclude that antibiotic therapy is not effective in atherosclerosis.

CONCLUSION

Some diseases that were previously considered to be metabolic, neoplastic, autoimmune or idiopathic have been linked to specific pathogens and reclassified as infectious diseases. There remain many unexplained diseases that may be caused by infectious agents. If we are to identify occult infectious causes of disease, then we must be open to the hypothesis of infection, while demanding rigorous evidence of microbial complicity. Although the search for microbial triggers of disease presents significant challenges, it also provides unmatched opportunities for improved diagnosis, prevention and treatment. Not all diseases are infectious, but only a fool would think that we have discovered all infectious diseases.

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Chapter 6 - Bioterrorism and Biodefense

Andrew W Artenstein

INTRODUCTION

Bioterrorism can be broadly defined as the deliberate use of microbial agents or their toxins as weapons against noncombatants outside the setting of armed conflict. The broad scope and mounting boldness of worldwide terrorism, exemplified by the massive attacks on New York City and Washington DC on 11 September 2001, coupled with recent revelations regarding the apparent willingness of terrorist organizations to acquire and deploy biological weapons, constitute ample evidence that the specter of bioterrorism will pose a persistent global threat.

Biological weapons have been used against both military and civilian targets throughout history. In the 14th century, Tatars attempted to use epidemic disease against the defenders of Kaffa by catapulting plague-infected corpses into the city.[1] British forces gave Native American tribespeople blankets from a smallpox hospital in an attempt to affect the balance of power in the Ohio River Valley in the 18th century.[1] In addition to their well-described use of chemical weapons, German forces purportedly infected livestock with anthrax and glanders to weaken Allied initiatives during World War I. Perhaps the most egregious period in the history of biological weaponry involved the Japanese program in Manchuria from 1932 to 1945. Based on survivor accounts and confessions of Japanese participants, thousands died as a result of experimental infection with a multitude of virulent pathogens at Unit 731, the code name for the biological weapons facility there.[2]

The USA maintained an active program for the development and testing of offensive biological weapons from the early 1940s until 1969, when the program was terminated by executive order of then President Nixon, although efforts continue with regard to countermeasures against biological weapons. The Convention on the Prohibition of the Development, Production, and Stockpiling of Biological and Toxin Weapons and on Their Destruction (Biological Weapons Convention; BWC), which was ratified in 1972, formally banned the development or use of biological weapons, with enforcement the responsibility of the United Nations.[1] Unfortunately, the BWC has not been effective in its stated goals; multiple signatories, including the former Soviet Union and Iraq, have violated the terms and spirit of the agreement. The accidental release of aerosolized anthrax spores from a military plant in Sverdlovsk in 1979, with at least 68 human deaths from inhalational anthrax reported downwind, was proved years later to have occurred in the context of Soviet offensive weapons production.

ASSESSMENT OF THREAT

Biological agents are considered weapons of mass destruction (WMD) because, as with certain conventional weapons, chemical weapons and nuclear weapons, their use may result in large-scale morbidity and mortality. In a World Health Organization (WHO) assessment model of the hypothetical effects of the release of 50g of aerosolized anthrax spores upwind from a population center of 500,000, it was estimated that the agent would concentrate in excess of 20km downwind and that nearly 85,000 people would be killed or incapacitated by the event.[3] Biological weapons also possess unique properties among WMD. Unlike other forms of WMD, biological agents are associated with a clinical latency period, in most cases, of days to weeks, during which time early detection is quite difficult. In addition, specific antimicrobial therapy and, in select circumstances, vaccines are available for the treatment and prevention of illness caused by biological weapons. In contrast, casualties from other forms of WMD can be treated only by decontamination, trauma mitigation and supportive care.

Nations adhering to democratic principles are vulnerable to bioterrorism because of the inherent freedoms that their citizens and visitors enjoy. Freedom of movement and access to public and private institutions can be exploited by rogue nations, terrorist organizations or malicious people acting individually and intent on untoward acts. This, coupled with worldwide cultural tensions, geopolitical conflicts and economic instability, provides fertile ground for terrorism.

Recent events have established bioterrorism as a credible and ubiquitous threat. The intentional contamination of restaurant salad bars with *Salmonella* spp. by a religious cult trying to influence a local election in The Dalles, Oregon in 1984,[4] the revelations that Aum Shinrikyo (the Japanese cult that released sarin nerve agent in the Tokyo subway system in 1995) had unsuccessfully experimented on multiple occasions with spraying anthrax from downtown rooftops before their successful chemical attack, and the findings of the United Nations weapons inspectors of massive quantities of weaponized biological weapons in Iraq during the GulfWar and its aftermath[5] served as sentinel warnings of a shift in terrorism trends. The anthrax attacks in the USA in October and November 2001, following the catastrophic events of 11 September, elevated bioterrorism to the fore of the international dialogue.

The aims of bioterrorism are those of terrorism in general: morbidity and mortality among civilian populations, disruption of the societal fabric and exhaustion or diversion of resources. A 'successful' outcome, from a terrorist standpoint, may be achieved without furthering all of these aims. The anthrax attacks in 2001 evoked fear and anxiety and diverted resources from other critical activities despite the limited number of casualties. Biological weapons offer other, significant advantages to terrorists: they are relatively inexpensive compared with conventional or nuclear weaponry; they can be deployed in a stealth fashion, owing to a variable clinical latency period, thus allowing the perpetrator an opportunity to escape if desired; and they clearly evoke anxiety and panic that is, in some instances, out of proportion to their physical effects. From a rogue government's standpoint, the technology for bioterrorism is 'dual use' in that it can serve legitimate functions, such as the production of vaccine or pharmaceutical agents, as readily as it can serve the production of biological weapons, thus making detection by inspectors all the more difficult.

To be employed in large-scale bioterrorism, biological agents must undergo complex processes of production, cultivation, chemical modification and weaponization. For these reasons, state sponsorship

or direct support from governments or organizations with significant resources, contacts and infrastructure would predictably be required in large-scale events. However, recent revelations have suggested that some agents may be available on the worldwide black market and in other illicit settings,[6] thus obviating the need for the production process. Although an efficient mode of delivery has traditionally been felt to be necessary, the anthrax attacks in the USA in late 2001 illustrated the devastating results that can be achieved with relatively primitive delivery methods such as high-speed mail sorting equipment and mailed letters.

Numerous attributes contribute to the selection of a pathogen as a biological weapon, including availability or ease of large-scale production, ease of dissemination (usually by the aerosol route), stability of the product in storage, in weapons and in the environment (biological entities differ in their physical properties), cost and clinical virulence. Clinical virulence refers to the reliability with which the pathogen causes high mortality, morbidity or social disruption. The Centers for Disease Control and Prevention (CDC) have prioritized biological agent threats on the basis of the characteristics mentioned above,[7] and this has influenced current preparedness strategies (Table 6.1). Category A agents are associated with high mortality and the greatest potential for major impact on public health. Category B agents are considered 'incapacitating' because of their potential for moderate morbidity but relatively low mortality. Most of the category A and B agents have been experimentally weaponized in the past and thus of proven feasibility. Category C agents include emerging threats and pathogens that may be available for development.

Another factor that must be addressed in assessing future bioterrorism risk is the historic track record of experimentation with specific pathogens, an area that has been informed from the corroborated claims of various high-level Soviet defectors and data released from the former offensive weapons programs of the USA and the UK.[1] [6] [8] It is apparent, from these sources combined with the burgeoning fields of molecular biology and genomics, that future risk scenarios may have to contend with genetically altered and 'designer' pathogens. To this end a miscellaneous grouping of potential threat agents to the extant CDC categories can be added (see Table 6.1). The most cautious approach to assessing risk may be to remain open to additional, novel possibilities.

BIOTERRORISM RECOGNITION

By definition bioterrorism is insidious. In the absence of an advance warning or specific intelligence information, clinical illness will manifest itself before the circumstances of a release event are known. For this reason, health care providers are likely to be the first responders to this form of terrorism. This is in contrast to the more familiar scenarios in which police, firefighters, paramedics and other emergency personnel are deployed to the scene of an attack by conventional weaponry or a natural disaster. Physicians and other health care workers must therefore maintain a high index of suspicion of bioterrorism and recognize suggestive epidemiologic clues and clinical features to enhance early recognition and to guide initial management of casualties. This remains the most effective way of minimizing the deleterious effects of bioterrorism on individual patients and on public health.

Early recognition is hampered for multiple reasons. It is likely that the circumstances of any event will only be known in retrospect, and therefore it may prove problematic to discern the extent of exposure immediately. Terrorists have an unlimited number of targets in most open, democratic societies and it is unrealistic to expect that, without detailed intelligence data, all of these can be secured at all times. Certain sites, such as government institutions,

TABLE 6-1 -- Agents of concern for use in bioterrorism.

AGENTS OF CONCERN FOR USE IN BIOTERRORISM	
Highest priority (category A)	
Microbe or toxin	**Disease**
Bacillus anthracis	Anthrax
Variola virus	Smallpox
Yersinia pestis	Plague
Clostridium botulinum	Botulism
Francisella tularensis	Tularemia
Filoviruses	Ebola hemorrhagic fevers, Marburg disease
Arenaviruses	Lassa fever, South American hemorrhagic fevers
Bunyaviruses	Rift Valley fever, Congo-Crimean hemorrhagic fevers
Moderately high priority (category B)	
Microbe or toxin	**Disease**
Coxiella burnetti	Q fever
Brucella spp.	Brucellosis
Burkholderia mallei	Glanders
Alphaviruses	Viral encephalitides
Ricin	Ricin intoxication
Staphylococcus aureus enterotoxin B	Staphylococcal toxin illness
Salmonella spp., *Shigella dysenteriae*, *Escherichia coli* O 157:H7, *Vibrio cholerae*, *Cryptosporidium parvum*	Foods and water-borne gastroenteritis
Category C	
Microbe or toxin	**Disease**
Hantaviruses	Viral hemorrhagic fevers
Flaviviruses	Yellow fever
Mycobacterium tuberculosis	Multidrug resistant tuberculosis
Miscellaneous	
Genetically engineered vaccine- and/or antimicrobial-resistant category	
A or B agents	
HIV-1	
Adenoviruses	
Influenza	
Rotaviruses	
Hybrid pathogens (e.g. smallpox-plague, smallpox-ebola)	

historic landmarks or large public events, may be predictable targets, but there are other, less predictable possibilities. Metropolitan areas are considered vulnerable, but owing to the expansion of suburbs and the increased number of commuters, and also to the clinical latency period between exposure and symptoms inherent with biological agents, casualties of bioterrorism are likely to present for medical attention in diverse locations and at varying times after a common exposure. An event in New York City on a Wednesday morning may result in clinically ill people presenting over the ensuing weekend to a variety of emergency rooms within a 60-mile radius. Additionally, modern modes of transportation ensure that there will also be affected people thousands of miles away at both

national and international locations. This adds layers of complexity to an already complicated setting and illustrates the critical importance of surveillance and real-time communication in this setting.

Further hindrance to the early recognition of bioterrorism comes from the fact that initial symptoms may be nondiagnostic. In the absence of a known exposure, many symptomatic people may not seek medical attention early on or may be misdiagnosed as having a flu-like illness. Once beyond the early stages many of these illnesses progress quite rapidly and treatment may be less successful. Most of the diseases caused by agents of bioterrorism are rarely, if ever, seen in clinical practice. Physicians are, therefore, likely to be inexperienced with their clinical presentation. Additionally, these agents, by definition, will have been laboratory-manipulated and may not present with the classic clinical features of naturally occurring infection. This was dramatically illustrated by some of the inhalational anthrax cases in the USA in October 2001.[9]

Early recognition of bioterrorism is facilitated by the recognition of epidemiologic and clinical clues. Clustering of patients with common symptoms and signs, especially if these are unusual or characteristic of bioterrorism agents, is suggestive and should prompt expeditious notification of local public health authorities. This approach will also lead to the recognition of outbreaks of naturally occurring disease or emerging pathogens. The recognition of a single case of a rare or nonendemic infection, in the absence of a travel history or other potential natural exposure, should raise the specter of bioterrorism and should prompt notification of public health authorities. Finally, unusual patterns of disease, such as concurrent illness in human and animal populations, should raise suspicions of bioterrorism or another form of emerging infection.

Infectious diseases specialists are uniquely suited to play pivotal roles in the recognition, investigation and mitigation of bioterrorism. This role is based on an understanding of epidemiologic principles and risk assessment, expertise in specific threat agents and their clinical presentations and diagnostic approaches, knowledge of communicability and infection control principles, and an understanding of the tenets of infection treatment and prophylaxis. Nonetheless, an effective response to bioterrorism requires co-ordination of the medical system at all levels, from the community physician to the tertiary care center, with public health, emergency management and law enforcement infrastructures all playing a part.

THREAT AGENTS

This section covers the biological threat agents felt to be of major current concern, largely the CDC category A agents. Extensive

TABLE 6-2 -- Infection control issues for selected agents of bioterrorism.

INFECTION CONTROL ISSUES FOR SELECTED AGENTS OF BIOTERRORISM			
Disease	**Incubation period (days)**	**Person-to-person transmission**	**Infection control precautions**
Inhalational anthrax (see Chapter 185)	2–43*	No	Standard
Botulism (see Chapter 25)	12–72 hours	No	Standard
Primary pneumonic plague (see Chapter 176)	1–6	Yes	Droplet
Smallpox (see Chapter 151)	7–17	Yes	Contact and airborne
Tularemia (see Chapter 177)	1–14	No	Standard
Viral hemorrhagic fevers (see Chapter 183)	2–21	Yes	Contact and airborne
Viral encephalitides (see Chapter 23)	2–14	No	Standard
Q fever (see Chapter 235)	2–14	No	Standard
Brucellosis (see Chapter 180)	5–60	No	Standard
Glanders	10–14	No	Standard

* Based on limited data from human outbreaks; experimental animal data support clinical latency periods of up to 100 days

coverage of specific pathogens can be found in related chapters in this text (cross-referenced in Table 6.2) and in other sources.[10] Data concerning clinical incubation periods, transmission characteristics and infection control procedures for agents of bioterrorism are provided in Table 6.2 . Syndromic differential diagnoses for select clinical presentations are detailed in Table 6.3 .

Anthrax

Anthrax results from infection with *Bacillus anthracis*, a Gram-positive, spore-forming, rod-shaped organism that exists in its host as a vegetative bacillus and in the environment as a spore (see chapter 185 and chapter 226). In nature, anthrax is a zoonotic disease of herbivores that is prevalent in many geographic regions; sporadic human disease results from environmental or occupational contact with endospore-contaminated animal products.[11] The cutaneous form of anthrax is the most common presentation; gastrointestinal and inhalational forms are exceedingly rare in naturally acquired disease. Cutaneous anthrax occurred regularly in the first half of the 20th century, in association with contaminated hides and wools used in the garment industry, but it is uncommonly seen in current-day industrialized countries, owing to import restrictions. The last known case of naturally occurring inhalational anthrax in the USA occurred in 1976.[12]

It was previously hypothesized that large-scale bioterrorism with anthrax would involve aerosolized endospores with resultant inhalational disease, but the recent attacks in the USA illustrate the difficulties in predicting modes and outcomes in bioterrorism; the attacks were on a relatively small scale and nearly 40% of the confirmed cases were of the cutaneous variety.[13] The serious morbidity and mortality, however, were related to inhalational disease, as was the case in the Sverdlovsk outbreak in 1979. Therefore, planning for larger scale events with aerosolized agent seems warranted.

The lesion of cutaneous anthrax may be similar in appearance to other lesions, including cutaneous forms of other agents of bioterrorism; however, it may be distinguished by epidemiologic as well as certain clinical features (see Table 6.3). Anthrax is traditionally a painless lesion, unless secondarily infected, and it is associated with significant local edema. The bite of *Loxosceles reclusa*, the brown recluse spider, shares many of the local and systemic features of anthrax but is typically painful from the outset and lacks significant edema.[14] Cutaneous anthrax is associated with systemic disease, and the mortality is up to 20% of untreated cases, although with appropriate antimicrobial therapy the mortality is less than 1%.[13]

Once the inhaled endospores reach the terminal alveoli of the lungs, generally requiring particle sizes of 1–5µm, they are phagocytosed by macrophages and transported to regional lymph

TABLE 6-3 -- Clinical presentations and syndromic differential diagnoses of selected agents of bioterrorism.

PRESENTATIONS AND DIFFERENTIAL DIAGNOSES OF BIOTERRORISM AGENTS		
Clinical presentation	**Disease**	**Differential diagnosis**
Non-specific 'flu-like' symptoms with nausea, emesis, cough with or without chest discomfort, without coryza or rhinorrhea, leading to abrupt onset of respiratory distress with or without shock, mental status changes, with chest radiograph abnormalities (wide mediastinum, infiltrates, pleural effusions)	Inhalational anthrax	Bacterial mediastinitis, tularemia, Q fever, psittacosis, Legionnaires' disease, influenza, *Pneumocystis carinii* pneumonia, viral pneumonia, ruptured aortic aneurysm, superior vena cava syndrome, histoplasmosis, coccidioidomycosis, sarcoidosis
Pruritic, painless papule, leading to vesicle(s), leading to ulcer, leading to edematous black eschar with or without massive local edema and regional adenopathy and fever, evolving over 3–7 days	Cutaneous anthrax	Recluse spider bite, plague, staphylococcal lesion, atypical Lyme disease, orf, glanders, tularemia, rat-bite fever, ecthyma gangrenosum, rickettsialpox, atypical mycobacteria, diphtheria
Rapidly progressive respiratory illness with cough, fever, rigors, dyspnea, chest pain, hemoptysis, possible gastrointestinal symptoms, lung consolidation with or without shock	Primary pneumonic plague	Severe community-acquired bacterial or viral pneumonia, inhalational anthrax, inhalational tularemia, pulmonary infarct, pulmonary hemorrhage
Sepsis, disseminated intravascular coagulation, purpura, acral gangrene	Septicemic plague	Meningococcemia; Gram-negative, streptococcal, pneumococcal or staphylococcal bacteremia with shock; overwhelming postsplenectomy sepsis; acute leukemia; Rocky Mountain spotted fever; hemorrhagic smallpox; hemorrhagic varicella (in immunocompromised patients)
Fever, malaise, prostration, headache, myalgias followed by development of synchronous, progressive papular leading to vesicular and then pustular rash on face, mucous membranes (extremities more than the trunk); the rash may become generalized, with a hemorrhagic component and systemic toxicity	Smallpox	Varicella, drug eruption, Stevens-Johnson syndrome, measles, secondary syphilis, erythema multiforme, severe acne, meningococcemia, monkeypox (with African travel history), generalized vaccinia, insect bites, Coxsackie virus infection, vaccine reaction
Non-specific flu-like, febrile illness with pleuropneumonitis, bronchiolitis with or without hilar lymphadenopathy; variable progression to respiratory failure	Inhalational tularemia	Inhalational anthrax, pneumonic plague, influenza, mycoplasma pneumonia, Legionnaire's disease, Q fever, bacterial pneumonia
Acute onset of afebrile, symmetric, descending flaccid paralysis that begins in bulbar muscles, dilated pupils, diplopia or blurred vision, dysphagia, dysarthria, ptosis, dry mucous membranes, leading to airway obstruction with respiratory muscle paralysis. Clear sensorium and absence of sensory changes	Botulism	Myasthenia gravis, brain stem cerebrovascular accident, polio, Guillain-Barré syndrome variant, tick paralysis, chemical intoxication

Acute onset fevers, malaise, prostration, myalgias, headache, gastrointestinal symptoms, mucosal hemorrhage, altered vascular permeability, disseminated intravascular coagulation, hypotension, leading to shock, with or without hepatitis and neurologic findings	Viral hemorrhagic fever	Malaria, meningococcemia, leptospirosis, rickettsial infection, typhoid fever, borrelioses, fulminant hepatitis, hemorrhagic smallpox, acute leukemia, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, systemic lupus erythematosus

nodes, where they germinate into vegetative bacteria and, subsequently, disseminate hematogenously.[12] Spores may remain latent in the host for extended periods of time — up to 100 days in experimental animal exposures.[11] This has translated into prolonged clinical incubation periods following exposure to endospores. Cases of inhalational anthrax occurred up to 43 days after exposure in the Sverdlovsk experience, although the average incubation period is 2–10 days, perhaps influenced by exposure dose.[11] [12]

Before the anthrax attacks on the USA in October 2001, most of the clinical data concerning inhalational anthrax derived from Sverdlovsk, the largest recorded outbreak. More detailed data are available from the recent experience in the USA. There were 11 confirmed cases of inhalational anthrax, five (45%) of whom died. Although this contrasts with a case-fatality rate of more than 85% reported from Sverdlovsk, the reliability of reported data from that outbreak are questionable. [11] Patients almost uniformly present an average of 3.3 days after symptom onset with fevers, chills, malaise, myalgias, nonproductive cough, chest discomfort, dyspnea, nausea or vomiting, tachycardia, peripheral neutrophilia and liver enzyme elevations.[9] [15] Many of these findings are nondiagnostic and overlap considerably with those of influenza and other common viral respiratory tract infections (see Table 6.3). Recently compiled data suggest that shortness of breath, nausea and vomiting are significantly more common in anthrax whereas rhinorrhea is uncommon but is noted in the majority of viral respiratory infections.[16]

Other common clinical manifestations of inhalational anthrax include abdominal pain, headache, mental status abnormalities and hypoxemia. Abnormalities on chest radiography appear to be universally present, although these may be identified only retrospectively in some cases. Pleural effusions are the most common abnormality; infiltrates, consolidation and mediastinal adenopathy or widening are noted in the majority (Fig. 6.1). Mediastinal abnormality is felt to be an early indicator of disease, but computerized tomography appears to provide greater sensitivity than chest radiographs for this finding.

The clinical manifestations of inhalational anthrax generally evolve to a fulminant septic picture with progressive respiratory failure. *Bacillus anthracis* is routinely isolated in blood cultures if

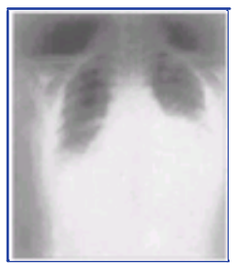

Figure 6-1 Imaging abnormalities associated with anthrax. (a) Chest radiograph demonstrating widened mediastinum due to inhalational anthrax. *Courtesy CDC and Dr PS Brachman.*

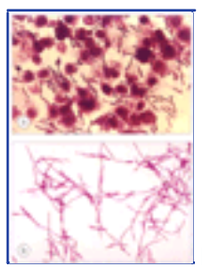

Figure 6-2 *Bacillus anthracis.* (a) *Bacillus anthracis* appearing as Gram-positive bacilli. (b) The typical 'jointed bamboo-rod' appearance of the organism from blood cultures. *Courtesy CDC and Dr William A Clark.*

the blood is obtained before the initiation of antimicrobial agents (Fig. 6.2). Pleural fluid is typically hemorrhagic; the bacteria can either be isolated in culture or documented by antigen-specific immunohistochemical stains of this material in the majority of patients.[9] In the five fatalities in the USA series, the average time from hospitalization until death was 3 days (range 1–5 days), consistent with other reports of the clinical virulence of this infection. Autopsy data typically reveal hemorrhagic mediastinal lymphadenitis and disseminated, metastatic infection. Pathology data from the Sverdlovsk outbreak confirm meningeal involvement, typically hemorrhagic meningitis, in 50% of cases.[17]

The diagnosis of inhalational anthrax should be entertained in the setting of a consistent clinical presentation in the context of a known exposure, a possible exposure or epidemiologic factors suggesting bioterrorism (e.g. clustered cases of a rapidly progressive illness). The diagnosis should also be considered in a single patient with a consistent or suggestive clinical illness in the absence of another etiology. The early recognition and treatment of inhalational anthrax is likely to be associated with a survival advantage.[9] Therefore, prompt empiric antimicrobial therapy should be initiated if infection is clinically suspected. Combination parenteral therapy is appropriate in the ill patient for a number of reasons:[9]

- to cover the possibility of antimicrobial resistance;
- to target specific bacterial functions (e.g. the theoretical effect of clindamycin on toxin production);
- to ensure adequate drug penetration into the central nervous system; and
- to have a possibly favorable effect on survival.

In the future it is likely that novel therapies, such as toxin inhibitors or receptor antagonists, will be available to treat anthrax.[18] Detailed therapeutic and postexposure prophylaxis recommendations for adults, children and special groups have been recently reviewed elsewhere.[12] Anthrax vaccine has been proven to be effective in preventing cutaneous anthrax in human clinical trials and in preventing inhalational disease after aerosol challenge in nonhuman primates.[19] The vaccine has generally been found to be safe but requires six doses over 18 months with the need for frequent boosting. Its availability is currently limited; it is hoped that second-generation anthrax vaccines will prove effective.

Smallpox

The last known naturally acquired case of smallpox occurred in Somalia in 1977. The disease was officially certified as having been eradicated in 1980, the culmination of a 12-year, intensive campaign by the WHO.[20] However, because of concerns that variola virus stocks may have either been removed from or sequestered outside their officially designated repositories, smallpox is considered to be a potential agent of bioterrorism. Multiple features make smallpox an attractive biological weapon and ensure that its re-introduction into human populations would be a global public health catastrophe: it is stable in aerosol form with a low infective dose; case fatality rates are historically high, approaching 30%; secondary attack rates among unvaccinated close contacts is 37–88% and are amplified; and much of the world's population is susceptible because routine civilian vaccination was terminated more than two decades ago, vaccine-induced immunity wanes over time, and there is no virus circulating in the environment to provide low-level, booster exposures.[21] Additionally, vaccine supplies are currently limited, although this problem has begun to be addressed, and there are currently no antiviral therapies of proven effectiveness against this pathogen.

After an incubation period of 7–17 days (average 10–12 days), the patient experiences the acute onset of a prostrating prodrome of fever, rigors, headache and backache that may last 2–3 days. This is followed by a centrifugally distributed eruption that generalizes as it evolves through macular, papular, vesicular and pustular stages in synchronous fashion over approximately 8 days, with umbilication in the latter stages (Fig 6.3 Fig 6.4 Fig 6.5 Fig 6.6). Enanthema in the oropharynx typically precede the exanthem by a day or two. The rash typically involves the palms and soles early on. The pustules begin crusting during the second week of the eruption; separation of scabs is usually complete by the end of the third week. The differential diagnosis of smallpox is delineated in Table 6.3 . Historically, varicella and drug reactions posed the most diagnostic dilemmas.[21]

Smallpox is transmitted from person to person by respiratory droplet nuclei and, less commonly, by contact with lesions or contaminated fomites. Airborne transmission by fine-particle aerosols has, in certain conditions, been documented. [21] The virus is communicable from the onset of the enanthema until all of the scabs have separated, although patients are felt to be most contagious during the first week of the rash, owing to high titers of replicating virus in the oropharynx. Household

members, other face-to-face contacts and health care workers have traditionally been at highest risk

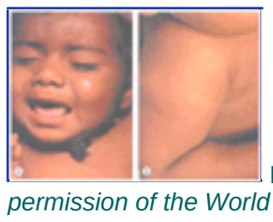

Figure 6-3 Third day of rash in smallpox. Additional lesions continue to appear and some of the papules are becoming obviously vesicular. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

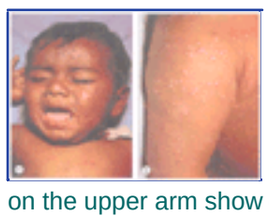

Figure 6-4 Fifth day of rash in smallpox. Almost all the papules have now become vesicular or pustular, the truly 'vesicular' stage usually being very brief. Some of the lesions on the upper arm show early umbilication. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

of secondary transmission. Thus, hospitalized cases are placed in negative-pressure rooms with contact and airborne precautions to minimize this risk, and those not requiring hospital-level care should remain isolated at home in order to avoid infecting others.

Issues related to the diagnosis and the initial approach to the management and containment of suspected smallpox cases are discussed in detail in Chapter 96d . The suspicion of a single smallpox case should prompt immediate notification of local public health authorities and the hospital epidemiologist. Containment of smallpox is predicated on isolating cases and the 'ring vaccination' strategy, which was successfully deployed in the WHO global eradication campaign and mandates the identification and immunization of all directly exposed people, including close contacts, health care workers and laboratory personnel. Vaccination, if deployed within 4 days of infection during the early incubation period, can significantly attenuate or prevent disease and may have a favorable impact on secondary transmission.[21] Because the occurrence of even a single case of smallpox would be tantamount to bioterrorism, an epidemiologic investigation would be necessary to ascertain the perimeter of the initial release, so that tracing of those initially exposed can be accomplished.

Botulism

Botulism, an acute neurologic disease resulting from intoxication with *Clostridium botulinum* toxin, occurs sporadically and in focal outbreaks throughout the world in relation to wound contamination by the bacterium or ingestion of food-borne toxin. A detailed discussion of botulism is found in Chapter 25 . Aerosol forms of the toxin, a rare mode of acquisition in nature, have been used in weapons designed for use in bioterrorism.[5] Botulinum toxin is considered

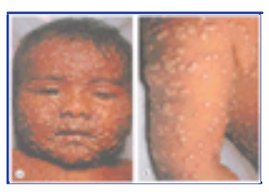

Figure 6-5 Eighth day of rash in smallpox. This case is now clearly classified as discrete ordinary-type smallpox. In the confluent subtype of ordinary-type smallpox the lesions would have been confluent on the face and forearms: in the semiconfluent subtype they would have been confluent on the face but not on the forearms. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

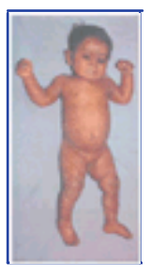

Figure 6-6 Twentieth day of rash in smallpox. The scabs have separated except on the palms of the hands and the soles of the feet, leaving depigmented areas. *From Fenner et al.,* [20] *with permission of the World Health Organization.*

to be the most toxic microbial molecule known; it is lethal to humans in minute quantities. It blocks the release of the neurotransmitter acetylcholine from presynaptic vesicles, thereby inhibiting muscle contraction.[22]

Botulism presents as an acute, afebrile, symmetric, descending, flaccid paralysis. The disease manifests initially in the bulbar musculature and is unassociated with mental status or sensory changes. Fatigue, dizziness, dysphagia, dysarthria, diplopia, dry mouth, dyspnea, ptosis, ophthalmoparesis, tongue weakness and facial muscle paresis are early findings seen in more than 75% of cases. Progressive muscular involvement leading to respiratory failure ensures. The clinical presentations of food-borne and inhalational botulism are indistinguishable in experimental animals. [22]

The diagnosis of botulism is largely based on epidemiologic and clinical features and the exclusion of other possibilities (see Table 6.3). Clinicians should recognize that any single case of botulism could be the result of bioterrorism or could herald a larger scale 'natural' outbreak. A large number of epidemiologically unrelated, multifocal cases should be clues to an intentional release of the agent, either in food or water supplies or as an aerosol.

The mortality from food-borne botulism has declined from 60% to 6% over the past four decades, probably as a result of improvements in supportive care, including mechanical ventilation. Because the need for mechanical ventilation may be prolonged, the limited availability of ventilators would be likely to be exceeded in the event of a large-scale bioterrorism event. Treatment with an equine antitoxin, available in limited supply from the national public health laboratories (the CDC in the USA), may ameliorate disease if given early.

Plague

Plague, the disease caused by the Gram-negative pathogen *Yersinia pestis*, presents in a variety of forms in naturally acquired disease (see Chapter 176). Plague is endemic in parts of South East Asia, Africa and the western USA. Aerosolized preparations of the agent, the expected vehicle in bioterrorism, would be predicted to result in cases of primary pneumonic plague outside endemic areas. As was the case with the anthrax attacks in the USA in 2001, however, additional forms of the disease, such as bubonic and septicemic plague, might also occur.

Primary pneumonic plague classically presents as an acute, febrile, pneumonic illness with prominent respiratory and systemic symptoms; gastrointestinal symptoms, purulent sputum production or hemoptysis occur variably.[23] Chest radiographs typically show

patchy, bilateral, multilobar infiltrates or consolidations. In the absence of appropriate treatment there may be rapid progression to respiratory failure, vascular collapse, purpuric skin lesions, necrotic digits and death. The differential diagnosis is largely that of rapidly progressive pneumonia (see Table 6.3). The diagnosis may be suggested by the characteristic small Gram-negative coccobacilli forms in stained sputum specimens with bipolar ('safety-pin') uptake of Giemsa or Wright stain.[24]

Confirmation by culture is necessary to confirm the diagnosis; the microbiology laboratory should be notified in advance if plague is suspected because special techniques and precautions must be employed.

Treatment recommendations for plague have been reviewed elsewhere. [24] Pneumonic plague can be transmitted from person to person by respiratory droplet nuclei, thus placing close contacts, other patients and health care workers at risk. Prompt recognition and treatment of this disease, appropriate deployment of postexposure prophylaxis and early institution of droplet precautions will interrupt secondary transmission.

Tularemia

Francisella tularensis, the causative agent of tularemia, is a small Gram-negative coccobacillus that would probably cause primary pneumonia if delivered as an aerosol agent of bioterrorism. Inhalational tularemia presents with the abrupt onset of a febrile, systemic illness with prominent upper respiratory symptoms, pleuritic chest pain, and the variable development of pneumonia, hilar adenopathy and progression to respiratory failure and death in more than 30% of those who do not receive appropriate therapy.[25] The diagnosis is generally based on clinical features, after ruling out other agents; laboratory personnel should be notified in advance if tularemia is suspected, because the organism can be very infectious under culture conditions. This agent is discussed in depth in Chapter 177 .

Viral hemorrhagic fevers

The agents of viral hemorrhagic fevers are members of four distinct families of RNA viruses that cause clinical syndromes with overlapping features: fever, malaise, headache, myalgias, prostration, mucosal hemorrhage and other signs of increased vascular permeability and circulatory dysregulation, leading to shock and multiorgan system failure in advanced cases.[26] Specific agents are also associated with specific target organ effects. These pathogens include the agents of Ebola hemorrhagic fever, Marburg disease, Lassa fever, Rift Valley fever and Congo-Crimean hemorrhagic fever (see Chapter 183).

Hemorrhagic fever viruses have been viewed as emerging infections in nature, owing to their sporadic occurrence in focal outbreaks throughout the world, thought to be the results of human intrusion into a viral ecologic niche. They are, however, potential weapons of bioterrorism because they are highly infectious in aerosol form, are transmissible in health care settings, cause high morbidity and mortality and are purported to have been successfully used in weaponry.[8] Blood and other body fluids from infected patients are extremely infectious, and person-to-person air borne transmission may occur; therefore, strict contact and air borne precautions should be instituted.[26]

Treatment is largely supportive and includes the early use of vasopressors as needed. Ribavirin is effective against some forms of viral hemorrhagic fevers but not those caused by Ebola virus and Marburg virus. Nonetheless, this drug should be initiated empirically in patients presenting with a syndrome consistent with viral hemorrhagic fever until the etiology is confirmed.

ASSOCIATED ISSUES AND SEQUELAE OF BIOTERRORISM

Quarantine

Quarantine, the physical separation and geographic restriction of groups of uninfected people potentially exposed to a communicable illness, has been variably considered to be one form of management strategy following bioterrorism. The potential effectiveness, feasibility, legality and consequences of quarantine have recently been reviewed.[27] The logistics of this approach are complex and impractical, and it can be associated with adverse consequences, such as increased risk of disease transmission among a quarantined group and an increased risk of riots. It seems clear that there are only limited scenarios in which the potential public health benefits of the imposition of quarantine may outweigh the potential problems engendered by this approach; these largely revolve around highly transmissible, lethal agents. In most situations a disease-specific containment strategy, based on transmission epidemiology and disease prevention approaches, is preferable.

Management of special patient populations

The approach to the management of diseases of bioterrorism must be broadened to include children, pregnant women and immunocompromised people. Specific recommendations for treatment and prophylaxis of these special patient groups for selected bioterrorism agents have recently been reviewed.[13] [24] [25] A general approach requires an assessment of the risk of certain drugs or products in select populations versus the potential risk of the infection in question, accounting for extent of exposure and agent involved. The issue extends to immunization because certain vaccines, such as smallpox, pose a higher risk to these special groups than to others. This will impact on mass vaccination strategies.

Psychosocial morbidity

An often overlooked but vitally important issue in bioterrorism is that of psychosocial sequelae. These may take the form of acute anxiety reactions and exacerbations of chronic psychiatric illness during the stress of the event, or post-traumatic stress disorder (PTSD) in its aftermath. Nearly half of the emergency department visits during the Gulf War missile attacks in Israel in 1991 were related to acute psychologic illness or exacerbations of underlying problems.[28] Data from recent acts of terrorism in the USA suggest that PTSD may develop in as many as 35% of those affected by the events.[29] In the early period after the 11 September attacks in New York, PTSD and depression were nearly twice as prevalent as in historical controls.[30] Although there were direct correlations between close proximity to the events and PTSD and between personal loss and depression, there was a substantial burden of morbidity among those indirectly involved. The psychologic impact of these events and of the ongoing international concern over terrorism can be expected to be significant and sustained for society as a whole.

CONCLUSION

The response to bioterrorism is unique among WMD because it necessitates the consequential management that is common to all disasters as well as the application of basic infectious diseases principles: disease surveillance, infection control, antimicrobial therapy and prophylaxis, and vaccine prevention. For these reasons physicians, specifically infectious diseases specialists, are likely first responders to bioterrorism and are expected to be reliable sources of information for their patients, colleagues and public health authorities.[31]

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Chapter 7 - Microbial Genomes

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INTRODUCTION

In the 1940s, Avery and his colleagues made an astounding and, at the time, controversial discovery. Heritable traits of the pneumococcus, specifically capsular serotype, could be transferred through the donation of DNA from one bacterial cell to another. In the 1950s, the structure of DNA was resolved by Watson and Crick, and with it was proposed a plausible (and as it turned out correct) basis by which heritable information could be accurately retained and transmitted to progeny cells. The genetic code by which DNA determines the order and specificity of the amino acids making up a particular polypeptide was solved. So began the era of molecular biology, a discipline that rapidly became a dominant force in biology. A biochemical method for establishing the order and content of the nucleic acids making up entire genes, even the entire genetic complement of viruses or free-living cells, was developed by Sanger. This opened up the possibility of determining the DNA sequence of complete genomes, the most ambitious project being the Human Genome Project. At this time, the only complete genome sequences were those of a few viruses (e.g. vaccinia in 1990 and cytomegalovirus in 1991). The first genome to be completed using automated technology was that of variola in 1993. However, at this time, there were no complete genome sequences of free-living organisms, including pathogenic bacteria, fungi and macroparasites.

A MILESTONE IN MICROBIOLOGY

The obvious single cell for which to obtain a complete genome sequence was *Escherichia coli*, but this project was languishing

Figure 7-1 The microbial revolution of the 1990s: bacterial genome sequencing. (1) Construction of a random gene library. (2) Random sequencing of thousands of clones. (3) Closure phase (which can be very labour-intensive). (4) Collation and annotation of the final sequence *(reproduced from Moxon[30] with permission from The Lancet)*

when, to the surprise of many, the Institute for Genome Research announced the completion of the entire genome sequence of a strain (Rd) of the bacterium *Haemophilus influenzae*.[1] This was immediately recognized as a conceptual and technical breakthrough of immense significance. It literally changed the face of microbiology and had a revolutionary impact on infectious diseases. This milestone was made possible, in part, through important technical innovations. Whole genome DNA was sheared into small fragments, each of which was cloned and sequenced (often referred to as shot-gun sequencing). Sophisticated computational methods, rather than methods that depended on the construction of physical genetic maps, were used to assemble the sequence. The approach relied on sequencing enough clones with unique DNA fragments to have a high probability that every nucleotide of the genome had been sampled. This posed an enormous challenge — the assembly of hundreds of thousands of overlapping partial DNA fragments of 300–500 nucleotides. A final step involved identifying and sequencing a relatively few remaining gaps in the sequence (closure; Fig. 7.1).

Once sequencing had been achieved for *H. influenzae*, numerous other bacterial genomes were sequenced using similar methods and more than 80 complete genome sequences have now been completed.[2] Of these, 38 strains represent pathogens (obligate, facultative and opportunistic) including the causative agents of important human diseases (pneumonia, meningitis and gastroenteritis) and agents of historically important pandemics and recurrent epidemics (plague, cholera, typhoid, typhus and meningococcal disease). Other completed genome sequences include the etiologic agents of nosocomial and community-acquired infections (*Staphylococcus*

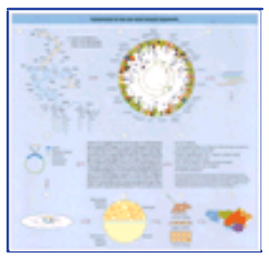

Figure 7-2 How a whole-genome sequence of a pathogenic microbe can be used to develop a candidate vaccine. (1) Representation of lipopolysaccharide (LPS), a major surface antigen of *H. influenzae* and a potential vaccine candidate. The lipid A (endotoxin) is inserted into the cell envelope of the bacterium to which are attached inner and outer core saccharides (uppermost in figure). These oligosaccharides are important in the interactions of the bacterium with host cells. (2) Whole-genome sequence of *H. influenzae* Rd that includes all of the genes involved in the biosynthesis of *H. influenzae* LPS of this strain (reproduced with permission from Fleischmann et al[1]). (3) DNA sequences (or their deduced amino-acid sequences), publicly available in databases (e.g. GenBank, EMBL), are used as probesto search and identify homologues (candidate LPS genes) in the *H. influenzae* complete genome sequence. (4) These candidate *H. influenzae* LPS genes can be further investigated to confirm or reject their role in LPS biosynthesis through appropriate experimental methods. (5) Oligonucleotide primers can be constructed to obtain multiple copies of each of the candidate LPS genes using polymerase chain reaction (PCR). (6) The PCR amplified candidate LPS genes are cloned into a suitable plasmid vector and mutations are constructed. In this example, the candidate LPS gene has been disrupted by an insertion of a cassette of DNA containing a gene for kanamycin resistance; this also acts as a selectable marker. (7) The cloned mutant gene is introduced into *H. influenzae* by transformation (allelic replacement); the phenotype of the parent strain and its mutant can then be compared to determine whether there are differences in LPS phenotype. (8) Immunoblotting is used to compare the reactivity of a monoclonal antibody (specific for LPS) in the parent and mutant strains; in this example, the LPS of the mutant has lost its capacity to bind to the monoclonal antibody, whereas colonies of the parent strain bind this antibody. This provides strong evidence in support of a function for this gene in LPS biosynthesis. (9) Further tests of biological function on parent and mutant can be done to further characterise the role of LPS, for example, its role in virulence. (10) Information on the many genes involved in LPS biosynthesis provides detailed information on the structure and its potential for use in vaccine development. *(Reproduced from Moxon[30] with permission from The Lancet.)*

aureus) and opportunistic infections (*Pseudomonas aeruginosa*). Importantly, complete genome sequences have become available for pathogenic bacteria, such as *Treponema pallidum* and *Chlamydia* spp., that could not be grown in vitro and for which few if any classical genetic methodologies are available. Ignited by the successes achieved in pathogenic bacteria, the entire genome sequence of all 14 chromosomes of the parasite *Plasmodium falciparum* have been sequenced,[3] a milestone in the quest to understand and control one of the most important causes of death and disability in the developing world.

IMPACT OF COMPLETE GENOME SEQUENCES

Complete genome sequences represent the most economical approach (less than 30 cents/base) to obtaining a vast body of information with which to investigate the biology of pathogenic microbes. However, the successful exploitation of these data is still dependent on the classical biologic methods of the pregenomic era. Indeed, many disciplines that had languished, such as microbial physiology, have been rejuvenated because of the wealth of information that can now be downloaded from a personal computer in minutes.

Complete genome sequences have not eroded any of the established fundamental tenets of biology. Our conceptual framework of the cell and its essential mechanisms, ranging from replication, genetic exchange and DNA repair to the complexities of commensal and virulence behavior of pathogens, remain unchanged. However, genome sequences have allowed a previously unattainable appreciation of genome content and of the very large number of distinct, even species-specific,

genes that exist. Many are of completely unknown function and these afford potentially completely novel approaches to understanding the biology of microbes, as well as opportunities for developing new diagnostics, therapeutics and vaccines. A critical aspect is the completeness of the information provided by genome sequences. This allows a comprehensive approach to determining which genes are essential to pathogenic or commensal behavior and whether they are deployed in colonizing, transmission, obtaining essential nutrients, evading host clearance mechanisms or damaging tissues. All the metabolic pathways available to the organism are apparent and these in turn can provide major clues as to the basis of the niche-specificity and tissue tropism of pathogens and facilitate the analysis of macromolecules (e.g. lipopolysaccharide)[4] of importance to virulence and the development of vaccines (Fig. 7.2). The nucleotide content (i.e. the ratio of adenine and thymidine to cytidine and guanine of a genome) is for the most part highly characteristic, and so it allows immediate identification of sequences within the genome that are foreign — DNA acquired by horizontal transfer, a mechanism responsible for conferring the genetic basis of antibiotic resistance or virulence. Examples of this kind of DNA include so-called pathogenicity islands (because they contain a whole suite of genes that are essential to causing disease) and large mobile resistance elements that confer multiple different resistances to antibiotics. Repetitive DNA (microsatellites), previously not considered a feature of prokaryotes, has been found in a number of major bacterial pathogens (*H. influenzae*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*) and is responsible for antigenic variation.

INFORMATION CONTENT OF COMPLETE GENOME SEQUENCES (ANNOTATION)

Any microbial genome sequence contains an extremely significant amount of information — the complete genetic blueprint for that organism. To make that information of use and easily accessible to researchers, a major task for bioinformaticians is the annotation of a genome sequence to identify the genes, their relative position and their likely function. An annotated genome constitutes a primary interface for researchers to access the information encoded within it.

Genome annotation requires coupling of the processes of identification of open reading frames (ORFs) and other genome features with prediction of function. Most genes in any genome have not been experimentally investigated, so the expedient method of determining the function of encoded proteins is through inference from genes of known or proposed function, on the basis of sequence similarity (Table 7.1). A majority of microbial genomes have been annotated using an ORF-based method of analysis. Open reading frames are first identified in a sequence defined by the predicted start and stop codons. As an example, the program currently available from The Institute for Genomic Research (TIGR) website,[2] GLIMMER (standing for gene locator and interpolated markov models), identifies coding regions and distinguishes them from noncoding DNA using interpolated Markov models. [5] A lower size limit must be assigned to reduce the number of spurious reading frames included for analysis. A derivative version, GLIMMERM, has been developed particularly for small eukaryotic genomes. Gene functions are then assigned after homology comparisons by automated searching of ORFs from the new genome (query sequence) against databanks of sequenced and published genes and other genome sequences. Various search algorithms are used, a majority of which are based on the BLAST and FASTA search programs. The best matches for each ORF are noted and a judgment is then made to assign a predicted function to a query gene sequence. Some more recent genome sequencing projects, such as that for *N. meningitidis* serogroup B strain, used GLIMMER in conjunction with a non-ORF based method.[6] This latter method searches database sequences using large tracts of genomic DNA and aligns homologies independent of reading frame. Start and stop codons can then be overlaid on the results of the search, which are viewed via a graphical interface, such as ACEDB, which runs on a UNIX platform.[7] This allows the operator to select the most appropriate reading frame in the genome sequence. This method is particularly valuable for identifying translational frameshifts, which are often found associated with phase variable genes and pseudogenes. Pseudogenes (genes not expressed because of the presence of stop codons or frameshifts that interrupt the translated reading frame) are found in many genome sequences. A further program, ARTEMIS (a JAVA-based tool) allows viewing of sequence and sequence features via a graphical interface.[8] It is generally considered that detailed interpretation of the raw output from an annotation procedure by researchers with an intimate knowledge of the relevant organism is the most appropriate means of resolving the many problems posed by annotation of any given genome.

CATEGORIZING AND ACCESSING INFORMATION (DATA-MINING)

The gene pool contains all of the genes required for the existence of the free-living organism. Riley has proposed a system to catalog genes into broad functional groups.[9] These 12 groups have proved useful as a framework for classifying genes and they allow researchers to access the DNA sequence information in a directed and informative manner relevant to any particular research interest. For many microbial genome sequences, around 60% of genes can be assigned to these broad functional categories. Importantly, typically about 40% of the ORFs at the time of annotation remain unassigned with respect to function by homology comparisons alone. The ORFs that appear only within one genome and that have no sequence similarity to other sequences in the databases are termed 'hypothetical' or 'function unknown' (FUN),[10] whereas those of unknown function but present in more than one genome are classified as 'conserved

TABLE 7-1 -- Useful website addresses for accessing information related to the genome sequences of pathogenic micro-organisms.

WEBSITES WITH INFORMATION OF GENOME SEQUENCES OF PATHOGENIC MICRO-ORGANISMS	
Database resource	**Website address**
The Institute for Genomic Research (TIGR) Database	http://www.tigr.org/tdb
TIGR Comprehensive Microbial Resource (CMR)	http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl
The Wellcome Trust Sanger Institute	http://www.sanger.ac.uk
The National Centre for Biotechnology Information (NCBI)	http://www.ncbi.nlm.nih.gov
Centre for Biological Sequence Analysis (DNA structural analyses)	http://www.cbs.dtu.dk/services/GenomeAtlas/index.html
Genome Channel	http://compbio.oml.gov/channel
Enzyme Nomenclature Page	http://www.chem.qmw.ac.uk/iubmb/enzyme
European Bioinformatics Institute	http://www.ebi.ac.uk
Kyoto Encyclopaedia of Genes and Genomes (KEGG)	http://www.genome.ad.jp/kegg
Microbial Genome Database (MBGD) for comparative analysis	http://mbgd.genome.ad.jp

hypothetical'. The number of FUN genes has decreased as the number of completed sequencing projects increases and homologous FUN genes are identified in other organisms. An important consideration following any genome annotation procedure, based solely upon homology comparisons, is that some gene functions listed in that genome database may remain erroneous. Homology predictions alone do not provide rigorous evidence for the biologic function of genes and their products. Existing data bank sequences upon which predictions are based may have putative, probable or confirmed functions, and each will be given equal weighting by most annotation teams. Good quality partial matches and lower quality more extensive matches may not always be distinguished but could be crucial in deciding likely gene function. When analyzing and comparing between genomes, useful terms for considering genes of comparable function are paralogs (homologous genes in the same organism whose products perform related but not identical functions) and orthologs (homologous gene functions from different organisms). Orthologs are likely to have the same function whereas paralogs are more likely to have diverged in their function.

For investigators, most microbial genome databases allow both the searching of the genome sequence annotation using key words and the genome sequence by a search algorithm (e.g. GRASTA for TIGR genome sequences). To permit more detailed analyses on the investigator's own computer system, the complete genome sequence can be downloaded, usually in a FASTA format, using the instructions at the appropriate website. Once downloaded, the sequence can be investigated or manipulated as required and viewed via a graphical interface using programs such as ACEDB and ARTEMIS, described above. Two widely used software packages are freely available for sequence searching — stand-alone BLAST[11] and FASTA.[12] Both packages contain a number of programs (or 'flavors') that are used, depending on the nature of the query and the database sequences. Each package has user information in an associated 'README' file. In general, comparative protein sequence searches are the most informative for homology comparison. The output from homology searches include an expectation (E) value for each match, a statistical estimate of the significance of the particular comparison against that expected purely by chance. This is important information for deciding the homology of a query sequence; the smaller the value the greater the confidence of a true match. Typically, E values of 10^{-5} to 10^{-6} are considered as the threshold for homology assignment.

Other useful programs for comparing sequence information are:

- PSI-BLAST (position-specific iterative BLAST) program,[13] which uses successive BLASTP searches of a database to identify distantly related sequence function;
- ProDom (protein domain),[14] Prosite[15] and Pfam (protein family)[16] for analysis of families of proteins of related structure and/or function — each of these databases contains proteins grouped by homologous domains using different automated searches; and
- the Expasy website,[17] which locates any putative enzyme function in a biochemical pathway by Enzyme Commission (EC) number and provides links to other

useful information on function.

COMPARING AND CONTRASTING INFORMATION ON PATHOGENS

The bias in the selection of micro-organisms of clinical significance for genome sequencing projects exists because of our need to understand and control infection. As the number of available microbial genome sequences has increased, many studies have focused on comparative genomics, comparing whole genome sequences from related bacteria with their different pathogenic potential or host ranges. This has the potential to identify genes associated with expression of particular disease traits. As examples, studies have compared hypervirulent and carrier isolates of *N. meningitidis* and *N. gonorrhoeae*, pathogenic and nonpathogenic *Listeria* spp., and the human gastric tumorigenic pathogen *Helicobacter pylori* with *Helicobacter hepaticus* (which is a cause of liver cancer in mice). A particular emphasis in comparative studies of microbial pathogens has been placed on identifying pathogenicity islands, regions of DNA normally containing clusters of virulence-related genes. These may be mobile and when acquired are sometimes all that is apparently required to convert a bacterium to a virulent phenotype.[18] Such 'foreign' DNA can often be identified by a divergence in DNA base (percentage of guanine plus cytidine) composition or the characteristic prevalence of particular nucleotides, the so-called dinucleotide signature. Innovative strategies to identify novel candidate virulence genes through computational approaches are continually being developed.

Analysis of genome sequences has led to an increase in our understanding of the population biology of some pathogenic bacteria. The availability of complete genome sequences has facilitated multilocus sequence typing methods for epidemiologic studies. Limited analysis of DNA sequence at multiple sites in the genome permits comparison of the relatedness of large numbers of strains for any organism. Such studies have advanced our understanding of the dynamics of the genome and the population structure of micro-organisms. *Streptococcus pneumoniae* and *N. meningitidis* are characterized by high rates of interstrain recombination whereas, in *S. aureus* and *Mycobacterium tuberculosis*, the major driving force for population variability within these species is mutation.

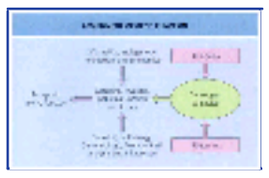

Figure 7-3 Assignment of gene function.

ADJUNCT METHODOLOGIES: MICROARRAYS AND PROTEOMICS

The assignment of gene function relies on a combination of sophisticated bioinformatic tools with the results of careful biologic experimentation (Fig. 7.3). Until the mid-1990s bacterial molecular biology was based on reductionist experiments designed to give information about individual genes. The genome sequence of the gastrointestinal commensal *Escherichia coli* was published in 1997 and revealed the limitations of these approaches. *E. coli* is the best understood of all organisms and detailed functions have been assigned to many of its genes. However, despite more than 60 years of research, it was found that one-third of the genome was made up of FUN genes.[10] Evidently, the development of functional understanding from genomic information posed a significant challenge. The necessity of looking at thousands of genes or proteins in a single experiment has spurred the development of 'functional genomics'. This methodology is largely based on identifying and characterizing messenger RNA (mRNA; transcriptomics) and translated peptides (proteomics) of individual genes.

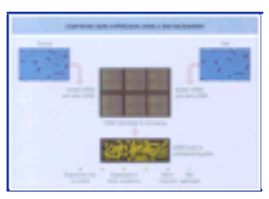

Figure 7-4 Comparing gene expression using a DNA microarray. The color of each spot relates to the level of expression of every gene on the microarray. *Further details can be obtained from http://www.ifr.ac.uk/safety/microarrays*

The transcriptome is the complement of mRNA species expressed in a bacterial cell under a particular condition, and it can be visualized with DNA microarrays (Fig. 7.4). Microarrays are microscope slides that have been spotted with DNA representing each of the gene sequences of a bacterium. They can be used to identify the activity of genes in a microbe, the so-called expression profile. To detect the changes in gene activity that occur when bacteria adapt to new environments, organisms are grown under different conditions, and the mRNA is extracted, fluorescently labelled and placed on the microarray. The degree of hybridization to specific genes reflects the abundance of mRNA and provides an indication of the level of gene expression. Microarray technology was originally developed to study eukaryotic RNA, which is extremely stable and easy to purify. In contrast, bacterial mRNA is very labile and this short half-life (as brief as 1 minute in some cases) poses technical challenges in recovering adequate amounts of mRNA to perform an analysis. These difficulties have been largely overcome, and reproducible gene expression profiles can now be obtained. Interpretation of these results has required the development of dedicated computer software methods to perform multivariate analyses so as to obtain data on bacterial gene expression from extremely large data sets.

Microarrays were first used to obtain gene expression profiles from in-vitro cultures of *M. tuberculosis* and *E. coli* in 1999. Subsequently, bacteria have been grown under defined conditions to determine the effect of factors such as acidity, anaerobicity, salinity and temperature on gene expression. Whole-genome expression studies can be used to identify genes differentially regulated in the host.[19] For example, it has been established that modulation of the heat shock response of *M. tuberculosis* promotes survival of the pathogen during the chronic phase of infection. Microarrays have been used to identify the effector regulatory genes (regulons).[20] These could be targets for the development of novel drugs.

Microarrays have been used to explain why *Vibrio cholerae* becomes more infectious during passage through the human gut; this has been done by isolating RNA from human stool samples and comparing it with RNA from laboratory-grown bacteria. Forty-four genes were more active and 193 were less active in the stool-derived sample. These findings provide new insights into the pathogenesis and transmission of cholera. In another example, gene expression

profiles showed significant changes in transcription during infection of murine macrophages with *Salmonella typhimurium*. One-quarter of the genome (919 genes), including many virulence genes and genes that respond to DNA damage (SOS response genes), had altered expression profiles.[21]

Microarrays are beginning to be used as a tool to study differences in the complement of virulence genes between bacterial strains of the same or related species. This 'genomic indexing' approach is based on analysis of chromosomal DNA rather than mRNA, and it gives information about the presence or absence of genes using microarray hybridization. It has already been used to determine the presence or absence of the major virulence determinants in a collection of strains of *Salmonella* spp.,[22] and in a range of other bacterial pathogens including *Campylobacter jejuni*, *H. pylori*, *M. tuberculosis*, *S. aureus* and *V. cholerae*. Genomic indexing could be the ideal way to assess the level of horizontal gene transfer between bacteria.

Much regulation occurs at the post-transcriptional, translational or post-translational levels; changes in mRNA transcription do not always lead to concomitant changes in protein expression. Consequently, the entire bacterial protein complement, the proteome, must be studied in parallel with the transcriptome. Current approaches for proteome analysis rely on a variety of two-dimensional polyacrylamide gel electrophoresis techniques. Proteins are separated according to their isoelectric point and their molecular weight. Mass spectrometry is then used to obtain partial peptide sequence, and information from comprehensive databases is then used to interrogate the relevant genome so as to identify the appropriate protein of interest.[23]

Most studies of bacterial proteomes have focused on the growth of particular pathogens in vitro. A large-scale study of *H. pylori* in which the bacteria were cultured on agar plates identified 175 proteins among which was the urease virulence factor.[24] Proteomic technology offers the hope of recognizing the proteins that are produced by bacteria during infection of mammalian cells, and two-dimensional gels have been used to show that the profile of bacterial proteins changes during infection. These approaches have generally relied on differential labelling methods to reveal the pathogenencoded proteins. Six abundant proteins that are expressed by *M. tuberculosis* during infection of human macrophages have been identified; they include two major chaperone proteins (GroEL-1 and GroEL-2) as well as two proteins that are components of the cell wall of *M. tuberculosis*. [25] However, this technology has been successfully only for the direct identification of bacterial proteins from infection of cultured cells in vitro; it is not currently applicable to whole-animal models because of the limited quantities of protein that can be obtained ex vivo.

One important and innovative application of immunoproteomics is the identification of subsets of bacterial proteins that are expressed during natural human infections.

This approach uses sera obtained from infected patients. The sera are used to probe two-dimensional gels comprising the proteome of the infecting pathogen. In one study, 310 antigenic proteins of *H. pylori* were identified and characterized by routine mass spectrometry.[26] Because these proteins raised an immune response, they were known to be expressed during infection and are therefore candidates for the development of specific diagnostic assays.

THE DISCOVERY OF NOVEL AGENTS FOR CONTROLLING INFECTIONS

The availability of complete genome sequences has dramatically changed the opportunities for developing new and improved vaccines or antimicrobial agents by facilitating the discovery of novel molecules and increasing the efficiency and rapidity of their development. Complete genomic databases provide an inclusive catalog of all potential candidate vaccines and drug targets. In conjunction with adjunct technologies, including bioinformatics, random mutagenesis, microarrays and proteomics, a systematic and comprehensive approach can be undertaken. Genomics must be used in conjunction with population biology to ensure that the vaccine or drug can target all pathogenic strains of a species. On a more cautious note, the genomic revolution has brought to the fore the immense difficulties inherent in evaluating the large number of potential candidates emanating from complete genome sequence data. How to arrive at a judicious decision on which of many candidates to take forward into human trials is an immense challenge. The costs of taking a new product from concept to market are huge (millions to billions of dollars) and the process lengthy (5–10 years). Given that genomic screens may yield scores, even hundreds, of candidates, there is a need to reduce to a handful the number of molecules that are taken forward into clinical testing.

Genomics and vaccines

Ideally, a vaccine should be effective against all pathogenic strains of a species because host immune responses to cell surface-exposed epitopes, through natural selection, result in both intra- and interstrain genetic diversity (diversifying selection). This is most effectively achieved by selecting strains that are representative of the genetic diversity within the natural population of that species through the use of, for example, ribotyping multilocus enzyme electrophoresis or multilocus sequence typing[27] [28] and determining its presence or absence and, if present, the degree of amino-acid variability. This amalgamation of population biology and genomics is critical because complete bacterial genome sequences are typically available for only one strain or a very few strains of a pathogen. Basing a genomic screen for vaccine antigen discovery on one or two strains of the species runs the risk of identifying candidate genes or epitopes that are absent in other genetically distinct pathogenic strains of the species. However, if a gene is present in some strains of the species but is absent from the genome of the sequenced strain, this is not a serious flaw in that any robust vaccine candidate should be present in all strains.

A proof in principle of the utility of genomics is provided by the recent exploitation of the complete genome sequence of *N. meningitidis* group B.[6] [28] This important pathogen is a major cause of meningitis and septicemia. Although group-specific capsular polysaccharide vaccines can prevent disease caused by most meningococcal strains, this approach is not easily applicable to the development of vaccines against group B strains.[29] As a result, there has been a substantial research effort over many years to identify alternative noncapsular antigens. This has proved to be a frustrating and costly process and one that has proved unable to deliver any convincing vaccine candidates suitable for routine immunization of infants. The availability of the complete genome sequence of *N. meningitidis* group B provided an opportunity to take a different, genomic-based, approach. Primary screening of the complete genome sequence for coding capacity was carried out using database and computer programs to exclude proteins with cytoplasmic functions and identify surface exposed or secreted proteins. This search identified 570 such ORFs, and the sequences of these predicted genes were amplified using polymerase chain reaction and then cloned into *E. coli* to express each polypeptide. Successful expression was achieved in the majority (61%) and included lipoproteins, inner membrane proteins, periplasmic proteins and outer membrane proteins. The recombinant proteins were used to immunize mice and the postimmunization sera were analyzed to verify the specific reactivity with, and surface localization of, each polypeptide. Out of 85 proteins that showed convincing evidence of surface localization, 22 also showed activity using the bactericidal

assay that has been shown to correlate with protection in humans. Several of these proteins are currently being prepared for testing in phase 1 human studies.

Perspective

The past 8 years have seen dramatic changes in the scientific approach to infectious diseases as the result of complete genome sequences, especially those of bacterial pathogens. This has revolutionized the study of individual microbes and pathogenesis of infectious diseases. Over 80 complete bacterial genomes have been published, half of which belong to strains that cause disease. Many more, probably hundreds of genomes are in draft form or are in the process of being sequenced,[19] and many of these are held privately. Increasingly, publications of complete genome sequences include innovative uses of sequence data, such as high-throughput experimental analyses (e.g. microarray studies). There will undoubtedly be an explosion of papers of this kind in the next few years.

The ability to integrate information derived from genomes with other sources of information will be central to our future ability to exploit bacterial genomes. The sequencing of pathogen genomes has proved beyond doubt the value of using genomes to discover new determinants of virulence. It has also proved the power of using genomes to elucidate the molecular basis of physiology, adaptation to niche, and the mechanisms by which genomes change over both short and long evolutionary time spans. In order to build upon this knowledge we must:

- find new ways to characterize the vast number of genes without known function, especially those conserved between organisms;
- continue to look for global patterns that help to define unusual features of specific sequences, strains and species;
- more effectively translate the results of genome sequence into hypotheses that can be tested by experiment;
- explore the extent and significance of population-level genetic diversity; and
- place each genome into its proper 'organismal' context by understanding its specific ecology and evolution.

Perhaps most importantly, this heterogeneous and complex information must be captured in next-generation databases that will improve the scope, quality and interconnectivity of our current invaluable electronic resources. By combining a sound knowledge of clinical manifestations of disease, classical microbiology and genome sequences, we will understand in more detail than ever before 'what makes a pathogen'.

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Section 2 - SYNDROMES BY BODY SYSTEM

Roger G Finch
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Chapter 8 - Viral Exanthems

Barbara A Bannister

INTRODUCTION

This chapter discusses those infections characterized by the eruption of widespread skin lesions (an exanthem). Although their etiologies differ, they share a number of features:

- many are highly infectious by the airborne route;
- the viral agents are shed in oropharyngeal secretions;
- exposure and infection usually occur in childhood;
- epidemics and large outbreaks are common in susceptible groups;
- infections in adults tend to be more severe, with complications; and
- infections in pregnancy can be detrimental to the fetus.

In spite of these similarities, each disease is discussed individually because each has its own etiology and clinical behavior.

MEASLES (RUBEOLA)

EPIDEMIOLOGY

Measles is highly infectious. In most populations, 95% or more of adults are seropositive. Transmission is usually by airborne droplet spread, which is important in hospitals and other institutions. As with other paramyxoviruses, including respiratory syncytial virus, transmission can also occur by direct transfer of infectious secretions, such as saliva, on hands, toys or other fomites. Viruses are shed from the respiratory mucosa during the prodrome and evolution of the rash; in adults excretion can persist for 6 days after the rash begins.

In nonimmune communities, large epidemics occur in the spring and early summer of alternate years. Maternally derived immunity is effective up to age 6–9 months. The peak age of infection is about 4 years. In small and isolated communities, epidemics occur at less frequent intervals; they affect a wider age range and result in a higher mortality.

Death occurs in approximately 1 in 4000 cases in developed countries, usually in debilitated or immunocompromised individuals. In malnourished populations fatality rates reach 10–15%, mainly as a result of bacterial complications.

PATHOGENESIS AND PATHOLOGY

Measles virus is a member of the Paramyxoviridae, a family of enveloped, negative single-stranded RNA viruses. Human signaling lymphocyte activation molecule (SLAM, or CDw150) acts as the major receptor for measles virus. SLAM is expressed on lymphocytes and dendritic cells and is important in T-cell and cytotoxic natural-killer-cell activation.[1] The involvement of SLAM may help to explain the significant immunosuppression that accompanies measles infection. The Edmonston strain of measles virus, progenitor of some commonly used vaccines, will also bind to CD46. Viral ribonucleocapsids are assembled in the host cell nucleus and accumulate as inclusion bodies in the cytoplasm. Virions acquire matrix protein and leave the host cell by budding, deriving their envelope from the host cell membrane. Infected host cells often coalesce to form multinucleate giant cells.

Once the virus has replicated in respiratory mucosal cells, it invades the local lymph nodes. Within 4–6 days it is detectable in the plasma and in blood mononuclear cells, particularly lymphocytes. By about 8 days the virus is detectable in the liver, spleen, lung, respiratory epithelia and eye, and has more recently been demonstrated in endothelium in the central nervous system. The rash reflects viral invasion of the skin, with accumulation of immune complexes and mononuclear cell infiltration.

In measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE), virus ribonucleocapsids are seen in neurons but the virus is replication-deficient; hemagglutinin is often absent, and membrane and fusion proteins are undetectable.[2] No virus release occurs and giant cells do not develop. In the laboratory these defects are rectified in the presence of interferon-a and the neuronal infection regains the ability to produce virus.[3]

PREVENTION

Pre-exposure prophylaxis

Live attenuated measles vaccines, and measles/mumps/rubella (MMR) vaccines, based on the Edmonston, Schwartz or Moraten virus strains, were introduced in the 1960s and 1970s. They rapidly controlled epidemic disease, including the rare neurological manifestations (Fig. 8.1). However, immunization before the age of 9–12 months produces short-lasting immunity, whereas immunization at the age of 1 year or more leaves a window of susceptibility between the loss of maternally derived protection and vaccine-induced protection; furthermore, vaccine uptake rates of over 95% are needed to prevent the spread of measles, emphasizing the need for effective immunization policies.[4]

A two-dose immunization program is used: the first dose at the age of 12–15 months (or at 6–9 months in developing countries in which measles threatens young infants), followed by a booster at 3–5 years of age. About 96% of recipients seroconvert satisfactorily. Outbreak investigations show that approximately 4% of exposed vaccinees suffer from clinical measles.

Common adverse effects of immunization include:

- a mild febrile illness, rarely with a transient rash ('mini-measles') after 7 days, in approximately 10% of first immunizations; and
- mild allergic reactions such as urticaria or other rashes (anaphylaxis is exceptionally rare).

All other adverse effects are rare. Although live vaccines are generally contraindicated in immunosuppressed individuals, measles vaccine has safely been given to children who had both asymptomatic and symptomatic HIV infection. There is some evidence that survival from severe measles infection is better in previously immunized HIV infected patients. However, in severe immunosuppression ($CD4^+$ cell count < 500) there is an increased risk of disseminated, pneumonic or neurological infections caused by the vaccine viruses,

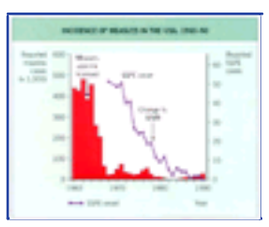

Figure 8-1 Incidence of measles in the USA 1960–90. The effect of measles and measles/mumps/rubella immunization on the incidence of measles and of SSPE in the USA, showing that both are prevented by the live attenuated vaccines.

and it is now recommended that immunization should be avoided in these cases.

Inactivated vaccine, available in the 1950s, was less effective; some recipients developed measles and suffered modified disease, with severe lung involvement and/or exfoliation. Other, highly immunogenic vaccines have been developed, and are effective at the age of 2–3 months, but there are concerns that their immunomodulatory effects may predispose recipients to other severe childhood infections.

Postexposure prophylaxis

Human normal immunoglobulin is effective in preventing measles. It should be given early, preferably within 3 days of exposure. Since universal immunization against measles, levels of protective antibody in pooled immunoglobulin products may have tended to decline. In a study from Japan, commercial products gave less than 50% protection. The recommended intramuscular doses for immunoglobulin to prevent an attack of measles are as follows:

- age under 1 year — 250mg immunoglobulin;
- age 1–2 years — 500mg immunoglobulin; and
- age 3 years and over — 750mg immunoglobulin.

All commercially available intravenous immunoglobulin preparations contain high levels of antibodies against measles.

CLINICAL FEATURES

The prodromal illness

After an incubation period of 9–11 days illness begins abruptly with high, swinging fever, irritability, nasal and conjunctival discharge, repetitive 'croupy' cough and, often, loose stools. Febrile convulsions are common in young children. Chest auscultation reveals many moist sounds but impaired respiratory function is rare.

Koplik's spots are pathognomonic of prodromal measles. They appear in the buccal mucosa, usually opposite the upper premolars, but may affect the whole inner cheek. White and irregular, their size varies from 'salt grains' to 'small breadcrumbs' and they are superimposed on a highly inflamed mucosa. They disappear by the second day of the rash and may be absent in the vaccinated individual.

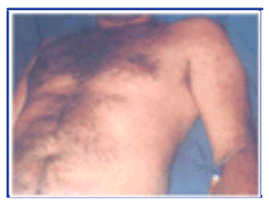

Figure 8-2 The acute rash of measles. Marked conjunctivitis accompanied the maculopapular skin lesions in this unimmunized adult.

Evolution of the rash

The rash begins on the third to fifth day of fever. It is maculopapular, extending downward from the ears and hairline, reaching the hips by the next day and the lower legs by the day after that (Fig. 8.2). After 4 days, it changes from pink to purplish and the fever abates quickly, marking the end of viremia. The rash fades without desquamation in 4–7 days.

In dark-skinned patients the rash may be invisible but the papular element causes a 'gooseflesh' appearance. In severe measles (which is more common in adults than in children) or in immunosuppressed patients there may be a hemorrhagic component, with desquamation on healing.

Other features

There is often mild neutropenia, with a white cell count of 3–4 × 10^9 /l.[5] Significant thrombocytopenia is rare. Liver transaminases and pancreatic amylase may be elevated, particularly in adults, although frank jaundice and pancreatitis are rare. More severe hematological and biochemical abnormalities indicate more severe disease.

Marked suppression of cell-mediated immunity occurs during the illness and for weeks afterward, with negative recall skin-test reactions.

Complications

Secondary bacterial infections

These are common and often severe. Acute suppurative otitis media and pyogenic bronchopneumonia are the most frequent. In addition to *Streptococcus pneumoniae* and *Haemophilus influenzae*, *Staphylococcus aureus* is an important secondary invader (Fig. 8.3). Rare cases of pyogenic mediastinitis are reported.

When poor hygiene, crowding or malnourishment exist, severe, destructive infection of macerated perioral or perinasal skin (noma, or cancrum oris) may be caused by pyogenic organisms, often accompanied by herpes simplex and/or anaerobic mouth flora (Fig. 8.4). A cloudy or punctate keratopathy with secondary bacterial keratoconjunctivitis may threaten sight. Tuberculosis may be unmasked or contracted during the immunosuppressed phase.

Measles is a dangerous infection in patients who have cell-mediated immunosuppression. Koplik's spots are often absent; the rash may be atypical and up to 30% of cases have no rash. Neutropenic patients have up to 70% mortality, and those who have severe HIV about 40%.[6]

Postinfectious encephalitis

Postinfectious encephalitis occurs in approximately 1 in 800–1600 cases and has a mortality of 12–15%. Up to 50% of survivors have neurologic sequelae of varying severity (see Chapter 27).

Measles inclusion body encephalitis

Measles inclusion body encephalitis, or subacute measles encephalitis, [7] is described most often in immunosuppressed children, with onset 1–7 months after acute measles. Occasional reports exist of cases with no known history of acute infection, and in one case a

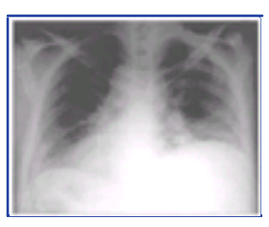

Figure 8-3 Secondary invasion by *Staphylococcus aureus* in measles. Ill-defined basal opacities were seen in a patient who had moderate respiratory failure; *S. aureus* was isolated from sputum (same patient as in Fig. 8.2).

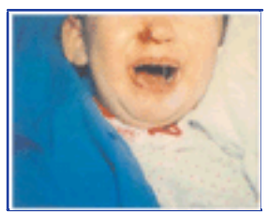

Figure 8-4 Secondary infections complicating measles. Perioral infection and paranasal herpes simplex lesion in a 2-year-old girl.

measles vaccine strain was identified in neural tissue.[8] Fever, loss of cerebral function and focal seizures develop progressively over 3 or more weeks. Fatality rates are about 75% and neurological sequelae are common in survivors.

Subacute sclerosing panencephalitis

Subacute sclerosing panencephalitis occurs in approximately 1 in 1,000,000 cases, particularly if the original measles infection occurred before the age of 2 years. After a 3- to 10-year interval, clumsiness and poor school performance herald the onset of myoclonic spasms with typical electroencephalographic changes; decerebration and death occur within 2 years.

Other associations of measles virus

Measles virus RNA has been demonstrated in the ossicles of patients who have otosclerosis. There is evidence that the age of onset of otosclerosis symptoms has increased since the availability of immunization for measles, suggesting that immunization may be protecting younger age cohorts and supporting measles as an etiological factor in otosclerosis. Paramyxovirus-like inclusions, which react with antibodies to measles virus, have been found in osteoclasts of patients who have Paget's disease.

In the 1980s a group of bowel specialists suggested that wild or vaccine strains of measles virus were an etiological factor in Crohn's disease. However, it is now known that the apparent presence of viral antigenic reactions in bowel mucosa was due to a cross-reaction with host protein and that the reaction was not specific to any particular type of inflammatory condition.[9] More recently, a further hypothesis — that measles vaccine virus, in the MMR formulation but not alone, could precipitate features of autism — has not been supported by epidemiological studies from several countries.[10]

DIAGNOSIS

Although the clinical features are often typical, the diagnosis should be confirmed by laboratory tests, particularly during measles eradication programs.

Measles virus is difficult to recover from cell cultures, although it will grow in primary primate or human cell lines. Serodiagnosis is sensitive using IgM enzyme-linked immunosorbent assay (ELISA) in serum or saliva samples. Positive results are obtained at presentation in approximately 70% of cases, and up to 100% are positive by the end of the first week. In SSPE there are very high levels of IgG antibodies, with evidence of local production in the cerebrospinal fluid (CSF), and measles nucleoprotein and genome are detectable in brain biopsy. In MIBE, CSF antibodies are not a reliable test; electron microscopy, antigen detection or genome detection must be performed on brain tissue. Bronchial lavage may demonstrate multinucleate giant cells, intracytoplasmic inclusion bodies and measles capsid antigen, which is helpful in detecting measles pneumonitis in immunosuppressed patients. High levels of measles-specific IgG are found in the perilymph in otosclerosis.

MANAGEMENT

Vitamin A

Measles mortality is inversely related to serum retinol concentrations. Children from developing countries or those from crowded urban areas of Western countries where health care and nutrition are suboptimal should receive oral vitamin A 10,000IU (age 1 year) or 20,000IU (age 1 year plus) at diagnosis.[11] Children who have keratoconjunctivitis should have further doses 1 day and 1 month later.

High-dose vitamin A can be given with measles vaccine, although there is some evidence of reduced seroconversion, possibly

because vitamin-induced immune enhancement reduces vaccine virus replication.

Antibiotics

The commonest febrile complications of measles are due to pyogenic bacterial infection. Empiric treatment for these should include antibiotics effective against common respiratory pathogens plus *S. aureus*. Co-amoxiclav, second-generation cephalosporins or trimethoprim-sulfamethoxazole are suitable choices. Treatment may be modified if necessary when laboratory results are available.

Antiviral drugs

Measles in the immunocompetent rarely demands specific treatment, although a single randomized, double-blind trial of ribavirin (tribavirin) therapy suggested that the course of the disease was slightly shortened and the occurrence of complications reduced in the treatment group.[12] Inhibitory levels of ribavirin can be demonstrated in serum and CSF during intravenous dosing. Reports of attempted treatment for severe or complicated measles are based on small numbers of cases. Although few adverse effects of ribavirin are reported in these cases, dose-related hemolysis, as well as nausea, malaise and agitation, are acknowledged adverse effects, which may limit longer courses of treatment.

Inhaled and intravenous (20–35mg/kg/day) ribavirin has been used successfully in patients who have severe viral pneumonitis.[13] However, experience suggests that specific therapy must be started early to derive significant benefit, preferably within 5 days. In the immunosuppressed, a 1-week course of intravenous ribavirin is usually given; if recrudescence occurs, this may respond to a further course.

Measles inclusion body encephalitis has also been treated with intravenous ribavirin, given in courses of 2 or 3 months.[7] An early fall in fever was seen in many cases, but treatment started later than 1 or 2 weeks after onset did not usually prevent severe or fatal disease.

Intrathecal interferon-a may significantly delay deterioration in SSPE but must be given intraventricularly in doses of around 3MIU, up to twice weekly. Sustained responses and some improvement in neurological deficit have been reported after the combined use of interferon and ribavirin.[14] However, the treatment is demanding and must be continued indefinitely to maintain its effectiveness.

Intravenous immunoglobulin has been used in conjunction with ribavirin in a few reported cases of severe pneumonitis and of disseminated measles. It is not possible to determine whether it contributed to a positive outcome.

RUBELLA

EPIDEMIOLOGY

Rubella virus (Rubivirus), a member of the Togaviridae, is transmitted from person to person by the airborne route and is only slightly less infectious than measles. An increase in cases occurs every 4–10 years, with larger epidemics every 10–20 years (Fig. 8.5). In susceptible populations the peak age of infection is between 5 and 9 years. In Western countries before the advent of immunization, 15–20% of individuals remained susceptible beyond the age of 20 years. In some tropical countries up to 40% of adults are susceptible.

Although rubella is mild in children and nonpregnant adults, in pregnant women the infection commonly causes severe disease and long-term damage to the developing fetus (see Chapter 65).

Recognition of rubella can be difficult, as it is often trivial, subclinical or atypical, mimicking other infections.[15]

Figure 8-5 Incidence of rubella in England and Wales. The effects of different immunization programs are shown. *Data from Bannister et al. Infectious disease, 2nd ed. Oxford: Blackwell Science; 2000: 234.*

PATHOGENESIS AND PATHOLOGY

Togaviridae are enveloped RNA viruses with cubical symmetry. Humans are the only known hosts of Rubivirus and only one serotype is recognized. Culture is possible in a variety of cell lines but rarely produces a cytopathic effect. Positive cell cultures are identified by immunofluorescence, antigen-detecting ELISA or interference with infection by other viruses such as enteroviruses.

The virus attaches to and invades upper respiratory and pharyngeal mucosal cells. Viral construction and assembly occurs in the cytoplasm, and virions bud into intracytoplasmic vesicles, acquiring their envelope from the host cell membrane. Viral hemagglutinin molecules are inserted into the envelope. Viremia and pharyngeal shedding are detectable 9 or 10 days after infection, and virus excretion continues for 14–21 days.

Most body tissues can be invaded, including lymphocytes, conjunctiva, synovium, uterine cervix and lymph nodes. Virus is excreted from the nasopharynx and in the stools and urine. The appearance of the rash marks the end of viremia and the production of neutralizing antibodies. Interestingly, men produce earlier and larger antibody responses than do women to several rubella antigens, but only women seem to produce antibodies to the second envelope protein E2.[16] Capsid antigens are important in eliciting cytotoxic cell-mediated immunity.[17]

PREVENTION

Live attenuated rubella vaccines became available in the late 1960s. About 95% of recipients seroconvert and develop hemagglutination-inhibiting antibodies (HI antibodies), which correlate with neutralizing antibody function. Vaccine virus is briefly excreted from the pharynx in the 4 weeks after immunization but transmission to close

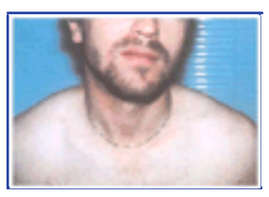

Figure 8-6 Rubella. This patient had a typical early maculopapular rash, irritating conjunctivitis and painful occipital lymphadenopathy.

contacts does not occur. Other members of a family may therefore be immunized even if a household member is pregnant.

Although HI antibodies decline over 10–16 years after immunization, resistance to re-infection by wild strains wanes little, indicating that vaccine-induced immunity is as durable as that which follows natural infection.[18] Between 1.5% and 4% of vaccinated individuals become re-infected (usually asymptomatically) and exhibit an IgM response after exposure to wild virus. Viremia is transient or absent during most re-infections. Congenital rubella rarely follows re-infection in naturally immune or vaccinated women. Effective immunization, vigilance and readily available screening and diagnostic testing are important in controlling outbreaks and protecting pregnant women.

Postexposure prophylaxis with human normal immunoglobulin has been attempted but even high doses have not been reliably effective.

CLINICAL FEATURES

Postnatally acquired rubella is usually mild, and in most studies about one-half of seroconversions are asymptomatic. The incubation period is about 15 days (range 14–21 days). The clinical features may appear in any combination, usually developing simultaneously, although joint involvement often follows after 4–7 days.

Rash

This usually coincides with fever, although adults may suffer 1 or 2 days' prodrome. The rash spreads from the face and chest (Fig. 8.6) to the peripheries over 1 or 2 days. It is maculopapular or macular, with small elements, often fading to a 'peach-bloom' appearance after the first day. It rarely lasts more than 3 or 4 days. It mimics the rashes of parvovirus infection (see below), echovirus infections and mild scarlet fever or early Kawasaki disease, although it does not desquamate on healing. Conjunctival itching, soreness and reddening often occur at the onset of illness.

Lymphadenopathy

Large, tender lymph nodes high in the occipital region are typical of rubella, although minor cervical lymphadenopathy may also occur. The patient may complain of localized head or neck pain. The nodes are hard and usually immobile, as they are tightly confined by the cervical fascia.

Arthralgia and arthritis

Many adults and some older children complain of marked joint pain late in the illness. There is also an increased incidence of arthropathy following rubella immunization. The proximal finger joints and wrists are most often affected. Frank arthritis with effusions, soft tissue swelling and erythema can occur; larger joints may

also be involved and women are affected more often than men.[19] In some individuals acute polyarthritis resembles rheumatoid arthritis but subsides over weeks or months. Some workers have found evidence of persisting rubella RNA in blood or peripheral blood mononuclear cells of patients who have arthropathy but others have failed to confirm this.[20] An auto-immune etiology is suggested by the increased prevalence of HLA-DR2 and DR5[21] in those affected, and by the interaction of cellular rubella-associated proteins with autoantigens such as Ro and La.[22]

Complications

Thrombocytopenia

A modest reduction in the platelet count is common and occasionally causes thrombocytopenic purpura, even if the original infection was subclinical. Severe bleeding is rare but may occur late in the course of the rash. The platelet count recovers completely within a few weeks. Thrombocytopenia rarely follows immunization with MMR vaccine but does not recur with further doses. Arthralgia may follow immunization in older girls and adults.

Encephalitis

Encephalitis is a rare complication, affecting approximately 1 in 5000 patients. It can occur up to 1 week after the appearance of the rash but very rarely before it. The onset is abrupt, with irritability, headache and, often, stiff neck. Lumbar puncture reveals lymphocytes and mildly elevated CSF protein. The electroencephalogram shows widespread slow-wave abnormalities. Convulsions, altered consciousness or neurologic signs may follow, with varying severity. Polyradiculitis occurs rarely.[23] Almost all patients recover fully within 1 week or so, although fatalities and a chronic SSPE-like syndrome have occasionally been reported.

Individual reports exist of rubella-associated cases of hepatitis, pancreatitis and retinal vasculitis. Rubella antigens have been demonstrated in the chondrocytes of patients who have Paget's disease.[24]

DIAGNOSIS

Rapid diagnosis depends on the detection of IgM antibodies, using antibody capture or sandwich ELISA tests and particle agglutination techniques. Immunoglobulin M antibodies are detectable very early in infection and are also useful in diagnosing re-infection in those already immunized. Many polymerase chain reaction (PCR)-based tests are under investigation but most laboratories still rely on serological tests.

Screening tests for immunity are important, especially in antenatal care. Radial diffusion precipitin or radial hemolysis tests are the most frequently used techniques.

MANAGEMENT

Management is symptomatic. Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in treating arthralgia and arthritis but should be substituted with simple analgesics if there is symptomatic thrombocytopenia.

Severe thrombocytopenia may be treated with prednisone (prednisolone), or intravenous immunoglobulin followed by a brief reducing course of prednisone.

Neither corticosteroids nor antiviral agents influence the course of rubella encephalitis.

PARVOVIRUS B19 INFECTION AND ERYTHEMA INFECTIOSUM

EPIDEMIOLOGY

Parvovirus B19, or human serum parvovirus, is the only member of the Parvoviridae pathogenic to humans. The Parvoviridae are small, icosahedral, nonenveloped viruses whose genome is a single 5kd strand of DNA. The family includes the adeno-associated viruses, which replicate only in the presence of co-infection with a helper virus. Serologic evidence suggests that some can infect humans asymptomatically. Parvovirus-like structures (small round viruses or small round featureless viruses) can be seen in the stools of patients who have acute gastroenteritis and have also been identified in some foods, but these have not been speciated.

Parvovirus B19 is common worldwide and is highly transmissible from person to person via respiratory tract secretions; it has an incubation period of 6–10 days. Epidemics occur, peaking in the spring, every 3–5 years, with smaller outbreaks in intervening years. Infection is commonest between the ages of 4 and 10 years and usually manifests as erythema infectiosum (fifth disease or slapped cheek syndrome). In those who have disorders causing short red blood cell survival, transient aplastic anemia occurs; in countries in which sickle-cell disease is common epidemics of aplasia occur. The high level of viremia in parvovirus infection means that transfusion-transmitted disease can occur. Infection probably confers lifelong immunity.

PATHOGENESIS AND PATHOLOGY

Viruses are adsorbed on to protein receptors of the host mucosa and are transported to the nucleus, where they uncoat. DNA replication can only occur if the host cell is in the S phase of mitosis. In the mature host, infection therefore affects rapidly replicating tissues, such as the bone marrow, reticuloendothelial system and, particularly, red-cell progenitors. The erythrocyte P-antigen acts as a virus receptor.[25] In the fetus, early infection may cause widespread damage and death of the conceptus, whereas later infections affect red-cell precursors (see Chapter 65).

Viral replication and assembly takes place in the host-cell nucleus; virions are released by lysis of the nuclear and cytoplasmic membranes. Virus is detectable in throat washings up to 1 week after infection, and an intense viremia (10^{10} –10^{12} virus particles/ml serum) occurs at the same time.

Infection of erythrocyte precursors causes a profound reticulocytopenia during the second week, whereas the total leukocyte count falls to a lesser extent. Between days 10 and 14, IgM antibodies to capsid antigens appear, terminating the viremia. They remain detectable for 2–3 months. Between the third and fourth week IgG antibody levels rise; these decline slowly over a number of years.

PREVENTION

Parvovirus B19 is extremely difficult to cultivate in the laboratory and this has delayed vaccine development. Attempts to engineer antigens, for instance in baculovirus systems, hold promise. Quarantine is not an effective means of interrupting transmission, as many infections are subclinical and the infectious period is over by the time typical clinical features develop.

Attempts at prevention by the use of intramuscular immunoglobulin have not been reported. Intravenous immunoglobulin preparations contain high levels of antibody to parvovirus B19 and might be effective in prophylaxis if the exposure was recognized.

CLINICAL FEATURES

At least 50% of parvovirus infections are unrecognized. Most recognized infections present with combinations of rash, anemia and arthritis but any feature can occur alone.[26]

Rash (erythema infectiosum)

After 2–4 days of malaise, fever and sweating, erythema of the cheeks appears, often with a slightly raised margin, as though the patient had been slapped (hence the name 'slapped cheek syndrome'). A macular rash appears 1 or 2 days later, spreading from the neck and shoulders to the peripheries in 2–3 days. Clear areas appear in the rash; these cause a reticulate pattern that can persist for 10–14 days, fading to violet and fluctuating with the skin temperature.

Other bizarre rashes may complicate the feverish, viremic phase of illness. Childhood papular-purpuric gloves and socks syndrome (PPGSS) is well described, consisting of a mildly itchy, confluent erythema with a marked purpuric element that sometimes resembles a large bruise. The author has seen similar rashes affecting the buttocks, perineum and thighs. Despite their intensity, these rashes resolve spontaneously in 4–6 days.

Arthropathy and arthritis

The incidence of arthritis is uncertain, as it can occur in the absence of rash or other features. In known cases it ranges from 10% to 33%, being highest in women and lowest in children. The commonest manifestation is polyarthralgia, with particular involvement of the wrists and proximal interphalangeal joints. Monoarthralgia and monoarthritis are also seen and may cause concern when they manifest as pseudopalsy in a child. Young women may suffer acute polyarthralgia or polyarthritis, with or without rash but usually with noticeable preceding fever. Although most individuals recover in 1–2 weeks, pain and stiffness can persist for several months. Evidence of parvovirus infection can be found in some cases of patients who have undifferentiated mono- and oligo-arthritis.[27]

Blood and bone marrow disorders

Stem-cell and erythrocyte infection profoundly affect erythropoiesis during the viremic phase. The reticulocyte count is usually negligible or undetectable for 4–6 days, and bone marrow examination shows cessation of red cell maturation and the presence of giant proerythroblasts. In normal patients, over 80% may exhibit mild anemia, but in patients who have sickle-cell disease, hereditary spherocytosis or severe nutritional anemias, there is transient, profound aplastic anemia. In tropical countries, epidemics of 'aplastic crises' can occur.

Other elements of the blood are less commonly affected; however, mild neutropenia and thrombocytopenia are common. Reticulocyte, platelet and neutrophil counts often rebound to levels well above normal in the 10 days after the fever.

Other clinical features

Individual reports of acute endocapillary proliferative nephritis have described parvovirus-containing immune complex deposits in the mesangium.[28] Meningoencephalitis has been described. Reports of hepatitis, myocarditis and systemic vasculitis have not been unequivocally attributed to parvovirus infection. Reports exist of systemic lupus erythematosus, apparently precipitated by acute parvovirus infection.

Complications

The most important complications are those affecting pregnancy, which include second-trimester fetal loss or hydrops fetalis (see Chapter 65)

Hemophagocytic syndrome has been reported to complicate acute parvovirus infection.

Persistent aplastic anemia can occur when an immunocompromised patient fails to clear replicating virus from the bone marrow stem cells.

Polyarticular juvenile arthritis and refractory rheumatoid arthritis have been associated with an increased likelihood of seropositivity to parvovirus B19.

DIAGNOSIS

The presence of fever plus cytopenias or a very low reticulocyte count should arouse suspicion of parvovirus infection, especially if a rash or arthralgia also exists.

In the febrile phase, high titers of virus in the blood permit confirmation by ELISA or immunofluorescence methods of antigen detection or by PCR and identification of parvovirus DNA.

Immunoglobulin M antibodies become detectable at about the onset of the rash, allowing a reliable serologic diagnosis by the sixth to 10th day of illness.

MANAGEMENT

There is no specific antiviral treatment. Most individuals recover without sequelae in 1–2 weeks with symptomatic treatment alone. NSAIDs are helpful in persisting arthralgia and arthritis.

High-dose intravenous immunoglobulin has been successful in treating immunosuppressed patients who have persisting aplastic anemia associated with parvovirus infection.

VARICELLA-ZOSTER VIRUS INFECTIONS (CHICKENPOX AND HERPES ZOSTER)

EPIDEMIOLOGY

Varicella-zoster virus (VZV) is an enveloped a-herpesvirus, with icosahedral symmetry and a linear, double-stranded DNA genome. Like other herpesviruses, it causes a primary infection (varicella or chickenpox) with seroconversion and subsequent lifelong latency. Reactivation causes localized neurologic disease with an associated skin eruption (herpes zoster or shingles). Both primary and reactivation diseases are infectious, although there is less virus shedding from the rash of herpes zoster.

VARICELLA

In developed countries varicella causes winter and spring epidemics, mainly affecting schoolchildren, with a peak incidence traditionally at the age of 5–9 years. In developed countries, there has been a downward shift in age prevalence from school age to pre-school age, which is likely related to an increase in attendance at out-of-home childcare increasing exposure at an earlier age. Maternally derived antibodies protect infants up to the age of 6–9 months. Likewise, about 90% of adults are seropositive and immune. By contrast, in the tropics, varicella is less common in children and seronegativity varies from 20% to 50% in adults. In the USA, introduction of varicella vaccine has been associated with a dramatic reduction in the incidence of varicella disease.[29]

Varicella-zoster virus is easily transmitted by respiratory secretions, on children's hands or by droplet spread. It also spreads along air currents in buildings such as hospitals.[30] In developed countries, exposure at home carries approximately an 80% risk of infection; in hospital or day care centers the risk is about 40–60%. The incubation period varies from 10–25 days, averaging 15 days.

The incidence of adult varicella is apparently increasing in several countries. In the UK reports of adult cases doubled between the 1970s and 1990s but have now stabilized. This is important, as the disease is more severe in adults and infection in pregnancy may cause fetal loss or damage (see Chapter 65). The fatality rate for varicella in the UK is about 1 per 6000 cases but this rises to approximately 1 per 600 for those aged over 55 years. In spite of the apparent increased severity in adults, it should be noted that children aged <15 yrs account for 90–95% of all cases of chickenpox, 80–90% of physician visits, 60–80% of hospital admissions and 25–45% of fatalities due to chickenpox.

HERPES ZOSTER

Herpes zoster reflects infection of a sensory nerve ganglion, its neurologic connections and associated dermatome. It complicates waning immunity and therefore affects elderly, debilitated and immunocompromised individuals. It may be precipitated by such stresses as another illness, trauma or bereavement, or by immunodeficiency as a result of HIV infection, malignancy or immunosuppressive chemotherapy. There is usually an interval of years between the occurrence of varicella and the eruption of herpes zoster, although varicella in a fetus or infant may be followed by neonatal or childhood zoster. Investigations into the cost of morbidity and mortality from VZV infections shows that the major cost to society is caused by herpes zoster.[31]

Close contact with a patient who has herpes zoster can lead to varicella in a seronegative person. It is possible that immunity is boosted throughout life by repeated exposure to varicella and zoster in the community.[32]

PATHOGENESIS AND PATHOLOGY

Varicella-zoster virus first infects the respiratory mucosa, where it replicates and invades the lymphatics, leading to asymptomatic primary viremia about 7 days after infection. Further viral replication occurs in most tissues; VZV DNA appears in the peripheral blood mononuclear cells and polymorphs at this stage. Virus replication and assembly takes place in the nucleus of affected cells, producing characteristic 'type A' intranuclear inclusions. Syncytium and giant cell formation occurs. In the skin, large, balloon-shaped 'Tzanck' cells, with typical nuclear inclusions, are seen in scrapings from vesicular skin lesions. VZV is strongly cell-associated and is more readily demonstrated by antigen detection in respiratory or skin specimens than by culture of secretions or vesicle fluid.

Clinical disease begins with a secondary viremia about 15 days after infection. Focal lesions develop, containing giant cells with intranuclear inclusions. Organs infected include the skin, lungs, gut, reticuloendothelial system and occasionally the brain, retina or joint synovia. In the skin, cell damage and fluid collection separate the layers of the epidermis to produce blistering, at the base of which Tzanck cells are found. Giant-cell vasculitis may play a part in rare neurologic events that precede or follow the classic illness.

Humoral immunity is demonstrable by fluorescence labeling of antibodies against membrane-associated antigens. These rise progressively in titer throughout the illness, and no clear cut-off predicts susceptibility rather than immunity. ELISA tests are now used to detect IgM and IgG antibodies.[33] Varicella-zoster virus can be cultured, with cytopathic effects in human embryo cell lines.

In spite of cellular and humoral immunity, VZV persists in the body after primary infection. Unlike herpes simplex, it is not detectable by the presence of DNA 'latency sequences'.[34] Messenger

ribonucleic acid has been demonstrated in trigeminal ganglion tissue. During reactivation, virus replication and assembly begins in sensory ganglion neurons, subsequently appears in glial cells and is accompanied by intense inflammation. Virus migrates along the dorsal root connections to the central nervous system, causing inflammation and nerve fiber degeneration, and along axons to the skin, where typical VZV skin lesions occur. Local meningeal inflammation surrounds the affected spinal segment and may proceed to a general, viral-type meningitis.

PREVENTION

Pre-exposure prophylaxis

Live attenuated varicella vaccine is derived from the Oka strain of VZV. It is immunogenic in 98–99% of children after a single dose and 94–99% of adolescents and adults when given in two doses 4–8 weeks apart. Breakthrough disease after exposure to wild varicella occurs in 12–20% of vaccinees 5–10 years after immunization, causing a similar secondary infection rate in immunized household contacts. However, the disease is mild, with a median of 20–40 vesicles. [35] A few recipients develop a sparse vesicular rash and may then infect close contacts with the vaccine strain.

The vaccine can be given to selected immunocompromised individuals, for example children in remission from steroid-responsive nephritic syndrome or from leukemia, or in the intervals between pulsed chemotherapy courses. The vaccine virus exhibits latency and can cause herpes zoster, earlier and more commonly in the immunosuppressed. Long-term follow-up will quantify the likelihood of later vaccine-related herpes zoster.

The vaccine is licensed for childhood use in some countries (including the USA), and in others for use in nonimmunes from special groups, including health care workers and household contacts of the immunosuppressed, or in immunosuppressed individuals.

Universal childhood immunization reduces the incidence of varicella in both immunized and nonimmunized children. Experience in the USA attests to its safety and effectiveness in reducing the incidence of varicella disease and morbidity. [29] [36] There is a concern that the resulting reduced circulation of wild virus may increase the susceptibility of the elderly to herpes zoster, by reducing the frequent boosting of immunity derived from exposure to varicella.[32] This remains to be confirmed clinically.

Postexposure prophylaxis

Human immunoglobulin derived from the plasma of individuals with high VZV antibody levels (usually after recent varicella or herpes zoster) is used to prevent or modify varicella after exposure. Varicella-zoster immune globulin is the product available in North America: zoster immunoglobulin (ZIG) is available in the UK. It prevents or modifies varicella even when given 7–10 days after exposure.[37] However, for maximum benefit, VZIG must be administered within 96 hours of exposure, and preferably within 72 hours. Immunoprophylaxis with VZIG may not prevent infection, but does effectively reduce disease severity. It also may prolong the incubation period a week, from 21 days to 28 days at the upper end. Zoster immunoglobulin is recommended for:

- immunosuppressed individuals exposed to varicella or disseminated herpes zoster;
- nonimmune pregnant women who have been exposed to varicella or disseminated herpes zoster; and
- the neonate whose mother developed varicella between 7 days before and 7 days after delivery.

The recommended dosages for intramuscular ZIG are as follows:

- age 0–5 years — 250mg;
- age 6–10 years — 500mg;
- age 11–14 years — 750mg;
- age 15 years and over — 1000mg.

VZIG is administered at a dosage of 125 units/10kg body weight to maximum dose of 625 units. If ZIG cannot be given, particularly if intramuscular injection is contraindicated, there is evidence that intravenous immunoglobulin contains sufficient antibody to prevent infection.[38]

Varicella can occur after immunoglobulin prophylaxis. In neonates about 30% of these infections are severe and in the immunosuppressed all infections are a threat. Antiviral chemotherapy should therefore be given to those who have breakthrough infections, especially those at risk for severe disease (ie newborns whose mothers rash onset 5 days before to 2 days after delivery, and immunocompromised patients). Such patients need to be treated at the first onset of rash, regardless of whether or not VZIG was given.

Varicella vaccine has been proven effective as post-exposure prophylaxis if given within 5 days of exposure and is recommended for such use in both Canada and the United States provided there are not contraindications to receipt of vaccine. [39]

Aciclovir alone has been given prophylactically, in household and institutional settings. A 10-day course prevented clinical disease in both, but larger studies are required to define the effective dose and duration of prophylaxis. [40]

CLINICAL FEATURES

Varicella

Childhood varicella is often first recognized when the rash appears. Adults more often have a 1–3 days' prodromal, influenza-like illness.

Rash

Lesions begin as papules but progress within hours to superficial, clear vesicles surrounded by a variable halo of erythema. Vesicles are often oval, with the long axis parallel to skin creases, and are commonly pruritic. New lesions appear progressively over 5–7 days. The head and upper trunk are affected first and most densely, whereas the limbs have fewer lesions and these appear later. The rash is exaggerated and appears earlier in hot areas of skin, for instance under a diaper or occlusive dressing.

The vesicular fluid opacifies and in 2 or 3 days a central dimple appears. A crust then forms from this center outward and falls away after about 5 days (Fig. 8.7). Unless secondary infection has occurred, scarring is limited to faint, pale outlines. The rash is accompanied by variable fever. Secondary cases in households are often more severely ill than the index case.

The density of the rash indicates the severity of varicella. Indicators of severe disease include confluence of the rash, multiple lesions in the mouth, pharynx and genital mucosae, and retrosternal or epigastric pain (presumably caused by tracheal and esophageal lesions).

Patients are infectious from the prodromal period until the skin lesions scab, although virus rapidly becomes undetectable after the sixth day of uncomplicated illness.

Varicella pneumonitis

Varicella is always accompanied by a giant-cell pneumonitis but this is not usually clinically significant. Cigarette smokers, women in late pregnancy and the immunosuppressed are at increased risk of respiratory disease. The first sign is a drop in arterial oxygen saturation (Fig. 8.8), followed within hours by respiratory symptoms, or abnormal sounds on auscultation. Pneumonitis varies widely in severity. In patients who have severe pneumonitis there is cough with mucoid or bloodstained sputum and respiratory failure, which may prove fatal. Co-existing bacterial infection must always be suspected. The chest

Figure 8-7 The lesions of varicella. Papules, vesicles and pustules, some of which are beginning to crust from the center, are seen.

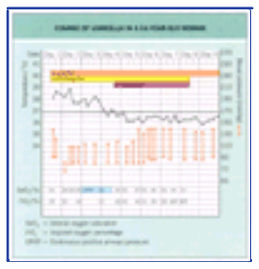

Figure 8-8 Course of varicella in a 54-year-old woman. Low arterial oxygen saturation precedes respiratory and hemodynamic failure.

radiograph may show ground-glass changes, widespread nodular opacities (chickenpox lung) or segmental shadows. However, reliance on chest radiographs will over-diagnose clinically relevant pneumonia, and they should not be obtained routinely, but reserved for those wih clinical respiratory distress.

Hepatitis

Elevation of transaminase levels to three times the upper limit or more is most often seen in adults, particularly men. Although transaminitis is common, clinically significant hepatitis or jaundice is rare.

Thrombocytopenia

Mild thrombocytopenia is common in adults, particularly men, and may cause a petechial rash and hematuria. The skin vesicles are then hemorrhagic. In severe

varicella, platelet and vascular damage may lead to disseminated intravascular coagulation.

Rare features of varicella

Rare features of varicella include:

- ¦ transient cerebellar disturbance with ataxia and vertigo; this occurs in convalescence in about 1 in 4000 cases and mostly affects males;
- ¦ encephalitis, which may be of rapid onset with focal signs, seizures or coma; most patients recover, although a few, often those who have imaging evidence of cerebral infarcts, suffer permanent sequelae; and
- ¦ retinitis, which can occur immediately or after several weeks' delay, is often severe or necrotizing and may be associated with lasting visual impairment.

Occasionally, cerebral or retinal disease precedes the rash or occurs alone. Since the availability of PCR-based tests in CSF, it is now recognized that VZV is among the common causes of meningitis and encephalitis, and is more common than herpes simplex virus in individuals of all ages.[41] VZV has also been linked with stroke-syndromes in healthy children and adults as well as HIV patients; rash is not always seen in such cases.[42]

Neonatal varicella

Neonatal varicella affects neonates born to seronegative mothers who therefore lack maternally derived immunity. The source of varicella is often the perinatally infected mother. The infant will not acquire adequate antibody levels until the mother is 7 days into her clinical illness. Babies born before this, or exposed in the first week of life, require varicella prophylaxis.

If acquired from exposure to their mother whose rash onsets from 7 days before to 2 days after delivery, it is a severe, multisystem disease with lung, kidney, bone marrow and brain involvement, and a resulting case-fatality rate of 25–40%. However, for infants exposed for the first time to infection more than 2 days after delivery, disease is often mild to inapparent. Premature infants who are considered at risk for severe disease throughout the first month of life. The risk of severe disease rapidly decreases: after 4 weeks of age varicella is no longer as dangerous; however, although most cases in the first year of life are mild-moderate in severity, disease can be severe as shown by a fatality rate (8/100000) that is 4 fold higher than for children aged >1 yrs, and ~¼ of that in adults.[43] Clinicians should consider iv aciclovir for infants whose rash is rapidly spreading and/or in whom complications other than secondary skin infections become apparent early in the course of disease.

Herpes zoster

Herpes zoster is heralded by pain in the dermatome served by the affected sensory root, which sometimes resembles pleurisy or abdominal pathology. Occasionally in children it manifests as a viral meningitis with increased lymphocytes in the CSF.

Groups of papules then appear at the sites where cutaneous nerves reach the skin. In a spinal nerve this is just lateral to the spine, in the midaxillary line and just lateral to the linea alba. This may not progress further; however, in adults it usually extends to fill the dermatome unilaterally. Some dermatomes, such as those of the supraclavicular and lumbar regions, are more often affected, while the arms are rarely involved, possibly reflecting the denser areas of the preceding varicella rash.

The papules progress to vesicles, pustules and crusts but, unlike varicella, lesions may become confluent and form large, flaccid bullae (Fig 8.9) that rupture to leave weeping bare areas. Uncomplicated

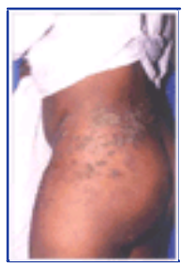

Figure 8-9 The rash of herpes zoster. It can be seen that the lesions occur in groups, with coalescence of lesions in the larger groups.

lesions can heal in 4–6 days but severe rashes may take 3–5 weeks. Nevertheless, altered skin pigmentation is often the only sequela. Scarring is rare.

Ophthalmic herpes zoster

The commonest cranial dermatome affected is the ophthalmic branch of the trigeminal nerve. The conjunctiva is usually inflamed and swollen, with associated edema of the eyelid; in severe cases, keratoconjunctivitis can cause prolonged blurred vision and, rarely, corneal scarring. Nasociliary involvement (evidenced by vesicles on the tip of the nose) indicates inflammation of the uveal tract, with pain, blurred vision and the risk of synechiae of the iris.

Geniculate herpes zoster (Ramsay Hunt syndrome)

In this rare syndrome the geniculate nucleus of the facial nerve is involved, affecting sensory neurons that serve the skin in the external auditory meatus. Vesicles are often seen in the ear, but inflammation and swelling also compress the facial and auditory nerves. There is unilateral facial palsy, deafness and severe vertigo, often with prostration and vomiting. If the chorda tympani is involved, there is also loss of taste sensation on the anterior two-thirds of the tongue.

Zoster-associated pain and post-herpetic neuralgia

The burning, tingling pain of herpes zoster recedes with healing, but older patients are at risk of persisting pain. This is often distressing, causalgic (not related in quality to the mild stimulus that causes it) and severe. Its average duration in those aged over 60 years is about 60 days, but it can last much longer and, rarely, persists permanently. This pain has been called post-herpetic neuralgia and has been given various definitions, for example pain after rash healing, or pain continuing more than 1 month after herpes zoster. It is now more correctly included in the whole continuum of pain caused by herpes zoster and is called zoster-associated pain (ZAP).

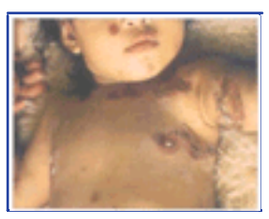

Figure 8-10 Secondary staphylococcal infection of varicella lesions. Staphylococcal pyrogenic exotoxin has caused a 'scalded skin' type of lesion surrounding the infected spots. *Courtesy of Dr MG Brook.*

COMPLICATIONS

Secondary bacterial skin infection

Secondary bacterial skin infection is the commonest complication of varicella. Children are twice as likely as adults to have significant skin infection, particularly severe or toxin-mediated disease (Fig. 8.10). Eczema does not predispose individuals to more severe varicella or to worse secondary infection.

Bacterial infection of individual spots causes pain, induration and often abscess formation. The usual pathogens are *S. aureus* or *Streptococcus pyogenes*. Local extension can cause cellulitis or erysipelas. Bacteremia occasionally co-exists. Children are particularly prone to staphylococcal or streptococcal toxic shock

syndromes, perhaps because they lack antibodies to the exotoxins. Necrotizing fasciitis is rare and affects adults or children. Nevertheless, children with varicella are at much higher risk of severe infection with group A *streptococcus*.[44]

Secondary bacterial infection can also complicate herpes zoster, especially when severe and in the elderly. In ophthalmic herpes zoster, bacterial conjunctivitis or keratoconjunctivitis can occur.

Bacterial lung infections

These can exist alone or complicate varicella pneumonitis. They are less important than skin infections in children, even in those who have a history of asthma, but are more common in adults.

Staphylococcus aureus is the commonest pathogen, but other chest pathogens occasionally occur. It is difficult to distinguish bacterial or mixed infection from viral pneumonia, especially as staphylococcal infection does not always cause an early neutrophilia. If doubt exists, bacterial infection should be assumed.

Motor paralysis in herpes zoster

Herpes zoster is occasionally associated with paralysis of muscle groups innervated from the affected dermatome. The quadriceps may be affected in lumbar zoster; diaphragmatic paralysis is sometimes seen in supraclavicular zoster. Transient paralytic ileus can occur in dorsal zoster. Electromyographic studies of affected muscles show evidence of axonal dysfunction. Gradual recovery is usual, but some residual weakness may persist.

Ascending myeloencephalitis

This is an exceptionally rare complication of herpes zoster, which is more common in immunosuppressed individuals. Successive involvement of higher spinal and cerebral levels first causes transverse myelitis, with pain, sensory and motor 'level's, and long-tract signs. These extend until cerebral irritability and coma supervene. There is a significant chance of recovery with vigorous antiviral treatment, depending on the severity of any underlying disease.

Varicella-zoster infection in the immunosuppressed

Cell-mediated immunosuppression predisposes individuals to severe VZV infections that result in progressive multisystem disease, often with a deceptively indolent onset. Herpes zoster is also recognized as one of the earlier opportunistic infections seen in patients who have AIDS (see Chapter 125). In severe immunosuppression, abnormal hemorrhagic lesions may develop without becoming vesicular, although the rash is distributed normally. Rarely, pneumonitis can exist without the rash, or retinitis occurs long after infection. Systemic corticosteroids during the incubation and prodrome are thought to predispose individuals to severe primary disease. A necrotizing cerebritis can appear in severely immunocompromised hosts, resulting in a syndrome resembling a brain abscess. A biopsy is necessary to establish the diagnosis.

Herpes zoster occurs earlier, more frequently and more severely in the immunosuppressed, often with deep, scarring lesions. Dissemination may occur, leading to varicella-like disease (Fig 8.11).

MANAGEMENT

Varicella

The nucleoside analogue aciclovir is licensed for the treatment of varicella. The oral dose is 800mg five times daily for 5–7 days but, even with this maximum dose, predose troughs below the mean

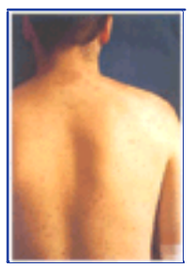

Figure 8-11 Varicella-zoster virus infection. Infection in a patient who had a history of varicella in childhood, had recently had a thymectomy and was taking high-dose prednisone (prednisolone): initially localized lesions on the neck were quickly followed by an extensive, varicella-type rash.

inhibitory concentration for VZV often occur. Valaciclovir, a prodrug of aciclovir, has much enhanced bioavailability, being well absorbed by the gut mucosa. It is hydrolyzed to release aciclovir. Famciclovir is similarly well absorbed and hydrolyzed to release penciclovir. Both prodrugs are licensed for the treatment of herpes zoster but as yet not for varicella.

Antiviral treatment has minimal impact on mild childhood infections and is not universally recommended (although it is cost-effective if loss of parental working time is included in the calculation). In adolescents and in uncomplicated adult disease, oral aciclovir reduces the severity of the rash and shortens the duration of illness by up to 1 day. It may reduce the risk of complications. Treatment commencing more than 1 or 2 days after the onset of rash is unlikely to influence the course of uncomplicated disease.

Intravenous aciclovir is the treatment of choice for severe or complicated VZV infections. A dose of 5–10mg/kg q8h is given by intravenous infusion over at least 1 hour. This avoids high peak blood levels and consequent renal impairment. Aciclovir is excreted by the kidney, and doses or dosing intervals must be altered if the creatinine clearance is below 60ml/min. Other side effects are mild and include nausea and occasional rashes. The dose may be increased to 15mg/kg in urgent cases, but renal function must be closely monitored.

Herpes zoster

In herpes zoster of the middle-aged and elderly, treatment started in the first 48 hours after the onset of the rash reduces the duration of the rash and the duration of ZAP. Recommended dosages are:

- aciclovir: 800mg five times daily orally for 1 week or 5mg/kg by intravenous infusion over 1 hour, q8h (may be increased to 10mg/kg in severe infection or in case of immunosuppression);
- valaciclovir: 1.0g orally q8h for 7 days; and
- famciclovir: *either* 250mg orally q8h *or* 750mg orally once daily, for 7 days.

Ophthalmic and geniculate herpes zoster should always be treated in order to reduce the risk of severe eye inflammation or permanent facial or vestibular nerve damage. Prednisone 0.5% eye drops 4–6 times daily are often added when there is evidence of uveitis. Atropine 2.5% eye drops may be used daily to dilate the pupil and avoid the development of synechiae, but carry a small risk of precipitating acute angle-closure glaucoma or atropine toxicity in the elderly, even at this low dosage.

Some immunosuppressed patients, particularly those who have had previous treatment with aciclovir, become infected with aciclovir-resistant VZV, which has an altered viral thymidine kinase. Ganciclovir (5mg/kg by intravenous infusion q12h for 14–21 days), which is activated by a different kinase, and foscarnet (60mg/kg by intravenous infusion q8h for 14–21 days) are useful in this setting. Valganciclovir is a prodrug of ganciclovir that is well absorbed and is hydrolyzed to release ganciclovir with much higher bioavailability than oral ganciclovir. It may reduce the need for regular injections in longer term therapy.

Ganciclovir has significant gastrointestinal and bone-marrow toxicity as well as a range of less frequent adverse effects, and foscarnet is nephrotoxic. Neither drug is therefore appropriate for the treatment of sensitive VZV infections. The use of corticosteroids with antiviral therapy is associated with a moderate acceleration in the

resolution of skin lesions and alleviation of acute pain.[45] Corticosteroids have no effect on the incidence or duration of ZAP.

Varicella retinitis

Varicella retinitis, with outer retinal necrosis, more often seen in HIV-infected patients and lymphoma sufferers, responds poorly to

aciclovir treatment. Case-series studies have shown that patients have a better long term visual outcome after treatment with ganciclovir or the combination of ganciclovir and foscarnet.[46]

Secondary bacterial infections

Secondary bacterial infections should be promptly treated. Skin lesions often respond to oral penicillins active against penicillinase-producing *Staph. aureus* or cefaclor. Severely painful or necrotic skin lesions need inpatient treatment with intravenous penicillins active against penicillinase-producing *Staph. aureus* for suspected staphylococcal infection. If streptococcal infection is suspected, then benzylpenicillin 2.4g q4h or q6h is appropriate; for necrotizing lesions, metronidazole 500mg q8h may be added or clindamycin substituted, and early surgery should be considered.

Mild chest infections in children may respond to oral flucloxacillin (or dicloxacillin) but infection in adults usually requires parenteral treatment. If a penicillin alone is not effective, an early change to a broad-spectrum cephalosporin such as cefuroxime or ceftriaxone should be considered.

Zoster-associated pain

In acute herpes zoster simple analgesics are often effective. Opiates should not be withheld if pain is severe, although nausea and confusion may limit their use in the elderly. Late, causalgic pain responds poorly to any analgesic but is often helped by gabapentin (300mg on day 1; 300mg q12h on day 2; 300mg q8h on day 3; then increased according to response as q8h doses up to a maximum of 1.8g daily) or amitriptyline (up to 25mg q8h). There is some evidence that this limits the duration of ZAP if given early. Antiviral agents have no proven benefit on chronic post-herpetic neuralgia.

HUMAN HERPESVIRUS TYPE 6 AND ROSEOLA (EXANTHEM SUBITUM)

Human herpesvirus type 6 (HHV-6) was recovered from B cells by cocultivation. It exists as two variants, HHV-6A and HHV-6B;[47] both can infect humans but only HHV-6B has been definitely associated with clinical disease. The virus is closely related to cytomegalovirus; a mononucleosis-like infection has occasionally been described. Infants are protected by maternally derived antibody detectable up to the age of 4–6 months. The virus can be found in the saliva of seropositive adults, suggesting that latent infection exists.

Almost all humans acquire infection and seroconvert in early childhood. Up to 60% of infected children develop roseola, in which 4–5 days of fever is followed by the sudden appearance of a macular or maculopapular rash, which quickly spreads across the neck, trunk and proximal limbs. The lesions fade in 1–2 days. Mild neutropenia is common in established infection. Occasional epidemics are recognized. The incubation period is about 10 days (range 5–15 days). Complications are few; febrile convulsions may accompany the high fever; occasional cases with encephalopathy or hepatic inflammation are described, rarely with lasting sequelae.[48] Some work suggests that the virus may be involved in the pathogenesis of multiple sclerosis.[49]

Treatment is symptomatic although the illness is usually trivial and self-limiting. Susceptibility to antiviral agents is similar to that of cytomegalovirus. Antipyretic medication is useful if the fever causes convulsions or other adverse effects.

HAND, FOOT AND MOUTH DISEASE

This is a systemic infection of young children that often occurs in local or household outbreaks and occasionally also affects adults. It is caused by Coxsackie viruses, most commonly type A16, although recent epidemics caused by enterovirus type 71 have occurred in eastern Asia.

After an incubation period of about 10 days, fever and mild generalized lymphadenopathy occur, followed after 2–3 days by the eruption of tense, clear vesicles on the palmar surfaces of the hands and feet (Fig. 8.12). Lesions also affect the mouth, tongue and pharynx. There is a papular or maculopapular rash on the buttocks and, rarely, on the back or thighs.

The illness is usually mild and self-limiting. Occasional cases, especially those caused by enterovirus 71, develop severe systemic disease with respiratory and/or encephalitic features and a significant mortality.

Specific treatment is not available. Skin lesions can be painful or tender and simple analgesics may help this. Prototype antiviral drugs directed at attachment proteins on the viral surface have not yet progressed beyond trials but may have a place in future in the treatment of systemic complications.

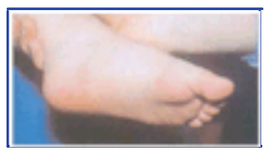

Figure 8-12 Hand, foot and mouth disease. Typical vesicles are seen on the foot of a 3-year-old child.

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Chapter 9 - Cellulitis, Pyoderma, Abscesses and Other Skin and Subcutaneous Infections

Dennis L Stevens

INTRODUCTION

Infections of the skin and/or subcutaneous tissues are highly diverse in respect to etiologic organisms, incidence, clinical manifestations, severity and complications. They may occur as single or recurrent episodes. Many cases are mild or self-limited, but some progress to cause scarring, loss of digits or limbs, or even death.

The terminology can be confusing because several different names, which are often not precisely defined, may be used to describe the same condition. Nomenclature for the most common infections is summarized in Table 9.1 .

When a patient presents with soft tissue infection, the clinician faces the challenge of establishing a specific diagnosis and prescribing definitive treatment. Important points in diagnosis are:

¦ the patient's symptoms;
¦ the general appearance of the infected site;
¦ historic clues such as contact with insects or animals, especially involving bites, travel to specific geographic areas, occupation or use of a hot tub (see Folliculitis, furuncles and carbuncles, below);
¦ the immune status of the host;
¦ chronicity; and
¦ anatomic distribution.

TABLE 9-1 -- Nomenclature, location and etiology of some common skin and subcutaneous infections.

NOMENCLATURE, LOCATION AND ETIOLOGY OF SOME COMMON SKIN AND SUBCUTANEOUS INFECTIONS			
Terminology	**Subgroups**	**Location**	**Etiology**
Pyoderma	Impetigo (impetigo contagiosa)	Skin	*Streptococcus pyogenes, Staphylococcus aureus*
	Bullous impetigo	Skin	*Staph. aureus* with group II phage
	Folliculitis (pustulosis)	Skin, hair follicles	*Staph. aureus*
	Folliculitis (sycosis) barbae	Skin, hair follicles of the beard	*Strep. pyogenes, Staph. aureus*
	Hot tub folliculitis	Skin	*Pseudomonas aeruginosa*
Abscesses	Furuncle (boil, subcutaneous abscess)	Subcutaneous tissue	*Staph. aureus*
	Hydradenitis suppurativa	Multiple furuncles in sweat glands: axilla, groins	*Staph. aureus* and other bacteria, including Gram-negative bacilli and anaerobes
	Carbuncle	Dense group of furuncles in areas of thick skin: back of neck, shoulders, buttocks	*Staph. aureus*
Cellulitis		Skin and subcutaneous tissue	*Staph. aureus, Strep. pyogenes*, Group C and G streptococci, *P. aeruginosa, Haemophilus influenzae* or Gram-negative bacilli; fungi can cause cellulitis in immunocompromised hosts
	Erysipelas	Skin	*Strep. pyogenes*
Ecthyma		Skin and subcutaneous tissue	*Strep. pyogenes, Staph aureus* or both; other bacteria *P. aeruginosa*
	Ecthyma gangrenosum	Skin and subcutaneous tissue in neutropenic patients	

If the diagnosis cannot be established based upon the history, symptoms and signs, then needle aspiration, biopsy or surgical exploration may be necessary to obtain specimens for appropriate staining and culture.

As the antimicrobial susceptibility of these microbes varies greatly, treatment (particularly for severe infections) should be based upon the results of microscopy, Gram stain and culture whenever possible.

EPIDEMIOLOGY

Although the exact incidence of these infections in the general population is unknown, they are among the most common infections occurring in all age groups. Some are age-related, for example impetigo is more common in children, erysipelas is more common in older adults.

Infections of the skin and soft tissues can be caused by bacteria (including rickettsiae), fungi, viruses, parasites and spirochetes.

TABLE 9-2 -- Probable etiology of soft tissue infections associated with some specific risk factor or setting.

PROBABLE ETIOLOGY OF SOFT TISSUE INFECTIONS ASSOCIATED WITH SOME SPECIFIC RISK FACTOR OR SETTING	
Risk factor or setting	**Likely etiologic agent**
Cat bite	*Pasteurella multocida*
Dog bite	*P. multocida, Capnocytophaga canimorsus* (DF-2), *Staphylococcus intermedius*
Tick bite followed by erythema chronicum migrans rash	*Borrelia burgdorferi*

Hot tub exposure	*Pseudomonas aeruginosa*
Diabetes mellitus or peripheral vascular disease	Group B streptococci
Periorbital cellulitis (children)	*Haemophilus influenzae*
Saphenous vein donor site cellulitis	Groups C and G streptococci
Fresh water laceration	*Aeromonas hydrophila*
Sea water exposure, cirrhosis, raw oysters	*Vibrio vulnificus*
Cellulitis associated with stasis dermatitis	Groups A, C and G streptococci
Lymphedema	Groups A, C and G streptococci
Cat scratch	*Bartonella henselae, B. quintana*
HIV-positive patient with bacillary angiomatosis	*B. henselae, B. quintana*
Fishmongering, bone rendering	*Erysipelothrix rhusiopathiae*
Fish tank exposure	*Mycobacterium marinum*
Compromised host with ecthyma gangrenosum	*P. aeuruginosa*
Human bite	*Eikenella corrodens, Fusobacterium* spp., *Prevotella* spp., *Porphorymonas* spp., *Streptococcus pyogenes*

TABLE 9-3 -- Differential diagnosis of bullous skin lesions.

DIFFERENTIAL DIAGNOSIS OF BULLOUS SKIN LESIONS	
Clinical condition	**Etiology**
Bullous impetigo	*Staphylococcus aureus* carrying group II phage
Erysipelas	*Streptococcus pyogenes*
Staphylococcal scalded skin syndrome	*Staph. aureus* producing exfoliative toxin
Necrotizing fasciitis	Type I: mixed aerobic and anaerobic bacteria
	Type II: *Strep. pyogenes*
Gas gangrene	*Clostridium perfringens, C. septicum*
Halophilic vibrio sepsis	*Vibrio vulnificus*
Pemphigoid	Immune-mediated
Toxic epidermal necrolysis	Drug-induced

TABLE 9-4 -- Differential diagnosis of crusted skin lesions.

DIFFERENTIAL DIAGNOSIS OF CRUSTED SKIN LESIONS	
Clinical condition	**Etiology**
Impetigo	*Staphylococcus aureus, Streptococcus pyogenes* or both
Ringworm	Dermatophytic fungi (e.g. *Tinea rubrum*)
Systemic fungal infections	*Histoplasma capsulatum*
	Coccidioides immitis
	Blastomyces dermatitidis
Cutaneous mycobacterial infection	*Mycobacterium tuberculosis*
	M. marinum
Cutaneous leishmaniasis	*Leishmania tropica*
Nocardiosis	*Nocardia asteroides*

Although there are hundreds of possible etiologic agents (Table 9.1 Table 9.2 Table 9.3 Table 9.4), two common species of Gram-positive cocci are the predominant causes of skin and soft tissue infections — *Staphylococcus aureus* and *Streptococcus pyogenes*. Skin and soft tissue infections caused by newly recognized or previously rarely encountered microbes are continually being described in immunocompromised patients, especially those who have AIDS.

Several noninfectious diseases can mimic infection of the soft tissues. For example, patients who have contact dermatitis, pyoderma gangrenosum, gout, psoriatic arthritis with distal dactylitis, Reiter's syndrome, relapsing polychondritis or mixed cryoglobulinemia secondary to immune complex disease from chronic hepatitis C or B virus infection may present with erythematous rashes, with or without fever.

PATHOGENESIS

The integument is an organ that reacts to noxious, infectious, external and internal stimuli in a limited number of ways. It is therefore

not surprising that infection can be mimicked by noninfectious inflammatory conditions. The rich plexus of capillaries beneath the dermal papillae provides nutrition to the stratum germinativum and the dermatocytes, which are bound together by tight junctions and form the barrier to microbial invasion. Once microbes have penetrated this barrier through a hair follicle, cut or bite, the dermal plexus of capillaries delivers the components of the host's defense — oxygen, complement, immunoglobulins, macrophages, lymphocytes and granulocytes — to the site of infection.

Perhaps the first clue available to the immune system to the presence of foreign material in the deep tissues is provided by the organisms themselves. Virtually all bacteria are comprised of proteins whose *N*-terminal amino acid sequence begins with an *N*-formylmethionine group and these proteins are chemoattractive to phagocytes such as macrophages and granulocytes. Other microbial cell wall components such as zymosan of yeast, endotoxins of Gram-negative bacteria and peptidoglycans of Gram-positive bacteria activate the alternative complement pathway,[1] yielding serumderived chemotactic factors. Chemotactic factors are therefore promptly produced at the site of infection by multiple mechanisms.

The efflux or diapedesis of phagocytes through endothelial cell junctions is dependent upon the orchestrated sequential expression of adherence molecules on the surface of the polymorphonuclear leukocyte (PMNL)[2] [3] such as L-selectin and CD11b/CD18 in association with counter-receptors (adhesins) on the endothelial surface.[4] *In vivo*, surface expression of these molecules results first in 'rolling' of PMNLs along the endothelial surface, followed by tethering, and finally firm adhesion of the PMNLs onto the surface of endothelial cells. Phagocytes actually leave the capillary through endothelial cell interstices bound by peripheral endothelial cell adherence molecules, which are found only at these junctional sites.

Once diapedesis has occurred, the PMNL follows the gradient of chemotactic factors derived from the bacteria and serum to the site of active infection. Recent studies suggest that the activated endothelial cells also produce chemotactic cytokines, such as interleukin (IL)-8. Finally, activated granulocytes synthesize leukotriene B_4 from

arachidonic acid, and this too is a potent chemoattractant for leukocytes.

Production of proinflammatory cytokines such as IL-1, tumor necrosis factor a, and IL-6 results in an augmentation of the immune functions described above. These cytokines induce fever, prime neutrophils, and increase antibody production and the synthesis of acute phase reactants, such as C-reactive protein.[5] [6] Cytokine-driven stimulation of endothelial cells also results in the generation of nitric oxide and prostaglandins, both of which cause vasodilation. The net physiologic effect is greater bloodflow to the tissue. These processes result in the cardinal manifestations of inflammation:

- heat,
- swelling,
- erythema, and
- tenderness or pain.

At some locations, factors such as pressure, thrombosis or drugs may reduce or stop bloodflow, resulting in inadequate oxygenation. Compounds such as corticosteroids, which inhibit phospholipase A_2 activity (necessary for releasing arachidonic acid from cell membranes), and nonsteroidal anti-inflammatory agents, which inhibit cyclo-oxygenase (the endogenous enzyme necessary for the synthesis of prostaglandins from arachidonic acid), reduce local bloodflow to tissues. These drugs are therefore useful in the treatment of noninfectious inflammatory conditions because they reduce pain and swelling. However, if the inflammation is secondary to undiagnosed bacterial infection, these drugs may predispose the patient to more severe infection or mask the clinical signs, so delaying the correct diagnosis.

If tissue perfusion is moderately attenuated, tissues may remain viable, but the threshold for progression of infection may be lowered. Predisposing conditions in this category include:

- peripheral vascular disease affecting large arteries,
- diabetes mellitus causing microvascular disease, and
- chronic venous stasis causing postcapillary obstruction.

Necrosis of the skin and deeper tissue may occur if there is severe hypoxia. Two examples are:

- pressure necrosis resulting in decubitus ulcers, and
- compartment syndromes resulting in hypoxia and then necrosis in muscles confined within tight fascial bundles.

When the host is physiologically, structurally and immunologically normal, only certain pathogens such as *Staph. aureus* and group A streptococci are able to cause disease by virtue of their potent virulence factors, such as toxins, capsules or dermonecrotic enzymes, which confer ability to withstand the barrage of host defenses and to induce clinical disease. This statement is supported by the observation that normal skin, although constantly exposed to many indigenous and exogenous microbes, rarely becomes infected.

In contrast, patients who have compromised skin integrity (e.g. patients with burns), vascular defects (e.g. those who have diabetes mellitus or pressure ulceration) or immunologic deficits may become infected with either virulent organisms (e.g. staphylococci or streptococci) or microbes that are usually saprophytic, such as *Pseudomonas aeruginosa, Escherichia coli,* enterococci or *Fusarium* spp. Other defects such as complement deficiency, immunoglobulin deficiencies or neutropenia attenuate the host response to the invading pathogen and predispose to infection.

PREVENTION

Avoidance of cuts, scratches and other forms of trauma that disrupt the natural barrier function of the skin helps to prevent skin and soft tissue infections. For example, stopping shaving may prevent recurrent folliculitis in the beard area (sycosis barbae). Prompt cleansing, debridement and disinfection of such lesions are important for preventing infection, particularly in the case of bite wounds. Treatment of eczema reduces the risk of secondary bacterial superinfection.

Prevention of recurrent folliculitis or furunculosis is difficult to achieve, but there has been some success using intranasal applications of bacitracin or mupirocin ointment. pHisohex (hexachloraphene) baths may be tried to eliminate or reduce staphylococcal carriage in adults.[7] Prophylaxis with systemic antibiotics is of doubtful efficacy and can result in the emergence of resistant strains; it should be tried only for severe cases (see Folliculitis, furuncules and carbuncles, below).

Recurrent bacterial cellulitis of the lower extremities can often be prevented by topical antifungal treatment for dermatophyte infections such as tinea pedis because even minor or inapparent superficial fungal infection can serve as a portal of entry for Gram-positive cocci.

CLINICAL FEATURES, DIAGNOSIS AND MANAGEMENT OF SPECIFIC SOFT TISSUE INFECTIONS

Folliculitis, furuncles and carbuncles

Pustules or abscesses can develop when organisms permanently or transiently resident on the skin surfaces are introduced into deeper tissues (Fig. 9.1). Pathogens can also seed the skin from hematogenous sources such as bacteremias, for example associated with staphylococcal endocarditis (Fig. 9.2) or by contiguous spread from infectious foci in the lung or gastrointestinal tract. Most commonly, small focal abscesses develop in the superficial layers of the skin,

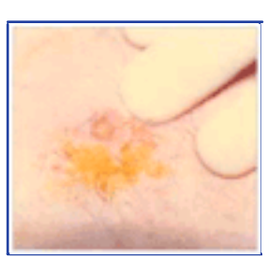

Figure 9-1 Cutaneous infection at the previous insertion site of an intravenous catheter. Organisms from the skin were likely introduced into the dermis and subcutaneous tissue at the time of catheter insertion. Many of these infections remain superficial, but in this patient suppurative thrombophlebitis with bacteremia ensued.

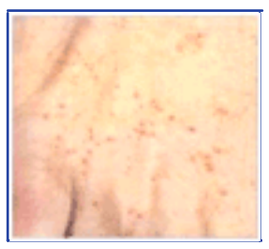

Figure 9-2 Diffuse skin involvement. Petechial lesions in a patient with *Staphylococcus aureus* bacteremia, endocarditis and acute aortic insufficiency.

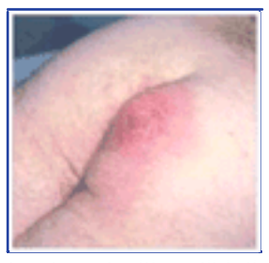

Figure 9-3 Carbuncle of the buttock caused by *Staphylococcus aureus.* This large carbuncle developed over the course of 7–10 days and required surgical drainage plus treatment with antibiotics. The patient had previously experienced numerous episodes of *Staph. aureus* cutaneous abscesses. He carried the staphylococci in his anterior nares.

where hair follicles serve as the portal of entry. Such lesions are called folliculitis. *Staphylococcus aureus* accounts for most of these infections, but many different

bacterial species can occasionally cause localized folliculitis.

Folliculitis can progress to form subcutaneous abscesses, called furuncles or boils, which usually drain and resolve spontaneously, but may progress to form a large, exquisitely painful group of contiguous furuncles, called a carbuncle (Fig. 9.3). Carbuncles require surgical drainage as well as antibiotic treatment.[6]

Recurrent furunculosis

Certain individuals seem to be predisposed to recurrent *Staph. aureus* skin infections (recurrent furunculosis). Although it has been suggested that diabetic patients are especially prone to boils and carbuncles,

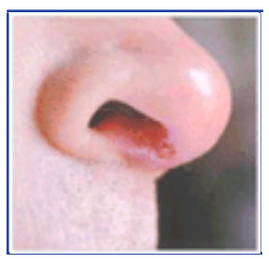

Figure 9-4 Staphylococcal nasal carriage. This patient had a small staphylococcal abscess beneath the mucosa of the nose, illustrating how *Staphylococcus aureus*, which colonizes the nares, can infect skin and submucosa. Intact mucosa is highly resistant to infection; such infections usually occur as a result of defects in the mucosal membranes or via hair follicles inside the nose.

few data support this concept. In contrast, it is well established that patients with Job's syndrome, who have eosinophilia and high levels of serum IgE antibody, are strongly predisposed to these focal *Staph. aureus* infections. However, most patients do not have immunologic or metabolic abnormalities, and the greatest predisposing factor has been the colonization of the anterior nares with *Staph. aureus* (Fig. 9.4). Thus, touching the nose or nasal secretions and then rubbing or scratching the skin results in autoinoculation and abscess formation. Breaking the cycle can be useful to prevent recurrences; however, it is important to document nasal colonization by appropriate techniques. Administration of intranasal mupuricin or bacitracin ointment for the first 5 days of each month has been shown to decrease colonization and reduce the frequency of recurrent infection by about 50%.[8] Treatment of recurrent furunculosis may also require surgical incision and drainage as well as antistaphylococcal antibiotics such as oral dicloxacillin or parenteral nafcillin.

Predisposing factors

Superficial dermal trauma such as insect bites or abrasions can result in cutaneous abscesses. Eczema may also serve as a portal of entry. Superinfected eczema may be difficult to distinguish from eczema itself because both result in crusted lesions, exudation and cutaneous erythema. The presence of lymphangitis, pustules or fever suggests infection. Because *Staph. aureus* is the most common cause of infected eczema, treatment with an oral antistaphylococcal antibiotic such as dicloxacillin is warranted.

Sebaceous glands empty into hair follicles; if the ducts become blocked they form sebaceous cysts, which may resemble staphylococcal abscess or become secondarily infected. Chronic folliculitis is uncommon except in acne vulgaris in which normal flora (e.g. *Propionibacterium acnes*) may play a role.

Recurrent folliculitis is most common in black males and associated with trauma from shaving (folliculitis barbae). Hidradenitis suppurativa occurs in either acute or chronic forms and can lead to recurrent axillary or pudendal abscesses.

Diffuse folliculitis

Diffuse folliculitis occurs in two distinct settings. The first, 'hot tub folliculitis' is associated with water maintained at a temperature between 98.6 and 104°F (37 and 40°C) that is insufficiently chlorinated and is caused by *P. aeruginosa*. The infection is usually self-limited, although serious complications of bacteremia and shock have occasionally been reported.

The second form of diffuse folliculitis, swimmer's itch (Fig. 9.5), occurs when the skin is exposed to water infected with avian freshwater

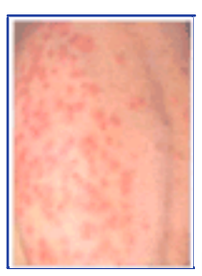

Figure 9-5 Swimmer's itch. Diffuse folliculitis can be caused by *Pseudomonas aeruginosa* (hot tub folliculitis), schistosomes (swimmer's itch) or *Staphylococcus aureus* (folliculitis). This young man had been fishing in an alkaline lake in the western part of the USA. He had been fishing from a 'float tube' and had exposed only his hands and arms to the water. The rash was associated with severe itching. Although his white blood count was not elevated 35% of the white cells were eosinophils.

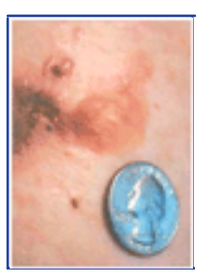

Figure 9-6 Staphylococcal scalded skin syndrome. Flaccid bullae occur as single or multiple lesions. Examination of a frozen tissue section reveals that the cleavage plane is at the stratum corneum. This disease must be distinguished from toxic epidermal necrolysis (see Fig. 9.7).

Figure 9-7 Toxic epidermal necrolysis. This picture shows a skin slough (Nikolsky's sign), which resulted when lateral pressure was applied by the thumb in a plane parallel to the skin surface. This disorder is more common in adults, has a high mortality rate and is usually caused by medications.

schistosomes. Warm water temperatures and alkaline pH are suitable for mollusks, which are the intermediate host between birds and humans. Free-swimming cercariae readily penetrate human hair follicles or pores, but quickly die. This triggers a brisk allergic reaction, causing intense itching and erythema. The infestation is self-limited, secondary infection is uncommon and antipruritics and topical corticosteroid cream promptly relieve the symptoms.

Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome has been described in all age groups, but it is usually seen in children under 5 years of age, including neonates. [9] The characteristic features are a faint erythematous rash with the formation of flaccid bullae (Fig. 9.6). *Staphylococcus aureus* of phage group II is the causative organism. These organisms produce the toxin exfoliatin, which appears to affect the cell junctions of young dermal cells. Specifically, there is intraepidermal cleavage at the level of the stratum corneum. A classic clinical feature is Nikolsky's sign, in which lateral pressure on the skin results in shearing off of the top layer of skin (Fig. 9.7). The

mortality of staphylococcal scalded skin syndrome is low, and fluid loss from the skin is minimal. Appropriate antibiotic therapy is the main component of treatment.

Staphylococcal scalded skin syndrome must be distinguished from toxic epidermal necrolysis, a condition that is more common in adults, is usually secondary to a drug reaction and is associated with high mortality rate (see Fig. 9.7). Frozen section examination of a punch biopsy readily distinguishes these two entities:

- staphylococcal scalded skin syndrome shows a cleavage at the level of the stratum corneum; and
- toxic epidermal necrolysis shows deeper cleavage, at the stratum germinativum.

Impetigo

Impetigo contagiosa is a form of superficial pyoderma caused by streptococci and/or staphylococci. Currently, about half of impetigo cases are caused by *Staph. aureus*.[9] Staphylococci and group A streptococci can be co-isolated from impetiginous lesions in many cases. Group A streptococci alone currently cause less than half the cases. Staphylococcal impetigo tends to occur sporadically, whereas epidemics of impetigo caused by group A streptococci have been well described. Epidemics occur throughout the year in tropical areas or during the summer months in more temperate climates. Impetigo caused by group A streptococci is sometimes complicated by the development of poststreptococcal glomerulonephritis. This important nonsuppurative complication is more likely to occur during epidemics of impetigo caused by certain M types such as M type 49 (see Chapter 225).

Impetigo is characterized by thick-crusted lesions with rounded or irregular margins, often located on the face (Fig. 9.8a).[10] Streptococcal pyoderma frequently has a golden brown or honey color, resembling a plaque of dried serum. Children between 2 and 10 years of age are most commonly infected. Impetigo is often associated with poor socioeconomic conditions and poor hygiene.

Initially, colonization of unbroken skin occurs either exogenously from other infected persons (hence the term impetigo contagiosa) or endogenously by contamination of the skin with organisms carried in the anterior nares or oropharynx. The development of impetiginous lesions takes 10–14 days and likely is initiated through lesions such as minor abrasions and insect bites, which serve as a means of intradermal inoculation. Initially, impetigo may appear as vesicular lesions, which then evolve into crusts (Fig. 9.8b).

Patients should receive penicillin treatment, particularly when numerous sites of the skin are involved, although treatment may not prevent poststreptococcal glomerulonephritis. Topical treatment with an agent effective against Gram-positive bacteria, such as bacitracin or mupirocin is also effective.

Bullous impetigo

Bullous impetigo is caused by strains of *Staph. aureus* harboring a group II bacteriophage that contains genetic elements coding for a

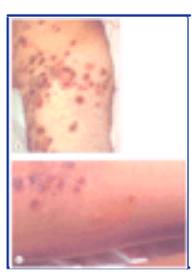

Figure 9-8 Impetigo. (a) Impetigo in a homeless man. Both *Staphylococcus aureus* and group A streptococci were cultured from these lesions. (b) Impetigo, with initial vesicles changing to crusts.

toxin, which causes cleavage in the epidermis. This results in separation of the cellular planes at the level of the stratum corneum and this is responsible for the superficial flaccid bullae that characterize this condition. Several of these lesions may coalesce and spread to form large reddish plaques, usually involving the neck, face or chin. The superficial flaccid bullae are easily ruptured and may not be apparent at the time when the patient is first seen. Because of the superficial nature of these infections, scarring does not occur. Appropriate treatment is an antistaphylococcal antibiotic, which may be given orally.

Ecthyma, paronychia and blistering distal dactylitis

Ecthyma, like impetigo, is characterized by dry crusted lesions of the skin and may be caused by *Staph. aureus*, group A streptococci or both. Unlike impetigo, this lesion extends into the dermis and may therefore lead to post-treatment scarring.[9]

Paronychia is an infection between the nail plate of a digit and the cuticle. It is associated with sucking of the fingers and occupations or hobbies involving prolonged immersion of the hands in water. Paronychia may occur in some immunocompromised patients. Staphylococci are the most common etiologic agents, although oral anaerobes and streptococci may also be isolated. Fungi such as *Fusarium* spp. may be isolated from paronychias in immunocompromised patients. Drainage is best accomplished between the nail-plate and the cuticle. Antimicrobial agents are rarely needed in otherwise healthy individuals.

Blistering distal dactylitis is characterized by painful blisters on the fingerpads of digits. It is most common in children. *Streptococcus*

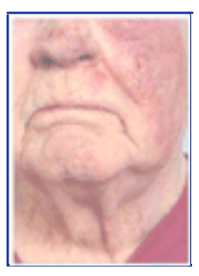

Figure 9-9 Erysipelas. This form of cellulitis is caused by *Streptococcus pyogenes* and is most common in the elderly. Unique characteristics include a fiery red or salmon color, well-demarcated edges, desquamation after 5–7 days and location on the face or lower extemities. This picture was taken 48 hours after treatment with penicillin when the brilliant red salmon color had evolved to a reddish blue color. On the second day of treatment patients usually have less pain and fever subsides, but swelling may be more extensive.

pyogenes is the most common organism isolated, although *Staph. aureus* can cause a similar lesion. Incision and drainage may be useful, in conjunction with an antibiotic appropriate for *Staph. aureus* or *Strep. pyogenes*.

Erysipelas

Erysipelas is a specific variant of cellulitis caused by *Strep. pyogenes,* and occasionally by streptococci of groups B, C and D.[11] [12] It is characterized by an abrupt onset of fiery red swelling of the face or extremities. Distinctive features are well-defined margins, particularly along the nasolabial fold, rapid progression and intense pain (Fig. 9.9). Flaccid superficial bullae may develop during the second to third day of the illness, but extension to deeper soft tissues is rare.

Surgical debridement is rarely necessary, and treatment with penicillin is effective. Swelling may progress for a time despite appropriate treatment, even while fever, pain and the intense red color are diminishing. Desquamation of the involved skin occurs after 5–10 days.

Erysipelas is most common in elderly adults, and the severity of systemic toxicity can vary from region to region. It seems to be less common and less severe now than in the past.

Cellulitis

The term 'cellulitis' is commonly used by physicians, but is not well defined in the literature. It is a localized area of soft tissue inflammation characterized by:

- leukocytic infiltration of the dermis,
- capillary dilatation, and
- proliferation of bacteria.

Clinically cellulitis is recognized as an acute inflammatory condition of the skin characterized by localized pain, erythema, swelling and heat.[6] The area of erythema is a paler pink than the flaming red of erysipelas, and has indistinct margins (Fig. 9.10).

Cellulitis caused by Staphylococcus aureus and Streptococcus pyogenes

Cellulitis is most commonly caused by indigenous flora such as *Staph. aureus* and *Strep. pyogenes,* which colonize the skin and appendages. Bacteria may gain access to the epidermis through cracks in the skin, abrasions, cuts, burns, insect bites, surgical incisions and intravenous catheters.

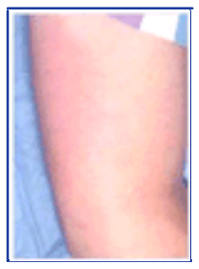

Figure 9-10 Cellulitis. In contrast to erysipelas, cellulitis is a pink color rather than brilliant red and has indistinct margins. *Staphylococcus aureus* and group A, C and G streptococci are the most common etiologies. Many other bacteria may cause cellulitis (see Table 9.1).

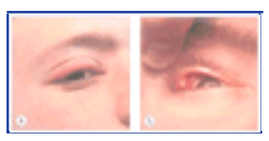

Figure 9-11 Cellulitis. (a) This case was caused by *Staphylococcus aureus* and is spreading centripetally from a central localized focus of infection. The redness and swelling characteristic of cellulitis are apparent over the upper eyelid. (b) The cellulitis has developed from a localized staphylococcal abscess formed in a meibomian gland (chalazion).

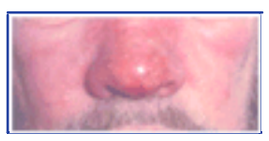

Figure 9-12 *Staphylococcus aureus* cellulitis of the nose. The focal lesion began in a hair follicle inside the nose, with redness, swelling and pain. Rarely, such lesions on the nose are complicated by extension into the cavernous sinus via veins draining the central part of the face.

Cellulitis caused by *Staph. aureus* spreads centripetally from a central localized infection such as an abscess (Fig. 9.11 and Fig. 9.12), folliculitis or foreign body (e.g. a sliver, prosthetic device or intravascular catheter).

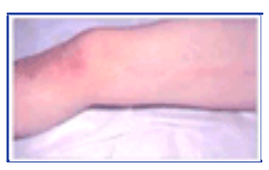

Figure 9-13 Lymphangitis. Cellulitis caused by group A streptococci began below the knee and rapidly spread; about 4 hours later lymphangitis had spread up the inner aspect of the thigh.

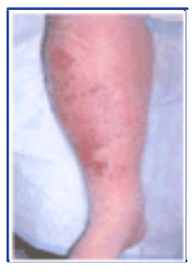

Figure 9-14 Cellulitis of the lower leg associated with chronic venous insufficiency. Streptococci of groups A, B, C and G are the most common isolates. Group B streptococci seldom cause cellulitis in previously healthy hosts, but should be considered in people who have peripheral vascular disease or diabetes mellitus.

In contrast, cellulitis due to *Strep. pyogenes* is a more rapidly spreading diffuse process, frequently associated with lymphangitis (Fig. 9.13) and fever.[13]

Recurrent cellulitis

Recurrent streptococcal cellulitis of the lower extremities may be caused by group A, C or G streptococci in association with skin lesions such as chronic venous stasis (Fig. 9.14), saphenous venectomy for coronary artery bypass surgery,[14] or healed burns, especially if the skin is colonized by dermatophyte fungi. Streptococci also cause recurrent cellulitis among patients with chronic lymphedema resulting from irradiation, lymph node dissection, Milroy's disease or elephantiasis.

Recurrent staphylococcal cutaneous infections occur in individuals who have eosinophilia and elevated serum levels of immunoglobulin E (Job's syndrome) and among chronic nasal carriers of staphylococci.

Cellulitis associated with predisposing conditions

A number of other conditions predispose to infection by endogenous or exogenous pathogens (see Table 9.2). For example:

- *Streptococcus agalactiae* cellulitis occurs in people who have diabetes mellitus or peripheral vascular disease; and[15]
- *Haemophilus influenzae* causes periorbital cellulitis in children in association with sinusitis, otitis media or epiglottitis and will presumably become less common, as has *Haemophilus* meningitis, due to the impressive efficacy of the *H. influenzae* type b vaccine.

Cellulitis associated with bites

Many other species of bacteria can cause cellulitis. These often occur in special settings, and the history can provide useful clues to the diagnosis (see Table 9.2). Bites of various types may introduce specific organisms into the deeper tissues, resulting in soft tissue infections. For example, cellulitis associated with cat bites and, to a lesser degree, dog bites is commonly caused by *Pasteurella multocida*, although in the latter case *Staphylococcus intermedius* and *Capnocytophaga canimorsus* (DF-2) must also be considered. Cellulitis and abscesses associated with dog and human bites also contain a variety of anaerobic organisms.[16] *Pasteurella multocida* is resistant to dicloxacillin and nafcillin, but sensitive to all other ß-lactam antimicrobials as well as quinolones, tetracycline and erythromycin. Ampicillin-clavulanate, ampicillin-sulbactam or cefoxitin are good choices for treating animal or human bite infections.

Soft tissue infections may result from the bites of mosquitoes, horse flies and spiders; usually they cause only local allergic reactions with itching, swelling and

erythema. Similarly, brown recluse spider bites may resemble acute infection at first, but later there is primary tissue destruction and central necrosis due to the action of dermonecrotic toxins. These infections may resemble pyoderma gangrenosum or may become secondarily infected with skin organisms. Mosquito bites may serve as a portals of entry for skin organisms such as *Staph. aureus* or *Strep. pyogenes*. Such infections are not uncommon in clinical practice, but given the number of individuals bitten by insects, infection is a relatively rare complication.

Cellulitis associated with water exposure

Aeromonas hydrophila causes a highly aggressive form of cellulitis in tissues surrounding lacerations that were sustained in fresh water lakes, rivers and streams. This organism is sensitive to aminoglycosides, fluoroquinolones, chloramphenicol, trimethoprim-sulfamethoxazole (co-trimoxazole) and third-generation cephalosporins, but is resistant to ampicilllin.

Fish food containing the water fleas of the genus *Daphnia* can be contaminated with *Mycobacterium marinum,* which may cause cellulitis or granulomas on skin surfaces exposed to the water in aquariums or following injuries in swimming pools. Rifampin (rifampicin) plus ethambutol has been an effective treatment for some, although no comprehensive studies have been carried out. In addition, some strains of *M. marinum* are susceptible to tetracycline or trimethoprim-sulfamethoxazole.

Pseudomonas aeruginosa causes four types of soft tissue Cellulitis caused by *Pseudomonas aeruginosa* and other Gram-negative bacteria infections:

- ecthyma gangrenosum in neutropenic patients,
- hot tub folliculitis,
- burn wound sepsis, and
- cellulitis following penetrating injury.

TABLE 9-5 -- Differential diagnosis of ulcerative skin lesions.

DIFFERENTIAL DIAGNOSIS OF ULCERATIVE SKIN LESIONS	
Clinical condition	**Etiology**
Anthrax	*Bacillus anthracis*
Cutaneous diphtheria	*Corynebacterium diphtheriae*
Ulceroglandular tularemia	*Francisella tularensis*
Bubonic plague	*Yersinia pestis*
Buruli ulcer	*Mycobacterium ulcerans*
Primary syphilis	*Treponema pallidum*
Chancroid	*Haemophilus ducreyi*
Lucio's phenomenon	*Mycobacterium leprae*
Decubitus (pressure) ulcer	Mixed aerobic and anaerobic bacteria
Leishmaniasis	*Leishmania tropica*
Ecthyma gangrenosum	*Pseudomonas aeruginosa*
Tropical ulcer	Idiopathic and nonspecific; mixed bacterial species

In the last of these *P. aeruginosa* is often introduced into the deep tissues by stepping on a nail, a scenario referred to as the 'sweaty tennis shoe syndrome'.

Treatment includes surgical inspection and drainage, particularly if the injury also involves bone or joint capsule. Choices for empiric treatment pending antimicrobial susceptibility data include aminoglycosides, third-generation cephalosporins such as ceftazidime, cefoperazone or cefotaxime, semisynthetic penicillins such as ticarcillin, mezlocillin or piperacillin, or fluoroquinolones. (The quinolones are not approved in children under 13 years of age.)

Cellulitis caused by Gram-negative bacilli, including *P. aeruginosa* as described above, is most common in hospitalized immunocompromised hosts. Recently, *Stenotrophomonas maltophilia* has emerged as an important cause of nosocomial cellulitis in patients who have cancer. [17] The bacterium has been isolated from incubators, nebulizers, humidifiers and tap water in hospitals. The cellulitis may be related to intravenous catheters and in some circumstances may be metastatic via the bloodstream.

Trimethoprim-sulfamethoxazole, or ticarcillin-clavulanic acid, with or without ciprofloxacin are reasonable treatment choices, although cultures and sensitivities are important because of the high prevalence of antibiotic-resistant organisms in the health care environment.

Other causes of cellulitis

The Gram-positive aerobic rod, *Erysipelothrix rhusiopathiae,* which causes cellulitis in bone renderers and fishmongers, remains susceptible to erythromycin, clindamycin, tetracycline and cephalosporins, but is resistant to sulfonamides and chloramphenicol.

Differential diagnosis

The etiology of cellulitis can be suspected on the basis of the epidemiologic data supplied above. If there is drainage, an open wound or an obvious portal of entry, Gram stain and culture can often provide a definitive diagnosis (Table 9.5). In the absence of these findings, the bacterial etiology of cellulitis may be difficult to establish. Even with needle aspiration from the leading edge or punch biopsy of the cellulitis itself, cultures are positive in only 20% of cases.[18] This suggests that relatively low numbers of bacteria may cause cellulitis and that the expanding area of erythema within the skin may be the direct result of extracellular toxins or the soluble mediators of inflammation elicited by the host.

Antibiotic treatment

Because many different microbes can cause cellulitis, the choice of initial empiric antibiotic therapy depends upon the clinical features described above. Once cultures and sensitivities are available, the

choice is easier and more specific. The physician must first decide whether the patient's illness is severe enough to require parenteral treatment, either in hospital or on an outpatient basis.

Presumed streptococcal or staphylococcal cellulitis

For presumed streptococcal or staphylococcal cellulitis, nafcillin, cephalothin, cefuroxime, vancomycin, or erythromycin are good choices. Cefazolin and ceftriaxone have less activity against *Staph. aureus* than cephalothin, although clinical trials have shown a high degree of efficacy. Ceftriaxone is a useful choice for outpatient treatment because it can be given once daily. Similarly, teicoplanin, like vancomycin, has excellent activity against *Strep. pyogenes* and both *Staph. aureus* and *Staph. epidermidis* and may be given once daily by intravenous or intramuscular injection. Because methicillin-resistant *Staph. aureus* has recently increased in prevalence throughout much of the world, vancomycin or linezolid should be used empirically in patients with severe soft tissue infections who are toxic or in those who have recently been hospitalized or received antibiotics.[19]

For patients being treated with oral antibiotics, dicloxacillin, cefuroxime axetil, cefpodoxime, erythromycin, clarithromycin or azithromycin are all effective treatments.

For known group A, B, C or G streptococcal infections, penicillin or erythromycin should be used orally or parenterally. For serious group A streptococcal infections such as necrotizing fasciitis or streptococcal toxic shock syndrome, clindamycin is more efficacious than penicillin.[20] This is probably because in this type of infection where there are large numbers of bacteria, streptococci are in a stationary growth phase and do not express a full complement of penicillin-binding proteins.[21] In contrast, the activity of clindamycin is not affected by inoculum size or growth phase. In addition, clindamycin suppresses the synthesis of many streptococcal exotoxins and surface proteins.[22] [23]

Other types of cellulitis

For cellulitis associated with *Eikenella corrodens* useful antibiotics are penicillin, ceftriaxone, sulfamethoxazole-trimethoprim, tetracyclines and fluoroquinolones. Interestingly, this organism is resistant to oxacillin, cefazolin, clindamycin and erythromycin.

Cellulitis associated with cat bites may fail to respond to treatment with oral cephalosporins, erythromycins and dicloxacillin. Reasons for failure include resistance of *P. multocida* to oxacillin and dicloxacillin and the inadequate serum and tissue levels attained with older oral cephalosporins and erythromycins.

Cutaneous ulcers

Infectious ulceration of the skin results from either:

- direct destruction of dermal cells by bacterial products, or
- an intense inflammatory reaction.

Cutaneous anthrax

This is an example of direct destruction of dermal cells by toxins produced by *Bacillus anthracis* (see Table 9.5). This disease is traditionally contracted by direct inoculation of the skin of animal handlers, especially goat and sheep herders or hide processors, [24] but recent cases have occurred as the result of deliberate bioterrorism (see Chapter 6). The lesion begins as a papule, which evolves into a bulla and then ulcerates. Sepsis may occur. The diagnosis is established by aspiration of the leading edge of the lesion, Gram stain and culture. Penicillin is appropriate therapy.

Cutaneous diphtheria

Since 1980 cutaneous diphtheria has been recognized in homeless individuals who present with chronic nonhealing ulcers with an overlying dirty gray membrane. These lesions may mimic those of psoriasis, eczema or impetigo, but have a deeper base. Appropriate cultures of the ulcer are mandatory because organisms growing from routine cultures may be misidentified as diphtheroids.[25]

Cutaneous tularemia (ulceroglandular tularemia)

Cutaneous tularemia occurs following a tick bite or handling of infected rodents or lagomorphs (rabbits). It most commonly presents with regional lymphadenopathy associated with suppuration and fever, although pneumonic, oculoglandular, oropharyngeal and typhoidal forms have also been described. The characteristic lesion is a small ulceration with an eschar, which develops 2–10 days after exposure. Treatment with streptomycin or gentamycin has been successful, and doxycycline, chloramphenicol or a fluoroquinolone are alternatives.

Buruli ulcer

Buruli ulcer is caused by *Mycobacterium ulcerans*. It presents as a shallow ulcer, which slowly expands centripetally. It is uncommon in the USA and Europe, but is endemic in tropical climates, particularly Africa. Diagnosis is easily established by biopsy, acid-fast staining or culture. The organism is susceptible to isoniazid, rifampin and *para*-amino salicylic acid (PAS). Oral treatment with isoniazid and rifampin for 2–3 months is usually successful.

Leishmaniasis

Leishmaniasis also presents as shallow ulcers with an expanding margin. Diagnosis should be suspected in patients residing in or returning from Central or South America. A biopsy from the raised edge stained with Giemsa or Wright's stain demonstrates the amastigote stage of *Leishmania tropica*. Treatment with antimony compounds is effective, but requires prolonged administration over 4–6 months.

Other causes of cutaneous ulcers

The differential diagnosis of cutaneous ulcers in genital areas should include:

- syphilis,
- chancroid,
- lymphogranuloma venereum, and
- herpes simplex virus infection.

Noninfectious causes of cutaneous ulceration include:

- Behçet's syndrome,
- cutaneous vasculitis, including lupus erythematosus,
- toxic epidermal necrolysis,
- pressure necrosis, and
- brown recluse spider bites.

Solitary shallow ulcers of skin and mucous membranes have also been described in disseminated histoplasmosis.

Herpes simplex can cause primary or recurrent cutaneous infections of the digits (herpetic whitlow) or head and neck. This viral infection is often misdiagnosed and mistreated as a bacterial condition, and occurs in those who are exposed to inoculation of the skin from oral secretions, such as from dentists, dental hygienists, nurses, anesthesiologists and wrestlers.

Orf is caused by a DNA virus similar to smallpox. It causes development of shallow ulcers (in general only one lesion) on the digits of animal handlers working with sheep or goats that harbor open mucous membrane lesions.[24]

Bacillary angiomatosis (see also Chapter 132)

Bacillary angiomatosis is a primary infection of endothelial cells that has important cutaneous manifestations.[26] The lesions may appear as purple nodules resembling Kaposi's sarcoma. They may also appear as scaly or ulcerated lesions and may have the appearance of superficial pink papules or plaques in black people. This disease usually occurs in people who have HIV infection and is caused by *Bartonella henselae* or *B. quintana*. The organisms can be acquired from cat bites and scratches or from cat fleas. The course and extent

Figure 9-15 Gram stain of purulent material demonstrating *Staphylococcus aureus.* The microbial etiology of cellulitis may be suspected based upon signs, symptoms and history; however, definitive diagnosis requires Gram stain and culture. If there is no portal of entry, aspiration or even punch biopsy of cellulitic skin yields a positive culture in only 20% of cases.

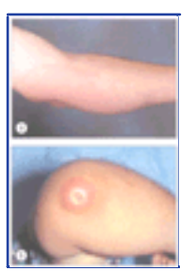

Figure 9-16 Cellulitis at the elbow associated with olecranon bursitis. (a) Pale pink erythema on the inner aspect of the elbow. (b) Careful inspection demonstrates a focal infection over the point of the elbow. Fluid aspirated from the olecranon bursa yielded a pure culture of *Staphylococcus aureus*.

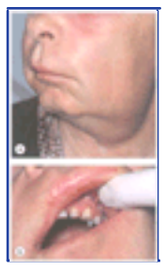

Figure 9-17 Erythema and swelling of the face due to a tooth abscess. (a) Swelling of the face, on inspection resembling periorbital cellulitis. (b) Further inspection reveals a gingival abscess above the patient's left upper canine tooth.

of infection is highly variable and depends upon the host's immune status.

Histopathology reveals capillary proliferation. The organisms can be visualized using Warthin-Starry silver stain or electron microscopy. Bacteriologic identification requires a special culture technique: lysed blood centrifugate or digested tissue is plated onto chocolate or Columbia agar and incubated for 10–14 days at 95°F (35°C) in 5–7% carbon dioxide. Small dry adherent oxidase-negative colonies of Gram-negative curved rods with twitching motility can be identified as *Bartonella* spp. by fluorescent antibody, gas-liquid chromatography or biochemical tests.[27]

Resistance to penicillin, cephalosporins, sulfonamides and vancomycin has been described. The recommended therapy is erythromycin 500mg q6h.[23]

Cutaneous manifestations of infections of deep soft tissues

Staphylococcal infections of deeper tissues may also cause superficial redness, warmth and swelling of the skin, even though the skin itself is not infected (Fig. 9.15). Examples include olecranon bursitis (Fig. 9.16), septic arthritis, osteomyelitis, staphylococcal parotitis and other deep infections of the head and neck, such as anaerobic infections, actinomycosis and tooth abscesses (Fig. 9.17).

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Chapter 10 - Necrotizing Fasciitis, Gas Gangrene, Myositis and Myonecrosis

Dennis L Stevens

INTRODUCTION

The spectrum of infections of the deep soft tissues ranges from localized bacterial, viral and parasitic lesions to rapidly spreading, tissue-destructive infections such as necrotizing fasciitis and myonecrosis. For example, pyomyositis, which is common in the tropics but rare in temperate zones, is a focal infection of skeletal muscle that is usually caused by *Staphylococcus aureus*; it generally remains localized and rarely causes systemic complications. In contrast, necrotizing fasciitis and myonecrosis may be caused by single or multiple pathogens and often give rise to extensive tissue loss, bacteremia, organ failure, shock and death. Even the experienced clinician may have difficulty distinguishing between the different forms of deep soft tissue infection during the early stages. Finally, despite early diagnosis and appropriate treatment, some patients will lose tissue, even limbs, whereas others will succumb to systemic complications. This chapter emphasizes the clinical clues that help to make early, specific diagnoses.

EPIDEMIOLOGY

Until the middle of the 20th century, wartime injuries were commonly complicated by gas gangrene caused by *Clostridium* spp. During the Civil War in the USA, nearly 50% of soldiers who sustained gunshot wounds developed infection and many of these developed gas gangrene. Clostridial gangrene is typically a sporadic infection but during the Civil War apparent epidemics of 'hospital gangrene' were described. Contributing factors included severe trauma, grossly contaminated wounds, crowded and dirty conditions, application of soiled dressings (often recycled from patients who had just died of infection) and primitive surgical techniques for debridement and fixation of open fractures. Group A streptococci undoubtedly caused some of these infections but other major bacterial pathogens, including *Clostridium perfringens*, Gram-negative bacteria and mixed aerobic-anaerobic bacteria, also contributed.

Gas gangrene was also common during the First World War, particularly in the European theater, where the soil was rich and well fertilized with animal feces containing large numbers of vegetative spores of clostridia. In contrast, in North Africa, cases of gangrene following gunshot wounds were far less common, presumably because the desert sand contained few clostridial spores.[1] Gas gangrene has become uncommon in modern warfare because wounded soldiers are evacuated rapidly to well-equipped hospitals for surgical intervention, arterial reconstruction and antibiotic treatment, all of which have greatly reduced the prevalence of this feared disease.

In modern times, these serious deep soft tissue infections have become less common. Sporadic cases in the general population most often occur as occasional complications of penetrating trauma, compound fractures or septic abortions. For the first time in history, spontaneous gas gangrene caused by *Clostridium septicum* may be more common than trauma-associated gas gangrene caused by *C. perfringens*, *C. histolyticum* or other *Clostridium* spp. (see Chapter 232). Recently, severe soft tissue infections caused by *C. perfringens*, *C. sordellii* and *C. novyi* have been described among intradermal ('skin popping') and intravenous drug users.[2] [3]

Necrotizing fasciitis is a life-threatening form of soft tissue infection. It can occur in association with gas gangrene as a part of generalized tissue necrosis or as a separate clinical entity.[4] Two types of necrotizing fasciitis, types I and II, are recognized. Type I necrotizing fasciitis occurs in patients who have diabetes mellitus or severe peripheral vascular disease, or both;[5] it is usually caused by mixed aerobic and anaerobic bacteria. Although the risk for an individual diabetic patient is low, this type of deep soft tissue infection is the most common form of necrotizing fasciitis in the general population, because the total number of people who have diabetes is large.

Type II necrotizing fasciitis, formerly called streptococcal gangrene, is caused by group A streptococci. Since the mid-1980s, this disease has been recognized with increasing frequency in many parts of the world, at a current annual incidence of 5–10 cases per 100,000.[6]

Morbidity and mortality

Before the availability of antibiotics, gas gangrene was usually fatal. Since then, mortality rates from gas gangrene caused by *C. perfringens* have improved, owing to aggressive antibiotic therapy, aggressive surgical therapy employing better surgical techniques, and hyperbaric oxygen therapy. The most important factors in determining outcome, reducing the need for amputations and preventing shock have been early recognition and aggressive treatment.

The mortality and morbidity of group A streptococcal necrotizing fasciitis has evolved differently. In the pre-antibiotic era this infection carried a mortality rate of about 25% when treated with surgery (such as 'bear claw' fasciotomies) alone.[7] In modern times, mortality due to group A streptococcal necrotizing fasciitis has not decreased and continues to range from 30% to 70% despite antibiotics, appropriate surgical debridement and intensive supportive care. This suggests that more virulent strains must be responsible.

CLINICAL FEATURES

Pain, either generalized or localized, is the most common reason for patients who have deep-seated infection to seek medical care (Fig. 10.1). Although myalgia may occur with any febrile illness as part of the systemic immune response, in certain infectious diseases myalgia may provide an invaluable clinical clue. For example, diffuse myalgia is one of the cardinal manifestations of influenza. Severe, localized pain in a febrile patient is a common presentation for deep-seated bacterial infection. Early in the course of necrotizing fasciitis caused by group A streptococci, patients may have a viral-like prodrome with nausea, vomiting, diarrhea and fever; however, later in the course of the disease, patients seek medical assistance because of increasingly severe localized pain with continuing fever.

A portal of entry can be defined in the majority of cases of deep bacterial soft tissue infections such as type I necrotizing fasciitis and

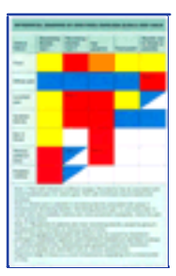

Figure 10-1 Differential diagnosis of infections involving muscle and fascia. Red, severe; orange, moderate; yellow, mild-to-moderate; blue, mild; white, none.

traumatic gas gangrene. In type I necrotizing fasciitis, infection begins at the site of a surgical incision, at a mucosal tear or at sites of skin breakdown in patients who have diabetes mellitus or peripheral vascular disease. Similarly, traumatic gas gangrene occurs at the site of major trauma such as crush injuries or penetrating injuries severe enough to cause arterial damage. In these cases, the clinician has reason to suspect infection as a cause of fever or increasing pain. In contrast, patients who have either type II necrotizing fasciitis or spontaneous gas gangrene may have no apparent portal of entry.[8] Early in the course of infection in such patients, the only physical signs of infection may be fever and localized tenderness. Fever and localized pain are the cardinal clues to this diagnosis but in some cases evidence of localized infection may not become apparent until after the development of systemic signs such as hypotension or organ failure.

Early in the course of deep infection associated with a defined portal of entry there is generally evidence of localized inflammation such as swelling, redness, warmth and tenderness. At that point the process may resemble simple cellulitis, which can be caused by any of a multitude of bacteria (see Chapter 9). In type I necrotizing

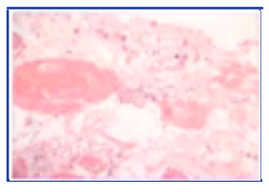

Figure 10-2 Histopathologic examination of tissue from a patient who has necrotizing fasciitis with extension into the underlying musculature. Note the absence of acute inflammatory cells in the area of muscle necrosis. When present, infiltrating granulocytes can be seen at the interface between normal and necrotic tissue and are often massed within small postcapillary venules.

fasciitis there is generally gas in the tissue, as there is in gas gangrene. Where there is no apparent portal of entry, the leading diagnoses to be considered are infection with group A streptococci (type II necrotizing fasciitis), *C. septicum* (spontaneous gangrene) or *Vibrio vulnificus*. The presence of gas in the tissue favors clostridial infection.

Gas may be detected by physical examination (crepitus) or by imaging (radiography, magnetic resonance imaging (MRI) or computerized tomography (CT) scan). Group A streptococci should be suspected if there is fever and severe pain and a history of blunt trauma or muscle strain. *Vibrio vulnificus* should be suspected in a patient who has cirrhosis of the liver, a history of ingestion of raw oysters or exposure to seawater in the south-east Atlantic Ocean or the Caribbean Sea.[9] Later, erythema is superceded by violaceous bullous lesions, massive local swelling becomes apparent and signs of systemic toxicity develop rapidly (see Chapter 230).

SPECIFIC TYPES OF DEEP SOFT TISSUE INFECTION

Necrotizing infections

Necrotizing infections of the skin and underlying soft tissues share the features of fulminant destruction of tissue and severe systemic signs of toxicity associated with high mortality (see Fig 10.1). Few areas of infectious diseases have a more confusing nomenclature. This is partly because authors have named necrotizing infections on the basis of clinical features, whereas these diagnoses should be based on surgical or pathologic findings. Thus, many different names have been used to describe processes that share common pathologic features:

- extensive tissue destruction;
- thrombosis of blood vessels;
- abundant bacteria spreading along fascial planes; and
- relatively few acute inflammatory cells, although small collections of polymorphonuclear leukocytes or microabscesses have been described (Fig. 10.2).

For patients who have evidence of an aggressive localized soft tissue infection, prompt surgical exploration of that site is of extreme importance to determine whether a necrotizing process is present. The same is true for patients who have milder local features associated

with severe systemic toxicity. In addition, although the clinical entity referred to as necrotizing fasciitis may occur alone, there is commonly also evidence of necrosis extending up to the dermis and down to underlying muscle (myonecrosis). Despite these common features, it is worth reviewing the many different types of necrotizing soft tissue infections that have been described in the literature because this may point to clinical clues leading to earlier surgical intervention and therefore an earlier diagnosis.

Necrotizing fasciitis

Necrotizing fasciitis is a deep-seated infection of the subcutaneous tissue that results in progressive destruction of fascia and fat, although it may spare the skin itself.[10] Two clinical types exist.

Type I necrotizing fasciitis

Type I necrotizing fasciitis is a mixed infection caused by aerobic and anaerobic bacteria. It occurs most commonly after surgical procedures, in diabetic patients or in those who have peripheral vascular disease (see Fig. 10.1). Non-clostridial anaerobic cellulitis and synergistic necrotizing cellulitis are both variants of the same syndrome. It may not be important to distinguish these entities from one another because all occur in diabetic patients and are caused by mixed anaerobic and aerobic bacteria.

Clinical features

These infections most commonly occur on or about the feet, with rapid extension along the fascia into the leg. Although cellulitis also occurs commonly in diabetic patients, necrotizing fasciitis should be considered in those who have cellulitis and systemic signs of infection such as tachycardia, leukocytosis, acidosis or marked hyperglycemia. In addition to its spontaneous occurrence in diabetic patients, type I necrotizing fasciitis may also develop as a result of a breach in the integrity of mucous membranes from surgery or instrumentation. In the head and neck region, bacterial penetration into the fascial compartments can result in a related syndrome known as Ludwig's angina (Fig. 10.3) or it may develop into necrotizing fasciitis. Group A streptococci may cause necrotizing fasciitis or a peritonsillar abscess, which can extend into the deep structures of the neck (Fig. 10.4).

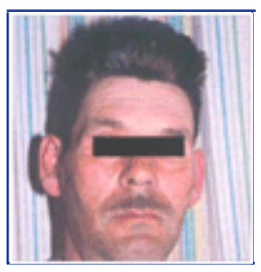

Figure 10-3 Ludwig's angina. Infection begins with a break in the mucosal lining in the oropharynx; oral bacterial flora invade the soft tissues at the base of the tongue and penetrate through the floor of the mouth and into soft tissue of the neck. The floor of the mouth is elevated and patients talk as though they have a 'hot potato' in their mouth. Potential airway obstruction is a major concern. Although patients usually respond to penicillin, surgical consultation should be obtained and CT or MRI scans are useful for determining whether a necrotizing process is present.

Diagnostic tests

The first goal of management is to determine the depth and extent of the infection. Computerized tomography or MRI scans are invaluable in this regard to determine whether the infection is localized or spreading along fascial planes. The second goal is to determine whether surgical intervention is necessary. Because of the proximity to vital structures of the neck, surgical consultation is of major importance because exploration, drainage and debridement may be necessary to prevent airway obstruction, to determine the level of soft tissue involvement and to establish which bacteria are involved.

Treatment

Both Ludwig's angina and necrotizing fasciitis of the head and neck are usually caused by mouth anaerobes such as *Fusobacterium* spp. anaerobic streptococci, *Bacteroides* spp. and spirochetes. Either penicillin or clindamycin is effective treatment largely because the Gram-positive aerobic cocci and anaerobes of the oropharynx are generally susceptible to both. In contrast, type I necrotizing fasciitis below the diaphragm requires ampicillin plus clindamycin and a fluoroquinalone to cover the *Bacteroides* spp. and enterobacteriacea.

Type II necrotizing fasciitis

Type II necrotizing fasciitis is caused by group A streptococci and was previously called streptococcal gangrene.[5] In recent years, there has been a dramatic increase in the number of invasive infections, including necrotizing fasciitis, caused by group A streptococci. In contrast to type I necrotizing fasciitis, type II may occur in any age group and among patients who do not have complicated medical illnesses. Predisposing factors include:

- a history of blunt trauma,
- muscle strain,

- childbirth,
- chickenpox,
- non steroidal anti-inflammatory agents,[11]
- intravenous drug abuse, or
- penetrating injury such as caused by a laceration or a surgical procedure.

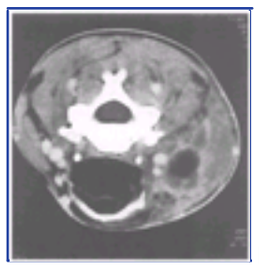

Figure 10-4 Computerized tomography of a soft tissue infection of the neck. This infection is caused by group A streptococci, which invaded as a rare complication of a previous 'strep throat'. Surgical drainage yielded a pure culture of group A streptococci and established a diagnosis. The patient was treated with intravenous penicillin for 10 days and made a good recovery.

In penetrating injuries, it is the skin rather than the mucous membranes that serve as the portal of entry for the streptococci. In contrast, among patients who do not have a defined portal of entry, hematogenous translocation of group A streptococci from the throat (asymptomatic or symptomatic pharyngitis) to the site of blunt trauma or muscle strain probably occurs.[12] The only other possibility, which is of course highly conjectural, is that group A streptococci reside in a dormant state in the deep tissue and trauma of various types reactivates their growth.

Group A streptococci are contagious microbes that in the past have caused epidemics of pharyngitis and scarlet fever in schools, rheumatic fever in military recruits and surgical wound infections in hospitalized patients. Thus, close contacts of a patient who has type II necrotizing fasciitis have a high likelihood of becoming colonized with a virulent strain. Clearly, the risk of developing a secondary case of fulminant necrotizing fasciitis is very low, but it is probably 50-fold higher than it is in the general population. In evaluating the risk to family members and hospital workers, the physician should consider the degree of exposure and the susceptibility of the host. Those contacts with conditions such as open wounds or chickenpox, as well as those family members and health care workers with frequent or continuous contact with a case of necrotizing streptococcal infection, should be treated with an agent to which the strain is sensitive (e.g. penicillin).

Pathogenesis

Pyrogenic exotoxins possess the unique ability to bind simultaneously to the MHC (major histocompatibility complex) class II portion of antigen-presenting cells, such as macrophage and specific Vß (variable part of ß chain) segments of the T-cell receptor in the absence of classic antigen processing by the macrophage. [13] Thus, pyrogenic exotoxins are superantigens that can cause rapid proliferation of T cells bearing specific Vß repertoires (see Chapter 2 and Chapter 56). Such stimulation of the host's immune cells is associated with production of both monokines (tumor necrosis factor (TNF)-a), interleukin (IL)-1 and IL-6)) and the lymphokines (IL-2, interferon and TNF-ß).[14] Expression of these cytokines in vivo probably contributes to shock, organ failure and tissue destruction.[15]

Clinical features

Necrotizing fasciitis exhibits a remarkably rapid progression from an inapparent process to one associated with extensive destruction of tissue, systemic toxicity, loss of limb or death.[8] [12] [16] Unexplained pain that increases rapidly over time may be the first manifestation of infection.[8] [12] The early signs and symptoms of infection may not be apparent, particularly in patients who have postsurgical infection, gunshot or knife wounds, or diabetes. In patients who have diabetes, the absence of pain may be related to neuropathy and anesthesia at the site of infection. In surgical patients, patients who have traumatic injuries and postpartum patients, the increasing pain may be assumed to be part of the normal convalescence rather than to be due to acute infection. Such a delay in diagnosis may allow the disease to progress to later stages before appropriate antibiotics and surgical invention are initiated.

In addition to pain, there may also be fever, malaise, myalgias, diarrhea and anorexia during the first 24 hours; erythema, which may be diffuse or local, may also be present. However, in most patients excruciating pain in the absence of any cutaneous findings

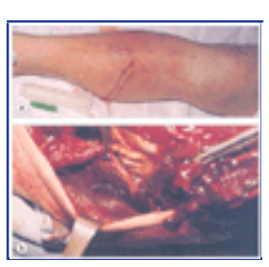

Figure 10-5 Type II necrotizing fasciitis caused by group A streptococci. (a) This patient was a 60-year-old man who had type II diabetes mellitus and who had a 3-day history of malaise, diffuse myalgia and low-grade fever. Over the course of 2–3 hours the pain became excruciating and was localized to the calf. During this time the calf swelled. Note that the skin over the anterior shin looks relatively normal, but that two small purple bullae are present. (b) Extensive necrotizing fasciitis was present on surgical exploration. In addition, myonecrosis was present beneath the fascia. The patient developed profound hypotension, acute respiratory distress syndrome and renal failure. He died despite aggressive surgical and medical management. There was no definable portal of entry, yet group A streptococci were grown from deep cultures and from blood.

may be the only clue of infection. Within 24–48 hours, erythema may develop or darken to a reddish-purple color, frequently with associated blisters and bullae. Conversely, erythema may be absent and the characteristic bullae may develop in skin of normal appearance. The bullae are initially filled with clear fluid and rapidly take on a blue or maroon appearance (Fig. 10.5). When the bullous stage is observed, there is already extensive necrotizing fasciitis (Fig. 10.5) and patients usually exhibit fever and systemic toxicity.

Although many different M-types of group A streptococci have been associated with necrotizing fasciitis in the past, M type I and 3 have been the strains most commonly isolated from patients throughout the world.[12] These strains can produce one or more of the pyrogenic exotoxins A, B and C.[8] [17] Necrotizing fasciitis caused by these strains is frequently associated with 'streptococcal toxic-shock syndrome'.[8] The hallmarks of this syndrome are the early onset of shock and multiple organ failure (see Chapter 225).

Diagnosis

Laboratory tests such as creatine phosphokinase, aspartate aminotransferase and serum creatinine usually show elevated levels and, together with leukocytosis with marked left shift, these findings should be sufficient to prompt surgical exploration. [8] Some experts have advocated punch biopsy and frozen section to establish the diagnosis; however, there may be false-negative findings if the deep tissue is not adequately sampled. Routine soft tissue radiographs, CT scans and MRI scans show soft tissue swelling.[18] Gas is not present and abscess formation is not apparent. These radiographic abnormalities

Figure 10-6 Postpartum sepsis due to group A streptococci. The patient was a 24-year-old woman who delivered a normal child. Thirty-six hours after delivery she developed fever, leukocytosis with marked left shift and increasing low abdominal pain. This MRI demonstrates swelling of the uterus, although not out of proportion for a recent delivery. There was no gas in the tissue. An emergency laparotomy revealed necrosis of the mucosa of the uterus, necrotizing fasciitis and myonecrosis of the uterus.

are not unusual in uninfected patients who have trauma or in postsurgical or postpartum patients (Fig. 10.6). In such cases, direct surgical exploration will determine whether necrotizing fasciitis is present, accomplish debridement of necrotic tissue and obtain material suitable for Gram stains and culture.

Management

The three main themes in treatment are surgical debridement, appropriate antibiotics and intensive supportive care. Some patients require mechanical ventilation and others need hemodialysis. Because of intractable hypotension and diffuse capillary leak, massive amounts of intravenous fluids (10–20 liters per day) are often necessary, although anasarca is a common complication. In some patients blood pressure improves with intravenous fluid alone. Pressors such as dopamine may be useful, but there is little controlled information from clinical or experimental studies in this specific infection. Although potent vasoconstrictors such as epinephrine (adrenaline) may improve blood pressure, symmetric gangrene may ensue, partly as a result of the drug and partly as a result of poor perfusion caused by the bacteria, toxins and endogenous mediators.

Antibiotic selection is difficult in patients who have rapidly progressing infection. Recent studies suggest that clindamycin is superior to penicillin for treatment of experimental necrotizing fasciitis or myonecrosis caused by group A streptococci.[19] This corroborates studies done by Eagle more than 40 years ago, which demonstrated the failure of penicillin in this setting.[20] It seems likely that penicillin failure is due to the reduced expression of critical penicillin-binding proteins during the stationary phase of bacterial growth.[21] Clindamycin may be more efficacious because:

- it is not affected by inoculum size or stage of growth,
- it suppresses toxin production,
- it facilitates phagocytosis of *Streptococcus pyogenes* by inhibiting M-protein synthesis,
- it suppresses the production of regulatory elements that control cell wall synthesis, and
- it has a long postantibiotic effect.[22]

Neutralization of circulating streptococcal toxins is a desirable therapeutic goal and is advocated by some experts.[23] [24] [25] [26] There seems little question that some batches of intravenous gammaglobulin contain neutralizing antibodies against some streptococcal toxins.[25] On

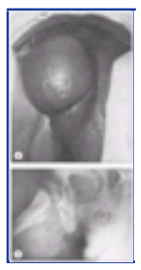

Figure 10-7 Type I necrotizing fasciitis. A 24-year-old man had been in good health but was awakened with severe perineal pain. (a) This photograph was taken 3 hours later. Note the massive swelling of the scrotum. (b) Soft tissue radiograph shows gas in the tissues of the thigh, buttocks, scrotum and anterior abdominal wall. Surgical inspection revealed brownish fluid in the scrotum, with gray, dull-colored, friable fascia but normal underlying musculature. Cultures grew *Enterococcus faecalis*, *Bacteroides fragilis*, *Escherichia coli* and anaerobic streptococci. The patient was treated with ampicillin, clindamycin and gentamicin for 3 weeks and surgical drains were placed in the scrotum, buttocks, thigh and anterior abdominal wall. There was an excellent clinical response. In some cases, surgery of a more radical nature may be necessary.

the basis of two case reports[23] [24] and a report of a non-randomized clinical trial, there is a suggestion that this treatment may affect the mortality and morbidity of this fulminant infection.[26]

Fournier's gangrene

In the perineal area, penetration of the gastrointestinal or urethral mucosa by bacteria may cause 'Fournier's gangrene', an aggressive infection caused by aerobic Gram-negative bacteria, enterococci and anaerobic bacteria such as *Bacteroides* spp. and peptostreptococci. These infections begin abruptly with severe pain and may spread rapidly to the anterior abdominal wall and the gluteal muscles; in males, infection frequently extends to the scrotum and penis (Fig. 10.7).

Surgical inspection, placement of drains and appropriate surgical debridement are necessary for both diagnosis and treatment. Antibiotic treatment should be based upon Gram stain, culture and sensitivity information when available. An appropriate empiric regimen would be either ampicillin or ampicillin and sulbactam combined with either clindamycin or metronidazole. Alternatively, broader Gram-negative coverage might be advisable if the patient has had prior hospitalization or if antibiotics have been used recently. This could be accomplished by substituting ticarcillin-clavulanic acid or piperacillin-tazobactam for ampicillin or by adding a fluorinated quinolone or an aminoglycoside.

Meleney's synergistic gangrene

This rare variant occurs in postsurgical patients. The lesion is a slowly expanding, indolent ulceration that is confined to the superficial fascia. It results from a synergistic interaction between *Staph. aureus* and microaerophilic streptococci. As in other forms of necrotizing infection, antibiotic therapy together with surgical debridement are the mainstays of treatment.

Non-clostridial anaerobic cellulitis

In non-clostridial anaerobic cellulitis, infection is associated with mixed anaerobic and aerobic organisms that produce gas in tissues. Unlike clostridial cellulitis, this type of infection is usually associated with diabetes mellitus and it often produces a foul odor. Surgical exploration is required to distinguish this condition from necrotizing cellulitis, myonecrosis and necrotizing fasciitis by *Clostridium* spp.

Clostridial cellulitis

In clostridial cellulitis, infection is usually preceded by local trauma or recent surgery. *Clostridium perfringens* is the most common species causing this entity. Gas is invariably found in the skin; the fascia and deep muscle are spared. Although clostridial cellulitis differs from clostridial myonecrosis in that there is less systemic toxicity, it is mandatory that thorough surgical exploration and debridement be performed to distinguish these entities. Magnetic resonance imaging or CT scans as well as a serum creatinine phosphokinase assay may also be useful for determining whether muscle tissue is involved. Treatment is discussed below under gas gangrene.

Clostridial gas gangrene

Three types of clostridial soft tissue infections have been defined: [1]

- simple wound contamination or colonization,
- anaerobic cellulitis, and
- clostridial gas gangrene.

The first type, simple wound contamination or colonization, does not progress to true infection for various reasons (e.g. there may be insufficient devitalized tissue to

promote infection or there may be effective host responses or effective medical and surgical management). Contamination is a very common occurrence; 30–80% of open traumatic wounds contain clostridial species.[27]

The second type, anaerobic cellulitis, occurs when there is devitalized tissue in a wound, sufficient for growth of *C. perfringens* or other strains. Although gas is produced locally and extends along fascial planes, bacteremia and invasion of healthy tissue do not occur. Appropriate medical and surgical management, including prompt removal of the devitalized tissue, is all that is necessary for cure and mortality is generally nil.[1]

The third type is clostridial gas gangrene or myonecrosis. This is defined as an acute invasion of healthy living muscle that is undamaged by previous trauma or ischemia.[27] It is divided into three different subtypes:

- traumatic gas gangrene,
- spontaneous or non-traumatic gas gangrene, and
- recurrent gas gangrene caused by *C. perfringens*.

Traumatic gas gangrene is the most common subtype. It develops when a deep, penetrating injury that compromises the blood supply (e.g. knife or gunshot wounds, crush injury or car accident) creates an anaerobic environment that is ideal for clostridial proliferation. This type of trauma accounts for about 70% of cases of gas gangrene. *Clostridium perfringens* is found in about 80% of such infections;[1] the remaining cases are caused by *C. septicum, C. novyi, C. histolyticum, C. bifermentans, C. tertium* and *C. fallax*. Other conditions associated with traumatic gas gangrene are bowel and biliary tract surgery, intramuscular injection of adrenaline, illegal abortion, retained placenta, prolonged rupture of the membranes and intrauterine fetal demise or missed abortion in postpartum patients.

Spontaneous or non-traumatic gas gangrene is less common. This is often caused by the more aerotolerant species *C. septicum*. As described below, most of these cases occur in patients who have a gastrointestinal portal of entry such as adenocarcinoma.

Third, and least common, is recurrent gas gangrene caused by *C. perfringens*. This has been described in people who have non-penetrating injuries at sites of previous gas gangrene; residual spores of *C. perfringens* may remain quiescent in tissue for periods of up to 20 years, and then germinate when minor trauma provides anaerobic conditions suitable for growth. [28]

Traumatic gas gangrene

Pathogensis

The initiating trauma introduces organisms (either vegetative forms or spores) into the deep tissues and produces an anaerobic niche with a sufficiently low redox potential and acid pH for optimal clostridial growth.[1] [27] Necrosis progresses within hours. At the junction of necrotic and normal tissues few polymorphonuclear leukocytes are present, yet pavementing of these cells along the endothelium is apparent within capillaries and in small arterioles and postcapillary venules.[29] [30] Later in the course of the illness there is leukostasis within larger vessels. Thus, the histopathology of clostridial gas gangrene is opposite to that seen in soft tissue infections caused by pyogenic organisms such as *Staph. aureus*, in which an early luxuriant influx of polymorphonuculear leukocytes localizes the infection without adjacent tissue or vascular destruction. Recent studies suggest that s-toxin (Fig. 10.8), when elaborated in high concentrations at the site of infection, destroys host tissues and inflammatory cells.[31] As the toxin diffuses into surrounding tissues or enters the systemic circulation, s-toxin promotes dysregulated adhesive interactions between polymorphonuclear leukocytes and endothelial cells and primes leukocytes for increased respiratory burst activity. [31] These actions lead to vascular leukostasis, endothelial cell injury and regional tissue hypoxia. Such perfusion deficits expand the anaerobic environment and contribute to the rapidly advancing margins of tissue destruction that are characteristic of clostridial gangrene.[1]

Figure 10-8 Colonies of *Clostridium perfringens* growing on an anaerobic blood agar plate. Theta toxin causes the clear zone of hemolysis closest to the colony. A second area of partial hemolysis is caused by a-toxin, an enzyme with phospholipase C activity.

Shock associated with gas gangrene may be attributable, in part, to direct and indirect effects of toxins. Alpha toxin (see Fig. 10.8), a phospholipase C, directly suppresses myocardial contractility ex vivo[32] and may contribute to profound hypotension via a sudden reduction in cardiac output.[33] In experimental models, theta toxin (a cholesterol-binding cytolysin) causes 'warm shock', defined as a markedly reduced systemic vascular resistance combined with a markedly increased cardiac output.[32] [33] It is clear that theta toxin accomplishes this indirectly by inducing endogenous mediators that cause relaxation of blood vessel wall tension, such as the lipid autacoids prostacyclin or platelet-activating factor.[34] Reduced vascular tone develops rapidly and, in order to maintain adequate tissue perfusion, a compensatory host response is required; this either increases cardiac output or rapidly expands the intravascular blood volume. Patients who have Gram-negative sepsis compensate for hypotension by markedly increasing cardiac output; however, this adaptive mechanism may not be possible in shock induced by *C. perfringens* due to direct suppression of myocardial contractility by alpha toxin.[32] The role of other endogenous mediators such as cytokines (e.g. TNF, IL-1, IL-6) as well as the potent endogenous vasodilator bradykinin have not been elucidated.

Prevention

Aggressive debridement of devitalized tissue and rapid repair of compromised vascular supply greatly reduce the frequency of gas gangrene in contaminated deep wounds. Intramuscular adrenaline, prolonged application of tourniquets and surgical closure of traumatic wounds should be avoided. Patients who have compound fractures are at particular risk of gas gangrene if the wound is surgically closed. Patients who have contaminated wounds should receive prophylactic antibiotics.

Clinical findings

The first symptom is usually the sudden onset of severe pain at the site of recent surgery or trauma.[2] [25] The mean incubation period is less than 24 hours, but it ranges from 6–8 hours to several days, probably depending on the degree of soil contamination or bowel contents spillage and the extent of vascular compromise.

The skin may initially appear pale, but it quickly changes to bronze then purplish red, becoming tense and exquisitely tender (Fig. 10.9). Bullae develop; they may be clear, red, blue or purple.

Gas in tissue may be obvious from physical examination, soft tissue radiographs, CT scan or MRI. Interestingly, none of these radiographic procedures have proved to be more specific or more sensitive than the physical finding of crepitus in the soft tissue.[10] However, radiographic procedures are particularly helpful for demonstrating gas in deeper tissue such as the uterus.

Signs of systemic toxicity develop rapidly; these include tachycardia, fever and diaphoresis, followed by shock and multiple organ failure. Shock is present in 50% of patients at the time they present to the hospital.[35] Bacteremia occurs in 15% of patients and may be associated with brisk hemolysis. In one patient, the hematocrit fell from 37% to 0% over a 24-hour period.[36] Subsequently, despite transfusion with 10 units of packed red blood cells over 4 hours, the hematocrit never exceeded 7.2%.[36] Based on my studies using recombinant alpha and theta toxins, it is clear that both toxins contribute to this marked intravascular hemolysis (unpublished). Not all cases of *C. perfringens* bacteremia have been associated with gas gangrene[37] but 90% of *C. perfringens* and 100% of *C. septicum* isolates from blood were associated with clinically significant infection.[38]

Additional complications of clostridial myonecrosis include jaundice, renal failure, hypotension and liver necrosis. Renal failure is largely due to hemoglobinuria and myoglobinuria, but it may be a

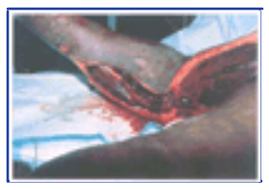

Figure 10-9 Extensive gas gangrene of the arm due to *Clostridium perfringens.* A 35-year-old man sustained a knife wound to the forearm. He did not seek medical care, but 36 hours later experienced severe pain in the upper arm and came to the emergency room. There was extreme tenderness of the arm and crepitus was easily demonstrated. A radiograph also demonstrated gas in the deep soft tissues. Surgical debridement and antibiotics were instituted, but later amputation at the level of the shoulder was necessary. A pure culture of *C. perfringens* was grown from the deep tissues.

result of acute tubular necrosis caused by hypotension. Renal tubular cells are probably directly affected by toxins, but this has not been proved.

Diagnosis

Increasing pain at a site of previous injury or surgery, together with signs of systemic toxicity and gas in the tissues, support the diagnosis. Definitive diagnosis rests on demonstrating large, Gram-variable rods at the affected site (Fig. 10.10). Note that although clostridia stain Gram positive when obtained from bacteriologic media, when visualized from infected tissues they often appear both Gram positive and Gram negative. In fresh specimens *C. perfringens* may appear to be encapsulated,[39] although this was not corroborated in gas gangrene associated with wartime trauma. [40] Surgical exploration is essential. The exposed muscle appears edematous, may be an abnormal reddish-blue to black color and does not bleed or contract when stimulated. Usually, some degree of necrotizing fasciitis and cutaneous necrosis are also present. Microscopic evaluation of biopsy material (see Fig. 10.10) demonstrates organisms among degenerating muscle bundles and characteristically an absence of acute inflammatory cells.[27] [41]

Management

Penicillin, clindamycin, tetracycline, chloramphenicol, metronidazole and a number of cephalosporins have excellent in vitro activity against *C. perfringens* and other clostridia. No controlled clinical trials have ever been conducted to compare the efficacy of these agents in humans. Based strictly on in-vitro susceptibility data, most textbooks state that penicillin is the drug of choice.[42] [43] However, experimental studies in mice suggest that clindamycin has the greatest efficacy and penicillin the least.[44] [45] Other agents with greater efficacy than penicillin include erythromycin, rifampin (rifampicin), tetracycline, chloramphenicol and metronidazole.[44] [45] Slightly greater survival was observed in animals receiving both clindamycin and penicillin; in contrast, antagonism was observed with penicillin plus metronidazole.[45] Because between 2% and 5% of strains are resistant to clindamycin, a combination of penicillin and clindamycin is

Figure 10-10 *Clostridium perfringens* in a patient who has extensive gas gangrene. (a) Tissue Gram stain of tissue removed from the arm of the patient described in Figure 10.9 . Note that the bacteria are rod shaped but gram variable. Note also that there are few if any acute inflammatory cells at the site of infection. (b) Transmission electron micrograph of *C. perfringens*. Note the endospores.

warranted. Based on his experimental studies and his vast clinical experience with gas gangrene, the late Dr William Altemeier recommended tetracycline and penicillin. [46] Thus, given an absence of efficacy data from a clinical trial in humans, the best treatment would appear to be clindamycin or tetracycline combined with penicillin. The failure of penicillin in experimental clostridial myonecrosis may be related to continued toxin production and filament formation rather than lysis.[47] In contrast, the efficacy of clindamycin and tetracycline may be related to their ability to inhibit toxin synthesis rapidly.[47]

Aggressive and thorough surgical debridement is mandatory to improve survival, preserve limbs and prevent complications.[42] [43] The use of hyperbaric oxygen (HBO) is controversial, although some non-randomized studies have reported good results with HBO therapy when combined with antibiotics and surgical debridement.[35] [48] [49] Experimental studies in animals have demonstrated that HBO alone can be effective treatment if the inoculum is small and treatment is begun immediately.[50] In contrast, other studies have demonstrated that HBO was only of slight benefit when combined with penicillin.[51] However, survival was better with clindamycin alone than with either HBO alone, penicillin alone or HBO plus penicillin together.[51] The benefit of HBO, at least theoretically, is to inhibit bacterial growth,[52] to preserve marginally perfused tissue and to inhibit toxin production.[53] Interestingly, Altemeier did not use HBO and was able to realize a mortality rate of less than 15% using surgical debridement and antibiotics (tetracycline plus penicillin) alone.[46]

Therapeutic strategies directed against toxin expression in vivo, such as neutralization with specific antitoxin antibody or inhibition of toxin synthesis, may be valuable adjuncts to traditional antimicrobial regimens. Currently, antitoxin is no longer available. Future strategies may target endogenous proadhesive molecules such that toxin-induced vascular leukostasis and resultant tissue injury are attenuated.

Prognosis

Patients presenting with gas gangrene of an extremity have a better prognosis than those who have truncal or intra-abdominal gas gangrene, largely because it is difficult to debride such lesions adequately.[42] [43] [54] Hyperbaric oxygen could be useful in such patients, yet there are few data on this subject. In addition to truncal gangrene, patients who have associated bacteremia and intravascular hemolysis have the greatest likelihood of progressing to shock and death. In one study, of those patients who developed shock at some point in their hospitalization, 40% died, compared with 20% mortality in the group as a whole.[35] In another study, those who were in shock at the time of diagnosis had the highest mortality.[54]

Spontaneous, non-traumatic gas gangrene due to *Clostridium septicum*

Pathogenesis

Predisposing factors include:[54] [55] [56]

- colonic carcinoma,
- diverticulitis,
- gastrointestinal surgery,
- leukemia,
- lymphoproliferative disorders,
- cancer chemotherapy,
- radiation therapy, and
- AIDS.

Cyclic or other forms of neutropenia are also associated with spontaneous gas gangrene due to *C. septicum* and in such cases necrotizing enterocolitis, cecitis or distal ileitis are commonly found. These gastrointestinal pathologies permit bacterial access to the bloodstream; consequently, the aerotolerant *C. septicum* can become established in normal tissues. [1]

Clostridium septicum produces four toxins:

- a-toxin (lethal, hemolytic, necrotizing activity),
- ß-toxin (deoxyribonuclease),
- ?-toxin (hyaluronidase), and
- ?-toxin (septicolysin, an oxygen labile hemolysin).

Clostridium septicum also produces a protease and a neuraminidase.[1]

The *C. septicum* a-toxin does not possess phospholipase activity and is thus distinct from the a-toxin of *C. perfringens*. Active immunization against a-toxin significantly protects against challenge with viable *C. septicum*. [57] The mechanism by which a-toxin contributes to *C. septicum* pathogenesis is unknown; however, the recent

cloning and sequencing of this toxin should facilitate studies in this area (see Chapter 232).

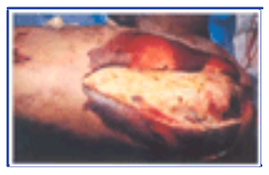

Figure 10-11 Spontaneous necrotizing fasciitis due to *Clostridium septicum.* This patient developed the sudden onset of severe pain in the forearm. Swelling rapidly ensued and he sought medical treatment. Crepitus was present on physical examination and gas in the soft tissue was verified with routine radiographs. Immediate surgical debridement revealed necrotizing fasciitis but sparing of the muscle. Note the purple-violaceous appearance of the skin. See also Figure 10.12 .

Clinical features

The onset of disease is abrupt, often with excruciating pain, although the patient may sense only heaviness or numbness.[1] [27] [54] [55] [56] The first symptom may be confusion or malaise. Extremely rapid progression of gangrene follows. Swelling advances and bullae appear; these are filled with clear, cloudy, hemorrhagic or purplish fluid. The skin around such bullae also has a purple hue (Fig. 10.11), perhaps reflecting vascular compromise resulting from bacterial toxins diffusing into surrounding tissues.[54] Histopathology of muscle and connective tissues includes cell lysis and gas formation; inflammatory cells are notably absent.[54]

Diagnosis

Unlike the situation in traumatic gas gangrene, bacteremia precedes cutaneous manifestations by several hours. In the absence of the usual cutaneous manifestations of gas gangrene, other causes of fever and extremity pain such as deep vein thrombophlebitis or cellulitis are naturally considered first, delaying appropriate diagnosis and treatment and as a consequence, increasing mortality.

Management

No comparative human trials have evaluated the efficacy of antibiotics or HBO for treating clinical cases of spontaneous gas gangrene. In-vitro data indicate that *C. septicum* is uniformly susceptible to penicillin, tetracycline, erythromycin, clindamycin, chloramphenicol and metronidazole. The aerotolerance of *C. septicum* may reduce the likelihood that HBO therapy would be effective.[52]

Prognosis

The mortality of spontaneous clinical gangrene ranges from 67% to 100%, with the majority of deaths occurring within 24 hours of onset. Unfavorable factors include underlying malignancy and compromised immune status. All patients who survive bacteremia or spontaneous gangrene caused by *C. septicum* should have appropriate diagnostic studies of the gastrointestinal tract (Fig. 10.12). Occasionally, this has led to detection and cure of an unsuspected malignancy that might otherwise have been fatal.[54]

Clostridium sordellii infections

Patients who have *C. sordellii* infection present with unique clinical features including edema, absence of fever, leukemoid reaction, hemoconcentration

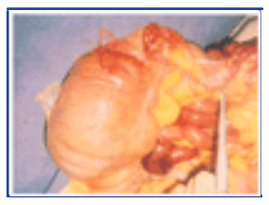

Figure 10-12 Colonic carcinoma in a patient who has spontaneous gas gangrene caused by *Clostridium septicum.* The patient described in Figure 10.11 was found to have a mass in the colon. Surgical resection revealed an adenocarcinoma, which probably served as a portal of entry for the *Clostridium septicum* bacillus. Hematogenous seeding of the forearm resulted in spontaneous gas gangrene.

and later shock and multiple organ failure.[43] Often *C. sordellii* infections develop after childbirth or after gynecologic procedures[58] and most represent endometrial infection. Rarely, other cases have occurred at sites of minor trauma such as lacerations of the soft tissues of an extremity. Recently, outbreaks of *C. sordellii* and *C. novyi* infections have been described among intravenous drug users in Scotland, Ireland and England. Patients have presented with severe soft tissue infections with shock with a case fatality rate of 20–30%.[3] Unlike *C. perfringens* and *C. septicum* infections, pain may not be a prominent feature of *C. sordellii* infections. The absence of fever and the paucity of signs and symptoms of local infection make early diagnosis difficult.[43] The mechanisms of diffuse capillary leak, massive edema and hemoconcentration are not well established but clearly are related to elaboration of a potent toxin or toxins. Hematocrits of 75–80% have been described and leukocytosis of 50,000–100,000 cells/mm^3 with a left shift is common.[41] [58]

Clostridium tertium infections

Clostridium tertium has been associated with spontaneous myonecrosis; however, it more commonly causes bacteremia in compromised hosts who have received long courses of antibiotics. Bacteremia probably arises from bowel sources, and the presence of the organism in the bowel may be partly related to its relative resistance to penicillin, cephalosporins and clindamycin. *Clostridium tertium* is, however, usually quite sensitive to chloramphenicol, vancomycin and metronidazole. Because this organism can grow aerobically, it may be mistakenly disregarded as a contaminant such as a diphtheroid or a *Bacillus* sp.[42] [43]

Pyomyositis

Most cases of pyomyositis occur in tropical areas. Local trauma is a common predisposing factor. Initially, seeding of traumatized muscle occurs and physical findings are not usually helpful. Within 10–20 days, fever, chills, muscle pain and tenderness are manifest (see Fig. 10.1). Most patients seek medical care at this stage and a diagnosis can be established by appropriate imaging

Figure 10-13 MRI scan showing high signal STIR sequence (consistent with marked edema) in the adductor muscles of the patient's left leg in a patient with *Staphylococcus aureus* bacteremia (arrow). At operation, necrotic and infected muscle was decompressed and debrided. This represents the 'woody' stage, prior to muscle liquefaction and the formation of frank abscesses.

studies (Fig. 10.13), needle aspiration or exploration. Patients in whom a diagnosis has not been made may progress to shock and organ failure, though these complications are uncommon. *Staphylococcus aureus* is the most common cause of pyomyositis in tropical and non-tropical areas, and among HIV-positive patients. Hospitalized immunocompromised patients who are HIV negative occasionally develop pyomyositis caused by Gramnegative bacteria.

Surgical drainage of the abscess and empiric administration of parenteral antibiotics such as nafcillin or cephalosporins are reasonable treatments since most cases

are caused by *Staph. aureus*. Definitive treatment can then be established based on cultures and sensitivities. Due to an increase in the prevalence of methicillin-resistant *Staph. aureus*, it may be necessary to use vancomycin or linezolid empirically pending sensitivity results.[59]

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Chapter 11 - Ectoparasites

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INTRODUCTION

Parasites depend on their hosts for sustenance; an ectoparasite is a parasite that lives or feeds on the surface of that host. Most ectoparasites of vertebrates are hematophagous, but a few feed on skin and tissue debris. Some spend their entire life on the host, others move from host to host as they develop and many simply alight on the host to feed. The definition of 'ectoparasite' is usually extended to include those parasites that burrow into the epidermis as well as those that remain on the surface.

Humans are the preferred or only host of some ectoparasites, but the majority are catholic in their choice or turn to humans only when their primary host is unavailable. Ectoparasites can cause local skin disease as a direct result of their bites, as a result of secondary infection or as a result of hypersensitivity reaction. They can cause systemic disease by inducing an allergic response or by toxin release and they can act as vectors for a large number of viral, bacterial and parasitic infections.

This chapter reviews the clinical syndromes caused by ectoparasites, outlining the key features associated with each family.

SKIN PROBLEMS

Transient ectoparasites

Clinical and pathologic features

The most common feature of ectoparasite infection is local reaction to the bite of a blood-feeding arthropod. Numerous species of insects and arachnids rely on blood meals from vertebrate hosts. Although the behavior and feeding methods differ by species, most uncomplicated arthropod bites produce a similar local reaction.

Initial contact in an unsensitized person may produce little or no response. After repeated bites typical raised pruritic lesions (papular urticaria) appear within 24–48 hours (Fig. 11.1), although delays of more than 1 week have been reported. Occasionally, these lesions are bullous. With further exposure and increased sensitization, an immediate weal skin response may be seen, followed after some hours by papular urticaria. Some hypersensitive people may develop a pronounced immediate reaction with large areas of superficial edema. After prolonged and frequent biting, the delayed reaction often diminishes and eventually there is no response to further bites. The term papular urticaria is also used to describe a condition (usually seen in children) in which widespread papular lesions occur distant from but temporally related to insect bites; this may represent reactivation of previously sensitized bite sites.

The lesions of papular urticaria consist of pruritic papules, often with a central puncture marking the site of the bite. Superficial erosion and ulceration resulting from scratching is common. Unlike most other types of urticaria, papular lesions may persist for several days, although this period tends to decrease with regular exposure. Histologically there is intense inflammatory infiltrate with a predominance of T cells; the response is thought to be a type 1 IgE-mediated reaction. [1] A variety of vasodilators, anticoagulants and other substances have been isolated from arthropod saliva; in some species a specific allergen has been identified as the cause of local hypersensitivity, but in others the stimulus remains obscure.

Treatment of uncomplicated bites is usually unnecessary, although severe pruritus may be relieved by systemic antihistamines. (Caution is needed with terfenadine and astemizole, which can cause prolongation of the cardiographic QT interval and have a synergistic cardiotoxic effect with certain antimalarial drugs.) Secondary bacterial infection is common in hot moist climates (Fig. 11.2) and this may require antibiotic therapy; infection is usually due to Gram-positive cocci. Local lesions caused by various blood-feeding arthropods are usually indistinguishable, and diagnosis of the precise cause relies either on epidemiologic knowledge or on detection and identification of the parasite. This may sometimes be necessary in order to eradicate the source of infection.

The major types of transient-feeding ectoparasites responsible for this type of bite are discussed below.

Flies

The most common cause of local reactions are biting flies of the order Diptera. Mosquitoes, blackflies, horseflies, sandflies and midges are ubiquitous, whereas tsetse flies are essentially tropical. Only certain species of each family tend to attack humans, and in all except the tsetse fly only the females are blood feeders. Culicid mosquitoes usually feed by inserting their proboscis directly into the capillary, whereas other species feed by inflicting local tissue damage and sucking the resultant blood (pool feeding). This may explain the relatively more severe irritation that complicates bites by some species, although host factors also play a significant part.

Fleas

Fleas occur worldwide and are a common cause of pruritic bites in humans. They are free living, only approaching their host in order to feed. The human flea, *Pulex irritans*, is found mainly in crowded and unhygienic living conditions and is relatively uncommon in the developed world. However, many species of animal and bird fleas will also feed on humans, and household infestations with cat and dog fleas (*Ctenocephalides felis* and *C. canis*) are increasing in frequency.

Flea bites provoke typical papular urticaria, although there may be a more severe reaction with bulla formation. The distribution of the lesions reflects the source of the infestation; for example, bites on the lower leg are usually due to cat and dog fleas, at least in adults. Diagnosis can be difficult; fleas are rarely seen on the human victim and excoriation may mask the original nature of the lesions. If flea bites are suspected, careful inspection of the home environment is needed, although other sources of exposure (e.g. school, the workplace) should be considered. Household pets and their bedding should be checked thoroughly for fleas and droppings. Flea bites need no specific treatment unless there is secondary infection. Prevention depends on identifying the source of the infestation; in

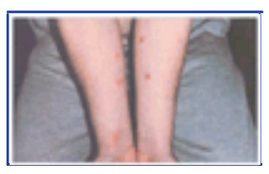

Figure 11-1 Typical lesions of papular urticaria, caused in this case by bedbug bites.

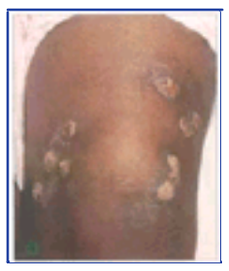

Figure 11-2 Mosquito bites with secondary staphylococcal and streptococcal infection.

the case of pets, insecticides must be applied to both the animal and its environment.

Tungiasis, caused by the flea *Tunga penetrans*, is described in Chapter 155 .

Bugs

Many members of the order Hemiptera (bugs) are blood feeders, but only two families include significant ectoparasites of humans. The Reduviidae cause relatively little local irritation, although hypersensitivity can develop with prolonged exposure; they are important principally as vectors of South American trypanosomiasis (see Chapter 173). Cimicidae (bedbugs) are voracious feeders and cause intense reactions. Bedbugs live and reproduce in crevices in walls and furniture, only approaching the host to feed (usually at night). They are found worldwide, the tropical bedbug (*Cimex hemipterus*) parasitizing humans in hot climates and the common bedbug (*C. lectularius*) in other areas. Bites cause papular lesions similar to those of other arthropods, but in unhygienic conditions infestations can be very heavy and secondary iron deficiency anemia has been reported. Personal insect repellants and permethrin-impregnated bed nets provide some protection, but decontamination of the environment with residual insecticides may be necessary.

Ticks

Ticks are cosmopolitan ectoparasites of mammals, birds and reptiles. All stages of tick (larva, one or more nymphal instars and adults)

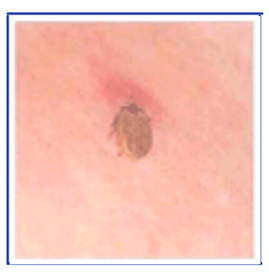

Figure 11-3 Ixodid tick after feeding on the host for several days.

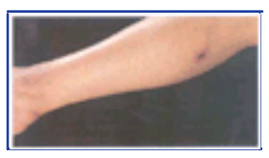

Figure 11-4 Tick-bite eschar associated with African tick typhus.

attach to vertebrate hosts to feed; some species spend their entire life cycle on a single animal (one-host ticks), while others drop off before moulting and then seek out a new host (multihost ticks). Although ticks are relatively host specific, most will attack humans in the appropriate circumstances. Worldwide, hard ticks (family Ixodidae) are the more important parasites of humans, but soft ticks (family Argasidae) can cause similar problems.

Most adult and immature ixodid ticks remain attached to the host for several days unless removed; argasid ticks feed more rapidly and may detach themselves after a few hours. The bite is often not irritating and passes unnoticed, especially in the early stages of attachment, which is an important factor in the role of the tick as a vector. Larval ticks are very small (<1mm in length) and are easily missed. In some cases, however (presumably when the host has been presensitized), tick bites can cause itchy papular lesions with evidence of a type 1 hypersensitivity reaction. Elevated levels of specific IgE against tick saliva can be demonstrated in such people.[2] Local skin reactions may occur when tick attachment is prolonged (Fig. 11.3). Hypersensitive people may experience more generalized urticarial skin reactions and even systemic features of anaphylaxis (see below).

A characteristic necrotic lesion may be seen when the tick bite is associated with the transmission of certain rickettsial infections — the so-called eschar or *tâche noire* (Fig. 11.4 ; see Chapter 235).

Avoidance and management of tick bites

Ticks usually prefer certain specific types of vegetation and most species are more commonly found during the spring and summer. In adults, ticks are generally found on the lower body, while children are often bitten on the head, neck and axillae. Some species of tick also have preferential sites of attachment. A knowledge of

the epidemiology of local tick species, as well as the wearing of long trousers and the use of permethrin-impregnated clothing, can decrease the incidence of tick bites and their complications.

Most ectoparasite vectors of infection feed rapidly, and length of time on the host probably does not influence the likelihood of infection. Ixodid ticks, by contrast, can spend many days feeding and the risk of transmission of some infections appears to be directly related to the duration of the bite. Tick paralysis is also dependent on prolonged feeding and both infectious agents and toxins may be inoculated into the host by careless removal, which can also leave the barbed mouthparts embedded in the skin. As well as predisposing to bacterial infection this can also generate a chronic granulomatous response; in some cases local surgical excision is needed to relieve the symptoms. Early detection and appropriate detachment of ticks is therefore essential, especially in regions where tick-borne diseases are endemic. The ideal method of removal is to grasp the tick mouthparts as close to the skin as possible with fine forceps or tweezers and gently lever the creature off. The body should not be squeezed, in order to prevent further inoculation. Any retained fragments should not be dug out, but the site cleaned and antiseptic applied. This method of removal is associated with a significantly lower incidence of rickettsial and borrelial infection following tick bites.[3] [4]

In the particular case of Lyme disease, early antibiotic therapy after infection with *Borrelia burgdorferi* may provide some protection against the development of disease. Tick bites are very common, and even in areas where the majority of vector species are infected the rate of human infection is very low.[4] Routine antibiotic prophylaxis following tick bites is not justified even in these areas. However, risk of infection rises dramatically once the tick has been feeding for more than 72 hours (from about 3% at <72 hours to 18–25% at >72 hours).[5] The approximate duration of attachment of the tick can be estimated from an index of tick engorgment (the scutal index), and antibiotics may be justified in cases where the tick had been feeding for more than 72 hours. Other attempts at predicting high-risk groups following tick bites (e.g. by testing the tick for *B. burgdorferi* infection) have not proved successful.[5] There is no evidence that empiric antibiotic prophylaxis following tick bites has a role in the prevention of rickettsial infections such as Rocky Mountain spotted fever.[6]

Mites

The majority of mites that parasitize humans are not blood feeders, but feed on tissue fluids and cell debris. However, the effect of their bites is similar to those of other transient feeders.

Trombiculid mites ('chiggers') are found worldwide. The adult and nymphal stages are free-living predators, but the larvae parasitize many animals, including humans. The mites inhabit areas of transitional vegetation (hence the alternative name of 'scrub mite'), often forming localized 'mite islands' in areas inhabited by a host species. Larval mites climb on to a human host who is passing through the vegetation and crawl over the host's body to find a suitable area of skin to bite, such as the axillae or groins or areas of skin that are constricted by clothing. They feed for several days if undisturbed and then drop to the ground. The resulting lesions are similar to those

caused by tick bites; the methods of prevention are similar.

Pyemotid mites are primarily predators of insects and their larvae, but they bite humans that come into contact with the grain or straw in which the mites live. The resulting papular urticarial rash, usually found on exposed surfaces or areas of thinner skin, goes under a number of occupational names, including 'straw itch', 'barley itch' and 'grain-shoveler's itch'.[7]

Cheyletiellid mites are ubiquitous tissue-feeding parasites of domestic dogs and cats. Bites follow close contact with infected animals and lesions are usually seen on the thighs and abdomen after a pet has sat on someone's lap. Itchy papules similar to other arthropod bites result and the diagnosis is made by finding mites on the animal. Some hematophagous animal mites also attack humans, causing typical lesions in areas that depend on the form of contact with the animal. Poultry, cage birds, wild and domestic rodents and snakes have all been incriminated. [7] In all these cases management is by removal or treatment of the principal animal host, with environmental acaricide if necessary.

Scabies and other resident mites are described below.

Infestations of resident hematophagous parasites

Head and body lice

Epidemiology

Head lice (*Pediculus humanus capitis*) and body lice (*Pediculus humanus corporis*) are morphologically almost indistinguishable from each other, but they each tend to keep within their own territory on the host. The latter species clings to and deposits eggs on clothing fibers rather than hair shafts. It parasitizes those who do not change or wash clothing, unlike the head louse, which is not associated with poor hygiene alone. Children are more commonly infected with head lice than adults and women are more frequently infected than men (at least in Western cultures). This is because the vast majority of infections are acquired by direct head-to-head contact, which is more likely in these groups. Overcrowding is also an important risk factor, but hair length is not. Head lice are more common in late summer, which probably reflects the need for high ambient temperature to hatch eggs. The role of fomites in transmission is controversial; there is little evidence that hats and brushes are important.

Clinical features

The main feature of head louse infestation is scalp itching, although secondary bacterial infection is not uncommon, and louse infection should be looked for in cases of scalp impetigo. Chronic infection can produce cervical lymphadenopathy. The diagnosis is made by finding empty egg-cases ('nits') stuck to the base of hair follicles; the highest concentration is usually in the occipital and parietal regions. In heavy infestations, developing eggs, nymphs and adult lice may be seen.

Management

The efficacy of traditional wet combing remains unproven and although some experts recommend dry combing, both methods are very time consuming and require good technique to be of any benefit.[8] Several insecticides are available for the treatment of head lice (Table 11.1).[9] The acetylcholinesterase inhibitors malathion and carbaryl both have reasonable ovicidal and pediculicidal activity, although some resistance has been seen. Malathion, unlike carbaryl, has a residual action of several weeks, but two applications 10 days apart are necessary whichever agent is used. The synthetic pyrethroids, such as permethrin, also have good activity, although there are concerns about the rapid development of resistance to these compounds. Topical ivermectin has also been used with good effect, although there is little evidence to suggest that it is preferable to other agents.

Malathion and carbaryl need to remain on the scalp for 8–12 hours before they are washed off and they are degraded by high temperatures (e.g. as occurs during the use of a hair dryer). Pyrethroids need only a short application. Lotion or mousse preparations are generally preferable to shampoos. Although there are numerous trials comparing different drugs and preparations, it is very difficult to draw general conclusions.[10] Resistance patterns vary from region to region and there are many different formulations and preparations of drugs available. If possible, the choice of therapy should be based on a local policy designed to take into account existing resistance patterns and to prevent the development of resistance.

TABLE 11-1 -- Commonly used topical preparations for the treatment of ectoparasite infestations.

COMMONLY USED TOPICAL PREPARATIONS FOR THE TREATMENT OF ECTOPARASITE INFESTATIONS				
Class	**Agent**	**Uses**	**Toxicity**	**Comments**
Acetylcholinesterase inhibitors	Malathion	Scabies		Good residual protection
		Head lice		Recommended for public lice infestation of eyelashes; safe in pregnancy
		Public lice		
	Carbaryl	Head lice	Carcinogenic in animals; minimal risk to humans in therapeutic doses	Prescription only in the UK
		Pubic lice		
Organochlorines	Lindane	Head lice	Neurotoxic (potential for systemic absorption)	Increasing resistance; no longer available in the UK
		Public lice		
Natural pyrethroids	Pyrethrin	Head lice		Less evidence of efficacy than synthetic pyrethroids
	Phenothrin	Public lice		
Synthetic pyrethroids	Permethrin	Scabies	Rarely rash and local edema	Probably safe in pregnancy and breast-feeding (limited data)
		Head lice		
		Public lice		
Others	Benzyl benzoate	Scabies	Skin irritation	Limited safety data in pregnancy; avoid in breast-feeding
	Ivermectin	Head lice		Available for topical and systemic use
		Scabies		
	Crotamiton	Head lice		Relatively poor efficacy
		Scabies		Avoid in pregnancy
	Mercury preparations	Head lice	Contact dermatitis	Available over the counter in some European countries
			Systemic toxicity	
	Monosulfiram	Scabies	'Antabuse' effect (alcohol should be avoided)	No longer available in the UK
	Sulfur ointment	Scabies	Skin irritation	Cheap, safe, reasonably effective

Systemic therapy has occasionally been used to treat head lice. Trimethoprim-sulfamethoxazole and ivermectin have been reported to be effective, although repeated doses are necessary because they affect only the feeding stages of the parasite. In most cases topical treatment should be adequate. Older drugs, such as organochlorines and mercury-based preparations, are less effective and more toxic.

Body lice are associated with poor hygiene and unwashed clothing and are principally parasites of vagrants and refugees. They can be found worldwide, but they are particularly found in cooler climates where clothing is rarely removed. Transmission is by direct body-to-body spread or by sharing infected clothing. Initially the bites are similar to those of other hematophagous ectoparasites, but the prolonged and persistent nature of the infestation leads to widespread excoriation and secondary infection and eventually to the hyperpigmented chronic skin condition known as 'vagabond's disease' or morbus errorum.

The diagnosis is made by finding lice and eggs in the clothing (particularly along seams), and treatment should be directed at the clothing rather than the patient. High-temperature washing, tumble-drying and malathion dusting powder are all effective at clearing garments of lice; permethrin-treated clothing may be protective for those at risk of infestation.

Pubic lice

Pthirus pubis (often written as *Phthirus pubis*) is morphologically different from *Pediculus humanus* and is unique to humans. Infection is usually confined to the pubic region, although lice and eggs are sometimes found in axillary and facial hair, eyelashes, eyebrows and (especially in children) the scalp. Infection is transmitted by close, usually sexual, contact and may be associated with other sexually transmitted diseases.

The main symptom is itching of the affected area, especially at night, although there may be few visible skin lesions. Close inspection may reveal eggs that are attached to the hair shafts, with adult lice clinging on close to the skin. Treatment is with the same insecticides as for head lice, with the proviso that alcohol-based preparations may irritate sensitive skin and mucous membranes. This is particularly true for the eyes, and eyelash infestations should always be treated with an aqueous formulation. It is usually advisable to treat the whole trunk and limbs in view of the possible spread to other hairy areas; where appropriate, sexual contacts should also be treated.

Other ectoparasite infestations

Scabies

Clinical features

Some infesting arthropod ectoparasites do not feed on blood at all, but cause disease in other ways. The most important of these is *Sarcoptes scabei*, the human scabies mite, which is found worldwide in conditions of poor hygiene. This skin-burrowing mite is not a blood feeder, but ingests predigested dermal cells as it tunnels through the epithelium. Female mites live in small burrows in the skin, which they extend by 2–3mm daily, leaving a trail of eggs and feces behind them. The burrows are intensely itchy and are responsible for the features of scabies. They are usually found around the wrists, web spaces, toes and genitalia, although they may be more widespread; small papules are sometimes seen at the distal end adjacent to the female mite. Infestations are relatively light, with an average of 12 adult females found on an infected patient. Crusted scabies, in which there is heavy mite infestation and gross hyperkeratosis, may be seen in immunocompromised and institutionalized patients.

Management

Diagnosis based on history and clinical features alone can be difficult and should be confirmed whenever possible by identification of mites. This is usually done by extracting parasites with a needle or in skin scrapings, but these techniques require operator experience and visible burrows. Newer methods of diagnosis include high-magnification videodermatoscopy, epiluminescence microscopy and polymerase chain reaction but none of these is widely available. Treatment should be given if scabies is suspected, even if the diagnosis cannot be confirmed.

Scabies is usually treated with topical acaricides (see Table 11.1). Comparative trials suggest that 5% permethrin is the most effective treatment, with 0.5% malathion the second choice; permethrin is considerably more expensive.[11] Oral ivermectin has been increasingly used in recent years, especially in crusted or recurrent infections. It is well tolerated and appears to be highly effective, although a second dose may be needed one week after the first. Although unlicenced, oral ivermectin is probably the treatment of choice in recurrent cases, institutional outbreaks and crusted scabies; combining systemic treatment with a topical preparation may give the best results in hyperkeratotic disease.[12] [13] Crusted scabies is highly contagious, and bedding and clothing should be boil-washed or treated.

Pseudoscabies

Sarcoptid mites are relatively host specific and mites acquired from animals do not usually cause prolonged infestations of humans. However, people in close contact with infected livestock (notably pigs, cows and dogs) may acquire temporary infestation, causing pruritic lesions but lacking the typical burrows of human scabies. In most cases no treatment is required except for avoidance of the source of infection.

Demodicidosis

Demodex folliculorum, the follicle mite, is a human ectoparasite that lives in the pilosebaceous follicles, where it feeds on cell contents. The mites are found in areas of high sebum production; the forehead, cheeks, nose and nasolabial folds. The role of follicle mites in skin disease is controversial; they are very common and unless they are present in large numbers do not appear to cause problems. However, they have been described as a cause of papulopustular eruptions in children and immunocompromised subjects, and they have been implicated in the pathogenesis of other skin conditions such as pityriasis folliculorum and rosacea.[14]

Storage mites

The so-called storage mites include a number of mite species that live in stored products (e.g. flour, grain, straw, dried meat, dried fruit and cheese). When a person handles infested stock, mites crawl onto exposed areas and migrate under the horny layer of the skin. Here they can cause an acute dermatitis, with erythema and small papulovesicles, which is known by a number of occupational names, for example 'grocer's itch', 'baker's itch' and 'copra itch'.[7] It is uncertain whether the mites actually feed on skin cells, so they may not be true ectoparasites of humans.

Skin lesions due to myiatic larvae

These parasites are covered in Chapter 155 .

NONINFECTIVE SYSTEMIC PROBLEMS

Tick paralysis/toxicosis

Epidemiology

Paralysis and death of domestic animals and occasionally humans following tick bites has been documented in Australia and South Africa since the end of the 19th century and more recently it has been observed in North America and Europe. This syndrome is not, as once thought, an infective process, but is caused by a toxin in the saliva of the pregnant females of certain ixodid species. Children are more often affected than adults and the condition is most commonly seen in spring and early summer.

Clinical features

Tick paralysis almost always results from bites on the head, typically behind the ear where the tick may not be noticed. Symptoms appear only after the tick has been feeding for at least 48 hours. The usual presentation is an ascending flaccid paralysis, frequently with cranial nerve involvement, although atypical features such as isolated nerve lesions and cerebellar signs are occasionally seen. There is no sensory loss or decrease in consciousness, but if the condition is untreated death from respiratory failure can supervene. In the North American form the paralysis starts to improve within a few hours of removing the tick, but in the Australian form of the disease deterioration may continue for a further 24–48 hours.[15]

Management

The neurotoxin involved probably varies between different tick species. It has not been fully characterized, but there are many clinical and neurophysiologic similarities to the effects of botulinum toxin.[15] The essential treatment is to remove the tick carefully to prevent further transfer of toxin, followed by supportive care until the neurologic signs resolve. Although rare, the diagnosis should be considered in any progressive flaccid paralysis in an endemic area.

Tick anaphylaxis

The local allergic urticarial reactions to tick bites have already been discussed. Rarely, in hypersensitive people, anaphylaxis can follow a bite, with bronchospasm, edema and hypotension. This is an IgE-mediated response to tick salivary antigens, which can occur soon after the tick first bites or following careless removal.[16] Treatment is as for any form of anaphylactic reaction and sensitive people in tick-infested areas may benefit from carrying epinephrine (adrenaline) for inhalation or self-injection.

Mite allergy

House dust mites (*Dermatophagoides* spp.) are cosmopolitan, free-living mites that feed principally on animal and human skin detritus. Although they are sometimes found on the skin surface they usually consume skin that has already been shed and are thus not true parasites. Their main medical importance is as a cause of allergic rhinitis and bronchial constriction.

ECTOPARASITES AS VECTORS OF INFECTION

The principal importance of many human ectoparasites is their potential for transmitting a wide variety of viral, bacterial and protozoal infections. Table 11.2 lists the major vector-borne human infections (see also Chapter 155).

Bedbugs do not appear in the table; their role as potential vectors of infection remains controversial. Viable infective hepatitis B virus and other pathogens have been found in the gut of bedbugs weeks after their last blood meal. However, community-based studies have failed to confirm that bedbugs have a significant role in hepatitis B transmission[17] and attempts at transmitting infection among a group of chimpanzees using bedbugs were unsuccessful when the bugs fed normally.[18] Although HIV has been isolated from bedbugs up to 8 days after feeding on heavily infected blood under experimental conditions, there is no evidence to suggest transmission to humans by this route.[19]

TABLE 11-2 -- Important ectoparasite vectors of human disease.

IMPORTANT ECTOPARASITE VECTORS OF HUMAN DISEASE				
Ectoparasite	**Vector**	**Infective organism**	**Disease**	**Distribution**
Flies (Diptera)				
Mosquitoes	*Anopheles* spp.	*Plasmodium* spp.	Malaria	Tropics and subtropics
	Anopheles spp. *Culex* spp. *Aedes* spp.	*Wuchereria bancrofti*	Lymphatic filariasis	Tropics
	Anopheles spp. *Mansonia* spp. *Aedes* spp.	*Brugia* spp.	Lymphatic filariasis	Southern Asia
	Aedes spp. *Culex* spp. *Anopheles* spp.	Arboviruses	Dengue, yellow fever Japanese encephalitis, West Nile virus St Louis encephalitis	Specific to each disease
Blackflies (Simuliidae)		*Onchocerca volvulus*	Onchocerciasis	Sub-Saharan Africa, Central and South America
		Mansonella ozzardi	Mansonelliasis	Amazon basin
Sandflies		*Leishmania* spp.	Visceral and cutaneous leishmaniasis	Sub-Saharan Africa, Asia and Mediterranean Europe
	Phlebotomus spp.	Bunyaviridae	Sandfly fever	Central Asia, Mediterranean and North Africa
		Leishmania spp.	Visceral and cutaneous leishmaniasis	Central and South America
	Lutzomyia spp.	*Bartonella bacilliformis*	Bartonellosis (Oroya fever, Carrion's disease, verruga peruana)	South America
Midges	*Culicoides* spp.	*Mansonella perstans*	Mansonelliasis	Sub-Saharan Africa
		Mansonella ozzardi	Mansonelliasis	Amazon basin
Tsetse flies (Glossinidae)		*Trypanosoma* spp.	African trypanosomiasis	Sub-Saharan Africa
Deer flies (*Chrysops* spp.)		*Loa loa*	Loiasis	West Africa
		Francisella tularensis	Tularemia	Western USA
Fleas (Siphonaptera)	Various species	*Yersinia pestis*	Plague	Worldwide (patchy)
	Various species	*Rickettsia mooseri*	Murine typhus	Worldwide (patchy)
Bugs (Hemiptera)	Reduviidae	*Trypanosoma cruzi*	South American trypanosomiasis	Central and South America

Lice	Body louse (*Pediculus humanus corporis*)	*Rickettsia prowazeki*	Epidemic typhus	Worldwide (patchy)
		Bartonella quintana	Trench fever	Worldwide
	Body louse (*Pediculus humanus corporis*) and head louse (*Pediculus humanus capitis*)	*Borrelia recurrentis*	Louse-borne relapsing fever	Worldwide (patchy)
Ticks				
Ixodidae	Numerous hard tick (ixodid) species	*Rickettsia* spp.	Tick typhus and various rickettsial spotted fevers	Worldwide
		Borrelia burgdorferi	Lyme disease	USA, Europe, Eastern Asia, Australia
		Francisella tularensis	Tularemia	USA, Europe, Japan
		Babesia spp.	Babesiosis	USA, Europe
		Arboviruses	Tick-borne encephalitis, Congo-Crimea hemorrhagic fever and other arbovirus infections	
		Ehrlichia spp.	Ehrlichiosis	USA, Europe, North Africa, Eastern Asia
Argasidae	*Ornithodoros* spp.	*Borrellia* spp.	Tick-borne relapsing fever	Africa, Central Asia, North and South America
Mites	*Leptotrombidium* spp.	*Rickettsia tsutsugamushi*	Scrub typhus	South East Asia, Pacific and (rarely) West Africa
	Liponyssoides sanguineus	*Rickettsia akari*	Rickettsialpox	USA, South Africa, Eastern Asia

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Chapter 12 - Dermatologic Manifestations of Systemic Infections

Anthony C Chu
Edward D Seaton

INTRODUCTION

The skin is the largest and most visible organ of the body. In addition to its role as a barrier separating the body from the external environment and its role in temperature regulation, the skin has a complex immune system that recognizes and attacks foreign antigens and microbes, but that also reacts to systemic disease to give characteristic clinical changes.

The skin may be affected by systemic infections in three ways:

- by direct involvement by the infectious agent,
- by specific reaction to an infection, and
- by non-specific reaction to an infection.

In addition, there are a number of inflammatory dermatoses that can mimic skin infections.

DIRECT INVOLVEMENT OF THE SKIN BY AN INFECTIOUS AGENT DURING A SYSTEMIC INFECTION

Viral infections

Chickenpox

Viral infection of the skin as part of a systemic infection is well demonstrated by chickenpox (see Chapter 8). After an incubation period of 14–21 days the patient develops 1–2 days of fever and malaise. This is followed by crops of unilocular vesicles, which quickly become pustular, appearing over 2–4 days (see Chapter 8). After the acute infection the virus persists in dorsal root nerve ganglion cells and on reactivation of the residual latent virus, herpes zoster or shingles develops.

Hand, foot and mouth disease

Hand, foot and mouth disease is caused by Coxsackie viruses A16, A5 and A10, and enterovirus 71[1] (see Chapter 8 and Chapter 213). It occurs predominantly in children. After an incubation period of 5–7 days the patient develops painful stomatitis with oral vesicles that ulcerate. A more variable feature is that of small, thin-walled vesicles on the fingers and toes (see Chapter 8 , Fig. 8.12). An uncommon feature is onychomadesis[2] due to nail matrix arrest leading to nail shedding from the proximal portion. Disease related to Coxsackie virus infection is generally mild and self-limiting. Cardiac and pulmonary involvement with enterovirus 71 hand, foot and mouth disease is associated with high mortality. Viral particles can be identified in the vesicles on electron microscopy.

Bacterial infections

Gonococcal infection

In disseminated gonococcal infection caused by *Neisseria gonorrhoeae*, characteristic skin lesions (called septic gonococcal dermatitis) may be observed. One or more crops of three or four macules or papules develop often over the extremities; these then become pustular or bullous. Gonococci can occasionally be cultured from the skin lesions.

Tuberculosis

In tuberculosis, skin involvement may occur as the result of contiguous involvement of the skin from underlying lymph nodes, joints or bones, a condition called scrofuloderma.[3] A bluish-red nodule develops over the affected bone, joint (Fig. 12.1) or lymph node and multiple fistulae develop. Diagnosis must be confirmed by biopsy, which shows tuberculous granulation tissue. *Mycobacterium tuberculosis* can often be isolated from involved tissue.

Skin involvement in tuberculosis may also occur secondary to hematogenous dissemination. In miliary tuberculosis, hematogenous dissemination can produce severe systemic symptoms and profuse crops of bluish papules, which become vesicular and then pustular. These often become necrotic, leading to ulceration of the skin.

Chronic hematogenous dissemination of tubercle bacilli in patients who have moderate or high degrees of immunity may present as one of the tuberculides:

- papulonecrotic tuberculid, in which there are symmetric crops of necrotic papules predominantly affecting the extremities;
- lichen scrofulosum, in which minute lichenoid papules appear predominantly on the limbs rather than on the trunk; and
- erythema induratum (or Bazin's disease), in which persistent or recurrent nodular lesions appear in the calves of the legs and may lead to ulceration (Fig. 12.2); these nodular areas are very well defined and are generally asymptomatic. [4]

The tuberculides respond rapidly to antituberculous therapy (see Chapter 202).

Spirochetal infections

Disease caused by spirochetes tends to affect the skin as part of the primary manifestation, but it may also involve the skin during subsequent, disseminated disease.

Syphilis

In syphilis, the primary lesion or primary chancre is often cutaneous or mucosal. Secondary syphilis starts approximately 3 months after the primary infection and gives rise to non-irritating, coppery red symmetric lesions, which start as macules and become papular (see Chapter 75 , Fig. 75.5). Secondary syphilis is the 'great pretender'[5] and lesions of secondary syphilis can mimic acne, psoriasis and a number of other non-specific dermatoses. Characteristically, the palms and soles are affected. When mucosal surfaces are involved, 'snail track' ulcers may develop. Later, condylomata may occur perianally and on the vulva or penis. Patchy hair loss is a characteristic sign of secondary syphilis, giving rise to a moth-eaten appearance of the scalp.

Late or tertiary syphilis occurs after a latent period of up to 20 years. Both skin and mucous membranes may be affected. Nodular syphilides present as nodular subcutaneous lesions appearing in groups and tending to develop a circinate arrangement. These are more common on the extensor surfaces of the arms, the back and the face (Fig. 12.3), but they may occur in the oral cavity. Gummas are masses of syphilitic granulomatous tissue; they may originate in the subcutis, underlying bone or muscle. These masses ulcerate to produce punched-out ulcers.

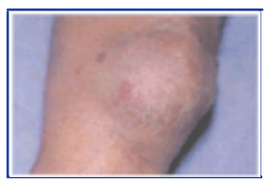

Figure 12-1 Scrofuloderma in a 60-year-old patient. A biopsy confirmed tuberculoid granulation tissue and the patient responded very well to antituberculous therapy.

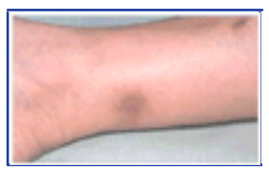

Figure 12-2 Erythema induratum on the back of the leg of a 45-year-old woman.

Yaws

Yaws is a disease caused by the spirochete *Treponema pertenue*. The primary lesion of yaws produces a cutaneous erythematous papule, which becomes papillomatous and resembles a raspberry, giving rise to its name, 'frambesia'. After 2–4 months the secondary eruption of yaws occurs, with multiple small papules developing into exudative papillomas (Fig. 12.4). Mucosal involvement does not occur in yaws. After 6 months to 3 years tertiary yaws occurs; this is characterized by ulcerated nodular and tuberous cutaneous lesions and keratoderma of the palms and soles.

Pinta

Pinta is caused by the spirochete *Treponema carateum*. The initial eruption starts in the skin as multiple erythematous papules and plaques. This is followed after months or years by generalized cutaneous lesions, where the skin becomes pale, pigmented or erythematosquamous. The late phase occurs 2–5 years after primary infection with irregular pigmentation, which can be grayish, steely or bluish in color. Areas of leukoderma, particularly around the elbows, knees, ankles and wrists, may develop. Hyperkeratosis occurs, particularly on the legs and arms, and is associated with areas of atrophy, particularly around the large joints (see Chapter 156 , Chapter 230).

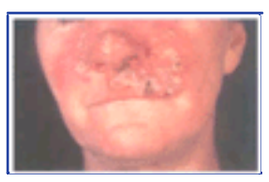

Figure 12-3 Tertiary syphilis on the face of a 56-year-old woman.

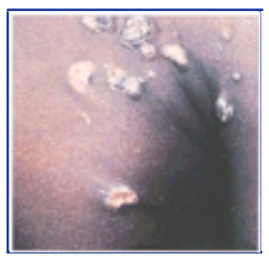

Figure 12-4 Secondary yaws showing papular and rather vegetative lesions on the anterior chest wall.

Lyme disease

In infections with *Borrelia burgdorferi*, the primary lesion occurs at the site of the Ixodes tick bite, with a characteristic eruption: erythema chronicum migrans.[6] The macular erythema starts up to 36 days after the bite and slowly increases in size by several centimeters each week (see Fig. 54.3 , Chapter 54). Subsequent dissemination leads to Lyme disease with involvement of the nervous system, heart and joints. One year or longer after the original infection, a late cutaneous manifestation may occur: acrodermatitis chronica atrophicans (see Chapter 54).[7] As this name suggests, this typically affects the hands and feet, but the elbows and knees may also be affected. Erythematous plaques develop and these slowly enlarge and become atrophic. *Borrelia burgdorferi* can be cultured from skin biopsies in this condition although often with difficulty. Hence, diagnosis relies on clinical features and serologic testing.

Fungal infections

A number of deep fungal infections may have cutaneous involvement in the course of systemic disease. These fungal infections include blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis and disseminated candidiasis.

Cutaneous lesions in these deep fungal infections tend to be nonspecific, with papules, nodules and ulcers developing on different parts of the skin. In disseminated blastomycosis cutaneous lesions start as papules or nodules, which then ulcerate and evolve into serpiginous lesions with raised warty borders.

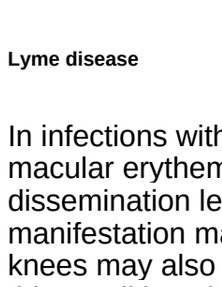

Figure 12-5 Cutaneous cryptococcosis in a renal transplant patient. These lesions started as nodules that then rapidly ulcerated. Computerized tomography of the patient's brain showed no abnormality but *Cryptococcus neoformans* was grown from the cerebrospinal fluid.

Disseminated coccidioidomycosis may result in cutaneous abscesses and granulomas and discharging sinuses. Disseminated cutaneous cryptococcosis is observed in patients on long-term immunosuppression. It presents as erythematous papules and nodules, which become exudative and eventually ulcerate (Fig. 12.5). Disseminated cutaneous cryptococcosis is seen most often in patients who have AIDS. The cutaneous lesions tend to be non-specific, but molluscum contagiosum-like lesions are a recognized feature of this disease. These typically occur around the nose and mouth and eventually ulcerate to leave punched-out, rolled edged ulcers.

SPECIFIC SKIN REACTIONS RESULTING FROM SYSTEMIC INFECTIONS

Systemic infections with viruses and bacteria can occasionally cause specific cutaneous reactions. These skin reactions can establish the diagnosis of the specific systemic infections and it is thus very important to recognize them.

Viruses

Roseola infantum and pityriasis rosea

Infection with human herpesvirus 6 in the first 3 years of life gives rise to a specific dermatitis: roseola infantum (exanthem subitum) (see Chapter 8). After an incubation period of 10–15 days, fever starts abruptly and lasts for 3–5 days.[8] As the fever subsides a maculopapular eruption, which is characteristically rose pink in color, develops on the neck and trunk. This later spreads to the arms, face and legs. The eruption subsides after 1–2 days, leaving no pigmentation or scaling of the skin. In common with other herpesviruses, human herpesvirus 6 is capable of establishing lifelong latent infection of its host.[9] Reactivation in the immunocompromised host is linked to various diseases, including encephalitis.

A similar eruption is observed in adults; this is called pityriasis rosea. There is generally no prodromal syndrome but patients develop a single erythematous macular lesion, which may reach several centimeters in diameter, most commonly on the trunk, thigh or upper arm. The macule has a characteristic collarette of fine scales. This herald patch is followed after 5–15 days by a widespread eruption of small erythematous, scaly macules, which typically form a Christmas-tree pattern on the trunk and eventually spread down the limbs, to subside after 6 weeks. Recent studies have implicated human herpesvirus 7 in pityriasis rosea[10] but these finding have been challenged by a number of studies that have failed to demonstrate an association between the disease and human herpesvirus 6 or 7.[11]

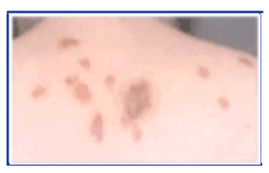

Figure 12-6 Kaposi's sarcoma in a 20-year-old man who had AIDS. One lesion on the patient's back had been treated with radiotherapy, resulting in disfiguring pigmentation at the site of treatment.

Cutaneous changes associated with HIV infection

Infection with HIV is associated with a number of cutaneous changes. Some of these are characteristic enough to alert the physician to the possibility of infection with HIV. Kaposi's sarcoma is seen in about 34% of patients who have AIDS. The great majority of these patients are homosexual or bisexual men in developed countries (see Chapter 130 and Chapter 132).

Kaposi's sarcoma is a multicentric endothelial cell neoplasm that often starts in the skin but may become disseminated to affect multiple organs.[12] It is associated with human herpesvirus 8 infection. Skin lesions range in color from red to purple-brown and may be macules, papules, nodules or plaques. Cutaneous Kaposi's sarcoma in those who have AIDS is often multiple and very disfiguring (Fig. 12.6).

Oral hairy leukoplakia presents as asymptomatic white plaques on the lateral borders of the tongue.[13] These may spread to involve the oral mucosa. Oral hairy leukoplakia is now considered to be due to a proliferation of Epstein-Barr virus within the epithelium of the tongue.

A common dermatosis in HIV-positive patients is a papulopruritic eruption or itchy folliculitis.[14] Small erythematous or skin-colored papules develop on the head, neck and trunk, and these are extremely pruritic. No infectious cause has been found for this and histologic examination shows only a non-specific perifollicular mixed cell infiltrate. This eruption does not respond well to topical corticosteroids, antihistamines or antimicrobials, but it may respond to ultraviolet light therapy.[15]

Another itchy papular eruption observed in HIV-positive patients is eosinophilic pustular folliculitis.[16] This condition starts as itchy erythematous papules on the head, neck, trunk and limbs and may develop into plaques with a papular or vesicular border. Histology shows an eosinophil-rich perifollicular infiltrate. No infectious cause has been found for this condition. As with the itchy folliculitis, topical therapies and antihistamines tend to be ineffective but the condition often responds well to ultraviolet light.

Bacillary angiomatosis presents as small angioma-like lesions affecting both the skin and the internal organs.[17] In the skin, lesions start as pin-point red papules, which then enlarge to become nodular. Although seen mainly in HIV-positive patients, bacillary angiomatosis has also been described in other immunodeficient patients and is now known to be caused by *Bartonella* spp (see Chapter 128 and Chapter 132 .

Bacterial infections

Specific bacterial infections may cause a variety of cutaneous syndromes as a result of toxin production.

Scarlet fever

Scarlet fever complicates acute infections by *Streptococcus pyogenes* strains that produce a pyrogenic exotoxin and *Staphylococcus aureus* strains that produce enterotoxins. Production of the exotoxin by *Strep. pyogenes* appears to depend on the presence of a temperate bacteriophage and is exclusive to the group A streptococci. Three antigenically distinct exotoxins can be produced: types A, B and C. Specific strains of *Strep. pyogenes* may produce one or more of these toxins.[18] After an incubation period of 2–5 days, fever develops with localized signs at the portal of entry (e.g. tonsillitis and lymphadenopathy or tenderness at a wound site).

The eruption of scarlet fever occurs on the second day of infection. It begins on the upper trunk with a punctuate erythema that becomes generalized over a few hours to 3–4 days. A characteristic sign is the development of transverse red streaks at the sites of skinfolds, owing to capillary damage; these are known as Pastia's lines. The face is erythematous but with a characteristic perioral pallor. After 7–10 days the eruption subsides with desquamation of the palms and soles.

Lyell's syndrome

Staphylococcal scalded skin syndrome, otherwise known as Lyell's syndrome, is caused by strains of *Staph. aureus* that produce a specific epidermolytic toxin. Phage group 2 staphylococci predominate, but other phage groups have been implicated.

The epidermolytic toxin cleaves the epidermis just below the granular cell layer, resulting in the typical scalded skin appearance. The eruption usually starts suddenly with erythema and tenderness of the skin. Flaccid blistering occurs, which often rubs off, leaving raw exudative areas. Staphylococci can usually be isolated from involved skin. This syndrome usually carries an excellent prognosis with resolution in 7–14 days.

The disease is one of infants and children. In the few reports of adults who have staphylococcal scaled skin syndrome there is generally an underlying medical problem, such as renal failure or immunosuppression, and it is thought that reduced clearance of the toxin by the kidneys in these patients may be important in the development of the disease.[19]

Toxic shock syndrome

The toxic shock syndrome is recognized as a complication of toxic shock toxin-1 production by selected strains of *Staph. aureus*. These have been incriminated in a variety of circumstances including tampon use, surgical or traumatic skin wounds and burns. Involvement of the skin, oral mucosa and conjunctiva is common. Dermatologic manifestations include diffuse erythema, punctate lesions, petechiae with subsequent desquamation and occasional nail loss.

Rheumatic fever

Group A ß-hemolytic streptococci are implicated in the pathogenesis of rheumatic fever. A specific (although now rare) cutaneous manifestation of rheumatic fever is erythema marginatum rheumaticum.[20] This consists of rings or arcs of pale or dull red erythema, which are either macular or slightly thickened. The rings make a discrete or enlarged polycyclic pattern. These rings characteristically fade over a few hours or days and appear in recurrent crops, usually at different sites, over many weeks.

Sepsis

Sepsis caused by a variety of different bacteria can cause disseminated intravascular coagulation. This results in hemorrhagic skin lesions, particularly of dependent

areas, followed by purpura and possibly ulceration of the skin. In acute meningococcal sepsis, 40–90% of patients develop these characteristic purpuric lesions, which are important diagnostic signs of this rapidly progressing infection.[21]

Henoch-Schönlein purpura

Henoch-Schönlein purpura has in the past been strongly associated with streptococcal infections but the link has become less impressive in recent years; other infectious agents, such as upper respiratory viruses, have been suggested. Henoch-Schönlein purpura has a characteristic appearance, with palpable purpuric papules developing on the lower legs and buttocks. This may be associated with arthritis, gastrointestinal syndromes and renal disease. Histologically, there is a leukocytoclastic vasculitis and characteristic deposition of IgA within the walls of affected blood vessels.[22]

Leprosy

Mycobacterium leprae affects the skin in all forms of leprosy. In tuberculoid leprosy the skin lesions tend to be few or solitary (see Chapter 154 , Fig. 154.2). They are often hypopigmented but they may have a rather indurated coppery color or a purple border. These patches tend to be hypoesthetic and dry with loss of hairs, and they show a negative histamine provocation test.

In lepromatous leprosy, multiple small macules, papules and nodules develop in a symmetric manner in all sites apart from the hairy scalp, the axillae and the groins, where the temperature tends to be higher. Patients often develop leonine facies because of diffuse involvement of the facial skin (Fig. 12.7). Borderline types of leprosy have clinical features between these extremes of immunologic reaction to the causative agent.

Three types of inflammatory reactions can occur during the course of leprosy. Type I leprae reaction occurs in borderline disease and is associated with upgrading of the condition. Existing skin lesions become more inflamed and painful or may ulcerate and new lesions may appear. This is associated with acute or insidious pain and tenderness of affected nerves.

Type II leprae reactions occur in patients who have lepromatous leprosy and borderline leprosy. These reactions may occur spontaneously or in response to treatment. The most common cutaneous manifestation is that of erythema nodosum leprosum, in which painful red nodules occur in the skin, most commonly on the face and extensor surfaces of the limbs.[23] Individual lesions are painful,

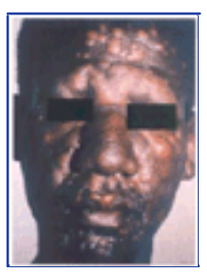

Figure 12-7 Lepromatous leprosy, with multiple symmetric lesions on the face, giving a leonine facies.

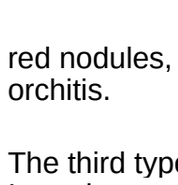

Figure 12-8 Erythema multiforme showing target lesions and bullous lesions on the palms of the hands.

red nodules, but they may ultimately ulcerate. Erythema nodosum leprosum may be accompanied by uveitis, myositis, lymphadenitis, neuritis, dactylitis, arthritis and orchitis.

The third type of reaction seen in leprosy is the so-called Lucio phenomenon.[24] This is due to deep cutaneous vasculitis leading to infarction of the overlying skin. Irregular erythematous patches develop and these may necrose to leave deep painful ulcers.

NON-SPECIFIC CUTANEOUS SIGNS OF SYSTEMIC INFECTIONS

Erythema multiforme

Erythema multiforme may occur at any age. The lesions are usually asymptomatic and start as dull red macules and papules that occur on acral sites (particularly the hands) and then spread more centrally. Typical target lesions occur; these have a central purpuric area and a raised, rather edematous border (Fig. 12.8). Less commonly, the feet, elbows, knees, face, neck and trunk are affected.

In severe eruptions, bullae may develop over the individual lesions. In the most severe form of the disease the oral mucosa shows extensive bullous formation with erosions affecting the lips and buccal mucosa. Genital lesions may also occur. This form is called the Stevens-Johnson syndrome. A variety of infectious agents have been implicated in causing erythema multiforme (Table 12.1).[25] The most common infectious cause of erythema multiforme is herpes simplex infection.

Erythema nodosum

Erythema nodosum is an acute type IV reaction to a number of different stimuli. The skin and subcutaneous fat are affected with a septal panniculitis. Clinically, there is a short prodrome of mild fever, myalgia and malaise. Erythematous nodules develop on the shins and more rarely on the arms, face and neck; these vary in diameter from 1cm to several centimeters (Fig. 12.9). The hallmark of erythema nodosum is pain and exquisite tenderness of the lesions. Initially, the lesions are bright red but as they subside over the next 3 weeks or so they undergo a bruise-like change from dusky purple-yellow and green before leaving mild scaling of the skin.

A number of infectious agents have been implicated in the etiology of erythema nodosum (Table 12.2). [26] These vary with the age of the patient and the country of residence. The most common infectious cause is an upper respiratory viral infection. Streptococcal sore throats are also a common cause in children and tuberculosis is still a common cause in children where the disease is prevalent. Infections with *Chlamydia psittaci* have been responsible for small outbreaks of erythema nodosum in adults in the UK,

TABLE 12-1 -- Infective causes of erythema multiforme.

INFECTIVE CAUSES OF ERYTHEMA MULTIFORME
Adenovirus
Coccidioidomycosis
Enterovirus
Hepatitis B virus
Herpes simplex virus
Histoplasmosis
HIV infection

Infectious mononucleosis
Legionnaires' disease
Lymphogranuloma inguinale
Mumps
Mycoplasma pneumoniae
Orf
Poliomyelitis
Pseudomonas aeruginosa
Psittacosis
Rickettsia spp.
Salmonella typhi
Streptococcus pyogenes
Syphilis
Tuberculosis
Tularemia
Typhoid
Vaccinia
Yersinia spp.

TABLE 12-2 -- Infections associated with erythema nodosum.

INFECTIONS ASSOCIATED WITH ERYTHEMA NODOSUM
Blastomycosis
Campylobacter spp.
Cat scratch disease
Coccidioidomycosis
Gonorrhea
Hepatitis C
Histoplasmosis
Inflammatory dermatophyte infections
Leptospirosis
Lymphogranuloma venereum
Psittacosis
Salmonellosis
Streptococcal infections
Tuberculosis
Tularemia
Upper respiratory tract viruses
Yersiniosis

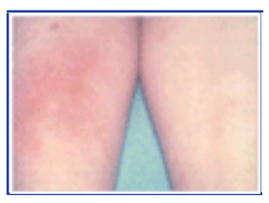

Figure 12-9 Erythema nodosum on the lower legs. On investigation this patient was found to have a negative Mantoux even at 1 in 100 but the erythema nodosum subsided when the patient was started on antituberculous therapy.

where contact with birds and poultry may be an important clue in identifying the cause of the reaction. Infections with *Yersinia* spp. are a common cause of erythema nodosum in France and Finland but are rare in other countries. Recent reports have shown that gonorrhea is a relatively common cause of erythema nodosum in Singapore.[27]

Erythema nodosum may be related to inflammatory tinea capitis in children, particularly in association with kerion formation. Deep fungal infection, particularly coccidioidomycosis, blastomycosis and histoplasmosis, has been associated with erythema nodosum. More rarely, erythema nodosum has been reported in association with tularemia, salmonellosis, *Campylobacter* spp. infection and leptospirosis.

Cutaneous vasculitis

The clinical features of cutaneous vasculitis depend on the size of the blood vessels affected and on whether the vasculitis is acute or chronic. Acute leukocytoclastic vasculitis is caused by immune complex deposition in cutaneous blood vessels with complement fixation and damage caused by polymorphonuclear leukocyte infiltration and activation. The targeted blood vessels tend to be small, superficial vessels and the clinical signs are of a purpuric macular or papular eruption on the lower legs and dependent areas; these eruptions may become bullous and ulcerate (Fig. 12.10). The most common bacterial infection associated with cutaneous vasculitis is a streptococcal sore throat,[28] [29] which precedes the vasculitis by 1–3 weeks.

Subacute and chronic cutaneous vasculitis is associated with chronic foci of infection such as dental abscesses or asymptomatic chronic pyelonephritis.[28] In nodular vasculitis, tuberculosis has been blamed for the reaction; leprosy and syphilis should also be considered. *Neisseria gonorrhoeae*, *Mycoplasma pneumoniae* and *Rickettsia* spp. may also cause an acute vasculitis (Table 12.3).

Viruses can cause endothelial cell damage, platelet agglutination and immune complex formation. The best studied causes of viral vasculitis are influenza vaccines, hepatitis B virus[30] and the Bunyaviridae-induced hemorrhagic fever. Hepatitis C virus should also be considered in any vasculitis work-up. Herpes simplex infection[31] and enterovirus infections[32] have also been implicated in cutaneous vasculitis.

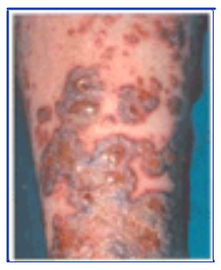

Figure 12-10 Acute leukocytoclastic vasculitis showing bullous lesions on the lower leg. This patient was found to have a high antistreptolysin titer.

TABLE 12-3 -- Infections associated with cutaneous vasculitis.

INFECTIONS ASSOCIATED WITH CUTANEOUS VASCULITIS
Streptococci
Dental abscesses
Chronic *Escherichia coli* infections
Tuberculosis
Leprosy
Syphilis
Gonorrhea
Hepatitis B virus
Hepatitis C virus
Enteroviruses
Hemorrhagic fever
Herpes simplex
Mycoplasma pneumoniae
Rickettsia spp.
Candidiasis
Cryptococcosis

Less commonly, fungal infections (including those with *Candida albicans*) have been reported in association with vascular damage and cutaneous vasculitis. Cryptococcosis may present with a widespread nodular vasculitis when complicating HIV infection.

Gianotti-Crosti syndrome

Gianotti-Crosti syndrome is a cutaneous reaction to virus infection, characteristically seen in children aged between 6 months and 12 years. The majority of cases are associated with hepatitis B virus infection, usually with subtype ayw. [33] More recently, a number of other viruses have been causally linked with this syndrome, including Epstein-Barr virus, Coxsackie viruses A16, B4 and B5, echoviruses 7 and 9, poliovirus, cytomegalovirus, respiratory syncytial virus, hepatitis A virus and parainfluenza virus.[34] [35]

The eruption presents acutely with dull red papules of 5–10mm diameter. These develop over 3–4 days, starting on the buttocks and thighs and spreading to the arms and face. The papules may become purpuric. In cases that are related to hepatitis B virus, the papules are not itchy, unlike those linked to other viruses. Lymphadenopathy of the axillae and groins is often present and may persist for several months after the eruption has settled, which generally occurs within 2–8 weeks to leave mild scaling but no scarring.

Kawasaki disease

Kawasaki disease is a diffuse vasculitic disease of unknown cause. A number of infectious agents have been implicated in its pathogenesis, including streptococci,[36] staphylococci,[37] *Leptospira* spp.,[38] *Pseudomonas* spp., *Rickettsia* spp., and Epstein-Barr virus and other viruses. In most cases no agent is identified. The disease generally affects children under the age of 4 years and although it most commonly occurs in Japan it has been reported throughout the world.

The disease is acute in onset with a remittent fever. The conjunctivae become infected and the lips and tongue are red. At the onset of fever, a generalized polymorphic eruption develops on the trunk and proximal limbs; this is associated with redness and induration of the palms and soles. Cervical lymphadenopathy develops in 50–80% of patients. The fever lasts for more than 5 days and as this subsides the skin scales and the patient may develop arthralgias and arthropathy. Cardiac involvement develops at this stage of the illness in 20% of patients, with myocarditis, aneurysm, stenosis and obstruction of the coronary arteries, which is usually responsible for the 1% mortality seen in this disease.

INFLAMMATORY DERMATOSES MIMICKING INFECTION

Acute febrile neutrophilic dermatosis (Sweet's syndrome)

Acute febrile neutrophilic dermatosis was first described by Sweet in 1964.[39] It is characterized by an explosive cutaneous eruption of raised violaceous plaques in association with constitutional symptoms and fever. Although the original series described a preceding upper respiratory infection in some patients, the majority of cases apparently are not associated with underlying infection. Approximately 50% of cases are associated with underlying malignancy, particularly myeloproliferative disorders and acute myelogenous leukemia. [40] Other reported associations include vaccination, endocrine disturbances (such as thyrotoxicosis and pregnancy), autoimmune diseases and inflammatory bowel disease. Women are more commonly affected.

Clinical presentation is an acute eruption of dull red elevated inflammatory nodules and plaques, which may pustulate in later stages or clear centrally to give an annular appearance. The majority of patients have a persistent fever and neutrophilia with elevated erythrocyte sedimentation rate and C-reactive protein. Diagnosis may be confirmed by cutaneous biopsy, which reveals a florid dermal polymorphonuclear cell infiltrate. Cases respond rapidly to systemic corticosteroid therapy, which is usually required for several weeks.

Toxic epidermal necrolysis

Toxic epidermal necrolysis (TEN) is a serious drug-induced skin disorder, which clinically may be difficult to distinguish from staphylococcal scalded skin syndrome (SSSS). Both conditions are characterized by an acute and widespread cutaneous erythema with sloughing and loss of the epidermis in sheets. As described, SSSS occurs mainly in childhood and is caused by staphylococcal toxin-mediated superficial cleavage of the epidermis below the granular layer. It has an excellent prognosis if appropriately treated. In comparison TEN is usually a drug-induced phenomenon causing full-thickness necrosis of the epidermis, with subsequent impairment of cutaneous barrier function and a high mortality (30%).[41] The precise pathogenesis is poorly understood, although increased circulating levels of fasL, a ligand for the fas (CD 95) keratinocyte death receptor, have been identified in patients with TEN.[42]

The most commonly implicated drugs are antibiotics, particularly sulfonamides, anticonvulsants, allopurinol and nonsteroidal anti-inflammatory drugs. Toxic epidermal necrolysis may be distinguished from SSSS by histologic examination of biopsied skin, allowing assessment of the level of epidermal splitting and identification of the presence or absence of micro-organisms. Treatment outcome is improved by intensive nursing in a specialist burns unit and by prompt withdrawal of all potential

precipitants. Corticosteroids do not appear to improve outcome and possibly increase mortality. Experimental evidence indicates that pooled human immunoglobulin inhibits fasL-CD95 interaction in vitro and beneficial clinical responses have been observed. [42]

Oral ulceration

Oral ulceration is a common clinical finding, with a wide differential diagnosis including infectious disease. It may be seen at any stage of syphilis, but affects up to one-third of patients with secondary disease. Ulcers are usually painless, shallow and may occur at any mucosal site.

Primary herpes simplex infection usually occurs in early childhood, producing a gingivostomatitis. Patients develop fever, malaise and restlessness followed by the development of a painful vesicular eruption on the lips, buccal mucosa, tongue and palate, with associated tender regional lymphadenopathy.

Aphthous ulceration is common, painful and occurs in a variety of clinical patterns. Crops of lesions typically occur. Minor aphthous ulcers are 2–4mm in diameter with a gray-white surface and a red margin. These are commonest on the lips, buccal mucosa or floor of the mouth and rarely occur on the gingiva, palate or dorsal tongue. Major aphthous ulcers may exceed 1cm in diameter and occur anywhere in the mouth. These are more painful, last longer than minor aphthous ulcers and may heal with scarring. Herpetiform aphthous ulceration produces very painful vesicles and multiple tiny ulcers less than 2mm in diameter. These may coalesce to produce larger lesions and tend to recur frequently. The syndrome closely resembles herpetic gingivostomatitis, although there is no evidence of viral etiology.

Behçet's syndrome is characterized by the presence of recurrent aphthous ulcers in association with genital ulcers, eye disease (particularly relapsing iridocyclitis), central nervous system disease (e.g. meningoencephalitis), arthropathy, skin lesions (e.g. pustules, pathergy, erythema nodosum) and other systemic manifestations. [43] Oral lesions in Behçet's syndrome start as small erythematous papules or pustules, which erode to form small ulcers. Pain is a variable feature. Recurrent ulceration may predate the syndrome by months or years.

Pemphigus vulgaris affects the oral mucosa in almost all patients and is the presenting feature in 50–70%. Bullae are fragile and therefore rarely seen. Ruptured bullae form painful large irregular erosions on any part of the oral mucosa. Most patients will also go on to develop flaccid cutaneous bullae which rupture easily.[44]

Eosinophilic cellulitis (Wells' syndrome)

Eosinophilic cellulitis is a rare syndrome, first described by Wells, which may closely mimic bacterial cellulitis.[45] Etiology is unknown. It is characterized by the development of indurated inflammatory areas of erythema, usually on a distal limb, which may be single or multiple. Systemic illness is unusual, although associated fever has been reported and peripheral eosinophilia is common. Early lesions are often pruritic and infiltrated but then flatten and resolve without scarring within a few weeks. Diagnosis is suggested on skin pathology by the demonstration of marked dermal eosinophilia with areas of granulomatous change surrounding aggregates of eosinophilic material, known as 'flame figures'. The condition responds to oral corticosteroid therapy.

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Chapter 13 - Superficial Fungal Infections

David W Warnock

INTRODUCTION

This chapter reviews the different fungal diseases of the skin, nails and hair. The most common of these diseases are dermatophytosis, candidiasis and pityriasis versicolor. Other, less frequent infections of the skin and hair include tinea nigra and piedra. In addition, there are a number of non-dermatophytic molds that can cause nail disease (onychomycosis). Superficial fungal infections, such as dermatophytosis and onychomycosis, have become an important problem in persons infected with HIV and other immunocompromised host groups. The diagnosis of these infections can be difficult because of atypical clinical manifestations. Furthermore, in such patients, skin and nail infections can be difficult to treat because the disease is often more extensive and severe.

EPIDEMIOLOGY

The organisms that cause dermatophytosis are molds belonging to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*.[1] Many of the 40 or so species that are recognized at present are worldwide in distribution, but others are confined to particular regions.[2] About 10 species are common human pathogens. The dermatophytes can be split into three ecologic groups depending on whether their usual natural habitat is the soil (geophilic species), animals (zoophilic species) or humans (anthropophilic species). Members of all three groups can cause human infections, but their different natural reservoirs have important implications in relation to the acquisition, site and spread of the disease. Infections originating from the soil are the least common. Infections having animal origins are more frequent and particular species are often associated with particular animal hosts. Anthropophilic dermatophytes account for most human infections; these species are contagious and are readily transmitted from person to person.

Tinea capitis (dermatophytosis of the scalp) is a common infection in children. The predominant etiologic agents differ from continent to continent, but the anthropophilic species *Trichophyton tonsurans* has replaced *Microsporum audouinii* as the dominant cause of this disease among urban populations in North, Central and South America. [3] Infections with this organism have also become much more common in the UK, particularly among black African or black Caribbean school children in London. The cause of this rise in anthropophilic infection rates is difficult to ascertain, but it is possible that it is associated with increased migration. In France, and in particular Paris, the main anthropophilic dermatophytes associated with tinea capitis are those that originate from Africa, particularly *Trichophyton soudanense*. Other less common causes of tinea capitis include the animal species *Microsporum canis* and *Trichophyton verrucosum*. Tinea pedis (dermatophytosis of the feet) is a contagious condition and is easily spread from person to person. The predominant agent is the anthropophilic species *Trichophyton rubrum*, but this disease can also be caused by *Trichophyton mentagrophytes* var. *interdigitale*. Transfer within households has been reported, but the main spread occurs in communal baths and showers.[4]

Cutaneous candidiasis is a less common disease than dermatophytosis. *Candida albicans*, the predominant etiologic agent, is a commensal organism found in the mouth and gastrointestinal tract of a significant proportion of the normal population. It is seldom recovered from normal skin, being much less prevalent than *Candida parapsilosis*, but it is a frequent colonizer of moist or damaged skin and nails.

Malassezia furfur is a common commensal organism that colonizes the normal skin of the head and trunk during late childhood.[5] In certain circumstances, such as hot humid climatic conditions, this lipophilic organism produces the disease pityriasis versicolor. In the tropics, up to 50% of the population may be affected. *Malassezia furfur* can be transmitted from person to person, either through direct contact or through contaminated clothing or bedding. In practice, however, infection is endogenous in most cases and spread between individuals is uncommon.

Tinea nigra is a chronic infection of the palms and soles. The disease is rare but has a worldwide distribution, although it is more common in the tropics and subtropics. The etiologic agent, *Phaeoannellomyces werneckii*, is a saprobic mold that is found in the soil and in decomposing vegetation. Human infection is thought to follow traumatic inoculation.

Black piedra is an uncommon hair infection that occurs in humid tropical regions. The natural habitat of the etiologic agent, *Piedraia hortae*, has not been identified. There are some reports of familial infection. White piedra is less common than black piedra. It is found worldwide, but it is more prevalent in the tropics and subtropics. The etiologic agent, *Trichosporon asahii*, has a widespread natural distribution and is sometimes found on normal skin.

Onychomycosis is a non-specific term used to describe fungal disease of the nails; tinea unguium is a more specific term used to describe dermatophyte nail infection. At least 80% of fungal nail infections and 90% of toenail infections are due to dermatophytes, in particular *Trichophyton rubrum*. [6] Between 5% and 10% of nail infections are due to *Candida* spp. and the remainder are attributable to non-dermatophytic molds. Most prominent among these are *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum* (*Hendersonula toruloidea*), *Aspergillus* spp. and *Fusarium* spp.[6] Unlike the dermatophytes, these molds are not contagious. Onychomycosis is more prevalent in older people and men are more commonly affected than women. Toenails are more frequently involved than fingernails.

PATHOGENESIS AND PATHOLOGY

The dermatophytes are keratinophilic fungi that are normally found growing only in the dead keratinized tissue of the stratum corneum, within and around hair shafts, and in the nail-plate and keratinized nail-bed. The clinical appearances of dermatophyte infections are the result of a combination of direct damage to the tissue by the fungus (mainly in the case of hair and nail infections) and of the immune

response of the host. The damage to tissue is due to a combination of mechanical forces and enzymatic activities. Dermatophytes produce a number of keratinolytic proteinases that function best at an acidic pH and these have been recognized as important virulence factors.[1]

The immune response to dermatophytes has been studied in human infections as well as in animal models.[7] The humoral response does not appear to help in the elimination of infection; the highest levels of antibodies are often found in patients with chronic dermatophytosis. Rather, it is the cell-mediated response that is important in ridding the stratum corneum of the infection.[8] Dermatophytes vary in their host interactions. Zoophilic species, such as *Trichophyton verrucosum*, often elicit intense inflammation in humans. This leads to enhanced epidermal proliferation and can result in spontaneous cure.[9] In contrast, anthropophilic species such as *Trichophyton rubrum* often produce chronic or recurrent lesions. Chronic dermatophytosis in otherwise healthy people may be mediated by fungal cell wall components, such as mannan, that diminish the local immune response.[9]

Except for neonatal infections, most cases of superficial candidasis result from infection of the host from his or her own commensal flora. This shift in the host-fungus relationship results from a number of influences, of which host factors appear to be the most important. Local tissue damage is a critical factor in the pathogenesis of cutaneous candidiasis; most infections occur in moist, occluded sites and follow maceration of the tissue. Chronic mucocutaneous candidiasis is a rare condition that results from inherited defects in the cell-mediated immune response.[10]

Malassezia furfur is present on the normal skin from late childhood. Hot, humid environmental conditions are among the factors that predispose to the development of the cutaneous lesions of pityriasis versicolor.

PREVENTION

Prevention of dermatophytosis must take into account the site of the infection, the etiologic agent and the source of the infection.

Anthropophilic tinea capitis is a common fungal infection in children. It is easily spread from child to child, both in the home and at school. To prevent this, contacts of children infected with *Microsporum audouinii* can be examined for infected fluorescent hairs with Wood's light (a source of ultraviolet light filtered through nickel oxide glass). In the more common non-fluorescent infection with *Trichophyton tonsurans*, detection is more difficult, but the scalp brush sampling method is often helpful in detecting subclinical disease. [11] All those found to be infected must be treated and the importance of good personal hygiene should be stressed. It is seldom practical to exclude infected children from school.

In the case of tinea capitis and tinea corporis caused by zoophilic species, such as *Microsporum canis* and *Trichophyton*

TABLE 13-1 -- Some characteristics of common dermatophytes causing scalp infection.

SOME CHARACTERISTICS OF COMMON DERMATOPHYTES CAUSING SCALP INFECTION			
Organism	**Arthrospore size**	**Arthrospore arrangement**	**Fluorescence under Wood's light**
Microsporum audouinii	Small (2–3µm)	Ectothrix	Yes
Microsporum canis	Small (2–3µm)	Ectothrix	Yes
Trichophyton mentagrophytes	Small (3–5µm)	Ectothrix	No
Trichophyton soudanense	Large (4–8µm)	Endothrix	No
Trichophyton tonsurans	Large (4–8µm)	Endothrix	No
Trichophyton verrucosum	Large (5–10µm)	Ectothrix	No
Trichophyton violaceum	Large (4–8µm)	Endothrix	No

verrucosum, it is important to locate the animal source. *Microsporum canis* infection of cats and dogs can often be detected with Wood's light examination. The subsequent course of action will depend upon the value placed on the infected animal. It is more difficult to detect and eliminate *Trichophyton verrucosum* infection in cattle, because infected hairs are not fluorescent and because the fungus can survive for long periods on hairs and scales that have been deposited on the walls of buildings and gates. Fungicidal washes have sometimes been effective in controlling this infection.

Tinea pedis is a contagious condition and is easily spread from person to person. Transfer within households has been reported, but the main spread occurs in communal baths and showers.[4] Educating infected people not to expose others to their infection by not walking barefoot on the floors of communal changing rooms and by avoiding public baths and showers can help to reduce the spread of this disease. Frequent hosing of the floors of public baths and antifungal foot dips near communal baths are helpful preventive measures. Prompt treatment of tinea pedis and the use of separate towels are sensible measures that can help to prevent tinea cruris, tinea manuum and tinea unguium.

Intertriginous candidiasis of the fingernails is often seen in people whose occupation necessitates frequent wetting of the hands. Wearing protective gloves can help to prevent this infection.

Good personal hygiene is important in preventing the spread of piedra. Infected people should not share hair brushes or combs with others.

CLINICAL FEATURES

The dermatophytes are the predominant causal organisms of fungal disease of the scalp, toe clefts, soles, palms and nails. In the temperate, developed countries, tinea pedis is the most common form of dermatophytosis. By contrast, in the tropics, tinea capitis and tinea corporis are the most prevalent.

Tinea capitis

The clinical manifestations of tinea capitis are varied and depend on the species of dermatophyte involved and the degree of host response (Table 13.1). The appearance of the lesions can range from mild scaling and hair loss with minimal inflammation to severe inflammation with kerion formation.

In *Microsporum audouinii* infection the lesions consist of well-demarcated patches of partial alopecia. Inflammation is minimal, but fine scaling is characteristic. Most of the hairs in these lesions are broken off near the surface of the scalp. In *Microsporum canis* infection the picture is similar, but there is usually more inflammation. In both these infections the hair surface is coated with small arthrospores (ectothrix infection). The affected hairs show green fluorescence under Wood's light.

In *Trichophyton tonsurans* and *Trichophyton violaceum* infections the lesions are often inconspicuous and inflammation may be minimal. The typical lesions are irregular patches of scaling. The affected hairs often break off at the surface of the scalp, giving a 'black-dot' appearance. The hairs are filled with arthrospores (endothrix infection) and do not fluoresce under Wood's light.

The most florid form of tinea capitis is a kerion. A kerion is a painful inflammatory mass in which the hairs that remain are loose. Thick crusting with matting of adjacent hairs is common. Pus may be discharged from one or more points. A kerion may be limited in extent, but a large confluent lesion may develop (a severe form of kerion) that involves most of the scalp. In most cases this violent reaction results from infection with an animal dermatophyte such as *Trichophyton verrucosum* or *Trichophyton mentagrophytes* var. *mentagrophytes*. However, geophilic or anthropophilic organisms are sometimes involved. In *Trichophyton verrucosum* infections the hairs are covered with chains of large arthrospores but they do not fluoresce under Wood's light.

Favus is now rare, but it is still a distinctive form of fungal scalp infection. The causal organism is *Trichophyton schoenleinii*, an anthropophilic dermatophyte noted for its persistence. Favus presents with hair loss and the formation of cup-shaped crusts known as scutula. These give off a fetid odor and can amalgamate to form dense mats on part or all of the scalp. Long-standing favus can lead to permanent patches of cicatricial alopecia. Infected hairs give off a dull green fluorescence under Wood's light.

Tinea capitis must be distinguished from seborrheic dermatitis, psoriasis, bacterial folliculitis and cicatricial alopecia.

Tinea barbae

The animal species *Trichophyton verrucosum* and *Trichophyton mentagrophytes* var. *mentagrophytes* are the principal causes of dermatophyte infection of the beard and moustache areas of the face. *Microsporum canis* is a less common cause. The characteristic appearance is of a highly inflammatory pustular folliculitis (Fig. 13.1). Some infections are less severe and consist of circular, erythematous, scaling lesions.

Tinea faciei

The more common causes of dermatophyte infection of the face are *Trichophyton rubrum* and *Trichophyton mentagrophytes* var. *mentagrophytes*, but many other species may be involved, including *Trichophyton tonsurans* and *Microsporum canis*. The typical annular lesions are erythematous, but scaling is often absent. The lesions are often pruritic and exacerbation after exposure to the sun is common.

Tinea corporis

The clinical manifestations of tinea corporis are varied and often depend on the species of the infective organism. The disease often

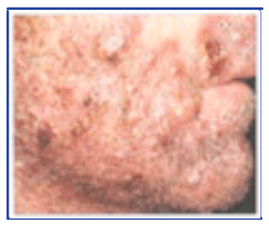

Figure 13-1 Tinea barbae due to *Trichophyton verrucosum.*

follows contact with infected animals, but occasional cases result from contact with contaminated soil. *Microsporum canis* is a frequent cause of human infection and *Trichophyton verrucosum* infection is common in rural districts. Infections with anthropophilic species, such as *Trichophyton rubrum*, often follow spread from another site, such as the feet. Infections with *Trichophyton tonsurans* are sometimes seen in children with tinea capitis.

The characteristic lesion is an annular scaling plaque with a raised erythematous border and central clearing. In their most florid form the lesions can become indurated and pustular (Fig. 13.2). This is more common in infections with zoophilic organisms. The differential diagnosis includes discoid eczema, impetigo, psoriasis and discoid lupus erythematosus.

Tinea cruris

Infection of the groin and the perianal and perineal regions is more common in men. The predominant causes are the anthropophilic species *Trichophyton rubrum* and *Epidermophyton floccosum*. The infection often follows spread from another site in the same person, but person-to-person spread (e.g. through contaminated clothing) is not uncommon.

In color, the lesions are erythematous to brown. They have raised scaling margins and radiate from the groin down the inner border of the thigh. Patients often complain of intense pruritus. The differential diagnosis includes intertriginous *Candida* spp. infection, bacterial intertrigo, psoriasis and seborrheic dermatitis.

Tinea imbricata

This is a chronic infection that is characterized by the development of homogeneous sheets or concentric rings of scaling that can spread to cover large parts of the affected person. Most reports of tinea imbricata have come from the Pacific Islands and Melanesia but there have been occasional reports from South East Asia and Central and South America. The etiologic agent is the anthropophilic species *Trichophyton concentricum*.

Tinea pedis

Infection of the feet is the most common form of dermatophytosis in the UK and North America. The main organisms involved are the anthropophilic species *Trichophyton rubrum* and, less commonly, *Trichophyton mentagrophytes* var. *interdigitale*.

The most common clinical presentation is interdigital maceration, peeling and fissuring, mostly in the spaces between the fourth and fifth toes. Itching is a common symptom.

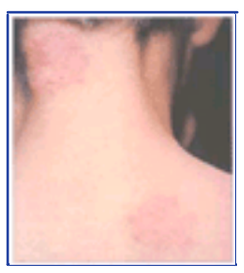

Figure 13-2 Tinea corporis due to *Trichophyton mentagrophytes* var. *mentagrophytes.*

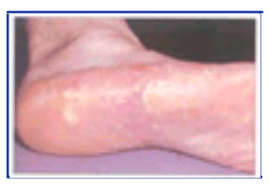

Figure 13-3 Moccasin tinea pedis due to *Trichophyton rubrum.*

Another common presentation associated with *Trichophyton rubrum* is hyperkeratosis of the soles, heels and sides of the feet. The affected sites are pink and covered with fine, white scales. This form of the disease is often chronic and resistant to treatment. If there is extensive involvement of the foot, then the term 'moccasin tinea pedis' is often applied (Fig. 13.3).

A third form of tinea pedis, associated with *Trichophyton mentagrophytes* var. *interdigitale*, is an acute vesicular infection of the soles. This severe form of the disease may resolve without treatment, but exacerbations tend to occur under hot humid conditions. There is often associated hyperhidrosis.

Tinea pedis can be difficult to distinguish from other infectious causes of toe web infection, such as *Candida* intertrigo and erythrasma. Noninfectious conditions that mimic tinea pedis of the soles include psoriasis and contact dermatitis.

Tinea manuum

Tinea manuum is usually unilateral, the right hand being more commonly affected than the left. Lesions on the dorsum of the hand appear similar to those of tinea corporis, with a distinct border and central clearing. Infection of the palms is more common. This presents as a diffuse scaling hyperkeratosis, with accentuation of the fissuring in the palmar creases. *Trichophyton rubrum* is the most common cause of tinea manuum. The differential diagnosis includes contact dermatitis, eczema and psoriasis.

Tinea unguium

The most common causes of dermatophyte infection of the nails are *Trichophyton rubrum* and *Trichophyton mentagrophytes* var. *interdigitale*, but many other species may be involved. Three clinical forms of tinea unguium are recognized. Distal (or lateral) subungual disease is the most common presentation (Fig. 13.4). This usually begins as a discoloration and thickening of the nail and it can result in destruction of the entire nail-plate and separation of the nail from the nail-bed (Fig. 13.5). In superficial white onychomycosis, crumbling white lesions are evident on the nail surface, particularly the toenails. This condition is most commonly caused by *Trichophyton mentagrophytes* var. *interdigitale*. Proximal subungual disease is the least common presentation of dermatophyte nail infection. In the USA *Trichophyton rubrum* is the principal cause of proximal subungual onychomycosis.

The differential diagnosis includes eczema, lichen planus, onychogryphosis and lichen planus. Unlike dermatophytosis, *Candida* infections of the nails often begin in the proximal nail-plate and are associated with nail-fold infection.

Candidiasis

The lesions of cutaneous candidiasis (intertrigo) tend to develop in warm, moist sites such as the folds of the skin under the breasts and the groin. The infection is more common in overweight or diabetic people. The initial lesions are papules or vesicopustules that later enlarge and become confluent. The larger lesions are erythematous

Figure 13-4 Patterns of fungal nail disease.

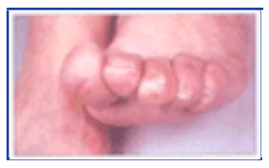

Figure 13-5 Total dystrophic onychomycosis due to *Trichophyton rubrum.*

and have an irregular margin. Smaller, satellite lesions are often present. Soreness and itching are usual. The differential diagnosis includes dermatophytosis, seborrheic dermatitis, bacterial intertrigo and psoriasis.

Infection of the skin between the fingers or toes can also occur. Infection of the webs of the fingers presents as a macerated, erythematous lesion (Fig. 13.6). It is often uncomfortable and may be painful. This condition is usually seen in people whose occupations necessitate frequent immersion of the hands in water. Infection of the webs of the toes mimics tinea pedis and many cases do occur in conjunction with this form of dermatophytosis.

Chronic mucocutaneous candidiasis is a rare condition that affects people with underlying endocrinologic or immunologic disorders. The disease often develops during the first 3 years of life. The mouth is usually the first site to be affected, but lesions then appear on the

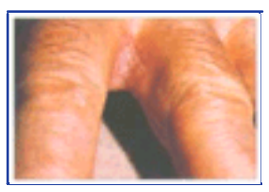

Figure 13-6 Interdigital candidiasis.

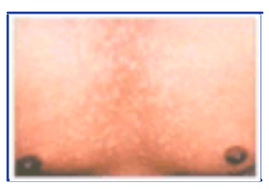

Figure 13-7 Pityriasis versicolor showing depigmented lesions.

scalp, hands, feet and nails. In some patients, disfiguring hyperkeratotic lesions develop on the scalp and face.

Three forms of *Candida* nail infection are recognized: infection of the nail-folds (paronychia), distal nail infection and total dystrophic onychomycosis. The last is a manifestation of chronic mucocutaneous candidiasis. Infection of the nail-folds is more common in women than in men. The periungual skin is raised and painful and a prominent gap develops between the fold and the nail-plate. White pus may be discharged. The infection usually starts in the proximal nail-fold, but the lateral margins are sometimes the first site to be affected. The nail-plate may be invaded.

Distal *Candida* nail infection presents as onycholysis and subungual hyperkeratosis. It is often difficult to distinguish from dermatophytosis, but candidasis tends to affect the fingernails rather than the toenails. In patients with chronic mucocutaneous candidiasis, the nail-plate is invaded from the outset, causing gross thickening and hyperkeratosis.

Pityriasis versicolor

Pityriasis versicolor is a disfiguring but otherwise harmless condition. The characteristic lesions consist of patches of fine brown scaling that are found particularly on the upper trunk, neck, upper arms and abdomen. In light-skinned people the affected skin may appear darker than normal. The lesions are light pink in color but grow darker, turning a pale brown shade. In dark-skinned or tanned people, the affected skin becomes depigmented (Fig. 13.7).

Hyperpigmented lesions must be distinguished from erythrasma, seborrheic dermatitis, pityriasis rosea and tinea corporis. Hypopigmented lesions can be confused with pityriasis alba and vitiligo.

Tinea nigra

The lesions of tinea nigra, which are found on the palm or sole, consist of one or more flat, dark brown or black, non-scaling patches with a well-defined edge. Inflammation is absent. The lesions, which are small at first, expand and become confluent. The disease is asymptomatic and may remain undiagnosed for long periods. Tinea nigra must be distinguished from malignant melanoma and chemical stains.

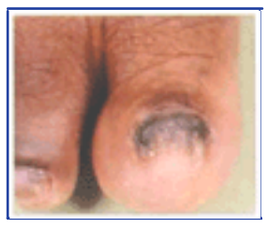

Figure 13-8 Onychomycosis due to *Scytalidium dimidiatum* (*Hendersonula toruloidea*).

Piedra

Black piedra is most often seen on the scalp hair. Small, brown or black, hard nodules, which are difficult to remove, are formed on the distal hair shafts. The appearance of white piedra is similar, but the nodules are softer and pale in color. This condition affects the hairs of the beard and moustache. Less commonly, it involves the scalp or pubic hair.

Onychomycosis

Up to 5% of cases of onychomycosis are due to non-dermatophyte molds. With the exception of *Scytalidium dimidiatum*, these molds usually affect nails that have previously been diseased or damaged. This may account for the fact that these infections often affect only one nail. There is nothing specific about the clinical appearance of the lesions (Fig. 13.8). Distal subungual hyperkeratosis with onycholysis of the distal nail-plate is common (see Fig. 13.4). Superficial white lesions are

another presentation.

Superficial fungal infections in immunocompromised patients

In general, fungal infections, such as dermatophytosis and onychomycosis, are no more common in immunocompromised persons than in immunocompetent individuals. [12] [13]

The clinical manifestations of dermatophytosis in the immunocompromised patient are often similar to those seen in normal individuals. However, the clinical presentation can be atypical, particularly in patients with T-cell defects, such as organ transplant recipients and persons who have AIDS. The major features of dermatophytosis in these groups are loss of obvious lesions, minimal scaling and the presence of follicular papules or pustules. In addition, the lesions can be more extensive than normal.

Tinea pedis has been described in both organ transplant recipients and persons who have AIDS. The lesions are often indistinguishable from those seen in normal individuals but can be extensive, with involvement of the dorsum of the foot. In tinea corporis and tinea cruris, the lesions can be extensive but the inflammation is mild and the margin is indistinct. Facial dermatophytosis has been noted in persons who have AIDS, where it can be confused with seborrheic dermatitis. This is because the rash is diffuse and can spread across both cheeks.

Although proximal subungual onychomycosis is the most infrequent form of fungal nail disease in the general population, it is common in persons who have AIDS and has been considered a useful clinical marker of HIV infection.[14] Infection of the toenails is much more frequent than fingernail infection. *Trichophyton rubrum* is the usual etiologic agent. In AIDS patients, it can spread rapidly from the proximal margin and superior surface of the nail to produce gross white discoloration of the plate without obvious thickening.

Malassezia furfur infections of the skin can take a number of different clinical forms in immunocompromised persons, including pityriasis versicolor, *Malassezia* folliculitis and seborrheic dermatitis.[12] [13] The clinical manifestations of pityriasis versicolor in immunocompromised persons are similar to those seen in normal individuals. However, the lesions are usually more erythematous and may appear raised. *Malassezia furfur* can also cause folliculitis in immunosuppressed individuals. This is characterized by scattered itching follicular papules and pustules on the chest and back.

Seborrheic dermatitis is chronic relapsing scaling dermatosis of the face, scalp and trunk. It is the commonest cause of dandruff (scalp scaling). The role of *Malassezia furfur* in the pathogenesis of seborrheic dermatitis is controversial and is based on the fact that most cases respond to azole antifungal treatment. Improvement is associated with disappearance of the organisms and relapse with recolonization. Seborrheic dermatitis is a common and troublesome problem, estimated to occur in up to 80% of persons with HIV infection. The lesions take the form of an erythematous scaling rash on the scalp, face, ears, chest and upper back. Scaling of the eyelid margins and around the nasal folds is a common presentation. The rash is often more extensive than in other individuals.

DIAGNOSIS

Superficial fungal infections often present with characteristic lesions but where this is not the case, mycologic investigation can assist in diagnosis. Material should be collected from cutaneous lesions by scraping outward from the margin. Cleansing the site with 70% alcohol before sampling will increase the likelihood of detecting fungus on direct microscopic examination. Nail specimens should be taken from discolored or dystrophic parts of the nail and should include the full thickness of the nail. If distal subungual lesions are present, debris should be collected from underneath the nail. If there is superficial nail-plate involvement, the scrapings should be taken from the nail surface. Specimens from the scalp should include hair roots and skin scales. Wood's light can sometimes be useful for the selection of sites of active infection, especially if the lesions are inconspicuous or atypical.

Direct microscopic examination of skin and nail material is often sufficient for the diagnosis of a dermatophyte infection, but it gives no indication as to which species is involved. With hair specimens, the size and disposition of the arthrospores can give some indication as to the etiologic agent (see Table 13.1 ; Chapter 240).

Culture is a more reliable method of diagnosis than microscopic examination. It permits the species of dermatophyte to be determined and this can aid the selection of the most appropriate form of treatment. If possible, both microscopic examination and culture should be performed on all specimens. If, however, there is insufficient material for both, microscopic examination should be performed.

Cutaneous candidiasis is often difficult to diagnose if the lesions are other than typical in appearance. Isolation of *Candida albicans* from scrapings is of doubtful significance because the organism is a common colonizer of cutaneous lesions in moist sites. Microscopic demonstration of the organism in scrapings is much more significant. Isolation of *Candida albicans* from nails is seldom significant unless the organism is seen on direct microscopic examination.

Microscopic examination of scrapings from lesions will permit the diagnosis of pityriasis versicolor if there are clusters of round or oval cells together with short broad filaments (which are seldom branched). Because this appearance is pathognomonic for pityriasis versicolor, and because *Malassezia furfur* is part of the normal skin flora, its isolation in culture is not helpful. Direct microscopic examination and culture of scrapings or epilated hairs will permit the diagnosis of tinea nigra and piedra.

It is not unusual to isolate molds other than dermatophytes from abnormal nails cultured on media from which cycloheximide has been omitted. In many cases, these molds are casual, transient contaminants and direct microscopic examination of the material is negative. However, if filaments are seen on microscopic examination but no dermatophyte is isolated, it is possible that the mold is the cause of the infection.

MANAGEMENT

There is now a good selection of topical and systemic agents for the treatment of superficial fungal infections. The choice of treatment and its duration depends on the causative organism, the site of infection and the extent of the disease, as well as on other factors for each individual patient, such as concurrent disease and medication. Topical agents can be used for localized skin infections, but they are seldom successful for sites with a thick keratin layer. The palms and soles and certainly the nails and hair often require systemic antifungal treatment. Although they respond well to many topical and systemic antifungal agents, persons who have AIDS often suffer from recurrent episodes of superficial fungal infections. If the disease is chronic and extensive, systemic treatment is required.

Tinea capitis

Topical treatment is ineffective on its own in tinea capitis. Terbinafine 250mg/day and itraconazole 100mg/day are both effective oral treatments for scalp infection. In adults, either agent should be given for 2–4 weeks. Terbinafine is licensed in some countries for use in children and it appears to be a safe and effective agent in this group.[15] In other countries, the older drug griseofulvin must still be used in children. The recommended dose is 10mg/kg/day for at least 6–8 weeks.

Tinea corporis and tinea cruris

The choice of topical or systemic treatment in these conditions depends on the extent of the disease. Localized lesions can be treated with topical antifungal preparations. Numerous imidazoles and allylamines are available in different formulations. These agents should be applied morning and evening for 2–4 weeks. To prevent relapse, treatment should be continued for at least 1 week after the lesions have cleared.

If the disease is widespread or the patient fails to respond to topical preparations, oral treatment is usually indicated. Terbinafine 250mg/day for 2–4 weeks and itraconazole 100mg/day for 2 weeks are more effective than griseofulvin 10mg/kg/day for 4–6 weeks. Unlike itraconazole, oral terbinafine has a low potential for drug interactions, making it a useful agent for the treatment of dermatophytosis in persons who have AIDS and other immunocompromised individuals. Terbinafine has been reported to be a safe and effective drug for tinea corporis and cruris in these patient groups.[16] [17]

Tinea pedis

Tinea pedis is a chronic infection that seldom clears if left untreated. Infection of the webs of the toes will often respond to topical terbinafine, applied morning and evening for 1–2 weeks. Topical imidazoles can also be used, but they are less effective and must be applied for at least 4 weeks.[18] The recurrence rate following

topical treatment is quite high and it is not uncommon for chronic infection to persist despite treatment.

If the disease involves the soles or if there is acute inflammation, oral treatment should be given. Terbinafine 250mg/day for 2 weeks has been shown to be effective in tinea pedis. Itraconazole 100mg/day is an alternative, but it must be given for 4 weeks.[19] Chronic tinea pedis is often associated with nail infection. Inadequate treatment of onychomycosis may result in reinfection of

the feet. Terbinafine has been reported to be a safe and effective drug for tinea pedis in persons who have AIDS.[16] [17]

Tinea manuum

Local treatment with an imidazole or an allylamine will often suffice to clear tinea manuum. In cases that fail to respond to topical preparations, oral treatment is usually indicated. Infections of the palms are difficult to clear with griseofulvin, but oral terbinafine 250mg/day for 2–6 weeks has been shown to be highly effective.[20] Itraconazole 100mg/day for 4 weeks is an alternative.

Candidiasis

Most patients with cutaneous candidiasis will respond to topical treatment with terbinafine or an imidazole such as clotrimazole or micronazole, applied for 2–4 weeks. However, relapse is common if any underlying problem is not controlled. If the infection is associated with an underlying skin disease, such as flexural eczema or diaper dermatitis, treatment with a combination preparation containing an azole agent together with hydrocortisone is often helpful.

Long-term treatment with itraconazole or fluconazole has helped many patients with chronic mucocutaneous candidiasis. However, protracted treatment has led to the development of azole-resistant stains of *Candida albicans* in some cases.[21]

Pityriasis versicolor

If left untreated, pityriasis versicolor will persist for long periods. Most patients with this disease respond to topical treatment with terbinafine, azole agents or selenium sulfide shampoo, but more than half relapse within 12 months. Oral treatment with itraconazole 200mg/day for 1 week is indicated for extensive or recalcitrant lesions. Oral terbinafine and griseofulvin are ineffective.

Seborrheic dermatitis

This is a difficult condition to treat in persons who have AIDS. Milder forms can often be managed with topical ketoconazole 2% cream with or without a topical corticosteroid. Terbinafine cream may also be effective. Patients with inflamed lesions often benefit from topical application of combined hydrocortisone 1% and antifungal drugs. However, relapse is common once treatment is discontinued.

Tinea nigra

Benzoic acid compound ointment or 10% thiabendazole solution can be applied morning and evening for 3–4 weeks. Topical imidazoles are also effective.

Piedra

Black piedra can be treated with a topical salicylic acid preparation or an imidazole cream. However, relapse is common. Shaving or clipping the affected hairs is often sufficient to clear white piedra. To help prevent recurrence, an imidazole lotion can be applied to the scalp after shampooing.

Onychomycosis

Topical agents should only be used where the infection is confined to the distal ends of the nails. Topical applications of tioconazole or amorolfine should be continued for at least 6 months for fingernails and 12 months or longer for toenails.

Oral terbinafine 250mg/day is the treatment of choice for proven dermatophyte infections of the nail (tinea unguium).[22] However, it is not appropriate for *Candida* infections, non-dermatophytic mold infections or mixed infections.[23] The optimum treatment period is 6–12 weeks for fingernails and 3–6 months for toenails. Treatment with terbinafine will also clear any associated skin infection without the need for additional topical treatment. Terbinafine 250mg/day for 3 months has been proven to be effective in the treatment of dermatophyte infections of the nail in persons who have AIDS. No drug interactions or significant adverse effects related to the drug have been reported.[24]

Itraconazole is also licensed for the oral treatment of nail infections at a dose of 200mg/day for 3–6 months. Pulsed treatment with itraconazole (in which 1 week of treatment is alternated with 3 weeks without treatment for 3–4 months) has also been advocated. It is at least as effective as continuous treatment while offering potential improvements in patient compliance and cost-effectiveness. Itraconazole has a broader spectrum than terbinafine and is more appropriate for patients who present with non-dermatophyte or mixed nail infections.[23] However, itraconazole interacts with a number of other drugs and this can limit its usefulness in certain situations. It is not recommended for simultaneous use with protease inhibitors in persons who have AIDS. Fluconazole is not licensed for use in fungal nail disease, but it is sometimes useful in severe *Candida* nail infections.

Oral griseofulvin is only effective in dermatophytosis. It is no longer regarded as a first-line choice for toenail infections, but it works quite well in fingernail infections if given over a 6–9 month period. It must be taken until the affected nail has fully grown out. It should be borne in mind that it has a number of side-effects and can interact with other medications.

Other interventions include chemical dissolution of the nail using 40% urea paste and, in rare instances, surgical removal of the nail. Surgical removal is a painful and disfiguring procedure that should be reserved for cases where there are either contraindications to the use of systemic antifungal agents or a drug-resistant fungus is present.

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Chapter 14 - Spotted Fever due to Rickettsiae

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INTRODUCTION

Rickettsioses are caused by bacteria of the order Rickettsiales, which are Gram-negative micro-organisms that grow only in association with eukaryotic cells.[1] Molecular phylogenic studies based on 16S rRNA analysis have helped to provide a basis for the reclassification of these bacteria. All rickettsiae belong to the family Rickettsiaceae. The genus *Rickettsia* is subdivided into the typhus group, whose members are *Rickettsia typhi* and *Rickettsia prowazekii*; the spotted fever group (SFG), which comprises about 15 different species of bacteria pathogenic for humans; and the scrub typhus group, whose only member is *Orientia tsutsugamushi*, the agent of scrub typhus.[1] The SFG comprises principally *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, and *Rickettsia conorii*, the agent of Mediterranean spotted fever.

Rickettsiae are associated with arthropods and are transmitted to humans principally by the bite of infected arthropods or by infected feces. Ixodid or hard ticks are the vectors of most SFG rickettsiae, mites are vectors of *Rickettsia akari* and fleas are the vectors of *Rickettsia felis*. [1] Prior to 1984, only eight rickettsioses were recognized and in the subsequent 13 years, seven new rickettsioses were described. [2] Strains of unknown pathogenicity and new rickettsiae strains have been isolated from arthropods, particularly in ticks, and their roles as human pathogens have yet to be determined.

The four main symptoms that may be observed during spotted fever due to rickettsiae include fever, a rash that is usually maculopapular but can be purpuric or sometimes vesicular, an inoculation black eschar, named 'tache noire', at the site of the arthropod bite, and lymphadenopathies draining this lesion or more generalized.

HISTORY

The Rocky Mountain spotted fever caused by *Rickettsia rickettsii*[2] was first recognized in the 19th century by American Indians in the Bitter Root Valley of western Montana. [3] At the beginning of the 20th century, Ricketts proved that the wood tick, *Dermacentor andersoni*, was involved in the transmission of this disease.

The first case of Mediterranean spotted fever (MSF) was reported in 1910, in Tunisia.[3] In 1925, the inoculation eschar at the site of the tick bite was reported in Marseilles (France). In 1930, the role of *Rhipicephalus sanguineus* was established. In 1932, the causative agent *Rickettsia conorii* was described by Brumpt.[3]

EPIDEMIOLOGY

The main characteristics of spotted fever due to rickettsiae are presented in Table 14.1 .

Rocky Mountain spotted fever (RMSF) is due to *Rickettsia rickettsii*.[1] [4] Most cases are diagnosed during late spring and summer in America. Two to 14 days after a tick bite, the patient presents with high fever, headaches and unspecific symptoms such as nausea, vomiting, anorexia, diarrhea and myalgia. The classic triad of fever, headache and rash is present in only 44% of confirmed cases. The inoculation eschar is very rarely observed. The rash, which is absent in 34% of cases, is macular. The disease can be associated with general manifestations related to increased vascular permeability and can lead to a multiple organ dysfunction syndrome (MODS). This malignant form is particularly observed in old people, in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and in alcoholics. The clue to the diagnosis is unexplained fever in a patient with a history of tick exposure, in an endemic area. Untreated patients worsen progressively with a high level of mortality (20–25%) when treatment is delayed.

Rickettsia conorii has different but related serotypes; the strain Malish is the most common (Europe and Africa) and the other serotypes are Israel (Israel and southern Europe), Astrakhan (on the Caspian sea) and Indian (India). Many names are given to the infection caused by *R. conorii*, including Mediterranean spotted fever, boutonneuse fever, Marseilles fever, Astrakhan fever, Israeli spotted fever, Indian tick typhus and Kenya tick typhus.

Mediterranean spotted fever is due to *Rickettsia conorii sensu stricto* and is transmitted by the dog tick *Rhipicephalus sanguineus* (Fig. 14.1), which seldom bites humans.[1] Most cases are observed in summer in the Mediterranean area. Approximately 7 days after a tick bite, the typical clinical presentation is that of a patient with fever, a rash and a single inoculation eschar at the site of the tick bite. Rash is observed in 97–99% of cases. It is clearly papular (Fig. 14.2). The inoculation eschar is found in 50–80% of cases. The spontaneous evolution is milder than in RMSF, but a fatality rate of 1% of diagnosed patients is still observed. The malignant form of the disease with shock and MODS occurs in 6–7% of patients. This form is particularly observed in old people, in patients with G6PD deficiency and in alcoholics. Israeli spotted fever, due to *Rickettsia conorii* serotype Israel, is transmitted by *Rhipicephalus sanguineus*. It is clinically very similar to MSF but the inoculation eschar is more rarely observed.[1] The disease, which occurs in Israel, is mild. Astrakhan fever, due to *Rickettsia conorii* serotype Astrakhan, is transmitted by *Rhipicephalus pumilio*. This disease, which occurs in Astrakhan, is very similar to MSF[1] but the inoculation eschar is rarely observed. The spontaneous evolution is mild. Indian tick typhus, due to *Rickettsia conorii* serotype Indian, is transmitted by *Rhipicephalus sanguineus*. This disease has been described in India[5] and is similar to MSF. The rash is frequently purpuric but an inoculation eschar at the bite site is rarely found. The disease is mild to moderately severe.

African tick bite fever, due to *Rickettsia africae*, is transmitted in sub-Saharan Africa by *Amblyomma* spp. It is characterized by a rash in 46% of patients after 1–10 days of incubation. [1] [2] [6] The rash is vesicular in half of the cases. Nearly all patients have an inoculation eschar and 54% have multiple eschars (Fig. 14.3). Eschars are frequently found on lower limbs. The tick vector, typically *Amblyomma*, attacks humans in groups, which explains why grouped cases and multiple eschars are observed. The evolution is much milder than that in MSF. Fever is often absent but draining lymphadenopathy in the groin is frequently observed.

TABLE 14-1 -- Main characteristics of spotted fever due to rickettsiae.

MAIN CHARACTERISTICS OF SPOTTED FEVER DUE TO RICKETTSIAE									
Bacteria	**Disease**	**Vectors**	**Geographic distribution**	**Spotted exanthema**	**Rash specificity**	**Inoculation eschar**	**Multiple eschars**	**Draining adenopathy**	**Current mortality %**
R. rickettsii	Rocky mountain spotted fever	*Dermacentor andersoni Dermacentor variabilis*	North, Central and South America	Yes	Purpuric (half of cases)	Very rare	No	No	2–5%
R. conorii sensu stricto	Mediterranean spotted fever	*Rhipicephaleus sanguineus*	South Europe, Africa, Georgia, Central Asia	Yes	Purpuric (rarely)	Yes	No	Rare	1
R. conorii (Israel)	Israeli spotted fever	*Rhipicephalus sanguineus*	Israel, Italy, Portugal	Yes	Rarely purpuric	No	No	No	<1

R. conorii (Astrakhan)	Astrakhan fever	*Rhipicephalus pumilio*	Caspian Sea	Yes	No	Rare	No	No	No
R. conorii (Indian)	Indian tick typhus	*Rhipicephalus sanguineus*	Indian subcontinent	Yes	May be purpuric	Rare	No	No	Low
R. africae	African tick-bite fever	*Amblyomma variegatum Amblyomma haebraeum*	Sub-Saharan Africa, West Indies	Rare (less than half)	Vesicular	Yes	Yes	Yes	Very low
							(Half of cases)		
R. slovaca	Spotted fever	*Dermacentor marginatus*	Europe	No	-	Yes	No	Yes	No
R. aeschlimannii	Spotted fever	*Hyalomma marginatum*	Africa	Yes	-	Yes	No	Yes	?
R. sibirica	Siberian tick typhus or North Asian tick typhus	*Dermacentor marginatus Haemophysalis concinna*	Central Asia, China	Yes	No	Yes	No	Yes	Low
R. australis	Queensland tick fever	*Ixodes holocyclus*	Australia	Yes	Vesicular	Half of cases	No	Yes	Low
R. honei	Flinders Island tick typhus	Unknown	Thailand, Australia	Yes	-	Yes	No	Yes	Low
R. japonica	Japanese or Oriental spotted fever	*Haemophylasis longicornis, Dermacentor taiwanensis*	Japan, China	Yes	No	Yes	No	No	Low
'R. mongolotimonae'	Spotted fever	*Hyalomma asiaticum*	China, Europe, Africa	Yes	Few spots	Yes	No	Yes	No
R. felis	Flea-borne spotted fever	*Ctenocephalides felis*	USA, Mexico, Europe, Africa	Yes	-	Yes	Possible	Maybe	?
R. akari	Rickettsialpox	*Allodermanyssus sanguineus*	USA, Korea, Eastern Europe	Yes	Vesicular	Yes	Yes	Yes	Low

Spotted fever due to *Rickettsia slovaca*, also named TIBOLA (TIck BOrne LymphAdenopathy), is apparently common in Europe.[1] [2] [7] [8] Its tick vectors, *Dermacentor marginatus* (Fig. 14.4) and *Dermacentor reticulatus*, bite preferentially in cold months and on the scalp as the vectors prefer hairy prey. The disease is more prevalent in children and women, contrary to the other tick-borne rickettsioses. The incubation period is usually 1 week. The inoculation eschar is an erythematous lesion ranging from 2 to 8cm in diameter associated with a neck adenopathy that may be painful (Fig. 14.5). Fever and rash are rarely present. Postinfectious asthenia and residual alopecia at the site of the tick bite have been reported.

Queensland tick typhus, due to *Rickettsia australis*, presents with fever, headache and myalgia.[1] [9] It is transmitted by *Ixodes holocyclus* in Australia. The patients develop a rash, which can be vesicular 10 days after the onset of symptoms. An inoculation eschar is frequently noticed. It is usually mild. Japanese or Oriental spotted fever, due to *Rickettsia japonica*, is transmitted by *Haemophylasis longicornis* and *Dermacentor taiwensis* in Japan but also probably in eastern China. The patient presents with fever, headache, inoculation eschar and a maculopapulous rash.[1] [2] Flinders Island spotted fever, due to *Rickettsia honei*, is a febrile illness associated with an erythematous rash. An inoculation eschar is observed in 25% of cases and locoregional adenopathy in 55%.[1] [2] This disease may also be observed in continental eastern Australia.

Siberian tick typhus is due to *Rickettsia sibirica*, which is transmitted by *Dermacentor marginatus* and *Haemophylasis concinna*. It is found in Siberia and China. After an incubation period of 4–7 days after the tick bite, an ulcerated necrotic lesion appears at

Figure 14-1 Adult *Rhipicephalus sanguineus.*

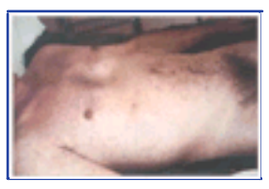

Figure 14-2 Maculopapular rash in Mediterranean spotted fever.

the inoculation site, often accompanied by regional lymphadenopathy.[1] Fever, headache and myalgias occur and can last for 6–10 days without treatment. The central nervous system is sometimes affected. *Rickettsia mongolotimonae*, related to *Rickettsia sibirica*, is prevalent in *Hyalomma asiaticum* ticks from inner Mongolia (China) and sub-Saharan Africa. Cases of spotted fever caused by this rickettsia have been seen in Marseilles (France). Patients show a discrete rash, an inoculation eschar and satellite lymphadenopathy.[2] [10] [11]

Rickettsia aeschlimannii has been isolated from *Hyalomma marginatum* ticks from Africa. [12] The first human case was recently reported. [12] The disease was described as a typical *Rickettsia conorii* infection in a patient who had traveled to Morocco.

Rickettsialpox or smallpox rickettsia is due to *Rickettsia akari*, which is transmitted by *Allodermanyssus sanguineus*. [1] Seven to 10 days after the mite bite, a vesiculous rash and a painless red papula appear at the inoculation site and become vesicular over the following days. Fever and myalgia are present at the beginning of the disease. A rash appears after 3 days. Even untreated, patients recover spontaneously in 1–3 weeks with no sequelae.

Flea-borne spotted fever is due to *Rickettsia felis*, which is transmitted by cat fleas. *Rickettsia felis* was first detected by molecular biology techniques in 1990 as the ELB agent from the midgut epithelial cells of cat fleas (*Ctenocephalides felis*). The bacterium was isolated in 2000, allowing the development of serologic tests.[13] *Rickettsia felis* has been observed in fleas or patients in the USA, Mexico, Brazil, Ethiopia, Spain, France and Germany. The disease is characterized by fever, rash, headache and central nervous system involvement.[13] [14] [15]

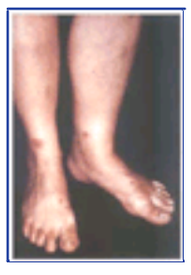

Figure 14-3 Two Inoculation eschars on the legs of a patient who presented with African tick bite fever.

Figure 14-4 Adult *Dermacentor marginatus.*

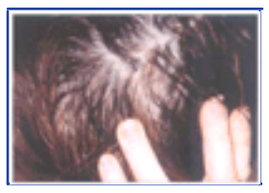

Figure 14-5 Eschar inoculation on the scalp of a patient who presented with *Rickettsia slovaca* infection.

DIAGNOSIS

Clinical features

The usual clinical manifestations of spotted fever are fever, rash and eschar inoculation. The epidemiology is important, with a history of tick or flea bite in an endemic area. Transmission is unlikely when a tick feeds for less than 20 hours. The differential diagnosis of spotted fever includes diseases caused by closely related organisms (*Rickettsia typhi, Rickettsia prowazekii, Orientia tsutsugamushi* and *Ehrlichia*), other bacteria, viruses, parasites (*Toxoplasma gondi*i) or fungi (*Penicillium marneffei*).

Laboratory diagnosis

The leukocyte count is usually normal but leukopenia and leukocytosis can be observed.[3] Thrombocytopenia occurs in 30–50% of cases.[3] Acute phase proteins (C-reactive protein, fibrinogen) are increased. Hepatic enzyme levels (ASAT and ALAT) can be raised.[3] Coagulopathy with a decrease in clotting factors can be responsible for bleeding.

Serology

Currently, the laboratory diagnosis of rickettsioses is based on serology. Two serum samples should be tested (early and convalescent). Samples can be obtained by using blood dried on blotting paper.[16]

The indirect immunofluorescence assay (IFA) is the reference test of choice, and is useful both for diagnosis of acute cases and for seroepidemiology. [17] It can simultaneously detect antibodies to several rickettsial antigens with the same drop of serum in a single well containing multiple dots. The IFA allows the detection of IgG and IgM anti-bodies and is highly specific and sensitive, but requires a fluorescence microscope. This is the reference technique in most laboratories. The early serum is often negative. A cut-off value of 1/64 for total immunoglobulins and 1/32 for IgM is usually required for the diagnosis. Cross-reactive antibodies have been observed with infections caused by the other rickettsioses, *Ehrlichia, Bartonella, Legionella* and *Proteus*. These cross-reacting antibodies, observed between species, appear to be directed against lipopolysaccharide. False positives, including those for IgM, have also been reported when rheumatoid factor is present and in patients with viral infection generating non-specific lymphocyte B proliferation (cytomegalovirus, Epstein-Barr virus) in the serum.

Latex agglutination is a simple and commercially available test that can be used in a non-equipped laboratory but the kit is expensive.[17] Enzyme-linked immunosorbent assay (ELISA) is both specific and sensitive and is useful for seroepidemiology.[17] The immunoperoxidase assay is specific and sensitive, and does not require a fluorescence microscope. [17] It may be used as an alternative to IFA and allows permanent slide records. The Western immunoblot assay, with sodium dodecyl sulfate gel electrophoresed and electroblotted antigens, is especially useful in differentiating true-positive from false-positive results created by cross-reacting antibodies.[17] This technique is the most specific and sensitive serologic test, allowing detection of the earliest antibodies. However, it is time consuming and lacks reproducibility.

Cross-absorption is used to discriminate cross-reacting antibodies between two or more antigens.[17] This technique is performed by mixing separately the serum studied with the bacteria involved in the cross-reaction. The homologous and heterologous antibodies both disappear when absorption is performed with the bacterium responsible for the disease, whereas disappearance of only homologous antibodies is observed when absorption is performed with the antigen responsible for the cross-reaction. This technique is limited by the large amount of antigen needed.

Techniques such as the complement fixation test, which lacks sensitivity, and the Weil Felix test, which lacks specificity, should not be used.

Immunodetection of Rickettsia[1] [17]

In skin biopsies, the bacteria can be detected prior to seroconversion. Biopsy specimens of the skin, preferably petechial lesions and 'tache noire', may be tested using the immunofluorescence and immunoperoxidase techniques performed on frozen or fixed, paraffin-embedded material, allowing a retrospective diagnosis. A positive result can be obtained 2 days after sampling, even for patients with prior antibiotic therapy.

Genomic amplification by polymerase chain reaction

In our laboratory, amplification of the gene encoding outer membrane protein A (*ompA*) and the gene encoding citrate-synthase (*gltA*) is used for the polymerase chain reaction (PCR). Several samples are suitable for use in PCR amplification of rickettsial DNA including skin biopsy specimens and peripheral white blood cells. Suspected arthropods should also be sampled and can be tested by PCR. Skin biopsy testing is probably the best method for early diagnosis before seroconversion. The blood may be collected in tubes containing EDTA or sodium citrate. Sera can also be tested. For this purpose, we recently introduced suicide PCR, which corresponds to a nested PCR using single-use primers.[6] Testing was done in a blinded fashion with one negative control used for every seven samples. All positive PCR products were sequenced for the identification of the pathogenic rickettsiae.

Culture

Culture is restricted to specialized laboratories with biohazard facilities. Currently, cell culture is the method used for isolating rickettsiae from samples.[17] This can be performed from human samples (decanted plasma or skin biopsies) and hemolymph from arthropods. The best sample is a biopsy from the inoculation eschar. Usually, culture of rickettsiae takes 3–7 days. This technique is essential for the identification of new rickettsial pathogens.

TREATMENT

Doxycyline is the treatment of choice for rickettsioses.[18] It can be prescribed in both adults and children[19] but not in pregnant women and allergic patients. Prognosis

depends on early antibiotic treatment and it should be prescribed in any suspected cases. The usual dosage is 100mg twice a day. The treatment should be given orally. The exact treatment duration is not fully determined but treatment should continue for 2–3 days after defervescence. In MSF, a single dose of 200mg of doxycycline is adequate. In pregnant women, chloramphenicol is the only available treatment for RMSF and the macrolide josamycin has been used successfully for MSF. Several antibiotics are effective in vitro against *Rickettsia rickettsii*, including rifampin (rifampicin), fluoroquinolones and new macrolides (except erythromycin) but clinical experience is lacking except for MSF, where quinolones and newer macrolides give results comparable to those with doxycycline but with longer regimens.[20] ß-Lactams, antibiotics, aminoglycosides and trimethoprim-sulfamethoxazole are ineffective in vitro and in vivo.

PREVENTION

Prevention is based on the avoidance of tick and flea bites. Repellents and/or protective garments can be used. After a possible exposure, people could be checked for ticks, with careful examination of axilla, groin and scalp. The tick can be removed by forceps followed by skin disinfection.

CONCLUSION

The diagnosis of rickettsioses must be suspected on the basis of clinical symptoms and epidemiology. The current diagnosis is frequently based on serology. The only exception is infection due to *Rickettsia slovaca* in which the serologic response is weak, perhaps because it does not cause a general infection. Polymerase chain reaction of a skin lesion or a lymph node aspirate gives an earlier positive test. The appropriate treatment should be started without waiting for laboratory confirmation.

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Chapter 15 - Practice Points

15.a Approach to the acutely febrile patient who has a generalized rash

Edmund L Ong

Introduction

In assessing patients who have fever and rash, the following four points are essential.

¦ Is the patient well enough to give a further history?
¦ Is immediate cardiorespiratory support required?
¦ From the nature of the rash, does the patient require isolation precautions?
¦ Is immediate empiric antimicrobial therapy required?

The history obtained should give the following information:

¦ drugs taken within the past month,
¦ geographic itinerary of travel,
¦ immunizations,
¦ occupational exposure,
¦ sexually transmitted disease exposure and risk factors for HIV,
¦ the immunologic status of the patient,
¦ whether the female patient is pregnant
¦ any history of valvular heart disease,
¦ recent exposure to other ill febrile patients,
¦ exposure to wild or rural habitats and wild animals,
¦ exposure to domestic animals,
¦ prior medical history including allergies, and
¦ sun exposure.

Pathogenesis

Virtually any class of microbe can induce a local skin rash with fever if the microbes are allowed to penetrate the stratum corneum. The systemic effects of micro-organisms on the skin, however, can also produce cutaneous eruptions by:

¦ multiplying in the skin,
¦ toxin-mediated effects,
¦ inflammatory responses, and
¦ altering the vasculature of skin.

Microbiology

The range of organisms causing systemic infections with prominent cutaneous manifestations is described in Table 15a.1 (see Chapter 8 , Chapter 9 , Chapter 12 and Chapter 14). There are other noninfectious causes of fever with a generalized rash and these need to be borne in mind.

Clinical features

Physical examination should include the following:

¦ vital signs,
¦ general appearance,
¦ signs of toxicity,
¦ evidence of adenopathy,
¦ presence of mucosal, genital or conjunctival lesions,
¦ presence of hepatosplenomegaly,
¦ evidence of arthropathy, and
¦ signs of meningismus or neurologic dysfunction.

The rash should be assessed with regard to:

¦ its distribution,
¦ its pattern of progression,
¦ the timing of its development relative to the onset of illness and fever (Table 15a.2), and
¦ its characteristics.

The morphology of skin lesions includes macules, papules, plaques, nodules, vesicles, bullae and pustules. Skin lesions are also characterized by their color and particularly by the presence or absence of hemorrhage. Lesions may also be hyperpigmented or hypopigmented. Blanching erythematous lesions are due to vasodilation, whereas nonblanching erythemas may be due to extravasation of blood. Purpuric lesions are hemorrhages into the skin and they may be small, petechial or large and ecchymotic. Lesions of erythema multiforme usually begin as round or oval macules and papules that vary in size and have central erythema surrounded by a narrow ring of normal skin, which is also surrounded by another thin ring of erythema to form target lesions. Most cases are idiopathic, but the common infective causes are shown in Table 15a.3 . The lesions of erythema nodosum are characterized by tender, erythematous nodules that vary in diameter from 1cm to several centimeters. Infectious agents are a major cause of this lesion (Table 15a.4).

Investigations

Establishing the microbiologic diagnosis is of great importance in managing the patient. Blood cultures should form part of the essential investigations, along with full blood count and differential white cell count, liver function tests and renal function tests. Skin lesion aspirates or biopsy should be considered, particularly for the identification of meningococcal, staphylococcal and gonococcal infections. A punch biopsy of the maculopapular skin lesion of disseminated candidemia is sometimes diagnostic. Occasionally, a Gram stain of a routine buffy coat may reveal the responsible organisms (e.g. staphylococci, meningococci or *Candida* spp.) in a

septic patient. Isolation of the causative organisms may be difficult, particularly with viruses and some bacteria. Serologic methods (e.g. serologic test for syphilis, paired viral complement fixation tests), molecular techniques (e.g. polymerase chain reaction for dengue fever virus, cytomegalovirus and HIV) and immunofluorescence microscopy are useful methods for establishing the difficult culturable organisms.

TABLE 15.a-1 -- Microbiology of cutaneous manifestations associated with systemic infections.

MICROBIOLOGY OF CUTANEOUS MANIFESTATIONS ASSOCIATED WITH SYSTEMIC INFECTIONS	
Macular or papular rash	
Viruses	Adenovirus
	Atypical measles
	Colorado tick fever
	Coxsackie viruses
	Cytomegalovirus
	Dengue virus
	Echoviruses
	Epstein-Barr virus
	Hepatitis B virus
	HIV-1
	Human herpesvirus 6
	Lymphocytic choriomeningitis virus
	Parvovirus B19 (erythema infectiosum)
	Rubella (German measles)
	Rubeola (measles)
Bacteria	*Bartonella bacilliformis*
	Bartonella henselae
	Bartonella quintana
	Borrelia burgdorferi (Lyme disease)
	Borrelia spp. (relapsing fever)
	Chlamydia psittaci
	Francisella tularensis
	Leptospira spp.
	Mycobacterium haemophilium
	Mycoplasma pneumoniae
	Pseudomonas aeruginosa
	Rickettsia akari (rickettsialpox)
	Rickettsia prowazekii (epidemic/louse-borne typhus)
	Rickettsia rickettsii (Rocky Mountain spotted fever)
	Rickettsia tsutsugamushi (scrub typhus)
	Rickettsia typhi (endemic/murine typhus)
	Salmonella typhi
	Spirillum minus (rat-bite fever)
	Staphylococcus aureus
	Streptobacillus moniliformis (rat-bite fever)
	Streptococci group A (scarlet fever)
	Treponema pallidum (secondary)
Fungi (disseminated)	*Blastomyces dermatitidis*
	Candida spp.
	Coccidioides immitis
	Cryptococcus neoformans
	Fusarium spp.
	Histoplasma capsulatum
Vesicobullous eruptions	
Viruses	Coxsackie viruses
	Echoviruses
	Herpes simplex virus (disseminated)
	Vaccinia
	Varicella (chickenpox)
	Varicella-zoster virus (disseminated)
	Variola (smallpox)
Bacteria	*Listeria monocytogenes*
	Mycoplasma pneumoniae
	Rickettsia akari (rickettsial pox)
	Vibrio vulnificus
Petechial purpuric eruptions	

Viruses	Adenovirus
	Atypical measles
	Congenital cytomegalovirus
	Coxsackie viruses
	Dengue virus
	Echoviruses
	Epstein-Barr virus
	Rubella (German measles)
	Viral hemorrhagic fevers
	Yellow fever
Bacteria	*Borrelia* spp. (relapsing fever)
	Capnocylophaga canimorsus
	Neisseria gonorrhoeae
	Neisseria meningitidis
	Rickettsia prowazekii
	Rickettsia rickettsii
	Staphylococcus aureus
	Streptobacillus moniliformis
Protozoa	*Plasmodium falciparum* (malaria)

TABLE 15.a-2 -- Skin lesions and systemic infections.

SKIN LESIONS AND SYSTEMIC INFECTIONS		
Lesion	**Common pathogens**	**Time of appearance after onset of illness**
Toxic erythema	*Staphylococcus aureus*, *Streptococcus pyogenes*	At presentation
Rose spots	*Salmonella* spp.	5–10 days
Purpuric lesions (in critically ill patients)	*Neisseria meningitidis*, *Rickettsia* spp., *Capnocytophaga canimorsus*, Gram-negative bacteria	12–36 hours
Macronodular lesions	*Candida* spp., *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Fusarium* spp.	Days
Erythema multiforme, bullous lesions, ecthyma gangrenosum	*Pseudomonas* spp., *Vibrio vulnilicus*, Gram-negative bacteria	Days

TABLE 15.a-3 -- Infective causes of erythema multiforme.

INFECTIVE CAUSES OF ERYTHEMA MULTIFORME
Adenovirus
Coccidioidomycosis
Enterovirus
Hepatitis B virus
Herpes simplex virus
Histoplasmosis
HIV infection
Infectious mononucleosis
Legionnaires' disease
Lymphogranuloma inguinale
Mumps
Mycoplasma pneumoniae
Orf
Poliomyelitis
Pseudomonas aeruginosa
Psittacosis
Rickettsia spp.
Salmonella typhi
Streptococcus pyogenes
Syphilis
Tuberculosis
Tularemia
Typhoid
Vaccinia
Yersinia spp.

TABLE 15.a-4 -- Infections associated with erythema nodosum.

INFECTIONS ASSOCIATED WITH ERYTHEMA NODOSUM
Blastomycosis
Campylobacter spp.
Cat scratch disease

Coccidioidomycosis
Gonorrhea
Hepatitis C
Histoplasmosis
Inflammatory dermatophyte infections
Leptospirosis
Lymphogranuloma venereum
Psittacosis
Salmonellosis
Streptococcal infections
Tuberculosis
Tularemia
Upper respiratory tract viruses
Yersiniosis

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15.b Management of foot ulcer

Sanjeev Handa

Introduction

Foot ulceration is a relatively common problem in clinical practice and one that sometimes poses difficult diagnostic and therapeutic dilemmas. Major complications are infection and, in more severe cases, the development of dry and wet gangrene. In certain cases making a determination of superinfection versus colonization can try even the most astute clinician.

Pathogenesis

The pathogenesis depends on the type and etiology of foot ulcer. The differential diagnosis includes:

¦ ischemic arterial ulceration;
¦ venous ulceration, due to increased venous hydrostatic pressure causing local edema, with its low exchange of oxygen and metabolites; edematous tissue, particularly skin, is more vulnerable to trauma than healthy tissue and is far less able to combat infection;
¦ neuropathic ulcers caused by diabetes, tabes dorsalis, leprosy, syringomyelia or hereditary sensory (radicular) neuropathy; the protective pain sensation is ablated, resulting in loss of awareness of trauma, which can cause further deterioration of the ulcer;
¦ vasculitis; and
¦ infection, including acute pyogenic infections, tuberculous infections, tropical ulcer (chronic phagedenic ulcer secondary to Vincent's organisms), syphilis and yaws.

Clinical features

The ischemic arterial ulcer is typically located on the toes, heel, dorsum of the foot or lower third of the leg. The pain is severe and

persistent and worsens at night. The ulcer is generally 'punched out' with a pale or necrotic base.

Venous ulcers are located in the 'gaiter' distribution around the ankle, especially around the medial malleoli. They are less painful, more diffuse and shallow and usually have some evidence of granulation tissue at the base.

Diabetic ulcers are usually located in the plantar or lateral aspect of the foot. They resemble arterial ulcers morphologically but are characteristically painless. Diabetic neuropathy (sensory, motor and autonomic), microvascular and macrovascular lesions, and diminished neutrophil function all conspire to generate diabetic foot ulcers.

Diabetic foot ulcers may be classified under Wagner's grades as follows.

```
Grade 0     No ulcer in a high-risk foot
Grade 1     Superficial ulcer involving the full skin thickness but
            not the underlying tissues
Grade 2     Deep ulcer, penetrating down to the ligaments and
            muscle but not involving the bone and no formation
            of an abscess
Grade 3     Deep ulcer with cellulitis or abscess formation, often
            with osteomyelitis
Grade 4     Localized gangrene
Grade 5     Extensive gangrene involving the whole foot
```

All ulcers may become secondarily infected. Features of infection range from minimal cellulitis with lack of systemic toxicity to extensive cellulitis with associated lymphangitis, purulent drainage, sinus tract formation, osteomyelitis, septic arthritis, abscess formation and sometimes the development of gangrene. Systemic signs and symptoms often occur late and suggest severe infection.

Infected ulcer — diagnosis

A peripheral blood count may demonstrate a leukocytosis (this may be absent in severe cases, especially in diabetes); the erythrocyte sedimentation rate is usually raised. Blood cultures may be positive, especially if the patient is febrile and has not received prior antibiotic treatment.

Obtaining a swab culture of the ulcer itself is an unreliable means of establishing the causative organism(s) in superinfected ulcerations. Ulcers are typically colonized by a multitude of organisms that may or may not be pathogenic. Deep tissue cultures that avoid contact with the ulcer surface or other draining lesions are preferable. In osteomyelitis, bone cultures obtained by percutaneous biopsy or surgical excision are the best specimens for determining the etiology provided the incision site is away from the ulceration itself.

Plain radiographs of the affected area are useful in determining the presence of foreign bodies or air in the soft tissues, which may suggest the presence of gas-forming bacteria. Computerized tomography scanning and magnetic resonance imaging are useful for looking for abscesses as well as early osteomyelitis. The performance of technetium bone scanning for the diagnosis of osteomyelitis in the impaired foot is poor and a 24-hour indium-111 leukocyte scan is more sensitive than a bone scan in diagnosing osteomyelitis associated with a diabetic foot. However, this test is expensive and it may be difficult to interpret in the presence of local soft tissue inflammation (Chapter 52).

Microbiology

Mild ulcers may be infected by single organisms. Organisms frequently involved include:

¦ *Staphylococcus aureus*,
¦ *Streptococcus pyogenes*, and
¦ facultative Gram-negative bacilli and anaerobic organisms (which are isolated infrequently).

Severe ulcers, especially the diabetic foot, are usually polymicrobial with aerobic and anaerobic bacterial isolates, including:

¦ *Staph. aureus*,
¦ coagulase-negative staphylococci,
¦ aerobic streptococci and enterococci,
¦ Enterobacteriaceae (e.g. *Escherichia coli, Klebsiella* spp. and *Proteus* spp.),

- *Pseudomonas* spp.,
- *Corynebacterium* spp.,
- *Bacteroides* spp., and
- *Clostridium* spp.

Therapy

Antibiotics are the mainstay of treatment (Chapter 9) and are recommended in the presence of a surrounding cellulitis, a foul-smelling lesion, fever or deep tissue infection. Empiric antibiotics are necessary until culture results are available (see Table 15b.1).

The optimal duration of therapy is unclear; however, for infections that are limited to soft tissue, intravenous therapy may be administered for 7–10 days followed by oral therapy for an additional 14 days. For those in whom osteomyelitis is identified, a minimum of 6–8 weeks' parenteral therapy is recommended if the offending tissue is not removed in its entirety (Chapter 52). Limb-threatening infections require immediate hospitalization, bed rest and a strict non-weight-bearing regimen, even if signs and symptoms of systemic infection are absent. Although medical stabilization, metabolic and glycemic control (in diabetic patients) and antimicrobial therapy are important, debridement should not be delayed. Failure to debride necrotic, infected tissue and to drain purulent collections increases the risk of amputation. The initial debridement must be performed

TABLE 15.b-1 -- Selected empiric antimicrobial regimens for infected foot ulcers.

SELECTED EMPIRIC ANTIMICROBIAL REGIMENS FOR INFECTED FOOT ULCERS	
Non-limb-threatening infection	
Oral regimen	Cephalexin
	Clindamycin
	Dicloxacillin/flucloxacillin
	Amoxicillin-clavulanate
	Quinolones
	Metronidazole
Parenteral regimens	Cefazolin
	Oxacillin or nafcillin
Limb-threatening infection	
Oral regimen	Clindamycin or metronidazole with a quinolone
Parenteral regimens	Ampicillin-sulbactam with or without an oral quinolone or aminoglycoside
	Ticarcillin-clavulanate with an oral or parenteral quinolone or aminoglycoside
	Piperacillin-tazobactam with an oral or parenteral quinolone or aminoglycoside
Life-threatening infection	
Parenteral regimens	Imipenem-cilastatin
	Meropenem
	Ertapenem
	Vancomycin, metronidazole with either aztreonam or quinolone
	Piperacillin-tazobactam with an oral or parenteral quinolone or aminoglycoside
Note that if methicillin-resistant *Staphylococcus aureus* is a concern, then vancomycin or linezoid should be included in the regimen.	

independently of the status of the arterial circulation and revascularization should be postponed until sepsis is controlled (Chapter 10).

It should be noted that definitive management of the ulcer will require treatment of the underlying cause. For example, up to 60% of diabetic patients with nonhealing ulcers have associated arterial insufficiency. Therefore, the arterial circulation must be critically evaluated in all diabetics presenting with a foot ulcer. Once the ulcer has healed, a lifelong program of proper footwear, education and close follow-up for routine callus and nail care must be maintained. In addition, the tetanus vaccination status must be ascertained in all patients presenting with ulceration or infection.

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15.c Role of hyperbaric oxygen in the management of gas gangrene

Athena Stoupis

Introduction

Gas gangrene (clostridial myonecrosis) is one of the most serious limb-threatening and possibly life-threatening infectious diseases (see Chapter 10). It may occur as a complication of surgery or trauma or it may occur spontaneously. Rapid surgical decompression and excision of necrotic tissue along with antibiotic therapy have been the mainstay of treatment. Over the past 30 years, the use of hyperbaric oxygen (HBO; 100% oxygen at two to three times the atmospheric pressure at sea level) as an adjunct therapy has demonstrated diminished mortality rates and diminished tissue loss. However, its use remains controversial, given the paucity of controlled clinical trials that have examined the specific efficacy of this modality.

Pathogenesis

Clostridial myonecrosis occurs when the oxygen tension (PO_2) of a necrotic wound is low, allowing the germination of clostridial spores and subsequent release of lethal toxins, which initiate the fulminant phase of hemolysis, loss of local host defenses and tissue necrosis.

Over 20 exotoxins are produced by *Clostridium perfringens*. The most virulent toxin appears to be the a-toxin. A tissue PO_2 of 250mmHg inhibits the production of a-toxin in vitro. Tissue oxygen levels of 300–400mmHg have been measured in patients during HBO therapy at two atmospheres. Once toxin production is halted, the disease cycle is broken and clinical improvement follows. Some investigators have shown direct inhibition of *Clostridium perfringens* in vitro by HBO therapy.

In addition, HBO increases the oxygen supply to the surrounding tissues of the wound, allowing normal phagocytosis and free radical formation by granulocytes, further assisting in the tissue repair process.

Low tissue oxygen tension levels reduce collagen synthesis. An increased oxygen supply enhances the rate of collagen synthesis and wound healing. Finally, although hyperoxia initially reduces the rate of capillary growth, a sharp increase in angiogenesis follows within the first 24 hours of oxygen therapy, allowing migration of cells into the previously hypoxic areas and further tissue proliferation.

Microbiology

Clostridium spp. are Gram-positive, obligate anaerobic organisms. They are widespread in the environment and can be cultured from soil, clothing and the intestinal flora of humans.

Clostridium perfringens is the most commonly isolated organism in gas gangrene (80–95%). Other organisms less commonly implicated as the cause of gas gangrene include *Clostridium novyi* (10–40%) and *Clostridium septicum* (5–20%; Chapter 232).

Clinical features

The incubation period of gas gangrene is usually less than 24 hours. The most significant clinical sign is intense pain that is out of proportion to the pain usually associated with the preceding injury or surgical procedure (Table 15c.1). The patient rapidly becomes ill with fever, tachycardia, hemodynamic compromise and change in mental status.

Tense 'woody hard' edema in the vicinity of the wound develops. Bullae and vesicles may appear. Putrid serosanguinous drainage may develop in the overlying skin blebs and often drains from open wounds. Crepitus may develop, but is a late sign of true gas gangrene.

Investigations

Early diagnosis is critical and is usually based on the clinical appearance of the patient. Demonstration of Gram-positive rods in the wound exudate gives rapid microbiologic identification that can be confirmed on subsequent anaerobic wound cultures. Blood cultures are usually negative and may not be helpful in establishing the diagnosis. Computerized tomography scanning may show involvement of muscle and fascial planes.

Management

Early initiation of antibiotic therapy and rapid surgical decompression and removal of necrotic tissue are essential for a favorable outcome.

There is no consensus over the timing of adjuvant HBO therapy in relation to surgical intervention. The paucity of controlled randomized clinical trials for the use of HBO in gas gangrene has created controversy among investigators. Several studies have indicated that the combination of surgery, antibiotics and antecedent HBO therapy have reduced morbidity and mortality rates from 80–90% to 20–30% in both human and animal models.

In one study, a dog model of clinical gas gangrene infection was used. All of the infected controls and dogs randomized to surgery alone or HBO therapy alone died. Survival was 50% with antibiotics alone, 70% with antibiotics and surgery, and 95% with the combination of antibiotics, HBO therapy and surgery.

TABLE 15.c-1 -- Clinical findings in acute gas gangrene.

CLINICAL FINDINGS IN ACUTE GAS GANGRENE
Severe soft tissue pain
Disproportionate tachycardia
Skin changes (bullae, bronze discoloration)
Gram-positive rods found on tissue smears
Demonstrable myonecrosis and gas formation in imaging studies

However, in human studies, the efficacy of HBO therapy was often determined retrospectively and the inclusion criteria for some of the cases were variable. Some investigators also argue that the large medical centers that have HBO chambers readily available also provide aggressive surgical and intensive care support, which may have an impact on morbidity and mortality rate.

However, most investigators advocate that HBO therapy before surgery because of the following potential benefits:

- clearer demarcation of the borders between viable tissue and devitalized tissue, permitting more conservative tissue debridement; and
- substantial improvement and stabilization of the severely ill patient before surgery, with inhibition of toxin production.

Others advocate that surgery is indicated before HBO therapy, given the fulminant, life-threatening course of gas gangrene and the need for rapid debridement of necrotic tissue. Most clinical experience is weighted toward antecedent HBO therapy.

Surgical debridement should not be delayed when a HBO chamber is not readily available. Arrangements should be made for transfer to the appropriate institution that can provide HBO therapy after initial surgical debridement. If fasciotomy is indicated for relief of compartment syndrome, then it should be performed before HBO therapy.

To be effective, HBO must be inhaled directly through the atmosphere, through an endotracheal tube in a monochamber, or through tight-fitting masks or hoods or endotracheal tubes in a multiple occupancy chamber. For HBO, pressure is expressed in multiples of the atmospheric pressure at sea level, which is 1 atmosphere (760mmHg).

The standard HBO therapy protocol consists of multiple early treatment sessions administered at 3 atmospheres for 90 minutes or 2.5 atmospheres for 120 minutes. Three treatments are given during the first 24 hours, then 2 treatments per day for an average of 7 chamber treatments.

Complications of hyperbaric oxygen therapy

The most common complaint during HBO treatment is the experience of ear or sinus pain (Table 15c.2). Persistent pain may require myringotomy. Patients who have sinus infection also require nasal decongestants to avoid barotrauma.

Generalized seizures have also occurred with oxygen toxicity and are usually controlled with anticonvulsants. During the immediate seizure episode removal of the oxygen mask may promptly terminate seizures. Patients may also develop transient myopia, the cause of

TABLE 15.c-2 -- Complications of hyperbaric oxygen therapy.

COMPLICATIONS OF HBO THERAPY
Air embolism
Combustion
Confinement anxiety
Ear or sinus pain
Generalized tonic-clonic seizures
Pneumothorax
Transient myopia
Tympanic membrane rupture

which is unknown. It is usually reversible within 2 months of completing therapy.

Another significant side-effect is the development of tension pneumothorax secondary to barotrauma. Immediate recompression with the placement of a chest tube is required.

Claustrophobia is also a common side-effect. Many patients require tranquilizers for relief of their confinement anxiety.

Complications of HBO therapy used for gas gangrene treatment are fortunately rare with the use of relatively low oxygen pressures (2–3 atmospheres) and with the short duration of HBO treatments.

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15.d Managing the patient with recurring skin infections

Stephen T Green
Ravi Gowda
Ben Clark

Background

Skin infections come in many forms. Most commonly, troublesome skin infections is synonymous with cellulitis, an entity that perfectly illustrates the cardinal signs of inflammation. Cellulitis is therefore an acute, usually noncontagious inflammation of the connective tissue of the skin, resulting from bacterial infection and characterized by localized warmth, erythema, pain and tenderness, swelling and reluctance to mobilize the affected area (Fig. 15d.1). When such a problem is recurrent, this can become extremely tiresome and even disabling for the afflicted individual.

Cellulitis is usually consequent upon a break developing in the skin surface or its appendages, such as a laceration, cut, fissure, puncture wound, insect bite, animal or human bite, scratch, abrasion, blisters or friction burn, such as might occur with shoes that are too tight. Organisms normally confined to the skin surface are admitted to the dermis where they proliferate and lead to cellulitis.

Recurrent cellulitis

Recurrent cellulitis implies that factors facilitate the recurrent entry of organisms into the dermis. Effective management of recurrent cellulitis involves identifying these factors and, if possible, remedying them. Recurrent cellulitis may cause local persistent lymphedema, resulting in permanent hypertrophic fibrosis.

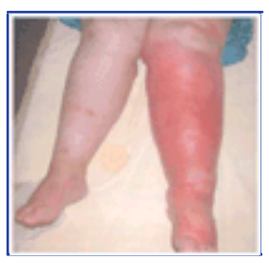

Figure 15.d-1 Severe recurrent cellulitis associated with obesity.

With respect to location, most often it is the lower limbs that are involved in recurrent cellulitis. The site may be the arm if, for example, the patient has received radiotherapy to the axillary area as part of breast cancer treatment. Other sites, such as the vulva and perianal region (sometimes in association with *Enterobius vermicularis*) can also be problematic.

To make matters more complex, cellulitis of the lower extremities is more likely to be complicated by thrombophlebitis in elderly patients, which in turn can encourage recurrence of cellulitis.

A number of clinical scenarios and risk activities render patients particularly vulnerable to recurrent episodes of cellulitis. These include:

¦ tinea pedis or onychomycosis;
¦ diabetes mellitus — there may be a family history;
¦ peripheral vascular (arterial) disease — there may be a history of smoking, angina pectoris or hypertension;
¦ ischemic or venous ulceration of the skin (including sickle cell disease);
¦ post-deep venous thrombosis;
¦ eczema and dermatitis;
¦ immunodeficiency states, for example patients with HIV infection (who may be more prone to recurrent staphylococcal skin sepsis), neutropenia (granulocytopenia), Job's syndrome (hyper IgE syndrome with recurrent staphylococcal cellulitis) or use of immunosuppressive or corticosteroid drugs — always establish the medication history;
¦ lymphatic obstruction, for example post-radiotherapy (e.g. post-mastectomy), post-block dissection of lymph nodes for cancer (Fig. 15d.2), elephantiasis (e.g. due to infections *by Wuchereria bancroftii*, *Brugia malayi*, *Onchocerca volvulus*) or Milroy's disease;
¦ scar cellulitis (e.g. previous burn or skin graft sites and in areas from which veins were harvested for coronary artery bypass grafting);
¦ trauma related, for example cosmetic piercings (studs, rings), injecting drug users, recurrent localized trauma, self-harm or Munchausen's syndrome;
¦ nasal carriage of staphylococci;
¦ lepromatous leprosy;
¦ underlying occult osteomyelitis;
¦ very poor personal hygiene (e.g. associated with alcoholism); and
¦ morbid obesity — largely associated with recurrent lower limb cellulitis (Fig. 15d.1).

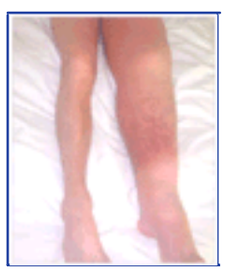

Figure 15.d-2 Severe recurrent cellulitis in a lymphedematous leg following radical surgery for rhabdomyosarcoma.

Microbiology

In immunocompetent individuals, cellulitis is usually the result of Gram-positive aerobic cocci, particularly *Staphylococcus aureus* and *Streptococcus pyogenes*, or sometimes a combination of both. It can be clinically difficult to decide which of them is causing the problem.

Non-group A streptococci, particularly groups B, C and G, are sometimes implicated in cellulitis, occurring in patients with lymphatic obstruction, or post-vein harvesting for coronary artery bypass grafting.

Recurrent cellulitis due to streptococci may be seen in association with chronic lymphedema (e.g. from lymph node dissection, post-irradiation, Milroy's disease, elephantiasis).

Neutropenic patients may develop cellulitis due to other organisms, such as Gram-negative bacilli (e.g. *Proteus, Serratia, Enterobacter* spp.) and fungi (e.g. *Cryptococcus neoformans*).

Other organisms may be involved as part of a mixed picture, depending upon the source of the organisms. Incontinent patients may contaminate their lower limbs with urine and feces while injecting drug users can inoculate their own tissues (Fig. 15d.3) with a variety of organisms from contaminated needles. Patients whose cellulitis

is the result of deliberate self-harm may also yield multiple organisms on culture — this is an extremely difficult diagnosis to make and requires the highest levels of clinical acumen. For example self-inoculation with milk has been reported as the cause of recurrent cellulitis.

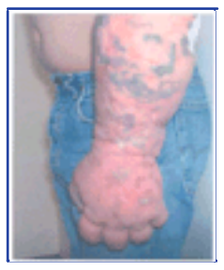

Figure 15.d-3 Injecting drug user with severe recurrent cellulitis of the left arm.

Is it really cellulitis?

Sometimes the apparent recurrent cellulitis problem may not in fact be cellulitis, and the following should be considered:

- acute gout can resemble recurrent cellulitis and certain diuretics may predispose to gout;
- recurrent deep venous thrombosis;
- migratory necrolytic erythema associated with underlying neoplasia, particularly glucagonoma of the pancreas;
- inflammatory carcinoma of the breast, which produces a picture of localized cellulitis unresponsive to antibiotics;
- herpes zoster, which can cause recurrent rash that may be complicated by superinfection;
- erythema nodosum, especially if it recurs;
- palmoplantar pustulosis and pyoderma gangrenosum, such as that associated with inflammatory bowel disease, can be mistaken for cellulitis;
- scurvy and pellagra; and
- fixed drug eruptions.

Assessment and diagnosis

Unless pus has formed or an open wound is present, it is often difficult to isolate the responsible organism from a case of cellulitis. Aspiration of material from the advancing edge of the lesion, skin biopsy and blood cultures yield potential pathogens in only about 25% of cases. The etiology of most cases of cellulitis will usually be *Staphylococcus aureus* and *Streptococcus pyogenes*.

In unusual circumstances, such as patients who are immunocompromised or those not responding to empiric therapy, or indeed whenever the clinical history points toward other infective or noninfective diagnostic options, further investigations may be warranted. This may become particularly important where the patient is suffering recurrent attacks. For example, among those with peripheral vascular disease or diabetes mellitus, minor injuries or cracked skin in the feet or toes can serve as an entry point for recurrent infection.

Attention should accordingly be directed toward establishing the presence or absence of factors that might be supporting the development of recurrent cellulitis and might be amenable to correction. The following range of tests can be applied selectively according to circumstances:

- microbiologic — samples for microscopy, Gram staining, culture and sensitivity, swabs from areas of abscess or bullae formation, needle aspiration of the advancing edge of cellulitis, full skin biopsy, interdigital skin and/or nail scrapings (especially where tinea pedis is present), blood culture (positive in only a few patients), nasal swabs (especially for *S. aureus* carriage, including methicillin-resistant *S. aureus* (MRSA)), perianal cellophane tape (for *Enterobius* ova), throat swab (for *S. pyogenes* in those with erythema nodosum) and bullous fluid (for immunofluorescence antibody test for varicella zoster);
- imaging — tissue scanning (plain radiographs, ultrasound, computerized tomography scanning, magnetic resonance imaging and indium leukocyte scanning) may identify collections of pus meriting drainage, foreign bodies or underlying osteomyelitis (if gas is seen in the tissues, the differential diagnosis then includes gangrene and fasciitis, which are generally considered to be surgical emergencies), Doppler scans (which may assist in identifying deep venous thrombosis or peripheral arterial disease);
- hematologic and immunologic — blood films (macrocytosis associated with alcohol excess and microfilaria in suspected filariasis), differential white cell count (to identify neutropenia, eosinophilia, e.g. in filariasis), hemoglobin electrophoresis in sickle-cell disease, immunoglobulin levels and subsets, complement levels, T-cell subsets;
- serology — HIV-1 and HIV-2, antistreptolysin titer (may point toward erythema nodosum as the diagnosis), hepatitis C, hepatitis B (may point toward occult injecting drug abuse), filariasis, onchocerciasis if patient is at risk;
- biochemistry — blood glucose studies (to exclude diabetes mellitus including hemoglobin A_{1C}), urate levels, liver function tests; and
- skin biopsy — may help with rarer causes of cellulitis.

Management

Managing the acute phase of recurrences

Tissue penetration sufficient to achieve adequate local antibiotic concentrations can be problematic. For acute exacerbations, intravenous therapy may therefore be necessary. Useful combinations include (flucl)oxacillin-benzylpenicillin, (flucl)oxacillin-amoxicillin and clindamycin-ciprofloxacin.

Other antibiotics may be indicated, depending upon the clinical scenario:

- where allergy to ß-lactam drugs is an issue — macrolides, levofloxacin, or moxifloxacin;
- where MRSA is an issue — doxycycline, rifampin (rifampicin), linezolid, or vancomycin; and
- where anaerobes are an issue — metronidazole or clindamycin-dalfopristin.

Surgical care includes debridement of devitalized tissue. Incision and drainage may be indicated if suppuration occurs. Treat the local effects of the cellulitis by elevating the affected limb.

Prevent recurrences

Adequate patient education and training are essential.

Skin and foot care for tinea pedis and onychomycosis includes:

- patient training regarding proper skin hygiene and suitable footwear;
- treating affected toe webs or feet with topical antifungals;
- consideration of oral antifungals such as itraconazole or terbinafine for severe chronic tinea pedis or onychomycoses; and
- expert podiatry — cuts and fissures should be washed and kept clean while healing.

For cases caused by edema treat any underlying cause (e.g. cardiac failure) and relieve edema using support stockings, specialized bandaging and nocturnal elevation of the affected area. Diuretics may have a role.

There is no convincing evidence for the value of antibiotic prophylaxis and there is a risk of antibiotic resistance. Penicillin, erythromycin and clindamycin have all been

advocated. Early institution of antibiotics may help in cellulitis of the lower extremities. The patient must be trained to spot the early signs of a recurrence, and given a supply of antibiotics (such as amoxicillin or flucloxacillin) to take. They should be advised to seek medical advice as soon as possible.

Nasal carriage of *S. aureus* can be treated with mupiricin if it is thought to be associated with recurrent disease.

Conclusion

Recurrent cellulitis is responsible for much morbidity. Diagnosis is not always straightforward and it presents a significant management challenge.

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Chapter 16 - Generalized and Regional Lymphadenopathy

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INTRODUCTION

The lymph nodes are major components of the body's surveillance system against foreign invaders; they function as a filter to trap micro-organisms, cancerous cells and immune complexes. The lymphoid system grows rapidly during childhood and achieves twice the adult size by early adolescence. Although lymphoid tissue begins to regress during mid-adolescence, it does not reach adult maturity until the age of 20–25 years. Peripheral lymphadenopathy, therefore, is a common finding throughout late childhood, adolescence and young adulthood.[1] Lymphadenopathy (i.e. disease of lymph nodes) may be due to primary lymphoproliferative diseases as well as to secondary reactive (infectious and noninfectious) or infiltrative diseases. Table 16.1 summarizes the differential diagnosis of lymphadenopathy. Lymphadenopathy occurring in returning travellers is discussed in Chapter 152 .

EPIDEMIOLOGY

In children, the cause of lymphadenopathy is clinically apparent in most cases. In approximately 80% of cases it is benign, reactive and most commonly due to an infectious cause. In contrast, lymphadenopathy in adults more often reflects serious disease. The probability of neoplasm affecting enlarged peripheral lymph nodes increases steadily with age; in those older than 50 years, more than 60% of cases of lymphadenopathy are due to malignancy.[2] [3] In tropical and subtropical parts of the world, lymphadenopathy requires other considerations.

PATHOGENESIS AND PATHOLOGY

Lymph nodes are widely distributed throughout the body, especially at potential portals of entry into the body (Fig. 16.1). The normal lymph node is an oval, encapsulated, soft structure; the size ranges from 1cm to 2cm in diameter. The node contains a reticular network packed with lymphocytes, macrophages and dendritic cells. A single lymph node weighs about 1g and contains approximately 2000 million lymphocytes. The lymph node is a dynamic structure and the exchange rate of lymphocytes between blood and the node is extremely high; lymphocytes equivalent to approximately three times the weight of the lymph node pass into the lymph each hour.[4]

Histologically, the lymph node can be divided into three regions (Fig. 16.2):[5]

- the cortex, which is the outermost layer and is composed mainly of B lymphocytes and macrophages arranged in primary follicles;
- the paracortical region, below the cortex, which is composed mainly of T lymphocytes and dendritic cells; and
- the medulla, which is the innermost region and has fewer lymphocytes than the other regions but more plasma cells that actively secrete antibodies.

Afferent lymphatic vessels empty the lymph drained from the tissues into the subcapsular sinus; from there the lymph flows through the cortex, paracortex and medulla, allowing phagocytic and dendritic cells to trap any foreign material. The efferent lymphatic vessels carry lymph enriched in lymphocytes and antibodies; this lymph reenters the circulatory system.

The lymph node has two main functions:

- it acts as a defensive barrier; and
- it serves as a factory for lymphocyte maturation and differentiation and for antibody production during antigenic challenge.

After antigenic stimulation, the primary follicle enlarges into a secondary follicle, which contains a germinal center in which large proliferating lymphoblasts and plasma cells are interspersed with macrophages and dendritic cells surrounded by packed lymphocytes. The germinal center is a site where B lymphocytes are intensively activated and differentiated into plasma and memory cells. The dendritic cells in the paracortex are rich in major histocompatibility class II molecules and act as antigen presenting cells to T-helper cells, which in turn activate B lymphocytes.

Lymphadenitis

Lymphadenitis (Table 16.2) represents inflammation of the lymph node. The initial response to acute inflammation consists of swelling and hyperplasia of the sinusoidal lining cells and infiltration by leukocytes. The process may progress to abscess formation depending on the micro-organism involved and the host response.

Acutely inflamed nodes are most commonly caused by local trapping of microbes; acute inflammation commonly affects the cervical nodes in association with infections of the teeth or tonsils or the axillary or inguinal nodes in association with infections in the limbs. Generalized acute lymphadenopathy is characteristic of viral infections, bacteremias or diseased caused by exotoxins, as well as a variety of other noninfectious diseases. In acute lymphadenitis the lymph node becomes enlarged owing to cellular infiltration and edema. As a consequence of distension of the capsule, the node becomes tender. Abscess formation causes the node to become fluctuant. Penetration of the infection through the overlying subcutis and skin surface may produce draining sinuses, particularly when nodes have undergone suppurative necrosis. Control of the infection with resolution of the inflammatory changes leads to shrinkage of the node. Nodes resume their former macroscopic and microscopic appearance if the infection has not caused extensive tissue destruction. If severe scarring and fibrosis ensue, nodes may remain firm and palpable.

Chronic lymphadenitis is typically a proliferative response, with either follicular hyperplasia or paracortical lymphoid hyperplasia, depending on the cause of inflammation. Characteristically, the nodes are not tender.[6]

CLINICAL FEATURES

Regional lymphadenopathy

Acute suppurative lymphadenitis

Acute suppurative lymphadenitis is commonly caused by a pyogenic infection. The inflammatory neutrophilic reaction arises due to

TABLE 16-1 -- Differential diagnosis of lymphadenopathy.

DIFFERENTIAL DIAGNOSIS OF LYMPHADENOPATHY

Reactive	**Infectious diseases**
	Viral (e.g. infectious mononucleosis syndrome, rubella)
	Bacterial (e.g. pyogenic, cat-scratch disease)
	Mycobacterial (*Mycobacterium tuberculosis*, atypical mycobacteria)
	Spirochetal (e.g. syphilis, leptospirosis)
	Chlamydial (e.g. lymphogranuloma venereum)
	Fungal (e.g. coccidioidomycosis)
	Parasitic (e.g. toxoplasmosis)
	Noninfectious diseases
	Sarcoidosis
	Connective tissue diseases (e.g. systemic lupus erythematosus)
	Kawasaki's disease
	Rosai-Dorfman disease
	Kikuchi's disease
	Castleman's disease
	Drug hypersensitivity (e.g. to phenytoin)
	Silicone breast implantation
Infiltrative	**Malignant diseases**
	Metastatic carcinoma
	Metastatic melanoma, germ cell tumor
	Leukemia
	Nonmalignant diseases
	Lipid storage diseases (e.g. Gaucher's disease, Niemann-Pick disease)
	Amyloidosis
Primary lymphoproliferative diseases	Lymphoma (e.g. Hodgkin's, non-Hodgkin's)
	Angioimmunoblastic lymphadenopathy
	Lymphomatoid granulomatosis
	Malignant histiocytosis

drainage of bacteria — *Staphylococcus aureus* or group A streptococci — from an infected site. The most common sites of involvement are the submandibular, cervical, inguinal and axillary lymph nodes. The affected lymph node is extremely tender and firm, although it may be fluctuant, and the overlying skin may be red and warm. There are usually systemic manifestations. Acute cervical lymphadenitis due to a pyogenic infection is more common in children than adults. In both children and adults it is commonly due to staphylococcal infections of the face or neck and, uncommonly, it may be a complication of streptococcal pharyngitis. [7] In adults, anaerobic bacteria, of which the predominant species are *Prevotella* spp., *Peptostreptococcus* spp., *Propionibacterium acnes* and *Fusobacterium* spp., are recovered in 30% of cervical lymphadenitis, 13% are anaerobes alone and 17% are mixed anaerobic-aerob bacteria.[8] Acute pyogenic cervical lymphadenitis is unilateral. In contrast, acute bilateral cervical lymphadenitis is commonly due to viral upper respiratory infection, infectious mononucleosis, streptococcal pharyngitis or localized periodontal infections. Acute suppurative axillary lymphadenitis is a severe infection with prominent systemic manifestations and axillary pain that radiates to the shoulder and down to the arm. The axilla, arm, shoulder and supraclavicular and pectoral areas are markedly

Figure 16-1 The human lymphatic system.

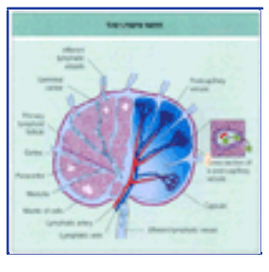

Figure 16-2 The lymph node.

TABLE 16-2 -- General histologic correlates of some diseases that cause lymphadenitis.

GENERAL HISTOLOGIC CORRELATES OF SOME DISEASES THAT CAUSE LYMPHADENITIS	
Histologic feature	**Type of disease and examples of causative organism**
Acute suppurative lymphadenitis	Pyogenic infections (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Yersinia pestis*)
Lymphadenitis with caseating necrosis	Tuberculosis (*Mycobacterium tuberculosis*)
	Atypical mycobacteria
Necrotizing granulomatous lymphadenitis	Cat-scratch disease (*Bartonella henselae*)
	Tularemia (*Francisella tularensis*)
	Leishmaniasis (*Leishmania braziliensis*, *L. major*)
	Lymphogranuloma venereum (*Chlamydia trachomatis*)
Non-necrotizing granulomatous lymphadenitis	Histoplasmosis (*Histoplasma capsulatum*)
	Coccidioidomycosis (*Coccidioides immitis*)
	Sarcoidosis

Necrotizing nongranulomatous lymphadenitis	Kikuchi's syndrome
	Systemic lupus erythematosus

edematous, but there are no signs of skin infection or lymphangitis. The portal of entry of the infecting bacteria (group A streptococci or *S. aureus*) is often a traumatic lesion of the arm.[9] Acute suppurative inguinal lymphadenitis due to group A streptococci has been reported in patients infected with HIV with or without chronic lymphadenopathy. Rapidly enlarging lymph nodes may be accompanied by systemic manifestations, including toxic shock syndrome, without obvious genital or skin lesions.[10] [11]

Patients who have chronic granulomatous disease experience recurrent pyogenic infections, of which the most common manifestations are lower respiratory tract infections, suppurative lymphadenitis, subcutaneous abscesses and hepatic abscesses. [12] The infecting pathogens are catalase-positive organisms such as *S. aureus*, *Serratia marcescens*, *Burkholderia* (*Pseudomonas*) *cepacia* and *Aspergillus* spp. The histologic appearance of the lymph node is one of inflammation with granuloma formation and necrosis.[12] [13]

Cat-scratch disease

Cat-scratch disease typically manifests after a cat scratch or bite as regional lymphadenopathy distal to the involved lymph node. The mode of transmission is presumably direct contact with the causative agent, primarily *Bartonella henselae*. The disease occurs worldwide, with healthy children and adolescents being most frequently affected.[14] A history of a trivial cat scratch or a bite by a kitten can

TABLE 16-3 -- Oculoglandular syndromes.

OCULOGLANDULAR SYNDROMES		
Disease	**Infecting organism**	**Features**
Cat-scratch disease	*Bartonella henselae*	Parinaud's sign in 3%
		Conjunctivitis in 6%
Tularemia	*Francisella tularensis*	Parinaud's sign in 5%
Lymphogranuloma venereum	*Chlamydia trachomatis*	Parinaud's sign in <1%
Pharyngoconjunctival fever	Adenovirus 3, 7	Common in children
Epidemic keratoconjunctivitis	Adenovirus 8, 19, 37	Occasionally seen in adults
Chagas' disease	*Trypanosoma cruzi*	Romaña's sign

be elicited in most cases.[15] Rarely, a dog or monkey is implicated. Occasionally, typical cat-scratch disease case can be caused by other pathogenic *Bartonella* sp. (i.e. *Bartonella claridgeiae*).[16]

Tender lymphadenopathy develops within 1–3 weeks after inoculation. Commonly, an erythematous papule at the site of inoculation precedes the development of lymphadenopathy and may last for several weeks. Regional lymph node enlargement is the sole manifestation in one-half of the patients. Most commonly the cervical, axillary or epitrochlear lymph nodes are involved, but any peripheral nodes at multiple sites may be enlarged. In one-third of the patients, low grade fever is present, and about 15% have systemic manifestations such as malaise, headache, splenomegaly and sore throat. Unusual clinical manifestations occur in 10% of patients; the most frequent of these is the oculoglandular syndrome of Parinaud,[15] [17] which is conjunctivitis with ipsilateral preauricular lymphadenitis (Table 16.3). The adenopathy subsides spontaneously within several months. Occasionally, aspiration of a suppurative lymph node is needed to relieve pain.

The diagnosis is based on epidemiologic exposure and can be confirmed by detection of serum antibody to *B. henselae*.[15] Occasional cat-scratch disease cases caused by other pathogenic *Bartonella* may, however, he serologically negative.[16]

In atypical presentations or whenever a neoplastic or mycobacterial process is suspected, a lymph node biopsy or aspirate may be needed. Early in the course of the infection the involved lymph node shows lymphoid hyperplasia; later, granuloma formation with central areas of necrosis give the distinctive histopathologic appearance of necrotizing granulomatous lymphadenitis. The histologic reaction cannot differentiate cat-scratch disease from diseases such as tularemia, lymphogranuloma venereum and fungal and mycobacterial infections. Sometimes, small pleomorphic bacteria can be visualized with the Warthin-Starry silver stain in necrotic foci of early-stage microabscesses. A polymerase chain reaction (PCR) assay for detecting *B. henselae* DNA in clinical specimens has been found to be a sensitive and specific tool,[18] but it is not widely available.

Toxoplasmosis

Acute acquired infection with *Toxoplasma gondii* is common worldwide. The prevalence of seropositivity by the fourth decade of life in the USA is 30–50%, and higher than 90% in certain areas of western Europe.[19] [20] Acute infection occurs primarily in patients in the second to fourth decades of life. In the immunocompetent host the infection is most often asymptomatic. In 10–20% of patients the infection is self-limited, with lymphadenopathy being the most frequent clinical manifestation. In 90% of patients who have clinically apparent infection, regional lymphadenopathy, usually of the head and neck, is the sole manifestation. A single node is usually involved, most commonly a posterior cervical, anterior cervical, axillary or

suboccipital node; occasionally an inguinal node is involved. Retroperitoneal or mesenteric lymphadenopathy may occur and can cause abdominal pain. Cases of toxoplasma hilar lymphadenopathy and unusual sites of lymph nodes such as intramammary,[21] parotid[22] and chest wall (our experience) have also been reported.

On palpation, the lymph nodes are discrete, of varying firmness and may or may not be tender; they rarely suppurate and they do not ulcerate. The histologic appearance is distinctive but may sometimes be confused with lymphoma, cat-scratch disease or Kikuchi's lymphadenitis. Lymphadenopathy is associated with fever, headache, sore throat and myalgia in approximately 15% of patients. Toxoplasmosis may rarely cause a syndrome resembling mononucleosis. In uncommon situations lymphadenopathy may persist or recur for months and pose a diagnostic challenge.[19]

Toxoplasmosis has been estimated to cause between 3% and 7% of clinically significant lymphadenopathy. Of major importance is the diagnosis of infection in pregnant women and the subsequent management of the fetus, and the need to differentiate toxoplasmosis from neoplasia (Hodgkin's and non-Hodgkin's lymphoma and carcinoma). Failure to consider toxoplasmosis in the differential diagnosis frequently results in unnecessary surgical biopsies.[19]

In the immunocompetent host, serologic testing is sufficient for the diagnosis of toxoplasmic lymphadenitis because it occurs almost exclusively as a manifestation of the acute acquired infection. A negative result in the Sabin-Feldman dye test or a comparable test for detecting *Toxoplasma* IgG in the first 3 months practically excludes the diagnosis. Acute infection is likely if elevated IgM antibody titer on enzyme-linked immunosorbent assay is present. In patients who have equivocal IgM antibody results (after 3 months), detection of IgA or IgE antibodies or an acute pattern in the differential agglutination titers may be helpful.[20]

Mycobacterial lymphadenitis

Tuberculous lymphadenitis is the most frequent form of extrapulmonary tuberculosis and in Western countries it accounts for 5% of cases of tuberculosis. [23] In areas where both AIDS and tuberculosis are endemic (e.g. in central Africa), tuberculous lymphadenitis is the presenting sign of tuberculosis in 50% of young children and is often associated with intrathoracic disease; a contact with an index case is often apparent.[24] In the past, *Mycobacterium bovis* was a common cause of cervical lymphadenitis in countries with a high incidence of bovine tuberculosis. [9] Currently, most cases of mycobacterial cervical lymphadenitis are caused by *Mycobacterium tuberculosis* or by atypical mycobacteria, especially *Mycobacterium scrofulaceum* and *Mycobacterium avium* complex (MAC). In the USA an abrupt change in the predominant etiologic agents from *M. scrofulaceum* to MAC was noted in the 1970s.[25] The latter is also seen in children in areas where *M. bovis* has been eradicated and *M. tuberculosis* is rare. Recent reports have documented rare cases of childhood lymphadenitis caused by other mycobacteria (*Mycobacterium interjectum* or *Mycobacterium malmoense*).[26] [27] [28]

Tuberculous cervical lymphadenitis (scrofula) is caused by spread of the infection from the lung, usually by the hematogenous or lymphatic route. When the source of

infection is milk contaminated with *M. bovis*, the primary focus is in the tonsils or the pharynx.

Scrofula most often presents as a unilateral firm, red, painless mass located along the upper border of the sternocleidomastoid muscle or in the supraclavicular area.[27] Occasionally, tuberculous lymphadenitis may be found in the axilla (Fig. 16.3). The process progresses indolently and is usually not accompanied by systemic symptoms. Miliary tuberculosis should be suspected when the lymphadenopathy is generalized, localized outside the cervical chain or accompanied by systemic symptoms.

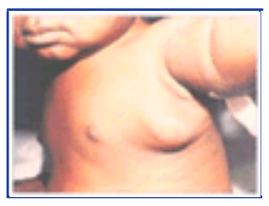

Figure 16-3 Tuberculous lymphadenitis of the axilla.

In HIV-infected or otherwise immunocompromised patients, the course of the disease is severe, with bilateral lymphadenopathy and systemic symptoms such as fever, night sweats and weight loss. Patients infected with HIV are more likely to develop extrapulmonary disease, often in conjunction with pulmonary tuberculosis. [29]

The diagnosis of mycobacterial lymphadenitis is confirmed by lymph node histology and bacteriology examinations. Fine-needle aspiration reveals the presence of granuloma but only rarely yields positive smears or cultures.[26] Bacteriology and histopathology examinations are complementary diagnostic tools, because *M. tuberculosis* has been cultured from lymph nodes without caseating granuloma and atypical mycobacteria have been cultured from lymph nodes with classic histopathologic appearance for tubercu-losis. Acid-fast bacilli are only rarely seen in smears except in HIV-positive patients, in whom positive smear results are much more frequent because of the high density of organisms. Therefore, a specific culture of aspirated lymph node should be performed routinely. New diagnostic tools such as PCR and hybridization and blotting techniques hold promise for rapid and precise diagnosis (see Chapter 37).

Inguinal lymphadenopathy

Sexually transmitted diseases (STDs) and metastatic genital neoplastic disease are the most common causes of inguinal lymphadenopathy. The differential diagnosis of infectious inguinal lymphadenopathy is shown in Table 16.4 .

Chancroid

Chancroid is caused by *Haemophilus ducreyi* and in some parts of the world (e.g. Thailand) it is one of the most common causes of genital ulcer with inguinal lymphadenopathy. The chancroid ulcer is a painful, nonindurated lesion that appears 1 day to several weeks after inoculation. Inguinal lymphadenitis occurs in between one-third and one-half of untreated cases. The lymph nodes are enlarged, painful and tender. The process is most commonly unilateral and, without treatment, can progress to suppuration with periadenitis and involvement of the overlying skin (bubo). Coinfection

TABLE 16-4 -- Differential diagnosis of infectious inguinal lymphadenopathy.

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS INGUINAL LYMPHADENOPATHY	
Sexually transmitted diseases	**Other diseases**
Syphilis	Pyogenic infections
Lymphogranuloma venereum	Cellulitis
Chancroid	Plague
Genital herpes	Filariasis
Granuloma inguinale	Onchocerciasis

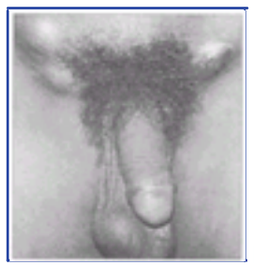

Figure 16-4 Groove sign of lymphogranuloma venereum. There is cleavage of extensive lymphadenopathy by the inguinal ligament.

with other organisms (e.g. herpes simplex virus (HSV) or *Chlamydia trachomatis*) is not uncommon. The differential diagnosis includes other STDs, especially syphilis, herpes simplex and lymphogranuloma venereum, secondary pyogenic infections of traumatic lesions and neoplasia.

Culture provides the definitive diagnosis; however, *H. ducreyi* is a fastidious organism and immediate direct inoculation into specific culture media is required for bacterial growth and isolation. Chemotherapy is sufficient in most uncomplicated cases but abscesses that are more than 5cm in diameter may need surgical drainage.[30] (See also Chapter 78).

Lymphogranuloma venereum

Lymphogranuloma venereum is a rare STD caused by *C. trachomatis* serovars L1, L2 and L3. The typical vesicular lesions appear 1–2 weeks after inoculation but the incubation period varies between 5 and 20 days. The lesions often go unnoticed by the patient. Inguinal lymphadenopathy appears 1–6 weeks after the vesicles disappear. The lymphadenopathy is most commonly unilateral but is bilateral in 30–40% of patients. The nodes are painful and the groove sign (cleavage of the enlarged nodes by the inguinal ligament) is seen in 25% of patients (Fig. 16.4). The involved lymph nodes frequently coalesce to form a bubo. If untreated, the nodes, which are filled with bacteria, rupture and a nonhealing fistula is formed. The anorectal syndrome, which occurs mainly in women and homosexual men, results from involvement of the pelvic lymph nodes. In the male, abscess formation may occur in the dorsal lymphatic of the penis and cause tissue destruction and elephantiasis of the penis. *Chlamydia trachomatis* can be isolated from both blood and aspirates of lymph nodes in approximately 30% of cases. Incision of the bubo is not warranted for diagnosis and positive serologic tests (complement fixation antibody test or microimmunofluorescence test) in the appropriate clinical setting are highly sensitive and specific for the diagnosis.[31] (See Chapter 78).

Syphilis

The lymphadenopathy of primary syphilis is easily differentiated from chancroid and lymphogranuloma venereum because nodes involved in syphilis are firm, only moderately enlarged, nonsuppurative and painless. The classic primary chancre appears 14–30 days after inoculation and is a nonexudative, painless ulcer. In the immunocompetent patient only one chancre appears but in the immunocompromised patient, especially in patients who have AIDS, multiple chancres may be seen. In women and homosexual men, the chancre may be located in the perianal region or in the anal canal. Regional painless lymphadenopathy is characteristic at this stage of disease. The chancre usually heals and disappears after 3–6 weeks, but the lymphadenopathy may persist for longer. The symptoms of secondary syphilis appear 2–8 weeks after the chancre has healed, with generalized lymphadenopathy and various skin lesions in the majority of patients. Epitrochlear lymphadenopathy suggests the diagnosis.[32]

Genital herpes

The typical vesicles associated with inguinal lymphadenopathy usually suggest the correct diagnosis. Rarely, lymph node enlargement may appear before the rash

develops.[33] Herpes simplex virus is the most common cause of genital ulcers in the Western world. The incidence of genital herpes rose in the pre-AIDS era but has been stable in the past decade. Women are reported to have higher rates of infection than men. Both HSV-1 and HSV-2 can cause genital herpes but from epidemiologic studies it is estimated that HSV-2 causes more than 90% of genital herpes. The distinction between the two types of the virus is significant because HSV-1 infection is less severe and is less prone to recur than infection with HSV-2.

Primary herpes genitalis infection is usually more severe than recurrent attacks. Genital lesions begin as macules and papules, followed by vesicles and ulcers that may appear and last for 2–3 weeks. Occasionally, primary infection is accompanied by systemic manifestations such as fever, malaise, aseptic meningitis (in 10% of patients) and extragenital lesions as well as dysuria and painful lymphadenopathy. In women, primary lesions appear on the vulva, with involvement of the cervix. Lesions can also appear in the perineum, vagina, buttocks and perianal region. Urinary retention has been described in 15% of women. In males, the lesions are vesicular and are superimposed on an erythematous base. In homosexual men the lesions may appear on the buttocks and in the anal and perianal areas.

The lymphadenopathy associated with primary herpes is either unilateral or bilateral and in severe cases generalized lymphadenopathy may also occur. Genital lesions and lymphadenopathy are common in genital herpes in the immunocompetent host; however, massive lymphadenitis is frequently seen in the immunocompromised patient. The diagnosis is based on isolation of HSV from a skin lesion, preferably from a vesicle.[34]

Granuloma inguinale

Granuloma inguinale (donovanosis) is caused by *Calymmatobacterium granulomatis*. The disease is rare in the Western world but is a major cause of genital ulcer in southeast India, Brazil and some parts of Africa. The penile papules of granuloma inguinale appear within days of inoculation and rapidly ulcerate to form a red, granulomatous, painless ulcer with a characteristic surface that bleeds easily on contact. Subcutaneous spread into the inguinal region results in swellings (pseudobubos) that are not a true adenitis. Lymphedema and elephantiasis occasionally result from scarring and blockage of the lymphatics. Granuloma inguinale should be differentiated from other genital ulcerative lesions with inguinal lymphadenopathy. The diagnosis is established through the demonstration of the typical intracellular Donovan bodies in stained smears obtained from the lesions.[35] (See Chapter 78 and Chapter 229).

Plague and tularemia

Plague and tularemia are both zoonotic infections that produce similar diseases, mostly fever and regional lymphadenitis.[36] These infections have regained interest after the events of 11 September 2001 as airborne transmission is considered to be a potential biologic weapon.[37] [38]

Plague is caused by *Yersinia pestis*. It is distributed worldwide (with the exception of Europe and Australia). Most human plague is of the bubonic form and is transmitted by the bite of infected fleas. After an incubation period of 2–8 days, patients are affected

by the sudden onset of fever, chills, malaise and headache. Usually at the same time or after a few hours, a painfully swollen regional lymph node appears in the draining area of the inoculation site, commonly in the axilla, neck or groin. The primary lesion is occasionally found at the bite site and may later develop into extensive cellulitis or abscess. Over the next few days, the discrete nodes become matted together to form the characteristic bubo (Fig. 16.5). If untreated, infection spreads hematogenously and results in a 'septic' phase with organ involvement, including secondary pneumonic plague (see Chapter 176).

Diagnosis of plague should be suspected in an acutely ill patient who has an extremely tender cluster of lymph nodes when there has been the appropriate epidemiologic exposure. Isolation of *Y. pestis* from bubo aspirate, blood and any other involved organ is possible but growth is slow and rapid identification of the bacteria is possible by Gram stain or Wayson stain. A patient suspected of having plague should be isolated and treatment should begin immediately. The prognosis is favorable in patients who are treated early.

Tularemia is restricted to the northern hemisphere. Over 80% of infections are acquired by handling infected animals or by tick or deer fly bites. In the USA, cases are mostly sporadic or occur in small clusters; in Eurasia, waterborne, arthropod-borne and airborne outbreaks involving hundreds of persons have been reported. [37]
Infection commonly manifests as ulceroglandular syndrome. The most common portal of entry is the skin, with an ulcer or pustule developing 1–10 days after exposure; regional lymphadenopathy, usually axillary or epitrochlear, follows. The lymph nodes may suppurate. Systemic manifestations are common, but severe endotoxemia as seen in plague is uncommon. The oculoglandular syndrome is seen when the portal of entry is the conjunctiva (as happens when the eyes are contaminated with infected fluids). Conjunctivitis, conjunctival ulcerations and papules on the eyelids appear, and lymph nodes of the head and neck become inflamed. Diagnosis of typical ulceroglandular tularemia in a patient who has the appropriate epidemiologic exposure is made on clinical grounds. In other less obvious settings, diagnosis relies on serologic studies (see Chapter 177).

Filariasis and onchocerciasis should also be considered in the differential diagnosis of inguinal bubo formation; these are discussed below (see Lymphadenopathy of presumed tropical origin).

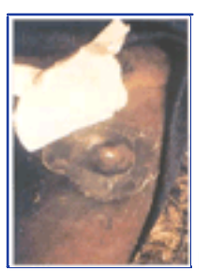

Figure 16-5 Bubonic plague. Femoral lymph nodes matted together to form the classic bubo.

Mediastinal and hilar lymphadenopathy

Mediastinal or hilar lymph node enlargement may be detected because it causes symptoms (e.g. cough, dyspnea, hoarseness caused by recurrent laryngeal nerve compression, superior vena cava syndrome caused compression of the superior vena cava); on other occasions it is detected during routine chest radiographic screening. The presence of mediastinal lymphadenopathy is usually indicative of a significant disease and is frequently seen in lymphoma. In contrast, bilateral hilar lymphadenopathy is more often benign and is seen in tuberculosis, sarcoidosis and endemic mycoses. [39]

Tuberculosis

Isolated mediastinal or hilar lymphadenopathy is an uncommon manifestation of tuberculosis, although tuberculosis should be considered, especially in high-risk patients such as those infected with HIV or people from Asia or Africa. Mediastinal and hilar lymphadenopathy can be a manifestation of primary disease or of reactivation of tuberculosis (Fig. 16.6). Isolated lymphadenopathy is relatively rare in postprimary tuberculosis.[40] Associated ipsilateral hilar or mediastinal adenopathy is almost universal in children who have primary tuberculosis but is less common in adults. In patients infected with HIV, mediastinal or hilar lymphadenopathy are usually present in tuberculosis even in the advanced stages of immunosuppression (see Chapter 129).

Anthrax

Inhalational anthrax is characterized by mediastinal lymphadenopathy. On CE 02 X-rays of suspected cases this may be the earliest diagnostic clue, appearing in 100% of the cases. Histology discloses diffuse hemorrhagic lympadenitis.

Endemic mycoses (also see Chapter 39)

Histoplasmosis

Histoplasmosis is a fungal infection acquired through inhalation. It is endemic in the USA in the great river valleys of the Mississippi and Ohio. More than 90% of the primary infections are asymptomatic or only mildly symptomatic, although sudden enlargement of the hilar lymph nodes may cause substernal pain. Non-specific

systemic symptoms such as fever, headache, malaise and anorexia are common. Most patients recover within 2–6 weeks.

Typical findings on chest radiography in symptomatic patients include patchy pneumonic infiltrates. Mediastinal and hilar lymphadenopathy

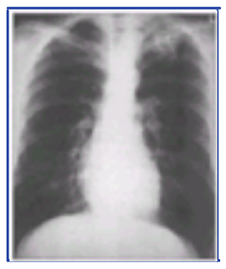

Figure 16-6 Pulmonary tuberculosis.

is common in patients with or without parenchymal involvement. At times, extension of the infection from the pulmonary parenchyma to the adjacent mediastinal lymph nodes causes central caseating necrosis and granuloma formation with multinucleated giant cells. Resolution causes fibrosis of the affected nodes, which usually causes no symptoms. Rarely, the fibrotic lymph nodes invade mediastinal structures, resulting in esophageal stricture or compromise to the mediastinal blood or lymph vessels (fibrosing mediastinitis).[41]

Coccidioidomycosis

Coccidioidomycosis is endemic in the south west USA, Mexico and Central and South America. The lymph nodes most commonly involved are the mediastinal and hilar lymph nodes. When there is involvement of the scalene or supraclavicular nodes, the organism can be isolated from these sites.[42]

Paracoccidioidomycosis

Paracoccidioidomycosis is endemic in Mexico and Central and South America. Most primary infections are asymptomatic. In 10% of patients there is lymphadenopathy, especially of the cervical, axillary and mediastinal nodes, with or without fistula formation. Characteristic budding yeasts may be demonstrated in aspirated material from lymph nodes.[43]

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disease of unknown etiology. Bilateral symmetric hilar lymphadenopathy, usually with paratracheal adenopathy, is characteristic. Peripheral lymphadenopathy is rare. In the mild form of the disease (radiologic grading stage 1) mediastinal or hilar lymphadenopathy is usually discovered inadvertently in asymptomatic persons undergoing a chest radiography for an unrelated cause. Computerized tomography scanning can detect anterior mediastinal and subcarinal lymph nodes that are undetected by chest radiography. Biopsy of lung tissue, even without radiologic findings, is superior for histopathologic diagnosis to lymph node biopsy because it has a higher specificity. The lymph node often shows only non-specific granuloma and therefore has a low diagnostic yield.[44]

Abdominal and retroperitoneal lymphadenopathy

Abdominal and retroperitoneal lymphadenopathy are not usually inflammatory in origin but are frequently due to neoplasia. In the patient who has HIV infection abdominal or retroperitoneal lymphadenopathy is commonly due to persistent generalized lymphadenopathy, disseminated MAC infection or lymphoma. Fever, night sweats and an elevated alkaline phosphatase serum level suggest disseminated MAC infection, whereas an elevated lactic dehydrogenase serum level is more suggestive of the presence of lymphoma.

Mesenteric lymphadenitis is frequently caused by *Yersinia enterocolitica*; some cases have been described with *Yersinia pseudotuberculosis*. The disease needs to be differentiated from appendicitis because it is manifested by right iliac fossa pain. In a 10-year follow-up study of 458 patients who had *Y. enterocolitica* infection [45] it was found that the majority of cases had diarrhea and abdominal pain; vomiting and weight loss were also common. Mesenteric lymphadenitis was diagnosed in 43 of 56 patients who had undergone laparotomy for severe right iliac fossa pain. The histologic appearance of resected mesenteric lymph node may vary from non-specific changes of inflammation and microabscesses to frank granulomatous changes.

Other causes of mesenteric lymphadenitis are talcum powder spread during abdominal surgery, viral diseases in children and adolescents, and inflammatory bowel disease.

Generalized lymphadenopathy

Generalized lymphadenopathy is identified whenever three or more anatomically discrete groups of nodes are involved. Numerous infectious and noninfectious diseases cause generalized lymphadenopathy. Viral diseases predominate but systemic bacterial diseases, including tuberculosis, typhoid fever, brucellosis and leptospirosis, are not uncommon causes of generalized lymphadenopathy. The clinical setting is important in the differential diagnosis. The age of the patient, epidemiologic factors, accompanying physical findings (e.g. rash, organomegaly) or laboratory findings (e.g. atypical lymphocytes, eosinophilia) usually guide further diagnostic investigations.

In intestinal anthrax the lymphnodes draining the ileocecal valve and caecum are also enlarged.

Infectious mononucleosis

Infectious mononucleosis is a syndrome that appears most commonly in children and young adolescents. It is classically characterized by the acute onset of fever, tonsillopharyngitis, lymphadenopathy, splenomegaly and the appearance of atypical lymphocytes in peripheral blood.[46] In children and adolescents, Epstein-Barr virus (EBV) is the most frequent cause (80–90%), followed by cytomegalovirus (CMV; 8–16%).[47] It is usually a benign and self-limited process. Patients may exhibit generalized lymphadenopathy, localized lymph node enlargement in unusual sites (e.g. the inguinal nodes) and lymphadenopathy without systemic manifestations. Rarely, a progressive, fatal disease develops in patients who have X-linked lymphoproliferative disorder and in other immunocompromised patients.[46]

Complete recovery generally occurs within 1–26 weeks after onset of disease, although postinfectious asthenia is not an uncommon complication. Serious complications, sequelae and death are exceedingly rare, occurring in fewer than one case in 3000. Other complications are meningoencephalitis with seizures, splenic rupture, upper airway obstruction, interstitial pneumonitis with hypoxemia and severe hepatitis with liver failure.

Lymphadenopathy is present in the vast majority of children and young adults but in only 45% of patients older than 40 years.[46] Lymph nodes are usually moderately enlarged throughout the body, principally in the posterior cervical, axillary and groin region. They are usually nontender or only minimally tender (Fig. 16.7). Lymph node histology demonstrates paracortical immunoblastic proliferation, as seen in most viral infections.[48]

Mononucleosis-like syndrome may occasionally be caused by *T. gondii* (1% of infectious mononucleosis cases)[19] and by other viruses, such as hepatitis A virus, HSV, rubella, adenovirus and human herpes virus type 6; it may also be caused by allergic reactions to various drugs. [46] Acute retroviral syndrome caused by HIV

Figure 16-7 Posterior cervical lymphadenopathy in infectious mononucleosis.

TABLE 16-5 -- Lymphadenopathy in patients infected with HIV.

LYMPHADENOPATHY IN PATIENTS INFECTED WITH HIV	
Generalized lymphadenopathy	
HIV associated	Acute retroviral syndrome
	Persistent generalized lymphadenopathy
Opportunistic infections	*Mycobacterium avium* complex
	Cytomegalovirus
	Tuberculosis
	Toxoplasmosis
	Cryptococcosis
	Bartonellosis
Malignancy	Lymphoma
	Kaposi's sarcoma
Drugs	Phenytoin
	Pyrimethamine
	Sulfonamides
	Abacavir
Others	Multicentric Castleman's disease
Localized lymphadenopathy	
Infections	Tuberculosis
	Cryptococcosis
	Syphilis
	Lymphogranuloma venereum
	Chancroid
	Other localized infection
Malignancy	Kaposi's sarcoma
	Lymphoma
	Metastasis

infection may manifest as mononucleosis-like syndrome and is discussed below.

Lymphadenopathy in HIV disease

Lymphadenopathy associated with HIV disease may have a multitude of origins, including lymphadenopathy related to HIV infection itself, opportunistic infections, STDs, malignancies and drugs (Table 16.5).

Lymphadenopathy related to HIV infection

Since key pathogenesis events and most viral replication take place in lymphoid tissues, it is not surprising that lymphadenopathy is common in patients who have HIV. Up to 90% of patients who become infected with HIV develop an initial mononucleosis-like illness, referred to as primary HIV infection or acute retroviral syndrome.[49] Illness typically occurs between 1 and 4 weeks after infection. Lymphadenopathy is common, usually appears in the second week of illness and most often involves posterior cervical, anterior cervical, occipital, submandibular and axillary groups. Enlarged nodes are symmetric and painless, with a diameter of 2cm or less.[49] [50] Following seroconversion, lymphadenopathy gradually declines but enlarged nodes may persist throughout the course of disease. Persistent glandular lymphadenopathy, once considered a marker of disease progression, apparently has no prognostic value.[51] In later stages of disease, lymph node architecture is disrupted, germinal centers involute and lymphadenopathy becomes less prominent.[52]

At times, a patient who has AIDS develops lymphadenopathy caused by a neoplastic disease (e.g. lymphoma or Kaposi's sarcoma) or an opportunistic infection. Sudden enlargement, pain or tenderness of a lymph node warrants aspiration or biopsy. Enlarged mesenteric or retroperitoneal lymph nodes can be part of the persistent lymph nodes enlargement seen during HIV disease, but if nodes are large or if there are symptoms such as fever, abdominal pain or diarrhea then a mycobacterial disease or lymphoma should be suspected. Mediastinal or hilar lymphadenopathy is usually not part of the syndrome of persistent generalized lymphadenopathy, and whenever isolated intrathoracic lymphadenopathy is detected a thorough investigation for mycobacterial disease is recommended. In such cases tuberculosis is found in more than half of the patients.[53]

Fine-needle aspiration cytology in HIV-positive patients is most useful in the diagnosis of opportunistic infections, obviating the need for tissue biopsy and allowing prompt initiation of treatment.[54] Whenever lymphoma is suspected biopsy with appropriate studies is preferred over fine-needle aspiration cytology.

Human T-lymphocyte leukemia virus 1

Lymphadenopathy is the most common finding in patients who have adult T-lymphocyte leukemia or lymphoma caused by human T-lymphocyte leukemia virus (HTLV)-1. Other characteristic findings include skin lesions, hepatomegaly, splenomegaly, hypercalcemia, lymphocytosis with abnormal circulating lymphocytes, hyperimmunoglobulinemia and rapid clinical deterioration. Histologic diagnosis of the disease requires immunophenotypic analysis, and these high-grade tumors often show loss of pan-T antigens.[55] Peripheral lymphadenopathy may also be observed in association with tropical spastic paraparesis and myelopathy caused by HTLV-1, which is endemic in some tropical areas. [56] (See Chapter 29).

Lymphadenopathy of presumed tropical origin

Leishmaniasis

Enlarged lymph nodes (mean diameter 3.6cm and up to 10.5cm) occur in 77% of patients who have parasitologically confirmed cutaneous leishmaniasis in South

America and in patients who have *Leishmania major* infection in equatorial Africa.[57] [58] Lymphadenopathy precedes the skin lesion by some 2 weeks in two-thirds of cases. Cultures of the enlarged lymph nodes for *Leishmania* spp. are more frequently positive (86%) than cultures of the skin. Lymph node histology frequently shows necrotizing or suppurative granulomas, sometimes with discharging sinuses. Patients who have leishmanial lymphadenopathy often have fever, hepatomegaly, splenomegaly and more intense leishmanin skin reactions and lymphocyte proliferation in response to antigenic stimulation, but fewer previous infections. Therefore, in endemic areas unexplained lymphadenopathy should prompt a search for leishmaniasis.

Leptospirosis (see Chapter 181)

Lymphadenopathy may appear in up to one-third of patients who have leptospirosis. Other common signs of this infection include fever, headache, myalgia and the classic clinical findings of conjunctival irritation, jaundice and impairment of renal function.[59]

Filariasis (see Chapter 170)

The most common symptomatic manifestations of filarial infection are recurrent episodes of high fever, occasionally with shaking chills, accompanied by lymphadenitis and a distinctive lymphangitis extending retrogradely from the lymph node where the filaria resides to the periphery. The attacks may last for 3–7 days and occur between six and 12 times a year. The attacks subside spontaneously without any specific therapy. The affected lymph nodes are characteristically enlarged and painful and the lymphatic vessels around them appear inflamed and indurated. Sometime local lymphedema appears as well. Occasionally, local thrombophlebitis may also occur. With infection by *Brugia malayi*, an abscess of the entire local lymphatic apparatus may develop with characteristic scars. The involved area is most commonly in the inguinal region.

With the continuation of lymphangitis and lymphadenitis, pitting edema of the skin develops; this is transformed into brawny edema of the involved area, resulting in thickening of the subcutaneous tissue and hyper-keratosis. There is coarsening of the skin, with deep fissuring and nodular and papillomatous hyperplastic changes. The damage to the lymphatic vessels leads to the development of elephantiasis, particularly in the legs but also in the lower arms and breasts.[60]

Onchocerciasis

The major manifestations of onchocerciasis are dermatitis, onchocercomas, lymphadenitis and visual impairment or blindness. The frequency and distribution of these symptoms vary according to the duration of exposure and the age and geographic location of the patient. Mild to moderate lymphadenopathy is common, particularly in the inguinal and femoral areas. Involved nodes are firm and nontender; at times they may reach gigantic proportions and be associated with local lymphatic obstruction and elephantiasis ('hanging groin'). In a recent survey of 770 patients in Uganda, [60] the most common manifestations of onchocerciasis were troublesome skin itching, chronic papular onchodermatitis and depigmentation; lymphadenopathy appeared only in conjunction with bacterially infected skin lesions associated with the itch.

Wuchereria bancrofti *infection*

In contrast to the situation in onchocerciasis, lymphadenopathy may be a striking feature of infection with *Wuchereria bancrofti*. Filaria are frequently present in the lymph nodes. The histology of the lymph node may not show the intense eosinophilia present in the peripheral blood smear or in other invaded organs. At times the genitalia may also be involved, with funiculitis, epididymitis, scrotal pain and anatomic disfiguration of the penis and scrotum. Scrotal lymphedema, hydrocele and elephantiasis may ensue. In addition, characteristic swelling of the leg below the knee and of the arm below the elbow develop as a result of lymphatic involvement and subsequent elephantiasis. Occasionally, obstruction of the retroperitoneal lymphatics occurs, leading to rupture of lymphatic vessels into the kidneys and the appearance of chyluria.

African trypanosomiasis (see Chapter 157)

Lymphadenopathy is observed in up to 80% of patients who have African trypanosomiasis. The supraclavicular or posterior cervical lymph nodes are the most frequently involved. The nodes are usually soft, rubbery, discrete, painless and nontender. They appear at the second stage of the disease. The skin chancre, the hallmark of the first stage of the disease, appears 1 week after the bite and lasts for 2–3 weeks; the skin chancre disappears without leaving a scar. During the second stage, when enlarged lymph nodes are present, pyrexial episodes lasting 1–6 days occur and alternate with afebrile intervals lasting several weeks. Anemia, monocytosis, elevated serum IgM and involvement of the central nervous system with elevated protein and immunoglobulins in the cerebrospinal fluid are characteristic of African trypanosomiasis in endemic areas. In light-skinned travelers to endemic areas, a characteristic extensive erythematous skin rash with circinate patches on the chest or back may appear and disappear several times and therefore suggest the correct diagnosis.[61]

American trypanosomiasis (Chagas' disease) (see Chapter 173)

Lymphadenopathy is an integral part of the first two stages of American trypanosomiasis, which is caused by *Trypanosoma cruzi*. The initial cutaneous lesion (the so-called 'inoculation chagoma'), which consists of a small raised reddish tender nodule with an indurated base, is accompanied by swelling of the regional lymph nodes.

Figure 16-8 Romaña's sign in acute Chagas' disease. There is unilateral edema of the eyelid accompanied by conjunctivitis and auricular lymphadenopathy.

Symptomatic acute Chagas' disease, which usually affects children and adolescents, is manifested by a lesion at the portal of entry in 50% of vector-infected patients. Regional lymphadenitis (iliac, axillary or cervical) may occur. Romaña's sign (Fig. 16.8) is a painless, unilateral indurated erythematous edema of the eyelids, accompanied by conjunctivitis and auricular lymphadenopathy. Local symptoms may be accompanied by rash, mild fever, hepatomegaly and acute myocarditis.

Occasionally, fatal meningoencephalitis occurs in young infants. Generalized enlargement of the lymph nodes, liver and spleen begins to develop during the second week of the infection, with accompanying subcutaneous edema of the face, legs and feet. The lymph nodes are usually isolated and discrete, nontender, nonadherent and painless. During this stage, myocarditis ensues; this is the hallmark of the disease and manifests itself with sinus tachycardia and a variety of conduction defects. [62]

Noninfectious causes of lymphadenopathy

Postvaccination lymphadenopathy

Postvaccination lymphadenopathy is rare nowadays. In the past, smallpox (vaccinia) vaccination was the most common cause of postvaccination lymphadenopathy. Regional lymphadenopathy may occur from several days up to 2 weeks after measles vaccination; it is not usually accompanied by systemic manifestations. The histopathologic findings of an excised node are similar to primary viral lymphadenitis[63] and characteristic Warthin-Finkeldey multinucleated giant cells can be seen. An outbreak of axillary lymphadenitis has been documented in children receiving bacillus Calmette-Guérin (BCG) vaccination. *Mycobacterium bovis* was isolated from a minority of these patients.[64]

Drug-induced lymphadenopathy

Certain drugs are known to cause hypersensitivity reactions associated with lymphadenopathy associated with fever, rash, arthralgia and eosinophilia. Liver enzyme abnormalities and pancytopenia are occasionally seen as well.[65] Phenytoin is the most common drug responsible for such cases.[65] [66] Moreover, some anticonvulsants (e.g. phenytoin and carbamazepine) can cause a pseudolymphoma syndrome, which is characterized by fever, erythematous rash, marked lymphadenopathy and organomegaly.[67] Most patients have taken the implicated drug for only a short time, usually less than 3–4 months, but in some patients many months elapse between initiation of the drug and the appearance of the clinical syndrome. In most patients lymphadenopathy spontaneously regresses 1–2 weeks after stopping the drug.

Lymphadenopathy and autoimmune disease

Generalized lymphadenopathy is common in some autoimmune disease. Studies have shown that lymphadenopathy occurs in up to 75% of patients who have rheumatoid arthritis, 43% of patients who have juvenile rheumatoid arthritis and up to 65% of patients who have active systemic lupus erythematosus.[48]

Kawasaki disease (mucocutaneous lymph node syndrome)

Kawasaki disease is an acute inflammation of small and medium-sized arteries that affects young children. The clinical manifestations include fever, conjunctivitis, oral mucositis, skin rash and cardiac involvement. Lymphadenopathy occurs in 75% of the patients, most frequently in the cervical area.[68] The most devastating complications of this disease include coronary artery aneurysm, coronary thrombosis and myocarditis. The histopathologic features of the lymph nodes are not characteristic; there are scattered necrotic foci associated with thrombi of the adjacent microvasculature.[48] The diagnosis is made on clinical grounds and at least five features should be present. The differential diagnosis of Kawasaki disease includes staphylococcal scalded skin syndrome, scarlet fever and hypersensitivity reactions. The presence of lymphadenopathy, however, suggests Kawasaki disease.

Rosai-Dorfman disease

Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy) is a rare, idiopathic and generally benign, self-limited disease that affects children and adolescents. Massive lymphadenopathy, most commonly involving the cervical nodes, is the hallmark of the disease. Nearly 50% of the patients have some extranodal involvement, most of them occurring in the head and neck where they involve the nose, paranasal sinuses and parotid gland. About 10% of the patients have an associated immunologic disorder.

The involved lymph nodes show a marked sinus expansion by numerous histiocytes with abundant clear cytoplasm containing engulfed small lymphocytes and a small bland nucleus. The clinical setting is usually a child or adolescent presenting with marked cervical adenopathy associated with fever, neutrophilic leukocytosis, elevated erythrocyte sedimentation rate and polyclonal hypergammaglobulinemia. Although the disease is usually self-limited, the prognosis is unfavorable in patients who have immunologic disorders.[48] [69]

Kikuchi's disease

Kikuchi's disease is a histiocytic necrotizing lymphadenitis that was first described in 1972 in Japan as a benign lymphadenopathy of the neck.[70] Kikuchi's disease affects women more often than men and, owing to its association with systemic lupus erythematosus and Still's disease, it is suspected of being an autoimmune disorder. The clinical features of Kikuchi's disease include fever and lymphadenopathy, which is usually cervical.[71] The differential diagnosis includes infectious disease and malignant disease. Biopsy is important for diagnosis and exclusion of lymphoma.

Castleman's disease

This condition, originally called angiofollicular lymph node hyperplasia, was first described as a rare cause of a mediastinal mass seen on chest radiography. It causes mild systemic symptoms of fever and night sweats, and is associated with an elevated erythrocyte sedimentation rate. More recently a variant has been described in which there is generalized lymphadenopathy and more systemic signs. There may be an association with the so-called POEMS syndrome: peripheral neuropathy, organomegaly, endocrinopathy a monoclonal paraprotein and skin lesions. The etiology of Castleman's disease has recently been linked to infection with HHV8 (human herpesvirus 8). Severe cases may require corticosteroid therapy.

Infiltrative lymphadenopathy

Inborn errors of metabolism (Gaucher's disease, Niemann-Pick disease), amyloidosis, histiocytosis X and metastatic malignancy cause infiltration of various organs, including the lymph nodes. The nodes in these cases are characteristically firm and nontender; they may constitute the first clue to a systemic infiltrative process.

Lymphadenopathy and silicone breast implant

In recent years, there has been a growing body of evidence concerning the immune response to silicone and its breakdown products. In women who have silicone breast implants, leakage of silicone causes axillary lymph node enlargement. The histopathology is characteristic for foreign body reaction.[72]

MANAGEMENT

Lymphadenopathy may be the presenting sign in many diseases. Techniques for palpation and assessment of lymph nodes are essential for providing the physician with useful information on which diagnostic and therapeutic decisions can be based. In adults, small lymph nodes can normally be palpated in the inguinal area and in children in the suboccipital area. Enlarged supraclavicular, scalenal, axillary and epitrochlear lymph nodes are usually pathologic and require appropriate investigation with possible aspiration or biopsy of the node. The investigation of lymphadenopathy can be organized according to the following categorizations:

- the mode of presentation (an acutely ill, chronically ill or asymptomatic patient, and any pertinent associated clinical manifestation);
- the patient's age, epidemiologic exposure and immune status;
- the physical characteristic of the enlarged node or nodes; and
- the location of the lymphadenopathy.

Mode of presentation

In acutely ill patients who have a tender, enlarged lymph node a pyogenic bacterial infection is most commonly found. A search for skin and soft tissue infection in the region drained by the involved lymph node is indicated. A thorough ear, nose and throat examination is mandatory if the adenitis is in the head and neck area. Fluctuant abscesses should be aspirated, Gram stained and cultured, and appropriate antibiotic treatment be instituted. Group A streptococcal and *S. aureus* infections cannot be differentiated on the basis of clinical presentation. In endemic regions plague should be suspected and treated promptly. The acutely ill patient who is found to have generalized lymphadenopathy should be evaluated for systemic infections such as streptococcal and staphylococcal bacteremia, infectious mononucleosis, typhoid fever, rickettsiosis, leptospirosis, miliary tuberculosis and reactive noninfectious diseases, depending on any associated clinical features. In the asymptomatic or mildly symptomatic young patient who has generalized lymphadenopathy and no localizing clinical features, the most plausible etiology is a viral disease, usually caused by EBV, CMV or HIV; appropriate serologic or culture studies are called for.

In the elderly, acute EBV or CMV infections cause lymphadenopathy less frequently than they do in younger patients. The differential diagnosis includes primary lymphoproliferative and metastatic disease as well as reactive processes.

Table 16.6 summarizes the clinical presentation of infectious lymphadenitis.

Disease progression

Lymphadenopathy of long duration mandates further evaluation. Although lymphadenopathy due to cat-scratch disease or toxoplasmosis may persist for months, a presumptive diagnosis of toxoplasmic

TABLE 16-6 -- Clinical presentation of infectious lymphadenitis.

CLINICAL PRESENTATION OF INFECTIOUS LYMPHADENITIS					
Disease		**Infecting organism**	**Lymphadenopathy**		**Systemic manifestations**
			Regional	**Generalized**	
Bacterial	Pyogenic	Group A streptococci	Yes	No	Prominent
		Staphylococcus aureus			
	Cat-scratch disease	*Bartonella henselae*	Yes	No	Occasional, mild
	Scrofula	*Mycobacterium tuberculosis*, nontuberculous mycobacteria	Yes	No	No, unless AIDS is present
	Miliary tuberculosis	*Mycobacterium tuberculosis*	No	Yes	Prominent
	Syphilis	*Treponema pallidum*	Yes	Yes	Variable
	Plague	*Yersinia pestis*	Yes	No	Prominent
	Tularemia	*Francisella tularensis*	Yes	No	Common, mild to moderate
	Chancroid	*Haemophilus ducreyi*	Yes	No	No
	Leptospirosis	Leptospira	No	Yes	Prominent
Viral	Infectious mononucleosis	Epstein-Barr virus, cytomegalovirus, HIV	Yes	Yes	Common, mild to moderate
	Rubella	Rubella virus	Yes	Yes	Common, mild
	Genital herpes (primary infection)	Herpes simplex virus 2	Yes	No	Common, mild to moderate
	Persistent generalized lymphadenopathy	HIV	Yes	Yes	Variable
	Pharyngoconjunctival fever	Adenovirus 3, 7	Yes	No	Common, mild
	Epidemic keratoconjunctivitis	Adenovirus 8, 19, 37	Yes	No	Occasional, mild
Chlamydial	Lymphogranuloma venereum	*Chlamydia trachomatis*	Yes	No	Common, moderate
Rickettsial	Spotted fever	*Rickettsia rickettsii*, *R. conori*	No	Yes	Prominent
Fungal	Histoplasmosis	*Histoplasma capsulatum*	Yes	Yes	Uncommon
	Coccidioidomycosis	*Coccidioides immitis*	Yes	No	Uncommon
Protozoan	Toxoplasmosis	*Toxoplasma gondii*	Yes	No	Uncommon
	Chagas' disease	*Trypanosoma cruzi*	Yes	Yes	Common, mild to moderate
	African trypanosomiasis	*Trypanosoma brucei*	Yes	No	Common, mild to moderate
	Leishmaniasis	*Leishmania* spp.	Yes	No	Variable
Helminthic	Filariasis	*Brugia malayi*	Yes	No	Common
		Wuchereria bancrofti	Yes	No	No
	Onchocerciasis	*Onchocerca vulvulus*	Yes	No	No

lymphadenitis should be questioned if adenopathy persists longer than 6 months, and lymph node biopsy or aspiration should be performed. Fine-needle aspiration is suitable for diagnosing infection or metastatic disease but a lymph node biopsy is required for the diagnosis of lymphoma. Analysis of biopsied lymph node yields a diagnosis in 50–60% of patients. Among patients in whom a diagnosis is not established by biopsy, 25% will develop a definable disease, mostly lymphoma, within a year. Therefore, close follow-up and repeated biopsies should be performed in patients who have a nondiagnostic initial biopsy.

Age

Lymphadenopathy is common in the pediatric age group and represents a benign process in approximately 80% of cases. Therefore, in a child who has lymphadenopathy and other features suggesting a possible bacterial infection, a trial of antibiotic may be appropriate. In adults, especially in patients older than 50 years, any finding of lymphadenopathy requires a thorough evaluation and consideration of a lymph node biopsy.

Physical characteristics of the enlarged lymph node

The size, consistency and relation to underlying tissue are important clues to the etiology of the enlarged nodes. Lymph nodes involved by an infective process tend to be large, soft and tender. Signs of local inflammation may be present, and draining sinuses are commonly seen in mycobacterial lymphadenitis. This presentation may be mistaken for orocervicofacial actinomycosis, which seldom spreads to the lymphatic vessels to cause lymphadenopathy. Nodes involved in lymphomas are rubbery, matted together and nontender. Metastatic lymph nodes due to carcinomas are usually firm, non-tender and fixed to the underlying tissues.

Location

Specific locations of enlarged lymph nodes are associated with specific etiologies (Table 16.7). Intra-abdominal or intrathoracic lymphadenopathy is not accessible to palpation, and imaging should be undertaken whenever it is suspected. Suggested investigations for regional lymphadenopathy are discussed below according to site.

TABLE 16-7 -- Correlation between the site of lymphadenopathy and disease

CORRELATION BETWEEN THE SITE OF LYMPHADENOPATHY AND DISEASE	
Enlarged lymph node	**Associated disease or condition**
Occipital	Scalp infections, insect bites, head lice
Posterior auricular	Rubella, ear piercing, HIV
Anterior auricular	Eye or conjunctival infections
Posterior cervical	Toxoplasmosis
Submental	Dental infections
Anterior cervical or submandibular	Oral cavity infections, Epstein-Barr virus, HIV, tuberculosis
Supraclavicular	Neoplasia
Mediastinal	Sarcoidosis, tuberculosis, histoplasmosis, neoplasia (Hodgkin's or non-Hodgkin's lymphoma, metastases), anthrax

Axillary	Cat-scratch disease, pyogenic infection of the upper arm, neoplasia (breast, lymphoma)
Epitrochlear	Viral diseases, cat-scratch disease, tularemia, hand infection, secondary syphilis
Abdominal/retroperitoneal	Tuberculosis, neoplasia, yersiniosis
Inguinal	Genital herpes, syphilis, lymphogranuloma venereum, filariasis, neoplasia

Head and neck lymphadenopathy

Any lymph node enlargement in the head and neck region should stimulate a thorough search for an infection in the oropharyngeal and nasal cavities. Symmetric lymph node enlargement usually suggests self-limited viral disease, and unilateral node enlargement may be viral in origin but evokes a broader differential diagnosis, including cat-scratch disease, toxoplasmosis and neoplasia. Unilateral enlarged tender lymph nodes usually indicate an acute infection but may be due to a rapidly proliferating process. If asymptomatic cer-vical node enlargement persists and serologic testing for toxoplasmosis is negative, a lymph node biopsy or aspiration should be performed. An abnormal chest radiograph in children and young adults who have cervical lymphadenopathy has been found strongly associated with malignant neoplasm, mostly lymphoma. Furthermore, 80% of patients who have cervical lymphadenopathy, abnormal chest radiograph and nondiagnostic lymph node biopsy are subsequently found to have malignant or granulomatous disease. We recommend a lymph node biopsy, and not aspiration, as the diagnostic procedure of choice when asymptomatic cervical lymphadenopathy persists in the presence of an abnormal chest radiograph. Supraclavicular lymphadenopathy without any other enlarged nodes, particularly in adults, is often of neoplastic origin and should therefore be biopsied.

In children and adolescents other causes of lumps in the neck, such as epidermoid thyroglossal or branchial cysts and parotid gland enlargement, should be differentiated from cervical lymphadenopathy.

Axillary lymphadenopathy

Infectious causes of unilateral axillary lymphadenopathy include local infectious processes of the arm and hand, hidradenitis suppurativa, streptococcal and staphylococcal lymphadenitis, cat-scratch disease, toxoplasmosis and tularemia. Conventional bacteria are easily differentiated from cat-scratch disease and toxoplasmosis on clinical grounds and should be treated accordingly. Asymptomatic unilateral axillary lymphadenopathy should be evaluated for neoplasia in both adults and adolescents. In the younger age group, however, a serologic test for the presence of *Toxoplasma* antibodies should precede the biopsy. Bilateral axillary lymphadenopathy can by caused by a variety of viral and bacterial infections as well as by neoplasms and immunologic disorders.

Thoracic lymphadenopathy

The principal differential diagnosis of intrathoracic lymphadenopathy includes neoplasia, tuberculosis, anthrax sarcoidosis and endemic mycoses. Unilateral or bilateral mediastinal lymphadenopathy in children without additional findings is the characteristic feature of primary tuberculosis. However, malignant lymphoma in the pediatric age group may also manifest in this way. Therefore, if a tuberculin skin test is found to be strongly reactive in such circumstances, antituberculous therapy should be started. However, if the tuberculin skin test is nondiagnostic or nonreactive and remains so on retesting 10–14 days later, an invasive diagnostic procedure is indicated.

Mediastinal lymphadenopathy in adults who have no accompanying complaints or findings is usually indicative of a neoplasm requiring an invasive diagnostic procedure. An exception to this is the patient who has HIV infection or one who arrives from an endemic area, in whom tuberculosis may be as frequent as a neoplasia. In the appropriate epidemiologic settings, endemic mycosis is also a diagnostic possibility requiring specific tests.

These considerations also apply to patients who have unilateral hilar lymphadenopathy. Bilateral hilar lymphadenopathy in the asymptomatic young patient is commonly caused by sarcoidosis. In patients who have parenchymal infiltration on radiography in addition to intrathoracic lymphadenopathy, bronchoscopy should be performed if sputum smear for *M. tuberculosis* is negative, regardless of age.

In the future, it is possible that new laboratory procedures such as PCR may reduce the need for invasive procedures. In patients who have no lung parenchymal involvement the diagnostic yield of sputum examination is low. Bronchoscopy increases the diagnostic yield of tuberculosis to 50–75%, especially if mucosal biopsy specimens are taken from ulcerating granuloma seen during the procedure.[54] Video-assisted thoracic surgery and mediastinotomy increase the diagnosis to 100% of patients, whereas mediastinoscopy supports the diagnosis of tuberculosis in 85–90%. [54]

Abdominal lymphadenopathy

Abdominal lymphadenopathy may be detected as an isolated finding during the investigation of abdominal complaints. In such cases, etiology of lymph node enlargement lies in the abdominal organs that drain into the enlarged nodes. The appropriate diagnostic approach should be directed at investigating and, if appropriate, biopsying a particular organ. Exceptions to this recommendation are intraabdominal lymphoma and abdominal tuberculosis (mesenteric lymphadenitis), in which the abdominal lymph nodes may be the primary site involved. In cases with abdominal lymphadenopathy, a careful search for peripheral lymph node enlargement is mandatory.

Inguinal lymphadenopathy

The main causes of inguinal lymphadenopathy include infectious diseases (STDs and others) and neoplasm (lymphoma or metastatic carcinoma). If an STD is suspected or a genital ulcer is detected, serologic tests (e.g. Venereal Disease Research Laboratory, complement fixation test, microimmunofluorescence test for *Chlamydia* spp.), bacteriologic investigations (dark field, immediate culture) and virologic investigations (herpesvirus culture) should be performed. According to epidemiologic considerations, the presence of one STD could indicate the possibility of another STD. Therefore, it is recommended that multiple infectious etiologies such as HIV and hepatitis B virus be ruled out.

PRACTICAL SUMMARY

¦ The size of an enlarged peripheral lymph node is often not helpful in determining the cause of lymphadenopathy or the need for biopsy.
¦ Empiric antibiotic treatment for suspected supperative lymphadenitis is indicated and should be primarily directed toward *Streptococcus pyogenes* and *Staphylococcus aureus*.
¦ In immunosuppressed patients (e.g. patients who have acute leukemia) or in diabetic patients who have foot ulcers and inguinal lymphadenitis, aspiration of pus from the involved node should be performed and therapy modified accordingly.
¦ Do not attempt drainage of a scrofula; this may cause fistula formation. Total excision is the treatment of choice in such cases. If in doubt, excision is the preferred approach.
¦ Consult a hematologist before processing an excised lymph node. It is essential that the biopsy material be handled properly to allow possible ancillary tests, such as immunoperoxidase staining, cytogenetic analysis and molecular clonality studies.
¦ Aspirated or excised material should be of sufficient amount to ensure recovery of suspected organisms.
¦ Specimens for bacterial and fungal diagnosis should be processed for Gram stain, acid-fast stain, Giemsa or Wright stain and methenamine silver stain.
¦ Specimens for parasitic diseases should be fixed in 100% alcohol and not in formalin.
¦ Specimens for viral cultures should be transported in appropriate transport media.
¦ For PCR assays, consult the local laboratory.
¦ The patient who has acute HIV infection manifesting as mononucleosis-like syndrome is typically HIV-antibody-negative. In order to rule out the possibility of acute retroviral syndrome, a negative serum p24 antigen or HIV PCR are required.
¦ In suspected tropical lymphadenitis or lymphadenopathy, appropriate serologic tests are mandatory and tissue specimens should be processed accordingly.
¦ Lymphadenopathy caused by dual infections or by an infectious process and a noninfectious one has been described; if, for example, lymphadenopathy attributed to toxoplasmosis on the basis of serologic investigations persists for more than 6–8 months, lymph node biopsy should be performed to exclude other diagnoses.

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Chapter 17 - Practice Point

Management of the solitary enlarged lymph node

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Pathogenesis

Enlargement of a lymph node may result from:

- infiltration by metastatic malignancy;
- acute inflammation secondary to infectious agents being filtered from afferent lymphatics or blood; or
- proliferation of lymphocytes and other mononuclear cells in response to antigenic stimuli or due to a primary lymphoproliferative disorder.

In certain infections there is nonspecific abscess formation and suppuration, whereas in others there is a more distinctive histologic

TABLE 17-1 -- Infectious causes of a solitary enlarged lymph node.

INFECTIOUS CAUSES OF A SOLITARY LYMPH NODE			
Infecting agent		**Site**	**Comments**
Viruses	Herpes simplex virus, type 2	Inguinal	Not always associated with mucocutaneous lesions
	Adenoviruses	Cervical or preauricular	Associated with pharyngitis or conjunctivitis
Bacteria	Group A streptococci	Cervical	Tender, may suppurate
	Staphylococcus aureus	Regional	Search for skin focus
	Mycobacterium tuberculosis	Regional (particularly cervical)	Particularly in certain ethnic groups and in people who have HIV infection
	Atypical mycobacteria	Cervical	Scrofula; particularly in children
	Treponema pallidum (syphilis)	Inguinal	In primary disease; painless lymphadenitis; search for chancre
	Haemophilus ducreyi (chancroid)	Inguinal	Painful papules and suppurative nodes
	Yersinia pestis (plague)	Inguinal or lemoral	Extremely tender node and acute systemic illness; exposure to rodents or fleas
	Francisella tularensis (tularemia)	Regional	Painful ulcer at site of inoculation; tender node; exposure to rodents
	Bartonella henselae (cat-scratch disease)	Regional (usually axillary, epitrochlear or cervical)	History of cat scratch or bite; skin lesion may have healed; not usually systemically ill; node moderately tender
Rickettsia/chlamydia	*Rickettsia tsutsugamushi* (scrub typhus)	Regional	Travel to Asia or Australasia; history of mite bite with eschar at site
	Rickettsia akari (rickettsialpox)	Regional	Travel to South Africa, Korea or North America; papule at site of mite bite and generalized vesicular rash
	Rickettsia aricae		
	Chlamydia trachomatis (lymphogranuloma venereum)	Inguinal	Fixed, tender, matted nodes above and below inguinal ligament (groove sign); foci of suppuration and fistulas
Fungi	*Histoplasma capsulatum* var. duboisii	Regional	West Africa; cutaneous lesion
	Paracoccidioides brasiliensis (paracoccidioidomycosis)	Cervical	Central and South America: usually chronic mucocutaneous lesions present
Protozoa	*Toxoplasma gondii*	Cervical	Mononucleosis-like illness; often generalized lymphadenopathy
	Trypanosoma brucei (African trypanosomiasis)	Cervical	African travel or residence; often generalized lymphadenopathy

appearance. Caseation necrosis suggests mycobacterial (or occasionally fungal) infection; a granulomatous reaction with or without stellate necrosis is typical of sarcoidosis, cat-scratch disease, tularemia, lymphogranuloma venereum and Kikuchi's disease; and follicular hyperplasia with epithelioid histiocytes is seen in toxoplasmosis. In other cases it is usually not possible to determine the infecting organism from the histologic appearances.

Microbiology

Many infections can result in a solitary enlarged lymph node, but most (particularly viral and protozoal infections) are much more

TABLE 17-2 -- Noninfectious causes of a solitary enlarged lymph node.

NONINFECTIOUS CAUSES OF A SOLITARY ENLARGED LYMPH NODE	
Immunologic	Rheumatoid arthritis (Felty's syndrome)
	Systemic lupus erythematosus
Malignant	Leukemia
	Lymphoma
	Metastatic malignancy

Miscellaneous	Sarcoidosis
	Drugs (carbamazepine, phenytoin)
	Castleman's disease (possibly related to human herpesvirus-8)
	Kikuchi's disease (histiocytic necrotizing lymphadenitis; possibly an infectious etiology)
	Histiocytosis X
	Kaposi's sarcoma

likely to cause a generalized lymphadenitis. The organisms listed in Table 17.1 are those that need particular consideration. Causes of lymphadenopathy other than infection are listed in Table 17.2 .

Clinical features

A patient may present with an enlarged lymph node or it may be a chance finding. Palpable lymph nodes do not always have a pathologic cause; a submandibular or cervical node less than 1cm in diameter or an inguinal node less than 2cm in diameter in an adult may be considered to be normal. Solitary nodes in other areas are more likely to have a pathologic cause. Evaluation requires an assessment of the duration and progression of the enlargement and any associated systemic or local (related to the area drained by the enlarged lymph node) symptoms or signs. The patient's occupation, exposure to animals, sexual behavior and travel history will indicate whether diseases listed in Table 17.1 and Table 17.2 can be excluded or should be considered more seriously.

Examination of the mass should first focus on ensuring that it is a lymph node (Table 17.3) and an enlarged node should be assessed for its size, texture, tenderness and any discharge. An acutely enlarged, very tender (perhaps fluctuant), cervical, submandibular, axillary or epitrochlear node with overlying skin erythema and fever, particularly in a child, suggests a streptococcal or staphylococcal etiology. Suppurative iliac lymphadenitis in a child is also usually caused by *Staphylococcus aureus*, but in an adult several sexually transmitted diseases, particularly lymphogranuloma venereum, also need to be considered. In an appropriate geographic location and with animal

TABLE 17-3 -- Masses often confused with enlarged lymph nodes.

MASSES OFTEN CONFUSED WITH ENLARGED LYMPH NODES		
Name of mass	**Usual site**	**Comment**
Branchial cyst	Over the midpoint of the sternomastoid muscle	Contains sterile pus and many cholesterol crystals
Dermoid cyst	In the midline at any line of embryologic fusion	Fluctuant mass containing cheesy epithelial debris
Thyroid nodule	In region of the thyroid gland	Moves on swallowing
Thyroglossal cyst	In midline of throat	Moves on protruding the tongue
Onchocercal nodule	Associated with bony prominence such as the iliac crest or occiput	Travel or residence in Africa or Latin America; may be associated skin changes or visual problems
Femoral hernia	Below inguinal ligament	Cough impulse; reducible

exposure, plague and tularemia should be considered in an acutely ill person who has inguinal buboes.

In the absence of fever and tenderness, an indolent suppurative painless lymphadenitis, particularly in the cervical or submandibular region, suggests mycobacterial infection. In children this is is often caused by atypical mycobacteria such as *Mycobacterium scrofulaceum* or *M. avium-intracellulare*. Peripheral tuberculous lymphadenitis occurs particularly in young adult women immigrants from areas where tuberculosis is endemic. Similar slowly progressive regional lymphadenitis is a feature of cat-scratch disease, which is caused by *Bartonella henselae*, and most patients who have this infection have been scratched or bitten by a cat. Hard immobile nodes are suggestive of malignancy and rubbery painless nodes may indicate a lymphoproliferative process. The area drained by any enlarged node should be examined for signs of skin inflammation or malignancy. Careful genital examination for mucosal ulcers and urethral discharge are indicated if the node is in the inguinal region.

For people who have HIV infection, especially those who originate or who have spent time outside Europe and North America, there should be a high index of suspicion for tuberculosis. Kaposi's sarcoma should also be considered, even in the absence of cutaneous lesions.

Investigations

Investigations should be tailored to the clinical findings. Basic tests include a full blood count and blood film and an antibody test for mononucleosis. Swabs should be taken from the throat (for cervical nodes), and any ulcerative lesions or sinus tracts. A fluctuant node can be aspirated and the pus stained with Gram and Ziehl-Neelsen stains and cultured for bacteria, including mycobacteria, and fungi. Any material from a submandibular swelling can also be sent for cytology because this may be helpful in differentiating a submandibular node from a branchial cyst containing cholesterol crystals. If the patient is febrile blood cultures should be obtained. A chest radiograph is useful if the node is supraclavicular because it may show a pulmonary infiltrate, mediastinal or other masses, or hilar adenopathy due to sarcoidosis. Patients who have an enlarged cervical node without a visible cause should have a thorough ear, nose and throat examination and any suspicious mucosal lesions should be biopsied. For an inguinal node in an adult a full sexually transmitted infection (STI) screen should be offered, including counseling for HIV testing if any risk factors are identified. It should be remembered that STIs can be asymptomatic.

Serologic tests can be helpful in supporting a diagnosis of streptococcal, viral or rickettsial infection, toxoplasmosis and cat-scratch disease. Immunologic tests such as antinuclear antibody, double-stranded DNA and rheumatoid factor may be indicated if systemic lupus erythematosus or rheumatoid arthritis are thought likely. A tuberculin test (Mantoux or Heaf) can suggest tuberculosis as a cause, but needs to be interpreted carefully, particularly for patients who have lived in Asia and other countries where the disease is endemic and previous infection is likely, and in those who have had previous bacille Calmette-Guérin (BCG) immunization. It is often falsely negative in patients who have HIV infection.

Other investigations such as computed tomography should be guided by clinical suspicion. A single stony-hard supraclavicular node suggests an intra-abdominal malignancy.

Management

If a cause for the enlarged node is found on examination or after initial investigations (including microscopy) of any purulent material obtained after aspiration, the management is usually straightforward and directed at the underlying disease. An acutely ill patient who has

suppurative lymphadenitis or an enlarged node accompanied by lymphangitis can be treated with a penicillinase-resistant penicillin such as oxacillin or flucloxacillin. Cervical nodes secondary to presumed pharyngotonsillar infection can be treated with penicillin, which may need to be given intravenously at first until the patient is able to swallow. More chronic suppurative lymphadenitis might be treated empirically as tuberculosis, an atypical mycobacterial infection or cat-scratch disease, depending upon the epidemiologic features. A single lymph node infected by atypical mycobacteria is usually treated by surgical excision alone.

If no specific diagnosis is suggested by the history and initial investigation a decision has to be made about performing an excision biopsy. If a node is less than 1cm in diameter and there are no suspicious features (hard, fixed, painless node or supraclavicular location) in an otherwise well patient under 40 years of age it can usually be observed on a regular basis every few weeks and in many cases will regress or the disease process will manifest itself. Biopsy is indicated for:

- nodes that fail to regress or have enlarged despite empiric antibiotic therapy directed at the disease thought to be the most likely cause;
- a node over 2cm in diameter; and
- a progressively enlarging hard node or one with associated local symptoms or signs (e.g hoarseness, nasal obstruction or mucocutaneous ulceration and

induration).

It needs to be stressed, however, that if an enlarged node is thought to due to metastastic malignancy the patient needs a thorough evaluation for the primary before any lymph node biopsy.

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Chapter 18 - Conjunctivitis, Keratitis and Infections of Periorbital Structures

Luke Herbert

CONJUNCTIVITIS

Conjunctivitis is inflammation of the conjunctiva. It is not a diagnosis but a description of a clinical syndrome. There may be redness, dilated blood vessels, follicles, papillae and a watery-to-purulent discharge. Follicles are germinal centers of conjunctival lymphoid tissue and are predominant in viral conjunctivitis. They are small (typically <1mm) pale bumps under the tarsal conjunctiva and sometimes the limbal conjunctiva. Small papillae are non-specific and give the tarsal conjunctiva a velvety appearance. Giant papillae are a sign of chronic inflammation, give the upper tarsal conjunctiva a cobblestone appearance and may lead to reticular scarring on resolution.

Decreased vision or photophobia are associated with keratitis or uveitis, which can accompany conjunctivitis.

NONINFECTIVE CONJUNCTIVITIS

Endogenous causes of noninfectious conjunctivitis include dry eye and acute and chronic inflammatory conditions of the conjunctiva such as Stevens-Johnson syndrome or mucous membrane pemphigoid. Exogenous causes include pollution and medication (conjunctivitis medicamentosa). Rarely conjunctival or eyelid tumors can cause conjunctivitis (masquerade syndrome). Molluscum contagiosum lesions near the eye can shed viral particles into the eye and cause a reactive follicular conjunctivitis, without evidence of conjunctival infection. This is more common in children than in adults. Large crops of periocular molluscum lesion can be seen in patients with AIDS.

The distinction between noninfective and infectious causes is usually clear from the history. Except for chlamydial conjunctivitis, infectious conjunctivitis rarely lasts longer than 3 weeks, beginning to resolve after 10 days. Conjunctivitis medicamentosa can be a difficult condition to diagnose and may follow an infectious conjunctivitis. Clues include a history of initial improvement on starting a new type of eye-drop followed by worsening and complaints that the eye-drops sting. Thorough history taking is necessary because patients may not recall all the different eye-drops that they have been using during an episode of conjunctivitis. Withdrawal of all topical medication and reassessment 2 or 3 days later is helpful.

In allergic conjunctivitis there is often a history of atopy and chronic mucus discharge, and giant papillae are seen.

INFECTIOUS CONJUNCTIVITIS

Bacterial conjunctivitis

Epidemiology

Bacterial conjunctivitis is ubiquitous and is more common in warmer months and regions. It is transmitted by contact with ocular or upper respiratory tract discharges of people who have the infection, fomites, medical equipment or shared cosmetics. In some areas insect vectors are involved.

Clinical features

Tearing, irritation and sticky discharge without preauricular lymphadenopathy are early clinical features. The conjunctiva is pink (not red). The lids may be stuck together by a mucopurulent exudate on awakening.

Diagnosis

Microbiologic investigation is necessary only in neonatal, hyperacute, severe, unusual or chronic cases. Microscopy of conjunctival smears can be useful, and conjunctival swabs on blood agar and (especially in children) chocolate agar are used. *Haemophilus influenzae* biogroup *aegyptius* and *Streptococcus pneumoniae* are common causes. *Haemophilus influenzae* type b and *Moraxella* and *Branhamella* spp., *Neisseria meningitidis* and *Corynebacterium diphtheriae* are also involved. In infants the main organisms are *H. influenzae* biogroup *aegyptius*, *Neisseria gonorrhoeae*, *S. pneumoniae*, viridans streptococci, enterococci and rarely *Pseudomonas aeruginosa*.[1] [2]

Management

Topical broad-spectrum antibiotic drops are used 2-hourly until the symptoms subside, which should occur rapidly. In Europe, chloramphenicol is used; in the USA, neomycin, polymyxin and bacitracin are used. Gentamicin and tobramycin are useful against Gramnegative organisms, but cause a higher rate of local toxic reactions. Erythromycin or the quinolones may also be used.

Hyperacute conjunctivitis

Hyperacute conjunctivitis is characterized by copious discharge, chemosis and lid swelling. Membranes may form on the tarsal conjunctiva and there may be a tender preauricular lymphadenopathy. Corneal involvement is common and severe, and corneal perforation can occur within 24 hours. It is caused by *N. gonorrhoeae* and *N. meningitidis*.

Management

The drug of choice is an extended-spectrum cephalosporin such as ceftriaxone 1g (25–40mg/kg) every 12 hours for 3 days. Repeated irrigation of the conjunctival sac is recommended to reduce inflammatory microbial mediators to the cornea.

Brazilian purpuric fever

This is caused by a rare invasive clone of *H. influenzae* biogroup *aegyptius*. Systemic disease occurs in children 1–3 weeks after an episode of conjunctivitis. It is seen over a widespread area in Brazil and there is a case mortality of 70%. The clinical picture is similar to that of meningococcal sepsis.

Management

The Brazilian purpuric fever clone of *H. influenzae* biogroup *aegyptius* is resistant to trimethoprim but is sensitive to chloramphenicol and ampicillin. During epidemics systemic rifampin (rifampicin) (20mg/kg/day for 2 days) may be used as prophylaxis against Brazilian purpuric fever for children who have conjunctivitis.

Ophthalmia neonatorum

Ophthalmia neonatorum is any conjunctivitis in the first 3 weeks of life. The most serious cause is gonococcal conjunctivitis, which can be life-threatening. More common causes are *Chlamydia trachomatis*, chemical irritation and herpes simplex virus.

Epidemiology

Ophthalmia neonatorum occurs worldwide, but is uncommon where there is adequate infant eye prophylaxis. Globally gonococcal ophthalmia neonatorum is an important cause of blindness. The risk of ophthalmia neonatorum for a child born to an infected mother is 30–50% for gonorrhea[3] and 15–35% for chlamydial infection.[4] Transmission usually occurs in the birth canal.

Prevention

Topical prophylaxis was described in 1881 by Credé. His silver nitrate eye drops substantially reduced the incidence of gonococcal conjunctivitis; however, they are inactive against chlamydia, and toxicity is common. Recently, 2.5% aqueous povidone-iodine has been shown to be safer, cheaper and more effective. [5] [6] Maternal treatment before birth is the best prevention.

Clinical features

Gonococcal conjunctivitis presents 24–48 hours after birth and earlier after premature rupture of membranes. Lid edema, chemosis and a discharge, which is serosanguineous and rapidly becomes purulent, progress to corneal ulceration. Perforation can occur soon afterward.

Chlamydial conjunctivitis presents 5–12 days after birth with a watery discharge, which becomes purulent more slowly. Follicles are absent because the conjunctival lymphoid tissue in infants is immature. Untreated the condition usually resolves in 3–4 weeks, but can take 1 year. Rarely membranes and micropannus form, resulting in significant stromal scarring in later life. Pneumonitis, rhinitis, vaginitis and otitis can follow the conjunctivitis.

Diagnosis

Cultures on blood and chocolate agar for gonococci, and viral culture and immunofluorescent testing, enzyme immunoassay or DNA probing for chlamydial antigen should be performed.

Management

Neonates who have a purulent discharge are presumed to have gonococcal infection and are treated with ceftriaxone 25–50mg/kg intramuscularly or intravenously to a maximum of 125mg. Frequent irrigation removes bacteria and microbial products. A single intramuscular dose of 125mg of ceftriaxone is effective therapy for gonococcal ophthalmia neonatorum.[7] Parents should be screened for sexually transmitted diseases.

Treatment of chlamydial ophthalmia neonatorum comprises 2 weeks of erythromycin 10mg/kg q12h for the first week and q8h for the second week of life.

Chlamydial conjunctivitis: trachoma

Chlamydial infection results in three distinct clinical pictures. Trachoma is a scarring condition of the conjunctiva and cornea in adults caused by recurrent childhood infection with *C. trachomatis*. Adult inclusion conjunctivitis is an acute condition associated with sexually transmitted chlamydial infection. Neonatal chlamydial conjunctivitis is described above.

Epidemiology

Trachoma occurs worldwide and is endemic in poorer rural areas of developing countries. Blindness due to trachoma is still common in parts of the Middle East, northern and sub-Saharan Africa, parts of the Indian subcontinent, South East Asia and China. There are pockets of infection in South America, among Australian Aborigines, in the Pacific islands and in native American reservations in southwest USA.

Prevention

The infection is transmitted by contact with ocular or nasopharyngeal secretions, either directly through fomites or insect vectors (the flies *Musca sorbens* in Africa and the Middle East and *Hippelates* spp. in the Americas). Untreated active lesions can be infectious for years. Prevention of transmission is the most important public health measure.[8] Education about personal hygiene and regular washing of the face is very important, and these hygiene measures require only a tiny amount of water. There has been mass treatment of the population with topical tetracycline or erythromycin in hyperendemic areas.

Clinical features

The acute conjunctivitis is characterized by a diffuse follicular reaction in the conjunctiva of the superior tarsal plate and at the limbus with soft follicles. Papillary hypertrophy is a non-specific sign. With resolution of the follicles there is subconjunctival scarring and a loss of conjunctival mucin-producing goblet cells. Blinding complications result from chronic re-infection,[9] severe dry eyes and entropion leading to corneal scarring, bacterial superinfection and ulceration.

Diagnosis

The diagnosis is made on clinical grounds in endemic areas.
Expressed follicles have a characteristic microscopic appearance involving macrophages (Leber's cells), plasma cells and lymphoblasts.

Chlamydia trachomatis types A–C are involved. Secondary bacterial superinfection is common and causes severe disease. Antibodies to *C. trachomatis* can be demonstrated in the tears and serum.

Management

Prevention of the chronic sequelae by treatment in childhood is a public health priority. Once scarring has occurred management is with lubricants and surgery.

Chlamydial conjunctivitis: adult inclusion conjunctivitis

Clinical features

The conjunctivitis presents with a mucopurulent discharge after a 4- to 12-day incubation period. It is often monocular and develops into a chronic follicular conjunctivitis, often with epithelial keratitis and limbal follicles. Tender preauricular lymphadenopathy is common. Untreated the disease has a chronic course and may progress to keratitis and possibly iritis.

Diagnosis

Enzyme-linked immunoassay or immunofluorescent monoclonal antibody stains of conjunctival scrapings are rapid and convenient ways to make the diagnosis. [10]

Management

Treatment is with 3 weeks of systemic doxycycline 100mg q12h or erythromycin stearate 500mg q6h. Topical treatment is relatively ineffective.

VIRAL CONJUNCTIVITIS

Epidemiology

Viral conjunctivitis is common and occurs worldwide. Epidemics frequently occur and may be propagated by eye clinics. Transmission is

by direct or indirect contact with ocular or upper respiratory tract secretions. Airborne transmission occurs. Viral particles can remain infectious on surfaces for more than 1 month.

The incubation period is from 2 days to 2 weeks, and infected people remain contagious for up to 2 weeks after the symptoms begin.

Prevention

Control of epidemics associated with eye clinics involves setting up a triage system for those suspected of viral conjunctivitis.[11]

These patients are seen in a separate room with dedicated personnel. I strongly recommend the use of gloves and noncontact examination techniques. Staff infected with viral conjunctivitis should be furloughed until 2 weeks after the start of symptoms, by which time they are considered to be no longer contagious.

Clinical features

The main patterns of viral conjunctivitis are:

- epidemic keratoconjunctivitis (EKC),
- pharyngoconjunctival fever (PCF), and
- acute hemorrhagic conjunctivitis (AHC).

The symptoms and signs of these patterns of infection are similar, but vary in degree. All of these patterns present as an acute follicular conjunctivitis, with watering, grittiness, redness, ecchymosis and lid edema, often with flu-like symptoms. Preauricular lymphadenopathy and bilateral involvement are common.

In EKC most patients have some focal epitheliopathy and photophobia by 2 weeks. Subepithelial opacities appear after 2 weeks in 50% of patients and occasionally impair vision. These lesions cause occasional recurrences of grittiness. The natural history is of slow resolution, which can take more than 1 year.

In PCF there is a similar ophthalmic picture, but the systemic features are more pronounced.

In AHC ecchymosis is more prominent and the onset of symptoms more rapid. Rarely a polio-like radiculopathy follows AHC.

Diagnosis

Several methods are available for typing the viruses that cause acute conjunctivitis; most of these methods are time-consuming and expensive. More recently, polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis have allowed faster diagnosis. Immunochromatography promises almost instant results.[12] These tests will be useful for epidemiology.

Most epidemics of viral conjunctivitis are caused by adenoviruses, commonly serotypes 8, 19 and 37 for EKC, and types 3 and 8 for PCF. Picornaviruses such as enterovirus type 70 and Coxsackie virus type A24 cause AHC. Conjunctivitis can be a feature of many other viral diseases, including influenza, rubella, rubeola, chickenpox and glandular fever.

Management

The management of viral conjunctivitis is supportive, with warm or cold compresses reducing symptoms of itch, plus lubrication and cycloplegics if there is an element of keratitis (see below). Antibacterial prophylaxis is probably unnecessary and can cause an allergic or toxic conjunctivitis.

KERATITIS

Keratitis is an inflammation of the cornea. Microbial keratitis is suppurative inflammation of the cornea produced by a replicating micro-organism. It carries a high risk of visual loss and can be caused by bacteria, fungi, viruses or parasites. A large variety of bacteria and fungi can infect the cornea.[13]

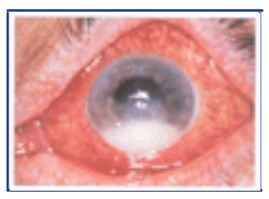

Figure 18-1 Typical microbial keratitis. Note accumulation of inflammatory cells at the dependent part of the anterior chamber of the eye (hypopyon) and mid-corneal defect. *Courtesy of Myron Yanoff.*

Typical acute microbial keratitis is the major syndrome. Patients present with a corneal epithelial defect and a stromal infiltrate (Fig. 18.1). The other main clinical problems are herpesvirus infections, with recurrent inflammation leading to scarring, and infection in a 'compromised' cornea (i.e. after local or systemic immunosuppression, injury or chronic disease). *Acanthamoeba* keratitis, which used to be rare, is a problem in contact lens wearers. Noninfectious keratitis is important in that it can be confused with infectious keratitis.

Algorithms for the initial division of keratitis into the main clinical situations are shown in Figure 18.2 .

INFECTIOUS KERATITIS

Typical acute microbial keratitis

Epidemiology

Microbial keratitis is responsible for 30% of cases of blindness in some developing countries. In hot climates fungal infection is more frequent, although bacterial keratitis still accounts for 60% of cases.[14]

The wearing of contact lenses increases the risk of developing bacterial keratitis.[15] The annual incidence is 5/10,000 for daily wear of soft lenses and 20/10,000 for overnight wear of soft lenses, although estimates 10-fold higher than this have been made. It has been estimated that over 65% of all new cases of keratitis in London, UK are due to the wearing of contact lenses.[16] The largest risk factor is the overnight wearing of soft lenses. This increases the risk 5-fold compared with the daytime wearing of lenses and 20-fold compared with the wearing of rigid lenses.[17]

Surgery, particularly corneal grafts and corneal sutures, and concomitant use of topical corticosteroids increase the risk of infection. Other than vitamin A deficiency, systemic predisposing conditions are relatively unimportant clinically. Vitamin A deficiency is particularly important in children who are malnourished and who have a concomitant infection, especially measles, in which keratomalacia (corneal melting) can occur suddenly.

Pathogenesis and pathology

Four principal groups of bacterial pathogens are responsible for most cases of infective keratitis. Micrococci (*Staphylococcus* and *Micrococcus* spp.), *Streptococcus* and *Pseudomonas* spp. and the Enterobacteriaceae (*Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia* and *Proteus* spp.) accounted for 87% of cases of bacterial keratitis in one series.[18]

Pseudomonas infection is recognized for its swift suppurative course to perforation secondary to proteolytic enzyme release. It is particularly common in contact lens wearers (hard and soft). *Serratia marcescens* corneal ulcers have been associated with the wearing of contact lenses and with contaminated eye drops. *Neisseria gonorrhoeae*, *C. diphtheriae* and *Haemophilus* and *Listeria* spp. are

Figure 18-2 Diagnosis of keratitis.

capable of penetrating an intact corneal epithelium. Other bacteria and fungi produce disease only after a loss of corneal epithelial integrity.

Following invasion by micro-organisms, several chemotactic substances are released, leading to an inflammatory cell infiltrate and consequent phagocytosis, cell death, release of proteolytic enzymes and corneal stromal damage.

Clinical features

The clinical appearance is not pathognomonic for any particular infecting organism, although some patterns are characteristic. Stromal ring infiltrates imply a massive immune response characteristic of infection by *Bacillus* and *Pseudomonas* spp. They are also seen in infections by *Streptococcus*, *Listeria*, *Proteus* and *Acanthamoeba* spp.

Staphylococcal infection can present early without an infiltrate but with pain, localized edema and a ring of keratic precipitates (inflammatory cells adherent to the endothelium). Developing ulcers caused by *Staphylococcus aureus* infection are round or oval and tend to progress in depth rather than area.

Diagnosis

Diagnosis is by microscopy and culture of a corneal scrape. In contact lens wearers the lenses and the lens case should be cultured.

In some parts of the world broad-spectrum therapy is used without initial investigation and it has been suggested that this practice could be extended. [19] If microbiologic services are not available there is little choice. However, the current high success rates of this treatment may not continue and in some areas antibiotic resistance is developing. Corneal scraping also debrides the ulcer, allows antibiotics to enter the base of the ulcer and provides microbiologic data (provided that antimicrobial therapy has not been given within the previous 24 hours).

The cornea is anesthetized with nonpreserved amethocaine 1%, five or six drops at 2-minute intervals. Using a slit-lamp, necrotic material is removed from the center of the ulcer. A 23 gauge needle tip is scraped parallel to the cornea to remove infected tissue. A fresh needle is used for each specimen. If a slit-lamp is not available scraping with a scalpel blade is safer than using a needle. The diagnostic yield is greater from the sides of the ulcer than from necrotic areas.

Slides should be made for Gram stain and Giesma stain, and appropriate bacterial cultures (and fungal cultures if suspected) should be prepared directly from the needle. Bending the tip of the needle before use facilitates this. The sharp side is used to scrape the cornea and the smooth surface used to plate out the specimen without penetrating the surface of the agar.

Management

Either gentamicin 1.5% with cefuroxime 5% or the fluoroquinolones ofloxacin 0.3% or ciprofloxacin 0.3% are effective against 90% of expected isolates in most clinical settings.[20] [21]

Aminoglycosides are toxic to the epithelium in moderate doses and quinolones are preferred as monotherapy. In cases in which streptococci are likely (e.g. chronic ocular surface disease, children) there may be quinolone resistance, and combination with cefuroxime 5% is recommended. There are increasing reports of quinolone resistance in some settings.[22]

Initial treatment is with drops every hour day and night for 2 days, followed by review.[23] If the clinical picture has improved or not worsened, treatment is then reduced to drops every hour by day for 3 days. After this time the ulcer should be sterile. Treatment is reduced to prophylactic levels q6h until the epithelium has healed. One trap for the inexperienced is that intensive treatment with ciprofloxacin 0.3% can cause a white ciprofloxacin precipitate to form in the corneal stroma adjacent to an epithelial defect. This may be confused with progression of the infection.

If the clinical picture progresses after 2 days of treatment the patient should be admitted to hospital for supervised treatment. If after the first week there is no response to therapy, consider stopping therapy and reculturing or switching to a different antibiotic guided by antibiotic sensitivity testing.

Fungal keratitis

Fungal infections are seen in two distinct clinical settings. In hotter climates the cornea is inoculated with infected vegetable matter as a result of agricultural or other trauma. Infection can then occur in otherwise healthy eyes. In temperate climates infection most often occurs in association with chronic ocular surface disease or prolonged corticosteroid use.

The septate filamentous fungal species, especially *Fusarium* and *Aspergillus* spp., are the most common cause of fungal keratitis. With increasing distance from the equator the relative incidence of candidal infection increases. Dematiaceous filamentous fungi such as *Curvularia* spp. are of low virulence and cause indolent infections.

Prevention

The main cause of fungal keratitis worldwide is trauma. In the developed world risk factors include protracted epithelial ulceration, therapeutic wearing of soft contact lenses, corneal transplant and topical corticosteroid therapy. Patients who have exposure keratitis and a history of previous herpes simplex or herpes zoster keratitis are at risk.

Clinical features

Fungal keratitis usually presents as a typical microbial keratitis. It is not possible to diagnose the type of infection on clinical grounds. Clinical context is the most important guide to appropriate investigation and therapy. In filamentary fungus keratitis the corneal surface is gray or dull. Satellite lesions are common in the surrounding stroma and may be seen with an intact epithelium (Fig. 18.3).

Figure 18-3 Fungal keratitis. The corneal surface looks rough, and there are several satellite lesions best seen here at the periphery on the left side of the cornea. *Courtesy of Myron Yanoff.*

Candidal lesions appear in corneas that are already abnormal. They are often quite localized at first with a collar button configuration.

Diagnosis

Diagnosis is by culture of corneal scrapes. In indolent cases corneal biopsy is indicated.

Management

Historic data are the best guide to selecting antifungal therapy. [24] The greatest experience in the use of antifungals for keratitis comes from India. However, this experience may not apply to situations where there is a local or systemic disorder of immunity.

No currently available antifungal agent has a favorable profile of activity and toxicity. Response to antifungal treatment is poor compared with that to antibacterial treatment. Surgical management (corneal transplant) may be necessary.

Combined topical and systemic therapy is often used and is usually prolonged. Corneal drug penetration is helped by daily scraping of the epithelium and necrotic stroma.

The choice of antifungal agent is often dictated by availability. Most topical antifungal agents must be made locally from tablets or intravenous preparations.

Among the polyenes, amphotericin B is fungicidal, but is toxic to the eye in high concentrations. It is used diluted in sterile water at 0.1–0.15% two to four times every hour for the first 1–2 days. Natamycin 5% has poor corneal penetration, but is useful for superficial filamentary fungal infections.

Among the pyrimidines, flucytosine 1% can be prepared from a commercially available intravenous or tablet form and is used in combination only (early resistance occurs) with amphotericin B for candidal keratitis. Concomitant systemic use may be helpful.

Among the imidazoles, miconazole 10mg/ml in polyethoxylated castor oil (intravenous preparation) was effective in more than 60% of cases in a prospective trial in India[25] when used topically every 2 hours. Ketoconazole 2% prepared as an aqueous solution from tablets is probably effective against *Aspergillus flavus*, but not *Aspergillus fumigatus* or *Fusarium* spp. Systemic and topical administration is often combined. Econazole 1% is used topically in combination with systemic itraconazole, and clotrimazole 1% vaginal cream has also been used. Fluconazole is given systemically or as a 0.2–0.5% topical aqueous solution. Itraconazole used orally has an some activity against filamentous fungi (see Chapter 208). It has poor tissue penetration, but a good clinical response when used against candidal infections. *Fusarium* spp. respond less well.

Silver sulfadiazine 1% is used for antibiotic prophylaxis in patients who have burns. It has moderate antifungal activity in vitro, but has been widely used in developing countries as a topical antifungal with good rates of success, particularly against *Fusarium* spp.

Chlorhexidine gluconate 0.2% solution is at least as effective as natamycin in treating fungal infections and is suggested as an inexpensive agent for use in the

developing world.[26]

Initial treatment for fungal keratitis depends upon local experience. In London, UK we use topical econazole 1% (or amphotericin B 0.15 or 0.3%) hourly for 48 hours, then 2 hourly for 72 hours and then reduce the dosage depending upon the initial response. Long-term treatment is required. Systemic treatment is used as an adjunct: itraconazole 200mg daily initially, reduced to 100mg for longer term use, or fluconazole 200mg daily for *Candida* infections. If surgical debridement is performed, a 2mm clear margin is advised. The inflammatory reaction results from live organisms and fungal debris. Because most antifungal agents are fungistatic, reduction of inflammation also depresses local immunity so that the organisms are not killed. Concomitant corticosteroid use is therefore not recommended for fungal keratitis.

Acanthamoeba keratitis

Epidemiology

Acanthamoeba histolytica was not recognized as a cause of keratitis until the 1970s, and the incidence has increased since. *Acanthamoeba histolytica* live in soil and water, including swimming pools, water storage tanks and contact lens cases (see Chapter 244).[27] Approximately 10–15% of cases are associated with agricultural and other trauma, but most are associated with contact lens use. The annual incidence in 1985–7 was estimated at 1.65–2.01/million contact lens wearers.[28] Poor lens hygiene by soft contact lens wearers and the use of homemade saline- or chlorine-based disinfection systems are risk factors. Disposable lens wearers have a particularly high risk, although this may be related to care systems used with the lenses.[29]

In the UK the use of tank-stored water by contact lens wearers to rinse lenses or lens cases may have introduced *A. histolytica* into their lens care systems.[30] The free-living protozoa have been seen grazing on bacterial slime on the bottom of contact lens cases.

Acanthamoeba histolytica can also cause scleritis and chorioretinitis.

Prevention

Good lens hygiene, the use of hydrogen peroxide 3% for at least 3 hours[31] and avoidance of tap water in contact lens care are important preventive measures.

Clinical features

In early epithelial disease there may be punctate keratopathy, pseudodendrites, epithelial wrinkling, diffuse or focal subepithelial infiltrates and radial perineural infiltrates. Ring infiltrates and corneal ulceration may present later.[32] [33] Pain out of proportion to the clinical signs is common, but not constant. The clinical picture may resemble herpetic or fungal keratitis, and a high index of suspicion is warranted in contact lens wearers who have apparent herpetic disease. Bacterial coinfection or superinfection in late disease occurs in around 10% of cases and causes an atypical presentation.

The clinical course of *A. histolytica* keratitis is prolonged. Early diagnosis is important, but treatment started before proper diagnostic tests are carried out only confuses the clinical picture. Unlike bacterial keratitis there is no justification for starting therapy without investigation. A delay of a 1–2 days while a patient is referred for expert opinion and investigation is less harmful than early inappropriate therapy. The grave prognosis of *A. histolytica* keratitis in the past was due to the slow inexorable progression of disease rather than fulminant corneal destruction.

Diagnosis

Diagnosis is made by microbiologic and histopathologic examination of tissue specimens. The epithelium is removed for examination by epithelial biopsy. In cases in which only the stroma appears to be involved corneal biopsy (described above) is necessary. If culture is negative, tear fluid PCR has been suggested as an adjunctive investigation.[34]

For microbiologic analysis, specimens[35] can be plated on nonnutrient agar, which is later overlain with *Escherichia coli* in the laboratory. Tracks of bacterial clearing show where the amebae have been grazing. Bacterial and fungal cultures should also be obtained.

Microscopy techniques vary. Fluorescent microscopy can be carried out using calcofluor white, which stains the walls of cysts, with acridine orange or with an immunofluorescent antibody. An immunoperoxidase test is used in some laboratories. Confocal microscopy of a wet preparation can be used to identify motile trophozoites, which have a large karyosome and a contractile vacuole.

Biopsy specimens should also be stained with hematoxylin and eosin, periodic acid-Schiff and methenamine silver to demonstrate *A. histolytica* cysts.

Sensitivity testing, although useful in screening for amebicides, is less useful in the management of individual cases,[36] [37] perhaps because *A. histolytica* encysts in infected tissues.

Management

The diamidines, propamidine isethionate 0.1% and hexamidine 0.1% and the cationic antiseptics, polyhexamethyl biguanide (PHMB) 0.02% and chlorhexidine 0.02% are probably the most effective medications,[33] [38] although availability can be a problem. Local manufacture of chlorhexidine or PHMB may be possible from 20% disinfectant preparations. The azoles (clotrimazole, fluconazole, ketoconazole and miconazole) have also been suggested for use as a third agent.

Initial treatment starts immediately after the epithelium is debrided using a cationic antiseptic and a diamidine, each hour day and night for 48 hours, then hourly by day for 3 days. Dosage is then reduced to 3-hourly to reduce local toxicity. If toxicity is suspected the frequency of the diamidine should be reduced.

It may take 2 weeks to achieve a response to treatment. Pain relief with systemic nonsteroidal anti-inflammatory agents and cycloplegia is very important.

The use of corticosteroids in controlling inflammation is controversial. Some authorities say that they are contraindicated at any stage. Others advocate the use of a weak topical corticosteroid to control inflammation after at least 2 weeks of antiamebic therapy, emphasizing the importance of continued antiamebic therapy until several weeks after the corticosteroid is stopped.

Viral keratitis: herpes simplex virus type 1 or 2

Epidemiology

Herpes simplex keratitis has an annual incidence of new cases of 8/10,000. The incidence peaks at ages 5–10 years and 35–40 years. Males are affected twice as often as females. Approximately 50% of patients have a history of herpes labialis. Primary ocular infection occurs in 5%.[39]

Corneal infection is usually with herpes simplex virus type 1 (in 98% of cases), except in neonatal herpes infection, when 80% of cases are due to herpes simplex virus type 2.

Pathogenesis and pathology

The pattern of dendritic ulcers is caused by direct infection of corneal epithelium. It is thought that delayed-type hypersensitivity to viral antigens causes the inflammation that leads to disciform keratitis.[40]

Clinical features

Primary ocular infection is usually asymptomatic, although a vesicular reaction may be seen. Occasionally there is a follicular conjunctivitis. Corneal damage is caused by recurrent disease.

Recurrence occurs in around 10% of cases at 1 year and 50% by 20 years. Triggers include fever, trauma and ultraviolet light. Recurrence is a consequence of latency.[41] Latency develops as virus spreads to a sensory ganglion and remains dormant there. It is thought that infection can spread across a ganglion, for instance from the mandibular to the ophthalmic division of the trigeminal nerve, and hence from lip to eye. Spread from eye to eye is uncommon except in atopic patients in whom delayed-type hypersensitivity is impaired. These patients are at risk of bilateral disease, larger geographic ulcers and disseminated infection.

With each recurrence corneal scarring increases, and it is thought that viral particles remaining within the cornea cause the continuing inflammation and edema seen in disciform keratitis.

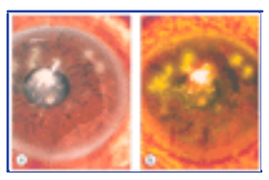

Figure 18-4 Herpes simplex virus dendritic keratitis, showing branching epithelial lesions seen (a) without staining and (b) with rose bengal staining. Rose bengal stains the devitalized cells at the edges of the dendritic lesions. *Courtesy of Myron Yanoff.*

Diagnosis

Viral culture or immunofluorescence[42] can be used to confirm infection, but in practice the diagnosis is clinical. In epithelial disease there is a characteristic dendritic ulcer (Fig. 18.4). Corneal sensation is often impaired, and there is usually a history of similar attacks in the past.

Management

Untreated herpes simplex keratitis usually resolves within 1–2 weeks, but it can progress, resulting in the development of geographic ulcers. Resolution is more rapid if the dendritic ulcer is debrided. Antiviral treatment also speeds up resolution, and no benefit has been shown for a combination of debridement and antiviral agents when compared with the use of antiviral agents alone. The choice of antiviral agent depends on availability. In Europe aciclovir 3% ophthalmic ointment five times a day is almost universally preferred because it is effective and has a low incidence of toxicity.[43] In the USA, trifluridine 1% 2-hourly is used.

Oral aciclovir or famciclovir is effective but comparatively expensive. It may be useful when there is a desire to reduce corneal exposure to topical agents. Systemic antiviral treatment is recommended as prophylaxis against encephalitis for infants who have primary infection.

Prophylactic treatment with oral aciclovir 400mg q12h reduces the recurrence rate of stromal (severe) disease from 28 to 14%.[44]

The management of the chronic sequelae of herpes simplex keratitis may require topical corticosteroid therapy under close ophthalmic supervision. Topical antiviral treatment reduces recurrence rates in these patients.[45]

Viral keratitis: herpes zoster ophthalmicus

Epidemiology

The varicella-zoster virus is a herpesvirus that causes chickenpox as a primary infection and shingles on recurrence. Around 90% of adults are seropositive for varicella-zoster virus. The rate of recurrence increases with age, and second recurrences can occur in those who have impaired immunity.

Pathogenesis and pathology

Reactivated virus replicates in the ganglion, producing local inflammation and premonitory pain before it travels down peripheral nerves to the skin or eye. A perineuritis and vasculitis occurs, and in the eye a disciform keratitis, iritis and cyclitis are common manifestations.

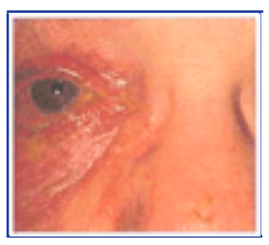

Figure 18-5 Herpes zoster ophthalmicus. Inflamed right periorbital skin, with conjunctivitis and a lesion on the nose. *Courtesy of Myron Yanoff.*

Clinical features

The clinical appearance is typical. Symptoms of eye involvement include photophobia and decreased vision. Hutchinson's sign, a rash on the side of the nose, is associated with ocular inflammation (Fig. 18.5).

Management

Oral aciclovir 800mg five times a day for 7 days accelerates skin healing and reduces the incidence of episcleritis, keratitis, iritis, postherpetic neuralgia and probably acute pain.[46] The newer antivirals famciclovir and valaciclovir have improved bioavailability and are increasingly used instead of aciclovir. There is little role for topical antivirals.

Systemic corticosteroids used with antiviral therapy have some benefit in shortening the clinical syndrome, but they do not reduce the incidence of postherpetic neuralgia.[47] There is no clinical consensus on whether the benefits of treatment outweigh the risks (see Chapter 8).

Disciform keratitis, iritis and cyclitis respond to conventional topical corticosteroid treatment, but prolonged therapy, sometimes for years, is required.

AIDS: varicella-zoster epithelial keratitis

A chronic, painful epithelial keratitis has been described in patients with AIDS and severe immunodeficiency ($CD4^+$ T cell counts of less than 0.1×10^9 /l). Clinically characterized by a variable dendrite-like keratitis, the associated severe, burning pain is the most dramatic feature. Response to treatment is variable; some patients have responded to oral or topical aciclovir, but such patients tend to relapse when treatment stops. [48]

INFECTIONS OF PERIORBITAL STRUCTURES

PERIORBITAL CELLULITIS

The orbit is the bony structure surrounding the eye. Infection of the contents of the bony orbit is called orbital cellulitis. Orbital infection commonly occurs as a result of contiguous spread from adjacent structures. Periorbital cellulitis is divided into preseptal or postseptal (orbital) depending on the site of infection. Orbital cellulitis is sight-threatening and occasionally life-threatening. Preseptal cellulitis is five to 10 times more common than orbital cellulitis in infants and toddlers and is not sight-threatening or life-threatening, but it can, rarely, spread to become orbital cellulitis. It is commonly caused by *H. influenzae* or *Streptococcus* spp. and follows an upper respiratory tract infection. In adults it is most often seen after minor trauma such as an infected bite or scratch.

The source and agents of infection causing orbital cellulitis vary with age. The paranasal sinuses (the ethmoid, maxillary and frontal sinuses) are the main sources. Orbital cellulitis is uncommon in neonates, but when it occurs it is usually secondary to conjunctivitis or a developmental abnormality such as a ruptured dacryocele. In infants respiratory tract infections may cause preseptal cellulitis. In older children and adults dental abscesses and trauma become important causes. Endogenous orbital cellulitis is rare.

Clinical features

In adults there is frequently a history of sinusitis, headache or recent tooth extraction or abscess. Children often have an antecedent upper respiratory tract infection.

Preseptal cellulitis is characterized by eyelid swelling and edema, which is usually unilateral mainly involves the upper lids. Lower lid swelling occurs when the cellulitis is secondary to dacryocystitis. Edematous conjunctiva can prolapse between the lids.

In orbital cellulitis features of preseptal cellulitis are variably present, but there is also proptosis, decreased ocular mobility and even decreased vision or a relative afferent pupil defect. These signs are caused by increased intraorbital pressure due to edema or abscess. If a subperiosteal abscess forms there may be nonaxial displacement of the globe and a palpable fluctuant mass in the orbit. Headache, fever and leukocytosis are common.

Diagnosis

Blood and local cultures are mandatory. A computerized tomography (CT) scan can distinguish between preseptal and orbital cellulitis and show the site of an orbital or subperiosteal abscess:[49]

- preseptal cellulitis produces edema of the lids and tissues anterior to the orbital septum, and
- orbital cellulitis produces edema of the orbital tissues and proptosis.

Orbital cellulitis is often associated with signs of primary or secondary sinus disease.

Staphylococcus and *Streptococcus* spp. and, in those under 4 years of age, *H. influenzae* are the main pathogens that cause preseptal and orbital cellulitis in children. In subperiosteal abscesses in children over 9 years of age and adults there is often a mixed infection of aerobes and anaerobes[50] from extending sinus or dental infections.

Management

Preseptal cellulitis responds well to systemic antibiotics. One or more broad-spectrum agents that cover *Staphylococcus* and *Streptococcus* spp. and *H. influenzae* should be used. A response is usual within 24 hours.

Orbital cellulitis requires prompt diagnosis and inpatient treatment with intravenous antibiotics. Monitoring of vision, pupillary reaction, extraocular movements and central nervous system function should be carried out during the first 1–2 days until the infection begins to resolve. If a subperiosteal abscess or sinusitis is identified and the clinical picture is not improving after 24 hours, surgical management is required. Orbital or brain abscesses are less common and should be drained immediately.

LACRIMAL SYSTEM INFECTIONS

Dacryoadenitis

Dacryoadenitis is usually due to a viral infection of the lacrimal gland. Patients present with adenopathy, fever, malaise and leukocytosis. The causes include infectious mononucleosis, herpes zoster, mumps, trachoma, syphilis, tuberculosis and sarcoidosis. Dacryoadenitis occasionally occurs in dehydrated patients as an ascending staphylococcal infection associated with a purulent discharge. On CT scanning there is diffuse lacrimal gland swelling without bony defects.

Diagnosis

The condition is usually self-limiting. Investigation other than CT scanning is reserved for chronic dacryoadenitis, and such patients should be referred for specialist investigation to exclude neoplasia.

Management

Treatment is generally symptomatic. Corticosteroids can help speed up resolution. It can be difficult to distinguish dacryoadenitis from idiopathic lacrimal gland inflammation (pseudotumor), although the presence of enlarged preauricular lymph nodes makes a viral diagnosis more likely.

Canaliculitis

There are chronic and acute forms. Acute dacryoadenitis may be caused by herpes simplex or herpes zoster and is often unrecognized except as a conjunctivitis and by its sequelae: scarred closed canaliculi and a punctum.

Chronic canaliculitis is usually unilateral and is characterized by pain or tenderness at the inner canthus. A chronic conjunctivitis may mask the more specific signs. The lacrimal punctum may pout and 'sulfur granules' may be expressed. These sulfur granules are pathognomonic of infection with *Actinomyces israelii*, an anaerobic Gram-positive branching filamentous bacterium. Less common causes are *Aspergillus* and *Candida* spp.

Treatment is by incision of the infected canaliculus and wash-out of all 'sulfur' material, usually with a penicillin-containing irrigation fluid.

Dacryocystitis

Pathogenesis and pathology

This condition occurs in chronic and acute forms. In infants it is usually an indolent condition resulting from incomplete development of the lacrimal drainage system. There is a mucopurulent discharge and recurrent conjunctivitis. Colonization is usual with *H. influenzae*, *Streptococcus pneumoniae*, staphylococci and *Klebsiella* and *Pseudomonas* spp.

In adults an acquired blockage of the lacrimal drainage system can cause an acute or chronic infection. An acute infection can be precipitated by instrumentation for investigation of a suspected blocked lacrimal duct. For this reason, mucoceles or chronic dacryocystitis should not be probed or syringed. Acute dacrocystitis presents with a painful swelling over the lacrimal sac.

Treatment is with warm compresses and systemic antibiotics. A large abscess should be drained by a stab through the skin or inferior fornix conjunctiva. A dacryocystorhinostomy will prevent recurrence.

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Chapter 19 - Endophthalmitis

Michael Whitby

Endophthalmitis is fortunately an uncommon condition; however, it may result in severe visual impairment or loss of an eye.

EPIDEMIOLOGY

Definition and nomenclature

Endophthalmitis is an infection within the vitreous and may involve the cornea and, in severe cases, the sclera (panophthalmitis). A number of classifications of this condition have been published but from a practical point of view, categorization by the clinical setting, taking into account such factors as the events preceding infection and the time to diagnosis, is most appropriate. Categories include postoperative endophthalmitis (acute (within 2 weeks of operation), delayed onset (more than 2 weeks after operation), conjunctival filtering bleb associated), post-traumatic endophthalmitis and endogenous endophthalmitis. Each of these subtypes may have characteristic clinical features and a spectrum of common causative pathogens (Table 19.1).

Incidence and prevalence of endophthalmitis

Although recent eye surgery is the most common cause of endophthalmitis, accounting for more than 70% of cases, the incidence of infection after cataract extraction, the most commonly performed eye surgery, continues to decline. Reported infection rates over the past few decades after extracapsular cataract extraction, with or without intraocular lens implantation, are very low, at 0.07–0.25%. [1] Endophthalmitis may occur after any other form of ocular surgery, but appears to be more common after glaucoma filtering procedures.

Endophthalmitis after penetrating ocular trauma is common, representing 7–30% of all endophthalmitis cases; 3–26% of penetrating eye injuries develop infection. It is more common when trauma is associated with a retained intraocular foreign body or when the injury is contaminated with vegetable matter.[2] The leading organisms in this setting are staphylococci, especially *Staphylococcus aureus*, and *Bacillus* spp.

Endogenous bacterial and fungal endophthalmitis are the least common forms, accounting for less than 2–8% of cases; they usually follow bloodstream spread of organisms and are commonly associated with a number of chronic medical conditions, such as diabetes mellitus, chronic renal failure, chronic immunosuppression, invasive medical procedures (including urinary catheterization and intravascular central lines) and intravenous drug abuse.

PATHOGENESIS AND PATHOLOGY

Although a broad range of organisms can cause endophthalmitis, the most common causative infectious agents are bacteria. Virtually any bacterium, including those usually accepted as saprophytes, can cause infection, although members of the normal ocular microflora are the most commonly implicated.

Acute postoperative endophthalmitis

In over 70% of cases, the pathogenic organism is a Gram-positive bacterium. *Staphylococcus epidermidis* and other coagulase-negative staphylococci are now the most frequently isolated bacteria from postsurgical endophthalmitis, representing 50–55% of all culture-positive cases. [3] *Staphylococcus aureus* and *Streptococcus* spp. are cultured from 10–30% of postoperative infections, whereas Gram-negative organisms, including *Pseudomonas* spp., *Proteus* spp. and *Citrobacter* spp., are implicated in only 7–20%. The change in prevalence of *Staph. epidermidis* probably represents, at least in part, a past failure to recognize coagulase-negative staphylococci as potential ocular pathogens.

Infecting organisms are usually introduced into the eye via incisions at the time of surgery. Nosocomial outbreaks of endophthalmitis caused by contaminated irrigation fluids, intraocular lenses and donor corneas have been recognized. Infiltration of pathogens in the immediate postoperative period may be associated with inadequately buried sutures, suture removal or the presence of vitreous wicks.

Delayed-onset postoperative endophthalmitis

Delayed-onset endophthalmitis is often caused by less aggressive organisms, including *Staph. epidermidis*, *Corynebacterium* spp. and *Candida* spp. A specific syndrome of chronic localized infection may occur with *Propionobacterium acnes*.

Filtering bleb endophthalmitis

Filtering bleb endophthalmitis is frequently caused by streptococci (60%) and *Haemophilus influenzae* (20%), although *Staph. aureus* remains a prominent pathogen.[4]

Post-traumatic endophthalmitis

Post-traumatic infection may be polymicrobial (10–40%), particularly in rural settings. Rarely, although more commonly than seen in postoperative infection, it can be caused by anaerobic organisms, especially *Clostridium* spp. *Staph. aureus* remains a common agent, although saprophytes such as *Bacillus* spp., especially associated with intraocular foreign bodies, may induce fulminating endophthalmitis.[5] Spread of organisms through corneal abrasions and penetrating corneal ulcers, particularly those involving *Staph. aureus* or *Pseudomonas aeruginosa*, may lead to endophthalmitis.

Endogenous endophthalmitis

Endogenous infection may be associated with a recognizable infective focus elsewhere in the body and this may provide an indication as to the likely causative organism. Ocular involvement, however, may also be the first and only manifestation of systemic infection. Two to three decades ago, the most commonly associated bacteria were meningococci and pneumococci related to meningitis and infective endocarditis, respectively; more recently, *Streptococcus* spp. other than *Strep. pneumoniae*, *Staph. aureus* and Enterobacteriaceae from gastrointestinal sources have become more prominent.[6] Intravenous drug use may be associated with infection with *Candida* spp., *Aspergillus* spp. and *Bacillus cereus*, although more common pathogens, including *Staph. aureus*, may be involved.

TABLE 19-1 -- Microbial etiology of endophthalmitis.

MICROBIAL ETIOLOGY OF ENDOPHTHALMITIS	
Category of endophtalmitis	**Common causative organisms**

Postoperative	Acute	Coagulase-negative staphylococcus
		Staphylococcus aureus
		Streptococcus spp.
		Gram-negative bacilli
		Pseudomonas spp.
	Delayed	*Staphylococcus epidermidis*
		Propionobacterium acnes
		Candida spp.
	Filtering bleb	*Streptococcus* spp.
		Haemophilus influenzae
		Staphylococcus aureus
Post-traumatic	Bacterial	*Staphylococcus aureus*; other staph spp.
		Bacillus spp.
		Pseudomonas spp.
		Other Gram-negative bacilli; anaerobes; corynebacteria; streptococci
	Fungal	*Penicillium* spp.; *Fusarium* spp.; *Acremonium* spp; other filamentous fungi
Endogenous	Bacterial	Enteric Gram-negative bacilli
		Fungi (including *Candida albicans*, *Aspergillus* spp.)
		Streptococcus spp.
	Fungal	Yeasts (*Candida albicans*, *Cryptococcus* spp.)
		Filamentous fungi (*Aspergillus* spp., *Acremonium* spp., *Fusarium* spp., *Paecilomyces* spp.)

Fungal endophthalmitis

Fungal endophthalmitis may occur as exogenous or endogenous infection. Postoperative fungal infection is fortunately exceedingly rare; however, after trauma, fungal endophthalmitis may represent up to 10% of cases, particularly if penetration with vegetable matter has occurred.[7] Extension of a fungal corneal ulcer may also lead to endophthalmitis. Fungi most commonly identified in this situation are usually saprophytic and may include *Aspergillus* spp., *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. Endogenous fungal endophthalmitis has been seen with increasing frequency over the past two decades, concurrent with an increased recognition of systemic fungal infections. *Candida albicans* is the most frequently reported causative agent after hematogenous dissemination from other infected body sites, particularly infected central venous catheters, and often in immunocompromised patients.[8] Direct intravenous inoculation as a result of narcotic abuse or contaminated infusion solutions has also been reported. Other fungi less commonly implicated in endogenous fungal endophthalmitis include *Cryptococcus neoformans*, *Aspergillus* spp. and *Paecilomyces* spp.

PREVENTION

The prevention of endophthalmitis is based on identification and pre-treatment of high-risk patients, and reduction in the conjunctival commensal flora.

Preoperative precautions

High-risk patients

Host factors that lower resistance to infection, such as chronic immunosuppression or diabetes mellitus, have been reported as significant risk factors for postoperative endophthalmitis. Reduction in immunosuppressive medications, when possible, and optimal control of blood glucose are essential in such groups. Pre-existing infection of external ocular tissue, for example chronic blepharitis, conjunctivitis and lacrimal outflow obstruction, should be identified and treated with appropriate topical antibiotics.

Antimicrobial prophylaxis

The aim of preventive treatment is to reduce eyelid and ocular surface microflora; this may be achieved by using topical antibiotics, topical antiseptic agents or subconjunctival antibiotics at the time of surgery.

Topical antibiotics

Although there is no consensus as to the optimal use of preoperative topical antibiotics in intraocular surgery, several studies have demonstrated significant falls in bacterial colonization of the conjunctiva with the application of topical antibiotics and have thus suggested a reduction in the incidence of postoperative endophthalmitis with the use of such antibiotics preoperatively. Topical antibiotics have been reported to be most effective in decreasing conjunctival bacterial colony counts when administered 2 hours before surgery.[9] Until the early 1980s, gentamicin (3mg/ml) was consistently found to be the most effective antibiotic in this situation compared with other agents such as chloramphenicol (5mg/ml), bacitracin (10mg/ml), neomycin (5mg/ml) and polymixin (2.5mg/ml).[10] However, the increase in gentamicin resistance among *Staph. epidermidis*, now the most common cause of postoperative endophthalmitis, suggests that it may no longer be the optimal agent for prophylaxis.[11] Vancomycin, when used prophylactically, has been shown to be active against staphylococci, but the potential risk of emerging resistance in enterococci and to a lesser extent in methicillin-resistant *Staph. aureus* has led some authorities to discourage this practice. Although no specific recommendations have been developed for ophthalmology, it seems appropriate to restrict the use of vancomycin to the treatment of, for example, serious keratitis, endophthalmitis or orbital cellulitis caused by ß-lactam resistant Gram-positive organisms or alternatively to the treatment of enterococci and *Staph. aureus* in patients unable to tolerate ß-lactam antibiotics. More recently, fluoroquinolones (all compounded at a concentration of 3mg/ml), for example ciprofloxacin, norfloxacin and ofloxacin, have been shown to be very effective in reducing conjunctival and eyelid bacterial flora when used preoperatively.[12]

Subconjunctival antimicrobials

Subconjunctival antibiotics can be administered after intraocular surgery based on the rationale that, at the completion of the ocular procedure, it is appropriate to inhibit growth of any bacteria that may have gained entry into the eye during surgery. During routine cataract surgery, aqueous fluid samples have been demonstrated to be culture positive in up to 43% of cases.[13] Conflicting results as to the value of this modality have been reported and penetration into the vitreous is relatively poor.

Topical antiseptics

Application of 5% aqueous povidone-iodine solution alone has been shown to be nontoxic and to decrease perioperative conjunctival bacterial colony counts and reduce the incidence of postoperative endophthalmitis significantly. In combination with topical antibiotics, povidone-iodine has been found to sterilize the conjunctiva in more than 80% of treated patients.[14]

Intraoperative precautions

Although the judicious use of preoperative antibiotics can reduce infection rates considerably, they do not replace meticulous aseptic technique in intraocular procedures. An appropriate operating room

environment, with efficient ventilation to reduce bacterial contamination, is essential; surgical techniques should be modified to minimize entry of ocular surface microbes into the eye during the surgical procedure, and adhesive-backed plastic drapes to isolate the eyelids and lashes from the operative field are recommended.

Implantation of intraocular lenses with prolene haptics appears to increase the risk of endophthalmitis, probably because coagulase-negative staphylococci bind well to this particular plastic. Binding to polymethylmethacrylate material is less, and therefore its use may reduce risk.[15] Care must be taken to minimize contact with the external eye during insertion of an intraocular lens to prevent contamination with conjunctival flora. A foldable lens insertion device has been developed to facilitate this. There is always the threat of infection from personnel and equipment in the operating room, and from contaminated irrigation solutions.

CLINICAL FEATURES

Clinical signs of endophthalmitis vary greatly depending on the preceding events, the nature of the infecting organism, the degree of tissue inflammation and the duration of disease. Early diagnosis requires the maintenance of a high index of suspicion as classic features of infection may be absent.

Acute postoperative endophthalmitis

Acute postoperative endophthalmitis usually occurs within 2 weeks of surgery, whereas the presentation of infection after penetrating trauma will often be more rapid. As a general principle, the more rapid the onset of symptoms, the more virulent the organism, with *Staph. aureus*, *Streptococcus pyogenes*, *Bacillus* spp. and Gram-negative bacilli being implicated in very rapid onset of infection within 24–72 hours of surgery. This presentation is characterized by marked anterior chamber inflammation, by the rapid development of a fibrinous anterior chamber exudate with hypopyon (which produces severe pain, more prominent than general postoperative discomfort) and by a progressive decrease in visual acuity. A marked vitreous inflammatory reaction, often obscuring visualization of the retina, frequently follows (Fig 19.1 , Fig 19.2 and Fig 19.3). Associated features may include marked conjunctival, lid and corneal edema, but systemic features are virtually never seen.

Delayed-onset postoperative endophthalmitis

Delayed-onset endophthalmitis has a more insidious course and frequently overlaps with acute postoperative endophthalmitis if less virulent organisms are implicated. Symptoms may not manifest until weeks or even months after surgery, although early mild clinical features with progressive worsening over time are not uncommon (Fig. 19.4). When delayed-onset endophthalmitis is caused by *P. acnes*, it usually develops months after cataract extraction; patients will often have a history of corticosteroid-responsive postoperative inflammation with a fluctuating course over many months. The

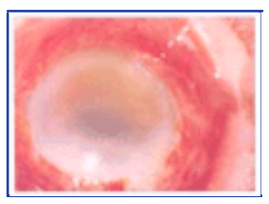

Figure 19-1 Corneal edema and fibrinous anterior chamber exudate in a traumatic foreign body-induced endophthalmitis. Any posterior vitreal or retinal view is obscured by the anterior corneal and aqueous haze.

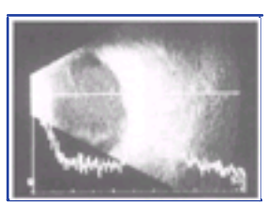

Figure 19-2 B-scan ultrasound of the eye showing total vitreous opacity of a severe endophthalmitis in patient seen in Figure 19.1 . This horizontal 'cut' through the eye shows the normally 'transparent' vitreous cavity to be filled with inflammatory debris, but there is no obvious retinal detachment.

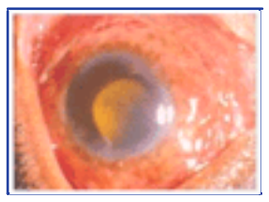

Figure 19-3 A dense vitreous abscess in advanced endophthalmitis. This partially treated postoperative endophthalmitis has vitreous cellular and protein deposits obscuring the retinal view.

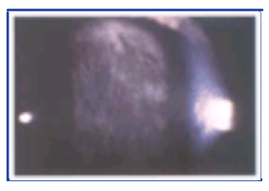

Figure 19-4 The typical posterior capsular opacities seen in a late-onset *Staphylococcus epidermidis* endophthalmitis. These deposits are actual coccal colonies, which are frequently removed at subsequent vitrectomy surgery to open up the capsular bag to intraocular antibiotics.

Figure 19-5 A 'quiet' endogenous fungal endophthalmitis with small hypopyon. This eye is relatively quiet with little chemosis, injection and pain, but has a small hypopyon and some small fungal 'balls' on the temporal iris.

most common clinical signs are posterior capsular deposits and chronic iridocyclitis. [16]

Fungal infection may also have a delayed clinical onset. Anterior chamber reaction is seen with progressive white infiltrates often adherent to the iris and posterior corneal surface (Fig 19.5 and Fig 19.6). Fluffy white fungal ball infiltrates ('string of pearls') occurring in the vitreous are characteristic (Fig. 19.7). Patients who have chronic postoperative endophthalmitis caused by coagulase-negative staphylococci may present with a hypopyon and diffuse vitritis, which occasionally obscures the view of the fundus. Visual loss is usually more severe than that in endophthalmitis caused by *P. acnes* or fungi.

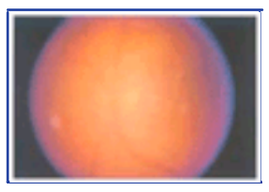

Figure 19-6 The degraded ophthalmoscopic retinal view obtained in patient seen in Figure 19.5 . The corneal edema and anterior chamber activity make vitreous and retinal observation difficult.

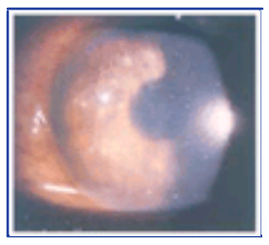

Figure 19-7 Fungal 'fluff balls' on the iris seen in a fungal endophthalmitis. Although these are not pathognomonic, their appearance raises the real possibility that the infection is of fungal origin.

Filtering bleb endophthalmitis

A conjunctival filtering bleb is a collection of fluid under the conjunctiva resulting from the formation of a fistula at operation through the sclera from the anterior chamber in an endeavor to reduce pressure in the anterior chamber. Endophthalmitis associated with a conjunctival filtering bleb is actually an acute presentation of endophthalmitis, but may occur months to years after the operation with rapid development of symptoms. Intraocular spread occurs from an initial bacterial penetration through the mucosa of the bleb, often in association with bacterial conjunctivitis. Infection is often associated with late bleb leakage and is facilitated by antimetabolites (e.g. 5-FU or mitomycin) used to ensure bleb survival.[2] A purulent discharge and an injected bleb are commonly seen, ultimately in association with typical signs of endophthalmitis. Viridans streptococci, *H. influenzae* and *Staph. aureus* are commonly implicated.

Post-traumatic endophthalmitis

Presentation will vary depending on the nature and severity of the ocular trauma or the type of retained foreign body, for example steel or vegetable matter, and the virulence and concentration of the organism initially deposited into the intraocular tissues (Fig. 19.8). The more virulent this organism, including *Staph. aureus* and *B. cereus*, the more rapid the onset of pain and associated ophthalmic features of infection. *Bacillus cereus*, fungi and to a lesser extent Nocardia spp. and atypical mycobacteria should be considered when injury to the eye is related to plant or vegetable matter.[17]

Endogenous endophthalmitis

Endogenous endophthalmitis usually has an insidious onset with a slow decrease in visual acuity caused by vitritis and localized areas of chorioretinitis. It may be suspected when other systemic symptoms of infection are present or in certain groups of patients, including those in whom bacteremia or fungemia is common, such as those with infective endocarditis and those with intravascular or urinary catheters, or patients who abuse intravenous drugs. Rarely, a fungal endophthalmitis, with a predilection for *Paecilomyces* infection, may occur in otherwise healthy individuals with no antecedent trauma.[18]

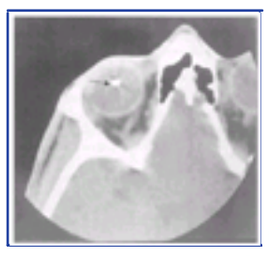

Figure 19-8 Horizontal computerized tomography scan section of eye seen in Figure 19.1 and Figure 19.2 revealing metallic intraocular foreign body in vitreous cavity (arrow). This CT scan demonstrates that the vitreous opacity seen by ultrasound is 'invisible' to this investigation.

Differential diagnosis

The differential diagnosis of postoperative inflammation includes sterile uveitis related to retained lens cortex; operative complications such as vitreous loss, hemorrhage and iris trauma; pre-existing uveitis; and toxicity of foreign material such as irrigation solutions introduced during surgery. These presentations are often difficult to distinguish from similar symptoms caused by infective endophthalmitis and careful sequential monitoring of such eyes or intraocular sampling for culture is appropriate to facilitate early diagnosis and treatment.

DIAGNOSIS

Microbiologic investigations in endophthalmitis

Confirmation of the diagnosis of infective endophthalmitis is essential to rational management. Because most postoperative infections are caused by normal ocular flora, there is some correlation between the results of external swabs of the eyelid margins and conjunctivae with intraocular isolates.[19] These should not be used to determine causative pathogens; routine preoperative cultures have a low predictive value and are not recommended. The optimal specimen for laboratory processing in endophthalmitis is an intraocular aspirate; aqueous and vitreous specimens should be obtained, although the latter appears the most reliable, as some 30–55% of concomitant aqueous specimens are negative in the presence of positive vitreous isolates.[20] In the case of late endophthalmitis, aqueous vitreous and some capsule material should also be cultured. Foreign bodies should be processed in traumatic endophthalmitis, whereas a swab of the bleb may assist in bleb-associated infections.

Because specimen volumes are often very limited, the traditional approach of direct inoculation onto culture media for aerobic and anaerobic bacteria, and fungi, remains important. Direct inoculation of specimens into blood culture bottles is of more limited value, but is useful in circumstances in which appropriate media are not readily accessible. Molecular diagnostic techniques are increasingly reported, but require further study of their utility and are not yet widely available. Rapid results by microscopy of Gram stains are useful, with positive results occurring in some 50–70% of well-collected vitreous aspirates.[21] An appropriate clinical history should accompany specimens to the laboratory so that culture can be prolonged for fastidious organisms and so that skin commensals such as *P. acnes* are not routinely discarded as contaminants.

MANAGEMENT

Antibiotic chemotherapy

Early institution of antimicrobial therapy is essential to optimal outcome in the management of infective endophthalmitis. Controversy continues as to the best therapeutic approach.

Intravitreal antibiotics

Intravitreal injection of antibiotics is the most effective way of rapidly achieving high intraocular antibiotic levels. Direct intravitreal injections of nonpreserved (i.e. intravenous) formulations of antibiotics are now the recommended route of administration in endophthalmitis treatment. The rationale of such therapy is that, although antibiotics when administered topically, subconjunctivally or systemically may attain therapeutic concentrations in the anterior chamber, concentrations in the vitreous are much lower. Intravitreal injections may produce significant retinal toxicity, although single injections have been shown to be safe and effective.

The optimal combination of antibiotics in empiric therapy normally covers Gram-positive and Gram-negative organisms. Over recent years, vancomycin has replaced first-generation cephalosporins for Gram-positive activity because of the increasing incidence of *Staph. epidermidis* infection resistant to methicillin and other ß-lactam drugs. Reports of retinal toxicity caused by gentamicin led to the use of amikacin to cover Gram-negative organisms, as this later antibiotic was believed to be less toxic. Amikacin has now been used widely in controlled and uncontrolled clinical situations. However, more recently, gentamicin and amikacin have been associated with macular infarction[22] and this has led to increased use of other broad-spectrum Gram-negative antimicrobial agents, particularly ceftazidime. An initial single injection of 0.2mg vancomycin maintains therapeutic concentration for at least 3 days and a repeat injection at that time for at least 4 days.[23] Repeat injections of aminoglycosides should be avoided, except in severe cases. Intravitreal amphotericin B injections (1–5µg) can be utilized in fungal endophthalmitis, with a single repeat inoculation after 72–96 hours in progressive infection; again, however, toxicity limits longer duration of therapy.

Subconjunctival antibiotics

The data regarding subconjunctival antibiotic penetration is conflicting, possibly because of varying degrees of inflammation in the eye or poor and variable sampling techniques. Penetration is affected by the transcleral and transcorneal permeability of the agent, but high aqueous levels can be achieved with vancomycin, gentamicin and ß-lactam antibiotics for up to 4 hours; however, vitreous concentrations are generally poor. Subconjunctival injections are an irritant and are painful, thus limiting duration of this form of therapy to a few days.

Topical antibiotic therapy

The efficacy of topical antibacterial applications in endophthalmitis is not well studied, although significant concentrations of antibiotics in the anterior segment can be obtained with frequent administration of highly concentrated (fortified) solutions. A combination of vancomycin and fortified gentamicin, e.g. 1.5%, provides a broad-spectrum cover, but solutions must be prepared by a qualified pharmacist as they are not commercially available. Third-generation cephalosporins, such as cefotaxime and ceftazidime, and fluoroquinolones, such as ciprofloxacin, may be used to replace gentamicin to provide appropriate Gram-negative cover. The use of collagen shields to produce frequent topical application of antibiotic solutions has been explored, but is limited by potential corneal toxicity. Iontophoresis also increases anterior chamber concentrations, but its efficacy remains unproved.

Systemic antibiotics

In humans, intraocular penetration of systemically administered antibiotics is generally poor. However, intravenous antibiotics are a common adjunctive therapy in endophthalmitis, justified in that they may enhance concentrations of antibiotics achieved with intravitreal agents and extend the duration of therapeutic activity. Intraocular inflammation and/or performance of a vitrectomy may alter the blood-retina barrier to allow improved intraocular penetration.

Posterior chamber concentrations of newer and broad-spectrum agents, including ceftazidime, imipenem and ciprofloxacin, appear improved, particularly in terms of efficacy against Gram-negative bacilli such as *Pseudomonas* spp. However, none is reliably active against *Staph. epidermidis*, the most common cause of postoperative endophthalmitis, and resistance may develop rapidly.

A multicenter randomized trial, the Endophthalmitis Vitrectomy Study (EVS), sponsored by the National Eye Institute of the National Institutes of Health, followed a cohort of 420 patients who had clinical evidence of endophthalmitis within 6 weeks after cataract surgery or secondary intraocular lens implantation. [24] Patients were randomly assigned to therapy, with or without pars plana vitrectomy and with or without systemic antibiotics (ceftazidime and amikacin), but each patient underwent intravitreal injection of vancomycin and amikacin. When outcome was assessed 9–12 months after the operation, no difference in final visual acuity or media clouding between groups with and without systemic antibiotics could be determined. Well conducted as this study was, a number of questions remain unanswered, particularly in relation to generalization of the results. Do the results, for example, extrapolate to Gram-negative infection or to other categories of ocular surgery? Moreover, a significant percentage of coagulase-negative staphylococci and *Streptococcus* spp., the most common Gram-positive pathogens, are resistant to ceftazidime and amikacin, although vancomycin was active against all.

Duration of therapy is very variable. In spite of the presence of an ocular foreign body such as an intraocular lens, endophthalmitis caused by *Staph. epidermidis* will settle rapidly with appropriate management. Length of therapy can be assessed by a reduction in cellular activity in both the anterior and posterior chambers and by improvement in visual acuity.

Fungal endophthalmitis poses a particular problem. [25] Itraconazole, a lipophilic antifungal imidazole, has been shown to be efficacious in *Aspergillus* endophthalmitis. Fluconazole, a hydrophilic imidazole, achieves useful concentrations in intraocular tissue and has also proved successful in *Candida* endophthalmitis. A newer imidazole, voriconazole, shows promise in fungal endophthalmitis, though further experience is needed. Amphotericin B, often the only available therapy in filamentous fungal infections other than those caused by *Aspergillus* spp., does not achieve significant concentrations in intraocular tissue. Nevertheless, it is widely used in both conventional and liposomal formulations, in fungal endophthalmitis with some evidence of success.

Specific recommendations

Endophthalmitis may cause irreparable visual loss within 24–48 hours. Initial therapy must frequently be empiric, because of the low sensitivity of Gram stain film and the 24–48 hour delay until culture results become available. Antibiotic therapy should be chosen to cover the spectrum of pathogens likely to be implicated. In this situation classification of endophthalmitis by clinical setting provides useful information. Suggested initial antibiotic therapy for acute postoperative, filtering bleb and post-traumatic endophthalmitis is illustrated in Table 19.2 .

Localized bleb infection without endophthalmitis can usually initially be managed with topical therapy. Because of the increased prevalence of *H. influenzae* in this infection, a combination of antibiotics

TABLE 19-2 -- Initial empiric recommendations for antimicrobial therapy of endophthalmitis.

INITIAL EMPIRIC RECOMMENDATIONS FOR ANTIMICROBIAL THERAPY OF ENDOPHTHALMITIS				
Category of endophthalmitis		**Antimicrobial therapy**		
		Topical	**Intravitreal**	**Systemic**
Postoperative	Acute	Cefazolin (5mg/ml) + gentamicin (8–15mg/ml) or amikacin (25–50mg/ml)	Cefazolin (2.25mg) + amikacin (400mg)	Cefazolin (2g q8h) + gentamicin (4–5mg/kg q24h)* or amikacin (15mg/kg q24h)*
		Vancomycin (50mg/ml) + gentamicin or amikacin or ceftazidime (5mg/ml) or ciprofloxacin (3mg/ml)	Vancomycin (1mg) + amikacin or ceftazidime (2.25mg)	Vancomycin + gentamicin or amikacin or ceftazidime (2g q8h) or ciprofloxacin (400mg q12h)
	Delayed	Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin	Vancomycin + amikacin or ceftazidime	Vancomycin + gentamicin or amikacin or cefotaxime (2g q6h) or ceftazidime or ciprofloxacin
	Filtering bleb	Vancomycin + ceftazidime or ciprofloxacin	Vancomycin + ceftazidime	Vancomycin + /or cefotaxime, ceftazidime, ciprofloxacin
Post-traumatic		Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin	Vancomycin + amikacin or ceftazidime	Vancomycin + gentamicin or amikacin or ceftazidime or ciprofloxacin
Dosages given in brackets apply to all citations of each specific drug within the relevant column.				

*Dosages must be adjusted to reflect the patient's age, body weight and renal function.

that covers this pathogen and provides broad-spectrum activity against Gram-positive and Gram-negative organisms should be chosen.

Adjunctive therapy

Vitrectomy

The place of pars plana vitrectomy remains a controversial issue in the management of endophthalmitis. The theoretic rationale for such a procedure is that it offers a reduction in organism load, a reduction in traction effect on the retina with less potential for detachment, collection of adequate culture material and possibly improved distribution of intravitreal antibiotics. Evidence for its efficacy has been conflicting, often as a result of poorly controlled studies. The EVS was designed to determine

definitively the value of vitrectomy in the presence of intravitreal antibiotics. The final conclusions of this study were that visual acuity was improved significantly in patients treated with vitrectomy only if the initial vision was light perception, but not if initial vision was hand movements or better; that is, the most severe cases on presentation benefited most from vitrectomy. [24]

Corticosteroids

The use of corticosteroids to reduce the inflammatory response to infection and thus preserve ocular tissue is widely practiced with administration by several routes, including intraocular, periocular, topical and systemic, but its place in therapy remains contentious. Experimental animal studies demonstrate superior outcomes utilizing corticosteroids with antibiotics even in endophthalmitis caused by pseudomonads and fungi. In general, clinical studies do not report deterioration in outcomes if corticosteroids are used in combination with antibiotics, at least at the ocular level.[26]

Management of intraocular lens

In most cases of acute postoperative pseudophakic endophthalmitis, removal of the intraocular lens is not necessary and does not influence outcome. In fact, it may be hazardous and predispose to anterior segment hemorrhage and retinal detachment. Exceptions may occur when the pathogen is a fungus and in cases of late-onset endophthalmitis caused by *P. acnes* when conservative treatment with intravitreal vancomycin and corticosteroids is unsuccessful. In such cases, complete capsulectomy and lensectomy may be necessary to provide cure.

Outcome

Up to 50% of patients who have endophthalmitis suffer major visual loss within 24–48 hours of onset, emphasizing the essential need for early diagnosis and prompt treatment.[26] Approximately 30% of patients in recent studies of endophthalmitis achieved a final visual acuity of 20/60 or better after treatment.[27] [28] Certain factors are highly correlated with poor visual outcome; these include severity of infection, delay in time to diagnosis and institution of treatment, virulence of infecting organisms and intraocular complications such as vitreous hemorrhage and retinal detachment. Poor vision at the time of diagnosis correlates with either a virulent organism, such as *Bacillus* spp., *Streptococcus* spp. or Gram-negative bacilli or fungi, or a delay in diagnosis even with a low-virulence organism. Normally, however, *Staph. epidermidis* endophthalmitis has an excellent outcome, although even in this situation some 10% of patients develop blindness.[29] Culture-negative cases of endophthalmitis generally have a better visual outcome than do culture-positive groups, which may relate to the lower virulence of more fastidious organisms or to the veracity of diagnosis.

The outcome of post-traumatic endophthalmitis and endophthalmitis related to a conjunctival filtering bleb is poor, probably because of the intrinsic virulence of organisms implicated; however, for converse reasons, prognosis in delayed ophthalmitis, including that caused by *P. acnes*, is usually more favorable.

The primary complication of endophthalmitis is retinal detachment that may occur at any time before, during or after treatment. Prognosis in this situation is poor, although surgical repair can salvage useful vision in a substantial number of patients.[30]

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Chapter 20 - Infectious Retinitis and Uveitis

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Michael Barza

INTRODUCTION

Strictly speaking, uveitis is inflammation of one or more parts of the uveal tract of the eye, namely, the choroid, the iris and the ciliary body. In practice, uveitis has come to mean any inflammation of the intraocular structures, regardless of the precise anatomic sites involved. Inflammation localized to certain structures can be denoted by more specific terms. For example, inflammation of the iris (iritis) or ciliary body (cyclitis) is called anterior uveitis. Inflammation of the vitreous (vitritis), retina (retinitis) or choroid (choroiditis) is called posterior uveitis. Inflammation of the entire globe is called panuveitis. Inflammation localized to the outer coats of the eye or optic nerve without accompanying intraocular inflammation (e.g. scleritis, keratitis, optic neuritis) does not usually fall under the heading of uveitis.

The anterior segment of the eye consists of the cornea, anterior chamber, lens, iris, posterior chamber and ciliary body; the posterior segment refers to the vitreous cavity and posterior structures including the retina and optic nerve (Fig. 20.1).

The location, distribution and ophthalmoscopic appearance of inflammatory lesions is useful to the ophthalmologist in suggesting likely causes of uveitis (Table 20.1).[1] Intermediate uveitis centers about the equator of the eye between the anterior and posterior parts of the uveal tract, whereas anterior uveitis involves the anterior chamber and posterior uveitis involves the posterior segment of the eye. Chronic intermediate uveitis with certain morphologic characteristics may be termed the pars planitis syndrome.

A diagnosis of retinal vasculitis is made by finding inflammatory sheathing of the retinal vessels on ophthalmoscopic examination and leakage of dye from the involved vessels on intravenous fluorescein angiography (Fig. 20.2).[1] Retinal vasculitis is sometimes isolated, but more commonly it occurs in conjunction with posterior uveitis. It is a nonspecific finding and can occur in ischemic conditions as well as infection. Endophthalmitis, although it overlaps to some degree with uveitis and retinitis, is considered separately (see Chapter 19).

TABLE 20-1 -- Anterior, posterior and intermediate uveitis.

ANTERIOR, POSTERIOR AND INTERMEDIATE UVEITIS			
	Anterior uveitis	**Intermediate uveitis**	**Posterior uveitis**
Ophthalmoscopic signs that define the type of uveitis	Inflammatory cells in the anterior chamber with or without keratic precipitates or iris lesions	Inflammatory cells more highly concentrated in anterior vitreous than in anterior chamber	Inflammation of retina, choroid, retinal vessels, posterior vitreous humor, or a combination of these
Additional clinical signs	Ciliary flush (perilimbal injection of the sclera)	Macular edema	Retinal vasculitis
	Posterior synechiae	Inflammatory exudate on pars plana	Optic disk edema
			Macular edema
Symptoms	Pain	Floaters	'Floaters'
	Redness		Blurred vision
	Photophobia		
Definitions and common symptoms.			

There are many known causes of uveitis, including infections, autoimmune disorders, various other systemic diseases and trauma, including surgical trauma. In some instances, the ocular lesions are only one manifestation of an underlying multisystem disorder, whereas in others the eye represents the only site of overt disease. Many cases of uveitis remain of uncertain origin despite extensive investigation. In trying to determine the cause of any particular case

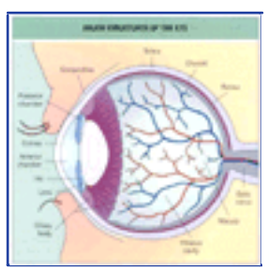

Figure 20-1 Major structures of the eye.

Figure 20-2 Retinal inflammatory vascular sheathing (vasculitis). This case occurred secondary to sarcoidosis.

of uveitis, the ophthalmologist considers a number of features, including the distribution and morphologic characteristics of the lesions, the chronicity of the disorder and the presence of underlying systemic diseases. In select cases, invasive diagnostic testing in the form of anterior chamber paracentesis, vitreous biopsy or even retinal biopsy may be necessary to rule out an infectious etiology.

Among the diagnostic tests commonly carried out to determine the cause of uveitis are:

- chest radiograph to detect sarcoidosis and tuberculosis,
- blood count,
- erythrocyte sedimentation rate,
- serologic tests for syphilis,

- HLA-B27 antigen, and
- angiotensin-converting enzyme concentration

Within the broad categories of retinitis and uveitis, there are certain distinctive syndromes, such as acute retinal necrosis (ARN), progressive outer retinal necrosis (PORN) and pars planitis, which allow the ophthalmologist to make a syndrome diagnosis on clinical grounds alone.

This chapter describes the major manifestations of uveitis and retinitis, with an emphasis on the infectious causes of these syndromes. Uveitis and retinitis in immunosuppressed patients and neonates are considered separately because the causative agents differ from those in immunocompetent and adult patients. Only the infectious aspects pertinent to the eye will be discussed. Because significant uveitis and retinitis must be managed by an experienced ophthalmologist, detailed regimens of locally applied anti-infective and anti-inflammatory agents have not been given.

EPIDEMIOLOGY

Uveitis and retinitis are uncommon in clinical practice. In Minnesota, USA, the annual incidence of new cases of uveitis has been found to be 17/100,000 population.[2] A general ophthalmologist is likely to see only a dozen patients with uveitis or retinitis each year. A specific cause can be identified in only about half of the patients with uveitis. In some instances, uveitis provides the first evidence of an underlying systemic disease. Uveitis and retinitis can occur at any age and the incidence is about equally divided between the sexes. In one study, the mean age of patients with uveitis was 45 years.[1]

Infectious uveitis or retinitis in neonates is almost always the result of congenital infection — toxoplasmosis, rubella, cytomegalovirus (CMV), herpesvirus (TORCH) syndrome (see Chapter 65). Each of the TORCH agents can involve the uvea or retina. Among immunosuppressed patients, uveitis or retinitis is found most commonly in patients who have AIDS. However, hematogenous fungal endophthalmitis occurs primarily in patients with other forms of immunosuppression.

Although uveitis does not appear to be more prevalent in any particular parts of the world, there is geographic variation in the underlying causes. For example:

- acquired ocular toxoplasmosis is quite common in Brazil, but rare in the rest of the world;
- Behçet's disease is prevalent in Turkey and the Middle East, but unusual elsewhere;
- leprosy has been eradicated in most developed countries, but can still be found in less developed regions;
- CMV retinitis associated with AIDS occurs in patients from developed countries, but is rarely seen in developing areas of the world; and
- onchocerciasis (river blindness), which primarily affects the cornea, but can produce retinitis and choroiditis, is seen only in equatorial Africa and Central America.

CLINICAL FEATURES

Common symptoms of intraocular inflammation, irrespective of the cause, are ocular pain, photophobia, 'floaters' (specks that appear to float in the visual field) and impaired vision (see Table 20.1). Both eyes may be affected simultaneously, but unilateral involvement does not rule out a systemic cause. Anyone with these symptoms should have an ophthalmologic evaluation with pharmacologic dilatation of the pupil with examination by slit lamp and indirect ophthalmoscopy. Because most of the uveal tract can be readily visualized in this fashion, the morphology of the lesions can be precisely defined. The findings allow the process to be characterized as anterior, intermediate or posterior uveitis, or panuveitis (see Table 20.1).

Additional clinical signs include conjunctival injection, anterior segment cells and 'flare' (protein floating in aqueous fluid, seen on slit lamp examination), iris nodules (granulomas), posterior synechiae, posterior segment cells, optic disk edema, retinal vasculitis, retinitis and choroiditis. A hypopyon refers to layered inflammatory cells that settle gravitationally in the inferior aspect of the anterior chamber (Fig. 20.3). In cases of severe acute uveitis, there may be so much intraocular inflammation that a cloudy media results. This may

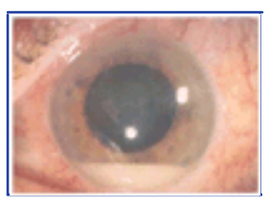

Figure 20-3 Hypopyon. The finding of a hypopyon (layered inflammatory cells in the anterior chamber of the eye) usually denotes a severe anterior uveitis.

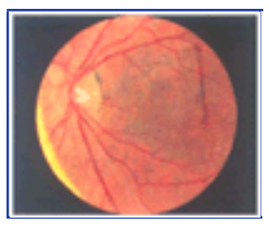

Figure 20-4 Salt-and-pepper fundus. The pigment alterations in the macula give a 'dirty' appearance to the retina. The lesion occurred following congenital rubella infection. The vertical black line across the fovea is a focusing stick.

preclude visualization of the inner aspects of the eye. Ocular ultrasonography should be performed in such cases.

Chronic uveitis can lead to:

- cataract formation,
- epiretinal membrane formation,
- iris, retinal or choroidal neovascularization, and
- retinal detachment.

TABLE 20-2 -- Uveitis in community-based and tertiary ophthalmology centers.[D]

UVEITIS IN COMMUNITY-BASED AND TERTIARY OPHTHALMOLOGY CENTERS		
	Patients in community-based ophthalmology practice	**Patients in tertiary referral center**
Mean age of patients (y)	46	45
Males (%)	49	52
Percentage with:		
Anterior uveitis	91	60
Intermediate uveitis	1	12
Posterior uveitis	5	15
Panuveitis and other	3	13
Percentage of cases of anterior uveitis that are chronic	9	63
Percentage in which specific diagnosis was made	47	58
Percentage attributable to infection	14	21

Percentage associated with systemic disease (including infections)	13	9
Most common causes of uveitis	HLA-B27-associated anterior uveitis (15%)	CMV retinitis (33%)
	CMV retinitis (7%)	HLA-B27-associated anterior uveitis (8%)
	Traumatic uveitis (5%)	Pars planitis syndrome (6%)
	VZV uveitis (4%)	HSV-associated uveitis (5%)
	Toxoplasma retinochoroiditis (4%)	*Toxoplasma* retinochoroiditis (4%)
Most common causes of posterior uveitis	CMV retinitis	CMV retinitis
	Toxoplasma retinochoroiditis	*Toxoplasma* retinochoroiditis

The characteristics of uveitis seen in a community-based ophthalmology practice and in a tertiary referral center. The figures for the proportion of cases in which a specific diagnosis was made are based on data gathered at, or requested at, the first visit; cases of CMV retinitis and 'masquerade' syndrome are omitted. The figures for the proportion of cases associated with systemic disease include cases due to varicella, candidiasis, coccidioidomycosis and syphilis.

Data from McCannel et al., 1996.[1]

A rare sequel of healed diffuse retinitis or choroiditis is known as salt-and-pepper fundus because of the stippled appearance of the retinal pigment epithelium. The lesions are nonspecific and may be seen following a variety of infectious and inherited disorders (Fig. 20.4).

The characteristic causes and clinical presentations of uveitis seen in a general community-based ophthalmologic practice and in a tertiary referral center have been found to differ (Table 20.2).[1] In the community ophthalmic practice, about 90% of uveitis was anterior and most cases of anterior uveitis were acute. By contrast, in the tertiary referral center, intermediate and posterior uveitis and panuveitis were more prevalent and much of the anterior uveitis was chronic. In both settings, a specific diagnosis could be made in only about half of patients from data gathered at the initial visit. An infectious cause was documented in 21% of the referred patients and 14% of the community-based patients. The uveitis was attributed to a systemic disease, usually rheumatologic, in a smaller number of patients. The most common causes of uveitis in both settings were:

- HLA-B27-associated anterior uveitis;
- CMV retinitis;
- herpesvirus-associated uveitis, caused either by herpes simplex virus (HSV) or varicella-zoster virus (VZV); and
- toxoplasma retinochoroiditis (see Table 20.2).

Thus, three of the four most common causes of uveitis were infectious, but infectious causes accounted for well under half of all cases of uveitis in either setting.

Anterior uveitis is much more common than posterior uveitis, and the specific cause is more likely to be identifiable. When infectious, anterior uveitis commonly arises from systemic disease that is

clinically obvious, whereas posterior uveitis may arise from an infection that has no extraocular manifestations. This is the case in uveitis caused by *Toxoplasma gondii*, *Toxocara canis* and *Histoplasma capsulatum*. The lesions of anterior uveitis not uncommonly remit without treatment, whereas those of posterior uveitis tend to persist or progress.

PATHOGENESIS AND PATHOLOGY

Most of the infectious agents discussed in this chapter gain access to the ocular structures via hematogenous spread of micro-organisms from other sites. The uveal tract is highly vascular, offering a ready target for seeding by blood-borne microbes. Some organisms (e.g. herpesviruses, *T. gondii*) seem to have a propensity for the retina itself. Furthermore, circulating inflammatory cells and mediators of inflammation have a potent impact on the uveal tract and adjacent structures. These inflammatory cells include T and B cells, macrophages and monocytes, mast cells and eosinophils. Inflammatory mediators include cytokines, complement and antibodies. In some types of infectious uveitis (e.g. tuberculous and possibly spirochetal uveitis), the inflammation may be produced by a combination of microbial invasion and immunologic mechanisms. Occasionally, uveitis occurs as a 'sympathetic' reaction to an adjacent infection (e.g. anterior uveitis in patients with HSV keratitis). It is hypothesized that other infections gain access to the eye by spreading along nerves (e.g. the ARN syndrome associated with VZV).

The eye is unique among organs in showing a neovascular response to certain stimuli, especially prolonged inflammation. New vessels may form in the cornea, iris, retina, optic nerve and choroid, presumably as a result of the production of various protein growth factors such as vascular endothelial growth factor. These newly formed, acquired vessels themselves can cause severe loss of vision. Together with specific treatment of the underlying cause, laser photocoagulation or ocular photodynamic therapy may be needed to treat ocular neovascularization.

Many of the pathogens that produce uveitis are found preferentially in an intracellular location (viruses, spirochetes, mycobacteria, fungi, parasites). Some tend to persist indefinitely and have the ability to produce episodic exacerbations. The eye may be the only site of clinically evident inflammation.

PREVENTION

Patients who have systemic infections associated with an appreciable risk of intraocular infection should be screened by an ophthalmologist for early detection and treatment. An example is the periodic

TABLE 20-3 -- Viral causes of retinitis and uveitis.

VIRAL CAUSES OF RETINITIS AND UVEITIS			
Viral infection	**Systemic infection**	**Features of retinitis or uveitis**	**Other ocular disease**
Measles	Uveitis occurs 1–2 weeks after onset of rash	Common: anterior uveitis	Common: conjunctivitis, keratitis
		Rare: chorioretinitis or neuroretinitis	Rare: optic neuritis
SSPE (see Fig. 20.5)	Uveitis occurs some years after infection	Chorioretinitis involves macula	Papillitis; motility disturbances
Herpes simplex virus	Primary infection; lids and conjunctiva	Common: anterior uveitis	Dendritic corneal ulcer common
		Rare: uveitis, retinitis, ARN	
Varicella-zoster virus	Convalescence from varicella or trigeminal zoster (often with nasociliary branch involved)	Common: mild, anterior uveitis, self-limited; rarely, chorioretinitis or ARN	Granulomatous keratic precipitates; synechiae; glaucoma, cataract
Epstein-Barr virus	Infectious mononucleosis	Rare: mild anterior uveitis or chorioretinitis	Common: follicular conjunctivitis
Influenza, adenovirus, mumps	Systemic infection usually evident	Rare: bilateral, mild, self-limited, anterior uveitis	None
		Rare: neuroretinitis or optic neuritis	
These ocular problems are seen in immunocompetent adults and children.			

examination of patients who have HIV infection and a low $CD4^+$ lymphocyte count to detect CMV retinitis. Likewise, patients who have HIV infection who develop ophthalmic zoster are at risk of developing necrotizing herpetic retinitis and are candidates for ophthalmologic screening examination. Patients who have *Candida*

fungemia merit ocular examination to detect metastatic retinitis. Neonates who have congenital HSV infection must be screened carefully for ocular lesions, which may first appear up to several months after birth.

MANAGEMENT

There are two major principles of therapy for intraocular infections. The first is to treat the infection. Drugs for the treatment of ocular diseases may be given by topical administration, periocular injection, systemic administration (orally or intravenously, or both) and intravitreal injection.

The second principle is to suppress intraocular inflammation, lest there be persisting damage to the retina and other crucial structures. This is usually accomplished by using corticosteroids, which may be given by various routes. Rarely systemic immunosuppression is employed.

Uveitis in immunocompetent adults and children

Viral causes of uveitis

Uveitis, especially anterior uveitis, may occur in the course of many viral infections, most commonly those caused by rubeola virus and the herpesvirus family (Table 20.3). The uveitis is generally self-limited and does not require treatment.

Measles often causes conjunctivitis and keratitis; the keratitis rarely leads to bacterial ulceration and perforation of the cornea. There may be anterior uveitis. Other rare complications are chorioretinitis or neuroretinitis; these may occur, together with measles encephalitis, 1–2 weeks after the onset of the rash.[3]

The ocular lesions of rubeola are generally self-limited and no specific treatment is available. Involvement of the optic disk (optic neuritis) may cause severe visual loss, but this may improve spontaneously over subsequent months. There may be residual pigmentary retinopathy with the salt-and-pepper appearance (see Fig. 20.4).

Subacute sclerosing panencephalitis (SSPE), which results from measles infection in very early life, commonly causes chorioretinitis, usually about the time that the neurologic signs of the disease become evident. The chorioretinitis is often focal, involving the macula, and there is mild vitritis (Fig. 20.5). Cortical blindness may occur. The prognosis of the ocular lesions is poor. There is no specific treatment.[4]

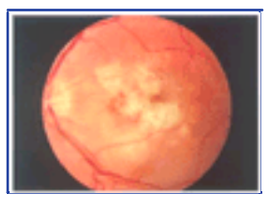

Figure 20-5 Macular retinitis. This patient has subacute sclerosing panencephalitis.

Herpes simplex virus infection of the eye in children and adults usually presents as recurrent keratitis with characteristic dendrites (see Chapter 215). There may be an associated anterior uveitis (iridocyclitis). Rarely, HSV keratitis may spread along the axons to produce retinitis and posterior uveitis. [5] Another rare manifestation of HSV ocular infection is ARN (see below). Retinitis and posterior uveitis are usually treated by antiviral agents such as aciclovir given intravenously in high dosage. Anterior uveitis alone may be treated topically except, perhaps, in immunosuppressed patients.

Varicella, especially in the convalescent stage, may cause a mild, self-limited anterior uveitis. Treatment is not usually necessary, but corticosteroid drops may be applied. A more serious lesion, keratitis, may develop in patients who have trigeminal zoster, especially during convalescence from zoster that has involved the nasociliary branch of the trigeminal nerve. The keratitis may be dendritiform or geographic. It may be accompanied by anterior uveitis with granulomatous keratic precipitates (clumps of inflammatory cells on the corneal endothelium) and posterior synechiae (adhesions between the iris and the anterior surface of the lens).[6] The uveitis of varicella infection may be treated with 1% trifluridine ophthalmic drops or oral aciclovir. Rarely, trigeminal zoster may lead to chorioretinitis and a form of necrotizing herpetic retinitis referred to as the ARN syndrome (see below). In such cases, intravenous aciclovir and corticosteroids should be administered. Cases have been reported with primary varicella infection of childhood (chickenpox). [7]

TABLE 20-4 -- Bacterial causes of retinitis and uveitis.

BACTERIAL CAUSES OF RETINITIS AND UVEITIS			
Bacterial infection	**Systemic infection**	**Features of retinitis or uveitis**	**Other ocular disease**
***Yersinia* spp.**	Infection occult, not proven	Suggested important cause of anterior uveitis in patients with HLA-B27 antigen	None
Borrelia burgdorferi	Features of extraocular	Nonspecific anterior or posterior uveitis	Conjunctivitis
	Lyme disease		
Treponema pallidum	Uveitis usually during secondary syphilis; interstitial keratitis is delayed manifestation of congenital syphilis	Common: bilateral anterior uveitis	Acute bilateral intersitial keratitis (age 5–10 years, after congenital infection)
		Rare: choroiditis (large while lesions), retinal vasculitis, papillitis	
***Bartonella* spp.** (see Fig. 20.6)	Nonspecific systemic symptoms antedate ocular symptoms	Bilateral papillitis: optic disk edema; white retinal lesions; vitritis	None
Metastatic endophthalmitis	Often extraocular source is evident	Often bilateral: focal or diffuse uveitis or retinitis	None
These ocular problems are seen in immunocompetent adults and children.			

During infectious mononucleosis caused by Epstein-Barr virus (EBV) infection, a bilateral, mild, follicular conjunctivitis may be seen. Rarely, a mild anterior uveitis can occur, as well. Recently, a choroiditis resembling that seen with histoplasmosis, but with vitritis, has been ascribed to acute or chronic EBV infection.[8] [9]

Other viral infections may produce uveitis on occasion. Influenza virus infection may cause a mild, transient, bilateral anterior uveitis during the acute infection or neuroretinitis during convalescence.[10] No treatment is needed.

One of the characteristic manifestations of adenovirus infection is epidemic keratoconjunctivitis. There may be concomitant anterior uveitis, for which either no treatment or topical corticosteroid treatment may be given.

Mumps virus infection may cause a mild, evanescent, bilateral anterior uveitis, which may appear up to 4 weeks after the onset of clinical infection. Optic neuritis, sometimes with neuroretinitis, may occur after mumps infection, but nearly always resolves spontaneously and no treatment is needed.[11]

HLA-B27-associated uveitis

An autoimmune form of recurrent, bilateral anterior uveitis is quite common in patients who harbor the HLA-B27 antigen. HLA-B27-associated uveitis is the most common type of nonidiopathic anterior uveitis. Although usually considered immune-mediated rather than infectious, it may be triggered by systemic infection with Gram-negative bacteria, *Mycoplasma pneumoniae* or *Chlamydia trachomatis*, as seen in Reiter's syndrome. Reiter's syndrome consists of the triad of conjunctivitis, urethritis and uveitis. [12] Several serologic studies from Scandinavian countries and Australia suggest that *Yersinia* infections may be important contributors.[13]

Bacterial causes of uveitis

Many bacterial species are associated with ocular infections (Table 20.4). *Borrelia burgdorferi* infection may cause a wide variety of ocular problems. Conjunctivitis and keratitis occur commonly in the early stages of infection, probably on an immunologic basis. In the later stages of Lyme disease there may be iridocyclitis, retinal vasculitis, exudative retinal detachment, vitritis and optic disk edema. Orbital pseudotumor and orbital myositis have also been described.[14] [15] [16] The treatment is with systemic antibiotics, using regimens appropriate for the stage of the disease (see Chapter 54).

Ocular lesions are common in the course of secondary syphilis (*Treponema pallidum* infection). The most common manifestation is a bilateral anterior uveitis, sometimes with iris nodules (granulomas). Less common is a choroiditis with large, white, 'geographic' lesions. There may also be retinal vasculitis, vitritis and papillitis.[17] [18] [19] Early

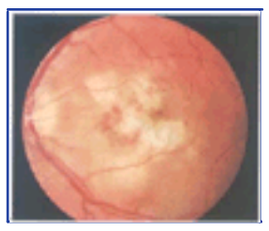

Figure 20-6 Neuroretinitis with a macular star associated with *Bartonella* infection. Note the swelling of the optic disk with hard exudate in the macula in the so-called 'stellate' pattern.

syphilis tends to be associated with a florid uveitis and Venereal Disease Research Laboratory (VDRL)-positive syphilitic meningitis, often in HIV-infected patients whereas late syphilis produces a chronic posterior uveitis with associated subclinical neurosyphilis.[20]

Syphilis should be considered in the differential diagnosis of any posterior segment inflammation or any bilateral anterior segment inflammation. The diagnosis is made serologically and treatment is according to the stage of the syphilis (see Chapter 75).

Bartonella henselae in immunocompetent patients has recently been reported as a cause of stellate neuroretinitis, also known as macular star (Fig. 20.6), and of Parinaud's oculoglandular fever with retinitis. Macular star can also seen in *Toxocara canis* infection in children and as a benign postviral illness. Cats are thought to be an important reservoir of *B. henselae*, and most infections have occurred in patients with a clearcut history of exposure to cats.[21] *Bartonella* infection should be suspected in any patient with optic disk edema and intraocular inflammation, especially if retinal lesions are seen.[22]

The infectious agent of Whipple's disease, *Tropheryma whippelii*, is associated with a multisystem disorder, which can include panuveitis, retinitis and choroiditis. The diagnosis can be made by biopsy of affected tissue (see Chapter 45). [23]

Rarely, other bacterial infections have been associated with uveitis distinct from endogenous endophthalmitis. The list includes leptospirosis,[24] brucellosis[25] and tularemia.[26]

Mycobacterium tuberculosis infection can cause anterior uveitis, posterior uveitis or isolated choroiditis. This infection should be a consideration in every case of nonspecific uveitis of unknown cause. There is nearly always active extraocular disease. The anterior uveitis may be acute or chronic, unilateral or bilateral, and there may be granulomatous keratic precipitates. A periphlebitis resembling that seen in sarcoidosis may occur, along with choroidal infiltrates; these

TABLE 20-5 -- Fungal causes of retinitis and uveitis.

FUNGAL CAUSES OF RETINITIS AND UVEITIS			
Fungal infection	**Systemic infection**	**Features of retinitis or uveitis**	**Other ocular disease**
***Candida* spp.** (see Fig. 20.7)	Risk factors for candidemia	Whitish fluffy patch of retinitis and some vitritis	Anterior segment cells with hypopyon; corneal abscess
Histoplasma capsulatum (see Fig. 20.8)	History of residence in an endemic area; no extraocular infection evident	Choroidal granulomas, scars; optic neuritis; no vitritis	No anterior segment inflammation
These ocular problems are seen in immunocompetent adults and children.			

infiltrates represent miliary tubercles. The uveitis usually improves within 2 weeks of the start of specific antituberculous treatment.

Infection with *Mycobacterium leprae* also may result in ocular inflammation. Corneal complications caused by peripheral nerve damage is the most common ocular complication, and uveitis is seen in only 2% of cases.[27]

Bacterial endophthalmitis is considered in detail in Chapter 19 . Whereas most instances of bacterial endophthalmitis are exogenous, following surgery or trauma, between 2 and 8% of cases arise from metastatic (endogenous) infection of the eye in the course of bacteremic illness.[28] The possibility of metastatic endophthalmitis should be considered in all patients with unexplained ocular inflammation, especially in those with significant underlying medical diseases. The ophthalmic findings are variable, but generally fall within the following patterns:

- one or more focal areas of inflammation within the uveal tract or retina;
- diffuse involvement of the anterior segment but sparing of the retina, choroid and vitreous;
- diffuse involvement of the retina, choroid and vitreous, but sparing of the anterior segment; and
- panophthalmitis.

The diagnosis can usually be made by blood cultures, identification of a primary source of infection, and, if necessary, aspiration and culture of the vitreous. Treatment typically does not salvage vision.

Fungal causes of uveitis

The most common fungal species that cause metastatic endophthalmitis are *Candida* spp. (Table 20.5), especially *C. albicans*. The hallmark lesion is a yellow-white, fluffy patch of retinitis with indistinct borders (Fig. 20.7), almost always associated with vitritis.

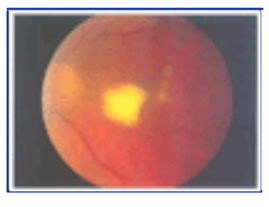

Figure 20-7 A focal area of superficial retinitis and vitritis. This occurred secondary to *Candida* albicans.

Classic 'puff balls' of the vitreous cavity may not been seen if the infection is limited to the choroid. The chorioretinal lesions may mimic those of toxoplasmosis, but inactive retinal scars are not seen in *Candida* infection. However, some cases of *Candida* endophthalmitis have an indolent course and some even improve spontaneously. The diagnosis is suspected from the appearance of the lesions in a typical clinical setting. Blood cultures may assist in the diagnosis, and vitreous

culture may prove it. Patients with *Candida* fungemia should be considered candidates for routine ophthalmoscopic screening. Treatment of *Candida* infections is discussed in Chapter 208 .

Whereas metastatic *Candida* infection usually occurs in a setting of active fungemia, *Histoplasma* ocular infection usually occurs without evident extraocular infection (see Table 20.5). The lesions arise from previous hematogenous spread to the choroid, producing a granuloma that usually becomes an inactive scar.[29] Vitritis is supposedly never seen, but disk edema with optic neuritis can occur acutely. As a late complication, choroidal neovascular membranes can emanate out of the old choroidal scars leading to retinal edema, hemorrhage and permanently decreased central vision.

A diagnosis of ocular histoplasmosis is suspected by finding the characteristic choroidal lesions (Fig. 20.8) in a patient who has resided in an area endemic for the fungal infection. Serologic or skin tests for histoplasmosis are usually positive but do not prove the diagnosis. The organisms have never been cultured from an intraocular source so the relation to *Histoplasma* infection is inferential. Pseudohistoplasmosis syndromes also exist because the characteristic chorioretinal scars are rarely seen in patients who have never resided in an endemic area and who are *Histoplasma* antibody negative. The treatment of ocular histoplasmosis depends on the stage of infection. Corticosteroids may be used to treat the acute choroidal granulomas, but have little role in the chronic, neovascular stage when there is no active inflammation. Laser photocoagulation is usually employed if there is a choroidal neovascular membrane that is not subfoveal; ocular photodynamic therapy or surgery may be carried out for subfoveal lesions because the laser would cause a permanent blind spot. There is no indication for antifungal treatment in these eyes.

Rarely, other fungi such as *Coccidioides*, *Blastomyces* and *Aspergillus* spp. and *Cryptococcus neoformans* may cause chorioretinitis by hematogenous spread. *Nocardia* spp. (Fig. 20.9) also occasionally cause chorioretinitis. Although quite rare overall, these

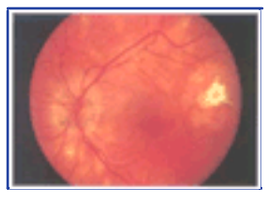

Figure 20-8 The classic ocular findings of previous histoplasmosis. Note the peripapillary atrophy and punched-out yellowish chorioretinal scars. An old choroidal neovascular membrane is present in the center of the histoplasmosis scar temporal to the macula.

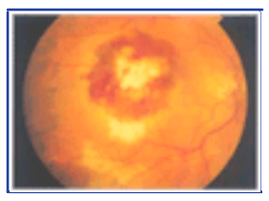

Figure 20-9 A *Nocardia* chorioretinal abscess. This abscess is in the macula of a patient on systemic immunosuppression following heart transplant.

infections develop more commonly in immunosuppressed individuals. The diagnosis should be suspected on confirmation of the associated systemic infection. Systemic antifungal treatment is required.

Parasitic causes of uveitis

Ocular infection by *Toxocara canis*, the dog roundworm, occurs in young children, especially boys, aged from 6 months to 4 years (Table 20.6). *Toxocara cati*, the cat roundworm, has been implicated, but the organisms have never been positively identified in the human eye. The typical presenting complaint is decreased vision, strabismus, or leukocoria (a whitish lesion in the pupil). *Toxocara* infection is acquired by the ingestion of soil contaminated with embryonated eggs (see Chapter 246). There may be a history of pica. The larvae are believed to reach the eye by hematogenous spread. However, most affected children do not have a history of or current evidence of visceral larva migrans. The ocular findings are generally unilateral.

There are several ophthalmoscopic presentations of ocular toxocariasis (Fig. 20.10). The features they have in common are a whitish or yellowish retinal mass, representing a granuloma surrounding the larva, and the eventual formation of traction bands between the vitreous and the granuloma, which may result in retinal folds and exudative retinal detachment. [30] There may be diffuse posterior uveitis, sometimes called nematode endophthalmitis. There is usually dense vitreous inflammation. Unlike bacterial endophthalmitis, there is little or no pain or anterior segment

TABLE 20-6 -- Parasitic causes of retinitis and uveitis.

PARASITIC CAUSES OF RETINITIS AND UVEITIS			
Parasitic infection	**Systemic infection**	**Features of retinitis or uveitis**	**Other ocular disease**
Toxocara canis (see Fig. 20.10)	Children 6 months to 4 years of age; no extraocular infection	Usually unilateral; pale granulomatous mass or focal retinitis; traction bands; vitritis	No anterior segment inflammation
Toxoplasma gondii (see Fig 20.11 , Fig 20.12)	Usually acquired *in utero* but not evident systemically	Recurrent self-limiting attacks of chorioretinitis and vitritis ('headlight in the fog')	May be keratic precipitates (granulomatous reaction) in anterior chamber
These ocular problems are seen in immunocompetent adults and children.			

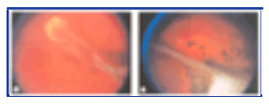

Figure 20-10 Ocular toxocariasis. (a) The posterior pole of a left eye affected by toxocariasis. There is severe macular distortion and dragging of the retina toward a granuloma in the inferotemporal retinal periphery. (b) The periphery of the inferotemporal retina in the same eye showing the granuloma.

inflammation. Other presentations include localized retinitis affecting the macula or the peripheral retina, and acute optic neuritis, in which the granuloma overlies the optic disk. A macular star (neuroretinitis), which is characteristic of toxocariasis, certain viral infections and bartonellosis, may be seen as a result of leakage from vessels in the optic disk.

The major differential diagnoses of ocular toxocariasis are noninfectious diseases such as retinoblastoma and Coats' disease, a congenital vascular disorder. Peripheral blood eosinophilia is rare in ocular toxocariasis. A serum enzyme-linked immunosorbent assay (ELISA) antibody titer to the parasite of 1 to 8 or more supports the diagnosis. A positive ELISA titer of the intraocular fluid, which may be found even if the serum titer is negative, is highly indicative of the infection. Treatment is directed toward stemming the intraocular inflammation, clearing any media opacity and preventing permanent distortion in the retinal architecture. Corticosteroids, usually applied locally in the eye, and cataract and/or vitrectomy surgery are the mainstays of treatment.

Toxoplasma gondii infection is a common cause of posterior segment infection in children and adults (see Table 20.6). In the immunocompetent, it represents the most common cause of infectious

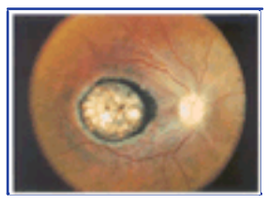

Figure 20-11 An old, inactive congenital macular toxoplasmosis scar. The patient's vision was 20/400.

posterior uveitis worldwide. In many countries, most cases of ocular toxoplasmosis are thought to represent reactivations following primary intrauterine infection (see Chapter 65). However, in some countries, such as Brazil, postnatally acquired infection is the more common antecedent of ocular toxoplasmosis. Postnatally acquired toxoplasmosis is followed by ocular toxoplasmosis in fewer than 5% of cases. The infection as it presents in immunosuppressed patients is discussed later in this

chapter.

The lesions of ocular toxoplasmosis are found preferentially in the nerve fiber layer of the retina, but can affect any layer including the choroid. [31] Healed lesions of congenital toxoplasmosis are flat, atrophic, variably pigmented chorioretinal scars that have a propensity for the macular area of the fundus (Fig. 20.11). There may be recurrent bouts of uveitis, caused by the rupture of cysts, releasing trophozoites. Most patients have their first reactivation before 30 years of age. Ophthalmoscopic examination shows vitritis and one or more white retinal lesions that are usually round or oval and up to 5mm in diameter. There may be many such lesions surrounding an old scar (Fig. 20.12). Aggregates of vitreous cells over the active lesions are the rule. The appearance of the vitreous haze and the white granuloma has been likened to a 'headlight in the fog'. There

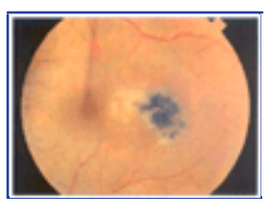

Figure 20-12 A reactivated area of retinal toxoplasmosis. The lesion is the area of whitening and is adjacent to an old scar, just temporal to the macula of the left eye.

TABLE 20-7 -- Characteristics of some infections causing posterior uveitis/retinitis without extraocular manifestations.

CHARACTERISTICS OF SOME INFECTIONS CAUSING POSTERIOR UVEITIS/RETINITIS WITHOUT EXTRAOCULAR MANIFESTATIONS				
	Usual number of lesions	**Appearance of lesions**	**Distribution of lesions**	**Accompanying vitritis**
Toxoplasma gondii (see Fig. 20.11 & Fig. 20.12)	One to a few	'Headlight in fog'	Random, but heavier in macular area	Almost always
Toxocara canis (see Fig. 20.10)	One	Granuloma	Macular, optic disk or periphery	Always
Histoplasma capsulatum (see Fig. 20.8)	Multiple	Choroidal granulomas	Various	Almost never
Treponema pallidum	Diffuse retinochoroiditis	Bilateral anterior uveitis; large white geographic choroidal lesions	Random	Always
Borrelia burgdorferi	Focal or diffuse	Anterior uveitis, retinal vasculitis, exudative retinal detachment	Random	Usually
Mycobacterium tuberculosis	One or a few	Retinitis or chorioretinitis	Random	Usually

may be retinal hemorrhages, sheathing of arterioles and venules, and papillitis. There may be a granulomatous reaction in the anterior chamber with keratic precipitates and conjunctival injection.

A diagnosis of ocular toxoplasmosis is based on the characteristic appearance of the lesions together with a positive serum antibody test for *T. gondii* (see Chapter 245). Other possible causes of localized necrotizing retinitis with vitritis, such as syphilis or tuberculosis, should be considered (Table 20.7). Further support for the diagnosis can be obtained by determining the ratio of *Toxoplasma* antibody between serum and a sample of ocular fluid.[32]

In otherwise healthy persons, flare-ups of ocular toxoplasmosis tend to be self-limited over a period of weeks to months. Not all active lesions need to be treated. The highest priority for treatment is for lesions that threaten the fovea or the optic nerve or large areas of the nerve fiber layer, as well as those that produce enough vitritis to impair vision. Once the fovea is directly involved, visual acuity is usually permanently compromised.

Treatment is the same as for other systemic forms of toxoplasmosis — pyrimethamine, sulfadiazine and clindamycin (see

TABLE 20-8 -- Infectious retinitis and uveitis in neonates.

INFECTIOUS RETINITIS AND UVEITIS IN NEONATES			
	Systemic infection	**Features of uveitis/retinitis**	**Other ocular disease**
Viral infections			
Herpes simplex virus	Disseminated infection usually evident	Anterior uveitis early; optic neuritis, retinal hemorrhages and necrosis later; sparing of choroid	Conjunctivitis, keratitis common
Varicella-zoster virus	Features of congenital varicella-zoster (rare)	Chorioretinitis	Microphthalmia; cataracts
Cytomegalovirus (CMV)	Features of congenital CMV infection	Retinitis in 20–25% of infants with symptomatic CMV infection; resembles that seen in adults with AIDS	None
Rubella	Congenital rubella syndrome	Mild, self-limiting chorioretinitis + vitritis in 25–50% with congenital rubella syndrome; unilateral or bilateral; eventual salt-and-pepper fundus	None
Bacterial infections			
Treponema pallidum	Stigmata of congenital syphilis	Salt-and-pepper fundus; occasionally optic atrophy, retinal vascular sheathing	Delayed manifestation: acute interstitial keratitis (age 5–10 years)
Fungal infections			
Candida spp. (see Fig. 20.7)	*Candida* fungemia; risk factors	Whitish fluffy patch of retinitis, some vitritis	None
Parasitic infections			
Toxoplasma gondii (see Fig. 20.11 , Fig. 20.12)	Rarely, encephalitis, hydrocephalus at birth	Rarely, chorioretinitis at birth	None

Chapter 209). Many ophthalmologists avoid pyrimethamine initially because of its marrow-suppressive effects, preferring to rely on the other two agents. Corticosteroids are often used concomitantly, but not without the anti-infective drugs. Laser photocoagulation may be used for active lesions unresponsive to medication and pars plana vitrectomy may be used to clear vitreous opacities.

Rarely, uveitis occurs in other parasitic infections such as cysticercosis, myiasis and onchocerciasis.

Uveitis in neonates

There are several causes of uveitis in neonates (Table 20.8).

Herpes simplex virus

Congenital infection by HSV is usually acquired directly from the infected birth canal, but sometimes occurs transplacentally or through the amniotic fluid. The congenital infection may mainly affect:

- the skin, eyes and oral cavity;
- the internal organs ('disseminated disease'); or
- the central nervous system.

The eyes may be involved in any of these presentations. Ocular findings may first appear from 1 week to several months after birth, and must be carefully sought by periodic ophthalmoscopic examination of infants at risk. About 80% of isolates are HSV-2 and this strain is associated with more severe ocular infections than HSV-1.

Congenital HSV infection may cause a wide variety of ocular lesions. In the anterior segment, conjunctivitis and keratitis (punctate, dendritic or geographic) are most common, and are usually seen in the acute phase of the infection. There is often an associated anterior uveitis. Peripheral anterior and posterior synechiae and secondary cataracts may be seen.

Posterior segment changes tend to occur later in the infection. [33] There may be disk edema with optic neuritis. Retinal involvement ranges from scattered hemorrhages to widespread retinal necrosis similar to that seen in the ARN syndrome. There may be retinal vasculitis and vitritis, and retinal detachments in severe cases. Residual changes include pigment migration and clumping (salt-and-pepper appearance), macular chorioretinal scars, optic atrophy and pre-retinal neovascularization.

The diagnosis and treatment of neonatal HSV infection are reviewed in Chapter 205 . Because the ocular lesions are part of a generalized infection, systemic treatment is required. In the exceptional instances in which the anterior segment of the eye is the only evident site of the suspected infection, viral cultures of the conjunctiva should be carried out and topical treatment alone may be given.

Varicella-zoster virus

Congenital infection by VZV is very rare (see Chapter 65). The ocular findings in the neonate are microphthalmia, chorioretinitis and cataract formation. If there is active chorioretinitis, an antiviral agent such as aciclovir should be given intravenously.

Cytomegalovirus

Congenital infections by CMV are usually asymptomatic (see Chapter 65). With symptomatic infections, retinitis occurs in 20–25% of cases. If retinitis is not evident at birth, it is unlikely to occur later. The retinitis resembles that seen in adult patients with HIV infection (see below).

Rebella

In the congenital rubella syndrome, ocular manifestations occur in 25–50% of affected children.[34] The classic finding is a mild, self-limited

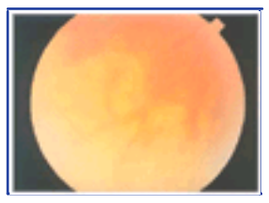

Figure 20-13 Typical appearance of the retina in the acute retinal necrosis syndrome. There is dense peripheral retinal whitening with a geographic border. Satellite lesions are common. The view is hazy owing to vitritis.

chorioretinitis that may be unilateral or bilateral. There may be vitritis. Vision is usually not impaired. A common sequel is a salt-and-pepper fundus caused by changes in the retinal pigment epithelium (see Fig. 20.4). A similar appearance is produced by congenital syphilis and influenza. Other ocular manifestations of congenital rubella include cataracts and a mild anterior uveitis that may cause posterior synechiae. There is no specific treatment.

Syphilis

The most common ocular manifestation of congenital syphilis (see Chapter 75) is salt-and-pepper fundus. The lesions are almost always bilateral, but may be sectoral or exclusively peripheral. They are the result of chorioretinitis, which may be evident at birth or appear in the first few years of life. Visual acuity is rarely affected. In more severe cases, there may be diffuse sheathing of the retinal vessels, optic atrophy and migration of the retinal pigment epithelium in a manner resembling retinitis pigmentosa.[35] Acute interstitial keratitis is a delayed manifestation of congenital syphilis. It may be accompanied by anterior uveitis and secondary glaucoma may develop. The lesions of keratitis are self-limited, but treatment of the syphilitic infection is indicated.

Other causes

Candida spp. may cause retinitis and vitritis in neonates. The infection is acquired after birth and the ocular infection is usually hematogenous. Risk factors are prematurity, low birth weight, sepsis, malnutrition and treatment with broad-spectrum antibiotics. The clinical presentation and treatment are as for adults (see Chapter 208).

Toxoplasma chorioretinitis is usually acquired in utero. Although the ocular complications usually present long after the neonatal period, they are sometimes evident at birth in the TORCH syndrome (see Chapter 65). Manifestations include encephalitis, hydrocephalus and bilateral chorioretinitis. Treatment is described in Chapter 209 .

Acute retinal necrosis syndrome

The ARN (acute necrotizing herpetic retinitis) syndrome is a recently described, rare syndrome of a vaso-obliterative retinal and choroidal vasculitis, diffuse retinal necrosis and vitritis. It is bilateral in one-third of patients, although the two eyes need not be affected simultaneously. It has been reported in children as young as 8 years of age, but it occurs most often in adults. Conclusive evidence now exists that VZV is the primary cause; HSV is a less common cause.[36] Patients infected with HIV who develop herpes zoster of the first division of the trigeminal nerve appear to have a high risk of subsequent ARN, often bilateral, over the next few weeks to months.[37]

Acute retinal necrosis causes diffuse arteritis and phlebitis with sheathing of the retinal vessels and striking white areas of retinal necrosis (Fig. 20.13). Broad areas of the peripheral retina are involved early, but the macula is usually spared initially. There is often mild inflammation of the anterior segment. As the disease progresses, there is increasing vitritis. Optic neuropathy with disk edema may appear.

Retinal detachment follows in up to 75% of patients. The detachments are notoriously difficult to repair. Previous prophylactic laser treatment of healthy retina posterior to the areas of necrosis reduces the incidence of retinal detachment.

The mainstay of treatment for ARN is aciclovir, given in high dosage intravenously (e.g. 12–15mg/kg q8h for 7–14 days). Oral aciclovir or one of its prodrugs should be continued for 2–3 months to lessen the risk of fellow eye involvement. Systemic corticosteroids are given as well, to decrease inflammation. Retinal detachments require complex vitreoretinal surgery for successful repair. It has been suggested that patients who have AIDS and who develop ophthalmic zoster are given long-term oral prophylaxis with aciclovir, but there is no proof of benefit from this approach.[37]

Uveitis and retinitis in immunosuppressed patients

Because of the AIDS pandemic, ophthalmologists are now encountering a variety of intraocular infections that were previously either unknown or extraordinarily rare.

Cytomegalovirus retinitis

The most common ocular infection in HIV-positive adult patients is CMV retinitis. In developed countries prior to 1996, CMV retinitis occured in about 35% of adult patients who had AIDS. Since highly active antiretroviral therapy (HAART) has become widely available in developed countries, the incidence of CMV retinitis has dropped dramatically. CMV retinitis rarely occurs in patients who have $CD4^+$ lymphocyte counts over 50 cells/mm^3 .[38] Pediatric patients who have AIDS have a much lower incidence of CMV retinitis than adults, presumably because most children have not been exposed to CMV. In one study of African patients who had AIDS, there were no cases of CMV retinitis.[39]

Symptoms of CMV retinitis tend to be absent or minimal at the start. 'Floaters', decreased peripheral vision or metamorphopsia are occasionally seen. Often, an active infection is noted on a routine ophthalmologic screening examination. In 40% of patients with CMV retinitis, the lesions are bilateral at presentation. Nearly 75% of patients present to the ophthalmologist with disease that is considered immediately sight-threatening due to the proximity of active lesions to the optic nerve or the macula. Pain, redness or more than a mild anterior uveitis are unusual with CMV retinitis.

The hallmark lesion of CMV retinitis is a necrotizing, full-thickness retinitis resulting in retinal cell death (Fig. 20.14). Retinal tissue adjacent to major retinal blood vessels or the optic disk is often affected initially, consistent with the theory that CMV is spread hematogenously. The areas of active retinitis have a granular appearance and are dirty-white in color. Hemorrhage is common, owing to damage to vascular endothelial cells. The appearance has been likened to a 'brush fire' and to 'ketchup and cottage cheese'. The retinitis spreads contiguously as well as by producing satellite lesions. It is common to see areas of healed retinitis alongside areas of active necrosis. Areas of burned-out necrosis show an absence of retinal tissue while the underlying retinal pigment epithelium assumes a salt-and-pepper appearance.

Loss of vision occurs because of both the death of retinal cells and retinal detachment. About 10–20% of eyes with CMV retinitis can be expected to suffer detached retina. The risk is time dependent; after 1 year the risk approaches 50%. Although vitreous surgery to

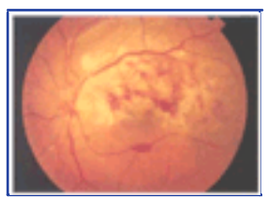

Figure 20-14 Cytomegalovirus infection with granular retinal whitening along the major blood vessels with mild hemorrhage. The view is clear because there is only mild vitritis.

repair the detached retinas is successful in more than 90% of instances, visual results are often limited by the underlying disease process.[40]

Three agents are available for systemic administration for the treatment of CMV retinitis: ganciclovir, foscarnet and cidofovir.[41] Although all three are typically effective initially, they all have potential side-effects and disadvantages that limit their long-term use. The mean time to ophthalmoscopic progression of the retinitis is only 2 months for all three medications, making long-term efficacy the major problem with intravenous therapy. An orally administered ganciclovir prodrug (valganciclovir) apprears to be equally effective as the intravenous preparation. Systemically administered agents are most useful for preventing progression of the extraocular infection that may accompany or follow CMV retinitis (see Chapter 125).

Direct intraocular treatment in the form of intravitreal injections of ganciclovir or foscarnet or intravitreal insertion of a sustained release ganciclovir implant can achieve much higher intraocular concentrations of drugs than intravenous therapy. The long-term efficacy of such local forms of therapy is superior to that of intravenous therapies. The mean time to progression of newly diagnosed CMV retinitis treated with a ganciclovir implant is 220 days (7 months), which is approximately the designed life span of the device. If the devices are replaced before the end of their estimated life span, however, at least 75% of eyes can be expected to suffer no progressions.[42] [43] High-dose (2mg per dose) weekly intravitreal injections of ganciclovir appear to have similar long-term efficacy.[44]

Implants and intravitreal injections carry the risks of invasive ocular procedures. Intravitreal injections must be given at least once a week, whereas ganciclovir implants only need to be placed at intervals of 6–8 months. If no concurrent systemic anti-CMV medication is given to patients treated with local therapy, there is a risk of systemic CMV. The incidence of systemic CMV infection in the year following initiation of exclusively local treatment for CMV retinitis is reportedly 11–30%.[43] Ongoing treatment of healed CMV retinitis or prophylaxis against end-organ infection can generally be stopped in patients who gain immune reconstitution with highly active antiretroviral therapy.[45] [46]

Other causes of uveitis and retinitis

About 75% of patients with HIV-1 infection have a non-sight-threatening retinopathy that may be caused by the HIV-1 itself.[47] The lesions are multiple, bilateral, cotton-wool spots and scattered retinal

Figure 20-15 HIV retinopathy. There are multiple superficial white patches in the retina (cotton-wool spots). These do not affect vision and typically wax and wane over time.

hemorrhages (Fig. 20.15). If there is concern that such lesions may be due to CMV, close observation over a period of days to weeks with documentation by photographs is recommended. CMV retinitis will invariably progress, whereas cotton-wool spots caused by HIV will resolve.

Opportunistic infections with *Pneumocystis carinii*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and atypical mycobacteria, may produce multifocal choroiditis in patients who have AIDS. These infections are rarely sight-threatening, but they should alert the clinician to the presence of a systemic infection. Unfortunately, it is generally not possible for the ophthalmologist to distinguish between these possible opportunistic infections on the basis of the eye examination alone.[47]

Other important posterior segment infections that can occur in HIV-positive patients include toxoplasmosis, syphilis and infection with HSV and VZV. Whereas toxoplasmosis in immunocompetent patients produces a slowly progressive, focal, relapsing chorioretinitis, in immunocompromised patients it can produce a severe, diffuse retinitis.[48] Serologic testing may be helpful. Nearly one-third of patients who have AIDS and *Toxoplasma* retinitis have concurrent toxoplasmosis of the central nervous system. Ocular syphilis can also mimic CMV retinitis, but the lesions of secondary syphilis are usually a choroiditis, rather than a retinitis.[49] Syphilis is diagnosed serologically. Neuroretinitis caused by *Bartonella* infection has been reported in a patient with AIDS.[21]

Acute retinal necrosis syndrome

The ARN syndrome (see above) occurs in both healthy and immunosuppressed people, including those with AIDS. Acute retinal necrosis differs from CMV retinitis in that the lesions of ARN are typically peripheral and the course is much more rapid. In addition, significant vitreous cells are a prominent feature of ARN, but not of CMV retinitis. Patients who have HIV infection and who develop zoster of the first division of the trigeminal nerve appear to have a high risk of subsequent ARN, which is often bilateral, over the next few weeks to months.[37] Therefore, when a rash of ophthalmic zoster appears, patients who have AIDS should be followed carefully for

signs of ARN.

A specific type of rapidly progressive ARN (PORN) due to VZV infection has been described in patients who have AIDS and a low $CD4^+$ lymphocyte count.[50] Without aggressive therapy, PORN results in bilateral loss of vision within days to weeks. The recommended treatment is a combination of foscarnet, together with either ganciclovir or aciclovir, given intravenously or via intravitreal injection.

Perhaps the most common cause of noninfectious anterior uveitis in HIV-positive patients is a dose-related reaction to rifabutin. Patients receiving rifabutin for prophylaxis against *Mycobacterium avium-intracellulare* in conjunction with another agent that interferes with the metabolism of rifabutin (such as clarithromycin or ritonavir) and patients weighing less than 65kg who are receiving 600mg of rifabutin are at particular risk.[51] [52] The incidence of rifabutin-induced uveitis is 20% among AIDS patients taking the medication, but it has been reported in HIV-negative patients as well. Treatment with corticosteroids and discontinuation of the medication usually results in prompt reversal of the inflammation.[53] Systemic cidofovir has also been reported to cause uveitis in patients who have AIDS.

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Chapter 21 - Practice Point

Eye problems and the patient in the intensive care unit

Harminder S Dua

Introduction

The critically ill patient in an intensive care unit (ICU) is particularly vulnerable to bacterial corneal infections, which are by far the most serious ocular surface problems encountered in these patients. Exposure keratitis and associated corneal abrasions are other, relatively minor problems, although they are significant because they are often the precursors of serious corneal infection (see Chapter 18). Despite considerable awareness of the problem and various protocols followed by ICUs all over the world, bacterial keratitis with sequelae of corneal scarring and visual impairment continues to occur in ICU patients. Critically ill patients are also susceptible to endogenous, blood-borne intraocular infections (endophthalmitis), which, although rare, can result in rapid and permanent blindness in the affected eye(s).

Ocular surface defense

The exposed position of the eyes ensures constant encounters with environmental pathogens. However, the ocular surface is equipped with a wide range of protective mechanisms. The lids provide physical and mechanical protection. The blink reflex aids flow of tears across the eye surface and helps in flushing contaminating organisms down the nasolacrimal passages into the nasopharynx, where they are effectively dealt with by the lymphoid tissue. The conjunctiva is also provided with lymphoid tissue, which can mount a competent humoral and cell-mediated immune response against invading organisms. Constant evaporation of tears keeps the ocular surface temperature down and so makes it less favorable to bacterial growth. Furthermore, tears have several important antimicrobial constituents such as immunoglobulins, lysozyme, lactoferrin, complement components, ceruloplasmins, defensins and betalysins. The integrity of the corneal surface epithelium is the single most important defense against bacterial invasion. In critically ill ICU patients, several of the above factors are breached or compromised, predisposing to infection.

Risk factors for eye infection in intensive care unit patients

Important risk factors for bacterial keratitis in the setting of an ICU are listed in Table 21.1 . Exposure, inadequate blinking and drying (leading to epithelial erosions and abrasions) are important factors that make a critically ill patient susceptible to ocular surface infection. When intermittent positive-pressure ventilation is employed, it leads to venous stasis, body fluid retention and conjunctival edema. Edematous and chemosed conjunctiva prolapses through the lids with consequent drying and bacterial contamination. Infected respiratory tract secretions are a common source of bacterial contamination of the eyes. Suction of copious tracheobronchial secretions when carried out over the patient's head, across the eyes, is known to cause bacterial dispersion and ocular contamination. *Pseudomonas aeruginosa* is the most common pathogen isolated from corneal ulcers in ICU patients. Risk factors for endophthalmitis include chronic nutritional deficiency, especially related to alcoholism, diabetes mellitus, intravenous drug abuse, AIDS and major surgical interventions, particularly on the bowels or infected tissue or organs. *Pseudomonas aeruginosa, Neisseria meningitidis, Streptococcus pneumoniae* and *Bacillus cereus* are the common and important organisms isolated from eyes with endophthalmitis.

Management

Prevention, by eliminating or addressing the risk factors, is the key to management of ocular surface infections in critically ill patients. The eyes should be frequently inspected, every 4–6 hours at least (Table 21.2). Sterile artificial tear drops should be instilled at each inspection and proper lid closure should be maintained at all times. This prevents drying of the corneal surface.

Lid closure can be achieved by placing a strip of microspore adhesive tape horizontally across the closed lid margins. Constant removal and reapplication of adhesive tape can lead to excoriation of the lid skin. The Apache eye patch (BID Instruments Ltd, Aberdeen, UK), which is shaped like a 'T' with an adhesive horizontal arm applied to the upper lid and the long arm provided with a Velcro mechanism, allows repeated opening and closure of the lids without any abrasive effect. Alternatively a temporary 'Frost suture' can be passed through the upper or lower lid. A 4.0 or 5.0 silk suture mounted on a cutting needle is passed horizontally through the skin of the lid, close to the lid margin (upper or lower). At this site, the skin is fairly firmly adherent to the underlying tissues and provides good anchorage to the suture (Fig. 21.1). The two ends are left 2–3 inches (4.5–7.5cm) long and taped to the cheek (or brow). The suture is used as a handle to open or close the lids. Eye patches or sutures can be discontinued when the blink reflex returns to normal. When the lids cannot be apposed owing to rigidity or loss of tissue, as occurs after injury or burns, or when chemosed conjunctiva protrudes through the palpebral aperture, polyacrylamide gel patches of high water content or cling wrap can be used. These provide adequate cover and protection and conserve moisture. Gel patches need to be kept constantly hydrated with sterile normal saline. Instruments used to cut gel patches or cling wrap to appropriate size must be sterile.

Suction of secretions should be carried out from the side of the head rather than over the top of the head. At all times, the eyes

TABLE 21-1 -- Risk factors for corneal and conjunctival infection in patients in intensive care units.

RISK FACTORS FOR CORNEAL AND CONJUNCTIVAL INFECTION IN PATIENTS IN ICUs
Dry eye
Inadequate tear production
Poor or absent blink reflex (e.g. because of coma or the use of paralyzing and sedating drugs)
Exposure as a result of proptosis or poor closure of lids (lagophthalmos), leading to excessive evaporation
Microtrauma
Tips of eye medication dispensers
Cotton wool or gauze wipes or patches applied over open lids
Conjunctival contamination
Nosocomial pathogens, commensals
Infected body fluids (droplets) related to suction from respiratory tract
Conjunctivitis, conjunctival edema (chemosis as a result of ventilation support), with protrusion of conjunctiva through lids
Retained contact lens
Poor host resistance
Malnutrition, chronic alcoholism, immunodeficiency, prolonged corticosteroid medication, diabetes mellitus, overwhelming systemic infection

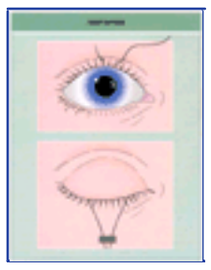

Figure 21-1 Frost suture. A 5.0 silk suture is placed at the upper lid margin through half the tissue thickness (above) and used to tape the lid shut (below).

TABLE 21-2 -- Suggested protocol for eye care in patients in intensive care units.*

SUGGESTED PROTOCOL FOR EYE CARE IN PATIENTS IN ICUs
At the outset
Assess condition of eyes (A–C)
Ensure patient is not wearing contact lenses (soft or rigid)
In road traffic accident patients, exclude conjunctival sac foreign bodies (seek ophthalmic opinion if necessary)
A Eyes are clean and moist, and lids close adequately
If blink reflex preserved:
Inspect eyes at regular intervals (4–6 hours)
Ensure that eyelids remain closed at all times
Keep eyes moist by instillation of simple eye ointment or artificial tear drops at each inspection
If blink reflex is absent:
The lids can be lightly taped shut
B There is conjunctival edema, drying of ocular surface or eyes tend to remain open
Nurse patient with head up (if possible) to encourage dispersion of conjunctival fluid
Remove any obstruction to jugular venous drainage (e.g. with endotracheal tube harness)
Instil drops or ointment as above
Close eyes fully with adhesive tape or polyacrylamide gel patches
Ensure polyacrylamide gel patches are kept hydrated with sterile normal saline
Inspect at 4–6 hours intervals for first 24 hours
If eyes remain moist and satisfactory, continue as above
If dryness increases or epithelial abrasions develop, increase frequency of simple paraffin eye ointment and consider topical antibiotic prophylaxis (gentamicin or ciprofloxacin), particularly if the patient has infection at any other site
C The eyes are/become red, inflamed and sticky
Suspect conjunctivitis or keratitis
If there is conjunctival discharge, the eyes should not be patched or taped shut
Allow free drainage of discharge, irrigate with sterile normal saline, culture swabs from conjunctival sac and commence (prophylactic) antibiotics
Because eyes are open, risk of drying becomes greater
Increased frequency of inspections and instillation of ocular lubricants
Refer to an ophthalmologist
Avoid
Touching tips of drop or ointment applicators to ocular surface
Using the same drop or ointment units for right and left eyes
Placing patch or tape over partially open eyes
Patching of eyes with discharge
Suction of secretions across the head of the patient and suction without covering the eyes
Routine eye swabs for culture in all patients in ICUs
Embarrassment of leaving contact lens in situ

** Modified from protocol used in the ICU of the University Hospital, Queen's Medical Centre, Nottingham, UK (Dr Bernard Riley, personal communication).*

should be adequately covered and shielded during suction maneuvers. In the presence of respiratory or other infections, eye swabs should be taken every 1–2 days to detect early colonization of the conjunctival sac. In the presence of a positive swab, prophylactic antibiotic drops should be commenced. Chloramphenicol or ciprofloxacin drops provide a broad range of cover. Gram staining helps to tailor the choice of antibiotic. Antibiotic ointments smeared on the surface of the lid skin will make it difficult to retain adhesive tape.

Early detection of infection is the next best step in management. Appearance of lid swelling and conjunctival swelling and redness are

important signs. Any discharge or crusting of the lid margins should be viewed with suspicion. Early signs of corneal involvement include loss of the normal shine or luster, corneal haziness and localized white infiltrates. Corneal staining with 2% fluorescein is a useful bedside test and, if positive, indicates corneal abrasion or ulcer. Instil a drop or two of 2% fluorescein, close and open the eyelid to ensure even spread, irrigate with a few drops of sterile saline 20–30 seconds later and view the cornea with a blue light (pen lights with cobalt blue filter attachments are widely available). Any area of the cornea denuded of epithelium will fluoresce green.

Treatment of established corneal infection constitutes an ocular emergency and an ophthalmologist must be involved at the outset. Corneal ulcers, particularly those caused by *Pseudomonas* spp., can progress rapidly with perforation of the cornea and loss of the eye. The usual protocol is to take corneal scrapes for Gram stain and culture (aerobes and anaerobes and fungi) and sensitivity. Eye drops of gentamicin (15mg/ml) and cefuroxime (5% or 10%) (or vancomycin 25mg/ml if the patient is hypersensitive to ß-lactams) are commenced at 1-hourly intervals, each drop alternating every half hour, round the clock, for 24–48 hours. Treatment is tapered or modified according to clinical response and sensitivity results. Ciprofloxacin 0.3% is a useful option, especially if *Pseudomonas* spp. are suspected. For all drops, an initial intensive loading regimen of 1–2 drops every 15 minutes for 4–6 hours is beneficial.

Features that should alert the physician to onset of endophthalmitis include sudden visual impairment, ocular pain and a diminished or altered fundus red reflex on ophthalmoscopy. Pus in the anterior chamber, hypopyon, can occur with both endophthalmitis and keratitis. Treatment of endophthalmitis includes intravitreal (such as amikacin 0.4mg in 0.1ml and vancomycin 1 or 2mg in 0.1ml) and intravenous injection of appropriate antibiotics and vitrectomy. An ophthalmologist should be involved

in the management at a very early stage.

Further reading

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Chapter 22 - Acute and Chronic Meningitis

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INTRODUCTION

Meningitis is the most common serious manifestation of infection of the central nervous system (CNS). Inflammatory involvement of the subarachnoid space with meningeal irritation leads to the classic

TABLE 22-1 -- Microbial causes of meningitis.

MICROBIAL CAUSES OF MENINGITIS		
Acute bacterial meningitis		
Common etiologic species	*Haemophilus influenzae*	*Listeria monocytogenes*
	Neisseria meningitidis	*Streptococcus agalactiae*
	Streptococcus pneumoniae	
Other etiologic species	*Staphylococcus aureus*	*Flavobacterium* spp.
	Staphylococcus epidermidis	*Moraxella* spp.
	Escherichia coli	*Propionibacterium acnes*
	Klebsiella pneumoniae	*Enterococcus faecalis*
	Pseudomonas aeruginosa	*Salmonella* spp.
	Nocardia spp.	Group A streptococci
	Viridans streptococci	*Serratia* spp.
	Enterobacter spp.	*Acinetobacter* spp.
	Proteus spp.	*Pasteurella multocida*
	Citrobacter spp.	*Aeromonas* spp.
Other organisms	Spirochetes	*Leptospira* spp.
	Mycobacteria	*Brucella* spp.
	Borrelia spp.	*Naegleria* spp.
	Rickettsiae	*Acanthamoeba* spp.
Chronic meningitis		
Viruses	Lymphocytic	Varicella-zoster
	Choriomeningitis virus	Arbovirus
	Mumps virus	Flavivirus
	Herpes simplex virus	Echovirus
Bacteria	*Mycobacterium tuberculosis*	*Leptospira* spp.
	Brucella spp.	*Nocardia* spp.
	Treponema pallidum	*Actinomyces* spp.
	Borrelia spp.	*Listeria monocytogenes*
Fungi	*Cryptococcus neoformans*	*Blastomyces dermatitidis*
	Coccidioides immitis	*Sporothrix schenckii*
	Histoplasma capsulatum	*Aspergillus* spp.
	Candida spp.	
Parasites	*Cysticercus* spp.	*Schistosoma* spp.
	Angiostrongylus cantonensis	*Echinococcus* spp.
	Paragonimus westermani	*Strongyloides* spp.
	Gnathostoma spinigerum	
Aseptic meningitis		
Viruses	Enteroviruses	HIV
	Mumps	Morbillivirus
	Herpesviruses	Rubivirus
	Lymphocytic choriomeningitis	Epstein-Barr virus
	Adenoviruses	Arboviruses

triad of headache, fever and meningism, and to a pleocytosis in the cerebrospinal fluid (CSF). Meningitis is divided clinically into acute and chronic disease; acute meningitis develops over hours or days, whereas the symptoms of chronic meningitis evolve over weeks or even months.

Acute meningitis is caused by a variety of infectious agents (Table 22.1). The most serious form of acute meningitis is caused by pyogenic bacteria, such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*. [1] [2] [3] Group B streptococci (*Streptococcus agalactiae*), Enterobacteriaceae and *Listeria monocytogenes* are the major pathogens in the neonatal period (Table 22.2).[3] [4]

TABLE 22-2 -- Bacterial causes of acute meningitis related to age and predisposing factors.

BACTERIAL CAUSES OF ACUTE MENINGITIS RELATED TO AGE AND PREDISPOSING FACTORS		
Age	**Pathogen**	**Predisposing factors**
0–4 weeks	*Streptococcus agalactiae*	Birth complications
	Escherichia coli	Birth complications
	Listeria monocylogenes	Maternal infection
	Streptococcus pneumoniae	CSF leak, asplenia
1–3 months	*Escherichia coli*	Nosocomial colonization
	Haemophilus influenzae	CSF leak, sinusitis, otitis
	Listeria monocytogenes	Immunodeficiency
	Neisseria meningitidis	Complement deficiencies, immunodeficiency
	Streptococcus agalactiae	Nosocomial colonization
	Streptococcus pneumoniae	CSF leak, immunodeficiency
3 months–18 years	*Haemophilus influenzae*	Age 3 months–6 years, CSF leak, otitis media, sinusitis
	Neisseria meningitidis	Epidemics, terminal complement deficiencies
	Streptococcus pneumoniae	CSF leak, otitis media, sinusitis, asplenia
18–50 years	*Neisseria meningitidis*	Epidemics, immunodeficiency
	Streptococcus pneumoniae	CSF leak, otitis media, sinusitis, asplenia, alcoholism
>50 years	*Listeria monocytogenes*	Immunodeficiency, diabetes mellitus
	Streptococcus pneumoniae	CSF leak, otitis media, sinusitis, asplenia, alcoholism
Not age-related	Enterobacteriaceae	Neurosurgery, nosocomial acquisition
	Staphylococcus aureus	Neurosurgery, CSF leak, endocarditis, abscesses
	Propionibacterium acnes	Neurosurgery, CSF leak, dermal sinus

Patients who have aseptic meningitis show signs of meningeal inflammation and CSF pleocytosis with predominance of lymphocytes, but no bacterial pathogen can be isolated from CSF or blood. The term 'aseptic meningitis' predates the routine use of tests to detect viruses and other difficult to culture organisms and includes cases caused by highly diverse etiologic agents. Many cases of aseptic meningitis are caused by viruses, primarily enteroviruses (Table 22.3), including Coxsackie viruses (see Chapter 23). Nonviral causes of aseptic meningitis include spirochetes, rickettsiae, *Mycobacterium tuberculosis*, *Leptospira* spp., *Brucella* spp., fungi and noninfectious etiologies (see Table 22.1).[5] While signs and symptoms overlap between bacterial and aseptic meningitis, the latter has a much more favorable prognosis, particularly when caused by viruses.

Chronic meningitis is defined by symptoms of meningeal inflammation with CSF pleocytosis that persist for more than 4 weeks.[5] The diagnosis is based on the history, clinical evidence of meningitis, CSF examination, and often on imaging studies.[6] The differential diagnosis is broad (see Table 22.1). The predominant CSF cell type can provide clues to the underlying disease (Table 22.4).

EPIDEMIOLOGY

Incidence

Acute bacterial meningitis

The incidence of acute bacterial meningitis in the USA is 5–10/100,000 persons per year, resulting in 15,000–25,000 cases annually.[1] The very old and the very young are more commonly affected. The organisms that cause bacterial meningitis vary with the age of the patient (see Table 22.2). Neonatal meningitis has been reported in 1/200–500 live births; at present more than 50% of these cases are caused *by Strep. agalactiae*.[3] [4] Until recently, young children aged 1 month to 2 years have had the highest incidence of meningitis, with *H. influenzae* type b as the predominant pathogen. The use of new conjugate *H. influenzae* type b vaccines has reduced the incidence of invasive *H. influenzae* infection, including meningitis, by more than 90% in developed countries, where the vaccine is widely used.

The incidence of bacterial meningitis shows a peak in winter and early spring and varies greatly in different areas of the world. This variability is accounted for primarily by the epidemiology of *N. meningitidis*, which can cause either sporadic cases or epidemics of meningitis. Small outbreaks typically occur in populations of young adults living in close quarters, such as dormitories of military camps or schools. Major epidemics, which dramatically increase the incidence of the disease, have occurred periodically in certain parts of the world, including sub-Saharan Africa (the so-called 'meningitis belt'), Europe (particularly Scandinavia), Asia and South America. During these epidemics, attack rates can reach several hundred per 100,000 people, with devastating consequences, particularly in areas with limited medical resources.[7]

Special clinical circumstances affect the spectrum of bacterial pathogens likely to cause meningitis in a particular patient. Age is the single most important determinant in this regard (see Table 22.2). Other important factors include surgery, trauma and focal suppurative infections of the head, various forms of immunosuppression and genetic predisposition (see Table 22.2).[8] [9]

Bacterial meningitis can occur in hospitalized patients (nosocomial meningitis). In a study of acute meningitis seen in a large city hospital, almost 40% of cases were nosocomial.[10] Most cases of nosocomial meningitis occur in patients undergoing neurosurgical procedures, including implanting of neurosurgical devices, and in patients who have focal infections of the head. The organisms responsible for nosocomial meningitis differ markedly from those causing community-acquired meningitis and include Gram-negative rods (*E. coli, Klebsiella* spp. *Pseudomonas aeruginosa, Acinetobacter* spp., *Enterobacter* spp. and others), staphylococci and streptococci other than *S. pneumoniae*.[11]

The extent to which alteration of host defenses, such as chemotherapy-induced neutropenia, increases the incidence of meningitis is not known, but it is clear that the organisms causing meningitis in these patients are markedly different from those found in community-acquired meningitis in immunocompetent patients. Bacterial pathogens causing meningitis in neutropenic patients include Gram-negative bacilli, staphylococci, non-pneumococcal streptococci (viridans and ß-hemolytic streptococci) and rare pathogens such as *Stomatococcus mucilaginosus* and *Bacillus cereus*. In patients who have T-cell deficiencies, intracellular pathogens (*Listeria* spp., *Salmonella* spp. and *Nocardia* spp.) must be considered. In addition to bacteria, fungi (*Aspergillus* spp., *Cryptococcus* spp., *Candida* spp.) and viruses (herpes simplex, cytomegalovirus, JC [JC are the initials of the patient from whom the virus was first isolated]) are well-recognized causes of meningitis in patients who have severe immunodeficiencies. Some cases of bacterial meningitis in immunocompromised patients have been rapidly fatal, but in general clinical aspects of meningitis in these patients appear to be similar to those in immunocompetent patients.[12]

Recently, several examples of genetic predisposition to infections with *N. meningitidis* have been identified.[13] Deficiencies of one of the terminal components of complement, albeit rare, are associated with recurrent neisserial infections or infections with uncommon serogroups. Other genetic traits have been associated with either increased risk for infection, risk for fatal infections or risk for septic shock as opposed to meningitis. These include variants of mannose-binding lectin, Fc?-receptor, interleukin (IL)-1 and IL-1 receptor, IL-10, tumor necrosis factor a (TNF-a) and plasminogen activator inhibitor.[14] [15] These observations point to the importance of hereditary traits of innate immunity in determining the susceptibility to and the course of invasive infections with *Neisseria* spp.

Aseptic meningitis

In the USA, aseptic meningitis has an incidence similar to that of bacterial meningitis — up to 10/100,000 people per year. A recent report noted 8000–13,000 cases of aseptic meningitis over a 7-year period in the USA. [5] The true incidence rate is likely to be higher because aseptic meningitis is often not reported. The disease

preferentially affects children and young adults. Other populations at risk are those prone to acquire sexually transmitted diseases, including HIV, herpes simplex and syphilis, and those exposed to mosquitoes (which transmit arboviruses), ticks (Lyme disease, Colorado tick fever), or animals or animal products (lymphocytic choriomeningitis, *Brucella* spp.). The incidence of aseptic meningitis is often four to seven times higher in the summer to autum months than in winter. This is a direct reflection of seasonal variations in the acquisition of systemic enteroviral infections that can lead to aseptic meningitis and of arbovirus infections, whose insect vectors are most prevalent in summer and fall (see also Chapter 23).

Chronic meningitis

The epidemiology of chronic meningitis is quite varied, being determined by the specific etiologic agent (see Table 22.4).[16] Meningitis caused by *M. tuberculosis* predominantly affects young children and the elderly, with an increased incidence in developing countries and in patients who have low socioeconomic status or HIV infection.[17] [18] Some causes of chronic meningitis have defined geographic distributions, such as coccidioidomycosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, Lyme disease, cysticercosis, angiostrongyloidiasis and sarcoidosis. Those exposed to animals and animal

TABLE 22-3 -- Characteristic features of aseptic meningitis by etiology.

CHARACTERISTIC FEATURES OF ASEPTIC MENINGITIS BY ETIOLOGY			
Agent	**Season**	**Clinical signs**	**Special features**
Enteroviruses	Summer–autumn	Nonspecific	Children and young adults; culture throat and stool
Coxsackie virus	Summer–autumn	Petechial rash, pleurodynia, herpangina, myopericarditis, conjunctivitis	Children and young adults; culture throat and stool
Echovirus	Summer–autumn	Dermatomyositis	Children and young adults; culture throat and stool
Arboviruses	Spring, autumn (vector dependent)	Encephalitis prominent	Serology and local epidemiology important for diagnosis
Herpes simplex virus	No seasonal pattern	May accompany primary genital herpes	CSF glucose may be low; CSF polymerase chain reaction positive
Cytomegalovirus	No seasonal pattern	Very rare complication of CMV infection	
Epstein-Barr virus	No seasonal pattern	Complication of mononucleosis	
Mumps	Spring	Orchitis, oophoritis, pancreatitis	Children and young adults; CSF glucose may be low
Lymphocytic choriomeningitis	Autumn–winter	Exposure to rodents; alopecia, orchitis, arthritis, myopericarditis	CSF glucose may be low; CSF pleocytosis may be > 1000/mm^3
HIV	No seasonal pattern	Mononucleosis-like syndrome	Check serology; if negative, recheck in 3 months
			Check p24 antigen or HIV PCR if strong suspicion
Syphilis	No seasonal pattern	During acute infection and in neurosyphillis	CSF serology
Lyme disease	Summer–autumn	History of tick bite or erythema migrans	Check blood serology
Leptospirosis	Summer–autumn	Biphasic illness after rodent or water exposure	First phase — check blood and CSF cultures; second phase — check urine cultures and serology

TABLE 22-4 -- Pathogens and clinical features of chronic meningitis.

PATHOGENS AND CLINICAL FEATURES OF CHRONIC MENINGITIS				
Pathogen		**Predominant type of CSF pleocytosis**	**Predisposition and risk factors**	**Associated clinical manifestations**
Bacteria	*Actinomyces* spp.	Neutrophils	Mouth and ear lesions	CNS lesions, endophthalmitis
	Borrelia burgdorferi (Lyme disease)	Lymphocytes	Tick bite	Cranial nerve palsy (VII nerve)
	Brucella spp.	Lymphocytes, neutrophils	Unpasteurized dairy products	Undulant fever, hepatomegaty
	Leptospira spp.	Neutrophils	Exposure to urine of infected animals	Hepatomegaly, hepatitis, thrombocytopenia
	Mycobacterium tuberculosis	Neutrophils, monocytes, lymphocytes	Immunodeficiency, high endemic prevalence	Cranial nerve palsy (VI nerve)
	Nocardia spp.	Neutrophils	Immunodeficiency	Abscesses
	Treponema pallidum	Eosinophils, lymphocytes	Sexually transmitted diseases	Cranial nerve palsy (VII and VIII nerves)
Viruses	Cytomegalovirus	Lymphocytes; neutrophils (in HIV)	Immunodeficiency	Fever, retinitis
	Echovirus	Lymphocytes	Agammaglobulinemia	Dermatomyositis
	Lymphocytic choriomeningitis virus	Lymphocytes	Exposure to rodents	Orchitis, leukocytopenia, thrombocytopenia
	Mumps virus	Neutrophils	No vaccination	Parotitis, orchitis, oophoritis
	HIV	Lymphocytes	HIV risk factors	Mononucleosis-like illness
Fungi	*Aspergillus* spp.	Lymphocytes or neutrophils	Immunodeficiency, surgery	Lung involvement
	Candida spp.	Neutrophils	Antibiotics, surgery, immunodeficiency	Disseminated disease
	Coccidioides spp.	Lymphocytes	Endemic areas	Lung involvement
	Cryptococcus spp.	Lymphocytes	Immunodeficiency	Encephalitis, headache
	Histoplasma spp.	Lymphocytes	Endemic areas, immunodeficiency	Fever, oral lesions, hepatosplenomegaly
	Pseudallescheria spp.	Neutrophils	Immunodeficiency	Skin lesions, endophthalmitis
	Sporothrix spp.	Neutrophils	Immunodeficiency	Skin lesions, endophthalmitis
Parasites	*Taenia solium*	Eosinophils	Endemic areas	Elevated intracranial pressure, calcified lesions on head imaging
	Angiostrongylus spp.	Eosinophils	Raw seafood	Fever

products are at risk of brucellosis and other zoonoses. Areas with high rates of HIV infection also have higher incidences of chronic meningitis associated with AIDS and the specific etiologies of those cases may be influenced by the geographic location (e.g. cryptococcosis, coccidioidomycosis, histoplasmosis and tuberculosis).

Agents of meningitis

Acute bacterial meningitis

Gram-positive pathogens

Streptococcus pneumoniae, an encapsulated Gram-positive diplococcus, causes a severe form of bacterial meningitis that often leaves neurologic sequelae in survivors and is fatal in up to 30% of patients. The organism can affect all age groups and causes the most severe disease in the very young and the very old. There are over 90 serotypes identified among pneumococcal isolates, but a few serotypes predominate as the causes of meningitis. Different serotypes cause invasive disease in children (14, 6, 18, 19, 23, 4, 9) and in adults (1, 3, 4, 7, 8, 9, 12, 14).[19] [20] A group B streptococcus (*Strep. agalactiae*) is a pathogen of the neonatal period and often causes a devastating sepsis and meningitis. It colonizes the birth canal of women, from where it is transmitted to the child. The colonized newborn can develop group B streptococcal disease of early onset (developing at less than 7 days of age; median 1 day) or late onset (developing later than 7 days of age). Early-onset disease presents as a sepsis-like disease with a very high mortality rate; late-onset disease presents primarily as meningitis.[21] Group B streptococci are also increasingly found as cause of meningitis in older patients, particularly those who have chronic underlying diseases.[22] *Listeria monocytogenes* is a Gram-positive rod that causes meningitis preferentially in neonates, in adults who have underlying conditions such as alcoholism and on long-term treatment with corticosteroids, and in pregnant women. There is often an encephalitic component to presentation, with early mental status alterations, neurologic deficits and seizures.[23]

Staphylococci rarely cause meningitis, except in the setting of intraventricular shunts, or as a consequence of staphylococcal bacteremia in patients who have endocarditis, intravascular devices or suppurative foci.[24] Streptococci (other than *Strep. pneumoniae*), enterococci and Gram-positive anaerobes are rare causes of bacterial meningitis.

Gram-negative pathogens

Haemophilus influenzae is a Gram-negative coccobacillus that is serotyped based on its capsule. Of the six encapsulated serotypes (a–f), type b causes almost all cases of invasive disease, including meningitis. *Haemophilus influenzae* meningitis is a disease of young children.[25]

Neisseria meningitidis, a Gram-negative diplococcus, is mainly responsible for bacterial meningitis in young adults; it causes both sporadic cases and epidemics. The organism is transmitted from person to person by droplets, a form of transmission favored by crowded conditions.[13]

Enterobacteriaceae (e.g. *Escherichia coli*, *Klebsiella* spp. and *Serratia* spp.) cause meningitis in neonates and in patients undergoing neurosurgical procedures.

Pseudomonas aeruginosa can cause meningitis in neutropenic patients and in patients after neurosurgery.[21]

Acute aseptic meningitis (see also Chapter 23)

Viruses

Viruses are the most common cause of aseptic meningitis (see Table 22.3).[5] Enteroviruses account for more than 80% of cases in which the cause is identified. Enteroviruses that cause meningitis include Coxsackie viruses A and B, echovirus and poliovirus. Enteroviruses are transmitted by the fecal-oral route and are spread through close contact in households and day care centers. Affected groups include infants, young children and those looking after them. Mumps, a paramyxovirus, is spread by respiratory droplets and is usually seen in the late winter and early spring. Neurologic complications range from encephalitis to meningitis. Young children are most commonly affected, and boys are more often affected than girls by a ratio of between 2 to 1 and 5 to 1. Mumps meningitis follows mumps parotitis, and there is often no salivary gland involvement at the time of presentation of aseptic meningitis.[5]

Lymphochoriomeningitis virus is an arenavirus spread by contact with rodent urine or feces. The disease was most prevalent in young adults and in impoverished populations, but the virus has become a rare cause of meningitis. Patients present with the typical symptoms of acute meningitis. After an initial improvement, some patients relapse into a second phase of meningitis, which is believed to be immune-mediated.[26]

Patients infected with arboviruses (St Louis encephalitis virus, Western equine encephalitis virus, California encephalitis virus and Eastern equine encephalitis virus) usually present with symptoms of encephalitis rather than meningitis. However, a minority of patients have meningitis, with a paucity of symptoms suggestive of encephalitis.

Many of the herpesviruses cause neurologic complications, including aseptic meningitis. Primary genital herpes caused by herpes simplex virus type 2 is the most common cause. Aseptic meningitis is much less likely to complicate recurrent outbreaks of herpes. Herpes aseptic meningitis is a benign and self-limited illness that must be distinguished from herpes encephalitis, which is a serious illness that often has devastating neurologic consequences and often causes death.[27]

Varicella-zoster virus, cytomegalovirus and Epstein-Barr virus can also cause aseptic meningitis. HIV-1 has been identified as a cause of aseptic meningitis. Seroconversion of HIV-1 often causes a mononucleosis-like illness with fever, malaise, rash, myalgias and arthralgias, and this is sometimes associated with aseptic meningitis (see Chapter 122).[28] Other less important viral etiologies of aseptic meningitis include adenovirus, measles and rubella.

Bacteria

Some bacterial infections can cause an aseptic meningitis syndrome. [29] Patients who have bacterial meningitis and who have been partially treated with antibiotics may have symptoms of meningitis with a CSF profile very similar to that of aseptic viral meningitis. Parameningeal bacterial foci (e.g. brain abscesses and epidural abscesses) can be associated with culture-negative meningitis, usually with a predominantly granulocytic CSF pleocytosis. Symptoms of meningitis are generally mild or absent.

Bacterial endocarditis can cause a cerebritis, which is characterized by vasculitis of the small cerebral vessels; it can also be associated with aseptic meningitis.

Spirochetes commonly cause brain infections with meningeal inflammation and a CSF profile similar to that of viral meningitis. Secondary syphilis rarely (in about 1% of all cases) causes acute, aseptic meningitis that can be associated with hydrocephalus, cranial nerve palsies and encephalitic changes.

Aseptic meningitis is also a manifestation of early Lyme disease and is frequently associated with cranial and peripheral neuropathies.[30]

Both the early and second phase of leptospirosis can cause an aseptic meningitis.[31] The CSF shows a moderate lymphocytic pleocytosis and is sterile. The meningitis resolves without specific therapy over the course of a few weeks. Rickettsiae, including *Coxiella burnetii* and *Ehrlichia* spp., can cause an aseptic meningitis with lymphocytic pleocytosis and elevated protein concentrations as part of

the meningoencephalitis that characterizes infections by these intracellular pathogens.

Amebae

Naegleria fowleri, and rarely *Acanthamoeba* spp., can cause an acute meningoencephalitis that occurs most commonly in children and young adults and resembles bacterial meningitis with signs indicating severe brain involvement. The organism is acquired by swimming in fresh water. The CSF shows a polymorphonuclear pleocytosis with increased protein and decreased glucose concentrations, many erythrocytes and a negative Gram stain. A fresh, warm sample of CSF should be examined microscopically for evidence of motile amebae. The disease is fatal in more than 95% of cases.[32]

Noninfectious causes

Neurosurgery involving the posterior fossa can result in aseptic meningitis ('posterior fossa syndrome'). Signs of meningitis appear rapidly, but patients often do not look very ill. The CSF shows a polymorphonuclear pleocytosis with elevated protein and low glucose mimicking bacterial meningitis, but cultures remain negative. The syndrome is diagnosed after excluding infectious causes (particularly bacteria) and is treated with high doses of corticosteroids for several days.

Other noninfectious diseases causing aseptic meningitis include carcinomatous meningitis, sarcoidosis, systemic lupus erythematosus and Behçet's disease. Many drugs have been linked to aseptic meningitis, most importantly trimethoprim-sulfamethoxazole (cotrimoxazole), nonsteroidal anti-inflammatory drugs and OKT3, an antibody directed against T cells.[33] Mollaret's meningitis is a recurrent lymphocytic meningitis, usually seen in young women. The cause is unknown but is thought by some to be related to herpes simplex virus.[34] The episodes are benign and self-limiting, the prognosis is good.

Chronic meningitis

Bacterial

Worldwide, tuberculous meningitis, which results from the rupture of a tubercle into the adjacent subarachnoid space, is the most important cause of chronic meningitis. The presentation is typical for chronic meningitis — slowly progressive headache and signs of meningeal irritation, followed by cranial nerve involvement, other neurologic deficits and progressive mental status changes over a period of weeks. Tuberculous meningitis may be a consequence of either primary infection or reactivation of disease. The diagnosis can be confirmed by a positive CSF culture; however, *M. tuberculosis* is recovered from the CSF in only 38–88% of cases. A moderate lymphocytic pleocytosis is most common. The glucose can be very low; the protein is often very high. Cerebrospinal fluid smears for acidfast bacilli are positive in only a minority of cases (10–20%). Skin tests for delayed hypersensitivity to tuberculin are frequently negative in tuberculous meningitis, whether resulting from primary infection or reactivated disease. [18]

Chronic meningitis is an unusual complication of brucellosis. Symptoms of the meningitis tend to progress over an extended period of months to years. The diagnosis should be entertained when there is a history of exposure to farm animals or consumption of undercooked meats or unpasteurized dairy products from endemic areas.[35]

Secondary syphilis may cause chronic meningitis. The disease is slowly progressive, and generally symptoms have been present for more than 1 month before presentation. Cranial nerve palsies are common; the facial and acoustic nerves are the most frequently affected. Diagnosis is based on a positive Venereal Disease Research Laboratory (VDRL) test in CSF.[36]

The diagnosis of meningitis associated with Lyme disease, caused by *Borrelia burgdorferi*, should be considered in patients who live or have travelled through endemic regions, particularly those with a history of a tick bite or erythema chronicum migrans. Meningitis may persist for weeks and may be associated with cranial nerve palsies and peripheral neuropathies. [30] Syphilis, other spirochetal diseases and collagen vascular diseases may result in a false-positive Lyme serology.

Fungi

Presentations of chronic meningitis caused by *Cryptococcus neoformans*, an encapsulated, ubiquitous fungus, range from a subacute meningoencephalitis to fever of unknown origin. People at highest risk are those with defects in cellular immunity such as occurs in AIDS, hematologic malignancies and prolonged use of high-dose corticosteroids, even though a significant number of patients with cryptococcal meningitis in the pre-AIDS era had no identifiable immune defect. The CSF shows a moderate lymphocytic pleocytosis, but in patients who have AIDS, inflammation may be virtually absent. Cryptococcal antigen latex agglutination is positive in more than 90% of cases, whereas microscopy of India ink preparations to visualize the yeast in CSF is less sensitive.[37]

Coccidioides immitis grows in the dry sandy soils of the southwest USA, and Central and South America. Acute infection is acquired by inhalation of the spores, and meningitis develops within a few months. There are few distinguishing features of the disease; some patients who have generalized disease have erythema nodosum; hydrocephalus is a common complication. Cerebrospinal fluid eosinophilia in patients who have lived or traveled though endemic regions should alert the clinician to the possibility of *Coccidioides* meningitis. Complement-fixing antibodies are present in the CSF in 75–95% of cases, and CSF cultures are positive in more than 50%.[38]

Histoplasma meningitis is a rare complication of histoplasmosis. The diagnosis should be considered in patients who live or have traveled through endemic regions — the Ohio River Valley of the USA, the Caribbean and South America. Cerebrospinal fluid cultures are positive in 27–65% of cases. Blood should be cultured and a buffy coat of the blood should be examined for the presence of the fungus. *Histoplasma* polysaccharide antigen is found in the urine, blood or CSF in 61% of patients and in an even higher proportion of patients who have AIDS.

Candida meningitis is rare and is most commonly a result of disseminated infection or placement of a ventricular shunt. Risk factors for candidemia are the prolonged use of antibiotics or corticosteroids, hyperalimentation, neutropenia, abdominal surgery, intravenous drug use and intravenous catheterization. Neonates are particularly prone to disseminated infection. Cerebrospinal fluid cultures are diagnostic.

Parasites

Neurocysticercosis is endemic in Mexico, South America and Asia. Infection is acquired by eating food contaminated with eggs of *Taenia solium*. Seizures are the most common manifestation. Intraventricular and basilar cysts (racemose cysticercosis) may present with signs of obstructive hydrocephalus. The CSF shows a lymphocytic pleocytosis with eosinophils. Computed tomography (CT) scans of the head show multiple calcified lesions. Serology of blood and CSF may provide support for the diagnosis.

Angiostrongylus cantonensis, the rat lung worm, is most prevalent in Asia and the Pacific Islands and is acquired by the ingestion of raw or inadequately cooked shellfish or snails. Symptoms are typical of chronic meningitis, and rash with pruritus is also common. Infection results in peripheral eosinophilia and chronic eosinophilic

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meningitis, which resolves spontaneously within 2 months. There is no effective therapy.

Other less common causes of infectious chronic meningitis are organisms that usually cause abscesses, which may leak into the subarachnoid space to cause chronic meningitis. These conditions include blastomycosis, paracoccidioidomycosis, phaeohyphomycoses, mucormycosis, actinomycosis, nocardiosis and toxoplasmosis. Less common fungi causing this syndrome include *Sporothrix schenckii*, chromoblastomycoses and *Aspergillus* spp.

Noninfectious causes

Meningeal carcinomatosis may cause a chronic meningitis that is difficult to distinguish from infectious causes. Neurosarcoidosis is an uncommon complication of sarcoidosis. Basilar inflammation is a prominent feature, resulting in cranial nerve palsies. The CSF shows a lymphocytic pleocytosis and usually a normal glucose. Less common noninfectious causes include systematic lupus erythematosus granulomatous angiitis, Behçet's disease and Vogt-Koyanagi-Harada syndrome.

PATHOGENESIS AND PATHOLOGY

Pathogenesis

The pathogenesis of meningitis is best known from studies on bacterial meningitis, where the role of mucosal colonization, bloodstream invasion, CNS colonization and multiplication within the CSF have been elucidated in experimental systems (Fig. 22.1).

Figure 22-1 Pathogenesis of meningitis.

Nasopharyngeal colonization and invasion

Specific bacterial virulence factors for meningeal pathogens include specialized surface components (e.g. the polysaccharide capsule with specific epitopes and fimbriae or pili). These factors are crucial for adherence to the nasopharyngeal epithelium, the evasion of local host defense mechanisms and subsequent invasion of the bloodstream. Hydrogen peroxide produced by *Strep. pneumoniae* has a bactericidal effect on the microbial flora in the respiratory tract, suggesting that the ability to produce hydrogen peroxide might provide the organism with a competitive advantage for colonization. The invasion of pneumococci needs the activation of nasopharyngeal epithelial cells and the presentation of receptors suitable for pneumococci. The presence of the polymeric immunoglobulin A receptor on human mucosa, which binds to a major pneumococcal adhesin, CbpA, correlates with the ability of pneumococci to invade the mucosal barrier. Lack of specific mucosal antibodies correlates with an increased risk of invasive disease. Viral infection of the respiratory tract may also promote invasive disease. From the nasopharyngeal surface, encapsulated organisms cross the epithelial cell layer and invade the small subepithelial blood vessels.[39]

Intravascular survival

In the bloodstream, bacteria must survive host defenses, including circulating antibodies, complement-mediated bactericidal mechanisms and neutrophil phagocytosis. Encapsulation is a shared feature of the principal hematogenous meningeal pathogens (*H. influenzae, N. meningitidis, Strep. pneumoniae, E. coli* K1 and group B streptococci). The

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capsule is instrumental in inhibiting neutrophil phagocytosis and complement-mediated bactericidal activity.

Several defense mechanisms counteract the antiphagocytic activity of the bacterial capsule. Activation of the alternative complement pathway results in cleavage of C3 with subsequent deposition of C3b on the bacterial surface, thereby facilitating opsonization, phagocytosis and intravascular clearance of the organism.

Impairment of the alternative complement pathway occurs in patients with sickle-cell disease and those who have undergone splenectomy, and these groups of patients are predisposed to the development of pneumococcal meningitis. Recently, functional deficiencies of several components involved in the activation and function of complement-mediated defenses have been identified (i.e. mannose-binding lectin, properdin, lack of terminal comple-ment components), which increase the susceptibility for invasive meningococcal infections.[13]

Meningeal invasion

Studies on the pathogenesis of experimental bacterial meningitis show that cells in the choroid plexus and cerebral capillaries possess receptors for adherence of meningeal pathogens. For *E. coli*, a complex interplay between endothelial factors and microbial genes orchestrates the crossing of the blood-brain barrier by bacteria.[40] Experimental evidence has identified the choroid plexus as a potential site for the invasion of meningeal pathogens, and this may be facilitated by its exceptionally high rate of bloodflow (200ml/g per minute) and the presence of pathogen-specific receptors.

Pneumococci are thought to enter the CNS by crossing the blood-brain barrier or the blood-CSF barrier either by local tissue damage or by transcytosis through microvascular endothelial cells.[41] Pneumolysin, a major pneumococcal virulence factor, was shown to damage endothelial cells and to be an important component for compromising the blood-brain barrier.[42]

Nonhematogenous invasion of the CSF by bacteria occurs in situations of compromised integrity of the barriers surrounding the brain (e.g. in otitis media, mastoiditis, sinusitis). Direct communication between the subarachnoid space and the skin or mucosal surfaces as a result of malformation or trauma give rise to meningeal infection with bacterial species that vary with the site of the abnormal communication. Bacteria can also reach the CSF as a complication of neurosurgery, spinal anesthesia or ventriculostomy placement.[43]

Host defense mechanisms in subarachnoid space inflammation

Pathogens reaching the CSF are likely to survive because of a paucity of resident macrophages and deficient opsonization due to low concentrations of capsule-specific immunoglobulins and complement in the CSF. Lack of opsonization greatly reduces the effectiveness of incoming granulocytes and allows largely unrestricted multiplication of the meningeal pathogens. Bacterial multiplication is associated with the release of bacterial products (fragments of cell wall, lipopolysaccharide) that:

- ¦ trigger the inflammatory response in the subarachnoid space by inducing the production and release of inflammatory cytokines (e.g. TNF-a, IL-1 and IL-6) and chemokines (e.g. IL-8, the growth-related oncogene a [GRO-a], the CC chemokine monocyte chemoattractant protein-1 [MCP-1] and others), and lipid inflammatory mediators such as platelet activation factor;
- ¦ upregulate adhesion molecules on brain vascular endothelial cells; and
- ¦ promote the recruitment of granulocytes into the CSF.

The granulocytic inflammation appears to be primarily responsible for the complex pathophysiologic CNS alterations associated with bacterial meningitis.[44] [45]

Blood-brain barrier

The blood-brain barrier separates the brain from the intravascular compartment and maintains homeostasis within the CNS. The permeability of the blood-brain barrier increases in meningitis at the level of the choroid plexus epithelium and the cerebral microvascular endothelium. Separation of intercellular tight junctions and increased pinocytosis contribute to the increased permeability. Matrix metalloproteinases (MMP), zinc-dependent enzymes produced as part of the immune response to bacteria that degrade extracellular matrix proteins, also contribute to the increased permeability of the blood-brain barrier.[46] The resulting increased extravasation of serum components, including antibiotics leads to higher concentrations in the CSF during meningitis, with an approximately fivefold increase over the uninfected state for most antibiotics.[47] [48]

Increased intracranial pressure

The major element leading to increased intracranial pressure in bacterial meningitis is the development of cerebral edema, which may be vasogenic, cytotoxic or interstitial in origin. Vasogenic cerebral edema is primarily a consequence of increased blood-brain barrier permeability.

Cytotoxic edema results from an increase in intracellular water following alterations of the cell membrane and loss of cellular homeostasis. Cytotoxic mechanisms include ischemia and the effect of excitatory amino acids. Secretion of antidiuretic hormone also contributes to cytotoxic edema by making the extracellular fluid hypotonic and increasing the permeability of the brain to water.

Interstitial edema occurs by an increase in CSF volume, either through increased CSF production via increased bloodflow in the choroid plexus, or decreased resorption secondary to increased CSF outflow resistance.[49] [50]

Cerebral vasculitis and alterations in cerebral bloodflow

Bacterial meningitis is associated with marked changes in cerebral bloodflow. In the early phase of the disease, an increase in bloodflow is observed, and this appears

to be mediated by nitric oxide and oxidative radicals. Formation of oxidative radicals in meningitis has been documented to occur together with the presence of inflammatory cells in the subarachnoid space and along penetrating cortical blood vessels, and leads to oxidative alterations of the cerebral vasculature, which may contribute to the cerebral bloodflow reduction observed in advanced meningitis. [51] [52] [53] [54]

Other factors involved in bloodflow reduction in advanced meningitis include the vasoconstrictor peptide family of endothelins, the levels of which are greatly increased in the CSF during bacterial meningitis. An antagonist of endothelin receptors normalized cerebral bloodflow and prevent cortical injury in experimental meningitis.[52] [55]

Several clinical studies have found an association between severe cerebral bloodflow reduction and adverse outcomes in children and adults with meningitis, suggesting that ischemia is an important mediator of brain damage in meningitis. Cerebral bloodflow reduction during meningitis can be global, as a result of reduced cerebral perfusion pressure (resulting from increased intracranial pressure, systemic hypotension, or both), or focal, as a result of the vascular involvement of cerebral arteries and veins by the subarachnoid space inflammation (Fig 22.2 , Fig 22.3). [51]

Neuronal injury

The exact mechanisms that lead to permanent brain injury are incompletely understood. There is converging evidence, however, that cerebral ischemic necrosis contributes to this process, particularly regarding damage to the cerebral cortex. Acute breakdown of the blood-brain barrier, intrathecal production of cytokines, and accumulation of blood-derived leukocytes in the CSF are key events leading to brain edema, cerebral vasculitis and ultimately neuronal

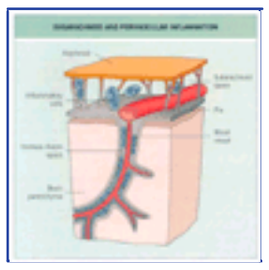

Figure 22-2 Subarachnoid and perivascular inflammation in meningitis. The inflammation in the subarachnoid space extends into the Virchow-Robin spaces along the vasculature.

Figure 22-3 Arachnoid membrane in purulent meningitis. Close-up view of the arachnoid membrane covering the purulent subarachnoid space, with penetrating blood vessels exhibiting inflammatory vasculitis and thrombosis. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

injury. These events are in part mediated by MMPs (see 'Blood-brain barrier', above). CSF levels of specific MMPs (i.e. MMP-9) were significantly higher in patients who developed neurological sequelae than in those who recovered fully. The metalloproteinase TNF-a converting enzyme (TACE), a close relative of the MMPs, may contribute to the pathophysiology of bacterial meningitis by its ability to release cytokines and their receptors, thus increasing stimuli that trigger further MMP release.[46] Given as adjuvant therapy in addition to antibiotics, combined inhibition of MMP and TACE reduced the extent of cortical and hippocampal damage and protected from learning disability resulting from meningitis.[56]

While the mechanisms of necrosis in the cortex have only partially been explored, even less is known regarding the causes of neuronal injury in the dentate gyrus of the hippocampus. In patients dying from bacterial meningitis and in corresponding animal models, neuronal cell death in the hippocampal dentate gyrus fulfils criteria for apoptosis that appears to be induced by activation of the effector caspase-3 and other caspase-independent mechanisms.[57] [58] [59]

Pathology

Important pathologic findings in patients with meningitis include:

- subarachnoid space inflammation;
- inflammatory involvement of the cerebral vasculature; and
- parenchymal brain damage.

The subarachnoid space inflammation appears as a grayish yellow-to-green exudate covering the base and convexities of the brain (Fig 22.3 , Fig 22.4). The exudate consists predominantly of granulocytes in acute bacterial meningitis, and of a mixture of lymphocytes, macrophages and granulocytes in subacute and chronic forms of meningitis. On the surface of the brain, the involvement of the vasculature is often macroscopically obvious (see Fig. 22.3). Histologic examination shows infiltration of vessel walls by inflammatory cells with consecutive thrombosis of the vessel lumen (Fig. 22.5). Vascular involvement is most prominent in acute bacterial meningitis, but it is also seen in chronic, especially tuberculous meningitis.

Inflammation also involves the inner ear, to which it gains access via the cochlear aqueduct connecting the subarachnoid space with the endolymphatic space. Toxic effects of the inflammation on hair cells of the inner ear appear to be responsible for the hearing impairment associated with bacterial meningitis (Table 22.5).

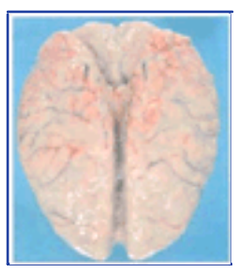

Figure 22-4 Brain with inflammatory exudate covering the cortical hemispheres in purulent meningitis. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

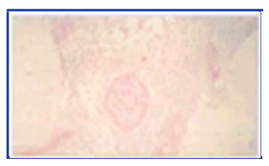

Figure 22-5 Histopathology of the subarachnoid space in meningitis. Note the inflammatory involvement of the blood vessels and the small vessel leading into the brain parenchyma surrounded by inflammation in the Virchow-Robin space. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

TABLE 22-5 -- Common neurologic sequelae of bacterial meningitis.

COMMON NEUROLOGIC SEQUELAE OF BACTERIAL MENINGITIS	
Deficit	**Approximate frequency (%)**
Hearing loss	15–30
Parenchymal damage	5–30

Cerebral palsy	5–10
Learning disabilities	5–20
Seizure disorder	<5
Cortical blindness	<5
Cerebral hemiation	3–20
Hydrocephalus	2–3

Damage to the brain parenchyma is evidenced by the presence of brain edema (including signs of cerebral herniation), by areas of cerebral infarction resulting from ischemia and by histologic changes. Loss of neurons is associated with a marked reaction of astrocytes and microglia. Apart from the damage to the cortex, neuronal injury is evident in the dentate gyrus of the hippocampus in patients dying from meningitis, and damage to the hippocampus is further suggested by a decreased hippocampal volume in patients surviving meningitis.[60] [61] Given the function of the hippocampus, injury to this structure may represent the substrate for the learning and memory deficits in survivors of meningitis. The extent and localization of neuronal loss probably determines the type of neurologic sequelae resulting from meningitis (see Table 22.5).[40] [52]

Neurologic sequelae

Neurologic sequelae after bacterial meningitis include hearing impairment, mental retardation, focal sensorimotor deficits, epilepsy and cortical blindness. In a retrospective survey of survivors of bacterial meningitis, 31 and 24% reported attention and memory deficits, respectively, which affected their ability to work.[62] Three more recent studies have found significant functional impairment in survivors of bacterial meningitis.[63] [64] [65] Among 1584 children aged 5 years who survived bacterial meningitis during the first year of life, there was a 10-fold increase in the risk of having moderate-to-severe disabilities, including learning diffculties and behavioral problems, neuromotor disabilities, seizure disorders and hearing deficits.[63] Infection with *Strep. pneumoniae* was associated with a higher rate of disabilities than infection with *H. influenzae* or *N. meningitidis*. In a second study, many of the deficits identified at a 7-year follow-up persisted 12 years after bacterial meningitis, with 9% of the patients showing major neurologic, auditory or intellectual impairments, and 30% having less severe disabilities, compared with 11% of the controls.[64] In the third study, 16 of 22 adult patients had neurologic and psychopathologic symptoms 3 years after the acute stage of bacterial meningitis.[65] The speed of cognitive processes and psychomotor performance, concentration and memory functions were reduced in patients compared with controls. Again, sequelae were particularly pronounced in patients after meningitis caused by *Strep. pneumoniae*.

PREVENTION

The most effective prevention for bacterial meningitis is provided by vaccination. This has been most impressively documented with *H. influenzae* type b, for which the new conjugated vaccines have led to a reduction of more than 90% in the number of cases in vaccinated populations.[1] Current recommendations are to vaccinate all children against *H. influenzae* type b with one of several available vaccines, beginning at 3 months of age. Unfortunately, this is not possible in many countries for economic reasons.

A 23-valent polysaccharide vaccine and newly developed conjugate vaccines are available for vaccination against invasive pneumococcal disease, including meningitis.[66] The polysaccharide vaccine, which is cheaper and covers more serotypes than the currently available conjugate vaccines, should be offered to adults at increased risk for invasive pneumococcal infections, such as the elderly and those with underlying disease, including immunocompromising conditions, asplenia, sickle-cell disease and anatomic defects predisposing to meningitis. Some countries, including the USA, have adopted recommendations to routinely vaccinate all children under 24 months of age with the conjugate vaccine.[67] Older children at increased risk for invasive diseases should also be immunized with a conjugate pneumococcal vaccine.

In the case of meningococcal meningitis, polysaccharide vaccines are available for all major serotypes except group b, which has a poorly immunogenic capsule. For group c, new conjugate vaccines that are highly immunogenic in children under the age of 2 years have recently been licensed. Vaccination against meningococci is currently recommended for children and young adults in some countries in the setting of ongoing epidemics and for travelers to countries with high endemic infection rates. [67]

Although no vaccines to prevent transmission of group B streptococci from colonized mothers to their newborn are yet available, preventive strategies using antibiotics have been widely implemented and have substantially reduced the risk of group B streptococcal infections in neonates. Typically, intrapartum administration of penicillin is offered to women who have been shown to be colonized with group B streptococci before delivery, to women who have risk factors for neonatal infection (prematurity, prolonged rupture of the membranes, signs of infection during labor) or to both groups. A recent study suggests that screening with subsequent intrapartum treatment of colonized women may be the preferred approach.[68]

Antibiotics are widely used for the prevention of meningitis associated with neurosurgery and the placement of intraventricular shunts. In patients undergoing craniotomy, perioperative antibiotic prophylaxis appears to reduce the number of postoperative infections. Studies examining the effect of periprocedural or prophylactic antibiotics in patients receiving various types of intraventricular shunts or intracranial pressure monitors have not consistently shown a benefit. In some cases, prophylactic antibiotics have been associated with infections caused by difficult-to-treat organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and fungi. Given the increasing problems with resistant organisms and the diagnostic problems caused by prophylactic antibiotics in patients who have suspected device-associated infection, it seems preferable to refrain from routine use of long-term prophylactic antibiotics in these patients.[43]

CLINICAL FEATURES

Bacterial meningitis

Meningitis is the most likely diagnosis in patients who present with the classical triad of fever, headache and a stiff neck, which is present in at least 80% of patients. Other signs and symptoms occur less frequently (Table 22.6), and patients complaining of headache or presenting with altered mental status must be carefully examined for evidence of meningeal irritation (i.e. meningism, with Kernig's sign, Brudzinski's sign, or both).

Bacterial meningitis can be present in patients in whom the clinical diagnosis is not obvious. This is particularly true in small children and in the elderly. In children under 2 years of age, signs of meningeal inflammation are frequently absent, and the most common clinical presentations include fever and altered mental status (irritability, lethargy), which are present in over 90% of

TABLE 22-6 -- Common signs and symptoms in acute meningitis and encephalitis.

COMMON SIGNS AND SYMPTOMS IN ACUTE MENINGITIS AND ENCEPHALITIS			
Meningitis	Adult	Headache	Kernig's sign
		Fever	Brudzinski's sign
		Neck and back pain	Photophobia
		Meningism	Lethargy or coma
		Nausea and vomiting	Seizures

	Infant	Fever	Bulging fontanelles
		Irritability	Convulsions
		Lethargy	Opisthotonus
		Refusal to feed	Seizures
		Strange cry	
Encephalitis	Fever		Stupor or coma
	Vomiting		Seizures
	Psychiatric alteration		Electroencephalographic changes
	Focal neurologic deficits		

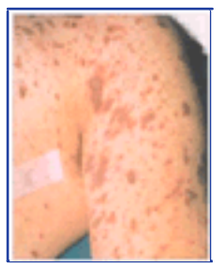

Figure 22-6 Skin lesions in acute meningococcemia. Characteristic purpura with petechiae and ecchymoses in a patient who has fulminant sepsis and meningitis due to *Neisseria meningitidis*. Courtesy of Professor W Zimmerli, University of Basel, Switzerland.

patients. Similarly, in elderly patients, fever may be minimal, and mental status changes may be the most obvious symptom. In patients who have suspected meningitis, the skin should be examined carefully for the characteristic purpuric or petechial skin rash of *N. meningitidis* (Fig. 22.6). Other clues derived from the history and physical examination may help in the differential diagnosis of meningitis (Table 22.7).

Untreated bacterial meningitis is characterized by progressive loss of consciousness, which is commonly associated with other neurologic signs including seizures and focal deficits, leading to coma and death. The extent of mental status changes, with profound coma as the most extreme, provides a clinical indication of the severity of the disease. Patients presenting in coma have a very high mortality rate (up to 50%). Systemic complications of the infectious process include septic shock, disseminated intravascular coagulation (particularly with meningococcal infections) and acute respiratory distress syndrome.

Aseptic meningitis

The clinical manifestations of aseptic meningitis are often indistinguishable from those of bacterial meningitis. Acute onset of

TABLE 22-7 -- Clues from the history and physical examination in the differential diagnosis of meningitis.

CLUES FROM THE HISTORY AND PHYSICAL EXAMINATION IN THE DIFFERENTIAL DIAGNOSIS OF MENINGITIS	
Clinical feature	**Likely organism**
Purpura, petechiae	*Neisseria meningitidis*
Cellulitis of the face	*Staphylococcus aureus*, *Haemophilus influenzae*
Otitis media, sinusitis, mastoiditis	*Streptococcus pneumoniae*, *Haemophilus influenzae*
Cerebrospinal fluid fistula	*Streptococcus pneumoniae*
Parotitis	Mumps
Endocarditis (peripheral stigmata, murmur)	*Staphylococcus aureus*
Pericarditis	*Neisseria meningitidis*, *Streptococcus pneumoniae*
Pneumonia	*Streptococcus pneumoniae*, *Haemophilus influenzae*
Spondylodiscitis	*Staphylococcus aureus*, *Mycobacterium tuberculosis*
Septic arthritis	*Staphylococcus aureus*, *Streptococcus pneumoniae*
Focal signs	*Listeria monocytogenes*, brain abscess, encephalitis
Alcoholism, liver cirrhosis	*Listeria monocytogenes*, *Streptococcus pneumoniae*
Pregnancy	*Listeria monocytogenes*
Neurosurgery	Staphylococci, Enterobacteriaceae, *Pseudomonas aeruginosa*
Kidney, heart transplantation	*Listeria monocytogenes*, *Cryptococcus neoformans*
Neutropenia	Gram-negative rods, fungi
HIV infection	*Cryptococcus neoformans*, *Nocardia* spp., *Listeria monocytogenes*, *Mycobacterium tuberculosis*

fever, headache, photophobia, nausea with vomiting and meningism are most common. On the other hand, severe neurologic findings, including seizures, are very uncommon in most forms of aseptic meningitis. Overall, symptoms are generally milder than those of pyogenic meningitis. Most cases of aseptic meningitis are self-limiting and do not lead to sequelae although constitutional symptoms can persist for several weeks. Depending on the causative pathogen, signs of meningeal inflammation may be associated with other clinical findings (see Table 22.3).

Chronic meningitis

Symptoms and signs of chronic meningitis evolve over several days to weeks. Most prominently, patients complain of headaches, often associated with constitutional signs of infection (fever, anorexia). Nuchal rigidity may be subtle or absent. Many forms of chronic meningitis involve the base of the brain and lead to cranial nerve palsies, often affecting eye movements and facial musculature. As the syndrome progresses, signs of brain involvement with seizures, mental status changes, confusion or hallucinations, and focal neurologic deficits develop. Hydrocephalus and increased intracranial pressure may accompany the syndrome.

TABLE 22-8 -- Differential diagnosis of meningitis based on cerebrospinal fluid findings.

DIFFERENTIAL DIAGNOSIS OF MENINGITIS BASED ON CEREBROSPINAL FLUID FINDINGS						
Diagnosis	**Pressure (cmH_2O)**	**White blood cells ($\times 10^6$ /1)**	**Polymorphonucleocytes (%)**	**Glucose (ratio)**	**Protein (g/l)**	**Lactate (mmol/l)**
Normal	<20	1–2	<1	>0.5	<0.45	<2
Acute bacterial meningitis	>20	>1000	>50	<0.4	>1	>4
Chronic meningitis	Variable	>1000	Variable	<0.4	>0.45	>2
Aseptic (viral) meningitis	<20	<1000	<50	>0.4	Variable	<2

DIAGNOSIS

Cerebrospinal fluid examination

Examination of CSF is of paramount importance for the diagnosis of all forms of meningitis (Table 22.8). Accordingly, a lumbar puncture should be performed in patients who have suspected meningitis once a mass lesion that may lead to cerebral herniation has been ruled out on clinical grounds or by CT scan of the head. Evidence for mass lesions consists of the combination of focal neurologic signs (focal seizures, sensorimotor and visual defects) and clinical evidence of increased intracranial pressure (headache, vomiting, impaired mental status, papilledema). In patients who have suspected bacterial meningitis who show no evidence for a focal lesion or increased intracranial pressure, imaging studies before lumbar puncture are usually not necessary. While performing the lumbar puncture, CSF pressure should be recorded.

Immediate examination of the CSF provides valuable information. A Gram stain of uncentrifuged CSF (if the CSF is turbid) or centrifuged CSF (if it is not) indicates the presence of white blood cells, their approximate differential count (mononuclear versus polymorphonuclear) and whether bacteria are present.

Cultures for bacteria and fungi should always be performed, even in patients already treated with antibiotics. Tests for the detection of bacterial antigens by immunologic methods, such as latex particle agglutination, have sensitivities in the range of the Gram stain or culture and are of doubtful benefit when used routinely, but sometimes identify organisms in patients with partially treated bacterial meningitis and negative Gram stain and culture.

Cerebrospinal fluid concentrations of protein, glucose and lactate, in addition to the number and type of white blood cells are helpful in the differential diagnosis of various forms of meningitis (see Table 22.8).[69]

In addition to the routine tests carried out on all CSF samples, specific tests may be indicated under special circumstances. Viral cultures are mostly negative in CSF from patients who have aseptic meningitis and should not be performed routinely. However, nucleic acid detection by polymerase chain reaction and related tests is highly sensitive for detecting selected pathogens, particularly herpes viruses such as herpes simplex virus and cytomegalovirus. Serologic tests of CSF can establish the etiology of cases of chronic meningitis (syphilis, coccidioidomycosis). Isolation of enteroviruses from throat washes or stool should be attempted because after the first 3 days of symptoms enteroviruses are recovered only from these sites.

Systemic parameters

Signs of inflammation are most pronounced in acute bacterial meningitis, and in some cases of chronic meningitis (e.g. tuberculosis, fungal meningitis). In bacterial meningitis, the differential blood count will frequently show a leukocytosis with a left shift. The erythrocyte sedimentation rate and other acute phase reactants are typically elevated.

Blood cultures should be performed in all patients who have suspected meningitis before initiation of antibiotic therapy. They:

- yield the infecting organism in more than 60% of cases of acute bacterial meningitis;
- should be sterile in aseptic meningitis; and
- rarely reveal the organism in chronic meningitis (*Brucella* spp., *Nocardia* spp., fungi).

Patients who have acute meningitis should be examined for evidence of electrolyte imbalance. Hyponatremia is common and may indicate dehydration (in which case the urine sodium concentration is low) or the syndrome of inappropriate antidiuretic hormone (SIADH), in which case the urine sodium concentration is high, an assessment that is important for making the correct choice of fluid substitution, particularly in children who have acute meningitis.

Evidence for disseminated intravascular coagulation is most commonly seen in patients who have meningococcal meningitis. Platelet count, prothrombin time, partial thromboplastin time, fibrinogen levels and fibrin d-dimers should be measured in patients who have suspected disseminated intravascular coagulation.

Imaging studies

In patients who have bacterial meningitis, the possibility of focal infections of the head (sinusitis, otitis media) must be considered, and in selected cases, an appropriate radiologic test (e.g. a CT scan of the head) may be necessary. A CT scan of the head is also indicated in patients who have suspected intracerebral mass lesions or parameningeal foci. Chest radiographs in patients who have acute bacterial meningitis may reveal pneumonia, and in patients who have chronic meningitis, it may show evidence of pulmonary involvement by tubercle bacilli or fungi.

MANAGEMENT

Treatment of meningitis includes two main goals:

- eradication of the infecting organism; and
- management of CNS and systemic complications.

Bacterial meningitis represents a medical emergency, particularly in patients who have rapidly progressive disease and severely impaired CNS function. In these patients, we recommend initiation of empiric therapy without delay. One or two blood cultures should be obtained before administering the first antibiotic dose. We also administer adjunctive therapy with dexamethasone in these severely ill patients concomitantly with the first antibiotic dose (for more detail, see below). Once empiric therapy has been initiated, further diagnostic investigation is carried out. Further therapy depends on the CSF findings. In patients who are clinically stable and are unlikely to be adversely affected if antibiotics are not administered immediately, including those who have suspected viral or chronic meningitis, a lumbar puncture represents the first step, unless there is clinical suspicion of a mass lesion. Findings in the CSF and on CT scan, if

TABLE 22-9 -- Recommendations for empiric antibiotic therapy of bacterial meningitis.

RECOMMENDATIONS FOR EMPIRIC ANTIBIOTIC THERAPY OF BACTERIAL MENINGITIS			
Patients and special modifying circumstances	**Antibiotic**	**Dosage (intravenous)**	
		Children	**Adults**
Neonate or infant under 3 months	Ampicillin plus	50mg/kg q6h	
	cefotaxime or	50mg q6–8h	
	gentamicin	2.5mg/kg q8h	
Neonate (pre-term, low birth weight)	Vancomycin plus	10mg/kg q12h	
	ceftazidime	50mg/kg q12h	
3 months to 50 years	Ceftriaxone or	50mg/kg q12h or 100mg/kg q24h	2–4g q24h
	cefotaxime	50mg/kg q6h	2g q4–6h
Over 50 years or impaired cellular immunity	Ceftriaxone or		2–4g q24h
	cefotaxime plus		2g q4–6h
	ampicillin or		2g q4h
	penicillin G		3–4 million units q4h

Drug-resistant *Streptococcus pneumoniae*	Ceftriaxone plus	50mg/kg q12h or 100mg/kg q24h	2–4g q24h
	rifampicin	10–20mg/kg q24h	600mg q24h
	or vancomycin	10–15mg/kg q6h	0.5g q6h
Neurosurgery, CSF shunt, or head trauma	Ceftazidime	50mg/kg q8h	2g q8h
	plus nafcillin	50mg/kg q6h	2g q4h
	(or vancomycin plus	10–15mg/kg q6h	0.5g q6h
	aminoglycoside)	2.5mg/kg q8h	1.5–2mg/kg q8h

performed, will guide further diagnostic investigation and therapy in all patients.

Antibiotic chemotherapy

Empiric antibiotic therapy

Table 22.9 summarizes empiric antibiotic regimens designed to cover the likely pathogens in different patient populations with suspected bacterial meningitis. Antibiotics should be administered intravenously by bolus infusion at the highest clinically validated doses, corrected for age and renal function. High doses are needed because only a small fraction of the serum concentration (between 3 and 15% for most ß-lactam antibiotics) penetrates into the CSF and because only antibiotic concentrations that exceed the minimum bactericidal concentration (MBC) by a factor of 10–30 are rapidly bactericidal in the CSF.

Empiric therapy is primarily based on the age of the patient, with modifications if there are positive findings on CSF Gram stain or if the patient presents with special risk factors (see Table 22.2). It is safer to choose regimens with broad coverage because they can usually be modified within 24–48 hours when the antibiotic sensitivities of the infecting organism become available.

An important factor in the choice of empiric antibiotic therapy is the emergence of organisms with increasing resistance to antibiotics. Most importantly, pneumococci that are relatively resistant to penicillin — mean inhibitory concentration (MIC) 0.1–1.0µg/ml — or highly resistant to penicillin (MIC >1.0µg/ml) are increasingly important in many parts of the world. Many penicillin-resistant organisms have reduced sensitivity to cephalosporins, and failure of these drugs in the treatment of pneumococcal meningitis caused by resistant organisms has occurred. Empiric therapy with dual antibiotic coverage effective against ß-lactam-resistant pneumococci may therefore be prudent, unless a patient is highly unlikely to have pneumococcal meningitis (e.g. a Gram stain indicates other etiology) or the local incidence of highly resistant pneumococci is very low.

Patients at risk of infection with *L. monocytogenes* must be covered with ampicillin. For patients at risk of infections caused by difficult-to-treat Gram-negative bacilli with a high likelihood of resistance to many ß-lactam drugs, inclusion of an aminoglycoside in the empiric therapy regimen is recommended (see Table 22.9).[70]

In patients who have suspected shunt infections, empiric therapy should include vancomycin to cover for coagulase-negative staphylococci, the most common cause of infection in these patients. The

TABLE 22-10 -- Treatment recommendations for common treatable causes of nonpyogenic meningitis.

TREATMENT RECOMMENDATIONS FOR COMMON TREATABLE CAUSES OF NONPYOGENIC MENINGITIS			
Agent	**Therapy**	**Dose**	**Route**
Herpesviruses	Aciclovir	10mg/kg q8h	iv
Mycobacterium tuberculosis	Isoniazid	10mg/kg/day (up to 300mg/day)	po or iv
	Rifampicin	10mg/kg/day (up to 600mg/day)	po or iv
	Ethambutol	25mg/kg/day	po or iv
	Pyrazinamide	25mg/kg/day (up to 2.5g/day)	po or iv
Brucella spp. (>8 years)	Doxycycline plus gentamicin	100mg q12h	po
		1.7–2mg/kg q8h	iv
Brucella spp. (<8 years)	Trimethoprim-	5mg/kg trimethoprim q12h	po
	sulfamethoxazole	25mg/kg sulfamethoxazole q12h	po
	plus gentamicin	2mg/kg q8h	iv
Treponema pallidum	Penicillin G	2g q4h	iv
Borrelia spp.	Ceftriaxone	2–3g q24h	iv
Cryptococcus spp.	Amphotericin B	0.5–0.8mg/kg/day	iv
	plus flucytosine	37.5mg/kg q6h	po
Coccidioides spp.	Fluconazole	400–600mg/day	po
Note that the dose of flucytosine must be reduced or the drug omitted in patients who have AIDS because of the increased bone marrow toxicity (see Chapter 126).			

TABLE 22-11 -- Recommendations for antibiotic therapy for patients who have a positive cerebrospinal fluid Gram stain or culture.

RECOMMENDATIONS FOR ANTIBIOTIC THERAPY IN PATIENTS WITH A POSITIVE CSF GRAM STAIN OR CULTURE			
Bacteria		**Antibiotic**	**Alternative antibiotic (in the case of allegy)**
Gram stain	**Culture**		
Gram-positive diplococci	*Streptococcus pneumoniae*, penicillin-resistant or sensitivity unknown	Ceftriaxone plus vancomycin or rifampicin	Vancomycin plus rifampicin
	Streptococcus pneumoniae, penicillin-sensitive	Penicillin G	Ceftriaxone or meropenem or chloramphenicol
Gram-positive cocci	ß-hemolytic streptococci	Penicillin or ampicillin	Cefotaxime, chloramphenicol, or vancomycin
Gram-negative coccobacilli	*Haemophilus influenzae*	Ceftriaxone or cefotaxime	Meropenem or chloramphenicol
Gram-negative diplococci	*Neisseria meningitidis*	Penicillin G	Ceftriaxone or chloramphenicol
Gram-positive bacilli	*Listeria monocytogenes*	Ampicillin plus gentamicin	Trimethoprim-sulfamethoxazole
Gram-negative bacilli	Enterobacteriaceae	Ceftriaxone plus gentamicin	Quinolones or meropenem
Gram-negative bacilli	*Pseudomonas aeruginosa*	Ceftazidime plus tobramycin	Quinolones or meropenem

drug is given parenterally, often combined with intraventricular administration. For cure, infected ventricular shunts must be removed in most patients. If a new permanent shunt is needed, it should only be implanted after a few days of adequate antibiotic therapy, during which time the ventricles are drained externally.

In patients who have chronic meningitis, empiric antibiotic therapy should be directed at the suspected pathogen (Table 22.10). Often, a definite diagnosis is not available for several days, in which case empiric therapy may have to be initiated. It is important to cover the treatable causes of chronic meningitis, for which the outcome is poor if treatment is delayed. For example, empiric antituberculous therapy should be instituted promptly for cases of suspected tuberculous meningitis.

Definitive antibiotic therapy

Identification and sensitivity testing of the causative organism is followed by adjustment of antibiotic therapy to provide optimal but narrow coverage (Table 22.10 , Table 22.11). Information on antibiotic sensitivities is crucial in the case of the pneumococci (see above), *H. influenzae* and Gram-negative rods or staphylococci that commonly cause bacterial meningitis in neurosurgical patients. Optimal duration of treatment of bacterial meningitis has not been carefully studied, but a total of 7 days appears adequate if the disease is caused by *H. influenzae* type b or *N. meningitidis*, although treatment should be extended to 10–14 days for the other common organisms, including pneumococci, and to 21 days for neonatal meningitis. Duration of treatment of chronic meningitis depends on the pathogen and can vary from days (syphilis) to months (tuberculosis, cryptococcosis) to indefinitely (coccidioidomycosis).

Adjunctive therapy

Several recent clinical trials have indicated that adjunctive therapy with dexamethasone improves the neurologic and audiologic outcome in bacterial meningitis. This has been shown primarily in children who have *H. influenzae* meningitis; the data are more limited for children who have pneumococcal and meningococcal meningitis, and only scant for adults who have bacterial meningitis.[71] [72] [73] Experimental and clinical data suggest that the maximum benefit of dexamethasone is achieved when the drug is given either shortly before or at the same time as the first antibiotic dose. This is probably related to the fact that corticosteroids reduce the release of proinflammatory cytokines, which are stimulated by bacterial products liberated from the pathogen by the bactericidal action of antibiotics.

Based on the available data, dexamethasone is recommended for all children over 6 weeks of age who have bacterial meningitis, beginning if possible 10–15 minutes before the first antibiotic dose. The recommended dose is 0.15mg/kg intravenously q6h, for a duration of 2–4 days. A study of dexamethasone in neonates with meningitis, caused in a majority of patients by *Klebsiella pneumoniae*, has yielded negative results and dexamethasone is not recommended in this patient group.[74]

In the absence of sufficient controlled data for adult patients, a conservative approach could consist of giving dexamethasone (in the same dose as in children) to patients who have clinical evidence of severely impaired CNS physiology with altered mental status, high CSF pressure, signs of brain edema on CT scan or rapidly progressive disease. Dexamethasone in standard doses (Table 22.12) had a significant beneficial effect on the mortality rate in patients with severe pneumococcal meningitis admitted to intensive care units in a recent French study, supporting its use in high-risk populations.[75] A small controlled study in India and the preliminary results from a large, controlled European study also indicate beneficial effects of dexamethasone

TABLE 22-12 -- Measures to reduce intracranial pressure in acute meningitis.

MEASURES TO REDUCE INTRACRANIAL PRESSURE IN ACUTE MENINGITIS
Intracranial pressure monitoring
Normalization of systemic blood pressure
Dexamethasone, 0.15mg/kg q6h
Head elevation of more than 30° to the horizontal
Mannitol, 1–1.5g/kg iv over 15 min; repeat once
Intraventricular shunt with CSF drainage in cases where there is evidence of hydrocephalus

on the neurologic outcome in adults who have bacterial meningitis.[73] When instituting dexamethasone therapy, it is important to confirm the bacterial etiology of meningitis. If this cannot be achieved within 24–48 hours, it is safer to stop the anti-inflammatory drug and to reassess the appropriateness of the chosen antimicrobial therapy. At present, there is no evidence that treatment with dexamethasone for 1–2 days has any adverse effects on the outcome of viral meningitis. It is important that patients who are given dexamethasone are closely monitored for evidence of gastrointestinal blood loss.

There is no recognized benefit from either prophylactic or full anticoagulation in the management of patients who have bacterial meningitis, and the involvement of the cerebral vasculature by the inflammation is likely to increase the risk of cerebral hemorrhages in anticoagulated patients who have bacterial meningitis. In fulminant disseminated meningococcal sepsis, there may be a benefit on outcome from the administration of protein C or activated protein C.[76] However, there are no data to suggest that protein C has a beneficial effect on the outcome of meningitis caused by *N. meningitidis* or other meningeal pathogens.

Supportive care

In critically ill patients who have bacterial meningitis, intensive supportive care may be needed, indicating admission to a critical care unit. Patients who have severe meningitis are often neurologically depressed and prone to seizures; they may need intubation for airway protection or assisted ventilation.

Children may have complex requirements for fluid supplementation, with the need for a careful clinical assessment of fluid status, repletion of fluid deficits and monitoring for SIADH. If SIADH is present (indicated by falling serum sodium and reduced urine output with a urine sodium concentration of more than 50mmol/l), fluids should be reduced to two-thirds of the maintenance level until the SIADH has resolved. Resolution within 1–2 days is usual.

In addition, active attempts to reduce intracranial hypertension should be undertaken in patients who have increased intracranial pressure and severely impaired CNS function. The potential importance of aggressively controlling intracranial hypertension was documented in a recent study in patients who had severe intracranial hypertension, in whom ongoing intracranial pressure monitoring and a standardized approach to lower intracranial pressure with antihypertensive drugs, establishment of normovolemia and normal plasma colloid osmotic pressure, and sedative therapy was used ('Lund Concept').[77] Interestingly, even patients with imminent cerebral herniation survived in this small series.

Other means of lowering intracranial pressure that have been described are listed in Table 22.12 . With the exception of dexamethasone, none of these approaches has been validated in controlled studies in patients who have meningitis.[78] Hyperventilation, which is used to lower intracranial pressure in other settings, may be harmful in patients who have increased intracranial pressure due to bacterial meningitis by exacerbating focal cerebral ischemia.[75]

Monitoring during therapy

Repeat CSF examination should be carried out in patients in whom there is doubt about the success of therapy or the accuracy of the initial diagnosis, but not in patients who respond promptly to therapy. Recurrent fever after an initial response to therapy is common in patients who have meningitis, particularly in children, and is most commonly due to infected intravenous lines, secondary infectious foci (e.g. septic arthritis, purulent pericarditis, pleural or intracranial empyema) or drug fever. Sterile subdural effusions, which occur in approximately one-third of children who have meningitis, do not require drainage, unless symptoms or signs of intracranial hypertension are present. Obstructive hydrocephalus, which occurs in less than 5% of patients, usually manifests within the first few weeks of infection and should be treated with ventriculoperitoneal shunting. Neurologic sequelae, including hearing impairment, cranial nerve palsies and motor deficits, can improve for several months after the acute illness, and appropriate, individually tailored supportive therapies should be arranged for patients who are left with sequelae from the disease.

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Chapter 23 - Viral Infections of the Central Nervous System

Richard J Whitley

INTRODUCTION

Central nervous system (CNS) symptoms (e.g. headache, lethargy, impaired psychomotor performance) are frequent components of viral infections; however, viral meningitis and encephalitis are unusual manifestations of human disease. Thus, while many individuals develop systemic viral illnesses, disease is usually mild and self-limiting with only a few developing symptomatic involvement of the brain.

Viruses vary widely in their potential to produce CNS infection. For some virus infections, CNS involvement is a common but a relatively benign component of the clinical syndrome (e.g. mumps). For others (e.g. Japanese encephalitis), neurologic disease is the most prominent clinical feature of illness. A third group of viruses commonly cause infection but only rarely cause encephalitis (e.g. herpes simplex virus (HSV)). Lastly, there are viruses for which human infection inevitably and exclusively results in CNS disease (e.g. rabies). In addition to acute pathology, numerous other viruses (e.g. influenza and measles) can cause syndromes of postinfectious encephalopathy.

When infection involves the CNS, there is potential for neurologic damage and, in some instances, death. Neural tissues are exquisitively sensitive to metabolic derangements and injured brain tissue recovers slowly and often incompletely. [1] Clinical presentation and patient history, while frequently suggestive of a diagnosis, remain unreliable methods for determining the specific etiology of CNS disease.[2] [3] Tumors, infections and autoimmune processes in the CNS often produce similar signs and symptoms.[3] Different diseases may share a common pathogenic mechanism and, therefore, result in a similar clinical presentation. Furthermore, understanding the pathogenic mechanism of a disease provides a rational basis for the development of antiviral medications as well as strategies for the prevention of viral CNS infections.

DEFINITIONS

Definitions of CNS viral disease are often based on both virus tropism and disease duration. Inflammation can occur at multiple sites within the brain, accounting for the myriad clinical descriptors of viral neurologic disease. Inflammation of the spinal cord, leptomeninges, dorsal nerve roots or nerves results in myelitis, meningitis, radiculitis and neuritis, respectively. Aseptic meningitis is a misnomer frequently used to refer to a benign, self-limited, viral infection that results in inflammation of the leptomeninges.[4] [5] The term hinders epidemiologic studies as the definition fails to differentiate between infectious (fungal, tuberculous, viral or other infectious etiologies) and noninfectious causes of meningitis. Encephalitis refers to inflammation of brain parenchyma and is usually accompanied by an altered level of consciousness, impaired cognition, seizures and/or focal neurologic signs. Acute encephalitis occurs over a relatively short period of time (days) while chronic encephalitis presents over weeks to months. The temporal course of slow infections of the CNS (kuru, visna, variant Creutzfeldt-Jakob disease (vCJD)) overlaps the chronic encephalitides. Slow viral infections of the CNS are distinguished by their long incubation, eventually resulting in death or extreme neurologic disability over months to years.[6]

Viral disease in the CNS can also be classified by pathogenesis. Neurologic disease is frequently categorized as either primary or postinfectious. Primary encephalitis results from direct viral entry into the CNS that produces clinically evident cortical or brainstem dysfunction.[7] Subsequent damage results from a combination of viral-induced cytopathic effects. Viral invasion, however, remains the initiating event.[7] The parenchyma exhibits neuronophagia and the presence of viral antigens or nucleic acids.[7] Postinfectious encephalitis produces signs and symptoms of encephalitis, temporally associated with a systemic viral infection, without evidence of direct viral invasion in the CNS. Pathologic specimens demonstrate demyelination and perivascular aggregation of immune cells, without evidence of virus or viral antigen, suggesting an autoimmune etiology.[7]

Meningitis and encephalitis represent separate clinical entities; however, a continuum exists between these distinct forms of disease. Nevertheless, a discussion of meningitis and encephalitis provides a useful format for this review.

VIRAL MENINGITIS

Epidemiology

Acute viral meningitis and meningoencephalitis represent the majority of viral CNS infections and frequently occur in epidemics with a seasonal distribution. [8] As illustrated in Figure 23.1 , enteroviruses cause an estimated 90% of cases (in countries that immunize against mumps), while arboviruses constitute the majority of the remaining reported cases in the USA.[4] [9] [10] Mumps is an important cause of viral CNS disease in countries that do not immunize against this agent. In a Japanese study, mumps was the second leading cause of aseptic meningitis, accounting for approximately 30% of the cases.[11] Viral meningitis is not a reportable disease to the Centers for Disease Control and Prevention (CDC); however, there are probably over 74,000 cases annually in the USA.[10] [12] Most cases occur from late spring to autumn, reflecting the increased incidence of enteroviral and arboviral infections during these seasons.[10] [13] A retrospective survey performed in the 1980s found that the annual incidence of 'aseptic meningitis' was approximately 10.9/100,000 persons or at least four times the incidence passively reported to the CDC during the same period.[8] Virus was isolated in only 11% of patients in this study, likely reflecting the technologic limits of the period, the infrequency with which viral cultures were performed and the decreased incidence of viral CNS disease resulting from widespread vaccination against mumps and polioviruses.[8] With the advent of molecular-based diagnostic techniques, namely polymerase chain reaction (PCR), identification rates now approach 50–70%.[9] [11]

Pathogenesis

The pathogenesis of viral meningitis is not completely understood. Inferences regarding the pathogenesis of viral meningitis are largely

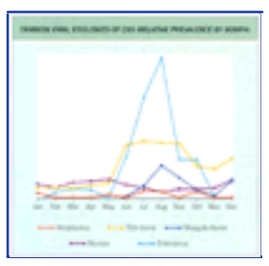

Figure 23-1 Common viral etiologies of central nervous system infection (relative prevalence by month).

derived from data on encephalitis, experimental animal models of meningitis and clinical observations.[4] Viruses use two basic pathways to gain access to the CNS, namely hematogenous and neuronal, regardless of the resulting clinical syndrome (i.e. meningitis or encephalitis). A combination of host and viral factors along with seasonal, geographic and epidemiologic probabilities influence the likelihood of CNS infection. For example, arboviral infections occur more frequently in epidemics and show a seasonal variation, reflecting the environmental prevalence of the transmitting vector.[13] Enteroviral meningitis occurs with greater frequency during the summer and early autumn months, reflecting the seasonal increase in enteroviral infections. Enteroviral infections also exemplify the difference host physiology plays in determining the extent of viral disease. In children less than 2 weeks of age, enterovirus infections can produce a life-threatening infection, including meningitis or

meningoencephalitis.[10] Ten per cent of neonates with systemic enteroviral infections die and 76% of survivors have permanent sequelae. In children over 2 weeks of age, however, enteroviral infections are rarely associated with severe disease or significant morbidity.[10]

For hematogenous spread to the CNS, as illustrated in Figure 23.2 , the virus must first either bypass or attach to and enter host epithelial cells to produce infection. Virus then spreads and initially replicates in the regional lymph nodes (e.g. measles, influenza) or, alternatively, it enters the circulatory system where it seeds other tissues (e.g. arboviruses, enteroviruses, varicella). Primary viremia allows virus to seed distant locations of the body, especially the reticuloendothelial system, and frequently marks the onset of clinical illness. In rare circumstances, such as disseminated neonatal HSV infection, virus infects the CNS during primary viremia. The liver and spleen provide ideal locations for secondary viral replication due to their highly vascular structure and reticuloendothelial network. Secondary viremia results in high titers of virus in the bloodstream, facilitating viral CNS spread. The pathophysiology of viral transport from blood to brain and viral endothelial cell tropism is poorly understood. Virus infects endothelial cells, passively channels through endothelium (pinocytosis or colloidal transport) or

Figure 23-2 Hematogenous spread of viral pathogens to the central nervous system.

bridges the endothelium within migrating leukocytes. This trans-endothelial passage occurs in vessels of the choroid plexus, meninges and/or cerebrum.

Numerous host defense barriers limit viral dissemination to the CNS. The skin and mucosal surfaces possess mechanical, chemical and cellular defenses that protect the cells from viral infection. Leukocytes and secretory factors (interleukins, interferons, antibodies) further augment these defenses and help eliminate viruses that bridge the epithelial layer. Local immune responses are crucial in limiting systemic viral infection. A prompt inflammatory response can limit viremia and subsequent symptoms of infection. In the liver and spleen, the high degree of parenchymal contact and large number of fixed mononuclear macrophage cells provide an excellent opportunity for host eradication of viremia. The blood-brain or blood-cerebrospinal fluid (CSF) barrier, a network of tight endothelial junctions sheathed by glial cells that regulate molecular access to the CNS, further limits virus access to the brain.[14]

Viral meningitis is a relatively benign, self-limited illness; thus, tissue specimens are rarely available for pathologic study.[10] The CSF, however, is frequently sampled and demonstrates a mononuclear immune cell response to most viral infections. Certain viral infections, most notably mumps and some enterovirus infections, elicit a polymorphonuclear cell infiltrate in the CSF early during disease. The initial CSF formula mimics bacterial meningitis and later shifts to a mononuclear predominance. Viral antigen presentation by mononuclear histiocytes stimulates the influx of immune cells. Recruited immune cells release soluble factors (cytokines, vasoactive amines) that mobilize other cells and change the permeability of the blood-brain barrier.[15] [16] Physical and chemical changes in this barrier allow the entry of serum proteins (i.e. immunoglobulins and interleukins), further augmenting host immune responses. The cell-mediated immune response is important for eliminating virus from the brain; however, immunoglobulin also has a role in protecting the host in some viral infections. This is best illustrated by the devastating clinical course of enteroviral meningitis in agammaglobulinemic patients as well as X-linked hyper IgM syndrome (see Chapter 98).[10] [17] Patients with impaired cell-mediated immunity have a higher incidence of CNS infections with certain viruses (e.g. varicella-zoster virus (VZV), measles, cytomegalovirus (CMV)).

Clinical manifestations

Age, immune status and viral etiology influence the clinical manifestations of viral meningitis. Patients with enteroviral meningitis often present with non-specific symptoms such as fever (100.4–104.00°F; 38–40°C) of 3–5 days duration, malaise and headache.[10] [18] Approximately 50% of patients have nausea or vomiting.[18] While nuchal rigidity and photophobia are the hallmark sign and symptom for meningitis, 33% of patients with viral meningitis have no evidence of meningismus.[18] Importantly, clinical presentation does not lead to the suggestion of etiology. Less than 10% of children younger than 2 years of age develop signs of meningeal irritation; they present with fever and irritability.[19] Children may also present with seizures secondary to fever, electrolyte disturbances or the infection itself. In the immunocompromised host, enteroviral infection is both a diagnostic quandary and a potentially life-threatening disease. Immunocompromised patients frequently do not mount brisk immune cellular responses; therefore, CSF analyses do not necessarily reflect evidence of CNS involvement.

Symptoms of meningitis (stiff neck, headache and photophobia) occur in approximately 11% of men and 36% of women with primary HSV-2 genital infection. In one study, 5% of patients with primary HSV genital infection had severe enough meningitis to require hospitalization. All hospitalized patients had evidence of a CSF lymphocytic pleocytosis. In another study, HSV-2 was cultured from the CSF of 78% of patients with meningismus during primary genital infection. These patients also exhibited a CSF leukocytosis and subsequent increases in CSF HSV antibody titers. [20] Recurrent HSV-2 meningitis (with or without genital lesions) occurs, although cases associated with primary infection are more common.[21] Herpes simplex virus meningitis may spread to the CNS neuronally along the sacral nerves. Alternatively, the virus may reach the CNS hematogenously, as virus has been cultured from the blood buffy-coat layer.[4] Varicella-zoster virus, CMV, Epstein-Barr virus (EBV) and parainfluenza virus have all been cultured or detected by PCR in the CSF of patients with meningitis.[4] [11] [22]

Laboratory findings

Initial CSF samples, while frequently suggestive of the diagnosis, are neither sensitive nor specific enough to differentiate viral from bacterial meningitis. [23] Instead, epidemiologic trends, patient history and accompanying laboratory information are important adjuncts in assessing the etiology of meningitis. The CSF in patients with viral meningitis typically exhibits pleocytosis with 10–500 leukocytes and a slightly elevated protein level (<100mg/dl). The glucose level in the CSF is typically greater than 40% of a simultaneously drawn serum sample. Tremendous variation in CSF formulas exists, with significant overlap between viral and bacterial findings.[23] In a retrospective review of over 400 patients with acute viral or bacterial meningitis performed before the *Haemophilus influenzae* B conjugate vaccine, investigators found that approximately 20% of the CSF samples that grew bacteria exhibited a CSF pleocytosis <250 WBC/mm^3 .[4] Of the patients with bacterial meningitis 15% had a CSF lymphocytosis, while 40% of the patients with viral meningitis had a predominance of polymorphonuclear cells. Some investigators recommend repeating the lumbar puncture 6–12 hours later, as the CSF profile of patients with viral meningitis will shift from a polymorphonuclear to a lymphocytic pleocytosis over this period.[24] A retrospective study found that during an enterovirus outbreak, 51% of patients demonstrated PMN predominance in their CSF profile despite symptoms of greater than 24 hours duration but the etiology of meningitis could not be confirmed in most cases. [23] Other investigators have confirmed that the change to a lymphocytic CSF profile occurs 18–36 hours into the illness. Most clinicians do not obtain CSF viral cultures.[25] [26]

Etiologic diagnosis

Historically, the techniques for identifying viral meningitis were insensitive and often impractical. While virus can be cultured from CSF during the early stages of the infection, this has little utility in the acute management of a patient who has CNS disease.[20] Depending upon the study cited and diagnostic methods used, investigators identify an agent in only 25–67% of presumed CNS infections.[3] Now, however, molecular techniques have advanced identification of agents. Recently, PCR and reverse transcription (RT)-PCR have been used to diagnose enteroviral meningitis in both normal and agammaglobulinemic patients, indicating both sensitivity and specificity. [25] Polymerase chain reaction provides a rapid and reliable test for verifying the etiology of certain types of meningitis. These techniques provide results within 24–36 hours and therefore may limit the duration of hospitalization, antibiotic use and excessive diagnostic procedures.[26] As it relates to the diagnosis of arboviral disease, the use of molecular techniques is fraught with greater variability.[10] [20] [26] Because of the diverse viral etiologies of arboviral infection, the development of specific, conserved primers that hybridize across multiple viral families (alphaviradae, flaviviradae, bunyaviradae) has been difficult. Currently, there is an emphasis on the development of improved 'universal group primers' to perform an initial group screening followed by RT-PCR using higher specificity primers as a second viral diagnostic test.[27] For other infections, molecular techniques are the standard for diagnosing viral meningitis.[9] [12]

Differential diagnosis

Unusual but treatable infections should always be considered and investigated in patients with a CSF pleocytosis and negative conventional bacterial cultures. Spirochetes (*Treponema, Borrelia, Leptospira*), mycoplasma, bartonella and mycobacteria can produce a pleocytosis with negative Gram stain and negative bacterial cultures. Fastidious bacteria (*Listeria*) may fail to grow in culture and occasionally produce a mononuclear pleocytosis similar to viral meningitis, as can occur in infants, the elderly and immunocompromised patients. Some bacteria, while not directly infecting the CNS, can release toxins that create a change in the level of consciousness, specifically *Staphylococcus aureus* and *Streptococcus pyogenes* exotoxin-mediated toxic shock syndrome. Parameningeal infections, especially from infected sinuses, produce a pleocytosis and CNS symptoms; however, these infections more frequently present as encephalitis with focal neurologic changes and altered mental status. Similarly, partially treated bacterial infections can have CSF findings resembling those of viral meningitis. Fungal and parasitic infections can produce CNS infections although they uncommonly produce only meningitis. The exceptions to this rule are Coccidioides and Cryptococcus. These pathogens

characteristically produce meningitis rather than any focal CNS disease. Cryptococcus, for example, produces subacute meningitis in both normal and immunosuppressed patients and remains the leading cause of fungal meningitis.[5] Such fungal infections as *Candida*, *Aspergillus*, histoplasmosis and blastomycosis frequently cause focal parenchymal disease of the CNS. These fungi, while often weighed in the differential diagnosis for an immunocompromised host, also cause disease in the normal host. Parasites such as *Naeglaeria fowleri* produce meningoencephalitis with purulent CSF findings. A history of recent summertime swimming in a stagnant pond raises suspicion for this infection.

Noninfectious processes that can produce true aseptic meningitis include hematologic malignancies, medications, autoimmune diseases and foreign material and proteins. Leukemia produces a CSF pleocytosis with cancerous cells and occurs most frequently with acute lymphocytic leukemia, although subarachnoid involvement can also occur in acute myelogenous leukemia.

Immunomodulatory drugs such as intravenous immunoglobulin or antilymphocytic globulin (OKT-3) can cause aseptic meningitis. Of the medications associated with meningitis, nonsteroidal anti-inflammatory agents, sulfa-containing drugs and cytosine arabinoside are the most common offenders. Drug-induced aseptic meningitis fre-quently occurs in patients who have underlying connective tissue or rheumatologic diseases.[4] [5] Epithelial or endothelial cysts can rupture and spill their contents (keratin, protein), producing a brisk inflammatory response, mimicking meningitis.

Those common viral agents responsible for CNS infection are listed in Table 23.1 . Notably, all of these agents cause both meningitis and encephalitis with two exceptions — rabies and B virus.

Treatment and prognosis

The fundamental principle of therapy for viral meningitis lies in the identification of potentially treatable diseases. Until recently, no therapy existed for most cases of viral meningitis. Efforts instead focused on preventive strategies (largely through vaccination) as well as identification of treatable nonviral etiologies of meningitis. Antiviral therapies are emerging for the treatment of enteroviral meningitis.[28] [29] Despite recent chemotherapeutic and diagnostic advances in the approach to enteroviral infection, patients who have meningitis warrant careful assessment for treatable, nonviral etiologies of meningitis. The clinician must also anticipate and treat the complications of viral CNS disease (seizures, inappropriate ADH secretion, hydrocephalus, raised intracranial pressure). Supportive therapy includes hydration and antipyretics/analgesics.

As noted above, in the normal host, viral meningitis is a relatively benign self-limited disease. A prospective study in children less than 2 years of age, for example, found that even in the 9% of children who develop evidence of acute neurologic disease (complex seizures, increased intracerebral pressure or coma) long-term prognosis is

TABLE 23-1 -- Viral causes of meningitis or encephalitis.

VIRAL CAUSES OF MENINGITIS OR ENCEPHALITIS	
Togaviridae alphavirus	Eastern equine
	Western equine
	Venezuelan equine
Flaviviridae	West Nile
	St Louis
	Japanese B
	Murray Valley
	Tick-borne complex
Bunyaviridae	California/LaCrosse
	Rift Valley
Adenoviridae	Adenovirus
Arenaviridae	Arenavirus
Rhabdoviridae	Rabies
Reoviridae	Colorado tick fever
Ortho- and paramyxoviridae	Influenza
	Mumps
	Measles
Picornaviridae: enterovirus	Poliovirus
	Coxsackie virus
	Echovirus
Herpesviridae	Herpes simplex viruses 1 and 2
	Epstein-Barr virus
	Varicella-zoster virus
	Cytomegalovirus
	Human herpesvirus 6
Retroviridae	HIV

excellent. During follow-up (42 months), children with acute CNS complications performed neurodevelopmental tasks and achieved developmental milestones as well as did children with an uncomplicated course.[19]

Currently, antibody preparations and an antiviral agent, pleconaril (see below), have shown activity against enterovirus infection in case reports and animal studies. Randomized, controlled trials, however, have not supported their routine use in enterovirus meningitis. In case reports, immunoglobulin preparations, given systemically or intrathecally, retarded mortality and morbidity in agammaglobulinemic patients with enteroviral meningitis. Despite the administration of immunoglobulin, patients do not eliminate virus from the CSF and in turn develop chronic enteroviral meningitis.[30] As noted previously, enteroviral infections in neonates frequently produce over-whelming viremia and CNS disease. A blind, randomized controlled trial did not demonstrate clinical benefit for enterovirus-infected neonates with severe life-threatening disease who received intravenous immunoglobulin. [31]

The antiviral agent (3-[3,5-dimethyl-4-[[3-(3-methyl-5-isoxazolyl)propyl]phenyl]-5-trifluoromethyl-1,2,4-oxadiazole), pleconaril, is a bioavailable, small-molecule inhibitor of picornavirus replication that binds the capsid and prevents uncoating of viral RNA. Because of homology among the picornaviruses, the drug has activity against enteroviruses and rhinoviruses as well. Randomized controlled, double-blind clinical trials, while demonstrating slight improvements in adults with enteroviral meningitis, do not demonstrate the substantial efficacy initially anticipated and have not been published in peer-reviewed literature. In 32 adults with aseptic meningitis, duration of headache was decreased in the pleconaril group to an average 6.5 days of headache versus 18.3 days for placebo recipients.[28] The duration of headache in the placebo group was greater than previously published. Similarly, if one uses the objective measurement of duration of analgesic use (historical average control = 5 days) versus that reported in the pleconaril study (placebo group = 11.5 days), the statistically significant 5.3 days of analgesic use by the pleconaril group is less dramatic.[28] [32] A multicenter randomized controlled trial is evaluating pleconaril in the treatment of severe neonatal infection but preliminary information is unavailable at this time.

Specific antiviral agents are available for several other viral etiologies of meningitis. Although no definitive clinical trials have been conducted, most authors recommend the use of intravenous aciclovir for HSV meningitis, as it decreases the duration of primary disease and may limit meningeal involvement.[33] There are no data on benefit of antiviral treatment or on suppressive therapy for recurrent HSV CNS disease.[21] Effective antiviral therapy exists for VZV infections of the CNS and should be instituted in these patients. [34] The issue of therapy for CMV CNS infection in the immunocompromised host is more problematic and therapy should be tailored based upon the clinical likelihood of infection.

VIRAL ENCEPHALITIS

Epidemiology

Similar to viral meningitis, passive reporting systems underestimate the incidence of viral encephalitis,[8] as reviewed. [35] An estimated 20,000 cases of encephalitis occur annually in the USA; however, the CDC received only 740 (0.3/100,000) to 1340 (0.54/100,000) annual reports of persons with encephalitis from 1990 to 1994.[7] [36] A review of the cases in Olmsted County, Minnesota, from 1950 to 1980 found the incidence of viral encephalitis to be twice that reported by the CDC.[8] A prospective multicenter study in Finland provided results similar to the Olmsted study, indicating an encephalitis incidence of 10.5/100,000.[37] Herpes simplex virus CNS infections occur without seasonal variation, affect all ages and constitute

the majority of fatal cases of endemic encephalitis in the USA.

Arboviruses, a group of over 500 arthropod-transmitted RNA viruses, are the leading cause of encephalitis worldwide and in the USA. Arboviral infections occur in epidemics and show a seasonal predilection, reflecting the prevalence of the transmitting vector.[38] Asymptomatic infections are vastly more common than symptomatic infections. Patients who have disease may develop a mild systemic febrile illness or viral meningitis.[39] Encephalitis occurs in a minority of persons with arboviral infections, but the case fatality rate varies from 5% to 70%, depending upon viral etiology and patient age. LaCrosse encephalitis is the most commonly reported arboviral disease in the USA, while St Louis encephalitis is the most frequent cause of epidemic encephalitis, although this may be replaced by West Nile encephalitis if epidemics continue.[38]

Japanese B encephalitis (JE) and rabies constitute the majority of cases of encephalitis outside North America. JE, a member of the flavivirus genus, occurs throughout Asia and causes epidemics in China despite routine immunization. In warmer locations, virus occurs endemically. As with the other arboviral infections, asymptomatic infections occur more frequently than symptomatic infections. However, the disease has a high case fatality rate and leaves half of the survivors with significant neurologic morbidity. Rabies is endemic around much of the world. Human infections in the USA decreased over the last decades to 1–3 cases per year due to the immunization of domesticated animals. In areas outside the USA, human cases of rabies number in the thousands and are caused by bites from unvaccinated domestic animals following contact with infected wild animals.

Postinfectious encephalitis, an acute demyelinating process, has also been referred to as acute disseminated encephalomyelitis (ADEM) or autoimmune encephalitis and accounts for approximately 100–200 additional cases of encephalitis annually.[36] The disease historically produced approximately one-third of the encephalitis cases in the USA and was associated with measles, mumps and other exanthematous viral infections.[40] Postinfectious encephalitis is now associated with antecedent upper respiratory virus (notably influenza virus) and varicella infections in the USA.[7] Measles continues to be the leading cause of postinfectious encephalitis worldwide and complicates 1 of every 1000 measles infections.[7]

Slow infections of the CNS or transmissible spongiform encephalopathies (TSEs) occur sporadically worldwide (Table 23.2). While not

TABLE 23-2 -- Transmissible spongiform encephalopathies.[6]

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES	
Animals (excluding experimentally transmitted diseases)	Scrapie in goats and sheep
	TME in mink
	Chronic wasting disease in deer and elk
	BSE in cattle
	Feline spongiform encephalopathy in cats and zoo felines, probably from BSE
	TSE of zoo ruminants, probably from BSE
	TSE in zoo monkeys, probably from BSE
Humans	Kuru
	CJD
	Iatrogenic
	Sporadic
	Familial
	vCJD, probably from BSE
	Gerstmann-Straussler-Scheinker syndrome
	Fatal familial insomnia
	Sporadic fatal insomnia

caused by a classic infectious agent, brief consideration is warranted (see also Chapter 26 and Chapter 223). The prototypical TSE is Creutzfeldt-Jakob disease (CJD), occurring at high rates within families and having an estimated incidence of 0.5–1.5 cases per million population.[6] In 1986, cases of a TSE in cattle, namely bovine spongiform encephalopathy (BSE), were reported in the UK. In addition to affecting other livestock throughout Europe that were fed supplements containing meat and bone meal, cross-species transmission of BSE has been documented, leading to a ban on the use of bovine offal in fertilizers, pet food and other animal feed.[6] A decrease in the recognized cases of BSE has occurred since the institution of these restrictions. Concomitant with the increased cases of BSE in Europe, an increase in cases of an atypical CJD also occurred, suggesting animal-to-human transmission. The report of atypical CJD (unique clinical and histopathologic findings) affecting young adults (an age at which CJD rarely has been diagnosed) led to the designation of a new disease, nvCJD (see Chapter 26). According to the November 2002 fact sheet of the World Health Organization, there have been 93 confirmed cases of vCJD reported in the UK, six in France and one each in Italy, Canada, and the USA.[41]

Pathogenesis

The pathogenesis of encephalitis is similar to that of viral meningitis and requires that viruses reach the CNS by hematogenous or neuronal spread. Viruses most frequently access the CNS after a high-titer secondary viremia and cell-free or cell-associated CNS entry.[4] Other than direct entry via cerebral vessels, virus can initially infect the meninges and then enter the parenchyma across either ependymal cells or the pial linings. Viruses exhibit differences in neurotropism and neurovirulence.[42] For example, enteroviruses with similar receptors produce very different diseases. Five coxsackie B viruses (B1–B5) readily produce CNS infections while type B6 rarely produces neurologic infection.[5] Similarly, viral genes have been discovered that influence the neurovirulence of HSV-1. Mutant HSV-1 viruses in the $?_1$ 34.5 gene have a decreased ability to cause encephalitis and death following intracerebral inoculation in mice as compared with wild-type virus.

In addition to viral factors, host physiology is also important in determining the extent and location of viral CNS disease. Age, sex and genetic differences between hosts influence viral infections and clinical course.[10] Host age influences the clinical manifestations and sequelae. Variations in macrophage function between individuals

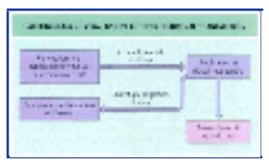

Figure 23-3 Pathogenesis of viral central nervous system infections: neuronal transmission.

can result in clinically distinct infections and disease. Moreover, macrophage-antigen response can change with age and is important in limiting spread of infection within a patient. In addition to age, physical activity may be another important host factor that determines the severity of infection. Exercise has been associated with increased risk for paralytic poliomyelitis and may result in an increased incidence of enteroviral myocarditis and aseptic meningitis.[43] [44] Increasingly host differences are recognized as equally important determinants of disease at the cellular and molecular levels.

In addition to blood-borne spread of virus to the brain, neuronal transmission plays an important role in pathogenesis; HSV and rabies are the best examples, as illustrated in Figure 23.3 . Sensory and motor neurons contain transport systems that carry virion or virion components along the axon to (retrograde) and from (anterograde) the nucleus of the sensory ganglion. Peripheral or cranial nerves provide access to the CNS and shield the virus from immune regulation.

Rabies classically infects by the myoneural route and provides a prototype for peripheral neuronal spread.[45] Rabies virus replicates locally in soft tissue following a rabid animal bite. After primary replication, the virus enters the peripheral nerve by acetylcholine receptor binding. Once in the muscle, virus buds from the plasma membrane, crosses myoneural spindles or enters across the motor endplate.[45] The virus travels by anterograde and retrograde axonal transport to infect neurons in the brainstem and limbic system and spreads from the diencephalic and hippocampal structure to the remainder of the brain, killing the animal.[45] Virus also infects the CNS through cranial nerves, as suggested from animal studies but without supporting human data.

The pathologic findings of encephalitis are unique for each virus and reflect differences in pathogenesis and virulence. In the case of typical HSV encephalitis, a hemorrhagic necrosis[7] occurs in the inferomedial temporal lobe with evidence of perivascular cuffing, lymphocytic infiltration and neuronophagia.[46] Pathologic specimens with rabies encephalitis demonstrate microglial proliferation, perivascular infiltrates and neuronal destruction. The location of the pathologic findings can be limited to the brainstem areas (dumb rabies) or the diencephalic, hippocampal and hypothalamic areas (furious rabies) based on the immune response mounted against the infection.[45]

Some viruses do not directly infect the CNS but produce immune system changes that result in parenchymal damage. Patients with postinfectious encephalitis exhibit focal neurologic deficits and altered consciousness associated temporally with a recent (1–2 week) viral infection or immunization.[40] Pathologic specimens, while showing evidence of demyelination by histologic or radiographic analysis, do not demonstrate evidence of viral infection in the CNS by culture or antigen tests. Patients with postinfectious encephalitis have subtle differences in their immune system and some authors have proposed an autoimmune reaction as the pathogenic mechanism of disease.[7]

The TSEs are noninflammatory CNS diseases involving the accumulation of an abnormal form of a normal glycoprotein, the prion protein (PrP).[47] These encephalopathies differ in their mode of transmission. While most of the TSEs are experimentally transmissible by direct inoculation in the CNS, this mode rarely occurs except for iatrogenic transmissions.[6] The scrapie agent spreads by cell-to-cell contact. There is no evidence for lateral transmission in the case of BSE or vCJD and all cases appear to have occurred following parenteral transmission or ingestion of affected materials. The transmissible agents remain infectious after treatments that would normally inactivate viruses or nucleic acids (detergent formalin, ionizing radiation, nucleases).[47]

Most of the experimental work on TSEs has involved analysis of the scrapie agent. The current working model is that post-translational alteration of the normally a-helical form of the PrP protein results in a protease-resistant ß-pleated sheet structure that accumulates in neurons, leading to progressive dysfunction, cell death and subsequent astrocytosis. In studies on the scrapie agent, gastrointestinal tract involvement with infection of abdominal lymph nodes occurs first, followed by brain involvement a year or more later.[6] Experimental subcutaneous inoculation in mice and goats also leads to local lymph node involvement followed by splenic spread and then CNS involvement. The mode of transmission to the CNS (direct versus hematogenous) and the infectivity of body fluids at different stages of infection are not known.

Clinical manifestations

While physical examination of the patient usually does not suggest an etiologic diagnosis, a few considerations are essential. For example, encephalitis caused by West Nile virus is usually accompanied by a rash and can result in severe disease. In 2001, there were 66 human cases in the USA with nine deaths reported. Fatalities have been greater among the elderly. However, in general, for patients with acute viral encephalitis, the distinction between generalized and focal neurologic findings is important. In a nonepidemic setting, the most common viral cause of focal encephalopathic findings is HSV.[3] [48] However, when signs and symptoms of patients with biopsy-proven herpes simplex encephalitis (HSE) are compared with those who did not have HSV CNS infection, there were no distinguishing clinical characteristics (see Table 23.1). Viruses that usually cause diffuse encephalitic diseases can, on occasion, localize to one area of the brain and mimic HSE,[3] as summarized in Table 23.3 .

A distinction must be made clinically between viral encephalitis and postinfectious encephalomyelitis. Postinfectious encephalomyelitis generally follows a vague viral syndrome, usually of the respiratory tract, and is most common in children. Neurologic findings vary and reflect the areas of the brain involved. Demyelination is a prominent pathologic finding. The distinction between postinfectious encephalomyelitis and acute viral encephalitis is crucial, since the management and prognosis are often quite different.

Most patients have a prodromal illness with myalgias, fever and anorexia reflecting systemic viremia. The clinical hallmark of acute viral encephalitis is a triad of fever, headache and altered level of consciousness. Other common clinical findings include disorientation, behavioral and speech disturbances, and focal or diffuse neurologic signs, such as hemiparesis or seizures. These clinical findings distinguish a patient with encephalitis from one with viral meningitis, who may have headache, nuchal rigidity and fever but not altered sensorium or focal neurologic findings. Clinical findings reflect the specific areas of CNS involvement, which are determined, in large part, by

TABLE 23-3 -- Diseases that mimic herpes simplex encephalitis.[*]

DISEASES THAT MIMIC HERPES SIMPLEX ENCEPHALITIS	
Diseases	**No. of patients**
Treatable	**46**
Infection	
Abscess or subdural empyema	
• Bacterial	5
• Listeria	1
• Fungal	2
• Mycoplasma	2
Tuberculosis	6
Cryptococcal	3
Rickettsial	2
Toxoplasmosis	1

Mucormycosis	1
Meningococcal meningitis	1
Other viruses	
• Cytomegalovirus	1
• Influenza A*	4
• Echovirus infection*	3
Tumor	5
Subdural hematoma	2
Systemic lupus erythematosus	1
Adrenal leukodystrophy	6
Nontreatable	**49**
Vascular disease	11
Toxic encephalopathy	5
Reye syndrome	1
Viral	40
Arbovirus infection	
• St Louis encephalitis	7
• Western equine encephalitis	3
• California encephalitis	4
• Eastern equine encephalitis	2
Other herpesviruses	
• Epstein-Barr virus	8
Other viruses	
• Mumps virus	3
• Adenovirus	1
• Progressive multifocal leukoencephalopathy (JC virus)	1
• Lymphocytic choriomeningitis virus	1
• Subacute sclerosing panencephalitis (measles virus)	2

* *Adapted from Whitley et al.*[3]
* Investigation drug therapy

the tropism of different viruses for different cell types. For example, polioviruses preferentially infect motor neurons, rabies selectively infects neurons of the limbic system, while mumps can infect epithelial cells of the choroid plexus. Infection of cortical neurons results in abnormal electrical activity and can be associated with seizures or focal deficits. Demyelination may follow destruction of oligodendroglial cells, while involvement of ependymal cells can result in hydranencephaly. The predilection of HSV for temporal lobe involvement, as illustrated in Figure 23.4 , leads to clinical findings of aphasia, anosmia, temporal lobe seizures and other focal abnormalities. As noted, only two viruses, namely rabies and B virus, produce encephalitis without significant meningeal involvement; however, most patients with encephalitis have concomitant meningitis.

Laboratory findings/diagnosis

Establishing an etiologic diagnosis of encephalitis is just as difficult as for viral meningitis. As with the latter, epidemiologic features such as the season of year, prevalent diseases within the community, travel, recreational activities (e.g. caving or hiking), occupational exposures and animal contacts (e.g. insect or animal bite) may provide helpful clues to the diagnosis. Late summer and early fall are seasons when enteroviral infections are encountered in temperate climates. Similarly, during warm summer months, mosquito propagation may enhance the likelihood of transmission of arthropod-borne viruses.

A CSF pleocytosis usually occurs in encephalitis but is not necessary for the diagnosis. White blood cell counts typically number in the 10s to 100s in viral encephalitis, although higher counts occur. The CSF glucose levels are usually normal although some viral etiologies (Eastern equine encephalitis) produce findings consistent with acute bacterial meningitis. Some viruses (HSV) produce a hemorrhagic necrosis and the CSF exhibits this with moderately high protein levels and evidence of red blood cells. Supratentorial and cerebellar tumors can produce increased intracranial pressure and can mimic encephalitis. A careful fundoscopic examination should be performed to rule out any evidence of papilledema and increased intracranial pressure prior to obtaining CSF.

Unlike meningitis, encephalitis often requires additional laboratory and radiologic tests to establish the diagnosis. The clinical circumstances of the patient and the likely etiologies dictate specific laboratory and radiologic evaluations. Historically, the standard for diagnosis has been brain biopsy and viral culture. However, PCR has, for the most part, replaced this approach. For some viruses (HSV, enterovirus, VZV, JC virus), PCR detection of viral nucleic acids from the CSF has replaced culture and brain biopsy as the standard for diagnosing encephalitis.[9] [11] [25] [50] [51] [52] Radiographic studies that support the diagnosis of focal encephalitis are computerized tomography (CT) scanning and magnetic resonance imaging (MRI). The increased sensitivity of MRI to alterations in brain water content and the lack of bone artefacts make this the neuroradiologic modality of choice for CNS infections.[53] The MRI detects parenchymal changes earlier than CT scan and better defines the extent of a lesion.[53] Furthermore, MRI is more sensitive for detecting evidence of demyelinating lesions in the periventricular and deep white matter, thus allowing the differentiation of parainfectious from acute viral encephalitis.[53] Patients who have viral encephalitis frequently have diffuse or focal epileptiform discharges with background slowing.[2] These electroencephalogram (EEG) changes precede CT scan or MRI evidence of encephalitis and provide a sensitive, although non-specific, diagnostic test. Electroencephalogram changes in the temporal lobe area strongly suggest a diagnosis of HSE but absence of these changes does not rule out HSE.

Historically, patients who had viral encephalitis required a battery of different diagnostic tests. Herpes simplex encephalitis, for example, could be diagnosed acutely by brain biopsy and viral culture or retrospectively by CSF antibody and convalescent serologic tests.[46] Routine evaluation of acute and convalescence sera to demonstrate either seroconversion or sero-boosting is of no practical value in the decision to institute treatment, but remains useful for retrospective diagnosis of some infections (e.g. arboviral encephalitis). New diagnostic assays have simplified the diagnosis of viral infections of the brain. For example, an ELISA assay that detects IgM antibodies in the CSF from patients with presumed Japanese encephalitis (JE) is both sensitive and specific, as most patients have antibodies at the time of hospitalization and virtually all acquire them by the third day of illness.

A CSF PCR is used to diagnose enterovirus, HSV, VZV, human herpesvirus (HHV)-6, EBV and CMV,[54] as well as a few arboviruses (California encephalitis group, JE, West Nile virus, St Louis encephalitis (WNV), dengue fever virus 1–4 and yellow fever virus); however, the development of universal arboviral primers has been difficult.[9] [25] [27] [52] The successful detection of viral DNA in the CSF is influenced by the duration, extent and etiology of disease. Application

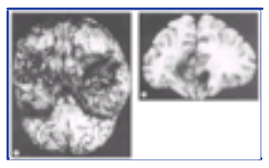

Figure 23-4 Herpes simplex encephalitis. Basilar view of herpes simplex encephalitis showing (a) hemorrhagic necrosis of temporal lobes. (b) Coronal section of brain from patient with herpes simplex encephalitis. [49]

of PCR is rapid and sensitive and provides a less invasive means to diagnose encephalitis. For example, only 4% of CSF cultures are positive in patients with sporadic HSE; however, 53 of 54 patients with biopsy-proven HSE had evidence of HSV DNA in the CSF by PCR. Cerebrospinal fluid PCR has a sensitivity in excess of 95% and a specificity approaching 100% in patients with HSE.[55] Interestingly, in the three cases where the CSF PCR was positive but the brain biopsy negative, biopsy samples were improperly prepared prior to viral culture or the biopsy site was suboptimal.[55] Efforts now focus on correlating viral nucleic acid copy number with clinical outcome.

The TSEs are currently only diagnosed by histologic examination of brain tissue, characteristic electroencephalography (EEG), MRI changes and clinical correlation. A CSF examination shows normal values or slightly elevated protein levels. The EEG in classic CJD reveals generalized slowing early in the disease, punctuated by biphasic or triphasic peaks late in the disease with the onset of myoclonus. The MRI changes late in the illness reveal global atrophy with hyperintense signal from the basal ganglia.[6] Fluid attenuation inversion recovery (FLAIR) MRI provides greater sensitivity and demonstrates signal intensity changes in the cortex that T2-weighted spin-echo MRI cannot detect. Histopathologic examination of the brain using a specific antibody to the PrP-res protein confirms the disease. In addition, evidence of gliosis, neuronal loss and spongiform changes supports the diagnosis. In cases of vCJD characteristic amyloid plaques (so-called florid plaques) microscopically define the disease. The florid plaques are not seen in other TSEs and consist of flower-like amyloid deposits surrounded by vacuolar halos. The detection of PrP-res in the tonsillar tissue by immunohistochemical staining is also strongly supportive of vCJD diagnosis.[6]

Differential diagnosis

Identifying treatable disease expeditiously is a priority in patients presenting with neurologic changes. In patients who have suspected HSE undergoing brain biopsy for confirmation of disease, alternative diagnoses are frequently found. Of 432 patients, only 45% had biopsy-confirmed HSE and 22% had another etiology established by brain biopsy. Of these, 40% had a treatable disease (9% of the biopsy group) including bacterial abscess, tuberculosis, fungal infection, tumor, subdural hematoma or autoimmune disease. The majority of the remaining 60% identifiable but nontreatable causes for encephalitis were of viral etiology. A third group of 142 patients (33%) went undiagnosed even after brain biopsy and the conventional diagnostic tests,[3] as summarized in Table 23.3 .

More specifically, mass lesions in the CNS (tumor, abscess or blood) can cause focal neurologic changes, fever and seizures, similar to encephalitis. Metabolic (hypoglycemia, uremia, inborn errors of metabolism) and toxin-mediated disorders (ingestions, tick paralysis or Reye syndrome) can cause decreased consciousness, seizures and evidence of background slowing on EEG. Limbic encephalitis can produce protracted encephalitis and is caused by paraneoplastic phenomena. Treatable infectious causes of encephalitis must be vigorously investigated. Mycoplasma produces demyelinating brainstem encephalitis in approximately 0.1% of infections.

PREVENTION

Prevention remains the mainstay of therapy. Historically, the most frequent cause of viral CNS disease, namely mumps, has largely been eliminated through vaccination. Live attenuated vaccines against measles, mumps and rubella have resulted in a dramatic decrease in the incidence of encephalitis in industrialized countries. Measles continues to be the leading cause of postinfectious encephalitis in developing countries, complicating 1 of every 1000 measles infection.[7] Vaccination has also changed the incidence of previously common viral CNS disease. In 1952, poliomyelitis affected 57,879 Americans. Widespread vaccination has eradicated

the disease currently from the Western hemisphere. Vaccines exist for some arboviral infections. Vaccination against JE virus has reduced the incidence of encephalitis in Asia; however, in China, where 70 million children are immunized for JE, 10,000 cases still occur annually.

Vaccination is not cost-effective for preventing all viral infections. For example, vector avoidance, the use of mosquito deterrents and mosquito abatement programs provide less costly strategies for preventing arboviral encephalitides in the USA.[2] [38] Pre- and immediate postexposure prophylaxis are the only ways known to prevent death in rabies-exposed individuals.[45] Case reports exist of patients surviving symptomatic rabies, but all of these patients had some prior immunity or received postexposure prophylaxis prior to developing symptoms, as discussed above. The US Food and Drug Administration has implemented guidelines eliminating whole blood or blood components prepared from individuals who later developed CJD or vCJD. Changes in agricultural practices in Europe and bans on infected cattle led to a decline in cases of vCJD. In the USA one case of vCJD has been reported and the US Department of Agriculture has programs in place to monitor for TSEs in livestock.

APPROACH TO THE PATIENT WHO HAS VIRAL CENTRAL NERVOUS SYSTEM DISEASE

The approach to a patient who has a presumed CNS viral infection must be tailored to the severity and distribution of neurologic involvement. The degree of diagnostic as well as therapeutic intervention differs based on the type of CNS disease. For example, a patient who has photophobia and nuchal rigidity but a nonfocal neurologic examination does not require invasive intracranial pressure monitoring like a patient with encephalitis and evidence of increased intracranial pressure. After establishing the degree of CNS disease by history and physical examination, and stabilizing the patient (airway, breathing, circulation), the clinician then must ascertain a diagnosis. A simplified flow diagram reviewing the way to approach the patient who has viral CNS disease is presented in Figure 23.5 . The first step of any intervention hinges on establishing the correct diagnosis. A history and physical examination are logical first steps. The thoroughness of the initial history and physical examination is tailored to the stability of the patient. A comatose individual with apneustic respirations requires immediate intervention whereas the individual with nuchal rigidity and photophobia can afford a more detailed investigation for the etiology of symptoms prior to instituting any therapy.

Treatable causes of CNS dysfunction require rapid evaluation and intervention in an effort to prevent permanent or further CNS damage. Potentially treatable disease (fungal CNS infections, partially treated bacterial meningitis, tuberculous meningitis, parameningeal infection, mycoplasma and fastidious bacterial infections) can mimic viral CNS disease and should be vigorously investigated before attributing the illness to an untreatable viral etiology. The same logic applies to treatable viral infections and noninfectious etiologies. The radiographic and laboratory studies available for establishing a diagnosis must be prioritized based on the likely etiology and the stability of the patient.

Currently, few antiviral medications are available to treat CNS infections. Antiviral therapy exists for HSV-1, HSV-2, VZV, CMV and HIV. The introduction of aciclovir and vidarabine has resulted in a sharp decline in mortality and morbidity from herpes infections. Neonatal mortality from disseminated HSV disease and HSE has declined from 70% to 40% since the development of aciclovir and vidarabine. Varicella-zoster immunoglobulin (VZIG) and aciclovir have reduced the complications from primary VZV infection and zoster in the neonate and immunocompromised patient. Although

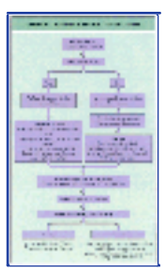

Figure 23-5 Approach to the patient who has presumed viral central nervous system disease.[56]

controlled trials have not evaluated the efficacy of aciclovir in VZV encephalitis, the medication is routinely used to treat this complication. With the increase of HIV infection, diseases previously limited to the neonatal and postnatal period now occur with increasing frequency in the adult population. Ganciclovir and foscarnet are used for the treatment of CMV encephalitis although controlled clinical trials have not confirmed the efficacy of treatment. In cases of postinfectious encephalitis, no randomized controlled trial has confirmed the benefit of immunomodulatory drugs. In practice clinicians often treat postinfectious encephalitis with different immunomodulators in an attempt to limit T-cell-mediated destruction of the CNS.[40] Importantly, no placebo-controlled studies have been performed and immunomodulatory therapy is based simply on isolated case reports. As with most case reports, clinical failures and iatrogenic morbidity from a therapeutic modality are rarely reported.

After establishing a presumptive diagnosis and instituting therapy, the clinician must also vigilantly anticipate and treat complications associated with the viral CNS disease or the therapeutic interventions. Seizures secondary to direct viral CNS damage, inflammatory vasculitis

and electrolyte changes require anticonvulsant therapy with benzodiazepines, phenytoin and barbiturates.[2] Patients with cerebral edema may require intracranial pressure monitoring and hyperventilation, osmotic therapy and CSF removal in an attempt to maintain cerebral pressures less than 15mmHg.[2] The ultimate goal of intracranial pressure monitoring is to maintain adequate cerebral perfusion. While a physician struggles to maintain an adequate intravascular blood volume, intracranial pressures can rise to dangerous levels as capillary leak complicates the patient's course. The risks of increased intracranial pressure from aggressive fluid resuscitation or the syndrome of inappropriate antidiuretic hormone release necessitate fastidious fluid management and frequent electrolyte monitoring. Cardiac arrhythmias can also develop in patients who have encephalitis secondary to electrolyte changes or brainstem damage. Cardiac and respiratory arrest can occur early in disease, and so equipment for intubation and cardioversion should be readily available for a patient who has encephalitis.

In addition to the direct damage the virus can cause in the CNS, certain viruses can also produce systemic damage and complicate the management of the CNS disease. Patients can develop overwhelming hepatitis, pneumonitis, disseminated intravascular coagulation and shock. Patients in coma from encephalitis can recover after long periods of unconsciousness. The physician should limit the amount of iatrogenic damage and vigorously support the patient during the acute phase of the illness.

CONCLUSION

Numerous factors influence the clinical manifestations of viral CNS infections. An individual's age, immune history, cultural practices and genetic make-up can influence the clinical expression of viral infection as readily as the viral serotype, receptor preference, viral load and cell tropism. Changes in behavior or cultural beliefs, increased travel and the modification of environment alter disease patterns and expose individuals to new infectious agents. Infections of the CNS, therefore, must be examined in a geographic, cultural and environmental context as well as the cellular, molecular and genetic levels.[57] [58]

Contemporary disease changes are relevant. The past 5 years have seen an outbreak of a deadly and previously unknown encephalitis virus (Nipah), dramatic extension of the range of a well-known arbovirus (WNV)[59] [60] and unexpected neurovirulence from a common pediatric pathogen (enterovirus 71).[61] [62] Each of these outbreaks suggests the high probability of future epidemics of encephalitis caused by previously unknown pathogens or novel manifestations of known agents. The Nipah virus epidemic highlights the potential for amplification of zoonotic viruses when agricultural practices force huge numbers of animals into close quarters, creating an ideal environment for exchange of pathogens. The WNV outbreak in the USA demonstrates how a pathogen can suddenly appear on the other side of the world. Although WNV is naturally spread to new regions by migratory birds, there is at least the suspicion that the New York outbreak could have originated with illegal importation of infected birds. These scenarios suggest the need for clinicians to develop a broad differential diagnosis when evaluating a new patient who presents with the acute onset of viral encephalitis. Improvements in our ability to diagnose CNS infections will produce a better understanding of the pathogenesis and true extent of CNS viral disease.

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Chapter 24 - Brain Abscess and Other Focal Pyogenic Infections

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Focal pyogenic infections of the central nervous system (CNS) include brain abscess, spinal cord abscess, subdural empyema, epidural abscess and suppurative intracranial phlebitis. The year 1993 marked the 100th anniversary of the publication of *Pyogenic Infective Diseases of the Brain and Spinal Cord* and *Atlas of Head Sections*, by Sir William Macewen, which were landmarks in the descriptions of the anatomy and natural history of focal suppurative brain processes and of their management.[1]

These conditions are characterized by the presence of one or more localized and well-defined collections of purulent material within the cranial vault or the paraspinal space. They exert their effects largely by direct involvement and destruction or encroachment of the brain or spinal cord parenchyma, by elevation of intracranial pressure or by interference with flow of blood or cerebrospinal fluid.

Infections in contiguous structures tend to lead to infections in certain areas of the cranial vault due to their anatomic relationships (Fig. 24.1). The frontal and ethmoidal paranasal sinuses underlie the anterior cranial fossa, so that infections in these sinuses often lead to infections in or near the frontal lobe. The sphenoid sinuses adjoin the sella turcica, temporal lobes and cavernous sinuses; sphenoid sinusitis may lead to infections in the frontal or temporal lobes or pituitary gland or to cavernous sinus thrombosis. Infections in the middle ear and mastoids may spread to the temporal lobe, cerebellum or brainstem. The focal nature of these infections often manifests by focal neurologic deficits, rather than by more global CNS dysfunction. This chapter focuses on the more common of these diseases (brain abscess, subdural empyema and epidural abscess), touching only briefly on the others.

BRAIN ABSCESS

Brain abscess is a focal suppurative process of the brain parenchyma. The diagnosis and management of brain abscess have undergone considerable changes during the past several years as a result of the widespread availability of noninvasive radiographic diagnostic techniques, the use of antimicrobial agents that demonstrate adequate penetration across the blood-brain barrier and into abscesses, and the refinement of minimally invasive surgical procedures.

EPIDEMIOLOGY

Brain abscess is an uncommon condition. A large series reported a cumulative lifetime incidence of approximately 1.3 per 100,000 person-years, although the overall incidence decreased from 2.7 per 100,000 person-years in 1935–44 to 0.9 per 100,000 person-years in 1965–81. The highest rates were observed in children 5–9 years old and adults over 60 (approximately 2.5 per 100,000 person-years each).[2] Recent reports have indicated that brain abscesses account for approximately 1 in 10,000 hospital admissions in the USA.[3]

The predominant age of patients with brain abscesses tends to vary somewhat with the predisposing factors; brain abscesses following otitis media are most common among young children and older adults, while those due to paranasal sinusitis are most common among older children and young adults.[3] [4] [5] [6] Although there have been conflicting reports on the possibility of a male predominance, three recent series indicated that brain abscesses are approximately 2–3 times as common among males as among females.[3] [5] [7]

The major factors that predispose to brain abscess are:

- an associated contiguous focus of infection (e.g. sinusitis, otitis media),
- trauma (e.g. penetrating head injury, post-neurosurgery),
- hematogenous spread from a distant focus (e.g. in association with lung abscess), and
- cryptogenic (no recognized focus).

Brain abscesses associated with a contiguous focus account for approximately 40–50% of the total; the percentage of cases without an identified predisposing factor has been reduced to approximately 15% with the use of newer, more sensitive imaging procedures. [7] [8] [9] In addition, brain abscesses have been reported in immunocompromised individuals or after a cerebrovascular accident.

The most common underlying conditions in patients in developed countries with brain abscess are otitis media and mastoiditis. However, it appears that the percentage of cases associated with otitis media and mastoiditis has decreased in association with early antimicrobial therapy for suspected otitis media. Recent data indicate that the risk of brain abscess in cases of otitis media is less than 0.5%.[9] [10] Brain abscess may also be associated with cyanotic heart disease and trauma at least as often as with otitis media and mastoiditis.[7] [11] Other head and neck infections associated with brain abscesses include paranasal and dental infections. In a recent report from Turkey, the most common predisposing factor in children was bacterial meningitis.[12]

As noted above, the location of focal CNS infections is often determined by proximity to a contiguous focus of infection. Thus, brain abscesses associated with otitis media and mastoiditis are most common in the temporal lobe and cerebellum (Table 24.1). Brain abscesses associated with sinus infections occur primarily in the frontal lobe or the temporal lobe. The frontal lobe is also most commonly affected following dental infections. Post-traumatic brain abscesses usually occur in the setting of a penetrating wound, but may also occur in closed head injuries. These injuries have included penetrating pencil tip and lawn dart injuries in children. Presentation of the abscess may occur months to years after the precipitating event. In one study, the median time to development of brain abscess was 113 days[13] and there have been recent reports of brain abscesses occurring at the site of war-related penetrating head injuries up to 52 years after the injuries occurred.[14]

Brain abscesses occurring in the setting of a distant primary site with hematogenous spread are often multiple; approximately 10–15% of patients with brain abscesses have multiple abscesses. They tend to occur in the distribution of the middle cerebral artery at the junction of the gray and white matter, where microcirculatory flow is poorest. Cyanotic congenital heart disease and chronic pyogenic lung diseases (e.g. lung abscess, bronchiectasis) are common predisposing factors.

Figure 24-1 Anatomic relationships between potential contiguous sources of infection and sites at which focal pyogenic central nervous system infections may occur.

Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) is also associated with brain abscess; it is thought that in these cases pulmonary arteriovenous malformations allow septic microemboli to bypass the normal pulmonary filter and gain access to the cerebral circulation. Brain abscesses have been reported following dental extractions and other manipulations, dilation of esophageal strictures and endoscopic sclerosis of esophageal varices.

PATHOGENESIS AND PATHOPHYSIOLOGY

The main pathogenetic factors responsible for the development of brain abscesses are a source of virulent micro-organisms and the presence of ischemic or devitalized brain tissue. The vulnerability of compromised tissue to brain abscess is evidenced by the occurrence of brain abscesses after trauma or cerebrovascular accident, in association with cyanotic heart or lung disease and in areas of poor local perfusion such as the junction of gray and white matter.

TABLE 24-1 -- Site of brain abscess based on predisposing condition.

SITE OF BRAIN ABSCESS BASED ON PREDISPOSING CONDITION	
Predisposing condition	**Site**
Otitis media or mastoiditis	Temporal lobe
	Cerebellum
Paranasal sinusitis	Frontal lobe
	Temporal lobe
Dental infection/manipulation	Frontal lobe
Trauma/neurosurgery	Related to wound
Meningitis	Cerebellum
	Frontal lobe

Cyanotic heart disease	Middle cerebral artery distribution
Pyogenic lung disease	
Bacterial endocarditis	
Gastrointestinal source	
T-cell deficiency	
Neutropenia	

Experimental models have demonstrated differences in the abilities of various micro-organisms to induce brain abscesses. These differences may be inter- or intraspecific. For example, *Escherichia coli* has been demonstrated to be more effective in producing brain abscesses than are *Staphylococcus aureus, Streptococcus pyogenes* or *Pseudomonas* spp.; encapsulated strains of *E. coli* were more infective than nonencapsulated strains.[15] Differences in infectivity have also been demonstrated among strains of *Citrobacter diversus*.[16]

There are two main postulated mechanisms by which brain abscess may occur in association with a contiguous focus of infection: direct extension through infected bone, or hematogenous spread through emissary or diploic veins or spread through local lymphatics. Infections associated with otogenic infections may also spread through the internal auditory canal, between suture lines or through cochlear aqueducts. Brain abscesses developing after trauma or neurosurgical procedures may follow deep wound injury with direct inoculation into the brain parenchyma or may be a result of extension of a superficial infection through compromised tissue.

The areas of the brain most commonly involved by solitary brain abscess are the frontal and temporal lobes, followed by the frontoparietal region, the parietal, cerebellar and occipital lobes.[17] These areas are those most likely to be associated with a contiguous focus or hematogenous seeding. Although rare, abscesses in other areas, such as the pituitary gland, thalamus, basal ganglia and brainstem, may occur and may be associated with specific predisposing conditions. For example, abscesses of the pituitary are often associated with pre-existing pituitary adenomas and with sphenoidal sinusitis.

Experimental animal data, surgery and autopsy findings and radiographic examinations indicate that brain abscesses develop in a four-stage process:[18] an early and late cerebritis (days 1–3 and 4–9, respectively); and an early and late capsule formation (days 10–13 and day 14 and later, respectively). These represent a continuum rather than discrete steps. The evolution of this process is dependent upon the causative organism, local factors, host immunologic status and antimicrobial therapy.

The microbiology of brain abscess is dependent upon the site of the initiating infection, the patient's underlying condition and the geographic locale (Table 24.2). The organisms most commonly isolated are streptococci, the Enterobacteriaceae, anaerobes, *Staph. aureus* and fungi; approximately 30–60% are polymicrobial. Fungi

TABLE 24-2 -- Likely pathogens and suggested empiric therapy for brain abscess based on predisposing condition.

LIKELY PATHOGENS AND SUGGESTED EMPIRIC THERAPY FOR BRAIN ABSCESS		
Predisposing condition	**Likely pathogens**	**Empiric therapy**
Otitis media or mastoiditis	Streptococci (anaerobic and aerobic)	Third-generation cephalosporin + metronidazole
	Bacteroides spp.	A penicillin
	Enterobacteriaceae	
Paranasal sinusitis	Streptococci	Third-generation cephalosporin ± metronidazole
	Bacteroides spp.	
	Enterobacteriaceae	
	Staphylococcus aureus	
Dental infection or manipulation	Streptococci	Penicillin ± metronidazole
	Fusobacterium spp.	
	Bacteroides spp.	
Trauma or neurosurgery	*Staphylococcus aureus*	Antistaphylococcal penicillin + third-generation cephalosporin
	Coagulase-negative staphylococci	
	Enterobacteriaceae	
	Streptococci	Vancomycin
	Pseudomonas aeruginosa	
Cyanotic heart disease	Streptococci	Third-generation cephalosporin
	Haemophilus spp.	
Pyogenic lung disease	Streptococci	Penicillin or third-generation cephalosporin + metronidazole
	Nocardia asteroides	
	Actinomyces spp.	
	Fusobacterium spp.	
	Bacteroides spp.	
Bacterial endocarditis	Viridans streptococci	Ampicillin and gentamicin ± antistaphylococcal penicillin
	Staphylococcus aureus	
	Enterococci	
	Haemophilus spp.	
Gastrointestinal source	Enterobacteriaceae	Third-generation cephalosporin
T-cell deficiency	*Toxoplasma gondii*	Variable
	Nocardia spp.	
Neutropenia	Enterobacteriaceae	Third- or fourth-generation cephalosporin, meropenem
	Pseudomonas aeruginosa	
	Fungi, especially *Aspergillus* and *Mucor*	Amphotericin B

were the organisms most commonly isolated in a recent report from Saudi Arabia[19] and are particularly common causes of brain abscesses in immunocompromised patients; *Aspergillus* spp. are especially common in patients who have bone marrow and solid organ transplants.[20] Patients who have defects in T-cell immunity (including AIDS patients) are predisposed to infections with intracellular organisms such as *Toxoplasma gondii*, *Nocardia* spp., *Cryptococcus neoformans*, *Mycobacterium* spp. and fungi.

PREVENTION

The primary means of prevention of brain abscesses and other focal CNS infections is the appropriate use of antibiotics in patients who have predisposing infections such as otitis media and mastoiditis. Other preventive measures include surgical correction of cyanotic congenital heart disease, maintenance of dental hygiene, management of pyogenic lung infections and attention to proper sterile techniques during neurosurgical procedures. In patients who have underlying T-cell defects, measures to prevent exposure to *T. gondii* should be recommended.

CLINICAL FEATURES

The clinical manifestations of brain abscess and other focal suppurative CNS processes are due largely to the presence of a space-occupying lesion.[17] [21] [22] The most common symptom is headache, which is usually hemicranial. Other common symptoms include fever, focal neurologic findings (especially hemiparesis), nausea and vomiting, and seizures (usually generalized). Nuchal rigidity may occur if the abscess is near the meninges.

Other signs and symptoms vary depending on the stage, size and anatomic location of the abscess. Abscesses of the frontal lobe are characterized by global mental status changes, hemiparesis and expressive speech disturbances. Temporal lobe abscesses may present with headache and aphasia (if the abscess involves the dominant hemisphere). Cerebellar abscesses are associated with vomiting, ataxia, nystagmus and dysmetria. Vomiting, hemiparesis, dysphagia and facial weakness may be seen with brainstem abscesses. Rapid deterioration with nuchal rigidity suggests the possibility of rupture of an abscess into the intraventricular or subarachnoid space.

Laboratory findings may include a peripheral leukocytosis and a left shift, but approximately 40% of patients with brain abscess have normal leukocyte concentrations. The erythrocyte sedimentation rate is often elevated. An elevated C-reactive protein has been found to be both sensitive (77–90%) and specific (77–100%) when used to distinguish brain abscess from cerebral neoplasms.

The differential diagnosis of brain abscess includes subdural empyema, epidural abscess, bacterial meningitis, cerebral neoplasm, cerebrovascular accident and encephalitis.

DIAGNOSIS

Radiographic imaging with contrast-enhanced computerized tomography (CT) or magnetic resonance imaging (MRI) has contributed greatly to diagnosis and management of brain abscess. These have obviated the need for more invasive techniques such as pneumoencephalography and myelography. Plain skull radiographs are insensitive but the presence of air indicates the need for further evaluation. Technetium-99 (^{99m}Tc) brain scanning is very sensitive and is the procedure of choice where CT or MRI is unavailable; there is some evidence that ^{99m}Tc scanning may be more sensitive than CT in the early cerebritis stage. ^{99m}Tc-HMPAO labeled leukocyte single photon emission CT (SPECT) has been examined as a potential means of distinguishing brain abscess from other focal cerebral parenchymal lesions, such as neoplasms. Ultrasonography may also be used if other techniques are unavailable.

The characteristic appearance of brain abscess on CT scan varies with the stage of the disease.[23] During the cerebritis stage, cerebral edema is prominent; no abnormalities may be seen in the early cerebritis stage. As capsule formation progresses, the abscess appears as a lesion with a hypodense center composed of necrotic debris surrounded by ring enhancement, which may in turn be surrounded by hypodense cerebral edema (Fig. 24.2). Although highly sensitive, CT scanning is not specific. These findings may also be seen in patients who have cerebral neoplasms, cerebrovascular accidents or granulomas.

Magnetic resonance imaging appears to be more sensitive than CT in the early cerebritis stage of brain abscess, where it appears as

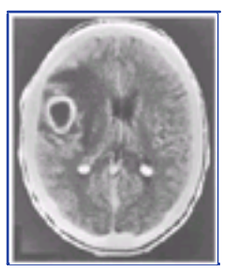

Figure 24-2 Contrast-enhanced CT scan of the head in the coronal projection of a 43-year-old man with an atrial septal defect that persisted after attempts at surgical repair. The patient presented with seizures after undergoing dental work for which he did not receive antimicrobial prophylaxis. Note the ring-enhancing lesion in the right frontoparietal region with edema and mass effect.

slightly low intensity on T1-weighted images and very low intensity on T2-weighted images and may be more sensitive in diagnosing lesions in the posterior fossa due to the absence of bone artefact[24] (Fig. 24.3). Also, MRI may allow distinction of abscess fluid from cerebrospinal fluid, which may be important if intraventricular rupture is suspected. Enhancement with gadolinium-DTPA allows evaluation of disruption of the blood-brain barrier and permits greater distinction of the radiographic appearance of the central abscess, capsule and surrounding edema. Examination by ^{1}H magnetic resonance spectroscopic imaging has been proposed as a means of distinguishing brain abscess from other focal cerebral parenchymal lesions.

It must be noted that the appearance of edema and contrast enhancement on CT and MRI may be diminished or absent in immunocompromised patients, possibly due to poor host inflammatory response.

Lumbar puncture should be avoided in patients who have known or suspected brain abscess. The yield of cerebrospinal fluid culture is low (less than 10% positive) and the risk of herniation is considerable (approximately 15–30%). In patients in whom both diagnoses are considered, blood cultures should be obtained and appropriate

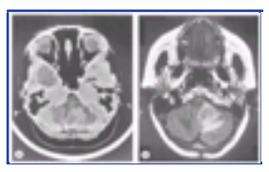

Figure 24-3 Contrast-enhanced CT and MRI scans of the head in the coronal projection of a 43-year-old woman with headaches after a recent fall on her head. (a) CT scan image reveals a cystic ring-enhancing lesion in the left cerebellum. Note the prominent bone artefact. (b) T1-weighted MRI scan image reveals an enhancing cystic lesion in the left cerebellum with significant surrounding edema. Bone artefact is absent. Both CT and MRI scans were felt to be most consistent with a primary or metastatic neoplasm, but culture of material obtained at stereotactically guided aspiration grew *Staphylococcus aureus*.

empiric therapy should be initiated, after which an imaging procedure should be performed. Lumbar puncture may be performed if there is no evidence of a mass lesion or signs of raised intracranial pressure.

MANAGEMENT

Most patients who have a brain abscess require surgical drainage. In addition to its role as a therapeutic measure, drainage also permits microbiologic evaluation of abscess material, which may be important in guiding antimicrobial therapy. It is now clear that aspiration is as effective as excision in most cases and is less invasive, and thus it has become the procedure of choice. Stereotaxic CT-guided aspiration permits accurate access even to areas that had been difficult to reach by aspiration, such as the brainstem, cerebellum and thalamus.[25] Multiple abscesses may thus be drained. Neuroendoscopic aspiration has also been used with success in a small number of patients.

Abscess material should be examined by Gram stain and by aerobic and anaerobic cultures as a minimum; in one study, the Gram stain revealed organisms in 82% of cases and the culture was positive in 88%.[26] The clinical setting may also dictate the use of special stains and cultures for fungi, mycobacteria and protozoa. In patients who have HIV infection, polymerase chain reaction (PCR) examination of cerebrospinal fluid may be useful in diagnosing tuberculous brain abscesses.

Antibiotics used in the management of brain abscess should be parenteral, have activity against the pathogens that are likely in a given clinical scenario, penetrate into abscess fluid (and into the site of any underlying infection) in adequate concentrations and be bactericidal. The combination of penicillin or a third-generation cephalosporin (cefotaxime or ceftriaxone) plus metronidazole is effective as empiric therapy in most cases (see Table 24.2). An antistaphylococcal penicillin (such as flucloxacillin, nafcillin or oxacillin) should be used if staphylococci are suspected. Vancomycin should be used instead if methicillin resistance is suspected or identified or if the patient is allergic to ß-lactam antimicrobial agents. In the case of intraventricular rupture of brain abscess, it has been recommended that optimal management include open craniotomy with debridement, intraventricular lavage and intraventricular as well as intravenous antibiotics. Changes in therapy in all cases should be guided by results of microbiologic examination and by clinical and radiographic progress.

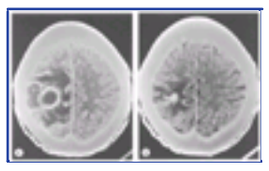

Figure 24-4 Contrast-enhanced CT scans of the head in the coronal projection of a 66-year-old woman with a group B streptococcal brain abscess demonstrating evolution of the abscess during and after surgical and antimicrobial therapy. (a) The original scan demonstrates a hypodense necrotic center surrounded by an enhancing capsule and hypodense edema. (b) Seven weeks later, after stereotactically guided aspiration and a full course of antimicrobial therapy, the central cavity can no longer be seen, although the enhancement and surrounding edema persist to a small degree.

There is a great deal of evidence that there are certain circumstances under which brain abscess may be treated without surgical drainage. Small abscesses (less than 3cm diameter) and abscesses in the cerebritis stage may respond to antimicrobial therapy alone.[8] Medical therapy alone may also be indicated if the patient is a poor surgical candidate. In these cases, prolonged courses of antibiotics (at least 8 weeks of parenteral therapy) and close monitoring with sequential CT or MRI scans are necessary; MRI may be especially useful here because of its lack of ionizing radiation.

Although the optimal duration of antimicrobial therapy for brain abscess after surgical drainage has not been established, many authorities recommend 4–6 weeks of parenteral antibiotics. Radiographic imaging procedures should be used for monitoring of therapy. Radiographic abnormalities may persist for months after successful therapy of brain abscesses (Fig. 24.4).

Adjunctive therapy with corticosteroids, mannitol and hyperventilation may be indicated where there is evidence of increased intracranial pressure. The routine use of corticosteroids in the absence of increased intracranial pressure cannot be recommended.

The prognosis for patients who have a brain abscess has improved considerably, particularly since the introduction of CT scanning. Mortality is now less than 30% in most series. Long-term sequelae may occur in about one-third of patients and include mental retardation, seizures and focal neurologic deficits. Poor prognostic factors include delayed or missed diagnosis, multiple lesions, deep-seated lesions, intraventricular rupture, severe impairment of mental or neurologic status (including coma), fungal etiology and extremes of age.[5]

SUBDURAL EMPYEMA AND INTRACRANIAL EPIDURAL ABSCESS

Subdural empyema and epidural abscess are focal collections of purulent material between the dura mater and arachnoid mater, and outside the dura mater, respectively. Subdural empyema accounts for approximately 15–20% of all focal intracranial infections.

EPIDEMIOLOGY

Subdural empyema in adults is most often a complication of acute or chronic bacterial paranasal sinusitis, otitis media or mastoiditis.[27] [28] It is the most common intracranial complication of sinusitis, accounting for approximately 60% of such cases.[29] The frontal and ethmoidal sinuses are the foci in well over half of the cases. Hematogenous spread from a distant source may also occur. In children the most common predisposing condition is bacterial meningitis. Other predisposing conditions include trauma (including neurosurgical procedures) and infection of a pre-existing subdural hematoma. As with brain abscess, there is a male predominance among patients who have subdural empyema.[30]

Intracranial epidural abscess usually follows paranasal sinusitis (particularly frontal), otitis media, mastoiditis or cranial trauma. Males are again more commonly afflicted than females.[31]

PATHOGENESIS AND PATHOPHYSIOLOGY

As with brain abscesses, extension of infection into the epidural or subdural space from a contiguous focus may occur by extension through infected bone or by hematogenous seeding through emissary veins. Intracranial epidural abscess is almost always associated with subdural empyema and with overlying osteomyelitis. In patients with subdural empyema, infection can spread rapidly through the subdural space until limited by its natural boundaries. These include the falx cerebri, tentorium cerebelli, base of the brain, foramen magnum posteriorly and the anterior spinal canal. Within the compartments defined by these boundaries, as the infection progresses it behaves as an expanding mass lesion.

As the lesion expands, intracranial pressure increases and the cerebral parenchyma is compromised. Interference with flow of blood or of cerebrospinal fluid may cause cerebral edema and hydrocephalus. Septic thrombosis of veins within the affected subdural or epidural space may lead to thrombosis of cavernous sinuses or cortical veins, leading to infarction of brain tissue.

Organisms commonly isolated from adult patients with subdural empyema and intracranial epidural abscess include anaerobes, aerobic streptococci, staphylococci, *Streptococcus pneumoniae, Haemophilus influenzae* and other Gram-negative bacilli. Polymicrobial infections are common; in one study, more than 50% of infections were polymicrobial.[30] In children, the most common causative agents are those that are responsible for the underlying meningitis. In the past, this has been *H. influenzae* in children outside the neonatal age, but as the relative frequency of *H. influenzae* meningitis declines it is likely that its role in subdural empyema will diminish as well.

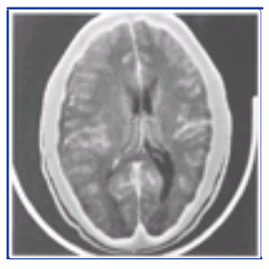

Figure 24-5 Contrast-enhanced CT scan of the head in the coronal projection of a 23-year-old man with fever and headache. There is a small isodense extra-axial fluid collection in the subdural space on the right, with significant mass effect shown by right-to-left midline shift and effacement of the right lateral ventricle. There was also opacification of the frontal and ethmoid sinuses, suggesting sinusitis as the source of this subdural empyema.

CLINICAL FEATURES

The most prominent early symptoms associated with subdural empyema and intracranial epidural abscess are fever and headache. The headache is often focal at onset but may become generalized. These are usually followed by focal neurologic defects. Abscesses near the petrous portion of the temporal bone may be associated with fifth and sixth cranial nerve palsies, causing unilateral facial pain and lateral rectus muscle weakness. Periorbital edema and subgaleal abscess (Pott's puffy tumor) may be found in up to about one-third of patients. Signs of increased intracranial pressure (such as vomiting, gait disturbances and mental status changes) and meningeal irritation may follow and may be accompanied by seizures, hemiparesis and hemisensory defects.[30]

DIAGNOSIS

Computerized tomography or MRI scanning are the procedures of choice in the diagnosis of subdural empyema and epidural abscess. Imaging reveals a hypodense lesion with displacement of the arachnoid mater in both entities, with accompanying displacement of the dura mater noted in patients who have subdural empyema. Mass effect is more common with subdural empyema than with epidural abscess (Fig. 24.5). Capsule formation with contrast enhancement may be seen in either condition, but is more common with epidural abscess (Fig. 24.6). Cranial osteomyelitis may also be noted in patients who have underlying contiguous foci of infection. Gadolinium-enhanced MRI may detect lesions not noted on CT (because the lesions may be isodense with the cerebral tissue on CT). As with brain abscess, lumbar puncture is contraindicated in patients with known or suspected subdural empyema or epidural abscess. Clinical deterioration

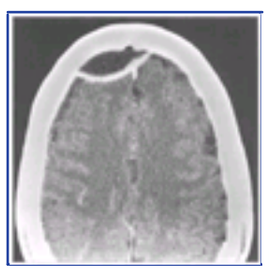

Figure 24-6 Contrast-enhanced CT scan of the head in the coronal projection of a 19-year-old man with otitis media who presented with sinus congestion 1 week earlier. Plain films of the sinuses revealed opacification of the right maxillary and ethmoidal sinuses and an intracranial air-fluid level. Note the intracranial gas in the right frontal region abutting a hypodense region in the epidural space with ring enhancement and surrounding edema, representing an intracranial epidural abscess.

was reported in 33 of 280 patients (11.3%) who underwent lumbar puncture with subdural empyema[30] and in 1 of 12 patients (8.3%) with epidural abscess.[31]

MANAGEMENT

Surgical evacuation is necessary for management of most patients who have subdural empyema and intracranial epidural abscess. This should be accomplished by craniotomy or by the use of burr holes. Although the optimal surgical procedure has not been established, in one study of 699 patients with subdural empyema, the use of limited procedures such as burr holes or craniectomies was associated with a worse prognosis than more extensive procedures such as craniotomies.[32] It may also be necessary to debride the primary source of infection. Samples should be submitted for Gram stain and aerobic and anaerobic cultures. Antimicrobial therapy alone may be used for a limited number of patients with very small fluid collections. [30] [31]

The choice of empiric antimicrobial therapy is dependent upon the age of the patient and the site of the primary infection (Table 24.3). In adults, the wide variety of

possible pathogens and the potential for polymicrobial infection dictate the use of broad-spectrum therapy. In children, therapy should be directed against the likely causes of meningitis. Parenteral antimicrobial therapy should be continued for 3–6 weeks, with close monitoring of clinical status and radiographic appearance.

SUPPURATIVE INTRACRANIAL PHLEBITIS

Suppurative intracranial phlebitis is inflammation of the blood vessels within the cranium as a result of infection.[33]

TABLE 24-3 -- Pathogens and suggested empiric antibiotic regimens for subdural empyema and intracranial epidural abscess based on underlying condition.

PATHOGENS AND SUGGESTED EMPIRIC ANTIBIOTIC REGIMENS FOR SUBDURAL EMPYEMA AND INTRACRANIAL EPIDURAL ABSCESS		
Predisposing condition	**Likely pathogens**	**Empiric therapy**
Paranasal sinusitis	Streptococci	Third-generation cephalosporin + metronidazole ± antistaphylococcal penicillin or a penicillin
	Bacteroides spp.	
	Staphylococcus aureus	
	Enterobacteriaceae	
	Haemophilus influenzae	
Otitis media or mastoiditis	Streptococci	Third-generation cephalosporin + metronidazole ± antistaphylococcal penicillin or a penicillin
	Bacteroides spp.	
	Staphylococcus aureus	
	Enterobacteriaceae	
	Pseudomonas aeruginosa	
Trauma	*Staphylococcus aureus*	Vancomycin + third-generation cephalosporin
	Coagulase-negative staphylococci	
	Enterobacteriaceae	
Dental infection	*Bacteroides* spp.	Penicillin or third-generation cephalosporin + metronidazole
	Streptococci	
	Fusobacterium spp.	
Neonate	Enterobacteriaceae	Third-generation cephalosorin + ampicillin
	Group B streptococci	
	Listeria monocytogenes	
Infant or child	*Streptococcus pneumoniae*	Third-generation cephalosporin ± Vancomycin
	Haemophilus influenzae	
	Neisseria meningitidis	

EPIDEMIOLOGY, PATHOGENESIS AND PATHOPHYSIOLOGY

These infections usually follow infections of the paranasal sinuses, middle ear, mastoids, face and oropharynx. They may also occur in association with subdural empyema, epidural abscess or bacterial meningitis. Conditions associated with increased blood viscosity or hypercoagulability increase the risk of suppurative intracranial phlebitis.

Spread generally occurs along emissary veins. The venous sinuses most commonly involved are the cavernous sinus, lateral sinus and superior sagittal sinus. If there is sufficient involvement of the vasculature, then cerebral edema and hemorrhagic infarction may result. The infarcts tend to occur in venous watershed regions. Involvement of the superior sagittal sinus or of the lateral sinuses may block reabsorption of cerebrospinal fluid and lead to hydrocephalus and increased intracranial pressure. There may also be subsequent involvement of contiguous structures leading to brain abscess, subdural empyema, epidural abscess or meningitis, or distant seeding and infection of the lungs and other organs.

The microbiology of suppurative intracranial phlebitis is similar to that of subdural empyema and intracranial epidural abscess, with *Staph. aureus*, streptococci and anaerobes being most commonly identified.

CLINICAL FEATURES

The clinical manifestations of suppurative intracranial phlebitis vary with the location of the involved venous sinuses or cortical veins.

Cavernous sinus thrombosis is associated with palsies of cranial nerves III, IV, V and VI, producing loss of corneal reflexes, ophthalmoplegia and hypesthesia over the upper part of the face. Papilledema and visual loss may result from obstruction of retinal venous return.

Lateral sinus thrombosis involves cranial nerves V and VI, resulting in altered facial sensation and lateral rectus muscle weakness. Obstruction of venous cerebrospinal fluid resorption may cause communicating hydrocephalus and increased intracranial pressure. Cranial nerves IX, X and XI may also be affected.

Involvement of the superior sagittal sinus may also diminish CSF resorption. In addition, obstruction of venous drainage from the motor cortex region of the cerebral hemispheres may lead to weakness of the legs.

Cortical vein thrombosis may be neurologically silent or produce only transient defects if collateral venous drainage can compensate for thrombosis. If collateral flow is inadequate, the lesion will manifest as progressive neurologic defects. The precise nature of the defects depends on the location of the veins involved. Unilateral or bilateral extremity weakness, hemiparesis, aphasia, seizures and mental status changes may be seen.

DIAGNOSIS

Magnetic resonance imaging is more sensitive than CT and is the diagnostic procedure of choice. The appearance on MRI is that of increased signal within the involved vessel (Fig. 24.7). Sensitivity of MRI can be enhanced by the use of magnetic resonance imaging angiography. Computerized tomography may be used when MRI is unavailable. If CT or MRI is unremarkable and suppurative intracranial phlebitis is still suspected, angiography should be performed.

MANAGEMENT

Empiric antimicrobial therapy is similar to that employed for subdural empyema and intracranial epidural abscess (see Table 24.2). It may be necessary to control

increased intracranial pressure with adjunctive measures such as corticosteroids, hyperosmolar agents

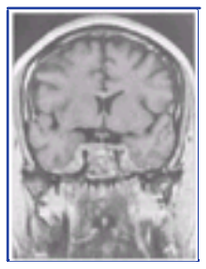

Figure 24-7 Contrast-enhanced MRI scan of the head in the sagittal projection of a 29-year-old man with sinus congestion and headache. There is non-uniform signal intensity of the cavernous venous sinuses, indicating cavernous sinus thrombosis. The sphenoid, ethmoidal and maxillary paranasal sinuses also demonstrated abnormal signal intensity.

and hyperventilation. Anticoagulant therapy has been used with some success, but carries the risk of hemorrhagic infarction. Surgery may be required for drainage of associated abscesses.

SPINAL EPIDURAL ABSCESS

Spinal epidural abscess is a focal infection of the paraspinal epidural space.

EPIDEMIOLOGY, PATHOGENESIS AND PATHOPHYSIOLOGY

Spinal epidural abscess may be secondary to a contiguous source of infection, such as vertebral osteomyelitis, penetrating trauma or decubitus ulcers, or may arise by hematogenous spread from a distant source.[27] [34] [35] [36] A history of back trauma, including spinal and paraspinal procedures, is common. Diabetes mellitus, alcoholism and injection drug use are common risk factors. In addition to pulmonary infections and infective endocarditis, as seen in patients who have brain abscess and subdural empyema, possible sources for hematogenous seeding of the spinal epidural space include cutaneous, intra-abdominal, pelvic and genitourinary infections. Spread from these sources occurs via the paravertebral venous plexus.

In most cases in adults, the thoracic spine is involved, while cervical and lumbar involvement are more common in children. There is a male predominance. [36]

The organism responsible for the majority of cases of spinal epidural abscess is *Staph. aureus*, accounting for almost three-quarters of all cases in a meta-analysis of 915 cases reported.[36] Other staphylococci, aerobic and anaerobic streptococci, *Escherichia coli* and *Pseudomonas aeruginosa* are also common. Polymicrobial infections may occur, but are uncommon.

CLINICAL FEATURES

Four clinical stages have been described for spinal epidural abscess:

- fever and focal back pain;
- nerve root compression with nerve root pain;
- spinal cord compression with accompanying deficits in motor, sensory and bowel and bladder sphincter function; and
- paralysis (respiratory compromise may also be present if the cervical cord is involved).[37]

Figure 24-8 Contrast-enhanced MRI scan of the spine in the coronal projection of a 28-year-old man with a 1-week history of headache, fever and sweats. Physical examination demonstrated meningismus but no focal neurologic deficits. Scans in the sagittal section demonstrated a substance nearly isointense with the spinal cord and running nearly the length of the cord. This scan clearly demonstrates impingement and anterior displacement of the cord by the spinal epidural abscess.

Progression tends to be rapid, with infection due to direct hematogenous spread, and may be accompanied by severe pain.

Progression to the second stage tends to occur slowly in patients who have abscesses secondary to vertebral osteomyelitis, but may accelerate after that.

Headache, meningismus and focal tenderness are common signs and symptoms. Laboratory evaluation often reveals leukocytosis and elevated erythrocyte sedimentation rate in acute cases.

DIAGNOSIS

Gadolinium-enhanced MRI has supplanted CT and myelography as the diagnostic procedure of choice for spinal epidural abscess[38] [39] (Fig. 24.8). Magnetic resonance imaging is highly sensitive (91%) and noninvasive and can identify osteomyelitis and intramedullary spinal cord lesions. If MRI is not available, then myelography should be performed.

MANAGEMENT

Surgical drainage is indicated for the management of most patients with spinal epidural abscess. This should ordinarily be achieved by laminectomy, but CT-guided aspiration may be performed in selected patients. Antimicrobial therapy alone may be considered in patients who have not progressed to the third stage of the illness (i.e. those without neurologic deficits). Such patients should be monitored closely for worsening pain, fever or appearance of neurologic deficits.

An antistaphylococcal penicillin should be instituted as empiric therapy in all patients; vancomycin may be used in patients who are allergic to penicillin. Antibiotics with antimicrobial activity against Gram-negative organisms (such as a third-generation cephalosporin) and anaerobes (such as metronidazole) should be added in patients whose underlying source might have been an intra-abdominal, pelvic or genitourinary infection. The duration of parenteral therapy should be at least 3–4 weeks; if osteomyelitis is present, then therapy should be continued for a total of 8 weeks.

SPINAL CORD ABSCESS

Intramedullary spinal cord abscess is a rare condition that can be defined as a focal suppurative processes of the spinal cord parenchyma. It usually involves the thoracic segment of the spinal cord and is generally hematogenous in origin. The lungs are usually the source of infection and there have been reports of such abscesses in injection drug users. Spinal cord abscesses may also arise secondary to congenital dermal sinuses. The organisms that have been isolated in patients with spinal cord abscesses have included *Staph. aureus*, streptococci, *Listeria monocytogenes* and *Burkholderia cepacia*.[40] The symptoms of spinal cord abscess mimic those of spinal epidural abscess. Diagnosis may be made by CT, MRI or myelography. Treatment consists of surgical debridement and prolonged antimicrobial therapy. Antibiotics should be directed against *Staph. aureus* and possibly Gram-negative bacilli.

CONCLUSION

The diagnosis of focal pyogenic infections of the CNS has been revolutionized in the past two decades with the introduction and widespread use of CT and MRI scanning. Because of its high sensitivity, ability to delineate infections in surrounding tissues and absence of ionizing radiation, MRI is generally preferable to CT for diagnosis. Management has also been altered with refinement of minimally invasive surgical techniques such as CT-guided aspiration and by studies demonstrating the efficacy of medical management alone in defined situations. It is hoped that further advances in management will lead to greater improvements in patient outcomes.

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Chapter 25 - Toxin-mediated Disorders: Tetanus, Botulism and Diphtheria

Martin J Wood

The three diseases described in this chapter are caused by exotoxins that conform to the general A–B model, each being composed of an enzymatic (A) portion and a binding (B) portion. The biologic activity resides in the A portion whereas the B subunits may bind to target cells but are biologically inactive. Common to all the three toxins described, A and B are domains of a single protein that is cleaved by proteolytic activity of the bacterium.

TETANUS

Tetanus is caused by tetanospasmin, a neural toxin that interferes with inhibition of spinal cord reflexes and is produced by the obligate anaerobic bacterium *Clostridium tetani*.

EPIDEMIOLOGY

Tetanus is still common in developing tropical countries, where it is an important cause of death, particularly in neonates. The World Health Organization estimated in 1990 that there were about 715,000 deaths worldwide from neonatal tetanus. In the developed world, however, active immunization and better hygiene, wound care and management of childbirth have meant that the disease is now rare. The annual incidence of tetanus has fallen from nearly 4 to 0.2 per million population in the USA since 1947 (Fig. 25.1); mortality has dropped even more, with the death:case ratio falling from about 50% to below 30%.[1] In England and Wales there were 83 cases of tetanus (and 19 deaths) in the period from 1988 to 1997.

Neonatal tetanus usually occurs within 3–14 days of birth in infants delivered under nonsterile conditions to nonimmunized women. The umbilical cord stump is the usual portal of entry, particularly if cultural practices dictate the application of animal dung to the stump. At other ages acute wounds are the portal of entry for *C. tetani* in about 80% of cases, with the remainder associated with chronic decubitus ulcers, gangrene, abscesses or parenteral drug abuse. Tetanus is more likely to occur in the summer months when gardening and other pastimes bring people into contact with soil.

PATHOGENESIS AND PATHOLOGY

Spores of *C. tetani* contaminate a wound and germinate under anaerobic conditions. The proliferating organisms elaborate tetanospasmin (tetanus toxin), one of the most potent of the known poisons, with an estimated lethal dose of 2.5ng/kg body weight. It is produced by a plasmid-encoded gene and is synthesized as a 151kDa polypeptide. As with botulinum and diphtheria toxins, this polypeptide is then split by clostridial proteolytic cleavage into the light (L) chain (approximately 50kDa and containing the enzymatic (A) domain) and heavy (H) chain, containing the binding (B) domain (approximately 100kDa).

Most of the toxin gets into the bloodstream, but it must then gain entry into the central nervous system (CNS) via neurons to exert its toxicity. The effect of tetanus toxin is via a three-stage process: binding, internalization and induction of paralysis. The H chain interacts with the ganglioside GT_1 of neurons at neuromuscular junctions, both locally and distally (via the bloodstream), and enables the L chain to enter the cytoplasm of the neuron. The tetanospasmin is then transported intra-axonally at 75–250mm/day in a retrograde manner to the cell body in the ventral horns of the spinal cord and the motor nuclei of cranial nerves and then, via trans-synaptic spread, to other neurons within the CNS (Fig. 25.2). Within the neurons tetanospasmin acts as a zinc-dependent protease that cleaves synaptobrevin, a protein component of synaptic vesicles,[2] and prevents release of neurotransmitters at the presynaptic membrane.

The predominant adverse effect is disinhibition of spinal cord reflex arcs as a result of interference with the release of the neurotransmitters glycine and ?-aminobutyric acid (GABA) from presynaptic inhibitory Renshaw cells and 1a fibres of a motor neurons. Excitatory reflexes, freed from inhibition, thus lead to multiple, intense muscle spasms. Tetanospasmin also interferes with presynaptic acetylcholine release at the neuromuscular junction (similar to the effect of botulinum toxin; see below) and disinhibits sympathetic reflexes at the spinal level, producing autonomic dysfunction.

There are no specific gross or histologic abnormalities in tetanus. Any changes described in fatal cases reflect terminal hypoxia and autonomic dysfunction.

PREVENTION

Tetanus is a preventable disease. Generally, concentrations of antibody to tetanospasmin as low as 0.01IU/ml are regarded as protective against clinical tetanus, although cases have occurred in patients who have antibody concentrations at least 10-fold higher than this. Active immunization with tetanus toxoid is extremely effective. A primary series of three doses of tetanus toxoid given in infancy, either with diphtheria toxoid and acellular or whole cell pertussis (DTaP or DTP) or diphtheria toxoid alone (DT), with a booster at school entry, is virtually 100% effective for 5–10 years. Another dose should be given to school leavers. Five doses of vaccine are now considered to give adequate immunity and routine boosters every 10 years are not recommended. Neonatal tetanus can be prevented by ensuring that all pregnant women are immune. Owing to lack of a primary course of vaccination, many elderly individuals do not have protective levels of tetanus antibodies.[3]

The need for both active and passive immunization against tetanus, with toxoid and specific human tetanus immunoglobulin (HTIG)[4] should be reviewed after any injury that brings an individual to medical attention (Table 25.1).

Clean, minor wounds do not need any special treatment. Previously nonimmunized persons over 7 years of age should receive a three dose series of Td, the first two doses 4–8 weeks apart and the third after 6–12 months. Although protocols suggest that Td should be given if the patient has not previously completed a primary series or it is more than 10 years since the last dose of tetanus toxoid, there is little justification for any boosting if the individual has ever

received five doses of toxoid.[5] All other wounds, including frostbite, burns and others, should be considered to render the patient prone to tetanus. Foreign bodies and ischemic tissue should be removed. If the patient has not received a primary series then Td and HTIG (250 units intramuscularly), or equine tetanus antitoxin if HTIG is

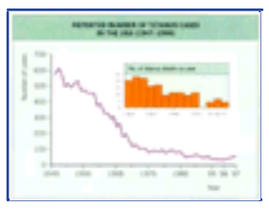

Figure 25-1 Reported number of tetanus cases in the USA (1947–97).

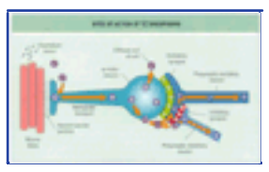

Figure 25-2 Sites of action of tetanospasmin. Tetanospasmin (TS) is produced by *Clostridium tetani* at the site of the wound and binds and internalizes at the neuromuscular junction into the a motor neuron. It then travels by retrograde axonal flow to the cell body and diffuses out into the synapses and extracellular space of the CNS. It enters other neurons and travels further into the CNS. Its major effect is to inhibit transmitter release from the glycinergic presynaptic inhibitory neuron but it can also inhibit release of transmitters at the excitatory synapses and of acetylcholine at the neuromuscular junction.

TABLE 25-1 -- Recommendations for use of tetanus prophylaxis in wound management.

RECOMMENDATIONS FOR USE OF TETANUS PROPHYLAXIS IN WOUND MANAGEMENT				
History of tetanus toxoid administration	**Clean, minor wounds**		**All other wounds**	
	Tetanus toxoid*	**Immunoglobulin**	**Tetanus toxoid***	**Immunoglobulin**
Unknown or less than three doses	Yes and proceed with basic immunization	No	Yes and proceed with basic immunization	Yes (250IU HTIG or 3000IU equine tetanus antitoxin)

Three or more doses	No, unless >10 years since last dose	No	No, unless >5 years since last dose	No

*Administered as Td (i.e. low dose of diphtheria toxoid) in adults

not available within 24 hours, should be given. Patients who have previously received five or more doses of vaccine probably need no immunization although some countries (notably the USA, many European countries and Australia) still recommend that a Td booster be given if it is more than 5 years since the last dose of tetanus toxoid.

CLINICAL FEATURES

Although tetanus can occur at any age, those over 60 are at greatest risk in the developed world since they have lowest immunization levels;[3] elderly women are at greatest risk since they are less likely to have been immunized during earlier military service.

The incubation period to the first symptom ranges from 1 day to several months but most cases start between 3 and 21 days after an acute injury. There is a correlation between the distance of the injury from the CNS and the duration of the incubation period. The time between the first symptom and the first reflex spasm is termed the 'period of onset'.

There are four clinical forms of tetanus: neonatal disease and localized, cephalic and generalized tetanus, depending on the predominant site of toxin action.

Localized tetanus consists of fixed muscle rigidity and painful spasms, sometimes lasting weeks or months, confined to an area close to the site of the injury. It is rare and generally mild but may herald generalized tetanus. Cephalic tetanus is a particular form of localized tetanus associated with wounds to the head or face or with

Figure 25-3 Facial spasm and risus sardonicus in a Filipino patient who has tetanus.

chronic otitis media, and manifested by atonic palsies involving the motor cranial nerves. The incubation period is often only 1–2 days and generalized tetanus may follow.

Generalized tetanus (which is by far the most common form) typically starts with rigidity and spasm of the masseter muscles, causing trismus or lockjaw and the characteristic risus sardonicus — a grimace through clenched teeth and closed mouth with wrinkled forehead and raised eyebrows (Fig. 25.3). Other muscles, first in the neck, then the thorax, back and extremities, become rigid and go into spasms, producing opisthotonos, abdominal rigidity and apnea. Tetanospasms are intermittent, irregular and unpredictable, although they are often triggered by external stimuli, sometimes very trivial such as a sudden noise or puff of cold air, or even the internal stimulus of a distended bladder or bowel. Each spasm is sudden, painful and generalized, resulting in opisthotonos, leg extension and arm flexion; pharyngeal spasm causes dysphagia and spasm of the glottis may cause immediate asphyxiation and death. Cognitive functions are not affected. Severe tetanus is accompanied by abnormalities of the autonomic nervous system, including hypo- or hypertension, arrhythmias and flushing.

Neonatal tetanus typically starts with poor sucking and irritability, followed by trismus and tetanospasms. It has a higher death:case ratio than tetanus at other ages.

With intensive care the death rate from tetanus (which is due to respiratory dysfunction or autonomic cardiovascular instability) may be as low as 10–20%, with higher rates in infants and in the elderly. A rating scale for the severity and prognosis of tetanus (Table 25.2) may be used. In general, the more rapid the evolution of symptoms and signs, the worse the prognosis but the belief that a short incubation period leads to a worse prognosis has been challenged.[6]

Complications related to spasms include vertebral and long bone fractures, glottic obstruction and asphyxia, and intramuscular hematomas. Rhabdomyolysis is common in generalized tetanus. Other complications are those related to general debility and prolonged intensive care.

Strychnine poisoning is the only true mimic of tetanus although there are several other diseases that may overlap to some extent. Strychnine poisoning develops more rapidly than tetanus and there is usually no muscle rigidity between spasms; serum analysis for strychnine should be performed in suspect cases. Other causes of trismus include dystonic reactions to phenothiazines, which may be ruled out by administration of benztropine 1–2mg intravenously or diphenhydramine 50mg intravenously, and dental abscesses. Tetany

TABLE 25-2 -- Rating scale for severity and prognosis of tetanus.

RATING SCALE FOR SEVERITY AND PROGNOSIS OF TETANUS		
Score 1 point for each of the following		
• Incubation period <7 days		
• Period of onset <48 hours		
• Acquired from burns, surgical wound, compound fracture, septic abortion		
• Narcotic addiction		
• Generalized tetanus		
• Pyrexia >104°F (40°C)		
• Tachycardia >120 beats/min (>150 beats/min in neonates)		
Total score provides indication of severity and prognosis		
Score	**Severity**	**Mortality**
0–1	Mild	<10%
2–3	Moderate	10–20%
4	Severe	20–40%
5–6	Very severe	>50%
NB: Cephalic tetanus is always scored as severe or very severe, and neonatal tetanus as very severe.		

from hypocalcemia or alkalosis tends to affect the extremities rather than the axial muscles and there is no trismus.

DIAGNOSIS

The diagnosis of tetanus depends upon clinical features, and epidemiologic history and laboratory tests are usually unhelpful. There is often a moderate leukocytosis but the CSF is normal, except for increased pressure. Neither electroencephalography nor electromyography is helpful. Occasionally, characteristic Gram-positive bacilli with terminal or subterminal spores may be visualized in aspirates from a wound but anaerobic cultures are rarely positive and the organism may be grown from

wounds in the absence of disease.

MANAGEMENT

A guide to the general management of the patient with tetanus has been published.[7] Human tetanus immunoglobulin 500IU as a single intramuscular injection[4] should be given at the time of diagnosis in order to prevent further circulating toxin from reaching the CNS. The use of intrathecal HTIG to neutralize toxin that has entered but is not yet fixed to nervous tissue has not been consistently beneficial and is not routinely recommended; intrathecal injections are potent stimuli for tetanospasms.

The source of toxin should be removed by wound debridement and removal of foreign bodies. Only vegetative forms of *C. tetani* will be susceptible to antibiotics. Therapy with metronidazole (15mg/kg intravenously followed by 20–30mg/kg/day intravenously for 7–14 days) should be used to eradicate *C. tetani*, even though antibiotics are not likely to penetrate into the anaerobic conditions that support growth of the organism.[8] Penicillin is theoretically less suitable (it acts as a central GABA antagonist) although it is still used in much of the world.

A benzodiazepine (midazolam administered intravenously at 5–15mg/h is suitable) should be used to produce sedation, decrease rigidity and control spasms. Airway protection during spasms is paramount. If the patient's ventilation is compromised then he or she should be sedated, intubated, provided with a soft nasal feeding tube and transferred to a quiet and darkened area. A tracheotomy may be needed. If benzodiazepines do not adequately control the spasms then the patient will need long-term neuromuscular blockade.

TABLE 25-3 -- Characteristics of groups of *C. botulinum* and the toxins that produce human disease.

CHARACTERISTICS OF GROUPS OF *C. BOTULINUM* AND THE TOXINS THAT PRODUCE HUMAN DISEASE				
Group	**Toxins produced**	**Proteolysis**	**Heat resistance of spores**	**Disease severity**
Group I	A, B, F	Yes	High	Severe
Group II	B, E, F	No	Low	Less severe

The management over the next few weeks is that of any ventilated patient plus specific therapy for autonomic nervous system complications and control of spasms.[9] Sympathetic hyperactivity is treated with combined a- and ß-blockade or morphine. Intrathecal baclofen has been shown to be effective in controlling muscle rigidity.[10] Epidural blockade with local anesthetics may be needed. Hypotension requires fluid replacement and dopamine or norepinephrine (noradrenaline) administration. Parasympathetic overactivity is rare but if bradycardia is sustained then a pacemaker may be needed.

Clinical tetanus does not induce immunity against further attacks of the disease and all patients should be fully immunized with tetanus toxoid (in the form appropriate for their age) during convalescence.

BOTULISM

Botulism is caused by neurotoxins (antigenic types A, B, E and F cause human disease) produced by groups I or II of *Clostridium botulinum*, an anaerobic, spore-forming bacillus (Table 25.3). There are three forms of the illness: food-borne botulism from ingestion of preformed toxin; wound botulism; and botulism from intestinal colonization, usually, but not universally, in infants.[11] [12]

EPIDEMIOLOGY

All three forms of botulism occur throughout the world but for food-borne botulism, the causative strains, the responsible foods and the resulting illness vary in different geographic areas.

Accurate data about the incidence of botulism are difficult to obtain but it is estimated that, in France, there were about 300 food-borne outbreaks between 1979 and 1988, and in the USA there were 474 outbreaks (a large proportion from Alaska) involving nearly 1050 persons between 1950 and 1990. Outbreaks were most frequent in the summer or autumn. In the UK the incidence is much lower than in Europe or the USA, with only nine outbreaks between 1922 and 1988. In 1989 the largest ever outbreak of food-borne botulism in the UK affected 27 people who had eaten hazelnut yoghurt. The illness was caused by type B toxin formed by bacteria growing in canned hazelnut conserve used to flavour the yoghurt and that had been inadequately heat treated.[13]

Nearly 1000 cases of infant botulism (roughly equally divided between type A and type B *C. botulinum*) were reported in the USA between 1976 and 1990. Almost half the cases were reported from California, with an incidence of 7 per 100,000 live births. Cases occur most frequently in the second month of life and 95% of cases are in infants less than 6 months old.[11]

Most cases of food-borne botulism are associated with home-preserved meats, fish and vegetables but the common vehicles are often idiosyncratic to a country or culture.[14] In the USA (apart from Alaska), Spain, Italy and China most cases follow consumption of home-preserved vegetables (home-canned asparagus, beans and peppers in the USA, home-fermented bean curd in China)

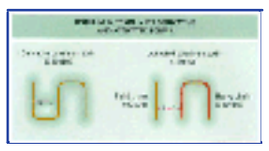

Figure 25-4 Botulinum toxin in its derivative and activated forms.

contaminated with type A *C. botulinum*. In Alaska, Japan, Canada and Scandinavia cases are usually due to type E toxin and follow eating fermented foods or preserved fish products; in central continental Europe cases typically arise from home-cured meats and are caused by nonproteolytic strains of type B *C. botulinum*. Commercially prepared foods are only rarely implicated. There is a significant association between infant botulism and ingestion of bee honey.

Occasionally, botulism can follow wound infections with *C. botulinum*. The wounds are often compound fractures or penetrating wounds; cases have also been reported in intravenous drug users[15] and complicating sinusitis in chronic cocaine sniffers.

PATHOGENESIS AND PATHOLOGY

In food-borne botulism there is ingestion of preformed toxin, but in other forms of the syndrome the toxin is produced in vivo and released when vegetative cells lyse. The toxin is not released from spores. Botulinum toxins are the most poisonous substances known to man and are synthesized as a polypeptide of molecular weight 150–165kDa, which is then broken into an H chain of about 100kDa and an L chain joined by a disulfide bond (Fig. 25.4). The mechanism of action is similar or identical to that of tetanospasmin, resulting in cleavage of synaptobrevin and inhibition of release of acetylcholine at peripheral cholinergic synapses.[2] The H chain of the toxin binds rapidly to the membrane of the presynaptic a motor neuron and then some or all of the toxin molecule translocates through the membrane. Finally, there is a slow paralytic step that may partially depend upon temperature and activity of the neuron. The process is irreversible and the synapse is permanently damaged; recovery of function depends upon the budding and growth of new presynaptic end-plates.

Infant botulism[16] results from colonization of the infant's gastrointestinal tract with as many as 10^8 proteolytic *C. botulinum* organisms per gram of feces. The mechanisms that relate to colonization and toxin absorption from the infant gut are unclear, and both the organism and toxin may sometimes continue to be excreted in the feces for several months after the illness has resolved. Wound botulism typically results from soil contamination of severe head wounds, as occurs in warfare.

Death results from respiratory paralysis and there are no specific pathologic findings on gross or histologic examination in any of the forms of botulism.

PREVENTION

The key to the prevention of botulism is adequate processing and storage of food to destroy spores and prevent their germination and

toxin production. Spores are not killed by boiling at 212°F (100°C) but are destroyed by heating at 250°F (121°C) for 2.5 minutes (the type of temperature achieved under pressure processing of low-acid foods). Once toxin is formed, it can be inactivated by boiling or heating at 176°F (80°C) for 30 minutes.

Toxin production by strains of *C. botulinum* is inhibited at a pH below 4.6, in saline and at low temperatures (below 38°F (3.3°C)); the respective values differ somewhat for different strains. Commercial canneries pay particular attention to less acid (pH >4.6) fruit and vegetables; the canning and curing of meats relies on a reduced heat treatment to kill vegetative bacteria and sodium chloride and nitrite to inhibit spore growth. Vacuum packaging of food may encourage the growth of anaerobes and there are concerns that botulinum toxin may be produced before spoilage is obvious. Toxin has been detected in mushrooms and coleslaw kept in modified atmosphere packaging. This should be preventable by piercing the packaging of fresh vegetables with air holes to allow sufficient oxygen to be present.

Honey has been associated with infant botulism and honey is not recommended as a food for infants less than 1 year old.

CLINICAL FEATURES

Food-borne botulism usually develops 12–36 hours after ingestion of the toxin, although the interval may be as short as 6 hours or as long as 10 days. Patients who have type E toxin-mediated disease tend to have shorter, and those with type B tend to have longer incubation periods. Wound botulism occurs at a mean of 7.5 days (range 4–18 days) after the injury.[17]

Typically, botulism first affects the muscles supplied by the cranial nerves with disturbances of vision and difficulties in swallowing and speech followed by descending weakness of muscles of the trunk and extremities that is bilateral but not necessarily symmetric. Cardiovascular, gastrointestinal and urinary autonomic dysfunction may follow. The presentation may be related to the type of toxin: autonomic symptoms occur earlier and are more prominent in intoxication with type B and E toxins.

Common presenting symptoms are diplopia, dysphagia, dysarthria, dry mouth and fatigue (Table 25.4). [18] Ptosis and ophthalmoplegia are common physical signs, together with facial weakness and a decreased gag reflex. The pupils are dilated or fixed in less than 50% of cases. Frequently, there is weakness of the extremities, although deep tendon reflexes are usually normal. Patients are usually afebrile and have no

TABLE 25-4 -- Frequency of symptoms in types A, B and E food-borne botulism.[18]

FREQUENCY OF SYMPTOMS IN TYPES A, B AND E FOOD-BORNE BOTULISM			
Symptoms	**Type A disease (% of cases)**	**Type B disease (%)**	**Type E disease (%)**
Dysphagia	25–96	77–100	63–90
Dry mouth	26–83	96–100	55–88
Diplopia	50–90	57–100	85
Dysarthria	25–100	69–100	50
Fatigue	8–92	69–100	Not known
Weakness of arm	16–86	64–86	Not known
Constipation	73	17–100	25–38
Weakness of leg	16–76	64–86	Not known
Dyspnea	35–91	34	88
Vomiting	70	50–100	88–100
Dizziness	8–86	30–100	63
Diarrhea	35	8–14	10
Paresthesiae	20	12–14	Not known

sensory deficits. Patients who have wound botulism have a similar presentation but acute gastrointestinal symptoms are lacking.

Constipation is the first sign of infant botulism, with neurologic signs developing either concurrently or up to several weeks later. The neurologic signs progress in a similar fashion to those in other forms of botulism but they may be overlooked by the parents, who merely note the infant is irritable, lethargic or unable to suck. There is a wide range of clinical illness associated with infant botulism; 50% of cases develop ventilatory failure. Although some studies suggested that it was responsible for 5% of cases of the sudden infant death syndrome (SIDS) in California, no evidence of botulism has been found in any SIDS cases elsewhere in the USA.

COMPLICATIONS

The severity and duration of food-borne botulism are related to the amount of toxin ingested. Respiratory failure occurs in 20–35% of patients; the mean duration of respiratory support is 7 weeks for those requiring mechanical ventilation. Recovery from botulism is usually complete but persistent dysphagia, diplopia and prolonged weakness are rare complications of severe cases.[19] [20]

There has been a steady decline in mortality associated with botulism over the past century; the rate was about 70% in the period 1910–19 and about 9% during 1980–89. The mortality is higher in type A disease than in type B.[21]

The prognosis for infants hospitalized with botulism and given meticulous supportive care is very good, with less than 1.3% case fatality and full recovery.

DIFFERENTIAL DIAGNOSIS

The diseases most often confused with botulism are Guillain-Barré syndrome (particularly the Miller-Fisher variant confined to the cranial nerves), myasthenia gravis and the Eaton-Lambert myasthenic syndrome, other forms of food poisoning and tick paralysis. Guillain-Barré syndrome frequently has sensory complaints and the Miller-Fisher syndrome includes prominent ataxia. The myasthenias lack autonomic dysfunction and are less fulminant than botulism. In tick paralysis a careful search will reveal the *Dermacentor* tick still attached.

DIAGNOSIS

Routine laboratory tests are not helpful in the diagnosis of botulism. The diagnosis is best confirmed by assay of botulism toxin in the patient's blood, gastric washings or feces by means of toxin neutralization tests in mice. Toxin may also be demonstrated in the incriminated food. This test takes anything from 6 to 96 hours to perform and the initial diagnosis must therefore be based on clinical findings.

Clostridium botulinum may be cultured or the toxin detected by an enzyme-linked immunosorbent assay in the patient's feces, particularly in infant botulism and other cases resulting from intestinal colonization.

Electrophysiologic studies show normal nerve conduction velocities but the electromyogram is often abnormal with facilitation (an incremental increase) of the M-wave amplitude when high-frequency (20–50 per second) repetitive stimuli are applied. These abnormalities may persist for several months after the onset of illness.

MANAGEMENT

Elimination of any unabsorbed toxin from the gastrointestinal tract should be encouraged in patients who have suspected botulism. Administration of an emetic or gastric lavage is recommended if ingestion of the suspect food has occurred within the preceding few

hours and (unless there is a paralytic ileus) purgation or high enemas should be administered even several days after food ingestion.

The mainstay of therapy for botulism is meticulous supportive care. Patients should be admitted to an intensive care unit and their respiratory function monitored by repeat vital capacity measurements. Intubation should be performed if vital capacity falls below 12ml/kg.

Equine antitoxin, containing antibodies to types A, B and E toxin, is available through public health services in many countries. There are few data concerning its use in humans but it is clearly effective in experimental animals. It has to be given as early as possible in the course of the illness but its use needs careful consideration in view of the risk of serious anaphylaxis or serum sickness. A test dose is administered into the skin or the conjunctiva. If there is no hypersensitivity, then one vial is given intravenously and one vial intramuscularly for an average adult; further doses may be given 2–4 hours later if the symptoms persist. In view of the good prognosis in infant botulism, the risks of equine antitoxin probably preclude its use. The potential role of human or monoclonal botulism immune globulin is still unclear in infant botulism.

Although guanidine hydrochloride (which increases release of acetylcholine from nerve terminals in response to nerve stimulation) has theoretical advantages, there was no improvement in recovery in a controlled study of patients with type A disease. Guanoxan and 3,4-diaminopyridone are investigational. Antibiotics do not help except as part of meticulous debridement of the wound in wound botulism.

The relevant public health authorities should be notified promptly of a suspected case of botulism so that the necessary investigation may be conducted.

DIPHTHERIA

EPIDEMIOLOGY

The incidence of diphtheria has fallen dramatically in the technically developed world over the past 50 years but the disease remains endemic in many parts of the Third World, including India, Nigeria, Brazil, Indonesia and the Philippines. [22] Between 1990 and 1995 there was a considerable epidemic of diphtheria in Russia and other parts of the former Soviet Union; nearly 50,000 cases occurred, including some in tourists and other visitors.[23] [24]

Spread of *Corynebacterium diphtheriae* takes place via respiratory droplets or by direct contact with infected respiratory secretions and 3–5% of healthy persons may harbor the organism in their throats. Epidemics have also resulted from contaminated milk. Skin infections are important reservoirs of infection and sources of dissemination of diphtheria in tropical countries and among alcoholic persons in urban areas of the West; person-to-person spread by exudate from infected skin lesions is relatively easy.[25] Three biotypes (mitis, gravis and intermedius), producing different quantities of toxin, can be differentiated. Occasional cases of diphtheria are caused by toxigenic strains of *Corynebacterium ulcerans*, which is usually transmitted by unpasteurized dairy products or contact with farm animals.[26]

Although immunization with toxoid does have a dramatic effect upon the incidence of diphtheria, the disease incidence started to decline well before the widespread use of immunization and other factors are also important. Toxoid after all only attenuates the effects of toxin and does not prevent colonization with the organism; carriage rates should therefore remain high and there should be frequent epidemics among the relatively large proportion of individuals with subprotective antitoxin levels. The evidence, however, points to an extremely low carrier rate and disease is very rare in most developed countries. It is still unclear why this should be so.

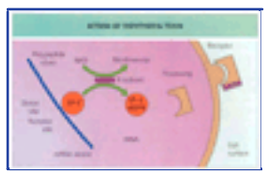

Figure 25-5 Action of diphtheria toxin. The binding subunit attaches to the cell surface and the toxin enters the cell. After endocytosis the toxin is cleaved and the active A subunit is released. This then catalyzes the cleavage of nicotinamide adenine dinucleotide (NAD) and the transfer of adenine diphosphate ribose (ADPR) to EF-2. EF-2 is essential for ribosomal reactions at the acceptor and donor sites, whereby the mRNA code is transferred via tRNA to an amino acid sequence and the building of a polypeptide chain. EF-2-ADPR is incapable of adding amino acids to a polypeptide chain and protein synthesis is stopped.

PATHOGENESIS AND PATHOLOGY

Corynebacterium diphtheriae is not very invasive and organisms remain in the superficial layers of the respiratory mucosa or skin. Its major virulence is the result of the exotoxin, which inhibits protein synthesis in mammalian cells. Exotoxin production by *C. diphtheriae* is the result of a lysogenic ß-phage that carries the *tox*+ gene into the bacterium. Toxin production in a phage-bearing strain depends upon genetic and nutritional factors, especially a deficiency of iron in the environment. The toxin is secreted as a 62kDa polypeptide comprising two segments: the B segment binds to specific receptors on the cell surface and, after endocytosis, the toxic segment A is released by proteolytic cleavage. Segment A catalyzes the cleavage of nicotinamide adenine dinucleotide leading to ribosylation and inactivation of elongation factor-2 (EF-2), which is necessary for the interaction of mRNA and tRNA. Its inactivation prevents the addition of further amino acids to the developing polypeptide chain (Fig. 25.5).[27] The toxin is very potent; a single molecule can inactivate all the EF-2 in a cell and stop protein synthesis within a few hours.

The toxin affects all cells but the major effects are local in the vicinity of bacterial growth and distant on the heart, nerves and kidneys.

Within a few days of respiratory infection the toxin produces a local necrotic coagulum of fibrin, dead epithelial cells and cellular infiltrate, which forms the adherent pseudomembrane. This gray-green or black membrane can be local or extend widely to cover the entire pharyngeal or tracheobronchial mucosa. There is a marked underlying soft tissue edema and regional lymphadenitis producing the bull neck appearance. Palatal paralysis is an early local effect of the toxin.

Myocarditis, renal tubular necrosis and demyelination and axon degeneration within cranial or peripheral nerves are prominent features of the more severe infections with *C. diphtheriae*.

PREVENTION

Diphtheria can be prevented by active immunization with formalin-detoxified diphtheria toxin (toxoid). Mass immunization with four

or five doses of diphtheria toxoid-containing vaccine is given to infants/children in most developed countries but immunity declines over time and 20–80% of adults have antibody titers below the level of 0.01IU/ml that is considered protective. Indeed, epidemic diphtheria may occur when more than 70% of a population have antitoxin titres below 0.01IU/ml. In order to maintain population immunity to diphtheria a booster dose (usually given with tetanus toxoid) is recommended every 10 years in adults. Persons over age 7 years have a higher incidence of constitutional symptoms to the concentration of diphtheria toxoid in infant vaccines and hence an adult vaccine (Td), with low dose purified diphtheria toxoid, is used. The resurgence of diphtheria in the former Soviet Union emphasizes the need to ensure that population immunity is maintained by booster immunization of adults.[23] [28] (See also Chapter 178).

CLINICAL FEATURES

Symptoms of diphtheria may be divided into two groups: those that occur locally as a result of noninvasive infection of the respiratory tract or skin, and those that occur at distant sites secondary to dissemination of diphtheria toxin.

After an incubation period of 2–4 days the onset of diphtheria is usually insidious with a mild sore throat and fever; local symptoms and signs of inflammation may then develop at various sites within the respiratory tract. Infection limited to the anterior nares is generally a mild disease, with a thin blood-stained or purulent nasal discharge and a delicate membrane on the nasal mucosa. Toxin absorption from the nasal mucosa is poor and there are usually few systemic symptoms or toxin-mediated problems. The most common site for clinical diphtheria is the posterior structures of the mouth and pharynx. The membrane develops typically on the tonsil(s) and spreads locally. The membrane is initially translucent, thin and white but rapidly becomes thicker and develops a gray-green color with patches of necrosis. It is adherent to the underlying tissues and its removal produces bleeding. The patient is feverish and there are enlarged lymph glands in the neck. In severe cases the membrane spreads rapidly over the uvula, palate, oropharynx and nasopharynx; the extent of the membrane correlates with the severity of the local and systemic symptoms. In the most severe cases there is a weak thready pulse, profound exhaustion and muscle weakness. Severe cervical lymphadenopathy and edema of the anterior cervical tissues creates the bull neck appearance and may cause respiratory embarrassment.

Spread of the membrane downward leads to laryngeal diphtheria; the voice becomes hoarse and there is a bovine cough and stridor. The patient becomes cyanosed and anxious and requires urgent intubation and removal of the membrane if death is to be prevented.

In tropical areas *C. diphtheriae* is a cause of skin infections, which may be impetiginous or appear as chronic ulcers with a grayish slough or membrane. Outbreaks of cutaneous diphtheria have been recorded among vagrants and other impoverished, homeless groups.[29] Cutaneous infection rarely causes clinical disease but is an important reservoir for the organism.

It is the later effects of diphtheria toxin on the heart and the nervous system that produce the most severe complications. Some degree of cardiac toxicity is detectable in most cases of diphtheria with a widespread membrane and about 50% of those with a moderate local exudate. Myocarditis characteristically begins during the first 1–2 weeks of the illness, often as the local respiratory disease is settling. First-degree heart block or ST-T wave changes on the electrocardiogram and raised serum aspartate aminotransferase concentrations develop early and warn of more severe forms of heart block or life-threatening arrhythmia. Myocarditis is a poor prognostic sign, increasing the mortality 3- or 4-fold. Bundle branch block or complete heart block has a mortality rate of up to 90% and the survivors are often left with conduction

defects.

Some form of neuritis occurs in between 10% and 20% of patients who have diphtheria and in up to 75% of those who have severe primary disease. Bilateral paralysis of the palate and posterior pharyngeal wall often appears in the first week of the illness and produces rhinolalia, dysphagia and nasal regurgitation of swallowed liquids. Other manifestations of neuritis are oculomotor and accommodation defects, facial paralysis and a peripheral motor neuropathy. These are usually delayed effects, developing 5 weeks or more after the onset of diphtheria. The degree of dysfunction is variable but footdrop is particularly common. Sensory neuropathies are rare complications of diphtheria. Although it may be slow, recovery of diphtheritic neuropathy is usually complete.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes infectious mononucleosis, streptococcal tonsillitis and acute epiglottitis. The tonsillar exudate of infectious mononucleosis is creamy in color, does not extend beyond the tonsil and does not produce bleeding if removed. Streptococcal pharyngotonsillitis is associated with more severe local symptoms and a higher fever and epiglottitis is more acute in onset and is not associated with a local membrane.

DIAGNOSIS

The need to initiate antitoxin therapy as soon as possible means that the diagnosis of diphtheria needs to be presumptive and made on clinical grounds. It must be suspected whenever there is a membrane in the throat or nares. Many individuals have diphtheroids in their throats and hence microscopy for *C. diphtheriae* is unreliable; confirmation of the diagnosis of diphtheria depends upon isolation of a toxin-producing strain of *C. diphtheriae*. The organism can be cultured on selective media and isolates tested for toxin production by inoculation of guinea pigs or by the Elek's plate method (immunoprecipitation of toxin in agar cultures by overlaid diphtheria antitoxin-containing filter paper strips). Polymerase chain reaction testing for the *tox* gene can be performed at the Centers for Disease Control and Prevention in the USA and at central public health laboratories in other countries.

MANAGEMENT[30]

Administration of diphtheria antitoxin (DAT) is the crucial therapeutic measure in diphtheria. It will only neutralize extracellular toxin and hence needs to be administered as early as possible, often before the disease can be microbiologically confirmed. It is an equine antiserum, however, and all patients must be tested for hypersensitivity to horse protein; this is done by instilling diluted DAT into the conjunctival sac or injecting 0.1ml of a 1:100 dilution subcutaneously, while taking appropriate precautions for anaphylaxis. Even if hypersensitivity is shown, DAT will need to be administered but only after desensitization procedures. The therapeutic dose and route of administration of DAT depend upon the extent of the disease. The recommended doses (the minimum therapeutic dose has never been determined) are as follows: for mild disease of less than 48 hours' duration, 20,000–40,000IU (or 500IU/kg body weight) given half intramuscularly and half by intravenous infusion over 30 minutes; for more severe disease or illness more than 3 days old, 80,000–120,000IU (up to 2500IU/kg) by intravenous infusion.

Erythromycin (2g/day for 6 days, then 1g/day for 8 days (50% of these dosages for children less than 6 years old)) or procaine penicillin (1.2 million IU/day for 6 days, then 600,000IU/day for 8 days (50%

of these doses for children less than 6 years old)) should be administered to eradicate *C. diphtheriae* and terminate toxin production. The patient should be kept in strict isolation during this period and until three throat swabs taken after therapy are culture negative. Patients with diphtheria due to *C. ulcerans* do not need isolation since person-to-person spread has never been documented. Supportive measures are also important. Patients should be confined to bed and their electrocardiogram monitored daily until the danger of myocarditis has passed; those who develop cardiac toxicity need full support and effective therapy for any conduction abnormalities. Corticosteroid therapy does not prevent myocarditis or neuritis in diphtheria.[31] Respiratory monitoring needs to be vigilant and early tracheotomy and intubation is sensible for those who have laryngeal diphtheria.

Because clinical infection does not always induce immunity, a course of toxoid should be commenced at the end of the first week of illness and completed during convalescence.

Those exposed to a case of diphtheria who have been fully immunized need to have throat cultures taken and to be observed for 7 days. Those with incomplete or unclear immunity should be started on erythromycin (7 days of oral therapy) or benzathine penicillin (a single intramuscular dose of 1.2 million units) and a course of toxoid. The antibiotic can be stopped if the cultures are negative; those initially carrying the organism need to have eradication confirmed 2 weeks after completing therapy.

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Chapter 26 - Transmissible Spongiform Encephalopathies of Humans and Animals

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John Collinge

The prion diseases or transmissible spongiform encephalopathies are a group of closely related transmissible neurodegenerative conditions of humans and animals. In recent years prion diseases have captured the public attention with the emergence of the bovine spongiform encephalopathy (BSE) epidemic in Europe and more recently with the appearance of a novel phenotype of Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD) in humans, which is experimentally linked to dietary exposure to BSE.

The nature of the transmissible agent in these diseases has been the subject of intense controversy. The understandable initial assumption that the agent must be some form of virus was challenged by the failure to directly demonstrate such a virus (or an immunological response to it) and by the remarkable resistance of the transmissible agent to treatment expected to inactivate nucleic acids (such as ultraviolet radiation or treatment with nucleases). Such findings had led to suggestions as early as 1966 by Alper and others that the transmissible agent may be devoid of nucleic acid[1] [2] and led Griffith to suggest in 1967 that the transmissible agent might be a protein. [3] Progressive enrichment of brain homogenates for infectivity resulted in the isolation of a protease-resistant sialoglycoprotein, designated the prion protein (PrP), by Prusiner and co-workers in 1982.[4] This protein was the major constituent of infective fractions and was found to accumulate in affected brains and sometimes to form amyloid deposits. The term prion (from *pr*oteinaceous *in*fectious particle) was proposed by Prusiner in 1982[5] to distinguish the infectious pathogen from viruses or viroids. Prions were defined as 'small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids'.

The unifying hallmark of the prion diseases is the aberrant metabolism of the PrP, which exists in at least two conformational states with different physicochemical properties. The normal form of the protein, referred to as PrP^{C} , is a highly conserved cell surface protein attached via a glycophosphatidyl inositol anchor (Fig. 26.1). It is expressed in a wide range of cell types and particularly in neuronal cells. PrP^{C} is a sialoglycoprotein of molecular weight 33–35kDa with a high content of a-helical secondary structure that is sensitive to protease treatment and soluble in detergents (Fig. 26.1). The diseaseassociated isoform, referred to as PrP^{Sc} , is found only in infected brains as aggregated material, is partially resistant to protease treatment and insoluble in detergents and has a high content of ß-sheet secondary structure.

Due to its physicochemical properties, the precise atomic structure of the infectious particle or prion is still undetermined but considerable evidence argues that prions are composed largely, if not entirely, of an abnormal isoform of PrP. According to the protein-only hypothesis of prion replication, PrP^{Sc} recruits PrP^{C} into the infectivity-associated isoform, an event that is central to prion propagation. Prion propagation may involve recruitment of an alternately folded form of PrP^{C} , ß-PrP, into such aggregates, the process being driven thermodynamically by intermolecular interactions[6] (Fig. 26.2).

EPIDEMIOLOGY

Animal prion diseases

Scrapie is the prototypic prion disease (Table 26.1). It has been recognized as an enzootic disease of sheep and goats for more than 250 years. Present in many countries, its prevalence in the UK has been estimated as 0.5–1% of the sheep population. The etiology of natural scrapie has been the subject of intense debate for many years, but it is now clear that it is an infectious disease,[7] for which susceptibility is genetically modulated by the host.

Following its discovery in 1985, BSE reached epidemic proportions, with over 180,000 confirmed cases in UK cattle and much smaller numbers in many other European countries. It has been estimated that up to 1 million cattle were infected with BSE in the UK.[8] Smaller epidemics have also been described in Switzerland, Ireland, Portugal and France and in September 2001 the first case was reported in Japan.[9] In May 2003, cases were reported in Western Canada. Epidemiologic studies point to contaminated offal, used in the manufacture of meat and bone meal and fed to cattle, as the source of prions responsible for BSE.[10] Because the UK has a relatively large sheep population in which scrapie is endemic, it was hypothesized that scrapie-contaminated sheep offal was the initial source of BSE. An alternative view is that BSE prions originated spontaneously in cattle and that infection was subsequently amplified by recycling of infected cattle that had subclinical disease. As sheep were also fed meat and bone meal, it is not unreasonable to expect that a BSE-like disease with characteristics different from conventional scrapie strains might also appear in the sheep population, although there is currently no confirmation that this has occurred. However, this is currently of concern in the countries where BSE is prevalent.

The host range of BSE appears to be unusually wide, affecting many other animal species (Table 26.1). Foodstuffs contaminated with BSE appear to have caused disease in several other animal species in the UK, including feline spongiform encephalopathy (FSE) in domestic cats, exotic ungulates and captive cats in zoos. In addition to these 'natural' infections, BSE has been experimentally transmitted to a wide variety of species. Outbreaks of transmissible mink encephalopathy and chronic wasting disease in captive populations of mink, mule deer and elk in certain regions of the USA have also been attributed to prion-infected foodstuffs, although the origin of infection is less certain in these diseases. Epidemiological studies suggest lateral transmission as the most plausible explanation for the spread of chronic wasting disease in captive populations of Rocky Mountain elk. Chronic wasting disease has also been diagnosed in free-ranging mule deer, Rocky Mountain elk and white-tailed deer from north-central Colorado.

Human prion diseases

The human prion diseases are unique in biology in that they manifest as sporadic, genetic and infectious diseases (Table 26.2). The majority of cases of human prion disease occur sporadically as

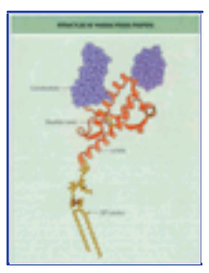

Figure 26-1 Structure of the human prion protein. Model of glycosylated human prion protein indicating positions of *N*-linked glycans (blue), the single disulfide bond linking helixes 2 and 3, and the glycosylphosphatidylinositol (GPI) anchor to the outer surface of the cell membrane. *Courtesy of Dr Richard Sessions and Mr Ray Young.*

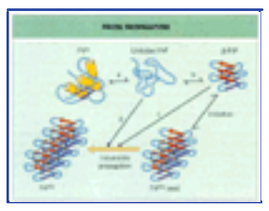

Figure 26-2 Prion propagation. Schematic representation of a possible mechanism for prion propagation. The predominantly a-helical form of the normal prion protein (PrP^{C}) proceeds via an unfolded state (a) to refold into a predominantly ß-sheet form (ß-PrP) (b). Prion replication may require a critical 'seed' size. Further recruitment of ß-PrP (c) or unfolded PrP (d) occurs as an essentially irreversible process. *Courtesy of Prof Tony Clarke and Mr Ray Young.*

TABLE 26-1 -- Animal prion diseases.

ANIMAL PRION DISEASES		
Disease	**Host**	**Etiology**
Scrapie	Sheep and goats	Thought to involve both horizontal and vertical transmission
Transmissible mink encephalopathy	Captive mink	Probably food-borne, although the origin of infectious prions is uncertain
Chronic wasting disease	Captive and free-ranging mule deer and Rocky Mountain elk	Origin unknown There is evidence for horizontal transmission
Bovine spongiform encephalopathy (BSE)	Cattle	Food-borne in the form of contaminated meat and bone meal
Feline spongiform encephalopathy	Domestic and zoo cats	Feed contaminated with bovine spongiform encephalopathy prions
Exotic ungulate encephalopathy	Captive bovidae	Feed contaminated with bovine spongiform encephalopathy prions

Creutzfeldt-Jakob disease (sporadic (s) CJD) at a rate of roughly 1 per 10[6] population across the world, with an equal incidence of disease in men and women. However, very recently, an increased incidence of sCJD has been reported in Switzerland, with a twofold increase in 2001, and figures from the first quarter of 2002 indicated that it continues to rise.[11] These cases are clinically definite sCJD and vCJD was excluded on histopathological and laboratory analysis. Several scenarios have been proposed to account for this increase in sCJD in Switzerland, including improved reporting, iatrogenic transmission and transmission of a prion zoonosis similar to that of CJD which is thought to be caused by exposure to bovine prions. The etiology of sCJD is unknown, although hypotheses include somatic mutation of the PrP gene (referred to as *PRNP*) and the spontaneous conversion of PrP^{C} into PrP^{Sc} as a rare stochastic event. There is a common coding polymorphism at codon 129 of *PRNP* encoding either methionine or valine (Fig. 26.3). Homozygosity at this position predisposes people to the development of sporadic and iatrogenic CJD.[12] [13] Additionally, a *PRNP* susceptibility haplotype has been identified indicating additional genetic susceptibility to sporadic CJD at or near the *PRNP* locus.[14]

Approximately 15% of human prion diseases are inherited with an autosomal dominant mode of inheritance. Inherited human prion diseases have been shown to segregate with more than 30 different missense and insertion mutations in the coding sequence of *PRNP* (see Fig. 26.3).[15] [16] [17] [18] Although the human prion diseases are experimentally transmissible, the acquired forms have, until recently, been confined to rare and unusual situations. For example, kuru was caused by cannibalism among the Fore linguistic group of the Okapa district of the Eastern Highlands in Papua New Guinea.[19] The disease had its origins at the beginning of the 20th century and was the leading cause of death in this population by the middle of the century, killing over 3000 people in the total population of 30,000. Mainly adult women as well as children of both sexes were affected, to give an annual disease-specific mortality of approximately 3%. It is thought that the roughly sevenfold higher incidence of disease in adult women than adult men was the result of higher exposure of women to infectious brain material. Since the cessation of cannibalistic

practices around 1956, the disease has all but died out, with

TABLE 26-2 -- Human prion diseases.

HUMAN PRION DISEASES			
Disease	**Incidence**	**Etiology**	**Age of onset or incubation period and duration of illness**
Sporadic Creutzfeldt-Jakob disease	1 case per 1 million population	Unknown but hypotheses include somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}	Age of onset is usually 45–75 years; age of peak onset is 60–65 years; 70% of cases die in under 6 months
Inherited prion diseases (GSS, FFI, CJD)	10–20% of cases of human prion disease	Autosomal dominant *PRNP* mutation	Onset tends to be earlier in familial CJD compared to sporadic CJD. Can be wide phenotypic variability between and within families
Kuru	>2500 cases among the Fore people in Papua New Guinea	Infection through ritualistic cannibalism	Incubation period 5->40yrs; duration of illness 12 months
Iatrogenic Creutzfeldt-Jakob disease	About 90 cases to date	Infection from contaminated human growth hormone, human gonadotropin, depth electrodes, corneal transplants, dura mater grafts, neurosurgical procedures	Incubation periods of cases from human growth hormone 4–30 years; duration of illness 6–18 months
Variant Creutzfeldt-Jakob disease	128 young adults in the UK and France*	Infection by bovine spongiform encephalopathy-like prions	Mean age of onset 26 years; mean duration of illness 14 months
PrP^{C} , normal form of prion protein; PrP^{Sc} , disease-associated isoform of prion protein; *PRNP*, prion protein gene.			

* to Oct 2002

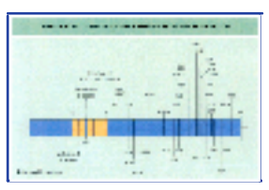

Figure 26-3 Pathogenic mutations and polymorphisms in the human prion protein. The pathogenic mutations associated with human prion disease are shown above the PrP coding sequence. These consist of 1, 2 or 4–9 octapeptide repeat insertions within the octarepeat region between codons 51 and 91, a deletion of 2 octapeptide repeats; and various point mutations causing missense amino acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single-letter amino acid conventions. Polymorphic variants are shown below the PrP coding sequence. Deletion of one octapeptide repeat is not associated with disease.

only a handful of cases currently occurring in older people who were presumably exposed to kuru as young children, indicating an incubation time in these cases of greater than 40 years. Other examples of acquired human prion diseases have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation and surgical operations using contaminated instruments or apparatus. In addition, iatrogenic CJD has occurred after implantation of dura mater grafts and treatment with growth hormone or gonadotropin derived from the pituitary glands of human cadavers.[20] [21] [22]

The appearance of CJD cases in teenagers and young adults in the UK during the mid-1990s prompted considerable concern that they might have acquired the illness as a result of exposure to BSE. By March 1996, it became clear that the unusual clinical presentation and neuropathology were remarkably consistent in these new cases.[23] Up to October 2002, 128 cases of probable and pathologically confirmed vCJD were reported in predominantly teenagers and young adults in the UK, six cases in France, one case in Hong Kong in an ex-UK resident, one case in the Republic of Ireland, two cases in the USA in ex-UK residents and one case in Italy.[24] Molecular strain typing, which focuses on the biochemical properties of PrP^{Sc} from the brains of BSE-infected cattle and patients who have CJD, has demonstrated that vCJD is different from sporadic CJD but similar to BSE.[25] [26] Moreover, the incubation times and profile of neuropathologic lesions of vCJD and BSE prions are indistinguishable in inbred lines of mice.[27] These data argue that BSE and vCJD are the same strain. All reported cases of vCJD have been homozygous for methionine at the polymorphic codon 129, a genotype

shared by ~40% of the British Caucasian population.[28] Polymorphisms in the human *PRNP* gene are not the sole genetic influence on disease susceptibility and incubation time. Studies with inbred lines of mice show that large differences occur even with the same amino acid sequence of the prion protein, suggesting that other genes may contribute to the observed variation.

Studies of quantitative trait loci (QTL) linked to prion disease incubation periods in mice have identified susceptibility loci on chromosomes 2, 4, 8, 11 and 12 and 15.[29] [30] These QTL studies provide strong evidence that genetic loci other than the coding region of *PRNP* have a major influence on scrapie incubation time in experimental prion disease. These findings suggest the need for caution in interpreting estimates of vCJD epidemic sizes utilizing existing genetic epidemiological studies which may result in overly optimistic predictions of the size of the vCJD epidemic because these models assume that only methionine-homozygous individuals are susceptible to vCJD.[31] [32] This is unlikely given the evidence from another acquired human prion disease, kuru, which occurred in all codon 129 genotypes as the epidemic evolved, with codon 129 heterozygotes having the longest mean incubation time.[33] [34] Therefore the patients identified to date with vCJD represent those individuals most genetically susceptible to the disease.

Iatrogenic secondary transmission of vCJD prions from asymptomatic carriers is of considerable concern in the UK. If secondary transmission does occur, the mean incubation time is likely to be much shorter than in primary cases because transmission does not involve a species barrier. As there is no diagnostic test for preclinical carriers of vCJD, estimates of asymptomatic carriers relies on screening of surgical lymphoreticular tissue to assess the prevalence of preclinical disease. [35] [36] Therefore a large prospective longitudinal study of surgical tonsil samples is currently being undertaken in the UK.

CLINICAL FEATURES

The human prion diseases can be divided etiologically into inherited, sporadic and acquired forms with CJD, Gertsmann-Straussler-Scheinker syndrome (GSS) and kuru now seen as clinicopathological syndromes rather than individual disease entities. The identification of one of the pathogenic *PRNP* mutations in a patient with neurodegenerative disease allows the diagnosis of an inherited prion disease and subclassification according to mutation.[37] Over 30 pathogenic mutations have been described in two groups:

- point mutations resulting in amino acid substitutions in PrP or, in one case, production of a stop codon resulting in expression of a truncated PrP;
- insertions encoding additional integral copies of an octapeptide repeat present in a tandem array of five copies in the normal protein (see Fig. 26.3).

They are all autosomal dominantly inherited conditions. Kindreds with inherited prion disease have been described with phenotypes of classic CJD and GSS and also with a range of other neurodegenerative disease phenotypes. Some families show remarkable phenotypic variability which can encompass both CJD- and GSS-like cases as well as other cases which do not conform to either CJD or GSS phenotypes.[38] Such atypical prion diseases may lack the classic histologic al features of a spongiform encephalopathy entirely although PrP immunohistochemistry is usually positive.[39] Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features are seen in variable combinations. *PRNP* analysis is also used for

presymptomatic genetic testing in affected families.[40]

Classic CJD is a rapidly progressive dementia accompanied by myoclonus. Decline to akinetic mutism and death is rapid and often occurs within 3–4 months. Cerebellar ataxia, extrapyramidal and pyramidal features and cortical blindness are also frequently seen The EEG may show characteristic pseudo-periodic sharp wave activity which is helpful in diagnosis but present only in around 70% of cases. To some extent, demonstration of a typical EEG is dependent on the number of EEGs performed and serial EEG is indicated to try and demonstrate this appearance. Cerebrospinal fluid (CSF) immunoassay for the neuron-specific 14-3-3 protein may be helpful.[41] A combination of both 14-3-3 CSF analysis and EEG is recommended in the investigation of suspected classic CJD cases to increase the sensitivity of pre-mortem case definition. [41] A raised 14-3-3 protein is not, however, specific for classic CJD and is raised in viral encephalitis or recent stroke; it is a marker of neuronal injury and loss. Of more concern with respect to the differential diagnosis is that it may also be raised in rapidly progressive forms of Alzheimer's disease, which may be confused with CJD. MRI scanning may show signal changes in the basal ganglia that, although not specific, can be diagnostically helpful.[42] Atypical cases of classic CJD are well recognized, however, and can still present diagnostic difficulties.

The clinical features of kuru consist of a progressive cerebellar ataxia accompanied by dementia in the later stages and death, which usually occurs within 12 months. Iatrogenic prion disease arising from intracerebral or optic inoculation usually manifests clinically as classic CJD, whilst those arising from a peripheral route of inoculation, such as pituitary growth hormone, commonly present like kuru with a progressive ataxia. GSS commonly presents as a chronic cerebellar ataxia with pyramidal features and dementia occurs much later in the clinical course, which is longer than that seen in classic CJD. Fatal familial insomnia (FFI) is characterized by progressive untreatable insomnia, dysautonomia and dementia and selective thalamic degeneration and is most commonly associated with a missense mutation at codon 178 of *PRNP*.[43] The FFI phenotype has also been described as occurring sporadically with no causative mutation in *PRNP* identified.[44]

The early clinical presentation of vCJD resembles kuru more than classic CJD and consists of behavioral and psychiatric disturbances, peripheral sensory disturbance and cerebellar ataxia. Common early psychiatric features include dysphoria, withdrawal, anxiety, insomnia and apathy. Neurological symptoms preceded psychiatric symptoms in 15% of cases studied and were present in combination with psychiatric symptoms in 22% of cases from the onset of disease. No common early neurological features were noted, but paresthesiae and/or pain in the limbs is seen in around half of the cases. However, a significant proportion of patients exhibited neurological symptoms within 4 months of clinical onset and these included poor memory, pain, sensory symptoms, unsteadiness of gait and dysarthria. Disorientation, hallucinations, paranoid ideation, confabulation, impaired self-care and the most common neurological features (cerebellar signs, chorea, dystonia, myoclonus, upper motor neuron signs and visual symptoms) developed late in the course of the illness.[45] The duration of disease is longer in vCJD with mean patient survival times of about 13 months, compared with about 4 months for classic CJD. Moreover, whereas classic CJD is predominantly a late-onset disease with a peak onset between 60 and 65 years, the median age of onset of vCJD is 26 years.[45]

The EEG is not helpful in the diagnosis of vCJD; whilst generalized slowing is usually present, the characteristic periodic changes associated with classic CJD are not. The CSF 14-3-3 protein is not helpful and may often be negative. Magnetic resonance imaging, however, is useful in the diagnosis of vCJD; in the majority of cases high signal is noted in the posterior thalamus (pulvinar) bilaterally on dual echo (T_2 - or proton density-weighted) MRI (Fig. 26.4).[46] Other common MRI features of vCJD are medial thalamic and periaqueductal gray matter high signal and the notable absence of cerebral

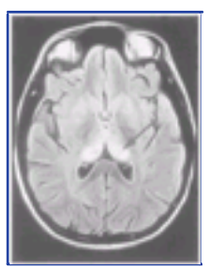

Figure 26-4 Axial T_2 -weighted MRI brain demonstrating high signal bilaterally in the posterior thalamus (arrowed) — the 'pulvinar sign' in a patient with vCJD.

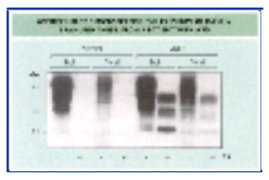

Figure 26-5 Western blot demonstrating the presence of PrP^{Sc} in brain and tonsil from a patient with vCJD. The presence of PrP^{Sc} is revealed after proteinase K (PK) treatment, which digests the normal form of PrP (PrP^{C}) but not the pathological form (PrP^{Sc}). *Courtesy of Ms Susan Joiner and Dr Andy Hill.*

atrophy.[46] All cases to date are homozygous for methionine at *PRNP* codon 129.[28] [35] vCJD can be diagnosed by detection of characteristic PrP^{Sc} immunostaining on tonsil biopsy.[35] Importantly, PrP^{Sc} is only detectable in tonsil and other lymphoreticular tissues in vCJD and not other forms of human prion disease, indicating that it has a distinctive pathogenesis.[35] [47] The PrP^{Sc} type detected on Western blot in vCJD tonsil has a characteristic pattern designated type 4t. Tonsil is the tissue of choice for diagnostic biopsy in the investigation of possible vCJD. Tonsil biopsy is well tolerated, with minor discomfort, and has, so far, shown 100% sensitivity and specificity.[35] If the tonsil biopsy is positive with the specific vCJD banding pattern (Fig. 26.5), then a more invasive brain biopsy becomes unnecessary.

PATHOGENESIS AND PATHOLOGY

Molecular strain typing

The marked clinical heterogeneity observed in sporadic CJD has yet to be explained. However, it has been clear for many years that distinct isolates, or strains, of prions can be propagated in the same host and these are biologically recognized by distinctive clinical and pathological features in experimental animals (for review see[48]). It is therefore likely that a proportion of the clinicopathological heterogeneity in CJD, and other human prion diseases, relates to the propagation of distinct human prion strains. The identification of these prion strains would allow an etiology-based classification of CJD by typing of the infectious agent itself.

The existence of prion strains has been difficult to accommodate within the protein-only model of prion propagation. As they can be serially propagated in inbred mice with the same *PRNP* genotype, they cannot be encoded by differences in PrP primary structure. Furthermore, strains can be re-isolated in mice after passage in intermediate species with different PrP primary structures.[49] Conventionally, distinct strains of conventional pathogens are explained by differences in their nucleic acid genome. However, in the absence of such a scrapie genome, alternative possibilities must be considered. A wealth of experimental evidence now suggests that PrP^{Sc} itself may encode strain-specific phenotypic properties. Different subtypes of PrP^{Sc} were associated initially with two strains of transmissible mink encephalopathy in hamsters.[50] [51] Recently, several human PrP^{Sc} types have been identified which are associated with different phenotypes of CJD.[25] [52] The different fragment sizes seen on Western blots, following treatment with proteinase K, suggest that there are several different human PrP^{Sc} conformations, referred to as 'molecular strain types'. These types can be further classified by the ratio of the three PrP bands seen after protease digestion, representing di-, mono- and unglycosylated fragments of PrP^{Sc} . Sporadic CJD is associated with PrP^{Sc} types 1–3, while type 4 human PrP^{Sc} is uniquely associated with vCJD and characterized by glycoform ratios which are distinct from those observed in sporadic CJD.[25]

Importantly, these biochemical changes in PrP^{Sc} are transmissible to PrP in a host. This has been demonstrated in studies with CJD isolates, with both PrP^{Sc} fragment sizes and the ratios of the three PrP glycoforms maintained on passage in transgenic mice expressing human PrP.[25] Furthermore, transmission of human prions and bovine prions to wild-type mice results in murine PrP^{Sc} with fragment sizes and glycoform ratios which correspond to the original inoculum.[25] Variant CJD is associated with PrP^{Sc} glycoform ratios which are distinct from those seen in sporadic CJD. Similar ratios are seen in BSE, and BSE when transmitted to several other species. [25] Recently, it has been demonstrated that BSE propagated in transgenic mice expressing the human prion protein can induce two different prion strain types — the type 4 PrP^{Sc} pattern associated with vCJD and the type 2 PrP^{Sc} pattern which is commonly associated with sporadic CJD. This finding has important potential implications as it raises the possibility that some humans infected with BSE prions may develop a clinical disease indistinguishable from sporadic CJD associated with type 2 PrP^{Sc} .[53] All these data strongly support

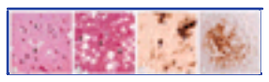
Figure 26-6 Neuropathology of prion disease — histopathological findings in vCJD. (a) Florid plaques, a characteristic feature of vCJD pathology. They consist of a round amyloid core (arrowed) surrounded by a ring of vacuoles (hematoxylin and eosin stain). (b) Spongiform degeneration in prion disease. This area shows severe vacuolization (spongiosis), there is severe neuronal loss and many strongly reactive astrocytes (arrowed) (hematoxylin and eosin stain). (c) Immunostaining of the pathological prion protein. The specimen is pretreated to denature the normal PrP and staining with a prion protein antibody reveals the presence of plaques (P) and synapses (S) staining positively for PrP^{Sc} . (d) Detection of pathological prion protein in the follicular dendritic cells in a tonsil. Accumulation of prion protein in lymphoreticular organs, such as spleen, tonsils or appendix, is a specific finding in vCJD and is not present in other forms of CJD. Therefore tonsillar biopsies can be used to specifically diagnose vCJD when clinical symptoms are only emerging. *Courtesy of Dr Sebastian Brandner.*

the 'protein-only' hypothesis of infectivity and suggest that strain variation is encoded by a combination of PrP conformation and glycosylation pattern.

Pathology

The animal and human prion diseases share a number of characteristic features, the most consistent being the neuropathological changes that accompany disease in the central nervous system. Indeed, it was the neuropathological similarities between scrapie and kuru that strongly suggested that the two diseases might be closely related and that kuru, like scrapie, might also be transmissible by inoculation. [54] Subsequently, brain extracts from patients who have kuru produced a progressive neurodegenerative condition in inoculated chimpanzees after a prolonged incubation period of 18–21 months.[55] The neuropathologic similarities between kuru and CJD prompted similar transmission experiments from CJD patients.[56]

Although the brains of patients or animals who have prion disease frequently show no recognizable abnormalities on gross examination, microscopic examination of the central nervous system typically reveals characteristic histopathologic changes, consisting of neuronal vacuolation and degeneration, which gives the cerebral gray matter a microvacuolated or 'spongiform' appearance (Fig. 26.6b), and a reactive proliferation of astroglial cells (Fig. 26.6b), which is often out of all proportion to the degree of nerve cell loss. Although spongiform degeneration is frequently detected, it is not an obligatory neuropathologic feature of prion disease; astrocytic gliosis, although not specific to the prion diseases, is more constantly seen. The lack of an inflammatory response is also an important characteristic. Although it is by no means a constant feature, some examples of prion disease are characterized by deposition of amyloid plaques composed of insoluble aggregates of PrP. Amyloid plaques are a notable feature of kuru and GSS but they are infrequently found in the brains of patients who have classic CJD.

Although there is wide variation in the neuropathologic profiles of different forms of human prion disease, the histopathologic features of vCJD are remarkably consistent and distinguish it from other human prion diseases. Large numbers of PrP-positive amyloid plaques are a consistent feature of vCJD but they differ in morphology from the plaques seen in kuru and GSS in that the surrounding tissue takes on a microvacuolated appearance, giving the plaques a florid appearance (Fig. 26.6a).[23] It is noteworthy that transmission of BSE to three macaques produced disease with neuropathologic features similar to those reported in cases of vCJD in humans.[57] vCJD is clearly very different in its pathogenesis from other human prion diseases and this is reflected in the tissue distribution of PrP^{Sc} in vCJD. AS mentioned, it is readily detectable in lymphoreticular tissue and, using highly sensitive immunoassays, PrP^{Sc} has been found in retina, optic nerve, rectum, adrenal gland and thymus in vCJD postmortem tissue (Fig. 26.7).[47]


Figure 26-7 The tissue distribution of PrP^{Sc} in vCJD compared to classic CJD. In vCJD PrP^{Sc} is found in lymphoreticular tissue as well as brain and spinal cord. Using highly sensitive immunodetection methods, PrP^{Sc} has also been found in the optic nerve, retina, adrenal gland and rectum. *Courtesy of Dr Jonathon Wadsworth and Mr Ray Young.*

Pathogenesis

Detection of PrP^{Sc} in brain material by immunohistochemical or immunoblotting techniques is considered to be diagnostic of prion disease (Fig. 26.6c). However, certain examples of natural and experimental prion disease occur without accumulation of detectable protease-resistant PrP^{Sc} ,[17] [58] [59] and the time course of neurodegeneration is not equivalent to the time course of PrP^{Sc} accumulation in mice expressing lower than normal levels of PrP^{C} .[60] Moreover, PrP^{Sc} is not toxic to cells that do not express PrP^{C} . [61] Additional evidence that PrP^{Sc} may not be the sole neurotoxic species has been demonstrated in mice inoculated with^{Sc} 237 hamster prions. These mice replicate prions to high levels in their brains but do not develop any signs of clinical disease during their normal lifespan. [62]

The essential role of host PrP^{C} for prion propagation and pathogenesis is demonstrated by the fact that mice in which the PrP gene has been disrupted (referred to as $Prnp^{0/0}$) are resistant to scrapie infection[63] [64] and that reintroduction of the murine PrP^{C} transgene restores susceptibility to infection.[65]

Gene-targeted $Prnp^{0/0}$ mice have also been studied to probe the normal function of PrP^{C} . Two independently generated lines of gene-targeted $Prnp^{0/0}$ mice developed normally and appeared to suffer no gross phenotypic abnormalities. [64] [66] The relative normality of these PrP null mice was thought to result from effective adaptive changes during development. However, data from *Prnp* conditional knockout mice suggest this is not the case;[67] these mice undergo ablation of neuronal PrP expression at 9 weeks of age. The mice remain healthy without evidence of neurodegeneration or an overt clinical phenotype, demonstrating that acute loss of neuronal PrP in adulthood is tolerated and that the pathophysiology of prion diseases is not due to loss of normal PrP function. [67] The normal function of PrP is not known but evidence from PrP knockout mice reveals defects in neurophysiological and biochemical function. Electrophysiological studies have demonstrated that fast inhibition and long-term potentiation mediated by d-aminobutyric acid receptors were impaired in hippocampal slices from $Prnp^{0/0}$ mice [68] [69] and that calcium-activated potassium currents were disrupted.[67] [70] These abnormalities of synaptic inhibition are reminiscent of the neurophysiological defects seen in patients who have CJD and in scrapie-infected mice[68] and suggest a direct role for PrP in the modulation of neuronal excitability. Normal PrP has also been shown to bind copper ions,[71] [72] with femtomolar affinity,[73] and a role for PrP in copper metabolism or transport has also been suggested.

Thus, it appears that neither accumulation of PrP^{Sc} , nor loss of normal PrP function, is the cause of the neurodegeneration in prion diseases. It is possible that a toxic intermediate species is produced in the conversion of PrP^{C} to PrP^{Sc} and that the steady-state level of such an intermediate could then determine the rate of neurodegeneration.[74]

Although the pathological consequences of prion infection occur in the central nervous system and experimental transmission of these diseases is most efficiently accomplished by intracerebral inoculation, most natural infections do not occur by these means. Indeed, administration to sites other than the central nervous system is known to be associated with much longer incubation periods, which may extend to 20 years or more. Experimental evidence suggests that this latent period is associated with clinically silent prion replication in the lymphoreticular tissue, whereas neuroinvasion takes place later.[75] The *M* cells in the intestinal epithelium mediate prion entry from the gastrointestinal lumen into the body[76] and follicular dendritic cells (FDCs) are thought to be essential for prion replication and for accumulation of disease-associated PrP^{Sc} within secondary lymphoid organs. Inhibition of the lymphotoxin (LTß) signaling pathway with a soluble receptor that depletes FDCs abolishes prion replication in spleens and prolongs the latency of scrapie after intraperitoneal challenge.[77] B cell-deficient mice are resistant to intraperitoneal inoculation with prions, [78] possibly because of impaired FDC maturation. [77] [79] Opsonization by complement system components may also be important in peripheral neuroinvasion as mice genetically engineered to lack complement factors[80] or mice deleted of the C3 complement component by the administration of cobra venom [81] are resistant to peripheral prion inoculation.

PREVENTION

Because there are currently no treatments for these invariably fatal diseases, prevention is particularly important. Perhaps the most effective example of prevention was the cessation of cannibalistic practices among the Fore people of Papua New Guinea in the 1950s, which resulted in the disappearance of kuru. The replacement of growth hormone derived from the pituitary glands of human cadavers with recombinant growth hormone was implemented to avoid the continued iatrogenic transmission of CJD to young children who have growth hormone deficiency. Similarly, because CJD has resulted from the use of prion-contaminated surgical instruments or apparatus after neurosurgical or ophthalmic procedures, it is advised that surgical instruments be incinerated in cases where CJD is confirmed so as to avoid future iatrogenic transmission of prion disease. Current policy in the UK is to quarantine surgical instruments until a suspected diagnosis is confirmed, and instruments used on a confirmed case are destroyed. Recent experimental studies have confirmed that prions adhere readily to metal following a contact time with infected brain of as little as 5 minutes.[82]

When it was realized that BSE was caused by feeding prion-contaminated foodstuffs to cattle, a number of preventive measures were introduced in the UK. In July 1988 a ban on feeding ruminant-derived protein to other ruminants was introduced to break the cycle of infection via feed. Because the available evidence indicates that vCJD has resulted from human exposure to bovine prions via the food chain, the BSE epidemic prompted concerns over the safety of prion-contaminated foodstuffs. A ban on specified bovine offals was introduced in the UK in 1989 to prevent inclusion in the human food chain of bovine tissues thought to contain the highest titer of prions; these included tissues from the lymphoreticular system and the central nervous system. The European Union imposed a worldwide ban on the export of British cattle, products derived from them (with the exception of products for technical uses) and mammalian meat and bone meal in March 1996 after the announcement that BSE and vCJD might be linked. Since then, more than 1.35 million cattle over 30 months old have been culled in the UK in a further attempt to limit human exposure to BSE. The 'over thirty month' (OTM) rule is one of the UK BSE controls used to prevent further BSE-infected cattle from entering the human food chain because cattle over 30 months are more likely to develop BSE than younger animals. Therefore, since 1996 there has been a ban on selling meat in the UK from slaughtered cattle over 30 months old. The cost of tackling BSE to the British and European taxpayer has been estimated at over £3500 million (approximately $US5600 million). These measures appear to have been effective in reducing the incidence of BSE in the UK and the number of newly identified BSE cases is in sharp decline.[9] The EU-imposed worldwide ban on British beef exports was lifted in late 1999 after the EU was satisfied that appropriate measures had been taken to counteract the likelihood of BSE-infected animals getting into the human food chain.

Although there is no current evidence that vCJD can be transmitted via blood or blood products, the long incubation period of prion diseases and the possibility of increased numbers of future cases of vCJD as a result of exposure to BSE have raised the issue of blood as a possible vehicle for iatrogenic disease. There have been no reported cases of prion transmission in cases where humans have

been transfused with blood from a patient who has gone on to develop either classic CJD or vCJD. During the past 20 years more than 20 patients have received blood components from donors who have later developed vCJD.[83] Although to date there is no evidence of disease transmission, incubation periods are likely to be prolonged and these recipients continue to be carefully monitored.

Many animal experiments have addressed the question of whether blood, buffy coat, plasma or blood extracts from diseased animals or animals incubating the disease can transmit the disease to healthy animals via the intracerebral, intravenous or intraperitoneal route and they have recently been reviewed.[83] As a protective measure against the theoretical risk of iatrogenic transmission of vCJD via blood transfusions, a number of policies have been implemented in the UK. The UK government decided in 1998 that all blood donations should be leukodepleted. Since then the majority of European countries have followed this strategy. The UK National Blood Transfusion Service now imports all plasma and plasma derivatives from BSE-free countries and blood donors are screened to exclude anyone with a blood relative with classic CJD or vCJD. Several countries have instituted policies of deferral of blood donors who have resided in the UK for a cumulative period of 6 months or more from 1980 until the end of 1996. In view of the potential exposure to the vCJD agent in other European countries in addition to the UK, the US Food and Drug Administration has a blood donation deferral policy for cumulative 10-year residence in France, Portugal and Ireland. The American Red Cross Blood Banks have adopted a deferral policy of 3 months' residence in the UK and 6 months' residence in any other European country. The efficacy of these risk reduction procedures is not known and a screening test for blood infectivity is urgently needed.

DEVELOPMENT OF THERAPIES

Prion diseases are invariably fatal and whilst curative therapies for prion infection are conceivable, such therapies, if developed, will not be available for some years.[74] Such approaches may involve targeting PrP itself.[74] However, the development of neuroprotective agents and pre- and postexposure prophylaxis is also important. In addition, early firm diagnosis will be crucial to allow such treatments to be initiated before extensive brain damage occurs.

A number of compounds have been shown to be effective at clearing PrP^{Sc} in cell culture systems.[84] These include the acridine and phenothiazine derivatives quinacrine and chlorpromazine, Congored, sulfated polyanions and anti-PrP antibodies (reviewed in[84]). A few compounds have been shown to prolong survival in animal models after intraperitoneal inoculation with prions. These include pentosan polysulfate, cyclic tetrapyrroles[85] and CpG oligodeoxynucleotides.[86] Recently vaccination with recombinant mouse prion protein, before and after intraperitoneal inoculation of prions in mice, demonstrated delayed disease onset in both groups but this was more prolonged in animals immunized before prion exposure.[87] Only amphotericin B and dapsone prolonged life when animals were prion inoculated by the more challenging intracerebral route, in which neuroinvasion is directly initiated. However, amphotericin B was not effective in prolonging survival in a CJD patient and the results with dapsone were not substantiated in another rodent model of prion disease. Currently clinical trials using quinacrine and chlorpromazine treatment in CJD and vCJD patients are under way in both the UK and USA, but results are not yet published. However, there is no evidence that these drugs are useful against prion disease *in vivo* and recently quinacrine treatment in a rodent model of CJD demonstrated no efficacy.[88]

Currently a huge international research effort is attempting to develop therapies aimed at both pre- and postexposure prophylaxis, in addition to neuroprotective agents that may slow disease progression.

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Chapter 27 - Postinfectious and Vaccine-related Encephalitis

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INTRODUCTION

Infections or immunizations can cause disease by aberrant host responses directed against brain, spinal cord or peripheral nerves. Pathogenesis is divided by anatomic differences in myelin into diseases of the central nervous system (CNS) and peripheral nervous system (PNS). The CNS syndromes discussed below are encephalomyelitis, transverse myelitis, cerebellar ataxia, optic neuritis, Sydenham's chorea, encephalopathy and Reye syndrome. The PNS syndromes are Guillain-Barré syndrome (GBS), brachial neuritis and cranial neuropathies.

ENCEPHALOMYELITIS, TRANSVERSE MYELITIS AND RELATED CONDITIONS

EPIDEMIOLOGY

The most important of the various postinfectious CNS syndromes are:

¦ acute disseminated encephalomyelitis (ADEM), an inflammatory demyelinating disease, probably autoimmune in nature, that characteristically follows a monophasic course.[1] Acute hemorrhagic leukoencephalitis is a hyperacute necrotizing form of ADEM;
¦ postinfectious encephalomyelitis (PIE), a subset of ADEM affecting brain and spinal cord after an infection;
¦ postvaccinal encephalitis (PVE) follows various immunizations;
¦ acute cerebellar ataxia, characterized by predominant cerebellar dysfunction;
¦ acute transverse myelitis, a distinctive syndrome affecting the spinal cord; and
¦ optic neuritis, inflammation of the ophthalmic nerve, which can occur in isolation or with multifocal CNS involvement.

Although postinfectious CNS syndromes are differentiated by their predominant anatomic involvement, they probably represent similar pathologic mechanisms; overlapping syndromes and variants may occur. Encephalitis associated with antecedent bacterial infections displays a predilection for basal ganglia, producing Sydenham's chorea or stereotypic behaviors.

The incidence of ADEM varies by country as a function of endemic diseases, intercurrent epidemics and use of international or locally developed vaccines. Seasonality, reported in some case series, might reflect underlying epidemics that trigger these rare diseases. With the introduction of vaccines against common childhood diseases, the proportion of PIE decreased from 33% to 15% of reported acute encephalitis cases in the USA and Europe.[2] The incidence of PIE in the USA is approximately 1/100,000 population. The incidence after immunization is generally much lower than after natural infection (Table 27.1). Annual incidence of acute transverse myelitis in the USA is 0.8/100,000 population.[3] The incidence of Sydenham's chorea in the USA and Europe is approximately 0.1/100,000 population. There are no population-based estimates for optic neuritis or acute cerebellar ataxia.

The association of optic neuritis with subsequent development of multiple sclerosis is well established. The autoimmune, demyelinating but multiphasic phenotype of multiple sclerosis is an invariable contrast to these monophasic postinfectious syndromes. The prevalence of multiple sclerosis is 60/100,000 population. For comparison with ADEM, adrenoleukodystrophy, a rare genetic demyelinating syndrome, occurs with a prevalence of 2/50,000 males.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of postinfectious syndromes of the CNS has been best delineated following measles infection and immunization against rabies.[4] [5] The pathology of encephalomyelitis following measles infection is perivenular mononuclear inflammation, edema and demyelination, with relative sparing of axons. Lipid-laden macrophages are present in areas of demyelination. Almost identical pathology is seen in PVE and experimental models of allergic encephalomyelitis. The pathology of acute cerebellar ataxia, which is benign and self-limited, is rarely described; the pathology of optic neuritis and acute transverse myelitis is similar to that of PIE. The pattern of demyelination observed in postinfectious syndromes is distinct from the demyelination seen in progressive multifocal leukoencephalopathy due to papovavirus infection, human T-cell leukemia/lymphoma virus (HTLV)-1 infection or multiple sclerosis.[6] Repeated attempts to recover infectious agents from brain tissue (culture, viral antigen or nucleic acid) have been mostly unsuccessful. Intrathecal production of interferons or antibodies, which are indicators of CNS infection, are frequently absent.[1] [4]

Patients who have major neurologic complications after rabies vaccine or measles infection have elevated levels of antibody reactive to brain white matter or myelin basic protein (MBP), as well as increased lymphoproliferative responses to MBP.[4] [5] The animal model of experimental allergic encephalomyelitis, using repeated immunization with brain tissue, induces inflammatory demyelinating lesions in the CNS similar to those in PIE or PVE. The pathogenesis is by cell-mediated attack on CNS myelin. The incidence of PIE is low relative to the prevalence of associated infectious agents (Table 27.2). Genetic factors predisposing to autoimmunity or enhanced CNS inflammation are important in experimental allergic encephalomyelitis and may determine which individuals develop ADEM.[7]

The pathogenesis of CNS autoimmunity following bacterial infections is less clear. Mycoplasmas are associated with PIE; mycoplasmal antigens cross-react with brain tissue.[8] Limited pathologic descriptions in Sydenham's chorea indicate neuronopathy rather than demyelination. Antibodies of IgG subclass reactive against subthalamic and caudate nuclei are detected more frequently in patients who have acute rheumatic fever or Sydenham's chorea than in controls. Children who have tics or obsessive-compulsive disorder with attention deficit-hyperactivity disorder can have antibodies to caudate and putamen.[9] The role of cell-mediated immunity has not been defined.

TABLE 27-1 -- Incidence of postinfectious and postvaccinal encephalomyelitis.

INCIDENCE OF POSTINFECTIOUS AND POSTVACCINAL ENCEPHALOMYELITIS		
Disease	**Disease-associated**	**Vaccine-associated**
Smallpox	1/2000	1/20,000
Rabies	Fatal disease	Semple vaccine 1/400
		Suckling mouse vaccine 1/7500
		Duck embryo vaccine 1/50,000
		Human diploid vaccine: none
Measles	1/1000	1.2/million
Rubella	1/6000	<1/million
Mumps	1/6000	<1/million
Pertussis	1/125	1/140,000
Varicella	1/4000	<1/100,000

TABLE 27-2 -- Infections associated with postinfectious central nervous system syndromes.

INFECTIONS ASSOCIATED WITH POSTINFECTIOUS CNS SYNDROMES
Non-specific upper respiratory infections
Non-specific gastrointestinal infections
Measles
Mumps
Rubella
Varicella-zoster virus

Epstein-Barr virus
Herpes simplex virus
Influenza
Smallpox (variola)
Mycoplasma pneumoniae
Streptococcus pyogenes
Campylobacter jejuni
Vaccines
Smallpox (vaccinia)
Rabies
Measles
Oral poliovirus
Diphtheria-tetanus (DT and Td) and tetanus toxoid vaccines
Haemophilus influenzae type b
Plasma-derived hepatitis B
Inactivated *Vibrio cholerae*
Japanese B encephalitis

Common infections are frequently associated with postinfectious ADEM (see Table 27.2). Acute cerebellar ataxia is especially common after chickenpox, occurring in 35% of cases of ADEM associated with varicella-zoster virus (VZV).[10] Abnormalities of the CNS and PNS are common in children and adults who have HIV and AIDS. Involvement of the CNS in primary HIV infection includes periventricular demyelination resembling ADEM. Other retroviruses, such as HTLV-1, have also been linked to tropical spastic paraparesis and myelopathy, both chronic and progressive demyelinating neuropathies. The overall risk of tropical spastic paraparesis/myelopathy after HTLV-1 infection is 25/100,000 in endemic areas.

PREVENTION

The incidence of PIE has decreased over the past 30 years, probably because of successful immunization against many viral diseases as well as development of more purified vaccines (see Table 27.1). Although vaccines can trigger ADEM, the probability of this event is much higher after wild-type virus infection. Although vaccinia virus vaccines are no longer in use, Semple rabies vaccines are still produced in some countries. A change to human diploid cell rabies vaccines virtually eliminates the risk of PVE. New vaccine technology, including recombinant proteins and DNA vaccines, should further reduce the incidence of PVE.

CLINICAL FEATURES

The age-specific incidences of infectious encephalitis and PIE are distinct. The incidence of encephalitis is highest in infancy (22.5/100,000), although PIE is rare in children under 2 years of age.[11] The age distribution of PIE and PVE generally coincides with the epidemiology of associated pathogens or vaccines. Most patients who have acute cerebellar ataxia are 1–6 years of age; a viral prodrome is present in 64%.[10] The incidence of acute transverse myelitis has distinct peaks in adolescence, middle age and the elderly; a viral prodrome is present in 30%.[12] The incidence of optic neuritis is highest in young to middle-aged adults; a viral prodrome is present in 50%.[13] Unlike most postinfectious CNS syndromes, the sex-specific incidence of optic neuritis is unequal, favoring females with a ratio of 2:1. Sydenham's chorea affects school-aged children; a similar age distribution is reported for the onset of childhood tics and obsessive-compulsive disorders.[9]

The onset of PIE is usually abrupt, occurring 5–14 days after infection. The illness begins with a recurrence of fever and a depressed level of consciousness. Neurologic manifestations are classically multifocal. Signs range from lethargy and irritability to convulsions (50%), involuntary movements (18%), ataxia (10%), hemiplegia (12%), visual disturbances and cranial nerve deficits. Recovery can begin within days but complete resolution occurs over weeks or months. The mortality ranges from 5% to 20%, with highly variable morbidity. A poor prognosis is associated with coma, focal neurologic deficits and extreme or persistent fever.

Postvaccinal encephalitis

This is best characterized for Semple rabies vaccines.[5] Prodromal fever, headache and myalgia develop 6–14 days after the first immunization. Within 1–4 weeks, patients develop neurologic signs of lethargy (50%), meningisms (33%), focal neurologic deficits (50%) and sphincter disturbances (33%). The duration of illness is less than 2 weeks and 80% of patients recover completely; the associated mortality is 15%.

Distinguishing between infectious encephalomyelitis and PIE can be challenging, especially when they are caused by the same infectious agent.[8] [14] The differential diagnosis of PIE and PVE includes infectious encephalitis, progressive multifocal leukoencephalitis (papovavirus), multiple sclerosis, vasculitis, cerebral emboli, cerebral vein thrombosis, chronic meningitis, sarcoidosis, intracranial hemorrhage, malignancy, mitochondrial disorders (mitochondrial encephalomyopathy-lactic acidosis-stroke-like episodes syndrome) and metabolic disorders (adrenoleukodystrophy).

The risk of PVE has recently been highlighted by the reemergence of the need to provide smallpox immunization to counteract the threat of bioterrorism. Postvaccinal encephalitis is the most serious complication of smallpox immunization, with an estimated incidence of approximately 1/100,000 and a case-fatality of 50%. [15] This problem, plus the increased risk of disseminated vaccinia in immunocompromised patients (especially those who have HIV), greatly complicate the public policy considerations of mass smallpox immunization.

Acute cerebellar ataxia

This develops suddenly with vomiting, inco-ordination, truncal ataxia and dysarthria. Refusal to walk and mutism are common presenting signs in children.[10] Cerebellitis is a frequent component of general encephalomyelitis and can be complicated by acute hydrocephalus. Acute cerebellar ataxia must be distinguished from drug ingestion, GBS, benign paroxysmal vertigo associated with migraine, peroxisomal disorders, metabolic diseases, inherited ataxias, Wernicke's encephalopathy, paraneoplastic syndromes (myoclonus-opsoclonus syndrome) and posterior fossa tumors.

Acute transverse myelitis

This is an acute syndrome mimicking transection of the spinal cord. Prodromal symptoms include fever, rash and pain in the legs, interscapular region or back. Peak neurologic dysfunction is reached within 2 hours in 15% of the patients, and within 24 hours in 50%, but progression up to 14 days has been reported. Neurologic deficit is commonly localized to the thoracic region but 20% of patients are affected in the cervical or lumbar area. Muscular weakness is accompanied by decreased or absent deep tendon reflexes. Some degree of asymmetry can be observed during early evolution of the disease; significant asymmetry suggests other diagnoses. Loss of sensation to pain and temperature below a clear demarcated level is universal; loss of perception of touch and proprioception are common. Sphincter disturbances and dysautonomia are usually described. Recovery to independent ambulation occurs in 50% of pediatric and 35% of adult cases. Improvement may continue beyond 6 months. Pulmonary emboli, urinary tract infections and decubitus ulcers are common complications.

Transverse myelitis must be differentiated from multiple sclerosis, acute epidural abscesses, hematomas or arteriovenous malformations, tumors, anterior spinal artery syndrome associated with lupus, syphilis or schistosomiasis, dissecting aneurysm and tropical paraparesis (HTLV-1), as well as infections by VZV, cytomegalovirus (CMV) and toxoplasmosis in immunocompromised hosts.

Optic neuritis

This is heralded by pain above or behind the eye aggravated by movement, loss of color discrimination and progressive central visual

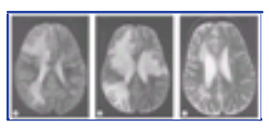
Figure 27-1 Postinfectious encephalomyelitis. (a, b) MRI of brain at presentation showing asymmetric demyelination of cortical white matter. Basal ganglia were also involved bilaterally (not shown). (b) At 7 weeks, the distribution is more symmetric. (c) Residual lesions at 4 months, with full clinical recovery.

impairment. Total blindness of the eye may follow within a few hours to days. Single eye involvement is present in 90% of adult cases; bilateral neuritis occurs in up to 40% of pediatric cases. Most patients recover within 4 weeks. The reported incidence of multiple sclerosis following optic neuritis varies widely (5–57%) but is less in children.[13]

The differential diagnosis of optic neuritis includes multiple sclerosis, hereditary retinal diseases, vasculitis (giant cell arteritis, lupus), intoxications, parasellar tumors, granulomatous meningitis, neuroborreliosis, syphilis and infection with *Bartonella henselae*.

Sydenham's chorea

Chorea is a smooth rapid movement that flows from joint to joint. Sydenham's chorea, which is associated with 15% of cases of rheumatic fever, can include dyskinesias such as tics, athetosis (worm-like movements) and ballismus (violent flinging movements). The prodrome includes emotional lability, obsessive-compulsive symptomatology and hyperactivity. The onset is insidious, with a latency of from 2 weeks to several months after streptococcal pharyngitis. Recovery varies, taking from several weeks to more than 6 months. Rheumatic carditis recurs in 20% of patients who have chorea.

The differential diagnosis of basal ganglia lesions includes metabolic disease (Wilson's disease, organic acidemias), cerebral palsy, postpump cardiac surgery, adverse drug reactions, cerebral vein thrombosis and lupus erythematosus (lupoid sclerosis).

DIAGNOSIS

It is challenging to differentiate postinfectious syndromes from infectious encephalitis (see Chapter 23). Magnetic resonance imaging (MRI) is more sensitive than computerized tomography (CT); CT scans are frequently normal;[16] MRI findings are large white matter lesions with initially asymmetric distribution and variable enhancement with contrast (Fig. 27.1). There is limited correlation between clinical signs and neuroradiologic imaging. White matter lesions may take up to 18 months to resolve, and new lesions may appear despite clinical improvement.[17]

Cerebrospinal fluid (CSF) findings are not specific. Elevated intracranial pressure is uncommon and the CSF profile is often

normal. Pleocytosis, if present, is mononuclear and rarely exceeds 200 cells/µl. With extreme inflammation (hemorrhagic leukoencephalitis), neutrophils and erythrocytes are seen. A mild elevation of CSF protein is common. The presence of MBP in the CSF is indicative of oligodendroglial damage and demyelination; the sensitivity is 60%.

Magnetic resonance imaging is recommended for acute cerebellar ataxia or optic neuritis to delineate multifocal CNS disease and to exclude vascular and space-occupying lesions. For acute transverse myelitis, exclusion of treatable causes of cord compression is essential. Computerized tomography scan or MRI of the spine has replaced myelography in the diagnostic approach; myelograms are usually normal in acute transverse myelitis. Despite clinical 'transection', MRI shows cord inflammation extending along many segments. Cerebrospinal fluid analyses are similar to those in PIE. Testing for syphilis, schistosomiasis, HIV and HTLV-1 (often coincident) is important in patients from endemic areas who have unexplained myelopathy. Polymerase chain reaction (PCR) best detects CMV, VZV and toxoplasmosis in the CSF of immunocompromised hosts.

Culture of CSF is indicated, but rarely informative in cases of ADEM. Results of genetic amplification assays such as PCR can be useful when interpreted properly. DNA or RNA derived from infectious agents can persist for weeks and should not necessarily be construed as evidence of active infection; it can identify antecedent infection.[18] [19] Genetic material can mislead when detected as false positives or 'innocent bystanders' in areas of inflammation.[20] Cerebrospinal fluid antibody titers are of limited use. An absence of CSF antibodies is consistent with PIE but CSF antibodies may be long-lived and unrelated to the acute process.[4] [21] Simultaneous seroconversions to several pathogens are common, and antibodies to *Mycoplasma pneumoniae* are cross-reactive with brain tissue.[8]

MANAGEMENT

There is no proven treatment for PIE. It is difficult to distinguish postinfectious myelitis from neurosurgical emergencies; early use of CT or MRI is indicated. Lumbar puncture should include pressure measurement. Antiviral therapy is not indicated but initial exclusion of treatable infectious encephalitis can be difficult and empiric therapy is often given. Immunoglobulin and corticosteroid therapies have no demonstrated benefit in ADEM, although corticosteroids have been used to treat swelling of the cord.[22]

Management of seizures with anticonvulsants and administration of antipyretics is recommended; autonomic instability should be treated with short-acting drugs. Protection of the airway and control of intracranial pressure are often necessary. Management of acute transverse myelitis requires meticulous care to avoid complications during prolonged recovery. Pneumonia, sepsis, renal failure and autonomic disturbances, including arrhythmias, are acute life-threatening complications. Urinary tract infection is very common; intermittent catheterization is preferable to use of an indwelling catheter to minimize this risk. Good nursing and, in some cases, anti-coagulation should minimize the risk of pulmonary embolism. Psychologic support is necessary for both the patient and his/her family. Corticosteroids are sometimes given in high doses for optic neuritis to obtain a rapid improvement but have no significant effect on long-term outcome.[23]

ENCEPHALOPATHY AND REYE SYNDROME

EPIDEMIOLOGY

The term encephalopathy is used when CNS dysfunction is not associated with histologic or indirect evidence (CSF pleocytosis, MRI findings) of inflammation. Encephalopathy following infection or immunization does not appear to be a single syndrome. The Vaccine Safety Committee of the Institute of Medicine, Washington, DC, has modified case definitions used in the National Childhood Encephalopathy Study (NCES; Table 27.3). Reye syndrome is an encephalopathy associated with liver dysfunction.

The incidence of postinfectious and postvaccinal encephalopathy varies by country, reflecting national immunization practices and sanitation. The incidence of postvaccine encephalopathy is less than 10.5/million immunizations. [24] Age-specific incidences of vaccines and postvaccinal encephalopathy overlap the ages of onset of many childhood neurologic conditions. [25] [26] The incidence of encephalopathies associated with typhoid and other enteric pathogens is highest during the summer or the rainy season. The seasonality of Reye syndrome corresponds to antecedent varicella or influenza infections. The incidence of Reye syndrome was 0.31/100,000 individuals under 18 years of age in 1980; milder forms of the encephalopathy occur in 5.6/100,000 children.[27] Reye syndrome associated with salicylate use has become less common since the educational campaigns discouraging aspirin use in children.[28]

Whole-cell pertussis (wP) is the vaccine most frequently associated with encephalopathy. Controversy over the neurologic complications of the diphtheria, tetanus and wP (DTwP) vaccine reduced the immunization rate in the UK to 31% in 1977; this in turn gave rise to the largest epidemic of pertussis observed in 20 years. The NCES, a case-control study of the association of DTwP immunization with serious neurologic diseases, was undertaken from 1976 to 1979. Estimated attributable risk for acute encephalopathy, atypical seizures, infantile spasms and Reye syndrome was 1/110,000 doses.[25] A 10-year follow-up study confirmed that all serious acute neurologic events, irrespective of association with DTwP, might be associated with important neurologic sequelae.[26] Evidence is consistent

TABLE 27-3 -- Definition of encephalopathy.*

DEFINITION OF ENCEPHALOPATHY
Acute encephalopathy
Children <24 months:
Significantly decreased level of consciousness (stupor or coma) lasting for at least 24 hours, not attributable to postical state or medication
Children = months:
Condition lasting for at least 24 hours, characterized by two of following:
Confusional state or a delirium, or a psychosis, not medication related
Significantly decreased level of consciousness (stupor or coma), independent of seizure and not attributed to medication
Seizure associated with loss of consciousness
Increased intracranial pressure is consistent with the diagnosis at any age
Excluded conditions:
Sleepiness
Persistent inconsolable crying
Bulging fontanelle
Seizures
Chronic encephalopathy
Persistence of acute findings over several months to years beyond the acute episode
Excluded conditions:
Return to normal, followed by chronic encephalopathy
Chronic encephalopathy secondary to genetic, prenatal or perinatal factors

** Modified from Vaccine Safety Committee, Institute of Medicine.[3]*

with, but does not establish, a causal relationship of DTwP vaccine with encephalopathy; there were no associations of DTwP with infantile spasms or Reye syndrome. Acellular pertussis vaccines will change the incidence of this syndrome.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of postinfectious or postvaccinal encephalopathy is not known. Latency between infection or vaccination and encephalopathy is highly variable, ranging from hours up to 6 weeks. Autopsy series of postinfectious encephalitis include cases of encephalopathy; findings demonstrate cerebral edema without inflammation. Hypoxic insult, electrolyte disorders and endogenous toxins are often invoked to explain this pathology.[29] [30] Some cases of postvaccinal encephalopathy may result from unmasking or precipitation of an underlying neurologic condition by the vaccine.[24] Postinfectious encephalopathy is associated with non-specific respiratory and gastrointestinal tract infections, measles, pertussis and typhoid infections.[31] [32] Whole-cell pertussis, DT/Td/tetanus toxoid, and measles, mumps and vaccinia vaccines have been associated with encephalopathy. [24]

Liver pathology in Reye syndrome is pathognomonic. Panlobular accumulation of small lipid droplets occurs without evidence of cholestasis or inflammation. By electron microscopy, the mitochondria are large and irregular, with diminished matrix granules. There may be a proliferation of peroxisomes and smooth endoplasmic reticulum, and depletion of glycogen. The pathogenesis is not known. Underlying metabolic disorders cause some cases of Reye syndrome.[33]

PREVENTION

Repeat immunization with the specific vaccine is contraindicated in instances of postvaccinal encephalopathy not due to another identifiable cause. Avoidance of aspirin use for common fevers and for analgesia in children effectively minimizes the risk of Reye syndrome. Reye syndrome following the administration of live varicella vaccine to patients on chronic salicylate therapy has not been described. The decision of whether to give this vaccine to such patients must be individualized.

CLINICAL FEATURES

Postinfectious encephalopathy is a disease of early childhood, occurring at a mean age of 18 months. In contrast, PIE is rare in children under 2 years of age. Encephalopathy after pertussis infection occurs mostly during the first 6 months of life.[30] In contrast to postinfectious and postvaccinal encephalopathy, Reye syndrome affects school-aged children and adolescents.

Encephalopathy is indistinguishable clinically from encephalitis. [11] [29] Fever and seizures are common but the presence of focal neurologic deficits is unusual. No specific neurologic syndrome has been described after DTwP vaccination. The onset of encephalopathy after wP vaccination typically occurs in the first 3 days and is rare after 7 days, although encephalopathy after pertussis infection occurs in the second or third week. Reye syndrome is heralded by severe repetitive vomiting refractory to common interventions, followed by altered consciousness.

The differential diagnosis of encephalopathy is similar to that for PIE. In infants, shaken baby syndrome, cerebral vein thrombosis and hyperpyrexic shock with encephalopathy must be considered. In many countries, shigellosis, acute typhoid fever and malaria cause diagnostic confusion.

Encephalopathy is frequently complicated by aspiration pneumonia. Intracranial hypertension, inappropriate antidiuretic hormone secretion, electrolyte disorders and hypoxic-ischemic injury may exacerbate it and produce secondary brain damage. Cerebral edema can be severe and lead to cerebral herniation. Children suffering severe neurologic syndromes temporally associated with wP vaccine administration carry a 5-fold relative risk for chronic neurologic disease over asymptomatic vaccine recipients. [26]

DIAGNOSIS

There is no diagnostic test for postinfectious encephalopathy. The CSF is frequently under increased pressure but is otherwise normal by routine analysis. In Reye syndrome there must be evidence of liver disease consisting of elevated liver transaminases or elevated plasma ammonia. The blood glucose concentration is frequently low; bilirubin is normal.

A diagnosis of postvaccinal encephalopathy is by temporal association; proof of causation is impossible in individual cases.

MANAGEMENT

Therapy is supportive. Electrolyte or metabolic disorders and intoxications are excluded by history and laboratory analysis. Magnetic resonance imaging may reveal encephalomyelitis, cerebral edema or other pathology. Plasma and urine samples for metabolic analysis should be obtained early. Intravenous glucose is important for brain metabolism and reversal of gluconeogenesis and hyperammonemia. Complications of encephalopathy include aspiration and cerebral herniation. Intubation and mechanical ventilation are commonly indicated.

GUILLAIN-BARRÉ SYNDROME

EPIDEMIOLOGY

Guillain-Barré syndrome is an acute, ascending, symmetric paralytic disorder diagnosed by consensus clinical criteria. Acute inflammatory demyelinating polyneuropathy (AIDP) is the classic form of GBS. Variants include:

- acute motor axonal neuropathy (AMAN; Chinese paralysis syndrome);
- acute motor sensory axonal neuropathy (AMSAN);
- hyperacute GBS; and
- Miller-Fisher syndrome.

Other forms may exist.

Guillain-Barré syndrome has replaced poliomyelitis as the most common cause of acute flaccid paralysis worldwide. In the temperate Americas, Europe and Australia, GBS occurs without seasonality.[33] [34] In China, the middle latitudes of the Americas, and possibly the Indian subcontinent, the number of cases increases during summer months.[35] Geographic or familial clustering is rare with either form. Guillain-Barré syndrome and axonal variants are coincident and the incidence of GBS is estimated to be 0.4–1.5/100,000 population in temperate climates.

Guillain-Barré syndrome is associated with most antigenic triggers of PIE, including minor respiratory infections in 50% of cases. *Campylobacter jejuni* infections are associated with 26–40% of cases in industrialized nations and in up to 90% of cases in China.[35] Fewer than 5% of cases are vaccine-associated.[36] Guillain-Barré syndrome has been reported with primary HIV infection and as part of the immune reconstitution syndrome during therapy.[37]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of GBS is heterogeneous.[38] [39] Peripheral nerve myelin is distinct from CNS myelin and mechanisms of demyelination

in the CNS and PNS may differ. Major proteins in peripheral myelin include P_0 , MBP and P_2 . The G_{M1} ganglioside is enriched in paranodal regions of both nerve and myelin, although the G_{Q1b} ganglioside is similarly enriched in oculomotor nerves.

Classic GBS (AIDP) is an acute monophasic demyelinating syndrome of peripheral nerves.[34] It is multifocal, with a predilection for nerve roots and internodes. Demyelination is associated with deposits of antibody and complement as well as macrophage 'stripping' of outer myelin lamellae. Inflammation at the nerve roots is believed to lead to disruption of the CNS blood-brain barrier, with leakage of serum proteins into the CSF. In AIDP, axonal loss is correlated with severe inflammation.

Experimental allergic neuritis, caused by repeated immunization with peripheral nervous tissue, is clinically, electrophysiologically and pathologically similar to GBS. Immunologic mechanisms may differ between experimental allergic neuritis and GBS. Passive transfer of lymphocytes sensitized to P_0 or P_2 myelin proteins reproduces experimental allergic neuritis; transfer of serum does not. In contrast, human T cell activation against myelin proteins is variable, although sera from GBS patients causes nerve demyelination in tissue culture. Antibodies against G_{M1} and related sialylated gangliosides are detected in up to 60% of patients with *C. jejuni*-associated GBS. Strains of *C. jejuni* contain membrane glycolipids with identical glycoconjugate structures to those of peripheral nerve gangliosides. This is antigenic identity rather than mimicry. Anti-ganglioside G_{Q1b} antibodies against the neuromuscular junction are described in 90% of cases of Miller-Fisher syndrome, a GBS variant with ophthalmoplegia.

Acute motor sensory axonal neuropathy and AMAN variants, although clinically similar to demyelinating GBS (AIDP), are characterized by axonal degeneration with or without sensory nerve involvement, respectively.[38] [39] Both syndromes are frequently associated with *C. jejuni*. Axonal degeneration correlates with macrophage infiltration of internodal and periaxonal spaces.[38] A chicken model of *C. jejuni*-associated AMAN has been reported.

Infectious and other antigenic triggers associated with GBS are common (Table 27.4). *Campylobacter* can induce several autoimmune conditions; reactive arthritis and GBS do not cosegregate. Specific matches of bacterial serotypes (Penner serotypes O:19 in Japan and O:41 in South Africa) and host genotype may be required; these may be specific for each geographic region.[40] [41]

TABLE 27-4 -- Conditions associated with Guillain-Barré syndrome.

CONDITIONS ASSOCIATED WITH GBS
Non-specific respiratory tract symptoms
Campylobacter jejuni
Mycoplasma pneumoniae
Cytomegalovirus
Epstein-Barr virus
HIV-1
Japanese encephalitis virus
Hodgkin's disease
Lymphoma
Systemic lupus erythematosus
Surgery
Parturition
Vaccines
Vaccinia
Rabies
Influenza A/New Jersey/76
Oral poliovirus in Finland

PREVENTION

Guillain-Barré syndrome associated with *C. jejuni* infections is prevented by improvements in local sanitation, water and food supplies. Control of common diseases

such as CMV, HIV, *Mycoplasma* infection and Japanese encephalitis should reduce the incidence of GBS and other postinfectious syndromes. Replacement of whole-cell or tissue-derived vaccines with purified component vaccines may also diminish the incidence of GBS.

CLINICAL FEATURES

The annual incidence of GBS in the USA increases with age, from 0.8/100,000 in individuals younger than 18 years to 3.25/100,000 in those over 60. [37] There is an overall male to female preponderance of 1.5:1 for GBS occurring in later life.[35] Acute motor axonal neuropathy in China affects children and young adults, with a mean age of 19 years.[36]

Guillain-Barré syndrome is an acute afebrile paresis that progresses over a few days to weeks. Diagnosis is by consensus criteria; there is no specific laboratory diagnosis (Table 27.5).[42] Paresthesias in toes or fingertips variably precede motor findings. Weakness is generally more profound in the legs but may predominantly affect the arms or cranial nerves in up to 10% of cases each. Weakness begins distally and progresses centrally; progression may be quite rapid. As GBS evolves, general symmetry of paresis is the rule. Dysautonomia may be prominent but bowel or bladder incontinence is rare. Pain is common, involving the large muscles of the legs and back.

Examination shows relatively symmetric motor weakness, absent or greatly diminished tendon reflexes and minimal loss of sensation despite sensory complaints. In severe cases, respiration, airway control and autonomic function are affected.

Miller-Fisher syndrome comprises ophthalmoplegia, ataxia and areflexia, with little weakness. Axonal neuropathy (AMAN or AMSAN) is clinically indistinguishable from demyelinating disease (AIDP). Curiously, axonal involvement is important for the prognosis of the adult but not the pediatric form of AMAN.[36] [43]

Phases of GBS include:

- progression (2–4 weeks);
- plateau (2–4 weeks); and
- recovery (weeks to months).

Respiratory failure develops in 20% of children and 40% of adults; pneumonia occurs in 25% of patients and urinary tract infections in 40%. Mean duration of intubation is 15–30 days with current therapy. Guillain-Barré syndrome is complicated by lability of blood pressure, cardiac arrhythmias or thrombosis in 20%, 25% and 3% of adults, respectively. Mortality with optimal supportive care and treatment is less than 5%.

A rapid onset of severe paresis, need for mechanical ventilation, increasing age, antibodies to *C. jejuni* and G_{M1} and axonal neuropathy are associated with slow or incomplete recovery.[44] Mean time to ambulation with current therapy is 40–50 days; 15% of cases do not walk at 48 weeks. Most (80%) eventually pursue normal activities but show mild residual effects under careful examination. The outcomes for children and adults are probably similar.

Guillain-Barré syndrome must be distinguished from a variety of diseases of the CNS and PNS. Chronic inflammatory demyelinating polyneuropathy, a relapsing demyelinative disease, and chronic progressive demyelinating polyneuropathy are clinically indistinguishable at onset from GBS.

DIAGNOSIS

Diagnosis requires examination of the CSF and nerve conduction studies (see Table 27.5). Cerebrospinal fluid pressure should be

TABLE 27-5 -- Criteria for diagnosis of Guillain-Barré syndrome.*

CRITERIA FOR DIAGNOSIS OF GBS	
Required	Muscle weakness
	Progressive motor weakness of more than one limb, and/or bulbar and facial paralysis (30%) and/or external ophthalmoplegia (6%)
	Areflexia
Supportive	Clinical
	Progression over 2–4 weeks
	Relative symmetry
	Mild sensory signs
	Cranial nerve involvement (without sphincter involvement; 50%)
	Recovery after interval of 2–4 weeks after nadir
	Autonomic dysfunction (20%)
	Absence of fever at onset
	Cerebrospinal fluid
	CSF protein rising after first week
	CSF leukocytosis less than 10/µl (mononuclear cells)
	Nerve conduction studies, in two or more motor nerves
	Partial conduction block or decreased M responses, or both (75%)
	Nerve conduction velocity <70% of normal, several weeks into illness (60%)
	Abnormalities in late responses (F waves, H reflexes; 46%)
	Features discordant for GBS
	Marked persistent asymmetry of weakness
	Sphincter dysautonomia at onset or persistent
	CSF leukocytosis >50/µl or neutrophils in CSF
	Sharp sensory level
	Systemic illness or constitutional symptoms
Exclusionary	-
Required criteria must be present for diagnosis. Supportive criteria strongly support the diagnosis, but may not be present initially and in all cases. Exclusionary criteria suggest an alternate diagnosis is likely.	

* *Modified from Asbury and Cornblath.* [42]

normal and contain fewer than 10 leukocytes/ml. Cerebrospinal fluid protein may be normal during the first 48 hours of illness and is not reliably elevated until the second week of illness. Nerve conduction studies must include several nerves because demyelination is patchy. Conduction studies may not become abnormal until

several weeks into the illness; up to 20% of patients never have abnormal studies. Conduction velocity is usually less than 60% of normal. F-wave responses, which measure nerve root disease, are a useful indicator of the disease.[42]

Stool cultures for *C. jejuni* are often positive in patients who have GBS, especially those who have axonal neuropathy. Diarrhea is an insensitive (70%) predictor of carriage of *C. jejuni*. Enrichment methods may improve yield from culture.[35] Serology for recent *C. jejuni* infection is of limited use. Cases may be culture-positive for *C. jejuni* but not mount an antibody response; IgM antibodies are cross-reactive with *Salmonella* and *Yersinia* spp. Approximately 50% of cases colonized with *C. jejuni* have serum antibodies against G_{M1} ganglioside. Anti-G_{M1} antibodies of IgM class are present in acute and chronic motor neuropathies, including axonal GBS; IgG class antibodies are more specific for classic GBS (AIDP).[45] Serologic evidence of infections with Epstein-Barr virus (EBV), CMV and *Mycoplasma* are reported in 15%, 8% and 5%, respectively, of GBS cases occurring in childhood and young adulthood.[46] [47] Cold agglutinins are often positive and result from EBV, CMV or *Mycoplasma* infection. Rheumatoid factor may cause false IgM seropositivity.

MANAGEMENT

The management of GBS is a challenge to the clinician because laboratory features are often absent until the second week of illness. It is essential to exclude competing diagnoses requiring emergent management, especially epidural abscess of the spine. Initial evaluation should include CT or MRI of the spine. Lumbar puncture is performed and repeated a week or two later. Nerve conduction studies are necessary for diagnosis and have prognostic significance for the course of recovery. Stool cultures with enrichment for *C. jejuni* provide corroborative data. Analysis for antiganglioside and anti-*Campylobacter* antibodies remains a research tool. Serology for syphilis and HIV are frequently ordered; some experts test for antibodies against EBV, CMV and *Mycoplasma*.

During evolution of the illness, it is imperative to monitor evolving respiratory paralysis by objective measures of vital capacity. In children unable to comply with standard testing, range of serial counting, alphabets or song in a single breath is useful at the bedside. Intensive care is indicated for rapidly declining vital capacities or those below 18ml/kg. Disease is intermittently progressive up to 4 weeks after onset.

Plasma exchange has proved to be superior to supportive treatment alone in GBS. Plasma exchange is more beneficial when started within 7 days of disease onset but is still beneficial up to 30 days after onset.[43] [44] [48] Intravenous immunoglobulin has similar ability to speed the recovery from GBS. Use of corticosteroids is not effective.[34] The natural history of GBS, with spontaneous full recovery in 80% of cases, limits statistical power for detecting differences in therapy. Therapy for Miller-Fisher syndrome has not been well studied; pediatric representation in studies is low. Complications limit therapy in 14% of plasma exchange and 3% of intravenous immunoglobulin therapies. Combination therapy with plasma exchange and intravenous immunoglobulin (IVIG) results in comparable efficacy but additive toxicities.[43] Anecdotal reports suggest better outcome after treatment with IVIG over plasma exchange in cases with both G_{M1} and *C. jejuni* antibodies. [49] Relapses occur after either therapy in 10% of cases.[34] Although therapy with IVIG can be performed at many clinics, most experts agree that GBS requires expert diagnosis and management available at tertiary referral centers. An international support group, GBS Foundation International, is accessible on the internet at http://www.webmast.com/gbs/.

BRACHIAL NEURITIS

EPIDEMIOLOGY AND PATHOGENESIS

Brachial neuritis (brachial plexus neuropathy, neuralgic amyotrophy) is a well-described but poorly understood axonopathy. Annual incidence is approximately 1.6/100,000 individuals.[3] About 15% of cases are associated with immunizations or administration of antiserum; outbreaks have been reported. Administration of tetanus toxoid carries an excess risk of 0.5–1.0/100,000 doses. Latency from immunization to disease is 6–21 days, consistent with an immunologic mechanism.[3]

CLINICAL FEATURES

Brachial neuritis begins with severe aching pain of the shoulder and upper arm. Weakness develops as pain subsides. Motor and

sensory deficits are consistent with lesions in the brachial plexus. There is no correlation of laterality of brachial neuritis and antecedent immunization; the syndrome is bilateral in up to one-third of cases. Recovery, requiring regeneration and collateral innervation of axons, begins within 1 month of onset and requires 2–3 years. Brachial neuritis can be complicated by paresis of the diaphragm.

Brachial neuritis must be distinguished from injection damage to the nerve, poliomyelitis at the site of an antecedent injection, cervical ribs, cord compression and Lyme disease. In the infant, perinatal traction injury, syphilitic pseudoparalysis and occult trauma or osteomyelitis must also be considered.

DIAGNOSIS AND MANAGEMENT

Nerve conduction studies are consistent with axonal neuropathy without a conduction block. Radiologic study of the affected arm and shoulder is often performed to exclude alternate diagnoses. Serologic testing for syphilis and Lyme disease may be indicated. There are no controlled studies on the therapy of this uncommon disorder. Psychologic support is essential during prolonged rehabilitation.

CRANIAL NEUROPATHIES

EPIDEMIOLOGY AND PATHOGENESIS

Peripheral cranial nerves can be affected singly or as a multifocal process following infections or immunizations. Facial palsy is the most common cranial neuropathy, with incidence of 35/100,000 population. Bell's palsy or isolated acute peripheral facial paralysis of unknown etiology accounts for 73% of facial palsies. Similar etiologies for GBS and Bell's palsy were suggested in one study.[37] Although a postinfectious etiology is presumed, recent molecular data implicate reactivation by herpes simplex virus (HSV)-1.[50] Bulbar nerve palsies and ophthalmoplegia occur most commonly in association with GBS (30% and 6% of GBS cases, respectively).

CLINICAL FEATURES

Mean age of onset of facial palsy is 46 years, with equal sex distribution. The onset frequently follows a respiratory tract infection. The paresis is sudden, without systemic illness. Simultaneous, bilateral disease occurs in less than 1% of cases in adults and may be associated with subclinical neuropathy of other cranial or peripheral nerves. Bilateral disease is more frequent in children. Resolution occurs within 6 weeks in 80% of cases. Bulbar nerve palsies present with a weak voice or cry, nasal intonation, nasal reflux and difficulty handling oral secretions. Ophthalmoplegia can present as torticollis or diplopia and be associated with retro-orbital pain.

In areas of endemicity, *Borrelia burgdorferi* causes 30% of facial, and most bilateral, palsies.[51] Herpes simplex virus and VZV cause facial palsy by primary infection or reactivation. Although HIV-1 is associated with facial palsy, paresis during severe immunodeficiency usually indicates another etiology. Causes of facial paresis include GBS, multiple sclerosis, skull fractures, suppurative otitis, acoustic neuroma, carcinomatous or granulomatous meningitis, sarcoidosis, leprosy, diphtheria and Kawasaki disease. [52] Causes of ophthalmoplegia include chronic meningitis, intracranial hypertension and botulism. Bulbar paresis occurs in GBS, diphtheria, botulism, poliomyelitis and paraneoplastic syndromes.

DIAGNOSIS

Polymerase chain reaction detected HSV-1 genome in 79% of endoneural fluids from a surgical series with Bell's palsy.[50] Extrapolation to PCR testing of other sterile fluids, such as CSF, is uncertain. Serologic diagnosis of Lyme borreliosis or antecedent HSV or VZV infection in facial palsy is complicated by limitations of test sensitivities and specificities.[51] Simultaneous seroconversions to several viruses are common.[52] In areas endemic for Lyme disease, physicians frequently evaluate facial palsy after empiric oral therapy against *B. burgdorferi*. Given the self-limited nature of Bell's palsy, it is poor logic to make a causal diagnosis based on the response to therapy for Lyme disease.

MANAGEMENT

Management of isolated Bell's palsy varies. Evaluation of bilateral facial palsies or other cranial neuropathies includes investigation for GBS and CNS demyelinating disease. There are few controlled therapeutic trials to address therapeutic strategies; corticosteroid therapy is no more effective than placebo. Favorable responses to aciclovir await further confirmation.

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Chapter 28 - Infections in Hydrocephalus Shunts

Roger Bayston

Hydrocephalus shunts drain excess cerebrospinal fluid (CSF) from the cerebral ventricles to the peritoneal cavity (ventriculoperitoneal, VP), to the right cardiac atrium (ventriculoatrial, VA) or less commonly to other sites (Fig. 28.1). Cerebrospinal fluid is also sometimes drained from the lumbar spinal theca to the peritoneal cavity (lumboperitoneal, LP).

EPIDEMIOLOGY

The incidence of infection varies according to the age at which the shunt is inserted. Up to 25% of operations in premature infants with hydrocephalus after periventricular hemorrhage result in infection, whereas in older children the incidence is 3–8%.[1] [2] [3] There is no difference in infection rates between VA and VP routes.[4] Although the incidence of shunt infections has generally fallen, it is still unacceptably high. Some centers have reported rates of infection near to zero[5] but this is rare.

PATHOGENESIS AND PATHOLOGY

The source of the organisms is almost invariably the patient's skin, from which they gain access to the device during its insertion.[6] [7] Where there is a serious breakdown in surgical asepsis or where the operating room environment or air is grossly contaminated, these may be alternative sources. Although the bacterial population on the surface of the patient's skin can be reduced to almost zero by agents such as alcoholic chlorhexidine, recolonization by resident bacteria occurs rapidly. It is therefore usual to find coagulase-negative staphylococci in the incision during the procedure. In nonimplant surgery these are irrelevant, but where a biomaterial or device is inserted they are highly likely to adhere to and colonize the device. Coagulase-negative staphylococci, and particularly *Staphylococcus epidermidis*, predominate in shunt infections. After adhering to the shunt material, they multiply and produce copious amounts of exopolysaccharide ('slime'), enabling the formation of a biofilm. Because of nutrient depletion, growth is very slow and this accounts for the often long periods between surgery and clinical presentation of infection. *Staphylococcus aureus* causes a different clinical picture and is more frequently involved in external shunt infections (Fig. 28.2).[4] Unlike *Staph. epidermidis*, it produces a-toxin, which protects it from phagocytosis. A very active inflammatory response is also evoked, leading to erythema and suppuration.

The clinical presentation of infection in VA shunts differs from that in VP shunts (Table 28.1). In the former, bacteria enter the bloodstream directly to cause intermittent fever which, in infections caused by *Staph. epidermidis*, propionibacteria or coryneforms, may continue for months or years with little other evidence of infection. However, antibody to bacterial components is produced in large quantities and immune complex disease may ensue, with deposits of C3, C4, IgG and IgM on the synovial and glomerular basement membranes. Hypertension, renal failure (shunt nephritis) and arthropathy may result.[8] In VP and LP shunt infections, the bacteria are discharged into the peritoneal cavity, provoking the greater omentum to seal off the distal catheter. This and associated adhesions give rise to shunt obstruction and raised CSF pressure (Fig. 28.3). Occasionally, peritoneal abscesses are seen. In all types of shunts ventriculitis is seen in most cases, although the inflammatory response is usually feeble. Only a few shunt infections are due to causes other than surgery. In babies or adults whose nutritional status is poor, erosion of the skin over the shunt can take place, leading to secondary infection with *Staph. aureus* or Gram-negative bacteria. An unusual but well-documented cause of VP shunt infection is visceral perforation by the distal catheter, which results in polymicrobial infection of the cerebral ventricles.[9] However, peritonitis from this cause is rare.

No cases of hematogenous spread, including to VA shunts, have been documented. Cerebrospinal fluid shunts appear to be unusually free of risk from this source. However, VP shunts can become infected during abdominal surgery or continuous ambulatory peritoneal dialysis.

PREVENTION

Studies have shown that knowledge of the causes of shunt infections and surgical experience are very important and shunt surgery should be carried out only by experienced personnel or by fully supervised trainees. In addition, the principle that surgical infection rates can be reduced by shorter preoperative hospitalization should be applied to shunt surgery wherever possible. In view of the source of infection, most attempts at prevention have been targeted at the surgical procedure. Alcohol-based povidone-iodine and chlorhexidine should be used for skin preparation, the latter having the greater activity. Assiduous surgical technique is extremely important, but infection rates are rarely reduced below 3–5% for adults and 10% for infants. The use of prophylactic antibiotics might appear to be reasonable, but they have not been found to have a statistically significant beneficial effect in properly designed trials.[10] Those who feel obliged to use them despite this are advised to administer 10mg vancomycin hydrochloride in 1–2ml sterile water for injection intraventricularly as soon as the ventricular catheter is inserted (this should be inserted first), and 1.5g cefuroxime (25mg/kg for children) intravenously at induction of anesthesia. Intravenous antibiotics alone are not recommended.

External shunt infections (involving the tissues around the outside of the shunt) are not sufficiently common to merit the routine use of prophylaxis, but where a special problem exists a first- or second-generation cephalosporin should be administered intravenously at induction. Because of the general lack of success in further reducing the infection rate beyond acceptable minima, several innovative processes have been developed for treatment of biomaterials and devices in order to reduce bacterial adherence. To date these have not proved effective outside the laboratory and very few are suitable for central nervous system (CNS) implantation. Often, only the outside surface of the shunt is modified, so

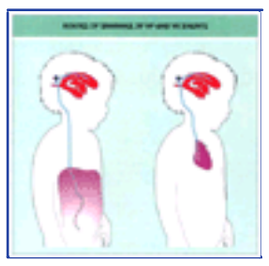

Figure 28-1 Routes of drainage of ventriculoperitoneal and ventriculoatrial shunts. Ventriculoperitoneal shunts drain CSF from the cerebral ventricles to the peritoneal cavity via catheter tubing implanted superficially over the rib cage. The lower end of the peritoneal catheter lies free in the abdomen. Ventriculoatrial shunts drain CSF via a convenient neck vein such as the jugular and the superior vena cava to the right atrium.

Figure 28-2 External shunt infection in a premature infant with poor nutritional status. The infection can be caused by organisms introduced at surgery or they may gain access through minor skin abrasions and pressure necrosis. Differing from the more common internal shunt infections, they are usually caused by *Staphylococcus aureus* and constitute a wound infection enhanced by a foreign material.

no significant effect on the majority of shunt infections can be expected. One process (Bactiseal) consists of introducing rifampin (rifampicin) and clindamycin into the molecular matrix of the silicone in such a way that high-level protection of all surfaces can be conferred for over 2 months in long-term in-vitro tests. Antibacterial activity is undiminished in the presence of protein conditioning film, such as that found in infants with high CSF protein levels after hemorrhage or meningitis, who are at greatest risk of shunt infection.[11] The results of clinical trials are awaited.

CLINICAL FEATURES

The clinical features of VA shunt infection differ considerably from those of VP and LP shunts (see Table 28.1). Although the shunt lumen becomes colonized at implantation, in VA shunts symptoms

TABLE 28-1 -- Clinical features of ventriculoatrial and ventriculoperitoneal shunt infections of surgical origin.

CLINICAL FEATURES OF VA AND VP SHUNT INFECTIONS OF SURGICAL ORIGIN		
	VA shunts	**VP shunts**
Time from surgery to presentation	Weeks, months, several years	<9 months
Intermittent fever	75%	<50%

Anorexia, lassitude, poor sleep pattern	>80%	>50%
Shunt obstruction	<1%	>75%
Other features	Chills, rigors: 20%	Abdominal pain, bloating: >75%
	Arthralgia: 50% (late onset cases (1–15 years))	Swelling, erythema over shunt tubing: >60%
	Rash: 70% (late onset cases (1–15 years))	Headache, vomiting, etc. (i.e. recurrence of hydrocephalus): 75%
	Nephritis: 30% (late onset cases (1–15 years))	
Percentages indicate the approximate proportion of cases in which features are present. It is important to realize that each case is different and that many of these features may be absent or modified		

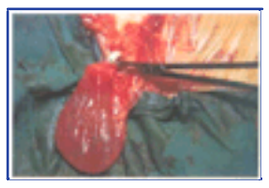

Figure 28-3 Cystic obstruction of a ventriculoperitoneal shunt caused by shunt infection with *Staphylococcus epidermidis.* Bacteria and bacterial products entering the peritoneal cavity via the shunt catheter evoke an inflammatory response involving the greater omentum, which seals off the catheter outlet. The resulting cyst fills with CSF, giving rise to recurrence of the hydrocephalus. Cystic obstruction can occur from noninfective causes but unlike those cases caused by infection, which present within 6–9 months of surgery, they can arise at any time.

considered serious enough to warrant specialist medical attention may not appear for months or years.[8] Many patients have intermittent low-grade fever, but some do not. Some report chills and occasionally rigors. Transient rashes are common. Sore throat and muscular and joint pains are common complaints. Anemia is almost universally found and there is increasing lassitude, anorexia, irritability and poor sleep. Dyspepsia may also be a problem. In later stages, arthralgia becomes more common. Unfortunately, these features are non-specific and are often mistaken for those of other conditions. As the disease progresses, nephritis and vasculitis may

appear. The vasculitic rash is usually confined to the lower extremities and can be frankly hemorrhagic and can ulcerate. Nephritis is indicated by hypertension, hematuria and proteinuria, edema and often loin pain. Endocarditis has been reported only rarely.

In contrast, infections in VP and LP shunts almost always present within 6 months of surgery.[12] [13] Fever is present in fewer than 50% of cases and is usually intermittent and mild. Chills and rigors are rare. There may be abdominal discomfort, bloating, pain or tenderness and occasionally persistent flatulence. In cases presenting within 1 or 2 weeks of surgery, there may be failure of the abdominal wound to heal, with CSF leak and sometimes catheter protrusion. In LP shunts there is often spinal pain. However, the most constant symptoms are those of hydrocephalus caused by obstruction at the distal end and the differential diagnosis is between infective and noninfective shunt obstruction. In the former there is often erythema and tenderness over the lower shunt track, whereas these features are absent in noninfective obstruction. In addition, distal VP shunt obstruction occurring more than 9 months after shunt surgery is very unlikely to have an infective cause.[13] [14] A very small number of cases present as acute abdomen, with fever, abdominal pain and tenderness suggesting appendicitis or peritonitis. [15] These may present at any time and may lead to unnecessary laparotomy. The tenderness is not necessarily associated with the location of the shunt tip.

Ventriculoperitoneal shunt infections can also present at any time after perforation of the bowel or vagina. The distal catheter often protrudes from the anus or vagina and CSF leaks from these sites. They usually present as meningitis rather than shunt obstruction or peritonitis, with few abdominal features. Considering the often large numbers of bacteria seen in the CSF in these cases, the patients are not usually severely ill and recovery is often uneventful after shunt removal, without need for laparotomy.

DIAGNOSIS

Blood should be drawn for culture in all cases of suspected shunt infection. However, in infected VP and LP shunts the positive culture rate is less than 5%, except where *Staph. aureus* or Gram-negative bacilli are involved. In VA infections the positivity rate is much higher and blood should be drawn for culture on several occasions. However, in longstanding infections blood cultures may remain negative, possibly because of high antibody and opsonin titres. All isolates, however doubtful, should be saved until a definitive diagnosis is made. Attempts should be made to compare consecutive isolates, by antibiograms and by proprietary kits such as API Staph, or by molecular typing techniques if available. Differentiation between contaminants and pathogens, however, remains a problem. In addition, despite claims that a positive test for adherence or 'slime' production indicates a likely pathogen rather than a contaminant,[16] [17] critical evaluation shows that these tests, as currently formulated, are of no value in the diagnostic laboratory.[18]

Aspiration of CSF from the shunt reservoir carries little risk of introducing infection. The CSF sample should be examined promptly and a portion should be centrifuged for Gram film and culture, whatever the cell count, because bacteria are not infrequently found in the absence of a significant cellular response. As with blood cultures, isolates should be kept and identified, although the isolation of an organism from a shunt aspirate, particularly if it is also seen on Gram film, is diagnostic of shunt infection. It is important to realize that no isolate should be disregarded, whatever its identity.

It should be noted that the shunt can be infected only distal to the reservoir. In such cases reservoir aspiration may yield normal CSF that is culture negative.

In the presence of symptoms and a suggestive history, negative blood and CSF cultures cannot rule out shunt infection completely and a high index of suspicion should be retained. If symptoms persist consideration should be given to removing the shunt empirically and it is then imperative that it is sent immediately to the laboratory in a sterile container for Gram stain and culture. Cultures should be incubated for at least 5 days. The Gram stain is very important; where organisms are isolated on culture without being seen on microscopy they are almost invariably contaminants, except where antimicrobials have been given immediately before shunt removal. Also, Gram-positive bacilli seen on microscopy often indicate propionibacterial infection, requiring anerobic culture for up to 2 weeks.

In view of the difficulties both of laboratory and of clinical diagnosis, serologic tests have been developed. For example, a whole-cell agglutination test using *Staph. epidermidis* has proved useful in diagnosing VA shunt infections.[8] The agglutinin titer in individuals without shunts rises with age. In patients who have VA shunt infection caused by coagulase-negative staphylococci, the titer rises before symptoms appear and, over several months, it can rise to 15–30 times the normal level for age. It can therefore be used as a screening test. When used in this way, shunt nephritis is not seen because a diagnosis is invariably made sufficiently early for it to be avoided.[8] Ventriculoperitoneal and LP shunts do not discharge directly into the bloodstream, which may explain why the agglutination test is not useful in these cases. The plasma C-reactive protein can also be helpful in distinguishing between infective and noninfective distal VP shunt obstruction.[19]

MANAGEMENT

Three factors are important in the antimicrobial chemotherapy of shunt infections. The first is the inherent multiresistance of many strains of coagulase-negative staphylococci. Almost all are resistant to penicillin and at least 50% are resistant to methicillin and therefore to cephalosporins. Resistance to aminoglycosides is also common. However, all clinical isolates of *Staph. epidermidis* are currently susceptible to vancomycin, although some strains of *Staph. haemolyticus* and a few of *Staph. epidermidis* are resistant to teicoplanin. Similarly, the incidence of resistance to rifampin and lincosamines is low in most centers.

The second factor is the lack of a vigorous inflammatory response in the CNS to most shunt infections and most systemically administered antimicrobials fail to penetrate the CSF, this being particularly true of aminoglycosides, ß-lactams, glycopeptides and streptogramins. Of the few drugs that give acceptable CSF concentrations in such circumstances, chloramphenicol is bacteriostatic and ineffective in treating shunt infections; rifampin is highly active against most organisms causing shunt infections but cannot be given alone because of rapid development of resistance; and trimethoprim is active against fewer Gram-positive bacteria than rifampin.

The third factor is the mode of growth of the organisms in the shunt lumen. Bacteria that grow as a biofilm have much lower growth rates and produce exopolymers. The concentration of antimicrobials required to kill biofilm organisms is often several logs higher than the conventional minimum inhibitory concentration. [20]

These factors explain the generally disappointing results achieved whenever attempts are made to treat shunt infections without shunt removal. The shunt should therefore be removed early and an external ventricular drain (EVD) inserted to control CSF pressure (Table 28.2). This course of action is supported by many reports,

clearly indicating that shunt retention is associated with a greater chance of relapse, a longer hospital stay and a greater risk of death.

Antimicrobials should be begun as soon as the diagnosis is confirmed. Vancomycin is recommended for coagulase-negative staphylococci, *Staph. aureus*, coryneforms and for those enterococci that are susceptible. As the drug does not give adequate CSF

TABLE 28-2 -- Treatment of shunt infections caused by *Staphylococcus epidermidis*, other coagulase negative staphylococci and other susceptible Gram-positive bacteria

TREATMENT OF SHUNT INFECTIONS CAUSED BY *STAPH. EPIDERMIDIS*, OTHER COAGULASE NEGATIVE STAPHYLOCOCCI AND OTHER SUSCEPTIBLE GRAM-POSITIVE BACTERIA
Shunt removal, insertion of external drain
Intraventricular vancomycin 20mg daily plus intravenous/oral rifampin 15mg/kg per day (pediatric) or 600mg/day (adults), both in two divided doses
After 7–10 days of treatment, reshunt if necessary. Stop both antibiotics on this day

concentrations when given intravenously,[4] it should be given intraventricularly via a reservoir or through the clamped EVD tube. A Rickham reservoir should be incorporated in the system if the chosen EVD does not have a reservoir or injection port. Alternatively, an Ommaya reservoir can be inserted contralaterally. The standard dose of intraventricular vancomycin is 20mg daily, although this should be reduced to 10mg daily for those with small ventricles. It should be noted that the dose depends on ventricular volume rather than on age or body weight. The vancomycin should be diluted in 1–2ml sterile water for injection. In addition to vancomycin, rifampin should be given intravenously in a total dose of 15mg/kg per day (two divided doses) for children or 300mg q12h for adults along with intravenous or intramuscular flucloxacillin (250–500mg q6h or 60–125mg q6h for children) or oxacillin (500mg q6h; for children >40kg body weight, use adult dose, <40kg 25–50mg/kg q4h). The drugs can be given orally in most cases after a few days. Alternatively, trimethoprim can be given intravenously, 3mg/kg q8h for children and 250mg q12h for adults. Teicoplanin offers no obvious advantage over vancomycin. Intraventricular vancomycin given in the doses recommended above leads to CSF concentrations that commonly reach 5–10 times the expected plasma concentrations, but no toxicity has been encountered. Attempts should not be made to titrate the dose to keep the CSF concentrations below the toxic plasma levels. There is no indication for the use of intravenous vancomycin in addition to that given by the intraventricular route except in the case of methicillin-resistant *Staph. aureus* shunt infections, for which vancomycin might be the only available agent. The drug may be given by the intravenous route alone if the CSF cell count and protein concentration are sufficiently raised to indicate a vigorous inflammatory response, but this is the case only with *Staph. aureus* infections.

Using this regimen, coagulase-negative staphylococci should no longer be detectable in the CSF on microscopy or culture by day 4 and any fever should have resolved. A new shunt, if needed, can be inserted by day 7–10 of the regimen, the last dose of antibiotics being given on that day. It is unwise to wait for a few days after stopping treatment, as this is the period of greatest risk for secondary infection from the EVD. Using this regimen for coagulase-negative staphylococci, *Staph. aureus*, coryneforms and propionibacteria, successful eradication and reshunting within 10 days without relapse can be expected in almost all cases.[21]

For shunt infections caused by Gram-negative bacilli, the shunt should again be removed and the treatment for Gram-negative meningitis (Chapter 22) instituted.

A notable exception to the rule of shunt removal is community-acquired bacterial meningitis in shunted persons. Such patients should be treated in the same way as those without shunts and can be expected to respond at least as well. On no account should these patients be subjected to shunt removal.[4]

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Chapter 29 - Neurotropic Virus Disorders

Martin J Wood

The diseases described in this chapter are all the result of infections of or infection-mediated damage to, cells within the spinal cord, the dorsal root ganglia adjacent to the cord, or the brainstem. Rabies is a zoonosis and produces a fatal encephalomyelitis and is discussed in Chapter 219 .

POLIOMYELITIS

EPIDEMIOLOGY

The incidence of acute paralytic poliomyelitis has fallen dramatically over the past four decades as a result of mass vaccination campaigns and the global poliomyelitis eradication initiative set up by the World Health Organization (WHO) in 1988. From 1988 to 2001 reported cases of paralytic polio declined by over 99% from about 35,000 to less than 1000 (Fig. 29.1).[1] The American and Western Pacific Regions of the WHO have been declared free of indigenous wild poliovirus, but India, Pakistan and Nigeria still represent major poliovirus reservoirs. Within the European region importations of wild virus have occurred: in 1996 a large outbreak occurred in Albania, which had been free of polio since 1985, confirming the high risk of importation and transmission of polio in unvaccinated and incompletely vaccinated populations that had been seen elsewhere.[2]

In countries where poliovirus is endemic, paralytic disease is caused by serotypes 1 or, to a lesser extent, 3; wild type 2 poliovirus has not been detected worldwide since 1999. Polio cases attributed to circulating vaccine-derived polioviruses now account for many reports: in 2000–2001 such cases were reported from the Caribbean island of Hispaniola (Haiti and the Dominican Republic) and the Philippines.[1] In the USA, apart from six imported cases, all reported cases of paralytic poliomyelitis since 1979 have been vaccine-associated (Fig. 29.2).[3]

Poliovirus is usually transmitted by direct fecal-oral contact, indirect contact with saliva or feces or from contaminated water. Disease occurs all year round in the tropics, and during the summer and autumn in temperate countries. Children are more susceptible to infection, but the risk of paralytic disease is higher in adults.

PATHOGENESIS AND PATHOLOGY

After exposure poliovirus multiplies in the lymphoid tissue of the tonsils and, particularly, in the Peyer's patches of the ileum. The virus passes to the regional lymph nodes, and then a primary viremia subsequently distributes the virus to the entire reticuloendothelial system. In most instances the immune system then controls the infection and viral replication ceases at this point. In a minority of persons, however, further replication occurs in the reticuloendothelial system and there is a major disseminating viremia. It is believed that virus enters the central nervous system (CNS) as a result of this hematogenous dissemination but this may be only part of the story. The cell surface receptor for poliovirus is a member of the immunoglobulin-like superfamily of proteins.[4] Experiments in transgenic mice containing the human gene for this receptor suggest that poliovirus spreads from muscle to the anterior horn cells via neural pathways. [5] Within the CNS poliovirus primarily targets motor neurons in the brainstem and the anterior horn of the spinal cord. Neurons at these sites, and to a lesser extent, those in the mesencephalon, cerebellum and precentral gyrus of the cerebral cortex are destroyed and surrounded by an inflammatory infiltrate of polymorphonuclear leukocytes and mononuclear cells (Fig. 29.3). Gliosis develops when the inflammation has subsided.

PREVENTION

Poliovirus causes an acute nonpersistent illness; humans are the only reservoir and virus survival in the environment is finite. These facts, combined with the ability of polio immunization to interrupt virus transmission, made poliovirus a candidate for eradication by the WHO.[6]

Two types of poliovirus vaccine are available:

- the orally administered Sabin vaccine (OPV) consisting of live attenuated strains of all three serotypes of poliovirus; and
- the Salk-inactivated poliovirus (IPV) vaccine.

After one dose and three doses of OPV, 50 and >95% of recipients, respectively, develop longlasting immunity. Administration of OPV interferes with subsequent infection with wild poliovirus.

After two doses of IPV 90–100% of children develop protective antibody to all three serotypes of poliovirus. Persons vaccinated with IPV may still be infected with and excrete wild-type strains of poliovirus, although epidemiologic studies have confirmed that IPV reduces circulation of wild-type virus considerably.

The strategies for global eradication of poliomyelitis eradication include the following measures aimed at interrupting transmission of wild poliovirus in endemic countries:

- high vaccination coverage of children younger than 1 year of age with three doses of OPV;
- effective surveillance systems; and
- supplemental vaccination on national immunization days, when two doses of OPV are given to all children younger than 5 years of age, irrespective of previous immunization.

The scale of this undertaking can be judged by the fact that, in India alone, more than 125 million children were immunized on 18 January 1997.

Orally administered Sabin vaccine can be complicated by paralytic poliomyelitis; the risk is one in 2.4 million doses, although for children receiving their first dose of OPV it is one in 750,000 doses. People who are immunodeficient, particularly those with hypogammaglobulinemia are at greatest risk (a 3000–7000-fold higher risk than in immunocompetent recipients). Where routine immunization has been widespread and wild poliovirus has been almost eradicated (e.g. the USA), most cases of paralytic poliomyelitis are caused by OPV. In these circumstances IPV has been recommended. Since 2000, for example, the USA guidelines for routine vaccination of children recommend exclusive use of IPV given at 2 months, 4 months, 6–18 months and 4–6 years.[7]

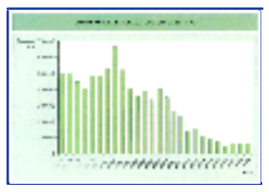

Figure 29-1 Global annual poliomyelitis cases reported to the World Health Organization 1974–99.

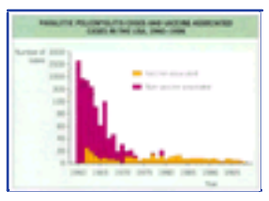

Figure 29-2 Total number of cases of paralytic poliomyelitis and vaccine-associated poliomyelitis in the USA 1960–98.

CLINICAL FEATURES

The incubation period of poliomyelitis is probably about 10–14 days but most (at least 95%) poliovirus infections are asymptomatic. Indeed, even most symptomatic cases only suffer a nonspecific febrile illness corresponding to the enteric and primary viremic phase of viral replication. This illness lasts a few days and is followed in a small percentage of cases by the major illness of aseptic meningitis and paralytic disease. The onset of this phase is abrupt, with meningitic symptoms and muscle pains, often in the neck or back, followed a few days later by the gradual onset, in less than 1% of poliovirus infections, of paralytic disease. Risk factors for paralytic

disease are a large inoculum of virus, increasing age, pregnancy, tonsillectomy, strenuous exercise and intramuscular injections during the incubation period.[8]

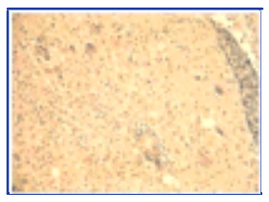

Figure 29-3 Anterior horn in poliomyelitis. Damaged and destroyed anterior horn neuron cell bodies are surrounded by an inflammatory infiltrate (hematoxylin & eosin stain).

The typical clinical features of paralytic poliomyelitis result from viral lysis of motor neurons of the anterior horn of the spinal cord and/or the brainstem. There is fever and muscle pain, and rapid progression over 2–4 days to maximal paralysis. Spinal paralysis is typically asymmetric and is more severe proximally. Bulbar paralysis may affect swallowing and respiration. Fasciculation is often evident and the deep tendon reflexes are absent or diminished. Autonomic disturbance is often evident. Although sensory symptoms are common, the presence of sensory signs should alert the clinician to an alternative diagnosis.

The mortality rate from paralytic poliomyelitis is 2–10% and is generally caused by bulbar involvement or respiratory failure. Following the acute illness there is often a degree of recovery of muscle function over the subsequent 6 months or more.

After many years of stable neurologic impairment, new neuromuscular symptoms (weakness, pain and fatigue) develop in 25–40% of patients, a disorder termed the postpolio syndrome.[9] It has been suggested that postpolio syndrome is the result of decompensation of a chronic denervation and reinnervation process, whereby surviving motor neurons no longer maintain new sprouts. It has also been suggested that reactivation of latent virus accounts for the foci of inflammation seen in some biopsies.[10] Others suggest that postpolio syndrome is caused by orthopedic factors, radiculopathies, or merely normal functional deterioration in muscles that are already weak after polio.

The most important disease likely to be confused with paralytic poliomyelitis is the Guillain-Barré syndrome (GBS). The illnesses can usually be distinguished by the symmetric paralysis and sensory signs of GBS and the elevated protein, but relatively normal cell count in the CSF of patients who have GBS.

DIAGNOSIS

In the preparalytic phase there is nothing to distinguish poliomyelitis from other causes of viral meningitis. There is an increased number of leukocytes in the CSF, but poliovirus is rarely isolated from the CSF. The diagnosis may be confirmed by serologic testing of paired acute and convalescent sera or by isolation of poliovirus from throat swabs taken during the first week of the disease, or from feces cultured up to several weeks after the onset. Polymerase chain reaction methods have been developed for detecting poliovirus, but are not yet used in routine diagnostic laboratories.

MANAGEMENT

Management of the acute phase of paralytic poliomyelitis is supportive and symptomatic. Patients need hospitalization and bed rest during the first week or so. Light splints and passive physical therapy to prevent contractures, moist hot packs for muscle pain and spasms and frequent turning to prevent bedsores are important. Nutrition and fluid balance need to be maintained, and close monitoring of respiration is vital. If the vital capacity falls below 50% of predicted values or hypoxia occurs, or there is pooling of pharyngeal secretions, assisted ventilation should be started.

When the fever subsides active physiotherapy and mobilization is started: 80% of eventual recovery is attained within 6 months, although recovery of muscle function may continue for up to 2 years.

Long-term management of the paralyzed patient is complex and outside the scope of this account. The management of postpolio syndrome includes pacing of activity and nonfatiguing exercise. No consistent benefits have been obtained with anticholinesterases[11] or corticosteroids.

HERPES ZOSTER IN THE NORMAL HOST

EPIDEMIOLOGY

Studies of the incidence of herpes zoster in the USA and UK suggest that there are about three cases per 1000 persons each year.[12] These figures suggest that in the USA there may 900,000 cases of herpes zoster and that in the UK there may be 200,000 cases of herpes zoster annually. There are few reliable data for other areas of the world. The incidence increases dramatically after middle age (Fig. 29.4). There is no sex difference, but owing to population demographics in developed countries most cases are seen in women in their sixth and seventh decades of life. Children or adolescents who acquire varicella-zoster virus (VZV) infection in utero or in the first year of life have an up to 20-fold greater risk of developing herpes zoster before 20 years of age. Almost 20% of individuals in the UK

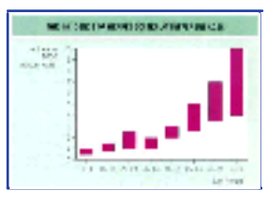

Figure 29-4 Incidence of herpes zoster at different ages.[12]

will develop herpes zoster at some stage; about 5% of immunocompetent patients can expect to suffer a second, and less than 1%, a third, episode.[12]

Typically, herpes zoster occurs unexpectedly with no seasonality. There is no evidence that it is more common during chickenpox outbreaks.

PATHOGENESIS AND PATHOLOGY

Identification of VZV nucleic acids within sensory ganglia and demonstration that VZV isolates from varicella and herpes zoster in the same patient are identical have proved that the disease is caused by reactivation of VZV from within the sensory ganglia, where it has been resident since the primary attack of chickenpox, usually many years earlier. During this latent period it is believed that host immunity to VZV is repeatedly boosted by re-exposure to VZV antigens, either from exogenous sources or from episodes of endogenous replication that did not lead to herpes zoster. The molecular mechanisms that establish and maintain VZV latency are not fully understood, but reactivation of the virus is clearly related to declining VZV-specific cell-mediated immunity (CMI). In the elderly the risk of herpes zoster is proportional to the marked decline in VZV-specific CMI associated with advancing age.[13]

After reactivation of VZV from latency, the virus replicates within the neuron and travels down the nerve to the skin that nerve innervates. As it does this the neurons are destroyed and there is a marked inflammatory neuritis with cellular infiltrate and hemorrhage. Once it reaches the skin the virus replicates within the epidermis and produces the characteristic vesicular rash. Histologically, the lesion is indistinguishable from that of varicella or herpes simplex, consisting of an intra-epidermal blister with multinucleate giant cells in its floor.

The pathogenesis of postherpetic neuralgia (PHN) is poorly understood, but the increased transmission of nociceptive impulses during the period of acute neuritis induces central sensitization and hyperexcitability of spinal neurons. This is then maintained by a changed peripheral input and by excitotoxic damage in the dorsal horn of the spinal cord.[14]

PREVENTION

The development of herpes zoster in individuals previously infected with VZV might be prevented by measures designed to boost their

declining CMI to the virus. This can be accomplished by the live attenuated VZV_{Oka} strain vaccine: when this vaccine is given to seropositive elderly adults (mean age 67 years), most have a long-lasting boost in their VZV-specific CMI to a level similar to that of normal 40-year-olds, a group with a relatively low risk of herpes zoster.[15] [16] Whether this will reduce the frequency and severity of herpes zoster in those who are vaccinated is still to be tested in a controlled trial.

CLINICAL FEATURES

Herpes zoster is almost always unilateral. The dermatome most frequently affected (10–15% of cases) is the ophthalmic division of the trigeminal nerve. Otherwise, each dermatome is affected at a similar rate. Hence, more than 50% of herpes zoster involves one or more of the thoracic dermatomes and the cervical and lumbosacral dermatomes are each affected in 10–15% of cases.

The chief clinical features of acute herpes zoster are pain and rash.[12] Systemic symptoms and signs may also occur, including headache, malaise, nausea and vomiting, fever and regional lymphadenopathy. Pain and paresthesia within the affected dermatome often precede the rash by several days: prolonged periods of prodromal pain have been reported. The pain is extremely variable in its character, periodicity and severity, and because it is similar to that occurring in a wide range of other conditions, the true nature of the cause is not usually recognized until the rash appears. The pain usually increases for a few days and then declines in severity somewhat slowly, often in parallel with skin healing.

The rash of herpes zoster (Fig. 29.5) generally appears proximally in the involved dermatome and spreads distally over the following few days. It begins as erythematous maculopapules that vesiculate within 12 hours or so. After 3–4 days, the vesicles become cloudy pustules and these then gradually dry and crust over the subsequent 7–10 days, thereafter persisting for a further 1–2 weeks. New lesions continue to appear for a mean of 2–3 days and only 10–15% of normal individuals have new lesions beyond 4 days. Virus can be cultured from the vesicles for only a few days, although in 15% of cases it is recoverable from lesions for 1 week. Occasionally, especially in the very elderly or those with poor nutrition, the rash may become necrotic, ulcerative and gangrenous. The appearance of a score or so of lesions outside the primary dermatome within a few days of rash onset is not unusual in otherwise healthy adults, but widely disseminated cutaneous disease is rare.

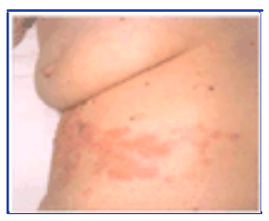

Figure 29-5 Typical dermatomal rash of herpes zoster.

Although most cases of herpes zoster in the immunocompetent host are self-limiting, one or more complications occur in 15–20% of patients (Table 29.1). The major complication and cause of morbidity after herpes zoster in the immunocompetent host is chronic pain or PHN. Postherpetic neuralgia is an arbitrarily defined term; some definitions include any pain after rash healing, while others limit PHN to pain persisting for 30 days or longer.[17] Pain persists for more than 4 weeks after the resolution of the rash in 10–15% of adult patients with herpes zoster; only 5–10% are still in pain after 3 months, and 2–5% after 12 months.[17] In the elderly, prolonged pain is more common. At 1, 3 and 6 months after the illness only 50–60%, 25% and 9–13%, respectively, of patients over 60 years of age are still suffering pain. The other factors that influence the likelihood of prolonged pain after herpes zoster are the severity of the acute pain, prolonged prodromal pain and psychological distress and disease conviction.

Some ocular involvement is common in patients with ophthalmic zoster. Up to 85% of patients with involvement of the nasociliary branch of the trigeminal nerve clinically suggested by Hutchinson's sign (the rash involving the lateral tip of the nose, Fig. 29.6) will develop ocular complications, but ocular involvement can occur

even if this sign is absent. Every ocular tissue can be affected by VZV.[18] Although conjunctivitis is the most frequent complication, anterior uveitis and keratitis are of greater significance. Keratitis may

TABLE 29-1 -- Complications of herpes zoster in the immunocompetent individual.

COMPLICATIONS OF HERPES ZOSTER IN THE IMMUNOCOMPETENT INDIVIDUAL	
Complication	**Examples**
Postherpetic neuralgia	
Ocular complications	Conjunctivitis
	Uveitis
	Keratitis
	Glaucoma
	Retinal necrosis
Motor weakness	Ramsay Hunt syndrome
Encephalitis, transverse myelitis, etc.	
Cerebral angiitis	

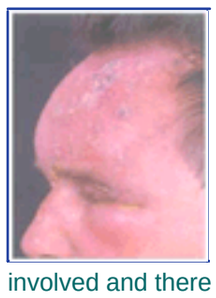

Figure 29-6 Hutchinson's sign. When the rash of herpes zoster involves the skin at the tip and side of the nose it indicates that the nasociliary branch of the trigeminal nerve is involved and there is an increased risk of uveal tract inflammation and ocular damage.

manifest as epithelial or subepithelial punctate changes, dendritic or disciform ulceration, or corneal vascularization. Eyes with stromal disease may be rendered blind.

Localized motor paralysis is observed in less than 5% of patients with herpes zoster, predominantly in cases involving the trigeminal nerve or the cervical or lumbosacral dermatomes. The true incidence of motor weakness is much greater, but there is great difficulty in assessing weakness of intercostal or abdominal musculature, the most common sites of a herpes zoster rash. Motor signs usually develop abruptly with or shortly after the rash and reach a peak within a few days; in most cases there is complete functional recovery, although this may be prolonged. A particularly common motor complication is the Ramsay Hunt syndrome (vesicles in or around the external auditory meatus and a lower motor neuron facial palsy).

Encephalitis and myelitis are uncommon complications of herpes zoster in otherwise healthy patients and are probably caused by direct extension of virus from the dorsal root ganglion to the meninges and the brain. A variety of other neurologic complications, such as aseptic meningitis, transverse myelitis, necrotizing myelopathy, cerebral angiitis and GBS have been described in association with clinical herpes zoster.

There is no evidence of any risk to fetal development if a pregnant woman has herpes zoster.[19]

DIAGNOSIS

The diagnosis of herpes zoster is essentially clinical, based on the characteristic appearance and distribution of the rash. The only condition that is likely to be confused with it with any regularity is herpes simplex virus (HSV) infection. Zosteriform rashes may be caused by HSV, but HSV usually causes a much less extensive rash than herpes zoster; the individual lesions of HSV infection are smaller and tend to recur. Patients with recurrent herpes zoster of the buttocks or thighs almost always have HSV type II infection. Confirmation of VZV infection can be obtained by polymerase chain reaction examination or culture of the vesicular fluid.

MANAGEMENT

Although steps should be instituted to ease the inflammation and irritation caused by the skin lesions, the management of herpes zoster is primarily aimed at reducing the pain and complications of the illness.[20] Adequate analgesia is very important and antiviral therapy limits the degree of neuronal damage by VZV. Placebo-controlled studies have shown that oral aciclovir (800mg five times daily for 7 days), if started within 72 hours of rash onset, reduces the severity and duration of the acute illness and also reduces the duration of the pain in the normal host with herpes zoster.[21] The newer, more bioavailable drugs, valaciclovir (1000mg q8h for 7 days) and famciclovir (250mg, the dosage licensed in much of Europe, or 500mg, the dosage licensed in the USA, q8h for 7 days) are of similar efficacy to aciclovir for rash healing, but somewhat better than aciclovir at reducing the duration of pain in individuals who have herpes zoster and who are immunocompetent.[22] As the clinical efficacy of the three drugs is very similar,[23] a choice between them may depend on fiscal constraints and personal experience. Each of the three drugs reduces the incidence of ocular complications in ophthalmic zoster. All patients with ophthalmic zoster should also be examined by an ophthalmologist.

Whichever drug is used the benefits have only been demonstrated if treatment is started within 72 hours of the appearance of the herpes zoster rash (except in ophthalmic zoster, in which the ocular complications are reduced even if aciclovir is started 7 days after the onset of the rash).

HUMAN T-CELL LEUKEMIA VIRUS I (HTLV-I)

Human T-cell leukemia virus I (HTLV-I) was the first human retrovirus to be discovered. In addition to its role in adult T-cell leukemia (ATL), it causes a progressive myelopathy termed tropical spastic paraparesis (TSP) in the West Indies and several other countries, and HTLV-I-associated myelopathy (HAM) in Japan.

EPIDEMIOLOGY

Human T-cell leukemia virus-I infection is found endemically in southwestern Japan, where 20% of the population is seropositive, and in the Caribbean basin (including northern South America and the southeastern USA) where seropositivity approaches 5%. Studies have also shown high seroprevalence rates of HTLV-I in West Africa, the islands of Melanesia in the Pacific and the Middle East. Infection is also found in immigrant populations from these areas, including West Indians in the UK.

Human T-cell leukemia virus-I can be transmitted by sexual intercourse, inoculation of infected blood or blood products and perinatal exposure. Sexual transmission is primarily via semen, from which it can be isolated. Epidemiologic data from Japan suggests a very low rate of transmission from females to males in serologically discordant couples.[24] Breast-milk is an important vehicle of transmission and seroconversion is rare in infants who are not breast-fed, even if their mother is infected. The risk of seroconversion after receiving blood infected with HTLV-I is high (up to 80% after receipt of fresh blood products).[25] In endemic populations the prevalence of infection increases with age and clusters in families. Infection is lifelong. Not unexpectedly, HTLV-I is more prevalent in intravenous drug users and homosexual males than in the general population.

PATHOGENESIS AND PATHOLOGY

Human T-cell leukemia virus-I can infect a variety of human cells but only $CD4^+$ T cells are transformed by the virus: the specific receptor has not been identified but the HTLV-I envelope glycoprotein (gp46) is the probable attachment molecule. Within the cell, reverse transcription, integration of proviral DNA, and transcription and virus replication are typical of retrovirus replication (Fig. 29.7).

There are two hypotheses for the pathogenesis of HAM/TSP.[26] In one, HTLV-I infects glial cells and the cytotoxic T-cell response against infected cells causes demyelination. In the second the HTLV-I infection induces an autoimmune process. Although indirect evidence favors the first hypothesis, direct demonstration of HTLV-I infection of CNS cells is lacking.

Gross pathology shows spinal cord atrophy but a normal brain. The histology shows a diffuse inflammatory encephalomyelitis, with predominantly midthoracic cord involvement. The inflammatory infiltrate of mononuclear cells is mostly perivascular and there is hyaloid thickening of the vascular adventitia and media.[27] Demyelination and significant axonal loss is the final result of the inflammatory process.

PREVENTION

Prevention of HTLV-I infection depends upon screening blood to minimize the risk of transfusion-related disease. This is policy in France, the UK, the USA, Canada and Japan. Health educational programs should also promote condom use and warn seropositive mothers of the risks of transmission by breast-feeding. No effective vaccine has been developed.

Figure 29-7 Life cycle of HTLV-I. HTLV-1 infection is initiated by cell-free virions or, more commonly, by cell-to-cell virus transmission. The two RNA genome copies are converted into double-stranded DNA provirus by the viral enzyme reverse transcriptase and the proviral DNA is integrated into the host chromosome. Transcription is activated by the viral Tax protein. In the early stages of infection both Tax and Rex proteins are produced. The Rex protein directs the preferential transport of unspliced or singly spliced viral messages to the cytoplasm for translation into structural proteins for virion assembly.

CLINICAL FEATURES

Only a small proportion of those infected with HTLV-I develop ATL or HAM/TSP: the lifetime risk of these diseases in HTLV-I infected Japanese is estimated to be 2–4% and 0.25%, respectively.[26] The usual age at onset of HAM/TSP is the fifth decade of life and more women than men are affected.

The myeloradiculopathy produced by HTLV-I mainly affects the pyramidal tracts and, to a lesser extent, the sensory system. Both TSP and HAM have identical features: they are clinically characterized by a chronic syndrome with a combination of upper and lower motor neuron signs. Patients often complain of difficulty walking, dragging pains and stiffness (together with numbness and paresthesia) of the legs, urinary retention and/or incontinence and impotence. About one-third of patients have weakness in the upper limbs, but the cranial nerves are only very rarely involved. Examination reveals a symmetric spastic paraparesis with mild sensory abnormalities indicative of posterior column involvement (diminished vibration and proprioception). Most patients progress gradually over months or years.

There may be confusion between HAM/TSP and multiple sclerosis. There is, however, a lack of optic neuritis or ocular movement problems in HAM/TSP while multiple sclerosis tends to run a relapsing and remitting course. The WHO has published diagnostic guidelines for HTLV-I myelopathy.[28]

DIAGNOSIS

The hallmark of HTLV-I infection is the presence of 'flower lymphocytes' (T-helper cells with multilobulated nuclei that are similar to the cells of ATL) in the blood. These cells comprise only about 1% of the circulating white cells, however, and the diagnosis of HTLV-I infection requires the demonstration of specific antibodies in the serum.

In HTLV-I CNS disease, the CSF examination may be normal or show a slightly elevated protein concentration and a mild lymphocytosis. Flower lymphocytes are found in a minority of cases. A definitive diagnosis of HAM/TSP requires detection of HTLV-I DNA in the CSF by polymerase chain reaction or evidence of intrathecal synthesis of HTLV-I antibody.

Myelography and CT scanning are usually normal apart from spinal cord atrophy. The imaging of choice is magnetic resonance imaging (MRI), which shows diffuse high-intensity signals in the thoracic cord on T_2 -weighted images. Similar lesions are sometimes seen in the periventricular white matter. Visual evoked potentials and somatosensory evoked potentials from the legs are also delayed.

MANAGEMENT

No therapy has been proven to be of benefit in TSP/HAM. Occasional patients have improved while receiving oral corticosteroids, cyclophosphamide or systemic a-interferon, and it has also been claimed that plasmapheresis leads to a temporary benefit. A potentially useful approach for ATL (which has a much poorer prognosis than neurological disease caused by HTLV-I) is a monoclonal antibody to the interleukin-2 receptor, which is upregulated by HTLV-I infection. Its potential in HAM/TSP needs to be evaluated.

At present the management of HAM/TSP is similar to that of myelopathies of any cause, with supportive therapy of spasticity and urinary sphincter disturbance.

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Chapter 30 - Practice Points

30.a Neuroradiology — what and when?

Barbara McKeown
Verka Beric

The mortality from central nervous system infections has fallen since the introduction of antibiotics. The continued reduction in mortality has been attributed as much to advances in imaging, particularly computerized tomography (CT) and magnetic resonance imaging (MRI), as to improvements in chemotherapy and surgical techniques. Computerized tomography and MRI have facilitated earlier and more accurate diagnosis and can guide stereotactic biopsy and drainage. Computerized tomography remains the first choice for excluding intracranial emergencies. It is more readily available and is not subject to specific contraindications or limitations imposed by requiring compatible life support systems. However, MRI is emerging as the procedure of choice in evaluating suspected intracranial infections because of its inherent contrast resolution, multiplane capability, sensitivity in the posterior fossa, sensitivity to the presence of subacute and chronic hemorrhage and sensitivity to the detection of meningeal disease on postcontrast images. Magnetic resonance imaging is far superior to CT in detecting and evaluating lesions in the spinal cord and brain stem and it often demonstrates lesions in the brain at an earlier stage in the disease process.

Meningitis

A wide spectrum of infectious diseases can affect the cranial meninges. Imaging may be indicated in three situation: to detect increased intracranial pressure prior to lumbar puncture, to characterize the condition and to detect complications.

When meningitis is suspected, cerebrospinal fluid sampling for microbial and biochemical analysis is indicated as a matter of urgency. If the patient's conscious state is depressed or if focal neurological signs or papilledema are present, imaging should be performed before lumbar puncture. However it is critical that antimicrobial therapy be initiated rapidly — before imaging if necessary. Withdrawal of cerebrospinal fluid in the presence of raised intracranial pressure may result in coning (or cerebral herniation). This is attributed to impaction of the cerebellar tonsils with consequent compression of the medulla. In most centers CT is available more rapidly than MRI. The characteristic imaging findings include obliteration and enhancement of the basal subarachnoid cisterns, sulci or fissures. In practice, imaging studies are usually normal, especially early in the disease process. Gadolinium-enhanced T1-weighted MRI, especially in the coronal plane, and fluid-attenuated inversion recovery (FLAIR) MRI acquisitions have substantially improved the ability to detect and differentiate between the subcategories of intracranial infection. Magnetic resonance imaging also better demonstrates the extent of the infectious process.

One of the major roles of imaging in patients who have meningitis is in identifying potentially serious complications and associated phenomena, including hydrocephalus, extra-axial collections, infarcts, ventriculitis and parenchymal abscess or granuloma formation.

Brain abscess

Brain abscesses are most commonly bacterial in origin but they may be tuberculous, parasitic or fungal. They arise in the cerebral parenchyma 10–14 days after diffuse infection (cerebritis), which is usually limited to the white matter. Early cerebritis is rarely seen on CT, but MRI can demonstrate increased signal on T2-weighted images. Unfortunately, this finding is of limited clinical value as patients rarely present at this early stage. A developing abscess is well demonstrated on both CT and MRI. Necrosis develops in the region of cerebritis and becomes walled off by a fibrovascular capsule that is in turn surrounded by edema. The capsule enhances intensely with CT and MRI contrast agents and tends to be thinner on its medial side, so that it appears to 'point' toward the ventricles (Fig. 30a.1). Complications such as ependymitis and ventriculitis or daughter abscesses may occur if the abscess ruptures. If the diagnosis is in doubt, or there is a poor response to empiric treatment, CT- or MRI-guided stereotactic needle aspiration may be performed to obtain a specimen for microbial analysis. Response to treatment is best assessed by serial cross-sectional imaging, for which CT is usually adequate, and is seen as a gradual reduction in the size of the abscess and surrounding edema.

A tuberculous abscess (tuberculoma) is known to follow an episode of tuberculous meningitis; it may also arise through hematogenous spread from an extracranial source, usually of pulmonary origin. It may be solid or cavitating with a thick capsule, is often small and multiple, and is demonstrated on both contrast-enhanced CT and MRI.

Worldwide, cysticercosis (caused by *Taenia solium*) is the most common parasitic infection to affect the central nervous system. Patients may have hundreds of larvae-containing cysts scattered throughout the brain, ventricles and subarachnoid spaces. Acute illness occurs many months after initial infection when the larvae die and incite an intense local inflammatory reaction. At this stage, CT

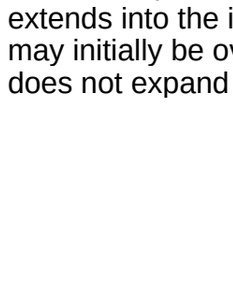

Figure 30.a-1 Cerebral abscess. Contrast-enhanced axial CT demonstrating two cerebral abscesses with surrounding edema and mass effect. Thin, smooth, enhancing capsules surround cavities of nonenhancing necrotic tissue. *Courtesy of Dr I Colquhoun.*

and MRI reveal contrast-enhancing nodular or ring lesions surrounded by extensive localized edema. The edema is most clearly seen on T2-weighted MRI. Later in the disease process, the dead larvae appear as punctate calcifications without a surrounding mass effect and are most readily visible on CT.

Hydatid disease (caused by *Echinococcus* spp.) results in a large cyst that contains fluid of the same density as cerebrospinal fluid; the cyst does not display contrast enhancement or surrounding edema. Magnetic resonance imaging confers no particular advantage over CT.

Toxoplasma gondii is a common opportunistic infection in patients who have AIDS. Multiple ring-enhancing and nodular lesions are the hallmark of toxoplasmosis but the appearances are non-specific and are similar to those of multiple brain abscesses, lymphoma or metastatic disease. Because toxoplasmosis is the most common cause of a mass lesion in the central nervous system in HIV-positive patients, empiric treatment is usually commenced if CT or MRI suggest an abnormality. Biopsy may be required for definitive diagnosis if clinical or radiologic improvements are not demonstrated.

Intracranial empyema

An intracranial empyema is an abscess that has developed in the subdural or epidural space. Subdural empyemas are more common, rapidly progressive and result in significant mortality if a delay in diagnosis occurs. Extradural empyemas cause less neurologic deficit because the dura mater minimizes the pressure exerted on the brain. Computerized tomography scans and MRI demonstrate similar features. A subdural empyema is crescent-shaped, following the contour of the skull, and often extends into the interhemispheric space, where it appears linear. This causes pressure and mass effect on the underlying cerebral hemisphere. Subdural empyemas may initially be overlooked on CT but the mass effect in the absence of a focal lesion is a clue to their presence. An extradural empyema has a lentiform shape and does not expand into the interhemispheric fissure (Fig. 30a.2). Empyemas may be readily detected on coronal MRI scans, even when small and difficult to visualize

on axial CT.

Encephalitis

Encephalitis refers to a generalized and diffuse inflammation of the brain. It is usually of viral origin. Acute infective encephalitis may be caused by the herpesviruses, rabies, arthropod-borne viruses and enterovirus (polio). Of these, herpes simplex virus type 1 is the most common. It commonly results in necrosis of the temporal lobes and

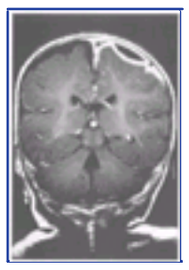

Figure 30.a-2 Extradural empyema. Contrast-enhanced T1-weighted coronal MRI demonstrating a lentiform extradural collection surrounded by enhancing dura mater. *Courtesy of Dr K Chong.*

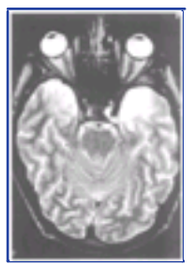

Figure 30.a-3 Herpes simplex encephalitis. T2-weighted axial MRI demonstrating characteristic involvement of medial temporal lobes with high-signal edema. *Courtesy of Dr K Chong.*

posterior frontal gyri. Involvement is usually bilateral and a may be symmetric. Computerized tomography may demonstrate hypodense temporal lobe lesions with or without involvement of the frontal lobes. This hypodensity may be difficult to detect early on in the disease process. Parenchymal enhancement as seen by contrast-enhanced CT is infrequent. Magnetic resonance imaging can demonstrate the early edematous changes on T2-weighted images with characteristic high signal in the temporal lobes and inferior frontal lobes (Fig. 30a.3). FLAIR imaging is even more sensitive in the depiction of gray matter and white matter changes. Gyriform enhancement occurs with disease progression. Parenchymal hemorrhage is more readily detected with MRI, especially gradient echo images.

Acute disseminated encephalomyelitis (ADEM) presents with neurologic signs and symptoms 5 days to 2 weeks after a viral illness or

vaccination. Perivenous demyelination is the pathological hallmark. Magnetic resonance imaging is the imaging study of choice in detecting demyelinating plaques in the brain and spinal cord. They can be seen scattered throughout the white matter of the posterior fossa and cerebral hemispheres. Gray matter involvement is common. These hyperintense T2 lesions are more conspicuously seen with FLAIR images. Contrast enhancement is variable. Differentiation from multiple sclerosis is possible only by the clinical course of the disease.

Subacute encephalitis can be caused by various agents and includes subacute sclerosing panencephalitis caused by the measles virus, progressive rubella panencephalitis, progressive multifocal leukoencephalopathy as a result of infection by the JC virus, HIV encephalitis, cytomegalovirus and Creutzfeldt-Jakob disease. HIV encephalopathy is caused by the HIV itself. It presents as a progressive subcortical dementia and is usually a manifestation of end-stage AIDS. Magnetic resonance imaging shows global atrophy and diffuse high-signal lesions in the deep white matter on T2-weighted images. Creutzfeldt-Jakob disease also presents with dementia as the predominant feature, accompanied by sensory changes, confusion and inappropriate behavior or cerebellar ataxia. Magnetic resonance imaging shows diffuse atrophy and may be useful in identifying gray matter lesions on T2-weighted images, FLAIR images and diffusion-weighted images.

Brain stem and spinal infection

Brain stem abscess is an uncommon condition that in the past was invariably fatal. Although the abscess may be visible on CT, MRI using T1-weighted gadolinium-enhanced images is superior. A cystic mass with ring-like enhancement is seen. Management of brain stem abscess always includes antibiotic therapy. Magnetic resonance imaging-guided stereotactic aspiration may be required to obtain a sample for microbial analysis and to decompress the lesion.

Staphylococcus aureus is the most common cause of bacterial spinal infection. This may be manifested as osteomyelitis, diskitis, epidural spinal abscess, perispinal abscess or myelitis. These phenomena may occur as a solitary manifestation or in combination with one another. Magnetic resonance imaging is the imaging modality of choice. T2-weighted and precontrast and post-gadolinium-enhanced T1-weighted images are obtained in the sagittal, axial and occasionally coronal planes.

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30.b When to do a lumbar puncture for evaluation of meningoencephalitis

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Case presentation

A 25-year-old man presents to the emergency department with a 2-day history of severe headache, fever and neck stiffness. On examination he has a temperature of 101°F (38.3°C), no rash and a normal mental status and neurologic examination. He has pain on neck flexion but is able to flex his neck fully. Kernig and Brudzinski signs are absent.

Discussion

Should a lumbar puncture be performed?

The clinical history and physical examination alone are not sufficient to make the diagnosis of infective meningitis. Findings on clinical examination that have been shown to be of value in assessing the possibility of acute meningitis in adults include fever (sensitivity 85%), meningism (sensitivity 70%) and altered mental status (sensitivity 67%); however, the specificity of each of these findings is poor. In sum, short of open meningeal biopsy, the analysis of cerebrospinal fluid (CSF) is the only test to establish reliably the diagnosis of infective meningoencephalitis.

The most common indication for a lumbar puncture (LP) is as a diagnostic tool for analysis of CSF when acute infectious meningitis is suspected. It is also employed when subarachnoid hemorrhage is suspected and the results of cranial computerized tomography (CT) scan are normal, in the investigation of demyelinating diseases and Guillain-Barré syndrome, and for performing spinal anesthesia. Instillation of radiologic contrast media for myelography, delivery of antimicrobial or chemotherapeutic agents to the subarachnoid space and therapeutic removal of CSF for the treatment of pseudotumor cerebri are less frequent indications.

What are the contraindications to lumbar puncture?

Contraindications to LP are as follows:

- ¦ LP is absolutely contraindicated if there is a known or suspected space-occupying lesion with mass effect, such as an intracranial tumor, hematoma or brain abscess, or massive brain edema, each of which greatly increases the risk of catastrophic post-LP rostral-caudal herniation — if intracranial mass lesions or brain edema are suspected clinically, then LP should be deferred until a CT scan can be done;
- ¦ severe uncorrected coagulopathy (e.g. International Normalized Ratio (INR) >1.5) or thrombocytopenia (platelet count <50,000/mm^3) is also a relative contraindication to LP, which should be deferred until the coagulation deficiency has been corrected — in a retrospective review of 956 children with acute leukemia who underwent LP, no hemorrhagic complications occurred if the platelet count was higher than 50,000/mm^3 ; and
- ¦ infection at the puncture site, such as a large lumbosacral decubitus ulcer, is also a contraindication to LP because of the risk of producing iatrogenic meningitis — in this circumstance, CSF can be safely obtained by a neuroradiologist through a C1–C2 cisternal puncture.

Complications of lumbar puncture

In general, LP is a safe procedure with rare serious complications, if performed properly. Post-LP headache is the most common complication, occurring in 11–25% of patients; it typically presents within hours after the procedure and can persist for weeks. Prospective studies have shown that use of a small styleted needle (20- or 22-gauge), directing the bevel of the needle parallel rather than perpendicular to the dural fibers, and replacing the stylet in the

TABLE 30.b-1 -- Complications of lumbar puncture.

COMPLICATIONS OF LP		
Complication	**Risk factors**	**Preventive measures**
Rostral-caudal herniation	Known intracranial mass lesion or suspicion of intracranial mass lesion: coma, papilledema, focal neurologic abnormalities, immunocompromised state, >60 years of age	Perform head CT before LP to rule out intracranial mass lesion
Iatrogenic meningitis	Passage of needle through infected tract	Avoid LP if soft tissue infection in lumbar area; in this circumstance, CSF may be obtained through a C1–C2 cisternal puncture
Post-LP headache	Use of a large-gauge needle	Use small (20- or 22-gauge) needle
	Directing needle perpendicular to dural fibers	Direct needle parallel rather than perpendicular to dural fibers
	Withdrawing needle without stylet	Replace stylet in needle before withdrawing
Epidural or subdural hematoma	Uncorrected coagulopathy (INR >1.5, platelet count <50,000)	Correct coagulopathy before performing LP
Trauma to periosteum, spinal ligaments and interverterbral disks	Traumatic tap, poor technique	Careful positioning of patient, attention to landmarks before performing LP, well-trained procedurist

needle before withdrawing can lessen the risk of post-LP headache. Although widely recommended, there is no evidence to suggest that prolonged recumbency after an LP or increased intake of oral fluids decrease the risk of post-LP headache.

In the absence of soft tissue infection at the puncture site, the risk of iatrogenic meningitis during an LP is extremely low, even in severely granulocytopenic or other immunocompromised patients, and fear of iatrogenic infection should never dissuade physicians from performing an LP for suspected meningitis.

Other rare complications of LP include subdural or subarachnoid hemorrhage, epidural hematoma (which can produce paraparesis), trauma to the spinal ligaments, periosteum or intervertebral disk, entrapment of nerve roots through the dural tear and pyogenic discitis or spondylitis. Correction of pre-existing severe coagulopathy (INR >1.5, platelet count < 50,000/mm^3) is mandatory before performing an LP.

The most feared complication of LP, although rare, is acute neurologic deterioration from brain herniation, which is commonly fatal.

Table 30b.1 summarizes the complications of LP and measures to prevent them.

When should a computerized tomography scan precede a lumbar puncture?

Concern for herniation prompts many physicians to consider a head CT scan as mandatory before an LP. When CT should be performed before LP remains a

controversial and hotly debated issue.

In a prospective study in 301 patients presenting to an emergency department and suspected of bacterial meningitis, Hasbun *et al.* (2001) used the following clinical features to define a group of patients in whom CT scan was most likely to yield abnormal findings and in whom CT should be considered mandatory before performing an LP:

- aged over 60 years;
- immunocompromised state;
- history of primary neurologic disease;
- history of seizure within the past week; and
- altered mental status and focal neurologic examination.

Of 235 patients who underwent cranial CT scan before LP, 76% had normal CT scan results; 34% of the ordering emergency room physicians stated that they considered doing a CT scan before any LP as a standard of care. In a subset of 96 patients none of whom were older than 60 years of age, had altered mental status, previous history of CNS disease or focal findings on examination, 93 had a normal CT scan, yielding a negative predictive value of 97%; in contrast, all four patients with significant mass effect were identifed by one or more of these clinical markers and LP was not carried out. It is noteworthy that there was a 1 hour delay in initiation of empiric antimicrobial therapy in patients undergoing CT before LP for suspected meningitis.

Another recent prospective study in 113 adults identified three statistically significant predictors of intracranial mass lesions:

- altered mentation (positive likelihood ratio (LR) 2.2, 95% CI 1.5–32);
- focal neurologic abnormalities (LR 4.3, 95% CI 1.9–10); and
- papilledema (LR 11.1, 95% CI 1.1–115).

These studies provide support for a more selective approach to the use of CT scan before LP. The vast majority of patients with symptoms suggestive of meningitis can safely undergo LP without a preceding CT scan; a CT scan is strongly recommended before performing an LP in patients with papilledema, altered mental status, focal neurologic abnormalities, an immunocompromised state or over 60 years of age.

It must be stressed, however, that if meningitis is strongly suspected clinically but LP will be delayed because of need for CT scan, empiric anti-infective therapy (i.e antibacterial or aciclovir) should be initiated without delay, after blood and other indicated non-neurologic cultures have been obtained.

What tests should be ordered on the spinal fluid?

The CSF should be collected in four sterile tubes. A minimum of 1ml of CSF is needed for the tests needed to diagnose bacterial meningitis reliably: cytospin Gram stain and culture, cell count and differential, glucose and protein. In most cases, the laboratory would prefer at least 3–5ml; if additional studies, such as antigen tests, fungal, mycobacterial or viral cultures, polymerase chain reaction (PCR) or cytology are desired, then up to 10–15ml will be needed.

Table 30b.2 summarizes the characteristics of CSF seen in selected intracranial infections and related conditions. Normal CSF should have less than 3–5 cells/ml, all of which should be lymphocytes or mononuclear cells, a protein of 15–45mg/dl and a glucose concentration two-thirds the concomitant serum value. The typical CSF profile in acute bacterial meningitis is polymorphonuclear pleocytosis, hypoglycorrhachia and elevated protein; a Gram stain of the cytospin CSF has a sensitivity of 90% if the LP is carried out before the administration of antibiotics. Table 30b.3 summarizes the tests on CSF of greatest value to diagnose neurologic infections.

In a retrospective review of 128 children with bacterial meningitis, Kanegaye *et al.* (2001) compared 39 patients who received empiric antimicrobial therapy before LP with 55 who underwent LP before receiving antimicrobial therapy. The authors found that

TABLE 30.b-2 -- CSF characteristics in selected neurologic conditions.

CSF CHARACTERISTICS IN SELECTED NEUROLOGIC CONDITIONS				
Condition	**Opening pressure (mmH_2O)**	**Cell count**	**Protein (mg/dl)**	**Glucose**
Normal	50–150	0–5 lymphocytes	15–45	2/3 of serum glucose
Bacterial meningitis	Elevated	200–10,000 cells, 95% PMNs	Elevated, usually >100	Low (<50mg/dl; <2.8mmol/l)
Viral meningoencephalitis	Elevated	6–1000 cells, lymphocyte predominance but may show PMNs early	Elevated, usually <100	Normal
Tuberculous or fungal meningitis	Elevated	10–500, lymphocyte predominance	Greatly elevated, 100 to >3000	Low (<50mg/dl; <2.8mmol/l)
Brain abscess	Elevated	Elevated, usually <500, lymphocyte predominance, PMNs if rupture into ventricle	Elevated, usually <100	Normal
Subarachnoid hemorrhage	Normal or elevated	Mildly elevated, lymphocyte predominance, xanthochromia	Elevated	Normal or low
Guillain-Barré syndrome	Normal	Normal	Elevated	Normal
PMN, polymorphonuclear cell				

TABLE 30.b-3 -- Diagnosis of neurologic infections by analysis of CSF.

DIAGNOSIS OF NEUROLOGIC INFECTIONS BY ANALYSIS OF CSF			
Condition	**Diagnostic test**	**Sensitivity (%)**	**Specificity (%)**
Bacterial meningitis	Cytospin Gram stain	60–90	100
	Culture	90	100
	Antigen detection assays*	50–100	100
Viral meningoencephalitis	Culture	<50	100
	PCR†	94	94–100
Tuberculous meningitis	Acid-fast stain	10–22	100
	Culture	38–88	100
	PCR	27–85	95–100
Fungal meningitis	Cryptococcal antigen	85	99–100
	Coccidioides immitis antibody	55–95	100
	Histoplasma capsulatum antigen	60	Moderate-high
	H. capsulatum antibody	Moderate	Moderate
	B. dermatidis antibody	Low	Moderate

Toxoplasma encephalitis	*Toxoplama gondii* antibody	50–70	99
	PCR	11–77	100

* Available for *Hemophilus influenzae* type B, *Neisseria meningiditis, Streptococcus pneumoniae, Streptococcus agalactiae*
† Available for enterovirus, herpes simplex virus, Epstein-Barr virus, cytomegalovirus, West Nile virus and varicella-zoster virus

in the case of meningococcus, CSF sterilization occurred within 2 hours of receipt of antimicrobial therapy, whereas with pneumococcus, CSF sterilization occurred within 4 hours. While antimicrobial therapy should never be withheld if there is a delay in performing LP, every effort should be made to expeditiously obtain CSF for an early definitive diagnosis. Previous antibiotic therapy is unlikely to alter the biochemical and cellular CSF abnormalities in bacterial meningitis.

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30.c Approach to the patients who has fever and headache

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Introduction

Fever and headache are common presenting signs with a wide differential diagnosis. The physician needs to be able to recognize the early features of bacterial meningitis and initiate appropriate treatment without delay as the consequences of a missed diagnosis or inadequate treatment can be fatal. Investigations for other causes of fever and headache can be initiated once meningitis has been excluded.

Pathogenesis

Inflammation of the meninges may result from a number of pathologic processes including infection, acute vasculitis, malignant infiltration and subarachnoid hemorrhage. Organisms enter the meninges via the nasopharyngeal mucosa or after blood-borne spread. Direct invasion can follow skull fracture or neurosurgery. Relatively poor host defenses and low complement levels in the cerebrospinal fluid (CSF) may facilitate the spread of infection.

Bacterial meningitis is associated with acute inflammation stimulated by bacterial antigens and cytokines. This results in a fibrinous neutrophilic exudate across the leptomeninges and may be associated with the systemic features of sepsis syndrome and circulatory collapse. The increased permeability of the blood-brain barrier, raised intracranial pressure and vasculitic changes around the vessels traversing the subarachnoid space may result in brain ischemia. Tuberculous and fungal infections tend to have a more insidious onset but they may begin fairly abruptly. A CSF pleocytosis with negative Gram stain and culture is termed an aseptic meningitis (Table 30c.1). Viral infections cause disease either by direct invasion of the leptomeninges and brain or indirectly by postinfective immune-mediated phenomena. Brain abscess should also be considered (see Chapter 24) and can have a variable microbial etiology. Occasionally, an abscess may rupture and involve the meninges to cause meningitis.

Microbiology

Fever and headache may be nonspecific features of many infections but they may result from infection of the meninges (meningitis) or brain tissue (encephalitis). The organisms causing bacterial meningitis vary with age and geographic region (Table 30c.2). *Neisseria meningitidis*,

TABLE 30.c-1 -- Some causes of lymphocytic cerebrospinal fluid.

SOME CAUSES OF LYMPHOCYTIC CEREBROSPINAL FLUID	
Viruses	Enteroviruses
	Mumps virus
	Herpes simplex virus
Bacteria	Partially treated bacterial meningitis
	Early bacterial meningitis
	Cerebral abscess
	Tuberculosis
	Brucellosis
	Spirochetes (treponemes, leptospira, *Borrelia* spp.)
Fungi	Cryptococcosis
	Histoplasmosis
Protozoa	Toxoplasmosis
	Amebiasis
Inflammatory conditions	Seropositive conditions (e.g. lupus)
	Seronegative conditions (e.g. Behçet's syndrome, Kawasaki disease)
Chemicals	Irritants
Carcinomatous conditions	Usually secondary deposits

Streptococcus pneumoniae and *Haemophilus influenzae* type b cause about 80% of cases of adult meningitis.

The incidence of *H. influenzae* meningitis in children has declined dramatically following the introduction of *H. influenzae* type b vaccine. Pneumococcal meningitis may follow an initial otitis media, sinus infection or pneumonia and most cases occur in infants and the middle-aged or elderly. Meningococcal meningitis is most common in infants, children and young adults. Outbreaks of infection occur in nurseries, schools, universities and residential accommodation. Cases of meningococcal infection occur more frequently during the winter months and may be associated with influenza infection. In Europe and North America meningococci belonging to groups B and C most

TABLE 30.c-2 -- Likely organisms and possible empiric treatment regimens for bacterial meningitis.

LIKELY ORGANISMS AND POSSIBLE EMPIRIC TREATMENT REGIMENS FOR BACTERIAL MENINGITIS		
Group of patients	**Likely organisms**	**Treatment**
Neonates	Group B streptococci	Ampicillin and gentamicin
	Escherichia coli	Cefotaxime
	Listeria monocytogenes	
Children	*Neisseria meningitidis*	Ceftriaxone
	Streptococcus pneumoniae	Cefotaxime (vancomycin if resistant pneumococcus)
	Haemophilus influenzae	

Adults	*Neisseria meningitidis*	Ceftriaxone (vancomycin if resistant pneumococcus)
	Streptococcus pneumoniae	
	Haemophilus influenzae	
	Listeria monocytogenes	Ampicillin
Elderly persons/underlying malignancy	*Neisseria meningitidis*	Ceftriaxone (vancomycin if resistant pneumococcus)
	Streptococcus pneumoniae	
	Haemophilus influenzae	
	Listeria monocytogenes	Ampicillin
	Gram-negative organisms	
Posttraumatic surgery	*Streptococcus pneumoniae*	Ceftriaxone
	Haemophilus influenzae	Ceftazidime
	Escherichia coli	Carbapenem
	Klebsiella pneumoniae	
	Enterobacter spp.	
	Pseudomonas spp.	
	Staphylococcus aureus	

commonly cause disease, whereas group A strains have been responsible for serious outbreaks of infection in sub-Saharan Africa. Typing of infection is relevant because the current meningococcal vaccine gives protection against groups A and C strains but has no effect on group B disease.

Rare causes of bacterial meningitis include *Listeria monocytogenes*, which is associated with pregnancy and underlying immunosuppression or malignancy. Following trauma or neurosurgery, meningitis caused by Gram-negative organisms such as *Escherichia coli, Pseudomonas* spp., *Klebsiella pneumoniae* and *Enterobacter* spp. can occur.

Viral meningitis is predominantly caused by the enteroviruses (70%), including Coxsackie viruses A and B and the echoviruses. The mumps virus causes about 10% of the diagnosed cases of viral meningitis in the UK with the remainder being caused by herpes simplex virus, varicella-zoster virus, measles virus, adenoviruses or Epstein-Barr virus. Herpes simplex encephalitis can cause necrotic edematous changes that may be asymmetric and localized in the temporal lobes.

Clinical features

The cardinal features of meningeal inflammation are:

- headache,
- neck stiffness,
- photophobia.

Meningococcal meningitis and sepsis are associated with a petechial or purpuric rash. An exanthem may be present in viral infections. The history can often localize the source of infection or may suggest a generalized febrile illness. The presence of intracranial shunts, previous head trauma, recent travel history and underlying immunosuppression will influence the range of potential pathogens. Symptoms and signs of meningitis may be nonspecific in very young or elderly patients. Wakening or early morning headache, with or without vomiting, that is worse on coughing and bending forward or that is associated with visual disturbance are symptoms of raised intracranial pressure.

On examination, vital signs must be documented and the skin should be fully exposed to look for a rash and for the presence of cervical or other lymphadenopathy. Signs of meningism should be sought, including evidence of neck stiffness, photophobia and the presence of Kernig's sign. To elicit Kernig's sign, the patient is placed supine and the lower limb is flexed at the hip and the knee is extended. Patients who have meningism resist by contracting the hamstrings. However, a prospective study of 297 adults admitted with suspected meningitis found that the diagnostic accuracy of nuchal rigidity, Kernig's and Brudzinski's signs was of limited value and not correlated with the presence of moderate meningeal inflammation, highlighting the need for clinical vigilance in presenting patients of all ages. Assessment of focal neurological signs, including cranial nerve assessment and fundoscopy for papilledema, is important. Examination of the tympanic membrane may reveal an underlying otitis media. Urinary dipstick examination should be performed. Acute meningococcal meningitis associated with a florid purpuric rash is usually unmistakable, but patients may be relatively well with equivocal signs or have a more insidious illness.

Investigations

The aim of investigation is to establish the cause of fever and headache and to identify any infecting organisms. A blood count is helpful because bacterial infections are usually accompanied by a neutrophilia. Biochemical and clotting profiles can indicate the development of systemic complications. Cultures of body fluids (blood, sputum, urine, CSF, throat swab and skin scrapings) may reveal the causative pathogen. Antigen detection kits are also available and give rapid results. Meningococcal, tuberculosis, herpes simplex and enterovirus genome detection by the polymerase chain reaction (PCR) is now available from reference laboratories. Comparison of meningococcal antibody titers in acute and convalescent serum samples can yield a retrospective diagnosis.

Chest and sinus radiographs may be helpful in revealing underlying infection. Computed tomography or MRI scans of the head can identify the presence of cerebral edema, hydrocephalus or a mass

effect and exclude alternative diagnoses of intracranial hemorrhage, subdural collection or abscess formation. Examination of the CSF remains important in many cases; routine analysis includes cell count, protein and glucose estimation, culture and Gram stain.

Acute bacterial meningitis is usually associated with polymorphonuclear leukocytosis in the CSF with raised protein and low glucose levels. Viral meningitis characteristically gives a lymphocytosis with normal protein and glucose levels. However there is a wide differential diagnosis of a lymphocytic CSF, which can be investigated by Ziehl-Neelsen and India ink staining, cytology, direct immunofluorescence and viral PCR. A low CSF glucose (<2/3 serum glucose) usually indicates a bacterial, fungal, tuberculous or carcinomatous cause.

Management

Immediate management

The possibility of meningitis should be considered in all patients who have fever and headache. Patients who appear reasonably well with no obvious source of infection need to be observed closely because the signs of meningitis may evolve very rapidly.

If bacterial meningitis is suspected, immediate hospital admission should be arranged and blood cultures and intravenous antibiotic therapy should be instituted without delay. Resuscitation may be required and all patients who have suspected or confirmed bacterial meningitis should be closely monitored, preferably in a high-dependency or intensive care unit.

There is compelling evidence that immediate empiric antibiotic therapy can improve the outcome of meningococcal sepsis and meningitis. In the UK, general practitioners are advised to give intravenous or intramuscular benzylpenicillin to any patient who has suspected bacterial meningitis. The only contraindication is a

previous anaphylactic reaction to penicillin.

Following admission and any immediate resuscitation, the major question for the clinician managing the patient is whether to do a head scan (CT or MRI) or a lumbar puncture.

Role of head scanning

Computed tomography and MRI scanning are unhelpful in uncomplicated viral meningitis, when it is safe to perform a lumbar puncture.

A prospective study of 301 adults admitted with suspected meningitis identified that immunocompromised status, age >60 years and presence of neurological abnormalities or seizure were associated with abnormal CT head scans. CT scanning in those without these features had a negative predictive value of 97% and of the misclassified patients, all underwent safe lumbar puncture, suggesting that the use of CT screening could be better targeted. The indications for performing an urgent scan before lumbar puncture include focal neurological signs, altered level of consciousness, papilledema or symptoms suggestive of raised intracranial pressure, convulsions and suspected subarachnoid hemorrhage.

Role and safety of lumbar puncture (see Practice Point 30b)

The safety of lumbar puncture in patients who have bacterial meningitis has recently been questioned because occasional patients who have unsuspected cerebral edema have developed brainstem coning and death after the procedure. This occurs most commonly in patients who have fulminant meningococcal disease and early lumbar puncture should therefore be avoided in patients who have purpuric rash. Lumbar puncture may be considered at a later stage in these patients if there has been no improvement on empiric treatment.

The value of lumbar puncture is also declining with increasing use of antibiotics before the procedure and the availability of newer diagnostic techniques. Nevertheless, the lumbar puncture remains a very important investigation in patients who have suspected viral meningitis, in whom it allows the diagnosis to be established, and in the atypical causes of meningitis. The Consensus Statement on diagnosis, investigation, treatment and prevention of acute meningitis in adults published by the British Infection Society recommends lumbar puncture in all suspected patients except where there is a clear contraindication (including signs of raised intracranial pressure, focal neurological deficit, severe shock, depressed conscious level or coagulation disorder) or a confident clinical diagnosis of meningococcemia. If the CSF is clear, further investigations should be performed to establish the cause of fever and headache. In these circumstances a chest radiograph is helpful to exclude pneumonia and urinalysis is useful to exclude a urinary tract infection, because these conditions may present with signs of meningism.

Choice of antibiotic therapy (see also Chapter 22)

Empiric therapy should cover all likely pathogens; treatment can then be modified if any organisms are identified. When considering antibiotic choice, the agent used must penetrate the CSF to achieve an adequate inhibitory concentration. Antibiotic penetration is initially good because the blood-brain barrier is impaired owing to the inflamed meninges, but as the disease resolves penetration becomes less. Ceftriaxone and cefotaxime are third-generation cephalosporins that will cover the meningococcus, the pneumococcus, *H. influenzae* and most other organisms (see Table 30c.2) and they are frequently used as empiric therapy. Cephalosporins have little activity against *Listeria* spp. and ampicillin should be added if listeriosis is suspected. Penicillin-resistant pneumococci are occurring with increasing frequency in many areas and benzylpenicillin is therefore inadequate as empiric therapy for bacterial meningitis. Vancomycin should be added in communities where there is significant resistance to penicillin among isolates of *S. pneumoniae*.

The treatment of lymphocytic meningitis is difficult because the diagnosis is often uncertain and there is a wide range of potential pathogens. It is occasionally necessary to treat patients with empiric antibiotics, antiviral therapy (aciclovir), antituberculous therapy and antifungal therapy, with the possible addition of corticosteroids. Patients who have lymphocytic meningitis can present a major diagnostic and therapeutic challenge.

Role of corticosteroids

Early dexamethasone treatment has been shown to be associated with an improved outcome in meningitis caused by *H. influenzae*, and possibly *S. pneumoniae*, but the effect of corticosteroids is less clear in other forms of bacterial meningitis. Clinicians differ widely in their practice; however, it seems sensible to give corticosteroids as early as possible to have a maximal effect.

Management of the complications of meningitis

Bacterial meningitis may be accompanied by all the features of sepsis syndrome, with multiple organ failure requiring intensive care support. It has been suggested that hemofiltration may have a therapeutic role by removing bacterial toxins and unwanted cytokines, although there are no controlled studies. A sensible approach is to consider early hemofiltration in seriously ill patients who have hypotension and oliguria. The role of extracorporeal membrane oxygenation is also uncertain, but this should be considered if the patient is failing to respond to conventional ventilation and circulatory support.

Seizures are managed with anticonvulsants or sedation and ventilation. Metastatic seeding of infection can form cerebral or epidural abscesses and progress to a subdural empyema. The blockage or interruption of CSF circulation at the foramina, aqueduct or subarachnoid granulations can produce hydrocephalus. Raised intracranial pressure can result in sinus thrombosis, cerebral edema with herniation, cranial nerve palsies or brain ischemia. Sensorineural deafness occurs in

about 10% of patients who have pneumococcal meningitis and a smaller proportion of patients who have meningococcal meningitis, and audiologic assessment is recommended for all patients who have recovered from bacterial meningitis.

Public health issues

Public health physicians should be notified immediately of any patients who have known or suspected meningitis so that appropriate action can be taken. Cases of meningitis are often associated with considerable local publicity and anxiety in the community. The role of the public health physician is to provide appropriate information and to organize antibiotic prophylaxis for close contacts of patients who have meningococcal or *Haemophilus* meningitis and more extensive prophylaxis and vaccination campaigns if these are indicated. There are published national guidelines for the management of clusters or epidemics of meningococcal disease.

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30.d Empiric antimicrobial therapy for suspected infection of the central nervous system

Marc A Tack

Introduction

Bacterial infections of the central nervous system (CNS) can vary from rapidly life-threatening illnesses such as pneumococcal meningitis to more insidious infections such as neuroborelliosis and cryptococcal meningitis. The selection of an appropriate initial antimicrobial therapy is frequently empiric. The key clinical determinants of antimicrobial selection are the age of the patient, the immune status, a history of trauma or instrumentation and the clinical presentation. A detailed history including the use of immunosupressing agents such as corticosteroids is necessary to adequately assess a patient's risk of immune dysfunction as this will result in an expanded list of potential etiologic agents and thus alter the selection of empiric therapy. A previously healthy adult who has acute bacterial meningitis poses different etiologic considerations from those of a patient with asthma on long-term prednisone complaining of fever and a headache. Similarly, if the blood-brain barrier has been compromised, such as in the case of placement of a ventriculostomy or epidural pump or other surgical manipulation, empiric antimicrobial therapy must be targeted toward pathogens that gain entry by these routes.

Pathogenesis

There are several mechanisms of infection that account for most CNS infectious processes. Hematogenous spread in patients with bacteremia can lead to bacterial seeding and acute meningitis. Direct extension of local infections, such as sinusitis and otitis, have been implicated as frequent causes of brain abscesses. Surgical manipulation such as the placement of a ventriculoperitoneal shunt may allow direct seeding of the CNS. Lastly, trauma may provide a portal of entry for direct inoculation of pathogens.

Microbiology

The most frequent pathogens associated with bacterial meningitis in a previously healthy host are *Streptococcus pneumoniae* and *Neisseria meningitidis*. The emergence of strains of *Strep. pneumoniae* highly resistant to penicillin and ceftriaxone has major implications in the selection of empiric treatment of meningitis. *Haemophilus influenzae*, formerly a frequently isolated organism, is now rarely implicated owing to routine pediatric immunization. In neonates, *Streptococcus agalactiae* is frequently isolated. In elderly or immunocompromised patients, *Listeria monocytogenes* must be included in the differential diagnosis of acute bacterial meningitis (Table 30d.1).

Patients with inplanted foreign bodies or who have undergone recent neurosurgical procedures are at increased risk for infections caused by *Staph. aureus* and coagulase-negative staphylococci, although a host of other organisms including fungi have been reported. Diphtheroids and other common skin flora may cause infection in patients with recent manipulation of the CNS, including epidural injections. Aerobic Gram-negative bacilli have also been encountered in posttrauma and postoperative patients. A detailed review of bacterial meningitis is found in Chapter 22 .

Clinical features

The most common presenting clinical features of acute bacterial meningitis are fever, headache and meningismus. This triad of symptoms is seen in over 85% of cases and should be easily recognizable to the experienced clinician. Confusion and altered sensorium are also frequently noted. Physical examination frequently reveals nuchal rigidity, a positive Kernig's or Brudzinki's sign (although see comment in practice point 30c, above) and the presence of photophobia.

TABLE 30.d-1 -- Common bacterial pathogens associated with predisposing factors for meningitis.

COMMON BACTERIAL PATHOGENS ASSOCIATED WITH PREDISPOSING FACTORS FOR MENINGITIS	
Predisposing factor	**Common bacterial pathogens**
CFS shunt	Coagulase-negative staphylococci, *Staphylococcus aureus*, Gram-negative bacilli
Postneurosurgery or post-trauma	*Staphylococcus aureus*, coagulase-negative staphylococci, Gram-negative bacilli
Immuncompromise	*Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes*, Gram-negative bacilli
Skull fracture	*Streptococcus pneumoniae*, group A streptococci, *Staphylococcus aureus*

Presumptive evidence of meningococcal meningitis may be apparent on physical examination. Of patients who have meningococcemia, with or without meningitis, 50% manifest a macular erythematous papular rash early in the course of the disease. This rash rapidly appears petechial and then becomes purpuric.

In neonates, the presentation of acute bacterial meningitis may be subtle. In this group, meningismus is infrequently noted and irritability and temperature instability may be the only indications of meningitis. Refusal to feed, lethargy, a high-pitched cry, vomiting, diarrhea and respiratory distress should alert the clinician to this diagnosis.

Elderly patients, especially those who have co-morbid disease, as well as immunosuppressed hosts often present with insidious manifestations. Fever is frequently absent and headache, confusion or altered level of consciousness may be the only clinical finding.

Other less common causes of meningitis may frequently present with varying clinical syndromes. Cryptococcal meningitis in a patient who has AIDS typically presents as an insidious illness; persistent fever and headache over several days or even weeks is often the only clinical complaint. Lyme meningitis, more common in highly endemic areas, often presents as a subacute basilar meningitis with cranial nerve palsies and radiculopathy as common findings. Tuberculous meningitis may also present in an insidious manner, necessitating a high index of suspicion for early recognition and successful treatment.

The clinical manifestations of a brain abscess are usually more attributable to the space-occupying effect of the lesion than to the actual infection. The classic triad (fever, headache and a focal neurologic deficit) is seen in only 50% of patients. Headache, which is usually moderate or severe, is the most common manifestation; the next most common is fever. Eventually, altered mentation, ranging from confusion to coma, ensues. Neurologic findings may be focal, but this depends on the size and location of the abscess. Seizures as well as papilledema are more common in brain abscess than in meningitis.

Infections of the CNS in patients with recent neurosurgical manipulation or with foreign inplanted devices may vary in clinical presentation depending upon the etiologic agent. A patient with a foreign body infection caused by *Staph. aureus* infection will likely appear toxic with fever and other common signs of meningeal inflammation while infection from coagulase-negative staphylococci may only cause complains of a mild headache over several days. Clinical obstruction of a previously functioning venticular drainage shunt should raise suspicion for infection and warrants evaluation of the CSF.

Investigations

Early diagnosis and rapid initiation of appropriate antimicrobial therapy are essential in patients who present with manifestations of acute CNS infection. A lumbar puncture should be performed in all patients unless there are specific contraindications to this procedure. Patients who present with papilledema or focal neurologic findings should undergo a CT scan before lumbar puncture to determine whether there is a space-occupying lesion under increased intracranial pressure; it will determine the presence of a brain abscess in most cases as well. In patients who have a shunt or reservoir, cerebrospinal fluid (CSF) samples may be obtained from

these sites.

Samples of CSF should be examined, preferrably using a cytospin technique, for the presence of bacterial and fungal pathogens using appropriate stains. Glucose and protein levels in the CSF should be obtained together with a simultaneous serum glucose level for comparison. Cultures of CSF for bacterial, fungal and mycobacterial organisms should be obtained. If cryptococcal meningitis is suspected, a cryptococcal antigen assay should be performed on CSF as well. Bacterial latex agglutination tests are frequently used but are of limited benefit in clinical practice owing to their high false-negative rate.

TABLE 30.d-2 -- Common bacterial pathogens causing meningitis according to the age of patient.

COMMON BACTERIAL PATHOGENS CAUSING MENINGITIS ACCORDING TO THE AGE OF PATIENT	
Age of patient	**Most common bacterial pathogens**
0–4 weeks	*Streptococcus agalactiae, Escherichia coli, Listeria monocytogenes, Klebsiella pneumoniae*
4 weeks to 2 years	*Streptococcus agalactiae, Streptococcus pneumoniae, Haemophilus influenzae*
2–18 years	*Haemophilus influenzae* (decreasing), *Neisseria meningitidis, Streptococcus pneumoniae*
18–60 years	*Streptococcus pneumoniae, Neisseria meningitidis*
Over 60 years	*Streptococcus pneumoniae, Listeria monocytogene*s, others

In addition to studies of the CSF, blood cultures should be sent in all cases because of the high rate of concomitant bacteremia. Other specimens, such as sputum, urine and sinus aspirates, should be obtained and evaluated when clinically appropriate.

Management

The rapid initiation of appropriate antimicrobial therapy in acute bacterial meningitis is imperative. The initiation of antibiotics should not be delayed while awaiting an imaging study before lumbar puncture. In general, the patient should receive the first dose of antibiotics within 30 minutes of arrival at the hospital if bacterial meningitis is suspected. Appropriate therapy should be based on the age, clinical presentation and predisposing factors and therapy should be modified if indicated when the etiologic agent is identified (Table 30d.2).

The empiric antibiotic of choice for acute bacterial meningitis in adults is a third-generation cephalosporin (ceftriaxone or cefotaxime) and vancomycin. In patients aged under 3 months or over 60 years, ampicillin should be included in the empiric regimen for the treatment of potential listeriosis. In the past several years the incidence of resistance of community isolates of *Strep. pneumoniae* to penicillin has risen to the point where I believe that vancomycin should be included in the initial empiric treatment. Therapy can be adjusted when antimicrobial sensitivities of the cultured isolate become available.

Patients who develop a CNS infection after trauma or postoperatively are at higher risk of Gram-negative bacillary infections. An antipseudomonal cephalosporin (ceftazidime or cefepime) in combination with vancomycin is an appropriate empiric regimen. Vancomycin is used because of the frequent ß-lactam resistance seen with coagulase-negative staphylococci, which are often identified as the pathogen in this setting. This is also the empiric regimen I suggest for

TABLE 30.d-3 -- Empiric antibiotic regimen based on clinical presentation.

EMPIRIC ANTIBIOTIC REGIMEN BASED ON CLINICAL PRESENTATION	
Acute meningitis — previously healthy adult	Third-generation cephalosporin and vancomycin
Post neurosurgery or trauma	Antipseudomonal cephalosporin and vancomycin
Brain abscess	Third-generation cephalosporin and metronidazole
Foreign body or recent manipulation	Antipseudomonal cephalosporin and vancomycin

patients who have foreign bodies in place. In patients with a reservoir, direct administration of vancomycin may be utilized (5–10mg initially, diluted in preservative-free saline) and in patients with organisms difficult to eradicate, intrathecal antimicrobial treatment should be considered.

In patients who have a brain abscess, streptococci, Gram-negative bacilli and anaerobes are frequent pathogens and empiric antimicrobial therapy must target these pathogens and penetrate purulent collections. A third-generation cephalosporin (ceftriaxone or cefotaxime) in combination with metronidazole is useful in this clinical setting. The diagnosis and treatment of brain abscess is presented in detail in Chapter 24 .

Although there are no controlled clinical trials, most clinicians treat acute bacterial meningitis for 7–14 days. The duration of therapy for brain abscesses is prolonged and determined by clinical and radiographic response. AIDS patients who are infected with cryptococcal meningitis required prolonged therapy followed by chronic suppression (see Chapter 126 and Chapter 237 for further discussion).

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Chapter 31 - Pharyngitis, Laryngitis and Epiglottitis

Dennis A Clements

This chapter describes some of the most common infections of mankind, the upper respiratory infections, which include pharyngitis, laryngitis and epiglottitis. Although viruses play a significant role in the pathogenesis of many of these infections, bacteria and other organisms are responsible for many others. This chapter details the importance of these infections and provides a practical guide to diagnosis and management.

EPIDEMIOLOGY

Definition and nomenclature

Pharyngitis, commonly called 'sore throat', is an inflammatory process of the pharynx, hypopharynx, uvula and tonsils that can be caused by viral or bacterial pathogens, and occasionally both (Table 31.1). Pharyngitis can be separated into one group of illnesses with associated nasal symptoms (which are most commonly viral in origin) and another that cause only pharyngitis. It is important to distinguish between these infections because rheumatic fever and acute glomerulonephritis may complicate untreated group A ß-hemolytic streptococcal infections, but they can usually can be prevented by appropriate antibiotic treatment. Parapharyngeal abscesses and Ludwig's angina are discussed in Chapter 41 .

Laryngitis, or inflammation of the larynx (subglottic area), is almost always secondary to a viral infection. Lower voice pitch, hoarseness and aphonia frequently occur. It is important to differentiate laryngitis from other infectious forms of obstructive airway disease, such as epiglottitis, which may be life threatening. Laryngitis in small children often gives a 'croup' syndrome. Children with laryngitis are often noted to 'bark' like seals. These illnesses are generally self-limiting and require only supportive care.

Epiglottitis (bacterial cellulitis of the epiglottis), or supraglottitis, is an inflammatory process of the epiglottis and/or supraglottic structures that is almost always caused by *Haemophilus influenzae* type b (HIB). It is usually rapidly progressive, particularly in young children, and needs immediate antibiotic treatment and often intubation to avoid possible respiratory obstruction.

Incidence, prevalence and seasonality

Infections of the upper respiratory system are more common than any other acute infectious malady. The self-limiting viral infections are most frequently caused by adenoviruses, rhinoviruses, coronaviruses, enteroviruses and parainfluenza viruses (see Table 31.1). Other viral infections, such as respiratory syncytial virus (RSV) and Epstein-Barr virus (EBV), are less common but still frequently occur. Bacterial causes of upper respiratory infections are led by group A ß-hemolytic streptococci (GAS), but can also be caused by *Haemophilus influenzae, Bordetella pertussis, Chlamydia pneumoniae, Corynebacterium haemolyticum, Mycoplasma pneumoniae* and *Yersinia enterocolitica* among others.

These upper respiratory infections are often difficult to differentiate, and hence difficult to diagnose, frequently leading to futile overtreatment in many cases. Further complicating the issue is the fact that primary viral infections are often succeeded by secondary 'opportunistic' bacterial infections, making undertreatment a problem in a significant minority of infections. Additionally, individuals with allergies or structural defects are sometimes more prone to secondary bacterial infections. The clinician is therefore challenged to weigh multiple factors involved in deciding whether an infection is viral or bacterial in origin, and whether antibiotic treatment is warranted.

It is estimated that children in day care in the USA have an upper respiratory infection approximately every 3 weeks from the age of 6 months to 2 years. [1] The incidence then decreases so that by the time of school entry a child has about 3–6 episodes of upper respiratory infection per year. Most of these infections include pharyngitis and/or laryngitis. Young children often have pharyngitis on inspection but do not complain of sore throat; this symptom is more common in adolescents and adults. Often the only sign of pharyngitis in young children is refusal to eat and/or drink.

Viral upper respiratory infections frequently occur in miniepidemics (RSV, parainfluenza, influenza, varicella, measles). They are more common in the winter except for those caused by enteroviruses, which are more common in the summer. [2] Some viral infections occur year round, with no seasonal pattern (adenoviruses). Group A ß-hemolytic streptococcal infections are more common in the winter, but many other nonviral respiratory infections do not appear to be seasonally linked (*Chlamydia* and *Mycoplasma* spp.). Some bacterial infections appear to be linked to preceding viral infections and hence occur more commonly in the winter. Pharyngeal colonization may occur throughout the year. Fortunately, epiglottitis is rare now that HIB immunization is routine for infants in many countries.

Morbidity, mortality, historic change and risk factors

Influenza infections vary significantly from year to year. In the USA one subtype was predominant each year until about 1990, since which time both H3N2 and H1N1 strains have been circulating simultaneously. When there is a major shift in antigen type, significant excess morbidity occurs as the new strain infects the community. Increasing air travel has accelerated the rate at which the influenza viruses travel around the world and has perhaps been responsible for the increasing frequency with which the viruses are detected. The shifted strain outbreaks have most affected the elderly and children with congenital heart and lung disease. Minor influenza drifts have occurred also, but cause less disease. There has also been a recent decrease in the average age at which children acquire upper respiratory diseases because of the increasing use of day care for children. The long-term effect of this is unknown. In the short term it appears that it has been responsible for a significant increase in the number of ear infections in children less than 2 years old.[3]

Rheumatic fever, a complication of group A ß-hemolytic streptococcal pharyngitis, has waxed and waned in importance.[4] After a century of prominence, the disease was in considerable decline in developed countries for 40 years. Recently, clusters of rheumatic

TABLE 31-1 -- Common causes of pharyngitis, laryngitis and epiglottitis.

COMMON CAUSES OF PHARYNGITIS, LARYNGITIS AND EPIGLOTTITIS							
	Organism/entity	**Disease/syndrome**	**Pharyngitis**	**Nasopharyngitis**	**Laryngitis**	**Epiglottitis**	**Complications**
Bacteria	*Arcanobacterium haemolyticum*	Pharyngitis +/- scarlatiniform rash	++				
	Corynebacterium diphtheriae	Diphtheria with pseudomembrane	+++				
	Corynebacterium spp. (other)	Pharyngitis	++	+++			
	Haemophilus influenzae	Pharyngitis	+++				
	Legionella pneumophila	Pharyngitis	++	++		+++ (type b)	Epiglottitis, meningitis
	Neisseria gonorrhoeae	Pharyngitis	++				Pneumonia
	Neisseria meningitidis	Pharyngitis	++				Septic arthritis
	Streptococcus pyogenes (group A ß-hemolytic)	Pharyngitis (scarlet fever)	+++++				Meningitis, sepsis
	Streptococcus spp. (groups B, C and G)	Pharyngitis	+++		+		Rheumatic fever, acute glomerulonephritis
	Treponema pallidum	Secondary syphilis	+		+		
	Yersinia entercolitica	Pharyngitis and enterocolitis	++		+		

Viruses	Adenoviruses	Nasopharyngitis		+++			Pneumonia
	Coronavirus	Nasopharyngitis		++			
	Coxsackie A virus	Herpangina	++	++			
	Cytomegalovirus	Mononucleosis syndrome		+++			Prenatally: birth defects
	EBV	Mononucleosis syndrome	++	+++			
	HSV	Pharyngitis with ulcerations	+++		++		Systemic disease
	HIV-1	Pharyngitis and lymphadenopathy	++				AIDS
	Influenza A and B	Nasopharyngitis, myalgia, headache		++			Pneumonia
					+++		
	Measles	Measles disease	+++	+++			Conjunctivitis, pneumonia, meningitis
	Parainfluenza viruses	Cold and croup		++	+++		Pneumonia
	Reoviruses (1–3)	Nasopharyngitis (uncommon)	+	+			
	RSV	Pharyngitis		+++			Bronchiolitis
	Rhinovirus	Nasopharyngitis		+++			
Other organisms	*Candida* spp.	Pharyngitis	++				
	Chlamydia pneumoniae	Pharyngitis	++		+		Pneumonia
	Coxiella burnetii	Nasopharyngitis		+			
	Mycoplasma pneumoniae	Pharyngitis	++	+			Pneumonia
Unknown etiology	Aphthous stomatitis	Ulcerative gingivitis	+				
	Behçet's syndrome	Pharyngitis with ulcerations	+++				
	Kawasaki disease	Pharyngitis, conjunctivitis	+++				Coronary artery aneurysms
	Stevens-Johnson syndrome	Pharyngitis, stomatitis, ulcerations	+++				Shock

fever cases have occurred, for example in Salt Lake City, Utah, USA, where it is hypothesized that the re-emergence of certain M-types has been responsible.

Before HIB vaccination was instituted there were approximately 20,000 cases of HIB disease each year in the USA. Since the advent of HIB immunization the incidence of this disease has decreased by 95%.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of the sore throat due to pharyngitis is poorly understood. Volunteers given rhinoviral infections produce bradykinin and lysylbradykinin, which are known inflammatory mediators that can excite nerve endings in the pharynx to cause pain.[5] There is also suggestive evidence from laboratory animals that adenovirus, RSV and other viral infections directly invade the pharyngeal cells and produce an inflammatory response. This leads to the well-described 'red, sore throat'. Additionally, adenovirus and EBV often produce lymphoid hyperplasia and tonsillar exudation. Herpes simplex virus (HSV) and coxsackievirus infections frequently lead to ulcerations of the oral mucosa. Herpes simplex virus ulcers are more common in the anterior part of the mouth and coxsackievirus ulcers occur more frequently in the posterior part of the pharynx, but this is only a guide and both viruses can cause ulcers in any part of the oropharynx. Herpes simplex often produces a significant gingivitis as well.

Streptococcal pharyngitis often inflames the posterior pharynx, with petechiae on the uvula and soft palate.[6] When one sees this clinical sign, GAS are often isolated by throat culture. A confusing factor is that up to 10% of patients who have EBV infections will

have a secondary group A ß-hemolytic streptococcal pharyngitis during their illness: *Corynebacterium diphtheriae* can also cause pharyngitis, producing a characteristic gray membrane across the structures of the posterior pharynx. This is seldom seen today except in a few geographic areas where diphtheria outbreaks have occurred recently, such as Russia. There are also noninfectious causes of pharyngitis, such as Behçet's syndrome, Kawasaki disease and Stevens-Johnson syndrome.

Laryngitis may be an isolated event, but more commonly is part of a more extensive upper respiratory infection. Young children with croup cannot inform us of the extent of their symptoms and refusal to swallow and/or eat may be their only sign of difficulty. Adults with laryngitis often had croup as children and these two entities may be one and the same illness, the only difference being that the adult subglottic airway is larger and thus adults are less likely to develop stridor. Parainfluenza viruses are the most common cause of croup,[7] but adenoviruses, influenza and RSV also cause laryngitis/croup. Normally there is a mild coryza and sore throat followed by an inflammatory process of the larynx, trachea and subglottic area. There can be significant pain during coughing. The subglottic area swells and because it is located in an area of the nondistensible cricoid cartilage, it can only swell into the airway. This gives the characteristic croupy cough and stridor in a child and laryngitis in an adult. It is of interest that croup is more pronounced after sunset and at night, when the child is lying down. During the daytime the symptoms are often markedly improved. Occasionally the distention into the airway progresses enough to cause airway compromise and intubation is necessary. Fortunately, this is a rare event.

Epiglottitis is an acute cellulitis of the epiglottis and/or surrounding tissue that has the potential to cause complete obstruction of the airway. It is almost always caused by HIB. In Melbourne, Australia, HIB was isolated from 114 (93%) out of 123 blood cultures collected from epiglottitis patients and no other pathogens were isolated.[8] The usual patient is 1–5 years old (Fig. 31.1) and onset is sudden, with sore throat and fever, with head forwardly extended, often with drooling. Respirations appear delicate, with little movement of the head. There may be a raspy sound when breathing. Fever may cause tachycardia. The mood of some affected children may seem dull or anxious. Visualization of the pharynx reveals a 'cherry red' epiglottis, but sometimes the epiglottis is less red than the surrounding periepiglottic structures or base of the tongue. Visualization of the

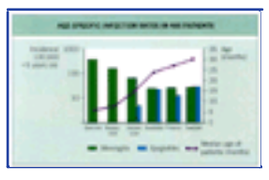

Figure 31-1 Incidence and median age of HIB disease. Disease type and geographic location per 100,000 children less than 5 years of age are shown.

epiglottis must be performed with care because respiratory arrest may occur if there is laryngeal spasm while probing the mouth.

PREVENTION

Preventing pharyngitis is desirable, but difficult to achieve. Viral pharyngitis is spread mostly by aerosolized oral secretions, hand-to-mouth contact with multiple individuals and the use of common utensils, glassware, etc. Certain viruses are known to be particularly resilient; RSV has been cultured from table tops hours after being inoculated there.[9] Measles has been known to be contracted from the air in a physician's waiting room, as long as 1 hour after the child with measles had left the

room. Other viruses may be less durable and less contagious, but close contact is obviously not necessary to transmit many of these agents. Prevention of disease depends mainly on good handwashing and preventing the spread of oral secretions. Masks and handkerchiefs inoculated with antiviral drugs have been used in experimental trials, but after several minutes of breathing, when the mask becomes wet, the benefit seems to diminish. There are vaccines available to prevent some of these diseases. Effective measles vaccines have been used for approximately 30 years, so the disease has decreased dramatically in most countries. Certain adenoviral vaccines have been used with some degree of success, mostly in military personnel. Vaccines for RSV and parainfluenza viruses are currently under development. These vaccines could have a significant effect on the population's health, particularly on that of the youngest children.

Transmission of streptococcal pharyngitis seems to require closer contact than for most viruses. Studies performed in the military during World War II showed that soldiers in barracks sleeping on either side of the index case were more likely to have disease than those further away.[10]

To date, there are no immunizations available to prevent streptococcal disease, although trials evaluating group B and group A vaccines are under way. For patients who have had prior group A disease and subsequent rheumatic fever, penicillin prophylaxis is recommended. Most patients receive intramuscular benzathine penicillin, 1.2 million units, once per month, although oral regimens are acceptable but have poorer compliance rates.

Vaccines against parainfluenza viruses would have the most impact on preventing laryngitis and croup. These are still experimental. The ability of influenza vaccine to prevent laryngitis has not been studied. To date, no other preventive measures against laryngitis are available.

In the only study to date seeking specific risk factors for epiglottitis, day care attendance was the strongest predictor for disease but the association was modified by whether the subject had had an upper respiratory illness in the previous 4 weeks.[11] There was also the suggestion that northern European ancestry was a risk factor as well. Fortunately, the incidence of epiglottitis (and meningitis) has decreased markedly since the advent of HIB vaccination. Whether the incidence of HIB disease in adults may change in the future is unknown, because long-term immunity from vaccination may prove to be either more or less effective than that due to natural infection.

CLINICAL FEATURES

Pharyngitis

Pharyngitis is a ubiquitous infection. A 'sore throat' affects most people at least once every year. Most cases of viral pharyngitis are associated with an upper respiratory infection (nasopharyngitis), as shown in Table 31.1 . Generally nasopharyngitis has a prodrome that may include malaise, diaphoresis, fever, headache and general aches and/or pains. Coryza and sore throat then begin. Many infections

will progress to produce a cough and/or laryngitis. Some viral infections produce predominantly coryza, others more pharyngitis, and others more cough or laryngitis. Coxsackieviruses often cause ulcers in the posterior pharynx along with a sore throat. Measles can cause a severe pharyngitis, but the associated symptoms of conjunctivitis, rash and Koplik's spots make the disease easily diagnosable. Parainfluenza and influenza viruses can give a particularly painful pharyngitis, with frequently associated symptoms of cough and laryngitis.

The DNA viruses (EBV (Fig. 31.2), adenovirus (Fig. 31.3), cytomegalovirus and HSV) can produce significant pharyngitis. They also tend to last longer than the other viral causes of pharyngitis. These viruses produce other upper respiratory symptoms such as non-tender cervical adenopathy (Fig. 31.4) of, in the case of HSV, tongue

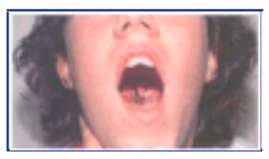

Figure 31-2 Epstein-Barr virus (mononucleosis or glandular fever) pharyngitis.

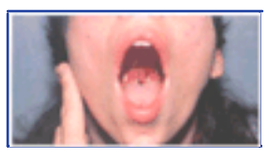

Figure 31-3 Adenoviral pharyngitis.

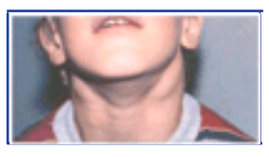

Figure 31-4 Adenopathy associated with EBV.

and mouth ulcers (Fig. 31.5). Herpes simplex virus pharyngitis has been described as a disease in which 'the gums swell up and swallow the teeth'. Rhinoviruses and RSV infections give upper respiratory symptoms as well as pharyngitis in infants.

The syndrome of acute HIV infection ('seroconversion illness') is well described and may cause symptoms in up to 50% of patients (see Chapter 122). It is a mononucleosis-like illness with pharyngitis being a prominent feature. Patients will also have fever, lymphandenopathy, rash and myalgias. The symptoms are nonspecific.

It is important to diagnose bacterial causes of pharyngitis because, unlike viral causes, many can be treated specifically with antibiotics. Proper treatment can avoid significant morbidity and/or mortality. Pharyngitis caused by GAS is the most common infection causing significant pharyngeal edema, frequently with petechiae on the soft palate and uvula (Fig. 31.6). Tender cervical nodes are common. Small children may complain of abdominal pain, which may be due to mesenteric adenitis. Headache and raised temperature are also common. Some patients who have a streptococcal sore throat have a characteristic red 'scarlet fever' rash that begins in the groin and axillary areas and spreads over the body (Fig. 31.7). The rash is sandpaper-like and may itch. A strawberry tongue is also often present. Other patients have a characteristic rash on the face (Fig. 31.8). Without treatment the illness usually resolves over 3 or 4 days, but rheumatic fever may ensue.

The recommended treatment is penicillin, or clindamycin, erythromycin or azithromycin for those allergic to penicillin. There is concern about the recurrence rate of streptococcal pharyngitis in adequately treated patients but there is no evidence for microbiological resistance to therapy. It is more likely that the organism is reacquired or sequestered in a nonapproachable site and simply reemerges after therapy is discontinued. Some have used rifampin at

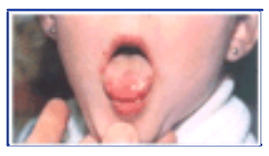

Figure 31-5 Herpes simplex virus stomatitis.

Figure 31-6 Pharyngitis associated with GAS infection. Exudates are not always present.

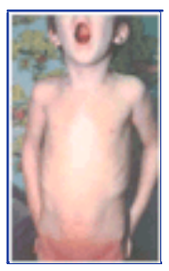

Figure 31-7 Scarlet fever. Skin rash and pharyngitis associated with GAS infection.

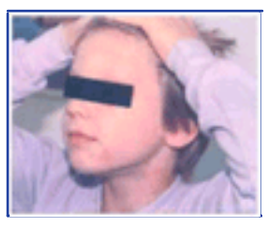

Figure 31-8 Facial rash associated with GAS infection.

the end of therapy in an attempt to alleviate this possibility. While all therapies have similar treatment profiles, azithromycin has a higher culture-positive recurrence rate. The risk of rheumatic fever without antibiotic treatment is complex. Rates as high as 3% were documented by Wannamaker[12] in 1952; however, more typical rates of 0.4% were found in a pediatric population in 1961.[13] Certainly M protein type, genetic susceptibility and previous infection play a part in this diversity of recurrence rates. Other ß-hemolytic streptococcal infections (groups C, G and B) can cause pharyngitis but not rheumatic fever. For these, antibiotic treatment may provide symptomatic relief. Occasionally, pharyngitis can be secondary to an abscess in the peritonsillar area. This is usually easily diagnosed by an asymmetry of the tonsillar pillars. The affected side is asymmetrically enlarged and protrudes anteriorly into the mouth.

Haemophilus influenzae (nontypable and types a–f) can cause pharyngitis and type b can also cause epiglottitis or meningitis. The appearance of pharyngitis is nondiagnostic. Many individuals are carriers but are not ill. *Corynebacterium diphtheriae* causes diphtheria, which is easily diagnosed because of the gray pseudomembrane in the posterior pharynx along with pharyngitis. The disease has recently become endemic in parts of the former Soviet Union. *Arcanobacterium* (previously *Corynebacterium*) *haemolyticum* is a common cause of pharyngitis and can also cause a scarlatiniform rash. It is the cause of many non-GAS throat infections.[14] *Neisseria gonorrhoeae* can also cause pharyngitis. The appearance of the pharyngitis is nondiagnostic, so a heightened awareness is required to make this diagnosis.[15]

Chlamydia pneumoniae and *Mycoplasma pneumoniae* can cause pharyngitis, but generally will go on to cause cough also, often with wheezing and pneumonia.[16] [17] *Candida albicans* can cause pharyngitis but normally only in the immunocompromised host. The pharyngitis is hyperemic, with white plaques on the buccal mucosa.

Aphthous stomatitis is a common cause of mouth ulcers. The etiology is unclear. Small painful ulcers appear on the buccal mucosa, but can also appear in the posterior pharynx. The ulcers are usually stress related and last approximately 1 week. Very extensive aphthous ulceration can also be seen as a complication of HIV infection. Behçet's syndrome may cause pharyngitis. Kawasaki disease, most common in children, can cause significant pharyngitis. Most children with Kawasaki disease also have fever, a strawberry tongue and, importantly, conjunctivitis. This condition is frequently confused with streptococcal disease. Stevens-Johnson syndrome can result in pharyngitis, stomatitis and perioral swelling and ulcerations.

Laryngitis

Laryngitis is inflammation of the subglottic area and is generally of viral etiology although there is evidence that some individuals with *M. pneumoniae* infection can also develop hoarseness.[18] The most common cause of laryngitis and croup in infants is parainfluenza virus. The order of frequency is type 3>type 1>type 2. Because the infant airway is relatively narrower, croup with a 'barky' cough is much more common in infants. Older children, adolescents and adults tend to have laryngitis only. The hoarseness lasts from 2 to 5 days. A sore throat is common. Other common causes of laryngitis are influenza, RSV and adenovirus infections.

The feared complications of laryngitis are respiratory arrest, particularly in children with croup, and bacterial tracheitis in anyone with croup/laryngitis, particularly those that might have had recent trauma to the subglottic area.[19] Croup is worse at night and when lying down. Cool air and an upright posture are the treatments of choice. Temperature may be slightly elevated, but fever is not a characteristic of the disease. Bacterial tracheitis, when it occurs, is usually heralded by the sudden onset of fever and dyspnea.[20] The syndrome may be clinically indistinguishable from epiglottitis, except that the epiglottis is normal. The most common causes of the infection are *Staphylococcus aureus*, GAS and HIB. Immediate antibiotic treatment is indicated. Sometimes intubation is necessary.

Epiglottitis

Patients with epiglottitis often have an underlying illness, presumed to be viral. They then have sudden onset of fever, with the neck extended forward, drooling and air hunger. Affected children are anxious and lean forward to open their airway (Fig. 31.9). The diagnosis is easily made by viewing the epiglottitis, which is swollen and red (Fig. 31.10). Intubation is often required. *Haemophilus influenzae* type b is almost always obtained by culturing swabs from the epiglottis in children. Some children have been discharged without intubation after receiving only one dose of ceftriaxone when the epiglottis did not appear reddened, but subsequent epiglottic and blood cultures have been positive for HIB. They were cured completely. The duration of hospital treatment averages 3 days. Intubation is needed for less than 24 hours in most cases.[21] In adults other pathogens may be obtained.[22] In most adults the disease is less severe and of slower onset. The airway obstruction occurs because of a progressive cellulitis of the supraglottic area. Thus at presentation,

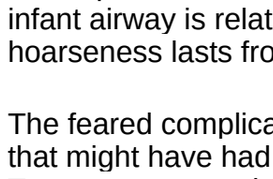

Figure 31-9 Child with epiglottitis. *Courtesy of Intensive Care Unit, Royal Children's Hospital.*

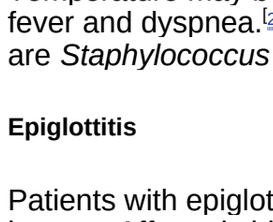

Figure 31-10 Acutely inflamed epiglottis associated with HIB. The epiglottis protrudes upwards and is cherry red from the bottom of the figure. *Courtesy of Intensive Care Unit, Royal Children's Hospital.*

antibiotic treatment and intubation at the first sign of increasing respiratory compromise may avert the need for tracheostomy. The use of steroids to reduce inflammation and decrease the need for tracheostomy is appealing but unproven.

DIAGNOSIS

The most common treatable cause of pharyngitis is GAS infection. This can be diagnosed with a simple latex antigen test directly from a throat swab, but this procedure is not widely used outside North America. The latex test has a high specificity and an adequate sensitivity,[23] but bacterial culture is the gold standard. Group

B, C and G streptococci can also cause significant morbidity, but only group A leads to rheumatic fever, so the reason for treatment is not only to eliminate the pharyngitis but also to prevent the subsequent rheumatic disease. Viral causes of pharyngitis do not normally require specific diagnosis, but serologic tests are available for mononucleosis (EBV) and cytomegalovirus. Adenoviruses, RSV and parainfluenza viruses can be diagnosed using rapid antigen tests, which are available but rarely used in uncomplicated community-acquired infections.

Laryngitis is not normally diagnosed by laboratory tests and radiographs are of little use except to exclude foreign body aspiration or epiglottitis.

The white blood cell count in epiglottitis is often elevated, with an increase in the percentage of neutrophils and band forms. A culture of the epiglottis is usually positive for HIB but the result may not be available until the child is ready for discharge. Blood cultures are frequently positive for HIB in children, although there are usually fewer of these organisms per milliliter than in children with meningitis. Efforts to determine whether there are organism subtype differences that predispose to meningitis versus epiglottitis have been equivocal. In adults, the disease is reported to be caused principally by HIB, but pneumococci, *H. parainfluenzae* and streptococci are also reported. At the time of admission, if time permits, a radiograph of the lateral

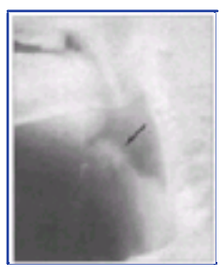

Figure 31-11 Lateral neck radiograph of a child with acute epiglottitis demonstrating an enlarged hypopharynx due to forward neck extension and an enlarged 'thumb-shaped' epiglottis (arrow). *Courtesy of Dr Donald Frush.*

TABLE 31-2 -- Antibiotic dosages for the treatment of respiratory infections.

ANTIBIOTIC DOSAGES FOR THE TREATMENT OF RESPIRATORY INFECTIONS			
Antibiotic	**Dosage**	**Oral maximum dose**	**IV/IM maximum dose**
Azithromycin	10mg/kg 1st day, 5mg/kg days 2–5	500mg	NA
Cefotaxime	100–200mg/kg/day im/iv, q6-8h	NA	12g/24h
Ceftriaxone	50–75mg/kg/day im/iv, q12-24h	NA	4g/24h
Clindamycin	20–40mg/kg/day, q6-8h	1.8g/24h	4.8g/24h
Doxycycline	3–5mg/kg/day, q12h	300mg/24h†	NA
Erythromycin	20–40mg/kg/day, q6-8h	2.4g/24h*	2.4g/24h*
Penicillin	penV-potassium 25–50mg/kg/day, q6h	500mg/dose	NA
Tetracycline	25–50mg/kg/day, q6h	500mg/dose†	NA

† Preferable to use other antibiotics in children under age 8 because of tooth enamel staining
* Erythromycin base

neck show may the 'thumb sign' (Fig. 31.11), demonstrating an enlarged epiglottis. Absence of such a finding does not eliminate epiglottitis as a diagnosis, but it provides reassurance that the pharynx can be visualized without threat of airway obstruction.

Visualization of the posterior pharynx is the best way to confirm the diagnosis of epiglottitis. Because airway obstruction is the most feared complication of this disease, this examination should be done in a manner and place where immediate intubation can be performed if necessary.[21] At hospitals where the disease has been seen frequently it is common to give an inhaled anesthetic to allow a quick examination of the pharynx with an anesthesiologist, anesthetist or intensive care specialist standing by. Culture of the epiglottis should be performed by obtaining a swab during the examination.

MANAGEMENT

Antibiotic dosages are shown in Table 31.2 . For group A streptococcal pharyngitis the recommended therapy is 7–10 days of oral penicillin or amoxicillin. Erythromycin and clindamycin are acceptable alternatives. There have been studies showing that one dose of ceftriaxone intramuscularly or oral azithromycin for 5 days is equally effective at eliminating carriage of GAS, but recurrent pharyngeal

colonization occurs with all treatment regimens. Viral causes of pharyngitis can be most suitably treated with supportive measures: gargles, lozenges, etc. *Mycoplasma* and *Chlamydia* spp. infections can be treated with erythromycin or tetracycline (depending on age). Diphtheria and *Arcanobacterium* spp. infection should be treated with erythromycin or penicillin. *Legionella* spp. infections should be treated with tetracycline. *Haemophilus influenzae* type b and *Yersinia enterocolitica* should be treated with a third-generation cephalosporin.

Causes of laryngitis are generally viral, therefore having no specific treatment. For supportive care, cool air and humidity often relieve some of the symptoms. Patients with bacterial superinfection (tracheitis) should be treated for presumed staphylococcal superinfections with nafcillin (flucloxacillin) or vancomycin, depending on whether the infection was acquired while in the hospital and therefore more likely to be methicillin resistant.

Treatment of epiglottitis in children is with cefotaxime or ceftriaxone and immediate intubation if needed. Ampicillin should not be used due to the high frequency of ampicillin-resistant strains of HIB. Even if the airway is patent at the time of diagnosis, intubation is recommended because progression to airway obstruction is common until antibiotic therapy has begun. Most children can be successfully extubated after 24 hours of antibiotic therapy and some extubate themselves before that time has expired. Family members and daycare contacts should receive rifampin (rifampicin) prophylaxis (300mg q12h for 2 days) to avoid secondary infection.

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Chapter 32 - Otitis, Sinusitis and Related Conditions

Stephen I Pelton

OTITIS MEDIA

EPIDEMIOLOGY

Acute otitis media (AOM) is a common and frequently recurrent illness associated with upper respiratory tract infection. It has been diagnosed more frequently over the past decade than previously. Although many risk factors, such as male sex, bottle feeding, an 'immature' immune system and familial predisposition, have been associated with an increased incidence,[1] the change in child rearing patterns resulting in early entry into the day care setting has been considered to be the critical factor in the increased incidence of AOM. [2] This hypothesis has been supported by an observed 2- to 3-fold increase in AOM among children in day care compared with home care.

A prospective study of children followed from birth up to 7 years of age has described the occurrence of AOM and its recurrent nature.[3] The peak incidence of disease was in the second 6 months of life. Almost two-thirds of children had at least one episode by the age of 1 year, and age at first episode was highly predictive for recurrent otitis media. Three or more episodes was relatively common during each of the first 4 years of life but became unusual by year 6 or 7. A more recent prospective study of a birth cohort suggests that the incidence of disease as well as the proportion of children with three or more episodes has increased in the first year of life, presumably as a result of the ongoing changes in entry into day care settings.[4]

PATHOGENESIS

Evidence suggests that host factors as well as infectious agents contribute significantly to the occurrence of AOM (Fig. 32.1). Two well-established host factors are eustachian tube dysfunction and immunologic abnormalities.[5] [6] Otitis media is nearly universal in children with cleft palate and the associated functional eustachian tube obstruction. In other children with recurrent disease, reflux of nasopharyngeal secretions (and presumably bacterial pathogens) into the middle ear has been demonstrated. Children who have immunologic deficiencies (especially hypogammaglobulinemia) suffer recurrent mucosal surface infections, including otitis media. More recently, recurrent otitis has been reported in children who have IgG subclass deficiency, IgA deficiency and HIV disease.[7] [8] Finally, passive immunization with antibacterial polysaccharide immunoglobulins has been demonstrated to reduce the incidence of type-specific pneumococcal otitis media.[9] These studies suggest that children who have humoral immune deficiencies are more susceptible to AOM. Respiratory viral pathogens also appear to be important in the pathogenesis of AOM, most often as a cofactor rather than as a direct invader. During seasonal respiratory syncytial virus outbreaks, the incidence of AOM increases significantly.[10]

PREVENTION

Strategies for the prevention of AOM and recurrent otitis media have focused on reducing disease caused by specific bacterial pathogens or on immunoprophylaxis against respiratory viral infection. Studies of pneumococcal polysaccharide vaccine in the early 1980s demonstrated that immunization with an octavalent or 14-valent pneumococcal polysaccharide resulted in a reduction in disease caused by serotypes that were associated with a good serum antibody response.[11] [12] Unfortunately, no reduction was observed in children less than 7 months of age, and in one of the studies the effect was limited to the first 6 months after immunization.

Recently, studies of passive immunoprophylaxis with bacterial polysaccharide immunoglobulin, both in experimental animals and in infants, have confirmed that high serum levels of antibodies to bacterial surface antigens are sufficient to provide protection against acute middle ear infection.[8] [13] [14] This has been demonstrated in experimental models for disease caused both by nontypable *Haemophilus influenzae* and *Streptococcus pneumoniae*, and in clinical trials for disease due to *S. pneumoniae*.

New formulations of pneumococcal polysaccharide conjugated to protein haptens (pneumococcal conjugate vaccine) have demonstrated enhanced immunogenicity in children immunized at 2, 4 and 6 months of age, protection against serotype-specific invasive disease and a modest effect on nasophayngeal carriage of *S. pneumoniae*.[15] [16] [17] Recent clinical trials have demonstrated a modest reduction (of about 6–8%) in episodes of AOM in children immunized with pneumococcal conjugate vaccine (heptavalent pneumococcal polysaccharide vaccine conjugate to CRM, or PCV7).[16] [18] Children immunized with PCV7 had a 57% reduction in episodes of AOM caused by one of the seven serogroups contained within the vaccine; however, a modest increase in disease caused by nonvaccine serogroups and nontypable *H. influenzae* diminished the overall benefit. Currently, PCV7 is licensed for prevention of otitis media and would be appropriate for high-risk children; however, its primary use is the prevention of invasive pneumococcal disease.

The concept of prevention of AOM through immunoprophylaxis against respiratory virus infection has been demonstrated using influenza vaccine.[19] [20] A reduction in cases of influenza virus A infection as well as a 36% decline in otitis media was observed in a day care center during a community outbreak of influenza. However, in studies documenting the role of viruses as a cofactor in the pathogenesis of AOM, influenza virus has been identified in about 5% of all episodes and therefore immunization against this pathogen will have, at most, a small benefit in the prevention of otitis media.[10] Respiratory syncytial virus (RSV) is the viral pathogen most frequently associated with AOM. Considerable progress has been made in the immunoprophylaxis of RSV disease, and two approaches have been shown to be effective.[21] [22] First, the use of immunoglobulin with high titers of neutralizing antibody against RSV has been shown to reduce incidence and severity of lower respiratory tract disease caused by RSV. It has also been shown to reduce the frequency of AOM from 0.78 episodes per child to 0.15 episodes. These studies did not permit discrimination between the anti-RSV effect and a nonspecific effect of passive administration of immunoglobulin with antibody directed against a spectrum of pathogens. Most recently, studies of an RSV monoclonal antibody demonstrated protection against

Figure 32-1 Pathogenesis of acute otitis media.

lower respiratory disease caused by RSV but failed to diminish episodes of AOM in recipients. This finding has been interpreted as showing that the reduction in AOM is most likely a result of the passive administration of antibodies against bacterial pathogens.

Antimicrobial prophylaxis has been used successfully in preventing recurrent episodes of AOM and sinusitis. However, its use should be limited to those at high risk.[23] [24] Selection criteria for patients most likely to benefit include multiple episodes within a recent 6-month time period or recognized immunologic defects that predispose to bacterial complications of respiratory infection and history of recurrent disease. Amoxicillin (20–40mg/kg/day) and sulfisoxazole (500mg q12h) have both been used successfully. This approach must be used cautiously as it is likely to contribute to the selection of resistant otopathogens both in the individual child and in the community.

Another approach has been the insertion of tympanostomy tubes to prevent AOM.[25] Although only a limited reduction in the number of episodes of acute otitis has been reported, time spent with middle ear effusion and the associated conductive hearing loss is significantly reduced.

DIAGNOSIS AND CLASSIFICATION

Optimal diagnostic criteria and classification for middle ear disease (Table 32.1) are critically important because children who have middle ear effusion and signs and symptoms of acute illness may be candidates for antibiotic therapy, whereas those lacking signs or symptoms are more likely to suffer from otitis media with effusion and are not likely to benefit significantly from antimicrobial therapy.

The hallmark of middle ear disease is the presence of middle ear effusion or otorrhea. Therefore, pneumatic otoscopy has become the diagnostic method of choice. The pneumatic otoscope permits visualization of the tympanic membrane as well as assessment of its mobility. The healthy tympanic membrane moves briskly inward when pressure is applied to the attached rubber bulb and it returns with the release of the bulb pressure. When middle ear effusion is present, the tympanic membrane has reduced or absent mobility on both positive

TABLE 32-1 -- Classification of otitis media.

CLASSIFICATION OF OTITIS MEDIA			
Diagnosis	**Middle ear effusion or otorrhea**	**Inflammation of tympanic membrane**	**Symptoms**
Acute otitis media	Effusion	Erythema	Fever, irritability, vomiting, earache
Otitis media with effusion	Effusion	Usually absent, may be opaque	Asymptomatic, may have difficulty sleeping
Chronic suppurative otitis media	Otorrhea	Perforated	Frequently painless, diminished hearing

and negative pressure. When AOM is present, the tympanic membrane usually has signs of inflammation (erythema, diminished translucency and loss of light reflex) and the child has localized clinical manifestations (earache) or systemic manifestations (e.g. fever, irritability, vomiting). It is critically important to distinguish AOM from otitis media with effusion because antimicrobial therapy is not considered effective against otitis media with effusion.

Acute otitis media with otorrhea must be distinguished from external otitis media. The history of relief from earache when drainage begins and the presence of a perforation of the tympanic membrane helps to distinguish these two diagnoses. The pain in external otitis usually continues to increase even after drainage begins, and the canal is frequently swollen so that its diameter is significantly reduced.

Chronic suppurative otitis media without cholesteatoma is a condition with persistent drainage and chronic perforation, with or without a myringotomy tube, lasting for longer than 6 weeks. The disease usually occurs in particular populations such as the Inuit, native Americans, Australian Aborigines, young infants and children who have immunodeficiency, or postmyringotomy and tube insertion. The pathogenesis and treatment for chronic suppurative otitis media is very different from that of AOM.

MICROBIOLOGY

The pathogens isolated in AOM as defined by aspiration and culture of middle ear fluid are most frequently *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis* and group A streptococci. The relative frequency of each pathogen varies throughout the world (Fig. 32.2).[26] A higher frequency of infection with group A streptococci is observed in Europe than in the USA, specifically in children older than 2 years of age. An increased proportion of cases caused by *M. catarrhalis* has recently been reported from Finland, mainly in infants less than 2 years of age. [27] *Haemophilus influenzae* has been demonstrated as an important pathogen in all age groups, including adults. [28]

Specific microbiologic diagnosis is rarely made because it requires needle apsiration through the tympanic membrane or culture of middle ear drainage from the site of perforation of the tympanic membrane or from the orifice of a ventilation tube. Therefore treatment is empiric, based on the likely microbial pathogen — clinical signs or symptoms, such as pain, fever and appearance of the tympanic membrane, do not distinguish etiology.[29] However, epidemiologic features are helpful in suggesting an enrichment for a given pathogen. Children with recurrent episodes of AOM are at increasing risk of nontypable *H. influenzae* whereas initial episodes are more likely to be due to the pneumococcus. [30]

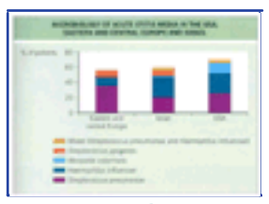

Figure 32-2 Microbiology of acute otitis media in the USA, eastern and central Europe and Israel. Culture results of middle ear aspirates from children. *Adapted from Jacobs et al.*[26]

Viruses, *Mycoplasma* spp., *Chlamydia* spp. and less common bacterial pathogens have on occasion been identified as etiologic agents in AOM. Recent studies have identified viral antigens or viruses in some children who have AOM, most frequently in combination with bacterial pathogens. Respiratory syncytial virus has been found most commonly,[31] but influenza virus, enteroviruses and rhinoviruses have also been reported. Polymerase chain reaction technologies have identified RNA from *Chlamydia pneumoniae* in middle ear fluid from children who have AOM and otitis media with effusion.[32] *Mycoplasma pneumoniae* has been cultured from one case and has been proposed as the etiology of AOM associated with bullous myringitis in patients who have concomitant pneumonia.[33]

Less common bacterial pathogens include *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Both of these are frequently found in children who have chronic suppurative otitis media but they have only occasionally have been isolated from children with intact tympanic membranes. *Mycobacterium tuberculosis* and *Pneumocystis carinii* have been isolated from the middle ear in unusual cases of otitis media.[34]

Otitis media in the first 6 weeks of life warrants special consideration. Although most children will have the usual respiratory pathogens (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*), enteric bacteria have been identified in 15% of cases and *S. aureus* in 10% of cases (Table 32.2).[35] Thus, neonates, especially those less than 2–3 weeks of age, require a different management strategy with careful evaluation, parenteral administration of broad-spectrum antimicrobial agents and close observation.

EFFECT OF ANTIMICROBIAL THERAPY ON THE OUTCOME OF ACUTE OTITIS MEDIA

The rapid increase in isolates of *S. pneumoniae* with reduced susceptibility to penicillin and a recognition of the dramatic rise in antibiotic prescriptions written each year for otitis media has resulted in a renewed evaluation of the role of antibiotics in the treatment of AOM. Studies comparing antibiotic and control-treated children who have AOM, historic reflection on the frequency of suppurative complications of AOM, and limited data from isolated areas where antibiotic therapy is often initially deferred for the diagnosis of AOM have provided evidence that antimicrobial treatment is associated with a shorter duration of local and systemic signs and symptoms such as fever, irritability and earache. [36] [37] The use of antibiotics for

TABLE 32-2 -- **Microbiology of otitis media in the first 6 weeks of life.***

MICROBIOLOGY OF OTITIS MEDIA IN THE FIRST 6 WEEKS OF LIFE	
Respiratory pathogens	**49.7%**
Streptococcus pneumoniae	18.3%
Haemophilus influenzae	12.4%
Streptococcus pneumoniae and *Haemophilus influenzae*	3.0%

Staphylococcus aureus	7.7%
Streptococci group A or group B	3.0%
Moraxella catarrhalis	5.3%
Enteric pathogens	**18.3%**
Escherichia coli	5.9%
Klebsiella spp. and *Enterobacter* spp.	5.3%
Pseudomonas aeruginosa	1.8%
Other	5.3%
No pathogens recovered	**32.0%**
Microbiology in 169 infants less than 6 weeks of age who have AOM.	

** Data from Shurin.[35]*

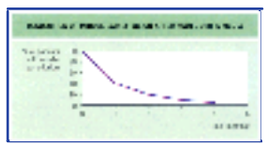

Figure 32-3 Resolution of middle ear effusion after acute otitis media. *Adapted from Klein et al.[1]*

AOM has also been associated with a reduction in acute suppurative mastoiditis over the past three decades and is further supported by recent reports of a rising incidence of mastoiditis associated with the withholding of initial antimicrobial therapy in children who have AOM.[38] Outcome measures such as resolution of signs and symptoms at 2 weeks after diagnosis, presence of middle ear fluid at 4–6 weeks and frequency of recurrent infection appear to be unrelated to initial antibiotic therapy. In most studies, middle ear effusion is found in up to 40% of children 1 month after an episode of AOM; this gradually resolves over several months in most children (Fig. 32.3).

Studies comparing antibiotic-treated children with untreated controls demonstrate that age is a critical feature in identifying the patients who are most likely to benefit from antimicrobial therapy.[39] Children under 30 months of age are at highest risk of having persistence of signs and symptoms and suppurative complications if untreated, whereas children over 30 months of age are likely to have resolution of their signs and symptoms without complications even if not treated with antibiotics. These observations have led to dichotomy in the management of children with AOM. In the USA the use of antibiotics for the treatment of AOM is standard. However, in Europe it is common to use symptomatic treatment initially (in children over 1 year of age) and to institute antibiotic therapy only when symptoms persist. It appears that this strategy reduces the need for antibiotic therapy by as much as 75%.[40] From recent studies it would appear that persistence of 'distress' (night sleep disturbance) beyond

3 days was associated with the presence of fever (over 99.5°F (37.5°C)) or vomiting or cough at diagnosis. The relative risk for persistence of symptoms following placebo was approximately 5 for children over 2 years of age with 'severe' disease, defined as temperature over 103°F (39.5°C) and substatial otalgia.[41]

Initial symptomatic intervention while reserving antimicrobial therapy for those with persistent symptoms is currently being considered by the American Academy of Pediatrics for children over the age of 2 years who have minimal signs and symptoms at presentation. The challenge will be to identify symptoms that are likely to be associated with persistent 'distress' so that parents can be included in the decision process as to the importance, within the context of the family structure, of the small differences in outcome between children treated with initial antimicrobial therapy compared with those for whom antibiotic treatment is reserved for persistence.

COMPLICATIONS

Mastoiditis is an uncommon complication of AOM that is seen predominantly in children under 2 years of age. Affected children are usually acutely ill with toxicity and localized pain and tenderness over the mastoid process. Bulging of the external ear secondary to subperiosteal abscess or inflammation or paralysis of the facial nerve are classic manifestations. Partially treated (subacute) mastoiditis may manifest itself as persistent fever, tenderness of the mastoid process and otorrhea. In contrast to uncomplicated AOM, the bacterial pathogens of mastoiditis are more virulent. Group A streptococci and *S. pneumoniae* are the two most common. *Haemophilus influenzae* type b was seen frequently before universal immunization, and it should be considered in children who have not been adequately vaccinated. *Staphylococcus aureus* and Gram-negative enteric bacteria have been identified in patients who have subacute mastoiditis.

Therapy for acute mastoiditis is parenteral antibiotics with close observation for the development of possible complications such as intracranial abscess, venous sinus thrombosis, osteomyelitis or hydrocephaly. Myringotomy is necessary if facial nerve palsy is present. Prolonged therapy for 2–4 weeks is generally required.

There is significant controversy about whether an increased risk of mastoiditis occurs in children when a strategy of delayed antibiotic therapy is used for the treatment of AOM. It is generally accepted that acute mastoiditis is most common in young children and that routine antibiotic treatment of AOM is not absolute for prevention.[42] When rates of mastoiditis in children (<14 years) are compared by country, the incidence where prescriptions for AOM exceed 96% are 50% lower than in countries where prescribing for AOM is <76%. [43]

SINUSITIS

EPIDEMIOLOGY

Sinusitis in children

The recognition and diagnosis of sinus disease in children remains difficult. The signs and symptoms frequently lack specificity and overlap with nasal allergy or airway obstruction. Physicians often have divergent views on what criteria are sufficient to warrant intervention. There is a wide variance in the monthly incidence of acute and chronic sinusitis reported by pediatric practitioners.[44] Table 32.3 summarizes the mean number of episodes and range reported by different physicians. The wide disparity and incidence probably reflects differences in diagnostic criteria rather than true differences in incidence.

CLINICAL FEATURES

The presentation of acute sinusitis changes with increasing age. In young children, persistent rhinorrhea (which is often purulent),

TABLE 32-3 -- Variability in reported incidence of sinus disease in pediatric practice.

VARIABILITY IN REPORTED INCIDENCE OF SINUS DISEASE IN PEDIATRIC PRACTICE		
	Cases per month	
Clinical diagnosis	**Mean**	**Range**
Acute sinusitis	18	1–200
Recurrent sinusitis	13	4–100
Chronic sinusitis	7.5	1–40

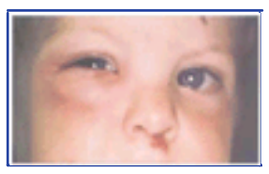

Figure 32-4 Acute sinusitis with facial swelling and periorbital edema.

daytime and night-time cough, foul breath and, less commonly, fever are the hallmarks of sinus disease. Less frequently, high fever, purulent rhinorrhea and facial tenderness or swelling signal the likely presence of acute sinusitis (Fig. 32.4). The overlap between uncomplicated upper respiratory tract infection and sinusitis is large. Most children who have signs and symptoms of less than 7 days duration have uncomplicated upper respiratory tract infection. Concern for sinusitis is appropriate when signs and symptoms persist beyond 7–10 days or when localized signs are present. In a prospective study of over 1000 children presenting to a pediatric office during winter months, 9.3% met the criteria of more than 9 days of symptoms for the diagnosis of sinusitis.

In older children and adults, symptoms and signs are more localized. Frontal headache, facial pain or pressure, and nasal congestion are frequent complaints. Facial tenderness or swelling over the maxillary or frontal sinus may be present. Nasal congestion or even obstruction is almost universally present except when sinus disease is of dental origin.

In chronic sinusitis, cough is especially prominent. The cough is usually present throughout the day, and it occasionally precipitates post-tussive emesis, especially soon after awakening. Chronic headache may also be part of the typical cluster of symptoms reported by patients. The pain is often dull; it often radiates to the top of the head or it may be bitemporal in nature. Nasal congestion and mucopurulent or purulent nasal discharge often complete the cluster of signs.

DIAGNOSIS

Defining the microbiology of paranasal sinus disease requires sampling of sinus secretions without contamination from normal

TABLE 32-4 -- **Bacterial species cultured from sinus aspirates performed in children.**[*]

BACTERIAL SPECIES CULTURED FROM SINUS ASPIRATES PERFORMED IN CHILDREN			
Bacterial species	**Single isolate**	**Mixed culture**	**Total (%)**
Streptococcus pneumoniae	14	8	22 (37.9)
Moraxella catarrhalis	13	2	15 (25.9)
Haemophilus influenzae	10	5	15 (25.9)
Streptococcus spp.	2	3	5 (8.6)
Eikenella corrodens	1	0	1 (1.7)

* *Data from Wald et al.* [45]

respiratory flora. Sampling involves a transnasal approach and attempted sterilization of the area through which the trocar will be passed. Because complete sterilization is usually impossible, investigators have frequently used a colony count of $=10^4$ colony forming units/ml to define infection.

Wald *et al.* have contributed greatly to our understanding of the microbial pathogenesis.[45] *Streptococcus pneumoniae* is the most frequent pathogen isolated from children who have acute sinusitis (Table 32.4). Other pathogens are bacterial species commonly found as part of the normal respiratory flora; these include nontypable *H. influenzae*, *M. catarrhalis* and group A streptococci.

Two distinct clinical settings require special knowledge, because the bacterial pathogens are likely to differ from there in patients who have concomitant upper respiratory tract infection. The microbiology of sinusitis in the intensive care unit includes *S. aureus*, *P. aeruginosa* and other Gram-negative enteric bacteria. In addition, anaerobes and yeasts are frequently isolated in combination with aerobes. In this setting, sinusitis is recognized as a cause of cryptogenic fever or in association with fulminant sepsis. Disease in this setting requires sinus aspiration to identify the specific pathogen. Initial antimicrobial therapy should be directed against nosocomial pathogens and then modified once the specific bacterial etiology has been identified.

A foul-smelling discharge and a recent history of dental pain or a dental procedure should suggest an odontogenic etiology for sinus disease. Bacterial pathogens from the oropharynx invade devitalized tissue in the gingiva and spread to the sinus. Mixed anaerobic infection with *Bacteroides* spp. and anaerobic streptococci are

commonly identified. Antimicrobial therapy, in combination with debridement or drainage of devitalized tissue or periapical abscess, is usually necessary.

A potential role for gastroesophageal reflux has been suggested as a contributing factor in the pathogenesis of sinusitis in children. A limited study of the prevalence of gastroesophageal reflux in children with chronic sinus disease identified esophageal reflux in 19 (63%) of 30 and nasopharyngeal reflux in six (32%) of the 19 using dual pH probe studies. Seventy-nine percent demonstrated improvement in their sinus disease after treatment for gastroesophageal reflux.[46] Further prospective evaluation of a nonreferral patient cohort is necessary to define the prevalence and significance of gastroesophageal reflux in children with chronic or recurrent sinus disease.

Imaging

Sinus radiographs must be interpreted with the knowledge that at birth and through early childhood only the maxillary and ethmoid sinuses are aerated. The frontal sinuses do not appear routinely until the age of 5–7 years and may be further delayed in some children. Sinus opacification without clinical signs or symptoms of sinus disease has been seen in some young infants undergoing radiologic

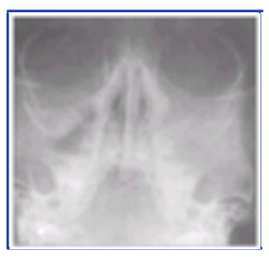

Figure 32-5 Left maxillary sinusitis.

TABLE 32-5 -- Natural history of acute paranasal sinusitis in 35 children.*

NATURAL HISTORY OF ACUTE PARANASAL SINUSITIS IN 35 CHILDREN		
	Number (%)	
Outcome	**Day 3**	**Day 10**
Cure	4 (11)	15 (43)
Improvement	14 (40)	6 (17)
No change	11 (32)	0
Failure (worsening or persistence of symptoms)	6 (17)	14 (40)

** Data from Wald et al.[48]*

studies for alternative diagnoses such as head trauma. One study reported a 60% incidence of sinus opacification in normal children under 1 year of age.[47] These observations limit the usefulness of routine sinus radiographs in the first year of life and demand that radiographic interpretations should be performed with consideration of clinical parameters.

Opacification (especially when asymmetric), mucosal swelling or air-fluid levels are all potential abnormalities in patients who have sinus infection (Fig. 32.5).

Computerized tomography (CT) and magnetic resonance imaging provide anatomic detail of the sinuses. These techniques are especially revealing when complications or extension of disease are suspected.

COMPLICATIONS

The course of untreated sinus disease and response to therapy in nontoxic children has been evaluated in placebo-controlled trials.[48] In these trials, half of the children have persistent or worsening signs or symptoms 3 days after diagnosis, and 40% are considered 'failures to improve' 10 days after diagnosis.

The American Academy of Pediatrics has published clinical practice guidelines for the management of sinusitis. These guidelines support the use of antibiotics with a strong recommendation based on limited scientific evidence but a strong consensus of their advisory panel.[49] In comparison with children treated with antimicrobial agents such as amoxicillin or amoxicillin-clavulanate, placebo-treated children are less likely to have a rapid resolution within 3 days of their signs and symptoms and are also less likely to achieve ultimate complete cure by day 10 (Table 32.5).[48] However, if end points beyond 10 days are selected, many investigators have been

TABLE 32-6 -- Classification of orbital cellulitis.*

CLASSIFICATION OF ORBITAL CELLULITIS		
Group	**Classification**	**Descriptions**
I	Preseptal	Erythema and edema of eyelids, normal vision and full range of motion
II	Orbital cellulitis without abscess	Diffuse edema (of orbit) but no abscess
III	Orbital cellulitis with subperiosteal abscess	Abscess adjacent to lamina papyracea; clinically there is proptosis, changes in vision and possibly pain on movement of the eye
IV	Orbital cellulitis with abscess in the orbital fat	Proptosis, limited motility of globe, loss of vision
V	Cavernous sinus thrombosis	Bilateral disease

** Data from Chandler et al.[50]*

unable to demonstrate any beneficial effect of antimicrobial agents on the outcome of sinus disease.

The complications of sinus disease frequently cause substantial morbidity and may require surgical intervention. The most common complications of sinusitis arise primarily from bacterial spread to the orbit, frontal bone or central nervous system. Extension of infection is usually direct through a complex network of venous channels and not the result of hematogenous spread. Occasionally, hematogenous dissemination produces bacterial complications at distant sites.

Orbital cellulitis is the most common serious complication of sinusitis and is most frequently associated with ethmoiditis. The clinical manifestations represent a spectrum of severity and they often predict the response to antibiotic therapy or the need for surgical drainage. A widely accepted classification system is that proposed by Chandler *et al.* (Table 32.6).[50] Proptosis, motility of the globe and visual acuity are the key features that differentiate the various stages of disease. A Marcus-Gunn pupil in the swinging flashlight test (pupillary dilatation when the light is moved from the normal eye to the affected eye) is diagnostic of optic nerve compression and a hallmark of advanced disease. Even with this classification scheme, it is often very difficult to distinguish cases that require surgical intervention from those that will respond to medical management. Computerized tomography scans should be performed if the patient has ophthalmoplegia or visual loss or if the patient is not responding to treatment. Differentiating 'inflammatory phlegmon' from abscess may still be difficult (Fig. 32.6).

Medical management requires a team approach and involves the infectious disease specialist, the otolaryngologist and the ophthalmologist. Initial therapy is directed against likely pathogens. A history of trauma or facial cellulitis can be helpful in predicting whether *S. aureus* is likely, whereas previous or concomitant sinusitis suggests *S. pneumoniae*, *H. influenzae*, group A streptococci or *M. catarrhalis*. However, the severity of illness and potential for complications mandate broad-spectrum parental therapy such as ceftriaxone, cefotaxime, amoxicillin-clavulanate or clindamycin in combination with aztreonam. The need for surgical intervention varies, and the decision is based on the response to antimicrobial therapy, the visual acuity and motility, and the results of CT scanning. Drainage of the involved sinus may also be necessary for rapid healing.

Intracranial complications from sinusitis are most common in adolescents and adults.[51] Brain abscess in the frontal lobe or subdural abscess are the most common. These complications are often difficult

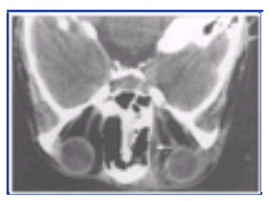

Figure 32-6 Computerized tomography scan of a patient who has right subperiosteal abscess (arrow) adjacent to the lamina papyracea. Note the partial opacification in the right ethmoid sinus.

to diagnose clinically and may present without signs or symptoms of increased intracranial pressure or toxicity. Fever, signs of meningeal irritation without toxicity, and focal neurologic abnormalities are the hallmarks. Less specific signs and symptoms include headache, behavioral changes and seizures. Currently, the diagnosis is best made by CT scan with contrast. Magnetic resonance imaging may be more sensitive for detection of small abscesses and cerebritis and for differentiation of an epidural abscess from a subdural abscess.

The mucosa of the frontal sinus and the marrow of the frontal bone have a common venous drainage. Thus, bacterial invasion of the marrow and subsequent osteomyelitis (Pott's puffy tumor) are recognized complications of frontal sinusitis. This entity was first described clinically by Sir Percival Pott in 1795, but it was not until 1879 that Lamel Oryne defined its pathology. Computerized tomography scanning is useful for defining the extent of frontal bone involvement and whether intracranial extension has occurred.

Allergic fungal sinusitis

Allergic fungal sinusitis has been increasingly described recently. Potentially mistaken for a paranasal sinus tumor, allergic funal sinusitis appears to be an allergic reaction to aerosolized, environmental fungi that occurs in immunologically normal hosts. The clinical presentation is often chronic nasal congestion and nasal obstruction. Facial abnormalities such as proptosis and malar flattening are frequently present. Unilateral disease is often present in children and bony erosion of the sinuses may be present. At the time of surgery, allergic mucin identified by the presence of degenerating eosinophils and the absence of histopathologic evidence of invasion is characteristic. Most disease is due to *Bipolaris spicifera*, *Aspergillus* spp., *Alternaria* spp., *Curvularia* spp. or *Exserohilum* spp. Surgical debridement is usually sufficient; however, a small benefit has been observed in patients treated with oral corticosteroids.[52] [53]

MANAGEMENT FOR ACUTE OTITIS MEDIA AND ACUTE SINUSITIS

The goal of antibiotic therapy is sterilization of the middle ear. Eradication of the bacterial pathogen in the middle ear is highly correlated with a successful clinical response.[54] Antibiotics should be chosen for their ability to achieve drug concentrations in the middle

TABLE 32-7 -- Antimicrobial agents commonly used in the therapy of acute otitis media.

ANTIMICROBIAL AGENTS COMMONLY USED IN THE THERAPY OF AOM				
Drug	**Pediatric dosage**	**Penicillin-sensitive *Streptococcus pneumoniae***	**ß-Lactamase-producing *Haemophilus influenzae***	**Advantages and concerns**
Amoxicillin	13.3mg/kg per dose q8h; maximum dose 500mg q8h	++++	-	1. Low cost, well tolerated
				2. Emerging high-level (MIC=2.0µg/ml) resistance among *Streptococcus pneumoniae* isolates
Amoxicillin-clavulanate	15mg/kg amoxicillin per dose with 2.4mg/kg clavulanic acid q8h; maximum dose 875mg q12h	++++	+++	1. Broad spectrum
				2. Frequent diarrhea
				3. Emerging high-level (MIC=2.0µg/ml) resistance among *Streptococcus pneumoniae* isolates
Cefaclor	20mg/kg per dose q12h; maximum dose 1g/day	++	+	1. Infrequent occurrence of serum sickness
				2. Limited activity against penicillin-intermediate (MIC 0.12–1.0mg/ml) and highly resistant isolates
Cefdinir	7mg/kg per dose q12h or 14mg/kg per dose q24h	+++	+++	1. Limited activity against resistant *Streptococcus pneumoniae* (MIC=2.0mg/ml)
				2. Diarrhea
Cefixime	4mg/kg per dose q12h or 8mg/kg per dose q24h; maximum dose 400mg q24h	++	++++	1. Limited activity against penicillin-intermediate (MIC 0.12–1.0mg/ml) and highly resistant isolates
				2. Potent activity against ß-lactamase-producing *Haemophilus influenzae*
				3. High cost
Cefprozil	15mg/kg per dose q12h; maximum dose 1g q24h	++++	+	1. High cost
				2. Active against penicillin-intermediate *Streptococcus pneumoniae*
				3. Less active against *Haemophilus influenzae*

Ceftibuten	9mg/kg per dose q24h; maximum dose 400mg q24h	+	+++	1. Not approved for AOM caused by *Streptococcus pneumoniae* 2. High cost
Ceftriaxone	50mg/kg/dose im	+++	++++	1. Parenteral administration 2. Multiple doses required for resistant *Streptococcus pneumoniae*
Cefuroxime axetil	15mg/kg per dose q12h; maximum dose 1g/day	++++	++	1. Significant after taste 2. Active against penicillin-intermediate *Streptococcus pneumoniae* 3. High cost
Cefpodoxime	5mg/kg per dose q12h or 10mg/kg per dose q24h; maximum dose 400mg q24h	++++	++++	1. Significant after taste 2. Active against penicillin-intermediate *Streptococcus pneumoniae* 3. High cost
Erythromycin sulfisoxazole liquid	12.5–16.6mg/kg erythromycin with 42.5–50mg/kg sulfisoxizole per dose q6h or q8h; maximum dose 2g erythromycin per day	++++	+++	1. Low cost 2. Gastric distress frequent 3. Four times daily dosing 4. Emerging macrolide resistance reported among *Streptococcus pneumoniae* with increasing use
Loracarbef	15mg/kg per dose q12h; maximum dose 800mg/day	+++	+	Limited activity against penicillin-intermediate *Streptococcus pneumonia*
Trimethoprim-sulfamethoxazole (co-trimoxazole)	4mg/kg trimethoprim with 20mg/kg sulfamethoxazole per dose q12h	++++	++++	1. In-vitro resistance among *Streptococcus pneumoniae* reported at 20–50% 2. Rare occurrences of Stevens-Johnson syndrome
Clarithromycin	7.5mg/kg per dose q12h; maximum dose 1g/day	++++	+	1. Undesireable after taste 2. Emerging resistance among *Streptococcus pneumoniae*
Azithromycin	10mg/kg per day on day 1, 5mg/kg per day on days 2–5; maximum dose 500mg on day 1	++++	+	1. Undesireable after taste 2. Emerging resistance among *Streptococcus pneumoniae*

ear that exceed the MIC for the likely pathogens. Successful sterilization of middle ear infection occurs when drug concentrations exceed the MIC for approximately 50% of the dosing interval for penicillins and cephalosporins and when the achieved area under the curve is sufficient for macrolides and azolides.[55] Presumptive therapy should be directed against nontypable strains of *H. influenzae*,

S. pneumoniae and *M. catarrhalis*. In AOM with perforation, activity against group A streptococci should be included routinely.

Table 32.7 lists the antimicrobial agents commonly used for therapy of AOM and acute sinusitis. Although studies of clinical outcome have rarely demonstrated significant differences, studies with microbiologic end points often suggest that sterilization of the middle ear may be more frequently achieved with antimicrobial agents that attain adequate levels in the serum and middle ear and are active in vitro against nontypable *H. influenzae* and *S. pneumoniae*. Amoxicillin remains the drug of first choice because of its ability to sterilize middle ear infection that is caused by most isolates of *S. pneumoniae* and nontypable *H. influenzae*, its low cost and its excellent safety record. None of the antimicrobials listed in Table 32.7 has been demonstrated, in appropriate trials, to be more effective than amoxicillin as initial therapy of AOM or acute sinusitis. Controvery persists about whether the initial dose of amoxicillin should remain at 40–45mg/kg/day administered in two or three doses or whether an increased dose of 80–90mg/kg/day administered in two doses is more appropriate. The use of a higher dose is intended to provide increased serum and middle ear concentrations more appropriate for the therapy of isolates of *S. pneumoniae* with reduced susceptibility to penicillin and other classes of antibiotics. In general few additional adverse events have been reported with the increased dosing of amoxicillin, and the higher dosing has gained popularity because of its enhanced pharmcokinetic profile.

Duration of therapy

Decreasing the duration of therapy reduces cost, may diminish the emergence of resistance among respiratory pathogens and should be associated with fewer adverse events. Several studies have demonstrated that by day 5 the middle ear can be successfully sterilized by antimicrobial agents with appropriate in-vitro activity.[56] Therefore, 'short-course' therapy is likely to be successful in resolving signs and symptoms caused by highly susceptible isolates. Unfortunately, the duration of acute therapy appears not to affect either the resolution of effusion at long-term follow-up or the risk of recurrence.

Recent studies that have compared short courses (5 days) with traditional courses have consistently demonstrated higher failure rates with the short course in children younger than 2 years of age, especially those attending group day care.[57] [58] These studies suggest that recommendations for short-course therapy should be limited to children older than 2 years of age. Further studies in children who have infection caused by multidrug-resistant *S. pneumoniae* suggest that short-course therapy is inadequate and should not be recommended in this setting.

Treatment failures

When a child's symptoms fail to respond within 3 days or the tympanic membrane remains bright red and bulging or full in appearance, the likelihood of persistent middle ear infection is high. Increasingly, a pathogen resistant to the initial antimicrobial agent prescribed is the likely problem. A broader spectrum antimicrobial agent that is active against ß-lactamase-producing nontypable *H. influenzae* and isolates of *S. pneumoniae* with reduced susceptibility to penicillin should be considered next (see Table 32.7). Studies suggest that high dose amoxicillin-clavulanate and cefuroxime are effective against the majority of these pathogens.

Emergence of penicillin-resistant isolates of *Streptococcus pneumoniae*

In South Africa, France and Spain, and now in the USA, strains of *S. pneumoniae* with MIC to penicillin of 2.0µg/ml or more have been isolated from the middle ear or sinus of children who have

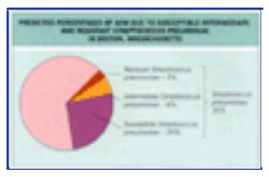

Figure 32-7 Predicted percentages of acute otitis media due to susceptible, intermediate and resistant *Streptococcus pneumoniae* in Boston, Massachusetts, USA.

AOM or sinusitis.[59] [60] These children have often already failed treatment with an antibiotic. The risk features that characterize these children include:

- recent antimicrobial treatment, most often with expanded spectrum cephalosporins or once daily macrolides;
- age under 2 years; and
- attendance at a day care center.

Clusters of cases have occurred in well defined geographic areas.[61]

Limited information is available about therapeutic regimens effective for children who have AOM caused by these isolates. Experience from Kentucky with a large cluster of cases and from Europe suggests high-dose amoxicillin (80–150mg/kg/day), amoxicillin (40mg/kg/day) plus amoxicillin-clavulanic acid (45mg/kg/day), ceftriaxone (50mg/kg/day) and clindamycin (50mg/kg/day) will successfully sterilize the middle ear and resolve symptoms.[61] More recently, studies of higher dose amoxicillin-clavulanic acid (90mg/kg/day) have demonstrated successful eradication of isolates of *S. pneumoniae* with MIC to amoxicillin as high as 4µg/ml.[62]

Disease caused by resistant isolates of *S. pneumoniae* remains infrequent among unselected cases of AOM and sinusitis. In Boston, we estimate that 3% of current unselected cases are due to resistant *S. pneumoniae* (Fig. 32.7). Initial therapy targeted at this pathogen is not recommended. However, in cases where initial and second courses of treatment fail, as well as in cases with significant toxicity, antimicrobial agents effective against resistant isolates of *S. pneumoniae* should be selected.

Penicillin allergy

For the child who has hives or anaphylaxis to penicillin, there is a limited selection of antimicrobial agents for therapy because aminopenicillins and cephalosporins have significant cross-reactivity. Macrolides such as azithromycin are effective against disease caused by susceptible isolates of *S. pneumoniae*; however, they are likely to be less effective against nontypable *H. influenzae* and multidrug-resistant isolates of *S. pneumoniae*. Trimethoprim-sulfamethoxazole (TMP-SMX; co-trimoxazole) has been effective, is low in cost and is relatively safe. However, resistance rates of 20–50% among isolates of *S. pneumoniae* have raised questions about the potential for clinical failure.

Alternatives to TMP-SMX and azithromycin include combination therapy with a macrolide and sulfisoxazole. Erythromycin sulfisoxazole has a profile of efficacy, low cost and safety, but suggested dosing is three to four times a day. Another potential selection is clindamycin in combination with sulfisoxazole.

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Chapter 33 - Bronchitis, Bronchiectasis and Cystic Fibrosis

James R Yankaskas

INTRODUCTION

Genetic, environmental and infectious factors can contribute to acute and chronic inflammation of the airways of the lung. The nature, severity and duration of these insults may produce acute or chronic inflammation with associated cough, dyspnea, sputum and obstructive lung disease. Clinically, these are classified as *bronchitis* (acute or chronic) when the main symptoms are cough and sputum production or *bronchiectasis* when the airways are structurally damaged, dilated and abundant (>60ml/day) sputum is expectorated. The diagnosis and clinical management of these various airways diseases are related to the underlying pathogenic processes and to differences among patients.

This chapter describes the pathophysiology, clinical features and management of bronchitis and bronchiectasis. Guidelines for diagnosis and therapy are provided. Cystic fibrosis (CF) is a common genetic disease that leads to progressive bronchitis and bronchiectasis. The molecular, cellular and organ-level pathogenesis of CF has been elucidated and new treatments are being developed. Some of these advances are applicable to bronchiectasis caused by other diseases. It must be emphasized that clinical outcomes are highly variable and treatment decisions must be based on the presentation and responses of the individual patient.

BRONCHITIS

EPIDEMIOLOGY

Bronchitis is defined as inflammation of the bronchial mucous membranes. Acute bronchitis is manifest by the development of a cough, with or without sputum, that typically occurs during the course of an acute viral illness. Such cough commonly develops in the first week of upper respiratory tract infectious (URIs) induced by rhinoviruses in 30% of patients.[1] Acute bronchitis develops in 60% of patients during influenza A infections.[2] In the United States over 34 million annual office visits are for acute sinusitis, bronchitis or URIs. A majority of these patients are treated with antibiotics and such prescriptions comprise 31% of the total antibiotic prescriptions written.[3]

Chronic bronchitis is defined by the clinical criteria of productive cough for more than 3 months per year for at least 2 years.[4] More than 12,000,000 Americans (about 5% of the population) have chronic bronchitis. The male-to-female distribution is about 2 to 1, but the prevalence is increasing in females. Chronic bronchitis is a major category of chronic obstructive pulmonary disease (COPD) and accounts for significant morbidity and mortality, especially in individuals over age 55. COPD accounted for nearly 83,000 deaths in 1989 and was the fifth leading cause of death in the United States.[5] COPD was also a contributing factor in death due to heart disease and other illnesses.

PATHOGENESIS AND PATHOLOGY

Acute bronchitis is most commonly due to infection of the respiratory epithelium with viruses, such as rhinoviruses, adenoviruses and influenza. Acute bronchitis may also be caused by infections with *Mycoplasma pneumoniae, Chlamydia pneumoniae* or *Bordetella pertussis*. The pathogenic effects of these organisms are incompletely understood, but they infect and directly damage airway epithelia, cause release of proinflammatory cytokines, increase production of secretions and decrease mucociliary clearance. Airways damaged by such infections may be more susceptible to irritation by inhaled toxins or bacteria. The role of secondary bacterial infections in the development of symptoms is not clear.

Chronic bronchitis develops as the result of a recurring or persistent injury and the resultant inflammatory responses. Cigarette smoking is the principal etiologic factor. Air pollutants such as sulfur dioxide or occupational exposures may also contribute. The pathologic effects are an increase in the proportion of goblet cells in the surface epithelium and an increase in the size of submucosal glands (Fig. 33.1). The distribution of these pathologic changes along the airway tree depends in part on the composition of the inhaled toxins and may involve peripheral bronchioles as well as central bronchi. There is an influx of polymorphonuclear leukocytes (PMNs), surface epithelial cell hyperplasia and metaplasia, and inflammatory mucosal edema. Genetic diseases that impair airway defenses may amplify these effects. Primary ciliary dyskinesia[6] decreases mucociliary transport secondary to altered ciliary structure and function. a_1 -Antitrypsin deficiency produces an imbalance in the defenses against neutrophil elastase and leads to panacinar emphysema and bronchitis, particularly in smokers.[7]

PREVENTION

Chronic bronchitis primarily occurs in cigarette smokers. Avoidance of inhaled toxins, particularly cigarette smoke, is of paramount importance in reducing the incidence and progression of chronic bronchitis. The loss of lung function, as measured by spirometry, is more rapid in active cigarette smokers. Such individuals can gain significant benefits from stopping or significantly decreasing their cigarette consumption. Sputum production usually decreases within weeks. The accelerated decline of lung function seen in smokers slows to that of nonsmokers of the same age.[8] Thus, the importance of avoiding primary and secondhand cigarette smoke cannot be overemphasized.

CLINICAL FEATURES

Acute bronchitis typically develops during the course of an acute URI. Pharyngitis, coryza, low-grade fever and malaise precede the development of a cough with scanty sputum. Dyspnea is rare. In the absence of other lung disease, most symptoms subside over several days, although the cough may persist for weeks to several months. The quantity of sputum and frequency of cough decrease with time and no long-term sequelae occur.

The key symptoms of individuals with chronic bronchitis are chronic cough, production of sputum, wheezing and exertional

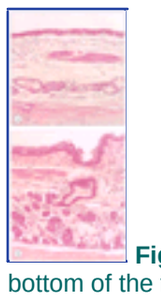


Figure 33-1 (a) Bronchial wall from normal patient. Normal pseudostratified columnar epithelium with few goblet cells overlies smooth muscle and a submucosal gland. Cartilage is at the bottom of the figure. H&E stain, 100× original magnification. (b) Bronchial wall from a patient with chronic bronchitis. Hyperplastic epithelium with mucous cell metaplasia overlies a hypertrophied submucosal gland. H&E stain, 100× original magnification.

dyspnea. These symptoms develop insidiously, often over many years. Presentation for medical care typically occurs during an acute exacerbation. Upon direct inquiry, patients often recall persistent dyspnea and sputum production following URIs for several years prior to presentation. The sputum is purulent, yellow or green, and may be blood-streaked. The daily volume ranges from scanty up to about 60ml. The cough and sputum are usually most severe soon after awakening. Exertional dyspnea and fatigue are first noticed during exacerbations and later become persistent.

Acute or subacute exacerbations are characterized by increases in cough, sputum production, dyspnea and wheezing.[9] These symptoms often follow acute URIs and tend to more frequent during winter months. The sputum often changes in color to darker yellow or green. Sputum cultures may identify one of the bacterial pathogens listed in Table 33.1 but the pathogenic role of these microbes is not certain.[10] [11] Pulmonary function decreases during such exacerbations and may lead to further complications. The progressive disease often leads to increasingly frequent exacerbations over time, further loss of lung function and worse symptoms.

Patients with severe obstructive airways disease may develop the serious complications of hypoxemic and/or hypercapnic respiratory failure. Hypoxemia develops initially during exercise or sleep and may cause morning headaches. Prolonged hypoxemia may cause cyanosis, polycythemia, pulmonary hypertension and cor pulmonale. Secondary signs of right heart failure, such as jugular venous distention, hepatomegaly and peripheral edema develop in late disease and may become chronic. The respiratory acidosis that signals hypercapnic

TABLE 33-1 -- Viral and bacterial pathogens in bronchitis.

VIRAL AND BACTERIAL PATHOGENS IN BRONCHITIS	
Common	**Uncommon**
Viruses: - rhinoviruses	*Chlamydia pneumoniae*
- adenoviruses	*Klebsiella pneumoniae*
- influenza A and B	*Mycoplasma pneumoniae*
- parainfluenza	*Pseudomonas aeruginosa*

Haemophilus influenzae	
Haemophilus parainfluenzae	
Moraxella catarrhalis	
Neisseria spp.	
Streptococcus pneumoniae	

respiratory failure may be of gradual onset and be compensated by a metabolic alkalosis through renal retention of bicarbonate. Individuals with scanty sputum production and greater degrees of emphysema often achieve adequate oxygenation and ventilation until late in the course of the disease. Exacerbations in the setting of severe disease often produce superimposed acute respiratory failure that is life threatening and requires intensive care.

DIAGNOSIS

The history of persistent cough and daily sputum is essential to the diagnosis of chronic bronchitis. The extent and duration of cigarette smoking quantify the major risk factor. The presence of antecedent reactive airways disease, for example, childhood asthma, may increase the risk for developing chronic bronchitis and COPD. Physical examination reveals tachypnea and late expiratory wheezes on auscultation. Patients with advanced disease develop hyperinflation of the lungs with increased anterior-posterior diameter of the thorax and a depressed diaphragm. In such patients, breath sounds and heart sounds are muted. Patients with severe disease may use accessory muscles of respiration and/or pursed-lip breathing. Medium or coarse inspiratory crackles appear in patients with bronchitis and excess airway secretions. Patients with severe disease may develop central and peripheral cyanosis or neck vein distention, hepatomegaly and peripheral edema as signs of hypoxemia and right heart failure.

Pulmonary function tests are essential to establish the diagnosis of obstructive lung disease, to measure the severity of airway obstruction and to follow the course of illness. Spirometry performed before and after inhaled ß-adrenergic agonists often reveals decreased FEV_1 and decreased FEV_1 : FVC ratio. Improved flows following inhaled ß-adrenergic agonists indicate the presence of reversible bronchoconstriction. Forced vital capacity (FVC) is also decreased in patients with very severe obstruction. In such individuals, lung volumes should be measured to distinguish restrictive and obstructive respiratory impairments and to quantify lung hyperinflation and gas trapping. The diffusing capacity for carbon monoxide (DLCO) may be reduced in patients with severe obstruction or emphysema. The chest X-ray shows increased lung volumes. In patients with emphysema the heart may appear small and the bronchovascular markings decreased. Patients with chronic bronchitis often have enlarged hearts, engorged apical vessels, bronchial cuffing and other signs of fluid overload. Arterial blood gases may reveal mild to severe hypoxemia. Patients with severe disease may develop respiratory acidosis and a compensatory metabolic alkalosis. The complete blood count may reveal polycythemia and the serum electrolytes a metabolic alkalosis. Sputum Gram stain and culture are important to assess the abundance of

PMNs and to help identify bacterial pathogens that may be associated with acute exacerbations.

MANAGEMENT

The symptoms of acute bronchitis are best managed with symptomatic treatment. Non-steroidal anti-inflammatory drugs and decongestants are useful for pharyngitis, sinusitis and coryza. Antibiotics are indicated for clinically significant bacterial bronchitis, but such a complication is difficult to distinguish from viral bronchitis. Antibiotics are prescribed for 53–66% of patients with acute sinusitis, bronchitis or URIs,[3] indicating excess usage. This practice promotes the development of antibiotic-resistant bacteria, which may lead to greater morbidity in the community. Therefore, antibiotics should be reserved for patients with acute bronchitis who have increased numbers of PMNs and numerous bacteria in Gram-stained sputum samples or who do not respond to symptomatic therapy.

The most important feature of managing chronic bronchitis is the avoidance of exposure to irritants, particularly cigarette smoke. Thus, smoking cessation is of primary importance for each individual. Support for smoking cessation can be provided by individual counseling, provision of smoking cessation literature or through smoking cessation groups. Nicotine gum or transdermal nicotine may be useful to reduce withdrawal symptoms.

No specific therapy is available to treat chronic bronchitis. Symptomatic therapy is directed at reducing mucosal edema, mucus hypersecretion, bronchial smooth muscle constriction and airway inflammation (Table 33.2). Inhaled ß-adrenergic agonists and anticholinergic drugs may be of benefit to patients with reactive airways disease, as demonstrated on pulmonary function tests.[12] Theophylline may be useful in patients with nocturnal symptoms or severe hyperinflation and respiratory muscle fatigue. In addition to relaxing bronchial smooth muscle, ß-adrenergic agonists enhance mucociliary clearance. Inhaled drugs with intermediate (4–6 hours) and long (8–12 hours) duration of action are available. These are generally preferred to systemic treatments. Metered dose inhalers (MDIs) appear to have comparable efficacy to nebulizers, but effective treatment requires co-ordination of MDI actuation and the breathing cycle. The beneficial effects can be enhanced by the use of a spacer to improve deposition of drugs in the lungs. Dry powder inhalers (DPIs) can be easier to use and move effective. Anticholinergic drugs relax bronchial smooth muscle and have an intermediate duration of

TABLE 33-2 -- Therapeutic options for acute exacerbations of chronic bronchitis.

THERAPEUTIC OPTIONS FOR ACUTE EXACERBATIONS OF CHRONIC BRONCHITIS		
Class/agent	**Examples**	**Notes**
Bronchodilators		
ß-Adrenergic agonists	Albuterol, salmeterol	Inhaled administration preferred
Anticholinergics	Ipratropium bromide	Inhaled administration mandatory
Theophylline		Second-line agent
Anti-inflammatory agents		
Corticosteroids	Prednisone, fluticasone	Administration by inhalation may reduce side-effects
Expectorants		
rhDNase	Dornase alfa	Efficacy in COPD not established
Iodinated compounds	Iodinated glycerol	Limited efficacy
Reducing agents	*N*-acetyl cysteine	Limited efficacy
Airway clearance measures		
Controlled coughing		Efficacy not established
Physical therapy		Efficacy not established
Supplemental oxygen		Corrects significant hypoxemia
Antibiotics		Indications and efficacy controversial

action (4–6 hours). Ipratropium bromide is available as an MDI. The effects of anticholinergics and ß-agonists appear to be roughly equivalent.[13] The responses of individual patients to such drugs must be assessed to determine the optimum treatment.

Systemic and inhaled corticosteroids provide a beneficial effect by reducing severity of airway inflammation. This typically results in decreased airway obstruction and decreased mucus secretion. Long-term use of systemic corticosteroids may be complicated by osteoporosis, central obesity and/or glucose intolerance. Once a beneficial steroid effect has been demonstrated with systemic therapy, these side effects can be minimized by use of moderate to high doses of inhaled steroids. Typical drugs include beclomethasone 800–1600µg per day and fluticasone 88–1760µg per day. Other forms of therapy such as cromolyn sodium, expectorants and chest physiotherapy have not been demonstrated to have significant effects in chronic bronchitis or COPD.

The role of bacterial infection in causing acute exacerbations of chronic bronchitis is controversial. It is likely that less than half the cases of acute exacerbation are due

to bacterial infection. A number of controlled studies have failed to show significant benefit of antibiotic therapy.[14] [15] Nevertheless, antibiotics have a role in patients who demonstrate a significant increase in the number of PMNs in expectorated sputum and dominant bacteria on Gram stain or in culture. Previous positive responses to antibiotic therapy may also support repeated use in individual patients. Antibiotics effective against the common bacterial species are listed in Table 33.3 . Patients with exacerbations of sufficient severity to require hospitalization should be treated with parenteral antibiotics with comparable antibacterial spectra, and the treatment modified based on sputum culture results.

Hypoxemia may be diagnosed by ambulatory, exercise or nocturnal pulse oximetry, as well as by arterial blood gases. Patients with significant hemoglobin desaturation (SaO_2 <90%) should receive supplemental oxygen. Nocturnal oxygen has been shown to improve survival[16] and continuous treatment has greater effects than nocturnal treatment alone.[17] Some patients with COPD also have obstructive sleep apnea and detailed sleep studies may be required to establish the effectiveness of oxygen and/or continuous positive airway pressure (CPAP) by nasal mask to prevent nocturnal hypoxemia.[18] Noninvasive assisted ventilation by nasal mask has been used for some patients with severe hypercapnic respiratory failure.[19] Patients with acute respiratory failure and significant respiratory

TABLE 33-3 -- Oral antibiotics for acute exacerbations of chronic bronchitis.

ORAL ANTIBIOTICS FOR ACUTE EXACERBATIONS OF CHRONIC BRONCHITIS	
Agent	**Dose**
Amoxicillin	250–500mg tid
Amoxicillin/clavulanic acid	875/125mg tid
Ampicillin	500mg qid
Azithromycin	500mg day 1, then 250mg qd
Cefaclor	500mg tid
Cephalexin	500mg qid
Ciprofloxacin	500–750mg bid
Clarithromycin	500mg bid
Doxycycline	100mg bid
Erythromycin	500mg qid
Ofloxacin	400mg bid
Tetracycline	500mg qid
Trimethoprim-sulfamethoxazole	160/800mg bid

acidosis or hypoxemia often require hospitalization, parenteral antibiotics, ICU care and mechanical ventilation.[20]

BRONCHIECTASIS

EPIDEMIOLOGY

Bronchiectasis is defined as abnormal dilatation of the bronchi. [21] [22] It typically involves medium-sized bronchi and results from destruction of the muscular and elastic components of the walls. Bronchiectasis is classified as cystic, cylindrical or varicose, based on the morphological structure of the airways. Chronic airway inflammation is the essential pathologic feature, resulting from genetic abnormalities that impair airway defense mechanisms or from chronic respiratory infections.[23] Such infections have become relatively rare in the United States; the current prevalence of bronchiectasis is less than 1 in 10,000.

PATHOGENESIS

The principal diseases that cause bronchiectasis are listed in Table 33.4 . The genetic diseases cause deficits in airway defense or immunological mechanisms that permit the development of chronic bacterial infections in the airways. Bacterial and inflammatory cell-derived proteolytic and oxidative molecules cause progressive airway wall damage that eventually produces bronchiectasis. Immune reactions to fungi can produce the central bronchiectasis that is associated with allergic bronchopulmonary aspergillosis (ABPA). Chronic infections with *Mycobacterium tuberculosis* [23] or nontuberculous mycobacteria (particularly *Mycobacterium avium* complex)[24] and *Bordetella pertussis* infections are recognized infectious causes. Bacteria that secondarily infect damaged airways following other injuries probably propagate airway damage, but the time course and relative contributions of the different organisms have not been established.

CLINICAL FEATURES

Daily cough and production of purulent sputum are the most typical symptoms of bronchiectasis. Sputum production can range from less than 10ml to greater than 150ml daily and tends to correlate with

TABLE 33-4 -- Principal causes of bronchiectasis.

PRINCIPAL CAUSES OF BRONCHIECTASIS
Genetic
Cystic fibrosis
Immunoglobulin deficiency
Primary ciliary dyskinesia
Infectious
Bordetella pertussis
Tuberculosis
Nontuberculous mycobacteria (especially *M. avium* complex)
Inflammatory
Allergic bronchopulmonary aspergillosis (ABPA)
a_1 -Antitrypsin deficiency
Bronchial obstruction
Other
Bronchopulmonary sequestration
Congenital cartilage abnormalities
Yellow nail syndrome

disease extent and severity. Bronchiectasis associated with cystic fibrosis, which becomes generalized and progresses relentlessly, is described in greater detail below. Occasional individuals have no discernible sputum production ('dry bronchiectasis'). The clinical course is usually of progressive symptoms and respiratory impairment. Airway obstruction progresses and leads to increasing exertional and resting dyspnea. Acute exacerbations may be precipitated by viral or newly acquired bacterial pathogens, as with chronic bronchitis. The main clinical differentiating feature is the quantity of sputum production. In addition to progressive respiratory failure, patients with bronchiectasis are prone to hemoptysis due to hypertrophied bronchial arteries that are closely apposed to the inflamed airways.[25] Hemoptysis from this source can be massive, even fatal.

DIAGNOSIS

Diagnosis is suggested by clinical symptoms and a physical exam with hyperinflated chest and medium- to low-pitched inspiratory crackles and sometimes expiratory wheezes. Chest X-ray may demonstrate hyperinflation and bronchiectatic cysts or dilated bronchi with thickened walls forming tram track patterns radiating from the lung hila. High-resolution chest computed tomographic (CT) scans readily demonstrate mild and severe forms of bronchiectasis. CT scans have largely replaced bronchography as a diagnostic examination. Sputum culture may identify characteristic pathogens, including *H. influenzae, Strep. pneumoniae* and/or *Pseudomonas aeruginosa*. Sputum AFB smears and cultures should be performed to evaluate mycobacterial disease. Spirometry is essential to determine the severity of airway obstruction and to evaluate the course of disease.

MANAGEMENT

Specific etiologies such as tuberculosis should be identified and treated whenever possible. Standard therapy includes measures to clear excess secretions from the airways. Chest physiotherapy based on chest percussion and postural drainage is accepted as the most effective technique. Alternatives such as pneumatic vests and aerobic exercise or flow interrupter valves may be effective, but their use must be individualized. Bronchodilators have a role in patients with objective spirometric or subjective clinical responses. Acute exacerbations are managed with intensification of airway clearance measures

and the use of antibiotics directed at pathogens identified in recent sputum cultures. The use of prophylactic oral or inhaled antibiotics has been advocated to control the major symptoms of bronchiectasis and such treatment must be tailored to the individual responses. Surgical resection of localized bronchiectatic lung is occasionally indicated. [26]

CYSTIC FIBROSIS

EPIDEMIOLOGY

Cystic fibrosis is the most common lethal genetic disease in Caucasians. [27] This autosomal recessive disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene located on chromosome 7. The incidence is one in every 3300 live Caucasian births, with a gene carrier rate of one in 29. Other ethnic groups have lower carrier rates, with the Hispanic birth incidence being one in 9500, the Native American one in 11,200, the African-American one in 15,300 and the Asian one in 32,100 live births.

When cystic fibrosis was first described in 1938, survival past infancy was rare. Improved treatments for pancreatic insufficiency, lung infections and other complications have increased the median survival from less than one year to more than 30 years (Fig. 33.2). Over 22,700 CF patients have been identified in the United States.[28] The median survival in 2001 was 33.4 years. Adults (=18 years old) now account for 39.5% of CF patients and survival can extend to 78 years.[28]

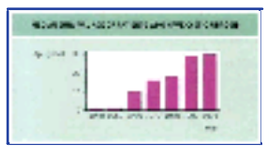


Figure 33-2 Median survival age of patients who have cystic fibrosis. The median survival age has increased dramatically. *Data from Cystic Fibrosis Foundation, Bethesda, MD.*

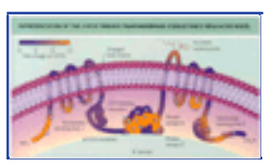


Figure 33-3 Representation of the CFTR model, based on structural, hydropathy and expression studies. The membrane-spanning domains are arranged in groups of six, each associated with a nucleotide-binding fold. These features are similar to those of the multidrug resistance 'P' glycoprotein. The 'R' domain is unique to CFTR.

PATHOGENESIS

The CFTR gene encodes a 1480 amino acid protein, with 12 membrane-spanning regions, two nucleotide-binding folds and a regulatory ('R') domain (Fig. 33.3). This protein is localized to the apical membranes of epithelia lining the organs affected by the disease, particularly the airways, pancreatic duct, sweat gland duct, intestines and reproductive tract. CFTR protein acts as a Cl^- channel[29] and as a regulator of epithelial Na^+ channels[30] and other Cl^- channels. [31] Over 700 different mutations in the CF gene have been identified, encompassing several functional abnormalities (Fig. 33.4). Class I mutations prevent protein production. Class II mutations produce proteins that fail to traffic to the apical cell membrane. The most common CF mutation, ?F508, is in this class; it accounts for 68% of US mutations. Class III mutations traffic properly but have defective regulation. Class IV mutations traffic properly but have defective Cl^- conductance. Some exon splice-site mutations have been labeled class V mutations and may permit transcription of some normal CFTR mRNA, conferring a less severe clinical

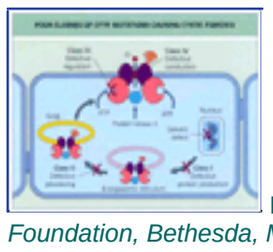


Figure 33-4 CFTR mutations are divided into classes based on the mechanisms of dysfunction. See text for detailed descriptions. *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.*

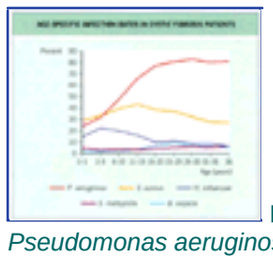


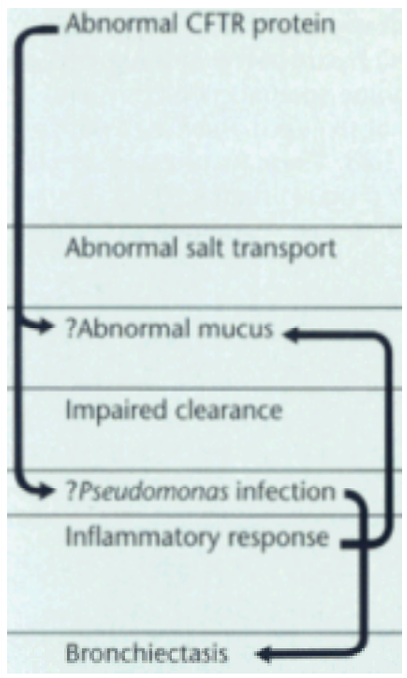
Figure 33-5 Age-specific infection rates in cystic fibrosis patients. Bacteria isolated from CF sputum samples vary with age and demonstrate the trend toward *Pseudomonas aeruginosa* as the dominant pathogen. *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.*

phenotype.[32] All classes of mutations alter Cl^- permeability and regulation of ion transport in the affected epithelial cells.

The pathogenesis of airways disease in cystic fibrosis is not completely understood. CFTR mutations produce decreased Cl^- permeability and increased net Na^+ absorption by bronchial epithelial cells. The effects on bronchiolar epithelial cells and on submucosal gland secretion are not fully understood. It has been suggested that CF mutations lead to relative dehydration, abnormal mucus sulfation or abnormal function of peptide antimicrobial molecules (defensins) in the airway surface liquid.[33] These abnormalities cause impaired

TABLE 33-5 -- Treatment approaches to CF airways disease.*

TREATMENT APPROACHES TO CYSTIC FIBROSIS AIRWAYS DISEASE			
		Approach	
Abnormality	**Solution**	**Available**	**Investigational**
Abnormal CF gene	Provide normal gene		Gene therapy

	Provide normal protein		Protein therapy
	Activate mutant form		?Phosphodiesterase inhibitors
			?Phosphatase inhibitors
			?Others
	Block Na^+ uptake		Amiloride
	Increase Cl^- efflux		UTP
	Decrease viscosity	Dormase a (rhDNase) (*in vitro*)	Gelsolin
	Augment ciliary action	Airway clearance techniques	
	Reduce bacterial count	Antibiotics	
	Decrease host reaction	Anti-inflammatory drugs (corticosteroids, ibuprofen)	Antiproteases
			Pentoxifylline
			IVIG
	Replace irreversibly damaged areas	Lung transplantation	

The pathogenesis of CF lung disease is based on a vicious cycle of airway infection, inflammation and obstruction (first column). Treatment can be directed at different pathogenic mechanisms (second column). Currently available and proposed treatment options are shown in final columns.

* *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD*.

mucociliary clearance, secondary bacterial infection and airways inflammation. This chronic infection and inflammation forms a vicious cycle that produces progressive airway obstruction, bronchiectasis and eventually respiratory failure. This scheme of pathogenesis is illustrated in Table 33.5 . Some existing and potential treatments, directed at the specific pathogenic processes, are indicated.

CLINICAL FEATURES

Cystic fibrosis is classically recognized from the triad of bronchiectatic airways disease, exocrine pancreatic insufficiency and elevated sweat chloride. Most patients have onset of cough and chronic respiratory tract infections during infancy or childhood. Early respiratory tract pathogens include *Staphylococcus aureus* and *Haemophilus influenzae*. *Pseudomonas aeruginosa*, particularly mucoid variants, appears in greater prevalence with increasing age and becomes the dominant pathogen by the teenage years (Fig. 33.5). It is common to isolate several different bacteria from the sputum of adolescent and adult CF patients. Pathologically, there is inflammation and obstruction of both bronchioles (Fig. 33.6) and bronchi, with submucosal gland hypertrophy (Fig. 33.7). Bronchiectasis tends to start in the upper lobes and becomes generalized. Chest X-rays show hyperinflated lungs, cystic bronchiectasis and occasionally upper lobe atelectasis (Fig. 33.8).

The clinical course of chronic cough, mucus hypersecretion and airway obstruction is progressive and is punctuated by acute exacerbations, characterized by the features listed in Table 33.6 . When such exacerbations are effectively treated, pulmonary function may return to baseline levels. With more severe disease exacerbations become more frequent and less reversible, culminating in fatal respiratory failure (Fig. 33.9).

DIAGNOSIS

The standard diagnostic criteria for cystic fibrosis are the combination of characteristic lung disease with airway obstruction, bronchiectasis and infection with typical bacterial pathogens; exocrine

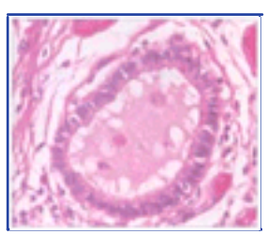


Figure 33-6 A CF bronchiole is completely occluded by mucoid secretions and surrounded by fibrotic tissue. H&E stain, original magnification 400X.

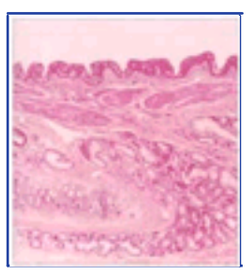


Figure 33-7 A CF submucosal gland demonstrates marked hypertrophy and dilated gland ducts with mucoid secretions. The surface epithelium has marked goblet cell metaplasia. H&E stain, original magnification 100X.

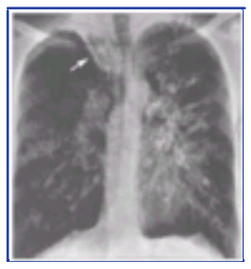


Figure 33-8 A typical postero-anterior chest X-ray of a 24-year-old man with CF. The lungs are hyperinflated due to airway obstruction and bronchiectasis. The right upper lobe atelectasis is chronic.

pancreatic insufficiency which occurs in more than 85% of CF patients; and elevated sweat chloride which occurs in >98% of patients. The diagnosis initially may be suggested by a family history of CF or by the presence of meconium ileus at birth, noted in 17% of cases. Of patients reported to the CF Foundation (CFF) Patient Registry, 51% presented with acute or persistent respiratory symptoms, 43% with failure to thrive or malnutrition, 35% with steatorrhea and 21% with meconium ileus or intestinal obstruction.

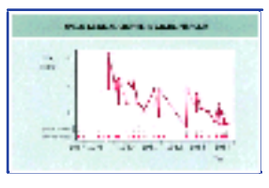


Figure 33-9 Typical clinical course in CF. Serial pulmonary function measurements (FEV_1) demonstrate a typical clinical course. Measurements are connected by solid lines

during therapy for acute exacerbations. The lower bars indicate periods of hospital treatment. Pulmonary function decreases and the exacerbations are more frequent and less responsive to treatment in advanced disease.

TABLE 33-6 -- Signs and symptoms of a CF pulmonary exacerbation.[*]

SIGNS AND SYMPTOMS OF A CF PULMONARY EXACERBATION
• Increased cough
• Increased sputum
• Increased dyspnea
• School or work absenteeism
• Reduced exercise tolerance
• Weight loss >1 kg or >5% of body weight
• Decreased FEV_1 (10% from baseline)
• New chest findings
Rales
Wheezes
• New radiographic findings

* *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.*

Developments in the understanding of the molecular and physiological pathogenesis of cystic fibrosis have led to additional diagnostic criteria. CFTR mutational analysis is offered by several companies that test for up to 70 common mutations and can detect mutations in about 95% of CF cases. Abnormal CFTR function in airway epithelia can be assessed by *in vivo* measurements of nasal electrical potential difference and its response to selected modulators of ion transport.[34] The clinical application of these tests has been summarized by a CFF-sponsored Consensus Committee.[35]

MANAGEMENT (see also Chapter 40g)

Exocrine pancreatic insufficiency and malnutrition are managed with oral pancreatic enzymes and dietary supplements. Pulmonary disease causes the major morbidity in CF and eventually death in 95% of patients. Daily clearance of airway secretions is essential (Table 33.7).[36] This can be accomplished by chest physiotherapy, which enhances sputum production and increases pulmonary function. [37] Physical exercise augments airway clearance and improves cardiovascular function. Special breathing techniques, including forced expiratory technique, autogenic drainage and active cycle of breathing,[38] have been useful in some individuals. Mechanical devices, including the flutter valve[39] and external thoracic compression devices, may improve patient independence, but their efficacy is less well established.

TABLE 33-7 -- Standard therapy for cystic fibrosis lung disease.

STANDARD THERAPY FOR CYSTIC FIBROSIS LUNG DISEASE
Airway clearance
Chest physical therapy
Physical exercise
Special breathing techniques
Mechanical devices
Antibiotics
Intravenous
Oral
Inhaled
Others
Bronchodilators
Supplemental oxygen
Anti-inflammatory agents (ibuprofen, corticosteroids)
Recombinant human deoxyribonuclease (rhDNase)

Antibiotics are used extensively. Acute exacerbations are treated with intravenous antibiotics directed at the major pulmonary pathogens, especially *Pseudomonas* species and *Staph. aureus*. Because of the high bacterial burden, two antibiotics with different mechanisms of action and with *in vitro* efficacy against each major bacterium are selected. Pharmacokinetic studies of ß-lactams, aminoglycosides and sulfa drugs demonstrate increased clearance in CF patients, necessitating the use of higher doses. Typical antibiotic choices are listed in Table 33.8 . Home intravenous antibiotic therapy has cost and convenience advantages, [40] but its clinical efficacy in this setting has not been rigorously established. The benefits of chronic oral antibiotics are controversial, but some aerosolized antibiotics have demonstrated efficacy.[41]

CF patients are particularly susceptible to the complications of massive hemoptysis and pneumothorax. Episodes of massive hemoptysis, defined as >240ml blood per 24 hours, are managed with antibiotics, transient cough suppression and reduction in chest physiotherapy, and bronchial artery embolization.[42] Such therapy is usually effective and does not compromise candidacy for eventual lung transplantation. Large pneumothoraces are managed by chest tube drainage. Recurrent pneumothoraces may require repeated chest tubes or abrasion pleurectomy. [43] Hypoxemia is best treated with supplemental oxygen plus standard pulmonary therapy. Ventilatory assistance can be effectively provided by mask ventilation.[44] [45]

Lung transplantation has become an effective form of therapy. [46] From the first heart-lung transplant for CF in 1983 to 2001, more than 1270 heart-lung or sequential double lung transplants (the preferred operation in the United States) for CF have been performed worldwide. Transplant evaluation is indicated when natural survival is expected to be slightly longer than the waiting time for donor organ availability, currently about 2 years. The highly variable progression of CF disease makes prediction difficult, but an FEV_1 less than 30% predicted or increasing functional impairment with frequent hospitalizations are accepted referral criteria. The 1- and 5-year survivals after lung transplantation for CF are 70% and 48%, respectively. These are comparable to the survival of patients who received lung transplants for other diseases. Deaths in the first year are primarily due to operative complications and infections. After

TABLE 33-8 -- Inhaled and parenteral antibiotics commonly used for cystic fibrosis.

INHALED AND PARENTERAL ANTIBIOTICS COMMONLY USED FOR CYSTIC FIBROSIS
Parenteral (normally two effective agents against each bacterial isolate)

Class/drug	Pertinent efficacy
Aminoglycosides*	
Gentamicin	*Staph. aureus, H. influenzae, Pseudomonas*
Tobramycin	*Staph. aureus, H. influenzae, Pseudomonas*
ß-lactams*	
Ceftazidime	*Pseudomonas*
Piperacillin	*H. influenzae, Pseudomonas*
Ticarcillin/clavulanate	*Staph. aureus, H. influenzae, Pseudomonas*
Monobactam	
Aztreonam	*Pseudomonas*
Carbapenem	
Imipenem-cilastatin	*Staph. aureus, H. influenzae, Pseudomonas*
Fluoroquinolones	
Ciprofloxacin	*Staph. aureus, H. influenzae, Pseudomonas*
Sulfa drugs*	
Trimethoprim/sulfamethoxazole	*Staph. aureus, B. cepacia*
Glycopeptides	
Vancomycin	Oxacillin-resistant *Staph. aureus*
Aerosolized	
Drug	**Common doses**
Tobramycin	80–300mg bid to tid
Colistamethate sodium	75–150mg bid to tid

* Higher doses required because of increased clearance in CF

1 year most deaths are caused by obliterative bronchiolitis, the pathologic marker of chronic rejection. The scanty number of donor organs limits the availability of lung transplantation and many patients wait more than 2 years after being accepted as a transplant candidate. Living donor transplantation (sequential transplantation of a lower lung lobe from each of two donors) has become an effective alternative in some centers.[47]

CONCLUSION

Inflammatory airways diseases are highly prevalent, causing significant morbidity and mortality. Different pathogenic factors cause distinct patterns of disease, including bronchitis and bronchiectasis. The ability to stop the progression of these diseases is often limited by chronic inflammation and by structural alterations in the airways. Nevertheless, antibiotics, anti-inflammatory drugs and other forms of therapy can modulate acute exacerbations and, potentially, the progression of these diseases. Elucidation of the pathogenic mechanisms, as is being done in cystic fibrosis, may uncover new and more effective means of treatment. The diagnostic tests, continued monitoring and choice of therapeutic options must be tailored to the individual clinical presentation and responses of each patient.

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Chapter 34 - Community-acquired Pneumonia

David R Baldwin
John T Macfarlane

INTRODUCTION

Pneumonia can be discussed in a number of different ways. We have chosen the scheme illustrated in Table 34.1 . This chapter concentrates largely on community-acquired pneumonia (CAP) and Chapter 35 on hospital-acquired pneumonia, including ventilator-associated pneumonia. Other subjects are covered elsewhere.

EPIDEMIOLOGY

Definition and classification

Pneumonia acquired outside hospital is termed CAP. It may be a primary disease occurring at random in healthy individuals or may be secondary to a predisposing factor such as chronic lung disease or diabetes mellitus. It is important to understand that community-acquired lower respiratory tract infection (LRTI) is not synonymous with CAP and only 5–10% of patients who have LRTI actually have pneumonia characterized by radiologic evidence of lung parenchymal disease, which may include lobar or segmental opacification, patchy nonsegmental shadowing or diffuse disease. Table 34.1 shows a clinically relevant classification of CAP; categories are based on severity and the presence of pre-existing disease. Management of patients in the different categories differs in terms of general supportive measures and specific antimicrobial therapy.

Incidence

Several large studies have measured the incidence of CAP and have reported rates that vary by more than 10-fold.[1] A large community study in Seattle, Washington, USA in the 1960s and 1970s showed an overall prevalence of 12 per 1000 population for all ages, which was

TABLE 34-1 -- Classification of pneumonia.

CLASSIFICATION OF PNEUMONIA
Community-acquired pneumonia (CAP)
Nonsevere CAP: no unusual risk factors present
Nonsevere CAP: risk factors present (related to host or environment)
Severe CAP
Hospital-acquired pneumonia (HAP)*
Nonsevere HAP: no unusual risk factors
Nonsevere HAP: risk factors present
Severe HAP: early onset
Severe HAP: late onset
Pneumonia in immunocompromised host
Pneumonia peculiar to certain geographic areas
This classification is designed to provide a practical approach to likely pathogens and their appropriate management.

* Includes ventilator-associated pneumonia (VAP)

similar to the rate in Finland in 1993 (11.6 per 1000).[2] In England in 1985, a lower rate of 1–3 per 1000 was found for adults, and in Spain in 1992 a similar rate of 2.6 per 1000 was found.[3] [4] This difference may in part be explained by differences in the criteria used to define pneumonia. However, when the same criteria are used, it can be shown that a variety of factors influence incidence: age, race, social deprivation indices,[5] recent admission for pneumonia and host defense factors.[6] In the North American study, prevalence was higher in 0 to 4-year-olds (12–18 per 1000) and in the Finnish study it was higher in 2 to 5-year-olds (36 per 1000) and in those aged over 70 years (34 per 1000). A more recent study from the Mayo Clinic has also shown a rate of pneumonia of 30 per 1000 for patients aged over 65 years. [7]

The attack rate is also influenced by the seasonal pattern of pathogens either causing or predisposing to pneumonia (such as viruses).[8] The seasonal pattern of common pathogens is shown in Figure 34.1 . Most respiratory pathogens are more common in the winter months but, notably, legionellosis is more common in the summer and *Mycoplasma* infection occurs in worldwide epidemics every 3–4 years, when it is responsible for approximately 20% of cases of pneumonia. At other times *Legionella* cases are sporadic and account for fewer than 10% of pneumonia cases. Bacterial pneumonia, including that caused by *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus*, are seen at times of peak activity of the influenza virus. Virus infections predispose bacteria normally contained in the nasopharynx to colonize the lower airway. Furthermore, viral infections may encourage more severe infection by impairing host defense mechanisms (see Pathogenesis and pathology). *Streptococcus pneumoniae*, *H. influenzae, S. aureus* and

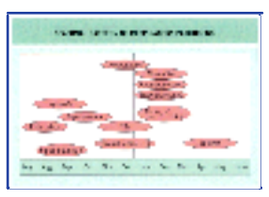

Figure 34-1 Seasonal pattern of respiratory pathogens in the northern hemisphere. Most respiratory pathogens are commoner in the winter months with the notable exceptions of *Legionella* spp. and *Coxiella burnetii* (Q fever). *With permission from Macfarlane J. Community-acquired pneumonia. Br J Dis Chest 1987;81:116–27.*

Moraxella catarrhalis are carried in the nasopharynx to varying degrees (50%, 50%, 30% and 5–50%, respectively). During epidemics of mycoplasmosis, pharyngeal carriage rates of 13.5% have been reported.[9] *Haemophilus influenzae* and *M. catarrhalis* can also colonize the lower respiratory tract in patients who have chronic obstructive pulmonary disease (COPD). This may reflect their ability to persist in a lower respiratory tract compromised by impaired defenses by producing only a low-grade inflammatory response and thereby not inducing a vigorous immune response.

When *Legionella*, psittacosis and Q fever infections occur in clusters, an environmental source should be sought. *Legionella* infections occur worldwide and are usually sporadic. Their occurrence in the summer months may partly be explained by foreign travel and exposure to risk factors such as water systems in public buildings and hotels. Debilitated or immunocompromised patients are particularly vulnerable. *Legionella* organisms are found in native waters, including rivers, lakes, thermal springs and under ice, in industrial coolant water and in water circulating in large buildings and domestic systems. *Legionella* spp. have been cultured from many hot water systems in homes, hotels and hospitals. The organisms are killed by drying but may survive for more than a year in domestic tap water. There is no human or animal reservoir, although algae and amebae may act as environmental hosts. Legionellosis epidemics have occurred in hotels and hospitals; indeed, the disease was first recognized in 1976 when an outbreak of severe pneumonia occurred among members of the Pennsylvanian branch of the American Legion who had stayed at a particular hotel. For an outbreak to occur, it is necessary to have a reservoir, such as an infected water system; bacterial amplification facilitated by stagnation, dirt or sludge; and a water temperature between 68° and 113°F (20–45°C). The bacteria then have to be disseminated via showers, fans, air conditioning, respiratory therapy equipment and, possibly, spray taps. Finally, the strain of *Legionella* must be pathogenic to humans and be inhaled in sufficient quantity to cause infection.[10]

Morbidity and mortality

Pneumonia is the sixth leading cause of death in the USA and the commonest fatal infectious disease. Mortality depends on the setting, being less than 1% in patients managed in the community and higher in those admitted to hospital. Rates reported in the UK and Europe vary from 5% to 14%. In-hospital mortality figures from the USA are higher, probably because more patients who have CAP are provided with ambulatory care and consequently only those who have more severe pneumonia or co-morbidity are admitted to hospital. If admission to intensive care is required, mortality rates may range from 22% to over 50%.[11] [12] Mortality, as well as attack rate, is influenced by co-morbidity. The majority of adult patients who die from CAP have one or more co-morbidities such as COPD, ischemic heart disease, diabetes mellitus, malignancy and neurologic disease. These are more often present in the elderly, which partly explains the rising mortality with age.

Economic implication

In the USA it has been estimated that CAP costs $8.4 billion. Half of the costs are for the 1.1 million patients treated in hospital and half for the 4.4 million outpatients.

PATHOGENESIS AND PATHOLOGY

The mode of acquisition varies by pathogen. Most bacterial infections result from aspiration of endogenous organisms resident in the nasopharyngeal secretions, although inhalation of infected droplets may occur from other patients (viral infections), from animals (psittacosis) or from environmental sources (legionellosis).

Host-pathogen interactions

The distal lung contains few commensal micro-organisms despite large numbers present in inspired air and upper airway secretions. A multifaceted defense system maintains this relatively sterile environment, which is overcome or subverted in pneumonia. Table 34.2 and Table 34.3 summarize these host defenses and the tactics used by pathogens to overcome them. The majority of bacteria entering the lungs are rapidly removed by local mechanisms such as the mucociliary escalator and cough reflex in combination with local secretory IgA and phagocytosis by macrophages. With more invasive infections, there is a progressive host response with recruitment of circulatory inflammatory cells, augmented by a specific immune response. In normal lungs, over 85% of the cells recovered from the lower airways by bronchoalveolar lavage are alveolar macrophages, but with infection this changes dramatically as outside cells are recruited. Greatly increased numbers of neutrophils are seen in conventional bacterial pneumonias such as those produced by the pneumococcus. In *Legionella* and viral infections, a lymphocyte infiltrate often predominates. Other inflammatory cells are found in lesser numbers, for example natural killer cells.[13] [14]

TABLE 34-2 -- Host defense mechanisms.

HOST DEFENSE MECHANISMS		
Host defense mechanisms		**Mechanism of lung defense**
Physical defenses	Nose	Filtering of larger particulate matter
	Cough	
	Mucociliary escalator	Bulk clearance of microbes in secretions
Non-specific cellular and humoral factors	Immunoglobulins (principally IgA)	Binding of microbes to facilitate removal by the mucociliary escalator or ingestion and destruction by alveolar macrophages or removal via pulmonary lymphatics
	Complement	
	Antiproteases	
	Opsonins	
	Lactoferrin	Depletion of essential nutrients for some bacteria
	Alveolar macrophage	Endocytosis of bacteria and release of toxic oxygen and nitrogen radicals and lysosomal enzymes
		Direct and antibody dependent cytotoxicity, secretion of IFN-?, TNF and transferrin
	Neutrophil	Endocytosis and generation of oxygen and nitrogen radicals, lactoferrin and lysozyme
Specific immune response	Specific antibody production	Enhanced production of a variety of cytokines, including IFN-?, IL-2, IL-12, TNF-ß, IgA, IgG, IgM, perforins and granzymes
	Macrophage-T-lymphocyte interaction	
	Recruitment of neutrophils, lymphocytes and natural killer cells	
TNF, tumor necrosis factor.		
The lung has several levels of defense, from mechanical barriers to a specific immune response. These processes have overlapping cellular and humoral effects.		

TABLE 34-3 -- Microbial strategies against host defense.

MICROBIAL STRATEGIES AGAINST HOST DEFENSE		
Microbial strategy	**Organism**	**Mediators/mechanisms**
Inhibition of ciliary function	*Pseudomonas aeruginosa*	Phenazine pigments
	Streptococcus pneumoniae	Pneumolysin
	Haemophilus influenzae	Unidentified low molecular weight compound
Damage to cilia	*P. aeruginosa*	Pyocyanin (a phenazine pigment)
	S. pneumoniae	Pneumolysin
	H. influenzae	Lipopolysaccharide (endotoxin)
Adherence to components of mucus	*H. influenzae, P. aeruginosa*	Pili, fibrils
Penetration of mucus and adherence	Influenza viruses	Viral neuraminidases

Inhibition of inflammatory cells	*H. influenzae*	Culture filtrates inhibit neutrophil migration
	Respiratory viruses	May induce host damage to phagocytes through cellular expression of foreign antigen effects on mobility, ingestion and microbial killing
Avoid detection	*Legionella*, fungi, mycobacteria	Survive intracellularly
	Respiratory viruses	
A knowledge of the mechanisms of microbial counter-defense can be useful in the clinical setting. For example, it is important to use antibacterial agents that are active in the intracellular environment when treating *Legionella* and mycobacterial infections.		

Inflammatory cell recruitment

Recruitment of cells from the lung interstitium and blood to the site of infection occurs through non-specific and specific immune reactions. Non-specific reactions include the local production of a variety of chemotactic and proinflammatory cytokines by resident lung macrophages and epithelial cells. In addition, a specific immune response is generated. Macrophages and dendritic cells process antigens to smaller molecules that are displayed on the cell surface as part of the major histocompatibility complex (MHC) locus. Antigen presentation requires direct $CD4^+$ T-cell-macrophage/ dendritic cell contact via intercellular adhesion molecules. The T-cell receptor binds to MHC class II molecules on the antigen presenting cell and at the same time binds the intercellular adhesion molecule (ICAM)-1 to its ligand lymphocyte function associated antigen-1. The latter provides additional co-stimulation of lymphocytes. Antigen presentation may occur in the lung interstitium, the bronchus-associated lymphoid tissue and the intrapulmonary lymph nodes. As a result a variety of cytokines are produced; interleukin (IL)-1 is secreted by antigen presenting cells, and this in turn induces IL-2 production by memory T cells. IL-2 is a potent inducer of T-cell activation and proliferation.[15]

Cell-mediated antimicrobial activity

T-cell activation and cytokine production stimulate other cells to increase antimicrobial function. For example, interferon (IFN)-? increases macrophage antimicrobial function by enhancing MHC expression and augmenting oxygen-dependent and -independent microbial killing. This mechanism acts on intracellular pathogens and inhibits growth of respiratory pathogens such as *Legionella* spp., mycobacteria, pathogenic fungi, *Pneumocystis carinii* and *Chlamydia* spp. There are many other host defense cytokines (see Table 34.2) that may interact and have common activities. They may act to stimulate or suppress host defense reactions. When viruses reach the lower airways they interact with macrophages that normally resist invasion and replication through the production of interferons. Macrophages can also reduce virus replication in neighboring cells. If a specific antibody to the virus is present, this can neutralize virus via induction of natural killer cells, T cells, IFN-? secretion and lysis by complement.

Counter-defense mechanisms employed by pathogens

Pathogenic organisms have a variety of virulence factors that may contribute to the inhibition of normal defense mechanisms. For example, many bacteria inhibit ciliary function, thus decreasing the efficacy of clearance from the lung (Table 34.3). Others may exist in the mucous layer without stimulating a significant host response, thus subverting detection and, when clearance is impaired as in COPD, may colonize the airway. Other bacteria, such as *Legionella* and *Mycobacterium* spp., may avoid host defenses by surviving and replicating intracellularly; the former exist inside membrane-bound vacuoles, which resist lysosomal fusion and acidification. Respiratory viruses can interfere with the mucociliary escalator and also produce factors such as neuraminidases, which facilitate penetration of mucus and attachment to the epithelial cells. This predisposes to bacterial colonization and secondary infection. Viruses affect phagocytic mobility, ingestion and microbial killing. Specific antibodies directed against defense cells expressing viral antigens may further impair their function.[16]

Gross pathology and histology

The interactions between host and pathogen result in the histopathologic features of pneumonia. Initially there is alveolar congestion and an exudate rich in fibrin and red blood cells. Macroscopically the consolidated lung appears rather like liver, hence the term 'red hepatization'. Later in the course of the illness the lung becomes more gray (gray hepatization) as a result of infiltration with neutrophils and other inflammatory cells. The consolidated lobe sometimes shows multiple abscess formation (Fig. 34.2) or, occasionally, frank necrosis.

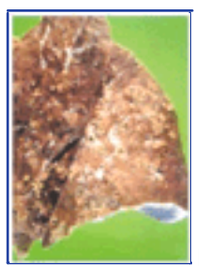

Figure 34-2 Multiple discrete areas of consolidation with abscess formation is a classic feature of *Staphylococcus aureus* pneumonia. *With permission from Macfarlane JT, Finch RG, Colton RE. A colour atlas of respiratory infections. London: Chapman & Hall; 1993.*

These features are more often present in *S. aureus*, *Klebsiella pneumoniae* and anaerobic infections.

Etiology

A key factor guiding therapy for CAP is knowledge of the spectrum of potential pathogens. Major factors that influence this spectrum are age (infection in children differs from that in adults and the elderly); severity (as defined by clinical criteria); and the presence of comorbidity, commonly chronic cardiorespiratory disease, diabetes mellitus, neurologic deficit or immunodeficiency as a result of drugs or disease. Geographic variation is also important; for example, tuberculosis is a relatively common cause of CAP in Hong Kong[17] and Q fever in Nova Scotia, Canada and northern Spain. The causative pathogens and their approximate relative frequencies are summarized in Table 34.4 .[18] [19] [20] [21]

In adults, 60–80% of CAP cases are caused by bacteria, 10–20% are 'atypical' and 10–15% are viral. *Streptococcus pneumoniae* is the commonest pathogen and is responsible for 30–50% of cases. In most series, no pathogen is identified in 30% of cases. The majority of these are also thought to be pneumococcal infections in which identification has not been made because of prior antibiotic therapy or lack of sputum for microbiologic investigations during the initial phase of the illness. The frequency of viral and atypical pneumonias may also be underestimated because the acute and convalescent serum samples required for diagnosis are often not obtained. During epidemics, *Mycoplasma pneumoniae* is the next most common pathogen, accounting for up to 23% of cases. Nontypeable (unencapsulated) *H. influenzae* is the next most common pathogen. It may

TABLE 34-4 -- Usual causes of CAP.

USUAL CAUSES OF CAP		
Age group	**Pathogens**	**Percentage of total**

Adults and the elderly	Core pathogens	*Streptococcus pneumoniae*	30–42
		Respiratory viruses	8–13
		Mycoplasma pneumoniae (during epidemics)	5–25
	Additional risk factors present	*Haemophilus influenzae*	5–10
		Staphylococcus aureus	0.8–4.0
		Moraxella catarrhalis	?
		Legionella pneumophila	0.5
		Influenza virus	5
Severe (adults and elderly)	Core pathogens	*S. pneumoniae*	22
		L. pneumophila	5–18
		S. aureus	7–8
	Unusual	*M. pneumoniae*	2
		H. influenzae	3–5
		Influenza A & B	2.3–5.4
		Enteric Gram-negative bacilli	1.6 (UK); 8.6 (Europe)
		Chlamydia psittaci	0.9–2.2
		Klebsiella pneumoniae	
		Varicella-zoster virus	
Children — less than 2 years	Core pathogens	Respiratory syncytial virus	25
		Other viruses	13
		Mixed viral and bacterial	25
		S. pneumoniae	8
		H. influenzae type b	2
Children — 2–15 years	Core pathogens	*S. pneumoniae*	25
		M. pneumoniae	10
		Mixed infections	15
		S. aureus	5
		H. influenzae type b	2
The most common pathogens in each category are shown with estimates of the overall percentage contributions. Core pathogens are those most commonly encountered and may be modified in severe adult CAP and by the presence of risk factors such as pre-existing lung disease and debility (see also Fig. 34.7).			

cause pneumonia in previously healthy individuals but is more common in patients who have pre-existing lung disease. In some series, in which subgroups of patients who have COPD have been studied, *H. influenzae* was more common.[22] *Chlamydia pneumoniae* is usually an uncommon pathogen except during epidemics, which may occur both in the community and in closed settings. It has also been described as a cause of severe pneumonia. Its usual role in pneumonia is less clear because other bacterial pathogens often coexist and patients may recover without antibiotics to which *C. pneumoniae* is sensitive. Laboratory diagnosis is usually in retrospect, employing paired serology.

Influenza virus is the most common cause of viral pneumonia and frequently precedes secondary bacterial pneumonia. Psittacosis, Q fever and *Legionella* spp. each account for 2% of cases. *Legionella* infection is more often found in hospital-based surveys of CAP and exhibits a geographic variation such that studies in Spain have shown up to 14% of cases caused by *Legionella* spp., slightly less than that for the pneumococcus (15%). Community-acquired pneumonia caused by *S. aureus* is uncommon but important because of its severity even in previously healthy individuals. A similar pattern of causative organisms is found in studies from Europe, Australia and New Zealand, but studies in North America more frequently report enteric Gram-negative bacilli (EGNB), aspiration and staphylococcal infections. This may be explained by the higher proportion of debilitated patients and drug and alcohol misusers in the study population. Two large studies from the USA and Canada found the pneumococcus to be a less frequent cause of CAP, but this may reflect different criteria for diagnosis of pneumonia. [23] [24]

In children, the spectrum of pathogens and disease is different (Table 34.4). The major LRTIs are bronchiolitis in infants under 6 months of age, pneumonia in the first 2 years of life and laryngotracheobronchitis in the second and third years of life. Respiratory syncytial virus (RSV) is the main cause of bronchiolitis, although adenovirus, parainfluenza virus type 3, rhinovirus and influenza A have been implicated. There are two important subtypes of RSV, types A and B. Epidemics occur yearly and coincide with the coldest winter month. RSV type A is the most common and tends to be the most severe. Pneumonia in children under the age of 2 years is usually caused by RSV, parainfluenza, influenza or adenovirus but less commonly by enterovirus, rhinovirus, measles virus, varicella-zoster virus and *Coxiella burnetii*. RSV is the most important cause in infants. Neonatal pneumonia usually follows premature rupture of the membranes, allowing the fetus to swallow amniotic fluid contaminated with vaginal organisms. Perinatal pneumonia is usually hospital-acquired secondary to invasive procedures and therefore is usually caused by EGNB and *S. aureus*.

In a study of 121 children hospitalized for LRTI in Helsinki, a cause was found in 69%: bacterial infection in 25%, viral in 25% and multiple organisms in 20%. *Mycoplasma pneumoniae* was more common with increasing age, as were bacterial infections. In Finland, 195 children hospitalized with pneumonia were surveyed over 12 months.[25] A viral infection alone was indicated in 19%, a bacterial infection alone in 15% and a mixed viral and bacterial infection in 16% of patients; 46% of the 69 patients who had viral infection and 52% of the 62 patients who had bacterial infection had a mixed viral and bacterial etiology. Respiratory syncytial virus was identified in 52 patients and *S. pneumoniae* in 21%. The next most common agents were nontypeable *H. influenzae* (9%), adenoviruses (5%) and *Chlamydia* spp. (4%). In Uruguay etiology was determined in 47.7% of 541 cases; 38.6% were viruses and 12.6% bacteria. Viral and mixed etiologies were more frequent in children under 12 months of age. Bacteria predominated in infants aged between 6 and 23 months. Among the viruses, RSV predominated (66%). The bacterial pneumonias accounted for 12.2% of the recognized etiologies. The most important bacterial agents were *S. pneumoniae* (64%) and *H. influenzae* (19%). [26]

In some geographic areas fungi such as *Histoplasma capsulatum* and *Blastomyces dermatidis* may also present as pneumonia (see Chapter 39 and Chapter 238 for further details).

PREVENTION

Methods for the prevention of CAP include vaccination, prophylactic antibiotic therapy, elimination of environmental sources and maintenance of good hygiene. In practice the only vaccines used to prevent CAP are pneumococcal, influenza, measles and *H. influenzae* b vaccines. Measles vaccine helps to prevent pneumonia, which may follow this disease, especially in malnourished children. Similarly, influenza may predispose to secondary bacterial pneumonia in addition to causing primary CAP. Influenza viruses A and B (especially A) regularly alter their surface hemagglutinin and neuraminidase antigens. It is essential, therefore, that influenza vaccine contains the components of the prevalent strains, which are defined each year by the World Health Organization. Influenza vaccine is recommended for all ages and especially the elderly who have the following conditions: chronic respiratory disease, including asthma; chronic heart disease; chronic renal failure; diabetes mellitus and other endocrine disorders; and immunosuppression as a result of disease or treatment. Residents of nursing or residential homes for the elderly or other long-term care institutions should also be vaccinated. Currently, influenza vaccines give 70–80% protection against vaccine strains, although this is less in the elderly.[27] Influenza vaccine is contraindicated in patients who have a history of allergy to hen's eggs.

A 23-valent capsular polysaccharide vaccine provides immunity against the most common serotypes causing pneumococcal pneumonia. It covers nearly 90% of those serotypes that cause severe disease in the UK. Overall efficacy in preventing bacteremic pneumococcal pneumonia is 60–70%, although the vaccine is not effective in

children under 2 years of age or in the immunocompromised. It has been relatively ineffective in patients who have multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma (especially during treatment) and in chronic alcoholism. It does not prevent otitis media in children or reduce the frequency of exacerbations of chronic bronchitis. A large retrospective study of 47 365 adults over 65 years showed a reduction in bacteremic pneumococcal pneumonia (hazard ration 0.56: 95% confidence interval 0.33 to 0.93) with a slightly increased risk of hospitalisation for pneumonia and no effect on outpatient pneumonia.[27A] This confirms efficacy in preventing bacteremia but suggests alternative strategies are required to prevent the more common form of pneumonia. In the UK, immunization is recommended to all those over the age of 2 years who are at special risk of pneumococcal infection or in whom infection might be unusually severe. This includes patients who have homozygous sickle-cell disease, asplenia or severe dysfunction of the spleen, chronic renal disease or nephrotic syndrome, immunodeficiency or immunosuppression as a result of disease or treatment (including HIV disease), chronic heart, lung or liver disease (including cirrhosis) and diabetes mellitus.[27] However, there is little evidence for efficacy in these groups. The vaccine should not be given during an acute infection or during pregnancy, and revaccination is contraindicated within 3 years. In the USA a 7-valent polysaccharide pneumococcal conjugate vaccine has been available since 2000 and it is recommended for use in all children under 2 years of age and for high risk groups aged 2–4 years. Controlled trials have shown this to be highly effective in preventing invasive disease when given as a four dose regimen.[27B] The rate of pneumonia in children under two years of age was 69% lower in 2001 than in 2000 based on a population study of 16 million.[27C] The same study also showed a reduction of 18% in persons aged 65 years or more, suggesting that the vaccine may result in reductions in pneumonia in adults.

Varicella-zoster immunoglobulin therapy is indicated to prevent infections in exposed, susceptible individuals, who may develop severe fulminating pneumonia. Such patients include the immunocompromised, neonates and pregnant women, and those who possess no antibodies to varicella-zoster virus and have had significant exposure to chickenpox or herpes zoster.[27] Oral aciclovir or famciclovir may also be used to prevent varicella-zoster infection in this population.

Prophylactic antibacterial drugs have only limited use in the prevention of CAP. Long-term oral penicillin is recommended in patients under 16 years of age at particular risk of pneumococcal infection, for example after splenectomy.

Prevention may also require identification and elimination of an environmental source of infection. If two or more cases of *Legionella* pneumonia, psittacosis or Q fever occur, a search for a potential reservoir of infection should be made. The relevant public health officials should be informed. For legionellosis, contaminated water systems should be cleaned and sterilized by hyperchlorination and heat. *Legionella* colonization can be prevented by the use of correctly designed water systems, including sealed tanks, and regular heat or hyperchlorination treatment.

CLINICAL FEATURES

General symptoms and signs

Symptoms may be constitutional and non-specific, such as malaise, anorexia, headache, myalgia, arthralgia, sweating and rigors. Specific

symptoms to pneumonia or a specific pathogen also occur. In viral and *Mycoplasma* infections, constitutional symptoms may be preceded by upper respiratory tract symptoms of sore throat, sneezing, nasal discharge and blockage. High pyrexia and rigors are common in the young who have pneumococcal and *Legionella* infections. In contrast, the elderly or seriously ill may have minimal or no fever. Cough is the most common respiratory symptom and is present in over 80% of cases, followed by dyspnea in 60–70%, pleural pain in 60%, new sputum production in over 50% and hemoptysis in 15%.[11] [28] Sputum is initially mucoid or absent, particularly with atypical and *Legionella* infections. Purulent sputum develops later, although in patients who have pre-existing chronic bronchitis or bronchiectasis it is present at the outset.

Nonrespiratory symptoms may be more prominent and mask the diagnosis. Marked confusion may occur with any pneumonia, especially in the elderly, who may have no physical signs other than an elevated respiratory rate and localizing chest signs. Confusion is also more common in *Legionella* and psittacosis pneumonias. A prominent headache in association with confusion or impaired consciousness raises the possibility of *Legionella* infection or co-existent meningitis, especially in pneumococcal pneumonia. Lower lobe pneumonia may present with abdominal pain mimicking acute abdominal or urinary tract pathology.

The duration of history is variable. Usually, symptoms have been present for several days before hospital admission; however, it is

TABLE 34-5 -- Pathogens and their usual pattern of clinical presentation.

PATHOGENS AND THEIR USUAL PATTERN OF CLINICAL PRESENTATION	
Clinical presentation	**Organism**
Abrupt onset of severe pneumonia, with fever, rigors and signs of lobar consolidation	*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Legionella* spp., *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Yersinia pestis* (plague), *Burkholderia pseudomallei* (rare)
Indolent or low-grade infection associated with pre-existing lung disease	*H. influenzae, S. pneumoniae, Moraxella catarrhalis, Chlamydia pneumoniae, Pasteurella multocida, Pseudomonas aeruginosa, S. aureus*
Prodromal flu-like or predominantly upper respiratory tract symptoms, followed by pneumonia	*Mycoplasma* spp., *Chlamydia* spp., *Legionella* spp., *Coxiella burnetii*, respiratory viruses
Extrapulmonary manifestations	*Mycoplasma* spp. (variety see Table 34.7), *Coxiella* spp. (variety see Table 34.7), Herpesviruses (skin and mucocutaneous), *Y. pestis* (lymphadenopathy), leptospirosis (renal and hepatic), *Legionella* spp.
Tuberculosis-like illness	*K. pneumoniae* (chronic form), *B. pseudomallei* (melioidosis), *Mycobacteria* spp., e.g. *kansasii*, *Cryptococcus neoformans*
Locally invasive disease with abscess and sinus formation	*Actinomycetes* spp., *Nocardia* (immunocompromised host), *Pseudomonas mallei* (glanders), *Eikenella corrodens*
Diarrhea	*Legionella* spp., *S. pneumoniae*, *C. burnetii*, tularemia (*Francisella tularensis*)
Organisms that may cause pneumonia as a rare complication of main infection	*Salmonella typhi or paratyphi* (enteric fever), *Brucella abortus* (brucellosis), *Bacillus anthracis* (anthrax), *Listeria monocytogenes*
Although it is usually not possible to say which pathogen is responsible for CAP on clinical criteria alone, it is helpful to have some understanding of the usual pattern of illness for common pathogens.	

important to realize that patients who have pneumococcal or staphylococcal infection may become critically ill within hours, whereas in *Mycoplasma* infection symptoms can be present for 2–3 weeks.

Classic signs of lobar consolidation are infrequent and it is more common to hear localized crackles. If pneumonia is complicated by parapneumonic pleural effusion or empyema, lower zone dullness to percussion and reduced breath sounds may be present. Abdominal

TABLE 34-6 -- Classic features of more common pathogens and clinical pointers to diagnosis.

CLASSIC FEATURES OF MORE COMMON PATHOGENS AND CLINICAL POINTERS TO DIAGNOSIS	
Organism	**Classic features**
Streptococcus pneumoniae	Abrupt onset over hours with rigors, fever, malaise, tachycardia and tachypnea. Dry cough initially, then productive of 'rusty' sputum. Less acute presentation (possibly modified by antibiotics). Worsening of cardiac or respiratory failure, confusion or general physical deterioration in elderly or debilitated. Herpes labialis.
Staphylococcus aureus	Rapid onset of fever, confusion and respiratory distress, often after influenza. May be secondary to infected intravenous cannulae or right-sided endocarditis. Multiple pulmonary abscesses, empyema. Look for a potential source.
Haemophilus influenzae	Two forms: typable strains cause acute severe infection with lobar consolidation in children; vaccination has dramatically reduced this. Nontypeable strains cause patchy bronchopneumonia with persistent purulent sputum and malaise. Subacute bronchopneumonia in patients with pre-existing lung disease.

Legionella spp.	Two forms: Pontiac fever — acute self-limiting flu-like illness. Pneumonia — abrupt onset with high fever, rigors, malaise and myalgia. Slight cough, in half, severe headache, confusion and delirium, diarrhea and abdominal pain. Neurologic (cerebellar) signs. Renal failure. Elevated creatine kinase.
Klebsiella spp.	Acute severe pneumonia with thick, tenacious bloodstained sputum, commonly affects the right upper lobe. Dense consolidation on chest radiograph with 'bulging fissures'.
Mycoplasma spp.	Upper respiratory tract symptoms such as sore throat and coryza followed after 4–7 days by fever, dry cough and dyspnea. In some cases erythema nodosum, erythema multiforme, Stevens-Johnson syndrome, myringitis, splenomegaly, generalized lymphadenopathy and salivary gland enlargement.
Chlamydia spp.	Psittacosis — mild flu-like illness to fulminating pneumonias with multiple organ failure. Macular rash (Horden's spots) rarely in psittacosis. Diarrhea occurs in 25%. *Chlamydia pneumoniae* commonly causes sore throat, prominent cough, prolonged bronchitis in young adults and mild pneumonia.
Coxiella spp.	Q fever manifests with a flu-like illness and pneumonia of varying severity. Multisystem involvement — arthritis, thromboplebitis, arteritis, pericarditis, myocarditis and chronic endocarditis, particularly of the aortic valve.
Influenza	Flu symptoms — fever, rigors, myalgias may be followed by severe pneumonia or by secondary bacterial pneumonia. Epidemics (occasionally pandemics) in winter months.
Viruses*	Coryzal infection, cough and rarely pneumonia.
Herpesvirus	Both varicella-zoster and herpes simplex may cause severe pneumonia, which follows 2–3 days after clinical evidence of infection (cutaneous lesions). Rash of chickenpox in varicella. Cold sore or primary oral lesions in herpes simplex.
Although classic clinical features are described in most texts, in practice there may be considerable overlap.	

* Parainfluenza, RSV, adenovirus, coronavirus, Coxsackie virus

tenderness is not unusual, especially in lower lobe pneumonia or if there is associated hepatitis. There are no specific symptoms and signs that permit a confident clinical diagnosis of a particular etiology, although some pathogens are more often associated with a particular pattern.[29] Commonly recognized patterns of pneumonic illness are described in Table 34.5 and the pathogens that may be responsible are listed. Occasionally, a specific clinical feature or characteristic pattern may suggest a specific pathogen. For example, herpes labialis is more common in pneumococcal pneumonia, and erythema nodosum and erythema multiforme in *Mycoplasma* infection. The rash of chickenpox is always present with varicella pneumonia. *Mycoplasma* pneumonia and Q fever have a variety of extrapulmonary

TABLE 34-7 -- History and examination findings that suggest a specific diagnosis.

HISTORY AND EXAMINATION FINDINGS THAT SUGGEST A SPECIFIC DIAGNOSIS	
History	**Pathogen**
Alcoholism	*Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, anaerobes
Chronic obstructive pulmonary disease	*S. pneumoniae, Haemophilus influenzae Moraxella catarrhalis*
Animal exposure	
Birds	*Chlamydia psittaci* (psittacosis)
Rats, squirrels, rabbits	*Yersinia pestis* (plague), tularemia
Rats, mice	*Leptospira* spp.
Horses	*Burkholderia mallei* (glanders)
Cats	*Coxiella burnetii* (Q fever), *Pasteurella multocida*
Cattle, sheep, goats	*C. burnetii*
Corticosteroid therapy	*S. aureus*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, *Legionella* spp.
AIDS/immunocompromised/iv drug abuse	*Mycobacterium* spp., *P. carinii*, fungal diseases, cytomegalovirus
Travel	
Thailand, South East Asia	*Burkholderia pseudomallei* (melioidosis)
Asia, Africa, Central and, South America	*Paragonimus westermani* (paragonimiasis)
Exposure to contaminated water systems	*Legionella pneumophila*
Examination	**Pathogen**
Skin	
Erythema multiforme	*Mycoplasma pneumoniae*
Maculopapular rash	Measles
Erythema nodosum	*Chlamydia pneumoniae*
Erythema gangrenosum	*M. tuberculosis, Pseudomonas aeruginosa, Serratia marcescens*
Mouth	
Dental caries	Anaerobic pneumonia
Herpes simplex	*S. pneumoniae*
Ears	
Bullous myringitis	*M. pneumoniae*
Neurology	
Poor gag reflex; altered conscious level; recent seizure	Aspiration
Cerebellar ataxia	*M. pneumoniae, L. pneumophila*
Encephalitis	*M. pneumoniae, C. burnetii*
Specific clinical features may occasionally point to a specific pathogen and may influence management.	

manifestations that, if present, may point to the diagnosis. Classic and some specific features of the more common pathogens are shown in Table 34.6 . Table 34.7 gives a more comprehensive list of clues in the history and examination that may suggest a specific pathogen.

Differential diagnosis of pneumonia

Pneumonia is most commonly confused with pulmonary infarction and pulmonary edema, and indeed may co-exist with either of these conditions. Careful attention to the exact sequence of the events in the history, for example establishing whether there was a prodrome of symptoms suggesting upper respiratory infection, may help point to pneumonia. Even with expert interpretation of examination findings and basic investigations the diagnosis may still be obscure and further investigations may be indicated. In practice, treatment is often given for more than one condition while further investigations are awaited. Pulmonary embolism may need to be excluded by ventilation perfusion scanning or pulmonary angiography. Less common differential diagnosis includes primary or metastatic lung cancer, pulmonary eosinophilia

and acute allergic or cryptogenic alveolitis. Hepatic or subphrenic abscess, pancreatitis or perforated peptic ulcer may mimic basal pneumonia.

DIAGNOSIS

The purpose of investigations in CAP are:

- to confirm the diagnosis of pneumonia;
- to determine etiology;
- to assess severity;
- to determine the impact of the illness on any underlying conditions, such as COPD or cardiac failure; and
- to provide data for epidemiologic purposes.

They can be broadly divided into general investigations, which help with the first three, and those that are mainly aimed at determining etiology.

Chest radiography, biochemistry and hematology

In relatively well patients who have suspected mild pneumonia seen in the community, investigations, including chest radiography, are not required but hypoxia should be excluded by means of pulse oximetry.

A chest radiograph is required for diagnosis of CAP and usually shows lobar or segmental opacification in bacterial pneumonias and in the majority of atypical infections. Less commonly, patchy peribronchial shadowing or more diffuse nodular or ground-glass opacification is seen, particularly in viral and atypical infections. The lower lobes are most commonly affected in all types of pneumonia, and small pleural effusions can be detected in about one-quarter of cases, especially if a lateral decubitus film is taken. Multilobar pneumonia is a feature of severe disease, and spread to other lobes despite appropriate antibiotics is seen in *Legionella* and *M. pneumoniae* infections. Hilar lymphadenopathy is unusual except in *Mycoplasma* pneumonia, particularly in children. Cavitation is uncommon but is a classic feature of *Staphylococcus aureus* and *Streptococcus pneumoniae* serotype 3 infections.

Clearance of radiographic changes usually occurs after about 8 weeks but varies from 2 weeks to many months depending on the age of the patient, the severity of pneumonia, the presence of preexisting lung disease and the pathogen. The rates of resolution of radiographic pulmonary shadows in different pneumonias are shown in Figure 34.3 . [30] Bacteremic pneumococcal pneumonia and *Legionella* infections are particularly slow to clear. After resolution of the illness it is important to arrange a further chest radiograph, as unsuspected malignant disease is surprisingly common. One study revealed the presence of unsuspected malignant disease in 7% of

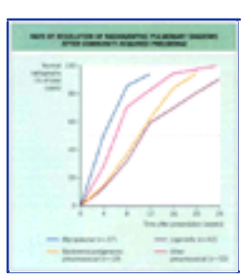

Figure 34-3 Rate of resolution of radiographic pulmonary shadows after CAP. Pneumonia can take 4–8 weeks to clear radiographically even in less severe cases such as *Mycoplasma* pneumonia. More severe pneumonias such as *Legionella* and bacteremic pneumococcal pneumonia take longer to resolve, sometimes up to 6 months. *Data from Macfarlane et al.*[30]

pneumonia cases (17% if limited to smokers) in patients over the age of 60 years. [3]

A full blood count, renal, electrolyte and liver biochemical profiles, a blood glucose and an erythrocyte sedimentation rate or C-reactive protein are useful general investigations. In most cases of pneumonia caused by *S. pneumoniae* or *H. influenzae*, the white blood cell count is above 15×10^9 /l; it is usually marginally raised in *Legionella* infections ($11–15 \times 10^9$ /l). Mildly raised hepatic enzymes and bilirubin are not uncommon in bacterial pneumonias, particularly if there is bacteremia. Raised blood urea, hyponatremia, hypokalemia, hypoalbuminemia, hyperglycemia, proteinuria and hematuria can be present in all severe pneumonias but none indicates etiology. Hyponatremia is seen in 50% of patients who have *Legionella* pneumonia.

Microbiologic investigations

For identification of the pathogen, sputum and pleural fluid (if an effusion is present) should be Gram stained and cultured. Sputum Gram staining can provide a specific and rapid diagnosis in about one-fifth of cases of pneumococcal and staphylococcal pneumonia, when large numbers of a predominant organism are usually seen. Sputum culture is less specific because it is often contaminated with upper respiratory tract organisms, and it is not produced in one-quarter to one-third of patients in the early stages of pneumonia. In addition, even a single dose of antibiotic can prevent a positive culture of *S. pneumoniae* and *H. influenzae*, and this occurs in over one-half of patients admitted to hospital in the UK. Even in the presence of untreated bacteremic pneumococcal pneumonia, sputum culture is negative in over one-half of patients. Blood cultures are positive in less than one-quarter of patients who have bacterial pneumonia who present to hospital, but when they are positive they are usually specific.

TABLE 34-8 -- Investigations aimed at determining etiology of community-acquired pneumonia.

INVESTIGATIONS AIMED AT DETERMINING ETIOLOGY OF CAP		
Investigation		**Comments**
General microbiology	Sputum Gram stain	Rapid and widely available
		Low sensitivity (10%)
		High specificity if positive (70–80%)
	Sputum culture	Prior antibiotics and contamination with oropharyngeal bacteria is a problem
	Blood culture	Positive in 20–25% of bacterial pneumonias and relates to etiology and prognosis
	Pleural fluid stain and culture	Essential to exclude empyema
	Nasopharygeal swabs	Useful in children, used to diagnose viral infections
Serologic tests	Acute and convalescent sera	Retrospective diagnosis of viral, atypical and *Legionella* infections
	Cold agglutinins	Positive in about 50% of cases of *Mycoplasma* infection
	Antigen detection	Pneumococcal-sputum positive in 80%, urine in 36–45%, serum in 9–23% of *Legionella* spp., encapsulated forms of *Haemophilus influenzae, Mycoplasma* spp., *Chlamydia* spp. and specifically *Chlamydia pneumoniae* and *Legionella pneumophila* serogroup 1
Invasive tests	Transtracheal aspiration, bronchoalveolar lavage, protected specimen bronchial brush (PSB), percutaneous needle biopsy, lung biopsy	Consider in patients who are immunocompromised, who fail to respond. Bronchoalveolar lavage and PSB are most often used
Immediate diagnosis is only possible in about 15% of cases of CAP. Even in research studies in which extensive investigations have been performed, no causative organism is identified in 30% of cases.		

Antigen detection, serology and other investigations

Antigen detection is useful for diagnosis of CAP caused by *S. pneumoniae*, encapsulated forms of *H. influenzae*, *Mycoplasma* and *Chlamydia* spp. (including specific identification of *C. pneumoniae*), and *Legionella pneumophila* serogroup 1. Detection of pneumococcal polysaccharide capsular antigen is particularly helpful as it may be detected in 80% of sputum samples, 36–45% of urine and 9–23% of serum. [31] [32] *Mycoplasma* antigen detection is a new test that improves the rate of diagnosis; *Legionella* antigen is present in urine in the first week of the illness before serum antibodies are detectable and is increasingly being found useful in early diagnosis. The most useful diagnostic tests are shown in Table 34.8 . Antigen detection adds to the cost of managing pneumonia and for the majority of patients does not influence outcome. It is therefore appropriate to reserve these tests for the more severely ill patient.

Serologic methods are used to diagnose viral, atypical and *Legionella* infections. A fourfold or greater increase in specific antibody titer in blood taken early in the course of the illness and after 10–14 days is evidence of recent infection, and high unchanging titers are suggestive. The main limitation of serology is that the diagnosis is obtained too late to influence initial management. This forces the adoption of an empiric approach to the choice of antibiotic. Specific

IgM antibody detection can be used to obtain a more rapid diagnosis in *Mycoplasma* and *Legionella* infections, although their sensitivity and specificity varies. Direct fluorescent antibody tests are available for staining respiratory secretions to detect *Legionella* spp. and some viruses, although these are not universally available. Cold agglutinins are present in about 50% of *Mycoplasma* infections but are a non-specific finding.

Viral isolation, culture and identification is a lengthy process and is of little practical value in most cases. However, in difficult cases when no diagnosis has been reached and there is no resolution it may provide a diagnosis, particularly in the immunocompromised host. Amplification of DNA from a variety of pathogens using the polymerase chain reaction has been attempted but these techniques remain under investigation.

In patients in whom the diagnosis is obscure, who fail to respond to empiric treatment or who are particularly ill or immunocompromised, invasive techniques may be indicated to sample lower respiratory secretions or lung tissue. These include fiberoptic bronchoscopy with lavage, quantitative culture and protected bronchial brush (see Invasive techniques, Chapter 35), percutaneous needle aspiration and lung biopsy (transbronchial or open). Whenever invasive techniques are used and when an unusual diagnostic test is indicated, it is most important for clinicians and microbiologists to liaise. This allows a decision about the most appropriate tests and the correct samples and ensures the laboratory is ready to process the samples.

MANAGEMENT

The management of CAP depends on the severity of the illness, the presence of underlying disease and the age of the patient. At best, immediate diagnostic tests may identify the pathogen in fewer than 30% of cases and therefore an empiric antibiotic regimen is necessary. The

TABLE 34-9 -- Features distinguishing severe pneumonia.

FEATURES DISTINGUISHING SEVERE PNEUMONIA		
Features associated with poor outcome	**Factors associated with increased mortality (from a large meta-analysis[11])**	**OR/CI (95%)**
Clinical	**Clinical**	
Age >60 years	Male sex	1.3 (1.2–1.4)
Pre-existing medical illness	Hypothermia	5.0 (2.4–10.4)
Confusion	Systolic hypotension	4.8 (2.8–8.3)
New atrial fibrillation	Tachypnea	2.9 (1.7–4.9)
Respiratory rate >30/min*	Diabetes mellitus	1.3 (1.1–1.5)
Diastolic blood pressure =60mmHg*	Neoplastic disease	2.8 (2.4–3.1)
Cyanosis	Neurologic disease	4.6 (2.3–8.9)
Investigations	**Investigations**	
White blood cells <4 × 10^9 /l or >30 × 10^9 /l	Bacteremia	2.8 (2.3–3.6)
Blood urea >7mmol/l*	Leukopenia	2.5 (1.6–3.7)
Hypoxia (Pao_2 < 8kPa)	Multilobar radiographic shadows	3.1 (1.9–5.1)
Multilobar or spreading radiographic shadows		
Severity of pneumonia has been determined by identifying those factors that are associated with increased risk of death. Some (*) are consistently identified in different studies and are used as a basis for guiding management.		

* Two or more of these three features increase risk of death by nine- to 21-fold.[29]

spectrum of organisms in the elderly and younger adult is similar, but it differs in children. In severe pneumonia, *Legionella* infection is more frequently found and hence different empiric antibiotics are required. Similarly, *H. influenzae* is more often found in patients who have pre-existing lung disease.

Assessment of severity

Studies of CAP have identified several prognostic factors on the basis of clinical and investigative features (Table 34.9).[29] [33] The American Thoracic Society guidelines[12] classify severe pneumonia on the basis of two or more minor criteria:

- low blood pressure (systolic =90mmHg, diastolic =60mmHg);
- severe respiratory failure (Pa_{O_2}/Fi_{O_2} ratio below 250; and
- bilateral or multilobe radiographic shadowing.

The British Thoracic Society Guidelines[34] suggest the CURB score for severity assessment: one point for each of confusion (new mental confusion with an Abbreviated Mental Test score of <9), urea (>7mmol/l), respiratory rate (=30 breaths per min) and blood pressure (systolic <90mmHg and/or diastolic =60mmHg).

Figure 34-4 Algorithm for the management of CAP. *Adapted from the Nottingham City Hospital CAP Guidelines, 1996.*

A modification of this score (CURB 65) has been shown by an international validation study to provide a useful way to stratify patients into different management

groups.[34A]

General management

All patients who have CAP, whether managed at home or in hospital, should rest in bed, have adequate oral fluids and should not smoke. Simple analgesics or nonsteroidal anti-inflammatory drugs can be given for pleural pain and fever. Chest physiotherapy is only useful if patients have a pre-existing lung disease, such as chronic bronchitis or bronchiectasis, that may lead to sputum retention. Patients who have moderate or severe CAP should be admitted to hospital and receive oxygen supplementation by face mask. Patients who have severe pneumonia should be monitored in a high-dependency unit and if necessary transferred to intensive care. The danger period is in the first 3–4 days after admission. In severe CAP the onset of progressive cardiorespiratory failure can be very rapid and assisted ventilation with inotropic support can preserve life while a response to antibiotics is awaited. Generally, previously fit adults will require ventilation if they are unable to maintain a Pao_2 of 8kPa with maximum supplemental oxygen. Assisted ventilation may be required for many days or weeks before successful weaning. In one study of severe CAP some patients weaned after 4 weeks of ventilation have survived.[35] Early attention to nutritional requirements is important in severe pneumonia. An algorithm for the management of CAP in adults is shown in Figure 34.4 .

TABLE 34-10 -- Empiric antibiotic therapy for CAP.

EMPIRIC ANTIBIOTIC THERAPY FOR CAP						
Choice	**Guidelines**	**Outpatients**		**Hospitalised**		
				Non-intensive care		**Intensive care**
		No modifying factors	**Modifying factors**	**No modifying factors**	**Modifying factors**	
First Choice	American	AZ or CLR	ß-lactam (CPD, CFX, high-dose AMX) + macrolide or DOX	iv AZ alone or (if allergic) DOX + ß-lactam	iv ß-lactam (CAX, CRX, AMP/sulbactam) + macrolide or DOX	iv ß-lactam (CAX, CRX) + either iv macrolide (AZ) or iv fluoroquinolone
	Canadian	Macrolide	Macrolide (AZ or CLR) or APF	APF	APF	APF + CAX, CRX or ß-lactam/ß-lactamase
	British	AMX	AMX	AMX (oral) or AMP (iv) or BPN iv + E or CLR	AMX (oral) or AMP (iv) or BPN iv + E or CLR	CAC or CFX or CAX or CRX + E or CLR ± rifampicin
	French	AMX	CAC			Macrolide + 3rd generation cephalosporin
	Spanish	PEN/AMP	CAC			Macrolide + 3rd generation cephalosporin
Second Choice	American	DOX	APF	PAF	APF (iv)	
	Canadian	DOX	CAC + macrolide or 2nd generation cephalosporin	2nd , 3rd or 4th generation cephalosporin + macrolide		iv macrolide + CAX, CRX or ß-lactam/ß-lactamase
	British	E or CLR	E or CLR	LEV (or any APF when more available)	LEV (or any APF when more available)	APF + BPN
	French	Macrolide	CAC			Macrolide + CAC
	Spanish	Macrolide	CAC			Quinolone + 3rd generation cephalosporin
AMP, ampicillin; AMX, amoxicillin; APF, antipneumococcal fluoroquinolone; AZ, azithromycin; BPN, benzyl penicillin; CAC, amoxicillin/clavulanate (co-amoxiclav); CAX, cefotaxime; CFX, cefuroxime; CLR, clarithromycin; CPD, cefpodoxime; CRX, ceftriaxone; DOX, doxycycline; E, erythromycin; LEV, levofloxacin; PEN, penicillin.						
A summary of recommendations from five countries is shown. Not all countries give specific recommendations for all categories of pneumonia						

Antibiotic therapy

Appropriate empiric antibiotic therapy choices are based on guidelines from five different countries (Table 34.10).[12] [34] [36] [37] The choices vary according to severity of illness, history of pre-existing chest disease and presence of penicillin hypersensitivity. In practice, most patients who have mild or moderate pneumonia can be managed with oral antibiotics, although there are large differences between oral and intravenous blood and tissue concentrations for some agents. Penicillins and quinolones are well absorbed and generally have an oral bioavailability of over 60% of serum. The newer third-generation cephalosporins have approximately 30% oral bioavailability, which may fall if the patient is not eating. Newer macrolides also have approximately 30% oral bioavailability but the most commonly used, erythromycin, has only 5–11%, depending on the preparation; thus, a 500mg dose will at best be equivalent to only 50mg given intravenously.

Antibiotics vary in their gastrointestinal side effects. The penicillins, quinolones and tetracyclines are relatively well tolerated, cephalosporins (especially oral third-generation) and new macrolides less so, and erythromycin causes enough side effects to warrant discontinuation of treatment in 10–30% of patients (depending on the preparation).[38] [39] Recently, newer fluoroquinolones with improved antipneumococcal activity have been included in guidelines and are being used widely. If they are used as first-line therapy, benzyl penicillin is added in severe infections. This has in part been prompted by the rising incidence of *Clostridium difficile* infection resulting in colitis, thought to be a consequence of use of broad-spectrum cephalosporins.

Adult nonsevere community-acquired pneumonia

In this category, *S. pneumoniae* is the most common organism, although other organisms such as *M. pneumoniae*, *C. pneumoniae* and *H. influenzae* may be a reason for a poor response. The latter is more likely if there is pre-existing lung disease. An aminopenicillin such as oral amoxicillin or intravenous ampicillin is an appropriate choice. In patients allergic to penicillin, a macrolide such as erythromycin can be substituted. If there is no improvement after 48 hours, the diagnosis should be reviewed and therapy adjusted if the pathogen has been identified. If pre-existing chest disease is present amoxicillin-clavulanate, a quinolone (levofloxacin or moxifloxacin) or a parenteral cephalosporin such as cefuroxime can be substituted to cover ß-lactamase-producing *H. influenzae* or *M. catarrhalis*. If there is no underlying pulmonary disease then a macrolide may be substituted to cover *M. pneumoniae* infections. Erythromycin is a less satisfactory choice for elderly patients because of the infrequency of atypical pathogens in this group and its poor activity against *H. influenzae*. Some recently updated guidelines specifically recommend newer macrolides and fluoroquinolones with antipneumococcal activity.[12] [36]

Adult severe community-acquired pneumonia

In severe infection, *Legionella* spp. becomes more important and therefore a macrolide and a ß-lactamase-stable ß-lactam (such as cefuroxime or amoxicillin-clavulanate) is recommended initially. This will cover all likely pathogens, including the commoner (pneumococcal, staphylococcal and *Legionella*) infections. A fluoroquinolone with antipneumococcal activity can be substituted for the macrolide. It is important to start antibiotic therapy as soon as possible and to use the intravenous route, although once there is clear clinical improvement this can be changed to the oral route. If there is no response to treatment, specialist advice should be sought. The diagnosis should be reviewed with all available clinical and laboratory data and consideration given to invasive diagnostic techniques. If *Legionella* infection is thought to be a possible cause, rifampin (rifampicin) or ciprofloxacin may be added to the macrolide. Some studies have suggested that clarithromycin may be more effective than erythromycin. Consideration should also be given to the possibility of secondary hospital-acquired pneumonia and appropriate antibiotics administered.

The duration of antibiotic therapy is debatable and is best dictated by clinical improvement associated with some knowledge of the half-life of the drug in plasma and tissues. In uncomplicated mild or moderate pneumonia a 5- to 7-day course is usually adequate. Patients started on intravenous therapy may be transferred to oral

therapy 24 hours after there has been a clear improvement with resolution of fever, and they should receive antibiotics for 5–10 days thereafter. Prolonged therapy may be required if lung cavitation occurs.

COMPLICATIONS

Most patients recover from CAP without complications. In about one-quarter of patients a small proteinaceous sympathetic effusion develops and resolves spontaneously. Such effusions may cause persistent fever after resolution of pneumonia even if all organisms have been eliminated. They should always be sampled to exclude empyema, which, if present, will require drainage (Fig. 34.5). Pulmonary abscess and pneumothorax are important complications that are discussed in Chapter 36 .

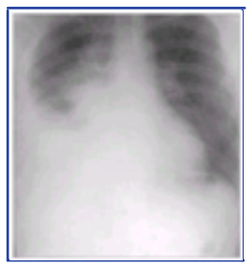

Figure 34-5 Chest radiograph showing a right sided lobar pneumonia with right empyema in a 25-year-old man who was training for the British rowing team. Prompt treatment with intercostal drainage ensured that he recovered without loss of lung function.

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Chapter 35 - Hospital-acquired Pneumonia

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EPIDEMIOLOGY

Definition

Hospital-acquired pneumonia (HAP) is traditionally defined as pneumonia that occurs more than 48 hours after hospital admission, excluding any infection incubating at the time of admission.[1] It has been suggested that this definition is no longer adequate because cases can occur within 48 hours of hospitalization, particularly as a consequence of emergency intubation, or cardiopulmonary resuscitation.[2] Ventilator-associated pneumonia (VAP) can be regarded as a particular subgroup of HAP for which the incidence, etiology, investigation and outcome are somewhat different. It should be remembered that patients recently discharged from hospital who develop pneumonia may have an illness with features more in keeping with hospital-acquired rather than community-acquired infection.

Incidence and size of the problem

Pneumonia is the second most common infection acquired in hospital after urinary tract infection and is associated with the highest mortality rate. Hospital-acquired pneumonia is estimated to occur in 300,000 hospitalized patients each year in the USA. It adds 5–9 days to the hospital stay of survivors[3] [4] and billions of dollars to health care costs. In the UK, around 9% of patients have a hospital-acquired infection at any one time, increasing health costs by £1 billion a year.[5] The incidence varies with type of hospital and ward and age of the patient. The incidence is lowest in district hospitals and on general medical and pediatric wards, and higher in teaching hospitals (presumably because of the increased complexity of medical cases)[3] [4] and in patients over 65 years of age. The highest incidence is found in the intensive care unit (ICU). The attack rate of pneumonia for patients receiving assisted ventilation rises progressively with duration of stay in the ICU;[6] [7] prevalences of up to 50% have been reported after 7 days of mechanical ventilation.[7] [8]

Mortality rate

Reported crude mortality rates for HAP range up to 70%.[9] [10] This is misleading because pneumonia may not be the true cause of death in patients who have multiple pathology. Pneumonia has been estimated to be the 'attributable cause' of death in one-third to one-half of patients who develop HAP, particularly in ventilated patients.[1] [3] [8]

PATHOGENESIS AND ETIOLOGIC AGENTS

Pathogenesis

Factors associated with the pathogenesis of HAP are summarized in Figure 35.1 .[11] Hospital-acquired pneumonia is usually caused by the aspiration or translocation of bacteria that colonize the upper respiratory tract, into the lungs. Aspiration of upper respiratory tract secretions is usually the result of impaired mechanical host defense; subsequent colonization of the lower airway is facilitated by debility, defective host defense and changes in bacterial mucosal adherence factors. Direct translocation of upper respiratory bacteria, and bacteria carried by health care workers into the lower respiratory tract by the act of intubation is also a risk factor, especially during emergency intubation. Less commonly, other mechanisms may be involved, including hematogenous spread of infection to the lungs from a distant focus and inhalation of pathogens aerosolized either from contaminated respiratory equipment (e.g. ventilator or nebulizer equipment) or from the hospital environment (e.g. showers and water systems colonized with *Legionella* bacteria).

Microaspiration of pharyngeal secretions is usually clinically silent and occurs even in healthy subjects during sleep, but becomes very frequent in patients who have reduced consciousness.[3] Aspiration is almost inevitable in intubated patients because the normal laryngeal barrier between the oropharynx and the lower respiratory tract is compromised. Secretions pool above the cuff of the endotracheal tube from where they leak into the lower airway. In the healthy individual, aspirated secretions can be dealt with effectively by lung defenses, including mucociliary clearance and alveolar macrophages. When host defenses are impaired, bacteria are able to proliferate and cause pneumonia.

The oropharynx of debilitated patients becomes colonized rapidly by enteric Gram-negative bacteria (EGNB). These bacteria are not normally present in the upper respiratory tract and the frequency of colonization increases with increasing severity of the underlying illness, use of antibiotics and duration of hospital stay. Up to three-quarters of critically ill patients become colonized within a few days of hospital admission.[3] [4] Oropharyngeal colonization is often a harbinger of subsequent pneumonia.[12] [13] The temporal sequence of bacterial colonization in patients receiving mechanical ventilation is reported as oropharynx (36 hours), stomach (36–60 hours), lower respiratory tract (60–84 hours) and interior of the endotracheal tube (60–96 hours).[14]

Colonization of the lower respiratory tract is facilitated by changes in respiratory epithelial cells that favor bacterial adherence. Alteration of cell surface carbohydrates, loss of surface fibronectin and alteration of epithelial cell bacterial receptors all contribute to enhanced colonization by pathogenic bacteria. [3] [12] [13]

Causative organisms

The pathogens associated with HAP have been studied extensively. However, variations in the patient populations studied, the methods used to obtain and analyze specimens and the definitions used for HAP have, until recently, made it difficult to obtain a sensible overview of the problem. Fortunately, guidelines from the American Thoracic Society (ATS),[1] the Canadian Consensus Conference[15] and other countries, [16] and information from other reviews[2] [10] [17] are now in existence; these propose a classification of the pathogens causing HAP that is both sensible and practical. The approach relies on assessing three factors, including disease severity, the presence of comorbid disease and other risk factors for specific pathogens, and the time of onset of the pneumonia, in considering likely pathogens and therefore guiding initial antibiotic selection.

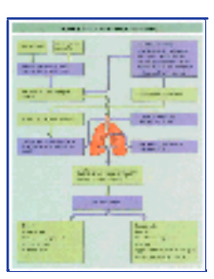

Figure 35-1 Factors involved in the pathogenesis of hospital-acquired pneumonia. *Reproduced from Macfarlane JT. Pneumonia. Medicine International 1986; 3 by kind permission of the Medicine Publishing Company.*

The Canadian Consensus document was the first to bring some order into the management of HAP, by accepting the problems inherent in the diagnosis of HAP and the difficulties in identifying an etiology. The committee argued that any initial therapy would be empiric and would need to take account of factors that would most likely

relate to outcome, specifically risk factors for specific pathogens and also severity of infection. In the ATS guidelines, the time of onset of pneumonia was introduced as the third variable.[16]

The spectrum of potential pathogens associated with HAP differs from that of community-acquired pneumonia. The bacterial pathogens most frequently associated with HAP are EGNB and *Staphylococcus aureus*. Mixed infections are not unusual, particularly in VAP. The role of viruses has not been widely studied, but is likely to be important, especially at times of community outbreaks of viral infection, when staff and visitors may transmit viral infection to hospitalized patients.

The potential pathogens can be categorized into three groups depending on the severity of the pneumonia, the presence of additional risk factors (e.g. comorbidity, previous antibiotic therapy and aspiration), and the duration of hospitalization. A group of 'core' pathogens must be considered in all cases of HAP. These include *Haemophilus influenzae* and Gram-positive organisms such as *Streptococcus pneumoniae* and methicillin-sensitive *S. aureus* and 'sensitive' EGNB (e.g. *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and *Serratia marcescens*). Other pathogens, in addition to the 'core' pathogens, must be considered in certain circumstances. These include resistant Gram-negative organisms such as *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella pneumoniae* and *Acinetobacter* spp., and methicillin-resistant *S. aureus* (MRSA) in cases of 'late-onset' severe pneumonia. Some pathogens will be more likely in the presence of additional risk factors such as aspiration (anaerobic organisms) or known exposure to a hospital source of *Legionella* infection (*Legionella* spp.).

For all grades of severity of HAP the duration of hospital stay before the development of infection has the same broad influence. If HAP occurs within 5 days of hospitalization, normal community respiratory commensals including *S. pneumoniae*, *H. influenzae* and *S. aureus* are the most frequently isolated pathogens. [1] [18] [19] Enteric Gram-negative bacteria become more common with increasing duration of hospital stay, presumably because the 'community'

Figure 35-2 Algorithm for classifying patients who have hospital-acquired pneumonia to provide a basis for empiric antibiotic management. *Adapted from Figure 1 in the American Thoracic Society consensus statement.*[1]

pathogens initially colonizing the oropharynx are replaced by 'hospital' pathogens. Resistant organisms such as *Pseudomonas* spp. are more likely after exposure to multiple antibiotics, or if the patient is nursed in an area of high risk of harboring such organisms, such as the ICU. An algorithm for classifying patients who have HAP is shown in Figure 35.2 . The three groups are considered below.

Patients who have mild-to-moderate HAP, no unusual risk factors and onset any time, or patients who have severe HAP with early onset

In this situation the 'core' pathogens are most likely and the antibiotic choice should reflect this possibility (Table 35.1).

Patients who have mild-to-moderate HAP, additional risk factors and onset at any time

In addition to the 'core' pathogens shown in Table 35.1 , other bacteria must be considered, depending on which risk factor is present

TABLE 35-1 -- A guide to likely 'core' pathogens and empiric antibiotic choice for patients who have non-severe HAP without any unusual risk factors, occurring at any time or early-onset severe HAP.*

A GUIDE TO LIKELY 'CORE' PATHOGENS AND EMPIRIC ANTIBIOTIC CHOICE FOR PATIENTS WHO HAVE NON-SEVERE HAP WITHOUT ANY UNUSUAL RISK FACTORS, OCCURRING AT ANY TIME OR EARLY-ONSET SEVERE HAP	
'Core' organisms	**'Core' antibiotics**
Escherichia coli	2nd or 3rd generation cephalosporins
Klebsiella spp.	or
Proteus spp.	
Serratia marcescens	ß-lactam/ß-lactamase inhibitor combination
Enterobacter spp.	
Haemophilus influenzae	Fluoroquinolone with enhanced
Streptococcus pneumoniae	pneumococcal activity (also if penicillin allergic)
Staphylococcus aureus (methicillin-sensitive)	
Specific antibiotics are mentioned in the text.	

** Adapted from Table 1 in the American Thoracic Society consensus statement.* [1]

TABLE 35-2 -- A guide to likely additional pathogens and antibiotic therapy required for patients who have mild-to-moderate HAP and additional risk factors present.*

A GUIDE TO LIKELY ADDITIONAL PATHOGENS AND ANTIBIOTIC THERAPY REQUIRED FOR PATIENTS WHO HAVE MILD-TO-MODERATE HAP AND ADDITIONAL RISK FACTORS PRESENT		
Core organisms plus:	**Core antibiotics plus:**	
Organism	**Risk factor**	**Antibiotics**
Anaerobes	Recent thoracoabdominal surgery	Clindamycin or ß-lactam ß-lactamase inhibitor combination
	Impaired swallowing	
	Witnessed aspiration	
	Dental sepsis	
Staphylococcus aureus	Coma	Consider adding vancomycin if MRSA possible
	Head trauma	
	Neurosurgery	
	Diabetes mellitus	
	Renal failure	
Legionella spp.	High-dose corticosteroids	MACROLIDE
	Organism endemic in hospital	+/- rifampicin
		+/- fluoroquinolone

Pseudomonas aeruginosa	Prior antibiotics	Treat as severe HAP (see Table 35.4)
	High-dose corticosteroids	
	Protonged ICU stay	
	Structured lung disease such as bronchiectasis	
Specific antibiotics are mentioned in the text.		

** Adapted from Table 2 in the American Thoracic Society consensus statement.*[1]

(Table 35.2). Aspiration of anaerobic organisms is more likely in certain patients, for example those with swallowing problems, poor dental hygiene or impaired consciousness. Using invasive techniques and specific anaerobic cultures,[20] such organisms can be identified in up to one-third of all patients who have HAP, but the true significance of these findings is debated in the absence of clear risk factors for anaerobic infection.[1] *Legionella* infection is well recognized as a sporadic and occasionally an endemic pathogen in the hospital environment. *Staph. aureus* infection is particularly associated with early infection in comatose patients, after multiple trauma or neurosurgical operations. The cause for this is not clear, but may relate to transfer of *S. aureus* from health care workers involved with resuscitation. However, MRSA is unlikely in this situation unless the patient has received multiple antibiotics before hospitalization.

Severe HAP

The definition of severe HAP is less developed than that for community-acquired pneumonia. Pointers to the presence of severe pneumonia are given in Table 35.3 .

When severe HAP occurs within 5 days of admission, the patient is likely to be infected by the core organisms, in particular *H. influenzae* and Gram-positive pathogens (see Table 35.1). With increasing duration of hospital stay, factors such as critical illness, mechanical ventilation, respiratory tract instrumentation, exposure to antibiotics, corticosteroids and other drugs and the ubiquitous presence of pathogenic organisms within the hospital, especially in the ICU, become increasingly important. In such circumstances resistant organisms such as *P. aeruginosa*, *Acinetobacter* spp. and MRSA must also be covered by initial therapy (Table 35.4). Infection with these organisms themselves denotes severe pneumonia because the mortality rate attributable to these organisms is greater than with other types of infection.

TABLE 35-3 -- Definition of severe HAP accepted by the American Thoracic Society.[A]

DEFINITION OF SEVERE HAP ACCEPTED BY THE AMERICAN THORACIC SOCIETY	
Chest radiographs	Multilobar, cavitating or rapidly progressing lung shadowing
	Admission required to ICU
Respiratory failure	Need for mechanical ventilation or
	Need for >35% oxygen to maintain arterial oxygen saturation >90%
Evidence of severe sepsis	Shock (systolic BP <90mmHg or diastolic BP <60mmHg)
	Need for vasopressors for >4 hours
	Urine output <80ml in 4 hours
	Renal dialysis required

Adapted from Table 4 in the American Thoracic Society consensus statement.[1]

TABLE 35-4 -- A guide to likely pathogens and appropriate empiric antibiotic therapy for patients with late-onset severe HAP, which develops after 5 days in hospital.[*]

A GUIDE TO LIKELY PATHOGENS AND APPROPRIATE EMPIRIC ANTIBIOTIC THERAPY FOR PATIENTS WITH LATE-ONSET SEVERE HAP, WHICH DEVELOPS AFTER 5 DAYS IN HOSPITAL	
Core organisms plus:	**Antibiotics**
Pseudomonas aeruginosa	Combination of:
	• Aminoglycoside or ciprofloxacin plus one of:
Acinetobacter spp.	• Antipseudomonal ß-lactamase stable ß-lactam antibiotic
	• Carbapenems
MRSA in some hospitals	• +/- Vancomycin
Those with early-onset severe HAP without unusual risk factors present are managed as shown in Table 35.1 .	

** Adapted from Table 3 in the American Thoracic Society consensus statement.* [1]

PREVENTION

With the enormous impact that HAP has on the workings and economy of hospitals, it is perhaps surprising that few evidence-based guidelines on prevention have been available until recently.[1] [3] [21] [22] A recent review has presented the evidence base for prevention of HAP[22] and highlighted the key areas to be addressed, as summarized below in descending order of importance:

- preventing colonization by hygiene, especially handwashing between patients;
- early enteral nutrition to enhance host responses;
- subglottic drainage of endotracheal tubes;
- selective digestive decontamination, especially in trauma patients;
- semirecumbent position and use of kinetic beds, to reduce aspiration of gastroesophageal contents;
- strict attention to best ventilator equipment techniques; and
- maintenance of low gastric pH, but a balance of reducing risk of infection and increased risk of gastrointestinal bleeding.

The principles of prevention relate to three factors including:

- reducing any predisposing factors for acquiring pneumonia,
- preventing colonization, and
- enhancing host defenses.

Reducing any predisposing factors for acquiring pneumonia includes cessation of smoking, weight reduction in the obese and improving control of coexisting disease

such as chronic obstructive pulmonary disease, diabetes mellitus or cardiac failure before elective surgery. Increasing the level of fitness for an operation is also likely to be of benefit, as are short preoperative hospital stays and operation times. Early nutrition is important in enhancing host defenses.

Prevention of colonization is currently the most practical approach to the general prevention of HAP.[22] The bacteria involved in nosocomial infection are frequently transmitted from patient to patient on the hands of health care workers and it is well established that use of handwashing, particularly with alcohol hand-rubs between patient contacts reduces cross-infection. Each staff member can contribute by adherence to a strict handwashing regimen. Unfortunately, compliance with handwashing strategies is a problem especially with medical staff.[23] Patient contact with staff harboring a viral respiratory infection should be avoided and influenza vaccination of staff during periods of high influenza activity should be ensured. Strict airway management during and after surgery is essential, and nasogastric and endotracheal tubes should be removed as early as possible. Noninvasive ventilation techniques carry less risk of VAP. Re-intubation increases the risk of infection, as does the use of nasal intubation, compared with the oral route. Aspiration of secretions that pool above the inflated endotracheal tube may be helpful in preventing lower respiratory colonization during prolonged mechanical ventilation.[22] Evaluation of swallowing should be a routine procedure for patients who have impaired consciousness or neuromuscular swallowing problems, with oral intake restricted as necessary. Avoidance of excess sedation may reduce the chance of silent aspiration.

It is now recognized that colonization of the gastroesophageal tract usually precedes subsequent contamination of the respiratory tree and gastric acidity plays an important part in preventing such colonization. Routine therapy with H_2 antagonists and proton pump inhibitors to reduce gastric acidity and 'prevent' stress gastric ulceration is no longer recommended, and meta-analyses support sucralfate therapy as being a safer alternative as far as reducing the incidence of HAP. Unfortunately the latter is a less effective antiulcer prophylaxis and H_2 antagonists and proton pump inhibitors should still be considered where the risk of ulceration is increased.[22] A recent meta-analysis concluded that selective decontamination of the gastrointestinal tract could reduce respiratory tract infections and overall mortality rate in some groups of critically ill patients, particularly after trauma.[24] However, there is risk of bacterial resistance and enthusiasm for selective decontamination of the gastrointestinal tract with topical and parenteral antibiotics has waxed and waned and has not become routine management in most ICUs.

One of the most important factors for prevention is probably the circumspect use of antibiotics and adoption of a strict and sensible hospital antibiotic policy, together with regular surveillance for HAP outbreaks and the antibiotic-resistant patterns of likely pathogens.

CLINICAL FEATURES AND DIFFERENTIAL DIAGNOSIS

The diagnosis of pneumonia may be obvious in the hospitalized patient who develops the classic symptoms of fever, malaise, cough, purulent sputum, localizing chest signs and consolidation on the chest radiograph. All too often, however, the situation is less straight-forward, with the list of differential diagnoses lengthening in proportion to the complexity of the underlying problem, for example:

- pulmonary infarction,
- adult respiratory distress syndrome,
- pulmonary edema,
- pulmonary hemorrhage,
- pulmonary vasculitis,
- underlying disease (e.g. malignancy),

- iatrogenic lung shadowing (e.g. drug toxicity or radiation pneumonitis), and
- pre-existing lung disease (e.g. fibrosing alveolitis).

Hospital-acquired pneumonia should be considered in the context of an illness developing after hospital admission and characterized by fever, leukocytosis, purulent sputum or tracheobronchial secretions and new or persisting infiltrates on the chest radiograph. However, the accuracy of a clinical diagnosis is poor compared with a microbiologic or pathologic diagnosis. In one study in an ICU less than one-half of the patients who have a fever and probable pneumonia diagnosed on clinical grounds had the diagnosis confirmed microbiologically.[25]

The difficulties in making an accurate diagnosis from simple clinical features has led to more complicated diagnostic criteria being developed, particularly for VAP. For example, a 'clinical pulmonary infection score' has been suggested to take account of temperature, white blood cell count, presence of purulent secretions, oxygenation requirements and chest radiograph infiltrates, combined with a clinical course consistent with a diagnosis of pneumonia, the lack of any alternative source of sepsis or histologic confirmation of pneumonia.[6] [26]

An alternative approach is to consider ventilated patients 'at risk' of pneumonia if they develop new lung infiltrates and have purulent tracheal aspirates. Daily sampling of tracheal secretions can give early warning of impending VAP because VAP is uncommon in the absence of positive tracheal cultures. A diagnosis of 'definite' VAP then requires microbiologic confirmation from quantitative culture from protected specimen bronchial brush (PSB) samples or the presence of intracellular bacteria in cells from a bronchoalveolar lavage (BAL) cytospin.[8]

DIAGNOSIS

General investigations (hematology, immunology and radiology)

Patients who have suspected HAP should have a full blood count; neutrophilia may point to infection as might a raised serum C-reactive protein. Biochemical tests are often indicated to assess the impact of the pneumonia on the underlying condition and to assess renal and hepatic function. Oxygenation should be assessed by pulse oximetry or arterial blood gas estimation. The chest radiograph will show new or worsening lung shadowing, although it is not usually diagnostic of infection. Cavitation is suggestive of infection, particularly by EGNB, anaerobes or fungi, but can be seen with pulmonary infarction.

General microbiologic investigations

Blood cultures should always be obtained. A positive culture identifies the pathogen and is equated with a worse prognosis. However, only about 8–20% of blood cultures from patients who have HAP are positive, indicating a low sensitivity.[27] The sources of the bacteremia, other than the lung, should always be considered. Up to 50% of patients with positive blood cultures may have an additional source of infection to HAP.[27] Pleural fluid should always be sampled to identify an impending empyema (Fig. 35.3). Serologic tests for viral and atypical pathogens are rarely of value, unless nosocomial *Legionella* pneumonia is a possibility or specific IgM tests are available. *Legionella* urinary antigen detection is a rapid, sensitive and specific test for nosocomial infection by *Legionella pneumophila* serogroup 1.

Special investigations and techniques to obtain lower respiratory tract samples

Ideally, there would be a widely available and accepted technique to obtain and culture uncontaminated secretions from the site of a lung

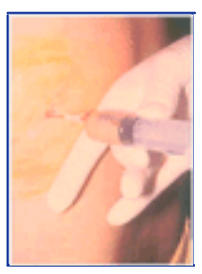

Figure 35-3 Pleural fluid, if present, should always be sampled in a patient who has pneumonia to assess etiology. In this case, purulent fluid was detected suggestive of an empyema. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

infection that was simple, safe and inexpensive to perform, the results of which would differentiate infection from colonization and were shown to aid management and improve outcome. Although the past 15 years has seen a rapid expansion in the use of different techniques for sampling in HAP, none fulfills the above criteria and controversy still exists among experts regarding the role of invasive and noninvasive investigations of HAP and VAP.[2] [27]

The techniques can be broadly divided into noninvasive and invasive groups (Table 35.5). A major limitation of studies that have attempted to validate these

techniques is the lack of a clear 'gold standard' for the diagnosis of pneumonia. In some studies, investigations have been performed just before death and the diagnosis of infection made after death. This is probably a better gold standard than lung biopsy, but does not reflect normal clinical practice, in which the diagnosis is attempted at an earlier stage of the illness. It is with these limitations in mind that the following techniques are described.

Noninvasive techniques

Expectorated sputum

The problems of sputum collection are well known and include contamination of the specimen by upper respiratory tract flora, making it unrepresentative of lower respiratory tract secretions. This is a particular problem in the nosocomial setting, when EGNB commonly colonize the upper respiratory tract and pathogens can be isolated with equal frequency in patients who have and do not have pneumonia.[4] The incidence of false-positive results for Gram-positive pathogens is relatively low but may rise to 50% for EGNB.[28] Only one-third to one-half of sputum cultures provide reliable information compared with blood cultures, transtracheal aspirates and PSB samples.[20] [28] [29] [30] Contamination of sputum by oral secretions should be suspected if the sputum Gram stain contains less than 25 polymorphonuclear neutrophils and over 10 squamous cell epithelial cells per low-power field. The presence of elastin fibers in potassium hydroxide preparations of sputum equates well with the presence of pneumonia, although the test is not widely available.[26]

Endotracheal aspirates

Aspiration via the endotracheal tube is the simplest method of obtaining secretions in patients on mechanical ventilation. Experience has indicated a high sensitivity but a very low specificity,

TABLE 35-5 -- Assessment of the advantages and disadvantages of the different techniques used to obtain respiratory secretions from patients who have suspected HAP.

ASSESSMENT OF THE ADVANTAGES AND DISADVANTAGES OF THE DIFFERENT TECHNIQUES USED TO OBTAIN RESPIRATORY SECRETIONS FROM PATIENTS WHO HAVE SUSPECTED HAP						
		Special equipment required (bedside + lab)	**Skill required**	**Risk of technique**	**Sensitivity**	**Specificity**
Noninvasive techniques						
	Expectorated sputum	0	0/+	0	+	+
	Endotracheal aspirate	+	+	0/+	++	+
	Blind distal airways sampling	++	++	+	++	++
Invasive procedures						
Perbronchoscopic	Protected specimen brush	+++	+++	++	+++	++++
	Bronchoalveolar lavage	+++	+++	++	++++	+++
	Protected bronchoalveolar lavage	++++	++++	++	++++	++++
Nonbronchoscopic	Percutaneous lung needle aspirate	+	+++	+++	++	++++
	Transtracheal aspiration	+++	++++	+++	+++	++
	Pleural fluid sampling	+	++	+	+	++++
Lung biopsy		++++	++++	+++	++++	++++
It is based on an arbitrary scale from 0 (very low/little) to ++++ (very high/much).						

suggesting that a negative rather than a positive culture is of greater value to the clinician. A negative culture effectively excludes VAP when the patient has not received antibiotics. Quantitative cultures with a high cut-off level improve specificity and adversely affect sensitivity, but can produce results comparable with invasive techniques without the need for special equipment or training.[27] Daily quantitative bacterial cultures may identify a rapid rise in bacterial counts a few days before the development of new pulmonary infiltrates; regular surveillance with endotracheal aspirates may, therefore, be helpful.[27] The identification of elastin fibers and antibody-coated bacteria may increase the ability to differentiate between colonization and pneumonia.[26]

Nonbronchoscopic techniques for sampling the distal airways

Nonbronchoscopic techniques have the advantage of being less invasive and simple to perform both by medical and nonmedical staff, such as respiratory therapists;[31] small endotracheal tubes (such as in children) can be used, although samples cannot be obtained reliably from the area of the lung where infection is suspected. The simplest technique involves distal, nondirected bronchial lavage through a standard aspiration catheter.[6] In one study mean bacterial colony counts increased significantly during the 2 days preceding the clinical onset of pneumonia, with counts falling significantly after appropriate antibiotic therapy. In another study, the sensitivity and specificity of nondirected bronchial lavage, cultured quantitatively, were both about 69% and comparable to those of bronchoscopic methods, if performed on patients with a clinical diagnosis of VAP.[32] Blind techniques using plugged catheters or PSB samples and quantitative culture have reported sensitivities between 61 and 100% and specificity at a similar level.[28]

Bronchoscopic invasive procedures

Much has been published about the value of invasive techniques for managing HAP. However, questions as to who, when and how to perform these tests and the reliability of the results remain unresolved, as does the applicability of published results to everyday clinical situations.[1] [2] [25] [26] [27]

Fiberoptic bronchoscopy provides direct visual access to the lower airways. Bronchoscopic techniques are relatively simple to carry out in patients receiving mechanical ventilation, but such patients have often received antibiotics, reducing the value of bronchoscopic studies. Invasive techniques might be considered in nonventilated patients who have moderate or severe HAP before antibiotic therapy is started. In practice immediate access to bronchoscopic techniques may be limited and the procedure itself may on occasions precipitate cardiorespiratory failure in a spontaneously breathing but hypoxic patient, necessitating ventilatory support earlier than anticipated. Consequently nonbronchoscopic techniques are generally used for such patients, including on occasions invasive techniques such as percutaneous lung needle aspiration. Transtracheal aspirates are rarely used.

Protected specimen brush

The double catheter PSB (Fig. 35.4) is effective in avoiding contamination during passage through the bronchoscopic suction channel. Attention to correct technique is important for obtaining the specimen and transferring the brush to transport medium for quantitative culture. [26] [27] Briefly, the brush catheter is passed bronchoscopically to the segment from which the sample is to be obtained; the inner sheath is then extended, thus expelling the wax plug. Next, the brush is extended and then withdrawn back into the inner sheath. The catheter is then removed from the bronchoscope, the inner sheath end wiped with a 70% alcohol swab, and the brush cut aseptically, once it has been advanced from the inner sheath, into 1ml sterile normal saline. The sample is then immediately vortex-mixed and plated out in dilutions for quantitative culture. Numerous studies have reported a sensitivity of 33–100% and a similar specificity, using a threshold concentration of $=10^3$ cfu/ml on quantitative bacterial culture.[27] [28]

Bronchoalveolar lavage

For BAL, the bronchoscope is wedged into a subsegmental airway and the bronchoalveolar area is lavaged; volumes of over 100ml sterile normal saline are required to

reach the distal alveoli (Fig. 35.5).

The advantage of this technique is that a large part of the lung is sampled and the specimen allows microscopic analysis to assess the presence of intracellular bacteria, neutrophils and elastin fibers and cytologic evaluation if required. This can be particularly useful if the diagnosis of pneumonia is in doubt and malignancy is a diagnostic

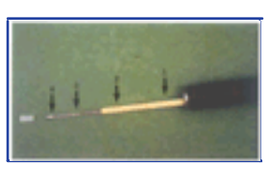

Figure 35-4 An extended protected specimen brush protruding from the end of a fiberoptic bronchoscope. Note the outer plastic sheath (arrow 1) and the inner protective yellow plastic cover (arrow 2) with the microbiologic brush pushed out (arrow 3). The protective gelatin plug occludes the end of the outer cover (arrow 4) until ejected before obtaining the specimen. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

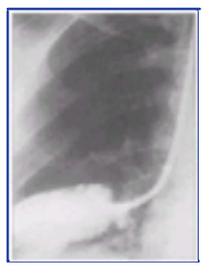

Figure 35-5 Limited bronchoalveolar lavage of right middle lobe using radiopaque solution. Note the alveolar filling pattern. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

possibility. Contamination of the BAL fluid in the bronchoscopic channel is one disadvantage and a careful technique is required to avoid this. After wedging the tip of the bronchoscope in the relevant subsegment, 50ml normal saline at 98.6°F (37°C) is instilled. This aspirate samples airways rather than the alveoli, is best referred to as a 'bronchial wash'[33] and is more likely to be contaminated with bacteria present in the suction channel. This sample should therefore be processed separately from the second, third and, if necessary, fourth aliquots of 50ml, which should be pooled. Sensitivity and specificity of BAL quantitative cultures have ranged from 42 to 100% and 69 to 100%, respectively, using threshold concentrations of $=10^4$ cfu/ml.

When compared with PSB performed at the same time, BAL has greater sensitivity but a marginally reduced specificity. Combining both techniques overcomes this problem and appears to provide the best results in experienced centers.[28] [34] An alternative is to perform a protected BAL using a balloon-tipped catheter (to allow effective wedging and isolation of a pulmonary subsegment) with a distal ejectable plug. This is reported to improve specificity compared with BAL and sensitivity compared with PSB, but adds to cost.[28] [35]

Percutaneous invasive techniques

Percutaneous lung needle aspirate

Percutaneous lung needle aspirate (PLNA) has been used successfully to investigate nonventilated patients who have lung shadowing. The technique can be performed at the bedside with minimal equipment. The use of a 25-gauge ultrathin needle reduces the chance of pneumothorax. In one study of 98 patients who had HAP outside the ICU, the sensitivity was 61%, the specificity 100% and the pneumothorax complication rate 3.5%. The latter reduces the usefulness of this technique in patients receiving mechanical ventilation. Animal models suggest a high sensitivity and specificity. [26] When compared with histologic diagnosis of pneumonia on lung biopsy immediately postmortem as a 'gold standard', PLNA had the lowest sensitivity (25%) but the highest specificity (79%) of the invasive techniques discussed. However, recent studies have questioned the value of immediate postmortem lung biopsies as the 'gold standard' for the diagnosis of pneumonia.[25] [36]

Transtracheal aspiration

Transtracheal aspiration is now rarely used due to the ready availability of fiberoptic bronchoscopy. However, its value was demonstrated for the investigation of patients who have suspected HAP on general medical and surgical wards.[20]

Influence of invasive techniques on management of HAP

The literature supports the view that PSB, BAL and tracheal aspirate specimens cultured quantitatively can provide useful and reliable information about the likely presence and cause of HAP in patients who have the clinical features of pneumonia, particularly in the ICU.[28] Their value is considerably diminished if the patient is already receiving antibiotics, which is the usual situation.[37] There is controversy as to whether these tests improve patient management and outcome in routine clinical practice. The effect of PSB results on antibiotic management was shown in one study of 110 mechanically ventilated patients who had suspected HAP.[38] Antibiotics were stopped in the majority before PSB was performed. Quantitative PSB culture supported the clinical diagnosis of pneumonia in only 41% of patients. The PSB results suggested that the initial treatment was appropriate in one-third of all patients, inappropriate in a further one-third (patients were receiving antibiotics that were not considered necessary) and the situation was unclear in the remaining one-third. Of the patients who had pneumonia, over one-third had their antibiotics changed because of the PSB result.

Although this study suggests that invasive techniques may influence immediate management, the literature does not generally show that using these techniques improves outcome. Randomized studies, summarized elsewhere, have also shown that invasive techniques lead to more antibiotic changes, increase costs, but do not affect mortality rate or morbidity.[2] [27] Only one randomized study, from France, has shown a bronchoscopic strategy including quantitative cultures of PSB and BAL specimens to be superior to a clinical strategy using qualitative tracheobronchial aspirates, in terms of 2-week mortality rate and overall morbidity.[39]

For now the clinician can consider using a diagnostic algorithm for patients with suspected VAP proposed by the recent evidence-based guidelines for the diagnosis of VAP,[27] [40] which provides an option of either starting directed treatment based on the results of quantitative culture of tracheobronchial samples obtained invasively or noninvasively, or starting immediate empiric treatment, obtaining tracheal samples for qualitative culture and then adjusting treatment according to culture results or response to treatment (Fig. 35.6). This suggests that invasive techniques and quantitative cultures should only be performed if the clinical and laboratory expertise is available and if the results are consistently applied to management decisions as part

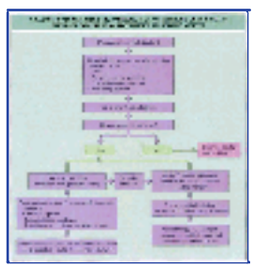

Figure 35-6 A diagnostic algorithm for the management of ventilator-associated pneumonia, depending on whether an empiric treatment approach or an investigation approach, (using quantitative culture of bronchoscopically or nonbronchoscopically obtained specimens), is adopted. *Adapted from Ioanas et al.[27] and Grossman et al.[40]*

of an agreed management protocol.[1] In practice, quantitative culture is time-consuming and demanding and is not performed routinely in all laboratories, which limits its usefulness in many hospitals.

MANAGEMENT

General factors

The general management of the patient who has HAP is important. Such patients frequently have underlying disease, which may be worsened by the infection and require additional therapy. Careful attention to fluid balance and oxygenation is essential, and chest physiotherapy may be helpful. Not infrequently the diagnosis of HAP may be uncertain and additional empiric therapy (e.g. anticoagulation for possible pulmonary embolus) may be started, pending results of appropriate investigations. Even with effective management, the prognosis may be poor because of the underlying disease or overwhelming infection. In only a proportion of patients dying with HAP is death directly attributable to the infection.

Guidelines for empiric antibiotic therapy

Guidelines from the ATS,[1] the Canadian Consensus Conference[15] and more recent reviews[2] have been published and provide a useful basis for the practical, empiric management of HAP. The decision tree is summarized in Figure 35.2 and Table 35.1 , Table 35.2 , Table 35.3 , Table 35.4 and depends on assessment of severity, the description of 'core' pathogens, the identification of risk factors for specific pathogens and time of onset to guide empiric antibiotic therapy. In a similar way to the empiric management of community-acquired pneumonia, patients who have severe infection are identified early and start by receiving combination antibiotic therapy to cover all likely pathogens. For nonsevere HAP, single antibiotic therapy is usually appropriate to cover the 'core' pathogens, unless additional risk factors are present.

Mild-to-moderate HAP; no risk factors for other pathogens; 'core' pathogens likely

In this situation a single agent effective against the 'core' pathogens, including Gram-positive organisms, *H. influenzae* and EGNB is appropriate.[10] [27] [41] Options include a second- or third-generation cephalosporin, such as cefuroxime, ceftriaxone or cefotaxime, or a combination of a ß-lactam antibiotic with a ß-lactamase inhibitor (e.g. amoxicillin or ticarcillin, together with clavulanic acid) or a new-generation fluoroquinolone with enhanced pneumococcal cover (e.g. levofloxacin or moxifloxacin; see Table 35.1). Oral therapy can be used if appropriate.

Mild-to-moderate HAP; risk factors present for specific pathogens in addition to 'core' pathogens

In this situation the specific risk factors will provide some guidance about whether additional antibiotic therapy may be required to cover pathogens such as anaerobes, more resistant EGNB and *Legionella* infection as well as the 'core' pathogens. Combination therapy may be required, including the antibiotic appropriate to cover the 'core' organisms (see Table 35.2).

Severe HAP

'Early' severe HAP occurring soon after hospital admission is likely to be caused by the 'core' organisms, in particular *S. pneumoniae*,

S. aureus and *H. influenzae* and is treated like nonsevere infection, except that the parenteral route should be used (see Table 35.1). For 'late' severe HAP occurring after 5 days of hospitalization, empiric therapy should cover *P. aeruginosa* and *Acinetobacter* spp. in addition to the 'core' organisms. Combination antibiotics providing antipseudomonal cover are required, including an antipseudomonal ß-lactam antibiotic (such as piperacillin/tazobactam, or ceftazidine or carbapenems (meropenem or imipenem/cilastatin) together with either an aminoglycoside or a fluoroquinolone (such as ciprofloxacin), or both. Methicillin-resistant *S. aureus* has become a problem in many institutions and requires the addition of vancomycin (see Table 35.4).[42]

Duration of therapy

There is no clear evidence on which to base a decision about the duration of treatment and clinical judgement is required, supported by improvement in observations, tracheobronchial secretions, inflammatory markers such as C-reactive protein and white cell count, and radiological shadowing. Generally there is a tendency to treat patients for longer than those with community acquired pneumonia (which is usually treated for 5–7 days). In particular, treatment for *Pseudomonas* or *Legionella* spp. may require up to 14 days or longer of antibiotics, particularly if lung cavitation is present with *Pseudomonas* infection. When possible oral therapy should be substituted for the parenteral route, if clinical progress in satisfactory and the oral route is available. Patients on the ICU or receiving mechanical ventilation or following surgery may have problems with enteral absorption and require continued intravenous therapy. A balance must be struck because prolonged antibiotics run the risk of promoting the selection of resistant organisms in the patient and in the unit, and also causing antibiotic side-effects such as *Clostridium difficile*-associated colitis.

Factors to consider when assessing response

Assessment of the response of the patient who has HAP is difficult because of the complex relationship between the infection and the underlying disease. Factors to consider if the patient is not responding to empiric therapy are shown in Table 35.6 , and have been reviewed elsewhere. *P. aeruginosa* and MRSA are the two pathogens most frequently associated with treatment failure.[1] [2]

TABLE 35-6 -- Factors to consider when a patient with hospital-acquired pneumonia is not improving with initial management.

FACTORS TO CONSIDER WHEN A PATIENT WITH HOSPITAL-ACQUIRED PNEUMONIA IS NOT IMPROVING WITH INITIAL MANAGEMENT	
Factor	**Action**
Improvement expected too soon	Continue therapy — review again
Deterioration in underlying disease	Optimize general management
Diagnosis of hospital-acquired pneumonia incorrect	Review situation
	Consider differential diagnosis
Additional pathologic process present	Review situation
	Consider differential diagnosis
Additional or unexpected pathogen present	Review microbiologic data
	Consider alternative or invasive tests
Pathogen resistant to antibiotic	
Local intrathoracic complication (e.g. empyema, lung abscess)	Review chest radiographs
	Consider CT scan, bronchoscopy
Metastatic infective complication (e.g. endocarditis, arthritis, abscess)	Detailed clinical examination and appropriate tests
Reason for pneumonia persisting (e.g. aspiration, bacteremia from distant focus)	Review history
	Repeat blood cultures
Secondary complications (e.g. intravenous line infection, pulmonary emboli)	Detailed clinical examination and further studies (e.g. V/Q scan)
General factors (e.g. dehydration, nutrition, hypoxia)	Manage appropriately
Allergic reaction to antibiotics (often after several days of therapy)	Look for rash and recurrence of fever
	Consider stopping/changing antibiotic
Patient not actually receiving or taking the antibiotic	Check

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Chapter 36 - Lung Abscesses and Pleural Abscesses

Julie E Mangino

INTRODUCTION

Lower respiratory tract infections (LRTIs) are a major indication for antimicrobial therapy in developed countries. Although many LRTIs are self-limiting, those caused by necrotizing organisms are invariably serious; they may lead to abscess formation in the lung and can spread to the pleural space.

EPIDEMIOLOGY

The etiologies of lung abscess and pleural abscess, or empyema, vary in different parts of the world. The common denominator is usually aspiration pneumonia, acquired either in the community or in the hospital. Nosocomial pneumonia is a major cause of morbidity and lengthened hospital stay, with enormous economic impact. Aspiration pneumonia leading to necrotizing pneumonia or lung abscess, with or without empyema, is a continuum; any stage or all stages may be encountered. Underlying diseases, associated trauma or surgery and the timeliness of appropriate therapy are the major factors in determining clinical presentation and prognosis.

Lung abscess

A lung abscess is arbitrarily defined as a localized area of pulmonary necrosis caused by infection, with a solitary or dominant cavity measuring at least 2cm in diameter. When cavities are multiple and smaller than 2cm, the infection is usually referred to as a necrotizing pneumonia.[1] [2] [3] Most abscesses are suppurative bacterial infections caused by aspiration.

Primary lung abscesses typically present in patients who have no predisposing disease other than a predilection to aspirate oral secretions; they are more common in males than in females. Secondary lung abscesses occur in patients who have an underlying condition such as a partial bronchial obstruction or lung infarct, or in those who are otherwise immunocompromised because of chemotherapy, malignancy, organ transplantation or HIV infection. Lung abscesses may be termed non-specific or putrid, referring, respectively, to the often unclear etiology and the offensive odor of the sputum.[3] [4]

Over the past five decades, the incidence of bacterial lung abscess in the USA has diminished considerably and the mortality rate has decreased from 30–40% to 5–10%. Factors associated with a worse prognosis include advanced age, prolonged symptoms, concomitant disease, nosocomial infection and (according to some studies) larger cavity size. In the past, tuberculosis was responsible for a higher proportion of lung abscesses. In recent years, more lung abscesses have been associated with pulmonary malignancies or other underlying conditions.[3] [4] [5] [6] [7]

Empyema

A pleural effusion associated with pneumonia, lung abscess, or bronchiectasis is referred to as a parapneumonic effusion. These occur in up to 40% of people who have bacterial pneumonia; they are the most common cause of exudative pleural effusions in the USA.[8] Empyema, or pleural pus, is an infected parapneumonic effusion with characteristic changes in the composition of the pleural fluid. It has been declining in frequency and changing in etiology. [6] Mortality ranges from approximately 2% to 50%, with the lowest rates in young, healthy people and the highest rates in the elderly and immunocompromised. The prognosis is poorer when pathogens are resistant to antimicrobial drugs or when appropriate treatment is delayed.[8] [9] [10]

PATHOGENESIS AND PATHOLOGY

Micro-organisms gain access to the lower respiratory tract by a variety of routes, including inhalation of aerosolized particles, aspiration of oropharyngeal secretions and hematogenous spread from distant sites (Fig. 36.1). Less frequently, infection occurs by direct extension from a contiguous site. Lung abscess is caused only by organisms that cause necrosis but empyema can result from infection by any pathogen that reaches the pleural space.

Lung abscess

Of the inhaled respiratory pathogens, only the mycobacteria (see Chapter 37 and Chapter 38) and the dimorphic fungi (see Chapter 39) commonly cause lung abscesses. Bacterial abscesses are usually caused by aspiration of oropharyngeal secretions or, occasionally, by hematogenous seeding. [1]

Aspiration of small quantities of oropharyngeal secretions occurs intermittently in everyone, particularly during sleep. Despite the frequency of aspiration, the airways below the level of the larynx are normally sterile. Highly efficient clearing mechanisms are in place; these include cough, a mucociliary system that carries particles cephalad to be swallowed, phagocytosis by alveolar macrophages and neutrophils aided by opsonizing antibodies and complement, and lymphatic trapping with sequestration in regional lymph nodes. Risk factors for pneumonia after aspiration include conditions that increase the inoculum of pathogenic organisms in aspirated secretions, conditions that increase the likelihood of aspiration and conditions that increase the volume of the aspirate (Table 36.1). Under these circumstances, aspirated oropharyngeal secretions are more likely to cause chemical irritation and infection. When an anaerobic pleuropulmonary infection occurs in an edentulous patient, the diagnosis of bronchogenic carcinoma should be considered.[1] [2] [3] [11]

The composition of the oropharyngeal flora at the time of aspiration determines the potential etiologic agents for LRTIs. Those organisms that are most numerous or virulent proliferate and emerge as single or predominant pathogens. Although the classic non-necrotizing respiratory pathogens *Streptococcus pneumoniae* and *Haemophilus influenzae* can cause disease by this mechanism, normal oropharyngeal secretions contain many more streptococci of various species and more anaerobes (approximately 10^8 organisms/ml) than aerobes (approximately 10^7 organisms/ml). Some of the streptococcal species are microaerophilic (i.e. they require supplemental carbon dioxide to grow on artificial media).[12] [13] The pneumonia that follows aspiration, with or without abscess formation, is typically polymicrobial with between two and four bacterial species present in large numbers. In

Figure 36-1 Causes of LRTIs in adults. Oropharyngeal streptococci and anaerobes, *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa*, the dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*) and mycobacteria frequently cause necrosis and subsequent abscess formation.

TABLE 36-1 -- Risk factors for aspiration pneumonia and lung abscess.

RISK FACTORS FOR ASPIRATION PNEUMONIA AND LUNG ABSCESS	
Increased bacterial inoculum	Periodontal disease, gingivitis, tonsillar or dental abscess, drugs that decrease gastric acidity
Impairment of consciousness	Drugs, alcohol, general anesthesia, metabolic encephalopathy, coma, shock, cerebrovascular accident, cardiopulmonary arrest, seizures, surgery, trauma
Impaired cough and gag reflexes	Vocal cord paralysis, intratracheal anesthesia, endotracheal tube, tracheostomy, myopathy, myelopathy, other neurologic disorders
Impairment of esophageal function	Diverticula, achalasia, strictures, disorders of gastrointestinal motility, neoplasm, tracheoesophageal fistula, pseudobulbar palsy
Emesis	Nasogastric tube, gastric dilatation, ileus, intestinal obstruction

general, 50% or more of these infections are caused by purely anaerobic bacteria, 25% are caused by mixed aerobes and anaerobes, and 25% or fewer are caused by aerobes only. Among hospitalized patients, progressive colonization with *Staphylococcus aureus*, Enterobacteriaceae and *Pseudomonas aeruginosa* occurs, and these aerobic organisms are frequent causes of nosocomial aspiration pneumonia and lung abscess.[6] [12] [14] [15]

The anaerobic organisms that are associated with pleuropulmonary infection, using current nomenclature,[16] [17] are shown in Table 36.2 . The primary pathogens are *Streptococcus* spp., *Peptostreptococcus* spp., *Fusobacterium nucleatum* and *Prevotella* spp. Additionally, *Porphyromonas* spp. are commonly associated with periodontal disease and may also be isolated. Although not consistently part of the normal oropharyngeal flora, members of the

TABLE 36-2 -- Anaerobic bacteria associated with pleuropulmonary infections.

ANAEROBIC BACTERIA ASSOCIATED WITH PLEUROPULMONARY INFECTIONS		
	Gram-negative bacteria	**Gram-positive bacteria**
Bacilli	*Bacteroides fragilis* group	*Actinomyces* spp.
	Fusobacterium nucleatum	*Bifidobacterium* spp.
	Fusobacterium necrophorum	*Clostridium* spp.
	Porphyromonas spp.	*Eubacterium* spp.
	Prevotella spp.	*Lactobacillus* spp.
		Propionibacterium spp.
Cocci	*Veillonella* spp.	*Gemella morbillorum*
		Peptostreptococcus spp.
		Streptococcus spp.
(Note: *Porphyromonas* spp. include organisms previously named *Bacteroides melaninogenicus* subsp. *asaccharolyticus*, *B. endodontalis* and *B. gingivalis*. *Prevotella* spp. include organisms previously named *B. melaninogenicus* subspp. *melaninogenicus* and *intermedius*, *B. oralis* and *B. denticola*. *Gemella morbillorum* was previously named *Streptococcus morbillorum*. *Peptostreptococcus* spp. include organisms previously named *Peptococcus* spp.)		

Bacteroides fragilis group of organisms are isolated from approximately 15% of patients.[2] [11] [12] [13] [14] [16]

A variety of virulence factors associated with oropharyngeal streptococci and anaerobes have been identified. Properties that facilitate attachment include capsular polysaccharides, fimbriae, hemagglutinin and lectin. Tissue breakdown and the metabolic activity of organisms provide reducing substances and a low redox potential; these factors facilitate bacterial proliferation. Volatile fatty acids, sulfur compounds, indoles, amines and hydrolytic enzymes (hyaluronidase, chondroitin sulfatase and heparinase) produced by damaged tissue lead to subsequent abscess formation.[18]

The pathology of aspiration pneumonia is characterized by alveolar edema and infiltration with inflammatory cells. Foci of aspiration pneumonia most commonly develop in the subpleural regions of the gravity-dependent segments of the lungs, particularly the superior segments of the lower lobes and the posterior segments of the upper lobes. The right lung is the more frequent location, presumably because of the less acute angle in the take-off of the right main stem bronchus. In general, the right upper and lower lobes are most commonly involved, followed by the left lower lobe and right middle lobe.[1] [3] [19] [20]

The degree and rate of progression of aspiration pneumonia vary considerably. These infections may be acute, subacute or chronic, depending on differences in the etiology, size of inoculum and host factors. If the process is indolent, fibrosis limits the spread of infection. Abscesses typically communicate with a bronchus, producing the familiar air-filled cavity, often with an air-fluid level that can be seen on radiographs. These are usually not apparent until the infection has been present for 1–2 weeks, when multiple adjacent microscopic abscesses filled with necrotic material (pulmonary gangrene) slough to form a gross cavity (Fig. 36.2).[5]

Although less common than aspiration, hematogenous seeding of the lung, or septic emboli, may also result in lung abscesses or necrotizing pneumonia. There may be a solitary infiltrate or cavity or, more often, multiple bilateral lesions. The most common etiologic agents are the nosocomial pathogens *S. aureus* and aerobic Gramnegative bacilli (see Fig. 36.1). When the primary focus is in the abdomen, anaerobes, particularly *B. fragilis*, may be present.

Infective endocarditis (predominantly right-sided), intravenous drug injection and indwelling right atrial catheters placed for vascular access are commonly associated with septic pulmonary emboli. Any organism that is part of the skin flora or contaminants in injected material may be responsible. *Staphylococcus aureus* and streptococci are the most common pathogens but *P. aeruginosa* and *Candida* spp. may also be responsible, causing serious infections.[21]

Some uncommon causes of lung abscess should be considered in appropriate circumstances. Inhaled micro-organisms such as *Legionella* spp., *Chlamydia* spp., *Mycoplasma pneumoniae* and viruses are rare causes of lung abscesses. *Cryptococcus*, *Aspergillus*

Figure 36-2 Cross-section of a lung abscess.

and *Rhizopus* spp. occasionally cause disease in normal hosts but are more commonly opportunistic pathogens. Patients who have advanced HIV disease may have cavitary lesions caused by atypical mycobacteria, particularly *Mycobacterium kansasii*, and other organisms such as *Rhodococcus equi* and *Nocardia asteroides*.[22] *Burkholderia pseudomallei* is endemic to South East Asia, particularly Thailand, and typically causes upper lobe cavities. In endemic areas, the parasites *Paragonimus westermani* and *Entamoeba histolytica* may cause abscess by contiguous extension.

Empyema

The pleural space is normally sterile. It is most commonly contaminated by direct extension from a contiguous focus of infection, usually pulmonary, or by direct inoculation at the time of trauma or surgery (Table 36.3).[8] [23] [24] The pleural space may also become involved through hematogenous seeding from a distant focus of infection, particularly in the presence of hemothorax or pleural malignancy.

The initial stage in the pathogenesis of empyema associated with pneumonia is the development of a sterile parapneumonic effusion, which has varying characteristics depending on its stage of evolution (Table 36.4).[8] [24] The effusion is initially transudative but rapidly becomes exudative with an influx of leukocytes and increasing permeability of the visceral pleura. Neutrophils, lactate dehydrogenase (LDH) and protein increase, and glucose and pH decrease. Fibrin is deposited on the pleural surfaces and loculations may occur. With time, a final organizing stage occurs in which pleural fibroblasts produce an inelastic membrane or pleural peel that encases the lung and restricts inflation. Invasion with bacteria accelerates the fibropurulent reaction. Empyema fluid is relatively deficient in opsonins and complement and it becomes progressively more acidic as the infection ensues. Occasionally, an empyema may spontaneously drain through necrotic lung tissue into a bronchus (bronchopleural fistula) or communicate through the chest wall (empyema necessitans).[8] [10] [24]

The various respiratory pathogens have different propensities to cause empyema. *Streptococcus pneumoniae*, the most common cause of pneumonia, is frequently associated with a parapneumonic pleural effusion, yet pneumococcal empyema is relatively uncommon. Aspiration pneumonia more frequently progresses to empyema.[8] Empyema is also more common with organisms, such as *S. aureus* and *P. aeruginosa*, that produce potent extracellular enzymes that breach the integrity of the pleura, allowing penetration to the pleural space.

Overall, the relative frequencies of various organisms causing empyema have changed over time. Prior to the antibiotic era, most

TABLE 36-3 -- Causes of empyema.

CAUSES OF EMPYEMA	
Pulmonary infection	Pneumonia
	Lung abscess
	Bronchiectasis
Mediastinal disease	Tracheal fistula
	Esophageal perforation
Subdiaphragmatic infection	Subphrenic abscess
	Hepatic abscess
Skeletal infection	Paravertebral abscess
	Vertebral osteomyelitis
Direct inoculation	Trauma
	Thoracentesis
Postoperative	Hemothorax (infected)
	Pneumothorax (infected)
	Bronchopleural fistula

TABLE 36-4 -- **Characteristics of pleural fluid associated with bacterial LRTIs.***

CHARACTERISTICS OF PLEURAL FLUID ASSOCIATED WITH BACTERIAL LRTI				
Pleural fluid characteristic	**Transudate**	**Exudate**		**Empyema**
		Uncomplicated parapneumonic effusion	**Complicated parapneumonic effusion**	
Appearance	Clear	Variable	Variable	Pus
White blood cell count (cells/ml)	<1000	Variable	Variable	>15,000
Differential cell count	Variable	Neutrophils	Neutrophils	Neutrophils
Protein (g/dl)	<3.0	>3.0	>3.0	>3.0
Glucose (mg/dl)	Same as serum	>60	40–60	<40
pH	Greater than serum	>7.2	7.0–7.2	<7.0
Lactate dehydrogenase (units/ml)	<200	<1000	>1000	>1000
Bacteria	Absent	Absent	Absent	Present
The values may overlap. Complicated pleural effusions are those with fluid characteristics that indicate the potential need for tube drainage. When the glucose is 40–60mg/dl or the pH is 7.0–7.2, repeat thoracentesis may be helpful. When the glucose is <40mg/dl or the pH is less than 7.0, a chest tube is indicated even if bacteria are not present by smear or culture.				

** Data from Light[8] and Sokolowski et al.[25]*

TABLE 36-5 -- frequency of organisms isolated from bacterial empyemas.

FREQUENCY OF ORGANISMS ISOLATED FROM BACTERIAL EMPYEMAS			
Organism	**% of isolates**		
	1971–1973[28] (*n* = 214)	**1969–1978[9] (*n* = 93)**	**1973–1985[29] (*n* = 343)**
Haemophilus influenzae	<1	0	3
Streptococcus pneumoniae	2	7	20
Other streptococci (including microaerophiles)	13	22	8
Staphylococcus aureus	8	8	17
Enterobacteriaceae	9	10	11
Pseudomonas spp.	5	9	3
Other aerobes	5	2	2
Bateroides spp.	11	14	8
Anaerobic cocci	15	9	10
Fusobacterium spp.	7	8	6
Prevotella spp.	6	4	6
Other anaerobes	19	7	6

empyemas were caused by *S. pneumoniae* and, to a lesser extent, by *S. aureus* and *Streptococcus pyogenes*. Between 1955 and 1965, penicillin-resistant *S. aureus*

was the predominant pathogen.[8] [26] In the early 1970s, coinciding with a surge of interest in anaerobic infections, anaerobic empyemas were recognized more frequently (Table 36.5).[27] [28] At that time, about 50% of empyemas were caused by aerobes, with Gram-positive cocci being more common than Gram-negative bacilli. About 25% were caused by anaerobes and about 25% were mixed aerobic-anaerobic infections. *Streptococcus pneumoniae* was most frequent in young ambulatory patients, anaerobes were most frequent after aspiration, and *S. aureus* and aerobic Gram-negative bacilli were most frequent after thoracotomy. [9] [28] [29] [30] Because one-quarter of all empyemas are now associated with trauma or surgery, there has been a relative increase in the proportion of staphylococcal infections and a decrease in anaerobic infections.[8] With the widespread use of *H. influenzae* type b vaccination, empyema due to this organism, previously common in children, has become rare.

PREVENTION

Minimizing the risks of aspiration in people who are unconscious, undergoing anesthesia or subject to seizures will reduce the incidence of pneumonia with subsequent abscess formation or empyema. If pneumonia does occur, the timely administration of appropriate antibiotics reduces the likelihood of progression. Pleural effusions should be aspirated for diagnosis and drained, if indicated, to abort progressive suppurative complications.

CLINICAL FEATURES

Lung abscess

Aspiration is usually subtle and unrecognized but may lead to pneumonia and lung abscess. If overt, it may be followed by symptoms and signs such as choking, cough, wheezing, cyanosis or asphyxia, which are related to the particulate, liquid and chemical nature of the material aspirated. Within hours, there may be fever, tachypnea, diffuse rales and hypoxemia.[11] [30] If pneumonia develops, patients usually present within 1 week with a productive cough, a temperature over 102°F (38.9°C) and a leukocyte count of more than 15,000 cells/mm^3 .[30] Aspiration pneumonia is not readily distinguishable from pneumococcal pneumonia, although true rigors are uncommon in aspiration pneumonia and symptoms have usually been present for longer before presentation to medical care. A condition predisposing to aspiration is also more likely to be present. Among community-acquired cases, common conditions that predispose to aspiration are alcoholism, seizures and drug overdose. Among hospital-acquired cases, patients tend to have neurologic disorders, such as cerebrovascular accidents and brain tumors, or metabolic disorders that result in stupor or coma. Only a minority of patients who have aspiration pneumonia have putrid sputum, which, if present, tends to develop 1–2 weeks into the course when an abscess has formed.[6] [12] [14] [30]

Lung abscesses may also present in a more indolent fashion with weeks to months of productive cough, malaise, weight loss, low-grade fever, night sweats, leukocytosis and anemia. The patient may become debilitated as if with tuberculosis. Findings that are suggestive of a suppurative lung abscess rather than tuberculosis include a shorter duration of symptoms, putrid sputum and leukocytosis. Lung abscess must also be distinguished from a necrotic neoplasm. Patients who have neoplasms often lack risk factors for aspiration, symptoms of respiratory infection, fever and leukocytosis. The possibility

of tuberculosis or a noninfectious cause of a lung cavity (neoplasm, infarct, or vasculitis) should be suspected in a patient treated for presumed lung abscess who does not respond to appropriate antimicrobial therapy.

In the pre-antibiotic era, lung abscess characteristically ran a chronic course with the potential for sudden, severe complications. These included brain abscess, massive hemoptysis, endobronchial spread to other portions of the lung, and rupture into the pleural space with the development of a bronchopleural fistula and pyopneumothorax. With modern antimicrobial therapy, these complications have become rare.

Empyema

Symptoms of empyema include fever, chills, cough, dyspnea and chest pain associated with a recent pulmonary or contiguous infection in the oropharynx, mediastinum or subdiaphragmatic area. The occurrence of persistent fever and leukocytosis with pleural effusion despite appropriate antibiotics should suggest the presence of empyema. There may also be severe constitutional manifestations such as shock, tachypnea, altered consciousness and respiratory failure. Typical findings on physical examination include diminished breath sounds, dullness to percussion and a pleural friction rub. Patients who have an empyema secondary to an aerobic pneumonia tend to present with an acute illness, whereas those who have an anaerobic pneumonia have subacute or indolent illness with findings such as putrid sputum to suggest the etiology. [1] [5] [30]

DIAGNOSIS

Radiography

Both lung abscess and empyema may be suspected from clinical symptoms but the chest radiograph is the primary tool for diagnosing these infections. Radiographs obtained soon after aspiration usually demonstrate localized or diffuse alveolar infiltrates within 1–2 days. There is nothing distinctive about the appearance of aspiration pneumonia except that infiltrates are usually in dependent segments of the lung.

The characteristic appearance of a lung abscess is that of a density or mass with a cavity, frequently with an air-fluid level indicating communication with the tracheobronchial tree (Fig. 36.3). The time required for cavitation after a known episode of aspiration is about 1–2 weeks. With necrotizing pneumonia, multiple small lucencies in circumscribed areas of opacification may develop more rapidly.[2] [5] [6] Abscesses

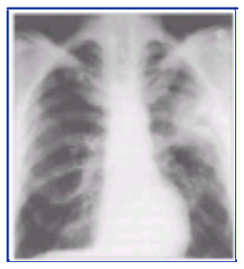

Figure 36-3 A lung abscess showing an air-fluid level.

due to tuberculosis are less likely to have an air-fluid level and are more likely to have a dense fibronodular infiltrate that surrounds the cavities.[20] Those associated with a malignancy may be more sharply defined or have an eccentric-shaped cavity with a thick, irregular wall (Fig 36.4).

A pleural effusion, visible on posterior-anterior and lateral upright chest radiographs, suggests the possibility of empyema. On a decubitus film, with the suspect side down, free pleural fluid can be visualized between the chest wall and the dependent lung. If the layer of pleural fluid is greater than 1cm thick, it should be aspirated for diagnostic studies. A decubitus film with the suspect side up is also useful because it permits assessment of any underlying parenchymal infiltrate, less obscured by the effusion.[8] [24]

Ultrasound and computerized tomography (CT) may be helpful in defining pleuropulmonary lesions. Ultrasound can define loculated collections of pleural fluid, and portable equipment is available for unstable or critically ill patients. Computerized tomography can define abscesses that are not apparent on plain radiographs and can distinguish between parenchymal and pleural disease (Fig. 36.5). Loculated empyema with a bronchopleural fistula may resemble a lung abscess. Features on CT that tend to favor lung abscess are the presence of thick walls and lesions that are round or oblong, whereas empyemas have thinner walls with a smooth luminal margin and exterior.[31] Both ultrasound and CT may be used to guide aspiration of fluid from abscesses or the pleural space.

Investigations

The first step in determining the specific etiology of any LRTI is the evaluation of lower respiratory tract secretions by Gram stain. These are most likely to be useful if they are obtained before the administration of effective antimicrobial treatment. If stains of expectorated specimens show neutrophils and alveolar macrophages, without

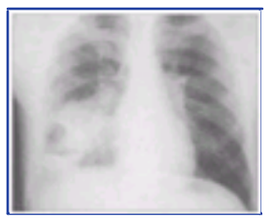

Figure 36-4 A lung abscess associated with a multinodular bronchogenic carcinoma.

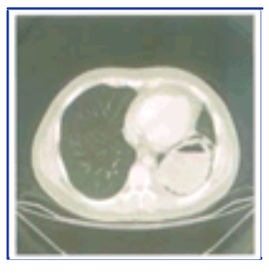

Figure 36-5 Computerized tomography scan of a lung abscess showing an air-fluid level.

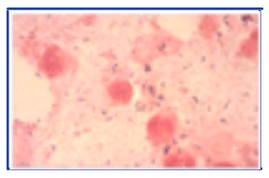

Figure 36-6 Gram stain of lower respiratory tract secretions. The patient had a lung abscess caused by oropharyngeal streptococci and anaerobes.

squamous epithelial cells (indicative of contamination with saliva), they are useful for defining the offending pathogen(s). In aerobic pneumonia there is usually a single predominant organism; in aspiration pneumonia there is usually a mixed flora, representing the diverse morphotypes of the oropharyngeal flora. Typically, there are various sizes of Gram-positive cocci and pleomorphic Gram-negative coccobacilli and bacilli, which may be tapered and are generally smaller and poorer-staining than the Enterobacteriaceae (Fig. 36.6). [1] [2] [11] [13] [32] Although some of the individual organisms may resemble pathogenic aerobes such as *S. pneumoniae*, there is no predominant pathogen.

Invasive procedures, such as endotracheal aspiration and bronchoscopy, may be useful to obtain lower respiratory secretions for evaluation. Tracheal aspiration through the nose or the mouth is of limited use because these specimens are often contaminated with oropharyngeal flora. Bronchoscopy with bronchoalveolar lavage is useful because large samples can be obtained with relatively little contamination. They can be concentrated by cytocentrifugation, permitting multiple microbiologic and cytologic evaluations. The bronchoscopic techniques of protected catheter aspiration and protected specimen brushing reduce contamination considerably but they provide relatively scanty specimens and rely on quantitative cultures to help distinguish the significance of cultures. In general, counts that indicate more than 10^5 cfu/ml of respiratory secretions for an appropriate organism (see Fig. 36.1 , Table 36.2) are indicative of infection.[1] [5] [15] [33]

Specimens of lower respiratory tract secretions that have passed through the mouth should be cultured for aerobes but not for anaerobes. Growth of recognized aerobic pathogens is helpful for interpreting Gram stains and for providing isolates for susceptibility testing. The absence of aerobic pathogens in specimens from untreated patients should indicate the possibility of anaerobic infection. When specimens are contaminated by saliva, the streptococci and anaerobes that comprise the normal oropharyngeal flora will always grow in culture, whether or not infection is present, and they provide no insight into the pathogenicity of the organisms isolated.

The problem of contamination of respiratory tract secretions by saliva can be avoided if specimens of lower respiratory secretions are obtained by transtracheal aspiration. A catheter is passed through the cricothyroid membrane and specimens are collected by suction.[1] [12] [14] Although infrequently used today, this technique established the role of anaerobes in suppurative pleuropulmonary infections in the early 1970s. Another method for obtaining uncontaminated material for culture is percutaneous transthoracic needle aspiration (percutaneous abscess drainage). Today, this procedure is more frequently performed under fluoroscopic or CT guidance for the diagnosis of malignancy than to obtain material for culture. It can be used, however, to aspirate peripheral abscesses, particularly if bronchoscopy does not provide an adequate specimen for microbiologic diagnosis.

If appropriate specimens from the lower respiratory tract are obtained for culturing anaerobes, they should be expeditiously transported to the laboratory, with minimal exposure to air, for proper processing. If tuberculosis or fungal infection is in the differential diagnosis, appropriate smears and cultures should be requested. If a malignancy is suspected, cytologic stains should be performed.

In addition to lower respiratory tract secretions, blood and pleural fluid, if present, should also be sent to the laboratory for microbiologic evaluation. In anaerobic lung infections, blood cultures are rarely positive. Pleural fluid, if present, requires analysis of protein, LDH and glucose, and determination of pH, as well as microbiologic evaluation. With empyema, the Gram stain usually indicates the pathogens.[8]

MANAGEMENT

Lung abscess

In the past, penicillin G was the preferred drug for treating aspiration pneumonia and lung abscesses, as well as for all anaerobic infections above the diaphragm caused by oropharyngeal flora. In a study of over 70 patients hospitalized with lung abscesses in the 1960s, nearly all responded to intravenous penicillin G. Oral penicillin V in a dose of 3g/day was also effective and those rare patients who failed to respond to penicillin could be satisfactorily treated with tetracycline.[6]

In the 1970s, when transtracheal aspiration was used to define the microbiology of pneumonia and lung abscesses, concern over the use of penicillin was raised because of occasional therapeutic failures and the isolation of penicillin-resistant *B. fragilis* from some patients.[1] [14] However, in one study that compared clindamycin, a drug that is active against *B. fragilis*, with penicillin G in the treatment of aspiration pneumonia and primary lung abscess, there was no difference in rates of defervescence, radiographic clearing or ultimate outcome. Notably, seven patients from whom *B. fragilis* was isolated responded to penicillin.[34]

Subsequently, in a prospective study published in 1983 of 39 patients who had lung abscess, there were 0/19 failures with clindamycin and 4/20 failures with penicillin G. Resolution of fever and putrid sputum was more rapid with clindamycin (4.4 days) than penicillin G (7.6 days). Patients who did not respond to penicillin G ultimately responded to clindamycin.[35] In a 1990 study of 37 patients who had lung abscess or necrotizing pneumonia,[36] only 1/19 patients failed to respond to clindamycin, whereas 8/18 failed with penicillin G. In this study, patients underwent transtracheal aspiration or protected specimen brushing to culture for anaerobes; 9/10 of the penicillin-G-resistant strains were ß-lactamase producers. It is now recognized that there has been a change in the susceptibilities of the oropharyngeal Gram-negative anaerobes since the 1970s. Many, in addition to *B. fragilis*, are now ß-lactamase producers and resistant to penicillin. In a study of 449 isolates, which included *Bacteroides* spp. other than *B. fragilis*, *Fusobacterium* spp., *Prevotella* spp. and *Porphyromonas* spp. from 28 US medical centers, 57.9% of isolates were ß-lactamase producers.[37]

Today, transtracheal aspiration and other invasive procedures are rarely performed to determine the microbiologic etiology of aspiration pneumonia and lung abscesses in non-immunocompromised patients. Treatment is usually empiric and largely effective.[6] Clinical outcome

for most anaerobic infections seems to correlate with *in-vitro* data as broadly applied, and detailed study of individual cases does not seem to be necessary. Monitoring trends in susceptibility patterns and detailed study in problematic individual cases suffices.[30]

The in-vitro spectrum and clinical utility of antimicrobials for the treatment of lower respiratory tract bacterial infections is summarized in Figure 36.7 . For community-acquired infections that result from aspiration, and where oropharyngeal streptococci and anaerobes are the likely pathogens, penicillin G (or ampicillin or amoxicillin) remains an excellent foundation for treatment, but the addition of a ß-lactamase inhibitor or metronidazole is advisable owing to the frequency of ß-lactamase production among Gram-negative anaerobes.[30] [37] [38] Clindamycin is also a primary therapeutic agent, despite in-vitro resistance among some

Bacteroides and *Fusobacterium* spp. Resistance rates vary significantly in different geographic regions, so surveillance of resistance is important in assessing the utility in a given area.[4] [6] [32] [38] [39] Metronidazole alone is

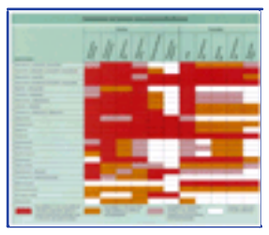

Figure 36-7 Antimicrobials for bacterial lung abscesses and empyemas. Information for *Streptococcus pneumoniae* is for penicillin-susceptible strains — selected cephalosporins or vancomycin should be used for resistant strains. Information for *Staphylococcus aureus* is for methicillin-susceptible strains — vancomycin should be used for resistant strains. Vancomycin is the drug of choice for ß-lactam-resistant Gram-positive organisms.

not effective because of its inactivity against the aerobic and microaerophilic streptococci. [6] [38] [40]

For hospital-acquired infections, where *S. aureus* and aerobic Gram-negative bacilli are common components of the oropharyngeal flora, piperacillin or ticarcillin (rather than penicillin or ampicillin) with a ß-lactamase inhibitor provide better coverage of likely pathogens. Appropriate alternatives are imipenem alone, clindamycin plus an aminoglycoside or ciprofloxacin, or an expanded spectrum cephalosporin such as cefotaxime, ceftizoxime, ceftriaxone or cefoperazone plus metronidazole. Many cephalosporins, for example ceftazidime, have little or no activity against anaerobes and should not be used unless the etiology has been defined and found to involve aerobic Gram-negative bacilli.[6] [10] [40]

Tetracyclines, which were used for lung abscess and empyema in the 1960s, are no longer recommended for the treatment of aerobic/anaerobic pleuropulmonary infections because of high rates of resistance. Chloramphenicol has an excellent spectrum of activity but it is

rarely recommended because of potential hematotoxicity and the availability of alternative agents.[6] [40] The macrolides and azalides have inconsistent in-vitro activity against oropharyngeal anaerobes and there is little clinical precedent for their use.[41] Aminoglycosides and quinolones should be used only for their activity against aerobic Gram-negative bacilli and need to be combined with a drug that is active against streptococci and anaerobes. A newer quinolone, such as moxifloxacin, has greater activity than ciprofloxacin or ofloxacin against oropharyngeal streptococci and anaerobes, and might prove to be more useful.[42] [43] Other agents that have suboptimal or no activity against oropharyngeal streptococci and anaerobes include aztreonam and trimethoprim-sulfamethoxazole. The antistaphylococcal penicillins and vancomycin should be reserved for staphylococcal infections.

The duration of antimicrobial therapy necessary to treat pleuropulmonary infections is variable: 1–2 weeks may suffice for simple aspiration pneumonia but necrotizing pneumonia and lung abscesses may require 3–24 weeks. Parenteral therapy is generally employed until the patient is afebrile (most are afebrile in 7 days) and able to have a consistent enteral intake.[30] For most community-acquired infections, amoxicillin-clavulanate and clindamycin are excellent oral drugs that can be used for continued treatment after initial parenteral therapy. A less costly oral alternative is penicillin V plus metronidazole. If aerobic Gram-negative bacilli are present, oral treatment is more problematic and must be based on the results of susceptibility tests; ciprofloxacin or ofloxacin plus a penicillin or clindamycin may be appropriate. Prolonged therapy is advisable, with treatment continued until the cavity is gone or until serial radiographs show considerable improvement or a small stable residual scar (Fig. 36.8). The time to cavity closure depends largely on the size of the cavity when treatment is initiated and the condition of the patient.

Surgical drainage of lung abscesses is rarely indicated because drainage occurs naturally via the tracheobronchial tree. If spontaneous drainage is not adequate, even with the aid of postural drainage and percussion, clinical improvement is impeded; CT-guided percutaneous abscess drainage may then be beneficial. The drainage catheter can be left in place until there is clinical improvement and drainage has diminished, usually within several days to 1 week. This

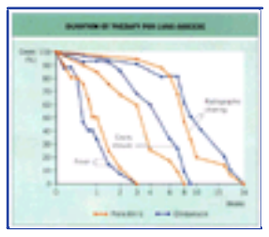

Figure 36-8 Duration of therapy for lung abscess. Response of patients who had lung abscess to penicillin G (17 patients) and clindamycin (16 patients).

technique may also be used to reduce the risk of endobronchial spread of infection to other areas of the lungs as well as in patients who are too ill to undergo lung resection to remove necrotic tissue, persistent cavities, or nonfunctional lung.

Empyema

The antimicrobial treatment of empyema is similar to that of aspiration pneumonia and lung abscess but single-organism infections with a defined etiology are more common, thus facilitating antibiotic choice. Antibiotics should be administered in full doses for 2–4 weeks. Therapy may need to be prolonged further, particularly if drainage is not optimal. Antibiotic levels in pleural fluid are comparable to those in serum, and so standard systemic doses provide adequate pleural fluid levels. [24]

The proper assessment and management of parapneumonic effusions associated with LRTIs is critical for a successful outcome. Most small effusions clear with antimicrobial treatment and need not be drained. However, if fluid persists more than a few days or layers to more than 1cm on a decubitus radiograph with the involved side dependent, it should be aspirated and analyzed. The characteristics of the fluid are used to determine the need for tube drainage (see Table 36.4).

Effusions are termed 'complicated' when characteristics indicate a potential need for tube drainage. Complicated effusions are termed 'empyemas' if they are frankly purulent and bacteria are present; tube drainage is mandatory. Chest tubes are placed using negative pressure until the lung is expanded, and then to an underwater drainage system until the fluid is scanty and clear, and then gradually withdrawn over a period of several days. Lack of prompt clinical improvement may indicate the need to reposition the tube to facilitate and continue drainage.

Chest tube drainage of complicated parapneumonic effusions is successful in most patients. It should not be delayed, because these effusions can progress from free-flowing to loculated fluid rapidly. When the fluid becomes loculated, drainage by repeated thoracentesis or tube insertion may not be adequate. The presence of loculated fluid should be suspected if the patient remains ill or febrile or has a persistent leukocytosis. After appropriate evaluation by ultrasound or CT, options for management include image-guided percutaneous drainage by an interventional radiologist [44] or instillation of thrombolytic agents (urokinase or streptokinase) through a catheter or chest tube.[45] If administered before fibrosis occurs, these agents attack the fibrin membranes causing the loculations. Successful therapy leads to an increase in the amount of drainage from the pleural space and can be administered for up to 2 weeks. Thoracoscopy to mechanically lyze adhesions and inspect the pleural cavity has also been used to assist in management.[8]

If patients do not respond to the above measures, contrast material can be injected into the chest tube to evaluate the empyema cavity. Open drainage, evacuation of all infected material and decortication of the pleura should be considered. This is a major surgical procedure that requires rib resection and may not be tolerated by debilitated patients. Open drainage, without decortication, may be better tolerated in these patients, but it is followed by a prolonged period of convalescence with an open chest wound.[8]

A serious complication of empyema is a bronchopleural fistula. The presence of a peripheral air-fluid level radiographically suggests the presence of a bronchopulmonary fistula, although such an air-fluid level may occasionally be due to the presence of gas-forming bacteria. Adequate tube drainage is mandatory to minimize spread of infection to other portions of the lungs. Empyema with a bronchopleural fistula after pneumonectomy is a disastrous surgical complication. The fistula often does not close with antibiotics, tube drainage and irrigation, and complex surgical procedures are usually necessary.[8]

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Chapter 37 - Tuberculosis

Jon S Friedland

INTRODUCTION

Mankind has been plagued throughout history by tuberculosis (TB). It has been identified in skeletons over 6000 years old and remains the most prevalent infectious disease in the world. Among a long list of notable patients are Samuel Johnson, Jean-Jacques Rousseau, John Keats and Fyodor Dostoyevsky. This chapter focuses on current understanding of the pathophysiology, epidemiology and clinical aspects of tuberculosis.

EPIDEMIOLOGY

Worldwide incidence and prevalence

Mycobacterium tuberculosis is estimated to infect 1.6 billion people worlwide or approximately one-third of the world's population.[1] Usually infection is contained by the immune system so that about 15 million people have clinical disease at any one time. Tuberculosis kills around 2–3 million people each year, which is more than any other single bacterial infectious disease. Recent data on TB incidence rates are shown in Figure 37.1 . In most countries, there is a statutory requirement to notify cases of TB, but collection of data is notoriously incomplete, with under-reporting of cases.[2] Confounding factors in the global collection of incidence and prevalence data include effects of treatment, difficulties in identifying extra-pulmonary disease and those associated with tuberculin testing (see 'Testing for exposure' later in chapter). However, the size of the TB problem is such that it has been uniquely identified as a 'global emergency' by the World Health Organization (WHO).

Over 96% of TB-related deaths occur in the poorer nations of the world and the disease has huge social and economic costs. In wealthier nations, the rates of TB have been falling over the past 80 years, partly as a result of the development of effective treatments, active case finding and use of the bacille Calmette-Guérin (BCG) vaccine. Recently, this trend has been halted in some countries because of the increased incidence of TB in high-risk population groups, including poorer communities, immigrants and patients who have HIV infection. Levels of disease in homeless populations in developed countries can be as high as 2%.[3]

Impact of HIV and immunosuppression

Approximately 8 million people are coinfected with HIV and TB, the majority of whom live in sub-Saharan Africa, the Indian subcontinent and South East Asia (Fig. 37.2). HIV-positive patients appear more susceptible to infection by *M. tuberculosis*. Subsequently, approximately 50% of people who have dual infection will develop clinical TB, and reactivation rates may be over 20 times greater than in similarly aged controls.[5] Clinical TB is associated with shorter survival in people who have AIDS.[6]

In many developed countries (e.g. USA, France, Germany and The Netherlands) HIV infection has led to well-documented increases in localized and disseminated TB. In the USA and elsewhere, people who have TB are offered screening for HIV. Miliary TB accounted for less than 1.5% of cases of TB in the USA for 20 years, but recently, in some areas, disseminated disease has accounted for up to 10% of cases.[7] In contrast, in the UK and some Scandinavian countries, the increased incidence of TB has been associated with economic deprivation rather than HIV infection.[8]

Diagnosis of dual infection can be difficult because HIV infection predisposes to atypical, nodal and extrapulmonary TB. In addition to HIV infection increasing the incidence of clinical TB, infection with *M. tuberculosis* appears to increase viral replication and HIV disease progression.[9] This is in part because tuberculosis activates nuclear factor kappa B (NF-?B)-dependent transcription of the HIV genome (see Chapter 120).[10] [11] In more affluent countries and in settings in which TB is unexpected, malignancies, chemotherapy and other systemic illnesses characterized by immunosuppression can lead to reactivation of TB. For example, the incidence of TB is very high in renal dialysis patients.[12] More recently, the use of specific antibodies to neutralize tumor necrosis factor (TNF) activity in rheumatoid arthritis has been associated with TB.[13]

Multidrug-resistant tuberculosis

Drug-resistant TB emerged rapidly. Isolated resistance to streptomycin therapy was noted in the 1940s almost immediately after the drug was introduced. The emergence of multidrug-resistant TB (MDR-TB), defined as resistance to a minimum of isoniazid plus rifampin (rifampicin), is a more recent phenomenon. It was noted in HIV-positive patients in the first part of the last decade in New York[14] and has since been widely reported. Resistance to second-line drugs has also emerged. [15] Multidrug resistance is caused by the sequential acquisition of single-drug resistance traits.[16] This has been a consequence of poor prescribing practice by doctors, patient non-compliance with treatment and the endogenous mutation rate of *M. tuberculosis*. The WHO has estimated the incidence of MDR-TB worldwide and this is shown in Figure 37.3 .

Spread of infection

Spread of infection is dependent on inhalation of aerosols from individuals who have pulmonary infection. Proximity to and duration of association with an index case are critical factors. There have been widely reported instances of spread of TB to airplane passengers from index cases traveling on the same flight. These cause considerable alarm, but the risk to fellow travellers is small and the WHO has issued guidelines for domestic and international flights, which are available on their web site.

Up to one-quarter of household contacts of an index case may acquire infection, although the extent to which individual genetic predisposition or immunologic impairment contributes to this is uncertain. The role of factors such as vitamin D deficiency and iron overload in the spread of TB are unknown. Spread of infection is quite separate to the development of disease, which occurs in less than 10% of infected persons and is significantly increased by impaired cell-mediated immunity. Congenital transmission of TB is not a significant factor in the natural spread of disease.

Figure 37-1 Estimated tuberculosis incidence rates worlwide. *Reproduced with permission from the World Health Organization.*

Figure 37-2 Estimated incidence rates of adults co-infected with TB and HIV. *Reproduced with permission from the World Health Organization.*[4]

Figure 37-3 Estimate of the incidence of multidrug-resistant TB (MDR-TB) in newly diagnosed cases of infection. *Reproduced with permission from the World Health Organization.*[17]

Transmission in closed institutions

Overcrowding contributes to the spread of TB among the poor. Close proximity to infected individuals is a significant issue in any closed institution and for health care workers. Many countries have specific guidelines for tuberculosis control in institutions (see 'Prevention'). Genetic techniques using restriction fragment length polymorphism (RFLP) analysis — often of the insertion sequence IS6110 — to provide DNA fingerprints has proved useful in documenting local outbreaks of TB, including those involving MDR organisms.[18]

In prisons, the situation is complicated by the fact that there is often an increased prevalence of HIV infection among inmates and inmates are regularly moved to other prisons or back into the community with little warning and are frequently poorly managed in terms of health services. Because prisoners are often released into poor circumstances and crowded hostels, the consequence of undetected or inadequately treated TB may be rapid spread of disease. An effective public health program with an active community care component can overcome such problems.

PATHOGENESIS AND PATHOLOGY

The pathogen

Mycobacterium tuberculosis, a restricted human pathogen, is a member of the order Actinomycetales, which is characterized by a complex cell wall rich in mycolic acids together with peptidoglycan and arabinogalactan, a complex polysaccharide molecule that surrounds the cell membrane. Many cell wall components are of pathogenic significance. Lipoarabinomannan (LAM) stimulates monocyte proinflammatory activity, principally by binding to the CD14 receptor, which is also the binding site for bacterial lipopolysaccharide. *Mycobacterium tuberculosis* was one of the first pathogens for which a complete genome sequence was obtained, which opens up the potential for novel diagnostic and therapeutic avenues.[19] The genome has a high G + C content (mean approximately 65%) and contains genes for aerobic, microaerophilic and anaerobic growth. Many genes are involved in lipid metabolism pathways. Details of other metabolic pathways within *M. tuberculosis* and of assimilation of nutrients such as iron, which is stored in association with mycobactins, are reviewed elsewhere.[20] Mycobacteria contain many proteins associated with growth, virulence and intracellular survival.[21] Among the most critical are the 10, 65, 70 and 90kDa families of heat shock proteins (hsps), the production of which is upregulated by environmental stress. Heat shock proteins are molecular chaperones involved in protein folding and assembly. Exactly how hsps confer survival advantage on *M. tuberculosis* is unknown. In addition, proteases that are involved in local tissue destruction have been isolated from mycobacteria.

Host immune factors

Monocytes, macrophages and phagocytosis

The principal tissue immune response in TB is the formation of granulomas comprising cells of the monocyte lineage, including multinucleate giant cells and T cells (Fig. 37.4). In the initial stages of the immune response, neutrophils are present, whereas more advanced disease is characterized by caseous necrosis and eventually deposition of calcium. After inhalation, *M. tuberculosis* is phagocytosed by the alveolar macrophage; similar fixed tissue cells are present elsewhere in the body. Pulmonary surfactant protein may enhance the process of phagocytosis. The phagocytosing macrophage initiates the host immune response (Fig. 37.5). Phagocytosis involves the complement receptors CR1, CR3 and CR4 as well as mannose receptors and

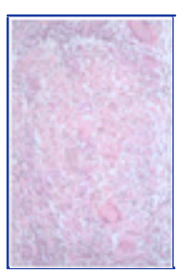

Figure 37-4 Detailed histology of the tuberculous granuloma. Monocytic cells and smaller T cells are shown together with multinucleate giant cells on the edge of an area of caseation.

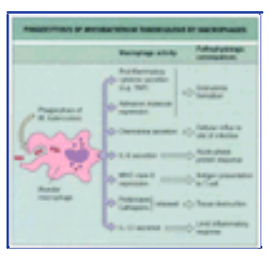

Figure 37-5 Phagocytosis of *Mycobacterium tuberculosis* by macrophages. Phagocytosis initiates many critical pathways involved in host defense to infection.

adhesion molecules.[22] Intracellular mycobacteria partly inhibit phagolysosome fusion, which prevents acidification of the vacuole. However, some mycobacterial components are detectable in the cytoplasm.

Phagocytosis is a potent stimulus to gene expression and secretion of proinflammatory cytokines such as TNF, interleukin (IL)-1 and IL-6. Phagocytosis of *M. tuberculosis* activates the transcription factors NF-IL-6 and NF-?B, both of which are important in proinflammatory cytokine gene activation.[23] The known consequences of TNF secretion include fever and cachexia, two prominent symptoms in TB. Tumor necrosis factor also has a pivotal role in granuloma formation and is found at sites of human infection. Injection of anti-TNF antibodies in a murine model decreased granuloma formation and increased replication of *Mycobacterium bovis*, BCG strain.[24] At early stages of infection, cellular recruitment to the granuloma is essential, and macrophage-derived chemokines are important in this process.[25] [26] The granuloma in TB is well circumscribed and it is likely that downregulatory cytokines such as IL-10 are involved in limiting inflammatory responses. Interestingly, when monocytes die by apoptosis there may be associated killing of intracellular mycobacteria, although how crucial this is in humans is unknown.[27] Surprisingly little is known about what exactly kills *M. tuberculosis* in humans.

Infected macrophages are probably involved in the tissue damage characteristic of TB although the exact cause of typical caseous necrosis remains unknown. Macrophages secrete proteases such as collagenase and gelatinase, the actions of which are opposed by tissue inhibitors of metalloproteinases (TIMPs). TIMP genes are closely regulated, with the transcription factor activator protein-1 having a central role, and their expression is increased by macrophage-derived cytokines TNF and IL-1ß. Other monocyte-derived proteins involved in tissue destruction include lysosomal proteases such as cathepsins, which function best at acid pH, and the plasminogen activator urokinase. Activation of the plasminogen system is followed by activation of the clotting system and laying down of fibrous tissue, a process in which macrophage-derived transforming growth factor ß has a central role.[28]

T cells

In immunity to TB aß and ?d $CD4^+$ T cells are critical.[29] Patients who have reduced T cell function or numbers are at higher risk of clinical TB. *Mycobacterium tuberculosis* may impair macrophage antigen presentation to T cells by downregulating the co-stimulatory molecule B7-1. Cells of the T-helper (T_H)1 subclass, which secrete interferon-? and IL-2, are central to the control of infection in murine models. In human disease the situation is more complex with T_H 1, T_H 2 (which produce

IL-4, -5, -6, -10 and -13) and intermediate cell types detectable,[30] although T_H 1 cells appear to concentrate at sites of infection such as pleura.[31] The dual presence of T_H 1 and T_H 2 cells may account for the fact that, although TB is limited within the granuloma, the organism is not usually completely killed off by the immune response.

The involvement of ?d T cells in immunity to human TB remains controversial, although they appear critical in murine models of infection. The percentage of ?d cells at sites of TB in the few studies performed in patients have been conflicting but their presence has been correlated with effective immune responses. These cells have the potential to recognize important, conserved mycobacterial antigens such as the 65kDa hsp.[32] ?d T cells may be involved in macrophage aggregation and activation, in lysis of infected cells and in cytokine secretion.[30] [33]

Other T-cell phenotypes involved in immunity to TB include $CD8^+$ lymphocytes,[34] although their exact role in humans is still to be defined. In addition, there are double-negative T cells lacking CD4 and CD8, which recognize mycobacterial lipoglycan antigens presented via CD1, proteins that have distant homology to the major histocompatibility complex.[35]

Humoral response

Many proteins, carbohydrates and lipids of *M. tuberculosis* contain epitopes that stimulate antibody production. Such antibodies have been used to characterize important antigens of *M. tuberculosis*, such as the 12, 65 and 72kDa hsps. However, in terms of pathogenesis, it has proved difficult to identify specific antibody responses of importance in host defense in the sera of patients who have TB.

Interest in antibody responses in TB has centered upon the development of diagnostic tests.

Local tissue responses

Granuloma formation is the consequence of a complex interaction between the organism and the immune system and the local release of tissue factors and proteases. Gross pathology reflects the relative influence of these factors in a particular patient. This process may be modulated by systemic or local production of hormones such as 1,25-dihydroxyvitamin D_3 .[36] Caseous necrosis, typical of mycobacterial granulomas, is probably caused by the delayed-type hypersensitivity response, although it is not known why it is so very rare in nontuberculous granulomas.

Host genetic factors

There is undoubtedly a genetic component to host resistance to *M. tuberculosis*. Identical twins have been shown to be concordant for TB and it has been suggested in the USA that black patients are more susceptible to infection.[37] Human leukocyte antigen (HLA) haplotypes associated with susceptibility to TB include A8, A10, B8, Bw15 and DR2. It is likely that many genetic polymorphisms influence susceptibility to TB to varying degrees. Much interest has focused on non-HLA-linked genes such as the *bcg* gene, located on human chromosome 2. Its product, the protein *Nramp* (natural resistance associated membrane protein), is a major determinant of disease susceptibility in murine systems[38] and is involved in human susceptibility to infection. Certain families that are susceptible to atypical mycobacterial infections have deletions in the interferon-? receptor gene.[39] However, it is likely that no single gene has a dominant effect in terms of population susceptibility to TB and this was confirmed in a genome-wide search in an African population.[40]

PREVENTION

Public health measures

Strategies to prevent the spread of TB aim:

- first, to identify and promptly treat infectious patients; and
- second, to prevent respiratory spread of infection.

Many countries have a system, often legally enforced, of infectious patient notification to a central body that traces infected contacts of index cases. In addition, high-risk patients or communities, such as intravenous drug users, may be screened so that definitive or prophylactic therapy may be instituted. In its simplest form, case finding involves clinical assessment and examination of sputum smears, although radiologic examination may be a useful adjunct. Skin testing may be appropriate in countries with low rates of infection and no routine immunization of the population. It is important to distinguish between disease relapse and reinfection, and RFLP analysis has proved useful.[41] Prevention programs may require incentives for successful implementation; they should be linked to educational initiatives and involve social services.

Infectious patients should be isolated until effective treatment has been instituted. In the USA, laminar airflow and negative pressure ventilation rooms are provided for patients who have known or suspected TB, particularly those who have MDR disease. Detailed guidelines have been produced in the USA from several sources which are useful,[42] but are potentially extremely costly to implement.[43] For example, expensive respirator masks have been advocated but are likely to represent a poor use of funds, particularly in developing countries. Shortwave ultraviolet illuminators to kill organisms in clinics and shelters have been used, although their efficacy has yet to be tested in comparative trials with other control methods. In many parts of the globe, basic patient isolation and possibly specified, ventilated rooms for procedures that generate aerosols is all that is feasible. Appropriate control measures should be defined in advance in high-risk procedure rooms, during patient transport and in all at-risk institutions.

Testing for exposure

Mantoux test

The Mantoux test is the commonest test used to screen for TB exposure and depends on the intradermal injection of a specified quantity of an internationally standardized purified protein derivative (PPD) of tuberculin. PPD solution should not be left in syringes because it may be variably adsorbed to their surface. Tuberculin positivity manifests as induration at the site of testing after 48 hours. Induration less than 5mm diameter after a standard injection of 10 units PPD is regarded as negative and greater than 15mm diameter as positive. However, PPD may have a booster effect on immunologic memory and a second Mantoux test should be performed a week after a negative test to confirm the finding.

Induration in the 5–15mm diameter range may be indicative of exposure to mycobacteria, previous immunization or disease in the immunosuppressed patient, all of which have unpredictable confounding effects on Mantoux testing. In the USA, induration greater than 5mm is taken as positive in patients who have HIV infection, a close contact with TB or a fibrotic chest radiograph, and induration above 10mm diameter is positive in any other high-risk group. Patients who are immunosuppressed, such as those who have HIV infection (particularly if $CD4^+$ T-lymphocyte count is <400 cells/mm^3) or who have measles or sarcoidosis, have a strong tendency to anergy. Systemic illness, including miliary TB, is also associated with anergy. The Centers for Disease Control and Prevention, Atlanta, USA, has recommended concurrent testing for generalized anergy, but the relationship between general anergy and tuberculin negativity is unclear. False-negative tests may also occur at the extremes of age in patients on high-dose corticosteroids, after the use of inadequately stored tuberculin or as a result of poor injection technique. Skin testing is not a useful exercise in low-risk children. PPD may restimulate previous hypersensitivity producing the booster Mantoux effect whereby a second Mantoux test 8 weeks after a negative test becomes positive. To avoid confusion negative Mantoux tests should be repeated within 2 weeks.

Heaf test

This Heaf test involves placing PPD on the skin of a subject and then using a multiple puncture gun to pierce the skin in six places; the response is then graded (Fig. 37.6 , Table 37.1). Heaf guns must be resterilized between patients; a disposable head is preferred.

Tine test

The tine test is similar to the Heaf test except that the puncture needles (usually four not six) are precoated with tuberculin. There has been considerable controversy

about the reliability of this technique and it is being used increasingly infrequently.

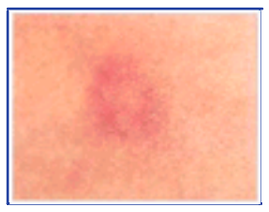

Figure 37-6 A Heaf test grade 2 response. *With permission from James DG, Studdy PR, A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

TABLE 37-1 -- The Heaf test grades.

THE HEAF TEST GRADES	
Heaf test grade	**Response**
0	No induration at puncture sites
1	Discrete induration at a minimum of four needle sites
2	Induration at needle sites merge to form ring but leave clear center
3	One large induration site seen (5–10mm diameter)
4	Induration over 10mm diameter

New approaches

In part as a result of increased understanding of the TB genome, immune responses to novel antigens are being investigated as potential ways to detect early infection. Enumeration of antigen-specific T cells specific for the antigen ESAT-6 which is specific to the *M. tuberculosis* group of organisms, has been proposed as a means of contact tracing and spatial tracking of TB, but to date studies are small.[44]

Chemoprophylaxis

Chemoprophylaxis may be primary in unexposed individuals or secondary in those exposed to *M. tuberculosis* who do not have clinical disease. Secondary prophylaxis is practiced most commonly and successfully in TB control programs in the USA. This approach, which is based on regular skin prick testing, is not possible when vaccination is common or resources are inadequate. Isoniazid, 300mg daily for 6–12 months, is the usual preventative regimen and, as in treatment schedules, is associated with a significant incidence of (principally hepatic) toxicity, which is particularly important because the subjects are well. In addition, with the increase in drug resistance, it is likely that significant numbers of organisms will not respond to isoniazid. A variety of regimens are used for prophylaxis of drug resistant infection and some authorities recommend full treatment courses for such patients. Chemoprophylaxis has been advocated for all HIV-positive patients because of the increased incidence of clinical disease in patients exposed to *M. tuberculosis* (see Chapter 129). However, if the diagnostic facilities are poor, there is a significant chance of inadvertently treating established TB, which would hasten the emergence of isoniazid resistance. The issues, which include economic factors, are complex and the validity of prophylaxis for HIV infection is vigorously debated.[45] In contrast, the use of secondary chemoprophylaxis in potential transplant recipients is established. Chemoprophylaxis should be deferred in pregnant women, a group who may be more prone to isoniazid hepatitis.

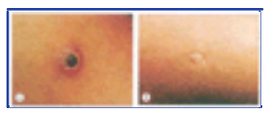

Figure 37-7 BCG response at 6 weeks. (a) Clinical evidence of a cell-mediated immune response is clearly apparent at 6 weeks. (b) The healed BCG scar. *With permission from James DG, Studdy PR, A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

Vaccines

Bacille Calmette-Guérin

The BCG vaccine, developed by Albert Calmette and Camille Guérin, was first used in 1921. Since then many strains have been used clinically, although the current reference type is the Pasteur strain. Vaccination leads to a local immune response and ultimately scar formation (Fig. 37.7). Keloid may form in susceptible individuals. Adverse reactions other than local irritation are uncommon and anaphylaxis is extremely rare. Local abscesses or ulcers usually reflect poor technique. Adenitis, sometimes suppurative, occurs at a rate of 25 cases per 1,000,000 vaccinations. Lupoid reactions, infected osteitis and disseminated BCG disease are very rare, the latter two necessitating therapy with rifampin and isoniazid (BCG is resistant to pyrazinamide).

The extent of immunity after BCG vaccination depends on the strain used, environmental factors, the genetics of the population being vaccinated and individual host factors such as age. There is a poor correlation between tuberculin reactivity after vaccination, which develops to maximal levels within 3 months, and protection against disease. Reviews indicate that BCG vaccine efficacy is about 60–80%,[46] although in certain parts of the world, such as Malawi, even repeated vaccination offered no protection against TB; it did, however, decrease *Mycobacterium leprae* infection rates.[47] Giving BCG to infants provides significant protection from TB in household contacts.

Novel vaccines

There are several novel vaccine approaches:

- the first of these involved using an environmental commensal *M. vaccae* to enhance protective immune responses, but it proved unsuccessful; [48]
- the second approach involved naked DNA vaccines encoding for immunogenic mycobacterial antigens — this has been successfully used in an animal model in which injection of the DNA coding for the mycobacterial 65kDa hsp was protective against challenge with virulent *M. tuberculosis*; [49]
- third, there is research interest in subunit, recombinant or genetically engineered vaccines in which either BCG serves as a vector for specific antigens of *M. tuberculosis*, or an attenuated *M. tuberculosis* is created by deletion of specific virulence factors, or antigens are administered, possibly with an adjuvant.

CLINICAL FEATURES

In this section, the diverse clinical presentations of primary, pulmonary and miliary TB are reviewed together with the characteristic changes found on radiologic examination. The principal extrapulmonary manifestations of TB are also covered.

Primary and childhood infection

Primary TB is acquired by inhalation of infected particles, usually in childhood, although in affluent countries the first encounter with TB may be as an adult. A single bacillus can cause disease but usually 50–200 organisms are required to develop active infection. Inhaled bacilli pass into the lung where damage is often but not always confined to one segment with concurrent involvement of draining, frequently hilar, lymph nodes. This gives rise to the primary Ghon complex. Clinical disease develops in less than 5% of people exposed to *M. tuberculosis*, although infection is established in about 30% of cases. After initial infection, the only sequelae may be scar tissue, which is often calcified and later may be identified on routine chest radiography. After the first year of infection, there is a 3–5% lifetime chance of

reactivation of the disease in individuals who do not have HIV infection. In addition, a patient exposed to TB may be infected by a second strain of *M. tuberculosis* later in life and this may account for up to one-third of adult clinical presentations.

Symptomatic patients present with cough with variable amounts of sputum and hemoptysis, together with localized pleuritic chest pain and dyspnea. In addition, systemic features such as fever, night sweats, anorexia and weight loss occur. A minority of patients have retrosternal pain, sometimes exacerbated by swallowing and increased by sternal pressure that is thought to relate to lymphadenopathy. Primary TB is seen as asymmetric hilar adenopathy, which may be associated with consolidation on chest radiography. In children, who like the very elderly are more susceptible to TB, isolated lymphadenopathy is more common. Less typical chest radiographs of primary infection include those that appear normal or have widespread disease, lobar consolidation and pleural effusions. An unusual complication of primary TB is bronchial obstruction caused by pressure of a node on a main bronchus. This phenomenon, sometimes called epituberculosis, may lead to secondary bronchiectasis. Untreated primary disease will progress to involve the entire lung and will also disseminate. Symptoms are present at this stage and may be severe, with continuous cough and sputum production, severe dyspnea, high fevers, drenching sweats and cachexia. Chest radiography reveals widespread patchy consolidation, with areas of collapse and cavitation.

Endobronchial TB is usually a complication of primary infection, although it may occur during reactivation. It may follow adhesion of inflamed lung parenchyma or lymph nodes to bronchi or may arise via lymphatic or hematogenous spread of infection and even from direct seeding of inhaled bacilli. Endobronchial TB probably frequently goes undiagnosed — it was found in over 400 of 1000 consecutive autopsies on patients who had TB.[50] The classic clinical presentation is with a barking cough and wheeze. Sputum production may be exacerbated when the mucosa is breached and caseous material extruded. Parasternal pain, dyspnea, symptoms caused by collapse and consolidation of distal lung tissue and systemic manifestations of TB may be found. The most important late complication of endobronchial infection is bronchiectasis.

Pulmonary infection

Risk factors for reactivation

Most cases of TB are caused by reactivation of infection acquired years earlier. The stimulus to reactivation may be frank immunosuppression or may be more subtle when factors such as malnutrition and vitamin D deficiency are involved. The role of corticosteroids in reactivation of TB has not been fully defined. High doses may cause reactivation, which often presents atypically or is detected belatedly because the symptoms of infection may mimic symptoms of the disease for which corticosteroids were being prescribed. However, TB does not appear more common in asthmatics maintained on daily low-dose prednisolone. Other conditions associated with reactivation of TB are end-stage renal disease, diabetes mellitus, silicosis, gastrectomy and transplantation; in the latter, disease usually results from immunosuppression, although occasionally infection is transmitted by the implanted organ. Although TB and lung malignancy may coexist and the symptoms of TB may mimic those of cancer, TB is not a risk factor for lung cancer. However, lung malignancy is a systemic risk factor for reactivation of TB and may invade and disrupt old tuberculous lesions leading to active infection. Tuberculosis has frequently been associated with sarcoidosis. Some workers have detected mycobacterial DNA in patients who have sarcoid but others have not, and whether there is any link between these diseases is unresolved.

Clinical presentation

In one prospective study, symptoms of reactivation of pulmonary TB were cough in 78% patients, weight loss in 74%, fatigue in 68%, fever in 60% and night sweats in 55%.[51] Some patients have only mild feelings of nonspecific malaise. Hemoptysis occurs in about one-third of patients. It may be massive from either enlarged bronchial arteries around tuberculous cavities (Rasmussen's aneurysms) or more frequently from erosions involving other bronchial or pulmonary arteries. Dyspnea suggests extensive disease and is a late symptom. Pleuritic chest pain indicates inflammation in and possibly infection of adjacent pleura. Clinical examination may be misleading in the early stages of reactivation and radiologically apparent changes of consolidation and cavitation hard to detect. Noninfectious complications of TB may be present (see below). It is important to recognize that patients can present with advanced disease in acute respiratory failure progressing rapidly to the acute respiratory distress syndrome (ARDS).

Chest radiography shows disease localized to the apical and posterior segments of the upper lobes of the lung in over 85% of patients, with other sites often secondarily affected. The apical segment of the lower lobe is also frequently involved. Infiltration and cavitation secondary to caseous necrosis may be associated with air-fluid levels. On treatment most cavities heal completely leaving residual scarring, which is often calcific. Tuberculous chest radiographs frequently show fibrotic changes with lobar atelectasis, elevation of hilar nodes and deviation of the trachea. A wide range of less common findings are described, for example pneumonic consolidation (Fig. 37.8). It is notoriously difficult to distinguish early reactivation from chronic healed lesions without follow-up radiographs. Computed tomography (CT) findings include cavitation with scarring as well as characteristic nodules and branching linear structures sometimes referred to as a 'tree-in-bud pattern'.

Other investigations in pulmonary TB may reveal leukocytosis or more specifically a monocytosis. It is common for the leukocyte count to be normal and rare for it to be decreased. Anemia, generally normochromic and normocytic, is typical. An acute phase response is almost invariably present with elevated C-reactive protein (CRP) concentrations in the plasma, raised erythrocyte sedimentation rate (ESR) and, in more chronic cases, decreased serum albumin. Hyponatremia occurs in about 10% of patients as a result of antidiuretic hormone-like activity. A much smaller percentage of patients have hypercalcemia, probably caused by abnormal vitamin D processing by macrophages within granulomas.[52]

Complications

The principal acute complications of pulmonary TB are hemoptysis, as discussed above, and pneumothorax. Bronchopleural fistulas may heal spontaneously, but more often require tube drainage and sometimes surgery. The prognosis is good because of the formation of scar tissue in TB. Chronic complications of lung TB relate to parenchymal damage and scarring. Aspergillomas may develop within healed cavitating

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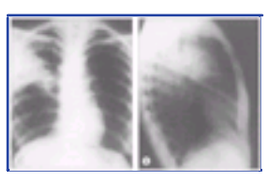

Figure 37-8 Chest radiograph of pulmonary tuberculous pneumonia. (a) Posteroanterior and (b) lateral chest radiographs of a patient with TB presenting as a consolidation. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

lesions in up to 20% of cases;[53] patients usually present with hemoptysis. Localized bronchiectasis (see Chapter 33) may only become clinically significant many years later and is best defined by high-resolution CT scanning. Tissue destruction may be severe enough to cause respiratory failure.

Pleural tuberculosis

Pleural TB classically occurs 3–6 months after primary disease, but the onset may be delayed and pleural disease may be the first sign of reactivation. Pleural disease may accompany lung infection or be the predominant feature. Tuberculosis and malignancy are the principal differential diagnoses of massive effusions that are exudates (protein concentration >3g/dl [>30g/l]). Effusions are usually straw colored but may be bloodstained and occasionally frankly bloody. Glucose concentrations in tuberculous effusions are frequently, but not always, less than 40mg/dl (2.22mmol/l). Pleural fluid lysozyme, lactate and pH may be elevated in tuberculous effusions, as in bacterial infections. Elevated adenosine deaminase (ADA), derived from CD4 lymphocytes, is characteristic. Low ADA levels are against a diagnosis of TB but high concentrations are nonspecific. The leukocyte count of tuberculous effusions is usually raised and may be as high as 5000 cells/ml (5,000,000 cells/l). Lymphocytes are generally the most prevalent cell type. Monocytes are also characteristic but mesothelial cells are scanty. Neutrophils may be present and may even predominate in acute disease.[54]

Chest radiography usually demonstrates a unilateral effusion, more often on the right. An effusion may be massive and bilateral effusions occur in approximately 10% of patients. Ultrasound may reveal loculated effusions containing fibrinous tissue, reflecting activation of the fibrinolytic system. Ultrasound and CT scanning are useful to document underlying disease and to aid diagnostic and therapeutic aspirations.

Miliary tuberculosis

Miliary TB follows blood-borne dissemination of *M. tuberculosis* and therefore clinical presentation is varied. Factors involved in the development of miliary infection include decreased immune responses, mycobacterial virulence factors, mycobacterial load and the number of organisms able to gain entry to the bloodstream. At least

50% of patients who have miliary disease in developed countries are immunosuppressed, most commonly by alcohol. Diabetes, chronic renal failure, underlying malignancies and immunosuppressive drugs are other risk factors. Disseminated infection caused by *M. bovis*, BCG strain, may follow instillation of the organism into the bladder when it is used as an adjuvant to chemotherapy for malignancy.

Miliary TB may manifest as fever of unknown origin or with symptoms attributable to involvement of one or more organ systems. Commonly there are widespread pulmonary granulomas and central nervous system (CNS) disease and occasionally cardiac involvement (pericardial, myocardial and endocardial manifestations have all been reported).

The differential diagnosis is often wide and clinicians must be alert to the possibility of miliary TB. Acute miliary TB tuberculosis presenting with shock and ARDS has a mortality rate that may approach 90%. In chronic cases, cachexia is prominent and localizing features may be few. Widespread macular and papular skin lesions (tuberculosis miliaris disseminata) are suggestive of miliary infection. Choroidal tubercles, 0.5–3.0mm in diameter, are essentially diagnostic of miliary disease (Fig. 37.9).

The chest radiograph of miliary TB shows well-defined nodules less than 5mm in diameter throughout both lung fields (Fig. 37.10). Radiographic changes may only develop after a patient has been admitted to hospital, so patients must be reassessed frequently. Larger nodules and a pulmonary focus occur in approximately one-third of patients. CT scanning, which is seldom of diagnostic value, may show smaller nodules not seen on radiography. Ultrasound scanning may show increased echogenicity in the liver but is not diagnostic. Hematologic investigations are similar to those found in tuberculous pneumonia but a neutrophilia may be seen and should

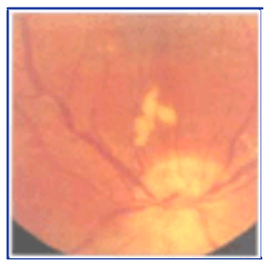

Figure 37-9 Choroidal TB. Choroidal disease is a manifestation of TB which is very highly suggestive of miliary disease. *With permission from James DG, Studdy PR. A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

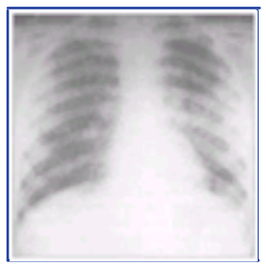

Figure 37-10 Miliary TB. Chest radiograph of miliary TB showing characteristic mottled shadowing throughout both lung fields. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

not put the diagnosis in doubt or cause empiric therapy to be restricted to antibacterials. Rarer abnormalities include disseminated intravascular coagulation and the hemophagocytic syndrome. Sterile pyuria and organisms in the absence of urinary leukocytes have been reported, but the exact frequency of such renal manifestations is uncertain. Delay in microbiologic diagnosis is a contributory factor to the high mortality rate in miliary disease.

Extrapulmonary tuberculosis

This section reviews those forms of extrapulmonary TB not considered in detail elsewhere and refers readers to other key chapters where further information is to be found.

Lymph node disease

Lymphadenitis or scrofula or the 'King's evil' (so-called because in Europe in the Middle Ages the royal touch was thought to be curative) is a very common extrapulmonary presentation of TB. In Europe, Australia and the USA, atypical mycobacteria (*Mycobacterium scrofulaceum* and *M. avium-intracellulare* complex) are

Figure 37-11 Vertebral TB. Tuberculosis of the spine or Pott's disease. Kyphosis is secondary to anterior destruction of vertebral bodies resulting in wedging of adjacent vertebrae and loss of disk space clearly seen by radiography. *(a) and (b) Courtesy of Professor J Cohen, Brighton, UK; (c) Courtesy of Dr A Wightman, with permission from Edmond RTD, Rowland HAK, Welsby PD, A colour atlas of infectious diseases, 3E. London: Mosby; 1995.*

also a frequent cause of lymphadenitis in children,[55] unlike the situation in adults in whom *M. tuberculosis* predominates. Peripheral nodes are usually infected through hematogenous spread of *M. tuberculosis* from the lung. The most commonly involved nodes are those in the cervical region, sometimes in association with axillary, inguinal or hilar lymphadenopathy. Patients may present with painless lymphadenopathy or with marked systemic symptoms. There may be associated sinuses and abscess formation. Hilar nodes may be associated with thoracic pain and, if untreated, may infrequently result in esophageal erosion or a bronchoesophageal fistula. Unusual symptoms such as obstructive jaundice have been reported in specific association with anatomically defined local lymph nodes.

Tuberculosis of the head and neck

Aside from cervical node disease, TB of the head and neck is relatively uncommon and usually arises secondarily to pulmonary infection. Laryngeal TB may manifest with hoarseness, pain on speaking or swallowing, hemoptysis and respiratory obstruction. Cough may reflect lung disease or involvement of the superior laryngeal nerve. Untreated, widespread local tissue destruction may occur with secondary laryngeal stenosis. A plain chest radiograph may be suggestive, but in one series radiographic changes were minimal in two-thirds of patients.[56] In this study from a specialist center, coexistent squamous cell carcinoma of the larynx was present in 10% of patients. Therefore, all laryngeal lesions should be biopsied to exclude malignancy.

Tuberculosis rarely involves the middle ear, where it may manifest with a local, painless discharge from which the organism may be cultured. There may be destruction of the ossicles, hearing loss and invasion of the facial nerve canal and sinuses and extension into the posterior cranial fossa. Secondary pyogenic infection may obscure the diagnosis. Tuberculosis of the nose, nasopharynx and adenoids is rare, even in areas in which infection is endemic, and symptoms such as epistaxis reflect local tissue damage. Tuberculosis in the oral cavity generally occurs as a solitary, often inflamed ulcer with irregular borders. There may be secondary infection of salivary glands.

Musculoskeletal infection

Vertebral infection (Pott's disease) is the commonest presentation of tuberculous osteomyelitis, accounting for about 50% of all bony TB (Fig. 37.11). The male to female ratio is approximately 2 to 1. The thoracic spine is the most frequently involved, followed by lumbar and then cervical regions. Presentation is usually with back or neck pain. Systemic symptoms tend to be less marked than in pulmonary disease. Neurologic symptoms, such as weakness, numbness and disturbances of gait, occur in about one-third of cases. Some patients have an associated flank mass or other evidence of extraspinal TB. In more advanced cases, vertebral body collapse and gibbus formation lead to kyphosis of the spine. Destructive lesions with invasion of the joint space and deformity may be seen on plain radiographs, although CT or

ideally magnetic resonance imaging (MRI) are better imaging modalities for spinal pathology.

Osteomyelitis is otherwise most frequently found in the metaphysis of long bones, although the ribs, pelvis and skull may be infected. In children in countries in which TB is prevalent, osteomyelitis is a significant cause of crippling deformity. Direct extension from the ribs to the lung is rare, as is meningitis or tuberculomas in patients in whom there is skull involvement. Bony TB may be accompanied by sinus tracts or soft tissue masses. Diagnosis may be difficult because lesions can appear osteolytic or sclerotic on radiography and malignancy may be suspected first. Like radiographs, ^{99m} technetium bone scans have no pathognomonic features. Trauma does not predispose to tuberculous osteomyelitis.

Tuberculous arthritis most frequently manifests in the hips and other weight-bearing joints, although any joint may be involved. Polyarticular disease occurs in less than 20% of patients, but evidence of TB elsewhere, generally the lung, is present in about 50% of cases. Synovial fluid is usually turbid with a high leukocyte count and up to 60% may be neutrophils rather than mononuclear cells. Organisms are found in less than one-quarter of cases, although positive cultures are more frequent and ideally a synovial biopsy should be examined histologically and microbiologically. Prosthetic joints may be infected with *M. tuberculosis* after either hematogenous spread or contiguous reactivation of infection. Tenosynovitis is rare and generally associated with adjacent osteomyelitis.

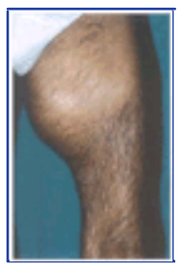

Figure 37-12 Abscess formation in TB. This patient had multiple TB abscesses particularly affecting the psoas and quadriceps muscle groups.

Tuberculous abscesses may form in most soft tissues, including muscle (Fig. 37.12). This is usually secondary to contiguous spread of infection but may follow hematogenous dissemination. The classic abscess site is in the psoas muscle, and such an abscess can occur with or without localizing signs, so a high index of clinical suspicion is necessary for the appropriate imaging to be arranged in advance of diagnostic aspiration.

Abdominal infection

The abdomen is a frequent site of TB, especially in patients from the Indian subcontinent, where rates are up to 50 times higher than in Europe, and in those immunosuppressed with HIV infection. Disease is usually secondary to hematogenous spread of mycobacteria, but can be secondary to local invasion or ingestion of organisms. However, any infected person can present with disease in the abdomen and as TB frequently mimics other pathologies, such as gastrointestinal malignancy, diagnosis is often delayed. In the gut, TB most frequently affects the ileocecal region, then the small bowel and then the colon, with involvement of the duodenum occurring in less than 2.5% of cases and involvement of the stomach and esophagus in less than 1% of patients. Approximately one-third of patients have evidence of TB elsewhere, usually in the lung.

Symptoms reflect site of involvement but may be non-specific with fever, weight loss, chronic abdominal pain, nausea and anorexia. Diarrhea occurs in less than 20% of cases and reflects either secondary overflow caused by obstruction or stimulation of the gastrointestinal tract by cytokines. Ileocecal TB may manifest as acute surgical abdomen secondary to obstruction of the bowel lumen or appendix, or after bowel perforation. Any tuberculous lesion, particularly those in the colon, may manifest with massive gastrointestinal bleeding, but this is rare. Lesions may be ulcerative or hypertrophic and can be associated with fistulas, which are probably partly the result of secondary bacterial involvement. Unusual sites of intra-abdominal TB include the pancreas and the adrenal glands; in the latter, disease occurs very rarely as an adrenal crisis, but more commonly with an insidious onset of symptoms that may be difficult to distinguish from those associated with infection. Tuberculous peritonitis is an important manifestation of disease and is discussed in Chapter 47 . Hepatobiliary infection is considered in Chapter 49 . Chapter 71 provides a detailed review of urogenital TB.

Central nervous system and eye disease

Tuberculous meningoencephalitis and CNS tuberculomas are extremely important, carrying high morbidity and mortality rates. They are considered further in Chapter 22 and Chapter 24 , respectively.

Ocular TB is relatively uncommon but important because, if overlooked, blindness may result. The commonest manifestation is choroidal disease (see Fig. 37.9) secondary to hematogenous spread in the context of miliary TB, which may rarely spread to the retina. Conjunctival TB may be caused by accidental self-inoculation by an infected person, spread from skin or very rarely by direct spread from another infected individual. Infection may be ulcerative or nodular, although occasionally focal tuberculomas, sometimes appearing polypoid, are seen. Tuberculosis of the sclera and cornea are rare, as is uveitis.

Pericardial infection

Tuberculous pericarditis is potentially fatal. A detailed description of this disease is provided in Chapter 58 .

Dermatologic disease

Lupus vulgaris is the best documented manifestation of dermatologic TB. Details of the dermatologic manifestations of primary and miliary infection can be found in Chapter 12 .

Clinical manifestations in people who have HIV infection

Tuberculosis is an AIDS-defining event in HIV-positive patients in the USA and a common secondary infection in HIV-positive patients living in countries in which TB is more prevalent. The clinical manifestations of TB in HIV-positive and -negative patients are generally similar. However, TB may be more widespread, have more atypical features and is more often extrapulmonary in HIV-positive patients (see Chapter 129). Many unusual or rare presentations of TB have been reported, bronchoesophageal fistula being one example.[57] *Mycobacterium tuberculosis* bacteremia is much more frequent in HIV-positive patients. [58] In Africa, CNS and disseminated TB occur more often in patients who have peripheral $CD4^+$ T lymphocyte counts less than 200 cells/mm^3 (200,000/1).

Making the correct diagnosis can be difficult when HIV and TB coexist. Non-specific systemic features of TB, such as fever, malaise and a prolonged acute phase protein response, are also characteristic of HIV infection. In Africa, both HIV and TB contribute to the wasting entity recognized as Slim disease. Many opportunistic infections in HIV, including those caused by the *M. avium-intracellulare* complex, may cause similar symptoms to those of TB, adding to diagnostic confusion. In addition, low peripheral T-cell counts may secondarily reduce leukocyte counts in TB-infected pleural fluid, which may be diagnostically confusing.

Noninfectious complications

Erythema nodosum is the most common noninfectious complication of primary TB; it also occurs in other granulomatous diseases (e.g. leprosy, sarcoidosis). It is particularly associated with primary disease. Arthritis occurs in up to 1% of patients who have acute infection and may also complicate reactivation. Poncet's disease is a reactive polyarthritis associated with TB, which resolves with antimycobacterial chemotherapy. Bazin's disease is a vasculitic skin reaction to TB that usually manifests as a purpuric rash on the lower extremities. Another important immunologic complication of TB is renal interstitial nephritis.

Bronchogenic carcinoma was thought for many years to occur more frequently in patients who had TB, but this has not been borne out in careful studies. Hypertrophic pulmonary osteoarthropathy, principally associated with bronchogenic carcinoma, has been reported in TB,[59] although some attribute the finding to undiagnosed lung malignancy. Another report described clubbing in 21% of patients in a Nigerian hospital, but this figure is much higher than experienced by most physicians.[60] The syndrome of inappropriate antidiuretic hormone secretion is more common in pulmonary and miliary disease.

DIAGNOSIS

Clinical approach

The definitive diagnosis of TB requires identification of the mycobacterial pathogen in a patient's secretions or tissues. Therapy is often initiated before a definitive diagnosis has been made, but one should always be pursued. Identification of the organism is necessary for drug susceptibility testing, which guides local treatment policies and individual therapeutic regimens. Sputum examination has a high diagnostic yield, identifies the majority of infectious patients and is cheap to perform. Sputum should be collected on three separate occasions, but additional specimens are not cost-effective.[61] Induced sputums obtained in properly ventilated and isolated areas can be useful, but bronchoscopy with alveloar lavage has the best diagnostic yield.[62] Routine screening of induced sputums for TB in HIV-positive patients is not useful. *Mycobacterium tuberculosis* is seen infrequently on aspiration of pleural fluid and is cultured in under 50%

Figure 37-13 2-Fluoro-deoxy glucose positron emission tomography (FDG-PET) scan in bilateral apical pulmonary TB (arrowed). Tracer is excreted via the renal tract and is clearly seen in the bladder.

of cases. However, pleural biopsy reveals granuloma or results in culture of the pathogen in over 90% of cases. Thoracoscopy may be indicated in exceptional circumstances because it allows visually guided biopsy of lesions. Tests such as an elevated pleural fluid ADA concentrations may support a diagnosis of TB but their specificity is limited. More recently 2-fluoro-deoxy glucose positron emission tomography (FDG-PET) scanning has been used to identify occult disease although such scans are also positive in malignancy (Fig. 37.13).

In extrapulmonary or miliary infection, appropriate body fluids and/or tissues must be obtained for microbiologic and histologic examination, with the aid of ultrasound, CT or MRI scanning if available. Liver biopsy and bone marrow aspiration are useful investigations in disseminated or occult disease.[63] An acid-fast stain of buffy coat leukocytes may be diagnostic in immunosuppressed patients.

For tuberculous lymphadenitis, fine-needle aspiration is the initial investigation of choice because it is easy to perform, and has a high specificity and sensitivity when both microbiologic and cytologic specimens are collected. Fine-needle aspiration does not preclude subsequent lymph node biopsy. Needle aspiration remains of value in countries in which TB is common but resources, including trained laboratory staff, are scarce.[64] It can also be used for other manifestations of suspected extrapulmonary disease such as tuberculous arthritis.

Specimens from potentially infected patients are normally analyzed in local laboratories, but drug sensitivity testing and more specialized facilities are ideally concentrated in centralized laboratories. All laboratories must be arranged to deal with hazardous, possibly drug-resistant pathogens. Safety measures include containment areas, approved safety cabinets, centrifuges, gowns, gloves, sinks and facilities for disposal of contaminated waste and treatment of spillages. Special training for the staff is essential.

The usual principles of microbiologic safety apply to the collection of specimens into sterile containers. Tissue biopsies should be divided and a fresh specimen sent for microbiologic and one in formalin for histologic examination. Tissue is better for culture than necrotic material and old pus, which may contain acids that are toxic to mycobacteria, a fact to be considered when selecting biopsy sites. Blood and bone marrow specimens can be injected directly into blood culture bottles. Examination of cerebrospinal fluid for *M. tuberculosis* is usually an emergency investigation, with the clinician forewarning the laboratory. Specimens from gastric lavage should be rapidly transported to the laboratory because stomach acidity may kill mycobacteria. Feces are not useful in the diagnosis of *M. tuberculosis* in HIV-negative patients.

If no diagnostic results are forthcoming in patients in whom TB is a possibility, the clinician may reasonably resort to a trial of therapy. Improvement of symptoms and a decrease in the acute phase response (monitored by ESR, CRP, etc.) are sufficient indication to move from the diagnostic trial to full therapy. Any diagnostic trial of therapy should last a minimum of 2 weeks and ideally 4 weeks.

Microbiologic diagnosis

The standard method for staining clinical specimens for *M. tuberculosis* is that of Ziehl-Neelsen (Fig. 37.14), which requires application of heat, whereas a modification, the Kinyoun method, does not. Both methods utilize the fact that the stained cell wall is resistant to decolorization by acid alcohol although the biochemical reason for this is unknown. Auramine staining has facilitated rapid screening of diagnostic specimens and has greater sensitivity (Fig. 37.15). Mycobacteria are often scanty and specimens should be reviewed by an experienced operator. Diagnostic microscopy is most successful in patients who have extensive disease and cavitation on chest X-ray and has an overall sensitivity of 50–90% depending on case mix.

Mycobacterium tuberculosis is traditionally cultured on egg and potato-based media such as Middlebrook 7H10/11. Such media are of limited value in the analysis of clinical specimens because of overgrowth by more rapidly dividing commensal organisms. Selective media such as Lowenstein-Jensen or Petragnani are used, with Middlebrook being reserved for subcultures. Cultures must be maintained for up to 8 weeks. *Mycobacterium tuberculosis* is

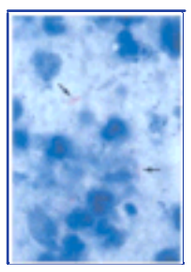

Figure 37-14 Ziehl-Neelsen-stained sputum specimens containing *Mycobacterium tuberculosis.* *Courtesy of Dr F Ahmed, Ealing Hospital, UK.*

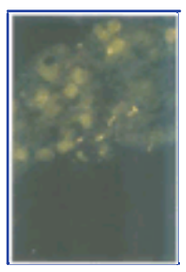

Figure 37-15 *Mycobacterium tuberculosis* in sputum detected by auramine staining. *Courtesy of Mr M Croughan, with permission from Edmond RTD, Rowland HAK, Welsby PD, A colour atlas of infectious diseases, 3E. London: Mosby, 1995.*

identified as slow-growing, rough, whitish colonies with a tendency to cord formation. More specific biochemical tests detect a range of characteristics, including the ability of the organism to produce niacin, reduce nitrate and produce a heat-sensitive catalase. To speed diagnosis, broth-based growth systems containing Middlebrook 7H12 with ^{14}C-labeled palmitic acid as the only carbon source are widely used. This is the BACTEC system, which detects release of radioactive $^{14}CO_2$ from dividing mycobacteria and approximately halves the time to diagnosis in smear-positive and negative patients. The BACTEC system has been refined for specific identification of *M. tuberculosis*. Failure of a mycobacteria to grow in a BACTEC NAP vial (containing p-nitro-a-acetylamino-ß-hydroxypropiophenone) indicates that it is either *M. tuberculosis*, *M. bovis* or *M. africanum*. *Mycobacterium tuberculosis* can also be identified from a fatty acid profile obtained by high-performance liquid, gas-liquid or thin-layer chromatography. A novel colorimetric diagnostic method is the mycobacteria growth indicator tube that contains 7H9 Middlebrook broth and an oxygen-sensitive fluorescent indicator that indicates bacterial growth. Once the indicator is positive, detection by genetic means is possible. Such methodology may

have a particular role in the rapid detection of drug-resistant organisms in clinical specimens. Several cheaper approaches to rapid microbiological diagnosis have been investigated and the use of an inverted microscope to detect mycobacterial growth within days rather than weeks is one of the most promising.[65]

There are four methods of susceptibility testing of *M. tuberculosis* to determine whether the majority of pathogens are sensitive to the drugs being prescribed:

- the absolute concentration method uses a series of cultures containing a graded range of dilutions of the drug being tested, as well as a control to determine the minimum amount of drug that inhibits growth of any isolate;
- the resistance ratio method, commonly used in the UK, is similar, but results are related to the sensitivity of a known strain of *M. tuberculosis* such as H37-Rv;
- the proportion method, frequently employed in the USA, compares the number of colonies growing on a standard medium with the number on a drug-containing medium; and

in many countries, BACTEC bottles containing various drugs are now used because of their rapidity and reliability.

Pyrazinamide-sensitivity testing presents specific problems in that it must be carried out at pH5.5 and this degree of acidity can inhibit the growth of some strains of *M. tuberculosis* independently of drug action. An alternative approach has been to screen isolates for production of pyrazinamidase.

In the future, genetic techniques screening for relevant mutations are likely to have a role in rapidly identifying resistant organisms, but will not detect novel mutations.

Genetic techniques

The polymerase chain reaction (PCR) has the potential to allow rapid detection of low levels of infection with the possibility of immediate probing for known drug resistance genes. The usual targets for amplification are the DNA insertion sequence IS6110, which is found in all the *M. tuberculosis* complex of organisms (*M. tuberculosis, M. bovis, M. africanum* and *M. microti*) or rRNA. The main current usage of PCR-based technology is for the rapid diagnosis of drug-resistant TB and MDR-TB once standard cultures are positive. Since nearly all rifampin drug resistance is due to mutations in the RpoB (RNA polymerase B) gene and because rifampin resistance is highly associated with isoniazid resistance, testing for this mutation is used for early identification of patients with MDR-TB. Considerable methodologic problems with PCR have been encountered so this has not yet replaced traditional microbiological techniques as a primary diagnostic tool.

Several large-scale studies have reported high sensitivity and specificity using culture positivity as the gold standard.[66] However, in one study only 70% of laboratories could make the diagnosis in samples containing 10,000 *M. tuberculosis* (lower limit for detection by microscopy) and 22% of laboratories identified the organism in negative control samples.[67]

PCR is potentially of most use in smear-negative culture-positive disease, for which it should be able to identify over 50% of cases.

Another problem is that PCR positivity in sputum may persist during treatment and after clinical cure.[68]

It is likely that PCR will be useful in diagnosing extrapulmonary TB, but large studies of sensitivity are awaited. One interesting observation is that about 50% of patients are positive on blood-based PCR analysis for *M. tuberculosis*, which suggests that a blood test might be diagnostically useful.[69]

PCR tests are good at distinguishing isolates from the *M. tuberculosis* family from other mycobacteria, which can be important in HIV patients who are susceptible to disseminated infection by *M. avium-intracellulare* complex organisms. Although PCR is finding an increasing role in the diagnosis of TB, it has not yet replaced microscopy and culture and, in view of its cost, it is unlikely to do so soon in most areas of the world in which TB is prevalent.

The use of reporter phages encoding genes for luciferase from the firefly *Photinus pyralis* represents the main alternative genetic approach to the diagnosis of TB. On introduction of a phage such as TM4 into mycobacteria in the presence of luciferin and ATP, oxidization results in the generation of luciferyl-AMP and then oxyluciferin, which gives out 0.85 photons per molecule of substrate. This is measured in a luminometer, thus allowing detection of mycobacterial growth in a few hours. Subsequently, efficacy of drug activity can be rapidly screened within 48 hours by monitoring changes in luminescence.[70]

Serodiagnosis

Serodiagnostic techniques are not in routine use in most laboratories. Enzyme-linked immunosorbent assays (ELISAs) have been developed to detect a number of mycobacterial proteins, such as the 16kDa hsp, the 30kDa antigen, the 38kDa antigens and LAM, but the sensitivity of antibody testing is not sufficiently high in ELISAs with adequate specificity to warrant their regular use. Local testing for antibodies at sites of infection, such as in pleural effusions, has not proved useful. Antibody levels are also often low in culture-negative individuals in whom alternative diagnostic approaches are needed. Alterations in antibody levels over time are not a useful guide to the clinical response to treatment. Finally, the impaired antibody response makes serology of negligible value in HIV-positive patients.

MANAGEMENT

First-line antimycobacterial drugs and their toxicities

This and the next section briefly outline the principal characteristics of the most important antituberculous drugs. More detail concerning mode of action and dosage regimens can be found in Chapter 202 .

Isoniazid

Isoniazid (INH or isonicotinic acid hydrazide: $C_6 H_7 N_3 O$) is a bactericidal drug (MIC generally <0.1mg/ml), the biologic activity of which resides in a pyridine ring and a hydrazine group. Isoniazid is well absorbed, minimally bound to plasma proteins, has a half-life of 1–3 hours depending on patient acetylator status and is excreted into urine. Concentrations achieved in most tissues are similar to those in serum, with the important exception of the CNS in which concentrations are approximately 20% of those in serum. Thus, the drug is used at double the normal dose in CNS disease. Isoniazid enters *M. tuberculosis* by diffusion and by oxygen-dependent active transport where it inhibits mycolic acid synthesis and the nicotinamide adenine dinucleotide pathway and interacts with the catalase-peroxidase enzyme system. Mutations in the *kat* G gene encoding the catalase-peroxidase enzyme confer resistance to INH and account for approximately 25% of cases of drug resistance.[71] Mutations in the *inh* A gene, which encodes an enoylacyl carrier protein reductase probably involved in mycolic acid synthesis, appear important in drug resistance to INH and ethambutol in some mycobacteria but not *M. tuberculosis*.

The most important, potentially fatal, side-effect of INH is hepatotoxicity, which is more common in patients over 60 years of age, in the presence of coexisting liver disease and possibly in pregnancy. Asymptomatic, usually transient, rises in transaminases occur in about 20% of patients. A greater than threefold increase in enzyme levels above the normal range is an indication to discontinue therapy. In practice, it is often impossible to separate INH hepatotoxicity from that caused by rifampin or pyrazinamide but, interestingly, reintroduction of these drugs singly rather than in combination usually avoids a second episode of hepatotoxicity. The other major side-effect is a peripheral and rarely optic neuritis caused by interference with niacin metabolism. This is prevented by concomitant administration of vitamin B_6 (pyridoxine) 10mg daily. Less common side-effects include nausea, vomiting and arthralgia and rarely hypersensitivity reactions, lupus-like reactions, cerebellar ataxia, convulsions, psychoses, hyperglycemia, agranulocytosis and in malnourished patients pellagra.

Rifampin

Rifampin is a bacteriocidal drug with excellent antituberculous activity. Rifampin undergoes first-pass metabolism in the liver where it is deacylated; it is excreted into bile and then into the gut where there is a minor degree of enterohepatic circulation. Rifampin is a potent inducer of hepatic enzymes and therefore has many clinically significant interactions with other drugs (Table 37.2). Rifampin targets the RNA polymerase ß subunit, blocking initiation but not

TABLE 37-2 -- Drug interactions with rifampin.

DRUG INTERACTIONS WITH RIFAMPIN	
Drug category	**Example**
Antibacterials	Chloramphenicol
Antifungals	Fluconazole
Corticosteroids	Prednisolone
Anticoagulants	Warfarin
Analgesics	Methadone
Immunosuppressive therapy	Ciclosporin
Ulcer-healing drugs	Cimetidine
Respiratory drugs	Theophylline
Cardiac drugs	
ß blockers	Propranolol
Calcium channel blockers	Diltiazem
Cardiac glycosides	Digitoxin (only member of class affected)
Antiarrhythmics	Disopyramide
Lipid-lowering drugs	Fluvastatin
CNS drugs	
Anti-epileptics	Phenytoin
Anxiolytics	Diazepam
Antidepressants	Tricyclic compounds
Antipsychotics	Haloperidol
Endocrine drugs	
Antidiabetics	Tolbutamide
Estrogens & progesterones	Combined & progesterone only contraceptive pill
Thyroid replacement	Thyroxine
These are the principal classes of drugs for which metabolism is increased (plasma concentration decreased) when taken with rifampin. The examples are not exhaustive and clinicians should check interactions with related drugs in appropriate formularies. (See Chapter 129 for interactions with drugs used to treat HIV.)	

elongation of mRNA transcripts. Mutations in the *rpoB* gene, which encodes this subunit, are associated with drug resistance. A worrying development has been the emergence of rifampin-resistant TB through an *rpoB* mutation in a patient taking rifabutin prophylaxis for *M. avium-intracellulare* complex infection.[72]

Patients should be warned that rifampin turns all body secretions, including urine and tears, orange (Fig. 37.16). This is a useful side-effect in terms of monitoring compliance. Rifampin may cause hepatic injury, particularly in the presence of pre-existing liver disease. Gastrointestinal side-effects seldom necessitate stopping therapy and include anorexia, nausea, vomiting, abdominal pain and diarrhea. Pseudomembranous colitis caused by *Clostridium difficile* toxin production is occasionally described. Immune-mediated problems associated with rifampin include rashes, urticaria, conjunctivitis and rarely hemolysis or thrombocytopenic purpura. A 'flu-like' syndrome may be troublesome, particularly in intermittent therapy regimens, and very rarely may be severe with circulatory collapse and respiratory failure.

Pyrazinamide

Pyrazinamide, a structural analog of nicotinamide, is a bactericidal drug that is well absorbed via the gut and distributed widely, including in the CNS where concentrations are the same as those in serum in patients who have tuberculous meningitis. Serum half-life is about 10 hours and excretion is urinary. Pyrazinamide principally acts intracellularly at relatively acidic pH, although it may also have some cidal action on extracellular bacteria. The drug penetrates macrophages where an enzyme from *M. tuberculosis* converts it into

Figure 37-16 Rifampin urine testing. Patients should be warned that rifampin turns urine and other body secretions orange. This fact can be helful in monitoring compliance with drug treatment. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

active pyrazinoic acid. Little is known about mechanisms of drug resistance to pyrazinamide, although some strains of *M. tuberculosis* produce an enzyme called pyrazinamidase.

Hepatotoxicity ranging from elevation of liver transaminases to frank jaundice and liver failure is the principal side-effect of pyrazinamide. More common are mild gastrointestinal problems and an arthralgia associated with raised serum urate concentrations secondary to inhibition of tubular secretion of uric acid by pyrazinamide; gout is rare. Sideroblastic anemia has been reported.

Ethambutol

Ethambutol is a bactericidal drug that is rapidly and well (over 80% of the dose) absorbed in the gut, with peak serum levels occurring 2 hours after a dose. It is then rapidly excreted in urine. Ethambutol appears to alter *M. tuberculosis* RNA synthesis and the transfer of mycolic acids into cell wall. Changes in cell wall lipids have been noted in organisms resistant to the drug.

The most important complication of ethambutol therapy is retrobulbar neuritis manifest by impaired visual acuity, color-blindness and restricted visual fields. Except in patients who have pre-existing ophthalmic disease, optic neuritis is extremely rare when ethambutol is used at standard doses (15mg/kg). In affluent countries, it is appropriate to have patients assessed by an ophthalmologist before treatment is begun, but lack of this facility should not prevent the use of ethambutol. Patients should be warned to report symptoms of visual change immediately and ethambutol should generally be avoided in children. Very rarely ethambutol causes a peripheral neuritis.

Streptomycin

Streptomycin, the first clinically useful drug discovered in the fight against TB, is an aminoglycoside that has to be given intramuscularly. Streptomycin penetrates cerebrospinal fluid and other remote tissues (e.g. prostate and eye) poorly. There is an immediate and a delayed pathway of excretion via the renal tract. The persistence of the drug at low doses is one factor in the development of side-effects. Streptomycin binds to the 30S rRNA subunit, which results in decreased protein synthesis and misreading of mRNA. Mutations, including those in the 30S subunit, arise readily in response to isolated streptomycin therapy and lead to drug resistance. The principal side-effects of aminoglycosides are ototoxicity and nephrotoxicity.

TABLE 37-3 -- Second-line therapy in TB.

SECOND-LINE THERAPY IN TB			
Drug (chemically closely related drug)	**Mechanism of action**	**Toxicity**	**Usual initial adult dose**
PAS	Competes with mycobacterial dihydropteroate synthetase	Gastrointestinal intolerance, hypersensitivity, hypothyroidism, crystaluria	12g/day (divided doses)
Ethionamide (prothionamide)	Inhibits cell wall mycolic acid synthesis	Gastrointestinal intolerance, hepatitis, hypersensitivity, convulsions, depression, alopecia	0.5–1.0g/day (divided doses)
Ciprofloxacin (ofloxacin)	Inhibits topoisomerase II	Gastrointestinal intolerance, crystaluria, tremor, convulsions, rash, hepatitis, renal failure	750mg q12h
Capreomycin (viomycin)	Binds 30S and 50S ribosomes	Nephrotoxic, ototoxic, hypersensitivity	1g/day
Kanamycin	Binds 30S ribosome	Nephrotoxic, ototoxic, hypersensitivity	15mg q12h*
Amikacin	Binds 30S ribosome	Nephrotoxic, ototoxic, rash, neuromuscular blockade, eosinophilia	7.5mg/kg q12h*
Cycloserine	Competitive D-alanine analog	Seizures, psychoses, various CNS effects	250mg q12h/q8h
Thiacetazone	Bacteriostatic ?exact mechanism	Gastrointestinal intolerance, Stevens-Johnson syndrome bone marrow depression, ototoxic, hepatitis	150mg daily
Second-line drugs used in treatment of TB are presented together with their mechanisms of action and toxicity.			

* peak drug levels should be less than 3mg/dl (30mg/l) and trough less than 1mg/dl (10mg/l)

Rarer side-effects are neuromuscular blockade and hypersensitivity reactions with maculopapular rashes, fever and eosinophilia.

Second-line drugs

Second-line agents are becoming increasingly important with the advent of MDR organisms. Information about these drugs is listed in Table 37.3 , although the use of many has not been confirmed in clinical trials. The value of proved efficacious drugs such as ethionamide is limited by toxicity. There is evidence to suggest that the role of other drugs such as imipenem in the treatment of TB should be reevaluated.[73] The potential second-line role of clofazamine, used in the treatment of *M. leprae*, is also uncertain. It remains a poor reflection on the wealthier nations that thiacetazone, which has a dangerous side-effect profile, continues to be widely used in developing countries because it is all that can be afforded. More details about these agents can be found in Chapter 202 .

Treatment regimens

Many trials, notably those involving the British Medical Research Council, have established short-course chemotherapy as the preferred treatment of pulmonary, pleural, nodal and most other forms of TB. Such regimens are dependent on using the potent antituberculous drugs isoniazid and rifampin throughout a 6-month course in addition to a 2-month initial treatment with pyrazinamide. An additional drug such as ethambutol or streptomycin is frequently used in

TABLE 37-4 -- Short-course chemotherapy.

SHORT-COURSE CHEMOTHERAPY			
Drug	**Adult dose (orally)**	**Duration of treatment**	**Modification of drug dose in renal failure**
1. Isoniazid*	5 mg/kg (maximum 300mg)	6 months	No
2. Rifampin*	10 mg/kg (maximum 600mg)	6 months	No
3. Pyrazinamide*	30mg/kg (maximum 2.0g)	2 months	? dose or ? dosage interval
4. Ethambutol	15mg/kg†	2 months	? dose or ? dosage interval
or			
Streptomycin	15mg/kg im (maximum 1.0g)	2 months	?? dose or ?? dosage interval
Short-course chemotherapy regimen for treatment of TB and necessity for drug dose modifications in renal impairment.			

* Isoniazid and rifampin are marketed as a single combination tablet ± pyrazinamide which may facilitate compliance
† Some authorities use ethambutol at 25mg/kg for 2 months only (longer courses should be at 15mg/kg)

the induction period and must be used if drug resistance is a possibility. Single drug resistance occurs at frequencies of up to 20% in West Africa and 10% in the USA.[74] The aim of therapy is to kill all *M. tuberculosis*, with multiple drugs countering the spontaneous emergence of drug-resistant mutants.

One standard treatment protocol is given in Table 37.4 . Alternative regimens are essential in the presence of drug resistance and are possible when drugs are in short supply. For example, 7 months' isoniazid and ethambutol therapy can substitute for isoniazid and rifampin in the period after 2 months' quadruple therapy. For sensitive organisms, drug therapy in compliant patients is very efficacious, with cure rates approaching 100%. However, it may be 2 weeks before clinical improvement becomes apparent, which is important in empiric trials of therapy and for appropriate patient expectations. Radiologic improvement lags further behind and it may take 3–5 months before all that remains is residual scarring on the chest radiograph. Drug therapy must be linked with the public health measures discussed above.

The necessary duration of treatment for extrapulmonary TB is debated. Limited trials in osteomyelitis, regarded as a difficult site to treat, indicate that 9 months' therapy is effective, providing both isoniazid and rifampin are used. In miliary disease, the 6-month regimens have been very successful. If TB involves the CNS, a minimum of 12 months of therapy is required. Complications may develop during the treatment of disease, partly as a result of an

influx of leukocytes. The consequences of this depend on the site of infection and include pneumothoraces, the expansion of intracerebral granulomas or discharge from subcutaneous nodes.

Patient isolation

It is critical that infectious patients admitted to hospital are nursed in full respiratory isolation. Specific assessment should be made of the likelihood of MDR-TB when negative pressure isolation is mandatory although the reality is that countries with a high incidence of MDR-TB are unlikely to have such facilities. Procedures that may induce aerosols such as sputum induction should be avoided if possible and bronchoscopy of suspected TB patients must be performed in appropriately ventilated facilities with staff trained in respiratory isolation procedures. Patients with drug sensitive TB are thought to become noninfectious within 2 weeks although recent genetic studies on disease transmission using various DNA fingerprinting techniques have cast some doubt on this and better data are required. Acid-fast bacilli may be recovered in sputum up to 1 month after the initiation of therapy, but growth in culture indicates noncompliance with treatment or drug-resistant disease. The identification of drug-resistance may be delayed in patients in whom initial culture is negative or if facilities for PCR testing are not available with the possible

consequence of transmission of MDR-TB. Isolation is not normally required for extrapulmonary disease.

Directly observed therapy

Compliance with therapy is critical for successful treatment and to limit the development of drug-resistant strains. Many social, personal, public health and economic factors influence patient noncompliance with treatments.[75] The WHO and others strongly advocate directly observed therapy, short-course (DOTS). When patients are in hospital, normal treatment regimens may be used, but for patients in the community intermittent therapy is preferred (Table 37.5). DOTS programs do not simply involve watching patients take their medications, but also incorporate case finding by smear microscopy, ensuring a good supply of effective drugs for short-course chemotherapy, reporting of treatment outcomes and ensuring government commitment to TB control which may be very effective in controlling TB.[76] Although there is little doubt that DOTS may be extremely useful in certain patient groups, randomized controlled trials suggest that not all patients benefit from DOTS.[77] DOTS programs often recruit from non-DOTS programs and the total detection of sputum-positive disease has only increased 5% despite the massive increase in DOTS use.[78]

TABLE 37-5 -- Intermittent chemotherapy regimens for TB.

INTERMITTENT CHEMOTHERAPY REGIMENS FOR TB		
Drug	**3 times/week dose**	**2 times/week dose**
1. Isoniazid	15mg/kg for 6 months (maximum dose 900mg)	15mg/kg for 6 months (maximum dose 900mg)
2. Rifampin	10mg/kg for 6 months (maximum dose 900mg)	10mg/kg for 6 months (maximum dose 900mg)
3. Pyrazinamide	50mg/kg for 2 months (maximum dose 3.0g)	70mg/kg for 2 months (maximum dose 4.0g)
4. Ethambutol	30mg/kg for 2 months	50mg/kg for 2 months (some authorities reduce this dose by 5mg/kg)
or		
Streptomycin	25mg/kg for 2 months (some authorities reduce this dose by 5mg/kg; maximum 1.5g dose)	30mg/kg for 2 months (some authorities reduce this dose by 5mg/kg; maximum 1.5g dose)

Treating multidrug-resistant organisms

Patients should never be prescribed less than three drugs initially and seldom less than four if there is any chance of resistance or a history of previous therapy, irrespective of the sensitivity of the organism initially isolated. Treatment protocols for MDR disease must be designed for the individual, but the aim is to use as many of the first-line drugs as possible before adding second-line drugs. In view of the fact that at least four drugs and possibly more should be given, options are frequently limited. A second-line therapeutic regimen should be continued for 18–24 months after cultures are negative. It is critical that affected patients are adequately isolated and that health care workers are protected from infection. Adjunctive surgical therapy may have a role in circumscribed lesions. Treatment of MDR TB can be difficult and response rates in HIV-negative patients vary from 56[79] to 96%.[80]

Patients with HIV infection

HIV infection does not adversely influence the response to short-course chemotherapy in the absence of drug resistance.[75] [81] The rate of relapse of TB treated with first-line drugs is less than 5% in compliant patients, but increases significantly if rifampin and isoniazid are not used. However, HIV-positive patients who have TB have a 5-to 14-fold increased risk of dying, although superinfection and disease caused by other opportunistic pathogens contribute to the mortality rate. In patients who have HIV infection and TB, four drugs should probably be given routinely in the induction phase of treatment. In contrast, the prognosis of patients who have MDR disease is poor and worse than that of HIV-negative patients. HIV-positive patients are more likely to have adverse reactions to antituberculous medication, particularly to thiacetazone, but also to other drugs.[82] Antituberculous and antiretroviral medications, including protease inhibitors and non-nucleoside reverse transcriptase inhibitors have numerous and complex interactions; the CDC provide updated guidance on this topic via their useful web site (see also Chapter 129).

Use of corticosteroids

Corticosteroid therapy is often advocated but seldom proved to be of benefit in TB. In pericardial TB, corticosteroids have been shown to decrease the acute mortality rate from pericarditis and reduce the need for pericardiocentesis. [83] Corticosteroids may have a role in pleural disease but further data are needed before they can be recommended.[84] Corticosteroids are used during the treatment of CNS tuberculomas to limit their expansion caused by cellular influx and to prevent raised intracerebral pressure. Evidence in favor of using corticosteroids in tuberculous meningitis is conflicting but they are probably beneficial in severe infection and spinal disease.[85] Retrospective studies investigating the role of corticosteroids in military TB have shown no benefit. In all cases, steroid metabolism is increased by rifampin. (See also Chapter 40.d .)

Pregnancy

Pregnancy probably does not increase the severity or reactivation rate of TB or responses to therapy.[86] However, there may be a significant increase in spontaneous abortions and labor difficulties. Congenital TB is very rare and usually presents with hepatosplenomegaly, respiratory distress and fever;[87] it carries a high mortality rate. Tuberculosis frequently requires treatment during pregnancy and this should not be deferred. Isoniazid, rifampin and ethambutol are not teratogenic but few data exist on pyrazinamide. Streptomycin is associated with fetal hearing loss and should be avoided. Little is known about second-line drugs in pregnancy except para-aminosalicyclic acid, which appears to be safe. The presence of antituberculous drugs in breast milk is seldom a problem unless both mother and

child are on treatment, in which case up to 20% more isoniazid than indicated may be taken by the child and bottle feeding is preferable. Breast-feeding children of mothers taking isoniazid require pyridoxine supplementation.

Surgical therapy

Surgical techniques such as artificial pneumothoraces, phrenic nerve paralysis, plombage and thoracoplasty are part of the history of TB before the era of chemotherapy. Now surgeons are most often involved in diagnostic rather than therapeutic procedures relevant to TB. However, resection of tissue may be necessary in patients who have MDR infection, massive hemoptysis (after embolization has failed) and in the management of tuberculous empyema, draining sinuses and bronchopleural fistulas. Surgery has been widely used in the treatment of spinal TB but is only indicated for the presence of progressive neurologic abnormality, spinal instability and to drain large paravertebral abscesses in which CT-guided drainage is not possible. Surgery may be required after destructive TB involving weight-bearing joints. Surgery may have an adjuvant role in nodal TB if a fluctuant mass persists. In laryngeal infection, temporary tracheostomy may be necessary and a few patients may require complex surgery such as partial laryngectomy.

Immunotherapy and the future

Drugs remain at the forefront in the treatment of TB but new compounds are urgently needed. The long-term aim must be to produce true short-course therapy of a few weeks' rather than months' duration. Novel approaches are required in the face of drug resistance. The cytokines interferon-? and IL-2 have been used with success in *M. leprae* infection and such compounds have potential in TB. At present, it is only possible to speculate on other options such as encouraging cellular recruitment to areas of infection, but it remains vital that the necessary basic research is supported.

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Chapter 38 - Nontuberculosis Mycobacteria

David E Griffith

INTRODUCTION

The nontuberculous mycobacteria (NTM) encompass all mycobacterial species that are not included in the *Mycobacterium tuberculosis* complex. There are more than 100 species, most of which do not or only rarely cause human disease. A few NTM species, however, are important pathogens in both immunocompetent and immunocompromised hosts. In the past, patients with NTM disease were described as having 'atypical tuberculosis', regardless of the site of disease or the NTM species isolated. Because of the need for specific therapy directed at individual NTM species, 'atypical tuberculosis' or even 'atypical mycobacteria' have become obsolete. Clinicians must now be knowledgeable about the virulence (i.e. potential for human disease), sites of infection and treatment of individual NTM species.

Nontuberculous mycobacteria classification systems have generally not been helpful to the clinician. The most widely used classification scheme in the past, the Runyon system, was based on microbiologic characteristics of the organisms, such as rate of growth and colony pigment formation. Familiarity with the Runyon system remains useful for presumptive laboratory identification of possible NTM pathogens; however, positive identification of NTM species is now largely based on biochemical and molecular biologic techniques. Classification of NTMs based on the organ system of primary involvement (lung, lymph node, disseminated, skin and soft tissue) is more useful to the clinician and is used in this chapter.[1] [2]

EPIDEMIOLOGY

The prevalence of NTM infection can only be estimated because NTM infections are not reportable and the clinical significance is not always clear. There is evidence that the prevalence of NTM disease is increasing, in both the AIDS and non-AIDS populations. In the USA, two surveys of NTM isolates during the years 1979–83 have been published.[3] [4] Using data from these national surveillance studies, the prevalence of NTM disease, largely pulmonary, was estimated to be 1.8/100,000 population. *Mycobacterium avium* complex (MAC) accounted for 1.1/100,000 population, followed in frequency by '*Mycobacterium fortuitum* complex' and *Mycobacterium kansasii*. These prevalence figures are, at best, estimates as the surveys were not comprehensive and the authors could only estimate the number of clinically significant NTM isolates.

A more recent study, covering the period 1991–92, demonstrated an apparent increase in the prevalence of NTM infection in the USA.[5] Despite the increase in *M. tuberculosis* isolates noted in the USA during these years, there were more isolates of MAC than of *M. tuberculosis* in this study. The emergence of disseminated NTM disease in AIDS patients probably had a significant impact on the increased number of NTM isolates in the early 1990s. A bimodal age distribution in NTM isolates was found, with peaks in young adults and in elderly patients, probably reflecting the age peaks in prevalence for disseminated and pulmonary NTM infections.[5] Before the advent of highly active antiretroviral therapy, disseminated NTM infection was almost entirely due to MAC and occurred in 20–40% of AIDS patients.[6] [7]

There is a consensus among investigators in the field that the number of significant NTM isolates continues to rise, especially MAC lung disease, and that the number of immunocompetent patients with NTM disease (primarily lung disease) is increasing. The factors that may be contributing to this increase include:

- an increased total number of clinical specimens submitted for acid-fast bacilli (AFB) analysis;
- better clinical recognition of NTM disease, especially chronic pulmonary disease, in immunocompetent patients; and
- improved laboratory techniques, which have made identification and isolation of NTM more accurate and rapid.

Nontuberculous mycobacterial disease prevalence worldwide is also difficult to estimate but may show geographic clusters (see Table 38.1). For instance, *Mycobacterium xenopi*, a rare pathogen in the USA, is the second most common cause of NTM lung disease in areas of Canada and the UK.[8] *Mycobacterium malmoense*, also a rare pathogen in the USA, is the second most common cause of NTM lung disease in Scandinavia and other areas of northern Europe.[9]

Although MAC disease has a worldwide distribution, disseminated MAC is rarely seen in people from central Africa who have AIDS, even though MAC can be recovered from that environment.[10] One possible explanation is that people who have AIDS in Africa may die from infection with other pathogens before their immunosuppression becomes severe enough for them to develop disseminated MAC.

PATHOGENESIS AND PATHOLOGY

Evidence is mounting to show that the environment is the major source of human NTM infection. Nontuberculous mycobacteria are ubiquitous in the environment and have been isolated from water, soil, dust, domestic and wild animals, milk and food. It is now generally accepted, however, that water is the source of infection for most NTM infections in both immunocompetent and immunocompromised hosts. [2]

DNA fingerprinting techniques that include restriction fragment length polymorphism analysis by pulsed field gel electrophoresis and major polymorphic tandem repeat probe sequence analysis have been especially useful for epidemiologic investigation (Fig. 38.1 and Fig. 38.2). These techniques also may provide clues to pathophysiologic differences between NTM species. For instance, there appears to be great genetic variability between MAC isolates from different patients and sometimes even from the same patient (see Fig. 38.2).[11] In contrast, clinical *M. kansasii* isolates generally have very closely related genotypes, suggesting that most clinical isolates are clonal.[12] This clonal nature of most clinical isolates of *M. kansasii* would seem very unusual for an environmental species (such as MAC), and suggests that their colonization of environmental sites of human disease acquisition (e.g. municipal water supplies) is quite recent and involves only select genotypes.

Pulmonary disease

The evidence for environmental transmission of MAC in pulmonary disease is mostly circumstantial. It grows well in natural waters and is readily aerosolized above bodies of water.[13] *Mycobacterium avium* complex strains with plasmids, possibly associated with virulence, have been shown to be preferentially aerosolized.[14] Recovery rates of MAC from the environment in the USA are highest in the states bordering the Atlantic Ocean and the Gulf of Mexico and this is also the area with the highest rates of MAC infection.[15] In a skin

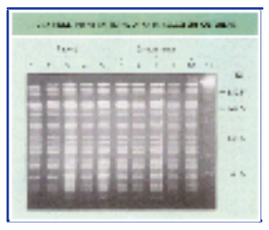

Figure 38-1 DNA fingerprint patterns of 10 Mycobacterium abscessus isolates from an outbreak of M. abscessus infection in a hemodialysis center. Lanes 1–5 are identical strains of *M. abscessus* recovered in blood cultures from five different patients, indicating a common source of infection. Lanes 6–10 are the same strain of *M. abscessus* recovered by sampling a water treatment system contaminated by *M. abscessus*, the source of the patients' isolates. *Courtesy of Dr Yansheng Zhang and Dr Richard J Wallace Jr.*

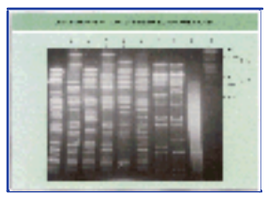

Figure 38-2 DNA fingerprint patterns of nine *Mycobacterium avium* complex (MAC) respiratory isolates (lanes 1–9) collected over an approximately 7-year period from a 67-year-old woman with nodular- bronchiectatic MAC lung disease. Each of the MAC isolates has a distinct DNA fingerprint pattern that suggests repeated reinfection by new MAC strains rather than relapse of infection from a single MAC strain.

test survey performed on 275,000 naval recruits, each of whom had resided from birth in a single county, positive reaction to an MAC skin test antigen (purified protein derivative (PPD)-B) was heavily distributed among those who had lived along the Gulf Coast and Southern Atlantic states.[16] Although the skin test is not specific for MAC, these results support the correlation between geographic environmental exposure and infection in humans.

Pulmonary infection by *M. kansasii* also probably occurs via an aerosol route. Whereas MAC appears to be acquired in rural areas, *M. kansasii* is more likely acquired in urban environments.[17] *Mycobacterium kansasii* isolates of the same phage type as those isolated from patients have been recovered from urban drinking water distribution systems.[18] Clinical isolates and tap water isolates of the same genotype, determined by DNA fingerprinting, have also been identified.[19]

Isolation of *M. kansasii* from tap water can be intermittent, which may explain why some investigators have failed recover it from that source. No other environmental (water or soil) source of *M. kansasii* has been identified.

Disseminated disease

Environmental exposure to NTM in people who have AIDS also probably occurs from aqueous sources, resulting in gastrointestinal or pulmonary acquisition of NTM, usually MAC. Following ingestion of NTM there is direct tissue invasion and, at some point with a declining host immunity, NTM bacteremia. One study using DNA fingerprinting directly linked an environmental source of MAC (hospital tap water) with clinical disseminated MAC disease in some people who had AIDS who were exposed to this water source.[20]

Localized skin and soft tissue infections

Skin and soft tissue infections in immunocompetent patients are probably the result of direct exposure to contaminated water sources through inoculation after accidental trauma, surgery or injection. *Mycobacterium marinum* inhabits water and marine organisms. Infection in humans may be introduced into the skin:

- during cleaning of fresh water fish tanks ('fish tank granuloma');
- by scratches or puncture wounds from organisms such as saltwater fish and shrimp and from fins; and

- through abrasions in nonchlorinated swimming pools ('swimming pool granuloma').

Mycobacterium xenopi has been recovered from hot water taps within hospitals, and in one study a clinical isolate and hospital water isolates were found to be identical by DNA fingerprint analysis.[21] Investigations of nosocomial outbreaks caused by *M. fortuitum, Mycobacterium chelonae* and *Mycobacterium abscessus*, including the use of DNA fingerprinting, have demonstrated that tap water, ice prepared from tap water, processed tap water used for dialysis and distilled water used for preparing solutions are the source of these organisms in nosocomially acquired infection (see Fig. 38.1).[22] [23]

Transmission

There is no firm evidence of human-to-human or animal-to-human transmission of NTM disease. Although family groupings of NTM infection have occasionally occurred in humans, these groupings have been assumed to be due to a common environmental exposure. *Mycobacterium avium* complex is an important cause of disease in poultry and swine, but recent DNA fingerprint studies have shown that strains infecting human and animals are different.[24] [25]

Histopathology

Nontuberculous mycobacterial disease is characterized histopathologically by the presence of caseating and noncaseating granulomatous inflammation, epithelial histiocytes and occasional giant cells. Nontuberculous mycobacterial infection cannot be differentiated histopathologically from tuberculosis. Poorly formed granulomas with histiocytic reactions are more commonly reported in immunodeficient patients, especially those who have AIDS, but they can be seen in immunocompetent patients because not all NTM stimulate granuloma formation equally well.

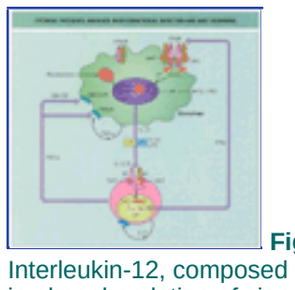

Figure 38-3 Cytokine pathways involved in mycobacterial infection and host response. Mycobacteria infect macrophages and stimulate production of interleukin (IL)-12. Interleukin-12, composed of p35 and p40 subunits, recognizes its receptor on T cells or natural killer (NK) lymhocytes, causing phosphorylation of the cytoplasmic kinases, Tyk2 and Jak2. This results in phosphorylation of signal transducer and activator of transcription (STAT)4, leading to production of interferon (IFN)-?, tumor necrosis factor (TNF)-a and granulocyte-macrophage colony stimulation factor (GM-CSF). Interleukin-12 also stimulates production of IL-2, which feeds back on the T or NK lymphocyte. Interferon-? binds to its receptor on the macrophage, causing aggregation of IFN-?R1 and IFN-?R2 and phosphorylation of the cytoplasmic Janus kinases, Jak1 and Jak2. Consequently, cytosolic STAT1 is phosphorylated, homodimerized and transported to the nucleus, where it upregulates IFN-? responsive genes. Interferon-? and GM-CSF stimulate macrophages to produce TNF-a, which in conjunction with IFN-? drives forward the production of IL-12 and reactive oxygen intermediates, such as superoxide and nitric oxide. *Adapted with permission from Holland SM. Host susceptibility factors in mycobacterial infection: genetics and body morphotype. Infect Dis Clin North Am 2002;16:163–86.*

Interaction between nontuberculous mycobacteria and host defenses

An understanding of the interaction between NTM and host defenses is emerging. The critical interaction, which determines whether NTM infection is established, is between the mycobacterium and the macrophage. Mycobacteria are successful intracellular pathogens that not only penetrate host defense cells but also replicate within them. The mycobacterial cell wall, composed of glycopeptidolipids, including lipoarabinomannan, is probably a key element responsible for the survival and multiplication of NTM within the hostile intracellular environment of the macrophage.[26] Organisms engulfed by the macrophages are taken into intracellular vacuoles, and the prevention of acidification of the intracellular vacuole may be an important mechanism of mycobacterial evasion of host defenses and a virulence factor.[27] If the organism persists and multiplies within the macrophage, then a lymphocyte-mediated immune response, including $CD4^+$ T cells and natural killer (NK) cells, is triggered by the infected macrophage. The role of neutrophils in controlling NTM infection is not clear. The expanded immune response may result in augmented killing of intracellular mycobacteria by the macrophage or destruction of the infected macrophage itself by NK cells. The macrophage-lymphocyte interaction may result in granuloma formation and ultimately determines whether the NTM infection is limited (i.e. controlled) or whether it progresses.

Infected macrophages and stimulated lymphocytes produce soluble factors, including cytokines and prostaglandins, that modulate a complicated immune interaction (Fig. 38.3). Important components of this immune response to mycobacteria include:

- interleukin (IL)-12;
- tumor necrosis factor (TNF)-a;

- interferon (IFN)-?;
- IL-2, IL-6 and IL-10; and

¦ granulocyte-macrophage colony stimulating factor.

Macrophages from normal hosts that phagocytose mycobacteria respond with production of IL-12,[28] which activates T-cells and NK cells through binding to the IL-12 receptor.[29] [30] Binding results in phosphorylation of associated cytoplasmic kinases, which in turn lead to transcription of IL-12 responsive genes, in particular IFN-?. Interferon-? activates neutrophils and macrophages to produce superoxide and nitric oxide, increase surface display of major histocompatibility complex (MHC) molecules and Fc receptors, decrease lysosomal pH and increase the intracellular concentration of certain antibiotics. [31] [32] [33] Interferon-? signals ultimately influence IFN-? responsive genes such as IL-12, MHC genes and TNF-a.[32] [34] The positive feedback loop between IFN-? and IL-12 is pivotal in the immune response to mycobacteria and other intracellular infections. Defects in any of these receptors or cytokine genes may negatively affect the production of IFN-? or IL-12 (or both) and consequently enhance mycobacterial susceptibilitly.[35]

Tumor necrosis factor-a is produced by activated macrophages and NK cells and is also an important immunomodulator for controlling mycobacterial infection. Tumor necrosis factor-a has a significant additive effect on macrophage killing of MAC.[36] The release of TNF-a is stimulated by and is probably responsible for the antimycobacterial effects of IFN-?.[36] Interleukin-2 greatly augments the capacity of NK cells to lyse MAC-infected monocytes, and IL-2-stimulated NK cells also enhance intracellular monocyte killing of MAC, again probably via TNF-a. [37] As noted above, IL-12 is a major stimulant for T cells and NK cells to produce IFN-? and TNF-a and, in turn, IL-12 release is stimulated by IFN-? and TNF-a.[38] [39] Granulocyte-macrophage colony stimulating factor is produced by MAC-infected monocytes and NK cells, and it appears to augment mycobacterial killing.[40] In contrast, IL-6 and IL-10 down-modulate the inflammatory response, especially the effect of TNF-a, and are therefore permissive factors for proliferation of intracellular mycobacteria.[41] [42]

These immune modulators act in a complicated fashion and it is difficult to isolate and measure in vivo any single aspect of the inflammatory cascade. Nevertheless, this line of investigation provides important clues to NTM disease pathogenesis. A practical application of this research is illustrated by a family with a monocyte defect in IL-12 production that was associated with disseminated MAC infection in the absence of HIV infection. This defect was overcome by exogenous administration of IFN-? in addition to other antimycobacterial therapy, resulting in successful treatment of the disseminated MAC infection.[43] [44] Recent identification of specific genetic defects in the IFN and IL-12 response pathways, especially receptor deficiencies, has elucidated key pathways in immune surveillance and NTM control and identified other patients at risk of disseminated NTM infection.[45] To date, however, no immune deficiency has been identified for patients with NTM lung disease.

Body morphotype is another possible predisposition to NTM lung disease. Some patients with pulmonary NTM infections (nodular disease associated with bronchiectasis) appear to have similar clinical characteristics and body types, including scoliosis, pectus excavatum, mitral valve prolapse and joint hypermobility. [46] [47] The constellation of phenotypic abnormalities seen in pulmonary NTM disease may represent a *forme fruste* of a connective tissue disease.

Because most NTM are nonpathogenic, there is intense interest in identifying virulence factors for NTM. Plasmids may encode virulence genes and are more common in MAC isolates from people who have AIDS than in environmental isolates. [48] [49] Other potential virulence factors include:

¦ prevention of acidification of phagocytic vesicles,[27]
¦ prevention of phagosome-lysosome fusion,[50]
¦ delay in TNF secretion by infected host cells,[51] and
¦ catalase activity.[52]

Which factor or factors are most important and whether they can be favorably modified remains to be determined.

CLINICAL FEATURES

Pulmonary disease

Chronic pulmonary disease in the most common clinical manifestation of NTM infection in the USA. It is unclear whether the increased numbers of respiratory NTM isolates recently observed is due to an increasing prevalence of disease or better recognition of existing disease.[4] Several NTM species can cause lung disease in the immunocompetent host (Table 38.1); however, MAC, *M. kansasii* and *M. abscessus* are the most common respiratory pathogens.[2] Whether these ostensibly immunocompetent hosts have an unrecognized defect in host defences is unknown.

Signs and symptoms of NTM pulmonary disease are variable and non-specific and include chronic cough, sputum production and fatigue. Malaise, dyspnea, fever, hemoptysis and weight loss can also

TABLE 38-1 -- Nontuberculous mycobacteria that cause pulmonary disease in the immunocompetent host.

NTM THAT CAUSE PULMONARY DISEASE IN THE IMMUNOCOMPETENT HOST		
Mycobacteria		**Clinical comment**
Common	*Mycobacterium avium* complex	Cavitary disease in male cigarette smokers with COPD; nodular/bronchiectatic disease in elderly nonsmoking women; southeast USA, worldwide
	Mycobacterium kansasii	Clinically and radiographically closely resembles tuberculosis; south central USA, UK, Europe
	Mycobacterium abscessus	Clinically resembles nodular/bronchiectatic form of MAC lung disease; southeast USA
Uncommon	*Mycobacterium fortuitum*	Single isolate usually not clinically significant; unusual lung pathogen except in the setting of gastroesophageal disease with chronic aspiration
	Mycobacterium xenopi	Canada, UK, Europe
	Mycobacterium simiae	Single isolate frequently not significant; southwest USA, Israel
	Mycobacterium szulgai	One culture positive specimen adequate for diagnosis
	Mycobacterium malmoense	One culture positive specimen adequate for diagnosis; Scandinavia, northern Europe
Rare	*Mycobacterium celatum*	
	Mycobacterium asiaticum	
	Mycobacterium shimodei	
NTM that usually represent contamination of respiratory specimens from immune competent hosts: *M. gordonae*, *M. genavense*, *M. smegmatis*, *M. nonchromogenicum*, *M. haemophilum*, *M. vaccae*, *M. thermoresistable*, *M. flavescens*		
COPD, chronic obstructive pulmonary disease.		

occur, usually with advanced disease, but are less common than with tuberculosis.

Nontuberculous mycobacterial lung disease produces variable radiographic features, ranging from apical cavitary lung disease, typical of reactivation tuberculosis, to nodular disease associated with bronchiectasis. Although there are differences in the radiographic features of NTM lung disease compared with those of *M. tuberculosis*, there are no radiographic findings that discriminate with certainty between tuberculosis and NTM lung infection. Similarly, it is difficult to differentiate, on either a clinical or radiographic basis, between NTM species causing lung disease.

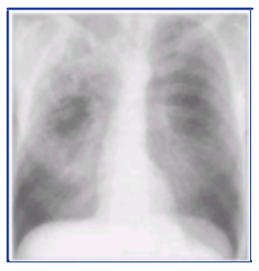

Figure 38-4 Apical cavitary infiltrates similar to those caused by pulmonary tuberculosis in a 60-year-old male cigarette smoker with *Mycobacterium avium* complex lung disease.

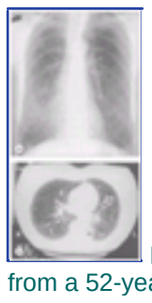

Figure 38-5 *Mycobacterium avium* complex lung disease. (a) Interstitial nodular midlung field infiltrates (right>left) in a 64-year-old female with MAC lung disease. (b) Chest CT scan from a 52-year-old woman with MAC lung disease demonstrating three abnormalities that are common in MAC lung disease: bronchiectasis, a cavity and small (<5mm) nodules.

Mycobacterium avium *complex infection*

Mycobacterium avium complex is the most common cause of NTM lung disease. In the majority of patients in the USA, MAC lung disease is due to *Mycobacterium intracellulare*; in other geographic areas *M. avium* infection is equally common.[53] [54] Lung disease caused by MAC has traditionally been diagnosed in middle-aged or older men, usually with a history of cigarette smoking and underlying lung disease such as chronic obstructive pulmonary disease, previous tuberculosis, pneumoconiosis or bronchiectasis.[17] [55] The majority of these patients have cavitary changes on chest radiography (Fig. 38.4). This form of disease can be aggressive and causes extensive lung destruction.

It is now clear that MAC lung disease has a more heterogeneous clinical presentation, in particular in elderly female nonsmokers who have no known underlying lung disease.[56] These patients present radiographically with mid- and lower-lung field disease characterized by a combination of discrete, small (<5mm) pulmonary nodules and accompanying bronchiectasis — abnormalities that are especially apparent with high-resolution computerized tomography (CT) of the chest (Fig. 38.5). [57] Because this form of disease is radiographically atypical for mycobacterial disease, diagnosis may be delayed, even in patients who have persistent cough and progressive radiographic abnormalities. Disease progression is usually indolent; however, this form of MAC lung disease can be associated with significant morbidity and mortality.[56]

It has also become apparent recently that patients with noncavitary MAC lung disease who are infected by one MAC genotype can be reinfected by another MAC genotype (i.e. polyclonal infections are possible; see Fig. 38.2).[11] These patients would previously have been considered treatment failures, but in fact they are actually reinfected by new MAC strains. This phenomenon complicates the evaluation of a patient who has successfully completed therapy but has sputum that again becomes culture-positive for MAC.

Mycobacterium kansasii *infection*

Mycobacterium kansasii produces pulmonary disease that most closely parallels clinical disease caused by *M. tuberculosis*. Patients with *M. kansasii* lung disease are characteristically older men from urban environments who are cigarette smokers with one or more underlying pulmonary diseases including chronic obstructive pulmonary disease, previous tuberculosis, bronchiectasis or pneumoconiosis.[17] The chest radiographic changes are also very similar to those of reactivated pulmonary tuberculosis, with an upper lobe predilection and cavitation in approximately 90% of patients (Fig. 38.6).

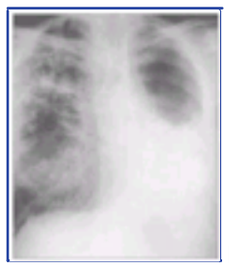

Figure 38-6 Far-advanced *Mycobacterium kansasii* bilateral lung disease in a 42-year-old male cigarette smoker with extensive cavitary destruction of the left upper lobe.

Some patients with noncavitary disease (similar to those with MAC lung disease) have also been identified with *M. kansasii* disease.

Mycobacterium abscessus *infection*

Patients who have *M. abscessus* lung disease are typically elderly female nonsmokers with no known underlying or predisposing lung disease.[58] This disease clinically and radiographically most closely resembles noncavitary (nodular bronchiectatic) pulmonary MAC disease. *Mycobacterium abscessus* and MAC are sometimes isolated concurrently or consecutively in some patients.[58]

Lymphadenitis

Mycobacterial lymphadenitis presents as an insidious, painless, unilateral process involving one or more nodes in a regional distribution with only rare associated systemic symptoms. Upper anterior cervical, submandibular, submaxillary and preauricular lymph nodes are the most commonly involved. Lymphadenitis is the most common disease manifestation of NTM in children. This presentation is most commonly due to MAC (in 60–80% of cases), whereas tuberculosis accounts for only 10–20% of mycobacterial lymphadenitis in this age group.[59] The predominance of MAC is a change from 20 years ago when most geographic areas reported *Mycobacterium scrofulaceum* as the most common etiologic agent.[46] In contrast, mycobacterial lymphadenitis in people over 12 years of age is due to *M. tuberculosis* in approximately 95% of cases. In children, and especially adults, the main differential diagnosis is therefore tuberculosis.

Nontuberculous mycobacterial lymphadenopathy is not usually associated with a history of tuberculosis exposure; tuberculin skin testing in the patient and family are negative and the chest radiograph is normal. Confirming the diagnosis may be difficult because aspiration, biopsy and culture of the involved nodes yields positive cultures in only approximately 50% of the cases.[60] Other NTM that are uncommon causes of lymphadenitis include *M. malmoense, M. fortuitum, M. chelonae, M. abscessus, M. kansasii* and *Mycobacterium haemophilum*.

Disseminated disease

Disseminated NTM disease is most commonly encountered in people who have AIDS with advanced immunosuppression, but it can also occur in people who have severe immunosuppression unrelated to AIDS, such as occurs as a result of chronic corticosteroid use, organ transplantation management or leukemia. More than 95% of disseminated NTM disease in people who have AIDS is due to isolates of MAC. In contrast to MAC pulmonary disease, 90% of MAC isolates in disseminated disease are *M. avium*.[54] [61] Polyclonal MAC infection

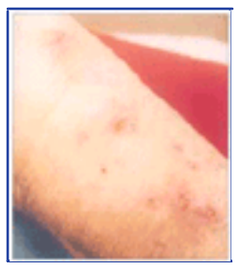

Figure 38-7 Disseminated *Mycobacterium chelonae* disease. This manifests itself here by subcutaneous nodules on the lower extremities in an 81-year-old woman receiving high-dose corticosteroids for rheumatoid arthritis.

in AIDS patients appears common and may occur in up to 20% of patients with disseminated MAC infection.[62] Other NTM species also produce disseminated disease, including *M. kansasii, M. chelonae, M. abscessus, M. xenopi, M. malmoense, M. genavense, M. conspicuum, M. gordonae* and *M. haemophilum*. Although disseminated MAC disease presents as a febrile wasting illness, disseminated disease caused by *M. chelonae, M. abscessus* and *M. haemophilum* may present as diffuse subcutaneous nodules or abscesses (Fig. 38.7). Some NTM isolates that cause disseminated disease in people who have AIDS have been considered nonpathogenic when isolated from immunocompetent hosts (see Table 38.1); all NTM isolates from people who are severely immunocompromised, and especially from people who have AIDS, should be regarded as potential pathogens, at least initially.

Disseminated MAC disease in people who have AIDS accompanies severe immunosuppression, with the average $CD4^+$ lymphocyte count at the time of dissemination being approximately 25/mm^3 . Patients who have less than 100 $CD4^+$ lymphocytes/mm^3 and who are not receiving prophylaxis develop disseminated MAC at a rate of approximately 20% per year.[61] Presenting symptoms usually include several weeks of fever, sweats, diarrhea and wasting. Nausea, vomiting and intractable abdominal pain are indicative of the gastrointestinal involvement that frequently occurs. Physical findings include weight loss, hepatosplenomegaly and intra-abdominal lymphadenopathy. Worsening anemia and an elevated alkaline phosphatase level that is out of proportion to hepatic transaminase elevation occur late with continuous bacteremia. Localized pulmonary disease caused by MAC occurs in fewer than 5% of people who have AIDS with MAC infection (see also Chapter 129).[63]

Mycobacterium kansasii infection in AIDS patients, unlike MAC infection, frequently causes disease with the same symptomatology as in people who are immunocompetent.[64] It will disseminate in approximately 35% of AIDS patients who have the infection, usually in association with far-advanced pulmonary disease. Radiographic manifestations of *M. kansasii* pulmonary disease in people who have AIDS are somewhat atypical, with noncavitary changes in approximately 50% of patients. Clinical symptomatology is non-specific and similar to that of disseminated MAC infection; however, disseminated *M. kansasii* may also present with multiple subcutaneous nodules. As in disseminated MAC disease, $CD4^+$ lymphocyte counts average 24/mm^3 in patients with disseminated *M. kansasii* disease, and not surprisingly these patients may have coexistent disseminated MAC infection.

Nontuberculosis mycobacteria rarely cause infection of the central nervous system (CNS) but the most common setting for NTM CNS involvement is as part of disseminated disease in a patient with severe immunocompromise, such as seen with advanced AIDS[65] in which MAC, *M. kansasii, M. gordonae* and *M. genavense* predominate. A very small number of cases of NTM CNS infections have been reported in nonimmunocompromised patients, especially as a result of *M. fortuitum* infection and associated with trauma, surgery, or chronic infections (otitis, mastoiditis). Presenting symptoms for NTM CNS infections are variable and include neuropsychiatric symptoms, headaches, altered mental status, meningismus, cranial nerve abnormalities and problems with co-ordination. Not suprisingly these patients also sometimes have concomitant CNS infections caused by other pathogens.

Differential diagnosis

Disseminated NTM disease must be differential from other infections complicating advanced AIDS. These include disseminated cytomegalovirus, histoplasmosis and, of course, tuberculosis. *Mycobacterium tuberculosis* infection tends to occur earlier in the course of AIDS than NTM infection does, but there are no reliable clinical indicators to distinguish

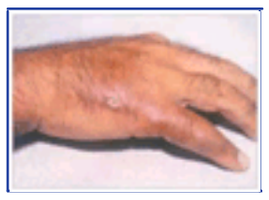

Figure 38-8 Nodular lesions on the hand caused by *Mycobacterium marinum.* This 45-year old man contracted *M. marinum* after penetrating trauma while cleaning his boat in salt water.

between disseminated NTM and disseminated tuberculosis. In general, a positive AFB specimen from a person who has AIDS must be assumed to be due to tuberculosis until proven otherwise (see Chapter 129).

Localized skin and soft tissue infection

Localized abscess formation after direct inoculation of organisms is most often due to *M. fortuitum, M. abscessus* or *M. chelonae*.[2] Nosocomial skin and soft tissue disease caused by these three species includes infections of long-term intravenous or peritoneal catheters, postinjection abscesses and surgical wound infections, including those after augmentation mammoplasty or cardiac bypass surgery. *Mycobacterium marinum*, MAC, *M. fortuitum, M. abscessus, M. chelonae* and *M. kansasii* have all been implicated in chronic granulomatous infection in tendon sheaths, bursae, joints and bones. *Mycobacterium haemophilum, M. nonchromogenicum* and *M. smegmatis* are rare causes of localized skin and soft tissue disease.

Mycobacterium marinum produces solitary papules on an extremity, especially on the elbows, knees and dorsa of the feet and hands, and subsequently progresses to shallow ulceration and scar formation (Fig. 38.8). Occasionally, multiple 'ascending' subcutaneous lesions resembling sporotrichosis may develop. *Mycobacterium marinum* requires low temperature incubation for isolation; if it is suspected, the laboratory should be notified. Disseminated infection by *M. marinum* has occurred in people who have AIDS and is presumably acquired in the same fashion by these patients as it is by immunocompetent hosts.

Mycobacterium ulcerans causes indolent necrotic lesions of the skin and underlying tissue. The lesions occur most commonly in children and young adults and can result in severe deformities of the extremities if left untreated. *Mycobacterium ulcerans* infections occur in tropical areas of the world, including tropical parts of Australia, but have not been reported in the USA (see Chapter 147).

DIAGNOSIS

All mycobacteria are 'acid-fast' and the fluorochrome method (auramine stain) is the preferred method for microscopic recognition of NTM in clinical samples. The appearance of NTM by microscopy is sometimes indistinguishable from that of *M. tuberculosis*, and therefore confirmation of the presence of NTM still requires cultures. Cultures should be inoculated onto one or more solid media (e.g. Lowenstein-Jensen or Middlebrook 7H10 or 7H11) and into a liquid medium as well, given the more rapid recovery of all mycobacteria in broth systems such as the BACTEC system. All skin or soft tissue samples should be incubated at 95°F (35°C) and at 82.4–89.6°F (28–32°C), because a number of pathogens that infect these tissues, including *M. haemophilum* and *M. marinum*, may grow only at the lower temperatures.

The majority of clinical and public health laboratories now use one or more rapid diagnostic methods for mycobacterial species identification. These rapid methods include high-performance liquid chromatography and commercial DNA probes, which are available for identifying isolates of *M. tuberculosis, M. gordonae, M. kansasii, M. avium* and *M. intracellulare*. These probes are highly sensitive and specific and can provide species identification using a culture directly from both media.

A variety of skin test reagents have been prepared from various species of NTM, including PPD-A from *M. avium* and PPD-B from *M. intracellulare*; however, they are not specific, lack standardization and are not clinically useful in the diagnosis of NTM disease. The use of NTM skin test reagents is currently confined to epidemiologic studies.

Pulmonary disease

Unlike the situation in tuberculosis, a single positive NTM culture from a respiratory specimen, especially if it contains only a small number of organisms, is not always

sufficient to diagnose NTM lung disease or initiate therapy. No single diagnostic algorithm is satisfactory for interpreting the clinical significance of all NTM respiratory isolates. For example, a single positive sputum culture for *M. kansasii* in the appropriate setting is diagnostic. The same is probably true for *M. abscessus, M. szulgai* and *M. malmoense*, and possibly even for MAC. For relatively nonvirulent pathogens, such as *M. fortuitum* and *M. simiae*, a single isolate from sputum is almost never adequate to confirm the diagnosis. Determining the significance of a respiratory NTM isolate requires knowledge of:

- the virulence of the NTM isolated;
- the frequency and quantity with which the NTM was isolated;
- the patient's symptoms, chest radiograph appearance and comorbid conditions; and
- the rapidity of disease progression.

With increasing experience, the process will inevitably evolve to the point that diagnostic criteria will emerge for each NTM species, thereby eliminating the need for 'universal' NTM diagnostic criteria.

Diagnostic criteria for NTM lung disease in the past discriminated between patients with cavitary and noncavitary disease, with more diagnostically rigorous criteria applied to noncavitary disease.[1] Recently proposed diagnostic criteria are applicable to all patients with suspected NTM lung disease regardless of radiographic presentation (Table 38.2).[2]

TABLE 38-2 -- Proposed diagnostic criteria for nontuberculous mycobacterial pulmonary disease.*

PROPOSED DIAGNOSTIC CRITERIA FOR NTM PULMONARY DISEASE
Compatible clinical presentation based on symptoms, radiographic findings (chest radiograph or high-resolution CT scan) and exclusion of other diagnoses
Collection of at least three sputum and/or bronchial wash specimens
One culture-positive sputum or bronchial washing that is either heavily (2+ or greater) smear positive or heavily (2+ or greater) culture positive
or
One culture-positive sputum or bronchial washing associated with multiple smear-positive specimens
or
Multiple positive cultures (at least three) over 1 year, regardless of smear positivity
For unusual radiographic presentation or nondiagnostic sputum analysis, lung biopsy (bronchoscopy with transbronchial biopsy) demonstrating granulomatous inflammation or culture positive for NTM
In questionable cases, obtain expert consultation.

* *Adapted from Wallace et al.*[2]

The term 'colonization' has been used to describe the intermittent or occasional isolation of NTM from respiratory specimens. The significance of 'colonization' in this setting, with its implied benignity, has never been rigorously tested or validated. It may not be clear, for instance, whether a single isolate of an NTM from sputum is due to 'colonization' (superficial or noninvasive infection), slowly progressive or indolent infection, or contamination of the cultured specimen by an NTM from the environment of the patient or of the laboratory. In the case of NTM species that are known to cause pulmonary disease, it is the responsibility of the patient's physician to ensure that a patient with slowly progressive disease is not misidentified as being 'colonized' by the NTM. Isolation of a potential pulmonary NTM pathogen may require the clinician to follow the patient with serial sputum AFB analysis and periodic chest radiographs over a long period of time, perhaps years, in order to judge the significance of the isolated NTM. The worst mistake a clinician can make in managing a patient with NTM lung disease is not a short-term delay in starting therapy, but a failure to provide appropriate clinical and laboratory follow-up for a patient who then has significant progression of NTM lung disease.

Lymph node disease

Nontuberculous mycobacteria disease in lymph nodes is diagnosed by culture of NTM infection in the presence of granulomatous inflammation found on biopsy of infected tissue. The use of fine-needle aspiration for obtaining diagnostic material is controversial because it will yield a positive culture in only 50% of cases.[60] Additionally, a simple diagnostic biopsy or incision and drainage of the involved lymph nodes can be followed by fistula formation with chronic drainage. Because complete excision of involved nodes constitutes adequate therapy for most NTM lymphadenitis, it may also be an appropriate diagnostic procedure.

Disseminated disease

Diagnosis of disseminated NTM infection in people who are immunocompromised, including those who have AIDS, is made by culture of organisms from sterile closed sites, such as blood, liver or bone marrow, or from biopsy of a skin lesion. A single positive blood culture is considered diagnostic of disseminated NTM in an AIDS patient. The isolation of MAC from the sputum or stool of a patient who has AIDS is not diagnostic for disseminated MAC infection; however, the presence of MAC in these specimens is a harbinger for disseminated MAC.[66]

The diagnosis of NTM CNS involvement is difficult, and therefore it is extremely important to suspect the diagnosis on the basis of the clinical setting. [65] Nontuberculosis mycobacteria are rarely found on routine AFB smear analysis of cerebrospinal fluid (CSF), so that a positive AFB culture is usually required to make the diagnosis. The role of rapid diagnostic techniques applied to CSF, such as high performance liquid chromatography analysis, is unknown. The CSF in NTM disease usually shows an elevated white blood cell count with either a neutrophilic or lymphocytic predominance. Cerebrospinal fluid protein and glucose values may vary widely from within normal limits to far outside the normal range. Radiographic evaluation of NTM CNS involvement is usually not diagnostic and can be complicated by radiographic abnormalities caused by concomitant CNS infections due to other pathogens.

Localized skin and soft tissue infections

Diagnosis of NTM infection in skin, soft tissue, bones and joints is made by culture of the specific pathogen from drainage material or tissue biopsy. It is important from a therapeutic standpoint to differentiate between skin and soft tissue lesions related to penetrating injury and those associated with disseminated NTM infection.

MANAGEMENT

The introduction of agents such as clarithromycin, azithromycin and rifabutin has dramatically improved the treatment outcome for some NTM infections. Treatment recommendations for NTM diseases in both immunocompetent and immunocompromised hosts will probably continue to evolve as new agents with activity against NTM are introduced; therefore, recommendations in this chapter may soon become outdated.

The treatment of NTM infections is complicated by the observation that response to therapy for some NTM infections does not correlate with in-vitro drug susceptibilities, especially to antituberculous drugs. Treatment of NTM infections is therefore not as simple or dependent on in-vitro drug susceptibilities as the treatment of tuberculosis; some familiarity with the treatment idiosyncrasies of each NTM is necessary. For instance, clinical response to multidrug antituberculous regimens for *M. kansasii* correlates only with in-vitro susceptibility to rifampin (rifampicin). Response of MAC disease to antituberculous drug regimens in the past frequently had no correlation with in-vitro drug susceptibilities. In contrast, there is a strong correlation between successful treatment of NTM infections, including MAC infection, with clarithromycin and azithromycin and the in-vitro macrolide susceptibility of the specific NTM. Patients with either pulmonary or disseminated MAC infection that is resistant to macrolides will not respond favorably to macrolide-containing regimens.[53] [67] Clarithromycin monotherapy for disseminated or pulmonary MAC disease is associated with a genetic mutation conferring resistance to macrolides and should therefore be avoided.[68]

Recommendations for treating NTM pulmonary disease are given in Table 38.3 . Potential toxicity and drug interactions of commonly used drugs are listed in Table 38.4 . Because of the duration of therapy required and the potential toxicity of the medications, not all patients with NTM lung disease will benefit from therapy. For some patients, the treatment is in essence worse than the disease. Elderly patients who have few symptoms and minimal or very slowly progressive disease or,

alternatively, have severe comorbid conditions and limited life expectancy may not benefit from drug therapy directed at some NTM pathogens, especially MAC. These patients should be selected to receive treatment only after very careful evaluation.

Patients undergoing therapy for NTM pulmonary disease require frequent follow-up:

- to evaluate symptomatic and objective response to therapy and medication toxicity, and
- to collect specimens for AFB analysis.

Serial sputum AFB analysis is the most important element of disease monitoring. The sputum analysis is a critical measure of medication efficacy and may provide evidence for treatment failure (which may be due to the emergence of selective drug resistance), disease relapse or reinfection. Additionally, the duration of therapy for some patients is determined by the duration of time sputum is AFB culture negative while on therapy. Patients who have NTM lung disease should not be placed on therapy for extended periods of time without repeated sputum AFB evaluation. Although periodic chest radiographs are also helpful, the chest radiograph is likely to improve only very slowly.

Drug treatment is not successful for all patients with NTM lung disease. Surgical resection of limited disease remains an important option, although surgical morbidity and mortality dictates that the surgical approach should be undertaken only by surgeons experienced with mycobacterial disease.[71] [72] Other approaches, such as cytokine therapy (especially IFN-?), are promising but remain investigational.[43] [73] Unfortunately, since clarithromycin and azithromycin

TABLE 38-3 -- **Treatment recommendations for selected nontuberculous mycobacteria causing lung and disseminated disease.**

TREATMENT RECOMMENDATIONS FOR SELECTED NTM CAUSING LUNG AND DISSEMINATED DISEASE			
NTM species	**Suggested drug regimen**	**Duration of therapy**	**Comments**
M. avium complex[69] [70] [75] 85,86	Clarithromycin 1g or azithromycin 600mg MWF plus rifabutin 300mg or rifampin 600mg MWF plus ethambutol 25mg/kg MWF	12 months of sputum AFB culture negativity for pulmonary disease, or lifetime therapy for disseminated disease unless immune status restored	Clarithromycin or azithromycin should not be used as monotherapy; consider surgical resection of limited pulmonary disease; add streptomycin initially (2–3 months) 500–1000mg im MWF or amikacin 400mg/day iv for severe disease; rifampin contraindicated with protease inhibitors (consider rifabutin 150mg/day with indinavir)
M. kansasii[1] [2] (rifampin susceptible in vitro)	Rifampin 600mg/day plus INH 300mg/day plus ethambutol 25mg/kg/day for 2 months followed by 15mg/kg/day	18 months and 12 months of sputum AFB culture negativity for pulmonary disease or lifetime for disseminated disease unless immune status restored	Add streptomycin 500–1000mg im MWF or clarithromycin 1g/day initially (2–3 months) for advanced disease; treatment success with this regimen is dependent upon in-vitro rifampin susceptibility; PZA is not effective
M. kansasii (rifampin resistant in vitro or patient on protease inhibitor)	Clarithromycin 0.5g q12h plus ethambutol 25mg/kg/day for 2 months followed by 15mg/kg/day plus INH 900mg/day (B6 50mg/day) plus sulfamethoxazole 1.0g po q8h plus streptomycin 500–1000mg im MWF (initial 2–3 months)	12 months of sputum AFB culture negativity for pulmonary disease or lifetime for disseminated disease unless immune status restored	In-vitro rifampin resistance occurs as a consequence of treatment failure (noncompliance) for rifampin-susceptible *M. kansasii* lung disease; rifabutin 150mg/day can be used with indinavir (see text for other options in AIDS patients)
M. abscessus	Clarithromycin 1g/day or azithromycin 500mg MWF ± cefoxitin, imipenem, amikacin	12 months of sputum AFB culture negativity	No drug regimen of proven efficacy; surgical resection of limited pulmonary disease most effective therapy; first-line antituberculosis drugs not useful
M. fortuitum	Two agents including: ofloxacin 800mg/day or ciprofloxacin 1500mg/day; doxycycline 100mg q12h; sulfamethoxazole 1g q8h; clarithromycin 0.5g q12h	6 months	Therapy based on in-vitro antibiotic susceptibility; only 50% of *M. fortuitum* isolates susceptible to clarithromycin; for severe disease amikacin 400mg/day iv or cefoxitin 12g/day iv until favorable clinical response; first-line antituberculosis drugs not useful
M. chelonae	Clarithromycin 1g/day	6 months	Macrolide monotherapy effective in this clinical situation
Other NTM respiratory pathogens that likely would respond to macrolide-containing regimens: *M. xenopi*, *M. malmoense*, *M. simiae*, *M. szulgai*			
Other disseminated NTM pathogens that likely would respond to macrolide-containing regimens: *M. gordonae*, *M. haemophilum*, *M. genavense*			
AFB, acid-fast bacilli; INH, isoniazid; PZA, pyrazinamide; MWF, Monday, Wednesday and Friday.			

became available, there have been few new drugs introduced with activity against NTM. Linezolid, a new oxazolidinone, has in-vitro activity against some NTM species, including *M. abscessus, M. chelonae* and MAC,[74] but this is not consistent or predictable for all isolates. Linezolid is also associated with frequent and severe side-effects, such as anemia. Clearly, better antibiotic agents for treatment of NTM infections are needed.

Recommendations for treating disseminated NTM disease in AIDS patients are listed in Table 38.3 (see also Chapter 129). Treatment of disseminated MAC, as well as other NTM pathogens such as *M. kansasii*, results in clinical and bacteriologic improvement as well as increased survival. Treatment of these infections has been complicated by the introduction of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of HIV infection, which interact with rifamycins.[76] There are several options for treating disseminated NTM disease for patients who are also undergoing therapy for HIV infection. One strategy is the use of three nucleoside reverse transcriptase inhibitors (NRTIs) as initial therapy for HIV disease, which would allow use of rifampin in a multidrug regimen for disseminated NTM infection. Efavirenz, with appropriate dosage adjustment (800mg/day if used with rifampin), can also be added to a multidrug HIV treatment regimen that contains NRTIs, and the patient could still receive a rifampin-containing regimen for NTM disease. For patients receiving the PIs indinavir, nelfinavir and amprenavir or the NNRTIs nevirapine or evafirenz, rifabutin could be used instead of rifampin in the NTM treatment regimen. Recently, a strategy for boosting PI levels by giving ritonavir has been developed, which might also allow concommitant administration of rifampin with the PIs, but dose adjustments are still required. Clearly, the treatment of NTM disease in HIV-infected patients can be very complicated. Inappropriate combinations of drugs may result in treatment failure of one or both infections as well as significant drug-related toxicity. Physicians who do not routinely treat HIV-infected patients or who are not familiar with the drugs involved should seek expert consultation for the management of these patients. Effective regimens for prophylaxis against disseminated MAC are outlined in Table 38.5 . The successful treatment of NTM CNS diseases is difficult because of the relative antibiotic resistance of the organisms, the poor CNS penetration of important agents, such as clarithromycin, and the usually far advanced underlying disease of the host. [65]

Treatment of skin and soft tissue infections due to *M. fortuitum, M. abscessus*, or *M. chelonae* unrelated to disseminated disease involves regimens similar to those recommended for pulmonary or disseminated disease (see Table 38.3).[81] [82] Surgical debridement is important for extensive or poorly responsive disease. Several regimens administered for 3 months are effective for the treatment of *M. marinum* infection, including:

- clarithromycin 500mg q12h,
- doxycycline 100mg q12h,

- trimethoprim-sulfamethoxazole (co-trimoxazole) 160/800mg q12h, and
- rifampin 600mg/day plus ethambutol 15mg/kg/day.

TABLE 38-4 -- Adverse events and drug interactions associated with medications commonly used to treat nontuberculous mycobacterial infections.

ADVERSE EVENTS AND DRUG INTERACTIONS ASSOCIATED WITH MEDICATIONS FOR NTM INFECTIONS

Drug	Adverse events	Drug interactions
Clarithromycin	Bitter taste, nausea, vomiting, abnormal liver enzymes, hearing loss	Blocks cytochrome p450 enzyme metabolism of multiple agents including rifabutin; rifamycins (rifamycin, rifabutin) accelerate hepatic metabolism of clarithromycin
Azithromycin	Nausea, vomiting, diarrhea, abnormal liver enzymes, hearing loss	No known effect on cytochrome p450 enzymes or hepatic metabolism of other drugs
Rifabutin	Nausea, vomiting, abnormal liver enzymes, polyarthralgia, polymyalgia, leukopenia, thrombocytopenia, anterior uveitis	Promotes cytochrome p450 enzymes (less than rifampin) with increased hepatic metabolism of multiple drugs, including clarithromycin; severe side-effects (uveitis) almost exclusively with combined clarithromycin therapy
Rifampin	Nausea, vomiting, abnormal liver enzymes, flu-like syndrome, thrombocytopenia, renal failure, hypersensitivity response	More potent cytochrome p450 enzyme promoter than rifabutin; increased hepatic metabolism of multiple drugs, including clarithromycin, protease inhibitors
Ethambutol	Optic neuritis with loss of red-green color discrimination, loss of visual acuity	

TABLE 38-5 -- Effective agents for prophylaxis against disseminated *Mycobacterium avium* complex lung disease in patients who have AIDS.

EFFECTIVE AGENTS FOR PROPHYLAXIS AGAINST DISSEMINATED MAC LUNG DISEASE		
Regimen	**Dose**	**Comments**
Rifabutin[77] [78]	300mg/day	Rifamycin (rifampin) resistance can emerge with rifabutin monotherapy in patients with occult active tuberculosis; not compatible with some protease inhibitors
Clarithromycin[79]	1g/day	Well tolerated; clarithromycin resistance will emerge if monotherapy used for active disseminated MAC infection
Azithromycin[80]	1.2g/week	Well tolerated; macrolide (clarithromycin) resistance will emerge if monotherapy used for active disseminated MAC infection
Azithromycin[80] plus rifabutin	1.2g/week plus 300mg/day	Very effective prophylaxis regimen; high incidence of rifabutin toxicity

Again, surgical debridement may be necessary for extensive disease.

Complete surgical excision is the standard treatment for NTM lymphadenitis and is usually curative.[83] Antimycobacterial therapy is seldom necessary, except in patients who are immunocompromised. Regimens that contain the newer macrolides are effective for eradicating disease in patients who are unable to have surgery or who undergo incomplete excision of MAC lymphadenitis. [84]

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Chapter 39 - Endemic Mycoses

L Joseph Wheat

INTRODUCTION

The endemic mycoses are restricted geographically to areas where environmental and other factors favor the growth of these organisms in the soil. Histoplasmosis and blastomycosis mostly afflict patients in the Mississippi and Ohio river valleys, while coccidioidomycosis occurs primarily in the desert south west USA. Infection occurs when humans are exposed to the organism in the soil, most often by inhalation of aerosolized spores but, in the case of sporotrichosis, also by direct inoculation into the skin and subcutaneous tissues. These mycoses are increasing in importance as causes of opportunistic disease in immunocompromised patients.

HISTOPLASMOSIS

MYCOLOGY

Histoplasma capsulatum var. *capsulatum* is a dimorphic fungus that grows as a mold in the soil and in culture at room temperature and as a yeast in the tissue and when cultured at 98.6°F (37°C). Other varieties of *H. capsulatum* cause African histoplasmosis (*H. capsulatum* var. *duboisii*) and infection of horses and mules (*H. capsulatum* var. *farciminosum*). This chapter will focus on histoplasmosis caused by *H. capsulatum* var. *capsulatum*. Mold cultures grown on Sabouraud's agar appear as white, tan or light brown colonies with abundant aerial hyphae, and require 2–4 weeks for isolation. Definitive identification requires transformation to the yeast at 98.6°F (37°C), exoantigen testing or nucleic acid hybridization. Yeast cells appear as oval structures measuring 2–3 × 3–4µm in diameter. Microscopically the mould is characterized by tuberculate macroconidia measuring 8–15µm and smaller microconidia (2–4µm).

EPIDEMIOLOGY

Histoplasmosis is the most common endemic mycosis and a major cause of morbidity in patients who live in endemic areas (Fig. 39.1). It has emerged as an important complication of AIDS. *Histoplasma capsulatum* is endemic in areas of North and Latin America but can be found throughout the world. Factors accounting for its geographic distribution include humid environmental conditions and acidic, permeable soil characteristics. Heavily contaminated soils, which may harbor up to 105 viable particles per gram, are more common in environments populated by birds or bats, such as bird roosts, chicken coops and chimneys with plentiful bird droppings, old uninhabited buildings and caves with large numbers of bats. Bird and bat excrement enhances growth of the organism in soil by accelerating sporulation.

PATHOGENESIS

Infection develops when conidia are inhaled and germinate into yeast in the tissues (Fig. 39.2) or when old foci of infection reactivate.[1] Cellular immunity plays the key role in defense against *H. capsulatum*. [2] With development of specific T-cell-mediated immunity, interferon-? and interleukin-12 arm macrophages to kill the fungus and halt progression of the disease. These defense mechanisms are sufficient to control the infection in immunocompetent individuals, explaining the subclinical or self-limited course characteristic of acute histoplasmosis. Reactivation of latent infection may account for some cases occurring in individuals who have had histoplasmosis in the past and who become immunocompromised.

CLINICAL FEATURES

The majority of patients remain asymptomatic or develop self-limited disease following exposure. Low inoculum exposure leads to symptoms in approximately 1% of individuals, but 99% have a clinically undetectable infection. Following a high inoculum exposure, the frequency of symptomatic infection is much higher (approximately 50–100%).[3]

Asymptomatic infection

In endemic areas, half to more than 80% of adults are infected with *H. capsulatum*, most cases being asymptomatic. Asymptomatic cases are usually identified by radiographs made for other reasons, which show calcified mediastinal lymph nodes or pulmonary or splenic granuloma.

Primary pulmonary histoplasmosis

The severity of illness correlates with the intensity of exposure. Following heavy exposure, patients may present with diffuse pulmonary involvement. Symptoms of fever, chills, sweats, headache, myalgia, anorexia, cough and chest pain characterize these illnesses, and respiratory failure and death may ensue. Chest radiographs show diffuse reticulonodular or miliary pulmonary infiltrates in 90% of cases, sometimes with mediastinal lymphadenopathy. Some patients may manifest progressive extrapulmonary dissemination. Although patients may recover from heavy-inoculum exposure without treatment, illness is often severe and recovery slow. Thus, treatment is advised. Adjunctive corticosteroid treatment may accelerate improvement in such cases, as the inflammatory response may contribute to the pathogenesis of the respiratory injury.

Following the more typical low-level exposure, the pulmonary illness is more commonly subacute and mild, or even asymptomatic. Chest radiographs show enlarged hilar or mediastinal lymph nodes with patchy infiltrates, but may be normal. While rapid improvement in 2–3 weeks is characteristic, fatigue may linger or inflammatory complications, including rheumatologic syndromes, pericarditis and granulomatous mediastinitis may develop.

Mediastinal granuloma

Enlarged mediastinal lymph nodes may impinge upon the airways, pulmonary vessels or vena cava, or the esophagus, occurring in fewer than 10% of patients who have acute pulmonary histoplasmosis.[3] These findings may first present years after the initial infection, because of smoldering inflammation and necrosis in the involved node. Symptoms include chest pain, cough, hemoptysis, dyspnea

Figure 39-1 Endemic distribution of histoplasmosis in the Americas. This is based upon skin testing surveys.

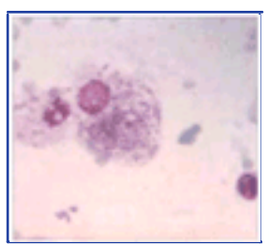

Figure 39-2 Cytologic specimen from bronchoalveolar lavage fluid showing intracellular *Histoplasma capsulatum.*

and dysphagia. Caseous necrosis may lead to fistula formation between the necrotic node and adjacent structures, including the pericardium, skin or esophagus. Calcification may be seen in the involved node, in other mediastinal nodes or in parenchymal granulomas, indicating that the infection occurred years earlier. The affected lymph nodes may form cystic masses containing a necrotic center surrounded by granulomatous tissue and encased in a fibrous capsule. Biopsy of such nodes may lead to drainage of the cheesy central core. Fungal stains and cultures of the node are often negative. Diagnosis usually is made on clinical grounds and supported by positive tests for antibodies to *H. capsulatum*, or by histopathology findings or cultures in patients who undergo node biopsy. Although enlarged mediastinal nodes usually shrink and symptoms resolve without treatment, obstructive syndromes may be severe and the masses may persist for years. Antifungal therapy may be helpful but studies of the effect of treatment have not been conducted. Mediastinal granuloma is not thought to progress to fibrosing mediastinitis and treatment to prevent such an outcome is not advised.

Pericarditis

Pericarditis is a local inflammatory or immunologic reaction to the adjacent mediastinal histoplasmosis, occurring in about 10% of symptomatic cases. [4] Pericarditis rarely may be a complication of disseminated histoplasmosis. Hemodynamic compromise occurs in 40% of patients. Patients usually respond to anti-inflammatory medications without antifungal therapy, while those who have hemodynamic compromise may require corticosteroid therapy or drainage of the pericardial fluid. Outcome is excellent, with rare progression to constrictive pericarditis. Resection of the pericardium to prevent later restrictive disease is not recommended.

Rheumatologic syndrome

This syndrome represents a systemic immunologic reaction following acute histoplasmosis.[3] Arthralgia or arthritis may be the sole finding, or may be associated with pulmonary complaints. Half of patients exhibit erythema nodosum. Joint radiographs are normal, while chest radiographs may show pulmonary histoplasmosis. The joint symptoms usually resolve in response to anti-inflammatory therapy.

Chronic pulmonary histoplasmosis

Patients present with chronic cough, dyspnea, chest pain, fatigue, fevers, sweats and fibrotic apical infiltrates, with cavitations on chest radiographs or computerized tomography (CT) scans. This manifestation occurs mostly in patients who have underlying emphysema.[5] [6] Sputum production is common, facilitating diagnosis by isolation of *H. capsulatum* from respiratory secretions. The illness is progressive, manifested by cavity enlargement, development of new cavities, spread to other areas of the lungs and formation of bronchopleural fistulas. In addition to underlying emphysema, vascular compromise, tissue necrosis and fibrosis are characteristic pathologically.

Fibrosing mediastinitis

Fibrosing mediastinitis represents a fibrotic response to a prior episode of histoplasmosis and is characterized by invasion and obstruction of mediastinal structures.[7] Some 90% of cases occur in persons less than 45 years old and half in women. Active infection is not felt to play a role in the pathogenesis of fibrosing mediastinitis, however, judging from the rarity with which *H. capsulatum* is isolated from the tissues and the lack of response to antifungal therapy.

The clinical features of fibrosing mediastinitis are varied and are caused by obstruction of mediastinal structures. Commonly reported symptoms include cough, dyspnea, chest pain, hemoptysis, pleurisy, facial or upper extremity swelling or congestion, varicose veins on the head, neck, arms or abdomen, dizziness, headache and rarely syncope caused by obstruction of the superior vena cava. Obstruction may involve the superior vena cava, airways, pulmonary arteries or veins, or esophagus. Recurrent and often serious hemoptysis may result from parenchymal damage caused by airway obstruction and vascular compromise. Very rarely the thoracic duct, recurrent laryngeal nerve, or atrium may be involved. Progressive right heart failure and respiratory insufficiency occur in one-third of cases. Recurrent pneumonia also may complicate fibrosing mediastinitis.

Subcarinal or superior mediastinal widening is seen on chest radiograph, while CT scans reveal restriction and invasion of mediastinal

structures.[8] Ventilation-perfusion lung scans may show reduced blood flow in patients who have pulmonary artery obstruction. Pulmonary artery or venous contrast studies may be required to define the extent of obstruction in patients who have severe manifestations and are under consideration for surgery. While fungal stains of tissues are positive in two-thirds of cases, cultures are positive in only 10%,[8] supporting the hypothesis that fibrosing mediastinitis is an excessive scarring reaction to a past infection. Serologic tests are positive in two-thirds of cases at titers of more than 1:32.

Broncholithiasis

Mediastinal nodes and pulmonary granulomas eventually calcify. Rock-like structures may erode into bronchi, causing ulceration or obstruction manifested by cough, hemoptysis and purulent sputum production.[9] Patients may expectorate 'stones', which represent calcified lung tissue or lymph nodes. Fungal stains of tissue may demonstrate organisms but cultures are usually negative and antifungal therapy is not felt to be helpful. Surgical resection of the damaged tissue may be required in patients who have significant hemoptysis, recurrent obstructive pneumonia or broncho-esophageal fistulas. Airway obstruction and lung damage predispose to bacterial pneumonia, necessitating antibiotic therapy.

Disseminated histoplasmosis

Progressive disseminated histoplasmosis occurs in about one in 2000 acute infections, usually in patients who are immunosuppressed or at the extremes of age.[10] [11] Severity varies with the degree of immune deficiency. An acute, rapidly fatal course with diffuse reticuloendothelial involvement characterizes the infection in infants and others who are severely immunosuppressed, while a chronic course with a more focal organ distribution is more typical in nonimmunocompromised children and adults. [10] Patients who have AIDS or those who are receiving immunosuppressive medications may present with shock, respiratory distress, hepatic and renal failure, obtundation and coagulopathy.[12] Severe manifestations may also occur if immunosuppressive medications are administered for a mistaken diagnosis of sarcoidosis. Fever and weight loss are the most common symptoms. Examination reveals hepatomegaly or splenomegaly in about half of cases and lymphadenopathy in a third. Meningitis or focal brain lesions occur in 10–20% of cases.[13] Other common sites of dissemination include the oral mucosa, skin and adrenal glands, seen in 5–10% of cases. Chest radiographs are abnormal in 70% of patients, usually showing diffuse interstitial or reticulonodular infiltrates.

DIAGNOSIS

Antigen detection

The approach to diagnosis has recently been reviewed.[14] Detection of antigen in the body fluids offers a valuable approach to diagnosis

TABLE 39-1 -- Laboratory tests for histoplasmosis.

LABORATORY TESTS FOR HISTOPLASMOSIS			
	Sensitivity (% true positives)		
Test	**Disseminated**	**Chronic pulmonary**	**Self-limited***
Antigen	92	21	39
Culture	85	85	15
Histopathology	43	17	9
Serology	71	100	98

* Self-limited manifestations include acute pulmonary histoplasmosis, rheumatologic syndromes and pericarditis.

in severe cases, including those with disseminated and extensive pulmonary histoplasmosis, providing results within 24–48 hours. Antigen is found in the blood, urine and bronchoalveolar lavage fluid of most individuals who have disseminated histoplasmosis (Table 39.1) and in up to 75% of those who have diffuse lung involvement during acute pulmonary histoplasmosis. Positive results caused by cross-reacting antigens occur in patients who have African histoplasmosis, blastomycosis, paracoccidioidomycosis and *Penicillium marneffii* infection.[15] Cross-reactions have not been recognized in patient who have candidiasis, cryptococcosis, aspergillosis or coccidioidomycosis.

Mycologic tests

Fungal staining permits rapid diagnosis but with a lower sensitivity than culture or antigen detection. *Pneumocystis carinii*, *Candida glabrata*, *Blastomyces dermatitidis*, *Toxoplasma gondii* and *Penicillium marneffii* may be misidentified as *H. capsulatum*. Cultures are most useful in disseminated and chronic pulmonary cases, positive in about 85% of cases if multiple specimens are submitted. In disseminated histoplasmosis, the highest yield is from bone marrow or blood. Cultures are usually negative in patients who have mild acute pulmonary, pericardial or rheumatologic manifestations, and need not be performed.

Serologic tests

Antibodies to *H. capsulatum* measured by immunodiffusion or complement fixation develop in most patients.[16] Enzyme immunoassay and radioimmunoassay methods for measurement of antibodies are not reliable and should not be performed. Limitations of tests for antibodies include: a 4- to 8-week delay in diagnosis while antibodies are being produced following acute infection; false-negative results in immunocompromised patients; and false-positive results in patients who have blastomycosis, coccidioidomycosis and paracoccidioidomycosis. In addition, antibodies persisting following an earlier episode of histoplasmosis may cause confusion in patients who have other diseases.

Histoplasmin skin test

Skin tests are not useful diagnostically because of high background rates of skin test positivity (50–80%) in endemic areas, false-positive results in patients who have other fungal diseases and false-negative results in patients who have disseminated disease. Furthermore, skin tests boost antibody levels, compromising the interpretation of serologic tests. The skin testing reagents are no longer available.

MANAGEMENT

Although histoplasmosis resolves without therapy in most individuals, severe illness may follow heavy exposure even in the absence of immunosuppression. In addition, some patients who have acute histoplasmosis experience persistent symptoms due to either the infection or the accompanying inflammatory response. Most patients who have underlying emphysema have chronic pulmonary infection that does not clear spontaneously. Patients who have disseminated histoplasmosis rarely recover without therapy. Treatment guidelines have recently been published.[17] [18]

Acute, diffuse pulmonary disease

Patients who have extensive acute pulmonary histoplasmosis and are dyspneic or hypoxic should receive antifungal therapy. Amphotericin B 0.7–1mg/kg per day, or the liposomal formulation at 3mg/kg per day,[19] is recommended in those who have more severe manifestations, followed by oral therapy with itraconazole after the patient has improved clinically. Corticosteroids (60mg daily tapered over 2–4 weeks) also are helpful in severe cases. Patients who have milder manifestations may be treated with itraconazole alone. A 3-month

course of itraconazole is recommended, but studies to support this duration of therapy are lacking.

Subacute, localized pulmonary disease

Antifungal therapy is unnecessary in most patients who have localized disease, as they recover without treatment within a month. Some patients, however, may remain symptomatic longer and may possibly benefit from therapy. Treatment is recommended in patients who have moderately severe symptoms and have not improved after 2–4 weeks of observation. Itraconazole given for 3 months is recommended in such cases.

Chronic pulmonary disease

Treatment is indicated in all patients who have chronic pulmonary histoplasmosis. The illness is slowly progressive, causing loss of pulmonary function and even death; and treatment halts progression and reduces mortality. Itraconazole 200mg daily was effective in 80% of cases.[20] Fluconazole 200–400mg daily is less effective (64% response).[21] Amphotericin B is also effective, with response rates of 59–100%. Relapse occurs in 10–20% of patients, however, supporting 12–18 months of therapy and close follow-up.

Mediastinal granuloma

The role of therapy in patients who have granulomatous mediastinitis is unclear. Improvement may occur spontaneously or in response to antifungal therapy. Surgical resection of obstructive masses may be helpful if symptoms do not respond to antifungal therapy but resection of enlarged nodes to prevent progression to fibrosing mediastinitis cannot be supported. A trial of itraconazole is recommended in patients who have symptomatic infection and those who have significant impingement on the esophagus, airways or vasculature.

Pericarditis or rheumatologic syndromes

These inflammatory or immunologic responses to the infection are not treated with antifungal therapy.[4] Symptomatic patients respond to anti-inflammatory therapy. If corticosteroids are used for more than a few weeks, itraconazole may be appropriate to reduce the likelihood of progressive dissemination.

Fibrosing mediastinitis

Many patients demonstrate nonprogressive disease without major disability, and do well without therapy.[7] Based upon the pathologic finding of extensive fibrosis, without inflammation or active infection, antifungal or anti-inflammatory treatment would not be predicted to be helpful. Nevertheless, a 3-month trial of itraconazole seems reasonable, particularly if complement fixation titers and the sedimentation rate are elevated. If follow-up imaging shows improvement, treatment should be continued for 6–12 months. Use of corticosteroids or other anti-inflammatory agents is not recommended.

The role of surgery remains controversial. Fewer than half of patients benefit and up to a quarter may die as a complication of surgery.[7] Placement of intravascular stents may be helpful in selected patients who have pulmonary vascular obstruction, and embolization may relieve pulmonary hemorrhage.

Disseminated histoplasmosis

In a study in AIDS patients who had disseminated histoplasmosis, improvement of symptoms occurred faster and mortality was lower in patients treated with liposomal amphotericin B 3mg/kg per day than with the standard deoxycholate preparation 0.7mg/kg per day.[19] Thus, liposomal amphotericin B is preferred as initial therapy in patients who have severe or moderately severe disseminated histoplasmosis. Once patients are stable (10–14 days), they can be switched to oral therapy. Itraconazole is recommended in patients who have less severe illness and following response to amphotericin B. In noncomparative trials, itraconazole was successful in 85–100% of cases.[22] If itraconazole is used as initial therapy for patients who have moderately severe manifestations of histoplasmosis, the intravenous formulation is preferred initially (3–7 days). Fluconazole is less effective than itraconazole[21] [23] and may lead to development of resistance, [24] causing relapse. Fluconazole also antagonizes the activity of amphotericin B in histoplasmosis,[25] [26] discouraging the use of the two drugs in combination. Patients who have AIDS may require lifelong therapy (see Chapter 126); other immunocompromised patients should be treated for at least 1 year.

PREVENTION

Prophylaxis in people who have AIDS with $CD4^+$ counts below 150 lymphocytes/mm^3 is highly effective and is recommended if the case rate exceeds 5 cases/100 patient-years,[27] but may encourage the development of resistance among *Candida* spp.[28]

COCCIDIOIDOMYCOSIS

MYCOLOGY

Coccidioides immitis exhibits saprophytic and parasitic life cycles. Arthroconidia measuring 2.5–4 × 3–6µm cause infection following inhalation. In the host tissue the arthroconidia transform into spherules (30–60µm), which mature with the formation of numerous endospores (Fig. 39.3). Transformation between the yeast and mold does not exhibit predictable temperature dependency. The mold phase grows rapidly on fungal media, and arthroconidia can be seen within 5–10 days.

EPIDEMIOLOGY

Coccidioidomycosis occurs in a spotty distribution in the south western USA, northern Mexico and Central America (Table 39.2). There are an estimated 100,000 new infections yearly, of which half or more are subclinical.[29] Bat and rodent droppings enhance growth in the soil. Hot summers, mild winters and arid conditions characterize climates in the endemic areas. Exposure is heaviest in the late summer and autumn, when the soil is dry and conditions are windy, especially following rainy winters. Experience following the Northridge earthquake in southern California indicated that exposure to subsequent dust storms increased the risk of coccidioidomycosis. [30] Workers exposed to soil are at increased risk of coccidioidomycosis.[31] It is notable that nearly 50% of cases in persons

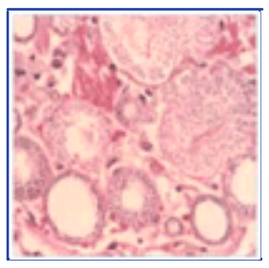

Figure 39-3 Pulmonary coccidioidomycosis. Granuloma showing typical spherules.

TABLE 39-2 -- Fungi causing infection in normal and compromised hosts.

FUNGI CAUSING INFECTION IN NORMAL AND COMPROMISED HOSTS				
Fungus	**Incidence**	**Geographic distribution**	**Epidemics**	**Form**
Histoplasma capsulatum var capsulatum	Common*	River valleys between latitudes 45°N and 30°S	Occasional	Dimorphic
Coccidioides immitis	Common*	South western USA, Central America, Argentina	Occasional	Dimorphic
Blastomyces dermatitidis	Uncommon	Ohio and Mississippi river valleys	Uncommon	Dimorphic
Sporothrix schenckii	Uncommon	Worldwide	Uncommon	Mold
Paracoccidioides brasiliensis	Common*	Mexico, 23°N to Argentina, 34°S	Uncommon	Dimorphic

* In endemic areas

who have AIDS are identified outside the endemic area,[32] [33] occurring in travelers who have visited these regions.[32] [34] Also of note is the increasing incidence in elderly persons who have moved to Arizona in recent years.[35]

PATHOGENESIS AND PATHOLOGY

Infection occurs following inhalation of arthroconidia of the mycelial phase of the fungus, which enlarge to form thick-walled spherules containing multiple endospores in the tissues (see Fig. 39.3). Spherules rupture, releasing endospores that spread locally and disseminate hematogenously. Cellular immunity and neutrophils are both involved in host defense in coccidioidomycosis. Pathologically, coccidioidomycosis is characterized by a pyogranulomatous reaction, often resulting in the development of large abscesses. Fibrosis may be prominent in the lungs or meninges.

CLINICAL FEATURES

About 40% of nonimmune individuals experience symptoms following exposure to *C. immitis*, while the others have subclinical infection.[36] Pulmonary illness is most common. Over 90% of symptomatic patients recover without treatment and fewer than 1% develop chronic pulmonary or extrapulmonary complications. The illness is more severe in immunosuppressed individuals, African-Americans and Filipinos. Unlike the normal hosts, two-thirds of immunosuppressed patients exhibit disseminated disease. Clinically recognized pulmonary involvement is present in most cases in transplant patients and in well over 90% of those who have AIDS.[37] Although coccidioidomycosis is reported to be more severe during pregnancy, recent reports indicate that the course is more favorable than was previously thought.[38]

Primary pulmonary disease

Flu-like symptoms, including chest pain, nonproductive cough, fever and malaise, develop within a few weeks following exposure.[34] [36] Patients who have more extensive pulmonary infection may experience dyspnea or respiratory failure. Chest radiographs show patchy infiltrates, often with mediastinal adenopathy. Nodular coin lesions (coccidiomas) and thin-walled cavities may follow in 5% of patients. Pleural involvement is uncommon (<10%), and may lead to pneumothorax, bronchopleural fistula or empyema. Pericardial effusions may also be seen. Joint pain occurs in a third of cases[34] and erythema nodosum is particularly common in women.[30] [38] A non-specific maculopapular rash also is common, especially in adolescents.[34] Airway involvement has been described,[39] involving the trachea, larynx or bronchi. Airway lesions may present as friable masses, small nodules with yellow centers, hyperemic patches or cobblestone patterns, and ulcers have been noted in autopsy reports.

Chronic pulmonary disease

Several types of pulmonary disease can develop after the initial infection. These include the formation of pulmonary nodules (coccidiomas), cavitary disease and progressive pneumonia. The nodules are typically solitary, measuring 2–3cm in diameter, representing areas of granulomatous organization of coccidioidal pneumonia and raising concern for lung cancer. Thin-walled cavities are seen in approximately 0.1% of all patients and often (35–50%) resolve spontaneously within 2 years. Thick-walled cavities are more likely to progress. Chronic fibrocavitary infection with progressive fibrosis and retraction similar to that seen with histoplasmosis may also be seen in coccidioidomycosis, occurring primarily in those with underlying emphysema.

Disseminated disease

Extrapulmonary dissemination occurs in fewer than 1% of persons who have coccidioidomycosis, typically during the first year following exposure. [36] Dissemination occurs more frequently in the very young or very old, certain racial or ethnic groups, those who have AIDS or other immunosuppressive disorders, and during the second half of pregnancy or postpartum. Pulmonary involvement is manifested as reticulonodular infiltrates in most cases. Tissues most commonly involved are skin, bone, joints and meninges, and tenosynovitis and sinus tract formation have been reported. A septic shock syndrome associated with respiratory failure has been described in patients presenting with focal or diffuse pulmonary coccidioidomycosis who are later identified as having disseminated disease.[40] [41] Meningitis is a

frequent complication, with a poor prognosis.[42] Hydrocephalus and neurologic deficits due to cranial nerve damage are common. Other sites of infection include lymph nodes, liver, peritoneum, kidneys, epididymis, prostate, testes, retina, ears, larynx, heart, thyroid, adrenal, pituitary, esophagus and pancreas.

DIAGNOSIS

Diagnosis of chronic pulmonary or disseminated coccidioidomycosis usually can be substantiated by positive culture. Fungal stains of respiratory secretions or tissues may show typical spherules, providing a more rapid diagnosis. Bronchoscopy improves the yield for isolation

of *C. immitis* from the respiratory tract. *Coccidioides immitis* may often be recognized by culture after only 3–5 days of incubation. In contrast to histoplasmosis, blood cultures are infrequently positive in coccidioidomycosis. It is of note that laboratory workers are at risk of acquiring coccidioidomycosis if the organism is not handled using biosafety level 3 precautions.

Serologic tests are useful in coccidioidomycosis, being positive in over 80% of cases.[43] High titers of complement fixing antibodies (1:16 or greater) or rising titers suggest more extensive infection and support the need to exclude dissemination. Detection of antibody in cerebrospinal fluid at titers of 1:2 or higher is valuable for diagnosis of meningitis.

Skin test reactivity to coccidioidin precedes development of antibodies by several weeks and may be useful in diagnosis of acute coccidioidomycosis.[43] Demonstration of skin test reactivity may provide the sole basis for diagnosis in up to 20% of patients who have self-limited illness and 95% of asymptomatic cases. Cross-reactions with other mycoses are uncommon and skin tests do not induce production of antibodies to coccidioidal antigens. The standard reagent should be diluted for use in patients who have erythema nodosum, however, as severe reactions may occur. [36]

MANAGEMENT

Most patients who have newly diagnosed coccidioidomycosis and no risk factors for complications or evidence of extensive disease may be managed without antifungal therapy.[33] During the first few weeks of infection, such patients should experience spontaneous improvement in fatigue, fever and weight loss; persistence of these findings should prompt reconsideration of the need for therapy. All patients should be monitored for 1–2 years to exclude progressive pulmonary or extrapulmonary disease. Treatment guidelines have recently been published.[29] A quarter of patients with disseminated coccidioidomycosis fail therapy and a similar proportion subsequently relapse. Lifelong therapy may be appropriate in those who are immunocompromised or have meningitis.[29] The role of therapy for asymptomatic, seropositive individuals who are to undergo organ or bone marrow transplantation is unknown but is recommended by some.

Amphotericin B is recommended for initial therapy for patients who are severely ill or have diffuse interstitial infiltrates because it acts more rapidly than triazole antifungal agents.[29] In severe cases (e.g. disseminated disease in patients who have AIDS), the combination of amphotericin B and fluconazole can be used initially. If amphotericin B is used as the sole therapy at least 35mg/kg is needed to achieve satisfactory and durable responses. Fluconazole induced a clinical response in 79% of patients who had meningitis, [44] 76% of those who had cutaneous manifestations and 86% of those who had skeletal manifestations of disseminated infection.[45] Response was poorer in patients who had chronic pulmonary infection (55%).[45] Itraconazole induced a response in 57–94% of patients who had chronic pulmonary or disseminated disease.[46] [47] The two agents were equally effective in a prospective trial in persons who did not have AIDS.[48] Prolonged therapy (more than 1 year) with high daily doses (fluconazole 400–800mg and itraconazole 400mg) would seem appropriate. Chronic maintenance therapy is essential for all patients who have severe immunodeficiency, including AIDS,[29] and perhaps in others who have relapsed after appropriate therapy.

Treatment of coccidioidal meningitis offers a special challenge. The relatively poor response to amphotericin B is partially explained by its poor penetration into the cerebrospinal fluid. Previously, to circumvent this problem, amphotericin B was given intrathecally. Now, fluconazole has replaced amphotericin B for treatment of coccidioidal meningitis. In total, 80% of patients who had coccidioidal meningitis responded to fluconazole, often after failing amphotericin B.[44] Lifelong therapy to prevent recurrence is recommended in coccidioidal meningitis. [29]

Adjunctive surgical therapy

Surgical debridement or resection of infected tissue often is necessary as an adjunct to antifungal therapy in coccidioidomycosis. Chronic foci of pulmonary necrosis or cavitations may require resection to prevent progression during therapy or recurrence following therapy. Soft tissue, joint or bony abscesses may require drainage or debridement.

PREVENTION

Antifungal prophylaxis in AIDS has not been recommended. However, if the attack rate is high during an outbreak, prophylaxis should be considered. Efforts to develop an effective vaccine have been intensified but none is available to date.[49] Immunosuppressed individuals should avoid areas experiencing active outbreaks and should perhaps avoid the desert during dry windy conditions.

BLASTOMYCOSIS

MYCOLOGY

Blastomyces dermatitidis exists as a mold at room temperature and in the soil and yeast at 98.6°F (37°C) and in the tissues. The mold can usually be isolated in the laboratory in less than 2 weeks, and conversion to the yeast occurs readily, usually within 1–3 weeks. Yeast cells measure 8–15µm, and occasionally up to 30µm, and exhibit thick refractile cell walls and broad-based buds (4–5µm).

EPIDEMIOLOGY

The organism may be found in microfoci enriched with animal excreta. Cases most often occur in the mid-western and south eastern USA in a distribution overlapping that of histoplasmosis. Up to 30% of men from endemic areas demonstrate immunologic evidence of previous blastomycosis.[50] Rare isolations from soil have occurred in samples from areas inhabited by farm animals and from beaver lodges or dams.[51] Common source outbreaks have been reported, often in association with outdoor activities in wooded or swampy environments such as hunting, camping or canoeing.

PATHOGENESIS AND PATHOLOGY

Cellular immunity plays a role in defense against *B. dermatitidis* but perhaps less than against other endemic mycoses. Infection is more extensive and outcome is worse in immunosuppressed individuals. Pathologically, blastomycosis is characterized by pyogranuloma formation with central microabscesses, but not by caseation as seen in histoplasmosis. Histologic changes may resemble those of cancer, leading to a mistaken diagnosis.

Reactivation of latent infection has been reported.[52]

CLINICAL FEATURES

In an outbreak setting, half of persons who were infected were asymptomatic.[51] The commonly recognized manifestations include acute and chronic pulmonary and disseminated disease, and the illness may be fulminant.[53] [54] The acute illness may be self-limited; in one outbreak only 18% of patients were treated.[51]

Pulmonary disease

Lung involvement may be localized or diffuse, each occurring in about half of patients.[55] Presenting symptoms include fever, cough, dyspnea, chest pain and weight loss. Tracheal and endobronchial involvement may occur. Pulmonary lymphadenopathy and calcification

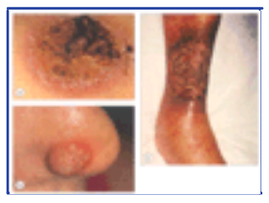

Figure 39-4 Cutaneous blastomycosis. (a,b) Skin lesions caused by *Blastomyces dermatitidis* in a normal host. (c) A large cutaneous ulcer in a patient who has multiple myeloma.

are uncommon.[56] Mass lesions may suggest cancer in about 15% of cases.[56] [57] Pleural involvement occurs in 20% of cases and may invade into adjacent tissues. About 10% of cases may present with diffuse infiltrates associated with respiratory failure. The illness resembles bacterial sepsis and carries a 50–90% mortality rate.[54] Such cases may have an underlying chronic disease in less than half of cases, and immunodeficiency in about a quarter.

Disseminated disease

Extrapulmonary dissemination is identified in a quarter to one-half of diagnosed cases of blastomycosis. Skin lesions, noted in half of patients, are usually painless erythematous nodules that develop verrucous or ulcerative surfaces (Fig. 39.4). Bone and joint lesions occur in less than half of patients. Central nervous system involvement, manifested as meningitis, brain lesions or epidural abscesses, is relatively common (~15%), occurring more often in immunocompromised individuals.[57] Laryngeal involvement may be mistaken for cancer, and cases have been described with involvement of the paranasal sinuses and ears. The kidneys, testes, prostate or epididymis, spleen, liver, pericardium, eyes, adrenal glands and thyroid may also be involved.

DIAGNOSIS

Mycology

Diagnosis usually is based on demonstration of organisms by culture or fungal stain.[57] Cultures are positive in over 90% of patients in the immunosuppressed host.[58] *Blastomyces dermatitidis* has been isolated most frequently from respiratory secretions or skin lesions but has also been detected in bronchoscopy specimens, bone, cerebrospinal fluid, brain or other less common sites of dissemination. Of note, fungal stains are often positive, providing a more rapid diagnosis.

Serology

The complement fixation test is neither specific nor sensitive and thus cannot be relied upon. The immunodiffusion test is more specific but less sensitive. Overall, the serologic tests are positive in about a quarter of cases. [51] [54] Tests for antibodies are of limited value in diagnosis of blastomycosis and are not recommended. [59]

Antigen detection

Diagnosis by antigen detection could be useful in patients who have extensive pulmonary or disseminated disease. An antigen cross-reacting with *H. capsulatum* may be detected in the body fluids in over half of patients, providing a rapid diagnosis in patients who have extensive disease.[15] Work is in progress to develop a specific antigen detection assay for blastomycosis.

MANAGEMENT

Observation would be appropriate in an individual who does not have underlying immunosuppression and was already improving when first evaluated. [60] Such patients are at risk for relapse, however, and should be monitored for at least a year. The main cause of death is overwhelming pneumonia, which may occur within the first few days following diagnosis, highlighting the importance of aggressive therapy in patients who have severe disease or underlying immunosuppressive disorders.

Amphotericin B has been effective in 70–90% of cases, with low relapse rates.[59] Amphotericin B 0.7–1mg/kg per day is recommended for initial therapy in the immunocompromised host, those who have severe manifestations or central nervous system disease, and those who have failed treatment with an azole.[60] Once the patient responds to amphotericin B (after about 1g), itraconazole should be given for at least 1 year. A higher total dose of amphotericin B of at least 2g is recommended in those who have central nervous system involvement, usually given at a dose of 0.7mg/kg q24h.[61]

Itraconazole 200mg q12h or q24h is the most effective azole, inducing remission in over 90% of cases.[59] It is recommended in patients who have mild to moderate disease and following response to a minimum dose of 500mg of amphotericin B. The duration of itraconazole therapy should be at least 6 months in most cases and 12 months in patients who have bone disease. It should be continued until all evidence of ongoing infection has subsided. Chronic suppressive therapy is recommended in those who have AIDS or ongoing severe immunosuppression.

Fluconazole at high doses of 400–800mg per day is also reasonably effective (response rate 87%[62]) and may be used in patients unable to be treated with itraconazole and perhaps in those who

have meningitis, after completing 2g of amphotericin B. In those who have central nervous system disease, the minimum fluconazole dose is 800mg daily, adjusted for renal function.

SPOROTRICHOSIS

MYCOLOGY

Sporothrix schenckii grows rapidly as a mold in the laboratory at 86°F (30°C) and transforms to yeast at 95–98.6°F (35–37°C). The yeast is characterized by cigar-shaped buds. Radial conidia of the mould are flower-like in appearance. Definitive identification requires conversion to the yeast at 95–98.6°F (35–37°C) on enriched media.

EPIDEMIOLOGY

Sporotrichosis, caused by the dimorphic fungus *S. schenckii*,[63] is most common in tropical and subtropical regions but occurs worldwide. The organism is often isolated from soil, sphagnum moss and plant debris such as thorns, timber, hay and straw. Infection usually occurs following cutaneous inoculation of the organism during outdoor activities such as forestry, gardening and farming, or manual labor in rural areas. Cases are common in children, who are exposed during outdoor play. Clusters of cases may occur following a common exposure, and outbreaks have been reported rarely.

PATHOGENESIS AND PATHOLOGY

Typically, there is a history of scratches from rose thorns, conifer needles or timber. Natural infection also occurs in wild and domesticated animals. Zoonotic infections have been traced to bites and scratches from cats, birds, rodents and armadillos. Cutaneous or lymphocutaneous disease follows local inoculation. Hematogenous dissemination can occur, leading to multifocal disease. Rarely, inhalation of organisms results in a granulomatous pneumonitis. The inflammatory response is characterized by an accumulation of neutrophils and macrophages, in the presence of giant cells and central necrosis.

CLINICAL FEATURES

Lymphocutaneous disease is the most common manifestation of sporotrichosis. Skin lesions appear at the site of inoculation and may progress to cause lymphangitis and regional lymphadenopathy. Systemic complaints are absent. The distal extremities are most often involved, although there may be lesions at any cutaneous site. These raised, nodular, erythematous lesions often ulcerate. They vary in size from tiny punctate lesions to 2–4cm in diameter. Infection advances through local lymphatic channels, producing secondary lesions that are identical to the primary lesions.

Deep inoculation can lead to the development of tenosynovitis and nerve entrapment or carpal tunnel syndromes, with or without joint involvement. Ocular infection has resulted from finger-to-eye transmission. Osteoarticular involvement most often involves the joints of the extremities. The infection is usually indolent and progresses slowly.

Pulmonary sporotrichosis may result from inhalation of the organisms or as a result of dissemination from a cutaneous focus. The usual presentation is that of a pneumonitis with non-specific respiratory symptoms, including cough, dyspnea, chest pain and sputum production, and occasionally hemoptysis. Hilar or paratracheal adenopathy may occur, and chronic pulmonary infection may evolve into fibrocavitary disease. Pleural involvement is rare.

Disseminated sporotrichosis may occur in a setting of impaired cellular immunity. Sites of dissemination include the skin, bones, joints, genitourinary tract, lungs and central nervous system. The organism has been recovered from blood and bone marrow only rarely.

DIAGNOSIS

Delay in diagnosis is common, because of the non-specific appearance of the skin lesions. Isolating the organism from the affected site generally makes a specific diagnosis. Periodic acid-Schiff staining may demonstrate cigar-shaped yeast forms, occasional hyphal forms and stellate eosinophilic material called asteroid bodies, but organisms often are scarce. Yeast may be mistaken for *H. capsulatum* var. *capsulatum*. There are no standardized serologic or antigen detection tests for diagnosis of sporotrichosis.

MANAGEMENT

Itraconazole is the treatment of choice in most patients who have sporotrichosis, reserving amphotericin B for those who have severe manifestations. [64] Amphotericin B can be changed to itraconazole once there is an improvement.[65] Patients who have meningitis or disseminated sporotrichosis should be treated with amphotericin B initially. Disseminated infection in severely immunosuppressed patients such as those who have AIDS requires lifelong therapy.[65] Surgery can play a useful adjunctive role for some localized sites of infection.

PARACOCCIDIOIDOMYCOSIS

MYCOLOGY

Paracoccidioides brasiliensis is thermally dimorphic, growing as yeast in the tissues and in culture at 95–98.6°F (35–37°C). Yeast cells measure 3–40µm and are described as 'pilot wheel'-like, resulting from small surface buds. Yeast may resemble *H. capsulatum* var. *capsulatum*. In culture at room temperature in the laboratory the mold grows slowly, requiring 3–4 weeks for isolation. Identification requires conversion from the mold to the yeast.

EPIDEMIOLOGY

Paracoccidioidomycosis (South American blastomycosis) is endemic to parts of Latin America, with most cases reported from Brazil, Columbia, Venezuela and Argentina. *Paracoccidioides brasiliensis* is found in the soil, and is most common in poor socio-economic, rural areas. Over 80% of cases are reported in Brazil, where 10% of the population exhibit skin test reactivity to paracoccidioidin. There appears to be an association ecologically with armadillo burrows. An increase in cases is predicted to accompany deforestation of rural areas of Latin America.

PATHOGENESIS

The infection is acquired by inhaling spores of the organism, causing localized pulmonary disease accompanied by hematogenous dissemination. As with the other endemic mycoses, cellular immunity is the most important host defense. Pathologically, the infection is characterized by granuloma formation, often with associated neutrophil response and suppuration.

CLINICAL FEATURES

Clinical manifestations of the disease are rarely seen in people less than 30 years of age, and men are 15 times more frequently affected than women. Most primary infections are subclinical. Although primary infection occurs in the lungs, disseminated disease involving the skin, mucous membranes, reticuloendothelial system and adrenals is common.[66] Two distinct forms of paracoccidioidomycosis have been described. A subacute form seen mostly in young adults

(approximately 5% of cases) is associated with severe manifestations and a high mortality rate. A more chronic form, which accounts for the majority of cases, is seen in older adults and is associated with a better prognosis. Pulmonary involvement predominantly involves the mid and lower portions of the lungs. Cavities and hilar adenopathy are less common. Longstanding cases often reveal fibrosis, and pulmonary hypertension may develop.

Disseminated disease often causes generalized symptoms, including fever, malaise, weakness, weight loss and anorexia. Mucosal lesions are common and involve the mouth, lips, gums, tongue, palate, larynx and pharynx. They are infiltrated or ulcerated and generally painful. Cutaneous lesions tend to be located around body orifices. They may appear warty, crusted or ulcerated, and occasionally granulomatous. Adenopathy often involves cervical, axillary, mediastinal or mesenteric lymph nodes and may be complicated by the formation of draining sinuses and fistulas. Adrenal involvement can range from minimal dysfunction to overt insufficiency. The liver, central nervous system, spleen, vascular system, bones and male genitourinary tract are involved less often.

DIAGNOSIS

Laboratory diagnosis can occasionally be made by potassium hydroxide examination of sputum or pus. Definitive diagnosis generally requires biopsy and demonstration of typical multiple budding yeast, which often produce a characteristic 'pilot-wheel' appearance. Fungal culture on Sabouraud-dextrose or yeast extract agar is also recommended. Several serologic tests are available, including immunodiffusion and the complement fixation test. Skin testing is unreliable. Based on the presence of a cross-reacting antigen in the *Histoplasma* antigen test, research is in progress to develop an antigen test for diagnosis of paracoccidioidomycosis.

MANAGEMENT

Amphotericin B is recommended in patients who have severe manifestations, and itraconazole in those who are less ill or who have improved with amphotericin B. Itraconazole induces response in about 90% of cases.[67] Limited experience with fluconazole also indicates that this drug might be useful.

PENICILLIOSIS

MYCOLOGY

Penicillium marneffei is yeast in the tissues and a mold in culture at room temperature. The yeast resembles *H. capsulatum* var. *capsulatum* but may exhibit characteristic sausage-shaped cells, some with septation. Yeast may be free or within macrophages. Identification requires differentiation from other *Penicillium* spp., based upon either morphologic differences or the exoantigen test.

EPIDEMIOLOGY

Penicillium marneffei is endemic to South East Asia, and is the fourth leading opportunistic infection in patients who have AIDS in Thailand.[68] [69] [70] [71] The natural habitat has not been clearly defined but it is thought to grow in the soil. *Penicillium marneffei* infects a variety of animals, including the bamboo rat, which serves as a healthy carrier of the fungus. It is more common in inhabitants of rural areas.

PATHOGENESIS AND PATHOLOGY

Penicilliosis is acquired by inhalation. Individuals who have impaired cell-mediated immunity are at risk for disseminated disease, supporting animal studies that demonstrate the importance of $CD4^+$ and $CD8^+$ T cells. The pathologic features include necrotizing granulomas and microabscess formation.

CLINICAL FEATURES

Most patients present with disseminated infection manifested by fever and weight loss often accompanied by lymphadenopathy, hepatosplenomegaly, skin lesions and bone and joint involvement.[68] [69] [72] A variety of skin lesions may be noted but are not characteristic. These include nodules, maculopapular eruptions, ulcers, folliculitis and maculopapular lesions. Localized pulmonary disease is uncommon but the lungs are frequently involved in patients who have disseminated infection.

DIAGNOSIS

A rapid diagnosis may be established by touch smears and Wright's stain of bone marrow aspirates or other tissue biopsies. The organism appears as oval, round or sausage-shaped yeasts. Central septation is characteristic of *P. marneffei*, differentiating it from *H. capsulatum*, which divides by budding. The diagnosis is confirmed by culture using routine fungal media.

MANAGEMENT

Penicillium marneffei is susceptible to amphotericin B, 5-flucytosine, ketoconazole and itraconazole.[73] Studies in AIDS have demonstrated the effectiveness of amphotericin B and itraconazole but not fluconazole.[74] [75] Amphotericin B is recommended for more severe cases and itraconazole 400mg/day in milder cases. Maintenance therapy with itraconazole is required to prevent relapse. [76] Prophylaxis with itraconazole may also be useful in highly endemic areas.

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Chapter 40 - Practice Points

40.a Aspiration of pleural fluid: pleural biopsy

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John T Macfarlane

Introduction

Pleural effusions result from diverse disorders ranging from malignancy to cardiac failure. In the context of infection, parapneumonic effusions (literally 'by the side of pneumonia') are common; they are present in up to 40% of cases of bacterial pneumonia. All such effusions of any significance should be sampled; one in ten will need specific treatment such as tube drainage or a surgical procedure.

Pathogenesis

Pleural effusions are divided into transudates and exudates. Transudates occur when increased systemic or pulmonary capillary pressures (e.g. in cardiac failure) or reduced systemic oncotic pressures (e.g. in hypoalbuminemia or liver failure) result in pleural fluid formation exceeding removal.

Exudates usually result from disease of the pleural surfaces. Three suggested mechanisms are:

- an increase in protein production (e.g. parapneumonic effusions);
- a decrease in lymphatic absorption (e.g. in malignancy); and
- a decrease in pleural pressure caused by bronchial obstruction.

More than one of these mechanisms may operate simultaneously.

Parapneumonic effusions progress seamlessly through three stages. The exudative stage is characterized by the accumulation in the pleural space of sterile fluid with a high protein content. It occurs in response to pleural inflammation. In the fibropurulent stage, leukocytes and fibrin accumulate in the pleural space and loculations eventually develop. In the final stage of organization, fibroblasts invade and produce an inelastic pleural peel, which encases the affected lung.

Microbiology

Empyema is defined as the presence of pus in the pleural fluid or a positive Gram stain or culture of the pleural fluid. Most empyemas (55%) develop as a complication of pneumonia; surgery and trauma account for the remainder of cases.

Pure aerobic infections account for 40–62% of empyemas, pure anaerobic infections for 15–30% and mixed aerobic and anaerobic infections for 8–20%. *Staphylococcus aureus*, *Streptococcus pneumoniae* and other *Streptococcus* spp. make up the majority of aerobic Gram-positive isolates, and *Pseudomonas* spp., *Escherichia coli* and *Klebsiella* spp. are the most common aerobic Gram-negative isolates. *Bacteroides* spp. are the most common anaerobic isolates, although microaerophilic streptococci may be isolated in up to 10% of anaerobic infections. In 28–43% of empyemas, multiple organisms are responsible (see Chapter 36).

The incidence of *Mycobacterium tuberculosis* as a cause of pleural effusion is related to local epidemiology.

Clinical features

Patients who have parapneumonic effusions may present with fever, chills, breathlessness or chest pain: symptoms very similar to those of pneumonia. The physical examination may be limited to findings of an effusion, which is sometimes not apparent on initial presentation and only develops later. An empyema should be suspected if there is a recrudescence or persistence of symptoms of infection after initial recovery from pneumonia. The patient may then also complain of malaise, weight loss, dull pleural pain and unresolving fever.

Investigations

The proper management of pleural effusions is crucially dependent on obtaining adequate samples for diagnosis. The analysis of both pleural fluid and pleural tissue is complementary in this regard.

Pleural aspiration

Large effusions can be safely aspirated at the bedside. Ultrasound guidance is useful when aspirating small or loculated effusions. The patient should be sitting comfortably, with arms stretched out in front and resting on a support. Aspiration is performed one interspace below the level of the effusion posteriorly or in the axilla using a 21-gauge needle attached to a 50ml syringe. The needle is passed perpendicular to the chest wall just above a rib, avoiding the neurovascular bundle. About 30ml of fluid should be aspirated for diagnostic purposes. Local anesthetic is not required if a single pass is

successful. If large-volume aspiration is intended, the skin and chest wall should be infiltrated with local anesthetic and an 18-gauge cannula used and connected to a three-way tap after removal of the needle.

We recommend that pleural fluid is sent for measurement of protein content, lactate dehydrogenase (LDH) level and for cytologic and microbiologic analysis, including mycobacterial culture. A serum sample for measurement of protein and LDH should be taken simultaneously.

The value of other tests, including pH, glucose level and amylase, is limited to specific circumstances (Table 40a.1). Samples for pH estimation need to be collected anaerobically in a heparinized syringe and analyzed promptly, the process being similar to arterial blood gas analysis.

Pleural biopsy

Pleural biopsy is a safe bedside procedure when performed by an experienced operator. It is important to obtain pleural biopsy specimens, if indicated, before complete removal of pleural fluid. The positioning of the patient and the approach is as for pleural aspiration. The use of an Abram's needle is described here, although a Cope's needle may also be used.

The biopsy site is cleaned and adequate local anesthetic infiltrated down to the pleural surfaces. A diagnostic pleural tap should be performed at this stage to confirm free entry into the pleural space and to avoid blood contamination after biopsy. A small vertical skin incision is made to allow easy passage of the Abram's needle through the skin. With the side hole in the closed position, firm but controlled pressure is applied in a twisting movement until the pleural space is breached. There is usually a sudden 'give' when the parietal pleura is first penetrated. Care must be taken not to plunge the needle into underlying lung. A 20ml syringe is attached to the

end of the needle and its positioning

TABLE 40.a-1 -- Pleural fluid examination.

PLEURAL FLUID EXAMINATION			
Investigation	**Result**	**Possible causes**	**Comment**
Visual inspection	Turbid (turbid supernatant)	Chylothorax, pseudochylothorax	Triglycerides >110mg/dl (1.2mmol/l), Cholesterol >200mg/dl (5.2mmol/l)
	Pus	Empyema	
	Blood	Malignancy, tuberculosis, trauma, embolism	Pleural fluid hematocrit >50% of that of peripheral blood
Smell	Putrid	Empyema	Anaerobic infection
Protein	<3g/dl (30g/l)	Transudate (but also seen in malignancy, parapneumonic effusions)	See Light's criteria
	>3g/dl (30g/l)	Exudate, effusion in diuretic treated cardiac failure	See Light's criteria
Lactate dehydrogenase	Pleural fluid to serum ratio >0.6	Exudate	Indicates pleural inflammation
	Pleural fluid level >two-thirds upper limit normal serum level		
Amylase	Raised salivary amylase	Esophageal rupture, malignancy	
	Raised pancreatic amylase	Acute or chronic pancreatitis	
Glucose	<60mg/dl (3.3mmol/l)	Malignancy, tuberculosis, rheumatoid arthritis, parapneumonic	
pH	In parapneumonic effusions:	Uncomplicated parapneumonic effusion	
	=7.3		
	>7.2 and <7.3	May progress to empyema	Requires close observation. Expert advice recommended
	=7.2	Empyema	Tube drainage recommended
Ultrasound	Loculations	Empyema	Consider tube drainage

in the pleural space is confirmed by opening the side hole and aspirating pleural fluid. Keeping the side hole open, the needle is now withdrawn slowly, at an acute angle to the upper chest wall, until it 'catches' on the parietal pleura. While continuing to apply outward and lateral pressure in order to hold the pleura in the open side hole, the inner cylinder is rotated clockwise thus closing the side hole and cutting a biopsy of the pleura (Fig. 40a.1). (A common error is to fail to maintain outward pressure when closing the side hole.) The entire needle is then quickly removed and the biopsy site covered to minimize air entry while the biopsy is placed in a suitable container. The procedure is repeated with the side hole of the needle in different positions until adequate tissue has been obtained. A biopsy is not taken with the side hole at 12 o'clock so as to avoid damage to the neurovascular bundle running underneath each rib.

We recommend obtaining at least six good samples, five to be placed in formalin for histology and the sixth in sterile normal saline for microscopy and mycobacterial culture. In some centres where the relevant expertise is available, medical thoracoscopy may replace blind pleural biopsy as the initial diagnostic procedure of choice.

Management

Initial management of a pleural effusion is based on differentiating between transudates and exudates. Using a pleural protein level of >3.0g/dl (30g/l) alone to identify exudative effusions will miss some parapneumonic and malignant effusions because these effusions exhibit a wide range of protein levels. With Light's criteria (sensitivity 98%, specificity 77%, accuracy 95%), an exudate is identified by one or more of:

- pleural fluid to serum protein ratio >0.5;
- pleural fluid to serum LDH ratio >0.6; or
- pleural LDH more than two-thirds the upper limit of normal serum LDH.

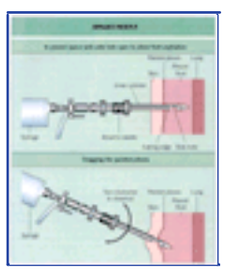

Figure 40.a-1 Abram's needle. The curved arrow shows clockwise rotation of the inner cylinder to close the side hole.

The management of parapneumonic effusions includes:

- sampling of the effusion to confirm the diagnosis and to determine whether it is complicated;
- deciding whether drainage is needed; and
- antibiotic therapy directed at likely or proven underlying infection.

Drainage is necessary in all cases of empyema or if loculations are seen on imaging. In the latter situation, ultrasound-guided catheter placement may be preferred. Ultrasound enables better visualization of loculations and small effusions, which enables better positioning and the use of smaller catheters, resulting in greater patient comfort. The effectiveness of tube drainage should be evident within 24 hours. Otherwise, the early use of intrapleural fibrinolytics should be considered before organization occurs.

Less evidence exists to aid the early identification of parapneumonic effusions that are likely to progress to empyema and hence require tube drainage. The pleural pH is of value in this situation. Sterile, nonpurulent effusions with pH >7.3 are very unlikely to develop a complicated course. A pleural pH of <7.2 predicts the need for pleural drainage with 80% sensitivity and 90% specificity. Tube drainage is generally recommended for these cases. Parapneumonic effusions with pH >7.2 but <7.3 fall in middle ground. Close observation with repeat thoracentesis according to clinical progress is a reasonable approach in some cases.

In all circumstances, antibiotic therapy should be guided by the underlying cause of pneumonia, if known. For ill patients in whom empiric therapy is required in the absence of positive microbiology, a second- or third-generation cephalosporin is recommended with the addition of metronidazole if anaerobic infection is suspected.

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40.b sternotomy wound infection

Michael T Poshkus

Definition of the problem

Sternotomy infections are serious complications of cardiac surgery, ranging from mild cases of cellulitis, subcutaneous tissue and fat necrosis, osteomyelitis, chondritis and more seriously, mediastinitis. The incidence ranges from 0.4% to 9.7% with mortality ranging from 7.2% to 14% for deep sternal infections. Early diagnosis and aggressive management are the important factors determining patients' survival. Most cases occur within 14 days after surgery but some cases have been reported more than a year following surgery.

Several studies have determined clinical risk factors for sternal wound infections, including: obesity, diabetes mellitus, smoking, peripheral vascular disease, a high NYHA heart failure classification, prolonged ventilator support, bilateral use of internal mammary arteries, skin shaving technique and emergency surgery. Nasal carriers of *Staphylococcus aureus* have a higher chance of developing *S. aureus* wound infections.

Most sternal wound infections are secondary to direct intraoperative contamination. Other sources include hematogenous spread from distant infections (e.g. infections of indwelling catheters, the lungs, gastrointestinal tract, genitourinary tract or leg wounds).

In addition, the body's defenses are compromised by cardiopulmonary bypass, which impairs both cell-mediated and humoral immunity. The use of bilateral internal mammary artery grafting increases the chance of wound infection to five times that of saphenous vein grafting and three times that of single internal mammary artery grafting. The presence of hematomas and foreign bodies and inappropriate timing of preoperative antibiotic prophylaxis all increase susceptibility to infections.

Most sternal wound infections are monomicrobial and about 90% are caused by *Staphylococcus* spp. — both *S. aureus* and coagulase-negative staphylococci (CNS). CNS is more often associated with wound dehiscence and *S. aureus* more often with a stable sternum. Patients who require reoperation more often have Gram-negative infections.

The clinical presentation of sternal wound infections varies according to the extent of infection. One must have a high index of suspicion for sternal wound infections in febrile postoperative cardiothoracic patients. Early signs and symptoms may be nonspecific and simply include fever and leukocytosis. Following cardiac surgery patients often have tenderness, erythema and serous drainage at the surgical site. Sternal instability with purulent discharge implies deeper tissue involvement. Sternal dehiscence suggests underlying infection. Hamman's sign, a 'crunching' sound appreciated upon ausculation of the chest wall, indicates mediastinal emphysema and possible infection.

Typical case

A 63-year-old man with history of poorly controlled diabetes mellitus, multivessel coronary artery disease and a 40 pack-year smoking history undergoes coronary artery bypass grafting. He received cefazolin prophylactically prior to surgery. Prolonged intubation and elevated body temperatures to 100.7°F (38.1°C) complicate his postoperative course. On the ninth postoperative day, his sternal wound, which had initially been described as 'clean, dry and intact', is noted to be erythematous with yellow-tinged drainage. Sternal instability is appreciated. His laboratory evaluation reveals leukocytosis with 16,000 cells/μl (1.6×10^{10} cells/l) with 10% bands. The patient is treated empirically with pipercillin/tazobactam. CT scan of the chest shows pneumomediastinum. Blood cultures and superficial cultures of the sternal wound grow methicillin-resistant *S. aureus* and the patient is treated with vancomycin.

The patient is taken to the operating room for a sternal wound debridement and a bilateral pectoralis major myocutaneous advancement flap with greater omental transposition. His recovery is uncomplicated and he is discharged 35 days postoperatively on vancomycin.

Diagnosis

The diagnosis of sternal wound infections is usually clinical. Patients generally will have fevers, increased pain over the sternum and increased erythema or drainage from the wound. Cellulitis or superficial infections can be easily diagnosed by examination. Deeper infections, including mediastinitis, often require imaging and CT is considered the 'gold standard', having a sensitivity of 93–100% and specificity of 33–100%, showing localized mediastinal fluid and pneumomediastinum. Patients with mediastinitis have bacteremia approximately 50% of the time. A deep substernal aspiration can also be done to help with the diagnosis. Patients with this picture are generally taken to the operating room where the diagnosis of a deep sternal infection is confirmed and an organism can be identified by Gram stain and culture. The fluid should be sent for Gram stain, aerobic/anaerobic culture, acid-fast staining and culture and fungal culture.

Management options

Other than cellulitis, most sternal wound infections need both medical and surgical treatment. Aggressive surgical debridement is necessary with removal of all foreign bodies. Depending on the extent of the involvement, more radical debridement may be necessary, including removal of the sternum. The wound is then either left open with irrigation and packing or closed with drainage. Using muscle or omental flaps following sternal debridement has been shown to decrease length of hospital stay, improve survival and decrease need for further debridement or reinfections.

Antibiotic therapy should begin when sternal wound infection is suspected and after appropriate cultures are taken, if possible (including blood cultures and wound drainage cultures). The initial regimen should be broad and cover likely organisms with local resistance patterns in mind. Hospitals with a high incidence of methicillin-resistant *S. aureus* should have a regimen containing vancomycin as the first choice. Gram-negative rods should also be covered initially, pending cultures, and treated empirically with a third-generation cephalosporin, a carbapenem, a quinolone or an aminoglycoside. If all foreign bodies or necrotic tissues have not been removed, rifampin can be added to provide additional synergy for staphylococcal spp. and to better penetrate into the biofilm layer surrounding those areas. Treatment can be narrowed down later once the causative organism is known. Duration of treatment ranges from 10–14 days for those with cellulitis to 4–6 weeks for those with deeper tissue involvement.

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40.c The pros and cons of antibiotics for pharyngitis and otitis media

Jenifer Leaf Jaeger

Acute upper respiratory infections are the most common illnesses of humans and occur with greater frequency in children than adults. Sore throat is one of the most common complaints and otitis media is the leading indication for outpatient antibiotic use in the USA. Although most infections are relatively mild and resolve spontaneously, most physicians agree that antimicrobial therapy for group A streptococcal (GAS) pharyngitis and acute otitis media (AOM) is useful and often necessary to prevent suppurative and nonsuppurative complications and data support this. However, controversy exists regarding optimal treatment practice. Understanding the epidemiology and natural history of these infections is an essential component in the decision to treat. It is the lack of rigor in adhering to criteria for

establishing these diagnoses that leads to overdiagnosis and promotes selection of resistant bacteria.

Pharyngitis

Group A streptococci (*Streptococcus pyogenes*), the leading bacterial cause of pharyngitis, accounts for approximately 5–17% of adult and 15–36% of pediatric cases of pharyngitis. The majority of patients therefore have a nonstreptococcal illness and will not benefit from therapy. During the 1990s, an estimated 6.7 million adults in the USA annually presented as outpatients with a complaint of sore throat. Seventy-three percent of these visits resulted in the prescription of an antibiotic. Concurrent with the emergence of resistant bacteria there was an increase in the use of more expensive and broader spectrum antibiotics, with 80% of those adults receiving antibiotics being given nonrecommended antibiotics as first-line agents.

Viruses are the most common causative agents identified in pharyngitis, accounting for up to 90% of all upper respiratory infections. Herpes simplex virus, Epstein-Barr virus, rhinovirus (usually in the setting of cough and coryza) and adenovirus infections commonly present as pharyngitis. Enteroviruses that are responsible for herpangina, lymphonodular pharyngitis and hand-foot-mouth disease are important in the differential diagnosis, as is the acute retroviral syndrome associated with primary infection with HIV, which may present with pharyngitis.

Bacterial causes of pharyngitis other than GAS are rare. Non-group A streptococci, particularly groups C and G streptococci, cause pharyngitis in older children and will respond to antimicrobial therapy. These ß-hemolytic streptococci can be isolated by throat culture but will not be identified by rapid antigen tests and have been associated with acute glomerulonephritis but not acute rheumatic fever (ARF). *Arcanobacterium* (formerly known as *Corynebacterium*) *haemolyticum* is an infrequent infection that may be indistinguishable from GAS infection. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infections may present with pharyngitis with concurrent lower respiratory tract involvement.

The peak age range for GAS pharyngitis is 5–15 years, accounting for 15–20% of cases. GAS is prevalent in the late winter/early spring and close contact with an infected person(s) increases the likelihood of true infection. Signs and symptoms suggestive of GAS infection, including sudden onset of sore throat, fever, headache, nausea, pharyngeal erythema with patchy exudate, and tender, enlarged anterior cervical chain lymph nodes, may be present in only 30–50% of patients. Antigen detection tests for GAS are highly specific (90–100%) but not as sensitive (55–90%) as throat culture. Neither can distinguish between acute infection with GAS and the carrier state in people who have pharyngitis due to some other etiology.

The goals of therapy are straightforward:

- to hasten clinical recovery,
- to prevent transmission to others,
- to avoid suppurative complications, and
- to prevent development of rheumatic fever.

Strategies for achieving these goals are less clear.

The earlier in the course of the infection that antibiotics are begun, the greater the potential impact. Of patients treated appropriately 95% will become noninfectious within 24 hours, allowing an earlier return to work or school. However, early therapy may be associated with an increased recurrence rate as a result of an abortive antibody response. The development of protective, type-specific antibodies is slow and thus early treatment may interfere with the immunologic response. Local infections, such as retropharyngeal and peritonsillar abscess as well as invasive GAS disease, including streptococcal toxic shock syndrome, are known complications of GAS pharyngitis. However, there is no proven benefit of early treatment in decreasing the risk of these complications. Antibiotic therapy is effective in preventing ARF even if therapy is delayed up to 9 days after disease onset.

Since most patients do not have a GAS infection and will not benefit from antibiotic treatment empiric therapy is not justified. However, the indiscriminant use of throat culture or antigen tests contributes to excessive cost of health care, increases the likelihood of obtaining a 'false-positive' result (i.e. identifying a carrier), leads to substantial overuse of antibiotics and encourages development of resistant bacteria. Group A streptococci may be carried in the pharynx of untreated patients for several weeks or months and approximately 25% of patients who have been adequately treated become GAS carriers. Carriers are at low risk of developing invasive GAS disease or acute rheumatic fever and are unlikely to transmit GAS to contacts. Identification and treatment of the carrier state may be necessary if the patient or household contact is at risk for rheumatic fever.

Accurate diagnosis is facilitated by employing algorithms which include epidemiologic and clinical factors to identify individuals at low risk of having GAS pharyngitis. In those patients in whom GAS infection cannot be ruled out, throat culture or rapid antigen detection is indicated. A properly performed throat culture has a sensitivity of >90% and the delay of 24–48 hours to obtain definitive results does not negatively impact on the efficacy of therapy. A cost-effective strategy is the initial use of the antigen detection tests; a positive test allows prompt specific therapy, but a negative result must be followed by culture held for 48 hours. Rapid antigen tests are not as sensitive as conventional culture, particularly in children and adolescents, and a negative rapid test should be confirmed by culture. In those patients who appear acutely ill and in whom there is a strong possibility of GAS, it may be appropriate to initiate therapy after culture has been obtained. However, it is imperative to discontinue therapy when a negative result is returned. Surveillance of physician practices in the USA has revealed that 40% of physicians admit to continuing therapy despite negative culture results and many acknowledge not following through with results.

Treatment of GAS pharyngitis is based on cost, efficacy, palatability and spectrum of activity. Penicillin remains the drug of choice in non-allergic patients. There have been no documented cases of penicillin-resistant GAS. Penicillin has a narrow spectrum of activity and is safe, well tolerated and inexpensive. Amoxicillin is an acceptable alternative. It has the advantage of improved taste and is inexpensive. Although there are no data specifically assessing its efficacy in preventing ARF it is likely to be as effective as penicillin. The broader spectrum of activity of the aminopenicillins may exert greater selective pressure on the development of resistant bacteria. Macrolides are the drugs of choice for allergic patients in regions where resistance rates are low. Resistance rates to erythromycin as high as 40% have been reported in Japan and Finland. Decreasing availability of erythromycin in Finland has been associated with subsequent decreases in the number of resistant organisms. Clarithromycin and azithromycin appear to be as effective as erythromycin, have wider spectrums of activity and are more expensive. Extended-spectrum macrolides have been shown to induce resistance among GAS isolates in vitro. For these reasons the use of these agents is discouraged. Oral narrow-spectrum cephalosporins may be another alternative in penicillin-allergic patients, although cross-reactivity occurs in 15–20%. Cephalosporins are slightly more effective in eradicating the carrier state than penicillin, but given the benign nature of the carrier state this therapy may not be worth the added expense. A 10-day course of therapy is effective in approximately 90% of children. Shorter courses of therapy are less effective, except for azithromycin, where a 5-day course is acceptable based on the extended half-life of this drug. A 5-day course of a cephalosporin has been suggested by some investigators, but further study is necessary to confirm initial results. Whether

use of a broader spectrum drug for a shorter period will impede the development of resistant organisms remains to be demonstrated. This topic is reviewed in Chapter 31 .

Otitis media (see also Chapter 32)

Acute otitis media is one of the most frequent infections in childhood and accounts for more than one-quarter of all antibiotic prescriptions in the USA. Although there is considerable evidence supporting the use of antimicrobials in the treatment of AOM, the treatment effect is small. Tympanocentesis or needle aspiration of middle ear effusion has demonstrated a bacterial pathogen in only two-thirds of children who have AOM (*S. pneumoniae* accounts for 25–50% followed by nontypable *Haemophilus influenzae* in 15–30%) and two-thirds of patients improve without intervention. However, there is no reliable method of determining which children require treatment. Overdiagnosis of AOM and unnecessary use of antibiotic therapy for otitis media with effusion (OME) contribute to the development of resistant organisms. Rigorous attention to distinguishing between AOM and OME and withholding antibiotics in the latter group alone would eliminate 6–8 million unnecessary prescriptions in the USA per year.

Acute otitis media is defined as the presence of fluid in the middle ear, with inflammation of the tympanic membrane in association with the rapid onset of signs and symptoms of local or systemic infection, whereas OME is characterized by an asymptomatic middle ear effusion in the absence of inflammation. Following appropriate therapy for AOM, middle ear effusion persists in 50% of patients at 1 month and in 10% at 3 months. Thus, the presence of middle ear fluid in a child recently treated for AOM does not indicate treatment failure and does not warrant therapy.

Of the 16 antibiotics labeled for use in the treatment of AOM in the USA, only two reach sufficiently high levels in middle ear fluid to be effective against penicillin-resistant pneumococci: high-dose amoxicillin (80–100mg/kg/day) and intramuscular ceftriaxone. Extended spectrum ß-lactamase-stable agents do not appear to improve clinical cure rates. Previously accepted second-line agents, including trimethoprim-sulfamethoxazole and erythromycin, may be ineffective and have been shown to permit the emergence of resistant organisms during treatment.

There is an increased risk of infection and colonization with penicillin-resistant pneumococci in children receiving antimicrobial agents. Therapy should be reserved for those children who have effusion in the setting of new onset of illness or who have evidence of middle ear effusions accompanied by bilateral hearing loss for at least 3 months. These guidelines reflect the concern that persistent middle ear effusion may contribute to hearing impairment and subsequent language and cognitive deficits. A causal relationship between the modest conductive hearing impairment associated with otitis media and subsequent developmental deficits, however, has not been established. Other disadvantages to therapy include cost and potential side-effects of antimicrobial agents.

Antibiotic prophylaxis for control of recurrent AOM is indicated in the setting of three or more episodes in a 6-month period or four or more episodes in a 12-month period. Numerous studies have demonstrated that continuous treatment with low-dose amoxicillin or sulfisoxazole significantly reduces the number of new infections compared with placebo. The greatest benefit from this regimen is derived in those patients who have frequent recurrences, those under 2 years of age and in those attending day care. An alternative approach to antimicrobial prophylaxis is myringotomy and placement of tympanostomy tubes. Although tympanostomy tubes are somewhat less effective than antibiotics in reducing the number of recurrences, they are more effective in reducing the period during which an effusion is present. Their use is recommended in the context of hearing loss. The latest development in the treatment of AOM is the use of quinolone ototopical solutions for the treatment of acute otitis media with tympanostomy tubes as well as for otitis externa and chronic suppurative otitis media. These preparations are safe with no demonstrable effects on hearing and have equal or greater bacterial eradication compared with cortisporin drops or amoxicillin/clavulanate. Additional strategies to prevent recurrent AOM include eliminating smoking in the household, encouraging of breast-feeding and removing the child from day care.

Several vaccination strategies have been shown to reduce the incidence of AOM. Vaccinating against *H. influenzae* in infancy and pneumococci in children over the age of 2 years has a limited role. The pneumococcal conjugate vaccine, licensed in the USA in 2000, has been shown to markedly reduce the incidence of AOM as well as to inhibit transmission of antibiotic-resistant pneumococci. Several studies have shown that vaccination of preschool children against influenza virus significantly reduces the risk of AOM. Yearly vaccine against influenza also reduces the number of antibiotic prescriptions and over-the-counter medications in adults. Otitis media is reviewed in detail in Chapter 32 .

In summary, the evidence supports treating acute GAS pharyngitis and AOM. It is the injudicious use of antibiotics for those infections that are not likely to benefit from therapy and that often resolve spontaneously which contributes greatly to the development of resistant bacteria. Pressure from patients/parents has been cited by physicians as the major reason for treating primarily viral infections. During the past 2 years multidimensional interventions such as community-wide education directed toward health care practitioners and the public have resulted in reduced antibiotic prescription rates. It is imperative that physicians continue to take the time to educate themselves and their patients regarding the problem of antimicrobial resistance.

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40.d When to use corticosteroids in noncentral nervous system tuberculosis

Anur R Guhan
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Introduction

Tuberculosis, a chronic necrotizing infection, can involve any organ in the body. The clinical manifestations represent breakdown in local or systemic immunity against the causative organism, *Mycobacterium tuberculosis*. In this context, it might appear paradoxic that corticosteroids, with their known immunosuppressive effects, should even be considered in the treatment of tuberculosis. Indeed, this topic continues to be controversial.

Both cellular immunity and cytokines are involved in the pathogenesis of tuberculosis. Caseating granulomas, the pathognomonic lesions of the disease, represent the attempt by the immune system to contain the bacilli. Cytokines (tumor necrosis factor-a and interferon-?) released by sensitized tissue macrophages and peripheral blood mononuclear cells are thought to be responsible for the necrosis of lesions, the tissue edema and the constitutional symptoms (such as fever, anorexia, weight loss and night sweats) that are seen in patients who have tuberculosis. Healing results in fibrosis, causing local architectural change, which may affect physiologic function.

Adjuvant role of corticosteroids

The anti-inflammatory properties of corticosteroids have been used to modulate these harmful immune-mediated changes. Supported by case-control studies, the adjunctive use of corticosteroids with antituberculous medication was widely practiced in the 1950s and 1960s, especially with fulminant disease. However, the advent of short-course regimens containing rifampin (rifampicin) in the 1970s and the general decrease in the prevalence of tuberculosis in the Western world led to reduced need for adjuvant corticosteroid therapy except in selected situations.

With the resurgence of tuberculosis worldwide and the prospect of florid disease in the immunocompromised host, it is important to reevaluate the adjuvant role of corticosteroids in the treatment of tuberculosis. The topic is discussed in this chapter, basing recommendations on supporting evidence from the literature.

The anti-inflammatory properties of corticosteroids are well known and their use in the treatment of tuberculosis is based on their known capacity to:

- control the systemic effects of cytokines and other immune mediators;
- reduce immune-mediated tissue edema that occurs before and with treatment;
- suppress the clinical manifestations of hypersensitivity to tubercular proteins; and
- reduce the extent of fibrosis associated with the healing process.

Corticosteroids should only be used together with effective antituberculous chemotherapy.

Evidence of efficacy

The strength of evidence supporting the adjuvant use of corticosteroids in the treatment of tuberculosis is based on a few prospective, randomized, placebo-controlled trials, several retrospective, case-controlled trials and numerous case reports. Most of these studies were conducted before the newer, more effective antituberculous medications became available. However, there is no reason why these results cannot be extrapolated to the newer drugs. The following clinical situations are supported by evidence from prospective, randomized, placebo-controlled trials (RCTs).

Patients with *extensive pulmonary tuberculosis* have shown a more rapid clinical improvement with adjuvant corticosteroids, with faster normalization of body temperature, weight loss and other constitutional symptoms and earlier radiological resolution of pulmonary cavities.

Conversely, a RCT from India of patients on short-course chemotherapy failed to show any benefit of additional corticosteroid, at a dose of prednisolone 20mg/day, which is half that recommended with the rifampin-containing regimen (see below). The dose of prednisolone employed is probably important.

Patients with *tubercular pericarditis* receiving adjuvant prednisolone in a large RCT from South Africa had significantly faster resolution of symptoms and effusion and significantly reduced acute mortality than those receiving placebo. The need for later pericardectomy was not affected. Similar evidence is available for *tubercular pleural effusions*.

However, recent Cochrane Reviews of the above concluded that the evidence was insufficient on account of low numbers and recommended further RCTs.

Children with *tubercular mediastinal lymphadenopathy* causing extrabronchial compression who received adjuvant corticosteroid made significantly better radiological and bronchoscopic recovery.

Risk of progressive tuberculosis with adjuvant corticosteroids

Studies have shown either a benefit or no benefit from adjuvant corticosteroids; there is no evidence of increased risk of disease progression when corticosteroids are given together with effective antituberculous chemotherapy. However, adjuvant corticosteroid therapy could have systemic side effects related to their influence on the metabolism of bone, protein and carbohydrate. Monitoring of blood sugar is important in patients who have diabetes mellitus as a comorbid condition.

Use of adjuvant corticosteroids in tuberculosis

There are no agreed standards on the corticosteroid dosage or the duration of adjuvant treatment. Most authors, including ourselves, use the regimen suggested by Crofton and Douglas for most clinical situations: prednisolone 30–40mg/day for 2 weeks, gradually tapered over 6 weeks. Higher initial doses are suggested for tuberculous pericarditis: prednisolone 60–80mg/day for 4 weeks, tapering over 8 weeks.

Rifampin increases the catabolism of corticosteroids by hepatic microsomal enzyme induction, effectively halving the bio-availability of any administered dose of prednisolone. It is important to bear this in mind, particularly when corticosteroid replacement is instituted for tubercular Addison's disease.

The use of corticosteroids as adjuvant therapy in tuberculosis can be thought of under two headings:

- supported by evidence and general agreement (Table 40d.1); and
- not supported by unequivocal evidence (Table 40d.2).

The doses and duration of treatment suggested are what we would use in a particular clinical situation with a chemotherapy regimen that contains rifampin.

There is as yet insufficient information on the effects of adjuvant corticosteroids in patients who have HIV infection and tuberculosis. Co-infection with HIV and *M. tuberculosis* modifies the manifestations of clinical tuberculosis, making it difficult to extrapolate the results of trials on the use of adjuvant corticosteroids in the HIV-negative population to the HIV-positive population. However,

TABLE 40.d-1 -- Adjuvant use of corticosteroids supported by evidence and general agreement.

ADJUVANT USE OF CORTICOSTEROIDS SUPPORTED BY EVIDENCE AND GENERAL AGREEMENT		
Clinical situation	**Aim and comments**	**Suggested regimen**
Fulminant pulmonary tuberculosis with toxemia	Buy time for chemotherapy to take effect	Prednisolone 30–40mg/day until improvement in constitutional symptoms and erythrocyte sedimentation rate, then gradually taper by 2.5mg/day and stop
Miliary tuberculosis	Buy time for chemotherapy to take effect.	Prednisolone 40mg/day until improvement in constitutional symptoms and erythrocyte sedimentation rate, then gradually taper by 2.5mg/day and stop
	Important to monitor for occult adrenal insufficiency	
Adrenal insufficiency	Corticosteroid replacement: monitor progress with short ACTH stimulation test	Prednisolone 60–80mg/day. Long-term maintenance therapy may be required
Tuberculous pericarditis and tamponade	Expedite fluid resolution, symptom control and improve acute survival	Prednisolone 60–80mg/day for 4 weeks, tapering over 8 weeks. Insignificant effect on need for later pericardectomy
Massive tuberculous effusion with toxemia	Expedite fluid resorption, temperature control and reduce risk of adhesions	Prednisolone 30–40mg/day for 2 weeks, tapering over 2 weeks
Extrabronchial compression or atelectasis caused by tuberculous mediastinal lymphadenopathy	Reduce lymph node enlargement and improve lumen and lobar aeration	Prednisolone 30–40mg/day until radiologic resolution of atelectasis, tapering over 6 weeks
Endobronchial tuberculosis	Reduce risk of post-treatment structures	Prednisolone 30–40mg/day for 3 weeks, tapering over 12 weeks
Drug hypersensitivity reactions	Suppress manifestations of hypersensitivity while sequentially reintroducing antituberculous drugs.	Prednisolone 20–30mg/day, while the suspected putative drug is reintroduced gradually in increasing doses. Cautious tapering of corticosteroid dose
	Dose and duration need to be titrated to the individual. At least two effective antituberculous drugs must be introduced before corticosteroids are added	
Hypersensitivity phenomenon — tuberculides, episcleritis, scleritis, Eale's disease (retinal periphlebitis)	Modulate immunopathogenesis and reduce risk of scarring. Lesions are manifestations of hypersensitivity to tuberculoprotein and can occur with or without overt tuberculosis. Antituberculous chemoprophylaxis is required; in case of active tuberculosis, full-course chemotherapy is required	Ocular tuberculides, episcleritis and scleritis: topical corticosteroids
		Cutaneous tuberculides: topical corticosteroids; systemic corticosteroids occasionally needed
		Eale's disease: in consultation with an ophthalmologist, prednisolone 30–40mg/day for 3 weeks tapering over 8–12 weeks

TABLE 40.d-2 -- Adjuvant use of corticosteroids not supported by unequivocal evidence.

ADJUVANT USE OF CORTICOSTEROIDS NOT SUPPORTED BY UNEQUIVOCAL EVIDENCE		
Clinical situation	**Aim and comments**	**Suggested regimen**
Tuberculosis of the upper respiratory tract	Reduce risk of stricture formation after healing	Prednisolone 30–40mg/day for 3 weeks, tapering over 6 weeks. Endoscopic monitoring advisable
Tuberculous peritonitis	Expedite fluid resorption and reduce risk of adhesions	Prednisolone 30–40mg/day for 3 weeks tapering over 6 weeks
Tuberculosis of the genitourinary system	Reduce the risk of stricture formation and deformity	Prednisolone 30–40mg/day for 3 weeks tapering over 6 weeks
Tuberculosis and AIDS	Modulate the deleterious effects of cytokines and other immune mediators and buy time for chemotherapy to take effect	Similar to non-AIDS population. Prednisolone 30–40mg/day until improvement in constitutional symptoms and erythrocyte sedimentation rate, then gradually taper by 2.5mg/day and stop.
		Essential to be aware that AIDS patients may have co-existing cryptogenic opportunistic infections, which could progress under cover of corticosteroids

evidence from cohort studies suggests that HIV-positive patients with all manifestations of tuberculosis fare better when receiving adjuvant corticosteroids compared with matched control groups. Further research is needed.

It is important to bear in mind that patients with AIDS may have other co-existing but unrecognized opportunistic infections, which may progress under corticosteroid cover unless specific therapy is instituted.

Conclusion

Adjuvant corticosteroid therapy is effective in selected patients who have tuberculosis, producing useful and faster clinical recovery from

the disease. Its role in pleural tuberculosis needs further elucidation. When used under cover of effective antituberculous chemotherapy, there is no risk of disease progression. It should never be used when resistance to more than one drug is suspected. The recommended corticosteroid doses with rifampin-containing regimens take into account increased hepatic metabolism and reduced bio-availability. Conversely, with regimens that do not contain rifampin the recommended doses should be halved.

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40.e How to manage a patient on anti-TB therapy with abnormal liver enzymes

L Peter Ormerod

Introduction

Although isoniazid, rifampin (rifampicin) and pyrazinamide are all potentially hepatotoxic, when pyrazinamide is added to the other drugs there does not seem to be an increase in the incidence of hepatitis. However, in unselected cases outside clinical trials, pyrazinamide is the drug with the highest monthly rate of hepatitis.

The incidence of hepatic reactions to antituberculosis drugs depends on the drug itself, age, and possibly sex and ethnic group. Hepatic reactions to antituberculosis drugs were reported in 4% of cases treated with isoniazid/rifampin with or without pyrazinamide in a UK trial, and in 3% in a large clinical series. The overall rate of adverse reactions increases with age. Liver function tests (LFTs), particularly serum bilirubin and transaminases — aspartate aminotransferase (AST) and alanine aminotransferase (ALT) — should therefore be checked before treatment. Pre-treatment liver function testing is not advised for children receiving treatment for latent infection (chemoprophylaxis) because of the very low incidence of reactions. This may, however, be required if rifampin/pyrazinamide for 2 months is used to treat latent infection in adults, a regimen not advised in the UK where rifampin/isoniazid for 3 months is the alternative to isoniazid.

Testing of liver function

Regular monitoring of liver function is not required for those with no evidence of pre-existing liver disease and normal liver function pre-treatment. Patients and their family doctors should, however, be informed of possible side-effects and the indications for stopping medication and seeking medical advice, preferably in writing in their native language. Tests of liver function only need to be repeated (and treatment stopped) if fever, vomiting, jaundice, malaise or unexplained deterioration occur. In such circumstances virologic tests to exclude coexistent viral hepatitis (A or B) should also be considered.

Management of elevated tests of liver function

Modest elevations of hepatic transaminases (AST/ALT) are not uncommon in tuberculosis patients even without known liver disease. They are also to be expected in patients with chronic liver diseases including alcoholism, chronic active hepatitis, cirrhosis and in chronic carriers of hepatitis B or C. Monitoring of such patients should be as in Figure 40e.1 . If the pre-treatment AST/ALT is more than twice normal, liver function should be monitored weekly for 2 weeks then 2-weekly until normal. If the pre-treatment AST/ALT is less than twice normal, liver function should be repeated at 2 weeks. If the transaminase levels have fallen further repeat tests are only needed for symptoms. However if the AST/ALT rises to above twice normal, management should be as above (see Fig. 40e.1).

If the AST/ALT rises to above five times normal, or the bilirubin becomes elevated then isoniazid, rifampin and pyrazinamide should be stopped. Immediate management then depends on the severity of the patient's illness, and whether they are infectious as judged by positivity on sputum smear-microscopy within 2 weeks of treatment commencement (Fig. 40e.2). If the patient is not unwell and had a form of tuberculosis that is non-infectious, no treatment is needed until liver function returns to pre-treatment levels. If the patient is unwell or the sputum is smear-positive within 2 weeks of commencing a rifampin/isoniazid-based regimen, some form of drug treatment needs to be given until liver function returns to pre-treatment levels, preferably as an inpatient because of the monitoring required. In such

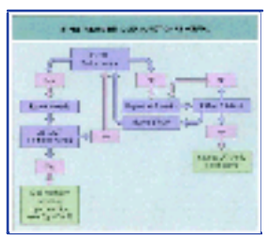

Figure 40.e-1 Management of elevated tests of liver function if pre-treatment liver function is abnormal. *Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.*

Figure 40.e-2 Management of elevated tests of liver function if antituberculosis drugs (rifampin/isoniazid/pyrazinamide) stopped because AST/ALT over five times normal or bilirubin elevated. *Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.*

cases a regimen of streptomycin and ethambutol with appropriate renal and visual checks is advised unless there are clinical contraindications or drug resistance to these agents is known or suspected. If second-line drugs are used, any potential hepatotoxicity (e.g. with macrolides, quinolones, prothionamide or ethionamide) should be considered. Occasionally patients with serious liver disease and active tuberculosis have very abnormal LFTs, with raised bilirubin and AST/ALT over five times normal pre-treatment levels. In these circumstances a risk/benefit analysis needs to be made, and often the risk of death (and infectivity) from untreated tuberculosis

TABLE 40.e-1 -- Order of re-introduction of antituberculosis agents following drug-associated hepatotoxicity.*

ORDER OF RE-INTRODUCTION OF ANTITUBERCULOSIS AGENTS FOLLOWING DRUG-ASSOCIATED HEPATOTOXICITY
Start:
Ethambutol (15mg/kg) plus isoniazid 50mg/day for 2–3 days then
plus isoniazid 150mg/day for 2–3 days then
plus isoniazid 300mg/day for 2–3 days and continue
If no reaction then:
Ethambutol (15mg/kg) plus isoniazid 300mg/day plus rifampin 75mg/day for 2–3 days then
plus rifampin 300mg/day for 2–3 days then
plus rifampin 450mg/day (under 50kg) or
rifampin 600mg/day (=50kg) for 2–3 days and continue
If no reaction then:
Ethambutol (15mg/kg) plus isoniazid 300mg/day plus rifampin 450/600mg/day and add sequentially:
pyrazinamide 250mg/day for 2–3 days then
pyrazinamide 1g/day for 2–3 days then
pyrazinamide 1.5g (<50kg) or
pyrazinamide 2g (=50kg) for 2–3 days and then continue if no reaction

Ethambutol can then be withdrawn if not required on drug resistance probability grounds
This is carried out with daily monitoring of clinical condition and regular LFTs.

** Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.*

significantly exceeds that of additional hepatotoxicity. Standard treatment may then be appropriate under corticosteroid cover with regular monitoring of liver function.

Once liver function has returned to normal or pre-treatment levels, challenge doses of the original drugs can be re-introduced sequentially (Table 40e.1). Such re-introduction is best carried out in parallel with treatment with ethambutol 15mg/kg to prevent the possible emergence of drug resistance during the re-introduction. The patient's condition should be monitored daily together with liver function. Sequential re-introduction of isoniazid, rifampin and pyrazinamide is advised. Isoniazid should be started initially at 50mg/day increasing to 150mg/day after 2–3 days and then 300mg/day after a further 2–3 days if no reaction occurs, which is then continued. After a further 2–3 days without reaction rifampin at a dose of 75mg/day can be added, increasing to 300mg/day after 2–3 days, and then to 450mg (<50kg) or 600mg (>50kg) according to patient's weight after a further 2–3 days without reaction, and then continued. Finally pyrazinamide can be added at 250mg/day, increasing to 1.0g after 2–3 days and then to either 1.5g (<50kg) or 2.0g (=50kg). If there is no further reaction standard chemotherapy can be continued and any alternative drugs introduced temporarily may then be withdrawn. Ethambutol may need to be continued in the initial phase if indicated by local drug resistance profile and national treatment guidelines.

Management of further reactions following re-introduction of therapy

If there is a further reaction during the re-introduction (Table 40e.3), the offending drug should be excluded and an alternative regimen constructed. If pyrazinamide is the offending drug then a regimen of rifampin and isoniazid for 9 months, supplemented by ethambutol for 2 months will be required. For other drugs an alternative regimen may need to be on the advice of an experienced TB physician. Sometimes, because for example of drug resistance, the choice of drugs is so limited that if reactions occur, desensitization and re-introduction of the offending drug may be necessary using conventional protocols.

Such densensitization may need to be carried out under the cover of two other antituberculosis drugs to avoid the emergence of drug resistance.

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40.f The place of antibiotics in the management of COPD

Robert Wilson

Chronic obstructive pulmonary disease (COPD) encompasses several conditions (airflow obstruction, chronic bronchitis, bronchiolitis or small airways disease and emphysema) that often co-exist. Tobacco smoking is the main etiological factor. Patients suffer exacerbations of their condition that are usually associated with increased breathlessness and they often have increased cough that may be productive of mucoid or purulent sputum. Exacerbations have different causes and several of these may be involved during a single exacerbation. There is considerable diversity in the severity of COPD and in the contribution made by the different pathologies listed above. Patients with chronic bronchitis are more susceptible to bacterial bronchial infections than those at the emphysema or asthma end of the spectrum. Although impairment of respiratory function does not in itself make patients more susceptible to infection, it does influence the outcome of a lower respiratory tract infection. Severe airflow obstruction, hypoxemia and the presence of hypercapnia all increase the risk of a poor outcome following an exacerbation. Therefore decisions about antibiotic treatment will be determined not only by the likelihood of bacterial infection and consideration of the species commonly involved, but also by the type of patient and concern about the outcome if bacterial infection is present and left untreated.

When COPD patients are admitted to hospital there is also concern about their susceptibility to nosocomial infections, even if bacterial infection was not the prime cause of the exacerbation. In a recently published study of severe exacerbations of COPD requiring admission to hospital and either noninvasive ventilatory support or mechanical ventilation, patients were randomized to receive either antibiotic (ofloxacin 400mg once daily) or placebo. Although additional antibiotics could be prescribed at any time at the physician's discretion, mortality was five times higher in the placebo arm. The timing of the deaths suggested that although some infections were present on admission, the majority were acquired in hospital.

Bacteriology

There is a general agreement that the bacterial species most commonly isolated from sputum during acute exacerbations of COPD are nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*, but several studies have shown that the same bacteria can also be isolated from patients during stable periods. However, since these species are upper respiratory tract commensals, bacteria may be picked up in the nasopharynx during expectoration of sputum and not represent true lower respiratory tract colonization or infection. A number of recent studies have used bronchoscopic techniques to avoid this contamination and have shed light on whether bacteria are pathogenic or simply commensal passengers.

Monso and colleagues studied 40 COPD patients during a stable phase of their illness and found positive protected specimen brush (PSB) cultures defined as $=10^3$ colony-forming units (cfu)/ml in one quarter. *H. influenzae* and *S. pneumoniae* were the predominant species isolated. Another group of patients were studied during an exacerbation. The number with positive PSB cultures was about 50% and although the same species were predominant, the bacterial counts were higher. Twenty-four percent of patients had more than 10^4 cfu/ml during an exacerbation, compared with only 5% of stable patients. Other studies have confirmed that higher bacterial numbers are associated with increased neutrophilic inflammation and that asymptomatic lower airway bacterial colonization (LABC) occurs in about 25% of COPD patients. One study has shown that LABC by itself is associated with airway inflammation.

Several recent studies have shown that Gram-negative species, more commonly associated with bronchiectasis, cause exacerbations in severe COPD. *H. influenzae* is still the most common isolate overall in these studies. In patients with mild airflow obstruction, *S. pneumoniae* and other Gram-positive cocci are more frequently isolated, whereas *Pseudomonas aeruginosa* and other Gram-negative bacilli account for a significant number of isolates in patients with severe airflow obstruction (defined as FEV1 =50% of predicted), but are rare in milder cases. This raises the possibility that unsuspected bronchiectasis may be present in some COPD patients prone to frequent infections. The role of atypical species such as mycoplasma and chlamydia in exacerbations of COPD is also debated. However, most studies have shown a low prevalence.

How often is bacterial infection involved in exacerbations of COPD?

The etiology of exacerbations is often multifactorial. Viral infections, particularly rhinovirus, probably trigger an exacerbation in about half of cases. Bacteria can also be isolated in about half of cases and this proportion increases to over 70% when there is more severe airflow obstruction. Furthermore, the more thoroughly a patient is investigated, the more often that infection, either viral or bacterial or both, is found to be implicated in the exacerbation. Air pollution (including tobacco smoke), cold weather and allergic responses will also contribute in some cases.

A cycle of events is illustrated in Figure 40f.1 . LABC occurs in COPD because the local airway defenses are impaired. Further studies are required to confirm whether LABC is associated with increased airway inflammation and a higher frequency of exacerbations. Bacterial infections can occur de novo or may represent an

Figure 40.f-1 A schematic representation of the role played by bacteria in COPD. There is limited evidence at the present time for the steps indicated by broken arrows which suggest that inflammation might be caused by lower airway bacterial colonization (LABC) and that airway infections might lead to progression of COPD.

increase in resident bacterial numbers when a new insult such as a viral infection reduces the host defenses even more. There is insufficient evidence to say whether repeated infections increase the rate of progression of airflow obstruction in COPD. The Lung Health Study which included large numbers of patients suggested that this might be the case, at least in those who continued to smoke.

Antibiotic treatment of exacerbations of COPD

In the study of Anthonisen and colleagues 173 patients with COPD were followed for 3.5 years during which time they had 362 exacerbations. Antibiotics or placebo were given in a randomized, double-blind, cross-over fashion. Three levels of severity of exacerbation were recognized: the most severe (type 1) comprised worsening dyspnea, increased sputum volume and purulence; type 2 was any two of these symptoms and the least severe grade (type 3) was any one of these symptoms with evidence of fever or an upper respiratory tract infection. Three antibiotics were used: amoxicillin, trimethoprimsulfamethoxazole and doxycycline, the choice being made by the physician. Overall there was a significant benefit from antibiotics that was largely accounted for by patients with type 1 exacerbations, whereas there was no significant difference between antibiotic and placebo in patients who had only one of the defined symptoms. However, even with type 1 exacerbations 43% of patients recovered in the placebo group within 21 days.

This emphasizes the important point that in most cases bacterial infections in COPD are mucosal infections which will resolve without antibiotic treatment when the host defenses overcome the infection. Therefore antibiotic treatment should have two aims. The most important is to prevent progression of an exacerbation into pneumonia or respiratory failure. The risk of this is greater in patients with more severe COPD. The second aim is to hasten recovery, although evidence that antibiotics have a major impact is lacking, particularly in those with mild disease. The Anthonisen study showed that for those patients with multiple exacerbations, which in other studies correlates with more severe COPD, the duration of antibiotic-treated exacerbations averaged 2.2 days less than those treated with placebo. However, when all

patients were considered and treatment failures were eliminated from the analysis, the benefit from antibiotics on speed of recovery was only 0.9 days.

The British Thoracic Society have adopted the Anthonisen criteria in their guidelines on the management of COPD exacerbations. My own opinion would be to give more weight to neutrophilic sputum purulence, which has been shown to accurately identify the presence of bacteria. In Figure 40f.2 I have outlined a simple scheme that might be used to decide whether antibiotics should be given. More difficult questions are which antibiotic to choose, what dosage and for how long; these questions are outside the scope of this chapter. There is little evidence in the literature to help in making these decisions.

In recent years there has been a dramatic rise in antibiotic resistance among common respiratory pathogens; for example, penicillin and macrolide resistance in *S. pneumoniae* and ß-lactamase production by *H. influenzae* and *M. catarrhalis*. The frequency with which antibiotics are used in a community is reflected in the amount of antibiotic resistance in a particular class. Antibiotics should not be prescribed for trivial illnesses which resolve spontaneously, including exacerbations of mild to moderate COPD, particularly if the sputum is not purulent. Although there is very wide variation in the prevalence of these strains from country to country, and even in different parts of the same country, there is some evidence to suggest that patients with a history of more frequent exacerbations, who have received several antibiotic courses beforehand, are more likely to carry resistant strains. I have used these principles in the choice of antibiotics outlined in Figure 40f.2 .

An antibiotic containing a ß-lactamase inhibitor, such as amoxicillin/clavulanate, or a respiratory quinolone, e.g. moxifloxacin, would be

Figure 40.f-2 A decision tree outlining how choices about whether or not to prescribe an antibiotic for patients with airway diseases can be made. There is very little evidence that one antibiotic is superior to another, but concern about antibiotic resistance will influence choice in severe cases. An antibiotic which is resistant to ß-lactamase enzyme and potent against the major pathogens should be chosen. Ciprofloxacin is indicated when there is concern about *Pseudomonas aeruginosa*. Evidence shows that this bacterium is usually only a consideration in patients with FEV1 =50% predicted, although in my own experience it is only patients with even more severe airflow obstruction in whom it occurs or in those patients who also have bronchiectasis.

appropriate for severe cases. In moderately severe cases, for patients who have not recently received antibiotics older agents such as amoxicillin or doxycycline are appropriate. In hospital most exacerbations of COPD will be treated with an antibiotic. A second-generation, e.g. cefuroxime, or third-generation, e.g. cefotaxime or ceftriaxone, cephalosporin given intravenously, an intravenous preparation of amoxicillin/clavulanate or a respiratory quinolone might be used for a few days until clinical improvement is evident when switch to an oral preparation should be made. An oral antibiotic throughout will be appropriate in some cases. *P. aeruginosa* is resistant to all oral antibiotics except ciprofloxacin.

There have been nine prospective placebo-controlled trials to investigate whether continuous antibiotic treatment reduces frequency of exacerbations. Five trials showed no reduction in the frequency, whereas four did show a benefit versus placebo. Two of the five trials that showed no benefit did show significantly less time lost from work in the antibiotic group. Patients most likely to benefit from continuous antibiotic treatment were found to be those suffering frequent exacerbations which was judged to mean =4 per year. My own practice is to only use antibiotic prophylaxis as a last resort and then only during the viral season from October to April. Much more commonly I prescribe a course of antibiotics for the at-risk patient to keep at home,

to begin when the symptoms of an exacerbation persist for a couple of days and immediately the sputum becomes purulent. The patient can then make an appointment for my clinic so that progress can be monitored.

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40.g Management of the infected cystic fibrosis patient

Daniel Rosenbluth

Definition of the problem

The airways of persons with cystic fibrosis (CF) are chronically infected with bacteria, usually *Staphylococcus aureus* and *Pseudomonas aeruginosa* (typically mucoid strains). This persistent infection and the accompanying inflammatory response result in mucus hypersecretion with obstruction of smaller airways, bronchiectasis and the symptoms of chronic cough, sputum production and dyspnea. While chronic symptoms are slowly progressive, intermittently patients may have a more accelerated decline associated with increases in the bacterial burden in the airways with additional symptoms, usually referred to as an acute respiratory exacerbation. Optimal management of the infected CF patient necessitates strategies to combat both the chronic airway infection and acute exacerbations.

Typical case

Persons with cystic fibrosis typically exhibit symptoms of chronic cough, sputum production and dyspnea on exertion if they have more advanced obstructive lung disease at baseline. Exacerbations of their lung disease are usually characterized by an increase in cough and sputum production, a change in the color (to darker or more green) or character (thicker and more viscous) of the sputum, dyspnea and worsening exercise tolerance. These exacerbations may also be accompanied by generalized fatigue, fevers, decreased appetite, weight loss and, in diabetic patients, worsening glycemic control. Acute exacerbations may be highlighted by massive hemoptysis, a true medical emergency (Table 40g.1).

Physical exam may demonstrate new physical findings such as wheezes or crackles, use of accessory muscles of respiration, tachycardia, cyanosis and decreased oxyhemoglobin saturation. Chest X-rays may reveal a new infiltrate or evidence of increased mucus plugging on a background of chronic bronchiectatic changes. Many patients with acute exacerbations will not demonstrate any change in their radiograph. On occasion the onset of an exacerbation may be insidious and occur over weeks. Under these circumstances patients may not fully appreciate the magnitude of their decline unless questioned carefully. In these cases pulmonary function testing (discussed

TABLE 40.g-1 -- Signs and symptoms of acute exacerbations of CF-related lung disease.

SIGNS AND SYMPTOMS OF ACUTE EXACERBATIONS OF CF-RELATED LUNG DISEASE
Increase in cough and sputum production
Change in the color or character of sputum
Dyspnea and worsening exercise tolerance
Fatigue
Fevers
Decreased appetite
Weight loss
Worsening glycemic control
Massive hemoptysis
New wheezes or crackles
Use of accessory muscles of respiration
Tachycardia
Cyanosis
New radiographic infiltrate
Decrease in FEV1

below) will usually detect a significant change from the patient's baseline and this by itself is an indication for intensification of therapy.

Diagnosis

Diagnosis of an acute respiratory exacerbation of cystic fibrosis-related bronchiectasis is best made by a clinician experienced in the care of patients with CF. It is usually made when a patient presents with a combination of some or all of the above-mentioned signs and symptoms, in association with a decrease in their forced vital capacity (FVC) and forced expiratory volume in one second (FEV1). These pulmonary function tests or spirometry are the best objective measure of a patient's status and during exacerbations will usually demonstrate worsening airway obstruction and a decline of at least 10%.

Management options

Optimal management of the infected patient with CF requires the identification of specific pathogens which infect the individual patient. Early in life infection with *Staphylococcus aureus* and *Haemophilus influenzae* is common but as patients age, infection with *Pseudomonas aeruginosa* and other Gram-negative organisms, such as *Achromobacter xylosoxidans, Stenotrophomonas maltophilia* and *Burkholderia cepacia*, predominates. Infections with *Burkholderia cepacia* can be difficult to treat, and have been associated with an accelerated decline in lung function in some patients and with epidemic outbreaks at some CF care centers. This organism may not grow readily in culture; therefore all respiratory samples from patients with CF should be cultured on media specific for this organism, in addition to the other organisms mentioned above.

Many patients will be infected with multiple organisms or multiple colony types of the same organism that may have different susceptibility patterns. It is therefore recommended that all organisms and their differing microcolonies be identified in culture and have their susceptibility patterns determined to facilitate the selection of the optimal antibiotic regimen. Persons with cystic fibrosis may become infected with nontuberculous mycobacteria. Therefore their respiratory secretions should be evaluated for the presence of these pathogens if they do not respond to routine antimicrobial therapy.

In the treatment of acute exacerbations (Table 40g.2) selection of specific antimicrobials should be guided by results of sputum culture and sensitivity results. Mild exacerbations may sometimes be treated with oral and/or inhaled antibiotics if effective agents are available. Response to therapy should be closely monitored and if ineffective, a switch should be made to parenteral therapy. More severe exacerbations should be treated parenterally and in general two antipseudomonal agents are chosen, usually a third- or fourth-generation cephalosporin or antipseudomonal penicillin, along with an aminoglycoside. Therapy should be modified based on culture results. The antipseudomonal penicillins or carbapenems may be more active against *Achromobacter xylosoxidans*, and carbapenems such as meropenem may have increased activity against *Burkholderia cepacia*. Infections

TABLE 40.g-2 -- Management of acute exacerbations of CF-related lung disease.

MANAGEMENT OF ACUTE EXACERBATIONS OF CF-RELATED LUNG DISEASE

Parenteral antimicrobial therapy based on respiratory culture results (typically two antipseudomonal agents)
Enhanced airway clearance
Bronchodilators
Mucolytics
Pulmonary rehabilitation
Nutritional support

with *Stenotrophomonas maltophilia* are sometimes best treated with ticarcillin/clavulanate, minocycline or trimethoprimsulfamethoxazole. Patients with CF often exhibit high volumes of distribution and increased antimicrobial drug clearance; therefore higher doses of antibiotics should be utilized. Aminoglycosides should be administered on an 8-hour dosing schedule with target peak levels of 8–12µg/ml and trough levels less than 2µg/ml. Some have reported successful therapy with single daily dosing regimens. Typically 2–3 weeks of parenteral therapy is required.

Therapy of the chronically infected CF patient who is not in the midst of an acute exacerbation may include inhaled suppressive antibiotic therapy. In clinical trials, a preservative-free formulation of tobramycin (TOBI®) has been shown to improve lung function and decrease the risk of hospitalization in patients colonized with *Pseudomonas aeruginosa* who inhaled this medication for three cycles of twice-daily administration for 28 days followed by 28 days off medication. Patients are often treated with other inhaled antibiotics such as colistin; however, the use of these agents is less well studied or standardized. A study of oral cephalexin prophylaxis in infants and young children with cystic fibrosis failed to demonstrate a clinical benefit.

In addition to antimicrobial therapy, airway clearance is an essential component in the management of the infected airways of patients with CF. This may be accomplished via traditional chest percussion and postural drainage or with the assistance of various devices such as percussors, pneumatic compression vests or the Flutter valve. Bronchodilators facilitate airway clearance and decrease airway obstruction and are commonly used by patients with CF. Dornase alpha (Pulmozyme®) degrades extracellular DNA in the airway secretions of patients with CF. The DNA is released from neutrophils which have entered the airway and contributes to the viscoelasticity of CF sputum. Daily inhalation of dornase alpha thins the CF sputum to allow improved airway clearance and has been shown to improve lung function and decrease the need for parenteral antibiotics in patients with CF.

Other adjunctive therapies for the infected CF patient include regular exercise and aggressive nutritional support to address the increased metabolic needs and malabsorptive problems which these patients may exhibit. Since the inflammatory response in the CF lung may contribute to the morbidity of the chronic airway infection, anti-inflammatory therapy may benefit some patients. Short courses of corticosteroids may benefit some patients; however, while long-term corticisteroid use may slow the decline in lung function, their benefit is outweighed by side effects of the therapy. High-dose ibuprofen may slow the decline in lung function in young patients with mild disease but this therapy should be closely monitored. Identification and evaluation of more specific and less toxic anti-inflammatory agents is currently being actively researched.

Optimal management of infected CF patients requires an effective infection control strategy to prevent patient-to-patient transmission of virulent organisms. This strategy should include both the inpatient and outpatient settings and conform to local infection control guidelines. Frequent handwashing is extremely important. Patients infected with *Burkholderia cepacia* should be segregated from other CF patients who are not infected with this organism. Since children infected with *Pseudomonas aeruginosa* have decreased lung function and survival when compared with children only infected with *Staphylococcus aureus*, some advocate segregating these groups of patients.

Conclusion

Management of the infected patient with cystic fibrosis requires strategies to address both the chronic infection and acute exacerbations of infection. This necessitates routine symptomatic and physiologic evaluation of patients, along with frequent microbiologic surveillance of airway secretions. These should guide aggressive but

appropriate use of oral, inhaled, and parenteral antibiotics, along with adjunctive therapies such as bronchodilators, mucolytics, airway clearance, pulmonary rehabilitation, and nutritional support. Finally an effective infection control policy should be in place to prevent patient to patient transmission of potentially harmful organisms.

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Chapter 41 - Orocervical and Esophageal Infection

Robert C Read

Infections of the oral cavity and neck include dental and periodontal infections, deep fascial space infections of the neck that are often odontogenic or caused by contiguous spread from pharyngeal foci, nondental oral infections, including ulcerative and gangrenous stomatitis, and infections of the salivary glands. Infections of the esophagus mostly occur in the context of severe underlying disease.

DENTAL AND PERIODONTAL INFECTIONS

EPIDEMIOLOGY

Dental caries is the commonest infectious disease in humans. The incidence of the disease is closely related to the use of derivatives of cane sugar and began to take on epidemic proportions in Europe in the 19th century. Dental caries first becomes evident in infancy and is most noticeable on the chewing surfaces of the molar teeth. The likelihood of dental caries is increased by high sugar intake, poor oral hygiene and any factors that reduce salivary flow — notably drugs (e.g. antidepressants).[1]

Periodontal disease, including gingivitis, is mainly related to poor oral hygiene and increasing age. Increased incidence of periodontal disease is also evident in diabetics and during hormonal disturbances, including puberty and pregnancy.[2] It is suspected that most periodontal disease arises from an inflammatory response to the accumulation of dental plaque in the gingival margin. Plaque contains mainly *Streptococcus* spp. and *Actinomyces* spp., which probably generate an early gingivitis leading ultimately to periodontitis.[3] These processes occur over many years with incremental destruction of periodontal tissue.[4]

PATHOGENESIS AND PATHOLOGY

The indigenous oral flora includes a large number of aerobic and anaerobic bacteria and varies by site within the oral cavity. There are of the order of 10[11] micro-organisms per gram wet weight of oral secretions, with the majority being obligate anaerobes. *Streptococcus* spp., *Peptostreptococcus* spp., *Veillonella* spp., *Lactobacillus* spp., *Corynebacterium* spp., *Bacteroides* spp., *Prevotella* spp. and *Actinomyces* spp. form the majority of oral flora.[5]

Dental caries is characteristically polymicrobial with no fixed pattern of microbial etiology, except that *Streptococcus mutans* has emerged as the only organism consistently isolated from carious teeth compared with normal teeth. In contrast, gingivitis has characteristic microbial specificity; the normal flora of the periodontium (i.e. *Streptococcus sanguis* and *Actinomyces* spp.) is replaced by anaerobic Gram-negative rods, notably *Prevotella intermedia*. With chronic gingivitis there is ulceration of the mucosa, loss of attachment of periodontal tissue, loss of enamel and necrosis of the dental pulp, and an increase in complexity of microbial flora with a preponderance of anaerobic Gram-negative rods, including *Porphyromonas gingivalis*.[2] When abscesses form, for example periapical abscesses, the flora is always polymicrobial with species such as *Fusobacteria*, pigmented *Bacteroides*, *Peptostreptococcus*, *Actinomyces* and *Streptococcus*.

In healthy individuals teeth are protected from decay by the cleansing action of the tongue and buccal secretions, the buffering effect of saliva and the acquired pellicle — the fluid microenvironment of the tooth surface. With poor dental hygiene the acquired pellicle is colonized and replaced by plaque that contains bacteria, notably *Strep. mutans*. This process is accelerated by intermittent exposure to carbohydrates and simple sugars. The presence of subgingival plaque leads to inflammation of the gingival epithelium and to destruction of the periodontium.[5] The normal resident polymicrobial flora provides an important defense against invasion of gingival epithelium by pathogenic bacteria. Normal saliva also protects the epithelium by irrigating it with enzymes, including lysozyme and lactoperoxidase, and other antimicrobial substances such as lactoferrin. Secretory IgA provides additional protection by aggregating organisms and preventing bacterial adherence. The importance of intact phagocytic defenses is reflected by the high prevalence of periodontal infections in patients who have cyclic neutropenia and chronic granulomatous disease.[3] Some oral micro-organisms involved in periodontal disease secrete IgA proteases. [6] Host factors credited with leading to periodontitis include psychosocial stress, diet, smoking, alcoholism and intercurrent disease. [7]

The usual cause of deep-seated odontogenic infection is necrosis of the pulp of the tooth followed by bacterial invasion through the pulp chamber and into the deeper tissues.[8] If a pulp abscess is allowed to progress, infection will spread toward the nearest cortical plate (Fig. 41.1).

PREVENTION

Prevention of dental and periodontal infections includes interference with transmission and suppression of *Strep. mutans* colonization once it has occurred. There is a strong correlation between maternal salivary *Strep. mutans* infection and the presence of this organism in children. Acquisition of *Strep. mutans* by infants has been prevented by aggressive treatment of *Strep. mutans* infection in mothers.[9] Existing infections can be suppressed by regular cleaning with agents that include fluoride and antimicrobial substances such as chlorhexidine. Periodontal disease can be prevented by good oral hygiene and regular rinsing with chlorhexidine. The use of controlled-release antibiotics is currently under investigation.[10]

CLINICAL FEATURES

Subgingival dental caries is asymptomatic, but destruction of enamel results in invasion of the pulp with subsequent necrosis, eventually leading to a periapical abscess. The tooth becomes sensitive to temperature and pressure once the enamel is penetrated, and toothache results.

In simple gingivitis there is usually discoloration of the gum margin with occasional bleeding after brushing of the teeth. There may be halitosis. If gingivitis is allowed to become chronic there may be destruction of periodontal tissue with loosening of the teeth. This

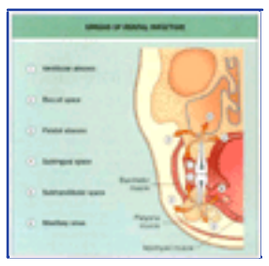

Figure 41-1 Spread of dental infection. A spreading tooth abscess will encroach upon the nearest cortical plate and its subsequent spread depends on the relationship of that site to muscle attachment. *Adapted from Peterson.*[6]

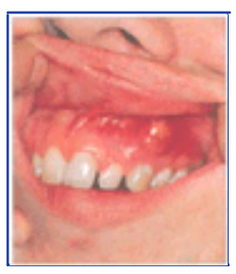

Figure 41-2 Painful vestibular abscess. *Courtesy of Professor I Brook.*

may be relatively asymptomatic or the patient may have itchy gums, temperature sensitivity and halitosis.

COMPLICATIONS

Dental pulp infections can lead to involvement of the maxillary and mandibular spaces (Fig. 41.1). Spread of infection from maxillary (upper) teeth most commonly leads to vestibular abscesses (Fig. 41.2). Erosion of canine pulp abscesses can lead to canine space abscesses if the abscess points above the insertion of the levator labii superioris. This results in swelling lateral to the nose, which usually obliterates the nasolabial fold. Buccal space abscesses can result when pulp abscesses of the molar teeth erode above or

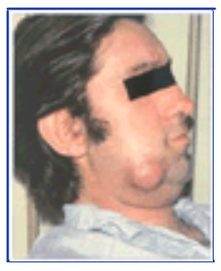

Figure 41-3 Buccal space abscess originating from right lower molar infection. The buccal space lies between the buccinator muscle and the overlying skin and fascia. *Courtesy of Professor I Brook.*

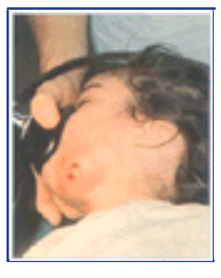

Figure 41-4 Submandibular abscess originating from a 2nd molar tooth infection. *Courtesy of University of Sheffield School of Dentistry, UK.*

below the attachment of the buccinator muscle; these point below the zygomatic arch and above the inferior border of the mandible (Fig. 41.3).

When infection spreads from mandibular (lower) teeth the commonest result is again vestibular abscess. Deeper abscesses may point into the sublingual and submandibular spaces. The sublingual space lies underneath the oral mucosa and above the mylohyoid muscle (Fig. 41.1). Posteriorly, it communicates with the submandibular space. Infection within the sublingual space results in swelling of the floor of the mouth, which may spread to involve both sides and be sufficiently pronounced to lift the tongue. This space is involved if the infected tooth apex giving rise to the disease is superior to the insertion of the mylohyoid (e.g. premolars and first molars).

The submandibular space lies between the mylohyoid muscle and the skin. It becomes involved if the apex of the infected tooth is inferior to the insertion of the mylohyoid muscle (e.g. third molar). Clinically, infection in this space causes extraoral swelling (unlike sublingual space infections) that begins at the inferior lateral border of the mandible and extends medially to the digastric area. Occasionally the abscess may point spontaneously and rupture (Fig. 41.4).

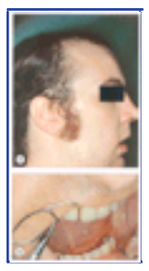

Figure 41-5 Ludwig's angina. (a) This patient had painful cellulitis within the submandibular and sublingual spaces. (b) Brawny edema was present within the floor of the mouth, pushing the tongue upwards. *Courtesy of University of Sheffield School of Dentistry, UK.*

Ludwig's angina refers to a severe cellulitis of the tissue of the floor of the mouth with involvement of the submandibular and sublingual spaces (Fig. 41.5). The source of infection is almost always the second and third mandibular molars. If the infection is allowed to continue there may be local lymphadenitis, systemic sepsis and extension of the disease to involve deep cervical fascia with a cellulitis that extends from the clavicle to the superficial tissues of the face. Other potential complications include asphyxia, aspiration and mediastinitis. The disease is almost always polymicrobial, including a-hemolytic streptococci and anaerobes such as *Peptostreptococcus* spp., *Prevotella melaninogenica* and *Fusobacterium nucleatum*.[11]

Very rarely, spread of infection from maxillary teeth may cause orbital cellulitis or cavernous sinus thrombosis (see Chapter 24). The latter is distinguished by toxemia, venous obstruction within the eye and orbital tissues (Fig. 41.6), involvement of the III, IV and VI cranial nerves and meningismus.

MANAGEMENT

Treatment of dentoalveolar infections includes elimination of the diseased pulp and deep periodontal scaling or tooth extraction. Any dentoalveolar abscess present should be surgically drained. If drainage is not complete, antibiotic therapy is appropriate. Treatment of periodontal disease includes appropriate debridement and short-term antimicrobial therapy with oral metronidazole 400mg q8h or oral phenoxymethylpenicillin 500mg q6h. Periodontal and vestibular abscesses should be treated by drainage.

Treatment of maxillary and submandibular space infections should always be by surgical drainage of pus. Ludwig's angina is a life-threatening condition and the first aim of treatment is protection of the airway, if necessary by emergency intubation or occasionally

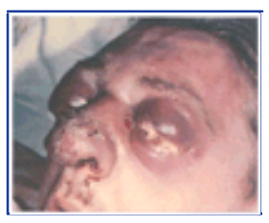

Figure 41-6 Cavernous sinus thrombosis. A patient who displays evidence of severe orbital swelling caused by obstruction of orbital veins is shown. In this patient, the originating focus was infection of soft tissues of the nose. *Courtesy of University of Sheffield School of Dentistry, UK.*

tracheostomy. Intravenous antibiotics should be administered. Benzylpenicillin 1.2g q4h plus metronidazole 400mg q8h or clindamycin 450mg q8h are appropriate.

Management of cavernous sinus thrombosis is by surgical decompression and high-dose intravenous antibiotics, the choice of which is influenced by whether the originating focus is dental or within soft tissues.

DEEP CERVICAL SPACE INFECTION

Infections of the lateral pharyngeal space, the retropharyngeal space and the prevertebral space are uncommon but life-threatening problems. The lateral pharyngeal space is funnel shaped, with its base at the sphenoid bone at the base of the skull and its apex at the hyoid bone. It is bounded by the medial pterygoid muscle laterally and the superior pharyngeal constrictor medially. Posteromedially it extends to the prevertebral fascia and communicates with the retropharyngeal space. The carotid sheath and cranial nerves are within the posterior compartment of the space. The retropharyngeal space lies posteromedial to the lateral pharyngeal space, between the superior constrictor muscle and the alar portion of the prevertebral fascia. Superiorly, it extends from the skull base of the pharyngeal tubercle down to the level of C7 where the superior pharyngeal muscle and the prevertebral fascia fuse.[8]

Unlike the lateral pharyngeal space it has few contents apart from lymph nodes, but its importance as a site of infection relates to its proximity to the airway and to the contents of the superior mediastinum. The prevertebral space extends from the skull base inferiorly to the diaphragm. It is bounded by the two layers of prevertebral fascia: the alar and prevertebral layers.

EPIDEMIOLOGY AND PATHOGENESIS

Parapharyngeal infections can complicate peritonsillar abscess (see Chapter 31), but a larger proportion of infections are odontogenic or secondary to intravenous drug abuse. [10] Rarer sources include parotitis, otitis and mastoiditis. The incidence of parapharyngeal infection has declined sharply in the antibiotic era and such infections now form less than 30% of all deep cervical infections. [11] [12]

Infections of the retropharyngeal and prevertebral spaces most commonly result from lymphatic spread of infection in the pharynx or sinuses, with subsequent suppuration of the retropharyngeal lymph nodes. Retropharyngeal infections are therefore commonest in children mainly because retropharyngeal lymph nodes are more numerous.[13] Occasionally retropharyngeal infections may be caused by accidental perforation of the pharynx, for example during emergency intubation. The bacteriology of deep cervical space infections reflects the microbial flora of the originating source. Thus, infections arising from the pharynx are often caused by *Streptococcus pyogenes*, whereas odontogenic infections are polymicrobial and include *Strep. mutans* and anaerobic pathogens such as *F. nucleatum, P. melaninogenica, Peptostreptococcus* spp., *Eikenella corrodens* and *Actinomyces* spp.

CLINICAL FEATURES

The characteristic feature of lateral pharyngeal space infection is severe trismus, which results from involvement of the pterygoid muscle and other muscles of mastication. There is also swelling of the lateral pharyngeal wall, which pushes the tonsil toward the midline. Occasionally there is lateral neck swelling below the angle of the mandible. The disease can be confused with peritonsillar abscess, although the latter should not produce trismus. The patient experiences fever, painful swallowing and pain that occasionally radiates to the ear. The infection tends to be severe and progresses rapidly. Posterior extension of the process into the carotid sheath can result in suppurative jugular thrombophlebitis, carotid artery erosion or interference with cranial nerves IX–XII. There is hyperacute sepsis, with rigors and high fever. There may be pain and swelling below the mandible, marked swelling of the lateral pharyngeal wall, torticollis and neck rigidity. There may be metastatic abscesses within the brain, lungs and bone. Suppurative thrombophlebitis of the internal jugular vein secondary to oropharyngeal infection was described by Lemierre and the syndrome bears his name.[14] The major organism associated with this complication is *Fusobacterium necrophorum*, which is usually obtained from blood cultures, but may require several days of anaerobic culture to grow.

Patients who have retropharyngeal abscess may present with fever and rigors that usually follow on from a streptococcal pharyngitis, but often there is no history of sore throat.[15] A child with a retropharyngeal abscess may be withdrawn and irritable. Adults may complain of sore throat, dysphagia, neck pain and dyspnea. The neck may be hyperextended, and there may be drooling and stridor. Examination of the throat by indirect laryngoscopy may reveal bulging of the posterior pharyngeal wall. Potential complications include upper airway obstruction as a result of anterior displacement of the posterior pharyngeal wall into the oropharynx, and spontaneous rupture of the abscess with aspiration pneumonia (which may complicate attempted insertion of an endotracheal tube). Other potential complications include purulent pleural effusion, pericardial effusion and posterosuperior mediastinitis.[16]

Patients who have AIDS, particularly intravenous drug users, have a higher incidence of deep neck infections, most commonly caused by *Staphylococcus aureus*, which is often methicillin resistant. In contrast to immunocompetent patients, there is often no leukocytosis.[17] Diabetics are also at increased risk of deep neck infections, and in addition to *Staph. aureus*, Gram-negative organisms, notably *Klebsiella* spp., may be isolated from these patients.[12]

DIAGNOSIS

If lateral pharyngeal space infection is suspected, the diagnosis is best confirmed by CT or MRI scanning. Plain radiographs are usually unhelpful. In contrast, a retropharyngeal space abscess can be diagnosed

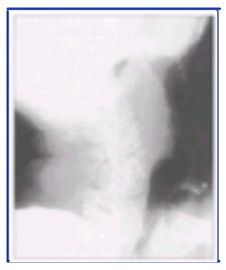

Figure 41-7 Retropharyngeal abscess. Lateral radiograph of the neck in a patient who has a retropharyngeal abscess, showing gross expansion of prevertebral soft tissue. *Courtesy of Mr R Bull.*

by a lateral radiograph of the neck (Fig. 41.7). The average width of the prevertebral soft tissue should be no more than 7mm (average 3.5mm) at C2 and no more than 20mm (average 14mm) at C6.[18] The neck should be fully extended during evaluation. The major clinical differential diagnosis of retropharyngeal abscess includes cervical osteomyelitis and meningitis. The latter can usually be discounted when there is obvious pharyngeal swelling, but cervical osteomyelitis may require MRI scanning of the cervical vertebral bodies for exclusion.

MANAGEMENT

In any patient with a suspected deep neck infection, maintenance of the airway is always the first consideration; about one-third of patients who have retropharyngeal abscess will require tracheostomy.[12] Incision and drainage of involved spaces and intravenous antibiotic therapy should be performed promptly in order to produce rapid and complete resolution of the infection with minimal likelihood of complications. Radiologic evidence of gas within soft tissues increases the urgency, because expansion of lesions containing anaerobes is usually rapid. Lateral pharyngeal and retropharyngeal abscesses can be drained by an incision along the anterior border of the sternocleidomastoid muscle followed by blunt dissection and drainage. Extensive surgery should be unnecessary if infections are treated promptly and high-dose intravenous antibiotics are used. Appropriate intravenous antibiotics include penicillin 1.2–1.8g q3h plus clindamycin 300–600mg q8h or metronidazole 400mg q8h, plus ceftriaxone 2g q12h.

CERVICAL NECROTIZING FASCIITIS

Cervical necrotizing fasciitis is a rare and extremely dangerous complication of odontogenic and deep cervical space infection. The disease is characterized by involvement of more than one neck space (usually bilaterally) and contiguously spreading necrosis of connective tissue, with cellulitis that extends below the hyoid bone to the chest wall, onto the face and into the mediastinum. Most cases are odontogenic, particularly after dental abscesses, but some cases follow on from tonsillar abscess or from surgical trauma to the oropharynx. Almost all cases are polymicrobial, often with a single aerobic isolate (e.g. *Streptococcus* spp.) plus two or more anaerobes (mostly *P. melaninogenica* and *F. nucleatum*), although any of the oral anerobes can be involved. The typical clinical presentation is

usually with dental pain and submandibular swelling over a few days, followed by rapid evolution of fasciitis, which is extremely tender on palpation and usually associated with crepitus. Mediastinal extension can be clinically silent and detectable only by CT of the chest, but can lead to pericarditis, pneumonia or empyema. Predisposing conditions include diabetes mellitus, alcoholism and malignancy. Management includes surgical drainage via incision along the sternocleidomastoid muscle followed by blunt dissection of the neck. Appropriate intravenous antibiotic therapy is benzylpenicillin 1.2–1.8g q3h plus clindamycin 600mg q8h.[19]

ACTINOMYCOSIS

Actinomycosis is a chronic suppurative bacterial infection that principally affects the head and neck but can involve almost any system. It spreads directly through tissue, skin and bone, and therefore is able to form sinuses and fistulas.

EPIDEMIOLOGY AND PATHOGENESIS

The agents that cause actinomycosis are facultative anaerobic Gram-positive commensals of the mouth. *Actinomyces israelii* is the most common pathogen, but *Actinomyces naeslundii*, *Actinomyces viscosis*, *Actinomyces odontolyticus* and *Arachnia propionica* may also cause the disease. These agents commonly inhabit carious teeth, dental plaque and cavities and also the normal intestinal tract. Head and neck infection usually occurs in the context of dental disease or dentistry, during which the normal mucosal barriers are broken down. Thoracic involvement usually follows aspiration of infected oropharyngeal secretions in patients who have poor dentition. Lesions of actinomycosis consist of areas of acute inflammation surrounded by fibrosing granulation tissue. Such material contains 'sulfur granules' (colonies of organisms forming an amorphous center surrounded by a rosette of clubbed filaments); these usually contain associated organisms, including *Actinobacillus actinomycetemcomitans*, *Haemophilus* and *Fusobacterium* spp., which probably contribute to the pathogenesis of the disease.

Any age group can be infected, including infants and children. Males outnumber females by three to one.

CLINICAL FEATURES

The most common manifestation of actinomycosis is soft tissue swelling of the head, face or neck, usually over or underneath the mandible.[20] Occasionally the swelling is very extensive and waxes and wanes over many months, spreading to involve other parts of the head and neck, including the scalp, palate, eyes, larynx, salivary glands, middle ear and paranasal sinuses. Sinuses and tracts develop that open into the mouth and the skin (Fig. 41.8). Involvement of local bone (e.g. the mandible) can result in periosteal reaction or frank osteomyelitis.

DIAGNOSIS

The diagnosis is usually obvious in patients who have head and neck swelling, particularly in the context of poor dentition and discharging sinuses yielding sulfur granules. The granules can be trapped in gauze placed over the sinus opening or by injecting and aspirating saline from the sinus; by shaking the aspirate, the granules can be seen with the naked eye. Sulfur granules can also be seen in sputum on microscopic examination. Any material obtained can be cultured under anaerobic conditions. In formalin-fixed tissues, immunofluorescence can be used to identify species. There is no reliable serologic test; laboratory diagnosis depends on microscopy and culture of material from the patient.

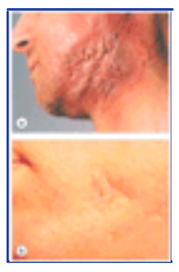

Figure 41-8 Actinomycosis. (a) This patient had chronic disease over the mandible which (b) healed with several months of antibiotics, leaving a residual chronic sinus. *Courtesy of Professor I Brook.*

MANAGEMENT

Most patients who have actinomycosis will respond to intravenous benzylpenicillin, 1.2–1.8g q3h for 3–6 weeks, followed by oral penicillin V, 2–4g/day for 6–12 months. Alternative treatments include intravenous amoxicillin or ampicillin, followed by oral amoxicillin. Chloramphenicol, erythromycin, tetracycline and clindamycin have also been used successfully. Prolonged treatment with penicillin results in complete resolution of the disease, although there may be some residual fibrosis or scarring (Fig. 41.8). Whilst intravenous benzylpenicillin has been the traditional treatment for this condition there have been reports of the use of intravenous agents that can be given in once-daily dosing for home therapy, including ceftriaxone and imipenem.

INFECTIONS OF THE ORAL MUCOSA: GANGRENOUS STOMATITIS

Acute necrotizing ulcerative gingivitis, or trench mouth, is an ulcerative necrosis of the marginal gingivae. The disease may spread to other oral structures, including the tonsils or pharynx, to cause Vincent's disease or may result in rapid necrosis and sloughing of facial structures, producing the classic features of cancrum oris (noma).

EPIDEMIOLOGY

The disease is mostly seen in developing countries in the context of severe debilitation and malnutrition. In addition, poor oral hygiene, HIV infection, measles, local irritation from food impaction and smoking are associated factors. [21]

PATHOGENESIS

Necrotizing gingivitis may begin as an aseptic necrosis secondary to mucosal capillary stasis. In infections in which the disease spreads superficially to involve the pharynx, it is most likely secondary to a combination of *F. nucleatum* and Gram-negative anaerobic organisms

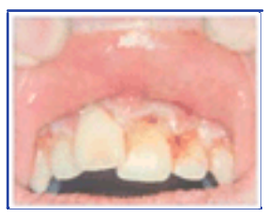

Figure 41-9 Acute necrotizing gingivitis. *Courtesy of Professor I Brook.*

(*Bacteroides* subsp. *intermedius*). If the disease spreads deeper into facial tissues to cause cancrum oris, fusospirochetal organisms such as *Borrelia vincenti* and *F. nucleatum* are consistently cultured. *Prevotella melaninogenica* may also be present. Biopsies of any advancing lesion often reveal a mat of predominantly Gram-negative thread-like bacteria that cannot be positively identified.[22]

CLINICAL FEATURES

The earliest feature is a small painful red lesion that may be vesicular on the attached gingiva and often in the premolar or molar region of the mandible, with sudden onset of painful gums (Vincent's disease; Fig. 41.9). The disease may then progress rapidly to produce halitosis and gingival bleeding. If there is involvement of the tonsils and pharynx (Vincent's angina) there is searing pain in the pharynx with high fever, regional lymphadenopathy and anorexia. If the disease spreads into deeper tissues (noma) a necrotic ulcer rapidly develops with painful cellulitis of the lips and cheeks, which often sloughs, exposing underlying bone, teeth and deeper tissues (Fig. 41.10).

DIAGNOSIS

Although the infection is usually polymicrobial, material should be obtained for Gram stain and aerobic and anaerobic culture. Debrided material is optimal for anaerobic culture. Gram stain may reveal fusospirochetal Gram-negative organisms as well as Grampositive cocci and Gram-negative rods.

MANAGEMENT

In early acute necrotizing ulcerative gingivitis (Vincent's infection), treatment with oral penicillin V 500mg q6h and metronidazole 400mg q8h is usually sufficient. In patients who have noma, high doses of intravenous penicillin and metronidazole are required, with the dose being dependent on the age and size of the patient. An antibiotic to treat aerobic Gram-negative rods, such as ceftriaxone, may be necessary. Gangrenous tissues should be removed and loose teeth extracted. The patient should be carefully rehydrated. Once the infection has been controlled, reconstructive surgery is often necessary.

INFECTIONS OF THE ORAL MUCOSA: PRIMARY HERPETIC GINGIVOSTOMATITIS

EPIDEMIOLOGY

Herpes simplex virus (HSV)-1 and HSV-2 can cause a primary infection of the oral cavity, although type 1 is much more frequently responsible. The disease can occur in infants, although this is becoming

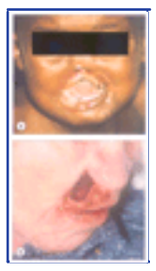

Figure 41-10 Noma. This is a destructive process extending from oral structures, which is a sequel of necrotizing gingivitis and (a) is seen most commonly in patients in developing countries, although (b) occasionally it is seen in the elderly debilitated in developed countries. *Courtesy of Professor I Brook.*

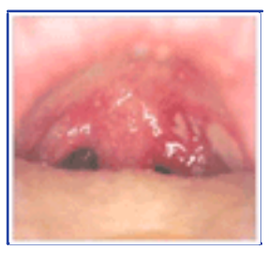

Figure 41-11 Primary HSV-1 stomatitis.

increasingly uncommon. Oral lesions caused by HSV-2 are seen in sexual contacts of patients who have genital herpes and are clinically indistinguishable from those caused by HSV-1.

CLINICAL FEATURES

The disease may be very mild, with a few painful ulcers and no systemic features, or it may be more severe with fever, sore throat, malaise, headache and regional lymphadenopathy. Oral lesions tend to appear 1–2 days after the onset of pain and lead to a painful, red gingiva or palate. These symptoms generally persist for approximately 2 days. The vesicles occur as 2–4mm ulcers on a red background. When lesions coalesce they can resemble aphthous ulcers (Fig. 41.11). At this point the disease is highly infectious. The clinical course of unmodified primary herpetic gingivostomatitis usually lasts 2 weeks.

DIAGNOSIS

The clinical differential diagnosis of oral herpetic gingivostomatitis includes herpangina, varicella, herpes zoster and hand, foot and mouth disease. These diseases can usually be distinguished on the basis of concomitant cutaneous features. Primary herpes infection of the mouth can occasionally be recurrent and several other recurrent diseases have similar oral lesions — these include minor aphthous ulcers, Behçet's syndrome, cyclical neutropenia and erythema multiforme. A laboratory diagnosis of herpes can be verified by direct immunofluorescence or viral culture of material obtained by swabbing the ulcers.

MANAGEMENT

In primary herpetic gingivostomatitis oral aciclovir 200–400mg q8h is appropriate therapy.

OTHER INFECTIONS OF THE ORAL MUCOSA

HERPANGINA

Herpangina produces characteristic oropharyngeal vesicles, generally at the junction of the hard and soft palates (Fig. 41.12). It primarily affects children and teenagers and generally occurs in epidemics during the summer. Several different coxsackieviruses, notably coxsackievirus A (types 1–10, 16 and 22) and less commonly coxsackievirus B (types 1–5), have been associated with this disease. Other enteroviruses, including echovirus, have been implicated. Patients usually have mild disease, but they can complain of sudden fever, anorexia, neck pain, extremely sore throat and headache. The lesions are often more vesicular than herpetic lesions and consist of multiple, small, white papules with an erythematous base that appears less inflamed than that with herpetic lesions. These lesions usually spontaneously rupture within 2 or 3 days and seldom persist for more than 1 week. There may be cervical lymphadenopathy but this is unusual. A laboratory diagnosis can be obtained by culturing swabbed material from the lesions. Herpes simplex virus infection can usually be distinguished on clinical grounds, but can be rapidly excluded by direct immunofluorescence. Management consists of topical analgesia only.

HAND, FOOT AND MOUTH DISEASE

Hand, foot and mouth disease is caused by systemic infection with Coxsackie group A viruses (usually serotype 16) and primarily affects children, but occasionally adults. The disease consists of vesicular eruptions on the hands, wrists, feet and within the mouth. Lesions

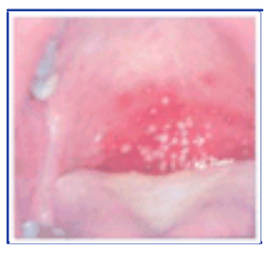

Figure 41-12 Herpangina in a teenager with severe throat pain.

on the hands are almost always present, but oral lesions are present in 90% of patients and can occasionally be the only manifestation of the disease.[23] The oral vesicles are often on the palate, tongue and buccal mucosa and may range from a few isolated lesions to a marked stomatitis. In addition patients may suffer fever, malaise, conjunctival injection, headache and abdominal pain and occasionally diarrhea. The lesions on the feet and hands are flaccid, grayish vesicles, most often on the sides of the fingers, instep and toes.[24] If the disease is confined to the oral cavity it is almost indistinguishable from primary herpetic gingivostomatitis. Laboratory diagnosis of the disease can be confirmed by culture of feces or swabs obtained from the lesions. Management is symptomatic. The disease is usually self-limiting and rarely persists for more than 2 weeks.

APHTHOUS STOMATITIS

The cause of aphthous ulceration is unknown but a number of infectious agents, including viruses, have been implicated. It usually manifests as small ulcers of the buccal and labial mucosa, often affecting the floor of the mouth or the inferolateral aspect of the tongue, almost always within the anterior part of the oral cavity; the palate and pharynx are rarely involved. The ulcers are characteristically exquisitely painful, particularly during eating, and in the most severe form can lead to anorexia. Humoral and cytotoxic T cell-mediated immune responses to oral mucosa have been demonstrated in some patients, suggesting an autoimmune process. The lesions are usually raised and appear grayish yellow, but in severe cases they may be herpetiform with secondary bacterial infection and cervical lymphadenopathy. Major aphthous ulcers may persist for months, but minor lesions usually heal over 2 weeks. They often recur, with periods of remission lasting as long as a few years. Cultures of swabs from aphthous ulcers are negative on viral culture. Treatment is usually symptomatic with mouth washes and anesthetic lozenges. Oral prednisolone has been used in some patients but is generally unhelpful. Severe aphthous ulcers have been successfully treated with oral thalidomide. The risk of teratogenicity precludes the use of this drug in women of child-bearing potential. Ulcers are particularly severe in HIV-infected patients.

PRIMARY SYPHILIS

Primary chancres can occur in the mouth approximately 3 weeks after oral sex. An ulcerating papule develops at the site of initial contact of *Treponema pallidum* with the oral mucosa. The papule is painless but is accompanied by significant regional cervical lymphadenopathy. At presentation patients are often seronegative, but dark-ground microscopy of material obtained from the ulcer may reveal spirochetes, although care should be taken to avoid contamination of the material obtained with saliva because other *Treponema* species inhabit the mouth and may be easily mistaken for *T. pallidum*. Treatment is discussed in Chapter 75 .

CANDIDA INFECTIONS OF THE MOUTH

Oral candidiasis is a common problem that usually signals local or generalized disturbance of host defenses.

EPIDEMIOLOGY

Most patients who have oral candidiasis are at the extremes of age, but any individual who has recently taken oral or inhaled steroids or broad-spectrum antibiotics is at risk. The disease is also seen in patients wearing dentures and patients who have diabetes mellitus.[25] Between 1980 and 1989, rates of oropharyngeal candidiasis in

hospitalized patients increased from 0.34 to 1.6 cases per 1000, caused mainly by the HIV epidemic.[26]

PATHOGENESIS

Yeasts are common colonizers of the oral cavity of healthy individuals. *Candida albicans* is the most common of oral yeast isolates (up to 50%).[27] *Candida albicans* can adhere to complement receptors, various extracellular matrix proteins and carbohydrate residues on oral epithelial cells and oral micro-organisms. [28] The organism exists in yeast and hyphal forms. Invasion of tissue is probably related to secreted hydrolytic enzymes and hyphal formation, each of which is possibly initiated by contact with epithelial cells. The immunopathology of mucosal candida infections is unclear, although suppression of normal oral microflora by antibiotics probably permits proliferation of yeasts. Salivary antibodies inhibit bacterial adherence to buccal epithelial cells and are protective in animal models.[29] Saliva also contains a number of antifungal proteins, including histatins and calprotectin, that protect the mouth in concert with local antibody and cell-mediated defense. A disturbance of cell-mediated immunity is partly responsible for overproliferation in patients who have HIV-1 infection and malignancy.

CLINICAL FEATURES

In patients using broad-spectrum antibiotics or who suffer candidiasis as a result of denture use, lesions are often erythematous with a burning sensation of the tongue, which displays diffuse redness of the entire dorsum. Most patients who have denture-related oral candidiasis are asymptomatic. Patients who have cell-mediated defects (i.e. diabetics, those on oral steroids or immunosuppressed patients) mostly have the characteristic syndrome of thrush, a pseudomembranous form of the disease in which there is a layer of white curd-like flecks of material that can be wiped off to leave an erythematous surface, beneath which there may be bleeding points.

DIAGNOSIS

The diagnosis is usually clinically obvious in patients who have thrush, but in patients who have erythematous lesions diagnosis can be made by scraping the mucosa and identifying characteristic ovoid yeasts with hyphal forms on microscopy. The organism can be cultured on Sabouraud's agar, but culture alone is insufficient to make the diagnosis since the organism can be recovered from the mouth of approximately 10% of completely normal individuals with no symptoms.

MANAGEMENT

In normal individuals the disease can usually be terminated by removing the cause — either inhaled steroids or broad-spectrum antibiotics — or by removing dentures at night. If necessary, patients can use 7–14 days of topical antifungal therapy, such as nystatin or clotrimazole, which is usually quite sufficient to ablate the infection. Immunocompromised individuals, particularly those with advanced immunosuppression, may require systemic therapy. For a full discussion of candidiasis in patients who have AIDS see Chapter 126 .

OTHER ORAL FUNGAL INFECTIONS

Histoplasma capsulatum is endemic in the midwestern USA and Central and South America. The organism is generally associated with lower respiratory tract infection, but oral lesions can occur, particularly in elderly, debilitated patients who have disseminated disease. The lesions tend to appear as erythematous areas that may ulcerate.[30] Biopsy is usually required to established a diagnosis. Because the infection is usually disseminated, systemic therapy with amphotericin B is generally required (see Chapter 39 and Chapter 238).

The dimorphic fungus *Paracoccidioides brasiliensis* is a major cause of systemic mycosis in Central America and South America and should be considered in patients originating from this region. Most patients have an oral mucosal ulcer with some surrounding edema. There may be perioral lesions that may be ulcerated or warty. Diagnosis can be made by smear and culture and treatment with oral imidazole compounds is generally sufficient (see Chapter 208 and Chapter 238).

ORAL LESIONS IN PATIENTS WHO HAVE MALIGNANCY

A common problem among cancer patients undergoing chemotherapy or radiotherapy is severe mucositis and stomatitis that occurs approximately 1 week after the onset of chemotherapy.[31] At this point, destruction of oral epithelium is at its height with an accompanying disturbance of immune surveillance of oral mucosal microorganisms. This leads to opportunist bacterial[32] or fungal infection. Patients nearly always complain of pain and tenderness in the mouth with or without formation of a pseudomembrane. Symptoms can persist long after chemotherapy has been terminated. Management should include a vigorous search for a microbial etiology; a short course of metronidazole is sometimes helpful. Some prevention can be achieved by careful oral hygiene and effective management of xerostomia associated with chemotherapy. Once there is established mucositis, topical therapy with antiseptic and anesthetic preparations is indicated. Aluminum hydroxide gel can be used to provide symptomatic relief of painful inflammation.

INFECTIONS OF THE SALIVARY GLANDS

The commonest cause of parotitis is mumps virus, but parotitis can occasionally be caused by bacteria or other viruses, including parainfluenza virus, coxsackievirus, echovirus, Epstein-Barr virus and HIV.

EPIDEMIOLOGY

The incidence of mumps has markedly decreased in the era of childhood measles, mumps and rubella (MMR) vaccination, which confers lifelong immunity. Despite this, mumps virus remains the most common cause of parotitis. It is highly contagious by air-borne droplet transmission. Mumps infections occur in late winter and early spring; enterovirus infections, including parotitis, are mostly seen in mid to late summer. Before the introduction of the MMR vaccine in the UK in 1988, the annual incidence of mumps was approximately 5 per 100,000 population, but in the postvaccine era this has declined to less than 0.5 per 100,000.[33]

Most patients who have primary bacterial parotitis are over the age of 60 years and are frequently debilitated because of chronic illness or have underlying diseases such as diabetes. Patients who have dehydration, whatever the cause, are at greatest risk. Medications that lead to xerostomia include anticholinergic and occasionally diuretic agents. Poor oral hygiene increases the chances of reflux of bacteria into the salivary gland.[34]

PATHOGENESIS

Mumps virus is a paramyxovirus and gains entry via the respiratory tract. The subsequent viremia allows access of the virus to tissues for which it has tropism, including salivary gland tissue, gastrointestinal tissue such as pancreas, testicular tissue and the central nervous system. The incubation period is 18–21 days.

Bacterial infection of the salivary glands is normally prevented by constant salivary flow, which removes contaminants from the ductal systems. Dehydration, xerostomia or obstruction of the ducts can lead to bacterial proliferation within the salivary glands and subsequent parotitis.

CLINICAL FEATURES

The most common clinical manifestation is gradual onset of painful swelling of either one or both of the parotid glands, which occurs 14–21 days after contact with an infected individual. Pain within the parotid gland can be initiated by salivation during meals, and the glands are tender. Occasionally submandibular salivary glands are involved, but inflammation of sublingual glands is extremely rare. Orchitis is present in approximately 10–20% of individuals and is bilateral in 5%, but there is no firm evidence that it causes male sterility. Mumps meningoencephalitis may occur in concert with parotitis, but patients who have mumps meningitis often do not have parotitis. In the pre-MMR era mumps was a relatively common cause of viral meningitis in children less than 15 years old in whom permanent unilateral deafness was a recognized complication. Pancreatitis is rare. On examination there is smooth tender swelling that obliterates the angle of the jaw and may raise the pinna. Rarely, the outlet of Stensen's duct may be inflamed. There may be generalized symptoms, including fever, arthralgia, malaise and headache, that generally persist for up to a week. Culturable virus is present in the saliva for up to 1 week after gland enlargement. Management is essentially symptomatic.

Recurrent episodes of glandular swelling, particularly of the parotid gland, can occur in children with a history of mumps. Clinical features include recurrent parotid swelling with general malaise and pain frequently after a meal. Viridans streptococci are usually cultured from exudate from the Stensen's duct.

In primary bacterial parotitis there is usually rapid onset of pain, swelling and induration of the involved gland (Fig. 41.13). Manual palpation of the gland is exquisitely painful and can result in discharge of pus from the duct. In addition there are usually systemic features, including fever, rigors and a neutrophilia. The most frequently isolated organisms are *Staph. aureus, Strep. pyogenes*, viridans streptococci and *Haemophilus influenzae*.

HIV-associated salivary gland swelling most commonly occurs as a bilateral cystic enlargement of the parotid glands, occasionally in

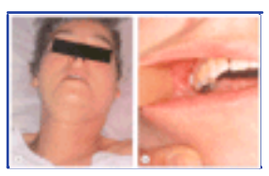

Figure 41-13 Suppurative parotitis (a) in a diabetic patient who had a recent history of dehydration secondary to diabetic ketoacidosis. (b) Pus was manually expressed from Stensen's duct from which *Staphylococcus aureus* was cultured. *Courtesy of Dr E Ridgway.*

association with xerostomia, dry eyes and arthralgia. Salivary gland involvement can occur very early on in HIV infection but is most commonly seen in late disease. Histologically, there are numerous epithelium-lined cysts, some up to several centimeters in size, containing macrophages and lymphocytes. The commonest identified opportunist infection of salivary glands is CMV; about 15% of postmortem submandibular glands of all patients who have AIDS have evidence of CMV inclusion bodies.[35] In children, there is a strong association between HIV-parotid swelling and lymphocytic interstitial pneumonitis. Examination usually reveals smooth bilateral swelling. Uneven swelling should be biopsied because 10% of salivary gland disease in HIV-infected patients is caused by lymphoma.[36]

DIAGNOSIS

In mumps, this can be achieved by detection of salivary IgM or by culture of salivary washings or of viral throat swab. A convalescent rise in complement-fixing antibody occurs. In established viral parotits there is elevation of serum salivary-type amylase. Rarely, there may be biochemical evidence of pancreatitis.

PREVENTION

The MMR vaccine consists of live attenuated measles, mumps and rubella viruses. Immunization provides protection for 90% of recipients for measles and mumps and over 95% for rubella. The antibody response to the mumps component is too slow for effective post-exposure prophylaxis. After the first dose of MMR, malaise, fever or rash may occur about 1 week after immunization, although this syndrome usually self-terminates within 3 days. Febrile convulsions occur in approximately 0.1% of children between 1 and 2 weeks after administration of the vaccine (similar to the attenuated live measles vaccine), and by the fourth week parotid swelling is seen in approximately 1% of infants.[37]

MANAGEMENT

Management of viral parotitis is symptomatic. Bacterial parotitis can usually be managed by prompt fluid replacement and parenteral antibiotic therapy using amoxicillin/clavulanate 1.2g q8h or intravenous cefuroxime 750mg q8h. Drainage of the duct should be assisted by manual massage. Occasionally steroids are necessary to

suppress inflammation and potentiate drainage. Surgical drainage of a salivary gland abscess is rarely necessary.

PAROTITIS CAUSED BY *MYCOBACTERIA* SPECIES

Nontuberculous mycobacterial infections of the parotid gland are now increasingly seen in children, in whom they present as unilateral painless indurated swellings that can be mistaken for neoplasm. Diagnosis can be made by fine needle aspiration with cytology and culture, which may reveal organisms such as *Mycobacterium scrofulaceum, Mycobacterium avium-intracellulare* or *Mycobacterium malmoense*. Management is conservative. *Mycobacterium tuberculosis* infection of the parotid gland is rare, but is one of the differential diagnoses of parotid tumor and should be rigorously excluded by histology of needle biopsy or fine needle aspiration cytology before unnecessary deforming surgery is undertaken.[38] The disease responds well to conventional antituberculous chemotherapy.

ESOPHAGEAL INFECTION

Normally there are relatively low numbers of micro-organisms within the esophagus; those present include a-hemolytic streptococci, lactobacilli, *Candida* spp. and low numbers of oral bacteria. Bacterial esophagitis is virtually unknown in the normal host, although occasionally the esophagus may be involved in generalized *M. tuberculosis* infection. Clinically important infections are fungal or viral and occur only in the context of immunosuppression or other underlying systemic diseases. The three most common causes of infective esophagitis in immunosuppressed patients are *Candida* spp. and, less commonly, HSV and CMV.

EPIDEMIOLOGY

Esophageal infection with *Candida* spp., HSV or CMV is generally seen in HIV-infected patients during the late stages of disease once the patient is profoundly immunodeficient, usually once the $CD4^+$ count is less than 200 cells/mm^3 . Each of these infections is an AIDS-defining illness in its own right. *Candida* esophagitis has been described in some patients during seroconversion illness as a result of the profound transient immunosuppression.[39] Patients who have solid tumors undergoing chemotherapy are at risk of *Candida* and HSV esophagitis. Mucositis caused by antineoplastic drugs predisposes patients to deep-seated candidiasis, including esophagitis. Solid organ transplant recipients are also susceptible to esophagitis caused by *Candida* spp., HSV and CMV.

PATHOGENESIS

Candida albicans is the predominant cause of superficial and deep-seated candidiasis, but other significant pathogens include *Candida tropicalis* (an important pathogen in neutropenic patients) and *Candida parapsilosisz*. *Candida glabrata, Candida lusitaniae* and *Candida krusei* have also been associated with esophageal disease and noted to be resistant to certain antifungal drugs.[40] Deep-seated candidiasis can be seen in patients rendered neutropenic as a result of an underlying malignancy or its treatment, or with significant cell-mediated immune defects as seen in AIDS.

CLINICAL FEATURES

Patients who have infective esophagitis can experience dysphagia or retrosternal esophageal pain (odynophagia), or both. The clinical features of AIDS-related esophagitis associated with *Candida* spp., CMV and HSV are similar, but patients who have AIDS-related esophageal candidiasis often complain of dysphagia rather than pain, whereas odynophagia and retrosternal episodic pain without swallowing, in addition to dysphagia, are more commonly encountered in patients who have HSV esophagitis and CMV esophagitis.[41]

DIAGNOSIS

In the context of oral candidiasis, radiologic or endoscopic investigation of esophageal symptoms is not absolutely necessary, because a therapeutic trial with antifungal agents will suffice. Otherwise, endoscopy is indicated to exclude HSV or CMV infection and also rarer causes of esophageal symptoms in AIDS, particularly lymphoma, Kaposi's sarcoma, mycobacterial infection, histoplasmosis or squamous cell carcinoma. The endoscopic appearance of *Candida* esophagitis ranges from diffuse mucosal hyperemia, with or without discrete white mucosal patches on the mucosa, to gross mucosal ulceration and perforation. In HSV and CMV disease there are usually discrete single ulcers of the distal third of the esophagus. With CMV disease these tend to be serpiginous, whereas in HSV they are small and punched out. These features can also be seen on barium studies of the esophagus; in candidiasis there is usually mucosal irregularity whereas in HSV disease ulcers can sometimes be seen in the distal third of the esophagus. At endoscopy, brushings are taken for detection of *Candida* spp. by direct microscopy or culture, for rapid diagnosis of HSV by direct immunofluorescence, and for viral culture of HSV and CMV. Biopsy of an ulcer edge may reveal cells with dense intranuclear inclusion bodies indicative of CMV disease, although their absence does not rule out the disease. Alternatively, esophageal biopsies may reveal epithelial cells with ballooning degeneration and occasional inclusion bodies suggestive of HSV infection.

MANAGEMENT

For a full discussion of treatment of esophageal *Candida* spp., HSV and CMV infection, see Chapters 125 , Chapter 126 , Chapter 215 and Chapter 237 .

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Chapter 42 - Gastritis, Peptic Ulceration and Related Conditions

John C Atherton
Andrew F Goddard

INTRODUCTION

Until recently the inclusion of gastritis and peptic ulcer in a textbook of infectious diseases would have seemed incongruous. However, in 1983, Marshall and Warren described the bacterium now known as *Helicobacter pylori* (see Chapter 230) and suggested that it may be important in the pathophysiology of chronic active gastritis and peptic ulceration.[1] They were proved correct, and it is now accepted that *H. pylori* infection:

- causes chronic active gastritis;
- is the main cause of duodenal and gastric ulceration; and
- is an important risk factor for gastric adenocarcinoma and lymphoma.

The term 'gastritis' is often erroneously applied to the macroscopic appearance of 'inflamed' (erythematous) gastric mucosa seen at endoscopy (Fig. 42.1a). However, these appearances correlate poorly with histologic inflammation, for which the term 'gastritis' should be reserved. Gastritis may be subtyped from the histologic appearance and distribution within the stomach, and these features often indicate etiology and risk of associated disease (Table 42.1). The main cause of gastritis is *H. pylori* infection. Two other helicobacters, namely *Helicobacter bizzozeronii* (formerly called *Helicobacter heilmannii* or *Gastrospirillum hominis*) and *Helicobacter felis*, are tight spiral bacteria present in 2–6% of the population of a developed country and thought to be zoonotically acquired, as both commonly colonize domestic pets.[3] [4] They usually cause mild gastritis and although there are case reports of associated disease and response to anti-helicobacter treatment, this is rare and a causal link is not well established.[3] [4]

Peptic ulcers may be associated with some types of gastritis. A peptic ulcer is a macroscopic break in the gastric or duodenal mucosa with obvious depth and definite size (usually defined as greater than 0.5cm; Fig. 42.1). Erosions are smaller breaks in the mucosal surface, which usually reflect the ulcer diathesis and should be managed similarly. Although gastric and duodenal ulcers share some characteristics, there are notable differences in their etiologies and pathogeneses (Table 42.2).

EPIDEMIOLOGY

Prevalence and incidence

The age prevalence of *H. pylori* differs markedly between countries, but two broad patterns are found (Fig. 42.2).

- In group 1 countries (predominantly developing countries), there is a rapid rise in prevalence before 20 years of age, after which point prevalence stabilizes at above 80%, implying that *H. pylori* is acquired in childhood and persists throughout life.
- In group 2 (usually developed) countries, the prevalence of infection increases steadily with age at a rate of roughly 1%/year of life. Most epidemiologic evidence suggests that this is the result of a birth cohort effect.[5] According to this theory, about 30% of 30-year-olds have acquired the infection in childhood, as compared with 60% of 60-year-olds, because of a changing incidence of infection in childhood over the past 60 years.

Associations

Aside from associations with age and geographic area, *H. pylori* is closely associated with socio-economic conditions, particularly in childhood. This may explain the different prevalences of infection found in different ethnic groups within the same geographic area.[6] Markers of childhood socio-economic status that have been correlated with prevalence of infection include general level of hygiene, water supply and sanitation and level of crowding in the household. These associations further support the view that most *H. pylori* acquisition is in childhood, from when it persists throughout life in the absence of effective treatment.

Although most infected adults became infected as children, there are well-documented examples of *H. pylori* being acquired de novo in adult life and several studies have suggested that crowding and poor sanitation are risk factors for this.[7] [8] However, marital status is only weakly associated with infection (and in some studies not associated), supporting the view that infection between adults is rare.

Transmission

Helicobacter pylori is thought to be acquired by direct human-to-human contact. Although monkeys and domestic cats may become infected, these are not an important reservoir for human infection. *Helicobacter pylori* DNA has been isolated from drinking water supplies in developing countries but *H. pylori* has not been cultured consistently from these sources and most data do not support an environmental source of infection. It is unclear whether *H. pylori* is transmitted by the fecal-oral or oral-oral route, or both. The bacterium has been cultured with difficulty from the feces of people who have *H. pylori* infection in both developing and developed countries, but it is more easily cultured from gastric refluxate into the mouth and from vomitus. However, it appears not to persist in the mouth as, although *H. pylori* DNA has been found in dental plaque, persistent colonization is not confirmed by culture. In past years, transmission of *H. pylori* has been documented following insufficient sterilization of endoscopy or gastric pH measuring equipment, although with adequate sterilization this is no longer a problem. Acute *H. pylori* infection by this route is thought to be the cause of the occasional outbreaks of epidemic acute hypochlorhydria[9] observed before the discovery of *H. pylori*.

PATHOGENESIS AND PATHOLOGY

Helicobacter pylori is primarily a gastric infection, although it may colonize areas of gastric metaplasia in the duodenum and esophagus, and rarely heterotopic gastric tissue elsewhere in the gastrointestinal tract. In the stomach, infection causes chronic active gastritis characterized by continuing neutrophil and lymphocyte infiltration, epithelial damage and thinning of the mucus layer (Fig. 42.3a). This is in

Figure 42-1 Endoscopic pictures of the stomach and duodenum. (a) Erythema of the gastric antrum. This appearance correlates poorly with histologic gastritis and may be a normal finding. (b) Duodenal ulceration. (c) Gastric ulcer. Note the clot in the base indicating recent bleeding and high risk of rebleed and the endoscope entering the stomach through the cardia.

TABLE 42-1 -- Classification of chronic gastritis according to the updated Sydney system.*

CLASSIFICATION OF CHRONIC GASTRITIS ACCORDING TO THE UPGRADED SYDNEY SYSTEM	
Type of gastritis	**Etiology**

Nonatrophic	*Helicobacter pylori*
Atrophic	
Autoimmune	Autoimmunity
Multifocal atrophic	*Helicobacter pylori* ± dietary and other environmental insults
Special forms	
Chemical	Aspirin/NSAIDs, bile and possibly other agents
Radiation	Radiation
Lymphocytic	Idiopathic, overt or latent celiac disease, drugs (ticlopidine), possibly *H. pylori*
Noninfectious granulomatous	Crohn's disease, sarcoidosis, vasculitides, foreign substances, idiopathic
Eosinophilic	Food sensitivity, possibly other allergies
Other infectious gastritides	Bacteria other than *H. pylori* (particularly *H. bizzozeronii* or '*H. heilmanii*' and *H. felis*, mycobacteria and syphilis), viruses (particularly cytomegalovirus), fungi (particularly *Candida* spp. *Histoplasma capsulatum* and Mucoraceae)
Non-helicobacter infectious gastritides are very rare, usually occur in immunocompromised patients and are not discussed in this chapter.	

** Data from Dixon et al., with permission.* [2]

contrast to gastritis caused by chemical agents, including nonsteroidal anti-inflammatory drugs (NSAIDs), which is characterized by regenerative epithelial changes and a paucity of inflammatory cells (Fig. 42.3b). *Helicobacter pylori* gastritis is associated with several important pathologic conditions including:

- duodenal ulceration,
- gastric ulceration,

- gastric adenocarcinoma arising from the distal stomach but not from the gastric cardia,
- gastric lymphoma, and
- a form of Ménétrier's disease.[10]

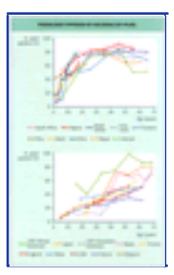

Figure 42-2 Prevalence patterns of *Helicobacter pylori*. Prevalence of *H. pylori* infection in 10 developing countries (Group 1) and 10 developed countries (Group 2). *Adapted with permission from Pounder and Ng.*[5]

TABLE 42-2 -- Causes of duodenal and gastric ulceration (with estimated proportions).

CAUSES OF DUODENAL AND GASTRIC ULCERATION (WITH ESTIMATED PROPORTIONS)			
Cause		**Duodenal ulcer (% of cases)**	**Gastric ulcer (% of cases)**
Infection	*Helicobacter pylori*	90	60–70
Drugs	Aspirin and NSAIDs	5–10	25–30
Neoplasms	Zollinger-Ellison syndrome	Rare	Rare
	Lymphoma	Rare	Rare
	Gastric adenocarcinoma	-	2–5
	Other adenocarcinoma	Rare	Rare
	Leiomyoma	-	Rare
Others	Crohn's disease	Rare	Rare
	Systemic mastocytosis	Rare	Rare
	Severe systemic illness	Rare	Rare

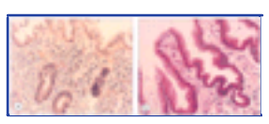

Figure 42-3 Appearances of *Helicobacter pylori* and NSAID antral gastritis. (a) Antral gastritis in *H. pylori* infection with active (neutrophil) and chronic inflammation of the lamina propria and glands. The epithelial surface is typically ballooned. *Helicobacter pylori* organisms are not readily apparent on a hematoxylin and eosin stain. (b) Antral gastritis associated with NSAID use. Foveolar hyperplasia with a mild chronic inflammatory infiltrate and smooth muscle cells are seen in the lamina propria. *Courtesy of Dr MM Walker.*

There is controversy over the association of gastric *H. pylori* infection with gastroesophageal reflux disease (GERD); it appears to offer a degree of protection in a subset of people.[11] There is also controversy over whether *H. pylori* is the cause of symptoms in a small subset of people with a macroscopically normal upper gastrointestinal tract (so-called 'functional dyspepsia').[12]

Approximately 80% of people who have *H. pylori* infection do not develop any of these diseases in their lifetime. Whether an infected person develops disease and which disease develops is becoming better understood and is dependent on a combination of bacterial strain virulence, host genetic susceptibility and environmental cofactors.

Duodenal ulcer disease

Approximately 90% of patients who have duodenal ulceration have *H. pylori* infection; the remaining 10% are aspirin or NSAID users or have other rare conditions (see Table 42.2). Few people now doubt that *H. pylori* causes ulcers; the best evidence is that eradication virtually abolishes ulcer relapse. [13] [14]

Several characteristics are found more commonly in ulcer-associated than in non-ulcer-associated *H. pylori* strains. The two best characterized are:

- presence of the *cag* (cytotoxin-associated gene) pathogenicity island, and
- production of an active vacuolating cytotoxin, VacA.

The *cag* pathogenicity island is a genetic region containing over 30 genes.[15] The island encodes a type IV secretory apparatus, best thought of as a syringe, through

which one *cag*-encoded protein, CagA, is 'injected' into epithelial cells.[16] CagA induces the epithelial cell to undergo several changes including the secretion of pro-inflammatory cytokines, which leads to increased gastric inflammation.[15] CagA is also highly immunogenic and anti-CagA antibody detection can be used as a serum test for the presence of the island. About 70% of strains in the USA are *cag*$^+$ and these strains colonize the gastric mucosa more densely, cause more inflammation and are more likely to be associated with ulcers than are *cag*$^-$ strains.[17]

The vacuolating cytotoxin, VacA, is a pore-forming toxin that increases epithelial permeability and causes massive epithelial cell vacuolation in vitro. It is particularly suited to the stomach because it is activated by acid, then becoming acid and pepsin resistant.[18] Although only about 40% of strains isolated in the USA exhibit cytotoxin activity, all have *vacA*, the gene encoding the cytotoxin. However, only some *vacA* genotypes are associated with the toxigenic phenotype and infection with strains of certain *vacA* genotypes is associated with increased prevalence of peptic ulcer disease.[19] Virtually all toxigenic strains are *cag*$^+$, although the relative importance of the toxin and *cag*-encoded proteins and how they interact are unclear.

There has been considerable recent research into other virulence factors present in only some strains and these may turn out to be as important as the *cag* island and VacA. They include an adhesin, BabA, a bacterial outer membrane proinflammatory protein, OipA, and a restriction enzyme, IceA, and its associated methylase.[20] Other bacterial factors are also thought to be important for pathogenesis in terms of colonization and the induction of inflammation, such as the enzyme urease and the ability to adhere to gastric mucosa, but these are present in all strains and so do not explain why only some strains cause ulcers.

Recently, the pathogenic link between infection in the stomach and ulceration in the duodenum has become better understood. Duodenal ulcers arise in patients with antral-predominant gastritis. Infection of the gastric antrum leads to a reduction in somatostatin-producing D-cells resulting in hypergastrinemia, as somatostatin inhibits gastrin production.[21] Both inflammation and hypergastrinemia are more marked in infection with *cag*$^+$ strains. High gastrin levels lead to increased stimulated acid output from parietal cells in the gastric corpus, which is most marked when the corpus is relatively spared of infection. The resulting increased acid load entering the duodenum leads to the formation of adaptive gastric metaplasia.[22] This can be colonized by *H. pylori* and local inflammation and release of toxic bacterial products can lead to ulceration.

Specific host factors predisposing to antral-predominant gastritis and duodenal ulceration have not been identified. However, environmental factors are important and, in particular, smokers who have *H. pylori* infection are much more likely to develop ulcers than are nonsmokers who have the infection. Aspirin and NSAIDs can cause ulcers independently of *H. pylori* infection and further increase the risk of ulcers in people with *H. pylori*.

Gastric ulcer disease

Helicobacter pylori-associated gastric ulcers usually arise in junctional mucosa between antral- and corpus-type tissue, typically on the lesser curvature. They usually occur in patients with pan-gastritis rather than antral-predominant gastritis and are not associated with increased stimulated acid output. Their pathogenesis is uncertain, but infection with virulent strains and smoking increase risk. Although *H. pylori* infection is the most common cause of gastric ulceration, the proportion caused by aspirin and NSAIDs is higher than for duodenal ulcers.

Gastric adenocarcinoma

The World Health Organization has classified *H. pylori* as a type 1 or causal carcinogen.[23] It is a risk factor for distal adenocarcinoma with a relative risk of 4–9, but it is unrelated to carcinoma of the cardia, which is now the most common type of gastric carcinoma in the USA. Distal gastric adenocarcinoma usually arises in patients with pan-gastritis and patients who have previous duodenal ulceration (usually with antral-predominant gastritis) are less likely than others to develop gastric cancer. Both *cag*$^+$ and cytotoxic strains are more likely to be associated with carcinoma than other strains. Host genetics are also important; people with genetic polymorphisms that lead to high-level secretion of the proinflammatory cytokine interleukin-1 in response to bacterial infection are more likely to develop gastric cancer.[24] Young age at infection, smoking and dietary factors (high nitrosamines, low antioxidants) have also been associated with increased risk. The pathogenesis of gastric carcinoma is unclear and may differ between the two main types, intestinal and diffuse cancer. Intestinal-type gastric cancer is thought to occur by a step-wise process from superficial gastritis through atrophy to intestinal metaplasia, dysplasia and ultimately carcinoma. [25] One possibility is that the hypochlorhydria associated with pan-gastritis and with atrophy may allow survival of DNA-damaging oxygen and nitrogen free radicals. Hypergastrinemia may also be important and is increasingly recognized as a risk factor for gastrointestinal tract tumors.

Gastric lymphoma

Primary gastric lymphomas arise in lymphoid tissue which is only present in the *H. pylori*-infected stomach. B-cell mucosa-associated lymphoid tissue (MALT) lymphomas are particularly interesting as they are driven by chronic stimulation by *H. pylori* antigens. When histologically low grade, the majority regress following *H. pylori* eradication.[26]

Protection against gastroesophageal reflux disease

There is increasing epidemiologic evidence that people with *H. pylori* infection, especially those with pathogenic strains, are less likely to develop GERD and its rare sequelae — Barrett's esophagus and esophageal adenocarcinoma.[11] This is of considerable interest because the incidence of these conditions is increasing rapidly in developed countries. Evidence suggests that reduced risk is associated with pan-gastritis and reduced acid production, presumably because gastroesophageal refluxate is less damaging. In those with *H. pylori* infection, treatment may improve, worsen or not affect GERD symptoms and esophagitis. Thus, there is no clinical reason not to treat *H. pylori* in those with an indication for eradication.

Functional dyspepsia

Most people with a macroscopically normal upper gastrointestinal tract do not benefit from *H. pylori* eradication. Most studies show that a small subgroup of about 10% will benefit, but this may be a group with undiagnosed ulcer disease. Thus, there is little evidence that *H. pylori* infection causes symptoms in the absence of ulceration or malignancy.

PREVENTION

Prevention of *H. pylori* infection is difficult in view of our paucity of knowledge about its transmission, although improved living conditions, clean water and improved health should reduce the incidence in developing countries. In developed countries *H. pylori* incidence is falling steadily[5] and specific preventive measures may not be necessary.

Helicobacter pylori vaccine research is ongoing, with encouraging early results in animal models. However, whether an effective human vaccine can be developed remains uncertain.

CLINICAL FEATURES

Acute *Helicobacter pylori* infection

The clinical features of acute infection in the community are unknown. However, where high doses of cultured *H. pylori* have been self-administered, upper abdominal discomfort and pain occurred 3 days after dosing, followed by vomiting and finally a resolution of symptoms by the end of the week.[27] A similar pattern of symptoms was observed in patients with acute epidemic hypochlorhydria,[9] an illness that occurred in volunteers undergoing nasogastric intubation for acid secretion studies in the 1970s and presumed to be iatrogenic acute *H. pylori* infection. *Helicobacter pylori* is most commonly acquired in childhood, but whether initial colonization is usually symptomatic or asymptomatic is not known

Chronic *Helicobacter pylori* infection

Chronic *H. pylori* infection is characterized by chronic active gastritis, but this condition is asymptomatic. Chronic infection is therefore only manifest symptomatically if complications develop, such as duodenal ulceration, gastric ulceration or gastric cancer.

DIAGNOSIS

Diagnosis of *H. pylori* infection can be made by endoscopic biopsy-based tests or by noninvasive tests. Most patients require endoscopy to assess indications for treatment, and so tests for *H. pylori* are usually performed at this time. The choice of endoscopic-based test depends upon the information required, cost and convenience; usually only a urease test on a mucosal biopsy is used. As infection is very likely in non-NSAID-associated duodenal ulcer, some regard testing for *H. pylori* in this context as unnecessary. Our practice,

however, is to make a positive diagnosis before prescribing potentially harmful multiple antibiotic treatment regimens. Nonendoscopic tests (the urea breath test (UBT), serology or the stool antigen test) are useful in primary diagnosis only if a treatment indication already exists (e.g. in a patient with demonstrated previous ulcer).

Following *H. pylori* treatment, some consider retesting unnecessary due to the high efficacy of modern treatment regimens. We prefer to monitor treatment success or failure as a prognostic guide and as an aid to managing recurrent symptoms. Repeat endoscopy is usually unnecessary and the UBT, which is well suited for assessing treatment success, is becoming more widely available. However, in situations where endoscopy is conventionally repeated (e.g. to check healing of gastric ulcers to exclude malignancy) biopsy-based tests are equally suitable. Tests must be delayed for at least 4 weeks after finishing treatment or false-negative results may occur; even in primary diagnosis, testing within 4 weeks of treatment with intercurrent antibiotics or bismuth compounds or within 2 weeks of dosing with proton pump inhibitor may give false-negative results. Serologic tests are not suitable for checking the success of treatment as specific antibody levels fall only slowly.

Endoscopic tests

Endoscopic tests are based on mucosal biopsy specimens. Infection may be patchy, and so if possible two biopsies should be taken from the usually more uniformly infected antrum to minimize sampling error. In some situations, notably after treatment, during acid suppressive therapy or when intestinal metaplasia and atrophy are likely (e.g. in the elderly), the infection may be more marked in the corpus and at least two additional biopsies should be taken from there.

Culture

Helicobacter pylori can be cultured from gastric biopsies, although sensitivity is often low compared with that of other tests. Biopsies should be put into a sterile solution and transferred as soon as possible to the laboratory. Methods for culture and identification are discussed in Chapter 230 . Cultured bacteria can be tested for antibiotic sensitivities and the main indication for culture is previous failed treatment.

Histology

Chronic superficial gastritis seen on standard hematoxylin and eosin staining is strongly indicative of *H. pylori* infection, but unless specialized stains are used (e.g. modified Giemsa, Gimenez (Fig. 42.4), Warthin-Starry or Genta) the infection may be missed. Histology is expensive, but sensitive in experienced hands, and may provide other useful information, such as the presence of epithelial dysplasia.

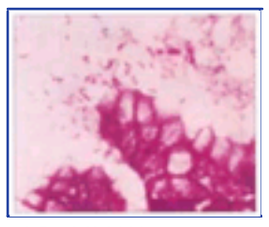

Figure 42-4 *Helicobacter pylori* (Gimenez stain). Other special stains that can be used are the modified Giemsa stain or a silver stain such as Warthin-Starry stain. *Courtesy of Dr MM Walker.*

Biopsy urease test

In this test, two gastric biopsies or one large biopsy are placed in a gel or solution containing urea and a pH indicator. If *H. pylori* is present, its urease enzyme catalyzes urea hydrolysis and a color change occurs. These tests can be performed in the endoscopy room and are sensitive, specific, cheap, convenient and quick. A positive result can be obtained in a few minutes, although for most commercial tests a 24-hour wait is necessary to ensure that the test is negative.

Nonendoscopic tests

Urea breath tests

Several protocols exist, but in essence the patient drinks urea solution isotopically labeled with either stable ^{13}C or ultra-low dose radioactive ^{14}C. If *H. pylori* is present, the urea is hydrolyzed and labeled carbon dioxide can be detected in breath samples. Both isotopes are safe, although it is sensible to avoid ^{14}C in pregnant women and children. The best UBT protocols are as specific as biopsy-based tests and are perhaps more sensitive as sampling error is avoided. The UBT is the most widely used and appropriate test to check for treatment success in situations where repeat endoscopy is unnecessary, but must be performed at least 1 month after any antibiotic, bismuth or proton pump inhibitor treatment.

Stool antigen tests

These newly described simple stool tests are an alternative to UBTs and indications for use are the same.[28] Whether they are as accurate remains to be determined.

Serology

Many commercial and in-house assays, usually enzyme-linked immunosorbent assays, are available to test for specific IgG and IgA in serum. The best are as sensitive and specific as other tests and serologic testing is more convenient and cheaper than even the UBT. This makes it the most suitable test for infection when there is a known indication for treatment and endoscopy is not required. A fall of over 40% in specific IgG titer 6 months after treatment accurately reflects treatment success, but testing paired pre- and 6 months posttreatment samples presents a storage problem and a long wait for results, and offers little cost advantage over the UBT. Rapid in-office tests for *H. pylori* are being heavily marketed to primary care physicians, but these usually perform poorly in independent studies and are not currently recommended.

MANAGEMENT

The medical and surgical management of gastric adenocarcinoma are not within the context of this book. Here we address the management of *H. pylori*-associated peptic ulcer disease and infection.

Peptic ulcer disease

Until recently, treatment of peptic ulcers was based upon acid-suppressing drugs. When the treatment was stopped, the ulcers often relapsed. However, eradication of *H. pylori* not only heals ulcers but also prevents their recurrence. Therefore, once an ulcer is diagnosed by endoscopy or barium meal, *H. pylori* infection should be sought and, if found, treated (Fig. 42.5). This is usually done immediately, but if there is a delay in performing diagnostic tests for *H. pylori*, ulcer healing can be started with acid-suppressing drugs and *H. pylori* treatment can be added when the infection has been confirmed.

Duodenal ulcers are virtually always benign, but gastric adenocarcinoma can present as gastric ulceration. Therefore, additional biopsies for histologic examination should be taken when there is gastric ulceration, with follow-up endoscopy performed to confirm healing.

Figure 42-5 Decision algorithm for the management of duodenal ulcer disease diagnosed at upper gastrointestinal endoscopy.

Uninvestigated dyspepsia

To reduce the number of endoscopies performed (and therefore cost) and to increase patient comfort and convenience, most national and other guidelines now recommend noninvasive *H. pylori* testing and, if the organism is present, immediate eradication in patients with persistent dyspepsia younger than a locally defined age cut-off.[29] The reason for performing endoscopy in older people is to avoid delay in diagnosing gastric cancer, and so age cut-off depends on the local incidence and demographics of this disease, but it is usually between 40 and 55 years. All patients with 'alarm' symptoms or signs such as weight loss, dysphagia, persistent vomiting, gastrointestinal bleeding, unexplained anemia, epigastric mass, previous gastric ulcer or gastric surgery should be referred for upper gastrointestinal endoscopy and/or other investigations regardless of age, both to exclude malignancy and to make a positive diagnosis.

***Helicobacter pylori* treatment regimens**

First-line treatment is with 'low-dose triple therapy' consisting of either a proton pump inhibitor, amoxicillin and clarithromycin, or a proton pump inhibitor, clarithromycin and metronidazole (see

TABLE 42-3 -- *Helicobacter pylori* treatment regimens.

***HELICOBACTER PYLORI* TREATMENT REGIMENS**	
First-line treatments	
Regimen 1[*]	
Omeprazole 20mg q12h[†]	7–14 days
Clarithromycin 500mg q12h	
Amoxicillin 1g q12h	
Regimen 2	
Omeprazole 20mg q12h[†]	7–14 days
Clarithromycin q12h	
Metronidazole q12h	
Second-line treatments[‡]	
Omeprazole 20mg q12h[†]	14 days
Bismuth subcitrate[§] 120mg q6h	
Tetracycline HCl 500mg q6h	
Metronidazole 400mg q8h	

* Many physicians use regimen 1 rather than regimen 2 for first-line therapy as it avoids use of metronidazole, which is an important constituent of second-line therapy.
† Other proton pump inhibitors are equally effective or, in regimens 1 and 2, ranitidine bismuth citrate 400mg.
‡ An alternative to 'blind' second-line therapy is to culture *H. pylori* and be guided by antibiotic sensitivities. Patients failing second-line therapy should be referred to a specialist and *H. pylori* culture will usually be necessary.
§ Bismuth subsalicylate may be used instead of bismuth subcitrate.

Table 42.3) .[29] Knowledge of local antibiotic resistance patterns is helpful in choosing an optimal *H. pylori* treatment as regimens are less effective if *H. pylori* has in-vitro resistance to a component antibiotic. Metronidazole-resistant *H. pylori* is common in:

- developing countries;
- in many ethnic populations within developed countries; and
- in individuals who have previously taken metronidazole for concurrent illnesses, even in the distant past.

Clarithromycin resistance is still rare, but increasing as the antibiotic becomes more commonly used for respiratory tract infections. Amoxicillin resistance is very rare.

For any *H. pylori* treatment regimen, patients should be warned of the importance of compliance and potential side-effects. Providing written instructions may be the most effective strategy. The success of treatment should be assessed by UBT or endoscopy, 4 weeks or more after treatment finishes.

Patients in whom treatment is unsuccessful often have antibiotic-resistant strains, and even if this is not the case unsuccessful treatment often induces antibiotic resistance. Two approaches are commonly used: administration of 'blind' second-line therapy (see Table 42.3) or endoscopy for *H. pylori* culture and sensitivity testing and guided therapy. After two treatment failures referral for endoscopy and culture is always indicated.

For whom is *Helicobacter pylori* eradication justified?

As discussed above, *H. pylori* treatment is now frequently recommended in young people with uninvestigated dyspepsia who do not have 'alarm' symptoms or signs. For patients who do undergo endoscopy, three groups of patients who have *H. pylori* infection should unequivocally receive eradication treatment:

- those with an active or previous duodenal ulcer;
- those with a nonmalignant gastric ulcer; and
- those with a MALT lymphoma, particularly if at an early stage.

In this last group, some patients will require additional chemotherapy.

The benefits of treating other groups of patients should be balanced against the potential risks of treatment.[30] These risks include:

- development of antibiotic resistance (both by *H. pylori* when treatment has failed and by other organisms within the body);
- adverse drug reactions; and
- rarely, pseudomembranous colitis.

Patients for whom there is some evidence that *H. pylori* eradication may be beneficial include those with reflux esophagitis requiring long-term proton pump inhibition

and those with high-grade gastric dysplasia. Some evidence suggests that people in the former group have an increased risk of developing accelerated atrophic gastritis, which is thought to be an early step in the progression to adenocarcinoma. [31] However, whether the risk of gastric adenocarcinoma is increased and whether *H. pylori* eradication prevents this are both unknown. Until the situation is clear, it may be prudent to err on the side of caution and treat patients in this group. Outside these situations there is no accepted indication to treat *H. pylori* infection for the primary prevention of gastric malignancy, although many physicians treat the infection if there is a family history of gastric adenocarcinoma. Occasional patients with nonucler dyspepsia have a good symptomatic response to *H. pylori* eradication.[12] However, there is no method for selecting which patients will respond and we do not recommend *H. pylori* treatment for this indication.

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Chapter 43 - Enteritis, Enterocolitis and Infectious Diarrhea Syndromes

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EPIDEMIOLOGY

Infectious diarrhea illness is a public health problem for both the industrialized and the developing countries. The highest prevalence occurs in the latter where it is associated with increased morbidity and mortality among children under the age of 5 years. Recurrent episodes of diarrhea occur routinely in these regions where each child may experience up to six or more bouts of diarrhea on an annual basis.[1] The highest rates of illness in these areas occur in infants younger than 2 years whose fatality rate is five times higher than older children. Over the past two decades and because of the introduction of oral rehydration therapy (ORT) and the promotion of breast-feeding and measles vaccination programs, there has been a significant decline in child mortality secondary to diarrhea. In 1980, an estimated 5 million children died secondary to the disease; these numbers decreased to 3.3 million cases in 1990 and to 1.5 million in 1999. [2] However, diarrhea still represents 12% of the causes of death in children worldwide, second only to the respiratory diseases. Many deaths still occur in developing tropical regions because of a number of factors, of which malnutrition is often the most important. Malnutrition is associated with about 50% of all death among children.[3] Some of the main factors associated with death in patients with diarrheal disease include:

- malnutrition;
- complications of diarrhea, including dehydration, pneumonia, sepsis and the hemolytic-uremic syndrome (HUS);
- Infection by an agent more likely to cause dehydration, such as *Vibrio cholerae* and rotavirus;
- infection by an invasive pathogen such as *Shigella* spp.;
- lack of access to rehydration and/or antimicrobial therapy;
- persistent diarrhea, defined as illness lasting 14 days or longer.

In the industrialized nations, the prevalence of diarrhea is lower in the general population. It is estimated that children under 5 and adults in the USA will experience about 1–2 bouts of diarrhea annually.[4] However, in certain individuals the rate of infectious diarrhea is substantially higher. These persons at higher risk include international travelers visiting tropical and semitropical regions, institutionalized elderly, infants in daycare centers, hospitalized patients, especially those exposed to broad-spectrum antibiotics, and military personnel deployed to conflict areas. Change in travel plans, work leave for parents caring of sick children and extended hospital stay are just a few examples of the clinical and financial burdens of diarrhea in the developed world.

It is estimated that the cost of dealing with *Clostridium difficile*-associated diarrhea in US hospitals exceeds $1.1 billion per year.[5] Outbreaks of acute gastroenteritis also affect military personnel. The most common cause of disability among soldiers was related to Norwalk-like virus (NLV) diarrhea outbreaks in Operations Desert Storm and Desert Shield. There have been recent similar reports during military operations in Afghanistan.[6]

People living with HIV-AIDS are also at a higher risk of developing diarrhea. This is true for all continents but more evident in Africa and South Asia where the prevalence is higher and the access to treatment and living conditions are lower. Table 43.1 gives general estimates of the expected frequency of diarrhea development for a variety of populations. Figure 43.1 shows the number of deaths secondary to diarrhea in the year 2000, by age group and world regions.

PATHOGENESIS AND PATHOLOGY

Pathogens known to cause diarrhea in humans include bacteria, viruses and protozoa. After overcoming the host's defense factors (e.g. gastric acids, intestinal mucus, gut motility, normal intestinal flora and immune mechanisms), pathogenic microbes produce diarrhea through one of four different but not exclusive mechanisms:

- increased active intestinal secretion of electrolytes that induces fluxes of water and ions, usually mediated by enterotoxins (watery diarrhea);
- malabsorption of nutrients and electrolytes secondary to damage to the brush border;
- increased intestinal osmolality secondary to disaccharidase deficiency when the brush border is damaged, with resultant lactose intolerance;
- altered intestinal motility.

The first mechanism is most frequently involved in acute diarrhea; the other three are most important in chronic forms of diarrhea.

The inoculum necessary to cause diarrhea illness varies among the different pathogens. Table 43.2 shows a few examples of the minimum number of viable organisms required to produce diarrhea in 25% of exposed persons (ID_{25}).

Bacterial enteropathogens

The pathogenesis of diarrheal illness caused by bacterial enteric organisms in the gastrointestinal tract involves attachment or adherence to the intestinal mucosa, production of toxins and mucosal invasiveness (Table 43.3).

Adherence to the intestinal mucosa

Adherence is the ability of the organism to attach to and colonize the intestinal mucosa where the disease is caused. It is the first step for production of an infection, which can be followed by toxin production, signal transduction or invasion. Adherence capacity is defined by the bacteria exhibiting mannose-resistant adhesion to HeLa or HEp-2 tissue culture cells. Using these cell lines and varying the conditions of the adhesion assay, three classic distinct patterns of *Escherichia coli* adherence have been observed.

- *Localized adherence.* This is the characteristic form of enteropathogenic *E. coli* (EPEC) where tight clusters of organisms can be seen in microcolonies attaching to the cell surface.
- *Diffuse adherence.* These *E. coli* (DAEC) attach in a scattered pattern onto the whole cell surface.
- *Enteroaggregative adherence*: The *E. coli* (EAEC) attach in clumps of organisms with a 'stacked brick' appearance to cells and cover slips.

TABLE 43-1 -- Rates of endemicity of diarrhea in various populations.

RATES OF ENDEMICITY OF DIARRHEA IN VARIOUS POPULATIONS	
Population	**Prevalence/incidence of diarrhea**
Infants (<2 years) in developing countries	3–6 bouts/person/year
Older children and adults in developing countries and all age children and adults worldwide	1–2 bouts/person/year

Travelers from industrialized regions during stays in developing tropical or semitropical areas	15–40% per trip
Patients who have AIDS:	
Africa	40%
North America	15%
Other high risk persons: elderly, gay men, infants in daycare centers, persons receiving antimicrobials	>1–2 bouts/person/year

The pattern of adhesion is directly related to the mechanism by which *E. coli* produce diarrhea. EPEC adheres to the intestinal mucosa and produces a characteristic histopathologic lesion, referred to as an attaching and effacing (A/E) lesion.[7] [8] The A/E lesion is characterized by dissolution of the intestinal brush border, loss of microvillus structures (effacement) and F-actin polymerization beneath the site of bacterial attachment as a result of localized

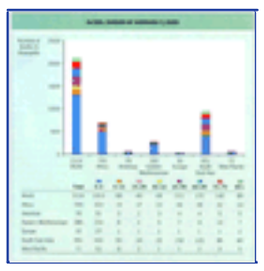

Figure 43-1 Global burden of diarrhea illness. Estimated number of deaths secondary to diarrhea in the year 2000, by age group and world regions. Numbers are expressed in thousands. *Source: Global Burden of Disease 2000, World Health Organization.*

cytoskeletal breakdown.[9] [10] The A/E lesion that appears to be a specific marker for EPEC and enterohemorrhagic *E. coli* (EHEC) can be detected by the fluorescent-actin staining reaction (FAS). The localized EPEC adherence property is mediated by bundle-forming pili (bfp), which cause the characteristic bacterial aggregation to each other and to the microvilli. This inducible adherence property is conferred by a 50–70mDa plasmid encoding the EPEC adherence factor.[11] [12] After adhering to the enterocyte, the next step is the induction of signal transduction pathways in the host cell. The bacterial genes are located on a 35kb pathogenicity island called the locus of enterocyte effacement (LEE), which encodes a bacterial adehesin called intimin. Without this intimate adherence process, the bacteria are unable to produce its effects on the enterocytes. These multiple signal transduction pathways originate an increment of the intracellular calcium levels which is responsible for the cytoskeletal changes observed and the inhibition of NaCl absorption and increment in Cl^- secretion producing the secretory component of EPEC diarrhea. The gene encoding intimin, eae (attaching and effacing), is also present in EHEC strains showing A/E histopathologic lesions. Finally A/E lesions are also responsible for severe losses of absorptive microvilli leading to diarrhea through malabsorption (Fig. 43.2)

EAEC does not hybridize with the EPEC adherence factor probe, is fluorescent-actin staining negative and does not produce A/E lesions. A lesion characterized by shortening of villi, hemorrhagic necrosis and mild inflammatory response of the submucosa is observed in rabbit and rat ileal loops.[13] EAEC possesses a 55–65mDa plasmid that encodes for fimbriae similar to bundle-forming pili of EPEC and mediates aggregative adhesion and hemagglutination.[14] EAEC

TABLE 43-2 -- Dose of viable microbes responsible for disease production (ID_{25}).

DOSE OF VIABLE MICROBES RESPONSIBLE FOR DISEASE PRODUCTION (ID_{25})	
Enteropathogen	**ID_{25}**
Shigella spp	10–100
Giardia and *Cryptosporidium parvum*	30–100
Shiga toxin *E. coli* 0157:H7	10–100
Norwalk-like virus	100
Salmonella	10^3 –10^5
Campylobacter	10^3 –10^6
Vibrio cholerae	10^6
ETEC	10^8

was isolated from US travelers to Mexico and from Mexican and Indian children with protracted diarrhea.[15] [16] [17] DAEC is distinct from localized adhering EPEC because it does not hybridize with the EPEC adherence factor probe and is negative in the FAS test.[18] [19] The pathogenic capability and epidemiologic significance of DAEC are controversial. [20]

The HEp-2 adherence is also exhibited by enterotoxigenic *E. coli* (ETEC), and some *Salmonella* and *Shigella* strains. ETEC produces a specific type of mannose-resistant pili that is important for the adherence and colonization of the mucosa. This adherence capacity is mediated by the production of bacterial surface adhesions called colonization factor antigens (CFA), which are genetically encoded by plasmids.

Toxin production

The toxins produced by enteric bacteria causing diarrhea can be classified as enterotoxins neurotoxins and cytotoxins.

Enterotoxins

These are bacterial products that act on the epithelial cells of the small intestine and produce a fluid secretion without any structural

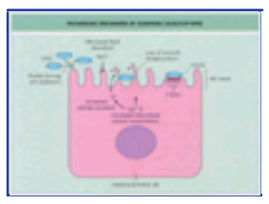

Figure 43-2 Pathogenic mechanism of diarrhea caused by EPEC. Figure shows attachment of EPEC to the enterocyte, villous destruction and formation of the A/E lesion.

TABLE 43-3 -- Pathogenic mechanisms of diarrhea caused by enteric bacteria.

PATHOGENIC MECHANISMS OF DIARRHEA CAUSED BY ENTERIC BACTERIA			
Pathogenesis	**Mode of action**	**Clinical presentation**	**Examples**
Mucosal adherence	Attachment, colonization and effacement of intestinal mucosa	Secretory diarrhea	Localized adhering EPEC, enteroaggregative *E. coli*, diffuse adhering *E. coli*, ETEC
Toxin production:			
Neurotoxin	Action on the autonomous nervous system	Enteric symptoms	Staphylococcal enterotoxin b, *Clostridium botulinum*, *Bacillus cereus*

Enterotoxin	Fluid secretion without damage to the mucosa	Watery diarrhea	*Vibrio cholerae*, ETEC, *Salmonella* spp., *Campylobacter* spp., *Clostridium difficile* toxin A, *Clostridium perfringens* type A
Cytotoxin	Damage to the mucosa	Inflammatory colitis, dysentery	*Shigella dysenteriae* serotype 1, *E. coli* 0157:H7, *Clostridium difficile* toxin B, *Salmonella* spp., *Campylobacter* spp.
Mucosal invasiveness	Penetration into the mucosa and destruction of epithelial cells	Dysenteric syndrome	*Shigella dysenteriae* serotype 1, *Shigella sonnei*, *Shigella flexneri*, EIEC, *Campylobacter* spp., *Yersinia* spp.

damage to the mucosa. *Vibrio cholerae* toxin (CT) and ETEC heat-labile toxin (LT) are oligomeric toxins that share very similar structure and mode of action and are similar to the enterotoxins produced by other watery diarrhea-causing bacteria, such as *Salmonella* spp., *Campylobacter* spp., *C. difficile* toxin A and *Clostridium perfringens*

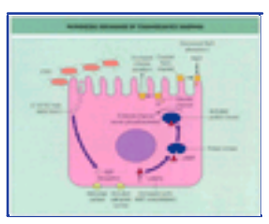

Figure 43-3 Pathogenic mechanism of toxin-mediated diarrhea.

type A. CT and LT are conformed of a single A-subunit surrounded by five identical B-subunits. The toxin binds via the B-subunit pentamer to the GM1 ganglioside receptor on the membrane of the enterocyte. The A-subunit enters the cell where it is proteolytically cleaved to yield the A1 and A2 peptides joined by a disulfide bond. A1 peptide activates adenylate cyclase located in the baso-lateral membrane, thereby increasing intracellular cAMP concentrations.[21] [22] [23] The cAMP activates a cAMP-dependent protein kinase, which subsequently leads to excess phosphorylation of chloride channels located in the apical epithelial cell membranes. It is postulated that the major chloride channel activated by LT and CT is CFTR, the ion channel defective in cystic fibrosis. [24] The end result is increased Cl^- secretion by cryptic cells and inhibition of coupled influx of Na^+ and Cl^- across the brush border of the enterocyte. The combination of oversecretion and underabsorption of ions by the small intestine leads to passive transport of water into the lumen, thus producing watery diarrhea (Fig. 43.3). One of the main differences between LT and CT is the failure of the former to release serotonin. In addition, prostaglandins, platelet-activating factor and the enteric nervous system appear to be involved in the secretory response demonstrated mostly by CT.[22] [25] [26]

ETEC can also produce another type of enterotoxin responsible for diarrheal illness: a low molecular weight, heat-stable enterotoxin (ST). ST is a monomeric toxin containing multiple cysteine residues with disulfide bonds accounting for the heat stability of this toxin. There are two different classes of ST named STa and STb. STa, which contains 18–19 amino acids, binds to small bowel mucosa cells via a receptor in the apical membrane of the enterocytes called guanylate cyclase C (GC-C) and increases intracellular levels of cGMP, which leads to the secretory effect of the enterotoxin. Other enteropathogenic bacteria also produce STa, including *Yersinia enterocolitica* and *V. cholerae* non-01.[22] Another type of enterotoxin produced mainly by ETEC, STb, causes noncyclic nucleotide-dependent bicarbonate secretion in piglet loops, but its role in human disease remains unclear.[27] The genes controlling the production of LT and ST reside on transferable plasmids.

Cytotoxins

Cytotoxins are defined by their ability to produce damage to the mucosa that often results in inflammatory colitis, usually by inhibition of protein synthesis. The prototype of this group is the Shiga toxin, which is produced by *S. dysenteriae* serotype 1. This toxin is immunologically and structurally related to the cytotoxin produced by all strains of EHEC 0157:H7, which is associated with HUS. Using DNA hybridization and planned restriction patterns, this cytotoxin has also been shown for other *E. coli* serotypes. These cytotoxins, also known as verotoxin or Shiga-like toxins, are composed of an enzymatically active A cytotoxic subunit and a B-subunit pentamer that binds to the glycolipid globotriosyl ceramide Gb3 cell receptor. The A-subunit cleaves the adenosine-4324 in the 28S rRNA of the 60S ribosomal subunit and permanently blocks protein synthesis by inhibiting elongation factor-1.[28] Histopathologic examination of human and animal tissues has suggested that vascular endothelial cells are a target for verotoxin. This finding is consistent with the capillary lesions of the gastrointestinal mucosa and renal glomerulus in the hemorrhagic colitis and hemolytic uremic syndromes, respectively.

Strains of *C. difficile* isolated from patients who have antibiotic-associated diarrhea produce a potent cytotoxin that causes cellular damage in tissue cultures. *C. perfringens* also produces a cytotoxin that displays a similar effect to Shiga-like toxin in HeLa cells and in animal models. *Salmonella* strain elaborate an enterotoxin that activates cAMP and a cytotoxin that inhibits protein synthesis.

Neurotoxins

These include staphylococcal enterotoxin b, *Clostridium botulinum* and *Bacillus cereus* emetic toxin. These toxins cause enteric symptoms, primarily because of their action on the autonomic nervous system.

Invasiveness

Invasiveness is defined by the ability to penetrate into the intestinal mucosa and destroy the epithelial cells, causing a dysentery syndrome. Enteroinvasive *E. coli* (EIEC) and *Shigella* spp. are the prototypes

of invasive enteric pathogens. The invasiveness of certain strains of *Shigella* and EIEC is demonstrated in the laboratory by the guinea-pig Séreny test, which manifests destruction and invasion of the superficial corneal epithelium in guinea pigs. This property is mediated by proteins encoded by 120–140mDa plasmids found in *Shigella sonnei, Shigella flexneri* and EIEC [29] [30] and appears to be dependent at least in part on additional chromosomally encoded products and a lipopolysaccharide structure.

Viral enteropathogens

The exact mechanisms by which viruses produce diarrhea are not completely understood. The most studied model of viral enteritis is rotavirus (RV). Animal models of RV infection and viral replication in human intestinal epithelial cells have demonstrated that a multifactorial process is involved. After attachment to the cell surface and subsequent entry to the enterocyte, RV induces multiple cell-signaling pathways. The glycoprotein receptor and exact mode of entry have not been identified yet.[31] [32] Diarrhea is produced partly by intestinal fluid secretion attributed to the enterotoxin NSP4, the first described viral enterotoxin. NSP4 induces intracellular Ca^{2+} mobilization, Cl^- secretion and secretory diarrhea.[33] There are no structural similarities between NSP4 and the bacterial enterotoxins and NSP4 does not involve cyclic nucleotide signaling. The loss of absorptive villous enterocytes, the site of RV replication, also contributes to the malabsorption component of RV diarrhea. It is also believed that RV stimulates the enteric nervous system (ENS), thus prolonging diarrhea. The adenovirus and the calicivirus (including the NLV) are noncultivable viruses and little is known about their pathogenesis.

Intestinal protozoa

Diarrhea caused by the intestinal protozoa like *Giardia* and *Cryptosporidium* is usually self-limited in the normal host. The virulence factors leading to persistent diarrhea in the immunocompromised host and in certain individuals are a matter of intense research. The main mechanisms by which protozoa induce diarrhea are attachment and colonization of the intestinal epithelial cells, loss of villous absorptive cells and subsequent malabsorption. [34] [35] [36] Additionally, *Entamoeba histolytica* is capable of disrupting the intestinal mucosa, producing invasive disease, localized ulcerations, ameboma, dysentery syndrome and hematogenous dissemination.

PREVENTION

The major measure for the prevention of enteric infections in the general population is continuous vigilance over water supply and sanitation facilities. The morbidity

caused by this type of illness has diminished notably in developed nations with improvement in public health measures. The manufacture and distribution of nutritional products require extraordinary precautions from the food industry and continuous surveillance from the health authorities. Personal hygiene, especially handwashing, is of vital importance, particularly after contact with animals or animal products or after contact with hospitalized patients.

Travelers to areas with high prevalence of enteric infections may be able to reduce the probability of developing diarrhea by careful selection of food and beverages. It is important to encourage the consumption of well-cooked food, pasteurized milk and potable or processed water or bottled drinks. There is vast evidence that contaminated foodstuffs and water are the most important vehicles of diarrheal illness transmission in developing countries. Many public restaurants and street vendors often fail to meet adequate food hygiene standards and food is frequently contaminated with bacteria like ETEC, *Shigella, Campylobacter, Salmonella* and *Aeromonas*. The benefit of using chemoprophylactic agents such as antimicrobials and bismuth subsalycilate (BSS) against travelers' diarrhea (TD) has been demonstrated in the past. The development of resistance to commonly used antibiotics in different areas of the world,[37] short-lived protection and the possibility of side effects of the drugs during travel preclude the widespread use of antimicrobials as prophylactic agents. Although BSS showed a protective efficacy of up to 65% when taken at high doses (2.1g/day), the amount of tablets (two four times daily) needed for optimal protection, the required level of compliance and possible side effects make BSS unattractive for prophylaxis of TD.[38] Therefore, chemoprophylaxis is currently recommended only for those travelers with concomitant serious medical illness or in very special cases like short-term travelers in whom temporary incapacity from TD would be unacceptable.

The development of vaccines to prevent diarrheal illness in the general population in areas with high prevalence and travelers has been studied extensively, with different outcomes. Two large field trials of the inactivated whole cell *V. cholerae* plus B-subunit oral cholera vaccine (WC/BS) were conducted in Bangladesh and Peru. Five-year follow-up data in the Bangladesh study demonstrated that the protective efficacy of three doses of WC/BS was age and biotype dependent (less protective in children less than 5 years old and in those infected with the biotype El Tor); protection was lost after the second or third year of follow-up. [39] The cholera vaccine in the Peruvian trial used a B-subunit produced by recombinant genetic technology (WC/rBS). Two initial doses of WC/rBS were insufficient to provide protection against cholera infection, but a boost dose after 10 months of the initial doses reduced the overall number of cholera episodes by 61% and the number of hospitalizations by 82%.[40] Other forms of cholera vaccines are still under development, including a live attenuated form.[41]

In 1998 a rotavirus vaccine was approved for routine childhood immunization. This vaccine, a tetravalent rhesus-based vaccine (RRV-TV), was found to have a protective efficacy of about 50% and 70–100% against all RV diarrhea and severe diarrhea/hospitalizations respectively. Unfortunately, after several case reports of intestinal intussusception in recipients of the vaccine, RRV-TV was withdrawn from routine use in the USA in November 1999.[42]

Two different typhoid vaccines are currently available for use. An oral live-attenuated vaccine made from the Ty21a strain of *Salmonella typhi* is given every other day for four doses. The second is a purified Vi capsular polysaccharide vaccine, Typhim Vi, given parenterally. These vaccines have demonstrated an overall efficacy of approximately 70%. Protection after vaccination with any of these two vaccines occurs 2 weeks after completion of the primary vaccine series; therefore, vaccination should not preclude meticulous attention to food and beverage hygiene precautions.

Novel methods of delivery of enteric vaccines are being explored, including transcutaneous immunization. A combined recombinant ETEC colonization factor 6 (CF6) with heat-labile enterotoxin (LT) has proved to be immunogenic in volunteers.[43]

The widespread and indiscriminate use of broad-spectrum antibiotics is not only leading to the emergence of resistant pathogens but also contributes to the increased incidence of hospital antibiotic-associated diarrhea, most commonly caused by *C. difficile*. Judicious use of antibiotic and hospital measures such as strict handwashing and patient isolation precautions may help to decrease these complications.

CLINICAL FEATURES

On the basis of the clinical-pathologic features, diarrheal illness may be classified into a number of syndromes:

- watery diarrhea;
- dysenteric syndrome;
- antibiotic-associated diarrhea;
- enteric or typhoid fever;
- travelers' diarrhea;
- HIV-AIDS-associated diarrhea;
- diarrhea in homosexual men (Table 43.4).

TABLE 43-4 -- Major clinical-pathologic features and causes of infectious diarrhea.

MAJOR CLINICAL-PATHOLOGIC FEATURES AND CAUSES OF INFECTIOUS DIARRHEA		
Watery diarrhea		Cholera
		ETEC
		EPEC
		Salmonella gastroenteritis
		Shigella (initial phase)
		Cryptosporidium parvum
		Clostridium perfringens
		Bacillus cereus
		Giardia lamblia
		Rotavirus
		Norwalk-like virus
Dysenteric syndrome	Acute dysentery	Shigellosis
		EIEC
		EHEC
		Vibrio parahaemolyticus
		Salmonella enteritidis
		Yersinia enterocolitica
		Campylobacter jejuni
	Parasitic dysenteric-like syndrome	*Entamoeba histolytica*
		Schistosoma japonicum
		Schistosoma mansoni
		Trichinella spiralis
		Strongyloides stercoralis
Antibiotic-associated diarrhea		*Clostridium difficile* (pseudomembranous colitis)
		Clostridium perfringens type A
		Staphylococcus aureus

Enteric fever	*Salmonella typhi*
	Salmonella paratyphi A
	Salmonella schottmuelleri
	Salmonella hirschfeldii
Enteric fever-like syndrome	*Yersinia enterocolitica*
	Yersinia pseudotuberculosis
	Campylobacter jejuni

Watery diarrhea

This is a noninflammatory process that is confirmed by the absence of fecal leukocytes. There is usually a large volume of stool and a modest increase in the number of stools because the colonic reservoir is intact. This disorder is often manifested by nausea and vomiting, but other associated symptoms include cramping, abdominal pain, arthralgias, myalgias, chills and rarely fever. The diarrhea is mediated by bacterial enterotoxins that alter fluid and electrolyte transport (*V. cholerae*, ETEC, EPEC, *Salmonella enteritidis, Salmonella typhimurium, Cryptosporidium* spp., *C. perfringens, B. cereus*) or organisms that characteristically affect the proximal small bowel (e.g. ETEC, *Giardia*, RV, NLV).

Cholera is the prototypic model of enterotoxigenic diarrhea. After an incubation period that varies from several hours to 5 days, the illness may begin with sudden onset of profuse, watery diarrhea or anorexia and abdominal discomfort followed by diarrhea. The stool has a characteristic 'rice water' appearance, because of the mucus content, and a mild fishy smell. Tenesmus is absent and vomiting often occurs a few hours after onset of diarrhea.[44] Signs and symptoms of cholera infection result from the severity of dehydration caused by the fluid and electrolyte losses from the intravascular and extracellular spaces into the gut lumen. Only 2–11% of patients infected with toxin-producing *V. cholerae* 01 develop cholera gravis as a result of rapid fluid loss (500–1000ml/h). This leads to tachycardia, hypotension and severe hypovolemic shock.[45] Patients are typically afebrile, but low-grade fever occurs in up to 20% of cases. Patients may experience abdominal pain, muscle cramps, nausea, vomiting, thirst and faintness as a result of the fluid loss. The electrolyte imbalance may also cause leg cramps. Most cholera complications are related to fluid and electrolyte losses and include altered consciousness, acidosis, hypoglycemia, hypokalemia (rarely resulting in intestinal ileus, weakness and cardiac arrhythmias), hypernatremia and renal failure.

Salmonella gastroenteritis is characterized by crampy abdominal pain, nausea, vomiting and diarrhea that begins 8–48 hours after ingestion of contaminated food. Diarrhea varies from cholera-like watery diarrhea to dysentery. Patients may have moderate fever. Approximately 1–4% of patients develop transient bacteremia. The most common serotypes that cause gastroenteritis are *S. typhimurium, S. enteritidis, S. newport* and *S. anatum*.

NLV diarrhea usually affects adults of all ages and is the most common cause of nonbacterial food-related diarrhea outbreaks. The onset of illness is abrupt (24–28h) and the duration is relatively short (12–60h). NLV symptoms include diarrhea, abdominal cramps and nausea but it is the high frequency of projectile vomiting that characterizes its presentation.

Acute dysentery

This inflammatory or invasive process involves the colon and occasionally the distal small intestine. The finding of numerous leukocytes in feces indicates the diffuse colonic inflammation or invasion of the colonic mucosa. *Shigella dysenteriae* is the prototypic pathogen of bacillary dysentery, particularly in the developing world. Elsewhere, other species may predominate, for example, *Shigella flexneri* in Mexico, South America and most other tropical countries, and *S. sonnei* in the USA. This condition is characterized clinically by fever, passage of low-volume stools that contain blood, mucus and leukocytes (dysentery), and variable degrees of fever, chills, abdominal cramping, tenesmus and vomiting. The patient feels the urge to defecate frequently, and may pass only small-volume stools, flatus or mucus. This is caused by the colonic infection and decreased reservoir capacity of the colon. [46] Occasionally young infants with early shigellosis will have hyperpyrexia and febrile convulsions, initially without enteric symptoms. *S. dysenteriae* 1 produces a more serious form of diarrhea and has been associated with HUS, disseminated intravascular coagulation and sepsis.

EIEC can cause a clinical illness that is indistinguishable from that produced by shigellosis. EAEC has been reported as causing diarrhea in 6% of travelers to Mexico and in 3% of US troops in the Middle East.[47] [48]

A form of dysentery that differs from that associated with EIEC is the bloody diarrhea (hemorrhagic colitis) caused by EHEC serotype 0157:H7 and, less commonly, a variety of other serotypes including 026:H11. This syndrome appears both as sporadic cases and in food-borne outbreaks characteristically associated with the consumption of undercooked ground beef in fast-food restaurants. *E. coli* 0157:H7 can cause asymptomatic infection, nonbloody diarrhea, hemorrhagic colitis, HUS and thrombocytopenic purpura. The disease begins with severe abdominal cramps and nonbloody diarrhea. By the second or third day of illness the individual may pass high-volume bloody stools. Nausea and vomiting are present in half of the patients. Unlike EIEC and shigellosis, high fever and abundant fecal leukocytes are not

a feature of *E. coli* 0157:H7 infection. The HUS, which is a major cause of acute renal failure in children, develops in about 6% of patients and is usually diagnosed 2–6 days after onset of diarrhea.[49] [50] The thrombocytopenic purpura, which is usually diagnosed in adults, has the clinical manifestations of the HUS, but the renal injury is less severe and the neurologic complications, including seizures, coma and hemiparesis, are more prominent. The clinical features of the hemorrhagic colitis can be confused with noninfectious diseases, such as ulcerative colitis, inflammatory bowel disease, intussusception, ischemic colitis, diverticulosis and appendicitis.

Other enterobacteria that cause dysentery syndrome are *Vibrio parahaemolyticus* and *C. difficile* (pseudomembranous colitis). *Salmonella enteritidis* and *Campylobacter jejuni* involve the small bowel and have the potential for developing acute inflammatory bowel disease. Some parasites may produce dysentery-like illness. Although *Entamoeba histolytica* is the best-known pathogen, *Schistosoma japonicum, Schistosoma mansoni, Trichinella spiralis* and *Strongyloides stercoralis* infections may all result in bloody diarrhea during the acute phase of infection.

Antibiotic-associated diarrhea

The onset of antibiotic-associated diarrhea is usually between 4 and 9 days after starting antibiotics. The most commonly implicated antibiotics are clindamycin, cephalosporins and penicillins. The clinical manifestations vary from mild watery or mucoid green diarrhea to dysenteric syndrome with bloody diarrhea, high fever, marked abdominal tenderness, the presence of fecal leukocytes, colonic thickening on computed tomography and findings of characteristic mucosal changes on endoscopy. *Clostridium difficile* is documented in about 20% of cases of antibiotic-associated diarrhea. Colitis results from cytotoxins produced by *C. difficile* that alter mucosal function and integrity. Sigmoidoscopy typically reveals the presence of small raised pseudomembranous nodules or plaques that may become confluent over an erythematous mucosa in the distal colon, sigmoid, or rectum (pseudomembranous colitis). Complications of pseudomembranous colitis include hypovolemic shock, toxic megacolon, peritonitis, fecal perforation, hemorrhage and sepsis. This disorder should be suspected when bloody diarrhea develops in hospitalized patients who are receiving antibiotics. Inflammatory bowel disease, ischemic colitis and other enteric pathogens such as *Salmonella* and *Shigella* spp., EIEC, *E. histolytica, Campylobacter, Yersinia* and *Strongyloides* spp. should always be considered in the differential diagnosis of pseudomembranous colitis.

Other causes of antibiotic-associated diarrhea include other enteric pathogens (*C. perfringens* type A, *Staphylococcus aureus*), direct effect of the antibiotics in the intestinal mucosa (erythromycin, clavulanate) or the metabolic consequences of the changes in the intestinal flora (see also Chapter 44).

Enteric fever

The enteric fever syndrome is an acute systemic illness characterized by fever, headache and abdominal discomfort. This syndrome is classically produced by *S. typhi* and referred to as typhoid fever; however, *Salmonella paratyphi* A, *S. paratyphi* B (*S. schottmuelleri*) and *S. typhi* C (*S. hirschfeldii*) may cause a similar but less severe clinical syndrome, referred to as paratyphoid fever. *S. typhi* is a food-borne and water-borne organism for which humans are the only natural hosts. *Salmonellae* are ingested orally and must traverse the acid barrier of the stomach as well as the various pancreatic enzymes, bile and intestinal secretions, and secretory IgA, which are effective antimicrobial factors. The postgastrectomy state, hypochlorhydria, altered intestinal motility and prior antibiotic use are known conditions predisposing to salmonellosis. In addition, salmonella infection is a common occurrence in patients who have sickle cell anemia, chronic liver disease and immunodeficiency of CD4

cell-mediated immunity accompanying neoplastic diseases and AIDS.

Salmonellae transcytose the infact distal small bowel mucosa, possibly via microfold cells over Peyer's patches. After replicating in the regional lymphatic nodules, organisms spread to the bloodstream through the lymphatic route and replicate in reticuloendothelial cells in lymph nodes, liver, bone marrow and spleen. The presence of mononuclear cells in the stool examination when diarrhea is present indicates that the intestinal mucosa is not damaged. The incubation period varies from 5 to 21 days, depending on the inoculum and the immune status of the patient. The onset is insidious, characterized by nonspecific manifestations of remittent fever, headache and abdominal pain. Diarrhea is present in approximately 50% of patients. Physical findings in patients who have typhoid fever include abdominal tenderness, splenomegaly, hepatomegaly, evanescent maculopapular rash on the upper abdomen and lower thorax ('rose spots'), relative bradycardia and mental confusion (at times delirium).[51] Complications of enteric fever occur during the third week of the illness and later as symptoms resolve. They may be related to recurrent bacteremia with dissemination of the organism, which may result in pneumonia, endocarditis, osteomyelitis, arthritis or meningitis, or to local organ involvement, such as erosion of blood vessels in Peyer's patches, which may result in intestinal hemorrhage or perforation of the ileum.[52] The case fatality rate in the USA is approximately 2%.

Yersinia enterocolitica, Y. pseudotuberculosis and *Campylobacter jejuni* may also be responsible for an enteric fever-like syndrome indistinguishable from that of typhoid fever. This syndrome has frequently been reported in patients who have underlying diseases such as chronic liver disease, thalassemia, kwashiorkor and amyloidosis.[53] [54]

Travelers' diarrhea

Travelers' diarrhea is defined as the passage of three or more unformed stools per day in a resident of an industrialized country visiting a developing nation. The onset of travelers' diarrhea usually occurs within the first 2 weeks after arrival in the foreign country, most often within the first week. Approximately 30–50% of the travelers from industrialized countries that spend at least 3 weeks in a less developed area will experience diarrhea. The illness is more common among young travelers.

Travelers' diarrhea is acquired through the ingestion of fecally contaminated food and/or water. A wide array of pathogens, including bacteria, viruses and protozoa, has been reported to cause it[55] [56] [57] (Table 43.5). ETEC is the most commonly isolated organism, responsible for 40–70% of cases of travelers' diarrhea in Latin America, Africa and Asia.[58] *Shigella* and *Salmonella* spp., *E. histolytica*, *G. lamblia*, *C. jejuni, Cryptosporidium, Vibrio, Aeromonas* and *Plesiomonas* spp. are other enteric pathogens responsible for travelers' diarrhea limited to certain geographic areas and seasons. Recently EAEC has been suggested as a cause of travelers' diarrhea. These findings represent about 19–33% of the cases in different world regions, second only to ETEC. [59] Giardiasis and cryptosporidiosis watery diarrhea are frequently seen in travelers to Russia and national parks in the USA. *Cyclospora cayetanensis* has been associated with prolonged and intermittent, but eventually self-limiting diarrheal illness in travelers to Nepal, in staff physicians at a Chicago hospital and in Peruvian infants and children living in the slums of Lima.[60] [61]

In summary, travelers' diarrhea is a bacterial illness and close to 80% of cases will respond to antibiotic therapy. On the other hand, viruses like rotavirus, NLV and adenovirus may represent about 10–12% of the etiology of travelers' diarrhea.

Travelers' diarrhea is almost always a self-limiting illness, rarely lasting more than 5 days even when untreated. However, 30% of travelers with diarrhea will be confined to bed and another 40% will have to modify their travel plans.[62] The diarrhea is accompanied by

TABLE 43-5 -- Etiologies of travelers' diarrhea in Latin America, Asia and Africa.*

ETIOLOGIES OF TRAVELERS' DIARRHEA IN LATIN AMERICA, ASIA AND AFRICA			
Organism	**Latin America (%)**	**Asia (%)**	**Africa (%)**
ETEC	40–70	20–34	36
EAEC	26–33	?	33
EIEC	6	3	2
Shigella spp.	2–30	2–13	2–15
Salmonella spp.	0–16	11–15	0–4
Campylobacter jejuni	1–7	2–15	1–28
Aeromonas hydrophila	2	1–57	1–8
Rotavirus	4–36	18	0–6
Giardia lamblia	0–9	0–6	0
Undiagnosed	23–30	33–53	15–33
Highest rates of ETEC occur in rainy summertime; highest rates of *Campylobacter jejuni* occur in dry wintertime.			

** (Modified from Arduino RC, DuPont HC. Travelers' diarrhea. In: Gracy M, Bouchier IAD, eds. Clinical gastroenterology: infectious diarrhea. London: Baillière Tindall; 1993;7:365.)*

abdominal pain and cramps in 20–60% of episodes. Nausea and vomiting are present in about 50% of the illnesses. Approximately 10% of patients develop fever. This clinical presentation is variable and depends on the etiologic agent, microbial virulence, size of the inoculum and host response. Travelers' diarrhea can frequently be separated into four syndromes:

- noninflammatory or watery diarrhea (ETEC, *Salmonella* spp., *C. jejuni*, *Shigella* spp. and *V. cholerae*);
- inflammatory or dysenteric diarrhea (*Shigella* spp., *C. jejuni*, nontyphi *Salmonella* spp., *E. histolytica*, *V. parahaemolyticus*, EIEC and *Aeromonas* spp.);
- vomiting out of proportion to diarrhea (*Staph. aureus*, *B. cereus*, rotavirus and NLV);
- and persistent diarrhea lasting longer than 2 weeks (*Giardia*, *Entamoeba*, *Cryptosporidium* spp. and *Cyclospora cayetanensis*).

Diarrhea may also occur in travelers within days of their return to their country of origin. In 1–2% of travelers, it lasts for longer

TABLE 43-6 -- Diarrhea in HIV-positive patients: causes of diarrheal disease in relation to the absolute $CD4^+$ lymphocyte count.*

CAUSES OF DIARRHEAL DISEASE IN RELATION TO THE ABSOLUTE $CD4^+$ LYMPHOCYTE COUNT			
Absolute $CD4^+$ lymphocyte count cells/ml	**Pathogen**	**Small bowel pathogens**	**Large bowel pathogens**
=200	Protozoa	*Glardia lamblia*	*Entamoeba histolytica*
	Viruses	Rotavirus	Herpes simplex virus
		HIV	Adenovirus
	Bacteria	*Salmonella* spp.	*Shigella* spp.
		Enteroadherent *E. coli*	*Campylobacter* spp.
		EPEC	*Yersinia* spp.
		Mycobacterium tuberculosis	*Clostridium difficile*
	Fungi		*Histoplasma capsulatum*

=100	Protozoa	*Cryptosporidium parvum*	
		Isospora belli	
		Cyclospora cayetanensis	
		Enterocytozoon bieneusi	
		Septata intestinalis	
		Microsporidia	
	Virus		Cytomegalovirus
	Bacteria	*Mycobacterium avium* complex	
		Enteroadherent *E. coli*	
		EPEC	
	Fungi	*Cryptococcus neoformans*	

** (With permission from Arduino RC. HIV/AIDS: approach to the patient with diarrhea and/or wasting. In: Kelly WN, ed. Textbook of internal medicine, 3rd ed. Philadelphia: JB Lippincott; 1996:1878–80.)*

than 1 month. Bacterial pathogens such as *Shigella, Salmonella* and *Campylobacter* spp. are the most common causes of chronic diarrhea or remitting symptoms. In addition to parasites such as *G. lamblia*, the most common parasite acquired by travelers worldwide, *Cryptosporidium* spp., *Cyclospora cayetanensis* and *E. histolytica* can cause prolonged diarrhea. An idiopathic condition known as Brainerd diarrhea may produce diarrhea among international travelers. This illness lasts on average 2 years before complete recovery.

Diarrhea in HIV-AIDS patients

Gastrointestinal symptoms are common in persons infected with HIV and diarrhea is one of the most frequent presenting complaints of patients with AIDS. Diarrhea occurs in up to 50% of patients who have AIDS in the USA and Europe, and in up to 90% of those in developing countries.[63] [64] [65] [66] A wide array of pathogens, including bacteria, viruses and protozoa, can be associated with diarrheal disease in HIV-infected patients. A pathogen can be identified in 50–80% of patients who have AIDS and diarrhea. Infection of the mucosa by HIV itself, infection with unrecognized or unidentified pathogens, dysregulation of the enteric immune system, fat malabsorption and small bowel bacterial overgrowth are potential causes of diarrhea and weight loss in some HIV-infected patients without detectable pathogens ('AIDS enteropathy'). Table 43.6 shows the potential small and large bowel pathogens that cause diarrheal disease in relation to the absolute $CD4^+$ lymphocyte count.

Parasitic infection is the most common form of pathogen-identified illness in patients with AIDS-associated diarrhea. *Cryptosporidium parvum* enteritis is commonly found in these patients. It occurs in up to 20% of AIDS patients in the USA and 50% of patients in Africa and Haiti who have AIDS and diarrhea. *C. parvum* primarily causes a small bowel disease characterized by chronic voluminous watery diarrhea. Patients may have anorexia, nausea, vomiting, crampy upper abdominal pain, low-grade fever and marked weight loss. Although *C. parvum* is generally restricted to the small bowel, it may involve the stomach and the colon, as well as the gallbladder, the pancreatic and biliary ducts or the respiratory tract. *Isospora belli* occurs in fewer than 3% of AIDS patients in the USA, but it is an

important cause of diarrhea in Haiti and Zaire. This parasite has also been reported in South America, Africa and South East Asia.[67] *Cyclospora cayetanensis* has been identified in stool specimens from HIV-infected patients who have chronic diarrhea in Haiti and Mexico [68] [69] but this pathogen is uncommon among AIDS-associated diarrhea in the USA. *Enterocytozoon bieneusi* and to a lesser extent *Septata intestinalis* are two microsporidia species associated almost exclusively with AIDS patients. Prevalence rates of *E. bieneusi* among patients with AIDS-associated chronic diarrhea range from 7% to 50%. Enteritis is observed in patients who have a $CD4^+$ lymphocyte count below 100/ml. The clinical features of isosporiasis, cyclosporiasis and microsporiasis are indistinguishable from those seen in patients who have cryptosporidiosis. *Giardia lamblia, E. histolytica* and *Blastocystis hominis* are not significantly more frequently found among AIDS patients than among HIV-negative homosexual men.

Mycobacterium avium complex infection may present with fever, night sweats, abdominal pain, diarrhea, severe weight loss and anemia. *Salmonella* and *Shigella* spp. may cause protracted, severe and bacteremic infections in HIV-infected individuals despite relatively minor gastrointestinal symptoms. EPEC, EAEC, or both, have been found on colonic biopsy from AIDS patients who have chronic diarrhea, malabsorption and weight loss.[70]

Disseminated fungal infections, such as those caused by *Cryptococcus*, *Histoplasma* and *Aspergillus* spp., may also be accompanied by diarrhea. The colon is the site most commonly involved by *H. capsulatum*.

The colon is also the gastrointestinal site most commonly involved by cytomegalovirus. The clinical presentation includes diarrhea with hematochezia, urgency, tenesmus, crampy abdominal pain and occasionally rebound tenderness. Fever, malaise, anorexia and weight loss frequently accompany the colitis. Herpes simplex virus is a common disease among HIV-infected patients, usually confined to the perianal region, rectum and esophagus. In addition to painful perianal ulcers, involvement of the distal rectum may result in low-volume bloody stools, constipation, painful defecation, inguinal lymphadenopathy, tenesmus and mucopurulent discharge that may be misinterpreted as diarrhea. Rarely, extension to the sigmoid colon leads to proctocolitis and mild diarrhea. Rotavirus and adenovirus have also emerged as significant potential pathogens in patients who have AIDS and diarrhea.

Diarrhea in homosexual men

There is a high prevalence rate of acute rectal and enteric infections in homosexual men. This high prevalence varies widely depending on promiscuity, certain sexual practices (receptive anal intercourse) and the incidence of asymptomatic and untreated infections. Where fecal contamination of the oral cavity commonly occurs, these enteric infections are often polymicrobial.[71] Oral-fecal contamination, which might occur during anal intercourse or oral-anal contact, appears to be the major risk factor for the transmission of enteric pathogens. In addition, anorectal infections with conventional venereal pathogens are caused by receptive rectal intercourse. The predilection of enteric infections for certain segments of the gastrointestinal tract and the patient's symptoms allow division of the disease into four syndromes. Enteritis is an inflammation of the small intestine; patients may present with symptoms of diarrhea, abdominal cramps and bloating. Pathogens commonly associated with enteritis include *G. lamblia, Cryptosporidium* spp. and *Strongyloides stercoralis*. Proctocolitis refers to an inflammation throughout segments of the colon and rectum that causes diarrhea and lower abdominal cramps in addition to the symptoms of proctitis. Sigmoidoscopy is often abnormal beyond 15cm. Proctocolitis is associated with isolation of *Campylobacter* spp., *Shigella flexneri, Salmonella* spp., *E. histolytica, C. difficile* and *Chlamydia trachomatis* lymphogranuloma venereum serovar. Proctitis implies inflammation limited to the rectum that causes anorectal pain, mucopurulent anal discharge, constipation, tenesmus and rectal bleeding. Anoscopy is often abnormal and evidences rectal exudate, mucosal friability, ulceration and bleeding. Proctitis is commonly associated with *Neisseria gonorrhoeae*, herpes simplex virus, *C. trachomatis* and *Treponema pallidum*. The perianal disease is a dermatologic disorder involving the anus and perianal area that causes perianal discomfort, pruritus and external lesions such as those produced by syphilis, herpes simplex virus, chancroid, granuloma inguinale, condyloma acuminata and human papilloma virus.

DIAGNOSIS

In the diagnostic approach to a patient who has diarrhea, the physician should obtain a careful exposure history and physical examination. Important diagnostic clues are history of antibiotic use, recent and remote travel, duration of diarrhea, weight loss, water supply, hobbies, pets, use of drugs that can cause diarrhea, other cases of diarrhea in the family and diet (e.g. lactose intolerance, intake of unpasteurized dairy products, raw or uncooked meat or fish). Once epidemiologic features of the illness and selective clinical characteristics are determined, it may be useful to distinguish between small and large bowel diarrhea. Large volume and relatively infrequent or nocturnal diarrhea suggests small intestine involvement. It is often associated with bloating, gas and periumbilical pain. On the other hand, frequent small-volume stools with infraumbilical pain on either side and rebound tenderness suggest colonic involvement. Rectal symptoms such as tenesmus, incontinence and urgency occur with colonic and anorectal inflammation. Fever is common, indicating an inflammatory process. Fecal leukocytes and blood in the stool (dysentery) result from diffuse colonic mucosal inflammation. Patients who have colitis can develop severe weight loss, often as a complication of the pathogens causing diarrhea rather than malabsorption.

Laboratory tests for stool samples are time consuming and expensive. Final identification requires a battery of media that is not available in all clinical laboratories.

Thus, in nonhospitalized patients who have mild to moderate diarrhea (five unformed stools or fewer without fever and/or cramps, pain, nausea and vomiting) routine stool culture is not justified from a cost-benefit point of view. Supportive treatment can be given. Fluid and electrolyte replacement and diet modification will usually be followed by spontaneous recovery. On the other hand, in patients who have more severe diarrhea (six unformed stools or more, presence of the aforementioned clinical symptoms and/or positive fecal leukocytes), persistent diarrhea (lasting >2 weeks), dehydration, requiring admission to hospital, who are very young, debilitated or with immunodeficiency diseases, or during an outbreak, laboratory tests can offer invaluable help in diagnosis and management (Table 43.7).

Direct examination

Microscopic examination of fecal smears can give rapid and useful information at a low cost. This test should be done in any patient who has moderate to severe diarrhea. One drop of stool, preferably including blood and mucus, is mixed with two drops of methylene blue on a glass slide and a cover slip is placed. The finding of numerous polymorphonuclear leukocytes, using high-power magnification (40×), indicates diffuse colonic (mucosal) inflammation caused by an invasive or inflammatory enteric pathogen, but does not specify the etiology. Mononuclear leukocytes may predominate in patients who have typhoid fever or amebic dysentery. The most common enteric pathogens that cause positive fecal leukocytes are *Shigella,*

TABLE 43-7 -- Indications for laboratory tests and findings.*

INDICATIONS FOR LABORATORY TESTS AND FINDINGS		
Test or procedure	**Indications**	**Positive findings**
Fecal leukocyte test or fecal lactoferrin	Fever and/or dysentery	Infectious:
	Moderate to severe diarrhea	*Shigella*, *Salmonella*, *Campylobacter* spp.; less frequently, *Clostridium difficile*, *Yersinia enterocolitica*, *Vibrio*, *parahaemolyticus*, *Aeromonas hydrophila*, EIEC, EHEC
	Hospitalized patients	
		Noninfectious:
		ischemic colitis, Crohn's disease, ulcerative colitis, diverticulitis, pseudomembranous colitis, necrotizing enterocolitis
Stool culture	Fever and/or dysentery	*Shigella*, *Salmonella*, *Campylobacter* spp.
	Moderate to severe diarrhea	
	Hospitalized patients or hospital admission	
	Diarrhea and dehydration	
	Diarrhea >1 week in duration	
	Patients with immunodeficiency diseases	
	Diarrhea outbreak	
Parasitic examination	Diarrhea >2 weeks in duration	*Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp.
	Bloody stool with few leukocytes	
	Recent travel to developing countries, Russia or Rocky Mountains	
	Diarrhea in homosexual men or in infants in daycare centers	
Proctosigmoidoscopy	Chronic diarrhea	White-yellowish plaques in pseudomembranous colitis, selective sampling of ulcers in amebiasis, biopsy to rule out other pathologies
	Severe antibiotic-associated diarrhea with equivocal test for *C. difficile* toxin	
	Amebiasis	
	Idiopathic inflammatory bowel disease	
	Hiv-positive with large-bowel diarrhea or acute proctitis	
	History of anal manipulation	
Gastroduodenoscopy	HIV-positive patients who have small-bowel diarrhea	*Giardia lamblia*, *Cryptosporidium parvum*, *Enterocytozoon bieneusi*, *Septata intestinalis*, *Mycobacterium tuberculosis*, *Mycobacterium avium* complex

** (With permission from Arduino RC, DuPont HL. Diarrhea in the critically ill: causes and treatment in five clinical settings. J Crit Illness 1992;6:715–24.)*

Salmonella and *Campylobacter* spp. Other disorders associated with feces containing leukocytes are *C. difficile*, *Y. enterocolitica, Aeromonas hydrophila, V. parahaemolyticus*, EIEC and EHEC. The finding of numerous fecal leukocytes is an indication for performing stool culture and giving antibiotic treatment. The examination of feces on saline wet mount preparations by dark-field or phase-contrast microscope for motility can reveal *Campylobacter* or *Vibrio* spp., which show 'darting motility'.

Examination for parasites is indicated in all patients who have diarrhea lasting longer than 2 weeks, diarrhea acquired while traveling to the Rocky Mountains, Russia or developing countries, persons in daycare centers, male homosexual patients or HIV-infected patients. Wet mounts can be performed using a drop of iodine solution. With low-power magnification (10×) eggs, larvae, cysts and trophozoites can be detected. Special stains are used when trophozoites of amoeba are suspected. *Giardia lamblia* is missed in half of these cases. If *G. lamblia* is suspected but the examination is negative, it is recommended to continue by collecting a small bowel fluid sample, performing a small bowel biopsy, using the nylon string test, or treating the patient empirically (see Chapter 209).

Modified Kinyoun's acid-fast stain and trichrome staining of stool should be done, particularly in immunocompromised patients, for cryptosporidiosis, isosporiasis and microsporidiosis. The oocytes stain red against a blue background with these stains. Stool concentration techniques and duodenal aspirates increase the sensitivity of examination for parasites. *Microsporidia* spores can be demonstrated in stool by light microscopy using Gram, Weber chromotrope-based, Giemsa or chitin-binding fluorochrome stains. However, small bowel aspirates or jejunal biopsy may be required for identification of these organisms (see Chapter 243). Electron microscopy is a valuable adjunct for the identification and taxonomy of *Microsporidia* in infected tissues and fluids.

Monoclonal-based immunofluorescence stains and enzyme-linked immunosorbent assay (ELISA) have been developed for the

direct detection of *Isospora, Giardia, Cryptosporidium* spp. and *E. histolytica* antigens in feces. Diagnosis of cyclosporiasis can be improved by concentrating oocysts from fecal samples without the use of formalin, to allow them to sporulate at room temperature in 5% potassium dichromate, and visualizing the sporocysts by autofluorescence using ultraviolet light or by modified acid-fast stain.

Stool culture

Bacteria cause 15–50% of cases of diarrhea in adults, depending upon the severity and duration of illness when studied. Clinical laboratories are able to identify the

most commonly recognized invasive pathogens, such as *Shigella, Salmonella* and *Campylobacter* spp. Routine use of media for the isolation of other pathogens varies depending on the geographic location and patient history. For example, history of exposure to coastal areas and seafood should prompt culture for vibrios (*V. cholerae, V. parahaemolyticus* and others). These organisms require a selective thiosulfate citrate bile salt sucrose agar. Culture of *Y. enterocolitica* may require the selective process of cold enrichment, alkali treatment or selective cefsulodin-irgasan-novobiocin agar, but these methods are not cost effective for routine diagnosis of diarrhea.

Unfortunately, there are no specific biochemical tests to detect any one of the different groups of diarrheagenic *E. coli* in stool. The ETEC, EPEC, EIEC, enteroaggregative *E. coli* and EHEC belong to different serotypes based on their O and H antigens. Although certain serotypes have been associated with these different types of *E. coli*, routine serotyping in sporadic cases is of limited value. Immunospecific tests including ELISA, receptor ELISA, latex and coagglutination have been developed to detect both STa and LT. Unlike most other *E. coli* serotypes, most strains of *E. coli* O157:H7 do not ferment sorbitol rapidly; here, sorbitol-containing MacConkey agar can be used as a culture medium for stool for patients who have bloody diarrhea, nonbloody diarrhea who may have been exposed to the organism or the HUS. Sorbitol-negative colonies that agglutinate with O157 antiserum can be presumptively identified as *E. coli* O157:H7. Commercial testing reactions are available to detect *E. coli*, which produces Shiga-like toxin. Diagnostic DNA probes from genes encoding virulence factors have been developed to find complementary sequences of DNA by hybridization, and to identify the different groups of diarrheagenic *E. coli*.

Stool cultures for *C. difficile* or tests for the presence of its toxins in stools are indicated in patients who have diarrhea and history of prior antibiotic use. An ELISA method to detect both toxins A and B is now available.

Stool studies to detect virus are not used routinely in medical practice. They are useful in investigation of nonbacterial diarrhea outbreaks. The recent advances in molecular laboratory methods like PCR and RT-PCR have changed what was known about the epidemiology of viral diarrhea. NLV, a noncultivable virus, has been identified as the cause of about 70–90% of nonbacterial diarrhea outbreaks in developed countries. These methods also help to detect viral particles in food and beverages and to better understand their way of transmission.

Blood culture

Blood cultures are recommended in patients ill enough to require hospitalization, in those in whom typhoid fever or bacteremia is suspected or in those with systemic enteric infections, nosocomially acquired diarrhea or who are immunocompromised.

Endoscopic examination

Proctosigmoidoscopy may be very helpful in the differential diagnosis of patients who have bloody diarrhea, prior antibiotic use, history of anal manipulation, who are HIV positive with large bowel diarrhea or acute proctitis. It is valuable in diagnosing pseudomembranous colitis (characterized by whitish yellow plaques), amebiasis (selective sample of ulcers) and idiopathic inflammatory bowel disease.

In HIV-positive patients who have no pathogen identified in stool and/or blood, gastroduodenoscopy is important in establishing the diagnosis in those with small bowel diarrhea, colonoscopy in those with symptoms suggesting colitis, or both, when it is not possible to differentiate between small and large bowel diarrhea. Examination of small bowel biopsies from the distal duodenum or proximal jejunum should include light and electron microscopy, viral (i.e. cytomegalovirus) and mycobacterial cultures, and special stains for acid-fast bacteria, viral inclusions, fungi and parasites. Examination of colonic biopsies includes light and electron microscopy, mycobacterial and viral cultures (i.e. cytomegalovirus, adenovirus, herpes simplex virus) and special stains for viral inclusion, acid-fast bacteria and fungi.

MANAGEMENT

There are four therapeutic approaches in the management of patients who have acute diarrhea: fluids and electrolytes, diet, symptomatic drugs and antimicrobial agents.

Fluids and electrolytes

Fluid and electrolyte replacement is the cornerstone of therapy for acute diarrhea.[72] It may be life saving for infants and the elderly with dehydrating illness. For severe cholera-like dehydrating diarrhea, fluid therapy has two phases: rehydration phase over the first few hours of treatment and then the maintenance phase to match continuing losses. Patients who have severe dehydration are ordinarily treated with intravenous fluids and electrolytes. Ringer's lactate is the preferred intravenous solution. For most patients who have diarrhea, oral rehydration solution can be used for both phases of fluid therapy. The World Health Organization's (WHO) oral rehydration salts (ORS) have contributed to the significant decrease in mortality secondary to diarrhea around the world.

The oral therapy usually recommended for adults and children with cholera diarrhea consists of one of the higher sodium-containing solutions (approximately 90mmol/l sodium) and plain water. For acute noncholera diarrhea, not associated with moderate or severe dehydration, the reduced osmolarity ORS (30–60mEq/l sodium) has been proved to decrease the amount of stool output and frequency of vomiting. A new formulation of the reduced osmolarity ORS, including 75mEq/l of sodium and 75mmol/l of glucose with a total osmolarity of 245mmol/l, has been tried in adults and children with cholera diarrhea and proved to be safe and to reduce the need for intravenous therapy in 33% of cases. The main concern is the development of hyponatremia; these studies demonstrated a small but statistically significant decrease in serum sodium in those patients receiving reduced osmolarity ORS. However, no symptoms were described. The WHO scheduled the use of this new ORS formula for all types of diarrhea in 2002.[73]

Diet alteration

During a bout of illness the diet should ordinarily be modified as the intestinal tract may have difficulty absorbing certain food items.[72] Milk other than breast milk for an infant should be withheld during the early stages of illness. To facilitate enterocyte renewal, calories should be taken in during a bout of acute diarrhea. For infants breast milk or lactose-free formula may be administered. For older children and for adults, appropriate foods include boiled starches and cereals such as potatoes, noodles, rice, wheat and oats with some salt added. Crackers, yogurt, bananas, soup and boiled

TABLE 43-8 -- Symptomatic therapy of older children and adult patients who have acute diarrhea.

SYMPTOMATIC THERAPY OF OLDER CHILDREN AND ADULT PATIENTS WHO HAVE ACUTE DIARRHEA		
Pharmacologic agent	**Dose**	**Comment**
Bismuth subsalicylate	30ml or two tablets each 30 minutes for eight doses for no more than 48 hours	Will turn stools and tongues black; is 50% effective in reducing number of stools passed
Loperamide	4mg initially, then 2mg after each unformed stool, not to exceed 8mg/day (over-the-counter dosage) or 16mg/day (prescription dosage) for no more than 48 hours	This agent is 80% effective in reducing number of stools passed; the drug may rarely worsen invasive forms of diarrhea and may produce post-treatment constipation
Rocecadrotil	Not established	In development
Zaldanide		
Provir		

TABLE 43-9 -- Empiric therapy of acute diarrheal disease.

EMPIRIC THERAPY OF ACUTE DIARRHEAL DISEASE		
Clinical syndrome	**Adults**	**Children**

Febrile dysenteric diarrhea in industrialized regions or moderate to severe travelers' diarrhea	Ciprofloxacin 500mg bid or levofloxacin 500mg qd for 3–5 days	Azithromycin 5–10mg/kg/d for 3–5 days Trimethoprim-sulfamethoxazole 5–25mg/kg/d in two equally divided doses for 3–5 days plus erythromycin 40mg/kg/d in four divided doses for 5 days
Persistent diarrhea (=14 days in duration) in industrialized countries	Consider anti-*Giardia* therapy: metronidazole 250mg tid for 7 days	Consider anti-*Giardia* therapy: metronidazole 20mg/kg/d in three divided doses for 7 days

vegetables may also be eaten. When stools are formed, diet may return to normal.

Symptomatic therapy

Although the major objective of therapy for diarrhea in young infants and in the elderly is amelioration of complications of illness such as dehydration, in most older children and nonelderly adults an important objective is amelioration of morbidity and suffering. Symptomatic drugs do play a role in reducing the number of stools passed and duration of illness in most forms of diarrhea. Nonspecific therapy may be employed where improvement in symptoms is the objective. Table 43.8 describes common agents used to improve symptoms of diarrhea. These drugs can be useful in nondehydrated patients to reduce unpleasant symptoms of enteric illness, helping to return patients to school, work or leisure activities. These drugs do not cure illness and they are not indicated for infants with diarrhea, particularly in developing regions. In these infants and in the elderly with severe diarrhea, the focus should be on fluid and electrolyte therapy alone.

Antimicrobial therapy

Although antimicrobial therapy is primarily used for the treatment of pathogen-specific illness, these drugs can be used in one of several clinical syndromes in which an etiologic agent is suggested by the resultant illness.[74] Patients living in industrialized regions with febrile dysentery (presence of fever and passage of bloody stools) often have enteric infection caused by an invasive enteric bacterial pathogen, such as *Shigella* spp. and *C. jejuni*. Empiric therapy in this setting is appropriate. Table 43.9 lists recommended drugs and dosage for children and adults. For adults one of the fluoroquinolones is recommended. For children azithromycin is recommended after infection with Shiga-like toxin producing pathogens has been excluded. The same drugs are used for adults and children with moderate to severe travelers' diarrhea, in which a variety of bacterial pathogens may be encountered. A single drug, furazolidone, may be used in children with travelers' diarrhea[75] although resistance to the drug has become common.[37] When diarrhea persists for 2 weeks or longer, a work-up for the etiology should be undertaken. Many would use empiric anti-*Giardia* therapy for a subset of these patients, usually metronidazole given for 7 days. For young children furazolidone is preferred because a pediatric suspension form of the drug is available.

In Table 43.10 , specific recommendations for therapy are provided according to the etiologic agent identified.

It is advisable to treat all patients who have proven shigellosis in view of the potential for transmission of the infecting organism to susceptible contacts. In the case of intestinal *Salmonella* spp. infection, the decision to use antimicrobials often depends on the severity of clinical illness together with the presence of certain host conditions known to predispose to more serious infection by the organism. Indications for treating intestinal salmonellosis include presence of high fever and systemic toxicity suggesting bacteremic illness, or one of the conditions known to predispose to bacteremic illness and higher risk for fatal illness. These conditions include: age greater than 65 years or less than 3 months, malignancy, inflammatory bowel disease, hemodialysis, uremia, renal transplantation, aortic aneurysm and patients who have AIDS. For these patients, treatment is given for 10–14 days. Other patients who have less severe cases of salmonellosis without underlying medical conditions need not be treated with antimicrobial agents. Other enteric infections where specific therapy may be given include *C. jejuni* diarrhea, diarrhea caused by diarrheagenic *E. coli* other than enterohemorrhagic *E. coli* (in which case antimicrobials may predispose to the HUS[76]), *Aeromonas* and *Plesiomonas* diarrhea, yersiniosis, giardiasis, amebiasis, isosporiasis and cyclosporiasis. Patients who have cryptosporidiosis are usually not treated, although those with advanced HIV infection may benefit from suppressive treatment with paromomycin.[77] New anticryptosporidial drugs are in development and may offer therapeutic advantages over paromomycin (see Chapter 127).

In cases of bloody diarrhea it is hard to make a decision about empiric therapy because of the fear of potentiation of HUS in patients with Shiga toxin-producing *E. coli* infection. The key aids here are the following: HUS is an important complication of Shiga toxin-producing *E. coli* infection in children and the elderly; the organism characteristically causes outbreaks of illness where the other causes of dysentery (*Shigella* and *C. jejuni* being the most important in the industrialized countries) frequently cause sporadic or single cases. Also, Shiga toxin-producing *E. coli* infection is rarely associated with important levels of fever. Thus, when we have a sporadic case of bloody diarrhea in an older child or young adult where there is fever, empiric therapy with a quinolone is appropriate. On the other hand, when an outbreak of dysentery occurs in a group of children or the elderly and fever is either absent or low grade, antimicrobial therapy should probably be withheld pending the establishment of etiology of the illness.

TABLE 43-10 -- Treatment of pathogen-specific diarrhea TMP-SMX: trimethoprim-sulfamethoxazole).

TREATMENT OF PATHOGEN-SPECIFIC DIARRHEA		
Pathogen-specific diarrhea	**Adults**	**Children**
Shigellosis	Ciprofloxacin 500mg bid for 3–5 days or levofloxacin 500mg/d for 3–5 days	Azithromycin 10mg/kg/d. If resistance suspected use ceftriaxone, cefixime or cefotaxime
Salmonellosis	Mild or asymptomatic: no therapy	If =3 months old use ceftriaxone 50mg/kg iv qd.
	With underlying illness (see text) use ciprofloxacin 400mg bid or levofloxacin 500mg qd for 5–7 days depending on response	If >3 months old and healthy with mild illness or asymptomatic, no antimicrobial therapy; with underlying illness (see text) use ceftriaxone 50mg/kg iv qd not to exceed 2g/d
	Alternative: azithromycin	
Campylobacteriosis	Azithromycin 500mg po qd for 3 days	Erythromycin stearate 40mg/kg/d in four divided doses for 5 days or azithromycin 10mg/kg/d
	Alternative: erythromycin stearate 500mg po bid for 5 days	
ETEC, EPEC, EAEC	Same as empiric therapy for febrile dysentery and travelers' diarrhea (see Table 43.9)	Same as shigellosis
EHEC (Shiga toxin and Shiga-like toxin producing *E. coli*).	No treatment (increased risk of increasing toxin release and HUS)	No treatment
Aeromonas and *Plesiomonas*	Same as empiric therapy for febrile dysentery and travelers' diarrhea (see Table 43.9)	Same as empiric therapy for febrile dysentery and travelers' diarrhea (see Table 43.9)
Yersiniosis	Same as empiric therapy for febrile dysentery and travelers' diarrhea (see Table 43.9). If severe: ceftriaxone 1g iv qd for 5 days	Ceftriaxone 50mg/kg qd for 5 days
Giardiasis	Metronidazole 250mg tid for 7 days or albendazole 400mg po qd for 5 days or tinidazole 2g po one dose	Metronidazole 20mg/kg/d in three divided doses for 7 days or furazolidone 6mg/kg/d divided in four doses for 7 days
Amebiasis	Metronidazole 500mg po tid for 10 days or tinidazole 1g po bid for 3 days. Follow with paromomycin 500mg po tid for 7 days	Metronidazole 50mg/kg/d in three divided doses iv plus di-iodohydroxiquin 40mg/kg/d in three divided doses for 20 days
Crytosporidiosis	Immunocompetent patients: nitazoxanide 500mg po bid for 3 days [78]	Nitazoxanide (children 4–11 years old) 100mg po bid for 3 days[78]
	AIDS: paromomycin 1g bid plus azithromycin 600mg qd for 4 weeks	

Isosporiasis	Immunocompetent: TMP-SMX 160–800mg po bid for 7 days	TMP-SMX 10–50mg/kg/d in two divided doses for 7 days
	AIDS: TMP-SMX 320–1600mg bid for 2–4 weeks then 16–800mg qd indefinitely	
Cyclosporiasis	Immunocompetent: TMP-SMX 160–800mg po bid for 7 days	TMP-SMX 10–50mg/kg/d in two divided doses for 7 days
	AIDS: TMP-SMX 160–800mg po bid for 10 days then once three times a week indefinitely	
Clostridium difficile colitis	Metronidazole 500mg po tid for 10–14 days. Repeat if relapse.	Metronidazole 20mg/kg/d in three divided doses for 7 days
	Alternative: iv metronidazole, po vancomycin	

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Chapter 44 - Antibiotic-associated Colitis/Diarrhea

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EPIDEMIOLOGY

Diarrhea may occur as a result of administration of any antimicrobial. The vast majority of such occurrences are self-limited and resolve with discontinuation of the drug. However, a much more serious antibiotic-associated diarrhea and pseudomembranous colitis (PMC) is caused by *Clostridium difficile* infection following antimicrobial treatment. It is the major diagnosed cause of infectious diarrhea that develops in hospitalized patients in developed countries. [1] *C. difficile*-associated diarrhea (CDAD) is unique in that it occurs essentially only in association with antimicrobials and is most frequent in hospitals and nursing homes where antimicrobial use is high and the environment and personnel are most likely to be contaminated by *C. difficile* spores. The incidence and severity of CDAD appear to be increasing and the annual cost of the disease to US hospitals is estimated at $1.1 billion.[2] [3]

Clindamycin, ampicillin and cephalosporins were initially the antibiotics most frequently associated with CDAD, but the second- and third-generation cephalosporins, particularly cefotaxime, ceftriaxone, cefuroxime and ceftazidime, are now most frequent.[4] Penicillin plus ß-lactamase inhibitor combinations such as ticarcillin-clavulanate and piperacillin-tazobactam have significantly less risk.[4] [5] All antibiotics, including vancomycin and metronidazole, the most common agents used to treat CDAD, have been found to have risk of a subsequent episode of CDAD. [4]

Clindamycin has a prolonged persistent effect on the colonic flora after the drug is stopped, explaining the clinical observation of CDAD well after discontinuation of clindamycin. Clindamycin resistance was a marker for a *C. difficile* strain implicated in epidemics in four geographically dispersed US hospitals. The identical epidemic strains carried the same *ermB* resistance gene.[6] Infection with the epidemic strain was associated with clindamycin use and the epidemic ended promptly with restriction of clindamycin in one hospital.

C. difficile is carried commonly in the stool of asymptomatic patients in the hospital.[1] The rate of colonization in hospitalized adults is often 20% or greater for patients hospitalized for more than 1 week, compared to 1–3% of community residents, and the risk of *C. difficile* colonization increases in proportion to length of hospital stay.[7] Asymptomatic fecal carriage of *C. difficile* in healthy neonates is very common, often exceeding 50% in the first 6 months of life. Spores of *C. difficile* are found on environmental surfaces (where the organism can persist for months) and on the hands of hospital personnel if they are not washed between patients. The incidence of community-acquired CDAD is low (7.7 cases per 100,000 person-years of observation) compared to rates of 5–35 per 1000 hospital discharges.

Genetic or phenotypic typing studies have shown that even the most virulent of *C. difficile* organisms produce asymptomatic colonization more often than CDAD, suggesting that factors in addition to organism virulence are necessary for CDAD to occur. Molecular typing has shown that recurrent CDAD is caused by both relapses from the original infecting strain and reinfection with a new strain of *C. difficile*. Hospital outbreaks of CDAD have been caused by a single *C. difficile* strain in some institutions whereas in others a variety of strains have been found. Over 500 unique types of *C. difficile* organized into >100 distinct toxin-negative or toxin-positive groups have now been identified by *Hin*dIII restriction endonuclease analysis (REA). PCR ribotyping has identified 116 different ribotypes, suggesting wide organism diversity.

Other risks for CDAD have been identified, including gastrointestinal surgery, older patient age, longer hospital stay, greater severity of illness, use of electronic rectal thermometers, enteral tube feeding and antacid treatment.[1] [7]

PATHOGENESIS AND PATHOLOGY

Spores of toxigenic *C. difficile* are ingested, survive the acidity and other upper gastrointestinal defense mechanisms, germinate and colonize the lower intestinal tract where they elaborate toxins. In addition to the enterotoxic and cytotoxic effects of toxin A and toxin B, toxin A is a potent chemoattractant for neutrophils *in vivo*, and both toxins induce cytokine release from monocytes.

Although patients colonized with *C. difficile* were thought to be at high risk for CDAD, four prospective studies have shown that colonized patients actually had a decreased risk of subsequent CDAD.[8] Many of these patients were colonized with nontoxigenic strains (explaining their lack of clinical illness), but 56% were colonized with virulent, toxigenic strains that caused CDAD in other patients. We now propose that at least three factors influence the development of CDAD and the timing of these factors or 'hits' appears to be critical (Fig. 44.1). Exposure to antimicrobials establishes susceptibility to *C. difficile* infection. The second hit is exposure to toxigenic *C. difficile*. Prospective observations have shown that the majority of patients do not become ill after the first two 'hits'. A third factor appears to be necessary for CDAD to occur, which may be related to *C. difficile* virulence, the type and timing of the antimicrobial exposure, or the host immune response. Serum antitoxin antibody levels (prior to colonization) were comparable in those patients who later became colonized and those who developed CDAD. However, following exposure to *C. difficile*, median antitoxin A IgG levels in the serum increased significantly higher in those who became asymptomatic carriers than in patients who developed CDAD.[9] Thus, one likely 'third hit' is the anamnestic response to toxin A at the time of exposure to *C. difficile* which is protective against CDAD but not colonization, and supports the earlier observation that asymptomatic *C. difficile* carriers are at decreased risk of subsequent CDAD. A second study found that the early antibody response in those patients who developed CDAD also influenced the risk of diarrhea recurrence. Antitoxin A IgM levels on day 3 and antitoxin IgG levels on day 12 were significantly higher in those patients who did not have a CDAD recurrence.[10]

The pseudomembrane found in association with antibiotic PMC is confined to the colon and initially appears as small (1–2mm), whitish-yellow plaques along the colonic wall. The intervening mucosa

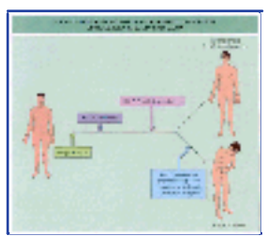

Figure 44-1 The pathogenesis model for hospital-acquired *Clostridium difficile*-associated diarrhea (CDAD). This 'three-hit' model of *C. difficile* pathogenesis shows that exposure to antibiotics establishes susceptibility to infection. Once susceptible, the patient may acquire nontoxigenic (nonpathogenic) or toxigenic strains of *C. difficile* (the second 'hit'). Acquisition of toxigenic *C. difficile* may be followed by asymptomatic colonization or *C. difficile*-associated disease, depending on one or more factors (the third 'hit'). Inadequate anamnestic IgG response to toxin A produced by toxigenic *C. difficile* strains is an important host factor determining disease outcome.[9]

appears unremarkable. As the disease progresses, the pseudomembranes may coalesce to form larger plaques and in advanced cases become confluent over the entire colon wall (Fig. 44.2). The distribution of lesions typically involves the entire colon, but rectal sparing occurs in about 10% of patients. Microscopically, the pseudomembranes contain necrotic leukocytes, fibrin, mucus and cellular debris with a point of attachment to the underlying mucosa. The superficial epithelium is commonly eroded and necrotic in focal areas and the

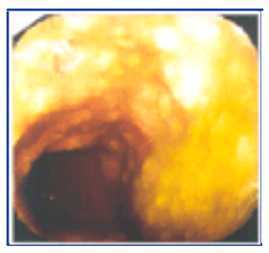

Figure 44-2 Endoscopic view of multiple pseudomembranes covering the colon in a patient with PMC.

mucosa beneath the pseudomembrane is inflamed, with a predominantly neutrophil infiltration.

PREVENTION

Control and prevention strategies for *C. difficile* infections can be divided into two types:

- barrier-isolation-disinfection procedures to prevent transmission of the organism to the patient;
- reduction of the risk of clinical illness if the organism does contact the patient.[1]

One of the latter highly successful methods is restriction of the use of specific antibiotics to reduce the risk of colonization and infection. Neither barrier precautions nor changes in antimicrobial use are easy to implement successfully, a factor that may explain the limited success of control and prevention guidelines to date.[1] Transmission prevention methods include barrier precautions, gloving, handwashing, environmental disinfection, replacement of electronic rectal thermometers and treatment of asymptomatic carriers of *C. difficile* with vancomycin or metronidazole. Of these interventions, only discontinuation of the use of contaminated electronic thermometers and wearing of gloves by personnel have reduced CDAD rates in actual hospital clinical practice. Environmental disinfection with hypochlorite solutions reduces *C. difficile* contamination in the hospital environment, presumably because hypochlorite is sporicidal; however, because of odor and bleaching of surfaces by hypochlorite, hospitals have not widely adopted its use.

Because prevention of organism transmission has been difficult, control measures have focused on reducing clinical illness if transmission cannot be prevented. Control of clindamycin, and second-and third-generation cephalosporins, has been the most effective prevention strategy.[5] [6] In the case of clindamycin, outbreaks of CDAD have correlated with *C. difficile* resistance to clindamycin and have resolved with restriction of the antibiotic.[6] Other prevention

approaches under development include a vaccine which has been successful in the hamster model, prophylaxis with the yeast *Saccharomyces boulardii*, which reduced antibiotic-associated diarrhea but not CDAD, use of *Lactobacillus* spp., that has not successfully prevented CDAD, and colonization with nontoxigenic strains of *C. difficile* that has succeeded in hamsters but is untested in humans.

CLINICAL FEATURES

Diarrhea is by far the most common symptom caused by *C. difficile*. Stools are almost never grossly bloody and range from soft and unformed to watery or mucoid in consistency with a characteristic odor. Stool frequency varies widely from three to 20 or more bowel movements per day. Clinical and laboratory findings from one large series are shown in Table 44.1 .[11] When adynamic ileus (which is seen on X-ray in about 1/5 of patients) results in cessation of stools, the diagnosis of *C. difficile* disease is frequently overlooked. Such patients are at high risk of complications of *C. difficile* infection, particularly toxic megacolon and colonic perforation. Overall, mortality associated with CDAD ranges from 0.6% to 3.5%. Extracolonic manifestations of *C. difficile* infection such as bacteremia and abscesses occur rarely, but reactive arthritis is not uncommon.

Recurrence of *C. difficile* diarrhea following treatment occurs in about 20% of patients. Recurrences may be either relapses with the same strain or reinfections with a new strain of *C. difficile*. Data from three studies suggest that nearly half of all recurrences are a result of reinfection with a new strain. Recurrence of clinical CDAD is likely a result of continued disruption of normal fecal flora by the treatment antibiotics and does not correlate with continued presence of the organism is stool following treatment (which occurs commonly), but does correlate with failure of the patient to develop serum antibodies to toxin A.[10]

DIAGNOSIS

The diagnosis of CDAD is based on a combination of clinical and laboratory criteria:

- diarrhea, e.g. a minimum of three unformed stools per 24 hours for a minimum of 2 days;
- no other recognized cause for diarrhea.

When this clinical definition is combined with either visualization of colonic pseudomembranes or toxin A or B in the stool or stool culture positive for a toxin-producing *C. difficile* organism, then the diagnosis of CDAD can be made.[1]

PMC is a more advanced form of CDAD and is visualized at endoscopy (Fig. 44.2) in only about 50% of patients with diarrhea

TABLE 44-1 -- Clinical and laboratory features of *Clostridium difficile*-associated disease.[11]

CLINICAL AND LABORATORY FEATURES OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE	
Clinical or laboratory finding	**Number of patients (percent positive)**
Diarrhea	109/109 (100)
Pseudomembranes on endoscopy	34/67 (51)
Leukocytosis (>10,000/mm^3)	54/109 (50)
Fever >100.4°F (38.0°C)	30/109 (28)
Mucus in stools	29/109 (27)
Occult blood in stools	28/109 (26)
Abdominal pain/cramping	24/109 (22)
Ileus (as determined by abdominal X-ray)	23/109 (21)

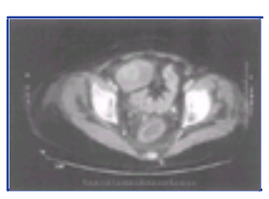

Figure 44-3 CT scan of the abdomen in a patient with fulminant PMC. The colonic and rectal walls are markedly thickened with fluid-filled colon and rectum.

who have a positive stool culture and toxin assay for *C. difficile* (Table 44.1). Endoscopy remains a valuable and rapid diagnostic tool in seriously ill patients with an acute abdomen and suspected PMC, but a negative examination does not rule out *C. difficile* disease. An abdominal CT scan may be more sensitive in this setting[3] (Fig. 44.3).

An array of tests are available for detection of *C. difficile* and its toxins (Table 44.2), but no single test has high sensitivity, high specificity and rapid results turnaround. For this reason, some clinical laboratories use two tests, often combining a specific test for toxin (cell cytotoxin or enzyme immunoassay) with stool culture to increase sensitivity; however, this approach is labor intensive and expensive. If the original specimen is negative and diarrhea persists, examination of additional stool

specimens increases the likelihood of diagnosis, but is time consuming and expensive.

The primary advantage of the cell cytotoxicity test, which can detect as little as 1pg of toxin B, is its sensitivity. Cell cytotoxicity is detected by a rounding of the cells and is confirmed as caused by *C. difficile* if cytotoxicity is neutralized by *C. difficile* (or *C. sordellii*) antitoxin. Enzyme immunoassays (EIA) use specific monoclonal or polyclonal antibodies to toxin A or toxin B (or both) to detect *C. difficile* toxin in stool specimens. These tests are more rapid than the cell cytotoxicity assay (1–3 hours), but EIA tests for toxin A will not detect some clinically important strains of *C. difficile* that do not produce toxin A. EIA tests (both toxin A and toxin A/B EIA tests) lack sufficient sensitivity to recommend these tests alone without back-up stool culture.

Stool culture for *C. difficile* is performed using a selective antibiotic-containing medium such as cycloserine-cefoxitin-fructose agar (CCFA). Colonies of *C. difficile* have a ground-glass appearance surrounded by a yellow halo on CCFA. *C. difficile* culture lacks specificity in diagnosing CDAD because nontoxigenic strains that do not cause CDAD may be detected. Specificity can be increased by testing isolates *in vitro* and reporting as positive only those specimens containing toxin producing *C. difficile*. Latex agglutination (LA) tests are rapid, but neither sensitive nor specific and are inferior to toxin assays and culture (Table 44.2).

MANAGEMENT

Treatment of uncomplicated first episodes of CDAD

CDAD will resolve in 15–23% of patients within 2–3 days after discontinuing the precipitating antimicrobial.[12] Withholding specific

TABLE 44-2 -- Sensitivity and specificity of tests for the diagnosis of *Clostridium difficile*-associated diarrhea.[*] [†]

SENSITIVITY AND SPECIFICITY OF TESTS FOR THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DISEASE[*]			
Test	**Sensitivity**	**Specificity**	**Utility of test**
Endoscopy	51%	~100%	Diagnostic of PMC and therefore CDAD
Culture for *C. difficile*	89–100%	84–100%	Most sensitive test; confirmation of organism toxicity necessary to improve specificity
Cell culture cytotoxin test	67–100%	85–100%	With clinical data is diagnostic of CDAD; highly specific but not as sensitive as culture
EIA toxin test	63–99%	75–100%	With clinical data is diagnostic of CDAD; rapid but not as sensitive as culture or cell culture cytotoxin test
Latex test for *C. difficile* antigen	58–92%	80–96%	Less sensitive and specific than other tests but gives rapid results
PMC, pseudomembranous colitis; CDAD, *Clostridium difficile*-associated disease; EIA, enzyme immunoasay; PCR, polymerase chain reaction.			

* Using both clinical and test-based criteria.
† *(Adapted from reference[1] with permission of Infection Control and Hospital Epidemiology, Slack Publishers.)*

TABLE 44-3 -- Summary of randomized, comparative trials of oral therapy for initial episodes of *Clostridium difficile*-associated diarrhea.[*]

SUMMARY OF RANDOMIZED, COMPARATIVE TRIALS OF ORAL THERAPY FOR INITIAL EPISODES OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA[*]					
Antibiotic	**Regimen**	**Number of patients**	**Resolution of diarrhea (%)**	**Recurrence (%)**	**Mean days to diarrhea resolution**
Metronidazole	250mg qid × 10d	42	40 (95)	2 (5)	2.4
	500mg tid × 10d	31	29 (94)	5 (17)	3.2
Vancomycin	500mg tid × 10d	31	29 (94)	5 (17)	3.1
	500mg qid × 10d	87	87 (100)	13 (15)	2.6–3.6
	125mg qid × 7d	21	18 (86)	6 (33)	4.2
	125mg qid × 5d	12	9 (75)	?	<5
Teicoplanin	400mg bid × 10d	28	27 (96)	2 (7)	2.8
	100mg bid × 10d	26	25 (96)	2 (8)	3.4
Bacitracin	25,000U qid × 10d	15	12 (80)	5 (42)	3.0
	20,000U qid × 7d	21	16 (76)	5 (31)	4.1
Colestipol	10g qid	12	3 (25)	?	<5
Placebo		14	3 (21)	?	<5

* *(Adapted from reference[17] with permission from Springer-Verlag)*

anti-*C. difficile* treatment may avoid the 20% risk of recurrence following treatment with vancomycin or metronidazole, but most patients will require specific treatment.[13] [14] General treatment guidelines include hydration and avoidance of antiperistaltic agents and opiates that may mask symptoms and possibly worsen disease. These agents have been used safely with vancomycin or metronidazole for treatment of mild to moderate *C. difficile* disease.[15] Finally, test-of-cure cultures or toxin assays following treatment are not recommended, as they do not predict subsequent recurrence, and treatment of asymptomatic patients has been ineffective.[16]

Prospective randomized clinical trials show no statistical differences in the outcome of CDAD treatment for the primary endpoint, cessation of diarrhea, among all regimens tested (Table 44.3). [17] Vancomycin has been shown to be superior to placebo. Colestipol is no better than placebo and is inferior to antimicrobials. The clinical response rate for bacitracin is 10–20% lower than for vancomycin so bacitracin use for first-line therapy should be discouraged (Table 44.3). Fusidic acid and teicoplanin are also effective agents. CDAD recurrence rates have been shown to be significantly different in only one study; the recurrence reduction for teicoplanin compared to fusidic acid.[18]

Antimicrobial therapy, particularly vancomycin, should be administered orally. Metronidazole achieves bactericidal fecal concentrations in the presence of acute diarrhea when used intravenously and anecdotal evidence supports intravenous treatment for CDAD; however, intravenous metronidazole has failed in the treatment of PMC in the presence of adynamic ileus. Response to oral therapy with vancomycin or metronidazole is =95%. Mean time to resolution of diarrhea ranges between 2 and 4 days and patients should not be deemed treatment failures until at least 6 days of treatment have been given.[19] Based on data from shorter courses of vancomycin (Table 44.3), we recommend treatment be given for 10 days, although no controlled comparisons are available. Metronidazole does not have US Food and Drug Administration (FDA) approval for *C. difficile* treatment, but is the current drug of choice in the USA and the least expensive agent. Metronidazole-resistant isolates have been reported rarely, but treatment failure has not been correlated with in vitro resistance.

Treatment of recurrences of CDAD

First recurrences of CDAD usually resolve after retreatment with metronidazole.[19] There is no standard treatment approach supported by comparative data for patients with more than one recurrence of CDAD. Biotherapeutic approaches include vancomycin or metronidazole followed by the yeast *Saccharomyces boulardii*;[20] metronidazole or bacitracin followed by *Lactobacillus* GG; vancomycin followed by synthetic fecal bacterial enema; and administration of a nontoxigenic *C. difficile* strain. *S. boulardii, Lactobacillus* GG, nontoxigenic *C. difficile* and fecal enemas are not available as pharmaceuticals in the USA and do not have FDA approval for use

in treating CDAD.

Other approaches include vancomycin in tapering doses over 21 days followed by pulse dosing for 21 days; no treatment with careful observation; vancomycin followed by the anion-exchange binding resin, cholestyramine; and combined treatment with vancomycin

and rifampin which is the approach we favor [10 days of vancomycin (125mg orally four times daily) and rifampin (300mg orally twice daily)], since it is a relatively simple and effective regimen. Treatment of antibody-deficient children with intravenous immunoglobulin (IVIG) has resulted in clinical and bacteriological improvement, but the high cost and limited availability of IVIG limit this approach to the few chronically *C. difficile*-infected antibody-deficient children.

Treatment of complications

The most difficult treatment issue is the management of the rare patient who presents with or develops toxic megacolon or ileus as a result of fulminant CDAD.[3] These patients often have no diarrhea and mimic an acute surgical abdomen. Sepsis (hypotension, fever, tachycardia, leukocytosis) is also a complication of severe *C. difficile* disease. Acute abdomen may occur with or without toxic megacolon and may have signs of obstruction, ileus, bowel wall thickening and ascites on abdominal CT (Fig. 44.3), accompanied by a marked peripheral blood leukocytosis that is often >25,000wbc/mm^3 . *C. difficile* infection should be included in the differential diagnosis of an acute abdomen, sepsis or toxic megacolon if the patient has received antibiotics within the previous 2 months, whether diarrhea is present or not. Rapid diagnosis of *C. difficile* disease can best be achieved by performing cautious sigmoidoscopy or colonoscopy to visualize pseudomembranes or by doing an abdominal CT exam[3] (Fig. 44.3).

There is no established effective treatment for these seriously ill patients. Medical management is suboptimal, in part because of the inability to achieve effective concentrations of metronidazole or vancomycin in the colon by the oral route if ileus is present. We have successfully treated six patients with severe ileus using vancomycin via nasogastric tube and by retention enema plus intravenous metronidazole.[19] Surgical intervention is indicated for patients with toxic megacolon, fulminant disease or acute abdomen who do not respond to medical management or have suspected colonic perforation. The incidence of fulminant CDAD requiring colectomy may be increasing.[3] Mortality in patients who require surgery for CDAD ranges from 38% to 80%,[3] emphasizing the need for earlier recognition and more effective diagnostic and therapeutic approaches in these patients.

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Chapter 45 - Whipple's Disease

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Michael JG Farthing

EPIDEMIOLOGY

Whipple's disease was originally described by George Whipple in the Johns Hopkins Medical Bulletin in 1907.[1] The disease is rare, with fewer than 1000 validated cases reported in the literature, although it has been estimated that two to three unpublished cases may exist for each one reported. The majority of cases have occurred in the USA, the UK, Europe, Australia, South America and South Africa, with a small number of additional cases from Japan, India and China.

The largest review to date, published in 1970, reported 19 patients who had Whipple's disease, and reviewed the literature from 1950 to 1969.[2] A further 114 patients were identified, of whom 88% were male. All 114 patients were white, with the exception of one native American and three black patients. Of the 19 patients reported, 18 were male and all were white. The mean age at diagnosis was 48 years (range 33–62 years). Other studies have confirmed this demographic distribution.

PATHOGENESIS AND PATHOLOGY

Whipple's disease is a multisystem disorder.[2A] It most commonly affects the small intestine and its lymphatic drainage, but the pathologic features of the disease have been demonstrated in most organs (Table 45.1). In the small intestinal mucosa the presence of numerous macrophages that stain strongly with periodic acid Schiff (PAS) is pathognomonic (Fig. 45.1). Whipple noted the presence of a great number of rod-shaped organisms in affected tissues, but these were only identified as bacilli when examined by electron microscopy. The bacilli are rod-shaped and approximately 0.2mm wide by 1.5–2.5mm long, with cell walls that consist of a trilaminar membrane (Fig. 45.2). These cell walls reflect the PAS-positive material within macrophages in affected tissues seen under light microscopy.

TABLE 45-1 -- Pathologic features of Whipple's disease.

PATHOLOGIC FEATURES OF WHIPPLE'S DISEASE	
System	**Feature**
Gastrointestinal tract	Transmural involvement of the esophagus, stomach, intestine and colon
	PAS-positive macrophages concentrated in lamina propria
Central nervous system	PAS-positive macrophages present throughout the brain
	Occasional microinfarcts reported as a result of microvascular occlusion
Cardiovascular system	PAS-positive macrophages in endocardium, myocardium and pericardium
	Fibrosis of heart valves
	Lymphocytic myocarditis
Respiratory system	PAS-positive macrophages predominantly in intra-alveolar septa and pleura of lungs
	Occasionally granulomatous 'sarcoid-like' reaction in tissues
Genitourinary tract	Few PAS-positive macrophages noted
Lymphoreticular system	PAS-positive macrophages in spleen and lymph nodes throughout body
Eyes	Uveitis
	PAS-positive macrophages in vitreous humor
Bone marrow	Focal collections of PAS-positive macrophages

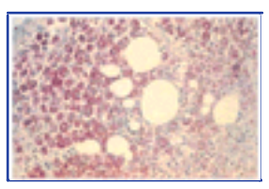

Figure 45-1 Section from a PAS-stained duodenal biopsy. There are numerous (pink-staining) PAS-positive macrophages in the lamina propria.

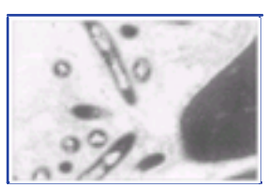

Figure 45-2 Electron micrograph of small intestinal mucosa demonstrating the typical appearance of the Whipple's disease bacillus. *Courtesy of Professor H Hodgson.*

Whipple's bacilli have been identified and classified phylogenetically using molecular genetic techniques. Bacteria can be identified by amplification of the bacterial small subunit (16S) rRNA sequence from infected tissue. rRNA is present in all living cells and is highly conserved. All genetic sequences accumulate mutations with time; the evolutionary distance between species is proportional to the number of nucleotide differences between two copies of the same gene and can be inferred from the number of rRNA sequence differences between them, thereby allowing a phylogenetic classification. The phylogenetic classification of an unknown organism can be determined by comparing its 16S rRNA with a catalog of rRNA sequences from known organisms. The polymerase chain reaction (PCR) is used to amplify and sequence minute quantities of rRNA from infected tissue. In 1991 this technique was applied to a small bowel specimen from a patient who had Whipple's disease.[3] On the basis of its 16S rRNA sequence the organism was found to be most closely related to *Rhodococcus*, *Arthrobacter* and *Streptomyces* spp. (see Chapter 226). In 1992 a unique 1321-base bacterial 16S rRNA sequence was identified in a patient who has Whipple's disease; from this sequence specific PCR primers that enabled detection of this bacillus in four other patients were designed.[4] The organism was not detectable in tissue from ten control patients. Analysis of the 1321-base 16S rRNA sequence revealed the organism to be a previously uncharacterized bacterium belonging to the subdivision of Gram-positive bacteria known as actinomycetes (Fig. 45.3). This newly characterized organism has been named *Tropheryma whipplei*. Using molecular techniques, three different types of *T. whipplei* have been identified; it remains to be clarified whether this represents three subtypes, or three closely related, species.[5] The complete genome of *T. whipplei* has recently been sequenced and analyzed.[5A]

Culture of *T. whipplei* has been elusive until recently. Successful growth in a human macrophage cell line was described in 1997,[6] but subsequent subculture was not achieved. More recently, the bacterium was grown in a human fibroblast cell line, and ongoing culture has been established.[7]

Whipple's disease bacillus has been identified using light or electron microscopy in all tissues affected by the disease. However, the pathogenesis of the disease is unknown. Electron microscopy studies of infected intestinal mucosa have demonstrated extensive epithelial cell invasion by bacilli;[8] the route of invasion appears to be from the lamina propria rather than the intestinal lumen. There is evidence of ultrastructural damage to the infected epithelial cells, but no signs of injury have been found in macrophages, polymorphonuclear leukocytes, mast cells or intraepithelial lymphocytes containing the bacilli. *T. whipplei* is cytopathic to IgA plasma cells,[9] and

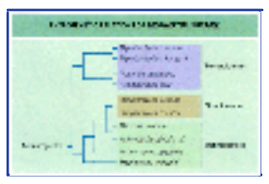

Figure 45-3 Phylogenetic relations of the Whipple's disease bacterium, *Tropheryma whipplei.*

this may explain the common finding of reduced plasma cells within the intestinal mucosa in infected patients.

Several attempts have been made to investigate whether there is a host immune defect in patients who have Whipple's disease. Subtle defects of cell-mediated immunity have been noted, with a reduction in the number of circulating lymphocytes and a decreased response to mitogens. It may be difficult to distinguish a primary immunologic defect from those that might arise as a consequence of nutritional deficiency. A decreased response to skin test antigens has been described, which persists after treatment. In addition there is a greater association with the HLA B27 haplotype in Whipple's disease patients than in controls.[10] Major histocompatibility complex (MHC) class I expression on small intestinal epithelial cells has been found to be normal, but MHC class II was markedly reduced compared with controls.[11] This defect was restored to normal after treatment, suggesting that the observed immune changes in the intestinal mucosa may occur as a result of infection rather than as a primary defect. *In vitro* culture of *T. whipplei* in macrophages required addition of interleukin (IL)-4 to the culture medium, indicating that a Th2 immune response may be necessary to sustain infection.[6] However, successful culture has been achieved in fibroblasts without addition of IL-4.[7]

The characteristic pathologic appearances of Whipple's disease are seen in the small intestine. Macroscopically the mucosa is often normal, although it may appear erythematous, with a friable mucosa and small erosions or yellow-white plaques. Microscopically the villi may be normal, reduced in height or flattened in severe cases. Enterocyte height is reduced and some show vacuolization of the cytoplasm. PAS-positive macrophages within the lamina propria and other infected tissues are characteristic but not specific. PAS-positive staining may persist after successful treatment as a result of bacterial cell wall remnants within the macrophages.

PREVENTION

The origin of the bacterium presumed to be the etiologic agent of Whipple's disease is unknown. To date there are no known preventative measures for this disease, but the detection of *T. whipplei* DNA in sewage[12] suggests a possible environmental source, which warrants further investigation.

CLINICAL FEATURES

In Whipple's original description of this disease he identified most of the important clinical and pathologic features.[1] The patient described in the original case initially presented with recurrent attacks of arthritis, progressive weakness and weight loss. This was followed by a cough and mild fever, then persistent diarrhea. The abdomen was swollen with a mass below the umbilicus and the skin was pigmented. The patient was found to be severely anemic and examination of stools revealed an excess of fat. At laparotomy, the mesenteric lymph nodes were enlarged and the diagnosis was considered to be tuberculosis. The patient died. At autopsy there was deposition of fat in the intestine and mesenteric glands, the heart was also enlarged and the aortic valve affected. The serosa of the heart, lungs and abdomen was affected and microscopy revealed extensive tissue infiltration by macrophages.

The most common symptoms and signs reported in Whipple's disease are listed in Table 45.2 . The onset is often insidious and several years may pass before a diagnosis of Whipple's disease is made. Intermittent arthralgia, low-grade fever and weight loss are common and a rheumatologic cause is often sought initially. Arthralgia may predate other symptoms by many years; in the series reported in 1970, 14 of the 19 patients suffered from arthralgia for more than 10 years before developing other symptoms.[2] Arthralgia is usually migratory, and largely involves the ankles,

TABLE 45-2 -- Common clinical features of Whipple's disease (in order decreasing frequency of occurrence).

COMMON CLINICAL FEATURES OF WHIPPLE'S DISEASE		
Symptoms	**Signs**	
Weight loss	Weight loss	Glossitis
Diarrhea	Hypotension	Abdominal tenderness
Arthralgia	Lymphadenopathy	Abdominal mass
Abdominal pain	Skin pigmentation	Ascites
Gastrointestinal bleeding	Fever	Splenomegaly
Central nervous system manifestations	Edema	Abnormal neurologic signs

knees, shoulders or wrists. The joints may become swollen and inflamed, but a chronic deforming arthropathy is rare. Weight loss may be the only presenting feature and may be profound.

Diarrhea may be watery or steatorrheic. Edema secondary to hypoalbuminemia may occur, and less commonly ascites. Tetany caused by hypocalcemia has been described, but not osteomalacia or bony fractures. Scurvy may occur. Occult gastrointestinal bleeding is common and may occasionally become frank. Five patients who had intestinal bleeding have been described; three presented with overt bleeding and the others with microcytic anemia and positive fecal occult blood tests; two showed an erythematous friable duodenal mucosa that bled on contact at endoscopy.[13] Small intestinal and rectosigmoid ulceration have also been described.

Neurologic features usually manifest late, occasionally in the absence of other symptoms, and may even develop after apparently successful treatment for other features of Whipple's disease. Central nervous system (CNS) manifestations include personality change, apathy, dementia and encephalopathy. Hypothalamic symptoms of insomnia, hypersomnia, hyperphagia and polydipsia may occur. Clonic movements, spastic paresis and hyperactive reflexes with extensor plantar responses are also described. Ocular manifestations include papilledema, nystagmus and gaze pareses. Isolated bilateral uveitis has also been described.[14]

Symptomatic cardiac involvement is rate at presentation, but congestive cardiac failure is a common terminal event in untreated disease. Sudden death caused by arrhythmia may occur in up to 20% of patients. At autopsy PAS-positive macrophages have been demonstrated in heart valves, endocardium, myocardium and pericardium. Lymphocytic myocarditis has been reported.[15] Cardiomyopathy in the absence of gastrointestinal symptoms has also been reported, although duodenal biopsy was diagnostic of Whipple's disease.[16] Endomyocardial biopsy failed to show the presence of PAS-positive macrophages, but electron microscopy revealed the presence of Whipple's bacteria within the endothelium of myocardial capillaries. Atrial tachycardia with right bundle branch block was observed. Treatment with antibiotics was associated with a recovery of cardiac function and a return to sinus rhythm.

Skin involvement in Whipple's disease is characterized by hyperpigmentation, most commonly in exposed areas and in scars. Various skin rashes have also been described. Lymphadenopathy is common and enlarged intestinal lymph nodes may occasionally result in a palpable abdominal mass.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of Whipple's disease is extensive because of the multisystem nature of the disease and the variable way in which symptoms may occur. An

initial presentation of fever, weight loss and arthralgia would be compatible with a diagnosis of rheumatoid arthritis or seronegative arthropathy. The combination of these symptoms together with skin rashes, cardiac or neuropsychiatric features may suggest a connective tissue disease such as systemic lupus erythematosus. When pulmonary manifestations are prominent, sarcoidosis may be suspected. A granulomatous inflammatory reaction with poor or absent PAS-staining may occasionally occur in Whipple's disease infected tissues, further confusing the diagnosis with sarcoidosis. Hyperpigmentation necessitates exclusion of Addison's disease. The combination of any of the above features with diarrhea, with or without frank malabsorption, should alert the clinician to the possibility of Whipple's disease. When diarrhea is the predominant symptom the differential diagnosis would include celiac disease, giardiasis or inflammatory bowel disease.

DIAGNOSIS

When the diagnosis of Whipple's disease is suspected a small bowel biopsy is the initial diagnostic investigation of choice and is usually obtained by endoscopic biopsy of the second part of the duodenum. Examination of formalin-fixed paraffin-processed biopsies stained with hematoxylin and eosin may demonstrate nonspecific enteropathic changes. However, PAS staining will demonstrate the presence of multiple PAS-positive macrophages within the lamina propria and is highly suggestive of Whipple's disease, but not specific. Systemic *Mycobacterium avium-intracellulare* infection associated with AIDS may mimic Whipple's disease clinically, with weight loss, fever and malabsorption, and histopathologically as a result of PAS-positive macrophages within an enteropathic small bowel. PAS-positive macrophages may also be found in the intestinal mucosa in histoplasmosis and macroglobulinemia, although other pathologic features may differ. The finding of Whipple's bacilli by electron microscopy of affected tissues is diagnostic.

Recently, the diagnosis of Whipple's disease has become possible by applying the molecular phylogenetic techniques described above.[3] [4] [5] PCR detection of *T. whipplei* 16S rRNA has been successfully applied diagnostically in a number of tissues including peripheral blood,[17] cerebrospinal fluid[18] and vitreous fluid.[14] PCR has become negative with successful treatment,[19] [20] but relapse with cerebral Whipple's disease has been described despite negative intestinal PCR.[19] False positive tests have been described.[21] Diagnostic PCR for the detection of *T. whipplei*, however, is a promising technique, and is cheaper, faster and potentially more readily available than electron microscopy. This technique is also likely to be particularly valuable in Whipple's disease patients without intestinal involvement, in whom the diagnosis is more difficult to establish. Serum antibodies to *T. whipplei* have been detected in a high proportion of infected patients, [7] and this may form the basis of a useful diagnostic test in the future.

Anemia is present in the majority of patients, with hemoglobin concentrations of <8g/dl in one-quarter of patients.[2] The anemia is usually microcytic and may be related to chronic gastrointestinal blood loss.[13] Megaloblastic anemia is rare. Serum vitamin B12 is usually normal, but serum folate is often reduced. The erythrocyte sedimentation rate is usually raised. Hypoalbuminemia is common and is likely to result from a combination of increased intestinal loss, decreased hepatic synthesis and protein malabsorption. Stool examination in the majority of patients will reveal an excess of fat.

Small bowel contrast radiology is abnormal in the majority of patients. The findings are non-specific but include coarsening and thickening of the duodenal and jejunal folds and occasional dilatation.

MANAGEMENT

Whipple's disease was inevitably fatal until clinical improvement was noticed in patients treated with antibiotics in the 1950s.

A number of antibiotic regimens have been used for the treatment of Whipple's disease with variable success. The antibiotic treatment and outcome of 88 patients with Whipple's disease was reviewed retrospectively;[22] of the 57 patients who responded to treatment [mean follow-up 8.2 years (range 18 months to 27 years)], 31 (54%) relapsed [mean time to relapse 4.2 years (range 2 months to 20 years)]. There were no significant differences in sex or age at diagnosis between those who relapsed and those who did not. Of those who relapsed 21 out of 31 were treated with tetracycline alone. Two patients who relapsed were treated with parenteral penicillin and streptomycin followed by oral tetracycline, but the latter drug was given for only 2 weeks. CNS relapse was common, occurring in 11 out of 31 patients — all occurred late — nine out of 11 occurred in patients treated with tetracycline alone and none in patients treated with antibiotics that penetrate the blood-brain barrier. Treatment of CNS relapse was ineffective when using drugs with poor CNS penetration. A combination of parenteral and oral penicillin and chloramphenicol followed by oral trimethoprim-sulfamethoxazole (co-trimoxazole) was effective. In a more recent review of treatment, trimethoprim-sulfamethoxazole was more efficacious than tetracycline in inducing remission (92 versus 59%) and resulted in fewer CNS relapses.[23] However, 40% of patients with cerebral Whipple's disease did relapse after treatment with trimethoprim-sulfamethoxazole alone. CNS relapse has been treated successfuly with oral chloramphenicol, although one case of CNS relapse occurring on long-term trimethoprim-sulfamethoxazole has been successfully treated with oral cefixime (400mg q12h) after failure of oral chloramphenicol. In two further cases, intravenous ceftriaxone (2g q12h) resulted in improvement of cerebral symptoms.[24] No randomized prospective trial of alternative treatment regimens has been published.

We would recommend intravenous benzylpenicillin (2.4g daily) and streptomycin (1g daily, reduced to 0.5–0.75g daily if the patient is elderly or has a body weight <50kg) for 2 weeks followed by oral trimethoprim-sulfamethoxazole (960mg q12h) for 1 year (Table 45.3). Patients allergic to penicillin and those with prominent CNS involvement at presentation should be treated with intravenous ceftriaxone (2g q12h) and streptomycin for 2 weeks, followed by oral trimethoprim-sulfamethoxazole (960mg q12h) for 1 year. The

TABLE 45-3 -- Current recommended treatment regimens for Whipple's disease.

CURRENT RECOMMENDED TREATMENT REGIMENS FOR WHIPPLE'S DISEASE		
Indication	**Antibiotic regimen**	**Duration**
No clinical CNS involvement at presentation	Benzylpenicillin 2.4g daily iv + streptomycin 15mg/kg daily iv then trimethoprim-sulfamethoxazole 960mg q12h po	2 weeks 1 year
Penicillin allergy or CNS symptoms at presentation	Ceftriaxone 2g q12h iv + streptomycin daily iv then trimethoprim-sulfamethoxazole 960mg q12h po	2 weeks 1 year
CNS relapse on treatment	Chloramphenicol 2g daily po	1 year
Streptomycin dose should be monitored and reduced as needed in patients who have impaired renal function, especially the elderly.		

duration of treatment remains empiric and relapse may occur after apparently successful treatment. Relapse may be confirmed by demonstrating the presence of Whipple's bacilli by electron microscopy in biopsy samples. The persistence of PAS-positive macrophages after treatment is common and is not diagnostic of relapse. CNS relapse on treatment should be treated with oral chloramphenicol (500mg q6h) for at least 1 year; a third-generation cephalosporin with good CNS penetration is the alternative.

In addition to specific antibiotic therapy, various symptomatic treatments and supportive measures may be required, depending upon the clinical manifestations of the disease. Pain caused by arthropathy may be treated with simple analgesics or nonsteroidal anti-inflammatory drugs. Diarrhea and malabsorption may necessitate nutritional support with protein-calorie enteral supplementation and vitamins. Antidiarrheal agents such as codeine or loperamide may provide symptomatic relief. Cardiac involvement with cardiac failure may require diuretic and angiotensin-converting enzyme inhibitor therapy. Antiarrhythmics may also be required. Occasionally mitral or aortic valve replacement has been required for those with advanced valvular involvement. The wide range of neurologic manifestations may require nursing support, physiotherapy and neurorehabilitation. Recovery may not be complete.

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Chapter 46 - Parasitic Infections of the Gastrointestinal Tract

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INTRODUCTION

This chapter examines the parasitic causes of gastrointestinal infection in the nonimmunocompromised host. The organisms involved are a very heterogeneous group of protozoa and helminths. They vary in size from microsporidia, which are barely visible using light microscopy, to long multicellular organisms such as *Taenia saginata*, which can reach 25m in length (Fig. 46.1). The clinical approach is emphasized here because the parasites themselves are discussed elsewhere (see Chapter 242 , Chapter 243 and Chapter 246), and diagnosis is covered in Chapter 165 . Parasitic gastrointestinal infections in HIV infection are discussed in Chapter 127 . The parasites discussed in this chapter are listed in Table 46.1 .

EPIDEMIOLOGY

In the developed world, parasitic infections appear to be uncommon causes of gastrointestinal illness. Certain populations are particularly likely to be affected:

- returned travelers,
- day care workers and patients,
- immigrant workers, and
- homosexual men.

The most common causes of parasitic gastrointestinal infection are *Giardia lamblia* and *Cryptosporidium parvum*.

In developing countries the impact of parasitic infections is of much greater significance in relation to morbidity, mortality and economic impact. Their epidemiology is varied and their control presents a complex sociopolitical problem. The most important infections are *Entamoeba histolytica*, *G. lamblia*, hookworms, *Ascaris* spp. and tapeworms.

Geographic distribution of parasites

The distribution of a parasite depends upon:

- human behavior,
- the physical environment, and
- the biological environment.

Human behavior

Behavioral factors are often dominant but the distribution of vectors, intermediate hosts and reservoir hosts is also to some extent under human control. Some parasites are worldwide in their distribution, such as *E. histolytica* and *G. lamblia*, whereas others are very localized.

Physical environment

The physical environment also affects the distribution of parasites. Temperature and humidity affect the viability of parasites in the external environment; viability is lost in cold or over-hot temperatures and most parasites require moist, aerobic conditions. Examples of parasites for which these factors are critical include:

- the cysts of protozoa with direct life cycles, such as *E. histolytica, G. lamblia, Balantidium coli* and the various gut commensal species (*Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Iodamoeba butschlii*);
- the oocysts of *Isospora belli*;
- the eggs and larvae of soil-transmitted nematodes (*Ascaris* spp., hookworm[1] and *Strongyloides stercoralis* (see Fig. 46.3));
- the eggs of trematodes (*Fasciolopsis buski* and *Heterophyes heterophyes*) and cestodes before their entry into the aquatic environment; and
- the eggs of cestodes before their ingestion by the intermediate hosts (*Taenia solium* and *Taenia saginata*).

Temperature and humidity also affect sporulation of oocysts, the rates of embryonation of nematode, cestode and trematode eggs and the development times for hookworm and *Strongyloides* larvae. Parasites that have a soil stage are affected by the particular physical properties of the soil, including its particle size and water-holding capacity, and by factors such as rainfall. Susceptibility to anaerobic conditions is important for some parasites when 'night soil' (human feces) is used raw or after composting as a fertilizer.

The aquatic environment is important in the life cycle of many parasites. In the trematodes, the cercariae of *Heterophyes* spp. must survive long enough to infect the fish or shrimp and the metacerariae of *F. buski* must survive long enough to infect the human or pig hosts. *Strongyloides* larvae live in the capillary water films in soil and on low vegetation.

Biologic environment

The biologic environment also affects the epidemiology of these gastrointestinal parasites. The distribution in nature of appropriate vectors, intermediate hosts and reservoir hosts can obviously affect the distribution of parasites. Examples of animal reservoirs are *F. buski* in dogs, pigs or rabbits; *B. coli* in pigs; *C. parvum* in domestic animals, particularly cattle; and *H. heterophyes* in fish-eating mammals. Secondary hosts include snails for *F. buski* and freshwater fish for *H. heterophyes*. An important biologic factor is the presence of dung beetles that take the parasitic ova or larvae underground to a more favorable physical environment.

Human factors

Population density and urbanization

The agricultural revolution in developing countries has produced large resident human populations with the potential for direct person-to-person spread of infection and greater environmental contamination by feces. In addition, animal husbandry has created other cycles for parasite transmission, for example *Cryptosporidium* spp. in calves. Rapid urbanization, especially in the tropics, is often associated with increased poverty, poorer housing and unsanitary conditions. The result is that people may be living in a more fecally polluted environment than in rural areas, encouraging such diseases as amebiasis and giardiasis. Epidemics, such as outbreaks of cryptosporidiosis, may occur when public water supplies become fecally contaminated.

Figure 46-1 Adult beef tapeworm (*Taenia saginata*) passed in a patient's feces.

Cyclospora cayetanensis is transmitted via contaminated produce and contaminated drinking water.[2] The soil-transmitted nematodes *Ascaris lumbricoides* and *Trichuris trichiura*[3] are often more common in towns and cities. Overcrowding favors direct transmission of *Hymenolepis nana* and *Enterobius vermicularis*, especially in children when levels of hygiene and sanitation are poor.[4]

Population movements

Population changes associated with mining, political unrest or industrialization may cause people to move into at-risk areas; travelers may also visit such areas.

Dams and irrigation

Development programs in the tropics frequently involve irrigation projects, where contaminated water supplies reach greater numbers of people and larger water-borne outbreaks may occur. Irrigation and poor drainage supplies favor the breeding of flies that may have a role in spreading fecal material.

Agriculture

Cattle raising may be complicated by bovine cysticercosis (*T. saginata*), which renders carcasses unsaleable, and calves may be a source of human infection. Pigs can allow *T. solium*, the pork tapeworm,

TABLE 46-1 -- Gastrointestinal parasites.

GASTROINTESTINAL PARASITES			
Intestinal protozoa	Amebae	*Entamoeba histolytica; Entamoeba dispar*	
		Commensals	*Entamoeba coli*
			Entamoeba hartmanni
			Endolimax nana
			Iodamoeba butschlii
		Blastocystis hominis	
	Flagellates	*Giardia lamblia*	
		Dientamoeba fragilis	
	Ciliate	*Balantidium coli*	
	Coccidia	*Cryptosporidium parvum*	
		Cyclospora cayetanensis	
		Isospora belli	
	Microsporidia	*Enterocytozoon bieneusi*	
		Encephalitozoon intestinalis (formerly *Septata intestinalis*)	
Intestinal helminths	Nematodes (round worms)	*Ascaris lumbricoides*	
		Enterobius vermicularis	
		Hookworms:	*Ancylostoma duodenale*
			Necator americanus
		Trichuris trichiura	
		Strongyloides stercoralis	
	Trematodes (flukes)	*Fasciolopsis buski*	
		Heterophyes heterophyes	
	Cestodes (tapeworms)	*Taenia solium*	
		Taenia saginata	
		Hymenolepis nana	
		Diphyllobothrium latum	

to be spread and *F. buski*, the intestinal fluke, to prosper. *Balantidium coli* is also acquired from close contact with pigs. Fish farms in which water plants such as water calthrop are grown transmit *F. buski*, especially if human or pig feces are used as fertilizer. Foods implicated in outbreaks of cyclosporiasis in the United States include fresh raspberries, mesclun lettuce and basil.[5] The use of untreated human night soil enables soil-transmitted helminths to enter the human food chain.

Domestic environment

Sanitation, water supplies and domestic customs in hygiene and food preparation are all very important. Children are at risk of parasitic infections because of poor hand washing after defecation, finger sucking and playing with soil. Local dietary behavior is critical for parasite transmission. Ingestion of certain fish, crustacea, molluscs and aquatic vegetation can lead to fluke infection. Tapeworms are contracted by ingestion of undercooked pork or beef (*Taenia* spp.) or certain fish (*Diphyllobothrium* spp.). People who have occupations involving sewage, water or soil contact are at increased risk of parasitic infection.

Host susceptibility

Host susceptibility is affected by many factors such as nutritional status, intercurrent disease, pregnancy, immunosuppressive drugs and malignancy. Previously mild or clinically inapparent infections can produce dangerous disease when host immunity falls, such as occurs in strongyloidiasis, in which the parasite is capable of multiplying by autoinfection within its host, and fatal amebiasis that may occur if corticosteroids are administered in error.

Some protective immunity is usually acquired by the host but its effectiveness is variable. The absence of symptomatic giardiasis in adults in places where the infection is common is good evidence for acquired immunity. Re-infection and superinfection, possibly by different gastrointestinal parasite strains, is certainly common in

TABLE 46-2 -- Anatomic location of gastrointestinal parasites.

ANATOMIC LOCATION OF GASTROINTESTINAL PARASITES		
Lumen only	Small bowel (normally)	*Ascaris lumbricoides*
	Large bowel	*Entamoeba histolytica/dispar*
		Balantidium coli
		Enterobius vermicularis
Mucosal attachment	Small bowel	*Giardia lamblia*
		Tapeworm
		Hookworm
		Fasciolopsis buski
		Heterophyes heterophyes
	Large bowel	*Trichuris trichiura*
Epithelial cell invasion	Small bowel	*Isospora belli*
		Cyclospora cayetanensis
		Cryptosporidium parvum
		Microsporidia
Mucosal invasion	Small bowel	*Strongyloides stercoralis*
	Large bowel	*Entamoeba histolytica*
		Balantidium coli

areas of endemic infection. Immunodeficiency associated with HIV infection is of paramount importance in some of the more recently recognized gastrointestinal parasitic diseases such as cryptosporidiosis and microsporidiosis (see Chapter 127).

PATHOGENESIS AND PATHOLOGY

Gastrointestinal parasites cause disease in a variety of ways. Most are present in the lumen of the gut or attached to the mucosa of the gut wall and are not capable of invasion. The coccidian parasites such as cryptosporidia can invade the epithelial cells of the small bowel. Others, such as *E. histolytica, S. stercoralis* and occasionally *B. coli*, do invade the mucosa (Table 46.2).

Gastrointestinal protozoa

Amebiasis

Amebic ulcers mostly develop in the cecum, appendix or adjacent ascending colon, although the sigmoidorectal region can be involved.[6] Amebic ulcers are formed on the mucosa. They are usually flask shaped with a small, raised opening and a larger area of mucosal destruction below. The mucosa between abscesses is normal but lesions can be confluent. Pathogenic amebae are able to resist complement-mediated lysis and they possess other virulence factors such as attachment lectins, cysteine proteases and other enzymes.[7] Amebae have tissue-lysing enzymes on their surfaces that can be released from lysosomes or after amebic rupture. In a study in Bangladeshi children, acquired immunity to amoebiasis was associated with the appearance of an intestinal IgA response to the parasite Gal/GalNAc lectin, the virulence factor required for attachment of *E. histolytica* trophozoites to human cells. An intestinal IgA response to this lectin was associated with a 70% reduction in infections in children over the 2-year study period. On the other hand, those children who developed anti-amebic IgG antibodies in the serum were more susceptible to amebiasis. Interestingly, serum anti-lectin IgG antibodies cluster in families, supporting the presence of an inherited component to susceptibility to amebic disease.[8] Based on work with *Entamoeba invadens*, a parasite of reptiles, Eichinger has suggested that interactions of amebae with intestinal mucin glycoproteins promote encystment of the parasite and thus prevent invasion. [9] This is in keeping with the finding that *E. histolytica* cyst passers are asymptomatic.[10]

Giardiasis

The histopathology of the upper small bowel varies from normal to subtotal villous atrophy in giardiasis. *Giardia* spp. seem unable to penetrate the mucosal wall in humans but are able to attach to the mucosa of the small bowel. In symptomatic cases there is increased mucus secretion and dehydration.[11] *Giardia lamblia* may undergo antigenic variation, thereby evading the human immune response.[12] Giardiasis is more common in the immunodeficiency syndromes, particularly in common variable hypogammaglobulinemia, although there is no particular increase in incidence among the HIV-infected population.

Balantidium coli

The trophozoite of *Balantidium coli* causes mucosal inflammation and ulceration, invading the distal ileal and colonic mucosa. Ulceration may be superficial or involve the full thickness of the bowel, leading to perforation.[13] Invasion may be enhanced by hyaluronidase produced by the parasite. Other products liberated by the parasite as well as host factors, such as the recruitment of mucosal inflammatory cells, may also be important.[14]

Cryptosporidiosis

Cryptosporidial infection in humans is most commonly due to *Cryptosporidium parvum*, but *C. felis, C. muris* and *C. meleagridis* have been identified in immunocompromised individuals. *Cryptosporidium parvum* has two genotypes: type 1 (human derived) and type 2 (animal and human derived) and it has been suggested that they may represent two distinct species. [15] *Cryptosporidium* infects the intracellular, extracytoplasmic area of host epithelial cells of the small bowel. The intracellular stage of *C. parvum* resides within a parasitophorous vacuole in the microvillus region of the host cell. Oocysts undergo sporogony while in the host cells. Approximately 20% of the oocysts do not form the usual environmentally resistant oocysts but are released as sporozoites that are capable of penetrating the microvillus regions of other cells within the intestine. This explains the ability of *C. parvum* to cause severe diarrhea in some patients, particularly in the immunocompromised. [16] Small bowel histology shows villous atrophy and crypt hyperplasia, usually with a mixed inflammatory cell infiltrate in the lamina propria. There is impaired absorption and enhanced intestinal secretion. There appears to be *Cryptosporidium*-associated apoptotic epithelial cell death. The precise molecular mechanism for cryptosporidial diarrhoea is not yet elucidated. There appears to be enterotoxin-like activity and it appears that attachment of *Cryptosporidium* to epithelial cells induces survival signals in those cells so that the organism is able to multiply, while at the same time causing alterations such as apoptosis in adjacent uninfected cells which impair absorption and secretion by those cells and result in clinical disease.[17]

Cyclosporiasis

The mechanism of diarrhea production has not been clearly established for *Cyclospora cayetanensis*. The organism is found within enterocytes. There is reduction in villus height with associated mucosal inflammation and increased numbers of intraepithelial lymphocytes, which suggests a direct effect on the intestinal mucosa.[18]

Isosporiasis

Mild to subtotal villous atrophy and crypt hyperplasia occurs in *I. belli* infection. Histologically, there may be infiltration of the lamina propria with large numbers of eosinophils, plasma cells, lymphocytes and polymorphs. Dilated lymphatics may be seen.[19]

Microsporidiosis

Intestinal microsporidia infect enterocytes in the small bowel and undergo sporogony, which leads to enterocyte degeneration,

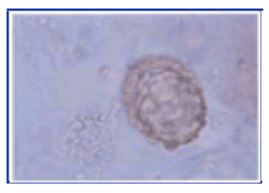

Figure 46-2 *Ascaris lumbricoides* ovum in feces. The ovum measures 50–70mm × 40–50mm and is elliptical. The rough albuminous coat gives it a mammillated appearance.

vacuolation and loss of the brush border. These cells are sloughed off and the spores are capable of infecting further enterocytes.[20] [21]

Gastrointestinal helminths

Nematode infections

Ascariasis (roundworm infection)

The embryonated eggs of *A. lumbricoides* are ingested and hatch in the stomach and duodenum, from where the larvae penetrate the intestinal wall (Fig. 46.2). They are carried to the lungs in the circulation and usually cause no symptoms unless there are a large number of larvae, in which case pneumonitis can ensue. The larvae then break out of the lung tissue and may cause some bronchial epithelial damage. Intense tissue reaction with infiltration of eosinophils, macrophages and epithelioid cells occurs.[22] Jejunal histology shows shortened villi, a decrease in the villus: crypt ratio and cellular infiltration of the lamina propria.[22]

Ancylostomiasis (hookworm infection)

Hookworm disease is caused by *Ancylostoma duodenale* and *Necator americanus*. Vesiculation and pustules can occur on the skin at the site of entry of the filariform larvae. Asthma and bronchitis occur during migration through the lungs, with small hemorrhages into the alveoli and infiltration of eosinophils and leukocytes. Adult hookworms attach firmly to the small bowel mucosa; *A. duodenale* does this by means of well-developed mouth parts and *N. americanus* by means of cutting plates. There tends to be chronic blood loss at the site where the worm attaches.

Trichuriasis (whip worm)

The egg of the nematode *T. trichiura* hatches in the small intestine and the larva penetrates the villi causing no pathologic reaction. It re-emerges after 1 week and migrates to the cecum and colorectum. When few worms are present there is little damage, but with heavy infections there is hemorrhage, mucopurulent stools and symptoms of dysentery, sometimes with rectal prolapse (the *Trichuris* dysentery syndrome).[23]

Strongyloidiasis

The life cycle of *S. stercoralis* is complex (Fig. 46.3). Human infection is acquired when filariform larvae in the soil penetrate the skin. This can cause petechial hemorrhages, congestion and edema at the site of entry. The larvae migrate into cutaneous blood vessels and are carried to the lungs, where they break out of the pulmonary capillaries and sequentially enter the alveoli, trachea, pharynx and then the mucosa of the duodenum and upper jejunum. There can be pathologic findings similar to those of bronchopneumonia with lobar consolidation. The females mature in the intestine, invade the tissues of the bowel wall and lay their eggs, which hatch and release first-stage (rhabditiform) larvae in the feces. In certain situations, the rhabditiform larvae mature in the intestine to the filariform stage, and these parasites bore into the wall of the duodenum and jejunum and initiate another cycle of infection, which eventually results in there being more adult worms in the small bowel. Filariform larvae that penetrate the bowel wall can spread throughout the lymphatic system to the mesenteric lymph glands and can enter the general circulation and hence the liver, lungs, kidneys and gallbladder. They can cause granulomas in the gastrointestinal tract and mesenteric glands. There are abscesses in the lungs and there may be granuloma in the liver. Migrating larvae may cause the patient to die from sepsis arising from the normal intestinal bacterial flora.

Trematode infections (flukes)

Fasciolopsis buski infection

The giant intestinal fluke *F. buski* is contracted by humans and pigs through eating metacercariae attached to water plants. The parasite excysts and attaches to the mucosa of the jejunal and duodenal wall causing mechanical injury and inflammation, which can lead to ulcers, bleeding or abscesses. There may be a mild anemia and low levels of serum vitamin B12, owing to the parasite's competition for the vitamin or impairing its absorption.

Heterophyes heterophyes infection

Heterophyes heterophyes is a trematode that infects humans and is found mainly in Asia. The pathology depends on the degree of infection acquired through ingestion of pickled or raw fish. The metacercariae excyst and attach to the walls of the small intestine. The adults may cause only a mild inflammatory reaction. Eggs may enter the circulation because the adults are attached deeply into the intestinal wall. Ectopic eggs can provoke granuloma formation, especially if they lodge in the heart or brain.[24]

Cestodes infections (tapeworms)

Taenia solium and *Taenia saginata*

The pork tapeworm (*T. solium*) and the beef tapeworm (*T. saginata*)[25] are acquired by the human host by ingestion of poorly cooked meat that contains the encysted larvae (cysticerci). The larva is digested in the stomach and the head of the tapeworm evaginates in the upper small intestine, attaches via the scolex to the intestinal mucosa and feeds by absorbing nutrients from the bowel. The scolex of *T. solium* has four suckers and a rostellum that contains a double row of hooks; *T. saginata* has the suckers only. Very little pathology is caused by these well-adapted adult worms, which may reach 7m and 25m in length respectively. The pathologically significant stage for humans is the cysticercus of *T. solium*. The eggs passed in feces are ingested and hatch when they are exposed to gastric juice. The oncospheres that are released penetrate the intestinal wall and are carried via the bloodstream and can potentially form a cysticercus in any organ. The cysticercus is an ovoid, milky-white bladder with the parasite head invaginated inside. The pathology is described in detail in Chapter 168 .

Dwarf tapeworm (*Hymenolepis nana*)

Once the ova of the dwarf tapeworm *H. nana* are ingested they encyst in the small intestinal villus mucosa. The adult worm reaches a length of 3–4cm and begins egg production. Heavy infections of more than 100 worms can cause some symptoms, and the competition

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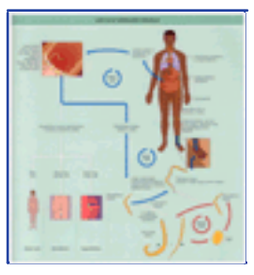

Figure 46-3 Life cycle of *Strongyloides stercoralis*.

for nutrients has been linked with growth retardation in children, although this may be more the result of insanitary conditions, poverty and malnutrition.

Fish tapeworm (*Diphyllobothrium latum*)

The fish tapeworm *D. latum* has a life cycle involving a first intermediate host of tiny aquatic invertebrates, a second intermediate host of fish and a definitive host that includes humans, other terrestrial mammals and marine mammals. The tapeworm attaches to the small intestinal mucosa by means of two longitudinal slit-like suckers (bothria). Infections are commonly multiple and may reach more than 100 individual worms, each measuring up to 10m in length. The pathology is minimal from the local effects. Tapeworm anemia has been described exclusively in Finland and has now all

but died out as a result of control measures. The anemia is caused by vitamin B12 deficiency, with worms competing for the limited dietary vitamin B12, and it is strongly associated with gastritis and achlorhydria; there is probably a genetic predisposition to this condition.

PREVENTION

The prevention and control of parasitic gastrointestinal disease can be achieved through improvement of living standards, personal hygiene and better sanitary conditions.[26] In immunocompromised patients, different recommendations need to be considered (see Chapter 123).

Public health hygiene measures

Disposal of human sewage and waste water is fundamental to the control of parasitic gastrointestinal infections. Fecal material contaminates agricultural food crops or water supplies if it is passed promiscuously in the fields or close to habitation or if it is deliberately used unprocessed on fields as fertilizer ('night soil'). Any of the gastrointestinal parasites can be transferred in this way. Following rain, fecal material is washed into rivers and pools. Giardia cysts are found in surface water in many parts of the world.

The provision of latrines can reduce these sources of environmental contamination but, unfortunately, unless they are well constructed and maintained, latrines can themselves become important foci of infection. The prevalence of *Ascaris* infection is often higher among urban latrine users than among rural non-users. The eggs of *A. lumbricoides* and also those of *T. trichiura* are very often resistant and can remain viable in the latrine environment for long periods. In addition, the moist soil around a latrine favors the survival of hookworm larvae[27] and the free-living cycle of *S. stercoralis*.

Domestic waste water (and the excreta that it accompanies) when used for irrigation puts crops and workers at risk. Sewage may enter lakes, rivers and ponds and the water then be used for drinking or irrigation. *Cryptosporidium* oocysts are particularly difficult to eliminate from water and require an efficient filtration system. Boiling water is the most reliable method of killing oocysts.

Composting is another reliable way of killing the infective forms of parasites. This takes 3–4 months. Cysts, eggs and larvae are quite rapidly killed at temperatures of 131°F (55°C) and die within a few days at 113°F (45°C). The compost heap needs turning and good maintenance or the periphery may become an intense transmission focus. Chemical treatment of excreta has been used, for example 12–24 hours in ammonium sulfate, but proper control is important to maintain efficacy and avoid toxicity to fish and plant life. Solid waste from sewage treatment plants is used as agricultural fertilizer and can contain viable parasites if inadequately processed. Sewage treatment removes or destroys parasites by sedimentation and the creation of completely anaerobic conditions. The eggs of *Taenia* spp. and *A. lumbricoides* are notoriously resistant and they sometimes survive in the solid wastes taken from sedimentation tanks or ponds.

Personal hygiene measures

Most gastrointestinal diseases could be prevented if it were easy to modify human behavior. In practice this is often very difficult, but health education can be very effective and it can take many forms. Schools can be targeted or the local press used for a health awareness campaign. The fecal-oral route of transmission is very important, especially for intestinal protozoa. For example, infected food handlers are disseminators of *G. lamblia* and *E. histolytica* cysts. There is high prevalence of infection in institutions for children or the mentally subnormal, where personal hygiene is poor. Those intestinal helminths whose life cycles can lead to fully embryonated eggs being released or formed very soon after entry into the environment (*H. nana* and *E. vermicularis*) are also spread in this way. Microsporidia are probably spread by the fecal-oral or urinary-oral routes. Simple hand-washing before preparing and eating food prevents transmission of these infections. The soil-to-skin route of infection can be inhibited by wearing shoes.[28] Infective forms of hookworm and *Strongyloides* larvae will be stopped from entering the skin. Persons dealing with composted human feces must use boots and gloves.

Good food hygiene is essential. Prevention of most of the parasites transferred by the fecal-oral route can be achieved by washing all salad vegetables and fruit before consumption. Kitchen utensils and hands should be washed frequently. Avoidance of wild-grown watercress and other water plants prevents *F. buski* infections. Proper cooking of meat and fish removes the risk of flesh-derived parasites such as *H. heterophyes* and tapeworms. Problems arise when cultural preferences dictate consuming these products raw, undercooked, salted, dried or pickled. The inspection of meat can detect the presence of cysticerci in the carcasses and allow infected meat to be condemned. Stopping the consumption of fish that is raw, pickled or salted is often impossible if it is part of a deep cultural tradition. Deep freezing of meat and fish at temperatures colder than -4°F (-20°C) for 24–48 hours kills all these parasites.

Prevention by chemotherapy

The treatment of these gastrointestinal parasites with appropriate chemotherapy is helpful in the prevention of further cases. This is particularly so in developed parts of the world. In areas where parasitic disease is endemic, generally only symptomatic patients are treated. Patients that are asymptomatic, those in whom the diagnosis is incidental and those who do not have heavy infections are usually not treated, as re-infection is probably inevitable. Mass chemotherapy has been used in amebiasis, soil-transmitted nematodes (*A. lumbricoides*, [29] hookworm, *T. trichiura*) and tapeworms (*T. saginata, T. solium*, *D. latum*).[30] Success depends upon several factors:

- the chemotherapeutic agent used should have a broad spectrum of activity and be given annually as a single oral dose;
- the drugs should be cheap to purchase and administer; and
- the drugs should have few side effects.

In the past, mass chemotherapy was used in an attempt to eradicate amebiasis but, because treatment required a prolonged course of luminal amebicides, compliance was poor and reinfection in endemic areas was high. *Fasciolopsis buski* infection in Indonesia has been treated by community-based praziquantel treatment but rapid reinfection followed. Selective or targeted chemotherapy may be of more use. *Enterobius vermicularis* infection in one child requires treatment not only of that child but also all family contacts together with education in personal hygiene.

Vaccines

As yet no vaccines have been used in humans for the control of gastrointestinal parasitic diseases.[31]

Role of prophylaxis

There is no evidence for prophylactic use of chemotherapy being helpful in prevention of these gastrointestinal parasitic diseases.

Advice for travelers on preventive measures is discussed in Chapter 143 .

CLINICAL FEATURES

The symptoms produced by gastrointestinal parasites are diverse. The most common presentation is of poorly localized abdominal pain; less commonly there is nonspecific diarrhea (Table 46.3). The severity varies from asymptomatic carriage to life-threatening

TABLE 46-3 -- Gastrointestinal parasites associated with diarrhea.

GASTROINTESTINAL PARASITES ASSOCIATED WITH DIARRHEA	
Diarrhea and fever	*Entamoeba histolytica*
	Cryptosporidium parvum
	Isospora belli
	Cyclospora cayetanensis (occasionally)
Diarrhea with blood in stool	*Entamoeba histolytica*
	Trichuris trichiura
	Strongyloides stercoralis (rarely)
Chronic diarrhea	*Entamoeba histolytica*
	Giardia lamblia
	Cryptosporidium parvum
	Isospora belli
	Cyclospora cayetanensis
	Microsporidia
	Trichuris trichiura
	Hookworm (*Necator americanus* and *Ancylostoma duodenale*)
	Strongyloides stercoralis

gastrointestinal disease, as occurs in amebiasis and cryptosporidiosis. It is important to emphasize that the identification of an infection by stool microscopy does not necessarily imply that it is the cause of a patient's symptoms. This is particularly so in the case of light helminth infections, which are often completely symptomless. Other pathologies should be excluded before attributing the patient's symptoms to the gut parasite. Some of these infections present with extraintestinal manifestations, such as iron-deficiency anemia, wasting or (in the case of hookworm infection) growth retardation.

Gastrointestinal disease caused by protozoa

*Amebiasis (*Entamoeba histolytica*)*

It is now recognized that there are two species of amebae that were formerly termed pathogenic and nonpathogenic *E. histolytica*. [32] Methods used to separate these include biochemical, immunologic and genetic data. These are now called *Entamoeba histolytica* and *Entamoeba dispar*. Only *E. histolytica* is capable of causing disease. Asymptomatic cyst carriers may excrete cysts for a variable length of time, usually only a few weeks. Confirmation that the parasite previously described as *Entamoeba histolytica* is in reality two species, *Entamoeba dispar* which is nonpathogenic and *Entamoeba histolytica* which is a pathogen, meant that the natural history of amebiasis needed reassessment. [10] Haque *et al* studied 300 preschool children in Dhaka, Bangladesh.[8] Over a 2-year period, new *Entamoeba histolytica* infections were found in half the children. Of those who were asymptomatic cyst passers, one in 10 subsequently developed diarrhea within 2 months of the start of infection; 10% of the children had diarrhea in association with *E. histolytica* and 4% were found to have amebic colitis.

Amebic intestinal disease

The incubation period of invasive amebiasis varies from a few days to 1–4 months. There is a good correlation with the presence of *E. histolytica* trophozoites containing ingested red blood cells. Patients may be asymptomatic or present with colicky abdominal pain, frequent bowel movements and tenesmus. Amebic dysentery is characterized by blood-stained stools with mucus occurring up to 10 times a day. The duration of the dysentery can be very variable and may last for only a few days or for several months with concomitant weight loss and debility. The symptoms may be confused with inflammatory bowel disease. In acute cases the clinical picture may mimic appendicitis, cholecystitis, intestinal obstruction or diverticulitis.

Amebic extraintestinal disease

Amebic dysentery progresses to invasive amebic disease in between 2% and 8% of patients. Symptoms can be gradual in onset, with right upper quadrant pain and fever (see Chapter 145). Weakness, weight loss, dry cough and sweating are less common. Tender hepatomegaly is often seen with liver function tests that are normal or only slightly abnormal. Jaundice is very unusual. The site most commonly involved is the upper right lobe of the liver, and abscesses are mostly solitary. A raised right diaphragm may be found on chest radiograph. The abscess is visualized by ultrasound, CT or MRI. Hematogenous spread to the brain (see Chapter 158), lung (see Chapter 165), pericardium and other sites is possible. Serologic tests are valuable in cases of suspected amebic abscess. The indirect fluorescent antibody test is positive in over 95% of cases after 14 days, but it should be confirmed by the cellulose acetate precipitin test.

*Giardiasis (*Giardia lamblia*)*

The clinical spectrum of giardiasis ranges from asymptomatic infection, through acute gastrointestinal infection to severe chronic diarrhea[33] with intestinal malabsorption.[34] The average incubation period is 9 days and the acute infection is self-limiting.[35] Common symptoms include nausea, diarrhea, flatulence and upper abdominal cramps with distention and nausea. Weight loss is common and there can be signs of malabsorption (steatorrhea, disaccharidase deficiency and vitamin B12 deficiency).

*Blastocystis infection (*Blastocystis hominis*)*

There is controversy surrounding *B. hominis*; it is unclear whether it causes gastrointestinal disease or not. When large numbers are found in stool in the absence of other parasites, bacteria or viruses it may be the cause of diarrhea, cramps, nausea, fever, vomiting and abdominal pain. Data from Canada indicate that, although it is commonly seen in stools, it is not pathogenic. [36] It is possible that a small subset of *B. hominis* organisms have virulence factors that are missing in most.[37]

Dientamoeba fragilis infection

Like *Blastocystis*, the pathogenicity of *Dientamoeba fragilis* is uncertain. It has been associated with a wide range of symptoms. In children, symptoms may include intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain and unexplained eosinophilia.[38]

*Cryptosporidiosis (*Cryptosporidium parvum*)*

The incubation period of *Cryptosporidium parvum* infection averages 3–6 days. Symptoms include a flu-like illness, diarrhea, malaise, abdominal pain, anorexia, nausea, flatulence, malabsorption, vomiting, mild fever and weight loss. In the 1993 Milwaukee water-borne outbreak of cryptosporidiosis, which affected around 403,000 people, the mean duration of illness was 12 days and the median maximum number of stools per day was 12.[17] Oocyst excretion generally occurs for 3–30 days (average 12 days) and occurs at the same time as the symptoms. Generally the symptoms are self-limiting, and prolonged disease in uncommon.[39] In immunocompromised patients the situation is different and there can be intractable, profuse, life-threatening diarrhea (see Chapter 127). [40] [41]

*Cyclosporiasis (*Cyclospora cayetanensis*)*

There is epidemiological evidence that *Cyclospora cayetanensis* is the cause of persistent diarrhea in immunocompetent patients as well as

in immunocompromised patients. Initially, the clinical findings do not distinguish cyclosporal diarrhea from other causes of diarrhea. Other common symptoms are abdominal pain, nausea, vomiting and anorexia. Cyclospora infection can last for 1–8 weeks.[18]

Isosporiasis (Isospora belli)

The predominant clinical symptom is diarrhea, which may be intermittent and last for months or even years. Stools are watery soft, foamy and offensive, which may suggest malabsorption. There is associated weight loss, abdominal pain and fever.[42] [43] In HIV-infected patients, chronic infection occurs (see Chapter 127).

Microsporidiosis

Microsporidia may cause acute self-limiting diarrhea in immunocompetent persons,[20] in patients who have immunodeficiency other than AIDS and in the elderly[21] . In HIV infection (see Chapter 127), chronic diarrhea and wasting are common.[44] *Enterocytozoon bieneusi* is one of the most important intestinal pathogens in severely immunodeficient HIV-infected patients; it is present in 7–50% of those who have otherwise unexplained chronic diarrhea. There are also increasing reports of intestinal microsporidial infections in immunocompetent people.[45] *Encephalitozoon intestinalis* (formerly *Septata intestinalis*) is a less commonly recognized cause of chronic diarrhea. *Encephalitozoon cuniculi* intestinal infection has been reported. These are covered in Chapter 127 and Chapter 243 .

Balantidiasis (Balantidium coli)

Balantidium coli, the largest and least common of the human protozoan pathogens, is capable of causing an infection resembling amebic colitis. It is particularly prevalent among people living in close association with pigs in South America, Iran, Papua New Guinea and the Philippines. Up to 80% of persons carrying the organism are asymptomatic carriers.[14] Acute diarrhea with blood and mucus begins abruptly and is associated with nausea, abdominal discomfort and marked weight loss. There can be inflammatory changes and ulceration in the proctosigmoid region but the rectum is usually spared. Peritonitis and colonic perforation can progress rapidly to death. A chronic infection occurs with intermittent diarrhea and infrequent bloody stools.

Helminthic gastrointestinal disease

The World Bank regards intestinal helminth infections as the main cause of disease burden in children from 5 to 14 years old in developing countries.

Ascariasis (Ascaris lumbricoides)

Most cases of ascariasis are asymptomic; symptomatic infections are more common in children than adults. When large numbers of larvae migrate to the lungs in a short time period, *Ascaris* pneumonitis can result. This is the clinical picture of Löffler's syndrome, which is characterized by dyspnea, dry cough, wheezing or coarse rales, fever up to 104°F (40°C), transient eosinophilia and a chest radiograph that is suggestive of viral pneumonia and that resolves within a couple of weeks. In addition, eosinophils, Charcot-Leyden crystals and larvae may rarely be found in the sputum. Symptoms of asthma and urticaria may continue during the intestinal phase of ascariasis. The adult worms occasionally migrate from the small bowel and cause biliary or hepatic ascariasis. Rarely, adult worms migrate into the biliary tree with secondary sepsis and abscess formation. Pancreatitis may result from migrating ascarids that obstruct the pancreatic duct. In people who have large numbers of adult worms intestinal obstruction can occur owing to the sheer bulk of worm bodies. Adult worms migrate more in the presence of a stimulus such as a fever of over 102°F (38.9°C) or the use of a general anesthetic, and they may block the bile duct or pancreatic duct or enter the liver or peritoneal cavity. In children, nutritional deficiencies such as reduced appetite, kwashiorkor (protein-energy malnutrition), vitamin A deficiency and growth deficit are related to the burden of the adult worms.[46]

Enterobiasis (Enterobius vermicularis)

Infection with *Enterobius vermicularis* (threadworm or pinworm) causes few or no symptoms in the vast majority of people. The predominant symptom is nocturnal pruritus ani, caused by migration of the female worms from the anus to the perianal skin in the process of laying their eggs. Scratching may be intense and secondary infection may ensue. Pruritus vulvi caused by pinworms entering the vulva is occasionally seen. In children, insomnia, loss of appetite, loss of weight, emotional instability, enuresis and irritability may also be found. [46]

Ancylostomiasis (hookworm infection, Ancylostoma duodenale, Necator americanus)

Hookworm causes ground itch, a moderate-to-severe pruritus of the skin, usually of the feet, as the hookworm larvae penetrate. Secondary infection can occur if the vesicular lesions are excoriated by scratching. A pneumonitis, caused by alveolar migration of larvae, is less common, less severe and causes less sensitization than that seen with *Ascaris* or *Strongyloides* infection. Symptoms of the intestinal phase are fatigue, nausea, vomiting, abdominal pain, diarrhea with occult bleeding, and weakness. Heavy worm burdens may have serious sequelae in young children.[47] This is particularly problematic with *A. duodenale* infection. Eosinophilia is usually present. According to the degree of worm burden, chronic infection leads to an iron deficiency anemia[27] and hypoproteinemia with pallor, edema of the face and feet, listlessness, koilonychia, cardiomegaly, heart failure and rarely mental retardation. Iron deficiency in children is associated with impaired academic performance at school and in adults with weakness and fatigue, leading to reduced productivity.

Trichuriasis (Trichuris trichiura)

Light infections with this common, ubiquitous infection rarely cause symptoms. In heavy infections (more than 10,000 eggs per gram of feces), epigastric pain, vomiting, distention, flatulence, anorexia and weight loss may occur. Rarely the *Trichuris* dysentery syndrome may occur, with blood and mucus in the stools and, in heavy infections, prolapse of the rectum. Reduced childhood growth rates, reduced food intake, iron deficiency and gastrointestinal protein loss are seen in heavy infections. The diagnosis is made when numerous worms are seen on the rectal mucosa. A 'honeycomb' effect of the small intestine has appearances similar to Crohn's disease. There may be deformity of the proximal colon and also the ileum or appendix.

Strongyloidiasis (Strongyloides stercoralis)

Strongyloides stercoralis largely causes asymptomatic infection of the small intestine, which can last for 30 years or longer.[48] The prepatent period from infection through the skin to the appearance of rhabditiform larvae in the stools is 1 month or more. Symptoms only develop with high intestinal worm loads, which can be the result of several factors. In people debilitated by concurrent disease or malnutrition, there may be massive invasion of the tissues by *S. stercoralis*. Treatment with immunosuppressive drugs in a patient harboring *S. stercoralis* can also lead to the same effect.[49] Infection with human T-cell leukemia virus 1 is important in predisposing to massive infection by *S. stercoralis*. Infection with HIV is not a common cause of the *Strongyloides* hyperinfection syndrome (see Chapter 127). Symptoms include watery mucous diarrhea, with the severity depending on the intensity of the infection. Sometimes diarrhea alternates with constipation. Malabsorption of fat and vitamin B12 with a chronic diarrhea and protein-losing enteropathy has also been described and is rapidly reversed by treatment.

There are two types of skin rash. The first is larva currens, which occurs on the trunk or near the anus and is a linear eruption in which the larvae migrate under the skin causing an itchy, non-indurated wheal with a red flare that moves rapidly and disappears in a few hours. This contrasts to the indurated and persistent track of nonhuman hookworm larvae (cutaneous larva migrans). The second type of rash is urticaria.

Features of the strongyloidiasis hyperinfestation syndrome (see Fig. 46.3) include severe diarrhea, malabsorption, edema, hepatomegaly and paralytic ileus. Gram-negative sepsis is a recognized complication. In very severe cases encephalopathy and even secondary pyogenic meningitis have been described.

Fasciolopsis buski *infection*

Fasciolopsis buski is confined to Asian countries, particularly Thailand. Symptoms are more frequent in children than adults owing to their greater exposure to water plants while at play. Like many other intestinal parasites, the majority of infected people have very minor symptoms or none at all. In heavier infections, symptoms can include diarrhea, abdominal pain, vomiting, flatulence, poor appetite, eosinophilia and fever. In very severe cases there may be ascites, edema of the face, abdomen and legs, anemia, anorexia, weakness and even intestinal obstruction. Intestinal ulceration may cause malabsorption and lead to malnutrition and wasting.[24]

Heterophyes heterophyes *infection*

Heterophyes heterophyes causes few symptoms unless the infection is heavy, which is dependent on the quantities of pickled or uncooked fish eaten in endemic areas (mostly the Middle and Far East). The adult worms produce abdominal pain, diarrhea with mucus and ulceration of the intestinal wall.

*Beef and pork tapeworm infection (*Taenia saginata *and* Taenia solium*)*

The clinical features of *T. saginata*, the beef tapeworm, and *T. solium*, the pork tapeworm, are similar. The adult worms in the gastrointestinal phase of both organisms usually cause no symptoms, but carriers can sometimes feel a proglottid emerging from the anus; the motile proglottid may be upsettingly obvious in the feces. Other associated symptoms, such as abdominal pain and distension, nausea and anorexia have been attributed to the tapeworm. There is occasionally a mild eosinophilia but there is no anemia, even in long-term infection. Cysticercosis complicates infection with *T. solium* only. Ingestion of *T. solium* eggs leads to the dissemination of oncospheres in the bloodstream; these can become lodged anywhere in the subcutaneous and intramuscular tissues,[50] where they become cysticerci; symptoms depend on the particular body site involved. The clinical findings of neurocysticercosis are discussed in detail in Chapter 168 .

*Dwarf tapeworm infection (*Hymenolepis nana*)*

As with other tapeworms, there are usually few symptoms in *H. nana* infection. Symptoms that may occur include abdominal pain, anorexia, irritability and headache. Eosinophilia is common. Symptoms are more common in heavy infections and may cause growth retardation in children.

*Fish tapeworm infection (*Diphyllobothrium latum*)*

There are few if any symptoms from infection with *D. latum*. Symptoms including diarrhea, headache and non-specific malaise all appear to be somewhat more common than in uninfected people. Tapeworm-associated anemia is probably related to vitamin B12 deficiency caused by competition for the vitamin between the tapeworm and a genetically predisposed host; however, this is now exceedingly rare.

DIAGNOSIS

Microscopic examination of the stool is fundamental to the diagnosis of all the gastrointestinal infections (see Chapter 165). A minimum of three stool specimens, examined by trained personnel using a concentration and a permanent stain technique, should be used.

Amebiasis is often suspected on clinical grounds but confirmation is always required by demonstrating cysts and trophozoites in the stools or trophozoites from the bowel mucosa. Fresh stools examined within 20 minutes for the presence of trophozoites containing ingested red blood cells enables *E. histolytica* to be distinguished from the nonpathogenic *E. dispar*. *Entamoeba hartmanni, Entamoeba coli, Iodamoeba butschlii* and *Endolimax nana* are nonpathogenic amebae, the cysts of which can be distinguished by their size and morphology (see Chapter 164). Material aspirated or scraped from mucosal surfaces at sigmoidoscopy needs microscopic examination. Culture of amebae is possible and allows zymodeme pattern analysis (a reference standard to diagnose and distinguish between *E. histolytica* and *E. dispar*). The polymerase chain reaction (PCR) can also distinguish *E. histolytica* from *E. dispar*. Monoclonal antibodies and DNA probes for this purpose are available in research centers. A recent rapid stool antigen enzyme-linked immunosorbent assay (ELISA) kit, based on antilectin antibodies, is 80% sensitive and 99% specific in diagnosing *Entamoeba* infection; another commercial test is 95% sensitive and 93% specific in distinguishing between *E. histolytica* and *E. dispar* when compared with culture and zymodeme analysis.[51] If this becomes widely accepted it may be useful in the management of asymptomatic carriers, because it would allow discrimination between pathogenic and nonpathogenic strains and avoid unnecessary treatment. Serologic diagnosis of invasive amebiasis is discussed below.

Giardial cysts and sometimes trophozoites are seen in fecal specimens. Multiple fecal specimens are required. A duodenal aspirate, biopsy or string test may sometimes be positive in the presence of negative stool microscopy. Giardial antigens can be detected in feces by a commercially available ELISA with reported sensitivity and specificity of 87–100% compared with microscopy; research laboratories can offer DNA probes or PCR diagnosis. An indirect immunofluorescence test using a cyst-specific anti-*Giardia lamblia* monoclonal antibody has been reported to detect twice the number of positive stool specimens than light microscopy.[52] This may allow more accurate diagnosis from fewer stool samples and obviate the need for biopsy or endoscopy.

Diagnosis of *Cryptosporidium, Cyclospora* and *Isospora* infections relies on identification of the oocysts in feces or on intestinal biopsy. For cryptosporidia, three staining methods are used: auramine, modified Ziehl-Neelsen and immunofluoresence using monoclonal antibodies to the oocysts.[53] Cryptosporidial antigen detection in feces and PCR techniques are research tools at present. Serologic tests are of limited diagnostic use. *Cyclospora* and *Isospora* spp. can be seen by light microscopy and identified by transmission electron microscopy *Isospora* oocysts are stained by modified Ziehl-Neelsen and they fluoresce with phenol auramine stain under ultraviolet light.

Microsporidia are so small that they are hard to detect in stool, but a modified trichrome stain or fluorescent chromotrope stain makes this possible. Histologists are able to visualize the spores in small bowel sections with Giemsa and other stains, but electron microscopy is needed for species identification. The parasitology is covered in detail in Chapter 243 .

The diagnosis of the gastrointestinal helminths depends on the finding and identification of ova, proglottids, larvae or worms in the feces on light microscopy (Fig. 46.4). Eggs are never uniformly distributed,

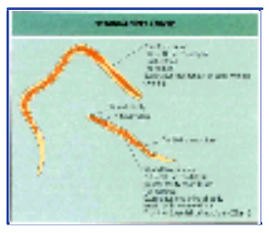

Figure 46-4 *Strongyloides* larvae.

TABLE 46-4 -- Treatment of gastrointestinal protozoal infection.

TREATMENT OF GASTROINTESTINAL PROTOZOAL INFECTION		
Condition	**Drug**	**Dosage**
Amebiasis		
Asymptomatic carrier of intestinal cysts		
1st choice	Diloxanide furoate	500mg q8h for 10 days
2nd choice	Paromomycin (aminosidine)	500mg q8h for 10 days

Intestinal infection (amebic dysentery or ameboma)	Metronidazole	750–800mg q8h for 5 days
	or	
	Tinidazole followed by	2g daily for 2–3 days
	Diloxanide furoate	500mg q8h for 10 days
	or	
	Paromomycin	500mg q8h for 10 days
Amebic liver abscess	Metronidazole	400–500mg q8h for 5–10 days
	or	
	Tinidazole followed by	2g daily for 3–5 days
	Diloxanide furoate	500mg q8h for 10 days
Giardiasis		
1st choice	Tinidazole	2g single dose
	or	
	Metronidazole	400–500mg q8h for 3 days
2nd choice	Albendazole	400mg daily for 5 days
3rd choice	Mepacrine	100mg q8h for 5–7 days
***Balantidium coli* infection**		
1st choice	Tetracycline	500mg q6h for 10 days
Alternatives	Ampicillin, metronidazole or paromomycin	
***Cyclospora cayetanensis* infection**	Trimethoprim-sulfamethoxazole (co-trimoxazole)	960mg q12h for 7 days
***Isospora belli* infection**		
1st choice	Trimethoprim-sulfamethoxazole (co-trimoxazole)	960mg [160mg (TMP)/800mg (SMX)] q12h for 7–10 days (q6h in immunosuppressed patients)
2nd choice (if intolerant to sulfonamides)	Furazolidone	100mg q6h for 10 days
All treatments are adult dosage and given orally unless stated otherwise.		

so a fecal sample should be mixed well before examination. The deposit made by a concentration method is usually used and examined by light microscopy at magnifications of 100 and 400. Chapter 165 discusses methods used in detail. The parasites are identified by appearance and size. Culture can be undertaken for *S. stercoralis*.

Serologic tests for the intestinal protozoa and helminths are helpful only in the diagnosis of amebiasis, strongyloidiasis, invasive giardiasis and cysticercosis (but not intestinal tapeworm infection). The amebic immunofluorescent antibody test is positive in 95% of cases of amebic abscess by the end of the first 14 days of illness. However, there are some false-positive results in nonamebic liver disease, so a cellulose acetate precipitin test is done to confirm the diagnosis. Anticysticercal antibodies have a useful role in the diagnosis of neurocysticercosis (see Chapter 168).

An ELISA for *Strongyloides* is useful in screening patients who have suggestive symptoms or eosinophilia. Cross-reactions occur occasionally with ascariasis and filarial infection. The filarial ELISA cross-reacts in some cases of strongyloidiasis. Serum antigiardial antibody detection is not clinically useful in highly endemic areas because people may be seropositive from past infection. The giardial immunofluorescent antibody test gives good titers if the disease has caused mucosal damage, and it is helpful in the investigation of parasite-associated malabsorption.

MANAGEMENT

Gastrointestinal protozoa

The management of gastrointestinal protozoa is summarized in Table 46.4 . Treatment regimens are discussed in detail in Chapter 209 .

Entamoeba histolytica (see Chapter 164)

Treatment of *E. histolytica* infection is divided into two types. Luminal amebicides, such as diloxanide furoate, act on organisms in the intestinal lumen and are not effective against organisms in tissue. Tissue amebicides, such as metronidazole and tinidazole are effective in treating invasive amebiasis but less effective in the treatment of organisms in the bowel lumen. There is some controversy about treating asymptomatic patients. Ideally, any amebic cysts should be tested to identify whether it is *E. histolytica* (in which case the infection should be treated to avoid the risk of developing invasive disease and to prevent secondary spread) or *E. dispar* (which does not require any treatment). However, until newer ELISA or monoclonal antibody tests become widely available it will continue to be usual to treat asymptomatic patients in nonendemic areas with diloxanide furoate 500mg orally q8h for 10 days (see Table 46.4). When asymptomatic cyst carriage persists after treatment for amebic dysentery or liver abscess, further treatment with a luminal amebicide is mandatory, otherwise relapse is frequent. The treatment of asymptomatic cyst carriers in endemic areas is of questionable value because of the high rate of re-infection. A second-choice intraluminal amebicide is paromomycin 500mg orally q8h for 10 days.[54] New broad-spectrum antiparasitic agents are being introduced as infection with multiple parasites is a common occurrence in the tropics. Nitazoxanide, a compound structurally similar to nitrobenzamide, is reported to act on a wide range of intestinal parasites, including some cestodes, nematodes and protozoa. In a study in Mexican children, Davila-Gutierrez reported a higher eradication rate for treatment of *E. histolytica/ dispar* with nitazoxanide 100mg twice daily for 3 days than with quinfamide 100mg as a single dose.[55] Further work is needed to determine whether or not nitazoxanide will become a first-line treatment for amebic cyst passage.

Proven amebic dysentery should always be treated. Drugs of choice are metronidazole (750–800mg orally q8h for 5 days) or tinidazole (2g daily for 2–3 days) followed by diloxanide furoate (see Table 46.4). Amebic liver abscess is treated with metronidazole, 400–500mg orally q8h, followed by diloxanide furoate as above. Tinidazole (2g daily orally for 3–5 days) is an alternative; chloroquine (150mg base q6h for 2 days then 150mg base q12h for 19 days) can also be used.

Giardia lamblia (see Chapter 242)

Treatment is often unnecessary because most healthy, immunocompetent patients have a self-limiting disease and recover by their own natural host defense mechanisms. Treatment of symptomatic patients reduces the duration and severity of symptoms. The treatment of asymptomatic cyst carriers is controversial in endemic areas. Generally, in a nonendemic area, asymptomatic *Giardia* cyst carriers are treated. The 5-nitroimidazole derivatives metronidazole or tinidazole are the treatment of choice and can be used in short courses.[35] Albendazole, 400mg daily for 5 days, has been shown to have useful antigiardial activity. Mepacrine, an acridine dye, has a similar efficacy but is generally less well tolerated with an incidence of 1.5% of acute psychosis. Furazolidone, a nitrofuran, has lower efficacy but is well tolerated. *In vitro* and *in vivo* resistance of *G. lamblia* has been demonstrated, although rarely, to conventional therapy, particularly to the 5-nitroimidazoles such as metronidazole and tinidazole.[56]

Blastocystis hominis

If *B. hominis* is present in the stool, the physician must not stop looking for another cause of diarrhea. Whether any treatment is required is controversial. Metronidazole

seems to be the most appropriate drug.

Dientamoeba fragilis

In adults infected with *D. fragilis*, improvement can be seen with tetracycline; in children, metronidazole is appropriate.

Balantidium coli

Tetracycline, 500mg four times a day for 10 days, is effective against *B. coli*. Doxycycline 100mg daily for 10–14 days is an alternative. Other drugs to which *B. coli* is sensitive are ampicillin, metronidazole and paromomycin. Surgery may be required for fulminant disease with perforation or abscess formation.

Cryptosporidium parvum (see Chapter 127)

Cryptosporidium parvum infection is self-limiting in those who have normal immunity. It presents a severe problem when it occurs in patients who have AIDS.[40] Where available, treatment is with HAART (highly active antiretroviral therapy) with or without antiparasitic agents. No currently available drug reliably eradicates cryptosporidial infection, but suppression of infection is possible. Agents currently in use are paromomycin, azithromycin and nitazoxanide.

Cyclospora cayetanensis

Many cases of *C. cayetanensis* infection are self-limiting. When treatment is felt to be necessary trimethoprim-sulfamethoxazole (co-trimoxazole) 960mg (160mg TMP/800mg SMX) q12h for 7 days has been found to be effective eradicating the oocysts from 94% of 16 patients in 7 days compared with 12% of 17 patients who received placebo.[57] Relapse is common in the immunocompromised but responds to a second course of treatment. Given the propensity of sulfonamide-containing drugs to cause side-effects in HIV-infected individuals, an alternative agent to co-trimoxazole is required. Verdier *et al* compared the activity of trimethoprim-sulfamethoxazole (TMP-SMX) 160/800mg orally twice daily for 1 week with ciprofloxacin 500mg twice daily for 1 week in HIV-positive patients infected with *Cyclospora cayetanensis*. [58] Nine received TMP-SMX and 11 received ciprofloxacin. Diarrhea ceased in all patients receiving TMP-SMX and in 10 of 11 patients receiving ciprofloxacin. At 7 days all those who received TMP-SMX had negative stool examinations, four of 11 treated with ciprofloxacin had persisting oocysts, one of whom still had diarrhea. All four responded to open treatment with TMP-SMX. Patients who had shown a complete response at 7 days were given maintenance therapy with TMP-SMX 160/800 three times weekly or ciprofloxacin 500mg three times weekly for 10 weeks. No recurrences were observed in the TMP-SMX group but one of seven patients who received ciprofloxacin experienced a recurrence after 4 weeks. Whilst ciprofloxacin is not as effective as TMP-SMX against *Cyclospora*, it provides an acceptable alternative for individuals who cannot tolerate a sulfonamide.

Isospora belli

Treatment of *I. belli* infection may be necessary in the immunocompromised, when oral trimethoprim-sulfamethoxazole 960mg (160mg TMP/800mg SMX) q6h daily for 7–10 days eliminates the parasite in most cases; relapse is common but retreatment is usually effective. Prophylactic trimethoprim-sulfamethoxazole may then be necessary. Pyrimethamine-sulfonamide combinations have also been proved to be effective.[59] If the patient is intolerant to sulfonamides, furazolidone 100mg four times daily for 10 days is an alternative. The macrolide antibiotic roxithromycin (2.5mg/kg every 12 hours) was reported to be successful in a single patient with AIDS and *I. belli* infection who had not responded to co-trimoxazole or pyrimethamine therapy. The 5-nitrothiazole derivative nitazoxanide is also reported to

TABLE 46-5 -- Treatment of gastrointestinal helminthic infection.

TREATMENT OF GASTROINTESTINAL HELMINTHIC INFECTION		
Condition	**Drug**	**Dosage**
Nematodes		
Round worms		
Ascaris lumbricoides	Albendazole	400mg, single dose
	Mebendazole	100mg q12h for 3 days
	Levamisole	150mg, single dose
	Piperazine hydrate	4.5g, single dose
Enterobius vermicularis	Mebendazole	100mg, single dose
	Piperazine phosphate	4g, single dose
	Pyrantel pamoate	10mg/kg, single dose
Hookworms		
Ancylostoma duodenale	Mebendazole	100mg q12h for 3 days
Necator americanus	Albendazole	200mg q24h for 3 days
	Levamisole	150mg, single dose (less effective against *N. americanus*)
	Pyrantel pamoate	10mg/kg, single dose
Trichuris trichiura	Mebendazole	100mg q12h for 3 days or 600mg, single dose
	Albendazole	400mg, single dose
Strongyloides stercoralis	Ivermectin	200µg/kg, single dose
	Albendazole	400mg q12-24h for 3 days
	Thiabendazole	25mg/kg (max 1.5g) q12h for 3 days
Trematodes (flukes)		
Fasciolopsis buski	Praziquantel	15mg/kg, single dose
Heterophyes heterophyes	Praziquantel	10–20mg/kg, single dose
Cestodes (tapeworms)		
Taenia solium	Praziquantel	10mg/kg, single dose
Taenia saginata	Niclosamide	2g, single dose
Hymenolepis nana	Praziquantel	20mg/kg, single dose
	Niclosamide	2g on day 1 then 1g/day for 6 days
Diphyllobothrium latum	Praziquantel	10mg/kg, single dose
	Niclosamide	2g, single dose
All treatments are adult dosage and given orally unless stated otherwise.		

be effective in isosporiasis in a dose of 7.5mg/kg (500mg for adults; 200mg for children less than 12 years of age) twice daily for 3 days. Biliary isosporiasis may require intravenous co-trimoxazole as both oral co-trimoxazole and oral nitazoxanide have been reported to fail in the presence of malabsorption and cholangitis due to

Isospora belli.[60] [61]

Microsporidia

The treatment of microsporidiosis in the immunocompetent is not required. Evidence and experience in treating these infections comes from HIV-infected patients (see Chapter 127) where albendazole has been shown to be useful with some species of microsporidia, notably *Encephalitozoon intestinalis*, and fumagillin has shown activity against *Enterocytozoon bieneusi* [62]

Gastrointestinal helminths

The management of gastrointestinal helminths is summarized in Table 46.5 (see Chapter 209).

Ascaris lumbricoides

Treatment is effective only against the adult worm. It is usual to treat any established infection. The drugs used are albendazole (400mg, single dose), mebendazole (100mg q12h for 3 days), levamisole (150mg, single dose) or piperazine in a single adult dose of 4g of piperazine phosphate or 4.5g of piperazine hydrate, or pyrantel pamoate in a single dose of 10mg/kg. *Ascaris* pneumonitis responds dramatically to prednisolone therapy and anthelmintics should be given for 2 weeks after lung involvement. Surgery is sometimes required for bowel perforation or obstruction.

Enterobius vermicularis

Enterobius vermicularis infection is treated with mebendazole (100mg, single dose, which is repeated if necessary after 2–3 weeks), piperazine phosphate (4g, single adult dose repeated after 14 days) or pyrantel pamoate (10mg/kg, single dose). The whole family should be treated simultaneously, fresh bedlinen and night clothes should be provided and the nails kept short and scrubbed. Repeat treatment may be required because recurrence is common.

Hookworm

Hookworm infection is treated by eliminating the adult worms and treating anemia if present; these treatments can be carried out concurrently. In endemic countries where re-infection is inevitable, light infections are treated only in children, not in adults. Mebendazole (100mg q12h for 3 days) or albendazole (400mg, single dose) are effective against both *A. duodenale* and *N. americanus*. In a single-dose comparison of albendazole and mebendazole, albendazole gave better cure and egg reduction rates. [63] Levamisole (150mg orally, single dose) is less effective against *N. americanus*, and pyrantel pamoate (10mg/kg orally) is preferred. In a study from Ivory Coast, Utzinger *et al* reported a reduction in the prevalence and intensity of hookworm infections in schoolchildren receiving praziquantel for *Schistosoma mansoni* infection. If their results are replicated they will have a significant impact upon helminth control programmes.[64]

Trichuris trichiura

In symptomatic patients and in asymptomatic carriers who have high numbers of eggs, trichuriasis is treated with mebendazole (100mg q12h for 3 days or 600mg, single dose) or albendazole (400mg, single

dose). In undernourished children who have moderate infection intensities in Jamaica, albendazole treatment also resulted in improvement in some tests of cognitive ability and in school attendance and school performance, even after controlling for socio-economic status.[65]

Strongyloides stercoralis

Strongyloides stercoralis infection should be treated in both symptomatic and asymptomatic people because of its ability to cause hyper-infection if immunosuppression occurs. Ivermectin is the drug of choice (200µg/kg orally, single dose). This regimen has proven very effective in a prospective randomized trial comparing the efficacy of ivermectin and thiabendazole in Cambodian refugees who had symptomatic chronic strongyloidiasis.[66] Ivermectin also looks very promising in HIV-positive patients infected with *S. stercoralis* (see Chapter 127). Albendazole, 400mg once or twice daily for 3 days, is also effective. Thiabendazole, 25mg/kg (maximum 1.5g) orally twice daily for 3 days, is effective but often poorly tolerated. Subcutaneous ivermectin (unlicensed in humans) has proven effective in a case of life-threatening *Strongyloides* hyperinfestation.[67]

Fasciolopsis buski

Fasciolopsis buski infection is treated with praziquantel (15mg/kg orally), which is highly effective. Niclosamide (2g orally, single dose) has also been used.

Heterophyes heterophyes

Heterophyes heterophyes infection is treated with a single dose of praziquantel (10–20mg/kg orally). Niclosamide is an alternative.

Taenia solium *and* Taenia saginata

Patients who have *T. solium* infection should be evaluated for the presence of cerebral cysticercosis before commencing therapy against the intestinal tapeworm. Praziquantel (10mg/kg, single dose) is effective therapy for the adult worm. Niclosamide (2g, single dose) has also been widely used.

Hymenolepis nana

Hymenolepis nana infection can be treated with a single oral dose of praziquantel (20mg/kg). Niclosamide (2g on day 1, then 1g daily for 6 days) is also successful.

Diphyllobothrium latum

Diphyllobothrium latum infections are treated with praziquantel (10mg/kg, single dose). Niclosamide (2g, single dose) was extensively used in the past.

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Chapter 47 - Peritonitis, Pancreatitis and Intra-abdominal Abscesses

Joseph S Solomkin

INTRODUCTION

Intra-abdominal infections are common problems, ranging in severity from acute appendicitis to peritonitis from colonic perforation. There have been important advances in supportive care, diagnostic methods, anti-infective therapy and interventional techniques and these have generated both improved care and new controversies in management. Most notable is the role of routine noninvasive imaging for suspected intra-abdominal infections and the role of percutaneous or laparoscopic intervention in place of formal laparotomy. The continuing development of new antibiotics has provided new opportunities for tailoring therapy to the requirements of the individual patient.

The primary effect of these innovations has been on more accurate diagnosis and reduced morbidity, measured as length of stay. It is likely that percutaneous abscess drainage for various diseases has lessened mortality. However, even with application of state-of-the-art care, these infections can result in considerable morbidity and mortality.

Additional complexity surrounds the care of hospitalized patients for whom intra-abdominal infection occurs following elective or emergency abdominal operation. These patients are typically infected with a more difficult to treat flora and represent the current frontier in therapeutic research.

CLINICAL ASPECTS OF CARE FOR PATIENTS WITH INTRA-ABDOMINAL INFECTIONS

Diagnosis of intra-abdominal infection

The history and physical examination of patients suspected of having intra-abdominal infection are pivotal in defining the urgency of intervention and for guiding decisions regarding use of the available diagnostic techniques. A brief history should be taken during the examination and should determine how long the patient has been ill, where the pain is located, whether it has changed location or character, whether associated with anorexia, vomiting or obstipation, and whether the patient has been aware of fever or chills. A pertinent past medical history, including recent hospitalizations, medications, chronic disease diagnoses and prior operations, should also be obtained.

Plain radiographs of the abdomen may reveal free air on an upright abdominal or lateral decubitus film if a ruptured hollow viscus is the cause of peritonitis. A left lateral decubitus view, with the patient in this position for 5 or more minutes, is a sensitive test for free air (Fig. 47.1a). Other findings from plain radiographs support the diagnosis of intra-abdominal infection include pneumatosis intestinalis (Fig. 47.1b), bowel obstruction and a mass effect. There are benign causes of pneumatosis. More dramatic but less common findings are air in the portal vein (Fig. 47.1c) or extraluminal gas collections indicative of an abscess; these radiographic signs are sufficiently specific to justify immediate intervention.

The radiologic picture of intra-abdominal infection otherwise mimics that of paralytic ileus. Inflammatory exudate and edema of the intestinal wall produce widening of the space between adjacent bowel loops noted on a flat film of the abdomen. Peritoneal fat lines and the retroperitoneal psoas shadow, however, may be obliterated because of edema.

Surgical management of diffuse peritonitis

Physical findings and the patient's history routinely provide sufficient diagnostic accuracy to obviate further testing beyond plain radiographs. The primary management problem in these patients is usually hemodynamic resuscitation prior to and during operation. In the presence of continuing soiling and continuing absorption of toxins through diaphragmatic lymphatics, hemodynamic stability will not be attained until an operation is performed, and resuscitation must continue intraoperatively.[1] [2]

Operative management of peritonitis involves evacuation of all purulent collections. Perforated bowel should be resected. Controversies in the operative management of peritonitis primarily surround whether or not to perform a colonic anastamosis or ostomy, and appropriate wound closure method.[3] [4] [5] [6] [7]

Wound closure following laparotomy for diffuse peritonitis

Patients with diffuse peritonitis secondary to colonic perforation or anastomotic dehiscence typically develop abdominal wall edema as part of a generalized syndrome of increased capillary permeability. Primary closure of the abdominal incision in such patients may be difficult or even unwise. Increased intra-abdominal pressure can result in compression of mesenteric and renal veins, leading in some instances to acute renal failure or bowel necrosis. This has been defined as the abdominal compartment syndrome and patients become at risk for this when intravesicular pressures (measured through a Foley catheter) exceed 25mmHg.[8] [9] This situation can be handled by insertion of a fascial prosthesis. Abdominal wall and visceral edema typically resolves over the first week, at which time definitive closure of the abdomen can be performed.

Planned relaporotomy for diffuse peritonitis

In the 1980s, considerable work was done exploring scheduled or planned relaporotomy for diffuse peritonitis.[10] [11] [12] The notion driving this was that repetitive laparotomy would allow abdominal irrigation and thereby prevent recurrent abscess formation. However, as experience accumulated, it became clear that mortality was not decreased and that there was a substantial increase in the incidence of intestinal fistulas.[13]

Postoperative peritonitis

Postoperative peritonitis is usually due to a leak from a suture line and is typically discovered only after some delay, as a rule between the fifth and seventh postoperative days.[14] Delay in diagnosis contributes to a high mortality rate. The high mortality of anastomotic leakage or suture line breakdown after duodenal operations is explained by the fact that the duodenum is fixed in the retro-peritoneum, cannot be exteriorized and the source of infection often

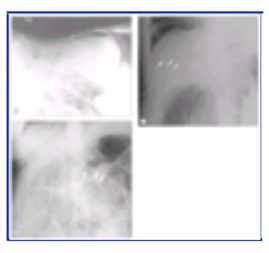

Figure 47-1 (a) Left lateral decubitus radiograph demonstrating free air outlining the liver. (b) Pneumatosis coli. (c) Air in the portal vein.

cannot be adequately controlled or closed. Consequently, infective material and proteolytic enzymes are continuously delivered into the peritoneal cavity, sustaining the infection. Drainage, controlled fistula formation, exteriorization, repair or resection and reanastomosis are employed as indicated.

Tertiary peritonitis

Patients in whom peritonitis and sepsis initially have been controlled operatively, and in whom bacteria have been eliminated by successful antibiotic therapy, may progress, a state in which host defense systems are hypothesized to produce a syndrome of continued systemic inflammation.[15]

The clinical picture is one mimicking occult sepsis, as manifested by a hyperdynamic cardiovascular state, low-grade fever and general hypermetabolism. The patient has the clinical picture of sepsis without radiographically demonstrable infection. Such patients sometimes are subjected to laparotomy seeking to provide drainage of anticipated recurrent or residual collections of infected fluid. On operation, no localized infection is identified. Management is generally supportive and any identified micro-organisms should be treated. It is important to note that this was first identified in patients managed by an open abdomen technique, patients with an ongoing serositis from such exposure.

Diagnostic imaging for suspected intra-abdominal infections other than peritonitis

In the absence of physical findings of diffuse peritonitis, diagnostic imaging with either computed tomography (CT) or ultrasound should be routinely performed in patients with clinically suspected intra-abdominal infection. The urgency is dictated by the degree of hemodynamic instability present, but in any case should be evaluated within hours.

Computed tomography is the single best modality for fully evaluating the extent of disease in most situations. Ultrasound is quite versatile and is portable, allowing procedures to be performed in the ICU. Ultrasonography is limited by bowel gas, body habitus and lower sensitivity for retroperitoneal processes and parenchymal infection. Usually the choice of modality is based on the experience and preference of the interventional radiologist.

When feasible, nonoperative (i.e. percutaneous) drainage of pus is preferable to open surgical intervention. This is because of the initial deterioration that almost always occurs following operative manipulation of intra-abdominal infection. Percutaneous drainage of an

intra-abdominal abscess is usually successful if the following criteria are met:

- there is a well-established fluid collection;
- a safe route of access is available;
- joint evaluation by a surgeon and a radiologist; and
- immediate operative back-up available in case of failure or complications.

Percutaneous drainage procedures for intra-abdominal abscesses

Percutaneous drainage and operative intervention are best viewed as complementary rather than competitive techniques. There are many situations for which percutaneous drainage is the definitive procedure of choice, others for which surgery alone is indicated and some for which both techniques are applicable, alone or in conjunction. Inflammation may manifest as a phlegmon (viable inflamed tissue), a liquefied abscess, infected necrotic (nonviable) tissue or a combination. Liquefied abscesses are drainable, whereas phlegmonous and necrotic tissue is not. Decisions regarding which modality to employ are largely based on CT findings. Specific indications for percutaneous drainage have expanded significantly over the past decade and now include multiple and/or multiloculated abscesses, abscesses with enteric communication and infected hematomas.[16]

It is important to define the goals of the procedure in evaluating indications and success. A cure is achieved when the abscess is resolved by the drainage procedure. Temporization refers to resolution of an abscess and clinical improvement, with operative intervention needed to treat the underlying cause.[17] Palliation refers to improvement in the patient's condition due to abscess drainage, despite the presence of a rapidly fatal underlying condition. We consider temporizing and palliative results to represent success.

It is important that the drainage route does not cross a sterile fluid collection or other infected space due to the risk of cross-contamination. Crossing the pleural space for thoracic and upper abdominal drainage carries the risk of empyema formation.

In most cases drainage is performed following fine needle (18–22 gauge) aspiration, with the aspirate being used to document infection and gauge the viscosity of the fluid. For most collections, a drain should be placed to ensure complete evacuation and minimize the chance of recurrence. The aspiration needle can be used for placement of a guide wire or as a guide for tandem insertion of the drain. If the patient is not already on antimicrobial therapy, this should be instituted prior to the drainage procedure to minimize the infectious complications of contaminating sterile tissue.

The choice of catheter size is determined primarily by the viscosity of the fluid to be drained. In the majority of cases, 8–12F drains are sufficient. Larger drains may be needed for collections containing debris or more viscous fluid. Drains of larger caliber can be placed, if needed, by exchange over a guide wire. There is no absolute limit on the number of drains that can be placed. While most abscesses can be drained with a single catheter, there should be no hesitation in placing as many drains as are needed to effectively evacuate the abscess(es).

Following catheter placement, the cavity should be evacuated as completely as possible and irrigated with saline until the fluid is clear. Initial manipulation of the catheter(s) and irrigation should be done as gently as possible to minimize the induction of bacteremia. Immediate imaging determines the need for repositioning of the catheter or for placing additional drains. For cavities that are completely evacuated at the initial drainage and for which there are no abnormal communications to viscera, simple gravity drainage generally suffices. For larger or more viscous collections and those with ongoing output due to fistulous connections, suction drainage with sump catheters is more effective.

MANAGEMENT OF SPECIFIC INTRA-ABDOMINAL INFECTIONS

Colonic diverticulitis

In cases with abscesses complicating diverticulitis, percutaneous drainage usually permits stabilization and allow time to optimally prepare the patient for operative therapy.[14] [18] [19] [20] Subsequent operation is indicated in most, but not all, patients and is generally simplified to a one-step procedure. In some patients who remain asymptomatic following drainage, such as those with other ultimately fatal diseases, subsequent colectomy may be avoided. It is important to perform follow-up radiographic studies to exclude the possibility of a perforated neoplasm. Initial concerns regarding persistent fecal fistulas have not been borne out by clinical experience.

The results of percutaneous drainage for abscesses complicating Crohn's disease are less positive.[21] Patients without fistulous communications to the bowel are usually cured by percutaneous drainage, whereas those with fistulas generally require bowel resection. Among

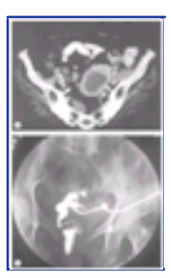

Figure 47-2 Diverticular abscess (a) before and (b) after drainage. A small fistula is seen that required further drainage but did not interfere with planned interval colectomy.

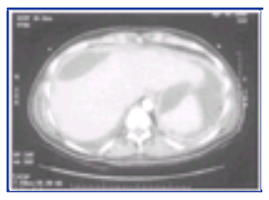

Figure 47-3 Multiple intra-abdominal abscesses following appendectomy of perforated appendicitis.

patients requiring operation, initial percutaneous drainage usually leads to significant clinical improvement and permits performance of a one-stage operation. There are no reports of iatrogenic enterocutaneous fistulas.

Low pelvic abscesses in contact with the rectum or vagina may be treated surgically by incision and drainage through these organs. The same approach can be taken using sonographic guidance and recent advances in endoluminal ultrasound techniques have facilitated such procedures. Experience with ultrasound-guided transrectal and transvaginal drainage is growing and these procedures appear to be effective and well tolerated. Good success also has been achieved in the management of tubo-ovarian abscesses complicating pelvic inflammatory disease that are refractory to medical management. In many such cases, the need for hysterectomy and oophorectomy has been obviated.

Infections complicating acute pancreatitis

Acute necrotizing pancreatitis is the antecedent cause of pancreatic infection in the majority of cases. Secondary infection of a pancreatic pseudocyst and abdominal trauma with pancreatic injury are other important causes. Abdominal pain, nausea, vomiting, distention and absent bowel sounds are frequently present. Abdominal tenderness along with a temperature greater than 102.2°F (39°C) is also very commonly present.

Pancreatic abscesses are polymicrobial, the common organisms being aerobic representatives of the fecal flora.[22]

The role of percutaneous and operative management of infection complicating necrotizing pancreatitis is in rapid flux. Pancreatic abscesses have a high failure rate with percutaneous drainage; therefore, open surgical drainage is currently preferred. The approach is transperitoneal and involves radical debridement of all necrotic tissues, followed by irrigation with saline and placement of large-bore sump suction drains. The availability of follow-up imaging and percutaneous drainage has done away with a strategy of repetitive laparotomy (every 24–48 hours). Patients are now most commonly managed with a single operative debridement, abdominal closure and expectant management. Percutaneous drainage, if needed, is utilized for peripheral or residual fluid collections.

However, there are reports that initial management by percutaneous drainage, followed by operative intervention for treatment failures, is associated with reduced mortality.[23] [24]

For localized (acute or chronic) fluid collections, percutaneous drainage is successful in most cases. Fistulous communications to the

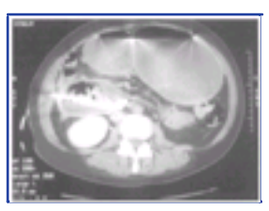

Figure 47-4 Pancreatic drainage for lesser sac infection during the course of acute necrotizing pancreatitis. The difficulty in removing necrotic debris through the narrow catheter is suggested by the abscess's persistence. Follow-up operative intervention was required.

pancreatic duct are commonly present but may be difficult to document radiographically. [25] To minimize the risk of recurrence with pancreatic fluid collections, it is especially important to document complete cessation of drainage (<25ml/day) prior to removing drains. Endoscopic retrograde cholangiopancreatography (ERCP) is valuable to document patency of the pancreatic duct, since fistulas associated with downstream obstruction are unlikely to heal and generally require operation.

Antimicrobial chemotherapy for patients with infections complicating pancreatitis should be guided by cultures of the pancreatic bed. Often patients are sequentially infected with Gram-negative enteric flora, then Gram-positive, methicillin-resistant organisms and finally *Candida* species.

Biliary infections, including acalculous cholecystitis

Ascending cholangitis, which results from biliary obstruction and secondary bacterial infection, typically presents with the clinical triad of fever, chills and jaundice. Relief of obstruction should be performed on an emergency basis. Imaging is performed to document biliary obstruction and identify the cause. The diagnosis of biliary obstruction is based on demonstration of abnormal dilatation of the common bile duct and/or its tributaries. Ultrasound is the best modality for demonstrating biliary dilatation and may also demonstrate ancillary findings such as ductal wall thickening or intraluminal gas or pericholecystic fluid collections. Intravenous contrast administration is needed to document biliary dilation with CT.

Common causes of biliary obstruction include ductal calculi, benign strictures, adenopathy and neoplastic diseases (pancreatic carcinoma, cholangiocarcinoma). Ultrasound is extremely sensitive in detecting stones in the gallbladder, but less sensitive (55%) in detecting common duct calculi. Computed tomography is less sensitive than ultrasound in identifying stones, as stones often have the same radiographic density as the surrounding bile, but superior to ultrasound for demonstrating underlying pancreatic diseases, whether inflammatory or neoplastic.

There are several pitfalls in the diagnostic imaging of cholangitis. Early obstruction may present without demonstrable biliary dilatation. Conversely, a dilated bile duct may not be functionally obstructed, but rather the dilation may be due to prior biliary obstruction with resultant ectasia. Clinical and laboratory signs of

biliary obstruction (i.e. jaundice, hyperbilirubinemia, elevated serum alkaline phosphatase activity) are generally adequate to distinguish between obstructive and nonobstructive biliary dilatation. Patients who have undergone prior biliary bypass or sphincterotomy may have gas in the biliary tree without infection.

Direct visualization of the biliary tree, via percutaneous trans-hepatic cholangiography (PTC) or ERCP, is often utilized for both diagnostic and therapeutic purposes. A thorough discussion of the relative merits of these two techniques is beyond the scope of this chapter. In most cases, cross-sectional imaging is sufficient for diagnostic purposes and intervention is utilized for therapy. If biliary obstruction is strongly suspected on clinical grounds and imaging does not demonstrate dilatation, direct visualization of the duct may be warranted to identify early nondilated obstruction. Both studies (ERCP and PTC) require transportation of the patient to a fluoroscopy suite. ERCP is less invasive and generally is the initial procedure of choice for distal obstruction, whereas PTC may be more useful for proximal obstructions. To a large extent, the choice of modalities rests with the expertise of available personnel.

Intra-abdominal infections in postoperative patients

Postoperative peritonitis generally is a consequence of anastomotic dehiscence. This is a highly lethal condition, in part because it is often diagnosed late because of reluctance to entertain the possibility of a suture line leak. This diagnosis should be considered in any patient with signs of sepsis who has undergone a gastrointestinal anastomosis. Typical findings of diffuse abdominal tenderness may be masked by incisional pain. Because laparotomy itself introduces free air into the abdominal cavity, pneumoperitoneum is a nonspecific finding in patients during the first few days after celiotomy. The most common error is to ascribe clinical deterioration to pulmonary processes that often are a consequence of peritonitis.

Ultrasound or CT will reveal peritoneal fluid which, if present, should lead to ultrasound-guided aspiration for diagnostic purposes. A Gram stain that reveals white cells or bacteria is an indication for immediate laparotomy. Surgical treatment should include either reanastomosis (small bowel) or end colostomy (colon). Postoperative

abscesses are managed as detailed above.

Management of drainage catheters

Drains should be checked regularly (at least daily) to monitor the volume and nature of the output, ensure adequate function and clinical response. Most authorities recommend periodic irrigation of the drains, once or several times per day, with sterile saline. Fibrinolytic agents may be useful for evacuation of fibrinous or hemorrhagic collections. The need for follow-up imaging studies should be determined on a case-by-case basis by monitoring clinical progress and drainage output.

Catheters should be removed when criteria for abscess resolution are met. Clinical criteria of success include resolution of symptoms and indicators of infection (fever, leukocytosis).[26] Catheter-related criteria include a decrease in daily drainage to less than 10ml and a change in the character of the drainage from purulent to serous. Radiographic criteria include documentation of abscess resolution and closure of any fistulous communications. If catheters are maintained until these criteria are satisfied, the likelihood of recurrence of the abscess will be minimized; we usually remove the drain in one step and have had no significant problem with recurrence. For sterile fluid collections, the drain should be removed as soon as possible, generally within 24–48 hours, to minimize the risk of superinfection.

Mechanical causes of failure

In evaluating the causes of percutaneous drainage failure, a number of factors are consistently identified. Among these are fluid that is too viscous for drainage or the presence of phlegmonous or necrotic debris. Technical modifications such as increasing the drain size and irrigation can salvage some of these procedures. Recognition of phlegmonous or necrotic tissue on follow-up imaging studies may lead to cessation of attempts at percutaneous drainage or a modification of the expected goal. Multiloculated collections and multiple abscesses are another cause of failure that can be minimized by using an adequate number of catheters along with mechanical disruption of adhesions with a guide wire. Fistulous communications, either unrecognized or persistent, are yet another potential cause of failure, as is drainage of a necrotic tumor mistaken by imaging to represent an abscess. Recognition of a significant soft tissue component, maintenance of a high index of suspicion and the use of percutaneous biopsies can minimize the risk of failing to appreciate the presence of tumor. Suspicious fluid also may be sent for cytology. The success rate for percutaneous drainage tends to be lower in immunocompromised patients. Lambiase *et al* found a cure rate of 53% in immunocompromised patients (including those with alcoholism, AIDS, diabetes, renal failure or steroid use) as compared to 73% in immunocompetent patients.[27]

Operative management of abscesses

The indications for open surgical drainage are failure of percutaneous drainage, inability to safely drain percutaneously, the presence of a pancreatic or carcinomatous abscess, an association with a high-output bowel fistula, the involvement of the lesser sac or the presence of multiple, isolated interloop abscesses. Abscesses in the pelvis usually are drained directly through the rectum or vagina, obviating the need for either percutaneous drainage or an abdominal operation.

Confirmation of the presence of pus is obtained by needle aspiration. It is essential to obtain specimens of the abscess content for Gram stain, aerobic and anaerobic culture and sensitivity studies. All of the abscess contents should be evacuated by suction.

After percutaneous or open drainage of an abscess, the drains are left in place until external drainage stops or is clear. The drainage track should then be irrigated and a sinogram obtained to document collapse of the cavity before the drains are moved. It may take 2–3 weeks for a large cavity to become small enough to permit drains to be removed.

THE MICROBIOLOGY OF INTRA-ABDOMINAL INFECTION

Normal bowel flora

The morbidity and mortality of intra-abdominal infection vary dramatically depending upon the level of GI tract perforation. This is because the number and type of micro-organisms vary throughout the GI tract.[28] Under normal circumstances the stomach contains less than 10^3 bacteria per mm^3 , a consequence of the very low pH within the stomach. When patients receive drugs such as H_2 blockers that raise gastric pH, the number of bacteria rapidly approaches the levels seen in the proximal small bowel. The number of bacteria per mm^3 increases as one moves down the GI tract. In the proximal small bowel there are ~$10^{4–5}$ bacteria per mm^3 , whereas the terminal ileum contains more than 10^9 per mm^3 . The highest absolute numbers of bacteria are found within the colon where there are between 10^{10} and 10^{12} bacteria per mm^3 .

Not only does the number of bacteria change, but also the type of bacteria. In the upper gastrointestinal tract facultative and aerobic Gram-negative bacteria predominate, while the colon contains many logs more anaerobic than aerobic bacteria. In the colon one finds both Gram-negative and Gram-positive anaerobic bacteria, along with facultative aerobes. Furthermore, the number of species of

TABLE 47-1 -- Organisms identified in three recently completed clinical trials in intra-abdominal infections.[56] [74] [75]

ORGANISMS IDENTIFIED IN THREE RECENTLY COMPLETED CLINICAL TRIALS IN INTRA-ABDOMINAL INFECTIONS			
	Ciprofloxacin v imipenem	**Clinafloxacin v imipenem**	**Ertapenem v piperacillin/tazobactam**
Study	**Cip/Imi**	**Clina/Imi**	**Erta/Pip/Tazo**
Number of patients	**330 (100%)**	**312 (100%)**	**396 (100%)**
Facultative/aerobic Gram-negatives	81%	84%	83%
Any anaerobes	50%	67%	-
Any Gram-positive cocci	61%	67%	50%
Escherichia coli	59%	68%	70%
Klebsiella spp.	19%	16%	13%
Pseudomonas aeruginosa	12%	15%	13%
Proteus spp.	7%	6%	4%
Enterobacter spp.	5%	5%	5%
Citrobacter spp.	5%	4%	-
Other Gram-negatives	12%	8%	12%
Bacteroides fragilis	31%	32%	36%
Bacteroides thetaiotaomicron	-	19%	20%
Bacteroides uniformis	-	14%	11%
Bacteroides vulgatus	-	9%	7%
Bacteroides distasonis	-	8%	11%
Bacteroides ovatus	-	-	11%
Other *Bacteroides* spp.	11%	13%	17%
Clostridium species	12%	20%	33%
Prevotella spp.		14%	10%
Peptostreptococci	9%	18%	16%

Fusobacterium spp.	2%	11%	7%
Eubacterium spp.		15%	18%
Other anaerobes	12%	24%	19%
Streptococci	16%	58%	22%
Viridans streptococci	17%	-	8%
ß-Hemolytic streptococci	5%	-	-
Staphylococcus aureus	5%	5%	2%
Other staphylococci	4%	-	6%
Coagulase-negative staphylococci	6%	-	-
Enterococcus, not speciated	17%	2%	12%
Enterococcus faecalis	4%	13%	11%
Enterococcus faecium	2%	4%	3%
Enterococcus avium		6%	-
Group D streptococcus	5%	-	-

bacteria isolated also is highest in the colon where there are more than 500 species of bacteria and fungi.

The vast majority of the bacteria in the colon are anaerobic species that contribute little to clinical intra-abdominal infection. The most common bacteria isolated in clinical infections are *Escherichia coli*, followed by *Enterobacter*, *Klebsiella* and *Pseudomonas* species (Table 47.1). These organisms make up less than 0.1% of the normal colonic flora. Even the most common anaerobic pathogen, *Bacteroides fragilis*, accounts for only 1% of the colonic flora. The presence of large numbers of nonpathogenic bacteria provides a measure of protection to the host by suppressing the growth of potentially pathogenic bacteria. Overgrowth of pathogenic Gram-negative aerobic bacteria is commonly seen following treatment with broad-spectrum antibiotics.[29] Many of these bacteria are associated with nosocomial infections which are much more difficult to eradicate.

Synergism involving aerobic and anaerobic bacteria

Among facultative and aerobic bacteria, Gram-negative species of the Enterobacteriaceae, particularly *E. coli, Klebsiella* and *Enterobacter* spp. predominate. Among anaerobic bacteria, the most common isolates are *Bacteroides* species. To gain insight into the relative contribution of each bacterial class experimental models were developed and investigations performed in the laboratory. [30] If pure cultures of *E. coli* were injected into animals, peritonitis developed in all animals, was associated with a high incidence of *E. coli* bacteremia and significant mortality. However, injection with *E. coli* alone did not cause any intra-abdominal abscesses, which are usually seen in patients who survive the diffuse inflammatory phase of peritonitis. In contrast, if *Bacteroides fragilis* was injected in the same experimental model, there was almost no mortality, very few *B. fragilis* recovered from the bloodstream, but nearly a 100% incidence of intra-abdominal abscesses. Injection of the combination of *E. coli* and *B. fragilis* resulted in a picture similar to that seen in human patients. There was significant mortality and virtually all survivors were found to have intra-abdominal abscesses.

The lipopolysaccharide present on the surface of Gram-negatives, including the Enterobacteriaceae, is a key virulence factor for these organisms. [31] This molecule interacts with a broad range of human cell types to induce an inflammatory response. This response consists of initial thrombosis, restricting blood supply to an area of contamination, and then induces an influx of cells which are profoundly cytotoxic for both the bacteria and resident tissue. The net effect of these responses is to reduce the numbers of organisms needed to

establish an infection, a paradoxical consequence of the inflammatory response. This paradox can be explained if the inflammatory response is considered a means of generating abscesses and tunneling to an external surface.

B. fragilis produces a capsular polysaccharide that interferes with complement activation and inhibits leukocyte function.[32] These phenomena are thought to restrict the delivery of phagocytes to the site of infection, permitting a more rapid rate of bacterial growth than would otherwise be seen.

ANTIMICROBIAL THERAPY FOR INTRA-ABDOMINAL INFECTIONS

The goals of antibiotic therapy for intra-abdominal infections that will be treated by either percutaneous or operative intervention are to hasten the elimination of infecting micro-organisms and thereby minimize the risk of recurrent intra-abdominal infection and (perhaps) shorten the clinical manifestations of infection. Since the surgical would is heavily contaminated by the infecting micro-organisms, it is important that effective antimicrobial therapy is begun prior to operation. Necrotizing fasciitis and other forms of extension of infection to the surgical wound represent catastrophic failures of antimicrobial treatment.

In patients with localized abscesses, antibiotics reduce fever and other manifestations of systemic response, but only over a 24–36-hour interval. Antibiotics should be administered after fluid resuscitation has been initiated to restore adequate visceral perfusion and provide better drug distribution. Particularly in the case of aminoglycosides, nephrotoxicity is exacerbated by impaired renal perfusion.

In practice, antimicrobial agents are often begun when the diagnosis of intra-abdominal infection is suspected. This is often prior to the establishment of an exact diagnosis and before results of appropriate cultures are available. Accordingly, the clinician must anticipate the pathogens most likely to be encountered at the site of infection. Antibiotics used for intra-abdominal infections should be active against enteric Gram-negative facultative and obligate anaerobic bacilli. The microbiology of intra-abdominal infection has been well defined. The identity and density of micro-organisms depend on the site of the gastrointestinal tract perforation. In general, gastric, duodenal and proximal jejunal perforations release small numbers of Gram-positive aerobic and Gram-negative anaerobic organisms into the peritoneal cavity. These organisms are generally susceptible to ß-lactam antibiotics and are rapidly eradicated by defense mechanisms in intact hosts. *Candida albicans* or other fungi are cultured from about 20% of patients with acute perforations of the gastrointestinal tract. Even when fungi are recovered, antifungal agents are unnecessary unless the patient has recently received immunosuppressive therapy for neoplasm, transplantation or inflammatory disease or has recurrent intra-abdominal infection.

Clinical bacterial isolates

The organisms recovered from peritonitis and intra-abdominal abscess are very similar. The organisms recovered include a mixture of aerobic and anaerobic species. Polymicrobial clinical isolates have been obtained in more than 66% of recent clinical series (Table 47.1). The precise number of bacteria isolated depends upon the vigor with which the microbiology laboratory pursues bacterial culture and on the quality of the clinical specimen (see below). In most clinical settings 2–3 aerobic species are identified and 1–2 anaerobic species. In the setting of a research-oriented microbiology laboratory, 7–10 aerobic organisms are identified, along with 10–15 anaerobic species.[33] The clinical utility of such extensive microbiologic efforts is dubious.

The concept of presumptive or empiric therapy: different standards for community-acquired and nosocomial infections

In the case of suspected community-acquired infection, knowledge of the endogenous flora giving rise to infection is a valuable guide to selection of antibiotic therapy while awaiting the results of cultures. In community-acquired infections, the encountered flora is routinely susceptible to commonly prescribed regimens and, particularly for anaerobes, there is no benefit in identification or susceptibility testing. This is also likely true for facultative and aerobic Gramnegative bacilli.

There is a strong case to be made against culturing perforated or gangrenous appendicitis. Several retrospective studies have examined the impact of such cultures on outcome and have failed to identify an association.[34] [35] [36] [37] [38] [39] [40]

There are, however, several concerns that prevent easy extrapolation of this observation to other intra-abdominal infectious settings. Firstly, all of these studies have been confined to pediatric populations with perforated, not abscessed appendicitis. Failure in this situation due to recurrent infection is extremely uncommon. In fact, this is more likely a prophylactic versus infectious setting: the inflamed viscus (the appendix) is removed and there remains no rim of an abscess cavity. The situation is

analogous to acute cholecystitis: the disease is an obstructive, inflammatory condition that is not commonly infected; the inflamed viscus is resected; and only brief antibiotic therapy is of benefit.

For other intra-abdominal infections, particularly involving the colon, failure rates are substantially higher and there is evidence that if empiric therapy is not active against any identified isolate, altering the regimen to cover identified isolates improves outcome.[41] In an observational study of *Bacteroides* bacteremia, Nguyen and colleagues found that the mortality rate among patients who received inactive therapy (45%) was higher than among patients who received active therapy (16%; $p = 0.04$).[42] Clinical failure (82%) and microbiologica persistence (42%) were higher for patients who received inactive therapy than for patients who received active therapy (22% and 12% respectively; $p = 0.0002$ and 0.06, respectively). The authors concluded that *in vitro* activity agents directed at *Bacteroides* species reliably predicts outcome: the specificity was 97% and positive predictive value was 82%. The National Committee for Clinical Laboratory Standards has now suggested that clinical laboratories strongly consider surveillance testing annually, with testing of individual solates performed in certain clinical settings.[43]

There are marked differences in susceptibility patterns in different communities and these epidemiologic data are of value.[44] Local or regional hospital antimicrobial susceptibility patterns should be heeded in selecting initial empiric therapy. This appears particularly true for facultative and aerobic Gram-negative organisms. Identification and susceptibility testing of anaerobes, a more tedious and expensive undertaking, appears unnecessary if broadly active anaerobic agents are used in settings where anaerobes are frequently encountered (colon-derived infections).

In postoperative infections, a more resistant flora is routinely encountered.[45] There is good evidence that not providing empiric therapy active against the subsequently identified pathogens is associated with significant increases in mortality and failure rates.[46] The organisms seen are similar to those seen in other nosocomial infections and anaerobes are rarely encountered. Antibiotic therapy for such infections should be guided by knowledge of the nosocomial flora seen at the particular hospital and its antimicrobial susceptibilities.

In vitro data, especially antimicrobial susceptibility tests, are predictive of the *in vivo* response of infecting bacteria to particular antibacterial agents.[41] Such data also allow selection of a specific agent or combination regimen and obviate the need for broad-spectrum

TABLE 47-2 -- Antimicrobials or combinations of antimicrobials effective for the treatment of intra-abdominal infections.

ANTIMICROBIALS OR COMBINATIONS OF ANTIMICROBIALS EFFECTIVE FOR THE TREATMENT OF INTRA-ABDOMINAL INFECTIONS
Single agents:
Ampicillin-sulbactam
Ticarcillin-clavulanic acid
Piperacillin-tazobactam
Imipenem-cilistatin
Meropenem
Ertapenem
Combination regimens:
Cefuroxime plus metronidazole
Third/fourth-generation cephalosporin (cefotaxime, ceftriaxone, ceftizoxime, ceftazidime, cefepime) plus metronidazole
Aztreonam plus metronidazole
Ciprofloxacin plus metronidazole
None of these regimens has been clearly demonstrated to be superior to another.

therapy. These are the primary reasons for obtaining cultures of sites of infection.

Selection of antibiotics

Perhaps the most common circumstance for surgeons is empiric treatment for suspected mixed flora infections, including intra-abdominal and soft tissue infections. The combination of evidence from *in vitro* data, animal studies and clinical trials has led to wide-spread acceptance of the need to provide empiric antimicrobial therapy directed against *E. coli* and other common members of the family Enterobacteriaceae and *B. fragilis*. See Table 47.2 for a list of recommended regimens. *B. fragilis* and *E. coli* are the most common isolates from intra-abdominal infections and are the organisms most likely to cause bacteremia in abdominal sepsis, further attesting to their pathogenicity.

The evidence in support of broadening therapy to cover organisms other than common facultative and obligate anaerobes such as *E. coli* and *B. fragilis* is more controversial. Initial empiric coverage of *Pseudomonas aeruginosa* is associated with a decreased likelihood of persistent or recurrent abdominal infection if these organisms are isolated from the site of infection.[47] Other clinical trials using anti-infectives not effective against *P. aeruginosa* have not found a high incidence of treatment failure with this organism.

There are a large number of agents which are broadly active against the bacteria found in intra-abdominal infection. These are best discussed as classes of drugs and include aminoglycosides, carbapenems, cephalosporins and penicillins plus ß-lactamase inhibitors, monobactams (aztreonam) and quinolones.

Aminoglycoside therapy for suspected or documented Gram-negative infection

Aminoglycosides have been the mainstay of therapy for serious Gramnegative infections for the last 30 years. Due to their potential for nephro- and ototoxicity, there has been considerable movement away from aminoglycosides as first-choice agents for community-acquired intra-abdominal infections. The use of antibiotics with clindamycin or metronidazole, ß-lactams combined with ß-lactamase inhibitors, or single agent imipenem/cilastatin or meropenem in mixed flora infections has produced clinical results equivalent to or better than those seen with aminoglycoside-based combinations. Aminoglycosides no longer represent a 'gold standard' of comparison and need not be used for community-acquired intra-abdominal infections.

The caveat to this is the use of adjunctive aminoglycoside therapy for bacteremia and shock. Approximately 10–20% of patients with nonappendiceal intra-abdominal infection are bacteremic. While Gram-negative organisms do not represent the same risk of endocarditis on normal valves or of metastatic abscess formation seen with *Staphylococcus aureus*, combination bactericidal therapy may result in more rapid clearance of organisms and abbreviate host deterioration and has been shown to improve survival in *Klebsiella* and *Pseudomonas* bacteremia.[48] [49] We believe aminoglycosides should specifically be used in the initial treatment of patients with major intra-abdominal infection and hypotension in combination with a ß-lactam agent likely effective against the anticipated Gram-negative organisms.

ß-Lactam antibiotics and ß-lactam/ß-lactamase inhibitor combinations

The terminology of first-, second- and third-generation cephalosporins is used. First-generation agents, including cefazolin, cephapirin and cephalothin, have excellent Gram-positive activity, moderate Gram-negative activity and no anaerobic activity. Cefonocid, cefamandole and cefuroxime may be grouped with these agents because none has anaerobic activity. The second-generation agents cefoxitin, cefotetan and cefmetazole all have some anaerobic activity, improved facultative Gram-negative activity and less Gram-positive coverage. The anaerobic activity of these agents against *Bacteroides fragilis* is unimpressive. In general surveys about a third to a half of tested isolates are resistant.[50] Because of the high incidence of *Bacteroides fragilis* and relatively large inoculum loads encountered in colon-derived infections, these agents are best used for prophylaxis and for treatment of low-inoculum infections such as appendicitis.

The third-generation agents, cefotaxime, ceftizoxime, cefoperazone, ceftriaxone, ceftazidime and cefepime, have considerable facultative and aerobic Gram-negative activity but no anaerobic and, excepting cefepime, limited Gram-positive coverage. Cefuroxime, commonly used in Europe in combination with metronidazole, has good Gram-negative and Gram-positive activity and is effective in combination with metronidazole. Aztreonam, termed a monobactam, has activity against facultative

Gram-negatives equivalent to third-generation cephalosporins. It has no Gram-positive or anaerobic activity. Metronidazole has remained highly effective against *Bacteroides* species, in contradistinction to clindamycin, and is now the preferred agent for combination therapy.

The choice of one extended-spectrum ß-lactam over another is not a major issue. As clinical experience with these agents has widened, it has become apparent that the differences between agents do not affect outcome results. Many hospitals have therefore taken the position that cephalosporins can be grouped into classes and that within each class the agents are therapeutically interchangeable. Commonly, acquisition costs now determine which cephalosporin is used within each class. Ceftazidime is no longer recommended for this role because broad usage is associated with decreasing susceptibility of nosocomial *P. aeruginosa*. Additionally, ceftazidime therapy is associated with an increased incidence of enterococcal superinfections.

Carbapenems

Imipenem, a carbapenem derivative, has broad activity against facultative and obligate Gram-negative anaerobes and excellent Gram-positive activity (excepting methicillin-resistant staphylococci). This agent is formulated with cilastatin, a renal dehydropeptidase inhibitor which prevents renal tubal epithelial metabolism of the drug.

This agent has been extensively tested in clinical trials of complicated infection and is highly effective.[51] [52] [53] The drug may cause seizures in situations where plasma accumulation of the drug occurs (high dose levels or renal failure). With lower dose levels (500mg) and appropriate adjustments for renal failure, this is not a problem.

Meropenem is a synthetic agent with typical broad carbapenem antibacterial spectrum, but with no evidence of an increased risk of seizures compared with cephalosporin control agents. Several clinical trials, in which serious intra-abdominal infections were treated with meropenem dosed at 1g every 8 hours, have been completed and have shown considerable efficacy.[54] [55]

Ertapenem is a newly licensed once-a-day parenteral carbapenem effective in the treatment of intra-abdominal infections. It is highly resistant to inactivation by a wide variety of ß-lactamases. It has limited activity against nonfermentative Gram-negative bacilli and enterococci, which more often are nosocomial pathogens. In a large clinical trial ertapenem was shown to be effective in comparison to piperacillin/tazobactam for complicated intra-abdominal infections. [56]

Fluoroquinolones

The currently marketed fluoroquinolones are active against facultative and aerobic Gram-negative bacteria. Streptococci and enterococci show *in vitro* susceptibility to quinolones but other, more effective agents are available. *Staphylococcus aureus* and coagulase-negative staphylococci have become progressively more resistant to fluoroquinolones.

Ciprofloxacin, levofloxacin and moxifloxacin have excellent activity against Gram-negative bacilli. For *P. aeruginosa*, ciprofloxacin has consistently shown greater *in vitro* activity.[57] [58] [59]

The pharmacodynamics of the fluoroquinolones are of some interest. A primary virtue of the quinolones is a very large volume of distribution.[60] Because of their relatively small molecular size and absence of localized electrical charges, these agents penetrate well into interstitial fluid and achieve high tissue levels, in excess of those seen with other, larger molecules.

The area of greatest concern with quinolones is the rapid development of resistance by anaerobes, in particular *B. fragilis*. The most common mechanism for this appears to be mutation on the DNA gyrase A target. Rates of resistance to these antibiotics in clinical populations are increasing. In an ongoing surveillance study, 16.4% of 1220 clinical *B. fragilis* group strains from 19 European countries were considered resistant to moxifloxacin, with minimum inhibitory concentrations (MICs) of 4µg/ml (M Hedberg, Karolinska Institute, Sweden, cited in[61]). In another broad survey, increased geometric mean MICs were observed with the fluoroquinolones and were usually accompanied by an increase in resistance rates.[62]

Importance of pharmacokinetics

Dosing of cephalosporin, carbapenem and quinolone antibiotics should be optimized based on the known pharmacodynamics of these agents.[63] [64] Cell wall active agents are effective at the MIC of the drug for the organism(s) being treated. Increasing the drug concentration substantially above about 2–4 times the MIC does not increase the rate of killing. Once the drug falls below the MIC, the organism(s) begins regrowth immediately. Dosage regimens for cell wall active agents in critically ill patients should involve dosing intervals sufficiently short to maintain serum levels above the MIC. The general rule with these agents is therefore to give relatively small doses frequently to maintain the trough level above the MIC and avoid the costs and toxicities seen with high doses. This is best accomplished by administering these drugs every four half-lives, with adjustments as needed for renal compromise. There has been interest in infrequent drug dosing with ß-lactams. This is likely to succeed primarily in mild to moderate infections in otherwise intact hosts.

Duration of therapy

Antimicrobial therapy of established infections should be limited to no more than 5 days unless it is impossible to achieve adequate source control.

Clinical signs of infection, such as fever and leukocytosis, should be used to judge the adequacy of antimicrobial therapy. Continued clinical evidence of infection at the end of the time period designated for antimicrobial therapy should prompt appropriate diagnostic investigation rather than continuation of antimicrobial treatment.

In patients who have persistent clinical evidence of infection but a negative investigation for a new or persistent infectious source, a trial of careful monitoring off all antimicrobial therapy may be warranted, since the persistent clinical symptoms and signs may be the result of ongoing tissue inflammation (the systemic inflammatory response syndrome) or a drug reaction.

Should combination therapy be employed for serious infections?

One result of the availability of multiple classes of effective antibiotics is the possibility of combination therapy with two effective agents. Combination therapy has several theoretical advantages. Firstly, a broader spectrum of pathogens may be covered, of particular importance when treatment is inititiated for suspected sepsis without an identified source. Use of agents with differing mechanisms of action may also delay or prevent emergence of resistance and superinfection. Combinations of such agents may act synergistically to enhance killing of organisms.

The time-honored tradition of providing an aminoglycoside along with a ß-lactam has recently gained support from recent studies of Gram-negative bacteremia.[48] [65] [66] These studies indicate that improved survival occurs in patients with severe acute illness treated with combination therapy as opposed to those treated with a single agent. In a similarly constructed study of *Klebsiella* bacteremia, a similar improvement in severely ill patients treated with combination therapy was noted. In both studies, survival approximated 70% in the combination therapy versus 50% in the monotherapy group.

Regimens employing two ß-lactams have been tried, primarily in the setting of febrile neutropenia. No benefit has been found and there is concern that coverage with such regimens is not as broad as that obtained with an aminoglycoside-based regimen since ß-lactams have similar mechanisms of action.

An alternative approach to a fixed duration of antimicrobial therapy is to discontinue antimicrobials when the patient's symptoms and signs of infection resolve. The risk of treatment failure appears to be quite low in patients who have no clinical evidence of infection at the time of cessation of antimicrobial therapy. This usually implies that the patients are afebrile, have normal white blood cell counts and are tolerating an oral diet.

Indications for antifungal therapy

Candida albicans or other fungi are cultured from about 20% of patients with acute perforations of the gastrointestinal tract.[67] Even when fungi are recovered, antifungal agents are unnecessary unless the patient has recently received immunosuppressive therapy for neoplasm, transplantation or inflammatory disease, or has recurrent intra-abdominal infection.[67] [68]

The antifungal of choice for confirmed intra-abdominal candidal infections is uncertain. For established *Candida* peritonitis, amphotericin B, azoles such as fluconazole or voriconazole, or an echinocandin such as caspofungin are all likely to be effective. The ultimate choice of antifungal therapy will be heavily influenced by the risks of toxicity in a given patient and presumed susceptibilities of the identified yeast.

Indications for antienterococcal therapy

Although the appropriate role of antienterococcal therapy is controversial, most authorities believe that specific antienterococcal therapy should be given only when enterococci are the only organisms isolated or are isolated from blood. Isolation of enterococci as part of a mixed Gram-positive and Gram-negative flora should not prompt addition of ampicillin or vancomycin to the antibiotic regimen.[69] The incidence of treatment failure for patients harboring enterococci and not treated for it is the same as for patients treated with imipenem or other agents effective against enterococci. Enterococci are very low-level pathogens, meaning that they incite little host response and do not cause invasive infection in intact hosts. [70] However, patients who have had one major episode of sepsis are sufficiently immunosuppressed so that isolation of enterococci from a second infectious site (including recurrent infection within the abdomen) should mandate specific antienterococcal therapy.

Identification of high-risk patients

Several attempts have been made to identify clinical features in patients with peritonitis that increase the risk of adverse outcomes.[71] The purpose of identifying such patients is to tailor both the surgical approach and antimicrobial therapy to minimize this risk. Unfortunately, most analyses have identified parameters prognostic of mortality rather than the risk of recurrent infection. As many patients die of their co-morbidities rather than as a direct result of infection, it becomes difficult to ascertain the precise role antimicrobials play in preventing mortality. In most analyses, factors independently associated with death include advanced age, higher APACHE II scores (a marker of physiologic derangement), poor nutritional status, significant cardiovascular disease and inability to obtain adequate source control.[72] [73] Prolonged prehospital length of stay may represent a marker for significant co-morbidity, previous antibiotic exposure or postoperative peritonitis, suggesting that organisms resistant to the empiric antimicrobial regimen may be responsible.

Recognition and management of treatment failure

Treatment response is defined as a diminution in the physical signs of infection, including decrease in fever, tachycardia and local findings of tenderness and organ dysfunction (such as ileus). The more localized the initial infection, the more rapidly response will occur. Conversely, extensive infections such as peritonitis or multilobar pneumonia may respond only slowly. As a general rule, infections should show definite evidence of response within 72 hours of treatment initiation. The absence of such a response is not an automatic indication that the wrong antibiotic was selected. In fact, most cases of apparent treatment failure call for continuation of the same antibiotic. A diligent search should be made for a septic focus requiring drainage, debridement or excision.

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Chapter 48 - Viral Hepatitis

Stephen D Ryder

INTRODUCTION

The clinical syndrome of acute hepatitis has been recognized since antiquity and is characterized by jaundice, usually after a prodromal illness. Acute hepatitis may cause severe sequelae, including fulminant hepatitis, but the major impact on human health is chronic liver disease and hepatocellular carcinoma resulting from chronic infection.

The nomenclature of the hepatitis viruses is somewhat eclectic, being based on the time of discovery of new or putative agents rather than on any consideration of modes of transmission or clinical problems associated with that agent. There are at present five primary human hepatotropic viruses, A, B, C, D and E, which are well characterized and known to account for approximately 90% of acute and 95% of chronic viral hepatitis. Other viruses, F and G, may cause human disease.

EPIDEMIOLOGY

Hepatitis A virus

Hepatitis A virus (HAV) is distributed throughout the world and causes outbreaks of infection, usually in association with direct fecal-oral contact [1] or contaminated water supplies.[2] Most food-related outbreaks of HAV infection are sporadic and due to poor food hygiene measures, but contamination of shellfish caused by sewage contamination is well described [3] and represents a continuing problem because mollusks are able to retain and concentrate viruses from water. Homosexual men and those working with newly imported nonhuman primates are high-risk groups for HAV infection. In the 1980s the proportion of cases of hepatitis A spread by blood contact increased to 19% of reported cases in the USA; this appears to have been due to intravenous drug use.[4]

In the developed world, the proportion of people who have immunity to HAV has declined over the past two decades, owing to improved sanitary conditions in childhood. Travelers from low-risk geographic areas to high-risk areas are at substantial risk of acquiring HAV infection.[5] In the developing world, infection rates are as high as 95% by the age of 16 years. Early infection usually produces a less severe clinical illness and immunity to future infection. The incubation period is short, ranging from 15 days to 50 days (mean 30 days).

Hepatitis B virus

Hepatitis B virus (HBV) is one of the most common chronic viral infections in the world, with estimates of approximately 170 million chronically infected people.[6] Hepatitis B virus infection is relatively rare in developed countries, with an incidence of 1 per 550 population in the UK and North America. Even in areas like the UK, where acute infection appears relatively uncommon, up to 2% of the population show evidence of past infection. In high-risk areas such as South East Asia and sub-Saharan Africa up to 20% of the population have evidence of previous infection.[7] A major mode of spread in high-endemicity areas is vertical transmission from carrier mother to child, and this may account for 40–50% of all HBV infections in such areas.[8] This mode of transmission is highly efficient; more than 95% of children of carrier mothers are infected and develop chronic viral infection themselves. In low-endemicity countries, the mode of spread is predominantly by sexual transmission or blood-borne transmission through intravenous drug use. In most of northern Europe, immigration accounts for a substantial proportion of new cases of hepatitis B infection presenting to the health care services. Many asylum seekers are from areas of the world where hepatitis B infection is very common. It is estimated that in the UK 90% of new hepatitis B diagnoses are in migrant populations with chronic rather than acute infection. Between 5% and 10% of acutely infected people develop chronic viral infection. Certain groups in the Western world are known to be at higher risk of HBV exposure; these include intravenous drug users, hemodialysis patients, homosexual men and institutionalized people, particularly those with mental handicap. Males are more likely to become chronic HBV carriers than females, for reasons that are unclear. The incubation period ranges from 28 days to 160 days (mean 80 days).

Hepatitis C virus

Hepatitis C virus (HCV) infection is common, with an estimated 500 million cases worldwide. Unlike infection with HBV, this infection is very common in the developed world, with 0.3–0.7% of the UK population infected. The virus is spread almost exclusively by blood contact. Since the introduction of screening of blood products in 1990 or 1991, intravenous drug use has become the almost exclusive mode of hepatitis C transmission in northern Europe and North America. In the early 1990s, of cases in northern Europe 35% had a past history of blood transfusion and a further 40% had used intravenous drugs. Of new cases seen in the late 1990s, intravenous drug use accounted for over 75% of cases. Sexual transmission does occur, but it is unusual, with less than 5% of long-term sexual partners becoming infected.[9] Vertical transmission also occurs, but again it is unusual. It is certainly not the predominant mode of spread of HCV, with the frequency of infection in children of viremic mothers less than 5%. The mode of delivery does not affect infection rates and breast-feeding is safe.[10] The rate of infection in children of infected parents may rise in the first 10 years of life but it seems relatively difficult to acquire this viral infection from close household contact.

This leaves a substantial minority of those identified in whom no specific risk factor is present. This proportion may be up to 20% of cases. It has been postulated that this group may have acquired the infection from medical interventions. This is based on the high prevalence of infection in areas such as southern Italy, where military vaccination programs were undertaken in the period immediately after the Second World War, and in Egypt, where up to 20% of the population have HCV markers in certain geographic areas and treatment for diseases such as schistosomiasis are commonly given by injection. Hospitalization for whatever reason appears to be a risk factor for HCV infection. The incubation period varies between 14 and 60 days (mean 50 days).

Hepatitis D virus

Hepatitis D virus (HDV) or delta virus is an incomplete RNA virus that uses hepatitis B surface antigen (HBsAg) to enable replication and transfer from cell to cell. Hence, its epidemiology is closely linked to that of HBV. There are, however, considerable differences in the frequency of delta infection or superinfection in different patient groups. Intravenous drug users have a relatively high incidence of HDV infection whereas homosexual men, a high-risk group for the sexual spread of HBV, have a low incidence of delta infection. The reason for this epidemiologic paradox is unknown. Transmission of HDV is parenteral,[11] either via transfusion or close personal contact. Screening of blood products for HBsAg effectively excludes HDV-positive donors. The commonest risk factor for the acquisition of HDV infection in the Western world is intravenous drug use, with between 17% and 90% of HBsAg-positive addicts also testing positive for HDV.[12] In developing countries, HDV infection generally parallels HBV infection, although there are exceptions, particularly in areas of Asia, where HDV is rare despite a high level of HBV carriage. In general, approximately 5% of people with chronic HBsAg carriage will be co-infected with HDV, giving an estimated worldwide figure in excess of 10 million. The incubation period is quite variable.

Hepatitis E virus

Hepatitis E virus is transmitted by the fecal-oral route. Its epidemiology correlates with the presence of contaminated water. It is responsible for outbreaks of epidemic-type hepatitis in the Indian subcontinent, but sporadic cases are seen throughout the world (Fig. 48.1). The incubation period is short, ranging between 15 and 45 days (mean 40 days).

Hepatitis F virus

Hepatitis F virus was described as the cause of a fulminant giant cell hepatitis. Its pathogenicity in humans is controversial and if it does cause human disease, it is rare.

Hepatitis G virus

Hepatitis G virus has only recently been described and its potential role as a pathogen and its epidemiology are largely unknown. In the USA and Europe, initial studies suggest that up to 1% of blood donors are viremic.

PATHOGENESIS AND PATHOLOGY

The molecular structure of the hepatitis viruses is considered in Chapter 214 . The mechanisms of pathogenicity of the hepatitis

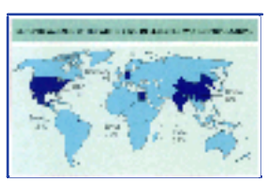

Figure 48-1 Seroprevalence of hepatitis E IgG in selected world populations.

viruses are complex and involve viral and host immune factors. The basis of any hepatitis is hepatocyte death, usually immunologically mediated in order to eliminate infected cells and to prevent further viral replication. It is highly likely that the mechanisms involved vary depending on the virus. Further differences in either the virus or the host response are involved in the development of chronic infection with HBV or HCV. Because these are the major cause of liver disease, these two viruses are considered in detail in this chapter.

Hepatitis B virus

Hepatitis B virus enters the hepatocyte by binding of determinants that are present on HBsAg. The virus replicates mainly in the hepatocyte (Fig. 48.2), although HBV DNA has been found in extrahepatic tissues, including skin, pancreas, kidneys, bone marrow and peripheral blood mononuclear cells. Hepatitis B virus has a highly unusual genetic structure, with a circular, partially ssDNA genome. When internalized in the hepatocyte, the genome is released and the negative strand is converted by ligation to a closed circular supercoiled form.[13] This form is present in the hepatocyte nucleus and forms the template for HBV RNA synthesis. Hepatitis B virus is almost unique in that its DNA is synthesized via an RNA intermediate; the same molecules are therefore used for protein synthesis and for reverse transcription to DNA.

Figure 48-2 Hepatitis B virus replication. Viral DNA present in the nucleus is transcribed to RNA, which then acts as a template for protein synthesis (viral coat-HBsAg and viral proteins essential for infectivity and replication-HBcAg). Viral particles are then assembled and secreted from the cell cytoplasm. For every complete virion a large number of incomplete particles derived from HBsAg alone are exported. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen.

The HBV RNA template is encapsulated in hepatitis B core antigen (HBcAg) particles and reverse transcribed to produce negative-strand DNA. This is then used to synthesize an incomplete positive DNA strand and the virion is encapsulated with HBsAg before excretion from the cell.

There are a number of host mechanisms deployed to prevent initial infection and then to remove infected hepatocytes. Hepatitis B virus is not thought to be directly cytopathic except in highly specific circumstances, such as fibrosing cholestatic hepatitis seen in reinfection of liver grafts, when the host is immunosuppressed. Liver cell damage in both acute and chronic HBV infection is thought to be immunologically mediated.

Cell-mediated and humoral immune responses occur in HBV infection and both are probably important in limiting and eliminating infection. There is invariably a humoral immune response directed against HBcAg and usually against HBsAg, [14] but this response alone is not the cause of hepatitis as evidenced by liver disease in agammaglobulinemic patients. The responses of HLA class I restricted cytotoxic T cells are thought to be the major mechanism of liver cell injury.[15] The fact that patients with production of HBsAg alone in hepatocytes usually have little inflammatory liver disease suggests that the target of this attack is likely to be core antigen.

Acute HBV infection can be self-limiting, with complete clearance of the virus, or it can develop into chronic infection with the potential for the development of cirrhosis and primary liver cell cancer.

Chronic HBV infection can be thought of as occurring in phases, depending on the degree of immune response to the virus. This is particularly true of patients infected in the first few weeks of life. If infected when the immune response is 'immature', there is initially little or no immune response to HBV. The levels of HBV DNA in serum are very high and the hepatocytes contain abundant HBsAg and HBcAg, but little or no ongoing hepatocyte death is seen on a liver biopsy because of the defective immune response. This state persists for a variable period of time; usually the degree of immune recognition increases over some years. When immune recognition starts to occur, the level of HBV DNA tends to fall and the liver biopsy shows increasing inflammatory liver disease. This inflammation and hepatocyte death produces hepatic fibrosis. Once this phase of infection is initiated there are two major possible outcomes: either

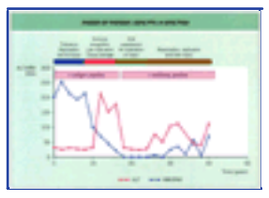

Figure 48-3 Phases of chronic hepatitis B infection. Initial infection is usually in childhood and has a long immune tolerant phase. Immune recognition then develops which can allow inactivation of hepatitis B by either clearance of infected cells (with associated liver cell damage) or suppression of viral antigen expression on infected hepatocytes. This produces viral inactivation. In a significant proportion of patients where this has occurred, a third phase develops, where replication of HBV resumes due to viral escape mutants, leading to HBV DNA again appearing in serum and the risk of chronic liver disease.

the immune response is adequate and the virus is inactivated and then removed from the system or the attempt at removal results in extensive fibrosis, distortion of the normal liver architecture and death from the complications of cirrhosis.

A third phase of hepatitis B virus infection is now recognized. This occurs late in the natural history of the immune response against HBV. In situations where the wild-type virus is inactivated but not eradicated, characterized by hepatitis B surface antigen positivity, e antigen negativity and undetectable levels of HBV DNA, there is still virus present in hepatocytes. This virus will be present in a number of forms, with viral mutants commonly produced. It is now well recognized that some of these mutant species can replicate in the presence of an adequate immune response against wild-type virus. This leads to re-emergence of HBV DNA in serum and can lead to progressive liver injury (Fig. 48.3). These mutant stains may account for up to 60% of all replicating HBV infection in Europe. Recognition of this situation clinically is both important and relatively simple, important because reactivation of HBV replication carries a significant risk of the development of hepatic fibrosis and cirrhosis and therapy is different from the usual 'wild-type', e antigen-producing HBV. It is simple to recognize as abnormal transaminases and significant levels of HBV DNA are present in the serum in patients who are HBsAg positive but e antigen negative.

Hepatitis C virus

Chronic HCV infection has a long natural history, with most patients discovered in the presymptomatic stage. In the UK, most patients are now screened because of an identifiable risk factor (previous intravenous drug use or blood transfusion) or because of abnormal liver biochemistry.

The mechanism by which HCV causes human disease is unclear. A very high proportion (80%) of acute infections go on to become chronic and the prognosis from chronic infection is very variable. Some infected people have normal liver histology,[16] which indicates that HCV is not directly cytopathic for hepatocytes. Immune mechanisms are again thought to be important in determining outcome.

Hepatitis C virus is an RNA virus. It is highly variable, with six major genotypes.[17] Genotypes are frequently geographically restricted, such as type 4 in the Middle East, or may vary with time

and mode of spread. This has been seen in Europe with genotype 1 occurring in older transfused individuals, with type 3 in younger drug users. There is some evidence that viral factors are important in the outcome of HCV infection. More severe fibrotic disease has been found in patients infected with genotype 1a or 1b than in those infected with other genotypes.[18] This is somewhat controversial as there are confounding factors, such as duration and mode of transmission, that make interpretation difficult.[19] The variability of the infecting HCV has also been postulated to affect outcome. The number of quasispecies present, a measure of genetic variability within a viral population, has been suggested as an important factor in evading the host immune response. Furthermore, quasispecies variability has been shown to be associated with increasing severity of liver disease, [20] whereas the number of quasispecies present in serum is higher in acute infection than in chronic infection.[21] More severe outcome appears to be correlated with selection of single species in the infecting HCV, which are presumably better adapted to survive in that particular immune environment.[22]

The level of viremia in a patient with HCV infection can be estimated using quantitative polymerase chain reaction (PCR) methods or branched chain DNA technologies, although these are somewhat less sensitive. High levels of viremia have been found to correlate with severity of liver disease.[23]

Evidence suggests that the host immune response is important in determining the outcome of HCV infection. There are HLA associations with viral clearance as evidenced by positive antibodies and negative tests for circulating viral genome (using PCR). In addition, patients with combined HCV and HIV infections and patients who were infected with HCV as a result of contaminated immunoglobulin given for hypogammaglobulinemia have more severe and more rapidly progressive liver disease.

PREVENTION

Hepatitis A

Primary prevention mechanisms are the avoidance of water contaminated by human urine or feces. As water purification has improved, the seroprevalence of HAV infection has fallen. In countries with a low seropositivity for HAV infection, protective immunization is recommended for certain groups at high risk (see Chapter 214). It should be remembered that the severity of the acute illness caused by HAV increases with age of exposure. Approximately 5% of those over the age of 65 years who are infected will develop fatal acute fulminant hepatitis.

Hepatitis B

The prevention of HBV infection is covered in Chapter 214 .

Hepatitis C

There is at present no effective vaccine against HCV infection. The difficulties in developing such a vaccine are numerous: the virus is highly variable, the mutation rate is high and the development of antibodies does not protect against reinfection with the same strain of HCV. The production of a clinically useful vaccine is likely to take many years.

Hepatitis D

No specific vaccine for HDV exists. In patients known to carry HBsAg, prevention is restricted to avoidance of risk factors. In populations at risk, the high level of effectiveness of HBV vaccination will also protect against HDV infection.

Hepatitis E

Prevention is restricted to avoidance of contaminated water. No vaccine is available.

CLINICAL FEATURES

Acute hepatitis

Anicteric disease

A large proportion of infections with any of the hepatitis viruses are asymptomatic or anicteric illnesses. Hepatitis A virus typically causes a minor illness in childhood, with more than 80% of infections being asymptomatic. In adult life infection is more likely to produce clinical symptoms, although only 30% of a cohort exposed to contaminated water experienced icteric illness.[2] Infections with HBV, HCV and HDV can also be asymptomatic. With HBV infection this again depends on the mode and time of transmission. Vertical transmission of infection from mother to child is almost always asymptomatic, although it produces chronic infection in the child. Transmission of HBV by other routes is much more likely to produce a symptomatic illness; about 30% of cases transmitted by intravenous drug use are icteric.

Clinically apparent acute hepatitis

Acute hepatitis presents with jaundice or elevated liver enzymes, usually preceded by a prodromal illness. The clinical features give little indication as to the likely etiologic agent. A history from patients who are suspected to have an acute hepatitis should be aimed at identification of specific risk factors for viral or other liver disease (Table 48.1).

Common symptoms in the preicteric phase include myalgia, nausea, vomiting, fatigue and malaise. There is often a change in the sense of smell or taste and right upper abdominal pain is common. Coryza, photophobia and headache are often seen and cough may be prominent in hepatitis A. Diarrhea with transient pale stools and dark urine may occur.

A serum sickness-like illness occurs in about 10% of patients who have acute HBV infection and in 5–10% of patients who have acute HCV infection. [24] This is characterized by an urticarial or maculopapular rash and arthralgia, typically affecting the wrist, knees, elbows and ankles. This illness is due to immune complex formation and rheumatoid factor is frequently positive. It is almost always self-limiting and usually settles rapidly after the onset of jaundice.

Viral hepatitis may produce other clinical or subclinical problems. Acute hepatitis B is rarely associated with clinical pancreatitis in the acute phase of the illness, although elevation of amylase is present in up to 30% of patients and autopsy studies in patients who have fulminant hepatitis B show histologic changes of pancreatitis in up to 50%. Myocarditis, pericarditis, pleural effusion, aplastic anemia, encephalitis and polyneuritis have all been reported.

Physical examination in the preicteric phase is usually normal although mild hepatomegaly (10%), splenomegaly (5%) and lymphadenopathy (5%) may be seen. Stigmata of chronic liver disease should not be present in patients who have an acute illness, and their detection suggests either that the episode causing presentation

is the direct result of chronic liver disease or that there has been an acute event superimposed on a background of chronic liver disease, as for example in HDV superinfection in a HBV carrier (Fig. 48.4).

TABLE 48-1 -- Important points in the history of a patient who has suspected viral hepatitis.

IMPORTANT POINTS IN THE HISTORY OF A PATIENT WHO HAS SUSPECTED VIRAL HEPATITIS
• Contacts with jaundiced patients
• Intravenous drug use
• History of blood transfusion
• Surgery or hospitalizations
• Family history of chronic liver disease
• Occupation

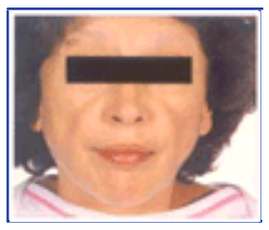

Figure 48-4 Facial stigmata of chronic liver disease. This woman presented with serologically proven acute hepatitis A virus infection. She has multiple stigmata of chronic liver disease including facial spider nevi and was shown to have pre-existing cirrhosis.

Biochemical confirmation of acute hepatic injury was seen with an elevated transaminase level which may reach 100 times normal.[25] No other biochemical test has been shown to be a better indicator of acute liver injury. There are a number of other laboratory tests that may be abnormal in patients who have acute viral hepatitis. Leukopenia is common, and in 10% of patients the white cell count may fall below 5000/mm^3 .[26] Both anemia and thrombocytopenia are described. Immunoglobulin levels may be nonspecifically elevated in viral hepatitis, with levels usually returning to normal within 2 weeks. In a small proportion of patients who have acute viral hepatitis, a profound cholestatic illness may occur. This is most frequently seen in patients who have hepatitis A and it may be prolonged, with occasional patients remaining jaundiced for up to 8 months.

Death from acute viral hepatitis is usually due to the development of fulminant hepatitis. This is usually defined as hepatic encephalopathy with an onset within 8 weeks of symptoms or within 2 weeks of onset of jaundice.[27] The risk of developing fulminant liver failure is generally low but there are groups with higher risks. Pregnant women with acute HEV infection have a risk of fulminant liver failure of around 15%, with a mortality of 5%.[28] The risk of developing fulminant liver failure in HAV infection increases with age [29] and with pre-existing liver disease.[30] Fulminant hepatitis B is seen in adult infection but it is relatively rare.

The primary clinical features of acute liver failure are encephalopathy and jaundice. Jaundice almost always precedes encephalopathy in acute liver failure, and the onset of confusion or drowsiness in a patient who has acute viral hepatitis is always a sinister development. The degree of the rise in transaminase values does not correlate with the risk of developing liver failure. Prolongation of coagulation is the biochemical hallmark of liver failure; it is caused by lack of synthesis of liver-derived clotting factors. Prolongation of the prothrombin time in acute hepatitis, even if the patient is clinically well without signs of encephalopathy, should be regarded as sinister and monitored closely. Hypoglycemia is seen only in fulminant liver disease, when it can be profound.

Chronic hepatitis

The agents that cause chronic hepatitis are HBV, HCV and HDV. Chronic hepatitis has been defined as abnormality of transaminase values persisting for more than 6 months. This has generally been a useful concept in patients who present with an acute illness. In clinical practice, the vast majority of patients will present with either an asymptomatic biochemical or serologic abnormality or the complications of cirrhosis, and it is reasonable to assume chronicity in these clinical settings at the time of initial presentation. Chronic viral hepatitis is characterized by the presence of inflammatory infiltrates in the liver associated with hepatocyte death.

The risk of developing chronic infection varies greatly with the virus implicated. Hepatitis A virus never causes chronic viremia or chronic liver disease. Hepatitis B virus causes chronic liver disease in a proportion of infected patients; this rate varies depending on the mode of transmission. Patients who are infected at or around the time of birth via a chronic carrier mother have infection rates of almost 100%, with the vast majority becoming chronic carriers of the virus and therefore at risk of long-term liver damage.[31] If HBV is acquired in later life, the risk of chronic infection falls considerably, with only 5% of such patients remaining HBsAg positive at 5 years.[32] The difference in the rate of chronic infection is probably related to the maturity of the host immune response.

Infection with HCV has, overall, the highest risk of chronicity. Posttransfusion studies indicate that at least 50% of patients with icteric disease develop chronically abnormal transaminase values, and most studies suggest that up to 80% of acutely infected people remain viremic.[33]

Hepatitis D virus can cause chronic liver damage, and there is evidence to suggest that the combination of HBV and HDV carries a particularly poor outlook.

Most patients who have chronic viral hepatitis either present with a complication of their viral liver disease or are detected by screening, either for viral serology or for abnormal biochemistry.

Symptoms

The majority of patients who have chronic viral hepatitis are asymptomatic and patients are often unaware of the infection. In HCV infection a number of symptoms are frequently reported by patients in the absence of severe liver disease. These include lethargy, inability to concentrate and pain over the liver. It is unclear if these symptoms are the direct result of the viremia or are related to depression as a result of the diagnosis. These symptoms, whatever their origin, contribute to a reduced quality of life for patients with hepatitis C compared to patients with chronic hepatitis B.[34] Other symptoms are due either to associated diseases affecting organs other than the liver or to end-stage liver disease.

Extrahepatic manifestations

Chronic hepatitis B can be associated with polyarteritis nodosa, with vasculitic rash, fever and polyarthralgia. Circulating HBsAg and anti-HBs complexes can be demonstrated, as can cryoproteins and HBsAg in blood vessel walls.[35] Cyclophosphamide appears to be effective treatment for HBV-associated vasculitis.

Glomerulonephritis is now known to occur with both HBV[36] and HCV infections.[37] This is a relatively rare association thought to be mediated by the deposition of immune complexes. There are reports of improvement of the renal lesion on treatment of the responsible virus.

Up to 60% of patients who have mixed essential cryoglobulinemia are anti-HCV positive;[38] the condition is again thought to be the result of immune complex formation and antigen-antibody deposition in the vasculature (Fig. 48.5). Successful therapy of the underlying hepatitis C may produce remission.

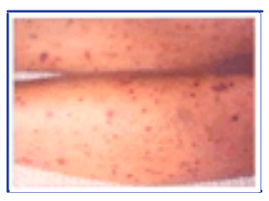

Figure 48-5 Vasculitic rash in a patient who has mixed cryoglobulinemia.

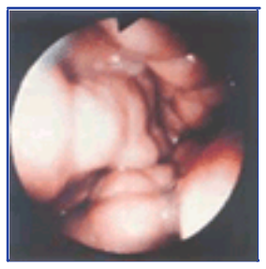

Figure 48-6 Endoscopic view of esophageal varices.

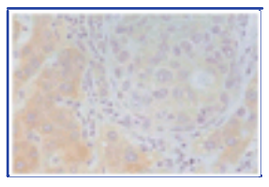

Figure 48-7 Surgical histology of a nodule of hepatocellular carcinoma in a patient undergoing liver transplantation for hepatitis C cirrhosis.

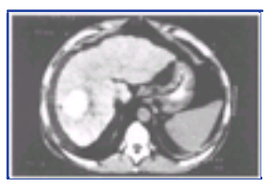

Figure 48-8 Computed tomography scan following lipiodol injection into the hepatic artery showing hepatocellular carcinoma in the cirrhotic liver of a hepatitis B virus-positive male. Lipiodol is selectively retained in the tumor.

Porphyria cutanea tarda is strongly associated with HCV infection, with up to 76% of Italian patients with porphyria cutanea tarda having antibodies to HCV.[39] Lichen planus is also associated with HCV infection.

Clinical signs

Patients who have decompensated cirrhosis caused by viral agents present with jaundice, encephalopathy, ascites or gastrointestinal bleeding as a result of portal hypertension (Fig. 48.6).

Cutaneous stigmata of chronic liver disease, spider nevi, leukonychia, gynecomastia, testicular atrophy and loss of body hair may be present. A late complication of chronic liver disease caused by HBV, HCV or HDV is hepatocellular carcinoma (Fig 48.7 , Fig 48.8).

Hepatocellular carcinoma may present as a sudden decompensation of previously stable chronic liver disease or as pain in the right upper quadrant of the abdomen, or there may be distant metastatic disease, with pulmonary or bone metastases being most common. Alphafetoprotein is often raised, although this is normal in up to 60% of small hepatomas.

All of these clinical signs occur late in the course of these viral infections and for most of the duration of infection no abnormal clinical signs will be present and the patient will be asymptomatic.

DIAGNOSIS

Acute hepatitis

Hepatitis A

Infection with HAV can be reliably diagnosed by the presence of anti-HAV IgM. This test has very high sensitivity and specificity.[40] Very occasional false-positive results can be seen in liver disease of other etiologies where very high levels of immunoglobulin are present but the clinical context usually makes this obvious.

Hepatitis B

Infection with HBV is usually characterized by the presence of HBsAg. In acute HBV infection the serology can be difficult to interpret. The reason that an acute hepatitis develops is the immune recognition of infected liver cells, which results in T cell-mediated hepatocyte killing. Active hepatocyte regeneration then occurs to replace those hepatocytes that have been lost. As well as a cell-mediated immune response, a humoral immune response develops and this is probably important in removing viral particles from the blood and thus preventing reinfection of hepatocytes. Because of the immune response attempting to eradicate HBV, at the time of presentation with acute hepatitis B, viral replication may already have ceased and the patient may be HBsAg positive, hepatitis B e antigen (HBeAg) negative, e antigen being a surrogate marker of HBV replication.

It is difficult in this situation to be certain that a patient's illness was due to acute hepatitis B and that the serology does not imply past infection unrelated to the current episode. To enable a clear diagnosis to be made, most reference centers now report the level of IgM antibody to HBcAg (IgM anti-core; Fig. 48.9). As core antigen does not appear at any time in serum, this implies an immune response against HBV within liver cells and has been shown to represent a sensitive and specific marker of acute HBV infection.[41]

Rarely, the immune response to HBV infection is sufficiently brisk that even HBsAg has been cleared from serum by the time of presentation with jaundice. This may be more frequent in patients who develop severe acute liver disease and has been reported in up to 5% of patients who have fulminant hepatitis in whom the diagnosis was made by an appropriate pattern of antibody response.

Diagnosing past HBV infection or establishing immunity to vaccine is easy serologically. If the virus is cleared after infection with HBV, antibodies to all viral antigens can be detected. The levels of most of these in blood, however, decline with time. This is particularly true of anti-HBsAg: after 1 year, most patients' level of antibody has fallen below the level of detection in most commercially available assays. Despite this low level, immunity is still sufficient to

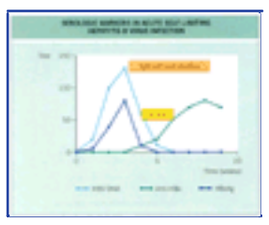

Figure 48-9 Serologic markers in acute self-limiting hepatitis B virus infection. HBV, hepatitis B virus; HBeAg, hepatitis B e antigen. Measurements in arbitrary units.

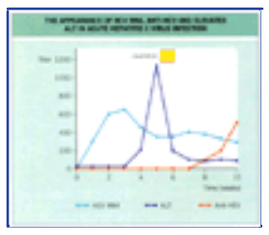

Figure 48-10 The appearance of hepatitis C virus RNA, anti-hepatitis C virus and elevated ALT in acute hepatitis C virus infection. HCV, hepatitis C virus; ALT, alanine transaminase. Measurements in arbitary units.

prevent reinfection. Natural immunity is proven by the presence of IgG antibodies to HBcAg. These are a highly reliable marker of past infection and remain at detectable levels for a long period. Vaccine-induced immunity is directed purely against HBsAg epitopes, and hence a vaccinated person will have detectable levels of anti-HBsAg but no anti-HBcAg.

Hepatitis C

Screening tests for HCV infection are enzyme-linked immunosorbent assay (ELISA) techniques that use recombinant viral antigens and patient's serum. Acute hepatitis C cannot be reliably diagnosed by antibody tests because these frequently do not become positive for up to 3 months (Fig. 48.10).

Hepatitis C virus used to be the cause of more than 90% of all posttransfusion hepatitis in Europe and North America[42] but acute HCV infection is now most commonly seen in intravenous drug users. Antibodies to HCV appear relatively late in the course of acute infection and therefore, if clinical suspicion is high and risk factors are present, testing of the patient's serum for HCV RNA is the only means of establishing the diagnosis (see Fig. 48.10). Identification of cases of acute HCV infection is important because there is strong evidence that early treatment with interferon-a may reduce the risk of chronic infection with HCV. The apparent rate of chronicity in untreated patients is approximately 80%; with interferon monotherapy given for 6 months, a recent study showed that more than 90% of treated patients with acute hepatitis C clear virus.[43]

Hepatitis D

As HDV requires the presence of HBsAg for infectivity, it occurs only in patients who are infected with HBV either previously (superinfection) or simultaneously (co-infection). Co-infection is usually relatively benign; the determining factor for severity of illness is the HBV infection. As this is usually self-limiting, with loss of HBsAg after a relatively short interval, chronic infection with HDV is rare (about 2%).

Superinfection with HDV, by contrast, is a much more severe disease. The presence of large amounts of HBsAg allows rapid replication of the HDV and establishment of both acute and chronic infection. Chronic infection is common in this setting because the continued production of HBsAg allows the delta agent to continue replication.

Diagnosis of delta hepatitis is not easy: the encapsulation of HDV by HBsAg masks much of the HDV antigenemia, and assays for the detection of delta RNA in serum are not widely available and are not always reliable. Conventional solid-phase immunoabsorbent tests for the detection of delta antigen in serum are only positive early in the infection, before the development of delta antibodies, which complex with antigen. Immunoblot assay is now more widely available and does not have the same drawback because it is independent of antidelta antibody. The IgG antidelta antigen antibody is not protective and elevated levels correlate with continued infection.[44]

A number of other viruses can cause hepatitis (Table 48.2). In the main, liver involvement is incidental and other features of the illness will suggest a diagnosis other than primary hepatitis. The exceptions are yellow fever, and disseminated herpes simplex and varicella-zoster infections in immunosuppressed patients, which in both cases may be associated with a severe hepatitis.

TABLE 48-2 -- Viral infections that may be associated with hepatitis.

VIRAL INFECTIONS THAT MAY BE ASSOCIATED WITH HEPATITIS	
Virus	**Comment**
EBV, CMV	Both these viruses cause an acute mononucleosis-type syndrome in the normal host. Mild elevations of the liver transaminases may be seen but are usually of little consequence
HSV, VZV, CMV	All may cause hepatitis in the immunosuppressed host as part of a disseminated infection. CMV is the least severe
Flaviviruses	Yellow fever causes severe hepatitis
Measles, rubella, rubeola, coxsackie B adenovirus	Mild hepatitis occurs occasionally

TABLE 48-3 -- Tests required in asymptomatic patients who have elevated transaminase levels.

TESTS REQUIRED IN ASYMPTOMATIC PATIENTS WHO HAVE ELEVATED TRANSAMINASE LEVELS
• Hepatitis B surface antigen
• Hepatitis C virus antibodies
• Antismooth muscle, antinuclear antibodies
• Immunoglobulins
• Copper studies (if patient >50 years)
• Ferritin
• a_1 -antitrypsin phenotype (if indicated)
EBV, Epstein-Barr virus; CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

Chronic viral liver disease

Chronic viral liver disease may present with abnormal liver biochemistry, by serologic testing in at-risk groups without symptoms or as a result of the complications of cirrhosis. Abnormal liver biochemistry in viral hepatitis is usually a sustained elevation of transaminases. In contrast to the situation in acute infection, the rise in the transaminase values is modest, characteristically only two or three times the upper limit of normal. In HCV infection, the ?-glutamyl transpeptidase values are also often elevated. The degree of abnormality of transaminases has little relevance to the degree of underlying hepatic inflammation. This is particularly true of HCV infection, in which the transaminase values are often normal despite active liver inflammation.

Further investigation in patients who have a positive serologic test for a hepatotrophic virus is to exclude other concomitant liver disease and assess the severity of liver damage induced by the hepatotropic virus. The risk of development of serious liver pathology is increased in the presence of multiple pathologies. Table 48.3 shows the required baseline serologic tests required. In HCV infection, autoantibodies are frequently positive, antismooth muscle antibodies being detected in about 10% of patients and low-titer antinuclear antibodies in 15–20%. These autoantibodies do not appear to play a part in pathogenesis and would only alter management if a high titer were present with a raised IgG, suggesting a primary autoimmune hepatitis, in which interferon therapy may be contraindicated.

Chronic hepatitis B

The vast majority of patients who have hepatitis B will be HBsAg positive. There are viral mutants that do not produce HBsAg detectable by the usual serologic tests (Table 48.4), but these are very rare except in patients treated with interferon or after liver transplant.

The traditional classification of those individuals who have chronic hepatitis B has been based on the presence of HBeAg. All patients who have HBsAg can be regarded as infected but HBeAg has been regarded as a marker of infectivity. Hepatitis B e antigen is produced as a truncated version of core protein (Fig. 48.11).

In the wild-type virus, HBcAg and HBeAg are transcribed together using overlapping bases. Hence HBeAg is an indirect marker of viral replication. The exact function of HBeAg is unclear but it appears to be involved in the downregulation of the T-cell response to HBV infection and may play a pivotal role in the development of the long

TABLE 48-4 -- Tests required for diagnosis in hepatitis B virus infection.

TESTS REQUIRED FOR DIAGNOSIS IN HEPATITIS B VIRUS INFECTION
• Hepatitis B surface antigen
• Hepatitis B e antigen
• Hepatitis B anticore antibody (HB total anticore)
• Hepatitis B DNA
• Hepatitis B IgM anticore

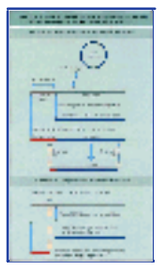

Figure 48-11 Production of hepatitis B core antigen and hepatitis B e antigen and the generation of e antigen-negative mutants. Hepatitis B virus (HBV) has a closed circular genome that contains insufficient bases to produce all its required proteins. It therefore uses different start points for transcription, enabling it to use the same base sequence to produce different proteins. Two important proteins, e antigen and core antigen, are produced by transcription of the same region with overlap; e antigen is produced from the core protein by cleavage at a specific site.

phase of relative immunologic tolerance seen in chronic HBV-infected carriers who acquire their infection in childhood. It has been shown that children infected with HBeAg-negative viral mutants do not have the immune tolerance that is almost universal in wild-type HBV transmission; most infected infants develop severe hepatic inflammation and cases of fulminant liver failure are described.

The discovery of HBeAg-negative mutant viruses has produced the need for a more specific marker of viral replication. The measurement of HBV DNA now provides direct detection of the circulating HBV genome.[45] This is the most sensitive measure of viral activity. There are two commonly used methods to estimate HBV DNA levels in serum: PCR-based techniques, which are very sensitive, and

branched chain DNA technology, which is rather cheaper to perform but less sensitive.

Patients who have persistence of HBsAg after 20 weeks of an acute episode can be assumed to have chronic carriage of the virus. Spontaneous loss of HBsAg after this time is unusual, being seen in only 1–2% of patients a year. Many people present with no obvious acute episode of hepatitis, and an apparently healthy person who is HBsAg positive can be regarded as chronically infected with HBV. The prognosis for that person is largely determined by the presence or absence of ongoing viral replication.

Chronic hepatitis C

Screening for HCV relies on antibody testing, with a second confirmatory test usually performed. Initial first-generation antibody assays were relatively unreliable with a high rate of false-positive results, but third- and fourth-generation ELISA-based tests are readily available and have high sensitivity and specificity. Confirmatory tests include radio-immunoblot assay (RIBA) and direct detection of viral RNA in peripheral blood using PCR. Initially there were major problems with the reliability of assays for detection of HCV RNA, with studies showing large variation in results between laboratories. This has been overcome to a large extent with the advent of commercially available kit systems for HCV RNA detection. These are reliable and sensitive. Hepatitis C virus RNA detection has largely replaced the use of RIBA testing in most centers. Detection of HCV RNA is regarded as the best test to determine infectivity and response to therapy. Hepatitis C antigen testing is now possible, and commercially available kit-based technology will make this test widely available. It has a very comparable specificity for the presence of HCV in blood to HCV RNA detection but is probably slightly less sensitive. Its major advantage is likely to be simplicity of assay and cost.

Patients presenting with detectable antibodies to HCV should be investigated (Table 48.5) to exclude other causes of chronic liver disease and to assess the severity of their HCV infection. Assessment of the severity of liver disease requires liver biopsy. Liver biopsy findings in hepatitis C are discussed later.

Chronic hepatitis D

Chronic infection with HDV has a high risk of producing severe liver disease. In the vast majority of cases of chronic infection, the HBV infection is nonreplicative as evidenced by HBeAg negativity and absence of HBV DNA.[46] This viral interference is seen in many combined viral infections (e.g. HBV and HCV infections) and it is rare to find patients with two replicating hepatotropic viruses. However, if the accompanying HBV is replicating, the prognosis is very poor with rapid progression to cirrhosis over as little as 2 years.[46]

Diagnosis of chronic HDV infection is usually by antibody testing in serum, where both IgM and IgG antidelta antigen antibodies are usually present in high titer, or by delta antigen staining on liver biopsy. Delta RNA testing is now more reliable and widely available

TABLE 48-5 -- Investigations required in anti-hepatitis C virus-positive patients.

INVESTIGATIONS REQUIRED IN ANTI-HEPATITIS C VIRUS-POSITIVE PATIENTS	
Tests to assess hepatitis C virus	**Tests to exclude other liver diseases**
PCR for hepatitis C virus RNA	Ferritin
Viral load	Autoantibodies and immunoglobulins
Genotype	Hepatitis B serology
	Liver ultrasound

and is useful for monitoring therapy. For patients who have chronic infection, delta antigen staining in liver biopsy material remains the most definitive diagnostic test.[47]

MANAGEMENT (see Chapter 207)

Hepatitis B

Assessment of chronic hepatitis B virus infection

Any patient who has detectable levels of HBsAg is chronically infected. Assessment of a patient who has chronic HBV infection should include more detailed HBV serology, including measurement of HBV DNA levels. In patients who have no evidence of viral replication and normal liver enzyme levels and a normal liver ultrasound, liver biopsy is not usually required. Such patients have a very low risk of developing symptomatic liver disease or hepatocellular carcinoma. Reactivation of HBV replication has been described due to development of viral mutants which escape immune surveillance and patients who are HBsAg positive should be followed with yearly serology and liver enzyme estimations. The low risk of hepatocellular carcinoma does not justify screening in this group. In patients who have abnormal liver biochemistry (even without detectable HBV DNA) or an abnormal liver texture on ultrasound, a liver biopsy is probably required, because such patients may either have superinfection with HBV or have had ongoing replication of HBV in the past, sustaining substantial liver damage in the process. It is estimated that around 5% of patients in developed countries who have only HBsAg carriage at presentation will have a posthepatitic cirrhosis on liver biopsy. This finding is important because they are at risk of developing the complications of cirrhosis, including variceal bleeding and hepatocellular carcinoma.

Assessment of patients who have HBV replication is carried out by assessing the degree of liver inflammation and the stage of the underlying liver disease. Patients who are still in the tolerant phase of their infection usually have normal transaminase levels and high serum levels of HBV DNA (in excess of 10^8 copies per ml). As immune recognition increases, the HBV DNA levels fall, the degree of hepatic damage increases and the transaminases become abnormal. This is important in the selection of patients for therapy. Patients who have viral replication and abnormal transaminases should have a liver biopsy. The need for liver biopsy in a patient who has high levels of HBV DNA and repeatedly normal transaminases is less clear because it is very unlikely that the biopsy will show advanced liver disease or considerable inflammatory activity.

Therapy for hepatitis B

There are two licensed drug treatments for hepatitis B and a substantial number of new antiviral agents which are in either clinical trials or advanced stages of drug development. Although the agents used to treat hepatitis B vary considerably in their mode of action, the basic aim of treatment is the same: to stop viral replication. The concept of 'viral eradication' is not possible in hepatitis B; it is clear that viral DNA remains present in infected individuals over their lifetime. This is exemplified by reactivation of HBV in patients undergoing chemotherapy or other profound immunosuppression whose only marker of infection was antibodies to core antigen. It is, however, clear that almost all the major sequelae of infection with HBV are a consequence of active viral replication. This is clearly key for infectivity with major implications for others but is also required to generate liver cell damage. Individuals who remain HBsAg positive but have no ongoing viral replication are not at risk of developing significant hepatic fibrosis and consequently have a dramatically reduced risk of developing decompensated liver disease compared to those with ongoing viral replication. The only serious complication of HBV infection which is not entirely dependent on viral replication

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TABLE 48-6 -- **Factors indicating the likelihood of response to interferon in chronic hepatitis B virus infection.**

FACTORS INDICATING THE LIKELIHOOD OF RESPONSE TO INTERFERON IN CHRONIC HEPATITIS B VIRUS INFECTION		
Factor	**High probability of response**	**Low probability of response**
Age	<50 years	>50 years
Sex	Female	Male
Hepatitis B DNA level	Low	High
Activity of liver inflammation	High	Low
Country of origin	Europe, North America, Australasia	Asia
Co-infection with HIV	Absent	Present
PCR, polymerase chain reaction.		

is cancer development where a risk remains, particularly in those with an established cirrhosis. Even there, the risk of developing hepatocellular carcinoma is substantially greater in patients where replication continues. Consideration of therapy is therefore only appropriate in patients where HBV is active as evidenced by detectable levels of HBV DNA. The choice of therapy is then determined by a number of factors: the stage of infection, the level of HBV DNA, the presence of mutant virus as the predominant strain (as evidenced by the presence or absence of e antigen), the degree of inflammatory liver disease and the degree of hepatic fibrosis.

Interferon therapy

Interferon-a was first shown to be effective for some patients who have HBV infection in the 1980s and it remains an effective therapy.[48] There are a number of commercially available variants of interferon-a, natural interferon, recombinant interferons and consensus interferons. In HBV infection there is little evidence that the type of interferon-a used has any substantial difference in effect or side-effect profile. Newer formulations of interferon-a, pegylated interferons, are now available. Clinical trials of these molecules in hepatitis B infection are being undertaken although there are few data available currently as to their effectiveness. They are more convenient for patients because of once rather than three times weekly administration. Trials are addressing their effectiveness as monotherapy but also in combination with other antiviral agents such as lamivudine. There are a number of factors that can help predict the likelihood of response to treatment with interferon-a and these aid selection of patients who have the best chance of response to therapy (Table 48.6).

TABLE 48-7 -- Common side-effects of interferon-a therapy for viral hepatitis.

COMMON SIDE-EFFECTS OF INTERFERON-a THERAPY FOR VIRAL HEPATITIS	
Side-effect	**Frequency (%)**
Flu-like syndrome	80
Depression	20
Local inflammation at injection sites	25
Hypothyroidism	10
Arthralgia or arthritis	10
Hair loss	10

Overall, the probability of response to interferon therapy in chronic hepatitis B is between 25% and 40%. A response to therapy is the cessation of viral replication; only a small number of patients lose all markers of infection with HBV and HBsAg usually remains in the serum. There is now good evidence that successful therapy with interferon, which renders the HBV nonreplicative, produces a sustained improvement in liver histology and a decrease in the risk of developing end-stage liver disease. The risk of developing hepatocellular carcinoma also appears to be reduced but it is not abolished in those who remain HBsAg positive. The likely reason why a number of patients with apparently successful responses to interferon therapy go on to develop significant liver disease is the emergence of mutant HBV strains which resume viral replication and hence liver cell injury.

Interferon therapy has a number of drawbacks: it is not effective in many patients, it is expensive and it has a large number of side effects (Table 48.7). In general, about 15% of patients on interferon therapy have no side-effects, 15% cannot tolerate therapy and the remaining 70% experience side effects but are able to continue therapy. Most patients are able to continue at work during therapy but many require substantial support. Depression can be a major problem and both suicide and admissions with acute psychosis are well described. Early use of antidepressants and close monitoring are essential, especially if there is a preceding history of

depression.

Treatment of hepatitis B in patients who have significant fibrotic liver disease is rewarding because they have a relatively high probability of response and have most to gain from cessation of viral replication. Many patients have a substantial improvement in liver function if viral replication is stopped, but treatment in such patients does carry an increased risk. Interferon therapy produces viral clearance, at least in part, by inducing immune-mediated killing of infected hepatocytes, and hence a transient hepatitis can cause severe decompensation requiring liver transplantation.

TABLE 48-8 -- Monitoring of interferon therapy in hepatitis B virus infection.

MONITORING OF INTERFERON THERAPY IN HEPATITIS B VIRUS INFECTION				
	Full blood count	**Liver function tests**	**Hepatitis B virus DNA**	**Hepatitis B surface antigen and e antigen**
Before treatment	+	+	+	+
Week 1	+	+	-	-
Week 2	+	+	-	-
Week 4	+	+	+	+
Week 8	+	+	-	-
Week 12	+	+	+	+
Week 16	+	+	+	+
Week 20	+	+	+	+

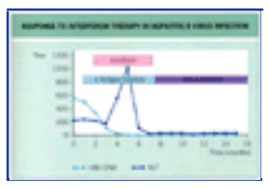

Figure 48-12 Response to interferon therapy in hepatitis B virus infection. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

The optimal dose and duration of interferon for hepatitis B remain somewhat contentious, but most clinicians use 8,000,000–10,000,000 units three times a week for 4–6 months. It is likely that most treating clinicians will use pegulated interferons in the future, with doses based on body weight for the 12kDa molecule and a standard dose of 180µg for the 40kDa molecule. No comparative data exist as to their relative effectiveness. Monitoring therapy is intensive, and appropriate schedules are shown in Table 48.8 .

Patterns of response and special situations

The most frequent pattern of response to interferon-a is shown in Figure 48.12 . The HBV DNA level falls rapidly after initiation of interferon therapy. This is followed by a marked rise in transaminase values. This represents immune-mediated clearance of virus, and HBV DNA levels quickly fall to undetectable levels. This is then followed within a few weeks by seroconversion to HBeAg-negative, HBeAb-positive status with complete normalization of transaminases. This type of response is seen in 25% of treated patients. Hepatitis B surface antigen usually remains positive, with a small proportion of patients clearing all markers of viral infection either during interferon therapy (2%) or many months after it (6%). A further proportion of patients show a late seroconversion after therapy (Fig. 48.13).

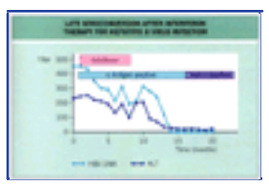

Figure 48-13 Late seroconversion after interferon therapy for hepatitis B virus infection. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

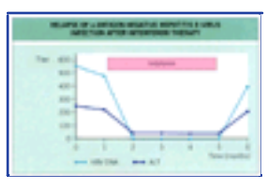

Figure 48-14 Relapse of eAg-negative hepatitis B virus after interferon therapy. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

In such patients there is often an initial fall in HBV DNA levels but these remain detectable throughout therapy together with persistently abnormal transaminase values. After the end of treatment there is then a progressive fall in HBV DNA and normalization of transaminases. Seroconversion from HBeAg to anti-HBeAb follows after a few weeks or months of HBV DNA negativity. Such delayed responses to therapy occur in 10–15% of treated patients and this emphasizes the need to persevere with therapy once the decision to undertake treatment has been made.

There are a number of specific situations in which the above treatment protocols may change. In Greece and Italy, the proportion of patients with HBeAg-negative mutant virus infection is relatively high, and this situation is also encountered elsewhere. There is compelling evidence that, although such patients show a good rate of initial response to interferon with disappearance of HBV DNA from serum, they very frequently relapse after cessation of treatment (Fig. 48.14). There is now evidence that giving interferon therapy for longer in such patients may improve the rate of loss of viral replication. A regimen of 6,000,000 units three times a week for 24 months has been shown to produce a loss of viral replication in 30% of patients.[49]

Lamivudine therapy

Lamivudine, a nucleoside analogue, is a potent inhibitor of HBV DNA replication. Lamivudine has a good safety profile and has now been widely tested in patients who have chronic HBV infection. It was initially shown to produce a rapid fall in HBV DNA levels in short-term trials, mainly in post-transplant, recurrent HBV infection. In this setting, it produces marked improvement in liver function and histology and there is little doubt that this agent represents a major improvement in the therapy of hepatitis B.

Both in the posttransplant setting and when given in trials in patients who have chronic HBV in their own livers (Fig. 48.15), lamivudine has been shown to produce rapid inhibition of HBV DNA production, but this rapidly returns when therapy is stopped. With a 6-month period of therapy, 80% of patients who have chronic HBV became HBV DNA negative.[50] In about 14% of transplant patients a breakthrough was seen in which viral replication started again despite continuation of therapy. This phenomenon has also been seen in patients treated in the nontransplant setting with similar frequency (14% at 1 year),[51] and has been shown to be due to selection of a mutation in the YMDD locus of the HBV polymerase gene,[52] which allows viral replication in the presence of lamivudine.

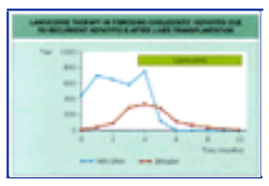

Figure 48-15 Lamivudine therapy in fibrosing cholestatic hepatitis due to recurrent hepatitis B after liver transplantation. HBV, hepatitis B virus. Measurements in arbitary units.

In longer term trials in patients of Asian origin, who have a relatively low probability of response to interferon therapy, almost all treated patients showed prompt inhibition of HBV DNA and, if therapy was continued for 12 months, a sustained inhibition of replication was seen in 35% with seroconversion to HBeAg negativity. This was associated with an improvement of inflammation and a reduction in progression of fibrosis on liver biopsy. Side-effects of treatment are generally mild. Breakthrough occurred in 6% of treated patients. A trial in Hong Kong looking at combination therapy with interferon and lamivudine failed to show any additional benefit from combining therapy, but this remains an area of ongoing study, particularly with the use of pegylated interferon and lamivudine. Lamivudine monotherapy, however, has an important role in the treatment of HBV, its main limitation being the development of viral resistance. Emergence of lamivudine-resistant HBV is increasingly common with prolonged treatment. Genotypic resistance is detectable in 14–32% of patients after 1 year and increases to 38%, 49% and 66% after 2, 3 and 4 years, respectively.[53] [54] The emergence of lamivudine-resistant HBV is not necessarily associated with phenotypic resistance, i.e. loss of clinical benefit. If this occurs, the decision whether to continue with lamivudine or stop treatment depends on the clinical setting. There is no doubt that lamivudine resistance can be associated with severe histological liver damage, particularly in the post-transplant setting. It is, however, clear that YMDD mutants of HBV are generally less replication competent than wild-type HBV, with lower levels of HBV DNA present in serum. This means that therapeutic benefit can continue even if viral replication returns.

The optimal duration of lamivudine therapy remains uncertain. For the majority of patients treated with lamivudine, inhibition of virus occurs with resumption on stopping therapy. In addition, the viral breakthrough means that a significant number of patients will still have active virus despite lamivudine. In patients treated with wild-type HBV, those with e antigen present in serum, the main aim of therapy once viral inhibition has been obtained is to see seroconversion to e antigen negative and e antibody positive. This switch is dependent on the immune recognition of HBV and if it occurs, usually represents an extension of host immunity and an immune environment where continued HBV replication will be either not present or present at much lower levels. If this occurs on therapy there is evidence that treatment with lamivudine can be stopped with a much reduced risk of the resumption of replication. Using available data therefore, approximately 35% of patients will have

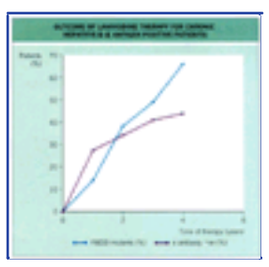

Figure 48-16 Outcome of lamivudine therapy for chronic hepatitis B (e antigen positive patients). e antigen seroconversion and the development of viral resistance both occur.

made this e seroconversion switch after a year of therapy and lamivudine can be stopped with continued monitoring (Fig. 48.16). There remains uncertainty as to the implications for an individual who has seroconverted to e antigen negativity but has also developed YMDD breakthrough and remains HBV DNA positive. In this situation, a trial period off therapy may be appropriate but monitoring will be required.

Factors predicting an initial response to lamivudine therapy (removing HBV DNA from serum initially) have been examined in large clinical trials. The most important predictors of initial response are the pre-treatment HBV DNA level and the degree of inflammatory response.[55] It is clear that patients in the 'immunotolerant' phase of their infection are those least likely to respond to lamivudine as they have very high levels of HBV DNA and minimal or no liver injury. Clearly this is the same group who will not respond to interferon therapy. This group are, however, not ill and can be safely monitored without therapy until the immune response changes. The only indication to try lamivudine in this context would be uncommon situations where the risk of transmission to others was high. The chances of removing viral replication in this group are, however, low.

The other clinical group where uncertainty remains about lamivudine therapy are those with significant liver disease. There is no doubt that in patients with decompensated cirrhosis and ongoing viral replication, lamivudine is effective at suppressing viral activity and that liver function recovers. The only issue is the potential for long-term viral breakthrough in this group which could preclude liver transplantation. This issue is likely to be resolved with the advent of alternative antiviral agents which can be effective in the pretransplant setting.

e Antigen-negative HBV and lamivudine

In Europe, up to 60% of all replicating hepatitis B infections now are in the late phase of infection, where initial inactivation of replication has occurred with later reactivation with so called precore mutant viruses which can presumably avoid the immune response. It is well

described that these mutants are generally much less responsive to therapy with interferon-a. Lamivudine has been shown to be effective at removing HBV DNA from serum and normalizing transaminases. Unfortunately this effect is not maintained in all patients, approximately 50% being HBV DNA positive again in 18 months. This virological breakthrough is progressive in terms of the level of HBV DNA and accompanied by the return of biochemical and histological hepatitis in the majority over time.[56] The management of precore mutant HBV therefore remains imperfect but lamivudine clearly has a role in patients with significant liver injury.

New therapies for HBV

There are a substantial number of new agents which have evidence of activity against HBV; most are nucleoside analogues. The best characterized is adefovir dipivoxil. Adefovir has been shown to produce a rapid decline in HBV DNA in the majority of treated patients and, unlike lamivudine, has shown little development of viral resistance in both wild-type and precore mutant HBV infection over a sustained period.[57] Histological improvement has been shown with this agent and it is a viable alternative for patients who have significant liver injury with lamivudine-resistant virus. Its safety profile is good but significant renal injury has been described with high doses. Entecavir and tenofovir are other agents with proven activity against HBV. Further studies are required to define their exact role (see Chapter 207). [58]

Treatment of HIV and HBV co-infection

With the advent of improved antiretroviral therapy, patients with HIV infection have good immune reconstitution and excellent long-term survival. Co-infection with hepatitis viruses is common and liver disease is now a leading cause of death in HIV-infected people. Hepatitis B infection in this group is challenging to manage. Many patients have been exposed to lamivudine as part of their antiretroviral therapy and have high levels of replication with lamivudine-resistant HBV. In this setting interferon has only a limited role, as the altered immune environment rarely allows clinical response to this agent. Adefovir dipivoxil is effective at reducing HBV DNA in lamivudine-resistant HBV in HIV-infected patients but there are few long-term outcome data (see also Chapter 125). [59]

Hepatitis C

Natural history of hepatitis C virus infection

In order to assess the need for treatment in a patient who has hepatitis C, it is important to have a clear understanding of the natural history of this infection and of the factors that may predispose to more severe outcome. In general, our knowledge is limited because of the relatively recent discovery of HCV. It is, however, clear that HCV-related liver disease is usually slowly progressive, taking many years to produce significant hepatic fibrosis. The most definitive studies to date suggest that the average time from infection to the development of cirrhosis is 33 years.[60] The rate of progression is, however, very variable with some 'rapid fibrosers' progressing to cirrhosis 11 years after infection and other 'slow fibrosers' who would take more than 40 years to progress to cirrhosis. The major factors associated with increased risk of progressive liver disease were age over 40 years at infection, high alcohol consumption and male sex (Table 48.9). Viral genotype may be important in progression but the data are controversial. This is illustrated by the epidemiology of HCV infection in Italy, which can be regarded as two separate outbreaks. The first outbreak, which involved HCV genotype 1, was spread by blood transfusion, infected older people and produced severe liver disease. The second, later outbreak was spread by intravenous drug use and was predominantly due to HCV genotype 3; it appeared to be associated with less severe liver disease. This emphasizes the difficulty of

extracting

TABLE 48-9 -- Factors influencing the progression of hepatitis C virus (HCV) infection.*

FACTORS INFLUENCING THE PROGRESSION OF HCV INFECTION	
Risk factor	**Time from infection to cirrhosis (years)**
Age >40 years	12
Age =40 years	35
Alcohol <50g/day	31
Alcohol >50g/day	24
Male	26
Female	36

** From Poynard et al. [60]*

single risk factors for progression of liver disease from epidemiologic studies because patients infected via transfusion are older as well as being infected by a different viral genotype.

In general, virologic factors have not proved as significant in predicting rates of progression; high viral load and genotype 1 have been proposed as markers of more serious liver disease but evidence that they are directly related to rapid progression of liver disease is lacking.

Management of the patient is usually based on the degree of liver damage as assessed by biopsy.

Liver biopsy

Assessing the need for therapy in hepatitis C is difficult. This is a disease which has a long natural history and an uncertain outcome. Liver biopsy is the only method of directly assessing the degree of inflammation in the liver and the stage of liver disease (fibrosis). All patients who are viremic with abnormal transminases need assessment by liver biopsy. There is a small group of patients who have documented viremia who have persistently normal transaminase levels. In this group, the incidence of serious disease on biopsy appears low.[61] However, the degree of elevation of alanine aminotransferase (ALT) in HCV infection is often minor and it fluctuates. Hence many patients with an initially normal ALT level will have abnormal values if they are followed carefully. In general it seems sensible to have liver biopsy evidence of the stage of disease in all viremic patients. Emerging therapies may alter this requirement. With the very high levels of cure seen with the latest therapies in patients infected with certain viral genotypes, the need for histology may lessen, particularly in young patients with no other risk factors for the development of severe fibrotic liver disease. Most treatment guidelines currently available still rely on assessment of liver histology.

Assessment of liver biopsies

There are two important features in the histologic assessment of hepatitis C. The first is disease stage (fibrosis); the second is the degree of necroinflammatory change. In order to improve the reproducibility of assessment there are a number of scoring systems that have been used to quantify viral hepatitis: the most widely used are the Knodell score[62] and a modification of this, the Ishak score.[63] These scores combine the two elements of assessment of chronic viral liver disease: fibrosis stage and inflammation. The difference between the Ishak and Knodell scores is that Ishak expanded the stage score to allow more discrimination of minor degrees of fibrosis.

Interferon-a therapy

The licensed treatment for hepatitis C is a combination of interferon-a and ribavirin. It is possible to predict response rates in patient populations, but wide variations are seen between patient groups.

Response to interferon-based therapy is defined by measurement of two parameters: serum transaminases and the detection of HCV RNA in serum. Many studies have also examined the effect of interferon therapy on the histologic progression of liver disease.

Interferon-a was first used to treat non-A, non-B hepatitis in the 1980s. Since the identification of HCV in 1989, a large amount of information has become available about the effectiveness of interferon therapy in HCV infection and its drawbacks. There is some evidence that the natural interferons may have slightly more antiviral effect than the recombinant interferons in HCV infection. This difference is, however, marginal at best and in practice there is little difference in effectiveness or side-effects with any of the commercially available variants. These 'standard' interferons have been superseded by the advent of pegylated molecules, which should now be regarded as the standard treatment for HCV infection when given in combination with ribavirin (see also Chapter 207).

Defining response to therapy

There are three potential responses to interferon-based therapy in patients who have HCV infection:

- no response in either biochemical or virologic markers (nonresponders);
- a suppression of HCV RNA and normalization of ALT on therapy but relapse after cessation of therapy (relapsers); or
- a disappearance of HCV RNA and normalization of ALT that is maintained after stopping therapy (sustained responders).

Overall, the chance of sustained response to interferon-a as monotherapy in HCV infection was approximately 20%.[64]

Ribavirin (1-b-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is an oral nucleoside analogue which inhibits replication of a number of viruses. Initial studies of ribavirin in chronic HCV infection showed a lowering of transaminase levels but no effect on viral load when given as monotherapy. The effect on transaminases was also transient, returning to baseline values when therapy stopped. These initial data encouraged trials of combinations of interferon-a and ribavirin.

A landmark study in 1998[65] showed that combining standard interferon-a (3 million units three times per week) with ribavirin (1–1.2g per day on bodyweight) increased overall cure rates from around 20% to 35–40%. This study in the USA and a parallel study in Europe[66] also underlines the critical response factors and established the vital importance of hepatitis C genotype in the prediction of response to therapy. Genotype 1 or 4 infection is more difficult to

TABLE 48-10 -- 'Response factors' in interferon and ribavirin therapy for chronic hepatitis C.*

'RESPONSE FACTORS' IN INTERFERON AND RIBAVIRIN THERAPY FOR CHRONIC HEPATITIS C.			
Factor	**IFN+ribavirin 48 weeks**	**IFN+ribavirin 24 weeks**	**IFN alone 48 weeks**
Genotype 2 or 3	64%	64%	33%
Genotype 1 or 4	31%	18%	11%
HCV RNA $<2 \times 10^6$	47%	44%	31%
HCV RNA $>2 \times 10^6$	40%	28%	13%
Age <40	49%	41%	28%

Age >40	34%	26%	11%
Fibrosis — minor	46%	38%	21%
Fibrosis — major	33%	17%	10%
Female	45%	43%	24%
Male	41%	31%	16%
All percentages relate to sustained virological response.			

** Adapted from Poynard et al.*[66]

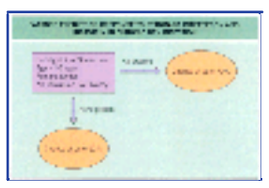

Figure 48-17 Factors predicting response to standard interferon and ribavirin therapy in chronic HCV infection.

treat and requires a longer duration of therapy to obtain its best results (Table 48.10). While genotype of virus remains the major determinant of response to therapy, other factors continue to have an influence (Fig. 48.17) and when planning duration of therapy should be taken into account. Ribavirin therapy does add to the toxicity of interferon. Ribavirin produces a hemolytic anemia in a substantial minority of patients treated. This effect is dose dependent and it is possible to keep patients on therapy by the use of dose reduction (Fig. 48.18). The mechanism of ribavarin's effect on hepatitis C remains unknown. It appears to act predominantly by immunomodulation, rather than as a direct antiviral effect. Its major action seems to be to prevent patients with relatively interferon-sensitive hepatitis C infection relapsing once initial viral clearance has occurred.

The combination of interferon and ribavirin was rapidly accepted as standard therapy. This has now been superseded by the advent of the pegylated interferons. Pegylation involves the attachment of an inert polyethylene glycol molecule to the active molecule, in this case interferon-a. The chemistry of pegylation is complex; there are many sites where the PEG chains can be attached and they have a variety of chain structures. There are two pegylated interferon-a molecules available and they do have substantial differences in their chemistry, if not in clinical effect. Interferon a-2a has been pegylated to form a 40kDa molecule whereas interferon-a-2b is pegylated into a 12kDa molecule. The difference in molecular size has two implications: the 40kDa molecule is excreted in bile and to a lesser extent renally, whereas the smaller molecule is cleared by the kidney. The 12kDa molecule is insoluble and is dispensed as powder for reconstitution whereas the larger is a liquid. Overall half-life in plasma is around 5–7 days with both molecules (Fig. 48.20).

Initial studies with these agents as monotherapy showed that effectiveness was enhanced compared to standard interferon monotherapy (Fig. 48.19).[67] One significant study with the 40kDa molecule showed sustained response rates of over 30% in patients with established cirrhosis (Fig. 48.20), [68] a dramatic change over previously published results. These studies also established that the fibrosis seen in patients with hepatitis C infection could reverse with successful therapy, underlining the fact that all patients with severe fibrotic liver disease should be considered for therapy.

The success with pegylated interferon monotherapy was followed by trials of combination therapy with pegylated interferon and ribavirin.[69] The only fully published data relate to the 12kDa pegylated interferon (Fig. 48.21). These data, using a year of therapy in all patient groups, showed that this combination had significant benefit for patients with genotype 1 infection and had no greater side effects

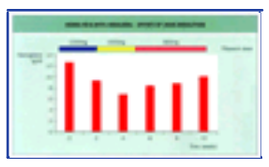

Figure 48-18 Hemolysis with ribavirin — effect of dose reduction.

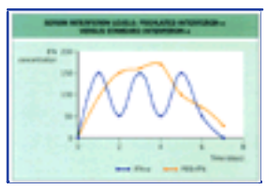

Figure 48-19 Serum interferon levels: pegylated interferon-a versus standard interferon-a.

for those with genotype 2 or 3 infection where effectiveness was similar. Hence with a better method of delivery for patients, this combination of treatments established the new standard of care and for the first time placed clinicians in a position to offer cure to more than 50% of infected patients. Trials of the 40kDa molecule have

Figure 48-20 PEG-interferons as monotherapy in interferon-naive patients with and without cirrhosis.

also been undertaken and results are available in abstract form only at present. The 40kDa molecule showed an overall sustained response rate of 57%, with a significant increment for both genotype 1 and genotype 2 or 3 infection. Results for the two differing interferons have been remarkably similar considering their different structure, with cure rates of 75% or more in genotype 2 or 3 infection and in excess of 40% in genotype 1 infection. The licence approved for the 12kDa molecule extrapolated from the previous interferon and ribavirin studies and gave 6 months therapy for patients with genotype 2 or 3 infection and 12 months for genotype 1 infection. Trials to confirm this are ongoing but most clinicians use these durations in clinical practice. The 40kDa molecule has been trialed in 6 versus 12 month settings and these data confirm that with this therapy there is no advantage for continuing therapy beyond 6 months in good response genotypes but that longer therapy produces worthwhile benefit in genotype 1 or 4 infection. Again these data are not yet fully published.

This great improvement in effectiveness of therapy has encouraged many patients to try treatment. The side-effect profile differs very little from standard interferon and ribavirin therapy, the only changes being a greater degree of injection site reactions. This remains a challenging therapy for patients to take. Compliance has been shown to be important; in an analysis of the 12kDa molecule with ribavirin, patients who were able to comply with therapy (by taking 80% of their medication for 80% of the time[70]) had better

Figure 48-21 Results or therapy with pegylated interferon and ribavirin compared to standard therapy. All based on 12 months' duration of therapy.

response rates than those who did not. Hence encouraging patients to complete the intended duration of therapy is important. This is somewhat easier in patients infected with genotype 2 or 3 infection as they have a high chance of cure with only a 6-month course of treatment required. It is more difficult for genotype 1 or 4-infected patients who have to have 12 months therapy to obtain the best result. About 15% of patients will be nonresponders to pegylated interferon and ribavirin; there is now good evidence to show that the results at week 12 of treatment are able to detect these individuals. The viral kinetics of responders show an initial very rapid decline in viral load, often complete in the first week or two of therapy. In a proportion, this initial decline slows and a second phase of more gradual decline starts. With both PEG-interferons, if the virus has not disappeared from serum at week 12, or at least declined by 2 logs from pretreatment samples, then the chance of a sustained response is almost nil (0% for the 12kDa molecule, 2% for the 40kDa molecule[71]). This allows patients who will not benefit from further therapy to be identified at the 3-month stage of treatment and therapy can be stopped. It also has the advantage of giving significant encouragement to patients who do make this cut-off; if they have responded at this stage their overall chance of cure rises.

Special patient populations

There are limited data as to the effectiveness of PEG-ribavirin in individuals co-infected with HIV. What information is available suggests that therapy with PEG and ribavirin is safe in patients on antiretroviral therapy and can be effective. Immune reconstitution seems important in response; if CD4 counts are normal response rates seem only slightly lower than non-HIV infected patients. Large trials in the USA and Europe are under way (see also Chapter 125 and Chapter 141).

In renal replacement therapy units or after renal transplantation, treatment is difficult. Ribavirin is renally excreted and effectively contraindicated in this setting. PEG interferon monotherapy can be given and has a reasonable chance of success prior to renal transplant but carries significant risks of rejection if used post transplant.

Hepatitis D

Delta virus infection is uncommon and the mainstay of therapy is to inactivate HBV replication in those with active co-infection. For patients with negative HBV DNA interferon-a therapy has been studied with only limited success. With standard interferon given at a dose of 3 million units three times per week, 29% will normalize ALT but most do not lose HDV RNA and relapse at the end of therapy. Higher dose treatment (9 million units three times per week) had a better biochemical and virological response but again most patients relapsed.[72] It is clear that if interferon therapy is given for delta infection a long duration of therapy is required, which few patients can tolerate. There is a case report of resolution of HDV replication after 12 years on interferon[73] but few patients will be so motivated. Studies of lamivudine[74] and famciclovir[75] have shown no benefit. There are as yet no trials of pegylated interferon in delta hepatitis.

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Chapter 49 - Hepatobiliary Infection

Janice Main

LIVER ABSCESSES

PYOGENIC ABSCESS

Pathogenesis and pathology

Bacteria can reach the liver via the portal vein, the systemic circulation, the biliary tree, through the skin (following trauma or trans-hepatic procedures such as liver biopsy) or directly following perforation of a viscus with an ingested fish bone or other sharp object (Fig. 49.1).[1] Pyogenic liver abscesses are usually associated with biliary tree obstruction or gastrointestinal (GI) tract infection such as missed appendicitis, diverticulitis, perforated peptic ulcers, colonic carcinoma or following colonic surgery. The usual organisms are therefore from the GI tract or biliary tree. Liver abscesses are often polymicrobial with Enterobacteriaceae and enterococci predominating. *Streptococcus milleri* is increasingly seen in liver abscesses and staphylococci are not uncommon. *Actinomyces* spp.,[2] *Bartonellae henselae* [3] and *Capnocytophaga* [4] spp. are more unusual isolates from liver abscesses; in immunosuppressed persons infections by *Nocardia* spp.,[5] mycobacteria[6] and fungi such as *Aspergillus* spp. can cause abscess formation. Abscesses can be solitary or multiple and range in size from several centimetres in diameter to microabscesses identified histologically.

Clinical features

Pyogenic liver abscesses are classically seen in elderly patients with underlying GI disease. The patient may complain of right hypochondrial discomfort that is insidious in onset or may present more acutely with feverishness or an acute confusional state. Interventions such as transarterial embolization and percutaneous laser ablation of hepatocellular carcinoma can be complicated by liver abscess[7] and the diagnosis may be missed because fever and discomfort are not uncommon following the procedure. The differential diagnoses include cholecystitis and pyelonephritis.

Diagnosis

Abdominal ultrasound is the simplest way of making the diagnosis. Single or multiple abscesses may be present and the ultrasound may also help identify the original source of the sepsis, such as an obstructed biliary tree. Smaller abscesses not detected on ultrasound may be visible on computed tomography (CT) scanning,[8] which can also help determine the source of sepsis if this is not apparent on the ultrasound. Aspiration of the abscess yields useful microbiologic material and is an important part of therapy. Cytologic examination of smears from the abscess are important to exclude underlying and malignancy.

Blood cultures may also help with the microbiologic diagnosis. The white count is usually elevated and liver function tests may be deranged with elevation of alkaline phosphatase. The presence of a microcytic hyphochromic anemia in an elderly patient, for example, may point to an underlying colonic carcinoma. The patient should also be screened for diabetes and iron overload syndromes should be considered when *Yersinia* spp. are identified. [9]

Management

Drainage of the abscess is important and the favored approach is needle aspiration under ultrasound guidance. This may need to be repeated on several occasions, particularly if there are multiple lesions. Percutaneous catheter drainage is another approach, but is generally only practicable if there is one large and accessible abscess.

Abscesses are often polymicrobial and broad-spectrum intravenous antibiotics are generally recommended. If the abscesses are thought to have occurred as a result of biliary sepsis it is advisable to give antibiotics that achieve good concentrations in bile. Positive blood cultures and cultures from the abscess should help direct antimicrobial chemotherapy. The combination of gentamicin and amoxicillin-clavulanic acid or ticarcillin-clavulanic acid are useful regimens and should be given with metronidazole to broaden the anaerobic cover and if there is concern about possible amebic infection. Piperacillin-tazobactam and the carbapenems are also useful.

Although liver abscesses are more commonly seen in hospital practice the mortality rate has fallen considerably over the past 40 years[10] and this is attributed to advances in imaging and anti-microbial chemotherapy.

AMEBIC LIVER ABSCESS

Pathogenesis and pathology

Entamoeba histolytica reaches the liver via the portal vein and causes necrosis of the liver parenchyma. This is a complication in up to 10% of cases of amebic colitis.

The necrosis leads to liquefaction of the liver and the characteristic thick reddish brown pus, which is said to resemble anchovy sauce. On microscopy, hepatocytes, neutrophils and red blood cells are evident. Host factors can influence outcome. Secondary bacterial infection can occur and can complicate the clinical picture and cause confusion with pyogenic liver abscess.

Clinical features

Clinical features of amebic and pyogenic abscesses are compared in Table 49.1 . An amebic abscess can develop many years after travel to tropical areas and the long latency period has not been adequately explained. Cases have been described 30 years after the initial bowel infection. The patient may complain of right hypochondrial pain and fever. Lesions near the diaphragm can cause referred right shoulder discomfort. Low-grade fever may be present. More acute presentations with rigors have been reported. An abscess encroaching on the biliary tree may cause some biliary obstruction and mild jaundice, but deep jaundice is unusual. Right hypochondrial tenderness may be present and rarely a swelling may be evident in the epigastrium or right hypochondrium. There may be elevation of the right hemidiaphragm or a pleural effusion and therefore reduced expansion,

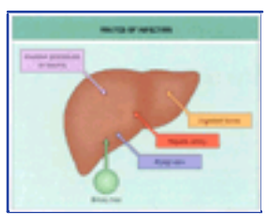

Figure 49-1 Routes of infection.

TABLE 49-1 -- Features of pyogenic and amebic liver abscess.

FEATURES OF PYOGENIC AND AMEBIC LIVER ABSCESS		
	Pyogenic	**Amebic**
Patients	Elderly, underlying gastrointestinal or biliary tract disease	Much more common in males than females
Imaging	Single or multiple abscesses	Solitary abscess right lobe
Pathogens	Polymicrobial, Enterobacteriaceae, enterococci	*Entamoeba histolytica*
Other tests	Blood cultures	Amebic immunofluorescent antibody test
Treatment	Broad-spectrum antimicrobials, aspiration	Metronidazole, diloxanide +/- aspiration

dullness to percussion and reduced air entry. Rarely an abscess can rupture within the lung or pericardium.

Diagnosis

An ultrasound examination will demonstrate the presence of an abscess, which is typically large and located in the right lobe of the liver. Smaller abscesses may be present. Abscesses can also be demonstrated on CT scanning or magnetic resonance imaging (MRI) of the liver. Aspiration may reveal the characteristics 'anchovy sauce' appearance, and although it is unusual to demonstrate amebae on microscopy, microscopy and culture are important to exclude a pyogenic liver abscess or secondary infection of an amebic abscess. A bleed into an underlying malignant lesion can mimic the symptoms of an amebic abscess. Cytology can demonstrate the presence of malignant cells and point to the diagnosis of an underlying metastatic lesion or primary hepatocellular carcinoma.

In patients presenting acutely it important to repeat the ultrasound a few days later if initially negative.

The chest radiograph may demonstrate elevation of the right hemidiaphragm or the presence of a pleural effusion.

The liver function tests may be normal, but there may be an elevation of the alkaline phosphatase and bilirubin and a large abscess can have a compressive effect on the intrahepatic bililary tree.

TABLE 49-2 -- Major causes of granulomatous liver disease.

MAJOR CAUSES OF GRANULOMATOUS LIVER DISEASE
Bacteria
Mycobacteria (*Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Mycobacterium leprae*)
Brucella spp.
Listeria spp.
Tropheryma whipplei
Yersinia spp.
Treponema pallidum
Viruses
Cytomegalovirus
Epstein-Barr virus
Hepatitis A and C viruses
Rickettsiae
Coxiella burnetii
Rickettsia conori, Rickettsia typhi
Protozoa
Leishmania spp.
Worms
Schistosoma spp.
Toxocara spp.
Fungi
Histoplasma spp.
Coccidioides spp.
Drugs
Primary liver disease (e.g. primary biliary cirrhosis)
Neoplasms (e.g. lymphoma)
Diseases of unknown cause (e.g. sarcoidosis, inflammatory bowel disease)

Treatment

Metronidazole followed by diloxanide to remove luminal cysts is the treatment of choice. In patients with large amebic abscesses aspiration with metronidazole hastens recovery,[11] but medical treatment alone is effective in over 90% of cases.[12] (See also Chapter 164 .)

DIFFUSE PARENCHYMAL INVOLVEMENT

The liver may be involved in many systemic infections. Viral infection has been described elsewhere (Chapter 48), but acute hepatitis has been described with several bacterial pathogens including meningococci, *Salmonella typhi*, *Listeria*, *Campylobacter*, *Borrelia* and *Brucella* spp.[13] [14] Septicemia and other causes of circulatory collapse can also cause hepatic ischemia, which can be associated with high transaminase values.[15] Infections account for many of the main causes of granulomatous liver disease (Table 49.2). Liver involvement and symptoms of liver disease can be a major part of the following infections:

MYCOBACTERIAL INFECTIONS

Tuberculosis of the liver

The liver is generally involved in miliary tuberculosis and the liver biopsy may help establish a diagnosis of tuberculosis in a patient with unexplained fever.

Pathology

Tuberculosis of the liver may present as an abscess[6] or more diffuse disease and liver biopsy may reveal nonspecific hepatitis or granulomatous

liver disease. Rarely tuberculomas may be present or enlarged intrabdominal lymph nodes can cause compression of the biliary tree and the patient presents with features of biliary tract obstruction.

Clinical features

Fever and weight loss are the usual symptoms. The patient may appear wasted. Hepatomegaly may be evident, but massive hepatomegaly is unusual. In severe tuberculosis infection the patient may present with liver failure.

Diagnosis

Elevated alkaline phosphatase levels are usually present and the albumin levels may be low if the disease has been longstanding. Anemia and elevation of the erythrocyte sedimentation rate and C-reactive protein are common. A chest radiograph is important to exclude active pulmonary disease, which is evident in a minority of cases.[16] The abdominal ultrasound may demonstrate intrabdominal lymphadenopathy or evidence of peritoneal involvement. Liver biopsy may be diagnostic, but caseating granulomas with acid-fast bacilli are demonstrable in less than 10% of cases. The appearances may be nonspecific and it is important to culture some of the liver biopsy material. It is hoped that rapid diagnostic methods such as polymerase chain reaction will facilitate diagnosis in the future.[17]

Management

Standard antimycobacterial therapy should be given (see Chapter 37 , Chapter 202). Liver function tests should be monitored as with standard therapy. As the patient improves the elevated alkaline phosphatase gradually returns to normal. Elevation in the transaminase level may be a sign of drug toxicity. Fever may persist for several weeks, but if the patient fails to improve then the possibilities of resistant *Mycobacterium tuberculosis* or atypical mycobacteria have to be considered.

Atypical mycobacteria

Atypical mycobacterial infection of the liver is usually seen in the setting of immunosuppression, particularly underlying HIV infection[18] or congenital immunodeficiency. Again culture of the biopsy material is important to establish the diagnosis, but the appearance of many organisms with poorly formed granulomas may be an important clue to atypical mycobacterial infection in an immunocompromised host.

SYPHILIS

The liver is involved with congenital, secondary and later stages of syphilis.

The standard screening tests usually confirm the diagnosis of congenital infection, but the diagnosis should also be considered in the setting of undiagnosed acute hepatitis in adults.[19] This is discussed in detail in Chapter 75 and Chapter 230 .

LEPTOSPIROSIS (see also Chapter 181)

Epidemiology

Several *Leptospira* species are associated with human disease, but *L. icterohaemorrhagiae* is specifically associated with Weil's disease. The main source of this pathogen is rat urine and it is therefore an occupational hazard for farm and sewage workers and those who use contaminated waters for recreational purposes such as rowing.

Pathology

The liver demonstrates cholestasis with swelling of hepatocytes, but very little necrosis. In classic Weil's disease renal disease is evident with acute tubular necrosis. There may be evidence of multifocal hemorrhage, particularly within skeletal and cardiac muscle.

Prevention

The use of protective clothing can help prevent occupational exposure and it has been recommended that sewage workers carry cards to remind clinicians of the possibility of leptospirosis should they become ill. Prophylactic antimicrobials such as doxycycline[20] have been suggested for those at risk of exposure through work or leisure activities such as canoeing, although this is not generally recommended.

Clinical features

The patient's occupation or leisure activities may point to the diagnosis.

In common with other spirochaetal infections there are several characteristic stages of the disease process. The severity varies from a minor influenza type illness to life-threatening multisystem failure. The incubation period ranges from two to 17 days.

The first (septicemic) stage is of a multisystem disease. It is abrupt in onset. Features may include:

- high fever,
- myalgia (often severe),

- abdominal pain,
- nausea,
- vomiting,
- severe headache and meningitis (CSF leukocytosis and an elevated protein level),
- pneumonitis,
- conjunctivitis,
- hepatomegaly,
- jaundice (said to be an ominous sign),
- bleeding (thought to be mainly related to capillary damage and leading to ecchymoses, gastrointestinal bleeding and on occasion intracerebral bleeding).

Urinalysis reveals proteinuria and bilirubinuria. The white count is usually elevated with mainly polymorphonuclear cells (it may be as high as 30,000/ml or 3×10^9 /l). Thrombocytopenia may be present. The liver function tests demonstrate elevated levels of bilirubin and alkaline phosphatase. In contrast to fulminant viral hepatitis the transaminase values may be normal or moderately elevated.

During the second stage or 'immune stage' the pyrexia generally resolves, but life-threatening disease may be evident. There may be signs of myocardial involvement with arrhythmias and nonspecific ECG changes. Blood tests confirm deteriorating liver function with evidence of muscle damage (elevated muscle enzymes). Worsening renal function is also a feature with elevations in the serum creatinine and proteinuria.

In the third (convalescent) stage a steady clinical and biochemical improvement occurs with resolution of the liver and renal failure and improved myocardial function. Minor relapses of symptoms can occur at this stage with further episodes of myalgia and spikes of fever.

Diagnosis

During the first phase of the disease leptospira may be found on examination of the blood film or grown from blood cultures. By the second phase the blood cultures are likely to be negative. Leptospira may be evident on urine microscopy, but the diagnosis is more likely to be made from the serologic tests for antileptospira antibodies, which are found in increasing titres in the convalescent stage of the disease.

At the time of the initial presentation, although the symptoms and jaundice may suggest a viral hepatitis, the high fever and leukocytosis

would be unusual for viral etiology, but similar hepatitic illnesses with or without renal failure have been described with hantaviruses.[21] [22]

Management

Most patients with leptospirosis are not seriously ill, but for those with Weil's disease full supportive care may be required with support and hemodialysis in an intensive care unit. High-dose intravenous penicillin is the treatment of choice, but the dose may need modification according to the renal function. A febrile reaction, similar to the Jarisch-Herxheimer reaction, can occur.

HELMINTHIC PARASITES OF THE LIVER

SCHISTOSOMIASIS (see also Chapter 167)

Schistosomal ova can reach the liver via the mesenteric veins and portal system. Liver involvement is particularly seen with *Schistosoma japonicum* and *Schistosoma mansoni*,, but can also occur with *Schistosoma haematobium*. In the early stages a granulomatous reaction is seen, but over years, if untreated, extensive collagen deposition can lead to portal fibrosis with the development of portal hypertension and splenomegaly (Fig. 49.2).

The diagnosis of schistosomal liver disease may only come to light when the patient presents with features of portal hypertension and, for example, has a variceal bleed.

Diagnosis

The diagnosis may be made from the serologic test — schistosomal enzyme-linked immunosorbent assay — or the histologic appearances of the granulomatous liver disease in the early stages and the fibrosis in the later stages. In the early stages a peripheral eosinophilia may be evident.

Management

Praziquantel is the treatment of choice. Patients should be warned that an influenza-like like illness can follow therapy. Supportive therapy is required for the patient with severe fibrosis and liver transplantation may be required in advanced cases.

HYDATID INFECTION (see also Chapter 169)

Hydatid cysts can follow infection with the *Echinococcus* tapeworm.

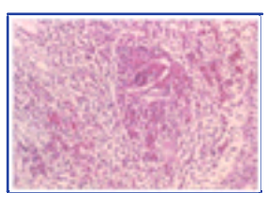

Figure 49-2 Schistosomiasis of the liver. A refractile schistosome ova is located in a portal tract and is associated with an eosinophil-rich granulomatous inflammatory reaction.

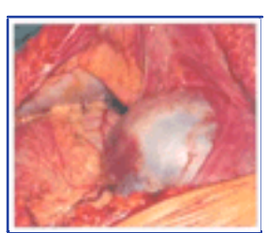

Figure 49-3 Hydatid disease. Hydatid cyst of the liver.

Epidemiology

The disease is widespread and man is typically infected by close contact with dogs which have become infected by the consumption of eggs from infected meat. Echinococcal infection is endemic in the main sheep farming areas of the world and is a particular concern throughout much of Europe, the Mediterranean littoral, Asia, South America and Kenya.

Pathology

The liver is the most frequent site for hydatid cysts (Fig. 49.3).

The ova enter the liver via the portal vein and lead to cyst formation within the liver parenchyma — classically involving the right lobe of the liver on the inferior surface. Rupture of a cyst, spontaneously or following trauma or surgical procedures, can lead to cardiovascular collapse. This is thought to be an inappropriate immune response to released hydatid antigens. Rupture of the cysts can also lead to infection into the biliary tree, the peritoneum, lungs and pleura.

Clinical features

Hydatid disease may be asymptomatic and liver cysts found incidentally on ultrasound examination, as calcified lesions on plain abdominal radiographs or at autopsy. Hepatomegaly may be present. The cyst may be diagnosed after ruptures or if secondary infection of the cyst occurs. Cysts can also be found within the brain, lungs, kidney and the heart (Fig. 49.4).

Diagnosis

The hydatid serology test is particularly useful although false-positive and -negative results can occur. A peripheral eosinophilia may be present. Several radiologic features can be helpful in making the diagnosis. Plain abdominal radiographs may reveal calcified

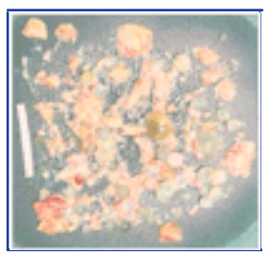

Figure 49-4 Hydatid disease. Hydatid 'daughter cysts'.

spherical structures within the liver. Ultrasound examination can often reveal several forms of the cysts, which can be single, multiple, thin- or thick-walled. CT scanning can detect smaller lesions and the presence of calcification. It may difficult to differentiate a single cystic lesion from a tumor. Biopsy or aspiration of the cysts may be dangerous in view of the risk of antigen leakage and immune response and should be performed only when there is appropriate backup to treat circulatory collapse and laryngeal edema.

Management

The management of hepatic hydatidosis remains controversial and many approaches have been tried. It is recognized that by itself antimicrobial therapy with albendazole, mebendazole or praziquantel is generally ineffective and should be combined with a drainage procedure. Percutaneous drainage has the advantage of avoiding major surgery, but in open surgical procedures peritoneal contamination is thought to be minimized by packing the surgical field with povidone-iodine swabs, decompressing the cysts by aspiration and then removing the cyst contents. Some surgeons advocate the use of injecting formalin or hypertonic saline into the cysts following decompression. In one study,[23] 50 patients with hepatic hydatidosis were randomized to receive either percutaneous aspiration and albendazole or cystectomy. Similar efficacies were demonstrated in both treatment groups, but the open surgical procedure was associated with greater morbidity. Puncture, aspiration and the use of alcohol and polidocanol as sclerosing agents are being increasingly used.[24]

ASCARIASIS

Rarely the large *Ascaris* roundworm can migrate up through the bile duct and cause biliary obstruction. More commonly the liver is involved as a result of ova invading the liver via the portal vein and setting up a localized granulomatous reaction.

Liver involvement is usually asymptomatic unless rare complications such as biliary obstruction, hemobilia or liver abscesses develop.

Worms within the biliary tree may show up as motile linear lesions on ultrasound or endoscopic retrograde cholangiopancreatography (ERCP).

TOXOCARA

Toxocariasis is a further cause of hepatic granulomas. The recommended therapy is thiabendazole.

LIVER FLUKES

Liver flukes are thought to invade the liver from the peritoneal cavity and migrate through the liver parenchyma to the biliary tree where an inflammatory reaction develops.

CLONORCHIS SINENSIS

Infection with *Clonorchis sinensis* is usually associated with the consumption of raw or undercooked fish in Asia. The flukes live within capillaries of the biliary tree and the inflammatory reaction can cause obstruction and encourage cholelithiasis. Cholangitis is a common complication and the ongoing inflammation and fibrotic reaction is thought to predispose the patient to the development of cholangiosarcoma.[25]

Diagnosis

Flukes may be evident on radiologic imaging such as percutaneous transhepatic cholangiography or ERCP. This diagnosis must be considered when atypical cholangiopathy is diagnosed in patients of eastern origin. The diagnosis is confirmed by finding the ova on stool microscopy.

Management

Praziquantel is the treatment of choice. Surgical or endoscopic approaches may be required to deal with associated cholelithiasis and biliary obstruction.

FASCIOLA HEPATICA

Infection with this common sheep fluke can follow consumption of contaminated watercress and is recorded throughout Europe, South America, the Caribbean, Africa and China.

Pathology

The picture is usually of biliary tract infection and obstruction, but hepatic granulomatous reactions can also occur.

Clinical features

In the early stages of infection the patient may complain of fever and right hypochondrial pain. Hepatomegaly may be present. The migration of the flukes within the biliary tree can lead to inflammatory and fibrotic reactions. Cholelithiasis can occur and the clinical picture may be of a bacterial cholangitis. In common with bacterial cholangitis the alkaline phosphatase may be elevated, but the presence of an eosinophilia should point to the possibility of a fluke infection.

Diagnosis

The diagnosis may be suggested by a history of watercress consumption and features of cholangitis and an eosinophilia. Praziquantel is the therapy of choice and surgical and endoscopic intervention may be required to deal with biliary tree obstruction.

PROTOZOAL INFECTIONS

LEISHMANIASIS (see also Chapter 172)

Visceral leishmaniasis or kala-azar can lead to massive hepatosplenomegaly.

Epidemiology

Leishmanial infection is transmitted by sandflies and is reported particularly around the Mediterranean basin, in South America and Asia.

Pathology

Host factors are thought to be important in determining the disease outcome following leishmanial infection. It is not known why some patients develop only localized lesions and others develop more serious systemic disease. Underlying HIV infection is associated with multisystem disease and reactivation of previous infections.[26]

Clinical features

The patient may present with fever or anemia. Massive hepatosplenomegaly may be present.

Diagnosis

The presence of organisms may be detected by bone marrow examination, liver biopsy or splenic aspiration. Hypergammaglobulinemia is often present. Serologic tests are often useful, but antibody tests may be negative in patients with HIV infection or other types of immunodeficiency.

Management

Therapy of leishmaniasis is somewhat limited by the toxicity of many of the antimicrobial agents. Antimonial compounds, for example, can

cause pancreatitis. Liposomal and other preparations of amphotericin are being increasingly used with success. In the setting of immunosuppression maintenance regimens are often required with regular administration of amphotericin B or intravenous pentamidine.

MALARIA

During acute malaria the parasitic load within the reticuloendothelial system and particularly the liver may be large and this may result in hepatic dysfunction. This is thought to predispose the patient with malaria to bacteremia. Mild elevation of the transaminase values may occur, but hyperbilirubinemia is mainly associated with the hemolysis of malaria rather than hepatic dysfunction.

TOXOPLASMA

Mild hepatitis or granulomatous liver disease has been reported with both primary and reactivation forms of toxoplasmosis infection.

FUNGAL INFECTIONS

The liver may be involved as part of a systemic fungal infection in a patient with candidemia.[27] Cryptococcal, histoplasmosis and pneumocystis infection of the liver are mainly seen in patients with HIV infection.

Aspergillus infection of the liver with abscess formation is mainly seen in patients with neutropenia as a result of chemotherapy.

BILIARY TREE INFECTIONS

ACUTE CHOLECYSTITIS

Pathology

Gallbladder infections are usually the results of gallstone formation and impaction within the cystic duct[28] with impaired biliary drainage leading to infection, edema and compressive effects on the local blood supply, which can cause gangrene of the gallbladder. Suppuration within the gallbladder can lead to bacteremia, septicemia, cholangitis and liver abscess formation. Acalculous cholecystitis can follow infection with *Salmonella* or *Campylobacter* spp. or can occur in acutely ill patients following major surgery or burns. In the setting of HIV infection, infection with *Salmonella* or *Campylobacter* spp. may be implicated or the gallbladder disease may be a feature of HIV cholangiopathy, [29] which is often associated with cytomegalovirus infection, cryptosporidiosis, microsporidiosis or lymphoma.

Clinical features

The patient may describe right hypochrondrial discomfort, which can occur in waves. Radiation to the right shoulder is common. Feverishness or rigors can occur and the patient may develop bacteremia and septicemia. Right hypochondrial tenderness is usually present and the gallbladder may be palpable in one third of cases. A degree of jaundice may be present depending on the extent of biliary tree obstruction.

Diagnosis

The diagnosis is usually made clinically, but ultrasound of the liver and gallbladder may demonstrate gallstones with thickening of the gallbladder wall or dilatation of the biliary tree in the presence of obstruction. Laboratory findings include an elevated white cell count, hyperbilirubinemia and elevated alkaline phosphatase. There may be modest elevations of the transaminase values. Impaired biliary drainage because of pancreatic carcinoma can also cause dilatation of the biliary tree and ERCP may be required to delineate the anatomy and to facilitate drainage if required. Magnetic resonance cholangiopancreatography (MRCP) is a helpful, noninvasive imaging technique useful in visualizing the biliary tract.[30]

Management

The priorities are to facilitate drainage of the biliary tree and to treat infection. Cholecystectomy, when performed by the open technique, has a high mortality and morbidity rate in patients with acute cholecystitis and the usual practice was for conservative management of the acute illness and elective cholecystectomy some weeks later. This, however, required two hospital admissions and the emergency readmission rate was high for these patients.[31] Laparascopic cholecystectomy is increasingly performed early in patients with acute cholecystitis[32] and appears successful, even when gangrenous cholecystitis[33] is present. For frail patients who are unfit for surgery ERCP can be both life-saving and diagnostic, with endoscopic removal of stones by sphincterotomy, basket or balloon removal and stent insertion. Where ERCP is not available or not technically feasible percutaneous biliary drainage can be performed.

Elective cholecystectomy can generally be performed subsequently, but emergency surgery may be required if the situation is complicated by a gangrenous gallbladder or poor response to antimicrobial therapy.[34]

Antimicrobial therapy is generally prescribed to cover Gram-negative bacilli, enterococci and anaerobes, although the importance of enterococcal infection is debated in this setting.[35] Piperacillin-tazobactam or ticarcillin-clavulanic acid are therefore useful. Aminoglycosides must be used carefully in this setting, particularly in the elderly because there is a high incidence of renal impairment in patients with biliary tree sepsis. Cephalosporins and quinolones do not adequately cover enterococcal infection. Antimicrobial prophylaxis is also advised for procedures involving an obstructed biliary system.

CHOLANGITIS

Pathology

Bacterial cholangitis generally results from infection of an obstructed biliary tree and is most commonly caused by gallstones obstructing the common bile duct.[36] Other predisposing causes include congenital biliary tract disorders, sclerosing cholangitis, HIV cholangiopathy and bile duct strictures.

Clinical features

The symptoms and signs are generally similar to acute cholecystitis, but the patient may have no history of abdominal pain and present with septicemia.

Diagnosis

Bacteremia has been reported in half the cases of cholangitis. Blood cultures are important investigations. The alkaline phosphatase and bilirubin are usually elevated. Ultrasound may show dilatation of the biliary tree with stones, but the bile duct is often difficult to visualize and other imaging techniques, such as endoscopic ultrasound and MRCP[37] may be required. ERCP is useful for diagnosis when the patient has been stabilized.

Management

As with cholecystitis the priorities are supportive care for the patient with the appropriate antimicrobial chemotherapy and drainage of the obstructed biliary system. The mortality rate when endoscopic sphincterotomy is used is lower [38] than with conventional surgical techniques.

CHRONIC CHOLECYSTITIS

Chronic cholecystitis is an unusual condition. Again gallstones are commonly seen. Chronic cholecystitis may be seen following infection with *Salmonella* or *Campylobacter* spp.

Management

Elective cholecystectomy is the treatment of choice. Laparoscopic cholecystectomy is increasingly used for gallstone disease and acalculous cholecystitis and is associated with lower morbidity and mortality rates.

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Chapter 50 - Practice Points

50.a Management of an outbreak of gastroenteritis

Hilary Babcock

It is estimated that 76 million people contract food-borne illnesses annually in the USA. The incidence of the most common bacterial gastrointestinal infections has been decreasing steadily since 1996.[1] Outbreaks of gastroenteritis are still frequent, however, and can be both disruptive and disturbing to the community where they occur. This era of increasingly global mobility has stretched the definition of 'community' as well, so that the guests at a wedding in Smalltown, Ohio, who all contracted *Salmonella* from the chicken salad, might be found 3 days later all over the country and all over the globe.

Investigating these outbreaks serves several functions. First, investigations can suggest both short- and long-term control measures, preventing further cases from occurring during an ongoing outbreak and preventing further outbreaks in the future. Any members of the wedding party who took home left-over chicken salad can be prevented from sharing it with their family and the caterer can be better educated about handling raw chicken so that future weddings will not suffer the same aftermath. Second, outbreak investigations can identify new pathogens or provide new information about transmission, natural history and management of known pathogens. Third, the information generated in these investigations can be used by epidemiologists locally, nationally and internationally to monitor trends in specific infections over time and to guide changes in food handling regulations to improve public safety in the future.[2]

Common causes of food-borne outbreaks of gastroenteritis

Table 50a.1 and Table 50a.2 summarize the major causes of gastroenteritis outbreaks and provide clinical and epidemiologic information on each one. More detailed information on these diagnoses is available in Chapter 93 .

Identification of an outbreak

An 'outbreak' is defined as an increase in the incidence of a given disease or symptom complex clearly in excess of the usual rate. An outbreak may be brought to attention by clinicians noticing an unusually high number of patients with similar complaints, by laboratory workers noticing an unusually high number of isolates of a particular organism, or by members of the public noting an unusual number of cases of illness among friends or family (or the wedding party). The first goal of the outbreak investigation is to verify that an outbreak is occurring. This verification requires establishment of a prior baseline rate of illness or disease and confirmation that the purported cases all have the disease of interest. For example, laboratory contamination of multiple cultures with the same organism represents a different problem than an outbreak of clinical disease in the community. Co-ordination with local health departments, infection control specialists, infectious disease physicians and laboratory directors is crucial for these investigations.

Epidemiologic investigation

The first step in evaluating a verified outbreak is to generate case definitions. Multiple definitions may be necessary. A definite case, or laboratory confirmed case, is usually a person who has a clinically compatible symptom complex as well as laboratory-confirmed evidence of a specific disease. In cases of gastroenteritis, not all subjects may have undergone stool testing and not all microbes are easily identified. These subjects may be labeled clinical cases or probable cases. Once the source of the outbreak is identified, cases may also be divided into primary cases, acquired directly from the source, and secondary cases, acquired later from person-to-person transmission.

Once cases have been identified, an 'epi curve' can be generated with the date of onset of each case's symptoms. This exercise can help to determine the timing of the exposure and the incubation period of the infection. The shape of the curve will also help to determine the type of outbreak, by transmission pattern. Outbreaks can be divided into three types by source of exposure:

- ¦ Point source: exposure occurs once, at a single time and place, although possibly to multiple subjects. Example: contaminated chicken salad at a wedding reception (Fig. 50a.1);
- ¦ Common source: there is a single source of exposure but the source is available over a period of time. Example: a food handler who has hepatitis A prepares contaminated food at a restaurant. All subjects patronizing that restaurant while the food handler is working there will be exposed (Fig. 50a.2); and
- ¦ Person-to-person/propagative/ongoing transmission: after the initial exposure, infected subjects can pass on the infection to 'unexposed' subjects (Fig. 50a.3).

A combination of these types is also possible.

Determining the exposure source in an outbreak of gastroenteritis requires eliciting careful and detailed food histories from all affected subjects. The Centers for Disease Control and Prevention have on their web site an 'Outbreak Investigation Toolkit' that includes an

TABLE 50.a-1 -- Types of food poisoning.

TYPES OF FOOD POISONING			
Infection	**Incubation period**	**Major symptoms**	**Outbreak pattern**
Clostridium perfringens	18–36 hours	Watery diarrhea and colicky abdominal pain	1
Norwalk agent (small, round-structured viruses)	24–48 hours	Vomiting, diarrhea, myalgias, headache	1, 2, 3
Scombrotoxic poisoning	1–4 hours	Histamine poisoning (headache, flushing, urticaria, swelling, tingling lips)	1
Diarrhetic shellfish poisoning	<1 day	Acute diarrhea (severity proportional to dose of toxin)	1, 2
Paralytic shellfish poisoning	4–6 hours (?)	Bradycardia, parasthesia, muscle weakness	1, 2
Salmonellosis	18–36 hours	Diarrhea, vomiting	1, 2, 3
Bacillus cereus toxin (rapid)	1–6 hours	Nausea, precipitant vomiting	1, 2
Bacillus cereus toxin (late)	10–12 hours	Abdominal pain, profuse watery diarrhea, nausea	1, 2
Clostridium botulinum	24–48 hours	Cranial nerve palsy followed by general paralysis	1
Bean hemagglutinins	Minutes after ingestion	Severe vomiting, profuse diarrhea	1
Shigellosis	1–3 days	Fever prodrome, colic and diarrhea, occasional hyperpyrexia in children	2, 3

Escherichia coli 0157, verotoxin producing *E. coli* organisms	1–2 days (up to 5 days)	Brisk watery diarrhea, bloody stools, abdominal pain	1
Food-poisoning *Vibrio* spp.	5–92 hours	Watery diarrhea and colicky abdominal pain	1, 2
Yersinia enterocolitica	3–7 days	Mild-to-moderate gastroenteritis, aching abdominal pain	1, 2
Staphylococcal toxin	30 minutes to 6 hours	Nausea, precipitant vomiting	1
Campylobacter spp.	2–4 days (maximum 8–9 days)	Diarrhea, vomiting, abdominal pain, blood *per rectum*	1, 2, 3
Outbreak patterns: 1, point source; 2, common source; 3, person-to-person.			

TABLE 50.a-2 -- Complications of food poisoning.

COMPLICATIONS OF FOOD POISONING			
Infection	**Complications**	**Patients who have complications (%)**	**Isolation needed**
Bacillus cereus toxin (rapid)	Dehydration, occasional shock	Unusual	No
Bacillus cereus toxin (late)	No direct complications	-	No
Bean hemagglutinins	Dehydration, shock	Rare	No
Campylobacter spp.	Prolonged symptom, potential for relapse, bacteremia, focal sepsis, rarely Guillain-Barré syndrome	10–20% ill for >1 week, 5–10% relapse, bacteremia in <1%	Yes
Clostridium botulinum	Full requirement for respiratory support	50–80%	No
Clostridium perfringens	Dehydration, occasional shock	Elderly and compromised	Yes
Diarrhetic shellfish poisoning	No complications	-	No
Escherichia coli 0157, verotoxin producing *E. coli* organisms	Hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura	2–8%	Yes
Food-poisoning *Vibrio* spp.	No complications	-	Yes
Norwalk agent (small, round-structured viruses)	Not recorded	-	Yes
Paralytic shelfish poisoning	Death	Rare	No
Salmonellosis	Sepsis, reactive arthritis, distant focal collection, osteomyelitis in sickle cell disease, meningitis in infants	5% sepsis, 2% reactive arthritis	Yes
Scombrotoxic poisoning	Hypotensive crisis	<1%	No
Shigellosis	Osteomyelitis in sickle cell disease	Rare	Yes
Staphylococcal toxin	Shock, occasional death	<1%	No
Yersinia enterocolitica	Postinfectious arthritis, erythema nodosum	Arthritis 10–30%, erythema nodosum 30%	Yes

extensive 'Standard Foodborne Disease Outbreak Case Questionnaire' that can be very helpful (www.cdc.gov/ncidod/dbmd/outbreak/). Travel and event (e.g. weddings, parties) histories, as well as names of specific restaurants, should also be obtained when possible. If a specific etiologic agent is known or suspected, the incubation period of that pathogen can guide the timeframe for the food history.

In cases with a point source outbreak, any remaining food products should be collected and saved for possible testing. In common source outbreaks, where exposure may be ongoing, collecting

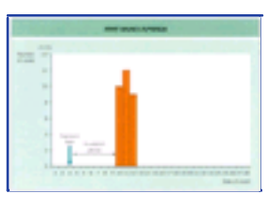

Figure 50.a-1 Point source outbreak.

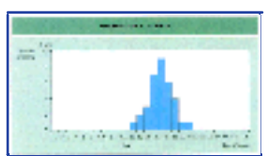

Figure 50.a-2 Common source outbreak.

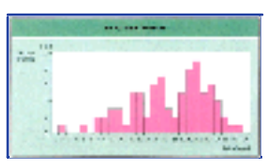

Figure 50.a-3 Propagative outbreak.

environmental and food samples from the suspected source location may confirm the etiology. Occasionally, the source of the infection can be confirmed with positive cultures from the incriminated food source. Negative cultures from food or other sources, however, should not be used to exclude potential sources as techniques of culture from disparate materials are not well standardized and may not be reliable. A positive source is not the end of the investigation either, as the infected food item may have been contaminated by a worker who has since prepared other items. Medical histories focused on compatible symptoms should be elicited from workers who prepared any implicated food items, if they are not already identified as cases.

From the elicited histories, multiple hypotheses may be made, evaluated and discarded. Comparison with unaffected individuals can be helpful in testing promising hypotheses. Often, histories are elicited from unaffected family members, work colleagues or guests of an event. From these histories a case-control study can be performed to analyze statistically the association of illness with specific exposures. Depending on the size of the outbreak and the number of cases and controls available, the case-control analysis may not have the power to demonstrate a statistically significant association. In those settings, other components of the analysis can be helpful in making a causal inference. These factors include the magnitude of the association, the temporal sequence of exposure and disease, any dose-response effect and the biological plausibility of the hypothesis.

Management of the outbreak

Management of the suspected source of the outbreak can be difficult. Witnessing new subjects becoming infected while information is being collected can be frustrating both to the investigators and to the public. However, announcing the inspection or closure of an individual food establishment or the recall of a food item can have profound legal and economic consequences. Management of the public announcement of any findings must also be handled carefully. Adequate but appropriate communication is necessary to ensure public health protection. A single designated media spokesperson is often helpful in the delivery of a coherent and co-ordinated message. Once the source is identified, prompt control measures must be instituted to prevent further exposures. In cases where the investigation reveals the potential for widespread effects, involvement of state and national agencies may be necessary. If the chicken salad at the wedding was contaminated by tarragon imported from another country, international co-operation may be required.

Practical arrangements for large scale outbreaks include ensuring adequate bed and staffing availability in local hospitals, emergency rooms and clinics; adequate communication of etiologic findings, presenting symptoms and clinical management suggestions to local clinicians; and co-ordination with state and local authorities.

Conclusions

Prior investigations of outbreaks of gastroenteritis have implicated a hepatitis-A-infected pastry chef who hand-glazed the pastry,[3] a food service worker infected with a Norwalk-like virus who prepared contaminated deli sandwiches,[4] cheese curds inadvertently made with unpasteurized milk contaminated with *Escherichia coli* 0157:H7,[5] a Komodo dragon at the zoo with *Salmonella* infection,[6] and lasagne contaminated with *Salmonella enteritidis*.[7] Each investigation is an opportunity to prevent further acute illnesses and to gather information helpful in preventing similar outbreaks in the future.

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50.b Management of persistent postinfectious diarrhea in adults

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Introduction

The presence of diarrhea beyond 2 weeks of a confirmed or presumed infectious exposure is a useful working definition of persistent diarrhea as this helps to exclude most common acute bacterial and viral infections, although protozoal infections may persist for longer. Patients who have persistent diarrhea who have recently returned from a tropical or developing country can also be considered as having postinfectious diarrhea.

Pathogenesis and clinical features

Postinfectious irritable bowel syndrome

In a prospective study, we found that 25% of previously healthy patients developed a diarrhea-predominant type of irritable bowel syndrome (IBS) (Table 50b.1) after an episode of acute infectious diarrhea. Psychologic disturbances such as anxiety and stressful recent life events were important predictors for the development of IBS. Other potential pathogenic factors are lactose malabsorption,

TABLE 50.b-1 -- Points to note in the history and physical examination of patients who have persistent diarrhea.

POINTS TO NOTE IN THE HISTORY AND PHYSICAL EXAMINATION OF PATIENTS WHO HAVE PERSISTENT DIARRHEA
• Onset of diarrhea in relation to confirmed or presumed infectious illness
• Travel to or residence in tropics or developing countries
• Weight loss
• Nature of the stools; frequency, consistency, estimated volume, steatorrhea
• Bowel symptoms suggesting irritable bowel syndrome: abdominal pain relieved by defecation, constipation, passage of mucus, feeling of incomplete evacuation, bloating
• Other abdominal symptoms: flatulence (suggesting giardiasis, lactose malabsorption), blood in the stool (suggesting colitis, dysentery)
• Risk factors for HIV if appropriate
• Examination for evidence of weight loss, anemia, malabsorption, abdominal masses, lymph nodes

TABLE 50.b-2 -- Organisms that may be involved in chronic diarrhea and their antibiotic treatment.

ORGANISMS THAT MAY BE INVOLVED IN CHRONIC DIARRHEA AND THEIR ANTIBIOTIC TREATMENT			
Organism		**Antibiotic**	**Suggested dosage**
Bacteria	*Shigella* spp.	Quinolones, e.g. ciprofloxacin, nonfloxacin	500mg q12h for 5 days
	Salmonella spp.		400mg q12h for 5 days
	Campylobacter spp.		
	Aeromonas spp.		
	Plesiomonas shigelloides		
	Spirochetes	Metronidazole	400mg q8h for 10 days
	Clostridium difficile toxin positive colitis	Metronidazole	400mg q8h for 10–14 days
		Vancomycin (oral)	125mg q6h for 10–14 days
		Cholestyramine	4g q8h for 10–14 days
Protozoa	*Giardia lamblia*	Metronidazole	2g q24h for 3 days
		Tinidazole	2g once daily for 1 day
	Entamoeba histolytica	Metronidazole	800mg q8h for 5 days
		Tinidazole	1g q12h for 3 days
	Cyclospora cayentensis	Trimethoprim-sulfamethoxazole	2 tablets q12h for 7 days
	Isospora belii	Trimethoprim-sulfamethoxazofe	2 tablets q6h for 14 days
	Blastocystis hominis	Metronidazole	800mg q8h for 10 days
Helminths	*Strongyloides stercoralis*	Thiabendazole	25mg/kg (maximum 1.5g) q12h for 3 days
		Albendazole	400mg q12h for 3 days (repeat at 3 weeks if required)
	Trichuris trichiura	Mebendazole	100mg q12h for 3 days
	Capillaria philippinensis	Mebendazole	100mg q6h for 20–30 days
	Mixed infection	Mebendazole	200mg q12h for 5 days
		Albendazole	400mg q24h for 3 days
Tropical sprue		Tetracycline plus	250mg q6h for 30 days (minimum)
		Folic acid	5mg q12h for 90 days

alterations in colonic motility and sensitivity, changes in the colonic microflora and bile acid malabsorption.

Inflammatory bowel disease

Some patients who have inflammatory bowel disease (IBD) in their initial presentation have a positive microbial finding. However, symptoms may persist or recur despite the eradication of the inciting organism. Commonly implicated pathogens are *Entamoeba histolytica, Shigella* spp., *Salmonella* spp., *Aeromonas* spp. and *Clostridium difficile*. Possible explanations for this association include bacterial infection added on to previously unrecognized IBD, and the precipitation of IBD by an

altered intestinal microflora or by an immunopathogenic effect of bacterial products of inflammation.

Human immunodeficiency virus-associated enteropathy

HIV-associated enteropathy may present with chronic diarrhea and significant weight loss or malabsorption. It is believed that in the majority of HIV-infected patients who have chronic diarrhea, a potential pathogen can be detected.

Postinfective malabsorption

Tropical sprue presents with chronic gastrointestinal symptoms and malabsorption following an acute diarrheal episode that is usually contracted in a tropical country (although it has also been described

in travelers to the Mediterranean area). Although a specific microbial agent has not been identified, the typical response to broad-spectrum antibiotics suggests an infectious pathogenesis. Intestinal infections with parasites, especially *Giardia lamblia*, may also present similarly.

Persistent intestinal infections

Occasionally infective colitis associated with *E. histolytica, Cryptosporidia, Campylobacter jejuni* or *Salmonella* spp. may persist beyond 6 weeks (Table 50b.2). The clinical spectrum of *G. lamblia* infection ranges from asymptomatic cyst excretion through chronic diarrhea with marked flatulence to intestinal malabsorption. *Cyclospora cayetanensis* is a newly recognized protozoal parasite that causes prolonged watery diarrhea in a characteristic relapsing cyclic pattern even in immunocompetent patients. Although cases of chronic diarrhea associated with *Blastocystis hominis, Aeromonas* spp. and *Plesiomonas shigelloides* have been reported, their pathogenic potential remains uncertain.

Diagnosis

Patients can be stratified to an appropriate level of testing on the basis of the points listed in Table 50b.1 .

Level 1 tests are:

- stool studies — microscopy, culture, ova, cysts and parasites, and tests for *C. difficile* toxin;
- blood studies — full blood count, erythrocyte sedimentation rate, biochemistry (electrolytes, protein, albumin, thyroid hormones) and studies for the detection of HIV antibodies; and
- sigmoidoscopy.

In a patient who has no history of travel to or residence in a tropical or developing country and no significant weight loss or blood in the stools, level 1 tests would usually be sufficient. To optimize the yield from stool examinations, three specimens, preferably when stools are loose, should be collected on separate days and processed rapidly. Sigmoidoscopy and rectal biopsy help to exclude colitis, although biopsies taken late in an infective colitis often show changes that are hard to distinguish from IBD. When there is doubt about the diagnosis, a follow-up biopsy 6–8 weeks later is helpful, as infective colitis usually reverts spontaneously to normal. In the absence of proctitis, a rectal biopsy can still provide an indication of mild but significant forms of proximal colitis. The decision to test further should be based on clinical indications and the results of the level 1 tests. For instance, in the presence of relevant exposure, or the finding of lymphopenia, the issue of testing for HIV should be addressed.

Level 2a tests are:

- a lactose hydrogen breath test or a 2-week trial of dietary exclusion; and
- selenium-75-labeled homotaurocholic acid test (SeHCAT) of bile malabsorption or trial of cholestyramine.

For patients whose presentation is consistent with IBS (see Table 50b.1), level 2a tests can be considered, depending on the clinical circumstances and the facilities available. Level 2b tests are:

- tests for malabsorption (serum folate, vitamin B12, iron and xylose absorption tests);
- endoscopy with small bowel biopsy or duodenal aspiration.

If there is a suspicion of malabsorption, evidence for this should be obtained as indicated for level 2b tests. Where there is positive evidence, and if stools are negative, further tests should be carried out to explain the malabsorption. Endoscopy with duodenal biopsy is preferred to duodenal aspiration. Histologic examination contributes to the diagnosis of conditions such as tropical and celiac sprue through the diagnosis of villous atrophy, as well as helps to isolate *Giardia* spp. trophozoites and other infectious and parasitic agents that reside on enterocytes. Duodenal aspiration was reported to add only 15% to the yield for giardiasis from three stool examinations.

Level 3 tests are:

- radiologic examinations (barium studies of the small and large bowel); and
- colonoscopy.

If the diagnosis remains uncertain, level 3 tests help to exclude Crohn's disease, colonic tumors, small intestinal diverticula (which may give rise to bacterial overgrowth) and intestinal lymphoma.

Management

If malabsorption or weight loss is present and a specific infective diagnosis can be made, the indication for antimicrobial therapy is clear. If the diagnosis remains uncertain after thorough investigation, a chemotherapeutic trial of metronidazole or tetracycline appears justified. In the presence of HIV, a persistent search for an enteric pathogen is important because it is recognized that a potentially treatable pathogen can often be detected. Nutritional supplements may occasionally be required.

Patients who have a positive stool isolate but no malabsorption, weight loss or dysenteric stools pose a therapeutic dilemma. Many of these patients are persistent excretors or carriers of the organism and are suffering from postinfectious IBS; antibiotic treatment will either not improve the diarrhea or will produce a temporary improvement, possibly as a non-specific effect of antibiotics on colonic flora. However, occasionally bacterial pathogens may give rise to protracted diarrhea and, as long as the pathogenic potential of an organism remains uncertain, a trial of antimicrobial chemotherapy can be justified. Repeated courses of antibiotics should not be pursued if the patient shows no improvement.

In patients who have postinfectious IBS, treatment with a lactose-free diet, loperamide, cholestyramine and low-dose tricyclic anti-depressant agents may be helpful.

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50.c Infectious diarrhea and antibiotic treatment

Philip Bejon

Acute diarrhea kills 3–4 million per year; 4 billion cases of acute diarrhea occur yearly worldwide, generating 8 million consultations and 250,000 hospital admissions per year in the USA. The role of antibiotics is unclear. Randomized controlled trials compare outcomes for those with self-limiting albeit incapacitating colitis. Personal and economic benefits may result from antibiotic use, although serious morbidity and mortality from infective diarrhea are rare.

Treatment

Between 30% and 90% of traveler's diarrhea and community-acquired diarrhea in adults is bacterial. In children, most diarrheal episodes are viral and antibiotics are not indicated. The role of antibiotics in treating acute gastroenteritis has been the subject of numerous randomized trials and a Cochrane Review meta-analysis. Most of these patients would have been infected with enterotoxigenic (ETEC) or enteropathogenic (EPEC) *Escherichia coli*; these strains are not identified by routine stool culture. Significant clinical benefit from antibiotic use was described; there were more patients cured at 72 hours, reduced stool frequency and a shorter time to last unformed stool. Different antibiotics have been compared to placebo, but comparative data between antibiotics are not available. Data for tetracyclines, ampicillin and trimethoprim-sulfamethoxazole should be interpreted cautiously given the increase in reported resistance of enteric pathogens. Quinolone and macrolide treatments, ranging from single doses to 5 days, are effective. Minor antibiotic side effects were frequent. The rare occurrence of serious adverse events, such as rash, anaphylaxis, tendon rupture or reduced seizure threshold with quinolones must also be considered. Antibiotic use is felt to promote resistant organisms in the community; this contests an economic benefit to the 30–60% of overseas travelers who acquire diarrhea.

While bacterial diarrhea is unpleasant and inconvenient for most travelers, it is life threatening for undernourished children in the tropics. These children have a high incidence of *Campylobacter* and *Salmonella* infection. The immediate danger is dehydration, but recurrent bacterial infection is linked to small bowel enteropathy, malabsorption and undernutrition. EPEC is frequently isolated in association with persistent diarrhea. The limited trials undertaken have not prevented these complications with antibiotics and observational data link persistent diarrhea with prior antibiotic use during acute gastroenteritis.

Prophylaxis

Antibiotic prophylaxis against traveler's diarrhea has been examined in randomized controlled trials. Doxycycline and trimethoprim-sulfamethoxazole were effective in trials conducted 20 years ago. Resistance to these agents has since increased and recent trials now suggest limited efficacy. Quinolone susceptibility has largely been retained but there are limited safety data for long prophylactic courses and the optimal dose is unknown. It may be more appropriate to advise early self-treatment of diarrheal episodes.

Enteric fever

Patients with bloody or mucous stool, high fever or tenesmus have a 60–70% chance of *Campylobacter, Salmonella* or *Shigella* infection. *Salmonella* and *Shigella* species, the main causes of complicated colitis and bacteremia, retain predictable ciprofloxacin susceptibility. On initial presentation, typhoid fever may be in the differential diagnosis; ciprofloxacin is the drug of choice. Ceftriaxone has also been used in disseminated disease. Scoring systems based on clinical parameters and inflammatory markers have been shown to correlate with the isolation of pathogens in stool, but do not predict the risk of complications or the need for antibiotics.

Campylobacter and *Salmonella*

Campylobacter jejuni is isolated seven times more frequently than *Salmonella* or *Shigella*. Quinolone resistance is becoming more frequent in *Campylobacter* isolates (20–80%). Macrolides retain activity against *C. jejuni* and randomized trials demonstrate their utility. The length and severity of symptoms were reduced only when patients were treated early, before the stool culture results were available. However, most enteric pathogens (i.e. *Salmonella, Shigella, E. coli*) display intrinsic resistance to macrolides and macrolides were not demonstrably superior to quinolones, even where quinolone resistance is frequent.

By contrast, patients with either symptomatic or symptomless enteric *Salmonella* infection derive no benefit from antibiotics given in randomized controlled trials, even when treated early. Antibiotics were often associated with more prolonged carriage and weakly associated with risk of relapse. These trials do not provide data on the immunosuppressed, the elderly or neonates. Case control data suggest individuals with bacteremia have a high odds ratio for medical co-morbidity and HIV infection. Nevertheless, most of these individuals presented with disseminated disease rather than gastroenteritis alone. While empiric treatment targeting *Campylobacter* and *Salmonella* can be justified, initiating treatment after isolating organisms in stool cannot.

Hemolytic uremic syndrome

E. coli 0157 is a rare cause of bloody diarrhea. In 1–14% of infected children, the hemolytic uremic syndrome (HUS) results. Retrospective and prospective studies have associated antibiotic use with subsequent HUS. This may not be causal. HUS is associated with a high white cell count, early presentation and vomiting; these factors may influence antibiotic prescribing. Animal models of antibiotic-treated mice demonstrate harm from increased toxin production. However, HUS is less frequent in adults and less often linked to *E. coli* 0157. Waiting for stool culture before antibiotic use necessarily precludes benefit; the risks from antibiotic use remain unclear.

Protozoa

Protozoa account for up to 12% of travelers' diarrhea. Amebic colitis is rare, but the most serious; afebrile, insidious worsening of bloody diarrhea is characteristic. Empiric metronidazole may occasionally be necessary pending endoscopy. A trial of metronidazole or tinidazole may also be necessary when persistent diarrhea with steatorrhea suggests *Giardia*, since parasites are not always seen in stool.

Cryptosporidium (3% of all travelers' diarrhea) is not antibiotic responsive and early diagnosis by direct stool microscopy will avoid unnecessary treatment. It is persistent only in the immunocompromised. Cyclospora infection responded to trimethoprim-sulfamethoxazole in a single clinical trial. It is endemic in Nepal and Guatemala (3–8% of returning travelers with diarrhea) and acquired from imported raspberries in the USA. Since infection is self-limiting, rarely severe and amenable to rapid diagnosis by direct microscopy, empiric trimethoprim-sulfamethoxazole should not be necessary. Although the protozoan infections isospora and microsporidia may respond to trimethoprim-sulfamethoxazole and albendazole respectively, they are self-limiting infections in the immunocompetent and there are insufficient systematic data on incidence and treatment outcomes.

Clostridium difficile

Patients recently discharged from hospital or taking broad-spectrum antibiotics are more likely to have either antibiotic-related diarrhea or *Clostridium difficile*-related colitis than *Salmonella* or ETEC: if severe, metronidazole or oral vancomycin should be used empirically pending analysis for *C. difficile* toxin.

Cholera

The profuse watery diarrhea associated with *Vibrio cholerae* infection lasts 1–2 days less with doxycycline or single-dose ciprofloxacin. Fluid replacement requirements fall and stool carriage is reduced. Limited data suggest that the severity of secondary cases is attenuated by antibiotic prophylaxis for close contacts.

Conclusion

In conclusion, data show that antibiotics reduce the length of symptoms for those with acute gastroenteritis in the developed world, and either a quinolone or a macrolide may be considered. The personal and economic benefits must be balanced against antibiotic side effects and a potential impact on resistance patterns. Where antibiotics are given to those with a severe, febrile illness or where bacteremia or metastatic infection is suspected, quinolones or third-generation cephalosporins are active against the likely etiological agents. Evidence opposes antibiotic prescribing consequent on stool culture results.

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Chapter 51 - Infective and Reactive Arthritis

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INTRODUCTION

Infective arthritis is an inflammation of the joint space caused by invasion of micro-organisms. Hematogenous seeding of the joint is the most common mechanism of infection in native joints. The incidence of infective arthritis in adults caused by bacteria other than *Neisseria gonorrhoeae* is relatively low, but these infections can cause major morbidity as a result of pain, immobility and loss of joint function. Successful treatment requires prompt drainage of the joint, using multiple arthrocenteses or open arthrotomy, and prolonged antimicrobial therapy to achieve sterilization of the joint space as well as a satisfactory functional result. This chapter discusses infective arthritis in adults, with the major emphasis on bacterial infective arthritis. Viral and reactive arthritis are discussed briefly. Infective arthritis caused by *Borrelia burgdorferi* is discussed in Chapter 54 and mycobacterial arthritis in Chapter 37 .

BACTERIAL ARTHRITIS

EPIDEMIOLOGY

In 1998, according to the Centers for Disease Control and Prevention (CDC), there were an estimated 23,000 cases of pyogenic arthritis in the USA, or 8.4 cases per 100,000 person years; 61% of the patients were male and 39% were 65 years of age or older.[1] The mean duration of hospitalization of the 11,000 patients who had a primary diagnosis of pyogenic arthritis was 7.2 days. These data are in agreement with other published incidence rates of bacterial arthritis in the general population.[2] [3] Disseminated gonococcal infection with associated gonococcal infective arthritis is the leading cause of hospital admission due to infective arthritis in the USA, with an estimated incidence rate of 2.8 per 100,000 person years (Chapter 227). [4]

RISK FACTORS

Rheumatoid arthritis, diabetes mellitus, malignancy, old age and HIV infection, alone or through their treatment, suppress the immune system and, as such, are risk factors for acquiring bacterial arthritis.[5] [6] [7] [8] Novel therapies such as etanercept and infliximab, targeting tumor necrosis factor for the treatment of rheumatoid arthritis, have come into widespread clinical use. These agents have been shown to be implicated as a possible risk factor for severe polyarticular infectious complications in this patient population.[9] They have also been linked with an increased risk of mycobacterial infections, including tuberculosis.[10] Local abnormalities of host defenses caused by previous joint damage or surgery also predispose the joint to infection, as do situations that increase the risk of bacteremia, such as injection drug use, indwelling intravenous catheters and skin infection, and situations that allow direct inoculation of micro-organisms, such as intra-articular injection or arthroscopy. Recently, several cases of infective arthritis due to *Clostridium* spp. were reported to the CDC. All these cases underwent tissue allograft reconstruction surgery. Anaerobic cultures of the nonimplanted donor tissue yielded *Clostridium* spp. in selected cases. It is believed that the donor tissue was hematogenously seeded by bowel flora and that the aseptic processing techniques used for the implicated donor tissues did not eliminate spores of *Clostridium* spp.[11]

Disseminated gonococcal infection is more common among sexually active, menstruating women, although it can also occur during pregnancy and the peripartum period.[4] The male:female ratio is approximately 1:4. Often, the microbiologic etiology of infective arthritis can be predicted on the basis of the specific risk factor predisposing to infection (Table 51.1).[12]

PATHOGENESIS

Nongonococcal bacterial arthritis most often results from hematogenous seeding of the joint space as a result of bacteremia. Synovial tissue has a rich vascular supply but no basement membrane, factors that favor ingress of blood-borne organisms.[8] [12] The bacteremia can be primary or secondary to an infection elsewhere in the body (e.g. pneumonia, cellulitis) or to injection drug use.[5] An identifiable focus of infection can be found in approximately 50% of cases.[8] [13]

Direct inoculation of micro-organisms into the joint space due to trauma, arthrotomy, arthroscopy or diagnostic and therapeutic arthrocenteses is another mechanism of infection. The risk of septic arthritis after arthrocentesis has been reported to be 0.002–0.007%; after arthroscopy it is reported to be 0.04–0.4%.[14] Infection of the joint space as a result of contiguous soft tissue infection or periarticular osteomyelitis is much less common.

Once bacteria have entered the joint space there is ingress of polymorphonuclear leukocytes, which results in hydrolysis of proteoglycans and collagen through stimulation of locally synthesized cytokines and release of enzymes.[14] If left untreated, destruction of the articular cartilage eventually occurs, leading to irreversible joint damage.[15] [16] [17]

Staphylococcus aureus is the most common etiologic agent of infective arthritis in adults (Table 51.2).[8] [18] [19] [20] [21] In young, sexually active persons, *N. gonorrhoeae* is the predominant pathogen. Infection in patients who have rheumatoid arthritis is due to *S. aureus* in as many as 80% of patients. Group B streptococcal infection is more likely to occur in patients who have diabetes mellitus. Coagulase-negative staphylococci may cause infection following arthroscopy and other medical procedures, including intra-articular injections. Infective arthritis due to Gram-negative bacilli is more common in the elderly and in patients who have co-morbid illnesses.[22]

Anaerobic infection is uncommon except in the setting of septic arthritis occurring after human or animal bite injuries or diabetic foot infections. Among injection drug users, *Pseudomonas aeruginosa* and *S. aureus* are common pathogens.[23] [24] Hypogammaglobulinemia is a risk factor for infective arthritis due to *Mycoplasma* spp.[25]

PREVENTION

Prevention of infective arthritis is obviously preferable to treatment of established infection. Examples of efforts to decrease the incidence

TABLE 51-1 -- Epidemiologic and clinical features associated with specific etiologic agents of infective arthritis.*

EPIDEMIOLOGIC AND CLINICAL FEATURES ASSOCIATED WITH SPECIFIC ETIOLOGIC AGENTS OF INFECTIVE ARTHRITIS	
Clinical or epidemiologic setting	**Likely etiologic agent**
Bacteria	
Staphylococcus aureus	Rheumatoid arthritis, injection drug use, arthroscopy, arthrotomy, polyarticular arthritis
Coagulase-negative staphylococci	Arthroscopy, arthrotomy, foreign material
Neisseria gonorrhoeae	Young, sexually active, history of sexually transmitted disease or unsafe sex, menstruation, pregnancy, multiple skin lesions
Pseudomonas aeruginosa and other aerobic	Injection drug use, elderly
Gram-negative bacteria	
Anaerobes	Human and animal bites, orthopedic allograft infection with allograft without sporicidal sterilization, anaerobic infection elsewhere in body
Usual oral flora	Human and animal bites
Eikenella corrodens	Human bite
Pasteurella multocida	Cat or dog bite
Streptobacillus moniliformis	Rat bite
Neisseria meningitidis	Multiple purpuric lesions
Other	
Mycoplasma spp.	Common variable hypogammaglobulinemia
Borrelia burgdorferi	Resident in endemic area, known or suspected tick exposure, history of erythema chronicum migrans
Troypheryma whipplei	Other manifestations of Whipple's disease
Mycobacteria	

Mycobacterium tuberculosis	Positive tuberculin skin test, resident in country where disease is endemic, known exposure history
Mycobacterium marinum	Exposure to aquatic environment
Mycobacterium avium intracelulare	HIV, T-cell suppression
Mycobacterium leprae	Resident in country where disease is endemic
Fungi	
Sporothrix schenckii	History of trauma with exposure to colonized soil (e.g. sphagnum moss)
Candida	Prior known or suspected candidemia or culture-negative central infection, neutropenia
Aspergillus	Prior known or suspected invasive aspergillus infection, prolonged neutropenia
Blastomyces dermatididis	Resident in area where disease is endemic, exposure to beaver dams
Coccidiomyces immitis	Resident in area where disease is endemic

** Data from Smith and Piercy.*[12]

of infective arthritis include the promotion of public health measures to prevent the acquisition of *N. gonorrhoeae*, measures to decrease the incidence of animal bites, prophylactic foot care in patients who have diabetes mellitus and eradication of injection drug use. Rapid and effective treatment of antecedent infections that may cause joint infections, such as catheter-associated bacteremia due to *S. aureus* or skin and soft tissue infection in patients who

TABLE 51-2 -- Etiologic agents of nongonococcal bacterial arthritis in adults.[*]

ETIOLOGIC AGENTS OF NONGONOCOCCAL BACTERIAL ARTHRITIS IN ADULTS	
Micro-organisms	**Cases (%)**
Staphylococcus aureus	68
Streptococci (including ß-hemolytic streptococci, viridans group streptococci and *Streptococcus pneumoniae*)	20
Haemophilus influenzae	1
Aerobic Gram-negative bacilli	10
Polymicrobial and miscellaneous	1
Unknown	<1

** Data from Roberts and Mock.*[21]

have rheumatoid arthritis and diabetes mellitus, as well as the administration of vaccines against *Haemophilus influenzae* and *Streptococcus pneumoniae*, are also effective preventive measures.[26]

CLINICAL FEATURES

Nongonococcal infective arthritis

Nongonococcal infective arthritis is typically monoarticular and has an acute presentation. Patients complain of pain and limitation of motion in over 90% of cases.[12] In one study, fever was present in 78% of patients within 24 hours of hospitalization, although it was rarely above 102°F (39°C).[17] Chills were uncommon. Physical examination usually reveals a large effusion and a marked decrease in active and passive range of motion of the joint. However, these findings may be minimal or absent in those patients who have rheumatoid arthritis and they may be difficult to discern in infections of the hip or shoulder.

The knee is the most commonly involved native joint in adults. In a case series from the Netherlands, the percentage of cases involving a particular joint was as follows: knee 55%, ankle 10%, wrist 9%, shoulder 7%, hip 5%, elbow 5%, sternoclavicular joint 5%, sacroiliac joint 2% and foot joint 2%.[3] Sacroiliac or sternoclavicular joint infection is more common among injection drug users and may be difficult to diagnose. Polyarticular infection occurs in approximately 15% of patients and is often due to *S. aureus*.[27] Thus, a polyarticular presentation does not always imply the presence of gonococcal, viral, reactive or noninfectious arthritis. It is more common among patients who have rheumatoid arthritis, and patients receiving corticosteroid therapy.

The case-fatality rate for patients who have nongonococcal bacterial arthritis is estimated to be between 10% and 16%, and as many as 50% of patients who survive their infection have some degree of permanent loss of joint function. [8] [18] [19] [20] Morbidity and mortality is dependent on a number of factors, including age, presence of rheumatoid arthritis, infection in the hip or shoulder, duration of symptoms before treatment, the presence of polyarticular arthritis, persistently positive joint fluid cultures after appropriate therapy, the presence of bacteremia and the virulence of the infecting organism.[2] [8] [28] [29]

Gonococcal arthritis

Disseminated gonococcal infection presents with two distinct clinical entities.[4] Early after dissemination from mucosal surfaces such as the cervix or urethra the patient presents with bacteremia, fever, polyarthralgia, tenosynovitis (typically of the hands and fingers) and multiple maculopapular, pustular, vesicular or necrotic skin lesions. Asymmetric joint involvement is common. The knee, elbow, wrist, metacarpophalangeal and ankle joints are the most

commonly involved. This presentation accounts for 60% of patients who present with disseminated gonococcal disease, and it has been described as the dermatitis-arthritis syndrome. If left untreated, the patient will present later with monoarticular arthritis, usually without tenosynovitis or skin lesions. Co-infection with HIV often leads to infection of unusual joints and an aggressive course.[30]

The outcome of disseminated gonococcal infection is almost always excellent (see Chapter 227).

DIAGNOSIS

Although the history and physical examination can lead to a high index of suspicion for infection, a synovial fluid culture that yields a causative micro-organism is the only definitive method for diagnosing bacterial arthritis. Fever and rigors in the setting of an inflammatory arthritis have a low positive predictive value for bacterial arthritis and have been reported in crystal-induced arthropathy.[14] The erythrocyte sedimentation rate (ESR), C-reactive protein and leukocyte counts are elevated in the majority of cases, although again the positive predictive value of these tests in the setting of a monoarticular inflammatory arthritis is low. A rise in the ESR may help in the differential diagnosis of new joint pain and effusion in those patients who have rheumatoid arthritis.[12] [31] Blood cultures are positive in up to 70% of all patients with nongonococcal arthritis. [20] [27]

In disseminated gonococcal infection the majority of patients have an elevated ESR, and only 50% will have an abnormal leukocyte count. Anemia and abnormal liver function tests also may occur but these findings are usually transient. [4]

In approximately 80% of patients who have disseminated gonococcal arthritis there is a positive culture or *N. gonorrhoeae* DNA can be identified by polymerase chain reaction or ligase chain reaction in samples from the cervix, urethra, rectum, pharynx or urine. Skin lesions yield *N. gonorrhoeae* in 30% of cases and blood cultures in 5%.

The diagnostic procedure of choice for bacterial arthritis is an arthrocentesis. This should be done immediately once the diagnosis of joint infection is suspected so as not to delay appropriate medical or surgical therapy. If synovial fluid cannot be obtained by blind needle aspiration (e.g. in the case of hip joint infection), then aspiration

should be done with the help of a radiologist under fluoroscopic guidance. In selected cases, an open arthrotomy should be performed to make a definitive diagnosis.

Synovial fluid is often cloudy or purulent in appearance. The synovial fluid should be routinely examined for uric acid and calcium pyrophosphate crystals, and a leukocyte count and differential should be obtained on each specimen. The leukocyte count is usually greater than 50,000/mm^3 and often greater than 100,000/mm^3 , with more than 75% polymorphonuclear leukocytes. These findings can also be seen in patients who have inflammatory arthritis and crystal deposition arthritis. The sensitivity and specificity of a synovial fluid leukocyte count of more than 20,000/mm^3 for the presence of inflammatory arthritis have been estimated to be 84% and 84%.[32] For a differential count of more than 75% polymorphonuclear leukocytes the sensitivity and specificity are 75% and 92%. Although many clinicians order synovial fluid glucose and protein levels, the results of these tests have been shown to be less informative than the leukocyte count and differential.[32] The synovial fluid lactic acid and lactate dehydrogenase levels are often elevated in patients who have infective arthritis but elevation of these can be seen in other inflammatory joint disorders as well.[8]

Synovial fluid should be cultured for both aerobes and anaerobes and other organisms, depending on the clinical circumstances. A recent study performed at the Mayo Clinic showed superior performance for the BACTEC Peds Plus/F bottle over the conventional agar plate method for the detection of clinically significant micro-organisms from synovial fluid specimens.[33]

The synovial fluid culture will be positive in 90% of cases of nongonococcal arthritis, assuming that antibiotic therapy has not been started before the sample has been collected.[17] The Gram stain is positive in 50% of patients.[8]

In patients who have disseminated gonococcal infection, the synovial fluid cultures are positive in 25–30% of all patients and 50% of patients who present with monoarticular arthritis. The role of the polymerase chain reaction in detecting bacterial pathogens in patients who have infective arthritis has not yet been well defined, although the technique seems a promising tool for the detection of infectious arthritis due to *N. gonorrhoeae*, *Borrelia burgdorferi* and *Tropheryma whippleii*. [34] Polymerase chain reaction can help to distinguish disseminated gonococcal infection from other inflammatory arthropathies such as Reiter's syndrome.[35]

Synovial tissue cultures are indicated only for chronic infective arthritis when mycobacterial or fungal arthritis is suspected or when synovial fluid cultures cannot be obtained by less invasive techniques.

Periarticular soft tissue swelling is the most common abnormality seen on plain radiography in patients who have bacterial arthritis. Periarticular erosions and osteoporosis as well as joint space narrowing due to cartilage destruction do not occur for several weeks. Thus, plain radiographs are not usually helpful in making a diagnosis of bacterial arthritis. Occasionally, in longstanding infection, periarticular osteomyelitis will be visible on plain radiograph (Fig. 51.1). It is often difficult to distinguish infection from inflammatory arthritis using radiographic methods in the setting of rheumatoid arthritis, but the development of a rapid destructive arthritis in one or two joints suggests infection. Computerized tomography (CT) scans and magnetic resonance imaging (MRI) are more useful than plain radiographs for identifying concomitant periarticular osteomyelitis, soft tissue abscesses and joint effusions, but they are expensive and most often are not necessary (Fig. 51.2). Sacroiliac or sternoclavicular joint disease is optimally evaluated with these modalities as well as with radionuclide studies. Indium-111 scans may be useful for identifying relatively asymptomatic septic arthritis in immunocompromised patients who have one or more known septic joints and in whom there is a high index of suspicion for polyarticular infection.

In adults, the differential diagnosis for patients who have an acute onset of fever, chills and an inflammatory arthritis of one or more joints includes bacterial arthritis, gout, pseudogout, rheumatic fever, reactive arthritis and rheumatic illnesses such as rheumatoid arthritis and psoriatic arthritis. Gout and pseudogout are the most common

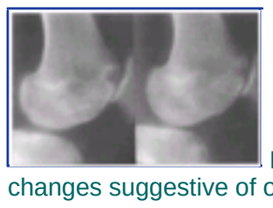

Figure 51-1 Tomogram of right knee of a patient who has *Staphylococcus aureus* septic arthritis and periarticular osteomyelitis. Note the mixed sclerosis and lytic changes suggestive of osteomyelitis.

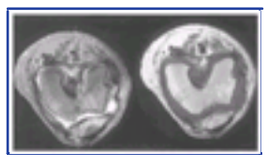

Figure 51-2 MRI scan of right knee of a patient who has *Staphylococcus aureus* septic arthritis. Note the soft tissue inflammation and a joint effusion.

noninfectious inflammatory arthritides that must be differentiated from bacterial arthritis.[8] They should be suspected when there is a history of previous episodes or there is chondrocalcinosis on plain film. Synovial fluid analysis using polarized light microscopy is the most useful diagnostic test for gout or pseudogout.

TABLE 51-3 -- Antibiotic therapy of infective arthritis in adults for selected bacterial micro-organisms.

ANTIBIOTIC THERAPY OF INFECTIVE ARTHRITIS IN ADULTS FOR SELECTED BACTERIAL MICRO-ORGANISMS			
Micro-organisms		**Antibiotic therapy**	**Alternative therapy**
Staphylococcus aureus	Methicillin-sensitive strains	Nafcillin or oxacillin 1.5–2.0g iv q4h	Vancomycin 15mg/kg iv q12h, not to exceed 2g in 24h unless serum levels are monitored
		or	
		Cefazolin (or other first-generation cephalosporins in equivalent dosages) 1 g iv q8h	
	Methicillin-resistant strains	Vancomycin 15mg/kg iv q12h, not to exceed 2g in 24h unless serum levels are monitored	Consult a specialist in infectious diseases
			Linezolid 600mg iv/po q12h
Penicillin-sensitive streptococci or pneumococci with an MIC=0.1 µg/ml		Aqueous crystalline penicillin G 20 × 10^6 U per 24h iv either continuously or in six equally divided doses	Vancomycin 15mg/kg iv q12h, not to exceed 2g in 24h unless serum levels are monitored
		or	
		Ceftriaxone 2g iv or im q24h	
		or	
		Cefazolin 1g iv q8h	
Enterococci or streptococci with an MIC =0.5µg/ml or nutritionally variant streptococci (all enterococci causing infection must be tested for antimicrobial susceptibility in order to select optimal therapy)		Aqueous crystalline penicillin G, 20 × 10^6 U per 24 iv either continuously or in six equally divided doses, plus gentamicin sulfate, 1 mg/kg iv or im q8h	Vancomycin 15mg/kg iv q12h, not to exceed 2g in 24h unless serum levels are monitored
		or	
		Ampicillin sodium 12g per 24h iv either continuously or in six equally divided doses	

Neisseria gonorrhoeae	Ceftriaxone 1g im or iv q24h for 24–48h after clinical improvement followed by	Ciprofloxacin 400mg iv q12h for 24–48h after clinical improvement
	Cefixime 400mg po q12h for 1 week	*or*
	or	Ofloxacin 400mg iv q12h for 24–48h after clinical improvement
	Ciprofloxacin 500mg po q12h for 1 week	
	or	*or*
	Ofloxacin 400mg po q12h for 1 week	Spectinomycin 2g im q12h for 24–48h after clinical improvement
		followed by
		Ciprofloxacin 500mg po q12h for 1 week
		or
		Ofloxacin 400mg po q12h po q12h for 1 week
Enterobacteriaceae	Ceftriaxone 2g iv q24h	Levofloxacin 500mg po q24h
	or	
Pseudomonas aeruginosa, Enterobacter spp.	Ciprofloxacin 750mg po q12h (based on in-vitro susceptibility)	
Dosages recommended are for patients who have normal renal function.		

The articular manifestations of rheumatic fever occur in approximately 75% of first episodes, last for several weeks and do not cause permanent joint damage. Typically, there is development of a migratory polyarthritis involving the knees, elbows, ankles and wrists that occurs within 1–5 weeks of the antecedent streptococcal pharyngitis. Joint symptoms can range from arthralgia without obvious physical findings to inflammatory arthritis indistinguishable from infective polyarthritis. Often more than six joints are affected. The diagnosis of rheumatic fever is dependent on satisfying the updated Jones criteria (see Chapter 225).[36]

Bacterial arthritis should be suspected in those patients at increased risk of infection. Fungal and mycobacterial infection is usually monoarticular but their presentation is usually over weeks to months instead of hours to days. Diseases that must be distinguished from disseminated gonococcal infection include viral and reactive arthritis, rheumatic fever and secondary syphilis.

MANAGEMENT

The keys to the management of infective arthritis are:

- drainage of the purulent synovial fluid;
- debridement of any concomitant periarticular osteomyelitis; and
- administration of appropriate parenteral antimicrobial therapy.

Experimental models of septic arthritis suggest that early drainage and antimicrobial therapy prevent cartilage destruction.[37] Local antimicrobial therapy is unnecessary and may cause a chemical synovitis.[8] Joint immobilization and elevation is useful for symptomatic relief of pain early in the course of the disease, but early active range of motion exercises are beneficial for ultimate functional outcome.

Synovial fluid drainage

The optimal method of drainage of an infected joint remains controversial, in part because no well-controlled randomized trials exist to guide therapy and because the therapy of each patient should be individualized.[38] Most adults who have septic arthritis have been managed with repeated joint aspirations instead of surgical debridement.[17] Patients who have disseminated gonococcal infection rarely require repeat joint aspirations, arthroscopy or arthrotomy.[4]

The use of arthroscopy has expanded in recent years because of the minimal morbidity of the procedure and the improved ability of arthroscopy to adequately drain purulent material from the joint compared with joint aspiration. [39] [40] [41] [42] [43] [44] A recent study by Smith *et al*[51] compared the efficacy of aspiration with arthroscopy in the therapy of septic arthritis in 61 children using a prospective randomized clinical trial. There was no statistical difference in the clinical outcome for the two treatment groups. Large multicenter, randomized trials are needed to further evaluate the comparative efficacy of these modalities.

Debridement

Recommended indications for surgical debridement have included effusions that fail to resolve with 7 days of conservative therapy and inability to adequately drain the infected joint by aspiration or arthroscopy either because of location (hip and shoulder) or loculations of pus[8] [12] (Fig. 51.3).

Antimicrobial therapy

Antimicrobial therapy should be administered as soon as the diagnosis is suspected and synovial fluid cultures obtained. Any delay in the administration of antimicrobial therapy may result in significant cartilage loss and functional limitation of the affected joint. To date there are no randomized studies to help guide the clinician in the antimicrobial therapy of septic arthritis. Initial antimicrobial therapy should be based on the results of the Gram stain and the specific clinical and epidemiologic setting. If no micro-organisms are seen on the Gram stain, empiric therapy for *S. aureus*, streptococci and gonococci (in young, sexually active adults) should be given. Antimicrobials with anaerobic activity should be added to this regimen if septic arthritis occurs after the use of an allograft.[11] Most experts

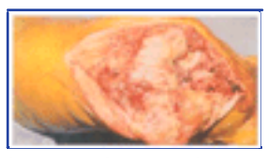

Figure 51-3 Intraoperative photograph of right knee of a patient who has ***Staphylococcus aureus*** **septic arthritis.** Note the damaged joint and dark brown, boggy and hyperemic synovium.

administer 2–4 weeks of intravenous antimicrobial therapy for the treatment of nongonococcal septic arthritis.[8] [12] In most cases this therapy can be administered on an outpatient basis after an initial period of hospitalization.

Suggested antimicrobials for specific pathogens causing infective arthritis are shown in Table 51.3 .

Oral antimicrobial therapy with an effective agent with excellent bioavailability, such as ciprofloxacin or linezolid, is also acceptable, particularly if compliance with oral therapy can be assured.

Current treatment guidelines for gonococcal septic arthritis recommend ceftriaxone as the initial drug of choice, followed by oral therapy with a cephalosporin or a quinolone after initial clinical improvement (see Table 51.3).

VIRAL ARTHRITIS

Arthritis is a common complication of infections with hepatitis viruses, parvovirus B19, rubella, HIV and alphaviruses, and is relatively rare with mumps virus, enteroviruses, adenoviruses and herpesviruses. The most common mechanism by which viruses cause arthritis is by invasion of the joint during the period of viremia. Other postulated mechanisms include immune complex deposition, insertion of the viral genome into the host DNA, thus promoting autoimmunity through an 'altered self,' and direct viral infection of the immune system, thus altering the immune response.[45] [46] [47] [48]

Typically, viral arthritis occurs during the prodromal stages of viral infection and is associated with a rash. Polyarticular involvement, including the small joints of the hands, is typical (Fig. 51.4). There is no specific pattern of joint involvement that is unique to a given viral etiology. Diagnosis is based on historic and clinical clues (Table 51.4) and diagnostic testing specific for each individual virus, the details of which are discussed in the relevant chapters. Viral arthritis is usually self-limited but may progress to a chronic arthropathy in certain instances. It is important that viral arthritis should be distinguished from rheumatic fever and the initial presentation of autoimmune disorders, including rheumatoid arthritis (Chapter 212 , Chapter 214 , Chapter 220 and Chapter 221).

Treatment is discussed in the chapters devoted to specific viruses. Prevention of viral arthritis is dependent on vaccination against the specific pathogen causing arthritis (e.g. mumps virus, rubella, hepatitis A virus, hepatitis B virus and varicella-zoster virus).

REACTIVE ARTHRITIS

Reactive arthritis describes the acute onset of an inflammatory arthritis soon after an infection elsewhere in the body in which micro-organisms cannot be cultured from the synovial fluid. However, genetic material may be found in the joint using molecular diagnostic techniques. Reiter's syndrome (the classic triad of arthritis, urethritis and conjunctivitis) is a common example of reactive arthritis. Many patients who develop reactive arthritis are HLA-B27-positive. The micro-organisms that have been associated with reactive arthritis are detailed in Table 51.5 .[49]

Typically, reactive arthritis begins several weeks after an antecedent infection. The initial clinical presentation is usually asymmetric oligoarticular arthritis without prominent constitutional symptoms. The syndrome also occurs without any identifiable symptom of infections, however, particularly in the case of *Chlamydia trachomatis*. In the case of Reiter's syndrome, extra-articular manifestations are also present.

Laboratory abnormalities are non-specific and include mild elevations in the leukocyte count, ESR and C-reactive protein. Radiographs usually show only soft tissue swelling in early disease, but juxta-articular osteoporosis and erosion may also be seen.

TABLE 51-4 -- Clinical or epidemiologic features of infective arthritis caused by selected viruses.*

CLINICAL OR EPIDEMIOLOGIC FEATURES OF INFECTIVE ARTHRITIS CAUSED BY SELECTED VIRUSES			
Viral agent	**Epidemiologic features**	**Clinical characteristics**	**Outcome**
Rubella (including rubella vaccine)	No prior vaccination; 51–61% of rubella cases; 0–14% of patients receiving vaccination. Less common with new vaccine.	Symmetric arthiritis of the metacarpal and proximal phalangeal joints, wrist, elbow, ankle, knee; onset variable in relationship to rash. May mimic rheumatoid arthritis. Post vaccination disease less symptomatic	Spontaneous resolution in days to weeks but may be chronic or recur.
	Ratio of women : men -9:1.		
Parvovirus B19	60% of adult cases, females > males.	Sudden severe polyarticular arthritis in small joints.	Spontaneous resolution most common. Chronic arthritis can occur
	May mimic rheumatoid arthritis	Child care providers or school teachers; unusual in children	
Hepatitis A	10–14% of cases of hepatitis A; typical risk factors for acquisition of hepatitis A	Often associated with rash	Resolves spontaneously
Hepatitis B	10–25% of cases of hepatitis B; typical risk factors for acquisition of hepatitis B	Severe arthritis of sudden onset, symmetric, polyarthritis involving hand and knee; morning stiffness is considerable; skin rash may be present including urticaria	May last 1–3 weeks. Typically resolves during the preicteric phase.
			Chronic arthritis may occur with chronic hepatitis B infection
Hepatitis C	Typical risk factors for acquisition of hepatitis C	Can occur with acute or chronic infection. Sudden onset; joint pain in hands, wrists and shoulders often greater than physical findings. May mimic rheumatoid arthritis. Distinct from cryoglobulinemia with hepatitis C	May resolve spontaneously. Has been reported to persist for months and recur
HIV	Typical HIV associated risk factors.	Most cases are monoarticular but monoarticular and polyarticular presentations occur. Distinct from Reiter's syndrome or psoriatic arthritis which also occur in HIV infected individuals. Occurs in multiple stages of HIV infection	Usually resolves in several weeks.
	Approximately 8% of HIV infected patients affected		May persist for months
Arthropod-borne alpha-virus infection		May occur in epidemics. May be associated with rash.	Spontaneous resolution is typical.
		Constitutional symptoms may be present.	Chronic arthritis is unusual except with Sindbis virus
Chikungunya	East Africa, India, South East Asia, Philippines		
O'nyong-nyong	East Africa		
Sindbis virus	Sweden, Finland, Russia		
Ross River agent	Australia, New Zealand, New Guinea		
Barmah Forest virus	Australia		

** Data from Smith and Piercy,[12] Siegel and Gall,[45] Veno et al,[52] Epinosa,[53] and Navdes.[54]*

TABLE 51-5 -- Micro-organisms associated with reactive arthritis.*

MICRO-ORGANISMS ASSOCIATED WITH REACTIVE ARTHRITIS
Definite association
Chlamydia trachomatis
Shigella flexneri
Salmonella enteritidis
Salmonella typhimurium
Yersinia enterocolitica
Yersinia pseudotuberculosis
Campylobacter jejuni

** Data from Hughes and Keat.[49]*

Reactive arthritis is normally a self-limited disease, but chronic arthritis and sacroiliitis can occur in up to 15–30% of patients. Treatment is with anti-inflammatory agents. The role of antibacterial therapy is controversial. Randomized trials evaluating the efficacy of antimicrobials in patients who have reactive arthritis have been limited by small numbers of patients. In one study there was a trend toward efficacy for acute reactive arthritis due to *Chlamydia* spp.[50] Prevention of infection is reliant on effective prevention and treatment of precipitating antecedent infections.

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Chapter 52 - Acute and Chronic Osteomyelitis

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Carl W Norden

EPIDEMIOLOGY

The character of osteomyelitis changed with the advent of antibiotics, evolving from a disease of high mortality to a disease with high morbidity. Certain trends are apparent. Bone and joint tuberculosis has become less common in the developed world, although the advent of HIV-related disease may bring about a reversal in that trend. An increasing number of chronic bone infections are now associated with trauma, surgery and joint replacement rather than being secondary to hematogenous spread.

The epidemiology of acute hematogenous osteomyelitis has been detailed.[1] The incidence is higher in males, it varies among geographic areas (Fig. 52.1) and, in some areas, classical acute hematogenous infection is in long-term decline.[2] The male-to-female ratio increases with age from 1.25 in the 0- to 4-year age group to 3.69 in the 13- to 19-year age group. There are substantially higher rates in Maori children from New Zealand and Aboriginal children from Western Australia compared both with white children living in the same areas and with children living in Europe. Although almost certainly socioeconomic in origin, these differences may also be influenced by host genetic factors.

There is less clear information on the epidemiology of chronic osteomyelitis, with the exception of diabetic foot infections.[3] There are an estimated 11 million people in the USA with diabetes; the majority of these have type 2 disease and hence are older adults. Some 3% of diabetic people develop a foot ulcer annually and 10–30% of patients with an ulcer will eventually need an amputation. Of all amputations in people with diabetes, 60% are preceded by an infected ulcer. Foot problems have been estimated to be responsible for 15% of the hospital admissions and 25% of the hospital bed usage among diabetic patients. The annual hospital costs for limb amputations that are related to diabetes amount to more than US$350 million.

PATHOGENESIS AND PATHOLOGY

Microbial factors

Adhesion is the initial event in the localization of infection. [4] The initial loose adhesion to bone is potentially reversible. However, if the solid phase offers a configuration that is acceptable to the receptors of the micro-organisms, a more permanent adhesion occurs. *Staphylococcus aureus* strains possess receptors for extracellular matrix components such as collagen, fibronectin, bone sialoprotein and osteopontin.[5] [6] It is unclear which are crucial for the genesis of osteomyelitis, with conflicting data on the role of the collagen receptor. The fibronectin-binding proteins appear to play a role in attachment to, and invasion of, endothelial cells, events that may be of relevance in the earliest stages of hematogenous seeding.[7] It is possible that trauma or injury may expose binding sites for strains of *S. aureus*.

Following adhesion, firm attachment and adherent growth occurs. For staphylococci and some Gram-negative organisms, synthesis of an exocellular polysaccharide (glycocalyx) produces a 'biofilm', within which bacteria can form microcolonies (Fig. 52.2 and Fig. 52.3). Adherent growth confers phenotypic resistance to antibiotics, probably as a result of changes in cellular metabolism, and the glycocalyx may confer protection against phagocytes and complement.[8]

Prostaglandins are potent bone resorption agents that enhance osteoclast activity and collagen synthesis. It was noted in studies of human bone, as well as in studies of experimental osteomyelitis in animals, that increased production of prostaglandin E2 (the most potent prostenoid in the resorption of bone) was present. [9] Cytokines, in particular tumor necrosis factor, are also potent stimulators of osteoclast action. Finally, it has been recognized that molecules released from a number of the pathogens that cause osteomyelitis are potent stimulators of bone resorption, via cytokine release from monocytes and by stimulation of osteoclast formation.[10]

Pathology

As in most organs, an insult to bone is followed by vascular and cellular responses. However, in bone this process is modified by the rigid nature of the bone, because the increased tissue pressure cannot be diffused into soft tissue. With increased intramedullary pressure, sinuses and capillaries are compressed in the marrow, producing infarction. At the infarction edge, there is reactive hyperemia, which is associated with increased osteoclastic activity. This in turn produces loss of bone and localized osteoporosis. An inflammatory process begins at the margin of the infarcted area and penetrates through the cortex into the subperiosteal area. Because there are few anchoring fibers in the periosteum of infants and children, the periosteum is readily stripped from the bone surface by the increased subperiosteal pressure. This can result in disruption of the periosteal blood supply to the cortex, and this leads to cortical bone infarction, bone death and sequestrum formation. (A sequestrum is a macroscopic piece of dead bone that is retained within the overall bone structure.) Stripping from underlying bone is an osteogenic stimulus to the periosteum, which responds by laying down new living bone. The end result is a shell of new bone around or above a dead segment, a response that preserves the mechanical strength of the bone, even though parts of it are now dead.

Classification systems for osteomyelitis

The most frequently used classification system is that of Waldvogel *et al.*[11] In this classification, infections are classified as hematogenous, secondary to a contiguous focus of infection or related to vascular insufficiency. For chronic long bone osteomyelitis, a second classification system, developed by Cierney and Mader,[12] combines four stages of anatomic disease and three categories of physiologic host (Fig. 52.4). This classification is useful for describing severity and location of infection and for planning treatment, and it is amenable to study. The three categories of host are:

- normal except for osteomyelitis
- systemic or local compromise, and
- treatment would be worse than the disease.

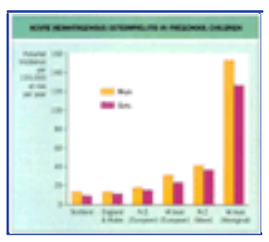

Figure 52-1 Acute hematogenous osteomyelitis in preschool children. *Data from Gillespie.*[1]

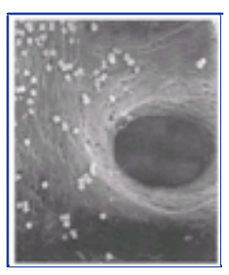

Figure 52-2 Endosteum of bone showing staphylococci near the endosteal haversian canal. In-vitro incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). *From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.*

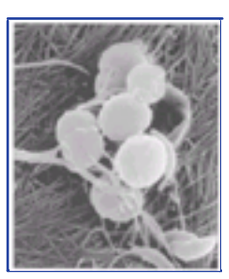

Figure 52-3 Staphylococci enmeshed in glycocalyx near the haversian osteum. In-vitro incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). *From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.*

Figure 52-4 Anatomic classification of osteomyelitis in adult long bones. *Adapted with permission from Mader JT, Calhoun J. Osteomyelitis. In: Mandel G, Bennet J, Dolin R, eds. Infectious diseases. New York: Churchill-Livingstone; 1995:1039–52.*

Causative agents of osteomyelitis

In acute hematogenous osteomyelitis in children, *S. aureus* accounts for more than half of the organisms isolated.[13] The next most frequent group of isolates are streptococci. In osteomyelitis or osteochondritis due to puncture wounds to the foot, *Pseudomonas aeruginosa* is isolated frequently and is associated with the wearing of sneakers. The organism is found in the sole of the sneaker and is presumably carried into the foot by the puncturing nail.[14] *Salmonella* spp., although an infrequent overall cause, are strongly associated with sickle cell disease. In diabetic patients with foot infections, *S. aureus*, *Staphylococcus epidermidis*, enterococci, other streptococci and *Corynebacterium* spp. are among the most frequent aerobic organisms that are isolated from bone. Anaerobic organisms are also frequently isolated, with *Peptostreptococcus* spp. being most common. Fungi, mycoplasma, mycobacteria, brucella, treponema, actinomycosis and parasites have also all been associated with osteomyelitis.

Pathogenesis of diabetic foot osteomyelitis

The pathogenesis of osteomyelitis in the diabetic foot is an important problem that merits additional consideration. Diabetic patients develop foot ulcers because of a combination of motor, sensory and autonomic neuropathy interacting with changes in the mechanical properties of the soft tissues of the foot. Motor neuropathy causes a high-arched foot with clawing of the toes, and this delivers excessive pressures to the metatarsal heads, the heel and the ends of the toes. Subluxation at the metatarsophalangeal joints not only brings the metatarsal head into a more prominent weight-bearing position but also causes the fibrous metatarsal pads to slip out from under the metatarsal heads. Sensory neuropathy reduces the response to pain, so that foreign bodies in the shoes are neglected, and clouds the recognition that it is time to change an ill-fitting pair of shoes or rest the feet. Autonomic neuropathy is associated with excessive fissuring and cracking from dry, poorly lubricated skin, an ideal portal of entry. Nonenzymatic glycosylation of collagen leads to cross-linking, with increased stiffness.

These factors together can readily lead to ulceration, which may lead down to a joint or bone. Loss of periosteum causes death of the superficial part of the cortex of the bone. Infection can track through the cortical bone into the medulla and spread rapidly up inside the long axis of the bone. Bone infarction and reaction to infection then proceeds just as in larger bones. Ischemia from peripheral vascular disease compounds the problem, as may diminished phagocyte function from poor glycemic control.

PREVENTION

There is no known effective method of preventing the development of acute hematogenous osteomyelitis. There is also no proven effective means of preventing the development of osteomyelitis secondary to bacterial seeding from an infected focus, such as an intravenous catheter. Even with rapid removal of the catheter and treatment for up to 6 weeks with an antimicrobial agent that is effective against the organism producing the bacteremia, osteomyelitis at a remote site has still been shown to develop on occasion.[15]

Antibiotic prophylaxis has been used successfully to prevent wound infections following surgery for noncompound hip fractures, and it has also been used successfully in the placement of total hip and knee prostheses.[16] The end point of these studies has been wound infections, but it is reasonable to presume that a certain number of patients who develop wound infections could go on to develop infection of the underlying bone and, therefore, antibiotic prophylaxis may play some role in preventing osteomyelitis. In trauma of long bones, aggressive debridement of contaminated and devitalized tissue, with appropriate stabilization and soft tissue cover, has been shown to reduce rates of infection.[17]

Prevention of diabetic foot osteomyelitis involves prevention of ulceration. Patients should have an annual review of their feet with reference to pulses, protective sensation, ulcers, callosities, evidence of infection, dermatophytosis and footwear. Those with a history of previous ulceration are at high risk of developing further ulcers and need more frequent review from a trained podiatrist. Ulceration must be promptly treated with the aim of healing the soft tissues to prevent the entry of new pathogens.

CLINICAL FEATURES

Acute hematogenous osteomyelitis

Early signs in children, particularly infants, are failure to move the affected extremity and pain on passive movement. These findings in an infant with an acute febrile illness should lead to suspicion of skeletal infection. Soft tissue changes of swelling, redness and heat occur late in osteomyelitis; if found early in the course of illness, one should suspect cellulitis. In older children, the diagnosis is often easier, but it may still be difficult to distinguish between bone and joint infection. Most radiographs do not show evidence of infection until at least 10–14 days after the onset, but they may show soft tissue changes.

In a large series, about 3% of children developed chronic infection as a complication.[13] However, most of these were associated with failure to treat adequately with antibiotics or with significant delays in treatment. Pathologic fractures are rare. If infection involves the growth plate, abnormal growth, resulting in either a shorter or longer limb, can occur. In young children, infection can track out of the focus in the metaphysis into the joint, because the joint capsule inserts distal to the growth plate. In general, the outcome of acute osteomyelitis in pediatric patients is good, as long as patients are seen within 7–10 days of the onset of illness and treatment is begun and continued for at least 3 weeks.

Subacute hematogenous osteomyelitis

Some studies suggest that, in temperate zones at least, an increasing proportion of cases present with longer, more insidious histories of pain of more than 2-week

duration, with minimal functional impairment and without systemic illness. The diagnosis of osteomyelitis is generally made when radiology shows a suspicious lytic lesion in the metaphysis, which biopsy shows to be infective. Cultures are usually negative, but the patient responds well to curettage of the lesion and antistaphylococcal antibiotics. One series of such cases represented 7% of all cases of osteomyelitis seen in the reporting hospital over a 9-year period.[18]

Chronic osteomyelitis in long bones

Chronic osteomyelitis in long bones usually occurs as a result of trauma; less frequently it occurs as a complication of acute hematogenous osteomyelitis. Patients usually report few systemic symptoms but are commonly troubled by persistent pain and drainage through sinus tracts. Following successful treatment, many patients comment on the dramatic improvement in overall physical well-being, and some patients gain weight. The fundamental problem is the prolonged persistence of viable pathogens. The process involves the consequences of continuing necrosis, such as sequestrum and sinus formation, versus repair with new bone formation and scar (Fig 52.5 and Fig 52.6).

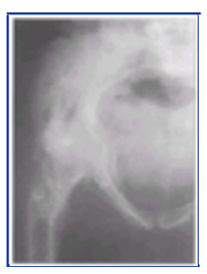

Figure 52-5 Chronic osteomyelitis. The patient is a 30-year-old man who was born in Pakistan and who, as a child, had chronic osteomyelitis caused by *Staphylococcus aureus*. He is asymptomatic now except for occasional pain in the hip and a limp. The radiograph shows destruction of the femoral head and acetabulum, chronic changes in the femoral shaft and fusion of the right hip joint. *Courtesy of Dr Joseph Mammone.*

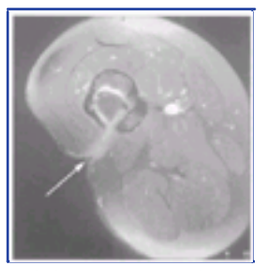

Figure 52-6 Chronic active osteomyelitis in the femur. This case of osteomyelitis was secondary to a fracture and open reduction and internal fixation 30 years before. This axial, contrast-enhanced, fat-suppressed T1-weighted MRI scan shows cortical thickening and a focal intraosseous fluid collection with an enhancing rim, communicating via a sinus tract to the surface of the thigh (arrow).

Potential complications of chronic osteomyelitis include septic arthritis if infection tracks into a joint, pathologic fracture, septicemia if a draining sinus becomes blocked and secondary amyloidosis, which is a rare occurrence (one series reported an incidence of about 1%).[19] A second rare complication, long recognized, is the development of squamous cell carcinoma in scar tissue. Again, the incidence is low (probably less than 1%) and those cases that have been reported occurred after an average of 27 years of osteomyelitis with drainage. The clinical features that are characteristic of malignancy include increased pain, increased drainage, odor and a mass. There was usually more radiographic evidence of bone destruction than is seen in patients with uncomplicated osteomyelitis.

Vertebral osteomyelitis

The most typical presentation of vertebral osteomyelitis is back pain. The pain is increased by loading the spine and relieved by rest. The degree of pain may seem out of proportion to the examination; it is unusually severe, and night pain is an important feature. In about 10% of patients, symptoms may be present for less than 1 week and

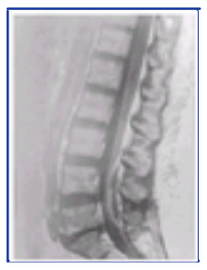

Figure 52-7 Vertebral osteomyelitis. A sagittal, contrast-enhanced convential spin echo MRI scan (T1-weighted) demonstrates a posteriorly located epidural abscess at the L4–L5 vertebral level with an enhancing rim and displacement of the nerve roots anteriorly. *Courtesy of Dr Joseph Mammone.*

the illness appears more severe with fever, night sweats and other systemic signs of infection. In such patients, blood cultures are usually positive. The majority have a subacute presentation with symptoms of back pain that are present for anywhere from 2 weeks to 2 years before diagnosis. Generally, only about half of the patients are febrile on initial evaluation.

The major complications of vertebral osteomyelitis are neurologic symptoms, caused by retropulsion of disc material, an inflammatory mass or an associated epidural abscess.[20] The classic clinical progression goes from spinal ache to root pain to weakness, followed by paralysis. Careful and repeated examination of patients with vertebral osteomyelitis is critical; if such symptoms begin, they should be investigated rapidly with radiologic studies, particularly magnetic resonance imaging (MRI; Fig 52.7 and Fig 52.8). Urgent surgical decompression is often needed.[21] Unfortunately, the neurologic complications of epidural abscess are not always reversible, so the goal of management should be detection at the earliest stage (Fig. 52.9).

Bone infections that underlie pressure sores

Patients with osteomyelitis beneath pressure sores are immobile, insensate at the pressure area or malnourished; they may be all of these things. Osteomyelitis presents as a failure of the patient to thrive, and a failure of the wound to heal, despite optimal nursing care and offloading of the sore.[22] There may be pain, but it is often not prominent. Systemic illness is ominous, implying the development of septicemia or the formation of an abscess. Depending on the degree of local sensory impairment and the location of the sore, underlying collections of pus can be very extensive. So too can be the extent of the wound, which may be deeply undermined and sloughy at presentation.

Special patient populations

Patients undergoing hemodialysis

In patients undergoing hemodialysis who present with bony pain or fractures, there must be a high index of suspicion of bone infections. Bone biopsy is necessary to make the diagnosis and to identify the infecting agent because the clinical signs, radiographic picture and symptoms can mimic those of renal osteodystrophy. [23] The usual infecting organisms are staphylococci (either *S. aureus* or *S. epidermidis*) or *P. aeruginosa*.

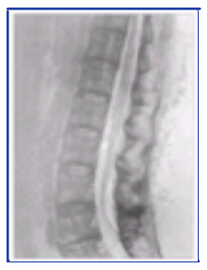

Figure 52-8 Vertebral osteomyelitis. A sagittal, turbo spin echo MRI scan (T2-weighted) from the same patient as the scan in Fig. 52.7 . *Courtesy of Dr Joseph Mammone.*

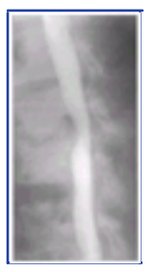

Figure 52-9 Vertebral osteomyelitis. A myelogram showing posterior compression of the spinal cord by an inflammatory mass. Note the involvement of adjacent vertebral endplates and the intervertebral disc. *Courtesy of Dr Joseph Mammone.*

Intravenous drug users

Although septic arthritis is more common than osteomyelitis in intravenous drug users, the diagnosis must be suspected if bone pain is present. Pain and tenderness are common. In general, the organisms isolated from bone are *S. aureus*, streptococci or *P. aeruginosa*; *P. aeruginosa* infection is presumably due to the use of nonsterile water for injecting drugs.

Osteomyelitis in the diabetic foot

Diabetic patients rarely manifest a fever with foot infections. Systemic illness indicates severe disease and at a local level, there is usually some accompanying necrosis, gangrene, fasciitis, severe

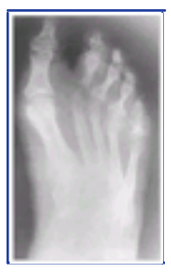

Figure 52-10 Osteomyelitis in a diabetic patient. Diabetic patient with osteomyelitis and destruction of proximal second phalanx and metatarsal as well as second metatarsal-phalangeal joint. *Courtesy of Dr Joseph Mammone.*

cellulitis or significant swelling of the foot indicating a deep abscess. Most patients appear well, although with some worsening of glycemic control. Despite neuropathy, there is often some pain to accompany an ulcer, with signs of soft tissue infection, purulence, erythema, swelling and local warmth. Depending on the depth and extent of the ulceration, bone, cartilage, joint capsule or tendon may be visible in the wound. Callosities indicate chronic excessive weight-loading on a particular area, and it is often beneath these that tissue breakdown occurs. Hemorrhage beneath a callosity is often associated with tissue breakdown or infection. Feet of this kind are often not properly evaluated during admission of the patient to a general hospital (Fig. 52.10).[24]

DIAGNOSIS

The diagnosis of osteomyelitis requires clinical suspicion, a consistent history and physical examination, and supportive laboratory studies (both radiographic and microbiologic). Certain conditions mimic osteomyelitis, and these differential diagnoses are reviewed briefly below.

Acute osteomyelitis

The diagnosis of acute hematogenous osteomyelitis is essentially a clinical one assisted by some of the studies discussed below. In the absence of a clear cause, limping or pain in an extremity should raise the suspicion of infection of bone. The sedimentation rate and C-reactive protein are frequently elevated in the presence of osteomyelitis (in 96% and 89% of cases, respectively), but normal values do not exclude the diagnosis.[25] Blood cultures are positive in just over 50% of cases. Plain radiographs may show soft tissue swelling but are otherwise usually normal because it takes anywhere from 10 to 14 days to destroy 50% of the bone (which is the amount of destruction required to show up as a lesion on conventional radiography).

Ultrasonography has been reported to be successful in detecting subperiosteal abscess in the presence of acute osteomyelitis; deep soft tissue swelling is the earliest sign of acute osteomyelitis, followed by periosteal elevation and a thin layer of periosteal fluid, which, in some cases, progresses to form a subperiosteal abscess.[26] [27] These later stages were marked by cortical erosion; this sign generally appears only in patients who have had symptoms for more than 1 week.

Technetium bone scans are exquisitely sensitive and are generally positive before lesions appear on radiograph; however, false-negative bone scans have been reported when the diagnosis of acute osteomyelitis has been confirmed by aspiration of pus.[28]

The ultimate diagnostic test in acute osteomyelitis is growth of the infecting pathogen in cultures of purulent material obtained by needle aspiration from the painful infected area. Complex tests such as indium-labelled white blood cell scan, computerized tomography (CT) scanning and MRI have little place in the management of acute hematogenous osteomyelitis unless for surgical planning or to confirm the diagnosis. In any even, treatment of the patient with suspected acute osteomyelitis must not wait for diagnostic imaging.

Chronic osteomyelitis

In contrast to acute osteomyelitis, investigation and diagnosis can generally precede antibiotic therapy. Given a clinical suspicion of chronic osteomyelitis, the clinician has a plethora of diagnostic studies to choose from. [28] Unfortunately, none is perfect (Table 52.1). An algorithm is offered for the approach to suspected osteomyelitis and its management (Fig. 52.11). In a nondiabetic patient, if the plain radiograph is positive for osteomyelitis, it is possible to proceed directly to bone biopsy for determination of the infecting organism and its antimicrobial susceptibility. The features of rapidly progressive,

TABLE 52-1 -- Tests for osteomyelitis.[*]

TESTS FOR OSTEOMYELITIS				
	Sensitivity (%)	**Specificity (%)**	**Positive predictive value (%)**	**Negative predictive value (%)**
Three-phase bone scan	95	33	53	90
Gallium scan	81	69	71	80

Indium-labeled white blood cell scan	88	85	86	87
MRI	95	88	93	92
Sensitivity, specificity, positive predictive values and negative predictive values of tests used to diagnose infection of bone.				

** Adapted from White et al. [30]*

Figure 52-11 Investigation and management of chronic osteomyelitis.

mixed destructive and reparative bone responses are highly distinctive. If the radiograph is normal and osteomyelitis is suspected, one may go directly to a three-phase bone scan, a labelled white cell scan or MRI. Indeed, MRI is particularly valuable in that it shows the extent of infection inside the bone, the presence of soft tissue abnormalities, including abscesses and sinus tracts, and erosions or breaches of the cortex. Its very high sensitivity and specificity have made it the imaging modality of choice in osteomyelitis,[29] although

its value is reduced in patients who have present or past metal work (because of signal void from implants or metallosis) and in patients with recent surgery, which causes marrow edema in its own right.

Ultimately, the procedure of choice is bone biopsy, often referred to as the gold standard for osteomyelitis. The test is easily done, but the rate of false-negative results have been reported in some series as being as high as 65%, probably because osteomyelitis has a patchy distribution in the bone. All specimens should be sent for both histology and microbiology. In one well-done study, in which 16 biopsy specimens demonstrated histologic evidence of osteomyelitis, only eight were also culture-positive.[30] In the same study, if either histology or culture was considered a positive criterion for osteomyelitis, the positive predictive value was 100% and the negative predictive value was 66%. Obviously, the larger the amount of bone sampled, the more biopsies taken and the better the imaging guidance, the more likely one is to get a positive biopsy. Finally, it should be noted that, in diagnosing osteomyelitis, sinus tract cultures have little value and correlate poorly with the organisms found in specimens taken in the operating room.[31] Therefore, the results of cultures of draining sinuses should not be relied on to identify the causative pathogen.

Bone infections underlying pressure sores

Confirming the diagnosis of osteomyelitis beneath a pressure sore can be difficult. Radiographic or nuclear imaging and soft tissue cultures can be abnormal in the area of a pressure sore and may suggest osteomyelitis when none is present. Such misdiagnosis can lead to prolonged and potentially toxic courses of antimicrobial agents.

A careful study of bone infections and pressure sores made several valuable points:[22]

- the diagnosis of underlying bone infection should be considered whenever a pressure sore does not heal;
- clinical evaluation of the depth of the sore or its duration is not helpful in determining whether bone infection is present;
- failure of the sore to close after pressure is removed is helpful in determining whether there is underlying osteomyelitis;
- nuclear scans are generally useful only if negative — the negative predictive value was high;
- Gram-negative bacilli, anaerobes and streptococci are most often cultured from infected bone; and
- bone biopsy histology and culture are the gold standard in diagnosing osteomyelitis — the procedure is rarely associated with complications. Biopsy must, however, be taken through uninvolved skin if done percutaneously, or after debridement of overlying tissue if at operation, to avoid culturing surface contaminants.

Osteomyelitis in the diabetic foot

In diabetic patients, the approach to diagnosing osteomyelitis in the foot (the usual site of the disease) is somewhat different. Conventional radiographs may be diagnostic if there is rapid progression of changes (e.g. over 2–3 weeks), but it can be extremely difficult to distinguish diabetic osteopathy from osteomyelitis. Because osteopathy will not respond to antimicrobial agents, this distinction is critical. Nuclear medicine scans are often difficult to interpret because there is soft tissue infection and it is difficult to localize infection to bone as opposed to the soft tissue (Fig. 52.12 and Fig. 52.13). One of the simplest tests is to take a steel probe and insert it into the ulcer; contact by the probe with bone has a high correlation with the presence of osteomyelitis. [32] If the probe does not hit bone, the negative predictive value of the test is not adequate to exclude osteomyelitis; in this instance, MRI should be used because it has an extremely high predictive value for osteomyelitis (Fig. 52.14). An algorithm for the diagnosis and management of osteomyelitis in the diabetic patient with infection of the foot is given in Figure 52.15 .

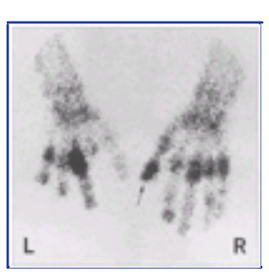

Figure 52-12 Twenty-four-hour bone scintigram of the hands. The patient is a 50-year-old diabetic with a draining ulcer at the top of the right thumb (arrow). A biopsy grew *Staphylococcus aureus*. There is intense uptake in distal first phalanx and in multiple neuropathic joints. *With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.*

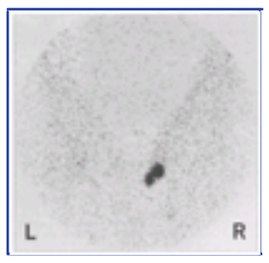

Figure 52-13 Leukocyte scintigram of the hands. This scan is from the same patient as the scan in Fig. 52.12 . Again there is intense uptake in distal first phalanx, but there is no accumulation of leukocytes in the multiple neuropathic joints. *With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.*

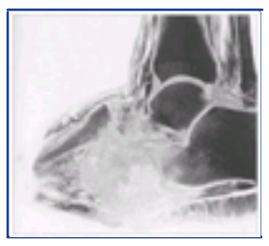

Figure 52-14 T1-weighted image of the foot. The scan reveals forefoot amputation and a normal signal in distal tibia, talus and posterior calcaneus. The interior portion of the calcaneus has edema. The remainder of the tarsal bones have been destroyed and replaced by a pale, heterogeneous inflammatory mass.

Differential diagnosis

Chronic recurrent multifocal osteomyelitis

Chronic recurrent multifocal osteomyelitis (CRMO) generally occurs in female children and presents a fascinating and bewildering spectrum of manifestations. [33] It is possible that a number of separate causes and conditions have been grouped together or that a single entity has a highly variable course. Patients present with pain and malaise, and they are found to have a bone lesion that is evidently chronic at presentation. Biopsy or exploration reveals sclerosis and some granulation tissue but very rarely any pus. Histology shows chronic inflammation including plasma cells (the condition has been called plasmacellular osteomyelitis), with no evidence of the bone tumor that is usually feared at first. Further radiographs or isotope scans may reveal multiple skeletal lesions, or these may develop over time as separate episodes. In contrast to the highly metaphyseal distribution of pyogenic osteomyelitis, the lesions are not only in the metaphysis but also in the clavicle, pelvis and spine (but in the vertebral body, not the disc space). Cultures are negative.

There may be an overlap with primary chronic osteomyelitis and chronic unifocal osteomyelitis. Both these terms describe osteomyelitis that has similar characteristics to CRMO but is only ever present at a single site. By adulthood some 50% of cases of CRMO also have an overlap with a syndrome called SAPHO (standing for synovitis, acne, pustulosis (psoriasis or palmoplantar pustolosis), hyperostosis and osteitis). More evident sacroiliitis, arthritis or spondylitis can develop over time. There is no link with HLA-B27, but a potential susceptibility gene exists on chromosome 18q21.3–18q22, analogous to a CMO gene in mice and identified in a human family study.[34] Management of these children is difficult because of persisting symptoms. Although the disease does not cause the rapid destruction and harm associated with pyogenic infection, 25–57% have been found to have persistent pain or flares at over 5 years and some children develop leg length inequality. Because antibiotics appear not to work, many therapies have been tried, including bisphosphonates. In some series, azithromycin has been shown to be helpful; it is not clear whether

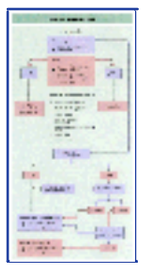

Figure 52-15 Diabetic foot infections. Algorithmic approach to diagnosis and management. *Adapted with permission from Lipsky et al.*[3]

this drug exerts an antimicrobial action or an anti-inflammatory action. Nonsteroidal anti-inflammatory drugs are often very helpful. It is important to remember the relatively good prognosis and to avoid giving repeated courses of antibiotics in the face of negative cultures.[35]

Histiocytosis X (eosinophilic granuloma)

Eosinophilic granulomas may present as one of several destructive foci within the skeleton, often without any constitutional indications of illness. The lesions generally respond to curettage or low-dose irradiation.

Gaucher's disease

Gaucher's disease is a hereditary metabolic disturbance in which cerebrosides accumulate in reticuloendothelial cells. The disease occurs mainly in Ashkenazi Jews. Constitutional symptoms include fatigue, weakness, weight loss, abdominal discomfort, hepatomegaly and splenomegaly. Bone symptoms include pain and limitation of motion, primarily of the hips and the knees. Vascular infarcts can mimic acute osteomyelitis; pathologic fractures may occur. The combination of necrosis of bone and subsequent repair produces a radio-graphic appearance similar to that of bone infection.

Ewing's sarcoma

A Ewing's sarcoma is rarely mistaken for osteomyelitis, although this error can occur, especially in locations with high prevalences of chronic osteomyelitis. [36] This emphasizes the importance of obtaining samples for histology and culture from suspicious lesions. More frequently, the biopsy of the suspected tumor is reported as infection, with no accompanying cultures.

MANAGEMENT

Acute hematogenous osteomyelitis

In the management of acute osteomyelitis, the experience of Nelson *et al.* is invaluable.[13] Optimal surgical management is controversial. In general, if frank pus is found in a diagnostic aspiration, surgical decompression is usually done. If the tap produces only bloody material, medical therapy will generally be sufficient. The choice of initial antibiotic agent can be guided by the results of Gram-stained specimens and modified if necessary after the results of culture are available. If the Gram stain is unrewarding, therapy must be empiric, based on the age of the patient and the most likely pathogen in that age group. The data obtained by Nelson *et al.* indicate that when patients are treated for a minimum of 3 weeks for infections caused by staphylococci or Gram-negative bacilli, the response is excellent and chronic osteomyelitis does not ensue. Longer periods of therapy may be necessary if the clinical response is slow or if the erythrocyte sedimentation rate remains elevated. Oral antibiotics may be given after a short course of parenteral therapy, provided that the patient and family are compliant, the drug is well absorbed and the organism is sensitive to the antibiotic.

Chronic osteomyelitis

The general principles of the treatment of chronic osteomyelitis are:

- to set realistic goals with the patient;
- to establish the microbiologic diagnosis and to stage the disease;
- to expect to need surgery to explore, to debride, to drain pus and to excise dead bone, sinus and scar tissue;
- to use antibiotics to support surgical management, according to the skill of the surgeon and the reliability of culture results and surgical excision;
- to preserve or restore physical function with further surgery if necessary; and
- to support the medical and psychologic state of the patient.

Multidisciplinary working with specialized surgical input is strongly recommended for success in chronic osteomyelitis. The condition is technically and emotionally demanding, although also enormously rewarding to treat.

Surgery

Surgery plays a major role in the management of osteomyelitis of long bones. The presence of dead bone or sequestra makes eradication of infection exceedingly

difficult because the dead bone is avascular and therefore poorly accessed by white cells or antibiotics. The goal of surgical management is to correct the underlying biology to the point where antibiotics can once again exert a useful effect. Staged procedures may be necessary (e.g. to perform bone grafting for defects) or segmental excisions, requiring stabilization with external fixators including circular frames (Ilizarov fixators). Bony reconstruction may require a free fibular transfer or bone transport. For complex wounds or areas with poor soft tissue cover, such as the front of the shin, collaboration with a plastic surgeon may be necessary to reconstruct the soft tissue defect that is left after bony debridement. The development of microvascular anastomosis allows muscle to be transferred with its vascular pedicle, thus providing a healthy soft tissue envelope to cover the operated bone.

Not all patients are suitable for surgery, either psychologically or because the procedure needed to treat their infection would create more morbidity than the infection. In such patients, the only option is long-term antibiotic use or intermittent antibiotic use to manage flares of activity. The condition is relapsing and remitting, and self-arrest is possible if a sequestrum is spontaneously discharged.

In vertebral osteomyelitis, surgery is generally not needed except for persistent pain, progressive deformity or neurologic compromise or to drain epidural or paravertebral abscesses. Scanning with MRI or CT is valuable in diagnosing epidural abscess, but clinical suspicion and careful observation of patients with vertebral osteomye-litis are critical to allow early detection by these sensitive imaging techniques.

Antimicrobial therapy

As noted above, establishment of an appropriate etiologic diagnosis either by bone biopsy or by blood cultures is critical to the management of osteomyelitis. Table 52.2 indicates our recommendations for antimicrobial therapy once the infecting organism has been identified.

The most common infecting organism is *S. aureus*. For methicillin-susceptible strains, treatment with nafcillin 2g q6h, flucloxacillin 2g q6h, cefazolin 1g q8h or clindamycin 600mg q6–8h parenterally for 4–6 weeks is appropriate therapy. Intravenous therapy may be administered at home; once daily ceftriaxone is effective. However, oral therapy, provided the drug is well absorbed and the organism is susceptible, has been shown to be highly effective following a short course of parenteral therapy. A few studies have suggested that an entire course of oral therapy will be as effective as parenteral therapy, but these studies have involved relatively few patients. A meta-analysis of all randomized antibiotic trials in bone and joint infection was unable to generate firm conclusions as to the best mode or choice of treatment.[37]

For osteomyelitis caused by methicillin-resistant strains of *S. aureus*, vancomycin remains the drug of choice. However, this agent has not always been effective, and rifampin (rifampicin) or rifampin plus gentamicin have been used in addition to vancomycin. Controlled studies are lacking in this area. For less common causes of osteomyelitis, including Gram-negative organisms, therapy is based on sensitivities of the infecting organism. Recommendations for duration of therapy for either Gram-negative or Gram-positive osteomyelitis are not based on controlled data, but we recommend a minimum of 4 weeks of therapy.

Quinolones have been used with increasing frequency in the treatment of chronic osteomyelitis. A total of seven randomized trials have been conducted, and despite methodologic issues, the overall view is that they are indistinguishable from conventional, ß-lactam-based therapy.[37] It is possible to draw certain conclusions regarding quinolones in osteomyelitis: [38] [39]

- the published success rate in osteomyelitis due to Enterobacteriaceae is sufficiently high (92%) to eliminate the need for further trials;
- for osteomyelitis caused by *P. aeruginosa* or *S. aureus*, success rates of 72% and 75% suggest a need for further comparative trials with larger sample sizes before quinolones can be recommended with confidence as sole treatment for these pathogens;

- development of resistance to quinolones has been observed with disturbing frequency among strains of *S. aureus*;
- the combination of rifampin and quinolones appears to offer promise; further studies are required in this area as well; and
- as newer quinolones with increased activity against Gram-positive organisms are developed, they may play an increasing role in the treatment of osteomyelitis due to *S. aureus*.

TABLE 52-2 -- Antimicrobial therapy for infections of bone.

ANTIMICROBIAL THERAPY FOR INFECTIONS OF BONE AND JOINTS		
	Antimicrobial agent of choice	
Organism	**Agent**	**Alternative agents**
Staphylococcus aureus (methicillin-sensitive)	Nafcillin or oxacillin 2g q6h iv	Cefazolin, vancomycin, clindamycin
Staphylococcus aureus (methicillin-resistant)	Vancomycin 1g q12h iv	Trimethoprim-sulfamethoxazole plus rifampin
Streptococcus pneumoniae	Penicillin G 5×10^6 U q6h iv*	Cefazolin, vancomycin, clindamycin
Group A ß-hemolytic streptococci	Penicillin G 5×10^6 U q8h iv	Cefazolin, vancomycin, clindamycin
Enterococci	Ampicillin 2g q4h iv†	Vancomycin
Haemophilus influenzae (ß-lactamase-negative)	Ampicillin 2g q4h iv	Trimethoprim-sulfamethoxazole, ceftriaxone
Haemophilus influenzae (ß-lactamase-positive)	Ceftriaxone 1g q12h iv	Trimethoprim-sulfamethoxazole
Klebsiella pneumoniae	Ceftriaxone 1g q12h iv	Ciprofloxacin, piperacillin, imipenem
Escherichia coli	Cefazolin 1g q8h iv	Ciprofloxacin, ceftriaxone, imipenem
Pseudomonas aeruginosa	Ciprofloxacin 400mg q12h iv or ceftazidime 1g q8h iv	Pipercillin plus aminoglycoside, aztreonam
Serratia marcescens	Ceftriaxone 1g q12h iv	Imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin
Salmonella spp.	Depends on sensitivity test; choose between ampicillin, ceftriaxone, imipenem, ciprofloxacin	
Bacteroides spp.	Clindamycin 600mg q8h iv	Imipenem, metronidazole

* If susceptible or intermediate resistance. If high level resistance, use ceftriaxone 1g q24h or vancomycin 1g q12h.
† If susceptible. If ampicillin resistant but vancomycin susceptible, use vancomycin. If resistant to both, check susceptibility to tetracyclines, chloramphenicol or experimental agents.

The role of rifampin may not be confined to its usefulness in combination with vancomycin. Animal data support the view that rifampin is of particular value in killing staphylococci present as biofilms, and human experience in the area of orthopedic hardware suggests that combinations of fluoroquinolone and rifampin are effective in some cases. This combination has been reported as effective in diabetic foot infections and other combinations (e.g., trimethoprim-sulfamethoxazole (co-trimoxazole) plus rifampin, fusidic acid plus rifampin) have also been studied. A randomized trial of nafcillin with or without adjunctive rifampin did not show superiority. Further studies would be appropriate, because the addition of rifampin clearly increases the incidence of side-effects.

Local therapy

Among the potential complications of parenteral antibiotic therapy with high serum concentrations (particularly of aminoglycosides) are toxic reactions involving the kidneys, hearing and vestibular function. In theory, these potentially adverse effects of systemic antibiotic therapy can be avoided by releasing antibiotics locally into the wound in high concentrations through either antibiotic impregnated cement or antibiotic impregnated beads.[40] [41] There is considerable experience with this technique

in parts of continental Europe, and major published series have reported success rates comparable to those of systemic therapy. A multicenter randomized controlled trial to study this question was undertaken in the USA, but massive protocol violations made interpretation impossible.[42] A range of antibiotics can be incorporated into bone cement, including gentamicin, tobramycin, cefuroxime, erythromycin, vancomycin and fusidic acid. Antibiotics can be delivered via 'high-tech' resorbable polymers and by structural osseointegrative materials such as ceramics, or even via plaster of Paris, a 'low-tech' solution that merits further study for resource-poor areas of the world.

Hyperbaric oxygen therapy

Hyperbaric oxygenation (HBO) has frequently been proposed as a treatment modality or adjunct therapy for chronic osteomyelitis. Experimentally, there is evidence that increasing intramedullary oxygen tensions allows increased effectiveness of leukocytes in killing staphylococci. Increased oxygen tensions may also improve wound healing. Several open studies have reported improvement with the use of hyperbaric oxygen in patients with chronic osteomyelitis who were refractory to prior therapy. There is currently no compelling controlled clinical evidence to support the use of HBO, and it probably has little to add to the treatment of osteomyelitis when adequate debridement produces a well-vascularized wound. However, in patients in whom this condition is not met, HBO may be of some therapeutic value. A number of studies have been carried out in diabetic patients with infected ulcers, but these studies have been largely retrospective. A single randomized controlled trial showed only 3/35 patients treated with HBO needed amputation, whereas 11/33 patients treated with standard care did. The need for controlled studies in this area is obvious.[43]

Foot infections in diabetic patients

Treatment begins with evaluation of severity. The patient should be assessed for systemic compromise and admitted to hospital if this is present. The foot should be examined for the extent of infection. Uninfected ulcers and those with <2cm of erythema (mild infections) and involvement of the skin only can be treated on an outpatient basis. Moderate infections, with no systemic compromise, but with >2cm erythema and a degree of deeper involvement (early tendon, joint or bone) may be suitable for outpatient treatment or need hospitalization. Patients with gangrene, gross necrosis, fasciitis or major soft tissue loss with exposed bone should be admitted.

The next steps are to debride the ulcer to determine its extent, obtain microbiology (from the base of the debrided ulcer) and physically to debulk the ulcer of dead and infected material. This can usually be performed by a podiatrist in the clinic, but for severe cases a surgeon is required. An assessment of the healing ability of the wound must now be made, by checking the peripheral pulses with palpation or a Doppler probe. Ischemia, measured by Buerger's test, impalpable pulses, or an ankle-brachial pressure index of <0.6, should prompt urgent vascular review, because the combination of infection and ischemia has a high risk of culminating in amputation. Finally, for patients who are admitted, bedrest is essential. Outpatients should be advised to rest, and must be given shoes that offload the ulcer when walking.

Only when all these steps have been taken is it appropriate to consider antibiotic treatment.

Until culture results are available, therapy must be empiric, ensuring that *S. aureus* is covered. ß-Lactam agents, clindamycin or vancomycin are most frequently used. For methicillin-resistant *S. aureus*, rifampin has been added to vancomycin. New agents such as the prototype oxazolidinone, linezolid, which is effective against methicillin- and vancomycin-resistant organisms, are showing promise.

Mild infections can be treated for 2 weeks. Moderate infections should be treated for 2–4 weeks if there is no bone involvement. Severe infections may need ablative therapy to save the patient's life, and minimal therapy (48 hours) is needed if the amputation is through uninvolved skin and bone. For osteomyelitis, a minimum of 6 weeks is generally appropriate.

Surgical resection of infected bone may be helped by delineating the area of infection. In one series of 35 patients, 21 underwent surgical resection of infected bone. In 13 of these, the surgery was limited to the specific region of infection demonstrated by MRI. In all patients, the surgical margin encompassed the region of bone with abnormal MRI signal intensity, and there were no recurrences at the surgical margins with an average follow-up of 9 months.[44]

There are data to support the need for an aggressive surgical approach. Early surgical intervention has been shown to be associated with a lower rate of above-the-ankle amputation and a shorter duration of hospitalization than delayed or less aggressive surgical intervention.[45]

Against this must be set a number of retrospective series showing cure rates of diabetic foot osteomyelitis with antibiotics alone, with success rates of the order of 70–80%. It is difficult to compare between series because of varying methodologies and case definitions, but the overall trend is hard to dismiss. [46] [47] Recent studies have suggested that adjunctive treatment with GCSF (lenograstim) can reduce the complications associated with diabetic foot infections.[48] Further work is necessary to better define the precise role of GCSF in this setting.

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Chapter 53 - Infections of Prosthetic Joints and Related Problems

Anthony R Berendt

INTRODUCTION

Infections of orthopedic devices are paradigms of device-related infection. Microbiological diagnosis is complicated by the important role of skin commensals as pathogens, but also as contaminants of diagnostic samples. Treatment requires a multidisciplinary approach that brings together the surgeon and the specialist in infection. Surgery is needed to remove infected foreign material and necrotic tissue, to restore skeletal integrity and soft tissue cover and, in some cases, to obtain diagnostic specimens. Antibiotics are essential — to prevent systemic illness and soft tissue involvement, to clear residual contamination after surgery or to suppress infection when further surgical intervention cannot be contemplated. The role of the infectious diseases specialist in prosthetic joint infection is therefore usually as a guide and supporter, helping the orthopedic surgeon to perform and interpret microbiologic tests appropriately and to choose and use antimicrobials rationally. This chapter therefore includes a strong emphasis on surgical management. Understanding the perspective of the orthopedic surgeon is crucial to arriving, through successful combined working, at the best plan for the individual patient.

EPIDEMIOLOGY

Reported infection rates for hip and knee replacements are approximately 0.5–3% and 1–2%, respectively.[1] [2] Most infection presents within the first 5 years of implantation, but all prosthetic joints are at a continuing low lifelong risk of infection. This risk probably rises if the joint begins to fail mechanically. Thus, rates of infection depend on the duration and diligence of follow-up and also on the criteria used for diagnosis. Lack of consensus definitions of prosthetic joint infection make such rates hard to compare between centers. Independent risk factors for infection, based on a large retrospective case-control study of hip and knee replacements,[2] are a history of superficial wound infection (OR, 35.9), a diagnosis of malignant disease (OR, 3.1), prior surgery on the replaced joint (OR, 2) and a National Nosocomial Infections Surveillance (NNIS) System patient risk index score of 1 (OR, 1.7). The latter is a composite score in which prolonged operation time (over 3 hours), wound status and an ASA score of >2 each contribute one point. Drainage of the postoperative wound at the fifth day or longer, and the presence of a hematoma, have both separately been shown to be independent risk factors for *superficial* wound infection (OR, 1.3 and 11.8 respectively).[3] In the same study, superficial wound infection was confirmed as a major risk factor for deep infection.

Rates of fracture-fixation infection vary widely depending on the site and the method of fixation adopted. Infection rates for closed fractures undergoing internal fixation by incisions through healthy soft tissue, for example, by intramedullary nailing, should be comparable to those of other 'clean' surgical procedures. Compound fractures are at higher risk, depending on the extent of environmental contamination, soft tissue injury and vascular injury.[4] In addition, delay in wound debridement and soft tissue closure leads to greatly increased infection rates.

PATHOGENESIS AND PATHOLOGY

Pathogenesis

Infection commences with the adhesion of bacteria to host tissues or biomaterials, either directly or via host plasma proteins, such as fibronectin, deposited on the biomaterial surface.[5] Bacteria express numerous structures to enhance adhesion, including proteinaceous cell wall associated adhesins and capsular polysaccharide adhesins. Furthermore, the surfaces of many bacteria are relatively hydrophobic, enhancing initial interactions mediated by van der Waals forces.

Adherent micro-organisms frequently produce exocellular polysaccharides that enmesh the bacteria. The resulting consortium of adherent bacteria is called a biofilm, which may be monomicrobial or polymicrobial. Compared with the same organisms cultured in liquid media, bacteria in biofilms are exceedingly difficult to kill with antibiotics or biocides or by host defenses. The possible mechanisms for this resistance include alterations in the growth rate of organisms within the biofilm and adherence-dependent differential gene expression. It does not appear to be due to impaired antibiotic access. Characterization of genes involved in coagulase-negative staphylococcal exopolysaccharide synthesis[6] indicates an essential role for a cluster denoted *icaABCD*, with a number of other genes involved in regulation and biofilm formation (reviewed in reference[7]). Biofilm formation is subject to phase variation, in part through integration and excision of insertion sequences close to regulatory genes. In human disease, the relationship between adherence to plastic or elaboration of exopolysaccharide and the clinical manifestations caused by isolates has been studied, with inconclusive results.

Host responses are impaired in the vicinity of biomaterials. This was dramatically demonstrated in the classic observations of Elek and Conen, who showed that the inoculum of *Staphylococcus aureus* required to cause a skin infection in human volunteers was reduced many-fold in the presence of a skin suture.[8] Neutrophils show defective phagocytosis, partly owing to the concentrations of metal ions or to the presence of monomeric or polymerized methacrylate (bone cement). In addition, bacterial exocellular polysaccharide may inhibit phagocytosis and other elements of the immune response,[9] though recent findings suggest that human leukocytes can interact with *Staphylococcus aureus* biofilms.[10]

Pathology

Once the organisms have established an association with the surface of the biomaterial, infection ensues with two major pathologic features:

- acute inflammation around the prosthetic material,
- activation of osteoclasts and recruitment of monocyte-derived bone-resorbing cells, leading to bone loss at the interface with the implant. This is probably in part due to inflammatory cytokines[11] and in part to direct triggering by bacterial antigens.[12]

The normally thin layer of poorly vascularized fibrous tissue between the host and the implant or its anchoring cement, generally termed

the 'membrane' by orthopedic surgeons, becomes markedly thickened and more vascular. Frank granulation tissue may be produced alongside the implant and eventually free pus is formed. In prosthetic joint infection, these processes exaggerate the pathologic mechanisms operating in aseptic loosening, in which a chronic inflammatory response is produced in response to wear particles derived from acrylic cement and polyethylene. This chronic inflammation, associated with mononuclear cells and giant cells, also leads to bone resorption (Fig. 53.1). A key feature of infection is thus the presence of an acute inflammatory infiltrate, but it may be superimposed on chronic inflammation (Fig. 53.2).[13]

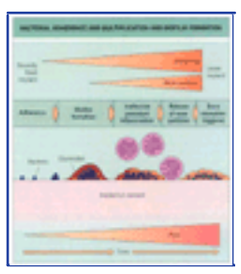

Figure 53-1 Bacterial adherence to the implant, bacterial multiplication on the surface and biofilm formation. An ineffective host response triggers bone resorption,

contributing to loosening, which is accelerated by wear particles and the host response to them.

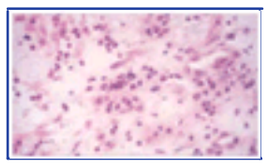

Figure 53-2 Histologic features of infection. Periprosthetic tissue from a clinically infected total hip replacement from which multiple specimens grew an indistinguishable organism. Numerous neutrophils are present in the tissue.

TABLE 53-1 -- Bacteria associated with infected implants presenting at different stages.

BACTERIA ASSOCIATED WITH INFECTED IMPLANTS PRESENTING AT DIFFERENT STAGES	
Early infection (up to 3 months)	*Staphylococcus aureus*
	Coagulase-negative staphylococci
	Aerobic Gram-negative rods
	ß-hemolytic streptococci
Delayed infection (4–12 months)	Coagulase-negative staphylococci
	Other skin commensals
	Staphylococcus aureus
Late infection, including hematogenous seeding (more than 12 months)	Coagulase-negative staphylococci
	Other skin commensals
	Staphylococcus aureus
	Aerobic Gram-negative rods
	Anaerobes
	Mycobacterium tuberculosis

Microbiology

Orthopedic implant infections are classified as early, delayed and late (Table 53.1). It can also be useful to distinguish between acute infections, which may arise early (postoperatively) or late (by hematogenous spread), and chronic ones, as this helps determine the management.

Early infections usually manifest as wound infections. They tend to be caused by higher grade pathogens able to exploit postoperative hematomas and breaches in the skin. *Staphylococcus aureus*, including methicillin-resistant strains, predominates. ß-Hemolytic streptococci and aerobic Gram-negative rods are also seen.

Delayed infections, in which pain and swelling develop in the context of a healed wound, are more often caused by coagulase-negative staphylococci and other skin commensals. This is presumably because, as lower virulence pathogens, they take longer to reach the critical number of organisms needed to produce clinical symptoms.

Late infections, which present more than 12 months postoperatively, are mostly caused by Gram-positive skin commensals that have been chronically infecting the prosthesis since implantation, but also include cases of hematogenous seeding with organisms capable of causing bacteremia.

Irrespective of the time of presentation, mixed infections are relatively common (accounting for approximately 20% of cases), with a variable proportion of aerobic Gram-negative rods, fastidious anaerobes and culture-negative cases. A very large number of individual organisms have been described as causes of prosthetic joint infection, including *Mycobacterium tuberculosis*, atypical mycobacteria, *Brucella* spp., *Listeria monoyctogenes, Campylobacter* spp., *Mycoplasma* spp and yeasts.

The high proportion of culture-negative cases in clinically suspected infected revisions (c.15%) is sometimes plausibly due to sampling error or prior antibiotic therapy, but there may also be fastidious organisms that cannot adapt to the conditions in the diagnostic laboratory. Furthermore, there is some evidence that sonication of implants leads to the release of bacteria from the prosthesis surface that can be cultured (yielding a high proportion of *Propionibacterium* spp.), detected visually with specific antisera or inferred from the presence of bacterial 16S ribosomal DNA identified by PCR.[14] The clinical spectrum of prosthetic joint infection and its microbiological causes may yet broaden.

PREVENTION

Prevention of all infections of orthopedic devices depends to a great extent on good surgical technique and operating room discipline. Ultraclean air in theaters reduces infection rates, as do prophylactic antibiotics. In most centers, one to three doses of a first- or second-generation cephalosporin are used; [15] where methicillin-resistant *Staph. aureus* is endemic, glycopeptide prophylaxis may be advised. Although there is clear evidence of the benefit of antibiotic prophylaxis, it is less clear as to whether a single dose will suffice, and whether intraoperative redosing is essential if the procedure is prolonged or accompanied by excessive blood loss, though many would advise this. Optimum prophylaxis specifically to prevent infection with methicillin-resistant organisms is also uncertain.

For joint replacement, specialized theater apparatus may also be used, including total body exhaust suits and the Charnley-Howarth sterile air enclosure, a plastic hood that excludes most of the operating theater environment from the vicinity of the surgical field. For prevention of fracture-fixation infection, energetic wound debridement and early soft tissue closure are essential, with initial stabilization by external fixation before proceeding to internal fixation once the soft tissues have recovered from the initial trauma. Free tissue transfer using muscle flaps allows debridement to be radical, removing all devitalized and contaminated tissue and replacing it with healthy tissue at the fracture site.

Late prophylaxis

The risk of hematogenous infection of prosthetic joints must be considered when planning procedures that are known to cause transient bacteremia. The risk is unclear, but the routine use of antibiotic prophylaxis for all procedures liable to cause bacteremia in all patients with prosthetic joints is probably not justified on risk-benefit or economic criteria. Patients at particular risk are likely to be those with newly implanted or loose prostheses and those with active infection elsewhere, including dental infection, and it may be reasonable to offer prophylaxis in these situations,[15] using protocols similar to those used for the prevention of infective endocarditis.

CLINICAL FEATURES

Orthopedic implant infections are almost always associated with pain, which may be mild at first but which usually becomes severe. 'Infection pain' is often distinguishable from other pains, even at the same site, but it does not have pathognomonic features. It is commonly present to some extent as soon as the infection is active. Even if presentation is delayed, many patients give a history that the implant was 'never right', that is, that it never became comfortable. Pain is due to inflammation but is also caused by loosening of the implant. This is characteristically 'start-up' pain, initially severe following rest (and therefore especially in the morning) but subsequently easing off with use of the joint. As loosening progresses, use of the joint or limb becomes increasingly painful at all times and night pain and unpredictable spasms develop.

Early infections are generally also associated with fever and marked inflammation in the operative wound. There may be purulent discharge (Fig. 53.3) or frank wound breakdown with skin necrosis when high-grade pathogens are involved. Rarely, sepsis results; this is also often a feature in hematogenous late infections. If infection

with less virulent pathogens presents early, the clinical features may be less florid, with a wound that continues to drain in a patient who is systemically well. In either case, inadequate treatment will lead to the formation of a sinus tract, which is also often seen with delayed and late infections (Fig. 53.4). It is not unusual for patients to give histories of recurrent wound infections, treated with repeated short courses of oral antibiotics because the underlying deep infection was

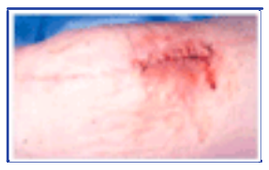
Figure 53-3 An acutely infected knee replacement. The site was washed out but the infection failed to resolve. At reoperation the implant was found to be loose and it needed to be removed. *Staphylococcus aureus* was grown from deep specimens.

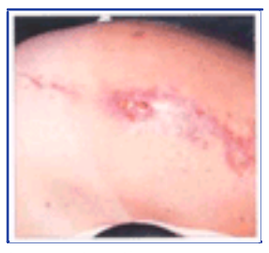
Figure 53-4 A sinus tract discharging from an infected total hip replacement. *Staphylococcus aureus* was grown from deep specimens. Note the Koebner phenomenon; this patient's psoriasis was probably a significant risk factor for infection.

not addressed. By the time such infections present to the specialist, the implant has usually loosened. A proportion of delayed and late infections simply present with rapid loosening and do not show any signs of local or systemic inflammation, although they do cause pain.

DIAGNOSIS

Diagnosis of infection requires clinical suspicion and appropriate investigation. When wounds develop purulence, spreading cellulitis, persistent erythema or persistent drainage (even with serosanguinous fluid), the implant should be considered infected until proved otherwise. Unless there are convincing alternative explanations, the same approach should be adopted for any implant that fails early or is persistently painful.

Early infections

The erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell count are generally elevated. Blood cultures may demonstrate a bacteremia. Ultrasound of the implant may reveal a fluid collection or a hematoma; if so, this should be aspirated for microscopy and culture. If unexplained fever is present, aspiration of the joint space or the region of the fracture should be considered. Plain radiographs are of little value in early infection, but they serve as a useful baseline for subsequent change.

Of central importance in making the diagnosis is exploration, which should be performed before starting antibiotic therapy if the overall condition of the patient and the state of the soft tissues permit it. Infections presenting at this time interval, and acute late

infections, generally have a high microbial load and are readily diagnosed from microbiological specimens. It is nonetheless recommended that a number of independent samples (up to 5) are taken from around the joint at debridement, to maximize the definition of the infecting flora and to assist interpretation of the results if skin commensals are isolated.

Late infections

These are accompanied by systemic features only in the small minority of patients in whom bacteremia or abscess formation occurs. The white blood cell count, ESR and CRP may all be normal. Plain radiographs usually demonstrate loosening (Fig. 53.5). Additional features, such as periosteal reaction, cortical scalloping and endosteal erosion (with intramedullary implants), may be seen but their specificity for infection is unknown.

The clinical context is helpful in interpreting plain film changes, as infection is generally associated with early failure and with more rapid loosening. For prosthetic hips, arthrography combined with aspiration or capsular biopsy for microscopy and culture may be useful for determining the extent of loosening. Arthrography can detect loosening that is not apparent on the plain radiograph. Even if this is not seen the prosthesis is likely to be loose if pain is immediately relieved by the instillation of local anesthetic. More elaborate methods of imaging have been disappointing. Metal artefact renders CT scanning and MRI of little value except to evaluate periprosthetic

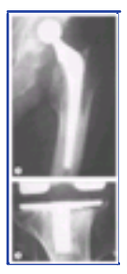
Figure 53-5 Implant loosening in late infection. (a) Radiograph of the infected hip shown in Figure 53.4 . There is an obvious radiolucent line at the bone-cement interface. This implant required revision. (b) Radiograph of a loose knee replacement, showing resorption of bone beneath the tibial component, a cause of instability. Coagulase-negative staphylococci were grown from multiple deep specimens.

soft tissue. Technetium bone scanning is sensitive but not specific and although indium-labeled white cells, gallium citrate, labeled immunoglobulins and even labeled antibiotic scans all have their proponents, there is no widely applicable and robust test with high sensitivity and specificity.[15] [16]

Molecular diagnosis, using the PCR or other molecular methods to detect bacterial DNA, offers the advantage of speed and potential sensitivity. Despite early studies that offer promise, it has not yet been validated as a practical diagnostic test.[17] Molecular methods would need to provide not only evidence of infection, but identification and antibiotic sensitivities, and this is likely to require considerable refinement.

Pathology has a useful role to play. In the absence of inflammatory joint disease, the presence of neutrophils is indicative of infection. Different authors have set different limits for an abnormal number of neutrophils, ranging from 10 to 1 cell per high power field, averaged over at least 10 fields. Often, however, the histologic changes are focal, with some specimens negative and others showing unequivocal acute inflammation. This variability, which was also observed in experimental studies of infected prosthetic joints in dogs, is probably due to infection with very low numbers of micro-organisms in a patchy distribution. Frozen sections can be used to make the diagnosis during surgery, again using as criteria the presence of neutrophils.[18] [19] [20] The specificity of the test is high and this makes it possible to identify at least some infected cases intraoperatively. The surgeon can then accordingly choose between one- and two-stage revision (see below).

If there is sinus tract drainage, the diagnosis of infection is effectively beyond doubt. However, because sinus tracts commonly acquire a complex mixed flora of both commensals and pathogens, including *Staph. aureus*, aerobic Gram-negative rods, enterococci and anaerobes, it has long been recognized that the predictive value of sinus tract cultures is poor. Such cultures should be discouraged except for the purpose of infection control in hospitals, where attempts are made to identify cases of methicillin-resistant *Staph. aureus*.

Aspiration for microscopy and culture is frequently performed, but published sensitivities and specificities vary widely. This may be explained by differences in techniques of aspiration, in sample handling and processing, and in the criteria used for the final diagnosis of infection when evaluating the test. With careful case selection, it was possible to achieve a sensitivity of 92% and a specificity of 97% in one study.[21] In general, specificity outperforms sensitivity, so the test is more useful at 'ruling in' infection than excluding it.

Definitive microbiologic diagnosis requires culture of periprosthetic tissue samples. For maximum diagnostic yield, deep specimens should be obtained from multiple

areas around the implant at debridement or excision and transported to the laboratory without delay. The surgeon must use separate instruments to prevent cross-contamination of samples and these should be processed in a laminar flow cabinet to avoid contamination in the laboratory. Enrichment cultures are essential. Unfortunately, published criteria for the diagnosis of infection based on operative samples are non-standardized (if stated at all), ranging from the isolation of organisms in four out of five or five out of six specimens to diagnosing infection on the basis of a single positive direct culture or on two or more positive enrichment cultures. [22] [23] [24] One prospective study of over 300 revision arthroplasties (known infected and predominantly believed uninfected) compared the results of culture with histology as the criterion standard for infection.[25] Using the strict protocols above, the sensitivity of a single sample from an infected patient was found to be approximately 60%. The false-positive rate for a single sample averaged 6%. Likelihood ratios were found to be

extremely high for the diagnosis of infection (positive histology) when three or more independent specimens of periprosthetic tissue yielded indistinguishable micro-organisms. The receiver-operator characteristic modeled from the data suggested that if five or six samples were sent a sensitivity of 91–95% and a specificity of 95–97% could be achieved (taking two or more samples positive with an indistinguishable organism as the cut-off). In agreement with other studies, the Gram stain was found to be of no value in diagnosing infection at revision arthroplasty.[25]

MANAGEMENT

Early infections of prosthetic joints

These should be considered to be emergencies, for two reasons:

- infection may lead to bacteremia or soft tissue loss or both;
- some cases of early infection have good long-term outcomes if aggressive debridement is combined with appropriate antibiotic therapy, whereas there is clear evidence that delay worsens outcome.[26] [27]

Hence, early infections (and the acute presentations of late infections) should be managed by urgent exploration, debridement of infected and devitalized tissue, and inspection of the prosthesis. If the implant is soundly fixed, salvage may be possible; if loose, the prosthesis should be removed. After specimens have been taken, intravenous broad-spectrum antibiotics should be given, active against methicillin-resistant staphylococci and aerobic Gram-negative rods. Antibiotic treatment can be rationalized according to culture results.

Many authors recommend treatment with intravenous antibiotics for 6 weeks. Outpatient parenteral antimicrobial therapy (OPAT) (see Chapter 191) may make this more acceptable to the patient and the surgeon and to health economists. Insertion of a central venous catheter (Hickman, Broviac or as a peripherally inserted line or PICC) allows assured long-term access for the outpatient and home setting. Once-daily dosing with ceftriaxone (for methicillin-sensitive organisms) or with teicoplanin (for methicillin-resistant Gram-positives) is particularly convenient. More frequent dosing or continuous infusion is possible, with a range of portable electrical or elastomeric devices available. It is essential that if such therapy is undertaken, there is a clear system of follow-up and patient support in place. A French study calculated that OPAT for 39 patients with osteomyelitis had saved $1,873,885 relative to conventional intravenous therapy.[28] The duration of therapy necessary to maximize the chance of salvage is unclear. There is anecdotal evidence that infection can be eradicated with finite courses of treatment in some cases but in others, prolonged suppression seems more appropriate rather than risking recurrence of infection.

An advance in this regard may be the recognition that combinations of a fluoroquinolone and rifampin (rifampicin) show excellent killing activity inside abscesses, inside cells and in experimental models of biofilms or infected foreign devices. This led to observational studies with ofloxacin and rifampin, or ciprofloxacin and rifampin, of a range of different infections of orthopedic hardware, including prosthetic joints.[29] [30] Subsequently a randomized controlled trial of ciprofloxacin and rifampin compared to ciprofloxacin alone showed that of 12 patients (with a range of orthopedic devices in situ) treated with combination therapy, all had cure of infection, whereas five of 12 failed in the placebo group. A further seven of nine patients who were randomized but dropped out of the trial subsequently took open label rifampicin combinations, and five of these were also cured.[31] Other regimens, including fusidic acid and rifampicin or high-dose trimethoprin-sulfamethoxazole, may also be effective with success rates of the order of 50%, according to sensitivity patterns.

In all cases, the key features associated with a good outcome appear to be:

- thorough debridement early after the onset of infection;
- infection with a sensitive organism (poorer outcomes occur with *Staph. aureus* and aerobic Gram-negative rods);
- the absence of a draining sinus; and
- a soundly fixed prosthesis.

If wound breakdown is a feature, implant salvage will also require the prompt restoration of soft tissue cover to prevent secondary colonization with other, more resistant flora. Plastic surgery may be necessary to cover large defects with free or rotational flaps, which obliterate the dead space generated by debridement and provide a mechanism for systemic antibiotics to reach to the site of infection.

In all situations of early infection, there is a need for randomized trials to address the question of whether long-term antibiotic therapy improves outcomes and, if it does, at what cost. Definitive identification of those patients who should proceed to immediate revision surgery would save patients and carers from the medical, psychological and financial costs of prolonged and ineffective attempts at salvage treatment.

Delayed and late infections of prosthetic joints

Excision arthroplasty

If the prosthesis is loose, revision surgery is usually needed. The simplest option is excision arthroplasty, which has a high cure rate provided that the debridement of cement and dead tissue is adequate. In the hip, where a Girdlestone's pseudarthrosis is the result, function is generally very much poorer than with a successfully reimplanted hip. However, it is an option in cases where attempted reconstruction is technically unrealistic or poses unacceptable risks of reinfection, other major morbidity or death. The affected leg is short, requiring a substantial shoe raise, the patient walks with a limp and at least one stick for life and function is particularly poor in patients of above average weight.

Excision arthroplasty alone is rarely employed for prosthetic knee infections because it leaves the knee flail and the limb unstable. An external brace is needed for ambulation and levels of discomfort are high. Nonetheless, in certain cases where limited demands are placed on the leg and poor bone stock makes reimplantation or fusion unlikely to succeed, this may still be preferable to an above-knee amputation.

One-stage revision

In one-stage procedures, the excision is followed by immediate reimplantation. Although systemic antibiotics may also be administered, in the largest published experiences of this method, great reliance was placed on a meticulous debridement and the use of gentamicin-impregnated bone cement. The high prevalence of gentamicin resistance among hospital-acquired coagulase-negative staphylococci has led to a decline in the popularity of this strategy.

It should, however, be noted that the benefits to the patient of a successful one-stage revision are enormous, making this a technique that would justify renewed efforts to improve its success. Published success rates vary, depending on both the skill of the surgeon and the completeness of the follow-up, but in the most reliable and largest series, approximately 80–85% of treated implants showed no signs of recurrence at 7 years.[32] [33]

One-stage revision can still be considered for patients for whom a staged procedure carries unacceptable anesthetic risks and there is prior knowledge of a highly sensitive pathogen. It is less successful if there are resistant organisms involved; furthermore, few surgeons would happily perform this operation in the presence of extensive bone loss, for which bone allografting is often used in reconstruction.

A retrospective review of patients referred to one center and managed with two-stage revision found that only four of 37 cases would have met accepted criteria for one-stage revision, and that preoperative definition of the pathogen was incomplete in 30% of cases.[34]

Two-stage revision

In two-stage revision, an excision arthroplasty is combined with intensive antibiotic treatment, often for 6–12 weeks, followed by reimplantation of a new prosthesis. Antibiotics may be administered systemically (intravenously or orally) or locally using antibiotic-impregnated cements. In the knee, some form of spacer is required to stop femur and tibia impacting.

Comparative studies between local or systemic routes of antibiotic administration are not available. Local delivery offers obvious advantages of convenience to patient and surgeon without the complications of systemic therapy, but it is necessary to know the infecting flora and antibiotic sensitivities in advance of the excision arthroplasty and this may necessitate an additional diagnostic procedure. Although again variable, the best results for two-stage revision of both hips and knees appear superior (90% success or greater) to one-stage revision, but these are in noncomparative, nonrandomized studies.[35]

One approach that is growing in popularity, especially in cases of severe bone loss from previous revisions, is to use some form of articulating spacer constructed with an outer surface composed entirely of antibiotic-loaded bone cement.[36] These can be fashioned intraoperatively and usually include both vancomycin and an aminoglycoside (gentamicin or tobramycin). Published results in specialist hands have been impressive.

Arthrodesis and amputation

The quality of bone stock at the distal femur and proximal tibia or the state of the soft tissues (including the extensor mechanism) may preclude an attempt at reimplantation of a functioning knee prosthesis. In these situations the options are external bracing (see above), a fusion or an above-knee amputation. In all series, a proportion of cases progress to these unsatisfactory outcomes and this proportion rises if the revision implant itself becomes infected.[37] Therefore, the risks should be discussed openly with all patients before knee revision surgery is embarked upon.

Suppressive therapy

For a small group of patients, the pursuit of radical cure and the surgery necessary to achieve it is inappropriate. This group includes the medically unfit patient in whom the risk of an anesthetic and an operation is unacceptable, patients with obvious infection (e.g. with sinus tract drainage or early infection) but a pain-free, mechanically sound implant, and patients in whom excision of a prosthesis would lead to an unreconstructable defect. Such cases may be suitable for long-term antibiotic suppression, particularly when infected with organisms that are sensitive to safe and well-tolerated antibiotics. In the majority of cases, revision will eventually be necessary.

Infections of fracture fixations and other orthopedic hardware

These pose related but different problems. Internal fixation devices are designed to stabilize the skeleton until mechanical strength is restored biologically by fracture healing or by the incorporation of bone graft. Infection may impair these processes, leading to nonunion, but mechanical instability also appears to play an important role in this. For the purposes of obtaining union or fusion, a stable but infected fracture (with implant retained) may be preferable to an unstable, uninfected fracture and it is definitely preferable to an unstable, infected fracture. Thus the goal of obtaining radical cure of infection may have to take second place to the need to allow the implant to perform its function (ideally with infection controlled), after which it may be removed and the infection treated definitively.

It follows that the key factor in management is whether or not union or fusion has occurred. Irrespective of the time of presentation, exploration and debridement should be carried out. If the fracture has not united but the implant is mechanically sound, the device can be retained, provided the organisms cultured are sensitive to a suitable antibiotic, the bone at the fracture or fusion site appears viable and soft tissue cover can be obtained. If the implant is loose or substantial amounts of dead bone are present, in which case fracture healing is unlikely, the implant should be removed and stability ensured, preferably with an external fixator. Once multiple samples have been obtained, an empiric antibiotic regimen can be commenced and refined according to results. Infections that are well established before the onset of suppression are liable to relapse once antibiotics are discontinued, whereas cure may sometimes be possible if very early infections are treated promptly. Elective implant removal and final debridement of poor-quality tissue once union has occurred have the advantage that the soft tissues (and the patient) are in an optimal state for the procedure while infection is suppressed. Oral suppression need not be stopped before removal of implants but samples should be sent for microbiology and histology. If organisms are cultured at the time of implant removal, antibiotic treatment as if for an osteomyelitis should be offered. As a minimum, 1 month of therapy should be given, even if the cultures are negative, while bone heals after the debridement. Implant removal is not always essential, though there is little information about late recurrence. This decision is best left to the surgeon and patient, who together must bear the risk of refracture, soft tissue breakdown or perioperative morbidity if the metalware is removed. The nature of the infecting organism is obviously an important factor in the risk-benefit equation. Intravenous therapy with a ß-lactam or glycopeptide, followed by oral therapy with bio-available drugs (depending on sensitivities) such as rifampicin with ciprofloxacin, doxycycline or fusidic acid, are standard regimens.

CONCLUSION

Infections of prosthetic joints and other orthopedic hardware continue to pose considerable challenges to the infectious diseases physician, just as they do to patients and surgeons. There is a pressing need to establish consensus definitions of infection that will allow comparison between separate studies and form a basis for multicenter trials. These can then address the challenge of finding the most cost-effective diagnostic and therapeutic strategies to benefit the relatively small, but hard-pressed group of patients beleaguered by these troublesome conditions.

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Chapter 54 - Lyme Disease

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HISTORY

Lyme borreliosis is caused by the tick-borne spirochete, *Borrelia burgdorferi* which is distributed throughout the Northern Hemisphere. The cutaneous manifestation, acrodermatitis chronica atrophicans (ACA) was first described in Germany by Buchwald. Afzelius subsequently described erythema migrans (EM) in Sweden. French physicians Garin and Bujadoux reported the first case of a neurologic manifestation, meningoradiculoneuritis.[1] [2] The disease was first treated with antibiotics in 1949 by Thyresson. In 1975, Steere and colleagues delineated the disease in the USA, while Burgdorfer isolated and cultivated the causative agent.[1] [2]

EPIDEMIOLOGY

Lyme disease is the commonest vector-borne disease in the USA [3] and occurs widely throughout Europe and Asia. [4] The infecting organism, *B. burgdorferi*, is maintained in and transmitted by ticks of the *Ixodes ricinus* complex, including *Ixodes scapularis* in north-east and north-central USA, *Ixodes pacificus* on the west coast of the USA, *Ixodes ricinus* in Europe, and *Ixodes persulcatus* in Asia (see Chapter 11).

In Europe, three genomospecies of the *B. burgdorferi* sensu lato complex are pathogenic, including *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*. *Borrelia burgdoferi* is the only pathogenic species in North America. The relative distribution of these genomospecies from region to region throughout Europe and Asia may account for the relative variability of disease syndromes associated with Lyme disease. Additional borrelial species that have been identified are *Borrelia valaisiana* and *Borrelia lusitaniae*; although the role of the former in promoting human disease is not entirely clear, it has been detected by polymerase chain reaction (PCR) in skin biopsies of a few patients with EM and ACA. However, the organism has not yet been cultured from these lesions. [1] [5] [21]

Lyme disease is increasing in both incidence and recognition. The known endemic range is expanding, but the precise incidence and geographic spread are uncertain due to difficulties in diagnosis and disagreement regarding diagnostic criteria. A National Surveillance Case Definition was adopted in the USA in 1990 to establish uniform diagnostic criteria for surveillance (Table 54.1). Over 90% of cases in the USA have been reported by ten states in the north-east, upper midwest and Pacific coastal regions.[3] Even within these endemic states, the regional distribution is highly variable. The recent upsurge in cases in the USA may be explained by the reforestation of land used for farming a generation ago, creating environments suitable for deer (for *I. scapularis*, at least, deer are important for maintenance of the tick life cycle),[6] and outward migration of residential areas from cities into these rural areas.

Although cases have occurred at all ages, individuals who are active outdoors in spring and summer months are at greatest risk.[7] [8] In north-east and north-central USA, where *I. scapularis* is the primary vector, most cases begin in summer with the occurrence of EM. Those people who do not manifest this marker of disease onset may come to medical attention months later with one or more symptoms of disseminated disease.

The seasonal variation of onset in temperate climatic zones is explained by the ecology of the predominant tick vectors. Among the ixodid tick vectors, the life cycle and feeding habits of *I. scapularis* are best understood (Fig. 54.1 , Fig. 54.2). This tick has a three-stage life cycle (larva, nymph and adult) that spans 2 years. Larvae hatch from fertilized eggs in late spring and feed once for 2 or more days in midsummer. Preferred hosts include a broad range of small mammals. The next spring they molt into nymphs, and feed again for 3 or 4 days, with the same host range. After this second blood meal, the nymphs molt into adults. Adult *I. scapularis* has a narrower host range, with a preference for deer. Mating occurs on deer, and the female deposits her eggs and the cycle begins anew.[9]

In endemic areas, 30% or more of nymphs may be infected with *B. burgdorferi*; the rate of infection in adult ticks may be even higher, but infection rates in unfed larvae are less than 1%,[10] a pattern suggesting that ticks acquire *B. burgdorferi* from a reservoir host in the environment rather than from congenital transmission. The white-footed mouse, *Peromyscus leucopus*, is the primary reservoir host for *I. scapularis*.[11]

An enzootic cycle of infection is maintained through passage of *B. burgdorferi* back and forth between ticks and their hosts. Infected nymphal ticks transmit *B. burgdorferi* to mice, which serve as a reservoir from which uninfected larvae may acquire infecting organisms. In this manner, a high rate of infection can be maintained in the tick population once the organism, ticks, mice and deer are all present in the environment.

The establishment and maintenance of an enzootic cycle requires a competent reservoir host in addition to a tick vector. Variation in vector-host relationships provide the primary explanation for wide regional variation in the rate of infection in the tick population in California,[12] south-east USA[13] and north-east USA.

Lyme disease occurs when an infected tick (most often a nymph) feeds on a susceptible individual and transmits the causative spirochete.[8] As yet unknown factors influence the risk of developing Lyme disease after a bite by an infected tick. In published studies, the risk of developing Lyme disease after a bite by an infected tick has been less than the rate of infection in the tick vectors alone would predict. The low risk of transmission after a known tick bite may relate to the duration of tick attachment before removal.[14] [15] A tick attached for less than 24 hours has a low likelihood of transmitting *B. burgdorferi*.

Other modes of transmission have been postulated including transfusion of infected blood products[16] and biting flies, [17] [18] but evidence strongly favors ixodid ticks as the primary and, most likely, exclusive vector of Lyme disease. Congenital transmission has been reported,[19] [20] but the evidence regarding clinical disease resulting from transplacental transfer is inconclusive.

TABLE 54-1 -- Lyme disease: a summary of the US National Surveillance Case Definition.

LYME DISEASE: US NATIONAL SURVEILLANCE CASE DEFINITION	
Definition	A systemic, tick-borne disease with protean manifestations: dermatologic, rheumatologic, neurologic and cardiac abnormalities. The initial skin lesion, erythema migrans, is the best clinical marker (occurs in 60–80% of patients)
Case definition	1. Erythema migrans present *or*
	2. At least one late manifestation and laboratory confirmation of infection
General definitions	

1. Erythema migrans (EM)	• Skin lesion typically beginning as a red macute/papule and expanding over days or weeks to form a large round lesion, often with partial central clearing
	• A solitary lesion must measure at least 5cm; secondary lesions may also occur
	• An annular erythematous lesion developing within several hours of a tick bite represents a hypersensitivity reaction and does not qualify as erythema migrans
	• The expanding EM lesion is usually accompanied by other acute symptoms, particularly fatigue, fever, headache, mildly stiff neck, arthralgias and myalgias, which are typically intermittent
	• Diagnosis of EM must be made by a physician
	• Laboratory confirmation is recommended for patients with no known exposure
2. Late manifestations These include any of the opposite *when an alternative explanation is not found*	Musculoskeletal system
	• Recurrent, brief attacks (lasting weeks or months) of objective joint swelling in one or a few joints, sometimes followed by chronic arthritis in one or a few joints
	• Manifestations not considered to be criteria for diagnosis include chronic progressive arthritis not preceded by brief attacks, chronic symmetric polyarthritis, or arthralgias, myalgias or fibromyalgia syndromes alone
	Nervous system
	• Lymphocytic meningitis, cranial neuritis, particularly facial palsy (may be bilateral), radiculoneuropathy or, rarely, encephalomyelitis alone or in combination
	• Encephalomyelitis must be confirmed by evidence of antibody production against *Borrelia burgdorferi* in CSF, shown by a higher titer of antibody in the CSF than in serum
	• Headache, fatigue, paresthesia or mildly stiff neck alone are not accepted as criteria for neurologic involvement
	Cardiovascular system
	• Acute-onset, high-grade (2nd or 3rd degree) antrioventricular conduction defects that resolve in days to weeks and are sometimes associated with myocarditis
	• Palpitations, bradycardia, bundle-branch block or myocarditis alone are not accepted as criteria for cardiovascular involvement
3. Exposure	• Exposure to wooded, brushy or grassy areas (potential tick habitats) in an endemic county no more than 30 days before the onset of EM
	• A history of tick bite is not required
4. Endemic county	• A county in which at least two definite cases have been previously acquired or in which a tick vector has been shown to be infected with *B. burgdorferi*
5. Laboratory confirmation	• Isolation of the spirochete from tissue or body fluid *or*
	• Detection of diagnostic levels of IgM or IgG antibodies to the spirochete in the serum or the CSF *or*
	• Detection of an important change in antibody levels in paired acute and convalescent serum samples
	• States may separately determine the criteria for laboratory confirmation and diagnostic levels of antibody
	• Syphilis and other known biologic causes of false-positive serologic test results should be excluded, when laboratory confirmation is based on serologic testing alone

CLINICAL FEATURES

Although the most recognizable feature of Lyme disease is the skin condition EM, it is a complex systemic illness with protean clinical manifestations. Like syphilis, Lyme borreliosis is a 'great imitator'; its symptoms range from cutaneous to musculoskeletal, cardiac to neurologic. It is interesting to note the differences in manifestations between North American and European disease arising from the endemicity of particular genomospecies in those regions.[5] Variations in disease presentation within regions of Europe and Asia may be attributed to local endemicity of disease vectors (ticks) and reservoirs (deer, rodents) harboring specific borrelial genomospecies.[2] Just as these genomospecies produce a wide range of clinical manifestations, they also induce differing immunologic responses, rendering clinical and serologic diagnosis a formidable challenge. [21] Reported seropositivity depends upon the borrelial strain employed as antigen. Further confounding this diagnostic dilemma is the issue of coinfection with other tick-borne illnesses (i.e. ehrlichiosis and babesiosis).

Lyme disease, human granulocyte ehrlichiosis, and babesiosis not only share a common tick vector (*I. scapularis*), but may also be difficult to clinically distinguish due to overlap of clinical features. [22] However, despite similarities in presentation, each illness tends to influence and alter the natural course of the other. Therefore, recognition of the state of co-infection is crucial to guide choice of antibiotic therapy.

Localized early disease

Lyme disease begins with the appearance of a characteristic skin lesion, EM, at the site of a tick bite. (Fig. 54.3 Fig. 54.4 Fig. 54.5 Fig. 54.6). In approximately one-third of cases, however, this skin lesion is missed or absent and patients present with symptoms of disseminated disease.

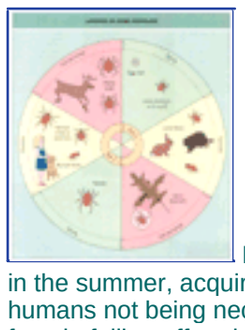

Figure 54-1 Life cycle of *Ixodes scapularis* (also known as *Ixodes dammini*). The life cycle spans 2 years. Eggs hatch in the spring; six-legged larvae develop and feed once in the summer, acquiring *Borrelia burgdorferi* from their preferred host, the white-footed mouse. Next spring, the larvae molt into eight-legged nymphs, which feed once; mice are the preferred host, humans not being necessary for the ticks' life cycle. The nymphs molt into adult male and female ticks; mating often occurs while the female feeds on a deer, and the male may remain on the deer, the female falling off and then laying eggs. Adapted, with permission, from an illustration by Nancy Lou Makris in Rahn and Malawista.[30]

Figure 54-2 *Ixodes scapularis.* Larva, nymph, adult male and adult female. *Courtesy of Pfizer Central Research.*

The interval between tick bite and appearance of EM varies from a few days to a month (median 7 days). The lesion begins as an erythematous papule and expands over several days to achieve a median diameter of 15cm; favored sites are the groin, buttock, popliteal fossa and axilla.

The lesions of EM are generally annular with a sharply demarcated outer border and an erythematous or bluish hue. They are warm to the touch, flat and minimally or non-tender. Lesions may show partial central clearing, but may also be indurated or even necrotic. This cutaneous lesion is the best clinical marker of Lyme disease.[8]

An annular erythema following a tick bite is insufficient evidence for a definitive diagnosis of Lyme disease, however, especially in nonendemic areas.

Early disseminated disease

Within several days of the appearance of EM, many patients develop evidence of dissemination of their infection by the appearance of prominent systemic symptoms, the occurrence of multiple secondary skin lesions, or both. Malaise, fatigue, lethargy, headache, fever and chills, arthralgia and myalgia are particularly common, each occurring in one-half or more of patients. Symptoms may fluctuate rapidly

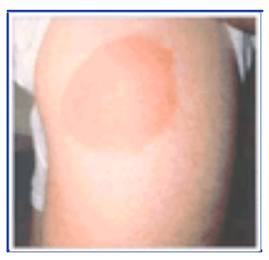

Figure 54-3 Erythema migrans. A typical annular, flat, erythematous lesion with a sharply demarcated border and partial central healing. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

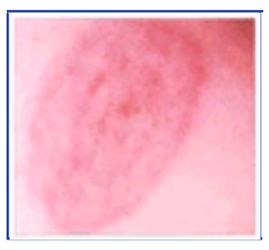

Figure 54-4 Erythema migrans. A lesion with variation in color and a target-like appearance. The bite site is visible in the center. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

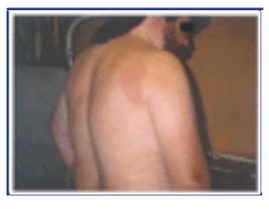

Figure 54-5 Erythema migrans. A lesion with a dusky center, a common variant. *Courtesy of Dr Steven Luger.*

and vary from a flu-like syndrome to a meningitis-like illness from day to day.

Secondary skin lesions may occur anywhere on the body and resemble primary lesions but are usually smaller, show less expansion with time and lack indurated centers. These manifestations of early disseminated infection appear to be more common in the USA than in Europe and may reflect biologic differences in infecting organisms. Erythema migrans, secondary skin lesions and associated symptoms resolve, even without antibiotic therapy, a median of

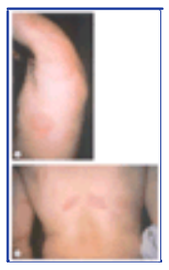

Figure 54-6 Multiple erythema migrans lesions. Lateral (a) and posterior (b) views of the same patient with multiple erythematous macules of EM. Secondary lesions result from hematogenous spread. They may occur anywhere in the body. Secondary lesions are usually of uniform color and lack in duration. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

28 days after onset, but recurrent crops of evanescent lesions may occur, and fatigue, intermittent musculoskeletal pain and headaches may persist for months.

Disseminated disease

After resolution of the signs and symptoms of early disease, some patients (20% in one series)[23] experience a long-lasting spontaneous remission, which may reflect cure. Most, however, subsequently develop other disease manifestations, with predominant involvement of the heart, nervous system and joints, although case reports have described involvement of multiple organs, including the liver,[24] subcutaneous tissue,[25] muscle,[26] eye structures[27] and spleen.[28] More than one organ system may be (and often is) affected simultaneously or sequentially in an individual patient.

Carditis

Fewer than 10% of patients with untreated early Lyme disease develop carditis, generally a few weeks to a few months after EM.[29] However, palpitations from an arrhythmia or unexplained syncope from high-degree atrioventricular block may be the presenting manifestation of Lyme disease.[30] The primary clinical manifestation of Lyme carditis is heart block, which may fluctuate from first-degree to

complete heart block over minutes to hours and generally resolves spontaneously in a few weeks or sooner, even in untreated patients.[31] Although temporary pacing is frequently required, permanent pacing is rarely needed. [30]

Much less commonly, congestive heart failure has been linked to Lyme disease. A case report suggested that Lyme carditis might be a cause of chronic dilated cardiomyopathy.[32] Endomyocardial biopsy and electrophysiologic testing have revealed direct spirochetal invasion of cardiac muscle and widespread abnormalities of cardiac conduction.[33]

Neurologic manifestations

Neurologic abnormalities were recognized as being associated with ixodid tick bites and EM in Europe many years before the initial description of Lyme disease in the USA.[7] [34] In the early series of Lyme disease cases described in the USA, frank neurologic abnormalities, including cranial neuropathy (particularly Bell's palsy), meningitis, radiculoneuropathy, myelopathy and encephalopathy, occurred in 15% of patients. Neurologic symptoms generally began a few weeks after EM (median 4 weeks), although some patients presented with neurologic manifestations alone.[8] [35]

Subtle neurologic complaints without objective deficits (headache, irritability, paresthesias, photophobia and lethargy) may accompany early disease dissemination and represent the mildest end of the spectrum of neurologic Lyme disease. Multiple neurologic abnormalities may coexist or occur sequentially. Peripheral nerve abnormalities may involve sensory and motor nerve roots, plexi and motor and sensory nerve fibers.[36] [37] [38] Meningitis and radiculoneuropathy often wax and wane for weeks to months but ultimately resolve spontaneously.

Borrelia burgdorferi has been isolated from the cerebrospinal fluid (CSF) of a patient whose only complaint was tinnitus,[39] and has been demonstrated by PCR in

patients with symptoms limited to headache, Bell's palsy or paresthesias, indicating that the symptoms associated with central nervous system (CNS) infection may be minimal. [40] The emergence of subtle neurologic manifestations years after onset of Lyme disease also supports the possibility of latent persistent infection in the CNS (see Late neurologic syndromes, below).

Rarely, cases of demyelinating encephalopathy mimicking multiple sclerosis have been reported.[41] [42]

Arthritis

Most people who have untreated Lyme disease develop arthritis. Brief, intermittent attacks of migratory musculoskeletal pain commonly begin while EM is present and may persist for months before the appearance of overt arthritis. Frank arthritis occurs in 60% of patients in the USA, at a median of 6 months after EM, but, as with other symptoms of disseminated disease, arthritis may be the presenting manifestation of Lyme disease.[43] Although the clinical expression varies, patients most often experience brief recurrent attacks of monoarticular or oligoarticular inflammatory arthritis involving large joints, particularly the knee.[23] [44] [45] Effusions may be massive (100ml or more), causing popliteal cysts, which may rupture and result in a pseudothrombophlebitis syndrome.

Attacks last a few days to a few weeks and over time (months to years) decrease in severity, frequency and duration.[23] Arthritis has been reported to be milder in children than in adults.[46] [47] Polyarthritis is decidedly uncommon. Synovitis becomes chronic in 20% of cases (see Chronic arthritis, below).

Late (persistent) disease

After the disseminated stage of Lyme disease during which multi-organ involvement occurs and symptoms are often changing and self-limited, inflammation may become chronic and persistent in some tissues. This has been been called 'late Lyme disease'. Late Lyme disease may involve the CNS, the joints and (particularly in Europe) the skin. Information is still emerging on this patient group. It is as yet unclear whether the clinical manifestations of late Lyme disease result from persistent infection in all cases.

Late neurologic syndromes

In Europe, radiculoneuritis is the predominant neurologic manifestation of *B. burgdorferi* infections in adults (as compared to the frequent occurrence of meningitis in children).[2]

Other reported CNS abnormalities include lymphocytic meningitis, peripheral neuropathy and cranial neuritis, collectively known as Garin-Bujadoux-Bannwarth (MPN-GBB) or Bannwarth's syndrome.[48] Garin and Bujadoux presented the first clinical description of such a disorder in 1922, 60 years before the disease itself became known as Lyme borreliosis.[2] Bannwarth's series of 26 patients in 1941 and 1944 with this constellation of findings led to the discovery of the link between this clinical entity and tick bite/EM.[2] Pfister and colleagues found that 73% of patients studied had a preceding arthropod bite or EM and that 73% developed radicular pain within the dermatomal distribution of the known area of an arthropod bite or EM.[48]

Both a mild, predominantly sensory, peripheral neuropathy and subtle encephalopathy may occur months to years after the onset of Lyme disease. [38] [49] [50] The diagnosis of encephalopathy hinges on the presence of cognitive deficits involving primarily short-term memory and concentration. Chronic fatigue, headaches and sleep disturbance may accompany these abnormalities, but these are not sufficient, without a documented neurologic deficit, to support the diagnosis of neurologic Lyme disease. Rarely, severe encephalomyelopathy has occurred with impairment of higher cortical function, seizures and spinal cord lesions.

Chronic neuropathy in patients with ACA has been described. The most common manifestations include peripheral neuropathy with sensory deficits in a patchy distribution.[51]

Chronic arthritis

Arthritis becomes chronic in 20% of patients who have untreated Lyme disease, resulting in a syndrome that is clinically indistinguishable from other forms of monoarticular or oligoarticular inflammatory arthritis. However, chronic Lyme arthritis is preceded by recurrent brief attacks of joint inflammation in most cases. The knee is by far the most commonly affected joint. Immunogenetic factors, particularly human leukocyte antigens (HLA-DR2 and DR4), may predict which people are at highest risk of developing chronic Lyme arthritis.[52] Even chronic Lyme arthritis may eventually remit spontaneously. Only a minority of patients develop radiographic evidence of erosions of cartilage or bone.[53] There have been no unequivocal cases of symmetric, peripheral, polyarticular, inflammatory arthritis with joint destruction resulting from Lyme disease. Differentiation from rheumatoid arthritis is rarely a problem.

Chronic skin involvement

Chronic skin involvement (acrodermatitis chronicum atrophicans) as a late manifestation of Lyme disease occurs primarily in Europe.[54] It usually occurs on the acral portion of an extremity and is characterized by violaceous discoloration and swelling of involved skin, often at a site where EM occurred years earlier. The lesion eventually becomes atrophic (Fig. 54.7). Other clinical manifestations include fibrotic nodules, ulnar bands, sensory disturbances, muscular weakness, myalgias, arthalgias and tenderness on impact over bony prominences.[51] In a study of 50 consecutive patients with ACM in Sweden, elevated antispirochetal antibody titers were found at indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA), and histologic biopsies demonstrated dermal lymphocytic infiltrates with plasma cells and telangiectasia.[51] Acrodermatitis

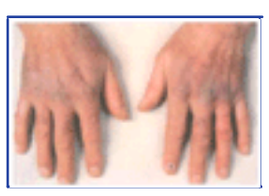

Figure 54-7 Acrodermatitis chronicum atrophicans. Typical inflammatory bluish-red lesions of acrodermatitis chronicum atrophicans. Lesions usually occur on acral portions of extremities. *Courtesy of Dr Eva Asbrink.*

chronica atrophicans is thought to result from local persistence of *B. burgdorferi*, which has been isolated from a lesion 10 years after onset. [55] Lichen sclerosis et atrophicus or morphea-like lesions have also been described.[54]

INVESTIGATIONS

It is often difficult to diagnose Lyme disease definitively because of the variation in clinical presentation and course, and because of the limitations of currently available diagnostic assays. The National Surveillance Case Definition of Lyme disease developed in the USA (see Table 54.1) requires either the presence of EM or a definite late manifestation of Lyme disease combined with laboratory confirmation of infection in a person who has had opportunity to be exposed to the causative agent. Definitive laboratory confirmation requires isolation of the causative organism from clinically involved tissue, but this is rarely achievable in clinical practice. Demonstration of specific serologic immune responsiveness against *B. burgdorferi* has been accepted as a substitute for bacteriologic isolation. Because the sensitivity and specificity of many tests has been poor,[56] [57] [58] Western blot confirmation of all positive and equivocal results is recommended.[59] The key to diagnosing Lyme disease rests with recognition of the characteristic clinical features of the illness. Laboratory testing should only be used for confirmation.

The laboratory abnormalities associated with early Lyme disease reflect the inflammatory nature of the illness. When the infection is localized to a single skin lesion, blood tests are typically normal. As the infection spreads, with development of systemic symptoms and secondary skin lesions, there may be a mild leukocytosis, anemia, elevation in the erythrocyte sedimentation rate, microscopic hematuria, elevation in liver enzymes and elevation in total serum IgM (which mirrors the specific IgM immune response).[8] These abnormalities resolve spontaneously when this phase of illness remits.

Subsequent localization of inflammation to specific target organs results in abnormalities of organ-specific tests. Patients who have carditis have fluctuating atrioventricular block and occasionally more generalized repolarization abnormalities, including ST-segment and T-wave changes on electrocardiography.[31] If carditis affects ventricular performance, the chest radiograph may reveal cardiomegaly, and cardiac ultrasonography and radionuclide ventriculography may reveal impaired

ventricular performance.

The laboratory hallmark of Lyme meningitis is a lymphocytic pleocytosis (generally a few to a few hundred cells)[37] in the CSF; the CSF protein may be elevated and, in protracted cases, the ratio of IgG to albumin may be elevated and oligoclonal bands may be present, reflecting the immunologic component of the inflammatory process within the CNS.[38]

Attacks of arthritis result in the accumulation of inflammatory joint fluid containing a few hundred to 50,000 white blood cells, mostly polymorphonuclear, elevation in protein and normal glucose.[45] Radiographs of affected joints most often show only soft tissue swelling, but when joint inflammation has persisted for many months, there may be pannus formation and erosions of underlying cartilage and bone.[53] Some patients develop enthesopathy with calcifications of tendon and ligament attachment sites.

Cognitive deficits associated with late neurologic involvement may be quantified by neuropsychologic testing, a very helpful modality in the formal assessment of cognitive complaints.[49] [50] A depressive overlay is often present, but findings should also reveal an organic encephalopathy primarily affecting short-term memory. Peripheral neuropathy and radiculoneuropathy cause abnormalities on electromyography and, less often, abnormalities of peripheral nerve conduction;[38] [50] sensory fibers are more frequently affected than motor fibers. Although these findings on tests of CNS and peripheral nervous system function are not specific for Lyme disease, abnormal findings indicate a definite disorder of the nervous system, and the combination of central and peripheral nerve abnormalities characteristic of Lyme disease is uncommon in other diseases.

Specific laboratory confirmation may be sought through either direct or indirect means. Efforts at direct confirmation have included culture of affected tissue, employing PCR on skin, urine, joint fluid, blood or CSF to amplify specific gene sequences unique to *B. burgdorferi*, and antigen testing of urine.[60] Although the causative organism has been cultured from affected skin,[55] [61] [62] blood, [63] [64] CSF[39] [61] and joint fluid,[65] the rarity with which it has been isolated limits the usefulness of culture. Polymerase chain reaction has been validated as a means of identifying the presence of *B. burgdorferi* both in vitro[66] and in tick vectors[67] using a variety of primers. Work on human tissues and fluids is in progress,[68] [69] [70] [71] [72] but PCR cannot be considered a routine diagnostic test for Lyme disease at present.

Detection of a specific immune response to *B. burgdorferi* remains the best means of confirming the diagnosis of Lyme disease. The first immune response in Lyme disease is mediated by T cells and is directed against a variety of epitopes.[73] [74] This T-cell response subsequently localizes preferentially to involved tissues. [73] [75] Unfortunately, tests to measure this response are not standardized, are technically demanding, require live cells and have varied in sensitivity and specificity.[76] For these reasons, measurement of the T-cell response cannot be recommended routinely for diagnosis.

The serologic response to *B. burgdorferi* has been well characterized (Fig. 54.8).[61] [77] Specific IgM antibody, directed initially primarily against the flagellae of the organism, is detectable a few weeks after disease onset. The response broadens to include additional antigens over time and peaks by 3–6 weeks. Generally IgM antibody falls to within the normal range by 6 months, but occasionally it may remain elevated for much longer. Specific IgG antibody is detectable a few weeks after IgM, but it may not peak until many months after disease onset. The IgG response is also initially directed primarily against flagellin and broadens with time to include an array of antigens on the outer surface of the organism.

Both immunofluorescence (IFA) and ELISA have been used to detect this antibody response; in general, ELISA is preferable because of better sensitivity, objectivity and reproducibility and because of its adaptability to automated systems. Serology results may be falsely positive or negative for various reasons, so the diagnosis of Lyme disease cannot be made by serologic test results alone. Immunofluorescence and ELISA may yield false-positive results

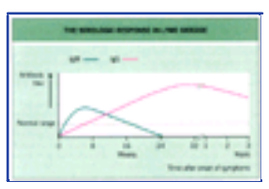

Figure 54-8 The usual serologic response in Lyme disease. Specific IgM becomes detectable 1–2 weeks after symptom onset and the appearance of erythema migrans. The later appearance of IgG is frequently concurrent with systemic manifestations. IgG is nearly always elevated with late disease. Typically, and even in untreated patients, IgM falls over 4–6 months; persistence for longer than this predicts later manifestations.

because of epitopes in the test antigen preparations that cross-react with other bacteria.[58] False-positive results may also occur in conditions associated with polyclonal B-cell activation. Specificity is increased by a two-step approach in which all positive or equivocal results are confirmed by Western immunoblotting. This two-step approach was recommended by participants in a national conference on serologic diagnosis of Lyme disease held in 1994 under the sponsorship of the Centers for Disease Control and Prevention.[59] A true positive ELISA is associated with immunoreactivity against polypeptides specific for *B. burgdorferi*, reactivity that is absent in the various conditions associated with false-positive ELISA results. The currently recommended criteria for a positive Western blot are given in Table 54.2 .

One special circumstance deserves mention. Antibiotic therapy administered early in the course of Lyme disease may result in a negative serology by curing the infection and eliminating antigen before systemic immune challenge. [78] One series indicated, however, that infection may persist after early antibiotic therapy despite persistently negative serologies. [74]

TABLE 54-2 -- Western blot criteria.

WESTERN BLOT CRITERIA
• All serum specimens found to be positive or equivocal by a sensitive enzyme immunoassay or immunofluorescent assay should be tested by a standardized Western blot procedure
• When Western immunoblot is used in the first 4 weeks of illness, both IgM and IgG procedures should be performed
• After the first 4 weeks of illness, IgG alone should be performed
• An IgM blot is considered positive if two of the following three bands are present: 24kDa (OspC), 39kDa (BmpA) and 41kDa (Fla)
• An IgG blot is considered positive if five of the following 10 bands are present: 18, 21 (OspC), 28, 30, 39 (BmpA), 41 (Fla), 45, 58, 66 and 93kDa
Recommendations of the Second National Conference on Serologic Diagnosis of Lyme Disease sponsored by the Centers for Disease Control and Prevention, the Association of State and Territorial Public Health Laboratory Directors and the Michigan Department of Health
Centers for Disease Control and Prevention Working Group recommendations for Western blot positivity. Osp, outer surface protein; Fla, flagella.

In a study of 17 patients with acute Lyme disease who received prompt treatment with oral antibiotics and subsequently developed 'chronic' illness, it was shown that none of them had diagnostic levels of antibodies to *B. burgdorferi* on either a standard ELISA or IFA.[74] On Western blot analysis, the level of immunoglobulin reactivity against *B. burgdorferi* was no greater than that of normal controls. These patients had a vigorous T-cell proliferative response to whole *B. burgdorferi* at levels similar to that of 18 patients with chronic Lyme disease with detectable antibodies. The T-cell response of both groups was greater than that of a control group. The investigators concluded that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against *B. burgdorferi* and that a specific T-cell response to *B. burgdorferi* is evidence of infection in seronegative patients with suggestive clinical parameters. [74]

This apparent dissociation between T-cell and B-cell immune responses may be attributed to the administration of antibiotics for ECM and therefore, the presence of low or undetectable levels of antiborrelia antibodies. Antibiotic therapy may have resulted in the elimination of most spirochetes at a critical early stage of the immune response, thereby hindering the development of a sustained B-cell response. [74] Persistent disease activity may result from harboring of *B. burgdorferi* in sites such as the CNS, which may not receive adequate concentrations of antibiotics to reach the minimum inhibitory concentrations (MICs) required against the majority of strains of *B. burgdorferi*. In support of this observation is the discovery of local production of antiborrelia antibody in the CNS in those suffering from neurologic symptoms of Lyme borreliosis in the absence of diagnostic serum antibodies levels. [79] [80] [81]

The concept of antigenic variation displayed by other borrelial species may represent an alternative explanation for the apparent absence of a specific antibody response in seronegative Lyme disease.[82] Classically, immunodominant 'variable major proteins' are replaced periodically by variant forms, enabling the organism to elude the host's antibody. Although *B. burgdorferi* does not precisely exhibit antigenic variation, antigenic diversity has been noted among strains found in humans,

animals, and ticks. These differences in strains must induce significant changes in antigenic profile to engender nonreactive Western blot assays.[82]

Although it may be challenging to diagnose definitively, Lyme disease is treatable with prompt and appropriate antibiotic therapy. Presently, chronic Lyme disease is a frequently used diagnosis, usually applied to patients with a constellation of symptoms including fatigue, arthralgias, aches, pains, memory disturbances, and impairment in concentration. Patients with persistent disease unresponsive to antibiotics have further compounded this clinical dilemma, creating anxiety and promoting phobias regarding the stigma of 'chronic' or lifelong disease. There is a lack of scientific medical literature to support the following misconceptions:

- antibiotics are often not curative in Lyme disease;
- long-term antibiotic therapy is always required;
- serologic tests are inaccurate yielding false-negative results; and
- the prognosis for patients who have Lyme disease is often poor.

To achieve an accurate diagnosis, one must elicit a history of EM with suggestive clinical features and ascertain a probable exposure in an endemic area. Serologic assays interpreted under these circumstances remain the best screening methods for exposure followed by confirmation with immunoblotting techniques. A lack of response to antibiotic therapy in presumed Lyme disease is most likely due to incorrect diagnosis, thereby warranting a continued search for the cause of illness.

These limitations notwithstanding, the greatest problem with serologic testing for Lyme disease results from interlaboratory variation. Standardization of procedures and performance as recommended by the Centers for Disease Control and Prevention is essential before

serologic response can be used reliably as a screening tool for Lyme disease. At best, serologic testing can only indicate immunologic exposure, not ongoing infection.

Measurement of immunoreactivity in CSF is a useful adjunct in the diagnosis of CNS Lyme disease.[83] [84] To have diagnostic value, a CSF index should be calculated comparing the ratios of specific antibody to total immunoglobulin in CSF and serum. A specific antibody response in CSF is not found uniformly in cases meeting other clinical criteria for CNS Lyme disease in the USA, but it is helpful if positive.

Until more specific serologic tests based on recombinant antigens are developed,[85] culture techniques are improved, or tests based on the PCR or antigen detection are available routinely, caution must be exercised in the use of diagnostic tests for Lyme disease. Serologic testing should be ordered only when the diagnosis is suspected from suggestive clinical data; in this circumstance, a positive serology and Western blot performed in a laboratory with high performance standards should be considered confirmatory of the diagnosis. As a corollary, a negative result should lead to serious doubt of the diagnosis. If testing is ordered indiscriminantly, many false-positive results must be expected, thus diminishing the predictive value of a positive test.

DIFFERENTIAL DIAGNOSIS

Erythema migrans is the classic skin lesion of Lyme disease and, in its typical appearance, can be confused with little else. However, atypical presentations may lead to confusion.[86] Not all annular erythemas associated with tick bites are indicative of Lyme disease, so particular caution must be exercised in nonendemic areas.[87] [88]

Lesions with indurated centers may be confused with cellulitis, and those with necrotic centers may look like spider bites, but EM lesions are generally minimally tender or nontender. Secondary skin lesions may be confused with erythema multiforme, but mucosal lesions do not occur and palms and soles are generally spared in Lyme disease. Secondary skin lesions may also have an urticarial appearance. The lesions of EM are generally nonpruritic, however, and other features of disseminated early Lyme disease aid in distinguishing it from a generalized allergic reaction or a systemic condition associated with generalized urticaria, such as the prodromal phase of hepatitis B.

An EM-like illness has recently been described in Missouri and North Carolina. The skin lesions in this illness are always single and are associated with *Amblyomma americanum*, a tick not known to transmit Lyme disease. Patients do not develop immunoreactivity against *B. burgdorferi*, and skin biopsy specimens have been negative for *B. burgdorferi* in culture and by PCR.[87] [88] The importance of this syndrome is that it demonstrates that EM-like skin lesions alone occurring in a patient in a nonendemic region are not sufficient to support a diagnosis of Lyme disease.

In later stages, Lyme disease may mimic a variety of cardiac, neurologic and arthritic disorders. Carditis can be confused with subacute bacterial endocarditis or acute rheumatic fever, but valvular lesions do not occur in Lyme disease. Blood cultures are negative.

The most helpful diagnostic feature of neurologic Lyme disease is the frequent simultaneous or sequential involvement of multiple levels of the CNS and peripheral nervous system. Bell's palsy caused by Lyme disease is indistinguishable from idiopathic Bell's palsy, but Lyme disease is one of very few causes of bilateral Bell's palsy (a helpful clue if present) and, by this stage of disease, patients are typically seropositive. Lyme meningitis presents a clinical picture similar to that of viral meningitis, but there is often an antecedent history of EM, the course is more protracted and Lyme meningitis is characterized by relapses and remissions. The peripheral neuropathy associated with chronic neurologic Lyme disease mimics other predominantly sensory neuropathies; a history of previous non-neurologic involvement is usually present, however. The rare patients who have demyelinating encephalopathy must be distinguished from patients who have multiple sclerosis; positive serology (particularly if present in CSF) combined with an exposure history is very helpful in this rare circumstance.

The typical patient with Lyme arthritis who has monoarticular inflammatory arthritis of a knee has a clinical appearance similar to that of reactive arthritis or septic arthritis. At initial presentation, bacterial infection must always be excluded. The pattern of brief, recurrent attacks is more common in Lyme disease than in either reactive or septic arthritis. Furthermore, patients who have Lyme arthritis are virtually always seropositive.

Patients who have chronic fatigue and non-specific symptoms (headaches, musculoskeletal pain, back pain, sleep disturbance and mood swings) have often been presumptively diagnosed as having Lyme disease with little or no substantiation of this diagnosis. The diagnosis of Lyme disease should not be made on the basis of non-specific complaints unsubstantiated by either objective neurologic deficits or inflammation of joints in a pattern known to be due to this illness, particularly if serologic confirmation is lacking. It is particularly important to differentiate fibromyalgia from chronic Lyme disease because the prognosis and response to therapy are quite different.[89]

PATHOGENESIS AND PATHOLOGY

Borrelia burgdorferi has been unequivocally established as being the cause of Lyme disease. Clinical isolates differ in their outer surface protein expression[90] [91] and genetic composition.[92] Three different genospecies have been described: *B. burgdorferi* itself, *B. garinii* and *B. afzelii*. All three genospecies have been isolated from patients who have Lyme disease and from ticks of the *I. ricinus* complex. There is some evidence that disease expression in humans may vary depending upon the genospecies of the infecting organism, but this is preliminary at present.

Unlike *Treponema pallidum*, *B. burgdorferi* can be grown in culture although a specialized medium is required. [61] [93] Serial passage in culture alters surface protein expression with loss of pathogenicity in mice,[92] but the specific factors conferring pathogenicity have not yet been identified. *Borrelia burgdorferi* can penetrate endothelial monolayers and survive intracellularly in cultured fibroblasts. [94] The implications for human infection of this intracellular persistence in vitro are unclear. Work in progress is providing insight into the mechanisms enabling the organism to cause persistent infection in humans despite a vigorous immune response.

Borrelia burgdorferi has been recovered from tissues or fluids of patients who have Lyme disease. It is most readily isolated by biopsy and culture of EM lesions,[62] but this is rarely indicated clinically. Later in the disease, organisms are scant in histologic sections[95] and have been recovered by culture only in research settings.

Borrelia burgdorferi is transmitted to the skin of the host by an infected tick. The organism may evade eradication through an initially delayed and ineffective immune response. It disseminates preferentially to certain target organs where it engenders an immunologically mediated inflammatory response, with tissue injury occurring as a result of the inflammatory response. Why this lesion becomes persistent in some people and whether live organisms persist in all cases of chronic disease remain to be elucidated.

An important question is how *B. burgdorferi* avoids destruction in the presence of the vigorous specific T- and B-cell responses that are

usually apparent within a few weeks of onset of disease. In humans, the early immune response is directed primarily against a flagellar antigen. Vaccine studies in mice have shown that specific antibodies against this antigen do not protect against subsequent infection. Vaccination with outer surface protein (Osp) A, an immunodominant surface antigen, to which antibodies appear much later in human disease, is protective.[96] It may be that, in human Lyme disease, *B. burgdorferi* is able to establish infection because of an ineffective initial immune response focused primarily against flagellae. Later, when the immune response broadens, infection may already be established and disease may be perpetuated through other mechanisms.

Immunogenetic studies have suggested that HLA-DR4 predisposes to the development of chronic Lyme arthritis and that DR4 is associated with a lack of response to antibiotics.[52] Patients who are HLA-DR4 positive and who have treatment-resistant Lyme arthritis have also been shown to have a strong immune response to an epitope on OspA that cross-reacts with human lymphocyte function antigen-1 (LFA-1), which may serve as an autoantigen.[97] This evidence suggests a possible autoimmune mechanism for chronic Lyme arthritis through a mechanism involving molecular mimicry between OspA and LFA-1 in HLA-DR4 positive individuals. No predictors of chronic neurologic disease have been described as yet.

A third potential mechanism through which organisms may evade antibiotic and immune-mediated destruction is by entering human cells.[94]

Histologic studies of affected tissues have provided evidence for immunologically mediated inflammation. Stains of EM lesions reveal a perivascular mononuclear infiltrate and fibrin deposition in the dermis, without epidermal changes except at the site of the bite.[43] Endomyocardial biopsies have revealed similar changes in the heart, with a focal perivascular infiltrate of mononuclear cells and fibrin deposition in both the endocardium and myocardium. [101] Biopsies of affected nerves, although few in number, have shown inflammatory infiltrates around endoneurial and perineurial vessels without vessel necrosis.[98] Both myelinated and unmyelinated fibers may be affected. Hematoxylin and eosin stains of synovium from arthritic joints have revealed synovial lining cell hyperplasia and hypertrophy, vascular proliferation and lymphocytic infiltration of the subsynovial areas. The intensity of the infiltrate varies, and fibrin deposition may be pronounced. Aggregates of T and B cells, often with lymphoid follicle formation, are common and may be concentrated in perivascular areas with obliteration of vessels but without vessel necrosis.[95] Levels of interleukin-1,[99] prostaglandin E_2 and collagenase[100] in joint fluid are elevated, similar to the situation in rheumatoid arthritis.

Spirochetes have been visualized in skin lesions,[61] heart tissue[32] [33] [101] and synovium,[95] but not in peripheral nerves, where it has been postulated that an autoimmune mechanism accounts for the inflammatory lesions.[102]

MANAGEMENT

The primary goals of therapy for Lyme disease are the control of inflammation and the eradication of the infection. Lyme disease is most responsive to antibiotic therapy early in the course of the disease. As with syphilis, some later disease manifestations do not seem to improve after administration of antibiotics.

Treatment regimens are based in part on data from controlled clinical trials and in part on clinical experience.[103] In-vitro antibiotic sensitivity testing does not reliably predict clinical response. Loose criteria for diagnosis accepted without critical review have led to widespread antibiotic use for presumptive Lyme disease in patients who almost certainly have other explanations for their symptoms. In one report, the leading reason for failure to respond to antibiotic therapy for Lyme disease was incorrect diagnosis.[104] In addition, the appropriate end-point of antibiotic therapy is often not clear because of the difficulty of proving when the infection has been eradicated and because of the common persistence of symptoms long after treatment. Current treatment recommendations (Table 54.3) represent a distillation of available evidence and will no doubt be refined in time.

Early localized or early disseminated disease

If antibiotic therapy is initiated early in the course of Lyme disease, EM typically resolves promptly and later stage disease is prevented.[105] [106] Early localized infection, limited to a single skin lesion, with mild or no systemic symptoms, is uniformly responsive to short-course oral antibiotic therapy with a number of agents. Of the antibiotics studied to date, amoxicillin (500mg q8h), doxycycline (100mg q12h) or cefuroxime axetil (500mg q12h) have been the most effective for this stage of disease.[107] [108] Although the optimal duration of therapy is unknown, most clinicians currently recommend 2–3 weeks for both early localized and early disseminated disease.

The appearance of systemic symptoms (fever, arthralgias, fatigue) and secondary skin lesions reflects dissemination of the organism beyond the site of inoculation. As long as no neurologic symptoms are present, 3 weeks of oral therapy is sufficient for this group of patients as well. In a recent study, 10% of patients with a single EM lesion and no systemic symptoms had a positive PCR on blood. This was interpreted as demonstrating that clinically silent blood-stream invasion may be relatively common early in the course of infection.[70]

Some experts have recommended the addition of probenecid to amoxicillin, but this combination has been associated with a relatively high frequency of rashes and is not known to be superior to amoxicillin alone. The pediatric dose range of amoxicillin is 30–40mg/kg per day in three divided doses. Herxheimer-like reactions, with intensification of fever and arthralgias, may occur shortly after initiation of therapy. If possible, doxycycline should be avoided in children under the age of 9 years and during pregnancy because of the possibility of staining of the teeth. Tetracyclines must be used with caution during summer in all patients because they may predispose the patient to sun-sensitive rashes or severe sunburn. Penicillin-allergic young children can be treated with cefuroxime or erythromycin, but results with macrolide antibiotics have been less satisfactory than those with penicillin, amoxicillin or tetracyclines.[105] Azithromycin has been studied systematically and found to be less effective than amoxicillin.[108]

Regardless of which agent is chosen, some patients experience a delayed resolution of systemic symptoms (headache, musculoskeletal pain, fatigue), which may persist as long as 3 months after completion of therapy. These symptoms usually resolve spontaneously and do not indicate continued infection requiring further antibiotic therapy.[106] A seemingly self-perpetuating fibromyalgia syndrome may develop as a sequel of Lyme disease; this too is unresponsive to antibiotic therapy.[109] The likelihood of delayed resolution of symptoms is greatest in patients who have prominent systemic symptoms or a delay in diagnosis before institution of antibiotics.[105]

Disseminated disease

With the possible exception of arthritis, which usually responds to oral therapy (see Arthritis, below), disseminated infection with target organ involvement other than skin should generally be treated with intravenous antibiotics. Carditis, meningitis, cranial neuropathy and radiculoneuropathy, and arthritis are discussed separately.

TABLE 54-3 -- Suggested antibiotic regimens for Lyme disease.

SUGGESTED ANTIBIOTIC REGIMENS FOR LYME DISEASE	
Early disease	• Doxycycline, 100mg po, q12h for 21 days, or
	• Amoxicillin (with or without probenecid) 500mg, q8h for 21 days, or
	• Erythromycin, 250–500mg po, q6h for 21 days, or
	• Azithromycin 500mg daily for 7 days, or
	• Cefuroxime axetil, 500mg po, q12h for 21 days
	Shorter courses (14 days) may suffice for localized early disease.
	Erythromycin and azithromycin less effective than other choices

Lyme arthritis	Initial treatment:
	• Doxycyline, 100mg po, q12h for 30 days, or
	• Amoxicillin and probenecid, 500mg each po, q6h for 30 days
	If initial treatment fails:
	• Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 14 days, or
	• Ceftriaxone sodium, 2g iv, daily for 14 days
Neurologic manifestations	For facial nerve paralysis alone:
	• Doxycycline, 100mg po, q12h for 21–30 days, or
	• Amoxicillin, 500mg po, q8h for 21–30 days
Additional signs (e.g. Lyme meningitis, radiculopathy, encephalitis)	• Ceftriaxone, 2g iv, daily for 30 days, or
	• Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 30 days
	Possible alternatives:
	• Cefotaxime sodium, 2g iv, q8h for 30 days, or
	• Doxycycline, 100mg po, q12h for 14–30 days, or
	• Chloramphenicol, 1g iv, q6h for 14–30 days
Lyme carditis	• Ceftriaxone, 2g iv, daily for 14 days, or
	• Penicillin G, 20 × 10^6 iv, daily in divided doses for 14 days
	Possible alternatives:
	• Doxycycline, 100mg po, q12h for 21 days, or
	• Amoxicillin, 500mg po, q8h for 21 days
During pregnancy	Localized, early disease:
	• Amoxicillin, 500mg po, q8h for 21 days
	Other manifestations:
	• Penicillin G, 20 × 10^6 IU iv, daily in divided doses for 14–30 days, or
	• Ceftriaxone, 2g, daily for 14–30 days

Carditis

Although carditis resolves spontaneously, observational data suggest that resolution of heart block may be hastened by treatment with salicylates, corticosteroids, oral penicillin, oral tetracyclines and intravenous ceftriaxone and penicillin.[31] [30] This response to either anti-inflammatory therapy or antibiotics suggests that the proximate cause of the heart block is the inflammatory reaction engendered by the Lyme spirochete rather than direct tissue destruction resulting from the infection. Control of the inflammatory response leads to resolution of clinical manifestations.

Heart block caused by carditis may progress suddenly, necessitating a temporary pacemaker, but permanent pacing is rarely necessary. Hospitalization with cardiac monitoring is prudent while antibiotic therapy is instituted. Although no comparative trials have been conducted, treatment with intravenous penicillin or a third-generation cephalosporin for a minimum of 2 weeks to eradicate systemic spirochetal infection is recommended. Salicylates or non-steroidal anti-inflammatory agents may hasten symptom resolution. Systemic corticosteroids may be dramatically effective in reversing heart block, but they may be best avoided during antibiotic therapy if possible.

Neurologic manifestations

Data on the treatment of neurologic manifestations are derived primarily from clinical experience. The tendency for spontaneous resolution of Bell's palsy, the fluctuating course of meningitis, the clinical variation of neurologic syndromes and the delayed emergence of the subtle deficits associated with late neurologic Lyme disease must all be considered in evaluating the clinical response to antibiotic therapy.[38] [50] [101] The emergence of chronic neurologic impairment years after remission of acute neurologic symptoms highlights the danger of complacency about the potential consequences of incomplete eradication of CNS infection.

Bell's palsy

Historically, Lyme facial palsy has been treated with oral antibiotics, whereas other neurologic syndromes have been treated intravenously. It is unclear whether this distinction is warranted. Facial palsy itself resolves completely or nearly completely in nearly all patients (121 of 122 patients in one series).[110] Patients who have facial palsy should undergo a careful neurologic evaluation, including a CSF examination. Cerebrospinal fluid invasion has been demonstrated by PCR in patients who have minimal CNS complaints and facial palsy, most of whom have clinically silent CSF pleocytosis.[40] [72] If facial palsy is the only clinical abnormality and CSF is normal, current practice is to administer oral antibiotics for 21–30 days, a practice that has resulted in favorable outcomes. Long-term follow-up of this group is important and, if CSF examination is not possible, the preferred course at present is to administer intravenous antibiotics.

Meningitis

Intravenous penicillin for 10 days has been shown in clinical trials to be effective treatment of meningitis. In one small series, intravenous ceftriaxone for 14 days was superior to a 10-day course of penicillin.[111] Most experts prefer a 30-day course of treatment, however, because of the occasional occurrence of late neurologic relapses after shorter courses of therapy. It is not necessary to document clearing of all CSF abnormalities before discontinuation of therapy because clearing of inflammation may lag behind bacteriologic cure. The co-occurrence of encephalopathy or encephalomyelopathy does not change this approach.

Radiculoneuropathy

Radiculoneuropathy is less clearly responsive to antibiotic therapy. Intravenous penicillin has not been shown to hasten resolution, and the response to ceftriaxone is unpredictable.[37] [38] Current practice is to administer intravenous antibiotic therapy, however, based on favorable long-term outcome with this approach and the belief that radiculoneuropathy is driven by systemic infection.

Resolution of neuropathy may be very gradual (taking place over months) and, with chronic involvement, this response may be incomplete. Doxycycline has been used orally and intravenously as an alternative to ceftriaxone or penicillin, but experience with this agent is limited.[112] One patient who had severe neurologic involvement, unresponsive to penicillin, responded favorably to chloramphenicol.[113]

Arthritis

Arthritis may respond to either oral[114] or parenteral[96] [103] [115] [116] antibiotic therapy, but antibiotic failures occur with either approach. Amoxicillin plus probenecid given orally for 4 weeks cures the majority of patients and those who fail oral therapy do not appear to respond to intravenous therapy. In a carefully carried out PCR study, no joint fluids were PCR-positive in patients who had received at least 8 weeks of antibiotic therapy. The optimal duration of therapy is unknown but an initial course for

4 weeks is recommended.

The role for parenteral therapy for Lyme arthritis is unclear. Patients who have concurrent neurologic involvement should receive intravenous treatment. In one randomized study, intramuscular benzathine penicillin given weekly for 3 weeks cured less than one half of patients, but intravenous penicillin for 10 days cured a higher percentage.[116] Ceftriaxone given for 14 days has been found to be more effective than 10 days of penicillin.[113] The primary reason for selection of intravenous therapy is concurrent neurologic involvement, in which case ceftriaxone or cefotaxine for 2–4 weeks are probably the agents of choice.

Resolution is commonly delayed, with synovitis persisting for months after completion of antibiotic therapy before eventually resolving. It has been suspected that administration of intra-articular corticosteroids during or before antibiotic therapy increases the risk of antibiotic failure, but a single intra-articular injection after completion of antibiotic therapy may hasten resolution. Patients who do not respond to a first course of therapy may respond to a repeat course, but there is no known rationale for a course of longer than 8 weeks. Adjunctive treatment measures should include evacuation of large effusions and limitation of weightbearing during acute attacks. Chronic inflammatory arthritis may occur through an autoimmune mechanism rather than as a result of persistent infection.

Late Lyme disease

Late Lyme disease, a designation reserved for those patients who have symptoms that persist for longer than 1 year, generally involves persistent inflammation in the CNS, joints or skin (ACA). Generally, patients who have ACA respond to oral penicillin. Late neurologic and arthritic involvement, however, are less predictably responsive. In one report, only one-half of patients who had late neurologic symptoms showed either resolution or sustained improvement after 6 months of follow-up after a 2-week course of ceftriaxone.[50] Those who did not respond, however, did not show progressive worsening. Long-term follow-up of this patient group is essential. In a European trial ceftriaxone and penicillin for 2 weeks were both effective for late neurologic involvement.[117]

Persistent arthritis after antibiotic therapy occurs most often in people who have HLA-DR4.[52] They are usually PCR negative and may be treated satisfactorily with arthroscopic synovectomy after failing to respond to either oral or intravenous antibiotics, suggesting that the pathogenesis of antibiotic-resistant arthritis may involve mechanisms other than persistent infection.[118] A recent study has provided strong evidence of an autoimmune mechanism for chronic Lyme arthritis.[97] Comparative trials of different antibiotics and varying durations of therapy are currently in progress for the treatment of late manifestations of Lyme disease, but there are no controlled data involving treatment periods longer than 4 weeks.

Pregnancy

Lyme disease acquired during pregnancy represents a special category because the health of the fetus must also be considered. Case reports have provided convincing evidence that *B. burgdorferi* can cross the placenta. Stillbirth[119] [120] and neonatal death[19] [20] have been attributed to *B. burgdorferi* transmitted from mother to fetus in utero, but the evidence to support this conclusion is still incomplete. The vast majority of pregnancies complicated by maternal Lyme disease have normal outcomes. *Borrelia burgdorferi* has not been linked statistically to congenital anomalies,[106] and no increased risk of an adverse outcome of pregnancy has been associated with asymptomatic seropositivity[121] or history of previous Lyme disease. It is appropriate to maintain a lower threshold for institution of aggressive antibiotic therapy for suspected Lyme disease during pregnancy, but women should be reassured that no cases of fetal Lyme disease have occurred with currently recommended antibiotic regimens.

PREVENTION

If possible, it is preferable to prevent Lyme disease by personal protection and the use of vaccine than to treat it. One obvious issue with regard to prevention is whether an individual with a known ixodid tick bite in a Lyme disease endemic area should be treated prophylactically with antibiotics. This question has been studied in a Lyme disease endemic area in Connecticut. A randomized, double-blind trial of amoxicillin therapy for tick bites[15] showed that, although the tick infection rate approached 15%, the risk of Lyme disease in untreated people was so low — 1.2% (95% CI 0.1–4.1%) — that prophylactic therapy (although probably effective) was not warranted. In a recent survey of pediatricians in an endemic area, 26% (70/267) routinely administered prophylactic antibiotics after tick bites.[122] Considerable controversy has arisen over this issue following a recent report by Nadelman *et al.*,[123] in which a single dose of oral doxycycline (200mg) within 72 hours after removal of an *I. scapularis* tick significantly reduced the incidence of Lyme disease (1/235 in the doxycycline group versus 8/247 in the placebo group). Many clinicians argue that this practice is unwarranted and may promote the development of antibiotic resistance.[124] [125] Prophylactic antibiotics may be cost effective in endemic regions if the risk of transmission following tick bites is greater than 0.01.[126] If the tick is preserved after it has been removed from its attachment site, a PCR for *B. burgdorferi* DNA can be done of the tick contents. This may allay fears of transmission risk and serve as a guide to preventative treatment in certain risk categories (e.g. pregnancy). Personal protection measures, including the wearing of protective

clothing, the use of insect repellent containing N,N-diethyl-metatoluamide (DEET) and the prompt removal of ticks, reduce the risk of Lyme disease.

Another area of active research involves efforts to develop a Lyme disease vaccine. A recombinant vaccine based on OspA, Lymerix, has been approved but was recently withdrawn from the market.[127] Efficacy was reported to be 79%[128] after three doses. New recombinant-based vaccines are under development; these are expected to have a broad range of specificity against the variants of *B. burgdorferi* distributed throughout the world.

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Chapter 55 - Practice Points

55.a Fever and arthralgia

Nigar Kirmani

Introduction

Fever and arthralgia is a common clinical presentation of a variety of infectious and noninfectious entities. Clinicians need to be familiar with a broad differential diagnosis in order to differentiate active infection requiring antimicrobial therapy from a systemic inflammatory process that may require immunosuppressive therapy. Arthralgia may often progress to frank arthritis. The pattern of joint involvement and presenting signs and symptoms help categorize patients and are useful clues in establishing the diagnosis. Clinical presentations include:

- acute monoarticular arthritis,
- oligoarticular arthritis,
- polyarticular arthritis, and
- fever and arthralgia or arthritis with associated skin eruption.

Table 55a.1 lists the common etiologic agents in each category. Major causes of fever and arthralgia/arthritis and their diagnostic approach are listed in Table 55a.2 . Synovial fluid profiles of selected diseases are shown in Table 55a.3 . Infectious and reactive arthritis are reviewed in detail in Chapter 51 .

Diagnosis

The first and most important step in narrowing down the extensive differential diagnosis is a thorough history and physical examination. The severity and duration of symptoms, fever pattern, sexual history, immunization status, dietary, transfusion and travel history, along with occupation, animal or other exposures should be obtained. Past illnesses such as diarrhea, sexually transmitted disease or flu-like syndromes would suggest reactive arthritis as an immune-mediated sequel. A complete and thorough physical examination is essential with special attention to the joints, skin and mucous membranes. Fever with pain and swelling of a single joint should be considered septic arthritis until proven otherwise. Chronic monoarticular arthritis suggests mycobacterial or fungal infection. Splinter hemorrhages, Osler's nodes or other peripheral embolic phenomenon help in diagnosing bacterial endocarditis. Pustular skin lesions on the trunk or extremities are seen in disseminated gonococcal infection; erythema migrans (Lyme disease) and the 'slapped cheek' rash of erythema infectiosum are helpful clues, although adults with parvovirus B19 infection do not usually have rash. Schnitzler's syndrome is characterized by chronic urticaria, intermittent fever, arthralgia and lymphadenopathy. It is often confused with adult Still's disease, although high fever and an evanescent rash are seen in the latter. A malar or

TABLE 55.a-1 -- Causes of fever and arthralgia.

CAUSES OF FEVER AND ARTHRALGIA
Monoarticular arthritis
Septic arthritis
Tuberculosis
Fungal infections
Bacterial endocarditis
Calcium pyrophosphate dihydrate deposition disease (pseudogout, pseudorheumatoid arthritis)
Gout
Oligoarticular arthritis
Disseminated gonococcal infection
Brucellosis
Fungal infections
Histoplasmosis
Coccidioidomycosis
Sporotrichosis
Reactive arthritis
Salmonella spp. infection
Shigella spp. infection
Campylobacter spp. infection
Chlamydial infection
Lymphogranuloma venereum
Bacterial endocarditis
Rheumatic
Still's disease
Inflammatory bowel disease
Psoriatic arthritis
Ankylosing spondylitis
Paraneoplastic syndromes
Serum sickness
Sarcoidosis
Strongyloidiasis
Dracunculiasis
Polyarticular arthritis
Viral infections
Hepatitis B virus

Hepatitis C virus
HIV
Cytomegalovirus
Epstein-Barr virus
Arboviruses
Rheumatic fever
Rheumatoid arthritis
Relapsing fever
Whipple's disease
Giardiasis
Loa loa infection
Toxoplasmosis
Paraneoplastic syndromes
Atrial myxoma
Fluoroquinolone induced
Familial Mediterranean fever
Arthralgia or arthritis with skin eruption
Lyme disease
Disseminated gonococcal infection
Chronic meningococcemia
Syphilis
Rocky Mountain spotted fever
Rat-bite fever (streptobacillary form)
Murine typhus
Parvovirus
HIV
Rubella (natural or vaccine)
Dengue fever
Systemic lupus erythematosus
Mixed cryoglobulinemia
Schnitzler's syndrome

TABLE 55.a-2 -- Clinical findings and diagnostic strategies in selected diseases that cause fever and arthralgia.

CLINICAL FINDINGS AND DIAGNOSTIC STRATEGIES IN SELECTED DISEASES THAT CAUSE FEVER AND ARTHRALGIA			
Disease	**Etiologic agent(s)**	**Associated clinical findings**	**Diagnostic strategy**
Bacterial endocarditis	***Staphylococcus aureus,* coagulase-negative staphylococci, viridans streptococci, other organisms**	Cardiac murmur, splenomegaly, Osler's nodes, splinter hemorrhages, petechiae. Polyarthralgias, oligo- and monoarthritis and back pain are common musculoskeletal symptoms	Blood cultures, echocardiography
Lyme disease	***Borrelia burgdorferi***	History of tick exposure, erythema migrans, headache, myalgia, malaise, fatigue. Arthralgias in early disease, oligoarthritis in late disease	Acute disease: characteristic rash; chronic disease: serology, confirmed by Western blotting
Disseminated gonococcal infection	***Neisseria gonorrhoeae***	History of sexual exposure, pustular or necrotic skin lesions in acral and juxta-articular distribution, more common in women and associated with menses. Two forms of arthritis occur — migratory polyarthritis with skin lesions and oligoarticular arthritis	Confirmatory culture from urethra, endocervix, rectum, pharynx or involved joint; characteristically prompt resolution of symptoms with antibiotic therapy
Rheumatic fever	***Streptococcus pyogenes***	History of recent pharyngitis, cardiac murmur, erythema marginatum. Migratory polyarthritis involving large joints	Throat culture or streptococcal antigen assay; antistreptolysin O titer, anti-DNAse B, anti-hyaluronidase; Jones criteria
Parvovirus	**Parvovirus B19**	Erythema infectiosum ('slapped-cheek') or reticular recrudescent rash, mild-to-severe pancytopenia. Rash uncommon in adults	Parvovirus IgM and IgG antibody titers, PCR on blood
Reactive arthritis (Reiter's syndrome)	***Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter* spp., *Chlamydia* spp.**	History of diarrheal illness or previous sexually transmitted disease, conjunctivitis, urethritis, dactylitis, enthesopathy, oral ulceration. Sacroiliitis, sausage digits and tendinitis are seen	No specific diagnostic test, haplotyping for HLA-B27 provides supportive evidence for diagnosis
HIV-associated arthropathy	**HIV**	Arthralgia, which may occur at any stage of disease, including acute seroconversion, may be severe and refractory to analgesics ('painful articular syndrome'), increased susceptibility to other forms of arthritis such as Reiter's arthritis and psoriatic arthritis	Testing for HIV antibodies; in acute infection, HIV RNA testing by PCR
Rubella	**Rubella virus (wild-type and live-attenuated vaccine strains)**	Erythematous, macular rash, spreading from face to trunk, which may occur 2–4 weeks after vaccination, particularly in adults. Polyarthritis involving small joints of hands and other peripheral joints	Viral isolation from throat swab or blood, fourfold rise in IgG antibodies from acute to convalescent period
Hepatitis B	**Hepatitis B virus**	In acute infection, immune complex-mediated serum sickness associated with malaise, anorexia, maculopapular rash, urticaria preceding onset of jaundice by about 10 days; in chronic infection, mixed cryoglobulinemia with associated palpable purpura, urticaria, hepatosplenomegaly and glomerulonephritis	In acute infection, hepatitis B surface antigen, elevated hepatic enzymes and bilirubin; in chronic infection hepatitis B surface antigen, core antigen, antihepatitis B core antibody, antihepatitis B surface antibody

Septic arthritis	***Staphylococcus aureus,* streptococci**	History of pre-existing arthritis or injury, injection drug use is a risk factor. Usually monoarticular; may be polyarticular in patients with rheumatoid arthritis	Arthrocentesis revealing high white blood cell count with polymorphonuclear predominance; Gram stain and culture of synovial fluid
Brucellosis	***Brucella melitensis***	History of contact with livestock or ingestion of unpasteurized dairy products, typically a unilateral sacroiliitis	Culture of blood and synovial fluid, serologic testing (standard tube agglutinin test)
Crystal-induced arthritis	**Urate crystals (gout), calcium pyrophosphate dihydrate crystals (pseudogout)**	Tophi, first episode frequently involves the metatarsophalangeal joint of the first toe; recurrence generally polyarticular	Arthrocentesis with identification of crystals in the synovial fluid
Systematic lupus erythematosus		Malar rash, serositis, proteinuria, anemia, leukopenia, thrombocytopenia, seizures	LE cells, antinuclear antibodies, antibodies to dsDNA, Sm antigen
Still's disease		High fever, evanescent rash, lymphadenopathy	Leukocytosis, elevated ESR, negative rheumatologic serologies

discoid rash, along with fever and arthralgia are suggestive of systemic lupus erythematosus.

Initial investigations include a complete blood count and liver function tests. Diagnostic arthrocentesis is critical in distinguishing infectious from noninfectious arthritis. A Gram stain, culture, cell count with differential and examination for crystals should be performed. Antigen detection by PCR has been useful for fastidious organisms. In addition to blood, other sites (oropharynx, genital secretions, stool) may need to be cultured and skin lesions biopsied. Serum antibody titers (acute and convalescent) against specific viral and bacterial pathogens may establish a late diagnosis; saving a serum sample at the time of presentation is extremely useful. Rheumatoid factor, antinuclear

TABLE 55.a-3 -- Synovial fluid findings in selected diseases that cause fever and arthralgia.

SYNOVIAL FLUID FINDINGS IN SELECTED DISEASES THAT CAUSE FEVER AND ARTHRALGIA				
Group	**Color**	**Clarity**	**WBC/µl**	**PMN**
Normal	Colorless	Transparent	<200	<25%
Noninflammatory	Straw colored	Transparent	50–1000	<25%
Systemic lupus erythematosus (occasionally inflammatory)				
Osteo-arthritis (not associated with fever)				
Inflammatory	Yellow	Transparent	1000–50,000	>50%
Rheumatoid arthritis; rheumatic fever; reactive arthritis; viral arthritis; Lyme arthritis; bacterial endocarditis; gout (needle shaped, negative birefringent crystals); pseudogout (rhomboid, positive birefringent crystals)				
Septic	Yellow-green	Opaque	>50,000 (often >100,000)	>75%
Staphylococcus aureus (culture positive 90%)				
Neisseria gonorrhoeae (culture positive <50%)				

and other autoantibodies may help distinguish the rheumatic diseases. Radiologic studies are rarely necessary; CT scan and MRI are more helpful than plain X-rays.

Management

Identification of the underlying etiology is a critical first step. Empiric antibiotics should be avoided, unless the patient is critically ill. Repeated needle aspirations, arthroscopy or surgical drainage may be necessary for septic arthritis. Corticosteroids for presumptive rheumatologic disease should not be started until an infectious process has been ruled out.

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55.b For how long should osteomyelitis be treated?

Andrew R Murry

Introduction

Bone infections vary considerably in their pathogenesis, microbiology and clinical presentation. They are usually classified as acquired by hematogenous or contiguous spread and as acute or chronic. The transition from acute to chronic evolves over a period of weeks to months. The antimicrobial treatment of osteomyelitis needs to be tailored to the characteristics of the individual infection.

Pathogenesis

Hematogenous osteomyelitis is most common in infants, children and the elderly. Bone involvement may become obvious during the acute illness or as recrudescent disease after the bacteremia has cleared. The preceding bacteremia may be inapparent, with osteomyelitis presenting as an isolated finding. Most cases of acute osteomyelitis in adults do not result from bacteremia, but are a consequence of contiguous spread from infected wounds, teeth, sinuses, ulcers, open fractures or implanted prosthetic devises. Early, vigorous antimicrobial treatment of acute bone infections reduces the risk of progression to chronicity. Chronic osteomyelitis is associated with devitalized bone, fibrosis, sinus tract formation and clinical recrudescence. Chronic infections are much more difficult to cure, particularly in the presence of diabetes mellitus, vascular disease or any condition that impairs host defenses.

Microbiology

The pathogens most associated with various types of osteomyelitis are shown in Table 55b.1 .

Clinical features

Acute infections may be associated with local symptoms and signs, including pain and local inflammation with or without fever, chills and other constitutional manifestations of infection. In those infections acquired by contiguous spread, the presence of bone involvement may be indeterminate by clinical criteria. Findings may be more vague in chronic infection, with only nonspecific pain and few constitutional symptoms. Vertebral osteomyelitis commonly presents with dull, constant back pain, spasm of the paravertebral muscles and tenderness over the involved vertebrae. Infected prosthetic joints may become painful or loosen. Sinus tracts may develop or increase their drainage.

Interventions

Plain radiographs of the suspected area can often confirm the presence of osteomyelitis, although abnormalities may not be apparent for about 2 weeks. Radiographs may be difficult to interpret when there are soft tissue infections, fractures or noninfectious skeletal diseases.

TABLE 55.b-1 -- Common pathogens in osteomyelitis.

COMMON PATHOGENS IN OSTEOMYELITIS			
Hematogenous spread	**Contiguous spread**		
	Acute skin and soft tissue infections	**Orofacial and dental infections**	**Diabetic foot and pressure sores**
Staphylococcus aureus	*Staphylococcus aureus*	*Streptococcus* spp.	*Staphylococcus aureus*
Streptococcus spp.	*Streptococcus agalactiae*	*Anaerobic cocci*	*Staphylococcus epidermidis*
Haemophilus influenzae[*]	*Streptococcus pyogenes*	*Fusobacterium* spp.	*Streptococcus* spp.
Enterobacteriaceae[†]		*Prevotella melaninogenicia*	*Enterobacteriaceae*
Staphylococcus epidermidis[‡] , [§]		*Pasteurella multocida*[‡]	*Pseudomonas aeruginosa*[*]
Candida spp.[§]		*Eikenella corrodens*[§]	*Enterococcus faecalis*
Pseudomonas aeruginosa[*]		*Actinomyces* spp.	Anaerobic cocci
Salmonella spp.[†]			*Bacteroides* spp.
Neisseria gonorrhoeae[¶]			*Clostridium* spp.

*Infants and unimmunized children
†Elderly and neonates
‡Bone and joint prostheses
§Intravenous devices
‡‡Dog and cat bites
**Injection drug use and foot puncture wounds
§§Human bites
††Hemoglobinopathies
¶Young adults

Radionuclide scans, computed tomography or magnetic resonance imaging (MRI) can be helpful early in the disease. They do not need to be obtained routinely if treatment is initiated on the basis of clinical suspicion, and follow-up plain radiographs will be subsequently performed. Computed tomography and MRI are particularly useful when planning surgery.

A specific microbiologic diagnosis is most helpful in choosing antimicrobial therapy. Bone biopsy, although not through infected soft tissues, provides the most reliable specimen for microbiologic study. Although the results from cultures of sinus tracts, ulcers or wounds are somewhat unreliable, they may be useful for antibiotic selection if a single pathogen is consistently found or when a biopsy cannot be performed. Blood cultures should be obtained when appropriate.

Management

Acute osteomyelitis may be treated with antibiotics alone, but chronic infection often requires surgery to drain abscesses, resect fistulas, debride ulcers or necrotic devascularized bone, remove foreign bodies (including prostheses), manage dead space, provide stability or restore vascular supply, as well as for reconstruction. Optimization of host factors such as control of diabetes mellitus is also important.

Initial antimicrobial therapy is based on the known or probable pathogens as determined by the nature of the infection and the available culture and susceptibility test results. The antimicrobial agents most commonly used to treat osteomyelitis are shown in Table 55b.2 . Preferred drugs include the ß-lactams, clindamycin and the fluoroquinolones. Vancomycin, trimethoprim-sulfamethoxazole (co-trimoxazole) and metronidazole have more limited indications. Rifampin (rifampicin) may be used with a ß-lactam to treat staphylococcal infections, but there is more favorable experience using clindamycin alone. Aminoglycosides may be used with a ß-lactam for

Gram-negative infections, but they are toxic and ineffective when used alone. Macrolides and tetracyclines are not very effective and should be avoided. Linezolid has been used successfully in several case reports to treat osteomyelitis due to methicillin-resistant staphylococci and vancomycin-resistant enterococci.

Osteomyelitis has traditionally been treated with relatively high doses of intravenous antimicrobial agents for 4–6 weeks, although shorter courses of treatment have proven adequate in some children who have acute infection. Children who have acute infection caused by *Haemophilus influenzae*, *Neisseria* spp. or streptococci should be treated for at least 10–14 days and infections caused by *Staphylococcus aureus*, Enterobacteriaceae or *Pseudomonas aeruginosa* for at least 3 weeks.

For acute infections in adults and chronic infections in patients of any age, the recommended duration of treatment of 4–6 weeks should be considered a minimum unless the infected bone is completely removed by debridement. If this is accomplished, treatment can probably be abbreviated and, in patients undergoing amputation of an infected limb, treatment may be discontinued postoperatively if there is no residual soft tissue infection. For patients who have chronic, recalcitrant osteomyelitis that cannot be completely debrided, treatment with an appropriate agent for 3–12 months may be beneficial. Such infections may 'burn out' over a prolonged period of time with continued antimicrobial suppression. Antibiotic-impregnated beads or spacers can provide high antibiotic concentrations locally and may also be useful to manage dead space.

Prolonged intravenous treatment can be problematic because of hospitalization costs, the inconvenience and complications of long-term intravenous lines. The availability of highly active, predictably absorbed oral antimicrobial agents provide an alternative mode of therapy. The first experiences with oral therapy, usually a penicillin, cephalosporin or clindamycin, after about 5–10 days of initial intravenous therapy, were in children. Intravenous, and then oral, ampicillin-sulbactam was subsequently used successfully. Switching from intravenous to oral therapy has also been successfully accomplished in adults using those drugs or trimethoprim-sulfamethoxazole. Ciprofloxacin and other fluoroquinolones have been extensively used to treat skeletal infections successfully; however, the half-life of levofloxacin may be too short to rely on when treating osteomyelitis. Quinolones should not be used to treat methicillin-resistant staphylococci because resistance frequently develops during treatment.

When using oral treatment, either after an initial course of intravenous treatment or from the start, it is necessary to have a predictably

TABLE 55.b-2 -- Comparison of intravenous and oral antimicrobial regimens to treat osteomyelitis in adults.

COMPARISON OF INTRAVENOUS AND ORAL ANTIMICROBIAL REGIMENS TO TREAT OSTEOMYELITIS IN ADULTS						
INTRAVENOUS				**ORAL**		
Drug	**Dose**	**Approximate peak serum concentration (mg/l)**	**MIC (mg/l) for susceptible pathogens**	**Drug**	**Dose**	**Approximate peak serum concentration (mg/l)**
ß-lactams						
Penicillin G	1–2mU q4-6h	10–20 (U/ml)	=0.12	Penicillin V	500mg q6h	5
Ampicillin*	1–2g q4-6h	20–50	=0.25, =8	Amoxicillin	500mg q8h	5–10
Ampicillin-sulbactam†	1.5–3.0 q6h	50–150	=2/1, <8/4	Amoxicillin-clavulanate	500mg q8h	5–10
	875 mg q12h	13				
Nafcillin	1–2g q4-6h	40–80	=2	Dicloxacillin	0.5–1g q6h	10–25
Cefazolin	1–2g q8h	80–150	=2	Cephalexin§	0.5–1g q6h	15–25
Ceftriaxone	1–2g q24h	100–250	=4	Cefixime‡	400mg q6h	4
Non-ß-lactams						
Clindamycin	600mg q8h	8	=0.5	Clindamycin	300mg q6h	5
Trimethoprim-sulfamethoxazole	160/800mg q8h	6/120	=8/256	Trimethoprim-sulfamethoxazole	160/800mg q8h	4/100
Ciprofloxacin**	400mg q12h	4	=1	Ciprofloxacin	500–750mg q12h	3–4
Metronidazole	500mg q6h	15–25	=4	Metronidazole	500mg q6h	12
Linezolid††	600mg q12h	15	=2, =4	Linezolid††	600mg q12h	21
Vancomycin	1g q12h	40	=2	No oral equivalent available§§		

*MIC <0.12mg/l for most susceptible organisms; =8mg/l for *Enterococcus faecalis*
†MIC <2/1 mg/l for ßactamase-positive *Haemophilus influenzae*, <8/4mg/l for susceptible Enterobacteriaceae
§Cephalexin is fourfold less active than cefazolin against *Staphylococcus aureus*
‡Cefixime is for Gram-negative organisms only
**Avoid using for methicillin-resistant staphylococci
††Linezolid may be active against methicillin-resistant staphylococci and vancomycin-resistant enterococci, MIC <2mg/l for enterococci, <4mg/l for staphylococci
§§Trimethoprim-sulfamethoxazole, clindamycin may be active against methicillin-resistant staphylococci

absorbed drug that is highly active against the pathogen being treated. Although ß-lactams are often considered the preferred drugs for the treatment of skeletal infections, oral derivatives may not be predictably absorbed and do not yield serum concentrations as high as parenteral derivatives, even at high doses with probenecid. Serum concentrations after both intravenous and oral clindamycin far exceed those necessary to inhibit most staphylococci and streptococci. Serum concentrations of trimethoprim-sulfamethoxazole after intravenous and oral administration are virtually identical and far exceed those necessary to inhibit most staphylococci and Enterobacteriaceae. Careful studies have shown oral ciprofloxacin to be equivalent to intravenous ciprofloxacin and serum concentrations with either route of administration far exceed those needed to inhibit most Enterobacteriaceae and *P. aeruginosa*. The same appears true for most other quinolones. Serum concentrations of metronidazole after intravenous and oral administration are nearly equivalent and exceed those needed to inhibit most anaerobes. Finally, linezolid serum concentrations are equivalent using either intravenous or oral dosing making it an attractive option for treatment of multidrug-resistant staphylococci and enterococci.

Appropriate follow-up is necessary to evaluate adherence to the therapeutic regimen and to monitor for clinical improvement and radiographic healing. Determination of serum antibiotic concentrations or performing serum bactericidal tests may be helpful in drug regimens that might be marginal, but these tests are usually not necessary. Normalization of the erythrocyte sedimentation rate (ESR) is reassuring. If the ESR has fallen but not normalized, further treatment might be appropriate, and a persistently elevated ESR often predicts relapse within months of discontinuing antimicrobial agents.

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55.c Management of chronic infection in prosthetic joints

Jonathan Cohen

Introduction

Joint replacement is one of the surgical success stories of recent years. Hips, knees, shoulders and elbows are all amenable to this approach. The major complication is infection, which occurs in less than 1–2% of cases. Acute infection of a prosthetic joint is unmistakable: there is pain, fever and physical signs of inflammation around the joint. A common and often more difficult problem is the patient who presents with features consistent with chronic prosthetic joint infection (PJI). Although there is no formal definition, chronic PJI typically presents 1–2 years after the placement of the prosthesis, although it may be much later.

TABLE 55.c-1 -- Microbial causes of prothestic joint infection.

MICROBIAL CAUSES OF PROSTHETIC JOINT INFECTION	
Pathogen	**Proportion of infected joints (%)**
Staphylococci	50
Coagulase-negative staphylococci	25
Staphylococcus aureus	25
Streptococci	20
Gram-negative aerobic bacilli	20
Anaerobes	10

TABLE 55.c-2 -- **Drugs for the treatment of prosthetic joint infections.** HAI, home antibiotic infusion.

DRUGS FOR THE TREATMENT OF PROSTHETIC JOINT INFECTIONS			
Antibiotic	**Spectrum**	**Preparation used**	**Comments**
Vancomycin	Gram-positive bacteria; used particularly for coagulase-negative staphylococci	Only as a parenteral agent	A single daily infusion is suitable for HAI therapy
			Nephrotoxic
Teicoplanin	Gram-positive bacteria; used particularly for coagulase-negative staphylococci	Only as a parenteral agent	Given as a single daily infusion is suitable for HAI therapy
	Do not assume that vancomycin and teicoplanin have interchangeable susceptibility patterns		Nephrotoxic potential
Isoxazolyl penicillins: nafcillin, flucloxacillin and related compounds	Gram-positive bacteria, particularly *Staphylococcus aureus*	Both oral and parenteral preparations	
	Most coagulase-negative staphylococci are resistant		
Fusidic acid (not available in USA)	*Staphylococcus aureus* and many strains of coagulase-negative staphylococci	Oral (intravenous also available)	Excellent record in bone infections, always used in combination with flucloxacillin
			Parenteral preparation has significant hepatotoxicity; avoid if at all possible
Clindamycin	*Staphylococcus aureus* and some strains of coagulase-negative staphylococci	Both oral and parenteral formulations	A large body of successful experience in bone and joint infections
	Has activity against some strains of anaerobic bacteria		Care in elderly people because of risk of antibiotic-associated colitis
Rifampin	*Staphylococcus aureus* and many strains of coagulase-negative staphylococci	Most useful as an oral agent	Little published experience in this setting, but widely used in tuberculosis
Cephalosporins	Broad activity which may include *Staphylococcus aureus* and many aerobic Gram-negative bacilli, depending on the particular agent chosen	Both oral and parenteral formulations	Most useful when the microbiology is unknown and it is necessary to treat empirically with broad-spectrum agents
	Not active against coagulase-negative staphylococci		Long-acting drugs such as ceftriaxone are useful for HAI
Ciprofloxacin (and other quinolones)	Wide activity including *Staphylococcus aureus* and many aerobic Gram-negative bacilli	Oral and parenteral formulations	Very valuable agents in mixed infections, known Gram-negative infection, patients with ß-lactam hyper sensitivity, or as second-line agents
	Not active against coagulase-positive staphylococci		
Trimethoprim-sulfamethoxazole	Broad spectrum includes many strains of *Staphylococcus aureus* and Gram-negative aerobic bacilli	Oral and parenteral formulations	Often forgotten; a useful reserve agent particularly in patients who have ß-lactam hypersensitivity
	Not active against coagulase-negative staphylococci		

Pathogenesis

Most PJIs are caused by exogenous infection by organisms that gain entry at the time of operation, and this is reflected in the type of organisms isolated from these cases (see Chapter 53), which are typically low-grade pathogens associated with skin contamination. However, prosthetic joints can become infected by the hematogenous route, and this is more common in late infections, more than 2 years after operation.

Microbiology

About 80% of infections are associated with a single organism, 10% are mixed infections and 10% are sterile. Gram-positive bacteria are the commonest isolates,

particularly coagulase-negative staphylococci (Table 55c.1). Infections may also be caused by anaerobic bacteria, typically peptococci and peptostreptococci, and by proprionobacteria.

Clinical features

The dominant complaint is of pain. It is usually aching in character, may be continuous or intermittent and is not necessarily of great severity. Local signs of inflammation are often absent, but there may be some erythema, induration or swelling. The finding of a sinus is very helpful; it may discharge very little material, and only intermittently. General systemic features of infection (fever, malaise, shaking chills) are very uncommon in chronic PJI.

The principal differential diagnosis is joint loosening without infection. Laboratory investigations are not very helpful (see below); only joint aspiration and isolation of the causative organism can establish the diagnosis.

Investigations

Establishing the microbiologic diagnosis is of great importance in managing these infections. Isolation of the causative organism will not only confirm the diagnosis of infection, but will also be crucial in guiding the choice of antimicrobial therapy. In this context, interpreting the microbiologic findings from a discharging sinus can present difficulties. While it may seem 'logical' to use this as a surrogate for direct joint aspiration (particularly as both the patient and the physician may be reluctant to do a further surgical procedure), there are pitfalls. The most obvious is that bacteria such as *Staphylococcus epidermidis* are common causes of PJI, but they are also common skin commensals and likely to be isolated from sinus swabs. On the other hand, isolation of *Staph. aureus*, *Proteus* spp. or *Pseudomonas* spp. from such a sinus is a very good indication of the likely cause of the underlying infection. A reasonable approach is to require:

- that the organism should be repeatedly isolated from the sinus; and
- that the organism is identical on each occasion by techniques such as antibiograms and, where appropriate, other more sophisticated forms of bacterial typing.

If there is any doubt, and in particular in cases where a patient presents after one failed course of therapy, joint aspiration should be carried out, ideally under computed tomography or ultrasound guidance. The laboratory should be forewarned, and anaerobic cultures should be included in the processing of the specimen. In the study by Tunney *et al.* (see Further reading, below) proprionobacteria were isolated from 60% of specimens submitted for investigation of possible PJI.

Measurement of C-reactive protein (CRP) is particularly helpful in gauging the response to treatment. It is nonspecific, but in my experience it is not significantly elevated in noninfected joints (even if they are loose and painful). Failure of the CRP to normalize is a reliable sign of persistent infection. Other investigations are less useful. The leukocyte count is rarely elevated in chronic PJI. Radiologic investigations have been particularly disappointing. Plain radiographs do not generally distinguish between infection and an uninfected loose joint. Scintigraphic investigations using gallium, technetium or indium are neither sensitive nor specific enough to be of real clinical value, particularly in the patient who has a low-grade, chronic infection. Ultrasound is useful for identifying collections that may be susceptible to surgical drainage, and it deserves further evaluation in chronic infections.

Management

Lasting cure almost always requires removal of all prosthetic material and extensive local debridement. The procedure of choice is a two-stage revision. First, the infected joint is removed and the patient is given 6 weeks of antimicrobial therapy based on the susceptibility pattern of the causative organism (Table 55c.2). In the second stage, a replacement joint is fitted. An alternative approach is a single-stage procedure involving removal of the infected prosthesis and immediate replacement with the new joint using antibiotic-impregnated bone cement.

A detailed discussion of the surgical aspects of the treatment can be found in orthopedic textbooks, but for the infectious diseases practitioner there are a number of difficult and unresolved questions.

Duration of antibiotic treatment

The advice to use 6 weeks of treatment is based on clinical experience rather than comparative clinical trials. A practical consequence of this is that the regimen chosen should either be available as oral agents, or else offered as part of a home-antibiotic treatment program. In a small number of cases in which further surgery is contraindicated, long-term suppressive therapy may be tried, although this is not generally recommended.

Choice of regimen

Despite the fact that a number of antibiotics have been used quite extensively in these infections, there are no good clinical trials that rigorously compare different regimens. For instance, there is no agreement as to whether one or two drugs should be used; many UK orthopedic surgeons favor the combination of flucloxacillin and fusidic acid, whereas in North America single-agent therapy is more common. Another issue is whether there is any merit in parenteral therapy; is high-dose oral therapy just as good?

In the absence of adequate data, the following recommendations are suggested, based on my experience; they will need modifying in the light of individual circumstances:

- for proven or presumed Gram-positive infection with coagulase-negative staphylococci, 6 weeks of parenteral vancomycin (or teicoplanin) plus oral rifampin (rifampicin);
- for proven or presumed *Staph. aureus* infection: 2 weeks of high-dose (2–4g/day) intravenous flucloxacillin (or nafcillin) plus oral fusidic acid (where available), followed by 4 weeks of oral therapy;
- for proven or presumed Gram-negative infection, 6 weeks of ciprofloxacin;
- for mixed infections or infections of uncertain etiology, the combination of vancomycin or rifampin plus ciprofloxacin is useful;
- reserve agent for Gram-positive infection: clindamycin; linezolid or quinupristin-dalfopristin are additional agents that may be useful but clinical experience in this setting is still very limited.
- reserve agent for Gram-negative infection: trimethoprim-sulfamethoxazole (co-trimoxazole).

Specific anti-anaerobic agents may be needed, depending on the microbiologic findings; penicillin, clindamycin and metronidazole are all useful for this purpose. The treatment of pseudomonal infections is particularly difficult; there may be benefit in adding gentamicin to ciprofloxacin, but the dangers of a long course of an aminoglycoside, particularly in the elderly, are such that this should only be done after very careful consideration.

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Chapter 56 - Sepsis

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This chapter examines the pathophysiologic consequences of the systemic effects of infection. This is broadly referred to as sepsis and is a heterogeneous syndrome resulting from a complex interaction between host defenses and invading pathogens. In this chapter, I describe the pattern of disease seen in bacterial sepsis and detail a logical approach to therapy.

EPIDEMIOLOGY

Definition and nomenclature

For many years physicians recognized that infection could lead to generalized circulatory collapse and death. The association of bloodstream infection with severe systemic illness was generally referred to as septicemia, and in the 1960s the work of McCabe *et al.* described in detail, for the first time, the spectrum of disease associated with Gram-negative bacteremia, including septic shock.[1] [2] Although sepsis and septic shock are terms that most practitioners recognize, if asked to define bacteremia, sepsis, septicemia and septic shock a considerable range of views would emerge. Driven by the need to adopt a common currency of terms, Bone initiated a debate that continues to provoke discussion.[3] Bone suggested that 'sepsis' be used for the systemic response to infection according to specific clinical criteria. Furthermore, he recognized that an identical syndrome could occur with noninfective inflammatory stimuli. The American College of Chest Physicians and American Society of Critical Care Medicine (ACCP/ASCCM) accepted much of what Bone suggested and also introduced the term the systemic inflammatory response syndrome (SIRS) to define a common set of physiologic changes occurring in infected (sepsis) and noninfected patients.[4] SIRS has been criticized for being non-specific and encompassing too wide a range of disease processes.[5] Thus, although these concepts have helped to improve our understanding of the pathophysiology of sepsis, it is important to remember that all different infections may not behave the same way in all patients. A pragmatic approach to the terminology of sepsis is defined in Table 56.1 .

TABLE 56-1 -- Working definitions associated with sepsis and related disorders.

WORKING DEFINITIONS ASSOCIATED WITH SEPSIS AND RELATED DISORDERS	
Disorder	**Definition/signs**
Bacteremia	Bacteria present in the blood, as confirmed by culture. May be transient or associated with sepsis and organ failure.
Sepsis (identical to SIRS criteria minus evidence of infection)	Confirmed or clinical evidence of infection plus evidence of a systemic response manifested by two or more of the following:
	Temperature >100.4°F (38°C) or <96.8°F (36°C)
	Heart rate >90 beats/min
	Respiratory rate >20 breaths/min or arterial CO_2 tension <32mmHg (4.3kPa)
	WBC >12,000 cells/ml, <4000 cells/ml or >10% immature forms (bands)
Severe sepsis	Sepsis with associated organ dysfunction with one or more of the following:
	Hypotension
	Confusion
	Oliguria
	Hypoxia — not explained by primary respiratory disease
	Metabolic (lactic) acidosis
	Disseminated intravascular coagulation (DIC)
	Hepatic dysfunction — not explained by primary liver disease
Septic shock	Severe sepsis plus hypotension despite adequate fluid resuscitation
SIRS, systemic inflammatory response syndrome	
WBC, white blood count	

There is no single classification system that will satisfy the requirements of clinicians and researchers alike, but the current definitions can be used as a basis for recognizing patients who have sepsis and directing both therapies and clinical resources. These processes are best considered as a continuum, with localized inflammation at one end and a severe generalized inflammatory response leading to multiorgan failure at the other. Patients may present anywhere along this spectrum or alternatively may visibly progress from early sepsis, through severe sepsis, to refractory shock and death. The challenge for physicians is to recognize the onset of sepsis, identify those at high risk of complications and intervene early to try to prevent progression along this continuum, because once shock and organ failure are established the mortality rate remains high despite intensive therapy.

Incidence and prevalence of sepsis

Many host and environmental factors interact in the development of sepsis, making it a heterogeneous condition with varying rates in different patient populations. Sepsis is not a notifiable disease and estimates of the incidence of sepsis and SIRS have been further confounded by the failure of multicenter studies of novel therapeutic agents to include all patients fulfilling the definition of sepsis or SIRS. However, it is clear that sepsis is a major cause of hospital morbidity and death and has been increasing in prevalence over the past 30 years.[6] [7]

Community-acquired bacteremia is responsible for approximately 7–12/1000 hospital admissions. Angus and co-workers have performed the most comprehensive estimate of the epidemiology of sepsis in the USA.[8] Using existing data bases they analyzed discharge records of over six million patients from seven states in the USA during 1995 and extrapolated the results for the whole USA population. Data collected in this way should be viewed with some caution but the sheer size of this study demands attention. They estimated an annual incidence for severe sepsis of 3/1000 (2.26/100 hospital discharges) equivalent to 751,000 cases each year. Incidence rose from 0.2/1000 in childhood to 26/1000 over the age of 85 years. In terms of resources severe sepsis was estimated to cost around US$16 billion per year. In Holland Kieft *et al.* measured the attack rate for SIRS at 13.6/1000 and septic shock at 4.6/1000 hospital admissions. [9] An audit study of 61,874 admissions to intensive care units (ICUs) in England and Wales in 1999 indicated that 27.7% fulfilled criteria for severe sepsis during the first 24 hours following admission.[10] Once in the ICU the prevalence of SIRS is high, and in one study fully 68% of 3708 patients in intensive care fulfilled the criteria for SIRS at some time during their ICU stay.[11]

Morbidity and mortality rates

The mortality rate of SIRS and sepsis varies between countries and between different patient groups, possibly because of variations in

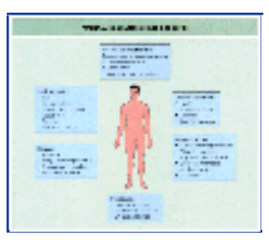

Figure 56-1 Potential risk factors leading to sepsis.

case definition, ICU facilities and patient populations. In an analysis of four large sepsis trials, 14-day mortality rate averaged 26% and 28-day mortality rate, 42%.[12] In

the Angus study cited above[8] overall mortality rate was 28.6%, equivalent to 215,000 deaths per annum in the USA. In this study mortality rate in childhood was around 10% rising to 38.4% in those over age 85 years.[8] Factors associated with early death include the number of organ systems involved, low arterial blood pH, shock and an unfavorable SAPS (Simplified Acute Physiology Score) or APACHE (Acute Physiology and Chronic Health Evaluation) score. Later deaths (within 28 days) are associated with the underlying disease process, pre-existing cardiac or liver disease, hypothermia, thrombocytopenia and multiple sources of infection. *Pseudomonas aeruginosa, Candida* spp. and mixed infections have a higher attributable mortality rate than other infectious agents.[13] Patients who have sepsis often have serious underlying medical or surgical problems that contribute to the mortality rate, but even when this is taken into account the attributable mortality rate from sepsis has still been measured as at least 25%. [13] Furthermore, a long-term follow-up of patients enrolled in the Veterans Affairs Co-Operative Study Of Corticosteroids In Systemic Sepsis performed in the early 1980s revealed a significant reduction in survival persisting for at least 5 years after the episode of severe sepsis.[14] Quality of life is also significantly impaired in survivors of sepsis.[15]

Risk factors for sepsis

Broadly speaking, risk factors for sepsis are those factors that weaken or breach host defenses, increasing the likelihood of bacterial invasion of otherwise sterile tissue as shown in Figure 56.1 . One of the most intriguing aspects of severe sepsis is why one individual will respond to a particular infection with devastating consequences of septic shock while another in apparently similar circumstances resolves the infection. It is apparent that there are numerous variations in our host response to all types of infecting organisms.

Figure 56-2 Positive blood cultures in severe sepsis. *Data from Bochud et al.* [42]

Genetic variations are known to be important in susceptibility to infection, but it is also clear that our genes may also hold the key to our vulnerability to severe sepsis.[16]

PATHOGENESIS AND PATHOLOGY

Etiologic agents of sepsis

Bacteria are the commonest underlying pathogens in patients presenting from the community and in hospital-acquired sepsis. McCabe *et al.*[1] identified Gram-negative bacteria as the predominant cause of septic shock in the early 1960s.[1] Over the past decade, however, there has been a shift in the etiology of nosocomial infections, with increased infection due to Gram-positive bacteria.[17] This is also reflected in the pattern of underlying infections in patients who have sepsis (Fig. 56.2). Staphylococci, *Escherichia coli* and enterococci remain the most commonly isolated pathogens.

Although it remains true that bacterial infections continue to be frequent, there has been a growing awareness that a wide range of nonbacterial pathogens may induce the pathologic responses necessary to lead to sepsis. This includes fungi (e.g. *Candida* spp.), rickettsia (e.g. *Rickettsia rickettsii* — Rocky Mountain spotted fever), protozoa (including *Plasmodium falciparum*) and certain viruses (e.g. those that cause dengue fever).

TABLE 56-2 -- Bacterial components involved in the pathogenesis of severe sepsis.

BACTERIAL COMPONENTS INVOLVED IN THE PATHOGENESIS OF SEVERE SEPSIS		
Category	**Source**	**Examples**
Structural cell wall constituent	Outer cell wall of all Gram-negative bacteria	Endotoxin (LPS, lipid A)
	Cell wall of all bacteria	Peptidoglycan
	Cell wall from Gram-positive bacteria	Lipoteichoic acid
Pore-forming exotoxins	*Staphylococcus aureus*	a-Hemolysin
	Streptococcus pyogenes	Streptolysin-O
	Escherichia coli	*E. coli* hemolysin
	Aeromonas spp.	Aerolysin
Superantigens	*Staphylococcus aureus*	Toxic shock syndrome toxin 1, enterotoxins A–F
	Streptococcus pyogenes	SPEA, SPEC, SMEZ
Enzymes	*Streptococcus pyogenes*	Interleukin-1ß convertase, proteases
	Clostridium perfringens	Phospholipase C
LPS, lipopolysaccharide		

As with other aspects of infectious diseases, the occurrence of sepsis is due to the combination of host and environmental factors. Thus the organisms responsible will vary geographically; for example, melioidosis due to *Burkholderia pseudomallei* is a common cause of community-acquired sepsis in South East Asia. Also there are large differences between specific patient groups, with certain pathogens occurring more frequently in vulnerable hosts, for example pneumococcal sepsis in asplenic patients.

Bacterial products involved in sepsis

Bacteria produce a variety of extracellular products (exotoxins) and endogenous cell wall products that have the capacity to induce proinflammatory responses in vitro that may culminate in sepsis in vivo (Table 56.2). Such products are released by bacteria during local or systemic infection and, in turn, may have local or distant proinflammatory effects. Thus, it is possible for a localized infection to induce the full inflammatory response leading to sepsis without the infecting organism itself actually disseminating. The most commonly recognized bacterial products are outlined in Table 56.2 (there are undoubtedly numerous others yet to be discovered or defined) and it is important to recognize that they may be additive or synergistic in their proinflammatory actions. For two bacterial toxins, namely endotoxin and toxic shock syndrome toxin 1 (TSST-1), considerable progress has been made in understanding their molecular and cellular interactions.

Cellular activation by endotoxin

The central role of endotoxin — lipopolysaccharide (LPS) — in the pathogenesis has been recognized for many years.[18] LPS is found only in Gram-negative bacteria, where it forms part of the outer leaflet of the bacterial cell wall (Fig. 56.3). It consists of a polysaccharide domain covalently bound to a unique diglucosamine-based phospholipid, lipid A, which is the key toxic moiety of LPS. Lipid A and LPS induce a wide range of both proinflammatory and counter-regulatory responses in vitro and in vivo. In human volunteers, administration of small amounts of endotoxin induces fever and release of cytokines including tumor necrosis factor (TNF)-a, interleukin (IL)-1 and IL-6.[19] That LPS alone can initiate severe sepsis in humans was reported in dramatic style in 1993 when a laboratory worker developed septic shock after deliberate self-administration of *Salmonella minnesota* endotoxin.[20]

So how does this lipid exert such widespread effects on a variety of cell types? The interaction of LPS with cells is complex, involving both serum LPS-binding proteins and cell-bound and soluble LPS

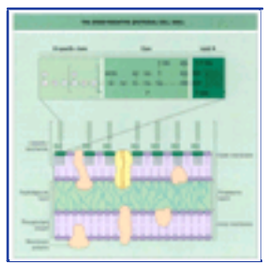

Figure 56-3 The Gram-negative bacterial cell wall. In the cell wall of a Gram-negative bacteria such as *Escherichia coli*, the inner membrane is composed of phospholipids and membrane proteins and is separated from the outer membrane by the periplasmic space and peptidoglycan. Lipopolysaccharide (expanded box) is found only in the outermost leaflet of the outer membrane with the lipid A moiety in the membrane and the polysaccharide (O) side chain directed outwards. Lipid A is highly conserved across Gram-negative bacteria and consists of a phosphorylated diglucosamine backbone decorated with six or seven acyl side chains. Dephosphorylation or deacylation of lipid A abrogates its toxicity. Lipid A is covalently linked to an inner core of sugar residues that is relatively well conserved across species and antibodies directed against the core may protect against challenge with heterologous Gram-negative bacilli. The core is followed by an outer polysaccharide chain, of repeating sugar residues, that varies between different of bacterial strains (O antigen). Antibodies directed against the O antigen will only protect against challenge with that individual bacterial strain. Gal, D-galactose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; KDO, 3-deoxy-D-manno-octuylosonic acid; Hep, L-glycero-D-mannoheptose.

receptors.[21] [22] When released from bacteria, LPS interacts with serum proteins that may either enhance or negate its bioactivity. One of these, LPS-binding protein (LBP) binds LPS with high affinity and presents it to LPS acceptor molecules including CD14, a glycosphatidyl-inositol (GPI)-anchored protein on the surface of monocyte-macrophages and neutrophils as shown in Fig 56.4 . CD14 is also present in soluble form in human serum and can complex with LPS and LBP to activate cell-types that do not express surface CD14, such as endothelial cells. CD14 does not have a transmembrane domain and LPS passes from CD14 to one of the toll receptor family, namely TLR4, which has an intracellular signalling domain with homology to the IL-1 receptor.[23] Following receptor binding there is rapid activation of a number of second messenger pathways including mitogen activated protein (MAP) kinases leading to translocation of nuclear factor-kappa B (NF-?B) to the nucleus and activation of many genes. Several other LPS receptors have been identified including the leukocyte integrin CD11d/CD18, the macrophage scavenger receptor and most recently triggering receptor expressed on myeloid cells (TREM-1).[22] The inter-relationships of these different LPS response pathways and their relation to the cellular and physiologic processes in sepsis remain to be elucidated.

The toll receptor family are worthy of further comment regarding their role in responding to infection and the development of sepsis.[24] Toll receptors were first identified as important in the recognition of bacteria in *Drosophila* spp. Toll receptors are found in widely diverse organisms, including plants, animals and insects, where they play a key role in innate immunity. Thus far in man ten toll-like receptors (TLR) have been identified that bind bacterial, viral and fungal products as follows:

- TLR2: peptidoglycan, and lipoteichoic acid and some LPS,
- TLR3: dsRNA, (viruses)
- TLR4: LPS
- TLR5: flagellin
- TLR6: lipoteichoic acid
- TLR9: CpG (deoxycytidylate-phosphate-deoxyguanylate) unmethylated bacterial DNA.

The role of toll receptors and other arms of the innate immune response in host responses to infection is likely to provide further insights into the pathophysiology of severe sepsis.

Superantigens and toxic shock

Superantigenic bacterial toxins can cause profound hypotension, organ failure and inflammation.[25] It is postulated that strains of *Staphylococcus aureus* and *Streptococcus pyogenes* that are able to express these toxins (see Table 56.2) are the causal agents of staphylococcal and streptococcal toxic shock. The principle behind immune activation by superantigens is outlined in Figure 56.5 . Essentially, the bacterial toxin is capable of bypassing the normal highly antigen-specific mechanisms of T-cell activation by directly linking major histocompatability complex (MHC) II on antigen-presenting cells to the Vß subunit of the T-cell receptor. Whether an individual infected with a superantigen-producing organism develops toxic shock is complex, depending on the quantity of toxin produced, the presence or absence of neutralizing antitoxin antibodies and the composition of an individual's MHC molecules and T-cell repertoire of Vß subunits. The interaction of these factors was clearly demonstrated in outbreaks of staphylococcal toxic shock epidemiologically linked to tampon use in the USA in the 1970s. The use of a particular type of

Figure 56-4 CD14 and toll receptor pathway of cellular activation by bacterial lipopolysaccharide. Schematic representation of events at the inflammatory cell surface. Lipopolysaccharide (LPS), either free or as part of lipoprotein complexes, is bound by LPS-binding protein (LBP) in the fluid phase. The LPS-LBP complex binds to the cell surface receptor CD14 on neutrophils and macrophages. CD14 lacks an intracellular domain and acts as a co-receptor presenting LPS to toll-like receptor 4 (TLR4), leading in turn to activation of intracellular signalling and gene activation. MYD88, myeloid differentiation factor 88; NF?B, nuclear factor kappa B; I?B, inhibitor of kappa B; IRAK, interlkeukin 1 receptor-associated kinase; TRAF6, tumour necrosis factor receptor-associated factor 6; MAP3K, mitogen-activated protein 3-kinase

superabsorbent tampon allowed proliferation and increased toxin production by staphylococci in the vagina, leading to a marked increase in cases of menstruation-associated cases of toxic shock syndrome.[26]

Role of specific proinflammatory mediators in sepsis

As already alluded to, severe sepsis is the result of a complex interaction of environmental, microbial and genetic factors. Central to the development of shock and organ failure is the concept that severe sepsis is the end-result of an excess proinflammatory response to infection. Numerous mediators and inflammatory pathways have been described, some of which are depicted in Figures 56.6 and Table 56.3 . It is important to remember that any attempt to consider one mediator in isolation is a simplification and many of the proinflammatory mediators have additive or synergistic effects.

Cytokines

The evidence that proinflammatory cytokines such as TNF-a and IL-1 play a causative role in sepsis stems from several lines of research. First, IL-1 and TNF-a can induce features of septic shock in experimental animals, and inhibitors of these cytokines can prevent the onset of sepsis in these models. Furthermore, mice deficient in the genes encoding receptors for TNF-a are resistant to septic shock induced by endotoxin. Finally, these cytokines are elevated in human sepsis, and high levels of cytokines correlate with a poor outcome. TNF-a is released early in response to LPS challenge, and the cooperative effects of TNF-a, IL-1 and interferon (IFN)-? in producing inflammatory responses have made these three cytokines prime targets for experimental intervention in sepsis.[27]

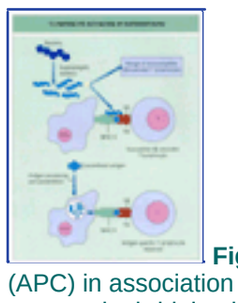

Figure 56-5 T-cell activation by superantigens. In the conventional response to bacterial antigens (bottom) the antigen is processed and presented by the antigen-presenting cell (APC) in association with MHC II. Only T cells with the correct antigen recognition site can then be activated (i.e. this is a highly antigen-specific process). Superantigens (top) are able to bypass this process by bridging between MHC II and the Vß subunit of the T-cell receptor. Thus the entire population of T cells expressing that particular Vß subunit can be activated; this can be up to 20% of the

total T-cell population. *Adapted from Sriskandan and Cohen. The pathogenesis of septic shock. J Infect 1995;30:201–6.*

Arachidonic acid metabolites

Thromboxane A_2 and prostaglandins are released via the cyclo-oxygenase pathway and leukotrienes through the action of lipoxygenases. LPS and other mediators can activate increased synthesis and release of cyclo-oxygenase and lipoxygenase products in sepsis.[28] Broadly speaking, prostaglandins (PGs), particularly PGE_1 and PGI_2 , have beneficial effects in sepsis by reducing procoagulant activity and improving tissue perfusion. Thromboxane and leukotrienes, however, are largely deleterious and have been particularly implicated in the pathogenesis of adult respiratory distress syndrome (ARDS). In the complicated setting of septic shock it is difficult to assess the contribution of individual arachidonic acid metabolites to the overall clinical picture.

Platelet-activating factor

Platelet-activating factor (PAF) is a potent phospholipid mediator produced by a variety of cell types — monocytes, neutrophils, platelets and endothelial cells — during severe sepsis. It enhances neutrophil chemotaxis, primes neutrophils for superoxide release and increases thromboxane synthesis in the lung. In animal models, infusion

TABLE 56-3 -- Important mediators in sepsis.

IMPORTANT MEDIATORS IN SEPSIS		
Target	**Proinflammatory mediators**	**Inhibitory or counter-regulatory mediators**
Monocyte/macrophage	TNF-a, IL-1, IL-8, IL-6, IL-12, IFN-?, tissue factor, prostanoids, leukotrienes, PAF, NO	IL-1ra, sTNFr, TGF-ß
Neutrophils	Integrin expression, superoxide production	BPI, CAP 57, defensins, acyloxyacylhydolase
Lymphocytes	IFN-?, IFN-ß, IL-2	IL-4, IL-10, sIL2r
Endothelia	Selectins, VCAM, ICAM, NO, tissue factor	TFPI
Platelets	Serotonin, prostanoids	PDGF
Coagulation pathway	Tissue factor, coagulation cascade leading to thrombin generation	TFPI, AT-III, PAI
Plasma components	Complement activation, bradykinin	Lipoprotein complexes, LBP, CRP

AT-III, anti-thrombin III	PAF, platelet-activating factor
BPI, bacterial/permeability-increasing protein	PAI, plasminogen activator inhibitor
CRP, C-reactive protein	PDGF, platelet-derived growth factor
ICAM, intercellular adhesion molecule	sIL-2r, soluble IL-2 receptor
IFN-?, interferon-?	sTNFr, soluble TNF receptor
IL, interleukin	TGF-ß, transforming growth factor ß
IL-1ra, interleukin-1 receptor antagonist	TNF-a, tumor necrosis factor a
LBP, lipopolysaccharide-binding protein	TFPI, tissue factor pathway inhibitor
NO, nitric oxide	VCAM, vascular cell adhesion molecule

Figure 56-6 Interaction of inflammatory pathways in severe sepsis. ARDS, adult respiratory distress syndrome; IFN-?, interferon-?; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; LBP, LPS-binding protein; LPS, lipopolysaccharide; NO, nitric oxide; TGF-ß, transforming growth factor-ß; sTNFr, soluble TNF receptor.

of PAF leads to a sepsis-like state, and in particular can cause the pathophysiologic changes of ARDS. Increased levels of PAF can be detected in sepsis, and experimental strategies directed against PAF have been developed.[28]

Complement

The complement system exists to provide an immediate and non-specific host response to a variety of invading pathogens. Activation of complement leads to a proteolytic cascade resulting in the liberation of molecules that can act as opsonins, chemoattractants and also mediate bacterial lysis. LPS and other bacterial products activate complement by the alternative pathway. Massive local activation of complement during sepsis will fuel the inflammatory response by recruiting neutrophils, and through the activation of kinins and histamine will contribute to increased endothelial permeability and capillary leakage.[29] In addition, complement-mediated lysis of bacteria, by releasing endotoxin and other bacterial products, can exacerbate the septic response to infection.[30]

Coagulopathy

Widespread activation of the coagulation pathways leading to disseminated intravascular coagulation (DIC) is a serious consequence of severe sepsis.[31] Endotoxin and other bacterial products directly activate components of the coagulation system and also indirectly activate coagulation via activation of tissue factor on endothelial surfaces and release of kinins and proinflammatory cytokines. In addition to the direct effects of DIC, the widespread activation of coagulation factors and damage to the microvasculature further impairs endothelial function and exacerbates capillary leak. The development of coagulopathy and endothelial dysfunction is recognized as central to tissue damage and organ failure in severe sepsis.

Nitric oxide

Nitric oxide (NO) may be involved in the pathophysiology of sepsis through a number of mechanisms.[32] First, NO is a free radical and has the ability to kill phagocytosed organisms in rodents, although the importance of this action in humans is not clear. Second, NO is a potent vasodilator and a basal level of NO is required for the maintenance of normal arteriolar tone. Synthesis of NO is increased by a number of bacterial products, including LPS and cytokines. In particular, TNF-a, IL-1 and IFN-? appear to increase NO synthesis synergistically. Sepsis is characterized by widespread peripheral vasodilatation and loss of the normal regulation of tissue blood flow. There is considerable experimental evidence in animals to suggest that the vasodilatation in sepsis is mediated by increased NO levels. In humans, elevated urinary nitrite levels are found in sepsis/SIRS, implying increased NO synthesis.

Neutrophil-endothelial cell interactions

An early response to the local production of inflammatory mediators is the upregulation of adhesion molecules on endothelial cells and the release of chemokines that recruit neutrophils and other inflammatory cells to the site of infection. This is a key event in the successful containment of infection; for example, patients with leukocyte adhesion deficiency have neutrophils that cannot cross the endothelium and suffer from recurrent bacterial infection. Exposure of neutrophils to LPS and

some cytokines enhances the ability of neutrophils to release inflammatory molecules and primes neutrophils for the generation of oxygen radicals and proteolytic enzymes. These mechanisms enable neutrophils to survive longer in an inflammatory environment and to kill more bacteria, but excessive activation of endothelial adhesion may result in the migration of primed activated neutrophils into sites distant from infection. The ensuring tissue damage stimulates a vicious cycle of re-recruitment of inflammatory cells that may ultimately lead to organ damage and failure.

Endogenous anti-inflammatory mediators in sepsis

In addition to the large number of proinflammatory mediators induced during severe sepsis a growing number of counter-regulatory mediators have been discovered (Fig. 56.6 and Table 56.3). They have specific actions and may neutralize or detoxify bacterial products, block the synthesis or release of cytokines, or downregulate cell responses to inflammatory stimuli. Indeed, the notion that sepsis resulted from an uncontrolled pro-inflammatory 'storm' has given way to the recogition that later in the disease it is anti-inflammatory response that dominates. Indeed, some investigators believe that septic patients become effectively 'immunocompromised' and that immunostimulatory therapy is indicated.[33]

Interaction of mediators in sepsis

As already discussed there is no single central mediator, but rather a complex network of inflammatory responses to infection, which can in certain circumstances result in sepsis as depicted in Figure 56.6 . Once activated, these mediators induce further inflammatory responses in what has been referred to as the 'sepsis cascade', leading to tissue damage and eventually death. However, these same inflammatory processes trigger another complicated network of counter-regulatory or anti-inflammatory mediators, such as the IL receptor antagonist (IL-1ra) and soluble TNF receptors (sTNFr). Thus, it is the balance between these opposing mechanisms that will determine whether inflammation is successful in eliminating infection and then 'shutting down', or progressing so that healthy tissues become victims of 'friendly fire'. The complexity of this interaction can be seen by examining cytokine patterns in meningococcal disease. The risk of death in meningococcal disease is closely related to cytokine levels at presentation and those with the highest TNF-a levels do poorly. Thus one could hypothesize that people who produce less TNF-a have a survival advantage. However, an elegant study that examined whole blood responses measuring TNF-a (proinflammatory) and IL-10 (anti-inflammatory) release in response to meningococcal LPS in the relatives of survivors and nonsurvivors of meningococcal disease,[34] found that the relatives of survivors had a high TNF-a/IL10 ratio in comparison to the relatives on the nonsurvivors. Therefore maintaining a strong proinflammatory response appears to protect against overwhelming meningococcal infection.

Pathophysiologic events leading to organ failure in sepsis

How do the complicated and often confusing inflammatory responses outlined above lead to tissue damage and eventually multiorgan failure? At the cellular level, the end-result of the inflammatory process is cell death and there is increasing evidence that apoptosis is an important pathological event.[33] In the whole organism the key to organ damage lies in the breakdown of normal vascular homeostasis, leading to tissue hypoxia as described in Figure 56.7 . All organs may be affected, but clinically the most important are depressed myocardial function, alterations in the peripheral vasculature and failure of oxygen exchange in the lung.

Cardiac function

Initially when peripheral vasodilatation occurs in sepsis there is a compensatory rise in cardiac output (Table 56.4). However, the increase in cardiac output is often less than that predicted and it is recognized that most patients who have severe sepsis have impaired myocardial function. Cumulative evidence suggests that the impairment of myocardial contractility seen in sepsis is a consequence of circulating cytokines. TNF-a directly reduces myocyte contractility

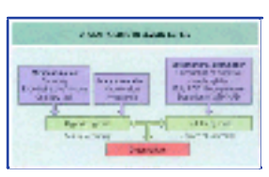

Figure 56-7 Organ failure in severe sepsis. Organ dysfunction and finally organ failure is the result of cellular hypoxia and acidosis. Hypoxia and acidosis result both from a failure of oxygen delivery due to disturbances in circulation and limitation of the cellular ability to use oxygen as a result of mitochondrial dysfunction.

TABLE 56-4 -- Hemodynamic changes in severe sepsis.

HEMODYNAMIC CHANGES IN SEVERE SEPSIS		
Parameter	**Normal range**	**Changes in severe sepsis**
Heart rate (HR)	72–88 beats/min	Sinus tachycardia
Mean arterial pressure (MAP)	70–105mmHg	Hypotension <60mmHg
Cardiac output (CO)	4–8L/min	Increased but not enough to compensate for low SVR
Systemic vascular resistance (SVR)	800–1500dyne/s/cm^2	Reduced (<600 if no pressor agents)
Oxygen delivery (DO_2)	520–720ml/min/m^2	Decreased
Oxygen consumption (VO_2)	100–180ml/min/m^2	Typically increased
CO = SV × HR, SVR = (MAP - CVP)/CO × 79.92		
Do_2 = CI × arterial oxygen × 10, Vo_2 = CI × (arterial - venous oxygen) × 10		
CI, cardiac index (CO/m^2 surface area); CVP, central venous pressure; SV, stroke volume.		

and induction of myocardial NO synthesis by cytokines also impairs myocardial function. Recent data suggest that elevated troponin levels occur in some patients with severe sepsis, indicating that direct myocardial damage may be a factor.[35]

Peripheral vasculature

The hallmark of severe sepsis is widespread peripheral vasodilatation, and, in refractory septic shock it may resist all attempts at pharmacologic intervention. As mentioned earlier, this is largely the consequence of increased NO production in response to cytokines. The most serious consequence of the vasodilatation is the loss of homeostatic regulation of tissue bloodflow. Thus, much of the circulating blood volume is shunted through capillary beds, bypassing deep tissues and reducing the opportunity for oxygen extraction. This in turn exacerbates tissue hypoxia and helps to drive the metabolic acidosis that is one of the prominent features of severe sepsis. Further local impairment of perfusion may occur as the result of aggregation of platelets and activation of coagulation in the microcirculation.

Respiratory failure

The lung is one of the most vulnerable organs in sepsis, and TNF-a, PAF, C5a, IL-8 and thromboxane appear to play prominent roles in the development of ARDS. The passage of neutrophils across the endothelium and subsequent degranulation in the lung interstitium leads to impairment of endothelial integrity and the accumulation of fluid and inflammatory cells in the alveolar spaces. Impaired gas exchange inevitably produces hypoxia, which further worsens the situation. The combination of hypoxia and increased pulmonary thromboxane synthesis increases pulmonary vascular resistance, in contrast to the fall in systemic vascular resistance. Thus the end-result of the pulmonary events in sepsis is the development of interstitial edema and pulmonary hypertension, the hallmarks of ARDS.

PREVENTION

The morbidity, mortality rate and hospital costs associated with sepsis demand that strenuous efforts must be made to reduce the incidence of this serious disease. These can be divided into three main areas:

¦ assiduous infection control for high risk patients;
¦ the use of prophylactic antibiotic therapies; and
¦ prompt recognition and treatment of infection

Details of infection control policies are outlined elsewhere (see Chapter 87), but it is worth re-emphasizing that many cases of sepsis are acquired in hospital, particularly in the ICU, and that nosocomial sepsis has a much higher mortality rate than community-acquired sepsis. It has been estimated that up to one-third of cases of nosocomial infections are preventable and a multidisciplinary approach to infection control can lead to significant reductions in ICU pneumonia and sepsis.

Antibiotic prophylaxis has a limited role in sepsis prevention, but it is important to recognize patients who have risk factors for developing sepsis, for example manipulation of the urinary tract in the presence of active infection, and to administer appropriate antimicrobial therapy. Principles of antibiotic prophylaxis are covered elsewhere (see Chapter 3).

Even where infection cannot be prevented it may still be possible to minimize the mortality rate associated with severe sepsis through the early recognition and management of patients. Intervening once refractory shock and multiorgan failure are established is simply too late for may patients.

CLINICAL FEATURES

A rigorous history, physical examination and directed investigations are essential to arrive at the correct diagnosis of any infectious disease. However, severe sepsis and shock is a medical emergency and therefore full assessment may have to be delayed until resuscitation and empiric antimicrobial therapy have been commenced. The aims of clinical evaluation are to establish the diagnosis of sepsis, estimate disease severity and prognosis, and elucidate the underlying cause. Although sepsis has many features common to most cases, the exact presentation will depend on the site of infection, the nature of the infecting organism, the host response and coexistent illness. Therefore, in the elderly and other immunocompromised groups the physical signs and some of the laboratory parameters that suggest sepsis may be absent; in this group it is important to consider sepsis in the differential diagnosis of any unexplained illness.

History and symptomatology

Symptoms that suggest the onset of sepsis are often nonspecific and include sweats, chills or rigors, breathlessness, nausea and vomiting or diarrhea, and headache. Confusion may be found in 10–30% of patients, especially the elderly.[36] There may be specific symptoms to suggest the underlying pathology, such as cough, dysuria or meningism, but in many cases there are no clues. Other important points to elicit that may give an indication of the diagnosis or help in choosing empiric therapy include recent travel, contact with animals, local infectious disease outbreaks, recent surgical procedures, indwelling prosthetic devices, previous antibiotics, underlying pathology and immunosuppressive illness or medication (see Fig 56.1). For the critically ill patient such details are often unavailable, and it may be helpful to speak to relatives or the patient's primary care physician.

Physical signs on examination

In its simplest form sepsis simply refers to the physiologic response to infection. With progression to severe sepsis and shock there is increasing evidence of organ dysfunction; the key physical signs and physiologic changes indicating this are encapsulated in Table 56.1 . Remember that sepsis is a dynamic evolving clinical picture and frequent evaluation and monitoring of the patient is essential.

The characteristic patient is febrile, tachypneic, tachycardic with warm peripheries and a bounding arterial pulse, hypotensive, disoriented

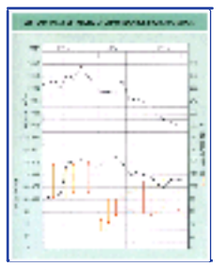

Figure 56-8 Systemic manifestations of Gram-negative bacterial sepsis. Observation chart of a 49-year-old woman admitted to hospital with a suspected drug fever. For the first 24 hours she was observed without antimicrobial chemotherapy and demonstrated a persistent fever and tachycardia (sepsis). At this point she suddenly became confused, hypotensive and oliguric indicating the development of severe sepsis. The underlying cause was an *Escherichia coli* bacteremia from an unsuspected urinary tract infection. She responded to fluid replacement and antibiotics and made a full recovery.

Figure 56-9 Toxic shock syndrome. A 30-year-old woman presented with a short history of fever, breathlessness, diarrhea and abdominal pain. She was pyrexial and hypotensive with a blanching macular rash noted on day 2. Blood cultures grew a Group A ß-hemolytic streptococcus. Laparotomy revealed 800ml of peritoneal pus with no intestinal perforation consistent with spontaneous bacterial peritonitis. She required ventilatory support plus inotropes to maintain blood pressure for 6 days and developed evidence of disseminated intravascular coagulation (DIC) and adult respiratory distress syndrome (ARDS). She made a gradual recovery complicated by an enterococcal urinary tract infection, finally leaving hospital 27 days after presentation. The group A streptococcal isolate from her blood was subsequently shown to release streptococcal mitogenic exotoxin Z (SMEZ) a potent superantigen. *Courtesy of P Gothard and S Sriskandan, Hammersmith Hospital, London.*

and oliguric. The observation chart of one such patient is shown in Figure 56.8 and the clinical course of another in Figure 56.9 . Some patients have a much more subtle presentation necessitating a high index of suspicion to recognize early disease. Although most patients are febrile, severe sepsis may present with hypothermia. A detailed physical examination is vital and it is important to examine the skin, all wounds and the fundi, and to perform full ear, nose and throat, and rectal and vaginal examinations because these sites are often overlooked and may hold valuable clues to the diagnosis (e.g. a retained vaginal tampon in the toxic shock syndrome). In the hospitalized patient and the ICU patient who develops sepsis, it is necessary to pay particular attention to indwelling intravenous and intra-arterial lines, insist on exposing and examining all wounds, and carefully review the pressure areas because these are frequently neglected sources of infection.

Occasionally, the physical examination will directly establish the diagnosis; helpful signs include the purpuric rash or peripheral gangrene of meningococcemia (Fig. 56.10a), peripheral emboli in endocarditis (Fig. 56.10b), the erythematous rash or desquamation in staphylococcal or streptococcal toxic shock (Figs. 56.11), ecthyma gangrenosum in patients who have neutropenia and *P. aeruginosa* bacteremia, or the retinal lesions of *Candida* endophthalmitis. Focal physical signs may help to identify the site of infection, for example renal angle tenderness, pulmonary consolidation, new cardiac murmur or finding an intra-abdominal mass, but in many cases the site of the infection remains uncertain.

If the patient is hypotensive, other causes of shock such as cardiac dysfunction (including myocardial infarction and cardiac tamponade), hypovolemia and redistributive shock from pancreatitis and physical injuries need to be considered. Invasive physiologic measurements are often used to distinguish between these (see below), but it is important to remember that the hypotension seen in sepsis is often multifactorial and sepsis may complicate or coexist with other causes of shock. Thus such invasive measurements must not be interpreted in isolation, but should be reviewed in the context of the whole clinical picture.

Figure 56-10 Cutaneous changes in meningococcal infection. (a) Petechial hemorrhages are the hallmark of meningococcal infection and may be found on the periphery or

as in this case the conjunctivae. (b) In severe disease the purpura may become confluent (purpura fulminans) and lead to severe digital gangrene. *Courtesy of J Cohen, Brighton.*

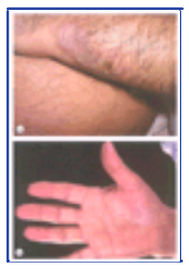
Figure 56-11 Cutaneous changes in toxic shock. (a) Localized infection at the edge of a patch of eczema in a patient presenting with staphylococcal toxic shock syndrome. (b) Desquamation of the palm following an episode of staphylococcal toxic shock. *Courtesy of M Jacobs, London.*

DIAGNOSIS

Laboratory investigations

Laboratory investigations can be broadly divided into those that help to confirm that the patient has sepsis and detect and follow complications such as organ failure, and those that establish the underlying cause.

Hematologic and biochemical evaluation in sepsis

Full blood count and blood film

Look for leukopenia or a neutrophil leukocytosis. The blood film may suggest bacterial infection with toxic granulation of neutrophils (increased band forms) even when the white cell count is within the normal range. Leukopenia at presentation is a poor prognostic sign. Low platelet count suggests DIC and evidence of microangiopathic hemolysis may be visible. In patients who have traveled to an endemic area, three separate thick and thin films for malaria parasites are required.

Coagulation screen

Look for evidence of DIC — prolonged prothrombin or activated partial thromboplastin time, low fibrinogen and elevated markers of fibrinolysis (fibrin degradation products or D-dimer levels).

Electrolytes and renal function

It is essential to monitor renal function closely. Elevated potassium may indicate rhabdomyolysis, which can occasionally complicate severe sepsis.

Liver function tests

Minor abnormalities are very common in patients who have bacteremia and sepsis, and do not necessarily signify a hepatic source of infection. Rises in bilirubin, alkaline phosphatase or transaminases of two to three times the normal level may be seen in up to 30–50% of patients.[37] These are generally transient and not of prognostic importance. More markedly abnormal liver function suggests the possibility of underlying hepatic or biliary tract infection, particularly if the pattern of liver enzymes suggests biliary obstruction. Progressively deteriorating liver function in patients who have sepsis suggests hepatocellular damage or acalculous cholecystitis, which may occur as complications of severe sepsis.

Plasma albumin

In patients who have severe sepsis an acute fall in albumin, to as low as 1.5–2.0g/dl (15–20g/l) over 24 hours, may occur as a result of widespread endothelial damage and capillary leakage of protein. In patients who have chronic underlying illness or prolonged infection, the albumin falls as a result of poor nutrition and a switch in hepatic metabolism towards acute-phase proteins.

C-reactive protein

C-reactive protein (CRP) is an acute-phase reactant that rises within a few hours of bacterial infection. High levels are detectable in patients who have septic shock although a raised CRP does not reliably distinguish septic from nonseptic causes of shock. Procalcitonin levels are also elevated in bacterial infections and may be a more sensitive marker than CRP and are preferred in some centers.

Blood glucose

Hypoglycemia or hyperglycemia may occur in severe sepsis. In patients who are diabetic, normalization of blood glucose is essential in helping to control the infection.

Plasma lactate

Plasma lactate is often increased three- to fivefold in severe sepsis (normal 1.0–2.5mmol/l) and relates to the degree of tissue hypoxia. Lactate estimations may be helpful in both confirming the diagnosis and in monitoring the response to therapy.

Arterial blood gases

Typically a respiratory alkalosis is seen early and metabolic acidosis late. The degree of acidosis is a marker of the severity of illness. The onset of hypoxia indicates severe disease and a high risk of ARDS. Measurement of venous and arterial oxygen content allows calculation of oxygen delivery and consumption (see below).

Other biochemical investigations

Amylase, creatinine phosphokinase, calcium and magnesium levels should be measured at baseline.

Endotoxin or cytokine levels

In some but not all studies plasma levels of endotoxin and cytokines such as TNF-a and IL-6 have correlated with outcome of Gramnegative sepsis. [38] [39] Variation in reported data along with technical difficulties in performing these assays in real time makes routine testing impractical at present.

Microbiologic investigations in sepsis

Ideally, cultures should be taken before initiating antibiotic therapy, but treatment is urgent and should not be unduly delayed.

Blood cultures are the most important microbiologic investigation. Two or three separate blood cultures (total 20–30ml of blood) should be inoculated into one of the standard commercial blood culture media. Culturing an inadequate volume of blood is the most common reason for not detecting bacteremia.[40] The bottles should be incubated aerobically and anaerobically at 98°F (37°C), preferably agitated, for 7 days. The development of automated blood culture analysis allows for more rapid

identification of positive cultures. Where there is a high risk of underlying fungal infection then specific fungal media may be helpful.

Sputum and urine microscopy and culture should be performed in all cases. All wounds should be swabbed and sterile body sites such as cerebrospinal fluid (CSF), joint or pleural fluid sampled as indicated. In cases in which toxic shock syndrome is suspected, wound, nose, throat and vaginal swabs should be taken. If staphylococci or streptococci are isolated in cases of toxic shock then the appropriate reference laboratories can assay the isolate for toxin production. Cultures should be repeated as directed by previous results and the condition of the patient.

Histopathologic changes in sepsis

Organ pathology in sepsis is due to the combined effects of hypoxia, impairment in tissue perfusion and severe acidosis. In the lung the changes are those of ARDS, with early findings of interstitial and alveolar edema, fibrosis developing at a later stage. The kidneys may show acute tubular necrosis that is generally reversible (Fig. 56.12). Hepatic changes are of an ischemic zonal necrosis and some cases may develop an acalculous cholecystitis. In the brain there may be areas of focal ischemia or hemorrhage. In severe cases of septic shock associated with *Neisseria meningitidis* infection there may be peripheral gangrene due to severe impairment of perfusion (see Fig. 56.10) and hemorrhage into the adrenal glands (Fig. 56.13). The gut is also affected by ischemia and may show mucosal ulcerations and areas of infarction.

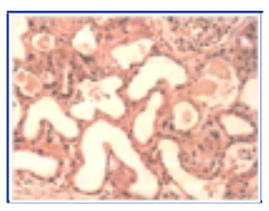

Figure 56-12 Acute tubular necrosis. The tubules are dilated with flattened epilithial cells, and contain debris; the glomerulus is not greatly affected. Hematoxylin and eosin. *With permission from Williams JD et al., Clinical Atlas of the Kidney. London: Mosby.*

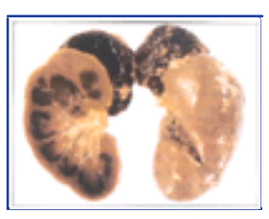

Figure 56-13 Acute hemorrhagic necrosis of the adrenal glands (Waterhouse-Friderichsen syndrome). Both adrenal glands of a child with meningococcal septicemia show hemorrhagic necrosis leading to acute adrenal failure. *With permission from Stevens A, Lowe J. Pathology. London: Mosby; 1995.*

Figure 56-14 Radiologic detection of occult foci of infection in severe sepsis. Plain abdominal radiograph in a diabetic with an *Escherichia coli* urinary tract infection and pyelonephritis who had developed hypotension and oliguria. The radiograph reveals gas around the kidney due to a perinephric abscess requiring urgent drainage.

Radiologic investigations

Chest radiography is necessary in all cases and may show signs of ARDS other studies may be indicated. Radiologic investigations can be of great help in identifying occult sites of infection (Fig 56.14), and close liaison with the diagnostic radiology department is vital. Ultrasound and computed tomography are particularly useful when trying to detect deep abscesses; in selected cases, nuclear medicine techniques may pinpoint the site of infection. Access to an interventional radiologist who can perform percutaneous sampling and drainage of sites of infection is invaluable.

MANAGEMENT

The mortality rate from sepsis and multiorgan failure remains high, but by early recognition and prompt therapeutic intervention patients can be prevented from progressing down this path. Guidelines on the management of severe sepsis and septic shock have been produced by the International Sepsis Forum.[41]

There are four therapeutic goals:

- to treat underlying infection;
- to preserve vital organ perfusion;
- to maintain tissue oxygenation; and
- to avoid complications.

Antibiotic chemotherapy

Studies of patients who have sepsis have identified the use of inappropriate empiric antibiotics (i.e. the infecting organisms were resistant to the antibiotics used) as being associated with a higher mortality rate.[42] In general it is accepted that the regimen used in severe sepsis should be bactericidal rather that bacteriostatic. There has been considerable debate regarding the use of monotherapy or combination therapy regimens in a number of infections, including sepsis. However, in studies in which appropriate empiric therapy has been employed it has been hard to demonstrate that any particular antibiotic or combination of antibiotics is superior. One exception may be in sepsis associated with necrotizing fasciitis due to streptococci where there are data to support the use of clindamycin in addition to penicillin.[25]

TABLE 56-5 -- Possible empiric antibiotic choice in severe sepsis.

POSSIBLE EMPIRIC ANTIBIOTIC CHOICE IN SEVERE SEPSIS	
Suspected site of infection	**Antibiotic**
Pneumonia	
Community-acquired	Cefotaxime + erythromycin
Hospital-acquired	Cefotaxime/ceftazidime alone or ureidopenicillin + aminoglycoside
Urinary tract	
Community-acquired	Amoxicillin + clavalunic acid (co-amoxiclav) or cefotaxime
Hospital-acquired	Ceftazidime alone or ureidopenicillin + aminoglycoside
Skin and soft tissue	
Community-acquired	Penicillin G (benzylpencillin) + nafcillin (flucloxacillin)
Hospital-acquired	Cefotaxime + nafcillin or cefotaxime + vancomycin
Intra-abdominal	Cefotaxime + metronidazole or ureidopenicillin + aminoglycoside or imipenem monotherapy
Biliary tract	Ureidopenicillin + aminoglycoside

Neutropenic	Ureidopenicillin + aminoglycoside or ceftazidime monotherapy

If the infecting organism has been identified and drug sensitivities determined, then the choice of antibiotics is comparatively easy. However, in most clinical situations empiric choice of appropriate antibiotic(s) is required. Table 56.5 provides a useful guide to antibiotic choice for the more common causes of sepsis, but it is not possible to recommend specific antibiotic regimens for all cases because of variations in infecting pathogens and antimicrobial resistance between hospitals. The decision regarding antibiotic choice should be made on the same principles as for any serious infection:

- clinical syndrome/likely microbiology;
- site of infection;
- risk of resistance based on local and national data;
- host factors including immunocompromise;
- comorbidities including organ function and drug allergy.

The antibiotic regimen should be reviewed every 24 hours in the light of the clinical situation and available microbiology data until the patient is clearly improving. There are few available data to give accurate guidance on the duration of therapy.

Antibiotic-induced endotoxin release.

Endotoxin is a major factor in the pathogenesis of shock due to Gram-negative bacteria. In experimental models, and some limited situations in humans, release of endotoxin from bacteria has been shown to increase the inflammatory response and different antibiotics vary in their capacity to induce endotoxin release from bacteria.[43] Thus, a school of thought has developed that antibiotics should be chosen on the basis of their potential to cause endotoxin release. However, beyond a handful of small studies there are few clinical data to support a major role for this hypothesis in severe sepsis. At present, antibiotics should be selected on the basis of efficacy for the specific clinical picture, rather than on the theoretic risk of endotoxin release.

Detection and removal of infected material

In addition to antimicrobial therapy it is essential to drain and remove all possible infective foci because these are often the cause

for treatment failure. Thus, abscesses should be drained, dead tissue resected and infected foreign material, such as an infected central venous catheter, removed. [44] The critically ill patient on intensive care may have repeated episodes of sepsis. Locating the underlying focus often requires considerable determination and patience and sometimes repeated discussions with surgical colleagues.

Supportive therapy

The central goal of supportive therapy is to try to maintain tissue oxygen delivery, a concept advanced by Shoemaker and others through the 1970s.[45] A full discussion of advanced techniques to preserve organ function in the critically ill patient is beyond the scope of this chapter, but the principles underpinning successful care are discussed here.[46] [47]

Ideally, patients who have sepsis should be closely monitored in an ICU or high-dependency unit. Indeed a review of 41 patients who had septic shock revealed an increased mortality rate when patients were managed outside an ICU, 70% versus 39%, even though the patients had less severe illness.[48] Minimal requirements for safe management include facilities for measurement of blood pressure, continuous cardiac monitoring, central venous pressure recording, rapid arterial blood gas analysis, continuous oxygen and facilities for assisted and mechanical ventilation or dialysis when required.

Invasive monitoring is useful in excluding other causes of shock such as hypovolemia and in directing supportive therapy. However, invasive monitoring is not a substitute for careful examination and assessment of the patient. Many factors such as cardiovascular disease, hypovolemia or inotropic drugs can confound such data; results must be interpreted in the context of the clinical situation. Normal values and the typical ranges for hemodynamic parameters in severe sepsis are given in Table 56.4 .

In the hemodynamic management of sepsis, the aim is to achieve sufficient arterial blood pressure for organ perfusion. This does not require normalization of blood pressure and generally can be achieved with a mean arterial pressure of 50–60mmHg. Sepsis is characterized by vasodilatation and widespread capillary leak and there is often significant depletion of intravascular volume, in which case correction of hypovolemia should be performed immediately. There is continuing debate about the ideal fluid replacement, and despite a number of systematic reviews this question is still unresolved.[49] Most clinicians use both colloid and crystalloid but avoid dextrans in sepsis because of an increased risk of bleeding. In practice, a balance must be kept between early aggressive fluid replacement, which is required to maintain tissue perfusion and organ function, and fluid overload, which may increase the risk of ARDS. Monitoring of central venous pressure and preferably pulmonary capillary wedge pressure is essential to achieve the correct balance. Pulmonary capillary wedge pressure measurements were routinely used but there has been some doubt over the safety and usefulness of pulmonary artery catheters in the critically ill patient.[50] As a result many ICUs have reduced their use of pulmonary catheters and monitor cardiac output and pulmonary artery pressure noninvasively. Blood transfusion is required in the event of hemorrhage or severe anemia (hemoglobin <9g/dl), but probably does not improve oxygen delivery in patients in whom hemoglobin is over 10g/dl.

If there is an inadequate response to volume replacement the use of inotropic/pressor agents is required. Impaired myocardial contractility responds to dobutamine (2–25µg/kg/min) or epinephrine (adrenaline, 2–8µg/min). However, it may be necessary to add pressor agents such as norepinephrine (noradrenaline, 2–8µg/min) or dopamine (2–25µg/kg/min) to raise peripheral resistance and increase blood pressure. No single regimen is effective for all patients and there is no consensus about the optimal combination or dosage of inotropic and pressor agents. Patients are best managed by repeated observation of response to different therapeutic measures.[47] Some patients develop resistance to catecholamines due to down-regulation of a and ß catecholamine receptors. Escalating concentrations of pressor agents are then needed to maintain blood pressure. In these patients low-dose corticosteroids may return catecholamine sensitivity. Vasopressin and terlipressin are also useful agents in this setting.

In established shock with multiorgan failure, poor perfusion and tissue oxygenation are the main factors. Patients require supplemental oxygen, and many need mechanical ventilation to maintain arterial oxygen saturation. Tissue hypoxia increases lactic acidosis, which further impairs cardiac and other functions. Experimental methods of extracorporeal oxygenation have been tried but none are in routine use.[51] Measurement of oxygen delivery (DO_2) and oxygen consumption (VO_2) (see Table 56.4) are employed in some ICUs to monitor the response to treatment. In severe sepsis VO_2 is increased and DO_2 is generally inadequate, which is often referred to as an 'oxygen debt'. If DO_2 and VO_2 are calculated then fluids, inotropes and oxygenation can be adjusted to attempt to achieve optimal oxygen delivery. Tissue hypoxia can also be measured directly using tonometry to follow gastric pH, by hepatic venous oxygen measurement and by muscle needle probes, but it is not clear whether routine use of these measurements will improve patient outcome.

The potential benefit of early aggressive supportive therapy is shown in a recent study by Rivers *et al.*[52] 263 patients with early sepsis seen in the emergency room were randomized to receive standard therapy or 'goal directed' therapy. Cardiac preload, afterload and contractility were actively managed to try and balance oxygen delivery with demand before admission to the ICU. The in-hospital mortality rate was reduced from 46.5% in the standard group to 30.5% in the goal-directed group ($p<0.009$). Further studies are required before these recommendations can be extended to other groups of septic patients.

Management of coagulopathy

Currently there is no universally accepted approach to the management of DIC in patients who have sepsis. For patients who have severe DIC, replacement of clotting factors with fresh frozen plasma or cryoprecipitate may be required to combat bleeding. However, it must be remembered that plasma also contains potentially pro-inflammatory components, such as complement, and that replacing the clotting factors may simply fuel the processes leading to DIC. Thus, patients should be treated with clotting factors not simply because of abnormal laboratory parameters but rather when there is a clear clinical indication.

Modulators of coagulation are protective in animal models of severe sepsis and some have entered human trials (Table 56.6). Of these recombinant activated protein C (rAPC) is most promising. In a phase III multicenter trial of rAPC versus placebo in 1690 patients with severe sepsis 28-day mortality rate was reduced from 30.8 to 24.7% ($p<0.005$). [53] This represents a relative reduction in risk of death of 19.4% and a number needed to treat to save one life of 16. Not surprisingly there was a

small but significant increase in serious bleeding in the active therapy group. On the basis of these results rAPC (drotrecogin alpha) was granted a limited product license for the treatment of severe sepsis in adults in the USA in early 2002. rAPC use is limited in most institutions to those with severe sepsis based on all of the following: a) known or suspected source of infection, b) evidence of a systemic response to infection and c) evidence of sepsis-related organ failure in two or more organ systems (using defination in Table 56.1). rAPC appears to have anti-inflammatory properties in addition to anticoagulant activity and the mechanism

TABLE 56-6 -- Adjunctive therapy for severe sepsis.

ADJUNCTIVE THERAPY FOR SEVERE SEPSIS	
Trial results	**Agents**
No benefit	Most anti-TNF monoclonal antibodies, soluble anti-TNF receptors, IL-1ra, pentoxyfylline
	Anti-LPS antibodies — ES, HA-1A
	Platelet factor antagonists, bradykinin antagonists, nonsteroidal anti-inflammatory drugs
	High-dose corticosteroids TFPI, AT-III
Possible benefit	Anti-LPS agents $rBPI_{21}$ in meningococcemia, E5331
	IVIG
	Anti-TNF MAb MAK 195f (Afelimomab)
	Plasma filtration, plasma exchange etc
Proven benefit	Insulin therapy
	rAPC
	Low-dose corticosteroids
AT-III, anti-thrombin III IL-1ra, interleukin-1 receptor antagonist IVIG, intravenous immunoglobulin LPS, lipopolysaccharide rAPC, recombinant activated protein C TFPI, tissue factor pathway inhibitor	
A multitude of approaches have been used to attempt to modify the course of sepsis and septic shock. The approaches listed above are those where sufficient human data are available to allow a judgment to be made.	

of protection in sepsis remains to be fully elucidated. The precise role for rAPC in patients with sepsis remains to be fully defined but it is likely that rAPC represents a major step towards effective immunotherapy.

Nutrition

Patients who have severe sepsis are extremely catabolic and it is very important to plan nutritional support and supplementation at an early stage. Unless there is a contraindication to oral feeding, nutrition should be given by the enteral route. Enteral feeding may help to protect the gut mucosa from ischemic damage in sepsis and to reduce bacterial translocation from the gut, which is thought to be involved in some episodes of sepsis. Parenteral nutrition increases the rate of secondary infections, particularly bacterial and fungal intravenous catheter infections, and so should be avoided if the gut is functional.

Corticosteroids in sepsis

There has been considerable controversy surrounding the use of corticosteroids in severe sepsis. In animal models pretreatment with corticosteroids protected against endotoxemia, and initial promising reports in humans lead to the widespread use of high-dose corticosteroids in sepsis. Subsequently two large multicenter trials failed to show benefit and meta-analyses of all suitable trials confirmed that the use of high-dose corticosteroids in sepsis is not of benefit and may potentially be deleterious by increasing rates of secondary infection.[54] However, impairment of the hypothalamic-pituitary-adrenal axis is seen in some patients with sepsis and is associated with a poor outcome. This has led to a re-evaluation of the role of lower-dose corticosteroid replacement therapy. Annane and colleagues have reported the results of a placebo-cntrolled randomized study of low-dose hydrocortisone and fludrocortisone in 300 patients with catecholamine-refractory septic shock.[55] 75% of patients in the active and placebo treatment groups were non-responders to synacthen. Beneficial effects of steroid replacement were confined to the synacthen non-responders with a significant reduction in pressor use (p=<0.001) and 28 day mortality (53% vs 63% P=<0.04). On the basis of this study it is reasonable to consider steroid replacement therapy in patients with catecholamine-resistant septic shock.

Intravenous immunoglobulin therapy

Intravenous immunoglobulin (IVIG) administration has been examined in bacterial sepsis with variable results.[56] In the case of toxic shock syndrome there is a rationale for the use of high-dose IVIG. Toxic shock is seen in persons with low levels of antitoxin antibodies and pooled IVIG contains neutralizing antibodies against a variety of superantigenic toxins. Experimental data and anecdotal reports would support the use of IVIG in this setting, but there are as yet no substantial clinical trials.[25]

Insulin therapy

As mentioned earlier assiduous control of the blood glucose level is essential in diabetic patients with severe sepsis. A recent study has suggested that insulin may be beneficial in nondiabetic adults with critical illness, including sepsis.[57] These are early data and should be viewed with caution, but if confirmed this approach could enter clinical practice.

Adjunctive therapy for sepsis

Despite all the therapies available, the mortality rate of established septic shock and organ failure remains high. The concept that organ damage in sepsis is the result of the host inflammatory response has lead to an enormous research effort to understand and intervene in this process. Many therapeutic strategies have been investigated in the laboratory setting and the past decade has seen large randomized clinical trials of some of the more promising agents. This has highlighted the difficulties inherent in performing interventional studies in sepsis and initial results have often been conflicting and disappointing. A full discussion of these approaches cannot be considered here but a brief summary of the current situation is given in Table 56.6 . The apparent success of rAPC and early results with other coagulation modulators suggests that this will be a fruitful therapeutic area. However, it must be stressed that attention to detail, good quality patient care and the early recognition and management serious infection can save many lives.

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Chapter 57 - Infections of Vascular Devices

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INFECTIONS OF PERCUTANEOUS INTRAVASCULAR DEVICES

The use of percutaneous intravascular devices (IVDs) for the delivery of fluids, blood products and medicines as well as for hemodynamic monitoring has become an essential component of modern health care. Unfortunately, these devices are associated with a considerable and often underappreciated risk of bloodstream infection (BSI). Data from hospitals participating in the US National Nosocomial Infections Surveillance system suggest that the risk of primary BSI, most of which derive from central venous catheters (CVCs) of one form or another, ranges from 2.9 to 9.7 BSIs per 1000 CVC-days, depending on the type of medical unit,[1] and it is estimated that 250,000 to 500,000 IVD-related BSIs (IVDR BSIs) occur in the USA each year.[2] The prevention of IVDR BSI must be a high priority as studies have shown that these infections prolong hospitalization, increase health care expenditures to nearly US$40,000 for survivors[3] and, most importantly, are associated with significant attributable patient mortality. [2]

TABLE 57-1 -- Rates of bloodstream infection caused by the various types of devices used for vascular access.*

RATES OF BSI CAUSED BY THE VARIOUS TYPES OF DEVICES USED FOR VASCULAR ACCESS				
Catheter type	**Rates of device-related BSIs per 100 catheters**		**Rates of device-related BSIs per 1000 catheter days**	
	Pooled mean	**95% CI**	**Pooled mean**	**95% CI**
Peripheral venous cannulas				
Plastic catheters	0.2	0.1–0.3	0.6	0.3–1.2
Steel needles	0.4	0.0–2.3	1.6	0.0–9.1
Cutdowns	3.7	0.1–20.6	8.8	0.2–49.3
Arterial catheters used for hemodynamic monitoring	1.5	0.9–2.4	2.9	1.8–4.5
Central venous catheters				
Standard noncuffed, nonmedicated	3.3	3.3–4.0	2.3	2.0–2.4
Antibiotic coated	0.2	0.0–1.0	0.2	0.1–1.4
Control nonmedicated	4.4	3.5–5.5	—	—
Antiseptic-impregnated	3.1	2.4–3.9	2.9	2.3–3.7
Control nonmedicated	3.8	1.5–7.8	—	—
Peripherally inserted central catheters (PICCs)	1.2	0.5–2.2	0.4	0.2–0.7
Silver-impregnated cuffs	3.3	1.9–5.4	3.2	1.9–5.3
Tunneled but noncuffed	12.4	10.7–16.6	1.8	1.4–2.0
Pulmonary-artery catheters	1.9	1.1–2.5	5.5	3.2–12.4
Heparin-bonded	0.4	0.2–0.9	2.6	1.1–5.2
Hemodialysis catheters				
Noncuffed	16.2	13.5–18.3	2.8	2.3–3.1
Cuffed	6.3	4.2–9.2	1.1	0.7–1.6
Tunneled and cuffed central venous catheters	20.9	18.2–21.9	1.2	1.0–1.3
Subcutaneous central venous ports	5.1	4.0–6.3	0.2	0.1–0.2
Peripherally inserted central ports	0.0	0.0–2.8	0.0	0.0–0.1
Intra-aortic balloon counter-pulsation devices	1.9	0.9–3.6	5.5	2.5–10.5
Left ventricular assist devices	22.5	17.5–28.5	4.5	3.6–5.7
Based on 206 published prospective studies in which every device was evaluated for infection.				

** From Kluger and Maki.[4]*

EPIDEMIOLOGY

A large variety of IVDs are employed in the care of hospitalized and, increasingly, nonhospitalized patients, including long-term devices for indefinite vascular access, which are used for total parenteral nutrition, outpatient antibiotic therapy or frequent drawing of blood. All IVDs carry some risk of causing BSI although the magnitude of risk varies by the type of device used (Table 57.1). Peripheral intravenous catheters, which typically remain in place for no more than 3–4 days, are associated with a very low risk, =0.2 BSIs per 1000 catheter-days.[4] In contrast, CVCs, which often remain in place for weeks, are associated with far higher rates of infection, in the range of 1.9–16.3 BSIs per 1000 CVC-days (see Table 57.1).[4] Surgically implanted IVDs, such as tunneled and cuffed Hickman or Groshong catheters as well as subcutaneous central venous ports, are associated with much lower rates of infection, in the range of 0.2–1.2 BSIs per 1000 IVD-days.[4] Contrary to popular belief, arterial catheters and peripherally inserted central catheters (PICCs) carry a considerable risk of infection (see Table 57.1) that may approach that seen with noncuffed and nontunneled CVCs. [4] The majority of

TABLE 57-2 -- Risk factors for intravascular device-related bloodstream infection.*

RISK FACTORS FOR IVDR BSI		
Risk factors (number of studies)		**Relative risk or odds ratio**

Patient characteristics	Underlying disease:	
	AIDS (2)	4.8
	Neutropenia (2)	1.0–15.1
	Gastrointestinal disease (1)	2.4
	Surgical service (1)	4.4
	Placement in intensive care unit or coronary care unit (3)	0.4–6.7
	Extended hospitalization (3)	1.0–6.7
	Other intravascular devices (2)	1.0–3.8
	Systemic antibiotics (3)	0.1–0.5
	Active infection at another site (2)	8.7–9.2
	High APACHE III score (1)	4.2
	Mechanical ventilation (1)	2.0–2.5
	Transplant patient (1)	2.6
Features of insertion	Difficult insertion (1)	5.4
	Maximal sterile barriers (1)	0.2
	Tunneling (2)	0.3–1.0
	Insertion over a guidewire (8)	1.0–3.3
	Insertion site:	
	Internal jugular vein (6)	1.0–3.3
	Subclavian vein (5)	0.4–1.0
	Femoral vein (2)	3.3–4.8
	Defatting insertion site (1)	1.0
	Use of multilumen catheter (8)	6.5
Catheter management	Routine change of iv set (2)	1.0
	1:2.0	61.5
	1:1.5	15.6
	1:1.2	4.0
	1:1	1.0
	Inappropriate catheter usage (1)	5.3
	Duration of catheterization >7 days (5)	1.0–8.7
	Colonization of catheter hub (3)	17.9–44.1
	Parenteral nutrition (2)	4.8

** Adapted from Safdar et al., 2002.*[69]

endemic nosocomial BSIs are primary, and most originate from an IVD, while a smaller number are secondary and stem from a local nonvascular site, such as ventilator-associated pneumonia or postsurgical site infection,

Whatever type of IVD is used, a variety of factors during IVD insertion and individual patient characteristics can have a profound impact on the risk for IVDR BSI (Table 57.2).

PATHOGENESIS

Micro-organisms that cause IVDR BSI may gain access to the blood-stream by a variety of routes (Fig. 57.1). A preponderance of evidence suggests that infections of short-term catheters (in place for less than 10 days) derive from cutaneous colonization of the insertion site, with centripetal migration along the extraluminal surface of the catheter. [3] Accumulating evidence suggests that long-term catheters (in place for 10 days or longer) most often become infected intraluminally, by micro-organisms that contaminate the hub or gain access to fluid infused through the catheter.[5] Less commonly, organisms may infect IVDs hematogenously, from remote sites of infection.[3] Outbreaks of IVDR BSI derive mainly from intrinsically or extrinsically contaminated infusate.[3]

An IVD is a foreign body that impairs local immune function and further provides an attractive surface to which micro-organisms can readily adhere to form a complex biofilm. After implantation, host proteins, including albumin, fibrinogen, fibrin, fibronectin and platelets, rapidly coat vascular catheters, providing an attractive surface for microbial adherence.[6]

Most micro-organisms that cause IVDR BSI produce large quantities of extracellular polysaccharide matrix (slime).[7] The combination of host proteins in intimate association with microcolonies of the infecting organism, embedded in exoglycocalyx, comprises a unique micro-ecosystem, the biofilm.[8] The extraordinary capacity of the biofilm to produce refractory infection has been demonstrated in studies showing that concentrations of a bactericidal antibiotic 100–1000 times those effective against the planktonic phase of the organism do not kill the micro-organisms within a biofilm.[9] Thus, although antimicrobial therapy is often effective in resolving the acute features of IVDR infection, such as local inflammation and fever, antimicrobial therapy alone rarely kills the indolent sessile organisms within the deepest layers of an implant-associated biofilm. This explains why it is usually necessary to remove an infected IVD to control the infection reliably.

MICROBIOLOGY

Figure 57.2 summarizes the microbial profile of IVDR BSI from 159 published prospective studies. [10] As might be expected, skin micro-organisms account for the largest proportion of IVDR BSIs.

PREVENTION

Considerable evidence has accumulated over the past two decades affirming the efficacy of specific infection control practices and novel technologies for reducing the risk of IVDR BSI. This information has been incorporated into the 2002 Centers for Disease Control and

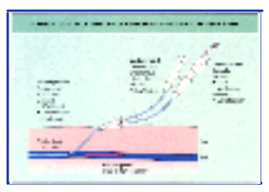

Figure 57-1 Potential sources of infection of a percutaneous intravascular device (IVD). These include contiguous skin flora, contamination of the catheter hub and lumen, contamination of infusate and hematogenous colonization of the IVD from distant, unrelated sites of infection. HCW, health care worker. *From Crnich and Maki.*[23]

Prevention Hospital Infection Control Practices Advisory Committee (HICPAC) Guideline for Prevention of Intravascular Catheter-Related Infections (Table 57.3).[11] With more consistent application of these guidelines, the incidence of CVC-related BSI in intensive care units in the USA has dropped by nearly 40% over the past decade.[12]

Given the pre-eminent role of skin micro-organisms in the pathogenesis of IVDR BSIs, it would seem that measures aimed at reducing the cutaneous microbial populations at the time of IVD insertion should have a great impact on the incidence of infection. Insertion of IVDs by trained and experienced staff, most reliably achieved with dedicated intravascular therapy teams, has been associated with greatly reduced rates of IVDR BSI.[13] However, if such personnel are unavailable, formal training programs for nurses and medical house officers can also reduce the risk of IVDR BSI.[14] Use of maximal barriers during insertion of CVCs, including sterile gloves, a sterile gown, a mask and a large sterile drape, has been shown to greatly cut the risk of IVDR BSI[15] and is considered mandatory for nonemergency insertion of all CVCs, including non-tunneled, noncuffed CVCs, pulmonary arterial catheters and hemodialysis catheters. Finally, multiple trials have shown that

Figure 57-2 Microbial profile of intravascular device-related bloodstream infection. Based on an analysis 159 published prospective studies, *from Maki et al.*[10]

chlorhexidine-based cutaneous antiseptics are associated with a 50% reduction in the risk of IVDR BSI compared with other cutaneous antiseptics, such as povidone-iodine or alcohol, making chlorhexidine the antiseptic of first choice when inserting an IVD.[16]

Although studies have shown that application of topical antibiotics such as mupirocin on the catheter insertion site can reduce the risk of CVC-related BSI, their use has been discouraged in the 2002 HICPAC Guideline because of the risk of emerging antibiotic resistance.[11]

The choice of the type of IVD, the location of its insertion and the duration of catheterization can also reduce the patient's risk of IVDR BSI:

- IVDs should have the minimum number of lumens needed for adequate access,
- CVCs are preferably inserted into the subclavian or internal jugular vein rather than the femoral vein, and
- the duration of IVD use should be limited to the absolute minimum.

Despite compliance with recommended guidelines, many centers continue to have high rates of IVDR BSI. Here, novel technology holds much promise (Table 57.4). Innovative technologies designed to reduce the risk of IVDR BSI have proved not only to be effective but also to reduce health care costs, both with short-term and long-term IVDs.

Studies of polyurethane dressings, which contain antiseptic, such as povidone-iodine or ionized silver, have been disappointing. However, on the basis of the demonstrated superiority of chlorhexidine for cutaneous disinfection of access sites, a novel chlorhexidine-impregnated sponge dressing has been developed that maintains a very high concentration of the antiseptic on the insertion site under the dressing. The largest study to date found that use of the chlorhexidine-impregnated sponge dressing was associated with a 60% reduction in catheter-related BSI (RR=0.37, p=0.01); there was no evidence of chlorhexidine resistance associated with use of the novel dressing.[17] Although there were no adverse side-effects associated with the use of this dressing in this trial in adults, a subsequent pediatric trial found that about 15% of low-birth-weight neonates developed local dermatotoxicity.[18]

Intravascular devices directly coated or impregnated with antimicrobials or antiseptics have been intensively studied over the past decade. A meta-analysis of prospective trials studying the most

TABLE 57-3 -- Guideline for the prevention of intravascular device-related bloodstream infection.*

GUIDELINE FOR THE PREVENTION OF IVDR BSI		
Recommendation		**Strength of evidence***
General measures	Educate all health care workers involved with IVD care and maintenance	IA
	Ensure adequate nursing staffing levels in intensive care units	IB
Surveillance	Monitor institutional IVD infection rates of IVD-related BSI	IA
	Express rates of CVC-related BSI per 1000 CVC-days	IB
At catheter insertion	Aseptic technique:	
	Hygienic hand care before insertion or manipulation of any IVD	IA
	Clean or sterile gloves during insertion and manipulation of noncentral IVDs	IC
	Maximal barrier precautions during insertion of CVCs: mask, cap, sterile gown, gloves, drapes	IA
	Dedicated IVD team strongly recommended	IA
	Cutaneous antisepsis: first choice, chlorhexidine; however, tincture of iodine, an iodophor or 70% alcohol are acceptable (no recommendations for use of chlorhexidine in infants less than 2 months, unresolved issue)	IA
	In adults, other than hemodialysis catheters (jugular site preference), use a subclavian site rather than a jugular or femoral site for CVC access (in pediatric patients, no recommendations for preferred site, unresolved issue)	IA
	Use of sutureless securement device	NR
	Sterile gauze or a semipermeable polyurethane dressing to cover site	IA
	No systemic or topical antibiotics at insertion	IA

Maintenance	Remove IVD as soon as no longer required	IA
	Monitor IVD site daily	IB
	Change dressing of CVC insertion site at least weekly	II
	Do not use topical antibiotic ointments	IA
	Change needleless iv systems at least as frequently as the administration set; replace caps no more frequently than every 3 days or per manufacturers' recommendations	II
	Complete lipid infusions within 12 hours	IB
	Replace administration sets no more frequently than every 72 hours. When lipid-containing admixtures or blood products are given, sets should be replaced every 24 hours; with propofol, every 6–12 hours	IA
	Replace peripheral IVs every 72–96 hours	IB
	Do not routinely replace CVCs or PICCs solely for prevention of infection	IB
	Do not remove CVCs or PICCs solely because of fever unless IVD infection is suspected but replace catheter if there is purulence at the exit site, especially if the patient is hemodynamically unstable and IVD-related BSI is suspected	II
Technology	Use antimicrobial-coated or antiseptic-impregnated CVC in adult patients if institutional rate of BSI is high despite consistent application of preventive measures and catheter likely to remain in place >5 days (no data or recommendations for pediatric patients)	IB
	Use chlorhexidine-impregnated sponge dressing for adolescent or adult patients with uncuffed CVCs or other catheters likely to remain in place >5 days (no recommendation for children, do not use in neonates <7 days old or gestational age <26 weeks)	NR
	Use prophylactic antibiotic lock solution *only* in patients with long-term IVDs who have continued to experience IVD-related BSIs despite consistent application of infection control practices	II

** Adapted from the Centers for Disease Control and Prevention. Guidelines for the prevention of intravascular catheter-related infections.*[11]

* IA, strongly recommended for implementation and supported by well-designed experimental, clinical or epidemiologic studies; IB, strongly recommended for implementation and supported by some experimental, clinical, or epidemiologic studies and a strong theoretical rationale; IC, required by state or federal regulations, rules or standards; II, suggested for implementation and supported by suggestive clinical or epidemiologic trials or a theoretical rationale — unresolved issue, an unresolved issue for which evidence is insufficient or no consensus regarding efficacy exists; NR, no recommendation for or against at this time.

widely used commercial CVC, the chlorhexidine-silver sulfadiazine catheter, found that use of the chlorhexidine-silver sulfadiazine CVC was associated with a 44% reduction in the rate of IVDR BSI (p=0.005).[19] Subsequent analyses estimated that US$59,000 could be saved and one patient death averted for every 300 such CVCs used.[20]

Comparably impressive results have been achieved with another CVC that is impregnated with minocycline and rifampin (rifampicin). In a large multicenter randomized trial, IVDR BSIs occurred in 5% of patients who received a nonmedicated CVC, compared with none of the patients who received a CVC impregnated with minocycline and

TABLE 57-4 -- Pooled analyses of prospective randomized clinical trials of novel technologies for prevention of intravascular device-related bloodstream infections with short-term and long-term devices.*

NOVEL TECHNOLOGIES FOR THE PREVENTION OF IVDR BSI WITH SHORT-TERM AND LONG-TERM DEVICES						
Technology		**Number of trials**	**Number of catheter-related bloodstream infections/number of central venous catheters studied**		**RR (95% CI)**	**p value**
			Technology	**Controls**		
Short-term devices	Chlorhexidine (vs povidone-iodine) cutaneous antisepsis	5	14/931	33/1213	0.55 (0.22–1.15)	0.07
	Dressings:					
	Polyurethane (vs gauze)	7	27/1070	20/725	0.97 (0.43–1.89)	0.76
	Chlorhexidine sponge	1	8/665	24/736	0.37 (0.17–0.81)	0.01
	Silver-impregnated cuff	5	10/283	14/247	0.62 (0.28–1.38)	0.30
	Anti-infective coated or impregnated CVC					
	Chlorhexidine-silver-sulfadiazine	15	68/2100	107/2135	0.65 (0.45–0.90)	<0.01
	Minocycline-rifampin	1	0/130	7/136	0.00 (0.00–2.80)	0.02
	Minocycline-rifampin (vs chlorhexidine-silver-sulfadiazine)	2	1/394	14/418	0.08 (0.00–0.81)	<0.01
	Needleless connectors	2	4/245	21/263	0.20 (0.07–0.59)	<0.01
Long-term devices	Securement device	2	1/144	13/135	0.07 (0.00–0.78)	<0.01
	Chlorhexidine sponge dressing	1	12/314	11/341	1.18 (0.39–4.06)	0.83
	Antibiotic-lock	6	13/257	40/267	0.34 (0.18–0.62)	<0.01

** Adapted from Crnich and Maki.*[23] [24]

rifampin.[21] A subsequent study comparing the CVC impregnated with minocycline and rifampin to the chlorhexidine-silver sulfadiazine CVC found that CVCs impregnated with minocycline and rifampin were far less likely to be associated with IVDR BSI (p<0.002).[22] Although neither clinical trial found resistance to minocycline or rifampin among clinical isolates, concerns over the emergence of resistance has limited the wide use of this catheter. Other coatings, including active and passive silver iontophoresis, have also been developed;[23] however, their role at this time is limited by inadequate clinical studies.

Given the importance of hub contamination and intraluminal colonization in the genesis of IVDR BSI with long-term IVDs, intraluminal installation of an antibiotic or antiseptic solution has the potential to reduce the risk of BSI associated with these devices. Six randomized, prospective trials have examined a vancomycin-containing antibiotic lock solution for the prevention of IVDR BSI.[24] The largest of these trials found that use of a vancomycin or vancomycin-ciprofloxacin lock solution reduced the risk of IVDR BSI by nearly 80% (p=0.005).[25] Concern about the emergence of resistance with prophylactic antibiotic-containing lock solutions has limited their

acceptance to date. However, their use is considered acceptable in the HICPAC Guideline if a patient with an essential long-term IVD has continued to experience recurrent BSIs despite compliance with published infection control practices.[11]

CLINICAL FEATURES

Infection of an IVD represents a continuum, beginning with colonization of the extraluminal or intraluminal surface of the catheter, occasionally producing local infection manifested by erythema, pain and purulent drainage, and potentially culminating in BSI, attended by signs and symptoms of sepsis (Table 57.5). Colonization of short-term catheters precedes catheter-related BSI but, in the absence of signs of local infection or BSI, is of itself not an indication for initiating antimicrobial therapy. Similarly, with any IVD, a single positive blood culture drawn from the device, with negative peripheral blood cultures, most often represents contamination rather than true IVDR BSI. In contrast, local erythema, especially when accompanied by purulence at the insertion site, is normally an indication for antimicrobial therapy and mandates removal of the device. Local infection may occur in isolation or in combination with BSI; however, it must be noted that most IVDR BSIs in short-term devices occur in the absence of any signs of local infection. [26]

The majority of patients with IVDR BSI show signs of the sepsis syndrome, with fever, tachycardia, tachypnea or leukocytosis. However, these signs are not specific and are encountered with other types of infectious and noninfectious inflammatory conditions. Moreover, systemic signs of infection may be absent or minimal with IVDR BSIs caused by organisms of low pathogenicity, such as coagulase-negative staphylococci or *Corynebacterium* spp.[2] Despite the challenge of identifying the source of a patient's signs of sepsis, [27] several clinical, epidemiologic and microbiologic findings point strongly toward an IVD as the source of a septic episode (Table 57.6).[3] [28]

It is likely that any patient with an IVD who develops high-grade bacteremia or candidemia that persists after an infected catheter has been removed has an infected thrombus in the recently cannulated vein (Fig. 57.3); such a patient may even have developed secondary endocarditis or seeding to other distant sites. Fortunately, suppurative phlebitis of peripheral intravenous catheters is now rare and the syndrome of intravenous suppuration is predominantly a complication of CVCs, typically catheters that have been left in place for many days in vulnerable patients in the intensive care setting. The micro-organisms most frequently implicated in suppurative phlebitis or septic thrombosis are the same organisms that cause uncomplicated catheter-related septicemia: *Staphylococcus aureus*, nosocomial aerobic Gram-negative bacilli and, in recent years, *Candida* spp.[29]

DIAGNOSIS

Removal and culture of the IVD has historically been the gold standard for the diagnosis of IVDR BSI, particularly with short-term catheters.[3] [28] Studies have demonstrated the superiority of semiquantitative

TABLE 57-5 -- Proposed definitions for intravascular device-related colonization, local infection and bloodstream infection, which are based on microbiologic confirmation of the intravascular device as the source.‡

DEFINITIONS FOR IVDR COLONIZATION, LOCAL INFECTION AND BSI	
IVD colonization	(i) A positive semiquantitative* (or quantitative†) culture of the implanted portion or portions of the IVD; (ii) absence of signs of local or systemic infection
Local IVD infection	(i) A positive semiquantitative* (or quantitative†) culture of the removed IVD or a positive microscopic examination or culture of pus or thrombus from the cannulated vessel; (ii) clinical evidence of infection of the insertion site (i.e. erythema, induration or purulence) but; (iii) absence of systemic signs of infection and negative blood cultures, if done
IVD-related BSI	If the IVD is removed: (i) A positive semi-quantitative* (or quantitative†) culture of the IVD or a positive culture of the catheter hub or infusate (or positive microscopic examination or culture of pus or thrombus from the cannulated vessel) *and* one or more positive blood cultures, ideally percutaneously drawn, concordant for the same species, ideally by molecular subtyping methods; (ii) clinical and microbiologic data disclose no other clear-cut source for the BSI.
	If the IVD is retained: (i) If quantitative blood cultures are available, cultures drawn both from the IVD and a peripheral vein (or another IVD) are both positive and show a marked step-up in quantitative positivity (5-fold or greater) in the IVD-drawn culture; (ii) clinical and microbiologic data disclose no other clear-cut source for the BSI
	or
	(i) If automated monitoring of incubating blood cultures is available, blood cultures drawn concomitantly from the IVD *and* a peripheral vein (or another IVD) show both are positive, but the IVD-drawn blood culture turns positive more than 2 hours before the peripherally drawn culture; (ii) clinical and microbiologic data disclose no other clear-cut source for the BSI.

‡ *Adapted from Crnich and Maki.*[2]
* Roll plate of cannula segment(s) >15 cfu
† Sonication culture of cannula segment(s) =10^3 cfu

or quantitative catheter tip culture methods for the diagnosis of IVDR BSI.[3] Using these techniques, catheter colonization is defined as recovery of more than 15 colony forming units (cfu) from a catheter segment using the semiquantitative roll-plate method[30] or more than 1000 cfu using a quantitative sonication method.[31] The diagnosis of IVDR BSI is completed when a colonized IVD is associated with concomitant BSI, with no other plausible source.[28]

Cultures of IVDs require their removal, a major problem in patients with long-term IVDs. Studies have shown that only 25–45% of episodes of sepsis in patients with long-term devices represent true IVDR BSI.[32] Thus, it would seem that in-situ methods for detecting IVDR BSI that do not require removal of the IVD would be of great value.

If a laboratory has available an automated quantitative system for culturing blood, quantitative blood cultures drawn through the IVD and concomitantly by venepuncture from a peripheral vein (or another IVD) can permit the diagnosis of IVDR bacteremia or fungemia to be made, with sensitivity and specificity in the range of 80–95%,[33] [34] without removal of the catheter, if empiric antimicrobial therapy has not yet been initiated. However, quantitative blood

TABLE 57-6 -- Clinical, epidemiologic and microbiologic features of intravascular device-related sepsis.‡

CLINICAL, EPIDEMIOLOGIC AND MICROBIOLOGIC FEATURES OF INTRAVASCULAR DEVICE-RELATED SEPSIS	
Non-specific features	**Suggestive of device-related etiology**
Fever	Patient unlikely candidate for sepsis (e.g. young, no underlying diseases)
Chills, shaking rigors*	
Hypotension, shock*	Source of sepsis inapparent, no identifiable local infection
Hyperventilation, respiratory failure	IVD in place, especially a CVC
Gastrointestinal*	Inflammation or purulence at insertion site
Abdominal pain	Abrupt onset, associated with shock
Vomiting	BSI caused by staphylococci (especially coagulase-negative staphylococci) or *Corynebacterium* spp., *Candida* spp., *Trichophyton* spp., *Fusarium* spp. or *Malassezia* spp.†
Diarrhea	
Neurologic*	

Confusion	
Seizures	
	High-grade candidemia (>25 cfu/ml)
	Cluster of cryptogenic infusion-associated BSIs caused by *Enterobacter cloacae*, *Pantoea agglomerans* or *Serratia marcescens*†
	Sepsis refractory to antimicrobial therapy or dramatic improvement with removal of IVD and infusion*

‡ *Adapted from Maki and Mermel.* [3]
* Commonly seen in overwhelming Gram-negative sepsis originating from contaminated infusate, peripheral suppurative phlebitis or septic thrombosis of a central vein
† Conversely, septicemia caused by streptococci, aerobic Gram-negative bacilli or anaerobes is unlikely to dervie from an intravascular device

cultures are labor intensive and expensive. The wide availability of automated radiometric blood culture systems, in which blood cultures are continuously monitored for microbial growth, has led to a clever application for the detection of IVDR BSI, the differential-time-to-positivity of blood cultures drawn through the IVD and concomitantly from a peripheral site. Positivity of a blood culture drawn from the IVD more than 2 hours before the culture drawn from a peripheral site turns positive has been shown to be highly predictive of IVDR BSI; in one study a sensitivity of 94% and specificity of 91% were reported. [35]

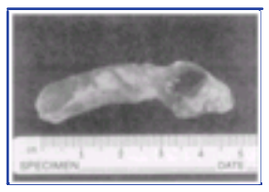

Figure 57-3 Thrombus extracted surgically from distal subclavian vein. *From Andes et al.* [29]

MANAGEMENT

Management of the device

Short-term intravascular devices

If a short-term vascular catheter is suspected of being infected because the patient has no obvious other source of infection to explain a fever, there is inflammation at the insertion site, or cryptogenic bacteremia or candidemia has been documented, blood cultures should be obtained and the catheter should be removed and cultured. Failure to remove an infected catheter puts the patient at risk of developing septic thrombophlebitis, septic thrombosis of a great central vein[36] [37] or even endocarditis. Continued access can always be established with a new catheter at a new site. A new catheter should never be placed in an old site over a guidewire if the first catheter is suspected of being infected, especially if there is purulence at the site.[38]

Long-term intravascular devices

Bloodstream infection originating from a cuffed and tunneled CVC does not automatically mandate removal of the device unless:

- there has been persistent exit site infection;
- the tunnel is obviously infected;
- there is evidence of complicating endocarditis, septic thrombosis or septic pulmonary embolism;
- the infecting pathogen is *S. aureus*, *Corynebacterium jeikeium*, a *Bacillus* sp., *Stenotrophomonas* spp., *Burkholderia cepacia*, any pseudomonal sp., a filamentous fungus or *Malassezia* spp. or a mycobacterium; or
- bacteremia or candidemia persists for more than 3 days despite adequate therapy.[39]

Further information on the management of an IVD in patients with suspected or confirmed IVDR BSI is covered in Chapter 61 .

Anti-infective therapy

If IVDR BSI is suspected (see Table 57.6), after cultures have been obtained, the combination of intravenous vancomycin (for resistant staphylococci) with a fluoroquinolone or cefepime or imipenem-meropenem (for multiresistant nosocomial Gram-negative bacilli) should prove effective against the pathogens most likely to be encountered.[39] Initial therapy should later be modified on the basis of the susceptibilities of the infecting organisms.

How long to treat IVDR BSI will be influenced by the infecting micro-organism and by whether the patient has underlying valvular heart disease, already has evidence of endocarditis or septic thrombosis, or shows evidence of metastatic infection. If endocarditis is suspected, transesophageal echocardiography offers superior sensitivity and discrimination for detecting vegetations.[40] In patients with high-grade bacteremia or fungemia but without clinical or echocardiographic evidence of endocarditis, septic thrombosis should be suspected. Central

TABLE 57-7 -- Rates of infection by location of arterial graft.*

RATES OF INFECTION BY LOCATION OF ARTERIAL GRAFT	
Location of arterial graft	**Rates of infection (%)**
Thoracic aorta	1.2–3.0
Aortoiliac	0.0–1.3
Aortofemoral	0.5–3.0
Femorofemoral	3.6–5.0
Femoropopliteal	1.0–4.6
Axillofemoral	5.3–6.0
Cervical	0.0–0.2

* *Adapted from Goëau-Brissonnière and Coggia.*[43]

venous thrombosis can now be diagnosed by venography, ultrasonography, magnetic resonance imaging (MRI) or computerized tomography (CT).[3]

Most uncomplicated coagulase-negative staphylococcal infections can be cured with only 5–7 days of therapy[28] [41] and most infections caused by other micro-organisms can be adequately treated with 10–14 days of antimicrobial therapy,[28] as long as there are no complications related to the infection and the BSI clears within 72 hours of initiating therapy.

All patients with a IVDR BSI must be monitored closely for at least 6 weeks after completing therapy, especially if they have had high-grade bacteremia or candidemia, to detect late-appearing endocarditis, retinitis or other metastatic infection, such as vertebral osteomyelitis.

INFECTIONS OF ARTERIAL GRAFTS

Surgical replacement of occluded or aneurysmal arterial segments became feasible with the availability of synthetic polymeric grafts in the 1950s. Over 200,000 ateriovenous shunts and vascular bypass procedures are performed in the USA each year, most requiring the placement of a prosthetic arterial graft.[42] The most feared complication of arterial graft implantation is infection, which may result in limb loss or death. Diagnosis of infection of arterial grafts is often a formidable challenge for clinicians but is imperative to avert a catastrophic outcome.

EPIDEMIOLOGY

Most studies quantifying the risk of arterial graft infection have been retrospective and further limited by incomplete follow-up; however, it would appear the risk of infection following implantation of an arterial graft ranges from 1% to 6%.[43] Risk varies greatly by location of the arterial graft (Table 57.7): [43] [44]

- aortic grafts within the abdomen, 0.5–1.0%;
- aortofemoral bypass grafts, 1.5–2.0%; and
- infrainguinal grafts needed for revascularization, 2.2–6.0%.

The greater risk associated with arterial grafts below the inguinal ligament may be related to surgical disruption of lymphatics in the inguinal region, placement of the graft in a highly mobile area of the body so that wound closure is compromised, and an anatomic area associated with heavy cutaneous microbial colonization.

Arterial grafts used for hemodialysis appear to be at much higher risk of infection than grafts implanted for vascular bypass; rates of infection with synthetic grafts used for hemodialysis have ranged from 11% to 16%.[45]

Additional factors associated with an increased risk of arterial graft infection include operative times longer than 4 hours, advanced age, diabetes mellitus, malignancy, malnutrition, lower extremity infection at the time of surgery and reoperation.[43]

The 1-year survival in patients with aortic graft infection is only 58–77%,[46] and although survival is higher in patients with arterial grafts in the infrainguinal region — in the range of 75–90%[47] — infection of extremity grafts is associated with prohibitive risk of limb loss, ranging from 30% to 50%. [48] Approximately 20% of patients with prosthetic hemodialysis fistulas lose their graft because of infection,[49] [50] and bacteremia in this patient population is associated with greatly increased risk of death.[51]

PATHOGENESIS

Infections of arterial grafts occasionally occur in the early postoperative period (less than 1 month after surgery) but more commonly much later. Infections of arterial grafts involving the groin occur, on average, 3 months after implantation[52] whereas infections of aortic

grafts occur much later, with a delay to onset of 10–29 months.[53] [54] Infection in the early postoperative period is rare (<1%) and most often arises as a consequence of infection of groin or lower extremity wounds.[46]

Intraoperative cultures have shown that as many as 55% of arterial bypass grafts are contaminated by cutaneous or bowel micro-organisms at the time of implantation, suggesting that most arterial graft infections derive from intraoperative microbial contamination.[55] Graft infection as a result of contiguous spread from local wound infections can also occur and most commonly involves the graft segment immediately beneath the surgical incision.[56] Hematogenous contamination of the graft from a remote site of infection, such as pneumonia or an infected IVD, can also occur but, fortunately, appears to be rare.

Regardless of the route of infection, micro-organisms that adhere to the arterial graft surface are able to evade the effects of the host immune system and antimicrobial therapy by mechanisms analogous to those described above with infected IVDs. The implications are clear; once established, arterial graft infection is very difficult, if not impossible, to control without removal of the prosthetic material.

MICROBIOLOGY

Data from published series show that *S. aureus* is implicated in the majority (about 30%) of arterial graft infections; less commonly infection is caused by coagulase-negative staphylococci (about 12% of cases), enterococci (about 9%) and streptococci (about 5%) (Fig. 57.4). The Enterobacteriaceae, as a group, account for 25% of arterial graft infections and *Pseudomonas aeruginosa* is isolated in approximately 7% of cases. Approximately 10% of arterial graft infections are polymicrobial, and no organisms are recovered from 5% of patients with clinically overt signs of infection at the time of surgery. Early postoperative infections appear to be caused primarily by *S. aureus* and nosocomial Gram-negative bacilli; late infections appear to be caused by by organisms of lower virulence, such as coagulase-negative staphylococci. Enterobacteriaceae are recovered far more frequently from aortoilac graft infections whereas *S. aureus* and coagulase-negative staphylococci predominate in infections of aortofemoral and femorodistal arterial grafts (see Fig. 57.4).[43]

PREVENTION

The disastrous outcome associated with uncontrolled infection of a prosthetic arterial graft mandates an aggressive approach to prevention. If possible, the patient's nutritional and immune status should be optimized before elective surgery. Every attempt must be made to eradicate (or at least to control) active infections, especially infected skin ulcers or gangrenous toes; 55% of patients who develop arterial graft infections have had an identifiable focus of infection present before vascular surgery.[56] Although preoperative showering with chlorhexidine has been shown to produce significant reductions in cutaneous microbial counts,[57] its true value in vascular surgery remains to be defined.[58] Finally, hair removal at the prospective surgical site should be done as close to the surgery as possible, with depilatories or clipping rather than shaving.[57]

Vascular procedures are considered clean operations; however, the added risk with implantation of prosthetic materials justifies the use of perioperative antibiotic prophylaxis.[57] Perioperative use of cefazolin in 462 patients undergoing vascular surgery has been shown to be associated with a significantly lower risk of surgical site infection compared with placebo (0.9% vs 6.8%, RR=0.12, p=0.001); arterial graft infections occurred in the placebo group but none occurred in patients randomized to receive cefazolin.[59] Hasselgren *et al.* also confirmed the benefit of antistaphylococcal prophylaxis perioperatively and further showed that extending the duration of prophylaxis beyond 24 hours was of no added benefit.[60]

The increasing frequency of coagulase-negative staphylococcal infections of arterial grafts and rising rates of methicillin resistance of nosocomial *S. aureus* have raised the question of whether glycopeptides should become standard agents for perioperative prophylaxis in vascular and cardiothoracic surgery. One well-designed prospective randomized study of cardiothoracic and vascular surgical patients found a significantly lower rate of postoperative wound infection with vancomycin compared with either cefazolin or cefamandole.[61] However, at this time the routine use of glycopeptides in vascular surgery is not recognized by HICPAC.[57] Therefore, it is recommended that an antibiotic with antistaphylococcal activity, such as cefazolin or cefuroxime, should be given perioperatively for not more than 24 hours after vascular surgery; prophylactic use of glycopeptides should be limited to high-risk patients — such as those with a history of methicillin-resistant *S. aureus* colonization, heavy antibiotic use or chronic skin ulceration — and to hospitals with high rates of methicillin-resistant *S. aureus* infections of surgical sites.[57]

Figure 57-4 Microbial profile of infections of prosthetic arterial grafts by location of the implanted graft. *From Goëau-Brissonniere and Coggia.*[43]

Although in-vitro studies have shown that micro-organisms adhere to grafts made of polyethylene terephthalate more easily than to grafts made of polytetrafluoroethylene, [62] differences in rates of clinical infection have not been seen. However, there has understandably been much interest in developing graft materials that are intrinsically resistant to infection. A rifampin-bonded polyethylene terephthalate graft has been studied in animal models[43] with encouraging results, and it has been used with apparent success by surgeons for in-situ replacement of infected arterial grafts.[63]

CLINICAL FEATURES

The clinical manifestations of prosthetic arterial graft infection are heavily influenced by the location of the graft, the elapsed time since implantation and the organisms involved. Early postoperative infections and infrainguinal arterial graft infections typically present with local signs, such as cellulitis, wound dehiscence or cutaneous erosion over the graft, in combination with systemic signs of sepsis. [47] If not promptly recognized, infections caused by virulent organisms, such as *S. aureus*, can progress rapidly to false aneurysm formation and catastrophic hemorrhage. In contrast, local signs of infection are often absent with infections caused by coagulase-negative staphylococci and enterococci, and patients often feel well; the presence of graft thrombosis or delayed wound closure may be the only signs to suggest infection.

Aortic graft infections most often present late as unexplained bacteremia or fever, but they can also manifest themselves as catastrophic gastrointestinal hemorrhage from graft-enteric erosion or fistula formation. It is estimated that 35% of patients who develop infection of an aortic graft infection will show evidence of a graft-enteric fistula; however, not all present with massive gastrointestinal bleeding.[47] Bleeding seen in patients with graft-enteric fistulas often manifests itself as chronic, intermittent bleeding rather than as massive life-threatening hemorrhage. As a result, it is impossible to recognize graft-enteric fistula solely by presentation and this entity must be suspected in all patients with an aortic graft who present with gastrointestinal hemorrhage.

DIAGNOSIS

Laboratory abnormalities include leukocytosis and elevation of the erythrocyte sedimentation rate and C-reactive protein, which are non-specific and may only be borderline abnormal in infections caused by coagulase-negative staphylococci or enterococci. When arterial graft infection is suspected, two blood cultures should be obtained before empiric antimicrobial therapy is initiated. Cultures from open wounds, sinus tracts or exposed grafts may also be helpful. The presence of fluid about the graft segment is highly suggestive, but not diagnostic, of infection; within the first 1–2 months after surgery, fluid may represent normal postoperative changes or sterile lymphocele formation. Deep fluid collections should always be aspirated and can usually confirm infection caused by *S. aureus*, *P. aeruginosa* or Enterobacteriaceae but may fail to identify graft infections caused by coagulase-negative staphylococci or anaerobes or infections in patients who have received empiric antibiotic therapy before sampling.

Imaging techniques are very helpful in identifying arterial graft infection. With infrainguinal arterial grafts, ultrasonography may show the presence of perigraft fluid, pseudoaneurysm formation or graft thrombosis. Contrast CT has become the most useful imaging modality for investigating suspected graft infection, especially if an aortic graft is involved, and has shown sensitivity and specificity approaching 90% in published series.[44] The failure of an arterial graft to become incorporated into contiguous tissues, as indicated by the presence of air or fluid about the graft (Fig. 57.5), or the presence

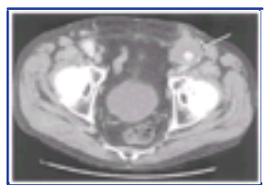

Figure 57-5 Contrast computerized tomography of the pelvis demonstrating perigraft fluid in a patient with an infected left-sided aortoiliac graft (arrow).

of a pseudoaneurysm is highly suggestive of graft infection, especially if these findings are present more than 6–7 weeks after graft implantation.[64] Bowel wall thickening on abdominal CT, especially in the fourth duodenal segment, is suspicious for graft-enteric fistula and may even abrogate the need for endoscopic evaluation.

Magnetic resonance imaging has been reported to be more sensitive than CT for the diagnosis of arterial graft infection;[65] however, it must be noted that neither CT nor MRI can differentiate between infection or a sterile fluid collection, and at this time there are insufficient data to recommend routine the use of MRI for the evaluation of suspected arterial graft infections.

There has also been considerable interest in use of functional imaging studies, such as Gallium-67 citrate, indium-111-labeled leukocyte or IgG, or technetium-99m hexametazime-labeled leukocyte scintography.[66] These techniques are of little value in the early postoperative setting, because normal postoperative changes will result in a positive scan.[67] However, they may be of some use in difficult late postoperative cases.

MANAGEMENT

The treatment of suspected arterial graft infection must take into account the patient's acuity of illness and the options for preserving perfusion distal to the graft. If the patient is not critically ill and information about the causative micro-organism is lacking, an expedited diagnostic work-up utilizing one or more of the imaging modalities discussed above, blood and wound cultures and aspiration of deep fluid collections should be performed before empiric antimicrobial therapy is initiated. In patients who are floridly septic on presentation, empiric therapy should be initiated with drugs that cover nosocomial Gram-positive and Gram-negative pathogens; vancomycin combined with an antipseudomonal ß-lactam — cefepime, pipericillin-tazobactam, imipenem or meropenem — should provide adequate coverage. Antimicrobial therapy is usually initiated before surgery and continued for 4–6 weeks postoperatively, guided by the results of operative cultures and susceptibilities, the clinical response and normalization of C-reactive protein levels.

Although effective antimicrobial therapy is essential, surgical removal of the infected prosthetic graft material is the most important aspect of management. The exact surgical approach taken is dependent on the location of the graft, the extent of the infection and the options for preserving distal perfusion. Infection of an infrarenal aortic graft is best managed by total excision of the

infected area of the graft, followed by extra-anatomic revascularization, most commonly through a right axillobifemoral bypass. With this approach, overall operative mortality approaches 15% and rates of distal limb loss approach 20%, with a 3–10% risk of recurrent infection.[44] Extraanatomic bypass of the suprarenal aorta is not technically feasible and the only option in such cases is surgical debridement with in-situ placement of a fresh graft; operative mortality approaches 30% and rates of distal limb loss approach 15%, with recurrent infection seen in up to 20% of cases.[44] Newer surgical approaches that utilize graft excision with in-situ placement of human aortic allografts or antibiotic-bonded graft material or surgical debridement with retention of the original graft have been reported but remain investigational.

Infection of infrainguinal arterial grafts almost always requires complete surgical resection of the infected graft (or its involved segment) with in-situ replacement utilizing an autogenous vein graft or extra-anatomic bypass with a new prosthetic graft. Several groups have reported successful management of infrainguinal graft infection without removal of the graft by surgically debriding the wound and graft bed, followed by placement of a muscle flap.[68] Most of these reports have been from small series and were most successful in the early postoperative period.

Management of graft-enteric fistula must take into account the degree of intestinal bleeding and the stability of the patient. Patients who are hemodynamically stable and have shown only intermittent bleeding can be evaluated by contrast CT of the abdomen, followed, if negative, by an upper endoscopic study that visualizes the third and fourth segments of the duodenum. In contrast, immediate upper endoscopy in the operating room should be performed in patients who present with massive gastrointestinal bleeding. If CT or endoscopic findings suggest a graft-enteric fistula, immediate surgery is mandatory, with resection of the involved bowel and infected graft, followed by in-situ placement of a fresh graft, often with an omental wrap. Mortality in patients with graft-enteric fistulas approaches 40%, even with experienced surgeons; however, mortality is universal if the diagnosis is missed. [69]

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Chapter 58 - Myocarditis and Pericarditis

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MYOCARDITIS

EPIDEMIOLOGY

The term myocarditis applies to a variety of disease states that produce inflammation of the myocardium. In its acute form, myocarditis ranges from an asymptomatic illness with reversible changes to fulminant myocardial necrosis and death. In its chronic form, lymphocytic infiltration of the myocardium may cause subacute deterioration of cardiac function; indeed, chronic myocarditis may predate the development of 'idiopathic' dilated cardiomyopathy (IDC). The possible association between viral myocarditis and IDC is intriguing and potentially important. Although frequently ascribed to inflammation caused by an infectious agent, myocarditis may also be seen in allergic reactions, drug reactions and in association with systemic inflammatory disease. A diagnosis of acute infectious myocarditis is suggested when unexplained heart failure or malignant arrhythmias occur in the setting of a systemic febrile illness or after symptoms of an upper respiratory tract infection.

The incidence of infectious myocarditis in the general population is unknown. In a prospective study of Finnish military recruits conducted over several years, a mean annual incidence of 0.02% was found.[1] The prevalence of clinically significant myocarditis is higher in children and young adults than in older adults, possibly due to different exposures to infectious agents and an absence of relevant immunity. Indeed, myocarditis is thought to be a major cause of sudden cardiac death in adults under the age of 40 years.[2] [3] [4] Within immunosuppressed patients, myocarditis is more prevalent, affecting approximately 50% of AIDS patients at autopsy.[5]

PATHOGENESIS AND PATHOLOGY

In myocarditis, damage to cardiac myocytes appears to involve one or more of four possible mechanisms:

- direct cytopathic effects of an infectious agent;
- cellular injury secondary to circulating exogenous or bacterial toxins;
- specific cell-mediated or humoral immunologic response to the inciting agent or induced neoantigens; and
- non-specific cellular injury caused by generalized inflammation.

Histologically, both myocyte necrosis and infiltration by inflammatory cells in the absence of ischemia are pathognomonic of the disease. The infiltrate may be composed of a variety of cell types, including neutrophils, lymphocytes, macrophages, plasma cells, eosinophils and/or giant cells (Fig. 58.1).

The pathologic abnormalities, location, severity and changes over time span a wide range depending on the etiologic agent and individual host response involved. Coxsackie virus, for example, appears to infect cardiac myocytes directly and can trigger either focal or widespread inflammatory infiltration and cellular necrosis. Infection with hepatitis B virus and varicella-zoster virus appears to involve vascular endothelium and thus can produce a different histologic pattern. The time after infection when tissue is obtained for analysis also greatly influences the histologic appearance. In acute enteroviral myocarditis, for example, few inflammatory cells may be seen, whereas cellular infiltrates are the hallmark of chronic enteroviral myocarditis. Analysis of endomyocardial biopsy specimens obtained during infection with different agents shows considerable overlap, however. Thus, an histologic diagnosis of myocarditis usually does not indicate the agent responsible.

Bacteria

Bacteria may cause myocarditis by a variety of mechanisms. Bacteremia caused by a wide variety of species may result in metastatic foci within the myocardium. Myocarditis has been noted in association with streptococcal and staphylococcal bacteremia, meningococcemia, bartonellosis, brucellosis, leptospirosis and Whipple's disease. However, the resulting myocardial dysfunction is only clinically significant in some patients who have overwhelming infections. In contrast, myocardial involvement in bacterial endocarditis is more common, and is often clinically significant. Bacteria (especially *Staphylococcus aureus*) may directly invade the myocardium from infected valves to cause abscesses, valvular failure and conduction abnormalities, or may embolize throughout the myocardium to cause global ventricular dysfunction. Cardiac infections caused by salmonellae are particularly serious; mural involvement responds poorly to antimicrobial agents and, without surgical therapy, mortality is 100%.

Bacterial toxin production can also be clinically significant. Subtle evidence of myocarditis can be detected in as many as two-thirds of patients who have diphtheria, with 10–25% of patients developing clinical cardiac dysfunction that is in direct proportion to the severity of the respiratory tract infection. [6] The virulence of *Corynebacterium diphtheriae* does not result from direct invasion of myocardium but from the effect of its potent exotoxin, which inhibits protein synthesis. Thus, evidence of cardiac toxicity may occur 1–2 weeks after the onset of diphtheria, often when the

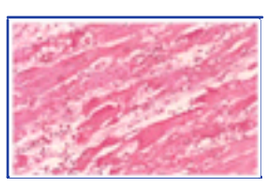

Figure 58-1 Acute viral myocarditis, with a characteristic mononuclear infiltrate.

oropharyngeal manifestations are improving. Patients who have electrocardiogram (ECG) changes of myocarditis have a mortality rate three to four times that of patients who have normal tracings, with atrioventricular nodal and left bundle branch block carrying a mortality rate of 60–90%.[7]

Finally, cardiac involvement (including myocarditis) occurs in up to 50% of cases of acute rheumatic fever (ARF). Pancarditis (e.g. endocarditis, myocarditis and pericarditis) in cases of ARF is likely the result of an over-exuberant host immune reaction to upper respiratory tract infection with rheumatogenic strains of group A streptococci. 'Molecular mimicry' (e.g. immunologic cross-reactivity to cardiac antigens elicited by streptococcal products) underlies the postulated pathogenesis of this disease, as investigators have failed to demonstrate the presence of streptococci within inflammatory cardiac lesions.

Spirochetes

Spirochetes such as *Borrelia burgdorferi*, the etiologic agent of Lyme disease, are important causes of myocarditis. The cardiac manifestations of Lyme disease may occur in an isolated manner, or coincident with other features such as erythema chronicum migrans or neurologic abnormalities. Within several weeks of infection, about 8% of patients will develop cardiac manifestations. [8] The most prevalent abnormality is fluctuating atrioventricular block, but some patients have evidence of more diffuse myopericardial involvement. Indeed, organisms have occasionally been demonstrated in endomyocardial biopsy tissue both pre-mortem[9] and at autopsy, [10] providing supportive evidence for direct spirochetal invasion. Myocardial involvement is common in cases of fatal leptospirosis (Weil's disease), where arrythmias or acute circulatory collapse may occur in conjunction with hepatorenal or central nervous system syndromes. At autopsy, myocardial inflammation, coronary arteritis and

aortitis are common findings.

Rickettsiae

Rickettsiae produce systemic vasculitis by endothelial invasion, which not infrequently involves the myocardium. Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*) and scrub typhus

TABLE 58-1 -- Infectious causes of myocarditis.

INFECTIOUS CAUSES OF MYOCARDITIS			
Region	**Normal host**		**Immunocompromised host**
Developed world	**Common and/or important**	Viruses: Coxsackie viruses (A and B), echovirus, CMV, Epstein-Barr virus, human herpes virus-6, influenza viruses (A and B), adenovirus, parvovirus, hepatitis B virus, ?hepatitis C virus	Viruses: Human immunodeficiency virus (HIV), CMVV, EBV, varicella zoster virus (VZV), adenovirus, parvovirus
		Bacteria: diphtheria, Lyme disease, any organism associated with infective endocarditis	Fungi: Candidiasis, aspergillosis, crytococcosis
		Parasites: American trypanosomiasis, trichinosis	Parasites: toxoplasmosis, American trypanosomiasis
	Uncommon	Viruses: adenovirus, parvovirus, respiratory syncytial virus, hepatitis B virus, ?hepatitis C virus	Fungi: histoplasmosis, blastomycosis, coccidioidomycosis, zygomycosis
		Bacteria: staphylococci, streptococci, meningococci, salmonellae, listeria, clostridia, rickettsia, bartonellosis, ehrlichiosis	
Developing world		Viruses: poliovirus, mumps, rubella, arenaviruses, dengue, rabies, chikungunya, ebola virus, yellow fever	
		Bacteria: leptospirosis	
		Parasites: American trypanosomiasis, African trypanosomiasis	

(caused by *R. tsutsugamushi*) infections may cause transient cardiac dysfunction in severe illness, which invariably clears with disease resolution. *Coxiella burnetii* (the agent of Q fever) is a rare cause of myocarditis, but this may progress to heart failure and death. [11]

Parasites

Many parasites are known to cause chronic myocarditis and sustained myocardial dysfunction, primarily in the developing world. Chagas' disease (American trypanosomiasis), which is widely distributed in Central and South America, is caused by the protozoan *Trypanosoma cruzi*. The organism enters the human host via the bite of the reduviid bug. Rarely, patients develop fever, myalgias, hepatosplenomegaly and myocarditis during acute infection, when myocardial parasite are abundant. Far more common is the development of biventricular failure from chronic myocarditis, which occurs in 30% of infected individuals. In chronic Chagas' disease the heart is enlarged and microscopic evidence of focal mononuclear cell infiltrates is commonly found, despite the fact that parasites may be demonstrated in only a minority of patients. A history of residence in Central or South America should increase clinical suspicion for this pathogen.

Assessment of epidemiologic exposure is also essential for the diagnosis of *Trichinella spiralis*, an important parasite with worldwide distribution that has been linked to fatal myocarditis. Myocarditis generally develops in severe infections, in which the cardinal features of periorbital edema, myositis, fever and eosinophilia are present. Recent consumption of poorly cooked pork enhances the likelihood of this diagnosis. Other parasites and/or their ova, including ascaris, schistosomes and *Taenia solium*, may lodge in the myocardium during their systemic phase. The presence of eosinophilia in the context of acute heart failure should prompt a search for their presence, although eosinophilia is an important distinguishing factor for patients who have hypersensitivity myocarditis as well.

Viruses

Although any infectious agent may produce inflammation within the myocardium (Table 58.1), viruses are thought to cause the majority

of cases of acute infectious myocarditis in the Western world. Historically, outbreaks of mumps, influenza, measles, poliomyelitis and enterovirus-associated pleurodynia were commonly associated with classic findings of myocardial involvement. In the present era of widespread immunization, the enteroviruses (e.g. echovirus, Coxsackie A and Coxsackie B viruses) have been most commonly implicated in clinically significant myocarditis. As the amplification of viral genome by polymerase chain reaction (PCR) is increasingly applied to explore the role of viruses in myocarditis and dilated cardiomyopathy, other pathogens have also received attention. Recent molecular studies have alternatively implicated[12] or dismissed[13] adenovirus as a cause of myocarditis in the immunocompetent host. Given the high worldwide rates of chronic infection with hepatitis C virus (HCV), recent report of a link between HCV and dilated cardiomyopathy[14] [15] have stirred considerable interest but also controversy.[16] [17]

Until recently, evidence of a causal link between enterovirus infection and myocarditis was primarily circumstantial. For example, many individuals who have acute myocarditis present with flu-like symptoms; in one prospective study of patients who had biopsyproved myocarditis, over 50% had experienced an antecedent viral syndrome in the previous 3 months.[18] Observations of increased enteroviral antibody titers or a fall in convalescent titers have been offered as further evidence that enteroviruses are the causative agents. Unfortunately, these infections are common in the general population and it has been difficult temporally to associate serologic changes with changes in cardiac function. In one study it was found that 34% of patients who had IDC had Coxsackie virus B titers of 1:40, but the same incidence was found in control individuals.[19] However, if a cut-off antiviral titer of 1:1024 was used, 30% of patients who had IDC were abnormal as compared with 2% of control individuals.[20] Viral culture from myocardial biopsies has not been revealing.

Given the lack of confirmatory culture or serologic data, animal models of viral myocarditis have been constructed to demonstrate the pathogenicity of the enteroviruses. Murine models of myocarditis induced by Coxsackie B virus have been extensively studied and have shed light on the possible immunopathogenesis of infection. In this model, acute viral infection is characterized by viral attachment, myocyte penetration and subsequent viral replication, with resulting scattered foci of cellular necrosis. This initial phase terminates with viral clearance by mononuclear cells and the expression of proinflammatory cytokines, including tumor necrosis factor and interferon-?.[21] The chronic phase of infection is characterized by the presence of macrophages and T cells, not replicating virus, within myocardial tissue. Interestingly, depletion of T cells prevents the later stages of myocardial injury. Further cellular injury or scars do not form, although replicating virus persists within the myocardium after T-cell depletion.

These experiments highlight the importance of T cells in the perpetuation of myocardial injury. The antigens to which they are directed, however, remain unknown. Segments of the viral genome itself have not been shown to induce chronic immune activation. Rather, it appears that autoimmune reactivity to a novel tissue antigen induced by infection may be involved.[22] In addition, circulating autoantibodies against mitochondrial, contractile and adrenergic receptor proteins have recently been demonstrated in humans,[23] although their role in the pathogenesis of myocardial damage remains unclear. Finally, it is important to consider that the host genetic background may determine whether viral exposure results in a self-limited infection or chronic myocarditis. Indeed, asymptomatic relative of patients with cardiomyopathy often have echocardiographic evidence of ventricular dysfunction[24] or circulating autoantibodies against cardiac proteins.[25]

Although it is clear that direct injury to cardiac myocytes is important in many cases of neonatal myocarditis and some cases of acute myocarditis in adults, the studies noted above have led to the hypothesis that acute viral myocarditis more commonly predates chronic inflammation within the myocardium. The resulting chronic myocarditis may be the final common pathway to IDC, which accounts for 25% of cases of heart failure in the USA. Studies using molecular techniques suggest that 18–53% of patients who have myocarditis or IDC may have persistent enteroviral infection.[26] In a representative study, enteroviral RNA was detected in six out of 19 patients who had IDC; no RNA could be detected in a control group of 21 patients who had other cardiac disorders.[27] Histologic evidence of myocarditis and persistence of enteroviral genomic sequences, however, often appear as independent variables in many studies, and the findings correlate poorly with one another. A recent study using nested PCR demonstrated enteroviral sequences in only 7% of the patient sample; sequence analysis of the amplified products casts doubt on the true positivity of even these samples.[28] Moreover, the isolation of intact replicating virus from 'infected' tissue remains elusive, suggesting that any detected sequences are viral fragments that may merely be markers of previous infection. Whether these fragments result in antigenic persistence, forming a nidus for chronic autoimmunity, remains to be determined. Perhaps more importantly, the absence of replicating virus in most cases of myocarditis limits the potential application of

antiviral agents (see below).

Immunocompromised patients

Immunocompromised patients are subject to the same infections as the immunocompetent; however, their risk for clinically significant myocarditis is higher than in the general population, and they are uniquely at risk for opportunistic pathogens. In end-stage AIDS, as many as 10% of patients will have clinically significant cardiomyopathy.[29] In a study of 33 patients infected with HIV who underwent cardiac biopsy, specific DNA hybridization demonstrated HIV in five out of 33 patients and cytomegalovirus (CMV) in 16, suggesting that cardiotropic viral infections may be important in pathogenesis.[30] Although HIV itself has been cultured from heart tissue[31] and shown to be present by in-situ hybridisation,[32] it is only rarely present in cardiac myocytes in patients with demonstrable pathology. Thus, whether the virus itself causes heart failure, sets the stage for other cardiotropic pathogens, or is a correlate for the causative nutritional wasting present in late stage AIDS is unknown. In children with AIDS, myocarditis is perhaps even more common post-mortem than in adults, and may be associated with different pathogens; in one study, adenoviruses were detected in over 30% of hearts with histologic evidence of myocarditis.[33] Both *Cryptococcus* neoformans[34] and *Toxoplasma* gondii[35] are also important opportunistic causes of myocarditis in the setting of advanced HIV disease. Whether the use of highly active antiretroviral therapy has decreased the incidence of myocarditis remains to be determined.

Other disseminated fungal infections (such as disseminated candidiasis, aspergillosis and histoplasmosis) or viral infections such as herpes simplex virus or varicella-zoster virus may also present with myocarditis in the immunocompromised patient, but can usually be identified by associated findings. Cytomegalovirus is an important pathogen in solid organ and hematopoietic stem cell transplant recipients with myocarditis. In heart transplant recipients, CMV infection is a risk factor for a form of immune-mediated cardiac rejection that presents as accelaerated coronary atherosclerosis; importantly, prophylactic ganciclovir significantly reduces the incidence of this complication.[36] Finally, the role of CMV in myocarditis of immunocompetent patients is also intriguing; a recent study found intramyocyte CMV DNA in 14% of patients who had myocarditis but none was found in control individuals.[37]

TABLE 58-2 -- Noninfectious causes of myocarditis.

NONINFECTIOUS CAUSES OF MYOCARDITIS	
Connective tissue disorders	Systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, dermatomyositis, polymyositis
Idiopathic inflammatory/infiltrative disorders	Kawasaki disease, sarcoidosis, giant cell myocarditis
Insect and arachnid stings	Wasp, scorpion, spider stings
Medications	Cocaine, ethanol, arsenic, cyclophosphamide, daunorubicin, adriamycin, sulfonamides, tetracycline, methyldopa
Post-irradiation myocarditis	
Peripartum myocarditis	
Pheochromocytoma	
Thrombotic thrombocytopenic purpura	
Thyrotoxicosis	

A wide variety of other diseases may cause myocardial inflammation. Important noninfectious causes of myocarditis are listed in Table 58.2 .

CLINICAL FEATURES

The clinical expression of acute myocarditis ranges from an asymptomatic state to rapidly progressive myocardial dysfunction and death. Complaints on presentation may include fever, fatigue, malaise, chest pain, dyspnea and palpitations; arthralgias and upper respiratory tract symptoms may be associated with viral myocarditis, but are non-specific. Chest pain may be vague, pleuritic (suggesting pericardial involvement) or angina-like. The majority of patients, however, have no precordial discomfort.

Physical examination may reveal tachycardia out of proportion to the height of fever or degree of heart failure. Cardiac auscultation may be unrevealing, or may demonstrate muffled heart sounds, extra beats, transient murmurs or loud ventricular gallops; friction rubs are uncommon and indicate pericardial involvement. In severe cases, signs of congestive heart failure (CHF) are present, and pansystolic apical pulses may be palpated.

The electrocardiographic manifestations of myocarditis are usually transient and occur far more frequently than clinical myocardial involvement. Indeed, mild aymptomatic myocardial involvement diagnosed by serial electrocardiographic changes was present in over 1% of military conscripts with acute viral syndromes. ST segment elevation and T wave inversions may be seen acutely, and reflect the focal nature of the myocardial inflammation. These changes usually return to normal within 2 months. Atrial and ventricular arrhythmias are common in severe cases. Atrioventricular nodal or intraventricular conduction defects denote involvement of the conduction system, and suggest more widespread disease or specific etiologies (e.g. Lyme carditis). Although usually transient and without sequelae, complete heart block may cause sudden death in these patients. Routine radiologic examination demonstrates cardiomegaly and pulmonary vascular congestion in severe cases.

DIAGNOSIS

The clinical diagnosis of myocarditis is often difficult, and requires a high index of suspicion. When unexplained heart failure or malignant arrhythmias occur in the setting of an acute febrile illness, the clinical diagnosis of infectious myocarditis is suggested. Heart failure of recent onset mandates that the physician first consider ischemic, valvular, primary pulmonary or congenital disease in the differential diagnosis. Other causes of acute myocardial dysfunction, such as rheumatologic disease, endocrinopathies, electrolyte disturbances and toxin exposure (e.g. ethanol, cocaine and heavy metals), must also be ruled out.

Electrocardiogram changes are important ancillary findings but, given the high incidence of non-specific ST segment and T wave changes seen in acute viral syndromes, they alone are often non-diagnostic. Similarly, laboratory abnormalities such as leukocytosis and an elevated erythrocyte sedimentation rate are also non-specific. Serum creatinine phosphokinase (CPK) elevations, on the other hand, do signify acute myocardial injury and thus are very important. Unfortunately, these elevations do not differentiate the cause of that injury (i.e. ischemic versus inflammatory). Myocardial enzyme release parallels myocyte necrosis, as in small-to-moderate myocardial infarctions. However, in contrast to ischemic necrosis, in which CPK levels return to normal within 72 hours, elevated CPK levels may persist for 6 days in myocarditis. [38] The CPK-MB is elevated in 70% of patients who have myocarditis and ST segment elevation on ECG, but is usually normal if only T wave changes are present. Although serum assays for troponin T (a cardiac contractile protein) offer greater specificity for myocardial damage and persist for 2–3 days longer than CPK-MB determinations, they also are not specific for myocarditis.

Echocardiography is a valuable tool in the initial evaluation of the patient and in follow-up. Echocardiograms in myocarditis commonly show variable degrees of cardiac dysfunction, often with striking focal wall motion abnormalities. Dyskinesia or akinesia is most often biventricular. Furthermore, the test may eliminate other anatomic causes of CHF and may demonstrate the presence of ventricular thrombi or pericardial effusions. Echocardiographic changes generally resolve within a few days in parallel with the clinical course; if progressive ventricular dysfunction is demonstrated, chronic myocarditis may be suggested.

Many other imaging modalities have been evaluated in acute myocarditis. Gallium scanning may demonstrate inflammation within the myocardium; the sensitivity and specificity of this test, however, is unknown. Indium-111 labeled antimyosin antibody scans have been shown to identify the degree of myocyte necrosis, but this finding does not help in determining the cause of this necrosis. Magnetic resonance imaging is sensitive to the alterations in the water content of tissue that occur in the inflammatory response. Although this modality has shown promise in children who have myocarditis,[39] it has not been formally evaluated in adults.

Despite advances in imaging techniques, the definitive diagnosis of myocarditis can only be made by endomyocardial biopsy. Because the inflammation involves both ventricles, a transvenous approach is generally employed in order to obtain a biopsy of the right ventricular septum. In experienced hands the procedure is relatively safe, although deaths have occurred. Biopsy studies in acute disease have generally been carried out in cases of fulminant myocarditis. Studies using serial biopsies have demonstrated findings similar to those seen in the murine model. In the majority of cases, histologic evidence of myocarditis has resolved 3–4 weeks after the onset of symptoms.[40] Late biopsy is thus unhelpful. Furthermore, because of the focal nature of the disease, many practitioners have recommended obtaining four or

five biopsies from different sites at initial catheterization. Repeat biopsies and/or left heart catheterization with biopsy have sometimes been necessary to establish the diagnosis firmly but increase the risk of complications such as ventricular wall rupture.

Disagreement among pathologists regarding interpretation of specimens has also been problematic. Recently, a working standard,

termed the Dallas criteria, was established; these guidelines are now used by the majority of investigators to define the disease.[40]

- Active myocarditis is defined as 'an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischemic damage associated with coronary heart disease'.
- Borderline myocarditis is present when infiltration is sparse, or when cardiac myocytes are infiltrated with leukocytes, but associated myocyte necrosis is not present; myocarditis cannot be diagnosed in the absence of inflammation.
- The absence of myocarditis indicates normal myocardium or pathologic changes of a noninflammatory nature (fibrosis, atrophy, hypertrophy).

The adoption of the Dallas criteria has led to 90% concordance among experienced pathologists. Application of the criteria in the Multicenter Treatment Trial for Myocarditis (MTT) suggests that the prevalence of 'active myocarditis' in patients who have recent onset (<12 months) CHF is approximately 10%;[41] a recent study demonstrated identical findings.[42] However, these patients are qualitatively different from those who have acute infectious myocarditis in which fever and acute ECG changes are present. They are best characterized as having IDC with myocardial inflammation.

The rationale for the development of the Dallas criteria was to identify patients prospectively who were likely or unlikely to benefit from immunosuppressive therapy. Given the results of the MTT (see below), many physicians have called into question the utility of the endomyocardial biopsy. In selected cases, however, the biopsy can provide valuable clinical data. Rarely, a biopsy will identify specific disease processes (i.e. toxoplasmosis, CMV, giant cell myocarditis, trichinosis, sarcoidosis) for which specific therapy is available or for which a prognosis can be given (i.e. Pompe's disease, amyloidosis). Because the prognosis in most cases of acute myocarditis is favorable (and an etiology is rarely discovered in cases of cardiomyopathy of gradual onset), we believe that the endomyocardial biopsy is unnecessary for the majority of patients with suspected myocarditis. Notable exceptions include transplant recipients and patients who have AIDS, for whom the discovery of a treatable etiology is more likely. In the setting of progresive clinical deterioration (such as arrhythmias or heart failure refractory to standard therapy) in which the diagnosis is unknown, endomyocardial biopsy should also be considered.

Thus, the approach for the immunocompetent patient who has acute myocarditis should focus on the management of CHF and its complications (see below). A thorough history (with attention to epidemiologic detail) and physical examination will unearth the majority of nonviral etiologies, in which signs and symptoms other than CHF frequently dominate the clinical picture. In the febrile patient, blood cultures should be obtained. Serologic testing for HIV should always be performed given the high prevalence of myocarditis in patients with AIDS; testing for Lyme's disease, Chagas' disease and autoimmune diseases should be performed in the appropriate clinical context. A complete blood count with differential should be performed to rule out eosinophilia (which may suggest parasitic infection or hypersensitivity myocarditis). Testing for CMV in blood (using assays such as pp65 antigenemia assay or PCR for CMV DNA) is unlikely to be helpful because CMV reactivation in the presence of unrelated acute febrile illnesses is relatively common. Athough enteroviruses are prominent pathogens, routine use of acute and convalescent enteroviral titers adds little to clinical management. Noninvasive testing for enteroviruses and adenoviruses by serum PCR is possible (although not widely available), but studies have not been performed to demonstrate that this information affects outcome. Perhaps future advances in virology and molecular biology coupled with targeted antiviral thrapy will provide more options for the diagnosis and treatment of myocarditis; for now, however, the majority of cases remain classified as 'idiopathic' and are treated expectantly.

MANAGEMENT

The natural history of acute infectious myocarditis is quite variable, although the majority of cases run a benign, self-limited course. Acute cardiac dysfunction does not predict chronic impairment, as most of these individuals demonstrate normalization of laboratory, echocardiographic and histologic parameters within 1 month of symptom onset. For this reason, supportive care in lieu of aggressive, invasive procedures is of primary importance.

General measures target CHF, arrhythmias and other derangements associated with myocarditis. Animal models have demonstrated that exercise during viral myocarditis is associated with higher mortality and more extensive histologic damage; thus, bed rest may be important. Conventional therapy has included oxygen, diuretics, digoxin and sodium restriction, as for any patient who has CHF. Most practitioners recommend anticoagulation for all patients who have documented intracardiac thrombi, and for those who have severely depressed myocardial function in order to prevent thromboembolic complications. In the murine model, early treatment with the angiotensin converting enzyme (ACE) inhibitor captopril decreased left ventricular mass and myocardial necrosis, suggesting benefits above and beyond afterload reduction. [43] Randomized trials in humans have yet to be conducted, although afterload reduction appears safe and effective in other settings. Rarely, fulminant heart failure requires the use of inotropic support, intra-aortic balloon pumps or ventricular assist devices as a bridge to the resumption of cardiac function. Unfortunately, there are no clinical or laboratory indicators to identify those patients who will spontaneously recover; indeed, patients who have fulminant myocarditis may have a better prognosis than those with acute, nonfulminant myocarditis. [44] For those who do not respond, cardiac transplantation is an option; however, patients who have myocarditis before transplantation have a significantly higher incidence of rejection and death in the first year.

As significant arrhythmias are probably associated with the majority of deaths in acute myocarditis, all hospitalized patients should be monitored on telemetry. Premature beats are common and do not require therapy. Supraventricular tachycardia, however, worsens heart failure and should be electrically converted. The use of antiarrhythmic agents for high-grade ventricular ectopy has not been studied. Care should be exercised with any antiarrhythmic agent (including digoxin, which has a low threshold for toxicity in these patients) but sustained ventricular arrhythmias may be cautiously treated, as in ischemic heart disease. Complete heart block is an indication for temporary venous pacing. The condition often resolves without a need for permanent pacemaker placement.

If a specific infection is identified (such as Lyme or staphylococcal carditis), antimicrobial therapy should be directed at the causative pathogen. As noted above, viruses are the likely cause of most cases of myocarditis, but patients who have putative viral myocarditis most commonly present after viral replication has ceased. Murine models of CMV-induced myocarditis confirm that ganciclovir or cidofovir improve outcomes in the acute (<24 hours) phase of infection, but are ineffective later.[45] Pleconaril is a novel compound that has antiviral activity against the enteroviruses (including Coxsackie viruses A and B) and thus has potential for treating the most common etiology of myocarditis. Clinical response was favorable in three-quarters of patients treated in one report, [46] but high rates of spontaneous recovery make conclusions speculative. Antiviral therapy with interferon-a for patients with enterovirus-induced myocarditis is also curently under study.[47]

Contrary to expectations, most studies of anti-inflammatory therapy and immunosuppression have not favorably influenced outcome. Nonsteroidal anti-inflammatory drugs (NSAIDs)[48] and ciclosporin[49] were associated with more severe histologic damage when used in the acute stage of murine viral myocarditis. For the most part, late stage immunosuppression has also been disappointing. In the MTT, the largest clinical trial of immunosuppressive therapy for myocarditis to date, 111 patients who had biopsy-proved myocarditis and CHF for less than 2 years were randomized to receive placebo or combination therapy with prednisone (prednisolone)/ciclosporin or prednisone/azathioprine. At 28 weeks, left ventricular ejection fraction improved in both groups from 0.25 to 0.34; there was no significant difference in mortality between the two groups at 1 year (20% in all patients) and 4.3 years (56%). Interestingly, markers of effective immune response were associated with a more favorable outcome.[50]

Intavenous immunoglobulin (IVIg) showed promise in early uncontrolled studies, but a recently conducted randomized trial of 62 patients with recent acute onset (<6 months) dilated cardiomyopathy showed no effect of IVIg when compared with placebo, even among the subgroup with histologic inflammation on biopsy.[51] However, a recently published study has opened the door for further studies on immunosuppression for myocarditis. The authors randomized 84 patients with evidence of HLA activation on endomyocardial biopsy to prednisone and azathioprine versus placebo. After 2 years, the primary end point of death, heart transplantation or readmission was not different between the two arms, but immunosuppressed patients had significantly higher ejection fractions and improvements in functional status when compared with placebo. [52] Interestingly, the Dallas criteria would have classified only 23% of the biopsies from these 84 patients with HLA unregulation as having either active or borderline myocarditis. Thus, it is quite possible that newer tests for inflammation will better identify subgroups appropriate for targeted therapy.

Ongoing studies of cytokine inhibitors such as Etanercept (which blocks tumor necrosis factor-a) in patients with CHF may provide additional options for treatment;[53] drugs that target other cytokines are likely to be tested in the future. Standardization of immunohistologic techniques to detect HLA activation in biopsy specimens, further studies on markers of immune activation, and the use of these markers to identify responsive subgroups of patients with CHF are needed before targeted immunosuppression (and the resulting revival of widespread endomyocardial biopsy) is again ready for primetime.

PERICARDITIS

EPIDEMIOLOGY

Interest in the pericardium dates to antiquity. The writings of Homer and Maximus relate the history of the 'hairy hearts of heroes' such as Aristomenes, the legendary Messinian warrior; his heart was cut out in battle and found to be 'stuffed with hair', probably the first recorded case of fibrinous pericarditis. Significant understanding of the etiology and pathophysiology followed, from Galen's first pericardial resection in the 2nd century AD to Lower's classic description of tamponade, constrictive pericarditis and pulsus paradoxus in the 17th century. Medical advances in antibiotic therapy, surgical technique, antineoplastic therapy and hemodialysis have substantially altered the spectrum and prognosis of pericardial disease in the 20th century. Imaging modalities have also made a significant impact; since the advent of echocardiography in 1955, pericardial effusions are now readily diagnosed. The etiologic determination of pericardial disease, however, remains difficult. Timely, directed therapy depends in large part on the diligence of the clinician.

The incidence of pericardial inflammation detected in several autopsy series ranges from 2–6%, whereas pericarditis is diagnosed clinically in only about 1 out of 1000 hospital admissions. The relative frequency of each etiologic process depends upon the clinical setting. Thus, viral or idiopathic pericarditis often presents in the outpatient clinic, whereas malignant or uremic effusions are more frequently seen in referral centers.

PATHOGENESIS AND PATHOLOGY

The human pericardium forms a strong, flask-shaped sac that encloses the heart and the origins of the great vessels. It is composed of a fibrous outer layer and an inner serous membrane formed by a single layer of mesothelial cells. This membrane is attached to the surface of the heart to form the visceral pericardium; it reflects upon itself, lining the inside of the collagen-based fibrous layer to form the parietal pericardium. The visceral pericardium continuously produces a clear pericardial fluid, which serves as a lubricant; it is also the source of excess fluid in disease states. The human pericardium normally contains up to 50ml of this fluid, which drains via the thoracic and right lymphatic duct into the central circulation.

Pericardial effusion may develop in response to pericardial injury (as in pericarditis) or may be secondary to other processes that alter the secretion and drainage of pericardial fluid. The pathologic changes seen in acute pericarditis are those of non-specific inflammation with cellular infiltration, fibrin deposition and the outpouring of pericardial fluid. These changes may resolve spontaneously over time, or may organize with fibrous adhesions between the epicardium and visceral pericardium, the visceral and parietal pericardium, or the pericardium and adjacent sternum and pleura (Fig. 58.2). Thus, inflammation, fluid exudation, and fibrin organization account for the cardinal manifestations of pericarditis: chest pain, pericardial effusion, and constriction.

The causes of this pericardial inflammation are numerous, including both infectious (Table 58.3) and noninfectious (Table 58.4) etiologies.

Noninfectious agents

The three most prevalent noninfectious causes of pericardial disease are malignancies, uremia and connective tissue disorders.

Figure 58-2 Heart at autopsy of a patient who had acute suppurative pericarditis. The parietal pericardium has been stripped from the specimen, revealing a 'bread and butter' appearance.

TABLE 58-3 -- Infectious causes of pericarditis.

INFECTIOUS CAUSES OF PERICARDITIS
Viruses
Cytomegalovirus
Herpes simplex virus
Coxsackie A virus
Coxsackie B virus
Echovirus
Adenovirus
Influenza
Mumps
Varicella-zoster virus
Epstein-Barr virus
HIV
Bacteria
Streptococcus pneumoniae
Streptococcus spp.
Staphylococcus aureus
Neisseria meningitidis
Listeria monocytogenes
Haemophilus influenzae
Francisella tularensis
Brucella melitensis
Enteric Gram-negative rods
Actinomyces spp.
Nocardia asteroides

Legionella pneumophila
Tropheryma whippelii
Salmonella spp.
Campylobacter spp.
Rickettsial/Q fever
Mycobacteria
Mycobacterium tuberculosis
Mycobacterium chelonae
Mycobacterium avium complex
Spirochetes
Borrelia burgdorferi
Mycoplasma
Mycoplasma pneumoniae
Ureaplasma urealyticum
Mycoplasma hominis
Fungi
Histoplasma capsulatum
Coccidioides immitis
Cryptococcus neoformans
Blastomyces dermatiditis
Candida spp.
Aspergillus fumigatus
Parasites
Toxoplasma gondii
Entamoeba histolytica
Echinococcus granulosus
Schistosoma spp.

TABLE 58-4 -- Noninfectious causes of pericarditis.

NONINFECTIOUS CAUSES OF PERICARDITIS
Idiopathic
Connective tissue disorders
Acute rheumatic fever, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, mixed connective tissue disease, Wegener's granulomatosis, polyarteritis nodosa, temporal arteritis
Metabolic
Uremia, hypothyroidism
Malignancies
Lung cancer, breast cancer, leukemia, lymphoma, melanoma, others
Acute myocardial infarction
Post-myocardial infarction syndrome (Dressler syndrome)
Dissecting aortic aneurysm
Traumatic
Chest trauma, post-surgical hemopericardium, pacemaker insertion, cardiac catheterization, esophageal rupture, pancreatic-pericardial fistula
Post-irradiation
Idiopathic infiltrative/inflammatory disorders
Sarcoidosis, amyloidosis, inflammatory bowel disease, Behçet's disease, FMF
Medications
Procainamide, hydralazine, isoniazid, phenylbutazone, dantrolene, doxorubicin, dilantin, methysergide, minoxidil
FMF, familial Mediterranean fever.

¦ Neoplasms may cause pericarditis or effusions by direct involvement of the pericardium (in which malignant cells are usually demonstrated within pericardial fluid) or by obstruction of the pericardial lymphatic drainage (with resulting 'benign' effusions).
¦ Uremic pericarditis, which affects up to 20% of patients on chronic hemodialysis, is characterized by the appearance of a shaggy, fibrinous exudate without cellular infiltration.
¦ Collagen vascular diseases (most commonly systemic lupus erythematosus and rheumatoid arthritis) are notable for their propensity to involve the pericardium; immune complex deposition is thought to be primary in the pathogenesis of pericardial disease.

Infectious agents

A variety of microbes have been reported to cause pericarditis. Chief among these are viruses, which can produce a clinical syndrome of myopericarditis, but often other infectious agents are also implicated.

Viruses

Coxsackie virus and echovirus type 8 have been most commonly identified. As is the case for myocarditis, these viruses have only rarely been isolated from pericardial fluid or tissue; as such, evidence for viral causation of pericardial inflammation is primarily based upon isolation of virus from other sites, such as stool, by demonstration of a four-fold rise in serum antibody titers and, more recently, by PCR. Historic evidence, however, favors a viral etiology for the majority of community-acquired 'idiopathic' pericarditis. For example, epidemic pleurodynia (Bornholm disease), caused by Coxsackie virus infection, has long been noted to be epidemiologically associated with outbreaks of self-limited pericarditis. In addition, most cases of idiopathic pericarditis seem to cluster in the spring and autumn, coincident with outbreaks of enteroviral infections.

Many other viruses have been associated with pericarditis (see Table 58.3). For example, CMV has been pathologically confirmed as a cause of pericardial disease in

the immunocompetent patient who has the CMV mononucleosis syndrome. One series reported five cases of culture-proved CMV infection in patients who had large pericardial effusions. In four of these CMV cases, the patients were not immunosuppressed at the time of diagnosis. [54] In addition, CMV may be a common pathogen in HIV-infected patients. Herpes simplex pericarditis has also been documented both in patients who have AIDS and in patients who have uremia. Most recently, PCR testing has found evidence of adenovirus, enterovirus, CMV in pericardial fluid and tissue from patients with acute pericarditis.[55]

Bacteria and other infectious agents

Bacteria may cause pericarditis by a number of different mechanisms. Hematogenous seeding of the pericardium may occur during the course of bacteremia caused by a variety of organisms. In the pre-antibiotic era, most cases of purulent pericarditis were seen as complications of bacteremia or pneumonia. Today, extension of infection from a contiguous focus within the chest is seen as a postoperative or post-traumatic complication. Subdiaphragmatic abscesses may also cause pericardial infection by direct extension. Highly invasive bacterial infections within the heart, such as acute staphylococcal endocarditis, may erode into the pericardium from a perivalvular abscess to cause purulent pericarditis. Pericardial effusions in patients who have subacute infective endocarditis provide another mechanism by which bacterial infection can lead to pericardial disease. Pathologic changes in the pericardium are caused by immune complex deposition, resulting in sterile exudates. Although common, these effusions are not correlated with prognosis, and most resolve without specific therapy.

The microbiology of bacterial pericarditis continues to evolve (see Table 58.3). Before antibiotics, uncontrolled pneumococcal, streptococcal or staphylococcal pulmonary infections were most frequently implicated. Streptococci and staphylococci remain important pathogens today (particularly in traumatic and post-thoracotomy pericarditis), with Gram-negative bacilli, atypical bacteria and *Candida* also assuming important roles. Pericardial involvement has also been documented in the course of such illnesses as tularemia, brucellosis, salmonellosis, legionellosis, meningococcal disease and Q fever.[56] In children, bacteria cause proportionately more cases of pericarditis than is the case in adults; *Staphylococcus aureus* and *Haemophilus influenzae* are the most common etiologic agents.

Pericarditis caused by *Mycoplasma* spp. deserves special mention. Although pericarditis has been recognized in the course of *Mycoplasma* disease since 1944, culture of the organism has proved difficult. Therefore, autoimmune phenomena have been invoked to explain the association. Recently *Mycoplasma pneumoniae*, *M. hominis* and *Ureaplasma urealyticum* were isolated from culture of pericardial fluid and/or tissue in five patients who had large pericardial effusions.[57] Treatment with doxycycline after drainage of the effusions resulted in complete resolution in all five cases. Pericarditis caused by *Mycoplasma* spp. is thus more common than previously recognized, and fluid obtained for culture should always be analyzed for the presence of these organisms.

Mycobacteria continue to be important causes of acute pericarditis, pericardial effusion and constrictive pericarditis, particularly in developing countries. The incidence of tuberculous pericarditis among patients who have pulmonary tuberculosis ranges from 1–8%.[58] In Transkei, South Africa, tuberculous pericarditis with secondary constriction is the second most common cause of 'heart failure' after rheumatic disease.[59]

Histoplasma capsulatum is the most common cause of fungal pericarditis; in large outbreaks, pericarditis was noted in 6% of patients who had symptomatic histoplasmosis. It most commonly develops as a noninfectious inflammatory response that resolves spontaneously without therapy. Occasionally, seeding of the pericardium occurs in the course of disseminated infection. In contrast, pericarditis has only rarely been reported in cases of severe coccidioidomycosis.

In the severely immunosuppressed or post-thoracotomy patient, infection caused by *Candida* spp., *Aspergillus fumigatus* or *Cryptococcus neoformans* has occasionally resulted from either fungemia or direct inoculation.

Pericarditis in AIDS

In contrast to other immunocompromised states, pericardial disease in patients who have AIDS is quite common. Effusions are frequently noted in end-stage AIDS (occurring in 16–40% of patients) and are associated with a poor prognosis. Etiologies include a variety of pathogens (including viruses, bacteria, fungi and mycobacteria), although in the majority of cases no causative agent can be defined. Malignant effusions secondary to lymphomatous involvement of the pericardium have also been noted. Furthermore, although extrapericardial disease suggested specific infectious or malignant etiologies in 55% of patients who had pericardial effusions in one trial, these assumptions proved incorrect for all those in whom pericardiocentesis was performed.[60]

CLINICAL FEATURES

Acute pericarditis is most often recognized by its chief presenting manifestation: chest pain. The pain is usually precordial or retrosternal, often with radiation to the trapezius ridge or neck; it is exacerbated by lying supine, coughing or deep inspiration, with relief upon sitting upright or forward. The patient's discomfort may be caused by inflammation of the adjacent pleura, accounting for the pleuritic component that often accompanies the pain. This pain is distinguished from the pain of myocardial ischemia by its quality, its duration (pain may last for days without therapy) and the absence of associated factors (i.e. pain is unchanged with exertion or rest). Patients may also report that splinting reduces the pleuritic discomfort.

A pericardial friction rub is the pathognomonic physical finding of acute pericarditis. Characterized as scratchy or grating, it is best appreciated along the left sternal border with respirations suspended and the patient leaning forward. The classic friction rub has three components, corresponding to atrial systole, ventricular systole and the rapid ventricular filling phase of early diastole, although one or more of these phases are usually absent. Of note, the friction rub frequently waxes and wanes in intensity and may disappear altogether with the accumulation of fluid within the pericardial sac. The pericardial rub may again become prominent in tamponade, in which the pericardium rubs against the adjacent pleura.

Pericardial effusions range from the asymptomatic to those causing cardiac tamponade. The rate of fluid accumulation is a major determinant in physiologic manifestations. When the effusion develops slowly, the pericardium may stretch to accumulate as much as 2 liters of fluid. The normal pericardium, however, can accommodate the rapid accumulation of only 100–200ml of fluid before signs and symptoms of tamponade develop. Patients may then complain of dyspnea or a dull retrosternal ache, and examination will reveal jugular venous distension, the most common physical finding in acute tamponade. A fall of 10mmHg or more in systolic blood pressure during inspiration (the pulsus paradoxus) is recognized as a hallmark of critical cardiac tamponade, although it may be absent if hypotension is already present.

Enlargement of the cardiac silhouette on routine radiography does not usually occur until at least 250ml of fluid have accumulated in the pericardial space. Other findings on chest radiogram, such as a 'water bottle' heart (Fig. 58.3) or a prominent fat stripe sign, are found only in large pericardial effusions; their absence does not rule out the presence of a hemodynamically significant effusion. Chest radiograms may also provide etiologic clues for pericardial disease, such as pneumonic infiltrates or mediastinal adenopathy.

Electrocardiographic changes in acute pericarditis imply inflammation of the pericardium. Thus, in uremic or neoplastic pericardial

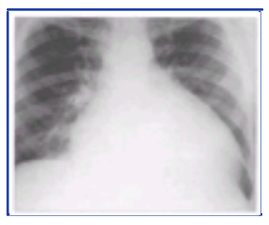

Figure 58-3 Cardiomegaly in a patient who has pericarditis. The presence of a 'water-bottle' heart on this plain film suggests a large pericardial effusion.

Figure 58-4 Electrocardiogram of a patient who has early acute pericarditis. Note the presence of diffuse ST segment elevation and PR depression in the inferolateral leads

(arrows). 25mm/s; 10.0mm/mV; F–W 0.05–100.

effusions, characteristic ECG changes are often absent. Cardiac arrhythmias are uncharacteristic in isolated pericardial disease; their presence implies myocardial involvement. The ECG typically evolves through four stages during acute pericarditis.

¦ Diffuse ST-segment elevation (usually concave up) with reciprocal ST depression in aVR and V1 accompanies the onset of chest pain and is virtually diagnostic of pericarditis; these findings are present in 50% of patients who have acute pericarditis. [61] PR depression in the inferolateral leads is frequently seen in this stage (Fig. 58.4).
¦ ST and PR segments normalize, typically several days later.
¦ Diffuse T wave inversions develop, generally after ST segments become isoelectric.
¦ Electrocardiograph changes normalize; long-term inversion of T waves suggests 'chronic' pericarditis.

Elevated CPK and troponin I levels are common in cases of acute pericarditis. Whether this signifies clinically relevant myocardial changes has not been adequately evaluated.

DIAGNOSIS

A number of studies have established the utility of using a stepped approach.[62] One study prospectively evaluated 231 consecutive patients who had acute pericardial disease of unknown cause.[63] Pericardiocentesis was performed in patients who had tamponade, suspicion of purulent pericarditis or symptoms and/or effusion persisting for more than 1 week after initiation of NSAID therapy. Pericardial biopsy was undertaken if clinical activity persisted at 3 weeks and the etiology was unknown. Despite this extensive evaluation, a specific diagnosis was confirmed in only 32 patients: neoplasia in 13, tuberculosis in nine, rheumatic disease in four, purulent pericarditis in two, toxoplasmosis in two and viral pericarditis in two. Diagnostic yield was substantial when pericardiocentesis or biopsy were performed to relieve tamponade, but poor when used solely for diagnostic purposes. Over a mean follow-up of 31 months, no patient diagnosed with idiopathic pericarditis and treated with NSAIDs showed signs of recurrent or chronic pericardial disease.

Because of the recent interest in subxiphoid pericardial biopsy and drainage of pericardial effusions, we recently undertook a prospective nonrandomized trial of all patients who had large pericardial effusions hospitalized at our institution. These patients underwent a similar stepped preoperative approach, with subsequent subxiphoid pericardial biopsy and drainage of their effusions.[64] Diligent handling and extensive microbiologic analysis (including cultures for aerobic and anaerobic bacteria, viruses, chlamydiae, mycoplasmas, fungi and mycobacteria) of pericardial fluid and tissue allowed specific diagnoses to be established in 53 out of 57 patients, confirming prior reports of high diagnostic yield when stepped algorithms are used for large effusions. More than one-third of the patients had malignancy or a history of irradiation to the thorax for malignancy. Infections (mostly viral), collagen-vascular disease and uremia were also frequently implicated (Table 58.5). Unexpected

TABLE 58-5 -- Etiology of large pericardial effusions[64] .

ETIOLOGY OF LARGE PERICARDIAL EFFUSIONS	
Etiology	**% of 75 diagnoses**
Malignancy	27
Viral	16
Collagen vascular disease	14
Radiation	11
Uremia	11
Mycobacterial	5
Mycoplasma	3
Bacterial	1
Idiopathic	5
Other	8

pathogens included CMV in three patients, herpes simplex virus 1 in one, *M. pneumoniae* in two, *Mycobacterium avium* complex in one, and *Mycobacterium chelonei* (see Table 58.3) in one patient. No patient showed evidence of Coxsackie A or B viral infection. Comparison of this study with two other important series shows a significant variation in etiologies as a result of different populations, diagnostic strategies and investigator interests (i.e. infectious disease specialist versus cardiologist).

A comparison of diagnostic yield between pericardial fluid and biopsy demonstrated that fluid analysis was far more sensitive for malignancy; tissue provided additional information only in infected patients in whom fluid was not available for analysis. Previous studies demonstrated similar utility of pericardial fluid analysis; in a retrospective study of 93 cases of malignant effusion with both pericardial fluid and tissue analysis, cytology was correct in 87 cases (diagnostic accuracy 94%) with 100% specificity.[66]

In summary, acute pericarditis is most often viral or idiopathic in etiology; as such, invasive work-ups are usually not necessary. For the patient who has large effusions, tamponade, or presentation suggestive of purulent pericarditis, early and aggressive intervention will often yield diagnostic and therapeutic rewards.

As noted previously, a wide variety of infectious and noninfectious agents can cause acute pericarditis and/or pericardial effusions (Table 58.6). For the patient presenting with acute chest pain, initial evaluation should focus on identifying conditions that may be rapidly fatal. Thus, myocardial infarction, aortic dissection, purulent pericarditis and cardiac tamponade should be systematically ruled out. An appropriate work-up includes a thorough history and physical examination, ECG and chest X-ray (to rule out intrathoracic malignancy, tuberculosis or a widened mediastinum suggestive of aortic dissection) and routine laboratory studies including complete blood counts, serum chemistries, serial CPK with MB fraction determination, thyroid function tests, rheumatoid factor and antinuclear antibodies; blood cultures should be obtained for the febrile patient. Due to the serious consequences of untreated tuberculous pericarditis, a tuberculin skin test with appropriate controls should be placed as well.

For the majority of individuals, a specific etiology will not be apparent, and a diagnosis of acute viral or idiopathic pericarditis will be made. Because either entity typically follows a brief and benign course, a full diagnostic evaluation is not appropriate. The confirmation of a particular viral agent is not necessary, as serologic titers and/or viral cultures are quite non-specific, the work-up is costly and a retrospective diagnosis is usually not helpful in management. Because

TABLE 58-6 -- Moderate-large pericardial effusion trials.[65]

MODERATE-LARGE PERICARDIAL EFFUSION TRIALS			
Etiology	**Corey *et al.*[64] (%)**	**Colombo *et al.*[67] (%)**	**Sagrista-Sauleda *et al.*[68] (%)**
Effusion	>5mm	>10mm	>10mm
n	57	25	322
Tamponade	Not reported	44	37
Idiopathic*	7	32	20
Chronic idiopathic effusion	?	?	9
Neoplastic	23	36	13
Uremia	12	20	6

Iatrogenic	0	0	16
Post acute myocardial infarction	0	8	8
Viral	14	0	0
Collagen vascular disease	12	0	5
Tuberculosis	0	0	2
Other	9	4	21
?, no distinction between acute idiopathic pericarditis and idiopathic chronic pericardial effusion.			

* Acute idiopathic pericarditis.

significant pericardial effusions may accumulate even in idiopathic disease, however, all patients should be carefully evaluated for their presence; hemodynamic compromise on physical examination, cardiac enlargement on chest radiography, or significant effusions on echocardiography necessitate rapid intervention. For patients who have large effusions, and for those in whom another diagnosis is suggested, a more thorough work-up often includes pericardial drainage with or without pericardial biopsy.

As noted above, noninvasive diagnosis of pericardial disease in patients who have AIDS is extremely difficult. Because specific etiologic diagnosis often has important treatment ramifications, pericardial effusions in these patients should be managed on an individualized basis, with invasive diagnostic testing employed in those whose baseline health would benefit from aggressive therapeutic measures.

Echocardiography has largely replaced other methods for the detection of pericardial fluid. With experience, operators can detect as little as 20ml of excess fluid posterior to the left ventricle. Echocardiography can also provide ancillary data in assessing the patient who has an effusion. Increased respiratory flow variation across the mitral valve with Doppler echocardiography is characteristic of cardiac tamponade. In addition, other etiologies of myocardial dysfunction such as left and right ventricular infarction can be ruled out. Finally, the echocardiogram can direct attempts at pericardiocentesis by identifying the location of pericardial fluid.

Computed tomography (CT) scans of the chest are primarily helpful in the diagnosis of pericardial thickening. Significant effusions are also readily demonstrated (Fig. 58.5). In addition, CT scans are more sensitive for the demonstration of small parenchymal nodules and mediastinal lymphadenopathy than is conventional radiography, and thus have clinical utility in the diagnosis of malignant pericarditis. Similarly, magnetic resonance imaging may be useful in the non-invasive characterization of pericardial fluid as well as pericardial thickness.

Cardiac catheterization is reserved for patients in whom pericardial constriction is believed to play a role in symptomatology. In those who have chest pain of undetermined etiology, catheterization is also useful for ruling out myocardial ischemia as a confounding diagnosis.

MANAGEMENT

Nearly all patients who have acute pericarditis should be hospitalized for relief of symptoms, diagnostic evaluation and observation for complications. Specific medical therapy is tailored to the cause of pericarditis.

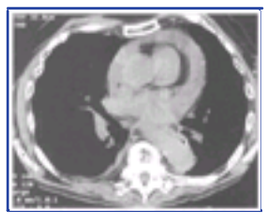

Figure 58-5 Chest CT of a patient who has a large crescent-shaped pericardial effusion.

Aspirin, at doses of 2–6g/day, or other NSAIDs are effective in reducing symptoms of pericarditis, and are the agents of choice for idiopathic or viral pericarditis. Corticosteroids should be reserved for symptomatic nonresponders. Prednisone, however, is the drug of choice in pericarditis associated with connective tissue disease.

Idiopathic or viral pericarditis generally follows a benign, self-limited course but the occasional patient will present with recurrent pericardial pain. Treatment for recurrences generally begins with NSAIDs; corticosteroids have been used successfully for NSAID-resistant cases. Colchicine has shown promise as a steroid-sparing agent in a number of small trials.[69] Pericardiectomy for recurrent pericarditis should be reserved for those who fail medical therapy. Interestingly, only one-third of patients will respond.[70]

Targeted intravenous antibiotics and surgical drainage of the pericardium remain the mainstays of therapy for purulent pericarditis. Pericardiocentesis urgently performed for the critically ill patient does not obviate the need for complete drainage and irrigation; fluid may re-accumulate rapidly, and sequelae such as constriction may develop in hours. There is no rationale for intrapericardial antibiotic administration, as pericardial penetration of antibiotic is excellent.

Tuberculous pericarditis remains a diagnostic and therapeutic challenge. Clinical features are non-specific, the disease course is confusing and laboratory evaluation is often nondiagnostic, particularly in low prevalence settings and in patients who have localized disease. For example, although large effusions are more likely to be tuberculous, up to 50% of tuberculous effusions resolve spontaneously despite ongoing tissue infection. The tuberculin skin test may be negative in up to 30% of patients as a result of cutaneous anergy yet may be positive in the patient who has acute idiopathic pericarditis and benign natural history. Suggestive, but not diagnostic, findings include a recent history of pulmonary tuberculosis, a positive sputum smear or culture, or a high pericardial fluid adenosine deaminase level (>45units/l). [71] Even granulomatous inflammation of the pericardium is not diagnostic, as this may be demonstrated in pericardial disease from other causes, such as histoplasmosis, sarcoidosis and rheumatoid arthritis. Additionally, a negative biopsy of the pericardium does not rule out tuberculous pericarditis, as removal of the entire pericardium may be necessary to demonstrate clear-cut evidence of tuberculosis.[72]

Definitive diagnosis rests upon the demonstration of the tubercle bacillus in pericardial fluid and/or tissue. However, the need for early therapy demands that treatment often be undertaken based upon a presumptive diagnosis. Initial treatment should consist of four drugs including isoniazid and rifampin (rifampicin) until sensitivities are known. The use of concomitant prednisone (at doses of 60mg/day initially) to reduce pericardial inflammation is supported by two large controlled trials in Transkei, South Africa. Clinical improvement occurred more rapidly, 2-year mortality was lower (4 versus 11) and pericardiectomy was required less often (21% versus 30%) compared with those treated with four-drug therapy alone.[73] These data also highlight the incidence of constrictive complications in this disease. Complete pericardiectomy is advocated for those who have recurrent effusions or cardiac compression with constrictive physiology after 4–6 weeks of oral therapy. Such early pericardiectomy is associated with a good outcome; mortality is substantially higher in patients who undergo pericardiectomy at the late stage of calcific pericardial constriction.[74] A recent review highlights these issues and the controversies that still remain.[75]

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Chapter 59 - Endocarditis and Endarteritis

Philippe Moreillon

INTRODUCTION

This chapter reviews infective endocarditis (IE) and other endovascular infections in the context of their evolving epidemiology, diagnostic tools and therapeutic strategies. These diseases were invariably lethal in the pre-antibiotic era. Fifty years ago, the introduction of penicillin followed by other antibiotics revolutionized the treatment and prognosis of IE. More recently, developments in clinical micro-biology and the availability of improved imaging techniques, especially transthoracic and transesophageal echocardiography, have led to new, more accurate diagnostic criteria. [1] [2] In industrialized countries, despite improvements in health care and the sharp decrease in the incidence of chronic rheumatic heart disease, IE has not disappeared and has even increased in some populations.[1] [3] [4] [5] [6] [7] New at-risk groups have emerged, including intravenous drug users, elderly people who have sclerotic cardiac valves and patients who have intravascular prostheses.

EPIDEMIOLOGY

Studies of the epidemiology of IE have been hampered by several factors: IE is a relatively rare disease, it is not officially a reportable disease and a precise case definition is lacking. This lack of a precise case definition is an important problem because it may be difficult to make a clinical diagnosis of IE with certainty. Therefore, many former studies were based on autopsy series. Fortunately, newer diagnostic criteria now allow a better assessment of IE in live patients.[1] [2]

Overall, the incidence of IE ranges between 2 and 6 per 100,000 population per year.[3] [4] [5] [6] [7] Recent series report a predominance in males, with a male:female ratio of approximately 2:1. Infective endocarditis has not disappeared in the modern era; it may even be increasing, according to recent reports from Europe and the USA. Moreover, its morta-lity remains high, and ranges between 20% and 30% in spite of aggressive antimicrobial treatment combined or not with surgical valve replacement.[3] [4] [5] [6]

The risk factors and the infecting micro-organisms have both changed over time. Chronic rheumatic heart disease, which was a prime risk factor in up to 75% of cases in the pre-antibiotic era,[8] is now rare in industrialized countries.[9] Classic pathogens such as pneumococci and gonococci have become uncommon, whereas there is an increasing number of cases due to *Staphylococcus aureus* and *Staphylococcus epidermidis*.[4] [5] [6] [7] These micro-organisms occur frequently in the newer risk groups of intravenous drug users, elderly people and patients who have prosthetic valves or pacemakers.

Infective endocarditis is commonly classified in four categories, which are discussed below:

- native value IE;
- prosthetic valve IE;
- IE in intravenous drug users; and
- nosocomial IE.

Native valve infective endocarditis

Risk factors for native valve IE include congenital heart disease and acquired abnormalities such as chronic rheumatic heart disease and degenerative heart disease. People who have cardiac abnormalities that result in high-to-low pressure gradients are at greater risk of infection. Turbulent blood flow may provoke damage or peeling of the endothelium and formation of vegetation. Circulating bacteria tend to adhere on the low-pressure side of such Venturi-like systems. These observations explain why left-sided valves and left-to-right ventricular or arterial shunts are the most common sites of IE.

Congenital heart disease

Congenital heart disease is a lifelong risk factor. It is a major risk factor in children, in whom it accounts for 30–40% of IE.[9] It is a less frequent risk factor in adults but still represents about 5% of cases. [10] This includes all of the cardiac abnormalities associated with turbulent blood flow. Tetralogy of Fallot carries the highest risk for IE, followed by bicuspid aortic valve, coarctation of the aorta and ventricular septal defect. In contrast, secundum atrial septum defects rarely put the patient at risk, probably because they result in a low-pressure shunt.

Surgical or medical closure of a patent ductus arteriosus usually eliminates the risk of endovascular infection. However, surgical correction does not exclude the risk of IE in patients suffering major congenital heart disease such as tetralogy of Fallot.[10] Importantly, the type of surgical correction may influence the risk of subsequent infection in this situation. In a long-term survey involving 1142 patient-years of observation, IE occurred in 23% of patients treated with anastomotic operations but in only 9% of patients treated with pulmonary valvulotomy or infundibular resection or both.[11] Because vascular anastomoses are likely to generate turbulent blood flow, this observation underlines the importance of hydrodynamic disturbances in promoting endovascular infections.

Rheumatic heart disease

Rheumatic heart disease was the most frequent acquired cardiac anomaly leading to IE in the pre-antibiotic era. In one series, the frequency of chronic rheumatic heart disease in patients who had developed IE decreased from approximately 22% between 1933 and 1952 to less than 1% between 1963 and 1972.[8] Although the prevalence of the disease has decreased to less than 10 per 100,000 population per year in industrialized countries, it remains the predisposing factor of IE in up to 50% of cases in some developing countries. Moreover, recent clusters of rheumatic heart disease are occasionally described in the USA, suggesting a possible resurgence of the disease. Thus, rheumatic heart disease remains an important risk factor for IE.

Mitral valve prolapse

Mitral valve prolapse is a relatively common condition, affecting 2–4% of the population. It has recently been shown to be inherited in an autosomal-dominant manner and linked to a region in chromosome 16p.[12] Patients who have valve regurgitation have a

10- to 100-fold increased risk of IE.[13] The risk of IE may be especially important in children and in patients over the age or 50 years.[14] Interestingly, mitral valve prolapse is associated with body leanness, lower blood pressure and lower prevalence of diabetes mellitus in Native Americans. Thus, the inherited valve alteration is ironically associated with some cardiovascular protective parameters and represents a Darwinian paradox.[15]

Degenerative valve lesions

Degenerative valve lesions are present in up to 25% of patients aged over 40 years and in 50% of patients over age 60 years who have IE.[16] Degenerative valve lesions leading to senile aortic stenosis or mitral regurgitation are frequent in patients aged over 70 years. Elderly people should be carefully examined for clinical

evidence of valve dysfunction.

Prosthetic valve infective endocarditis

Prosthetic valve endocarditis occurs in 1–5% of cases, or 0.3–0.6% per patient-year.[17] [18] The issue of whether mechanical or bioprosthetic valves are more prone to infection remains unresolved.[17] Prosthetic valve endocarditis is usually classified as either early infection or late infection, depending on whether the symptoms of infection occur within 60 days after surgery or later. The risk of endocarditis peaks during the 2 months after valve implantation; thus, infection probably results from perioperative contamination. Early prosthetic valve endocarditis is often due to *S. epidermidis* and less frequently to *S. aureus*. These organisms are commonly found on the skin and are frequently introduced into the heart or bloodstream during or soon after surgery.

Progressive endothelialization of the prosthetic material over 2–6 months eventually reduces the susceptibility of the implanted valve to infection. This is reflected in a change in the predominant pathogens over time. *Staphylococcus epidermidis* causes prosthetic valve endocarditis most often during the first year after a valve implantation; later, this organism is replaced by streptococci and sometimes by Gram-negative bacteria of the so-called HACEK group, including *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and

TABLE 59-1 -- Microbiology of infective endocarditis in the general population and in specific at-risk groups.£

MICROBIOLOGY OF IE IN THE GENERAL POPULATION AND IN SPECIFIC AT-RISK GROUPS				
Pathogens	**No. of episodes (% of total)**			
	Native valve	**Intravenous drug abusers**	**Prosthetic valve**	
			Early	**Late**
Staphylococci	124 (44)	60 (69)	10 (66)	33 (45)
Staphylococcus aureus	106 (38)	60 (69)	3 (21)	15 (20)
Coagulase-negative	18 (6)	0 (0)	7 (45)	18 (25)
Streptococci	86 (31)	7 (8)		25 (35)
Oral streptococci	59 (21)	3 (3)		19 (26)
Others (non-enterococcal)	27 (10)*	4 (5)	0 (0)	6 (9)
Enterococcus spp.†	21 (8)	2 (2)	1 (7)	5 (7)
HACEK group	12 (4)‡	0 (0)	0 (0)	1 (1.5)
Polymicrobial	6 (2)	8 (9)	0 (0)	1 (1.5)
Other bacteria	12 (4)§	4 (5)	0 (0)	2 (3)
Fungi	3 (1)	2 (2)	0 (0)	0 (0)
Negative blood culture	16 (6)	4 (5)	4 (27)	5 (7)
Total episodes	280 (100)	87 (100)	15 (100)	72 (100)

£ *Data from Watanakunakorn and Burkert[3] , Sandre and Shafran[4] and Bouza et al.[5]*
* Including 9 *Streptococcus agalactiae*, *6 S. bovis*, *3 S. pneumoniae*, *2 S. pyogenes*, 1 group G streptococcus, and 1 *Abiotrophia* spp.
† Mostly (>80%) represented by *Enterococcus faecalis*.
‡ Includes *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*.
§ Includes 4 *Escherichia coli, 2 Corynebacterium* spp., *2 Proteus mirabilis,* 1 *Mycobacterium tuberculosis* and 1 *Bacteroides fragilis*.

Kingella kingae.[6] Patients who have a prosthetic valve implanted during active IE also have a greater risk (up to seven times) of subsequent prosthetic valve endocarditis than patients undergoing elective valve replacement, although the infection may be due to a different organism.

Infective endocarditis in intravenous drug users

Intravenous drug users constitute a special risk group of relatively young people, with a median age varying between 30 and 40 years.[7] Men are more often affected than women. The prevalence of intravenous drug users among patients who have IE depends on the study population, representing up to 40% of cases of IE in San Francisco but less than 1% in Olmsted County, Minnesota, USA. [19] The tricuspid valve is infected in more that 50% of cases, followed by the aortic valve in 25% and the mitral valve in 20%, with mixed right-sided and left-sided IE in a few cases.[7] It has been suggested that repeated injections of impure drugs and particulate material might produce microtrauma to the tricuspid leaflets, thus facilitating microbial colonization and infection. However, 20–40% of intravenous drug users suffering IE have pre-existing cardiac lesions, often caused by previous valve infection. The responsible bacteria often originate from the skin, which explains the predominance of *S. aureus* infections (Table 59.1), but streptococci and other micro-organisms are also encountered; some pathogens, such as *Pseudomonas aeruginosa* and fungi, may produce severe forms of IE. Infection with HIV is not itself a risk factor for IE, except in rare cases caused by *Bartonella* spp. Nevertheless, the mortality of IE is higher in patients who have AIDS, especially in advanced cases, than in other patients.[20]

Nosocomial infective endocarditis

The incidence of nosocomial IE is increasing. In a recent series it accounted for 22% of 109 cases overall and 16% of cases excluding early prosthetic valve endocarditis in cardiac surgery patients.[5] Many patients had debilitating underlying conditions but fewer than 50% had obvious cardiac predisposing factors. In most circumstances a potential source of bacteremia could be identified, such as the presence of intravenous lines or invasive procedures. [5] [21] Pathogens usually originated from the skin or the urinary tract, staphylococci

and enterococci being the most common. In one study, up to 13% of nosocomial *S. aureus* bacteremia was responsible for subsequent IE.[22] However, other organisms may be encountered, including Gram-negative bacteria and fungi. Right-sided IE is increasingly recognized in association with central venous lines, pulmonary artery catheters and pacemakers. Possible right-sided IE was reported in 5% of bone marrow transplant recipients who had central venous catheters.

Thus, nosocomial IE must not be underestimated. Any procedures that produce transient bacteremia represent some degree of risk in hospitalized patients, especially when the circulating organism is *S. aureus*. [22] Such considerations are important because the mortality of nosocomial IE is greater than 50%.[5] [21]

PATHOGENESIS, PATHOLOGY AND INFECTING MICRO-ORGANISMS

The key issues in the pathogenesis of IE, discussed below, are:

- the predisposing host factors;
- the characteristics of the infecting micro-organisms;
- the role of transient bacteremia; and
- the inability of the immune system to eradicate micro-organisms once they are located on the endocardium.

Predisposing host factors

The importance of host factors is indicated by the fact that IE most often develops on pre-existing lesions of the layer of endothelial cells covering the valve or endovascular surfaces. Normally, this endothelium is resistant to colonization by circulating bacteria, but damage to this delicate protective layer increases susceptibility to bacterial colonization by several orders of magnitude (Fig. 59.1). Exposure of the underlying extracellular matrix proteins and the local production of

tissue factors trigger the deposition of platelets and fibrin as a normal host response initiating the healing process. Such a platelet-fibrin meshwork, referred to as nonbacterial thrombotic endocarditis (NBTE), is a favorable nidus for bacterial colonization during transient bacteremias.[23]

Endothelial damage may occur in several ways. Congenital cardiac abnormalities may cause turbulent blood flow, which in turn may provoke peeling of the endothelium. Valve scarring and calcification following rheumatic carditis or in the sclerotic valves of elderly patients result in endothelial lesions. Extrinsic intervention, such as

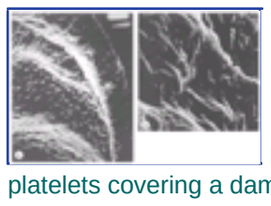


Figure 59-1 Scanning electron microscopy of a rabbit aortic valve leaflet. (a) A normal valve, covered by a monolayer of endothelial cells. (b) The meshwork of fibrin and platelets covering a damaged valve. Mechanical lesions of the valve were created by inserting a catheter through the right aortic carotid and across the aortic valve.

prosthetic valve replacement or indwelling electrodes or catheters, also promote endothelial lesions. Recently, the presence of *Chlamydia pneumoniae* or cytomegalovirus in endovascular locations has been linked to arteriosclerosis. [24] Whether these organisms also promote endothelial lesions that promote IE remains to be demonstrated.

Characteristics of the micro-organisms

The pathogenesis of IE was recently reviewed.[25] The organisms most frequently responsible for IE are also those that have the greatest ability to adhere to and colonize damaged values. Together, *S. aureus*, *Streptococcus* spp. and enterococci are responsible for more than 80% of all cases of IE (see Table 59.1). Infective endocarditis pathogens possess several surface ligands that mediate attachment to extracellular matrix proteins of the host. These proteins are abundant in the milieu of endothelial lesions. Such bacterial adhesins are collectively referred to as MSCRAMMs, for 'microbial surface component reacting with adhesive matrix molecules'.

In *S. aureus*, fibrinogen-binding proteins — also called clumping factors — and fibronectin-binding proteins are involved in valve colonization and infection.[26] Figure 59.2 presents a likely scenario in the case of IE due to this organism. The role of other *S. aureus* MSCRAMMs is currently being investigated. In streptococci, several adhesins as well as platelet-activating factors and exopolysaccharides seem to be involved in bacterial adherence to NBTE. This abundance of surface adhesins makes IE pathogens particularly prone to colonize damaged valve tissues.

Although bacterial colonization of pre-existing NBTE is a leading mechanism for establishment of IE, direct invasion of endothelial cells may also occur under certain circumstances. A few cases of IE are due to intracellular pathogens such as *Coxiella burnetii* (the agent of Q fever), *Chlamydia* spp., *Legionella* spp. and *Bartonella* spp.[27] Infection by these pathogens may occur by direct or indirect invasion of the cardiac endothelium. Direct invasion of endothelial cells has been demonstrated in vitro with *S. aureus* (reviewed by Moreillon *et al.*[25]). During inflammation, endothelial cells may express a variety of molecules, including integrins of the $ß_1$ family (very late antigen, VLA) that bind fibronectin. Endothelial-bound fibronectin may be a privileged ligand for pathogens expressing fibronectin-binding proteins, including *S. aureus*. Moreover, bridging endothelial cells and *S. aureus* via fibronectin triggers bacterial internalization by the host cell. Thus, local inflammation due to

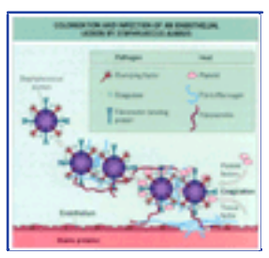


Figure 59-2 Colonization and infection of an endothelial lesion by *Staphylococcus aureus*. Exposure of the subendothelial matrix triggers the deposition of platelet-fibrin clots and other plasma-soluble and matrix proteins, including fibrinogen, fibrin, fibronectin and thrombospondin. Triggering of the coagulation cascade is also mediated by tissue factor, which contributes to platelet activation and the constitution of a nonbacterial thrombotic vegetation. *Staphylococcus aureus* is equipped with a wealth of surface determinants that may promote binding and colonization of nonbacterial thrombotic vegetations. The best known of these are fibrinogen-binding protein (or clumping factor), fibronectin-binding protein and coagulase. These factors are likely to mediate direct and/or indirect attachment to vascular lesions and promote infection.

degenerative lesions, arteriosclerosis or as yet undetermined conditions may promote fibronectin deposition on the endothelial surface and further infection. These events may be important in the pathogenesis of infection due to facultative or strict intracellular bacteria.

The role of transient bacteremia

Infective endocarditis has been modeled in rats and rabbits with catheter-induced valve lesions. These experiments have allowed the determination of a hierarchy of the infectivity of the various pathogens. Bacterial adherence is a critical factor and the magnitude and duration of the bacteremia after inoculation are also important determinants (reviewed by Moreillon *et al.* [25]).

Medical and surgical procedures in nonsterile anatomic sites may provoke transient invasion of the bloodstream with bacteria from the local flora. Such bacteremia is usually low-grade and of short duration (e.g. 1–100cfu/ml of blood for less than 10 minutes in the case of dental extraction). Depending on the characteristics of the circulating bacteria, even these transient bacteremias may put patients who have pre-existing cardiac lesions at greater risk of developing IE. In the case of dental procedures, postextraction bacteremia is more important in patients suffering from gingivitis than in individuals who have a healthy gingivodental status. This was simulated in rats with catheter-induced aortic NBTE.[28] Animals suffering from gingivitis were at a much greater risk of postextraction endocarditis than those with healthy gingivae. Most interestingly, although all the rats developed polymicrobial bacteremias during a dental procedure, only two types of organism — group G streptococci and *S. aureus* — colonized the valves to cause IE. This correlates with the ability of these bacteria to attach to NBTE.[29]

Transient bacteremias occur spontaneously during chewing, toothbrushing and other normal activities. These spontaneous bacteremias provide the likely explanation for the fact that most cases of IE are not preceded by medical or surgical procedures. Moreover, because streptococci are normal inhabitants of the mouth, spontaneous bacteremias arising from the mouth during chewing may explain why these bacteria are a predominant cause of IE. Thus, proper prophylactic measures during specific medical interventions will only marginally affect the overall frequency of IE.[30] Simple prevention strategies such as good dental hygiene provide the best method of prophylaxis in this context.

The role of host defenses

Infective endocarditis is most often due to Gram-positive organisms, and rarely to Gram-negative bacteria (see Table 59.1). The reason for this is probably multifactorial. Differences in bacterial adherence to NBTE may be one explanation. However, differences in the susceptibility of Gram-positive and Gram-negative bacteria to serum-induced killing may also account for these variations. The C5b–C9 membrane-attack complex of complement kills Gram-negative bacteria by perforating their outer membrane. In contrast, complement does not kill Gram-positive bacteria. They lack an outer membrane and their plasma membrane is protected from the membrane-attack complex of complement by the surrounding peptidoglycan.

The importance of serum was demonstrated in experimental IE induced by serum-susceptible *Escherichia coli*.[31] These organisms were spontaneously cleared from the vegetations even after infection with very large inocula. Nevertheless, some Gram-negative bacteria may carry thick capsules or other modifications of their outer membrane that help them resist complement-induced killing. An important subgroup of cases of IE are caused by Gram-negative bacteria, including micro-organisms of the HACEK group, as well as *P. aeruginosa* in intravenous drug users.[32]

Although Gram-positive bacteria are resistant to complement, they may be the target of another non-specific immune factor, namely the platelet microbicidal proteins (PMPs).[33] These are peptides produced by activated thrombocytes that kill bacteria by a mechanism that is as yet incompletely understood. Indirect evidence for the protective role of PMPs in IE came from experiments in which thrombocytopenic rabbits demonstrated greater bacterial densities in their vegetations than rabbits with normal platelet counts. Likewise, micro-organisms recovered from patients who had IE were consistently resistant to PMP-induced killing, whereas similar bacteria recovered from patients who had other types of infection were susceptible to PMP.[34] Therefore platelets, which are a major component of the vegetations, may be key

players in the non-specific defense against IE.

Humoral and cellular immunity seem to play only a limited role in defense against IE. Recent immunization studies in experimental endocarditis gave contradictory results. Immunization of rats against the streptococcal MSCRAMM FimA conferred cross-protection against IE due to several *viridans* group streptococci.[35] In contrast, immunization of rabbits against the enterococcal aggregation substance (AS) failed to protect the animals against experimental IE.[36] In this case, specific antibodies arising from vaccination could not appropriately penetrate inside the vegetation in vivo. Administration of granulocyte colony-stimulating factor did not influence the course of infection either.[37] Infective endocarditis is not noticeably more frequent in immunocompromised patients than in those who have no immune defects. In established infection, bacteria are clustered in amorphous platelet-fibrin clots (the vegetations), permitting little access to professional phagocytes to remove the bacteria (Fig. 59.3). This explains why successful treatment of IE relies primarily on the ability of antibiotics rather than host defenses to kill bacteria in situ in the vegetation. Figure 59.4 presents the

Figure 59-3 Microscopic appearance of a vegetation from a patient suffering mitral valve infective endocarditis due to *Streptococcus sanguis.* The purple area represents clusters of streptococci packed within a fibrin-platelet meshwork. Professional phagocytes are essentially absent from the lesion.

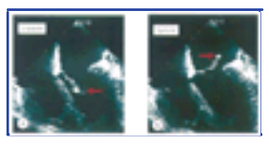


Figure 59-4 Transesophageal echocardiography of a mitral valve infective endocarditis. (a) Mass attached to anterior leaflet. (b) Prolapse of the leaflet due to rupture of chordae tendinae. The echo is from the patient shown in Figure 59.3 . Arrows indicate a large pediculated vegetation, which oscillates from the left ventricle to the left atrium during systole. *Courtesy of Dr X Jeanrenaud.*

echocardiographic picture of this very vegetation, which occurred in the context of a mitral valve endocarditis with a large, oscillating valve lesion.

PREVENTION

Because of its severity, it is generally agreed that IE should be prevented whenever possible. Choice of appropriate prophylactic measures requires:

- identification of patients at risk;
- determination of the procedures or circumstances that may result in bacteremias;
- choice of an appropriate antimicrobial regimen; and
- balancing of the known risks against the possible benefits of intervention.

In many countries, recommendations for prophylaxis have been established, based on underlying cardiac conditions and distinguishing between high-risk and low-risk situations depending on the anatomic defects and the medical or dental procedure in question (Table 59.2).[38] [39]

The practice of IE prophylaxis is not based on the results of randomized studies because these would require too many patients and raise difficult ethical issues. Therefore, antibiotic regimens used in humans are based upon their proven efficacy in animal models of IE. In contrast to therapy, successful prophylaxis does not require bactericidal antibiotics. The key factor is the period of time during which serum levels of antibiotics remain above the minimum inhibitory concentration (MIC) for the circulating pathogen. For amoxicillin, this optimal duration is 10 hours or more.[40] Therefore, prophylactic antibiotics are given in large single doses or in repeated doses, or both, in order to ensure the prolonged presence of the drug in the serum.

Antibiotics are chosen in relation to the most probable pathogens circulating in the blood during a given procedure (Table 59.3). For oropharyngeal manipulation, antibiotic prophylaxis should be aimed at streptococci. For gastrointestinal or urogenital manipulations, it should be aimed at enterococci. Indeed, although gastrointestinal procedures are more likely to produce Gram-negative bacteremia, these organisms are rarely responsible for IE. For drainage of skin or other infected lesions, antibiotic prophylaxis should be aimed at the most likely infecting organism present in the lesion and, as a minimum, it should cover staphylococci. It is noteworthy that, during dental manipulation, the risk of bacteremia correlates with the status of dental hygiene; patients who have healthy gums are at lower risk than those who have severe gingivitis. In cases of severe gingivitis, the local application of an antiseptic wash using chlorhexidine may be a useful additional measure.

CLINICAL FEATURES

Infective endocarditis may follow an acute or subacute course. The general clinical features (Table 59.4) are not usually specific.

Acute infective endocarditis

Acute IE is most frequently caused by *S. aureus*, followed by enterococci and certain streptococci, such as *Streptococcus milleri*. Infective endocarditis caused by *S. aureus* and other primary invasive pathogens can be devastating. Bacterial production of proteases and other exoproteins contributes to rapid destruction of valve leaflets and the development of abscesses located in the valve ring and the myocardium. Myocarditis and pericardial effusions are frequent. Patients are prostrate and have a high fever. Hypotension and shock may occur, caused both by the septic state and by cardiac failure. Cardiac vegetations may vary from a few millimeters in diameter to more than 1cm. Large vegetations are frequent in acute staphylococcal and fungal IE (Fig. 59.5) and are more likely to detach and give rise to septic emboli. Complications in peripheral organs mainly result from embolic lesions; these may include skin abscesses and retinal emboli (Fig. 59.6) and cerebral abscesses and splenic lesions (Fig. 59.7).

Major indications for urgent valve replacement include refractory cardiac failure due to valve destruction and persistent sepsis related to myocardial abscesses. A defect in atrioventricular conduction is often an early sign of septal invasion by a contiguous valve ring abscess, which usually requires urgent surgery.

Subacute infective endocarditis

Subacute IE is not usually due to *S. aureus* but it may be caused by any of the organisms listed in Table 59.1 . The course of subacute IE can mimic chronic wasting diseases. The duration between an identifiable event producing bacteremia (e.g. a dental procedure) and the diagnosis of IE can vary from a few days to 5 weeks or more. Fever is almost always present (see Table 59.4). Physical signs reflect the existence of cardiac or peripheral complications. These

TABLE 59-2 -- Pre-existing conditions associated with an increased risk of developing endocarditis.[*]

PRE-EXISTING CONDITIONS ASSOCIATED WITH AN INCREASED RISK OF DEVELOPING ENDOCARDITIS
Endocarditis prophylaxis recommended
High-risk patients
• Prosthetic cardiac valves, including bioprosthetic and homograft valves

• Previous infectious endocarditis
• Complex cyanotic congenital heart disease
• Surgically constructed systemic pulmonary shunts or conduits
• Other congenital cardiac malformations (except for those specified in the next column)
Moderate-risk patients
• Other congenital cardiac malformations (except for those specified in the next column)
• Acquired valve dysfunction (including rheumatic heart disease)
• Hypertrophic cardiomyopathy
• Mitral valve prolapse with valve regurgitation and/or thickened valve leaflets
Endocarditis prophylaxis not recommended
Patients who have identical risk to the general population
• Isolated secundum atrial septal defect
• Surgical repair of septal defect (atrial or ventricular)
• Surgical or medical repair of ductus arteriosus
• Previous coronary artery bypass
• Mitral valve prolapse without valve regurgitation
• Functional heart murmur
• Previous Kawasaki disease without valve dysfunction
• Previous rheumatic fever without valve dysfunction
• Cardiac pacemaker (intravascular or epicardial) and implanted defibrillator

** Adapted from recommendations in Europe[38] and the USA.[39]*

include a new or changing heart murmur and evidence of embolic events.

Immunologic stimulation during subacute IE causes hyperproduction of gammaglobulin. Rheumatoid factor is present in up to 50% of patients after 6 weeks of subacute infection; its level decreases after effective treatment. Immune phenomena may be the cause of petechiae, splinter hemorrhages, Osler nodes and Roth spots, arthritis and glomerulonephritis. Osler nodes are small and painful nodular lesions on the pads of the fingers or toes or on the thenar or hypothenar eminences (Fig. 59.8). They are caused by an allergic vasculitis. Although classic, they are not pathognomonic of subacute IE. Roth spots are rounded retinal hemorrhages with a white center. Focal or diffuse glomerulonephritis is present in most of the cases. Because these phenomena follow stimulation of the immune system, they are less common in acute IE.

Vascular complications

Embolic lesions result from vegetation fragments breaking off the valve and lodging in arteries serving peripheral organs. Other types of vascular manifestation are the consequence of immune-related vasculitis. Mycotic aneurysms are found in up to 15% of cases and are especially common in staphylococcal IE. They may arise either from direct invasion of the arterial wall by the infecting organisms, from septic embolization of the vasa vasorum or from the deposition of immune complexes that trigger local inflammation and weakening of the arterial wall. Mycotic aneurysms tend to be located at the bifurcation points of vessels. They may either heal during antibiotic therapy or become clinically evident later, even months after the clinical cure of the disease. Therefore, the true incidence of mycotic aneurysms during IE is probably underestimated. In right-sided IE, embolization occurs in the pulmonary circulation and gives rise to pulmonary infiltrates and lung abscesses.

Neurologic complications

Neurologic manifestations occur in up to 40% of cases.[3] [5] [41] However, because patients who have no neurologic symptoms do not undergo specific investigations, the true incidence of neurologic events during IE may be underestimated. Anatomic alterations include cerebral infarction, arteritis, abscesses, mycotic aneurysms, intracerebral or subarachnoid hemorrhage, encephalomalacia, cerebritis and menin-gitis. Such complications occur most often in staphylococcal or streptococcal IE but they are not restricted to IE caused by these pathogens. The frequency of stroke is similar between native valve and prosthetic valve IE.[41] [42] However, the frequency of both vegetations and stroke is significantly greater in patients who have mitral valve IE than in patients who have aortic valve IE.[5] [42]

Controlling the infection is essential. Embolization sharply decreases within 1–2 weeks of effective therapy.[43] [44] Recurrent embolization may necessitate urgent valve replacement. This decision is difficult because anticoagulation during extracorporeal circulation and after valve replacement puts the patients at increased risk of secondary intracerebral hemorrhage. Therefore, the tendency is often to postpone emergency surgery and wait for the patient to stabilize. On the other hand, ongoing studies suggest that earlier intervention, within the first 72 hours of stroke, may be beneficial in selected patients.[45] The best approach to these challenging issues needs continuing investigation.

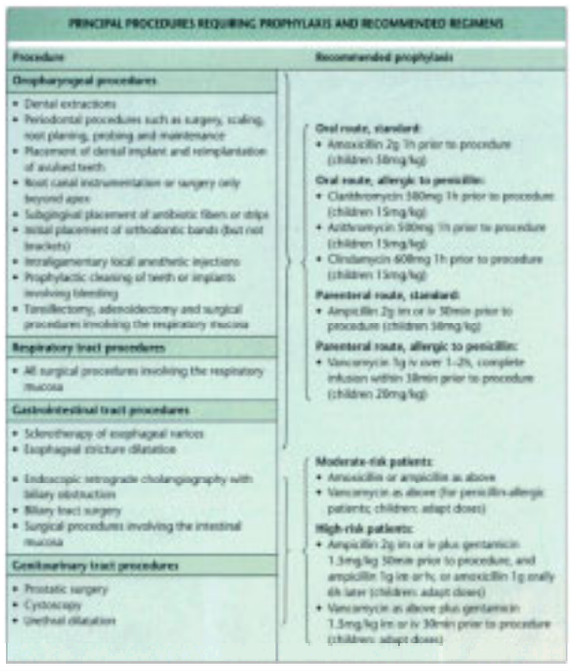
DIAGNOSIS

Whether acute or subacute, IE may result in life-threatening complications. Therefore, precise clinical and microbiologic diagnosis is mandatory in order to guide therapy. In theory, IE combines both persistent bacteremia and anatomic lesions of the valves. However, blood cultures may remain negative in up to 10% of cases (Table 59.1 and Table 59.5). Clinical and microbiologic diagnosis of IE is difficult in such blood-culture-negative cases, or when changes in the valve status cannot be assessed owing to lack of information on preexisting cardiac lesions.[1] [2]

The Duke criteria

In 1994 new diagnostic criteria based on both microbiologic data and echocardiographic imaging of cardiac vegetations were proposed.[1]

TABLE 59-3 -- Principal procedures requiring prophylaxis and recommended regimens.*



** Adapted from recommendations in Europe[38] and the USA.[39]*

Today, nearly all patients suspected of having IE should undergo at least one echocardiographic evaluation, including transesophageal echo in selected cases. These criteria, which are referred to as the Duke criteria, were validated in several studies worldwide. Recently, they were refined to more accurately detect patients who have IE in the case of negative blood cultures and *S. aureus* bacteremia (Table 59.6).[2]

Blood cultures

Blood cultures are of primary importance because they reveal the infecting organism and thus guide antibiotic therapy. For the main etiologic agents, the first two blood cultures will be positive in more than 90% of cases. However, certain micro-organisms may be difficult to isolate. Variables affecting isolation include the volume of blood cultured, the number of blood cultures obtained before antimicrobial therapy is started, the type of micro-organism involved and the techniques of blood culture.

The volume of blood cultured is critical because persistent bacteremia in IE is often low-level, representing only 1–100 bacteria/ml of blood. For each culture, 8–12ml of venous blood should be drawn with careful antiseptic precautions and distributed into a two-bottle system for incubation. Blood for culture should be drawn on two or three separate occasions over a 24-hour period. If possible, the interval between each culture should be at least 30–60 minutes.

Culture-negative infective endocarditis

Special attention must be given to culture-negative IE. Such cases are often associated with antibiotic consumption within the previous 2 weeks. In such patients it may be necessary to obtain additional blood cultures and to use media supplemented with ß-lactamase when appropriate. However, culture-negative IE may also be due to fastidious organisms or to intracellular pathogens that are not easily detected by standard culture conditions. The issue is important because these organisms often require alternate diagnostic procedures and respond to different therapies.

Infective endocarditis due to rare and fastidious organisms was recently reviewed.[27] Table 59.5 lists the principal organisms of this group and proposed diagnostic procedures and therapies. Since such cases are rare, most therapeutic recommendations are based on limited experience, including case reports and small series. Therefore, any of these conditions requires special case-by-case attention from the care giver.

Microbiologic cultures

Bacteria of the HACEK group are usually detected after prolonged (more than 1 week) incubation of the blood cultures. In contrast, *Abiotrophia* spp. grow well in blood but fail to grow when subcultured on conventional agar media. These so-called deficient bacteria do grow as satellite colonies if the plates are streaked with *S. aureus*, or if the growth medium is enriched with vitamin B6. One of these methods

TABLE 59-4 -- Clinical features of infective endocarditis in the general population and in specific at-risk groups.[*]

CLINICAL FEATURES OF IE IN THE GENERAL POPULATION AND IN SPECIFIC AT-RISK GROUPS				
Clinical features	**No. of episodes (% of total)**			
	Native valve	**Intravenous drug abusers**	**Prosthetic valve**	
			Early	**Late**
Symptoms				
Fever	170 (75)	42 (87)	7 (77)	47 (78)
Chills	104 (46)	35 (73)	5 (55)	32 (53)
Arthralgias/myalgias	44 (19)	13 (27)	-	10 (17)
Back pain	22 (10)	5 (10)	-	2 (2)
Pleuritic chest pain	10 (4)	17 (45)	-	3 (5)
Clinical signs				
Cardiac murmur	196 (86)	45 (93)	8 (88)	58 (96)
Petechia, emboli	80 (35)	3 (6)	3 (33)	30 (50)
Osler nodes, Janeway lesions, Roth spots	26 (11)	3 (6)	0 (0)	6 (10)
Splinter hemorrhages[*]	28/80 (35)	3/15 (30)	5/7 (71)	17/33 (51)
Palpable spleen[*]	15/80 (19)	3/15 (30)	2/7 (28)	2/33 (6)
Central nervous system manifestations[†]	38/148 (25)	4/33 (12)	0/2 (0)	5/27 (18)
Renal insufficiency[†]	45/148 (30)	5/33 (15)	1/2 (50)	8/27 (30)
Temperature (°F (°C))				
<100.4 (<37.8)	64 (28)	15 (31)	3 (34)	21 (35)
100.4–102 (37.8–38.9)	82 (36)	12 (25)	6 (66)	22 (37)
>102.2 (>39)	81 (36)	21 (44)	0 (0)	17 (28)

Total episodes	228	48	9	60

* *Data from Watanakunakorn and Burkert[3] and Sandre and Shafran.[4]*
*As reported by Sandre and Shafran.[4]
† As reported by Watanakunakorn and Burkert.[3]

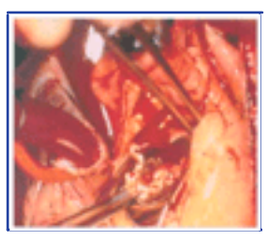


Figure 59-5 Aortic valve of a patient undergoing emergency valve replacement for acute endocarditis caused by *Staphylococcus aureus.* In this patient, emergency valve replacement was mandatory because of multiple embolizations and acute heart failure. Here, the aorta has been opened and the valve is viewed from its upper side. The lower tweezers are holding a valve leaflet covered with vegetations. Next to the upper tweezers, a portion of a cuspid with a normal appearance can be distinguished.

should be applied when bacteria are observed microscopically in blood cultures but fail to grow on the subculture plates.

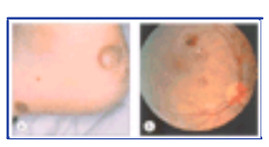


Figure 59-6 Skin lesions (Janeway spots) on the foot (a) and septic emboli of the retina (b), the results of peripheral emboli in acute endocarditis caused by *Staphylococcus aureus.* These occurred in the patient described in Figure 59.5 and were present on admission to hospital.

Brucella spp, *Bartonella* spp., *Legionella* spp. and *Mycobacteria* spp. are rare causes of IE that require special culture techniques. Serologic tests may help define both culture strategies and other diagnostic procedures. Tissue cell cultures may be performed in specialized laboratories for the detection of strict or facultative intracellular bacteria. These include *C. burnetii*, *Chlamydia* spp., *Legionella* spp. and *Bartonella* spp.

If the patient undergoes surgery it is of utmost importance to obtain valve samples for both cultures and other microbiologic tests. The presence of large numbers of bacteria in the vegetation increases the chance of positive culture and/or identification of the pathogen by other means. The importance of cultures cannot be emphasized enough, because they fulfill both diagnostic and therapeutic purposes, and in particular allow testing for antimicrobial susceptibility.

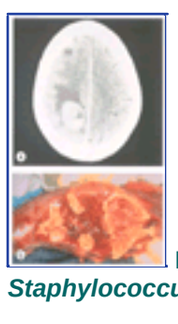


Figure 59-7 A cerebral abscess (a) and multiple abscesses and ischemic necroses of the spleen (b), the result of peripheral emboli in acute endocarditis cause by *Staphylococcus aureus.* Again these occurred in the patient described in Figure 59.5 ; they developed after admission to hospital.

TABLE 59-5 -- Rare causes of infective endocarditis associated with negative blood cultures.*

RARE CAUSES OF IE ASSOCIATED WITH NEGATIVE BLOOD CULTURES		
Pathogen	**Diagnostic procedure**	**Proposed therapy**
Brucella spp.	Blood cultures	Doxycycline plus rifampin or trimethoprim-sulfamethoxazole (treatment for >3 months*
	Serology	
	Culture, immunohistology and PCR of surgical material	
Coxiella burnetii (agent of Q fever)	Serology: IgG phase 1 > 1/800	Doxycycline plus hydroxychloroquine†
	Tissue culture, immunohistology and PCR of surgical material	Doxycycline plus quinolone (>18 months treatment)
Bartonella spp.	Blood cultures	ß-lactams or doxycycline plus aminoglycoside‡ (>6 weeks treatment)
	Serology	
	Culture, immunohistology and PCR of surgical material	
Chlamydia spp.	Serology§	Doxycycline
	Culture, immunohistology and PCR of surgical material	Newer fluoroquinolones¶ (long-term treatment, optimal duration unknown)
Mycoplasma spp.	Serology	Doxycycline
	Culture, immunohistology and PCR of surgical material.	Newer fluoroquinolones¶ (>12 weeks treatment)
Legionella spp.	Blood cultures	Macrolide plus rifampin
	Serology	Newer fluoroquinolones¶ (>6 months treatment)
	Culture, immunohistology and PCR of surgical material.	
Tropheryma whippelii (agent of Whipple's disease)	Histology and PCR of surgical material	Trimethoprim-sulfamethoxazole*
		ß-lactam plus aminoglycoside (long-term treatment, optimal duration unknown)
Because of the lack of large series on IE due to these pathogens, optimal treatment duration is mostly unknown. Treatment durations in the table are indicative and are based on selected case reports.		

* *Adapted from Brouqui and Raoult.[27]*
* According to Hadjinikolaou *et al.*[46]
† Doxycycline 100mg po q12h and hydroxychloroquine 200mg po q8h (hydroxychloroquine levels in the serum were monitored) was significantly superior to doxycycline.[47]
‡ Several therapeutic regimens were reported, including aminopenicillins and cephalosporins combined with aminoglycosides, doxycycline, vancomycin and quinolones (reviewed in Brouqui and Raoult[27]).
§ Beware of serologic cross-reaction with the more common IE pathogen *Bartonella* spp.
¶ Newer fluoroquinolones are more potent than ciprofloxacin against intracellular pathogens such as *Mycoplasma* spp., *Legionella* spp. and *Chlamydia* spp.
** Treatment of Whipple IE remains highly empiric. Successes were reported with long-term (>1 year) trimethoprim-sulfamethoxazole therapy. Gamma interferon plays a protective role in intracellular infections. It was proposed as adjuvant therapy in Whipple's disease.[50]

Histology

A number of special stains may help guide the etiologic diagnosis. Important techniques include Gram stain for Gram-positive and Gram-negative bacteria, periodic acid-Schiff stain for Whipple's disease, Giemsa and Warthin-Starry stains for numerous bacteria, including *Bartonella* spp., Ziehl-Neelsen stain for *Mycobacteria* spp, and Gimenez stain for *C. burnetii* and *Legionella* spp. While not strictly diagnostic, these techniques help to delineate more specific etiologic procedures, including cultures, immunohistology and molecular assessment.

Polymerase chain reaction amplification

Polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA gene has become a critical method for bacterial diagnosis in tissue samples. It has been successfully applied to IE surgical material,[48]

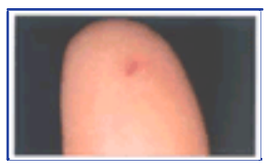


Figure 59-8 Osler node on the thumb during subacute endocarditis. This was a rounded, tender, inflamed mass about 5mm in diameter.

TABLE 59-6 -- Modified Duke criteria for diagnosis of infective endocarditis.*

MODIFIED DUKE CRITERIA FOR DIAGNOSIS OF INFECTIVE ENDOCARDITIS	
Definition terminology used in the criteria	
Major criteria	
1. Blood culture	Positive blood cultures (>2/2) with typical IE micro-organisms (*viridans* streptococci, *Streptococcus bovis*, HACEK group or community-acquired *Staphylococcus aureus* or enterococci in the absence of primary focus)
	Persistently positive blood cultures defined as two culture sets drawn >12h apart, or three or the majority of four culture sets with the first and last separated at least by 1h
	Single positive culture for *Coxiella burnetii* or anti-phase I antibody titer >1:800
2. Endocardial involvement	New valve regurgitation
	Positive echocardiogram for IE (transesophageal echo recommended in patients who have prosthetic valves and patients rated as 'possible' IE by clinical criteria) defined as:
	(i) oscillating intracardiac mass in the valve or supporting structure, *or* in the path of regurgitant jets, *or* on implanted material, in the absence of an alternative anatomic explanation, *or*
	(ii) abscess, *or*
	(iii) new partial dehiscence of prosthetic valve
Minor criteria	
1. Predisposing cardiac condition or intravenous drug use	
2. Fever: >100.4°F (>38°C)	
3. Vascular phenomena: arterial emboli, mycotic aneurysms, petechiae, Janeway lesions	
4. Immunologic phenomena: glomerulonephritis, Osler nodes. Roth spots, rheumatoid factor	
5. Microbiology: positive blood cultures, but not meeting major criteria, serologic evidence of active infection with plausible micro-organisms	
Diagnosis	
Definite	
• Pathology or bacteriology of vegetations, *or*	
• 2 major criteria, *or*	
• 1 major and 3 minor criteria, *or*	
• 5 minor criteria	
Possible	
• 1 major and 1 minor criterion, *or*	
• 3 minor criteria	
Rejected	
• Firm alternative diagnosis, *or*	
• Resolution of IE syndrome after <4 days of antibiotic therapy, *or*	
• No pathologic evidence at surgery or autopsy after <4 days of antibiotic therapy	
• Does not meet criteria mentioned above	

** Adapted with modifications from Durack et al. [1] and Li et al. [2]*

including cutaneous biopsies of peripheral septic emboli (personal experience). Polymerase chain reaction is invaluable to detect poorly cultivable or noncultivable bacteria such as *Tropheryma whippelii*.[50] Figure 59.9 depicts a case of culture-negative aortic valve IE necessitating valve replacement. *Tropheryma whippelii* was identified by histology and PCR amplification of the surgical material. Undiagnosed culture-negative IE is a genuine problem because unusual pathogens may not respond to empiric ß-lactam and aminoglycoside therapy.

Serology

Serologic tests are included in both the original and modified Duke criteria.[1] [2] Common tests include the agglutination test for *Brucella melitensis*, indirect fluorescence for *Legionella pneumophila*, enzyme-linked immunosorbent assay (ELISA) for *Mycoplasma pneumoniae*, and complement fixation, ELISA and indirect immunofluorescence for *Chlamydia* spp. Of note, cross-reaction may occur between *Bartonella* spp. and *Chlamydia* spp. Since culture-proven IE due to *Chlamydia* spp. is notoriously rare, *Bartonella* should be suspected in the case of positive *Chlamydia* serology. This point is important because antimicrobial therapy differs (see Table

59.5).

Other tests

Although many laboratory findings may be abnormal, most of them are non-specific. The erythrocyte sedimentation rate and the

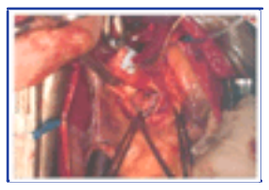


Figure 59-9 Blood culture-negative aortic valve endocarditis caused by *Tropheryma whippelii* in a 48-year-old patient. The patient was admitted to hospital for acute abdominal pain. Abdominal surgery revealed an ischemic necrosis of the transverse colon, presumably due to arterial embolization. An echocardiogram revealed exuberant vegetations on the aortic valve. All blood cultures were negative. Emergency valve replacement was performed for cardiac insufficiency. *Tropheryma whippelii* infection was identified by histology and by using broad-spectrum PCR amplification of the surgical material. The patient was treated with long-term (>1 year) trimethoprim-sulfamethoxazole (see Table 59.5). *With permission from Bugon and Moreillon.*[49]

C-reactive protein are elevated in 90–100% of cases. Acute IE may be accompanied by high or low leukocyte counts with increased polymorphonuclear cells and immature forms, as well as other blood and chemistry abnormalities related to active infection. In subacute IE, non-specific laboratory findings accompanying chronic infection are common but not universal. These findings include mild anemia with low serum iron concentration and low iron-binding capacity and thrombocytosis, as well as leukocytosis, hypergammaglobulinemia and the presence of circulating immune complexes. Urinalysis often reveals proteinuria and microscopic hematuria. Although none of these tests can prove the diagnosis of IE, their return to normal values during treatment is an indirect marker of therapeutic success.

MANAGEMENT

Successful treatment of IE relies primarily on antibiotic therapy. Combined medical and surgical treatment is needed in up to one-third of cases. Both experimental and clinical studies have indicated the importance of using bactericidal drugs in order to cure IE. For instance, 2 weeks penicillin treatment for streptococcal IE was insufficient to prevent relapses, whereas 2 weeks of the synergistic combination of penicillin plus an aminoglycoside successfully cured patients. Combination of bactericidal drugs is even more important against enterococcal IE, where the association of ß-lactams

TABLE 59-7 -- Suggested treatment for native valve endocarditis due to streptococci, enterococci and HACEK micro-organisms.‡

SUGGESTED TREATMENT FOR NATIVE VALVE ENDOCARDITIS DUE TO STREPTOCOCCI, ENTEROCOCCI AND HACEK MICRO-ORGANISMS			
Antibiotic	**Dosage and route**	**Duration (weeks)**	**Comments**
Penicillin-susceptible *viridans* streptococci and *Streptococcus bovis*			
Penicillin G Ceftriaxone*	6 × 2–3 million units/day iv	4	Preferred in patients older than 65 years or who have impaired renal function
	1 × 2g/day iv or im	4	
Penicillin G with gentamicin	6 × 2–8 million units/day iv	2	Studies suggest that gentamicin once daily might be adequate
	3 × 1mg/kg/day iv or im	2	
Ceftriaxone* with netilmicin	1 × 2g/day day iv or im	2	
	1 × 4mg/kg/day iv	2	
Vancomycin	2 × 15mg/kg/day iv	4	Recommended for ß-lactam-allergic patients
Intermediate penicillin-resistant (MIC 0.1–1mg/l) *viridans* streptococci and *S. bovis*			
Penicillin G with gentamicin	6 × 3 million units/day iv	4	Studies suggest that gentamicin once daily might be adequate
	3 × 1mg/kg/day iv or im	2	
Vancomycin	2 × 15mg/kg/day iv	4	Recommended againist highly resistant strains
***Enterococcus* spp.†**			
Penicillin G with gentamicin	6 × 3–5 million units/day iv	4–6	6-weeks therapy recommended for patients who have had symptoms >3 months
	3 × 1mg/kg/day iv or im	4–6	
Ampicillin with gentamicin	6 × 2g/day iv	4–6	Studies suggest that gentamicin once daily might be adequate
	3 × 1mg/kg/day iv or im	4–6	
Vancomycin with gentamicin	2 × 15mg/kg/day iv	4–6	Monitor drug serum levels and renal function
	3 × 1mg/kg/day iv or im	4–6	
Micro-organisms of the HACEK group			
Ceftriaxone*	1 × 2g/day day iv or im	4	
Ampicillin with gentamicin	6 × 2g/day iv	4	Studies suggest that gentamicin once daily might be adequate
	3 × 1mg/kg/day iv or im	4	

‡ *Adapted from Wilson et al.,*[52] *Francioli et al.*[53] *and Heldman et al.*[54]
* Preferred for outpatient treatment.
† Treatment of endocarditis due to vancomycin-resistant enterococci requires a careful assessment of susceptibility to alternative antibiotics, including the new streptogramin combination quinupristin-dalfopristin.

and aminoglycosides is mandatory to ensure treatment success.

Therapeutic schemes recommended for the most common pathogens are presented in Table 59.7 and Table 59.8 .[51] [52] [53] High concentrations of antibiotic in the serum are desirable to ensure penetration into vegetations. Moreover, prolonged treatment is mandatory to kill dormant bacteria clustered in the infected foci (see Fig. 59.3). Therefore, although outpatient and oral therapy has been proposed in specific conditions, [54] [55] prolonged parenteral therapy is usually recommended.

The choice of an optimal therapeutic regimen is based on antibiotic susceptibility testing. The MICs of the principal drugs for the infecting pathogens should be determined. More sophisticated tests, such as minimum bactericidal concentration (MBC) or serum inhibitory and bactericidal concentrations during drug therapy, are not usually needed, although they may be useful when the therapeutic response is inadequate. In such cases, it is important to exclude other causes of treatment failure, such as inadequate antibiotic administration, antibiotic resistance or the presence of a surgically removable focus.

Resistant pathogens and culture-negative IE may fail to respond to standard therapy. The three most problematic organisms in this respect are penicillin-resistant streptococci, methicillin-resistant staphylococci and multiple-drug-resistant enterococci. The organisms responsible for culture-negative IE are listed in Table 59.5 and are also described below.

TABLE 59-8 -- Suggested treatment for native valve and prosthetic valve endocarditis due to staphylococci.[†]

SUGGESTED TREATMENT FOR NATIVE VALVE AND PROSTHETIC VALVE ENDOCARDITIS DUE TO STAPHYLOCOCCI			
Antibiotic	**Dosage and route**	**Duration (weeks)**	**Comments**
Native valves			
Methicillin-susceptible staphylococci			
Flucloxacillin, or oxacillin, or nafcillin with gentamicin (optional)	6 × 2g/day iv	4–6	The benefit of adding gentamicin has not been demonstrated
	3 × 1mg/kg/day iv or im	3–5 days	
Cefazolin (or other first generation cephalosporins) with gentamicin (optional)	3 × 2g/day iv	4–6	Alternative for patients allergic to penicillins (not in case of immediate type penicillin hypersensitivity)
	3 × 1mg/kg/day iv or im	3–5 days	
Vancomycin	2 × 15mg/kg/day iv	4–6	Recommended for ß-lactam-allergic patients
Methicillin-resistant staphylococci			
Vancomycin	2 × 15mg/kg/day iv	4–6	Recommended for ß-lactam-allergic patients
Prosthetic valves			
Methicillin-susceptible staphylococci[*]			
Flucloxacillin, or oxacillin, or nafcillin with rifampin and gentamicin	6 × 2g/day iv	=6	Rifampin increases the hepatic metabolism of numerous of drugs, including warfarin
	3 × 300mg/day po	=6	
	3 × 1mg/kg/day iv or im	2	
Vancomycin with rifampin and gentamicin	2 × 15mg/kg/day iv	=6	Recommended for ß-lactam allergic patients
	3 × 300mg/day po	=6	
	3 × 1mg/kg/day iv or im	2	
Methicillin-resistant staphylococci			
Vancomycin with rifampin and gentamicin	2 × 15mg/kg/day iv	=6	
	3 × 300mg/day po	=6	
	3 × 1mg/kg/day iv or im	2	

† *Adapted with modifications from Francioli et al.*[53]

* Rifampin plays a special role in prosthetic device infection, because it helps kill bacteria attached to foreign material. Rifampin should never be used alone, because it selects for resistance at a high frequency (ca. 10–6).

Penicillin-resistant streptococci

Streptococci are becoming increasingly resistant to penicillin and other ß-lactams, owing to a decreased affinity of their membrane-bound penicillin-binding proteins (PBPs). Penicillin-resistant streptococci are classified as having either intermediate resistance (MIC 0.1–1mg/l) or high resistance (MIC >1mg/l).

Intermediately resistant streptococci may respond to standard therapy because the drug concentrations in the serum produced by intravenous ß-lactams are up to one or two orders of magnitude greater than the MIC for these bacteria (see Table 59.7). For instance, peak serum levels of penicillin G, amoxicillin or ceftriaxone are of the order of 100mg/l, as compared with the MICs of these drugs, which vary between 0.1mg/l and 1mg/l. Nevertheless, a ß-lactam should preferably be combined with an aminoglycoside in such situations.

Against highly resistant streptococci, on the other hand, alternative drugs must be considered. These include vancomycin, to which streptococci are still largely susceptible. In the future, newer quinolones with activity against Gram-positive bacteria, injectable streptogramins such as the quinupristin-dalfopristin combination, or newer oxazolidinones (linezolid) may prove useful.

Methicillin-resistant staphylococci

Staphylococci resistant to methicillin carry a new, low-affinity PBP called PBP2A. This protein allows cell wall assembly when normal PBPs are blocked by ß-lactams. PBP2A confers cross-resistance to most ß-lactam drugs. In addition, methicillin-resistant staphylococci are usually resistant to most other drugs, leaving only vancomycin to treat severe infections.

Vancomycin resistance has emerged among many strains of enterococci, and can be transferred experimentally to *S. aureus* via a transposable genetic element. Moreover, both *S. aureus* and coagulase-negative staphylococci with intermediate resistance to vancomycin have recently emerged in Japan and in the USA. The mechanism of resistance in these bacteria is different from that in enterococci, being mediated by chromosomal mutations affecting synthesis of the cell wall.[56]

Treatment of infections caused by vancomycin-resistant staphylococci will require new drugs. At the present time, few alternatives are available besides older ß-lactams with relatively good affinity for PBP2A, quinupristin-dalfopristin and oxazolidinones. Importantly, such organisms are most likely to be resistant to the newer quinolones.

Multiple-drug-resistant enterococci

These organisms are of major concern because they have become resistant to most available drugs, including vancomycin. Today, treatment of such organisms often relies on the combination of multiple drugs and the use of experimental antibiotics. Treatment of such infections requires precise determination of antibiotic susceptibilities,

testing for bactericidal activity and sometimes determination of serum inhibitory and bactericidal titers and monitoring of drug levels in the serum. Importantly, although aminoglycoside resistance is usually present, these antibiotics may still be synergistic with cell-wall inhibitors provided that the MIC for the aminoglycosides is 1000mg/l or less. Depending on the mechanism of resistance, streptomycin is worth testing because it may be active against enterococci that are resistant to other aminoglycosides.

Culture-negative endocarditis

Treatment of IE due to rare pathogens are summarized in Table 59.5 . Infective endocarditis due to *Brucella* spp. responds to prolonged (<3 months) treat- ment with doxycycline (100–200mg q12h) plus trimethoprim-sulfamethoxazole (960mg q12h) or rifampin (300–600mg/day) combined or not with streptomycin (16mg/kg/day) and surgery. Associated surgery may be required.[46] Cure is evaluated by antibody levels returning to less than 1:160.

Infective endocarditis due to *C. burnetii* is often treated with a combination of doxycycline plus a fluoroquinolone given for up to 3 years. However, recurrences are common. Recently, a regimen of doxycycline combined with hydroxychloroquine appeared more effective.[47] The proposition was based on in-vitro bactericidal tests performed in tissues cultures. Intracellular *C. burnetii* is poorly killed by tetracyclines in the acidic phagolysosomal environment. Hydroxychloroquine increases the phagolysosomal pH (from 4.7 to 5.8) and improves tetracycline-induced bacterial killing. Treatment success was assessed by the evolution of the serology. Patients are considered cured when the anti-phase I antigen IgG titer is less than 1:800 and IgM and IgA titers are less than 1:50.[47]

Infective endocarditis due to *Bartonella* spp. should be suspected in homeless at-risk patients. In a review of 48 *Bartonella* IE cases, patients were treated with a variety of regimens including primarily ß-lactams (amoxicillin and ceftriaxone) combined with aminoglycosides (netilmicin or gentamicin) for at least 2 weeks, or ß-lactams combined with other drugs (e.g. doxycycline) for a total of more than 6 weeks.[57] Importantly, more than 90% of these patients underwent surgical valve replacement, emphasizing the importance of an aggressive approach in such cases.

Optimum treatment of IE due to *Chlamydia* spp., *Mycoplasma* spp. and *Legionella* spp. is unknown. These organisms are highly susceptible to newer fluoroquinolones in vitro. Therefore, these drugs should probably be part of the therapeutic regimen.

Infective endocarditis due to *T. whippelii* is very rare. In non-IE Whipple's disease, trimethoprim-sulfamethoxazole (960mg q12h) given for more than 1 year seems the safest alternative.[50] Some authors also recommend sequential treatment, starting with penicillin plus streptomycin or ceftriaxone plus gentamicin, for 2–6 weeks, followed by long-term trimethoprim-sulfamethoxazole therapy. A recent review of 35 cases of Whipple IE supports this approach.[58] Moreover, it also suggests that surgical valve replacement might be a prerequisite for successful therapy (see Fig. 59.9).

In the absence of any positive microbiologic information the therapeutic approach to culture-negative IE must balance disease severity, risk factors for specific micro-organisms and the selection of a regimen that is likely to be effective against most of the likely pathogens.

In patients who have mild uncomplicated subacute IE, it may be appropriate to await the results of blood culture before initiating therapy. However, in acute fulminating disease, especially if occurring on a background of drug addiction, staphylococcal endocarditis is likely and must be treated promptly. A penicillinase-resistant penicillin (flucloxacillin, oxacillin or nafcillin) intravenously in high dosage (2g q4h), or a first-generation cephalosporin (intravenous cefazolin 2g q8h) in combination with intravenous gentamicin (1mg/kg q8h) or intramuscular streptomycin (0.5g q12h) is an appropriate choice pending culture results.

For the truly culture-negative patient, a regimen directed at the HACEK group, enterococci and non-viridans streptococci is appropriate. Intravenous penicillin 2–3 million units q4h (or intravenous ampicillin 2g q4h) combined with intravenous gentamicin (1mg/kg q8h) is recommended. A cephalosporin, such as intravenous ceftriaxone 2g q24h, may be substituted in patients who are allergic to penicillin. Vancomycin should be reserved for the truly ß-lactamintolerant patient, or where a resistant pathogen is suspected.

Treatment of culture-negative endocarditis should be continued for a total of 6 weeks, although, if the features are those of right-sided endocarditis complicating drug addiction, then the aminoglycoside may be discontinued after 2 weeks while continuing the other agents in full dosage.

Surgery

The leading indications for surgery are:

- ¦ refractory cardiac failure caused by valvular insufficiency;
- ¦ persistent sepsis caused by a surgically removable focus or a valvular ring or myocardial abscess; and
- ¦ persistent life-threatening embolization.

The decision of when to operate on an unstable patient is difficult and requires a multidisciplinary assessment of the situation.

ENDARTERITIS AND MYCOTIC ANEURYSMS

Endarteritis is the inflammation of the arterial wall. The term mycotic aneurysm was coined by Osler to define a nonsyphilitic aneurysm resulting from infective endarteritis. Arterial infection may result from:

- microembolization of bacteria in the vasa vasorum;
- hematogenous seeding of the arterial intima or of thrombi lining atherosclerotic plaques during bacteremia;
- extension from contiguous infected foci; or
- arterial trauma with direct bacterial contamination.

The first two mechanisms are most important during IE. Other risk factors include pre-existing endarterial lesions (e.g. congenital malformations such as patent ductus arteriosus and aortic coarctation, atherosclerosis of large vessels, vascular prosthesis and sometimes depressed host immunity due to diabetes, cirrhosis or corticosteroid therapy). Infected aneurysms represent 2.6% of all aneurysms, with a male:female ratio of about 3:1.[59]

MICROBIAL PATHOGENS AND CLINICAL MANIFESTATIONS

In the context of IE, the pathogens of endarteritis follow the pattern shown in Table 59.1 . In the absence of IE, on the other hand, Gram-negative bacteria are found more frequently. *Salmonella* spp., *E. coli* and other enterobacteria are isolated in more than 50% of cases of abdominal aortitis.[59] *Salmonella choleraesuis* and *Salmonella typhimurium* seem to have a particular tropism for atherosclerotic arteries. It was proposed that all patients over 50 years of age who develop *Salmonella* bacteremia should undergo abdominal imaging to detect possible aortic lesions. *Staphylococcus aureus* is the organism most often associated with extra-abdominal lesions and infections of vascular prosthesis, and it has been reported in about 30% of endarteritis overall. Other pathogens, including fungi, are occasionally reported.

Signs and symptoms depend on the anatomic site of the disease. Local signs include pain, erythema, manifestations due to compression of contiguous organs, and distal ischemia or embolization. Fever and leukocytosis are almost always present.

DIAGNOSIS AND MANAGEMENT

Diagnosis requires a high degree of clinical suspicion. Microbiologic documentation is essential. Blood cultures and cultures of surgical specimens should be performed, if possible before starting antibiotic treatment. Otherwise, special culture media should be used by the laboratory to inactivate antimicrobial drugs present within the samples. Ultrasonography, computerized tomography, magnetic resonance imaging and gallium- or indium-labeled leukocyte scans are helpful to delineate the lesions. None of these techniques will distinguish between sterile and infected fluids; however, they may guide more invasive diagnostic procedures such as percutaneous needle aspiration of the affected area.

Appropriate antibiotic therapy and surgical resection of the infected vessel with extensive local debridement is mandatory. The material should be carefully examined by both the pathologist and the microbiologist. Whenever possible, autologous vascular graft and bypass in uninfected tissue should be performed in order to decrease the risk of re-infection. Antibiotics should be given for at least 4–6 weeks. Certain situations are so complicated that therapeutic strategies must be adapted for each particular case. Sometimes, patients are kept on antibiotics for much longer periods of time, even for life. Despite antibiotic treatment, the morbidity of vascular infections remains high. Infections of arteries in the lower extrem-ities result in amputation in as many as 20% of cases.[60] Moreover, the mortality rate of patients who have infected aneurysms that are diagnosed late may exceed 70%.[61]

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Chapter 60 - Rheumatic Fever

Jonathan R Carapetis

Rheumatic fever is an autoimmune sequel to group A streptococcus infection and is characterized by damage to heart valves, brain, joints and/or skin and, less commonly, heart muscle, pericardium or lungs. All of these tissues eventually recover from the acute inflammation, with the exception of heart valves, which may be left with long-term or permanent damage. This is known as rheumatic heart disease, which remains the most common acquired heart disease of childhood in the world.

EPIDEMIOLOGY

Rheumatic fever is a disease of poverty. During the 1900s it became uncommon in industrialized countries but increasingly recognized in developing countries, which may represent an increasing incidence or better detection of cases. Most of the reduction in industrialized countries occurred prior to the availability of antibiotics[1] and is attributed to the effects of improved living conditions and hygiene infrastructure.

During the year 2000, there were estimated to be 2.5 million people in the world with congestive heart failure caused by rheumatic heart disease during 1994; of 340,000 deaths each year, only 6% occur in established market economies. [2] Others have estimated that about 12 million people suffered from either rheumatic fever or rheumatic heart disease.[3] The incidence of acute rheumatic fever is not well documented; the highest reported incidence was 508 per 100,000 children aged 5–14 years between 1987 and 1996, in the Aboriginal population of northern Australia.[4]

Approximately 50% of people with acute rheumatic fever will develop chronic heart valve damage. Rheumatic heart disease affects over 2% of all northern Australian Aboriginals[4] and is also common in the Pacific Island nations. Other regions with confirmed rheumatic heart disease prevalence >2 per 1000 include sub-Saharan Africa, the Indian subcontinent, Vietnam, Middle Eastern countries and some poorer countries in Latin America.[5] [6]

The prevalence of rheumatic heart disease is very low (usually <0.5 per 1000) in virtually all industrialized countries and is falling in many emerging market economies.[7] Since the 1980s, some areas of the United States have experienced outbreaks of acute rheumatic fever, thought to be due to the emergence of virulent group A streptococci belonging to M serotypes 1, 3 and 18.[8] [9]

First episodes of acute rheumatic fever occur most commonly in children aged between 5 and 15 years and are virtually unheard of in the first two years of life. First episodes may occur in adulthood, but are rare over the age of 35 years. Recurrent episodes also tend to occur in the 5–15 year age group but more frequently occur in adulthood, including some cases up the age of 60 years. [10] Although males and females were equally affected in most early studies, females now outnumber males in developing countries, particularly in rheumatic heart disease prevalence surveys (with ratios ranging between 1.3 and 2 to 1).

PATHOGENESIS

Acute rheumatic fever results from infection of a susceptible host with a 'rheumatogenic' group A streptococcus, leading to an abnormally vigorous, tissue-specific immune response. The exact nature of each of these components has yet to be unraveled but the recent development of improved animal models of rheumatic carditis is likely to be informative.

Immune response

Molecular mimicry — the presence of epitopes in human tissue that are immunologically similar to group A streptococcal antigens — is thought to be responsible for the autoimmune response. Epitopes cross-reacting with group A streptococcal antigens have been identified in human myosin, tropomyosin, keratin, actin, laminin, vimentin and *N*-acetylglucosamine. The cross-reactive epitopes in group A streptococci have been found in the cell wall and streptococcal membranes and in the A, B and C repeat regions of the surface M protein.[11] [12]

Antibodies reactive with both group A streptococci and human heart tissues in the sera of rheumatic fever patients have suggested that a humoral autoimmune response may mediate the tissue damage.[13] The subsequent finding that markers of cell-mediated immune responses were dramatically elevated early in acute rheumatic fever raised the possibility that T-cell activation may be critical and that humoral responses were secondary to antigens released from already damaged tissues. This has been supported by the identification of cytotoxic lymphocytes in the blood of rheumatic fever patients and the findings that M protein can stimulate cytotoxic lymphocytes, M5 protein contains T and B cell epitopes, T cells isolated from heart valve tissue of rheumatic fever patients previously infected with M serotype 5 organisms respond to several M5 peptides, and CD4 and CD8 T cells extravasate into rheumatic valves through the surface valvular endothelium.[11] [14] Finally, recent studies have demonstrated cross-reactive monoclonal antibodies cytotoxic for heart cells in culture, in the presence of complement. [15]

Host factors

There is an inherited susceptibility to rheumatic fever: there is weak concordance in monozygotic twins and inheritance may be autosomal recessive with limited penetrance.[16] [17] During outbreaks of group A streptococcal pharyngitis in US military camps during the 1950s and 1960s, up to 3% of recruits developed acute rheumatic fever.[18] More recent data suggest that approximately 3–6% of any population is likely to be susceptible to rheumatic fever and that this proportion does not vary substantially between populations.[4]

There has been little consistency in the association of HLA type with rheumatic fever. Using molecular techniques, HLA DRB1*0701 and DQA1*0201 alleles and DRB1*0701-DQA1*0201 and DRB1*13-DQA1*0501-3-DQB1*0301 haplotypes were associated with rheumatic heart disease in an Egyptian population, whereas the DQA1*0103 and DQB1*0603 alleles were found less commonly in rheumatic heart disease, suggesting that they may confer protection.[19]

In most populations in which it has been studied, the B-cell alloantigen, D8/17, is expressed in a high proportion of B cells in patients with rheumatic heart disease or a history of rheumatic fever, a moderate proportion of B cells in first-degree family

members of rheumatic fever patients and a low proportion of B cells in control subjects. [20] This suggests that D8/17 may be a marker of inherited susceptibility. Researchers in northern India have identified different B-cell alloantigens that appear to identify rheumatic fever patients in that population. [21]

Organism factors

Certain M serotypes of group A streptococci have been associated with rheumatic fever — particularly serotypes 1, 3, 5, 6, 14, 18, 19, 24, 27 and 29.[22] However, not all strains belonging to these serotypes cause rheumatic fever it therefore seems likely that the potential to cause rheumatic fever is not serotype specific and that any group A streptococcal strain could acquire this potential.

Group A streptococci associated with rheumatic fever have been characterized as class I, based on amino acid sequences within the C repeat region of the M protein (as opposed to class II organisms, which have been associated with poststreptococcal glomerulonephritis).[23] An alternative classification system, in which strains are separated into patterns (labeled a through e) based on the arrangement of genes within the *emm* region of the streptococcal chromosome, concluded that strains associated with rheumatic fever and/or throat infection came from patterns a, b or c, whereas strains associated with skin infection came from pattern d (pattern e strains had no particular tissue or disease association).[24] However, most of the initial studies associating class I or pattern a–c organisms with rheumatic fever came from populations with relatively low endemicity of rheumatic fever or primary group A streptococcal infections. Subsequent studies in Aboriginal Australian, Saudi and Thai populations have failed to confirm any strong association of certain groups or types of group A streptococci with rheumatic fever.[25] [26]

Other issues in pathogenesis

Classic studies from the first half of the 20th century established that acute rheumatic fever always followed a symptomatic or asymptomatic group A streptococcal infection of the upper respiratory tract.[27] This has been challenged in recent years.[28] In tropical developing countries where there is a high prevalence of pyoderma, group A streptococci originating in skin infections may have the potential to cause acute rheumatic fever, either de novo or by subsequently infecting the throat. An alternative hypothesis is that rheumatic fever may sometimes follow infection with group C or G streptococci, which may have acquired 'rheumatogenic' factors by horizontal

TABLE 60-1 -- Recommended doses of antibiotics to prevent rheumatic fever.

RECOMMENDED DOSES OF ANTIBIOTICS TO PREVENT RHEUMATIC FEVER					
Indication and antibiotic	**Route**	**Dose in children (=27kg)**	**Dose in adolescents and adults**	**Frequency**	**Duration**
Options for treatment of GAS tonsillitis/pharyngitis ('primary prophylaxis')					
Phenoxymethyl penicillin (penicillin V)	Oral	250mg	500mg	Twice daily	10 days
Benzathine penicillin G	IM	450mg (600,000U)	900mg (1,200,000U)	NA	Single dose
Erythromycin*	Oral	20mg/kg (max 250mg)	250mg	Twice daily	10 days
Options for prevention of rheumatic fever recurrences ('secondary prophylaxis')					
Benzathine penicillin G	IM	900mg (1,200,000U)	900mg (1,200,000U)	Every 4 weeks†	5 yrs since last episode or age 21 yrs (whichever is longer).
Phenoxymethyl penicillin (penicillin V)	Oral	250mg	250mg	Twice daily	To age 40yrs or for life if heart disease present (see text for details)
Erythromycin**	Oral	250mg	250mg	Twice daily	
IM, intramuscular; NA, not applicable; GAS, group A streptococcal					
Doses as recommended by the American Heart Association [32]					

* Erythromycin should only be used if patient is allergic to penicillin
† If possible, doses every 3 weeks provide superior protection

transmission from group A streptococci.[29] Both hypotheses have yet to be proved.

PREVENTION

Primary prevention

Primary prophylaxis — antibiotic treatment of group A streptococcal upper respiratory tract infection — has been the cornerstone of primary prevention since the mid-1900s. If commenced within 9 days of the onset of symptoms, oral or intramuscular penicillin treatment will usually prevent the subsequent development of rheumatic fever (see Table 60.1 for doses of antibiotics).[30] [31] Alternative regimens using shorter courses of macrolides or oral cephalosporins are effective in treating pharyngitis, but have not yet been proven to prevent rheumatic fever. Their increased cost and broader antimicrobial spectrum argue against their use as first-line agents.[32]

In populations with high rates of acute rheumatic fever, primary antibiotic prophylaxis is important. However, in settings where rheumatic fever is now rare, it is argued that the benefits of antibiotic treatment of group A streptococcal pharyngitis (prevention of rheumatic fever and suppurative sequelae and abbreviating symptoms) do not outweigh the cost, inconvenience, adverse reactions and potential for induction of resistance in other organisms.[33]

In developing countries, although the diagnosis and treatment of pharyngitis must remain priorities, primary prophylaxis has not been proven to substantially reduce the incidence of acute rheumatic fever. In recent studies, at least two-thirds of acute rheumatic fever cases have not been preceded by a sore throat, and therefore would not be preventable even if facilities were available to diagnose and treat group A streptococcal pharyngitis. This has heightened the importance of developing new approaches to primary prevention, whether based around reducing streptococcal transmission by improving housing and hygiene or combining sore throat treatment with skin disease control in tropical countries. The most effective control programs have combined secondary prevention, primary prophylaxis, control of skin infections and wider education of health staff and the community. [34]

A type-specific group A streptococcal vaccine targeting the *N* terminal region of multiple M serotypes is currently in human trials.[35] However, the potential effectiveness of this vaccine in developing countries, where new serotypes emerge rapidly, has been questioned.

Other vaccines in development contain epitopes conserved among all group A streptococci. [36]

While primary prevention remains a dilemma in developing countries, there is no doubt that secondary prophylaxis is feasible and cost effective.[7] Secondary prophylaxis (Table 60.1) is the long-term administration of antibiotics to those with previous acute rheumatic fever or rheumatic heart disease, to prevent rheumatic fever recurrences and the subsequent development, or worsening, of rheumatic heart disease. Benzathine penicillin G is the best option. Its effectiveness is optimal when administered every 3 weeks, although 4-weekly or even monthly dosing is acceptable. Oral penicillin is almost as effective as benzathine penicillin G, provided that adherence can be assured. Penicillin-allergic patients should be offered erythromycin.

The duration of prophylaxis is determined by the risk of recurrent rheumatic fever (which is greatest in the 5 years following the previous attack) and the potential consequences of a recurrence (which are most dangerous in patients with established valvular disease). The minimum duration is 5 years after the last attack or until age 21 years, whichever is longer. If at that time the patient has residual valvular disease, prophylaxis should continue for a minimum of 10 years or until age 40 years, whichever is longer. Patients with severe valvular disease, or any who have had valve surgery, should receive prophylaxis for life.[32]

In regions with high prevalence rates of rheumatic heart disease, it is recommended that a formal control program be established.[3] [37] This requires national commitment (including budgetary), a full-time co-ordinator, a multi disciplinary steering committee and a centralized register of patients. The priorities are case-finding and surveillance, co-ordinating secondary prophylaxis and ensuring good clinical care and follow-up of patients. Resources should be allocated to educational activities and primary prevention once the initial priority activities are in place.

CLINICAL FEATURES

The onset of rheumatic fever symptoms always follows a latent period after the preceding group A streptococcal infection. This latent period is usually 1–5 weeks (mean about 3 weeks) but may be shorter in recurrences or up to 6 months before the onset of chorea.[38] The preceding streptococcal infection is asymptomatic in about two-thirds of cases.

Although patients with acute rheumatic fever may have any or all of the following clinical features, the most common major manifestations are polyarthritis (found in 50–75% of cases) and carditis (40–60%). The most common combination of presenting clinical features in most countries is fever and polyarthritis, with or without a murmur of mitral regurgitation.

Carditis

Acute rheumatic fever may affect the pericardium (often asymptomatic, and sometimes with a pericardial rub and/or small effusion), the myocardium (usually detected only on echocardiography, and rarely contributing to cardiac failure) or the endocardium (the most common and important). Initially, cardiac valves become friable, inflamed and regurgitant, but with time become thickened, scarred, shortened and stenotic. Their function can be further affected by thickening and shortening of the chordae tendinae. The mitral and/or aortic valves are most commonly affected; pulmonary or tricuspid valve disease is rare and usually secondary to increased pulmonary pressures resulting from left-sided valve disease.

The most common clinical manifestation of carditis is a pansystolic murmur of mitral regurgitation, with or without a low-pitched mid-diastolic (Carey-Coombs) murmur.[7] Aortic valve disease may occur alone or accompany mitral valve disease. In general, younger patients

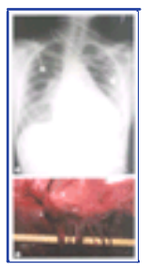

Figure 60-1 (a) Chest radiograph of a 15-year-old boy who had multiple recurrences of acute rheumatic fever, showing gross cardiac enlargement and failure. He had mitral regurgitation and stenosis, and aortic regurgitation and stenosis. He died 2 days after this radiograph was taken, of intractable cardiac failure. (b) Postmortem cardiac examination of the same boy, showing thickened, shortened mitral valve cusps with calcific vegetation and thickened chordae tendinae. *(Photographs kindly provided by Professor Bart Currie, Darwin, NT, Australia.)*

with carditis more commonly have pure mitral and/or aortic regurgitation, but in late adolescence and adulthood, stenotic or mixed regurgitant/stenotic lesions become more common (Fig. 60.1). Rarely, mitral stenosis occurs in younger children.

Rheumatic involvement of the myocardium, manifest by the pathognomonic 'Aschoff body' may disrupt the electrical conduction,

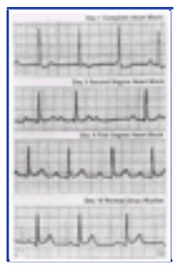

Figure 60-2 Electrocardiographic changes in a young adult with acute rheumatic fever, showing evolution over 18 days from complete heart block to second-degree (Wenckebach) block to first-degree block and then to normal sinus rhythm. *(Reproduced with permission from Bishop W, Currie B, Carapetis J, Kilburn C. A subtle presentation of acute rheumatic fever in remote northern Australia. Aus NZ J Med 1996;26:241–2.)*

leading to prolongation of the P–R interval (Fig. 60.2). Some healthy people have this finding, but a prolonged P–R interval that resolves over days to weeks may be a useful diagnostic feature in cases with atypical clinical features.

Joint manifestations

Rheumatic polyarthritis produces painful red, hot and swollen joints which may contain purulent (but sterile) effusions. The arthritis is asymmetrical and classically migratory, affecting large joints, most commonly knees, ankles, wrists or elbows. Usually, only one or two joints are affected at one time and each may be involved for just a few hours or up to one or two days. The arthritis is very responsive to nonsteroidal anti-inflammatory medication. Should joint symptoms and signs persist more than one to two days after starting high-dose salicylates, the diagnosis should be questioned.

Sometimes, the joint manifestations are milder. Arthritis may affect only a single joint or arthralgia only may occur.[10] As with polyarthritis,

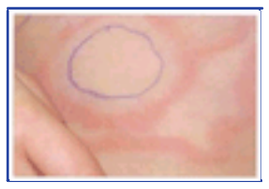

Figure 60-3 Erythema marginatum on the trunk of an 8-year-old Caucasian boy. The pen mark shows the location of the rash approximately 60 minutes previously. *(Photograph kindly provided by Associate Professor Mike South, Royal Children's Hospital, Melbourne, Australia.)*

polyarthralgia is usually asymmetrical, affects large joints and rapidly responds to nonsteroidal anti-inflammatory medication.

Chorea

This intriguing manifestation was named by the English physician Thomas Sydenham, who in 1686 initially called it St Vitus' Dance. Depending on the population, it is present in <10% to >30% of cases of rheumatic fever. Chorea may follow a latent period of up to 6 months following group A streptococcal infection, commonly occurs in the absence of other manifestations and is predominantly found in females (almost never occurring in postpubertal males). The choreiform movements most frequently affect the arms and face (particularly the tongue, which may dart around when protruded), but may be more generalized or asymmetrical. The classic signs include rhythmic squeezing when grasping the examiner's fingers ('milk-maid's grip'), flexion of the wrists and extension of the fingers when the hands are extended ('spooning') and out-turning of the arms and palms when held above the head (the 'pronator sign'). Chorea worsens with anxiety or purposeful movements, but always disappears during sleep. It is commonly associated with emotional lability, personality changes or obsessive-compulsive behaviors. The choreiform movements eventually disappear, usually within 6 weeks, and almost always within 6 months (although individual cases lasting up to 3 years have been described).

Erythema marginatum and subcutaneous nodules

Both manifestations are uncommon (<2% of cases). Erythema marginatum is rarely detected in dark-skinned people. It is a pink, serpiginous rash with a well-defined edge, which begins as a macule and expands with central clearing (Fig. 60.3). Multiple lesions can affect the trunk and sometimes the limbs (but rarely the face) and seem to move before the examiner's eyes. They usually appear early in the acute phase, but may recur for weeks or even months after the other symptoms have subsided: this does not signify ongoing rheumatic inflammation.

Subcutaneous nodules usually appear 2–3 weeks after the onset of rheumatic fever, last from days to 3 weeks and are commonly associated with severe carditis. They are painless, firm subcutaneous lumps between 0.5 and 2cm in diameter and found mainly over extensor surfaces or bony protuberances, particularly the hands, feet, occiput and back.

Fever

With the exception of chorea, most manifestations of acute rheumatic fever are accompanied by fever. Although the peak temperature is commonly >102.2°F (39°C), lower-grade temperatures are not uncommon. The fever, like arthritis or arthralgia, is very responsive to salicylate therapy.

Elevated acute-phase reactants

The C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) are usually dramatically elevated in the acute phase of rheumatic fever, except in chorea when they are commonly normal. The CRP often returns to normal more rapidly than the ESR. The peripheral white blood cell count may also be moderately elevated, although this is a less sensitive marker of rheumatic inflammation.

Other less common clinical features

Infrequently, the presenting complaint of acute rheumatic fever may be severe, central abdominal pain, which responds quickly to anti-inflammatory medication. Epistaxis was prominent in early descriptions of rheumatic fever, but has not been common in recent studies. 'Rheumatic pneumonia' refers to pulmonary infiltrates that

may be found in patients with acute carditis. Mild elevations of liver transaminases, microscopic hematuria, pyuria or proteinuria are found occasionally, but are non-specific and usually not severe.

Other associated poststreptococcal syndromes

Poststreptococcal reactive arthritis has a shorter incubation period after streptococcal infection than rheumatic fever, sometimes follows non-group A ß-hemolytic streptococcal infection, often affects small joints and is less responsive to anti-inflammatory medication. Because of the lack of cardiac involvement, these patients are said not to require secondary prophylaxis. However, this diagnosis should be considered with caution in populations with high rates of rheumatic fever, as some cases have subsequently developed carditis. Even in populations with low rates of rheumatic fever, patients diagnosed with poststreptococcal reactive arthritis should receive secondary prophylaxis for at least one year; it can then be discontinued if there is no evidence of carditis.

Some basal ganglia disorders other than chorea have also been associated with group A streptococcal infections. PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal) infections describes a constellation that may include tic disorders, Tourette's syndrome and obsessive-compulsive symptoms. Unlike rheumatic chorea, patients with PANDAS appear not to be at risk of developing carditis. These patients, and some children with autism, may have high proportions of circulating B cells expressing D8/17 antigen. It is not yet clear whether these syndromes are linked with acute rheumatic fever.

DIAGNOSIS

There is no definitive diagnostic test for acute rheumatic fever. In 1944, the American physician T Duckett Jones developed a set of diagnostic criteria. The clinical features were divided into those that were critical to the diagnosis ('major manifestations') and those that were less specific ('minor manifestations'). The 1992 updated Jones criteria (Table 60.2) for the first time specified that the criteria apply only to the initial diagnosis of acute rheumatic fever.[39] At least two major, or one major and two minor, manifestations are needed, in addition to evidence of a recent group A streptococcal infection to make the diagnosis. Recurrences may be diagnosed based only on the presence of a single major or multiple minor manifestations (together with evidence of recent group A streptococcal infection), provided that other diagnoses have been excluded. There are two exceptions: rheumatic chorea may be diagnosed in the absence of

TABLE 60-2 -- The Jones criteria for guidance in the diagnosis of the initial attack of acute rheumatic fever, updated 1992.*

THE JONES CRITERIA FOR GUIDANCE IN THE DIAGNOSIS OF THE INITIAL ATTACK OF ACUTE RHEUMATIC FEVER, UPDATED 1992	
Major manifestations	**Minor manifestations**
Carditis	Fever
Polyarthritis	Arthralgia
Chorea	Elevated acute-phase reactants
Subcutaneous nodules	Prolonged PR interval
Erythema marginatum	
Plus	
Supporting evidence of a recent group A streptococcal infection	
• Positive throat culture or rapid antigen test	
• Elevated or increasing streptococcal antibody titer	
The presence of two major or one major and two minor manifestations, plus evidence of a preceding group A streptococcal infection, indicates a high likelihood of acute rheumatic fever	

** Reprinted with permission from reference.[39]*

any other manifestations or evidence of a preceding streptococcal infection; and low-grade carditis may occur as a delayed presentation, after streptococcal antibody titers have returned to normal.

In experienced hands, Doppler echocardiography may help to confirm the diagnosis, by revealing subclinical valvular regurgitation or the characteristic appearance of affected valve leaflets.[8] However, echocardiographic criteria have not yet been standardized and the ability to distinguish acute carditis from previous rheumatic valve damage is not always definitive.[40]

A preceding group A streptococcal infection may be demonstrated either by a positive throat swab culture or rapid antigen test, or positive antistreptococcal serology. The serological tests most commonly used are antistreptolysin O, anti-DNase B and antihyaluronidase. At least one of any two of these tests will be positive following >90% of group A streptococcal infections. Serology is of limited value in regions with high prevalence rates of streptococcal impetigo, where children may have positive antistreptococcal titers most of the time.

The diagnosis of acute rheumatic fever may be difficult because of the nonspecific nature of many of the clinical features and the wide range of differential diagnoses, particularly of children presenting with fever and polyarthritis. Some possible differential diagnoses are listed in Table 60.3 .

MANAGEMENT

The main aims of management in acute rheumatic fever are to confirm the diagnosis, treat cardiac failure, shorten the duration of symptoms and ensure that ongoing secondary prophylaxis and clinical follow-up are assured. There is no evidence that treatment in the acute phase alters the likelihood or severity of long-term cardiac valvular damage.[41] Hospital admission is recommended for all cases, to ensure that relevant investigations are performed, provide adequate medical treatment, commence educational activities for the patient and family and ensure that a follow-up program (secondary prophylaxis, inclusion on rheumatic fever registers, etc.) is set in place.

Investigations

Initial investigations should include a throat swab and culture (or rapid streptococcal antigen test), streptococcal serology assays (at least two of antistreptolysin O, anti-DNase B or antihyaluronidase, if available), ESR, CRP, white blood cell count, electrocardiogram, chest X-ray and, if available, echocardiogram. Additional investigations may

TABLE 60-3 -- Differential diagnoses of the three most common major manifestations of acute rheumatic fever.*

DIFFERENTIAL DIAGNOSES OF THE THREE MOST COMMON MAJOR MANIFESTATIONS OF ACUTE RHEUMATIC FEVER.			
	Polyarthritis	**Carditis**	**Chorea**

Differential diagnoses	Connective tissue disease	Innocent murmur	Systemic lupus erythematosus
	Immune complex disease	Mitral valve prolapse	Drug reaction
	Septic arthritis (including gonococcal)	Congenital heart disease	Wilson's disease
	Viral arthropathy	Infective endocarditis	Tic disorder
	Reactive arthropathy	Hypertrophic cardiomyopathy	Choreo-athetoid cerebral palsy
	Lyme disease	Myocarditis: viral or idiopathic	Encephalitis
	Sickle cell anemia	Pericarditis: viral or idiopathic	Huntington's chorea
	Infective endocarditis		Intracranial tumor
	Leukemia or lymphoma		

** (Reprinted with permission from The Oxford Textbook of Medicine, 4th edn, Oxford University Press, 2003).*

be necessary to exclude alternative diagnoses, including blood cultures for endocarditis, antiviral serology and autoimmune markers for arthritis, and testing for systemic lupus erythematosus, Wilson's disease or acute drug ingestion in the case of chorea.

Bed rest

It is no longer recommended that all patients with acute rheumatic fever should routinely rest in bed for at least 4 weeks. Instead, as soon as the patient can walk, and provided that cardiac failure is controlled, bed rest can be relaxed.

Antimicrobial treatment

Although in most cases the causative group A streptococcal strain can no longer be isolated by the time rheumatic fever symptoms start, all patients should receive antibiotics to treat any possible ongoing streptococcal infection. The recommended regimens are listed in Table 60.1 . Intravenous penicillin is not required.

Anti-inflammatory medication

Although anti-inflammatory medication is used for most cases of acute rheumatic fever, there is no evidence that it alters the short- or long-term outcome.[41] In cases where the diagnosis is unclear, withholding anti-inflammatory medications for hours or even a day or two may allow the characteristic features (e.g. migratory polyarthritis) to appear. In these cases, pain can be controlled with codeine. Once the diagnosis is confirmed, patients with arthritis, arthralgia or fever may be treated with aspirin at a dose of 80–100mg/kg/day (4–8g/day in adults) in 4–5 divided doses for 2 weeks, reducing then to 60–70mg/kg/day for a further 2–4 weeks. The response to salicylate treatment is almost always dramatic: joint symptoms and fever resolve within 1 or 2 days. Sometimes nausea and vomiting occur with the initial high doses. In such cases, the dose can be reduced for 1–2 days and then gradually increased to treatment doses. If available, salicylate levels can be monitored if there are symptoms of toxicity. Arthritis or arthralgia may recur up to 3 weeks after cessation of salicylate therapy, sometimes accompanied by a raised CRP or ESR. In such cases a brief further course of salicylates will control symptoms. Although there are no data to compare salicylates with other nonsteroidal anti-inflammatory medications, good symptomatic responses to naproxen (10–20mg/kg/day) have been reported. [42]

Corticosteroids

A meta-analysis failed to confirm a long-term beneficial effect of corticosteroids in acute rheumatic carditis.[41] Although they have no proven benefit, most clinicians use steroids to treat acute rheumatic carditis associated with cardiac failure or more severe cases of rheumatic pericarditis. Oral prednisone or prednisolone is most commonly used at doses of 1–2mg/kg/day (max 80mg/day), tapering after 2 or 3 weeks. Intravenous methylprednisolone may be used in life-threatening carditis.

Management of cardiac failure

Diuretics, angiotensin-converting enzyme inhibitors (particularly in the presence of aortic regurgitation) and fluid restriction are the mainstays of treatment of cardiac failure due to rheumatic carditis.[43] Digoxin is mainly used when cardiac failure co-exists with atrial fibrillation. Cardiac failure in rheumatic carditis is due almost exclusively to valvular disease rather than myocardial dysfunction and is most common when recurrent carditis damages a previously affected valve. Rarely, life-threatening cardiac failure may necessitate urgent valve surgery during the acute inflammatory phase.

Valve surgery is more often required for chronic rheumatic heart disease. In recent years, there has been a tendency to undertake valve repair rather than replacement or to use homografts or xenografts rather than mechanical prostheses. These procedures avoid the need for anticoagulation, which is frequently associated with thromboembolic complications. There is an increasing tendency to identify candidates for valve surgery early in the illness, because valve repair is difficult when valves are extensively damaged, and preoperative left ventricular dysfunction and/or pulmonary hypertension are the most important negative prognostic factors. Ideally, a cardiologist should review all patients with rheumatic heart disease shortly after diagnosis. Surgery should be considered for patients with aortic or mitral stenosis, cardiac failure, left ventricular enlargement, evidence of pulmonary hypertension, tricuspid regurgitation or atrial fibrillation.

Treatment of chorea

Milder cases of chorea require no treatment. However, chorea that impairs normal daily activities, or that causes embarrassment or discomfort to the patient, may require intervention. Haloperidol, carbamazepine and diazepam have all been reported to be effective. Other medications sometimes used are chlorpromazine, sodium valproate and pimozide. The associated anxiety, emotional lability and behavioral abnormalities may require low-dose minor tranquilizers or even behavioral therapy. Salicylates and steroids have no role in treating chorea.

Immunomodulatory treatment

Because of the autoimmune nature of acute rheumatic fever, it has been postulated that immunomodulatory treatments may be beneficial. Small studies of intravenous immunoglobulin have suggested benefit in accelerating recovery from chorea, but have not demonstrated

reduced incidence of long-term valve disease in nonchorea acute rheumatic fever. [44] However, this and other similar treatments have not yet been properly assessed in large controlled trials.

Prognosis and follow-up

Acute rheumatic fever will resolve spontaneously, usually within 12 weeks, if left untreated. With treatment, most cases can be discharged from hospital within 2 weeks.

The likelihood of developing rheumatic heart disease relates to the severity of acute carditis and the number of episodes of recurrent acute rheumatic fever.[45] Overall, 30–50% of all people with acute rheumatic fever will eventually develop rheumatic heart disease, increasing to >70% in patients with severe carditis at the first episode or in those who have had at least one recurrence. Approximately 75% of recurrences occur within 2 years of the previous acute episode and >90% within 5 years. The reasons for this are unclear but probably related to immune sensitization that wanes with time following the acute attack. Although the clinical features of recurrent episodes tend to mirror those present at earlier episodes, this is not always the case and patients with no evidence of carditis initially may develop it with recurrences. [10]

Therefore, regardless of the clinical features of the initial episode, the priorities of long-term management are to ensure adherence to secondary prophylaxis, treat cardiac failure (including considering early surgical intervention if necessary) and educate about the need to treat streptococcal upper respiratory or skin infections. Angiotensin-converting enzyme inhibitors may delay the need for operation in asymptomatic patients with chronic aortic regurgitation. Regular echocardiography is

often useful to follow the progress of valve disease, especially where follow-up may be irregular or when communication or cultural differences make clinical assessment difficult. All patients with rheumatic valvular disease should receive antibiotic prophylaxis at the time of dental or surgical procedures (with a nonpenicillin antibiotic such as clindamycin if the patient is receiving penicillin secondary prophylaxis — see Chapter 59 on infective endocarditis). If heart failure is present, pneumococcal and influenza immunization should be offered.

If possible, all patients with carditis should be reviewed by their primary medical practitioner at least every 6 months, by a specialist (pediatrician, physician or cardiologist) every 1–2 years, by a dentist every year and have echocardiography every 1–2 years. These arrangements should be tailored to the requirements of each patient and according to local resources. Patients without clinical evidence of carditis may have less frequent specialist reviews and should not require echocardiography after the initial acute rheumatic fever diagnosis.

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Chapter 61 - Practice Points

61.a Role of white cell scans for deep-seated sepsis

Verka Beric

Introduction

White blood cell scans are used to locate areas of pyogenic infection or acute inflammation.

The neutrophil is the active component of the radiolabeled leukocyte preparation used during the scan. Lymphocytes play no part in the investigation as they are damaged by radioactive labeling; similarly, monocytes fail to respond to the labeling technique. In pyogenic infections, neutrophils are mobilized from their resting positions, along the margins of blood vessels, in order to migrate specifically to inflamed tissues. This migratory activity is most vigorous during the acute illness and it is this phenomenon that is exploited by the white cell scan. White cell scans have, however, a limited role in the investigation of chronic sepsis and inflammation, in which neutrophil migratory activity is reduced. Unless secondary pyogenic infection exists, a negative result is seen in viral, mycobacterial, fungal and parasitic infections because of the relative lack of neutrophil activity.

Technique

Mixed leukocytes from the patient and labeled in vitro with a chelated radioactive gamma-emitting isotope. Indium-111-oxine (^{111}In-oxine) remains the gold standard radiopharmaceutical used for labeling purposes but technetium-99m-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) is now preferred in certain clinical situations. The labeled leukocytes are then injected intravenously. The sites within the body to which the radioactive neutrophils migrate are detected by imaging the distribution of the isotope with a gamma camera. Sites of focal infection and acute inflammation appear as areas of abnormally increased isotope uptake.

The normal physiologic distribution of ^{111}In-oxine immediately after injection is the blood pool, lungs, liver and spleen, with activity decreasing over time. ^{99m}Tc-HMPAO has a similar distribution but has the disadvantage of additional renal and intestinal clearance. Renal, bladder, gallbladder and bowel activity may be seen within the first hour and are usually seen by 4 hours.

Despite the above limitation, ^{99m}Tc-HMPAO is less costly, more readily available, imparts a lower radioactive dose to the patient and yet provides images of better quality than ^{111}In-oxine. The relatively short half-life of ^{99m}Tc-HMPAO means that sensitivity is near maximal at 60 minutes. Indeed, the study may be completed by 2–4 hours, thus allowing abdominal imaging to be performed before signs of bowel clearance occur. By virtue of its longer half-life,^{111}In-oxine usually requires a 24-hour image for maximal sensitivity, which in turn becomes advantageous in situations in which the rate of neutrophil migration and turnover is reduced. With both agents, in order to limit errors in interpretation, serial images are acquired at approximately 1 hour, 3 hours and 24 hours. Despite such precautions, false-positive and false-negative results may be found in certain clinical conditions (Table 61a.1).

The decision to use the white cell scan and the choice between using technetium-99m and indium-111 for a particular clinical situation are both confusing and controversial issues. Suggested guidelines based upon current use and literature are outlined below.

Abdominal sepsis

The white cell scan is the next investigation of choice for the patient who has suspected abdominal or pelvic sepsis after an inconclusive ultrasound or computerized tomography scan.

Despite the normal physiologic uptake of ^{99m}Tc-HMPAO in the bowel, it is the preferred agent for imaging the abdomen in most situations, particularly when rapid diagnosis is required. Indeed, the main clinical role of^{99m}Tc-HMPAO in some centers is to detect acute relapses of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease.

For the diagnosis of abdominal abscesses that communicate with bowel (typically seen in diverticular disease, Crohn's disease and as a result of pancreatitis), ^{111}In-oxine is preferred for its lack of physiologic bowel excretion. Early images demonstrate focal abscess activity that declines with time as the abscess decompresses and discharges into the bowel. As a result, the isotope is first detected within the bowel lumen on the 24-hour image.

^{111}In-oxine is also preferred for the diagnosis of abscesses close to the liver and spleen, where they may be obscured by the intense physiologic uptake of these organs on early images. The abscess is identified by its increase in activity with sequential images over 24 hours, whereas the activity in the liver and spleen either reduces or remains constant during this period.

Thoracic sepsis

The white cell scan has no place in the routine investigation of pulmonary sepsis. It is usually negative for lobar pneumonia. Rarely, the white cell scan is used to determine the degree of inflammatory activity present in cavities thought to represent pulmonary abscesses or in a region of lung known to be bronchiectatic.

White cell scans are almost always negative for bacterial endocarditis and valvular vegetations. The investigation is only justified in these cases when pyrexia develops with associated positive blood

TABLE 61.a-1 -- False-negative and false-positive white cell scan results.

FALSE-NEGATIVE AND FALSE-POSITIVE WHITE CELL SCAN RESULTS
False-negative results
Chronic low-grade infection
Parasitic, fungal, mycobacterial and viral infections
Encapsulated nonpyogenic abscess
Acute vertebral osteomyelitis
Intrahepatic or intrasplenic abscess
Abnormal neutrophil function caused by chemotherapy or corticosteroid use
Appropriate antibiotic use
False-positive results

Drug-induced pneumonitis
Graft-versus-host disease
Inflammatory bowel disease
Hematoma and gastrointestinal hemorrhage
Pseudoaneurysm
Swallowed labeled leukocytes from oropharynx, esophagus or lungs
Surgical wounds, enterostomy or catheter sites

cultures, thereby raising suspicion of septic emboli or metastatic abscesses.

Musculoskeletal and soft tissue infection

Acute osteomyelitis has traditionally been diagnosed with the combination of a technetium-99m-methylene diphosphonate (^{99m} Tc-MDP) bone scan and plain radiography. The ^{111} In-oxine white cell scan is preferred for suspected acute relapses of chronic osteomyelitis or suspected prosthetic joint infection where plain radiography and the bone scan may appear abnormal, even in the absence of acute infection. In this situation, a map of the normal physiologic marrow uptake of neutrophils must be obtained in order to differentiate the areas of abnormal inflammatory uptake seen with the white cell scan. Normal marrow uptake is depicted by the 1-hour ^{111} In-oxine image or by a separate ^{99m} Tc-colloid scan.

Similarly, the combination of a ^{99m} Tc-MDP bone scan with a ^{111} In-oxine white cell scan may be used to determine whether soft tissue infection, such as a diabetic foot ulcer, has extended to involve the underlying bone. The scans are acquired simultaneously and superimposed to determine the precise anatomic relationship between focal neutrophil activity and the 'bone map' provided by the bone scan. Neutrophil activity overlying the bone scan image is taken to represent osteomyelitis. The combination of scans is required because the bone scan alone may be abnormal in the absence of osteomyelitis because of the effects of local hyperemia from the soft tissue infection.

Prosthetic vascular graft infection is associated with high morbidity and mortality rates if delay in diagnosis occurs. A high index of suspicion is required because the only indication may be a low-grade fever, although aortofemoral or iliofemoral graft infections may additionally present with groin pain or local soft tissue infection. White cell scans are particularly useful for demonstrating the extent of infection along the graft and for imaging abdominal grafts. ^{111} In-oxine is the preferred agent for detecting chronic graft infections.

Undiagnosed fever

In this situation, the white cell scan is used simply to locate the pathology causing the fever so that a diagnosis may be made with more conventional means. Determining the characteristics of the undiagnosed fever and using the white cell scan only if appropriate may optimize the yield of positive results.

A true 'fever of unknown origin' is defined as a fever of at least 3 weeks' duration where at least 1 week of in-hospital investigation has failed to reach a diagnosis. Most causes of fever of unknown origin are due to nonpyogenic infection, malignancy or connective tissue disease. Pyogenic infection is seen in only 10–20% of cases.

Any infection associated with an occult fever is likely to be subacute or chronic with reduced neutrophil migration, and the ^{111} In-oxine white cell scan is therefore preferred for its superior 24-hour images.

Neutropenic patients and HIV-positive patients may have fever and infection without localizing signs. Because the low neutrophil count will produce a suboptimal result with the white cell scan, fresh cross-matched donor leukocytes may be used instead and are often preferred in HIV-positive patients in order to avoid risk of contamination of staff handling the blood.

Discussion

The role of the white cell scan in deep-seated sepsis may be solely to localize or to confirm the presence of a pyogenic infection. A specific diagnosis is often regarded as a bonus. It is important to realize that this investigation may not be the most appropriate in chronic and nonpyogenic infections such as fever of unknown origin, sarcoidosis, tuberculosis and *Pneumocystis carinii* pneumonia. In these situations, more general inflammatory markers, such as gallium-67 and the newer agents, ^{99m} Tc- or ^{111} In-labeled human polyclonal immunoglobulin, may be more suitable. Further, other imaging modalities may be more appropriate in certain clinical situations; for example, magnetic resonance imaging in musculoskeletal infections. Advice from an imaging specialist should be sought in all but the most straightforward cases.

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61.b Should all infected intravascular devices be removed?

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Dennis G Maki

Case presentation

A 63-year-old woman patient in the intensive care unit (ICU) was admitted 5 days previously following a motor vehicle accident in which she sustained blunt chest trauma leaving her with a lung contusion and flail chest, mandating intubation and prolonged mechanical ventilatory support. Underlying co-morbidities include hypertension and non-insulin-dependent diabetes mellitus. Upon admission to the ICU, an arterial line and triple-lumen central venous catheter (CVC) were placed in the right radial artery and right internal jugular vein, respectively. The patient has been doing well, but has now developed fever of 102°F (38.6°C), associated with tachycardia and hypotension.

Discussion

How should patients with fever and an intravascular device be evaluated?

Intravascular devices (IVDs) in their many forms are essential to modern health care. Unfortunately, their use is associated with an often-underappreciated risk for IVD-related bloodstream infection (IVDR BSI). The majority of endemic nosocomial BSIs are primary, most of which originate from an IVD, while a smaller number are secondary BSIs and stem from a local nonvascular site, such as ventilator-associated pneumonia or postsurgical site infection. Prospective studies of IVDs show that every type of IVD carries some risk of causing BSI. It can be seen that when this risk is expressed per 1000 IVD days, the relative risk of IVDR BSI with standard noncuffed, nontunneled CVCs and hemodialysis catheters is approximately 2.3 and 2.8 BSIs per 1000 IVD days, respectively, whereas the risk with cuffed and tunneled CVCs (1.2/1000 IVD days) and subcutaneous central venous ports (0.2/1000 IVD days) is considerably lower. It should be noted that, contrary to popular belief, peripherally inserted central venous catheters (PICCs) (2.1/1000 IVD days) and arterial catheters (3.7/1000 IVD days) have a risk of IVDR BSI that approaches that seen with standard noncuffed CVCs.

Micro-organisms that cause IVDR BSI must first adhere to the intraluminal or extraluminal surface of the IVD before infection of the bloodstream can occur. With short-term IVDs (in place <10 days) — peripheral intravenous catheters, arterial catheters and noncuffed, nontunneled CVCs — most device-related BSIs are of cutaneous origin, from the insertion site, and primarily gain access extraluminally. In contrast, contamination of the catheter hub and lumen appears to be the predominant mode of BSI with long-term, permanent IVDs (in place >10 days), such as cuffed Hickman- and Broviac-type catheters, cuffed hemodialysis CVCs, subcutaneous central venous ports and PICCs.

Recent evidence-based guidelines provide the best current information on the evaluation of the ICU patient with fever or other signs of sepsis. Before any decision regarding initiation of antimicrobial therapy or removal of an IVD, the patient must be thoroughly examined to identify all plausible sites of infection, including ventilator-associated pneumonia, catheter-associated urinary tract infection, surgical site infection or antibiotic-associated colitis, as well as line sepsis. Although bacteriuria is extremely common in hospitalized patients with a urinary catheter, it is rarely the cause of fever or sepsis. The presence of inflammation or purulence at the catheter insertion site is now rare in patients with IVDR BSIs caused by short-term, noncuffed vascular catheters; however, the presence of purulence is highly suggestive of IVD-related infection.

Removal and culture of the IVD has historically been the gold standard for the diagnosis of IVDR BSI, particularly with short-term catheters. Studies have demonstrated the superiority of semiquantitative or quantitative catheter tip culture methods for the diagnosis of IVDR BSI. The diagnosis of IVDR BSI is completed when a colonized IVD is associated with concomitant BSI, with no other plausible source.

Cultures of IVDs obviously require their removal, which is a major problem in patients with long-term IVDs. If a laboratory has available an automated quantitative system for culturing blood, quantitative blood cultures drawn through the IVD and concomitantly by venipuncture from a peripheral vein (or another IVD) can permit the diagnosis of IVDR bacteremia or fungemia to be made with sensitivity and specificity in the range of 80–95%. The wide availability of automated radiometric blood culture systems, in which blood cultures are continuously monitored for microbial growth, has led to a clever application of this system for the detection of IVDR BSI: the differential-time-to-positivity (DTP) of blood cultures drawn through the IVD and concomitantly from a peripheral site. Detection of positivity in a blood culture drawn from the IVD more than 2 hours before positivity of the culture drawn from a peripheral site has been shown to be highly predictive of IVDR BSI in one study with long-term catheters, yielding an overall sensitivity of 94% and specificity of 91%.

Further assessment includes review of radiographic studies and, especially, Gram-stain and culture of sputum aspirated from the endotracheal tube, and cultures of urine and blood. It is ill-advised to routinely remove an IVD or start anti-infective drugs for suspected or presumed infection in the critically ill patient without first obtaining blood cultures, with at least one being drawn from a peripheral vein by percutaneous venipuncture.

What, if any, antimicrobials should be started in patients with suspected IVDR BSI?

Once the initial basic examination has been completed and cultures have been obtained, empiric antimicrobial therapy based on the most likely source of infection may be initiated. If an IVD is suspected as the source of sepsis — purulence is present at the insertion site or, more likely, no other plausible source of sepsis can be found — the combination of intravenous vancomycin (for staphylococci resistant to methicillin) and a fluoroquinolone, cefepime or imipenem/meropenem (for multiresistant nosocomial Gram-negative bacilli) should prove effective against the bacterial pathogens most likely to be encountered. Initial therapy can then be modified based on the microbiologic identification and susceptibility of the infecting organisms.

It should be emphasized that bacterial growth on culture of a vascular catheter or in a blood culture drawn from a retained device, with negative blood cultures drawn from a peripheral vein, is rarely an indication for initiation of antimicrobial therapy, especially if the culture is positive for coagulase-negative staphylococci. In the absence of local or systemic signs of infection, an isolated positive catheter culture usually reflects colonization of the device rather than true infection.

Does the type of catheter in place influence whether the IVD should be removed?

Short-term vascular catheters, such as noncuffed and nontunneled CVCs, arterial lines and noncuffed and nontunneled hemodialysis catheters, while essential for vascular access in critically ill patients,

are easily removed and can always be replaced with minimal additional risk to the patient. If a short-term, noncuffed CVC or arterial catheter is suspected of being infected because the patient has no obvious other source of infection to explain sepsis, there is inflammation at the insertion site or cryptogenic staphylococcal bacteremia or candidemia has been documented, blood cultures should be obtained and the catheter should be removed and cultured (Table 61b.1). Failure to remove an infected catheter puts the patient at risk of developing septic thrombophlebitis with peripheral intravenous catheters, septic thrombosis of a great central vein with CVCs, or even endocarditis. Continued access, if necessary, can always be established with a new catheter inserted in a new site. A new catheter should never be placed in an old site over a guidewire if the first catheter is suspected of being infected, especially if there is purulence at the site.

In contrast, suspected infection of long-term CVCs, such as cuffed and tunneled Hickman-like catheters and subcutaneous central venous ports, does not automatically mandate removal of the device (see Table 61b.1). These devices provide essential vascular access for patients who require total parenteral nutrition or chemotherapy, and

TABLE 61.b-1 -- Algorithm for diagnosis and management of line sepsis with intravascular devices.*

ALGORITHM FOR DIAGNOSIS AND MANAGEMENT OF LINE SEPSIS WITH IVDs
Examine the patient thoroughly, to identify unrelated sources of infection
Carefully examine all catheter insertion sites; Gram stain and culture any expressible purulence
Obtain two 10–15ml cultures:
• If standard (nonquantitative) blood cultures, draw one by percutaneous peripheral venipuncture and one through the suspect IVD
• If quantitative blood culture techniques are available (e.g. the Isolator system), catheter-drawn cultures can enhance the diagnostic specificity of blood culturing in the diagnosis of line sepsis. However, a peripheral percutaneous quantitative blood culture must be drawn concomitantly
Option regarding a peripheral intravenous or arterial catheter: remove and culture catheter
Options regarding a short-term CVC:
• Purulence at insertion site or no purulence, but patient floridly septic, without obvious source:
Remove and culture catheter
Gram stain purulence
Re-establish access at new site
• No purulence, patient not floridly septic:
Leave catheter in place, pending results of blood cultures
or
Remove and culture catheter, re-establish needed access at new site
Options regarding surgically implanted, cuffed Hickman-type catheters
• Remove at outset if:
Infecting organism known to be *Staphylococcus aureus, Bacillus* spp., *Corynebacterium jeikeium, Mycobacterium* spp. or filamentous fungus
Refractory or progressive exit site infection, despite antimicrobial therapy, especially with *Pseudomonas aeruginosa*
Tunnel infected
Evidence of septic thrombosis of cannulated central vein or septic pulmonary emboli
Evidence of endocarditis
• Remove later on if:
Any of the above become manifest
BSI persists =3 days, despite iv antimicrobial therapy through catheter
Options regarding surgically implanted subcutaneous central ports (e.g., Portacath):
• Cellulitis without documented bacteremia: begin antimicrobial therapy, withhold removing port
• Aspirate from port shows organisms on Gram stain or heavy growth in quantitative culture, or documented port-related bacteremia: remove port
Decision on whether to begin antimicrobial therapy, before culture results available, are based on clinical assessment or Gram stain of exit site or the blood drawn from a long-term IVD
With no microbiologic data to guide antimicrobial selection in a septic patient with suspected line sepsis, consider iv vancomycin and ciprofloxacin, cefepime, or imipenem/meropenem

** Adapted from Maki (2002).*

replacement of these devices is associated with considerably more risk and expense than with short-term IVDs.

What are the absolute indications for removal of an IVD, regardless of the type?

As summarized in Table 61b.1 , even long-term IVDs, despite their value, must be removed when:

¦ there has been persistent exit site infection;
¦ the tunnel is obviously infected;
¦ there is evidence of complicating endocarditis, septic thrombosis or septic pulmonary emboli;
¦ the infecting pathogen is *Staphylococcus aureus*, *Corynebacterium jeikeium*, a *Bacillus* sp., *Stenotrophomonas* spp., *Burkholderia cepacia*, any pseudomonal species, a filamentous fungus or *Malassezia* spp. or a mycobacterial species; or
¦ bacteremia or candidemia persists for more than 3 days despite adequate therapy.[4]

Staphylococcus aureus and *Candida* spp. are particularly virulent causative organisms of IVDR BSI.

How should IVD-related infections with Staphylococcus aureus and Candida spp. be managed?

Intravascular device-related BSI caused by *S. aureus* must always prompt removal of the IVD, even if signs of bacteremia have resolved following antimicrobial therapy because of the significant risk of infectious endocarditis (IE) or other metastatic infection if bacteremia recurs.

Historically, high rates of associated IE and late complications led to a universal policy of 4–6 weeks of antimicrobial therapy for all patients with *S. aureus* bacteremia. Earlier diagnosis and bactericidal therapy of nosocomial *S. aureus* in recent years has been associated with lower rates of IE and metastatic complications, prompting suggestions that short-course therapy (i.e. 14 days) is effective and safe for many cases of IVDR *S. aureus* bacteremia as long as the patient defervesces within 72 hours and there is no evidence of metastatic infection. In a study using transesophageal echocardiography (TEE) in 103 hospitalized patients with *S. aureus* bacteremia, 69 related to an IVD, Fowler *et al.* (1997) found a surprisingly high incidence of endocarditis, 23% with IVDR *S. aureus* BSI. In a more recent report, these authors have reported that the routine use of TEE with IVDR *S. aureus* BSI as a means to stratify patients into short-course or long-course therapy is cost-effective. However, at this time there are no prospective studies to affirm this approach. Until more data are available, short-course therapy for IVDR *S. aureus* bacteremia therapy should be approached with caution and employed only when the TEE is unequivocally negative and the patient has defervesced quickly — within 72 hours of removing the IVD and starting anti-infective therapy.

Likewise, all patients with IVDR candidemia should be treated, even if the patient becomes afebrile and blood cultures spontaneously revert to negative following removal of the catheter without antifungal therapy. Several studies have reported successful treatment of IVD BSI due to *Candida* spp. without IVD removal with prolonged courses of amphotericin B administered through the catheter; however, this is in contrast to the results of other prospective studies that have found an increased duration of candidemia and mortality rate in patients who retain their infected IVD. Until this issue is clarified by prospective randomized studies most episodes of candidemia caused by an infected IVD mandate early removal of the IVD. Intravascular device-related candidemia that responds rapidly to

TABLE 61.b-2 -- Formulations of various antibiotic-containing lock solutions published in the medical literature.*

FORMULATIONS OF VARIOUS ANTIBIOTIC-CONTAINING LOCK SOLUTIONS PUBLISHED IN THE MEDICAL LITERATURE

Drug	Dosage	Dwell time	Duration of therapy	Stability with heparin solutions
Vancomycin	1–5mg/ml	8–24 hours	7–15 days	10–100 units/ml has been shown to be safe when co-administered with low-dose vancomycin (1–5mg/ml)
				High dose vancomycin (83mg/ml) has been used successfully without co-administration of heparin
Teicoplanin	100–150mg/ml	24 hours	5–9 days	10 units/ml
Gentamicin	1–13mg/ml	8–72 hours	5–21 days	Gentamicin precipitates rapidly in heparin solutions when gentamicin doses of 5mg/ml are used
				A single study has reported the stability of 1 mg/ml of gentamicin in solutions with heparin concentrations as high as 2500 units/ml
Amikacin	1.5–2mg/ml	12–24 hours	6–27 days	Most studies have not addressed the issue of stability of amikacin with heparin
				A single study using amikacin concentrations as high as 40mg/ml reported no drug precipitation in heparin (100 units/ml) although formal stability studies were not performed

** Adapted, in part, from Berrington and Gould (2001).*

removal of the catheter and institution of intravenous amphotericin B can be reliably treated with a daily dose of 0.3–0.5mg/kg and a total dose of 3–5mg/kg. Fluconazole (400mg/day) has been shown to be as effective as amphotericin B in randomized trials in non-neutropenic patients, and has further been shown to be comparable to amphotericin B in observational studies of neutropenic patients with *Candida* IVDR BSI, but should not be used with IVDR BSIs associated with septic thrombosis and high-grade candidemia or, obviously, with infections caused by fluconazole-resistant organisms, such as *Candida krusei* and *Candida glabrata*. Although data on the use of caspofungin are limited, at least one study has demonstrated its equivalency to amphotericin B deoxycholate in 186 candidemic patients, and its use may be considered in cases where fluconazole resistance is likely or other antifungals are contraindicated.

Are there any proven methods that improve the chances of retaining a long-term IVD?

Studies using 7–21 days of antibiotics infused through the infected line, primarily with BSIs caused by coagulase-negative staphylococci, have shown success rates of 60–91% without catheter removal although there was considerable variability in the clinical response, depending on the infecting micro-organism; with coagulase-negative staphylococcal BSIs, the risk of recurrent bacteremia has been approximately 20%.

In small, uncontrolled clinical trials of antibiotic lock therapy (ALT), usually in conjunction with systemic antibiotic therapy, cure rates of infected IVDs in excess of 90% have been reported. The vast majority of IVDs reported in these studies were infected with coagulase-negative staphylococci and enteric Gram-negative bacilli and therefore, at this time, ALT cannot be recommended for the routine management of long-term IVDs infected by *S. aureus*, *Bacillus* spp., *Corynebacterium jeikeium*, *Stenotrophomonas* spp., *B. cepacia*, all pseudomonas species, fungi or mycobacterial species.

Antibiotic lock therapy is reasonable for the salvage of long-term IVDs infected with coagulase-negative staphylococci or enteric Gram-negative bacilli although commercially available standard lock solutions do not exist. Table 61b.2 lists the types of lock solutions that have been studied most extensively, although lack of data limits recommending one solution over another. Obviously, if IVDR BSI recurs after an attempt to salvage the IVD with ALT, the device should be removed.

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Chapter 62 - Vaginitis, Vulvitis, Cervicitis and Cutaneous Vulval Lesions

Jack D Sobel

VAGINITIS

Vaginal symptoms are extremely common, and vaginal discharge is among the 25 most common reasons for consulting physicians in private office practice in the USA. Vaginitis is found in more than one-quarter of women attending sexually transmitted disease (STD) clinics. Not all women with vaginal symptoms have vaginitis; approximately 40% of women with vaginal symptoms will have some type of vaginitis (Table 62.1).

EPIDEMIOLOGY

Bacterial vaginosis is the most common cause of vaginitis in women of child-bearing age. It has been diagnosed in 17–19% of women seeking gynecologic care in family practice or student health care settings.[1] The prevalence increases considerably in symptomatic women attending STD clinics, reaching 24–37%. Bacterial vaginosis has been observed in 16–29% of pregnant women. *Gardnerella vaginalis* has been found in 10–31% of virgin adolescent girls, but is found significantly more frequently among sexually active women, reaching a prevalence of 50–60% in some at-risk populations.

Evaluation of epidemiologic factors has revealed few clues of the cause of bacterial vaginosis. Use of the intrauterine device and douching was found to be more common in women with bacterial vaginosis. Bacterial vaginosis is significantly more common among African-American and sexually active women including lesbians.

PATHOGENESIS AND PATHOLOGY

Bacterial vaginosis is the result of massive overgrowth of mixed flora, including peptostreptococci, *Bacteriodes* spp., *G. vaginalis*, *Mobiluncus* spp., and genital mycoplasma.[2] There is little inflammation, and the disorder represents a disturbance of the vaginal microbial ecosystem rather than a true infection of tissues. The overgrowth of mixed flora is associated with a loss of the normal *Lactobacillus* spp. dominated vaginal flora. No single bacterial species is responsible for bacterial vaginosis. Experimental studies in human volunteers and studies in animals indicate that inoculation of the vagina with individual species of bacteria associated with bacterial vaginosis (e.g. *G. vaginalis*), rarely results in bacterial vaginosis. In support of the role of sexual transmission is the higher prevalence of bacterial vaginosis among sexually active young women than among sexually inexperienced women, and the observation that bacterial vaginosis-associated micro-organisms are more frequently isolated from the urethras of male partners of females with bacterial vaginosis.[1]

The cause of the overgrowth of anaerobes, *Gardnerella*, *Mycoplasma* and *Mobiluncus* spp. is unknown. Theories include increased substrate availability, increased pH and loss of the restraining effects of the predominant *Lactobacillus* spp. flora. It has been reported that normal women are colonized by hydrogen peroxide-producing strains of lactobacilli, whereas women with bacterial vaginosis have reduced population numbers of lactobacilli, and the species present lack the

TABLE 62-1 -- Causes of vaginitis in adult women.

CAUSES OF VAGINITIS IN ADULT WOMEN	
Common infectious vaginitis	Bacterial vaginosis (40–50%)
	Vulvovaginal candidiasis (20–25%)
	Trichomonal vaginitis (15–20%)
Uncommon infectious vaginitis	Atrophic vaginitis with secondary bacterial infection
	Foreign body with secondary infection
	Desquamative inflammatory vaginitis (clindamycin responsive)
	Streptococcal vaginitis (group A)
	Ulcerative vaginitis associated with *Staphylococcus aureus* and toxic shock syndrome
	Idiopathic vulvovaginal ulceration associated with HIV
Noninfectious vaginitis	Chemical/irritant
	Allergic, hypersensitivity and contact dermatitis (lichen simplex)
	Traumatic
	Atrophic vaginitis
	Postpuerperal atrophic vaginitis
	Desquamative inflammatory vaginitis (corticosteroid responsive)
	Erosive lichen planus
	Collagen vascular disease, Behçet's syndrome, pemphigus syndromes
	Idiopathic

ability to produce hydrogen peroxide.[3] The hydrogen peroxide produced by lactobacilli may inhibit the pathogens associated with bacterial vaginosis, either directly by the toxicity of hydrogen peroxide, or as a result of the production of hydrogen peroxide-halide complex in the presence of natural cervical peroxidase.

Accompanying the bacterial overgrowth in bacterial vaginosis is the increased production of amines by anaerobes, facilitated by microbial decarboxylases. Volatile amines in the presence of increased vaginal pH produce the typical fishy odor, which is also produced when 10% potassium hydroxide is added to vaginal secretions. Trimethylamine is the dominant abnormal amine in bacterial vaginosis. It is likely that bacterial polyamines together with the organic acids found in the vagina in bacterial vaginosis (acetic and succinic acid) are cytotoxic, resulting in exfoliation of vaginal epithelial cells and creating the vaginal discharge. *Gardnerella vaginalis* attaches avidly to exfoliated epithelial cells, especially at the alkaline pH found in bacterial vaginosis. The adherence of *Gardnerella* organisms results in the formation of the pathognomonic clue cells.

PREVENTION

Because the pathogenesis of bacterial vaginosis is obscure, preventive measures have not been forthcoming. Although not typically sexually transmitted, barrier contraception may reduce occurrence and avoiding douching is recommended.

CLINICAL FEATURES

As many as 50% of women with bacterial vaginosis may be asymptomatic. An abnormal malodorous vaginal discharge, often described as fishy, that is infrequently profuse and often appears after unprotected coitus, is usually described. Pruritus, dysuria and dyspareunia are rare. Examination reveals a nonviscous, grayish-white adherent discharge.

Bacterial vaginosis has been considered to be largely of nuisance value only. There is now considerable evidence of serious obstetric and gynecologic complications of bacterial vaginosis, including asymptomatic bacterial vaginosis diagnosed by Gram stain. Obstetric complications include chorioamnionitis, pre-term labor, prematurity and postpartum fever.[4] Gynecologic sequelae are postabortion fever, posthysterectomy fever, cuff infection and chronic mast cell endometritis. A more recent association is reported between untreated bacterial vaginosis and cervical inflammation and low-grade dysplasia.[5] Bacterial vaginosis is a risk factor for HIV infection.[6]

DIAGNOSIS

Signs and symptoms are unreliable in the diagnosis of bacterial vaginosis (Table 62.2). The clinical diagnosis can reliably be made in the presence of at least three of the following objective criteria:

- adherent, white, nonfloccular homogeneous discharge;
- positive amine test, with release of fishy odor on addition of 10% potassium hydroxide to vaginal secretions;
- vaginal pH >4.5; and
- presence of clue cells on light microscopy.

TABLE 62-2 -- Diagnostic features of infectious vaginitis.

DIAGNOSTIC FEATURES OF INFECTIOUS VAGINITIS					
		Normal	***Candida* vaginitis**	**Bacterial vaginosis**	***Trichomonas* vaginitis**
Symptoms		None or physiologic leukorrhea	Vulvar pruritus, soreness, increased discharge, dysuria, dyspareunia	Malodorous moderate discharge	Profuse purulent discharge, offensive odor, pruritus, and dyspareunia
Discharge	Amount	Variable, scant to moderate	Scant to moderate	Moderate	Profuse
	Color	Clear or white	White	White/gray	Yellow
	Consistency	Floccular nonhomogeneous	Clumped but variable	Homogeneous, uniformly coating walls	Homogeneous
	'Bubbles'	Absent	Absent	Present	Present
	Appearance of vulva and vagina	Normal	Introital and vulvar erythema, edema and occasional pustules, vaginal erythema	No inflammation	Erythema and swelling of vulvar and vaginal epithelium (strawberry cervix)
	pH of vaginal fluid	<4.5	<4.5	>4.7	5.0–6.0
	Amine test (10% potassium hydroxide)	Negative	Negative	Positive	Occasionally present
	Saline microscopy	Normal epithelial cell, lactobacilli predominate	Normal flora, blastospores (yeast) 40–50% pseudohyphae	Clue cells, coccobacillary flora predominate, absence of leukocytes, motile curved rods	PMNs + + +, motile trichomonads (80–90%), no clue cells, abnormal flora
10% potassium hydroxide microscopy		Negative	Positive (60–90%)	Negative (except in mixed infections)	Negative

These features are simple and reliable, and tests for them are easy to perform. The presence of clue cells is the single most reliable predictor of bacterial vaginosis. Clue cells are exfoliated vaginal squamous epithelial cells covered with *G. vaginalis*, giving the cells a granular or stippled appearance with characteristic loss of clear cell borders. Of observed epithelial cells, diagnostic significance is indicated by 20% clue cells. Occasionally, clue cells covered exclusively by curved Gram-negative rods belonging to *Mobiluncus* spp. can be demonstrated. The offensive fishy odor may be apparent during the physical examination or may become apparent only during the amine test. Gram strain of vaginal secretions is extremely valuable in diagnosis, with a sensitivity of 93% and specificity of 70%.

Although cultures for *G. vaginalis* are positive in almost all cases of bacterial vaginosis, *G. vaginalis* may be detected in 50–60% of women who do not meet the diagnostic criteria for bacterial vaginosis. Accordingly, vaginal culture has no part in the diagnosis of bacterial vaginosis. DNA probes for *G. vaginalis* are both sensitive (95%) and specific (99%), but have not been accepted by practitioners because of costs and lack of insurance reimbursement.

MANAGEMENT

Poor efficacy has been observed with triple sulfa creams, erythromycin, tetracycline, acetic acid gel and povidone-iodine vaginal douches.[7]

Only moderate cure rates have been obtained with ampicillin (mean 66%) and amoxicillin. The most successful oral therapy remains metronidazole. Most studies using multiple divided dose regimens of 800–1200mg/day for 1 week achieved clinical cure rates in excess of 90% immediately, and of approximately 80% at 4 weeks. Although single-dose therapy with 2g metronidazole achieves comparable immediate clinical response rates, higher recurrence rates have been reported. The beneficial effect of metronidazole

results predominantly from its anti-anaerobic activity and because *G. vaginalis* is susceptible to the hydroxymetabolites of metronidazole. Although *Mycoplasma hominis* is resistant to metronidazole, the organisms are usually not detected at follow-up visits of successfully treated patients. Similarly, *Mobiluncus curtisii* is resistant to metronidazole but usually disappears after therapy.

Topical therapy with 2% clindamycin cream once daily for 7 days, clindamycin ovules for 3 days or metronidazole gel 0.75% administered daily for 5 days have been shown to be as effective as oral metronidazole, without any of the side effects of the latter.[7]

In the past, asymptomatic bacterial vaginosis was not treated, especially because patients often improve spontaneously over several months. However, the growing evidence linking asymptomatic bacterial vaginosis with numerous obstetric and gynecologic upper tract complications has caused reassessment of this policy, especially with additional convenient topical therapies.[4] [8] Asymptomatic bacterial vaginosis should be treated before pregnancy, in women with cervical abnormalities and before elective gynecologic surgery. Routine screening for and treatment of asymptomatic bacterial vaginosis in pregnancy remains controversial, pending the outcome of studies proving that therapy of bacterial vaginosis reduces pre-term delivery and prematurity.[9]

Despite indirect evidence of sexual transmission, no study has documented reduced recurrent rates of bacterial vaginosis in women whose partners have been treated with a variety of regimens, including metronidazole. Accordingly, most clinicians do not routinely treat male partners.

After therapy with oral metronidazole, approximately 30% of patients initially responding experience recurrence of symptoms within 3 months.[1] Reasons for recurrnece are unclear, including the possibility of re-infection, but recurrence more likely reflects vaginal relapse, with failure to eradicate the offending organisms and re-establish the normal protective *Lactobacillus* spp. dominant vaginal flora. Management of bacterial vaginosis relapse includes oral or vaginal metronidazole, or topical

clindamycin, usually prescribed for 14 days. Maintenance antibiotic regimens have been disappointing and new experimental approaches include exogenous *Lactobacillus* spp. recolonization using selected bacteria-containing suppositories.

TRICHOMONIASIS

EPIDEMIOLOGY

Studies estimate that 2–3 million American women contract trichomoniasis annually, with a worldwide distribution of approximately 180 million annual cases.[1] The prevalence of trichomoniasis correlates with the overall level of sexual activity of the specific group of women under study, being diagnosed in about 5% of women in family planning clinics, in 13–25% of women attending gynecology clinics, in 50–75% of prostitutes and in 7–35% of women in STD clinics. In many industrialized countries, recent surveys indicate a decline in the incidence of trichomoniasis.

PATHOGENESIS AND PATHOLOGY

Sexual transmission is the dominant method of introduction of *Trichomonas vaginalis* into the vagina.[1] *Trichomonas vaginalis* was identified in the urethra of 70% of men who had had sexual contact with infected women within the previous 48 hours. There is also a high prevalence of gonorrhea in women with trichomoniasis, and both of these are significantly associated with use of nonbarrier methods of contraception.

Recurrent trichomoniasis is common and is indicative of lack of significant protective immunity. Nevertheless, an immune response to *Trichomonas* spp. does develop, as indicated by low titers of serum antibody, but this is insufficient for diagnostic serology. Antitrichomonal IgA has been detected in vaginal secretions, but a protective role is not defined.

Delayed hypersensitivity in natural infection can also be demonstrated. The predominant host defense response is provided by the numerous polymorphonuclear leukocytes (PMNs), which respond to chemotactic substances released by trichomonads and are capable of killing *T. vaginalis* without ingesting trichomonads. *Trichomonas vaginalis* destroys epithelial cells by direct cell contact and cytotoxicity. The urethra and Skeene's glands are infected in the majority of patients, and organisms are occasionally isolated from bladder urine.

PREVENTION

Sexual transmission of trichomonads is efficiently prevented by use of barrier contraception. Spermicidal agents such as nonoxynol-9 also reduce transmission. Re-infection of women is common, hence the mandatory requirement of treatment, preferably simultaneously, of all sexual partners with metronidazole.

CLINICAL FEATURES

Infection with *Trichomonas* spp. in women ranges from an asymptomatic carrier state to severe acute inflammatory disease. [10] [11] Vaginal discharge is reported by 50–75% of women diagnosed with trichomoniasis; however, the discharge is not always described as malodorous. Pruritus occurs in 25–50% of patients and is often severe. Other infrequent symptoms include dyspareunia, dysuria and, rarely, frequency of micturition. Lower abdominal pain occurs in fewer than 10% of patients and should alert the physician to the possibility of concomitant salpingitis caused by other organisms. Symptoms of acute trichomoniasis often appear during or immediately after menstruation. Although controversial, the incubation period has been estimated to range from 3 to 28 days.

Physical findings represent a spectrum depending on the severity of disease. Vulvar findings may be absent, but are typically characterized in severe cases by diffuse vulvar erythema (10–33%), edema and a copious, profuse and malodorous vaginal discharge, which is often described as being yellow-green and frothy, but is frequently grayish-white.[11] Frothiness is seen in a minority of patients and is more commonly seen in bacterial vaginosis.

The vaginal walls are erythematous and in severe cases may be granular in appearance. Punctate hemorrhages (colpitis macularis) of the cervix may result in a strawberry-like appearance that, although apparent to the naked eye in only 1–2% of patients, is present in 45% of cases on colposcopy.[11]

The clinical course of trichomoniasis in pregnancy is identical to that seen in the nonpregnant state, and when untreated it is associated with premature rupture of membranes and prematurity. Trichomoniasis is reported to facilitate HIV transmission.

DIAGNOSIS

None of the clinical features of vaginitis caused by *Trichomonas* spp. are sufficiently specific to allow a diagnosis of trichomonal infection based on signs and symptoms alone (see Table 62.2).[10] Definitive diagnosis requires the demonstration of the organism. Vaginal pH is markedly elevated, almost always above 5.0, and not infrequently 6.0. On saline microscopy, an increase in number of PMNs is almost invariably present. The ovoid parasites are slightly larger than PMNs and are best recognized by their motility. The wet mount is positive in only 40–80% of cases (low sensitivity). Gram stain is of little value because of its inability to differentiate PMNs from nonmotile trichomonads, and use of Giemsa, acridine orange and other stains

has no advantage over saline preparations. Although trichomonads are often seen on Papanicolaou smears, this method has a sensitivity of only 60–70% when compared with saline preparation microscopy, and false-positive results are not infrequently reported.

Several equivalent culture medium methods are available, and growth is usually detected within 48 hours. Culture is now recognized as the most sensitive method for detecting the presence of trichomonads (95% sensitivity) and should be considered in patients with vaginitis in whom an elevated pH, PMN excess, absence of motile trichomonads and clue cells are found. Several new rapid diagnostic kits using DNA probes are under investigation.

MANAGEMENT

Therapy consists of administering the 5-nitroimidazole group of drugs — metronidazole, tinidazole and ornidazole, — which are all of similar efficacy.[7] Oral therapy as opposed to topical vaginal therapy is preferred because of the frequency of infection of the urethra and periurethral glands, which provide sources for endogenous recurrence.

Treatment consists of oral metronidazole, 500mg q12h for 7 days, which has a cure rate of 95%. Comparable results have been obtained with a single oral dose of 2g metronidazole, achieving cure rates of 82–88%. The latter cure rate increases to greater than 90% when sexual partners are treated simultaneously. The advantages of single-dose therapy include better patient compliance, lower total dose, shorter period of alcohol avoidance and possibly decreased incidence of subsequent vaginitis caused by *Candida* spp. A disadvantage of single-dose therapy is the need to insist on simultaneous treatment of sexual partners.

The 5-nitroimidazoles are not in themselves trichomonacidal, but low-redox proteins reduce the nitro group, resulting in the formation of highly cytotoxic products within the organisms. Aerobic conditions interfere with this reduction process and decrease the antianaerobic activity of the 5-nitroimdazoles. Most strains of *T. vaginalis* are highly susceptible to metronidazole, with minimum inhibitory concentrations (MICs) of 1mg/l.

Patients not responding to an initial course often respond to an additional standard course of 7-day therapy. Some patients are refractory to repeated courses of therapy even when compliance is assured and sexual partners are known to have been treated. If re-infection is excluded, these rare patients may have strains of *T. vaginalis* that are resistant to metronidazole, which can be confirmed in vitro. Increased doses of metronidazole and longer duration of therapy are necessary to cure these refractory patients. The patients should be given maximal tolerated dosages of oral metronidazole of 2–4g/day for 10–14 days. Rarely, intravenous metronidazole, in dosages as high as 2–4g/day, may be necessary, with careful monitoring for drug toxicity. Considerable success has been observed in treating resistant infections with oral tinidazole; however, the drug is not readily available and the optimal dose to be used is unknown.[12] Most investigators use high-dose

tinidazole 1–4g/day for 14 days.[12] Rare patients not responding to nitroimidazoles can be treated with topical paramomycin.

Side-effects of metronidazole include an unpleasant or metallic taste. Other common side-effects include nausea (10%), transient neutropenia (7.5%) and a disulfiram-like effect when alcohol is ingested. Caution should be taken when 5-nitroimidazoles are used in patients taking warfarin. Long-term and high-dose therapy increase the risk of neutropenia and peripheral neuropathy. In experimental studies, metronidazole has been shown to be mutagenic for certain bacteria, indicating a carcinogenic potential, although cohort studies have not established an increase in cancer morbidity. Thus, the risk to humans of short-term low-dose metronidazole treatment is extremely small. Superinfection with *Candida* spp. is by no means uncommon.

Treatment of trichomoniasis in pregnancy is unsatisfactory.[7] Metronidazole readily crosses the placenta, and because of concern for teratogenicity some consider it prudent to avoid its use in the first trimester of pregnancy. More recently investigators have become more comfortable with the use of metronidazole throughout pregnancy. Topical clotrimazole and povidone-iodine jelly offer minimal benefit.

VULVOVAGINAL CANDIDIASIS

EPIDEMIOLOGY

Data from the UK reveal a sharp increase in the incidence in vulvovaginal candidiasis (VVC). In the USA, *Candida* spp. are now the second commonest cause of vaginal infections.[13] [14]

It is estimated that 75% of women experience at least one episode of VVC during their child-bearing years, and approximately 40–50% experience a second attack. A small subpopulation of women of undetermined magnitude, probably less than 5% of adult females, suffers from repeated, recurrent, often intractable episodes of *Candida* vaginitis.[14]

Point-prevalence studies indicate that *Candida* spp. may be isolated from the genital tract of approximately 20% of asymptomatic, healthy women of child-bearing age. The natural history of asymptomatic colonization is unknown, although animal and human studies suggest that vaginal carriage continues for several months and perhaps years. Several factors are associated with increased rates of asymptomatic vaginal colonization with *Candida* spp., including pregnancy (30–40%), use of oral contraceptives, uncontrolled diabetes mellitus and frequency of visits to STD clinics (Table 62.3). The rarity of isolation of *Candida* spp. in premenarchal girls, the lower prevalence of *Candida* vaginitis after menopause and the possible association with hormone replacement therapy emphasize the hormonal dependence of VVC.

PATHOGENESIS AND PATHOLOGY

The organism

Between 85 and 90% of yeast isolated from the vagina are *Candida albicans* strains. The remainder are other species, the commonest of which are *Candida glabrata* and *Candida tropicalis*. Non-*albicans Candida* spp. are capable of inducing vaginitis and are often more resistant to conventional therapy. Recent surveys indicate an increase in VVC caused by non-*albicans Candida* spp., particularly *C. glabrata*. [14] [15]

TABLE 62-3 -- Host factors associated with asymptomatic vaginal colonization by *Candida* and with *C andida* vaginitis.

HOST FACTORS ASSOCIATED WITH INCREASED ASYMPTOMATIC VAGINAL COLONIZATION BY *CANDIDA* AND WITH *CANDIDA* VAGINITIS	
Genetic	
Blood group antigen/secretor status	
Acquired	
Biologic	Pregnancy
	Uncontrolled diabetes mellitus
	Corticosteroids/immunosuppressive therapy
	Antimicrobial therapy (systemic, topical)
	HIV infection
Behavioral (sexual)	Oral contraceptives
	Intrauterine device/contraceptive sponge
	Nonoxynol-9 spermicide
	Receptive oral-genital sex
	Coital frequency (?)

Germination of *Candida spp.* enhances colonization and facilitates tissue invasion. Factors that enhance or facilitate germination (e.g. estrogen therapy and pregnancy) tend to precipitate symptomatic vaginitis, whereas measures that inhibit germination (e.g. bacterial flora and local mucosal cell-mediated immunity) may prevent acute vaginitis in women who are asymptomatic carriers of yeast.

Candida organisms gain access to the vaginal lumen and secretions predominantly from the adjacent perianal area. This finding is borne out by epidemiologic typing studies. *Candida* vaginitis is seen predominantly in women of child-bearing age, and only in the minority of cases can a precipitating factor be identified to explain the transformation from asymptomatic carriage to symptomatic vaginitis in individual patients.

Host factors

Host factors associated with increased asymptomatic vaginal colonization by *Candida* spp. and with *Candida* vaginitis are outlined in Table 62.3 . During pregnancy, the vagina is more susceptible to vaginal infection, resulting in higher incidences of vaginal colonization, vaginitis and lower cure rates. The clinical attack rate is maximal in the third trimester, and symptomatic recurrences are also more common throughout pregnancy. The high levels of reproductive hormones result in a higher glycogen content in the vaginal environment, which provides an excellent carbon source for growth and germination of *Candida* spp. A more common mechanism is where estrogens enhance vaginal epithelial cell avidity for *Candida* spp. adherence, and a yeast cytosol receptor or binding system for female reproductive hormones has been documented. These hormones also enhance yeast mycelial formation. Several studies have shown increased VVC associated with oral contraceptive use[16] and uncontrolled diabetes mellitus. Glucose tolerance tests have been recommended for women with recurrent VVC; however, the yield is low, and testing is not justified in otherwise healthy premenopausal women.

Symptomatic VVC is frequently observed during or after courses of systemic antibiotics. Although no antimicrobial agent is free of this complication, broad-spectrum antibiotics, such as tetracycline, and ß-lactams are mainly responsible, and are thought to act by eliminating the normal protective vaginal bacterial flora. The natural flora provides a colonization resistance mechanism and prevents germination of *Candida* spp. The provider of this protective function has been singled out to be *Lactobacillus* spp.[17] *Lactobacillus-Candida* interaction includes competition for nutrients, steric interference with adherence of *Candida* spp. and elaboration of bacteriocins that inhibit yeast proliferation and germination.

Other factors that contribute to an increased incidence of *Candida* vaginitis include the use of tight, poorly ventilated clothing and nylon underclothing, which increase perineal moisture and temperature.

Candida spp. may cause cell damage and resulting inflammation by direct hyphal invasion of epithelial tissue. It is possible that proteases and other hydrolytic enzymes facilitate cell penetration with resultant inflammation, mucosal swelling, erythema and exfoliation of vaginal epithelial cells, The characteristic nonhomogeneous vaginal discharge consists of a conglomerate of hyphal elements and exfoliated nonviable epithelial cells with few PMNs. *Candida* spp. may also induce symptoms by hypersensitivity or allergic reactions, particularly in women with idiopathic recurrent VVC (see Noninfectious vaginitis and vulvitis, below).[18]

Oral and vaginal thrush correlate well with depressed cell-mediated immunity in debilitated or immunosuppressed patients.[18] This is particularly evident in patients who have chronic mucocutaneous candidiasis and AIDS.

Pathogenesis of recurrent and chronic *Candida* vaginitis

Careful evaluation of women with recurrent vaginitis usually fails to reveal any precipitating or causal mechanism.[14] In the past, investigators attributed frequent episodes to repeated fungal reinoculation of the vagina from a persistent intestinal source or to sexual transmission.[19]

The intestinal theory is based on the report of recovery of *Candida* spp. on rectal culture in almost 100% of women with VVC. Typing of simultaneously obtained vaginal and rectal isolates almost invariably reveals identical strains. This theory has been criticized in the past few years because of lower concordance between rectal and vaginal cultures in patients with recurrent VVC. Moreover, long-term therapy with oral nonabsorbable nystatin is not effective in preventing recurrences.

Although sexual transmission of *Candida* organisms occurs via vaginal intercourse and orogenital contact, the role of sexual reintroduction of yeast as a cause for recurrent VVC is doubtful. Recurrent VVC frequently occurs in celibate women and only a minority of male partners of women who have recurrent VVC are colonized with *Candida* spp. Although most studies aimed at treating male partners have not reduced the frequency of recurrent episodes of vaginitis, reduction was achieved in recurrent VVC by treating colonized male partners.[19]

Vaginal relapse implies that incomplete eradication or clearance of *Candida* spp. from the vagina occurs after antimycotic therapy. Organisms persist in small numbers in the vagina and result in continued carriage of the organisms, and when host environmental conditions permit, the colonizing organisms increase in number and undergo mycelial transformation, resulting in a new clinical episode.

Whether recurrence is caused by vaginal re-infection or relapse, women with recurrent VVC differ from those with infrequent episodes in their inability to tolerate small numbers of *Candida* organisms re-introduced or persisting in the vagina. On the basis of typing of organisms, women with recurrent and infrequent infection have the same distribution frequency of *Candida* strains as women without symptoms.

Host factors responsible for frequent episodes are not clearly delineated, and more than one mechanism may be operative. There is no evidence of complement, phagocytic cells or immunoglobulin deficiency in these patients. Recurrent VVC is rarely caused by drug resistance.[20]

Current theories about the pathogenesis of recurrent VVC include qualitative and quantitative deficiency in the normal protective vaginal bacterial flora and an acquired, often transient antigen-specific deficiency in T-cell function that similarly permits unchecked yeast proliferation.[18] [21] Another theory is that of an acquired acute hypersensitivity reaction to *Candida* antigen, which is accompanied by elevated vaginal titers of *Candida* antigen-specific IgE. This theory has a clinical basis in that patients with recurrent VVC often present with severe vulvar manifestations (rash, erythema, swelling and pruritus) with minimal exudative vaginal changes, little discharge and lower numbers of organisms. Allergic responses to *Candida* spp. have been reported to involve the male genitalia immediately after coitus with a woman infected with *Candida* spp. and are characterized by the acute onset of erythema, edema, severe pruritus and irritation of the penis. As yet, only a minority of women with recurrent VVC have been shown to have elevated *Candida*-specific vaginal IgE. Limited studies using *Candida* antigen desensitization have been found to be helpful in reducing the frequency of recurrent episodes of vaginitis.

Women who are HIV seropositive have higher vaginal colonization rates than seronegative women, but the attack rate of symptomatic VVC appears similar. Reports of chronic, severe recurrent VVC are largely unsubstantiated. Recurrent VVC in the absence of other risk factors for HIV is not an indication for HIV testing. [14]

PREVENTION

In women with confirmed recurrent VVC linked to frequent courses of systemic antibiotics, prophylactic antimycotics are justified. A useful regimen is fluconazole 100mg once weekly for the duration of antibiotic therapy. No other dietary or alternative method has stood the test of time in preventing VVC. In women prone to VVC, avoiding use of oral contraceptives, intrauterine devices and the contraceptive sponge is prudent.

CLINICAL FEATURES

The most frequent symptom of VVC is vulvar pruritus because vaginal discharge is not invariably present and is frequently minimal.[14] Although described as typically cottage cheese-like in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia and external dysuria are commonly present. Odor, if present, is minimal and nonoffensive. Examination frequently reveals erythema and swelling of the labia and vulva (Fig. 62.1), often with discrete pustulopapular peripheral lesions and linear fissures (Fig. 62.2). The cervix is normal and vaginal mucosal erythema with adherent whitish discharge is present. Characteristically, symptoms are exacerbated in the week before the onset of menses, with some relief with the onset of menstrual flow.

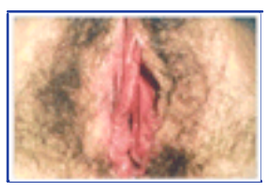

Figure 62-1 Typical *Candida* vulvovaginitis with bilateral symmetric erythema and edema of vestibule and labia.

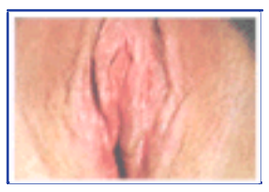

Figure 62-2 Severe *Candida* vulvovaginitis with bilateral painful fissure formation in the vulva.

DIAGNOSIS

The relative lack of specificity of symptoms and signs precludes a diagnosis that is based only on history and physical examination. Most patients with symptomatic VVC may be readily diagnosed on the basis of simple microscopic examination of vaginal secretions. A wet mount or saline preparation has a sensitivity of 40–60%. The 10% potassium hydroxide preparation is more sensitive in diagnosing the presence of germinated yeast. A normal vaginal pH (4.0–4.5) is found in *Candida* vaginitis, and the finding of a pH in excess of 4.5 should suggest the possibility of bacterial vaginosis, trichomoniasis or a mixed infection.[14]

Although routine fungal cultures are unnecessary, vaginal culture should be performed in the presence of negative microscopy. The Papanicolaou smear is unreliable, being positive in only about 25% of cases. There is no reliable serologic technique for the diagnosis of *Candida* vaginitis.

MANAGEMENT

Topical agents for acute *Candida* vaginitis

Antimycotics are available for local use as creams, vaginal tablets, suppositories and coated tampons (Table 62.4) There is little to suggest that the formulation of the topical antimycotic influences clinical efficacy.[20] Extensive vulvar inflammation dictates local vulvar application of cream.

The average mycologic cure rate of 7- and 14-day courses of nystatin is 75–80%. Azoles appear to achieve slightly higher clinical mycologic cure rates than the polyenes (nystatin): 85–90%. Although many studies have compared the clinical efficacies of the various azoles, there is little evidence that any one azole agent is superior to others.[22] Topical azoles are remarkably free of local and systemic side-effects; nevertheless, the initial application of topical agents is not infrequently accompanied by local burning and discomfort.

TABLE 62-4 -- Therapy for vaginal candidiasis — topical agents.[*]

THERAPY FOR VAGINAL CANDIDASIS		
Topical Agents		
Drug	**Formulation**	**Dosage regimen**
* Butoconazole	2% cream	5g/day for 3 days
* Clotrimazole	1% cream	5g/day for 7–14 days
	100mg vaginal tablets	1 tablet/day for 7 days
	100mg vaginal tablets	2 tablets/day for 3 days
	500mg vaginal tablets	1 tablet, single dose
* Miconazole	2% cream	5g/day for 7 days
	100mg vaginal suppository	1 suppository/day for 7 days
	200mg vaginal suppository	1 suppository/day for 3 days
	1200mg vaginal suppository	1 suppository, single dose
Econazole	150mg vaginal tablet	1 tablet/day for 3 days
Fenticonazole	2% cream	5g/day for 7 days
* Tioconazole	2% cream	5g/day for 3 days
	6.5% cream	5g, single dose
Terconazole	0.4% cream	5g/day for 7 days
	0.8% cream	5g/day for 3 days
	80mg vaginal suppository	80mg/day for 3 days
Nystatin	100,000U vaginal tablets	1 tablet/day for 14 days

* Drugs available over the counter, without prescription.

Systematic agents for acute *Canadida* vaginitis

There has been a major trend toward shorter treatment courses with progressively higher antifungal doses, culminating in highly effective single-dose topical regimens. Although short-course regimens are effective for mild and moderate vaginitis, cure rates for severe and complicated vaginitis are lower.

Oral systemic azoles available for the treatment of VVC include ketoconazole 400mg q12h for 5 days, itraconazole 200mg/day for 3 days (or q12h single-day regimen) and, finally, fluconazole 150mg single-dose.[23] All the oral regimens achieve clinical cure rates in excess of 80%; however, only fluconazole is approved for use in the USA. Oral regimens are generally preferred by women because of convenience and lack of local side-effects. None of the systemic regimens should be prescribed during pregnancy and the potential for systemic side-effects and toxicity exists. In particular, hepatotoxicity with ketoconazole precludes its widespread use in VVC.[22]

Vulvovaginal candidiasis is classified as uncomplicated or complicated on the basis of the likelihood of achieving clinical and mycologic cure with short-course therapy (Table 62.5). Uncomplicated VVC represents by far the most common form of vaginitis seen, is caused by highly sensitive *C. albicans* and, provided that the severity is mild to moderate, patients respond well to all topical or oral antimycotics, including single-dose therapy. In contrast, patients who have complicated VVC have a relatively resistant organism, a host factor or a severity of infection that dictates more intensive and prolonged therapy lasting 7–14 days. Most non-*albicans Candida* infections respond to conventional topical or oral antifungals provided they are administered for sufficient duration. However, vaginitis caused by *C. glabrata* often fails to respond to azoles and may require treatment with vaginal capsules of boric acid 600mg/day for 14 days. [24] Patients with severe vulvovaginitis require more prolonged systemic or topical therapy. The former can be provided by a second dose of fluconazole 150mg, 72 hours after the first dose.[25]

Treatment of recurrent vulvovaginal candidiasis

The management of women who have recurrent VVC aims at control rather than cure. The clinician should first confirm the diagnosis of recurrent VVC. Uncontrolled diabetes mellitus must be controlled and use of corticosteroids or other immunosuppressive agents should be discontinued where possible. Unfortunately, in the majority of women with recurrent VVC, no underlying or predisposing factor can be identified. Recurrent VVC requires long-term maintenance with a suppressive prophylactic regimen. Because of the chronicity of therapy, the convenience of oral treatment is apparent, and the best suppressive prophylaxis has been achieved with weekly oral fluconazole at a dosage of 150mg. An effective topical prophylactic regimen consists of weekly vaginal suppositories of clotrimazole 500mg.[14] [22]

ATROPHIC VAGINITIS

Clinically significant atrophic vaginitis is quite rare, and the majority of women with mild-to-moderate atrophy are asymptomatic. Because of reduced endogenous estrogen, the epithelium becomes thin and lacking in glycogen, which contributes to a reduction in lactic acid production and an increase in vaginal pH. This change in the environment encourages the overgrowth of nonacidophilic coliform organisms and the disappearance of *Lactobacillus* spp. Despite these major but usually gradual changes, symptoms are mostly absent, especially in the absence of coitus.

With advanced atrophy, symptoms include vaginal soreness, dyspareunia and occasional spotting or discharge. Burning is a frequent complaint and is often precipitated by intercourse. The vaginal mucosa is thin, with diffuse redness, occasional petechiae or ecchymoses with few or no vaginal folds. Vulvar atrophy may also be

TABLE 62-5 -- Classification of vulvovaginal candidiasis.

CLASSIFICATION OF VULVOVAGINAL CANDIDIASIS	
Uncomplicated	**Complicated**
Candida albicans	Non-*albicans Candida* spp.
	Resistant *Candida albicans* (rare)
+	or
Infrequent episodes	History of recurrent vulvovaginal candidiasis
+	or
Mild-to-moderate vaginitis	Severe vulvovaginal candidiasis
+	or
Normal host	Abnormal host, for example, uncontrolled diabetes, pregnancy, immunocompromised

apparent and discharge may be serosanguinous, thick or watery, and the pH of the vaginal secretions ranges from 5.5 to 7.0. The wet smear frequently shows increased number of PMNs associated with small, round epithelial cells. The latter parabasal cells represent immature squamous cells that have not been exposed to sufficient estrogen. The *Lactobacillus* spp.-dominated flora is replaced by mixed flora of Gram-negative rods. Bacteriologic cultures in these patients are unnecessary, and can be misleading.

The treatment of atrophic vaginitis consists primarily of topical vaginal estrogen. Nightly use of half or all the contents of an applicator for 1–2 weeks is usually sufficient to alleviate the atrophic vaginitis.

NONINFECTIOUS VAGINITIS AND VULVITIS

Women frequently present with acute or chronic vulvovaginal symptoms caused by noninfectious etiologies. Symptoms are indistinguishable from those of infectious syndromes, but are most commonly confused with those of acute *Candida* vaginitis, including pruritus, irritation, burning, soreness and variable discharge.

Noninfectious causes include irritants [physical (e.g. minipads) or chemical (e.g. spermicides, betadyne, topical antimycotics, soaps and perfumes, topical 5-fluorouracil)] and allergens, which are responsible for immunologic acute and chronic hypersensitivity reactions, including contact dermatitis (e.g. latex condoms, antimycotic creams). An enormous list of topical factors responsible for local inflammatory reactions and symptoms exists and many more have yet to be defined. Depending on the site of contact, symptoms may be vaginal or vulvar. Included in this category are systemic dermatoses that may present in the vulva (e.g. psoriasis, Fig. 62.3) or vulva-specific dermatosis (e.g. lichen sclerosus).

A noninfectious mechanism may coexist with or follow an infectious process, and should be considered when the three common infectious causes and hormone deficiency are excluded and in the presence of a normal vaginal pH, normal saline and potassium hydroxide microscopy, and, ultimately, a negative yeast culture. Unfortunately, given the anticipated 20% colonization rates in normal asymptomatic women, occasionally a positive yeast culture in a symptomatic patient reflects the presence of an innocent bystander and not the cause of the vulvovaginal symptoms. The only logical way of establishing the role of *Candida* spp. in this context is to treat with an oral antifungal agent and assess the clinical response.

Once a local chemical irritant or allergic reaction is suspected, a detailed inquiry into possible causal factors is essential. Offending agents or behaviors should be eliminated wherever possible, including

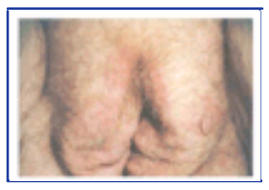

Figure 62-3 Vulvar psoriasis resulting in pruritus vulva and misdiagnosed as *Candida vulvovaginitis.*

avoiding chemical irritants and allergens (e.g. soaps, detergents). The immediate management of severe vulvovaginal symptoms of noninfectious etiology should not rely on topical corticosteroids, which are rarely the solution and frequently high-potency corticosteroid creams cause intense burning. Local relief measures include sodium bicarbonate sitz baths and oral antihistamines.

A syndrome of hyperacidity of the vagina causing overgrowth of lactobacilli has been described but not confirmed. A rebound increase in population numbers of lactobacilli is thought to occur after completion of topical antimycotics and is alleged to suppress population numbers of healthy resident flora. The proposed syndrome of cytolytic vaginosis is characterized by vulvovaginal burning, irritation, soreness and dyspareunia, and is usually incorrectly diagnosed as VVC. The finding of large numbers of lactobacilli on wet count and low pH, together with extensive squamous epithelial cell cytolysis is said to confirm the diagnosis. Recommended therapy for cytolytic vaginosis is daily alkaline douching using sodium bicarbonate to elevate the low vaginal pH and suppress growth of lactobacilli.

VULVITIS

Most of the important infectious and noninfectious causes of vulvitis have been described in the section on vaginitis. Human papillomavirus and genital herpes are described in Chapter 77 and Chapter 76 , respectively. Bacterial vulvitis due to streptococci, anaerobes and Gram-negative rods occurs infrequently and should be diagnosed by clinical features and bacterial culture. Specific antimicrobial treatment is indicated for patients with the diagnosis of bacterial vulvitis. Occasionally a Bartholin's abscess creates a painful swelling in the vulva. This can be due to *N. gonorrhoeae* or to a variety of pathogens, particularly Gram-positive organisms. A Bartholin's cyst infection should be treated with appropriate antibiotics and occasionally may require drainage. Other causes of vestibulitis or vulvitis include lichen sclerosus, erosive lichen planus and theoretically any cause of dermatosis or dermatitis such as psoriasis (see Fig. 62.3), eczema and pemphigus.

CERVICITIS

EPIDEMIOLOGY

The presence of a purulent exudate in the cervical os has been highly associated with cervical infection with *Chlamydia trachomatis, N. gonorrhoeae*, herpes simplex virus and cytomegalovirus. Infection with *T. vaginalis* correlates with colpitis macularis and inflammatory changes of the ectocervix.[26] [27] Not infrequently, mucopurulent endocervitis or ectocervicitis are seen in the absence of these pathogens, indicating that additional, as yet unrecognized causes exist. A role for disruption of vaginal flora, specifically overgrowth of anaerobes in causing cervical inflammation has been proposed. Rare causes of cervicitis include *Mycobacterium tuberculosis* and *Actinomyces israelii*, the latter almost invariably in the presence of intrauterine devices. Although the most important and prevalent infection of the cervix is undoubtedly human papillomavirus, this virus does not cause cervicitis and is discussed in Chapter 216 .

The prevalence of genital chlamydial infection ranges from 8 to 40%.[28] Risk factors include young age, unmarried status, lower socioeconomic conditions, number and recent change of sexual partner, ectopy, oral contraceptive use and concurrent gonococcal infection; the latter may reactivate latent chlamydial infection and increases shedding of chlamydia from the endocervix.[28] Risk factors for gonococcal mucopurulent cervicitis are identical to those of *Chlamydia* spp. but also include urban dwelling, prostitution, illicit drug use and minority racial status. Up to 60% of women with *N. gonorrhoeae* have co-infection with chlamydia.[28] Herpetic cervicitis is rare in the absence of genital lesions and is most commonly associated with first episode, primary disease with an 80% viral isolation rate.[27] Cytomegalovirus is thought to be responsible for approximately 5% of cases of cervicitis, is usually asymptomatic and when it is isolated from cervical secretions, the detection may not imply a causal relationship with present pathology.

CLINICAL FEATURES

Cervicitis is frequently asymptomatic and is detected on routine pelvic examination. Alternatively cervical inflammation is recognized because of signs and symptoms of concomitant infection (e.g. vaginal trichomoniasis, genital herpes or salpingitis). Mucopurulent cervicitis may result in a purulent vaginal discharge in its own right. Accordingly cervical speculum evaluation should be an essential part of vaginal examination in women with an abnormal discharge. Mucopurulent endocervicitis results in swelling and erythema of the zone of ectopy associated with friability, contact bleeding, spotting and a yellow or a green endocervical exudate. The purulent discharge is best appreciated by obtaining an endocervical swab specimen and observing the latter against a white background.

Trichomoniasis is associated with ectocervical squamous epithelial mucosal inflammation giving the cervix a 'strawberry' appearance due to microscopic focal patchy petechiae (colpitis macularis) in 5–20% of patients.[11] Primary herpes cervicitis may be associated with severe necrosis that is reminiscent of cervical cancer. Most commonly, primary herpetic cervicitis is characterized by increased surface vascularity, and micro- and macro-ulcerations with and without necrotic areas. Asymptomatic shedding of herpesvirus occurs in the absence of cervical lesions.

DIAGNOSIS

Mucopurulent cervicitis is confirmed when a Gram-stained specimen of green or yellow endocervical exudate reveals more than 30 PMNs per high power field. Microscopic examination of cervical mucus from a patient with mucopurulent endocervicitis reveals an overabundance of inflammatory cells, obliterating the background ferning pattern. A similar excess of inflammatory cells can be found in a Papanicolaou smear. These two microscopic studies are not reliable in identifying the underlying cause of mucopurulent cervicitis. For a diagnosis of *C. trachomatis* cervicitis, culture techniques to identify the obligate intraparasites served as the gold standard in the past.

Now the development of the more widely available enzyme-linked immunosorbent assay antigen detection tests has been replaced by the highly sensitive DNA amplification techniques, particularly ligase chain reaction, allowing diagnosis not only from cervical specimens, but also by screening urine specimens. Gram stain of cervical mucus may reveal intracellular Gram-negative diplococci, but has low sensitivity and specificity in the diagnosis of gonococcal cervicitis. Diagnosis relies mainly upon culture of the endocervix using a modified Thayer-Martin medium; however, diagnostic methodologies now include use of DNA probes, especially for screening purposes, given the high sensitivity of these newer techniques.

Although Papanicolaou smears in herpetic cervicitis are useful in revealing multinucleated giant cells, viral culture and fluorescein-conjugated monoclonal antibodies are the mainstay of clinical diagnosis; polymerase chain reaction is used for monitoring asymptomatic viral shedding in a research context.

The clinical differentiation of the various causes of cervicitis is not possible, but requires the aforementioned diagnostic tests, recognizing that frequently more than one etiologic agent may be present simultaneously because many of the pathogens share risk factors and behavior. The most important diagnostic problem is that of overdiagnosis of cervicitis. All too frequently physiologic changes in the appearance of the cervix, in spite of the use of colposcopy, are interpreted as reflecting pathologic cervicitis. Regrettably, after failed attempts to identify pathogenic micro-organisms, patients are needlessly treated with cervical ablative techniques. Cervical ectopy is often mistaken for cervicitis with eversion of endocervical columnar cells, and is commonly seen in women on oral contraceptives.[29] Other physiologic changes related to childbirth and dilatation of the cervical canal are mistakenly diagnosed as cervicitis. Equally important is the failure to recognize that a friable, abnormal cervix may reflect dysplasia and neoplasia. If the Papanicolaou smear reports inflammatory cells with or without atypia, the presence of atypical squamous cells of undetermined significance (ASCUS) should be considered in the differential diagnosis between a benign change in reaction to a stimulus and a low-grade squamous intraepithelial lesion. Accordingly, women with a Papanicolaou smear showing ASCUS should have their smears repeated. Persistence of an ASCUS smear should prompt colposcopy.

MANAGEMENT

Antimicrobial regimens for infectious cervicitis are provided in the chapters on gonorrhea and chlamydial infections and pelvic inflammatory disease (see Chapter 63 , Chapter 74 and Chapter 236).

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Chapter 63 - Infections of the Female Pelvis Including Septic Abortion

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Infections of the female pelvis constitute a diverse group. This chapter considers three groups of infections: pelvic inflammatory disease (PID), postpartum and postabortal infections (including postpartum endometritis and cesarean section, episiotomy infections and postabortion sepsis) and postsurgical gynecologic infections.

PELVIC INFLAMMATORY DISEASE

EPIDEMIOLOGY

Pelvic inflammatory disease refers to an acute clinical syndrome that results when vaginal or cervical organisms ascend into the upper structures of the female reproductive tract unrelated to pregnancy or surgery.[1] [2] The term PID includes the following: endometritis, parametritis, salpingitis, oophoritis, pelvic peritonitis, tubo-ovarian abscess, periappendicitis, perihepatitis (Fitz-Hugh-Curtis syndrome) and perisplenitis.

Pelvic inflammatory disease and sexually transmitted diseases (STDs) share many of the same risk factors and, in the USA, 40–80% of PID is attributed to STDs. Bacterial vaginosis may be an antecedent vaginal condition. [3] The identified risk factors for PID include younger age, unmarried status, lower socioeconomic status, sexual behavior (number of sexual partners, age of sexual debut, rate of acquiring new partners), substance abuse, poor health care behavior (treatment-seeking and compliance with treatment instructions), douching and intrauterine device insertion.[1] [4] In studies from the USA and Europe, about three-quarters of women who had PID were under 25 years of age, and about one-half had never been pregnant.[2] Oral contraceptive users tend to have clinically and laparoscopically milder infection than do nonusers and oral contraceptives appear to protect against chlamydial PID.[5]

PATHOGENESIS AND PATHOLOGY

The vast majority of PID cases result from a direct canalicular spread of organisms from the endocervix to the mucosa of the endometrium and fallopian tubes, although the precise mechanisms are poorly understood. Postinfectious scarring (e.g. intratubal adhesions, tubal occlusion, peritubal scarring and damaged fimbrial ostia) results in the long-term sequelae of PID.

Occurrence of PID is described only among sexually active women and its risk is associated with the numbers of sexual partners and the frequency of sexual acts among women with only one sexual partner. Multiple organisms have been implicated as etiologic agents of PID (Table 63.1). The rates of isolation of these organisms are variable. They may vary with geographic region, duration of infection and the site of sampling (i.e. cervix, fallopian tubes or endometrium). The most commonly recovered organisms are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, followed by other aerobic and anaerobic bacteria associated with bacterial vaginosis (e.g. *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella bivia*, and *Porphyromonas*, *Prevotella* and *Peptostreptococcus* spp.). *Mycoplasma genitalium* may also be an important etiological agent of PID but further studies are necessary. Rates of isolation of 10–20% in the upper genital tract and as high as 85% (5–27% in studies from Europe versus 44–70% in those from North America) in the lower genital tract have been reported for *N. gonorrhoeae*. For *C. trachomatis*, rates of 1.2–31% in the upper genital tract and 31% in the lower genital tract have been reported.[2] [3] [6] It is estimated that between 10% and 40% of women who have untreated gonococcal or chlamydial cervical infection will develop acute upper tract infection. However, PID is a polymicrobial infection, even in the setting of gonococcal or chlamydial cervicitis. It has been suggested that, in many cases of PID, STD organisms initiate the inflammation of the tubal mucosa and this process facilitates the invasion of the mucosa by organisms endogenous to the lower genital tract.[2]

PREVENTION

Prevention is directed at reducing a woman's risk of acquiring an STD, and the detection and treatment of lower genital tract infections. A recent study showed that women screened and treated for asymptomatic chlamydial infection were nearly 60% less likely than unscreened women to develop PID.[7] Prompt and correct treatment of upper tract infections will ameliorate some of the long-term sequelae.

CLINICAL FEATURES

The most common clinical complaint in a woman who has PID is bilateral lower abdominal or pelvic pain. Gonococcal PID tends to have an abrupt, fulminant presentation within 1 week of the onset of menses. Chlamydial PID is characterized by a subacute course with mild symptoms, often described as a dull pain. It is not unusual for a woman who has chlamydial PID to be unaware of her infection and to present as a contact of a male with urethritis, with right upper quadrant pain or with other genitourinary complaints such as dyspareunia, dysuria, dysmenorrhea, menorrhagia or abnormal vaginal discharge. Chlamydial PID represents the diagnostic challenge, as uterine and adnexal tenderness may be very mild. The clinician should be alert to adnexal masses or fullness as the major signs of chlamydial infection. Women who have HIV infection and PID may present with more clinically severe disease.[8]

These traditional clinical signs and symptoms of PID, however, are neither sensitive nor specific for the syndrome. Laparoscopy confirms salpingitis in 45–89% of women who have clinical PID. Of women who have acute clinical PID, 6–45% have normal fallopian tubes and 5–33% have other conditions, including ectopic pregnancy, appendicitis, hemorrhagic ovarian cysts, endometritis, pelvic adhesions and torsion of an adnexal structure.[6] [9] False-negative clinical diagnoses for PID range from 16% to 47%.[9] The diagnoses prior to laparoscopy in women who have laparoscopically confirmed PID included ectopic pregnancy, ovarian cyst, hemorrhagic ovarian cyst, endometriosis, fibroids, ovarian tumor, appendicitis, pyelonephritis and uncertain diagnosis. The common complications and sequelae of PID include

TABLE 63-1 -- Common etiologic agents of pelvic inflammatory disease.

COMMON ETIOLOGIC AGENTS OF PID	
Aerobic bacteria	*Neisseria gonorrhoeae*
	Chlamydia trachomatis
	Gardnerella vaginalis
	Escherichia coli
	Streptococcus spp.
	Haemophilus influenzae
Anaerobic bacteria	*Bacteroides* spp.
	Peptostreptococcus spp.
	Peptococcus spp.
	Prevotella spp.
	Porphyromonas spp.
Mycoplasmas	*Mycoplasma hominis*
	Mycoplasma genitalium

ectopic pregnancy, tubal infertility, recurrent PID, chronic abdominal pain, tubo-ovarian abscesses and pelvic adhesions. A woman's risk of ectopic pregnancy increases seven- to 10-fold after an episode of PID.

The number of episodes of PID, the woman's age and the severity of tubal inflammation determined at laparoscopy influences the fertility prognosis of PID (Table 63.2). It appears that PID caused by chlamydial infection may result in more infertility than PID caused by gonococcal infection. Recurrent pelvic infections will develop in up to one-third of women who have had PID. Chronic abdominal pain lasting more than 6 months occurs in 15–18% of women after PID. Pyosalpinx, tubo-ovarian abscesses and pelvic adhesions occur in 15–20% of women who have had PID and often require surgical intervention. Mortality from acute PID is rare. The most

common cause of death from PID is a ruptured tubo-ovarian abscess with subsequent peritonitis. The mortality rate from this complication of PID is 6–8%.

DIAGNOSIS

The clinical diagnosis of PID is imprecise. Laparoscopy can be used to obtain a more accurate diagnosis but is neither readily available in most cases nor justifiable in clinically mild disease.

Women who have lower abdominal tenderness, adnexal tenderness and cervical motion tenderness — the Centers for Disease Control minimum criteria — should be treated for PID if there is no other diagnosis that should be considered. [10] One analysis on a large number of women enrolled in a clinical trial showed that lower abdominal tenderness alone was the most sensitive of the physical

TABLE 63-2 -- Prevalence of post-infection tubal dysfunction (tubal factor infertility or ectopic pregnancy) infertility after pelvic inflammatory disease.*

PREVALENCE OF POSTINFECTION TUBAL DYSFUNCTION (TUBAL FACTOR INFERTILITY OR ECTOPIC PREGNANCY) INFERTILITY AFTER PID			
No. PID episodes	**Total patients**	**No. with tubal dysfunction**	**%**	**Relative risk**
0	448	10	2.2	1.0
1	1015	140	13.8	6.3
Mild	320	13	4.1	1.0
Moderate	459	45	9.8	2.4
Severe	236	82	34.7	8.5
<25 years	783	94	12	1.0
25–35 years	232	46	19.8	1.8
2	198	70	35.4	16.2
>3	69	45	65.2	29.6

** Data from Weström and Eschenbach.[2]*

findings.[11] Additional tests to document an inflammatory and infectious process in the lower genital tract should be used to increase the sensitivity of the clinical signs.

A ratio of more than one white blood cell to epithelial cell on vaginal wet mount or an endocervical Gram stain, obtained after cleaning the ectocervix, showing Gram-negative intracellular diplococci or 30 or more polymorphonuclear leukocytes per 1000× field is highly supportive of the diagnosis of PID in women with appropriate clinical criteria. Tests (culture or antigen) for the detection of *N. gonorrhoeae* and *C. trachomatis* are useful in supporting the diagnosis but not in the decision to start therapy. A pregnancy test should be done. Erythrocyte sedimentation rate, C-reactive protein and complete white blood cell count may also be useful. Pelvic and endovaginal ultrasound can detect findings consistent with severe PID, including tubo-ovarian abscesses, dilated fallopian tubes and cul-de-sac fluid. Ultrasound is less useful in mild or atypical clinical presentations. Computerized tomography (CT) has the same limitations as ultrasound. However, in severe cases of PID with atypical ultrasound findings CT can be useful.[12] For example, spiral CT scanning optimizes identification of small air bubbles that are specific for abscess.[13]

Endometrial biopsy documenting endometrial neutrophils or plasma cells confirms the diagnosis but requires at least 24 hours for processing. Purulent material from the peritoneal cavity obtained by culdocentesis, a painful procedure, may support the diagnosis of PID but may also occur with other intra-abdominal infections, such as appendicitis. Laparoscopy is the gold standard for the diagnosis and staging of acute PID. The minimum criteria for visual confirmation of PID include hyperemia of the tubal surface, edema of the tubal wall and a sticky exudate on the tubal surface and from the fimbriated end when patent.[14]

MANAGEMENT

Management of a patient who has PID includes therapy, education, careful follow-up and partner management. The goal is to cure the patient, prevent recurrences and, ultimately, to preserve fertility. Empiric treatment should be instituted as soon as the diagnosis is suspected. Based on the polymicrobial nature of PID, therapy must provide broad-spectrum coverage. Several antimicrobial regimens have proved to be highly effective in achieving clinical cure of PID but there are few data on the efficacy of recommended or tested regimens on preventing the late sequelae.[15] Studies from the pre-antibiotic era documented infertility rates after PID of 60–70%, indicating that prompt institution of antimicrobial therapy does influence the fertility outcome.[2]

Pelvic inflammatory disease should always be treated with at least two antibiotics for at least 10–14 days (Table 63.3). The combination

TABLE 63-3 -- Recommended treatment of pelvic inflammatory disease.*

RECOMMENDED TREATMENT OF PID		
Oral treatment		
Regimen A		Ofloxacin 400mg po q12h for 14 days
	Plus	Metronidazole 500mg po q12h for 14 days
Regimen B	Either	Ceftriaxone 250mg im, or
		Cefoxitin 2g im plus probenecid 1g po in a single dose concurrently, or
		Other parenteral third-generation cephalosporin (e.g. ceftizoxime or cefotaxime)
	Plus	Doxycycline 100mg po q12h for 14 days
	Plus	Metronidazole 500mg po q12h for 7 days
Parenteral treatment		
Regimen A	Either	Cefotetan 2g iv q12h, or
		Cefoxitin 2g iv q6h
	Plus	Doxycycline 100mg iv or po q12h
This regimen should be continued for 48 hours after substantial clinical improvement. Doxycycline 100mg po q12h should then be administered for a total of 14 days.		
Regimen B		Clindamycin 900mg iv q8h
	Plus	Gentamicin loading dose iv or im (2mg/kg body weight) followed by a maintenance dose of 1.5mg/kg
	Followed by either	Doxycycline 100mg po q12h, or
		Clindamycin 450mg po q6h
This regimen should be continued for 48 hours after substantial clinical improvement. Doxycycline 100mg po q12h or clindamycin 450mg orally q6h should then be administered for a total of 14 days.		

** Data from Centers for Disease Control and Prevention.[10]*

regimen of an extended-spectrum parenteral cephalosporin plus doxycycline provides good coverage for all potential pathogens, including ß-lactamase-producing strains. There are no data on the use of oral cephalosporins for the treatment of PID. An alternative parenteral inpatient regimen is the combination of clindamycin plus an aminoglycoside. Although this combination provides coverage against gonococcal and chlamydial infections, it is inferior to the cephalosporin-doxycycline combination for these pathogens. The combination of ofloxacin and clindamycin or metronidazole also provides excellent coverage.

Many experts recommend that all PID patients should be hospitalized to receive optimal therapy, although this is not always possible. Hospitalization is recommended for women whose tolerance or compliance with outpatient regimens is uncertain, or who have factors that complicate treatment, severe illness or an uncertain diagnosis (Table 63.4).

Supportive therapy includes hydration, bedrest in the semi-Fowler position to localize the infection to the pelvis, pelvic rest and pain relief. Intrauterine devices should be removed. The patient should abstain from sexual intercourse until test-of-cure studies and resolution of signs and symptoms. All sexual partners in the previous 30 days should be evaluated and presumptively treated for gonococcal and chlamydial infection. Explicit and clear patient education cannot be overemphasized in the treatment of PID, especially in the context of outpatient management.

The incidence of ascending infection among pregnant women who have intact membranes is unknown but is believed to be rare.[16] However, given the high-risk of miscarriage and pre-term delivery associated with PID, pregnant women who have PID must be hospitalized and given parenteral antibiotic therapy.[10]

Women who have tubo-ovarian abscesses should be hospitalized and begun on broad-spectrum antibiotics (aminoglycoside plus clindamycin or metronidazole). The vast majority of abscesses with a diameter of 4–6cm respond to antibiotics alone, whereas only 40% of those that are 10cm or larger respond to medical therapy alone.[17] Increasing abscess size or failure to defervesce 72 hours after administration of antibiotics suggests medical failure and requires surgical intervention (e.g. percutaneous or transvaginal drainage under sonographic guidance, laparoscopic drainage or laparotomy). Leaking or ruptured abscesses require immediate laparotomy after stabilization of the patient. Extensive surgery such as complete hysterectomy is rarely indicated except in life-threatening complications such as extensive necrotic myometrium.

POSTPARTUM ENDOMETRITIS AND CESAREAN SECTION

EPIDEMIOLOGY

Postpartum endometritis, an infection of the uterus, is the most common cause of maternal postpartum fever and includes the

TABLE 63-4 -- Recommendations for hospitalizing patients who have pelvic inflammatory disease

RECOMMENDATIONS FOR HOSPITALIZING PATIENTS WHO HAVE PID	
Uncertain tolerance or compliance with outpatient regimen	Adolescents
	Substance abusers
	Nausea and vomiting
	Follow-up at 72 hours after starting antibiotic treatment is problematic
Complicating factors	Pregnancy
	HIV infection or other immunosuppressive condition
	Intrauterine device use
	Suspected pelvic or tubo-ovarian abscess
	Recent history of intrauterine instrumentation
Severe illness	Temperature over 101°F (38.3°C)
	White blood cell count greater than 15,000/ml
	Peritoneal signs
	Septic
Uncertain diagnosis	Failure to respond clinically to outpatient treatment
	Inability to exclude surgical emergencies (ectopic pregnancy, appendicitis)

inflammatory conditions of endometritis, endomyometritis and endoparametritis. It can be categorized into early infection (i.e. onset within 48 hours of delivery) and late infection (i.e. onset 2 days to 2 weeks after delivery). The most significant risk factor for postpartum endometritis is cesarean section. The incidence of postpartum endometritis after vaginal delivery is 2–5% whereas the rate after cesarean section ranges from 20% to 55%.[18] Postpartum upper tract infections following vaginal delivery are about 10 times more common in developing countries than in developed countries as a result of unclean delivery practice, traditional birth practices and the high prevalence of STDs in some populations. [19]

PATHOGENESIS AND PATHOLOGY

Early postpartum endometritis usually is associated with nonelective cesarean section and is probably the result of direct uterine contamination by organisms in the amniotic cavity. This is in direct contrast to women who develop late postpartum endometritis, who usually deliver vaginally. The timing of these late infections suggests an ascending infection similar to the mechanisms for PID. Wound infections after cesarean section appear to be a result of a direct contamination of the wound by organisms in the endometrium at the time of surgery.

Postpartum endometritis is a mixed aerobic-anaerobic infection (Table 63.5). *Ureaplasma urealyticum* and *Mycoplasma hominis* have also been isolated from the endometrium and blood but their clinical significance is not clear. *Chlamydia trachomatis* is associated with the late form of postpartum endometritis.[20] Group A ß-hemolytic streptococcal endometritis is rare and clustered cases are probably related to a common source, often a care-giver. Herpes simplex endometritis has also been reported.[21] Bacteremia occurs in 10–20% of patients and most common blood isolates are group B streptococci, *Gardnerella vaginalis* and *Peptostreptococcus* spp.[22]

PREVENTION

The timely diagnosis and treatment of lower tract syndromes during pregnancy, especially bacterial vaginosis, would prevent some postpartum endometritis. Prophylactic antibiotic use for patients requiring a nonelective cesarean section after labor or rupture of membranes of any duration greatly reduces the incidence of post-cesarean endometritis.[18] [23]

TABLE 63-5 -- Common endogenous micro-organisms identified as potential etiologic agents in postoperative pelvic infections

COMMON ENDOGENOUS MICRO-ORGANISMS IDENTIFIED AS POTENTIAL ETIOLOGIC AGENTS IN POSTOPERATIVE PELVIC INFECTIONS	
Aerobic bacteria	*Streptococcus* spp.
	Enterococcus faecalis
	Staphylococcus aureus
	Staphylococcus epidemidis
	Escherichia coli
	Klebsiella pneumoniae
	Gardnerella vaginalis
Anaerobic bacteria	*Bacteroides* spp.
	Peptostreptococcus spp.
	Prevotella bivia
	Prevotella disiens
	Fusobacterium spp.
Mycoplasmas	*Mycoplasma hominis*
	Ureaplasma urealyticum

CLINICAL FEATURES

Risk factors for postpartum endometritis include duration of labor, length of time membranes remain ruptured, presence of STDs, presence of bacterial vaginosis, the number of vaginal examinations, the use of internal fetal monitoring and socioeconomic status.[22] [24] [25]

Postpartum endometritis should be suspected in any woman who develops significant fever (oral temperature 101.3°F (38.5°C) or higher in the first 24 hours after delivery or 100.4°F (38°C) or higher for at least 4 consecutive hours, 24 hours or more after delivery). The diagnosis of postpartum endometritis can be made on the basis of clinical features of fever and when signs on physical examination suggest an endometrial inflammatory process including abdominal pain, uterine tenderness, foul lochia, increased uterine bleeding and uterine subinvolution. Late-onset endometritis tends to have a mild, subacute clinical presentation.

Acute complications of postpartum endometritis include pelvic abscess and puerperal ovarian vein thrombophlebitis. Puerperal ovarian vein thrombophlebitis is an acute thrombosis of one or both ovarian veins postpartum, is usually associated with postpartum endometritis with an onset of 2–4 days after delivery, and can be associated with septic pulmonary emboli. The reported incidence is 1 in 2000 deliveries.[26] Chronic complications and sequelae result from postinfectious scarring.

DIAGNOSIS

Blood cultures should be obtained from all patients before starting therapy. Tests for the detection of cervical infection with *N. gonorrhoeae* and *C. trachomatis* should be obtained from all women at risk of STDs (in women failing initial therapy). Quantitative endometrial cultures obtained using a triple lumen catheter, which minimizes contamination, provides useful information. A complete white blood cell count should be done. If an adnexal mass is felt on examination, then ultrasound or CT can be used to confirm the diagnosis.

MANAGEMENT

Postpartum endometritis is commonly treated parenterally with a broad-spectrum antibiotic regimen with activity against *Bacteroides fragilis* and other penicillin-resistant anaerobic bacteria, including the second generation cephalosporins (cefoxitin or cefotetan) or the extended-spectrum penicillins (ticarcillin-clavulanate or sulbactam-ampicillin).[25] The combination of aminoglycoside plus clindamycin remains an appropriate regimen. [26] Once-daily administration of aminoglycosides appears to be effective and safe in the treatment of endometritis. Women should continue to receive parenteral therapy until fever has resolved, uterine tenderness and abdominal pain are gone, and white blood cell count has normalized. Subsequent oral antibiotic therapy with erythromycin or doxycycline need only be given to women who have documented chlamydial infection for a total of 10–14 days. Reasons for failure to respond to antimicrobial therapy include inappropriate antibiotics (enterococcal infection or resistant anaerobic infection), pelvic or wound abscess, or ovarian vein thrombophlebitis. When postpartum septic pelvic thrombophlebitis is suspected heparin should be given.[27] Late postpartum endometritis can be managed in the same way as PID (see Table 63.3).

EPISIOTOMY INFECTIONS

EPIDEMIOLOGY

Episiotomy infections are rare. The rate of infection of episiotomies is 0.1% overall but increases to 1–2% of episiotomies complicated

by third- or fourth-degree extensions. Episiotomy infections, however, can have severe and even fatal consequences.

PATHOGENESIS AND PATHOLOGY

Episiotomy infections have been classified into four categories based on the depth of infection in the soft tissue: simple infection, superficial fascial infection, superficial fascial necrosis and myonecrosis.[28]

Bacteria implicated in episiotomy infections include skin pathogens, streptococci and staphylococci, and bacteria associated with vaginal flora, Enterobacteriaceae and anaerobic bacteria, including *B. fragilis. Clostridium perfringens* and *C. sordellii* are likely if myonecrosis is present.

PREVENTION

Treatment standards should be introduced that reduce the liberal or routine use of episiotomies, as they appear to increase the risk of third- and fourth-degree tears. In the USA between 1980 and 1998 there was an overall decline of 39% in the use of episiotomy.[29]

CLINICAL FEATURES

The simple wound infection is a local infection limited to incision site in the skin and the superficial fascia. Clinically there is edema and erythema only along the incision. A superficial fascial infection involves two layers of the superficial fascia and resembles a cellulitis with erythema, edema and pain. This superficial infection is indistinguishable on the basis of skin appearance from an early superficial fascial infection with necrosis (necrotizing fasciitis). Necrotizing fasciitis involves all layers of the superficial fascia (and may involve the deep fascia). Skin anesthesia may precede the skin breakdown, because of nerve involvement. As nutrient vessels are occluded the skin may turn dusky and develop bullae and then frank necrosis. Subcutaneous gas may be present with the mixed infection. These patients often have evidence of marked toxicity out of proportion to the clinical findings. In myonecrosis, pain is often the dominant feature. Patients are extremely toxic, restless and confused or disoriented.

DIAGNOSIS

Prompt diagnosis is of paramount importance because necrotizing fasciitis and myonecrosis are rapidly progressive. Frozen section examination of full-depth biopsy specimens has been helpful in the diagnosis of necrotizing faciitis. [30] However, surgical exploration is warranted if necrotizing fasciitis or myonecrosis is suspected.

MANAGEMENT

Management of a simple episiotomy wound infection includes opening of the incision and exploration to ensure that there is no accumulated blood or a rectovaginal opening. Any superficial fascial infection should be managed with broad-spectrum antibiotic coverage (e.g. ampicillin/gentamicin/metronidazole or ampicillin/gentamicin/clindamycin) and observed closely. Surgical exploration should be undertaken if:

- erythema and edema extend beyond the incision site;
- there is no improvement in 24–48 hours after the start of antibiotics or if the patient deteriorates; or
- the patient has severe systemic manifestations.

In the case of necrotizing fasciitis the superficial fascia will separate easily from the deep fascia with a probe or finger (this does not occur in healthy tissue), the incisions will be bloodless and the exudate will be serosanguineous rather than purulent. Surgical debridement of all necrotic and pale tissue should be performed promptly and broad-spectrum antibiotic therapy should be instituted. The wound should be left open after debridement. A second-look procedure is often necessary after 24 hours.

Myonecrosis is an extremely rare event that is usually caused by *C. perfringens* or *C. sordellii* but may result from an extension of necrotizing fasciitis through the deep fascia to the muscle. Therapy includes high-dose penicillin and urgent surgical debridement. Hyperbaric oxygen therapy remains controversial and should be considered only as an adjunctive therapy.

POSTABORTION SEPSIS

EPIDEMIOLOGY

Postabortion sepsis is an ascending infection of the female pelvis after spontaneous or induced abortion. Inflammatory conditions associated with infectious complications of abortion are similar to those for PID but can be complicated by retained, poorly perfused tissue and uterine or bowel trauma. The mortality from abortion in developed countries is low (an estimated 0.6 per 100,000 cases), and abortion accounts for only 5% of all maternal mortality in the USA. Infection is the major cause when mortality does result from abortion complications. In a review of 107 deaths caused by abortion in the USA between 1975 and 1977, 33% were caused by sepsis.[31] In developing countries the World Health Organization estimates that illegal abortion accounts for 25–50% of the 500,000 maternal deaths that occur each year.

PATHOGENESIS AND PATHOLOGY

The bacteria associated with postabortion sepsis are similar to those associated with PID. However, the potential of direct uterine or bowel injury and retention of the products of conception after an abortion may result in injured and poorly vascularized tissue and an enlarged spectrum of enteric organisms, including *C. perfringens*. In developing countries, tetanus is a cause of mortality after abortion.

PREVENTION

Prevention measures include providing effective and acceptable contraception and appropriate medical management of abortion. Prophylaxis for cervical and vaginal infections before voluntary termination of pregnancy has been suggested as a preventive measure but there are few data to support this practice. Prompt diagnosis and effective treatment of endometritis after the procedure is extremely important as delayed treatment is a common feature in cases of death from septic abortion.

CLINICAL FEATURES

The diagnosis of septic abortion should be considered in any woman who has a temperature of 100.4°F (38°C) or higher on two occasions more than 24 hours after an abortion and in any woman of reproductive age who presents with fever, abdominal pain and bleeding. Additionally, details of the procedure, including microbiologic studies and pathology of the aborted tissues, should be obtained. Physical examination findings typically include uterine tenderness, foul or purulent cervical discharge and products of conception at the cervical os. In more severe infections the patient may be hypotensive or in shock. The presence of cervical or vaginal lacerations should be assessed. Women who have clostridial infection may have severe disseminated intravascular hemolysis.

Acute complications are seen in advanced stages of the disease process and include the respiratory distress syndrome, septic shock, renal failure, abscess formation, septic pelvic vein thrombophlebitis and septic emboli, and disseminated intravascular coagulopathy; death may also occur. The chronic complications are similar to those of PID and include infertility, chronic pelvic pain and ectopic pregnancy.

DIAGNOSIS

Except for women who have mild, early, uncomplicated postabortion endometritis, all women should have blood and cervical cultures as well as a complete white blood cell count and urinalysis. Upright and flat abdominal and pelvic radiographs should be done to assess the presence of air in the abdominal cavity, dilated bowel and gas in the uterus. Pelvic ultrasound can determine the presence of retained tissue and other fluid collections, and the disruption of the myometrium by fluid or gas. Computerized tomography is useful in assessing the entire abdomen. Laparoscopy can be used to examine the uterus for perforation but is suboptimal for a detailed examination of the bowel.[32]

MANAGEMENT

Any woman who has an incomplete or failed abortion or retained clotted or liquid blood (hematometra) should undergo immediate re-evacuation.[33] This tissue serves as a nidus for infection. The recommended treatment of PID (see Table 63.3) is appropriate for a woman who has early, uncomplicated postabortion infection limited to the endometrial cavity. The patient should be evaluated 48 hours after institution of therapy. If fever or pain persist then the patient should be hospitalized and evaluated as above.

Women who have more severe illness should be hospitalized and begun on broad-spectrum antibiotics such as ampicillin, gentamicin and clindamycin. If clostridial infection is suspected, high-dose penicillin therapy should be used. These patients should be monitored closely and aggressively evaluated for uterine perforation and bowel injury. Laparotomy with possible hysterectomy should be performed if there is failure to respond to uterine evacuation and medical therapy, uterine perforation with necrotic myometrium or suspected bowel injury, pelvic and adnexal abscesses, or clostridial myometritis. Indications for a total hysterectomy with removal of adnexae include a discolored, woody appearance of the uterus and adnexae, clostridial sepsis, pelvic tissue crepitation and gas in the uterine wall on radiographs.

POSTOPERATIVE GYNECOLOGIC INFECTIONS

EPIDEMIOLOGY

Hysterectomy is the most frequently performed elective surgical procedure among women of reproductive age in the USA.[34] The spectrum of postoperative infections after hysterectomy include vaginal cuff cellulitis, pelvic cellulitis, vaginal cuff abscess, phlegmon, pelvic abscess and wound infections.[23] Rates of infection after abdominal hysterectomy without antibiotic prophylaxis ranged from 11% to 38% and from 4% to 8% with antibiotic prophylaxis. For vaginal hysterectomies without antibiotic prophylaxis infection rates varied between 12% and 64% and with prophylaxis between 0% and 10%.[35] Risk factors for postoperative infection include duration of surgery, younger age, lower socioeconomic status and the presence of bacterial vaginosis.

PATHOGENESIS AND PATHOLOGY

Bacterial contamination of the operative site with flora of the lower reproductive tract occurs at the vaginal incision. The exposure in a vaginal hysterectomy occurs from the initial vaginal incision throughout the procedure and the exposure in an abdominal hysterectomy occurs near the end of the procedure. Hospitalization itself, regardless of whether antimicrobial prophylaxis is given, changes the vaginal flora, resulting in an increase in colony counts of *Escherichia coli*, *Enterococcus faecalis* and *Bacteroides* spp. and a decline in *Staphylococcus epidermidis* and *Streptococcus* and *Peptostreptococcus* spp.

Postoperative infections involve a mix of aerobic and anaerobic bacteria from the lower reproductive tract (see Table 63.5). *Bacteroides fragilis* and *Fusobacterium* spp. are more common in infections than when they are found in normal vaginal flora.

PREVENTION

Patients undergoing elective hysterectomy should be screened and, if necessary, treated for bacterial vaginosis several weeks before surgery. Preoperative single-dose antibiotic prophylaxis substantially reduces the rate of postoperative febrile morbidity in these procedures.[23] [35]

CLINICAL FEATURES

Postoperative fever itself does not indicate infection but should prompt the clinician to evaluate the patient for one. In pelvic cellulitis symptoms usually occur 2–3 days after surgery and include fever (temperature over 100.4°F (38°C)) and complaints of increasing abdominal and pelvic pain that may not be symmetric.[36] Parametrial tenderness without palpable mass is found on bimanual examination. Histologic vaginal cuff cellulitis will develop in all women postoperatively as part of the normal healing process and most cases resolve without antibiotic therapy. Women who have more severe cellulitis will complain of increasing central or lower abdominal pain, increasing vaginal discharge or low grade fever, usually within the first 2 weeks postoperatively. Bimanual pelvic examination may show only mild suprapubic tenderness to deep palpation without masses. Speculum examination will show a tender, indurated, hyperemic vaginal surgical margin. In a vaginal cuff abscess patients typically have fever 2–3 days postoperatively and may report vaginal fullness. On examination a tender, palpable collection will be found above the vaginal surgical margin. A phlegmon would be diagnosed if a tender mass were felt in one or both parametrial areas and if no abscess could be identified on radiographic studies. Pelvic abscesses are a late postoperative complication that present many weeks after surgery and most have a palpable mass in the pelvis. Wound infections are characterized by pain, marginal cellulitis and purulent exudate.

Septic pelvic vein thrombophlebitis and osteomyelitis pubis are both rare complications of gynecologic surgery.

DIAGNOSIS

Microbiologic cultures obtained from drained abscesses are useful in guiding therapy. Cultures of the vaginal cuff are likely to be contaminated with vaginal flora. Abdominal and pelvic ultrasound and CT scans are useful to confirm the presence of a fluid collection when pelvic abscesses are suspected.

MANAGEMENT

Patients who have pelvic cellulitis should be treated with a broad-spectrum parenteral antibiotic regimen, such as a second-generation cephalosporin (cefoxitin or cefotetan), extended-spectrum penicillin (ticarcillin-clavulanate or sulbactam-ampicillin), or an aminoglycoside plus clindamycin, for 24–36 hours after the patient becomes

afebrile. Cuff abscesses should be managed similarly and the abscess should be drained. Vaginal cuff cellulitis can be managed on an outpatient basis with oral antibiotics such as amoxicillin-clavulanic acid, but patients should have a follow-up evaluation 72 hours after starting therapy. Medical therapy that covers Gram-negative aerobes and Gram-negative anaerobes (aminoglycoside-clindamycin or aminoglycoside-metronidazole) is often successful in treating postoperative pelvic abscesses that are inaccessible to drainage. Patients who fail to respond to medical therapy alone (no defervescence in 72 hours or enlarging abscess) will require laparotomy and drainage, or excision. Abscesses accessible from a cutaneous surface should be drained. Patients should be treated with parenteral antibiotics until all signs and symptoms have resolved. Some clinicians give post-discharge outpatient treatment with metronidazole and amoxicillin for a week after discharge in those patients who responded to medical management. All patients who have pelvic abscesses should be re-evaluated 2 weeks after discharge to ensure no recurrence of the abscess has occurred.

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Chapter 64 - Complications of Pregnancy: Maternal Perspectives

Marleen Temmerman

EPIDEMIOLOGY

Medical progress, such as effective antibiotics and vaccines, in combination with improved living conditions has modified the sequelae of infections, yet infectious morbidity in pregnancy remains a serious problem.

Any acute or chronic infection may occur before conception, during pregnancy or during the puerperium and may have serious consequences for the mother, the fetus and the neonate. Some micro-organisms are known to cause congenital infections and are discussed in Chapter 65 . Others primarily influence the health of pregnant women and are described below.

The problem of maternal infections during pregnancy is addressed with emphasis on organ systems, including genitourinary tract infections, respiratory tract infections, gastrointestinal infections, puerperal sepsis, wound infection, mastitis, thrombophlebitis, endocarditis and meningitis. The infectious etiology of pre-term birth, premature pre-term rupture of membranes (pPROM) and chorioamnionitis deserves special attention. In addition, the implications of specific infections, including malaria, listeriosis, Lyme disease, varicella-zoster, HIV and other sexually transmitted diseases (STDs), are summarized in Table 64.1 and Table 64.2 .

The topic of infections in pregnancy is too wide to summarize in a single chapter. The interested reader will find excellent reviews by Sweet and Gibbs, Ledger, Hurley and Lamont.[1] [2] [3] [4]

Incidence and prevalence

Genital micro-organisms, particularly sexually transmitted organisms, are important in poor pregnancy outcome, including pre-term birth, pPROM, spontaneous abortion, perinatal morbidity and mortality, and maternal infections.[5] [6] [7] [8] [9] [10] [11] [12] Recent publications confirm the role of *Neisseria gonorrhoeae* in low birth-weight and in spontaneous abortion, of bacterial vaginosis in pre-term birth, and of genital mycoplasmas in pPROM and in postpartum febrile infections in women and neonates. Bacterial vaginosis has received renewed attention because of its role in pre-term delivery and because it is possibly prone to interventions. The prevalence of bacterial vaginosis varies between 15% and 25%. Group B streptococci (GBS), which are known to be a risk factor for neonatal infections and pre-term birth, also contribute to spontaneous abortion. The reported rates of GBS colonization in the genital tract range from 5% to 40%, with an average transmission rate to the neonate of 60%. The rates of early-onset GBS infection in the neonate, especially in pre-term and low-birth-weight babies, can be as high as 3 in 1000.

Prevalence rates of HIV in pregnant women are increasing all over the world but have reached endemic proportions in developing countries, where over 25% of pregnant women in some urban areas are HIV-seropositive. The impact of maternal HIV infection on pregnancy outcome is still debated but most data from large studies of pregnant women who do not use drugs show an increased risk of adverse obstetric outcome, including abortion, prematurity, low birth-weight and stillbirth, with perinatal HIV transmission rates of 15–40%.

Urinary tract infections (UTIs) are the most common infections in pregnancy, with or without clinical signs or symptoms. Asymptomatic bacteriuria is found in 4–7% of pregnant women, of whom 25–30% will develop pyelonephritis later in pregnancy.

Upper respiratory tract infections are common but of limited consequence for mother and child. In contrast, pneumonia is a serious illness for a pregnant woman.

Gastrointestinal infections caused by viruses are usually mild with no harm to the pregnancy and no need for specific medication. Meningitis is rare except for areas in which HIV and cryptococcal meningitis are endemic. Bacterial endocarditis is also uncommon and incidence rates vary from 1 in 4000 to 1 in 16,000 deliveries.

Febrile illness at delivery is uncommon in uncomplicated term pregnancies. Common underlying causes are chorioamnionitis, pyelonephritis, influenza and listeriosis.

The overall incidence of postpartum infections varies between 1% and 10%, depending on the definitions used, particularly for mastitis and postpartum endometritis. Postpartum infections consist of genital tract infections, puerperal mastitis, pelvic thrombophlebitis, UTIs, wound infections, complications of anesthesia and other infectious complications.

Genital tract infections of the uterus are the most common cause of puerperal infection and are categorized as endometritis, endomyometritis or endoparametritis depending on the extent of the infection. Wound and episiotomy infections occur frequently. After cesarean section wound infection defined as erythema, positive discharge and/or positive wound cultures varies between 5% and 10%, with emergency cases at higher risk of infection.

Septic thrombophlebitis is a rare complication of pregnancy with reported incidence rates of 1 in 2000 deliveries. The incidence of puerperal mastitis is estimated at around 1% in lactating women. Most have a mild disease.

Burden of disease, morbidity and mortality

In the general population the attributable risk of infections for adverse pregnancy outcome depends on the prevalence rates of infections in the population as well as on the socioeconomic and cultural factors that influence health, health behavior and health-seeking behavior. All infections that manifest with fever increase the risk of pre-term birth because of the release of pyrogens that increase myometrial activity.

Intra-amniotic infection diagnosed on clinical criteria occurs in 1–5% of pregnancies, with or without ruptured membranes. Consequences are pre-term birth, pPROM and postpartum and neonatal infections. The mother and the fetus are put at risk with pPROM, as it is associated with pre-term birth and frequent infectious morbidity. Ascending infections, either the cause or the result of pPROM, may lead to intra-amniotic infection, chorio-amnionitis, placentitis and fetal infections, including pneumonia and bacteremia.

The impact of asymptomatic UTI on pregnancy complications such as hypertension, anemia and poor obstetric outcome remains

TABLE 64-1 -- Implications of specific infections on pregnancy.

IMPLICATIONS OF SPECIFIC INFECTIONS ON PREGNANCY			
	Impact on mother and child	**Prevention**	**Management**

Malaria	More frequent, more severe in pregnancy, especially in nonimmune women; increased risk of hypoglycemia in the mother	Chemoprophylaxis in travelers to endemic areas	Prompt treatment with chloroquine or quinine according to resistance patterns
		If no *Plasmodium falciparum* resistance, chloroquine phosphate 500mg/week po	
	LBW, IUGR, pre-term birth, abortion and stillbirth increased	In case of resistance proguanil 200mg/day + chloroquine 500mg/week po	Similar treatment regimens to those in nonpregnant women
	Congenital malaria (fever, hepatosplenomegaly, jaundice, anemia)	Avoid exposure	
Listeriosis	Mild maternal infection, but increased susceptibility	Early diagnosis in any febrile illness in pregnancy, cervical and blood cultures for *Listeria monocytogenes*	Ampicillin 2g q6h iv + gentamicin 2mg/kg q8h for 1 week
	Serious impact on the fetus: amnionitis, pre-term birth, septic abortion, stillbirth, fatality rate 3–50%	Avoid implicated foods (e.g. unpasteurized cheese)	
Lyme disease	Erythema migrans in the mother, risk of transmission unknown, probably low	Protective clothes in tick-infected areas (rural forest); remove ticks	Early treatment with amoxicillin 500mg q6h po for 10–30 days or ceftriaxone 2g iv for 14 days
	Pre-term birth, stillbirth, syndactyle, cortical blindness, rash		
Varicella-zoster	Rare in adults, fever, malaise followed by rash, 20% risk of varicella pneumonia	IgG testing if exposed	In cases of pneumonia: admission, respiratory support, aciclovir 10–15mg/kg for 7 days
	Risk of abortion, stillbirth	If no IgG: varicella-zoster immunoglobulin 125 units/10kg, max 625 units im, <96h after exposure	Ultrasound assessment of the fetus
	Congenital varicella (limb hypoplasia, cortical atrophy, retardation, IUGR, cutaneous scars, microphthalmia)		
Measles	Increased maternal mortality secondary to pneumonia	Passive immunization in susceptible exposed women with pooled immunoglobulins 0.25ml/kg within 6 days of exposure	Symptomatic
	Risk of prematurity		
	Developmental abnormalities (e.g. congenital heart disease, cleft lip, cerebral leukodystrophy and cyclopia have been reported)	Avoid measles vaccine in pregnancy	
Group B streptococci	Sepsis in 1–3/1000 neonates, high mortality rates	No consensus	Penicillin G 5 million units iv followed by 2.5 million units q4h until delivery or ampicillin 2g followed by 1g q4h until delivery
		Antenatal case detection and treatment, or intrapartum treatment of women at risk?	

LBW, low birth weight; IUGR, intrauterine growth retardation

TABLE 64-2 -- Implications of some sexually transmitted diseases on pregnancy.

IMPLICATIONS OF SOME STDs ON PREGNANCY			
	Impact on mother and child	**Prevention**	**Management**
Neisseria gonorrhoeae	Ophthalmia nenatorum, pre-term delivery, puerperal infections	Silver nitrate 1% or tetracycline eye ointment	Spectinomycin 2g im, ceftriaxone 250mg im, or standard antimicrobial treatment
Chlamydia trachomatis	Ophthalmia neonatorum, puerperal infections, pre-term delivery	Tetracycline eye ointment	Erythromycin 500mg for 4–7 days
Bacterial vaginosis	Risk of pre-term birth	Case detection and treatment is still under study	Metronidazole 250mg q8h for 3–7 days or erythromycin base 333mg q8h for 3–14 days
Trichomonas vaginalis	Risk of pre-term birth	Case detection and treatment no proven effect	Metronidazole 2g single dose
Condylomata acuminatum	Risk of respiratory papillomatoses 1/80–1/1500	Case detection and treatment	Topical trichloroacetic acid (85%)/surgery
Herpes simplex	Neonatal herpes 50% in mother with primary herpes at delivery	History from pregnant woman, careful inspection of the genital tract on the day of delivery	In case of active lesions, cesarean section or vaginal delivery under aciclovir 200–400mg q8h (under study)
HIV	Transmission in 25–45%	Antiretroviral therapy according to the most recent guidelines	Avoid long labor, rupture of membranes <4h
	Risk of abortion, pre-term delivery, puerperal infections	Elective Cesarean section	Treat with antiretroviral therapy according to the most recent guidelines
		No breast-feeding	

controversial. In contrast, ascending UTIs clearly play a role in the etiology of pre-term delivery and neonatal death.

Lower respiratory tract infections, meningitis and bacterial endocarditis are all life-threatening conditions for the mother and should be treated without delay.

Although postpartum infections are seldom life-threatening, sepsis remains an important cause of maternal death worldwide. Maternal mortality rates of 1–5 per 100,000 live births are registered in the Western world, whereas 100–600 per 100,000 pregnant or childbearing women in developing countries die as a consequence of reproduction. In addition, for every woman who dies in childbirth another 30 women suffer from injuries, infections and disabilities. Overall, 25% of maternal mortality is considered to be caused by infections and this number can be lowered substantially by better health services and prompt treatment.

A number of reports estimating the role of infections in maternal death are summarized in Table 64.3 . Few etiologic studies have been carried out in developing countries but the role of infections is likely to be more important. My own personal observations from Nairobi, Kenya indicate that infections play a role in up to 40% of mothers dying in childbirth. The silent tragedy of maternal death should receive more attention from the international community, and also from the research world, because a substantial proportion of maternal deaths as a result of infections, bleeding and eclampsia are avoidable and interventions have to be tested to lower this unacceptable consequence of giving birth.

Postpartum genital infections may lead to chronic pain and discomfort, bleeding irregularities and infertility caused by ascending infections. Wound infections may increase pain and discomfort and prolong hospital stay. Pelvic vein thrombophlebitis may lead to serious complications such as septic pulmonary emboli.

Risk factors

Poverty is the most important risk factor for maternal infections during pregnancy. Poor women are more susceptible to malnutrition, infections, including STDs, less

adequate sanitary conditions and lower access to preventive and curative health care than those who are financially better off.

Risk factors for UTI in pregnancy include sexual activity, older age, history of UTIs, lower socioeconomic status, diabetes mellitus, sickle-cell disease and specific bacterial factors such as the serotype and the virulence determinants of the micro-organisms.

Risk factors for lower respiratory disease include low socioeconomic status and HIV infection, particularly for infections with *Streptococcus pneumoniae*. Bacterial endocarditis has been reported more often in urban settings and among drug users.

Risk factors for puerperal genital infections include socioeconomic variables, anemia, STD, obstetric factors such as length of rupture of membranes, pre-term delivery, Cesarean section and number of vaginal examinations. Puerperal fever caused by group A ß-hemolytic streptococci, once one of the most striking examples of iatrogenic

TABLE 64-3 -- Cases of maternal death from infections.[*]

CASES OF MATERNAL DEATH FROM INFECTIONS		
	Date of study	**%**
Michigan	1950–1971	23
Iowa	1926–1980	56
	1950–1980	16
South Carolina	1970–1984	14
Oklahoma	1950–1979	7

* *Data from Sweet and Gibbs.*[1]

infections in the 19th century, is a rare event in modern obstetrics, although sporadic outbreaks have been reported. A toxic-shock-like syndrome can occur caused by the release of pyrogenic exotoxins from streptococcal isolates (see Chapter 56).

Factors known to increase the risk for wound infections are age, obesity, bacterial contamination, operating time and duration of preoperative hospitalization, emergency procedures, number of vaginal examinations, duration of internal fetal monitoring, length of labor and underlying maternal disease. Puerperal mastitis can be related to poor nursing techniques and lack of strict hygiene measures.

PATHOGENESIS AND PATHOLOGY

Pathogenesis

The pathogenesis of most infections is similar in pregnant and nonpregnant women except for possible alterations in the immune system as noted below. Of specific interest is the role of infectious agents in the onset of labor or, more importantly, of pre-term labor. Although the exact mechanism of the onset of labor is still part of the human parturition puzzle, there is convincing evidence for the role of prostaglandins in the initiation of parturition. Arachidonic acid, one of the precursors of prostaglandins, is made available for prostaglandin synthesis by the enzyme phospholipase A_2 . This enzyme, produced by many micro-organisms but especially by anaerobes, might be one of the mechanisms of pre-term initiation of labor. Micro-organisms can stimulate the release of cytokines, such as interleukins and tumor necrosis factor, that stimulate prostaglandin precursors, thus leading to uterine contractions.

In theory, the amniotic cavity is sterile, protected by the placental membranes, with the cervical mucus serving as an effective barrier preventing micro-organisms from entering the uterine cavity. With the onset of labor and the rupture of membranes, bacteria may ascend and result in an amniotic infection. Pathogens may also gain access to the amniotic cavity through intact membranes or after invasive procedures such as amniocentesis, chorion villus sampling, umbilical blood sampling and cervical cerclage.

Urinary tract infections are caused by organisms that are part of the normal fecal flora, with *Escherichia coli* responsible for 80–90% of infections. Others are facultative Gram-negative bacteria, including *Klebsiella*, *Proteus*, *Enterobacter* and *Pseudomonas* spp., and Grampositive bacteria such as staphylococci and GBS. Symptomatic UTIs are more frequent in pregnancy for several reasons, including decreased ureteric muscle tone and activity, dilatation of the ureter and renal pelvis because of the progesterone effect, and mechanical obstruction caused by an enlarging uterus, changes of the bladder and alterations in the properties of urine during pregnancy. Bacteriuria is of concern because of the increased risk of pyelonephritis associated with pre-term labor caused by pyrogens, ureteric contractions leading to reflex myometrial contractions, the release of bacterial enzymes that may weaken the membranes and bacterial products that stimulate prostaglandin synthesis.

The most common organisms causing pneumonia in pregnant women are *S. pneumoniae*, *Haemophilus influenzae*, group A ß-hemolytic streptococci and coagulase-positive streptococci.

Postpartum endometritis seems to be a mixed infection with aerobic and anaerobic bacteria from the genital tract. Sexually transmitted diseases, including *Chlamydia trachomatis* and *N. gonorrhoeae*, are important risk factors for ascending infections. Cesarean section is the single most important predisposing factor for pelvic infection. Wound infections are determined by the surgical techniques used, the amount of bacterial contamination and the resistance of the patient. An adequate blood supply is necessary to avoid acidosis in the wound. Organisms involved are *Streptococcus faecalis*, *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus* spp.

and anaerobes. Predisposing factors for septic pelvic thrombophlebitis include changes in coagulation factors, alterations in the vein wall and stasis of blood flow.

In most cases of mastitis, *S. aureus* is the responsible organism, although *S. epidermidis* and viridans streptococci may also be isolated. Sporadic mastitis, usually the result of poor nursing technique, manifests as a cellulitis of the breast, primarily involving the interlobular connective tissue, to which the pathogens gain entry via a cracked or fissured nipple. In epidemic mastitis, however, infection occurs via the ductal system and spreads throughout the entire breast, resulting in mammary adenitis.

Immunity

The normal course of pregnancy is associated with a variety of changes in humoral and cellular immunity, such as a loss in $CD4^+$ cells and other alterations in T-cell subsets.[13] [14] [15] [16] Reports on T-cell subsets during pregnancy have been conflicting. Some studies have found a progressive fall in the $CD4^+$ count throughout pregnancy, from a mean of 950 cells/ml before 18 weeks to 720 cells/ml at term. Others have either reported a U-shaped $CD4^+$ cell count profile during pregnancy, with a minimum at approximately 32 weeks of gestation (CD4 of 30% and a $CD4^+$ count of 876 cells/ml), or have found stable CD4 levels and CD4:CD8 ratios during pregnancy with a rise (rebound) afterwards. Such differences may be attributable to different methodology (manual fluorescence microscopy versus automated flow cytometry), to differences in study populations or to the fact that blood was taken at different times during pregnancy. The altered immune status of pregnant women may also alter the response of the host to infectious agents.

Despite conflicting laboratory data, most studies agree that the humoral immune response in pregnancy is similar to that in nonpregnant women but that the cellular immune response is diminished. Mortality rates of, for example, pneumococcal pneumonia, malaria or influenza have been found to be higher in pregnant than in nonpregnant women.

PREVENTION

Elimination of poverty and improvement of antenatal and obstetrical care in deprived groups of society are primary strategies to prevent adverse pregnancy outcome.

The challenge of identifying women at risk of adverse obstetric outcome is still a subject of intensive research. Early markers of infection-related preterm birth are needed to identify a subset of women at risk for preterm delivery who could benefit from antimicrobial therapy. The finding of mucopurulent cervicitis defined by more than 30 polymorphonuclear cells per high-power field has been identified as a predictor of poor pregnancy outcome.[17] Elevated reproductive tract phospholipase A_2 concentrations were detected in pregnant women colonized with bacterial vaginosis, *Trichomonas vaginalis* or *C. trachomatis*.[18] Phospholipase A_2 is an enzyme that leads to prostaglandin synthesis and is important in the physiology of human parturition. Cervicovaginal interleukin-6 and elevated fetal fibronectin concentrations have been associated with preterm birth but their predictive value remains to be determined.[19]

Case detection and treatment of genitourinary infections is recommended in pregnant women who note an abnormal discharge. Screening for genitourinary pathogens with a potential negative impact on pregnancy outcome such as bacterial vaginosis, trichomoniasis, GBS, gonorrhea, Chlamydia and others, depends on the prevalence of infectious agents in the population, the expected outcome of the intervention, and the available resources. Recent intervention studies have provided convincing evidence of the benefit of identifying and treating bacterial vaginosis in pregnancy in women at high risk for pre-term birth.[8] [9] However, the practice of routine screening for bacterial vaginosis in asymptomatic women at low risk for pre-term delivery cannot be justified by current evidence from the literature.

Screening for GBS in pregnant women is still a subject of debate. Between 10% and 30% of pregnant women are colonized with GBS, an important source of perinatal morbidity and mortality. Recently, the Centers for Disease Control and Prevention issued recommendations for the active prevention of GBS, which have been adopted by the American College of Obstetricians and Gynecologists.[20] A strategy based on late prenatal cultures, followed by intrapartum treatment with penicillin or other broad-spectrum antibiotics is recommended. Others argue that routine antimicrobial treatment with a broad-spectrum antibiotic is optimal for pregnant women at high risk of adverse pregnancy outcome in order to reduce the incidence of infectious complications such as pre-term birth, neonatal infections and maternal infectious morbidity.[21] Risk may be based solely on laboratory findings or on clinical and obstetric factors (bad obstetric history, clinical signs and symptoms). However, many questions remain unanswered, especially the benefit of screening and treating low-risk groups and the ideal antimicrobial regimen. The existing evidence is hampered by methodologic weaknesses such as small numbers, use of combinations of antibiotics and differences in populations studied. Although practice guidelines have been established it is essential to prove that the costs and the benefits associated with screening and treating genital infections in pregnant women outweigh the potential risks and effects. Only prospective, randomized and blinded clinical trials with large study populations can determine the effect of antimicrobial therapy on infectious morbidity and mortality in pregnancy.

Intercourse during pregnancy has been implicated as a risk factor for pre-term birth. This could be because of the effect of STDs or because of increased myometrial activity and cervical ripening caused by the prostaglandins in sperm. After correcting for STDs, there is little evidence of a causal relation between sexual intercourse and pre-term birth. Randomized trials of condom use versus unprotected sex in high-risk groups for poor pregnancy outcome have not been carried out to date.

Detection and treatment of maternal bacteriuria in early pregnancy, preferably around 16 weeks, can reduce the risk of pyelonephritis, pre-term delivery and neonatal mortality.

Routine examination of stools for pathogens is not useful in pregnant women, except in populations that have high rates of anemia and malnutrition.

Prevention of puerperal infections is a major concern in obstetrics. Antenatal detection and treatment of STDs and hygienic standards during delivery decrease the risk for puerperal infection. The indications for cervical cerclage have to be carefully weighed against the risks, and antibiotic prophylaxis should be given. Active management of labor, including shorter labor, fewer vaginal examinations and reduced cesarean section rates, help to prevent genital infections. Antibiotic prophylaxis in cases of long, complicated or operative deliveries is effective, although resistance is a limiting factor.

Mastitis can be prevented through good nursing technique, including strict hygienic measures.

CLINICAL FEATURES

Infections in pregnancy can be asymptomatic but usually manifest with symptoms similar to those in nonpregnant individuals.[1] [2] [3] [4] The course of the infection may be worse because of alterations in immune response and because of the potential hazards for the outcome of pregnancy and the well-being of the fetus.

The clinical diagnosis of intra-amniotic infection is based upon fever, uterine tenderness, fetal tachycardia, leukocytosis and elevated C-reactive protein, with or without ruptured membranes.

Urinary tract infections are the most common infectious complications of pregnancy and can be asymptomatic or manifest with signs of cystitis such as frequency, urgency and dysuria. Fever, flank pain and chills occur with ascending UTIs.

Lower respiratory tract infections manifest with cough, fever and chest pain.

Gastrointestinal infections appear as diarrhea and are usually self-limiting and without complications. If the diarrhea persists beyond 24 hours, a stool specimen should be obtained for culture. Acute appendicitis can be a diagnostic dilemma as the clinical presentations differ from those seen in nonpregnant women because of the large uterus and the altered immune response. Appendicitis may manifest as upper right quadrant pain with nausea and vomiting, without leukocytosis or fever, and should be differentiated from acute cholecystitis and amebic liver diseases.

Meningitis should be considered in every patient with headache, malaise, nausea, vomiting and fever. The diagnosis of bacterial endocarditis in pregnant women should be considered in any febrile, lethargic patient with no signs of localizing infection. Cutaneous lesions and heart murmurs should be sought. Blood cultures are required for diagnosis.

Diagnostic criteria for postpartum endometritis include fever, uterine and/or adnexal tenderness, purulent or foul lochia and leukocytosis in the absence of other signs of infection within the first 5 days after delivery. Late postpartum endometritis may occur weeks after delivery. Retention of placental products has to be excluded.

Early wound infection starts usually within 48 hours postpartum and manifests with fever and cellulitis or edema of the wound. Early wound infection is often caused by group A or group B streptococci, or *Clostridium perfringens*. In clostridial infection, wound cellulitis is associated with a watery discharge and a bronze appearance of the skin. Late-onset wound infections occur about 4–8 days after surgery, and manifest with fever and an erythematous, draining wound.

Early recognition of life-threatening complications such as necrotizing fasciitis is crucial. Cutaneous findings can be minimal and include cellulitis, edema and sometimes crepitations. However, the patient may be critically ill and require prompt treatment. A surgical exploration of the wound may be necessary to make the diagnosis.

Puerperal mastitis occurs with breast engorgement and milk stasis, often in the second or third week after delivery. The onset of sporadic mastitis is rather sudden, with breast tenderness, chills, fever, malaise and headache mimicking a flu-like syndrome. The breast may show foci of local infection characterized by erythema, tenderness and warmth. The development of a breast abscess is rare in lactating women.

Patients with ovarian vein thrombophlebitis, which is more frequently present on the right, usually have distinct clinical findings. They present with fever and lower abdominal pain, and on examination are acutely ill with tachycardia and tachypnea and may be in respiratory distress. Abdominal examination usually shows direct tenderness, guarding and a tender abdominal mass. Pelvic pain thrombophlebitis without pulmonary emboli manifests less dramatically and has a more rapid response to therapy. These patients are more often not as critically ill, and just have fever and tachycardia.

DIAGNOSIS

Most infections that manifest with clinical signs and symptoms do not give rise to diagnostic difficulties, as they are related to specific infections of the urinary or reproductive tract, or to common infections in the community. Outlining a complete scheme of investigations is beyond the scope of this chapter, but a summary of the most important diagnostic leads and laboratory tests is presented in Table 64.4 . (See also Chapter 135).

TABLE 64-4 -- Clinical and laboratory criteria in the diagnosis of infection in pregnancy.

CLINICAL AND LABORATORY CRITERIA IN THE DIAGNOSIS OF INFECTION IN PREGNANCY	
History	Signs, symptoms, onset, specific localization, additional signs such as pain, rash, uterine tenderness, leakage of amniotic fluid, exposure to infections, pets, occupation, hobbies, travel, place of residence, history of infections
Clinical examination	Auscultation of heart and lungs, assessment of the uterus and cervix, examination of the breasts, detection of masses, enlargement of spleen, liver, lymph nodes, signs of thrombophlebitis
Laboratory tests	White blood cell count and differential, C-reactive protein, serum enzymes, blood smears, blood cultures, throat and vaginal swabs, specific antibodies, urine culture

Rapid and inexpensive tests for the early detection of intraamniotic infection in patients in pre-term labor, including amniotic fluid Gram stain, leukocyte esterase, amniotic fluid glucose concentration and the Limulus amebocyte lysate assay for endotoxin, have been tested in women admitted with pPROM or pre-term birth. The greatest sensitivity for predicting infection was demonstrated by a low glucose level in amniotic fluid but none of the tests had sufficient accuracy to allow clinical decisions.[21] [22] For women with cervical dilatation in the mid-trimester of pregnancy, amniocentesis to determine the microbiologic characteristics of the amniotic cavity should be considered before placing a cerclage because of the poor prognosis in women with microbial invasion of the amniotic cavity.

The diagnosis of UTI is based on quantitative cultures with more than 100,000cfu/ml clear-voided urine (midstream) in asymptomatic patients, or more than 100cfu/ml in symptomatic patients. Direct suprapubic bladder aspiration is a better technique for obtaining uncontaminated urine but is less readily accepted by patients and/or physicians. To avoid screening all pregnant women with expensive and time-consuming urine cultures, rapid screening tests such as leukocyte esterase dipstick, microscopy for pyuria, nitrite tests and enzymatic screening tests have been developed. The rapid enzymatic test seems to be a reliable alternative to culture screening with a sensitivity of 100%, a specificity of 81% and a negative predictive value of 100%.[23]

The key to the care of lower respiratory tract infections is an early diagnosis, with careful clinical evaluation and examination, if possible, of a sputum sample and a blood culture. One should not hesitate to take a chest radiograph, as well as an arterial Po_2 in pregnant women if a serious lower respiratory tract infection is suspected. In patients with meningitis, a spinal tap with Gram stain, culture and chemical analysis of the cerebrospinal fluid is usually indicated.

Postpartum endometritis is a clinical diagnosis supplemented by a cervical swab for aerobic culture to identify pathogens that may require additional measures besides the antibacterial therapy. Isolation of group A streptococci should lead to isolation of the patient whereas that of GBS should prompt further action in relation to the neonate. Culturing techniques of the endometrium with double- and triple-lumen devices have been hampered by vaginal and cervical contamination and are not used routinely.

The diagnosis of early wound infection is made clinically and confirmed by a Gram stain. Gram-positive rods are highly suggestive of clostridia, and Gram-positive cocci indicate the presence of group A streptococci or *S. aureus*.

The diagnosis of mastitis is a clinical diagnosis. Mammography and ultrasound can be useful in the early diagnosis of an abscess and

to differentiate infection from a breast malignancy. However, this technique is rarely used because of pain and discomfort to the patient.

Pelvic vein thrombophlebitis is a difficult clinical diagnosis often confused with acute appendicitis, torsion of an adnexa, urolithiasis, pyelonephritis, leiomyoma and pelvic abscess. In case of clinical suspicion of a pelvic vein thrombophlebitis, additional examinations such as venography, computerized axial tomography and sonography have to be performed.

MANAGEMENT

A number of interventions with proven value in the management of morbidity related to infection during pregnancy are discussed. The serologic screening and subsequent management of viral infections, including rubella, toxoplasmosis, cytomegalovirus, HIV and others, is discussed in Chapter 65 .

Although the initiating mechanism of labor, and particularly preterm labor, is unknown, the potential role of inflammation is clear. Consequently, attempts to prolong gestation and improve pregnancy outcome using antimicrobials have been made. A number of prospective, randomized clinical trials with antibiotics have been reviewed. Most authors agree that treatment of pre-term labor with antibiotic therapy can prolong gestation but the impact on neonatal morbidity and mortality has not been demonstrated. Antibiotics in women with pPROM seem to prolong the interval to delivery and neonatal outcome.[24] [25] [26] [27] [28] The reason for the continuing controversy might be that infection is the underlying cause of pre-term birth in only a fraction of women, the subset that may benefit from antimicrobial therapy. Hence, improved diagnostic methods are needed to identify those patients who will gain from antibiotics.

As bacteriuria predisposes the pregnant woman to pyelonephritis with potential hazards for mother and fetus, screening and management in pregnancy is justified. Antimicrobial treatment options of UTIs include 3-day courses of amoxicillin 500mg q8h, nitrofurantoin 100mg q6h, cephalexin 500mg q6h, or trimethoprim-sulfamethoxazole 160/800mg q12h. The last regimen should be avoided in the third trimester because sulfonamides cross the placenta and compete with bilirubin in the fetus. Trimethoprim is a folate antagonist and should be combined with folinic acid if given in high doses. In view of the high rate of recurrence of bacteriuria in pregnancy, suppressive therapy is recommended until 2 weeks postpartum in women with recurrent UTIs. Pregnant women with acute pyelonephritis require admission for parenteral administration of antibiotics and careful monitoring.

Penicillin is the drug of choice in women with lower respiratory tract infections caused by pneumococci. The fever may cause premature contractions. Because of possible cardiopulmonary complications, ß-mimetic tocolytic drugs should be avoided or, if necessary, administered with caution.

Treatment of gastrointestinal infections is usually not necessary during pregnancy except when the problems persist and interfere with the mother's health. Metronidazole should be prescribed for *Entamoeba histolytica* infection. Pregnant women with acute appendicitis should undergo surgical exploration by the obstetrician together with the surgeon, and antibiotics should be prescribed.

Treatment of postpartum genital infection includes appropriate antibiotics with good anaerobic coverage. After Cesarean section the desired results have been obtained with a combination of clindamycin and gentamicin. Newer antibiotics such as the monobactams may replace gentamicin in combination with clindamycin, and the newer cephalosporins with a wide spectrum of activity are increasingly used. Intravenous antibiotics should be continued for 24–48 hours after the patient has become afebrile, and can be stopped without changing to oral antibiotics unless a staphylococcal infection is present. The treatment of early wound infections may require excision of the necrotic tissue and aggressive antibiotic treatment with a cephalosporin or clindamycin. In late-onset (after 5 days) wound infection incision of the wound and drainage is required. If the patient does not become afebrile after 24 hours, antibiotics should be prescribed. Open wounds can be allowed to close spontaneously by granulation after wound debridement and packing. Surgical closure of Cesarean section has been shown to be successful and requires less healing time. The procedure may be carried out under general or local anesthesia, and antibiotic prophylaxis is generally used. Episiotomy incisions should not be resutured but given time to heal by granulation, unless the sphincter muscle or the rectal mucosa is involved. In rare complications such as necrotizing fasciitis or clostridial gas gangrene, treatment must be aggressive, including high doses of broad-spectrum antibiotics and extensive drainage and debridement.

Therapy of mastitis includes continuation of lactation and treatment with a penicillinase-resistant penicillin or a cephalosporin, given orally except in the case of a severely sick patient. Ice packs, breast support, analgesics and regular emptying of the infected breast may help to prevent abscesses. In case of abscess formation, surgical incision and drainage should be performed.

Treatment of pelvic vein thrombophlebitis includes broad-spectrum antibiotics, heparin for 7–10 days intravenously, followed by long-term anticoagulation with oral anticoagulants. Surgery, including bilateral ovarian vein and inferior vena cava ligation, may be required for patients who do not respond to treatment. Hysterectomy is seldom required.

Antibiotics in pregnancy

Antibiotics are frequently used during pregnancy. Several studies have shown that 25–40% of pregnant women take antibiotics, mainly in the second trimester, while the incidence of antibiotic intake is around 5% in the first trimester. Administration of a drug to a pregnant woman presents a unique problem. The pharmacologic

mechanisms must be well considered, and the fetus must always be kept in mind. Most drugs or chemical substances taken during pregnancy can cross the placenta to some extent throughout pregnancy, but the fetus is at highest risk during the first 3 months of gestation.

Risk factors have been assigned to all level of risk that a drug poses to the fetus (A, B, C, D and X). The definitions used for the risk factors, described by Briggs *et al.*,[29] are summarized below.

¦ Category A: Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote.
¦ Category B: Either animal-reproduction studies have not demonstrated a fetal risk but there are no controlled studies in pregnant women, or animal-reproduction studies have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters).
¦ Category C: Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other) and there are no controlled studies in women, or studies in women and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus.
¦ Category D: There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g. the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

¦ Category X: Studies in animals or human begins have demonstrated fetal abnormalities or there is evidence of fetal risk based on human experience or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant.

TABLE 64-5 -- Risk factor assignments for antibiotics in pregnancy.*

RISK FACTOR ASSIGNMENTS FOR ANTIBIOTICS IN PREGNANCY			
Agent		**Category**	**Potential toxicity**
1	Penicillin	B	
2	Cephalosporin	B_M	
3	Monobactam	B_M	
4.1	Aminoglycoside/group 1	C*	Fetal ototoxicity, maternal oto- and nephrotoxicity
4.2	Aminoglycoside/group 2 (spectinomycin)	B	
5	Chloramphenicol	C	Gray syndrome (cardiovascular collapse in babies) when given near term
6	Tetracycline	D	Adverse effects on fetal teeth and bones, congenital defects, maternal liver toxicity
7.1	Macrolide: erythromycin	B	! Estolate salt form can induce maternal hepatotoxicity
7.2	Other macrolides	$C\text{-}C_M$	
8	Clindamycin	B	
9	Fluoroquinolone	C_M	
10.1	Sulfonamides	B*	No teratogenic effect, to be avoided near term because of potential toxicity to the newborn (kernicterus)
10.2	Trimethoprim sulphamethoxazole	C_M	Megaloblastic anemia, folate activity

** The classification of commonly used antibiotics as set out by Briggs et al.[29]*

Many older drugs have not been given a letter by their manufacturers, and the risk factor assignments were made by Briggs *et al.*[29] In cases where the manufacturer has rated the product in its professional literature, the risk factor is shown with a subscript (e.g. C_M). Risk markers with an asterisk are drugs that present different risks for the fetus. The classification of the most commonly used antibiotics are summarized in Table 64.5 .[29]

CONCLUSIONS

Infectious diseases are important risk factors for maternal and neonatal morbidity and mortality and can be detected early with improved outcomes or prevented entirely. Maternal mortality due to infections is an unbearable tragedy and must be addressed by improved access to modern obstetric care for all pregnant women. This care must be affordable, even in resource-limited countries, and should be a priority for governments and international agencies.

Preventing pre-term birth is a global issue, including industrialized countries. A substantial portion of pre-term births can be prevented with improvements in the detection and management of infections during pregnancy. Further research is required to identify women and babies at risk, and to develop preventive, diagnostic and management strategies to enhance care with maximal benefits at minimal costs and adverse effects.

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Chapter 65 - Implications for the Fetus of Maternal Infections in Pregnancy

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The nature of congenital infections is changing rapidly with new opportunities to link pathogens to untoward events through advances in molecular technology (e.g. polymerase chain reaction (PCR)) and wider availability of intrauterine diagnostic testing (e.g. maternal serum a-fetoprotein screening, ultrasound, amniocentesis, fetal blood sampling). Congenital infection in pregnancy may come to clinical attention through:

- a history of maternal risk factors,
- known exposure,
- documented acute maternal infection,
- laboratory screening,
- detection of fetal abnormalities on clinical examination or ultrasonography,
- suggestive findings in the neonate, and
- suggestive findings in the child.

Depending on the scenario, one or a range of infections must be considered in the differential diagnosis.

Fortunately, the vast majority of maternal infections have no effect on the fetus, either because there is no transmission to the intrauterine site or because the fetal infection is asymptomatic. Fear and poor understanding of risk on the part of the parents or physician can lead to unnecessary termination of pregnancy.

EPIDEMIOLOGY

Although many micro-organisms are known to cause congenital (intrauterine) infection (Table 65.1),[1] only more common agents are discussed (see below). A brief summary of infections transmitted primarily at the time of delivery is given at the end of the chapter.

Because the use of appropriate diagnostic testing is highly variable and because many of these diseases are not reportable to public health departments, there are few data on incidence. Actual rates are highly variable and depend on the presence of specific risk factors; estimates are provided in Table 65.2 .

Geography

There is considerable geographic variation in risk of exposure. Although some variation is related directly to the presence of the pathogen (e.g. *Plasmodium* spp., *Trypanosoma cruzi*), in others it may be related to other practices (e.g. breast-feeding). Maternal toxoplasmosis exposure reflects culinary practices, including handling and ingestion of fresh raw meat.[3]

Other factors associated with infection

Annual seroconversion rates to cytomegalovirus (CMV) for health care workers, usually in the range of 2–4%, are generally lower than rates in day care workers or susceptible parents of children in day care (12–45%). [4] Syphilis in pregnancy generally affects women who are young, unmarried, of low socio-economic status and who receive inadequate prenatal care. Factors associated with maternal—fetal infection are summarized in Table 65.3 .

PATHOGENESIS AND PATHOLOGY

Infections occurring in the neonate may be acquired in the following ways:

- transplacentally in utero (congenital or intrauterine) by direct blood flow to the amniotic fluid or from the genital tract via the cervical amniotic route, during pregnancy or just before delivery. The placenta can be infected and even act as a repository for pathogen growth;
- at the time of birth (perinatal) through vaginal secretions and blood; and
- after birth but during the neonatal period (postnatal), from the mother, her breast milk or other sources.

Adverse effects include abortion, stillbirth, premature delivery, physical defects, intrauterine growth restriction (e.g. rubella, enterovirus, herpes simplex virus (HSV)) and postnatal persistence of infection (e.g. rubella, CMV, HSV). Association of these findings, particularly early gestational loss, with particular pathogens has been hampered by the lack of availability of molecular diagnostic testing of macerated and hydrolyzed fetal tissues.

General associations of the following sites of infection, with adverse effects, exist:

- embryo: malformations, spontaneous abortion;
- fetus: stillbirth, neurologic sequelae; and
- placenta: preterm birth, stillbirth and neonatal death.

Factors affecting fetal disease

Pathogen

The greater virulence of some microbes in the fetus and infant is probably caused by the hematogenous route of exposure and inoculum size, as well as by the status of the immune response derived from the infant and passively derived maternal antibody and postnatally, immunocompetent cells in colostrum and breast milk.[5] Primary mechanisms of damage include cell death, abnormalities of cell growth (mitotic inhibition), direct cytoxic effect (chromosomal injury, cell necrosis) and secondary inflammatory responses.

Certain organisms have a propensity for certain stages of organogenesis. Rubella virus inhibits cell growth and thus causes structural damage. Enterovirus, CMV, toxoplasma and HSV may also be associated with intrauterine growth restriction. The inflammatory response to HSV infection leads to intrauterine infection rather than any particular developmental anomalies. There is a receptor for parvovirus B19 on the red blood cell (p antigen), with the result that at the time of the rapid increase in erythrocyte numbers during the second trimester, anemia and hydrops occur. Postnatal persistence of infection and some further damage can occur with rubella, CMV, HSV and varicella-zoster virus (VZV) infections.

Maternal immunity

Before conception

Usually maternal antibody in the immunocompetent host is protective to the fetus. Exceptions include viruses with known latency,

TABLE 65-1 -- Infectious agents known to cause congenital infection.*

INFECTIOUS AGENTS KNOWN TO CAUSE CONGENITAL INFECTION

Viruses	Herpesviruses: cytomegalovirus, herpes simplex virus, varicella-zoster virus
	Parvovirus B19
	Rubella virus
	Measles virus
	Enteroviruses: Coxsackie B virus, echovirus, poliovirus
	HIV-1 and HIV-2
	Lymphocytic choriomeningitis virus
	Hepatitis B virus
	Vaccinia
	Smallpox
	Adenovirus
	Western equine encephalomyelitis virus
	Venezuelan equine encephalomyelitis virus
	Human herpesvirus 6
	West Nile virus
Bacteria	*Treponema pallidum*
	Mycobacterium tuberculosis
	Listeria monocytogenes
	Campylobacter fetus
	Salmonella typhi
	Borrelia burgdorferi
	Brucella sp.
	Coxiella burnetti
Protozoa	*Toxoplasma gondii*
	Plasmodium spp.
	Trypanosoma cruzi
Fungus	Coccidioidomycosis

** Adapted with permission from Guerina.[1]*

TABLE 65-2 -- General rates of selected congenital and perinatal infections.

GENERAL RATES OF SELECTED CONGENITAL AND PERINATAL INFECTIONS	
Infection	**Rate/live births**
Group B streptococcus	1–5/1000
Cytomegalovirus	2–24/1000 (10–20% have disease)[4]
HSV (intrauterine)	1–2/200,000
HSV (perinatal)	1/2000–5000 USA; 1/33,000 UK[2]
Toxoplasma	0.1–3.5/1000
Syphilis	0.05–6.1/1000 (varies with definition)
Rubella	0.02/1000

such as CMV and occasionally HSV, and rarely rubella, for which maternal immunity has waned, and untreated *Treponema pallidum*.

The rate of intrauterine transmission is about 40% after primary maternal CMV infection, with approximately 15% of affected infants ultimately developing clinically significant disease during childhood. Of the 10% of infants symptomatic at birth, 90% of survivors will have significant neurologic sequelae, including hearing

TABLE 65-3 -- Factors associated with maternal-fetal infection.

FACTORS ASSOCIATED WITH MATERNAL-FETAL INFECTION	
Association	**Pathogen**
Seasonality (in North America)	Parvovirus B19 (winter, spring)
	Rubella (winter, spring)
	Enterovirus (summer, autumn)
Handling/ingestion of uncooked, previously unfrozen meat	*Toxoplasma gondii*
Children:	
Day care	CMV, parvovirus
School	Parvovirus
Household	CMV, parvovirus
Exposure in travel to certain geographic regions	*Toxoplasma gondii*, *Mycobacterium tuberculosis*, *Plasmodium* spp., *Trypanosoma cruzi*, *Borrelia burgdorferi*, hepatitis B virus, *Brucella* sp.
	West Nile virus
Kitten/cat feces within 21 days of primary infection (handling animals, kitty litter, gardening)	*Toxoplasma gondii*
Number of sexual partners, sex industry worker/partner, illicit drug use	*Treponema pallidum*, herpes simplex virus, hepatitis B virus
Sexually active adolescents	CMV, herpes simplex virus, hepatitis B virus
Unimmunized (e.g. immigrant from developing world; World Health Organization Expanded Program of Immunization does not include rubella)	Rubella
Other incompletely immunized	All of measles, mumps, tetanus, diphtheria, poliomyelitis, hepatitis B, varicella

loss in 30–65%. Approximately 1% of previously seropositive women also transmit CMV in utero but there are no laboratory markers to identify those at greatest risk.

Symptomatic congenital CMV infection with long-term neurologic complications can thus occur after a nonprimary or recurrent maternal infection, probably due to reinfection with a different strain of CMV.[6]

Immunosuppressive disorders and immunosuppressive therapy will also alter the risk of fetal disease (e.g. HIV-1 infection facilitates the transmission of toxoplasma).

During pregnancy and before delivery

The length of time before conception during which infection may occur without causing later fetal damage is not known. In the immunocompetent mother, it is advisable to have an interval of 7–9 months between *Toxoplasma gondii* acquisition and conception. Over 50% of infants born to mothers with primary or secondary syphilis will have congenital infection, decreasing to 40% with early latent syphilis and 10% with late latent infection.[7] Although the risk of intrauterine syphilis increases during gestation and is particularly high in late pregnancy, it can occur at any time during pregnancy.

In some infections the mother may develop immunity to an infection, but the fetus is delivered before transplacental transmission of this protection. Essentially the infant experiences a massive viremia, but with cutting of the umbilical cord is left without protection. For example, the risk of acquiring maternal varicella in the 5 days

before delivery is associated with a 30% risk of congenital infection in the neonate.

Placenta

Placental infection is more common than fetal infection for a variety of infections, including rubella, malaria and tuberculosis. This may be because of specific placental immunologic defense, antimicrobial properties (e.g. interferon production), placental phagocytic capacity or a non-specific physical barrier function. In early gestation, the small developing placenta effectively excludes most pathogens. As the placenta matures, with expansion of the maternal-fetal interface, this barrier becomes more porous, rendering transplacental transmission more likely. Placental dysfunction may also be associated with hypoxia, fever, toxins, thrombosis and placentitis, and result in fetal injury or death indirectly.[5]

Stage of fetal gestation

Fetal disease may result from many pathogens at any time during gestation. In general, however, transplacental transmission (via umbilical blood flow or direct spread to the amniotic fluid) is less likely early in gestation, but the results of infection, if it occurs, are more likely to be severe. The deficiencies in immune function of the young fetus both in humoral and in cellular function contribute to tissue damage, organ dysfunction and teratogenicity. Detectable IgM is rarely produced before 20–24 weeks of gestation. Certain pathogens are associated with producing particular effects at certain stages of cell development. For example, maternal rubella infection in pregnancy after 16 weeks is not associated with defects. [8]

Congenital varicella syndrome occurs almost exclusively before 20 weeks of gestation; in seven studies of varicella infection in pregnancy, 14 out of 1063 babies had congenital anomalies (1.3%); a higher risk (2–9%) appears to occur between 13 and 20 weeks.[9] After 20 weeks of gestation, early childhood zoster is almost exclusively the presenting finding, occurring after maternal infections between 17 and 24 weeks in 0.8% of cases and after infection

TABLE 65-4 -- Summary of preventive antenatal strategies.

SUMMARY OF PREVENTIVE ANTENATAL STRATEGIES				
Pathogen	**Education/immunization**	**Routine screen**	**Selective screen**	**Maternal-fetal intervention**
CMV	Yes		Day care workers (pre-pregnancy only)	Handwashing after handling respiratory secretions and urine
HIV/AIDS	Yes	Yes	If high risk, third trimester delivery	Antiretroviral therapy, serologic follow-up
Parvovirus B19		Exposure, epidemic		Intrauterine transfusion(s)
Rubella	Yes	Yes	Exposure	If susceptible, postpartum immunization before discharge
VZV	Yes	Recommended by some experts	Exposure	If susceptible, some experts recommend varicella-zoster immune globulin =96 hours after exposure
Treponema pallidum	Yes	Yes	If high risk, third trimester delivery	Penicillin, HIV testing, monthly serologic follow-up
Toxoplasma gondii	Yes	Only if recommended by regional authority	If ingestion of raw, previously unfrozen meat; handling of kitten or litter	Spiramycin or pyrimethamine/sulfadiazine/folinic acid with monitoring

between 25 and 36 weeks in 1.7%.[10] While transmission of toxoplasmosis occurs in only about 15% of first trimester infections, severe disease occurs in about 40% of infected infants. In the third trimester the reverse is true, with 60% transmission, but the disease is generally milder or asymptomatic.

PREVENTION

Prevention of maternal infection in pregnancy requires a combination of approaches: education, immunization, screening and intervention (Table 65.4).

Prenatal prevention through immunization and education

Certain preventive efforts should antecede pregnancy, including documentation of rubella immunity and preventive education regarding optimal food handling practices (i.e. toxoplasma, enteric bacterial pathogens, *Listeria* spp.). All women should also be immune to measles, mumps, tetanus, diphtheria, poliomyelitis, hepatitis B and varicella, either by natural infection or by vaccination. Influenza and pneumococcal immunization are recommended for women who are at high risk of infection. Immune globulin or specific immune globulin may be indicated on exposure to measles, hepatitis A or B virus, tetanus, varicella or rabies and, in the case of certain travel, to poliomyelitis, yellow fever, typhoid and hepatitis B virus.[11] Although inactivated vaccines are generally considered safe, some physicians wait until after the first trimester to administer them. Live viral or bacterial vaccines should be avoided during pregnancy. However, the risk of congenital rubella syndrome after administration of vaccine to a pregnant woman is very low.

The efficacy of education regarding toxoplasmosis has been demonstrated. All women who are planning pregnancy should be given general information.

- ¦ Ensure complete adult immunization, including hepatitis B and ideally varicella.
- ¦ Ensure that you are immune to rubella by blood test. If you are not, you require immunization.
- ¦ Follow simple procedures to reduce the risk of infection with *Toxoplasma* spp. (Table 65.5).[1]
- ¦ Minimize the risk of other food-borne infections (Table 65.6).
- ¦ If you are exposed to erythema infectiosum (fifth disease, human parvovirus B19), chickenpox or shingles, or whooping cough (pertussis) during pregnancy, inform your physician promptly.
- ¦ If your partner is diagnosed with an infection other than a cold or flu, inform your physician.
- ¦ There is currently no effective strategy to prevent the complications of CMV or enterovirus during pregnancy. Although women who regularly handle the respiratory secretions or diapers of young children may wish to be tested for CMV immunity before pregnancy, the only current preventive strategy for susceptible women is good hygiene when they are with young children in their home or in the group child care environment.
- ¦ Symptoms of genital herpes may occur years after the original infection; it is usually the first infection, which may occur without any symptoms (asymptomatic primary), that affects the infant; such infections are rare.
- ¦ In pregnancy, your doctor routinely tests for rubella immunity, hepatitis B virus, HIV/AIDS and syphilis infection.

TABLE 65-5 -- Measures to reduce the risk of infection with *Toxoplasma* spp.

MEASURES TO REDUCE THE RISK OF INFECTION WITH *TOXOPLASMA* SPP.	
Source of infection	**Preventive measure**
Meat	Avoid eating undercooked meat in pregnancy; previously frozen meat is free of toxoplasma
	Wash hands thoroughly after handling; keep cooking utensils thoroughly cleaned
Cats	Avoid contact with materials potentially contaminated with cat excrement
	Avoid kitty litter boxes; infectious toxoplasma may be present if in use more than 24 hours
	Disinfect with boiling water for 5 minutes; avoid aerosolization
	(Even indoor cats may be in contact with mice and fresh, raw meats)
Vegetables	Wash raw fruit and vegetables thoroughly before eating; wash hands after handling
Gardening	Wear gloves

TABLE 65-6 -- Measures to reduce the risk of food-borne infections other than *Toxoplasma*.

MEASURES TO REDUCE THE RISK OF FOOD-BORNE INFECTIONS OTHER THAN *TOXOPLASMA*
• Wash hands thoroughly before eating and before and after food preparation
• Use separate or thoroughly cleaned surfaces for foods to be served raw and cooked
• Avoid unpasteurized milk or cheese
• Avoid soft cheese (e.g. Brie, Camembert)
• Reheat leftovers and pre-cooked foods until piping hot
• Wash and scrub raw vegetables before eating
• Use foods before the expiry date

Screening for infection during pregnancy

Rubella screening in pregnancy alerts the physician to which mothers require postpartum immunization. Also, if the susceptible woman has known exposure or disease, the physician can confirm acute infection through study of a second serum sample. Antenatal screening for syphilis is cost-effective at an incidence as low as 5 in 100,000 population.[12] Hepatitis B virus screening allows for preventive management of the infant of the hepatitis B surface antigen (HBsAg)-positive mother.

The interpretation of other serology in pregnancy in the absence of seroconversion is fraught with difficulty as the acuity of the infection often cannot be determined. A woman at high risk of CMV infection (e.g. a day care worker) would therefore be advised to establish her immune status before pregnancy. The general value of antenatal screening for CMV is debatable.[13]

Screening for toxoplasma requires testing each trimester to document seroconversion and to allow for prompt treatment before transplacental transmission has occurred. *Toxoplasma*-specific IgM antibody persists for more than a year in about one-third of infections. Screening may be worthwhile if the incidence of primary maternal infection exceeds 1.1 per 1000. Practitioner-based testing for acute toxoplasmosis in the absence of a community-based program should be discouraged in countries where screening and treatment are not routine.

Clinical and ultrasound findings

Clinical and ultrasound findings may provide an additional opportunity to make diagnoses in the fetus.

Maternal illness

Rash in pregnancy requires exclusion of syphilis, rubella, parvovirus B19 and enterovirus infection. Arthritis occurs with parvovirus B19 infection as well as with rubella. In the presence of acute mononucleosis-like symptoms of fatigue and lymphadenopathy, Epstein-Barr virus, CMV, *Toxoplasma* and HIV infection must be considered.

Fetal ultrasonography

Ultrasound findings of in-utero infection are provided in Clinical Features, below. For toxoplasmosis identified ultrasonographically, maternal antimicrobial therapy with spiramycin before 18 weeks of gestation or pyrimethamine and sulfadiazine thereafter may reduce the severity of disease. In nonresolving fetal hydrops caused by parvovirus B19 infection, intrauterine transfusion may be used to treat severe fetal anemia.

CLINICAL FEATURES

Fetal

The features of in-utero infections are summarized in Table 65.7 . Some fetal abnormalities detected on antenatal ultrasonography may be caused by infection. However, no ultrasonographic findings are pathognomonic for a particular agent.[14] [15] Postnatal follow-up of infants, including ophthalmologic examination, cranial neuroimaging (i.e. computerized tomography (CT) and magnetic resonance imaging (MRI) and head growth and developmental progress over at least the first 2 years of life, is important in identifying affected infants, particularly with CMV and *Toxoplasma* infections. Ophthalmologic examination and cranial neuroimaging studies are abnormal in 40–50% of apparently normal but *Toxoplasma*-infected infants at birth.[16]

Prematurity and low birth weight

Routine investigation of these infants for congenital infection only rarely yields positive results. Prematurity is typical of congenital

TABLE 65-7 -- Clinical features of in-utero infection.

CLINICAL FEATURES OF IN-UTERO INFECTION	
General	Intrauterine growth retardation: all etiologies
	Hydrops fetalis: parvovirus B19, CMV, syphilis, *Toxoplasma*, HSV, Coxsackie B3 virus[14]
	Placentamegaly: CMV, syphilis

Head and neck	Hydrocephalus: CMV, *Toxoplasma*, enterovirus, varicella
	Microcephalus: CMV, *Toxoplasma*, rubella, varicella, HSV
	Intracranial calcifications: CMV, *Toxoplasma*, HSV, rubella, HIV, parovirus, West Nile virus, lymphocytic choriomeningitis virus
Heart	Congestive heart failure: parvovirus B19, syphilis, CMV, *Toxoplasma*
	Pericardial effusion: parvovirus B19, syphilis, CMV, *Toxoplasma*
	Cardiac defects: rubella, parvovirus B19, mumps (not proved)
	Myocarditis: enterovirus
	Calcification of the pericardium and lungs: varicella
Lungs	Pleural effusion: parvovirus B19, syphilis, CMV, *Toxoplasma*
	Pulmonary hypoplasia: CMV
Abdomen	Hepatosplenomegaly: CMV, rubella, *Toxoplasma*, HSV, syphilis, enterovirus, parvovirus B19
	Hypoechogenic bowel: CMV, *Toxoplasma* [15]
	Hepatic calcifications: CMV, *Toxoplasma*
	Meconium peritonitis: CMV, *Toxoplasma*
	Ascites: parvovirus B19, CMV, *Toxoplasma*, syphilis
Extremities	Limb reduction, restriction: VZV

syphilis and common in perinatal HSV infection. Intrauterine growth retardation occurs with rubella, CMV infections, toxoplasmosis and, occasionally, enteroviral infection.

Spontaneous abortion and stillbirth

Pregnancy loss has been associated with infection with CMV, enterovirus, HSV, HIV, parvovirus B19, rubella, *T. pallidum* and *T. gondii*. Fetal loss occurring with any maternal viral infection requires comprehensive pathologic and microbiologic evaluation to determine the role of the infection in pathogenesis.

Syndromes of congenital infection

General

The majority of infected infants have no symptoms at birth although some will develop sequelae later in childhood (Table 65.8). The clinical findings in congenital infection are summarized in Table 65.9 .

Sepsis-like illness

Herpes simplex virus acquired just before delivery or intrapartum may present as a perinatal syndrome, often without skin lesions, and resemble neonatal sepsis or pneumonitis. An early laboratory clue is abnormal liver enzymes. Shock, coagulopathy, fulminant hepatitis and, often, skin lesions follow. Congenital tuberculosis can also present in this way.

Hepatitis

Enterovirus, HSV and *Toxoplasma* can cause overwhelming acute neonatal liver failure in the first week of life. Other infections, including CMV[20] and parvovirus B19, [21] can also present with hepatic findings.

Central nervous system

Central nervous system (CNS) involvement may occur with all of the congenital infections (as well as with perinatally acquired HSV and enteroviral infections), although initial findings may be very subtle. Occult findings in congenital neurosyphilis have led to general recommendations that neurosyphilis be assumed with any cerebrospinal fluid (CSF) abnormality.[22] With newer molecular techniques, CNS involvement may be better recognized (i.e. PCR detection of CMV DNA in the CSF). Clinical findings may be preceded by ophthalmologic and neuroimaging findings. Central nervous system involvement in congenital toxoplasmosis may be manifest only on neuroimaging at birth.[14] As opposed to the poorer prognosis of cranial calcifications in CMV infection, the cranial calcifications of toxoplasmosis may disappear during therapy in infancy or persist, with normal cognitive development.

Cardiac

Rubella infection causes structural defects including patent ductus arteriosus; parvovirus B19 may cause intrauterine congestive heart failure with resulting prenatal closure of the foramen ovale and Epstein's anomaly, and occasionally acute and chronic lymphocytic myocarditis.[23] Viral myocarditis is characteristically caused by Coxsackie B virus or other enteroviruses.

Ophthalmologic

Ophthalmologic abnormalities are seen in a variety of congenital infections (see Table 65.9). [5]

Deafness

Deafness is a common sequela of CMV and rubella infection, as well as of untreated *Toxoplasma* and syphilis infections. One-third of sensorineural hearing loss is caused by congenital CMV infection. Of rubella-infected infants, 80% or more may have deafness, often as the only significant consequence. Given the progressive nature of impairment, serial hearing evaluations to age 6–9 years are recommended for CMV-infected infants.

Specific infections

While characteristic presentations by etiologic agents are described below, it should be recognized that isolated and apparently uncharacteristic findings do occur (e.g. unilateral chorioretinitis following intrauterine HSV or esophagitis with CMV).

Cytomegalovirus

Up to 10% of CMV-infected infants will have typical findings of petechiae, hepatosplenomegaly, jaundice, microcephaly, inguinal hernia in males, chorioretinitis or other, atypical findings. Symptomatic infants are at high risk of significant neurologic and developmental dysfunction, particularly if abnormalities are noted on cranial CT or chorioretinitis exists.[24] Cerebral calcifications tend to be periventricular. Most children with an abnormal newborn CT scan (90%) develop at least one neurologic sequela as compared with 29% of those with a normal study, making a cranial CT scan a good predictor of an adverse neurodevelopmental outcome. Normal development at 12 months of age makes subsequent neurodevelopmental or intellectual impairment unlikely. Infants with asymptomatic infection have a 5–15% risk of hearing loss, mental retardation, motor spasticity and microcephaly evolving in the early years. While rates of hearing loss in symptomatic and asymptomatically infected infants are approximately 40% and 7%, respectively, in both the loss may be late in onset, fluctuating and progressive, necessitating follow-up (see Deafness above).

Enteroviruses (Coxsackie viruses, echovirus)

Although high rates of maternal infection in pregnancy (up to 25%) have been reported, disease is limited to case reports or series of

TABLE 65-8 -- Ratio of neonatal infection to neonatal and postnatal symptoms.

RATIO OF NEONATAL INFECTION TO NEONATAL AND POSTNATAL SYMPTOMS			
Micro-organism	**Infected fetuses/neonates with clinical manifestations (%)**	**Additional percentage with late-onset manifestations**	**Late-onset manifestations**
CMV	10%	5–15%	At =2 years sensorineural hearing loss, microcephaly, motor defects, mental retardation, chorioretinitis, dental defects
			>2 years hearing loss
Rubella	First 16 weeks, decreasing from 90% to 24%	>20%	Hearing loss, visual defects, multiple endocrinopathies, panencephalitis
Treponema pallidum	30%[17] (30–40% stillborn)	Unknown	Hearing loss, visual defects; abnormal dentition, bones and joints, CNS
Toxoplasma gondii	Severe organ damage in 20% of infants of untreated mothers and in 2% in treated mothers	Subclinical infection in 41% of infants of untreated mothers and in 17% in treated mothers	>85% have chorioretinitis

infected infants with a variety of entities including growth retardation, CNS malformations, blueberry muffin rash, hepatic necrosis, myocarditis or pericarditis.

Herpes simplex virus

Intrauterine HSV infection is characterized by the triad of skin vesicles or scarring, eye lesions and microcephaly or hydranencephaly. It may follow primary or recurrent, symptomatic or asymptomatic, HSV-1 or HSV-2 maternal infection at any stage of gestation. Acquisition just before delivery can lead to disease identical to that acquired at delivery, except that it occurs within the first 48 hours of life. The presence of even a single vesicle should prompt ophthalmologic examination and cranial imaging.

Parvovirus B19

The spectrum of infection in the fetus and neonate continues to expand and includes spontaneous abortion, nonimmune hydrops fetalis, stillbirth, congenital liver damage (portal fibrosis), transfusion-dependent congenital anemia, neutropenia and thrombocytopenia, prenatal closure of the foramen ovale and a syndrome of anemia, blueberry muffin rash and hepatomegaly.

The vast majority of maternal infections, both symptomatic and asymptomatic, are followed by delivery of a healthy term infant, perhaps in most cases because the fetus is not infected. Whereas the exact rate of loss in early and late pregnancy remains to be determined, there appears to be an excess loss in the second trimester. Studies of pregnant women with confirmed parvovirus infection have found a low rate of hydrops of 0–1.6%.

Rubella

The clinical features of congenital rubella syndrome (CRS) can be divided into the categories of transient, in newborns and infants; permanent, at birth or during the first year of life; and delayed, occurring in 10–20% of patients, usually in the second decade of life. In rare cases, reinfection with maternal rubella has resulted in CRS.

Varicella-zoster virus

The congenital varicella syndrome (or fetal varicella-zoster syndrome, better reflecting the pathogenesis), including cicatricial skin scars, eye abnormalities including microphthalmia, hypoplastic limbs, and autonomic nervous system damage causing gastroesophageal reflux with or without CNS abnormalities, occurs almost exclusively with maternal varicella infection acquired before 20 weeks of gestation. Cases have been reported at 26–28 weeks of gestation. Generally, after 20 weeks manifestations include skin scars, as with postnatal VZV infection, and childhood shingles. Only rarely has this syndrome been reported after gestational zoster. Subclinical maternal VZV infection is now recognized as a cause of neurologic symptoms and signs in children without other manifestations of congenital varicella syndrome, including dermatologic findings, suggesting the damage done by intrauterine varicella is underestimated. If the CNS is relatively spared, a good long-term outcome can occur.

Treponema pallidum

Most commonly physicians are required to investigate the asymptomatic infant whose mother had positive serologic testing for syphilis. Of the diverse findings, bone lesions are the most frequently encountered abnormality, occurring in 20% of symptom-free infected infants. Cerebrospinal fluid examination is required and some experts believe that minimal abnormalities in the CSF should be considered to indicate neurosyphilis. These include leukocyte counts of 5/mm^3 or more and protein concentrations of 100mg/dl or greater.[25]

Toxoplasma gondii

The diversity of findings may be classified according to timing of symptomatology as:

- symptomatic neonatal disease;
- disease in the first months of life, usually with neurologic and ophthalmologic findings;
- sequela of previously undiagnosed infection (i.e. chorioretinitis later in childhood); and
- subclinical disease.

Of asymptomatic infected infants, half will have abnormalities on cranial imaging or ophthalmologic examination. Among 23,000 mothers and infants in the Collaborative Perinatal Project, infants of IgG *Toxoplasma* antibody-positive mothers had a 2-fold increase in hearing loss, a 60% increase in the incidence of microcephaly and a 30% increase in the occurrence of low intelligence quotient (<70). [26]

Other

Intrauterine adenovirus infection has been associated with fetal myocarditis, pneumonia and encephalitis. Measles infection in pregnancy increases the risk of prematurity in the first 2 weeks after rash.

TABLE 65-9 -- Clinical findings in congenital infection.[5] [19]

CLINICAL FINDINGS IN CONGENITAL INFECTION	
Prematurity	Syphilis, HSV
Intrauterine growth retardation	All etiologies including tuberculosis
Anemia with hydrops	Parvovirus B19, syphilis, CMV, *Toxoplasma*
Bone lesions	Syphilis, rubella
Cerebral calcification	*Toxoplasma* (widely distributed)
	CMV and HSV (usually periventricular)
	Parvovirus B19, rubella, HIV, WNV, LCM
Congenital heart disease	Rubella, parvovirus B19, mumps (not proved)
Hepatosplenomegaly	CMV, rubella, *Toxoplasma*, HSV, syphilis, enterovirus, parvovirus B19
Hydrocephalus	*Toxoplasma*, CMV, syphilis, possibly enterovirus
Hydrops, ascites, pleural effusions	Parvovirus B19, CMV, *Toxoplasma*, syphilis
Jaundice	CMV, *Toxoplasma*, rubella, HSV, syphilis, enterovirus
Limb paralysis with atrophy and cicatrices	Varicella
Maculopapular exanthem	Syphilis, measles, rubella, enterovirus
Microcephaly	CMV, *Toxoplasma*, rubella, varicella, HSV
Ocular findings (see below)	CMV, *Toxoplasma*, rubella, HSV, syphilis, enterovirus, parvovirus B19
Progressive hepatic failure and clotting abnormalities	Echovirus, Coxsackie B virus, enterovirus, HSV, *Toxoplasma*
Pseudoparalysis	Syphilis
Purpura (usually appears on first day)	CMV, *Toxoplasma*, syphilis, rubella, HSV, enterovirus, parvovirus B19[18]
Vesicles	HSV, syphilis, varicella, CMV, parvovirus B19, enterovirus
Cataracts	Rubella, HSV, VZV, parvovirus B19, *Toxoplasma*, syphilis
Chorioretinitis	HSV, VZV, rubella, CMV, *Toxoplasma*,
Optic atrophy	HSV, VZV, rubella, CMV, *Toxoplasma*, syphilis
Microphthalmia	Rubella, HSV, parvovirus B19, *Toxoplasma*, CMV, varicella
Coloboma	CMV
Keratoconjunctivitis	HSV
Pigment retinopathy	Rubella
Glaucoma	Rubella, toxoplasmosis, syphilis
Iritis	HSV, rubella, syphilis
Anophthalmia	CMV
Peter's anomaly	CMV
Horner syndrome	VZV

DIAGNOSIS

Diagnosis of fetal infection

Attribution of adverse outcome to a particular micro-organism in early fetal life is problematic and frequently requires molecular diagnostic testing (e.g. DNA detection by PCR). Detection of micro-organism specific IgM is hampered not only by the testing method but also by the failure of the fetus to reliably produce IgM-specific antibody before 22–24 weeks. Fetal infection may follow maternal infection by at least 4–6 weeks, providing false reassurance if

TABLE 65-10 -- Laboratory evidence of clinically significant maternal infection.

LABORATORY EVIDENCE OF CLINICALLY SIGNIFICANT MATERNAL INFECTION		
Micro-organism	**Detection by culture, PCR (site)**	**Serology**
CMV	Infrequently blood/serum	Seroconversion
		Possibly CMV IgM capture enzyme-linked immunosorbent assay
Enterovirus	Yes (stool, throat, other)	Seroconversion
HSV	Yes (vesicle)	Seroconversion
		Research laboratory required to differentiate between HSV-1 and HSV-2
Parvovirus B19	Yes (blood)	Seroconversion
		Parvovirus B19-specific IgM
Rubella		Seroconversion
		Rubella-specific IgM
VZV	Yes (vesicle)	Seroconversion
Treponema pallidum	Yes (lesion, dark field)	VDRL/rapid plasma reagin =1/8 and positive treponemal test
Toxoplasma gondii		Seroconversion
		Toxoplasma-specific IgM with confirmatory timing of infection in reference laboratory

fetal diagnosis, through direct detection or antibody production, is attempted too soon after maternal infection.

Antenatal diagnosis of fetal infection requires a multidisciplinary approach to exclude noninfectious causes and to obtain maternal-fetal studies. Follow-up of the infant at birth and in the ensuing few months is also required to determine the presence, extent and damage, if any, resulting from the infection.[27]

In the absence of fetal disease, the prenatal diagnosis of fetal infection is not warranted because the predictive values of positive and negative results cannot be used as a basis for management decisions.[28] For example, in contrast to the prenatal diagnosis of genetic diseases for which the outcome is reasonably certain, most infants with congenital CMV infection are asymptomatic and do not suffer sequelae.

Maternal testing

Many maternal infections are asymptomatic and all infections, symptomatic or otherwise, can pose a risk to the fetus. Laboratory tests are listed in Table 65.10 . Routine broad screening of a single serum is unlikely to be helpful and is rarely indicated. The diagnosis of a primary CMV infection can be made by demonstration of seroconversion of CMV-specific IgG antibodies from negative to positive, but not by boosting of a titer, as this may occur with recurrent infection. Differentiation of the serofast state from inadequately treated syphilis is difficult. A serofast patient usually has a titer of =1:4; although titers can be as high as 1:8,[29] caution in interpretation is essential.

Maternal symptoms and their likelihood

Although fetal disease follows both symptomatic and asymptomatic maternal infection and maternal infections are frequently asymptomatic, the following symptoms should suggest a search for micro-organisms: flu or an acute mononucleosis syndrome-like illness (CMV, *Toxoplasma*, HIV) and arthritis or rash (parvovirus B19, rubella, *T. pallidum*).

Fetal testing

Detection of the micro-organism by culture or DNA by PCR amplification and product detection in amniotic fluid or fetal blood is promising, but should generally not be undertaken in the absence of fetal abnormalities because of the uncertain sensitivity and specificity. After week 18 of gestation, and at least 4 weeks after maternal infection, PCR testing of the amniotic fluid will detect 97% of toxoplasmosis-infected fetuses.[30] Fetuses infected with CMV are likely to have positive amniotic fluid cultures after 20–22 weeks of gestation because fetal kidney infection is common. Other sites such as effusions may also be cultured and tested by PCR; there are case reports of positive enteroviral cultures.[31]

Other indirect evidence of infection may be obtained through hematologic and biochemical profiles, including hepatic enzymes and, in the future, through study of lymphocyte subclass populations and cytokine production.

Neonatal testing

In evaluating the neonate with suspected congenital infection at birth, it is necessary to:

- review the maternal history including serologic screening (Table 65.10 and Table 65.11);
- review ultrasonography undertaken in pregnancy (Table 65.7);
- evaluate the infant (Table 65.9 and Table 65.12);
- attempt detection of the pathogen in the neonate; and
- undertake judicious maternal and infant serologic testing pertinent to the most likely diagnoses.

Detection of the micro-organism in the neonate

The best evidence for infection with CMV, enterovirus, HSV, parvovirus B19, rubella, syphilis and *Toxoplasma* comes from detection of the agent in the neonate (see below).

Maternal and infant testing pertinent to the most likely diagnoses

Diagnostic tests are summarized in Table 65.13 . Over- and under-diagnosis of congenital infection has arisen as a result of failure to:

- submit maternal serology for documentation of a source of infection;
- detect the organism in the infant through culture (or newer methods of antigen detection);
- sequentially test the infant serologically; and
- appreciate the enormous rate of false-positive and false-negative test results obtained through IgM-specific testing and use of cord blood.

False-negative IgM tests in infants are very common (e.g. only 25% of infants with clinical manifestations of intrauterine infection had VZ IgM).[10] An exception is the rubella-specific IgM test, which is highly sensitive and specific. Parvovirus-specific IgM is frequently negative when virus is detected by PCR.

Serologic documentation of maternal infection and acute and follow-up serology of the infant over the first months or year of life can be diagnostic, albeit not sufficiently quickly to facilitate decisions about therapy. Passively transferred antibody will disappear in the first 6–12 months, whereas antibody persists or rises in the infected infant. Follow-up serology is more complicated in the case of congenital CMV as infection may also be acquired at birth or postnatally through breast milk or other contact.

Cord blood is not acceptable for specific antibody testing for syphilis, *Toxoplasma* or CMV because of false-positive and false-negative results. Total cord IgM levels may be falsely elevated through contamination by maternal blood. Cases of congenital syphilis have been reported in which both mothers' and neonates' titers were negative at birth but the infants subsequently developed clinical syphilis at 3–14 weeks of age with strongly positive titers.[32]

TABLE 65-11 -- Maternal history relevant to congenital infections.

MATERNAL HISTORY RELEVANT TO CONGENITAL INFECTIONS
Woman's history
Underlying illness and medications
Previous history of sexually transmitted disease (HSV, syphilis, chlamydia, gonorrhea, HIV)
Drug or alcohol use, current and previous
Travel during pregnancy (consider culinary practices and other factors in region traveled)
Occupation
• Working with children wearing diapers or who have disabilities (CMV)
• Working with elementary school children (parvovirus, rubella)
• Working with animals or raw meat products (*Toxoplasma*)
• Working in the sex trade industry (HIV, syphilis, tuberculosis, hepatitis B virus, hepatitis C virus).
Household exposure to young children (CMV)
During her pregnancy
• Has she eaten raw meat or tasted it while cooking? (toxoplasmosis)
• Has she consumed unwashed vegetables? (food-borne pathogens, *Toxoplasma*)
• Has she changed kitty litter without wearing gloves? (*Toxoplasma*)
• Has she worked in soil/garden without wearing gloves? (*Toxoplasma*)
• Has she had any illness she might not have mentioned until now?

• Cold sore? (HSV)
• Dysuria, burning, itching? (HSV)
• Profound fatigue? (CMV, *Toxoplasma*, HIV)
• Swollen glands? (CMV, *Toxoplasma*, HIV)
• Arthritis? (rubella, parvovirus B19)
• Rash? (rubella, parvovirus B19, syphilis, enterovirus)
Has her husband/partner had any particular illness?
• Skin sores anywhere? (HSV, syphilis, HIV)
Routine serologic testing
HBsAg, rubella-specific antibody, syphilis serology (VDRL, rapid plasma reagin)
• First trimester
• If high risk, repeat third trimester, delivery
Antenatal care
• If absent, see Table 65.18

The futility of a single serologic (syphilis, toxoplasmosis, other agents, rubella, CMV, herpesvirus (STORCH)) screen is well known and a more directed approach is required. In preference to a single serum (STORCH titer), every effort should be made to recover the organism from the neonate and follow-up maternal and infant blood (Table 65.14).

Definitions of selected congenital infections

Because of delayed onset and/or recognition of signs of infection as well as the varied availability of diagnostic tests, definitions have been developed that take into consideration the likelihood of infection (Table 65.15).

MANAGEMENT

General

A thorough understanding of the consequences of infection in pregnancy is required to counsel parents regarding risks to the fetus and possible courses of action.

Selected maternal infections should be followed by ultrasonography to identify adverse effects. Antenatal diagnosis is generally attempted only if fetal abnormalities are present. After delivery, additional information

TABLE 65-12 -- Evaluation of the neonate with suspected congenital infection.

EVALUATION OF NEONATE WITH SUSPECTED CONGENITAL INFECTION	
Clinical	Physical examination (gestational age, height, weight, head circumference) to identify prematurity, intrauterine growth retardation, microcephaly
	Measure liver/spleen size
	Ophthalmologic examination (pediatric expert)
Laboratory	Complete blood count and smear
	Platelet count
	Liver transminase levels
	Bilirubin level, direct and indirect
	CSF examination (cells, protein, with pertinent antibody, detection; see Table 65.13)
	Laboratory testing (detection of agent, maternal and infant (not cord)) serology
	Immunoglobulin determinations
	Hold pretransfusion blood for possible additional tests
Other	Cranial CT scan with enhancement
	Long-bone radiographs (if syphilis or rubella likely)
	Placental pathology
	Audiology assessment
	Multidisciplinary follow-up

about contagiousness, breast-feeding and risk of transmission in subsequent pregnancies should be conveyed. Comprehensive long-term pediatric follow-up is required to identify and manage the spectrum of cognitive, motor, visual, hearing and other impairments that may be imparted by some of these infections.

Specific infections

Cytomegalovirus

Neurologic outcome cannot be reliably predicted until the infant is 2 or 3 years of age, during which head growth is monitored and achievement of developmental milestones is documented. Infants with abnormal cranial imaging are more likely to have cognitive and other deficits on follow-up.[24] Progression of existing retinal lesions or delayed development of chorioretinitis during childhood has been reported in about 20% of children, whereas hearing loss will evolve in about 30% of children, as late as school age. Children with asymptomatic congenital CMV infection show no differences in IQ score or other neuropsychologic test performance as compared with uninfected children.[33] [34]

The highly variable, unpredictable course of congenital CMV infection makes randomized controlled trials of therapy such as ganciclovir imperative before their widespread use. Infants with CNS disease at birth have already sustained enormous damage. Theoretically, therapy might be of some use because infection is active at birth and damage appears to continue for up to 2.5 years.[35] In a phase 3 randomly controlled trial, ganciclovir therapy (6mg/kg/dose twice daily for 6 weeks) protected infants from hearing deterioration beyond 1 year. Transient effects on growth and resolution of transaminase elevation were also seen. Approximately two-thirds developed neutropenia with half requiring dose reduction. Prevention through vaccination is urgently needed.

Herpes simplex virus

As intrauterine infection is rare, infected women should be assured that maternal infection is common but that fetal infection is unusual.

Parvovirus B19

After maternal exposure and documented infection, serial ultrasonography for 1–14 weeks will identify the hydropic fetus. For the fetus with nonimmune hydrops fetalis and a low hematocrit and reticulocyte count, one or more intravascular fetal transfusions of packed erythrocytes, until marrow aplasia resolves, have been used with good results. Hydropic fetuses with an intermediate hemoglobin and a high reticulocyte count, in whom the process is resolving spontaneously, need not be transfused and show no sequelae. The management of the neonate who has sustained red cell aplasia through a 'hit and run' effect of the virus on the bone marrow may respond to immunoglobulin and corticosteroids. There is no medical indication for termination of the pregnancy complicated by parvovirus B19 infection.[36]

Rubella

Infected infants should be considered infectious for the first year of life unless repeated nasopharyngeal and urine cultures are negative. Long-term follow-up will identify progressive hearing loss and visual deterioration, endocrinopathies and subacute sclerosing panencephalitis.

Treponema pallidum

All infected women should receive penicillin therapy appropriate to their stage of disease to minimize or eliminate the risk of transmission to the fetus. If serology has been positive for less than 1 year, the patient may be treated with intramuscular benzathine penicillin G 2.4 million units intramuscularly as a single dose. If the patient has been seropositive for 1 year or more, then benzathine penicillin G 2.4 million units in a single dose weekly for 3 successive weeks is required. Maternal CSF examination (protein, cell count, CSF-venereal disease research laboratory (VDRL) is required only if neurosyphilis is suspected; it is not routinely required in patients with infectious syphilis if the neurologic examination is negative. Retreatment during pregnancy is unnecessary if titers followed monthly continue to fall. In the presence of penicillin allergy, desensitization is the preferred management; any other therapy will necessitate treatment of the infant after birth. All women with positive serology require HIV testing and testing of their recent sexual contacts, as well as repeat testing in the third trimester and at birth. Cord blood should not be used for rapid plasma reagin/VDRL testing as it may be falsely negative or positive.[37] Long bone radiographs and usually CSF examination are required.

Indications for treatment include:

- ¦ symptomatic disease;
- ¦ inadequate maternal treatment (i.e. not documented, inadequate or unknown), use of a drug other than penicillin and treatment within 4 weeks of delivery (or last trimester, according to some experts) or poorly documented response to treatment;
- ¦ uncertain follow-up; and
- ¦ secondary or latent syphilis in the mother within 1 year of delivery.

Therapy with aqueous crystalline penicillin G 100,000–150,000IU/kg (given every 8–12 hours) intravenously for 10–14 days is recommended. After the second week of life, or with confirmed neurosyphilis or severe disease, aqueous penicillin 200,000–250,000U/kg per day may be used.[22] A preliminary study suggests that selected asymptomatic infants may be treated with intramuscular benzathine penicillin 50,000U/kg once, provided that there are no signs of congenital syphilis on physical examination, CSF cell count is normal and CSF-VDRL is negative, and radiographic studies of the long bones of the lower extremities, platelet counts and liver function tests are normal.[38] Follow-up of all infants is required up to 1 year or beyond to ensure that the appropriate falls in titers have occurred and

TABLE 65-13 -- Laboratory investigation and follow-up of neonate with suspected congenital infection.

LABORATORY INVESTIGATION AND FOLLOW-UP OF NEONATE WITH SUSPECTED CONGENITAL INFECTION			
Infection	**Mother at birth**	**Neonatal (not cord blood)**	**Follow-up of infant**
CMV	Antibody	Virus detection in urine, saliva, blood leukocytes, CSF	Repeated antibody testing to 6–12 months; passive maternal antibody disappears at 4–9 months; negative infant and maternal antibody rules out infection, although intrapartum cervical and postpartum breast milk transmission is common
		IgM capture enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay	
		Antibody	
Enterovirus	Virus detection in stool, throat, blood, of mother of infant with suspected congenital enteroviral infection	Virus detection in stool, throat, CSF, nose, blood, other	Selective testing of paired infant and maternal sera appropriate to infant, maternal or community isolates
	Bank serum	Bank birth and 2nd serum at 2–4 weeks	
HSV	Antibody	Virus detection in skin vesicles, throat, CSF, urine, nose, conjunctiva, rectal swab	Repeated antibody testing to 6–12 months (cannot differentiate between type 1 and type 2 viruses)
		Antibody	Negative infant and maternal antibody rules out infection
Parvovirus	IgM and IgG antibody	Detection of DNA (e.g. by PCR) in blood, bone marrow	Repeated antibody testing to 6–12 months
B19	Detection of DNA (e.g. by PCR) in blood	Parvovirus-specific IgM	Infected infants and their mothers may lack IgM antibody
Rubella	Rubella-specific IgG and IgM antibody	Rubella-specific IgM antibody	Repeated antibody testing to 6–12 months
		Virus detection in urine, nasopharynx, CSF, blood	Negative infant antibody at 6–12 months usually rules out infection
		CSF rubella-specific IgM antibody	
VZV	Antibody	Virus detection in skin lesions	Antibody testing 6–12 months postnatally
		Antibody	Intrauterine infection commonly manifest only as persistent antibody and childhood zoster
Toxoplasma gondii	*Toxoplasma*-specific IgM and IgG antibody	Reference laboratory testing of *Toxoplasma*-specific:	Repeated antibody testing to 6–12 months
	To determine acuity of infection in IgM positive mother, need reference laboratory testing	IgM-ISAGA, DS-IgM-ELISA, IgE-ELISA, IgA-ELISA	Negative infant antibody at 6–12 months usually rules out congenital infection
		or	
	Seroconversion or 4-fold rise in *Toxoplasma*-specific IgG in pregnancy	Blood culture (*Toxoplasma*-specific IgM falsely positive and negative)	
		Culture and histopathology of the placenta	

Treponema pallidum	Quantitative VDRL, rapid plasma reagin (RPR)	Detection of treponemes in nasal secretions, skin lesions, etc. by dark-field examination	Repeated quantitative VDRL and treponemal antibody testing to 12–15 months
	Treponemal antibody (e.g. fluorescent treponemal antibody absorption)	Quantitative VDRL, RPR	No test at birth can differentiate between the asymptomatic infected and uninfected neonate
	If positive, maternal HIV status	Serum treponemal antibody (e.g. fluorescent treponemal antibody absorbed (FTA) test)	Passively transferred antibody disappears at 6 months (VDRL, RPR) and 12–15 months (treponemal)

that treatment or retreatment is not required. Nontreponemal antibody titers, followed at 1, 2, 4 and 6 months, should be nonreactive at 6 months. At 12–15 months treponemal titers, if negative, rule out infection.

Toxoplasma gondii

If acutely infected women can be identified and treated in pregnancy through organization of a regional system of screening, there is evidence that the outcome is improved. Despite a large number of studies undertaken over the past 30 years, however, antenatal treatment of women with presumed toxoplasmosis remains controversial.[39]

Although maternal spiramycin therapy reduces the rate of transmission to the fetus, the combination of pyrimethamine and sulfadiazine is required once infection is established to improve fetal outcome. The regimen currently recommended for treatment in North America is given in Table 65.16 . (See also Chapter 66.b)

In the absence of antenatal diagnosis and fetal therapy,[40] the results of a 1-year course of therapy in infants in whom therapy was initiated within 2.5 months of birth have been reported in the Chicago Collaborative Treatment Trial.[41] Developmental, audiologic and visual performance was adequate for normal function in 75% of treated children with a follow-up of 33 months. The single most important predictor of poor outcome was hydrocephalus. Evidence of hydrocephalus must be actively sought and the condition managed aggressively. When infants are not treated, late sequelae after subclinical disease include active chorioretinitis in 85%, including some cases of blindness or impaired vision, developmental delay in 20–75% and moderate hearing loss in 10–30%.

TABLE 65-14 -- Appropriate specimens to diagnose congenital infection.[19]

APPROPRIATE SPECIMENS TO DIAGNOSE CONGENITAL INFECTION		
Specimen	**Tests**	**Interpretation**
Urine	Viral culture/detection (CMV, HSV, rubella)	Urine for CMV must be obtained at =2 weeks of age
		Positive is diagnostic
Throat swab	Viral culture (CMV, HSV, rubella, enteroviruses)	Positive is diagnostic
Blood	Agent detection (CMV, parvovirus B19)	Positive is diagnostic
Neonatal serum (single specimen)	Rubella-specific IgM	Positive is diagnostic, although determination of status at 10–12 months is confirmatory
Sequential neonatal, infant sera over 6–12 months	All	Passive maternal antibody in uninfected infant disappears at 4–9 months for CMV (unless peri-, postnatal transmission); 8 months for *Toxoplasma gondii*; 6 months VDRL, rapid plasma reagin; 12–15 months (treponemal)
		Positive specific antibody at 8–12 months suggests congenital toxoplasmosis, parvovirus B19, rubella, VZV infection
Single maternal serum at delivery	*Toxoplasma*-specific IgM	If IgM-specific antibody positive, reference laboratory testing of maternal, infant sera and placenta
Serology of both mother and infant	All	Negative maternal serology rules out source of infection
		Serial infant serology identifies passive maternal antibody (titers fall) and active infection (titers remain the same, rise over months)
CSF culture, detection	Detection of CMV, enteroviruses, HSV, *Toxoplasma* (reference laboratory), parvovirus B19	Positive is diagnostic
	Rubella-specific IgM antibody	
	VDRL (non-bloody tap)	
Skin lesions culture, detection	If active/vesiculated at birth: herpes, enteroviruses, VZV	Positive is diagnostic
	Syphilis dark-field	
Nasopharyngeal secretions	Syphilis dark-field	Positive is diagnostic
Stool cultures	Enteroviruses	Positive is diagnostic
Placenta	Variable	May be supportive of specific pathogen

Perinatal infections

These occur as a result of ascending infection and premature rupture of the membranes or through intrapartum transmission. Their management and prevention are summarized in Table 65.17 .

Specific infections

Enterovirus

Enteroviruses are among the commonest pathogens encountered by the newborn in the first months of life. The most common symptoms are undifferentiated fever and aseptic meningitis. Pleconaril is currently being studied in a National Institutes of Health-sponsored trial and should be considered in cases of severe hepatitis or sepsis.[42] Given the likelihood that an infected infant has received no passive maternal antibody, high-dose intravenous immune globulin may be reasonable in the acutely ill infant, although efficacy has not been demonstrated.

Herpes simplex virus

Because it is the primary infection, rather than recurrent infection in pregnancy, that is a risk to the fetus, transmission cannot be eliminated despite the best obstetric care. Primary infection is characterized by cervical infection in 80% of cases, protracted shedding for a mean of 11 days and a 33% risk of transmission to the newborn if shedding occurs at delivery. Conversely, in recurrent infection, there is only a 13% risk of cervical shedding, 2–4 days of shedding and a 3% risk of transmission if

there is shedding at delivery.

Neither a positive nor a negative clinical history will predict the neonatal risk for HSV infection. Of women with no previous history of genital HSV, 0.2% will have positive genital cultures at delivery.[43] Because viral shedding at the time of delivery cannot be predicted by third trimester cultures, screening is not recommended.

Women with recurrent disease should be reassured. With a risk of asymptomatic shedding of about 2% and a transmission rate of less than 5%, the risk of neonatal infection in this situation is about 1 in 2000 births. Cesarian section is generally recommended for women with active lesions at birth or a clinical primary infection during pregnancy, particularly in the last half of gestation; infection may occur in spite of Cesarian section. Fetal scalp monitors should be avoided. Viral cultures should be obtained from the maternal cervix at birth. Infant cultures (i.e. urine, mouth, nasopharynx, stool/rectum) should be obtained at 24 hours and the child carefully observed for symptoms of neonatal HSV requiring therapy.

Herpes simplex virus commonly presents without skin lesions as neonatal sepsis or pneumonitis, with one of the earliest clues being abnormal liver enzymes. Shock, coagulopathy, fulminant hepatitis and skin lesions follow. Prematurity is extremely common, occurring in one-quarter of infected infants. Infected infants may have progressive deterioration over the first year, especially with a history of multiple episodes of recurrent skin lesions in the first 6 months. Central nervous sytem relapse is common and involvement of the CNS, not initially evident, may occur. Initial evaluation of all infants with

TABLE 65-15 -- Definitions of selected congenital infections.

DEFINITIONS OF SELECTED CONGENITAL INFECTIONS		
CMV	Confirmed	Detection of CMV in the first 2–3 weeks of life from urine, throat or other sources in newborn with one or more of:
		• Small for gestational age
		• Hematologic findings (petechiae, purpura, splenomegaly, jaundice at birth)
		• Neurologic findings (microcephaly, chorioretinitis, neurologic abnormality, intracranial calcifications, hearing impairment)
		• Laboratory findings (direct hyperbilirubinemia >3mg/dl), thrombocytopenia (platelet count <75,000/ml), liver function abnormality (alanine aminotransferase >100mg/dl)
	Possible	As for Confirmed, except viral detection only after 3 weeks of age and other diseases ruled out
HSV	Congenital	Detection of HSV within 24 hours of birth or stable positive titer over 3 months in infants with one or more of skin, eye and brain lesions
	Perinatal	Detection of HSV after 24 hours and in first 6 weeks of life further characterized by clinical and laboratory findings as one of:
		• Disseminated
		• CNS
		• Skin, eye, mouth disease (with negative CSF pcv)
Rubella	Confirmed	Defects of congenital rubella syndrome present and one or more of:
		• Virus detected
		• Positive rubella-specific IgM antibody
		• Positive infant serology after disappearance of passively transferred maternal antibodies at 3–12 months
	Compatible	Insufficient laboratory data for confirmation of diagnosis but any two complications from (a) or one from (a) and one from (b):
		(a) Cataracts or congenital glaucoma, congenital heart disease, hearing loss, pigmentary retinopathy
		(b) Purpura, splenomegaly, jaundice, radiolucent bone disease, meningoencephalitis, microcephaly, mental retardation
	Possible	Presence of some compatible clinical findings, but insufficient criteria for either the Confirmed or Compatible categories
Syphilis	Confirmed	Identification of *Treponema pallidum* in nasal or skin lesions
	Presumptive	One or more of:
		• Infant born to mother with untreated or inadequately treated syphilis
		• Treated with drug other than penicillin and/or
		• <30 days to 3 months before delivery
		• Infant has reactive treponemal test with findings of one or more of abnormal physical examination, long bone radiographs, CSF (including reactive CSF-VDRL and/or a leukocyte count of 20ml or greater or a protein concentration of 100mg/dl or greater), 4-fold higher VDRL than mother has
		• Infant has documented 4-fold rise in titers and positive treponemal test
		• Infant has reactive treponemal test that does not revert by 12–15 months
Toxoplasma gondii	Confirmed	• *Toxoplasma*-specific IgM (or if available, Sabin-Feldman Dye test >300IU) in maternal sera with infant findings of chorioretinitis, cerebral calcifications or hydrocephalus in the absence of CMV infection
		• Positive antibody in infant at 8–10 months (after disappearance of passively transferred maternal antibody) with or without clinical findings
	Compatible	• Chorioretinitis with positive *Toxoplasma*-specific antibody after 8–10 months of age
		• Cerebral calcifications and/or hydrocephalus with positive *Toxoplasma*-specific antibody after 8–10 months of age in the absence of CMV infection
Parvovirus B19		Detection of parvovirus B19 by:
		• Direct electron microscopy or nucleic acid in blood and/or tissue obtained within the first 3 weeks of life in the presence of fetal or neonatal findings of hydrops and anemia
		• Parvovirus B19-specific IgM in the first 3 weeks or persistent IgG beyond 3–12 months with either documented maternal infection or fetal/neonatal findings of hydrops/anemia or cranial calcification
Varicella		One or more of:
		• Anomalies of congenital varicella syndrome (skin, eye, limb, neurologic)
		• Acute varicella at birth with viral detection
		• Herpes zoster in the first year of life with viral detection
		• VZV-specific IgM at birth, persistent IgG to 12 months or detection of specific lymphocyte transformation in response to VZV virus antigen

suspected or confirmed HSV infection must include CSF PCR to detect subclinical CNS disease. The role of long-term oral antiviral therapy in infected infants is under study.

Empiric therapy may be indicated for the infant who is unwell, especially if liver function tests are abnormal, if surface cultures are positive after 48 hours, if the CSF is

abnormal, if there has been prolonged

TABLE 65-16 -- **Treatment of toxoplasmosis in the fetus and infant.**[*]

TREATMENT OF TOXOPLASMOSIS IN THE FETUS AND INFANT			
Manifestation of disease	**Medication**	**Dosage**	**Duration of therapy**
In pregnant women with acute toxoplasmosis	Spiramycin	1g every q8h without food	Until fetal infection documented or excluded at 18–20 weeks; if documented, in alternate months with pyrimethamine, leukovorin and sulfadiazine until term (France)
If fetal infection confirmed after week 17 of gestation or if maternal infection acquired in last few weeks of gestation	Pyrimethamine and	Loading dose: 100mg/day in two divided doses for 2 days then 50mg/day	Until term (leukovorin is continued 1 week after pyrimethamine is discontinued)
	Sulfadiazine	Loading dose: 75mg/kg per day in two divided doses (maximum 4g/day) for 2 days then 100mg/kg per day in two divided doses (maximum 4g/day)	
	Leukovorin (folinic acid)	10–20mg/day	
Congenital *Toxoplasma* infection in the infant	Pyrimethamine and	Loading dose: 2mg/kg per day for 2 days, then 1mg/kg per day for 2–6 months, then this dose every Monday, Wednesday and Friday	1 year
	Sulfadiazine and	100mg/kg per day in two divided doses	1 year
	Leukovorin	10mg 3 times a week	1 year
	Corticosteroids (prednisolone) have been used when CSF protein is =1g/dl and when active chorioretinitis threatens vision	1mg/kg per day in two divided doses	Until resolution of elevated (=1g/dl) CSF protein level or active chorioretinitis that threatens vision

** Adapted with permission from Hohlfeld et al. [30] and Remington et al. [3]*

TABLE 65-17 -- Preventive strategies and intrapartum management.

PREVENTIVE STRATEGIES AND INTRAPARTUM MANAGEMENT			
Micro-organism	**Clinical situation**	**Preventive management**	**Comment**
Enterovirus	Active maternal enteroviral infection (i.e. fever, abdominal pain) at delivery	Attempt to defer delivery	May allow transmission of antibody
Hepatitis B virus	Maternal HBsAg status unknown	Neonatal immunization	Many experts recommend universal neonatal immunization
HSV	Maternal lesions at delivery	Active observation of neonate for signs of infection	See detailed guidelines
			Cesarian section for active lesions or primary infection in (late) pregnancy
HIV-1	HIV-positive mother	Single or combination antiviral therapy of infant	Antenatal delivery screening and therapy is advisable
Rubella	High risk, no antenatal care, no serology at delivery	Postpartum immunization of susceptible mother before hospital discharge	Failure to immunize prior to discharge has resulted in subsequent infected neonates
VZV	Maternal lesion within 1 week before or after delivery	Varicella-zoster immune globulin ± aciclovir therapy of neonate	Post-partum immunisation of mothers known to be susceptible
Treponema pallidum	High risk, no antenatal care, no serology at delivery	Evaluation and possible therapy with follow-up of neonate	In high-risk women repeat screen in third trimester, delivery

longed rupture of the membranes or if maternal primary infection has occurred near birth.

Aciclovir therapy is prescribed at a dose of 60mg/kg per day intravenously q8h for 14–21 days.[44] While mortality is certainly reduced, morbidity remains significant. The progression of cutaneous to CNS disease can be halted by antiviral therapy in the majority of cases. Emergence of resistant organisms has been reported. Isolation of the infant with lesions is required.

Varicella-zoster virus

A neonate whose mother has developed varicella 5 days before delivery to 48 hours after delivery should receive a dose of 125U (1.25ml or 1 vial) of zoster immune globulin as soon as possible. These infants carry a 30% risk of infection without the protection of passive maternal antibody and a 30% mortality rate without antiviral therapy. Aciclovir at a dose of 1500mg/m^2 per day is used to treat symptomatic neonates,[45] with dose adjustments for liver and renal failure or prematurity.[46]
Separation of an infected and contagious mother from the child may be advisable. Isolation of the exposed hospitalized infant is required.

HIV

Over 600,000 infants worldwide are infected with HIV from their mothers each year. There is evidence for both early and late

in-utero transmission. Fetal diagnostic testing may result in iatrogenic infection of the fetus and is generally contraindicated. Elective Caesarian section can reduce the risk of transmission by half but is not yet a realistic option for poor countries. Breast-feeding is a major contributing factor in mother-to-child transmission in Africa. Prevention of transmission is achievable through antiretroviral therapy including antenatal oral zidovudine, intrapartum intravenous zidovudine and 6 weeks of oral zidovudine in the babies (see Chapter 135). Shorter courses of lamivudine with zidovudine and nevirapine alone are effective in settings where such drugs are affordable and breast-feeding is not undertaken, although antiviral resistance, particularly with nevirapine, is concerning. Transmission rates reach 35% when there is no intervention and are below 5% when antiretroviral treatment and appropriate care are available. Routine use of zidovudine in pregnancy has reduced transmission rates in the USA by between a half and two-thirds to less than 5%. A similar pattern has been seen in Europe, where the rate of transmission has fallen from 15.5% in 1994 to 2.6% after 1998. Implementing programs to prevent mother-to-child transmission has been difficult and slow in poor countries. Non-breast-fed infants of infected mothers can be accurately diagnosed by 3 months of age (see Chapter 136).[47] Future research should focus on prevention of postpartum infection and operational issues. Studies in Africa have shown a 3-fold to 4-fold increased risk of death in children whose mothers have died in the first year of life.

Hepatitis B virus

In four studies of over 33,000 pregnant women in North America, a prevalence of HBsAg carriage of 0.8% was documented; 52% of women acknowledged no known risk factors. The risk of perinatal infection is greater if the maternal infection was acquired in the third trimester (80–90% versus less than 10% in the first trimester). If HBsAg positivity is accompanied by e-antigen positivity, the neonate is both more likely to become infected (70–90% versus 20–25%) and more likely to become a chronic carrier (85% versus 5%). The strongest predictor of transmission may be maternal hepatitis B virus DNA load.

Infants of carrier mothers should receive 0.5ml hepatitis B immune globulin within 12 hours of birth and either 5mg of the Merck hepatitis B vaccine (Recombivax HB) or 10mg of the SmithKline Beecham hepatitis B vaccine (Engerix-B). The second dose of vaccine is given at 1–2 months of age and the third dose at 6 months of age. Infants born to mothers of unknown status should receive either 5mg of Recombivax HB or 10mg of Engerix-B. The second dose of vaccine is given at 1–2 months of age and the third dose at 6 months of age.

Hepatitis C virus

In 20 studies the risk of perinatal transmission of hepatitis C virus was 14%, being 21% in infants born to HIV-infected mothers (65 out of 309), 10% in infants born to HIV-negative mothers (50 out of 495), 14% in those born to hepatitis C virus RNA-positive mothers and 2% in those born to hepatitis C virus RNA-negative mothers (Delages G, personal communication).

Group B streptococcus

Of the 10–35% of women asymptomatically carrying group B streptococcus, 50% of their infants will be colonized on their skin and mucous membranes. Of those colonized, 1–2% will develop early-onset disease. Two alternative approaches (i.e., screening, risk factor identification) to the prevention of group B streptococcal disease have been developed in the USA with a 70% reduction in

TABLE 65-18 -- Women with limited or no antenatal care.

WOMEN WITH LIMITED OR NO ANTENATAL CARE	
Mother	• Genital examination for findings suggestive of sexually transmitted diseases
	• Cultures for *Chlamydia trachomatis*, *N. gonorrhoeae*
	• Serologic testing for HBsAg, hepatitis C virus, HIV, syphilis (nontreponemal and treponemal testing), rubella
	• Adequate follow-up
Infant	• Prophylactic eye care
	• Serologic testing for HBsAg, hepatitis C virus, HIV, syphilis (nontreponemal and treponemal testing)
	• First dose of hepatitis B vaccine
	• Adequate follow-up

the incidence of early-onset disease.[48] According to the more effective screening-based approach, all pregnant women should be screened at 35–37 weeks' gestation for anogenital group B streptococcus colonization. Intrapartum antibiotics are offered to all culture-positive women, regardless of risk factors. If the results of cultures are not known at the time of labor, intrapartum antibiotics are used in the presence of risk factors, which include the following: less than 37 weeks' gestation; more than 18 hours of membrane rupture; and temperature higher than 100.4°F (38°C). Also, infants born to women with a previously infected infant and women with group B streptococcus bacteriuria during pregnancy or postpartum GBS sepsis are treated with intrapartum antibiotics (see Chapter 66.c). In geographic regions with differing epidemiologic data, this approach is not appropriate.

Absence of antenatal care

For women in whom there has been inadequate antenatal care, detection of infection is required at delivery, as summarized in Table 65.18 .

Breast-feeding

Breast-feeding during an active infection

Contaminated milk has been implicated in neonatal infection with *Staphylococcus aureus*, group B streptococci, *Mycobacterium* spp. and possibly *Salmonella* spp.

Although viral contamination of milk by rubella, HSV, hepatitis B virus and CMV has been reported, serious sequelae have not generally occurred. Mothers with herpetic lesions on their breasts should refrain from breast-feeding. They should cover other active lesions and wash their hands before breast-feeding.

Breast-feeding is estimated to confer an additional risk of 14% over transmission of HIV-1 in utero or at delivery (see Chapter 135).[47]

Breast-feeding and drug therapy

The American Academy of Pediatrics recommends that breast-feeding be discontinued while a nursing mother is being treated with metronidazole and warns about the use of nitrofurantoin and sulfa drugs, which can cause hemolysis in glucose-6-phosphatase-deficient infants.[49] Although it would be unusual for an effective maternal medication to be contraindicated because of risks to the infant through breast milk, physicians should be aware of information specific to agents being used. Frequent feeding exposes the infant to more drug than feeding at 4-hour intervals. Mothers can be encouraged to avoid frequent feedings to reduce drug exposure and the consequent changes in the infant's gastrointestinal flora and risk of oropharyngeal candidiasis.

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Chapter 66 - Practice Points

66.a Management of an HIV-positive pregnant woman who has a positive VDRL test from an area endemic for *Treponema* infection

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Introduction

The dual epidemics of venereal syphilis (*Treponema pallidum* subsp. *pallidum*) and HIV are responsible for increasing tolls of morbidity and mortality among pregnant women and their offspring. Syphilis and HIV infection are closely interrelated. As sexually transmitted diseases, each poses a risk for the other, and there is now substantial evidence that genital ulcerative diseases, including syphilitic chancres, facilitate the bidirectional transmission of HIV-1. Although a simple screening test for syphilis and effective antibiotics are available, the disease remains uncontrolled in resource-scarce regions where antenatal testing and treatment are not always accessible. Moreover, in many of these countries, the endemic (i.e. nonvenereal) treponematoses yaws, endemic syphilis and pinta, caused by *T. pallidum* subsp. *pertenue*, *T. pallidum* subsp. *endemicum* and *T. carateum*, respectively, have not only not been eliminated but are resurging in some places because of the breakdown of once successful control programs. In the pregnant female, regardless of HIV infection status, untreated syphilis poses a grave risk to the fetus or neonate on top of whatever morbidity, acute or chronic, it may inflict upon the mother. The possibility of infection with a nonvenereal treponematosis in such individuals poses a further diagnostic challenge inasmuch as it is impossible to distinguish these infections from venereal syphilis using currently available serologic tests. Health care professionals managing patients residing in or emigrating from areas endemic for the nonvenereal treponematoses need to be aware of the clinical and epidemiologic features that distinguish these diseases from venereal syphilis. They also need to know how HIV co-infection may influence the management and outcome of syphilis in pregnant women and their offspring (Table 66a.1).

Clinical features

A detailed discussion of the clinical features of the treponematoses is beyond the scope of this commentary (see Chapter 75 , Chapter 156 and Chapter 230); the salient clinical features of these diseases, however, are summarized in Table 66a.1 . Treponemal infections are divided into early (i.e. primary, secondary and early latent) and late (i.e. late latent and tertiary) stages. Early lesions, as well as blood and exudative body fluids, are infectious. Primary lesions of venereal syphilis (chancres) occur usually in the anogenital area of sexually active individuals; a substantially greater proportion of heterosexual men present with genital ulcers than women and gay men. By contrast, acquisition of nonvenereal treponematoses occurs earlier in life, usually in childhood, with primary lesions often occurring on an extremity. Although anecdotal reports indicate that syphilis manifestations in HIV-infected patients may be florid and/or predispose to central nervous system complications, the manifestations of syphilis in most HIV-infected patients appear to be essentially the same as in HIV-uninfected patients. No studies are available on the impact of HIV infection on the course or severity of the nonvenereal treponematoses. With both venereal and nonvenereal treponematoses, mucocutaneous lesions are the most common form of secondary disease; extracutaneous manifestations, occasionally severe, can occur in secondary syphilis (nonvenereal as well as venereal) and yaws. The gummatous lesions of late yaws can be extremely destructive. Venereal syphilis is the only treponematosis that involves the central nervous system or that causes congenital infection.

Serodiagnostic tests

Visualization of spirochetes in lesion exudate by darkfield examination is the definitive method for diagnosing primary treponemal infection. A presumptive diagnosis is possible with the use of nontreponemal and treponemal serologic tests. The nontreponemal tests (NTTs), represented by the Venereal Disease Research Laboratory (VDRL) test and the rapid plasma reagin (RPR) test, detect antibodies to a defined mixture of cardiolipin, lecithin and cholesterol. These tests have traditionally been designated as 'nontreponemal' based upon the belief that lipoidal antigens of host origin induce the antibodies being detected. Nontreponemal tests are used to screen for syphilis because they are easy to perform and have reasonably high sensitivity. Because they are titratable and usually decline in parallel with disease activity, they are also used to monitor the response to therapy. Falsely reactive NTTs are usually of low titer (<1:8) and may occur in a variety of conditions (see Chapter 75). Although pregnancy

TABLE 66.a-1 -- Overview of treponemal infections.

OVERVIEW OF TREPONEMAL INFECTIONS						
Disease	**Organism**	**Endemic areas**	**Primary lesion**	**Secondary lesions**	**Tertiary lesions**	**Congenital infection**
Venereal syphilis	*Treponema pallidum* subsp. *pallidum*	Worldwide	Chancre, usually in anogenital region	Mucocutaneous lesions (condyloma lata, papules, macules or maculopapules)	Gummas, including	Yes
				Visceral involvement	CNS	
				Central nervous system (CNS) involvement (usually) aseptic meningitis)	Carditis/aortitis	
					Neurosyphilis (meningovascular, tabes, paresis)	
Yaws	*Treponema pallidum* subsp. *pertenue*	Rural areas of Africa, Central and South America, the Caribbean, equatorial islands of South East Asia and remote parts of India and Thailand	Papule	Diffuse papules, papillomas and ulcers	Destructive gummas of skin and bone	No
			Papilloma	Osteitis		
			Ulcer, usually on an extremity	Dactylitis		
Pinta	*Treponema carateum*	Underdeveloped rural areas of Mexico and northern South America	Erythematous papule, usually on an extremity	Scaly papules	Areas of altered skin pigmentation	No
				Areas of altered skin pigmentation	Hyperkeratosis	
Endemic bejel	*Treponema pallidum* subsp. *endemicum*	West Africa, small foci in Zimbawe, Botswana, Arabian peninsula and central Australia	Oral mucosal ulcer	Oral and pharyngeal ulcers	Gummas of skin, bone and joints	No
				Mucous patches		
				Condyloma lata		
				Periostitis		

is a recognized cause of a falsely reactive NTT, one must exercise great caution in labelling a reactive NTT in a pregnant patient as a false-positive result.

The second type of serodiagnostic test, represented by the fluorescent treponemal antibody-absorption test and the microhemagglutination *T. pallidum* treponemal tests (MHA-TP), detects antibodies directed against *T. pallidum* antigens and hence is designated as 'treponemal'. Treponemal tests are used to confirm that reactivity in a NTT is indeed due to syphilitic infection. Because they are more difficult and expensive to perform than NTTs, treponemal tests may not always be available in underdeveloped countries. Molecularly based efforts to design serodiagnostic tests capable of distinguishing venereal syphilis from the nonvenereal treponematoses, principally yaws, have thus far been unsuccessful. It is hoped that the availability of the genomic sequence for *T. pallidum* and, eventually, those of other pathogenic treponemes may enable investigators to identify polymorphisms that can be exploited for developing disease-specific serodiagnostic or polymerase chain reaction (PCR)-based tests.

Both treponemal and nontreponemal serologic tests are generally reliable for the diagnosis and management of syphilis in patients co-infected with HIV. However, HIV-infected syphilis patients may on occasion show higher than expected NTT titers, false-negative treponemal or nontreponemal tests, or delayed seroreactivity. When serologic tests and clinical syndromes suggestive of early syphilis do not correspond, alternative tests such as biopsy of suspicious lesions should be performed. When available, lesions also can be analyzed by PCR for the presence of *T. pallidum* DNA. As with serologic tests, the PCR tests in widespread usage cannot distinguish between the pathogenic treponemes.

Syphilis testing during pregnancy

It is estimated that, of all pregnant women who have untreated syphilis, only 20% will both carry the fetus to term and deliver a normal child. Complications include stillbirth (30%), neonatal death (10%) and mental handicap (40%). Because of the seriousness of these complications, pregnant women should be screened serologically for syphilis early in pregnancy. For communities and populations

TABLE 66.a-2 -- Treatment of syphilis during pregnancy in HIV-infected women.

TREATMENT OF SYPHILIS DURING PREGNANCY IN HIV-INFECTED WOMEN	
Primary or secondary syphilis	Benzathine penicillin G 2.4 megaunits im (single dose)
Early latent syphilis	Benzathine penicillin G 2.4 megaunits im (single dose)
Late latent syphilis or latent syphilis of unknown duration	Benzathine penicillin G 7.2 megaunits in three doses each of 2.4 megaunits im at 1-week intervals
Tertiary syphilis	Benzathine penicillin G 7.2 megaunits in three doses each of 2.4 megaunits im at 1- week intervals
Neurosyphilis	Aqueous crystallin penicillin G 18–24 megaunits a day administered as 3–4 megaunits iv q4h for 10–14 days

in which the prevalence of syphilis is high or for patients at high risk, serologic testing should be performed twice during the first trimester, at 28 weeks gestation and at delivery. Pregnant women who are seropositive but who lack clinical manifestations should be considered to be infected unless an adequate treatment history is clearly documented and sequential serologic antibody titers have declined appropriately. Given the inability of serodiagnostic tests to distinguish between venereal and nonvenereal treponematoses, syphilis should be the presumed diagnosis in asymptomatic, seropositive patients unless a diagnosis of nonvenereal treponematosis can be established unequivocally (see Table 66a.1).

Treatment

Penicillin is the antimicrobial of choice in the treatment of syphilis during pregnancy (Table 66a.2). Treatment during pregnancy should consist of the penicillin regimen appropriate for the stage of syphilis. Some specialists recommend a second dose of benzathine penicillin

2.4 million units intramuscularly, 1 week after the initial dose. The present consensus is that alternative regimens are potentially too harmful to the fetus (e.g. tetracycline), lack efficacy because of the inability of the drug to cross the placenta (e.g. erythromycin) or are insufficiently studied (e.g. ceftriaxone). The current Centers for Disease Control and Prevention guidelines remind the practitioner of the importance of evaluating the HIV-infected patient both clinically and serologically for treatment failure. This is accomplished by blood draws at 3, 6, 9, 12 and 24 months after the course of therapy has been finished. The guidelines go on to suggest considering cerebrospinal fluid examination approximately 6 months after treatment, emphasizing that this is of unproven benefit. The treatment regimens for nonvenereal treponematoses are the same as those used for the comparable stage of venereal syphilis.

Because penicillin is clearly the preferred treatment, penicillin skin testing is recommended for reportedly penicillin-allergic pregnant women who have syphilis. If the penicillin allergy is confirmed, desensitization can be accomplished using incremental doses of oral penicillin V over 4–6 hours. A Jarisch-Herxheimer reaction, which presents with fever, headache and myalgia, can occur within hours of initiation of penicillin therapy in early syphilis. Women treated for syphilis during the second half of pregnancy may also experience self-limiting uterine contractions, decreased fetal activity and fetal heart rate abnormalities after penicillin treatment, but premature labor or fetal distress is rare. Women should be warned of the symptoms of the Jarisch-Herxheimer reaction and be instructed to use acetaminophen (paracetamol) to control these symptoms and to self-monitor uterine and fetal activity during the first 48 hours after penicillin therapy. In the second half of pregnancy, management and counseling may be facilitated by a fetal ultrasound but this should not delay therapy. Ultrasound signs of fetal syphilis (hepatomegaly, hydrops fetalis) indicate a greater risk to fetal health; such cases should be managed in consultation with specialists in high-risk obstetrics.

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66.b Treatment of a positive *Toxoplasma* titer in pregnancy

William R Bowie

Introduction

Clinically recognized toxoplasmosis is infrequent, but serologic evidence of toxoplasmosis is common. In immunologically competent people, even symptomatic toxoplasmosis is usually of minimal clinical significance. The exception is when symptomatic or asymptomatic infection is acquired just before or during pregnancy. *Toxoplasma gondi*ı readily crosses the placenta to infect the fetus, with immense clinical and financial implications. It is estimated that in North America, approximately one pregnancy in 1000 is affected, with higher reported rates in Europe. Although definitive proof is lacking, because early treatment of the mother has been thought to decrease the risk of infection of the fetus and diminish the sequelae in infected fetuses, appropriate management of the mother is essential.

Because most infected women are either asymptomatic or have non-specific and transient symptoms, the diagnosis is rarely made clinically. Rather, the health care provider and the woman are typically faced with a positive serologic test without clear information on when the infection was acquired. 'Positive' serology with routine tests does not reliably determine the acuteness of infection, which is what determines the management. Inappropriate response may result in unwarranted psychologic distress, unnecessary evaluations and treatments, and even unnecessary termination of pregnancy. At least in the USA, a recent study of obstetricians and gynecologists revealed that few were aware that 'positive' IgM antibody did not necessarily indicate acute infection, or that the US Food and Drug Administration had sent out an advisory about this problem with serologic tests for toxoplasmosis.

Pathogenesis

The definitive hosts of *T. gondii* are felines, and people are infected by direct or indirect contact with oocysts excreted by cats. Oocysts, spread for example in cat litter or soil or sand contaminated by cat feces, are highly infective. When ingested by animals or humans, they ultimately result in cysts in tissues. These also infect humans when uncooked or inadequately cooked meat containing viable cysts are ingested. Rarely, humans can also be infected by blood transfusions (see Chapter 65 and Chapter 245).

During acute infection of humans, toxoplasmosis is disseminated widely. Infection acquired immediately before conception or during pregnancy carries with it the risk of spread to the fetus. The risk of transmission is lowest in the first trimester (10–15%), but the consequences then are the most devastating (severe disease or death). By the second trimester, there is 25–40% transmission, with usually nonfatal sequelae. By the third trimester, over 60% of fetuses are infected, with typically mild or asymptomatic manifestations.

The classic triad of clinical features in severely infected infants who survive includes hydrocephalus, intracranial calcifications and chorioretinitis. Asymptomatically infected infants are typically not recognized at birth, but many if not most are thought to be at risk of subsequently developing chorioretinitis, hearing loss or subtle neurologic manifestations.

The economic impact of congenital toxoplasmosis is substantial, and screening programs to detect infection in pregnancy or at birth have been considered. In the USA, routine screening for toxoplasmosis in pregnancy is rarely recommended, but in some other countries screening is repeated serially in pregnancy, particularly in France.

Investigation

The focus of this Practice Point is on immunocompetent pregnant women who have had a 'positive' test for antibody to *T. gondii*. This will usually have been performed as a screening test rather than a diagnostic one. Depending on the laboratory, this will be either a positive IgG titer, or possibly a positive titer for both IgG and IgM.

Unless timing was fortuitous, even with repetition titers are likely to be stable rather than rising or falling.

Difficulties in interpretation of serologic results

There are several substantial problems in the interpretation of results.

- First, there are problems with sensitivity, and more frequently specificity of many of the commercially available tests.
- Second, potential markers of acuteness of infection, such as IgM and IgA, may persist long after the risk of transmission to the fetus ends, so that although positive IgM and IgA results may be true-positive results, they do not by themselves establish acuteness of infection. Thus, when the IgM is reported as being 'positive', it can be a false positive (with the rate of false positives dependent on the test and the laboratory), a true positive indicative of recent infection or a true positive but simply reflecting persistence of IgM antibody.
- Third, history and physical examination only rarely aid in the ascertainment of determination of the acuteness of infection.

*Negative anti-*Toxoplasma gondii *IgG and IgM*

Unless it is very early in infection, negative anti-*Toxoplasma gondi*ı IgG and IgM indicates absence of previous infection. Women with such results are susceptible to infection. Primary prevention messages should be strongly reinforced. Subsequent repeat testing is required if one wishes to exclude infection later on in the pregnancy.

IgG titer only available, and is positive

An IgM test should be requested on the same or a new serum specimen because a single positive IgG titer provides no information about acuteness of infection. The presence of IgG antibody with a negative IgM antibody excludes recent infection. If performed early in pregnancy, no further investigation is required for toxoplasmosis. If performed late in pregnancy, there is an outside possibility that acute infection may have arisen in pregnancy, with subsequent disappearance of IgM. Because of long-term persistence of IgM this is unlikely, but if there is any clinical concern of toxoplasmosis then the infant should be screened at birth.

Initial negative IgG titer with positive or equivocal IgM titer

When the IgG titer is negative, it is highly likely that the IgM titer is falsely positive. However, because the woman could be in the process of seroconverting, testing should be repeated in parallel on a second sample that is collected approximately 3 weeks after the first. If the IgG titer remains negative, then the result is probably falsely positive. Unless there are other features suggesting active infection, no further investigation is needed. Recent acquisition of infection is strongly suggested if the IgG titer becomes positive or there is a significant increase in the IgM titer.

Initial or subsequent positive IgG titer, and IgM titer is positive

Unless there is seroconversion or a significant increase in titers, interpretation at this stage is much more difficult because this information by itself does not establish acuteness of infection. This requires further clinical assessment and serologic and other studies in the mother and potentially in the fetus.

History and examination of the mother

Although symptoms are usually absent or so non-specific as to be unhelpful, a careful history and examination might detect symptoms that provide a clue to the onset of the disease. Most helpful would be development of lymphadenopathy, typically involving one node or a few nodes. A physical examination should be performed, looking in particular for abnormal lymphadenopathy and ocular disease.

Serologic studies in the mother

Pregnant women are often screened for a variety of processes (e.g. rubella, HIV), and this serum might have been stored. If available, prior stored serum should be tested for antibody to *T. gondii*. The results may be very helpful in determining the chronicity of infection.

Additional testing is required on available or newly acquired serum. If repeat testing on specimens run in parallel (that is, testing is performed on all specimens at the same time) shows or has shown seroconversion or a 4-fold or greater rise, then infection is acute. Usually titers are stable, and further testing is required in specialized or reference laboratories. A battery of tests is usually performed to assess acuteness of infection. Results should be interpreted in consultation with the reference laboratory. The two tests most often employed to assess acuteness of infection are IgG avidity and differential agglutination testing, but numerous other tests may be used by reference laboratories. If these additional results are consistent with acute infection or if they do not exclude acute infection, the woman should be managed as below.

Management

Studies diagnostic of or consistent with acute infection in pregnancy or immediately before conception

Management is in part determined by the time when infection was acquired. If results are consistent with infection immediately before or just after conception, the risk of delivering an infected baby is low. Either the fetus is not infected or if the fetus is infected it is likely to abort. When infection occurs later, the risk of having a viable but affected infant is much greater.

The mother should immediately be started on treatment (see below) for the duration of pregnancy and the fetus should be assessed by ultrasound. Amniocentesis for polymerase chain reaction (PCR) should be strongly considered.

Fetal ultrasound may show features consistent with infection, especially increased size of the ventricles. Negative studies do not exclude fetal involvement and studies may need to be performed serially.

With or without fetal abnormalities, amniocentesis should be considered at 18 weeks or later. If performed, as a minimum the amniotic fluid should be tested by PCR. Reference laboratories may do additional testing on amniotic fluid, such as mouse or cell culture inoculation. Although PCR can be falsely positive or falsely negative, if it is positive treatment of the mother should be switched to pyrimethamine and sulfadiazine.

Some authorities also sample fetal blood to detect the parasite or a fetal immunologic response, but PCR on amniotic fluid is probably safer.

Treatment of the mother

Initial treatment is with spiramycin 1g orally q8h. Spiramycin reduces the incidence and severity of fetal infection, but when the fetus is known to be infected, pyrimethamine and sulfonamides are more active than spiramycin. Hence, ultrasound or amniotic fluid findings suggestive of fetal involvement should prompt a change of treatment to pyrimethamine and a sulfonamide. A variety of dosage regimens have been used, but currently suggested is pyrimethamine 25mg/day and sulfadiazine 2g orally q12h, with supplemental folinic acid 5mg/day. When treatment of the mother is initiated, it should be continued for the duration of the pregnancy.

Spiramycin is a macrolide with possible adverse reactions including nausea, vomiting, anorexia and diarrhea. Sulfadiazine has typical adverse reactions associated with sulfonamides, including concerns of kernicterus. The major adverse reaction to pyrimethamine is dose-related bone marrow suppression. To assess hematologic abnormalities, testing is recommended at least once weekly to detect anemia, leukopenia and thrombocytopenia.

Although other drugs have been used to treat toxoplasmosis, there is minimal experience with them in the context of pregnancy and fetal infection.

Termination of pregnancy

Many women with 'positive' serology for toxoplasmosis have had inappropriate termination of pregnancy. Positive IgG and IgM serology is not of itself an indication for termination. Most of these women will not have infected fetuses, but even if the fetus is infected, with appropriate long-term treatment the infants often do well. Termination should only be considered when there is documented evidence of fetal involvement, especially if infection was acquired early in the first trimester. However, even most of these infants who survive have done well with treatment of the mother followed by treatment of the infant.

Initial management of the infant at birth

If there is any question about the possibility of fetal acquisition of toxoplasmosis, the infant should be evaluated, including by one or a series of serologic tests. If clinical, serologic or other testing suggests congenital toxoplasmosis, the infant should be treated for 1 year. On this treatment, even infants who have clinically apparent manifestations such as intracranial calcifications, meningitis or chorioretinitis are highly likely to have a favorable outcome.

HIV infection and toxoplasmosis in pregnancy

In contrast to immunocompetent women, women who have HIV infection whose antibody to *T. gondii* is IgG positive and IgM negative are at risk of transmitting HIV to the fetus. Appropriate management is unclear. Important variables probably include the degree of immunosuppression, concurrent *Pneumocystis carinii* prophylaxis with trimethroprim-sulfamethoxazole (co-trimoxazole), *Mycobacterium avium-intracellulare* treatment or prophylaxis with macrolides, *T. gondii* treatment or suppression, and infection acquired in pregnancy. Acute infection with *T. gondii* in pregnancy should be managed aggressively, probably with pyrimethamine and sulfadiazine, whether or not there is objective evidence of fetal involvement (see Chapter 134).

Other considerations

Pregnant women who have possible or proven toxoplasmosis are likely to feel guilty that they did something that put their fetus at risk. It is prudent for women who are or might be pregnant to avoid direct or indirect contact with cats or raw or inadequately cooked meat. However, most people with toxoplasmosis are totally unaware of when or how they became infected. Care should be taken to avoid adding guilt to the stress that women who have 'positive' serology undergo.

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66.C A pregnant patient with a previous pregnancy complicated by group B streptococcal disease in the infant

Upton Allen

Introduction

The group B streptococcus (GBS, *Streptococcus agalactiae*) remains an important cause of invasive disease in neonates and pregnant women. Among neonates, premature infants are at greatest risk of an adverse outcome from GBS infection. These premature infants account for 25% of the cases of GBS disease among neonates. In these infants, the disease manifests itself as an early-onset form (<7 days after birth) and a late-onset form (=7 days after birth). Disease among infants usually presents as bacteremia, pneumonia and meningitis. However, they may experience other syndromes, including soft tissue and bone infection. The mortality rate ranges from 5% to 10% and from 2% to 6% for early-onset and late-onset disease, respectively. However, higher mortality rates have been documented in pre-term and low-birth-weight infants. Improvements in survival rates in recent years have been attributed to advances in neonatal care.

Epidemiology

The organism colonizes the gastrointestinal tract of humans, with the genitourinary tract being the most common site for secondary spread. Colonization rates vary widely among different ethnic groups, geographic areas and age groups. These rates generally indicate that 10–30% of pregnant women have vaginal or rectal colonization with GBS. Approximately 1–2% of all infants born to colonized women develop early-onset GBS disease. Data from a multistate population-based study in the USA indicate that early-onset disease accounts for about 80% of neonatal GBS disease.

The incidence of early-onset disease is higher in babies born to women less than 20 years of age and in those who are of black race in the USA. Intrapartum risk factors include premature onset of labor (<37 weeks gestation), prolonged rupture of membranes (=18 hours) and intrapartum fever (<100.4°F/38°C). Additional risk factors include heavy vaginal colonization with GBS, previous

delivery of an infant who had GBS disease and the presence of low maternal levels of anti-GBS capsular antibody. Women who have GBS bacteriuria are at an increased risk of delivering an infected baby who has early-onset disease. This is related in part to the fact that women who have GBS bacteriuria are usually heavily colonized with GBS. Bacteriuria caused by GBS is associated with an increased risk of pre-term labor.

Microbiology

Group B streptococci are represented by several serotypes, including Ia, Ib, II, III, V–VIII. All serotypes may cause disease in humans but serotype III is the main cause of early-onset meningitis as well as of late-onset GBS disease among neonates.

In the determination of the GBS carrier status of a pregnant woman, culture techniques that maximize the recovery of GBS are essential. The optimal method for GBS screening involves collection of a single vaginal-anorectal swab or two separate swabs from the vagina and rectum. Swabs should be placed in a transport medium if the bacteriology laboratory is off-site and subcultured on to selective broth medium. After overnight incubation, the specimen is subcultured on to solid blood agar. Slide agglutination tests or other tests used to detect GBS antigen may be used to enable specific identification of GBS.

Prevention

Although research is being carried out to develop vaccines that could be administered to pregnant women to prevent GBS disease in neonates, this modality has not yet evolved as a preventive strategy. However, chemoprophylaxis has evolved as a useful preventive strategy. In this regard, because the majority of newborns who have GBS disease acquire infection in utero, the administration of antibiotics to neonates (postnatal prophylaxis) will not prevent the majority of GBS disease. Intrapartum chemoprophylaxis (administration of antibiotic during labor) has the potential of preventing neonatal as well as maternal GBS disease.

Guidelines on intrapartum chemoprophylaxis have been established by various groups. However, it should be noted that some countries do not have specific recommendations for prophylaxis against GBS disease. Guidelines are based on the collective evidence showing a beneficial effect of intrapartum prophylaxis in preventing early-onset GBS sepsis. The most widely accepted recommendations are those proposed by the US Centers for Disease Control and Prevention. Based on these recommendations, the following summary points can be made.

- ¦ Obstetric practitioners, in conjunction with supporting laboratories and labor and delivery facilities, should adopt a strategy for the prevention of early-onset GBS disease.
- ¦ Intrapartum chemoprophylaxis is recommended for women with GBS bacteriuria. Such women are usually heavily colonized with GBS. Women who have symptomatic or asymptomatic GBS urinary tract infections in pregnancy should be managed according to curent standards of care for urinary tract infections in pregnancy.
- ¦ Women who have previously given birth to an infant who had GBS disease should receive intrapartum chemoprophylaxis; prenatal screening is not necessary for these women.

In 1996, it was suggested that two approaches were appropriate for prevention of GBS disease, pending the availability of additional data: a screening-based and a risk-factor-based approach. As shown in Table 66c.1 , in the screening-based strategy, in which women are cultured at 35–37 weeks gestation and intrapartum antimicrobial prophylaxis is offered to those who have pre-term deliveries and all GBS carriers, theoretical modelling suggested that an estimated 86% of GBS disease could be prevented. The trade-off was that proportionately more women would have received antibiotics (26.7%) compared with a risk-factor-based approach (18.3%). Currently, revised

TABLE 66.C-1 -- Estimated impact of group B streptococcal prevention strategies.*

ESTIMATED IMPACT OF GROUP B STREPTOCOCCAL PREVENTION STRATEGIES.		
Strategy	**Early onset GBS disease prevented (%)**	**Deliveries receiving IAP (%)**
Recommended screening-based strategy: culture at 35–37 weeks; IAP for pre-term deliveries and all GBS carriers	86.0	26.7
Recommended risk-factor-based strategy: no prenatal cultures; IAP for all women who have intrapartum risk factors	68.8	18.3
Previous strategy recommended by the American Academy of Pediatrics: culture at 26–28 weeks; IAP for GBS carriers who develop risk factors	50.7	3.4
Boyer and Gotoff (1985) found that the proportion of deliveries among women who had positive prenatal cultures and who went on to develop intrapartum risk factors was 4.6%. IAP, intrapartum antimicrobial prophylaxis.		

** The figures are derived from the Centers for Disease Control and Prevention (1996) and Rouse et al. (1994).*

guidelines suggest that the former strategy is more effective and should be preferred. In order to put these revised guidelines in context, the 1996 guidelines are briefly summarized.

Screening-based approach

All pregnant women should be screened at 35–37 weeks gestation for anogenital GBS colonization. Patients should be informed of the screening results and of the potential benefits and risks of intrapartum antimicrobial prophylaxis for GBS carriers. Information systems should be developed and monitored to ensure that prenatal culture results are available at the time and place of delivery. Intrapartum prophylaxis should be offered to all pregnant women identified as GBS carriers by culture at 35–37 weeks gestation.

If the result of GBS culture is not known at the time of labor, intrapartum antimicrobial prophylaxis should be administered if one of the following risk factors is present:

- <37 weeks gestation;
- duration of membranes ruptured =18 hours; or
- body temperature =100.4°F (=38°C).

Culture techniques that maximize the likelihood of GBS recovery should be used. Because lower vaginal and rectal cultures are recommended, cultures should not be collected by speculum examination.

Laboratories should report results to both the anticipated site of delivery and the health care provider who ordered the test. Ideally, laboratories that perform GBS cultures will ensure that clinicians have continuous access to culture results

Risk-factor-based approach

A prophylaxis strategy based on the presence of intrapartum risk factors alone (e.g. <37 weeks gestation, duration of membrane rupture =18 hours or temperature =100.4°F/38°C) was considered as an acceptable alternative. However, in the revised guidelines below, the risk-factor-based approach is now considered to be inferior to the screening-based approach.

Women who have previously delivered a GBS-affected newborn and those who have GBS bacteriuria in pregnancy quality for prophylaxis regardless of their colonization status.

Revision of group B streptococcus prophylaxis guidelines, 2002

Data generated in the USA have suggested that the theoretical models are correct in indicating that the screening-based strategy is more effective than the risk-factor-based strategy. The revised recommendations suggest that all pregnant women should be screened at 35–37 weeks gestation for vaginorectal colonization. At the time of labor or rupture of membranes, intrapartum antimicrobial prophylaxis should be offered to all women identified as GBS carriers. If the results of cultures are not known at the time of labor, the risk-factor-based strategy becomes the default option.

If the results of GBS culture are not available and women present with onset of labour or rupture of membranes prior to 37 weeks gestation with a substantial risk of preterm delivery, intrapartum prophylaxis should be provided pending culture results.

As with previous guidelines, prophylaxis is recommended for women with GBS bacteriuria and those who have previously delivered an infant with invasive GBS disease, regardless of colonization status. Thus, screening at 35–37 weeks is not necessary for these women.

Figure 66.C-1 Management of neonates whose mothers received IAP. The flow chart summarizes the management of neonates born to mothers who have received IAP for early-onset GBS-disease (2002 revision). Full diagnostic evaluation includes full blood count and differential white cell count, blood culture and chest radiograph. A lumbar puncture is performed at the discretion of the clinician. Limited evaluation includes full blood count and differential white cell count plus blood culture. The duration of IAP stated applies only to penicillin, ampicillin and cefazolin prophylaxis.

Women who are colonized and have a planned cesarean section prior to rupture of membranes and the onset of labor do not warrant routine prophylaxis. These women are felt to be at an extremely low risk of delivering an infant who has early-onset GBS.

The recommendations for the prevention of GBS disease are accompanied by a suggested approach to the management of neonates born to mothers who have received intrapartum antimicrobial prophylaxis (Fig. 66c.1). This approach is based on the gestation age of the neonate, the presence or absence of symptoms and signs of sepsis and whether sufficient time has elapsed between intrapartum antimicrobial prophylaxis and delivery. Antibiotics must be administered at least 4 hours prior to delivery to allow for adequate antibiotic levels in amniotic fluid.

Management of a pregnant woman with a previous affected newborn

A woman who has lost an infant as a result of GBS disease requires the usual understanding and support given to any woman who has lost an infant during the neonatal period. Intrapartum prophylaxis is recommended regardless of screening cultures because of the previous delivery of a baby who had GBS disease. Routine vaginal-rectal screening for GBS is not necessary in this setting. However, it would be appropriate to obtain urine cultures at different antenatal visits to determine whether GBS bacteriuria is present.

Penicillin G (5 megaunits intravenously initially followed by 2.5 megaunits intravenously q4h) should be given until delivery. Ampicillin (2g intravenously initially and then 1g intravenously q4h until delivery) is an acceptable alternative. Penicillin G is preferred because it has a narrow spectrum and is thus potentially less likely to select out resistant bacteria. Women who are allergic to penicillin should receive clindamycin or erythromycin if they are considered to be at high risk of anaphylaxis. If they are thought to be at low-risk for anaphylaxis, cefazolin is recommended. Vancomycin should be reserved in women who are penicillin-allergic, but are unable to receive clindamycin due to drug resistance (Table 66c.2).

Group B streptococci are associated with various complications during pregnancy. These include septic abortion, urinary tract infections, chorioamnionitis, wound infection and endometritis. Although intrapartum antimicrobial prophylaxis may have a beneficial effect on endometritis, an assessment is necessary in the immediate postpartum period in order to guide further antibiotic therapy directed at the mother.

The newborn infant of a mother who has received intrapartum prophylaxis requires a special management approach. The approach outlined in Figure 66c.1 may be used as a guide to the management of neonate. However, if the infant is believed to have invasive GBS disease, the following apply.

- Penicillin G or ampicillin plus an aminoglycoside is the initial treatment. Penicillin G may be given alone when GBS is proved to

be the etiologic agent and clinical and microbiologic responses have been documented.
- In cases of meningitis, a second lumbar puncture at 24 hours after the start of treatment is recommended by some experts; this may have prognostic significance.
- Bacteremia without a defined focus should be treated for a minimum of 10 days.
- Uncomplicated meningitis should be treated for 14–21 days (longer periods of treatment are needed in infants who have complicated courses).

¦ Osteomyelitis should be treated for a minimum of 4 weeks.
¦ There is a high incidence of co-infection among twins, and the twin of an index case should be observed or evaluated for sepsis as clinically indicated.

TABLE 66.C-2 -- Alternative antibiotic regimens for intrapartum antimicrobial prophylaxis.

ALTERNATIVE ANTIBIOTIC REGIMENS FOR INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS	
Drugs	**Doses**
Penicillin	5 megaunits iv load, then 2.5 megaunits iv q4h
Ampicillin	2g iv load, then 1g iv q4h
Cefazolin	2g iv load, then 1g iv q8h
Clindamycin	900mg iv q8h
Erythromycin	500mg iv q6h
Vancomycin	2g iv load, then 1g iv q12h

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66.d Managing the pregnant woman exposed to or developing varicella

Barbara Law

Definition of the problem

The lifetime risk of becoming infected with varicella-zoster virus (VZV) is 95%. From 80% to 95% of infections occur before the age of 15 years with the highest rates among toddlers and primary school children. In temperate climates maternal chickenpox occurs 0.6–3 times per 1000 pregnancies giving rise to potentially serious consequences for both the mother and infant including:

- severe maternal disease complicated by potentially fatal pneumonitis;
- intrauterine infection resulting in fetal death, a characteristic fetal varicella syndrome or premature onset of labour; and
- if maternal infection onsets during the week before or after delivery, then severe, potentially fatal, neonatal infection.

These outcomes are preventable through the appropriate and timely use of immunoprophylaxis and antiviral therapy. The window for action is narrow, however, and it is essential to know who is at risk for infection and to manage both exposures and infection rapidly and appropriately.

A safe and effective live attenuated vaccine to prevent varicella is available in many countries and in the USA and Canada is recommended for universal use in childhood. Vaccine is also recommended for susceptible nonpregnant women of childbearing age provided they avoid pregnancy for 1 month following each dose of vaccine. In the USA, where vaccine coverage has been increasing steadily since 1996, significant reductions in the frequency of varicella are being observed. Nevertheless, chickenpox is still ubiquitous in the world community and women of childbearing age, especially those with young children at home, are likely to be exposed, giving rise to scenarios such as the one outlined below.

Perhaps because it is so frequent, nearly everyone, lay public and medical experts alike, has an opinion about the relative severity of disease in children versus adults. One of the more strongly held perceptions is that chickenpox is usually severe in adults and that pregnant women are particularly predisposed to a complicated course with pneumonia and high case fatality rates. Such beliefs stem from the inherent bias toward severity for data derived from hospitalized patients. Population-based data and prospective outpatient cohort studies suggest a different picture. Symptomatic pneumonia occurs in 3–10% of adults with chickenpox and the observed prevalence among pregnant women has been 3.4–5.2%. Most recover uneventfully with appropriate treatment. Risk factors for a more severe course include smoking and the presence of over 100 vesicles at the time of presentation. Nevertheless, while disease is mild to moderate in most cases, the mortality rate during pregnancy may be as high as 2–3% and thus appropriate and timely management of both exposures and proven infection is essential.

The risk to the fetus is also low but definite. The congenital varicella syndrome, of which the most notable and unique features are cutaneous cicatricial scarring (70%) and limb hypoplasia (50%), affects 1% of fetuses exposed during the first 20 weeks of gestation. The greatest risk, 2%, follows exposure between 13 and 20 weeks of gestation. From 20 weeks of gestation until just before delivery the risk of intrauterine infection rises, reaching 60% by the last month of the third trimester, but fetal infection is usually asymptomatic. In 0.8–1.7% of exposed infants, a mild case of herpes zoster occurs in infancy or early childhood. Of great concern, however, is when maternal rash onsets during the interval of 5 days before to 2 days after parturition. Their infants are exposed to the virus with no or minimal

ameliorating transplacental antibody transfer. From 30% to 60% will develop clinical infection and in the absence of appropriate management up to 30% may die.

Typical case

First consultation

During a routine prenatal visit at 28 weeks of gestation, a healthy 25-year-old pregnant with her second child mentions that her 3-year-old son broke out with chickenpox that morning. She cannot remember ever having had chickenpox as a child. She was known to be rubella immune from serology done during her first pregnancy but has never been tested for varicella. What is the appropriate management of this scenario?

Second consultation

Two weeks later the same patient calls to cancel her scheduled appointment because she is feverish, feels miserable and is very concerned that she is developing chickenpox. She decided against getting the immunoglobulin you recommended because it was a human blood product and she feared it might be contaminated. You suggest that she stays home but to call you back if she develops a rash similar to the one her son had, anytime over the next few days. Two days later she calls to confirm that she broke out in a rash overnight and is now feeling a bit short of breath. You arrange to see her at the local emergency department where appropriate isolation precautions can

Figure 66.d-1 Managing exposure to varicella during pregnancy.

be arranged. Physical examination confirms chickenpox based on the rash appearance. There are no other abnormalities, but because of her dyspnea you request a chest radiograph, which is normal. What treatment, if any, would you recommend at this point?

Third consultation

The next day the patient calls to say that her water has broken and she is in active labor. Ten hours later a 2000g infant girl is born by spontaneous vaginal delivery. The infant is transferred to the intermediate intensive care nursery where the attending staff judge her to be a normal premature infant of 30 weeks' gestation and in no acute distress. What is the appropriate management for the infant at this time?

Diagnosis

First consultation: establish varicella-zoster virus immune status

Blood should be drawn immediately for serum VZV antibody. Results should be available within 24 hours, leaving time for appropriate prophylaxis. Routinely available testing methods, usually by enzyme-linked immunosorbent assay (ELISA) or latex agglutination, are sufficiently sensitive to detect immunity. Ideally, all women should be screened for a history of varicella at the first opportunity during routine prenatal care. Chickenpox is still so prevalent and the course so typical that a personal history of typical infection is considered valid proof of immunity. For all others immunity should be

tested by serology. Among individuals with a negative or uncertain history of past chickenpox, 70–90% will be immune by currently available tests. Management of VZV exposures during pregnancy is relatively straightforward if the maternal immune status is known (Fig. 66d.1). To be effective varicella-zoster immunoglobulin (VZIG) prophylaxis must be given within 96 hours of the exposure. This window of opportunity is often compromised during last minute attempts, when more often than not, serology cannot be arranged, as at weekends and holidays.

Second consultation: confirm and determine severity of chickenpox

Chickenpox is usually a clinical diagnosis based on the characteristic pruritic papulovesicular rash with different lesion stages (macule, papule, vesicle, pustule, scab) present in the same area of involved skin. The presence of scalp lesions is especially characteristic of chickenpox. Onset of the rash during the usual incubation period (10–21 days) following a known exposure to chickenpox adds to the diagnostic certainty. Laboratory tests in individuals with known chickenpox should not be carried out to rule out suspected complications in the absence of specific symptoms or signs. Mild elevations of hepatic transaminases occur in 50% or more of adults but are transient in nature and do not predict a worse course. Mild thrombocytopenia (95,000 to <150,000) of no clinical consequence may be present in up to 25% of adults. Chest radiographs should be reserved for adults with dyspnea or other clinical evidence of respiratory distress because diagnosis based on radiographic changes alone can overestimate the incidence of pneumonia by a factor of 8. In the case above dyspnea was an appropriate indication to obtain a chest radiograph. The normal result was not unexpected based on a prospective

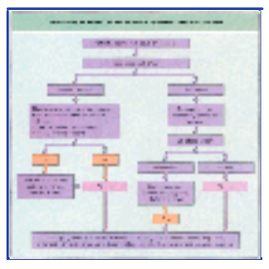

Figure 66.d-2 Managing chickenpox in the immunocompetent pregnant woman.

study by Harger *et al.* (2002) of 329 pregnant women with chicken-pox. Of 86 (26%) women with dyspnea, chest radiographs were normal in 68 (80%).

Management options

First consultation: varicella-zoster virus exposure

Figure 66d.1 presents an algorithm for management of VZV exposures during pregnancy. In this case serology revealed susceptibility to VZV, and VZIG should have been given as soon as possible after results were known. The likelihood of infection following household exposure to VZV is 80% or more. Varicella-zoster immunoglobulin is safe and significantly lessens the severity of maternal varicella. Steps in the manufacture of VZIG ensure the absence of risk of transmission of known blood-borne pathogens. Prevention of fetal infection is not guaranteed and this point should be made clear as part of counseling regarding the risks of infection.

Second consultation: varicella-zoster virus infection

Figure 66d.2 presents an algorithm for the management of VZV infection during pregnancy. Oral aciclovir may speed symptom and rash resolution if given within 24–48 hours of rash onset, but it is not routinely recommended for pregnant women with chickenpox because the risks to the fetus and mother are not clearly known. An ongoing registry of use in pregnancy, however, does suggest it is safe and can be considered in selected situations where the risk of pneumonia is high, as is true among smokers. Intravenous aciclovir is indicated for individuals with symptomatic pneumonia or other evidence of severe varicella. The earlier in the course of infection that treatment is started the better the outcome. Prior receipt of VZIG does not alter this recommendation although severe infection is much less likely to occur.

Third consultation: perinatal infant exposure to varicella-zoster virus

This infant is at risk of severe neonatal varicella because she was born within 4 days of the onset of maternal rash. She should be given VZIG at the earliest possible time after birth, preferably on the first day of life. Even with VZIG chickenpox is likely to occur and could be severe; thus, intravenous aciclovir should be started at the first sign of a vesicular rash.

Strict isolation precautions are required for the mother until her rash is completely crusted. Decisions regarding isolation of the infant are complex and should be made on a per case basis with input from neonatalogy, infectious disease, infection control and the parents.

Conclusions

Varicella is a treatable, and more importantly preventable viral infection. Evidence suggests that there is a great deal of room for improvement in the management of VZV exposures and infections during pregnancy. Screening for maternal immunity is simple but not routinely practiced. Prophylaxis with VZIG is available and effective but too often not used. Live varicella vaccine, contraindicated in pregnancy, has been mistakenly given to exposed pregnant women instead of VZIG. Intravenous aciclovir is live-saving for serious infection, but needs to be started promptly for maximum effect.

Establishing a woman's varicella immune status should be a routine part of early prenatal care. For the majority of individuals who do not remember having had chickenpox but are immune when tested, a great deal of needless anxiety can be avoided in the event of an exposure to chickenpox while pregnant. For the few individuals who are susceptible, advance knowledge is the key to appropriate management.

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Chapter 67 - Cystitis and Urethral Syndromes

Stephen T Chambers

Urinary tract infections (UTIs) are the second most common infectious cause for consultation and prescription of antibiotics among family physicians and are a common cause of morbidity in institutional care. Most infections are limited to the lower urinary tract but may cause pyelonephritis and bacteremia. The global incidence is estimated to be 2–3%, or at least 150 million cases per annum, costing billions of dollars annually.[1] Most cases of cystitis are uncomplicated and respond readily to antimicrobial treatment, but complicated infections, associated with anatomic or functional abnormalities, have an increased risk of therapeutic failure.

ACUTE CYSTITIS

EPIDEMIOLOGY

In the first 3 months of life UTIs are about three times more common in males than females, but thereafter infections occur more frequently in females. The prevalence of bacteriuria in preschool- and school-aged girls is 30 times higher than that in boys; 5–6% of girls will have had at least one episode of bacteriuria during their school-age years. Thereafter the prevalence of significant bacteriuria among females increases at about 1% per decade (Table 67.1). Men have low rates of bacteriuria until advanced age when rates rise dramatically.[2]

Among college women more that two-thirds of acute episodes may be attributable to intercourse.[3] Asymptomatic bacteriuria during childhood confers a risk of symptomatic UTI about the time women become sexually active. Other risk factors in this group include use of a diaphragm and spermicide. Less is known about the relationship between intercourse and UTI in older age groups, but previous symptomatic UTI is a risk factor for subsequent infections in both groups, with at least 20% of women developing a further infection within 6 months of the first.

In pregnancy asymptomatic bacteriuria occurs in 4–7% of women predisposing them to developing acute pyelonephritis (15–40% of cases) during the third trimester or in the puerperium. Up to 20% of such patients have significant abnormalities of the urinary tract. Bacteriuria of pregnancy has also been associated with increased risk of pre-eclampsia, lowered fetal birth weight, prematurity and increased perinatal mortality rates.[4]

In males UTI is associated with abnormalities of the urinary tract. In men with AIDS the risk is increased with a lowered $CD4^+$ count.[5]

In the elderly, both concurrent disease of the urinary tract and other medical conditions contribute to the high prevalence of UTI (Table 67.2). Instrumentation is associated with an increased risk of infection of 1% of ambulatory patients and 5–10% of hospitalized patients.

PATHOGENESIS

Infecting organisms

In uncomplicated cystitis more than 95% of infections are caused by a single organism. The most common pathogen is *Escherichia coli* (80–90% of cases) and *Staphylococcus saprophyticus* accounts for 10–20% of cases in young women during late summer and autumn (Table 67.3).[6] A small number of serotypes of *E. coli* account for most UTIs and some may be clonal.[7]

Complicated infections and infections among the institutionalized elderly may be polymicrobial (30% of cases), particularly when stones are present. [8] There is an increased incidence of resistant Gram-negative, *Pseudomonas aeruginosa* and yeast infections in this group. Urease-producing organisms *(Proteus, Providencia* and *Morganella* spp.) are of concern because urease leads to the conversion of urea to ammonia, an increase in the pH of urine, precipitation of struvite crystals ($MgNH_4PO_4.6H_20$), and stone formation.

Mechanisms

Cystitis is almost always caused by ascending infection, although *Staphylococcus aureus* may infect the urine from the bloodstream. The increased susceptibility of women to UTI is probably related to the shorter distance between the anus and urethral orifice, shorter length of urethra and the absence of the antibacterial barrier provided by prostatic fluid in males. In other respects the pathogenesis is similar in men and women and depends on a series of complex, interdependent host-parasite interactions that enable colonization of the periurethral area from the bowel, ascent of organisms into the bladder, growth in urine, tissue invasion and immune response (Fig. 67.1).

Colonization

The region between the anus and urethra is normally colonized by specialized flora. Lactobacilli, particularly hydrogen peroxide producing strains, inhibit colonization with enteric organisms. Spermicides (nonoxynol-9), diaphragms, estrogen deficiency and antibiotics (particularly ß-lactams) may cause a reduction in the number or concentration of these organisms and increase colonization by enteric organisms.[9]

Some women with recurrent UTI have an increased susceptibility to colonization of the periurethral zone with enteric organisms that persists between episodes.[10] *Escherichia coli* adheres more to vaginal epithelial cells from these patients than from controls. Nonsecretors of histo-blood group antigens (Lewis blood group [Le(a+b-)] and recessive [Le(a-b-)] phenotypes) are at greater risk of recurrent UTI.[11] Likewise, bowel carriage of *E. coli* possessing DNA sequences for P fimbriae, is more common among patients with the P blood group.[12]

Ascent

Bacteria normally enter the bladder by ascending along the mucosal sheath. This may be introduced mechanically by instrumentation or sexual activity. The mechanisms are poorly understood but include Brownian motion augmented by motile flagella.

Fimbriae: role in mucosal adherence and inflammation

Symptomatic bacteriuria is highly correlated with the presence of bacteria that mediate attachment to uroepithelial cells. Type I (mannose-sensitive) fimbriae are important in initiating colonization

TABLE 67-1 -- Prevalence of bacteriuria in different age groups.

PREVALENCE OF BACTERIURIA IN DIFFERENT AGE GROUPS		
	Group	**Prevalence (%)**
Females	Schoolgirls	1.2
	Sexually active young women	2–4
	Women	
	>60 years	6–8
	70 years	5–10
	80 years	20
	Institutionalized elderly	30–50

Males	Childhood to middle age	<1
	Men	
	60–65	1–3
	>80 years	>10
	Institutionalized elderly	20–30

TABLE 67-2 -- Risk factors for lower urinary tract infection.

RISK FACTORS FOR LOWER URINARY TRACT INFECTION			
Young adults	Women	Past history of UTI	Parity
		Sexual intercourse	Diabetes (women)
		Diaphragm use	Primary biliary cirrhosis
		Spermicide	Sickle cell anemia (pregnancy)
			Instrumentation
	Men	Lack of circumcision	Homosexual activity
		AIDS	
Elderly people	Women	Loss of estrogen effect	Abnormalities of urinary tract
		Incomplete emptying of bladder	Rectoceles
			Urethroceles
			Bladder diverticula
	Men	Strictures	Prostatic disease
		Instrumentation	Benign enlargement
			Calculi
			Loss of bactericidal secretions
	Both sexes	Neurologic disease	
		Alzheimer's disease	Cerebrovascular disease
		Parkinson's disease	

of the bladder, but are not expressed subsequently following phase variation of the organisms. This may prevent binding to Tamm-Horsfall protein and IgA, and decrease recognition by phagocytic cells, which possess type I receptors.[13] Type II fimbrial attachment (mannose-resistant) is mediated by P fimbriae (Gal-Gal), which are associated with pyelonephritis, and attach to a variety of receptors associated with the globose series of glycolipids, including Gal-Gal and Globo A structures. [14] Attachment of bacteria to the mucosa and cell wall components, such as lipid A, activate an inflammatory response, including production of tumor necrosis factor (TNF) and interleukin (IL)-2, IL-6 and IL-8, and attraction of inflammatory cells.

In contrast, asymptomatic bacteriuria is usually caused by organisms that neither possess fimbriae nor excite an inflammatory response unless tissue invasion occurs. The molecular mechanism by which they reach the bladder and maintain colonization is unknown.[15]

TABLE 67-3 -- Organisms associated with urinary tract infections.

ORGANISMS ASSOCIATED WITH URINARY TRACT INFECTIONS		
Common organisms	*Escherichia coli*	
	Staphylococcus saprophyticus	
Less common organisms	*Klebsiella* spp.	*Pseudomonas aeruginosa*
	Enterobacter spp.	*Acinetobacter* spp.
	Proteus spp.	*Serratia* spp.
	Morganella spp.	Yeasts
	Citrobacter spp.	*Corynebacterium urealyticum*
	Group B streptococcus	
	Group D streptococcus	
	Enterococci	
Rare infections	*Haemophilus influenzae*	*Salmonella* spp.
	Mycobacterium tuberculosis	*Shigella* spp.
	Anaerobes	Adenovirus (type 11)
Unproven causes	*Gardnerella vaginalis*	
	Ureaplasma urealyticum	
	Mycoplasma hominis	

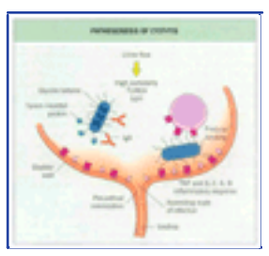

Figure 67-1 Pathogenesis of cystitis. Factors favoring bacterial persistence and infection include bacterial binding to bladder mucosa (fimbriae), and high bacterial growth rates despite high osmolarity and urea concentrations and low pH. Factors favoring bacterial elimination include high urine flow rate, high voiding frequency, bactericidal effects of bladder mucosa, secreted proteins that bind to fimbrial adhesins and the inflammatory response. IL, interleukin; TNF, tumor necrosis factor.

Urodynamics

Normal structure and function of the urinary tract promotes elimination bacteria from the urinary tract because voiding eliminates free organisms and the bladder mucosa is bactericidal to many organisms on contact. Similarly adequate urine flow promotes removal of bacteria from the upper urinary tracts. These defense mechanisms are potentially compromised by low urine flow rate,

infrequent voiding, residual bladder urine and reflux of urine.[16] Neurologic disease, diabetes, debility, and anatomic changes impair bacterial clearance by these mechanisms, particularly in the elderly.

Growth in bladder urine

Growth in urine is essential for invasion of the urinary tract. Gonococci, anaerobes and urethral commensals are inhibited whereas uropathogens grow well in urine. Extremes of pH (<5.5 and >7.5), high tonicity, high urea concentrations and dietary-derived weak organic acids have some inhibitory effects on growth. Bacteria derive protection from the inhibitory effects of high osmotic forces by the intracellular accumulation of osmolytes normally present in urine (e.g. glycine betaine). The osmolytes also counteract the toxicity of urea and low pH by stabilizing macromolecular structures.[17] Osmotic pressure also has a significant influence on the expression of fimbriae genes, but the importance of this is uncertain. Cranberry juice has a slight antibacterial effect due aromatization of quinic acid to benzoic acid by enteric bacteria and subsequent conversion to hippuric acid in the liver.

Immune response

Locally produced urinary antibodies produced in response to febrile UTI (monomeric and dimeric IgA and IgG) decrease adherence by interference with adhesion receptors and agglutination of bacteria. Hyperimmunization can protect animals against experimental UTI.[18]

PREVENTION

Normal urinary tract

Women who suffer recurrent or closely spaced symptomatic UTIs suffer considerable morbidity and anxiety. When no cause is found, advice is often given to empty their bladder completely, maintain a high fluid intake and to void after intercourse, but there is little evidence to support these measures. Application of antiseptic cream (e.g. 0.5% cetrimide) to the urethral oriface may prevent infections. Women who use a diaphragm and spermicide should consider alternative methods of contraception. Postmenopausal women with recurrent UTI have been shown to benefit from the application of estriol vaginal cream (0.5mg/day for 2 weeks then twice weekly). [19]

If these measures fail, chemoprophylaxis should be considered (Table 67.4). Low-dose antibiotics are effective if taken nightly three times weekly or after intercourse.[20] Efficacy continues for up to 5 years. Nitrofurantoin is an excellent agent because it does not alter the fecal flora, and resistance during treatment is rare. Self-initiated therapy is effective in selected groups and should be reserved for those who are not at risk of sexually transmitted infections (STIs) or pregnancy and who do not have significant co-morbid condition.[21] See Figure 67.3 for a treatment algorithm.

TABLE 67-4 -- Drug regimens for prophylactic therapy administered as a single dose at night.

DRUG REGIMENS FOR PROPHYLACTIC THERAPY ADMINISTERED AS A SINGLE DOSE AT NIGHT	
Drug	**Dose**
Nitrofurantoin	50mg
Trimethoprim	100mg
Trimethoprim-sulfamethoxazole (co-trimoxazole)	480mg
Norfloxacin	200mg
Cephalexin	125mg
Hexamine hippurate	1.0g

Abnormal urinary tract

Any lesions in the urinary tract should be corrected if possible. Urologic referral is essential. Large residual volumes and high pressure may require intermittent self-catheterization. Low-dose chemoprophylaxis is often effective.

Vaccine

There is no vaccine currently available, although there has been interest in the development of vaccines directed against type I and type II pili. These are more likely to be useful for the prevention of pyelonephritis than for the prevention of cystitis, but anatomic and functional abnormalities may limit their applicability.

CLINICAL FEATURES

Symptomatic infection

The dominant complaint in cystitis is usually painful micturition (dysuria), which may be associated with frequency, urgency, strangury, initial and terminal hematuria, suprapubic discomfort and voiding small amounts of turbid urine. Low-grade fever may occur, but is usually absent. Pyuria or hematuria are almost always present. Elderly patients usually have similar symptoms but occasionally present with incontinence or smelly urine, or in men, epididymo-orchitis.[22]

Dysuria caused by bacterial cystitis should be distinguished from that caused by vulvitis and urethritis (see Chapter 62). In vulvitis there is often labial discomfort as the stream of urine is passed. Genital herpes and candidiasis are common causes. In urethritis there may be a history of a new sex partner, urethral discharge, mucopurulent cervicitis or bartholinitis. *Chlamydia trachomatis,* and *Neisseria gonorrhoeae* are the usual causes.

Dysuria caused by chronic conditions such as interstitial cystitis and *Mycobacterium tuberculosis* infection may cause initial confusion but usually persists following attempts at treatment.

Asymptomatic bacteriuria

Asymptomatic bacteriuria is very common in elderly patients. It is poorly correlated with fatigue, poor appetite and urinary incontinence. Similarly, symptoms such as frequency, dysuria and hesitancy, which may be caused by infection are common and nonspecific and often fail to respond to treatment of coexisting bacteriuria. [23] Asymptomatic UTIs have been shown to increase morbidity in pregnancy but not in the elderly.

DIAGNOSIS

The diagnosis of UTI can only be proven by culture of an adequately collected urine sample. This is essential in all suspected cases in males, infants and children. A presumptive diagnosis can be made in sexually active women in the presence of typical clinical features together with the presence of pyuria if sexually transmitted infections are unlikely.

Pyuria

The preferred method for assessment of pyuria is microscopic examination of uncentrifuged fresh urine using a hemocytometer, although counts per microscopic field are reasonably reliable in the clinical laboratory. Urine from adult patients who have symptomatic UTI almost always (>96%) contains more than 10 leukocytes/ml.

Pyuria occurs less frequently in asymptomatic bacteriuria of pregnancy (50% positive) and the elderly (90% positive).

Pyuria alone is not a reliable predictor of infection. Specimens from women with vaginitis often contain white cells and there are many other causes of inflammation within the urinary tract (Table 67.5)

Urine dipsticks using esterase provide a simple inexpensive method for detecting pyuria. A positive test indicates a minimum of eight white blood cells per high power field and has a sensitivity of

TABLE 67-5 -- Conditions associated with pyuria but without culturable bacteria using standard bacterial isolation techniques.

CONDITIONS ASSOCIATED WITH PYURIA BUT WITHOUT CULTURABLE BACTERIA USING STANDARD BACTERIAL ISOLATION TECHNIQUES	
Recent treatment of UTI	
Organism not culturable on usual bacterial media	Adenovirus
	Anaerobes
	Chlamydia trachomatis
	Fungal infections
	Herpes simplex
	Leptospirosis
	Myobacterium tuberculosis
	Neisseria gonorrhoeae
Noninfectious causes	Cyclophosphamide therapy
	Foreign bodies
	Glomerulonephritis
	Interstitial cystitis
	Neoplasms
	Stones
	Transplant rejection
	Trauma
	Tubulointerstitial disease
	Vaginal contamination

88–95% and specificity of 94–98% compared with the counting chamber method. The presence of blood, rifampin (rifampicin), nitrofurantoin, bilirubin and ascorbic acid may result in a false-negative test, whereas trichomonads, imipenem and amoxicillin-clavulanate may give a false-positive test.[24]

Detection of bacteria

The presence of bacteria on microscopy of urine correlates well with culture results. Experienced laboratories can reliably detect 10^8 organisms per liter in unstained specimens. The nitrite test is reliable for detecting Gram-negative bacilli in first morning urines but not other specimens because prolonged incubation in the bladder is required to allow reduction of nitrate to nitrite.

Midstream urine specimens

The simplest method for obtaining urine for quantitative culture is to collect a clean midstream specimen. This minimizes the confounding effects of contamination from the first few milliliters of urine.

The sensitivity and specificity of quantitative culture for diagnosis of UTI is dependent on the presence of symptoms and pyuria:

- in asymptomatic women, a single count of 10^4 –10^5 /ml has a 95% chance of representing contamination;[25]
- in symptomatic women with pyuria (>10 white blood cells/ml) a single count of more than 10^5 /ml has a very high specificity (>99%) but a low sensitivity (51%) for UTI.[26]

Routine application of this criterion would fail to diagnose one-third of women with a UTI (Table 67.6).

Specimens from males are less likely to be contaminated and lower counts (>10^3 /ml) are highly predictive of infection. Grampositive bacteria and yeasts in urine tend to be associated with lower counts than Gram-negative bacilli.

In practice specimens are often poorly collected and delays in processing without refrigeration allow bacterial multiplication. In the

TABLE 67-6 -- Value of quantitative urine culture in diagnosis of UTI with Gram-negative bacilli in women.

VALUE OF QUANTITATIVE URINE CULTURE IN DIAGNOSIS OF URINARY TRACT INFECTION WITH GRAM-NEGATIVE BACILLI IN WOMEN				
	Number of specimens	**Organisms/ml of urine**	**Sensitivity (%)**	**Specificity (%)**
Asymptomatic women	two	>10^5	>95	>80
Symptomatic women with pyuria	one	>10^5	51	99
	one	>10^3	80	90
	one	>10^2	95	85

presence of symptoms and pyuria, a bacterial count greater than 10^3 /ml is a reasonable criterion for significant bacteriuria in routine laboratories, bearing in mind that this represents ten organisms on a plate if a 0.01ml loop is used. Others argue that over 10^4 /ml is more realistic. False-negative results can occur in the presence of obstruction, antimicrobial agents and, possibly, diuresis.

Suprapubic aspiration

Suprapubic aspiration of urine from a distended bladder is an efficient means of diagnosis. Any bacteria identified can be regarded as significant because the technique avoids contamination. In most infected specimens bacteria can be seen microscopically, and so treatment can be started promptly. Provided the patient has a full bladder it is safe and acceptable.

Catheter specimens

Catheterization specifically for a urine culture may be justifiable if the patient is unable to co-operate to obtain an uncontaminated sample or to hold urine in the bladder for a suprapubic aspiration. Catheterization rarely leads to false-positive results, but may introduce bacteria into the bladder. Straight plastic catheter or Alexa bag techniques are satisfactory.

Imaging of the urinary tract

All men, children and infants need investigations of the urinary tract if they have a UTI regardless of the clinical features at presentation. This is not cost-effective in women unless there is some evidence of an unusual clinical pattern,[27] such as urinary infection as a child, treatment failure and persistent microscopic hematuria or pyuria at follow-up. An ultrasound examination including postmicturition bladder volumes plus a plain abdominal radiograph, including the kidneys, ureters and bladder, or an intravenous urogram, are adequate in most instances. Cystoscopy rarely yields useful information in women with acute cystitis.

MANAGEMENT

Untreated 50–70% of lower UTIs will clear spontaneously although symptoms may persist for many months. Thus the goal of treatment is to eradicate the infection, and to reduce morbidity caused by relapse or recurrence with minimum toxicity, inconvenience and distress for the patient. The cornerstone of management is effective antimicrobial therapy (Table 67.7). Drinking large amounts of fluids may decrease bacterial counts and improve symptoms, but adds little to effective antimicrobial therapy. Likewise alkalinizing agents may decrease symptoms, but do not influence bacterial eradication.

Follow-up visits at 7–14 days after completion of therapy give the opportunity to obtain urine cultures and discuss the importance of

TABLE 67-7 -- Drug treatment regimens for a 3-day course of oral therapy for bacterial cystitis.

DRUG TREATMENT REGIMENS FOR A 3-DAY COURSE OF ORAL THERAPY FOR BACTERIAL CYSTITIS	
Drug	**Dose**
Trimethoprim	300mg q24h
Trimethoprim-sulfamethoxazole (co-trimoxazole)	960mg q12h
Ciprofloxacin	250mg q12h
Fleroxacin	400mg q24h
Lomefloxacin	400mg q24h
Nalidixic acid	500mg q8h
Norfloxacin	400mg q12h
Amoxicillin	250mg q8h
Amoxicillin/clavulanate	500/125mg q12h
Cephalexin	250mg q8h
Cephradine	250mg q8h
Pivmecillinam	200mg q8h

Figure 67-2 Treatment of uncomplicated cystitis in a nonpregnant woman.

the diagnosis. It is essential to relieve anxiety about sexual activity and perceived long-term consequences, and to discuss advice offered by the family and the popular press.

Acute uncomplicated bacterial cystitis

Short-course therapy has now become the standard for treatment in clinical practice for most treatment regimens.[28] Short-course therapy is contraindicated in complicated infections. Advantages of short-course therapy include better compliance, lower cost, fewer side-effects and decreased likelihood of the emergence of resistant strains. A treatment algorithm is given in Figure 67.2 .

Three-day therapy

A 3-day regimen has become the recommended approach for many clinicians because it provides the advantages of short-course therapy and has a slightly higher success rate than single-dose therapy, particularly in older women.[29] It is appreciated by patients because it may take several days for symptoms to abate. Three days of trimethoprim-sulfamethoxazole (TMP-SMX) (co-trimoxazole), trimethoprim alone, the quinolones or pivmecillinam are as effective as longer courses with fewer side-effects. ß-lactams as a group are less effective than trimethoprim, TMP-SMX or the quinolones. There is insufficient evidence to recommend nitrofurantoin for 3 days rather than 7 days

Single-dose therapy

Single-dose therapy is essentially 1 day of treatment given in a single dose that produces inhibitory concentrations of antibiotic over a 12–24-hour period. It is most suitable for cystitis in sexually active women and for younger patients who have normal urinary tracts and a short history (<7 days) (Table 67.8).

Amoxicillin is less effective than either TMP-SMX or trimethoprim alone and is no longer recommended in single-dose regimens. Fluoroquinolones are very effective, although they may be less effective against *S. saprophyticus* than Gram-negative bacilli. Fosfomycin trometamol is marketed specifically as single-dose therapy and is highly effective but is relatively expensive.

Those who fail to respond to single-dose therapy have an increased risk of abnormalities within the urinary tract.[30]

Therapeutic agents

Both TMP-SMX and trimethoprim (TMP) alone are highly effective, but as sulfonamides have occasional severe side-effects, especially in the elderly, TMP is preferred. There is no evidence that the combination with sulfamethoxazole prevents the emergence of resistance.

The fluoroquinolones have essentially superseded nalidixic acid and oxolinic acid. All these agents are extremely active and effective against most pathogens, including many hospital pathogens. Norfloxacin is usually the most inexpensive fluoroquinolone for the treatment of cystitis in some countries.

Nitrofurantoin is ineffective against *Proteus mirabilis*. The side-effects of nausea and vomiting can be minimized by treating with 50mg q8h without loss of efficacy, rather than 100mg q6h.

Mecillinam has been widely used in Scandinavia without apparent resistance problems developing in Gram-negative organisms. In-vitro studies show resistance in Gram-positive organisms, but clinical studies show that there is a high cure rate for *S. saprophyticus*,

TABLE 67-8 -- Suggested drug treatment regimens for single-dose therapy.

SUGGESTED DRUG TREATMENT REGIMENS FOR SINGLE-DOSE THERAPY	
Drug	**Dose**
Trimethoprim	600mg
Trimethoprim-sulfamethoxazole (co-trimoxazole)	1.92g
Ciprofloxacin	500mg
Fleroxacin	400mg
Norfloxacin	800mg
Fosfomycin trometamol	3g

presumably because of the very high concentrations achieved in the urine.

Amoxicillin is the treatment of choice for treating *Streptococcus faecalis,* but increasing resistance has limited its usefulness against other uropathogens. Combinations of ß-lactams with ß-lactamase inhibitors may be effective, but there is a high rate of diarrhea. Cephalosporins such as cephalexin, cephradine and cefaclor are useful, particularly in renal failure.

Choice of initial therapeutic agent

Trimethoprim and TMP-SMX have been regarded as the agents of first choice but are less appropriate if resistance rates in *E. coli* and other Gram-negative organisms exceed 10–20%, and the quinolones offer an alternative choice. In-vitro resistance reduces the clinical success of TMP-SMX from 95 to 60% and bacterial eradication from 93 to 50%. Thus the expected clinical and bacterial success rates for Gram-negative infections will be 92 and 89%, respectively, for a 10% resistance rate, and 88 and 84%, respectively, for a 20% resistance rate. [31] The net effect on success from intention to treat will be smaller, because Gram-positive uropathogens remain susceptible to TMP or TMP-SMX.

The decision to change policy may be made more difficult because reliable estimates of resistance rates in the local setting are often lacking. Resistance rates reported by clinical laboratories may be misleading because isolates from patients who have failed therapy or are at a high risk of resistance are over-represented. [32] Using

Figure 67-3 Treatment of recurrent bacterial cystitis in a nonpregnant woman.

quinolones as a first choice may be a short-term solution because resistance to these agents, although currently low, is increasing worldwide and has reached 23% in Spain and 18% in Bangladesh.

Where resistance to TMP-SMX is a potential problem a reasonable approach is to use it unless the patient has risk factors for TMP-SMX resistance. These include previous infection with TMP-SMX-resistant organisms, recent use of TMP-SMX or another antimicrobial agent, recent hospitalization or recurrent UTI in the past year. Alternatives include fluoroquinolones (3 days), nitrofurantoin (5–7 days), pivampicillin (3 days) and fosfomycin (single dose).

Recurrent infections

The major problem with uncomplicated acute cystitis is recurrence. About one-half of adult women will have another infection within 1 year, many within 3 months. Recurrence rates vary (0.3–7.6 episodes per year) and may occur in clusters. With treatment of each episode 20–30% will cease having recurrences. [33] If episodes are closely spaced, self-administered therapy, preferably after obtaining a urine specimen, postcoital therapy or prophylaxis can be considered. Self-initiated therapy should be reserved for those who are not at risk of STIs or pregnancy, and do not have significant co-morbid conditions.[21] A treatment algorithm is given in Figure 67.3 .

Complicated infections

Patients who fail short-course treatment often have abnormalities of the urinary tract (e.g. stones, diverticula, strictures, chronic bacterial

Figure 67-4 Treatment of asymptomatic bacteriuria in pregnancy.

TABLE 67-9 -- Possible toxicities of antimicrobial agents in pregnancy.

POSSIBLE TOXICITIES OF ANTIMICROBIAL AGENTS IN PREGNANCY	
Agent	**Toxicity**
Trimethoprim sulfonamides	Antifolate activity and megaloblastic anemia
Sulfonamides (protein bound)	Kemicterus of newborn

Sulfonamides/nitrofurantoin	Hemolytic anemia in glucose-6-phosphate dehydrogenase deficiency
Tetracycline	Fatty liver/hepatic necrosis in mother, stained teeth in baby
Fluoroquinolones	Not approved

prostatitis). These should be corrected where possible and infection treated with more prolonged courses of therapy of 10–14 days.

Urinary tract infections in pregnancy

The risks of symptomatic infection and pyelonephritis during the third trimester of pregnancy in mothers and prematurity in infants can be prevented by early detection and eradication of asymptomatic bacteriuria. In the first instance single-dose or a 3-day course of therapy are appropriate (Fig. 67.4). If relapse or re-infection occurs then the patients should be re-treated. At that stage the simplest strategy is to institute prophylaxis (e.g. nitrofurantoin 50mg at night), although some prefer close surveillance with repeated cultures and prompt treatment of each episode. All such patients need evaluation of the urinary tract with ultrasonography and a plain abdominal radiograph after delivery.

There is probably no absolute contraindication to any antimicrobial agent during pregnancy, but caution is urged with some agents.[34] The antifolate activity of trimethoprim and TMP-SMX is minimal in short courses and probably safe between 16 and 30 weeks of gestation (Table 67.9).

Males

Lower UTI in males may be complicated by infection of prostatic fluid, even if there is no clinical prostatitis.[35] For this reason there is reluctance to treat men with short regimens and studies of single-dose therapy in elderly men have been disappointing.[36] Long-term suppressive therapy may be helpful for frequent recurrences.

Asymptomatic bacteriuria

Asymptomatic bacteriuria is often best left untreated except in pregnancy. In elderly patients it is extremely common and there is no convincing evidence that treatment benefits the patient either in terms of recurrent symptomatic episodes or mortality rate. Antimicrobial therapy is associated with adverse effects, the potential for development of resistant strains, and financial cost.

Invasive procedures

Asymptomatic bacteriuria should be treated if a patient is to undergo an invasive procedure of the genitourinary tract. Mucosal trauma may cause postprocedural bacteremia, and occasionally septic shock and death. The antimicrobial agent should be selected on the basis of the sensitivity of the infecting organism. [37] Likewise, it is prudent to treat any urinary infections before the insertion of permanent indwelling devices, particularly prosthetic joints.

Treatment in the presence of renal failure

If treatment is truly indicated, drugs that achieve adequate urine concentrations in the presence of renal failure should be used. The best levels may be achieved with penicillins and cephalosporins. Trimethoprim and fluoroquinolones will probably achieve adequate concentrations, whereas nitrofurantoin, sulfamethoxazole and doxycycline are present in very low concentrations when creatinine clearance falls below about 0.16ml/s.

Infections with *Candida* spp.

Infections with *Candida* spp. may occur either from hematogenous spread or via the ascending route. There is an increased risk in those with diabetes, prolonged antimicrobial therapy and instrumentation of the urinary tract. The natural history has not been well-defined, but most infections are asymptomatic, limited to the lower urinary tract and may resolve in otherwise normal patients. Occasionally pyelonephritis, papillary necrosis or bezoars occur, especially in patients who have diabetes mellitus, obstructive uropathy or renal transplantation. Those at increased risk should be treated, but it is less clear for other patients. The treatment of choice for those without a catheter is oral fluconazole (50–100mg/day), although some species such as *Candida krusei* may be resistant.[38] A single dose of intravenous amphotericin B (0.3mg/kg) is usually effective, presumably because of prolonged renal excretion. Oral flucytosine and alteration of urinary pH are ineffective. If a catheter is present, bladder washouts with amphotericin B may be used, but are of questionable efficacy.

URETHRAL SYNDROMES

PATHOGENESIS AND CLINICAL FEATURES

Following the introduction of quantitative bacterial counts, about half of women with acute symptoms of cystitis did not have significant — $>10^5$ colony forming units (cfu)/ml — bacteriuria. These women were said to have acute urethral syndrome or dysuria-pyuria syndrome. Many women with acute urethral syndrome have periurethral colonization with uropathogenic organisms and low numbers of *E. coli, S. saprophyticus* and enteric Gram-negative organisms present in their urine. Furthermore many such patients responded to antibiotic therapy, suggesting that this was essentially a UTI. These patients probably have urethritis with little infection of the bladder and the diagnosis may be missed because of the low numbers of organisms present.

There remains a group with similar symptoms who do not have a low-count bacteriuria. It is possible that symptoms are caused by infection confined to the proximal urethra, especially if there is pyuria, but it is very difficult to diagnose. Various agents, including organisms that commonly cause UTIs (especially in women who suffer recurrent UTI), *Ureaplasma urealyticum, C. trachomatis* are possible pathogens. Multiple other causes have been suggested, including infection of the female paraurethral glands, lactobacilli, chemicals (e.g. bubble baths and deodorants), trauma and psychologic factors, but none have been proven.

MANAGEMENT

Management of the urethral syndrome is difficult. A pelvic examination should be carried out to exclude herpes simplex, gonorrhea and vaginitis. The symptoms usually settle in a few days, although some patients appear to benefit from a high fluid intake. Antimicrobial therapy may be helpful if pyuria is present, presumably reflecting bacterial urethritis or chlamydial infection. Antibiotics useful for treatment of UTI are often prescribed in the first instance. If these fail, doxycycline (100mg q12h) for 10 days may be effective.[39]

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Chapter 68 - Prostatitis, Epididymitis and Orchitis

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PROSTATITIS

The diagnosis of prostatitis syndrome refers to a variety of inflammatory and noninflammatory conditions probably not always affecting the prostatic gland itself. In 1978 a classification system[1] was developed to differentiate inflammatory from noninflammatory entities and was much used in the past. However, many aspects of chronic prostatic symptoms remained enigmatic. In 1999 a consensus conference at the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health developed a new classification system[2] [3] based on the clinical presentation of the patients, the presence or absence of leukocytes in the expressed prostatic secretion (EPS) or voided bladder urine after prostatic massage (VB_3), and the presence and absence of bacteria in the EPS or VB_3 . In addition, the inflammatory conditions are categorized into symptomatic and asymptomatic presentations identifying patients who are incidentally diagnosed from biopsies, evaluations for fertility disorders or routine examinations. The term chronic pelvic pain syndrome (CPPS) was chosen, because it has not been scientifically demonstrated either that CPPS is primarily a disease of the prostate or that it is an inflammatory process.[2] [3] Table 68.1 illustrates the most recent NIH concensus.

EPIDEMIOLOGY

Definition and nomenclature

Acute prostatitis is an acute febrile illness that may be characterized by intense pain in the perineum and rectum, fever, voiding difficulties,

TABLE 68-1 -- The National Institutes of Health consensus classification of prostatitis syndromes.

NIH CONSENSUS CLASSIFICATION OF PROSTATITIS SYNDROMES			
Category	**Characteristic clinical features**	**Bacteriuria**	**Inflammation***
I. Acute bacterial	Acute urinary tract infection (UTI)	+	+
II. Chronic bacterial	Recurrent UTI caused by the same organism	+	+
III. Chronic prostatitis chronic pelvic pain syndrome	Primarily pain complaints, but also voiding complaints and sexual dysfunction		
A. Inflammatory subtype†		-	+
B. Noninflammatory subtype‡		-	-
IV. Asymptomatic	Diagnosed during evaluation of other genitourinary complaints	-	+

* Objective evidence of an inflammatory response in EPS, postprostate massage urine or semen or by histology.
† formerly termed 'nonbacterial prostatitis'.
‡ Formerly termed 'prostatodynia'.

systemic symptoms of sepsis and a tender, swollen prostate on rectal examination. The chronic prostatitis syndromes (bacterial, nonbacterial and prostatodynia, or CPPS) cause symptoms that cannot be differentiated from each other (Table 68.2). In patients who have signs of inflammation, leukocytes (neutrophils, macrophages) are present in EPS or VB_3 . Pathogens must be present in EPS or VB_3 for a conclusive diagnosis of chronic bacterial prostatitis (CBP).[4] In noninflammatory CPPS (prostatodynia) no signs of inflammation are detectable. In asymptomatic inflammatory prostatitis detected either by prostatic histology or by the presence of leukocytes in seminal fluid or in prostate secretion during evaluation for other disorders, the patients have no subjective symptoms (see Table 68.1).

Incidence and prevalence

Because of classification difficulties, few data are available to determine the incidence of prostatitis. Acute prostatitis is infrequent, with a probable incidence of fewer than 1 in 1000 adult men per year. However, prostatic symptoms are common. In the USA approximately 30% of men between 20 and 50 years of age experience 'prostatitis-like' symptoms[5] and these symptoms are responsible for about 25% of physician office visits by men for genitourinary complaints.[5] Prostatitis is the most common urologic diagnosis in men under 50 years of age and the third most common urologic diagnosis in men over 50 years of age.[6] It has been demonstrated that a diagnosis of chronic prostatitis can have a quality of life impact similar to a diagnosis of angina or Crohn's disease.[7] Thus, prostatitis is a major health care issue, perhaps as important as the other two major prostatic diseases, namely benign hyperplasia and carcinoma.[8]

TABLE 68-2 -- Symptoms in patients with the chronic prostatitis syndromes.

SYMPTOMS IN PATIENTS WITH THE CHRONIC PROSTATITIS SYNDROMES	
Urethral symptoms	• Burning in the urethra during voiding
	• Discharge
	• Difficult urination
	• Stranguria
	• Frequency
	• Nocturia
	• Prostatorrhea
	• Leukocytospermia

Prostatic symptoms	• Pressure behind public bone
	• Perineal pressure tension in testes and epididymes
	• Inguinal pain
	• Anorectal dysesthesia
	• Diffuse anogenital syndromes
	• Lower abdominal discomfort
Sexual dysfunction	• Loss of libido
	• Erectile dysfunction
	• Ejaculatory dysfunction
	• Pain during or after orgasm
Other symptoms	• Myalgia
	• Headache
	• Fatigue

The term prostatitis implies inflammation, but only 5–10% of patients who have this diagnosis actually have a proven bacterial infection.[5] [9] The rest do not have 'significant' prostatic fluid bacterial counts. About half of these men have inflammatory CPPS/nonbacterial prostatitis (NBP) with an elevated leukocyte count in prostatic fluid.[4] [5] [9] [10] [11] [12] [13] [14] The rest are categorized as having noninflammatory CPPS/prostadynia. This is a diagnosis of exclusion and in most cases it cannot be proved that the symptoms arise from the prostate.

Risk factors

Urinary tract infections (UTIs) are the major underlying determinant of both acute bacterial prostatitis (ABP) and CBP. Strains of *Escherichia coli* responsible for both ABP and CBP appear to have similar urovirulence determinants to the *E. coli* strains that cause pyelonephritis. [15] Prostatic calculi can account for recurrences of CBP.[4] Bacterial micro-colonies enclosed within biofilms inside prostatic acini and ducts can be a foci for bacterial persistence. [12] Inflammatory CPPS/NBP may be due to intraprostatic reflux of urine causing inflammation.[16] Other presumed and unproven causes of inflammatory CPPS/NBP are immunologic reactions to spermatozoa and migration of sexually transmitted organisms from the urethra.

CLINICAL FEATURES

Diagnosis

Acute bacterial prostatitis is diagnosed by its clinical presentation. [14] It presents as an acute febrile illness with irritative and obstructive voiding symptoms. Prostatic massage is contraindicated and the diagnosis depends upon:

- urine and blood cultures;
- a gentle examination of the prostate that demonstrates acute inflammation; and
- a urinalysis, which usually demonstrates pyuria.

Prostatic abscess may occur in patients who have acute prostatitis. This diagnosis is made by clinical examination and transrectal ultrasonography. Focal hypoechoic zones with irregular internal echoes, septations and indirect borders with the surrounding parenchyma are typical patterns. The abscess may be distinct or more diffuse. Prostatic abscesses are usually due to the same uropathogens that are responsible for ABP, although a variety of anaerobes and fungi are implicated sporadically. Systemic mycoses, particularly *Cryptococcus neoformans, Blastomyces dermatitidis, Coccidioides immitis* or *Histoplasma capsulatum*, can involve the prostate gland and produce prostatic abscesses. *Candida albicans* can also cause prostatic abscesses.

Chronic bacterial prostatitis is a less precise diagnosis (see Table 68.2). Patients presenting with prostatic complaints should have a prostatic massage to localize the infection. The method of choice is the Meares and Stamey localization technique (Fig. 68.1).[17] Increased numbers of neutrophils and fat-laden macrophages are typical cytologic signs in the EPS. Although increased numbers of leukocytes may be found in EPS, it is generally accepted that over 10 neutrophils/high-power field indicates prostatitis.[14] [15] In patients for whom an EPS cannot be obtained increased numbers of neutrophils in the urine after prostatic massage (VB_3) is an indication of prostatitis if first voided urine (VB_1) and midstream urine (VB_2) do not contain these cells. In patients who have CBP, bacterial pathogens will be present in the EPS or VB_3 in larger numbers, usually a 10-fold higher concentration than in the VB_1 [14] [15] The exact technique for localizing infection with the Meares and Stamey technique is outlined in Figure 68.1 and should be followed carefully.[17]

The role of *Chlamydia trachomatis and Ureaplasma urealyticum* in bacterial prostatitis is uncertain and there are no widely accepted criteria for defining prostatitis due to these or other infrequently isolated pathogens (Table 68.3).[18] [19] [20]

Ejaculate analysis is sometimes recommended in men who have CBP to obtain further information but studies of seminal fluid are mostly unhelpful. A proportion of men who have CBP have bacteriospermia (>10^3 cfu/ml) and the organisms present are usually identical to those in the EPS.[21] Biochemical analysis of EPS has been used as an additional diagnostic criterion for CBP but these observations have not been shown to be sufficiently sensitive or specific to add to the diagnosis (Fig. 68.2).[14] [15] The pH is usually increased (>7.8) in the EPS from patients who have CBP.

Biopsy under ultrasonographic guidance, particularly if nodules are present, is used for histology and culture.[4] [10] [22] Inflammatory findings in the prostate are usually non-specific and the primary indication for biopsy is to exclude prostatic cancer.

Evaluation of bladder emptying by flow rate measurements and ultrasonography can be useful in patients who have voiding disturbances.[22] On occasion this diagnostic work-up should include a voiding cystourethrogram. Urodynamic changes are present in about one-third of patients who have CBP. In the presence of abnormal flow rate measurements, further studies should be performed to differentiate between functional and anatomic changes.

Urethrocystoscopy may reveal visible inflammatory changes in the posterior urethra. Prostatic sonography may demonstrate prostatic calculi (Fig. 68.3). Prostatic calculi may serve as nidi for pathogens and lead to CBP, but they are common and increase with age, and their role remains controversial. Figure 68.4 outlines the diagnostic investigation of patients who present with possible prostatitis.

Nonbacterial prostatitis is a less specific diagnosis. These patients have inflammatory cells in the EPS with negative cultures from both the EPS and VB_3 . Although numerous investigators have attempted to demonstrate that NBP is due to difficult-to-culture pathogens such as *C. trachomatis* or genital mycoplasmas, there is no consensus that these organisms cause NBP.[18] [23] [24] As a result, this diagnosis is currently poorly defined and is presumed to be caused by unknown etiologic and pathogenetic processes. This entity is classified as inflammatory CPPS according to the new definition of the NIDDK.[2] [3]

Figure 68-1 Meares and Stamey[17] localization technique to diagnose chronic bacterial prostatistis. Prostate secretion can be more readily obtained if the patient has not

ejaculated for approximately 3–5 days before the examination.

TABLE 68-3 -- Prostatitis infections by unconventional fastidious pathogens.

PROSTATITIS INFECTIONS BY UNCONVENTIONAL FASTIDIOUS PATHOGENS		
Species	**Clinical features**	**Comment**
Haemophilus influenzae		Single case reports
Neisseria gonorrhoeae	Associated with history of gonococcal urethritis	Decreasing due to effective antibiotic treatment
Mycobacterium tuberculosis	Urogenital manifestation	Associated with HIV infection
Anaerobes	Prostatic abscesses	
Candida spp.	In immunocompromised patients with indwelling urinary catheters	
Coccidioides immitis, Blastomyces dermatitidis, Histoplasma capsulatum	Disseminated disease	Associated with HIV infection
Trichomonas vaginalis	Chronic inflammation	May be associated with urethritis

Figure 68-2 Diagnostic criteria of chronic bacterial prostatitis by expressed prostatic secretion analysis. LDH, lactate dehydrogenase; PAF, prostatic antibacterial factor.

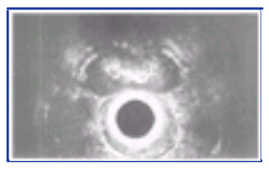

Figure 68-3 Transrectal ultrasonography of the prostate with diffuse calcifications (prostatitis calcarea).

Figure 68-4 Diagnostic management in patients who have prostatitis-like symptoms.

TABLE 68-4 -- Dissociation constants of fluoroquinolones.[28]

DISSOCIATION CONSTANTS OF FLUOROQUINOLONES [28]		
Quinolone	**Pk_{a1}**	**Pk_{a2}**
Ciprofloxacin	6.1	8.7
Enoxacin	6.3	8.7
Fleroxacin	5.5	8.1
Gatifloxacin	6.0	9.2
Lomefloxacin	5.8	9.3
Norfloxacin	6.3	8.4
Ofloxacin*	6.1	8.2
Pefloxacin	6.3	7.6
Sparfloxacin	6.3	8.8

* The disassociation constants of levofloxacin are similar.

MANAGEMENT

Treatment varies according to the severity of the patient's presenting symptoms and the probable etiologic agent. Antimicrobial treatment should be initiated immediately in patients who have acute bacterial prostatitis after blood and urine cultures have been obtained. Prostatic massage is contraindicated. Parenteral treatment with a fluoroquinolone or a ß-lactam with an aminoglycoside are all appropriate initial regimens. After initial improvement, a switch to an oral regimen, a fluoroquinolone, is appropriate and should be prescribed for at least 4 weeks.

Patients who have possible CBP require investigation for evidence of inflammation and an etiologic agent. Selection of an appropriate antimicrobial agent that has optimal pharmacokinetics for prostatic secretion and tissue is important. [25] Antibacterial diffusion into prostate secretion depends upon the lipid solubility, molecular size and pKa of the agent.[25] For example, trimethoprim, a weak base with a pKa of 7.4, penetrates well into vacid prostatic secretion. However, because the pH of prostatic fluid in patients who have CBP is often alkaline, concentrations in prostatic secretion may be inadequate. [26] [27] In contrast, the fluoroquinolones exist as zwitterions with a pKa in acid and alkaline milieus [28] (Table 68.4). This allows prostatic fluid levels that compare favorably with plasma levels, with ratios ranging from 0.12 to 1.02 (Table 68.5).[29] [30] [31] [32] The concentration of some fluoroquinolones in the alkaline seminal fluid may even exceed that in plasma (Table 68.5).[29] [30] [31] [32] Other studies have examined fluoroquinolone concentrations in prostatic tissue obtained at transurethral resection and they appear to be consistently at or above corresponding plasma concentrations.[31] Macrolides also penetrate into prostatic and seminal fluids very well.[25] [33]

Although it remains unproven, considerable evidence suggests that bacteria in prostatic tissue survive in a milieu protected by biofilms. Antimicrobial agents, particularly the fluoroquinolones and the macrolides, that penetrate through biofilm may be preferred drugs. Most studies in patients who have CBP have not been well controlled and have been variably designed.[31] [34] As a result, comparison is difficult. Duration of therapy has ranged from 14 to 150 days and follow-up investigation has not been standardized. An EPS should be obtained from all patients at 4–8 weeks and at 6 months after treatment to ensure that the pathogens have been eradicated.[34] Overall, it appears that 60–80% of patients who have *Escherichia coli* and other Enterobacteriaceae can be cured with a 4–6-week course of therapy (

Table 68.6). [35] [36] [37] [38] [39] [40] [41] [42] [43] However, prostatitis due to *Pseudomonas aeruginosa* or enterococci often fails to respond to treatment.

TABLE 68-5 -- Concentrations of fluoroquinolones in prostatic and seminal fluids (in case of split ejaculation, portion 2) of volunteers 2–4 hours after drug administration.[29] [30] [31] [32]

CONCENTRATIONS OF FLUOROQUINOLONES IN PROSTATIC AND SEMINAL FLUIDS						
Quinolone	**Dose (mg)**	**Plasma (mg/l)**	**Prostatic fluid (mg/l)**	**Ratio of prostatic:plasma concentration**	**Seminal fluid (mg/l)**	**Ratio of seminal:plasma concentration**
Norfloxacin	800 po	1.40	0.14	0.12	n.d.	-
Ciprofloxacin	200 iv	0.44	0.08	0.18	2.53	7.1
	750 po	0.88	0.23	0.23	6.57	7.7
Fleroxacin	400 po	3.71	1.00	0.28	5.80	1.7
Ofloxacin	400 po	2.00	0.66	0.33	4.09	4.0
Enoxacin	400 po	1.09	0.39	0.39	2.19	2.2
	428 iv	1.26	0.57	0.47	3.50	2.8
Lomefloxacin	400 po	1.81	1.38	0.48	2.04	1.3
Gatifloxacin	400 po	1.92	1.03	1.02	1.75	1.0
n.d., no data						

TABLE 68-6 -- Bacteriologic cure of prostatitis with fluoroquinolones.

BACTERIOLOGIC CURE OF PROSTATITIS WITH FLUOROQUINOLONES							
Quinolone	**Daily dosage (mg)**	**Duration of therapy (days)**	**Number of evaluable patients**	**Bacteriologic cure (%)**	**Duration of follow-up (months)**	**Year of study**	**Reference**
Norfloxacin	800	28	14	64	6	1990	Schaeffer *et al.*[35]
Norfloxacin	4–800	174	42	69	8	1991	Petrikkos *et al.*[36]
Ofloxacin	400	14	21	67	12	1989	Pust *et al.*[37]
Ciprofloxacin	1000	14	15	60	12	1987	Weidner *et al.*[38]
Ciprofloxacin	1000	28	16	63	21–36	1991	Weidner *et al.*[39]
Ciprofloxacin	1000	60–150	7	86	12	1991	Pfau[40] [41]
Ciprofloxacin	1000	28	34	76	6	2000	Naber *et al.*[42]
Ciprofloxacin	1000	28	78	72	6	2001	Naber
Lomefloxacin	400	28	75	63	6	2001	Naber
Eradication of pathogens (bacteriologic cure) in patients who have chronic bacterial prostatitis. Only studies are listed in which the diagnosis was derived from application of the Meares and Stamey technique and a follow-up of at least 6 months was available							

Chronic bacterial prostatitis can be a relapsing illness and recurrent episodes are best managed by either continuous low-dose suppressive therapy with an effective regimen such as fluoroquinolone, intermittent treatment whenever symptoms recur, or efforts to resect infected prostatic tissue, particularly prostatic calculi, in order to effect a surgical cure.[5] The latter is rarely successful and should only be carried out with very specific indications.

A prostatic abscess may require drainage in addition to antimicrobial treatment. Occasionally, anaerobes or mixed infections may be responsible for the abscess. Cultures should always be obtained and, if fungal infection is suspected, the laboratory should be informed. Most treatment regimens should include an agent effective against anaerobes. Prostatic abscesses can be drained through the urethra, the perineum and occasionally the rectum.

Inflammatory CPPS/NBP is managed empirically and no regimen has proved to be routinely successful. Occasionally, patients appear to have a very specific response to antimicrobial therapy and, whenever this occurs, a prolongation of therapy is indicated. However, most patients who have inflammatory CPPS/NBP do not experience any change in symptoms with antibacterial therapy. Other treatment regimens include anti-inflammatory agents, a-adrenergic blocking agents, regular prostatic massage and weekly ejaculation. However, all regimens are empiric and treatment is often unsatisfactory.

Prostatodynia is an imprecise diagnosis for which therapy is controversial and unproven. Although the symptoms can mimic those of CBP, the absence of inflammation or any signs of infection are presumed to mean that no microbial agent is involved. This entity is classified as noninflammatory CPPS according to the new definition of the NIDDK.[2] [3] Treatment regimens similar to those used for NBP can be tried empirically.

EPIDIDYMITIS AND ORCHITIS

Epididymitis is an acute painful swelling in the scrotum, which is usually unilateral.[44] The testes may be involved in the inflammatory process as 'epididymo-orchitis'. Inflammatory processes of the testes, especially viral orchitis, less often involve the epididymis.

EPIDEMIOLOGY

Orchitis and epididymitis are classified as acute or chronic processes according to their cause (Table 68.7). Chronic inflammation with induration develops in about 15% of patients following an episode of acute epididymitis. Viral and bacterial inflammation of the testes can lead to testicular atrophy and destruction of spermatogenesis.[45]

Epididymitis is common among individuals who have high-risk sexual behaviors (frequent change of sexual partners) and is one of the leading causes of acute admission to hospital among military personnel. It occurs in 1–2% of patients who have gonococcal and

TABLE 68-7 -- Classification of epididymitis and orchitis.

CLASSIFICATION OF EPIDIDYMITIS AND ORCHITIS		
Acute epididymitis or epididymo-orchitis	**Granulomatous epididymitis or orchitis**	**Viral orchitis**
Neisseria gonorrhoeae	*Mycobacterium tuberculosis*	Mumps
Chlamydia trachomatis	*Treponema pallidum*	Enteroviruses
Escherichia coli		
Streptococcus pneumoniae	*Brucella* spp.	
Klebsiella spp.	Sarcoid	
Salmonella spp.	Fungal	
Other urinary tract pathogens	Parasitic	
Idiopathic	Idiopathic	

chlamydial urethritis, with an equal risk from each. It is usually unilateral and is due to an extension of the urethral infection via the vas deferens to the epididymis (see Chapter 74).

In middle-aged and older men, epididymitis is usually due to the same organisms as those that cause UTI and is presumably a direct extension from the urinary tract. Epididymitis is more common in patients who have indwelling catheters. Bladder outlet obstruction and urogenital abnormalities are also risk factors for acute and chronic epididymo-orchitis.

Mumps orchitis was common before widespread vaccination. It is now rare. It occurs in 20–30% of postpubertal men who have mumps. Other viral infections can also cause orchitis, particularly enteroviruses. The testes can also be involved as a continuation of epididymitis, particularly when suppurative UTI pathogens are involved. Granulomatous orchitis is a rare condition of uncertain etiology.[46] With regard to chronic inflammatory conditions, a so-called 'low-grade autoimmune orchitis'[47] has been described.

Epididymo-orchitis can lead to abscess formation, testicular infarction, testicular atrophy, chronic epididymitis and infertility.[44] In men who have azoospermia, postinflammatory epididymal obstruction can sometimes be cured by reconstructive surgery.[48]

CLINICAL FEATURES

Inflammation, pain and scrotal swelling characterize acute epididymitis. [44] Frequently the tail of the epididymis is involved first. The spermatic cord is usually tender and enlarged. The testes may be spared or may be involved to produce a contiguous large painful mass. Acute epididymitis always requires immediate evaluation by Doppler duplex scanning to differentiate between acute epididymitis and spermatic cord torsion. The latter requires urgent surgical intervention to prevent testicular infarction.[49]

The microbiologic diagnosis of acute epididymitis must be made as specifically as possible. A urethral Gram stain, urine culture and other studies for identification of *Neisseria gonorrhoeae* and *C. trachomatis* should be obtained for all patients. Blood cultures are valuable if the patient is febrile or has systemic signs of toxicity. Ejaculate analysis according to World Health Organization criteria, including leukocyte analysis, may be of value. A transiently decreased sperm count or azoospermia is common. Infertility is a rare complication unless there is bilateral involvement.

Chronic epididymitis is characterized by thickening and induration of the epididymis. Especially in patients who are infertile, ejaculate analysis concerning semen quality is a necessary investigation to exclude azoospermia[47] and changes of sperm maturation.[50]

Orchitis, an isolated inflammation of the testis, is a rare event. Most frequently it occurs in association with epididymitis, as epididymo-orchitis (see Table 68.7). Testicular swelling, frequently accompanied by fever, is typical. Antibody and other specific serum investigations should be carried out to identify mumps, enteroviruses and other potential virus pathogens. In chronic infections, ejaculate analysis may demonstrate structural sperm defects providing reduced sperm motility and number.[47] Testicular biopsy in these cases may demonstrate focal inflammation, mixed atrophy and complete Sertoli-cell-only syndrome in the follow-up.[45]

MANAGEMENT

In acute epididymitis (epididymo-orchitis), antimicrobial agents should be chosen for initial empiric treatment based on the probability of the etiologic agent. In sexually active men who are at risk of *C. trachomatis* or *N. gonorrhoeae*, a therapeutic regimen that covers both these pathogens is mandatory. Details of the treatment of these specific pathogens is provided in Chapter 74 . Additional therapy includes scrotal support. Abscesses may require surgical drainage. If urinary tract pathogens are considered to be the probable etiologic agent, fluoroquinolones or trimethoprim-sulfamethoxazole are appropriate choices. Experimental[51] and clinical studies[52] suggest that the fluoroquinolones are very effective.

In acute mumps orchitis, interferon a has been prescribed. [47] Therapy with nonsteroidal anti-inflammatory agents has also been recommended,[53] as has treatment with long-acting gonadotrophin-releasing hormone agonists.[54]

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Chapter 69 - Pyelonephritis and Abscesses of the Kidney

James R Johnson

INTRODUCTION

Acute pyelonephritis, an acute infection (usually bacterial) of the kidney and renal pelvis, is one of the most common serious infectious diseases of otherwise healthy individuals, and is an even greater problem for compromised hosts. New approaches to the diagnosis and management of this disorder and its sequelae, including intrarenal and perinephric abscess, have resulted in improved outcomes for patients.

EPIDEMIOLOGY

Annually in the USA, approximately 200,000 adults are admitted to hospital for renal infection,[1] [2] [3] many others being managed as outpatients. In Manitoba, Canada, the annual risk of hospitalization for pyelonephritis is approximately 11/10,000 for women and 3/10,000 for men.[4]

Complicated versus uncomplicated pyelonephritis

Pyelonephritis can be stratified as 'complicated' or 'uncomplicated', depending on the presence of underlying urologic or medical conditions that predispose to kidney infection or that aggravate the severity or intransigence of such infections once they occur.[2] [5] Uncomplicated and complicated pyelonephritis have distinctive host substrates, microbial flora, pathogenetic mechanisms, clinical presentations and requirements for and response to therapy.

Risk factors

Although little is known about the specific risk factors for uncomplicated pyelonephritis, recognized risk factors for uncomplicated cystitis would be predicted to predispose to pyelonephritis also. Such associations include female sex and, among adult women, sexual intercourse, a history of previous urinary tract infections (UTIs), use of spermicide-diaphragm contraception, the postmenopausal state and being a nonsecretor of blood group substances (see Chapter 67).[6] [7] Among children, the P_1 blood group phenotype is associated with an increased pyelonephritis risk.[8] Pyelonephritis in compromised hosts, which by definition is 'complicated', is promoted by almost any anatomic or functional abnormality of the urinary tract, urinary tract instrumentation, diabetes mellitus, immunosuppression, pregnancy (during which the risk of pyelonephritis is 1–2%) and conditions associated with sensory impairment (such as diabetic or alcoholic neuropathies and spinal cord injury).[2] [5] Among the commonly implicated urologic conditions are posterior urethral valves (in infant boys), congenital vesicoureteral reflux (in girls), indwelling or intermittent urinary catheterization, other instrumentation of the urinary tract, neurogenic bladder, urolithiasis, ureteral diversions, any obstruction to normal urinary flow and kidney transplantation.

Renal abscesses, which can be intrarenal, intrarenal with perirenal extension or entirely perirenal, typically develop as a consequence of acute pyelonephritis and are among the most serious local complications of this illness. They occur predominantly in compromised hosts, notably patients who have diabetes mellitus or have undergone recent surgery or instrumentation of the urinary tract.[9] [10] [11] Urinary reflux and obstruction are prominent risk factors for renal abscesses. Rarely, renal abscesses may develop during a severe episode of otherwise uncomplicated pyelonephritis in an intact host.

PATHOGENESIS

Route of infection

Irrespective of the presence of predisposing host conditions, in almost all patients acute pyelonephritis arises via an ascending route of infection. [2] [5] The causative micro-organisms enter the urethra, colonize the bladder, then ascend the ureters to the renal pelvis and subsequently invade the renal parenchyma. In most cases, the pathogens arise from the host's own intestinal (and, in women, vaginal) flora, [12] although in patients who have indwelling catheters or nephrostomy tubes organisms may be transferred on the hands of health care workers and thus bypass the intestinal, vaginal and/or bladder colonization steps.

Microbial flora

Organisms must have substantial intrinsic virulence to overcome the many defense mechanisms of a healthy urinary tract and cause pyelonephritis in an intact host. In contrast, organisms of lesser intrinsic virulence can infect the kidney in patients who have impaired urinary tract defenses. Paradoxically, the less virulent organisms associated with complicated pyelonephritis are more often resistant to antimicrobial agents than are the more virulent ones that cause uncomplicated pyelonephritis. This, together with the impaired defense mechanisms of compromised hosts, makes such infections more difficult to treat and cure than uncomplicated pyelonephritis.

In uncomplicated pyelonephritis the distribution of micro-organisms is similar to that in uncomplicated cystitis, with approximately 80% of isolates being *Escherichia coli* and the remainder other Gram-negative bacilli (predominantly *Klebsiella* and *Proteus* spp.), *Staphylococcus saprophyticus* (especially in young women), *Enterococcus* spp. (especially in older men) and occasionally group B or other streptococci.[2] [5] [13] The *E. coli* strains that cause uncomplicated pyelonephritis exhibit multiple virulence properties that contribute to their ability to invade the urinary tract and stimulate inflammation and tissue damage (Fig. 69.1).[14] [15] [16] [17]

Among the various adhesins expressed by these strains, the most prevalent and pathogenetically important are type 1 fimbriae and P fimbriae. P fimbriae are strongly epidemiologically associated with pyelonephritis[14] [15] and contribute to kidney infection in a monkey model.[18] They recognize Gala(1–4)Gal-containing receptors on host epithelial surfaces, including the mucosal lining of the colon, vagina and urinary tract. The P fimbrial adhesin molecule PapG is situated at the tip of the fimbrial stalk and mediates attachment to receptors on the host cell. PapG occurs in three known variants, of which the class II variant is the most common among strains that cause uncomplicated pyelonephritis and bacteremic UTIs,[19] [20] [21] whereas the class III variant is associated with cystitis and with complicated UTIs.[20] [21] [22]

Figure 69-1 Uropathogenic strain of *Escherichia coli.* Note the typical virulence properties, including adhesive fimbriae, cytotoxins, lipopolysaccharide (LPS), capsular polysaccharide, the aerobactin system and outer membrane proteins important in serum resistance. Bacterial interactions with host cells trigger cytokine production, inflammatory cell infiltration and bacterial internalization within epithelial cells. Internalized bacteria can multiply intracellularly and stimulate sloughing, rupture, necrosis or apoptosis of host cells.

Type 1 fimbriae are structurally similar to P fimbriae but have a binding specificity for mannose-containing receptors on host cells. Since type 1 fimbriae are produced by almost all *E. coli*, epidemiologic associations of type 1 fimbriae with UTI or pyelonephritis are difficult to demonstrate. However, in the mouse model of UTI deletion of the type 1 fimbrial adhesin gene [16] or immunization against the corresponding FimH adhesin molecule[17] reduces both bladder and kidney infection. Other important virulence factors of pyelonephritogenic *E. coli* include cytotoxins such as a-hemolysin (which destroys or impairs the function of host epithelial cells, phagocytes and lymphocytes), iron sequestration systems such as the aerobactin system, polysaccharide capsules, lipopolysaccharide and serum resistance proteins (which protect

the organism against phagocytosis and/or complement-mediated lysis).[14] [15] [23]

In complicated pyelonephritis, although *E. coli* still is the single most common pathogen, it is less prevalent than in uncomplicated pyelonephritis and is represented by less virulent strains. Other Gram-negative bacilli are more commonly encountered, including *Pseudomonas aeruginosa*, *Enterobacter* spp. and other Enterobacteriaciae.[2] [5]

Factors promoting ascending infection

Vaginal colonization with urovirulent organisms is promoted by sexual intercourse, particularly with the use of a spermicide, which kills normal lactobacillus-based vaginal flora and permits overgrowth with *E. coli* and other coliform bacteria.[12] Similar changes in the vaginal flora occur after the menopause as a result of estrogen depletion, and are induced by the use of certain antimicrobial agents, notably ß-lactams.

In women, sexual intercourse promotes the entry of periurethral bacteria into the bladder on a mechanical basis. In catheterized patients, bacteria can be introduced into the bladder at the time of catheter insertion, or can migrate into the bladder along the external or luminal surfaces of catheters.[24] With improper catheter care, infected urine from the collecting bag and drainage tubing can reflux into the bladder. Catheter-associated organisms persist within the urinary tract in part by cementing themselves to the catheter within glycocalyx matrices that protect them against natural host defense mechanisms and antimicrobial agents.

Ascent of pathogens from the bladder up the ureters is facilitated by vesicoureteral reflux, which may be pre-existing or which in the intact host can result from a reversible ureteral aperistalsis induced by exposure of the ureteral wall to lipopolysaccharide from adherent bacteria.[25] Among several UTI-promoting physiologic alterations of pregnancy, ureteral hypotonia and some degree of ureteral obstruction may contribute to bacterial entry into the upper urinary tract in pregnant women and its persistence once there.[18] Once within the renal pelvis, micro-organisms migrate up the collecting ducts into the tubules, a process promoted by intrarenal reflux (if present in the particular host) and by bacterial adhesins that recognize receptors along this epithelial surface or in subjacent tissues.[14] [15] [25]

PATHOLOGY

Within the urinary tract, pathogenic bacteria adhere to the mucosa and trigger a local cytokine/chemokine network, with production of interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor-a, and recruitment of polymorphonuclear leukocytes (PMNLs) and lymphocytes.[26] [27] Triggers include the interaction of bacterial lipopolysaccharide with host cell Toll-like receptor 4 (TLR4),[28] P fimbrial binding to host membrane glycolipids, which activates an intracellular ceramide signaling pathway,[29] and type-1-fimbria-mediated bacterial internalization.[30] The influx of inflammatory cells leads to the generation of reactive oxygen species, leukotrienes, prostaglandins and other mediators of inflammation, which together with bacterial cytotoxins produce tissue damage, edema and, in the kidney, intense local vasoconstriction (Fig. 69.1).[2] [5] [25] These phenomena are responsible for the characteristic signs and symptoms of pyelonephritis, including dysuria and suprapubic pain from bladder involvement, flank pain and costovertebral angle tenderness from kidney involvement, and

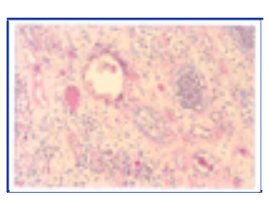

Figure 69-2 Acute pyelonephritis. Note interstitial edema, tubules packed with PMNLs and a diffuse interstitial acute inflammatory infiltrate in this autopsy specimen from a diabetic patient who had refractory *Escherichia coli* urosepsis.

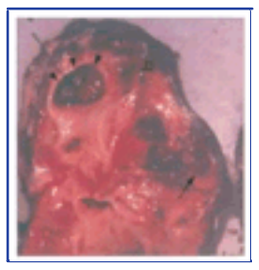

Figure 69-3 Emphysematous pyelonephritis. Cortical necrosis (solid arrow), diffuse cortical hemorrhage (open arrow) and dilatation of the collecting system (arrowheads) in a nephrectomy specimen from a diabetic patient who received combined medical/surgical therapy and survived emphysematous pyelonephritis due to an unusual pathogen, namely *Candida albicans*.

fever and malaise from inflammatory cytokines that enter the systemic circulation.

Histologically, in acute pyelonephritis the mucosa and submucosa of the collecting system, the tubules and the interstitium are edematous and infiltrated with PMNLs (Fig. 69.2). Tubules may necrose. Microabscesses form within the mucosa and interstitium, and can coalesce to form macroscopic abscesses.[2] [5] [25]

Grossly, the kidneys are diffusely or focally swollen and edematous. On sectioning, streaks of yellowish inflammatory infiltrate extend from the papillae and medulla toward the cortex, sometimes reaching the capsule and rupturing it.[2] [5] When macroscopic abscesses do form, typically they localize at the corticomedullary junction but they can be subcapsular or extend into the perirenal space.[9] In hyperglycemic diabetic patients, rapid fermentation of glucose by Gram-negative bacilli (or, rarely, yeasts) can produce gas within the renal parenchyma (emphysematous pyelonephritis; Fig. 69.3), within an abscess (gas abscess) or within the renal pelvis and collecting system (emphysematous pyelitis).[31] Papillary necrosis, which occasionally complicates acute pyelonephritis among diabetic patients, may make the infection worse because of the obstruction caused by sloughed tissue (Fig. 69.4).[2] [5]

Functionally, the intense interstitial inflammatory process leads to a reduction in urinary concentrating capacity. Decreased renal blood flow, functional tubular obstruction from inflammatory cells and

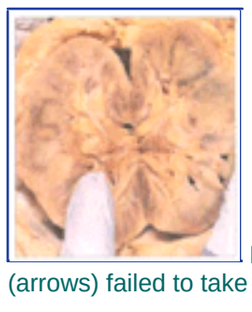

Figure 69-4 Acute papillary necrosis (arrows) in an autopsy specimen from a diabetic patient who died from refractory *Escherichia coli* urosepsis. Necrotic papillae (arrows) failed to take up formalin, so appear pink, in contrast to the grayish-tan formalinized tissue.

necrotic debris, and inflammation-induced tubular dysfunction result in delayed excretion of radiographic contrast dye[2] [5] but only rarely manifest as clinically apparent renal dysfunction.[32]

Bacteremia develops in between 10% and 65% of patients who have acute pyelonephritis, depending on the severity of infection and increasing in proportion to the age of the host. Bacterial entry into the bloodstream may be promoted by P fimbriae[19] and by tissue destruction mediated by microbial cytotoxins.[33] Systemic complications of pyelonephritis, which are more common among patients who have Gram-negative bacteremia, include septic shock, disseminated intravascular coagulation and the acute respiratory distress syndrome (ARDS). Pregnant women who have pyelonephritis are particularly prone to these complications, and also may develop premature labor as a result of the irritative effect of lipopolysaccharide on the uterus.[34]

Hematogenous renal abscesses

Intrarenal abscesses can also be caused by certain hematogenously borne pathogens, most commonly *Staphylococcus aureus*, *Candida* spp. and *Mycobacterium tuberculosis*.[9] In contrast to abscesses that form during acute ascending pyelonephritis, hematogenously derived abscesses are usually cortical in location, are not prone to rupture into the perinephric space and are not associated with the characteristic clinical syndrome of pyelonephritis. Conversely, the typical pathogens of acute

ascending pyelonephritis almost never cause renal abscesses in patients who have bacteremia arising from an extra-urinary-tract focus.[2] [5]

PREVENTION

Little is known about the prevention of pyelonephritis or renal abscess. Presumably, the same measures that can be recommended to noncompromised women who wish to reduce their risk of uncomplicated recurrent cystitis (e.g. avoiding spermicide-diaphragm contraception, use of chronic antimicrobial prophylaxis or early patient-initiated therapy for UTI symptoms) should decrease the risk of uncomplicated pyelonephritis.[35] Postmenopausal women can reduce their risk of bacteriuria with vaginal estrogen treatment;[36] this might also prevent pyelonephritis. Complicated pyelonephritis may be prevented by removing the precipitating factor. Urinary catheters should be avoided whenever possible, used according to current guidelines when unavoidable and removed as soon as no longer essential.[24] Correction of urologic abnormalities (whether surgically or medically) may reduce the associated infection risk but treatment decisions must be carefully individualized and based on the expected risks and benefits of the planned intervention(s). It is not known whether improved glycemic control among patients who

have diabetes reduces their increased risk of pyelonephritis but the other documented benefits of this therapy provide ample rationale for its use.

Prophylactic antimicrobial therapy is useful in women who have recurrent uncomplicated cystitis[37] and in certain compromised hosts, for example renal transplant recipients in the early post-transplant period.[38] However, in many other compromised hosts antibiotic prophylaxis is without clear benefit and often selects for resistant organisms and causes drug-related adverse effects.[39] Whether the experimental anti-UTI vaccines that are currently being evaluated will prevent cystitis or pyelonephritis is unknown.[17] [40] At present there is no medically defined role for vaccines, cranberry juice, receptor analogue therapy, *Lactobacillus* preparations or yoghurt in the prevention of UTI or pyelonephritis.[35]

CLINICAL FEATURES

The clinical manifestations of acute pyelonephritis vary considerably depending on the characteristics of the host and pathogen. A typical history for the classic pyelonephritis syndrome, which is most commonly observed with kidney infections in otherwise healthy young women, includes several days of progressive flank pain, malaise, fever and chills, prostration and possibly nausea and vomiting, often preceded and/or accompanied by symptoms of acute cystitis.[2] [5]

The physical examination characteristically shows an ill-appearing, febrile, tachycardic patient, often with evidence of volume contraction. The pathognomonic physical finding of acute pyelonephritis is tenderness to palpation or percussion over one or both costovertebral angles. Mild to moderate abdominal and suprapubic tenderness are often also present.

Atypical presentations are common. Even otherwise healthy young women who have pyelonephritis may not have all of the classic symptoms or examination findings, and infants or young children, elderly or debilitated patients and patients who have underlying systemic illnesses or neurologic impairment often have an even less characteristic clinical picture.[2] [5] Abdominal pain, headache, nonspecific constitutional symptoms, diffuse back pain, pelvic pain or respiratory complaints may predominate, obscuring the diagnosis and suggesting other processes. A deceptively benign presentation, including sometimes even the complete absence of suggestive symptoms, can mask the presence of severe renal infections in immunocompromised or sensory-impaired hosts.[2] [5] [41] On the other hand, even in patients who have a classic presentation for acute pyelonephritis, other entities must be considered in the differential diagnosis, including (in the appropriate setting) pelvic inflammatory disease, acute appendicitis, urolithiasis, basal pneumonia and acute pancreatitis or biliary tract disease. The decision whether to perform a pelvic examination in a woman suspected of having pyelonephritis must be individualized, taking into consideration the patient's demographic characteristics, the specifics of the history (including the sexual history) and the findings on general physical examination.

In addition to the varied combinations of symptoms and physical findings encountered in patients who have acute pyelonephritis, a wide range of severity of illness is seen. At one extreme, patients who seem healthy and have what otherwise appears clinically to be acute cystitis may demonstrate a slight elevation of body temperature or report mild malaise, suggesting early renal involvement. At the other extreme, patients may present in full-blown septic shock, with multisystem organ failure. The severity of illness has a significant influence on subsequent management, as described below.

Abscess

The initial history and physical examination usually provide few clues as to the presence of an intrarenal or perinephric abscess, although these entities should be kept in mind in high-risk patients. The presence of a palpable mass is suggestive of renal abscess but is neither a sensitive nor a specific finding. [11] Failure of a patient who is thought to have ordinary pyelonephritis to improve substantially after treatment for 48 hours increases the likelihood of abscess sufficiently to warrant further diagnostic studies.[2] [5]

DIAGNOSIS

Urinalysis and urine culture

Acute pyelonephritis is a clinical diagnosis based on a combination of characteristic symptoms and signs together with supporting laboratory tests.[2] [5] The minimal laboratory evaluation needed to make this diagnosis in the appropriate clinical setting is microscopic examination (whether by urinalysis or Gram stain) of a voided urine specimen to evaluate for the presence of pyuria, followed by quantitative urine culture. The Gram stain is also helpful by confirming the presence of bacteria in the urine (which are seen in unconcentrated urine specimens when the urine bacterial concentration is $>10^5$ cfu/ml) [42] and by suggesting the likely bacterial type, although effective empiric treatment often can be selected without this information. [43]

In the absence of prior antimicrobial therapy, the urine culture almost always shows high concentrations ($>10^5$ cfu/ml) of one or more bacterial species. Pure growth of a single uropathogenic organism is typical of infections in noncompromised hosts, whereas polymicrobial infections are more common in compromised hosts. Lesser bacterial concentrations are occasionally encountered, and in the appropriate clinical context (e.g. a patient who has typical symptoms and examination findings, plus pyuria) do not exclude the diagnosis of pyelonephritis. Antimicrobial susceptibility testing of urine isolates is essential, both to confirm that the empirically selected treatment regimen is appropriate, and for guiding selection of an effective oral agent for patients treated initially with a parenteral antimicrobial regimen.[43]

Ancillary tests

Other tests may be indicated depending on the severity of illness, the range of alternative diagnoses being considered and the presence of comorbid conditions. Pre-therapy blood cultures are commonly collected although, interestingly, bacteremia (if present) predictably clears with appropriate therapy directed toward the urinary infection, and clinical outcomes are similar regardless of the presence or absence of bacteremia.[5] A pregnancy test is useful if the patient might be pregnant and treatment is being considered with an agent (such as an aminoglycoside or a fluoroquinolone) that might be toxic to the fetus.[34]

Imaging studies

Imaging studies are not routinely indicated for the diagnosis or management of acute pyelonephritis.[30] For patients in whom the initial diagnosis is unclear, those who fail to respond appropriately to therapy and those in whom abscess or obstruction are suspected for other reasons, computerized tomography (CT) can be used to clarify the anatomy and guide a mechanical intervention.[2] [5] [44] [45] [46] [47] Of all urinary tract imaging modalities, contrast-enhanced CT provides the best anatomic definition of inflammatory processes in the urinary tract, including sensitive detection of abscesses and differentiation of abscesses (water density) from simple inflamed tissue (tissue density; Fig 69.5 Fig 69.6 Fig 69.7 Fig 69.8).[44] [45] [46] [47] Inflamed regions of the pyelonephritic kidney appear on enhanced CT as streaky or wedge-shaped hypodense areas that fail to concentrate contrast material normally in comparison with surrounding renal tissue. Focal bulges or diffuse swelling of the entire kidney are common, as is inflammatory stranding in the perinephric

Figure 69-5 Febrile urinary tract infection with white blood cell count of 36,000/ml (girl, 3 years). (a) Precontrast CT scan: left kidney is diffusely swollen; parenchymal attenuation is the same as that of the right kidney. (b) Postcontrast CT scan: wedge-shaped regions of hypoenhancing parenchyma in the left kidney are most pronounced in the posterior portion. Inflamed parenchyma enhances from 32 to 93 Hounsfield units (HU), whereas normal kidney enhances from 33 to 140HU. The right kidney shows normal cortical enhancement and pronounced medullary blush. *With permission from Talner.*[45]

Figure 69-6 Woman with clinical signs of acute pyelonephritis. (a) Precontrast CT scan: focal bulge present in anterolateral aspect of left kidney. Attenuation is the same as that of normal kidney parenchyma. (b) Postcontrast CT scan: rounded and streaky regions of hypoenhancing parenchyma in the left kidney are most pronounced anterolaterally. Attenuation in the region of interest (cursor) was 22HU on precontrast scans and increased to 93HU on postcontrast scans. Normal parenchyma increased from 25–130HU. *With permission from Talner.*[45]

Figure 69-7 Acute pyelonephritis with small intrarenal abscess. (a) Precontrast CT scan shows small region of low attenuation (arrows). (b) On the postcontrast CT scan, the abscess (A) fails to enhance at all. Surrounding inflamed parenchyma bulges and enhances less than adjacent normal parenchyma. (c) Follow-up CT scan obtained after prolonged antibiotic therapy. The abscess has resolved without drainage. The focal swelling is gone but the parenchyma still shows hypoenhancement. *With permission from Talner.*[45]

fat. Terms coined by radiologists in the 1980s for these changes, such as 'focal' (or 'lobar') nephronia and 'focal' (or 'diffuse') bacterial nephritis, were often confusing to the clinician and were applied inconsistently by different radiologists. The Society of Uroradiology has defined a new uniform terminology according to which all such changes are reported under the umbrella term 'acute pyelonephritis', with modifiers that describe the observed anatomic abnormalities.[45] The extent and severity of such CT findings at the time of presentation are predictive of the clinical course, including the likelihood of bacteremia, progression to abscess formation and death.[47]

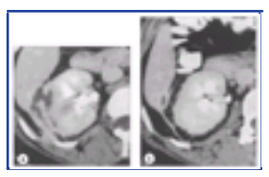

Figure 69-8 Renal abscess perforating into subcapsular and perinephric spaces (woman, 29 years). (a) Postcontrast CT scan. Dumbbell-shaped nonenhancing region laterally in right kidney represents parenchymal abscess breaking through subcapsular and perinephric spaces. Note marked thickening of perinephric fascia posterolaterally. (b) CT section obtained caudal to (a). Note thickening of perinephric inflammation. At this level there is a small pararenal abscess pocket adjacent to the liver. *With permission from Talner.*[45]

Ultrasonography, although commonly used as an initial imaging test for patients who have a suspected focal infectious complication during pyelonephritis, is comparatively insensitive[2] [5] [46] and is often followed by a CT scan irrespective of the ultrasound results. Consequently, it may be best to omit this test and proceed directly to CT. (However, serial directed sonographic examinations can be used subsequently to follow the response of abscesses or hydronephrosis to therapy, without the higher cost and exposure to radiation and contrast material of repeated CT scans.) Single photon emission CT (SPECT) using technetium-99 dimercaptosuccinate (^{99} Tc-DMSA), the newest imaging modality for use in pyelonephritis, is slightly more sensitive than CT for identifying areas of inflammation within the kidney, which can be advantageous if the initial diagnosis is in question.[45] However, it cannot distinguish between frank abscesses and inflamed but viable tissue, and so it is of little help in evaluating the patient who fails to respond to the therapy. Excretory urography and magnetic resonance imaging (MRI) have little role in the management even of complicated pyelonephritis.[2] [5] Nonenhanced spiral CT is more sensitive than excretory urography in detecting urinary calculi, and avoids exposing the patient to contrast material, and so may be the modality of choice (when available) if urolithiasis is a concern (Talner LB, personal communication). Occasionally, antegrade or retrograde ureterography may be indicated, usually when stent placement or calculus removal is needed to relieve obstruction.[46]

MANAGEMENT

In comparison with the treatment of acute cystitis, which has been extensively studied, there have been relatively few large, high-quality treatment trials for acute pyelonephritis on which to base therapeutic recommendations.[43] [48] [49] Much of the prevailing wisdom regarding the treatment of pyelonephritis comes from tradition, anecdotal experience, extrapolation from animal models or pharmacokinetic studies, small clinical trials involving heterogeneous patient populations and in-vitro susceptibility test results. Nonetheless, some guidelines can be suggested for key management issues.

Inpatient versus outpatient and parenteral versus oral therapy

Traditionally, most patients who have pyelonephritis have been hospitalized and given intravenous antimicrobial therapy, at least initially. However,

TABLE 69-1 -- Indications for hospital admission in patients with acute pyelonephritis.

INDICATIONS FOR HOSPITAL ADMISSION IN PATIENTS WITH ACUTE PYELONEPHRITIS	
Indication	**Rationale**
Severely ill, unstable (1)	Needs close monitoring, aggressive resuscitation
Moderate or severe host compromise	At risk of poor response to therapy, progression to 1
Suspected abscess, obstruction, stone	Needs diagnostic evaluation ± intervention; at risk of progression to 1
Pregnant women*	At risk of progression to 1
Children* , men*	At risk of poor response to therapy, progression to 1
Persistent vomiting (despite antipyretic therapy and intravenous hydration)	Needs iv or im therapy†
No suitable oral therapy available	Needs iv or im therapy†
Unsuitable home situation, unreliable follow-up, or unreliable/noncompliant patient	At risk of progression to 1

* Selected patients with mild illness and suitable home situations may be treated orally as outpatients, with or without a first iv dose in the emergency department or clinic.
†Home parental therapy acceptable (where available) for mildly or moderately ill patients.

evidence is accumulating that oral therapy on an ambulatory basis (with or without initial parenteral treatment and observation in the emergency department or short-stay unit) is acceptable for selected patients who have acute pyelonephritis.[49] [50] [51] [52] Outcomes with oral therapy for otherwise healthy ambulatory patients who are clinically stable and can take medications by mouth have been similar to those obtained with sicker patients given traditional in-hospital parenteral therapy, at a considerable cost saving.[52] Oral therapy has even been used successfully with pregnant women,[53] [54] in whom pyelonephritis has traditionally been considered to require in-hospital management.

Thus, there is no single right answer to the question of the optimal setting for treatment. The management plan must be individualized to the patient, taking into consideration the severity of illness (including the presence of nausea or vomiting), the patient's underlying host status and reliability level, and the availability of a support system at home and a mechanism for medical follow-up (Table 69.1). Women who have uncomplicated pyelonephritis and who are only mildly ill can sometimes be treated successfully from the outset with oral therapy alone (Table 69.2). Moderately ill patients can be can be rehydrated with intravenous fluids (if needed) in the clinic or emergency department, given an initial parenteral dose of antibiotic and observed. If after several hours their condition has failed to improve sufficiently they can be admitted to the hospital for continued parenteral therapy, whereas if they are feeling better and are able to take fluids by mouth they can be discharged to home with an appropriate oral antibiotic regimen (Table 69.2), with close follow-up arranged.

There is no published experience using oral therapy for pyelonephritis in men, children or women who have complicating factors other than pregnancy. Clinical judgment may identify suitable cases even within these populations (e.g. mildly ill patients who have only minor compromising conditions). However, most such patients should be admitted to the hospital initially for parenteral therapy, particularly if they are more than minimally ill.

TABLE 69-2 -- Suggested empiric initial treatment regimens for acute pyelonephritis.[§]

SUGGESTED EMPIRIC INITIAL TREATMENT REGIMENS FOR ACUTE PYELONEPHRITIS		
Modifying circumstances	**Treatment setting**	**Empiric treatment options**
Uncomplicated pyelonephritis		
Mild to moderate illness, no nausea or vomiting	Outpatient therapy acceptable	Oral[*] fluoroquinolone (not in children), TMP-SMX caution[‡] or amoxicillin-clavulanate (co-amoxiclav) caution[‡] for 7–14 days (Co-amoxiclav preferred if Gram-positive cocci present)
Severe illness or possible urosepsis	Hospitalization required	Parenteral[†] fluoroquinolone (not in children), third-generation cephalosporin, gentamicin (± ampicillin or piperacillin), piperacillin-tazobactam, aztreonam or carbapenem, TMP-SMX caution[‡] until patient is better; then oral[*] agent (see above) to complete 14 days of therapy (Initial regimen should include ampicillin or piperacillin if Gram-positive cocci are present)
Complicated pyelonephritis		
Pregnancy, mild illness	Outpatient therapy acceptable	Oral[*] co-amoxiclav, cephalosporin or TMP-SMX caution[‡] for 10–14 days (Co-amoxiclav preferred if Gram-positive cocci present)
Pregnancy with mild to moderate illness	Hospitalization required	Parenteral[†] third-generation cephalosporin, gentamicin (±ampicillin or piperacillin) caution;[‡] piperacillin-tazobactam or TMP-SMX caution[‡] until patient is better; then oral[*] amoxicillin, co-amoxiclav, a cephalosporin or TMP-SMX caution[‡] for 14 days (Initial regimen should include ampicillin or piperacillin if pre-therapy Gram stain shows Gram-positive cocci or no organisms, or is not done
Not pregnant, mild illness, no nausea or vomiting	Outpatient therapy acceptable	Oral[*] fluoroquinolone (not in children) for 10–14 days
Not pregnant with moderate to severe illness or possible urosepsis	Hospitalization required; imaging studies and urologic consultation often needed	Parenteral[†] gentamicin (±ampicillin or piperacillin), fluoroquinolone, third-generation cephalosporin, aztreonam, ticarcillin-clavulanate, piperacillin-tazobactam or carbapenem until patient is better; then oral[*] agent (see above) for 14–21 days (Initial regimen should include ampicillin, piperacillin or a carbapenem if pre-therapy Gram stain shows Gram-positive cocci or no organisms, or is not done
'Uncomplicated' is usually limited to noncompromised, nonpregnant adult women but can include carefully selected men and children who lack compromising conditions and are only mildly ill.		

§ *Adapted with permission from Stamm and Hooton.*[48]

* **Oral regimens:** TMP-SMX, 160mg + 800mg q12h; norfloxacin, 400mg q12h; ciprofloxacin, 500mg q12h; ofloxacin 200–300mg q12h;lomefloxacin 400mg q24h; levofloxacin, 500mg q24h; gatifloxacin, 400mg q24h; amoxicillin 500mg q8h; amoxicillin-clavulanate (co-amoxiclav) 850mg q12h or 500mg q8h; cefpodoxime proxetil, 200mg q12h

‡ **Cautions:** fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin, gatifloxacin, levofloxacin and lomefloxacin) should not be used in pregnancy or in young children. TMP-SMX, although not approved for use in pregnancy, has been widely used (but should be avoided in the first trimester and near term). TMP-SMX and co-amoxiclav should be used only if susceptibility of urine organism is known or is highly (>95%) likely. Gentamicin should be used with caution in pregnancy because of its possible toxicity to eighth-nerve development in the fetus. The fluoroquinolones norfloxacin and lomefloxacin can only be administered po; ciprofloxacin, gatifloxacin, levofloxacin and ofloxacin can be administered iv or po.

† **Parenteral regimens:** TMP-SMX, 160 + 800mg q12h; ciprofloxacin, 200–400mg q12h; ofloxacin, 200–400mg q12h; levofloxacin, 500mg q24h; gatifloxacin, 400mg q24h; gentamicin, 5mg per kg body weight q24h; ceftriaxone, 1–2g q24h; ampicillin, mezlocillin or piperacillin, 1–2g q6h; imipenem-cilastatin, 250–500mg q8h–q6h; meropenem, 1g q8h; ertapenem, 1g q24h ampicillin-sulbactam, 1.5–3g q6h; ticarcillin-clavulanate, 3.2g q8h–q6h; piperacillin-tazobactam, 3.375g q8h–q6h; aztreonam, 1g q12h–q8h

Antimicrobial regimen

Because urine culture and susceptibility testing takes several days to complete, the initial antimicrobial regimen for acute pyelonephritis is usually selected empirically (from among those agents that have suitable pharmacokinetic characteristics and a good 'track record' in pyelonephritis treatment trials) on the basis of the predicted susceptibility patterns of the expected organisms(s) (Table 69.2).[2] [5] [13] [43] [48] For all patients, activity against 'ordinary' Gram-negative bacilli is essential in the empiric regimen and, for patients who have complicated UTI or recent antimicrobial therapy, Gram-positive organisms and drug-resistant Gram-negative organisms must also be anticipated.

Suitable initial regimens are shown in Table 69.2 , which emphasizes aminoglycosides, fluoroquinolones, third-generation cephalosporins and ß-lactam-ß-lactamase inhibitor combination agents for parenteral use, and trimethoprim-sulfamethoxazole (TMP- SMX), fluoroquinolones and amoxicillin-clavulanate (co-amoxiclav) for oral use. Because of the high prevalence among uropathogens of resistance to ampicillin, other penicillins and first- or second-generation cephalosporins — as well as these agents' adverse pharmacokinetic properties and inconsistent performance in clinical trials[43] [48] — these drugs should be avoided as empiric monotherapy for even mild or uncomplicated pyelonephritis. Emerging resistance to TMP-SMX among uropathogens has diminished this drug's utility in the USA for empiric oral therapy of UTIs, particularly pyelonephritis, since in-vitro resistance is associated with clinical failure rates of more than 50%.[49] [55] Unfortunately, resistance to fluoroquinolones, which have traditionally been regarded as the 'fall-back' option for UTIs due to TMP-SMX-resistant Gram-negative uropathogens, is already quite prevalent in some places.[56] [57] How this will affect future recommendations for empiric therapy of pyelonephritis remains to be seen. Whether oral third-generation cephalosporins should have a role in the empiric therapy of pyelonephritis in outpatients has not been adequately studied.

Antimicrobial regimens can be simplified by using a single agent (there being little rationale for combination therapy except in patients who are thought to have both Gram-positive and Gram-negative pathogens)[58] and by using twice-daily dosing with ciprofloxacin, TMP-SMX and co-amoxiclar, or once-daily dosing with ceftriaxone, levofloxacin, gatifloxaci ertapenem and the aminoglycosides.

Conversion to oral therapy

Patients initially admitted to the hospital for intravenous therapy have traditionally been continued on parenteral therapy until susceptibility results are known. They are then placed on an oral agent selected on the basis of the susceptibility pattern of the urine organism, and are observed in the hospital for an additional 1–2 days to evaluate the success of oral therapy (Table 69.3). This approach leads to unnecessarily prolonged hospital stays in many patients.

TABLE 69-3 -- Criteria for conversion to oral therapy.

CRITERIA FOR CONVERSION TO ORAL THERAPY
Patient no longer severely ill or unstable
Patient taking fluids by mouth; no vomiting; adequate gut function
Suitable oral agent available:
• documented or predicted activity against causative organism(s)
• highly bioavailable
• good 'track record' in UTI therapy
• no contraindication to use (i.e. no history of previous adverse reaction), no drug-drug interactions, no fetal toxicity (pregnant women), no age-related toxicities (e.g. fluoroquinolones in children)

Conversion to oral therapy can be done safely as soon as the initial indications for parenteral therapy have resolved, as evidenced by the success of oral therapy for mildly ill ambulatory patients who have pyelonephritis. When the hospitalized patient is clinically ready for oral therapy before susceptibility results are available, an oral

regimen can be selected empirically, much as is done in the emergency department for patients treated with an oral agent from the outset.[43] [49] [50] In most locales in the USA, the fluoroquinolones are predictably active against *E. coli*. Thus, despite being slightly more expensive than TMP-SMX (which might be the preferred agent for a known susceptible organism), fluoroquinolones can yield a tremendous cost saving if they permit patients to be discharged sooner.

The practice of observing patients who have pyelonephritis in the hospital for 24 hours or longer on oral therapy before discharge is without empiric support. When examined retrospectively this approach was found to detect relapse in only 1% of patients and intolerance of the new oral agent in only 4%.[59] Thus, patients can usually be safely discharged once they have demonstrated tolerance of the first dose of an appropriate oral agent, whether the drug is selected empirically or on the basis of known susceptibility results.

Expected clinical course

Nearly all patients who have pyelonephritis and who will ultimately be cured by antimicrobial therapy alone experience substantial clinical improvement within the first 2 days of therapy, sometimes even after the first liter of intravenous rehydration fluid and before receiving any antimicrobial agent. Patients commonly continue to have fever and flank pain for several days on effective therapy but these manifestations should begin to wane and there should be improvement in the patient's energy level, appetite and sense of well-being. If after 48 hours there is no improvement in any of these parameters, aggressive re-evaluation is required.[2] [5] Possibilities to be considered include a mistaken diagnosis, a mismatch between the urine organism and the selected antimicrobial regimen, and an anatomic complication such as obstruction or abscess. A directed history and physical examination are indicated, as is repeated laboratory testing (including blood cultures and chemistries, urinalysis and urine culture plus Gram stain) and urinary tract imaging studies, beginning with enhanced abdominal CT. In some patients this evaluation will reveal a focal process in need of an invasive procedure, such as drainage of an abscess (see Fig. 69.8) or an obstructed collecting system; in some patients, continued medical therapy (with or without adjustment) will suffice (see Fig. 69.7). Consultation with an infectious diseases specialist and/or a urologic surgeon or interventional radiologist can be extremely helpful in problematic cases to ensure that all relevant options are considered and the appropriate procedures performed.

Complications

Supportive care for patients who develop septic shock, ARDS and multisystem organ failure during pyelonephritis, which is not specific to pyelonephritis, is discussed in Chapter 56 . When infection is present, obstruction to urine flow (e.g. by a stone or tumor) must be relieved, either by removal of the obstruction or by provision of alternative drainage. When possible, removal of urinary calculi from patients who have pyelonephritis is probably best delayed until the bacterial load can be reduced and the patient stabilized with medical therapy. Gas-forming UTIs have traditionally been managed surgically in most instances, often with nephrectomy in cases of emphysematous pyelonephritis (see Fig. 69.3).[60] However, reports of successful medical therapy of gas abscesses[31] and emphysematous pyelonephritis[61] indicate that even in these extreme situations therapy can be individualized.

Intrarenal (see Fig. 69.7) and perinephric (see Fig. 69.8) abscesses have also traditionally been managed with combined medical and surgical therapy.[9] [10] Recent experience with closed (catheter-assisted) drainage or medical therapy alone suggests the possibility of alternative approaches in this setting as well.[10] [62] Small abscesses, especially those occurring in otherwise intact hosts, are most likely to respond to medical therapy, whereas large collections, particularly in compromised hosts or in patients who have severe illness, are likely to require drainage. The cost and morbidity of a drainage procedure must be weighed against the cost and morbidity of the protracted antibiotic therapy that is usually required when abscesses are treated with antibiotics alone.[41] If an abscess is to be drained, the optimal method (open versus closed) depends in part on the anatomy, the host and local expertise. Perinephric abscesses (see Fig. 69.8) have been described as requiring a more aggressive interventional approach than intrarenal abscesses [9] but published experience suggests that drainage is not always needed even here.

Duration of therapy

The optimal duration of therapy for acute pyelonephritis, unlike that for acute cystitis, is largely undefined and remains a source of controversy. [58] As with other aspects of the management of pyelonephritis, because of the highly variable nature of the illness and the host substrate it is probably best to tailor duration of therapy to the individual patient. Clinical trial data demonstrate that 14 days of a traditional sequential regimen that includes an intravenous aminoglycoside initially, followed by oral TMP-SMX or ampicillin, eliminates the initial infection in 100% of women who have moderate or severe uncomplicated pyelonephritis, with no relapses at the 6-week follow-up visit. [63] Thus, courses of therapy longer than 14 days should be unnecessary when similarly potent regimens are used in comparable hosts. In other trials, approximately 90% of patients who had uncomplicated pyelonephritis and were treated for only 5 days with aminoglycosides, third-generation cephalosporins or fluoroquinolones were cured,[58] although some of the 10% failure rate was attributable to relapses with the initial pathogen.[64] Whether there is a real or clinically meaningful difference in success rates between 5–7 days and 10–14 days of therapy for uncomplicated pyelonephritis is unknown. Of note, a recent multicenter randomized clinical trial demonstrated that 7 days of oral ciprofloxacin (with or without an initial intravenous dose) was 96–99% effective for uncomplicated pyelonephritis of mild to moderate severity in ambulatory women.[49] Similarly, 14 days of oral TMP-SMX (with or without an initial intravenous dose of ceftriaxone) was 92–96% effective if the urine organism was susceptible to TMP-SMX.[49] However, these favorable findings are not necessarily applicable to women who have more severe uncomplicated infections or to patients who have complicating factors, for whom longer treatment duration may be preferable. Duration of therapy for abscesses must be individualized, taking into consideration

underlying host status, the nature of the abscess, adequacy of drainage (if undertaken) and response to therapy (both clinical and as revealed by serial imaging studies).

Follow-up

Routine repeat urine cultures are commonly performed during therapy for pyelonephritis to confirm sterilization of the urine but may add little beyond what is apparent from clinical evaluation and possibly from inspection of the urine for pyuria.[65] It is prudent to confirm at least by telephone that patients who are sent out from the emergency department with oral therapy are improving as expected. Whether routine post-therapy clinic visits, urine cultures and urinalyses contribute to favorable outcomes has not been studied. However, as it has been argued that in the setting of uncomplicated acute cystitis these measures are unnecessary,[66] it is possible that the same may be true with pyelonephritis, at least for uncomplicated cases in seemingly reliable and responsible patients. Post-therapy evaluations still are advisable in children, pregnant women[34] and probably also in other compromised hosts.

Urologic evaluation for predisposing conditions

In addition to the management of the acute pyelonephritis episode, in selected patients it is worth searching for an underlying urologic abnormality, as the surgical correction of such an abnormality might prevent future infections. The cost and morbidity of such a search must be weighted against the likelihood of finding a correctable abnormality, the morbidity of the possible corrective procedure itself and the infectious morbidity that can be averted by a successful procedure. In the absence of firm data, opinions differ as to the indications for imaging studies and corrective surgery after pyelonephritis.[66] One approach is to investigate all children and men who develop pyelonephritis, as they are the most likely to have an important correctable abnormality. Women probably should be studied if they have a second (same-strain) relapse of pyelonephritis despite an extended course of appropriate antimicrobial therapy for a first relapse. Whether women who have multiple episodes of pyelonephritis caused by diverse organisms will benefit from urologic investigation is unknown.

SUMMARY

Acute pyelonephritis is a diverse entity that challenges the clinician to intervene sufficiently but not excessively, and for which the management approach must be tailored to the individual patient. New developments in the field, such as the use of at-home oral therapy, shorter treatment courses, single-daily-dose intravenous aminoglycoside or ceftriaxone therapy and early hospital discharge provide opportunities for cost savings and enhanced patient convenience. Alertness is required to anticipate and detect complications in high-risk patients or in those who fail to respond to treatment as expected. Intrarenal and perinephric abscesses, gas-forming renal infections and infections superimposed on urinary obstruction are potentially lethal processes that require aggressive therapy, often including mechanical intervention.

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Chapter 70 - Complicated Urinary Infection, Including Postsurgical and Catheter-related Infections

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INTRODUCTION

The focus of this chapter is the group of urinary tract infections (UTIs) that is generally designated as 'complicated UTI'. This includes UTIs following urologic surgery. Infections associated with urinary catheterization, including intermittent catheterization and both short-term (<30 days) and long-term (>30 days) indwelling catheters, are also discussed.

EPIDEMIOLOGY

Urinary tract infection is the most common bacterial infection in adults. In the setting of structural or functional abnormalities of the genitourinary tract or after urologic interventions, its frequency may be exceptionally high (Table 70.1). For instance, for patients undergoing transurethral procedures with instrumentation or transurethral prostatectomy, the incidence of postintervention urinary infections is substantial for patients who do not receive antimicrobial prophylaxis.

Infection incidence on a population basis has not been reported. In a review of hospitalizations for acute pyelonephritis in Manitoba for 1989–92, [2] the total rate of admissions was 11 per 10,000 population for women and 3.3 per 10,000 for men. Of these, 34% of patients admitted to two tertiary care hospitals with pyelonephritis had complicating genitourinary factors. Of patients admitted to hospital for UTI other than pyelonephritis, 84% of subjects at one institution and 36% at a second had complicating factors.[7]

The urinary tract is also the most common source of infection in elderly individuals hospitalized with bacteremia and is responsible for about one-third of such bacteremic episodes. Most of these bacteremic elderly individuals have abnormalities of the urinary tract, primarily obstructing lesions and indwelling catheters.[8]

TABLE 70-1 -- Infection rates after genitourinary surgery, extracorporeal shock wave lithotripsy or catheterization.

INFECTION RATES AFTER GENITOURINARY SURGERY, EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY OR CATHETERIZATION	
Procedure	**Proportion infected postprocedure**
Genitourinary surgery	
Transurethral prostatectomy[1]	6–64%
Transurethral procedure with instrumentation for stone extraction [2]	25%
Extracorporeal shock wave lithotripsy (ESWL) [3]	
Negative urine culture before ESWL	1.5%
Positive urine culture before ESWL	21%
Sepsis	4.5%
Catheterization	
Urodynamic studies[4]	1.5–36%
Indwelling catheter[5]	5% per day
Intermittent catheterization[6]	4.06/100 patient days

Urinary tract infection is the most frequent hospital-acquired infection and is almost always associated with indwelling catheters. It accounts for 40% of all nosocomial infections and occurs at a rate of approximately 2 per 100 patient discharges. The catheterized urinary tract is the most frequent source of nosocomial Gram-negative rod bacteremia.[5] With short-term catheterization, acquisition of infection approaches 5% of exposed subjects per day.

Approximately 5% of individuals resident in long-term care facilities in North America have a chronic indwelling catheter. The prevalence of bacteriuria in these subjects is 100%.

Intermittent catheterization is also associated with a high frequency of infection. For individuals who have neurogenic bladders managed by intermittent catheterization, the reported rates of infection are 4.06 per 100 patient days[6] or 17.2 per patient year.[9]

Most catheter-associated infections are asymptomatic, but symptomatic infection, including bacteremia, sepsis syndrome and death, may occur. Although bacteremia occurs in only 2–4% of patients who have catheter-acquired UTI, the high frequency of indwelling catheter use means that the absolute number of episodes of bacteremia secondary to catheter-acquired UTI is high.

PATHOGENESIS

Risk factors

The normal genitourinary tract, apart from the distal urethra, is sterile. The usual colonizing flora of the distal urethra include *Staphylococcus epidermidis*, diphtheroids, streptococci and certain anaerobes. These organisms are rarely uropathogens. The sterility of the urine and genitourinary tract is primarily maintained through the flushing action of voiding of urine.

Obstruction to normal urine flow overwhelms all other factors in promoting infection. Other contributing factors to the development of UTI include the concentration and chemical composition of the urine, the bladder mucus layer and Tamm-Horsfall protein excreted from the kidneys. However, in complicated UTI, including postsurgical infection and catheter-related infections, the major factor contributing to the initiation and persistence of bacteriuria is an impaired ability to flush organisms from the urinary tract. This may be due to either:

- obstruction to urine flow with a pool of urine remaining in the urinary tract after voiding; or
- the presence of a protected environment, such as an infection stone or a bacterial biofilm on a catheter, from which organisms cannot be eradicated by usual antimicrobial therapy.

Many genitourinary abnormalities are associated with an increased incidence of UTI (Table 70.2). These are congenital or acquired functional, structural or metabolic abnormalities. The abnormality may be transient, for instance presence of a noninfected stone, a cystoscopy procedure or short-term catheterization. In this situation, the increased risk of UTI will resolve once the abnormality is corrected. If the abnormality cannot be corrected, as in a patient who has an ileal conduit or with a neurogenic bladder maintained on intermittent catheterization, there is a continued risk of recurrent UTI.

TABLE 70-2 -- Genitourinary abnormalities associated with an increased frequency of urinary tract infection.

GENITOURINARY ABNORMALITIES ASSOCIATED WITH AN INCREASED FREQUENCY OF UTI	
Type of lesion	**Examples**
Obstructing lesion	Tumor, stricture, urolithiasis, prostatic hypertrophy, diverticulum, pelvicalyceal junction obstruction, congenital abnormality, renal cysts
Foreign body	Indwelling catheter, ureteric stent, nephrostomy tube
Functional abnormality	Neurogenic bladder, vesicoureteral reflux
Metabolic illness	Diabetes mellitus, medullary sponge kidney, post renal transplantation
Urinary instrumentation and urologic surgery	Prostatectomy, cystoscopy
Urinary diversion	Ileal conduit

Where UTI occurs in a patient who has an indwelling urethral catheter, the organisms may have gained access to the bladder by two routes:

- ascending the mucous sheath from the periurethral area on the outside of the catheter; or
- intraluminally by ascension up the catheter.[10]

The intraluminal route appears to be more important in men than in women, in whom a shorter urethra probably facilitates extraluminal ascent.

When infection occurs in the presence of a foreign body in the genitourinary tract, such as a ureteral stent, nephrostomy tube or indwelling catheter, a bacterial biofilm usually forms on the inert material.[11] A biofilm is an adherent colony of organisms with individual organisms encased in copious extracellular matrix. This biofilm provides a relatively protected environment for the bacteria by interfering with the diffusion of antibiotics and host defenses and so contributing to relapsing infection.

Urinary tract infection may also be acquired in urologic practice as a result of organism transmission between patients on inappropriately cleaned diagnostic or therapeutic equipment.[12] In particular, contamination is a risk where instruments are not appropriately changed or cleaned between patients and where fluid is left standing for prolonged periods at room temperature. Multipatient use of urometers or urine collecting devices has also been repeatedly identified as a cause of nosocomial outbreaks of infection.[13]

MICROBIOLOGY

The spectrum of micro-organisms isolated from individuals who have complicated UTI is more varied than observed in patients who have uncomplicated UTI. Table 70.3 summarizes the organisms isolated in a number of studies of complicated UTI. Although *Escherichia coli* remains an important infecting organism, the frequency with which it is isolated is substantially lower than that reported for acute uncomplicated UTI. *Escherichia coli* has unique virulence characteristics, which promote symptomatic infection in the person with a normal genitourinary tract (see Chapter 67). Where abnormalities of the genitourinary tract bypass the important non-specific host resistance provided by complete voiding, organisms that do not possess unique virulence properties may also become important uropathogens. Therefore, there is a lower prevalence of genotypic or phenotypic expression of virulence factors by *E. coli* isolated from individuals who have complicated genitourinary infection than by *E. coli* isolated from acute uncomplicated UTI.[18]

TABLE 70-3 -- **Bacteria isolated in complicated urinary tract infection.** Shown is the frequency of isolation of different bacterial species.[14] [15] [16] [17]

BACTERIA ISOLATED IN COMPLICATED UTI	
Organism	**Proportion of total organisms isolated (%)**
Gram-negative organisms	
Escherichia coli	21–54
Klebsiella pneumoniae	1.9–17
Citrobacter spp.	4.7–6.1
Enterobacter spp.	1.9–10
Proteus mirabilis	0.9–10
Providencia spp.	1.9
Pseudomonas aeruginosa	2.0–19
Other Gram-negative organisms	6.1–23
Gram-positive organisms	
Enterococci	6.1–23
Coagulase-negative staphyloccoci	1.3–3.7
Staphylococcus aureus	0.9–2.0
Group B streptococci	1.2–3.5
Other Gram-positive organisms	1.9

A wide variety of bacterial species other than *E. coli* is isolated in UTI. The distribution of organisms is determined by factors such as:

- whether organisms are isolated from initial or recurrent infection;
- whether acquisition is nosocomial- or community-acquired; and
- previous antimicrobial exposure.

Common organisms include Enterobacteriaceae such as *Klebsiella, Citrobacter, Serratia, Proteus* and *Providencia* spp., other Gram-negative organisms such as *Pseudomonas aeruginosa* and other nonfermenters, and Gram-positive organisms such as *Enterococcus faecalis* and group B streptococci. Coagulase-negative staphylococci are frequently isolated, although rarely in symptomatic infection, and their pathogenicity is seldom clear. Yeasts, primarily *Candida* spp., may be isolated, usually in individuals who have had prolonged or repeated courses of antimicrobial drugs.[18A] Anaerobic organisms are isolated rarely, and then in the setting of highly complicated urologic abnormalities and abscess formation in the urinary tract.

The urease-producing organisms, principally *Proteus mirabilis, Providencia stuartii* and *Morganella morganii,* are important pathogens. Rarely, more unusual urease-producing organisms such as *Ureaplasma urealyticum* or *Corynebacterium* D2 may be isolated. These organisms maintain an alkaline environment, promoting persistence of infection and leading to the formation of struvite stones or catheter encrustation.

In addition to the much wider variety of infecting species in complicated UTI compared with uncomplicated UTI, there is also increased antimicrobial resistance among the infecting bacteria. Some of the infecting organisms, such as *P. aeruginosa*, are intrinsically more resistant to antimicrobials. Increased resistance is also promoted

by:

- repeated antimicrobial courses for previous UTI; and
- the high frequency of nosocomial infection.

PREVENTION

General measures

Urinary tract infection in the abnormal genitourinary tract occurs because of the presence of an underlying abnormality or intervention that breaches normal defenses and allows the introduction and persistence of micro-organisms. Therefore the most important interventions to prevent UTI are:

- to identify and, wherever possible, correct underlying abnormalities; and
- to avoid nonessential interventional procedures.

It is important to follow the appropriate aseptic technique for interventional procedures such as cystoscopy or urodynamic studies and for operative procedures. All fluids used in urologic procedures must be handled in a manner that ensures sterility. In particular, equipment must be disassembled after a procedure and reassembled using sterile components before the next procedure, and aseptic technique must be maintained. [12]

Institutions should establish and maintain appropriate infection surveillance programs to ensure that endemic infection rates are known and to facilitate early identification of potential outbreaks.

Catheter-acquired infection

The frequency of catheter-associated UTI in institutional settings has led to extensive study of specific interventions to prevent catheter-acquired infection (Table 70.4).[5] [10] The single most important practice in preventing infection in the catheterized patient is to maintain a closed urinary drainage system. In addition, use of an aseptic technique at insertion is important. Patients who have indwelling catheters who receive antimicrobial therapy have a decreased incidence of infection acquisition during the initial 4 days of catheterization compared with patients who do not receive antimicrobials. After the first 4 days, the infection rates are similar, but patients receiving antimicrobials develop infection with more resistant organisms. Therefore, antimicrobial therapy to prevent infection when an indwelling catheter is in situ is currently not recommended.

TABLE 70-4 -- Interventions to prevent catheter-acquired bacteriuria.[5] [10]

INTERVENTIONS TO PREVENT CATHETER-ACQUIRED BACTERIURIA	
Proven effective	Avoid use of catheter
	Limit duration of catheterization
	Aseptic insertion
	Maintain closed drainage system
	Antibiotics first 4 days (not recommended)
Possibly effective	Antibiotics last 48h of catheterization
	Antimicrobial decontamination of gut
Proven not effective	Daily meatal care with soap or antiseptic
	Disinfectant (formaldehyde, chlorhexidine, hydrogen peroxide) in drainage bag
	Silver-coated catheters
	Continuous antibiotic or antiseptic irrigation

TABLE 70-5 -- Prophylactic antimicrobial therapy in genitourinary surgery to prevent postoperative urinary tract infection.

PROPHYLACTIC ANTIMICROBIAL THERAPY IN GENITOURINARY SURGERY TO PREVENT POSTOPERATIVE UTI			
Procedure	**Regimen**	**Infection rate with prophylaxis (%)**	**Infection rate without prophylaxis (%)**
Transurethral instrumentation			
UTI with stone extraction[2]	Cefotaxime 1 g iv, one dose	8.5	25
Sepsis[20]	Cefotaxime 1 g iv, one dose	0	6.2
Prostatectomy			
Sterile urine preoperatively[22]	Various	3–22	6–70
Preoperative bacteriuria[22]	Various	35–41	65–92
Renal transplantation[23]	Trimethoprim-sulfamethoxazole 160mg–800mg daily for 4 months	8	35

Repeated evaluations of interventions using topical or local anti-infectives to prevent infection associated with indwelling catheters have consistently documented no benefit.[5] For instance, daily perineal cleansing with either soap or disinfectant does not decrease and may increase the rate of infection. Other measures that do not decrease the frequency of infection are the addition of disinfectants such as povidone-iodine or chlorhexidine to the drainage bag, the use of catheters impregnated with antimicrobial agents such as silver, and routine irrigation with normal saline. It is, in fact, remarkable how consistently local anti-infective measures have failed to modify the occurrence of catheter-acquired infection.

The use of antimicrobials for preventing infection in patients who have spinal cord injury and who are maintained on intermittent catheterization has also been controversial. Clinical studies report prevention of both asymptomatic and symptomatic infection in the early postinjury months, but at the cost of increased antimicrobial resistance when infection occurs. Prophylactic therapy in the long term is probably not effective. Therefore, prophylactic antimicrobials are currently not recommended for such patients.[19]

Postoperative infection

The perioperative use of antimicrobials encompasses two issues:

- treatment of pre-existing bacteriuria to prevent the complications of invasive infection; and
- prophylaxis to prevent postoperative infection in individuals without positive pre-intervention urine cultures.

Treatment of bacteriuria preoperatively in individuals undergoing genitourinary interventions is indicated to prevent postoperative bacteremia and sepsis. Postoperative

sepsis was reduced from 6.2% to zero in patients whose urine was infected preoperatively when appropriate antimicrobials were given 2–12 hours before operation.[20] The use of preoperative antimicrobials in this situation is most appropriately considered as therapy for UTI rather than prophylaxis, although it is prophylaxis for invasive infection. Antimicrobial therapy should be selected on the basis of the infecting organism and antimicrobial susceptibilities and initiated at least 1 hour before surgery.

There are some indications for the use of true prophylactic therapy in urologic surgery.[21] A summary of these indications is provided in Table 70.5 , with some reported rates of infection observed when selected prophylactic regimens are used. Most authorities[21] [22] now suggest that antimicrobial prophylaxis is appropriate for transurethral prostatectomy even if the pre-procedure urine culture is negative, although this recommendation was controversial in the past.[24]

There is no generally accepted 'standard' antimicrobial regimen for prophylaxis. Many different antimicrobials have been used.

Generally, an aminoglycoside, with or without a cephalosporin, or a fluoroquinolone is used. Studies have documented the efficacy of second- and third-generation cephalosporins, including cefotaxime, ceftriaxone, cefotetan, cefoxitin and ceftazidime, as well as fluoroquinolones. It is not clear, however, that these agents are superior to less costly alternatives such as aminoglycosides and trimethoprim-sulfamethoxazole.

The recommended dosing regimen is one dose 1–2 hours preoperatively. The appropriate duration of antimicrobial therapy has not been defined. If an indwelling catheter remains in situ postoperatively some authors recommend continuation of antibiotics until the catheter is removed. However, recent studies suggest that, at least for some agents, a single dose is as effective as multidose therapy.[25] [26] The shortest effective duration of therapy is preferred to limit cost, adverse effects and the emergence of antimicrobial-resistant organisms.

CLINICAL FEATURES

The clinical presentation of complicated UTI varies along a spectrum from asymptomatic bacteriuria without a measurable host response to septic shock and death.

In many clinical situations where chronic or recurring infection is anticipated, such as a patient with a chronic indwelling catheter or the individual who has a neurogenic bladder and is maintained on intermittent catheterization, asymptomatic bacteriuria is the most common presentation. When symptomatic infection occurs the clinical features are those usually observed with a lower UTI such as frequency, suprapubic discomfort, dysuria and urgency.

With renal infection the characteristic presentation of upper UTI infection, including fever and costovertebral angle tenderness, is observed.

Obstruction and trauma to the genitourinary mucosa at any site predispose to bacteremia and more severe infection. Other contributing factors in determining the clinical presentation for a given infectious episode have not been well studied.

Infection may occasionally present as a high fever without any localizing findings, particularly in individuals who have indwelling catheters or neurologic impairment. Therefore, the clinical presentation may suggest a urinary source of infection or be non-specific. A diagnosis of UTI in the febrile patient who has a positive urine culture and no localizing findings must, however, be viewed critically. In populations with a high prevalence of asymptomatic bacteriuria the majority of such episodes are not due to UTI.[27]

Infection may be localized to the bladder or may involve the upper tract or kidney. In addition, in males, bladder infection may be secondary to or lead to prostatic infection. Presenting clinical symptoms are generally unhelpful in localizing the site of infection unless renal or prostatic tenderness can be demonstrated. Infection may manifest with lower UTI irritative symptoms alone despite the presence of upper tract or renal infection. In individuals with uncomplicated UTI, fever is a reliable localizing symptom for upper UTI. This is not the case for complicated and postsurgical infection or infection in the presence of an indwelling catheter. In these cases trauma to the bladder mucosa can result in invasive infection and fever associated with lower UTI alone. In most cases, however, treatment decisions will not depend upon knowledge of the site of infection within the urinary tract.

Presentation in selected patient groups

Selected patient groups may demonstrate some variation in presentation.

For patients who have a spinal cord injury and a neurogenic bladder, the clinical presentation may differ from the usual irritative lower tract symptoms because of absent or altered sensation associated with the neurologic injury.[19] Signs and symptoms suggestive of UTI, in addition to fever, kidney pain or tenderness and bladder discomfort, may include a new onset of or increase in urinary incontinence, autonomic hyperreflexia, increased sweating, increased spasticity, cloudy or malodorous urine, and a general sense of being unwell.

In patients who have undergone renal transplantation, symptoms and signs may be absent or mild in the early post-transplant period, despite the presence of bacteremia. This lack of symptoms may be due to immunosuppressive therapy or uremia.

Occasionally, symptoms of the underlying genitourinary abnormality may be prominent. For instance, if a UTI occurs in the setting of a ureteral stone, symptoms of renal colic may predominate, and the bacteriuric patient who has diabetes mellitus and papillary necrosis may have prominent symptoms of renal colic. A man who has acute bacterial prostatitis may have prominent symptoms of urethral obstruction and even retention.

Presentation with specific infecting organisms

Infection by selected organisms may also produce a unique clinical presentation. *Corynebacterium* D2 infection is associated with the clinical syndrome of encrusted cystitis. This is encrustation of the bladder wall by struvite due to the urease production of the organism. Infections with Enterobacteriaceae, usually *E. coli* and *Klebsiella pneumoniae*, in patients who have diabetes mellitus and hyperglycemia and glycosuria may present as emphysematous cystitis or pyelonephritis. If a persistent fungal infection is identified, there may be a fungus ball in the bladder or kidney associated with obstruction.

Recurrent infection after antimicrobial therapy

Early recurrent infection after antimicrobial therapy is a characteristic clinical feature of individuals with persistent genitourinary abnormalities. It may be symptomatic or asymptomatic and may represent:

- relapse with recurrence of the pre-therapy infecting organism after therapy; or
- re-infection with a new organism.

Selected reports that document this high frequency of recurrent infection are summarized in Table 70.6 . Bacteriologic cure rates at 4–6 weeks (long-term follow-up) are consistently less than 50% (i.e. recurrent infection is the expected outcome). If the underlying abnormality

TABLE 70-6 -- Bacteriologic outcome after antimicrobial therapy of complicated urinary tract infection. NS, not stated.

BACTERIOLOGIC OUTCOME AFTER ANTIMICROBIAL THERAPY OF COMPLICATED UTI			
Regimen	**Follow-up after therapy**	**Cure (%)**	**Re-infection (%)**
Complicated urinary infection			
Lomefloxacin[15]	5–9 days	59	5.9
	4–6 weeks	43	19
Trimethoprim-sulfamethoxazole[15]	5–9 days	33	1.5
	4–6 weeks	28	9.2
UTI secondary to spinal cord injury			

Norfloxacin 14 days[28]	5–7 days	53	14
	8–12 weeks	16	NS
Varied 7–14 days[29]	1 week	47	NS
Varied =28 days[29]	1 week	41	NS

is transient or reversible, such as a single obstructing stone that is passed, permanent or long-term cure may, however, be achieved. If the underlying abnormality promoting infection cannot be corrected, recurrent infection with organisms of increasing antimicrobial resistance is a common outcome. Some patients may ultimately have infections for years with very resistant organisms such as *Pseudomonas* spp.

DIAGNOSIS

Clinical symptoms alone are not sufficient for a diagnosis of complicated UTI. For definitive diagnosis an appropriately collected urine specimen must be obtained for bacterial culture. The large variety of potential infecting organisms and the high likelihood of antimicrobial resistance in infecting organisms mean that a urine culture is essential for appropriate antimicrobial management of patients who have complicated UTI. The urine specimen must be collected before initiating antimicrobial therapy, using a urine collection method that limits contamination. A clean-catch voided specimen or, if a voided specimen cannot be obtained, a specimen obtained through in and out catheterization, is usually appropriate. For individuals who have indwelling catheters, urine is collected by aseptic aspiration from the catheter port. Specimens may also be obtained by ureteric catheterization or percutaneous aspiration of the renal pelvis but these invasive procedures are not recommended unless there is obstruction. There is no completely satisfactory way of collecting specimens for culture from people who have an ileal conduit. Specimens collected through the conduit will be contaminated with organisms colonizing the conduit.

Foreign material in the urinary tract, including indwelling urethral catheters, ureteric stents and nephrostomy tubes, are rapidly coated with a bacterial biofilm after insertion. Organisms isolated from urine specimens for culture obtained through such devices may be more representative of the microbiology of the biofilm on the inner surface of the catheter rather than of bladder urine. Therefore, it has been suggested that indwelling catheters should be changed before specimen collection and initiation of antimicrobial therapy.[10] The urine specimen is collected through the newly inserted catheter, which is free of biofilm, and is representative of bladder bacteriuria with a more reliable identification of the bacterial species responsible for symptoms.

Urine specimens should be forwarded promptly to the laboratory for semiquantitative culture and appropriate susceptibility testing. Blood cultures should also be obtained from patients who have evidence of sepsis, including fever, rigors, hypothermia and confusion, and from early post-transplant patients who may be significantly immunosuppressed.

TABLE 70-7 -- Quantitative bacteriology in the diagnosis of complicated urinary tract infection.

QUANTITATIVE BACTERIOLOGY IN THE DIAGNOSIS OF COMPLICATED UTI	
Clinical presentation	**Bacteriologic count**
Asymptomatic bacteriuria	=10^5 cfu/ml in two consecutive urine specimens
Symptomatic urinary infection	=10^4 cfu/ml in one specimen or =10^5 cfu/ml if collected by external catheter
Percutaneous aspiration in hydronephrosis	Any quantitative count
Diuresis, diuretic therapy, renal failure, selected infecting organisms (e.g. *Candida albicans*)	Lower quantitative counts (<10^5 /ml) may occur in these situations

Quantitative bacteriology

Current recommendations for quantitative bacteriology in the diagnosis of complicated UTI are provided in Table 70.7 . In the symptomatic patient, UTI may be diagnosed if the quantitative count of organisms in urine culture is 10^4 cfu/ml or more and there are symptoms consistent with genitourinary infection. For individuals who are asymptomatic, two specimens with a quantitative count of 10^5 cfu/ml or more with the same organism(s) isolated on two consecutive occasions are necessary for diagnosis. [30]

Infections usually involve a single infecting organism but there may be more than one bacterial species in the urine of patients who have frequent recurrent infections. In the patient who has a long-term indwelling catheter, isolation of two to five organisms is the norm.[5] In the presence of an indwelling catheter, small numbers of micro-organisms will usually increase to 10^5 cfu/ml or more in less than 24 hours and, therefore, any quantitative count may be significant.[31]

Rarely, less common organisms, such as yeast species, may not produce the usual quantitative counts. Unusual uropathogens such as *Mycoplasma hominis* or *Haemophilus influenzae*, which rarely cause infection, will not be isolated by routine laboratory methods for urine culture. Urine cultures may also be negative with symptomatic infection if the patient has had previous antimicrobial therapy or there is complete ureteric obstruction with infection localized proximal to the obstruction.

Pyuria

The presence of pyuria in a urine specimen may be useful in the diagnosis of UTI, but may be misleading. Pyuria reflects inflammation within the urinary tract, which is not necessarily caused by infection. Pyuria by itself is not sufficient to diagnose a UTI in the absence of a positive urine culture. Most UTIs have associated pyuria, but underlying abnormalities associated with complicated UTI, inflammation following a surgical procedure, or a chronic indwelling catheter may be associated with pyuria in the absence of infection. Pyuria does not discriminate between symptomatic and asymptomatic infection. Therefore, a dipstick test or urinalysis that shows evidence of pyuria is consistent with but not diagnostic of UTI.

MANAGEMENT

Essential elements in approaching the management of UTI in the setting of an abnormal genitourinary tract include:

- initial clinical evaluation and appropriate diagnostic specimen collection;
- initial antimicrobial therapy;
- appropriate supportive therapy;
- a review of urine bacteriology to ensure that the optimal antimicrobial regimen has been given; and
- an assessment of the need for genitourinary investigation and appropriate interventions to correct any abnormality.

Antimicrobial therapy

The empiric use of antimicrobials in this group of patients, who have a high likelihood of recurrent infection, will promote the emergence of organisms with increased antimicrobial resistance. Whenever possible, empiric therapy should be avoided and antimicrobial therapy should be specific for the infecting organism(s) identified in urine culture. This is frequently possible if the patient has mild symptoms. If the patient's symptoms are severe enough to warrant empiric therapy before the final culture results are available, a urine specimen for culture must be obtained before initiating therapy, and the antimicrobial therapy should be re-evaluated when the culture and susceptibility testing results are available, usually between 48 and 72 hours later.

TABLE 70-8 -- Oral therapeutic regimens for the treatment of complicated urinary tract infection.

ORAL THERAPEUTIC REGIMENS FOR THE TREATMENT OF COMPLICATED UTI	
Agent	**Dose**
Amoxicillin	500mg q8h
Amoxicillin-clavulanate	500mg q8h
Cephalexin	500mg q6h
Cefixime	500mg q24h
Nitrofurantoin	50mg q6h
Nitrofurantoin macrocrystals	100mg q6h
Norfloxacin	400mg q12h
Ciprofloxacin	250–500mg q12h
Ofloxacin	400mg/day or 200mg q12h
Lomefloxacin	400mg/day
Enoxacin	200mg q12h
Fleroxacin	400mg/day
Gatifloxacin	400mg q24h
Trimethoprim	100mg q12h
Trimethoprim-sulfamethoxazole	160mg trimethoprim-800mg sulfamethoxazole q12h

TABLE 70-9 -- Parenteral regimens for the treatment of complicated urinary tract infection.

PARENTERAL REGIMENS FOR THE TREATMENT OF COMPLICATED UTI	
Agent	**Dose**
Ampicillin	1g q6h
Piperacillin	3g q6h
Ticarcillin-clavulanate	3.1g q6h
Pipericillin-tazobactam	4g pipericillin-500mg tazobactam q8h
Cefazolin	1–2g q8h
Cefotaxime	1g q8h
Ceftriaxone	1g q24h
Ceftazidime	1g q8-12h
Imipenem	500mg q6h
Gentamicin	5mg/kg/day q12h or q24h
Tobramycin	5mg/kg/day q12h or q24h
Amikacin	15mg/kg/day q12h or q24h
Trimethoprim-sulfamethoxazole	160mg trimethoprim-800mg sulfamethoxazole q12h
Ciprofloxacin	400mg q12h
Ofloxacin	400mg q12h
Fleroxacin	400mg/day
Gatifloxacin	400mg/day

The antimicrobial agents appropriate for therapy are similar to those used in the treatment of acute uncomplicated UTI or acute nonobstructive pyelonephritis (Table 70.8 & Table 70.9). Initial parenteral therapy is preferred for individuals who have:

- hemodynamic instability;
- nausea and vomiting;
- questionable absorption of oral antimicrobials; or
- an infection by suspected resistant organisms for which oral therapy is not available.

In other situations, oral therapy can usually be initiated. The majority of patients can be managed without hospitalization or with a limited (24–72h) admission to a short stay unit for initial parenteral therapy. Oral therapy is then started as soon as the patient is stable, often when pre-therapy urine culture results are available to assist in selecting the appropriate medication.

The selection of a specific antimicrobial agent is based upon clinical presentation, the known or suspected infecting organism and its susceptibilities, patient tolerance, documented efficacy and, in some cases, cost. Comparative trials of different antimicrobials have not consistently reported that any one antimicrobial agent or class of antimicrobials is better than any other.[32] The selection of optimal antimicrobial therapy is further limited by a relative lack of relevant clinical trials. Although there are many published reports on the treatment of complicated UTI, few are useful in assisting with the selection of an antimicrobial in a specific clinical situation. One problem is the lack of an accepted 'standard' antimicrobial therapy with respect to agent, dose or duration to serve as a comparator for new agents.[30] In addition, studies have generally enrolled diverse and poorly characterized patient populations for whom different outcomes with therapy would be anticipated on the basis of underlying abnormalities. The severity of illness also varies, and subjects are enrolled into studies with clinical presentations ranging from an increase in incontinence or bladder spasms to a life-threatening illness with bacteremia.

In view of the very high frequency of relapse, therapeutic trials should provide both short-term (1 week post-therapy) and long-term (4–6 weeks post-therapy) outcomes.[30] Frequently, studies of therapy for complicated UTI have provided only short-term therapeutic outcomes. The many published studies therefore allow only a limited assessment of the comparative efficacy of different antimicrobials. They do, however, document the effectiveness of a wide variety of antimicrobial agents.

Oral therapy

For oral therapy, the quinolone antimicrobials in particular have been widely studied and promoted. Benefits of fluoroquinolones include a wide antibacterial spectrum and good patient tolerance. Problems include increasing antimicrobial resistance with widespread use of quinoline antimicrobials, and cost.

In studies of therapy for pyelonephritis, likely including some patients who had complicated infection, cell-wall-active agents such as amoxicillin or first-generation cephalosporins appear less effective than non-cell-wall-active agents such as trimethoprim-sulfamethoxazole or quinolones.[33] Appropriate comparative clinical trials have not shown whether these cell-wall-active agents have a lower efficacy for complicated UTI. For Gram-positive organisms such as group B streptococci and *Enterococcus* spp., amoxicillin orally or ampicillin parenterally would, of course, be the treatment of choice. Nitrofurantoin may be used for patients who have lower

tract symptoms. This agent should be avoided in subjects with renal impairment or pyelonephritis, and is not effective for *P. mirabilis* infection.

Parenteral therapy

For parenteral therapy, an aminoglycoside antimicrobial remains the treatment of choice because of the documented efficacy of this group of drugs over many years of use and the likelihood of effectiveness against more resistant organisms. The nephrotoxicity and ototoxicity of aminoglycosides are seldom a problem if the duration of therapy is limited, with an early switch to oral therapy. If *Enterococcus* spp. may be present, ampicillin should be added. The increasing prevalence of ampicillin resistance in *E. faecium* and *E. faecalis* in many institutions means that vancomycin may be necessary to treat nosocomial enterococcal infection. Empiric use of vancomycin is, however, to be discouraged because widespread empiric use will promote the emergence of vancomycin-resistant organisms.

Duration of therapy

Few reported studies have directly addressed the question of the appropriate duration of therapy. The usual recommended duration is

7–14 days.[30] There are selected clinical situations where alternative durations of therapy are appropriate:

- ¦ where relapsing infection is due to a prostate source, 6 or 12 weeks of antimicrobial therapy is appropriate;
- ¦ if symptomatic infection associated with an indwelling catheter is treated while the catheter remains in situ, it is recommended that the duration of therapy is as short as possible, usually 5–7 days, to limit the emergence of resistant organisms; and
- ¦ after successful extracorporeal shock wave lithotripsy for an infected struvite stone, antimicrobial therapy is continued for at least 4 weeks to prevent relapse and sterilize residual stone fragments.

Supportive therapy

This should be given as appropriate. It may include hemodynamic monitoring, parenteral fluids, measurement of urine output and antiemetic medication. If any obstruction in the urinary tract is suspected, urgent ultrasound or computerized tomography scan should be obtained. Immediate drainage may be necessary if an obstruction is identified. If a chronic indwelling catheter is present, catheter replacement prior to initiating antimicrobial therapy is associated with more rapid defervescence and a decreased frequency of symptomatic relapse[11] as well as providing a more accurate urine specimen for culture.

Renal failure

Patients who have abnormalities of the genitourinary tract are more likely to have impaired renal function. Renal function should be determined, if not already known, for patients who present with possible complicated UTI. If there is renal impairment:

- ¦ appropriate modifications in antimicrobial dose are necessary; and
- ¦ nitrofurantoin and tetracyclines other than doxycycline should be avoided.

Some quinolones have been shown to lead to a reversible increase in serum creatinine in renal transplant recipients receiving cyclosporin. Quinolones should be avoided, if possible, in these patients.

In renal failure, renal perfusion is decreased and antimicrobials may not reach infected renal tissue or achieve high urine levels. The aminoglycosides, in particular, may be less effective.[32] The fluoroquinolone antimicrobials, trimethoprim-sulfamethoxazole, trimethoprim and extended-spectrum ß-lactam antimicrobials appear, however, to be effective in treatment of UTI in the presence of significant renal failure. A more prolonged duration of antimicrobial therapy may also be necessary to cure UTI in patients who have renal failure.

An additional potential therapeutic problem is the patient who has normal measured renal function and satisfactory urinary antimicrobial levels but disparate kidney function.[32] If the function of one kidney is severely impaired relative to the other, blood flow is preferentially increased in the functioning kidney and, despite adequate bladder urine antibiotic levels, little antibiotic will be filtered by the poorly functioning kidney. Antimicrobial treatment may then not

TABLE 70-10 -- Treatment regimens for fungal urinary tract infection.[35]

TREATMENT REGIMENS FOR FUNGAL UTI		
Agent	**Dose**	**Cure rate (%)**
Amphotericin B: parenteral	0.3mg/kg single dose or 6mg/kg body weight total dose	75
Amphotericin B: bladder irrigation	Continuous, 50mg/l for 5 days	72–88
Fluconazole	50–400mg/day for 7 days	70–80
5-Flucytosine	50–150mg/kg/day q6h	70

eradicate infection localized to the poorly functioning kidney. This is one potential reason for relapsing infection.

Fungal infection

Treatment of asymptomatic funguria is not beneficial and is not recommended[34] .

Optimal treatment and expected outcomes for symptomatic fungal infection require further study.[35] The current treatment options and reported cure rates are listed in Table 70.10 . Currently recommended treatment for funguria is amphotericin B or fluconazole. Some non-albicans *Candida* spp., particularly *C. krusei* and *C. glabrata*, have decreased susceptibility or are resistant to fluconazole. Comparative trials of fluconazole and amphotericin B and, potentially, other azoles are necessary to understand the relative roles for different agents. Previously, bladder irrigation with amphotericin B has been recommended. This approach, however, is costly and time-consuming and is no longer considered optimal therapy.

If there is symptomatic fungal infection, the presence of a fungus ball in the bladder or kidneys should be excluded by ultrasound examination or other diagnostic imaging.

Suppressive therapy

Suppressive therapy is long-term antimicrobial therapy given to prevent recurrent symptomatic infection in individuals with underlying abnormalities that cannot be corrected (Fig. 70.1). [36] Long-term therapy, such as suppressive therapy, is infrequently indicated and should be used selectively because of the potential for inducing the development of resistant organisms.

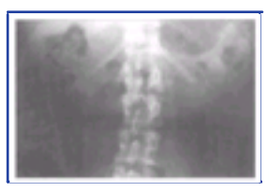

Figure 70-1 Nephrocalcinosis complicated by relapsing *Pseudomonas aeruginosa* infection. Plain film of the abdomen showing multiple bilateral renal stones. This 31-year-old woman has had recurrent stone formation since the age of 18 despite dietary manipulation and repeated lithotripsy. Urinary infection with *P. aeruginosa* was identified at 24 years of age with subsequent recurrent episodes of symptomatic upper tract infection. She has been maintained on suppressive ciprofloxacin therapy for years with control of symptomatic infection.

TABLE 70-11 -- Indications for the treatment of asymptomatic bacteriuria.

INDICATIONS FOR THE TREATMENT OF ASYMPTOMATIC BACTERIURIA	
Definite	Before an invasive genitourinary procedure[20]
	Pregnancy[38]
	Infection stone[37]
	Renal transplant[23]
Not indicated	In the elderly[39]
	For a schoolgirl[40]
	Intermittent catheterization[19]
	Indwelling urinary catheter[5]

Suppressive therapy is recommended for the few individuals with a struvite (infection) stone that cannot be completely removed.[37] Prolonged suppressive therapy in this situation will usually prevent further stone enlargement and preserve renal function. If a patient who has an underlying abnormality that cannot be corrected experiences recurrent invasive infection suppressive therapy may prevent symptomatic episodes. Another situation where suppressive therapy may be appropriate is in men who have frequent recurrent episodes of symptomatic urinary infection from a prostatic source when prolonged antimicrobial therapy has failed to cure the infection.

Asymptomatic bacteriuria

Indications for the treatment of asymptomatic UTI remain controversial. Table 70.11 summarizes current recommendations with respect to the treatment of asymptomatic bacteriuria in different patient populations. These recommendations for treatment or nontreatment are based on published studies. Where treatment of asymptomatic bacteriuria is not indicated, therapy has not been shown to decrease morbidity but is associated with a greater frequency of negative outcomes, including the emergence of resistant organisms and adverse drug effects. Studies in children, in fact, suggest that the treatment of asymptomatic bacteriuria may increase the frequency of symptomatic infection. Asymptomatic bacteriuria should not be treated except where clinical studies have demonstrated a benefit. Following from this, screening for bacteriuria is not indicated except in those for whom treatment of asymptomatic bacteriuria is indicated.

INDICATIONS FOR INVESTIGATION

An important aspect of the management of UTIs is determining when to carry out urologic or imaging investigations to determine whether there are abnormalities in the genitourinary tract (see also Chapter 69). Single infections that respond promptly to antimicrobials are unlikely to be associated with significant underlying abnormalities. On other occasions the abnormality may be obvious, such as the presence of an indwelling catheter. In selected other patients, genitourinary investigation should be considered. These clinical scenarios include:

- delayed or incomplete response to appropriate antimicrobial therapy; and
- early recurrence of infection after therapy.

Patients presenting with sepsis and hemodynamic instability may require urgent investigation to identify an abscess or obstruction requiring immediate intervention. Studies that define the relative efficacy of and optimal approach to investigation in selected clinical scenarios are, however, necessary.

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Chapter 71 - Tuberculosis of the Urogenital Tract

John David Hinze
Richard E Winn

The most common site for extrapulmonary tuberculosis (TB), next to lymphatic and pleural involvement, is the genitourinary tract.

EPIDEMIOLOGY

The incidence and prevalence of genitourinary TB are still uncertain. A large proportion of patients remain asymptomatic and symptoms that do arise are non-specific and easily attributable to other causes.[1] [2] The incidence of genitourinary TB has fallen over the past century, but at a slower rate than the decline of pulmonary TB. Extrapulmonary TB accounted for 22% of all TB cases in the USA in 1992.[3] When expressed as a proportion of extrapulmonary cases, genitourinary TB accounted for 18%.[4] Genitourinary TB varies from 1.3% of all TB cases in Europe[5] and less than 2% in the USA[6] to over 20% in developing countries.[6] [7] [8]

Most cases occur in sexually active adults aged 20–69 years. There is a male:female predominance of 2:1. The presence of TB at any site makes it mandatory to search for involvement at other sites.

PATHOGENESIS AND PATHOLOGY

Nearly all cases of genitourinary TB are caused by *Mycobacterium tuberculosis*. Renal infection with *M. tuberculosis* begins from hematogenous seeding of the renal cortex from a pulmonary source. Either primary or reactivated pulmonary TB infection, which may or may not be clinically apparent,[9] may seed the capillaries of the renal cortices. Other extrapulmonary sites should not, however, be excluded as potential sources. Infection has occurred in a native polycystic kidney after renal transplantation. [10] Given the high cardiac output delivered (20%) and therefore increased oxygen tension of the renal cortex, it is easy to understand the predilection of *M. tuberculosis* for the kidney among extrapulmonary sites. The vast majority of genitourinary TB cases involve the kidney bilaterally and may result in acute renal failure[11] but progression of disease is almost uniformly unilateral.

Once important, *Mycobacterium bovis* from cattle was transmitted via ingestion of infected cow's milk and caused gastrointestinal TB with the potential to spread to the genitourinary tract.

Mycobacterium kansasii and *M. avium-intracellulare* have been cultured from the urine of immunocompromised persons (i.e. AIDS patients), but these cases are rare. *Mycobacterium avium-intracellulare* in the urine is of uncertain clinical significance.

Once infected, host immune status determines the extent of destruction. Macrophages attracted to the site halt the progress of the tubercle bacilli, leading to necrosis and eventual granuloma formation. In an immunocompetent individual, tubercle bacilli are isolated and contained and may ultimately be killed by cellular enzymes. However, a less vigorous response by the host's immune system may not kill all the bacilli and these microscopic foci may remain dormant for decades. Later, if the host's cellular immunity becomes compromised secondary to age, malnutrition, diabetes mellitus, malignancy, corticosteroid use or chronic debilitating disease, reactivation TB may ensue. At this point, as infection spreads from the renal cortex, granuloma formation and subsequent necrosis advance with infection of the medulla and papilla (Fig. 71.1). As these foci coalesce, they form macroscopic cavitary lesions of caseation necrosis (Fig 71.2 and Fig 71.3).[6] [12] Infective debris can both infect and obstruct the calyces, ureters and bladder. Calyceal involvement by ulcerative and deforming lesions (Fig. 71.4) is responsible for many of the radiographic manifestations seen in genitourinary TB (Fig. 71.5). [6] If severe calyceal clubbing occurs with dilatation of the renal pelvis and ureters, then total destruction of the kidney ensues, termed 'autonephrectomy'.[13] Ureteral and bladder disease are secondary to renal involvement. During all stages of renal infection, tuberculous bacilluria is responsible for ureteral and bladder involvement, prostatitis and epididymitis. [14]

In men, urologic spread of renal foci will infect, in descending order of frequency, the prostate, seminal vesicles, epididymis and testes.[15] However, genital TB without renal involvement does occur, suggesting bacillemic spread. In addition, TB of the prostate, epididymis and glans penis can occur with conjugal contact. Primary TB of the glans penis is extremely rare, with about 150 cases reported[16] and, although transmission may be secondary to hematogenous dissemination, local direct sexual contact is most probable.

In women, as in men, genital TB is almost always hematogenous in origin. Direct spread from an intra-abdominal or intraperitoneal source is possible but genitourinary TB may also be part of a miliary process. The fallopian tubes are involved in 90% of cases[17] and chronic salpingitis is the most common manifestation. Fallopian TB is predominantly bilateral with a predilection for the ampullae.[18] From there the bacilli spread, in descending order, to the endometrium in 50–70% of cases, the ovaries in 30% and cervix in 5–15%; vaginal and vulvar disease are rare. [6] [19] [20]

In tuberculous endometritis, the typical lesion is the noncaseating granuloma formed by epithelial cells, lymphocytes and Langhans' giant cells.[18] Noncaseating granulomas are spread superficially and diffusely around the endometrium. Cases of vulvar or vaginal TB are usually secondary to hematogenous spread but, as in males, some may be sexually transmitted. Cases of sexually transmitted TB were reported at least as early as 1917 but remain uncommon.[21]

PREVENTION

The insidious nature of TB as well as the typical latency between onset of symptoms and medical therapy favors its communicability. Prevention of genitourinary TB mirrors that of pulmonary TB, involving identification of infected sources and institution of appropriate antimicrobial therapy. In addition, prevention of primary venereal transmission of TB should follow safe sex guidelines, as the true incidence, although small, is unknown. The Advisory Council for the Elimination of Tuberculosis in the USA recommends a national standard centered on three prevention strategies:

- identifying and treating patients who have active TB;
- screening contacts of active TB patients, determining whether they are infected and, if so, providing appropriate therapy; and

- screening high-risk populations and providing therapy to prevent progression to active TB.[20]

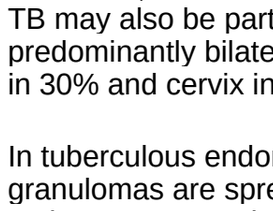

Figure 71-1 Granuloma formation in kidney biopsy. *Courtesy of Robert F Peterson.*

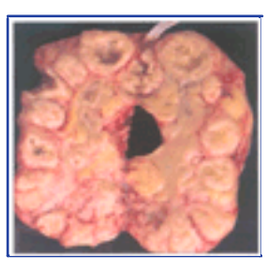

Figure 71-2 Chronic tuberculous nephritis with almost complete destruction of the kidney *Courtesy of Robert F Peterson*.

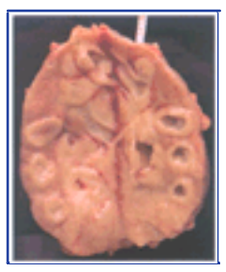

Figure 71-3 Diffuse acute tuberculous nephritis with abscess formation. *Courtesy of Robert F Peterson.*

Once at-risk contacts have been identified, the recommendations for chemoprophylaxis are the same as for pulmonary TB, especially for patients less than 35 years of age or in an 'at-risk' group.

CLINICAL FEATURES

It has long been known that 'generalized tuberculosis of the genitourinary tract may be present without the production of any grave symptoms'.[22] Genitourinary TB is a relentless destructive process that may lie dormant or clinically inapparent for decades. Many people who have pulmonary TB have coexistent asymptomatic genitourinary TB.[20] The most common clinical symptoms of genitourinary TB are:

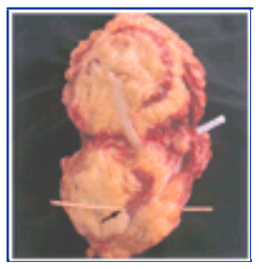

Figure 71-4 Chronic tuberculous nephritis with destruction of all landmarks and pelvic calculus. The arrow shows a fistulous tract delineated by the wooden probe. *Courtesy of Robert F Peterson.*

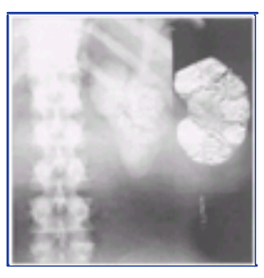

Figure 71-5 Intravenous pyelogram of left kidney with bivalved surgical pathology specimen.

- dysuria (including urinary frequency or urgency);
- recurrent urinary tract infections (UTIs); and
- pain (including back, flank, suprapubic and abdominal pain; Table 71.1).

TABLE 71-1 -- Symptom frequency in patients who have genitourinary tuberculosis.

SYMPTOM FREQUENCY IN PATIENTS WHO HAVE GENITOURINARY TB				
	Study of 160 patients[3]	**Study of 81 patients**[6]	**Study of 83 patients** [15]	**Study of 52 patients** [5]
Dysuria (with urgency and frequency included) (%)	52	65	46	31
Gross hematuria (%)	30	36	43	31
Frank pain/renal colic (%)	23	57	42	21
Fever (%)	12.5	35	23	ND
History of tuberculosis (%)	22.5	23.5	16	ND
ND, no data.				

Constitutional symptoms are generally uncommon but may be more frequent in patients who have advanced disease or those who are immunocompromised, and include fevers, chills, night sweats and weight loss. Hypertension is not generally a feature of tuberculous renal disease and its true prevalence is unknown, although case reports describe alleviation of hypertension after treatment of TB.[23]

In men, genital TB typically presents with a mildly tender or symptomless scrotal mass (epididymitis) and less commonly with prostatitis, orchitis or scrotal fistulas.

In women who have genital TB the most common presenting symptom is infertility[5] [13] [19] and it is estimated that 85% of patients have never been pregnant.[18] The next most common complaint is lower abdominal and pelvic pain, which occurs in 25–50% of patients. As the disease progresses, so does the intensity of the pain, which may be exacerbated by activity or coitus. The clinical presentation might mimic that of pelvic inflammatory disease unresponsive to usual therapy. The third most common complaint is abnormal menstrual bleeding.

DIAGNOSIS

A healthy clinical suspicion is mandatory to diagnose genitourinary TB early, given its insidious nature and the large proportion of patients who are asymptomatic. It should be considered in any patient who has unexplained fever. An evaluation for genitourinary TB is mandatory for any patient with a history of TB who develops dysuria or sterile pyuria from an unidentified cause. However, genitourinary TB may exist despite negative urine cultures. Historic queries regarding past exposure to TB, evening pyrexia, weight loss, asthenia, fatigue and past medical history are helpful. Physical examination findings are non-specific.

In over 50% of patients presenting with a UTI routine bacterial pathogens can be cultured from the urine, obscuring the underlying genitourinary TB. In men, a scrotal mass or nodular prostate may suggest TB. One study of 83 cases of genitourinary TB from Turkey revealed tenderness of the costovertebral angle in 55% of patients.[24]

After examination, initial laboratory investigation should include:

- renal function tests;
- at least three first-morning urine specimens for acid-fast bacillus (AFB) stain and culture; and
- tuberculin skin testing.

Positive AFB stains of urine are usually due to *M. tuberculosis* (R Wallace, personal communication). Of patients who have genitourinary TB, 77–92% will have positive urine cultures for *M. tuberculosis* [6] [7] [19] [24] and 90% will have positive tuberculin skin tests. [25]

Early in the course of genitourinary TB, radiologic manifestations are unremarkable. A chest radiograph should be obtained to assess for co-morbid pulmonary TB. Simple abdominal radiography is generally non-specific and commonly shows amorphous nephrocalcinosis or fine calcifications.

The 'gold standard' and most commonly used radiologic technique for evaluating the genitourinary system for *M. tuberculosis* remains the intravenous pyelogram (IVP) and excretory pyelogram.[26] The most valuable clue to renal TB is concurrent multiple abnormalities of both the upper and lower urinary tracts. Findings in progressive renal TB demonstrate abnormalities of the collecting system from the calyces to the bladder. Initially, an IVP may demonstrate irregular calyceal contours secondary to erosive inflammatory changes that later progress to delayed excretion, cavitation, 'moth-eaten' calyces or strictures with caliectasis. The characteristic finding of a 'phantom calyx' occurs when an IVP reveals an obstructed nonfunctioning calyx proximal to an infundibular stricture.[26] A 'hiked-up' renal pelvis is observed when the renal pelvis makes an acute angulation with the ureter, and is highly suggestive of renal TB. Another highly suggestive lesion is the 'putty kidney' consisting of a heavily calcified caseous mass surrounded by a thin parenchymal shell.[26] Autonephrectomy is the end result. Ureteral TB may manifest as different combinations of strictures and obstructions, giving the appearance of 'beading', 'corkscrewing', straight 'pipestems', focal calcification or hydronephrosis. Commonly, the bladder appears thickened and fibrotic with a small capacity.

In female genital TB, the hysterosalpingogram provides the most characteristic abnormalities, displaying a contracted deformed uterine cavity with associated intrauterine adhesions. The fallopian tubes may have ragged outlines and appear beaded or rigid.[18]

In the evaluation of genitourinary TB, ultrasound, computerized tomography (CT) and magnetic resonance imaging (MRI) are not usually necessary, although sometimes they have a role. A non-visualized kidney is best evaluated by ultrasound or CT.[26] Ultrasound has demonstrated small focal lesions in the kidneys in patients who have renal TB.[26] In women, ultrasonography may reveal predominantly solid adnexal masses containing small scattered calcifications bilaterally. Abdominal and vaginal ultrasound may have a role in delineating the features of tuberculous peritonitis.[26] In men, testicular ultrasound is a rapid and easy way for assessing scrotal contents; however, it is non-specific.[15] Transrectal ultrasound may also be helpful in delineating lower genitourinary tract pathology, but it is also non-specific. Sonography also has a use in providing a guide for fine-needle aspiration of genitourinary lesions.[19]

Computed tomography is superior to IVP and serial examination in delineating anatomic detail and may prove to be beneficial in contributing to therapeutic decisions.[24] Typical CT findings in genitourinary TB are caliectasis and focal cortical scarring (>80%).[26] Magnetic resonance imaging has also been used to describe tuberculous lesions because of its enhanced contrast resolution and multiplanar capabilities. Tuberculous foci appear hypointense on T2-weighted images.[26]

Although a positive purified protein derivative skin test with classic radiologic findings may be suggestive of genitourinary TB, diagnosis should only be made with bacteriologic or histologic evidence. If urine, menstrual blood or seminal fluid cultures are negative, then ultrasound- or CT-guided fine-needle aspiration or diagnostic laparoscopy can be used to provide appropriate tissue for bacteriohistologic diagnosis.

The use of diagnostic laparoscopy in women who have suspected genital TB may provide an earlier diagnosis (Fig. 71.6). The BACTEC system has reduced culture time to roughly 1 week. In addition, polymerase chain reaction (PCR) testing can, in just a few hours, detect

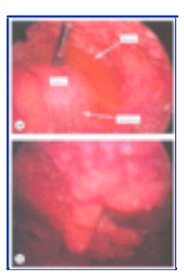

Figure 71-6 Laparoscopic views in genitourinary tuberculosis. (a) Free and loculated ascites and fine fibrous adhesions. (b) Miliary nodular exudate in the anterior wall.

the presence of *M. tuberculosis* in body fluids with exceedingly high specificity.[27]

Differential diagnosis

Differential diagnosis of genitourinary TB should include amebiasis as well as those entities that mimic granulomatous disease such as sarcoid, other mycobacterial disease (including leprosy), actinomycosis, histoplasmosis, tularemia, berylliosis, silicosis, foreign body reactions, malignancy and sexually transmitted diseases.[18] Other differential diagnoses include calyceal or diverticular stones, xanthogranulomatous pyelonephritis,[28] obstructive pyonephrosis and congenital multicystic dysplastic kidney.[17] The salpingitis and infertility of genital TB in women needs to be differentiated from that due to pelvic inflammatory disease.

MANAGEMENT (see also Chapter 37 and Chapter 202)

Many authors believe that there is little difference between the treatment decisions for extrapulmonary and pulmonary TB. Genitourinary TB should be easier to treat then pulmonary TB for several reasons:

- the lower bacillary load;
- the kidney's excellent blood supply;
- the high concentration of bactericidal medication in urine; and
- excellent drug penetration into closed cavities in lethal concentrations. [29]

Daily isoniazid, rifampin (rifampicin) and pyrazinamide for 2–3 months, followed by isoniazid and rifampin for the remainder of the therapeutic course have been used successfully (Table 71.2). If the patient comes from an area where drug resistance is common then a fourth drug, usually ethambutol, is added until drug susceptibility tests for *M. tuberculosis* have been completed.[19]

A 4-month regimen for genitourinary TB has been advocated, with excellent results.[30] However, a high incidence of surgical intervention was observed. A tailored regimen should be devised for each patient, based on susceptibility. The recommended treatment of

TABLE 71-2 -- Medications commonly used for renal tuberculosis, with dosages and common side effects.

MEDICATIONS COMMONLY USED FOR RENAL TB		
Medication	**Dose/day**	**Side effects**
Isoniazid	5–10mg/kg–300mg (max)	Hepatitis, peripheral neuropathy (pyridoxine may decrease the incidence), systemic lupus erythematosus, Dupuytren's contracture
Rifampin	10mg/kg–600mg po, single dose on empty stomach	Gastrointestinal irritation, abnormal liver function tests, stains body secretions orange, flu-like syndrome, multiple drug interactions
Ethambutol	25mg/kg for 2 months then 15mg/kg po	Optic neuritis manifested as changes in visual acuity and red-green color blindness, gastrointestinal discomfort
Pyrazinamide	25mg/kg to a maximum of 2.5g po	arthralgia, hyperuricemia
Streptomycin	15mg/kg im	Ototoxicity, nephrotoxicity
Capreomycin/amikacin	0.75/1.0g for 2–3 months then 1.0g 2–3 times a week	Low potassium, low magnesium

genitourinary TB, if sensitive, is a 9-month course of isoniazid and rifampin or, alternatively, a 6-month course with isoniazid, rifampin, pyrazinamide and ethambutol, as above. The mycobacterial burden is thought to be lower than in cavitary TB and short-course regimens are recommended for extrapulmonary disease in the same fashion.

A common problem in the management of genitourinary TB is the progression of nephroureteric disease despite appropriate antimicrobial therapy and negative sequential urine cultures. Obstructive fibrosis can occur anywhere along the urinary tract and is secondary to the healing process that begins after initiation of effective

chemotherapy.[23] Corticosteroids, endoscopic balloon dilatation and placement of stents for ureteral strictures have been advocated and may obviate the need for reconstructive or ablative surgery.

Prior to the advent of effective antimicrobial therapy, surgical resection of the 'more involved organ' was the standard of care.[31] Currently, nephrectomy should be restricted to patients who have intractable pain for longer than 1 year, uncontrollable or recalcitrant fever secondary to infection proximal to the stricture, life-threatening hematuria, uncontrollable hypertension secondary to renal TB and insurmountable drug resistance.[29] Surgical techniques vary, including laparoscopic, open and retroperitoneal approaches, and are usually employed for removal of nonfunctioning kidneys.[32] [33] [34] Occasionally percutaneous balloon stenting may be used to alleviate strictures.[35] It has been suggested that surgical intervention for pelvic TB is indicated for:

- recalcitrant disease;
- persistent pain;
- abnormal bleeding; and
- nonhealing fistulas.[17]

Re-evaluation of genitourinary TB should include urine cultures every 3 months; proof of cure must be documented by culture.[12] In addition, IVP or ultrasound should be considered every 6 months, to rule out obstructive uropathy, until the clinical regimen has been completed. Additionally, renal function should be assessed every 6 months for 2 years after therapy. No other specific intervention is necessary unless the patient experiences a recurrence of symptoms.

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Chapter 72 - Practice Points

72.a Asymptomatic urinary infection in women with diabetes mellitus

George Zhanel
Allan R Ronald

A 40-year-old woman with a 26-year history of insulin-dependent diabetes mellitus consults you for moderate renal impairment (creatinine 290µmol/l (3.3mg/dl), urea 23.5mmol/l (66mg/dl)), proteinuria (3.5g in 24 hours) and pyuria (100 leukocytes per high-power field). The patient's diabetes has been stable on twice-daily insulin and no other therapeutic agents. She denies any urinary tract symptoms except for nocturia three times each night. A first morning void urine culture obtained on two occasions showed >10^5 cfu/ml of *Escherichia coli*, which was susceptible to all antimicrobial agents tested. The patient has no previous history of urinary infection and has had no pregnancies.

Background

Asymptomatic bacteriuria is common in women with diabetes, with a prevalence three times that of controls. Among patients with diabetes and asymptomatic bacteriuria, upper tract urinary infection is often present, with 'localization studies' demonstrating renal infection in 50–70% of women. Asymptomatic infection occasionally progresses to acute pyelonephritis with bacteremia. Among women with diabetes, acute urinary tract infections (UTIs) are the second most frequent reason for hospital admissions. In a recent populationbased study, the rate of hospitalization for acute pyelonephritis was 10 times greater among both men and women with diabetes than in the nondiabetic population. The determinants of asymptomatic bacteriuria and its complications among women with diabetes are mostly unsubstantiated. It is assumed that hyperglycemia and glycosuria with resulting impairment of leukocyte function and enhanced microbial metabolism, diabetic neuropathy with a neurogenic bladder, and renal microangiopathy each contribute to increased susceptibility to bacteriuria among women with diabetes. However, additional investigation is required to understand fully the factors that facilitate bacteriuria among women with diabetes.

No well-conducted prospective studies have shown that asymptomatic bacteriuria in diabetes contributes significantly to end-stage renal function; rather deterioration in renal function is almost always due to changes relating to progressive glomerulosclerosis.

Among patients with renal failure, regardless of the cause, asymptomatic bacteriuria is common. In most instances, these infections are presumably not due to the renal impairment but rather to multiple factors common in patients with renal failure, including prior urologic investigation, failure to achieve adequate antibacterial concentrations in renal tissue or in the urine, and altered host defenses. Few large cross-sectional studies have been carried out in patients with asymptomatic bacteriuria and renal impairment, and no definitive prospective studies are published. As a result, there is no information on the significance of asymptomatic bacteriuria in patients with renal failure, the importance of its treatment or its contribution to further loss of renal function.

Diabetes is now the most common cause of renal failure, accounting for about one-third of patients in developed countries, and asymptomatic infection in patients with diabetes and impaired renal function is exceedingly common. As a result, the clinical scenario of a woman with either type 1 or type 2 diabetes, impaired renal function and asymptomatic bacteriuria is often encountered.

What are the appropriate recommendations for the management of this patient? Should the patient have been screened for bacteriuria? Does the presence of pyuria have any prognostic significance? Once she is discovered to have bacteriuria, should she have further investigation or treatment? Without more data, therapeutic regimens are empiric and unproven, and decisions must be based on anectodal experience and personal opinion.

Specific issues

Diagnosis

The definitive diagnosis of asymptomatic UTI requires at least two urine cultures obtained as clean-voided midstream urine samples in order to have at least 95% assurance of bacteriuria. However, no studies have validated these criteria in patients with renal impairment. The presence of white blood cells provides credence to the diagnosis of asymptomatic bacteriuria. The microbial etiology of asymptomatic bacteriuria in diabetes is predominantly *E. coli*, with more resistant organisms in patients who have had hospital admissions or prior instrumentation. Some pathogens, particularly *Proteus mirabilis*, may more often be associated with complications, and some physicians would treat these pathogens regardless of symptoms because of their propensity to cause struvite calculi. In some studies, they have also been associated with an increased occurrence of acute pyelonephritis.

In patients with renal failure and complicated diabetes, clinical symptoms caused by 'asymptomatic bacteriuria' can be difficult to exclude. Does asymptomatic infection cause non-specific illness such as fatigue, irritability or malaise? Some patients after treatment of 'asymptomatic' infection relate improvement of symptoms that are not usually attributed to bacteriuria.

Imaging studies in these patients are problematic. Ultrasound studies lack sensitivity and specificity. Intravenous pyelography is contraindicated by the presence of renal failure and diabetes. A helical computerized tomography (CT) scan is the most effective means of excluding obstructing lesions (particularly calculi), evaluating renal size and identifying other abnormalities. The net marginal cost of obtaining a noncontrast helical CT scan may be less than that of ultrasonography, and it should usually be the imaging procedure of choice. Contrast should not be used unless absolutely essential and, when it is necessary, the patient should be well hydrated and a low ionic formulation should be prescribed. Imaging, preferably with a helical CT scan, is indicated in order to ensure that no remedial causes of renal impairment are present.

Treatment

In the presence of an intact urinary system and significant renal impairment presumed to be due to diabetes, how would we treat this asymptomatic infection? At present, no one can be dogmatic. We would choose treatment empirically in the hope that within several years clinical studies will have demonstrated the possible benefit of treatment or perhaps have identified a subset within this population in whom treatment is worthwhile. In treating such patients, we are hoping to prevent the complications of urinary infection that do occur with increased frequency in this population. However, there is no conclusive evidence that presumptive treatment prevents progression of renal impairment or acute complications, including acute pyelonephritis.

The choice of treatment is important. A therapeutic agent should be selected that will achieve reasonable levels in both renal tissue and urine despite impaired function. Renal tissue may be even further impaired regionally, owing to altered perfusion with variable antimicrobial levels. Also, an agent should be selected that is not known to be toxic to the kidney. There is no urgency to treat asymptomatic infection, and susceptibility tests can be used to identify one or more potential 'safe' agents.

In this instance, we would choose a fluoroquinolone or trimethoprim alone. Sulfonamides, aminoglycosides and tetracyclines should not be prescribed. The fluoroquinolones, including ciprofloxacin, and the newer agents levofloxacin, gatifloxacin and others, are well tolerated and diffuse widely in renal tissue. Nitrofurantoin should never be used in patients with impaired renal function because it does not achieve adequate urine or renal levels and because metabolites rapidly accumulate and produce serious neurologic toxicity.

We would prescribe the antibacterial agent for 14 days, obtain a urine culture on the last day of therapy to ensure temporary eradication of infection, and follow the patient. If the pathogen recurred within 2 weeks, after discussion with the patient we might prescribe a longer course of therapy on the basis that, in this 'normal

functioning' urinary tract, a cure of asymptomatic bacteriuria could be obtained and that this objective is worthwhile. However, if the patient continued to have recurrences, particularly with different organisms, and these remain asymptomatic, we would not pursue treatment or prescribe ongoing suppressive regimens or prophylaxis unless the patient appears to have clinical improvement in objective or subjective symptoms during the course of therapy.

In summary, this example illustrates our lack of knowledge in proper management strategies for patients with asymptomatic bacteriuria in the presence of diabetes or renal impairment. Careful clinical studies are necessary for a further understanding of the pathogenesis of UTIs in these patients and to enable bacteriuria to be managed on the basis of evidence rather than empiricism.

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72.b Management of persistent symptoms of prostatitis

John N Krieger

Definition of the problem

A 40-year-old man is referred for persistent symptoms of prostatitis. The patient had an episode of acute urethritis when he was 20 that resolved during a course of antibiotics. He was well until 2 years ago when he experienced an acute onset of urinary frequency with perineal and penile discomfort radiating to his left hemiscrotum. This followed after a new heterosexual relationship. He has not responded to treatment with ciprofloxacin, doxycycline, trimethoprim-sulfamethoxazole and three other antibiotics whose names he cannot remember. The physical examination is unremarkable. Recent urinalysis and urine culture are negative and there are no additional laboratory studies.

Introduction

'Prostatitis' is the diagnosis given to men who present with varied complaints referable to the lower urogenital tract and perineum. By one estimate at least one-half of adult men experience symptoms of prostatitis at some time in their lives. The US National Health Center

data indicate that 76 of 1000 men each year seek a physician's opinion for genitourinary problems, and prostatitis accounts for approximately one-quarter of these visits. Patients may experience symptoms for prolonged periods. Management of patients who experience persistent symptoms following repeated courses of treatment is a challenge.

Pathogenesis

Most bacterial prostatic infections ascend through the urethra. The oblique courses of the ejaculatory and prostatic ducts may provide a mechanical defense. Other host defenses include the antimicrobial activity in the prostatic secretions, particularly a zinc-containing polypeptide known as prostatic antibacterial factor. The prostate has higher concentrations of zinc than any other organ and prostatic secretions from normal men contain high zinc levels. Men with chronic bacterial prostatitis have low prostatic fluid zinc concentrations, but their serum zinc levels are normal. Local immunoglobulin production by the prostate may also be an important host defense. Many patients with prostatitis have increased leukocyte numbers in their prostatic secretions or semen, but the role of cellular immunity in chronic prostatitis is uncertain.

Hematogenous dissemination may result in prostatic infection in patients with systemic infections, such as tuberculosis or other granulomatous infections. This route is especially common in patients who are immunosuppressed or who have HIV infection.

Microbiology

Uropathogenic bacteria

Bacteriuria is a hallmark of acute and chronic bacterial prostatitis. The agents are standard uropathogens associated with bacterial urinary tract infections. Recurrent infections caused by the same organism are the sine qua non of chronic bacterial prostatitis. Between episodes of bacteriuria these organisms may be 'localized' to the prostate as described below. Unfortunately, patients with well-documented acute and chronic bacterial prostatitis constitute less than 10% of patients presenting with prostatitis.

Other genitourinary pathogens

Other genitourinary pathogens have been implicated as causes of prostatitis. The best evidence supports a role for sexually transmitted pathogens. In the pre-antibiotic era, *Neisseria gonorrhoeae* was a recognized cause of prostatitis and the most common cause of prostatic abscess. However, in current practice *N. gonorrhoeae* is seldom implicated. Some studies suggest a role for the sexually transmitted pathogens *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma genitalium* and *Trichomonas vaginalis* but their role remains controversial.

Granulomatous infections

Granulomatous prostatitis is an uncommon syndrome with a characteristic histologic reaction. It is classified as 'specific' when associated with particular microbes such as *Mycobacterium tuberculosis*, atypical mycobacteria, BCG (after intravesical therapy for transitional cell carcinoma) and the deep mycoses. Causes of non-specific granulomatous prostatitis include acute bacterial prostatitis, prostatic surgery and connective tissue diseases.

Specific diagnostic studies are necessary to document pathogens in patients who may be at risk. Accurate diagnosis is a prerequisite for successful treatment.

Clinical features

Patients with prostatitis can be classified into four categories: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis/ chronic pelvic pain syndrome, or asymptomatic prostatitis (Chapter 68 , see Table 68.1).

Acute bacterial prostatitis

This patient does not have acute bacterial prostatitis. The clinical features of acute bacterial prostatitis are readily apparent. Characteristic complaints include acute symptoms of urinary tract infection, including urgency, frequency and dysuria, and occasionally gross hematuria or acute urinary retention. Patients may also have systemic symptoms or a 'flu-like' syndrome, with fever, chills or other symptoms associated with bacteremia. Patients may experience bladder outflow obstruction due to acute edema of the prostate. The rectal examination is often impressive, with an exquisitely tender, tense prostate. Urinalysis reveals pyuria and cultures will be positive for uropathogenic bacteria. Leukocytosis is common, with increased numbers of segmented cells.

Chronic bacterial prostatitis

It is possible, although unlikely, that the patient may have chronic bacterial prostatitis. The characteristic clinical feature of chronic bacterial prostatitis is recurrent episodes of bacteriuria caused by the same organism. Patients may be totally asymptomatic or have only minimal symptoms between episodes. The infected prostate remains a focus of organisms causing relapsing infection. The prostate is usually normal on examination. Thus, absence of a documented urinary tract infection makes chronic bacterial prostatitis unlikely. With acute exacerbations, bladder bacteriuria may result from the prostatic focus. This is especially true among older men, who may have both prostatic obstruction and infection.

'Chronic prostatitis'

This is the largest group, representing more than 80% of patients presenting with symptoms, and is the most likely diagnosis in this case. Chronic prostatitis/chronic pelvic pain syndrome is the new National Institutes of Health consensus term for these patients. Chronic pelvic pain symptoms are the most common presentation, especially perineal, lower abdominal, testicular, penile and ejaculatory pain. Other genitourinary tract complaints include sexual dysfunction and voiding complaints. Some patients have objective evidence of inflammation in their prostatic secretions, post-prostate massage urine or semen (inflammatory subtype of chronic

prostatitis/chronic pelvic pain syndrome, formerly termed 'nonbacterial prostatitis'), while others have no evidence of inflammation (noninflammatory subtype of chronic prostatitis/chronic pelvic pain syndrome, formerly termed 'prostatodynia').

Asymptomatic prostatitis

Asymptomatic prostatitis may be diagnosed among men undergoing evaluation for other genitourinary tract problems. For example, some patients undergoing evaluation for infertility have increased concentrations of leukocytes in their seminal fluid. 'Chronic prostatitis' is also a common 'benign' diagnosis among men who undergo prostate biopsy for evaluation of elevated prostate-specific antigen levels and have inflammatory infiltrates on histology.

Investigations

The critical practice point is to distinguish patients with lower urinary tract complaints associated with bacteriuria from the larger number of men without bacteriuria. Urine culture is essential for men with acute lower urinary tract symptoms. Men with documented bacteriuria should undergo lower urinary tract localization studies (Table 72b.1). This investigation may document a prostatic focus of infection when the patient does not have a bacteriuria.

TABLE 72.b-1 -- Lower urinary tract localization study.

LOWER URINARY TRACT LOCALIZATION STUDY		
Specimen	**Abbreviation**	**Procedure**
Voided bladder 1	VB_1	Initial 5–10ml of urinary stream
Voided bladder 2	VB_2	Midstream specimen
Expressed prostatic secretions	EPS	Secretions expressed from prostate by digital massage after midstream specimen
Voided bladder 3	VB_3	First 5–10ml of urinary stream immediately after prostate massage
Unequivocal diagnosis of chronic bacterial prostatitis requires a 10-fold higher concentration of a uropathogen in the VB_3 of EPS specimen when compared with the VB_1 specimen. The organism is identical to organisms causing repeated episodes of bacteriuria.		

Documenting persistent prostatitis infection supports the need for continued antimicrobial therapy.

Unequivocal diagnosis of bacterial prostatitis requires that the colony count of a recognized uropathogen in postmassage (VB_3) urine exceed the colony count in the first-void urine (VB_1) by at least 10-fold. However, many men with chronic bacterial prostatitis harbor only small numbers of pathogenic bacteria in their prostates. Direct culture of the expressed prostatic secretions (EPS) is useful in this situation because colony counts in EPS are often one or two logs higher than comparable counts in the VB_3 . The hallmark of chronic bacterial prostatitis is that the uropathogen present in VB_3 or EPS may be isolated on multiple occasions and is identical to the organism causing episodes of bacteriuria.

Patients with risk factors for sexually transmitted disease (STD) pathogens should have appropriate testing for *N. gonorrhoeae* and *C. trachomatis*. Serologic testing is recommended for both syphilis and for HIV infection. Patients who have clinical findings suggesting granulomatous prostatitis should have appropriate studies for specific agents associated with this condition.

Document inflammation

Microscopic evaluation is important to identify EPS inflammation because this provides objective support for the diagnosis. We define inflammation based on chamber counts with >1000 leukocytes/mm^3 . There appears to be little value in counting EPS leukocytes in patients with urethral inflammation, especially among men at risk for STDs. Therefore, we examine a urethral smear before proceeding with a localization study.

Other investigations

Our standard approach is to recommend noninvasive uroflow and postvoid ultrasound residual testing. Patients with abnormal flow rates or significant postvoid residual urine have additional evaluation with a retrograde urethrogram to evaluate the possibility of urethral stricture and video urodynamics is reserved for patients with abnormal uroflow findings and negative urethrograms. Cystoscopy is recommended if carcinoma in situ or interstitial cystitis is considered likely (e.g. in older patients, those with hematuria, a history of chemical exposure or prominent painful voiding complaints). Urinary cytology is obtained if transitional cell carcinoma in situ is considered. Prostate-specific antigen testing is useful because occasional patients with carcinoma of the prostate present with symptoms of prostatitis. However, such testing is not recommended for patients with acute symptoms, since temporary elevation of prostate-specific antigen is common following acute episodes. Transrectal ultrasound evaluation may also be considered in selected patients to evaluate possible ejaculatory duct obstruction or complications such as prostatic abscess.

Management

Acute bacterial prostatitis

Appropriate therapy results in dramatic improvement. Many antimicrobials that do not penetrate the uninflamed prostate have proved effective. Thus, drugs appropriate for Enterobacteriaceae, *Pseudomonas aeruginosa* or enterococci should be started once cultures are obtained. For men who require hospitalization, conventional therapy is the combination of an aminoglycoside plus a ß-lactam drug. The fluoroquinolones or third-generation cephalosporins are attractive alternatives for monotherapy and the fluoroquinolones are the agents of choice for outpatient management.

Patients with acute urinary retention require bladder drainage. In this situation we prefer a suprapublic cystotomy tube as an indwelling transurethral catheter passes through and may obstruct drainage of the acutely infected prostate, increasing the risk for bacteremia and prostatic abscess.

Chronic bacterial prostatitis

Trimethoprim-sulfamethoxazole has been the 'gold standard'. Long-term therapy with trimethoprim (80mg) plus sulfamethoxazole (400mg) taken orally twice daily for 6–16 weeks is superior to shorter courses. Such therapy results in symptomatic and bacteriologic cure in approximately one-third of patients, symptomatic improvement during therapy in approximately one-third (who relapse after stopping treatment) and no improvement in the remaining patients.

During the past decade the fluoroquinolones have proved useful for treatment of chronic bacterial prostatitis. In contrast to the ß-lactams, concentrations of many fluoroquinolones are relatively high in prostatic fluid, prostatic tissue and seminal fluid as compared with plasma levels. Good results were reported for men with bacterial prostatitis, including patients who failed therapy with trimethoprim-sulfamethoxazole. Our first choice for curative therapy for chronic bacterial prostatitis is an appropriate fluoroquinolone, at full dose for at least 3 months.

Patients who are not cured may benefit from long-term suppressive treatment using low-dosage antimicrobial agents. Since patients may be asymptomatic between episodes of bacteriuria, the goal of suppressive therapy is to prevent symptoms of urinary infection. Very low doses of drugs can be remarkably effective. Available agents include tetracycline, cephalexin and trimethoprim-sulfamethoxazole. Although effective, we seldom recommend fluoroquinolones for chronic suppression, because of cost and the potential for development of resistance.

Chronic prostatitis/chronic pelvic pain syndrome

Therapy is often unsatisfactory because the etiology of chronic prostatitis/chronic pelvic pain syndrome remains unclear. As outlined above, an etiologic role has been suggested for many infectious agents. Prostaglandins, autoimmunity, psychologic abnormalities, neuromuscular dysfunction of the bladder neck or urogenital

diaphragm, allergy to environmental agents, stress and other psychologic factors have all been suggested as causes. Antimicrobial drugs are often prescribed. However, for men without evidence of infection by recognized pathogens, antimicrobial treatment usually fails. For these reasons, we prescribe antimicrobial agents only for patients with documented infections rather than recommending repeated courses of empiric therapy.

Other recommended treatments include a-blockers, prostate massage, anti-inflammatory drugs, anticholinergic drugs, allopurinol,

muscle relaxants, transurethral resection of the prostate, sitz baths, diathermy, exercises, physiotherapy and psychotherapy. Some clinicians recommend increased frequency of ejaculation to relieve 'congestion'. Others recommend abstinence and avoidance of alcohol, coffee, tea and spicy foods. Prospective studies are currently evaluating the efficacy of some of these recommendations. However, at present, there is no objective evidence that any of these measures change the natural history of the chronic prostatitis/chronic pelvic pain syndrome.

Conclusion

Patients with documented bacteriuria may have acute or chronic bacterial prostatitis. These conditions are rare in patients with no history of bacteriuria. Specific diagnostic studies will document uropathogens, STD agents or granulomatous infections in patients with prostatitis. Other diagnoses should be excluded in selected patients. Accurate diagnosis is the prerequisite for successful treatment.

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72.c A positive urine culture with pyuria accompanied by bladder spasms in a 24-year-old woman who has spinal cord injury and an indwelling catheter

John Z Montgomerie
Kim Maeder

Introduction

Urinary tract infection (UTI) occurs in most patients who have spinal cord injury (SCI) during initial hospitalization and rehabilitation and may be a recurrent problem throughout their lives. Until methods of urinary drainage were improved, infections were the dominant cause of bacteremia and renal failure. These complications, with calculi and pyelonephritis, still occur and more frequently in patients who have indwelling urethral catheters. Indwelling catheters are used by more than 20% of patients with SCI in the USA.

Definition of the problem

A 24-year-old woman who has a 4-year-old spinal cord injury at T12 presents with increased leg spasms and feels generally unwell. She has had an indwelling Foley catheter since her injury. She has had these symptoms before and attributes them to recurrence of her urinary infection. She is on chronic trimethoprim-sulfamethoxazole for prophylaxis and suppression of urinary infection. Cultures from 6 weeks earlier grew both *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Pathogenesis

The pathogenesis of UTI depends on the type of bladder drainage. Most patients immediately following SCI have an indwelling urethral catheter, which is always associated with bacteriuria. In most SCI centers the catheter is removed within a few days and intermittent catheterization is the preferred method of urine drainage.

Changes in the bladder associated with long-term use of the indwelling catheter include squamous metaplasia, thickening and fibrosis of the bladder wall, bladder contraction, diverticuli, calculi, alkaline encrusting cystitis with urease producing bacteria and squamous cell carcinoma of the bladder. In male patients, penile and scrotal fistulas, abscesses and epididymitis are other complications.

Microbiology

Studies of UTIs from different SCI centers have suggested that a wide range of micro-organisms infect the urine with different bacteria predominating at different centers. *Escherichia coli*, *Pseudomonas*, *Klebsiella* and *Enterococcus* spp. have been the predominant micro-organisms causing UTIs in patients who have SCI. Some centers have noted a high prevalence of *Proteus* spp.; this may relate to the more frequent use of indwelling catheters, which are also associated with multiple organisms. The presence of urease producers (*Proteus*, *Providencia* and *Morganella* spp.) raises concerns about calculus formation.

Indwelling, urethral and suprapubic catheters are associated with calculi and multiple organisms and multiresistant Gram-negative bacilli. The patient's sex and level of injury may affect the microbiology of bacteriuria and colonization. At our institution, male patients have had a high incidence of infection with *Klebsiella* and *Pseudomonas* spp. that relates to the use of external condom catheters and colonization of the perineum, urethra, bowel flora and urine in the urine drainage bags. In female patients with SCI receiving intermittent catheterization, *E. coli* and *Enterococcus* spp. accounted for 71% of infections. It has not been possible to alter significantly the colonization of the perineal skin through increased bathing, the use of antiperspirants or antiseptics to clean the skin.

There are few studies of the modes of transmission of these micro-organisms. Although the patient's body sites and drainage bags are the immediate source of such infections, transmission on the hands of health care personnel is the most likely means of transmission among patients.

Clinical features

Because of loss of sensation, patients with SCI do not usually have the common symptoms of UTI such as frequency, urgency and dysuria. The clinical features of UTI may include fever, pyuria and 'soft' symptoms and signs such as discomfort over the back or abdomen during urination, onset of incontinence, increased spasticity, autonomic hyperreflexia, malaise, lethargy or observation of cloudy urine with increased odor. The term 'soft' is used because increased spasticity (and other symptoms) may occur in patients

without obvious cause. The presence of a catheter by itself may induce spasms. Identification of the infecting organism by urine culture is important. Blood cultures should be obtained if patients have a high fever. Calculus formation may occur with the infection and small stones called 'gravel' may be present in the urine of patients with indwelling catheters.

Investigations

The exclusion of obstruction and other factors that might influence response to treatment is important. In those patients voiding reflexly it is important to determine the residual volume. Ultrasound and/or intravenous pyelogram may be important to confirm that there is no obstruction to urine flow and that there is adequate drainage from the kidneys and bladder. In patients with indwelling catheters it is important to change the catheter if there is any question of obstruction of the catheter.

Management

We should be concerned that a 24-year-old woman with SCI has chosen to use an indwelling urethral catheter rather than intermittent catheterization. However, this can be a rational choice for women since there are no reasonable external collection devices. As mentioned above, 20% of patients with SCI in the USA use indwelling catheters to drain the bladder. The choice is made if the patient is quadriplegic and cannot use her or his hands and an assistant is not available. Others, particularly busy people with full-time jobs or persons who travel, make this decision because of the inconvenience of the repeated catheterizations. They use the indwelling catheter to improve their quality of life despite the increased risks to their health.

Unfortunately there are few studies of the optimal methods of care of the patient with a long-term indwelling catheter. The recommendations from our own institution and our own observations are listed in Table 72c.1 . Urethral catheters need to be changed on a regular basis to prevent obstruction of the catheter, which occurs with encrustation that includes struvite and apatite crystals and bacterial biofilm. Changes every 2–4 weeks are almost always adequate to prevent catheter obstruction. Occasional patients need a more frequent change of catheter. Silicone catheters have not been demonstrated to have sufficient advantages to be used routinely.

Studies of patients with long-term indwelling catheters indicate surprisingly few episodes of fever. Most episodes are of low-grade fever lasting for less than 24 hours and resolve without antibiotics. Because patients with indwelling urethral catheters are colonized with three or more bacterial species that change frequently, no useful purpose is served by routine culture. Culture and sensitivity tests should be reserved for patients who are starting on antimicrobial

TABLE 72.c-1 -- Care of long-term indwelling catheters.

CARE OF LONG-TERM INDWELLING CATHETERS
• Use the smallest-sized catheter and balloon consistent with minimal leakage

• Change catheters regularly every 2–4 weeks
• Prevent trauma to the urethra
• Maintain at least 2L of fluid intake daily
• Use nonrestrictive clothing
• Daily perineal care with soap and water
• Urethral antisepsis and routine irrigations are not recommended

therapy. The laboratory should be notified that the urine was obtained from a patient with an indwelling catheter, otherwise the technicians may consider the multiple bacteria to be contaminants.

Because of lack of evidence that treating asymptomatic bacteriuria reduces symptomatic bacteriuria or influences the long-term function of the urinary tract or kidneys, bacteriuria in all patients with SCI should only be treated when symptoms or signs are present. In the patient with SCI and an indwelling catheter, a combination of factors makes us even more reluctant to use antibiotics. Antimicrobial agents rarely eradicate micro-organisms in the presence of the catheter or other foreign bodies and the bacteria in the urine may become resistant or may be replaced by resistant flora. A recent meta-analysis has confirmed that prophylactic antibiotics provide no advantage to patients with SCI.

In considering the patient who is the topic of this discussion, bacteriuria and pyuria are usually present in the SCI patient with a long-term indwelling catheter. Occasional bladder spasms are also common, particularly because of the physical presence of the catheter. By themselves, these symptoms and signs do not constitute evidence of a need to treat with antibiotics. Fever is the main indication for treatment but the catheterized patient sometimes has definite symptoms of UTI without fever. Patients who have had previous episodes of UTI with fever may recognize early symptoms such as increased bladder spasms or sudden onset of cloudiness of the urine or change of odor. At the first evidence of infection patients should increase their fluid intake. A catheter change should be considered. If the symptoms persist these patients will respond to oral antibiotics active in vitro. Appropriate duration of treatment has not been well studied in patients with indwelling catheters but they usually respond to relatively short courses of therapy (5–7 days). If there is evidence of renal infection longer courses may be indicated. In symptomatic patients with high fever, who may have bacteremia, broad coverage may be necessary until the results of the cultures are available because these patients are frequently colonized with resistant bacteria. Bacteremia in patients with SCI has usually been the result of bacteriuria associated with catheterization or other bladder manipulation. In those episodes, enterococci, *E. coli* and *Pseudomonas aeruginosa* were the organisms most frequently isolated from the blood.

The formation of stones in the bladder is a not uncommon problem associated with urease-producing bacteria (*Proteus*, *Providencia* and *Morganella* spp.). Attempts to clear small stones (gravel) can be made by increasing fluid intake or blocking urease production with acetohydroxamic acid. Cystoscopy may be necessary to remove the stones.

There are a number of gaps in our understanding of the management of patients with indwelling catheters and further study of the optimal care of the catheter is needed.

Recommended reading

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Chapter 73 - Epidemiology and Public Health Issues in Sexually Transmitted Infections

Michael Adler

WHY SEXUALLY TRANSMITTED INFECTIONS ARE IMPORTANT

Sexually transmitted infections (STIs) are a major public health problem and are among the commonest cause of illness, and even death, in the world today. They have far-reaching health, social and economic consequences, particularly in the developing world. The World Bank[1] estimated that for women aged 15–44 years, the STIs (excluding HIV infection) were the second commonest cause of healthy life lost after maternal morbidity (Fig. 73.1). Other studies have estimated that 5% of the total discounted healthy life years lost in sub-Saharan Africa are due to sexually transmitted diseases (STDs) excluding HIV infection, and that HIV infection alone accounts for 10% of healthy life years lost.[2] Also, the total days lost due to HIV, chlamydia and syphilis is similar to the number of days lost to malaria and measles.

The STIs are usually easy to diagnose and cheap to treat. However, many infections remain unrecognized and undiagnosed, resulting in considerable long-term morbidity which can be costly in both human and monetary terms. The complications of untreated infections are far-reaching and include cancer and reproductive and pregnancy-related problems (Table 73.1). It has been calculated that reproductive ill-health (death and disability related to pregnancy and childbirth, STIs, HIV/AIDS and reproductive cancers) account for 5–15% of the global burden of disease.[4] Data on the monetary costs of the complications of STIs are sparse, particularly for the developing world. American data gives estimates of total direct and indirect

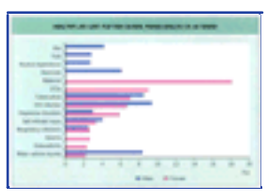

Figure 73-1 Healthy life lost — top ten causes in young adults aged 15–44 years.

costs attributable to STIs of US$ 9.9 billion annually, rising to US$16.6 billion if HIV infection and AIDS are included.[3] In the UK there are only limited data. For example, the prevention of unplanned pregnancy by National Health Service (NHS) contraception services probably saves over £2.5 billion per year, and the average lifetime treatment cost for an HIV-positive individual is £135,000–180,000 with a monetary value of preventing a single onward transmission of somewhere between £½ to 1 million in terms of individual health benefits and treatment costs.[5] Finally there are dramatic cost savings to be made by preventing infertility.

Few economic data exist in the developing world in relation to the consequences of STIs, which are considerable and personally devastating. Many women become infertile without even realising that they have suffered from pelvic inflammatory disease (PID). Estimates of the burden of infections for women in urban Africa have shown that chlamydial infection causes an average of 4.8 lost days of productive life, and syphilis 8.2 days per capita per year.[2] It has also been estimated that a high prevalence of syphilis among pregnant women, for example 10%, could result in up to 8% of all pregnancies carried beyond 12 week having an adverse outcome from syphilis.[6]

It is now recognized that there is a synergy between most STIs and HIV infection (particularly ulcerative and inflammatory conditions). STIs enhance infectivity in HIV-positive patients due to increased viral shedding and replication. STIs increase the possibility of HIV infection due to local trauma, ulceration and inflammation, and the increased presence and activation of cells receptive to HIV in the

TABLE 73-1 -- Major sequelae of STDs.*

MAJOR SEQUELAE OF STDs			
Health consequences	**Women**	**Men**	**Infants**
Cancers	Cervical cancer	Penile cancer	
	Vulval cancer	Anal cancer	
	Vaginal cancer	Liver cancer	
	Anal cancer	T-cell leukemia	
	Liver cancer	Kaposi's sarcoma	
	T-cell leukemia		
	Kaposi's sarcoma		
Reproductive health problems	Pelvic inflammatory disease	Epididymitis	
		Prostatitis	
	Infertility	Infertility	
	Ectopic pregnancy		
	Spontaneous abortion		
Pregnancy-related problems	Preterm delivery		Stillbirth
	Premature rupture of membranes		Low birth weight
			Congenital abnormalities
	Puerperal sepsis		
	Postpartum infection		Conjunctivitis
			Pneumonia
			Neonatal sepsis
			Acute hepatitis
Neurologic problems:	Neurosyphilis	Neurosyphilis	Mental retardation
			Herpes simplex virus
			Syphilis-associated neurologic problems

Other common health consequences:	Chronic liver disease	Chronic liver disease	Chronic liver disease
	Cirrhosis	Cirrhosis	Cirrhosis

** Adapted from Institute of Medicine, 1997.*[3]

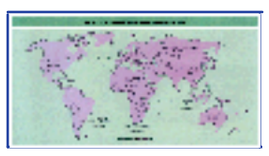

Figure 73-2 Estimated new cases of curable STD among adults, 1999.

presence of inflammation. The likelihood of risk per exposure to HIV in any sexual contact is in the order of 0.1%, which will increase considerably if an STI is present by the order of three to five. This synergy, and a realization that the control of STIs can have a profound effect on the incidence of HIV infection, has led to increased resources for STI control programs (see below). Clinical studies have

TABLE 73-2 -- Estimated new cases of STIs (millions), 1999.

ESTIMATED NEW CASES OF STIs (MILLIONS), 1999			
	Male	**Female**	**Total**
Chlamydia	41.95	50.03	91.98
Gonorrhea	28.7	33.65	62.35
Syphilis	10.24	12.96	23.2

TABLE 73-3 -- Estimated prevalence and incidence of STIs by region.

ESTIMATED PREVALENCE AND INCIDENCE OF STIs BY REGION		
Region	**Prevalence/million**	**Incidence/million**
Sub-Saharan Africa	32	69
South & South East Asia	48	151
Latin America & Caribbean	18.5	38
Eastern Europe & Central Asia	6	22
North America	3	14
Australasia	0.3	1
Western Europe	4	17
Northern Africa & Middle East	3.5	10
East Asia & Pacific	6	18
Total	**121.3**	**340**

shown that HIV-positive patients with a urethral infection have an eightfold increase in HIV-1 RNA in semen and this falls following treatment.[7]

SIZE OF THE PROBLEM

The size of the global burden of STIs is uncertain because of the lack of effective control and notification systems in many countries. The

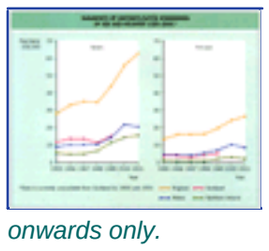

Figure 73-3 Diagnoses of uncomplicated gonorrhea by sex in England and Wales 1990–2000. ** Data for homosexually acquired infection in males available from 1994 onwards only.*

World Health Organization (WHO) has estimated a total of 340 million new cases of curable STIs in adults per year, mainly in South East Asia (151 million new cases per year), and sub-Saharan Africa (69 million) (Fig. 73.2 , Table 73.2). [8] In Eastern Europe and Central Asia the estimate is 22 million, and 17 million in Western Europe. The prevalence and incidence per million of the population varies regionally, for instance between sub-Saharan Africa and Western Europe, eight- and fourfold respectively (Table 73.3).

Gonorrhea

It is difficult to interpret differences between countries and even trends because of the variation in reporting practices and the provision of facilities. Rates of gonorrhea vary between countries in Europe. There was a peak in the number of cases of gonorrhea in most European countries during the early to mid 1970s. The subsequent advent of HIV and AIDS in the 1980s led to safer sexual practices and a reduction in the number of cases of gonorrhea, which has not been sustained in all countries. For example, recently there has been an increase in both male and female cases of gonorrhea in the United Kingdom between 1995 and 2001. In England there was a 128% increase in the number of cases in men from 6759 to 15,475, and in females a 95% increase from 3394 to 6641 (Fig. 73.3). The incidence of gonorrhea has increased since 1995 in homosexual men, particularly in those living in London, as have other sexually transmitted infections (Fig. 73.4). In 2000, 20% of gonorrhea diagnoses in males overall, and 24% of those in London, were homosexually acquired.

In Nordic countries, the annual incidence of gonorrhea declined very dramatically from over 100/100,000 of the population in most countries in the early 1980s to less than 10/100,000 by the late 1990s, but there have been recent slight increases. For example, in Sweden an all time low of 2.5/100,000 was achieved in 1996 but has risen again.

In the USA, the incidence of gonorrhea has declined, but there are marked differences between ethnic groups (Fig. 73.5). In 2000 there was a total of 358,995 reported cases, but the true number may be nearly double at 650,000 cases (Table 73.4).

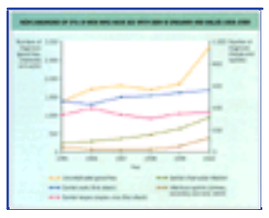

Figure 73-4 New diagnoses of selected STIs in men who have sex with men in England and Wales 1995–2000.

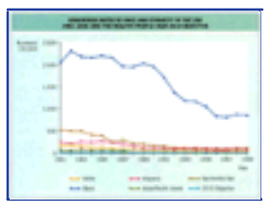

Figure 73-5 Gonorrhea rates by race and ethnicity in the USA 1981–2000 and the Healthy People Year 2010 objective. Nat Am/AK Nat, American Indian/Alaska Natives.

Eastern Europe, and particularly the newly independent states of the former Soviet Union have seen an epidemic of STIs, with high rates of gonorrhea in Estonia (166), Russia (138), Belarus (125) per 100,000 compared to France (18.5), Germany (5), and the Netherlands (8) per 100,000.

Syphilis

Syphilis is now rare in Western Europe and North America, largely due to the control of early acquired infectious syphilis in women and the screening of pregnant women for syphilis. In most Western European countries there has been a sustained decline in the incidence of syphilis to less than 5/100,000. As mentioned above, there has been an epidemic of most STIs in Eastern Europe with a recent epidemic of syphilis in all the newly independent states of the former Soviet Union. In 2000, the incidence of syphilis in these states ranged from 55 to 180/100,000 (Fig. 73.6). This epidemic is the vanguard of an HIV epidemic and already there have been outbreaks of HIV among intravenous drug users, particularly in Belarus, Russia and Ukraine. Likewise, syphilis is still a major clinical problem and cause of genital ulceration in the developing world. For

TABLE 73-4 -- Magnitude of the epidemics overall (USA).*

MAGNITUDE OF THE EPIDEMICS OVERALL (USA)		
STD	**Incidence Estimated number of new cases every year (millions)**	**Prevalence Estimated number of people currently infected (millions)**
Chlamydia	3	2
Gonorrhea	0.65	NA
Syphilis	0.7	NA
Herpes	1	45
Human papillomavirus	5.5	20
Hepatitis B	0.120	0.417
Trichomoniasis	5	NA
Bacterial vaginosis	NA	NA
NA, not available		

** From Cates 1999[9] .*

example, syphilis prevalence rates among pregnant women range from 17.4% (Cameroon) to 2.5% (Burkina Faso).

Chlamydia

In most of Europe and North America, chlamydia is still a major public health problem. In some countries, however, where widescale screening has been instituted, such as Sweden during the 1980s, the number of cases have declined dramatically. For example, in Sweden the number of cases in 1987 was 38,000 declining to 14,000 by 1997. In the UK no such trend has been seen, and *Chlamydia trachomatis* infection is now the commonest curable bacterial STI. There has been an increase in the number of cases since 1995 with females outnumbering males. In the year 2001, 71,055 people with chlamydial infections attended clinics: 30,725 males, 40,330 females. The condition is most commonly seen in young people; the peak age in men in 20–24 years and in women 16–19 years

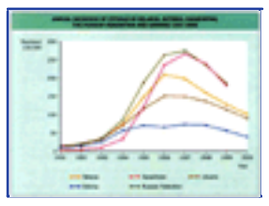

Figure 73-6 Annual incidence of syphilis in Belarus, Estonia, Kazakhstan, the Russian Federation and Ukraine 1991–2000.

(Fig. 73.7). Screening surveys carried outside normal STD clinic environments also show high levels in antenatal and gynecology clinics, general practice, family planning and termination of pregnancy clinics, prevalences ranging from 4.5 to 16%. Similar high rates have been seen in the USA. It should, however, be remembered that there has been increased availability of chlamydia testing and more sensitive detection tests, which to some extent account for the apparent increase in the number of cases seen.

Genital herpes and warts

In England and Wales, compared to gonorrhea and chlamydia, there has been a slowing down in the increase of both of these conditions in the past few years. It is estimated that the number of new cases of herpes per year in the USA is one million with a prevalence of approximately 45 million cases. For genital warts the number of new cases is 5.5 million with a prevalence of 20 million cases. Serologic surveys have shown an increase in prevalence of 17–21% in HSV-2 antibodies between 1976–1978 and 1988–1992 in population samples in the USA.[10]

It is often overlooked that high levels of herpes simplex virus (HSV) infection also exist in developing countries, for example in Mwanza, Tanzania, over 40% of teenage girls have serologic evidence of HSV infection. This becomes particularly important in relation to the increased risk of HIV acquisition.

Pelvic inflammatory disease

Pelvic inflammatory disease (PID) is one of the most serious complications of gonococcal and chlamydial infections and its prevalence is increasing in most countries. In western industrialized countries it is estimated that the annual incidence is 10/1000 women aged 15–39 years with a peak incidence of 20/1000 in age groups 15–24 years. Risk factors as well as STIs include the use of intrauterine devices and postpartum and puerperal infections. STIs cause most cases of PID, particularly in developing countries, and infertility as a result of PID is responsible for 50–80% of infertility among African women.[11]

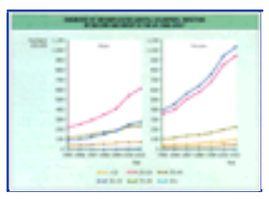

Figure 73-7 Diagnoses of uncomplicated genital chlamydial infection in genitourinary medicine clinics by sex and age group in the UK 1995–2000. *Data are currently unavailable from Scotland for 2000 and from Northern Ireland for 1996 and 1997. *Data from the PHLS and Scottish ISD(D)5 Collaborative Group (ISD, SCIEH and MSSVD).*

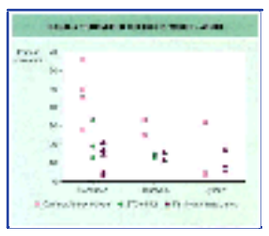

Figure 73-8 Sexually transmitted diseases in women in Africa.

Sexually transmitted infections in developing countries

STIs have a much higher incidence and prevalence in developing countries and are among the top five causes of consultation in general health services in many African countries. Routine and accurate surveillance data are often lacking, and an understanding of the burden of infection tends to come from ad hoc surveys, usually among high-risk groups. Particularly high rates of infections are seen in groups such as female prostitutes and their clients, and truck drivers. Prostitution continues to be an important factor in the transmission of STIs in developing countries. For example, in an urban Kenyan STD clinic, 60% of men with a diagnosis of gonorrhea or chancroid reported commercial sex exposure as the likely source of infection. Genital ulcer disease (syphilis, chancroid, lymphogranuloma venereum and granuloma inguinale) is more frequent than in developed countries. Fig. 73.8 illustrates the prevalence of three common STDs in women in an African setting taken from various surveys. In commercial sex workers, the prevalence of gonorrhea can reach nearly 50%, and the prevalence of syphilis ranges from 2–30% for acute or previous infection. Levels of chlamydia can be as high as 30%. The incidence of STI complications and their sequelae is much higher in developing countries due to lack of resources and adequate diagnosis and treatment. Particular complications that are seen relate to adverse pregnancy outcomes for both mother and the newborn, neonatal and infant infections, infertility in both sexes, ectopic pregnancy, urethral strictures in males, blindness in infants due to gonococcal and chlamydia ophthalmia neonatorum, and in adults due to gonococcal keratoconjunctivitis as well as genital cancers, particularly cancer of the cervix and penis.

CONTROL OF SEXUALLY TRANSMITTED INFECTIONS

Principles of effective STI control

The main principles of the control of STIs are to:

- prevent new infections;
- treat those with symptoms of infection and interrupt onward transmission — such treatment should prevent the development of disease complications and sequelae;
- identify and treat those without symptoms by screening and partner notification; and
- motivate health seeking behavior among those who may know they are infected but who delay or avoid seeking treatment.

Issues and problems in relation to control

Issues and problems in relation to control include:

- high rates of infection among young adults, adolescents and certain groups (e.g. commercial sex workers, truck drivers);
- asymptomatic infection;
- long-term morbidity, particularly in women;
- increased acquisition of HIV in transmission;
- disadvantaged and disempowered women;
- the complex mix of social, political, cultural, demographic and economic factors.

Approaches to prevention

It is theoretically possible to develop programs using the two approaches of primary and secondary prevention.

Primary prevention

The three basic elements of primary prevention are:

- health education,
- provision of condoms, and
- social, cultural and economic interventions.

Programs to promote safer sexual practices and increased condom use have been widely instituted throughout the developed and developing countries. Much of the cultural shift that has occurred in the past 20 years in relation to more explicit messages around sex and condom promotion have come with the advent of HIV infection and AIDS. Essentially, therefore, primary prevention should be concerned with entire communities and with efforts to prevent individuals from becoming infected. This approach requires a range of health promotion activities, both at national and local levels, to include sex education policies and programs, campaigns, and as mentioned previously, the promotion of condoms.

Encouraging the use of condoms has been a central part of many control programs, and is seen as particularly useful since their use reduces the acquisition and transmission of both STIs and HIV infection. There are now many examples in the literature of the effectiveness of condom use in both high-risk groups and the general population. For example, a 3-year program of condom promotion and STD control in Zaire saw an increase in consistent condom use from 11 to 68% among commercial sex workers.[12] This was associated with a decline in the incidence of HIV, gonorrhea, trichomoniasis and genital ulcer disease. Social marketing of condoms in Zaire saw sales increasing from 20,000 to 18 million in a period of 3 years. Multiple outlets were used, such as street traders, night clubs, commercial sex workers and pharmacists. More recent studies among commercial sex workers in the Côte d'Ivoire and Uganda confirm these earlier studies, namely that consistent use is related to a reduction in the prevalence of HIV infection and other STDs.[13] [14]

There are particularly problems in relation to the development of primary prevention initiatives. For example, even though initially health promotion and condom programs showed reductions of STIs and new HIV infections, for example in the UK and USA, the initial and profound changes of sexual behavior in homosexual men has not been universally sustained. This underlines the need for repeated reinforcement and continuation of health promotion programs. The particular problem in the developing world is the position of women, who are often poor and disempowered. To overcome this economic programs may be required that help reduce the necessity for women to work as commercial sex workers, and at the same time women need to be taught skills that help them negotiate safer sex with clients and their regular partners. Raising the status of women is a crucial factor in the effective control of STIs and HIV infection.

Secondary prevention

Health-seeking behavior

It is important to have a conceptual framework for the development of control programs to readily understand that not all those with infections seek care and are eventually treated successfully (Fig. 73.9). Thus, only half of those with an STD symptom recognize its significance, and only half of these present for treatment, of

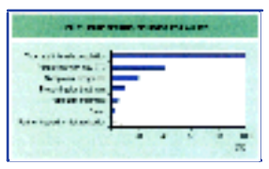

Figure 73-9 Health care-seeking behavior for an STD.

whom a further half receive adequate treatment. It is essential to encourage those with symptoms to seek treatment. It is therefore important to identify the large number of people who fail to obtain treatment or delay seeking treatment and may therefore continue to infect others and develop complications. The issue of health-seeking behavior is best addressed through public education, the provision of user-friendly services which do not stigmatize patients, and access to cheap confidential treatment.

Clinical services/case management

Once a patient attends for treatment, it is important that a correct diagnosis is made, effective treatment is instituted, compliance understood, risky behaviors discussed and partners notified. It is essential that services are nonjudgmental, sensitive, user friendly and easy to use.

Service models

Two types of approaches for control have been developed; vertical and integrated services:

- ¦ the vertical services model is the one that is usually developed in resource-rich countries and is based on etiologic diagnoses established by microscopy with laboratory back-up and run by specialist doctors;
- ¦ the integrated approach to services has been developed more in developing countries where services are run by nonspecialists using various facilities, for example outpatient clinics, primary health centers (PHCs), maternal and child health centers (MCHCs) and family planning clinics (FPCs) — in addition, private practitioners, pharmacists, traditional healers, unqualified practitioners and street vendors make a contribution to treatment.

Syndromic management

The WHO has put particular emphasis on integrated approaches and using the syndromic approach for patient management.[15] [16] Essentially this recognizes the limitation of resources for health care and of specialist trained medical personnel. It is used particularly in high prevalence areas where there are inadequate laboratory facilities, lack of trained staff, and large distances between rural primary health centers and specialist and laboratory facilities.

The syndromic approach is based on the use of algorithms developed for commonly presenting signs and symptoms, for example genital ulcers or urethral and vaginal discharge where laboratory support may or may not be present. One of the major advantages of this approach is that it can be integrated into other services such as MCHC, PHCs and FPCs. Unfortunately, not all the algorithms are

discriminatory and sensitive, for example that for vaginal discharge does not easily lend itself to making the distinction between vaginal infections and vaginal plus cervical infections. Work on improving this by incorporating risk assessment scores has been carried out in the last few years. The syndromic approach used in an integrated way received a considerable boost with the Mwanza Trial, which demonstrated the importance of an integrated STI program in rural communities on the incidence of HIV infection. [17] This trial showed that improved STI care integrated at PHC level resulted in a reduction of STI incidence of 42% over the 2-year period of the study.

Asymptomatic infection

Asymptomatic infection is a major issue in the control of STIs anywhere in the world. Strategies for dealing with asymptomatic infection are:

- ¦ encouraging check-ups for those at risk through mass media campaigns;
- ¦ promoting safer sex through health education and condom provision;
- ¦ screening and case finding, for example in antenatal clinics and FPCs;
- ¦ partner notification when a patient presents for care; and
- ¦ mass treatment.

The identification of asymptomatic infection by population screening requires an inexpensive and noninvasive, simple, available diagnostic test that can be widely used. Initial studies using the leukocyte esterase dipstick test in men on first-catch urine showed a low sensitivity. Newer tests using polymerase chain reaction techniques will help, but only if sensitive, specific and cheap.

Mass treatment

The attraction of mass treatment is the fact that a large number of asymptomatic and untreated infections can be eradicated and the Rakai Study carried out in Uganda showed the effectiveness of this approach.[18] In this study ciprofloxacin, azithromycin and metronidazole were used. The results showed that there was a failure to reduce the incidence of HIV infection despite good coverage of the study population. Considerable debate and analysis has ensued over this study, particularly since it contrasts with the Mwanza intervention. It is thought that the failure to have an impact on HIV infection was due to the fact that the HIV epidemic in Uganda was already mature and that STIs in such an epidemic are unlikely to have an important facilitatory role. On a more practical basis, even though it would appear that there was 80% coverage of the Rakai population, perhaps those at highest risk of infection, for example commercial sex workers and truck drivers, may not have been captured in the treatment intervention.

The principle disadvantages of mass treatment interventions are:

- ¦ the high cost of treating large numbers of people, many of whom do not require treatment;
- ¦ the potential danger of antibiotic resistance;
- ¦ the encouragement of unsafe sex in populations with lowered perception of risk;
- ¦ the danger of stigmatizing individuals or groups who are not diseased; and
- ¦ finally, ethical considerations of treating people who may not be infected and who may suffer ill effects from treatment.[19]

Interventions with core groups

Over and Piot have suggested that targeting core groups in relation to treatment would 'avert ten times as many cases of STIs as would have been averted by a policy directed at the non-core group'.[2] The rationale for this is that preventing STIs and HIV infection in individuals with high rates of partner change will avert many more infections than interventions among people with few partners. One of the stated disadvantages of this approach is the stigmatization of such groups. The approach of targeted interventions has been used, particularly in developing countries with commercial sex workers and their clients, and truck drivers. Evaluation of this approach is limited at this point in time.

Partner notification

Partner notification is an essential cornerstone of any control program, but is not always carried out. However, by offering treatment to sexual partners of patients whether they are symptomatic or not, reinfection can be halted and ongoing transmission curtailed. Partner notification can be carried out in a variety of ways:

- first, patient referral in which the index patient is encouraged to seek out his or her own sexual partners;
- second, provider referral in which the health team takes on the responsibility for identifying the partners; and
- finally, conditional referral — in this situation the health care workers of the index case obtain the names of the sexual partners but allow the patient a period of time to notify partners themselves — failure to do so in this time would result in the health care professionals taking on the responsibility for this mission.

CONCLUSION

STIs present a major public health problem throughout the world associated with significant morbidity and mortality rates. Well-designed control programs will help to reduce the incidence and prevalence of such diseases and reduce the morbidity, suffering and economic costs associated with them. Equally, eliminating STIs as a facilitatory factor in HIV transmission, and by contributing to behavioral changes towards safer sex, will play an important part in the prevention and control of HIV infection and AIDS.

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Chapter 74 - Gonococcal, Chlamydial and *Mycoplasma* Urethritis

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Myron S Cohen

Neisseria gonorrhoeae and *Chlamydia trachomatis* are the two major pathogens responsible for urethritis, a syndrome characterized by urethral discharge and dysuria. Urethritis is defined by its pathognomonic laboratory finding: an increased number of polymorphonuclear leukocytes (PMNs) on the Gram stain of a urethral smear. Urethritis has generally been classified as gonococcal urethritis or nongonococcal urethritis (NGU). Causes of NGU have only recently been elucidated; in addition to *C. trachomatis*, these include *Mycoplasma genitalium*, *Ureaplasma urealyticum*, other infectious agents and a variety of chemical and physical irritants.

EPIDEMIOLOGY

Neisseria gonorrhoeae and *C. trachomatis*, along with most other agents of urethritis, are sexually transmitted pathogens. Both pathogens have a worldwide distribution, although prevalences vary tremendously from region to region. In the USA, despite overall declines in the reported incidence of gonorrhea, rates remain high in adolescents and young adults (Fig. 74.1) and in minority groups. In 2000, rates among African-Americans were 30-fold higher than rates among non-Hispanic whites.[1] In addition, many cities in the USA have reported significant increases in gonorrhea among men who have sex with men (MSM) since the mid-1990s.[2]

Trends in chlamydial infection in the USA are less well documented because tests for *C. trachomatis* have only been widely available since the mid-1980s and only in 2000 was reporting of the infection mandatory in all 50 states. Chlamydial infection is probably several times more common than gonorrhea. However, currently reported rates of chlamydial infection (Fig. 74.2) reflect screening and reporting practices as much as they reflect the actual distribution of the disease. Testing for *C. trachomatis* in men has been especially limited. Specific testing for *M. genitalium* is not widely available, and so rates and trends for this infection have not yet been defined. Reported physician visits for NGU declined modestly during the 1990s.[1]

In Europe, gonorrhea rates declined during the 1980s and 1990s, reaching rates far lower than those in the USA. However, like the USA, many European countries saw increases in gonorrhea — especially among MSM — during the late 1990s. Chlamydial infection is still far more common than gonorrhea in most of Europe, as control programs were limited by the lack of sensitive diagnostic tests until the past decade.[3]

In the developing world, rates of gonococcal and chlamydial infection are less well known, but estimated incidence rates are several times higher than rates in many developed countries. The public health burden of these diseases is clearly tremendous. Complications such as pelvic inflammatory disease and its sequelae in women, urethral stricture in men and ophthalmia neonatorum in infants are common. [4]

Risk factors for urethritis are similar to those for other sexually transmitted diseases (STDs): multiple sexual partners, a recent new partner and other sexual behaviors that increase the likelihood of encountering a sexually transmitted pathogen. Young age, low socioeconomic status and minority race are recognized as risk markers for STDs, although these factors are more strongly associated with gonorrhea than with NGU.[5] [6] [7] Heterosexual men have higher rates of NGU and chlamydial infection than homosexual men, although rates of chlamydial infection among homosexual men may be increasing.[8] In addition, 10–30% of men with gonorrhea are co-infected with *C. trachomatis*. [9] [10]

PATHOGENESIS AND PATHOLOGY

The large majority of urethritis cases are caused by sexually transmitted infectious agents, most commonly *N. gonorrhoeae* and *C. trachomatis* (Table 74.1). For both pathogens, humans are the only natural host. *Neisseria gonorrhoeae* is a Gram-negative diplococcus that is highly adapted for growth on the mucosal membranes, infecting primarily columnar and cuboidal epithelium. Urethral infection requires that the organism first attach to the epithelium and then evade host defenses well enough to survive and multiply. The gonococcus uses a set of complex mechanisms to accomplish these goals (Fig. 74.3).[11] At least two outer membrane proteins, pilin and Opa, are important in adherence. Mechanisms for evading ingestion by PMNs appear to include production of the antioxidant catalase, competition for molecular oxygen and DNA repair mechanisms. Other mechanisms important in the evasion of host defenses include production of an IgA protease and blocking of antibody-mediated killing with sialylated lipo-oligosaccharide (LOS) or with blocking antibodies directed against reduction modifiable protein (Rmp). The purulent exudate characteristic of gonococcal infections is a result of the neutrophil response stimulated by LOS and other gonococcal antigens. Gonococci that cause disseminated infection have several unique characteristics, including particular nutritional requirements (arginine-, hypoxanthine- and uracil-requiring (AHU$^-$)), selected classes of the major outer membrane protein (IA serovars) and the ability to survive humoral defenses such as a complement-mediated bactericidal attack.[12]

Chlamydia trachomatis is the single most common cause of NGU, although it accounts for less than half of NGU cases in many populations. It is an obligate intracellular pathogen that primarily infects columnar epithelium. *Chlamydia trachomatis* serovars D–K cause ocular and genital disease; other serovars cause trachoma (A–C) and lymphogranuloma venereum (L1–L3). The pathogenesis of chlamydial infection is less well understood than that of gonorrhea. *Chlamydia trachomatis* has a unique life cycle involving an infectious stage, the elementary body; and a metabolically active stage, the reticulate body (Fig. 74.4). *Chlamydia trachomatis* evades host defenses by multiplying within a phagosome and preventing phagolysosomal fusion. Direct cytotoxicity and a host immune response to selected chlamydial antigens produce the clinical manifestations of infection. Repeated infection or chronic infection, with long-lived humoral and cell-mediated immune responses, are probably responsible for the complications of chlamydial infection such

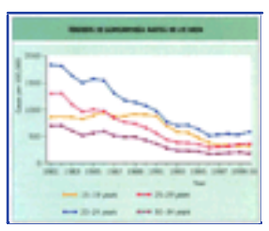

Figure 74-1 Trends in gonorrhea rates among men aged 15–34 years in the USA, 1981–2000. Rates are cases per 100,000 population. *Source: Sexually Transmitted Disease Surveillance, 2000.*[1]

Figure 74-2 Rates of chlamydial infection in the USA, 2000. Rates are cases per 100,000 population. *Source: Sexually Transmitted Disease Surveillance, 2000.*[1]

as Reiter's syndrome and tubal scarring leading to infertility. The immune response to a chlamydial 60kDa heat shock protein, which has substantial homology with human heat shock proteins, may be particularly important in producing these complications. [13]

Host factors involved in gonococcal and chlamydial infection are poorly understood. No racial and genetic factors predisposing to infection with these agents have been identified. Terminal complement deficiency predisposes to invasive, but not mucosal, gonococcal infections. Limited evidence supports the possibility of short-term, incomplete, strain-specific immunity to either agent. However, repeat infections with these agents argue against a longlived protective immune response after natural infection.

TABLE 74-1 -- Infectious and noninfectious causes of urethral discharge and dysuria.

INFECTIOUS AND NONINFECTIOUS CAUSES OF URETHRAL DISCHARGE AND DYSURIA		
Major infectious causes	**Other infectious causes**	**Noninfectious causes**
Neisseria gonorrhoeae	*Mycoplasma genitalium*	Chemical irritants (spermicides, bath products)
Chlamydia trachomatis	*Ureaplasma urealyticum*	Tumor
	Trichomonas vaginalis	Foreign body
	Herpes simplex virus	Stevens-Johnson syndrome
	Coliform bacteria	Wegener's granulomatosis

	Candida albicans	
	Treponema pallidum	
	Human papillomavirus	

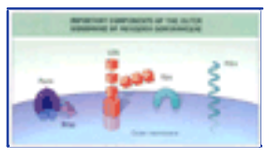

Figure 74-3 Important components of the outer membrane of *Neisseria gonorrhoeae*. Porin is the major outer membrane protein. Reduction modifiable protein (Rmp) is the target of blocking antibodies that prevent bactericidal antibodies from binding to porin. Pilin and opacity protein (Opa) are important in adhesion. Lipo-oligosaccharide (LOS) stimulates PMN response and, when sialylated, blocks antibody-mediated killing.

Nonchlamydial NGU is a group of syndromes caused by infectious and noninfectious agents. In the past decade, *M. genitalium* has been recognized as a relatively frequent cause of nonchlamydial NGU.[14] [15] [16] It exhibits strong adherence to eukaryotic cells and stimulates a neutrophil-dominated acute inflammatory response. The ability of *M. genitalium* to invade epithelial cells and persist in this environment may account for the association of this pathogen with persistent and recurrent urethritis.[14] [16] *Ureaplasma urealyticum* is found in 10–60% of all NGU cases, although its etiologic role has been controversial. It clearly causes symptomatic infection that responds to specific therapy in some men, but has also been found to colonize as many as 60% of men in STD clinic settings. [7] [15] *Mycoplasma hominis* is a frequent genital tract colonizer and was long suspected of causing urethritis, but studies have failed to confirm its role as a pathogen in men.

Approximately 10–20% of NGU cases are caused by agents other than *C. trachomatis*, *M. genitalium* and *U. urealyticum*. Herpes simplex virus can cause urethritis, usually in primary infection and in conjunction with external ulcerative lesions.[17] *Trichomonas vaginalis*

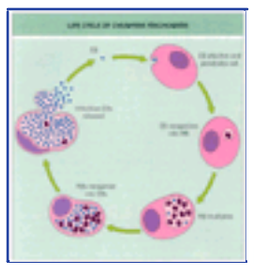

Figure 74-4 The life cycle of *Chlamydia trachomatis*. The elementary body (EB) invades the host cell and then reorganizes into the metabolically active reticulate body (RB) while in a phagosome. The reticulate body multiplies and the resultant reticulate bodies reorganize into elementary bodies, which are released by rupture of the host cell.

causes a variable proportion of NGU cases.[18] Coliform bacteria occasionally cause urethritis, especially if phimosis or urethral stricture is present or after urethral instrumentation. Distal urethritis may be caused by *Candida* spp. in association with yeast balanitis involving skin adjacent to the meatus. Urethral discharge without dysuria may be caused by an endourethral syphilitic chancre or by intraurethral condyloma acuminata. Limited evidence suggests that adenovirus, *Haemophilus influenzae*, *Clostridium difficile*, *Neisseria meningitidis* and *Bacteroides ureolyticus* may occasionally cause urethritis.

A small percentage of NGU cases do not have infectious causes. Spermicides and some bath products can cause a chemical urethritis. An endourethral tumor or intraurethral foreign body can cause a mucoid or bloody discharge and may become secondarily infected with local skin flora. Repeated vigorous urethral stripping (see Physical examination) may eventually cause the production of a clear urethral discharge. Heavy crystalluria or calculous gravel in the urine can produce dysuria and may have the appearance of a urethral discharge. Systemic illnesses such as Stevens-Johnson syndrome, Wegener's granulomatosis and Behçet's disease are occasionally associated with urethritis. Finally, the remnants of semen at the meatus or urinary incontinence may be misinterpreted by the patient as urethral discharge.

PREVENTION

The persistence of STDs such as urethritis in a community requires the spread of disease from each infected person to, on average, at least one other susceptible person. This concept is expressed in the equation, $R_0 = ßcD$, where the number of secondary infections arising from each case, or reproductive rate (R_0), depends on the efficiency of transmission (ß), the rate of sexual partner change (*c*) and the duration of infectiousness (*D*).[19] The value of each parameter varies by pathogen.

Gonococcal urethritis transmits infection with high efficiency, around 50–70% for a single act of vaginal intercourse.[20] Without treatment, gonococcal urethritis remains infectious for approximately 6 months, but most cases produce symptoms uncomfortable enough that men rapidly seek treatment, shortening the infectious period to several days. Given these parameters, the maintenance of gonorrhea in a community in which treatment is readily available requires an average partner change rate of 13 partners per year.[21]

Chlamydial urethritis is transmitted with less efficiency than gonorrhea — approximately 20–50% for a single contact or 70% for long-term partnerships.[22] Without treatment, chlamydial urethritis remains infectious for around 15 months and, because the majority of cases are asymptomatic, the infectious period remains long despite the ready availability of effective therapy. As a result of this long infectious period, the average partner change rate required to maintain chlamydial infection in a community is estimated at only four partners per year.[22] The transmission efficiency of *M. genitalium* urethritis has not been defined. Most persons with gonococcal or chlamydial infection in fact have lower rates of partner change; however, a small group of people — the core group — with higher rates of partner change are critical in maintaining the spread of infection.[23] This core group for chlamydial infection encompasses a broader population than that for gonorrhea because of the lower number of partners required.

Preventive measures targetting one or more of these key parameters can result in lowered rates of STDs (Table 74.2). Treatment of STDs reduces the duration of infectiousness, thereby limiting secondary spread of disease. Highly effective single-dose therapies are recommended for gonorrhea and single- or multiple-dose therapies for chlamydial infection (see Management). *Neisseria gonorrhoeae* is eliminated from the urethra within hours of oral or parenteral therapy;[24] elimination of *C. trachomatis* is slower. Treatment of the sexual partner is essential to prevent reinfection of the patient and spread to other individuals.

Screening, with prompt treatment of infected persons, is an important strategy to prevent secondary spread of asymptomatic or subclinical infections. Although the majority of gonococcal urethritis cases are symptomatic, men with asymptomatic infection contribute disproportionately to the spread of gonorrhea.[25] In addition, a large proportion of chlamydial urethritis cases are asymptomatic, and so screening of persons with high-risk behaviors is especially important in limiting the spread of this disease. A recent study of the general population in a major US urban center found that 7.9% of persons had untreated gonococcal or chlamydial infection.[26] Since chlamydial infection is highly prevalent in many areas, clinicians should have a low threshold for screening any sexually active person.

TABLE 74-2 -- Prevention of gonococcal and chlamydial infection.

PREVENTION OF GONOCOCCAL AND CHLAMYDIAL INFECTION	
Primary prevention	**Secondary prevention**
Male latex condoms	Screening of at-risk individuals
Sexual behavior change	Prompt treatment of infected individuals
Microbicides (under development)	Treatment of sexual partners
These preventive measures reduce the transmission effiency, the duration of infectiousness, or the rate of sexual partner change.	

Improving the accessibility and utilization of health care services to allow for prompt diagnosis and therapy can interrupt transmission and prevent complications of infection. Barriers to utilization include lack of awareness of STD symptoms, the cost of care, limited hours of service, lack of transportation and cultural differences between providers and patients.

Male latex condoms, when used correctly and consistently, are one of the most effective available tools for reducing the efficiency of transmission of gonorrhea and chlamydial infection.[27] The polyurethane female condom has been demonstrated to reduce transmission of trichomoniasis [28] and is being studied for its effectiveness in preventing transmission of other bacterial STDs. However, this product has had limited user acceptance in the USA.

Chemical barriers such as vaginal microbicides may have a future role in reducing the transmission efficiency of gonorrhea and chlamydia. The only currently available microbicide, nonoxynol-9 (also a spermicide), has poor efficacy against STDs and may increase the risk of HIV transmission.[29]

Finally, strategies to alter sexual behaviors to reduce the rate of partner change can decrease the spread of STDs. Changes in sexual behavior in the gay community were important in limiting the spread of HIV and other STDs in the 1980s[30] and recent relapses in risky sexual behaviors have been associated with increases in gonorrhea and syphilis in this population. [31] Effective strategies include enhanced risk reduction counseling for individuals or small groups and interventions that use peer opinion leaders to change community norms for sexual behaviors.

CLINICAL FEATURES

Urethritis classically produces urethral discharge accompanied by dysuria. The discharge may be scant or copious and may appear clear, white, yellow or green. Itching around the meatus is common. Frequency, urgency and hematuria are not generally part of this clinical syndrome and should lead to the consideration of alternative diagnoses.

The pattern of urethral symptoms and characteristics of the discharge can provide clues as to the etiologic diagnosis. In gonococcal urethritis, the incubation period is brief (2–6 days). The use of sub-curative doses of antibiotics during this time can prolong the incubation period. Symptom onset is abrupt. Gonococcal urethritis usually produces copious, purulent, often yellow-green discharge along with marked dysuria.[5] Inguinal lymphadenopathy is absent, although small nontender nodes, unrelated to the gonococcal infection, may be palpable in a majority of men. Up to 30% of men with gonorrhea are co-infected with *C. trachomatis*.[9] [10] The proportion of men with co-infection may be declining as dual treatment and, more recently, screening for *C. trachomatis* have become widely practiced.

Approximately 2–3% of men acquiring urethral gonococcal infection remain asymptomatic, especially those infected with strains that have selected serotypes and auxotypes.[32] [33] These infections may play a disproportionate role in the spread of *N. gonorrhoeae*, as they are identified only through partner notification or by the screening of high-risk populations. Symptomatic gonococcal urethritis becomes asymptomatic if left untreated over a period of months.[33]

Gonococcal urethritis and NGU can be accurately differentiated based on clinical grounds in three-quarters of patients.[5] Chlamydial urethritis has a longer incubation period (1–5 weeks) and produces more subtle symptoms than gonorrhea.[7] Onset of symptoms is subacute. The discharge is mucopurulent or mucoid and may be seen only after urethral stripping or in the morning before voiding. A small crust at the meatus may be the only visible discharge and may be associated with meatal itching. Dysuria is frequently present but may be less intense than in gonococcal infection. However, the presence of dysuria without urethral discharge is a very good (90%) predictor of NGU, including chlamydial infection.[5] As with gonorrhea, local lymphadenopathy is absent. One-quarter to one-half of men with chlamydial urethritis are asymptomatic.[7] The clinical manifestations of gonococcal and chlamydial urethritis appear to be similar in non-immunocompromised and immunocompromised patients, including those with HIV infection.

Urethritis caused by *M. genitalium*, *U. urealyticum* or *T. vaginalis* infection is clinically indistinguishable from chlamydial urethritis;[9] when available, laboratory testing for *T. vaginalis* may aid in diagnosis. Primary herpes simplex virus infection frequently results in urethritis accompanying external genital vesicle and ulcers.[17] Dysuria is severe and the mucoid discharge profuse; regional lymphadenopathy is common. Endourethral ulceration may result in localized tenderness along the urethra.

Several symptoms suggest diagnoses other than urethritis. Urinary frequency and urgency, with or without hematuria, suggest cystitis or upper urinary tract infection. Painless hematuria usually originates in the bladder or kidney from a variety of largely noninfectious causes. Hesitancy, dribbling and nocturia require evaluation for urologic and prostatic disorders. Prostate tenderness is not seen with simple urethritis, but may be found in the occasional case of prostatitis accompanying urethritis. Painful ejaculation without dysuria, blood in the ejaculate and pain radiating from the genitals to the pelvis or back are not seen in urethritis and mandate evaluation for other disorders.

Complications

Without treatment, gonococcal and chlamydial urethritis can lead to a variety of complications (Table 74.3). Epididymitis occurs in 1–2% of patients, with equal risk from *N. gonorrhoeae* and *C. trachomatis*. [7] [34] In this setting, epididymitis is unilateral and is caused by extension of the urethral infection via the vas deferens to the epididymis. Most cases of epididymitis in adult men under the age of 35 years can be attributed to *N. gonorrhoeae* and *C. trachomatis*. Homosexual men who practice and insertive intercourse may acquire urethritis and epididymitis caused by Gram-negative bacilli. Occasionally, epididymitis extends to the testis, producing epididymo-orchitis. Rapid differentiation of epididymitis and orchitis from testicular torsion is critical as the latter is a surgical emergency. Epididymitis and orchitis are discussed further in Chapter 68 .

Conjunctivitis, following accidental self-inoculation, complicates as many as 1–2% of cases of gonococcal or chlamydial urethritis.[34] In the pre-antibiotic era, prostatitis and urethral stricture often resulted from prolonged untreated gonococcal urethritis. Periurethral abscess occasionally complicates urethritis caused by coliform bacteria, especially if phimosis or pre-existing urethral stricture is present.

TABLE 74-3 -- Complications of urethritis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis.*

COMPLICATIONS OF URETHRITIS CAUSED BY *NEISSERIA GONORRHOEAE* AND *CHLAMYDIA TRACHOMATIS*
Neisseria gonorrhoeae
Disseminated gonococcal infection (0.5–2%)
Prostatitis (very rare)
Chlamydia trachomatis
Reiter's syndrome (<1%)
N. gonorrhoeae* or *C. trachomatis
Epididymitis (1–2%)
Conjunctivitis (1–2%)
Urethral stricture (rare)
Enhanced transmission of HIV (risk increased 4- to 5-fold)
Approximate rates for each complication are provided in parentheses.

Fewer than 1% of patients who have chlamydial urethritis develop Reiter's syndrome, with the triad of urethritis, conjunctivitis or uveitis and arthritis. [35] Other clinical manifestations include painless erythema and ulceration of the glans (circinate balanitis), pustular or hyperkeratotic lesions of the soles of the feet (keratodermia blennorrhagica), nonarticular body pain and oral mucosal ulcerations. The majority of patients who have Reiter's syndrome have the HLA-B27 histocompatibility antigen. Pathogenesis is thought to involve an abnormal host response to antigens of *C. trachomatis*, which have been found in the synovium of affected joints. *Mycoplasma genitalium* has also been identified in the joints of patients with arthritis. [14] Reiter's syndrome also develops after infection with certain gastrointestinal pathogens (see Chapter 43).

Disseminated gonococcal infection (DGI) arises primarily from asymptomatic gonococcal urethritis caused by strains with particular serotypes (IA) and auxotypes (AHU^-).[12] Strains associated with disseminated gonococcal infection are also resistant to killing by normal human serum, presumably allowing invasion of the bloodstream and dissemination to distant sites. Terminal complement deficiency predisposes to invasive gonococcal infections, but the majority of patients who have DGI have normal complement levels. Signs and symptoms of DGI include small numbers (usually fewer than 30) of papular, petechial or pustular skin lesions, usually on the hands, wrists and feet, tenosynovitis of the hands or feet or asymmetric polyarthritis involving few joints, and fever. Culture of joint fluid from a patient who has this dermatitis-arthritis syndrome is usually negative, although blood culture may be positive. A frank septic arthritis caused by *N. gonorrhoeae* may develop; in such cases, joint fluid culture is often positive and blood culture negative. Overall, around one-half of patients who have DGI have positive blood or joint fluid cultures. In those with negative sterile body site cultures, urethral, pharyngeal or rectal cultures frequently produce *N. gonorrhoeae* and thus support the diagnosis.

Increasingly, the importance of classic STDs in facilitating the sexual transmission of HIV infection has been recognized. The relationship between STDs and HIV is bidirectional; the presence of STDs in an HIV-negative person appears to increase the risk of acquiring HIV infection, whereas the presence of STDs in an HIV-positive individual appears to increase the potential for transmission of HIV. Gonococcal and chlamydial infection are associated with a 4- to 5-fold increased risk of acquiring HIV infection independent of sexual behaviors and other factors. [36] Limited serologic data link *M. genitalium* with HIV transmission.[14] Community-wide treatment of STDs with effective therapies reduced the rate of new HIV infections in one large study.[37] Recent data provide biologic evidence supporting the concept of increased potential for HIV spread in persons dually infected with HIV and urethritis. HIV-infected men with gonococcal urethritis shed much larger quantities of HIV in their semen than those without gonorrhea; this shedding is reduced dramatically by treatment of the urethritis.[38]

Persistent urethritis

Persistent or recurrent urethritis develops in a small proportion of patients who have NGU. In many cases, this represent re-exposure to an untreated sexual partner or infection with tetracycline-resistant *U. urealyticum* or with *T. vaginalis*. Repeat therapy, in the case of re-exposure, or empiric therapy directed at *U. urealyticum* and *T. vaginalis* is appropriate; success of the therapy confirms the diagnosis. *Mycoplasma genitalium* has also been implicated in persistent urethritis and may respond to repeated or prolonged therapy with tetracyclines, macrolides or fluoroquinolones.[14] Accompanying prostatitis may be responsible for persistence or recurrence of urethritis until a long course of therapy (3–6 weeks) is provided.[39] Other less common agents, such as *Candida albicans*, genital warts and coliform bacteria, should be considered in cases of persistent or recurrent urethritis. Noninfectious causes may explain persistent symptoms despite antimicrobial therapy. Urologic evaluation, including urethroscopy to identify intraurethral lesions or foreign bodies, may be necessary in select cases.

Urethritis in women

Urethral infection with *N. gonorrhoeae* and *C. trachomatis* occurs commonly in women; however, the clinical manifestations of concomitant cervicitis usually overshadow signs and symptoms related to urethral infection. In fact, nucleic acid amplification techniques identify *C. trachomatis* in the first-voided portion of the urine from the vast majority of women with chlamydial cervicitis. In a small proportion of these women, careful examination may reveal urethral discharge.

The diagnosis of acute urethral syndrome is made in women with symptoms of cystitis and pyuria but fewer than 10^5 bacteria/ml urine. When low-level bacteriuria is present, the etiology is probably coliform organisms typical of cystitis; when bacteriuria is absent, *C. trachomatis* is often found and the symptoms respond to appropriate therapy.[40]

Dysuria in women is more commonly caused by cystitis and vulvovaginitis. Cystitis produces urgency, frequency and internal dysuria characterized by a deep burning sensation. Vulvovaginitis results in external dysuria caused by the irritant effect of urine contacting an inflamed perineum.

Physical examination

Examination of the man with urethritis symptoms begins with assessment for urethral discharge. Ideally, the patient should not urinate for at least 2 hours before examination, as this washes discharge from the urethra. The examiner should look for evidence of spontaneous discharge and note the color, quality and quantity of the discharge. The only evidence of mild discharge may be crusting at the meatus; meatal erythema should also be sought. If no discharge is found, the urethra should be stripped to bring discharge forward. Stripping is accomplished by applying pressure along the underside of the penis from the base to the meatus. In symptomatic men, stripping frequently produces a small amount of discharge. Urethral discharge should be collected on a swab and then rolled onto a glass slide for Gram staining (Fig. 74.5). If no discharge is produced, a urethral swab should be inserted 2–4cm into the urethra for collection of the specimen. Next, the inguinal lymph nodes should be palpated;

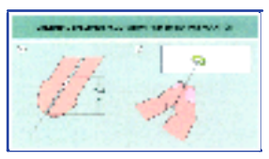

Figure 74-5 Procedure for obtaining a urethral specimen and preparing a smear for Gram stain. (a) If no discharge is present at the meatus, collect a specimen by inserting the urethral swab 2–4cm into the urethra and rotating for 5 seconds. (b) Roll the swab on a glass slide; rolling the swab preserves cell morphology.

small, mobile, nontender lymph nodes are commonly felt, but enlarged or tender lymph nodes suggest a diagnosis other than gonococcal or chlamydial urethritis. The scrotal contents should be evaluated for swelling, tenderness and warmth. Care should be taken to distinguish epididymitis and orchitis from testicular torsion, which requires prompt surgical attention (see Chapter 68).

Examination of the woman with urethritis symptoms should include a complete pelvic examination, during which cervical specimens may be obtained for diagnostic testing (see Chapter 62). In the absence of apparent cervical infection, a urethral specimen may be useful; urethral stripping may be accomplished by compressing the urethra against the symphysis pubis using a forward motion. A urethral swab should only be inserted into the most superficial part of the female urethra. However, the availability of diagnostic tests applied to urine (see Diagnosis) may obviate the need for urethral and cervical specimens in some settings.

DIAGNOSIS

Gram stain

Several laboratory studies are helpful in the diagnosis of urethritis. Once a specimen is obtained on a swab, the swab should be rolled on a slide for Gram staining (see Fig. 74.5). The presence of more than four PMNs per oil-immersion field averaged over five fields in the maximally dense part of the slide is considered objective evidence of urethritis (Fig. 74.6). Because recent urination washes away urethral discharge, fewer PMNs may be seen in this setting. In addition, variation in specimen collection technique and intraobserver variability in reading the smear mean that some men infected with agents of urethritis have fewer PMNs. As many as one-third of men with urethral chlamydial infection may not exhibit abnormal numbers of PMNs on the urethral smear.[7] The presence of Gram-negative intracellular diplococci (Fig. 74.7) indicates gonococcal urethritis with 95% sensitivity and 98% specificity and is adequate justification for therapy.[5] [20] However, sensitivity of the Gram-stained urethral smear may be lower in asymptomatic men and with specific

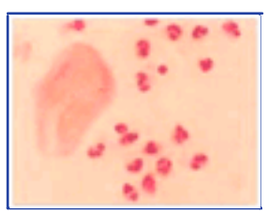

Figure 74-6 Nongonococcal urethritis. Gram-stained smear of urethral discharge containing many PMNs but no visible bacteria.

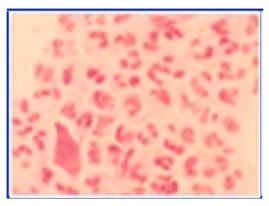

Figure 74-7 Gonorrhea. Gram-stained smear of urethral discharge containing numerous PMNs and Gram-negative intracellular diplococci consistent with *Neisseria gonorrhoeae*.

strains of *N. gonorrhoeae*.[41] Atypical intracellular or typical extracellular Gram-negative diplococci may represent other micro-organisms; gonorrhea cultures are positive in 20–30% of these cases. Other Gram-positive and Gram-negative organisms usually reflect colonization of the first centimeter of the urethra and are a normal finding.

Diagnostic tests for gonococcal urethritis and disseminated gonococcal infection

Gonorrhea culture or another test for gonorrhea should be performed if the Gram-stained smear is negative or equivocal. *Neisseria gonorrhoeae* is a fastidious organism, requiring specialized media and conditions for growth. Modified Thayer-Martin, Martin-Lewis or other media designed specifically for culturing *N. gonorrhoeae* should be used. Optimally, the specimen should be plated at the bedside and placed into a carbon dioxide incubator or candle jar immediately. Transportable culture systems using carbon dioxide-producing capsules have made culture possible in clinical settings when incubators are unavailable. Occasional strains sensitive to the concentrations of vancomycin used in the media result in false-negative cultures.

Culture has long been the gold standard for diagnosis of gonorrhea. However, the rigorous conditions necessary for successful culture have necessitated the development of new diagnostic methodologies (Table 74.4). The most important of these are the DNA probe and nucleic acid amplification assays. The DNA probe test for gonorrhea has a sensitivity of approximately 85% and specificity of 98–99%.[42] A DNA probe test is also available for *C. trachomatis*; a single swab can be submitted for both tests. Several nucleic acid amplification tests are available for the diagnosis of gonorrhea, including ligase chain reaction (LCR) and polymerase chain reaction (PCR). These techniques appear to offer no advantage over culture in terms of sensitivity and specificity for detection of urethral infection. [42] However, in situations in which optimal culture quality cannot be maintained, the DNA probe and nucleic acid amplification tests may be more sensitive than culture. In addition, nucleic acid amplification tests can be applied to the first-voided

TABLE 74-4 -- Diagnostic tests for *Neisseria gonorrhoeae.*

DIAGNOSTIC TESTS FOR *NEISSERIA GONORRHOEAE*			
Test	**Sensitivity (%)**	**Specificity (%)**	**Comment**
Gram stain*	95 (symptomatic) 60 (asymptomatic)	98	Results available immediately
Culture†	90–97	98–99	Requires careful handling and proper facilities
DNA probe†	85	98–99	Can test for *C. trachomatis* with same swab
DNA amplification (LCR, PCR, others)†	95	98–99	Can use first-voided portion of urine or urethral swab with equal performance, and test for *C. trachomatis* with same specimen
Sensitivity and specificity are given for the diagnosis of urethritis in men, using urethral specimens unless otherwise stated.			

* Compared with culture
† Compared with enhanced reference standard comprising culture and DNA amplification or probe competition assay

portion of urine or urethral specimens with equivalent performance. All nonculture tests have the disadvantage of not producing an isolate that can be used for antimicrobial susceptibility testing; this may become important in the near future if the prevalence of fluoroquinolone resistance increases.

The diagnosis of DGI generally depends on the constellation of clinical findings rather than on specific laboratory tests.[12] Patients suspected of having DGI should have genital, rectal and pharyngeal specimens cultured for *N. gonorrhoeae*. Joint fluid, if obtainable, should also be cultured. Blood cultures should be obtained for diagnosis and to exclude other infections associated with fever and petechial rash such as meningococcal sepsis. However, around 20% of patients who have DGI have no positive cultures. In the appropriate clinical setting, and with the exclusion of other bacterial infections of the joints or bloodstream, DGI is strongly suggested by the constellation of tenosynovitis, acral rash and fever.

Diagnostic tests for chlamydial urethritis

A wider variety of tests are available for the detection of *C. trachomatis* than for *N. gonorrhoeae* (Table 74.5). Although *C. trachomatis* can be cultured, the expense and complexity of this procedure have limited the clinical utility of culture as a diagnostic test. Several types of nonculture tests, including enzyme immunoassays (EIAs), direct fluorescent antibody tests (DFAs) and a DNA probe, have been widely used in chlamydial diagnostics; more recently, the nucleic acid amplification tests such as PCR and LCR have revolutionized the diagnosis of chlamydial infection by providing increased sensitivity and allowing the use of urine as a diagnostic specimen.

The EIAs and DFAs have sensitivities of 70–90% compared with culture and specificities of 95–99% when a confirmatory test is used. [43] Sensitivity in asymptomatic men is lower than that found in symptomatic men, presumably because of a lower organism burden. The DNA probe generally performs a little better than the EIA or DFA, with a higher sensitivity (86–93% compared with culture) and

TABLE 74-5 -- Diagnostic tests for *Chlamydia trachomatis.*

DIAGNOSTIC TESTS FOR *CHLAMYDIA TRACHOMATIS*			
Test	**Sensitivity (%)**	**Specificity (%)**	**Comment**
EIA*	70–90	95–99	Readily done in high volume
DFA*	70–95	95–99	Depends on skill of microscopist; not amenable to high volume
Culture†	65–80	>99	Requires expert laboratory
DNA probe†	85–93	98–99	Can test for *N. gonorrhoeae* with same swab
DNA amplification (LCR, PCR, others)†	94–99	98–99	Can use first-voided portion of urine or urethral swab with equal performance, and test for *N. gonorrhoeae* with same specimen
Sensitivity and specificity are given for the diagnosis of urethritis in men, using urethral specimens unless otherwise stated.			

* Compared with culture
† Compared with enhanced reference standard comprising DNA amplification plus culture or DFA test

high specificity.[44] The DNA probe test can be used to detect *N. gonorrhoeae* and *C. trachomatis* with the convenience of using a single swab. The performance of all of these tests is sensitive to the quality of specimen sampling and to the presence or absence of symptoms, as this may reflect organism burden. Rapid antigen detection tests that can detect *C. trachomatis* in less than 30 minutes and can be performed in the physician's office have been developed. However, until the sensitivity can be

improved from the current 50% (compared with culture), such tests will have very limited utility.

Comparisons of nucleic acid amplification tests with other tests for *C. trachomatis* suggest that the most sensitive test previously available, culture, detects only 65–80% of infections.[45] Nucleic acid amplification tests appear to detect 94–99% of urethral infections, based on a resolved gold standard.[45] [46] In addition to having higher sensitivities than any previously available test, nucleic acid amplification tests have very high specificities (98–99%) and can be performed using the first-voided portion of urine or urethral swabs with equivalent performance. All of the nonculture tests can detect nonviable organisms and accordingly should not be used within 2–3 weeks after treatment of chlamydial infection. A positive test for *C. trachomatis* using any of the above methodologies should be considered evidence of infection, although the clinician should recognize that even a highly specific test will yield some false-positive results, especially if the test is used to screen a low-prevalence population.

Other tests for urethritis

Tests for causes of urethritis other than *N. gonorrhoeae* and *C. trachomatis* have limited utility and are often not available to the clinician. Although *M. genitalium* is a frequent cause of urethritis, specific tests for this agent are not yet widely available. The role of *U. urealyticum* as a colonizer of the genital tract makes test results difficult to interpret; testing is also not widely available. *Trichomonas vaginalis* may rarely be seen in urethral Gram stains, but is better identified in a wet mount of the urethral specimen where motility can be appreciated (Fig. 74.8). However, most men infected with *T. vaginalis* have negative wet mounts. Culture and PCR for *T. vaginalis*, although not widely available, are much more sensitive than wet mount for urethral infection.[18] Reports of herpes simplex urethritis in the absence of external lesions suggest that testing for this virus in patients with NGU of unknown etiology may be useful. Despite use of all available diagnostic tests, 10–30% of NGU cases have no identified cause.

When the anatomic site of infection is in doubt, a urine sample can be used to distinguish urethritis from infection higher in the urinary tract. The presence of PMNs and mucous threads in the sediment of the first 10ml voided urine, while the remainder of the urine is clear, suggests urethritis;[47] equal numbers of PMNs in both parts suggests cystitis or pyelonephritis. The presence of at least 10 PMNs per high-power field in the sediment of first-voided urine is considered objective evidence of urethritis.

Figure 74-8 Trichomonal infection. Saline mount of *Trichomonas vaginalis* (arrow); characteristic ovoid shape and flagella can be seen.

Figure 74-9 A simplified approach to the diagnosis and management of symptomatic urethritis. hpf, high-power field; STS, serologic test for syphilis.

Diagnostic approach to symptomatic urethritis

An algorithm for the diagnosis and management of symptomatic urethritis is outlined in Figure 74.9 . The urethral smear classifies the patient as having gonococcal urethritis, NGU or no evidence of urethritis. The presence of typical intracellular Gram-negative diplococci is sufficient for the diagnosis of gonorrhea, requiring treatment for gonorrhea and possible co-infection with *C. trachomatis*. A test for *C. trachomatis* may be performed and partners should be treated. Since the presence of one STD increases the likelihood that another STD is present, a serologic test for syphilis is appropriate.

A patient who has a urethral smear showing five or more PMNs per high-power field, but no bacteria or only atypical diplococci, is treated for NGU. A test for *C. trachomatis* allows an etiologic diagnosis, but treatment should not be delayed pending test results. Gonorrhea testing should always be performed on patients thought to have NGU, as the Gram stain is less than 100% sensitive. As with all STDs, partners should be treated and a serologic test for syphilis performed.

If NGU persists or recurs after therapy, several possibilities should be considered. First, if the patient was noncompliant with therapy or was re-exposed to an infected partner, repeat therapy should be effective. Otherwise, empiric therapy directed at tetracycline-resistant *U. urealyticum* (erythromycin) and at *T. vaginalis* (metronidazole) is most likely to resolve the urethritis. Azithromycin may also be useful for *U. urealyticum* and for persistent or recurrent urethritis caused by *M. genitalium*. Less common causes of urethritis should also be considered at this time.

If four PMNs or fewer are seen per high-power field on the initial Gram stain, there is no objective evidence for urethritis. However, if a discharge is present, the patient should be managed as for NGU. Dysuria alone in a young sexually active man also suggests NGU; a single course of therapy directed at agents of NGU may be given before further evaluation. The presence of PMNs in first-voided urine supports the diagnosis of NGU. Whether or not treatment for NGU is given, testing for *N. gonorrhoeae* and *C. trachomatis* should be performed. If no discharge is present, and there are no PMNs in first-voided urine, then other diagnoses such as cystitis and prostatitis should be considered. Repeat examination after several hours without urination may also be helpful.

Screening for asymptomatic urethritis

A small percentage of men infected with *N. gonorrhoeae* and one-quarter to one-half of men infected with *C. trachomatis* are asymptomatic.[7] These men play a disproportionate role in spreading infection because they are unlikely to be identified and treated.[25] Attempts to improve identification of these men using leukocyte esterase testing of urine as a preliminary test have been unsatisfactory

TABLE 74-6 -- Therapeutic regimens for urethritis.[48]

THERAPEUTIC REGIMENS FOR URETHRITIS		
Gonorrhea	**Chlamydial infection**	**Nongonococcal urethritis (empiric therapy)**
Cefixime 400mg orally once*	Azithromycin 1g orally once*	Azithromycin 1g orally once
Ceftriaxone 125mg im once* †	Doxycycline 100mg orally q12h for 7 days	Doxycycline 100mg orally q12h for 7 days
Ciprofloxacin 500mg orally once† ‡	Ofloxacin 300mg orally q12h for 7 days	Ofloxacin 300mg orally q12h for 7 days
Ofloxacin 400mg orally once‡	Levofloxacin 500mg orally q24h for 7 days	Levofloxacin 500mg orally q24h for 7 days
Levofloxacin 250mg orally once‡	Erythromycin 500mg orally q6h for 7 days*	Erythromycin 500mg orally q6h for 7 days
Spectinomycin 2g im once*		
For treatment of recurrent or persistent urethritis, see discussion in text.		

* Considered safe for use in pregnancy
** Safety in pregnancy not proved
† Preferred for coexisting pharyngeal gonorrhea
‡ Not recommended for gonorrhea acquired in Asia or the Pacific, including Hawaii

owing to the poor sensitivity of this test. Gram stain testing of a urethral smear identifies urethritis in high proportions of asymptomatic men at risk for STDs and has the advantage of identifying urethritis of any etiology. Nucleic acid amplification tests, which can be performed on urine, simplify screening for *C. trachomatis* and *N. gonorrhoeae*. Urine-based testing for these pathogens allows identification of asymptomatic infected men without the discomfort of a urethral swab and can be performed in settings in which clinicians or examination facilities are unavailable. Infection at other sites (e.g. throat, rectum) is not identified by urine or urethral testing and requires additional testing; nonculture methodologies have not been fully evaluated for use outside the genitourinary tract.

MANAGEMENT

Therapeutic regimens for the management of urethritis are outlined in Table 74.6 . Gonococcal urethritis is generally treated with single-dose therapy; a regimen active against *C. trachomatis* is added because of the high likelihood of co-infection. Nongonococcal urethritis or chlamydial urethritis is treated with a single-dose or multidose regimen. Recurrent or persistent urethritis may require more than one antibiotic or a long course of therapy. Sexual partners must also be treated, as the likelihood of infection is high. To avoid reinfection, abstinence is necessary until the patient and partner have completed therapy.

The presence of an STD is a marker for high-risk sexual behavior. Therefore, all persons with an STD should have a serologic test for syphilis and should be counseled about STD and HIV prevention. Patients without immunity to hepatitis B should be vaccinated. Urethritis in HIV-infected persons is managed no differently from urethritis in persons not infected with HIV. The management of epididymitis complicating urethritis is discussed in Chapter 68 .

Management of gonococcal urethritis and disseminated gonococcal infection

A variety of highly effective single-dose therapies are available for the management of gonococcal urethritis. Therapies recommended by the Centers for Disease Control and Prevention in 2002 include single doses of cefixime 400mg orally, ceftriaxone 125mg intramuscularly, ciprofloxacin 500mg orally, ofloxacin 400mg orally or levofloxacin 250mg orally.[48] These therapies produce greater than 95% cure rates for genital and rectal infection and the first three have been demonstrated to eliminate *N. gonorrhoeae* from the urethra within a few hours of administration.[24] The discomfort of ceftriaxone injections may be reduced by the use of a 1% lidocaine solution as a diluent. Cefixime has a lower therapeutic index (ratio of peak serum level to MIC) than ceftriaxone, but has the advantage of being orally administered. Several other cephalosporins have demonstrated efficacy in the treatment of gonorrhea, but have no advantages over the recommended therapies and experience with them is more limited. The cephalosporins are safe for use in pregnancy and in children.

The fluoroquinolones ciprofloxacin, ofloxacin and levofloxacin provide alternatives for patients who are allergic to ß-lactams, but they are contraindicated in pregnancy and in those under 18 years of age. In addition, resistance to these fluoroquinolones in *N. gonorrhoeae* has been prevalent in parts of Asia and the Pacific Islands for several years and has recently appeared in the USA.[49] A clinically important prevalence of resistance has been reached in Hawaii and parts of California. As the prevalence and geographic range of these strains are likely to increase, knowledge of local prevalence should guide the use of fluoroquinolones to treat gonorrhea. Doses lower than those recommended should not be used. Fluoroquinolones not specifically recommended for the treatment of gonorrhea may be less effective and should not be used. Cephalosporins are preferred for treatment of gonorrhea acquired in Asia, Pacific Islands (including Hawaii), or California. If the prevalence of fluoroquinolone resistance in the USA increases, it may be necessary to determine susceptibility when treating patients with these therapies.

An alternative to the recommended therapies for gonorrhea is spectinomycin 2g intramuscularly once, which can be used in patients intolerant of ß-lactams and in pregnancy, but is expensive, requires injection and is relatively ineffective against pharyngeal gonorrhea. Spectinomycin-resistant *N. gonorrhoeae* is rare in the USA, but is prevalent in some parts of the world where spectinomycin has been widely used. Penicillins and tetracyclines are no longer used to treat gonorrhea because of the high prevalence of resistant strains (around 30% of US isolates are resistant to one or both drugs).[49]

There are several additional considerations in managing the patient who has gonorrhea. First, treatment for gonorrhea should be accompanied by a regimen effective for possible co-infection with *C. trachomatis* (see below), unless a concurrent test for chlamydial infection is negative. Second, all patients with an STD should be screened for syphilis with a nontreponemal serologic test such as the rapid plasma reagin (RPR). Patients who have incubating syphilis, however, have negative serologic tests and are accordingly difficult to identify; ideally, therefore, treatment for gonorrhea would also cure incubating syphilis. The cephalosporins, but not the quinolones or spectinomycin, have activity against *Treponema pallidum*. Azithromycin and doxycycline also have activity against *T. pallidum* and azithromycin appeared to be effective in curing incubating syphilis in a small study.[50] Third, ceftriaxone and ciprofloxacin have been proved to be effective in the treatment of pharyngeal gonorrhea, with greater than 90% cure rates; data for other therapies are insufficient to recommend their use in pharyngeal gonorrhea. Follow-up is not necessary after treatment for uncomplicated gonorrhea because of the effectiveness of current therapies; however, all patients should be instructed to return if symptoms do not promptly resolve and follow-up is advised for those who acquired infection overseas or when the diagnosis is unsure.

Disseminated gonococcal infection requires at least 7 days of antibiotic therapy, beginning with parenteral therapy and switching to oral therapy after 24–48 hours of improvement. Recommended parenteral therapies are ceftriaxone 1g intramuscularly or intravenously q24h. Alternatives are cefotaxime 1g intravenously q8h, ceftizoxime 1g intravenously q8h, ciprofloxacin 400mg intravenously q12h, ofloxacin 400mg intravenously q12h, levofloxacin 250mg intravenously q24h or spectinomycin 2g intramuscularly q12h. Therapy may be completed with cefixime 400mg orally q12h, ciprofloxacin 500mg orally q12h, ofloxacin 400mg orally q12h or levofloxacin 500mg orally q24h. Concurrent therapy active against *C. trachomatis* should be provided unless the absence of chlamydial infection has been documented.

Management of chlamydial urethritis

Chlamydial urethritis should be treated with a single-dose regimen of azithromycin 1g orally or with a multiple-dose regimen of doxycycline 100mg orally q12h for 7 days. Other effective regimens are ofloxacin 300mg orally q12h for 7 days or levofloxacin 500mg orally each day for 7 days. Azithromycin has the advantage of a single-dose administration, but is more expensive than doxycycline. Clinical experience and limited data suggest that azithromycin is safe and effective in pregnancy.[51] Doxycycline, ofloxacin and levofloxacin are not recommended for use in pregnancy or in children. Ofloxacin and levofloxacin are expensive and have no dosing advantages over doxycycline.

An alternative regimen for chlamydial infection is erythromycin 500mg orally q6h for 7 days. Erythromycin is inexpensive, but gastrointestinal upset may limit compliance. Amoxicillin 500mg orally q8h for 7 days is useful in pregnant women unable to tolerate erythromycin.[51]

Patients who have satisfactory resolution of symptoms generally do not require follow-up testing for *C. trachomatis*. However, if erythromycin or amoxicillin are used, post-treatment testing is advised. Testing should be delayed until at least 3 weeks after completion of therapy, as dead organisms may produce false-positive results on nonculture tests performed sooner.

Management of nongonococcal urethritis

Treatment of NGU is directed at the likely etiologic agents, including *C. trachomatis, M. genitalium* and *U. urealyticum*. Symptoms resolve slowly, sometimes lasting for several days after the completion of therapy. Doxycycline 100mg orally q12h for 7 days and azithromycin 1g orally in a single dose are equivalent in curing NGU. Ofloxacin 300mg orally q12h for 7 days or levofloxacin 500mg orally daily for 7 days is effective, but more expensive than other regimens. Erythromycin 500mg orally q6h for 7 days may be used as an alternative. For NGU that fails to respond to initial therapy, several alternatives may be considered. Repeat treatment with the same drug may be used if noncompliance or re-exposure to an untreated sexual partner is suspected. However, some doxycycline failures may be attributable to tetracycline-resistant *U. urealyticum*, which may be successfully treated with a 7-day course of erythromycin, or to *T. vaginalis*, which may be cured with a single 2g oral dose of metronidazole. Some isolates of *U. urealyticum* are resistant to erythromycin but may respond to a 7-day course of ofloxacin 300mg orally q12h.[52] *Mycoplasma genitalium* has also been implicated in persistent or recurrent Nongonococcal urethritis and may require repeated or prolonged therapy for eradication.[14] Azithromycin may be the drug of choice for *M. genitalium* and may also be effective against *U. urealyticum*. Nongonococcal urethritis persisting after two courses of therapy requires consideration of other diagnoses such as cystitis and prostatitis. Urologic evaluation may include urine flow measurements and urethroscopy or urethrography to look for foreign bodies, strictures and periurethral abscesses. If no cause can be found, then a 3-to 6-week course of doxycycline or erythromycin can be tried,[39] although the long-term effectiveness of this is unknown.

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Chapter 75 - Syphilis

George R Kinghorn

Syphilis is a chronic infectious disease caused by the spirochete *Treponema pallidum*, which is transmitted during sexual intercourse and other intimate contact; it may also be vertically transmitted by a pregnant woman to her fetus in utero or during birth.

The name of the disease was drawn from a poem, 'Syphilis sive morbus gallicus', written by Fracastoro of Verona in 1530, in which the mythical swineherd Syphilis refused to make sacrifices to Apollo and was smitten as a result. [1]

EPIDEMIOLOGY

An epidemic of sexually transmitted syphilis spread across Europe at the end of the 15th century. There are conflicting views about the origin of syphilis in Europe — some believe that it was brought back from the New World by Columbus, and others believe that it had been endemic throughout the Middle Ages and became sexually acquired at the time of the epidemic. The impact of syphilis in 16th century Europe was similar to the global impact of AIDS in the late 20th century. Syphilis had certainly become endemic in Europe by the 17th century, since when there have been several epidemic waves, notably during the Napoleonic wars and the period of industrialization in the 19th century, and during and after the two world wars of the 20th century.

In North America and the developed countries of northern Europe, syphilis had become predominately a disease of homosexual men by the 1970s. During the late 1980s, there were renewed outbreaks of heterosexual and congenital syphilis in North America in the wake of the HIV epidemic.[2] This resurgence was mainly observed in commercial sex workers, in whom it was often associated with selling sex for drugs (especially crack cocaine), and in other people of lower socioeconomic status. In 1994, there were 20,627 reported cases of primary and secondary syphilis in the USA (8.1 cases per 100,000 people, the rate being 60 times higher in non-Hispanic blacks than in Caucasians) compared with 304 cases in England (fewer than 0.6 cases per 100,000 people; Fig. 75.1).[3] Subsequently, incident syphilis cases have declined to fewer than 4 cases per 100,000[4] and syphilis is again being targeted for national elimination.[5]

Eastern Europe has experienced a 50-fold increase in reported syphilis cases between 1990 and 1997, with similar increases in the Ukraine, some central Asian countries, and the Baltic states. Neighbouring Scandinavian countries have witnessed an increase in imported cases.[6]

Although the overall incidence of syphilis in the UK was only 0.3 per 100,000 in 1998, there have since been outbreaks in several cities where there was previously a low prevalence.[7] The outbreaks have been characterized by rapid increases in sexual networks with high rates of partner change, travel or migration links with high incidence areas and an increasing predominance of homosexual transmission, with a high proportion of HIV co-infection among incident cases.

The annual incidence of congenital syphilis in infants aged less than 1 year in the USA increased from 3.0 per 100,000 live births in 1980 to a peak of 107.3 per 100,000 live births in 1990. The very large increase in reported cases has been artificially elevated by the introduction of a new reporting system,[8] [9] [10] which takes account of epidemiologic factors, especially maternal treatment status, in addition to cases showing characteristic clinical stigmata (Table 75.1). The annual incidence has since declined to 30.4 per 100,000 live births (in 1996).

The World Health Organization (WHO) estimates that the annual global incidence of syphilis is approximately 12.2 million cases, most of which occur in developing countries, where the disease has remained a prominent cause of genital ulcer disease in heterosexual men and women, of stillbirth, and of neonatal morbidity and mortality.[8] The prevalence of pregnant seropositive women is 0.1–0.6% in developed countries, but it may exceed 10% in many developing countries. In some parts of South Africa, seroconversion during pregnancy has been reported to occur in more than 2% of women.[11]

Transmission

The organism is transmitted from the early mucocutaneous lesions, and enters the body through small breaches in epithelial surfaces of genital, anorectal, oropharyngeal and other cutaneous sites.

Prenatal transmission is greatest in cases of maternal infection of short duration, but it may also occur during the latent stages of syphilis. Disease manifestations are unusual before 18 weeks in utero. Stillbirth caused by congenital syphilis has a maximum incidence at 6–8 months of gestation. Even when a previous pregnancy has resulted in an uninfected child, congenital syphilis may occur in subsequent offspring, and it may affect only one of twins. It is preventable with maternal treatment during pregnancy.

Syphilis is rarely transmitted during transfusion of blood or blood products or through needle sharing by intravenous drug abusers.

PATHOGENESIS AND PATHOLOGY

The causative spirochete of syphilis, *Treponema pallidum* subsp. *pallidum*, is microaerophilic with a unique wave-like cell body that is 6–15µm long and 0.15–0.20µm wide.[12] It contains a periplasmic flagellum and can be identified in darkfield microscopy by its characteristic morphology and movements, which typically include angling. The organism has evolved to become a highly invasive and persistent pathogen with little toxigenic activity and an inability to survive outside the mammalian host. It has extreme nutritional requirements, owing to deficiencies in biosynthetic pathways, and it has a narrow equilibrium between oxygen dependence and toxicity. It cannot be cultured on artificial media but it can be propagated in organ culture, such as rabbit testis. It has a slow growth rate, optimal at 91–95°F (33–35°C), with a doubling time of 30–36 hours. The organism is similar to and shares extensive DNA homology with three other pathogenic treponemes that cause yaws, bejel and pinta (see Chapter 230).

Analysis of the genome, which is contained on a single circular chromosome with 1,138,006 base pairs, shows that the organism

Figure 75-1 Diagnoses of syphilis in England and Wales. Primary, secondary and early latent infection seen in genitourinary medicine clinics. (a) 1931–2000 and (b) 1990–2000.

TABLE 75-1 -- Definitions of congenital syphilis.

DEFINITIONS OF CONGENITAL SYPHILIS	
Confirmed diagnosis	An infant in whom *Treponema pallidum* is identified by dark-field microscopy, fluorescent antibody, or other specific stains in specimens from lesions, placenta, umbilical cord, or autopsy material
Presumptive diagnosis	1. Any infant whose mother had untreated or inadequately treated syphilis at delivery, regardless of symptoms or signs in the infant
	2. Any infant or child who has a reactive specific treponemal test for syphilis and one of the following: evidence of congenital syphilis on physical examination evidence of congenital syphilis on long-bone X-ray reactive CSF VDRL elevated CSF cell count or protein (without other cause) reactive test for FTA-ABS-IgM using fractionated serum

lacks lipopolysaccharide and lipid biosynthesis mechanisms, as well as many metabolic pathways, including pathways for the tricarboxylic acid cycle, for components of oxidative phosphorylation, and for most amino acids and vitamins. It requires D-glucose, maltose and mannose but cannot utilize other sugars. It is able to use exogenously supplied amino acids and is dependent on serum components such as fatty acids.

Treponema pallidum initiates an inflammatory response at the site of inoculation and is disseminated during the primary infection. The organism has a surface-associated hyalouronidase enzyme, which may play a role in this process. Phagocytosis by cytokine-activated macrophages, as part of a predominant T-helper (Th)1-type early response, aids bacterial clearance and resolution of the primary lesion.[13] Virulent organisms promote adhesion of lymphocytes and monocytes to human vascular cells, and this is important in immunopathogenesis.[14] As with other organisms that cause chronic disease, *T. pallidum* has evolved mechanisms for evading immune responses. A Th1–Th2 switch occurs with macrophage suppression caused by prostaglandin E2 down-regulation; however, the molecular mechanisms remain poorly understood.[15] Depressed cell-mediated responses occur during the later stages of syphilis, and lowered $CD4^+$ lymphocyte counts have been reported.[16]

Relatively few genes are involved in pathogenesis.

It has been postulated that the immuno-evasiveness of *T. pallidum* is the result of the organism's unusual molecular architecture. The outer membrane lacks lipopolysaccharide and contains few poorly immunogenic transmembrane proteins; the highly immunogenic proteins are lipoproteins that are anchored predominately to the periplasmic leaflet of the cytoplasmic membrane.[17]

The dominant immunogen is a 47kDa membrane lipoprotein, which can induce synthesis of tumor necrosis factor-a. Immuno-blotting has also shown IgG responses to an antigen of 65kDa that is shared with nonpathogenic treponemes, and antigens of 44.5, 17 and 15.5kDa that are specific for *T. pallidum*.

Histologic appearances and pathology

Some manifestations of syphilis (e.g. neuropathy) are immune-complex mediated. In early lesions, perivascular infiltration by lymphocytes and plasma cells is accompanied by intimal proliferation in arteries and veins. This leads to ischemia and ulceration. Organisms are most numerous in the walls of capillaries and lymphatic vessels.

In late lesions, the characteristic lesion of mucocutanous surfaces is the syphilitic gumma. Granulation tissue forms with histiocytes, fibroblasts and epithelioid cells. Endarteritis obliterans and necrotic areas are pronounced. Gummas most often originate in subcutaneous tissues and spread in all directions. Spirochetes are not readily demonstrable in these lesions.

Heubner's arteritis occurs in cardiovascular and meningovascular syphilis. It is characterized by lymphocytic and plasma cell infiltration of the vasa vasorum and adventitia of large and medium-sized vessels. Occlusion of the vasa vasorum results in medial necrosis and fibroblast proliferation. There is associated subintimal proliferation, which leads to luminal occlusion and thrombosis.

PREVENTION

The risk factors for acquisition of syphilis mirror those of other sexually transmitted diseases (STDs), and primary prevention depends on similar methods, such as reducing the number of sexual partners and consistent use of condoms. Community outreach activities should target education at those who are at high risk.[18] Single-dose intramuscular benzathine penicillin G and oral azithromycin have been used as treatment for incubating syphilis.[19] Administration of treponemicidal antibiotics to treat other STDs, such as gonorrhea and chancroid, may also abort incubating concurrent syphilis. The development of a vaccine against syphilis has long been inhibited by the inability to grow the organism on artificial media, although the recent sequencing of the *T. pallidum* genome should advance new research efforts.

Secondary prevention by early diagnosis, treatment, partner notification, education and counseling remain the mainstay of prevention efforts. Access to prompt and appropriate services for infected persons is essential. In many developed countries, clinic-based specialist services have long been established. In developing countries, the WHO has recommended that STD management be integrated into basic health care and reproductive health services.

Serologic screening for syphilis remains a cost-effective measure for control. Congenital syphilis may be prevented by maternal screening and treatment during early pregnancy. In Europe, screening remains a

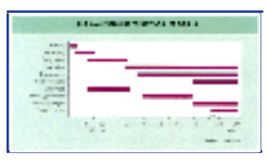

Figure 75-2 Clinical stages and presentation of syphilis.

routine part of antenatal care, usually at about 12 weeks of gestation. In developing countries, where congenital syphilis is more common, the disease occurs when there has been no maternal screening, when no treatment has been administered in response to positive tests or when primary infection occurs later in pregnancy. Repeat screening during the final trimester or at delivery is advocated in high prevalence regions. [20]

CLINICAL FEATURES

Incubation period

The time from transmission to the appearance of primary lesions averages 21 days (the range is 10–90 days); the incubation period varies inversely with the size of the spirochete inoculum.

The clinical presentation of syphilis is extremely diverse and may occur decades after initial infection. The time sequence of syphilis stages is shown in Figure 75.2 .

Primary syphilis

The primary chancre appears at the site of initial treponemal invasion of the dermis. It may occur on any skin or mucous membrane surface and is usually situated on the external genitalia (Fig. 75.3). Initial lesions are papular but rapidly ulcerate. They are usually single, but 'kissing' lesions may occur on opposing mucocutaneous

Figure 75-3 Primary chancre in coronal sulcus in primary syphilis. A typical solitary lesion with raised everted edges, central ulceration and undermined base. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

Figure 75-4 Chancre of upper lip in primary syphilis. The chancre shows the characteristic features of a raised, rolled and everted edge; central ulceration; and a granular base. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

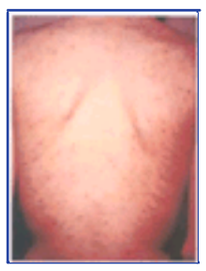

Figure 75-5 Maculopapular rash on trunk in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

surfaces. Typically, the ulcers are nontender (unless there is coexisting infection) and indurated and have a clean base and raised edges (Fig. 75.4). There is often surrounding edema, especially with vulval lesions. [21] Chancres of the cervix, anorectum or oropharynx are commonly silent. Nontender, nonsuppurative, rubbery inguinal lymphadenopathy appears 1 week later and usually becomes bilateral after 2 weeks. The chancre usually heals spontaneously within 3–6 weeks but leaves a scar.

The differential diagnosis includes other sexually transmitted causes of genital ulcer disease (which may co-exist) such as chancroid, lymphogranuloma venereum, donovanosis and genital herpes, as well as traumatic ulceration, pyogenic lesions, aphthous ulceration and malignancy.[22]

Secondary syphilis

The manifestations of generalized treponemal dissemination first appear about 8 weeks after infection. Constitutional symptoms consist of fever, headache, and bone and joint pains. There is wide diversity in physical features.

Skin rashes are the commonest feature. They are initially macular and become papular by 3 months. Lesions appear initially on the upper trunk (Fig. 75.5), the palms and soles (Fig. 75.6), and flexural

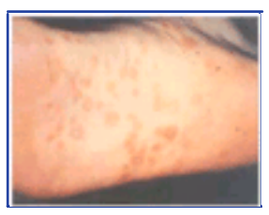

Figure 75-6 Plantar syphilid in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

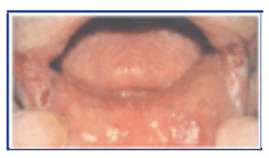

Figure 75-7 Split papules at angle of mouth and mucous patch on lower lip in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

surfaces of the extremities.[23] The later papulosquamous eruptions typically have a coppery color and often follow skin cleavage lines, as in pityriasis rosea. Facial lesions follow the hairline of the temporal and frontal scalp (the so-called corona veneris) and cause split papules at the angles of the mouth (Fig. 75.7). There may be hypopigmented lesions on the lateral neck (collaris veneris). Lesions in hairy areas cause moth-eaten alopecia of the scalp, beard, eye-brows and eye-lashes. Atypical facial plaques or ulcerated nodules (lues maligna) are more common with co-existing HIV infection.[24] Condylomata lata are moist flat-topped papules that appear about 6 months after infection in the moist intertriginous areas around the genitalia, anus and axillae (Fig. 75.8) and beneath the breasts. On mucous membranes, especially of the mouth, erythematous macules evolve into asymptomatic, slightly elevated flat-topped lesions covered by a hyperkeratotic grayish membrane. These mucous patches may coalesce to form 'snail-track' ulcers.

Generalized lymphadenopathy occurs in 50% of cases of secondary syphilis. It has similar characteristics to the localized lymphadenopathy of primary infection. Other systemic features of secondary syphilis include panuveitis,[25] periostitis and joint effusions, glomerulonephritis, hepatitis, gastritis, myocarditis and aseptic meningitis.

The lesions of secondary syphilis resolve spontaneously in a variable time period and most patients enter the latency stage within the

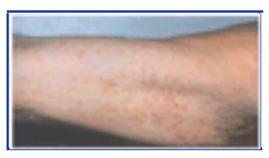

Figure 75-8 Maculopapular rash extending into axilla in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

first year of infection. In some, especially the immunocompromised, primary or secondary lesions may recur.

The differential diagnosis is broad and includes exanthema associated with many infectious diseases, including primary HIV infection, dermatoses (e.g. pityriasis rosea and guttate psoriasis) and connective tissue disorders such as systemic lupus erythematosus. Condylomata lata must be differentiated from viral warts, scabies and lichen planus.

Latent syphilis

In latent syphilis there are no clinical stigmata of active disease, although disease remains detectable by positive serologic tests. In early latency, within 2 years of infection, vertical transmission of infection may still occur, but sexual transmission is less likely in the absence of mucocutaneous lesions. The late manifestations of syphilis arise, often decades later, in about 25% of those who have latent syphilis.

Tertiary gummatous syphilis

The characteristic lesions of tertiary syphilis appear 3–10 years after infection and consist of granulomas or gummas. The granulomas appear as cutaneous plaques or nodules of irregular shape and outline (Fig. 75.9) and are often single lesions on the arms, back and face. Gummas are areas of granulomatous tissue, often arising from subcutaneous structures. They have a tendency for central necrosis and ulceration and for peripheral healing with tissue-paper scarring. Punched-out lesions appear most commonly on the scalp, face and sternoclavicular areas of the chest and on the lateral calf (Fig. 75.10). Gummas can also cause palatal perforation; destruction of nasal cartilage, producing saddle-nose deformity; painless testicular swelling, which may mimic a tumor; portal hypertension and portosystemic anastomoses; and diffuse interstitial glossitis and subsequent malignant neoplasms of the tongue.

Cardiovascular syphilis

The typical lesion of cardiovascular syphilis is aortitis affecting the ascending aorta and appearing 10–30 years after infection. The aortitis may be asymptomatic and detected as dilatation of the ascending aorta on chest radiography, often accompanied by linear calcification of the aortic wall, or it may lead to stretching and incompetence of the aortic valve, to left ventricular failure or to aneurysm formation. Aneurysms may be associated with a variety of syndromes caused by pressure on adjacent structures in the mediastinum, and they may cause sudden death from rupture. Other symptoms include angina pectoris from associated coronary ostial stenosis. Cardiovascular syphilis is more commonly associated with neurosyphilis than with gummatous disease.

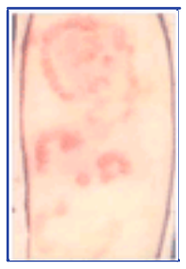

Figure 75-9 Cutaneous nodular gummas of upper arm in tertiary gummatous syphilis. The lesions have a serpiginous outline. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

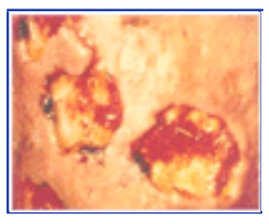

Figure 75-10 Multiple gummatous ulcers of lower leg in tertiary gummatous syphilis. The lesions have a punched-out appearance with 'wash-leather' slough overlying a base of granulation tissue. They show a tendency for peripheral healing with thin tissue-paper scars. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

The differential diagnosis of thoracic aortic aneurysm includes Behçet's disease, Tayakasu's arteritis, ankylosing spondylitis and atheromatous disease. Similar histologic lesions may appear in other large arteries.

Neurosyphilis

Neurosyphilis is characterized by a number of heterogeneous syndromes. The onset can occur weeks or decades after treponemal dissemination.[26]

Asymptomatic neurosyphilis precedes the development of clinically apparent disease and accounts for one-third of all neurosyphilis. It occurs in 10% of those with latent disease and has a peak

incidence at 12–18 months after infection. It reverts spontaneously in 70% of patients.

Meningeal neurosyphilis usually has its onset during secondary disease and is characterized by symptoms of headache, confusion, nausea and vomiting, neck stiffness and photophobia. There may be focal seizures, aphasia, delirium and papilledema. Cranial nerve palsies cause unilateral or bilateral facial weakness and sensorineural deafness.[27] Other manifestations include hydrocephalus, spinal pachymeningitis and spinal meningomyelitis.

Meningovascular syphilis occurs most frequenly between 4 and 7 years after infection. Symptoms usually begin abruptly, and focal neurologic signs are most common in the territory of the middle cerebral artery. Less commonly, focal ischemia affects the basilar or spinal arteries. The clinical features of hemiparesis, seizures and aphasia reflect multiple areas of infarction from diffuse arteritis.

Gummatous neurosyphilis is the least common syndrome. Lesions arise from the pia mater and subsequently invade the brain or spinal cord, resulting in features typical of a space-occupying lesion of these structures.

Parenchymatous syphilis appears later and has become rare in its classic forms in the antibiotic era. The peak incidence of general paralysis from parenchymatous disease of the brain used to be 10–20 years after infection. It was more common in males. The onset is insidious with subtle deterioration in cognitive function and psychiatric symptoms that mimic those of other mental disorders. As the disease progresses, neurologic signs develop, including pupillary abnormalities, hypotonia of the face and limbs, intention tremors and hyperreflexia. In late disease, there is progressive dementia, onset of seizures and increasing weakness leading to an incontinent bedfast state.

Tabetic neurosyphilis was the most common form of neurosyphilis before antibiotics were available. It has an onset 15–25 years after primary infection. The most characteristic symptom is of lightning pains — sudden paroxysms of lancinating pain affecting the lower limbs. Other early symptoms include paresthesia, progressive ataxia, and bowel and bladder dysfunction.

The clinical signs result from leptomeningeal infiltration of the preganglionic portion of the dorsal root ganglia, with subsequent atrophic change in the posterior columns of the spinal cord. The signs are hypotonia, areflexia, and impaired joint-position and vibration sense. Pupillary abnormalities are usual, and 50% of patients have the classic Argyll-Robertson pupil (small, irregular pupils that are unreactive to light but constrict normally to accommodation-convergence). Optic atrophy is also common.

Later features of tabes dorsalis include visceral crises in 10–15% of patients, characterized by recurrent episodes of epigastric pain and vomiting, mimicking an acute abdomen; acute urinary retention; progressive ataxia with a wide-based slapping gait; the appearance of neuropathic joints and perforating ulcers; and co-existing palsies of the third, sixth and seventh cranial nerves.

The differential diagosis of neurosyphilis covers the whole spectrum of neurologic and psychiatric conditions. Routine serologic screening tests for syphilis are indicated in patients that exhibit any of these features.

Congenital syphilis

Many infants with congenital syphilis are asymptomatic at birth. The placenta may show proliferative vascular changes and there may be acute inflammation of the umbilical cord (funisitis).[28] Early congenital syphilis manifests itself as rhinitis with serosanguinous nasal discharge, vesiculobulbous eruptions of the skin and oral mucous patches. Skin lesions on the lips, nostrils and anus heal with radiating scars (rhagades). In addition, there are often bone abnormalities, characterized by diaphyseal periostitis, osteochondritis and a positive

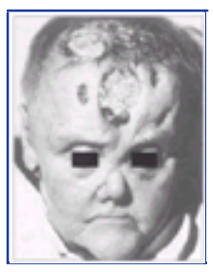

Figure 75-11 Typical facies in late congenital syphilis. There is frontal bossing, an underdeveloped maxilla, a prominent jaw and a depressed nasal bridge, with multiple gummatous ulcers of scalp. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

Wimberger's sign, which may present with limb pseudoparesis. Other features include chorioretinitis, visceral lesions causing pneumonia alba, hepatosplenomegaly associated with jaundice, and the nephrotic syndrome.

In late congenital syphilis (presenting after 2 years of age) there may be a variety of skeletal developmental defects, including a high-arched palate, a protruding mandible, frontal bossing of the skull and saddle-nose deformity resulting in a characteristic facies (Fig. 75.11); additionally, there can be bilateral hydroarthoses of the knees (Clutton's joints), sabre tibiae from osteoperiostitis, perforation of the palate and nasal septum, and sternoclavicular thickening. Dental abnormalities occur with peg-shaped permanent incisors (Hutchinson's teeth) and mulberry multicusped molars; together with interstitial keratitis and eighth nerve deafness, these dental abnormalities form Hutchinson's triad. Other features include hydrocephalus and mental retardation, as well as other typical lesions of gummatous syphilis and

neurosyphilis.

Syphilis and HIV infection

In most patients with early HIV infection, the clinical features, [29] serologic test results[30] and response to treatment are similar to those in non-HIV-infected people. With advancing immunosuppression, all of these may be significantly altered (Table 75.2).

DIAGNOSIS

Microscopic identification

Treponema pallidum can be identified from lesions of primary, secondary or early congenital syphilis by darkfield microscopy. The organism has a characteristic morphology and motility, with a sinusoidal profile with a wavelength and amplitude of 1.1µm and 0.4µm. Lesions are thoroughly cleansed with saline and scarified if necessary, and serum is allowed to collect for examination. Lymph node aspirates are also useful for microscopic examination in secondary syphilis.

The organism can also be identified by direct immunofluorescent antibody testing where no facilities for darkfield microscopy exist.

In biopsy specimens from late syphilis or in atypical early lesions, it may be possible to identify the organism by silver stains such as Warthin-Starry preparations or by direct immunofluorescent antibody testing.

Diagnosis using the polymerase chain reaction (PCR) has been based on primers and probes prepared from the 47kDa gene. After a

TABLE 75-2 -- Altered features of syphilis in HIV infection.

ALTERED FEATURES OF SYPHILIS IN HIV INFECTION	
Altered clinical features	Silent primary disease
	Presentation with secondary manifestations
	Persistence of primary lesions
	Lues maligna and atypical facial plaques
	Pneumonitis
	Accelerated course with early neurosyphilis
	Myelopathy, and auditory and ocular involvement
Altered serologic responses	Seronegative primary and secondary syphilis
	Increased incidence of prozone phenomenon
	Loss of reactivity to specific treponemal tests in previously treated disease
Altered therapeutic responses	Failure with benzathine penicillin treatment of early syphilis
	Relapse after iv penicillin G regimens for neurosyphilis

40-cycle series of denaturing, annealing and extension, the PCR products can be visualized by electrophoresis or Southern blot hybridization with a ^{32} P-labeled probe and then autoradiography. This technique should be of greatest value in detecting the low numbers of treponemal products in neurosyphilis; it should also be useful in congenital syphilis, in which the interpretation of serologic test results may be difficult.

Amplification of RNA is more sensitive than PCR, and positive results are indicative of living organisms. The reverse-trancriptase PCR uses the 16S ribosomal RNA of *T. pallidum* as the template.

Serologic testing

The serologic tests for syphilis[31] can be subdivided into two types: standard nontreponemal (reaginic) tests and the specific treponemal antibody tests (Table 75.3).

Standard nontreponemal tests

The nontreponemal tests detect IgM and IgG antibodies to lipoidal material released from damaged host cells and to lipoidal-like antigens of *T. pallidum*. There are four tests available that use the Venereal Disease Research Laboratory (VDRL) antigen (consisting of cardiolipin, cholesterol and lecithin) as the principal component. These tests are quantitative and are useful in assessing response to treatment. Reactivity to these tests does not develop until 1–4 weeks

TABLE 75-3 -- Serologic tests for syphilis.

SEROLOGIC TESTS FOR SYPHILIS					
	Sensitivity				Specificity
	Primary	Secondary	Latest	Late	
Non-specific reaginic tests					
VDRL	80	100	95	70	98
RPR	86	100	98	75	98
USR	80	100	94		98
TRUST	85	100	97		97
Specific treponemal antibody tests					
MHA-TP	76	100	96	95	99
FTA-ABS	84	100	100	96	97
FTA-ABS double-staining	80	100	100		98
The values for sensitivity are the mean values in CDC (Centers for Disease Control and Prevention) tests. The values for specificity are mean values in nonsyphilis populations in CDC tests.					

after the chancre appears in primary syphilis. Titers are highest in secondary syphilis. The prozone phenomenon occurs in 2% of sera; undiluted sera give negative results because of antibody excess or the presence of blocking antibodies, or both. The titer slowly declines after the 1- to 4-week period following the appearance of the chancre, and it may spontaneously become negative in some cases of late latent syphilis and neurosyphilis.

The VDRL Slide Test is widely used and requires the microscopic demonstration of antigen-antibody flocculations in heat-inactivated serum.

The unheated serum reagin (USR) test is similar to the VDRL test but does not require preheated serum, because the antigen has been stabilized.

The rapid plasma reagin (RPR) test and tuluidine red unheated serum test (TRUST) use either charcoal or red paint pigment added to the USR reagent to enhance visualization of the antigen-antibody flocculations. The flocculations are visible macroscopically.

Biologic false-positive reactions to these tests occur in a wide variety of conditions.[32] They can be subdivided into acute reactions, lasting for 6 months or less, and chronic reactions (Table 75.4).

Specific treponemal antibody tests

Specific treponemal antibody tests are used for confirmatory testing. They detect antibodies to antigenic determinants of treponemes. They are qualitative procedures and are not helpful in assessing treatment responses; once positive, they tend to remain positive for life, irrespective of treatment. They are used to differentiate true-positives from false-positives in the standard nontreponemal antibody tests.

The fluorescent treponemal antibody absorption (FTA-ABS) test and the FTA-ABS double-staining (FTA-ABS DS) test are both indirect immunofluorescent tests. The double stain test employs a fluorochrome-labeled counterstain for *T. pallidum* and an antihuman IgG conjugate labeled with tetramethyl rhodamine isothiocyanate to detect antibody in patient serum. False-positive results may occur in about 1% of sera; possible causes include technical error, Lyme borreliosis, pregnancy, genital herpes, alcoholic cirrhosis and connective tissue diseases such as systemic lupus erythematosus and scleroderma.

The microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP) detects passive hemagglutination of erythrocytes sensitized with ultrasonicated Nichol's strain *T. pallidum*. In many laboratories, the TPHA has been replaced by the similar *T. pallidum* particle agglutination (TPPA) test but uses gelatin particles rather than erythrocytes as the carrier. It is more sensitive than the FTA-ABS.

TABLE 75-4 -- Causes of biologic false-positive cardiolipin tests.

CAUSES OF BIOLOGIC FALSE-POSITIVE CARDIOLIPIN TESTS		
	Acute	**Chronic**
Physiologic	Pregnancy	Old age
Spirochete infections	Leptospirosis	
	Relapsing fever	
	Rat-bite fever	
	Lyme disease	
Other infections	Varicella zoster	Lepromatous leprosy
	Herpes simplex	Tuberculosis
	Infectious mononucleosis	Lymphogranuloma venereum
	Cytomegalovirus	Malaria
	Toxoplasmosis	Kala-azar
	Viral hepatitis	Trypanosomiasis
	HIV seroconversion illness	Tropical spastic paraparesis
	Mycoplasma infection	HIV or AIDS
	Malaria	
	Other acute viral or bacterial sepsis	
Vaccinations	Smallpox	
	Typhoid	
	Yellow fever	
Autoimmune diseases		Systemic lupus erythematosus
		Polyarteritis nodosa
		Rheumatoid arthritis
		Sjögren's syndrome
		Primary biliary cirrhosis
		Hashimoto's thyroiditis
		Autoimmune hemolyic anemia
		Idiopathic thrombocytopenic purpura
Other		Malignancy
		Malnutrition
		Injecting drug use

Treponemal enzyme immunoassay (EIA) commercial tests were initially designed as confirmatory tests for syphilis. Serum is added to microwells coated with a treponemal antigen. After incubation, an enzyme-labeled antihuman immunoglobulin conjugate and enzyme substrate are added to detect antigen-antibody reaction. The test can be modified to detect specific IgM antibody.

Treponema pallidum Western blot is available in some research laboratories and has similar sensitivity and specificity to those of the FTA-ABS test.[33] The presence of antibodies to the immunodeterminants with molecular weights 15.5, 17, 44.5 and 47kDa appears to be diagnostic for acquired syphilis. When an IgM-specific conjugate is used, the test has value in the diagnosis of congenital syphilis.

There are many commercial tests in any given format whose performance characteristics vary.

Guidelines for serologic screening

Guidelines have been developed for serologic screening for syphilis. [34] Recent studies suggest that a treponemal EIA used as a single test is an appropriate alternative to the combined VDRL-RPR and TPHA screen. It has a higher specificity than the FTA-ABS. The test also has advantages of automated or semiautomated processing and objective reading of results, and it can be interfaced with laboratory computer systems to allow electronic laboratory report generation.

Screening can be performed by either EIA or the combined VDRL-TPHA. Positive results are confirmed with a treponemal test of a different type. It is essential to

confirm the presumptive serologic diagnosis of syphilis on a second patient specimen.

Indications for examination of cerebrospinal fluid

Indications for examination of cerebrospinal fluid (CSF) in syphilis include:

- neurologic, ophthalmic or auditory symptoms and signs;
- other clinical evidence of active infection — aortitis, gumma, iritis;
- treatment failure;
- HIV infection;
- serum nontreponemal titer of more than 32 if duration of syphilis is more than 1 year; and
- non-penicillin-based treatment regimen planned.

The typical CSF findings of neurosyphilis consist of:

- moderate mononuclear pleocytosis (10–400 cells/ml),
- elevated total protein (0.46–2.0g/l), and
- positive CSFVDRL.

The CSFVDRL is highly specific, and false-positive results are rare in the absence of blood contamination. A negative CSFVDRL does not exclude neurosyphilis, although nontreponemal serologic tests usually remain positive in both serum and CSF in such cases.

Typical neuroimaging findings have been described for neurosyphilis. [35]

Evaluation of neonates for congenital syphilis

It is recommended that the following investigations be carried out in a child born to a seropositive mother if there has been no documented completion of treatment at least 4 weeks before delivery, if a nonpenicillin regimen was administered or if relapse or re-infection is suspected:

TABLE 75-5 -- Treatment of acquired syphilis.

TREATMENT OF ACQUIRED SYPHILIS			
Stage	**CDC-recommended regimens**	**Other penicillin regimens**	**Alternative regimens**
Early syphilis (primary, secondary, early latent of less than 2 years' duration)	Benzathine penicillin G 2.4 million units im single dose	Aqueous procaine penicillin 600,000–900,000 units im daily for 10 days	Tetracycline hydrochloride 500mg po q6h daily for 15 days
			Doxycycline 100mg po q12h for 15 days
			Erythromycin 500mg po q6h for 15 days
			Ceftriaxone 1g im daily for 10 days
Late syphilis (latent syphilis of more than 2 years' duration, cardiovascular, gummatous syphilis)	Benzathine penicillin G 2.4 million units im weekly for 3 weeks	Aqueous procaine penicillin 600,000–900,000 units im daily for 15 days	Tetracycline hydrochloride 500mg po q6h for 30 days
			Doxycycline 100mg po q12h for 30 days
Neurosyphilis	Aqueous crystalline penicillin G 3–4 million units iv q4h for 10 days	Aqueous procaine penicillin G 2.4 million units im daily for 10 days plus probenecid 500mg orally q6h for 10 days	No proven effective alternative — patients allergic to penicillin should be desensitized
	plus benzathine penicillin G 2.4 million units im weekly for 3 weeks	plus benzathine penicillin G 2.4 million units im weekly for 3 weeks	

- physical examination for stigmata of congenital syphilis,
- radiography of long bones for evidence of periostitis, and
- examination of the CSF.

Infection of the neonate is also suggested if the serum nontreponemal antibody titer is four or more times more than the mother's, or if specific IgM treponemal antibody tests are positive. Passively transferred maternal IgG antibody can persist in the infant's serum for up to 12 months.[36]

MANAGEMENT

Current treatment regimens are based on over 50 years of clinical experience with penicillin, expert opinion and open clinical studies rather than on randomized clinical trials.[37] [38] Many antibiotics, with the notable exceptions of the aminoglycosides and sulfonamides, have some treponemicidal activity, and their administration for other conditions may abort or modify the natural history of syphilis.[39]

Parenteral penicillin G is the preferred drug at all stages of syphilis. The preparations used, the dosage and the duration of treatment depend on the clinical stage and disease manifestations (Table 75.5). Adequate treatment requires the maintenance of serum concentrations in excess of 0.03 units/ml for at least 10 days.[40] A single intramuscular dose of 600,000 units of aqueous procaine penicillin gives an effective serum concentration for at least 24 hours; in comparison, a single intramuscular dose of 2,400,000 units of benzathine penicillin G maintains effective levels for about 2 weeks.

In patients who are hypersensitive to penicillin, regimens based on tetracycline, doxycycline, erythromycin, ceftriaxone and chloramphenicol have all been successfully used to treat syphilis; however, success is less assured than with penicillin. Azithromycin, given in dosages of 500mg daily for 7 days, has recently been successful but experience is limited.

Desensitization of penicillin-allergic patients is recommended for the treatment of pregnant women and neurosyphilis because parenteral penicillin G is the only treatment with documented efficacy in these situations.

Jarisch-Herxheimer reaction

The Jarisch-Herxheimer reaction is an acute febrile reaction that occurs in many patients within 24 hours of commencing treatment. It is mediated by cytokines. The fever may be accompanied by headache, myalgia, bone pains and an exacerbation of skin lesions. It must be differentiated from penicillin allergy. Patients should be advised that it may occur. Symptoms may be controlled by antipyretics.

In pregnant women the reaction may induce early labor or cause fetal distress. In late neurosyphilis and cardiovascular syphilis, the Jarisch-Herxheimer reaction can be more serious and may be associated with life-threatening sequelae. Many clinicians advocate a short course of corticosteroids to lessen its effects in these patients; one such regimen is to prescribe oral prednisone (prednisolone) 30–60mg daily for 3 days, beginning syphilis treatment on the third day, and then tailing off the

corticosteroid course by reducing the daily dosage by 10mg each day during the succeeding week.

Treatment of syphilis in HIV-seropositive patients

It is recommended that CSF examination be performed in all patients with syphilis who are seropositive for HIV and that a penicillin-based regimen appropriate for neurosyphilis, regardless of the stage of infection, be administered.[41] Because *T. pallidum* can persist in the central nervous system in spite of adequate antibiotic treatment, some clinicians have recommended that chronic maintenance treatment be administered after initial formal treatment. [42] Erythromycin has been reported to be ineffective. Close follow-up is essential, and CSF examination may be repeated after 6 months.

Treatment of congenital syphilis

The optimal treatment of congenital syphilis is unknown. Regimens that have been recommended for early congenital syphilis are intravenous crystalline penicillin G 50,000 units/kg every 8–12 hours for 10–14 days, or intravenous aqueous procaine penicillin G 50,000 units/kg once daily for 10–14 days. For infants with normal CSF findings, intravenous benzathine penicillin G 50,000 units/kg in a single dose has also been successful.[43] In children with late congenital syphilis presenting after 2 years of age, regimens should be the same as those recommended for late acquired disease.

Management of sexual contacts

It is recommended that attempts be made to identify, trace and offer further investigation to at-risk sexual contacts. In early syphilis, these are those contacts occurring within 3 months plus the duration

of symptoms for primary syphilis, within 6 months plus the duration of symptoms for secondary syphilis and within 1 year for early latent disease. All long-term partners of patients with late syphilis should be offered investigation.

Many clinicians recommend presumptive treatment of all sexual contacts within the 90-day period preceding patient presentation of early syphilis if serologic test results are not immediately available and if follow-up cannot be assured.

Follow-up

Follow-up for clinical and serologic assessment should be done at 3, 6 and 12 months after the completion of treatment in early syphilis. Recurrence is due more often to re-infection than to relapse.

In late latent or tertiary benign syphilis, a 2-year follow-up is adequate. Quantitative nontreponemal serologic testing are repeated at 3 and 6 months, and each 6 months after that. Follow-up of cardiovascular and clinical neurosyphilis should be for life.

Treatment failure is suggested by a 4-fold increase in titers, less than a 4-fold decrease in pretreatment titers within 12–24 months, and development of symptoms or signs attributable to syphilis. All treatment failures require CSF examination.

In neurosyphilis, it is usual to repeat the CSF examination every 6 months until the cell count has become normal. There is a slower response of the CSFVDRL and total protein. Retreatment should be considered if the cell count shows an inadequate response or if all of these parameters have not returned to normal by 2 years. In cases of serologic or clinical relapse, retreatment with double penicillin doses is recommended.

Prognosis

Cure rates with initial treatment of early syphilis are better than 95%. The long-term outcome of adequately treated cases is excellent. In late syphilis, infection can usually be arrested, although some treponemes may persist in less accessible sites (e.g. the eye and nervous system). As long as immune function is normal, this persistence of treponemes rarely has clinical sequelae. The outlook for HIV-positive and other immunocompromised patients appears to be less assured; however, long-term studies in these patients are needed.

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Chapter 76 - Genital Herpes

Diane Goade

Genital herpes is one of the most common sexually transmitted diseases. Of these, the agents of genital herpes — herpes simplex virus (HSV) type 2 and, less commonly, HSV type 1 — are among the most frequently encountered human pathogens.[1] [2]

EPIDEMIOLOGY

Genital herpes infections are common, with estimates of 500,000–700,000 cases of symptomatic first episodes/year in the USA.[3] [4] [5] [6] [7] Humans are the natural reservoir for HSV and virtually all cases of genital herpes are sporadic, acquired via person-to-person transmission. There are no reported epidemics of genital herpes.[2]

Herpes simplex virus infections have a worldwide distribution, with seroprevalence to either HSV-1 or HSV-2 approaching 90% in some age and sex groups.[3] [4] [5] [6] [7] Herpes simplex virus-1 infection is common early in life, typically as orolabial 'cold sores' with antibodies appearing in childhood and prevalence increasing with age; HSV-2 antibodies increase in prevalence with increasing age after the onset of sexual activity. Approximately one in every 3–4 adults in the USA is seropositive for HSV-2.[3] [4] [5] [6] [7] Herpes simplex virus-2 seroprevalence rates in the USA differ for some racial, sex and ethnic groups. Women acquire HSV-2 infections more readily than men and overall have a higher seroprevalence rate.[6] [8] Other risk factors for genital herpes include lower socio-economic status, increased number of sexual partners, African-Caribbean race and Hispanic ethnicity.[4] [5] [6] [7] The highest prevalence of HSV-2 antibodies is among commercial sex workers and up to 70% of prostitutes in the USA have the infection. The lowest HSV-2 prevalence is in sexually abstinent groups, including nuns, where seroprevalence is 3% or lower.[4] [5] [6] [7]

The incidence of seroconversion is approximately 5–10%/year when discordant couples are followed longitudinally. Among HSV-naive females with male partners who have the infection, seroconversion is as high as 15–30%/year. However, when the female partner has the infection first, less than 5% of male partners seroconvert/year.[8]

After infection with an HSV, antibodies to HSV-1 and HSV-2 type common antigens provide partial protection to infection with the counterpart virus. In prospective studies, women who have HSV-1 infection have a 5–20%/year lower rate of seroconversion to HSV-2 than women who do not have HSV infection.[8] Seroconversion to HSV-1 during childhood has decreased, particularly in upper and middle class socio-economic groups in the Western developed countries, whereas symptomatic infection with HSV-2 during adulthood has increased and first visit for medical care of genital herpes remains on the rise.[4] [5]

PATHOGENESIS

Herpes simplex viruses are large enveloped DNA viruses with a diameter of approximately 150nm, a dsDNA core, an icosahedral capsid composed of 162 capsomers, an amorphous tegument layer and a lipid envelope. There are 11 different glycoproteins projecting from the envelope that are crucial for virion-cell surface attachment and cell-to-cell spread (Fig. 76.1 ; see also Chapter 215). [1] [9]

Herpes simplex viruses are spread by direct contact, including contact with infected secretions:

- HSV-1 is typically spread through close contact with infected oral secretions, and genital herpes due to HSV-1 is usually due to oral—genital contact; and
- HSV-2 is primarily spread through intimate contact with infected genital secretions and tissues.

Intact skin is fairly resistant to virus infection, but abraded skin or mucous membranes are more susceptible.

The virus attaches to the cell surface and the viral envelope fuses with the cell membrane using a specific cellular receptor. Virion—cell surface attachment and virus intracellular penetration are mediated by viral surface glycoproteins. The viral nucleocapsid is released into the cytoplasm where it is transported to the nuclear pores of the cell. Following viral DNA replication and gene expression in the cell nucleus, the replicated nucleocapsid is assembled and buds through the nuclear membrane, acquiring an envelope. The enveloped nucleocapsid is translocated across the cytoplasm and cell membrane, acquiring surface glycoproteins at both the nuclear membrane and cell surface.[1] [9]

Herpes simplex virus then infects and replicates in parabasal and intermediate skin cells. Replication results in lysis of the infected cell. Infection may spread locally by direct cell-to-cell invasion or to more distant sites via sensory nerve pathways. As virus replication spreads to involve the local autonomic and sensory nerve endings, retrograde transmission of virions (or possibly nucleocapsids) occurs with transport of virus particles to the regional sensory ganglia. Transient virus replication may occur in the ganglia at this point. From the sensory ganglia, antegrade virion migration along sensory nerves allows viral spread to other sites. By this method of spread, crops of herpetic lesions may arise at nonadjacent sites such as the thighs or buttocks. Virus replication is associated with cell lysis, cell destruction and local inflammation of all tissues except the sensory ganglia.[2] [10] [11]

Central nervous system disease may occur as an aseptic meningitis with primary HSV-2 or as a necrotic focal encephalitis with HSV-1 via spread through the cribriform plate to the temporal horns. Recurrent aseptic meningitis has been described, but is rare. Rarely, hematogenous dissemination may occur with visceral organ involvement, most commonly in immune compromised patients.[2]

Immune response

Both cellular and humoral host immune responses appear to be elicited by genital herpes infection.[9] [10] [11] [12] [13] [14] [15] [16] The lysis of infected epithelial cells results in local inflammation, macrophage recruitment and T-cell activation. In a murine model, there is induction of natural killer lymphocytes. Lymphocyte activation results in the appearance of antibodies, including virus-neutralizing antibodies and antibodies that provide passive protection against infection when given to animals who are then challenged with virus.[1] [9] T-cell responses, including cytotoxic T-cell responses, appear to be crucial in limiting

Figure 76-1 The herpes simplex virion. This consists of a dsDNA core surrounded by a capsid, an amorphous tegument layer and a lipid envelope with numerous glycoprotein spikes. The overall diameter is 150–200nm. Virus replication takes place within the nucleus of the infected cell. The envelope is gained as the virion passes through the nuclear membrane. Replication of virus within the host cell results in cell lysis and destruction. Latent virus do not cause neural cell lysis within ganglia.

and clearing disease.[13] Murine models also indicate that the degree of macrophage function at the site of local invasion may contribute to limiting the spread of infection.[1]

Cytokine induction is less well described. In cell culture models, HSV-infected cells express the cytokines interleukin-2, tumor necrosis factor a and interferon-?.[13] Peripheral blood mononuclear cells have been shown to produce interferon-a within hours of exposure to HSV virions.[11] Individuals with limited cellular immunity, including those at the extremes of life, patients with AIDS and bone marrow transplant recipients, tend to have prolonged severe disease with extensive tissue involvement and a higher rate of dissemination.[14]

Human humoral immune responses to HSV include the rapid generation of IgM antibodies following infection, with the appearance of detectable IgG and IgA antibodies approximately 10–14 days later. Antibodies appear first to certain structural proteins, and then sequentially to the various surface glycoproteins. Seroconversion to all virus antigenic determinants after infection may require months as measured by Western blot.[5] [12] [15] Lifelong virus neutralizing antibodies and antibody-dependent cellular cytotoxic antibodies are detectable approximately 1 month after infection. Lack of antibody-dependent cellular cytotoxic antibodies has been correlated with a poor clinical outcome and may be a factor in severe disease in the newborn.[1] [16] [17]

Histology

The characteristic lesion of HSV infection is an erythematous macular or papular lesion, which progresses to a thin-walled vesicle on an erythematous base. Histologically, HSV infection is characterized by edema, multinucleate giant cells and Cowdry type A intranuclear inclusions. The inflammatory reaction present within HSV lesions consists of a mononuclear cell infiltrate of predominantly $CD4^+$ T cells (Fig. 76.2).[18]

Latency

Lifelong latency is a unique property of the herpesviruses and is characterized by persistence of virus or viral DNA in aquiescent state in sensory and autonomic ganglia.[1] Viral transcription is limited to three latency-associated transcripts[1] and antiviral medications are unable to eradicate latent virus.[1] [2] [9]

Periodic recurrences of symptomatic disease are due to reactivation of latent virus. Latent virus begins to replicate and form active virus particles. The virus particles are transported by antegrade axonal flow from the ganglion to the epithelium in the genital tract. Following reactivation, fully infectious virus may be detected in cutaneous lesions at the site of recurrence.

Reactivation of latent virus tends to be site and virus specific. HSV-2 reactivates much more readily from the sacral ganglia than

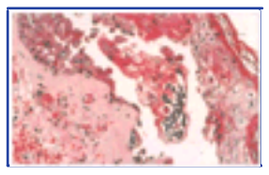
Figure 76-2 Histology. Section of human skin showing typical HSV virus effects: multinucleated giant cells (arrowhead) and intranuclear inclusion (arrow).

HSV-1 and HSV-1 reactivates more readily from the trigeminal ganglia.[2] [9] [19] How the virus remains latent within the ganglia and how reactivation occurs are not fully understood. In the epithelial cell, actively replicating virus DNA exists in a circular form. During latency the viral DNA assumes a linear configuration and latent viral DNA is episomal. Latency is not a wholly quiescent period, however, as some herpes simplex genes thought to be regulatory transcripts are expressed during this time.[1] [2] [9] Sequences within the herpes simplex genome specify 'latency-associated transcripts', which may be important in reactivation of virus replication. These sequences are highly divergent between HSV-1 and HSV-2 and may account for the preferential reactivation of virus type with its corresponding ganglion following latency.[2] [9] [19]

Virtually all recurrent genital herpes is due to reactivation of latent infection rather than to reinfection.[20] Several events may contribute to reactivation including trauma to the ganglia, immunosuppression, UV light, fever and possibly sexual activity.[2] [19]

PREVENTION

The only proven prevention strategy is avoidance of skin-to-skin contact when HSV is present in the genital tract. This includes avoidance of close physical contact during clinically symptomatic outbreaks when virus is present in high concentrations. Most patients can recognize symptomatic clinical outbreaks even when their symptoms are 'atypical' or mild and can avoid intimate contact during this time.

Frequent, intermittent aymptomatic or subclinical shedding of low titers of virus from the genital tract occurs in both men and women[21] and accounts for most cases of the transmission of genital herpes.[21] [22] [23] There is no current method to predict when asymptomatic virus shedding is occurring. It has been demonstrated that use of suppressive aciclovir decreases both the number of days of virus shedding and the quantity of virus shed.[24] Studies are under way to address the question of whether decreased shedding decreases transmission risk. Preliminary data indicate a 50% decrease in the amount of new disease among HSV discordant monogamous couples when the partner with HSV is on suppressive therapy (personal communication, L. Corey, 42nd ICAAC).

Vaccines

Protective vaccines may become important preventive options. The increasing incidence of genital herpes, the large number of HSV-2-seropositive individuals who are unaware of their infection status and the risk of disease transmission from asymptomatic shedding underscore the need for an effective vaccine.[22] [23]

In vaccine studies, various animal models, including a guinea-pig vaginal HSV-2 model and a mouse footpad inoculation or scarification model, indicate that certain vaccine candidates provide protection from acquiring infection or ameliorate disease.[25] [26] When studied in humans, these vaccine candidates have not been as effective as in the animal models. In a previous controlled trial in discordant couples, an inactivated HSV-2 glycoprotein vaccine failed to provide protection.[27] Recently, human trials have been completed or phase 3 trials are under way to evaluate recombinant surface glycoprotein vaccines.[28] [29] No vaccine for HSV-2 is currently licensed.

Barrier contraception

Barrier contraception such as latex condoms can reduce the risk of disease transmission by decreasing contact with the partner's infected skin or mucous membranes or infected secretions. Because herpesvirus may be present outside the portion of genital tract protected by barrier contraception, condom use is not infallible.

CLINICAL FEATURES

Genital herpes is a lifelong infection characterized by an initial infection followed by latency, and frequent recurrences. Infection may range from severe and symptomatic to asymptomatic. Herpes simplex virus disease is defined as:

- primary disease when a person lacking any antibodies to HSV acquires an infection with HSV-1 or HSV-2; and
- nonprimary first-episode disease when an individual with pre-existing antibodies to one serotype, typically HSV-1, acquires disease with the second type.

The presence of pre-existing type common antibodies and cell-mediated immune responses modifies the course of disease and so first-episode nonprimary disease is typically less severe than primary disease.[2] [9]

Genital herpes may recur from none to six or more times/year. Recurrent disease tends to be milder than symptomatic first-episode disease, with fewer vesicles, less discomfort and a shortened duration of symptoms.[2] [9]

Transmission of HSV-2 to a sexual partner or a neonate may be the first indication of the presence of genital herpes infection in an asymptomatic source partner.[23] [30] The time and source of disease acquisition are often difficult to prove conclusively because:

- genital herpes can have a prolonged asymptomatic phase after acquisition and may be transmitted via asymptomatic shedding; and
- clinically silent or unrecognized disease is common.

Primary first-episode genital herpes

Primary genital herpes is most often seen as a disease of sexually active teenagers and young adults. Following exposure, a clinically silent incubation period lasts for 2–7 days. Onset of clinically apparent disease may be heralded by fever, headache and local genital pain and burning. Patients may appear to be systemically ill. In general, females tend to have more severe disease than males, with estimates of urinary retention occurring in approximately 10% of females. Up to 25% of females manifest symptoms of aseptic meningitis.[2] [9]

The characteristic painful lesions in the genital area initially present as erythematous macules, which then progress to vesicles on an erythematous base, pustules, ulcers and finally to crusts (Fig. 76.3). Each crop of lesions takes an average of 8 days to heal completely, and successive crops of lesions may arise during the course of the disease. Untreated genital herpes may require weeks to resolve, averaging 3 weeks to cessation of lesions. Healing is usually complete, although particularly severe or large ulcers may result in scarring.[2]

Figure 76-3 Primary HSV. Multiple painful, erythematous, ulcerating lesions on shaft and head of penis. Exudative crust is visible over one lesion.

In the male, lesions typically appear on the penis and glans penis (Fig. 76.3). In females, lesions may be present throughout the genital tract including the perineum, vulva, labia, perianal regions and buttocks. The vesicles are typically distributed bilaterally in primary disease. Cervical involvement is usually present and may escape detection if limited external disease is present and a complete pelvic examination is not performed. A vaginal discharge may accompany cervical and, less commonly, vaginal herpes. In either sex, the perianal and rectal mucosa may be involved, especially if exposure was due to rectal intercourse. Tender bilateral inguinal adenopathy is generally present. Vesicles may also be present on the thighs.

If herpetic involvement of the urethra occurs, severe dysuria may result. Sacral radiculopathy may occur during the course of primary genital herpes, with urinary retention, neuralgias, dysesthesia and diminished rectal tone. Tenesmus and rectal pain may be present with rectal herpes.[2] [9]

In HSV-naive individuals, primary genital disease due to either HSV-1 or HSV-2 is clinically indistinguishable, making identification of infection by genital cultures and virus typing important for diagnosis. Atypical symptoms are quite common, with up to 30% of genital herpes presenting as paresthesias or urinary retention rather than the classically described vesicular genital lesions. [2] [9] [31]

Nonprimary first-episode disease

Nonprimary first-episode genital HSV-2 infection is typically intermediate in severity when compared with that of primary and recurrent genital herpes.[2] Untreated disease lasts for approximately 10–14 days, with fewer lesions and fewer crops of lesions than are typical for primary disease. Systemic symptoms, including fever, are less common than in primary genital herpes, and virus can be cultured less frequently from the cervix and genital tract.[2] [9]

Recurrent genital herpes

In approximately 50% of patients, a prodrome of symptoms heralds the onset of recurrent disease. Commonly reported prodromal symptoms include local burning and itching, tingling and dysesthesia. Patients may have their own recognizable cluster of symptoms. The prodromal symptoms may occur without the development of noticeable lesions or may be followed by the typical vesicular eruption lasting 6–10 days. In contrast to the widely distributed, bilateral lesions of primary genital herpes, the crop of vesicular lesions in recurrent disease tend to be localized and unilateral. Lesions are typically present on the vulva in women and the glans penis and penile shaft in men, although lesions may also present on the thigh or buttocks, in the rectum and at other sites. Paresthesia, dysesthesia, local edema, pain, regional adenopathy and local swelling may accompany recurrent cutaneous herpes.[2] [9]

Recurrence rates vary widely from none to six or more episodes/year, with some individuals having one or more recurrences each month. Recurrences may be triggered by sexual activity, with high rates of recurrent disease in commercial sex workers. Immunocompromised patients may experience frequent, prolonged and severe recurrences.[32] Genital herpes due to infection with HSV-2 reactivates much more readily than genital herpes due to HSV-1. Recurrent genital herpes has been reported in over 80% of individuals following primary genital HSV-2, whereas recurrence in patients who had HSV-1 primary genital herpes was less than 50%.[2] Recurrence of genital herpes averages 0.33/month with HSV-2 disease, but only 0.02/month for HSV-1 genital disease.[19]

Asymptomatic shedding

Landmark studies have demonstrated both by culture and by polymerase chain reaction (PCR) that herpesvirus is frequently present in the genital tract when lesions are absent and skin is intact, or when small or unrecognized lesions are present (Fig. 76.4). [33] [34] During periods of asymptomatic virus shedding, virus titers are typically lower than during a symptomatic acute outbreak. When detected by virus culture, asymptomatic shedding occurs on an average of 4% of days, whereas women with primary genital herpes may shed virus on up to 17% of days in the first few months following their primary outbreak. [21] [22]

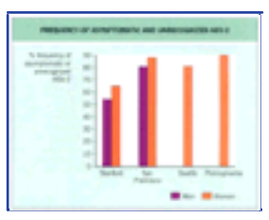

Figure 76-4 Frequency of asymptomatic and unrecognized HSV-2 in four major US cities.[4] [31] [37] [38] [39]

Uncommon sites of infection

Although the majority of herpes virus infections occur in the genital and orolabial regions, cutaneous disease may occur in virtually any site, causing localized to widespread disease. Genital herpes may present as:

- rectal herpes, with symptoms of proctitis, including rectal discharge, anal pain and pain on defecation;
- sacral paresthesia with urine retention; and
- painful anal fissures.

Males with rectal herpes may also present with impotence as a manifestation of neurologic involvement with HSV.[2] [9]

Neonatal herpes is a rare complication of genital herpes resulting from exposure of a newborn to HSV-2 from the maternal genital tract (see Chapter 65). Acquisition may occur during maternal primary genital disease via exposure to infectious virus in the birth canal during labor and delivery. Less commonly, the fetus acquires the infection during gestation. The incidence of neonatal herpes is approximately 1/3500 live births. An increased incidence is noted in preterm infants. Maternal acquisition of genital herpes during the third trimester of pregnancy increases the risk of neonatal transmission more than 10-fold compared with the risk of transmission from long-standing maternal HSV infection.[30] [35]

Disease in the immunocompromised host

Herpes simplex viruses may be significant pathogens in immunocompromised hosts (see Chapter 112 and Chapter 125). Patients who have HIV disease may have both frequent and increased duration of recurrences of HSV mucocutaneous disease (see Chapter 125). Delayed healing and continued virus replication lead to local spread and large, painful ulcers extending through the cutaneous and subcutaneous layers. In patients with $CD4^+$ lymphocyte count below 100 cells/mm^3 , treatment of

genital herpes may be complicated by emergence of aciclovir-resistant HSV strains.[2] [9] [32] [36]

DIAGNOSIS

Despite an overall increasing awareness of sexually transmitted diseases, recognition of genital herpes by both the patient and the health care provider is often limited, and the majority of infections are undiagnosed or unrecognized (Table 76.1).[4] [5] [31] Diagnosis of herpes simplex disease is important:

- to establish presence or absence of infection;
- to guide treatment considerations; and
- to allow the health care practitioner an opportunity to intervene in disease transmission.

Diagnosis may be accomplished by serologic and microbiologic methods.

Viral culture

Viral culture and typing is the preferred method for identifying infection, as it is the most accurate and most widely available diagnostic modality. It is recommended for all patients who have not had previous virologic confirmation of disease, including those with first-episode genital disease. Culture provides confirmation of the clinical diagnosis, and typing of virus predicts subsequent recurrence patterns. Culture of body fluids, vesicles and tissues from patients with symptomatic disease yields positive results in most cases when obtained early in the disease. Cervical cultures are positive in up to 90% of women with primary or first-episode genital herpes during the first week of symptoms, with decreasing rates of virus recovery during disease resolution. Virus may be recovered from mucocutaneous ulcers, cutaneous vesicles, CSF, rectum, urethra, urine and elsewhere in the genital tract. [2] [40]

In recurrent disease, virus may not be present in readily detectable quantities. Culture of multiple sites such as urethra, rectum and

TABLE 76-1 -- Differential diagnosis of genital herpes.

DIFFERENTIAL DIAGNOSIS OF GENITAL HERPES	
Disease	**Diagnostic clues**
Syphilis	Lesions usually single and painless; positive darkfield microscopy
Chancroid	Nonindurated ulcers, positive bacterial cultures for *Haemophilus ducreyi*
Lymphogranuloma venereum	Constitutional symptoms follow onset of lesions; responds to doxycycline
Genital warts	Chronicity of lesions: no vesiculation; minimal pain
Fixed drug eruption	History; viral culture negative
Contact dermatitis	History; lack of systemic symptoms; no adenopathy
Trauma	History; lack of systemic symptoms; no adenopathy
Psoriasis	Chronicity of lesions; lesions elsewhere on body

genital tract improves the chance of virus recovery. Growth and typing of virus requires less than 5 days, and modified culture techniques that detect herpes antigens (the shell-vial assays) yield preliminary results in 1–2 days.[33] [40]

Serologic assays

Type-specific serologic diagnosis is important:

- for serosurveys to gauge prevalence rates;
- for identification of asymptomatic patients;
- in situations where culture is not feasible or available; and
- for patient counselling in scenarios of sexually transmitted disease and other public health clinics.

Herpes simplex antibodies are readily detectable following infection, but it may take several months for complete antibody development to all antigenic determinants. Differentiating HSV-1 from HSV-2 antibodies is more problematic due to the antigenic similarity of the viruses.[1] [9] [15] Antibody responses in general are broadly crossr-eactive between HSV-1 and HSV-2.[40] Antibodies to glycoprotein G are type specific and antibodies to portions of glycoprotein B are type specific. [41] Recently developed tests using glycoprotein G-based serologic assays are now commercially available and include both ELISA and immunoblot formats. Sensitivities for HSV-2 using gG antibody development range from 80% to 98%, and perform worst in low-risk groups where false positives are more common. Specificity of gG assays is greater than 96%. Older assays do not discriminate between HSV type 1 and 2 and have little to no practical value.[41] [42]

Other diagnostic techniques

The advent of the PCR allows amplification and detection of HSV gene segments even when present in virus copy numbers too low for detection by culture. Assays based on PCR are rapid and sensitive, but strict control measures are necessary to avoid false-positive results. Detection of HSV by PCR is most useful for herpes meningitis, encephalitis and other central nervous system infections that are not readily diagnosed by cerebrospinal fluid culture or would other-wise require brain biopsy for diagnosis. The sensitivity of HSV PCR approaches that of biopsy for the diagnosis of herpes encephalitis. HSV-2 can be detected by PCR in a high proportion of people with recurrent aseptic meningitis.[1] [9] [43] [44]

Use of PCR to detect and quantify virus sequences on the skin and in the genital tract in the absence of lesions has provided important information about the duration and amount of asymptomatic virus shedding as the virus copy number is often below the threshold

of detection by culture.[21] Neonatal herpes infection may also be diagnosed by PCR amplification of HSV gene segments in the serum and CSF.

MANAGEMENT

Currently, the practitioner has a choice of effective, safe and well-tolerated medications (Table 76.2). Antiviral therapy is effective:

- in the treatment of primary and first-episode genital herpes;
- for episodic treatment of recurrences; and
- for suppression of frequent recurrences.

Continuous antiviral therapy has also been shown to decrease viral shedding. Antiviral therapy has not yet been shown to affect latency or 'cure' genital herpes. Preliminary data are encouraging that continuous antiviral therapy may decrease, but not eliminate, the risk of transmission.

Pharmacologic agents (see also Chapter 205)

Aciclovir is the acyclic analogue of the nucleoside guanosine. In order to be active, it must first be phosphorylated by the virus-encoded

TABLE 76-2 -- Antiviral therapy.

ANTIVIRAL THERAPY

Medication	Indication	Dose, route	Side-effects
Aciclovir	Primary genital herpes	200mg po five times daily or 400mg po q8h for 10 days	Nausea
	Recurrent genital herpes	200mg po five times daily or 400mg q8h for 5 days	Nausea
	Suppression of frequent recurrences	400mg po q12h or 200mg po q8h	Nausea
	Severe or disseminated disease	5mg/kg slow iv q8h for 10 days	Reversible crystalline nephropathy, tremors
	Encephalitis	10mg/kg slow iv q8h for 10 days	Reversible crystalline nephropathy, tremors
Valaciclovir	Primary genital herpes	1g q12h for 10 days	Nausea
	Recurrent genital herpes	500mg q12h for 5 days	
	Suppression of frequent recurrences	500mg q12h or 1g daily	
Famciclovir	Primary genital herpes	250mg po q8h for 10 days	Nausea
	Recurrent genital herpes	125mg po q12h for 5 days	
	Suppression of frequent	250mg po q12h	
Foscarnet	Aciclovir-resistant herpes simplex virus	60mg/kg q8h, infuse over 2 hours for 10 days	Azotemia, seizures, hypo- or hyperkalemia and hypo- or hyperphosphatemia
Note that the topical aciclovir 5% ointment available in the USA is of little value in the treatment of genital herpes and is not recommended as effective therapy. Valaciclovir 1g daily and 500mg daily are both approved for chronic suppressive therapy, but the authors recommend 500mg q12h.			

enzyme, thymidine kinase, to aciclovir monophosphate. The monophosphorylated form is then further phosphorylated by cellular enzymes to the di- and triphosphate form. The active form, aciclovir triphosphate, inhibits the HSV-specific DNA polymerase and terminates the replicating DNA chain by competing with its analogue deoxyguanosine triphosphate. Aciclovir lacks the 3'-hydroxyl group necessary for subsequent phosphodiester linkages, so that extension of the replicating DNA chain is no longer possible.[34]

Aciclovir has proved to be a well-tolerated and effective medication with a wide margin of safety. Its safety and specificity result from the relative inability of cellular kinases to phosphorylate aciclovir to its active form and from the more potent inhibition of HSV DNA polymerase than human DNA polymerase by aciclovir triphosphate. Resistance to aciclovir occurs when HSV strains develop that lack thymidine kinase or, less commonly, by strains with an altered thymidine kinase or DNA polymerase.[34]

Aciclovir is currently available as an oral, topical and intravenous agent. Clearance is via the kidney, with approximately 90% of the drug excreted unchanged in the urine.[34] Side-effects for the oral and topical forms are minimal. Topical aciclovir may cause burning and is not approved for use on mucous membranes.[45] Oral aciclovir may cause nausea, especially with high doses. These symptoms are generally mild and resolve over time with continued use of the drug.[46] Intravenous aciclovir may cause local pain and phlebitis if drug extravasates during administration.[34] The most common significant side effect of intravenous aciclovir is a reversible nephropathy secondary to crystallization of aciclovir in the renal tubules. Administration of intravenous doses slowly over 1 hour decreases the incidence of nephropathy. Aciclovir nephropathy is rarely seen in normal adults, but is more common in the elderly and in those with underlying renal dysfunction. Intravenous aciclovir may rarely cause neurologic complications, including lethargy, delirium and tremors.[34]

Other therapeutic agents

Antiviral medications that have recently become available include valaciclovir, penciclovir and famciclovir.

Valaciclovir

Oral bio-availability of aciclovir is limited, with only 15–20% of the oral dose absorbed. Valaciclovir, a prodrug of aciclovir, was developed to increase the oral bio-availability and is absorbed at levels 3–5 times greater than those for aciclovir. Following absorption, valaciclovir is then hydrolyzed to aciclovir. Oral administration of valaciclovir can lead to serum levels of aciclovir approaching those following intravenous administration of aciclovir.[47]

Penciclovir

Penciclovir is also an acyclic nucleoside analogue and is available in oral and topical forms. Topical penciclovir is a treatment option for orolabial HSV-1 but has not been approved for use in genital herpes. Like aciclovir, the drug must first be phosphorylated by the HSV-encoded thymidine kinase to penciclovir monophosphate. Cellular kinases then phosphorylate the compound to the di- and triphosphate forms. Like aciclovir triphosphate, penciclovir triphosphate is an inhibitor of HSV DNA polymerase. Although penciclovir is phosphorylated by thymidine kinase much more readily than aciclovir, this benefit is offset by reduced activity against the viral polymerase compared with that of aciclovir. DNA chain termination is not a significant property of penciclovir triphosphate. The intracellular half-life of penciclovir triphosphate is much longer than that of aciclovir triphosphate. It is unclear still whether these differences have an effect on the effectiveness or safety of penciclovir compared with those of aciclovir.[48] [49]

Famciclovir

Famciclovir is an oral prodrug of penciclovir. Similar to valaciclovir, famciclovir was developed in an effort to increase oral bioavailability. Following ingestion, famciclovir is rapidly converted by deacetylation to penciclovir. Approximately 60–70% of the dose is excreted in the urine as penciclovir.[50]

Foscarnet

Foscarnet is a pyrophosphate analogue that inhibits viral DNA polymerase.[51] Because it does not require phosphorylation to become active, its efficacy is not dependent on the presence of HSV-specific thymidine kinases. As such, foscarnet is one of the few drugs available for the treatment of aciclovir-resistant herpes simplex due to thymidine kinase or thymidine kinase-altered strains. Foscarnet is only available as an intravenous preparation. The most common side-effects include nephrotoxicity, electrolyte abnormalities, seizures and penile ulcers. Saline hydration decreases the renal toxicity and is recommended to diminish the risk of azotemia. Foscarnet-resistant HSV strains have occasionally developed in immunosuppressed hosts.[52]

Cidofovir or (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine

Cidofovir is an acyclic nucleoside phosphonate antiviral agent currently used in the treatment of cytomegalovirus retinitis. Cidofovir is active *in vitro* against HSV and has activity against thymidine kinase-negative and thymidine kinase-altered HSV strains. Trials

TABLE 76-3 -- Drug comparison trials.

DRUG COMPARISON TRIALS				
Medications	**Indication**	**Authors**	**Results**	**Conclusions**

Aciclovir 200mg po five times daily for 10 days versus placebo	First-episode genital herpes	Corey *et al.*[58]	With po or iv aciclovir, pain decreased by 4 days, viral shedding decreased by 7 days, time to healing reduced by 7 days, 60% fewer new lesions; topical aciclovir of limited value	Oral aciclovir significantly effective in treatment of first-episode genital herpes
Famciclovir various doses po q8h versus aciclovir 200mg po five times daily	First-episode genital herpes	Loveless *et al.*[60]	Famciclovir 125, 250 or 500mg q8h equal to aciclovir 200mg five times daily each given for 10 days; famciclovir 250, 500 or 750mg q8h equal to aciclovir 200mg five times daily each given for 5 days	Famciclovir equal in efficacy to standard dose aciclovir and required less frequent dosing interval
Valaciclovir 1g po q12h po versus aciclovir 200mg five times daily po	First-episode genital herpes	File *et al.*[73]	Valaciclovir q12h doses as effective as aciclovir five times daily	Valaciclovir as effective as aciclovir, but required less frequent dosing interval
Valaciclovir 1g q12h po versus aciclovir 200mg five times daily po versus placebo	Recurrent genital herpes	Smiley *et al.*[65]	Time to healing equivalent for valaciclovir and aciclovir (115–116 hours) and shorter than with placebo (144 hours); duration of viral shedding also decreased in treated group	Treatment with valaciclovir or aciclovir significantly more effective than with placebo, and no difference between the two drug treatments (valaciclovir 500mg q12h now shown to be as effective as 1g q12h in a separate study)
Famciclovir 125, 250 or 500mg q12h po compared with placebo	Recurrent genital herpes	Sacks *et al.*[74]	Time to healing reduced by 1.1 days in treated group; significant reduction in shedding	Famciclovir effective in treatment of recurrent genital herpes
Aciclovir 400mg q12h po versus placebo	Suppression of frequent recurrences	Mertz *et al.*[69]	Mean number of recurrences 11.4/year for placebo group, 1.8/year for treated group; number free from recurrences for 1 year: 2% for placebo group, 44% for treated group	Aciclovir highly effective at suppressing recurrences; minimal side-effects, frequent high patient compliance and satisfaction with treatment
Famciclovir various doses po versus placebo	Suppression of frequent recurrences	Mertz *et al.*[75]	Time to first recurrence 82 days for placebo, greater than 120 days for treatment; 250mg q12h most effective and 78% of patients with no recurrences at 120 days versus 42% of those treated with placebo	Famciclovir 250mg q12h effective for suppressing frequent recurrences
Aciclovir 400mg q12h po versus placebo	Suppression of asymptomatic viral shedding	Wald *et al.*[24]	Five of 34 treated shed virus during therapy compared with 25 of 34 in placebo group; days shed were 6.9% with placebo, 0.3% with aciclovir	Aciclovir dramatically decreases asymptomatic viral shedding

of cidofovir in humans for the treatment of resistant genital herpes have not been completed. The primary toxicity of cidofovir at doses used to treat cytomegalovirus is nephrotoxicity. The doses of cidofovir necessary to treat aciclovir-resistant genital herpes have not been established.[53] [54]

Ineffective therapies

Ineffective therapies include BCG vaccination, topical betadine, topical vidarabine, topical idoxuridine and gammaglobulin. Topical therapy with foscarnet has been disappointing, with recent studies of treatment of recurrent genital herpes showing little to no benefit.[55] Lysine is a popular over-the-counter supplement purported to decrease the symptoms of genital herpes. Well-controlled clinical trials of lysine, including a double-blinded cross-over study, have failed to demonstrate efficacy.[56] ,57

General treatment guidelines

Antiviral therapy decreases both the duration of symptoms and viral shedding in genital herpes. Maximum benefit occurs when antiviral therapy is initiated promptly, ideally with the first prodromal symptoms with recurrent genital herpes. With the wide margin of safety of the oral antiviral medications and the need to initiate therapy promptly, it is appropriate to begin medication before receiving culture results or other confirmatory test results.

Aciclovir, famciclovir or valaciclovir are all appropriate agents for the treatment of genital herpes. For some indications, valaciclovir and famciclovir offer more convenient dosing regimens. However,

for many health care providers, generic aciclovir remains the drug of choice for the treatment of genital herpes due to the extensive clinical experience with the drug, its excellent safety and efficacy profiles, wide availability and lower cost (Table 76.3).

Treatment of primary genital herpes

Antiviral therapy is clearly of benefit in the treatment of primary genital herpes. When compared with placebo it has been shown to reduce median:

- duration of viral shedding by 7 days;
- duration of pain by 4 days; and
- time to healing by 7 days.[58]

Antiviral therapy does not prevent the establishment of latency or affect the likelihood and frequency of recurrences when compared with placebo.[59] Higher than recommended doses of antiviral medications are no more effective in the treatment of genital herpes and may be associated with an increased risk of nausea. [46] Treatment for longer than 10 days in the normal host does not improve outcome when compared with the standard regimen.

Primary or first-episode genital herpes may be adequately treated with a variety of oral regimens including aciclovir at doses of 200mg five times daily or 400mg q8h for 10 days. Alternatively, valaciclovir 1g q12h for 7 days or famciclovir 250mg q8h for 7–10 days is also effective treatment for primary genital herpes.[57] [60]

Individuals with severe disease or who are unable to take oral medications should be treated with intravenous aciclovir at doses of 5mg/kg administered over 1 hour q8h. Topical aciclovir is of little value in the treatment of primary disease.[58] The combination of oral and topical aciclovir offers no therapeutic advantage.[57] [61]

Oral famciclovir and oral valaciclovir have been compared with oral aciclovir for the treatment of first-episode genital herpes in recent clinical studies. Oral famciclovir in doses of 125,250 or 500mg q8h for 10 days has been shown to be as effective as aciclovir 200mg five times daily for 10 days.[57] [62] Valaciclovir 1g q12h was also as effective as aciclovir 200mg five times daily in the treatment of first-episode genital herpes. [57] [63] Neither famciclovir nor valaciclovir appears any more effective than aciclovir.

Episodic treatment of recurrent genital herpes

Recurrent genital herpes is generally milder and of shorter duration than primary disease, so the expected benefits of treating individual episodes with antiviral therapy are not as profound as those seen in primary disease. Episodic treatment of recurrent genital herpes with antiviral therapy results in only a modest decrease in disease severity, with a mean decrease in the duration of symptoms of 1 day or less.[62]

Aciclovir at a dose of 200mg five times daily or 400mg q8h for 5 days remains the recommended therapy.[64] Patient-initiated therapy at the time of onset of prodromal symptoms has been shown to be of significantly greater benefit than therapy delayed to initial lesion onset.[62]

Both valaciclovir and famciclovir have been evaluated for effectiveness in the treatment of recurrent genital herpes. Recent clinical trials comparing valaciclovir (1g

q12h) with either aciclovir (200mg five times daily) or placebo showed the following in both treatment arms when compared with placebo:

- decreased time to lesion healing;
- decreased duration of pain; and
- reduced duration of viral shedding.[65]

Oral famciclovir at doses of 125, 250 or 500mg q12h reduced viral shedding and duration of symptoms and reduced time to healing when compared with placebo.[66] The lowest effective dose, 125mg q12h for 5 days, is the currently approved treatment dose. Neither famciclovir nor valaciclovir appears to offer any therapeutic advantage over aciclovir.

The 5% topical aciclovir ointment available in the USA is not effective at reducing viral shedding, symptoms or time to healing, and is not licensed or recommended for treatment of recurrent genital herpes.[67] A different preparation available in Europe, a 5% topical aciclovir cream, has been shown to be effective and may be an appropriate alternative to oral therapy if available to the patient.[68]

Suppression of frequently recurring genital herpes

Chronic antiviral therapy has proven to be a safe and well-tolerated mechanism for suppressing symptomatic recurrences in patients with moderate to frequently (more than five episodes per year) recurring genital herpes.

Aciclovir

Aciclovir was the first antiviral agent evaluated for effectiveness in suppressing recurrent disease. In one study, patients with frequent recurrences had a reduction of recurrences from more than 12/year to an average of 1/year when treated with aciclovir 400mg q12h.[69]

Suppressive therapy is safe and well tolerated, with no long-term effects on sperm motility, no noted laboratory abnormalities and minimal side-effects. [69] [70]

Aciclovir has now been used for suppressive therapy for up to 10 years and has not been associated with development of tolerance or with any significant development of resistant strains in the normal host.[69] [71] [72] However, interruption of therapy at 1- or 2-year intervals is suggested to assess the frequency of episodes and need for suppressive therapy. It has recently been shown that suppressive therapy with aciclovir decreases the frequency of asymptomatic viral shedding in women.[24]

Famciclovir

Famciclovir is now licensed in the USA for suppressive therapy at a dose of 250mg q12h, the most effective dose tested in a dose-ranging study of famciclovir compared with placebo for suppression of frequently recurring genital herpes in women.[75] In this trial, once-daily dosing regimens were not as effective as the q12h dosing regimens at equal or higher cumulative daily doses. A subsequent 1-year trial in men and women also found that the 250mg q12h dosage was effective and well tolerated. At present, there are no results available of trials comparing the efficacy of suppressive therapy with famciclovir to therapy with valaciclovir or aciclovir.

Valaciclovir

Valaciclovir has also been used for suppression of genital herpes and has recently been licensed in the USA at a dose of 500mg or 1g orally daily for suppression of genital herpes in the normal host. The 500mg daily dose appears to be effective in persons with a history of 6–10 episodes/year. This regimen appears to be less effective than aciclovir 400mg orally q12h or valaciclovir 1g daily or 250mg q12h in patients with very frequent recurrences (10 or more episodes a year). Once-daily dosing with valaciclovir 1g appears to have acceptable efficacy in the normal host, but should not be used in the immunocompromised host because there is evidence of reduced efficacy in people who have HIV infection. Valaciclovir 1g daily or 250mg q12h and aciclovir 400mg q12h appeared equivalent in efficacy, even among people with frequent recurrences. For maximum efficacy, we prefer to use valaciclovir at a dose of 500mg q12h for suppression. This dose was found to be safe and was also significantly more effective than valaciclovir 1g orally daily in suppressing genital herpes in people who have HIV infection and a median $CD4^+$ lymphocyte count of 320 cells/mm^3 .[76]

Summary

In summary, there are no data at present suggesting that suppressive therapy with either famciclovir or valaciclovir at the licensed doses is more effective than suppressive therapy with aciclovir 400mg q12h. In addition, cost comparisons favor the use of generic aciclovir rather than the newer agents.

CONCLUSION

Genital herpes is one of the most commonly encountered sexually transmitted diseases. Its management requires:

- recognition of disease;
- patient education about the unique features of the disease, including latency and virus shedding; and
- judicious use of antiviral medications.

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Chapter 77 - Papillomavirus Infections

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INTRODUCTION

Human papillomavirus (HPV) is probably the most common sexually transmitted viral infection in humans. Infected persons are usually asymptomatic. Mild disease includes genital warts, verruga or cytologically evident dysplasia of the cervix or anus. Persistent anogenital infection is associated with advanced cervical neoplastic disease and invasive carcinoma of the anogenital region, most notably the cervix.[1] High-risk genotypes of HPV are likely to be responsible for a high proportion of carcinomas of the cervix, vagina, vulva, anus and penis worldwide.[2] Other types of HPV are associated with lower grade squamous lesions such as low-grade neoplasia and abnormal squamous cells of unknown significance.[3]

Nongenital types of HPV cause benign epithelial warts of the hands, feet and elsewhere, as well as a number of other dermatologic conditions not reviewed in this chapter (see Chapter 216). Infection with HPV may be a risk factor for squamous cell carcinoma of the head and neck.[4] [5] However, further research is needed to elucidate the full role of HPV in the development of nongenital tract cancers. 'Low-risk types' such as HPV-11 can nonetheless cause severe non-neoplastic disease including juvenile laryngeal papillomatosis characterized by laryngeal warts in children. While benign from an oncologist's viewpoint (hence the term 'low risk'), juvenile laryngeal papillomatosis can compromise the respiratory integrity of a child and may require repetitive surgical intervention. The focus of this chapter is diagnostic, epidemiologic, molecular and therapeutic issues in anogenital disease.

Human papillomaviruses

Human papillomavirus represents a family of DNA viruses with more than 142 related genetic types of which more than 90 have been fully sequenced (Table 77.1).[6] The genome consists of a circular double-stranded DNA about 8000 base pairs long and is encapsidated in an icosahedral protein coat with no membrane envelope.[7] Most HPV genotypes have a similar organization consisting of:

- a transcription and replication control region;
- an early region encoding proteins for replication, regulation and modification of the host cytoplasm and nucleus; and
- a late region encoding capsid proteins.[6]

While HPV is related to other nonhuman papillomaviruses, there is considerable virus-to-species specificity among other vertebrates, including mammals, birds, amphibians and reptiles. [7]

EPIDEMIOLOGY, PATHOGENESIS AND PATHOLOGY

Epidemiology

Human papillomavirus is a common infection among sexually active adults and adolescents.[8] Incidence and prevalence estimates for HPV infection are imprecise due to diagnostic limitations, subclinical cases, repeated and multiple infections and the phenomenon of inconsistent HPV types noted at separate clinical encounters. In the latter circumstance, one cannot be sure whether a new viral type

TABLE 77-1 -- Human papillomavirus types and associated diseases.[6] [7]

HPV TYPES AND HPV-ASSOCIATED DISEASES	
HPV-associated diseases	**HPV types**
Skin warts	1,2,3,4,7,10,26,27,28,29,41,48,57,60,63,65,75,76,77,78
Epidermodysplasia verruciformis benign lesions	3,5,8,9,12,14,15,17,19,20,21,22,23,24,25,36,47,49,50
Epidermodysplasia verruciformis squamous cell carcinoma	5,8,14,17,20,47
Periungual squamous cell carcinoma	16,34,35
Laryngeal papillomas	6,11
Oral focal epithelial hyperplasia	13,32
Squamous cell carcinoma (tonsil)	16,33
Anogenital warts	6,11,40,42,43,44,54,55,74
Low-grade anogenital intraepithelial neoplasia	6,11,16,18,30,31,33,34,35,39,40,45,51,52,56,57,58,59,61,64,66,67,68,70,71,72,73,74
High-grade anogenital intraepithelial neoplasia	16,18,31,33,34,35,39,45,51,52,56,58,59,68
Squamous cell carcinoma (cervix mostly)	16,18,31,33,35,39,45,51,52,56,58,59,68
Adenocarcinoma (cervix mostly)	16,18

has been acquired or whether it was missed at a prior diagnosis. The estimates of prevalence based on visible warts will grossly underreport prevalence, while estimates based on molecular evidence of infection (presence of HPV DNA in exfoliated genital tract cells) show the highest rates (to 20% in a general or to 75% in a very high-risk population of women or adolescents). However, it is estimated that more than 50% of sexually active adult females show serologic evidence of prior infection with one or more genital HPV types.[8] The rate of active HPV infection decreases with age; sexually active women under age 25 years consistently show the highest rates. Both acquired genital tract immunity and diminishing behavioral risk with age may be responsible for this strong inverse prevalence-age relationship. A longitudinal study of HPV infection in a cohort of college women in the USA found an average annual incidence of 14% and about 60% of the women were infected with HPV at some time during the 3-year follow-up period.[9] Similar results were also observed in a slightly older cohort (mean age 32 years) of women with a cumulative incidence of 54% after 3 years.[10]

Age remains a significant predictor for HPV infection even adjusting for other factors such as number of sexual partners. Other risk factors associated with HPV infections include number of recent and lifetime sexual partners, frequency of sexual intercourse, immune status and characteristics of the sex partner, including genital warts

and the number of lifetime sex partners.[8] Estimates based on HPV DNA detection methods suggest that the prevalence of HPV in men may be similar to that found in women.[11] However, penile and urethral data on men are quite limited, due in large part to inadequate cell sampling modalities.

Infection with HPV in women is typically transient, with a 70% clearance rate in 12 months and greater than 90% clearance rate in 2 years.[9] [12] [13] [14] [15] Persistence of type-specific HPV infection is an important risk factor for cervical cancer development and progression.[16] [17] [18] Factors associated with persistence of infection include higher number of lifetime sexual partners, older age of the women, infection with multiple types of HPV, high-risk HPV type, higher HPV virus load and infection with multiple HPV types.[9] [10] Note that while both prevalence and incidence of HPV are higher among younger women, it is older women who seem to be at higher risk of persistent HPV infection.

Human papillomavirus infection and cervical cancer

Cervical HPV infection is the single most important risk factor for squamous intraepithelial lesions (SILs) of the cervix.[1] The association between cervical cancer and a sexually transmitted etiologic agent was hypothesized long before identification of genital HPV infection. For several decades, cervical cancer risk has been recognized in association with early onset of sexual activity and multiple sexual partners either of the woman herself or of her male sexual partner. Although most women with cervical cancer are over 45 years of age when diagnosed, HPV infection and disease pathogenesis are known to begin at the onset of sexual activity.[19] Early signs of cervical intraepithelial neoplasia (CIN) are detectable through cervical cytology screening programs that provide periodic Papanicolaou-type cervical cell examination testing (Pap smear). An overwhelming majority of cervical cancer cases occur in women receiving suboptimal cervical screening and treatment regimens. Hence, cervical cancer cases reflect failures in public health and preventive gynecology. Older women and women from ethnic or racial minorities are more likely to be diagnosed with advanced-stage disease as a direct consequence of fewer cervical testing opportunities. [20]

Cervical cancer is the second most common cancer among women worldwide. Globally, an estimated 468,000 new cases and 233,000 deaths occurred in the year 2000, with developing countries accounting for 80% of the cases.[21] In the absence of screening programs, 85% of the women with cervical cancer will die given the advanced stage of the disease at the time of diagnosis.[22]

Despite widespread screening in the USA, it is estimated that about 13,000 new cases and 4100 deaths from invasive cervical carcinoma were reported in the USA in 2002.[23] In 1997, the estimated cervical cancer prevalence in the USA was over 200,000 women, suggesting the long duration and under-recognition of most cervical cancers. In the USA, about 35% of invasive cervical cancers and 57% of deaths occur in women over 55 years of age. [20] Data from the Surveillance Epidemiology End Results (SEER) program indicate an overall age-adjusted incidence rate for invasive cervical cancer in the USA of 7.7/100,000 women during 1994–98. The lifetime risk for acquiring invasive cervical cancer in the USA is 0.85% and that for dying from it is 0.30%. However, black women are 61% more likely to develop invasive disease and have about twice the risk (0.56% vs 0.27%) of dying from invasive cervical cancer than white women in the USA.[20] The racial disparity in the incidence of cervical cancer becomes more prominent among women over the age of 40 years. Cytologic screening in the USA results in the treatment of at least 750,000 women each year for cellular abnormalities suspected to represent possible cancer precursor lesions.[24]

Oncogenesis

Molecular studies demonstrate that HPV has a direct mechanistic role in oncogenesis.[25] [26] The integration of viral DNA is critical for malignant transformation while continued expression of HPV proteins is necessary for maintenance of the transformed state. In benign warts and in preneoplastic lesions, the HPV genome is maintained in a circular non-integrated form, whereas in cervical cancer cells the previously circular viral DNA is often found integrated into the linear host cell genome.[27] Proteins from both HPV and endogenous origins can deregulate cell cycles and enable carcinogenesis to occur. The expression of some of the 'early' genes (E6/E7) of high-risk HPVs (16, 18 and others) seems to be an essential factor for malignant conversion of the cervical epithelium. The viral DNA will integrate into the host DNA within the E1/E2 open reading frame of the viral genome.[28] Because the E2 region of the viral DNA normally represses the transcription of the E6 and E7 early viral genes, HPV integration causes overexpression of the E6 and E7 proteins of high-risk HPV types.[28] Transformation can occur when these expressed E6 and E7 proteins bind the products of tumor suppressor genes p53 and *pRb*-1, respectively. This modifies or inactivates their normal cell-regulating functions, namely the transcription of genes involved in cell cycle control.[25] The E6/p53 and E7/*pRb*-1 interactions cause genomic instability, resulting in the accumulation of chromosomal abnormalities followed by clonal expansion of malignant cells.[6]

Gene mutations in the p53 region have been seen with high frequency in cervical and vulvar cancer specimens, although there is some uncertainty as to how central p53 is to the carcinogenic pathway.[6] The affinity of these transforming viral proteins for the products of the tumor suppressor genes differs depending upon the oncogenic potential of a given HPV type. E6 and E7 proteins derived from high-risk HPV bind to p53 and *pRb* with high affinity, whereas the E6 and E7 gene products of low-risk HPV bind with low affinity.[27] The weight of both the epidemiologic and molecular evidence suggests HPV as the primary carcinogenic initiator of cervical neoplasia. Additional cofactors and mutational events may be important through chromosomal rearrangements, proto-oncogene activation and other viruses acting as modulators. [28] Metastases and recurrences from cervical cancer usually have the same viral types that were present in the primary tumor unless clonal diversity existed at the primary site.

Cofactors for oncogenesis

Human papillomavirus DNA from oncogenic viral types is found in nearly all cervical cancer cells (99.7% in one study using polymerase chain reaction (PCR) for detection).[29] [30] Previously, it was thought that only squamous cell tumors were associated with HPV. However, low-risk HPV types do not appear to cause cancer. A study of cervical cancer samples from patients around the world identified HPV-16 as the most commonly found viral type (50%), followed by HPV-18 (15%), HPV-45 (8%) and HPV-31 (5%).[29] The remaining 20% of the samples in this global survey contained other high-risk HPV types. HPV-16 was predominant everywhere except Indonesia, where HPV-18 was more common. HPV-45 was noted more often in Western Africa, whereas HPV-39 and HPV-59 were comparatively common in Central and South America. In squamous cell tumors, HPV-16 was found in 51%, whereas HPV-18 predominated in adenocarcinomas (56%) and adenosquamous tumors (39%). The more extensive the geographic and molecular research effort in clinical studies, the more diversity and complexity are noted.[31] Since high-risk HPV type infection is common in women with normal cervical cytology and only a fraction of HPV-infected women develop cervical cancer, HPV infection is a necessary but not sufficient cause of cervical and many other anogenital cancers.

Carcinogenic risk is enhanced by factors for which detailed explication is beyond the scope of this brief chapter. These cofactors include viral pathogenicity, host immunogenetic factors, host immunosuppression, host sexual behavior, acquired mucosal immunity and health care access.[24] Women who are infected with more than one HPV type or have higher viral loads[32] [33] are probably at higher risk for progression of HPV infection to high-grade CIN or cancer. Persistence of HPV interacts with other key cofactors (e.g. HPV type, immunosuppression, multiple sexual partners) and may be the major factor mediating carcinogenesis.[16] [17] [18] Other genital tract infections such as herpes simplex virus type 2 have been implicated as cofactors in disease pathogenesis.[34] Douching and resultant bacterial vaginosis with high amine production, high vaginal pH and altered microbial ecology may increase HPV risk.[35] High parity is associated with higher risk of squamous cell carcinoma among HPV-infected women.[36] Those women with seven or more full-term pregnancies are four times more likely than nulliparous women, and two times more likely than women with 1–2 full-term pregnancies, to develop squamous cell carcinoma. Parity, however, is not associated with the risk of adenocarcinoma or adenosquamous carcinoma.[36]

Local inflammation from infectious or other causes may stimulate cytokine responses that impact on HPV expression.[34] Keratinocyte growth factor is a cytokine that downregulates HPV-16 expression and stimulates squamous epithelial growth. In contrast, a number of inflammatory cytokines may have a dysfunctional role by upregulating HPV expression. When inflammatory cytokines are present in high concentration associated with other sexually transmitted infections, increased HPV expression may ensue. Local inflammation from infectious or other causes may stimulate cytokine responses that impact on HPV expression. Vascular adhesion molecules may enable local recruitment of inflammatory cells.[37] Inflammatory cytokines from bacterial vaginosis or sexually transmitted infections may increase HPV expression.[34] [38]

The prevalence of HPV declines with age, perhaps due to reduced risk behavior and/or to increased acquired immunity at the systemic and mucosal level over time. Women who do not clear their virus at these older ages may not have successfully developed acquired immunity. Women at highest risk are those in whom HPV is persistent, suggesting the potential utility of therapeutic vaccine or immunotherapies if they could help the mucosal immune system to clear the HPV infection.

Smoking, diet and drugs

Smoking may be an independent risk factor for cervical cancer. [24] Toxic cigarette smoke components can be found in high concentrations in cervical mucus. A putative carcinogenic impact of smoking could be formation of DNA adduct mutations in the host cells.[39]

Protection of the integrity of mucosal immunology and structure may require selected nutritional factors. Retinoids such as ß-carotene, antioxidants such as vitamin C and methylation agents such as folic acid have been implicated in cervical disease.[40] Chemicals and drugs may also affect cervical risk. Corticosteroids may increase oncogenic risk, presumably through immunosuppression and facilitation of HPV persistence.[24] Long-term use of oral contraceptive hormones appears associated with

an increase in the risk of cervical cancer in HPV-infected women.[41] Whether parity and oral contraceptive use are fully independent risk factors is uncertain, however, since their association with cervical disease may be confounded by multiple sexual partners.

Host genetics and immunosuppression

A current research challenge is to investigate the role of host genetics in HPV acquisition, retention and disease pathogenesis. Theoretically, HLA type or other host cell receptor genetic profiles may affect the degree of host cell susceptibility, efficiency of viral replication, nature of host immune responses and likelihood that infection will persist.[3] Molecular techniques have improved the precision and affordability of immunogenetic assessments within epidemiologic and clinical studies.[25] Recent studies have suggested a role of HLA class II polymorphisms in genetic susceptibility to HPV infection and/or cervical cancer in various populations.[42] [43] [44] [45] [46] [47] [48] [49]

Impaired immunity increases susceptibility to many infections and malignancies; HPV and anogenital cancers are no exceptions. Immunosuppressed persons, including those with low $CD4^+$ T-cell counts due to HIV infection, cancer chemotherapy recipients, renal transplant patients or rheumatology patients on immunosuppressive drugs, may have an impaired ability to clear HPV, permitting longer duration of infection and increased likelihood of oncogenic integration of HPV into the host genome. Immunosuppression can exacerbate the severity of epithelial warts and facilitates the persistence, pathogenicity and progression of HPV-induced neoplasia.[50] [51]

Cervical and anal HPV infection and neoplastic changes are more common in HIV-infected persons than among persons with comparable behavioral risk who are not infected with HIV.[52] [53] [54] [55] [56] [57] In many (but not all) studies, the magnitude of increased risk is proportionate to the severity of immunosuppression.[10] [58] [59] [60] [61] Cervical intraepithelial neoplasia progresses more rapidly and recurs more often after ablative therapy in HIV-infected women than in other women. Human papillomavirus may also infect the vulva and the anus, especially in association with immunosuppression.[62] Cervical cytology is now deemed an adequate screening tool for CIN in HIV-positive women, but the high recurrence rate and multifocal nature of this disease reinforce the need for regular screening at least once a year.[55] [63] Among HIV-infected persons, it is plausible that the short-term risk of HPV-related 'opportunistic malignancies' will decline due to immune reconstitution from highly active antiviral therapies, but that later viral resistance resulting in drug failures as well as a longer duration of life in a relatively immunosuppressed state will result eventually in an increased cancer incidence. Hence, it remains most urgent that routine screening be maintained among at-risk persons.

Male cofactors and sexual behavior

A 'male factor' is apparent in increased cervical cancer risk to women. Women married to men whose first wives died of cervical cancer, monogamous women married to seafarers or travelling sales-men who frequently are away from home, and female sexual partners of men with penile cancer all have a higher than expected cervical cancer risk.[24] Lack of male circumcision is associated with higher penile HPV infection rates and increased risk of cervical cancer in their female sex partners.[64]

That the risk for cervical cancer can be influenced by characteristics of a woman's sexual partner is certainly consistent with its sexual form of transmission. Commercial sex workers with exceedingly high numbers of sexual partners have a very high cervical cancer risk, while celibate women such as nuns and virgins have a very low risk. While a history of multiple sexual partners is clearly associated with increased cervical cancer risk, early age of first coitus, early age of first pregnancy and a history of sexually transmitted diseases may also contribute. Unlike with other sexually transmitted pathogens, condom use is neither strongly nor consistently associated with HPV prevention, perhaps indicating higher infectiousness per contact of HPV or the possibility of genital transmission from perineal or other noncoital sexual contact. Sexual couples can be discordant in HPV infection status or type, demonstrating the transient nature of infection in many persons, its low level in at least one partner and/or resistance to HPV by the uninfected partner through local immune or genetic factors.[65] HPV-related genital

cancers can cluster in families due, perhaps, to shared risk factors and/or host immunogenetics.[66]

Transmission from mother to child

Human papilomavirus transmission can occur intrapartum due to vaginal delivery.[24] Conjunctival papillomas present during infancy may occur and mothers of infants with conjunctival papillomas should be examined for HPV infection.[67] Viral load in cervical and vaginal cells may be an important determinant for the risk of perinatal HPV transmission.[68] Whether acquisition of HPV during the perinatal period predisposes to an increased risk of CIN among female infants in later life is unclear as the evidence for high-risk HPV transmission and persistent infection throughout childhood is uncertain.[69] [70] Juvenile laryngeal papillomatosis can cause aphonia or severe respiratory obstruction; it is probable that vertical transmission results in laryngeal HPV-11 infection that manifests in warts years later. Genital warts in children, in contrast, do not seem to occur from perinatal transmission and are typically the consequence of child sexual abuse when seen in immunocompetent children.

Other genitourinary cancers

Anal cancers in women and men are strongly associated with HPV. A high level of HPV infection may be important for development of anal intraepithelial neoplasia.[71] Men and women who practice receptive anal intercourse have higher anal cancer rates, but anal disease in women can occur even without anal intercourse, presumably through perineal HPV spread. Prior to the era of highly active antiretroviral combination chemotherapies, the HIV epidemic was associated with a huge rise in anal cancer rates affecting both men and women. [72] Cytologic screening for anal disease with a Pap smear-type anal swab is advocated for high-risk persons, especially for sexually active homosexual men; the utility of HPV for anal disease screening is not established.

Invasive squamous carcinoma is not common in the vulva or vagina. When cancer does occur in these genital tissues, HPV infections of the same types as seen in cervical disease are often detected. Squamous carcinoma represents 74% of invasive vulvar malignancies and 71% of invasive vaginal malignancies.[73] Both HPV types 16 and 18 are associated with vulvar squamous cell carcinoma,[74] although the prevalence of HPV-16 is less than in cervical carcinoma.

Primary squamous cell carcinoma of the ovary is rare; most cases represent malignant transformation of ovarian teratomas and are not HPV related. [74] Oncogenic HPV types can be detected very often in carcinoma of the penis and urethra.[11] Other urologic malignancies (e.g. prostate and bladder) may not have an HPV etiology although this continues to be studied. It has been suggested, but not clearly demonstrated, that HPV can act as an oncogenic agent in persons who are already predisposed to bladder cancer for other reasons.[75]

PREVENTION

Primary prevention for HPV is similar to that for any sexually transmitted infection. This includes encouraging abstinence among sexual inexperienced youth, mutual monogamy, condom use (although good evidence of condom protection against HPV is lacking) and other sexual risk reduction strategies. However laudable primary prevention is, measurable successes in reducing the incidence of cervical cancer have actually been achieved in industrialized nations through secondary prevention, namely screening by cervical cytology (Pap smear) followed by clinical treatment when indicated. In countries where screening has been extensive, the mortality rate from cervical cancer has fallen markedly since the 1950s. Detection and treatment of cancer precursors and identification of invasive cancer at an earlier, more curable stage can save many lives. The number of abnormal Pap smears and the number of women receiving preventive medical intervention are orders of magnitude higher than the number of cervical cancer cases in industrialized countries. These health care screening, diagnosis and treatment costs are not borne easily by developing countries where cervical cancer rates are often extraordinarily high compared with rates in industrialized nations. This is most tragic since tens of thousands of women lose their lives yearly in resource-limited nations as a direct consequence of failure to use Pap smear screening. [76] [77] [78]

While the importance of the Pap smear needs no further validation, its complexity is also substantial. A proper Pap smear requires a pelvic examination, an adequate sample of cells including those from the transformation zone (the squamo-columnar junction), proper fixing of the cells, excellence in cytopathologic processing and assessment, and adequacy of the clinical response to the results (both definitive diagnosis with colposcopically directed biopsy and therapeutic intervention when indicated). This complexity, combined with the Pap smear's only moderate sensitivity and reasonably high specificity, has motivated a search for improved cervical cancer screening approaches. Human papillomavirus screening is now feasible to consider as a screening tool, with a commercial product in its second generation that virtually eliminates interlaboratory variation as a problem (Hybrid Capture II™, Digene Corporation, Gaithersburg, MD). Whether HPV has value as a stand-alone screening tool, can complement cytology screening or is too costly for use has been the subject of intense investigation. Screening for HPV to help with the management of ambiguous repeated Pap smears and for cytologic quality control has been recommended since the early 1990s.[79]

Current screening guidelines

In 2001 and 2002, results from an historic National Institutes of Health-sponsored, multicenter, clinical trial called the Atypical Squamous Cells of Undetermined

Significance (ASCUS) Low-grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS), a consensus report crafting revised guidelines for reporting cervical cytologies ('2001 Bethesda System'), another consensus report for management of women with abnormal cervical cytology and two cost-effectiveness models suggested logical roles for HPV testing in cervical cancer screening programs.[80] [81] [82] [83] [84] [85] [86] [87] [88] [89] Based on the ALTS results, the 2001 Bethesda System subdivides the previous ASCUS cytopathologic category into two: 'atypical squamous cells of undetermined significance' (ASCUS) and 'atypical squamous cells, cannot exclude high grade SIL' (ASC-H). The 2001 Consensus Guidelines for Cervical Cytological Abnormalities highlight the fact that liquid cytologic assessment makes possible retention of the Pap test specimen for subsequent HPV screening. Thus, a valuable role for HPV testing is use of the Pap test using the liquid cytology method and testing of the remaining sample should the cytology demonstrate ASCUS. Note that another visit and examination is not needed with this approach.

A decision analysis model from Georgetown University investigators developing cost-efficiency judgments on cervical screening strategies looked at three screening regimens: Pap test alone, HPV by hybrid capture technique alone, or a Pap test plus an HPV test. The combined approach was superior in quality-adjusted life-years saved using an every-2-years screening approach. Cervical cancer could be reduced by an additional 59% over either modality alone, according to the model. [88] The second decision analysis model looked at the cost efficiency of different approaches to managing women with an ASCUS cytologic screening result:

- immediate colposcopy,
- repeat Pap testing,
- an HPV DNA test on a subsequent visit,

- an HPV test done on the same cellular specimen as the original Pap test ('reflex' HPV test), and
- routine care ignoring the ASCUS result.

The 'reflex' HPV DNA test, in which the HPV test is done on the same specimen taken for the original Pap test, was judged superior in that the same or greater life expectancy was obtained as with other options but at lower cost.[89] The investigators suggested that every-other-year liquid-based Pap tests with 'reflex' HPV tested would save billions of dollars in an American or European setting compared to conventional yearly Pap testing with repeat visits for Pap smears and colposcopically directed biopsies since these would be eliminated for HPV-uninfected women. While much additional modeling work is needed, these studies suggest for the first time a cost-effective algorithm using HPV testing for cervical cancer screening.

Lower-income women may have higher sexual risk profiles, the lowest Pap smear screening rates, the highest cervical cancer rates and the poorest access to health care services, resulting in cervical cancer death rates that are orders of magnitude greater than that seen among women from higher socio-economic groups. More efforts and research are needed to increase overall screening rates among the indigent and the isolated. Application of 'reflex' HPV screening may be most helpful in such populations. Fiscal difficulties, challenges in health care organization and women's health traditions that emphasize health of others (children and men) rather than themselves have inhibited effective Pap smear screening programs both in developing countries and in selected poverty pockets of industrialized nations. [90]

Vaccines

A high public health priority is the development of HPV vaccines, especially those targeting HPV-16 and HPV-18.[12] Both preventive (for HPV-uninfected women) and therapeutic (for HPV-infected women) vaccine approaches look promising. Vaccines have been successful in several animal models. Safety and immunogenicity of a live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV-16 and HPV-18 (TA-HPV) have been evaluated in a phase 1 human clinical trial.[91] Studies to investigate the use of TA-HPV for immunotherapy of cervical cancer are underway.[92] Vaccines proposed as immunomodulators or therapeutic adjuncts to radiation therapy are termed therapeutic vaccines. Among the most promising vaccine approaches for prevention of sustained HPV infection (preventive or prophylactic vaccines) is the use of recombinant virus-like particles.[93] Virus-like particles are highly antigenic, protective in animal models and lack potentially carcinogenic viral DNA. Recently published results of a controlled clinical trial of HPV-16 L1 virus-like-particle found a very high efficacy (100%) in preventing HPV-16-related cervical e.g. intraepithelial neoplasia in 18 months of follow-up. [94] Immunization with HPV peptides of tumor origin will be tested both for therapy and prophylaxis with a goal of tumor regression or prevention.[95]

CLINICAL FEATURES

Human papillomavirus infection may be asymptomatic or may be manifested in various benign or malignant lesions on cutaneous and mucosal surfaces. Genital warts can be flat (condyloma lata), as is common in the cervix, or more papillary (condyloma acuminata), as is typical in the vulva, vagina or anus. Human papillomavirus is present in a wide variety of clinical circumstances — subclinical latent infection, clinically apparent warts, neoplasia or carcinoma — with normal or abnormal genital cytology.[96] Condyloma acuminata and condyloma lata are often caused by low-risk HPV types 6, 11 or related types. Most tend to regress naturally and are rarely associated with malignant progression. They are often multifocal, can be large and have a high rate of recurrence after treatment.[3]

Cervical intraepithelial neoplasia that exhibits nuclear atypia possesses potential for progression to invasive carcinoma if not removed. The transformation zone (squamo-columnar junction) of the cervix has neither the protective qualities of the keratinized squamous cells nor the vascular and immunologic milieu of the columnar epithelia and is particularly susceptible to HPV infection and subsequent neoplastic transformation. The usual cervical lesion is flat with warty colposcopic and cytopathic features and may be visualized when painted with 5% acetic acid to reveal aceto-white thickening. Both CIN I and II may precede CIN III and carcinoma in situ, and a small proportion progress to invasive carcinoma.[97] The majority of lower grade CIN regresses to normal. The trend in cytopathology and gynecologic pathology is to use full descriptions of biopsy results, acknowledging the limitations of the CIN system that may not capture the complexity of a given cervical specimen. In addition, the 2001 Bethesda System for cytology results (Table 77.2) merges CIN II and III, as did the older system, since interobserver reliability in distinguishing these is poor.[85] [98]

High-risk HPV types have been associated with all grades of CIN, whereas low-risk HPV types have segregated primarily in condylomata and CIN I. However, discrepancies between HPV type and morphology do exist, and cytology and histology provide variable and at times conflicting information.[97] Cells with inflammatory changes but no neoplastic characteristics are termed abnormal squamous cells or atypical cells (Fig. 77.2) and their management was the topic of the clinical trial (ALTS) described above.[99]

Human papillomavirus infections occasionally are clinically recognized by the characteristic raised appearance of the accompanying warts. Aceto-whitening (whitened color after application of 5% acetic acid) is helpful to clinicians examining the cervix, vagina, vulva, anus or penis, especially under magnification (colposcopy). This is done commonly following an abnormal cytology result in order to identify lesions requiring biopsy. A valid reference standard for grading HPV-related anogenital pathology remains elusive, as clinical and pathologic results are prone to observer interpretation and misclassification (Fig 77.3 , Fig 77.4 , Fig 77.5 , Fig 77.6 , Fig 77.7). [3]

On microscopic examination of a Pap test or biopsy, the diagnosis of HPV infection can sometimes be made by finding neoplasia or dysplasia. The term 'koilocytotic dysplasia', coined by Koss in the 1950s, is still used widely if cells pathognomonic for HPV infection are seen. Koilocytes are large cells with a clear enlarged cytoplasmic space. The nucleus is hyperchromatic, often irregular and larger than normal. It may appear smaller than normal but it is not, because the

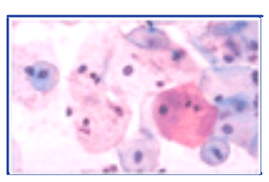

Figure 77-1 Normal squamous cells and inflammatory cells. (Pap stain). *Courtesy of Dr William H Rogers.*

TABLE 77-2 -- The 2001 Bethesda System terminology for reporting results of cervical cytology. [85] [98]

THE 2001 BETHESDA SYSTEM TERMINOLOGY FOR REPORTING RESULTS OF CERVICAL CYTOLOGY
SPECIMEN TYPE: *Indicate conventional smear (Pap smear) vs liquid based vs other*
SPECIMEN ADEQUACY

• Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g. partially obscuring blood, inflammation, etc.*)
• Unsatisfactory for evaluation ... (*specify reason*)
• Specimen rejected/not processed (*specify reason*)
• Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of ... (*specify reason*)
GENERAL CATEGORIZATION (*optional*)
• Negative for intraepithelial lesion or malignancy
• Epithelial cell abnormality: see Interpretation/Result (*specify 'squamous' or 'glandular' as appropriate*)
• Other: see Interpretation/Result (*e.g. endometrial cells in a woman 40 years of age*)
AUTOMATED REVIEW
If case examined by automated device, specify device and result.
ANCILLARY TESTING
Provide a brief description of the test methods and report the result so that it is easily understood by the clinician.
INTERPRETATION/RESULT
Negative for intraepithelial lesion or malignancy (*when there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report, whether or not there are organisms or other non-neoplastic findings*)
ORGANISMS:
• *Trichomonas vaginalis*
• Fungal organisms morphologically consistent with *Candida* spp.
• Shift in flora suggestive of bacterial vaginosis
• Bacteria morphologically consistent with *Actinomyces* spp.
• Cellular changes consistent with herpes simplex virus
OTHER NON-NEOPLASTIC FINDINGS (*optional to report; list not inclusive*):
• Reactive cellular changes associated with:
• inflammation (includes typical repair)
• radiation
• intrauterine contraceptive device
• Glandular cells status post-hysterectomy
• Atrophy
Other
• Endometrial cells (*in a woman 40 years of age*) (*Specify if 'negative for squamous intraepithelial lesion*)
Epithelial cell abnormalities
SQUAMOUS CELL
• Atypical squamous cells
• of undetermined significance (ASCUS)
• cannot exclude HSIL (ASC-H)
• Low-grade squamous intraepithelial lesion (LSIL) encompassing: HPV/mild dysplasia/CIN I
• High-grade squamous intraepithelial lesion (HSIL) encompassing: moderate and severe dysplasia, CIS/CIN II and CIN III
• with features suspicious for invasion (*if invasion is suspected*)
• Squamous cell carcinoma
GLANDULAR CELL
• Atypical
• endocervical cells (NOS *or specify in comments*)
• endometrial cells (NOS *or specify in comments*)
• glandular cells (NOS *or specify in comments*)
• Atypical
• endocervical cells, favor neoplastic
• glandular cells, favor neoplastic
• Endocervical adenocarcinoma in situ
• Adenocarcinoma
• endocervical
• endometrial
• extrauterine
• not otherwise specified (NOS)
Other malignant neoplasms (*specify*)
EDUCATIONAL NOTES AND SUGGESTIONS (*optional*)
Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included)

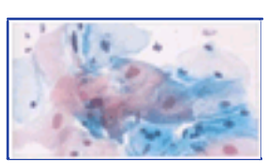

Figure 77-2 Atypical squamous cells of undetermined significance. Here the cells are slightly enlarged and irregular relative to the cells in Figure 77.1 and contain perinuclear clear areas suggestive, but not diagnostic, of HPV infection. *Courtesy of Dr William H Rogers (Pap stain).*

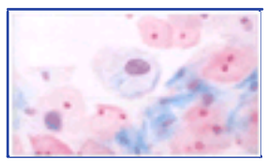

Figure 77-3 Low-grade squamous intraepithelial lesion. In this case, the lesion would classically be called a mild dysplasia; the cell in the center of the photograph has a nucleus that is enlarged more than four times the size of the surrounding normal squamous cells. In addition, the nucleus has irregular nuclear outlines and hyperchromasia. *Courtesy of Dr William H Rogers (Pap stain).*

nucleus is within a larger than normal cell and cytoplasmic space.[3] A cytologic or pathologic description of koilocytotic atypia alone implies that no dysplasia is seen, but the 2001 Bethesda System demands more detailed reporting to establish the proper classification.

DIAGNOSIS

Human papillomavirus cannot yet be grown in culture. Using molecular techniques, more than 90 HPV genetic types have been sequenced and more are certain to be identified in the future. Oncogenic, high-risk HPV types are found in a large majority of squamous cell cervical cancers. Genital tract HPV types include 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 66, 68 and 70. High-risk types of major public health significance include 16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58, 68 and 70.[6]

The sensitivity of PCR can result in DNA detection in a very large proportion of sexually active women (often 20–60%). Although it is the most sensitive technique for identifying HPV, a loss of specificity for predicting cervical disease means that the appropriate use of PCR in clinical screening remains to be determined. Older methods for molecular detection of HPV measure the presence of HPV DNA directly without PCR amplification, including Southern blot, in-situ

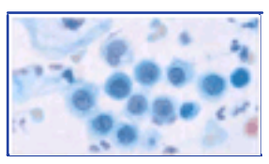

Figure 77-4 High-grade squamous intraepithelial lesion. This contains small cells with an increased nuclear to cytoplasmic ratio and marked nuclear hyperchromasia; in the classic terminology, this would be considered a severe dysplasia or CIN III. *Courtesy of Dr William H Rogers (Pap stain).*

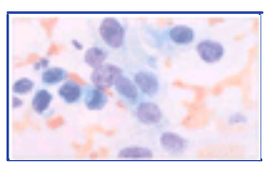

Figure 77-5 Carcinoma in situ. The abnormal hyperchromatic cells have indistinct cell borders and form a pseudosyncytial arrangement. *Courtesy of Dr William H Rogers (Pap stain).*

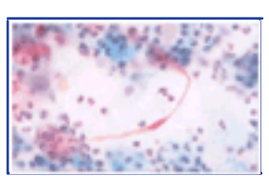

Figure 77-6 Squamous cell carcinoma. This shows highly atypical, enlarged, abnormal keratinized cells. *Courtesy of Dr William H Rogers (Pap stain).*

hybridization, filter in-situ hybridization and dot blot hybridization. These are hard to standardize across laboratories and are now used less commonly due to low sensitivity.[100]

Hybrid capture is a nonradioactive, rapid method of direct detection of HPV DNA. The technique allows semiquantitative assessment of major HPV types and can be used with a variety of cervical specimen collection methods. A second-generation hybrid capture technology

Figure 77-7 Genital HPV infection. (a) Vulvovaginal HPV infection. (b) Penile HPV infection.

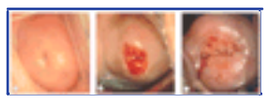

Figure 77-8 Cervigrams. (a) Normal cervix. (b) Cervix with ectopy. (c) Cervix with microglandular hyperplasia.

uses an enzyme-linked immunosorbent assay (ELISA)-type plate to enable lower cost and greater test volume without any loss in the sensitivity or high specificity of the test.[101] Hybrid capture is more reproducible across laboratories than PCR. Paradoxically, lower sensitivity of hybrid capture compared with PCR may be helpful in clinical screening by only detecting infections above a given quantitative threshold. It is probable that the 18 genotypes (including the 13 high risk) that the second-generation hybrid capture system can detect are associated with about 95% of HPV-related cancers.[102]

Cell sampling strategies include scrape, swab, brush, cervicovaginal lavage, home lavage performed by women themselves and even sampling from vaginal tampons. In many epidemiologic and clinical investigations, high sensitivity of HPV assessment is desired to avoid false-negative assessments. In such circumstances, use of the cervicovaginal lavage increases sensitivity method related directly to cellular yield.[103] In routine cervical screening, the Pap test-style approach of cervical scrape and swab, using DNA-based diagnostics, seems sufficiently sensitive for clinical purposes.

Serology may become a tool to evaluate treatment success, but it is not yet an adequate clinical diagnostic tool due to its suboptimal sensitivity and specificity and given the ubiquitous yet transient nature of HPV infection. Seroepidemiologic studies have assessed antibody response to HPV-16 and HPV-18, and HPV-6 and HPV-11 for most part. A variety of test formats have been employed, including ELISA, Western blot, radio-immunoprecipitation assays and IgG and IgA levels in serum and in cervical secretions.[3]

Innovative diagnostic approaches include infrared spectroscopy of cervicovaginal lavage fluid, automated PCR-ELISA-based techniques, laser-induced cervical fluorescence and semiquantitative techniques.[103] It is uncertain whether these technologies will reach the mainstream of clinical testing. Liquid-based cytology (Thin Prep™ Pap smear) is now the test of choice of many clinicians and laboratories for cervical cytology due to its relative ease of standardization

and sensitivity.[104] The role of cervicography, high-resolution photography of the cervix (Fig. 77.8), is unclear given its high cost and failure to demonstrate superiority to the currently recommended approaches.[86]

MANAGEMENT

An accurate diagnosis and appropriate treatment plan can help eliminate the long-term sequelae of HPV disease. Current forms of treatment attempt to ablate the

pathologic lesions and eliminate HPV. The first goal is realistic in a large majority of patients, whereas the second is elusive given multifocal infection and the ease of reinfection with HPV.

Ablation of frank warts or pathologic lesions identified visually or by biopsy can be achieved by topical application of chemicals, cold (cryosurgery), heat (loop electrosurgery, laser) or surgery (cone biopsy, hysterectomy). Topically applied chemicals and medications include salicylic acid, cantharidin, podophyllin liquid, podophilox gel, trichloroacetic acid and topical 5-fluorouracil.[105] Success in treating condylomata can be increased if the area is first soaked with 5% acetic acid to show the extent of the local infection more clearly. Topical therapies are more efficacious when applied on warts occurring on moist mucosal surfaces than on lesions found on heavily keratinized epithelia. Cryosurgery applies liquid nitrogen with a swab, freezing superficial squamous epithelium and killing many of the HPV inhabiting the cells that are killed.[106] Loop electrosurgical excision procedure (LEEP) is currently a popular option, especially for cervical lesions, due to the relatively high rates of cervical disease compared with other anogenital sites.[52] Warts of the vagina, urethral meatus, anus and oral mucosa are often treated with cryotherapy initially, while topical agents may be used to treat warts of the vagina, vulva, anus and urethral meatus, and penis in men, when the evidence implicates low-risk HPV types. Recurrence rates associated with all modalities are high because these methods often fail to eradicate the subclinical or latent reservoir of HPV remaining in adjacent epithelial cells and mucous membranes.[95] Invasive treatment modalities such as carbon dioxide laser and surgery are reserved for patients with either extensive or refractory lesions.[107]

During pregnancy, removal of visible warts is often advisable due to their propensity to proliferate and become friable. Similarly, high-grade CIN may need intervention even during pregnancy. In contrast, treatment for subclinical genital HPV infection and lower grade CIN during pregnancy is not recommended as spontaneous improvement postpartum is common.

Immunologic therapy with interferons can be directed against all sites of infection, including clinical, subclinical and latent disease. Interferons have been used successfully as monotherapy or in combination with traditional modalities to treat anogenital condyloma acuminata.[95] They function as cytokines, intercellular signaling proteins that help regulate cell proliferation, differentiation and immune function. Interferons indirectly perform their diverse biologic activities by binding tightly to specific cellular receptors, resulting in transmembrane signaling and synthesis of effector proteins. Cost and side-effects inhibit the widespread use of interferon for therapy. Persons with severe or recurrent disease are far more likely to receive interferon therapy in combination with other therapeutic modalities than monotherapy alone.[95]

In the absence of HIV co-infection, currently available therapeutic methods (all modalities) are moderately successful in the treatment of frank genital warts, with recurrence rates of 25% within 3 months, and in clearing HPV. Fortunately, cytologic successes are higher than HPV clearance rates. Patient follow-up is based on colposcopy and cytology, while the use of HPV diagnostic use for posttherapeutic prognosis and management remains to be defined.[108] All screening and therapeutic contacts with patients or their partners should emphasize the importance of primary prevention (i.e. health care providers should counsel on how to reduce sexual risk taking).[108]

Chemoprevention

Chemoprevention is the use of agents to prevent or retard neoplastic progression. The desired effect for chemopreventive agents is complete regression or at least the prevention of progression. However, spontaneous high regression rates, the subjective nature of CIN diagnosis, the impact of biopsy on regression and the low threshold for ablative intervention complicate evaluation of such approaches. [109]

Several processes in the HPV infection cycle are appropriate targets for the development of antiviral and/or antineoplastic agents. The development of chemical compounds active against HPV could prevent the benign and malignant diseases associated with HPV infection.[110] Use of retinoids such as dietary vitamin A (retinol) and carotenoids is being studied early in the neoplastic process (either systemically or locally) to maintain normal cervical cell function and inhibit the disease progression. Retinoids do not seem to inhibit proliferation of HPV-immortalized cervical cells via effects on HPV E6 and E7, but they may act to inhibit cervical proliferation by suppressing the activity of epidermal growth factor and insulin-like growth factor signaling pathways. Combined interferon-retinoid therapy might provide enhanced anticancer benefits due to the fact that each agent inhibits cervical cell proliferation.[111]

CONCLUSION

Human papillomavirus is a ubiquitous, often transient infection that can cause anogenital carcinoma under conditions favoring persistence of high-risk genotypes. Our understanding of viral pathogenesis has improved markedly in the past three decades. Diagnosis, therapy, screening policies and vaccine development are all evolving at a rapid pace. However, immediate progress in control of HPV-mediated genital tract cancers depends on expanded screening and neoplasia treatment programs both for high-risk populations in the industrialized world and for persons in developing countries.

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Chapter 78 - Lymphogranuloma Venereum, Chancroid and Granuloma Inguinale

Virginia R Roth
D William Cameron

Cutaneous lesions are common on the external genitalia.[1] [2] [3] Such lesions may be restricted to the genital region, part of a generalized skin eruption or a local manifestation of a systemic disorder (see Table 78.1). Genital lesions are often a source of great anxiety, although many are benign or inconsequential. This chapter focuses on penile lesions. Vulvar lesions are discussed in Chapter 62 .

A thorough medical history often provides the key to correctly diagnosing genital lesions. A history of multiple sexual partners, a recent new sexual contact or contact with commercial sex workers increases the likelihood of venereal disease. HIV infection also increases the risk of sexually transmissible genital ulcer diseases; conversely, genital ulcers are associated with a higher rate of HIV transmission. HIV-infected persons may present with genital Kaposi's sarcoma or Norwegian scabies or with an atypical presentation of a common disease such as herpes genitalis (due to herpes simplex virus, HSV).

A history of recent drug intake or systemic illness is useful in diagnosing fixed drug eruption or erythema multiforme. Although almost any drug can be implicated, antibiotics and antivirals are common causes of such reactions. A lesion that recurs at the same location upon reintroduction of the causative drug is characteristic of a fixed drug eruption. A history of trauma, such as a 'zipper accident', or genital manipulation may suggest traumatic ulcers. Trauma may also precipitate psoriasis or recurrent HSV and a history of trauma or inflammation helps to distinguish the white lesions of postinflammatory hypopigmentation from those of vitiligo. History of an autoimmune disorder or vitiligo elsewhere may confirm vitiligo. Minor trauma, such as shaving or scratching, can provide portals of entry for molluscum contagiosum and other infections. Contact dermatitis is usually accompanied by a history of contact with an irritant or allergen. These can include soaps, antiseptics, urine, feces, topical medications, lotions, lubricants, condoms or spermicides. In some cases, a history of contact is not obvious if the irritant was inadvertently transferred to the genital region by the hands (e.g. poison ivy). A history of pruritus may be obtained with contact dermatitis, scabies, lichen planus or lichen sclerosis. Local tingling or paresthesia may precede the appearance of recurrent HSV lesions. A history of systemic involvement should be sought, although genital lesions are rarely the sole manifestation of systemic disorders such as Reiter's syndrome, Behçet's syndrome and Crohn's disease.

The chronicity of a genital lesion may indicate a noninfectious cause such as malignancy. Occasionally the age of onset may guide the diagnosis, as certain lesions are more likely to appear in adolescence (e.g. hidradenitis suppurativa, pearly penile papules), after puberty (e.g. Fordyce spots, Beçet's syndrome, psoriasis), in middle age (e.g. lichen planus) or in the elderly (e.g. seborrheic keratosis, malignancy). A family history is rarely helpful, although up to 10% of patients with lichen planus may have a positive family history.

On physical examination, genital lesions can be broadly categorized as raised, flat or ulcerative (see Table 78.1). Raised lesions such as condylomata lata, pearly penile papules or molluscum contagiosum may be mistaken for genital warts. Hyperpigmented lesions, though usually benign, should be biopsied to rule out squamous cell carcinoma, Kaposi's sarcoma or malignant melanoma. Flat hypopigmented lesions that are not morphologically characteristic of vitiligo or postinflammatory hypopigmentation also should undergo biopsy to seek a diagnosis of lichen sclerosis or lichen planus. Ulcerative lesions may be classified as painful or painless. Painful ulcers include chancroid, HSV, aphthous ulcers and Behçet's syndrome. Syphilis and granuloma inguinale are typically painless, unless complicated. A general physical examination may provide diagnostic clues to a systemic disorder. Reiter's syndrome classically involves a triad of urethritis, conjunctivitis and asymmetrical polyarticular arthritis. Behçet's syndrome may manifest with oral ulcers, panuveitis, large joint arthritis, erythema nodosum or chronic meningoencephalitis.

The management of cutaneous genital lesions generally depends on making an accurate diagnosis. However, a syndromic approach to the management of venereal diseases has proved both feasible and useful in light of the cost and logistics required to make a definitive microbiological diagnosis in resource-limited settings.[4] The management of syphilis and HSV is discussed elsewhere in this text. The remainder of this chapter will deal with lymphogranuloma venereum, chancroid and granuloma inguinale.

LYMPHOGRANULOMA VENEREUM

Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by three serovars of *Chlamydia trachomatis*: L1, L2 and L3.

EPIDEMIOLOGY

Lymphogranuloma venereum is endemic in parts of Africa, India, Asia, South America and the Caribbean. It occurs sporadically in North America and Europe, usually in returning travelers or military personnel. Missed diagnosis and underreporting of LGV are thought to be common. Men are six times more likely than women to have a clinically evident infection, and are only infectious until the primary ulcer heals. In contrast, women may harbor persistent asymptomatic cervical lesions that serve as reservoirs of infection.

PATHOGENESIS AND PATHOLOGY

Lymphogranuloma venereum is acquired sexually, but transmission through direct contact with infected tissues or fomites has been documented. After exposure, epithelial abrasions allow the organism to penetrate the mucosal barrier. Replication within the macrophages is followed by spread via the lymphatic system. The histopathological changes on biopsy resemble those of other bacterial infections.

CLINICAL FEATURES

Lymphogranuloma venereum begins in the genital region and spreads through the lymphatics. Clinical disease occurs in three stages. The

transient primary lesion is a small, painless genital ulcer or papule. It appears 3–21 days after inoculation and generally goes unnoticed. It usually occurs on the coronal sulcus in men and on the cervix, posterior vaginal wall or vulva in women. Urethral involvement may cause urethritis.

The second stage occurs days to weeks after the primary infection. It is characterized by painful regional lymphadenopathy and systemic symptoms. In men, the inguinal lymph nodes are affected and node enlargement on either side of the inguinal ligament produces the characteristic 'groove sign'. In women, lymph drainage from the rectum and vagina results in pelvic lymphadenopathy. Involvement of these deep nodes causes lower abdominal and back pain. Initially, the lymph nodes are mobile and discrete, but with progressive inflammation they become fixed and suppurative with bubo formation. Buboes may spontaneously rupture or form chronically draining sinuses. Subsequent scarring and fibrosis results in lymphatic obstruction and genital edema (Fig. 78.1). Regional spread of LGV to the pelvis may cause salpingitis, pelvic adhesions and infertility. Hematogenous dissemination has been documented by the recovery of organisms from the blood and cerebrospinal fluid. Such patients experience nonspecific constitutional symptoms or, less commonly, present with meningoencephalitis, pneumonitis, arthritis or hepatitis. Skin lesions such as erythema nodosum classically follow surgical manipulation of infected tissue.

The tertiary or anorectal stage is predominantly seen in women and homosexual men. Rectal infection may result from anal intercourse, lymphatic spread or spread from vaginal secretions. Patients present with fever, rectal pain and mucopurulent or bloody discharge. The appearance on sigmoidoscopy resembles that of inflammatory bowel disease with mucosal ulceration and friable granulation tissue. Fibrosis can cause rectal strictures and bowel obstruction.

Conjunctival or oropharyngeal infection may be a result of autoinoculation or orogenital sex. The regional mandibular and cervical lymph nodes are involved and subsequent spread to supraclavicular and mediastinal nodes has been reported to cause pericarditis.

DIAGNOSIS

When LGV is suspected clinically, other causes of genital ulcer disease should be excluded. Growth of the organism in cell culture is the most specific diagnostic test but it is labour intensive, with a recovery rate of only 50%.[5]

Serology has been the mainstay for the diagnosis of LGV. Complement fixation is currently the most sensitive and widely used test and a titer of 1:64 or greater in the appropriate clinical setting is considered diagnostic. Other *Chlamydia* infections may result in a positive complement fixation test, but a titer of less than 1:16

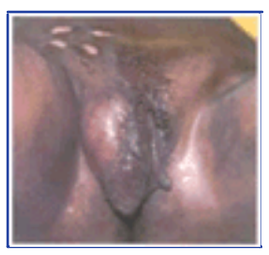

Figure 78-1 Lymphogranuloma venereum causing unilateral vulvar lymphedema and inguinal buboes.

essentially excludes acute LGV. Microimmunofluorescence is more specific but less sensitive than complement fixation. More recently, enzyme-linked immunosorbent assay (ELISA) and direct fluorescein-conjugated antibody tests have become available. Polymerase chain reaction (PCR), though highly sensitive and specific, is costly and not routinely available.

MANAGEMENT

Although LGV infections resolve spontaneously, this occurs over several weeks and may be complicated by fibrosis, stricture formation or superinfection. Antimicrobial therapy decreases the incidence of complications, but has not been shown to affect the rate of healing. The recommended therapy is oral doxycycline 100mg twice daily for 3 weeks. Alternative regimens are oral erythromycin 500mg four times daily or oral trimethoprim-sulfamethoxazole (TMP-SMX; cotrimoxazole) twice daily.[4] [6] Asymptomatic sexual partners should also be treated. Patients should be followed to document clinical resolution. Repeated aspiration of buboes may be necessary to relieve pain and prevent rupture. Surgical intervention may be required for fistula or stricture or for management of chronic local edema, genital elephantiasis.

CHANCROID

Chancroid is a classic sexually transmitted genital ulcer disease. The etiologic agent is *Haemophilus ducreyi*, a fastidious Gramnegative coccobacillus.

EPIDEMIOLOGY

Chancroid occurs worldwide. It is endemic in tropical climates and has been the commonest cause of clinically presenting genital ulcer disease in many developing countries. It is much less frequent in North America and Europe where it occurs in sporadic outbreaks associated with prostitution, illicit drug use, travel and returning military personnel. The prevalence is much higher in men than women, reflecting its association with prostitution. Uncircumcised men are apparently more susceptible to infection. A low clinical suspicion and a lack of readily available diagnostic tests have resulted in underreporting of the disease, particularly where the disease is uncommon.

Outbreaks of chancroid are related to frequent sexual partner change and the number of sexual partners of an infected person, as occurs in prostitution, which consistently appears to be the main reservoir of infection. Women are often asymptomatic and commercial sexual activity may continue despite active infection and ulceration. Also, PCR testing on cervical specimens from high-risk women without genital ulcers has confirmed that asymptomatic carriage of *H. ducreyi* does occur.[7]

Chancroid and other genital ulcer diseases are significantly associated with an increased rate of HIV transmission.[8] [9] Conversely, the presence of HIV is also associated with an increased risk of genital ulcer and chancroid in some populations. The significance of epidemiological synergy between chancroid and HIV, which is the result of these complex bidirectional clinical and ecological interactions, was emphasized in an epidemiologic study that estimated that genital ulcer disease increases the risk of HIV transmission per sexual exposure by 10–300-fold.[10]

PATHOGENESIS AND PATHOLOGY

Haemophilus ducreyi is a bipolar-staining, pleomorphic Gramnegative coccobacillus. In the past, three distinct histological zones have been described on biopsy of the chancroid ulcer. The superficial

zone, in which the organism is most readily seen, contains necrotic debris, fibrin and degenerated neutrophils. The middle zone is characterized by edematous inflammatory tissue with neovascularization, and the deep zone exhibits a dense cellular infiltrate. This inflammatory infiltrate contains $CD4^+$ lymphocytes and macrophages, which HIV-target cells may contribute to the facilitation of HIV transmission.[11]

Chancroid is transmitted from person to person by direct contact. A break in the skin may allow the organism to penetrate, establish infection and evoke humoral and cellular immune responses. Despite the host immune response, reinfection and serial autoinfection can occur. Virulence factors have not been well defined. Pili have been demonstrated, but their role in cellular adhesion is unknown. The production of lipo-oligosaccharides, heat shock proteins and cytotoxins may contribute to tissue damage and ulceration. Evasion of bacterial killing by phagocytosis and of other immune defences may be mediated in part through lipo-oligosaccharide, cytotoxins and outer membrane proteins. Virulence factors and studies of inducible immunity are sought in *in vivo* models of animal and human experimental infection.

PREVENTION AND CONTROL

The role of chancroid in HIV transmission makes it a great public health concern and its proven potential control an opportunity for HIV control. Thus, treatment of infected persons and their sexual contacts and the examination and treatment of commercial sex workers in targeted medical health care are important public health control measures. In certain settings, effective treatment of sexually transmitted diseases (STDs) can decrease the population incidence of HIV.[12] The future development of vaccines for prevention of genital ulcer diseases including chancroid may also be highly effective in reducing the spread of HIV. Global chancroid eradication is gaining recognition as a feasible and worthwhile effort for preventing HIV transmission.[4] [13]

CLINICAL FEATURES

Patients who have chancroid generally present with painful genital ulcers within 4–7 days of exposure. An initial painless papule, usually unnoticed, develops into a pustule and progresses to form a painful ulcer about 1–2cm in size (Fig. 78.2). The ulcer margins are raised, irregular and sharply demarcated. Because the ulcer edge is not indurated, it is known as 'soft chancre'. The friable, granular base is often covered with a necrotic exudate. Approximately one-third of patients develop multiple lesions, which may coalesce to form giant ulcers. Autoinoculation may result in 'kissing lesions' on opposing surfaces.

The clinical picture varies with gender and men are more symptomatic than women. In men, the ulcer is usually located on the prepuce, coronal sulcus, penile shaft, glans or urethral meatus (Fig. 78.3). The scrotum and perineum are less frequently involved. In women, the clinical presentation may be atypical, with symptoms of dyspareunia or dysuria. Ulcers in women are more likely to be painless and are found on the forchette, labia, perineum, perianal region and the medial aspect of the thigh. Involvement of the vagina and cervix is rare. The ulcers may resemble those of syphilis, genital herpes or granuloma inguinale (GI). Co-infection with herpes simplex or syphilis commonly occurs, in as many as 10%.

Inguinal lymphadenitis is seen in about 50% of men and 35% of women and may be bilateral. Lymph nodes progressively enlarge to become necrotic and fluctuant (buboes). Systemic symptoms are characteristically absent. Extragenital ulcers are rare, but have been described in the mouth, fingers and breasts. Co-infection with HIV

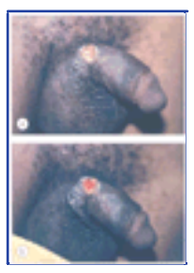

Figure 78-2 Chancroid ulcer. (a) Before and (b) after the performance of a swab, demonstrating the friability of the ulcer base.

Figure 78-3 Typical chancroid ulcer. Unilateral lymphadenitis and demonstration of the aspiration of a bubo.

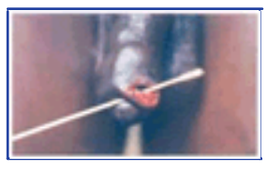

Figure 78-4 Phagedenic chancroid with extensive tissue destruction.

may alter the clinical presentation, as these patients are more likely to have multiple genital lesions, to develop extragenital ulcerations and may experience a delayed or reduced response to treatment. However, disseminated infection does not occur even in the immunocompromised. Although untreated ulcers usually heal spontaneously, they can be complicated by secondary bacterial infection, tissue destruction, scarring, fistula and stricture formation (Fig 78.4 , Fig 78.5).

DIAGNOSIS

Laboratory methods for the identification of *H. ducreyi* have developed rapidly over recent years.[14] Because the accuracy of clinical diagnosis may be as low as 33%,[14] laboratory confirmation of

Figure 78-5 Healed inguinal bubo with scar formation from previous chancroid infection.

infection should be obtained when possible. In addition, all patients who have suspected chancroid should be tested for HIV and for other or co-existing causes of genital ulcer disease.

Specimens are collected by swabbing the ulcer base. Bacterial contamination reduces the accuracy of the Gram stain and its overall sensitivity and specificity are less than 50%. Before the development of nucleic acid testing methods, culture of the organism was the standard diagnostic test. *H. ducreyi* is a fastidious, temperature-sensitive organism requiring an experienced laboratory and specific culture methods to achieve a sensitivity of 70–80%. A variety of culture media have been used (most commonly Mueller-Hinton or gonococcal agar base) and recovery rates can be improved by using more than one type of medium concurrently. Specimens should be inoculated onto culture plates within 1 hour of collection or stored in an enriched medium and refrigerated for up to 1 week. Culture plates are incubated at 91.4°F (33°C) in maximal humidity and high CO_2 . Growth is usually seen within 72 hours, but plates should be kept for 5 days before being reported negative. Colonies are raised; they are nonmucoid, yellow-gray in color and very cohesive. A positive culture may be confirmed by demonstrating the presence of nitrate reductase, cytochrome oxidase, alkaline phosphatase and the requirement for heme.

PCR techniques have become the preferred diagnostic test, with a sensitivity of 83–96% and a specificity of 100%.[14] A commercially available multiplex PCR assay for the simultaneous detection of *H. ducreyi, Treponema pallidum* and herpes simplex viruses simplifies the diagnosis and management of genital ulcer disease. Antigen detection techniques are of little clinical use. Serologic tests are available, but are primarily useful in epidemiologic studies.

MANAGEMENT

The treatment of chancroid has been complicated by antimicrobial resistance and HIV co-infection. There are wide regional variations in antimicrobial susceptibilities, but increasing global resistance to tetracycline, aminoglycosides, sulfonamides and amoxicillin-clavulanic acid has been documented.

Current recommendations for treatment include oral azithromycin 1g in a single dose, intramuscular ceftriaxone 250mg in a single dose, oral ciprofloxacin 500mg twice daily for 3 days or oral erythromycin base 500mg four times daily for 7 days.[4] Ease of administration and compliance make single-dose therapies preferable and there is good evidence that single-dose ciprofloxacin 500mg orally is effective regardless of HIV serostatus.[15] Intramuscular spectinomycin 2g is an alternative regimen. Response to treatment is characterized by a decrease in pain within 48 hours and complete ulcer healing may take approximately 10 days, depending on the size of the ulcer. The development of fluctuant buboes may occur on treatment and these should be aspirated by needle or drained by incision.

GRANULOMA INGUINALE

Granuloma inguinale (or donovanosis) is caused by the Gramnegative bacterium *Calymmatobacterium granulomatis*. It is a fastidious organism that is difficult to culture and thus continues to evade taxonomic classification and definition of its biochemical characteristics.

EPIDEMIOLOGY

Granuloma inguinale is endemic in tropical and subtropical regions such as Papua New Guinea and India. It can also be found in South East Asia, parts of Africa, the Caribbean, central Australia and South America. Granuloma inguinale is rare in North America and Europe. Underreporting probably occurs in nonendemic areas because of a low clinical suspicion and diagnostic difficulties.

The prevalence is highest among adults aged 20–40 years, although it does occur in children and in the elderly. The male to female ratio is about 2.5:1. Affected individuals are usually sexually active and may have a history of multiple sexual partners or contact with prostitutes. A significant association between granuloma inguinale and HIV seropositivity has been documented, particularly in men with genital ulcers of long duration.[16]

PATHOGENESIS AND PATHOLOGY

The exact mode of transmission is not known. However, it is widely believed to be sexually transmitted because of its predominance in sexually active persons, its predilection for the genital region, the high incidence of co-infection with other STDs and the association of anal lesions in men with receptive anal intercourse. The possibility of nonsexual transmissibility has been inferred from its occurrence in persons such as children presumed sexually inactive.

On histopathology, large mononuclear cells containing inclusion bodies (Donovan bodies) are characteristic. The associated epithelial changes include ulceration, microabscesses, acanthosis, irregular elongation of the rete pegs and pseudoepitheliomatous hyperplasia. Untreated lesions may become hyperkeratotic. The dermal layer exhibits inflammatory changes, with a dense cellular infiltrate and varying degrees of fibrosis and edema.

PREVENTION

Serious morbidity is best prevented by early diagnosis and treatment. Sexual contacts should be examined, as the rate of infection in this group is as high as 50%. It has been suggested that annual screening may reduce the incidence of GI in endemic areas and that condom use may decrease sexual transmission. As with other genital ulcer diseases, reducing the incidence of GI may be important in controlling the spread of HIV.[17]

CLINICAL FEATURES

Granuloma inguinale is a chronically progressive, ulcerative disease with no systemic symptoms. The incubation period appears to range from 3 to 90 days. Patients usually present with a nonsuppurative genital lesion. Less common presentations include vaginal bleeding or discharge, hematochezia, hematuria, pelvic inflammatory disease or a pelvic mass. The initial lesion is a small firm papule at the site of infection, which erodes to form a painless ulcer (Fig. 78.6). The ulcer is granulomatous and 'beefy-red' with a nonpurulent base. Multiple lesions may develop on opposing surface

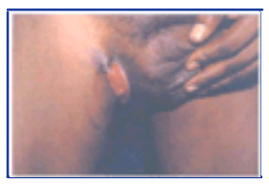

Figure 78-6 Granuloma inguinale. The chronic, granulomatous, beefy-red ulcer without suppuration is typical. *Photo kindly supplied by J K Maniar.*

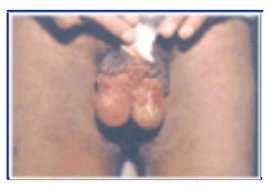

Figure 78-7 Granuloma inguinale. Lack of treatment has permitted progressive destruction of the scrotum. *Photo kindly supplied by J K Maniar.*

or along skinfolds. Lesions are usually found in the genital, perianal and inguinal regions. In women, cervical lesions may mimic dysplasia. Less frequently, extragenital sites such as the mouth, face or neck are involved. Abscess formation in the groin mimics lymphadenitis (pseudobuboes), but the lymph nodes themselves are rarely involved. This typical clinical picture may be altered if the genital lesions become secondarily infected, resulting in pain, purulent exudate and tender lymphadenopathy.

Without treatment, GI is slowly progressive, often resulting in soft tissue destruction and extensive scarring (Fig. 78.7). Possible sequelae include genital adhesions, stenosis of the urethral, vaginal or anal orifices, rectovaginal fistulas and lymphatic obstruction with genital pseudoelephantiasis. Contiguous pelvic spread may result in a 'frozen pelvis' or in hydronephrosis. Rarely, hematogenous spread to the bones or liver may occur. Even with treatment, there is a tendency for recurrence.

Granuloma inguinale may be confused with other causes of genital ulceration. Co-infection with other STDs is common. Carcinoma of the penis and vulva may occur simultaneously with GI or in areas of previously healed ulcers. It should be recognized that ulcers that do not respond to treatment or which appear recurrent may represent a malignancy. During pregnancy, GI is often more aggressive, with a higher rate of dissemination and a slower response to treatment. Perinatal infection is rare, but prophylactic antibiotics should be administered to the infant.

DIAGNOSIS

In nonendemic areas, a high index of suspicion is required to make a diagnosis of GI. Alternative or concomitant diagnoses must be ruled out. Clinical suspicion is confirmed by the demonstration of Donovan bodies on smear or biopsy. Reliable culture techniques and serologic tests are not available. To obtain a smear for diagnosis, the ulcer is scraped and the granulomatous tissue is spread directly onto a slide. The slide is air dried and stained with Wright or Giemsa stain. Donovan bodies are seen as darkly staining, ovoid organisms with or without a capsule. In the appropriate clinical setting, the 1-minute 'Quick test', in which the specimen is stained with eosin and thiazine dyes, is diagnostic.[18]

The smear is more likely to be negative if the lesion is early, sclerotic or secondarily infected. In such cases, a histological diagnosis should be sought. The diagnosis may also be established using transmission electron microscopy to identify Donovan bodies. PCR techniques, including a colorimetric detection system, have been developed recently but are not yet routinely available.[19]

MANAGEMENT

Treatment regimens are empiric, as *in vitro* susceptibility testing is not available. Currently recommended regimens include oral azithromycin 1g followed by 500mg daily, oral doxycycline 100mg twice daily, a double-strength tablet of TMP-SMX twice daily, oral ciprofloxacin 750mg twice daily or erythromycin 500mg four times daily.[4] [6] Clindamycin, the newer fluoroquinolones, chloramphenicol and aminoglycosides are also effective. Clinical resistance to tetracycline, ampicillin, TMP-SMX and streptomycin have been reported. The rapid clinical response achieved with azithromycin has made this drug the treatment of choice.[4] [20] Intravenous or intramuscular gentamicin may be added briefly in treatment for lesions that resolve slowly, and for HIV-infected persons. Combination therapy is used in more severe

disease. Response to treatment is usually seen within 1 week but antibiotics should be continued until all the lesions have resolved, which may take months in advanced disease.

Relapses are common and may occur up to 2 years after apparently successful treatment, requiring an additional course of antibiotics. Complications such as strictures, sinus formation, extensive superinfection or disfiguration may require surgical intervention.

TABLE 78-1 -- Differential diagnosis of cutaneous genital lesions.

DIFFERENTIAL DIAGNOSIS OF CUTANEOUS GENITAL LESIONS				
Category		**Lesion**	**Usual morphology**	**Comments**
Flat lesions	Erythematous	Contact dermatitis	Red edematous patch, may be vesicular	Recent contact with an irritant or allergen
		Seborrheic dermatitis	Ill-defined, erythematous, scaly patches or plaques with crusting	Idiopathic inflammation of the sebaceous glands
		Tinea cruris (dermatophytosis)	Well-demarcated scaling plaques, with an erythematous border, over the inner thighs	See Chapter 240
		Psoriasis	Well-demarcated erythematous plaques with scales on keratinized skin, and without scales on nonkeratinized skin	Typically involves the scalp, elbows, knees, back and buttocks
		Candidal balanitis	Red papules or plaques on penile shaft	See Chapter 237
		Plasma cell (Zoon's) balanitis	Glistening, moist, erythematous patch over the glans penis or coronal sulcus	Benign, chronic balanitis
	Hyperpigmented	Pigmented nevi	Well-demarcated lesions with regular borders and homogeneous pigmentation	Biopsy to rule out malignant melanoma
	Hypopigmented	Lichen sclerosis	Hypopigmented plaques of atrophic skin with progressive scarring over the vulva or penis (balanitis xerotica obliterans)	Benign chronic dystrophic disease, more common in postmenopausal women
		Lichen planus	Reticulated, white branching striae on nonkeratinized skin	Presents as inflammatory papules on keratinized skin
		Vitiligo	Depigmented well-demarcated patches with no skin surface changes	Absence of melanocytes, possibly autoimmune
		Postinflammatory hypopigmentation	Flat ill-defined demarcated patches with partial or total loss of pigment	Follows healing of some inflammatory lesions
Raised lesions	Normal pigmentation	Genital warts	Cauliflower-like (condylomata acuminata), flat-topped or rounded papules	See Chapter 77
		Secondary syphilis	Moist, hypertrophic, flat-topped papules (condylomata lata)	May also be ulcerative See Chapter 75
		Molluscum contagiosum	Smooth, firm, shiny umbilicated nodules	See Chapter 218
		Pearly penile papules	Crownlike arrangement of dome-shaped papules around the corona of the penis	Benign angiofibromas usually appearing in adolescence
		Prominent sebaceous (Tyson's) glands	Clustered pale papules on the corona and inner surface of prepuce	Aberrant sebaceous glands, normal anatomic variant
		Cysts	Dome-shaped nodules or papules	Common, benign growths
		Fordyce spots	Purplish or reddish telangiectatic papules on scrotum, penis or vulva	Occurs in late puberty, normal anatomic variant
		Sclerosing lymphangitis	Firm translucent cord encircling the penis proximal to the corona	Result of trauma or friction
	Erythematous	Scabies	Elongated papules (female's burrow) with surrounding excoriation	Look for corresponding lesions on wrists or in fingerwebs; see Chapter 11
		Hidradenitis suppurativa	Tender inflamed nodules at base of hair follicles	Recurrent abscesses of apocrine glands
		Pyogenic granulomas	Pedunculated vascular masses usually on the scrotum	Benign tumors
	Hyperpigmented	Seborrheic keratosis	Brown, keratotic, verrucous lesions	Benign lesions usually occurring after age 40
		Lichen planus	Pruritic, flat-topped violaceous papules on keratinized skin	Idiopathic, inflammatory eruption, often associated with oral lesions
		Squamous cell carcinoma in situ (3 forms)	Large, red-brown scaly or crusted verrucous lesion, usually solitary	Bowen's disease
			Smaller, flat verrucous lesions, usually multifocal	Bowenoid papulosis
			Erythematous, raised, irregular plaques	Erythroplasia of Queyrat
		Kaposi's sarcoma	Violaceous indurated plaques or nodules	See Chapter 132

Ulcerative lesions	Nonvenereal diseases	Aphthous ulcers	Painful irregular ulcers with erythematous borders and a white fibrin base	Commonly associated with oral ulcers
		Behçet's syndrome	Painful, shallow, irregular ulcers with erythematous borders and a white fibrin base on the glans penis or labia minora	Associated with oral ulcers, uveitis, arthritis, vasculitis or chronic meningoencephalitis
		Reiter's syndrome	Painless, serpiginous, shallow erosions of the penis with raised, erythematous borders (circinate balanitis)	Typically associated with urethritis, arthritis or conjunctivitis
		Erythema multiforme	Superficial vulvar, vaginal or penile erosions	Associated with stomatitis or bullous eruption over palms and soles
		Fixed drug eruption	Well-demarcated dusky red shallow erosion	History of drug intake
		Erosive lichen planus	Non-specific shallow ulcerations	Commonly associated with oral lesions
		Trauma	Variable morphology	History of recent trauma
		Crohn's disease	Ulcerative perineal granulomas; may form scars, sinuses or fistulas	Inflammatory bowel disease, anal fissures may be present
		Squamous cell carcinoma	Irregular, friable ulceration	Elderly patients with an indolent nonhealing ulcer
	Venereal diseases	Primary syphilis	Single painless ulcer with indurated edges and clean base	See Chapter 75
		Secondary syphilis	Multiple painless shallow ulcers	See Chapter 75
		Herpes simplex virus	Painful shallow ulcers, may be crusted	See Chapter 76
		Chancroid	Painful irregular, nonindurated ulcer with necrotic base; may have multiple ulcers	Commonly associated with inguinal buboes
		Granuloma inguinale	Painless nonindurated, 'beefy-red' ulcer with a clean base; may have multiple ulcers	May develop groin abscesses (pseudobuboes)
		Lymphogranuloma venereum	Primary lesion usually unnoticed but may present as a small painless ulcer or papule	Usually presents with painful inguinal lymphadenopathy

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Chapter 79 - Practice Points

79.a Persistent/recurrent vaginal discharge

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Introduction

Vaginal discharge is one of the cardinal symptoms of lower tract gynecologic exudative infections. These disorders are classified by anatomic site of origin. For example, gonorrhea and chlamydial infections are cervical infections; bacterial vaginosis (BV), trichomoniasis and vaginal yeast infections are vaginal disorders. Herpes simplex and human papillomavirus can cause epithelial lesions on the external genitalia, but they can also cause cervicitis with discharge (see Chapter 62).

Initial steps

The first step in assessing a complaint of recurrent vaginal discharge is to differentiate between a normal physiologic discharge, a vaginal discharge, and cervical infections. Many women have a small amount of vaginal discharge (physiologic leukorrhea), which is clear or white, does not have an odor, and is composed predominantly of squamous epithelial cells; this may vary with the menstrual cycle.

Patients do not differentiate from cervical and vaginal disorders, but often report 'vaginal discharge', which may represent either cervical or vaginal pathology. Therefore, initial evaluation of a patient who has recurrent or persistent discharge should include assessment for cervical infection. 'Recurrent' chlamydial and gonococcal infection is most often due to either patient noncompliance with treatment regimens, re-exposure to an untreated sexual partner, or re-exposure (i.e. new-incident infection). New-incident gonococcal and chlamydial infection is particularly common among adolescents.

Assuming that gonococcal and chlamydial infection have been ruled out and that the problem has been localized to the vagina, the next step in managing recurrent vaginal discharge is to determine the specific etiology of the vaginitis. This involves taking a careful history, with attention to recent douching, antimicrobial use (including use of over-the-counter antifungal medications) and reproductive history, followed by a clinical examination, which should include microscopic evaluation of vaginal discharge and determination of vaginal pH. The differential algorithm proposed by Sobel (see Further reading) is the standard in clinical assessment. The clinical and microscopic evaluation of recurrent vaginitis is shown in Figure 79a.1).

Recurrent vaginitis due to infection

Recurrent trichomoniasis

Recurrent infection with *Trichomonas* spp. is most commonly due to re-infection from an untreated male partner or to re-exposure. Trichomoniasis in men is usually asymptomatic, and it is therefore imperative that all male partners of women who have trichomoniasis be treated with metronidazole, 2g orally, as a single dose.

Metronidazole-resistant trichomoniasis is rare (<1% of clinical isolates) and should be considered only after re-infection has been ruled out and the patient has failed two courses of therapy. In these patients, metronidazole resistance should be confirmed, and they will respond frequently to a prolonged course of metronidazole (2–4g daily for 10–14 days).

Recurrent candidal vaginitis

Candidal vaginitis is extremely common, and recurrences occur frequently, in up to 45–50% of cases in some studies. Recurrent candidiasis is found more frequently in women who are taking antimicrobial agents and in those who have uncontrolled diabetes mellitus. Immunosuppressed patients, such as persons who have advanced HIV disease, are also at higher risk of recurrent candidiasis as well as recurrent BV (see below).

After potential precipitating factors have been considered, there remains a subset of women who continue to get frequent recurrences. Careful microbiologic evaluation, with identification of the fungal species, may occasionally reveal a yeast that is resistant to the commonly used imidazole therapies, such as *Candida glabrata* or *Candida tropicalis*. These investigations are expensive and should be reserved for use only after the clinical and epidemiologic risks have been identified. Persons who have chronic, recurrent candidiasis that is known to be caused by *Candida albicans* often benefit from a course of long-term prophylaxis with fluconazole, 100–200mg weekly (see also Chapter 62).

Recurrent bacterial vaginosis

Bacterial vaginosis is a disorder due to ecologic disturbances among the vaginal flora. Bacterial vaginosis is particularly prone to relapse. As above, the management of recurrent BV should account for the following:

¦ assurance that appropriate antimicrobial treatment was given initially — for example, many practitioners currently use a single dose regimen of metronidazole (2g orally), and this has a treatment failure rate of up to 20%; persons who have suspected recurrence should be treated with a full multiday regimen, of metronidazole 500mg orally q12h, or metronidazole vaginal gel (0.75%) 5g q12h for 5 days, or 2% clindamycin cream 5g q12h for 7 days;

¦ removal of exogenous factors that contribute to the pathogenesis of BV, including douching or long-term antimicrobials — douching is particularly associated with the development of BV because of the disruption of the local mucosal surfaces and flora and women who use spermicides are at higher risk for BV and must balance the risk and benefits of their use;
¦ ruling out the presence of cervical infection — infections in the lower genital tract induce an inflammatory response, which in turn causes BV, for example, therefore, as above, ruling out cervical gonococcal and chlamydial infection is important early in the evaluation of persons who have recurrent BV.

Figure 79.a-1 Clinical and microscopic evaluation of recurrent vaginitis.

There is a large population of women who have primary BV (i.e. BV without an identifiable cause). Primary BV resolves with treatment, but BV recurs frequently in these patients. Recurrence rates are as high as 20–30% 1 month after treatment. In these cases, long-term treatment with metronidazole should be considered. In nonpregnant women, treatment should take into account the impact of symptoms on the patient's lifestyle. In pregnant women, because of the potential for perinatal complications, periodic evaluation and treatment for recurrences is recommended.

Other causes of recurrent vaginitis

Bacterial vaginosis and yeast vaginitis are the most common types of recurrent vaginitis. Uncommon causes include hypersensitivity vaginitis, especially to latex, which is managed by avoiding latex exposure. This is one situation in which natural membrane condoms may be appropriate. Desquamative interstitial vaginitis is an uncommon disorder that is diagnosed by a high vaginal pH, a negative amine test, the absence of clue cells and the presence of polymorphonuclear leukocytes. These patients may respond to intravaginal 2% clindamycin cream, 5g q12h for 1 week; however, they are prone to frequent recurrence.

Use of biologic remedies

Women who have frequently recurrent vaginitis occasionally turn to biologic remedies obtained from health food shops or over the internet. These have included (among others) yoghurt douches, lactobacilli for vaginal instillation and prescriptions for eating large quantities of yoghurt. Controlled studies to date have failed to demonstrate any benefits of these remedies.

Patient counseling

By the time they see a medical specialist, patients who have recurrent vaginal discharge have typically been seen by many medical care providers with limited results, and they are therefore often frustrated. Counseling on the etiology and pathogenesis of the disorder with frank explanation of the expected impact of therapy is a critical component of management. Issues of sexuality should also be explored, because patients may find intercourse either uncomfortable or embarrassing.

Summary

Important points to remember are:

- take a complete history,
- account for exogenous factors,
- account for systemic diseases,
- rule out cervical sexually transmitted diseases,
- perform a complete evaluation including microscopy,
- consider episodic management and suppressive management, and
- counsel the patients intensively.

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79.b A couple with difficulty conceiving: is it due to previous sexually transmitted diseases?

John W Sellors
John A Collins

Definition of the problem

Pelvic infection and epididymo-orchitis are serious consequences of sexually transmitted disease (STD) that have known effects in the female on the function of the fallopian tubes (Chapter 63) and in the male on the ability to produce an adequate ejaculate. Although not all cases of STD have such clearly damaging outcomes, silent injury to the relevant tissues can occur, and the worry about fertility is a legitimate concern.

It has been estimated, from longitudinal studies among Swedish women, that about 8% of women develop laparoscopically proven salpingitis after infection caused by either *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. Less than 30% of cases of acute pelvic inflammatory disease have been proven to be due to *C. trachomatis* (by tubal specimens or serology), and a much smaller percentage has been linked to *N. gonorrhoeae*. The risk of tubal infertility in women is directly related to the number and severity of the episodes of pelvic inflammatory disease. Serologic studies in North American women have shown that at least half of the cases of tubal infertility and ectopic pregnancy are attributable to *C. trachomatis* infection.

Chlamydial and gonococcal infections frequently cause urethritis in young men. Spread into the upper genital tract can cause epididymo-orchitis, particularly in those who do not receive effective treatment. Epididymo-orchitis is invariably accompanied by pain with tenderness and swelling of the structures involved, while urethritis and other infections in men may be asymptomatic and may not lead to overt clinical manifestations in the infertile male partner. Although a link between epididymo-orchitis and infertility would be logical, a causal association has not yet been established. In epidemiologic studies, chlamydial serology was more often positive among infertile men but (with one exception — anti-chlamydial IgA in semen) the differences were not significant. A recent review found that studies linking prior STD infections and semen parameters among infertile men were methodologically flawed, and found a need for further research to demonstrate a link between STD and male infertility.

Typical case

A couple in their late twenties have not conceived after 2 years of coitus during which they did not use contraception. The history reveals no contributory information other than the concern that both partners had a history of STD as adolescents. They wish to know about any possible relationship between their infertility and STD and request further investigation and management.

Their question is not uncommon. In the average Western country, up to 10% of married couples are infertile. In those under 30 years of age, the prevalences of *C. trachomatis* and *N. gonorrhoeae* infections are approximately 7% and less than 1%, respectively, in asymptomatic women. The reported rates in men are less than one-sixth of these levels. In less developed countries, prevalence rates of these infections are generally higher and so too are the attributable fractions of infertility due to these preventable causes.

Diagnosis

The diagnostic assessment of infertility is designed to evaluate the couple for the presence of defects in ovulation, seminal or tubal function or the presence of endometriosis. These diagnostic categories (Fig. 79b.1) occur with the following approximate frequencies: ovulation defect 25%, seminal defect 25%, tubal defect 20% and endometriosis 5%. The remaining 25% have no apparent abnormality and are categorized as unexplained infertility. Of the 20% with a tubal defect, total tubal obstruction accounts for only one-quarter, and of the 25% with a seminal defect, azoospermia accounts for only one-fifth. Although oligospermia may occur in men who have a history of STD and infertile men who do not have such a history, it also occurs among fertile men. Even in the presence of an apparent deficiency such as oligospermia, however, it is possible that some other undetectable defect is the true cause of infertility.

Infertile couples should be managed together and both should undergo the testing that is deemed necessary in a given case. Initial testing includes general health screens in both partners, and cervical cytology and rubella antibody in the female partner. The investigation of infertility in the female partner includes a menstrual history and a midluteal progesterone assay to confirm ovulation. Follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and prolactin should be evaluated if the woman has amenorrhea. Semen analysis is necessary for the male partner and the semen analysis will be within normal limits in the majority of infertile male partners with a history of STD infection. If there is azoospermia, the FSH level should be estimated. Normal FSH levels are typical with obstructive azoospermia, which may be due to epididymo-orchitis or a related infection, while elevated FSH levels are common with non-obstructive azoospermia, which may be due to testicular failure.

For the evaluation of tubal disease, most clinicians prefer a hysterosalpingogram because it is a visual test that distinguishes between total and partial tubal obstruction. A laparoscopy is indicated as a primary procedure if there is a high risk of tubal disease or endometriosis and also whenever the hysterosalpingogram is abnormal. More than 50% of women who have tubal infertility have no history of pelvic inflammatory disease, however, and would not appear to be at high risk for tubal disease. The characteristic findings with either silent or manifest tubal disease include visible changes (adhesions, loss of fimbriae, tubal occlusion and hydrosalpinx) and microscopic changes, which range from minor impairment of the cilia to complete loss of epithelial function with evidence of chronic infection. When combined with laparoscopy, salpingoscopy allows an assessment of endotubal morphology.

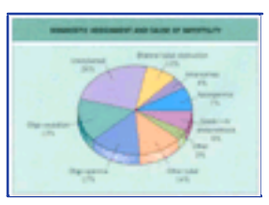

Figure 79.b-1 Diagnostic assignment and cause of infertility.

Genital culture should be carried out as a screening test before invasive testing. The most accurate tests for *C. trachomatis* are amplified nucleic acid assays (based on the polymerase chain reaction or the ligase chain reaction). *Neisseria gonorrhoeae* is best isolated by routine culture. Endocervical and urethral swab specimens are advisable to rule out infection in the woman and either urethral or first void urine specimens are acceptable in men. Chlamydial and gonorrheal serologic studies are not helpful in the investigation of infertility in either gender. Cultures for *Mycoplasma* spp. have no proven clinical use. Testing for antibodies to HIV may sometimes be warranted but there is a duty to provide counseling to the couple both before and after HIV testing.

Management options

Notwithstanding the diagnostic test findings, three characteristics of infertile couples are associated with a good prognosis:

- shorter duration of infertility;
- a previous pregnancy in the partnership (secondary infertility); and
- a younger female partner.

On average, untreated couples with only 2 years of primary infertility have a 22% likelihood of having a conception within 12 months that will lead to live birth. The rate

per month is just over 2% in the first 6 months.

If chlamydial, gonorrheal or trichomonal infections are found, the couple should be adequately treated with the appropriate antimicrobials. There is no evidence that presumptive treatment (of unproven infection) is an effective intervention for infertility. Similarly, the use of empiric antimicrobial therapy if leukocytospermia or endocervical leukocytosis is detected, in the absence of any defined pathogen, is of no proven benefit to the couple. For men who have oligospermia with or without a history of STD, empiric treatments such as clomiphene and antibiotics have not been demonstrated to be effective. In the presence of a varicocele, surgical repair is not an effective treatment for infertility. Intrauterine insemination of prepared sperm is associated with a small but statistically significant improvement in pregnancy rate, compared with intracervical insemination or timed intercourse. Intracytoplasmic sperm injection (ICSI) in an in-vitro fertilization (IVF) cycle is an effective but costly treatment for severe male infertility achieving pregnancy rates in excess of 25% per cycle. When the male partner has azoospermia, donor insemination is the most common treatment. Typical couples experience pregnancy rates as high as 15% per cycle of insemination. With obstructive azoospermia, sperm may be retrieved from the epididymis or the testis and even small numbers are sufficient for ICSI.

Many infertile women who have a history of STD have no evidence of tubal disease, but there may be either unilateral or bilateral occlusion and a variable extent of adhesions. When one fallopian tube is patent, the treatments include ovulation stimulation and intrauterine insemination, surgery and IVF. Bilateral tubal occlusion is optimally treated by IVF achieving pregnancy rates in excess of 25% per cycle, while microsurgery is associated with pregnancy rates of 15–20%.

Conclusions

Only 40–50% of infertile couples are successful in having a child and only 1–2% undergo IVF with or without ICSI, which is the most effective treatment for tubal infertility, severe male infertility or persistent infertility after the failure of conventional treatment. Not all couples choose to continue through every available investigation and treatment and therefore it is helpful for the physician to consider the wishes of the couple. Optimal infertility treatment decisions blend evidence from medical care research with the patients' preferences.

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Section 3 - SPECIAL PROBLEMS IN INFECTIOUS DISEASE PRACTICE

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Chapter 80 - Pathogenesis of Fever

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HISTORICAL CONSIDERATIONS

Akkadian cuneiform inscriptions confirm that fever has been recognized as a sign of disease since at least the 6th century BC. [1] Although various theories to explain the source of fever were advanced over the centuries, it was not until the 1850s that Claude Bernard correctly attributed the source of fever to metabolic processes within the body itself.

Our ability to measure body temperature progressed more quickly than our understanding of its source and regulation. Early devices used the expansion property of air to measure temperature, probably first in the 2nd century BC. In 1592, Sanctoria Sanctoria, a colleague of Galileo's at Padua, employed such a device in clinical studies, leading him to hypothesize the existence of a 'normal' body temperature. These early devices were prone to changes in barometric pressure until a closed thermometer employing alcohol was invented by Cornelius Drebbel in 1608. Some of the earliest closed liquid thermometers used scales based upon the freezing point of water and the oral temperature of a healthy human.[2] It was not until the early 18th century that Gabriel Daniel Fahrenheit popularized a reliable mercury-based thermometer with a scale based on the freezing and boiling points of water.

In spite of these advances, clinical thermometry was not fully integrated into medical practice until Carl Reinhold August Wunderlich published his sentinel work, *Das Verhalten der Eigenwärme in Krankenheiten* (The course of temperature in diseases) in 1868. In it, he reportedly examined some 25,000 patients from whom he obtained nearly 1 million individual measurements. These observations led him to propose 98.6°F (37°C) as the 'normal' body temperature and temperatures of 100.4°F (38°C) or above as fever.[3] [4] Despite the fact that these observations were made more than 130 years ago, were based on axillary temperatures and were obtained using thermometers calibrated 2.6–4.0°F (1.4–2.2°C) higher than contemporary thermometers, many clinicians continue to regard Wunderlich's observations as definitive.[5]

FEVER VERSUS HYPERTHERMIA

Fever is defined as 'a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (micro-organisms) or inanimate matter recognized as pathogenic or alien by the host'.[6] More simply, fever is a regulated rise in core temperature in response to a physiologic threat to the host. The febrile response, of which fever is a component, is characterized by a cytokine-mediated rise in core temperature, accompanied by increases in acute-phase reactants and a host of other immunologic, endocrinologic, neurologic and physiologic changes.

Fever and the febrile response should be differentiated from hyperthermia, which involves an unregulated increase in core temperature in which inflammatory cytokines play only a minor role. Hyperthermia is characterized by a sustained elevation of core temperature lacking the diurnal fluctuations characteristic of both fever and normal body temperature, and does not respond to antipyretic drug therapy.[7] [8]

Heat stroke, a cause of hyperthermia, often occurs in association with exertion and drugs, such as phenothiazines, anticholinergics or cocaine, that alter the body's ability to regulate heat. Classic heat stroke is most often seen in individuals at the extremes of age with co-morbid conditions. It is associated with a lack of sweating, moderate rhabdomyolysis and lactic acidosis, and rarely causes renal failure. In the exertional form of heat stroke, sweating is marked, making its detection more difficult. Rhabdomyolysis may be severe in exertional heat stroke, and, along with marked lactic acidosis, leads to a significant risk of renal failure. Common to both types of heat stroke is extreme hyperthermia, in which core temperatures may exceed 106°F (41.1°C).

Malignant hyperthermia, induced by general anesthetics in predisposed persons, and the closely related neuroleptic malignant syndrome are other types of hyperthermia. Malignant hyperthermia is a rare autosomal dominant disorder, usually triggered by inhalational anesthetic agents, which manifests as elevated core temperature, acidosis and muscular rigidity. Muscular rigidity appears to follow the development of hyperthermia in a majority of cases, suggesting that, at least initially, the increased core temperature is due to increased metabolism, not muscle contraction. Temperature increases rapidly, approximately 1.8°F (1°C) each minute. Core temperatures reaching 114.8°F (46°C) have been reported.[9] Hyperthyroid storm can also lead to hyperthermia, although in such cases temperatures rarely exceed 104°F (40°C).

CLINICAL THERMOMETRY

In clinical practice, fever is defined as a core temperature above the normal range. Presently, no physical examination is considered complete without a measurement of body temperature. Nevertheless, when interpreting the significance of such measurements, clinicians frequently disregard important variables such as the anatomic site at which the measurement is obtained, the time of day it is taken and the type of instrument used to obtain the temperature recording. Thermometers must be tested periodically according to the manufacturer's recommendations to ensure proper calibration.[10] [11] When measurements are taken, thermometers must be allowed adequate time to equilibrate with the test site. For example, when mercury-in-glass thermometers are used, rectal measurements require 1–5 minutes for optimal readings, whereas axillary and oral measurements require up to 11 minutes.[12] [13] Newer thermistor-based electronic thermometers have shorter equilibration times than traditional mercury-in-glass thermometers. Tympanic membrane thermometers equilibrate in seconds and are extremely convenient to use. Unfortunately, their results are inconsistent and correlate poorly with concurrent rectal and oral measurements.[2]

Core temperature is an amalgam of temperatures derived from the various internal organs, all having different metabolic rates. Under normal physiologic conditions such differences are small. However,

during shock or in the face of extreme thermal stresses, regional and organ-specific differences in temperature can be marked.[14] Because core temperature readings

are difficult to obtain, peripheral temperature measurements are used to approximate core temperature. Studies of the effect of anatomic site, oral stimulation and body position on estimates of core temperature have shown that mean rectal temperatures exceed oral by 0.8°F (0.4°C) and tympanic membrane temperatures by 1.6°F (0.8°C).[15] [16] During shock, rectal temperature may underestimate an elevated core temperature because of poor rectal perfusion.[17] Moreover, rectal measurements are associated with a small but significant risk of infection due to cross-contamination.[18] Oral temperature measurements have long been standard in clinical practice, largely because of accessibility but also because oral temperatures change quickly in response to changes in core temperature. Mastication and smoking both increase oral temperature. Ice-water ingestion decreases oral temperature, although only for a brief period.[16] Two recent studies of the effect of tachypnea and openmouth breathing on oral temperature found that open mouth breathing, but not tachypnea *per se*, lowers oral temperature.[19] [20] Because oral measurements require the subject's cooperation, they are difficult to obtain in critically ill and uncooperative patients.

NORMAL BODY TEMPERATURE

Despite the widespread application of thermometry in clinical medicine for over a century and a half, the definition of normal body temperature is still debated. A temperature of 98.6°F (37°C) is regarded as normal by most physicians and health care professionals, in all probability because of Wunderlich's work mentioned above. 'Normal body temperature', however, is not a specific temperature but a range of temperatures, the parameters of which vary from one person to another, as well as according to anatomic site and all those other factors noted above.[21] One of the largest studies of oral temperature in healthy subjects yielded a mean temperature of 98.2°F (36.8°C)[21] . Only 8% of the temperatures recorded were 98.6°F (37°C). In the subjects examined, the mean temperature varied diurnally, with a nadir at 0600h and a peak between 1600h and 1800h, a mean amplitude of variability of 0.9°F (0.5°C), and a range of 0.1–2.4°F (0.05–1.3°C). Women had slightly higher mean temperatures than men — 98.4°F (36.9°C) versus 98.1°F (36.7°C), $p < 0.001$ — but did not have greater diurnal variability. In this study population, 98.6°F (37°C) had no special significance with regard to normal body temperature (Fig. 80.1). The upper limit of normal temperature (i.e. greater than the 99th percentile), varied according to the time of day from an early morning low of 99.0°F (37.2°C) to an evening peak of 100°F (37.8°C).

THERMOREGULATION

Mammals employ various thermogenic mechanisms to increase heat production during episodes of cold exposure and during the initiation of fever. Shivering is a principal thermogenic mechanism, except in neonates. Nonshivering thermogenesis is most closely linked to brown adipose tissue, so named because of its brown color, which is due to a profuse vascular supply and a high concentration of mitochondria. Brown adipose tissue is most prominent near the shoulder blades, neck, adrenals and deep blood vessels. Noradrenaline (norepinephrine) induces enzymatic hydrolysis of triglycerides to glycerol and free fatty acids in brown adipose tissue. These free fatty acids are the primary substrate oxidized by mitochondria to produce adenosine triphosphate (ATP) and heat. They also act as a signal to uncouple oxidative phosphorylation, thereby generating excess heat.

In the resting state, approximately 72% of basal metabolic heat is produced by vital organs. Heat is distributed throughout the body

Figure 80-1 Distribution of baseline oral temperatures in healthy men and women. Frequency distribution of 700 baseline oral temperatures obtained during two consecutive days of observation in 148 healthy young volunteers. Arrow indicates location of 98.6°F (37°C). *With permission from Mackowiak et al.[21] Copyright 1992, American Medical Association.*

via the circulatory system. Variations in cutaneous blood flow determine the amount of heat lost at the skin surface by radiation, evaporation, convection and conduction. [22] When environmental temperature exceeds body temperature, evaporation is the most effective means of heat dissipation. The balance between heat production and heat loss, largely under control of the autonomic nervous system, is the means by which body temperature is maintained near the hypothalamic set-point of 98.2°F (36.8°C). When it is necessary to decrease core temperature, blood flow is preferentially directed to the skin and sweating mechanisms are activated. In cold environments or when fever dictates an increase in core temperature, blood flow is directed away from the surface towards the internal vital organs to minimize heat transfer to the environment.

More than 60 years of investigation have suggested that no single, central location is responsible for temperature regulation. Rather, a series of hierarchical structures appear to control body temperature (Fig. 80.2). This hierarchical system includes a continuum of structures from the hypothalamus and limbic system extending through the brain stem, reticular formation, spinal cord and sympathetic ganglia. The rostral hypothalamus, referred to as the 'preoptic' region, is actually composed of the medial and lateral aspects of the preoptic area, the anterior hypothalamus and the septum. This preoptic region plays a pivotal role in thermoregulation and appears to have greater thermosensitivity than other structures, which allows it to detect subtle changes in body temperature. The preoptic region orchestrates thermoregulatory responses in other effector areas via signals transmitted through the median forebrain bundle, a bidirectional pathway within the lateral hypothalamus.[23] Indeed, the median forebrain bundle has been shown to manage efferent signals controlling shivering and cutaneous blood flow.[24] Afferent signals ascend to the brain stem reticular formation via the lateral spinothalamic tract. The preoptic region of the hypothalamus, in turn, receives this information and integrates afferent and efferent signals to maintain the appropriate body temperature.

The rostral hypothalamus coordinates thermoregulation via a subset of temperature-sensitive preoptic neurons. There are three basic types of such neuron, which differ according to their responses to temperature changes. So called 'warm-sensitive neurons', accounting

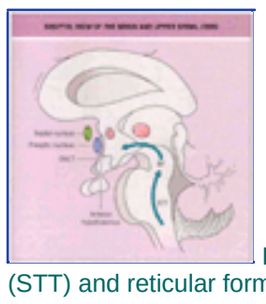

Figure 80-2 Sagittal view of the brain and upper spinal cord. The figure shows the multisynaptic pathway of skin and spinal thermoreceptors through the spinothalamic tract (STT) and reticular formation (RF) to the anterior hypothalamus, the preoptic region and the septum. *Redrawn with permission from Mackowiak.[1]*

for approximately 30% of the preoptic neuronal population, increase their firing rates in response to increases in preoptic temperature. Their efferent output initiates heat loss responses proportional to increases in body temperature over a certain threshold. A smaller number of 'cold-sensitive neurons' present in the preoptic region comprise less than 5% of total neurons. These neurons increase their firing rates as body temperature decreases. Cold-sensitive neurons are under synaptic inhibition from warm-sensitive neurons and, as the firing rates of warm-sensitive neurons decrease, so does the synaptic inhibition on cold-sensitive neurons. The net result is an increase in the firing rates of the cold-sensitive neurons[23] (Fig. 80.3). It may be that only the warm-sensitive neurons are truly thermosensitive and that thermoregulation is accomplished through their inhibitory and excitatory signals.[25] [26] [27] The majority of preoptic neurons do not demonstrate changes in firing rates in response to changes in preoptic temperature.

Pyrogens

Although peripheral neuronal input is of paramount importance during normal homeostatic thermoregulation, soluble 'endogenous pyrogens' (pyrogenic cytokines) exert a pronounced effect on temperature regulation during fever. These pyrogens are thought to mediate fever by elevating the hypothalamic set-point temperature (see also Chapter 2).

Pyrogens are artificially divided into 'endogenous' and 'exogenous' varieties. Pyrogenic cytokines are endogenous immunoregulatory polypeptides, such as interleukin (IL)-1ß, IL-6 and tumor necrosis factor (TNF)-a. They are primarily derived from stimulated mononuclear phagocytes and are capable of direct interaction with the anterior hypothalamus to elevate the core temperature set-point. Exogenous pyrogens are the usual stimuli that induce mononuclear cells to produce and release pyrogenic cytokines. They include substances such as lipopolysaccharide (LPS), peptidoglycan, lipopeptides

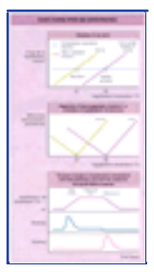

Figure 80-3 Events during fever and defervescence. When pyrogens are present in the preoptic region the whole body and neuronal responses are as shown by the purple lines. Upper: Normally the firing rates of warm-sensitive and temperature-insensitive neurons functionally overlap at 98.6°F (37°C), the set-point of thermoregulatory neurons. During pyrogen inhibition of warm-sensitive neurons this overlap occurs at the raised set-point of 102.2°F (39°C). Center: During fever, heat production is initially great but, as body temperature rises towards 102.2°F, heat production diminishes and should cease at 102.2°F. Lower: During fever initiation, shivering causes an increase in hypothalamic temperature and then ceases as the temperature reaches 102.2°F. During defervescence the hypothalamus activates heat loss responses such as sweating. *Adapted with permission from Boulant.*[23]

and numerous other microbial products. The distinction between endogenous and exogenous pyrogens and their ability to initiate fever is artificial, since some endogenous molecules can themselves induce the production of pyrogenic cytokines.

Figure 80-4 Production of fever. Microbial agents, inflammatory agents and some cytokines induce the synthesis and release of pyrogenic cytokines from a variety of cells. These cytokines, in turn, trigger specialized endothelial cells of the hypothalamic vascular organs, which release PGE_2 . Elevated PGE_2 then brings about increases in cyclic adenosine monophosphate (cAMP) monoamines and calcium in the thermoregulatory center of the anterior hypothalamus, resulting in a resetting of the thermostatic temperature from normothermia to febrile levels. These neurotransmitters then activate the vasomotor center, which brings about vasoconstriction (heat conservation) and increased heat production, both resulting in an increase in blood temperature (fever). Regardless of the cause of fever, antipyretics inhibit this pathway by preventing the increase in cytokine-mediated PGE_2 production in the hypothalamus. IFN, interferon.

Antigen- antibody complexes, complement, inflammatory bile acids and androgenic steroid metabolites are examples. Pyrogenic cytokines have numerous activities other than pyrogenesis, including autocrine, paracrine and endocrine functions that determine the character of the febrile response. Finally, the recent recognition that Toll-like receptors (TLRs) are expressed in the organum vasculosum laminae terminalis (OVLT) means that it is likely that exogenous pyrogens can induce fever directly, independently of proinflammatory cytokines.

Current concepts maintain that fever is initiated by exogenous pyrogens, such as LPS which exert their pyrogenic effect by increasing circulating pyrogenic cytokines, thereby elevating the thermal set-point of the thermoregulatory center in the preoptic region of the anterior hypothalamus (Fig. 80.4). However, being large, hydrophilic molecules, pyrogenic cytokines do not easily cross the blood-brain barrier, and it is unclear how they interact with central regions in the brain. One way in which they might gain access to thermoregulatory sites within the brain is through the relatively leaky blood-brain barrier associated with the OVLT. However, even in this region, and with the assistance of active transport, passage of pyrogenic cytokines across the blood-brain barrier is too slow and modest to account for the initial rapid onset of fever seen following the intravenous administration of LPS, IL-1 or IL-6.[28] Endothelial and microglial cells at the blood-brain barrier have IL-1 receptors and, in response to IL-1 and LPS, synthesize TNF-a, IL-1ß and IL-6, as well as cyclo-oxygenase (COX)-2 mRNA.[26] [29] Interaction of pyrogenic cytokines with cells at the OVLT might also induce responses that route the febrile signal inward toward the preoptic area via secondary cytokine production, phospholipase A_2 /COX-2 activity, a nitric oxide secondary messenger, or via direct neural input to the preoptic region.[30] Prostaglandin $(PG)E_2$, on the other hand, is able to cross the blood-brain barrier. Moreover, inhibiting PGE_2 synthesis blocks cytokine-induced fever, supporting its primary role in the pathogenesis of fever. Nitric oxide is generated by endothelial cells in the blood-brain barrier in response to IL-1 and IL-6. However, its role as a secondary messenger of the fever response is uncertain. [31]

It is more than likely that there are alternative mechanisms for activating the OVLT. In certain experimental models, circulating levels of IL-1 do not correlate with the initial fever peak, although IL-6 may correspond to subsequent peaks.[26] [32] Such observations suggest that IL-1 and TNF-a are paracrine modulators and IL-6 an endocrine modulator.[33] Yet other models suggest that bacterial superantigens, such as staphylococcal enterotoxin B, induce cell-associated cytokines that activate endothelial and microglial cells at the OVLT in the absence of high levels of circulating, cell-free cytokines.[34] Moreover, hepatic vagal fibers, stimulated by PGE_2 produced by Kupffer cells, appear to induce fever through neural input to the preoptic area.[28] Thus, it is possible that different combinations of mechanisms control fever in different situations.[34]

Acute-phase response

Fever is just one component of the febrile response, which also encompasses a host of other physiologic alterations collectively referred to as the 'acute-phase response'. Often fever and the acute-phase response are linked, as in trauma or infection. However, they can also exist independently, suggesting that, although pyrogenic cytokines have the capacity to elicit both responses, fever and the acute-phase response are, nevertheless, independently regulated entities (see also Chapter 2).

A wide array of physiologic and endocrinologic alterations occur during the febrile response. These include anorexia and sleepiness, and altered synthesis of glucagon, insulin, adrenocorticotropic hormone, cortisol, catecholamines, growth hormone, thyroid stimulating hormone, thyroxine, aldosterone and arginine vasopressin. Inhibition of bone formation, negative nitrogen balance, gluconeogenesis and altered lipid metabolism also occur. Serum levels of zinc and iron fall, whereas copper levels rise. Decreased erythropoiesis, in part responsible for 'anemia of chronic disease' (or, more appropriately 'anemia of chronic inflammation'), thrombocytosis and leukocytosis are additional features.[35]

Like fever, the acute-phase response is a tightly regulated response activated by a variety of stimuli (e.g. surgery, trauma, infection, cancer, burns). It is further characterized by production of an array of acute-phase proteins. These proteins are designated 'positive' or 'negative', depending on whether their levels rise or fall during the acute-phase response (Table 80.1). Those belonging to the former category are substantially more prominent than those belonging to the latter. The major positive acute-phase proteins are C-reactive protein (CRP) and serum amyloid A. Negative acute-phase proteins include albumin, pre-albumin (transthyretin) and transferrin. C-reactive protein and serum amyloid A normally exist in only minute concentrations but increase more than 1000-fold during the acute-phase response. C-reactive protein enhances phagocytosis by binding phosphocholine, present on foreign pathogens and damaged host cells, and activates complement. In

TABLE 80-1 -- Acute phase proteins.[‡]

ACUTE PHASE PROTEINS	
Positive acute-phase proteins[*]	
CRP	Ferritin
Serum amyloid A	Phospholipase A_2
Haptoglobin	Plasminogen activator inhibitor-1
a_1 -Acid glycoprotein	Fibronectin
a_1 -Protease inhibitor	Hemopexin
Fibrinogen	Pancreatic secretory trypsin inhibitor
Ceruloplasmin	Inter-alpha protease inhibitor

Complement (C3 and C4)	Mannose binding protein
C-1 esterase inhibitor	LPS binding protein
C4b binding protein	
a_2 -Macroglobulin	
Negative acute-phase proteins†	
Albumin	Transferrin
Transthyretin	a_2 -HS glycoprotein

‡ *Adapted from Kushner and Rzewnicki.*[36]
* Proteins exhibiting increased plasma concentrations during the acute-phase response.
† Proteins exhibiting decreased plasma concentrations during the acute-phase response.

certain situations, CRP stimulates thrombosis and promotes inflammatory cytokine production and the release of tissue factor by monocytes. Nevertheless, considerable evidence suggests that, in vivo, CRP functions primarily as an anti-inflammatory mediator.[37] Serum amyloid A enhances phagocyte adhesion and chemotaxis. Complement components, another group of acute-phase proteins, influence chemotaxis, opsonization and vascular permeability. The antioxidants and anti-inflammatory molecules haptoglobin and ceruloplasmin also appear to function as both positive acute-phase proteins and modulators of the inflammatory response. The thrombocytosis, decrease in iron and zinc levels and other features of the acute-phase response all appear to be important components of the defense against bacterial pathogens. [38]

Cryogens

Core temperature rarely if ever reaches, far less exceeds, 106.0°F (41.1°C) during fever. This suggests that fever has a thermal ceiling designed to protect the host against the deleterious effects of temperatures higher than 106.0°F (41.1°C).[32] Indeed, the warm-sensitive neurons in the preoptic region reach their peak firing rates and the cold-sensitive neurons in the same region are maximally suppressed at 107.6°F (42°C), suggesting that the anterior hypothalamus is incapable of thermoregulation at temperatures higher than 107.6°F (42°C).[39] Various mechanisms, including pyrogen tolerance, circulating cryogens and modification of effector synthesis, appear to work to prevent core temperature from rising to 107.6°F (42°C). The pyrogenic effects of IL-1 and TNF-a are attenuated after repeated or prolonged exposures. Moreover, an important feature of tolerance to LPS is a decrease in pyrogenic cytokine production by macrophages.[32] [40]

The best characterized endogenous cryogens are a-melanocyte stimulating hormone (aMSH), arginine vasopressin (AVP), lipocortin-1, glucocorticoids and IL-10.[41] [42] [43] IL-10 has been shown to prevent LPS-induced fever by attenuating the production of TNF-a, IL-6 and IL-1 and increasing release of soluble IL-1-receptor antagonist, and through direct interaction with microglial cells in the central nervous system.[44] Arginine vasopressin appears to exert its cryogenic effects as a neurotransmitter of specialized neurons projecting into the ventroseptal area. Firing of such neurons increases during fever.[45] Moreover, neutralization of AVP during experimental fever is associated with increases in both the height and the duration of fever, all of which suggest a cryogenic role for AVP.[43] The melanocortin aMSH is a neurotransmitter of neurons with projections to the hypothalamic paraventricular nucleus, the integrative output nucleus for fever and numerous autonomic stress responses. Under experimental conditions, peripheral and intracerebral administration of aMSH blocks IL-1- and LPS-induced fever. Centrally administered aMSH-receptor blockers inhibit the cryogenic effect of aMSH.[46]

Arachidonic acid liberated from cell membranes by the action of phospholipase can be metabolized either by COX to pyrogenic PGE_2 or via an alternative cytochrome-p450-mediated pathway to cryogenic eicosanoids (epoxyeicosanoids). Induction of cytochrome p450 shifts arachidonic acid metabolism away from COX and toward the cytochrome p450 pathway, thereby reducing core temperature through a combination of diminished PGE_2 production and an increase in production of cryogenic epoxyeicosanoids.[47]

ANTIPYRETICS

Aspirin and acetaminophen (paracetamol) have been a mainstay of antipyretic drug therapy since the turn of the 20th century. Release of the fenamates in the 1950s and indomethacin in 1963 was followed by introduction of a host of nonsteroidal anti-inflammatory drugs (NSAIDs), all with antipyretic properties. Despite the popularity of these drugs among both health care workers and the general public, it is still not clear that reducing core body temperature benefits febrile patients. Although antipyretic therapy assumes that fever is, at least in part, noxious and that suppression of fever will eliminate or reduce its noxious effects, neither assumption has been validated experimentally. Consequently, rational guidelines for suppressing fever have been slow in coming.

Fever creates a number of potential metabolic challenges for the host. During the chill or ascending phase of fever, activation of the sympathetic nervous system causes peripheral vasoconstriction and an associated increase in mean arterial pressure.[48] Oxygen consumption increases, as does carbon dioxide production.[49] External cooling can attenuate these effects, but only if shivering is prevented.[50] If shivering is not prevented, external cooling reduces skin temperature faster than core temperature and initiates vasoconstriction and shivering, which can paradoxically increase core temperature, oxygen consumption and the respiratory quotient. Importantly, external cooling can also cause vasospasm of diseased coronary arteries.[51] Unfortunately, certain antipyretic drugs also cause coronary vasoconstriction in patients who have coronary artery disease. Friedman *et al*. [52] observed significant increases in mean arterial pressure, coronary vascular resistance and myocardial arteriovenous oxygen difference after administration of intravenous indomethacin (0.5mg/kg) in such patients. Mean coronary blood flow decreased simultaneously from 181 ± 29 to 111 ± 14ml/min ($p < 0.05$). Thus, in this investigation, myocardial oxygen demand increased in the face of a fall in coronary blood flow following indomethacin administration.

Aspirin, acetaminophen and the NSAIDs all inhibit COX-mediated synthesis of inflammatory thromboxanes and prostaglandins from arachidonic acid. Cyclo-oxygenase has central and peripheral forms, as well as at least two distinct isoforms, COX-1 and COX-2. Cyclo-oxygenase-1 is expressed constitutively by cells and is involved in various housekeeping functions. Cyclo-oxygenase-2 is an inducible enzyme produced by a variety of cell lines, including macrophages, synoviocytes and endothelial cells. Pyrogenic cytokines and LPS induce COX-2 expression. Prostaglandin E_2 formed by COX-2 in microvascular endothelial cells is responsible for hypothalamus-mediated fever.

The distinctive affinities of the various categories of antipyretic drug for the different COX variants are thought to determine their

relative antipyretic and analgesic potencies. Acetaminophen and aspirin, for example, are equally potent inhibitors of central COX but only 10% as potent in this regard as indomethacin. Acetaminophen is only 0.02% as potent an inhibitor of peripheral COX as indomethacin. Its relatively poor activity against peripheral COX is thought to explain acetaminophen's weak systemic anti-inflammatory activity.[53] Only aspirin irreversibly inhibits COX and does so via acetylation within the active site of the enzyme. Other NSAIDs and acetaminophen inhibit COX reversibly.

Recent studies have shown that aspirin and the NSAIDs have COX-independent antipyretic activity. Aspirin induces cytochrome p450, which might augment its antipyretic effect by shifting arachidonic acid metabolism toward cytochrome-p450-mediated production of cryogenic epoxyeicosanoids. Additionally, acetylation of COX-2 by aspirin increases the production of 15R-hydroxyeicosatetraenoic acid, which neutrophils use to form aspirin triggered lipoxins (ATL). These lipoxins have potent anti-inflammatory activity, independent of aspirin. Heat shock proteins have been shown to reduce transcription of IL-1ß in vitro, and therapeutic doses of aspirin and certain NSAIDs increase heat shock factor-1 concentration in vitro. These same drugs also diminish the activity of transcriptional activator nuclear factor-?B.[54] The latter is involved in the transcription of pyrogenic cytokines, adhesion molecules, inducible nitric oxide synthase and COX-2 in certain cell lines. Production of adenosine, an anti-inflammatory mediator produced by leukocytes, is enhanced by aspirin and NSAIDs. The clinical implications of these alternative antipyretic pathways remain to be determined.

Antipyretic therapy is commonly administered to enhance patient comfort. General experience with antipyretic drugs, which are for the most part also analgesic agents, seems to support this rationale. However, carefully controlled efficacy studies have never quantified the degree to which antipyretic therapy enhances the comfort of patients who have fever. Moreover, the relative cost of such symptomatic relief, in terms of drug toxicity and adverse effects of the antipyretic agents on the course of the illness responsible for the fever, has never been determined. The importance of these negative drug effects is underscored by studies that demonstrated prolonged time to crusting of varicella skin lesions in children,[55] increased rhinovirus shedding and decreased neutralizing antibody formation following acetaminophen use in adults.[56] [57] Paracetamol use has been reported to prolong parasite clearance time in children infected with *Plasmodium falciparum*, presumably by decreasing

production of TNF and oxygen radicals.[58] The risks of antipyretic drug therapy, including direct and indirect drug toxicity, increased morbidity from infection and the masking of a possible underlying infection, must be weighed against any possible benefit.

Antipyretic therapy has not yet proved effective in preventing febrile seizures.[59] Camfield *et al*.[60] conducted a randomized double-blind study comparing single daily-dose phenobarbital plus antipyretic instruction with placebo plus antipyretic instruction in preventing recurrent febrile seizures following an initial simple febrile seizure. In children treated with phenobarbital and antipyretics, the febrile seizure recurrence rate was 5%, whereas in those receiving placebo and antipyretics the rate was 25%, suggesting that a single daily dose of phenobarbital is more effective than counseling parents about antipyretic therapy in preventing recurrent febrile seizures. More recent studies in children have shown that, whether given in moderate doses or in relatively high doses, both acetaminophen[61] and ibuprofen[62] fail to reduce the rate of recurrence of febrile seizures.

Finally, there has been considerable recent interest in the use of antipyretic drugs to modulate the activity of pyrogenic cytokines during bacterial sepsis.[63] In certain animal models, antipyretic drugs that inhibit COX confer protection when given soon after bacterial challenge, presumably by blunting the adverse effects of TNF-a and IL-1. In a recent large clinical trial, Bernard *et al*.[64] reported that 48 hours of intravenous therapy with the COX inhibitor, ibuprofen, lowered core temperature, heart rate, oxygen consumption and blood levels of lactic acid but did not decrease the incidence of organ failure or 30-day mortality rate in patients who have sepsis. Despite promising results in some experimental models, ibuprofen has not yet been shown to be of clinical value in treating bacterial sepsis.

Although clinicians have used various forms of antipyretic therapy since time immemorial, there is a dearth of data concerning the benefits and relative risks of such treatments. Nevertheless, several tentative conclusions regarding antipyretic therapy seem justified in light of the limited data available. It is clear, for instance, that short courses of approved doses of standard antipyretic drugs carry a low risk of toxicity. Most antipyretic drugs have analgesic as well as antipyretic properties. Therefore, if not otherwise contraindicated (as is, for instance, aspirin in young children because of the risk of Reye syndrome), such drugs can be used to provide symptomatic relief in patients who have fever, to reduce the metabolic demands of fever in chronically debilitated patients and possibly to prevent or alleviate fever-associated mental dysfunction in the elderly. To minimize antipyretic-induced fluctuations in temperature and the risk of recurrent shivering and its increased metabolic demands, antipyretic agents should be administered to patients who have fever at regular intervals to preclude abrupt recurrences, rather than as needed for temperatures above some arbitrary level. When prescribing such medication, physicians must recognize that each carries its own risk of toxicity and may prolong the course of at least some infections. It should be noted further that there is no compelling evidence that a response to antipyretic medications is useful diagnostically in distinguishing serious from self-limited illnesses, nor is there evidence that such medications are effective in suppressing febrile seizures, even if given prophylactically.

In view of the capacity of external cooling measures to induce a cold pressor response, it is questionable whether this form of antipyretic therapy should ever be administered to patients who have fever. If external cooling is used to treat fever, care must be taken to prevent shivering because of its associated increase in oxygen consumption. Unfortunately, even if shivering is prevented, there is no guarantee that a cold pressor response will be averted. In view of indomethacin's capacity to cause coronary vasoconstriction in patients who have coronary artery disease, NSAIDs should be used with caution, if at all, to suppress fever in such patients.

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Chapter 81 - Clinical Approach to the Acutely Febrile Patient

Harold Lambert

Fever is one of the most frequent symptoms that leads to consultation with a health professional. Vast numbers of febrile illnesses are of short duration and benign outlook, and few of these are diagnosed. However, in the midst of this mass of minor illness are patients whose illness is serious or likely to become so. Some of these serious illnesses also present a danger to others, and thus have a significance beyond that of the individual patient. This is the challenge of acute fever: to distinguish the threatening from the trivial in acute illnesses in which fever is a main feature.

In some cases, of course, the fever becomes prolonged and this topic of more longlasting fever (fever of unknown origin; FUO) is discussed in Chapter 82 . The distinction between fevers of short and those of longer duration is important in considering diagnostic possibilities. Many acute and short-lived fevers are of viral origin, and conversely viral fevers without particular diagnostic features rarely last longer than 1 or 2 weeks; thus, prolonged fever without distinguishing features is rarely caused by a virus, at least in immunologically normal patients.

HISTORY

As with any other medical problem, the history is the most productive component of the initial encounter. Hypotheses are generated from the patient's account and the physician's observations, and are successively pursued or rejected in the light of emerging data. In the case of acute fever, however, a few special features of the history stand out. One of these is the common difficulty in reaching agreement on the meaning of words and phrases used in this context. It is rare for the phrase 'I have a fever' to mean that the body temperature has been measured by a reliable method. More often, the phrase means that the patient has a subjective sensation of warmth, or a feeling of chilliness or undue sweating. 'Flu' is another word in common use and usually describes aching muscles, chills and shivering, but sometimes is used to denote upper respiratory symptoms such as a runny nose or scratchy throat, and sometimes to indicate a fever. And, of course, different meanings are attached to these and other words in different cultures and languages.

A second distinctive point when taking the history of a patient with acute fever relates to the time-honored 'systems review'. This is usually employed, if at all, at the end of a history taking, but often has so little to contribute that it is discarded. In the case of acute fever, however, because the range of possibilities is so much wider than for many presenting symptoms, the systems enquiry is useful early in the history taking; it often reveals the only relevant localizing evidence, as patients may have forgotten or thought insignificant symptoms that provide valuable clues. Among the apparently minor clues that may emerge on direct enquiry are minor respiratory, abdominal or urinary symptoms, a transient rash or previous episodes of illness of a similar nature. Apparent localizing features that are actually symptoms of the raised temperature may, however, give false leads. Thus, dark urine in a febrile patient may simply denote dehydration, and some patients, especially women, experience burning and discomfort passing urine when they are febrile. Muscle and joint pains are also hard to interpret during fever: severe pains suggest viral infections such as influenza or dengue, but they are also a feature of some enteroviral infections (Bornholm disease) and of leptospirosis. Neurologic features are especially difficult. Some patients regularly experience headache when febrile, and many children and some older patients become delirious with a high fever; whether such clinical features indicate a specifically neurologic involvement obviously needs careful observation.

A history of medication may be important in acute febrile disease. A large number of drugs may themselves cause fever, often without a rash or other clear indicators. Among antimicrobial agents, penicillins, cephalosporins and sulfonamides are especially notable, but many others may be implicated. Anti-infective agents may suppress or modify infections, and so confound a diagnosis, and antipyretic agents may greatly modify the pattern of fever in an infection.

Travel history

As rapid movements of vast numbers of people throughout the world have been made possible in the era of air travel, so have the possibilities of an infection developing in one country when it was acquired in another. A travel history must never be omitted in a patient who has fever and many tragic deaths from *Plasmodium falciparum* malaria testify to its importance. Acute fever and respiratory symptoms after a recent trip to Southern China may point to a diagnosis of the newly recognized severe acute respiratory syndrome (SARS). The history should be accurate as to time and place. The name of a country is not enough; a stay in a four-star hotel in a capital city presents different risks from those of a camping trek in rural areas of the same country. Timing is especially helpful, even though many infections have a wide range of recorded incubation periods. For example, an illness beginning more than about 10 days after return is unlikely to be one of the common acute respiratory infections, with the exception of *Mycoplasma pneumoniae* and perhaps Q fever; dengue, too, and other arbovirus infections would have developed by this time. An incubation period of more than 3 weeks excludes the hemorrhagic virus infections, such as Lassa fever, and almost excludes typhoid. On the other hand, longer periods still leave open the possibility of viral hepatitis, Katayama fever (acute schistosomiasis) and primary HIV infection. As to malaria, the incubation period of *P. falciparum* infection may be as little as 1 week, but after 6 or 8 weeks a first presentation of this form of malaria becomes uncommon. *Plasmodium vivax* and *Plasmodium malariae* infections may develop months or years after travel to a malarial area.

Other important aspects of the travel history are the immunization record and an account of medications, with special emphasis on antimalarial prophylaxis.

It is sometimes necessary to begin treatment before a definite diagnosis has been made, or when the causal organism but not its antibiotic susceptibility is known. The travel history is important here too, as the pattern of antibiotic resistance in many pathogens

varies greatly from country to country, and will determine an appropriate choice of therapy. Notable examples are the differences in drug resistance in malaria in different countries, the spread of multi-resistant typhoid and shigellosis and the erratic distribution of pneumococcal resistance to penicillin. In each of these examples, the area in which infection was acquired may limit the options available for chemotherapy.

Every physician who sees a febrile traveler cannot be expected to have an up-to-date knowledge of the precise infective risks, let alone the antibiotic resistance patterns of possible pathogens, and therefore early communication with a Tropical and Infectious Diseases Unit and with one of the specialized information services that deals with travel medicine, which are available in many countries, is essential.

What are the actual causes of acute fever in returning travelers? (As this chapter focuses on illnesses with a large element of fever, primarily diarrheal diseases, or sexually transmitted diseases with mainly local symptoms and signs, and many other health risks of travel are not discussed here, see Chapter 145 .) Contrary to popular myth, the 'classic' tropical diseases are rarely acquired by short-term travelers, with the vital exceptio of malaria. This ranks first among diagnoses of acute fever in returning travelers, followed by a large group of short-lived fevers for which no etiology is ever established. Other diagnoses obviously vary in their frequency with the pattern of travel to and from a particular country. As the traveler is exposed not only to exotic pathogens, but also to a changing ecologic background of widely distributed pathogens, it is not surprising that ordinary respiratory infections, ranging from colds to pneumonia, are common. So too are initially febrile presentations of diarrheal diseases and prodromes of hepatitis. Urinary infection, as one of the most common causes of fever in women not always accompanied by localizing symptoms, must also be remembered. Some diagnoses encountered with widely variable frequency in different units are listed in Table 81.1 .

Contact history

Contact history may be relevant in travelers and in people staying at home. Information about local endemic or epidemic infections is to be sought. Most frequent of all, especially in the winter months, is a history of contact with acute respiratory infection, which is possibly relevant because so many respiratory infections begin with 1 or 2 days of indeterminate fever, but the very frequency of such infections

TABLE 81-1 -- Diagnoses made in travelers returning from tropical countries with fever as a principal symptom.

FEVER IN RETURNING TRAVELERS
Common
Malaria
No diagnosis made
Respiratory infection
Diarrheal disease (fever before or accompanying gut symptoms)
Urinary tract infection
Viral hepatitis; febrile prodrome
Uncommon
Dengue
Typhoid
Tuberculosis

Acute HIV infection
Acute schistosomiasis
Rickettsial infections
Amebiasis

makes this aspect of the history difficult to interpret. Even in places with high uptakes of routine immunization, measles is encountered, and tends then to be missed because of its low prevalence and because it may occur in older subjects in highly immunized populations. Measles is especially important when the patient, or a contact, is immunosuppressed. Rubella, a more difficult and uncertain clinical diagnosis, is clearly of the greatest import if the patient or a contact is pregnant. Known contact with meningococcal disease obviously demands immediate attention, although, owing to the vagaries of meningococcal carriage and immunity, few patients who have this disease have a direct contact history. A story of 'food poisoning' or diarrheal disease in contacts may be relevant because shigellosis, salmonellosis and *Campylobacter jejuni* infections may all begin with a febrile phase, while ingestion of raw milk and some cheeses raises the possibilities of brucellosis and listerial infection. The long incubation periods of most forms of viral hepatitis should be remembered in exploring possible contact history. Of those most commonly associated with travel, the incubation period of hepatitis A is 2–6 weeks and that of hepatitis B from 4 weeks to many months.

Occupational history

Many occupational exposures are not particularly relevant to acute febrile presentations, but many of the points about contact history just discussed are especially applicable to health workers and to those involved in child care. Other specific risks arising from occupation that may present as an acute fever include leptospirosis in sewage workers and fish farmers, and the many infective risks in veterinary and abattoir work, including brucellosis and *Streptococcus suis* infection. Fever may be the first clinical manifestation of tuberculosis relevant to health professionals, especially those involved with the care of patients who have HIV, and to carers of the homeless (see Chapter 90).

Some fevers associated with occupation are not infective. Fever and chills may be caused by inhalation of metal fumes or breakdown products of polymers. Many occupational lung diseases manifest with primarily respiratory features, but in extrinsic allergic alveolitis fever and influenza-like symptoms may dominate the picture. These conditions are characterized by recurrent episodes related to the particular exposure.

Bioterrorism and fever

Several agents that might be involved in bioterrorism are likely to cause non-specific fever as their initial manifestation (Table 81.2). In smallpox, after the incubation period of 12 days, there is nearly always a period of 2 or 3 days of fever and chills, perhaps rigors, headache and backache before the focal rash begins to emerge. In the most severe forms, hemorragic manifestations appear and the patient

TABLE 81-2 -- Bioterrorism and fever.

BIOTERRORISM AND FEVER	
Agent	**Main early features**
Smallpox	Non-specific fever for 2–3 days followed by rash slowly progressing to vesicles or pustules OR hemorrhages, system failure and death within a few days
Pulmonary anthrax	Rapidly progressive systemic illness with fever, dyspnea, sometimes wide mediastinum and pleural effusions
Pneumonic plague	Rapidly progressive systemic illness with respiratory features prominent from the second day. Extensive, often bilateral, pneumonia

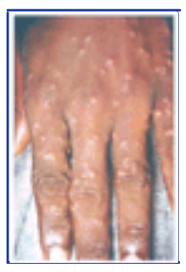

Figure 81-1 The early focal rash of smallpox. This is the fifth day of the fever and the third day since the rash began to appear.

dies without ever developing the focal rash. An early focal rash is illustrated in Figure 81.1 . The onset of plague is also very acute, with chills, rigors, headache and generalized pains. Signs of system failure including generalized hemorrhage become apparent early in the septicemic form, but are also often seen together with enlarged nodes in bubonic plague. Pulmonary anthrax usually has a very acute onset, with early respiratory and systemic symptoms, but less acute presentations are now known to occur. Chest radiography may show mediastinal widening. (For a further discussion of bioterrorism see Chapter 6 .)

PHYSICAL EXAMINATION

Temperature

Depending on the duration of the illness, few temperature measurements may be available for evaluation. Recorded temperatures of higher than 102.2°F (39.0°C) are more likely to be caused by a significant infection than are lesser degrees of fever, but very high fever must raise suspicion of a noninfectious cause such as heat stroke or substance abuse. The pattern of fever is much less valuable than is commonly supposed. This aspect is discussed more fully in Chapter 82 , but a few points relevant to short-term fevers may be mentioned. A dramatic fever with wild swings between readings is suggestive of pyogenic infection and especially of abscess formation, and of acute pyelonephritis, but may also be seen in other conditions, including malaria and disseminated tuberculosis, also in Still's disease and occasionally in drug fever. Perhaps the most common reason for this kind of chart, however, is the use of antipyretics in a febrile patient, which often gives rise to this feature.

The most important caveats relate to malaria, and they cannot be emphasized enough. The temperature pattern in *P. falciparum* infections is often quite erratic, and this diagnosis must be considered in all febrile and some nonfebrile patients coming from a malarial area. Regular tertian or quartan fevers (meaning every other day and every third day, respectively) are not found in the early stages of malaria, and are a feature of relapse rather than initial infection. On the other hand, when present they are very characteristic of malaria.

Rashes

Many acute febrile illnesses are accompanied by a rash, which aids greatly in establishing a diagnosis. A few, notably those of meningococcal sepsis, are of vital importance in determining the need for urgent treatment or the protection of contacts (Fig 81.2 , Fig 81.3). Some are pathognomonic, others only indicative (Fig 81.4 , Fig 81.5), and the features of the rash must be placed in context with other features of the illness. In hand, foot and mouth disease the distribution is pathognomonic. Table 81.3 Table 81.4 Table 81.5 provide information about rashes associated with acute fevers, including those encountered in

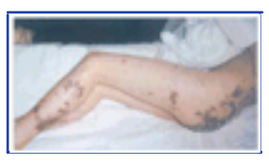

Figure 81-2 Fully developed, almost pathognomonic hemorrhagic rash of meningococcal sepsis.

Figure 81-3 Very early rash of meningococcal sepsis. A few petechiae only, but meningococcal sepsis nonetheless. It can progress to the appearance of Figure 81.2 within minutes or hours. This is the window of opportunity for early treatment.

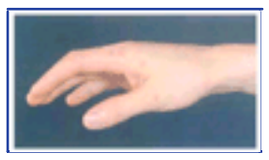

Figure 81-4 Hand, foot and mouth disease. This shows the scanty lax vesicles found at these sites. There is often a maculopapular rash too, especially on the buttocks.

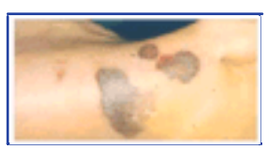

Figure 81-5 Purpuric skin lesions in staphylococcal endocarditis.

returning travelers, but a few specific points are worth emphasizing. In measles, easily forgotten in well-immunized populations but by the same token important to remember in older age groups, fever precedes the Koplik spots and the exanthem by 2 or 3 days, but sometimes by as much as 1 week, although respiratory features are prominent for most of this time. In rubella and in enteroviral infections, general symptoms only rarely precede the rash, and then only by 1 day or so. In dengue, the rash characteristically appears in the second phase of the biphasic illness. Conditions associated with pathognomonic or at least characteristic rashes may, however, also occur with nonspecific rashes; the early rash of meningococcal sepsis may be macular or maculopapular, and Lyme disease may exhibit nonspecific rashes in addition to erythema migrans.

TABLE 81-3 -- Rashes associated with acute viral infections.

RASHES ASSOCIATED WITH ACUTE VIRAL INFECTIONS		
Virus	**Syndrome**	**Comment**
Measles	Measles	Maculopapular followed by staining
Rubella	Rubella/German measles	Macular, often general facial flush
Herpesvirus 6	Roseola infantum	Macular or maculopapular after several days of fever
Parvovirus B19	Erythema infectiosum	Slapped cheeks, lacy on trunk and limbs, often rubelliform or hemorrhagic
Varicella-zoster	Chickenpox	Vesicular, rarely hemorrhagic
	Shingles	Neurologic distribution, premonitary pain, erythema
Herpes simplex	Disseminated herpes	
	Eczema herpeticum	
Epstein-Barr virus		Occasionally macular rash; severe rashes usually ampicillin-induced
Enteroviruses		Usually macular or maculopapular; sometimes hemorrhagic and/or vesicular (hand, foot and mouth disease; see Fig. 81.4)
Primary HIV	Mononucleosis-like illness	(Maculopapular rashes in chronic HIV infection; see Chapter 122)
Viral hemorrhagic fevers	(See Chapter 183)	Purpura, ecchymoses

TABLE 81-4 -- Rashes associated with acute bacterial infections.

RASHES ASSOCIATED WITH ACUTE BACTERIAL INFECTIONS		
Agent	**Rashes**	**Comment**
Neisseria meningitidis	Petechial/purpuric	Also non-hemorrhagic early rashes
Neisseria gonorrhoeae	Hemorrhagic vesicles, pustules	
Staphylococcus aureus	Pyogenic skin lesions	
	Scalded skin syndrome	
	Peripheral purpura	In staphylococcal endocarditis
	Erythema	In toxic shock syndrome
Streptococcus pyogenes	Erysipelas	Local erythema, bullae
	Erythema	In toxic shock syndrome
Salmonella typhi	Rose spots	
Pseudomonas aeruginosa	Ecthyma gangrenosum Cellulitis ± blebs	Also in *Aeromonas* and other Gram-negative bacillary infections
Haemophilus aegyptius	Brazilian purpuric fever	

TABLE 81-5 -- Rashes associated with acute spirochetal and rickettsial infections.

RASHES ASSOCIATED WITH ACUTE SPIROCHETAL AND RICKETTSIAL INFECTIONS		
Agent	**Rashes**	**Comments**
Leptospirosis	Hemorrhages	Weil's disease
	Also other rashes	
Borrelia recurrentis (relapsing fever)	Petechiae	Often no rash; sometimes severe hemorrhages
Borrelia burgdorferi (Lyme disease)	Erythema migrans	Sometimes secondary annular or nonspecific rashes
Spirillum minus (rat-bite fever)	Blotchy macular, papular and urticarial rashes, beginning near the bite and spreading	Rashes also in the *Streptobacillus moniliformis* form of rat-bite fever
Rickettsial infections	Macular, papular petechial	Primary eschar (*tache noir*) in some syndromes

Mouth

The mouth may show useful signs in a febrile patient (Table 81.6 , Fig. 81.6). Especially in infancy and childhood, the tongue and mouth give some indication of dehydration, although mouth breathing and tachypnea often produce a similar appearance. The tongue is notably raw and red in scarlatina, in Kawasaki disease and in toxic shock syndrome. Vesicles are found, especially on the soft palate and anterior fauces, in some enteroviral infections (hand, foot and mouth disease). Palatal petechiae are fairly non-specific, but are common in infectious mononucleosis and rubella. Many important signs in HIV infection are to be found in the mouth and these are discussed in Chapter 132 .

Eyes

Some degree of conjunctival suffusion is common in people with a high temperature. This is often prominent in measles, rubella, some

TABLE 81-6 -- Oral signs in acute fever.

ORAL SIGNS IN ACUTE FEVER	
Sign	**Diagnosis**
Dehydration	Any fever
Herpes simplex	Common in meningococcal and pneumococcal infection
Raw tongue	Scarlatina
	Kawasaki disease
	Toxic shock syndrome
Ulcers, vesicles	Varicella
	Herpes simplex
	Enteroviruses (herpangina, hand, foot and mouth disease)
	Aphthous stomatitis
	Secondary syphilis
	Erythema multiforme
Palatal petechiae	Non-specific, but common in infectious mononucleosis and rubella

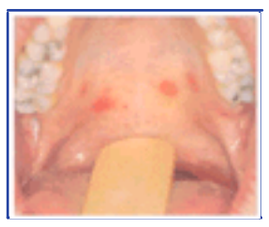

Figure 81-6 Oral signs in hand, foot and mouth disease.

adenovirus infections and in leptospirosis. In infections with a hemorrhagic rash, conjunctival hemorrhages may be present in addition to skin petechiae or purpura; this is especially helpful in patients with dark skin, when petechiae are difficult to see. Other ocular signs that may be important in acute febrile illness are uveitis in acute sarcoid (although many patients who have acute sarcoid do not show ocular involvement) and in Still's disease. Choroiditis occurs in histoplasmosis and in toxoplasmosis. Although choroidoretinitis in toxoplasmosis is most frequently a late marker of congenital infection, it is now clear that a few patients with acute acquired toxoplasmosis (and not HIV-infected) do have acute choroiditis. Miliary tuberculosis, with or without tuberculous meningitis, sometimes manifests as fever and general ill health; the diagnosis is immediately established if choroidal tubercles are seen.

Lymph nodes

Generalized node enlargement is relatively uncommon in acute febrile illness. Among the common infections of children and young adults, rubella, Epstein-Barr virus mononucleosis and cytomegalovirus infection are notable causes, to which must be added cat-scratch disease and, in those at risk, secondary syphilis and primary HIV infection. These latter infections are especially important to remember in returning travelers. General node enlargement is also seen in some more specifically tropical diseases, of which dengue is the most likely to affect a short-term traveler. Acute histoplasmosis is another possibility after a first visit to an endemic area.

Focal nodes must direct a careful search of the relevant drainage area. For example, tender enlarged inguinal nodes may be more prominent than the source of infection, which may be insignificant looking streptococcal lesions of the feet, perhaps superimposed on insect bites or fungal infection. Acquired toxoplasmosis in the immunocompetent host, although usually subclinical, manifests as a febrile illness with localized lymphadenopathy, most often in one or other cervical group but sometimes in nodes elsewhere. The persistent fallacy that toxoplasmosis is a cause of a 'glandular fever' syndrome must be firmly laid to rest. It is rare for the lymphadenopathy of acquired toxoplasmosis to be generalized, and atypical mononuclear leukocytes, if found at all on the blood film, are few in number.

Spleen

Acute and longer-term febrile illnesses make an interesting contrast here. Splenomegaly is found in so many of the infections and other conditions that can cause FUO that it is of little diagnostic value. By contrast, splenomegaly in a patient who has fever of a few days' duration certainly merits further attention. It may indicate a particular infection, such as infectious mononucleosis, rubella, a hepatitis prodrome, in which splenomegaly is especially common in children, or, in a returning traveler, malaria. Splenomegaly may be attributable to an underlying hematologic condition, perhaps a hemolytic anemia or a lymphoma, itself the cause of the fever or a reason for increased susceptibility to infection.

MAKING A DECISION

Some specific factors in the history and examination suggest the need for a plan of management that goes beyond symptom relief. This may mean repeated observation, investigation or investigation combined with provisional treatment. The factors are:

- recent travel, especially to a malaria-risk country (see Chapter 166);
- chills and rigors;
- height of fever;
- fever and rash;
- extremes of age;
- any known or suspected immunosuppression;
- neurologic features;
- dehydration;
- parental or partner concern; and
- physician's impression.

Chills are common enough at the onset of respiratory infections, particularly at the onset of influenza. Nevertheless, the rapid rise in body temperature that they denote is also common in some more serious infections, and this caution applies with greater force if the patient has rigors. A temperature of more than 102.2°F (39°C) is

common enough in the early stage of an ultimately minor infection, but higher temperatures sustained for more than a short time are more likely to be associated with serious infections.

The combination of fever and any kind of hemorrhagic rash, be it only a few petechiae, is especially important.

Many patients on immunosuppressive therapy are living and working normally in the community but are at increased risk of infection. In some conditions, notably asplenia from any cause,

infection may take a fulminant course and any fever demands very prompt attention (see Chapter 109). In addition, some forms of immunosuppression may initially manifest with a febrile illness, and the possibility of a first presentation of HIV infection is especially to be borne in mind.

Depression of consciousness, meningism or localizing neurologic signs are clearly important. Children with high fever may exhibit mild confusion and experience hallucinations; this is seen less commonly at older ages. Whether such clinical features denote specific neurologic involvement needs careful assessment.

Children and the elderly are at greater risk of dehydration, but patients of any age with fever, especially in warm conditions and if anorexic or vomiting, may become fluid deficient.

Whatever the level of anxiety in patient or carer, someone close to the patient may well have an accurate notion of whether the illness is out of the ordinary, and their opinions should be carefully considered.

The physician may form the impression that the illness is unusual, or serious, or likely to become serious. This is so common an issue as to merit more detailed discussion.

Is the patient ill?

Even after the most meticulous history taking, physical examination and attention to the issues just discussed, there are large numbers of patients with fever of short duration in whom no particular warning features are present. Fortunately, most patients who have fever of a few hours or a few days duration recover uneventfully without sequelae and without a diagnosis other than a meaningless attribution to 'viral infection'. How should one judge, in the home, in the health center or practice premises, or in the hospital emergency room, which of these patients should be further investigated, or investigated and given provisional empiric treatment? This must be one of the most frequent decisions that has to be made by clinicians all over the world and yet it is ignored in books about diagnosis. Decision theory is largely silent in this context because the elements of the decision involve multiple factors, usually heuristic in character, few of which can be assigned a numeric value.

It seems that the most common factor affecting the physician's decision is the impression that the patient has an infection with systemic and potentially hazardous features — for which the word 'toxic' is often used as shorthand — and this is the main determinant of further investigation and treatment in patients who have acute febrile illness. Indeed, algorithms on this problem often begin with the question 'Is the patient toxic?'

Attempts to analyze the physician's impression of illness have been more often pursued in children than in adults; this work had its origin in the increased use of blood culture in infants and children, most of whom did not look ill, taken to 'walk-in' clinics or hospital emergency rooms in the USA. Positive blood cultures were found in 2.8–8% of these infants. In most places the main organisms were *Streptococcus pneumoniae, Neisseria meningitidis* and, before general immunization against this organism, *Haemophilus influenzae*. A few of these children, perhaps 4–7%, went on to develop meningitis or other focal infections.[1] These findings spawned a vast amount of work on the early detection of potentially serious illness in febrile infants and on developing management plans for their care. Observations which commonly raise concern in doctors and parents about the possibility of serious illness are the child's reaction to handling and to social overtures, the skin color and state of hydration,[2] while in one recent study, the most sensitive predictors of serious illness were poor feeding and restlessness. [3] A qualitative study of 83 cases of meningococcal disease seen in general practice in South Wales showed how, even in the absence of a rash, clinical and contextual features helped to differentiate these patients from the many with acute self-limiting febrile illnesses.[4] Many of the patients showed 'features not normally expected in children with acute self-limiting illnesses'; these included lethargy, poor eye contact, altered mental state and abnormal cry.

Assessments attempting to systematize what experienced physicians do without conscious thought have thus confirmed the value of clinical judgment in predicting or excluding a high risk of serious disease in older infants and children and it seems impossible to dispense with the elusive but crucial clinical impression of illness, although even this may deceive in either direction. One reason for disasters associated with *P. falciparum* malaria is the apparently good condition of the patient that may precede a rapid decline, whereas, conversely, patients may look much more ill than they are after an exhausting long journey or a lively party. When, as so often, there is doubt about the assessment, a total leukocyte count of greater than 15×10^9 /l has been valuable in some but not all studies, in indicating an increased post-test probability of bacteremia. It is also sensible, if further investigations are decided upon, to include urine microscopy at this stage. Assessment is particularly difficult in infants younger than 3 months, and this problem is not addressed here.

Beyond the basic need for time and care in clinical assessment, a confident relationship between patient and physician and easy access for further consultation are perhaps the most important factors in ensuring that major illness is not missed.

A decision tree for the management of acute febrile illness is given in Figure 81.7 .

LABORATORY INVESTIGATIONS

With few exceptions, among them the classic infectious diseases such as measles and varicella, most acute and short-lived infections have no specific features that enable a clinical diagnosis to be made. Few of these illnesses are investigated. Facilities to do so are not available in most of the world and, when they are available, are unnecessary in patients who have mild and self-limiting illnesses. It follows that most of these infections remain undiagnosed, although a specific diagnosis can be assigned to a substantial number of them in research projects in which full virologic investigations are undertaken. This is the case especially in acute infections in children, who may experience a remarkably large number of infections, most of them viral, in the course of a year.

Investigations are certainly indicated when any of the 'danger points' just described are present, and in any febrile patient whose general condition gives concern. The precise range of investigations chosen will obviously vary depending on the facilities available and on the vagaries of the clinical situation, but even a small and frequently accessible range of investigations (Table 81.7) greatly increases the possibility of establishing a diagnosis. When the features of the illness give no specific direction, the most useful investigations are a routine blood count together with careful examination of a stained blood film, blood culture, urine examination, and posteroanterior and lateral radiograph of the chest. When blood is taken, a serum specimen should be saved for later study.

Initial investigations are often, of course, much more extensive depending as they do on available resources and initial clinical clues. These will most commonly include liver function tests, stool microscopy and culture, antigen detection and serologic tests for particular pathogens, and relevant imaging especially abdominal ultrasound scanning and computed tomography or magnetic resonance imaging. The more extensive range of investigations is fully discussed in the context of FUO (see Chapter 82).

Figure 81-7 Decision pathway for the management of acute febrile illness.

Total and differential leukocyte count

Modest degrees of neutrophilia, up to about 15×10^9 /l are of little help. More definite neutrophilia is principally found in pyogenic bacterial infections, but also in amebiasis, leptospirosis and in many noninfectious conditions such as thromboembolism, rheumatic fever, Still's disease, exacerbations of chronic liver damage and mechanical tissue damage (Table 81.8).

Neutropenia is common in many viral infections, including rubella and influenza, and, among infections of travelers, is often found in malaria, typhoid, brucellosis, rickettsial diseases and visceral leishmaniasis. Leukopenia is also a feature of severe and overwhelming sepsis, but the serious condition of patients who show this feature is

TABLE 81-7 -- Basic laboratory investigations in patients who have acute fever.

BASIC INVESTIGATIONS IN ACUTE FEVER
• Routine blood count
• Stained blood film
• Urine microscopy and culture
• Chest radiograph
• Save serum

TABLE 81-8 -- Value of total and differential leukocyte count in acute fever.

VALUE OF LEUKOCYTE COUNT IN ACUTE FEVER	
Neutrophilia	**Neutropenia**
Sepsis	Severe sepsis
Abscess	Malaria
Amebiasis (usually)	Typhoid
Leptospirosis (usually)	Brucellosis
Still's disease	Visceral leishmaniasis
Lymphoma (uncommon)	Rickettsial infections
Atypical mononuclear cells	**Eosinophilia**
Epstein-Barr virus	Schistosomiasis (Katayama fever)
Cytomegalovirus	Visceral larva migrans (toxocariasis, etc.)
This table concerns acute illnesses in which fever is a principal feature; it does not include conditions such as tropical eosinophilia in which respiratory features are dominant.	

usually only too evident. Thrombocytopenia is a very frequent feature of the blood film in malaria and in sepsis.

A substantial number of atypical mononuclear cells is found most commonly in acute Epstein-Barr virus and cytomegalovirus infections, whereas eosinophilia points to the tissue-invasive stage of many parasitic infections.

Specific diagnoses from the blood film

Malaria is by far the most important finding from examination of the blood in those at risk, but other diagnoses that can sometimes be made in this way are listed in Table 81.9 . In addition to examination of the blood, a diagnosis may occasionally be aided by direct examination of material from a skin lesion, for example in meningococcal sepsis.

TABLE 81-9 -- Specific diagnoses from the blood film.

DIAGNOSES FROM BLOOD FILM
• Malaria
• Babesiosis
• Trypanosomiasis
• Filariasis
• Leptospirosis (dark field)
• Relapsing fever (dark field or staining)
• Bartonellosis
• Ehrlichiosis
• Meningococcemia
• Histoplasmosis, candidemia
Etiologies of acute fever are sometimes established by examination of the blood film.

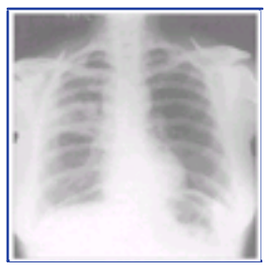

Figure 81-8 Chest radiograph of a patient with *Mycoplasma* pneumonia. Respiratory symptoms are often scanty or absent in the first few days of the illness.

Urine examination

Small degrees of proteinuria are of no significance in febrile patients. Dipsticks can be used to detect Gram-negative infections and pyuria, but should not be used alone in the diagnosis of fever because of the high false-negative rate of the nitrate test for bacteriuria. Urinary infection is best diagnosed by direct microscopy of a drop of urine with the finding of heavy pyuria, often some hematuria and visible organisms. The presence of organisms in association with pyuria in a freshly obtained specimen indicates significant bacteriuria and a Gram stain can help in distinguishing positive cocci from negative bacilli. Urinary infection is one of the most common infections that sometimes manifests as a febrile illness with no localizing symptoms or signs.

Chest radiograph

The most important findings in a febrile but previously healthy patient are areas of consolidation, since general symptoms and fever may precede respiratory symptoms and signs by several days and radiographic changes may be surprisingly prominent in patients with few or no respiratory symptoms. This is especially likely in 'atypical' pneumonia, such as that associated with Q fever or *Mycoplasma pneumoniae* infection (Fig. 81.7). The other crucial finding is that of pulmonary tuberculosis, in some communities to be suspected throughout the population, and in others more especially in the indigent and those with HIV infection. Other diagnoses may also be made, such as *Pneumocystis carinii* infection in HIV infection before respiratory features become evident, allergic pneumonias, visceral larva migrans and pulmonary emboli.

Blood culture

The importance of blood culture in febrile patients thought ill enough to need investigation is evident but, important as it is, the information gained is necessarily delayed and thus irrelevant to the immediate management decisions.

Serum

It is always sensible, if blood is drawn, to save a serum sample, especially for later comparative tests if the illness proves to be prolonged and remains undiagnosed.

ISOLATION

A few patients who have infections present a risk to other people. Because the diagnosis is often obscure at the time of admission to hospital of an acutely febrile patient, isolation is often advisable initially, if facilities are available. Sometimes, as for example in suspected Lassa fever, more elaborate measures involving the control-of-infection team in liaison with the public health authorities are indicated. Similar levels of precautions may be indicated for newly described and highly transmissible pathogens such as the SARS epidemic in Southern China and other regions in Asia. These aspects of the management of infection are discussed in Chapter 87 .

MANAGEMENT

Symptomatic treatment

Drug therapy is unnecessary in many acute fevers, but discomfort can be alleviated by agents such as aspirin, paracetamol and nonsteroidal anti-inflammatory agents, which act as cyclo-oxygenase inhibitors. There is little to choose between their effect on fever, but aspirin is avoided in infants because of its association with Reye's syndrome, and in many older patients because of its effects on the gastric mucosa.

The effectiveness of antipyretics in reducing fever does not seem to correlate with its cause. In a study of 1559 children with a temperature of more than 101°F (38.4°C) on arrival at the emergency room, reductions of temperature 1 and 2 hours after a single dose of paracetamol (acetaminophen) were slightly greater in patients who had a serious bacterial infection than in those who did not,[5] although it had been thought that fever with a serious cause might be less responsive to an antipyretic.

Sponging is still sometimes used as a method of reducing fever. It certainly does this, but it is often very uncomfortable, particularly if done with iced rather than tepid water. The vasoconstriction and shivering causing distress do not, however, produce a rise of core temperature. Combining paracetamol (acetaminophen) with sponging gives a slightly greater reduction of temperature than sponging alone. [6]

It is useful to know the timing of antipyretic action, especially if the aim is to reduce the likelihood of febrile convulsions. Both antipyretic drugs and sponging show an appreciable effect within about 30 minutes and have their maximal effects in 2–3 hours.

Corticosteroids are effective antipyretics but should be used only for specific indications in conjunction with appropriate anti-infective therapy for the known or presumed cause of the fever.

Maintaining hydration is important and not always easy in a warm climate as patients who have fever are often anorexic and sometimes vomiting.

Empiric treatment

One of the most taxing and common decisions that has to be taken in acute febrile illness is whether to start empiric treatment based on one or more hypotheses about the diagnosis. On one side is the fear of rapid and perhaps irreversible deterioration, especially in *P. falciparum* malaria and in septic shock. On the other are the confounding effects of possibly inappropriate treatment, and the added problem of adverse drug reactions. If immediate treatment is given, it is nearly always possible to take blood before starting and this can be used for diagnostic tests.

TABLE 81-10 -- Some dangerous features in patients who have acute fever.

DANGER POINTS IN ACUTE FEVER
• Petechial/purpuric rash
• Travel involving risk of malaria
• Chills and rigors
• Extremes of age
• Neurologic signs
• Asplenia
• Hypogammaglobulinemia
• Post bone marrow transplant

Some indications for immediate treatment (Table 81.10) in acutely febrile patients can be firmly stated; others are contingent and depend on the precise details of the clinical and epidemiologic situation. The most urgent are the possibility of meningococcal sepsis and that of serious infection in asplenic subjects. Next in urgency are indications pointing toward streptococcal sepsis and, in returning travelers, the possibility of *P. falciparum* malaria with clinical deterioration. Immediate treatment should also be seriously considered if there is evidence of any immunologic disorder and, more generally, if other indicators of severe illness such as those already discussed are present. Table 81.11 gives some recommendations for action in these circumstances. The antibiotic choice, as always, must take account of local susceptibility patterns, and for this reason, more than one option is given. For example, penicillin is no longer the agent of first choice in many places for serious pneumococcal infection, and the spread of meningococcal resistance to penicillin may make future changes necessary in the national policy in the UK for immediate treatment of suspected meningococcal sepsis.[7]

TABLE 81-11 -- Suggested empiric regimens for use when urgent treatment is indicated in patients who have acute fever.

EMPIRIC TREATMENT IN ACUTE FEVER	
Presumed diagnosis	**Action**
Meningococcal sepsis	1. Blood culture if possible
	2. Penicillin G (benzylpenicillin)
Septic shock in asplenia	1. Blood culture if possible
	2. Penicillin G (benzylpenicillin) or cephalosporin*
Streptococcal sepsis	1. Blood culture if possible
	2. Local lesion, Gram stain and culture if possible
	3. Penicillin G (benzylpenicillin) +/- clindamycin
Staphylococcal sepsis	1. Blood culture if possible
	2. Local lesion, Gram stain and culture if possible
	3. Flucloxacillin or similar agent*
Severe malaria	1. Blood films
	2. Quinine (or artemether or artesunate; see Chapter 166)
Lassa fever	1. Strict isolation
	2. Tribavirin (ribavirin)
	3. Inform public health authorities

* Knowledge of local resistance patterns and antibiotic policy needed

It has been written (by Garrison) that Wunderlich, the founder of clinical thermometry, 'found fever a disease and left it a symptom'. This chapter shows that fever remains an important and challenging symptom that demands meticulous analysis so as to achieve the best prospects for accurate diagnosis and treatment.

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Chapter 82 - Fever of Unknown Origin in the General Population and in HIV-infected Persons

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INTRODUCTION

Fever of unknown origin (FUO) is one of the most challenging tests of the clinical acumen of the physician. The clinical characteristics of 1315 cumulative cases of FUO in the general population have been published in 15 selected reports that span 77 years, from 1930 to 1997 (Table 82.1).[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] Since the early case series from the USA on FUO were published,[1] [2] [3] [4] [5] [6] important sociologic and technologic changes have occurred; for example, new microbiologic techniques[16] and radiographic diagnostic tools[17] have become routinely available, a growing number of people use intravenous drugs[18] or have implanted prostheses,[19] and patterns of immigration and travel destinations have expanded.[20] The incidence of newly described diseases such as AIDS[21] and Lyme disease[22] has risen. In developed countries, certain 'older' diseases such as rheumatic fever remain uncommon,[23] yet the frequency of tuberculosis has risen again after a decline during the 30 years preceding 1985.[24]

The Petersdorf and Beeson criteria for FUO, which standardized its definition in 1961,[7] are:

- a body temperature of more than 101°F (38.3°C) for at least 3 weeks; and
- failure to establish a diagnosis after 1 week of investigation.

These criteria were designed to eliminate self-limited diseases that might have been represented in the five series on FUO reported before 1961.[1] [2] [3] [4] [5] [6] Each of the eight subsequent published case series on FUO[8] [9] [10] [11] [12] [13] [14] [15] used the classic Petersdorf and Beeson criteria. Some of the recent series[13] [14] use a revised Petersdorf definition of FUO[25] [26] that permits the investigation to take place in the ambulatory setting as well as in hospital, the setting originally specified. Furthermore, a subclassification of HIV-associated FUO has been added to the classification of FUO in the general population to incorporate this new disease.[27] Fever of unknown origin associated with HIV infection has now been reviewed in several series. Five selected series are detailed totalling 257 cases;[28] [29] [30] [31] [32] HIV-associated FUO has been defined as fever that lasts more than 4 weeks in outpatients or 3 days in hospitalized patients and remains unexplained despite investigation. These series are reviewed later in this chapter.

CLASSIC FEVER OF UNKNOWN ORIGIN

Disease categories

An etiology of FUO is identified in the majority of cases in each published series on FUO (average 73%, range 30–93% of cases). In early reports, the individual diseases causing FUO were limited to a small number of predominantly infectious diseases.[1] [2] [3] [4] [5] In contrast, recent series show that the diseases responsible for FUO are much more extensive, involving over 100 disorders.[12] [13] [14] [15] Consequently, recent case series use disease categories rather than enumerating individual diseases to describe the clinical spectrum of FUO. The principal disease categories include infection, neoplasms and collagen diseases. For the sake of simplicity, the remaining diverse range of cases can be grouped together as 'miscellaneous' (see Table 82.1).

Certain changes in disease categories have occurred since the original description of FUO. Infections remain the most common cause of FUO in all but three series. Overall, 30% (range 6–70%) of cases of FUO are due to an infection. Neoplasms are responsible for 18% (range 7–34%) of cases, and the proportion of cases due to malignancy has remained constant. In contrast, there has been an increase in cases caused by collagen disorders. Although the overall percentage of cases in this category of diseases is only 12% (range 0–22%), series published within the past 10 years indicate an increase in the number of cases caused by collagen diseases. Diseases falling into the category of miscellaneous disorders have been identified in 14% of cases (range 0–28%). The individual diseases represented by these categories are discussed below.

Infections

The major infections represented in older papers — tuberculosis, endocarditis and abdominal abscesses — continue to make up a significant proportion of FUO in recent series — 5%, 5% and 10% respectively.[13] [14] However, compared with earlier series, the types of infection causing FUO have become more diverse.[11] [12] [13] [14] [15] Recently described infections, including HIV infection and Lyme disease; immunodeficiency-related infections such as *Pneumocystis carinii* pneumonia and atypical mycobacterial infection; and diseases such as typhoid fever and amebiasis in patients who have resided in developing countries were described for the first time in series reported within the past decade.[12] [13] [14] [15] In addition, these reports describe infections involving critically ill patients whose lives have been prolonged in an intensive care setting and who have developed superinfections with *Candida* spp. Other infections that make up a small percentage of FUOs include pyelonephritis, perinephric abscess, toxoplasmosis in the immunocompetent host and mononucleosis caused by Epstein-Barr virus or cytomegalovirus (CMV).

There are several reasons why tuberculosis, abdominal abscesses and infectious endocarditis make up the majority of the infectious causes of FUO. For example, tuberculosis continues to be prevalent in Africa and South East Asia and afflicts intravenous drug users, the elderly and HIV-infected persons.[20] [21] [22] Furthermore, the increase in the numbers of people who have prosthetic heart valves[19] [23] has affected the number of cases of FUO caused by infectious endocarditis.[15] In addition, advances in microbiologic techniques permit the identification of fastidious organisms that cause valvular vegetations.[33] Finally, modern radiologic studies permit the detection of abdominal abscesses in cryptic locations such as the spleen.[34]

The first appearance of newly discovered diseases such as HIV infection[21] and Lyme disease[22] as causes of FUO reflects the increased recognition of these diseases. The increase in the number of immunocompromised persons reflects the increase in the incidence of opportunistic infections, such as *P. carinii* pneumonia, *Mycobacterium avium* and disseminated candidiasis, as causes of FUO.[35]

TABLE 82-1 -- Diagnoses in selected series of fever of unknown origin reported in the literature.

DIAGNOSES IN SELECTED SERIES OF FUO REPORTED IN THE LITERATURE								
Study	**Number of patients with indicated diagnosis**							
	Total	**Country**	**Infection**	**Neoplasm**	**Collagen**	**Miscellaneous**	**Undiagnosed**	
Alt and Barker 1930[1]	57	USA	14	6	0	1	36	
Hamman and Wainwright 1936[2] [3]	54	USA	32	12	0	0	10	
Keefer 1939[4]	80	USA	51	19	0	10	0	
Bötigger 1953[5]	68	Sweden	16	11	4	4	33	
Geraci *et al.* 1959[6]	70	USA	15	21	0	20	14	

Petersdorf and Beeson 1961[7]	100	USA	36	19	15	23	7
Petersson 1962[8]	81	Finland	15	5	5	0	56
Sheon and Van Ommen 1963[9]	60	USA	12	11	8	6	23
Fransen and Böttiger 1966[10]	60	Sweden	8	19	2	4	27
Jacoby and Swartz 1973[11]	128	USA	51	26	19	22	10
Larson *et al.* 1982[12]	105	USA	32	33	9	18	13
Knockaert *et al.* 1992[13]	199	Belgium	45	14	42	47	51
Kazanjian 1992[14]	86	USA	28	21	18	11	8
De Kleijn *et al.* 1997[15]	167	Netherlands	43	21	33	20	50
Total	**1315**		**398**	**238**	**155**	**186**	**338**
Percentage			**30**	**18**	**12**	**14**	**26**

Neoplasia

Hodgkin's and non-Hodgkin's lymphoma have remained the most common neoplastic diseases responsible for FUO, and lymphoma is the most common individual disease causing FUO.[12] [13] [14] [15] Its ability to present in an insidious and protean fashion may explain why lymphoma continues to account for a sizable number of cases despite new diagnostic modalities.[36] For example, fever in some cases persists for months before a gland may become enlarged. In addition, it may be difficult to locate an involved lymph node on physical examination when the axilla is involved, and it is impossible when the retroperitoneal nodes or bone marrow are the only site of disease. Furthermore, a directed percutaneous needle biopsy of an involved gland or bone marrow may contain insufficient material to reveal diagnostic histopathology.[37] Thus, lymphoma may elude clinical detection for a long time. Malignant histiocytosis, [38] angio-immunoblastic lymphadenopathy with dysproteinemia [39] and Kikuchi's disease,[40] although uncommon, are causes of FUO with lymphadenopathy that were first recognized within the past 15 years.

Other neoplasms and solid tumors are less common causes of FUO than lymphoma.[12] [13] [14] [15] The peripheral blood smear of certain leukemias and myelodysplastic syndromes may reveal no definitive findings (aleukemic leukemia); establishing the diagnosis requires bone marrow examination. Renal cell carcinoma may present with prolonged fevers and no other symptoms in a minority of cases.[41] Metastatic adenocarcinoma in the liver, regardless of the primary site, may also cause FUO.[41] In most situations, but not all, there is elevated hepatic alkaline phosphatase, hepatomegaly or both. Other solid tumors that can cause FUO include leiomyosarcomas of the gastrointestinal tract, sarcomas and atrial myxomas.[42]

Rheumatologic disorders

Adult Still's disease, for which there is no definitive laboratory test, remains the most common rheumatologic cause of FUO.[43] The typical clinical features are myalgias, leukocytosis, lymph node enlargement, splenomegaly and an evanescent rash that appears with febrile episodes; other causes of this clinical presentation must be excluded.

Temporal arteritis, with or without polymyalgia rheumatica,[44] should be considered in those aged over 50 years; the erythrocyte sedimentation rate (ESR) is nearly always elevated. The classic presentation of temporal arteritis — headache, jaw claudication, visual loss — or a palpable temporal artery may be absent in one-third of patients who have temporal arteritis.

Other less common rheumatologic causes of FUO include Wegener's granulomatosis and cryoglobulinemia; polyarteritis nodosa should be considered if there is mononeuritis multiplex, myalgias, skin lesions, abdominal pain (which is due to small intestinal ischemia) and azotemia.[45]

Miscellaneous

A diverse array of conditions, including granulomatous diseases, inflammatory illnesses and drug-related fevers, are classified as miscellaneous. Crohn's disease, sarcoidosis and granulomatous hepatitis are examples of granulomatous diseases.[46] Patients who have granulomatous hepatitis require a biopsy of involved tissue and exclusion of other causes of this histologic pattern, such as infections with *Mycobacterium* spp., fungi or bacteria (e.g. brucellosis, tularemia, cat-scratch disease), and lymphoma.

Other conditions that must be considered include alcoholic hepatitis, [47] pulmonary emboli,[48] subacute thyroiditis, Sweet's syndrome and familial Mediterranean fever.[49] Leukocytosis may occur with these conditions but it is often especially prominent in alcoholic hepatitis and familial Mediterranean fever.[48] Fever most often resolves within a few days following anticoagulation in patients who have pulmonary emboli, but it may take longer in certain instances.[48] Familial Mediterranean fever causes recurrent fever, peritonitis and leukocytosis. Subacute thyroiditis may be difficult to diagnose because there are usually no systemic features of thyrotoxicosis and the thyroid function tests are usually normal. The thyroid gland may be nontender; however, it is commonly diffusely enlarged.[49] Schnitzler syndrome (hyperostosis, lymph node enlargement and monoclonal IgM gammopathy) is a rare cause of FUO.[49] In children, a new syndrome, PFAPA (periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis), has been described.[50] This syndrome can be difficult to distinguish from familial Mediterranean fever but lacks serositis and episodes respond to steroids.

Hematomas and drug fever may cause FUO.[51] Hematomas causing FUO may occur as a result of hemorrhages into the abdominal

TABLE 82-2 -- Drugs associated with fever of unknown origin.

DRUGS ASSOCIATED WITH FUO	
Common	**Less common**
Atropine	Allopurinol
Amphotericin	Hydralazine
Penicillins	Isoniazid
Cephalosporins	Rifampin (rifampicin)
Phenytoin	Macrolides
Procainamide	Clindamycin
Quinidine	Vancomycin
Sulfonamides	Aminoglycosides
Interleukin-2, interferon	

cavity or retroperitoneal space, but bleeding within the wall of an aneurysm or dissection of the thoracic or abdominal aorta has also been reported as being responsible.[51] In these cases, persistent fever and anemia typically follow an episode of chest, back or abdominal pain that spontaneously resolves.[51] Trauma may predispose to the formation of hematomas in extravascular spaces.

Drug fever can occur with virtually any medication, even those administered for long periods without previous problems (Table 82.2).[52] There may be no eosinophilia.[52] There are no distinctive clinical features associated with drug fever that help to distinguish it from the other causes of fever mentioned above.

Other miscellaneous causes of FUO are rare. Endocrinologic causes of FUO include hyperthyroidism and adrenocortical insufficiency.[49] Factitious fever does not always follow a specific pattern of a characteristic patient population, although many have the features of Munchausen syndrome.[53] In order to provide objective evidence of fever, some patients may warm the thermometer when the health care worker is not in attendance. Others may subject themselves to mutilation through such maneuvers as injecting themselves with specimens contaminated with micro-organisms.

Undiagnosed fever of unknown origin

Between 7% and 30% of cases remain undiagnosed despite intensive evaluations.[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] The long-term follow-up, available in two FUO series, shows that fever resolves in the majority of these patients within a short time.[54] In one series, follow-up was available for eight cases in whom no diagnosis was established.[14] Fever resolved within 3 weeks of the period of FUO in 87% of cases and within 4 months in all patients. The other series investigated the long-term follow-up of 49 cases of undiagnosed FUOs taken from a larger cohort of 199 cases.[54] Fever resolved within a few weeks of discharge in 31 of the 49 cases (63%) and within 2 years of discharge in 83%. In the remaining eight patients (17%), fever recurred and required repeated courses of nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Despite the lack of diagnosis in these patients, the mortality rate 5 years after discharge was small — only 3%. Thus, in most of these patients the fever abated without treatment, and rarely did a serious disorder emerge later. [14] [54]

FEVER OF UNKNOWN ORIGIN IN OTHER GEOGRAPHIC REGIONS

Table 82.1 details several large studies characterizing the spectrum of FUO as defined by strict inclusion criteria. All these studies, however, were performed in the USA or in northern Europe. The spectrum of disease in developing countries differs from that in the developed world. However, the literature on FUO in developing countries is limited. Several series have been published that characterize the specific diagnoses associated with prolonged fevers in other countries. Few of these series meet the revised Petersdorf and Beeson criteria requiring absence of a diagnosis despite an initial work-up. Several constraints have been identified that limit application of strict inclusion criteria to these studies.[55] These include presentation of the patient late in disease, limited access to diagnostic tests, including accurate blood culture methods, limited treatment facilities and poor patient follow-up. Accurate diagnosis can be further complicated by the presence of endemic disease that may not be the etiology of the prolonged fever under investigation. For example, typhoid fever is often diagnosed by serologic methods (the Widal test). However, in endemic areas, 12–29% of patients may have a positive titer at baseline. Convalescent titers are difficult to obtain because of poor follow-up, and misinterpretations of these tests frequently occur.[55]

Two large series have been published in the developing world that selected patients based on Petersdorf and Beeson criteria.[56] [57] The first, with 159 patients from Bangladesh, noted infectious diagnoses in 63% of patients, while the second with 233 patients in India reported infectious etiologies in 46%, suggesting a greater contribution of infectious causes when compared with the series in Table 82.1 . In each series, two of the three leading infectious diagnoses were typhoid fever and tuberculosis. In addition, malaria and chloroquine-responsive fever were common. Other diagnoses included visceral leishmaniasis, amebiasis, syphilis, leprosy and filariasis. In addition, conditions seen commonly in the developed world such as endocarditis and intra-abdominal abscesses were present. The suggestion that infectious diagnoses comprise a greater proportion of cases of FUO in developing countries is supported in other series as well.[55] [58] [59]

The clinician must consider diseases more common in specific geographic regions when evaluating patients from these areas. For example, a compilation of all FUO series in Spain over 25 years, including 914 patients, demonstrated that brucellosis accounted for 16% of infectious diagnoses and that 8% were due to parasitic causes, including hydatid disease, leishmaniasis and *Fasciola* infection.[60] Studies conducted in hospitalized patients who had fever in India and in Nigeria reported a *Brucella abortus* seroprevalence of 0.8–5.2%, leading the authors to speculate that brucellosis might account for a percentage of cases of undiagnosed FUO in these regions as well.[61] [62] Studies of prolonged fever in Egypt note that 16% of cases were diagnosed with a parasitic infection.[63] In addition to typhoid fever and tuberculosis, melioidosis has been reported as a common cause of FUO in South East Asia.[64] [65] HIV infection is an important consideration throughout the developing and developed world. Specific diagnoses to consider in the HIV-infected patient who has fever are discussed below.

GENERAL APPROACH TO DIAGNOSIS

Although some disorders may be associated with a distinct pattern of fever (Table 82.3), the charted fever curve is rarely helpful in establishing an individual diagnosis. The following approaches should be pursued, because most disorders that cause FUO do not have a specific fever pattern.

Routine noninvasive tests

Recent series have shown that a growing number of cases of FUO are being identified by a variety of noninvasive tests.[12] [13] [14] [15] Several factors may explain this observation. The number of diseases diagnosed by microbiologic methods has risen, possibly because of advances in diagnostic capabilities. [16] For example, blood

TABLE 82-3 -- Diseases associated with distinct patterns of fever.

DISEASES ASSOCIATED WITH DISTINCT PATTERNS OF FEVER		
Term	**Description**	**Disease**
Intermittent	Temperature elevation returns to normal at least once during most days	Abscesses, falciparum malaria, Still's disease
Remittent	Fevers do not return to normal each day	Tuberculosis, endocarditis, typhoid fever
Relapsing	Recurrent over days or weeks	Relapsing fever, brucellosis, malaria (tertian or quartan fever pattern), lymphoma (Pel-Ebstein fever pattern)
Biphasic	Recurs only once	Leptospirosis, dengue, Colorado tick fever, lymphocytic choriomeningitis
Continuous	Fever varies less than 1.8°F (1°C) over several days	Encephalitis, drug fever, salmonella, factitious fever

cultures using the lysis-centrifugation technique may identify *Mycobacterium* spp. and obviate the need for bone marrow aspiration.[66] The practice of incubating blood cultures in a carbon-dioxide-enhanced environment and subculturing broth even in the absence of turbidity has led to the detection of infectious endocarditis caused by fastidious organisms.[33] In other instances, the available serologic techniques have supplanted the need for pathologic analyses of surgically obtained tissue;[28] for example, elevated antibody titers to *Entamoeba histolytica* in patients who have hepatic masses seen on computerized tomography (CT) scans supports the diagnosis of amebiasis and avoids the need for invasive procedures.[14] Advances in serologic testing are relevant for 'older' diseases as well; for example, systemic lupus erythematosus can now be detected in a person who is negative for antinuclear antibody.[14] Also, newly described diseases that have made their first appearance as causes of FUO, such as Lyme disease or HIV infection, can be diagnosed by serologic methods.

The number of cases of FUO diagnosed by noninvasive techniques is likely to increase in the future as noninvasive diagnostic testing improves. Blood culture with use of the lysis-centrifugation technique is a sensitive method of detecting common mycobacterial and fungal infections that cause prolonged fever in patients who have AIDS.[66] The development of assays such as the polymerase chain reaction (PCR) for disseminated *M. avium* infection may further reduce the need for invasive procedures by identifying the organism far in advance of the time that is required to isolate it from blood cultures. The application of the PCR for diagnosis of other infections, such as acute HIV infection[67] or *Legionella* infection, could also avoid the need for an invasive procedure in selected circumstances. Finally, advances in obtaining expectorated sputum and analyzing it for the presence of *P. carinii* will drastically reduce the need for bronchoscopy for the diagnosis of *P. carinii* pneumonia in persons who have AIDS and have had prolonged fevers.

Testing guided by abnormal findings

In most instances of FUO, diagnostic testing is guided by notable findings on the physical examination or by abnormal values in a routine laboratory test.[68] The careful evaluation of patients necessary to reach a diagnosis cannot be replaced by the early use of new diagnostic tests such as transesophageal echocardiography and magnetic resonance imaging (MRI) in an effort to hasten a diagnosis.

A uniform diagnostic algorithm is not useful because studies are most helpful when performed in a guided fashion determined by each individual case. One study has identified the amount of time and resources required to establish the diagnosis.[14] This study showed that diagnosis required a mean time of 19 days (range 1 day to 8

months), including 11 days of hospitalization (range 3 days to 5 weeks) and four outpatient visits (range: 0–11). A substantial part of the evaluation should take place in an outpatient setting[69] if the patient's condition allows.

The approach to FUO is influenced by whether abnormal findings are present in the early or later stages of FUO. Several studies have reaffirmed the paramount importance of repeated evaluation of patients who have FUO in whom no abnormal findings are present in the early stages of the illness.[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14] [15] [68] Repeated histories and physical examinations are crucial for detecting new developments. If no group of findings occur early in the febrile course, then prolonged and meticulous observation is the most successful approach to discovering the cause of FUO. This approach yields crucial findings that do not become evident until a late stage of the illness. In one series, repetition of the physical examination, selected laboratory tests or observation after specific therapeutic intervention led to a diagnosis in 28 of the 86 cases.[14]

This study has also shown that the late appearance of abnormalities on physical examination helps to direct further diagnostic testing.[14] In this report, excisional biopsy of a skin lesion that appeared late in the course of the fever in two patients showed polyarteritis nodosa in one and Sweet's syndrome in the other. In addition, the visual appearance of keratoderma blenorrhagicum was diagnostic of a case of Reiter syndrome. In other situations, neither the gross nor the microscopic appearance was specific, but the diagnosis of Still's disease, systemic lupus erythematosus, POEMS (polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes) syndrome and essential mixed cryoglobulinemia was aided by the late appearance of a skin eruption. In some cases of lymphoma and in one case of angioimmunoblastic lymphadenopathy, fever persisted for months before a lymph gland became enlarged.

One case series has also demonstrated that the etiology of FUO may be established when diagnostic laboratory results first appear in the later stages. [14] Examples of cases diagnosed in this fashion include viral infections that were identified by a significant rise in serologic titers for CMV, Epstein-Barr virus or HIV. Blood cultures facilitated a diagnosis of infectious endocarditis caused by *Actinobacillus actinomycetemcomitans* and *Enterococcus faecium* and of abdominal abscesses caused by *Listeria monocytogenes*. Eosinophilic pneumonia, bronchial adenoma and cranial subdural empyema were diagnosed by evolution of findings on radiographic studies.

One series showed that abnormal findings may be present in the early stage of the febrile illness.[14] In these cases, the abnormal findings were discovered by the initial examining physician, who decided to initiate treatment for the most likely condition before testing for the presence of less likely diseases. When fever persisted, cessation of therapy and investigation for alternative explanations of the original clinical pattern proved to be an effective clinical approach. Examples of diagnoses established in this fashion included tuberculosis in a person who had pulmonary infiltrates that did not respond to empiric antibiotics. In another case, an open thoracotomy with pleural and lung biopsy was performed when fever persisted despite antimycobacterial therapy in a man who had a bloody lymphocytic exudate and a reactive tuberculin skin test. Histopathologic stains of tissue revealed lymphomatoid granulomatosis. In these cases, the true disease process was identified only when fever persisted despite initiation of therapy for the most likely cause of the condition. It is conceivable that diagnostic riddles solved by this approach may not be considered true FUOs in the future. Rapid

diagnostic tests may become available and lead to a prompt identification of the illnesses considered in the differential diagnosis prior to the initiation of treatment.

The laborious process of investigating FUOs is difficult to accomplish in today's medical environment; carefully listening to and examining a patient, contemplating the problem in a quiet environment and collaborating with others is an arduous task. Frustration, weariness and impatience intervene when a diagnosis is not promptly reached, and they render the physician vulnerable to over-looking the appearance of a valuable new finding. The physician must expect to devote a large amount of time to pacifying impatient relatives of the patient and justifying the length of stay to insurers and administrators who would prefer a quicker solution.

Blind testing in absence of abnormal findings

Although previous series and reviews have stressed the fact that tests that are not guided by abnormalities found on physical examination or laboratory testing have a low diagnostic yield, one series showed that diagnoses were established by such tests in 10% of cases.[14] An example from this series is a biopsy of a temporal artery that revealed temporal arteritis in an elderly woman who had an elevated ESR but no localizing symptoms for that disorder. In addition, abdominal CT scanning led to a specific diagnosis in 6% of total cases; for example, one such CT scan showed extensive liver metastases in a man who had stomach cancer but no liver enlargement and normal liver function tests. In other cases, abdominal CT scans identified retroperitoneal lymphadenopathy despite the absence of abnormalities detected on physical examination; specific diagnoses were established by biopsies of the enlarged nodes.

Of note, percutaneous CT-guided techniques are useful for obtaining diagnostic specimens in patients who have abnormalities identified by abdominal CT scan.[70] The quantity of the material obtained by these procedures may be inadequate to demonstrate the characteristic histopathologic changes of neoplasms[26] but it is often sufficient for microbiologic analysis to be able to establish the diagnosis of an infectious process. One series showed that the yield of these procedures is high for infection but low for tumor.[14] Biopsies of tissue that are found to be abnormal by physical examination (e.g. lymph node) or diagnostic imaging techniques (e.g. lung) have been shown to have a higher yield (38%) than routine biopsies of bone marrow or liver (see below).

In contrast, 'blind' serologic and biopsy procedures are often fruitless at best and may actually prove misleading, resulting in unnecessary diagnostic procedures. For example, serologic testing for zoonotic infections such as tularemia, Lyme disease or Rocky Mountain spotted fever should be performed only if the patient has been exposed to a relevant animal or tick; similarly, review of serial blood smears for malaria or serologic testing for conditions such as amebiasis or leishmaniasis should be undertaken only if the patient has been in an endemic region (Table 82.4).

The yield of routine bone marrow examination and liver biopsy has been mentioned in several case series.[12] [13] [14] The diagnostic yield of bone marrow examination varies but is low in most series, ranging from 0% to 14%.[12] [14] Liver biopsy has been commented upon as being useful in several series[71] [72] but these series do not list the diagnostic yield. For example, Petersdorf's two series state that needle biopsy of the liver is of particular value in certain cases, even when hepatomegaly or jaundice are absent, because of the frequent involvement of this organ in systemic disease.[7] [12] More recent series[13] report that the diagnostic yield of liver biopsy is 14%.

The previously useful diagnostic 'blind' laparotomy has been supplanted by the abdominal CT scan[14] and recent studies suggest that there is no longer an indication for routine exploratory laparotomy in the evaluation of FUO.[73] In contrast, exploratory laparotomy continues

TABLE 82-4 -- Evaluation of fever of unknown origin in patients with recent travel, occupational or recreational exposure.

EVALUATION OF FUO IN PATIENTS WITH RECENT TRAVEL, OCCUPATIONAL OR RECREATIONAL EXPOSURE.		
Exposure	**Disease**	**Method of evaluation**
Recent residence in or travel to an endemic region	Tuberculosis	Skin test, sputum smear
	Malaria	Blood smear
	Brucellosis	Serology, blood culture
	Hepatitis A, hepatitis E	Serology
	Typhoid fever	Blood culture
	Dengue	Serology
	Leptospirosis	Serology, urine culture
	Amebiasis	Serology, smears
Tick exposure	Relapsing fever	Blood smear
	Rocky Mountain spotted fever	Serology
	Lyme disease	Serology
	Tularemia	Serology, blood culture

Animal contact	Tularemia	Serology, blood culture
	Leptospirosis	Serology, blood culture
	Brucellosis	Serology, blood culture
	Q fever	Serology
	Psittacosis	Serology
	Murine typhus	Serology
All the conditions that are endemic in a particular region have their own unique geographic distribution. All the conditions associated with animal contact are caused by a unique pattern of animal exposure.		

TABLE 82-5 -- Distribution of causes of fever of unknown origin in the elderly.

DISTRIBUTION OF CAUSES OF FUO IN THE ELDERLY	
Cause	**Percentage of cases**
Infection (e.g. abdominal abscess, bacterial endocarditis, tuberculosis)	25–35
Connective tissue disorders (e.g. giant cell arteritis, polymyalgia rheumatica)	25–31
Neoplasia (e.g. lymphoma, carcinoma)	12–23
Unknown	9–16

to be a useful method for characterizing known lesions when percutaneous procedures are not feasible or are nondiagnostic.

The yield from biopsy of the temporal artery in elderly patients who have an ESR above 40mm/h is not available in any case series. However, reports on temporal arteritis estimate that up to 30% of patients who have this illness do not have the classic localizing symptoms. Moreover, several series comment that biopsy of the temporal artery should be performed early in the evaluation of an elderly patient who has an ESR above 40mm/h, because giant cell arteritis is a common cause of FUO in this age group and also because early recognition and treatment of giant cell arteritis can prevent the sudden blindness that it sometimes causes. Table 82.5 lists the distribution of conditions causing FUO in elderly patients. [74]

The usefulness of screening with radionuclide scans in patients who have FUO remains unestablished.[75] [76] [77] Several reports suggest that the value of these scans in FUO has been over-rated and that the overall value of the test has been disappointing and potentially misleading. In one large series, approximately 14% of gallium-67 scans were helpful in providing a diagnosis but false-positive results that were potentially misleading and led to unnecessary testing occurred in 21% of patients.[12] This series was performed before the

widespread use of abdominal CT scanning. Because abdominal CT scanning has proved to be of value in the evaluation of FUO, it may further reduce the value of gallium-67 scans in this setting. The diagnostic yield of indium-labeled leukocyte scanning, [76] technetium-labeled antigranulocyte antibody and indium-labeled polyclonal IgG[77] has not been systematically evaluated. Indium scans may be even less useful than gallium scans because tumors may not take up indium-labeled leukocytes. More recently, the value of positron emission tomography using 2-(^{18} F) fluoro-2-deoxy-D-glucose (FDG-PET scanning) has been evaluated in patients who had FUO, with inconsistent results. Although one study noted that 41% of scans aided in establishing a diagnosis, 48% of abnormal scans were noncontributory and potentially misleading.[78] In contrast, in another study, 11 of 12 abnormal scans led to the diagnosis.[79] PET scanning may prove to be more useful than other radionuclide scans. At present, however, because radionuclide scans may give misleading results, they should not be part of the standard evaluation of FUO. Because of false-positive test results, physicians should be cautious in interpreting the findings and should not recommend invasive procedures solely on the basis of positive radionuclide scans.

Drug-related fevers

When a drug-related fever should be considered, necessary drugs can be changed to alternatives of a different class. After stopping the agent that is responsible for the fever, the fever will usually resolve within 3 days, although it may take as long as 2 weeks. Persistence of fever beyond this should direct the clinician to investigate an alternate source. If the fever remits, the clinician can definitively confirm the diagnosis by re-instituting the agent, which characteristically elicits fever again within a few hours. This procedure is safe unless drug-induced organ damage, such as interstitial nephritis or hepatitis, has occurred.

THERAPEUTIC INTERVENTIONS

In most instances therapeutic trials should be discouraged in the early course of FUOs. The purpose of such trials is to establish a diagnosis by noticing an abatement of fever following the use of a therapeutic agent such as an antibiotic, a corticosteroid or a NSAID. The empiric use of agents such as these may be misleading for several reasons:

- medical intervention makes it difficult to determine whether a new finding has resulted from the treatment or the underlying disease;
- fall of temperature may be fortuitous or it may result from the antipyretic effects of corticosteroids or NSAIDs; and
- improper use of antibiotics may lead to a false sense of therapeutic and diagnostic security and interfere with finding a diagnosis.

The spontaneous resolution of fever in stable patients is another argument against the empiric use of therapeutic trials. Employing empiric antibiotics except in the most urgent situations ultimately creates more frustration, confusion and despair for the physician and the patient.

Prolonged empiric therapy may have multiple deleterious consequences beyond unnecessary expense and inconvenience for the patient. When fever in a patient who has a self-limited illness coincidentally abates while the patient is receiving treatment for an unproven diagnosis (e.g. antibiotics for possible culture-negative endocarditis, antimycobacterial therapy for possible tuberculosis or corticosteroid therapy for a collagen disorder) the patient may be exposed to unnecessary iatrogenic complications resulting from the treatment. In addition, complications resulting from unnecessary interventions may confuse further diagnostic strategies based on abnormalities on physical examination or laboratory findings. For these reasons, therapeutic interventions should be discouraged in stable patients. Nevertheless, there are occasional circumstances in which a patient has a rapidly progressive, potentially fatal illness in which empiric therapy becomes unavoidable. In such cases, for instance a fulminant vasculitis and even, rarely, tuberculosis, high-dose corticosteroid therapy can be life-saving.

FEVER OF UNKNOWN ORIGIN IN HIV-INFECTED PERSONS

Fevers of unknown origin are not infrequent in the late stage of HIV infection[27] (see Chapter 124 , Chapter 125 , Chapter 126 , Chapter 127 , Chapter 128 , Chapter 129 , Chapter 130 , Chapter 131 , Chapter 132 , Chapter 133). One prospective study showed that fever occurred in 46% of patients who had advanced HIV infection.[28] The number of FUO cases caused by AIDS-related diseases is likely to increase as HIV infection becomes more prevalent.

Several HIV-associated illnesses may present with fever before the onset of specific organ-related symptoms.[28] [29] [30] [31] [32] [80] [81] [82] [83] Examples include lymphoma, *P. carinii* pneumonia, leishmaniasis and infections due to CMV, *Cryptococcus neoformans*, *Toxoplasma gondii* and *Mycobacterium tuberculosis*. Other illness, such as disseminated *Mycobacterium avium* infection and histoplasmosis, may present with constitutional symptoms alone in the absence of specific symptoms of organ involvement. Five large series of FUO in HIV-infected persons, with a total of 257 cumulative patients, are summarized in Table 82.6 . The mean $CD4^+$ lymphocyte counts, reported in four of the five series, were 58, 71, 94 and 98. Thus, in HIV-infected persons, FUO tends to occur in the late stage of HIV infection. A modified criteria for FUO was used in four of the five series: a fever in excess of 101°F (38.3°C) that persists for more than 4 weeks as an outpatient or 4 days as an inpatient and that has no obvious source.

Infections are the most common cause of FUO in HIV-infected persons, accounting for more than 70% of cases (see Table 82.6). Mycobacterial infections, both *M. tuberculosis* and *M. avium*, account for the majority of these cases. The remaining infections are due to pyogenic infections and to infections caused by *T. gondii*, *P. carinii*, *C. neoformans* and *Leishmania* spp. Cytomegalovirus and HIV itself account for a small percentage of FUO. The cause of FUO was attributed to HIV alone

when the diagnostic evaluation did not reveal a specific etiology and when the fever abated following antiretroviral therapy. Tumors, principally lymphomas, are responsible for 7% of cases. Miscellaneous causes, including drug fever, adenoviral pneumonia and bleomycin lung toxicity, account for 13% of cases. The diagnosis remains unestablished in approximately 15% of cases. In 20% of cases there was found to be more than one disease causing the unexplained fever.

As with classic FUO, the spectrum of infections causing HIV-associated FUO differs according to geographic location. In the USA, the most common cause of HIV-associated FUO is disseminated *M. avium* infection. In contrast, outside the USA, tuberculosis accounts for the majority of cases of FUO.[32] Leishmaniasis is a common cause of FUO in series from outside the USA, and salmonellosis and cryptococcosis are more common in some non-US series.[31] [32] Histoplasmosis and Chagas' disease are prevalent in Central and South America and infection with *Penicillium marneffei* has been reported from South East Asia.[32]

Diagnosis

The utility of the available tests for FUO has not been determined from direct studies. Nevertheless, indirect information on their usefulness may be derived from studies that establish their sensitivity for the specific opportunistic infections that are responsible for causing FUO. The diagnostic modalities and the studies that establish

TABLE 82-6 -- Selected reports of fever of unknown origin in HIV-infected patients.

SELECTED REPORTS OF FUO IN HIV-INFECTED PATIENTS							
Report	**Sepkowitz *et al.*[28]**	**Bissuel *et al.*[29]**	**Miralles *et al.*[30]**	**Lambertucci *et al.*[31]**	**Armstrong *et al.*[32]**	**Total no. cases**	**% of total**
Country	**USA**	**France**	**Spain**	**Brazil**	**USA**		
Total no. patients	**25**	**57**	**50**	**55**	**70**	**257**	
Mycobacterium tuberculosis	0	10	21	18	4	53	21
Mycobacterium avium	6	7	7	5	22	47	18
Other mycobacteria	0	6	0	0	1	7	3
Pneumocystis carinii pneumonia	4	3	1	6	10	24	9
Cytomegalovirus	0	5	1	0	8	14	5
HIV	1	1	0	0	0	2	1
Pyogenic infections	0	0	1	5	4	10	4
Lymphoma	4	4	2	4	5	19	7
Cryptococcosis	0	1	0	3	1	5	2
Leishmaniasis	0	4	7	0	0	11	4
Toxoplasmosis	1	2	1	1	1	6	2
Other diagnosis	5	6	3	3	16	33	13
Unknown	4	8	6	10	14	42	16
More than one causative disease	0	0	0	38	13	51	20

In some cases, HIV itself as a cause of FUO was defined as a response to antiretroviral therapy. Included in the category of 'other diagnosis' are factitious fever, zidovudine toxicity, *Isospora belli* enteritis, *Candida* sepsis, aspergillosis, varicella-zoster encephalitis, drug fever, bleomycin pneumonitis, hepatitis B, malaria, disseminated histoplasmosis, pulmonary nocardiosis, disseminated *Penicillium marneffei* infection, disseminated cryptosporidiosis, Kaposi's sarcoma, Castleman's disease, hepatitis C, adenovirus pneumonia and herpes simplex esophagitis.

their usefulness in the evaluation of HIV-associated FUO are discussed below.

Noninvasive tests

Blood cultures for bacteria, *Mycobacterium* spp. and fungi are valuable diagnostic tests in the evaluation of FUO. In one report, blood cultures had a sensitivity of 75% for disseminated *M. avium* infection; other reports suggest a sensitivity of 64–85% for *Mycobacterium* spp.[29] Available systems include Bactec, Dupont Isolator and lysis-centrifugation systems; the comparative yield for each of these systems for FUO in HIV-infected persons is unknown. *Histoplasma capsulatum* has been grown from lysis-centrifugation system when it failed to grow in the routine biphasic blood culture system. In rare instances, other organisms may be recovered from isolator blood cultures, such as *Nocardia* spp. Buffy-coat culture for CMV infections has not been proved to be a useful test in establishing the diagnosis of disseminated CMV infection, both because patients may be viremic without having disease due to CMV infection and because fewer than 50% of patients who have documented CMV disease are viremic.[84]

Serum and urine antigen techniques are useful in identifying certain fungal and viral infections. Serum cryptococcal antigen is sensitive and specific for detecting dissemination with *C. neoformans*; it is therefore a useful test for this purpose.[85] Recent reports suggest that serum antigen for CMV and molecular testing to detect CMV DNA are sensitive for detecting dissemination due to CMV disease.[86] [87] Antibodies for CMV are not helpful in establishing a diagnosis because 90% of HIV-positive patients have antibodies to CMV. Diagnosis of CMV esophagitis, colitis, gastritis or encephalitis requires pathologic evidence of the effects of the virus; retinitis may be diagnosed by funduscopic examination. *Histoplasma* antigen in urine or serum is a very sensitive and specific finding for the diagnosis of disseminated histoplasmosis; [88] serial antigen assessment has also been found to be useful in monitoring the response to therapy.

For the diagnosis of *P. carinii* pneumonia, expectorated sputum is rarely diagnostic, because in most cases the cough is not productive. Nebulizer-induced sputum is a technique that is available in the ambulatory setting and is diagnostic in many cases because of the high number of organisms that are present in the alveoli. Published studies show that the sensitivity of the test for evaluating *P. carinii* pneumonia is 75–90%.[89] [90] The technique involves centrifugation of liquified sputum and staining the specimen with a direct fluorescent monoclonal antibody that reacts with the cyst wall. This test may establish the diagnosis of *P. carinii* pneumonia in a noninvasive fashion and avoid the need for bronchoscopy.

Invasive procedures

The introduction of sensitive noninvasive tests for diagnosing many conditions responsible for FUO in HIV-infected persons has reduced the need for invasive procedures. However, invasive procedures, such as biopsies of bone marrow, liver and abnormal lymph nodes, continue to have a role in evaluating FUO, especially when patients remain febrile despite a prolonged noninvasive evaluation.

The yield of bone marrow examination for FUO in HIV-infected persons has been investigated in several series and has ranged from 13% to 42%.[91] [92] [93] One series showed that, in 86% of patients in whom a cause of the fever was identified by bone marrow examination, the same diagnosis had been established by a noninvasive diagnostic modality.[93] This series also showed that in 53% of patients the diagnosis was made by bone marrow examination as rapidly as or sooner than it was by other modalities and that in 16% of patients the diagnosis was made exclusively by bone marrow examination. The authors concluded that bone marrow examination is indicated when a diagnosis is urgently sought or when an evaluation by other diagnostic modalities has been unsuccessful.[93]

The usefulness of liver and lymph node biopsy has been evaluated in several studies.[94] [95] [96] [97] The yield from liver biopsy in evaluating FUO in HIV infected persons has varied between 40% and 58%. Biopsy of enlarged lymph nodes has been reported to have a high yield. [98] [99] [100] In a population at high risk for HIV, the yield of lymph node biopsy for tuberculosis was 57%. Fine-needle aspiration was also reported in one study to have a high diagnostic yield.[100]

Approach to the diagnosis

An initial approach in the evaluation of FUO in HIV-infected persons should be to discontinue medication, especially antiviral agents and sulfonamides. The clinical features associated with opportunistic infections that cause prolonged fever often overlap with those associated with drug reactions (e.g. cytopenia and elevation of liver enzyme tests). If there is no response after 3 days, blood culture using the lysis-centrifugation technique is a sensitive method of identifying intracellular organisms that cause disseminated infections (e.g. *H. capsulatum, C. neoformans, M. avium*). However, because the mean time for cultures to turn positive is 18–25 days for *M. avium*, [83] other noninvasive tests should be performed simultaneously. Induced sputum for *P. carinii* and *M. tuberculosis* should be obtained because prolonged fevers may precede the onset of respiratory symptoms in both *P. carinii* pneumonia and tuberculosis. Tuberculin skin testing and antigen testing for *H. capsulatum* in serum and urine also should be performed. If the serum cryptococcal antigen is reactive at a titer of less than 1:16, a lumbar puncture should be performed, even if the patient does not complain of headache or demonstrate nuchal rigidity.[85] A dilated ophthalmologic examination should be performed to investigate retinitis due to CMV.

If the initial approach does not yield a specific diagnosis, the subsequent evaluation should include repeated physical examinations and routine laboratory work. Because abnormal findings of diagnostic importance (e.g. skin nodules, asymmetric and enlarging lymphadenopathy, and rapid and significant rise in serum alkaline phosphatase levels) may make their first appearance long after the onset of fever, abnormal organs should be biopsied and organisms looked for using special stains.

Other invasive procedures should not be performed while waiting for an organism to be isolated from lysis-centrifugation cultures if the patient remains stable, because cultures of material aspirated from bone marrow or lymph nodes or biopsied from the liver are no more sensitive than isolator blood cultures. If cultures of blood are still negative after 3 weeks, a bone marrow examination should be performed, even if the patient is stable, because the bone marrow examination has been shown to be the sole method of identifying an illness in approximately 5% of patients in whom all noninvasive tests have been unrevealing.[92] [93] Outside the USA, bone marrow examination may be considered earlier in the diagnostic evaluation, given the enhanced prevalence of leishmaniasis and tuberculosis in other countries.

In the evaluation of prolonged, unexplained fevers, the patient's previous exposures, stage of HIV infection and present epidemiologic setting often provide important clues. For example, tuberculosis should be suspected in anyone who has HIV infection and has been exposed to an infected person or who has resided in an endemic region. Similarly, histoplasmosis or coccidioidomycosis may occur in persons who have been in an endemic region.[82] Leishmaniasis should be considered in people who have been in South America or the Mediterranean area. Infections caused by *P. marneffei* should be considered in people who have lived in southern Asia. Extrapulmonary *P. carinii* infections may be identified in patients in the late stages of HIV infection who have been receiving aerosolized pentamidine.[81] Infections due to *Bartonella henselae* should be considered in people who have had exposure to cats; the organism may be identified by serology or by isolator blood cultures. Lymphoma should be considered in patients who have no specific exposure history. Finally, patients who have very low $CD4^+$ counts and have recently commenced highly active antiretroviral therapy who experience marked immunologic improvement are at risk for immune reconstitution disease. Unusual presentations of opportunistic infections should be considered in these persons; for example, *M. avium* or cryptococcal lymphadenitis can occur in the absence of disseminated disease and with a low burden of organism on biopsy specimens. Immune reconstitution illness also occurs in patients who have recently started antiretroviral therapy who are also being treated for an active opportunistic infection. Fever in this setting does not represent progression of the opportunistic infection and does not indicate a need for change of antimicrobial therapy. Rather patients should be treated with anti-inflammatory agents and, if necessary, corticosteroids.

When the initial and subsequent evaluation for FUO has been unrevealing, abdominal CT scan occasionally proves to be a useful diagnostic tool. Occasionally, peritoneal masses or a group of enlarged retroperitoneal lymph nodes may be identified on the scan; a directed CT-guided biopsy of these abnormal areas on CT scan may reveal the cause of fevers. Gallium scans and indium-labeled leukocyte scans are frequently performed in the setting of prolonged unexplained fevers but the tests are rarely of diagnostic value and are potentially misleading. Another test of uncertain significance is the buffy-coat culture of blood for CMV, because viremia may occur without symptoms and the test is insensitive for the detection of CMV disease.[86] A thorough evaluation of FUO as outlined above yields a diagnosis in approximately 85% of cases. Multiple causes for FUO will be identified in up to 20% of cases — the feature that distinguishes FUO in HIV-infected persons from those who are not infected with HIV.[32]

CONCLUSION

Since its original description in 1930, case series have shown that a careful, organized approach to the problem on the part of the physician remains of pre-eminent importance in identifying the cause of FUO. This remains true even though new diagnostic modalities that are useful in the evaluation of FUO have been introduced and the number of illnesses that are responsible for FUO has expanded. How to employ the available diagnostic tools must be individualized for each patient and remains a challenge for the physician. The role of exploratory laparotomy is limited to characterization of known lesions that remain undiagnosed after percutaneous procedures and of those that are not accessible by the percutaneous approach. Because blood culture with the use of the lysis-centrifugation technique is a sensitive method of detecting many of the infectious causes of HIV-associated FUO, invasive procedures may be avoided if the patient remains stable. Despite the introduction of new diagnostic modalities, the investigation of FUO in the general population or in HIV-infected persons remains one of the most challenging tasks for the clinician.

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Chapter 83 - Health Care-associated Infections

Didier Pittet
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EPIDEMIOLOGY

Trends and complexity in health care

Health care-associated infections (HAIs), whether acquired during home, ambulatory, institutional or hospital care, constitute one of the greatest challenges of modern medicine. According to the Institute of Medicine, Washington DC, USA, preventable hospital-related adverse events in the USA, including nosocomial infections, are responsible for 44,000–98,000 deaths annually and represent a cost of $17–29 billion. Among these, nosocomial infections now affect 5–15/100 hospitalized patients and can lead to complications in 25–50% of those admitted to intensive care units (ICUs). Costs for nosocomial infections related to hospital stay were estimated at $4.5 billion in 1992, which translates to $7.5 billion in 2001 using the Consumer Price Inflator.[1] In the UK, hospital-acquired infections cost around £1 billion a year. Importantly, these estimates only concern infections acquired in acute care hospitals and ignore those resulting from ambulatory care or acquired in other settings.

Infection characteristics and risks are strongly related to changes in health care. Whereas the hospital has traditionally been the center of health care systems, it only constitutes one piece of the jigsaw today. Similarly, while physicians and nurses have generally been considered as the primary health care workers (HCWs), they have been progressively assisted and even replaced in some instances by other health care providers, professionals or technicians. The economic pressures on health care are important drivers of such a splinter effect. One of the main results is the shift of patient care from acute care hospitals to other facilities or systems. Two main avenues can be identified:

- long-term care facilities (LTCFs), mainly due to the increasing elderly population; and
- different forms of ambulatory care (Fig. 83.1).

In the USA, around 1.5 million persons reside in approximately 17,000 nursing homes and 40% of those over 65 years of age will spend some time in a nursing home before dying.[2] Because of frailty and the closed environment, HAI rates in this setting probably equal those in acute care.[3] In 1985, they were estimated at 1.5 million annual infections,[4] or an annual incidence of five to six infections per 1000 resident days.[5]

Over the past two decades, a spectacular increase in specialized ambulatory clinics has been observed. In 1980, approximately 15% of all surgical interventions were carried out on an outpatient basis. Today, outpatient surgery represents almost three-quarters of all procedures. Similarly, there has been an increase of nearly 200% in the number of outpatient surgical procedures being performed in ambulatory care centers over the past decade.[6] Experts agree that this trend is likely to continue with a parallel economic impact on hospitals.

Major changes have occurred in the way in which health care is delivered and financed over the past few years. The patient demand for health care services has also substantially increased. In 1999 for example, 82.5% of the entire USA population had paid at least one visit to a physician's office, an emergency department or a clinic, or had received care at home. In 2000 in the USA, 31.7 million hospital discharges stood against 83.3 million outpatient department visits.[7] [8] Patient care is moving from hospital, to outpatient clinic, ambulatory care and finally home care. Some patients are not treated by physicians or even nurses for their conditions, and there has been a greater emphasis on providing quality health care at a low cost.

Health care-associated infections are becoming increasingly important as a result of multiple converging trends. Shortening of hospital stay has become an issue of survival for hospitals, especially under capitation, leading to an aggregation of the sicker patients who must remain in acute care hospitals,[9] and to the risk of early discharge of patients to sometimes poorly prepared health care settings or structures. Technical and scientific progress introduce more risk for acquiring infection, such as by the increase in device use, care complexity, immunosuppressive therapy and organ transplantation. This is shown by the data of repeated prevalence studies in a large tertiary care center over 10 years (Fig. 83.2).[10] As illustrated, while average length of stay decreased over time, patient case-mix and device use rates increased, resulting in an increased risk of device-associated infections despite stable overall infection rates. Aging of the population increases the problem further and treatments are increasingly aggressive, even in the elderly; for example, advanced age is no longer an exclusion factor for ICU admission.

Spiralling health costs fuel these changes, leading to early patient discharge and downsizing in staff number and expertise. There is no reason why this trend should be reversed in the near future. The various components of the health care system remain less separated than previously and overlap because of accelerating patient transfer activity. Of note, transferred patients have been found to be infected four times more frequently than new admissions from the community.[11]

Not all of this evolution has been anticipated and the necessary adaptations of very basic structures such as patient data update have not been made or lag behind in progress. This applies also for infection prevention.

Leading infections in different health care settings

The traditional focus for nosocomial infection is the acute care setting. Of equal importance is infection in lower level care institutions such as LCTFs. The increasing recourse to ambulatory care would suggest that this setting has an important potential for infection, but very few data exist on this topic. We discuss below the most common types of HAIs.

In contrast to acute care, LTCFs feature a higher proportion of soft tissue and urinary tract infections, but have less bloodstream and surgical site infections (Fig. 83.3). Note the considerable proportion of respiratory infections in all settings. Importantly, although infection rates were higher in critical care, they were no higher in acute-than in long- or intermediate-care beds, stressing the need to implement infection prevention strategies in all health care settings.

Bloodstream infections are the most easily defined because they are mostly related to the very specific event of a positive blood culture. The majority of these are due to intravascular device use.

Figure 83-1 Complexity of modern health care systems.

Although these events are rare in the long-term setting, they may be important and under-recognized in the outpatient setting among patients with long-term intravascular catheters, such as those with cancer or osteomyelitis. Bloodstream infection is associated with an increased mortality rate, an increased hospital and ICU

stay, and resource use. Attributable mortality rate averages 25%, and additional costs range from $10,000 to $45,000 in acute care settings. However, such data remain unknown for infections acquired in LTCFs or in the ambulatory or outpatient setting.

Infections at the surgical site complicate 1–10% of operations. They are associated with substantial morbidity and mortality rates, double the duration of hospitalization, and increase the cost of health care. Importantly, over 50% of these infections become manifest only after hospital discharge. Again, infection rates associated with ambulatory practices remain unknown; outbreaks of infections have been reported, however, further stressing the need for closer surveillance and control outside the hospital setting.

Pneumonia is the leading infection in the critical care setting, but its importance in the nonacute setting is often underestimated. In the ICU, it is mostly related to invasive respiratory support and associated with high crude and attributable mortality rates, and significant extra costs. In LTCFs, respiratory tract infections are associated with multiple risk factors and mostly treated with broad-spectrum antibiotics because of the high prevalence of antimicrobial resistance. [12] In this setting, mortality rates have been estimated to be up to 20%.[13]

Overall, there is a lack of comprehensive statistical data on infections acquired in the outpatient setting or during home therapy, but the literature abounds with reports of health care-associated events, in particular, infections. For example, a cluster of hepatitis B transmission at a physician's office, bloodstream infections associated with home infusion therapy, HIV transmission from a dentist to his clients, and transmission of tuberculosis in an ambulatory waiting room.

Trends in microbial pathogens and resistance

In contrast to the acute care hospital, data are scarce on trends in pathogens for HAIs outside this setting. Table 83.1 provides an overview of the importance of different multiresistant pathogens in different health care institutions.

Antimicrobial resistance is mostly the product of health care intervention and has become a worldwide problem. Together with globalization, issues related to resistance in one continent become relevant in almost any other. Similar trends in health care development (risk factors, antibiotic use) and spread due to increased travel activity might be the major determinants for this evolution. As an example, the proportion of methicillin resistance among *Staphylococcus aureus* infections is increasing globally, and identical strains are found at distant places at short intervals.

International air traffic has increased from 2 million passengers per year in the 1950s to over 1.4 billion in 2000. Over half of all European adults travel abroad during the same year, a large proportion to developing countries. This constant population movement facilitates global dissemination of infectious diseases, and carriage of micro-organisms from one corner of the earth to another occurs in a matter of hours. Asymptomatic carriers disseminate antimicrobial resistance. As an example, some major hospital outbreaks of methicillin-resistant *S. aureus* (MRSA) infection in Canada could be traced to a small village in North India.[14]

There are striking differences in the epidemiology of multiresistant pathogens between different continents, countries or hospitals. Antimicrobial prescription outside the hospital also dramatically differs from country to country (Fig. 83.4). Infection control practices such as the use of alcohol-based hand disinfection and barrier precautions have an effect on the transmission of nosocomial pathogens. Unfortunately, compliance with recommendations varies greatly, both among individual care-givers and institutions, and even among different countries.

Impact of health care-associated infections

Medicine was an art for over 3000 years, a science for the past 100 years, and has become a business for 30 years. Economic concerns have taken on increasing importance in health care over the past three decades. There is a major interest in determining the financial impact of HAIs to better tailor and target prevention efforts under current resource restraints. In acute care hospitals, the impact concerns mortality rates and morbidity, mostly expressed in additional length of stay and direct costs. The impact of infections on the health care system or the society must also be considered, but has been poorly studied so far. Although infections acquired in chronic care facilities or in the ambulatory or home care setting might be associated with fatal outcome in rare instances, they mostly impact on patient morbidity and extra costs to the society. For example, following ambulatory surgery, surgical infection may prolong patient absence from work. Furthermore, costs associated with extra help at home to take care of the family when a parent is sick need to be considered. Most of these costs are not 'billed' to the health care system, but certainly have an effect on other areas of society.

Another unique currency in which the costs of HAI can be expressed is the emergence of antimicrobial resistance, again further increasing hospital costs, as well as ambulatory and home care. The financial implications of antibiotic resistance in the hospital setting have been discussed in two review articles, which summarized examples of studies on the economic impact of transmission of antibiotic-resistant organisms.[16] [17] Direct medical costs of antibiotic resistance include, but are not limited to, the cost of more expensive

Figure 83-2 Trends in average length of hospital stay, patient case-mix, and nosocomial infection; Yale-New Haven Hospital, 1988–1995. (a) Trends showing the decrease in average length of hospital stay and the increase in the mean diagnosis-related group case-mix index. During the study period, there was a significant decrease in the mean length of stay, from 7.3 to 6.0 days (p = 0.01), and a concomitant increase in the mean diagnosis-related group case-mix index, from 1.03 to 1.24 (p = 0.001). (b) Nosocomial infection rates, assessed by repeated prevalence surveys, remained unchanged (p = 0.43) over the study period, but rates of nosocomial bloodstream infection increased (p = 0.05) corresponding to increased medical device use (p = 0.001, not shown). *From Weinstein et al.[10] , with permission.*

antibiotics, labor costs, laboratory costs, the cost of extra hospital days because of failure of initial therapies, and the cost of isolation and other infection control measures.[13] Accordingly, the annual direct cost of infections caused by antibiotic-resistant organisms in the USA are estimated to be $4–5 billion.[16]

PATHOGENESIS

Infection results from an interaction between host, micro-organism and health care setting-related factors (Fig. 83.5). A prerequisite is the presence of a microbial pathogen, be it from an endogenous patient colonizer or an exogenous source. Whereas some microbial pathogens are intrinsically virulent, others infect as a result of previous host colonization and a break in host vigilance, thus seizing the chance to multiply after disruption of physiologic flora or a break in the anatomic barrier. Pathogens are undesirable either because of their special virulence to the exposed population or because of their resistance pattern. Examples of breakdown in host defenses include a break in the skin barrier, the bypass of mucosal defense mechanisms and introduction of foreign bodies. The immune system may be hampered by immunosuppressive agents, pre-existing health conditions or simply by age.

To fully acknowledge possible risk factors, patient care must be seen in its larger sense, including:

- the patient environment (air and water quality, architecture, device sterilization);
- technical aspects of care (engineered solutions, device material, design);
- organizational structures (bed occupancy, transport of patients and material, ward exchange rate of patients and personnel); and
- health care worker and patient behavior toward procedures of care and health in general (Table 83.2).

Whereas procedures and quality of care are more closely attributable to individual patients, environment and, even more so, organizational factors are rather associated with the health care system and thus are common to an entire group of patients. Since therapeutic interventions such as immunosuppressive therapy or the nature and quality of surgical technique are a prerequisite to potential infection, they may be regarded as quality issues from the angle of infection outcome. Once preventive interventions are known, they become part of quality of care or departure from it (antibiotic prophylaxis in surgery to prevent surgical site infection, restriction in sedatives in elderly patients to prevent aspiration pneumonia, and skin care to prevent soft tissue infections). Importantly, even when procedure, environmental and organizational related parameters are optimal, patient or health care personnel behavior might interfere with patient safety.

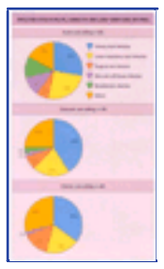

Figure 83-3 Leading sites in acute, subacute and long-term care settings. *From Sax et al.,[3] with permission.*

TABLE 83-1 -- The importance of multiresistant pathogens in different health care settings.

IMPORTANCE OF MULTIRESISTANT PATHOGENS IN DIFFERENT HEALTH CARE SETTINGS				
	Intensive care unit	**Acute care**	**Long-term care facility**	**Outpatient**
Methicillin-resistant *Staphylococcus aureus*	+++	++	+++	+
Vancomycin-resistant *Enterococcus* spp.	+++	++	++	-/+
Penicillin-resistant *Streptococcus pneumoniae*	-	+	?	++
Gram-negative rods with extended spectrum ß-lactamase	+++	++	+	-
Multiresistant *Pseudomonas* spp.	+++	++	+	-
-, no problem; +, small problem; ++, intermediate problem; +++, important problem.				

PREVENTION

History

Although many historic examples could be used to recall the milestones of HAI prevention, only two are cited here.

In 1846, the Hungarian physician Ignaz Philipp Semmelweis observed, at the Lying-In Women's Hospital of the General Hospital in Vienna, that women whose babies were delivered by students and physicians consistently had a higher mortality rate than those whose babies were delivered by midwives. He also noted that physicians and medical students who went directly from the autopsy room to the obstetrics ward had a disagreeable odor on their hands despite washing them with soap and water upon entering the delivery room. After thorough investigation, he postulated that the high rate of puerperal fever was caused by cadaverous particles transmitted from the autopsy to the delivery room via the hands of students and physicians. As of 15 May 1847, Semmelweis insisted that students and physicians scrubbed their hands in a chlorinated lime solution before every patient contact. The maternal mortality rate subsequently dropped dramatically and remained low thereafter. This intervention by Semmelweis represents the first evidence suggesting that cleansing hands with an antiseptic agent between patient contacts may reduce cross-transmission of infectious agents. Semmelweis' difficulties in modifying hand hygiene behavior among his peers and colleagues can be considered as the first step of the crusade of most hospital epidemiologists whose objective is to improve compliance with infection control practices.

Florence Nightingale, considered to be the first nurse-epidemiologist, published in 1863 the third edition of her book, *Notes on Hospitals*, which had a major impact on health care in Great Britain. She reported mortality rates for the country's main hospitals, concluding that 'most unhealthy hospitals are those situated within the vast circuit of the metropolis' and also made the statement that 'in all probability a poor sufferer would have much better chance of recovery if treated at home'. This famous book also contains arguments for a direct relationship between sanitary conditions and postoperative complications, such as gangrene and pyemia, as well as a detailed description for ward construction and the concept of air control. Furthermore, information is provided on an observed higher mortality rate among hospital personnel (matrons, sisters, nurses) compared to the female population of London, and possibly resulting from contagious conditions. Nightingale was also the first person to suggest that nurses could survey hospital-acquired infection. Together with William Farr, the Registrar General and premier British health statistician, she showed that contagious diseases and crowding could explain the excess mortality rate among soldiers in the Crimea, and pleaded for improved general hygiene practices.

Figure 83-4 Outpatient antibiotic use in different developed countries. *From Harbarth et al., [15] with permission.*

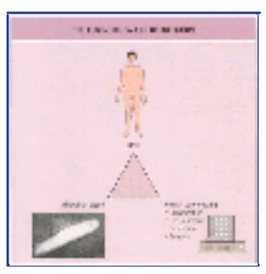

Figure 83-5 The essential causes of infection.

Structure, process and outcome

Infection prevention relies on measuring parameters integrated in the health care structure, process or outcome as categorized by Donabedian.[18] Once faults are recognized, action may be directed at improving structure- or process-related issues. For the latter, infection prevention should include influence on health care partner and patient behavior to achieve optimal outcome.

Semmelweis chose an outcome-based approach, measuring maternal mortality rate from puerperal sepsis. By modifying the process of care through the implementation of an intervention (system change), he changed HCW hand hygiene behavior with a subsequent impact on the outcome measure. Florence Nightingale worked on both issues — improvement of: the structure of hospitals and wards by modifying the architecture and introducing patient charts; and the process by collecting outcome data to justify the implemented changes.

As a very comprehensive approach, the seminal SENIC study for infection control in hospitals[19] (see Chapter 87) issued quality standards regarding structure (personnel) and process (feedback of surveillance results) based on outcome measurement.

Attempts to ameliorate process quality range from guidelines to improve device use and care, to water and air quality controls. For their efficient implementation, behavior control plays a strategic role but also represents the most challenging and important element because it may interfere with any prevention strategy.

Outcome measurement is typically considered the gold standard and motivates the major investment in infection surveillance. Yet, because of the rarity of some infectious outcomes or technical difficulty associated with assessing these events, structure or process measurement may often be more adequate to guide prevention. In this case, pre-existing knowledge must be available about the relation between structure or process and the outcome. The following serves as an illustration. An ever-increasing number of gastrointestinal and pulmonary endoscopic procedures are being carried out in physicians' consulting rooms and outpatient clinics.

Numerous outbreaks of infections associated with the improper use or cleansing of endoscopes have been reported. Reusable accessories that break the mucosal barrier should be cleaned and sterilized or submitted to high-level disinfection between each patient. Personnel assigned to reprocess endoscopes must receive device-specific reprocessing instructions and training to ensure proper management and patient safety. Competency testing of such personnel is not conducted on a regular basis among most private practice physicians and there are no regulations governing this issue. Appropriate control of endoscopic procedures in private practice would be an example of patient safety promotion outside the hospital; process control in this case would certainly be more effective than outcome-based surveillance, which is extremely difficult and time-consuming in this setting.

The art of intervention: two examples

By the following two examples, we attempt to explain the way interventions might be performed successfully. We believe that the fundamental elements will not change according to the health care setting.

Catheter-associated infection: implementation of evidence-based guidelines

Despite continued technologic progress, infection related to the use of intravascular catheters remains a major concern. It has recently been estimated that the annual cost of intravenous catheter-related

TABLE 83-2 -- Summary of risk factors by category and selected examples.

SUMMARY OF RISK FACTORS BY CATEGORY AND SELECTED EXAMPLES					
Category	**Issues**	**Examples**			
		Acute care	**Chronic care**	**Outpatient**	**Home care**
Host associated	Genetic background	HLA type, leukocyte function, polymorphisms of immune response genes, etc.			
	Real-life experience	Lacking immunity due to pathogen exposure or vaccination, poor living conditions, homeless, lack of adequate insurance coverage			
	Chronic health conditions	Diabetes, cirrhosis, renal insufficiency, immunosuppressive medication	Pressure ulcers, incontinence, dependency, age, poly-medication	Smoking, alcohol or intravenous drug use	Poor nutritional state, negligence
	Acute health condition	Acute physiologic imbalance, severe trauma, extensive burn, acute hyperglycemia	Reduced alertness after stroke	Viral respiratory infection	Falls
Microbial associated	Pathogen virulence	Multiplication rate, invasion strategies, adhesion, intracellular survival, colonization advantage			
	Pathogen transmission route	Airborne transmission route, environmental survival			
Environment associated	Space concept	Poor operating theater ventilation, deficient hospital architecture, lack of space between patients	Poor compromise between conviviality and hygiene needs	Poor waiting room concept, lack of workspace, attending day care center	Poor room adaption, impractical hygiene installation
	Inanimate environment treatment	Poor cleansing, disinfection, sterilization and dust removal	Like acute care	Uncontrolled instrument reprocessing	Poor hygiene conditions
	Air and water treatment	Lacking filter maintenance, uncontrolled water quality	Lacking negative pressure rooms for tuberculosis patients	Lacking aeration	
Procedure and care associated	Indication for procedure	Overuse of vascular access devices	Overuse of urinary catheters	Overuse of invasive diagnostic procedures	Prolonged use of vascular access
	Device and procedure design/technique	Lack in justified application of minimal invasive surgery, intravenous catheter coating, tunneled catheter, noninvasive ventilation	Open urinary drainage systems	Poor endoscope design	
	Operator performance	Prolonged surgical intervention time, poor antisepsis in catheter placement	Poor antisepsis in urinary catheter placement	Poor antisepsis in joint puncture	Poor compliance with hand hygiene between patients
Health care system organization	Staffing	Excessive workload, lacking expertise, training, inadequate nurse-to-patient ratio	Shortage in trained staff	Lack in basic training in endoscope reprocessing	Lack in basic training in infection prevention issues
	Patient allocation	Overcrowding, early transfer, wrong specialty attribution, highly specialized wards lacking experience in basic care outside of expertise	Mistaken transfer from and to acute care	Wrong specialty attribution	Missed hospitalization
	Guidelines	Missing written guidelines, no antibiotic use policy	Antibiotic use guidelines not adapted to local resistance or to patients' specificity	Lacking guidelines for 'standard precautions'	No guidelines
	Communication	Lack of surveillance scheme for legionellosis between public health and hospitals	Lack of communication of multiresistant organism carriage at patient transferral	Lack of access to patient record at time of consultation	Lack of stop order for intravascular device
Behavior	Patient behavior	Noncompliance with prescriptions	Mental impairment	Unhealthy lifestyle	Noncompliance with peritoneal dialysis procedure
	Health care personnel behavior	Noncompliance with vaccination guidelines, hand hygiene practice, lack of motivation			

bloodstream infections in USA ICUs is between $296 million and $2.3 billion and that approximately 2400–20,000 patients die annually of this infection (see Chapter 84).[20] Intravascular access is, however, essential for patient care and the search for effective preventive measures is an active field of clinical research.

Device-related infections can be reduced by specific prevention strategies and improved guidelines for their use. Strategies are multiple and include not only those dealing with catheter insertion and maintenance but also the choice of device material. According to recent studies, education and reinforcement strategies are of paramount importance. Physician-in-training education has been proven to decrease the risk of catheter infection.[21] A 1-day course on infection control practices and on procedures of vascular access insertion was shown to reduce the infection rate by 73%.[21] A multiple-approach prevention program to decrease vascular access infection was used in a medical ICU;[22] 3154 critically ill patients were included in a cohort study with longitudinal evaluation of an overall catheter-care policy

associated with an educational program including detailed information on clinical pathways for vascular access insertion, and device maintenance and use, based on previously identified risk factors. Following the intervention, the incidence of exit-site catheter infection and that of bloodstream infection decreased by more than 60%. Overall, the incidence of all infections acquired in the ICU was reduced by 35%.[22] Although infection rates before the intervention were within the accepted limits, the results of this study confirm that a large proportion of infections related to extrinsic factors are still preventable and that interventions targeted at vascular access can have a significant effect on the overall incidence of infections. The impact in terms of reduction of nosocomial infections in these two studies[21] [22] was impressive. Sherertz *et al.*[21] estimated that their program was associated with cost savings of at least $63,000 and may have exceeded $800,000. Using conservative estimates, the savings associated with the global strategy described[22] would correspond to the annual salary of three to six full-time infection control nurses. One of the important implications of the results is the possibility of extending the designed strategy outside the critical care arena, and including all patients with vascular access in health care settings. The program's cost-effectiveness would allow its generalization.

Hand hygiene: applied behavior science

Hand hygiene is the most effective measure to prevent cross-transmission and reduce the spread of antimicrobial resistance. Despite considerable evidence of its importance, compliance with hand hygiene by HCWs is unacceptably low. Factors predicting noncompliance have been extensively studied and include, among others, excessive workload. Reasons for noncompliance with recommendations at individual, group and institutional levels should be considered. Easy access to hand hygiene in a timely fashion is mandatory for appropriate hand hygiene behavior. In particular, in high-demand situations, in high-stress working conditions, and at times of overcrowding or understaffing, handrub with an alcohol-based solution appears to be the only alternative to allow decent compliance. Alcohol-based handrub is superior to traditional handwashing with (un)medicated soap, because it requires less time, acts faster and irritates hands less often. Furthermore, it was used in the only program that reported a sustained improvement in hand hygiene compliance associated with decreased infection rates. [23] The availability of alcohol-based handrub is, however, not sufficient to obtain sustained improvement with hand hygiene practices. Promotion strategies should be multimodal and multidisciplinary, and easy access to fast-acting hand hygiene agents should be viewed as the main tool of the strategy. Time constraint is the leading factor for noncompliance, but also the easiest to modify. System change must be addressed in most health care settings where waterless hand disinfection has not become a standard of care. In addition, HCW education and motivation are obviously important and must be part of multimodal strategies to enhance compliance during patient care. Successful promotion and behavioral changes will result in reduced infection rates and antimicrobial resistance spread, and enhance patient safety.

Guidelines for hand hygiene in health care settings were recently developed (www.cdc.gov/handhygiene/). They are not limited to indications for hand hygiene and technical recommendations, selection of hand hygiene agents and HCW skin care, but equally contain strategies for motivational programs, administrative measures and recommended outcome or process measurements. Importantly, administrative measures include:

- making improved hand hygiene adherence an institutional priority;
- providing appropriate administrative support and financial resources; and
- implementing a multidisciplinary program that includes a readily accessible waterless antiseptic agent.

Although the guideline has been prepared for hospitals, the majority of its content is valid for any health care setting; private physicians, home care nurses as well as those working in LTCFs will benefit from it.

CLINICAL FEATURES

Microbiologic phenomena are of widespread epidemiologic importance and demonstrate the complexities associated with new trends in health care. As examples, we have chosen the epidemiology and control of vancomycin-resistant enterococci (VRE), MRSA, penicillin-resistant *Streptococcus pneumoniae* (PRSP) and legionellosis. Table 83.3 summarizes the linkage between different health care settings and the emergence and dissemination of pathogens. These pathogens are discussed in detail in the following chapter on ICU infections (see Chapter 84).

Vancomycin-resistant enterococci

In the USA, VRE are among the feared multiresistant pathogens produced by acute care hospitals. The problem was detected in the late 1980s, and by 2000 28% of ICU-acquired enterococcal infections were caused by resistant strains. [24] The healthy population without contact with the health care system is not usually colonized. Vancomycin-resistant enterococci were initially transmitted in high-risk units such as ICUs and hemodialysis, hematology and transplant units, but then spread to the entire hospital and, consequently, to the community. This makes it a typical hospital-generated problem pathogen that is similar to MRSA in its reservoir (high-risk patients) and risk factors for transmission (the hands of HCWs and possibly the environment).[25]

In Europe, the situation is quite the opposite. There is a high prevalence of stool carriage of VRE in the healthy community who have no previous contact with the health care system. In contrast with this, VRE cause only singular outbreaks in hospitals, and have never reached threatening levels of appearance in HAIs. By indirect proof, it has become clear that this widespread colonization is introduced by the food chain.[26] Avoparcin, a glycopeptide antibiotic, was introduced in livestock as a growth promoter and has induced high-level intestinal colonization. Livestock is therefore the main reservoir for VRE in Europe; outbreaks only occur when colonized patients are exposed to antibiotic selection pressure and shed a high amount of pathogen, which is then transmitted within the hospital to other patients.

After the ban of avoparcin in various European countries in the mid-1990s, resistance in the animal and human population decreased.[27]

Decreasing genetic variability in VRE over time in US inpatients [28] leads to the assumption that the first VRE may have been imported from Europe at around 1990. Additional support for this hypothesis

TABLE 83-3 -- Interlinkage health care system components by a selection of indicator pathogens.

INTERLINKAGE HEALTH CARE SYSTEM COMPONENTS BY A SELECTION OF INDICATOR PATHOGENS				
	Origin	**Reservoir**	**Transmission**	**Effect**
Methicillin-resistant *Staphylococcus aureus*	Acute care hospitals (wards, ICU); patient-to-patient transmission, antibiotic pressure	Acute care hospitals, LTCF; patients Secondary: community; discharged patients	Acute care hospitals dialysis units, (physician's office, community?); hands of HCWs, (devices, environment)	Endemic, outbreaks in acute care hospitals, LTCF, dialysis units
Vancomycin-resistant *Enterococcus* spp. (USA)	Acute care hospitals; patient-to-patient transmission, vancomycin and cephalosporin overuse	Acute care hospitals; patients, devices, (environment)	Acute care hospitals, dialysis units, (physician's office, community?); hands of health care workers, devices, environment	Endemic, outbreaks in acute care hospitals, LTCF, dialysis units
Vancomycin-resistant *Enterococcus* spp. (Europe)	Avoparcin in livestock husbandry	Livestock, human gut	Food chain, (acute care hospitals)	Sporadic outbreaks in acute care hospitals
Penicillin-resistant *Streptococcus pneumoniae*	Pediatric and adult outpatient office; penicillin overuse	Community; children, adults	Community (schools, kindergarten); interhuman spread	Acute care hospital; community-acquired pneumonia
***Legionella* spp.**	Ubiquitous; man-made water systems	Ubiquitous; man-made water systems	Hospital, (community); exposure of lower respiratory tract with contaminated water	Acute care hospitals, LTCF; community and nosocomial pneumonia

comes from the fact that European VRE have similar resistance patterns as animal VRE and are polyclonal, whereas USA VRE are monoclonal.[29] Vancomycin-resistant enterococci serve as a good example of interaction between different conditions in separated areas of health care with global linkage of HAI problems spinning off from antibiotic abuse in agriculture. Control seems only possible when these interactions are revealed and intervention is multiorganizational.

Methicillin-resistant *Staphylococcus aureus*

Staphylococcus aureus is one of the leading causes of community-and hospital-acquired infection. Methicillin-resistance acquisition and spread is one of the most typical results of recent trends and the complexity of health care. Since its initial detection in Europe in 1960, MRSA has become a leading cause of HAI worldwide. According to molecular typing techniques, five MRSA clones account for almost 70% of over 3000 MRSA isolates recovered in hospitals in southern and eastern Europe, South America and the USA. These five pandemic clones have evolved from only two distinct ancestral genetic backgrounds, one of which can be traced back to the very first European MRSA isolates and also to related methicillin-susceptible *S. aureus* isolates identified in Danish hospitals in the mid-1950s. Isolates close to the epidemic MRSA 16 (EMRSA-16) were also detected in the early Danish isolates. EMRSA-15 and EMRSA-16 are currently responsible for the dramatic increase in the rate of nosocomial infections, in particular bacteremia, observed in the UK. Several MRSA clones are widespread worldwide. Methicillin-resistant *S. aureus* is highly endemic in most hospitals and LTCFs in the USA, the latter being considered major reservoirs of the burden of infection. High endemicity is associated with an increased nosocomial infection rate, increased use of glycopeptides with subsequent risk of suboptimal treatment and emergence of resistance in Gram-positive bacteria, and higher health care costs. Furthermore, since the first clinical isolate of *S. aureus* with intermediate susceptibility to vancomycin (VISA) was reported from Japan in 1996 and subsequently from four different continents, the first case of vancomycin-resistant *S. aureus* (VRSA) infection was confirmed in July 2002 in a patient in the USA.[30]

Patients who require hospitalization in today's modern health care industry tend to be very ill. Not surprisingly, the accumulation of critically ill patients increases the risk for MRSA infections because host factors are undoubtedly serving as indirect markers of frequent patient-staff contact (i.e. risk of transmission of MRSA by hands of personnel). Increased workload, understaffing and overcrowding have been associated with an increased risk for cross-transmission, and MRSA acquisition in particular. The inconsistent application of infection control guidelines, in particular hand hygiene, by hospital personnel largely accounts for the dissemination of MRSA in most institutions, whether acute, chronic or long-term care facilities.

Epidemic MRSA strains imported by unrecognized carriers from one geographic region to another have been repeatedly documented. On-admission screening of high-risk patients and implementation of barrier precautions pending results ('modified quarantine') is practiced systematically in countries where MRSA has been eradicated (e.g. The Netherlands). It is also practiced in certain endemic hospitals and is a cost-effective strategy for controlling transmission; the critical risk factor is direct or indirect exposure to a health care system.

Finally, MRSA has become an incipient community pathogen in many geographic regions with reports from Canada, Australia, the USA and Europe. Thus, unfortunately, after being considered for 40 years as a typical hospital problem, MRSA is now challenging the entire worldwide health care system. To combat this, an urgent need exists for international strategies for MRSA control to be applied by all partners of health care systems.

Penicillin-resistant pneumococci

Resistance of *S. pneumoniae* to penicillin is a widespread and increasing problem and illustrates the individual and community-level effects of antibiotic use on the spread of antimicrobial resistance. Studies of the association between antibiotic use and resistance in pneumococci vary in design, setting and quality.[31] Nevertheless, present evidence suggests that:

- ¦ although recent antibiotic treatment by itself, in particular with ß-lactams or cephalosporins, is not associated with an increased risk for PRSP infection in the individual patient, it makes carriage of these strains more likely than that of susceptible strains; and
- ¦ treatment of carriers of susceptible organisms reduces their infectiveness and carriage duration, but increases the carriage of resistant strains.

In other words, antibiotic treatment indirectly promotes cross-transmission of resistant organisms in the community by reducing the carriage of susceptible strains.

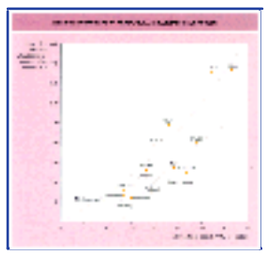

Figure 83-6 Relationship between antibiotic consumption and penicillin-resistant pneumococci. Total use of antibiotics (in daily defined dose (DDD) per 1000 population) is indicated (horizontal axis) and percent of penicillin-resistant *Streptococcus. pneumoniae* isolates. *Data aggregated from several studies and kindly provided by Dr S Harbarth. From Harbarth et al.,[15] with permission.*

Figure 83.6 illustrates the relation between the overall use of antibiotics in different countries and the reported proportion of low-level penicillin-resistant strains among *S. pneumoniae* isolates. This figure cumulates results from aggregated and cross-sectional data from different sources and illustrates the impact of antimicrobial use on resistance acquisition and spread at the population-level.[15] In countries with a significant proportion of PRSP, treatment with broad-spectrum antibiotics, such as oral third-generation cephalosporins, has been used extensively. [32] These drugs, considered as 'reserve drugs' for hospital use only a few years ago, are now recommended for the empiric treatment of community-acquired pneumonia by many experts.[33] Thus, overuse of antibiotics in the community has been the driving force for a major change in prescribing practices at all levels of the health care system, further accelerating the vicious cycle of antibiotic resistance.

Legionella spp.

The ubiquitous nature of *Legionella* spp. in natural aquatic environments, the intracellular survival in protozoa within biofilm, and their multiplication in a wide temperature range around 98.6°F (37°C) make them ideal invaders of water systems.[34] From these reservoirs, pulmonary exposure to aerosols and microaspiration from contaminated water sources constitute the main routes of transmission. Impaired cough and deficient immune function are the major patient-related risk factors, which explains why lower density of water contamination will have a major impact within hospitals, particularly in transplant and ICU patients.[34]

Because of the possibility of a low incidence of cases over a prolonged time related to the same source,[35] legionella surveillance must be active and prospective. On an inter-regional, statewide or international level, this requires an important, rapid and accurate flow of information to detect the common source of seemingly sporadic cases. An international effort has been made in Europe by the EWGLI (European Working Group for Legionella Infections) to detect sources of travel-associated cases (http://www.ewgli.org).

Importantly, as the length of hospital stay decreases, even in the domain of transplantation, high-risk patients are increasingly subject to immunosuppressive treatment at home. Control of the home environment may have to be revised together with other preventive aspects.[36] Surveillance and control of *Legionella* spp. as purely environmental pathogens challenges the linkage between the health care system and public health.

DIAGNOSIS

Surveillance — diagnosing health care defaults

Surveillance for nosocomial infections has been the foundation of infection control in the USA since the 1960s, whereas in Europe and elsewhere it has only been introduced over the past two decades. In the USA, surveillance has become such an integral part of infection control that professionals have to be reminded of its original primary purpose (i.e. to measure the outcome of interest, nosocomial infection), and to design and control interventions targeted at its reduction. [32] In Europe, infection control has been oriented since its inception toward intervention and procedure quality, sometimes on unfounded grounds.

Outpatient infection surveillance is very challenging because of the need to monitor multiple health care systems. Even if infected patients could be traced, denominator data are entirely lacking.[37] Construction of outpatient infection surveillance is only possible

after evaluation of the risk factors and applicability of infection definitions. [38] Novel approaches must be sought that include data already available on public health databases.

Data collection of infection rates always implies comparison. On a microepidemiologic level, this implies comparison of endemic rates over time within the same population before and after an intervention or system change or outbreak detection. Over the past few years, interinstitutional comparison, or 'benchmarking' as it is termed in analogy with industrial quality efforts, is on the rise. Two challenges emerge with benchmarking: case-mix adjustment and standardization of the method.

Health care-associated infections are considered to be one of the most accurate indicators of the quality of patient care. Interhospital benchmarking of nosocomial infection rates is being performed with the aim of improving the effectiveness of health care and promoting patient safety. Similar comparisons might soon be made between different health care settings. However, benchmarking among health care structures requires meticulous adjustment for case-mix. In a recent nationwide study, higher crude infection rates in larger compared with small hospitals suggested a health care quality problem.[39] Further analysis revealed this difference to be due to case-mix; hospital size turned out to be a surrogate marker, but not a risk factor for nosocomial infections. Failure to adjust adequately for infection-associated factors will erroneously punish commitment to more challenging medical tasks and, thus, hinder quality improvement. Hence, it is increasingly vital to have adjustment at hand for comparing infection rates between different health care structures and procedures. This is a field of intense research and expanding knowledge.

Standardization of surveillance method is a second challenge that must be addressed in order to make comparison possible. This obviously clashes with the will to improve and adapt definitions to medical progress and local specificities. Currently, even if standard definitions are applied, it is difficult to compare results due to methodologic variations.[40]

Adjustment should be implemented according to variations in the use of microbiologic investigation within the different health care settings and type of diagnostic techniques applied. Diagnostic power and accuracy largely impact on infection rates. This holds especially true for LTCFs because of budgetary constraints and for private practices because of the use of independent laboratories of uncertain standardization and quality assurance. This concern increasingly includes hospitals because of the trend to outsource microbiologic laboratory tasks.

Voluntary participation in a surveillance network, confidentiality and adequate feedback of the results are the prerequisites for health care settings' adherence to the method and dedication to data quality. Exchange of successful strategies in implementing quality initiatives based on surveillance data are often shared among participants in national networks, and this may represent the major impact of surveillance on infection rates.

MANAGEMENT

The understanding of infection as an indicator of flaws in patient safety and as being an often preventable event concerning all components of the health care system raises the issue of new organizational structures. It is not yet clear how this challenge will be met. Two conceptually different solutions are possible:

- ¦ expanding current duties and activities of hospital-based infection control teams (Fig. 83.7) regarded as centers of excellence to the remainder components of the health care system; or
- ¦ revising the structure entirely and creating government-associated infection prevention units.

However, any combination between these two scenarios can be imagined.

Exporting the product 'infection prevention and control' from hospitals considered as centers of excellence has several attractive aspects. A longstanding experience has accumulated within these services through exposure to everyday problems and the successful implementation of interventional strategies in real life conditions. The entire range of necessary efforts has been met, such as surveillance, outbreak investigation, procedure revision and standardization, and counseling in process and structure design, and has been shown to be cost-effective.

Furthermore, some issues might only be resolved in the controlled environment of the hospital. As an example, assessment of outcome as the gold standard of quality measurement might not be possible beyond this setting for a prolonged period because of the lack of reliable data sources and standardization. However, evidence about the linkage between structure, procedure and infection as the outcome of interest could be substituted by what was learned in the hospital setting. Still, central governmental-funded structures are necessary to ensure co-ordination, communication and guidance as much on a national as on an international level.

Some solutions for out-of-hospital surveillance have been driven by the need to meet the inter-regional, if not global, threat of bioterrorism. There is a parallel between these needs and the expanding interlinkage of the health care system over state and national boundaries. A report describes the use of a commercially available medical record system used by many large medical practice groups in the USA (Epicare; Epic Systems Corporation, Madison, Wisconsin). This system provides real-time data about each outpatient medical contact of a large group of people, which make it suitable as an online bioterrorism alert if a given clinical picture were to appear more frequently.[41] Payment information, which is more readily available in outpatient clinics and physician's offices than in hospitals, represents a further valuable data source for infection follow-up.

In the hospital, the development of computerized patient record systems featuring a standardized data structure provides valuable data more close to the patient (e.g. information on individualized medication, clinical signs and symptoms, nonmedical prescriptions and workload). Eventually, this technical support will be available for the entire health care system. Exploitation of these very heterogeneous data in an even more heterogeneous health care setting calls for powerful tools in data compilation and analysis. Whereas multinational enterprises already make use of more advanced techniques such as data mining, their value for infection control are only just being discovered.[42]

On the front of antibiotic resistance management, a new horizon will open with genetic diagnosis on the pathogen level as much as the host level. Microarray technology, once price and specificity issues are resolved, will provide instant data on carrier state and antibiotic resistance patterns of each subject in contact with the health care system to determine tailored measures for transmission precautions and infection prevention. To cut down on price, modeling will single out the population at risk to be screened. Widespread use of these techniques will deliver more accurate information about microepidemiology and local resistance patterns.

The CDC is planning a new HCW safety network to ultimately include noninfectious adverse events such as medication errors and patient falls. After almost a decade of discussion, this development could finally formalize widespread expansion of infection control beyond its traditional mandate. The aim of the CDC is to promote health care quality in a wide variety of health care settings with the vision to create a web-based knowledge system for accumulating, exchanging and integrating relevant information and resources among private and public stakeholders that support local efforts to

Figure 83-7 Current and future structure of infection prevention programs.

protect patients and promote health care safety. Users are from different types of health care settings, including clinics, health plans and consumers of care. The ultimate goal is to improve patient and HCW safety by providing protocols for monitoring adverse events associated with devices, procedures and medications. Performance improvement will be promoted by providing performance feedback and a free access to prevention tools, lessons learned and best practices. In our opinion, however, such a system would still need infection control practitioners to support care-givers at the bedside in their daily challenge to prevent HAI in today's increasingly complex health care delivery system.

To conclude, events on a local, micro-environmental level determine the evolution of HAIs in a much wider area. Although bacterial resistance is a global problem, its origins can be traced to local events. As René Jules Dubos declared, 'Think globally — act locally'.[43]

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Chapter 84 - Prevention of Infection in ICU Patients

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Intensive care units (ICUs) are synonymous with cutting-edge, high-tech medicine; mechanical ventilatory support; hemodynamic monitoring; total parenteral nutrition; hemodialysis; intracranial pressure monitoring; innovative forms of surgery; and a huge arsenal of drugs, especially anti-infectives of every genre. They have improved the care and outcome of patients with trauma, shock states and other life-threatening conditions. However, the complexities of ICU care and the fragile condition of many ICU patients yield rates of hospital-acquired (nosocomial) infection that are 3–5 times higher than rates in other hospital wards. Infection, usually nosocomial, is the most common cause of death, directly or indirectly, of patients who survive major trauma or full-thickness burns and it is the most commonly identified cause of multiple organ dysfunction syndrome.[1]

In the past 15–20 years, we have learned a great deal about the epidemiology of nosocomial infections acquired in the ICU.[2] [3] Evidence-based guidelines for prevention are now available (e.g. at www.cdc.gov/ncidod/hip/GUIDE/guide.htm) and, if applied consistently, these guidelines can reduce the risk of nosocomial infection, in some estimates by over a third. In this chapter, we review the epidemiology, prevention and control of ICU infections in general and the features of three areas of special concern: vascular access-related infections, ventilator-associated pneumonia and antibiotic resistance (Chapter 189). Additional site-specific infection topics and programmatic infection control issues (Chapter 87) and Employee Health Service concerns (see Chapter 83 , Chapter 88) are covered in other chapters.

EPIDEMIOLOGY

Nosocomial infections are estimated to affect more than 2 million patients in US hospitals annually, are a factor in more than 88,000 hospital deaths each year and, in 1992, cost more than $5 billion in excess health care costs. Considering that nosocomial infections acquired by ICU patients account for nearly half of all infections in most US hospitals — and more than half of all nosocomial epidemics now occur among the 10% of hospitalized patients confined to an ICU — progress in reducing the incidence of infection acquired within ICUs could produce substantial economic benefits. The epidemiology of ICU infections and its economic consequences is remarkably similar in many European countries and developed countries in Asia. The situation in ICUs in developing countries is less well characterized and likely to be highly variable depending on the nature of the patient population and access to potentially costly infection control techniques (disposable needles, syringes, gloves, medical devices, hand wash basins, etc.).

The US Centers for Disease Control and Prevention (CDC) has published widely recognized definitions for the purpose of surveillance of nosocomial infection, [4] [5] although for research purposes more stringent definitions are often used, especially for pneumonia. The incidence of nosocomial infection is most commonly expressed as the number of infections per 100 patients hospitalized and is highest in burn, surgical and neonatal ICUs (5–30%), intermediate in medical and pediatric ICUs (5–7%) and lowest in coronary care units (1–2%).[1] [2] [3]

Since the risk of nosocomial infection is heavily influenced by patients' lengths of stay and to facilitate interhospital comparisons, the CDC has advocated the use of infection rates expressed per 1000 patient-days.[3] Because the majority of infections acquired in ICUs are related directly to the use of invasive devices, the CDC has further recommended specific surveillance of device-associated nosocomial infections, reported as infections per 1000 device-days. [3] [6] [7] [8] Representative rates of ICU device-associated nosocomial infection in US hospitals that are members of the CDC's National Nosocomial Infection Surveillance System (NNIS); (Table 84.1) can be used for interhospital comparisons. Such comparisons are an increasing focus of attention from hospital accrediting organizations and third party payers.

Nosocomial infection site-pathogen tropisms in ICUs are well recognized (Table 84.2). Approximately 50% of nosocomial infections in the ICU are caused by aerobic Gram-negative bacilli, especially *Pseudomonas aeruginosa, Enterobacter* spp. or *Serratia marcescens*; 20% are caused by Gram-positive cocci, most commonly coagulase-negative staphylococci, *Staphylococcus aureus* or enterococci; 5–10% are caused by *Candida* spp.; and filamentous fungi such as *Aspergillus* and *Zygomycetes* spp. are being seen increasingly in patients with hematologic malignancy or those who received solid organ transplants. Viruses such as respiratory syncytial virus (RSV) and rotaviruses are important pathogens in pediatric ICUs. *Legionella* spp. now account for up to 10% of nosocomial pneumonias in some centers.

PATHOGENESIS

Patients admitted to ICUs are usually more susceptible to infection because of underlying diseases or conditions associated with impaired immunity, such as cancer, trauma or advanced age, or because of immunosuppression associated with malnutrition or therapy with corticosteroids, cancer chemotherapeutic agents or immunosuppressive drugs. Broad-spectrum antimicrobial agents — administered to the majority of ICU patients for treatment of community-acquired or prophylaxis or treatment of nosocomial infections — increase the likelihood of subsequent colonization or infection by antibiotic-resistant pathogens.[9] Even transfusion therapy may produce immunosuppression and increase the risk of nosocomial infection.[10]

In most cases, colonization is the first step in the progression to nosocomial infection. Understanding the reservoirs and modes of transmission of pathogens and colonization and infection risk factors (see Table 84.2) is essential to developing effective prevention strategies. In the ICU, the major reservoir of nosocomial organisms is the infected or colonized patient and these organisms, most often, are spread on the hands of medical personnel. A few pathogens, most notably *Mycobacterium tuberculosis* and varicella, can be spread by the airborne route.[11] Occasionally, ICU outbreaks result from the

TABLE 84-1 -- Rates of device-related nosocomial infection in US hospital intensive care units, expressed per 1000 device-days.†

RATES OF DEVICE-RELATED NOSOCOMIAL INFECTION IN US HOSPITAL ICUs, EXPRESSED PER 1000 DEVICE-DAYS*			
Type of infection	**Type of ICU**	**Rate (no. of cases per 1000 device-days)**	
		Median	**Range for 10th–90th percentile of hospitals**
Catheter-associated urinary tract infection	PICUs	4.9	0.9–11.1
	MICUs	6.8	1.9–12.2
	SICUs	4.9	1.1–9.3
Ventilator-associated pneumonia	PICUs	4.0	0–11.4
	MICUs	7.4	1.8–15.5
	SICUs	12.3	5.4–25.1
Central line-associated bloodstream infection	PICUs	7.1	1.7–12.8
	MICUs	5.4	1.5–10.2
	SICUs	4.9	1.3–9.8
MICU, medical ICU; PICU, pediatric ICU; SICU, surgical ICU.			

† *Adapted from Alonso-Echanove and Gaynes.*[3]
* *From the CDC's NNIS, January 1992-October 1998; the data include 62 PICUs, 125 MICUs and 146 SICUs.*

TABLE 84-2 -- Profile of common nosocomial infections in the intensive care unit.†

PROFILE OF COMMON NOSOCOMIAL INFECTIONS IN THE ICU		
Infection (% by site*)	**Major pathogens (% within site)**	**Common colonization and infection risk factors**
Urinary tract (17–35)	*Pseudomonas aeruginosa* (7–13) and *Escherichia coli* (14–28)	Urinary catheter
	Klebsiella (6) and *Enterobacter* spp. (5)	Monitoring of urine output
	Enterococci (14)	Other urologic manipulation or bladder irrigations
	Staphylococcus epidermidis	Renal transplantation
	Candida albicans (6–21)	Diabetes
		Female > male

Pneumonia (24–39)	*P. aeruginosa* (14–22)	Tracheostomy
	Klebsiella (7) and *Enterobacter* spp. (2–13)	Endotracheal tube, reintubation
	Serratia marcescens (1–4)	Nasogastric tube
	Acinetobacter spp. (3–6)	Intracranial pressure monitoring
	Staphylococcus aureus (17–21)	Stress ulcer prophylaxis with H_2 blocker or antacids
	Oral anaerobes	Immunosuppression, granulocytopenia
Postsurgical wound or intra-abdominal (5–13)	*S. aureus*	Trauma, especially penetrating abdominal injury
	Escherichia coli and other Gram-negative bacilli	Gastrointestinal or radical gynecologic surgery
	Enterococci	Prolonged operation
	Bacteroides fragilis and other bowel anaerobes	Immunosuppressive therapy
Bloodstream (14–19)		
Catheter related	Coagulase-negative staphylococci (37)	Heavy colonization of insertion site skin
	S. aureus (9–24)	Hemodynamic monitoring
	Candida albicans (3–6)	Exposure of catheter to remote-source bacteremia
Contaminated infusate	*Enterobacter* spp.	Intrinsic (i.e. manufacturer) or extrinsic (i.e. in use) contamination
	Serratia marcescens	
	Citrobacter spp.	
	Pseudomonas cepacia or *Stenotrophomonas maltophilia*	

† *Adapted from Maki and Weinstein[1] and Alonso-Echanove and Gaynes.[3]*
* *Site and pathogen percents are presented, where available, from the CDC's NNIS data[3] ; ranges represent data from pediatric, medical, surgical and coronary ICU.*

presence of a health care provider who is a carrier of an epidemic strain, usually *S. aureus* or group A streptococcus.

Many nosocomial infections acquired in the ICU derive from resistant enteric organisms carried by patients at the time of ICU admission. This explains the failure of conventional infection control practices that are directed at preventing extrinsically acquired infection.[12] Food and even enteral feeding preparations are often heavily contaminated by micro-organisms but studies have only rarely linked such contamination to disease.[13] Nosocomial organisms originating from colonized or infected patients are readily perpetuated and spread in contaminated medical equipment or devices, such as urine collection receptacles, respiratory therapy equipment, chamber domes or transducers used for hemodynamic monitoring, dialysis machines and fiberoptic bronchoscopes. [1]

PREVENTION AND INFECTION CONTROL

Infection control programs (Table 84.3)

The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in the USA and similar regulatory agencies in many other countries mandate that every hospital have an active program for surveillance, prevention and control of hospital-acquired infections. Surveillance of nosocomial infections is the cornerstone of an effective control program. Many hospitals focus their surveillance activities on infections that are associated with high morbidity (e.g. nosocomial pneumonia), that greatly increase health care costs (e.g. postcardiac surgery sternotomy infections), that are caused by antibiotic-resistant organisms with potential for spread (e.g. *C. difficile* colitis) or that are highly preventable (e.g. intravascular device-related bloodstream infections). American hospitals are also regulated by the Occupational Safety and Health Administration (OSHA) in terms of institutional standards and programs to protect health care workers from blood-borne pathogens and tuberculosis; the Environmental Protection Agency provides regulations for disposing and tracking medical waste, only a small fraction of which is truly biohazardous.

TABLE 84-3 -- Aspects of hospital infection control programs that impact intensive care units.[*]

ASPECTS OF HOSPITAL INFECTION CONTROL PROGRAMS THAT IMPACT ICUs
✚ Active Infection Control Committee, with representation from major departments and services, including the ICU(s)
✚ Surveillance of nosocomial infections, especially in each ICU
✚ Comprehensive and regularly updated institutional policies and procedures for:
¦ surveillance of nosocomial infections
¦ isolation precautions
¦ sterilization and disinfection
¦ indications for and management of invasive procedures and devices:
• all types of intravascular catheters
• hemodynamic monitoring
• tracheostomy and endotracheal intubation
• mechanical ventilation and other respiratory therapy
• bronchoscopy and gastrointestinal endoscopy
• anesthesia and the operating room
• hemodialysis
• intra-aortic balloon pumps
• cardiopulmonary bypass
• intracranial pressure monitoring
¦ investigation of an epidemic
✚ Guidelines for use of antibiotics, including ongoing utilization review
✚ Strong liaison with clinical microbiology laboratory:
¦ representation on the Infection Control Committee
¦ laboratory-based surveillance
¦ monitoring and reporting of trends in antimicrobial susceptibility

¦ saving of important isolates
¦ microbiologic support of all infection control activities
¦ subtyping of isolates for investigations or studies
✚ Educational programs for new employees and periodic updates dealing with nosocomial infection control
✚ Active employee health department:
¦ free immunizations (hepatitis B, influenza A, measles, mumps, rubella, varicella)
¦ tuberculin screening
¦ postexposure protocols
✚ Quality assurance review of implementation of infection control policies and practices

** Adapted from Maki and Weinstein.*[1]

All health care personnel working in an ICU must receive training in the epidemiology and control of nosocomial infections. This may be most important for trainees in teaching hospitals, who often have only a rudimentary knowledge of asepsis but have hands-on contact with many patients each day.[14] Caregivers in ICUs must be especially familiar with their hospital's protocols for management of invasive devices, such as intravascular lines, urinary catheters, endotracheal tubes, tracheostomies and external ventricular drains.

Health care workers

Health care workers in general, but especially those working in ICUs who are exposed daily to critically ill patients, many of whom have contagious but undiagnosed infections, are at increased risk of acquiring occupationally related infections. These include tuberculosis, herpetic whitlow, varicella-zoster virus infection, hepatitis A or B, HIV infection, influenza A infection, measles, rubella and viral conjunctivitis. Essential components of employee health activities for ICU staff include pre-exposure vaccination (e.g. for hepatitis B, influenza, measles, mumps, rubella and varicella), postexposure prophylaxis (e.g. for HIV or tuberculosis) where indicated, and education about hand hygiene and isolation precautions (see Chapter 87 for detailed discussion).

Environmental issues

Although the hospital's inanimate environment (e.g. surfaces and walls) does not contribute measurably to the occurrence of most nosocomial infections, some infections, such as those caused by airborne *Aspergillus* spp. and other filamentous fungi in seriously immunocompromised patients, may derive from environmental contamination. Moreover, in ICUs, where virtually all patients are heavily exposed to invasive devices and have a very high risk of infection, the inanimate environment may be a reservoir of resistant nosocomial organisms, such as methicillin-resistant *S. aureus* (MRSA), *Clostridium difficile* and vancomycin-resistant enterococci (VRE).[1] Although the ICU environment cannot be made microbe free, certain architectural and environmental issues warrant attention.[1] [15]

¦ ICUs should be located in areas that limit traffic flow to essential ICU personnel.
¦ Single-patient rooms theoretically may increase the likelihood of hand hygiene being practiced and may improve adherence to isolation practices, reducing the risk of cross-infection.
¦ Because of the large amount of equipment required for the care of many patients, it has been recommended that each cubicle or room should provide a minimum of $11 m^2$ per bed.[16]
¦ A centralized, filtered air-handling system that provides at least six room air exchanges per hour has been recommended.
¦ There must be an adequate number of sinks and dispensers of sinkless alcohol degermer for convenient hand hygiene by all entering personnel who will have or have had contact with the patient or the immediate environment.[17]
¦ Separate areas and sinks should be used for cleaning, for storage and for reprocessing contaminated equipment.
¦ All ICUs should have airborne infection isolation room(s) (i.e. negative pressure and 100% exhaust or HEPA filtration for any recirculated air) for patients with tuberculosis or other airborne infections, such as varicella or measles.
¦ For ICUs that house bone marrow transplant patients or other patients with prolonged severe granulocytopenia, positive-pressure isolation rooms using HEPA filters should be available.
¦ All surfaces contiguous to the ICU patient should be wiped down with the general hospital disinfectant at least daily and urine measuring devices, a frequent reservoir of antibiotic-resistant Gram-negative bacilli, should be rinsed with a disinfectant after each use.

¦ Each ICU patient should have a dedicated stethoscope and sphygmomanometer.
¦ The use of electronic measuring devices on multiple patients in an ICU needs re-evaluation, unless stringent efforts are made to decontaminate the devices after each use.

Disinfection and sterilization

Policies and procedures for disinfection and sterilization are a mundane but essential and often breached component of ICU infection prevention. [18] [19] Numerous epidemics of Gram-negative infection have stemmed from failure of a chemical disinfectant to disinfect reusable medical apparatus, such as respiratory therapy equipment, or failure of an antiseptic to disinfect the skin before insertion of invasive devices. In many outbreaks, the epidemic organisms were cultured from a working solution of the germicide. Most of these outbreaks were traced to use of dilute aqueous solutions of chlorhexidine, quaternary ammonium or phenolic germicides. Even iodophors such as povidone-iodine, in the past the most widely used antiseptics for cutaneous degerming in US hospitals and clinics, have been implicated in epidemics of nosocomial infection due to *Pseudomonas* organisms that contaminated the product in the manufacturer's plant.[20] Numerous epidemics have been traced to contaminated bronchoscopes or endoscopes[21] that were not reliably decontaminated after clinical use, usually because of the use of an unreliable chemical disinfectant, design flaws of automated endoscope washing machines or lack of attention to detail by personnel processing the scopes.

Hand hygiene

The role of hand hygiene in ICU infection prevention must be stressed at all times.[17] Handwashing rates are abysmally low (<30–50%) because of the personnel time required — up to 90 minutes per shift when handwashing is performed by ICU nurses as often as recommended by the CDC — and because of perceived and real damage to the skin of health care workers as a result of repeated soap and water washing. A potential solution, and an opportunity to fulfill the infection control 'prime directive' — use technologic advances to improve behavior — is the use of alcohol-based sinkless hand rubs. Studied extensively in Europe, alcohol-based hand rubs are highly effective degermers that can reduce the time spent on hand hygiene up to 4-fold and can improve the condition of health care workers' hands, thus overcoming the two major barriers to adequate hand hygiene.[17] Although alcohol, when used for handwashing or scrubbing, is perceived by health care workers as causing dry skin, use of alcohol hand rubs without rinsing is beneficial to skin condition, most likely because the protective oils are left in place on the hands when the alcohol dries and because of emollients and moisturizers in the products. In comparisons of alcohol hand rub and medicated soap and water handwashing, health care workers found that the hand rub caused less skin dryness and was readily accessible and convenient to use.

Aggressive hand hygiene campaigns, with adherence monitoring and feedback of ward and individual results, may achieve compliance rates as high as 70%. For some situations (e.g. when there is a large, endemic antibiotic resistance problem and extensive patient colonization by antibiotic-resistant bacteria[9] [12] [22]), these levels of adherence may not be sufficient to control cross-infection. A response to this problem has been to consider use of 'universal gloving', in addition to use of alcohol-based hand rubs (a 'belt-and-suspender' approach), to bridge the gap that is left by incomplete attention to hand hygiene even in the best of circumstances. Use of universal gloving has been successful in controlling spread of aminoglycoside-resistant Gram-negative bacilli in ICUs and *C. difficile*-related diarrhea.[22] Because patients' intact skin and the environment in patient rooms may be a source of resistant bacteria such as VRE, we recommend that disposable examination gloves be worn for all contact with ICU patients or their environment. Because gloves are not a complete barrier, they must be removed and hands disinfected by an alcohol hand rub between patient contacts.

Hand hygiene adherence rates, especially for nursing personnel, decrease as provider-to-patient ratios increase (e.g. when individual nurses are required to care for

two or more critically ill patients).[17] To improve nursing care and the likelihood of hand hygiene adherence, ICUs must have an adequate number of staff. Although the optimal nurse-to-patient ratio in an ICU is not known, one-to-one nursing may reduce risks of cross-infection considerably.

Isolation precautions

Isolation precautions for patients colonized or infected with problem pathogens are used to curtail cross-infections. The CDC's *Guideline for isolation practices in hospitals*[23] separated precautions into 'standard precautions', designed for the care of all patients in hospitals, regardless of their diagnosis or presumed infection status; and additional 'transmission-based precautions', designed for the care of specified patients who are known or suspected to be infected with highly transmissible or epidemiologically important pathogens. Standard precautions are designed to reduce the risk of transmission of micro-organisms from patient to patient and from patient to health care worker, from both recognized and unrecognized sources of infection in the hospital. Transmission-based precautions are divided into three subgroups, based on the mode of transmission: contact precautions, droplet precautions and airborne precautions. Contact precautions are recommended with multidrug-resistant bacteria that can be acquired by contact with the colonized patient or environmental surfaces or objects. Droplet precautions provide additional measures for preventing transmission by large-particle droplets, such as use of face masks by personnel during suctioning or bronchoscopy. Airborne precautions are added to standard precautions for care of patients with tuberculosis and other micro-organisms transmitted by the airborne route.

To contain the spread of certain resistant organisms in the ICU (e.g. MRSA, VRE), 'cohort nursing' is strongly recommended based on empiric evidence and on mathematical models of spread of infection in ICUs.[22] In cohort nursing, the care of patients known to be infected (or determined by surveillance cultures to be colonized) by problem organism(s) is provided by nurses and respiratory therapists who will not provide care during that shift for noninfected patients, and the nursing care of noninfected patients is restricted to personnel who will not have contact with infected patients, except in an emergency.

CLINICAL FEATURES

Vascular catheter-related infection

Intravascular devices are a major source of iatrogenic disease in ICUs, especially bloodstream infections originating from colonization of the device or, less often, from contamination of infusate. Vascular catheter-related bacteremia or candidemia in hospitalized patients is associated with two to three times increased attributable mortality. Most epidemics of nosocomial bloodstream infection derive from vascular access in some form. Probably more than any other nosocomial infection, intravascular device-related bloodstream infection is preventable.[1] [24] [25] [26] [27]

One of the most serious forms of intravascular device-related infection occurs when a thrombus surrounding the catheter becomes infected, creating a septic (suppurative) thrombophlebitis, which often produces overwhelming sepsis with high-grade bacteremia or

fungemia that characteristically persists despite removal of the device. The catheter insertion site is devoid of signs of inflammation more than half the time. The micro-organisms most commonly implicated are the same organisms that cause uncomplicated catheter-related bloodstream infection, namely *S. aureus*, enterococci, nosocomial Gram-negative bacilli and *Candida* spp.

Certain clinical, epidemiologic and microbiologic findings point toward an intravascular device as the source of a bloodstream infection.[1]

- A patient without significant underlying diseases, who is an unlikely candidate for sepsis.
- Lack of another source of infection to account for the picture of sepsis.
- Local inflammation, especially purulence, at the catheter insertion site.
- Abrupt onset, associated with fulminant shock, suggestive of heavily contaminated infusate.
- Sepsis refractory to antimicrobial therapy or dramatic improvement with removal of the device or discontinuation of the infusion.
- Nosocomial bloodstream infection caused by skin flora (e.g. staphylococci, especially coagulase-negative; *Corynebacterium*, especially JK, or *Bacillus* spp.) or by certain fungi (e.g. *Candida, Fusarium, Trichophyton* or *Malassezia* spp.). In contrast, bacteremia caused by streptococci, aerobic Gram-negative bacilli (especially *P. aeruginosa*) or anaerobes is less likely to have originated from infected intravascular devices.
- Bacteremia caused by strains that grow well in infusates — members of the tribe Klebsielleae (e.g. *Enterobacter cloacae* or especially *Enterobacter agglomerans*), non-*aeruginosa* pseudomonads (particularly *Pseudomonas (Burkholderia) cepacia* or *Pseudomonas pickettii*), *Stenotrophomonas maltophilia* or *Flavobacterium* or *Citrobacter* spp. — suggests the possibility of contaminated intravascular fluids. A cluster of cases mandates full-scale investigation, which may include culturing in-use infusions and informing the local, state and federal public health authorities.
- Primary (i.e. no source site identified) nosocomial bacteremia caused by psychrophilic (cold-growing) organisms, such as non-*aeruginosa* pseudomonads, *Achromobacter, Flavobacterium, Enterobacter, Serratia, Salmonella* or *Yersinia* spp., should raise suspicion of a contaminated infusion that is stored at refrigerator temperature, such as a blood product.

TABLE 84-4 -- Rates and risk factors for intravascular catheter-related infections.‡

RATES AND RISK FACTORS FOR INTRAVASCULAR CATHETER-RELATED INFECTIONS			
Type of catheter	**Mean no. of bloodstream infections/100 catheters (95% CI)**	**Risk factors for catheter infection**	**Approximate magnitude of increased risk***
Peripheral iv	0.2 (0.1–0.3)	Cutaneous colonization of site > 10^2 cfu	3.9
		Contamination of catheter hub	3.8
		Moisture on site, under dressing	2.5
		Placement > 3 days	1.8
		Systemic antimicrobial therapy	0.5
Arterial	1.5 (0.9–2.4)	Cutaneous colonization of site > 10^2 cfu	10.0
		Second catheter in site, placed over guide wire	-†
Central venous	3.3 (3.3–4.0)	Exposure of catheter to unrelated bacteremia	9.4
		Cutaneous colonization of site > 10^2 cfu	9.2
		Placement >4 days	-†
Swan-Ganz pulmonary artery	1.9 (1.1–2.5)	Cutaneous colonization of site > 10^3 cfu	5.5
		Internal jugular vein cannulation	4.3
		Duration > 3 days	3.1
		Placement in operating room under less stringent barrier precautions	2.1
cfu, colony-forming units.			

‡ *Adapted from Maki and Weinstein*[1] *and Crnich and Maki.*[25]
* Relative risk or odds ratio
† Indeterminate

Culturing a catheter segment semiquantitatively on solid media or quantitatively in liquid media by removing organisms by sonication provides a strong correlation between high colony counts and catheter-related bloodstream infections.[24] Direct Gram or acridine orange stains of intravascular segments of removed catheters also show excellent correlation with quantitative techniques for culturing catheters and can permit rapid diagnosis of catheter-related infection. Diagnosing infection caused

by contaminated infusate requires that a sample of fluid is aspirated from the line and cultured quantitatively.

Blood cultures are essential to the diagnosis of device-related bloodstream infection and, in any patient suspected of infusion-related sepsis, two blood cultures should be drawn, ideally from peripheral veins by separate venepunctures. If available, quantitative blood cultures drawn by venepuncture from a peripheral vein and concomitantly through the device, that show a =10-fold step-up, can permit the diagnosis of central venous device-related bacteremia or fungemia to be made with sensitivity and specificity in the region of 90%. A shorter incubation time to positivity for blood cultures drawn via the contaminated vascular device versus a simultaneous venepuncture blood sample using automated blood culture systems can also be used as a diagnostic clue to a vascular catheter-related infection.[24]

The intravascular device associated with the greatest risk of bloodstream infection today is the central venous catheter (Table 84.4). These catheters have been shown to be the single most important risk factor for nosocomial candidemia, exceeding serious underlying disease, and 80–90% of intravascular device-related bloodstream infections originate from them.

Considerable evidence suggests that the largest proportion of catheter-related bloodstream infections derives in some fashion from the cutaneous microflora of the insertion site. Hubs of central

venous catheters also are frequently contaminated, particularly by coagulase-negative staphylococci, and can cause catheter-related bacteremia. However, contaminated hubs do not appear to be as important in the pathogenesis of catheter-related sepsis with short-term, non-cuffed catheters as micro-organisms from the skin that invade the intracutaneous catheter tract.[1] [25] [26] With long-term, surgically implanted, cuffed Hickman or Broviac catheters, micro-organisms colonizing the hub and lumen may be the most important source of bloodstream infection deriving from these devices.

Although most catheter-related bloodstream infections originate from infection of the percutaneous catheter tract or contamination of the catheter hub, contamination of infusate is the most common cause of epidemic device-related bloodstream infection and of

TABLE 84-5 -- General recommendations for the prevention of intravascular device-related (IVDR) bloodstream infections (BSIs).†

GENERAL RECOMMENDATIONS FOR THE PREVENTION OF INTRAVASCULAR DEVICE-RELATED BLOODSTREAM INFECTIONS	
Recommendation	**Strength of evidence***
General measures	
Educate all health care workers involved with vascular access regarding indications for use, proper insertion technique and maintenance of IVDs	**IA**
Surveillance	
Routinely monitor ICU rates of IVDR BSI	IA
Determine rates of CVC-related BSI, using standardized definitions and denominators, expressed per 1000 CVC-days	IB
At insertion	
Use aseptic technique	
Observe proper hand hygiene before insertion or manipulation of any IVD	IA
Wear clean or sterile gloves during insertion or manipulation of non-central IVD	IC
Use maximal barrier precautions (mask, cap, long-sleeved sterile gown, sterile gloves and large sterile sheet drape) during insertion of CVCs	**IA**
Use cutaneous antisepsis (chlorhexidine is preferred; however, tincture of iodine, an iodophor or 70% alcohol can be used)	**IA**
Use sterile gauze or a sterile semipermeable polyurethane film dressing to cover the catheter insertion site	IA
Do not use prophylactic systemic antibiotics before insertion or during catheter use to prevent catheter colonization or infection	IA
Maintenance	
Designate trained personnel for insertion and maintenance of IVDs	IA
Remove IVDs as soon as their use is no longer essential	**IA**
Monitor the IVD site on a regular basis, at least daily	IB
Change dressing of CVC insertion site at least weekly	II
Do not use topical antibiotics at insertion sites (except for dialysis catheters)	IA (II)
Clean injection ports with 70% alcohol or an iodophor before accessing the system	IA
Cap all stopcocks when not in use	IB
Replace PIVCs every 72–96 hours in adults	IB
Replace administration sets no more often than every 72 hours (unless lipid-containing admixture or blood products given, in which case administration sets should be replaced within 24 hours)	IA (IB)
Technology	
No recommendation can be made for use of chlorhexidine-impregnated sponge dressing (but do not use in neonates)	NR (II)
If, after consistent application of comprehensive infection control precautions, the institutional rate of CVC-related BSI is above the goal set based on benchmark rates[24] and local factors, use a CVC coated with an anti-infective agent (i.e. chlorhexidine-silver sulfadiazine or minocycline-rifampin) in adults whose catheter is expected to remain in place > 5 days	IB
Do not routinely use antibiotic lock solutions to prevent IVDR BSIs. For individual patients with long-term IVDs in place who have had recurrent IVDR BSIs, despite consistent application of infection control practices, consider the use of a prophylactic antibiotic lock solution (e.g. heparin with vancomycin (25 µg/ml) with or without ciprofloxacin (2 µg/ml))	II
CVC, central venous catheter; PIVC, peripheral iv catheter. Performance indicators that individual institutions should use to monitor their progress in implementing, and the impact of, these recommendations are shown in bold print.	

† *Adapted from CDC.*[24]
* IA, strongly recommended for implementation and strongly supported by well-designed experimental, clinical or epidemiological studies; IB, strongly recommended for implementation and supported by some experimental, clinical or epidemiologic studies and a strong theoretical rationale; IC, required by state or federal regulations, rules or standards; II, suggested for implementation and supported by suggestive clinical or epidemiologic studies or theoretical rationale; NR, no recommendation for or against use at this times.

approximately half of the endemic bloodstream infections caused by arterial infusions used for hemodynamic monitoring. Arterial set-ups may be particularly at risk because their infusions consist of a stagnant column of fluid subjected to frequent manipulations, especially entries for drawing blood specimens.

Prevention of vascular catheter- and infusion-related infections must be a primary focus of ICUs. Recommended, evidence-based measures from the CDC's Healthcare Infection Control Practices Advisory Committee (HICPAC) and from other expert groups[24] [25] [26] [27] should inform these efforts (Table 84.5). The HICPAC has recommended that hospitals use four specific 'performance indicators' (highlighted in Table 84.5) tom monitor their success in implementing the vascular catheter prevention guidelines. [24]

Ventilator-associated pneumonia

Nosocomial pneumonia in ICU patients is largely ventilator associated (VAP), although aspiration pneumonia related to surgery or other procedures in nonventilator patients may occur occasionally. The diagnostic criteria and tests for VAP include clinical criteria; qualitative and quantitative endobronchial cultures; bronchoalveolar lavage (BAL) or culture of protected specimen brush samples obtained by bronchoscopic techniques; and specimens, including bronchial washings, mini-BAL and protected specimen brush samples, obtained by blind nonbronchoscopic procedures.[1] [28] [29] Clinical criteria (e.g. fever, leukocytosis, purulent secretions, new or changing radiographic infiltrate) have high sensitivity but relatively low specificity. They are most useful for initial screening for VAP and for selecting patients for invasive procedures, such as BAL, that have sensitivities and specificities in the region of 80%.

Rates of VAP in NNIS hospital ICUs, based largely on clinical diagnostic criteria, range from 0.7 cases per 1000 ventilator-days in coronary care units to 26 cases per 1000 ventilator-days in trauma units.[30] [31] It has been estimated that nosocomial lower respiratory tract infections, which largely represent VAP, prolong hospital stay for an average of 6 days and increase hospital costs by approximately $6000, based on 1992 dollars. Although mortality rates in patients who have VAP are 20–50%, the attributable mortality rates are in the range 6–14%, suggesting that a patient's risk of dying from VAP is affected greatly by other factors, most importantly comorbid conditions, inadequate antimicrobial therapy and specific nosocomial pathogens (particularly *P. aeruginosa* and *Acinetobacter* spp.). Of note, most interventions that have been effective in reducing VAP rates have not decreased ICU mortality, further suggesting that patients who die during the course of VAP may be dying with, rather than because of, the pneumonia.[1] [28] Nevertheless, VAP is associated with more nosocomial deaths than is infection at any other body site.

Based on clinical diagnosis, the most common pathogens isolated from pneumonia in patients in ICUs are *S. aureus, P. aeruginosa, Enterobacter* spp. and *Klebsiella pneumoniae*, with varying prevalences depending on the type of ICU.[30] [31] Early-onset VAP, which manifests within the first 4 days of hospitalization, is more often caused by community-acquired pathogens, such as *S. pneumoniae* and *Haemophilus* spp. When invasive techniques are used to diagnose VAP, the frequency of recovery of enteric Gram-negative bacilli decreases from 50–70% of isolates to 35–45%. Ventilator-associated pneumonia is polymicrobial in as many as 20–40% of cases. The role of anaerobic bacteria in VAP is not well defined.

Pathogens that cause VAP fall into two broad categories: endogenous and exogenous organisms. Endogenous organisms, largely Gram-negative bacilli and typical community-acquired pulmonary pathogens such as *S. pneumoniae* and *Haemophilus* spp., colonize the oropharyngeal mucosa. The role of the 'gastro-pulmonary' axis in causation of VAP has been controversial. In general, it appears that oropharyngeal bacteria, rather than gastric bacteria, are the major source of pathogens in VAP. Exogenous organisms are brought to patients mostly on inadequately disinfected hands of health care workers, who transmit bacteria and fungi between patients. Other potential exogenous sources include inadequately disinfected or sterilized respiratory therapy equipment, contaminated multidose medication vials and inadequately disinfected nebulizers or other devices or equipment that may be shared among patients. Risk factors for the development of VAP include those events that increase the risk of colonization by potential pathogens, that heighten the possibility of aspiration of oropharyngeal contents into the lower respiratory tract and that reduce the host defense mechanisms in the lung and permit overgrowth of aspirated pathogens.[1] [28] [29]

TABLE 84-6 -- Recommendations for the nonpharmacologic prevention of ventilator-associated pneumonia.[?]

RECOMMENDATIONS FOR THE NONPHARMACOLOGIC PREVENTION OF VAP			
Prevention strategy	**Recommended for clinical use**	**Grade***	**Recommended by CDC†**
Effective strategies			
Removal of nasogastric or endotracheal tube as soon as clinically feasible	Yes	C	Yes
Use of a formal infection control program	Yes	C	Yes
Proper hand hygiene between patient contacts	Yes	B	Yes
Semirecumbent positioning of the patient	Yes	B	Yes
Avoidance of unnecessary reintubation	Yes	C	NSA
Provision of adequate nutritional support	Yes	C	NSA
Avoidance of gastric overdistention	Yes	B	Yes
Oral (non-nasal) intubation	Yes	D	No
Scheduled drainage of condensate from ventilator circuits	Yes	C	Yes
Continuous subglottic suctioning	Yes‡	A	No
Maintenance of adequate pressure in endotracheal tube cuff	Yes	C	Yes
Ineffective strategies			
Routine changes of ventilator circuit	No	A	No
Daily changes of heat and moisture exchangers	No	A	Yes
Chest physiotherapy	No	A	No
Strategies of equivocal or undetermined effectiveness			
Use of protective gowns and gloves	Yes‡	B	Yes‡
Humidification with heat and moisture exchanger	Yes§	A	Yes§
Humidification with heat and moisture exchanger with bacteriologic filter	-	U	USA
Postural changes	Yes‡	B	No

? *Adapted from Kollef*[28] *and Hospital Infection Control Practices Advisory Committee, CDC.*[29]
* A, supported by at least two randomized, controlled investigations; B, supported by at least one randomized, controlled investigation; C, supported by nonrandomized, concurrent cohort investigations, historical cohort investigations or case series; D, supported by randomized, controlled investigations of other nosocomial infections; U, undetermined or not yet studied in clinical investigations.
† NSA, not specifically addressed. CDC recommendations are described in reference. [29]
‡ This strategy is recommended for specific groups of patients described in the studies cited.
§ This strategy is recommended for clinical use but has not been clearly established to reduce the incidence of VAP.

A number of VAP prevention measures have been recommended for clinical use in ICUs (Table 84.6 and Table 84.7). Those measures that

TABLE 84-7 -- Recommendations for the pharmacologic prevention of ventilator-associated pneumonia.[‡]

RECOMMENDATIONS FOR THE PHARMACOLOGIC PREVENTION OF VAP			
Prevention strategy	**Recommended for clinical use**	**Grade***	**Recommended by CDC†**
Effective strategies			
Avoidance of unnecessary antibiotics	Yes	C	Yes

Chlorhexidine oral rinse	Yes§	B	NSA
Granulocyte colony-stimulating factor for neutropenic fever	Yes	D	NSA
Antibiotics for neutropenic fever	Yes	D	NSA
Vaccination against *S. pneumoniae, H. influenzae* type b strains and influenza virus	Yes	D	NSA
Ineffective strategies			
Aerosolized antibiotic prophylaxis	No	B	No
Selective digestive decontamination	No	A	No
Routine parenteral prophylactic antibiotics for patients with coma	No	B	NSA
Limitation of stress-ulcer prophylaxis to high-risk patients (e.g. coagulopathy)	No	B	NSA
Strategies of equivocal or undetermined effectiveness			
Routine antibiotic class rotation	-	C	NSA
Combination antibiotic therapy	-	U	NSA
Prophylactic immune globulin	Yes§	D	NSA
Acidification of enteral feeding solutions	-	U	No
Use of peri-intubation systemic antibiotic prophylaxis	-	C	NSA

‡ *Adapted from Kollef*[28] *and Hospital Infection Control Practices Advisory Committee, CDC.*[29]
* The grading scheme is described in Table 84.6 .
† NSA, not specifically addressed. CDC recommendations are described in reference. [29]
§ This strategy is recommended for specific groups of patients described in the studies cited.

are aimed at reducing rates of colonization by potential pathogens, such as by use of selective digestive decontamination, have been relatively problematic. For example, selective decontamination has reduced rates of VAP caused by Gram-negative bacilli, without consistently altering mortality rates in ICUs. Use of sucralfate, rather than histamine type 2 blockers or proton pump inhibitors, for stress ulcer prophylaxis, with a goal of maintaining gastric pH and thereby suppressing gastric colonization by potential VAP pathogens, initially appeared to be a promising preventive strategy but this was not effective in a large, multicenter, randomized trial.[28] [29] Preventive measures aimed at reducing the risk of aspiration, particularly by semirecumbent positioning of patients, have been among the more successful and less costly strategies.

Antibiotic resistance and control

There is a global crisis in antibiotic resistance that reflects in large measure the heavy use of systemic antibiotics worldwide over the past 30 years.[9] [22] [32] Antimicrobial therapy has its greatest ecologic impact in the close confines of the ICU. Antibiotic pressure, which promotes the exchange of genes encoding drug resistance by a variety of transfer mechanisms, has been shown to be the single most important factor predisposing patients to nosocomial infection with resistant organisms. [32] Modern-day ICUs are the breeding grounds for MRSA, VRE and *Enterobacter, Serratia, Citrobacter, Proteus-Providencia* spp. and *P. aeruginosa* that are resistant to aminoglycosides, fluoroquinolones or extended-spectrum ß-lactams. Broad-spectrum antimicrobial therapy is the root cause of antibiotic-associated diarrhea and colitis caused by *C. difficile*. The recent report of a clinical isolate of a fully vancomycin-resistant MRSA should be a wake-up call for even the most complacent physicians. The ICU component of the CDC's NNIS system demonstrates the major rates of bacterial resistance in ICUs (Fig. 84.1).

In epidemiologic and clinical studies of antibiotic resistance, there are a proportion of patients in whom resistance emerges without exposure to the problem antibiotic. These patients usually have other important risk factors, such as increased severity of underlying disease, extremes of age, presence of invasive devices, recent surgery or proximity to patients who are infected or colonized with antibiotic-resistant bacteria. In these cases, the presence of antibiotic-resistant strains is most often attributed to indirect patient-to-patient spread, usually on contaminated hands of health care workers; occasionally, spread results from a contaminated common source, such as an inadequately cleaned piece of equipment or a colonized worker.[1] [9] [22] [32]

Stemming the tide of antimicrobial resistance requires a multifaceted approach (Table 84.8), especially in ICUs, where antibiotic pressures, lapses in hospital hygiene and the close proximity of high-risk patients are usually greatest.

CONCLUSION

It is clear that nosocomial infection is one of the most important causes of iatrogenic morbidity and mortality in patients who require prolonged life support care in an ICU. Strategies to increase adherence to hand hygiene, to prevent patient colonization and to prevent infection once colonization has already occurred should be a major focus of ICU staff attention and a research priority. The importance of hand carriage of pathogens by hospital personnel, the role of airborne transmission in the ICU and the relevance of contamination of the inanimate hospital environment by resistant pathogens need to be better delineated. Larger and more sophisticated studies, using multivariate techniques of statistical analysis to define risk factors and attributable morbidity and mortality for the major *endemic* nosocomial infections and pathogens in the ICU, are needed to guide allocation of infection control resources and to target future research efforts. More effective ways to communicate essential information on nosocomial infection control to hospital personnel, especially with regard to hand hygiene, aseptic use of devices and antibiotic therapy, and to apply control measures more consistently in all hospitals would have vast immediate benefits.

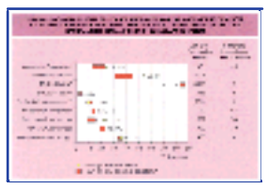

Figure 84-1 Antimicrobial resistance. Summary of antimicrobial resistance among common pathogens identified from ICU patients with nosocomial infections in hospitals participating in the CDC's NNIS. *From: http://www.cdc.gov/ncidod/hip/NNIS/ar_surv99.pdf*

TABLE 84-8 -- Multifaceted approach to control of nosocomial antimicrobial resistance.*

MULTIFACETED APPROACH TO CONTROL OF NOSOCOMIAL ANTIMICROBIAL RESISTANCE
✚ Active surveillance for resistance
✚ Molecular typing (e.g. pulsed-field gel electrophoresis) of resistant bacteria if rates increase
✚ Aggressive campaigns to improve hand hygiene
¦ Alcohol hand rub between patient (or environmental) contacts
¦ Universal gloving; gloves must be changed between patients (an adjunct and not a substitute for hand hygiene)
¦ Monitor adherence and give ward and health care worker-specific feedback of adherence rates
✚ Prevention of device-related infections (see Table 84.3 , Table 84.5 , Table 84.6 , Table 84.7)
✚ Stewardship of antimicrobial use
¦ Clinical guidelines for therapy of common infections
¦ Monitor appropriateness and need for continuation of therapy; give concurrent feedback
¦ Antibiotic restriction program
¦ Computer-based provider order entry
¦ Consider antibiotic cycling for ICUs (for control of specific resistance problems)
¦ Monitor adherence; give feedback
✚ For continued, increasing or difficult-to-control resistance
¦ Routine surveillance cultures to detect colonized patients (the 'resistance iceberg')
¦ Contact precautions for colonized and infected patients
¦ Cohort nursing for colonized and infected patients if contact precautions do not control spread

* *Adapted from Weinstein.* [22]

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Chapter 85 - Infection in Burn Patients

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EPIDEMIOLOGY

Thermal burns are less common than other forms of trauma but are unique in the production of the most severe physiologic stresses seen in any form of traumatic injury. It is estimated that 2 million people are burned annually in the USA, resulting in the need for 500,000 emergency department visits, 74,000 hospital admissions and 20,000 admissions to specialized burn treatment facilities.[1] Approximately 6500 people die each year from burns or exposure to fire in the USA.

Burn trauma is classified by depth of injury, extent of body surface area involvement and associated injuries:

- first-degree burns involve only the epidermal layer of skin, usually heal without medical intervention and normally do not become infected — they are not included in estimations of burn size for the purposes of determining triage, need for fluid resuscitation or for survival estimates;
- second-degree (partial thickness) burns involve varying layers of the dermis, and whether they heal without operative intervention depends upon depth of injury; and
- third-degree (full thickness) burns involve the full thickness of dermis and normally require operative debridement followed by split thickness skin grafting or other techniques to achieve wound closure.

The mortality rate of burn injury is proportional to the age of the patient and the size of the cutaneous second- and third-degree burn. Burn injury is poorly tolerated in the young, the elderly and in those with pre-existing chronic medical illness. For example, a burn sustained by an elderly diabetic while soaking a neuropathic foot in hot water may represent less than 3% total body surface area but can easily evolve into a limb-threatening or life-threatening injury if treatment is inadequate or delayed.

The mortality rate from burn injury has decreased significantly over the past half century, primarily because of improved control of burn wound infection through the use of topical antimicrobial agents and aggressive surgical debridement. The LA_{50} (percentage body surface burned associated with a 50% mortality rate) now exceeds 80% in selected age groups. The combined effects of burn size and age on predicted mortality rate are demonstrated in Figure 85.1 .

Infection remains the most frequent cause of morbidity and death in burn patients.[2] Although the incidence of invasive burn wound infection has significantly decreased, other infections, particularly pneumonia, remain a problem. Injury to the lungs from exposure to smoke is a significant co-morbid factor, and predisposes the patient to nosocomial pneumonia.[3]

The American Burn Association has established criteria for the referral of patients who have thermal injury to specialized care facilities (Table 85.1). These criteria, endorsed by the Advanced Trauma Life Support and Advanced Burn Life Support programs,[4] [5] represent the standard of care in developed countries. The infectious disease specialist is often the first consultant to see the burn patient in a community hospital setting. Referral to a designated burn center may be the optimal approach in this situation.

PATHOGENESIS AND PATHOLOGY

Burn injury produces profound alterations in homeostasis, which are proportional to the size of the cutaneous injury. Virtually every organ system is affected, and changes in the cardiovascular and immunologic systems are particularly pertinent.

Thermal injury results in a significant and sustained hypermetabolic response. The causes of post-burn hypermetabolism are poorly understood but may be related to a centrally mediated release of catecholamines, glucagon and cortisol. [2] Severe burn injury can result in resting metabolic rates that are twice normal levels, causing nutritional depletion if sufficient exogenous calories are not supplied. Burn hypermetabolism persists until the burn wounds are closed,[2] a process that may require several weeks.

The initial cardiovascular response to burn injury is a decrease in cardiac output along with an increase in systemic vascular resistance secondary to hypovolemia. With appropriate fluid resuscitation cardiac output returns to a normal level within 24 hours of injury. During the second 24 hours, the patient becomes hypermetabolic and cardiac output increases to supernormal (2–2.5 times predicted normal) levels. The elevation of cardiac output is accompanied by a reciprocal drop in systemic vascular resistance to 40–80% of normal levels.[6] Cardiac output remains elevated until the burn wounds are closed.[2] The stress on the cardiovascular system may precipitate myocardial infarction, particularly at the peak of the hypermetabolic response (1 week post-burn).[2] It cannot be overemphasized that the pattern of elevated cardiac output and decreased systemic vascular resistance is a normal response to thermal injury, and is not, of itself, indicative of sepsis.

The hypermetabolism of burn injury results in increased heat production, elevation of core temperature and a central upregulation of the thermoregulatory set point. Fever in the burn patient is thus often physiologic and not related to infection. Burn patients have poor temperature autoregulation while their wounds are still open. Loss of skin integrity results in high heat losses to the environment. Despite the hypermetabolic and thermogenic nature of burn injury, such patients may become hypothermic if the ambient temperature is not increased to compensate for these losses. The evaluation of hypothermia in the burn patient should start with consideration of an environmental cause.

A final consequence of burn hypermetabolism is the increased clearance of many medications, including antibiotics and anticonvulsants. Patients treated with standard doses of medications pre-injury cannot be assumed to retain therapeutic levels on the same doses post-injury. Drug dosing should be based upon repeated serum measurements if these are available. Likewise, administration of therapeutic antibiotics at dosages effective for nonburn populations will often result in subtherapeutic levels in burn patients. Antibiotic administration, particularly of aminoglycosides and vancomycin, must be guided by frequent measurements of peak and trough serum levels.

Thermal injury suppresses cell-mediated immunity. One benefit of this immunosuppression is the ability to use allogeneic skin grafts (cadaver skin) as a temporary skin substitute without fear of early

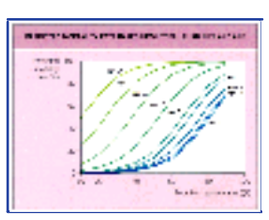

Figure 85-1 Predicted mortality rate based upon total burn size and age.

TABLE 85-1 -- American burn association criteria for referral to a burn center.[4] [5]

AMERICAN BURN ASSOCIATION CRITERIA FOR REFERRAL TO A BURN CENTER
Second- and third-degree burns >10% body surface area (BSA)
Third-degree burns of any size
Second- and third-degree burns involving face, eyes, ears, hands, feet, genitalia, perineum, or overlying major joints
Electrical burns, including lightning injury
Chemical burns
Inhalation injury
Burn injury in patients with pre-existing medical conditions that could complicate management, prolong recovery or affect mortality rate
Burn injury with associated trauma
Burn injury in patients with special social, emotional or rehabilitative needs, including suspected child abuse or neglect

graft rejection.[2] The multiple immunologic consequences of burn injury are well described[2] [7] [8] [9] [10] [11] and are summarized in Table 85.2 . An important point is that leukocyte counts in the range of 14,000–18,000 cells/ml or higher may be seen in burn patients in the absence of infection. The trend of white blood cell elevation rather than an isolated white blood cell elevation should alert the clinician to the possibility of infection.

A final immunologic consequence is the need for multiple transfusions of blood products. Transfusion is known to produce immunosuppression and may serve as a source of blood-borne pathogens. A study of 594 burn patients with a burn size over 10% body surface area surviving for more than 10 days showed an average transfusion rate of 19.7 units of packed red cells per patient.[12] A significant association exists between infectious morbidity and number of transfusions, independent of burn size or patient age.

In summary, a variety of metabolic events conspire to make the timely diagnosis of infection difficult in the burn patient. Hyperthermia, hypothermia, leukocytosis, tachypnea, tachycardia, disorientation, glucose intolerance and positive wound surface cultures are all seen in the absence or the presence of infectious processes, and are not sufficient to diagnose burn wound infection.[2]

TABLE 85-2 -- **The impact of thermal injury on the immune system.**

THE IMPACT OF THERMAL INJURY ON THE IMMUNE SYSTEM		
Circulating	IL-1	Initial increase in serum levels followed by decreased production
		Increased local production at sites of inflammation
	IL-2	Suppressed production
	TNF-a	Increased serum levels in severely infected burn patients
	IL-6	Increased serum levels in severely infected burn patients, increased local production at sites of inflammation
	Neopterin	Serum levels increased (non-specific marker of macrophage stimulation)
	Immunoglobulins	Decreased serum levels in first week
	Prostaglandin E_2	Increased serum levels
	Thromboxane B_2	Increased serum levels
	Activation/depletion of alternative complement pathway	
	Secondary elevation of fibronectin levels	
	Reduction in serum opsonic activity	
Cellular	Initial leukopenia (margination) followed by leukocytosis	
	Generalized activation of circulating granulocytes by multiple pathways	
	Depression of neutrophil chemotaxis, phagocytosis and bactericidal activity	
	Depression of helper T cells	
	Generation of suppressor inducer T cells in animal studies	
	Increased production of suppressor effector T cells in animal studies	
	Suppression of IL-2 production by lymphocytes	
	Activation of macrophages	
Other	Loss of cutaneous skin barrier to infection	
	Smoke inhalation — increased risk of pneumonia (impaired mucociliary clearance mechanisms, defective alveolar macrophage function, distal airway obstruction, alveolar collapse, segmental atelectasis, increased requirements for airway intubation and mechanical ventilation)	
	Requirement for long-term intubation of bladder and vascular system	
	Nutritional deficits	
	Impairment of reticuloendothelial system function	
	Multiple blood transfusions	
IL, interleukin; TNF, tumor necrosis factor.		

PREVENTION

Several steps may be taken to reduce the risk of infectious complications in the burn population. As with any intensive care unit patient, infectious complications are reduced by:

- the prompt extubation of respiratory, cardiovascular and genitourinary systems when clinical condition allows;
- the provision of adequate nutrition; and
- the timely mobilization of the patient to prevent pressure sores and atelectasis.

The use of single-room isolation for burn patients delays the onset of colonization with *Pseudomonas aeruginosa* and reduces the incidence

of wound infection, bacteremia and pneumonia associated with this pathogen.[13] Topical use of mafenide acetate in association with the avoidance of any pressure on the external ear reduces the incidence of suppurative chondritis.[14] The incidence of suppurative thrombophlebitis is decreased by the regular rotation of intravenous cannulation sites. At burn centers all indwelling venous lines should be replaced at the time of admission (if started outside the burn center) and every 3 days thereafter by fresh venipuncture.[15] Line changes over guidewires are not performed. The incidence of nosocomial pneumonia is decreased by the use of high-frequency percussive ventilation, which facilitates the removal of endobronchial secretions and allows adequate ventilation at lower airway pressures than conventional techniques.[15] [16] A bronchopneumonia incidence of 29.3% was reported in burn patients treated with high-frequency percussive ventilation compared with 52.3% in a

matched cohort treated with volume-cycled ventilation.[16]

Burn patients are prone to stress ulcers of the gastrointestinal system, and this process can be minimized by treatment with antacids and H_2 -blocking agents. In one study nosocomial Gram-negative pneumonia occurred more frequently in patients requiring mechanical ventilation who received antacids and H_2 -blocking agents than in a similar cohort receiving sucralfate.[17] Gram-negative colonization of the stomach secondary to elevation of gastric pH was a postulated mechanism. The study group was predominantly patients who had nontraumatic illness. A second study by Cioffi and associates was limited to burn patients.[18] Antacid and H_2 -blocker therapy was prospectively compared with sucralfate. No differences in rate of colonization of the respiratory or gastrointestinal tracts were observed, but a higher incidence of nosocomial pneumonia and upper gastrointestinal bleeding occurred in the sucralfate group.[18] A drug that could prevent gastric ulceration while maintaining a sufficiently low gastric pH to prevent colonization would be ideal for the prophylaxis of stress ulceration in burn patients. At present, a drug with these characteristics remains to be developed.

CLINICAL FEATURES

Organisms causing infections

The history of burn wound infection has been largely influenced by therapeutic and environmental factors. Before the development of antimicrobial agents and the use of fluids to resuscitate the burn patient, essentially all those with serious burns died within a short period from the consequences of hypovolemic (burn) shock. Those patients who remained in hospital after the initial shock period were subject to streptococcal infection. It is this group of organisms that was targeted by Lister and others for topical protection for open burns with antiseptics. With the recognition of the requirement for resuscitation, patients who have severe burns began to survive the initial post-burn period. This new patient population with large wounds that remained open for months led to the development of specialized burn centers, which were established at large hospitals and by the military. The traditional hospital open-ward design was adapted for burn care and little could be done to prevent cross-contamination. The situation of general ward care, large open wounds and the introduction of expanding generations of antimicrobial agents often resulted in wound infection by antimicrobial-resistant microbial pathogens. By the 1960s burn centers worldwide experienced infections from *P. aeruginosa* and other opportunists with intrinsic resistance to many antibiotics and with the capacity to acquire and propagate resistance mechanisms against newer generations of antimicrobials. Burn centers often gained notoriety for having a high incidence of infection and antimicrobial resistance.

With the recognition of the significance of cross-contamination and the necessity to improve patient isolation, the design of most

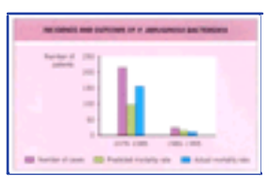

Figure 85-2 Incidence and outcome of *Pseudomonas aeruginosa* bacteremia over a 20-year period.

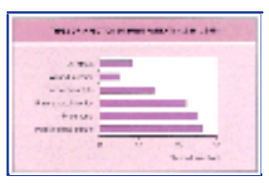

Figure 85-3 Types of infections in burn patients (1986–1995).

modern burn centers has changed to single rooms for the intensive phase of burn care. This change occurred at our burn center in 1983 and markedly changed the incidence, etiology and outcome of burn wound infections.[13] [19]

In subsequent reviews the continued prevention of previously common infections with multiply resistant organisms and improved survival has been documented. [20] An example of such change is presented in Figure 85.2 . These data document the incidence and outcome of *P. aeruginosa* bacteremia during the past two decades. In the first decade, the incidence of this infection was 9.4% and the mortality rate in bacteremic patients was 73%. When this mortality rate is compared with that predicted for severity of injury alone, an excess attributable mortality rate of 61% is realized.[13] During the current decade, the incidence of this infection has dropped to 1.2% of admissions and the mortality rate noted is not different from that predicted based on severity alone.[20]

The review of infections and their causes in the past decade is presented in Figure 85.3 and Figure 85.4 . As can be seen, wound invasion, the hallmark of burn infections for many decades, has been reduced to 5% of infections.

Coincident with a decline in bacterial wound infection, an increase in the incidence of fungal infection has been seen. A histopathologic review of all burn autopsy data at our institution between the years 1960 and 1969 showed a 10-fold increase in the incidence of wound infections caused by *Phycomycetes* and *Aspergillus* spp. coincident

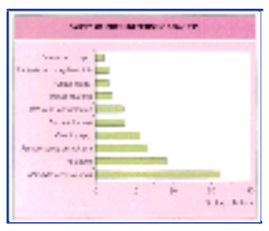

Figure 85-4 Causes of infection.

with the introduction of mafenide acetate burn cream in 1964.[21] More recent experience between the years 1979 and 1989 demonstrates a marked decrease in the incidence of bacterial wound infection, attributable to patient isolation, topical chemotherapy and surgical wound excision.[22] Interestingly, the incidence of fungal wound infection did not change during the same period. Bacterial and fungal wound infections were associated with large body surface area burns (mean 62.4% total body surface area (TBSA) burn for fungal infection and 54.4% TBSA for bacterial infection) and an increased incidence of smoke inhalation injury (74.5% of patients with fungal infection vs 70.6% of patients with bacterial infections). The immunosuppression of burn injury may predispose patients to fungal infection, which is difficult to prevent given the ubiquitous nature of fungi in the environment.[22] Indiscriminate use of systemic antibacterial agents in the absence of documented infection may suppress normal bacterial flora and result in fungal superinfection.

The burn wound

An understanding of the burn wound is key to an understanding of the burn patient. The physiologic, metabolic and immunologic changes seen in burn injury are proportional to the size of the cutaneous wound and do not return to normal until the burn wound is successfully closed. The burn wound is the first site to examine when sepsis is suspected.

The thick leathery nonviable coating of a burn is termed eschar. Burn eschar is warm, protein rich, moist and avascular, and represents an excellent culture medium that is unaffected by parenterally administered antibiotics or by circulating elements of the immune system.[2] [15] For this reason, the eschar of full thickness burns should be surgically excised as quickly as possible. Burn eschar normally becomes colonized with the patient's own flora (Gram-positive bacteria) within 3–5 days of injury.[9] This initial colonization is subsequently replaced by Gram-negative flora present in the hospital unit over a variable interval. The principal concern is eventual colonization of the subeschar space. When bacterial densities at the interface of eschar and underlying viable tissue reaches a level of 1×10^5 /g of tissue, wound infection with systemic microbial spread is likely.[2] The goals of burn wound management are to contain microbial colonization to a manageable level with topical agents pending surgical debridement and wound closure with skin autografts or other modalities.[2]

Figure 85-5 Contact plate used for wound surveillance cultures. The culture media is lifted out of the Petri dish by the attached sterile gauze, placed in contact with the burn wound, and then returned to the Petri dish for incubation.

Because the presence of bacteria in the burn wound per se is not a pathologic finding, a number of specialized procedures and terms have been developed to quantify the potential for wound infection. Burn wound colonization is the term given to the presence of microorganisms within the eschar. As the eschar is avascular, colonization 'does not imply an unavoidable and active local or systemic infection'. [9] Burn wound invasion occurs when micro-organisms invade viable tissue adjacent to the burn eschar. True invasive burn wound infection is rarely seen in most burn centers when topical antimicrobial agents are properly and promptly employed. Invasion accompanied by a positive blood culture or by distant spread of micro-organisms or toxic products is termed burn wound septicemia.[9]

Diagnostic modalities include swab or plate cultures of the burn wound surface, cultures of debrided tissue, quantitative wound cultures and histologic examination of the burn wound. Cultures of the burn surface or of debrided tissue are useful for epidemiologic purposes to document resident flora in the event of true infection.[2] [7] Surface cultures should be performed with contact plates (Fig. 85.5) rather than with swabs in order to sample a larger and more representative area and to avoid errors relating to prolonged incubation of the swab in transport media.[23] Quantitative wound cultures[24] or counts of colony forming units (cfu) per gram of tissue have limited the diagnostic modality in burn wound care. A negative quantitative culture (bacterial density $<10^5$ cfu) correlates well (96.1% negative predictive power) with absence of invasive infection on histopathologic tissue evaluation; however, the agreement between positive cultures and positive histologic examination is only 35.7% (positive predictive power).[25] Thus, only a negative quantitative culture has clinical significance.

The *sine qua non* of wound evaluation is histopathologic examination of a biopsy specimen to determine the presence of micro-organisms in viable tissue.[2] This is performed as a bedside procedure under local anesthesia. A 500mg sample of eschar (ellipse measuring 1–2cm) is excised to include the underlying viable tissue and submitted for microbial and pathologic analysis.[2] [23] Using a frozen section technique, results are available in 30 minutes.[26] The frozen section technique has a 3.6% false-negative rate, and should be confirmed by examination of permanent sections.[23] [26] A rapid section technique that produces permanent sections in 4 hours has been described. [27] Results with either technique are communicated to the clinician using standardized nomenclature (Table 85.3).

TABLE 85-3 -- Histologic classification of burn wound colonization and infection.

HISTOLOGIC CLASSIFICATION OF BURN WOUND INFECTION		
Class 1 - Colonization	1A Superficial	Superficial bacterial colonization of the wound
		Micro-organisms present on wound surface
	1B Penetration	Penetration of the eschar by bacteria in variable thickness of eschar
	1C Proliferation	Multiplication of micro-organisms present in the subeschar space
Class 2 - Invasion	2A Microinvasion	Micro-organisms present in viable tissue immediately subjacent to subeschar space
	2B Generalized	Multifocal or widespread penetration of micro-organisms deep into viable tissue
	2C Microvascular	Involvement of small vessels and lymphatics

The use of effective topical antimicrobial agents has decreased the incidence of true invasive wound infection. The percentages of infection-related deaths attributed to wound infection has decreased from 25.5% in 1979 to as low as 5.1% between 1987 and 1991.[15] [28]

Pneumonia

As the incidence of wound infection decreases, pneumonia has emerged as the most frequent septic complication of burn injury.[15] With better microbial control of the burn wound, the route of pulmonary infection has changed from hematogenous to airborne, and the predominant radiographic pattern has changed from nodular infiltrates to bronchopneumonia.[15] Smoke inhalation injury results in mucociliary dysfunction, atelectasis, ventilation-perfusion mismatch and impairment of polymorphonuclear leukocyte function. These defects in pulmonary function increase the risk of pneumonia nearly 5-fold.[2] [7] The effects of inhalation injury and pneumonia on predicted mortality rate in burn injury are independent and additive: inhalation injury increases expected mortality rate by up to 20%; pneumonia increases mortality rate by up to 40%; and the combination of inhalation injury and pneumonia increases mortality rate by up to 60%.[3] For this reason, prevention of pneumonia has become a prime concern in patients who have smoke inhalation injury. As discussed earlier, the use of single-room isolation, volume diffusive ventilation techniques and certain stress ulcer prophylaxis regimens may be associated with a lower risk of nosocomial pneumonia.

Criteria used at our burn center for the diagnosis of pneumonia in the burn patient are presented in Table 85.4 . Cases that meet criteria for diagnosis but lack radiographic evidence of an infiltrate are termed tracheobronchitis. Surveillance sputum cultures obtained three times per week provide an indication of the predominant organism when pneumonia or tracheobronchitis is diagnosed, allowing timely administration of an appropriate antibiotic. Pathogens commonly associated with bronchopneumonia in burn patients include *P. aeruginosa* and *Staphylococcus aureus*. The emergence of Gram-positive organisms as the predominant cause of bronchopneumonia has been a recent trend in many burn centers.[15] Staphylococcal pneumonia usually responds well to vancomycin. Our

TABLE 85-4 -- Diagnostic criteria for pneumonia or tracheobronchitis.

DIAGNOSTIC CRITERIA FOR PULMONARY INFECTION	
Pneumonia	1) Clinical findings consistent with pneumonia (i.e. pleuritic chest pain, fever, purulent sputum or other signs of sepsis)
	2) More than 25 polymorphonuclear leukocytes on methylene blue stain of endotracheal secretions with less than 25 squamous epithelial cells per 100× field
	3) Radiographic findings consistent with pneumonia
	4) Positive sputum cultures (confirmatory but not essential for diagnosis)
Tracheobronchitis	As above without radiographic evidence of infiltrate

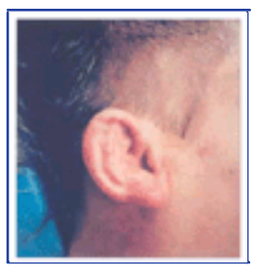

Figure 85-6 Clinical appearance of suppurative chondritis.

preference is to treat Gram-negative pneumonia with double antibiotic coverage, usually an aminoglycoside along with a broad-spectrum ß-lactam antibiotic (see Chapter 35).

Suppurative chondritis

Burns to the ears may result in secondary infection of the cartilage. This process, termed suppurative chondritis, occurs in partial and full thickness burns, has a peak incidence between 3 and 5 weeks post-burn, and may occur after skin healing is completed.[14] Before the widespread use of mafenide acetate, chondritis occurred in

20–25% of patients who had ear burns.[14] [29] This incidence has decreased to 0–3.3% when burned ears are treated with this agent.[14] Silver sulfadiazine does not penetrate eschar well and is not as effective in the prevention of chondritis.

Suppurative chondritis has a rapid onset, manifesting as a throbbing ear pain poorly relieved by narcotics.[29] Over several hours the external ear becomes edematous with loss of the normal surface features of the antihelix and scapha (Fig. 85.6). Edema may create the appearance of the external ear being more prominent or anterior displaced compared with the contralateral side. The pathogens responsible may be polymicrobial, with *Pseudomonas* spp. involved in 83–95% of cases and *Staphylococcus* spp. involved in 55%.[14] Intravenous antibiotics are ineffective because the ear cartilage does not have an intrinsic blood supply and diffusion from the perichondrium is impaired by edema. Treatment of chondritis is surgical.

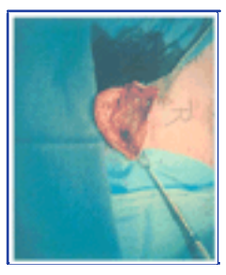

Figure 85-7 Bivalve excision of infected cartilage.

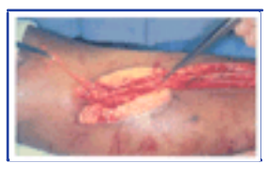

Figure 85-8 Vein resection for suppurative thrombophlebitis.

Early cases may respond to local debridement, whereas established chondritis often requires a disfiguring bivalve incision with removal of a majority of the ear cartilage (Fig. 85.7). Wounds are packed open with fine mesh gauze soaked in an antimicrobial solution, then allowed to close by granulation.

Suppurative thrombophlebitis

Suppurative thrombophlebitis occurs in up to 1.4–4.2% of burn patients.[30] Although the overall incidence is low, burn patients are at particular risk secondary to the need for long-term intravenous cannulation and to skin colonization with burn pathogens. The diagnosis is suggested by a positive blood culture in the absence of any obvious source. Clinical examination of current and previous intravenous cannulation sites is undertaken, although local signs of infection, including cellulitis, are present in only 35% of patients who have infected vessels. When the diagnosis is in doubt, surgical exploration is indicated. Infection is most frequently found at the vein site adjacent to the catheter tip; however, proximal migration and skip lesions are common, and the suspected vein should be excised proximally to the point at which the vessel becomes a tributary of the next larger order of veins (Fig. 85.8). In selected patients, resection may be terminated at a point at which the vein appears normal, has a lumen free of clot or intimal thickening and demonstrates brisk bleeding. The resected vessel should be sent for histologic and micro-biologic analysis (Fig. 85.9). Failure to clear bacteremia after vein

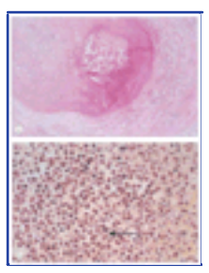

Figure 85-9 Suppurative thrombophlebitis. (a) Histologic section of excised vein demonstrating thrombus. (b) Micro-organisms (Gram-positive cocci) present within the thrombus (arrow).

excision should prompt consideration of exploration of other vessels. Septic thrombophlebitis of central vessels is usually treated with systemic anticoagulation and intravenous antibiotics because surgical excision is impractical.

Other infections

Sinusitis is a concern in burn patients because of the need for prolonged intubation of one or both nostrils. Nasotracheal intubation is the preferred airway access in burn patients for reasons of comfort and safety,[31] and nasojejunal tube feeding remains the method of choice for delivery of nutrients. The presence of sinusitis is suggested by headache, facial pain or purulent nasal discharge, although the diagnostic accuracy of clinical examination has been estimated to be only 60%. Plain film radiographs of the sinuses, particularly when taken with portable equipment, are difficult to interpret and add only 10% diagnostic accuracy to the clinical examination; for this reason the preferred diagnostic modality is computerized tomography scan of the sinuses. Treatment of sinusitis is removal of nasal tubes and administration of decongestants and culture-specific antibiotics. Antral puncture is infrequently required and should be reserved for patients who do not respond to conventional treatment (see Chapter 32 for further discussion on sinusitis).

Acute infective endocarditis is diagnosed in 1.3% of burn patients. [15] The right side of the heart is more commonly affected than the left; the offending organism is usually *S. aureus*. Heart murmurs are difficult to hear in burn patients, who are normally hyperdynamic, tachycardiac and frequently have their chest covered with dressings.

The diagnosis is usually made with transesophageal echocardiography. Intravenous antibiotics are instituted and continued for 6 weeks after the last positive blood culture. The management of endocarditis is reviewed in detail in Chapter 59 .

MANAGEMENT

Burn patients are prone to infection by virtue of loss of immune function and skin integrity. The daily care of burn patients requires meticulous infection control practices to avoid nosocomial infection. A microbial surveillance system is important to allow timely and exact diagnosis of infection and to guide antibiotic use. Because of the danger of superinfection from antibiotic-resistant organisms, the administration of antibiotics should strictly follow established protocols. The empiric administration of systemic antibiotics for fever, leukocytosis or non-specific 'sepsis' in the absence of a known infectious source should be strongly discouraged. [15]

The nursing care of burn patients requires adherence to barrier precautions and reverse isolation techniques appropriate for the immunosuppressed. The use of an open dressing technique (application of topical antimicrobial to the burn without overlying dressings) allows earlier identification of potential wound infection and is the standard of practice at many burn centers. The use of open dressings or the changing of closed dressings requires all personnel entering the patient's room to wear gowns, head covers and gloves.[13] Barrier precautions have the added benefit of protecting the staff from occupational exposure to blood-borne pathogens. Medical supplies and equipment should not be moved from room to room, and stethoscopes are provided in each room to avoid cross-contamination.

The use of single-bed isolation for burn patients is desirable. Compared with open-ward housing, single-bed isolation significantly delays the onset of colonization with *P. aeruginosa* by 10 days and decreases the incidence of bacteremia, pneumonia and burn wound invasion.[13] A delay in colonization may decrease morbidity and the mortality rate because the patient may be more likely to resist infection later in their hospital stay as wound healing progresses.

Resistance isolation is used when a patient is identified as harboring a bacterial organism with multiple antibiotic resistance or when a patient is transferred after being

an inpatient in another hospital for 7 days or more. Under resistance isolation, nursing staff are assigned to the same patient every day and normally do not enter the room of any other patient. Supplies are arranged to maximize patient care with minimal traffic in and out of the room, and physician care proceeds from non-isolated to resistance-isolated areas as the patient condition allows. Personnel providing resistance-isolation care must change all garments, including scrub clothing, before entering other areas of the hospital. Under optimal conditions, nurses providing resistance-isolation care are not reassigned to other patients until 48 hours after exposure to the isolated patient.

The microbial surveillance system in use at this and certain other burn centers[9] consists of thrice weekly cultures of sputum, wound surfaces and urine of all patients. The surveillance system allows timely identification of patterns of antibiotic resistance, identifies nosocomial spread of flora and facilitates the rational choice of antibiotic when infection is identified. In general, the antibiotic sensitivities of flora seen in the burn center are different from similar species isolated from other nursing care units, and the regular hospital antibiogram should not be relied upon when choosing antibiotics. If a full burn surveillance system cannot be established, an alternative is to produce an antibiogram specific for isolates from the burn treatment unit.

Care of the burn wound centers on debridement of nonviable tissue and prevention of infection. Wounds are cleansed twice daily with chlorhexidine gluconate solution and then placed in 'alternating agents' consisting of mafenide acetate cream during the day and silver sulfadiazine cream at night. The combination of mafenide and silver sulfadiazine reduces the incidence of complications and drug resistance compared with that seen when either agent is used alone. Mafenide acetate provides the best penetration into nonviable eschar and may be employed as a single agent when the wound is obviously full thickness or when invasive infection is suspected. The advantages and disadvantages of this and other topical antimicrobial agents are summarized in Table 85.5 .

The entire wound surface must be inspected at least daily for signs of infection.[2] Wound care should be provided in the individual patient's room to avoid cross-contamination between patients. Hydrotherapy or wound debridement in a Hubbard tank is no longer performed at most burn centers.

The ultimate goal of burn care is permanent wound closure. To this end, surgical excision of full thickness and deep partial thickness wounds commences 3–5 days after the burn injury and continues on a frequent basis until the entire burn is excised. Split thickness autografts harvested from unburned skin surfaces are used to cover the excised area. When donor sites are insufficient, temporary wound closure is performed with cadaver allograft or biosynthetic dressings. Donor sites may generally be reharvested every 10–14 days, allowing eventual replacement of temporary coverage with the patient's own skin.

Special considerations

Surgical prophylaxis

Topical antimicrobial agents such as silver sulfadiazine and mafenide acetate do not sterilize the burn wound and are intended only to keep microbial colonization of the burn eschar to manageable levels. Surgical excision and grafting therefore involves manipulation of colonized tissue, and perioperative antibiotic coverage would seem prudent. Despite this, there is little agreement among burn practitioners regarding the need for surgical prophylaxis or choice of agent, and few recent studies upon which to base recommendations.

A survey of 51% of all USA burn centers (*n*=68) conducted by Worrell *et al.* demonstrated that 46% use surgical prophylaxis in all patients and 20% do not use prophylaxis for any patient. [32] The remaining 34% base selective prophylactic decisions on factors such as cellulitis, suspected soft tissue infection or type of surgical procedure. Cefazolin as a single agent is the most common antibiotic used for prophylaxis. This drug provides poor coverage of the flora commonly present in the burn eschar. Burn excision and grafting procedures commonly result in significant blood loss, requiring intraoperative transfusion of multiple units of blood products. Despite this, only 20% of respondents re-dose antibiotics intraoperatively based upon length of procedure, and 2% re-dose based upon blood loss.

Studies in the 1970s demonstrated that the incidence of bacteremia following burn excision and grafting procedures ranged from 20.6 to 46%.[33] [34] The surgical management of burns has changed considerably since the time of these studies. More recently, Mozingo *et al.*[35] prospectively studied the incidence of bacteremia during burn wound care and during surgical excision procedures. The incidence of new bacteremia following wound cleansing and wound excision was 9.5 and 15.8%, respectively. No bacteremia occurred in any patient with total burn size under 40% of total body surface area when excision was undertaken within the first 10 days post-burn.

Clinically, there is no question that burn wound excision and grafting procedures may result in postoperative circulatory instability and a 'septic' picture in certain patients, even when appropriate perioperative antibiotics are used. On this basis, we advocate perioperative coverage with antibiotics based upon specific culture results of the patient in question or upon surveillance cultures or burn center

TABLE 85-5 -- Topical antimicrobial therapy of the burn wound.

TOPICAL ANTIMICROBIAL THERAPY OF THE BURN WOUND		
Agent	**Advantages**	**Disadvantages**
Mafenide acetate 11.1% cream	Best eschar penetration, most widely studied agent; broad spectrum, bacteriostatic against Gram-positive and Gram-negative, especially effective against *Pseudomonas aeruginosa* and *Clostridia* spp.; no Gram-negative resistance	Painful to apply on partial thickness burns; metabolic acidosis from carbonic anhydrase inhibition; accentuates hyperventilation; minimal coverage of yeasts; poor coverage of *Providencia* spp.; most effective when utilized with open dressing technique
Mafenide acetate 5% aqueous solution	Good eschar penetration; useful in wet dressings to facilitate debridement; especially effective on wound bed after eschar removal; effective dressing for open granulating wounds or over meshed autografts	Requires wet dressings; may contribute to hypothermia; not Food and Drug Administration approved in USA
Silver sulfadiazine 1% cream	Painless on application; good Gram-negative and yeast coverage; infrequent hypersensitivity; may be used with open or closed dressings; may be combined with nystatin to increase yeast coverage	Poor eschar penetration; transient leukopenia; poor or no coverage of *Enterobacter, Klebsiella, Clostridia* and some *Pseudomonas* spp.; plasmid-mediated resistance to this agent may extend to other antimicrobials
Silver nitrate 0.5% solution	Bacteriostatic against a broad spectrum of Gram-positive, Gram-negative and yeast-like organisms; effective for patients with toxic epidermal necrosis syndrome or burn patients allergic to sulfa drugs	Little to no eschar penetration; precipitates on tissue contact; works best on minimally colonized or debrided tissue; stains tissue, clothing and bedlinens; causes hyponatremia, hypokalemia and hypocalcemia; wet dressings may contribute to hypothermia; poor coverage of *Klebsiella* and *Providencia* spp.
Sodium hypochlorite 0.025% solution	Bactericidal against a broad spectrum of Gram-positive and Gram-negative organisms; inexpensive	Must be freshly compounded; requires wet dressings, which may contribute to hypothermia
Gentamicin sulfate 0.1% cream	Broad spectrum	Rapid emergence of resistant organisms; no longer in common use
Nitrofurazone 0.2% cream	Effective against *Staphylococcus* spp. (including methillin-resistant *Staphylococcus aureus* (MRSA)) and nonpseudomonad Gram-negative organisms	Poor coverage of *Pseudomonas* spp.
Mupirocin 2% cream	Effective against Gram-positive organisms, including MRSA; effective against some Gram-negative enteric organisms; useful for graft infection secondary to *Staphylococcus* spp.	Expensive; not a first-line therapy
Nystatin 100,000 units/g	Effective against *Candida* spp. and most true fungi	No antibacterial coverage
Clotrimazole 1% cream	Broad-spectrum antifungal effective against *Candida, Trichophyton* and *Microsporum* spp.; minimal systemic absorption from topical use	No antibacterial coverage; poorly absorbed through normal skin; eschar penetration unknown

antibiogram indicating the flora and sensitivities likely to be present. In our current practice, perioperative antibiotic coverage is usually undertaken with vancomycin added to an appropriate aminoglycoside.

Use of vancomycin in the burn center

The emergence of Gram-positive pathogens that are resistant to vancomycin is a growing concern. As a result, many institutions have instituted special methicillin-resistant *S. aureus* (MRSA) isolation procedures or restricted the prescription of vancomycin. Neither policy is necessary nor desirable in the burn center. In most cases, antibiotic use in the burn center is both selective and conservative, and in most burn centers effective isolation procedures are already in place. In addition, the methicillin sensitivity of *S. aureus* recovered from burn patients does not impact on outcome. Furthermore, at least one study (see discussion below) has demonstrated that the appropriate use of vancomycin in a burn center is not associated with the emergence of significant bacterial resistance to this drug.

Burn patients are immunosuppressed and at increased risk of infection secondary to large open wounds. For this reason, most burn care providers take exceptional care with infection control measures. Effective isolation and barrier techniques have been used in burn centers for over 50 years, both preceding and exceeding those mandated by universal precautions, body substance isolation or MRSA isolation. As an example, the resistant organism isolation techniques elsewhere mentioned in this chapter are instituted when a patient is transferred to the burn center following hospitalization in another facility. These additional infection control measures are continued until surveillance cultures demonstrate absence of pathogens that are multiple-drug resistant.

The significance of MRSA colonization and infection was examined in 1100 consecutively admitted, seriously burned patients in whom vancomycin was used to treat all staphylococcal infections.[36] Colonization with *S. aureus* was identified in 658 patients, including 319 patients with MRSA. Infection with *S. aureus* was documented in 178 patients, including 58 with MRSA. Survival of either group was the same as predicted solely on basis of age and burn size; there was no increase in mortality rate attributed to MRSA infection. On this basis, the study questioned the economic or clinical usefulness of added infection control practices for MRSA.

The long-term use of vancomycin in burn patients is not associated with the development of significant resistance of either *S. aureus* or *Enterococcus* spp. strains. McManus *et al.*[37] reported resistance patterns in burn patients from the USA Army Institute of Surgical Research/Burn Center at Brooke Army Medical Center. As part of a microbial surveillance program, all patients are cultured three times a week, including wounds, sputum, urine and gastrointestinal aspirate or stool. In a study of 2266 consecutive admissions between 1986 and 1995, there were 15,125 Gram-positive isolates, including 957 enterococci. Vancomycin resistance was found in only six of the 15,125 isolates, including three isolates positive for vancomycin-resistant

enterococci, two *Corynebacterium* and one *Lactobacillus* spp. Vancomycin was not used in three of these patients and none of the vancomycin-resistant organisms was associated with infection. In the absence of routine surveillance cultures, none of these organisms would have been clinically apparent. The lack of vancomycin resistance in this population is more remarkable for the fact that vancomycin had been used in this burn center for virtually every Gram-positive infection and most surgical prophylaxis since 1978.

Vancomycin remains a first-line antibiotic in the burn center. In view of the fact that methicillin sensitivity of *S. aureus* infection in burn patients does not appear to impact upon outcome, and because burn isolation techniques are routinely used for all patients, the usefulness of the term 'MRSA' in contemporary burn practice is questionable.

Diagnostic investigation of the potentially septic burn patient

As previously mentioned, the presence of fever, leukocytosis, elevated cardiac output, low systemic vascular resistance and positive wound surface cultures all represent 'normal' physiology in the burn patient. These clinical features may exist in the absence of sepsis, and thus are not diagnostic per se of infection. Nevertheless, given the fact that infection remains the major source of burn morbidity and death, careful and repeated examination for septic foci is indicated in this population.

Acute infection may be suggested by the presence of any of the above findings, or more commonly by a change in hemodynamic status, body temperature or clinical condition. Increased need for ventilatory support or the new onset of ileus, glycosuria or glucose intolerance also indicates the need for a 'septic workup'. We do not consider acute burn patients to be febrile until core (rectal or pulmonary artery catheter thermistor) temperature exceeds 102.5°F (39.2°C) and do not perform 'fever workups' below this level. An exception is the patient with nearly closed burn wounds who has previously returned to a 'normal' (98.6°F/37°C) body temperature; such patients are considered febrile by the usual criteria.

A 'septic workup' starts with a complete physical examination. In addition to the usual examination of the ears, oropharynx, sinuses, lungs and abdomen, specific attention should be directed at the burn wound (see below) and to any current or previous site of intravenous cannulation. Rectal examination should be performed to rule out prostatitis in males and perirectal disease in both sexes. Cultures of blood, sputum and urine are obtained, along with a chest radiograph. A time frame of initial presentation of infection by site is presented in Figure 85.10 .

Intra-abdominal processes producing sepsis include acalculous cholecystitis, bowel ischemia or necrosis, pancreatitis and, rarely, perforation of a gastric or duodenal ulcer. Pancreatitis or cholecystitis is evaluated by abdominal ultrasound. Triple contrast (nasogastric/rectal and intravenous) computerized tomography scan of the abdomen may be useful. We have found diagnostic peritoneal lavage, as normally performed for acute abdominal trauma, to have a sensitivity of 0.86, a specificity of 1.00 and an overall accuracy of 94% in the diagnosis of intraperitoneal infection in burn patients;[38] this technique remains our primary diagnostic modality for the abdomen.

Children may develop idiopathic fevers in the 103°F (39.5°C) range early in the course of burn care. The fever usually resolves without a source being identified; however, all febrile burned children should be carefully examined with particular attention paid to the middle ears, oropharynx and urinary system. Asymptomatic colonization of the pediatric oropharynx with Lancefield Group A ß-hemolytic streptococci is a theoretic concern because spread of streptococci to the burn wound can result in a rapidly spreading cellulitis

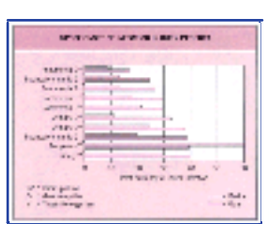

Figure 85-10 Day of onset of infection in burn patients.

or loss of previously placed autograft. Some advocate the prophylactic administration of penicillin to pediatric[39] or 'most' (pediatric and adult) burn patients.[40] Given the low incidence of ß-hemolytic streptococcal infection in burn patients, such routine prophylaxis is probably unjustified.[41] [42] A middle course is to obtain pediatric throat cultures or antigen tests at the time of admission, and to administer penicillin to only those children for whom group A streptococci are isolated.[29]

Electrical injury is a special category of burn trauma where extensive muscle necrosis may occur along the route of current passage. Because the external position of the skin allows rapid heat dissipation, it is common to encounter necrotic muscle covered by entirely normal appearing skin. The investigation of fever or sepsis in the electrical injury patient should include prompt surgical exploration of body regions known or suspected to be involved if another septic source is not immediately apparent.

Positive blood cultures in the absence of wound infection should prompt examination of intravenous cannulation sites and consideration of endocarditis. When a single positive blood culture is inconsistent with the clinical picture, treatment may be deferred pending repeat culture results.[2] Two blood cultures positive for the same organism that are uncontaminated by passage through the burn wound should be treated with systemic antibiotics even in the absence of systemic signs.[2] Gram-negative bacteremia, but not Gram-positive bacteremia, in burn patients is significantly associated with an increased mortality rate.[43] The association of fungemia with an increased mortality rate is equivocal.[43]

Radionucleotide studies such as indium or tagged leukocyte scans, in general, are of little use in the burn patient. Areas of open burn wound or skin donor sites will frequently concentrate isotope, making interpretation difficult.

Evaluation and treatment of the burn wound as a possible source of sepsis

The burn wound should be examined on a daily basis at least and at the first sign of systemic infection. Cellulitis is diagnosed by the presence of expanding erythema at the margins of the burn wound. Erythema surrounding second- or third-degree burn injury that does not increase in size may also represent areas of first-degree burn. Burn wound cellulitis usually responds well to a penicillin. [2]

True burn wound infection is suspected based upon signs such as early eschar separation, conversion of partial thickness to full thickness

Figure 85-11 Invasive pseudomonal burn wound infection, stage 2C.

injury, subeschar hemorrhage, degeneration of granulation tissue, dark red, brown or black discoloration of eschar (Fig. 85.11), violaceous discoloration of the unburned skin at the wound margins or ecthyma gangrenosum (green discoloration of subcutaneous fat due to pseudomonas infection).[2] [7] Diagnosis of invasive wound infection is confirmed by wound biopsy and histologic examination. When invasive bacterial infection (stage 2A, 2B or 2C) is diagnosed, the wound should be continuously covered with mafenide acetate cream and all other agents discontinued.[7] Systemic antibiotics are then instituted based upon the predominant organism in the biopsy specimen or on surveillance cultures; Gram-negative organisms are most frequently implicated.[2] The wound should be surgically excised as soon as possible.[7] Subeschar injection of systemic antibiotics (clysis) is a useful adjunct and should be performed upon diagnosis and repeated both 6 hours prior to and immediately prior to surgical excision of the burn eschar.[2] [44] One half of the daily dose of a semisynthetic penicillin (piperacillin or carbenicillin) may be mixed in sufficient diluent (1 liter of normal saline) for injection through a 20-gauge spinal needle.[7] [44] Clysis may be repeated twice a day for patients too unstable to tolerate surgical excision.[2] [44]

Fungal infection

Aspergillus, Candida, Mucor and *Rhizopus* spp. are the most common fungal infections of the burn wound. Wound colonization involving *Candida* spp. is commonly treated with topical nystatin or clotrimazole although few studies have addressed the effectiveness of these agents in burn care.[2] [22] *Aspergillus* spp. characteristically produce superficial colonization or infection amenable to local surgical excision.[2] *Mucor* spp. may produce a rapidly spreading fascial infection with early microvascular involvement. [2] Radical surgical debridement or amputation may be required to control this type of infection.[2]

We reserve the use of systemic antifungal therapy for cases where disseminated or systemic fungal infection is known or suspected, or when wound biopsies are positive at a 2C level.[22]

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Chapter 86 - Infectious Complications Following Surgery and Trauma

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Whether inflicted by accident, with malevolence, or as a consequence of treatment, tissue injury substantially increases the risk of infection for several reasons. The host response to injury is immunosuppressive — transgression of natural epithelial barriers (e.g. skin, respiratory mucosa, gut mucosa) creates a portal of entry for invasion by potential pathogens; tissue injury and ischemia provide optimal conditions for bacterial proliferation, especially if cofactors such as blood or a foreign body are present; well-intentioned therapies can have adverse consequences; and lapses of infection control often occur under emergency conditions. These risks can be apportioned as patient-derived risks, environment-derived risks or treatment-derived risks. Owing to the multiplicities and complexities of trauma and critical surgical illness, it can be appreciated that these risks are additive and that the danger for patients is high.

Some infective complications are unique to surgery (e.g. surgical site infections (SSIs), infection of traumatic wounds and burns, postoperative intra-abdominal infections), whereas others potentially affect all patients, although especially so in the surgical setting (e.g. nosocomial pneumonia (NP)). The approaches to diagnosis and management reflect the general principles described throughout this book; what is different in the surgical environment is, first, that it can be particularly difficult in surgical patients to distinguish sterile inflammation with or without bacterial colonization from invasive infection, and, second, that prevention of infection is of particular importance, especially after trauma and before high-risk elective surgery.

The incidence of infection following surgery or trauma is context-sensitive. The incidence of SSI following minor, clean, elective surgery is approximately 2%, and that of other infectious complications is practically nil.[1] In contrast, the overall incidence of infection following major trauma is about 25%.[1] [2] Infection is common, and the leading cause of late deaths after injury (i.e. among patients who die neither from exsanguination nor massive central nervous system injury) is the development of multiple organ dysfunction syndrome (MODS).[3] [4] [5] It is generally estimated that the development of nosocomial infection, especially with a multidrug-resistant pathogen, increases the risk of death 3- to 5-fold,[5] but the attribution of mortality to infection is controversial and not universally accepted.[6] In short, do critically ill or injured surgical patients die *of* infection or *with* it? Is nosocomial infection possibly just a marker of host immunosuppression and organ dysfunction?

PATIENT-DERIVED RISK FACTORS

Risk factors that result from intrinsic attributes of the host are listed in Table 86.1 . Among these factors are age, chronic medical conditions (especially diabetes mellitus) and hypocholesterolemia. The contribution of age to infection risk is increasingly being recognized.[7] [8] In a multicenter, preoperative risk assessment for the development of postoperative pneumonia, age was found to be the most powerful independent predictor of risk.[7] Substantial evidence indicates that perioperative hyperglycemia increases the risk of infection,[9] [10] [11] even if mild or transient. Failure to maintain euglycemia during open heart surgery triples the risk of sternal wound infection, [9] whereas a serum glucose concentration over 220mg/dl (12.2mmol/l) at any point on the first postoperative day quadruples the risk of SSI after major non-cardiac surgery.[10] A randomized study of critically ill surgical patients demonstrated that tight control of blood glucose (<120mg/dl (6.6mmol/l) vs 180–220mg/dl (10–12.2mmol/l)) reduced mortality by nearly 50%.[11] Hypocholesterolemia is rapid and dramatic after surgical stress,[12] and it has been associated with an increased risk of SSI and pneumonia[13] and independently with an increased risk of death in critically ill patients with systemic inflammatory response syndrome (SIRS).[14] The possible mechanisms underlying this risk are several, including dysfunction of lipid-laden macrophages or decreased binding of endotoxin by apolipoproteins.

The surgical stress response

Surgical stress is associated with a stereotypical response.[15] The stress hormone response to surgical stress or tissue injury augments cardiovascular function through the sympathetic nervous system, enhances glycogenolysis, mobilizes peripheral lean tissue and fat for use as fuel, enhances coagulation to limit hemorrhage, and stimulates a proinflammatory cytokine response to begin the process of tissue repair. Cellular immunity is depressed in large part by the actions of cortisol. Three major events characterize the initial inflammatory response:

- activation of coagulation;
- increased microvascular endothelial permeability with tissue edema formation; and
- chemotaxis, margination and transvascular migration of neutrophils.

The first step in the process is recognition of a foreign antigen, either by elements of the adaptive immune system (e.g. T cells, preformed antibodies) or by innate immune elements (e.g. phagocytic cells, alternative pathway of complement, coagulation). The development of inflammation is then amplified in a complex process regulated by cytokines, plasma enzymes (e.g. complement, coagulation, kinin and fibrinolytic pathways), lipid mediators (e.g. prostaglandins, leukotrienes) and mediators derived from mast cells and platelets. Fast-acting mediators, such as vasoactive amines and bradykinin, modulate the immediate response. Several hours later, mediators such as leukotrienes, chemokines and platelet-activating factor are involved in the accumulation and activation of phagocytes. Once neutrophils have arrived at a site of inflammation, they release mediators that, in turn, control the later accumulation and activation of monocytes and macrophages.

The acute-phase response is a dynamic homeostatic process that involves all of the major systems of the body, notably the immune, cardiovascular and central nervous systems.[16] Normally, the acute-phase response lasts only a few days. Two physiologic responses are closely associated with acute inflammation. The first involves the alteration of the temperature set point in the hypothalamus and the generation of fever. The second involves alterations in metabolism and gene expression in the liver. Interleukin (IL)-1, IL-6 and tumor necrosis factor-a regulate the febrile response through the induction of

TABLE 86-1 -- Host factors that contribute to the development of nosocomial infection.

HOST FACTORS THAT CONTRIBUTE TO THE DEVELOPMENT OF NOSOCOMIAL INFECTION
Ascites
Chronic inflammation
Corticosteroid therapy (controversial)
Obesity
Diabetes mellitus
Extremes of age
Hypocholesterolemia
Hypoxemia
Peripheral vascular disease (especially for lower extremity surgery)
Postoperative anemia
Prior site irradiation
Recent operation
Remote infection
Skin carriage of staphylococci
Undernutrition

prostaglandin E2, possibly as a protective mechanism against bacterial infection (see Chapter 80). At the same time, IL-1 and IL-6 act on the hypothalamic-pituitary-adrenal axis to generate corticotropin and stimulate cortisol production. This provides a negative feedback loop, because corticosteroids inhibit cytokine gene expression.

The second important aspect of the acute-phase response is altered hepatic protein synthesis. Normally, the liver synthesizes numerous plasma proteins at steady state concentrations. In the acute phase of tissue injury or inflammation, production of several proteins is upregulated, whereas synthesis of others is suppressed. Acute-phase reactants (APRs) thus may be 'positive' or 'negative'. Most positive APRs are induced several-fold over normal concentrations. This group includes, among others, fibrinogen, clotting elements, protease inhibitors, complement components, serum amyloid A and C-reactive protein. Negative APRs (e.g. albumin) are decreased in plasma concentration during the acute phase response to allow an increase in the capacity of the liver to synthesize the induced APRs. Although most APRs are synthesized by hepatocytes, some are produced by other cell types such as myeloid cells, endothelial cells, fibroblasts or adipocytes.

Acute-phase reactants contribute to host defense in several ways, including direct neutralization of inflammatory mediators, thereby minimizing tissue damage and facilitating tissue repair. For example, increased synthesis of complement proteins mobilizes neutrophils and macrophages. Fibrinogen plays an essential role in hemostasis and the promotion of wound healing. Protease inhibitors (e.g. a_1 -antitrypsin) neutralize the lysosomal proteases released following the infiltration of activated neutrophils and macrophages, thus mitigating the activity of the proinflammatory enzyme cascades. Increased plasma concentrations of metalloproteases help to prevent iron loss during infection and injury, minimize the amount of heme iron available for bacterial metabolism, and scavenge reactive oxygen species. There is evidence that elderly patients have a blunted acute-phase response,[16] and that minimally invasive surgery attenuates the response (see Chapter 2).[17]

PATHOGENESIS OF INFLAMMATION AND ORGAN DYSFUNCTION

The acute response to injury is characterized by a syndrome of:

- fever (=100.4°F (38°C)) or hypothermia (=96.8°F (36°C));
- leukocytosis (>12,000/µl), leukopenia (<4000/µl) and excess bands (>10% immature neutrophils);

¦ increased heart rate (<90 beats/minute); and
¦ increased respiratory rate (>24/minute) or, if mechanically ventilated, $P_a CO_2$ <32mmHg.

Figure 86-1 Conditions associated with systemic inflammatory response syndrome. The complex, overlapping relationship between infection and inflammation.

Systemic inflammatory response syndrome[18] is the name given to this constellation if at least two of the parameters are present. Among surgical patients, there are many causes of tissue injury-induced inflammation (e.g. trauma, burns, pancreatitis) (Fig. 86.1) that have no relation to infection on initial presentation. Recovery from general anesthesia can produce symptoms indistinguishable from true SIRS, but the effect is transitory. Although SIRS has been derided conceptually as being too non-specific, in the surgical setting the presence and especially the persistence of SIRS is important prognostically. The presence and magnitude of SIRS in the trauma bay has been associated with increased risk of nosocomial infection.[19] Moreover, among critically ill surgical patients, persistent SIRS was associated with prolonged stay in intensive care units (ICU), worsening SIRS was associated with higher mortality, and a greater magnitude of SIRS was associated with both a higher incidence and greater degree of MODS (see also Chapter 56).[20]

The proinflammatory response can be incited by tissue injury or infection. Inflammation is modulated by a complex inter-relationship of activation of the coagulation, kinin, complement and other systems, generating numerous already-known and probably yet-to-be-discovered mediators, including prostaglandins, leukotrienes, reactive oxygen and nitrogen species, lipid peroxides, coagulation factors, adhesion molecules and cytokines. Consequently, phagocytic cells, platelets and endothelial cells are activated, in part to contain the inflammatory response (e.g. to localize and eradicate a nascent infection). Containment is also the likely role of the counter-regulatory anti-inflammatory response, downregulating the proinflammatory response so that the patient will not be immolated on the pyre of inflammation. The anti-inflammatory response is mediated by cortisol, IL-4, IL-10, myeloid colony stimulating factors, transforming growth factors and other mediators. The counter-regulatory response results in the activation of genetic programs ('suicide' genes) that cause phagocytic cells to undergo apoptosis (i.e. programmed cell death). Apoptosis causes inflammation to subside, whereas several adverse clinical events such as hypoxemia[21] or blood transfusions[22]

may delay apoptosis, promote inflammation and lead to adverse outcomes. None of the putative anti-inflammatory mediators are measured with ease or regularity by the modern clinical chemistry laboratory. Therefore, the anti-inflammatory response is difficult to quantify at the bedside, and the clinically relevant relationships between proinflammatory and anti-inflammatory responses are hypothetical.[23]

Do nosocomial infection and organ dysfunction result from exuberant inflammation unchecked by an inadequate or even normal anti-inflammatory response? Does the anti-inflammatory response overwhelm the proinflammatory response (which is a necessary component, for example, of normal tissue repair), or does it supplant inflammation as it subsides as part of an inflammatory ebb and flow (Fig. 86.2). There is substantial experimental evidence that an accentuated neutrophil proinflammatory response occurs by neutrophils that are first 'primed' by an inflammatory stimulus, and then respond in an exaggerated, deleterious manner in response to a second insult.[24] Examples of a primary insult include a period of hypoxia[21] or blood transfusion,[22] or the initial injury (e.g. a femur fracture), whereas an example of a second insult would be a postoperative nosocomial pneumonia. The exaggerated proinflammatory response results in release of mediators that, among other effects, induce adherence of neutrophils and platelets to microvascular endothelium; entrap microthrombi of fibrin, platelets, neutrophils and cellular debris; and cause tissue hypoperfusion, ischemia and edema as a result of loss of microvascular integrity.[25] Resuscitation and natural fibrinolysis may restore microvascular flow before tissue necrosis occurs, but re-introduction of molecular oxygen to the ischemic bed may result in the generation of reactive oxygen species. These highly toxic mediators have innumerable deleterious effects, including the ability to oxidize lipid components of cell membranes.

The splanchnic bed is especially susceptible to ischemia, and ischemia and reperfusion of the gut may be central to the pathogenesis of inflammation and organ dysfunction.[26] The intestine is an immunologically active organ that serves to protect the host from ingested bacteria as well as the potentially lethal bacterial inoculum contained in feces. Ischemic reperfused intestine becomes dysfunctional, with ileus and the ensuing putative release of mediators, bacterial toxins (e.g. endotoxin) or live bacteria ('bacterial translocation'). Although the existence of bacterial translocation is unproved in humans, it is plausible that the gut is the source of a proinflammatory stimulus and that under catabolic

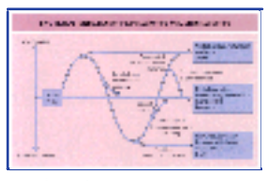

Figure 86-2 Hypothetical interactions of proinflammatory and anti-inflammatory responses. Pathogenesis of immunosuppression, nosocomial infection, organ dysfunction and outcome.

conditions the dysfunctional gut no longer provides trophic support of hepatic function, if indeed gut-derived mediators and toxins are the cause of the disrupted hepatocyte-Kupffer cell interactions that can no longer maintain hepatic reticuloendothelial host defense function.

Interleukin-1 and IL-6 are important regulators of the hepatic acute-phase response, bile secretion and (with other cytokines) the intercellular signaling that maintains normal hepatocyte-Kupffer cell interactions. When Kupffer cells (fixed tissue macrophages that are the critical component of hepatic host defenses) become dysfunctional, clearance of circulating particulates is decreased and other microcirculatory beds, notably the pulmonary microcirculation, become susceptible to microembolization and tissue injury.

The clinical manifestations of MODS are protean (see Fig. 86.2) and the consequences are grave. No longer conceptualized as an 'all-or-nothing' phenomenon in which organ 'failure' is recognized after a threshold of dysfunction is reached, MODS can affect several organs to varying degrees, and the effects are cumulative. Several methods exist for quantifying organ dysfunction; one of these methods, that of Marshall *et al.*,[27] correlates in magnitude with severity of illness on admission to the ICU, length of ICU stay and mortality.[4] As many as 50% of critically ill surgical patients and nearly 100% of critically ill nonsurvivors develop some degree of MODS. The development of MODS prolongs ICU stay even among patients who develop only minimal organ dysfunction, and it increases mortality exponentially as it manifests itself fully.

ENVIRONMENT-DERIVED RISK FACTORS

The importance of colonization with bacteria or active remote infection as a risk factor for subsequent infection cannot be over-emphasized. Patients who are chronic carriers of staphylococci and streptococci are at increased risk of SSIs after elective surgery,[28] as are patients with active soft tissue infections at tissue sites remote from the surgical wound. In hospitalized patients, colonization of the skin, lower airway and gut with pathogenic bacteria and fungi is common and occurs rapidly even among immunocompetent hosts. Aided and abetted by many standard health care practices (or lack thereof, in the case of breaks in infection control), these colonists wait for vulnerable hosts or their care providers to facilitate invasion and the establishment of infection. The risk of SSI[29] or failure of management of intra-abdominal infection[30] is increased by prolonged

pre-event hospitalization. Nosocomial pneumonia is promoted by pre-existing nosocomial sinusitis[31] or colonization of the lower airway by the same pathogen.[32] The same can be said for invasive fungal infections of surgical patients, which are often preceded by multiple-site colonization.[33] Numerous studies indicate that invasive infection with vancomycin-resistant enterococci is associated with prolonged hospitalization, repetitive ICU admission and prior exposure to antibiotics such as vancomycin and third-generation cephalosporins among debilitated, immunosuppressed patients.[34] Although bacterial colonization of hospitalized patients can occur regardless of exposure to antibiotics, there is no doubt that antibiotic selection pressure leads to nosocomial infections with multidrug-resistant bacteria, which are a threat to vulnerable patients.

TREATMENT-DERIVED RISK FACTORS

Modern therapy is often invasive and disruptive of homeostasis even as treatment tries to restore it (Table 86.2). Clinicians must be cognizant of and vigilant for the adverse consequences of therapies that may impair host defenses or disrupt commensal flora, increasing the risk of nosocomial infection. Among several factors of importance are the control of the serum glucose concentration, the avoidance of blood transfusion, maintenance of normoxia and normothermia, minimized duration of mechanical ventilation, and rational antibiotic prophylaxis and therapy. Also crucial are maintenance of the operating room environment and adherence to the principles of infection control and universal precautions. There are published guidelines for the prevention of SSI[35] (see also Chapter 84 and Chapter 87) and vascular catheter-related infection (see also Chapter 57);[36] the US Centers for Disease Control and Prevention produces updates and new guidelines.

Blood transfusion

Evidence is substantial and increasing that allogenic transfusion of red blood cell concentrates is immunosuppressive and increases the risk of nosocomial infection. Transfusion requirements are understandable in the trauma and surgery environment, and alternatives are few. Nonetheless, optional transfusions can be avoided safely,[37] and they should be avoided, especially in hemodynamically stable patients who are not bleeding.

Protection of the hypoxic wound

A fresh surgical incision is a hypoxic, ischemic environment. Blood vessels are divided at the wound margins, and use of electrocautery leaves a surrounding margin of coagulation necrosis. Maneuvers to support the oxygenation and perfusion of the incision have demonstrated benefit. Hypothermia (body temperature under 94.1°F (34.5°C)) leads to MODS and increased mortality after elective abdominal aortic aneurysm repair[38] and to an increased incidence of

TABLE 86-2 -- Examples of clinical interventions and potential deleterious effects upon host immunity.

EXAMPLES OF CLINICAL INTERVENTIONS AND POTENTIAL DELETERIOUS EFFECTS UPON HOST IMMUNITY	
Incisions	Drain pus but create portal of entry
Catheters	Help in the monitoring of the patient but create portal of entry and an artificial surface for colonization by bacteria
Transfusions	Improve oxygen transport but promote immunosuppression and increased risk of nosocomial infection and organ dysfunction
Nutrition	Encourages wound healing but promotes immunosuppression (parenteral nutrition) and carries a risk of aspiration (enteral feedings)
Antibiotics	Kill bacteria but disrupt host commensal flora

SSI after elective colon surgery.[39] Intraoperative hypothermia should be avoided at all costs. Avoidance can be challenging in trauma surgery, where operations are often prolonged, a large skin surface area may require exposure and open body cavities induce prodigious heat loss (especially if multiple, as in a thoracoabdominal incision). Avoidance should be absolute during elective surgery with the use of fluid warmers, forced-air blankets and the like.

ANTIBIOTIC PROPHYLAXIS AND THE RISK OF SURGICAL SITE INFECTION

The administration of antibiotics before surgery is commonplace and of proven benefit for the minimization of postoperative SSI. However, it is only the surgical incision that is afforded protection, and antibiotics are not a panacea. If not administered properly, antibiotic prophylaxis will not be effective and may be harmful (see also Chapter 190).

Selection of patients for antibiotic prophylaxis

Most commonly, the risk of SSI, as categorized by the National Nosocomial Infections Surveillance program, is defined by three risk factors:[1]

- a contaminated or dirty wound,
- poor overall medical condition of the patient, and
- a prolonged operative time (longer than the 75th percentile for operations of the type).

Surgical incisions are classified as clean (class I), clean-contaminated (class II), contaminated (class III) or dirty (class IV). A poor overall medical condition of the patient is categorized by an American Society of Anesthesiologists score of more than 2 (Table 86.3). Prolonged surgery has implications for the degree of tissue injury, antibiotic pharmacokinetics and intraoperative repeat dosing, and it provides a prolonged opportunity for wound inoculation. An increased risk of SSI occurs with an increasing degree of wound contamination (e.g. clean wounds have less risk than contaminated wounds), regardless of other risk factors (Table 86.4), [40] and as the number of risk factors increases for a given type of operation (Table 86.5).

TABLE 86-3 -- ASA physical status score.

ASA PHYSICAL STATUS SCORE		
Score	**Description**	**Examples**
ASA 1	A normal healthy patient	
ASA 2	A patient with a mild to moderate systemic disturbance that results in no functional limitations	Hypertension, diabetes mellitus, chronic bronchitis, morbid obesity, extremes of age
ASA 3	A patient with a severe systemic disturbance that results in functional limitations	Poorly controlled hypertension, diabetes mellitus with vascular complications, angina pectoris, prior myocardial infarction, pulmonary disease that limits activity
ASA 4	A patient with a severe systemic disturbance that is life-threatening with or without the planned procedure	Congestive heart failure, unstable angina pectoris, advanced pulmonary, renal or hepatic dysfunction
ASA 5	A moribund patient not expected to survive with or without the operative procedure	Ruptured abdominal aortic aneurysm, pulmonary embolism, head injury with increased intracranial pressure
E	Any patient in whom the procedure is an emergency	

TABLE 86-4 -- Incidence of surgical site infection as a function of wound classification.

INCIDENCE OF SURGICAL SITE INFECTION AS A FUNCTION OF WOUND CLASSIFICATION					
	NNIS risk index				
Traditional class	0	1	2	3	All
Clean	1.0%	2.3%	5.4%	NA	2.1%
Clean-contaminated	2.1%	4.0%	9.5%	NA	3.3%
Contaminated	NA	3.4%	6.6%	13.2%	6.4%
Dirty	NA	3.1%	8.1%	12.8%	7.1%
All	1.5%	2.9%	6.8%	13.0%	2.8%
NNIS, National Nosocomial Infections Surveillance Program; NA, not applicable (by definition, because the traditional wound classification is one of the risk factors, patients with clean wounds cannot have three risk factors, nor can those with dirty wounds have no risk factors)					
From Martone and Nichols[40]					

TABLE 86-5 -- SSI percentage rates for selected procedures.

SSI PERCENTAGE RATES FOR SELECTED PROCEDURES					
Procedure	**Time cutpoint (hours)**	**Number of risk factors**			
		0	**1**	**2**	**3**
Coronary artery bypass graft (chest or leg)*	5	1.20	3.57	5.68	9.63
Laparotomy	2	1.71	3.29	5.16	7.77
Open reduction and internal fixation of fractures†	2	0.73	1.35	2.51	4.85
From National Nosocomial Infections Surveillance System[1]					

* Pooled incidence of surgical site infection for both incisions
† Overall incidence

Antibiotic prophylaxis is indicated clearly for most clean-contaminated and contaminated (or potentially contaminated) operations. Dirty operations are those in which surgery and antibiotics represent treatment for an infection, not prophylaxis against it. An example of a potentially contaminated operation is lysis of adhesions for mechanical small bowel obstruction — intestinal ischemia cannot be predicted accurately before surgery, and the possibility exists of an enterotomy during adhesiolysis, which increases the risk of SSI 2-fold. An example of a clean-contaminated operation in

TABLE 86-6 -- Risk of the development of surgical site infection relative to the timing of antibiotic prophylaxis.

RISK OF THE DEVELOPMENT OF SSI RELATIVE TO THE TIMING OF ANTIBIOTIC PROPHYLAXIS				
Timing	**No. patients**	**No. (%) infections**	**RR (95% CI)**	**OR (95% CI)**
Early	369	14 (3.8)*	6.7* (2.9–14.7)	4.3* (1.8–10.4)
Preoperative	1708	10 (0.59)	1.0	
Perioperative	282	4 (1.4)	2.4 (0.9–7.9)	2.1 (0.6–7.4)
Postoperative	488	16 (3.3)*	5.8* (2.6–12.3)	5.8* (2.4–13.8)
All	2847	44 (1.5)		
Early, administration more than 2 hours preoperatively; Preoperative, administration during the recommended interval (=2 hours before skin incision); Perioperative, administration within 2 hours after the skin incision; Postoperative, administration more than 2 hours after the skin incision				
From Classen et al.[45]				

* $p<0.0001$ compared with preoperative group

which antibiotic prophylaxis is not always indicated is elective cholecystectomy. Antibiotic prophylaxis is indicated only for high-risk biliary surgery; patients at high risk include those over the age of 70 years, diabetic patients and patients whose biliary tract has recently been instrumented (e.g. during biliary stenting). [41] The vast majority of patients who undergo laparoscopic cholecystectomy do not require antibiotic prophylaxis.[42] Laparoscopic clean-contaminated operations are generally at decreased risk of infection. [1] The potential reasons are several, including a diminished surgical stress response, decreased tissue injury and smaller incisions.

Antibiotic prophylaxis of clean surgery is controversial. When bone is incised (e.g. in a craniotomy or sternotomy) or a prosthesis is inserted, antibiotic prophylaxis is generally indicated. The controversy exists in the case of clean surgery of soft tissues (e.g. breast, hernia). A randomized prospective trial has shown some benefit of prophylaxis,[43] but the results are confounded by higher than expected infection rates in the control group.

Choice of antibiotic

Most SSIs are caused by Gram-positive cocci. Most common is *Staphyloccoccus aureus*, followed by *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Escherichia coli*, the latter two of which are common pathogens after clean-contaminated surgery. The antibiotic chosen should be directed against staphylococci for clean cases and high-risk clean-contaminated elective surgery of the biliary and upper gastrointestinal tracts. A first-generation cephalosporin is the preferred agent for most patients, with clindamycin preferred for patients with a history of anaphylaxis to penicillin. Although methicillin-resistant *S. aureus* (MRSA) has been isolated in the community from never-hospitalized patients, vancomycin prophylaxis is appropriate only in institutions where the incidence of MRSA is high.

Elective colon surgery is a special circumstance, and one in which practices are in evolution. Mechanical bowel preparation to reduce bulk feces made colon surgery safe for the first time. Antibiotic bowel preparation, standardized in the 1970s by the oral administration of nonabsorbable neomycin and erythromycin base, reduced the risk of SSI further to its present rate of approximately 4–8%, depending on the number of risk factors. Although outpatient mechanical preparation is now the norm, three doses of oral antibiotics are still given to most patients at approximately 18, 17 and 10 hours preoperatively so that fecal bacterial counts are minimal at the time of surgery. A dose of parenteral second-generation cephalosporin (or a quinolone or monobactam plus metronidazole for the penicillin-allergic patient) is given before skin incision, and the benefit appears to be additive.[44] Moreover, there is less reticence to operate on the less-than-perfectly-prepared colon, extrapolating from good results from primary repair of penetrating colon injuries.

Timing and duration of parenteral antibiotics

When should parenteral antibiotics be given for optimum effect? It is firmly established that the optimal time to give cephalosporin prophylaxis is within 2 hours before the time the incision is made (Table 86.6).[45] Antibiotics given sooner (except possibly for longer half-life agents such as quinolones and metronidazole) are not effective, or are agents that are given after the incision is closed. Antibiotics with short half-lives should be re-dosed during surgery if the operation is prolonged or bloody,[46] and there is still some benefit if the initial antibiotic dose is given intraoperatively.

Considering the exigencies of surgical hemostasis, and that the fresh surgical incision is ischemic, the postoperative administration of antibiotics is questionable owing to hypoperfusion that results from divided vessels. Single-dose prophylaxis should be standard, with intraoperative dosing as noted above, but excessively prolonged usage is pervasive. A 24-hour regimen is often standard for orthopedic and cardiac or vascular surgery, owing in part to a lack of randomized, prospective studies. Other than in solid-organ transplant surgery, in which therapeutic immunosuppression has made 48-hour regimens standard, there is no indication for prolonged antibiotic prophylaxis. In particular, antibiotics should not be administered to cover indwelling drains or catheters or to mitigate operative technical shortcomings.

Topical antiseptics and antibiotics may also help prevent SSI. A preoperative shower with an antiseptic soap (e.g. povidone-iodine) is a mainstay of perioperative preparation. Topical mupirocin ointment applied to the nares of patients who are chronic carriers of *S. aureus* reduces the increased incidence of SSI that is characteristic of chronic staphylococcal carriage. [28] [47]

Prolongation of antibiotic prophylaxis beyond 24 hours not only provides no benefit, but can also be associated with a number of complications. *Clostridium difficile*-associated disease (CDAD) has been associated with disruption of the normal balance of gut flora and overgrowth of the enterotoxin-producing *C. difficile*. The incidence and possibly the severity of nosocomial CDAD is increasing.[48] The spectrum of disease is broad, ranging from asymptomatic to life-threatening pancolitis with infarction or perforation. Although virtually every antibiotic has been implicated in the pathogenesis of CDAD, even after administration of a single dose, prolonged antibiotic prophylaxis clearly increases the risk. Prolonged prophylaxis also increases the risk of later nosocomial infections unrelated to the surgical site, and the emergence of multidrug-resistant pathogens. Both pneumonia and catheter-related infections have been associated with prolonged antibiotic prophylaxis,[49] [50] as has

the emergence of SSI caused by MRSA. [50]

ANTIBIOTIC PROPHYLAXIS OF TRAUMA-RELATED INFECTIONS

Traumatic injury is profoundly immunosuppressive, and injured patients are at very high risk of infection. The overall incidence of infection after trauma is approximately 25%,[51] with infection of a wound (or an incision made as treatment) and nosocomial infection equally likely. Certain patterns of injury are independently associated with infectious morbidity in particular, including hemorrhagic shock, the need for blood transfusion, heavy wound contamination, central nervous system injury, colon injury, combined thoracoabdominal injuries, four or more organs injured, and increasing injury severity.[3] Co-morbid factors of importance include hyperglycemia, hypothermia and hypoxemia.

Certain characteristics of trauma make the situation more complex. Antibiotics are administered after injury, but there is a period when injured tissues may be vulnerable before antibiotics are administered. Trauma patients may be hypotensive and vasoconstricted owing to shock, and tissue penetration may be decreased. Ongoing blood loss may result in ongoing antibiotic loss, especially if the agent is highly protein-bound and tissue redistribution is slow, or if the antibiotic is administered before major hemorrhage is controlled. Postinjury fluid shifts and hypoalbuminemia can cause major fluctuations in volume of distribution that can be difficult to estimate. As a result, it has been postulated that higher doses of antibiotics should be administered for the prophylaxis of post-traumatic infection. Although older work with aminoglycosides suggest that this theory may be correct,[52] aminoglycosides are now seldom used except for the prophylaxis of high-grade open fractures, and the few studies performed more recently suggest otherwise.

Despite the high risk, the basic principles of antibiotic prophylaxis still apply — use a safe, narrow-spectrum agent for a defined brief period (no more than 24 hours), preferably one that has a limited role in the therapy of infection (e.g. a first- or second-generation cephalosporin).[3] Multiple studies indicate unequivocally that 24 hours of prophylaxis with a second-generation cephalosporin is all that is necessary following penetrating abdominal trauma, even in the presence of a colon injury and shock.[51] Other injuries in which appropriate antibiotic prophylaxis is beneficial include high-grade open fractures,[53] animal bites[54] and, possibly, chest injuries when an emergency tube thoracostomy is required.[55] Following a human bite, the risk of infection is so high that 7–10 days of therapy with an agent effective against oral anaerobic flora is commonplace.[56] Injuries in which there is demonstrated to be no benefit of antibiotic prophylaxis include clean lacerations (even if primary repair is delayed)[57] and skull fracture with or without leakage of cerebrospinal fluid.[58]

Tetanus prophylaxis is administered almost routinely despite widespread shortages of tetanus toxoid and despite the fact that most wounds are not tetanus-prone. Certainly, tetanus prophylaxis should be administered for true high-risk wounds, such as soft tissue wounds with fecal contamination (e.g. injury caused by farm implements), but if the injury truly poses a high risk then administration of tetanus toxoid will be insufficient and tetanus immune globulin should be co-administered (see Chapter 232).

Prophylaxis of postsplenectomy sepsis is another special circumstance, albeit an increasingly rare one owing to the development of successful protocols for the nonoperative management of blunt splenic injuries. Postsplenectomy sepsis can be fulminating and lethal, and is most commonly caused by encapsulated organisms such as *Streptococcus pneumoniae* (see Chapter 109).[59] The incidence is sufficiently low in adults (<1%) for long-term antibiotic prophylaxis not to be indicated. Children have a higher incidence, and daily oral penicillin prophylaxis until the age of 18 years is a standard recommendation. Polyvalent pneumococcal vaccine is administered both to adults and to children, usually postoperatively, although the asplenic host has a blunted immune response to vaccination; moreover, the optimal timing of administration is unknown, and whether booster immunization is ever required is also unknown. Although there is no evidence that vaccination of these patients against *Haemophilus influenzae* or *Neisseria meningiditis* is beneficial, many clinicians choose to vaccinate for these pathogens because of the safety and probable effectiveness of these vaccines. There is no evidence that patients with splenic injury who are managed nonoperatively need to be vaccinated (see Chapter 109).

THE POSTOPERATIVE PERIOD

Several circumstances conspire to make fever the most common postoperative complication. A proinflammatory response follows tissue injury. Pulmonary defense mechanisms are impaired during anesthesia and recovery, and small-volume pulmonary aspiration of

gastric contents unquestionably occurs. Wound hematoma is one of many known noninfections causes of fever,[60] and other potential postoperative complications, including venous thromboembolic disease, pancreatitis, myocardial infarction, visceral ischemia and atelectasis, can also cause fever in the absence of infection. In this milieu it is challenging, but crucial, to distinguish inflammation from infection so as to treat patients appropriately. Confounding the evaluation of patients further is that hospitalized patients become colonized rapidly with potential pathogens.[61] Colonization is known to precede invasive infection. However, the high incidence of colonization, especially of the upper aerodigestive tract, makes it imposible to equate the mere isolation of a pathogen with the diagnosis of nosocomial infection, especially in critically ill patients.

Postoperative prophylaxis of infection

Surgical patients are at especially high risk of nosocomial infection. Judicious preoperative antibiotic prophylaxis decreases the risk of infection, but this prophylaxis in the absence of other techniques will not accomplish much. Proper infection control practice cannot be emphasized enough. Handwashing is the most effective infection control practice of all, yet studies continue to demonstrate poor compliance.[62] The use of fast-drying alcohol gels for hand disinfection improves compliance, can be used in the operating room [63] and should be used at the bedside.[64]

Surgery is invasive and surgical patients are often immunosuppressed owing to illness or injury; the surgical stress response augments the immunosuppression. Many standard interventions impair host defenses (see Table 86.2). Surgical care must minimize the possibility of iatrogenic infection, while supporting the patient until host defenses can recover.

Catheters and drains should be removed as soon as possible. Each time a natural epithelial barrier to infection (e.g. skin, respiratory tract mucosa, gut mucosa) is breached, a portal is created for potential invasion of the host by pathogens. Prolongation of the time that drains and catheters are in place increases the risk of infection and is a factor that is controllable, to a degree, by the surgeon. Subcutaneous drains increase the risk of SSI.[65] Prolonged central venous catheterization increases the risk of bacteremia.[36] Prolonged intubation and mechanical ventilation increase the risk of pneumonia. [66] If prolonged catheterization is essential, silver-impregnated urinary drainage catheters,[67] antibiotic-coated central venous catheters[68] and aspiration of subglottic secretions via an endotracheal tube with an extra lumen [19] can all decrease the risk of nosocomial infection (see Chapter 83).

Although cumbersome and therefore not popular, topical antiseptics and antibiotics appear to decrease the risk of nosocomial infection in seriously ill, high-risk patients. Data demonstrate that topical 0.12% chlorhexidine mouthwash (a bactericidal, viricidal and fungicidal antiseptic), applied to the oropharynx and the exposed surface of the endotracheal tube, decreases the incidence of postoperative pneumonia. Selective digestive decontamination, described in various forms nearly two decades ago, utilizes an oral paste of multiple antibiotics, enteral administration of the same antimicrobial agents by gavage, and sometimes a short course of intravenous antibiotics. Infection rates are reduced unequivocally,[69] but critics point out the possibility of the development of resistance and also that the mortality rate is sometimes not reduced. At this point, it cannot be recommended universally, but it may be appropriate in high-risk situations.[70] [71]

Prophylaxis of fungal infections is controversial. Although surgical patients are immunosuppressed and are frequently exposed to antibiotics, invasive fungal infections are unusual. Although *Candida* is a frequent isolate from the peritoneal fluid of patients with peritonitis, specific antifungal therapy is generally not indicated unless *Candida* is isolated in pure culture from blood, from peritoneal fluid or from an abscess. Fungemia can be associated with indwelling central venous catheters (see below), but on the rare occasions where fungemia complicates surgery (solid-organ transplant patients excepted), the patient is usually debilitated and in the throes of a protracted serious illness and has already been exposed to multiple courses of antibiotics. Most surgical patients who are potential candidates for antifungal prophylaxis, apart from commonplace administration as part of organ transplant protocols, are those who become colonized with yeast at two or more sites (e.g. skin and urine). Colonization does precede invasive infection when it does occur in the absence of a gastrointestinal tract perforation, and therefore some have advocated prophylaxis with fluconazole as a means of decreasing the risk of invasive infection.[20] However, disciplined antibiotic prescribing may also be effective.[72] Fluconazole has numerous drug interactions (e.g. cyclosporine, macrolides, quinolones) and widespread use of fluconazole has been associated with the emergence of fluconazole-resistant strains of *Candida*.[73]

Blood transfusion

In surgery and trauma, blood transfusions are given commonly and may be life-saving; alternatives to transfusions in the acute setting are few, but for hemodynamically stable postoperative patients hemoglobin concentrations >7g/dl are well tolerated.[37] Erythropoietin administration may decrease transfusion

requirements of the 'chronically critically ill' patient. An expanding body of evidence suggests that blood transfusion should be avoided, if possible. The immunosuppressive effects of allogeneic blood transfusions have been demonstrated in both solid-organ transplant recipients (in whom graft survival has been prolonged) and colon cancer surgery patients (in whom survival is decreased).

Observations that blood transfusions are associated with increased rates of nosocomial infection are numerous. Blood transfusions have been associated with an increased risk of infection following penetrating abdominal trauma independent of related factors such as shock or acute blood loss,[74] and they have been related to increasing injury severity and increasing transfusion volume in unselected trauma patients.[75] Data suggest that blood transfusion therapy of 6–20 units in the first 12 hours following multiple trauma is associated with an increased risk of nosocomial infection. [76] The risk of infection increased as the total transfusion volume increased, especially when units were transfused after more than 14 days of storage.[76] The postulated 'storage lesion' is complex but includes changes in oxygen affinity, red blood cell deformability, shortened circulation time, and the biologic consequences of cytokine generation and release. Recently, observational studies have suggested that transfusion of critically ill patients increases the risk of nosocomial infection,[77] and may worsen MODS and increases mortality.[78]

Hyperglycemia and control of blood sugar

Hyperglycemia has several deleterous effects on host immune function, most notably impaired function of neutrophils and mononuclear phagocytes. It is possible also that hyperglycemia is a marker of the catabolism and insulin resistance associated with the surgical stress response, and that exogenous insulin administration may ameliorate the catabolic state. Increasing evidence indicates that poor control of blood glucose during surgery and in the perioperative period increases the risk of infection and worsens outcome from sepsis. Diabetic patients have a higher risk of infection of both the sternal incision and the vein harvest incisions on the lower extremities.[9] Tight control of blood glucose by the anesthesiologist during surgery must be accomplished to decrease the risk, and that control must extend into the immediate postoperative period as well. Moderate hyperglycemia (>200mg/dl (>11mmol/l)) at any time on

the first postoperative day increases the risk of SSI after noncardiac surgery.[10] Hyperglycemia during the immediate phase of trauma resuscitation increases the risk of nosocomial infection and death after trauma. Exogenous insulin administration to keep blood glucose concentrations under 120mg/dl (6.7mmol/l) was associated with a 40% decrease of mortality among critically ill surgical patients with sepsis[11] (see Chapter 56).

The need to manage carbohydrate metabolism carefully has important implications for the nutritional management of surgical patients. Gastrointestinal surgery may render the gastrointestinal tract unusable as a route for feeding, sometimes for prolonged periods. Ileus is common in surgical ICUs, whether from traumatic brain injury, narcotic analgesia, prolonged bed rest, inflammation in proximity to the peritoneal envelope (lower lobe pneumonia, retroperitoneal hematoma, fractures of the thoracolumbar spine, pelvis, or hip) or other causes. Parenteral nutrition is relied on for feeding, despite evidence of a lack of efficacy[79] and the possibility of hepatic dysfunction; hyperglycemia may be an important complication as well. Every effort should be made to provide enteral feeding, including the use of promotility agents such as erythromycin.[80] Substantial evidence indicates that enteral feeding reduces the risk of nosocomial infection by nearly one-half among critically ill and injured patients.[81]

The diagnosis of postoperative infection

The evaluation of a patient for possible infection usually begins with the report of a fever. The clinical response is too often the reflexive ordering of multiple cultures of various body fluids, even at times when the likelihood of infection and therefore the yield of the cultures is low.[82] The only intervention mandated by new-onset fever is a history and physical examination. [60] All additional evaluations, whether radiologic or microbiologic, should be dictated by the findings of the evaluation at the bedside. [83] Fever related to infection in the immediate postoperative period (<48–72 hours after the operation) is rare unless the patient has been operated on to treat a febrile illness. The exception is necrotizing SSI caused by *Streptococcus pyogenes* or *Clostridium* spp., for which inspection of the incision is mandatory. The yield of blood cultures is known to be very low in the immediate postoperative period,[74] and obtaining cultures to evaluate fever less than 72 hours postoperatively is not recommended.[60] After 72 hours, the possibility of nosocomial infection increases, but even then there is still the possibility that fever may be due to a noninfectious cause (e.g. venous thromboembolic disease, wound hematoma), and diagnostic rigor must be maintained. Overall, postoperative fever may be due to a noninfectious cause in as many as one-half of circumstances.[75]

Intra-abdominal infection

Intra-abdominal infection is a recognized complication following abdominal surgery, but it can, rarely, occur after other types of surgery as well. In contrast, nosocomial intra-abdominal infection is rare in medical and pediatric ICUs. Better patient selection and preparation before high-risk elective surgery, appropriate resuscitation, early nutritional support and prophylaxis of stress-related gastric mucosal hemorrhage have all contributed to improved outcomes (see also Chapter 47).

After an abdominal operation, infection may persist or recur. Potential causes include severe illness, inadequate antibiotic dosing, resistant pathogens, inadequate source control, technical shortcomings, tissue ischemia and complete failure of intra-abdominal host defenses. Most patients with community-acquired intra-abdominal infections (e.g. secondary peritonitis from, for instance, appendicitis or diverticulitis) are infected by sensitive Enterobacteriaceae and anaerobic Gram-negative bacilli such as *Bacteroides fragilis*. Such patients rarely harbor multidrug-resistant pathogens; if surgery is required for a good-risk patient and is performed properly, the patient will usually recover regardless of which of several appropriate antibiotic regimens may be chosen.[84]

Source control is an emerging concept in the management of intra-abdominal infection.[85] Simply stated, adequate source control is the correct operation performed at the correct time in the correct manner. In practice, though, it is much more difficult to define. Whereas the surgical management of complicated appendicitis has relatively few permutations, this is not the case for complex entities such as perforated diverticulitis. Issues surrounding whether to resect, perform an anastomosis or a colostomy, or place drains make it impossible to declare that there is one 'correct' surgical approach, but it may be possible to describe management attributes that are clearly not appropriate. Studies suggest that source control is inadequate in approximately 10% of cases of complicated intra-abdominal infection; therefore, failure of source control is more likely as a cause of failure of treatment of intra-abdominal infection than any shortcoming of the antibiotic regimen.

With nosocomial infections (e.g. fecal peritonitis after dehiscence of a colon anastomosis), antibiotic selection is more crucial in addition to the corrective surgical procedure, because organisms such as *Enterococcus* spp., *Pseudomonas aeruginosa* and sometimes *Candida* spp. may be encountered. In circumstances in which involvement of the peritoneal cavity is generalized and at least one source control procedure has failed to control a nosocomial intra-abdominal infection, the patient may be considered to have tertiary peritonitis.[86] Diffuse peritonitis, infected serosanguinous fluid rather than pus, poorly localized collections and isolation of enterococci, coagulase-negative staphylococci, yeast and *P. aeruginosa* are characteristic. Local peritoneal host defenses are nonfunctional. The management of tertiary peritonitis is controversial. Some experts believe that the peritoneal cavity is colonized rather than infected, and that peritoneal toilet should be provided by daily saline lavage with the abdomen left open,[87] but randomized studies are nonexistent. What is clear, however, is that the typical mortality rate of about 30% is more than 10-fold higher than that usually reported for secondary peritonitis.

Ischemic enteropathies (e.g. acute pancreatitis, acalculous cholecystitis, ischemic colitis, ischemic hepatitis) can complicate the management of critical surgical illness. Patients have usually sustained a period of splanchnic hypoperfusion, and the consequences can be devastating. Such patients usually manifest SIRS owing to tissue ischemia, and whether the patient is infected or becomes infected at some point can be very difficult to discern. Acute pancreatitis can complicate cardiopulmonary bypass or upper abdominal surgery (e.g. gastrectomy, splenectomy); most patients do not become infected and the administration of prophylactic antibiotics to patients with acute pancreatitis is controversial.[88]

Outcomes of postoperative pancreatitis vary widely, depending on the severity of the attack. Acute acalculous cholecystitis has been reported as complicating virtually any operation but has a predilection for patients with trauma, burns, shock and emergency cardiac and peripheral vascular surgery.[89] The diagnosis is made most efficiently by bedside ultrasonography, and the treatment of choice is evolving to percutaneous cholecystostomy. The mortality rate of acute acalculous cholecystitis is approximately 30%.

Ischemic colitis is the most dangerous of these entities. The distribution and severity vary widely; sometimes, the presentation can be as subtle as occult blood in the stool or an unexplained fever. When it does manifest itself, it usually does so within 72 hours of the insult that puts the patient at risk. The diagnosis is made most frequently by flexible lower gastrointestinal endoscopy but, because only the mucosa is inspected, endoscopy is not quantitative. Mild cases are probably noninfectious - there are no data to demonstrate that antibiotic therapy alters the course of mild ischemic colitis. Transmural

colitis can lead to perforation; at worst, when severe ischemic colitis necessitates an emergency colectomy in the context of critical illness, the mortality rate may be as high as 80%.[90]

Computerized tomography (CT) is probably the most useful modality for imaging the abdomen of seriously ill patients, particularly when there is substantial uncertainty as to the precise diagnosis, but it is not a panacea. The benefits of imaging must be weighed against the formidable logistics and inherent risk of transporting a critically ill patient within the hospital for an imaging study. Moreover, it is possible to image the abdomen too soon (before about 7 days postoperatively) and thereby achieve a false-negative result. Fewer than one-half of abdominal-pelvic CT studies performed on critically ill patients yield meaningful information about diagnoses that are amenable to further intervention.

Sinusitis

Nosocomial sinusitis is a closed-space, often occult infection that is recognized increasingly as a major cause of morbidity and mortality among critically ill patients.[18] Major risk factors include prolonged nasotracheal intubation (30–35% incidence after 1 week), nasogastric intubation, facial fractures, prolonged recumbency and corticosteroid administration. Maxillary sinusitis is the most recognized type (and the most treatable, by a drainage procedure), but infection can also involve the ethmoid or sphenoid sinuses. The pathogenesis is believed to be related to poor natural drainage in recumbency, combined with obstruction of sinus ostia by transnasal tubes. Outcomes can be adverse because the diagnosis must be sought specifically. An occasional patient will present with purulent or foul-smelling nasal drainage, but most cases of sinusitis are found in the context of evaluation for an occult source of fever. Considering that clinical manifestations are usually non-specific, it is important to remember the diagnosis when faced with a patient who has persistent fever with an elusive source.

The diagnosis of sinusitis is best made by CT of the facial bones, looking for thickened mucosa, an air-fluid level within the sinus or opacification. If any of those criteria are found in a maxillary sinus, the sinus should be punctured by needle, aspirated for culture and irrigated with saline.[91] Sinus fluid can be accessed easily via the alveolar ridge or the bicuspid ostium, but attention to disinfection before aspiration is crucial lest the sample be contaminated by pathogens colonizing the oral mucosa.

The potential list of pathogens is long,[18] but among surgical patients the most likely pathogens are *P. aeruginosa* and *S. aureus*. Fungal sinusitis has been reported. Most antibiotics achieve high concentrations in sinus fluid, so the choice of antibiotic therapy may be guided by the microbiology. Most episodes will respond to the initial course of drainage and antibiotics, but the possibility exists that the sinus may need to be aspirated again to achieve cure. In rare circumstances, formal surgical drainage either by endoscopy or by an open procedure may be necessary (see Chapter 32).

Although not a prerequisite, sinusitis is a recognized risk factor for the development of nosocomial pneumonia.[18] Microbiologic concordance between sinus cultures and subsequent sputum cultures is high, and a common pathogenesis has been postulated (notably prolonged endotracheal intubation and a large inoculum of potential pathogens in the upper airway waiting to be aspirated).

Pneumonia

Although SSI is probably the nosocomial infection most closely associated with surgery, nosocomial pneumonia (NP) is probably more common and certainly more dangerous. Long-term observational studies suggest that NP is more common than SSI among surgical patients, and indicate clearly that the incidence of NP is higher in surgical ICUs than in medical or pediatric ICUs.[1] In surgical subspecialty

TABLE 86-7 -- Incidence of ventilator-associated pneumonia.

INCIDENCE OF VENTILATOR-ASSOCIATED PNEUMONIA			
Type of ICU	**Ventilator utilization**	**Pooled mean**	**Median**
Medical	0.49	7.3	6.0
Pediatric	0.45	4.9	3.9
Surgical	0.47	13.2	11.6
Cardiothoracic	0.47	10.5	9.5
Neurosurgical	0.38	14.9	11.9
Trauma	0.58	16.2	15.3
Burn	0.33	15.9	
Utilization, number of ventilator days per number of patient days; rates are per each 1000 days of indwelling artificial airway (e.g. endotracheal tube, tracheostomy tube)			
From National Nosocomial Infections Surveillance System[1]			

units (e.g. burns units, trauma units, neurosurgical units, cardiothoracic units), the incidence of NP exceeds 15 cases/1000 days of mechanical ventilation (Table 86.7).[1] The increased incidence of NP among surgical patients occurs despite comparable rates of mechanical ventilation in the ICU, which may reflect that intraoperative mechanical ventilation is not captured in ICU statistics if the patient is extubated early after surgery. However, surgical patients are at increased risk with respect to airway reflexes (because of anesthesia, analgesia and sedation), hypoventilation and atelectasis (because of the above factors plus painful chest or upper abdominal incisions), gastric intubation, ileus and numerous other factors. Trauma patients are at especially increased risk if they sustain a head or chest injury (e.g. pulmonary contusion, rib fractures) or are intoxicated with drugs or alcohol. The risk of NP is increased by aspiration during endotracheal intubation, high severity of injury, blood transfusions, hyperglycemia, prolonged mechanical ventilation and the acute respiratory distress syndrome.

Elective surgical patients are at risk of pneumonia as well, with an estimated incidence of approximately 3%. Observational study has identified preoperative risk factors among elective surgical patients and allowed the development of a validated prediction model. Patients at highest risk include those over the age of 70 years and those who are to undergo thoracic surgery, upper abdominal surgery or aortic surgery.[7]

If increased risk can be defined, then effective targeted prevention strategies should be utilized to the fullest extent possible. Meticulous infection control to minimize the spread of pathogens around the unit, minimized sedation, daily sedation 'holidays', keeping the head of the patient's bed at an angle of 30° at all times, antiseptic or antibiotic decontamination of the oropharynx, endotracheal extubation at the earliest possible opportunity, early tracheostomy if extubation cannot occur promptly, early enteral nutrition and meticulous pulmonary toilet, including continuous aspiration of subglottic secretions, can all be part of a comprehensive program for the prevention of pneumonia (see Chapter 35 , Chapter 84 and Chapter 87).[92]

The diagnosis of NP can be challenging, especially if the patient is receiving mechanical ventilation and the diagnosis of ventilator-associated pneumonia (VAP) is being pursued. The clinical constellation of fever, leukocytosis, purulent sputum and a new or changed chest radiographic infiltrate is unreliable for the accurate diagnosis of NP-VAP,[93] considering that numerous noninfectious process can produce fever or pulmonary infiltrates, or both. Studies indicate that both clinical acumen[94] and chest radiography are non-specific for the diagnosis of VAP. Sputum cultures obtained by routine endotracheal suctioning without quantitative microbiology are often contaminated

by upper airway colonists, and the diagnosis may be made in error on the basis of such false-positive cultures. Such patients are often treated when they need not be, without the possibility of benefit but with the potential for the adverse consequences of antibiotic therapy. Methods to improve the accuracy of the diagnosis of NP-VAP have been developed to reduce the likelihood that the sputum specimen is contaminated in the collection process. Quantitative sputum cultures increase diagnostic specificity. However, the methods of 'high-technology' diagnosis of NP remains controversial (i.e. protected-specimen brush or bronchoalveolar lavage, or not; with or without quantitative microbiology; with or without bronchoscopy), owing to limited data that better diagnoses translate into better outcomes. Studies performed in surgical patients indicate that routine suctioning for sputum collection results in numerous false-positive cultures, and that sputum Gram stain is unreliable.[95] Because of the prevalence of SIRS and the substantial proportion of noninfectious febrile episodes among surgical patients, bronchoscopic bronchoalveolar lavage is employed increasingly in surgical critical care practice. Although imperfect, diagnostic accuracy is increased from about 50% to about 80%. If the diagnostic threshold to consider quantitative cultures to be positive is not reached ($>10^3$ cfu/ml of a predominant organism by protected-specimen brush, 10^4 cfu/ml for bronchoalveolar lavage), empiric antibiotic can be discontinued. One international multicenter trial of suspected VAP that included substantial numbers of surgical patients has shown that management according to bronchoscopic quantitative microbiology, as opposed to routine care, reduced mortality, exposure to antibiotics and the emergence of *Candida* spp.[72]

Bloodstream infection

Bacteremia is an unusual complication of most surgical infections. Bacteremia accompanies only 8% of intra-abdominal infections overall, although less common infections such as cholangitis are characterized by positive blood cultures in about 90% of cases. Clostridial bacteremia can complicate emphysematous cholecystitis or occult perforation or neoplasm of the gastrointestinal tract.[96] Surgical site infections have been described as potential sources of staphylococcal [97] or enterococcal bacteremias, and staphylococcal or pseudomonal bacteremia can complicate pneumonia caused by those organisms. Suppurative phlebitis, a bacteremic complication of infected vascular access sites, usually a peripheral intravenous site, is rare but dangerous.[98]

Catheter-related bloodstream infection is relatively uncommon in surgical units compared with medical or pediatric units, despite the high reliance on central venous access and monitoring (Table 86.8).[1] However,

TABLE 86-8 -- Incidence of central line-associated blood stream infections, National Nosocomial Infections Surveillance System, 1995–2001.

INCIDENCE OF CENTRAL LINE-ASSOCIATED BLOOD STREAM INFECTIONS			
Type of ICU	**Central line utilization**	**Pooled mean**	**Median**
Medical	0.51	5.9	5.2
Pediatric	0.46	7.6	6.8
Surgical	0.66	5.3	4.9
Cardiothoracic	0.79	2.9	2.4
Neurosurgical	0.44	4.7	4.5
Trauma	0.63	7.9	7.0
Burn	0.49	9.7	
Utilization, number of central venous catheter days per number of patient days; rates are per 1000 days of indwelling catheter			
From National Nosocomial Infections Surveillance System[1]			

the threat is high and the danger is real for surgical patients, and therefore strict adherence to principles of infection control for central venous catheter insertion and care must be observed. According to the US Centers for Disease Control and Prevention, the operator must wear a cap, mask and sterile gown and gloves for all insertion procedures, and the operative field must be draped widely.[36] Central venous catheter insertion under emergency conditions (e.g. trauma resuscitation, cardiac arrest) almost always has lapses of infection control, and therefore catheters inserted under such conditions should be removed (and replaced at a different site, if needed) as soon as the patient has been stabilized. Ironically, the area of the hospital where these principles are most often overlooked is the operating room.

The most common organisms causing catheter-related bloodstream infections are *S. epidermidis, S. aureus* and *E. faecalis*, implying that the skin is the source for most of the bacteria. Unfortunately, *S. epidermidis* is also the most common contaminant of false-positive blood cultures as well as being the major pathogen for infection of prosthetic implants such as joint prostheses and synthetic vascular grafts. A blood culture that is positive for *S. epidermidis* in only one bottle is most likely contaminated, and antibiotics can be withheld safely with close observation in most cases when there is neither a prosthetic implant nor an implanted vascular access device. When such a device is present, prudence often dictates a course of therapy, usually with vancomycin[99] (see Chapter 57 and Chapter 84).

PRINCIPLES OF ANTIBIOTIC THERAPY

Although the decision to start empiric antibiotic therapy is a clinical one, it is important for the decision to be an informed one. An occasional patient will develop an early infection, but when it occurs it is usually the delayed manifestation of a community-acquired infection that was occult at the time of admission. In the case of pneumonia that develops within 3 days after surgery, the clinician must be alert to the possibility of a pneumococcal pneumonia. However, 'atypical' pneumonia is almost unheard of in surgical practice unless the patient is profoundly immunosuppressed, and so there is seldom a consideration to administer a macrolide antibiotic for that indication.

It can be useful to distinguish NP as 'early-onset' NP or 'late-onset' NP (within 5 days of hospitalization, or in the case of VAP, of endotracheal intubation) with respect to likely pathogens and appropriate initial empiric therapy.[100] Early-onset pneumonia is more likely to be caused by antibiotic susceptible strains of *S. aureus*, *H. influenzae*, *E. coli*, and *Klebsiella pneumoniae*, whereas late-onset pneumonia is more likely to be caused by multidrug-resistant pathogens such as MRSA, *P. aeruginosa*, *Acinetobacter* spp. and others. Considering that *S. aureus* and *P. aeruginosa* are the two most common health care-associated pneumonia pathogens, initial empiric antibiotic therapy must account for both pathogens, meaning two-drug anti-MRSA and anti-pseudomonal treatment in institutions with a high prevalance of MRSA, until data have been generated by the microbiology laboratory to inform the prolongation of therapy.

The decision to truncate a course of antibiotics once it has been started or to narrow the spectrum of coverage is crucial. Although there are few data regarding the appropriate duration of therapy for NP-VAP, or for any other nosocomial infection for that matter, the typical decision to continue antibiotics until some time after the patient becomes afebrile and the leukocytosis has resolved may not be appropriate in every circumstance. Because the consequences of overtreatment (primarily later nosocomial infections with multidrug-resistant bacteria, or opportunistic pathogens such as fungi) can be as severe as undertreatment, ongoing antimicrobial therapy should be reviewed every day in every patient with a bias to stopping treatment as soon as the patient improves. It may be possible to withhold

or truncate antibiotic therapy by the use of a propensity score, such as the Clinical Pulmonary Infection Score.[101] [102]

The pharmacokinetics of antimicrobial agent dosing can be challenging in surgical patients. Shock and hypoperfusion, blood loss, hypoalbuminema and marked changes in volume of distribution owing to 'third-space' fluid shifts to the extravascular interstitial space are important but unpredictable factors. If anything, these factors conspire to cause antibiotic underdosing, which must be taken into consideration. It is unknown, for the most part, whether novel antibiotic administration strategies such as single-daily-dose aminoglycoside administration or continuous-infusion ß-lactam antibiotic administration have utility in the surgical setting.[103] It is also unknown whether high-dose, short-course antibiotic regimens might be better than the conventional-dose, prolonged (e.g. for 10–14 days) regimens that are commonplace.[104]

It has been hypothesized that 'antibiotic heterogeneity' — changing the pattern of antibiotic use either randomly (as may occur when choice is unfettered), by computerized decision support or by scheduled change of antibiotic class (e.g. antibiotic 'rotation' or 'cycling' programs) — may be a valid alternative to antibiotic control or restriction programs to prevent the emergence of multi-drug-resistant bacteria. Initial data in surgical patients provide support for the 'cycling' hypothesis. Quarterly cycling of cefepime, ciprofloxacin, imipenem-cilastatin and piperacillin-tazobactam resulted in decreased use of aminoglycosides, vancomycin and antifungal agents.[105] Moreover, infections caused by resistant bacteria were reduced, and so was mortality. It is not known which attribute or attributes (drug, order of administration, duration of cycle, omission of other drugs for potential empiric use) contribute to the effect.

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Chapter 87 - Hospital Infection Control

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EPIDEMIOLOGY

Definitions of hospital infections

Hospital infection, also known as nosocomial infection, encompasses all types of infections acquired by patients while being cared for in an acute care institution, and those acquired by health care personnel and visitors. The rationale for categorizing these infections separately lies in our ability to prevent them through adequate organization of patient care in the hospital setting.

To study the epidemiology of hospital infection and to evaluate the efficacy of prevention strategies, hospital epidemiologists must use standardized definitions. An extensive set of criteria that are widely used internationally for the detection and classification of hospital infection has been published by the Centers for Disease Control and Prevention (CDC; Table 87.1).[1] [2] These definitions include a combination of clinical criteria and, when available, documentation by laboratory test results, pathologic findings and imaging data. Although these standard case definitions provide a useful basis for recording hospital infections, their application inevitably entails some degree of interobserver bias and variability, because several criteria (e.g. visual detection of purulence) are based on subjective clinical judgment whereas others (microbiologic findings) rely on laboratory test usage and performance.

Incidence and public health impact

Hospital infections are an important public health problem because of their frequency, attributable morbidity and mortality, and cost. In the USA and in Europe, approximately 5–10% of hospitalized patients develop an infection during their hospital stay.[3] [4] [5] Higher incidence rates are reported in hospitals in developing countries.[6] [7] The risk of infection varies by type of patient population and clinical area of care. For example, among critically ill patients the prevalence of hospital infection can reach 50% in intensive care units where patients stay for prolonged periods and undergo invasive therapeutic support, such as mechanical ventilation.[8] Incidence rates of hospital infection are higher in larger and tertiary care hospitals, where patients are more severely ill and are exposed to more intensive care. The most common types of hospital infection include (in order of decreasing frequency) urinary tract infection, surgical site infection, pneumonia and bacteremia. Record trends indicate that the incidence density rates of some hospital infections have increased over the years, as illustrated by the rise of bacteremia incidence rates reported in Belgian hospitals in the past decade (Fig. 87.1), whereas other risks appear to be better controlled, as shown by a decrease in adjusted incidence rates of device-associated infections in critically ill patients in the USA (Fig. 87.2). [9] [10] [11] [12] [13]

Although the majority of hospital infections are minor, there is a wide range in the severity of clinical illnesses they cause; some have serious consequences, as in patients with infections of implanted prostheses who develop permanent disability or who need reoperation. The hospital length of stay is prolonged in infected patients, on average by 5–10 days.[11] The risk of death approximately doubles in patients who acquire hospital infection. [11] [14] The mortality rates attributable to bloodstream infection and pneumonia are 25–50% and 7–27%, respectively, depending on the etiologic agent and underlying disease.[10] [14] [15] [16] Estimates of direct and indirect costs associated with hospital infection vary widely according to differences in epidemiologic and econometric cost measures, patient population and health financing system. However, all studies consistently show that hospital-acquired infections are very expensive and contribute significantly to the escalating costs of health care.[16] [17] It has been argued that, even if moderately effective, a hospital infection control program is one of the most cost-effective and cost-beneficial preventive medical interventions currently available.[17] [18]

Modes of acquisition of hospital infections

Infection can be acquired in the hospital by either endogenous or exogenous routes. The relative contributions of these routes can be determined by epidemiologic investigation coupled with intensive microbiologic surveillance and molecular typing (see below). It is of key importance to identify the major routes of colonization and infection for the design of effective control strategies. A majority of hospital infections (approximately 80%) are acquired by the endogenous route through translocation of micro-organisms from the patient's mucocutaneous flora (Fig. 87.3). This autoinfection occurs as a result of predisposing host conditions (Table 87.2) and by exposure to invasive diagnostic and therapeutic procedures that lead to disruption of mucocutaneous barriers (Table 87.3 ; Fig 87.4 and Fig 87.5). These factors interfere with the normal balance between host defenses and the invasive properties of the commensal microflora. Examples of endogenous translocation leading to common hospital infections include *Escherichia coli* urinary tract infection in a patient who has an indwelling bladder catheter and *Staphylococcus epidermidis* bacteremia in a patient who has a percutaneously inserted intravenous catheter. The micro-organisms will migrate from their mucosal or skin habitat along the external surface of the catheter and across the meatal or skin barrier to gain access to the site of infection. In addition, the commensal microflora changes during hospitalization because of mucosal modifications (pH, expression of surface receptors, etc.) and the selective pressure of antibiotics, favoring the multiplication of resistant organisms.

The second mechanism of acquisition of hospital infection is by the exogenous route, from a microbial reservoir in the hospital. This includes other patients, hospital staff or the inanimate environment. The most common route is cross-infection or cross-colonization, for which the reservoir is the microbial flora of other patients. This flora is frequently transmitted by the hands of health care workers (see Fig. 87.3). Cross-infection may account for approximately 10–20% of hospital infections. This proportion can reach 40% in the intensive care unit where there is frequent interaction between health care workers and patients who have altered host defenses. [19] Contact spread is a mode of nosocomial transmission of environmentally sturdy micro-organisms that can be difficult to control, such as *Clostridium difficile* and *Candida*. [20] [21] Examples of pathogens

TABLE 87-1 -- Keypoints in Centers for Disease Control and Prevention Surveillance definitions of major nosocomial infections in adult patients.*

KEYPOINTS IN CDC SURVEILLANCE DEFINITIONS OF MAJOR NOSOCOMIAL INFECTIONS IN ADULT PATIENTS
Surgical site infection (SSI)
Infection affecting the surgical site and occurring within 30 days of the operative procedure, or within 1 year after implant surgery
Superficial incisional SSI
At least one of the following:
• Purulent drainage from the skin or subcutaneous tissue above fascia layer
• Organism isolated from aseptically collected fluid or tissue from the superficial incision closed primarily
• Surgeon deliberately opens the superficial incision, unless the culture is negative
• Diagnosis by the surgeon or attending physician
Deep incisional SSI
At least one of the following:

• Purulent discharge from the deep incision (i.e. fascia and muscle)
• Deep incision that spontaneously dehisces or is deliberately opened by the surgeon when the patient has fever and/or localized pain or tenderness, unless culture is negative
• Abscess or other evidence of infection of deep incision seen on direct examination, during reoperation or by histopathologic or radiologic examination
• Diagnosis by the surgeon or attending physician
Organ/space SSI
Infection of any organ/space located below the incision and opened or manipulated during the operative procedure, which meets at least one of the following:
• Purulent drainage from a drain that is placed through a stab wound into the organ/space
• Organism isolated from an aseptically obtained culture of fluid or tissue from organ/space
• Abscess or other evidence of infection of the organ/space seen on direct examination, during reoperation or by histopathologic or radiologic examination
• Diagnosis by the surgeon or attending physician
Bloodstream infection (BSI)
Laboratory-confirmed BSI infection was not present or incubating at time of admission and occurred during or after hospital stay, which meets one of the following:
• Recognized pathogen(s) isolated from blood culture and pathogen is not related to infection at another site or is related to an intravascular device (primary BSI) or related to nosocomial infection at another site (secondary BSI)
• Fever, chills or hypotension and isolation of a common skin contaminant from two blood cultures or from a single blood culture in a patient with an intravascular device if physician institutes appropriate antibiotic therapy, or positive antigen test on blood
Urinary tract infection (UTI)
Symptomatic UTI
Infection not present or incubating at the time of hospital admission that meets one of the following:
• Fever, urgency, frequency, dysuria or suprapubic tenderness and urine culture with $=10^5$ colonies/ml with no more than two organism species
• Two of the preceding symptoms and pyuria, or positive leukocyte esterase or nitrate test, or positive Gram stain, or two urine cultures with repeated isolation of organism with $=10^2$ colonies/ml, or one urine culture with $=10^5$ colonies/ml if physician institutes appropriate antimicrobial therapy, or diagnosis or appropriate therapy by physician
Asymptomatic bacteriuria
One of the following:
• Urine culture with $=10^5$ colonies/ml with no more than two organism species in a patient with an indwelling catheter and no symptoms
• Two urine cultures with $=10^5$ colonies/ml of the same organism in a patient who had no indwelling catheter in previous 7 days and no symptoms
Pneumonia
Infection not present or incubating on admission, which meets one of the following:
• Rales or dullness on chest percussion and new onset of purulent sputum, or pathogen isolated from blood, transtracheal specimen, bronchial brushing or biopsy
• New lung infiltrate, consolidation, cavitation or pleural effusion on chest radiograph and one of above criteria, or positive direct or serologic diagnostic test for respiratory pathogen, or histopathologic diagnosis of pneumonia

** Adapted from Garner[1] and Horan.[2]*

spread by this route are given in Table 87.4 . In fact, most micro-organisms that cause endogenous hospital infection have been shown to transiently contaminate the hands of hospital staff and to be disseminated in this way. Vehicles of cross-infection also include contaminated medical devices (such as inadequately disinfected endoscopes,[22] thermometers, electrodes or respirators), infected blood products or organ transplants (see Table 87.4).

Person-to-person spread of pathogens can also follow the fecal-oral route, secretion droplet route and airborne route (see Table 87.4). Pathogens transmitted from person to person in the hospital include community-acquired infectious agents, such as viruses (e.g. varicella-zoster virus, respiratory syncytial virus (RSV), influenza virus, herpes simplex virus, hepatitis A virus, rotavirus, adenovirus), bacteria (e.g. *Staphylococcus aureus, Neisseria meningitidis, Haemophilus influenzae, Mycobacterium tuberculosis*) and parasites (e.g. *Cryptosporidium* spp.). The risk of transmission of these pathogens in the hospital can vary depending upon the age and immune status of hospitalized patients, the staff-to-patient ratio, the number of patients sharing a room, the availability of handwashing facilities and geographic location of the health care facility. Seasonal and environmental changes may affect the prevalence of locally endemic infections in the community. Because many of these pathogens are virulent for normal, nonimmune hosts, these hospital outbreaks can involve infection of hospital personnel and visitors as well as patients.

Occasionally, healthy carriers or infected individuals among hospital personnel may be the source of outbreaks of nosocomial infections (see Table 87.4). Well-documented examples include operating room personnel who are mucosal or skin carriers of *Streptococcus pyogenes, Staph. aureus* or *Staph. epidermidis* and become the source of epidemics of surgical site infection.[23] [24] [25] Certain conditions, such as upper respiratory tract infection or dermatitis, appear to predispose individuals to become efficient airborne disseminators of these organisms.[26] Nosocomial outbreaks of adenovirus keratoconjunctivitis, of RSV and of influenza pneumonia have also been linked to infected health care workers.

Environmental acquisition is an uncommon route of hospital-acquired infection, accounting for less than 5% of cases. Common-source outbreaks can result from exposure of susceptible patients to

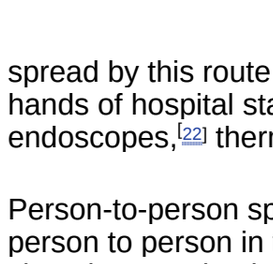

Figure 87-1 Record trend, 1992–2001, in bloodstream infection in Belgian hospitals by pathogen group.

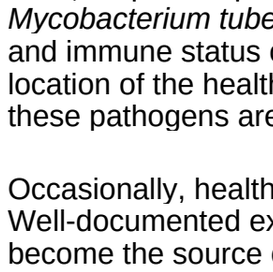

Figure 87-2 Trends in bloodstream infection rates by intensive care unit type and year. National Nosocomial Infection Surveillance System, USA, 1990–99.[13]

air, water, food, medication, disinfectant or medical devices contaminated with micro-organisms originating from environmental reservoirs inside or outside the hospital (see Fig. 87.3 and Table 87.4). Airborne pathogens include *Legionella* spp., *Aspergillus* spp. and other filamentous fungi[27] and *Nocardia* spp. These organisms represent a particular hazard to immunocompromised patients, especially those with cancer and transplant recipients. Waterborne outbreaks of infections in hospitals have been linked to patient bathing in hydrotherapy pools or enteral feeding with water contaminated with *Pseudomonas aeruginosa*, *Acinetobacter* spp. or *Legionella*

spp. Foodborne outbreaks are most frequently caused by *Salmonella* spp. Enteric feeds contaminated with *Enterobacter* spp., can be a source of sepsis. Contamination of intravenous fluids, medications, antiseptics, blood products or medical devices with a variety of aquatic

Figure 87-3 Endogenous and exogenous sources of hospital infection. Hospital infection can originate from an endogenous source or from an exogenous route. Endogenous infection may occur by translocation of resident microflora secondary to a breach in host defense (top). Exogenous infection may result from transmission from patient to patient or from health care worker to patient. It can also occur after exposure of a susceptible patient to a contaminated environmental source, such as inadequately disinfected medical devices (bottom).

micro-organisms, such as *Serratia* spp., *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Mycobacterium chelonae*, have caused hospital outbreaks of bacteremia, wound infection and pneumonia.[22] [28] Extrinsic contamination occurs during hospital handling of medications and can lead to a local outbreak[29] or, less commonly, intrinsic contamination takes place during manufacturing of the product, often triggering multi-hospital epidemics.[30] A wide spectrum of agents cause hospital outbreaks, as reported in 228 investigations published from 1997 to June 2002 and identified by Medline searching (Fig. 87.6).

TABLE 87-2 -- Host factors predisposing to hospital infection.

HOST FACTORS PREDISPOSING TO HOSPITAL INFECTION	
Factor	**Example**
Age	Neonates; elderly patients
Underlying disease	System or organ failure (e.g. liver cirrhosis, diabetes mellitus, chronic obstructive pulmonary disease, renal failure), cancer, neutropenia
Immunodeficiency	Congenital or acquired (e.g. AIDS, immunosuppressive therapy, malnutrition)
Specific immunity	Susceptibility to viral infections
Breach of mucocutaneous barriers	Trauma, burns, surgery, endoscopy, indwelling devices
	Mucosal and skin diseases
Anesthesia, sedation	Suppression of cough and peristalsis, hypoventilation
Antibiotics, antacids	Alterations of resident microflora and decrease of resistance to colonization by hospital flora
	Selection of antibiotic-resistant mutants and naturally resistant bacteria and yeasts
Colonizing flora	Carriage of opportunistic bacteria and fungi
Latent infection	Latent infection with intracellular pathogens reactivated by immunosuppression
A variety of factors can alter host defenses and predispose hospitalized patients to infection.	

TABLE 87-3 -- Infections associated with invasive devices and procedures.

INFECTIONS ASSOCIATED WITH INVASIVE DEVICES AND PROCEDURES	
Device/procedure	**Type of infection**
Intravascular catheter	Bacteremia; catheter site infection
Bladder catheter	Urinary tract infection
Mechanical ventilation	Pneumonia; sinusitis
Stents	Pyelonephritis; cholangitis; meningitis
Surgery	Surgical site infection; pneumonia
Endoscopy	Bacteremia; pneumonia; gastroenteritis and cholangitis
Blood transfusion	Bacteremia and fungemia; viral infections
Examples of important hospital infections that are directly related to the use of invasive diagnostic and therapeutic devices and procedures.	

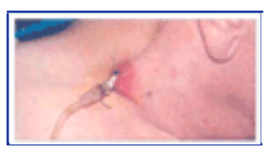

Figure 87-4 Catheter exit site infection in a patient with central venous catheterization through the jugular vein. *Courtesy of Dr F Jacobs.*

ETIOLOGIC AGENTS

A selection of important nosocomial pathogens and their major reservoirs and sources is presented in Table 87.4 . The majority of infections are caused by commensal bacteria, with *E. coli* and *Staph. aureus* being the most common.[7] [8] [9] [10] Micro-organisms isolated from patients who have nosocomial bacteremia and surgical infection reported by the National Nosocomial Surveillance of Hospital Infection in Belgian hospitals are illustrated in Figure 87.7 . The pattern of agents causing hospital infection can vary greatly according to the site of infection, the age and underlying conditions of the patients, and their exposure to medical procedures, invasive devices and antimicrobials.

The spectrum of hospital pathogens has changed over the past decades, reflecting the evolution of medicine. In the pre-antibiotic era, most infections were caused by *Strep. pyogenes* and *Staph. aureus*. Gram-negative bacteria emerged as leading pathogens in the 1960s and 1970s in response to the increased use of antistaphylococcal antibiotics. In the 1980s and 1990s, the massive use of broad-spectrum antimicrobials, the improved survival of critically ill patients, the growing population of compromised patients and the increased use of indwelling medical devices all contributed to the current emergence of multiple antimicrobial-resistant nosocomial pathogens. Well-known examples are methicillin-resistant *Staph. aureus* (MRSA)[31] and *Staph. epidermidis*,[9] multiply resistant *Enterococcus faecium*[32] [33] and *Enterobacter* spp.,[34] *Candida* spp.[9] and *Clostridium difficile*. [20] These changes are illustrated by the modification of the rates of antimicrobial resistance among pathogens identified from intensive care unit patients with nosocomial infections (Fig. 87.8).[35] A dramatic example of the resurgence of a major pathogen is the occurrence of outbreaks of multiply resistant *M. tuberculosis* in hospitals caring for HIV-infected patients and in which adequate diagnostic and patient isolation facilities were not available.[36]

The increasing prevalence of antibiotic-resistant bacteria as agents of hospital infection has reached alarming proportions.[37] [38] Occasional strains of organisms such as *E. faecium*, *P. aeruginosa* or *Acinetobacter baumannii*, that are resistant to all available antimicrobials with established clinical efficacy, cause infections that leave few therapeutic options. Patients infected with resistant pathogens are more likely to receive ineffective therapy, require hospital care, stay in for a longer time, develop complications and die of the infection.[39] The cost of care is also increased for such patients, due to the need for second-line drugs, longer duration of hospital stay, increased need for intensive care and diagnostic testing, and expenses of isolation precautions.

To provide a sound basis for the control of this problem, research efforts have focused on determining the respective roles of selection by antibiotic treatment of naturally resistant micro-organisms from the endogenous flora, selection and transfer of resistant mutants and of mobile genetic determinants, such as transposons and plasmids, and dissemination of resistant clones between hospitalized patients. [40] A number of recent studies, performed in intensive care units in which antibiotic-resistant bacteria were frequent and clinically significant, showed that these various determinants generally interact in a multifactorial fashion. [33] [41] [42] [43] [44] [45] [46] Although variations are observed between bacterial strains and different hospitals, general trends in the relative contributions of these mechanisms can be recognized as typical of each resistant pathogen (Table 87.5). Clonal dissemination from patient to patient is a predominant mechanism of increased prevalence for the majority of resistant nosocomial pathogens. Transmission occurs most commonly via the contaminated hands of health care personnel and, less commonly, by contaminated equipment. Risk factors for colonization (which generally precedes infection) or infection with

Figure 87-5 Severe clinical conditions are often associated with invasive life-support devices (a) causing multiple disruptions of mucocutaneous barriers. (b) Oropharyngeal and nasogastric tubes; (c) wound drainage; (d) urinary indwelling catheter; (e) central venous catheter.

resistant bacteria include the local 'colonization pressure' (or the proportion of colonized patients on admission to the unit), the severity of underlying disease, the duration of stay in intensive care and in the hospital, and the intensity of exposure (number and duration) to broad-spectrum antimicrobial drugs and invasive devices such as intravascular and urinary catheters.[33] [41] [42] [43] [44] [45] [46] Cost containment in hospital management has led to increased patient turnover and transfer between institutions as well as to chronic understaffing, both of which are factors that enhance nosocomial transmission of antibiotic-resistant bacteria.

CONTROL STRATEGIES

Before we examine the different components of an effective infection control program, it is worth considering exactly what the targets and objectives of the prevention strategies are, based on our

TABLE 87-4 -- Modes of transmission of nosocomial pathogens.

MODES OF TRANSMISSION OF NOSOCOMIAL PATHOGENS		
Mode of transmission	**Reservoir/source**	**Examples of pathogens**
Contact	Patients/health care workers, fomites, medical devices	*Staphylococcus aureus*
		Enterococcus spp.
		Enterobacteriaceae
		Clostridium difficile
		Respiratory syncytial virus
		Rotavirus
		Adenovirus
		Candida spp.
Droplet spread	Health care workers, patients	*Staphylococcus aureus*
		Respiratory syncytial virus
		Influenza virus
Device-related	Water/respiratory equipment, endoscopes	*Pseudomonas aeruginosa*
		Acinetobacter spp.
		Stenotrophomonas maltophilia
Medication-related	Water/iv fluids, disinfectants	*Burkholderia cepacia*
		Acinetobacter spp.
		Serratia marcescens
Transfusion, needlestick	Patients/blood	Hepatitis B virus, hepatitis C virus, HIV, etc.
Transplantation	Patients/donor tissue	Cytomegalovirus
		Toxoplasma gondii
		Creutzfeldt-Jacob agent
Airborne	Patients	*Mycobacterium tuberculosis*
	Hot water/showers	*Legionella* spp.
	Soil/dust	*Aspergillus* spp.
Food-borne	Animals/food products	*Salmonella* spp.
	Water/enteral feeding	*Enterobacter* spp.
		Pseudomonas aeruginosa
Modes of transmission and examples of sources of infection for selected nosocomial pathogens.		

understanding of the pathogenesis and epidemiology of hospital infection (Table 87.6). This classification is of course somewhat simplistic, as a single strategy may meet several objectives. For example, perioperative antibiotic prophylaxis reduces not only the bacterial inoculum released from the patient's own flora but also the bacteria released into the operative site from the surgical team's flora. However, this approach underlines the fact that further research into the mechanisms of acquisition of hospital infection is needed to design optimal prevention methods.

Functions and organization of the hospital infection control program

Major differences among countries in their health care resources and organization and different medical cultures explain the diversity of approaches to the organization of hospital hygiene and infection control in hospitals around the world. The best known approach is the paradigm of hospital epidemiology developed in the USA.[47] In the hospitals of industrialized countries today, the co-ordination of infection control typically operates on two levels: an executive body — the infection control team — and an advisory body to the hospital management — the infection control committee — which adopts the 'legislative' role of policy making. The functions of these

bodies are to design, revise and recommend policies and procedures; foster good hygienic practices through continuing education of and interaction with health care personnel; to perform epidemiologic surveillance and outbreak management; and to monitor and advise on the utilization of

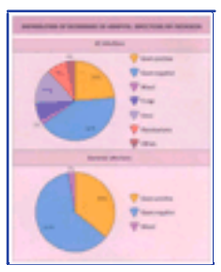

Figure 87-6 Distribution of outbreaks of hospital infection by pathogen. Distribution of outbreaks of hospital infection identified by Medline search from 1997 to June 2002, by category of pathogen. Bacteria caused 65% of outbreaks, of which 61% were Gram negative, 36% Gram positive and 3% mixed bacterial pathogens.

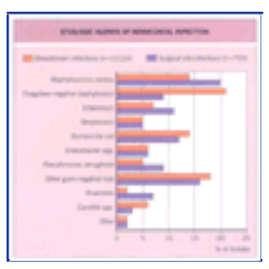

Figure 87-7 Etiologic agents of nosocomial bloodstream and surgical site infections in Belgian hospitals. National Program for the Surveillance of Infection in Hospitals, 1992–95.

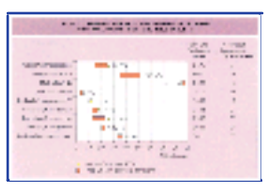

Figure 87-8 Selected antimicrobial resistant pathogens associated with nosocomial infections in intensive care unit patients. Comparison of resistance rates from January-December 2000 with 1995–99, NNIS Surveillance. CNS, coagulase-negative staphylococci; 3rd ceph, resistance to 3rd generation cephalosporins.

antimicrobial agents, safety of new medical devices, sterilization, disinfection, waste management and housekeeping in the institution. Evaluation of the program must ensure institutional compliance with policies, recommendations, regulations and accrediting requirements.

To achieve these broad missions cost-effectively, the infection control program requires adequate human and material resources proportional to the size and sophistication of the health care institution and the vulnerability of the patient population. In general, the team should include at least one or more physician and nurse specially trained in infectious diseases, microbiology, epidemiology and infection control. The hospital infection control physician may be an infectious diseases specialist or a clinical micro-biologist. Beyond the availability of infection control professionals, an extensive infrastructure is required, including microbiology laboratory support and access to integrated computing systems linking administrative, laboratory and clinical data.[48] Expert system software integrated in the microbiology laboratory information system can provide early warning of outbreaks by identifying the abnormal occurrence of clusters of patients colonized or infected with similar micro-organisms. Access to all sources of useful outcome data needs

TABLE 87-5 -- Relative importance of mechanisms that contribute to the increasing frequency of antimicrobial resistance of selected nosocomial pathogens.

MECHANISMS OF INCREASED ANTIMICROBIAL RESISTANCE OF NOSOCOMIAL PATHOGENS								
Pathogen	**Resistance pattern**	**Selection of mutation**	**Gene spread**	**Clonal spread**	**Reservoir**		**Transmission**	
					Human	**Environment**	**Direct**	**Indirect**
Staphylococcus aureus (MRSA)	ß-Lactam, multiple	+	+	+++	+++	+	+++	+
Enterococci	Glycopeptides, multiple	+	++	++	+++	+	+++	++
Klebsiella spp.	Extended spectrum ß-lactamase	+	++	+++	+++	-	+++	-
Enterobacter cloacae	Derepressed chromosomal ß-lactamase	+++	-	+	+++	-	+	-
Enterobacter aerogenes	Multiple	++	+	++	++	+	++	+
Acinetobacter baumannii	Multiple	++	+	+++	++	++	++	++
Pseudomonas aeruginosa	Multiple	+++	+	+	++	+	+	++

to be developed such as, for example, results of post-mortem examination to detect cases of nosocomial aspergillosis.

The landmark Study on the Efficacy of Nosocomial Infection Control, conducted in the 1970s in US hospitals, showed that the incidence of infection could be reduced by 32% in hospitals with an adequately staffed infection control team (defined as one physician per 1000 beds and one nurse per 250 beds) and performing active surveillance activities, compared with hospitals without such programs.[4] The infection control committee is typically composed of the physician hospital epidemiologist, hospital manager, hospital infection control practitioners (often nurses), medical and nursing directors, leading medical and nursing representatives of clinical departments, and representatives of other departments such as the microbiology laboratory, pharmacy, sterilization services, employee health clinic and safety committee. This advisory body develops and regularly updates infection control policies and reviews the progress achieved toward the institution's objectives in this field. Policies are designed based on national regulations, the published data documenting prevention strategies, guidelines and standards provided by national advisory committees and consensus conferences, and the local epidemiologic data and patient care resources. Extensive and detailed guidelines

TABLE 87-6 -- A rational classification of hospital infection prevention strategies.

A RATIONAL CLASSIFICATION OF HOSPITAL INFECTION PREVENTION STRATEGIES		
Target	**Objective**	**Example of strategy**
Endogenous infection	To prevent or neutralize the translocation of commensal flora	• Antibiotic prophylaxis in surgery
		• Skin antisepsis before surgery
		• Antiseptic-bound iv catheter
		• Intestinal decontamination of neutropenic patients
		• Pneumococcal immunization before splenectomy

Exogenous infection	To prevent cross-infection	• Hand hygiene for patient care procedures
		• Isolation and decolonization of carriers of transmissible pathogens
		• Sterilization or disinfection of invasive devices
		• Cleaning and disinfection of fomites
		• Outbreak detection and molecular epidemiologic studies to determine the mode and vehicles of spread
	To prevent common-source infection	• Ultra-clean air for prosthesis surgery or bone marrow transplant recipients
		• Disinfection of *Legionella* spp. in water systems
		• Environmental, water and food hygiene
		• Sterile parenteral drugs and implantable material
		• Outbreak detection and molecular epidemiologic studies to identify the source
Antimicrobial resistance	To prevent the emergence, and spread of resistance genes	• Restricted usage of broad-spectrum antimicrobial agents
		• Optimized anti-infectious therapy (agents, dosage and duration)
	To prevent the spread of resistant strains of micro-organisms	• Detection, monitoring and timely reporting of antimicrobial resistance
		• Isolation precautions and treatment of carriers of transmissible resistant strains
		• Molecular epidemiologic studies to distinguish between mutant selection, gene or clone dissemination

TABLE 87-7 -- Aims of hospital infection surveillance.

AIMS OF HOSPITAL INFECTION SURVEILLANCE
• To identify high-risk patients and procedures and assign infection control priorities
• To monitor trends over time of incidence and patterns of infection
• To detect outbreaks of hospital infection
• To evaluate the efficacy of prevention and control interventions
• To evaluate quality assurance programs
• To educate and motivate heath care providers and decision makers

have been published by the CDC and the Hospital Infection Control Practice Advisory Committee (HICPAC). The latest version of these recommendations is available at: www.cdc.gov/ncidod/hip/HICPAC. Each recommendation is categorized according to supporting scientific evidence, theoretical rationale and applicability.

TABLE 87-8 -- **Surveillance methods for the evaluation of infection control program outcomes and processes.**

SURVEILLANCE METHODS FOR THE EVALUATION OF INFECTION CONTROL PROGRAM OUTCOMES AND PROCESSES		
Scope	**Objective, indicators and method of data collection**	
Outbreak warning	Objective	Detection and cluster analysis of transmissible organisms
	Indicators	Numerator: colonization or infection by specific micro-organism
	Case-finding	Laboratory-based
Prevalence of hospital infection	Objective	Periodic assessment of prevalence of infection: hospital-wide or selective
	Indicators	Numerator: infection by site, ward, procedure or device
		Denominator: patient census on day of survey, procedures or devices
	Case-finding	Laboratory and/or ward liaison, chart review, computerized patient record
Incidence of hospital infection	Objective	Continuous monitoring of incidence of infection: hospital-wide or selective
	Indicators	Numerator: infection by site, ward, procedure or device
		Denominator: patient admissions, hospital-days, procedures, device-days
	Case-finding	Laboratory and/or ward liaison, chart review, computerized patient record
Antibiotic usage	Objective	Continuous monitoring and quality assessment of antibiotic utilization patterns
	Indicators	Numerator: type of antimicrobial cures, doses and duration used for prophylaxis and treatment
		Denominator: surgical interventions, documented infections (e.g. bacteremia), patient admissions
	Case-finding	Pharmacy records, operating room record, medical and nursing records, chart review, computerized patient record
Hygiene practices	Objective	Monitoring of compliance with, and quality assessment of, hygiene practices
	Indicators	Numerator: patient care procedures performed with appropriate hygiene precautions
		Denominator: procedures requiring standard hygiene precaution, patient census, density of care index
	Case-finding	Ward observations, pharmacy and central supply records (gloves, disinfectants, etc.)
Surveillance methods can vary in scope and objectives. The choice of indicators and case-finding methods will depend on the surveillance strategy selected.		

As a result of ongoing re-engineering of health care systems to improve cost-effectiveness, acute care hospitals increasingly operate as partners of larger consortia of health care programs including long-term care facilities, day-care surgery, outpatient clinics and home care. Likewise, infection control programs must increasingly assess and manage risk through these various levels of care. Furthermore, as nosocomial epidemics easily spread with transfer of

patients between acute and long-care facilities, and as the development of evidence-based control strategies requires substantial investment and data sample size, communication and co-ordination at regional and national levels has become an important part of infection control programs. The increasing demand for public accountability of health care providers also means that validated process and outcome indicators must be determined for performance assessment of the infection prevention.[11]

Surveillance

Epidemiologic surveillance is a systematic, ongoing process of data collection, analysis, interpretation and reporting, which is performed to monitor the temporal trends in disease frequency and associated risk factors in a population. The basic principle of its application to hospital infection surveillance is to measure the reduction of risk of infection as an outcome indicator of the effectiveness of the prevention efforts. Some of the objectives that can be assigned to surveillance activities are described in Table 87.7 . As a tool to evaluate the quality of care, outcome surveillance (i.e. monitoring the risk of hospital infection) can be coupled with the auditing of processes regulated by the infection control program, such as compliance with standards of antimicrobial usage and hygienic care practice.

A key element to the usefulness of any surveillance system is to achieve a consensus with the patient care providers on what will be its objectives, indicators and methods of case-finding and validation. Likewise, the involvement of clinical staff in data collection and interpretation of results is necessary for ensuring the credibility of the surveillance data as a guide to optimal care practices.

Because surveillance is a costly and time-consuming activity, each hospital must carefully tailor its system to its priorities and resources. Except in some US hospitals, staffing levels of infection control teams do not allow for comprehensive, hospital-wide surveillance of incidence rates of all infection types. Therefore, selective surveillance must be targeted to high-risk populations or procedures, particularly if specific control interventions are implemented. Haley provided a scheme to select surveillance objectives by site of infection based on the avoidable costs and morbidity associated with each infection type.[49]

Depending on the scope and objectives of the surveillance activities, different indicators and data collection methods can be used (Table 87.8). Outbreak warning can be largely accomplished by time-and-place cluster analysis of numerator laboratory data. This analysis will focus on patients colonized or infected by micro-organisms known to be transmissible by cross-infection or common-source acquisition, such as *C. difficile*, MRSA or *Legionella* spp. (alert organisms).

For the monitoring of overall infection risk, repeated prevalence surveys are more cost-effective than continuous incidence surveillance and can be used successfully to evaluate the efficacy of control measures.[50] [51] Data sources and collection methods used for the detection of patients who have hospital-acquired infection vary in their efficiency (i.e. the balance between accuracy and workload). A comparative evaluation of selective surveillance methods in a UK hospital reported a sensitivity of 76% for a time of 6.4 hours per 100 beds per week by using a laboratory-based and ward liaison method. [52] This method consists of daily review of case records of patients who have a positive microbiology report and of patients reported by nursing staff to have an infection. The reference method, based on exhaustive record review and staff interview, required 18.1 hours per 100 beds per week. In that study, laboratory-based detection had a sensitivity of 51% for a time requirement of only 3.1 hours per 100 beds per week. In US or Belgian hospitals this method achieved a sensitivity of 80–84%,[53] whereas it had only a 20% sensitivity in a Brazilian hospital.[54] These discrepancies illustrate the fact that the efficiency of a surveillance method will depend on local factors, such as the rate of utilization of microbiology tests. Other case detection methods based on review of temperature and drug prescription charts or on patient risk factors also appear to be efficient.[52] Ideally, the sensitivity, specificity and interobserver repeatability should be evaluated by comparison with an exhaustive case-finding reference method to validate the method used.

The efficiency of hospital infection surveillance and quality assessment of antimicrobial prescription can be greatly enhanced by the development of computerized patient records and integrated hospital information systems that link clinical information, laboratory, radiology and pharmacy records into a single database.[55] [56] Standardization of criteria for codification of risk factors, interventions and definitions of hospital infection is a first step toward inter-hospital comparison of rates.[57] [58] National surveillance systems can provide baseline data for hospitals willing to compare the rate of infection in their institution with risk-adjusted rates in similar institutions. However, methodologic issues remain unsettled on how best to adjust for confounding factors and to define similar institutions. National surveillance networks can also be set up to monitor the rate of nosocomial transmission of major pathogens, such as the incidence of MRSA acquisition in hospitals in which co-ordinated control policies have been implemented.[58]

When surveillance is used to assess the effectiveness of infection control interventions, a quantitative assessment of compliance with the recommended methods is a valuable component of the policy evaluation (see Table 87.7). Direct methods of practice auditing (e.g. observation of patient care procedure such as glove use, hand disinfection or reprocessing of flexible endoscopes) are preferred for data collection but require significant staffing resources. Indirect methods, for example measuring the rate of material usage (e.g. gloves, disinfectant, isolation facilities), are more efficient but provide only partial and crude indicators of patient care practices.

Outbreak management

An epidemic or outbreak can be defined as a significant temporal increase in the prevalence of infection above the expected baseline prevalence in a given population. The early detection and prompt management of outbreaks of hospital infection are important because of the frequent occurrence, clinical impact and preventable nature of epidemic infections.[59] The prevalence of outbreaks has been reported at approximately 1 per 10,000 admissions and the etiologic fraction can be estimated to account for 5–10% of all hospital-acquired infections. [59] [60] In addition, even small clusters of infection that do not cross the threshold of statistical significance can be worth investigating, because identification of the mode of transmission can lead to effective prevention. The true magnitude of small-scale clustering caused by cross-infection remains to be defined. In a study employing molecular subtyping [19] 38% of episodes of nosocomial infections detected in patients admitted to intensive care units were found to be cross-transmitted. Although many outbreaks resolve spontaneously, delay in the detection and control of an outbreak may be associated with considerable excess morbidity, mortality or costs.[61] The steps involved in the investigation and control of outbreaks have been well defined (Table 87.9).[59]

A pseudo-epidemic associated with extrinsic contamination of clinical samples during collection or processing should be ruled out, as well as any spurious rise in incidence due to observation bias, such as improvement in diagnostic sensitivity by change in laboratory methods. The first step to confirm the suspected epidemic event is the elaboration of a case definition on the basis of which additional epidemic cases should be sought. The definition is based on clinical signs, etiologic agents or both. As many nosocomial epidemics involve transmission of bacteria from asymptomatic

TABLE 87-9 -- Steps in the investigation and control of outbreaks.*

STEPS IN THE INVESTIGATION AND CONTROL OF OUTBREAKS
1. Establish a case definition and define the population at risk
2. Confirm existence of a true outbreak
• rule out pseudo-outbreaks (contamination of cultures during clinical sample collection or processing)
• rule out surveillance artefacts (increase in laboratory utilization, or improved laboratory or surveillance methods)
• determine space-time clustering and/or significant increase of incidence rate versus baseline period in exposed cohort
3. Determine clinical severity of infections and number of patients affected to define the degree of emergency; allocate time and resources for further investigation accordingly
4. Complete case-finding retrospectively and prospectively; ensure accurate microbiologic sampling and processing for optimal ascertainment of etiology; ensure storage and plan the typing of clinical isolates
5. Review the literature about risk factors and potential sources and compare with host and exposure factors revealed by reviewing the medical charts of infected patients
6. On the basis of descriptive epidemiology of epidemic cases in time, place, patient characteristics, and common exposure to devices, treatments and procedures, formulate tentative hypotheses about host factors, hospital exposure factors, reservoirs, source and mode(s) of transmission
7. Reinforce standard hygiene precautions and initiate temporary control measures based on hypotheses defined in step 6; follow-up impact on rate of transmission
8. If necessary, test hypotheses defined in step 6 by case-control, cohort and/or intervention studies and by epidemiologic typing of representative isolates
9. If necessary, initiate targeted culture surveys from potential reservoirs/sources (patients, personnel, environment, as appropriate); confirm the suspect epidemiologic link by comparative typing of isolates
10. Review results of the investigation with all concerned authorities and staff; report to the Infection Control Committee
11. Conduct follow-up surveillance to evaluate efficacy of control measures; update control measures if necessary; follow-up evaluation reports for the Infection Control Committee and all staff concerned

* *Adapted from Wendt and Herwaldt.*[59]

carriers, it may be useful to include both infected and colonized patients in the epidemic case definition. The epidemic is confirmed by comparing the incidence of cases during the outbreak period with the expected incidence using historic controls in the same population. Some rare and clinically severe nosocomial infections such as pneumonia caused by *Legionella* or *Aspergillus* surgical site infection should trigger immediate investigation and corrective action after a single case is detected. More generally, at the initial phase of epidemic investigation, it will be useful to reinforce standard hygiene procedures as insidious decline in compliance with these often

contributes to outbreaks.

The distribution of cases over time (the epidemic curve) may be suggestive of the mode of acquisition of the infection. The collection of data on demographic characteristics of cases, underlying disease, exposure to invasive procedures and devices is essential to complete the descriptive analysis and to formulate hypotheses of transmission. When needed, these hypotheses should be tested by performing analytic studies, often of case-control design, to identify the risk factors for transmission. Microbiologic investigations, including culture surveys and molecular typing, may be required to identify

TABLE 87-10 -- Role of the clinical laboratory in the investigation of hospital infection outbreaks.[*]

ROLE OF THE CLINICAL LABORATORY IN THE INVESTIGATION OF HOSPITAL INFECTION OUTBREAKS		
Investigation step	**Laboratory method**	**Aim**
Outbreak detection	Identification and susceptibility testing of clinical isolates	Case finding, cluster detection
Outbreak confirmation and descriptive epidemiology	Epidemiologic typing of clinical isolates	Delineation of clonal spread
Identification of reservoirs and mode of transmission	Culture surveys of reservoirs and vehicles	Documentation of reservoir of epidemic clone(s)
	Typing of environmental isolates	Test of transmission hypotheses
Follow-up of efficacy of control measures	Typing of clinical isolates, as part of post-outbreak surveillance	Test of interruption of transmission hypothesis

** Adapted from Struelens.*[60]

the reservoirs of the epidemic micro-organism and confirm the routes of transmission. The rapid evolution of medical technology results in the continuous introduction of new medical devices and modification of diagnostic and therapeutic procedures. Because the infection control team is often incompletely informed of these modifications, discussion with clinical staff and direct observation of patient care practices is useful for the detection of violation of infection control standards or problems with inadequate product design, maintenance or utilization.

After an outbreak is controlled and at regular intervals for endemic transmissible pathogens such as MRSA, the infection team should provide a report on the outbreak management to the infection control committee and local health authorities. Moreover, it is essential to provide feedback to all concerned clinical staff to consolidate compliance with the recommended procedures and trust in the infection control team (see also Chapter 50a).

Role of the clinical microbiology laboratory

The microbiology laboratory plays a key role in the detection, investigation and control of outbreaks of nosocomial infections (Table 87.10). This role includes accurate detection, species identification and susceptibility testing of micro-organisms causing hospital infection; archival and ongoing epidemiologic analysis of clinical test results; performing targeted microbiologic surveys of the hospital environment; and storage and epidemiologic typing of microbial isolates to support outbreak investigations.[60] [62]

Rapid laboratory detection and reporting of 'alert' organisms known for their potential to cause outbreaks, for example *C. difficile*, MRSA or multidrug-resistant *M. tuberculosis* (see Table 87.8), lead to the timely implementation of specific infection control precautions to reduce the risk of secondary spread.[60] In the past decade, 63% of outbreaks were detected in our institution by prospective or retrospective reviews of laboratory data. Certain types of epidemics are difficult to identify, like those caused by organisms that are also common causes of endemic infections or those caused by multiple strains. This type of epidemic can follow a break in disinfection technique, as observed in an outbreak of postendoscopy biliary sepsis in which only molecular typing could distinguish multiple strain cross-infection from endogenous infections.[22]

Epidemiologic typing

Epidemiologic typing is used for discrimination between unrelated isolates of the same microbial species and related isolates, derived by clonal descent from the same ancestor cell, as part of a common chain of transmission.[63] [64] This can be achieved by scoring appropriate phenotypic or genotypic markers that exhibit sufficient intraspecies diversity.[65] Typing systems can be used for different purposes, including to delineate the extent and patterns of dissemination of epidemic clone(s); to test hypotheses about the reservoirs, sources and vehicles of transmission; and to verify the interruption of transmission by application of control measures.[63]

A rapidly expanding number of laboratory techniques are available for the epidemiologic analysis of nosocomial pathogens. Table 87.11 gives an overview of the underlying principles and performance of selected typing methods. For the evaluation of performance, several criteria are used, including typeability, reproducibility, stability, discriminatory power and versatility:

- *typeability* refers to the proportion of isolates that can be assigned a type, ideally all isolates;
- *reproducibility* is the ability of the system to assign the same type on repeat testing of the same strain;
- *stability* is the probability that clonally derived isolates express the same type over time;
- *discriminatory power*, or the ability to distinguish epidemiologically unrelated isolates, is particularly important because it conditions the probability that isolates sharing the same type or clonal group of types are epidemiologically linked; and
- the *versatility* of a typing method, or its ability to type different pathogens, given minor technical modifications, is an important additional practical advantage for clinical laboratories involved in the study of nosocomial infections.

TABLE 87-11 -- Conventional and molecular typing systems for the epidemiologic analysis of nosocomial pathogens.

CONVENTIONAL AND MOLECULAR TYPING SYSTEMS FOR THE EPIDEMIOLOGIC ANALYSIS OF NOSOCOMIAL PATHOGENS						
Typing system	**Principle**	**Versatility**	**Typeability**	**Discrimination**	**Reproducibility**	**Stability**
Antibiogram	Susceptibility profile to a panel of antimicrobials	Broad	Excellent	Variable	Good	Variable
Biotype	Phenotypic characteristics (e.g. biochemical reaction profile)	Narrow	Excellent	Variable	Good	Good
Serotype	Pattern of surface antigens, defined by a set of specific antibodies	Narrow	Good	Moderate	Good	Good
Phage type	Lysis susceptibility pattern to a panel of specific bacteriophages	Narrow	Moderate	Good	Moderate	Moderate
Immunoblotting	Molecular weight patterns of protein antigens, separated by PAGE and identified with labeled antibodies	Narrow	Excellent	Good	Good	Moderate
Plasmid RFLP analysis	Pattern of restriction fragments of plasmid DNA by agarose electrophoresis	Moderate	Variable	Variable	Good	Moderate
Ribotyping	Pattern of restriction fragments of genomic DNA by Southern blot analysis with ribosomal RNA (or ribosomal DNA)-labeled probe	Broad	Excellent	Moderate	Excellent	Excellent
Southern blot RFLP analysis of genomic DNA	Pattern of genomic DNA restriction fragments by Southern blot analysis with specific DNA-labeled probe (e.g. insertion sequence element, structural gene sequence)	Narrow	Excellent	Variable	Excellent	Excellent
PFGE of macrorestriction fragments of genomic DNA (PFGE analysis)	Pattern of large genomic DNA restriction fragments generated by rarely cleaving endonucleases and resolved by PFGE	Broad	Excellent	Excellent	Good	Good

PCR-mediated gene RFLP analysis	Pattern of restriction fragments of PCR-amplified polymorphic gene region (e.g. variable size tandem repeats)	Narrow	Excellent	Variable	Excellent	Excellent
AP-PCR and randomly amplified polymorphic DNA analysis	Pattern of low-stringency PCR-amplified genomic DNA segments lying between motifs partly homologous to short, arbitrary sequence primers	Broad	Excellent	Good	Moderate	Good
Inter-repetitive element spacer length polymorphism analysis (rep-PCR analysis)	Pattern of high-stringency PCR-amplified genomic DNA segments lying between repeat motifs (e.g. insertion sequence elements, ERIC sequences) by using outwardly oriented primers	Variable	Variable	Good	Good	Good
AFLP analysis	Pattern of high-stringency PCR-amplified segments of adaptor-tagged genomic DNA restriction fragments	Broad	Excellent	Excellent	Excellent	Excellent
Nucleotide sequence analysis	Determination of DNA sequence of variable genomic regions	Universal	Excellent	Excellent	Excellent	Good
A tentative assessment of the performance characteristics of the methods is proposed. Many methods are still under evaluation and their performance may vary according to the pathogen analyzed.						

As yet there is no universally applicable, optimally discriminating, well-standardized and easily interpretable system. It is recommended that a combination of typing systems be applied to confidently assess microbial relatedness. In contrast to most phenotypic typing systems, many methods based on the analysis of genomic DNA polymorphism have broad applicability. Similar reagents (e.g. restriction enzymes) and equipment (e.g. electrophoresis systems and pattern recognition software) can be used for typing different micro-organisms. The majority of these methods provide results within 1–3 days (i.e. rapidly enough to be useful in outbreak management). In general, molecular typing systems are more discriminating than

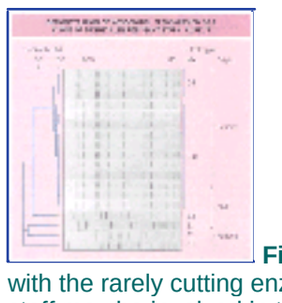

Figure 87-9 Demonstration of nosocomial transmission of a clone of methicillin-resistant *Staphylococcus aureus.* PFGE pattern of macrorestriction fragments cleaved with the rarely cutting enzyme *Sma I* of genomic DNA from MRSA. The same genotype (type D4) is found in isolates from 12 patients and 2 colonized health care staff, indicating that some members of staff may be involved in transmission in an intensive care unit department. Unrelated genotypes are also observed (B2–G1). Size marker, kb: kilobase pairs.

phenotypic methods (Table 87.11), but the results produced, typically as an electrophoretic pattern of DNA fragments (Fig. 87.9), can be complex and difficult to interpret. Guidelines have been proposed for definitions of commonly used terms, such as strain and clone, and of criteria for epidemiologic relatedness, based on the results from some DNA typing systems, such as pulsed-field gel electrophoresis (PFGE) analysis.[63] [66] Molecular typing systems have contributed greatly to our understanding of the mechanisms of acquisition of hospital infections. Pathogens that are difficult to type such as *Enterococcus faecalis* and *Candida albicans*, that were until recently believed to arise solely from an endogenous origin, have been shown to cause common-source or cross-infection outbreaks in hospitals by molecular epidemiologic studies.

Commonly used conventional and molecular methods are outlined below and their advantages and limitations are briefly reviewed. It should be stressed that adequate comparative evaluations of these methods are not available for many of the newer methods or less well-studied pathogens, making any comparative overview indicative only.

Phenotyping methods

Antibiogram

This conventional phenotypic method, particularly if it provides quantitative susceptibility data, is a useful and routinely available first-line technique used to recognize outbreaks of hospital infection with resistant bacteria, including *Staph. aureus*[67] and *A. baumannii*.[68] However, as phenotypic characters that influence fitness undergo periodic selection, unrelated clones of nosocomial pathogens exposed to the selective pressure of antimicrobials can display evolutionary convergence to the same advantageous resistance phenotype through mutations and multiple genetic exchanges. The level of discrimination of antibiogram varies according to the battery of test drugs used and the prevalence of acquired resistance traits in the study population.

Biotyping

This is based on diversity of colonial morphology, biochemical activity and other phenotypic characters, which tend to be unstable and poorly reproducible, unless highly specialized biotyping schemes are used.

Serotyping

Surface antigens are characterized using a panel of species-specific polyclonal or monoclonal antibodies. It is a moderately discriminating tool used for strain delineation of several hospital pathogens, including *Legionella* spp., *P. aeruginosa* and *C. difficile*. For a number of reasons, surface antigens are not, however, reliable markers of population structure. Clonally related strains may show antigenic variation, as occurs, for example, among highly genomically related *Legionella pneumophila* strains isolated from an outbreak of legionellosis.[69]

Phage typing

This highly specialized technique is based on susceptibility to cell lysis by a defined set of bacteriophages. Although very useful in past investigations and still used in some reference laboratories for typing important pathogens such as *Salmonella* spp. and *Staph. aureus*, it is being gradually supplanted by molecular techniques because of incomplete typeability, poor reproducibility and difficulties in pattern interpretation. [60] [70]

Immunoblot analysis

Also known as Western blot analysis, this technique involves the separation of bacterial proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and their subsequent transfer onto a membrane and reaction with labeled, broad-spectrum antibodies. It has been applied successfully to different bacterial pathogens, including *C. difficile*,[20] *Staph. aureus* and *A. baumannii*, [70] [71] but is now supplanted by genotypic methods. Immunoblotting shows good discrimination, but interpretation of pattern differences is not well defined. Other phenotypic methods that can be valuable for epidemiologic typing, such as multilocus enzyme electrophoresis and pyrolysis mass spectrometry, are less widely used.

Genotyping methods

Plasmid profile analysis

The first DNA-based typing method available for epidemiologic studies, plasmid typing has now largely been replaced by methods that determine chromosomal DNA polymorphism because of technical variability and biologic limitations. Restriction endonuclease analysis of plasmid DNA improves reproducibility and discrimination and is useful for strain differentiation of important pathogens such as *Staph. aureus*.[70] However, plasmids can be lost or acquired by conjugation and can recombine internally or into the chromosome by transposition. The in-vivo instability of plasmids must be taken into account when interpreting typing results.[72] Plasmid analysis is essential for the epidemiologic analysis of hospital infections with multiple antibiotic-resistant organisms and for tracing dissemination of mobile antibiotic resistance

genes.

Restriction fragment length polymorphism analysis

Genotyping can be performed by cleavage of chromosomal DNA with restriction endonucleases and electrophoretic separation of DNA fragments (Fig. 87.10). Restriction endonucleases that have frequent recognition sites produce complex fragment patterns by agarose or PAGE separation. The number and length of restriction fragments are affected by sequence variations that create or delete

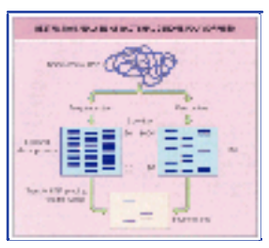

Figure 87-10 Restriction analysis of bacterial genome polymorphism. Chromosomal DNA is released after lysis of the bacterial cells. Restriction endonucleases that recognize commonly occurring sites will cut DNA in many small fragments. After conventional electrophoresis, these fragments are transferred onto a membrane and revealed by hybridization with labeled probes (Southern blot analysis). Alternatively, DNA can be restricted by endonucleases that recognize only rarely occurring sites. The few large, or macrorestriction, fragments are then separated by PFGE analysis.

recognition sites and by recombinational events that occur at or between restriction sites. To improve the resolution and facilitate the interpretation of genomic restriction fragment length polymorphism (RFLP) analysis, two approaches are used:

- transfer of restriction fragments onto membranes, followed by Southern blot hybridization with DNA probes, and
- use of endonucleases that have infrequent (<30) recognition sites in the chromosome, followed by separation of these macrorestriction fragments by PFGE (see Fig. 87.9).

Genome RFLP analysis by Southern blot hybridization

This can be applied by using different types of nucleic acid probes, including randomly cloned chromosomal fragments;[69] structural gene sequences;[73] insertion sequences and transposons;[74] and ribosomal RNA or cloned ribosomal DNA sequences, also called ribotyping.[75] The performance of these systems depends on the organism and DNA probe. Probe sequence, restriction endonucleases, electrophoresis and hybridization conditions must be optimized for each species. In general, these RFLP typing techniques are less discriminating than other genotyping systems such as PFGE and arbitrarily primed polymerase chain reaction (AP-PCR), described below.

Ribotyping

This is the most versatile and the most widely used RFLP typing strategy. The evolutionary conservation of ribosomal RNA makes *E. coli* ribosomal RNA applicable as a universal bacterial probe. Most bacteria have more than five ribosomal operons per chromosome and produce ribotype patterns of 5–15 bands. An automated ribotyping system is commercially available. Ribotyping exhibits excellent reproducibility and stability, but only moderate discriminatory power.[75] In addition, no consensus has been achieved on an optimal procedure and rules for pattern interpretation.

Genome macrorestriction analysis resolved by PFGE analysis

This method is currently preferred for molecular typing of most nosocomial pathogens.[60] [66] Low-frequency cleaving enzymes cut the bacterial chromosome into fewer than 30 fragments, typically 10–700kb in size. Periodic change in the orientation of electric field during agarose electrophoresis, or Pulsed Field Gel Electrophoresis (PFGE), allows separation and size determination of these macrorestriction fragments (see Fig. 87.10). It can be applied to any bacteria or yeasts, with minor technical modifications. In comparison with other typing methods, PFGE analysis shows equal or greater discriminatory power. Consensus rules for interpretation of PFGE patterns in outbreak investigation are available.[66]

PCR-based strategies

In PCR-mediated gene RFLP typing, a polymorphic DNA target sequence is PCR-amplified at high stringency, cut with restriction endonucleases, separated by electrophoresis and isolates are compared by RFLP pattern. Careful identification of polymorphic gene sequences and selection of discriminant enzymes must be performed for each species. Polymerase chain reaction-RFLP typing is a rapid, simple and reproducible technique, but it gives only moderate discrimination

Arbitrarily primed PCR typing and similar methods such as random amplified polymorphic DNA (RAPD) analysis are based on low-stringency PCR amplification and use a single primer of arbitrary sequence. In the early cycles of the PCR, the primer anneals to multiple sequences with partial homology and segments of DNA lying between closely spaced annealing sites are amplified to produce a strain-specific array of DNA fragments. This simple and rapid technique can be used for strain typing of bacteria, fungi and protozoans.[76] The discriminatory power varies according to the number and sequence of arbitrary primers and amplification conditions. Arbitrarily primed PCR typing is, however, limited by problems in reproducibility and the lack of consensus rules for interpretation of pattern differences. It is best used as a first-pass, efficient screening method to assess the genomic similarity of organisms during outbreak investigations.

Repetitive element PCR (rep-PCR) typing consists of high-stringency PCR amplification of spacer fragments lying between repeat motifs of the genome by the use of two outwardly directed primers. Targets for rep-PCR analysis include the repetitive extragenic palindromes, the enterobacterial repetitive intergenic consensus (ERIC) sequences, insertion sequences and other species-specific repeat elements.[77] Repetitive element-PCR typing shows moderate discriminatory power but a better reproducibility than does AP-PCR analysis.

The amplified fragment length polymorphism (AFLP) analysis has high resolution and excellent reproducibility.[78] A restriction-ligation reaction produces restricted genomic DNA fragments tagged with specially designed adapters. Primers complementary to these adapters and adjacent nucleotides are used to amplify various parts of the tagged restriction fragments.

Binary probe typing

Binary probe typing systems detect polymorphic genomic regions by solid-phase hybridization with a reference panel of sequence-variant specific probes. The resulting binary types are highly reproducible and portable. A validated binary type system is commercially available for *Staph. aureus*. [79] This approach is currently being developed based on DNA microarray technology.

Determination of nucleotide sequence

This is the most accurate method for comparing strains based on localized genomic polymorphism. It is completely portable between laboratories. This method is important in the investigation of transmission

of viruses, such as hepatitis C virus.[80] Sequence analysis of polymorphic loci is also applicable to bacterial pathogens, such as allelic variants of the protein A gene of *Staph. aureus*. Multilocus sequence typing (MLST) of bacteria consists of direct analysis of the nucleotide sequences of internal fragments of seven housekeeping genes following the concept of multiallele scoring established by multilocus enzyme electrophoresis (MLEE).[81] It is a phylogenetically robust and precise method that indexes genomic polymorphism at representative, evolutionary neutral loci. The sequence type data are relevant for population genetics, evolutionary genetics and

global epidemiology. This method is validated for *Staph. aureus*. Direct MLST database sharing between laboratories is possible over the Internet (www.mlst.net). Progress in the efficiency of automated sequence analysis systems will enlarge the application of this approach.

Interpretation of typing results

Molecular typing systems are used to determine whether isolates are clonally derived and thus likely to belong to the same chain of transmission. The level of genomic similarity that can be used to define clonally or epidemiologically related organisms depends on the resolving power of the typing system used, the genomic plasticity of the organism and the time scale of the study.[63] Rules for interpretation of differences in PFGE patterns, as applied to outbreak investigations, correlate increase in the number of restriction fragment mismatches with increasing number of genetic differences and with decreasing probability of epidemiologic relatedness.[66] Restriction pattern similarity coefficients are also useful for interpretation, particularly for large-scale studies and surveillance data.

Implementing molecular typing systems

Given the diversity of nosocomial pathogens, broad-range typing systems are to be preferred for hospital epidemiology. Confident assessment of clonality often requires the use of two or three methods in combination. The methods used should be highly discriminatory and give reproducible results, at least within the laboratory. Use of standard, 'library typing' systems is recommended for surveillance programs, especially if interlaboratory data comparisons or pooling for multicenter surveillance is the aim.[82] The methods currently best suited for hospital epidemiology, especially outbreak investigation, are the antibiogram and, when appropriate, serotyping, followed by PFGE analysis and/or PCR-based methods, particularly AFLP. Methods used for surveillance typing also include binary probe typing, MLST and other sequence-based analytic methods. Whatever method is chosen, typing should always be undertaken with a clear purpose in mind. Specific hypotheses to be tested by typing will guide the selection of the appropriate method(s) and sample of isolates. Interpretation of results requires careful comparison of clinical, epidemiologic and typing data.

Ideally, molecular typing services should be integrated into the clinical laboratory to ensure a close dialogue between the infection control team and the microbiologist. This joint approach is important to define the questions asked, to ensure the appropriate selection of isolates for typing and to interpret the results. Another option is to refer isolates for typing to a central reference laboratory. Although this may delay the availability of results, it will offer the advantage that standard 'library typing' methods are more often available to such centers and therefore the results refer to a surveillance database. Quality assurance is essential to ensure reproducibility and discrimination of typing methods. External proficiency surveys through networks of laboratories are one of the means to achieve this goal. Establishing a molecular typing service linked to the infection control and antimicrobial resistance surveillance programs appears to be cost-effective and cost-saving by contributing to a significant reduction in the incidence of nosocomial infection.[83] Additional benefits to consider include the sparing of unnecessary investigation when a suspected outbreak is not confirmed by typing and the local identification of multiresistant bacteria that need special isolation precautions.

STERILIZATION AND DISINFECTION

To minimize the risk of transmission of micro-organisms during invasive procedures that involve penetration of mucosa or skin, complete or nearly complete removal of micro-organisms from invasive devices must be achieved by sterilization or disinfection. In addition, skin antisepsis is essential for reducing the risk of autoinfection in surgery and for preventing cross-colonization between patients via contaminated hands of health care personnel.

Sterilization is the process of removing or destroying all viable micro-organisms from an object. Disinfection consists of eliminating or killing the majority of potentially pathogenic micro-organisms from a contaminated item. [84] These processes can be accomplished by using a variety of physical, chemical or physicochemical methods that denature proteins and nucleic acids (Table 87.12). Sterilization is most commonly performed by using steam heated under pressure (autoclaving), ethylene oxide gas, plasma sterilization or prolonged immersion in liquid sterilizing chemicals. Disinfection is usually achieved by using liquid chemicals for a shorter contact time. Different types of micro-organisms show varying levels of susceptibility to disinfectants. The most resistant forms are bacterial spores followed by (in decreasing order of resistance) mycobacteria, nonlipid viruses, fungi, vegetative bacteria and lipid viruses.[85] Medical devices can be classified according to the level of invasiveness of their intended use and are processed accordingly to ensure safe procedures. For example, critical devices that enter sterile tissues, such as surgical instruments or implants, require sterilization. Semicritical devices that come into contact with mucous membranes, such as endoscopes and rectal thermometers, must undergo high-level disinfection. This process should kill all micro-organisms, except for high levels of bacterial spores, and is achieved by using chemical or thermochemical methods (see Table 87.12). Noncritical items and surfaces that come in contact with intact skin, such as stethoscopes, can be submitted to low-level disinfection procedures to kill the majority of pathogenic micro-organisms, with the exception of mycobacteria and bacterial spores.

Sterilization and disinfection must be preceded by meticulous cleaning to eliminate organic material from the device and to reduce the biologic burden. A number of technical factors are important to ensure adequate sterilization and disinfection of medical material: the type and load of contaminating micro-organisms, the nature and physical configuration of the object, the concentration of chemical agent used, and the temperature and pH of the process.[85] To provide effective processing, it is preferable that high-level disinfection and sterilization are performed by specialized personnel in adequately centralized facilities. To monitor effectiveness, initial validation and regular quality control must be performed. Physical and chemical monitoring of each process cycle is necessary. Additional quality assurance can be achieved by periodically incorporating biologic indicators, generally a known inoculum of bacterial spores, and by testing their nonviability by culture after processing. Flexible fiberoptic endoscopes are especially difficult to clean internally and disinfect adequately and specific reprocessing guidelines must be followed.[86]

In recent years, new disinfection and sterilization technologies have been developed.[87] Ortho-phtalaldehyde, a new aldehyde compound, has several advantages compared with glutaraldehyde as it requires no activation, is less irritating to the eyes and nasal passage,

TABLE 87-12 -- Sterilization and disinfection techniques commonly used in hospital practice.

STERILIZATION AND DISINFECTION TECHNIQUES COMMONLY USED IN HOSPITAL PRACTICE			
Objective	**Method**	**Principle of microbial inactivation**	**Example of use**
Sterilization	Steam autoclaving	Thermal denaturation	Metallic surgical instruments
	Ethylene oxide	Thermochemical denaturation	Heat-labile surgical instruments
	Gamma-irradiation	Ionizing denaturation	Implantable medical devices (catheters, protheses)
	Gas plasma	Generated free radical induced denaturation	Heat-labile instruments
Disinfection	Chlorine	Chemical denaturation	Water disinfection
	Alcohols	Chemical denaturation	Skin antisepsis
	Iodophors	Chemical denaturation	Skin and mucosae antisepsis
	Aldehydes	Chemical denaturation	Flexible endoscopes
	Peracetic acid	Chemical denaturation	Flexible endoscopes

has excellent stability, does not require exposure monitoring and has a barely perceptible odor. In the field of sterilization, hydrogen peroxide gas plasma sterilization was recently cleared by the US Food and Drug Administration.

The most important and unresolved problem remains the effective disinfection and sterilization of material contaminated with unconventional agents such as prions. Prions indeed exhibit unusual resistance to conventional chemical and physical decontamination methods. Decontamination of medical devices must take into account a determination of the risk related to the patient (e.g. those with known or suspected Creutzfeldt-Jakob disease (CJD)) and the nature of the tissue in contact with the instrument, separated into high risk (e.g. brain, eyes), low risk (e.g. CSF, kidney, liver, spleen) and no documented risk (e.g. blood, serum, skeletal tissue).[88] Specific disinfection and sterilization procedures have been recommended for decontamination of these unconventional agents.[88] The infectivity of tissue remains controversial and, in particular, the new variant form of CJD may differ from sporadic and familial forms in terms of tissue infectivity.[89] [90] In Europe, strict recommendations have been made considering the potential risk associated with the emergence of this new variant form of CJD.[91] [92]

HAND HYGIENE AND ISOLATION PRECAUTIONS

To prevent transmission of potentially infectious micro-organisms from colonized or infected patients to other hospitalized patients, visitors or health care personnel, systematic hand hygiene precautions must be taken during patient care. In addition, patients who have certain conditions must be placed in special isolation to prevent transmission of pathogens by other routes. Isolation methods include special room requirements and the use of protective equipment, generally worn by patient care personnel.

Between 1970 and 1996, the CDC formalized a series of guidelines to implement isolation precautions. Category-specific precautions are based on the classification of infectious diseases into six categories according to their route of transmission: strict, contact, respiratory, tuberculosis, enteric and drainage-secretion precautions.[93] Disease-specific isolation is tailored according to the mechanism of transmission of each infectious disease. Although logical, these strategies are complex to implement. They tend to be difficult to apply to newly admitted patients who present with an undiagnosed, presumably infectious, syndrome.

In the face of the HIV pandemic, universal precautions were proposed to complement the disease-specific isolation precautions.[93] The goal was to protect health care workers from infection with blood-borne viruses such as HIV. Universal precautions required the use of gloves and other barrier precautions to avoid contact with blood, internal body fluids and genital secretions. To reduce the risk of percutaneous exposure to blood and body fluids, minimal handling of needles and sharp instruments and their safe disposal was recommended. At the same time, a simplified system, called body substance isolation, was proposed as an attempt to merge the objectives of contact isolation and universal precautions. [94] This system is based on the premise that any contact with tissue, body fluids, wounds, mucous membranes and secretions is potentially contaminating and a source of transmission of infectious agents to health care workers and other patients. The systematic use of gloves, and other barrier precautions when necessary, was advocated for any contact described above with all patients. The latest CDC guidelines incorporate this strategy with the additional need for handwashing after removing gloves, which was shown to recontaminate hands, as 'standard precautions' (Table 87.13). [95] These systematic precautions are supplemented by patient-specific isolation precautions, grouped into three categories, for preventing contact, droplet and airborne transmission of specific pathogens. In addition to physical isolation in a private room and use of barriers such as gloves and gowns, cohort nursing can contribute to effective isolation, as shown for the prevention of nosocomial RSV infection.[96]

The growing importance of nosocomial transmission of multiple antibiotic-resistant pathogens has led national advisory bodies to issue specific guidelines for the early detection, isolation and decolonization of patients with infection or colonization with MRSA,[31] [97] vancomycin-resistant enterococci,[98] vancomycin-resistant *Staph. aureus*[99] and extended-spectrum ß-lactamase producing Enterobacteriaceae.[100]

Since the seminal intervention studies of Semmelweis 150 years ago, it has been known that adequate hand hygiene is the most effective measure to reduce cross-infection resulting from patient care. The optimal methods of hand hygiene in hospital practice remain under debate.[101] This relates to major problems with health care

TABLE 87-13 -- Key points in the 1996 Centers for Disease Control and Prevention guidelines for isolation precautions in hospitals.*

KEY POINTS IN THE 1996 CDC GUIDELINE FOR ISOLATION PRECAUTIONS IN HOSPITALS				
Feature	**Standard precautions**	**Contact precautions**	**Droplet precautions**	**Airborne precautions**
Patient room	Standard	Private	Private	Private; door closed; well ventilated (minimum 6 air changes per hour; negative pressure)
Gloves	Contact with blood, body fluids, mucous membranes, secretions, excretions or broken skin	Before entering room	Standard	Standard
Handwashing	After glove removal; between patients	Standard; with antiseptic soap	Standard	Standard
Gown	Before procedure likely to generate projections of blood, body fluids, secretions or excretions	Contact with patient; if patient has diarrhea or open drainage of wounds or secretions	Standard	Standard
Mask	Before procedure likely to generate projections of blood, body fluids, secretions or excretions	Standard	If within 3 feet of patient	Before entering room
Examples of conditions	All patients	• Multidrug-resistant bacteria of special clinical and epidemiologic significance (e.g. MRSA, VRE)	• Meningitis	• Tuberculosis, or suspected tuberculosis
		• Major abscess, cellulitis or decubiti	• Diphtheria	• Measles
		• *Clostridium difficile* infection	• Pertussis	• Varicella, disseminated zoster
		• Acute diarrhea, in an incontinent or diapered patient	• Influenza	
		• RSV infections, bronchiolitis and croup in young infants	• Mumps	
			• Rubella	
			• Streptococcal pharyngitis, pneumonia or scarlet fever in young children	

* *Adapted from Garner.*[95] Refer to the original guideline for complete information.

worker compliance and tolerance with traditional handwashing. The latter is generally practiced only about 30–50% of the time it should be.[102] With the increasing use of gloves to prevent soiling of hands during contaminating procedures, greater emphasis is placed today on hand antisepsis, particularly in Europe. Handrub with alcohol-based disinfectants, such as 70% isopropyl-alcohol, is more effective, faster, easier to use and better tolerated compared with handwashing and medicated soap antisepsis (Table 87.14). Promoting hand cleansing with an alcohol-based handrub solution by multimodal educational programs that include observation of health care workers' hand hygiene practices and regular feedback of compliance rates can improve these practices and should be encouraged.[103]

TABLE 87-14 -- Hand hygiene methods.

HAND HYGIENE METHODS			
Feature	**Handwashing**	**Hand alcohol antisepsis**	**Gloves**
Advantages	• Removes soiling	• High efficacy for removal of transient microflora (3–4 log)	• Prevents soiling
	• Low cost	• Low cost	• Protects health care worker
		• Fast and easy to use	
Disadvantages	• Low efficacy for removal of transient microflora (2 log)	• Efficacy decreased on soiled hands	• Contamination during removal
	• Time consuming	• Skin intolerance	• Skin intolerance
	• Skin intolerance		• Cost
The use of gloves to prevent hand soiling and to reduce hand contamination can be combined with alcohol antisepsis after glove removal. Frequent handwashing with plain soap and water or with disinfectant/detergent and water leads to considerable problems of skin intolerance.			

CONTROL OF ANTIBIOTIC RESISTANCE

Learned societies have published guidelines for optimizing antibiotic use and controlling antibiotic resistance in hospitals by multimodal strategies. [104] [105] Action plans based on similar approaches have been formulated by public health agencies. [37] Important components of these guidelines include better medical training; education and regulation of prescribers by consultant specialists; multidisciplinary co-operation between hospital management, clinicians, infectious diseases specialists, infection control team, microbiologists and hospital pharmacists; formulary-based guidelines on anti-infective therapy and prophylaxis; monitoring and auditing drug use; surveillance

of resistance in the hospital flora; early detection and investigation of outbreaks by molecular typing; detection and notification of patients colonized with transmissible resistant bacteria for patient isolation and/or decolonization; and promotion of standard precautions and hand hygiene. These guidelines are mostly based on limited evidence from analytic studies and uncontrolled intervention studies. Few strategies have been tested for cost-effectiveness.[106] The applicability of guidelines may be limited by the ecologic heterogeneity in health care organizations. Because each hospital has its own ecosystem that evolves rapidly, effective solutions should be tailored to local needs and resources. Mathematical modelling can contribute to the prediction of the most effective interventions based on local ecology and epidemiology.[46] [107] On the other hand, regional co-ordination can lead to successful control of emerging antibiotic-resistant nosocomial pathogens.[108]

Antibiotic policies, including restriction, substitution and cycling, can help contain the prevalence of resistance in hospitals, particularly in outbreak settings. Selective antibiotic restriction has been followed by decreased transmission of resistant nosocomial pathogens, such as *C. difficile* [109] and *Klebsiella pneumoniae*. [110] Controlling the antimicrobial usage by restriction based on antibiotic formularies has been met with mixed success in the long term. [111] Studies linking antibiotic control to lower prevalence of resistance are difficult to interpret because of methodologic problems. [111] [112] [113] Computer-assisted support to prescribing antibiotics, based on locally defined consensus guidelines, can improve antibiotic use, reduce drug costs and limit the rise of antibiotic-resistant pathogens.[114] As antibiotic prescribers, physicians in all disciplines contribute to antimicrobial resistance and must strive for prudent antibiotic use. Innovative strategies are needed to improve the control of antibiotic-resistant nosocomial infections.

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Chapter 88 - Employee Health Service

Kent A Sepkowitz

An active hospital employee health service (EHS) or occupational health service is crucial to control of nosocomial infection. This is accomplished in several ways:

- identification and vaccination of workers susceptible to vaccine-preventable diseases;
- active surveillance for diseases such as tuberculosis (TB); and
- prompt diagnosis of transmissible illnesses such as respiratory syncytial virus (RSV) or hepatitis A virus infection in symptomatic workers.

Several reviews address specific infectious disease problems frequently encountered by an EHS.[1] [2] [3] [4]

ORGANIZATION OF EMPLOYEE HEALTH SERVICE

The organization of the EHS varies according to the size and administrative structure of a hospital. At larger hospitals, the EHS is staffed by full-time nurses and physicians, as well as by clerical staff. The model at smaller hospitals is characterized by fewer staff, limited hours and more limited capabilities (Fig. 88.1). The administrative reporting mechanisms for the EHS also vary considerably from hospital to hospital. The employee health service may be part of infection control, the Department of Nursing, or may report primarily to a senior hospital administrator.

Few studies have defined the scope of EHS activity.[5] [6] [7] In one analysis of 21,886 annual visits:

- 25% were for preventive services, such as vaccination and routine examinations;
- 25% were for occupationally related illnesses, including infectious diseases, stress and accidents; and
- 15% were for administrative procedures, such as prescription renewal, requests for letters and completion of forms.[5]

Another study reviewing 3000 annual visits found 30% of visits to the EHS were for occupationally related illness or injury, and of these less than 3% were for fever. [6]

ROUTINE TESTS FOR NEW EMPLOYEES

The medical evaluation of new employees is a fundamental job for the EHS. In general, all new employees should have tests performed to evaluate for immunity to various potentially transmissible diseases (Table 88.1). These include serologic tests and a tuberculin skin test for TB. Routine chest radiography should be reserved only for those employees with known pulmonary problems, a previously abnormal chest radiograph, or a positive tuberculin skin test. Recent concern about potential bioterrorism has resulted in some EHSs requesting information about previous vaccinia vaccination as well.

Serologic testing for numerous potential pathogens is essential, both for the safety of the health care worker and for patient safety. The Centers for Disease Control and Prevention (CDC) has published recommendations on immunization of health care workers[8] and infection control in hospital personnel.[9]

Since 1992 the US Occupational Safety and Health Administration has required demonstration of seropositivity to hepatitis B virus (HBV) for all health care workers with occupational exposure to blood or body fluids.[10] Seronegative health care workers must either receive vaccine or formally decline the three-vaccine series, which is offered free of charge. The rates of vaccination have subsequently increased, but remain low, at about 50%.[10] The observed decrease in HBV-associated morbidity and mortality among health care workers is attributed to improved vaccine coverage.[11] Varicella-zoster virus (VZV) antibody status should also be determined. In some countries (e.g. the USA) vaccination of susceptible employees is recommended but not required.[12] Specific countries and regions may require evidence of immunity to other pathogens, such as measles, mumps and rubella.

Few hospitals track pneumococcal vaccine status of employees and none mandate or even suggest routine vaccination of health care workers. However, reports have appeared of outbreaks of pneumococcal disease,[13] including those caused by penicillin-resistant strains.[14] Although pneumococcus has not been firmly established as a nosocomial pathogen, additional reports of possible nosocomial spread may force a careful reconsideration of the issue. Finally, as new vaccines eventually become available for such illnesses as RSV and cytomegalovirus (CMV), the EHS will be charged with assuring vaccine program implementation and delivery (Table 88.2).

Because of the USA cases of intentionally spread anthrax, concern has grown about other agents, especially smallpox. The potential role of vaccinia vaccine to prevent an outbreak of smallpox is being actively discussed. At issue is the risk of rapid spread of smallpox throughout a hospital, as has been described, versus the risk of the vaccine itself which is estimated to cause a fatal reaction in one per million persons and various other adverse events less commonly. Should the decision be made to vaccinate health care workers, the EHS will be entrusted with identifying, educating, and vaccinating the employees, as well as monitoring them for adverse events.

REGULAR FOLLOW-UP TESTING AND REVACCINATION

Routine surveillance to exclude infectious diseases is required for only a few illnesses, of which TB is a classic example. With the sudden rise in TB cases in many USA cities in the late 1980s and with the recognition of several dramatic nosocomial outbreaks of the disease, hospitals scrambled to implement effective TB control programs. These were plagued by poor or absent baseline data, nonspecific boosting as a result of frequent skin testing of employees, and concerns about the lack of effective prophylaxis against multidrug-resistant TB (MDR-TB). Because of this experience, effective tuberculosis control measures were developed. Control programs have waned in recent years in many developed nations, coincident with the national decrease in numbers of cases of TB. Of looming concern is development of an approach to protecting workers in less developed nations, where tuberculosis is much more common and resources are much more scarce.

Figure 88-1 Example of organization and interdisciplinary roles in an EHS.

TABLE 88-1 -- Immunizations recommended for hospital workers.*

IMMUNIZATIONS RECOMMENDED FOR HOSPITAL WORKERS			
Immunization against:	**Indications**	**Vaccine dose and schedule**	**Contraindications**
Hepatitis A virus	Employees working in high-risk areas (e.g. food service, neonatal intensive care unit) without serologic evidence of previous hepatitis A virus infection	1.0ml im at 0 and 6–12 months	Known hypersensitivity to any component of the vaccine.
Hepatitis B virus	All employees at risk for occupational exposure to blood or body fluids	1.0ml im (deltoid) at 0, 1 and 6 months	Hypersensitivity to yeast
Influenza	All hospital employees	0.5ml im annually	History of anaphylactic reaction to eggs
Measles	Employees with no history of physician-diagnosed measles or no laboratory evidence of immunity	0.5ml subcutaneously of trivalent measles, mumps and rubella vaccine	Pregnancy, history of anaphylactic reaction to eggs or neomycin, severe febrile illness, immunosuppression, recent receipt of iv immunoglobulin
Mumps	Employees without a history of physician-diagnosed mumps, laboratory evidence of immunity, or proof of vaccination on or after their first birthday	As for measles (above)	As for measles (above)

Pneumococcus	Employees over 65 years of age or with underlying cardiac, pulmonary, liver, renal or immunocompromising disease	0.5ml subcutaneously or im; booster dose every 6–10 years	Safety in pregnancy unknown
Rubella	Employees without verification of live vaccine delivery on or after their first birthday or proof of laboratory immunity	As for measles (above)	As for measles (above)
Tetanus-diphtheria	Employees who have not completed their initial series or who have not received a booster dose within 10 years	Initial series: 0.5ml im at 0.1 and 6–12 months; booster dose for immunized employees: 0.5ml im every 10 years	History of neurologic or hypersensitivity reaction following a previous dose; first trimester of pregnancy
Varicella	Employees with patient contact who have no history of chickenpox and negative varicella titer	0.5ml at 0 and 4–8 weeks	Hypersensitivity to vaccine, gelatin, neomycin; immunosuppression or immunodeficiency; active tuberculosis; febrile illness; pregnancy

** Data from Diekema and Doebbeling, 1995.*[4]

The best approach is to maintain an effective ongoing TB control program, both during outbreaks and, as importantly, when no outbreaks are occurring. [15] Such a program should test all tuberculin-negative employees every year. Consideration should be given to 'two-step' testing of new employees, especially those who have received bacille Calmette-Guérin (BCG) vaccination or are older than 40–50 years. This minimizes the potential effect of the booster phenomenon, which is particularly seen in these groups (in serial skin tests the reaction may be 'boosted' in subsequent tests). The CDC has recently updated recommendations and advises that hospitals with a modest rate of tuberculosis annually (six cases or less) may decide to test only new employees and as needed for outbreak investigation; routine annual testing in this circumstance is no longer recommended.[15]

Chemoprophylaxis with isoniazid should then be offered according to standard guidelines.[16] Chemoprophylaxis for health care workers exposed to patients with isoniazid-resistant MDR-TB is complex and not standardized. This topic is discussed in detail in Chapter 37 .

In the USA, the EHS plays a central role in the annual drive to revaccinate against influenza. Many countries in Europe do not routinely vaccinate workers against influenza. In the USA, experts feel that an adequate number of studies have demonstrated benefit to both health care workers and to patients; despite this, acceptance rates for influenza vaccination among healthcare workers in the USA remain poor, generally well below 50%.[17] Compliance rates for vaccinations

TABLE 88-2 -- Other potential health care worker vaccines: controversies and needs.

OTHER POTENTIAL HEALTH CARE WORKER VACCINES: CONTROVERSIES AND NEEDS	
Controversies Vaccine	**Issue**
BCG	Lack of proven efficacy in preventing pulmonary disease
	Compromise of the specificity of the purified protein derivative (tuberculin) test
Vaccinia	Threat of smallpox unknown but probably quite low
	Adverse reaction in 1/100,000; fatal reaction in 1/million
Meningococcus	Immunity not long-lived so protection from occupational exposure is unlikely
	Does not protect against several strains
Anthrax	Vaccine must be given yearly and requires multiple injections to establish immunity
	Risk for nosocomial spread (e.g. from patient clothes) very low
Needs Vaccine	**Issue**
HIV	Occupational risk remains
	Hospitals spend substantial funds on 'safer needle' systems and management of exposures
Hepatitis C	Treatment of disease not established
	Hospitals spend substantial funds on 'safer needle' systems and management of exposures
RSV	May cause significant morbidity and mortality rates among transplant recipients
	Symptoms non-specific in adults and cannot be distinguished from common cold
CMV	Would allay fears of CMV-seronegative workers
Norwalk virus	Rapid spread may affect up to 90% of those exposed, potentially interfering with a hospital's ability to care for patients

given during identified outbreaks, such as of measles or pertussis, are much higher.

Measles outbreaks in the early 1990s among previously vaccinated persons led to the recognition of waning vaccine-induced immunity and gave rise to the new recommendation that an additional measles vaccine was necessary before or during teenage years.[18] Evidence that the new requirement is in fact effective is lacking, although there have been no outbreaks of measles in USA hospitals in over 20 years.

Because of the issue of waning vaccine-induced measles immunity, many have wondered about the durability of vaccine-induced immunity to infections such as varicella, pertussis and HBV. At present, in the USA there is no recommendation extant for routine follow-up surveillance to determine immunity to these vaccine-preventable diseases. In many European countries, a booster dose of HBV vaccine is given to workers at risk every 5–10 years. However, should outbreaks of diseases continue to occur in well-vaccinated populations, as has been described with pertussis,[19] routine follow-up surveillance to demonstrate immunity may be advisable.[20] With measles programs, repeat serologic testing with targeted revaccination is significantly cheaper than simply vaccinating all workers.[21]

OUTBREAKS CAUSED BY HEALTH CARE WORKERS WHO ARE CARRIERS

Health care workers who are carriers may be responsible for the spread of infections to other health care workers and to patients (Table 88.3).

Reports have documented the spread of HBV[22] and hepatitis C virus (HCV)[23] from health care workers to patients. This emphasizes the need for careful guidelines for the potentially infectious health care worker[33] that do not interfere with that person's individual rights.[34] These thorny issues surfaced in the USA in the early 1990s, when the possibility of mandatory testing for HIV of all health care workers was considered by the CDC.[35]

Spread of HBV from an occupationally infected surgical resident has been well documented by routine and molecular epidemiologic

TABLE 88-3 -- Infections spread by health care workers to patients or other health care workers.

INFECTIONS SPREAD BY HEALTH CARE WORKERS TO PATIENTS OR OTHER HEALTH CARE WORKERS	
Infection	**Comment**
Hepatitis B virus[22]	e-Antigen positivity and high level of viremia associated with transmission

Hepatitis C virus[23]	Surgeon resumed work following medical control of his hepatitis C infection
Methicillin-resistant *Staphylococcus aureus* [24] [25]	'Cloud adult' and chronic sinusitis may facilitate spread
Group A streptococci[26]	Carriers may harbor the organism in throat, vagina, rectum or skin
Salmonella[27] [28]	Routine surveillance for dietary workers of unproven benefit
Tuberculosis[29] [30]	Health care workers may spread disease through hospitals
Measles, rubella[31] [32]	Unvaccinated medical students are source of many outbreaks

methods.[22] In one report, 19 (13%) of 144 susceptible persons became infected after receiving surgery from a surface- and e-antigen-positive thoracic surgery resident. Molecular analysis of 13 available strains showed identity with the surgeon's strain. A semiquantitative polymerase chain reaction test of the surgeon's serum revealed more than one billion infectious particles per milliliter, suggesting viral load may be an important factor determining transmissibility. More importantly from the EHS perspective, the index case had chosen to decline HBV vaccine 2 years before he himself became infected via occupational exposure. Thus the entire episode may have been averted with a more persuasive HBV vaccination program.

Probable transmission of HCV from a cardiac surgeon to at least five patients who underwent valve replacement was reported from

Spain.[23] In this study, molecular analysis showed significant homology between the surgeon's and the patients' virus. The surgeon was treated with interferon-a2b and ribavirin until his HCV RNA level, as measured by polymerase chain reaction, became undetectable. At that point, he was allowed to resume performing surgery.

Nasal carriage of *Staphylococcus aureus*, which is found in 20–90% of health care workers, has been associated with outbreaks of infection, often in intensive care units. Poor handwashing by workers is frequently revealed as the cause. Chronic sinusitis may also contribute.[24] An additional means of transmission — the 'cloud adult' — has also been described.[25] This refers to the occurrence of a viral upper respiratory infection in a patient with established nasal carriage of *S. aureus*. The associated sneezing results in aerosolization of the resistant bacteria, hence the term. In one study, a single 'cloud adult' surgeon was associated with spread of methicillin-resistant *S. aureus* to eight of 43 persons in an intensive care unit. The amount of bacteria aerosolized was significantly decreased when the surgeon wore a mask.[25] Nasal carriage can often be eradicated by local application of mupirocin ointment combined with oral ciprofloxacin and rifampin (rifampicin). Early recurrences are seen with this regimen, however, as has occurred with other regimens that have been used through the years. Selective screening of health care workers during nosocomial outbreaks of *S. aureus* infections is recommended. [36]

Outbreaks of group A streptococci traced to a health care worker have frequently been reported.[3] In many circumstances the health care worker is asymptomatic, but cultures of throat, vagina, rectum, or skin yields group A streptococci. When investigating a possible outbreak, it is essential to culture all potential sites routinely. The mode of spread may be by direct contact, via droplet transmission, or, in one report, through infected food. [26]

Dietary workers who are salmonella carriers have spread infection via the hospital kitchen, as may occur in restaurants.[31] [32] In one study routine surveillance of all dietary staff failed to prevent a nosocomial outbreak, bringing into question the value of surveillance.[27] Interruption of another outbreak required treatment of all dietary staff with trimethoprim-sulfamethoxazole (co-trimoxazole). [28]

OUTBREAKS DUE TO HEALTH CARE WORKERS WHO ARE ILL

A significant problem is the health care worker who continues to work despite feeling ill and thus spreads disease to patients and other staff. Younger doctors may be particularly likely to be associated with outbreaks of vaccine-preventable infections, such as measles and rubella.[31] In one study, 12% of all health departments had reported that medical students or interns were probably the source case of a nosocomial outbreak of measles or rubella.[32]

Varicella may also be spread by health care workers. In one report, a susceptible pediatric resident failed to report an exposure and continued to work until he himself developed chickenpox.[37] This resulted in exposure of 250 patients or workers and a cost of about $US10,000. No secondary cases of varicella occurred. The CDC and the Advisory Committee on Immunization Practices have recommended but not required varicella vaccination for health care workers.[12] Other methods of decreasing the likelihood of transmission of varicella from health care worker to patient, including wearing masks during the potentially infectious days and taking aciclovir pre-emptively, have also been suggested.[38]

Tuberculosis has been transmitted from staff to patients and to other staff.[29] [30] In one report eight health care workers developed active TB, and tuberculin conversion rates ranged from 30 to 48% on wards housing patients with TB.[29] On one ward, transmission from health care worker to health care worker was strongly implicated by molecular fingerprint analysis. Distinction between community and occupational transmission may be difficult in health care workers who also socialize together.[30] Although older reports suggest that compliance with isoniazid prophylaxis in health care workers, particularly physicians, is poor, one study showed that more than half of eligible health care workers completed the recommended course of therapy.[39] Furthermore, rates of completion were higher among physicians (74%) than among nonphysicians (48%). The occurrence of several cases of TB among health care workers at this medical center might have resulted in higher compliance rates.

The well-publicized case of a Florida dentist who apparently spread HIV to at least four patients became the subject of a national debate regarding patients' right to know the health status of their physicians and dentists.[40] Numerous thorough 'look-back' studies of surgeons, other dentists and other practitioners, however, failed to demonstrate transmission.[26] In France, a probable case of transmission from an infected orthopedic surgeon to a patient has forced a re-evaluation of this issue.[41]

OCCUPATIONALLY ACQUIRED INFECTIONS IN HEALTH CARE WORKERS

Similar to many other occupations, health care workers are at risk for a wide variety of occupationally acquired illnesses (Table 88.4).[38] [42] Three relatively simple interventions have been shown through the years to ensure the safety of workers. These include handwashing, vaccination and appropriate isolation of persons with known or suspected infectious diseases. More detailed consideration of this topic has been published.[38] [42]

Blood-borne transmission

Transmission of blood-borne infection has been the subject of intense scrutiny. Studies have demonstrated that HIV is transmitted in about 0.3% of exposures overall.[43] [44] To date, at least 57 health care workers have developed occupationally-acquired HIV and another 137 have probably developed disease from an occupational exposure. [45] Five confirmed cases have been transmitted through mucocutaneous exposure, whereas the others have involved a percutaneous injury. [45]

A retrospective case-control study determined that zidovudine prophylaxis reduced transmission by about 80% compared with placebo.[46] Factors associated with increased risk of transmission included a deep (intramuscular) injury [adjusted odds ratio (OR) 15], visible blood on the sharp device (OR 6.2), injury from a needle that had been used to enter a blood vessel (OR 4.3) and a source patient who has terminal AIDS (OR 5.6). New US Public Health Service recommendations have suggested that therapy be tailored to the likelihood of transmission and that a two- or three-drug combination be selected and given for 1 month (Table 88.5).[47] The recommended drugs include zidovudine, lamivudine and indinavir. This regimen costs about $US1000 for medications and monitoring.[48] Side-effects resulting in changes or discontinuation of the regimen are common (57%), but seldom severe (see also Chapter 117).[49]

The risks and rates of occupationally acquired HBV infection are even more disturbing. The CDC estimates that 120–195 health care workers die each year in the USA as a result of occupationally acquired HBV.[50] The rate of transmission is determined by the e-antigen status of the source case (about 3% for an e-antigen-negative and 30% for an e-antigen-positive source case). [51] A more recent estimate has suggested that the annual number of deaths among health care workers is decreasing as vaccine coverage increases.[11]

The morbidity and mortality rates of occupationally-acquired HCV infection are not yet well defined. Transmission occurs in about 3% of percutaneous exposures.[42] [52] The rate of progression of HCV

TABLE 88-4 -- Infectious diseases transmitted from patient to health care worker.[*]

INFECTIOUS DISEASES TRANSMITTED FROM PATIENT TO HEALTH CARE WORKER			
Infection		**Transmission rate**	**Comment**
Blood-borne	HIV	0.3%	New recommendations include up to three-drug therapy for needlestick injuries
	Hepatitis B virus	e-Antigen negative: 3%	More than 100 health care workers die annually from hepatitis B virus complications
		e-Antigen positive: 30%	Vaccination rates improving
	Hepatitis C virus	3%	No known therapy or prophylaxis though interferon appears promising for acute disease
	Cylomegalovirus	Very low	Studies do not demonstrate transmission risk to health care workers
	Ebola	Very high	Health care workers account for >30% of cases in recent outbreaks
Air-borne	Tuberculosis	20–50% in outbreaks	Several deaths from occupationally acquired multidrug-resistant tuberculosis and treatment
	Varicella	5–15%	New vaccine should decrease rates
	Measles	Very high	Physicians and nurses account for majority of occupational cases
	Rubella	13%	Some affected health care workers have opted to terminate pregnancy
	Parvovirus B19	>25%	Spread is less dramatic than in schools
	Respiratory syncytial virus	>40%	Infection control interventions decreased spread to patients but not to health care workers
	Adenovirus	>20%	May be spread more efficiently if source case intubated
	Pertussis	43%	Spread in one outbreak to 87 health care workers (2% of all health care workers)
Enteric	Hepatitis A virus	20%	Vaccine now available
	Salmonella	5–20%	May spread to health care worker from food, excretions or from patient
	Helicobacter pylori	Unknown	Implications of higher seroprevalence among endoscopists unknown
	Norwalk virus	>50%	Extremely high rates of spread for nursing assistants
	Cryptosporidia	>30%	Animal handlers at particular risk
	Clostridium difficile	Unknown	One health care worker died of apparent occupationally acquired *Clostridium difficile* infection

** Data from Septkowitz, 1996.*[36] [42]

TABLE 88-5 -- Recommended HIV postexposure prophylaxis for percutaneous injuries.[£]

RECOMMENDED HIV POSTEXPOSURE PROPHYLAXIS FOR PERCUTANEOUS INJURIES					
Exposure type	**Infection status of source**		**Source of unknown HIV status[†]**	**Unknown source[§]**	**HIV-negative**
	HIV-positive class 1[*]	**HIV-positive class 2[*]**			
Less severe[¶]	Recommend basic two-drug PEP	Recommend expanded three-drug PEP	Generally, no PEP warranted; however, consider basic two-drug PEP[?] for source with HIV risk factors[‡]	Generally, no PEP warranted; however, consider basic 2-drug PEP[?] in settings where exposure to HIV-infected persons is likely	No PEP warranted
More severe[¢]	Recommend expanded 3-drug PEP	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP[?] for source with HIV risk factors[‡]	Generally, no PEP warranted; however, consider basic 2-drug PEP[?] in settings where exposure to HIV-infected persons is likely	No PEP warranted

£ From CDC.[47]

† Source of unknown HIV status (e.g. deceased source person with no samples available for HIV testing).

§ Unknown source (e.g. a needle from a sharps disposal container).

* HIV-positive class 1 — asymptomatic HIV infection or known low viral load (e.g, <1500 RNA copies/ml); HIV-positive class 2 — symptomatic HIV infection, AIDS, acute seroconversion or known high viral load. If drug resistance is a concern, obtain expert consultation. Initiation of postexposure prophylaxis (PEP) should not be delayed pending expert consultation, and, because expert consultation alone cannot substitute for face-to-face counseling, resources should be available to provide immediate evaluation and follow-up care for all exposures.

¶ Less severe (e.g. solid needle and superficial injury).

? The designation 'consider PEP' indicates that PEP is optional and should be based on an individualized decision between the exposed person and the treating clinician.

‡ If PEP is offered and taken and the source is later determined to be HIV-negative, PEP should be discontinued.

¢ More severe (e.g. needle, deep puncture, visible blood on device, or needle used in patient's artery or vein).

to chronic liver disease, including cirrhosis, appears in excess of 80% for all patients. [53] The lack of an effective vaccine, therapy or prophylaxis makes this disease of increasing concern for health care workers.[54] A recent study examined the effectiveness of interferon in treating acute symptomatic HCV infection.[55] Included in the study were 14 health care workers who acquired the disease by needlestick. The authors demonstrated a rate of disappearance of detectable virus in the bloodstream of more than 95%. The fact that all patients were symptomatic was an extremely unusual feature of this study and may make generalization of the findings difficult. Evaluation of any percutaneous injury should be thorough and systematic, with attention to the injury and the source (Table 88.6 Table 88.7 Table 88.8).

Outbreaks of various viral hemorrhagic fevers, including Ebola virus, typically affect large numbers of health care workers in the treating hospitals. [42] Cytomegalovirus is a concern to pregnant health care workers, but studies have not demonstrated an increased rate of disease transmission to pediatric nurses or to other health care workers.[9] [42] The CDC does not recommend that any CMV seronegative worker avoids work among patients with high rates of CMV disease, such as those with AIDS or bone marrow transplant recipients. Rather, adherence to universal precautions is sufficient to protect the worker.[9]

B virus (simian herpes B virus) is a risk for researchers who work with old world monkeys, such as macaques and rhesus monkeys. At

TABLE 88-6 -- Recommendations for the contents of the occupational exposure report.[*]

RECOMMENDATIONS FOR THE CONTENTS OF THE OCCUPATIONAL EXPOSURE REPORT
Date and time of exposure
Details of the procedure being performed, including where and how the exposure occurred; if related to a sharp device, the type and brand of device and how and when in the course of handling the device the exposure occurred
Details of the exposure, including the type and amount of fluid or material and the severity of the exposure (e.g. for a percutaneous exposure, depth of injury and whether fluid was injected; for a skin or mucous membrane exposure, the estimated volume of material and the condition of the skin, for example chapped, abraded, intact)
Details about the exposure source (e.g. whether the source material contained HBV, HCV, or HIV; if the source is HIV-infected, the stage of disease, history of antiretroviral therapy, viral load, and antiretroviral resistance information, if known)
Details about the exposed person (e.g. hepatitis B vaccination and vaccine-response status)

Details about counseling, postexposure management and follow-up

** From CDC.*[47]

TABLE 88-7 -- Factors to consider in assessing the need for follow-up of occupational exposures.*

FACTORS TO CONSIDER IN ASSESSING THE NEED FOR FOLLOW-UP OF OCCUPATIONAL EXPOSURES
Type of exposure
Percutaneous injury
Mucous membrane exposure
Nonintact skin exposure
Bites resulting in blood exposure to either person involved
Type and amount of fluid/tissue
Blood
Fluids containing blood
Potentially infectious fluid or tissue (semen; vaginal secretions; and cerebrospinal, synovial, pleural, peritoneal, pericardial and amniotic fluids)
Direct contact with concentrated virus
Infectious status of source
Presence of HBsAg
Presence of HCV antibody
Presence of HIV antibody
Susceptibility of exposed person
Hepatitis B vaccine and vaccine response status
HBV, HCV, and HIV immune status

** From CDC.*[47]

least 40 cases have occurred from occupational exposure (usually bites) and many have died of progressive encephalomyelitis.[56] The CDC has established guidelines for prevention and management, including serologic screening of all imported monkeys.[57] Cases should be treated with an active agent, such as aciclovir. Lifelong suppression may be necessary because relapse has occurred even after prolonged courses.

Air-borne transmission

The risk of caring for patients with TB was demonstrated in the USA during several large hospital-based outbreaks of MDR-TB.[58] In these outbreaks at least 20 health care workers developed clinical MDR-TB,[59] whereas hundreds of others became latently infected and will remain at risk for re-activation disease during the decades ahead. At least one health care worker has died because of drug toxicity from

TABLE 88-8 -- Evaluation of occupational exposure sources.*

EVALUATION OF OCCUPATIONAL EXPOSURE SOURCES
Known sources
Test known sources for HBsAg, anti-HCV, and HIV antibody
Direct virus assays for routine screening of source patients are *not* recommended
Consider using a rapid HIV-antibody test
If the source person is *not* infected with a blood-borne pathogen, baseline testing or further follow-up of the exposed person is *not* necessary
For sources whose infection status remains unknown (e.g., the source person refuses testing), consider medical diagnoses, clinical symptoms, and history of risk behaviors
Do not test discarded needles for blood-borne pathogens
Unknown sources
For unknown sources, evaluate the likelihood of exposure to a source at high risk for infection
Consider likelihood of blood-borne pathogen infection among patients in the exposure setting

** From CDC.*[47]

a second-line regimen given for her MDR-TB[60] and several others have died of MDR-TB itself.[59] Recommendations for preventing transmission of TB in hospitals stress the need to place suspected cases into effective isolation immediately.[9] [15]

The introduction of the varicella vaccine has changed the infection control strategy for containing VZV infection at many hospitals.[2] [12] Transmission rates are poorly defined, but the incidence of new cases among susceptible individuals may exceed 10% per year if no precautions are taken.[2] [38] In addition, the problem of a potentially infectious health care worker is discussed above.

Measles virus may enter a hospital from the community or spread to the community from a hospital-based outbreak. Attack rates among susceptible individuals are high, vaccine failures occur, and physicians and nurses are at highest risk. Outbreaks of rubella[61] and parvovirus B19 [62] are of particular concern to pregnant employees because both infections may affect fetal development. Some health care workers in rubella outbreaks have opted to terminate pregnancies.[61] Transmission of RSV, adenovirus and pertussis to health care workers has been well-documented and probably occurs much more frequently than has been reported.[38]

Fecal-oral transmission

Nosocomial outbreaks of hepatitis A virus with spread to health care workers have occasionally been reported, often from neonatal intensive care units. One report described an outbreak traced to a child with an immune defect that prevented a detectable antibody response to hepatitis A virus.[63] This delayed serodiagnosis resulted in spread to 15% of the staff, a rate similar to that in other outbreaks.

Salmonella may spread in hospitals in numerous ways:

- from a point source, such as contaminated food;
- from patient to health care worker; and
- from contact with contaminated excretions.

As noted above, routine surveillance of hospital dietary employees may not be effective. [27] [28] In addition to nurses, laundry workers appear at increased risk.[64]

Other common enteric infections such as Norwalk virus and cryptosporidiosis may spread to workers.[42] Norwalk virus, a small round structured virus, is particularly contagious, and may affect more than 90% of health care workers in close contact. The clinical implications of elevated seroprevalence to *Helicobacter pylori* among persons who perform endoscopy are unknown.[42]

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Chapter 89 - Recreational Infections

Alastair Miller

INTRODUCTION

Although the arrival of the 21st century does not seem to have produced the predicted 'Age of Leisure' people continue to spend their leisure time in more adventurous and imaginative ways. This chapter examines how these activities expose them to increased risks of infection and disease. As with many other factors that predispose to clinical infection, recreational behavior may either expose the host to infective organisms or modify the host's immune response, thereby increasing susceptibility to infection and disease. Recreational infection can be classified by recreational activity or according to the particular infections (or systems infected — Fig. 89.1) Inevitably there is considerable overlap with other sections of this book, such as geographic and travel medicine (see Chapter 142 , Chapter 143 , Chapter 144 , Chapter 145 , Chapter 146 , Chapter 147 , Chapter 148 , Chapter 149 , Chapter 150 , Chapter 151 , Chapter 152 , Chapter 153 , Chapter 154 , Chapter 155 , Chapter 156 , Chapter 157 , Chapter 158 , Chapter 159 , Chapter 160 , Chapter 161 , Chapter 162 , Chapter 163 , Chapter 164 , Chapter 165 , Chapter 166 , Chapter 167 , Chapter 168 , Chapter 169 , Chapter 170 , Chapter 171 , Chapter 172 , Chapter 173 , Chapter 174 , Chapter 175 , Chapter 176 , Chapter 177 , Chapter 178 , Chapter 179 , Chapter 180 , Chapter 181 , Chapter 182 , Chapter 183 , Chapter 184 , Chapter 185 , Chapter 186), sexually transmitted diseases (see Chapter 73 , Chapter 74 , Chapter 75 , Chapter 76 , Chapter 77 , Chapter 78 , Chapter 79) and zoonotic infections (Chapter 222).

TRAVEL

Travel is a common recreational activity either as an end in itself or in order to participate in other recreations. Travelers may be exposed to infection either during the journey or at their destination. During travel there may be exposure to gastrointestinal pathogens in mass-produced food or exposure to respiratory pathogens from air-conditioning units and fellow travelers. In addition the general fatigue of long-distance travel may perhaps lower resistance to infection in a nonspecific manner.

Outbreaks of food poisoning from airline food occur frequently and are well described,[1] and even cholera has been transmitted in this way.[2] An estimated 4.5 million North Americans travel on cruise ships each year and a recent review has drawn attention to the incidence of respiratory and gastrointestinal infection associated with this activity.[3] Outbreaks of gastrointestinal illness have been caused by contaminated food and water,[4] and in addition there have been descriptions of more prolonged outbreaks of infection associated with the possibility of person-to-person transmission and environmental contamination. This type of outbreak is usually thought to be due to viral infection with organisms such as Norwalk agent or small round structured viruses.[5] [6] Historically a number of bacterial infections, including cholera and typhoid, have been associated with shipborne spread.[3]

Respiratory infection, including multidrug-resistant pulmonary tuberculosis, has also been transmitted during aircraft flight,[7] [8] and there is a well-recognized association between outbreaks of *Legionella* infection and air-conditioning systems in holiday hotels.[9]

Western travelers are increasingly seeking more exotic destinations where, as a result of poverty and poor infrastructure in the local population, they may be at risk of infection with common pathogens (particularly of the gastrointestinal and respiratory tracts). They may also be at risk of more exotic infections that do not exist in their own country (e.g. malaria). By the nature of the travel, the patient with infection often may not have access to the level of diagnostic and therapeutic interventions that would be regarded as standard in the developed world, so empiric treatment of presumed infections is often required. These risks are discussed in detail in Chapter 143 .

ZOONOSES

Zoonoses are infections of animals that can be transmitted to humans, who may act either as a dead-end host or may propagate the infection further. The resulting infection may or may not be clinically apparent. This topic is addressed in Chapter 164 , Chapter 174 , Chapter 175 , Chapter 176 , Chapter 177 , Chapter 178 , Chapter 179 , Chapter 180 , Chapter 181 , Chapter 182 , Chapter 183 , Chapter 184 , Chapter 185 , Chapter 219 and Chapter 222 , but issues specifically related to recreation are discussed here. Many leisure activities increase the opportunity for contact between humans and animals, with consequent increased risk of infection.

Hiking and camping (particularly light-weight backpacking) increase the risk of zoonoses. People may hike in a temperate climate or, increasingly, may choose to trek in a tropical or developing country. These activities increase the potential for contact with infected animals. Infection can then be transmitted by a number of possible routes such as:

- inhalation (e.g. Q fever, anthrax);
- ingestion of contaminated food or water (e.g. infections with *Salmonella* and *Brucella* spp.);
- animal bites (e.g. rabies, skin infections);
- exposure of skin to contaminated water (e.g. leptospirosis, schistosomiasis); and
- arthropod vectors (e.g. arboviruses, Lyme disease).

Zoonotic infection acquired by inhalation

Inhaled zoonoses that can be acquired by the intrepid outdoor explorer include Q fever (caused by *Coxiella burnetii*) and brucellosis (more commonly acquired by ingestion). Rarer problems include plague, anthrax, tularemia and psittacosis.[10]

Zoonotic infection acquired by ingestion

Many of the common 'food poisoning' organisms are zoonoses, and these are discussed in detail in Chapter 43 , Chapter 144 , Chapter 161 , Chapter 163 and Chapter 164 . Certain recreational activities particularly expose participants to increased risks of ingesting pathogenic organisms (which are often zoonotic, although they may be exclusively human parasites). Backpackers drinking inadequately boiled or purified water may become infected with *Cryptosporidium* spp., *Giardia* spp., hepatitis A, *Aeromonas* spp., and *Salmonella* spp. Barbecues are particularly notorious for leading to the ingestion of inadequately cooked meat (or fish) and consequent infection with *Salmonella* spp.,[11] *Campylobacter* spp.,[12] and other more exotic organisms such as *Trichinella* spp. There have been well-documented outbreaks of cryptosporidial infection in children enjoying recreational visits to farm open days.[13] [14]

Arthropod-borne zoonoses

Viruses that must spend some of their life cycle in a blood-sucking arthropod are known as arboviruses (see Chapter 222). Over 200 such viruses have been identified and over 70 have been reported as affecting humans. In 1994, 100 cases of presumed or confirmed arboviral disease were reported from 20 states of the USA.[15] These

TABLE 89-1 -- Recreational activities associated with risk of infection.

RECREATIONAL ACTIVITIES ASSOCIATED WITH RISKS OF INFECTION	
Activity	**Risk**
Travel	Infection during travel: gastrointestinal respiratory pathogens
	Geographic (tropical) infection
'Outdoor' recreation: hiking, backpacking, trekking, barbecues, etc.	Zoonoses: ingestion, inhalation, inoculation, arthropod spread
Water contact: jacuzzi, bathing, nonbathing water activity (wind surfing, canoeing, sailing, etc.)	Inhalation, inoculation, ingestion (see Table 89.2)
Contact sport: rugby, football, etc.	Skin infections, blood-borne pathogens
Strenuous exercise	Upper respiratory infection (possibly; see Table 89.3)

were all encephalitis viruses (mainly Californian and St Louis encephalitis), principally spread by mosquitoes. In 2001 there was a large outbreak of West Nile virus in the Eastern US with 48 human cases and numerous infections of birds (principally crows). [16] By 2002, the outbreak had spread to most of the contiguous United States, with several thousand human cases, and over 100 deaths (see Chapter 4). Yellow fever is a life-threatening mosquito-borne, zoonotic viral infection and the illness remains a risk for travelers and residents during outdoor activities in endemic regions in Africa and South America. In Europe, tick-borne encephalitis is regularly reported from Austria and southern Germany.[17] A major risk factor is outdoor recreation (in particular, walking through long grass while wearing short trousers). An inactivated vaccine against tick-borne encephalitis virus is available.

Tick-borne rickettsiae (see Chapter 14 , Chapter 179 and Chapter 235) are also potential pathogens among those who enjoy 'the great outdoors'.[18] They are mainly of the spotted fever group. In southern Europe, Africa and India, the disease is called tick typhus or boutonneuse fever and is caused by *Rickettsia conorii*. In the USA, it is Rocky Mountain spotted fever, caused by *Rickettsia rickettsii*. New rickettsioses identified during the past decade include Japanese spotted

TABLE 89-2 -- Infections spread by recreational contact with water.

INFECTIONS SPREAD BY RECREATIONAL CONTACT WITH WATER				
Mode of spread	**Organisms**			
	Bacteria	**Viruses**	**Protozoa**	**Helminths**
Fecal-oral spread (accidental ingestion)	*Vibrio* spp. *Salmonella* spp. *Campylobacter* spp. *Aeromonas* spp. *Escherichia coli* *Shigella* spp.	Enteroviruses (including polio) Hepatitis A Small round structured viruses	*Cryptosporidia* *Giardia*	
Spread by direct inoculation	*Pseudomonas* spp. *Aeromonas* spp. *Vibrio* spp. *Mycobacterium marinum* *Leptospira* spp.		*Acanthamoeba* *Naegleria*	*Schistosoma*
Spread by aerosol or aspiration	*Legionella* spp. *Pseudomonas* spp.	Adenoviruses		

fever, Astrakhan fever, Flinders Island spotted fever, California flea typhus, African tick-site fever and *Rickettsia slovaca* infections in central France.

Scrub typhus may affect the trekker in eastern Asia. The infective organism is *Rickettsia tsutsugamushi*. The reservoir is rodents and the vector is the larva (chigger) of the trombiculid mite. Clinically the disease resembles other rickettsial infections, and prevention and treatment strategies are similar.

Lyme disease (see Chapter 54), caused by infection with *Borrelia burgdorferi*, is another condition that may be acquired by recreational exposure. The reservoir consists of mammals such as rodents and deer, with infection being spread by hard ticks (the *Ixodes ricinus* complex).

INFECTIONS CAUSED BY EXPOSURE TO WATER

A large number of infections can be caused by exposure to water (Table 89.2), and some of these have already been discussed in the section on zoonoses. Exposure to water can take place in a variety of recreational contexts. Trekkers and fishermen may wade through infected water, people may bathe in fresh water or sea water, and people may undertake other non-bathing recreational activities in water (e.g. water skiing, sailing, canoeing). There is also an increasing popularity of spa baths, whirlpool baths and jacuzzis.

As with arthropod-borne infections, infections related to water may be acquired by a number of routes, including ingestion, aspiration, inhalation of aerosols, and penetration of skin or mucous membranes by invasive organisms. A variety of clinical infections, including gastrointestinal infection, hepatitis, conjunctivitis, pneumonia, skin and soft tissue infection, may result, and numerous diverse organisms have been implicated.

Pathogenic organisms may enter the water from exogenous sources such as human contamination (e.g. sewage), animal and bird contamination, and farm effluent. Organisms may also come directly from aquatic animals or protozoa or be free living in the water supply.

Infection associated with whirlpools

Jacuzzis, whirlpool baths, and spa baths, which are increasingly found in leisure resorts, are all based on the principle of being bathed in warm water through which jets of water and bubbles of air are blown in order to produce feelings of relaxation and pleasure. They therefore share the potential for the transmission of cutaneous,

mucosal and respiratory infection. The main pathogens implicated in these infections are *Pseudomonas aeruginosa* and *Legionella pneumophila*. *Pseudomonas* infections of the skin were initially described in the early 1980s.[19] The first reports were of folliculitis, but infection of wounds, eyes, ears and urinary tract have now been described.[20] Fatal *Pseudomonas* pneumonia in an immunocompetent male has been associated with jacuzzi exposure.[21]

Legionella spp. (mainly *L. pneumophila*, but other species are also implicated) cause two distinct syndromes:

- legionnaires' disease (or legionnaires' pneumonia), which is usually a severe pneumonic illness requiring appropriate antibiotic treatment; and
- Pontiac fever, which is generally a more benign self-limiting illness causing myalgia, fever and headache.

The latter syndrome has frequently been associated with whirlpool use, although a prolonged outbreak of legionnaires' pneumonia among cruise ship passengers associated with exposure to a contaminated whirlpool spa has been described.[22]

Infection from bathing

Numerous case reports and reviews have associated bathing in swimming pools, natural fresh water and the sea with gastrointestinal, respiratory and cutaneous infection. In swimming pools there have been reports of infection with *Shigella*, *Giardia* and *Cryptosporidium* spp., and various viruses including hepatitis A virus.[23]

The association of sea bathing and disease is a major political issue because millions of dollars are spent in the developed countries in an effort to improve sewage disposal and enhance the 'quality' of bathing water. Microbiologic standards now exist for bathing water in Europe and North America. There is certainly a risk of infection from swimming in heavily contaminated water, but the risk of minor symptomatic infection from swimming in less heavily polluted water remains contentious.[24]

In the 1950s the UK Public Health Laboratory Service used a case-controlled method and showed no link between polio and sea bathing.[25] Cabelli's classic work under the aegis of the US Environmental Protection Agency in the 1970s suggested a dose-response relationship between the microbiologic contamination of bathing water and self-reporting of gastrointestinal symptoms.[26] These studies have been criticized for looking at self-reported symptoms in self-selected groups, with no control for other risk factors for gastrointestinal symptoms.

More recently, a large UK study attempted to address these criticisms by randomizing holiday makers to be 'swimmers' or 'non-swimmers'.[27] This study showed a significantly higher rate of gastroenteritis in the swimmers and demonstrated a dose-response relationship between occurrence of symptoms and concentrations of fecal streptococci (although only with the concentration measured at chest height). Currently, coliform counts are used to assess water quality and the authors of the above study could not demonstrate a correlation between symptoms and the coliform count.

In addition to gastroenteritis, an Australian study showed increased reporting of respiratory, eye and ear symptoms among swimming beach-goers as opposed to non-swimming beach-goers. The incidence of the reported symptoms increased with increasing levels of pollution.[28] The authors of the UK study have now published their results of non-enteric illness acquired during bathing and their results are in broad agreement with those of the Australian study.[29]

Infection in non-swimming recreational water activities

In addition to the hazards of bathing detailed above, many people are exposed to infection by recreational use of water where swimming is not the primary purpose. Such activities include angling, canoeing, water skiing, sailing and white water rafting.

Leptospirosis (see Chapter 181 and Chapter 230) is traditionally regarded as a significant risk. It is estimated that on average in the UK there are five million recreational water users each year exclusive of bathers, and yet among this at-risk population there are only 2.5 cases of leptospirosis a year.[30] The annual total incidence of leptospirosis in England and Wales is more than ten times that figure; it occurs principally among agricultural workers. Leptospirosis is a zoonotic infection that is mainly carried by rodents. It is estimated that about 25% of the rats in UK are infected. The risk of contracting infection relates less to the overall water quality than to the density of the local rodent population.

By the nature of the sport, canoeing involves high level exposure to water and, in addition to leptospirosis, other infections can be acquired. There is an increased incidence of gastrointestinal symptoms, and it has been shown that more than 50% of canoeists had experienced 'flu-like' symptoms shortly after canoeing. [31] An outbreak of blastomycosis has also been reported in canoeists along Wisconsin rivers in northern USA.[32]

Miscellaneous water-related infections

Naegleria and *Acanthamoeba* spp. are free-living amebae with no insect vector or human carrier state. They have been isolated on a worldwide basis from water and soil, and rarely they produce a severe amebic meningoencephalitis that is usually fatal (see Chapter 244). Schistosomiasis is dealt with in detail in Chapter 167 . The cercariae of human schistosomes penetrate intact human skin and then migrate to their favored site to commence their maturation. Within 24 hours the penetration of the skin can produce a pruritic papular rash that is called 'swimmer's itch'. Avian schistosomes are found in temperate climates, including in the Great Lakes of North America, and although they are unable to mature past the cercarial stage in a human host and therefore cannot give rise to later stage schistosomiasis, they can be responsible for producing a significant 'swimmer's itch'.

Katayama fever occurs typically 4–8 weeks after infection and is associated with fever, chills, headache and cough. There is hepatosplenomegaly and lymphadenopathy, and usually a significant eosinophilia. It is caused by both *Schistosoma japonicum* and *Schistosoma mansoni*, the latter being particularly recognized in swimmers who have bathed in lake Malawi and the other rivers and lakes in East Africa (see Chapter 67).

INFECTION SPREAD BY DIRECT CONTACT

Many sports require close physical contact on the sports field and may also involve close contact in the changing rooms with for example shared towels and shaving equipment. Tetanus is caused by contamination of a wound by the spores of *Clostridium tetani*. After contamination, the organism then elaborates a toxin that produces the clinical syndrome of tetanus. Although immunization against tetanus is widely practised there is still a risk to those playing contact sports (especially rugby and football), as well as to those pursuing more leisurely activities such as gardening.

The close contact in the scrum of rugby football may transmit herpes simplex virus and cause a condition called scrumpox or herpes gladiatorium. This is highly infectious and may spread rapidly between players. Aciclovir is effective treatment.

The moist atmosphere of changing rooms may promote the transmission of respiratory infections as well as a number of cutaneous infections such as verrucas, athletes foot (*Tinea pedis*) and dhobie itch (*Tinea cruris*). The spread of these tineal infections may be facilitated by sharing towels and washing equipment.

Gardening is usually considered a fairly safe past-time, but tetanus is a potential risk and sporotrichosis (see Chapter 239) can be acquired by scratches from rose thorns and similar injuries.

Blood-borne infection transmission in contact sport

The risk of transmission of blood-borne pathogens during contact sport is thought to be extremely low. There were large outbreaks of hepatitis amongst orienteers in Sweden from 1956 to 1966, and on the basis of the clinical and epidemiologic picture these were assumed to be due to hepatitis B virus (HBV), although a serologic test was not available.[33] As part of these outbreaks, 568 cases of hepatitis occurred between 1957 and 1963; several modes of transmission were postulated, including twigs contaminated with infected blood inoculating subsequent competitors, contaminated water in stagnant pools and transmission during washing after competition. It was established that 95% of orienteers received scratches or wounds during the competition. The outbreak was curtailed by the introduction of regulations that banned competitors who had hepatitis from competing for 15 months and that specified compulsory protective clothing. More cases were reported when these regulations were relaxed. There has also been a report of an outbreak of HBV infection among sumo wrestlers in Japan.[34] There is one report from Italy of an HIV-positive football player transmitting infection to another player during a collision when both players were bleeding profusely,[35] but the risks are generally considered to be negligible.

Numerous guidelines exist to limit the risk still further[34] — in rugby football, for example, a player with an open or bleeding wound must leave the field until the wound is covered and the bleeding controlled. Similar policies now apply for many college level and professional sports settings.

EFFECT OF EXERCISE ON THE IMMUNE SYSTEM

Although it is clear from the above that recreational activity can expose participants to numerous infective agents that they might not otherwise encounter, it is by no means so obvious whether recreation (in particular, vigorous exercise) has any clinically significant effect on immune function.[36] There is increasing evidence that physical exercise may bring benefit in terms of cardiovascular health. However, the evidence from the immunologic perspective is less obvious.

There are numerous anecdotal reports of increased incidence of upper respiratory infection in athletes and there have been attempts to examine this systematically. However, despite numerous reviews there is no consistent association between physical activity and incidence of clinical upper respiratory infection. Nor has any consistent immunologic abnormality been demonstrated in high-level athletes. This inconsistency has many parallels with the situation in chronic fatigue syndrome, and many top athletes who have recurrent infections develop a clinical condition indistinguishable from chronic fatigue syndrome.

The overall message from anecdotal reports, from case-controlled studies of symptoms, from animal studies and from laboratory tests of immune function seems to be that moderate regular exercise enhances immunity whereas sudden unusual exertion or consistent, very high-grade training may have a deleterious effect. This is described as the

TABLE 89-3 -- Effects of exercise on the immune system.

EFFECTS OF EXERCISE ON THE IMMUNE SYSTEM

Symptoms/self-reported infections (anecdote and case-control)	Most studies suggest that moderate regular exercise reduces frequency and severity of upper respiratory tract infections but excessive training increases it
	Many studies are subjective and it may be that athletes are more aware of their symptoms than are controls
	Some increase in infection may be due to local factors such as mouth breathing rather than to any change in systemic immunity
Animal studies	Exhaustive exercise during experimental viral infection increases mortality and morbidity from that viral infection; the effect may be attenuated by exercise prior to infection
	Similar results have been shown in pneumococcal infection — exercise prior to infection protected against mortality but forced exercise after infection enhanced mortality
Immune function studies	Moderate exercise in HIV-positive people has been shown to produce some increase in $CD4^+$ lymphocyte count Excessive training in non-HIV-infected people has been shown to suppress $CD4^+$ lymphocyte counts
	Heavy exercise decreases lymphocyte proliferation and levels of IgA; the decreased levels of IgA may correlate with increased incidence of upper respiratory tract infections
	Regular moderate exercise will increase levels of natural killer lymphocytes
	Exercise generally increases the release of proinflammatory cytokines and acute phase proteins

'J-shaped curve' correlating exercise and immunity.[36] [37] Animal studies suggest that exercising before infection is beneficial, whereas exercising when infected is harmful. Some of the reported immunologic effects of exercise are listed in Table 89.3 .

It is generally advised that people who are suffering from acute infections do not participate in vigorous exercise. This seems common sense and most people would probably not feel like doing so, although definite evidence of harm remains contentious.

CONCLUSION

Recreational activities can expose participants to novel infectious agents that they are less likely to encounter in other contexts. In many of these the diagnosis may not be very obvious unless the condition is considered. Physicians need to add 'recreational history' to the already extensive list of travel, occupational and animal exposure details about which they need to enquire when evaluating a patient with a suspected infection. Whether recreational activity can alter immune function remains more controversial, although there seems to be increasing consensus that regular physical exercise may be of benefit but that excessive exercise may increase the risks of infection.

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Chapter 90 - Occupational Infections

Rodrigo L C Romulo

INTRODUCTION

Individuals may have an increased risk for acquiring certain infections as a result of their occupation. These include workers whose occupations involve significant exposure to potentially infectious material such as:

- health care workers exposed to patients who have communicable diseases;
- workers in contact with live animals (veterinarians, livestock handlers, animal trainers, hunters, trappers, zoo workers, laboratory animal workers, fishermen);
- workers in contact with animal carcasses or byproducts (butchers, abattoir workers, meat inspectors, raw animal product processers);
- biomedical laboratory workers exposed to infectious clinical specimens, cultures or infectious laboratory animals; and
- workers exposed to infections in outdoors environments such as arthropod-borne infections or major mycoses (forestry workers, agricultural workers, sewer and irrigation workers, wildlife workers).

Infections in health care workers are discussed in Chapter 88 . Those whose livelihood requires travel to and work in endemic areas have increased risk for malaria, leishmaniasis, trypanosomiasis, diarrheal diseases and other travel-related illnesses (see Section 6).

Groups of individuals working together or living as a result of their occupation may be at risk for outbreaks of airborne infections such as tuberculosis, pharyngoconjunctival fever or meningococcal disease. Such groups include military personnel living in barracks and factory shift workers.

This chapter provides a quick reference for clinicians faced with patients in whom an occupational infection is suspected. The information is presented entirely in tables which offer possible diagnoses for specific clinical presentations considering the patient's occupational exposure. The occupations have been loosely categorized as workers with animal contact; biomedical laboratory workers; farm, forestry and other outdoors workers; and sewer and irrigation workers.

TABLE 90-1 -- Workers who have animal contact: infections presenting with skin lesions.

WORKERS WHO HAVE ANIMAL CONTACT: INFECTIONS PRESENTING WITH SKIN LESIONS			
Clinical presentation	**Possible animal sources**	**Possible infectious agent**	**Comments**
Papular or vesicular lesions			
Enlarging pruritic papule that ulcerates and develops eschar and edema	Herbivores, particularly cattle, goats, donkeys, horses, water buffalo	*Bacillus anthracis* (cutaneous anthrax)	Transmission via contact with animals sick with or that have died from anthrax or with contaminated raw animal materials; bioterrorism-related outbreak in postal workers[1] (see Ch 9 , Ch 147 and Ch 226)
Papular or pustular lesions on arms or hands	Domestic and wild mammals, birds, fish, ticks, crustaceans	*Listeria monocytogenes* (primary cutaneous listeriosis)	Cases usually mild, resolve with treatment[2] (see Ch. 226)
Annular erythematous lesions with scaling, or erythematous scaling of scalp (tinea capitis, tinea corporis, tinea barbae)	Cattle	*Trichophyton verrucosum* (cattle ringworm)	Cases reported in dairy and cattle farmers, abattoir worker and veterinarian in Australia[3] (see Ch. 240)
Solitary papule on extremity with shallow ulceration and scar formation, followed by multiple subcutaneous nodules 'ascending' proximally	Fish, shellfish, other marine animals	*Mycobacterium marinum*	Risk factors include trauma while practicing water/fish-related occupation or hobbies (see Ch. 233)
Indurated papule, then ulcer, then sinus formation	Monkeys	*Mycobacterium simiae*	Case in wild animal handler acquired in Peruvian jungle[4] (see Ch. 233)
Vesicular lesions resembling smallpox	Monkeys from western and central Africa	Monkeypox virus	Rare in humans, most cases in Zaire (see Ch. 218)
Vesicles progressing to pustules, which coalesce and scab	Sheep and goats	Parapoxvirus (orf)	Transmitted via direct contact with animal lesions; lesions mild, but can become painful and pruritic and may persist for weeks (see Ch. 218)
	Cattle	Parapoxvirus (pseudocowpox, paravaccinia, milker's nodule)[5]	
	Gray seals	Parapoxvirus (sealpox)[6]	
Cellulitis/abscesses			
Localized painful cellulitis, slightly raised violaceous, peripheral spread with central fading	Wide range of vertebrates and invertebrates; mainly fish, swine, turkey, ducks, sheep	*Erysipelothrix rhusiopathiae* (erysipeloid)	Greatest risk in fishermen, fish handlers, butchers, abattoir workers, veterinarians[7] (see Ch. 226)
Acute painful cellulitis following animal bite or scratch	Mostly cats and dogs; other wild and domestic mammals	*Pasteurella* spp.	Must consider if cellulitis develops within 24 hours of bite or scratch (see Ch. 91)
Cellulitis of the hand	Freshwater fish grown by aquaculture	*Streptococcus iniae*	Outbreak in Canada from *Tilapia* fish imported from USA[8] (see Ch. 225)
Necrotizing cellulitis, fasciitis following fish-associated percutaneous injury	Fish and shellfish[9]	*Vibrio vulnificus*	Seen in fish 'farmers' or fishermen, often immunocompromised (see Ch. 230)
		Vibrio (Photobacterium) damsela	Severe cases from catfish puncture wound, knife cut while filleting bluefish, after rabbitfish bite (see Ch. 230)
		Edwardsiella tarda	Associated with fish spine injury (see Ch. 228)
		Aeromonas hydrophila	Not necessarily fish-related but may infect water-associated wounds (see Ch. 229)
Cellulitis, fulminant sepsis following bite or scratch	Mainly dog (also cat)	*Capnocytophaga canimorsus; C. cynodegmi* [10]	More rapid and severe progression in asplenic individuals, alcoholics and patients on corticosteroids (see Ch 91 and Ch 229)

Cutaneous or subcutaneous abscesses, sepsis syndrome in most cases	Animals not reservoir for human disease	*Burkholderia pseudomallei* (melioidosis)	Farmers in South East Asia and northern Australia; acquired by soil contamination of skin abrasions and possibly ingestion, inhalation or intranasal inoculation[11] (see Ch. 229)

TABLE 90-2 -- Workers who have animal contact: infections presenting with acute fever.

WORKERS WHO HAVE ANIMAL CONTACT: INFECTIONS PRESENTING WITH ACUTE FEVER			
Clinical presentation	**Animal exposure**	**Possible infectious agent**	**Comments**
Non-specific fever			
Fever, chills (endocarditis)	Pigs, wild boar	*Streptococcus suis*	Rare in humans; mostly in pig farmers, others who have close contact with pigs;[12] meningitis in hunter who butchered wild boar[13] (see Ch. 225)
	Horses	*Streptococcus equinus*	Case in UK farmer[14] (see Ch. 225)
	Fish	*Streptococcus iniae*	Endocarditis, meningitis and arthritis in diabetic who had rheumatic heart disease, osteoarthritis, renal failure after percutaneous injury while preparing fish[8] (see Ch. 225)
Fever, sweats, anorexia, headache, back pain	Mainly cattle, also buffalo, camels, yaks	*Brucella abortus*	Worldwide distribution, increased risk in veterinarians, livestock farmers, abattoir workers, meat inspectors (see Ch 180 and Ch 231)
	Goats, sheep, camels	*Brucella melitensis*	
	Swine	*Brucella suis* biovars 1–3	
	Reindeer, caribou	*Brucella suis* biovar 4	
	Dogs	*Brucella canis*	
Malaise, fatigue, fever, shaking chills, myalgias, arthralgias, dark urine	Cattle (in Europe)	*Babesia bovis, Babesia divergens* (babesiosis)	Thought to be transmitted to humans in Europe by cattle tick *Ixodes ricinus* (see Ch. 245)
Fever, myalgias, bacteremia	Cattle	*Bartonella vinsonii* subsp. *arupensis*	Case in rancher in Wisconsin, USA[15] (see Ch. 235)
Fever, chills, headache, vomiting, migratory arthralgias	Rats, mice, squirrels; also cats, dogs, pigs, ferrets, weasels	*Streptobacillus moniliformis* (USA), *Spirillum minus* (Asia) (rat-bite fever)	Increased risk in animal laboratory workers[16] (see Ch. 230)
Hemorrhagic fever			
Abrupt-onset fever, headache, rigors, nausea, vomiting, petechial rash or ecchymosis, generalized hemorrhage	Sheep, cattle	Rift Valley fever virus	Herdsmen, farm workers, wildlife rangers, abattoir workers and veterinarians exposed to infected animals or their products in Africa [17] (see Ch. 183 and Ch. 222)
	Cattle, sheep, goats, antelopes	Crimean-Congo hemorrhagic fever	Outbreak involving abattoir workers, livestock market employees, and animal skin processors in United Arab Emirates[18]
Fever and cough			
Undifferentiated febrile illness or atypical pneumonia	Most common reservoirs are cattle, sheep, goats	*Coxiella burnetii* (Q fever)	Worldwide distribution; usually affects those who have direct contact with infected animals[19] (see Ch. 235)
	Birds (poultry, parrot family, finches, pigeons, pheasants, egrets, seagulls, puffins); sheep	*Chlamydia psittaci* (psittacosis)	Increased risk in poultry farmers, pet shop workers, abattoir and processing plant workers (see Ch. 236)
Fever, chills, cough, chest pain, dyspnea, occasionally hemoptysis	Animals not reservoir for human disease	*Burkholderia pseudomallei* (melioidosis)	Farmers in South East Asia and Northern Australia; acquired by soil contamination of skin abrasions and possibly ingestion, inhalation or intranasal inoculation (see Ch. 175 and Ch. 229)
Other febrile syndromes			
Fever, chills, headache, myalgias, abdominal pain, conjunctival suffusion, muscle tenderness, jaundice	Wide range of mammals; rats most common source worldwide; also dogs, wild mammals, cats, pigs, other livestock	*Leptospira* spp. (Leptospirosis)	Worldwide distribution; cases usually result from contact with water or soil contaminated with infected urine; outbreak in Missouri, USA in humans exposed to infected swine[20] (see Ch. 181 and Ch. 230)
Recurrent fever, pleuropericardial effusion		*Campylobacter fetus* ssp. *fetus*	Case in slaughterhouse worker in UK[21] (see Ch. 230)
Abrupt-onset fever, chills, headache, malaise, anorexia, fatigue, tender lymphadenopathy, skin ulcer	Voles, squirrels, rabbits, hares, muskrats, beavers, hamsters	*Francisella tularensis* (tularemia)[19]	Transmitted via insect bite, aerosol, contact with contaminated water or mud, animal bites; increased risk in farmers, animal laboratory workers, veterinarians, hunters, trappers, meat handlers (see Ch. 176 , Ch. 177 and Ch. 231)
	Rats most important reservoirs worldwide; also squirrels, prairie dogs and other urban and sylvatic rodents	*Yersinia pestis* (plague)	

TABLE 90-3 -- Workers who have animal contact: infections presenting with acute fever and neurologic abnormalities.

WORKERS WHO HAVE ANIMAL CONTACT: INFECTIONS PRESENTING WITH ACUTE FEVER AND NEUROLOGIC ABNORMALITIES			
Clinical presentation	**Animal exposure**	**Possible infectious agent**	**Comments**
Fever, severe headache 3–5 days after flu-like illness	Rodents	Lymphocytic choriomeningitis virus	Increased risk in laboratory workers handling mice, hamsters (see Ch. 222)
Fever, headache, meningismus, declining sensorium (meningitis)	Pigs, wild boar	*Streptococcus suis*	Close contact with pigs documented in most cases; case in hunter who butchered wild boar[13] (see Ch. 225)
Fever, anorexia, nausea, vomiting, headache, pain or paresthesias at site of animal bite followed by hyperactivity, disorientation, bizarre behavior	In developing countries mostly dogs; farm animals (e.g. cattle, horses, sheep), wild mammals (e.g. raccoons, skunks, foxes, coyotes, wolves, jackals, mongoose, bats, cats)	Lyssavirus genotype 1 (rabies virus), genotype 3 (Mokola virus), genotype 4 (Duvenhage virus), genotype 5 (European bat lyssavirus 1), genotype 6 (EBLV 2), Australian bat lyssavirus	Incubation period usually 20–90 days: bites on head 25–48 days; on extremity 46–78 days; genotypes 3–6 found in Africa and Europe and associated with fatal encephalitis following bat bites (see Ch. 153)
High fever, myalgia, sore throat, dizziness, drowsiness, progressive encephalitis	Pigs	Nipah virus	Outbreak among pig farmers in Malaysia[22] and abattoir workers in Singapore;[23] also presents as atypical pneumonia (see Ch. 222)

Fever, progressive meningo-encephalitis following monkey bite or scratch	Macaque monkeys	Cercopithecrine herpesvirus 1 (herpes B virus)	Acquired from monkey bites or scratches or handling monkey tissues or cell cultures[24] (see Ch. 215)
Influenza-like prodrome, then progressive encephalitis	Horses	Hendra virus	Three cases reported in Queensland, Australia; all exposed during epidemics in horses[25] (see Ch. 222)

TABLE 90-4 -- Workers who have animal contact: infections presenting with diarrhea.

WORKERS WHO HAVE ANIMAL CONTACT: INFECTIONS PRESENTING WITH DIARRHEA			
Clinical presentation	**Animal exposure**	**Possible infectious agent**	**Comments**
Diarrhea or abdominal pain (pancreatitis)	Farm animals (cattle, sheep), laboratory animals (calves, rodents, rabbits)	*Cryptosporidium* spp.[26]	Transmission occurs animal to person, person-to-person, water-borne (see Ch. 243)
Acute diarrhea (watery or bloody), fever, abdominal pain	Wild and domesticated cattle, sheep, goats, swine, dogs, cats, rodents, fowl	*Campylobacter jejuni*	Worldwide distribution, transmission via ingestion of contaminated material, direct contact with infected animals (see Ch. 230)
	Swine	*Campylobacter coli, C. hyointestinalis*	
	Dogs	*Campylobacter upsaliensis*	
	Sheep, cattle, swine, poultry, reptiles	*Campylobacter fetus*	
	Cynomolgus macaque monkey	*Shigella* spp.	Outbreak of *S. flexneri* reported among workers in primate research unit (see Ch. 228)
	Rodents, rabbits, sheep, pigs, horses, cattle, dogs, cats	*Yersinia enterocolitica*	Transmission mainly by ingestion of contaminated food or drink, less commonly direct contact with infected animals; Finnish butchers documented at higher risk[27] (see Ch. 228)
Chronic diarrhea	Coho salmon, other salminoid fish	*Nanophyetus salmincola* (nanophyetiasis)	Transmission usually by ingestion; also by handling infected fish[28] (see Ch. 246)

TABLE 90-5 -- Workers who have animal contact: other infectious syndromes.

WORKERS WHO HAVE ANIMAL CONTACT: OTHER INFECTIOUS SYNDROMES			
Clinical presentation	**Animal exposure**	**Possible infectious agent**	**Comments**
Septic arthritis	Fish (catfish)	*Edwardsiella tarda*	Reported following catfish spine injury in Australia[29] (see Ch. 228)
Chronic upper abdominal pain or discomfort; jaundice weight loss	*Echinococcus granulosus* (hydatid disease, liver)	Mainly dogs	Worldwide distribution, predominantly South and Central America, European and African parts of Mediterranean area, Middle East, some sub-Saharan countries, Russia and China. Consider in workers having contact with dogs in endemic areas (e.g. ranchers, farm workers) (see Ch. 169 and Ch. 246)
	Echinococcus multilocularis (hydatid disease, liver)	Red and arctic foxes, domestic dogs and cats	Restricted to northern hemisphere. Human disease frequently reported in central and eastern France, Switzerland, Austria and Germany. Occurs in tundra zone of Russia, China and Japan. Cases reported in Manitoba, Minnesota and in Alaskan Eskimos. Consider in trappers, hunters, ranchers, farm workers, veterinarians and other workers in contact with foxes and dogs in endemic areas
Chronic cough, hemoptysis, pleuritic pain	*Echinococcus multilocularis* (hydatid disease, lungs)	Same as for *E. multilocularis* above	Same as for *E. multilocularis* above (see Ch. 169 and Ch. 246)

TABLE 90-6 -- Biomedical laboratory workers: infections presenting with skin lesions. [30] [31]

BIOMEDICAL LABORATORY WORKERS: INFECTIONS PRESENTING WITH SKIN LESIONS		
Clinical presentation	**Possible infectious agent**	**Exposure**
Cutaneous papules, pustules, nodules, ulcers or abscesses	*Coccidioides immitis* [32]	Direct percutaneous inoculation of contaminated material (see Ch. 185 , Ch. 226 and Ch. 238)
Verrucous skin lesions with peripheral microabscesses or initial pustule that spreads into ulcer with red granulation tissue	*Blastomyces dermatitidis* (cutaneous blastomycosis)[33]	
Enlarging pruritic papule, which ulcerates, develops eschar and edema	*Bacillus anthracis* (cutaneous anthrax)[34]	
Painless nodule at site of inoculation which ulcerates, develops satellite lesions around ulcer and along draining lymphatics	*Sporothrix schenckii* (cutaneous sporotrichosis)[35]	Percutaneous inoculation (see Ch. 239)
Enlarging papule that forms ulcer with a raised violaceous border	*Leishmania* spp. (cutaneous leishmaniasis)	Percutaneous inoculation, transmucosal, bites from insect vector in research laboratory workers (see Ch. 172 and Ch. 245)
Pseudotumoral destructive lesion in nasal or oropharyngeal mucosa	*Leishmania braziliensis* (mucocutaneous leishmaniasis)	
High fever, eschar, regional adenopathy, headache, myalgia, rash including palms and soles	*Rickettsia rickettsii* (Rocky Mountain spotted fever)[36]	Infectious aerosols, percutaneous inoculation, vector-borne in research laboratory workers (see Ch. 179 and Ch. 235)
High fever, headache, backache, generalized pain; eschars, adenopathy unusual	*Rickettsia prowazekii* (epidemic typhus) *Rickettsia typhi* (murine typhus)[37] [38]	
High fever, eschar, regional adenopathy, headache, myalgia, rash	*Orientsia tsutsugamushi* (scrub typhus)[39]	

TABLE 90-7 -- Biomedical laboratory workers: infections presenting with acute fever.[30] [31]

BIOMEDICAL LABORATORY WORKERS: INFECTIONS PRESENTING WITH ACUTE FEVER		
Clinical presentation	**Possible infectious agent**	**Exposure**
Gradually increasing fever, chills, headache, malaise	*Salmonella typhi*, *S. paratyphi* A, B	Oral, occasionally percutaneous inoculation (see Ch 228)
Fever, fatigue, malaise, night sweats, weight loss, lymphadenopathy, visceral abscesses	*Burkholderia mallei* (glanders) [40]	Infectious aerosols, possibly cutaneous inoculation (see Ch. 229)
Fever, severe headache 3–5 days after flu-like illness	Lymphocytic choriomeningitis virus	Parenteral inoculation, transmucosal, infectious aerosols (see Ch. 222)
Fever, sweats, sore throat, myalgias, maculopapular rash, headache, photophobia, meningismus	HIV (acute infection)	Percutaneous or mucous membrane exposure while processing infected blood or body fluid (see Ch 214 and Ch 221)
Fever, anorexia, malaise, then jaundice	Hepatitis viruses	
Swelling and/or redness at inoculation site, fever, rash adenopathy, myocarditis	*Trypanosoma cruzi* (North American trypanosomiasis, Chagas disease)	Needle, wound, transmucosal (aerosol?) (see Ch. 173)
Swelling and/or redness at inoculation site, fever, rash adenopathy, headache, fatigue, neurologic signs	*Trypanosoma brucei rhodesiense* and *gambiense* (African trypanosomiasis)	Needle, wound, transmucosal (aerosol?) (see Ch. 157)
Fever, shaking chills, fatigue, anemia	*Plasmodium* spp., *Babesia* spp.	Percutaneous inoculation, transmucosal (see Ch 166 and Ch 245)
Fever, chills, headache, vomiting, migratory arthralgias	*Streptobacillus moniliformis*, *Spirillum minus* (rat-bite fever)	Bite of laboratory rat (see Ch. 230)
Fever, chills, headache, myalgias, abdominal pain, conjunctival suffusion, muscle tenderness, jaundice	*Leptospira* spp. (leptospirosis)	Oral, percutaneous (see Ch 181 and Ch 230)
Fever, adenopathy, malaise, rash	*Toxoplasma gondii*	Oral, percutaneous, transmucosal (aerosol?)
Hemorrhagic fever	*Sabia virus*[41]	Aerosol (see Ch. 222)
Hemorrhagic fever with renal syndrome	Hantaan virus	Probable transmission via infectious aerosols, wound infection during animal handling;[42] [43] reported case probably due to contact with immunocytoma[44] (see Ch. 222)
Fever, purpuric/ecchymotic skin lesions, meningitis, shock	*Neisseria meningitidis*	Airborne; fatal cases described in laboratory workers[45] (see Ch. 227)
Fever, sweats, anorexia, headache, back pain	*Brucella* spp.[46]	Direct contact; air-borne spread among laboratory workers documented (see Ch 180 and Ch 231)
Abrupt-onset fever, chills, headache, malaise, anorexia, fatigue, tender lymphadenopathy, skin ulcer	*Francisella tularensis*	Infectious aerosols, percutaneous, transmucosal (see Ch 177 and Ch 231)

TABLE 90-8 -- Biomedical laboratory workers: infections presenting with fever and cough. [30] [31]

BIOMEDICAL LABORATORY WORKERS: INFECTIONS PRESENTING WITH FEVER AND COUGH		
Clinical presentation	**Possible infectious agent**	**Exposure**
Fever, chills, cough, pleuritic chest pain, soft tissue abscesses	*Burkholderia pseudomallei* (melioidosis) [47]	Infectious aerosols (see Ch 175 and Ch 229)
Mild fever, headache, chills, sweats, cough, maculopapular rash	*Coxiella burnetii* (Q fever)[19]	Infectious aerosols, percutaneous inoculation (see Ch. 235)
Fever, dermatitis, cough, hepatosplenomegaly, adenopathy	*Schistosoma* spp. (acute schistosomiasis)	Percutaneous (see Ch. 167)
Fever, cough, fatigue, arthralgias, pulmonary nodular lesions	*Blastomyces dermatitidis* (pulmonary blastomycosis)	Infectious aerosols (see Ch. 238)
Chronic cough, fever, night sweats, anorexia, weight loss	*Mycobacterium tuberculosis*	Infectious aerosols; laboratory-acquired case presenting as endometrial tuberculosis reported[48] (see Ch. 233)
	Coccidioides immitis	Inhalation of arthrospores from culture (see Ch. 238)

TABLE 90-9 -- Biomedical laboratory workers: other infections.[30] [31]

BIOMEDICAL LABORATORY WORKERS: OTHER INFECTIONS		
Clinical presentation	**Possible infectious agent**	**Exposure**
Diarrhea	*Shigella* spp., nontyphoidal *Shigella* spp., nontyphoidal *Salmonella* spp., *Campylobacter* spp., *Vibrio cholerae*	Oral, occasionally percutaneous inoculation (see Ch 228 and Ch 230)
	Cryptosporidium parvum	Oral, transmucosal (aerosol?) (see Ch. 243)
	Giardia lamblia	Oral (aerosol?) (see Ch. 242)
	Isospora belli	Oral (see Ch. 243)
Right upper quadrant pain, biliary colic, obstructive jaundice	*Fasciola hepatica*	Oral (see Ch. 246)
Conjunctivitis	*Neisseria gonorrhoeae*	Conjunctival contamination (see Ch. 227)

TABLE 90-10 -- Infections in farm, forestry and other outdoor workers.

INFECTIONS IN FARM, FORESTRY AND OTHER OUTDOOR WORKERS		
Clinical presentation	**Possible infectious agent**	**Exposure**
Red macule or papule with expanding borders and central clearing or migratory pain in joints, bursae, tendons, muscles; arthritis	*Borrelia burgdorferi* (Lyme borreliosis)	High seroprevalence in forestry workers, but asymptomatic infection more common (see Ch 54 and Ch 230)

Abrupt fever, headache, photophobia, vomiting, meningismus, seizures, altered sensorium	Russian summer-spring encephalitis virus/central European encephalitis virus (tick-borne encephalitis virus, TBEV)	Higher antibody levels to TBEV found in Polish forestry workers [49] (see Ch. 222)
Erythematous papulonodular lesion with several painless secondary nodules proximally along lymphatic channels	*Sporothrix schenckii* (sporotrichosis)	Outbreaks among forestry workers in USA associated with sphagnum moss;[50] higher skin-test positivity among those engaged in horticulture, gardening, woodwork and farming in northern India[51] (see Ch. 239)
Hemorrhagic fever with renal syndrome	Puumala virus (nephropathia endemica)	Higher seroprevalence among forestry workers in Sweden, but not in The Netherlands; high risk in farmers in Finland
	Hantaan virus	Increased seroprevalence in Dutch animal trappers and forestry workers (see Ch. 222)
Fever, chills, cough, chest pain, dyspnea, occasionally hemoptysis, soft tissue abscesses	*Burkholderia pseudomallei* (melioidosis)	Farmers in South East Asia and northern Australia; acquired by soil contamination of skin abrasions and possibly ingestion, inhalation or intranasal inoculation (see Ch 175 and Ch 229)
Fever, myalgias, jaundice	*Leptospira* spp.	Antibodies to *Leptospira* spp. found in 10–12% of farmers and forestry workers in Italian alpine region; only few clinical cases (see Ch 181 and Ch 230)
Fever, cough, fatigue, arthralgias, bilateral pulmonary nodular lesions	*Blastomyces dermatitidis* (pulmonary blastomycosis)	Two cases acquired during prairie dog relocation in Colorado; spores probably aerosolized during vigorous digging[52] (see Ch. 238)
Chronic cough, fever, night sweats, anorexia, weight loss	*Coccidioides immitis*	Cases among workers and students at archaeologic sites[53] (see Ch. 238)
Corneal ulcers	Fungal keratitis (organisms not specified)	Cases in onion harvesters in Taiwan[54]

TABLE 90-11 -- Infections in sewer and irrigation workers.

INFECTIONS IN SEWER AND IRRIGATION WORKERS		
Clinical presentation	**Possible infectious agent**	**Exposure**
Fever, malaise, anorexia, then jaundice	Hepatitis A	Sewer workers at higher risk than general population in developed countries (see Ch. 214)
Fever, chills, headache, myalgias, abdominal pain, conjunctival suffusion, muscle tenderness, jaundice	*Leptospira* spp. (leptospirosis)	Increased risk in sewer and public cleansing workers; in the Philippines associated with wading in flooded city streets (see Ch 181 and Ch 230)
Intestinal parasitism	*Ascaris lumbricoides, Entamoeba histolytica, Enterobius vermicularis*	More common in Egyptian sewer workers than in controls (see Ch. 246)
Fatigue, abdominal pain, intermittent diarrhea, hepatosplenomegaly	*Schistosoma japonicum, S. mansoni, S. mekongi* (schistosomiasis)	Rice farmers and freshwater fishermen in endemic areas (see Ch 167 and Ch 246)
Terminal hematuria, dysuria	*Schistosoma haematobium*	

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Chapter 91 - Infections from Pets

Ellie JC Goldstein

INTRODUCTION

Domestic pets, particularly dogs and cats, can be dated back prior to Ancient Egypt. Currently, more than one-third of USA households keep some pet including 53 million dogs, 57 million cats and a plethora of other animals from birds to fish to reptiles.[1] In Australia, 66% of households have a domestic pet.[2] It is therefore not surprising that transmission of infection from animals to humans and humans to animals is common.

DOG-ASSOCIATED INFECTIOUS DISEASES

Dog bites

Table 91.1 lists some of the infectious organisms associated with dogs and canine contact. Of the over four million Americans bitten annually by a dog, only 15–20% will seek, or need, medical attention and 3–18% will become clinically infected. Dog bites account for 1% of all emergency department visits and result in 10,000 hospitalizations and 1–10 deaths annually.[1] Most dog bites are inflicted by the victim's own pets or an animal known to them, often while separating the dog in a fight with another dog. Half of dog bite wounds are to the hands and pose a special risk for penetration into the bones and joints and potential spaces of the hand. It is possible for nerves and/or blood vessels to be damaged. Approximately 15% of dog bites are to the head and neck, 20% to the leg or foot and 15% to the upper extremity.[1] [2]

The incidence of dog bite wounds peaks in the summer months and on weekends. The median age of dog bite victims is 28 years old and over 60% are male. Most dog bite wounds are punctures (approximately 60%) with the remainder being lacerations (10%) or combinations of the two (30%). Most patients attempt some form of self-therapy before presenting for medical attention, which include washing with soap and water, applying a topical iodophor or alcohol product. The median time for presentation for medical care is 35 hours post-injury, usually after the onset of clinical signs of infection.

TABLE 91-1 -- Infections transmissible from dogs.

INFECTIONS TRANSMISSIBLE FROM DOGS			
Bacteria	**Fungi**	**Parasites**	**Viruses**
Normal flora	*Blastomyces* spp.	*Giardia* spp.	Rabies
Pasteurella spp.	*Microsporum* spp.	*Babesia* spp.	LCM (lymphocytic choriomeningitis)
Capnocytophaga canimorsus	*Trichophyton* spp.	*Toxocara* spp.	Influenza
Tularemia		*Dipylidium canium*	Mumps
Ehrlichiosis		Echinococcosis	
Brucellosis		*Ancyclostoma* spp.	
Mycobaterium fortuitum		Scabies	
Campylobacter spp.		*Cheytelliella*	
Anaerobiospirillum thomasii			
Yersinia spp.			

At presentation approximately 60% of wounds exhibit a purulent exudate, 30% have signs of infection but are without exudates and 10% are abscesses. Approximately one-third of patients reporting to an emergency department will require hospitalization for intravenous antibiotics. When cultured, these wounds yield an average of five isolates, usually three aerobes and two anaerobes. Mixed aerobic and anaerobic infection occurs in 50% of dog bite wounds and 35% grow only aerobes. Common aerobic isolates include *Pasteurella* species, 50% (*Pasteurella canis*, 26%; *Pasteurella multocida* subspecies *multocida*, 12%; *Pasteurella stomatis*, 12%; *P. multocida* subspecies *septica*, 10%), streptococci, 46%, staphylococci, 46% (half of which are *Staphylococcus aureus*), and *Neisseria* spp., 16%.[1] *Capnocytophaga canimorsus* has been associated with fatal sepsis, especially in asplenic patients and those with liver disease. Common anaerobes isolated include *Fusobacterium* spp., 32%, *Bacteroides* spp., 30%, especially *Bacteroides tectum*, *Porphyromonas* spp., 28%, *Prevotella* spp., 28%, especially *Prevotella heparinolytica*, and peptostreptococci 16%.[1] [2] [3]

Table 91.2 outlines the susceptibility patterns of common bite isolates. For those patients hospitalized, the mean duration of stay is 3 days, with approximately 30% requiring incision and drainage and 25% requiring debridement of the wound. Severely penicillin-allergic patients may need a combination of agents. Fluoroquinolones, tetracyclines and sulfa drugs are contraindicated in pregnant patients; tetracyclines and fluoroquinolones are relatively contraindicated in patients younger than 18 years old. Agents (mono-therapy) associated with therapeutic failure include first-generation cephalosporins and macrolides. The duration of therapy will depend upon the severity of the wound. For wounds that are seen less than 8 hours post-injury and require prophylaxis (moderate-to-severe wounds) the duration of prophylaxis is usually 3–5 days (see also Chapter 96e). Therapy for a cellulitis is usually 7–10 days, while the therapy for septic arthritis and osteomyelitis is often 4–6 weeks. Cases of endocarditis and infection of prosthetic joints following bites have been reported, albeit rarely. The principles of wound care are noted in Table 19.3.

TABLE 91-2 -- Activity of selected antimicrobials against animal bite isolates.

ACTIVITY OF SELECTED ANTIMICROBIALS AGAINST ANIMAL BITE ISOLATES					
	Pasteurella multocida	***Staphylococcus aureus***	**Streptococcus spp.**	***Capnocytophaga***	**Anaerobes**
Penicillin	+	-	+	+	V
Ampicillin	+	-	+	+	V
Amoxicillin-clavulanate	+	+	+	+	+
Ampicillin-sulbactam	+	+	+	+	+
Dicloxacillin	-	+	+	-	-
Cephalexin	-	+	+	-	-

Cefuroxime	+	+	+	+	-
Cefoxitin	+	+	+	+	+
Tetracyclines	+	V	-	V	V
Fluoroquinolones	+	V	-	+	-*
Erythromycin	-	+	+	+	-
Azithromycin	+	+	+	+	-
Clarithromycin	V	+	+	+	-
Trimethoprim-sulfamethoxazole	+	+	V	+	-
Clindamycin	-	+	+	-	+
+, Active.					
-, Poor or no activity.					
V, Variable activity against listed pathogen.					

* Newer fluoroquinolones, such as moxifloxacin, and desfluoroquinolones such as garenoxacin, with anaerobic activity are under clinical development.

TABLE 91-3 -- Components of care for animal bites.

COMPONENTS OF CARE FOR ANIMAL BITES	
History	Situation, pet ownership/identity
Geographic location	
Examination	Nerve function
	Tendon function
	Blood supply (pulses)
	Presence of edema, crush injury
	Proximity to joint
	Bone penetration
Diagram (or photograph) of wound(s) for the record	
Wound care	Irrigation (important)
	Debridement (cautious)
	Elevation (important)
Antimicrobials	Prophylaxis, 3–5 days (oral)
	Therapy for established infection (oral vs im initial dose)
	Empiric vs specific (animal specific)
Management:	
Culture (if infected)	
Baseline radiograph if in proximity to a bone or joint	
Tetanus toxoid (0.5ml im) if required	
Rabies prophylaxis (rabies immune globulin/human diploid cell vaccine) if needed	
Health Department report (if required)	
Indications for hospitalization	Fever >100.5°F (38°C)
	Sepsis
	Compromised host
	Advance of cellulitis
	Patient noncompliance
	Acute septic arthritis
	Acute osteomyelitis
	Severe crush injury
	Tendon/nerve injury or severance
	Tenosynovitis

Rabies

Approximately 7000 animals per year test positive for rabies in the USA. Most are wild animals, such as raccoons, foxes and bats. Dog-associated rabies is uncommon in developed countries as a result of stray animal control programs and widespread rabies vaccine use by dog owners. It has occurred in areas where endemic foci of infected raccoons and skunks are common. Canine transmission of rabies to humans infrequently occurs in the USA, but it is not uncommon in Latin American countries, Africa and particularly in the Indian subcontinent and other regions in Asia. The World Health Organization reported over 50,000 cases of animal rabies worldwide annually over the past decade (see Chapter 153 and Chapter 219). [4]

Enteric diseases from dogs

Giardiasis

Giardia lamblia is a flagellated protozoan parasite that is an important cause of diarrheal disease worldwide and can cause diarrhea in many mammalian species including dogs and humans. In many hospitals that have pet visitation programs, *G. lamblia* is the most frequent infectious agent identified on screening. The disease is transmitted by fecal-oral spread and causes flatulence, foul smelling stools, abdominal discomfort and malaise in humans. Diagnosis is by stool exam. Therapy is with metronidazole; relapses are not infrequent.

Echinococcosis

Echinococcus granulosis is a dog tapeworm found in the small intestine whose ova are shed into the stool. The illness is associated with dogs found in sheep raising

areas, especially if the dogs are fed offal. These ova may remain viable for up to 1 year in appropriate climatic conditions. Humans can ingest the ova, which hatch in the small intestine into oncospheres that penetrate the bowel wall. Humans will act as an intermediate host. Usually these oncospheres reach the liver and occasionally the lung, but may also reach the systemic circulation and be spread widely.

While most infestations are asymptomatic in humans, symptomatic disease, when it occurs, can be found in the liver (60%), and lung (25%). Rupture of a hydatid cyst can cause catastrophic disease and anaphylaxis. A variety of serologic tests are available to assist in diagnosis. However, the presence of septation in a liver cyst (indicating daughter cysts) is considered diagnostic. Therapy for this disease includes surgery. Some suggest aspiration is acceptable, although there is a risk of spillage and complications. Scolicidal agents such as albendazole and mebendazole remain as adjunctive therapies.

Echinococcus multilocularis causes a severe form of alveolar hydatid disease, again often involving the human liver. This parasitic infection is found primarily in the artic regions of the Northern Hemisphere and often necessitates aggressive surgical resection for successful removal. *Echinococcus vogeli* is found in canines of Columbia, Ecuador, Panama and Venezuela and can also cause human disease. The diagnosis and management of echinococcosis is further reviewed in detail in Chapter 168 .

Miscellaneous infections acquired from dogs

Isospora spp. infection of the gastrointestinal tract is common in dogs and while transmission to humans is not proven, it remains possible, especially in the immunocompromised patient. *Trichuris vulpis*, the whipworm of dogs, which resembles the human whipworm *Trichuris trichuria* but whose ova is twice its size, have been reported in children, institutionalized patients and rarely in immunocompetent adults associated with dog contact.

Toxocara canis is a ubiquitous roundworm in dogs that causes both cutaneous larva migrans and visceral larva migrans in humans, and affects many puppies (often less than 6 months old) and adult dogs (via ingestion of embryonated ova). Infection in humans is usually associated with children who have pica (1–6 years old) who acquire it from backyards and contaminated sandboxes. Most infection in humans is asymptomatic and eosinophilia (=30%) may be the only sign. However, the larva may migrate anywhere in the human body and their final location determines the associated symptoms. Some patients develop an asthma-like illness others pallor, weight loss, hepatomegaly or pruritic skin lesions. Diagnosis is based on a compatible history coupled with specific serology. Dogs may also transmit *Ancyclostoma canium* and *Ancyclostoma braziliense*, which also cause cutaneous larva migrans.

Transmission of infections from contact with dog urine

Leptospirosis

Leptospires are finely coiled, motile spirochetes that can be carried asymptomatically for many months by dogs. Dogs may also become ill manifested by fever, jaundice, conjunctivitis and hematuria. Dogs are often vaccinated for leptospirosis, but vaccine failures (over 1 year postvaccination) have been reported. Humans may become infected through contact from the urine of an infected dog, from contact with infected tissues or indirectly from contaminated soil or water. Slightly alkaline or neutral pH, temperature of 22°C (71.6°F) or slightly higher and moist soil favors survival of leptospires in the environment.

Most humans will have subclinical infection or an anicteric 'viral type' illness. Some may develop the classic biphasic illness and proceed to Weil's disease. During the first phase, leptospires may be isolated from the blood, urine and spinal fluid. Later, antibodies may develop and recrudescent fever arthralgia, hepatitis, skin rash and conjunctival suffusion may appear along with other complications (see Chapter 181 and Chapter 230). Some studies suggest that therapy with penicillin G (benzlpenicillin) or doxycycline may shorten the course of disease if started within the first 4 days of illness. Jarisch-Herxheimer reaction can occur after the first dose of therapy.

Brucellosis

Brucellosis is a disease of both wild and domestic animals that is transmitted to humans. Dogs may be infected with *Brucella canis*, a small Gram-negative coccobacillary organism that is found in kennel-raised dogs and is the least common cause of human brucellosis. Many infections with *B. canis* are laboratory animal acquired. Dogs may have persistent carriage of the organism and shed it in urine, and gestational tissues. It can be transmitted between dogs during mating. Human disease occurs 2–4 weeks after exposure and has protean manifestations with both an acute and chronic form (see Chapter 180).

Tick/flea-borne infectious diseases associated with dogs

Ehrlichiosis

Canine ehrlichiosis was first described in 1932 in Algeria. Since then it has been noted that dogs can be hosts to a variety of tick-borne, intraerythrocytic parasites including *Ehrlichia canis*, *Ehrlichia chaffeensis* (associated with human monocytic ehrlichiosis), *Ehrlichia phagocytophilia* (associated with human granulocytic ehrlichiosis), *Ehrlichia ewingii*, and *Ehrlichia platys* that cause canine pancytopenia and have a worldwide distribution. Although dog-to-human transmission is unlikely, the disease has this potential. In humans, ehrlichiosis is contracted by a tick bite that inoculates the organism into the skin and then spreads via the lymphatics. In humans it causes fever, headache, chills and malaise with an associated leukopenia, thrombocytopenia and elevated liver enzymes. Therapy in humans is tetracycline or doxycycline.

Infections acquired from direct contact with dogs

Canine scabies due to *Sarcoptes scabiei* var. *canis* is also known as mange. The mites feed on the stratum granulosum of the skin and deposit the eggs in a burrow. After hatching the larval form migrates to the surface. Transmission to humans has been noted and is manifested as an erythematous nonfollicular dermatitis, in part due to hypersensitivity. Skin scrapings will not demonstrate the mite. Diagnosis is by history and clinical response to scabicides. Dogs should also be treated with the application of a scabicidal agent. A similar condition known as 'walking dandruff' in dogs is due to *Cheyletiella* (mites). These mites do not burrow and disease is manifested in humans with erythematous macules, which may become pustular or vesicular or have other manifestations of allergic reaction such as urticaria and erythema multiforme (see Chapter 155).

CAT-ASSOCIATED INFECTIOUS DISEASES

Cat bites

Cats can transmit a number of diseases to humans as listed in Table 91.4 . Most physicians will encounter cat bites as the most common cat-related disease, with approximately 500,000 cat bites in the USA alone on an annual basis, usually when owners handle their own cat. Most wounds are trivial and do not need medical care. Cat bites affect more women (72%) than men, with an average age of 39 years for the victim. [1] At the time of presentation a non-purulent wound with cellulitis (42%) is the most common condition encountered; however, the wounds may also develop into a purulent cellulitis (39%) and even abscess formation (19%). An associated lymphangitis may be present in 28% of cases. An average of six bacterial species are isolated in the typical wound (range 0–13). Approximately 85% of cat bite injuries involve puncture wounds, less than 5% are lacerations and approximately 10% are combinations of both.[2] [3] Cats' teeth are small but sharp and easily penetrate the bones, tendons and joints of the hand and lead to osteomyelitis, tendonitis and septic arthritis, respectively.

The bacteriology of these wounds reflects the normal oral flora of the biting cat. Several recent studies[1] [5] [6] have shown *Pasteurella* species to be present in 75% of cases with *P. multocida* subspecies *multocida* in 54% of wounds, *P. multocida* subspecies *septica* in 28%. These two subspecies are associated with more severe injury and have a propensity for bacteremia and central nervous system infections, respectively. Other common aerobic isolates include oral streptococci (46%), staphylococci (35%) but not *S. aureus* (4%), *Neisseria* spp. (especially *Neisseria weaverii*), *Moraxella* spp. (35%), and *Corynebacterium* spp. (28%). *Capnocytophaga canimorsus* is an unusual nonfermentative Gram-negative aerobic rod that is associated

TABLE 91-4 -- Possible infections transmissible from cats.

POSSIBLE INFECTIONS TRANSMISSIBLE FROM CATS				
Inhalation	**Vector-borne**	**Fecal-oral**	**Bites, scratches**	**Direct contact**

Bordetellosis (*Bordetella bronchiseptica*)	Ehrlichiosis	Campylobacterosis	Normal flora isolates (including *Pasteurella* spp.)	Dermatophytosis
Plague	Cat-scratch disease	*Helicobacter* spp.	Rabies	Histoplasmosis
Q fever	Bacillary angiomatosis	Cryptosporidiosis	*Erysipelothrix rhusiopathiae*	Dermatophytosis
		Toxoplasmosis	Anthrax	Scabies
		Salmonellosis	Tularemia	*Cheyletiella* mites
		Anaerobiospirillum diarrhea		
		Yersiniosis		
		Toxocariasis		
		Opisthorchiasis		
		Dipylidiasis		

with bacteremia and significant mortality rates in compromised patients such as those with asplenia or liver disease.

Anaerobes are present in 63% of cases, usually in mixed culture, and when present were associated with more severe infection and abscesses. Common anaerobic isolates include *Bacteroides tectum* (28%), *Fusobacterium* species (33%), especially *Fusobacterium nucleatum* and *Fusobacterium russii*, *Porphyromonas* spp. (30%), and *Prevotella* spp. (19%).[5] [6] [7] Most anaerobes isolated from cat bites are not producers of ß-lactamase, but unusually compared to human oral isolates, the cat-associated *F. nucleatum* species are often resistant to fluoroquinolones and a new subspecies (*F. nucleatum* subspecies *canifelium*) has been proposed (personal communication, G. Conrads, DM Citron, EJC Goldstein).

In addition, *Erysipelothrix rhusiopathiae* has been isolated from cat bites. Although usually a localized infection manifested by painful ulceration and a papular skin lesion, a disseminated form exists.

Anthrax uncommonly infects cats and results from contact with infected soil, especially associated with herbivores. The differential diagnosis of the cutaneous papular lesion that starts as a vesicle and can progress to brawny edema and central necrosis also includes brown recluse spider bite, cat-scratch disease, [8] tularemia and plague. Tularemia has been associated with cat bites.

Antimicrobial selection is delineated in Table 91.2 . Severely penicillin-allergic patients may need a combination of agents. Fluoroquinolones, tetracyclines and sulfa drugs are contraindicated in pregnant patients; tetracyclines and fluoroquinolones are relatively contraindicated in patients younger than 18 years old. Agents (monotherapy) associated with therapeutic failure include first-generation cephalosporins and macrolides. The principles of wound care are noted in Table 91.3 . Pain, out of proportion to the injury and in proximity to a bone or joint suggests periosteal penetration (see also Chapter 96e).

Rabies

Rabies is a widespread zoonotic infection affecting numerous wild animals in many regions of the world. Domestic cats account for less than 5% of rabid animals and usually acquire it from a 'spillover effect' from exposure to infected wildlife. This disease is covered in Chapter 153 and Chapter 219 .

Cat-scratch disease

Bartonella henselae is a fastidious Gram-negative rod that is the etiologic agent of cat-scratch disease in the general population and bacillary angiomatosis in individuals with HIV infection. Cat-scratch disease is of worldwide distribution, has an autumn and winter prevalence in temperate climates and may be transmitted by direct

inoculation (scratch or bite) or by fleas. Over 80% of cases are in people under 21 years of age; children under 14 years of age are typically infected after exposure to a newly-acquired pet cat or kitten. Strays and cats from pounds have a higher frequency of infection and may be bacteremic (>40% in one San Francisco study) compared to household cats (6% in one New York City study). Most infections in cats are asymptomatic and can last for several months.[8]

Approximately 1 week (range 3–10 days) after injury or exposure, 25–60% of patients may develop a primary inoculation papule at the site of injury, which may become vesicular or crust. In approximately 2 weeks (5–120 days) tender, regional lymphadenopathy may develop. Scratches on the face may involve the eye (Parinaud's oculoglandular syndrome). Regional nodes may be the only manifestation in approximately half of the cases, often lasts over 3 weeks (6–12 weeks) and usually resolves spontaneously, but may suppurate in approximately 15% of cases. Accompanying symptoms include fatigue and malaise (28%), fever of 101–106°F (38.3–41.1°C) (12%), exanthem (4%), parotid swelling (2%) and seizures (<1%). Other manifestations may include ocular granuloma, erythema nodosum, thrombocytopenic purpura and osteomyelitis. Endocarditis due to *Bartonella quintana*, a related organism, and rarely *B. henselae*, both associated with cat fleas, has been reported in homeless men (see Chapter 235).

Diagnosis is usually on clinical grounds coupled with a history of cat exposure. The organism is very difficult to isolate (chocolate media or CDC agar after 2–3 weeks) except in the research setting and serologies are of variable reliability. Biopsies are sometimes performed to exclude diseases such as Hodgkin's lymphoma, and show granuloma formation with stellate microabscesses. While the Warthin-Starry stain has been recommended, it is difficult to interpret, especially in the absence of a positive control specimen.

Therapy is usually supportive. Antimicrobial therapy with azithromycin has been reported to diminish the size and duration of the adenopathy. Doxycycline and rifampin (rifampicin) have been used for cat-scratch disease-associated retinitis. In-vitro resistance to first-generation cephalosporins has been correlated with clinical therapeutic failure. Prevention is by control of fleas in pets and sometimes treatment of the pet at the time of acquisition.

Bacillary angiomatosis is cat-scratch disease in a compromised host (usually with HIV infection) that manifests either as purplish skin lesions resembling Kaposi's sarcoma or as colorless subcutaneous nodules. Biopsy of the lesions yields a characteristic histopathologic picture of vascular proliferation. DNA probes have also been used to diagnose this disease. These patients may also be symptomatic from the disease and have associated fever and even peliosis hepatis. Antimicrobial therapy, although its efficacy is poorly defined, is usually employed in this situation and includes macrolides, quinolones or doxycycline (see Chapter 128).

Cat-associated enteric infections

Cats may acquire salmonellosis from infected foods, especially offal, live prey (such as songbirds), uncooked meat or fishmeal, or contaminated water. Kennel transmission has also occurred. Cats may shed *Salmonella* spp. via the feces, the conjunctiva or the oral route. In addition their fur may become contaminated and pass infection as can their water dishes. Newly acquired young cats are more likely to carry *Campylobacter jejuni* than older cats. *Helicobacter bizzozeronii* and *Helicobacter felis* are found in cat stomachs and association with human cases has been noted, albeit rarely. *Helicobacter pylori* has also been isolated from cats and is thought to have been transmitted to them by human caretakers. Cryptosporidiosis can affect cats that, along with other animals, are definitive hosts. Naturally infected cats exhibit watery diarrhea and some cats can be colonized. Transmission between cats and humans has been reported.[1] [2]

Cats are definitive hosts for *Toxoplasma gondii* and millions of oocysts may be excreted daily in their feces. Approximately 1% of cats in the USA are thought to be excreting oocysts on any given day. Although ingestion of undercooked meat is the usual mode of transmission of toxoplasmosis, infection may develop from exposure to fecal oocysts when changing litter boxes or gardening in soil contaminated with oocysts by cat feces. The oocysts that pass from the feces are noninfectious and nonsporulated. However, 2–3 days after shedding, the oocysts may sporulate depending on temperature (>39.2 and <98.6°F; >4 and <37°C) and climactic factors. These may remain infective in soil for up to 1 year.

Less common isolates include *Anaerobiospirillum thomasii*, which causes diarrhea in cats and is associated with bacteremia in humans. Cats may be asymptomatic carriers or infected with *Yersinia pseudotuberculosis*, which can cause diffuse diarrhea and abdominal pain in humans. *Toxocara cati* is a helminthic parasite that affects cats with which humans may become incidentally infected when ingesting infected cat feces (usually children in the ages of pica). Most human infection is asymptomatic but can present as asthma, abdominal pain, hepatomegaly and eosinophilia. The disease is usually self-limiting. The cat liver fluke, *Opisthorchis felineus* can be transmitted to man from ingestion of rare or raw fish infected with the parasite. *Dipylidium caninum* is a cat tapeworm that can be transmitted to humans, usually

children, when they ingest infected fleas. Patients develop mild abdominal discomfort and eosinophilia. Demonstrating proglottids upon parasitologic examination of the feces makes the diagnosis.

Infectious diseases associated with direct contact with cats

Dermatophytosis

Domestic cats can harbor a wide variety of dermatophytes on the hair of their coat and skin. They may also acquire human dermatophytosis from their owners and transmit it to others with an incubation period of 1–3 weeks. Asymptomatic, as well as symptomatic carriage occurs. *Microsporum canis* has been found in up to 90% of longhaired show cats. Up to 50% of exposed humans will develop symptomatic infection, including ringworm and tinea capitis. Other organisms isolated include *Epidermophyton floccosum*, *Microsporum* spp. and *Trichophyton* spp. Infectious arthrospores can disseminate from the hair and skin to the local environment where they remain viable for months. Contaminated fomites may also act as vectors of transmission. In cats, dermatophyte infection can manifest as patchy alopecia or even a scaly dermatitis. To break a cycle of transmission, cats may be treated with topical antifungals and on occasion oral antifungals. In addition, cleaning areas of cat hair and removal of dander from carpets and restriction of pet cats from the bedroom may facilitate control. Diagnosis is by skin scrapings and microscopic examination after potassium hydroxide addition or by use of a Wood's lamp. Human infection can be treated by topical or oral antifungals.

Cats may also directly transmit *Dermatophilus congolensis*, an actinomycete that can cause cutaneous exudative or pustular dermatitis. In cats, the hair around the lesion should be clipped and the lesion kept dry. The human dermatitis is usually self-limiting but on occasion antimicrobial therapy (penicillin) may be required if extensive skin involvement with cellulitis develops.

Cryptococcus neoformans can infect cats and cause feline disease that sheds viable organisms into the environment. However, human transmission from infected cats has not, as yet, been documented. [9]

Mites

Cats may become infected with *Sarcoptes scabiei* (scabies) and transmit this to humans. The mites cause a hypersensitivity reaction in humans manifested by pruritic papular lesions and nocturnal

itching. As cat scabies do not burrow into human skin, skin scrapings will not be diagnostic and diagnosis is by clinical presentation. Therapy is by removal of the mites from the cat and laundering the household bedding and clothing. *Cheyletiella* spp. are another type of animal mite that can be transmitted from cats to humans.

Cat infections acquired by inhalation

Bordetella bronchiseptica is a Gram-negative coccobacillus that can be found in the respiratory tracts of domestic cats and for which they should be vaccinated. In dogs it has been associated with 'kennel cough' but its clinical manifestations are often less prominent in cats. When cough appears in cats there may be an associated pneumonia. This organism can cause a pertussis-like (whooping cough) illness in humans, especially children or compromised hosts (e.g., those with HIV infection). Although cross-immunity may exist in humans from 'whooping cough' vaccination as children, the immunity probably wanes by the time adulthood is reached. Human illness may range from a mild upper respiratory tract infection to frank pneumonia. The organism can be cultured on special media but is often not thought of and occasionally misidentified by routine clinical laboratories.

Yersinia pestis is the Gram-negative coccobacillus that causes plague. Cat fleas are considered poor vectors for transmission, but the cats themselves may contract the illness, especially in the summer months by exposure to rat fleas. Reports of fatal cases in humans of inhalation plague and exposure to infected cats have been reported.

Direct exposure of humans to infected material from parturient or aborted tissue from cats infected with Q fever (*Coxiella burnetii*) may also acquire disease. Cats may acquire infection from a tick bite or from infected material in the environment.

INFECTIOUS DISEASES ASSOCIATED WITH BIRD EXPOSURE

Contact with pet birds may vary from kissing or feeding the bird from the owner's mouth to cleaning cages or allowing the bird free range of a home or a yard. It is difficult to determine how often some of the organisms, such as *Campylobacter jejuni*, *P. multocida* or *Mycobacterium tuberculosis*, are passed to humans, but *Chlamydia psittaci* is regularly transmitted, as are *Salmonella* spp.

Inhalation infections from birds

Psittacosis

Chlamydia psittaci can be carried by any bird, pet or wild, not just psittacine birds such as parakeets or parrots, and all birds that carry the organism can pass it on to humans. The disease is better called ornithosis rather than psittacosis. Ducks and turkeys have been responsible for outbreaks of ornithosis in humans as well as birds kept in the home. Respiratory symptoms are the usual result of transmission from bird to humans, and there have been reports of human-to-human transmission, but apparently this is not common. Too little is known about the incidence of infection with *C. psittaci* because few studies have been done of different human populations using modern, accurate serologic techniques. If the respiratory symptoms are mild, the infection is often undiagnosed. Even in patients who have pneumonia, the diagnosis needs to be confirmed by showing a fourfold rise in acute and convalescent serum titers 2–4 weeks apart, but this is seldom pursued.

The diagnosis of acute disease is dependent on a high index of suspicion, and therapy must be empiric because rapid diagnostic techniques are not available. A history of contact with a sick bird is highly suggestive, but well birds can carry the disease and a number of cases have been reported without a history of bird contact, although presumably there has been inapparent contact with bird excreta in the environment. Pigeon and other birds' feces abound in many urban environments, as do chicken and duck feces in rural environments.

The pneumonia in humans can be severe, and is often lobar accompanied by high fevers and chills. The sputum may be purulent with a lack of potential pathogens on smear because *C. psittaci* does not take the Gram stain.

Once empiric therapy is decided upon, tetracycline is preferred, although erythromycin has been reported to be effective. A safe duration of therapy is 2 weeks, although a shorter period may be adequate. Complications such as meningoencephalitis, arthritis or endocarditis may occur.

A specific diagnosis is important because epidemiologic factors may need to be investigated. A bird dealer may be importing carriers without appropriate quarantine or treatment and cases of human-to-human transmission may be uncovered, including possible nosocomial spread. Control measures for *C. psittaci* infection of birds and humans have been detailed by the US Public Health Service.

Histoplasmosis

Histoplasma capsulatum has been found in bird droppings, especially from chicken and blackbirds. *Histoplasma capsulatum* has been found throughout the world, but it is especially common in river valleys of central North America, parts of Mexico and in the Caribbean and is thus considered a 'regional' fungus. Histoplasmosis is usually not due to exposures to pets, with the exception of exposure to chickens, which are sometimes kept and regarded as pets. The fungus grows in the feces of chickens, but does not infect them. Most human infections are asymptomatic but others may result in an influenza-like illness or rarely a progressive pneumonia (see Chapter 39 and Chapter 238). The majority of symptomatic infections are self-limiting; however, treatment when necessary (especially in the immunocompromised host) can begin with amphotericin B followed by itraconazole or fluconazole for acute progressive infections or itraconazole or fluconazole alone for more indolent disease. Prevention strategies should include advising immunocompromised people to avoid bird feces, especially from chickens.

Cryptococcosis

Cryptococcus neoformans in found in soil throughout the world, but it appears to thrive in pigeon feces. Pigeon fanciers who keep flocks of pigeons for sport are exposed to *C. neoformans* more than the general population, as demonstrated by serologic testing; however, an increased incidence of disease has not been documented in these people. Cryptococcosis initially begins as a self-limiting respiratory infection. The fungus may disseminate widely to multiple organs including the central nervous system. It has a predilection for people who have T-helper-4 lymphocyte defects, and if untreated the meningitis it causes is associated with a high mortality rate (up to 50%). There may be significant morbidity and sequelae (e.g. noncommunicating hydrocephalus, blindness) (see Chapter 111 and Chapter 126).

The diagnosis is aided by the presence of cryptococcal polysaccharide antigens in the blood or cerebrospinal fluid, and a decrease in antigen titer is usually associated with a response to therapy in patients who do not have AIDS. In patients who have AIDS, high levels of antigen may persist indefinitely despite an excellent clinical response to treatment. Amphotericin B with or without flucytosine is the treatment for the acute episode, followed by fluconazole for more chronic therapy. Prevention should include advising immunocompromised people to avoid contact with pigeons.

Tuberculosis has been seen in patients in households where tuberculosis has been recognized in macaws.[10] It is difficult to determine whether spread is from bird to humans or vice versa. Other psittacine birds may become infected with *M. tuberculosis*.

Enteric diseases associated with bird exposure

All of the organisms listed in Table 91.5 have been isolated from birds; some, such as *Salmonella* spp. and *Giardia lamblia*, are clearly implicated in transmission to humans. Parasites that may spread to humans from birds include *G. lamblia* and *Cryptosporidia* spp, although direct spread from the latter is not as well documented as the former. Methods of diagnosis and treatment are covered in detail in Chapter 163 , Chapter 242 and Chapter 243 . Mites may be spread by direct and indirect contact with infested birds.

Viral diseases from pet birds

The alphaviruses and flaviviruses that pass from birds to humans are carried to humans by mosquitoes and ticks (see Chapter 222). These birds are almost always wild and not pets. Identical strains of influenza virus have been found in both humans and ducks where contact has been documented. The incidence of ducks or other birds serving as reservoirs for influenza is not certain. In 1998, a large out-break of influenza in Hong Kong was associated with chickens and caused several deaths. Thousands of birds were slaughtered in an attempt to control the infection.

SMALL MAMMAL PETS

Small mammal pets include mice, rats, hamsters, gerbils, guinea pigs and rabbits. People may also keep more exotic animals such as mink, ferrets and ocelots. These animals carry organisms similar to those carried by mice and rats; ocelots carry organisms similar to those carried by cats. Table 91.5 lists organisms carried by small mammal pets.

Most pets, regardless of type, can carry *Salmonella* spp. and *Campylobacter* spp. Many, including rabbits, carry *P. multocida* as part of their mouth flora. There are some that are more likely to carry certain organisms.

Rat-bite fever

Rats can carry *Spirillum minus* and *Streptobacillus moniliformis*. Rat-bite fever or spirillarly fever due to *S. minus* is seen worldwide, but is most common in Asia. A rash with reddish or purplish plaques accompanies the fever. The healed bite wound may reactivate when fever develops. The diagnosis requires highly specialized laboratories for confirmation. Treatment consists of penicillin or a tetracycline.

Rate-bite fever due to *S. moniliformis* (also called Haverhill fever) may be due to a rat bite or to exposure to contaminated milk or water during an outbreak. The fever is usually accompanied by a

TABLE 91-5 -- Organisms carried by small mammal pets.

ORGANISMS CARRIED BY SMALL MAMMAL PETS			
Bacteria	**Fungi**	**Parasites**	**Viruses**
Campylobacter spp.	*Sporothrix schenckii*	*Crytosporidium* spp.	Lymphocytic choriomeningitis virus
Spirillum minus			
Streptobacillus moniliformis	*Penicillium marneffei*		Hantavirus
Salmonella spp.			
Leptospira interrogans			
Francisella tularensis			
Yersinia pestis			
Listeria monocytogenes			
Pasteurella multocida			
Burkholderi pseudomallei			

maculopapular or petechial rash that is most pronounced on the extremities. Arthritis of large joints is common, as are relapses. Focal abscesses and endocarditis may occur. A specialized laboratory confirms diagnosis unless a sterile site is positive on culture. Treatment is with penicillin or a tetracycline.

Penicillium marneffei is endemic in South East Asia and southern China. It is carried by the bamboo rat and is found in water inhabited by these rodents. It is usually contracted in a natural setting and not through pets. In humans it causes a granulomatous disease resembling pulmonary tuberculosis, except that skin lesions are common in penicilliosis. It is usually seen in immunocompromised hosts and, when diagnosed early, responds well to itraconazole or amphotericin B.

Lymphocytic choriomeningitis virus is carried by mice and other rodents, including pet Syrian golden hamsters. It has been isolated from guinea-pigs and dogs. Infection in humans results from exposure to the urine, feces or saliva of the rodent and may result in no symptoms, although a flu-like syndrome or meningitis may occur. The flu-like syndrome may be followed by recovery and then relapse with meningitis. Orchitis, parotitis and thrombocytopenia have also been observed. Diagnosis is made by isolation of the virus from a sterile site such as the cerebrospinal fluid or acute and convalescent serum specimens showing a fourfold rise in titer. There is no treatment. If a case occurs in one pet such as a hamster, the virus should be looked for in others in the hamster colony.

Hantavirus is spread from rodents to humans through urine and feces throughout the world (see Chapter 222). It is possible, but highly unlikely that pet rodents in cages in homes would be exposed.

MISCELLANEOUS PETS

Monkeys

The most insidious and disastrous viral infection that a pet monkey can pass to a human is herpes simiae (herpes B virus). This latent infection of often-asymptomatic monkeys can be passed to humans by saliva or by a bite. It is almost always fatal in humans, in whom it causes progressive encephalitis, which if not fatal will leave

severe sequelae in most cases (see Chapter 215). If diagnosed early, treatment with aciclovir or other antiviral herpes agent, may result in improvement and even recovery. Although it is a legal requirement to screen imported pet monkeys, this does not always occur and may even miss carriers. Nonhuman primates can also transmit hepatitis A and B, monkeypox (which is clinically indistinguishable from smallpox), salmonellosis, shigellosis, campylobacteriosis, amebiasis, strongyloidiasis, giardiasis, yersiniosis and dermatophyte infection.

Reptiles and amphibians

Any reptile or amphibian may carry *Salmonella* spp., which are excreted in the feces and may infect humans caring for the pet. This has been best exemplified by outbreaks in humans who have pet turtles.

Fish

Fish may harbor *Salmonella* spp., *Aeromonas hydrophila*, *Citrobacter diversus*, *Pseudomonas aeruginosa*, *Providencia stuartii* and *Vibrio* spp. including *Vibrio vulnificus* (which can cause a rapidly fatal infection in patients with liver dysfunction) and other halophilic bacteria (salt water). In addition, *Mycobacterium marinum* has been found in fish tanks (both fresh and salt water) and can cause a purplish rash with granulomatous lesion usually of the

hands in those cleaning the tanks and handling the fish. The diagnosis is made when the skin lesion is biopsied and cultured. Treatment with rifampin and ethambutol, minocycline or trimethoprim-sulfamethoxazole (co-trimoxazole) have all been successful in eradicating this infection.

Erysipelothrix rhusiopathiae is an uncommon infection of food handlers, especially fishmongers. It causes erypsieloid, painful, indurated, irregular skin lesions, usually on the hands. It is very susceptible to penicillin. Penicillin-allergic patients can be treated with clindamycin.

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Chapter 92 - Infections Acquired From Animals Other Than Pets

Daniel S Shapiro

Zoonotic infections may be acquired from farm animals, beasts of burden, fish and wild animals via a number of routes (Fig. 92.1). Many of the emerging infectious diseases are zoonotic in origin.

The approach to the patient with a potential zoonotic infection involves the generation of a reasonable differential diagnosis that includes those infectious agents that are potentially transmissible from the specific animal to which the patient was exposed. This may be a more challenging task for the clinician when the exposure has been to a rarely encountered animal than is the case for a commonly encountered pet, as there may be limited literature upon which to generate a differential diagnosis. Historical points worth considering are summarized in Table 92.1 .

Although the number of infectious agents potentially transmissible from a specific animal to humans may be great, many of these infections are limited geographically and need not be considered in the differential diagnosis. Examples include the lack of plague transmission outside endemic areas such as the Western USA, countries that are free of brucellosis and the limitation of tularemia to the Northern hemisphere. In some cases a good history of animal exposure will be elicited but a review of the medical literature will not be able to identify a published reference on zoonotic transmission of any relevant diseases from that specific animal. Although a search of the veterinary literature may identify the animal as a potential reservoir of a zoonotic agent, access to these journals is not routinely available to nonveterinarians. One additional difficulty is that the lack of an effective veterinary or human public health system in a given country may result in a lack of knowledge of zoonotic infections transmitted from even commonly encountered animals. In such cases, it is worthwhile to consider similar animals with known flora or from which zoonoses have been acquired and infections of the animal with known zoonotic agents. Examples include the following.

- *Escherichia coli* O157:H7 infections have been most commonly transmitted to humans via the ingestion of undercooked ground beef. Deer, like cattle, are grazing herbivores and have transmitted this infection to humans via the consumption of venison.
- Camels have been noted to have serologic evidence of infection with *Coxiella burnetii*, but human cases of Q fever as a result of contact with camels have not been documented. It is possible that this is, in part, due to the lack of active case finding in those parts of the world in which camels are common.
- The environment of the animal, which may include exposure to fresh water or salt water, is important. For example, shark bite wounds may be infected with *Vibrio* spp., which are commonly found in salt water and as part of the normal oral flora of sharks, whereas fresh-water alligator bites are most commonly infected with *Aeromonas hydrophila*, an organism that is found in fresh water and as part of the normal alligator oral flora.
- Consider the diet of the animal. Cattle that have been fed material that includes nervous tissue are at increased risk of having bovine spongiform encephalopathy (BSE).
- Consider other species with which the animal has had contact, including contact with humans while in captivity. Tuberculosis, measles and shigellosis are not thought to normally be infectious agents of nonhuman primates. Rather, they are acquired from human contact. Similarly, the housing of camels indoors with cattle increases the risk that the camels will acquire bovine tuberculosis.

Good communication with the clinical microbiology laboratory is essential in the diagnosis of zoonotic infections. In some cases the diagnosis is established serologically, whereas in others a particular pathogen, perhaps one that requires special culture media or handling, may be found. In addition to increasing the probability of correctly identifying the etiology of the patient's illness, good communication is essential for safety, especially when infections due to *Francisella tularensis, Brucella* spp., cercopithecine herpesvirus type 1 (herpesvirus simiae; B virus) and other highly biohazardous agents are under consideration.

The following discussion is organized by type of animal, as this is helpful for the clinician who is attempting to generate a reasonable differential diagnosis.

DOMESTICATED HERBIVORES (CATTLE, SHEEP, GOATS, PIGS, CAMELS, HORSES AND RELATED ANIMALS)

Bacterial infections

Brucella melitensis is most commonly acquired from goats but has been acquired from sheep and dromedary camels. *Brucella abortus* is associated with cattle and, although horses can occasionally become infected, transmission to humans from horses, if it occurs, is very rare. *Brucella suis* can be transmitted to humans from both domesticated and feral pigs. Of note, the specificity of the association between the species of *Brucella* and the animal host is far from absolute. Examples include the occurrence of *B. melitensis* in cattle in parts of southern Europe, Israel, Kuwait and Saudia Arabia and the establishment of *B. suis* biovar 1 in cattle in Brazil and Colombia. [1] Many other animals have contracted brucellosis. Infection of bison in Yellowstone Park has been of concern to cattle ranchers in the Western USA. There have not, however, been documented cases of transmission of brucellosis from bison to humans. *Brucella* infections of caribou have been known to spread disease to humans.

Transmission of anthrax by this group of animals is the normal means by which the infection is acquired by humans. Cutaneous anthrax, inhalation anthrax (woolsorter's disease) and gastrointestinal anthrax all are most commonly associated with the domestication of sheep, goats and cattle. In parts of the world in which water buffalo are domesticated, these animals have served as the source of outbreaks of human anthrax, as have oxen. Animal products can transmit the disease. For example, during the early 1900s cutaneous anthrax was transmitted via contaminated shaving brushes made of animal hair.

Yersinia pestis has been (rarely) transmitted to humans from both camels and goats in Libya and from camels in endemic areas of the

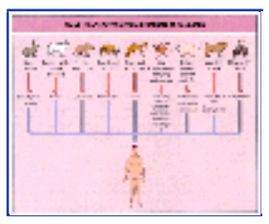

Figure 92-1 Examples of routes by which zoonoses are acquired.

TABLE 92-1 -- Selected historical points in patient exposure history.

SELECTED HISTORICAL POINTS IN PATIENT EXPOSURE HISTORY	
Historical finding	**Worth adding to differential diagnosis**
Contact with any vertebrate, especially reptiles	Salmonellosis
Exposure to urine, either directly or via contaminated water	Leptospirosis, as essentially all mammals can become infected with *Leptospira interrogans* and shed infectious organisms in the urine
Bites from wild mammals, with the exception of those from rodents other than groundhogs (*Marmota monax*)	Evaluate risk of rabies and the potential need for rabies prophylaxis
Itching and a history of cutaneous contact with a mammal	Allergic reaction, dermatophyte infection or infestation with ectoparasites, such as species-specific varieties of *Sarcoptes scabiei*
Consumption of undercooked wild mammals	Trichinellosis and toxoplasmosis
Consumption of fermented fish or marine mammals	Botulism, most commonly due to the type E toxin
Consumption of uncooked fish	Any of more than 50 parasitic infections, depending upon the species of fish eaten and the geographic locale

former Soviet Union via direct contact and in individuals who consumed the infected animals.[2]

Epizootics of tularemia, associated with heavy infestation by the wood tick, *Dermacentor andersoni*, occur in sheep. Human cases of tularemia have included cases in sheep shearers, owners and herders. In a review published in 1955, 189 human cases of tularemia had been reported in association with the sheep industry.[3] Tularemia has rarely been transmitted from pigs to humans.[4]

Tuberculosis due to *Mycobacterium bovis* subsp. *bovis* was the impetus for pasteurization of cow's milk. *Mycobacterium tuberculosis* infection can occur in cattle, but does not result in systemic infection. Infection with *M. bovis* is also associated with occupational exposure, as with slaughterhouse workers. The related *M. bovis* supsp. *caprae* has caused human infections after contact with cattle.[5]

Infection with *Listeria monocytogenes* may result from the ingestion of contaminated meat and dairy products or via direct cutaneous exposure during parturition. In the latter case, cutaneous listeriosis has been reported.[6] Infections transmitted by ingestion of animal milk are listed in Table 92.2 .

Yersinia enterocolitica, normally found in the fecal flora of pigs, has been transmitted from pigs to humans via contact and by ingestion of chitterlings (pig intestines).[7]

Erysipelothrix rhusiopathiae has been transmitted to humans from many different animals and animal products. It typically is an occupational illness, acquired via a hand wound while handling animal material. Alerting the clinical microbiology laboratory to its possibility is of help, as the organism's identification is not difficult if it is suspected.[8]

Streptococcus suis, especially type 2, a pathogen of pigs, is a common cause of bacteremia and bacterial meningitis among individuals working with pigs in Asia. *Rhodococcus equi* is commonly found in the feces of horses and in the soil. Exposure to farm animals, including horses, has been reported in some cases of human infection. The association of leptospirosis with swine is well known and one name for the illness is swineherd's disease. Cattle, goats and camels are also potential sources of human infection.

Exposure of pregnant women to the birth products of sheep and goats that are infected with *Chlamydophila abortus* (*Chlamydia psittaci*, serotype 1) has resulted in illness. Human infection with this organism has been reported in both Europe and the USA and can be severe, resulting in abortion.[9]

TABLE 92-2 -- Agents transmitted via milk products and cheese.

AGENTS TRANSMITTED VIA MILK PRODUCTS AND CHEESE	
Disease	**Source**
Clostridium botulinum toxin	Yogurt, cheese

Brucella spp.	Many animal's, milk and cheese
Campylobacter fetus	Cow's milk
Campylobacter jejuni	Cow's milk, cheese from goats
Campylobacter laridis	Cow's milk, contaminated by birds
Central European tick-borne encephalitis	Goat's milk, cheese from goats and sheep
Corynebacterium diphtheriae	Cow's milk
Corynebacterium ulcerans	Cow's milk
Escherichia coli O157:H7 and other strains	Cow's and goat's milk, cream, cheese
Listeria monocytogenes	Cow's milk, cheese
Mycobacterium bovis subsp. *bovis*	Cow's milk
Salmonella spp.	Many animal's, milk, cheese, ice cream
Staphylococcus aureus	Cow's milk
Streptobacillus moniliformis	Cow's milk (single outbreak in 1926)
Streptococcus zooepidemicus	Cow's milk, cheese
Toxoplasma gondii	Goat's milk
Yersinia enterocolitica	Cow's milk

Salmonellosis has been transmitted to humans by each of these animals, including camels. Pigs have been documented as a source of human cases of multidrug-resistant *Salmonella enterica* serotype *typhimurium* definitive phage type 104 (DT104) infection. [10]

Escherichia coli O157:H7 is often present in the gastrointestinal tract of cattle. The most common route of transmission is via ingestion of undercooked ground beef. Transmission due to fecal contamination of food products can occur, such as via unpasteurized apple cider prepared from apples that were on the ground in a cattle pasture and used for cider production. Deer, like cattle, are grazing herbivores and have been reported to transmit this infection to humans who have consumed venison. Outbreaks have been associated with visits to petting zoos.

Pasteurella aerogenes is the most commonly isolated organism from human infections following the bites of swine.[11] A number of other Gram-negative organisms have also been isolated from these infections. Camel bite injuries are typically infected and are particularly likely from male camels during the rutting season. Members of the genus *Actinobacillus* have been recovered from bites from horses and cattle and *Pasteurella caballi* has been isolated from wounds following horse bites. Rabies has been reported in all of these animals as well as in llamas.

Pig-bel, or enteritis necrotans, has been associated with the consumption of large quantities of pork in populations that have a low protein diet, such as in the highland Melanesians of Papua New Guinea and in Germany immediately following the Second World War. Evidence suggests that the major etiologic agent is *Clostridium perfringens* type C. Human cases of Q fever are acquired from sheep, goats and cattle. Infection can be transmitted for significant distances via the wind. The data on human acquisition via contaminated milk are less compelling.

Glanders, due to *Burkholderia mallei*, has been transmitted to humans via equids. The disease is now quite limited geographically and the isolation of this agent from a patient in North America or Europe must be assumed to be due to bioterrorism until proven otherwise.

Viral infections

Localized cutaneous involvement can be due to infection with the parapoxviruses orf virus (which causes contagious ecthyma and is transmitted by sheep and goats either directly or via fomites), bovine papular stomatitis virus and pseudocowpox virus; and by the orthopoxviruses cowpox virus (which is more commonly transmitted to humans via cats than cattle) and buffalopox virus. The host range of influenza A virus includes many other mammals, including swine and horses.

Variant Creutzfeldt-Jakob disease has been reported from the UK, France, Japan and other countries and appears to be associated with the consumption of meat from cattle that were infected with BSE. No cases of BSE have been identified in the USA. Prion diseases of large herbivores in the USA, including chronic wasting disease of cervids, have raised the possibility of the introduction of prion diseases into the human food supply. A detailed discussion of the molecular aspects of prion-associated disease is found in Chapter 223 and the clinical manifestations of the spongiform encephalopathies are found in Chapter 26 .

Rift Valley fever, which infects domestic ruminants, can be transmitted to humans both by mosquitoes and by contact with the tissues of slaughtered, infected animals such as sheep.[12] Similarly, Crimean-Congo hemorrhagic fever infects a variety of animals, including cattle and sheep, and is transmitted to humans via ticks (especially *Hyalomma* spp.), via contact with blood of infected animals and, if appropriate precautions are not taken, in the hospital setting.

Hendra virus, a paramyxovirus, caused an infection of horses and a few individuals in contact with these horses in Australia. The natural reservoir has been identified as a flying fox (bat). Nipah virus was the cause of an epidemic of encephalitis that affected more than 250 people in Malaysia and Singapore, killing 100 people. The vast majority of those infected had contact with pigs, which were culled in order to stop the epidemic. The natural reservoir of Nipah virus, a paramyxovirus that is related most closely to Hendra virus, has been identified as a bat. Menangle virus, a recently described paramyxovirus, caused an infection of pigs and infection in humans in contact with the pigs in Australia. The natural reservoir has been identified as a flying fox (bat).

Recent concern has been generated over the possibility of certain endogenous porcine retrovirus infections causing disease in humans following xenotransplantation of organ tissues from pigs. Some of these retroviruses can propagate in human cell lines and they could potentially induce immunodeficiency in experimental systems. [13] This poses a potential risk of activation of porcine retroviruses in the setting of an unnatural host such as an immunosuppressed, solid organ, human transplant recipient. Fortunately, it appears that commonly utilized porcine heterografts for heart valve replacement surgery are unlikely to be complicated by inadvertent activation of porcine retroviruses. The currently employed glutaraldehyde fixation and sterilization method for porcine heart valves eliminates infectivity of endogenous retroviruses.[14]

Parasitic infection

An epidemic of cryptosporidiosis occurred in 1993 in Milwaukee, Wisconsin, in which the public water supply was contaminated and more than 400,000 people were infected. The epidemic was traced to untreated water from Lake Michigan, from which the causative organism was incompletely removed by the water filtration process. Possible sources included cattle along two rivers, slaughterhouses and human sewage.[15] In addition to water-borne epidemics, human infections occur via direct contact with cattle and sheep (the disease primarily occurs in lambs), including infection as a result of farm visits.

Echinococcal disease, although not transmitted to humans from sheep, occurs in areas of the world in which sheep serve as an intermediate host and in which dogs ingest sheep viscera, subsequently excreting infective eggs in their feces. A different echinococcal strain occurs in dromedary camels.

The demonstration of the eggs of *Dicrocoelium dendriticum*, the lancet liver fluke of cattle, sheep and goats, in human feces may be due to a true infection (dicrocoeliasis) but is more often due to a pseudoinfection. In pseudoinfections, eggs obtained via the consumption of liver (sometimes eaten raw) are passed per rectum without causing an actual infection in the human.

The pig ascarid *Ascaris suum* has been reported to cause human infection. Illness, including pulmonary infiltrates as a result of larval migration, resulted in intubation in students who ingested intestinal contents of pigs as a result of a fraternity 'prank'.[16]

Taenia solium, the pork tapeworm, is acquired via the ingestion of undercooked infected pork. *Trichinella spiralis* is most commonly acquired from eating undercooked pork, but has also been acquired following the ingestion of horsemeat.[17] *Taenia saginata*, the beef tapeworm, is acquired via the ingestion of undercooked beef. Toxoplasmosis can be acquired via the ingestion of undercooked meat, especially lamb, as well as from contaminated goat's milk.

Dermatophyte infection

Infection with zoophilic dermatophytes commonly occurs in people in contact with these animals. These include, for example, *Trichophyton verrucosum* spread from cattle to humans, and *T. equinum* from horses,[18] among others.

BATS

Rabies virus is known to occur in many species of bat and transmission to humans via bite, scratch and inhalation of aerosolized saliva has been reported. Bats also account for many cases of rabies in livestock. Other lyssaviruses that have been transmitted to humans from bats include European bat lyssavirus-1, European bat lyssavirus-2 and, most recently, Australian bat lyssavirus.[19] Most recent reports of human rabies from bat exposure find no clear evidence of a documented bat bite. Transmission apparently occurs from inadvertent bites or from unrecognized contact with the bat saliva. This is the rationale for the administration of rabies immune globulin and rabies vaccine when a bat is found in the room upon awaking from sleep, in the room of a small child or in the room of an intoxicated or mentally challenged person[20] (see Chapter 219). As noted above, bats have been found to be reservoirs of the zoonotic paramyxoviruses Nipah virus, Hendra virus and Menangle virus.[21]

Many outbreaks of histoplasmosis due to *Histoplasma capsulatum* have been associated with exposure to bat guano in caves. Other bat-associated outbreaks have been the result of disturbing piles of bat guano from old buildings[22] and clearing debris from a bridge.[23]

NONHUMAN PRIMATES

The numerous pathogens that have been isolated from nonhuman primates (NHPs) include many human pathogens. Documented transmission of human pathogens include bacterial (*Shigella* and *Salmonella* spp.), mycobacterial (*M. tuberculosis*), viral (hepatitis A virus), parasitic (*Entamoeba histolytica*) and fungal (dermatophyte) agents. In addition, there are infectious agents of human origin that infect NHPs and that have not been reported to be transmitted back to humans. These include measles virus and (human) herpes simplex virus type 1.

Of concern are those infectious agents that are not normally regarded as human pathogens. Many of these are particularly virulent. Historically, it is worth noting that molecular evidence suggests that HIV-1 was originally a pathogen of chimpanzees, *Pan troglodytes troglodytes*, and that HIV-2 was originally a pathogen of sooty mangabees. There are numerous simian immunodeficiency virus (SIV) strains and it is possible that one or more might be transmitted to humans via contact, ingestion or by growing the pathogen. Transmission of SIV to a human has occured in a laboratory accident.[24]

The possibility of life-threatening infection with cercopithecine herpesvirus-1 must be considered in bites, scratches and contact with saliva from Asiatic macaques, especially from the rhesus monkey, *Macaca mulatta*. It is hypothesized that there are distinct genotypes of the virus and that the isolates from different species vary in their pathogenicity for humans. Institutions that work with macaques should have plans in place to prevent and treat infections with cercopithecine herpesvirus-1[25] and have access to testing by laboratories with special expertise in the isolation and serologic testing of this virus. The NIH B Virus Resource Laboratory at Georgia State University (phone (404) 651-0808, fax (404) 651-0814, e-mail bvirus@gsu.edu) is the reference laboratory for the USA.

Filovirus infections with both Ebola and the Reston strain of Ebola, which is less pathogenic for humans than are other strains of Ebola, have been transmitted from NHPs to humans. Marburg virus, a filovirus causing hemorrhagic fever with high mortality, has been transmitted from vervet, or green monkeys, to humans. The reservoir in nature of Ebola and Marburg viruses remains unknown.

Monkeypox, an orthopoxvirus, was initially identified in human cases of illness that were clinically consistent with smallpox. It is found in NHPs and in squirrels in Africa and has been transmitted from human to human. Tanapox (benign epidermal monkeypox) has been transmitted to humans both via mosquitoes and by direct contact with monkeys in primate centers in the USA, but has not been transmitted from human to human. Yabapox virus has, rarely, caused subcutaneous growths at the site of inoculation.

Kyasanur forest disease virus, a member of the tick-borne encephalitis subgroup, is found in Karnataka State, India, and has a number of NHP reservoirs. The presence of dead monkeys in the endemic area may precede an epidemic.

Rabies has been reported in NHP but, with the exception of a recent report in which the white-tufted-ear marmoset (*Callithrix jacchus*) was the source of eight human cases of rabies in Brazil,[26] there are only isolated case reports of transmission of rabies from NHPs to humans.

MUSTELIDS (FERRETS, SKUNK, OTTER, MINK, WEASEL, BADGER, MARTENS)

Influenza A virus has been transmitted in a laboratory setting in which a researcher was infected by a ferret that had been infected with a strain of influenza A virus and which 'sneezed violently at close range' while it was being examined.[27] Ferrets are susceptible to influenza A and B viruses. Mink on mink farms have been found to be infected with influenza A viruses.[28]

There is a report of *M. bovis* infection of the right palm more than 20 years following a ferret bite.[29] *Mycobacterium bovis* is known to infect wild ferrets and badgers. There is a case report of sporotrichosis complicating a badger bite. Rabies infection is known to occur in skunks, otters, badgers, weasels, mink and ferrets, including pet ferrets, in the USA. Transmission of rabies from skunks to humans has been documented.[30] A rabies vaccine has been licensed for use in ferrets and the National Association of State Public Health Veterinarians recommendations are for primary immunization at 3 months and booster immunizations annually.[31] The recommendations

regarding a healthy ferret that bites a human are the same as those for dogs and cats with respect to confinement and observation for 10 days, with evaluation by a veterinarian at the first sign of illness.[31]

Rat bite fever as a result of ferret and weasel bites was reported in the medical literature between 1910 and 1920. Only in a report of a weasel bite was there isolation of an organism from the patient's blood.[32] Trichinellosis has been reported in people who ate inadequately cooked or raw liver, spleen, blood and muscle of a badger.[33]

RODENTS

Yersinia pestis is transmitted in epidemics from rats to humans via the rat flea, *Xenopsylla cheopis*. Numerous rodents other than rats serve as reservoirs, some of which have been responsible for human plague. Similarly, tularemia is widely distributed in nature and has been transmitted to humans by many different rodents.

Leptospirosis is commonly associated with skin or mucous membrane exposure to water contaminated by the urine of rodents, including rats, mice and voles. It has rarely been reported to be transmitted via rodent bite.[34] Other uncommonly reported bacterial infections following rodent bites include *Pasteurella multocida*, the *Pasteurella* 'SP' group and sporotrichosis. Rat bite fever can be due to either *Streptobacillus moniliformis* or *Spirillum minus*. The former has been transmitted to humans not only by wild rats but also laboratory rats, mice and other rodents.

It is unclear how often rodents cause cases or outbreaks of human salmonellosis. However, given that *Salmonella* spp. are commonly recovered from rodent feces, the serotypes commonly recovered from rodents are similar to those recovered from cases of human disease, and rodents often infest human dwellings, restaurants and food production facilities, it is likely that they account for some fraction of human illness.

Many of the tick-borne relapsing fevers have wild rodents as reservoirs. This is also the case for *Babesia microti*, Lyme disease and human granulocytic ehrlichiosis. The reservoirs of Colorado tick fever include a number of squirrels, chipmunks and other rodents. Similarly, Powassan encephalitis, tick-borne encephalitis and Omsk

hemorrhagic fever virus are transmitted via ticks and have small mammals as reservoirs. *Leishmania* spp. are transmitted by sandflies and often have rodents as reservoirs.

Those members of the Hantavirus genus that are known to cause hantavirus pulmonary syndrome (HPS) are carried by New World rats and mice, family Muridae, subfamily Sigmodontinae, and are transmitted via the inhalation of rodent excreta or saliva or, rarely, via rodent bite. In the USA and Canada, the viruses include Sin Nombre virus, the main cause of HPS, transmitted by the deer mouse (*Peromyscus maniculatus*); New York virus, transmitted by the white-footed mouse (*Peromyscus leucopus*); Black Creek Canal virus, transmitted by the cotton rat (*Sigmodon hispidus*); and Bayou virus, transmitted by the rice rat (*Oryzomys palustris*).[35] In South America, viruses include Andes virus in Argentina, Chile and Uruguay transmitted by the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*), a virus for which there is epidemiologic evidence of person-to-person transmission; Juquitiba virus in Brazil; Laguna Negra virus in Paraguay, transmitted by the vesper mouse (*Calomys laucha*); and Bermejo virus in Bolivia.[36] Hantaviruses that are associated with hemorrhagic fever with renal syndrome in Europe and Asia include Hantaan virus, transmitted by the murine field mouse (*Apodemus agrarius*); Dobrava virus transmitted by the murine field mouse (*Apodemus flavicollis*); Seoul virus, transmitted by the Norway rat (*Rattus norvegicus*) in Asia; and Puumala virus transmitted by the bank vole (*Clethrionomys glareolus*).[37]

Arenaviruses are transmitted from rodents via the excreta and urine. These include lymphocytic choriomeningitis virus, which is found worldwide and has been transmitted to humans by hamsters[38] as well as mice; Machupo virus, which causes Bolivian hemorrhagic fever and is transmitted by *Calomys callosus*; Junin virus, which causes Argentinian hemorrhagic fever and is transmitted by *Calomys* spp.; Guanarito virus, which is found in Venezuela; Lassa fever virus, which is found in Africa and is transmitted by the multimammate rat, *Mastomys natalensis*; and a recently described New World arenavirus that caused three fatal infections in California and shared 87% identity with the Whitewater Arroyo virus at the nucleotide level.[37]

Reservoirs of cowpox virus include several rodents. This is consistent with the epidemiology of cowpox in which cat contact is implicated. Cowpox, or a similar virus, has also been transmitted via rat bite.[39]

Rickettsialpox has been associated with infestation of mice (*Mus musculus*) with mites which serve as the vector for human disease.[40] Rodents serve as reservoirs for many other rickettsial diseases, including murine typhus in which rats are the principal reservoir and the flea *X. cheopis* the principal vector; *Rickettsia prowazekii*, which has been associated with flying squirrels;[41] scrub typhus, in which rats are hosts of the trombiculid mite vectors; and members of the spotted fever group.

Although the issue of whether giardiasis is commonly zoonotic in origin is debated, beavers may have been the source of an outbreak of water-borne giardiasis.[42]

Ingestion of rodents has been associated with rare cases of trichinellosis, such as due to the ingestion of squirrel and bamboo rat.[43] There has been speculation on whether consumption of squirrel brains causes a spongiform encephalopathy, but data are limited.[44] Eating fermented beaver has resulted in botulism.[45]

Trichophyton mentagrophytes var. *mentagrophytes* is a common zoophilic dermatophyte, infecting humans and domestic animals. Rodents are regarded as the reservoir of infection.

LAGOMORPHS (RABBITS, HARES)

Tularemia, also known as rabbit fever, is acquired from rabbits and hares as a result of cutaneous contact and skinning of rabbits, presumably entering via microabrasions in the skin or conjunctiva, and via ingestion.[4] [46] Transmission via infectious aerosol has also been reported as a result of mowing over a rabbit.[47] Tularemia transmission to humans has not been reported from domesticated rabbits. Although uncommon, eight cases of human bubonic plague from 1950 to 1974 were reported as a result of contact (e.g. skinning) with rabbits and hares[48] in plague-endemic areas of the USA. Q fever has been transmitted to humans following contact with wild rabbits.[49]

A patient with *Bordetella bronchiseptica* respiratory infection was shown to have a strain that was indistinguishable by pulsed- field gel electrophoresis from the strain isolated from a respiratory tract isolate from one of 20 farm rabbits that slept with a cat with which she had contact.[50]

RACCOONS

The raccoon ascarid, *Baylisascaris procyonis*, has caused cases, including fatal ones, of meningoencephalitis, usually in young children who accidentally ingest infectious ova.[51] Ocular involvement has also been reported. Leptospirosis has been reported from contact with raccoons.[52] Rabies is common in raccoons, although transmission to humans in the USA has not been reported.

MONGOOSES

Leptospirosis is common among mongooses in Hawaii[53] and a number of Caribbean islands.[54] Rabies is quite common among many species

of mongoose and accounts for a significant number of cases of human exposure to rabies in the Caribbean, it is the principal rabies reservoir in South Africa and it may be an important source of wildlife rabies in India.[55]

INSECTIVORES

Hedgehog contact has transmitted salmonellosis[56] and dermatophyte infections due to *Trichophyton erinacei*. [53] In an outbreak of lepto-spirosis in Italy in which 32 of 33 confirmed cases were contracted by drinking water at the same water fountain, a dead hedgehog was found in a water reservoir connected to the system, although isolation of *Leptospira* spp. from the hedgehog was not attempted.[57]

The Asian house shrew, *Suncus murinus*, has been found to be infested with the oriental rat flea, *Xenopsylla cheopis*, and infected with *Yersinia pestis*. It may well be important in the maintenance of plague between epidemics. Insectivores also appear to be reservoirs of tick-borne encephalitis and tularemia.

MARINE MAMMALS (SEALS, SEA LIONS, WALRUS, WHALES, DOLPHINS, PORPOISES, MANATEES)

At the case report level, there are several infections that have been transmitted from marine mammals to humans. Leptospirosis, which is commonly encountered in seals and California sea lions, has been transmitted from an infected sea lion pup to a human. Two people developed leptospirosis after performing a necropsy on a sea lion that died of leptospirosis.[58] Human infection with *Erysipelothrix rhusiopathiae* has been reported on a few occasions among veterinarians and veterinary students caring for or performing autopsies on cetaceans.[59] In these reports, the isolation of the organism was not made from the human cases. Two of three people who cared for affected gray seals developed 'single milker's nodule-like lesions' on the fifth finger of the right hand. The lesions from the seal handlers demonstrated virus particles that were identical with the virus particles from the seals' pox lesions and were characteristic of the paravaccinia subgroup of poxviruses. [60]

Pulmonary tuberculosis due to *M. bovis* has been transmitted from seals in a marine park in Western Australia to a seal trainer who developed pulmonary tuberculosis 3 years after his last exposure to the animals with an isolate of *M. bovis* that could not be distinguished from the seal isolates on the basis of DNA restriction endonuclease analysis.[61] Seal trainers are in very close contact with seals who, by barking and coughing, are potentially able to transmit infection via the aerosol route.

Four people involved in necropsies of harbor seals from which influenza A virus, A/Seal/Mass/1/80 (H7N7), was isolated developed purulent conjunctivitis but did not have detectable antibodies in single serum samples 3–6 months after the exposure to the influenza A virus isolated from the seals.[62] A seal that was known to be infected with the influenza A virus sneezed into the face and right eye of a person who subsequently developed conjunctivitis from which the virus was isolated.[63] Influenza A virus has also been isolated from cetaceans.

Numerous cases of 'seal finger' have been reported in people who have been bitten or scratched by seals and from skinning or handling seals. Seal finger often responds to tetracycline therapy. The etiologic agent has not been established. Other organisms that have been transmitted via the bite of marine mammals include a single case report of *Mycoplasma phocacerebrale*, which was isolated from the drainage material from a patient's fingers and swabs from the seal's front teeth.[64]

Consumption of whale, seal and walrus meat is not uncommon among the Inuit in Canada, Alaska, Greenland and Siberia. There have been large epidemics of

salmonellosis resulting from consumption of whale meat from floating and beached whale carcasses that have been used as the source of food. Trichinellosis (trichinosis) has been acquired following the consumption of raw or undercooked walrus meat. The clinical presentation in arctic trichinellosis due to *Trichinella nativa* differs from that of classic trichinellosis caused by *Trichinella spiralis* in that the most prominent clinical symptoms in arctic trichinellosis are gastrointestinal, with prolonged diarrhea.[65] Food-borne botulism, typically due to *Clostridium botulinum* type E, has been acquired from the consumption of fermented foods included beluga whale meat, seal meat, seal flippers and walrus meat.

ARMADILLOS

Both experimental and naturally occurring leprosy in nine-banded armadillos has been noted and there has been a body of literature (reviewed by Blake *et al.*[66]) that suggests that contact with armadillos may have been the source of leprosy in some patients in the USA and Mexico. Sporotrichosis has been found to be highly associated with armadillo contact in Uruguay.[67]

BIRDS

Psittacosis is transmitted to humans not only via pet birds but also via turkeys, wild and domestic pigeons, ducks and other birds.[68]

Salmonellosis has been acquired from contact with birds and from consumption of birds (e.g. chicken, turkey) and eggs.[69] *Campylobacter jejuni* and *C. laridis* infections have been associated with both the consumption of birds and, interestingly, consumption of milk that has been pecked by magpies (*Pica pica*) and jackdaws (*Corvus monedula*). [70] *Erysipelothrix rhusiopathiae* has been acquired from bird contact. Newcastle disease virus of fowl, an occupational disease, causes an acute conjunctivitis which may be associated with pre-auricular adenitis.[71]

Histoplasmosis, often in large outbreaks, has been the result of inhalation of bird excreta.[72] Infection with *Cryptococcus neoformans*, which is known to be found in bird droppings, has at the case report level been linked to exposure to pet birds [73] and fancy pigeons.[74] Q fever has been reported in five members of a family as a result of exposure to either aerosolized, contaminated pigeon excreta or infected ticks, or both.[75]

Avian strains of influenza A virus represent a global concern, as the host range of the viruses may include humans. There exists the potential for pandemic influenza as a result of the introduction of an avian virus with a hemagglutinin to which humans lack immunity.[76] In 1997 in Hong Kong, there were 18 human cases of influenza A H5N1 infection and six deaths. The outbreak ended after the institution of control measures that included the culling of poultry in Hong Kong. In 1999 an H9N2 strain, closely related to a quail virus which cocirculated with H5N1 viruses in live poultry markets in Hong Kong in late 1997 and shown to possess a set of internal genes similar to those of the H5N1 viruses, infected two children.[77]

The recent epidemic of West Nile virus infection in the USA is largely attributable to the introduction of this flavivirus into a new ecologic niche in wild birds in North America. Black birds, crows, other wild birds and domestic chickens are susceptible to this viral illness and this forms the reservoir for this mosquito-transmitted infection that is responsible for a potentially lethal form of viral encephalitis.[78]

Tularemia has been acquired from wild birds. A case of Crimean-Congo hemorrhagic fever in an ostrich farm worker who was involved in the slaughter of ostriches, *Struthio camelus*, and handled the fresh blood and tissues of the birds has been reported. There were numerous adult *Hyalomma* ticks on the ostriches and he

likely was infected either directly due to skinning the ostriches or as a result of the presence of the ticks on the ostriches.[79]

FISH

In addition to the normal flora of the fish, a wound can become infected with environmental bacteria. The species of bacteria that live in water are dependent on both salinity and temperature. Freeliving estuarine and fresh-water bacteria include the genera *Vibrio, Aeromonas* and *Plesiomonas.* As a result, the etiologic agents isolated from an infected wound from a fish bite, spine or fin injury that occurs in salt water may well be different from one that occurs in fresh water. The normal flora of teeth in salt-water sharks includes, for example, *Vibrio* spp., including *V. carchariae*, an organism that was the cause of infection following the bite of a great white shark.[80] By contrast, *Edwardsiella tarda* is commonly isolated from catfish injuries occurring in fresh water. Other organisms that have caused wound injuries as a result of injuries from fish include *Aeromonas hydrophila, Erysipelothrix rhusiopathiae, Mycobacterium marinum, Mycobacterium terrae, Streptococcus iniae, Vibrio vulnificus* and *Vibrio vulnificus* serovar E (biotype 2; indole-negative) from eels.[81] *Vibrio alginolyticus, Photobacterium damselae* subsp. *damselae (Vibrio damsela), Shewanella putrefaciens, Pseudomonas aeruginosa* and *Halomonas venusta* have all been isolated from fish bites and injuries. It is not always clear whether the source of the organism is the fish or the water.

Ingestion of fish or fish products can pose a significant risk of acquiring both bacterial and parasitic infections unless the fish has been well cooked.

Vibrio spp., including *V. fluvialis, V. hollisae, V. parahaemolyticus* and *V. cholerae* O1,[82] have all been associated with fish consumption, as has *P. shigelloides*. Eel consumption has been associated with *Photobacterium damselae* subsp. *damselae (Vibrio damsela).*[83] *Listeria monocytogenes* infections have been associated with the consumption of fish, including vacuum-packed salmon and cold-smoked rainbow trout.[84]

Fish-associated botulism is usually due to type E toxin and in the USA is most common among Alaskans. Fermented fish eggs, fish eggs, home-marinated fish and dry salted fish have all been implicated. Consumption of apparently fresh (unpreserved and unfermented) fish in Hawaii resulted in three adults with botulism due to type B toxin.[85] Numerous parasitic infections have been reported following the consumption of raw, undercooked, pickled and lightly or cold-smoked fish. Selected cestodes, trematodes and nematodes acquired from the consumption of fish are listed in Table 92.3 .

AMPHIBIANS

Contact with amphibians has transmitted sparganosis, due to *Diphyllobothrium (Spirometra) mansoni*, which has been transmitted by the use of contaminated frog flesh as a poultice (reviewed by Huang and Kirk[86]), and intraocular *Alaria* spp. in a woman with a long history of frog collection and food preparation.[87]

Ingestion of amphibians has transmitted sparganosis. Infection with the trematode *Fibricola seoulensis* has been reported from Korea, including a report in which 10 Korean soldiers who ate raw or undercooked flesh of snakes or frogs during survival training acquired the infection. [88] A single case of a fatal infection in a 24-year-old man due to *Alaria americana* has been attributed to the consumption of undercooked frogs' legs, although there was no documentation

TABLE 92-3 -- Selected parasites transmitted via consumption of fish.

SELECTED PARASITES TRANSMITTED VIA CONSUMPTION OF FISH		
Parasite	**Type of parasite**	**Types of fish**
Diphyllobothrium latum	Cestode	Salmon, pike, perch, burbot
Diphyllobothrium pacificum	Cestode	Marine fish
Diphyllobothrium ursi	Cestode	Salmon
Nanophyetus salminicola	Trematode	Usually salmonids
Heterophyes heterophyes	Trematode	Mullet, tilapia, mosquito fish
Haplorchis yokogawai	Trematode	Mullet
Haplorchis taichui	Trematode	Mullet
Clonorchis sinensis	Trematode	Fresh-water fish
Opisthorchis viverrini	Trematode	Fresh-water fish
Opisthorchis felineus	Trematode	Fresh-water fish

Metorchis conjunctus	Trematode	Fresh-water fish
Anisakis simplex	Nematode	Salmon, tuna, herring, mackerel, others
Pseudoterranova decipiens	Nematode	Cod, pollock, haddock, salmon, Pacific rockfish
Eustrongyloides spp.	Nematode	Killfish, estuarine fish, minnows
Dioctophyma renale	Nematode	Fresh-water, estuarine fish
Capillaria philippinensis	Nematode	Fresh-water, estuarine fish
Gnathostoma spinigerum	Nematode	Fresh-water fish

of consumption. Two cases of intraocular infection with an *Alaria* spp. were reported in Asian-Americans in California who consumed cooked frogs' legs in Chinese dishes.[89] Although frogs' legs have a very high rate of contamination with *Salmonella*, there are no published reports of salmonellosis attributed to their ingestion. *Salmonella* infections remain a potential risk when handling reptiles, particularly turtles, lizards and snakes.

BEARS

There is a published report of transmission of leptospirosis to two zoo employees in which the most likely source was an ill polar bear cub.[90] There is a notable lack of published reports on infections following the bites of bears. A case report in which a man shot and killed a grizzly bear in Alaska and scratched his left index finger on one of the bear's teeth while removing the bear's tongue resulted in an infection with *Mycobacterium chelonae* subsp. *abscessus*.[91]

Consumption of undercooked bear meat has been associated with trichinellosis. Bears are known to have a high rate of toxoplasmosis and the possibility of a dual infection (trichinellosis and toxoplasmosis) in a person who ingested undercooked bear meat has been reported.[92] It is worth noting that acute hypervitaminosis A has been reported following the ingestion of polar bear liver.

LARGE HERBIVORES (ELEPHANTS, RHINOCEROSES)

Few infections have been transmitted from elephants and rhinoceroses to humans. These include documented transmission of *M. tuberculosis* from elephants,[93] *M. bovis* from rhinoceroses[94] and an orthopoxvirus (possibly cowpox). It is likely that cases of tuberculosis in elephants, which are almost all reportedly due to *M. tuberculosis*, are the result of human-to-elephant transmission.

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Chapter 93 - Food-borne and Water-borne Infections

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INTRODUCTION

Food and water are essential for human existence. However, there is a constant risk of food and water becoming contaminated with potentially pathogenic organisms. Every human being risks exposure to disease when ingesting either food or water that may be vehicles of infection. A large number of such infections simply result in acute diarrhea and/or vomiting, a syndrome usually called 'food poisoning'. The term 'food-borne illness' is used whether the vehicle of infection is food or water. Also included in the definition of food poisoning are gastrointestinal illnesses caused by natural plant toxins, by heavy metals and by toxins such as scombroid that are associated with eating some types of fish.

There are other food-borne and water-borne illnesses that are distinct from food poisoning. In these diseases, although food or water are the vehicles of infection and the gastrointestinal tract is the portal of entry, gastrointestinal symptoms are minimal and infection results in systemic symptoms or distant foci of disease.

Each year the average adult in a developed country drinks more than 500 liters of water and eats over 450kg of meat and vegetables, not to mention other foods. It is rare to find anyone who has not, at some time, been a victim of food poisoning. Many infections result from organisms derived from the gastrointestinal tract of other humans that have led to contamination of food or water, so-called fecal-oral transmission. Increasingly recognized, however, is the role of other species in food- and water-borne illness. Zoonoses such as brucellosis are well recognized and more unusual problems, such as trichinella infection related to wild boar meat, are occasionally reported.[1] Much recent interest has focused on prions crossing species barriers, with good evidence that new variant Creutzfeld-Jakob disease is related to the consumption of beef from animals suffering from bovine spongiform encephalopathy (see Chapter 26 and Chapter 223).

This chapter outlines the problems related to infection caused by what we eat and drink.

EPIDEMIOLOGY

Food

The provision of food and water supplies has become increasingly complicated as society has become more urbanized and as the global economy has become more complex. In developed countries, the production and distribution of food have become highly sophisticated so that it is frequently consumed a long distance from its source and a long time after it has been produced. Often there are large, centralized facilities for food production with the risk that contamination early in the production process may lead to widespread distribution of infected food to many different regions. In addition, there is an increasing tendency to eat food away from home and, in particular, there has been a huge increase in the consumption of convenience and 'take-away' foods. In contrast, in less developed countries food is usually consumed close to its source with much less centralization because refrigeration and other means of preservation are limited and food soon spoils if transported over any distance. Restaurants are less common and most food is prepared and consumed in the home. Thus, point source outbreaks of food poisoning, often on a massive scale, are relatively common in developed countries but rare in developing countries. In England and Wales between 1995 and 1999 there were 2374 reported outbreaks of food poisoning, and in the USA there were 2751 outbreaks over a similar time period.[2] [3] However, most cases of food-borne infection are sporadic (i.e. not part of an outbreak) whether they occur in the tropics or in temperate regions.

Almost every type of food has been associated with carrying infectious agents and some have been associated with noninfectious toxins (Table 93.1). There are numerous organisms that can cause food-borne infection, with some being recognized as very common causes of food poisoning. The data on the incidence and etiology vary geographically, largely because different countries have developed different surveillance systems. Most surveillance is passive, based on reports by clinicians and on laboratory reports of isolation of food-borne pathogens. In England and Wales, where food poisoning is notifiable and where laboratories report to the Public Health Laboratory Service, there were over 50,000 cases of food poisoning in 1991, giving a notification rate of 103 per 100,000 population. *Campylobacter* spp. were the most commonly reported cause of gastrointestinal illness, with *Salmonella* spp. infection second. The latter, however, was more commonly implicated in outbreaks. Similar figures are found in the USA, where it is estimated that over 76 million cases of food-borne disease occur each year, with about 3000 deaths. A program of active surveillance of food-borne diseases began in the USA in 1996. Called FoodNet, it surveys seven bacterial and two parasitic food-borne diseases in nine states, covering about 10% of the US population.[4] *Campylobacter* spp. are the most common cause of food-borne illness, with an incidence rate of about 25 per 100,000 population, and *Salmonella* spp. were second, with a rate of 16 per 100,000. There are regional and seasonal differences in the incidences of certain infections but the reasons for these are not clear. Information about food-borne illness in developing countries is scanty because few developing countries have any surveillance systems in place because of resource limitations.

The morbidity of food poisoning is considerable but, in developed countries, the mortality is relatively low. Most deaths occur in debilitated people, often at the extremes of age. Studies in the USA show that salmonellosis is the most likely infection to lead to death, although listerial infections are more likely to lead to hospitalization than other causes of food poisoning.[5] Statistics from the developing countries are not readily available but it is estimated that diarrheal disease (much of which is related to food-borne infection) is respons-ible for 3 million deaths a year in the tropics, mainly in children.[6]

Water

Water becomes a vehicle for infection when contaminated by human or animal feces or by organisms from another source. The probability

TABLE 93-1 -- Types of foods associated with various pathogens that cause food poisoning.

TYPES OF FOODS ASSOCIATED WITH VARIOUS PATHOGENS THAT CAUSE FOOD POISONING	
Pathogen	**Foods**
Staphylococcus aureus	Cream pastries, salads, meat products, cold foods
Bacillus cereus	Fried rice, vegetables, meat dishes, vanilla sauce
Clostridium perfringens	Cooked meats, gravies
Vibrio cholerae, *Vibrio parahaemolyticus*, *Vibrio vulnificus*	Shellfish, seafood
Campylobacter jejuni	Milk, poultry
Salmonella enteritidis	Eggs, poultry, other meats
Shigella spp.	Salads, milk, cold foods
Yersinia enterocolitica	Milk, pork products
Escherichia coli	Ground beef, milk, lettuce, unpasteurized cider

Listeria monocytogenes	Soft cheese, paté, milk, coleslaw
Clostridium botulinum	Meats, home-canned fruit and vegetables
Hepatitis A and enteric viruses	Shellfish, various foods

of becoming infected with an organism through this fecal-oral route depends on the availability of potable water. Thus, water-borne infection is relatively rare in developed countries where there are usually municipal supplies of treated drinking water and reliable sewage disposal systems to prevent contamination of the drinking water supply. However, failure of these sophisticated systems can result in large outbreaks of disease.[7] In recent years there have been outbreaks of illness caused by *Cryptosporidium* spp. from cattle feces contaminating reservoirs at times when water treatment procedures were inadequate. Sporadic water-borne infection may occur in developed countries as a result of poor hygiene but this is relatively uncommon. More common is the sporadic infection resulting from exposure to water inadvertently swallowed during leisure activities such as swimming, fishing, canoeing and surfing (see Chapter 89).[8] Some of these infections result from the dumping of raw sewage into rivers and into the sea (Table 93.2).

Water-borne infection is more common in developing countries, particularly in rural areas where basic sanitation may be rudimentary and access to clean water is limited. This can be a particular problem in times of drought. Contamination of drinking water is common and may even occur at the communal taps when piped water is supplied to a village. Firewood is usually scarce so drinking water is not boiled and water filters are usually too expensive. Constant exposure to unclean water not only leads to a large burden of acute and chronic diarrhea, but also increases the risk of parasitic disease (Fig. 93.1).

PATHOGENESIS AND PATHOPHYSIOLOGY

Host factors

The ability of the human gastrointestinal tract to withstand infection by contaminated food or water depends on a variety of host defenses, including human behavior. If food looks, smells or tastes bad because of contamination it may not be ingested. Most things we ingest are not sterile, even if they seem safe, but acid (pH<4) gastric secretions kill ingested bacteria relatively easily. Usually, a large inoculum of organisms is required to overcome this acid barrier. However, relative achlorhydria, whether due to disease or to drugs

TABLE 93-2 -- Micro-organisms associated with water-borne infections.

MICRO-ORGANISMS ASSOCIATED WITH WATER-BORNE INFECTIONS	
Bacteria	*Vibrio cholerae*
	Vibrio parahaemolyticus
	Campylobacter jejuni
	Shigella spp.
	Escherichia coli (especially enterotoxigenic *Escherichia coli*)
Viruses	Rotavirus
	Norwalk virus
	Small round-structured viruses
	Hepatitis A virus
	Hepatitis E virus
Protozoa	*Giardia lamblia*
	Entamoeba histolytica
	Cryptosporidium parvum
	Isospora belli
	Cyclospora cayetanensis
	Microsporidia spp.
	Dientamoeba fragilis
	Balantidium coli

Figure 93-1 Water source in a developing country at a refugee camp.

(e.g. proton pump inhibitors or antacids), may allow bacteria to multiply within the stomach so that a relatively smaller initial inoculum is needed to cause disease.

Additional protection is provided by the normal bowel flora. In the human gut there are several hundred species of bacteria, almost all of which are anaerobes, and these organisms may physically prevent pathogenic bacteria from adhering to enterocytes, often a key prerequisite for causing disease.[9] This is sometimes termed colonization resistance. Normal gastrointestinal motility ensures a regular distribution of the bowel flora and may help to eliminate potential pathogens.

The human gastrointestinal tract is also extremely active immunologically. Lymphocytes in the lamina propria, intraepithelial lymphocytes and lymphoid nodules, such as Peyer's patches in the small bowel, make up what is known as the gut-associated lymphoid tissue. Plasma cells in the lamina propria make specific antibody and most importantly produce secretory IgA, which can effectively block bacterial adhesion to enterocytes. Neonates receive immunoglobulin and lactoferrin in the colostrum of breast milk, which provides

extra protection as compared with formula feeds. Patients who are immunodeficient, such as those who have HIV infection or transplant recipients, are at greater risk of infection by enteric pathogens. Debilitated people, the very young and the very old are also at increased risk.

Microbial factors

Food- and water-borne organisms that cause disease must either cause damage to the intestinal mucosa or must be able to invade via the gastrointestinal tract to cause systemic or distant infection. The specific pathogenetic mechanisms for individual organisms are detailed in Chapter 1 , Chapter 43 and Chapter 46 , but the general principles are outlined below.

Toxins

A large variety of toxins may be produced by enteric pathogens and these fall into three main categories:[10]

- enterotoxins,
- cytotoxins, and
- neurotoxins.

Enterotoxins

The best example of an enterotoxin is cholera toxin, which binds to the enterocyte by means of its five ß-subunits, thus facilitating the entry of the a-subunit into the cell, where it can activate adenyl cyclase; this in turn leads to net secretion of chloride ions and water into the gut lumen. This excess secretion overwhelms the normal resorptive capacity of the small and large bowel, leading to diarrhea. Most enterotoxins act in a similar manner, causing net secretion into the lumen but causing relatively little inflammation in the mucosa (see also Chapter 161).

Cytotoxins

Cytotoxins, such as those produced by *Shigella* spp. or by enterohemorrhagic *Escherichia coli*, bind to enterocytes and lead to inflammation and usually to mucosal damage. Such cytotoxins are often associated with bloody diarrhea or dysentery, because of the mucosal damage they cause.

Neurotoxins

Neurotoxins are less commonly implicated in food-borne illness. The most common example is staphylococcal food poisoning. The preformed toxin is ingested and causes profuse vomiting by stimulating the emetic center in the brain. Staphylococcal enterotoxins may also cause concomitant diarrhea. Much more rare but more serious is botulism. The preformed toxin is extremely potent and once absorbed it is widely disseminated, binding to nerve endings and inhibiting the release of acetylcholine, particularly in skeletal muscle. This leads to neuromuscular paralysis and, if not recognized and treated, death from respiratory failure.

Adherence

Most organisms cannot cause disease unless they can adhere to enterocytes. Some bacteria adhere using various adhesions, or fimbriae, which can be encoded by plasmids and are thus potentially transferable to other species.[11] Some protozoa have specially adapted ways of sticking to the gut mucosa. *Giardia* spp. have suction plates on their ventral surface and microsporidia have a polar element that is inserted into the enterocyte. The helminth *Taenia solium* has a sucker and small hooklets that allow it to remain attached to the mucosa. Viruses, such as rotavirus, stick to the mucosa via ligands such as hemagglutinin protein.

Invasion

The ability of some organisms to invade mucosal cells and damage them may be associated with antigens in the bacterial cell wall. Virulence in *Shigella* spp. is related to fully expressed, smooth O antigen in the lipopolysaccharide cell wall, which may make it resistant to attack by complement. The production of some outer membrane proteins may enable the bacteria to survive intracellularly. Some of the genes controlling these attributes are chromosomal but others are on plasmids.

Other factors

The ability of bacteria to multiply quickly also provides an advantage against host defenses and this rapid multiplication allows for the exchange of genetic material, possibly with the acquisition of new virulence characteristics. Motility of enteric pathogens may be important in causing disease, either by aiding colonization of the mucosa or by evading phagocytes. Some viruses appear to cause selective damage to epithelial cells in the intestinal villi while leaving secretory cells in the crypts intact, thus favoring net secretion and the production of watery diarrhea.

PREVENTION

Food poisoning and other food-borne illnesses are often preventable through a combination of public health measures, personal hygiene and immunization and, sometimes, by chemoprophylaxis.

Public health

The provision of adequate housing and sanitation along with education about basic hygiene and food handling are major reasons why diarrheal illness has become much less common in developed countries than in underdeveloped ones. Modern sewage treatment plants and well-organized drinking water treatment and distribution dramatically reduce the risk of transmission of infection via the fecal-oral route. Good standards of food production, refrigeration and distribution along with proper food handling in shops and restaurants are also important. For such systems to remain safe there need to be regular inspections, training of staff and registration of premises, all backed up by legislation. All of these methods are more likely to be found in developed countries but progress is also being made in less developed countries. Flush toilets are common in cities in the tropics and well-designed pit latrines, such as the Blair privy, are increasingly being built in urban townships and rural villages (Fig. 93.2).

These preventive efforts need to be strengthened by surveillance of food-borne illness based both on clinical case reporting and on laboratory reports. A system of compulsory notification of certain infections is essential. Such surveillance should lead to the prompt recognition of outbreaks, and the timely investigation of such outbreaks can often identify the source of the problem and lead to control measures aimed at preventing further infections (see Chapter 50a).

Personal measures

The individual can play a major role in preventing food-borne illness. Attention to personal hygiene, especially hand washing, is an important way to reduce fecal-oral transmission. Proper food handling involving careful storage and preparation of both raw and cooked food is essential and, of course, easier to achieve in developed countries.

In countries with unreliable supplies of potable water, individuals may treat their personal drinking water in a variety of ways to minimize the risk of infection. Boiling and filtering water are time-honored methods of reducing contamination. Water can also be treated by chemicals such as chlorine or iodine to attempt to sterilize it. These methods are often used by travelers to less developed countries but can also be used by local residents if the methods are available and affordable. Travelers and others can reduce the chances of food-borne infection by careful eating, avoiding uncooked and unwashed fruit and vegetables, ice cubes and unpasteurized dairy

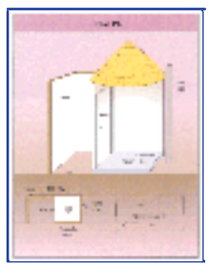

Figure 93-2 An example of a pit latrine.

produce. Chemoprophylaxis with drugs such as trimethoprim-sulfamethoxazole quinolones or doxycycline can reduce the risk of travelers' diarrhea (see Chapter 43 and Chapter 143). [12]

Immunization

Polio vaccination is the most successful example of prevention of a food-borne illness, the disease having been virtually eliminated from many parts of the world. Hepatitis A virus infection, formerly prevented by repeated doses of gammaglobulin, is now easily preventable with the new vaccines, although the cost of these is prohibitive in most parts of the world where the virus is endemic. The risk of acquiring typhoid can also be reduced markedly by immunization, either with a polysaccharide parenteral immunization or a live attenuated oral vaccine.

The old parenteral cholera vaccine offered less than 50% protection against infection and had serious side-effects. However, new oral vaccines directed against cholera toxin have looked extremely promising in field trials.[13] Because the toxin is very similar to the heat-labile toxin of enterotoxigenic *E. coli* (ETEC), immunization may protect against disease caused by this organism as well. Such a vaccine against infection with ETEC may also be used to protect against travelers' diarrhea.

CLINICAL FEATURES

The clinical consequences of food-borne infection depend, to some extent, on the infecting organism. However, with those organisms that cause food poisoning, with predominantly gastrointestinal symptoms, most patients develop diarrhea or vomiting or both and the clinical features relate to whether the small or large intestine is affected. Invasive organisms can cause a variety of clinical features. In these diseases, gastrointestinal symptoms are mild or nonexistent but fever is often a feature.

Bacterial food poisoning

Most patients who have food poisoning develop acute diarrhea, often with vomiting. Isolated vomiting may also occur but this is more unusual. Many infecting organisms, such as *Bacillus cereus*, elaborate enterotoxins that affect the small bowel, causing little mucosal inflammation and leading to a secretory diarrhea. Patients usually present with watery diarrhea without blood in the stool. There may be vomiting early on but this tends to settle before the diarrhea resolves. Other organisms such as *Shigella* spp. cause diarrhea via cytotoxins that cause inflammation of the intestinal mucosa and usually affect the distal small bowel and the large intestine. These patients may also have vomiting but diarrhea, often with blood (reflecting the mucosal inflammation), is the main feature and may last longer than with cases of secretory diarrhea. Generally, the incubation period from ingestion of the infected food or water until the onset of symptoms is longer with bacteria causing an inflammatory diarrhea. Some of the clinical features of individual infections are outlined below.

Infections with secretory diarrhea

Staphylococcal food poisoning

Often associated with cream-filled pastries and some tinned meats, staphylococci multiply in the food, producing various staphylococcal toxins. These preformed toxins are ingested and cause symptoms within a few hours. There is usually initial nausea followed quickly by severe vomiting. Diarrhea appears later and fever is rare. Most cases are self-limiting and recovery occurs within 24–48 hours. However, dehydration can be quite severe and, rarely, fatalities occur as a result of marked hypotension. In rare cases, other staphylococcal toxins may cause a toxic shock syndrome (see Chapter 224).

Bacillus cereus

There are really two syndromes associated with *B. cereus* infection, both toxin mediated.[14] In the first, ingestion of the preformed toxin, often in fried rice or vanilla sauce, leads to profuse vomiting within a few hours, accompanied by severe abdominal cramps. Diarrhea occurs in a minority of cases. The history of eating fried rice helps to differentiate this disease from staphylococcal food poisoning. The second syndrome has a longer incubation period (median 9 hours) and is characterized by watery diarrhea. In these cases, the bacteria multiply in the gut lumen and produce toxin. Both forms of *B. cereus* food poisoning are mild and resolve within 24 hours.

Clostridium perfringens

The clinical features of clostridial food poisoning are virtually indistinguishable from the diarrheal form of *B. cereus* infection. Most cases of infection due to *Clostridium perfringens* result from eating some form of cooked meat. The toxins are potent so there are often small outbreaks associated with a particular meal. This organism can sometimes cause a more serious infection, known as pigbel in the South Pacific, where it is associated with the consumption of pork.

Vibrio parahaemolyticus

This organism grows best in a salt-rich environment and is thus usually associated with food poisoning following the consumption of seafood and, especially, shellfish.[15] The incubation period can range from a few hours to several days, depending on the inoculum. The illness starts with explosive watery diarrhea followed by abdominal cramps and vomiting. Headache is often present and occasionally there is sufficient inflammation of the bowel to cause fever and,

rarely, some blood in the diarrhea. Most cases are self-limiting but may take several days to resolve.

Cholera

This disease, caused by *Vibrio cholerae*, is the classic example of a toxin-mediated secretory diarrhea.[16] Cholera usually follows the consumption of contaminated water but has been associated with eating shellfish. It is characterized by moderate to severe watery diarrhea without fever and with little vomiting. In severe cases, liters of fluid can be lost over 24 hours and severe prostration and even death may result from severe dehydration. In outbreaks, many infected people have few or no symptoms. With adequate fluid replacement, most cases are self-limiting. Almost all cases of cholera occur in the tropics and imported cases are rare, partly because of the short incubation period and the severity of symptoms and partly because the risk to travelers is small (Fig. 93.3).

Inflammatory diarrhea

Infections with *Campylobacter* spp., *Salmonella* spp. and *Shigella* spp.

Campylobacter jejuni, *Salmonella enteritidis* and other nontyphoidal salmonellae, *Shigella* spp. and enteroinvasive *E. coli* all produce an enterocolitis. *Campylobacter* spp. are sometimes associated with the consumption of milk or chicken and have also been associated with exposure to pets, such as puppies.[17] *Salmonella* spp. are particularly linked with poultry and eggs.[18] *Shigella* spp. have been found in association with a variety of foods and with contaminated water but these *Shigella* infections are rare in developed countries. The incubation period is often several days. Diarrhea is the principal symptom and there may be blood in the stool as a result of colonic inflammation. Vomiting is more common with *Salmonella* infection but can occur with any of these organisms. Abdominal pain is frequently severe and, particularly in *Campylobacter* and *Salmonella* infections, may mimic an acute abdomen. Most cases are mild and resolve over the course of about a week. However, sometimes symptoms can be prolonged, raising the possibility of inflammatory bowel disease as a differential diagnosis. *Campylobacter* spp. and *Salmonella* spp. occasionally become invasive and cause an enteric fever syndrome with positive blood cultures. This is more likely in the elderly and the immunosuppressed. Both *Salmonella* and *Campylobacter* infections are associated with extraintestinal symptoms in the absence of bacteremia, such as arthritis. Specific serotypes of *Campylobacter* species gastroenteritis are also strongly associated with Guillain-Barré syndrome (see Chapter 230).[19]

Figure 93-3 A cholera ward in Peru. *Courtesy of Dr J Sanchez.*

Yersinial infections

Yersinia enterocolitica can present in a number of ways. Most of the time it causes an entercolitis, presenting with acute diarrhea. Although it mainly affects young children, adults may sometimes be infected. The diarrheal illness may be severe and often resolves more slowly than that caused by other organisms. This organism may also cause a mesenteric adenitis and terminal ileitis in young children, which can mimic appendicitis. In such-cases, diarrhea is rarely a feature. Sometimes infection with *Y. enterocolitica* is associated with extragastro-intestinal features, such as arthritis, and rarely bacteremia occurs.

Escherichia coli 0157:H7

Infection with *E. coli* 0157:H7 causes an inflammatory colitis and is associated with hemolytic-uremic syndrome. It has become much more common in the past two decades and has been particularly associated with the consumption of hamburger meat.[20] This ground beef is often contaminated by intestinal contents and the production process may facilitate dissemination of the bacteria in large batches of meat that are then widely distributed. Sporadic cases occur but major outbreaks have highlighted the seriousness of this infection. In 1996 a large outbreak in Scotland caused 21 fatalities. The majority of cases present with diarrhea, which often contains frank blood and is usually occult blood positive. Most of these infections are self-limiting. However, a proportion of infected patients develop hemolytic-uremic syndrome, which occurs 5–7 days after the onset of diarrhea and which carries a significant risk of death from renal failure, bleeding or cerebral infarction (see also Chapter 43).

Botulism

This disease is caused by food poisoning with a preformed neurotoxin produced by *Clostridium botulinum.* Many cases are related to home canning of produce but outbreaks related to commercially prepared food also occur from time to time.[21] The neurotoxin causes predominantly bulbar and ocular palsies, and therefore swallowing difficulties, double vision, blurred vision and ptosis are common. Limb weakness and respiratory difficulty are often present. The differential diagnosis thus includes myasthenia gravis, Guillain-Barré syndrome and brainstem stroke.

Viral infections

Gastrointestinal disease

Viral gastroenteritis is common and is usually mild and self-limiting. [22] Sporadic cases are most commonly due to rotavirus, which mainly affects infants and small children. There is usually an abrupt onset of fever and vomiting, followed later by watery diarrhea. Severe dehydration can result, particularly if the diarrhea is profuse or prolonged, but most cases resolve within a week. Adenoviruses and astroviruses cause similar, although usually milder, symptoms.

Norwalk virus and other small round-structured viruses affect adults and older children and may cause sporadic disease, but they are often associated with outbreaks (Fig. 93.4). Infection may occur from eating contaminated food or infected shellfish or from drinking water contaminated by sewage. These viruses may cause diarrhea or vomiting or both. There are often other symptoms such as mild fever, myalgia and headache. Although the symptoms tend to be quite debilitating they resolve quickly, usually within 24–48 hours. Symptomatic disease with any of these viruses leads to excretion of large numbers of viruses in the stool, and so secondary cases are common.

Protozoal infections

Giardiasis

Giardia lamblia is the most common protozoal cause of diarrhea and is particularly common in travelers.[23] Affected people often develop

Figure 93-4 Electron micrograph of small round-structured viruses.

nausea and abdominal bloating, frequently accompanied by foul-smelling flatulence and belches tasting of hydrogen sulfide. The parasite affects the proximal small bowel so watery diarrhea is the main symptom; the diarrhea is often explosive. Steatorrhea and even malabsorption can occur and, if untreated, symptoms may persist for up to 6 weeks. In some patients sufficient damage occurs to the villus brush border enzymes that a secondary lactose intolerance occurs, with prolongation of the diarrhea.

Amebiasis (see also Chapter 164)

Infection with *Entamoeba histolytica* occurs mainly in the tropics and, as with giardiasis, many people are asymptomatic carriers of the parasite.[24] Ingested cysts mature into trophozoties in the bowel lumen and symptoms usually occur between 2 and 6 weeks after exposure. Amebic trophozoites damage the large bowel mucosa, leading to dysentery (Fig. 93.5). Most patients have blood in their stools but some may not — some do not even have fecal occult blood. In adults, dehydration is uncommon and the systemic symptoms, such as fever, are mild. Bacillary dysentery and a first episode of ulcerative colitis need to be considered in the differential diagnosis. Children tend to get more severe diarrhea and fluid loss and often have severe abdominal pain. Sometimes colonic perforation occurs and rarely, usually in debilitated or immunocompromised patients, a fulminant colitis may occur; this is clinically indistinguishable from severe ulcerative colitis. An uncommon complication (occurring in less than 1% of cases) is the development of an ameboma, usually in the ileocecum, when there is marked inflammation and bowel thickening that may resemble a malignant tumor or even Crohn's disease. Infection with the large protozoan parasite *Balantidium coli* may also produce a syndrome resembling amebic dysentery.

Figure 93-5 Amebic dysentery. A postmortem specimen. Note discrete flask-like ulcers with areas of hemorrhage.

Amebic trophozoites may also disseminate from the bowel and invade other tissues. Most commonly the parasite travels via the portal venous system to the liver, causing a mild hepatitis and, usually, an amebic liver abscess. These patients usually complain of right upper quadrant pain, often with shoulder tip pain as well, and are frequently febrile. Less common sites of spread are the lungs and brain. More than half of the patients presenting with amebic abscess have no history of previous dysentery. Sometimes there is direct extension of the infection from the bowel to the overlying skin.

Cryptosporidiosis

The coccidian protozoan *Cryptosporidium parvum* causes a secretory diarrhea. Infection is almost always associated with drinking contaminated water and it is a not infrequent cause of travelers' diarrhea.[25] In addition to the diarrhea, nausea and anorexia are common. The incubation period ranges from a few days to a couple of weeks. Most people clear the parasite easily and symptoms abate within a few days. However, this can be a serious disease in people who have HIV infection and in children who have severe congenital immunodeficiency syndromes, in whom it results in severe, prolonged cholera-like diarrhea that is associated with malabsorption and profound weight loss. Some patients who have AIDS develop an ascending cholangitis with upper abdominal pain, abnormal liver function tests and, occasionally, frank jaundice. The inability of immunocompromised patients to clear this parasite is a particular problem because no effective treatment exists (see Chapter 127 and Chapter 243).

Cyclospora cayetanensis

This coccidian parasite has only relatively recently been discovered and described as a human pathogen.[26] Most cases are related to water consumption but outbreaks associated with imported, contaminated soft fruit have occurred in the USA. Symptoms initially include watery diarrhea and anorexia and often vomiting. These infections are usually self-limiting but some patients may take several weeks to recover and many of these suffer anorexia, profound fatigue and marked weight loss even after the diarrhea has resolved. Immunocompromised patients, such as those who have HIV infection, may develop chronic symptoms of diarrhea that are indistinguishable from those seen in cryptosporidiosis (see Chapter 243).

Natural toxins

Patients may present with gastrointestinal or neurologic symptoms as a result of eating or drinking naturally occurring toxins. Such illnesses often mimic food- or water-borne infection.

Plant toxins

There are various alkaloids and other plant toxins that may produce symptoms in humans who consume them. People who have glucose-6-phosphate dehydrogenase deficiency are unable to reduce oxidants contained in some legumes or beans. These toxins accumulate and lead to a mixture of symptoms known as favism. Early symptoms are headache, nausea and vomiting with a mild fever. The main problem, however, is marked hemolysis with hemoglobinuria and jaundice.

If casava is not prepared and cooked properly, its naturally occurring cyanide precursors remain and are ingested.[27] In heavy, acute exposure breathlessness, paralysis and coma ensue. More commonly, chronic exposure leads to tropical spastic neuropathy. This is a particular risk for poor communities in times of severe drought.

Fish toxins

Neurotoxins associated with the consumption of shellfish lead to two distinct syndromes. Paralytic poisoning occurs within minutes to hours of ingestion and is characterized by breathlessness, muscle weakness and increasing respiratory difficulty.[28] Neurotoxic poisoning is milder, with some muscle weakness and paresthesiae but no respiratory problems.

Ciguatera poisoning follows the eating of fish that have consumed toxic microalgae. Such fish, like barracuda, feed around coral reefs where these algae are most commonly found. Symptoms include acute diarrhea and vomiting and there is often a macular erythematous rash. Neurologic symptoms are common with circumoral paresthesiae and sometimes paralysis. Most cases are mild and fatalities are rare.[29]

Scombroid poisoning resembles acute histamine toxicity. Coarse feeding oily fish, such as mackerel and tuna, are usually implicated.[30] The scombroid toxin is thought to be derived from the action of bacteria in the fish guts on histones in the flesh of the fish. The formation of the toxin is favored by heat, and so the risk is highest in poorly cleaned fish that have been inadequately stored before cooking. Symptoms include headache, diarrhea, erythema and, usually, a marked urticarial rash.

Mushrooms

Some mushrooms, such as *Psylocybe* spp., cause hallucinations and may be eaten intentionally for this effect. Other mushrooms may be eaten in error with grave consequences.[31] *Amanita phalloides* contains a deadly toxin that initially causes abdominal cramps and diarrhea. These symptoms improve somewhat to be followed by inexorable liver and renal failure, which is often fatal.

Other mushrooms contain muscarine-like toxins leading to cholinergic symptoms such as excessive salivation, blurring of vision, sweating and diarrhea.

Heavy metals

Ingestion of either cadmium or thallium may cause acute diarrhea and vomiting, somewhat resembling staphylococcal food poisoning. The symptoms may be severe enough to cause acute dehydration and collapse. More chronic ingestion of cadmium results in nephropathy; chronic ingestion of thallium causes a peripheral neuropathy.

Food-borne infections with systemic rather than gastrointestinal symptoms

Some organisms, after ingestion, invade through the intestinal mucosa and cause little in the way of gastrointestinal upset; rather, they result in myriad systemic symptoms. Many of these infections have fever as a prominent symptom and usually have longer incubation periods than those associated with food poisoning, so it is more difficult to recognize food-borne outbreaks with these organisms.

Typhoid

Typhoid, or enteric fever, is the classic example of a food-borne infection leading to systemic disease. This condition is characterized by a chronic *Salmonella typhi* bacteremia and fever is the main presenting complaint. Although older texts highlight relative constipation, diarrhea also occurs, although many patients have little in the way of bowel symptoms early in the course of the illness.[32] There is usually non-specific malaise and headache, and a nonproductive cough is common. Also common is the so-called 'typhoid facies', a rather lethargic and apathetic facial expression. Most patients appear subacutely unwell; very few are acutely toxic or look as if they have a Gram-negative sepsis. When the bacilli reinvade the bowel, particularly the Peyer's patches, inflammation results and may lead to intestinal hemorrhage, which is sometimes torrential. Occasionally, severe ulceration results in small bowel perforation. Less common complications include myocarditis and meningitis.

Some cases are mild and self-limiting but most require specific antimicrobial therapy. Rarely, infected people become chronic carriers and excretors of *Salmonella typhi*. Carriage is particularly associated with the presence of gallstones. Sometimes other organisms, such as *Salmonella paratyphi, Campylobacter fetus* and non-typhi *Salmonella* can produce an enteric fever-like illness (see also Chapter 163).

Brucellosis

This disease is usually caused by *Brucella melitensis* acquired by eating unpasteurized cheese or milk. Most cases present with a fever without localizing symptoms or signs, although orchitis is a well-recognized problem.[33] Associated symptoms such as headache, myalgia and chills are common. A less common presentation that has a more insidious onset is spondylitis; the main symptoms are back pain, related to the paraspinal inflammatory mass, and a milder fever. This may need to be differentiated from tuberculosis. Spinal cord compression is extremely rare in brucellar spondylitis. Rarely, both forms of brucellosis may have acute orchitis as a relatively early feature. Chronic brucellosis leading to chronic fatigue probably does not exist, but occasionally metastatic foci of infection in the joints may lead to chronic joint symptoms if appropriate antibiotics have not been administered (see Chapter 180).

Listeriosis

Infection with *Listeria monocytogenes* most commonly presents with an acute meningoencephalitis, which may be associated with a bacteremia. Patients present with headache, confusion and, sometimes, vomiting. Most cases have neck stiffness and other signs of meningeal inflammation. In the more encephalitic presentations, differentiation from herpes simplex virus encephalitis is impossible clinically, especially as both conditions may have lymphocytes and red cells in the cerebrospinal fluid. Central nervous system infection is most common in the elderly and the immunocompromised.

Less commonly, patients are bacteremic and present with mild influenza-like symptoms, although occasionally there is shock and renal impairment. Some cases may have impaired liver function tests as a result of a granulomatous hepatitis. Pregnancy carries an increased risk of bacteremia if *Listeria* is ingested, and infection in

early pregnancy may lead to spontaneous miscarriage. Late in pregnancy infection can result in stillbirth or in severe neonatal sepsis or meningitis. Intrauterine infection is almost always fatal even when the illness in the mother has been mild.[34]

Over the past few years it has become apparent that *Listeria* can lead to a syndrome of febrile gastroenteritis in immunocompetent people. In this setting, there is usually not invasive disease and routine stool analysis may not detect the organism. If *Listeria* is suspected as a cause of gastroenteritis, then the laboratory should be informed so that cultures can be kept longer and the organism specifically sought. *Listeria* gastroenteritis is usually associated with a large inoculum and is most commonly seen in the setting of a food-borne outbreak.[35]

Mycobacteria

Mycobacterium bovis

Although the majority of intestinal tuberculosis results either from swallowed infected sputum or from bacteremic spread from a pulmonary focus of *Mycobacterium tuberculosis*, in some areas of the tropics cases still occur from consuming unpasteurized milk and dairy produce contaminated by *M. bovis*, a closely related species.[36] Intestinal tuberculosis may present in a variety of ways, ranging from malabsorption if the proximal small bowel is affected to intestinal obstruction due to inflammatory strictures. Diarrhea may occur, as may intestinal hemorrhage, but both are rare presenting features. Intestinal tuberculosis may mimic Crohn's disease.

Mycobacterium avium-intracellulare

Mycobacterium avium-intracellulare (MAI) has become an important pathogen in the AIDS era. Late-stage HIV disease has been associated with an increased risk of disseminated MAI infection, which commonly presents with fevers, accelerated weight loss, anemia and chronic diarrhea. The organism is ubiquitous in the

environment.[37] Animal models of MAI infection suggest that the gastrointestinal tract is the portal of entry (see Chapter 129). Fortunately, with the advent of potent anti-HIV drugs that lead to improved immunity in those with HIV, MAI infection is now a relatively rare diagnosis.

Mycobacterium pseudotuberculosis

This organism is usually regarded as nonpathogenic. However, because of the similarities between intestinal tuberculosis and Crohn's disease, many researchers have sought a mycobacterial cause of Crohn's disease. Recently, molecular techniques have provided some evidence for the presence of *M. paratuberculosis* in the bowel of patients who have Crohn's disease, although this is controversial.[38] A causal link has yet to be proven.

Hepatitis viruses

Two of the hepatitis viruses, namely A and E, are acquired by the fecal-oral route. Hepatitis A virus is more common and is endemic in many countries, although its prevalence is highest in the tropics where it is a risk for nonimmune travelers. Hepatitis E virus is endemic in Asia, particularly in the Indian subcontinent, and is only rarely encountered as a disease of the returning traveler.[39] [40] Hepatitis A has an incubation period of 2–6 weeks; the incubation period of hepatitis E is less certain. Both hepatitis A and hepatitis E present, like most cases of acute hepatitis, with a relatively acute onset of malaise, nausea, anorexia and mild fever followed soon after by jaundice. In the prodrome, many patients who normally smoke find they no longer feel like smoking. The jaundice usually only lasts about 2 weeks but some patients develop marked cholestasis with prolonged jaundice.

Hepatitis A virus infection is usually asymptomatic in children below the age of about 8 years. It always causes symptoms in adults, which are usually mild but characterized by marked fatigue. Death from hepatitis A is very rare unless there is pre-existing liver disease and there is a case for immunizing those patients with chronic hepatitis B or hepatitis C infection. Hepatitis E virus infection may cause a fatal fulminant hepatitis in pregnant women. Recovery from hepatitis A results in lifelong immunity to reinfection, and the same is probably true for hepatitis E (see Chapter 48).

Prions

In the past decade an epidemic of bovine spongiform encephalopathy in cattle was recognized in the UK and some other European countries, linked to the consumption by the cattle of feeds made up partly of protein derived from other cattle. Although control measures have been introduced and such feeds stopped, there is evidence that the prions causing bovine spongiform encephalopathy in cattle have entered the food chain and resulted in human disease. An unusual form of Creutzfeld-Jakob disease has occurred in young people and is believed to be linked to the consumption of beef from cattle infected with bovine spongiform encephalopathy. These cases differ from 'classic' Creutzfeld-Jakob disease epidemiologically, clinically and histologically, and have a distinct molecular configuration of the prion protein. [41] Such cases of new-variant Creutzfeld-Jakob disease have aroused widespread public concern, but it remains uncertain whether a major epidemic of new-variant Creutzfeld-Jakob disease will occur or whether prions from species other than cattle will infect humans (see Chapter 223 for a detailed discussion).

Parasites

Protozoa

Most enteric protozoa, like *Giardia lamblia*, are associated with water or food contaminated with feces and are confined to the gut after ingestion. However, other protozoa with more complex life cycles are parasites of other species and may cause disease in humans when the meat of the intermediate host species is eaten. The most common example is infection with *Toxoplasma gondii* when tissue cysts are ingested with undercooked meat.[42] Many infections are asymptomatic. Acute symptoms include a febrile illness with or without generalized lymphadenopathy. This is usually self-limiting. Infection in pregnancy carries a risk of vertical transmission and congenital toxoplasmosis. Patients who have HIV infection may reactivate dormant cysts in the brain, develop toxoplasmal abscesses and present with the features of a space-occupying lesion (see Chapter 127). Toxoplasmosis is also a problem for some patients taking immunosuppressive medication, such as solid organ transplant recipients.

Helminths

Numerous helminths are ingested as infective ova, after which they undergo a complex life cycle that includes larval migration around the human host and then re-entry into the gastrointestinal tract, where they develop into adult worms that may or may not cause symptoms (Table 93.3).[43]

Other worms are ingested as ova or larva but migrate out of the gut and cause problems unrelated to the gastrointestinal tract. Table 93.4 outlines the main features of these infections, most of which are asymptomatic. Further details can be found in Chapter 46 , Chapter 165 and Chapter 246 .

TABLE 93-3 -- Food-borne helminthic infections that are confined to the gut.

FOOD-BORNE HELMINTHIC INFECTIONS THAT ARE CONFINED TO THE GUT
Ascaris lumbricoides
Ancylostoma duodenale
Trichuris trichiura
Enterobius vermicularis
Fasciolopis buski
Taenia saginata
Taenia solium
Hymenolepis nana

Diphyllobothrium latum

TABLE 93-4 -- Systemic helminthic infections that are transmitted by food or water.

SYSTEMIC HELMINTHIC INFECTIONS THAT ARE TRANSMITTED BY FOOD OR WATER	
Helminth	**Food vehicle**
Dracunculis medinensis (guinea worm)	Crustacea in drinking water
Trichinella spiralis	Pork
Toxocara canis	Contaminated soil ingestion
Opisthorchis viverrini	Raw fish
Opisthorchis sinensis (Clonorchis sinensis)	Raw fish
Fasciola hepatica	Contaminated vegetables, watercress
Paragonimus westermani	Freshwater crabs, crayfish
Taenia solium (cystlicercosis)	Pork
Echinococcus granulosis	Contaminated food or water (with eggs in animal feces)
Echinococcus multilocularis	

DIAGNOSIS

Food poisoning

Microbiology

When a patient presents with symptoms suggesting food poisoning there may be clues about the infecting organism in the dietary history. A recent restaurant or take-away meal may highlight suspect foods whereas a story of recent tropical travel may raise the possibility of a protozoan parasite. Recent consumption of mayonnaise, soft-boiled eggs or barbecued chicken, for example, hint that *Salmonella* spp. may be the cause.

The microscopy and culture of a stool specimen is probably the most useful test and the diagnostic yield can be maximized by examining at least three specimens obtained at different times. Most causes of secretory diarrhea will produce negative stool tests as the diarrhea is usually caused by toxins and there is little inflammation in the intestinal mucosa. The presence of pus cells in the stool suggests infection and also indicates an inflammatory colitis. Red blood cells in the stool indicate colitis and may occur with any cause of inflammatory colitis. Infection with *E. coli* O157:H7 usually causes a hemorrhagic colitis and so the presence of red cells in a stool specimen should alert the laboratory to the need to screen for this pathogen. If there is a history of tropical travel, blood in the stool may indicate shigellosis or amebic dysentery. Amebic trophozoites may be seen engulfing red cells but only if the stool is examined fresh or 'hot' because the trophozoites soon degrade in vitro.

Parasites, such as *Giardia* spp. and cryptosporidia, may be seen in stool specimens in heavy infections or in immunocompromised patients. However, because they infect the small bowel and are shed intermittently in small numbers in the stool they may be missed on microscopy.[44] Microscopic examination of duodenal aspirates or the use of the entero-test, or string test, is more likely to yield results with these small bowel parasites; however, these investigations are more invasive. It should be noted that the nonpathogenic *Entamoeba dispar*, found in up to 50% of people in endemic areas, has cysts that are indistinguishable from those of *E. histolytica*, so the finding of amebic cysts should be interpreted with caution in endemic areas or in patients returning from such areas (see Chapter 165).[45]

Electron microscopy has a role to play in the investigation of gastroenteritis caused by viruses. The characteristic appearances of rotavirus, astrovirus or small round-structured viruses such as the Norwalk virus clinch the diagnosis. Electron microscopy is particularly useful during outbreak investigations but it is not often routinely available for diagnosing individual cases.

Stool culture using a variety of selective media can identify the common bacterial causes of diarrhea unless the symptoms are due to preformed bacterial toxins in the food. The disadvantage is that the identification process can take several days. Stool cultures are relatively insensitive and hence not very cost-effective. Culture methods may also be used to investigate water or food vehicles in suspected outbreaks.

There are now several commercially available latex and enzyme-linked immunosorbent assay (ELISA) kits for the identification of rotavirus in stool or vomit. These allow prompt diagnosis without the need for electron microscopy. Increasingly, reverse transcription polymerase chain reaction is being used to identify a variety of viruses in stool lysates.[46] Although not used in routine laboratories, ELISA can be used to identify bacterial toxins and is important when investigating outbreaks.

Serology has a minor role in the diagnosis of invasive amebiasis. [47] There are several tests available with variable predictive values. In endemic areas, up to one-quarter of the population may have positive tests because of previous asymptomatic infection. Other than for epidemiologic studies, the current IgG ELISA is most useful in patients from nonendemic areas but it is usually used, because of its turnaround time, as a confirmatory test.

Hematology, biochemistry and immunology

Blood tests have little role in the specific diagnosis of food poisoning. The peripheral leukocyte count may be non-specifically raised but it is commonly normal. Eosinophilia rarely occurs with invasive amebiasis; rather it is a feature of metaozoan (i.e. worm) infection, not protozoan infection. There may be mild anemia in hemorrhagic colitis. One key finding on the blood film is fragmented red cells, which is indicative of intravascular hemolysis in hemolytic-uremic syndrome in association with enterocolitis caused by *E. coli* O157:H7.

Biochemical tests are useful for gauging the extent of dehydration or renal impairment. Serum albumin may be low in more chronic diarrhea or if there is severe colonic inflammation or sepsis. The C-reactive protein may be significantly elevated in cases of inflammatory colitis due, for example, to *Campylobacter* spp. None of these tests is in any way specific.

Radiology

A plain abdominal radiograph is useful for assessing the severity of infective colitis. Infection with organisms causing inflammatory diarrhea may mimic ulcerative colitis and lead to large bowel dilation and, sometimes, to a toxic megacolon. Plain radiographs may also show small bowel dilatation or fluid levels when there are inflammatory strictures in the ileocecum caused by infections with *Yersinia* spp. or amebae.

Barium enema examination can be used to demonstrate inflammation of the colon and may show ulceration in amebic dysentery, but this has largely been superseded by fiberoptic endoscopy. Computerized tomography (CT) scans, magnetic resonance imaging (MRI) and labeled leukocyte scans have no role in the diagnosis of food poisoning.

Other investigations

Although stool culture and microscopy are the most important diagnostic tools, a useful adjunct is flexible sigmoidoscopy and large bowel biopsy. Endoscopy may show an active colitis, which can be macroscopically similar to that seen in ulcerative colitis, although the finding of frank pus points more toward infection. Amebic colitis causes inflamed mucosa around discrete ulcers with patches of normal mucosa in between, a useful feature that distinguishes it from ulcerative colitis. Rectal or

colonic biopsy may show histologic features that differentiate between infection and inflammatory bowel disease (Fig. 93.6).

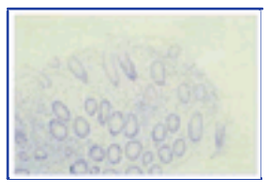

Figure 93-6 Histology of infective colitis caused by *Campylobacter jejuni.*

Microbiology

Many of the bacterial infections described above share the feature that, although they enter via the gastrointestinal tract, they disseminate from there. Many of these spread via the bloodstream and hence, particularly early in the course of disease, blood cultures are essential. Typhoid is most easily diagnosed by blood culture, although bone marrow culture is even more sensitive.[48] Stool culture is less sensitive but is still valuable, particularly in areas where blood culture facilities are less readily available. Even a rectal swab can be used to diagnose typhoid with a sensitivity of about 30%. Brucellosis and listeriosis are also commonly diagnosed by positive blood culture. Brucella spondylitis may result in a paravertebral mass, which may be positive on culture if aspirated or biopsied. *Listeria* may present with a meningoencephalitis, in which case the cerebrospinal fluid culture should be positive.

Mycobacterial infections affecting the gut are best diagnosed by tissue biopsy; specimens may show granuloma and, occasionally, acid-fast bacilli on histology, but they should always be sent for mycobacterial culture. In the setting of HIV infection, infection with MAI is associated with large numbers of mycobacteria and very poorly formed granulomas. Patients may be bacteremic or have positive bone marrow cultures. Not infrequently, large numbers of acidfast bacilli are seen in the stool, which will also be positive on culture. Intestinal biopsies will often show numerous acid-fast bacilli with little in the way of inflammatory reaction and certainly no caseation.

Serologic tests for brucellosis are useful in patients from nonendemic areas but even in endemic regions a rising titer can be diagnostic. The Widal test for typhoid is widely used in the tropics and is only partially useful in endemic areas if the test patterns in the population have been well studied and validated. It cannot be relied upon to exclude typhoid in returning travelers or in patients in nonendemic areas. Previous typhoid immunization will lead to false-positive results.

Hepatitis due to enterically acquired viruses is diagnosed by appropriate serologic tests, usually in the form of an ELISA.

There are now a variety of commercial and research tests to detect pathogens by means of molecular biologic techniques.

Most diagnostic laboratories are not able to culture protozoa. Diagnosis of invasive protozoa such as *T. gondii* rely on serologic tests such as an IgM seroconversion. In many cases there is detectable IgG to *Toxoplasma* spp., but this only defines previous exposure and does not prove that the current clinical problem is a disease caused by toxoplasmal infection. Usually, tissue samples are needed for histologic diagnosis.

Helminthic infections may be diagnosed by finding eggs in the stool or by the identification of adult worms passed in the stool (Fig. 93.7). Tapeworm infections may be diagnosed by finding segments, or proglottids, in the stool. Serologic tests may help identify invasive parasites causing problems such as cysticercosis or hydatid disease. However, these tests are best suited to returning travelers and are relatively unhelpful in endemic areas where large numbers of the population may be asymptomatically exposed.

Hematology and biochemistry

So-called routine blood tests are really of little value in the diagnosis of invasive food-borne infections and only serve an indirect purpose of assessing disease severity. Invasive helminths will commonly cause a peripheral blood eosinophilia. Viral hepatitis will result in raised levels of aminotransferase, such as aspartate aminotransferase, and in severe cases liver synthetic function is affected and hypoalbuminemia and abnormal clotting occur. Liver function tests may be abnormal in hydatid disease affecting the liver or if the biliary tree is obstructed by worms such as *Opisthorchis sinensis*.

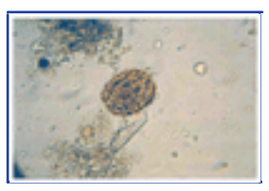

Figure 93-7 Unfertilized egg of *Ascaris lumbricoides*.

Immunology

There are no specific immunologic tests that help in diagnosis. There are proponents of tuberculin testing to aid in the diagnosis of mycobacterial disease but in practice this is of limited use and is of no value in diagnosing atypical mycobacterial infections, such as MAI.

Radiology

Plain radiographs are of little value in the diagnosis of these invasive diseases. Sometimes spondylitis caused by brucellosis may be seen on plain vertebral radiographs. Partially calcified hydatid cysts may be seen on plain radiographs but this usually represents inactive disease. Contrast radiology using, for example, a barium enema may find areas of disease, such as ileocecal abnormalities in intestinal tuberculosis, but will not yield a specific diagnosis.

Computed tomography scanning and MRI are particularly useful in delineating intracerebral lesions. In AIDS, cerebral toxoplasmosis appears as (usually multiple) ring-enhancing lesions or abscesses on CT scans or as multiple lesions on MRI. Neurocysticercosis presents with discrete, multiple lesions on these scans or with hydrocephalus if a cyst is blocking the fourth ventricle.

Histopathology

Tissue biopsy remains an important means of reaching a diagnosis in invasive disease. In addition to the histologic findings, specimens can be sent for culture (for *M. bovis*, for example). In-situ hybridization is increasingly used to demonstrate the infecting pathogen in tissue sections.

MANAGEMENT

Fluids

The mainstay of medical management for acute gastroenteritis is the administration of adequate fluid and electrolyte replacement to correct the intravascular volume and prevent cardiovascular collapse and renal impairment. For the vast majority of patients, oral dehydration is adequate but it should include electrolyte replacement as well as water.[49] [50] Many commercial preparations of oral rehydration salts exist with flavorings to make them more palatable. They are based on the World Health Organization recommendations for oral rehydration therapy but those for use in developed countries tend to have a lower sodium content (Table 93.5) because fecal sodium losses are lower in temperate climates than in the tropics. The glucose content of these solutions is essential to allow active transport of water and electrolytes across the mucosa from the lumen (see also Chapter 144).

TABLE 93-5 -- **Oral rehydration solution — World Health Organization formula.**

ORAL REHYDRATION SOLUTION — WHO FORMULA
Sodium 90mmol/l
Potassium 20mmol/l
Chloride 80mmol/l
Citrate 10mmol/l
Glucose 110mmol/l
Total osmolality 310mmol/l
Note that commercial oral rehydration solutions for use in developed countries have a lower osmolality (240mmol/l) with a lower sodium content (60mmol/l) because fecal sodium losses are lower in developed countries than in the tropics.

Intravenous fluid replacement is required for those who are too frail and for those who are vomiting too much to tolerate oral therapy, as well as for those who have severe dehydration and near circulatory collapse. Crystalloid solutions are usually appropriate, and Ringer's lactate with 5% dextrose is the preparation recommended by the World Health Organization. In difficult settings, infusion of fluids subcutaneously can be life saving.

Antidiarrheal agents

Drugs that inhibit intestinal motility have a small role to play in patients who have gastroenteritis. Loperamide, diphenoxylate and codeine phosphate all act in this way. Calmodulin, an antisecretory drug, may also have a role in reducing diarrhea. Only codeine is absorbed, with the advantage that it can act as an analgesic for those who have abdominal pain, but it carries the risk of side-effects, such as nausea, and the danger of accumulation in patients who have renal impairment. Kaolin-pectin has no role in the management of diarrheal illness but some studies have shown a beneficial role of bismuth subsalicylate. Although one report suggested that diphenoxylate-atropine use is associated with an increased risk of bacterial invasion in acute shigellosis, no other studies have found this.[51] Equally, there is little evidence to suggest that these agents significantly prolong the carriage of infecting organisms or that they increase the risk of the development of toxic megacolon.[52]

Other supportive measures

Patients who have severe shock from fluid loss may require intensive support, but this is very rarely needed with gastrointestinal infections. Some patients, particularly the elderly, may develop acute renal failure and require temporary renal support with hemofiltration or hemodialysis. This has been particularly true for those who have hemolytic-uremic syndrome following infection with *E. coli* O157:H7. There is also anecdotal evidence to suggest that patients who have hemolytic-uremic syndrome benefit from plasmapheresis.[53]

Some food poisoning, such as botulism or paralytic shellfish poisoning, results in neurologic disease and impaired respiration. These cases often require respiratory support with artificial ventilation along with intensive physiotherapy.

Antimicrobial therapy

Most cases of food poisoning resolve spontaneously and do not require specific therapy. There is controversy about the role of antibiotics in community-acquired bacterial diarrhea but recent guidelines help to clarify some of these issues.[54] Specific therapy may shorten the duration of illness and prevent complications but this is unproven in most cases. However, tetracycline treatment may reduce the duration of diarrhea in cholera both by killing bacteria and by interrupting toxin synthesis, and its use is important in epidemics. One problem is that therapy for most cases of diarrhea usually has to be empiric initially because it may take several days to isolate and identify a causative organism. Widespread use of empiric antibiotics for diarrhea may encourage the spread of resistant organisms, promote the development of diarrhea caused by *Clostridium difficile* and increase adverse reactions, such as rash. Studies have shown that the use of ampicillin for *Salmonella* enterocolitis may prolong excretion of the organism. There are also case-control study data to suggest that children who have hemolytic-uremic syndrome caused by *E. coli* O157:H7 who receive antibiotics may have a poorer outcome than those who do not, but this continues to be controversial.

Empiric antibiotics are appropriate for the elderly (aged over 70 years), those who have severe systemic symptoms, such as fever or joint inflammation, and the immunocompromised. In these cases, initial therapy is with a quinolone, such as ciprofloxacin (500mg q12h orally or 200mg q12h intravenously), because these drugs are well absorbed orally but are also available as intravenous preparations. They are effective against pathogens that can be invasive, such as *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp.; however, quinolone-resistant *Campylobacter* isolates are already well described.[55] [56] There is also concern about increasing quinolone resistance in nontyphoidal *Salmonella* in some parts of the world.[57] It is widely recognized that resistance in these common enteric pathogens frequently derives from the increased use of antibiotics in animal husbandry. Such use can lead to resistant organisms becoming established in animals destined for human consumption.[58]

Most protozoa that cause food poisoning are treated with specific agents. Benzimidazoles, such as metronidazole or tinidazole, are effective against *Giardia* spp. (metronidazole 400–500mg q8h orally for 5 days) and amebae (metronidazole 750–800mg q8h orally for 5 days), although some strains of *Giardia* are relatively resistant to metronidazole. This resistance can be a problem because culture and sensitivity methods are not routinely available. In practice, those giardial infections that do not respond to metronidazole or tinidazole usually respond to mepacrine (100mg q8h orally for 7 days). It should be remembered that patients who have *E. histolytica* dysentery may reinfect themselves with amebic cysts carried in the gut. Following killing of trophozoites by a benzimidazole, these patients should be given a luminal cysticide, such as diloxanide furoate (500mg q8h orally for 10 days). Infections with *Isopora belli* and *Cyclospora cayetanensis* respond to 10–14 days of oral trimethoprim-sulfamethoxazole (160mg TMP/800mg SMX q12h). There is no specific treatment for cryptosporidiosis.

Viral infections are self-limiting. Rarely, patients who have acute hepatitis A virus infection may become deeply jaundiced with prolonged cholestasis. In such cases, a short course of oral prednisone (prednisolone; 0.5mg/kg daily reducing to nothing over 4 weeks) may reduce inflammation around the bile canaliculi and lead to resolution of the jaundice.

Most helminthic infections need specific therapy, which can be tailored to the parasite that is detected.

Most invasive food-borne infections, such as typhoid, need to be specifically treated, so it must be re-emphasized that every effort should be made to obtain samples for culture and sensitivity testing. This is increasingly important as antibacterial resistance of enteric organisms increases. The specific treatments for these conditions are described in Chapter 43 .

Surgery

Surgery has little role in the management of these infections. Infections caused by *Yersinia* spp., *Salmonella* spp. and other

organisms may present in a manner resembling acute appendicitis, leading to laparoscopy or laparotomy. In severe, infective enterocolitis a toxic megacolon may arise. This sometimes leads to colectomy or to a decompressing colostomy. Surgery may also be required for the complications of typhoid, such as acute hemorrhage or intestinal perforation. Although surgical intervention may also be used to obtain tissue biopsies for histology and culture, such specimens are more often obtained by percutaneous biopsies guided with imaging techniques such as ultrasound or CT scanning.

Public health issues

Hospitalized patients who have diarrhea or who are infected with an organism that can be spread by the fecal-oral route should be isolated in single rooms. Gloves and aprons should be worn when dealing with the patient's excreta to prevent nosocomial spread.

The diagnosis or suspicion of a food- or water-borne illness should be notified to the relevant public health officer (see also Chapter 50a). This is particularly important

in suspected outbreaks, in institutions or when a food-handler is involved. Public health aspects have also come more to the fore in recent years, following September 11 2001 and the use of anthrax as a tool of bioterrorism. Organisms that cause food-borne disease can and have been used intentionally to contaminate food.[59] Systems must be in place to provide surveillance for such a scenario, to recognize the risk and to initiate an appropriate public health and clinical response.[60] One example of this is the establishment of PulseNet, a network to type food-borne organisms by pulse field electrophoresis to more rapidly identify point source outbreaks.[61] The establishment of good public health surveillance and good communication with clinicians and the public can help to provide reassurance during food-borne outbreaks, whatever the origin.

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Chapter 94 - Chronic Fatigue

Nelson M Gantz

Fatigue is one of the most common complaints in ambulatory care and in one report in an adult primary care clinic, 24% of patients indicated that fatigue was a major problem.[1] Laboratory tests are not helpful in determining the cause of the fatigue in the majority of patients and at 1-year follow-up, about 75% of patients have persistent fatigue. Over the past decade there has been a worldwide increase in attention to patients reporting fatigue, cognitive difficulties, malaise, weakness, myalgias, arthralgias, headache and low-grade fever. This disorder has in recent years been called the chronic fatigue syndrome (CFS), myalgic encephalomyelitis and the postviral fatigue syndrome.[2] [3] [4] In the press, the disorder has been referred to as 'yuppie flu'. Many patients and some physicians believe that the current name, CFS, does not adequately characterize the illness and is disparaging and promotes misunderstanding.

The diagnosis of CFS continues to stir controversy. It is a waxing and waning illness, the fundamental nature of which remains uncertain. Some physicians deny its existence, while others specialize in the disorder. Management strategies also evoke a diverse spectrum of responses. Some physicians use symptomatic treatment and others immediately prescribe a variety of exotic therapies, ranging from antiviral drugs to vitamins and herbal agents. Difficulty in identifying the cause of CFS has complicated the disorder's diagnostic definition, which, in turn, has introduced a measure of uncertainty when considering treatment options. The diagnosis is made by exclusion.

In my experience, about 20% of patients gradually improve and 80% have intermittent exacerbations. Unfortunately, some patients fail to respond to symptomatic therapy and must be considered disabled. Among those who fare poorly, some have a history of psychiatric illness, particularly depression. Unless an etiologic agent or unifying pathophysiology for CFS is identified, diagnosis will remain empiric and treatment largely supportive. It is important that clinicians recognize that CFS can be disabling, and provide support as the patient and family go through the difficult process of seeking disability benefits.

HISTORY

Chronic fatigue syndrome is not a new medical disorder. Patients who have a syndrome of fatigue, malaise and other somatic complaints have been recognized in the literature for more than 200 years (Table 94.1). In 1750, Sir Richard Manningham described febricula or little fever, an illness in which patients had weariness all over, pain, forgetfulness and low-grade fever.[5] In 1869, Beard coined the term neurasthenia to describe patients who lacked 'nerve strength', which he believed was the cause for the chronic fatigue.[6] Shortly after Beard's work was published, DaCosta described a syndrome of fatigue, breathlessness, palpitations, digestive problems, chest pain, sleep difficulties and dizziness in Civil War soldiers. DaCosta attributed the syndrome to an 'irritable heart'.[7] This syndrome was subsequently called the effort syndrome and the diagnosis continued to stir controversy in the 20th century, even though it was supported on psychologic and physiologic grounds. In the 1940s, DaCosta's syndrome lost support as a distinct clinical entity.[15] Since then, numerous reports have appeared in the literature describing patients similar to those characterized by Beard with neurasthenia. Some of these outbreaks are listed in Table 94.1 .[8] [9] [10] [11] [12] [13] [14] In the British Commonwealth, because neurologic symptoms have been prominent in some of the case clusters, the syndrome has been called benign myalgic encephalomyelitis.

Reports of patients who have fatiguing illnesses have also been attributed to various pathogens and to metabolic and environmental factors. In the 1930s, *Brucella* was erroneously linked as a cause of CFS.[16] Similarly, hypoglycemia, *Candida albicans* and the 'total allergy syndrome' have also been popular but unproved causes of this syndrome.[17] [18] In the 1980s, chronic infection with Epstein-Barr virus (EBV) was thought to be associated with CFS.[19] [20] However, when appropriate control populations were studied, EBV was not found to be a cause of CFS.[14] Other pathogens such as *Borrelia burgdorferi*, human herpesvirus-6 and human retroviruses have also been erroneously implicated as having a causative role in this disorder.[21] [22] [23] [24] [25]

Fibromyalgia, formerly called fibrosis, is a disorder that also has a long history and is seen commonly by rheumatologists.[26] The illness was described in the French literature in the 1850s and the clinical features overlap those seen in patients who have CFS.[27]

EPIDEMIOLOGY AND CASE DEFINITION

In 1987, the Centers for Disease Control and Prevention (CDC) convened a group of experts to develop a case definition for CFS for research and epidemiologic purposes. The definition required severe fatigue to be present for at least 6 months and no evidence of various illnesses that could produce chronic fatigue. In addition to these major criteria, the definition required the presence of eight criteria from the following symptoms or physical signs:

- the symptom criteria were fever and chills, sore throat, swollen neck or arm glands, muscle weakness, myalgias, post-exertional malaise, headaches, arthralgias, sleep disturbance, neuropsychiatric symptoms such as problems with memory, concentration and depression, and an acute onset;[2] and
- the physical sign criteria were low-grade fever, nonexudative pharyngitis and cervical or axillary lymphadenopathy.

In 1991, a conference organized by the National Institutes of Health (NIH) proposed that certain patients be excluded:

- those who have chronic medical conditions, such as malignancy or autoimmune disease, psychotic depression, bipolar disorder or schizophrenia; those prone to substance abuse; and
- those who have ongoing infectious diseases, such as chronic active hepatitis B or C, untreated Lyme disease or HIV infection.[28]

Patients who have a diagnosis of fibromyalgia, nonpsychotic depression, somatoform disorders, or anxiety or panic disorders were to be included. The original case definition and the modifications proposed by the NIH conference were developed by consensus based on the anecdotal experience of the participants. Using this case definition, the CDC implemented a surveillance system in four US cities. The

TABLE 94-1 -- Selected fatiguing disorders.

SELECTED FATIGUING DISORDERS	
Condition	**Year reported**
Febricula[5]	1750
Neurasthenia[6]	1869
DaCosta's syndrome[7]	1871
Atypical poliomyelitis[8]	1934
Icelandic disease[9] (Akureyri disease)	1948
Epidemic of poliomyelitis-like illness[10] , Adelaide, Australia	1949
Royal Free disease[11]	1955
Punta Gorda illness, Florida[12]	1956
Myalgic encephalomyelitis[13]	1956
Lake Tahoe illness[14]	1987

case records of 565 patients were reviewed and 23% were found to have fulfilled the case definition, 18% had unexplained chronic fatigue plus insufficient symptoms to meet the case definition, 42% had psychiatric disease before the onset of the fatiguing illness and 18% were found to have other medical disorders (unpublished CDC data). In this and another study, the physical examination criteria were found infrequently and did not contribute to the diagnosis.[29] The purpose of the definition was to select a homogeneous group of patients who have 'true CFS'. However, although the case definition did distinguish CFS cases from healthy controls and patients who have multiple sclerosis or depression, the criteria failed to separate CFS patients from other persons who have prolonged fatigue.[29]

At about the same time, a UK case definition and an Australian case definition were published.[30] [31] [32] The UK definition required the presence of severe fatigue and cognitive impairment. Patients who have medical conditions known to produce chronic fatigue and psychiatric disorders, such as schizophrenia, manic depressive illness, substance abuse, eating disorders, or organic brain disease, were excluded. Patients who have a fatiguing illness with a documented postinfectious onset should be identified.[30] The Australian case definition is similar to that of the UK;[31] [32] three subtypes are recognized — a postinfectious type, a neuropsychologic type with depression and one associated with fibromyalgia.

In 1993, a meeting was held at the CDC to revise the original case definition published in 1988. The study group consisted of worldwide experts including persons involved in the earlier UK and Australian case definitions. The criteria for diagnosis are given in Table 94.2 .[33] Unexplained fatigue must be present for at least 6 months and CFS remains a diagnosis of exclusion. Four symptom criteria must be concurrently present for 6 months. Selected laboratory tests and mental status examination are performed to identify disorders that should be excluded (Table 94.3). Patients who have severe fatigue for 6 months, the absence of other conditions to explain the fatigue, but less than four symptom criteria are designated as having idiopathic chronic fatigue. Patients who have anxiety and mild depression are not excluded. Fibromyalgia and depression are important overlapping disorders. The revised case definition has flaws and an international group plans to propose a new case definition in the future.

Chronic fatigue syndrome has been reported worldwide, with most of the studies from the UK, Australia and the USA. The prevalence varies from study to study, reflecting differences in surveillance methods and in the case definition used. In a study from Australia, the prevalence rate was 37/100,000 persons.[32] A CDC study that collected data from four USA cities found a prevalence rate of 2–7.3/100,000 persons using the 1988 case definition.[35] In a large community survey of fatigue in Washington state, USA, the

TABLE 94-2 -- Revised (1994) CFS case definition criteria.*

REVISED (1994) CFS CASE DEFINITION CRITERIA
1. Clinically evaluated, unexplained, persistent or relapsing fatigue for at least 6 months that:
• Is of new or definite onset
• Is not the result of ongoing exertion
• Is not substantially alleviated by rest
• Results in substantial reduction in previous levels of occupational, educational, social or personal activities
2. Four or more of the following concurrent symptoms on a persistent or recurrent basis during 6 or more consecutive months of illness, none of which may predate the fatigue.
• Self-reported impairment in short-term memory or concentration that is severe enough to cause substantial reduction in previous levels of occupational, educational, social or personal activities
• Sore throat
• Tender cervical or axillary lymph nodes
• Muscle pain
• Multijoint pain without joint swelling or redness
• Headaches of a new type, pattern or severity
• Unrefreshing sleep
• Postexertional malaise lasting more than 24 hours
Both 1 and 2 are required conditions for a diagnosis of CFS

** With permission from Quadrant Healthcom.*[34]

TABLE 94-3 -- Conditions that exclude the diagnosis of CFS.*

CONDITIONS THAT EXCLUDE THE DIAGNOSIS OF CFS
• Any active medical condition that may explain the presence of chronic fatigue (e.g. untreated hypothyroidism, sleep apnea, narcolepsy, adverse effects of medications, HIV disease)
• Any previously diagnosed medical condition without resolution documented beyond reasonable clinical doubt, and for which continued activity may explain the chronic fatiguing illness, (e.g. previously treated malignancies and unresolved cases of hepatitis B or hepatitis C virus infection)
• Any past or current diagnosis of major depression with melancholic or psychotic features, bipolar affective disorder, schizophrenia of any subtype, delusional disorders of any subtype, dementias of any type, anorexia nervosa or bulimia
• Alcohol or other substance abuse within 2 years before the onset of the chronic fatigue and any time afterwards
• Severe obesity as defined by a body mass index (BMI) =45: $BMI = \frac{\text{weight in kg}}{(\text{height in m})^2}$
• Any unexplained physical examination finding or laboratory or imaging test abnormality that strongly suggests the presence of an exclusionary condition

** With permission from Quadrant Healthcom.*[34]

prevalence rate of CFS ranged from 98 to 267/100,000 persons and the frequency of fatigue alone was much higher.[36] In a small study from Scotland, the prevalence of CFS was much higher with a rate of 560/100,000 persons. [37] In a large community based study in Chicago, the prevalence of CFS was 422/100,000 which compares with a rate of 373/100,000 based on a CDC study conducted in Wichita, Kansas (Reyes M, personal communication).[38] Most studies show a female (75%) predominance with the average age ranging from 30 to 40 years. Disease also occurs in adolescents and the

elderly.[39] All socioeconomic groups are affected. Cases occur sporadically and in outbreaks or clusters. No differences have been noted in sporadic cases compared with epidemic cases. Patients report three patterns of symptom onset: an acute onset of illness without precipitating cause, postinfectious onset or gradual onset of symptoms. Further studies are needed to compare the type of onset with the course of the illness. Chronic fatigue syndrome does not appear to be contagious.

PATHOGENESIS AND PATHOLOGY

The etiology and pathogenesis of CFS are unknown. Patients who have CFS often report an infectious illness such as a presumed viral respiratory tract infection as the inciting event. Much attention has focused on EBV as a possible cause of CFS in response to two reports in 1987.[19] [20] However, other studies failed to demonstrate a difference in EBV antibody levels between cases and controls.[14] [40] One UK report found higher levels of enterovirus in the stools of patients who have postviral fatigue syndrome than in those of control patients.[41] In another study, enteroviral RNA was identified in muscle biopsies from 20% of CFS patients compared with zero in control patients.[42] However, persistent enterovirus infection was not supported by a report from The Netherlands and another case-control study from the USA.[43] [44] In one report, human T-cell leukemia/lymphoma virus (HTLV)-1 antibodies were detected in 50% of adults who had CFS and in none of the controls.[23] In the same study, HTLV-II gag sequences were found in the sera of 83% of adults who had CFS, 72% of children who had CFS and in none of the controls. These results were not confirmed by a study of 21 patients conducted by the CDC.[24] Other investigators have also failed to confirm the retrovirus finding and have not detected antibody to spumavirus, another CFS implicated retrovirus.[25] Antibody levels of other agents, including arboviruses, cytomegalovirus, human herpesvirus-6, varicella-zoster virus, respiratory viruses (adenovirus, parainfluenza virus types 1, 2 and 3, respiratory syncytial virus), hepatitis viruses, measles virus, *Rickettsia* spp., *Bartonella* spp., *B. burgdorferi*, *Chlamydia* spp. and *C. albicans*, were not found more frequently in CFS patients than in matched controls.[44] Although many different infectious agents have been suspected of having an etiologic role in CFS, none qualifies as the sole cause of the illness. It also appears that after certain acute infections, such as influenza, brucellosis or adequately treated Lyme disease, CFS can develop.[45] [46] [47] The mechanism that accounts for CFS in this setting after an acute infection is unknown.

Chronic fatigue syndrome may be a neuroendocrine rather than an infectious disorder. There is a clear association between stress, the immune system, the endocrine system and CFS. Symptoms of CFS such as fatigue, myalgias and sleep problems occur in patients who have adrenal insufficiency. Patients who have CFS have been reported as having reduced serum levels of basal evening glucocorticords and decreased 24-hour urinary free cortisol excretion compared with controls.[48] Plasma adrenocorticotropic hormone (ACTH) levels were not reduced and a decreased response of ACTH to ovine corticotropin-releasing hormone was noted. These results do not suggest either adrenal or pituitary insufficiency, but a possible hypothalamic problem. A controlled study that compared normal replacement doses of hydrocortisone with placebo doses in patients who have CFS showed no significant improvement in symptoms in those treated with drug and a high frequency of adrenal suppression. [49] Low-dose hydrocortisone, 5–10mg/day, was found to be beneficial in one study when given for 1 month but this finding has not been confirmed.[50] Plasma levels of 5-hydroxyindoleacetic acid, a metabolite of serotonin, were noted to be increased in patients who have CFS.[51] In another study, prolactin levels were normal.[52] The interpretation of these various findings is unclear.

In addition to fatigue, patients who have CFS often complain of lightheadedness and cognitive dysfunction. Reports have shown abnormal tilt table tests in patients who have CFS; 96 versus 29% for controls.[53] Various therapies using increase in dietary salt intake, fludrocortisone, ß-adrenergic blocking agents and disopyramide alone or in combination were reported to cause a reduction in symptoms in an uncontrolled study, but a controlled trial using drugs to treat patients who have abnormal tilt

table tests found no difference in symptoms in those patients treated with fludrocortisone compared with placebo.[54]

A high frequency of atopy and positive immediate skin tests has been reported in patients who have CFS.[55] [56] Immunologic abnormalities similar to those reported with acute viral infections have frequently been noted in CFS patients.[57] [58] [59] Alpha-interferon and other cytokines such as interleukin (IL)-6 may be elevated in some patients who have CFS.[60] Flu-like symptoms have been associated with increased levels of various cytokines. The finding of persistent cytokine abnormalities in some studies along with an increase in $CD8^+$ lymphocyte cell subsets showing activation markers is intriguing;[61] however, they do not provide a diagnostic test for CFS. It is important not to overinterpret the lymphocyte phenotyping data because the results have not been consistent, nor identified in the majority of patients who have CFS. These abnormalities of increased cytokines and evidence of immune activation in many cases of CFS have led some to suggest that the illness be named 'chronic fatigue immune dysfunction syndrome'. This name or illness should not be confused with immune deficiency.

Possible factors in the pathogenesis of CFS are summarized in Table 94.4 . Likely predisposing factors include psychiatric illness, and genetic and environmental factors such as allergy. Delayed recovery from influenza virus infection was noted more frequently in patients who have pre-existing psychiatric illness.[46] Some studies have found a higher prevalence of psychiatric disorders such as depression in CFS patients before the onset of the illness compared with controls.[62] [63] [64] In contrast, another study reported the prior prevalence of major depression (12.5%) and of all psychiatric disorders (24.5%) in patients who have CFS to be no higher than in the general community.[65] Not surprisingly, depression and anxiety were common symptoms after the onset of the illness. A twin study is underway to evaluate the role of genetic factors in patients who have CFS. Infection or stress are often historically the precipitating or triggering factors. Possible perpetuating factors include physical deconditioning, concurrent psychiatric illness, misattribution of physical symptoms and increase in cytokines.

PREVENTION

There are no specific preventative measures for CFS because the etiology and mode of transmission are unknown.

TABLE 94-4 -- Possible pathogenesis of CFS.

POSSIBLE PATHOGENESIS OF CFS		
Predisposing factors	**Precipitating factors**	**Perpetuating factors**
Psychiatric illness	Infection	Physical deconditioning
Genetic	Stress	Concurrent psychiatric illness
Environment (e.g. allergy, chemicals)		Misattribution of physical symptom
		Raised cytokines

CLINICAL FEATURES

Fatigue is the hallmark of CFS and by definition should be present for at least 6 months (see Table 94.2). The fatigue refers to a state of severe mental and physical exhaustion that is not caused by activity or lack of rest. The fatigue is usually made markedly worse by activity and is not readily relieved by rest. This should not be confused with sleepiness, which indicates that the patient may have a sleep disorder such as sleep apnea, narcolepsy or depression. The fatigue is so severe that approximately 25% of patients report being often bedridden and unable to work.[66] Unfortunately, the precise measurement and definition of fatigue are difficult. The natural history of fatigue is poorly understood. Fatigue is usually measured using self-report instruments but these have limitations.

The majority of patients are female (about 75%) with a mean age of 35–40 years. Most patients (85%) report that the illness began suddenly, often with an initial 'flu-like' illness, although some report a gradual onset of symptoms with no triggering event. The disorder affects persons in all socioeconomic groups, but in most studies the patients are middle class.

In addition to the fatigue, patients note low-grade fever, myalgia, sleep disturbance, impaired cognition, depression, headache, sore throat, post-exertional malaise, arthralgia and dizziness.[66] The symptoms are present most of the time in the majority of patients and the illness typically waxes and wanes. As there is no diagnostic laboratory study, the clinical diagnosis rests upon the CFS case definition. Patients who have a diagnosis of fibromyalgia made by a rheumatologist often fit the case definition for CFS. Persons who have severe fatigue but fewer than four symptoms are classified as having idiopathic chronic fatigue.

The history is key in establishing the diagnosis. Epidemiologic clues related to travel, occupational and animal exposures, and substance abuse or alcohol misuse should be carefully sought. Patients should be questioned for HIV risk factors and, if positive, evaluated by testing.

A mental status examination is important to look for psychiatric disorders; if abnormalities are noted then a psychiatric or neurologic evaluation can be invaluable. Particular attention should be directed to depression, anxiety, suicide thoughts and a history of sexual abuse.

The diagnosis is based on self-reports and the physical examination is only helpful in identifying other causes of the patient's symptoms. The physical examination is usually normal and no pathognomonic physical findings have been reported. If a patient is found to have a temperature higher than 38.4°C (101°F), then another cause for the fever should be pursued. Similarly, generalized lymphadenopathy or a single large lymph node suggest another diagnosis.

The differential diagnosis of fatigue is extensive (Table 94.5). Psychologic disorders, particularly depression, are the most common causes of chronic fatigue. Other causes for fatigue such as multiple sclerosis, systemic lupus erythematosus or hypothyroidism should be considered.

DIAGNOSIS

No diagnostic tests are specific for CFS and the laboratory testing is to exclude possible causes for the fatigue and other symptoms. Laboratory studies should investigate the various diagnostic possibilities suggested by the history and physical examination. A minimum set of recommended screening laboratory tests to evaluate a patient who has suspected CFS is given in Table 94.6 . The erythrocyte sedimentation rate is a key test, and results tend to be normal or low in patients who have CFS. Neuropsychologic testing to measure cognitive function as well as tests to screen for the presence of psychiatric disease may be beneficial. Various immunologic

TABLE 94-5 -- Most common differential diagnoses of chronic fatigue.

MOST COMMON DIFFERENTIAL DIAGNOSES OF CHRONIC FATIGUE	
Habit patterns	Caffeine habituation
	Alcoholism
	Other substance abuse
Psychosocial	Depression
	Anxiety
	Stress reaction
Medications	Corticosteroids
	Sedatives
	Chemotherapy
Sleep disorders	Sleep apnea
	Narcolepsy

Pregnancy	Anemia
	Weight gain
	Fluid retention
Infectious diseases	Mononucleosis, cytomegalovirus, or EBV infection
	HIV infection
	Chronic hepatitis B or C virus infection
	Lyme disease
	Fungal disease
	Chronic parasitic infection
	Tuberculosis
	Brucellosis
	Subacute bacterial endocarditis
	Occult abscess
Autoimmune disorders	Systemic lupus erythematosus
	Multiple sclerosis
	Thyroiditis
	Rheumatoid arthritis
	Myasthenia gravis
Occult malignancy	Lymphomas
	Gastrointestinal malignancy
Endocrine disorders	Hyperparathyroidism
	Hypothyroidism
	Apathetic 'hyperthyroidism'
	Adrenal insufficiency
	Cushing syndrome
	Hypopituitarism
	Diabetes mellitus
Hematologic problems	Anemia
	Myeloproliferative syndromes
Metabolic disorders	Hyponatremia
	Hypokalemia
	Hypercalcemia
Cardiovascular disease	Low-output states
	Silent myocardial infarction
Hepatic disease	Alcoholic hepatitis or cirrhosis
Renal disease	Chronic renal failure
Respiratory disorders	Chronic obstructive pulmonary disease
Miscellaneous	Sarcoidosis
	Wegener's granulomatosis
This list is not meant to include every illness that can cause chronic fatigue. Rather, it is intended to highlight some of the illnesses that most commonly do so.	

tests can yield abnormal findings, but they are not indicated in clinical practice. A search for a diagnostic marker to identify patients with CFS has not been successful. One study found increased levels of a 37kDa 2-5A binding protein in patients with CFS but further data are needed.[67] Studies measuring cytokine levels and natural killer cell activity are best obtained in the research environment.

TABLE 94-6 -- Minimum recommended tests to evaluate suspected CFS.

MINIMUM RECOMMENDED TESTS TO EVALUATE SUSPECTED CFS
Complete blood count
Erythrocyte sedimentation rate
Alanine aminotranferase
Total protein
Albumin
Globulin
Alkaline phosphatase
Creatinine
Thyroid-stimulating hormone
Calcium
Phosphorus
Electrolytes
Glucose
Blood urea nitrogen
Urinalysis
Other tests are based on history and results of physical examination

TABLE 94-7 -- Managing the patient who has CFS.

MANAGING THE PATIENT WHO HAS CFS
1. Establish the diagnosis
2. Provide emotional support and refer patient to support groups
3. Prevent further disability by establishing a graded exercise program
4. Treat symptoms with appropriate medications, avoiding exotic untested remedies, and with cognitive behavior therapy
5. Follow up regularly and re-evaluate

There is no value in obtaining a battery of viral serologies, such as EBV titers, when chronic fatigue is suspected. Magnetic resonance imaging and single-photon emission computed tomography studies of the head of CFS patients are under investigation as diagnostic studies in such patients.

MANAGEMENT

Strategies for managing the patient who has CFS are outlined in Table 94.7 .[68] The objectives of therapy are:

- to help the patient develop realistic goals and expectations through education;
- to provide symptomatic relief; and
- to preserve and improve the patient's ability to function.

At the outset it is very important to acknowledge the sense of illness and debility expressed by these patients. Patient support groups can play an important role in helping patients and their families cope with this frustrating chronic illness.

Nonpharmacologic therapies

Patients with CFS often avoid activity because they fear exacerbating their symptoms. Complete bed rest should be avoided because of the problems associated with physical deconditioning. I believe that a balance between moderate levels of exercise and rest, dictated by common sense, is essential and that physical activity should be gradually increased as tolerated.

Cognitive behavior therapy attempts to alter attitudes, perceptions and beliefs that can contribute to maladaptive behavior. Controlled trials using cognitive behavior therapy with a graded exercise program show reduced symptoms and increased activity in patients who have CFS and fibromyalgia, an illness that overlaps with CFS.[69] In some patients hypnotherapy and physical therapy are helpful for morning stiffness and muscle pain.

Pharmacologic therapy

Many therapeutic agents have been used to treat CFS (Table 94.8). Information on the efficacy of most of these medications in treating CFS patients is limited to anecdotal reports, often in publications that are not peer-reviewed.[70] [71] [72]

Antiviral medications

Current data do not indicate the use of antiviral drugs. In a well designed placebo-controlled trial of 27 patients who have CFS, 46% of those given high-dose intravenous aciclovir and 41% given placebo responded favorably (P >0.05).[73] In the aciclovir-treated group, most persons reported a return of symptoms soon after treatment was discontinued; immunologic tests and EBV serologic assays were unaffected and 12% developed reversible renal failure.

Amantadine, an agent used for the treatment of Parkinson's disease and influenza A virus, has been reported to decrease fatigue in patients who have multiple sclerosis. Only anecdotal information is available regarding its use in patients with CFS, however.

Immunologically active medications

Immunoglobulin has been given to patients who have CFS based on the theory that CFS results from an immunoregulatory defect and because specific immunoglobulin subclass deficiencies have been found in some CFS patients. Two well-designed controlled studies of high-dose intravenous immunoglobulin in CFS patients were conducted. In the USA, 28 adult CFS patients were given either placebo or 1g/kg intravenous immunoglobulin each month for 6 months; about 20% in each group improved symptomatically. [74] In Australia, 49 adult CFS patients were given either placebo or 2g/kg intravenous immunoglobulin each month for 3 months; 43% of those given immunoglobulin reportedly felt better compared with 12% of those given placebo.[75] Symptomatic improvement, however, was noted only at 3 months after the final infusion, and symptoms and disability returned 6 months after the end of therapy. Phlebitis occurred in 55% of patients and constitutional symptoms, such as headache, fatigue and diminished concentration, occurred in 82% of patients. Possible explanations for the different results between these studies include the smaller sample size and lower doses of immunoglobulin used in the USA study, and differences in study populations and outcome assessments. There are no controlled studies on the value of immunoglobulin administered intramuscularly.

Transfer factor is a component of leukocytes that can transfer delayed-type hypersensitivity. In a double-blind trial, transfer factor given intramuscularly over a 4-week period was no more effective than placebo in improving immunologic parameters or the functional status of CFS patients.[61]

Poly(I)-poly(C_{12} U) (Ampligen) consists of double-stranded RNA molecules with possible antiviral and immunomodulatory effects.[76] In a double-blind placebo-controlled study of 92 patients who have CFS, poly(I)-poly(C_{12} U) given intravenously twice weekly for 6 months was associated with an enhanced capacity to perform activities of daily living and an improvement in memory.[76] Adverse effects included hepatic toxicity. This drug cannot be recommended until more data are available on its safety and efficacy. Controlled studies are in progress to evaluate this drug.

Trials of interferon-a given subcutaneously have been associated with improvement in some patients but the data are limited. Inosine pranobex, IL-2 and prednisolone in doses of 10–60mg/day have been attempted without beneficial effect.[34]

Antidepressant medications

Depression is common in patients with CFS. In addition to their antidepressive effects, antidepressants may help improve sleep,

TABLE 94-8 -- Selected list of agents used to treat CFS patients.

SELECTED LIST OF AGENTS USED TO TREAT CFS PATIENTS	
Antidepressants	Tricyclic antidepressants
	Amitriptyline
	Desipramine
	Nortriptyline
	Monoamine inhibitors
	Phenelzine
	Moclobemide

Selective serotonin reuptake inhibitors	Bupropion
	Fluoxetine
	Nefazodone
	Paroxetine
	Sertraline
	Venlafaxine
Immune modifiers	Transfer factor
	Poly(I)-poly(C_{12} U)
	Interferon-a
	Corticosteroids
	Cyclophosphamide
	IL-2
	Isoprinosine
	Thymic extract
Antibacterials	Ceftriaxone
	Ciprofloxacin
	Doxycycline
	Fusidic acid
Antivirals	Aciclovir
	Amantadine
	Ganciclovir
Anti-inflammatory agents	Hydroxychloroquine
	Nonsteroidal anti-inflammatory drugs
	Cyclobenzaprine
Opium antagonists	Naltrexone
Calcium channel blockers	Nifedipine
	Nimodipine
Stimulants	Amphetamines
	Modafinil
Vitamins and minerals	Cyanocobalamin
	Ascorbic acid
	Zinc
	Magnesium
Psychoactive agents	Benzodiazepines
	Alprazolam
	Clonazepam
	Non-benzodiazepines
	Carbamazepine
	Buspirone
Antifungals	Ketoconazole
	Fluconazole
	Nystatin
Antihistamines	H_2 -receptor antagonists
Other agents	Germanium
	Kutapressin
	Primrose oil
	Vasopressin
	Herbs
	Pentoxifylline
	Essential fatty acids
	Hydrogen peroxide
	Galanthamine hydrobromide
	Quinacrine
	Fludrocortisone
	Atenolol
	Disopyramide
	NADH

fatigue and pain symptoms, and are a mainstay in the treatment of CFS (Table 94.9). Anecdotal reports suggest that antidepressant doses lower than normal may be effective; dosing can be increased gradually if there is no effect. The chosen agent should be used for 4–6 weeks before therapeutic failure is considered. The choice of agent depends, to a large extent, on expected side effects.

Although large controlled studies are limited, tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs) appear to be beneficial. Tricyclic antidepressants are associated with sedative and anticholinergic effects. Anecdotally, patients who have difficulty sleeping sometimes respond well to agents such as amitriptyline or doxepin taken daily at bedtime.

In a study of patients who had fibromyalgia, 25mg amitriptyline at bedtime (rather than the usual dosages of 100–150mg) decreased fatigue and myalgias and improved sleep compared with placebo. Responses were usually seen in 3–4 weeks.[77] Desipramine is a less sedating tricyclic antidepressant. When sedation is not desirable, SSRIs, such as fluoxetine, citalopram, sertraline or paroxetine, can be helpful. In a study of 79 patients with CFS treated with 50mg sertraline daily for 6

months, 65% showed improvement in fatigue, myalgias, sleep disturbance and depression. Adverse effects, limited

TABLE 94-9 -- Antidepressants used to treat CFS/fibromyalgia.

ANTIDEPRESSANTS USED TO TREAT CFS/FIBROMYALGIA	
Drug	**Usual dose (mg/day)**
Amitriptyline	100–150
Citalopram	20–40
Doxepin	100–150
Desipramine	150–200
Nortriptyline	75–100
Fluoxetine	20–40
Sertraline	50–150
Paroxetine	20
Bupropion	200–300
Venlafaxine	75–225
Nefazodone	200–500

mainly to nausea and diarrhea, occurred in 8% of the patients.[78] Bupropion or another drug, venlafaxine, may be effective in patients who are unable to tolerate a tricyclic agent or SSRI.[79] However in a

small placebo-controlled trial, fluoxetine or venlafaxine were not beneficial in treating patients with CFS.[80]

Anxiolytic medications

Panic disorders and anxiety occur frequently in patients who have CFS.[81] Although controlled studies in CFS patients are lacking, alprazolam plus ibuprofen were more effective than placebo in reducing pain and anxiety in patients who have fibromyalgia.[82]

Anecdotally, clonazepam, other benzodiazepines and buspirone also appear beneficial. Alprazolam may be particularly helpful in managing panic attacks; however, a dosage schedule of 3–4 times a day is required and the potential for habituation is substantial.

Pain medications

Acetaminophen, aspirin and other nonsteroidal anti-inflammatory drugs are often used to treat myalgias and arthralgias in CFS patients. In a study of patients who have fibromyalgia, naproxen (500mg q12h) plus amitriptyline were more effective than placebo in decreasing muscle aches.[77] In another randomized trial, amitriptyline (10–50mg at bedtime) was more effective than placebo in reducing myalgia in fibromyalgia patients.[83] Response was seen as early as 1–2 weeks after beginning therapy. In a placebo-controlled trial of the muscle relaxant cyclobenzaprine, a dosage of 10–40mg at bedtime for 12 weeks resulted in decreased pain and improved sleep in fibromyalgia patients. [84] In a small controlled trial of patients with fibromyalgia, tramadol decreased pain.[85]

Sleep medications

Problems falling asleep and maintaining sleep, as well as awakening unrefreshed from sleep, are very common in CFS patients. Low doses of amitriptyline (25–50mg) or cyclobenzaprine (10–20mg) at bedtime may be beneficial. Other agents that have been anecdotally reported to be helpful are trazodone (25–50mg), doxepin (10–50mg) and clonazepam (0.5–1mg). In a controlled study, 10mg zolpidem improved sleep in patients with fibromyalgia.[86]

Allergy medications

New allergies or exacerbation of old allergies are commonly reported by patients with CFS. Food elimination diets, royal jelly, herbs and dietary supplements have not been shown to be helpful, however. Non-sedating antihistamines such as terfenadine, astemizole or loratadine can be tried, but in a small trial oral terfenadine versus placebo did not have a clinical benefit in alleviating CFS symptoms.[87] In one controlled study, nystatin did not reduce symptoms in patients who have the 'yeast connection'.[88] The yeast connection is a hypothesis that the *Candida* spp. present in the body produce products that cause symptoms of fatigue; however, there is no proof linking yeast with CFS.

Vitamins, minerals, fatty acids and stimulants

Liver extract, folic acid and vitamin B12 given intramuscularly were no better than placebo in adults who have CFS.[89] In a controlled trial intramuscular magnesium sulfate given weekly for 6 weeks was associated with increased energy, less pain and improved emotional state in patients who have CFS. In this study, red blood cell magnesium levels were lower in CFS patients than in healthy controls and became normal after treatment.[90] Other investigators have not found lower red blood cell magnesium levels in patients with CFS.[91] I do not recommend magnesium therapy in CFS unless abnormally low red blood cell magnesium levels have been documented.

The treatment of CFS patients who have essential fatty acids has led to conflicting results. In one study, a combination of evening primrose oil and fish oil led to improvements in 85% of patients after 15 weeks, compared with 17% of those treated with placebo.[92] In another study, no difference was found between patients receiving essential fatty acids and those receiving placebo.[93] Amphetamines are not usually beneficial because of the development of tolerance. Another stimulant, modafinil, appears useful for selected patients and controlled trials are in progress. Modafinil is well tolerated and does not have the problem of tolerance.

Treatment of neurally mediated hypotension

Despite the lack of controlled trials, patients who have CFS and a positive tilt table test should be tried on an increased dietary salt intake and fludrocortisone, 0.1–0.2mg/day, with the addition of a ß-blocker or disopyramide if no response occurs with fludrocortisone. Midodrine, an alpha-1 agonist, may be beneficial but controlled trials are lacking.[53]

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Chapter 95 - Psychological Aspects of Infectious Diseases

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The commonly held belief that psychosocial factors can influence susceptibility to disease dates back to antiquity and is by no means unique to Western culture. Yet although early research provided some interesting empiric support for this notion (see review by Cohen and Williamson),[1] it is only relatively recently that advances in immunology have enabled us to explore in detail the possible role of the immune system in mediating this process. The study of the complex bi-directional interactions between the immune system and the central nervous system (CNS) that are central to the influence psychosocial factors exert on physical health has been termed 'psychoneuroimmunology' (PNI).

An ever-growing number of PNI studies have now identified a wide variety of psychologic states and traits as being associated with dysregulation of the immune system.[2] [3] Perhaps the best documented of these is stress, the cognitive and emotional reaction experienced when we perceive that environmental demands exceed our ability to cope, [4] although other psychosocial factors including psychiatric illness, depression, social disruption, personality and coping style have also been linked to changes in immune status.[2] [5] Despite being of obvious theoretic interest, however, the clinical significance of such changes is not always clear. The immunologic sequelae of psychosocial factors are typically small in magnitude and rarely result in any given immune parameter moving outside its normal range of values. Given the high degree of overlap within the immune system it is not always clear whether such alterations have any real clinical significance. Nevertheless, several well-conducted studies have noted that host susceptibility to infectious diseases, as well as the progression of these diseases can be affected by alterations in psychosocial status. In this chapter we review some of the more compelling examples of this evidence in humans and provide a brief description of the various pathways through which psychologic factors can exert their influence on physical health. Clinical features of the host, stress-inducing stimulus and pathogen that can moderate the impact of psychologic stress on disease status are also summarized, as are potential techniques for diagnosing, preventing and managing stress-related downregulations of immunocompetence.

EPIDEMIOLOGY

As noted, although a large body of work has confirmed that a multitude of psychologic processes have the potential to downregulate various aspects of the immune system,[3] much less evidence is available regarding the clinical significance of this for infectious diseases in man. Nevertheless, several important studies have revealed that psychosocial factors can alter host susceptibility and disease progression in acute viral infection, HIV infection, other latent viral infections and bacterial infection.[6]

Acute viral infections

Due to their relatively benign nature, acute viral infections such as common colds and influenza have proved popular paradigms for experimental studies examining the role of PNI in infectious diseases. Early research, although frequently suffering from methodologic drawbacks,[1] suggested that higher levels of emotional disturbance may predict longer convalescence from such infections.[7] These findings have been confirmed and extended by more recent research. Of particular note is the seminal work of Cohen, Tyrrell and Smith[8] who questioned 394 healthy volunteers about current and recent stress levels before exposing them via nasal droplets to one of five respiratory viruses. Patients were subsequently quarantined and closely monitored for 1 week. The results showed that pre-infection stress levels had a dose-response relationship to the risk of succumbing to a verified clinical infection, regardless of the specific virus examined. This finding has since been replicated for several more respiratory viruses using similar methodologies, with the additional finding that pre-infection stress levels also predict greater postinfection symptom severity (mucus secretion and self-reported symptoms).[8] Limited diversity in a participant's social network has also been shown to predict risk of infection, and extroverts appear to be less likely to develop clinical cold symptoms than more introverted participants.

Human immunodeficiency virus infection

The progression of HIV following initial infection remains relatively unpredictable and can only be partially explained by, for example, specific viral strain, demographics and lifestyle. This has resulted in speculation about the possible role of psychosocial variables in determining disease progression, and over 50 studies have now been published that examine this issue in some detail (see review by Cole and Kemeny).[9] These have typically failed to identify any significant effect of general negative life events or personality traits on immune status or clinical outcome in HIV-positive individuals, although some inconclusive evidence does exist to suggest that both bereavement and a general tendency to inhibit expression of significant thoughts or feelings may be associated with a decline in clinical and immunologic status. By contrast, highly specific aspects of an individual's emotional and cognitive response to personally important negative events have been reported to predict disease progression. For example, self-reproach or denial in response to bereavement or notification of HIV status have been shown to predict increased decline in $CD4^+$ T-cell levels and faster progression to AIDS in HIV-positive gay men, while 'finding meaning' in bereavement and demonstrating an active coping response to HIV are both associated with an attenuated decline in $CD4^+$ T-cell levels and slower disease progression.

Several studies have also reported that large social networks and high perceived social support may predict increased rate of $CD4^+$ T-cell decline and a higher risk of symptom onset in HIV-positive gay men.[9] This somewhat counter-intuitive finding possibly reflects the psychologic drain that an extensive social support network can represent for this population, particularly in terms of the need to provide care-giving for other HIV-positive individuals and the increased risk of bereavement. In line with this theory, results from nonhuman primate studies have demonstrated that although disruption of a stable social environment accelerates disease onset in Rhesus macaques inoculated with simian immunodeficiency virus (SIV), living within an unstable social hierarchy is also associated with significantly reduced survival times.[10]

Other latent viral infections

Numerous studies have found that episodes of stress are associated with an increase in antibody levels to latent viruses such as the herpes simplex viruses (HSV) and Epstein-Barr virus (EBV).[1] Rather than implying improved immunocompetence, such results are normally taken as indicative of a decreased capacity of the cellular arm of the immune system to control viral replication. In terms of the clinical significance of this phenomenon, there is some evidence that for genital and oral herpes, negative mood and low social support can significantly predict onset of disease recurrence.[6] Not all studies have confirmed these findings, however, and it is possible that the link between mood and disease recurrence may be at least partially mediated by an increased susceptibility to other minor infectious diseases such as common colds.

Psychosocial variables have also been reported to increase susceptibility to, and severity of, primary infection with latent viruses. For example, in a 4-year follow-up study of trainee army officers initially seronegative to EBV, high motivation to achieve, poor academic success and having an 'overachieving' father were found to be significant risk factors for seroconversion, clinical mononucleosis and longer hospitalization following clinical infection.[11]

Bacterial infections

Good quality research into the role of psychosocial variables in the development of bacterial infections is limited,[6] although the data available suggest that stress may well be an important risk factor. One early prospective cohort study[12] noted that higher stress levels significantly predicted the development of streptococcal pharyngitis, while in a second study[13] 'psychologically vulnerable' individuals were found to experience greater severity of illness (number of hours of fever and self-reported symptoms) following experimental inoculation with typhoidal-type tularemia than those defined as 'psychologically nonvulnerable'. More recently, depression has also been associated with altered bactericidal activity of leukocytes in children infected with *Staphylococcus aureus*. [14]

PATHOGENESIS

The major pathways through which psychosocial factors can affect the onset or course of an infectious disease are illustrated in Figure 95.1 . Perhaps the most obvious of these is via the increased exposure to pathogens that can result from stress-related changes in sexual behavior, for example, or interaction with one's social support network. Other behavioral changes common during stress, including increased smoking, higher caffeine and alcohol consumption, poor

Figure 95-1 Potential health effects of stress. Indirect (behavioral) and direct (neural and neuroendocrine) mechanisms through which psychologic distress can alter the onset and course of infectious disease.

diet and altered sleep patterns, can also alter the course of an infection through their own direct effects on various aspects of immunocompetence.[1]

Even carefully controlling for such behavioral pathways, however, several studies have demonstrated that psychosocial factors remain significantly associated with the onset or course of infectious diseases, suggesting that more direct, biologically based pathways also exist. Of these, the interactions between the CNS and the immune system are believed to be the most important.[3] The impact of the CNS on the immune system is principally mediated by changes in the activity of the hypothalamic-pituitary-adrenal (HPA) and sympathetic adrenal-medullary (SAM) axes. Activation of these systems as part of the stress response causes an upregulation in plasma levels of glucocorticoids and catecholamines, classes of immunomodulatory hormones capable of binding to cytoplasmic receptors on leukocytes and altering their functioning and distribution. Thus animals administered glucocorticoids following experimental inoculation with a pathogen typically demonstrate reduced resistance to infection,[6] while administration of adrenergic blockers to prevent catecholamines from binding to immune cells has been shown to reduce the effects of stress on the immune system.[15] As well as these hormonal pathways, direct innervation by the sympathetic nervous system of lymphoid organs such as the spleen is also known to exist, providing a further mechanism through which neuroimmunomodulation can occur.

In addition to affecting immunocompetence, it is possible that psychologic processes can also produce changes in an organism's anatomic and functional barriers to infection. For example, it has been suggested that the direct effects of stress on host susceptibility to respiratory viruses may be partly mediated by an alteration in the nasal mucosa, while stress-induced increases in glucocorticoid and catecholamine levels may help to protect female mice against bladder infection by increasing

vesical epithelial shedding.

Finally, another biologically based pathway between psychologic processes and infection has been highlighted in several in-vitro studies with reports that glucocorticoids can directly promote viral replication in latent viruses including HIV and EBV,[6] presumably through encouraging alterations in the genetic expression of these agents.

PREVENTION

A limited number of randomized controlled trials (RCTs) have investigated whether psychologically preparing an individual for an impending stressful event can help to buffer against resulting

immunologic dysregulation. Two RCTs examined this issue by training medical students in self-hypnosis/relaxation therapy and subsequently comparing the degree of immunologic changes that occurred in the run-up to stressful academic examinations in these groups with those in students randomized to no-treatment control groups. Disappointingly, although both interventions were found to significantly reduce the stress of sitting an examination, neither study was able to identify any major effect of the training on stress-induced immune changes. These results may partially reflect the nature of the stressor and samples that were used, however. Examination stress is normally relatively short-lived and tends to provoke transient immune changes that may be qualitatively different to those caused by more chronic stress (see Clinical features, below). Furthermore, it may be that a more severely distressed sample would have been more motivated to learn the techniques on offer and would have had more to gain through their effective use.

In an attempt to redress these issues, one RCT assessed whether group training in cognitive-behavioral stress management and progressive muscle relaxation had any benefit for asymptomatic gay men awaiting notification of their HIV serostatus. [16] Among men subsequently informed that they were HIV positive, the intervention package significantly reduced post-notification distress and enhanced some immunologic markers including $CD4^+$ T-cell and natural killer (NK) cell counts in comparison to an assessment-only control group. A 2-year follow-up to this study further revealed that men who took part in the intervention continued to show reduced declines in $CD4^+$ T-cell counts over this period and were less likely to have progressed to AIDS. Well-structured interventions for people awaiting highly stressful events may thus have good justification from a clinical point of view, although further research is still required in this area.

CLINICAL FEATURES

Certain important features of potentially stressful situations, or 'stressors', have been identified that increase the likelihood that the stressor will provoke immunologic dysregulation. Attempts have also been made to identify what differentiates people who are likely to suffer immunocompromise as a result of stress from those who are not.[17] Finally, it would appear that even when a particular stress-related alteration of the immune system is apparent, its clinical significance is likely to be determined by various characteristics of the infectious pathogen under consideration.[6] Thus clinical features

TABLE 95-1 -- Aspects of the stressor, host and pathogen that may alter the clinical significance of a stressful event.

ASPECTS OF THE STRESSOR, HOST AND PATHOGEN THAT MAY ALTER THE CLINICAL SIGNIFICANCE OF A STRESSFUL EVENT	
Stressor	Appraisal of stressor and own resources
	Acute vs. chronic stressor
	Severity
	Perceived predictability
	Perceived controllability
Host	Age?
	Race?
	Culture?
	Sex?
	Personality
	Coping style
	Availability and quality of social support
	Pre-existing stress levels
Pathogen	Virulence

of the stressor, host and pathogen may all be important in determining whether psychosocial factors play a role in the course or onset of any particular infectious disease (Table 95.1).

Stressors

Stress is a fundamentally subjective phenomenon and it is possible for an experience that one person finds highly traumatic to represent a relatively mundane matter for someone else. Rather than being intrinsic to any specific situation or event, the experience of stress is instead determined by an individual's own cognitive appraisal of the stressor and their beliefs regarding their ability to cope with it.[4] Hence, changing the way we think about a situation and the resources we have available to us can frequently transform a highly stressful occurrence into something much more benign, a fact that has clear implications for our immunologic response to the stimulus.

Having said this, certain stressor characteristics do appear to increase the likelihood that they will elicit dysregulation of the immune system as well as influencing both the quantitative and qualitative nature of this response. The duration of the stressor is one such determinant. Acute stressors, such as public speaking, anticipation of a mild electric shock or sitting an important examination, typically elicit transient immune activation, including temporary upregulation of NK-cell numbers and function, increased $CD8^+$ T-cell numbers and, more uncertainly, decreased T-helper and B-cell numbers.[3] In contrast, chronic stressors, such as caring for a relative or partner with Alzheimer's disease, divorce or bereavement, are more likely to lead to downregulated immunity (e.g. reduced T-cell numbers, decreased number and function of NK cells and decreased proliferative response to mitogens).[3] This acute/chronic distinction almost certainly has important implications for the clinical significance of a stressor, and it is typically chronic stressors that have been linked to altered health outcomes in the literature. For example, a clear dose-response relationship would appear to exist between the duration of a previous stressful life event and the risk of developing a common cold following experimental inoculation.[8]

Unsurprisingly, a similar relationship also seems to exist between stressor severity and the likelihood of a clinically relevant alteration in immunocompetence, with stressors causing greater psychosocial turmoil resulting in increased chance of infection.[2]

The perceived controllability and predictability of a stressor are further characteristics that may partially determine an individual's immunologic response. Numerous laboratory studies have demonstrated that biologic and emotional reactions to a stressor are reduced when, for example, a buzzer is sounded before the onset of an unpleasant stimulus or participants are able to terminate the stressor by pressing a button. Immunologic responses seem to obey similar rules. Thus, although exposure to an apparently uncontrollable acute stressor may decrease NK cell activity, the same level of exposure to a stressor that is perceived as controllable does not. [18] Similarly, presenting a warning noise before inescapable footshocks attenuates decrements in lymphocyte reactivity in rats. By necessity, studies investigating these issues have typically used short-lived and somewhat artificial stressors. How these factors affect chronic stress and what their clinical implications may be in such settings therefore remain unclear.

Hosts

Unfortunately, the study of how individual differences affect the immune response to stress has been somewhat neglected in the PNI literature. Little is known about how age, race or culture might affect immunologic responses, for example, although it is conceivable that all three may be important moderators of the pathways linking

stress to infection.[3] Similarly, although some evidence exists to suggest that the immunologic response to stress in humans may be accentuated

by female sex hormones,[3] more research is required before any firm conclusions can be drawn regarding the importance of gender in this context.

In contrast, the role of personality traits and coping styles as moderators of the CNS-immune system interactions have received a greater degree of attention. Both clearly play an important role in shaping our appraisal of potential stressors and our cognitive and emotional reactions to stress, and accordingly there is some evidence to suggest that these variables can affect an individual's immunologic response to stress as well as having an impact on the immune system in their own right. For example, individuals who scored highly on measures of trait worrying were found to show a far greater decline in peripheral blood levels of NK cells following an earthquake than low worriers, a difference that persisted for 4 months after the event.[19] Similarly, higher levels of trait hostility have been found to predict larger increases in NK cell cytotoxicity following a brief social confrontation, while optimism appears to predispose individuals to greater decreases in NK-cell activity following more prolonged exposure to a stressor.[17] In terms of coping style, repressive and escape-avoidance coping when faced with stress have both been associated with decreased immunocompetence, although denial has been found to have some protective effects for gay men awaiting notification of HIV status.[2]

There is some evidence that individuals with more good quality social support available to them may be at less risk of suffering clinically significant immune changes as a result of stress than those with less support. For example, high-quality social support has been linked to a low incidence of oral herpes recurrence, reduced susceptibility to common colds and attenuated SIV progression in monkeys, [6] although it is not always clear whether this is due to social support acting as a buffer against stress having some direct effect independent of the presence of a stressor, or a combination of the two.

Finally, several studies have noted that high baseline levels of stress may predispose individuals to increased reactivity in response to a novel stressor, predicting greater declines in NK-cell function following a stressful mental arithmetic task,[20] for example. Individuals already suffering from high levels of chronic stress may therefore be most at risk of succumbing to an infection following a new stressful event.

Pathogens

Whether immune dysregulation as a result of stress is likely to alter infectious disease processes in other animals is partly dependent on the specific species and strain of the pathogen.[6] For instance, whilst high levels of stress may increase mortality rates in mice infected with a mildly virulent strain of *Staphylococcus aureus,* it does not appear to have the same effect in those exposed to a highly virulent strain.[21] Similar factors are likely to be important in determining the clinical significance of stress in humans.

DIAGNOSIS

No diagnostic test exists for assessing whether psychologic factors are affecting the course of a patient's infectious disease. However, where a full psychosocial assessment of the patient reveals that stress or negative mood is a problem, these deserve to be treated in their own right. Useful tools for measuring negative affect in the context of physical illness include the 12-item general health questionnaire[22] and the perceived stress scale. [23]

MANAGEMENT

The potential for psychologic interventions to modify the human immune response, and hence the potential for them to prove clinically useful in the management of infectious diseases have been examined in over 85 published studies and been the subject of an excellent review and meta-analysis by Miller and Cohen. [24] They characterized this literature as assessing five basic approaches to 'psychoimmunotherapy':

- stress management,
- relaxation,
- disclosure,
- hypnosis with immune suggestion, and
- classical conditioning.

The possible clinical usefulness of these are outlined below, together with the role that pharmacologic interventions may have in reducing dysregulation of the immune system due to stress or depression. Clearly, other psychologic approaches that do not rely on psychoneuroimmunomodulation but instead seek to reduce the risk of infection or alter the course of disease by encouraging health behaviors such as dental hygiene or adherence to drug regimen are also possible and can be highly effective, but fall beyond the scope of this chapter.

Stress management and relaxation

Stress management interventions typically attempt to reduce the distress associated with an event or illness by employing a complex therapeutic package consisting of any of a number of components including:

- education about a particular illness;
- cognitive therapy aimed at identifying and reducing cognitive distortions and automatic thoughts;
- anger management training;
- coping skills training; and
- help in identifying sources of social support.

Relaxation interventions tend to be slightly more straightforward and normally provide training in one of several techniques such as progressive muscle relaxation, biofeedback-assisted relaxation or relaxation-focused self-hypnosis.

By altering the recipient's cognitive appraisals of a stressor and of their ability to cope with it or by altering their emotional response to a stressor, these types of intervention certainly have the prima facie potential to reduce immunologic dysregulation, particularly in people suffering from chronic stress. In practice, however, they have usually been tested on individuals suffering from chronic illness, a fact that can make interpretation of the results difficult: chronic illness does not necessarily equate to chronic stress and many people are able to adapt to their condition and often retain or even exceed their pre-morbid quality of life.[24] Despite this caveat, however, several studies have identified positive effects of both stress management and relaxation therapies in individuals living with HIV,[9] with interventions such as bereavement-specific and generalized stress management programs, and progressive muscle relaxation showing the capacity to attenuate declines in $CD4^+$ T-cell levels.[9] These results are by no means universal for this population, however, and more work is required to identify which characteristics of the interventions or recipients might alter the therapeutic potential of such techniques.

Stress management and relaxation approaches may also be effective in other chronic infections and there are indications that they might be useful in the treatment of herpetic recurrences[25] and recurrent childhood upper respiratory tract infections[26] in particular.

Disclosure

Disclosure interventions require the patient to reveal traumatic events from earlier in their lives that had not previously been extensively discussed with anybody else. This normally takes the form of writing or speaking about the event for short periods (e.g.

15–20 minutes) over the course of a week and is believed to be able to affect the immune system by altering the individual's cognitive appraisal of the traumatic event and instilling an improved sense of personal control. Thus, one major RCT on the PNI effects of this intervention identified a significantly greater immunologic response to hepatitis B vaccination following 4 days of writing about a traumatic topic when compared to writing about an innocuous topic.[27] Disclosure has also been found to reduce EBV antibody titers, suggesting that it may have some clinical usefulness for herpes recurrences and HIV infection.

Hypnosis with immune suggestion

Hypnosis incorporating suggestions about immunomodulation has been attempted by several authors with varying degrees of success.[24] Reliable increases have been reported in salivary immunoglobulin A levels and neutrophil adherence following this technique, suggesting that it may have some clinical usefulness. In particular, there have been some interesting demonstrations of the efficacy of hypnosis in the treatment of warts and recurrent herpes.[6] [28] The precise mechanism through which hypnosis can affect the immune system remains unclear, however, and further work is required in this area.

Classical conditioning

A large body of research has confirmed that the immune system is receptive to classical conditioning such that after pairing an initially non-immunomodulatory stimulus such as saccharin-flavored water (the conditioned stimulus) with administration of an immunosuppressant drug (the unconditioned stimulus), subsequent presentation of the conditioned stimulus by itself is sufficient to cause downregulation of the immune system.[29] Upregulation through classical conditioning has also been demonstrated in humans, with upregulated NK-cell function, for example, having been elicited by a sweet flavoring that had previously been paired with an epinephrine (adrenaline) injection. The therapeutic effects of immunomodulatory conditioning have yet to be properly explored within the context of infectious diseases, however, although this approach may eventually prove useful as an adjunct to conventional therapy in the treatment of HIV infection.

Pharmacologic treatments

Benzodiazepines have the potential to attenuate negative mood and may be useful in patients in whom high levels of stress may be affecting the course of their infection. The immunomodulatory effects of benzodiazepines depend upon the specific molecule and dose used, however, and alter according to the immune parameters under consideration.[3] The effects of antidepressants are similarly inconsistent, and treatment of depression in HIV-positive patients using sertraline, imipramine or fluoxetine does not appear to affect $CD4^+$ T-cell levels.[9] Further research is required into the precise immunologic effects of these drugs before any recommendations can be made regarding their use in patients who have infectious diseases.

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Chapter 96 - Practice Points

96.a Management of candiduria in the intensive care unit

Shiranee Sriskandan

Introduction

Candiduria can be defined as the presence of greater than 10^5 fungal cfu/ml urine, though as little as 10^3 cfu/ml can result in disease in certain 'at-risk' groups. The prevalence of candiduria varies between 6.5% and 20% amongst hospitalized patients and presents a dilemma to clinicians, who must decide if the finding represents colonization alone or is a feature of invasive fungal infection. Probably only 3–4% of cases of candiduria lead to candidemia, but 10% of all cases of candidemia are associated with a prior episode of candiduria. Indeed, studies based in the intensive care unit (ICU) have shown that candiduria can be associated with a rise in mortality from 19% to 50%.

Pathogenesis

Candiduria can arise in several ways: simple contamination of specimens at the time of procurement can account for many such cases, hence the need for a confirmatory second specimen. Colonization of the urinary tract may occur in the catheterized patient. Local infection of both the lower urinary tract (cystitis, urethritis) and upper tract (pyelonephritis) with *Candida* spp. is encouraged by urological instrumentation, in particular catheterization. Other factors predisposing to such infections include ongoing broad-spectrum antibiotic therapy, diabetes mellitus, renal insufficiency and anatomic anomalies of the urinary tract. finally, patients with disseminated candidiasis may seed the urinary tract from bloodstream spread (Table 96a.1).

Microbiology

The majority (50–70%) of *Candida* isolates from urine in the ICU are *C. albicans*, which is sensitive to fluconazole. Indeed, provided that patients have not previously been exposed to fluconazole, it is reasonable to assume that any germ tube-positive yeast will be sensitive to fluconazole. However, increasing numbers of yeasts other than *C. albicans* occur in the ICU setting and the prevalence varies between units. In particular, *C tropicalis* and *C glabrata* account for 10–20% of such isolates, the latter species being notable for its resistance to azole drugs.

Clinical features

Candiduria alone does not cause symptoms; local infection can cause classic cystitis or urethritis and pyelonephritis may lead to flank pain. Patients who have candiduria as a feature of disseminated candidiasis may have evidence of systemic candidal disease, which should be assiduously checked for: clinical features include sepsis, fever, lesions of the optic fundi, skin lesions and hepatosplenomegaly.

Investigations

From Table 96a.1 , it is clear that a repeat fresh urine sample must be sent to the microbiology laboratory to confirm candiduria. Microscopy for the presence of white blood cells may be useful in differentiating colonization from urinary tract infection, but the finding can be nonspecific in a catheterized patient; the presence of granular casts containing hyphae is a rare finding which would confirm true renal infection. If the patient has not been catheterized or had

TABLE 96.a-1 -- Etiology of candiduria and laboratory investigation.

ETIOLOGY OF CANDIDURIA AND LABORATORY INVESTIGATION	
Source of candiduria	**Laboratory investigations**
Inadvertent contamination	Repeat sample: clear
Colonization of lower tract	No WBCs in urine, patient well
Infection of lower tract	WBCs in urine
	Ultrasound if not instrumented
	Screen for diabetes, renal disease
Infection of upper tract	WBCs + casts in urine
	Ultrasound
	Screen for diabetes, renal disease
Disseminated *Candida* infection	Blood cultures/other sterile site: positive for *Candida*
	CXR, abdominal ultrasound
	High CRP

TABLE 96.a-2 -- Management of candiduria in the ICU.

MANAGEMENT OF CANDIDURIA IN THE ICU		
Suspected cause of candiduria	**Action**	**Additional considerations**
Contamination	None	-
Colonization	No antifungal	If patient is at risk of infection, e.g. neutropenia or undergoing urological procedure, fluconazole 200mg/day for 7–14 days
	Remove/replace catheter	
	Stop antibacterials if possible	
Lower UTI	Fluconazole 200mg/day for 7–14 days if *C. albicans*	
Upper UTI	Fluconazole 6mg/kg/day for 2–6 weeks if *C. albicans*	Surgical drainage may be needed.
		Amphotericin B if patient unstable or if non-*albicans*

Disseminated infection likely	Fluconazole 6–12mg/kg/day (if *C. albicans*) *or* amphotericin B 0.7–1.0mg/kg/day, depending on severity, for at least 2–6 weeks	If dissemination confirmed: follow up for at least 3–6 months after discharge from ICU in case of distant seeding Use of lipid formulations of amphotericin B necessitates doses of 3–5mg/kg/day

urological instrumentation recently, it is prudent to screen for diabetes mellitus and renal insufficiency by biochemical testing, and anatomic anomalies using ultrasound. Ultrasound of the renal tract can also demonstrate the presence of fungal balls in patients who have persistent candiduria. Simple tests to screen for the possibility of disseminated candidiasis would include a chest X-ray, abdominal ultrasound, C-reactive protein, cultures of other potentially infected sites (e.g. tracheal aspirate or bronchial lavage, bile, surgical drains, intravascular line tips) and blood cultures.

Management

The modern management of candiduria in the ICU setting is determined by the likely source of fungi (see Table 96a.2). It is clear that colonization can be treated by simply replacing the urinary catheter or, better, permanent removal. In all cases, rational reduction in the spectrum of antibacterial agents administered to patients will help eliminate fungal colonization and infection. These simple measures allow up to 40% of patients with candiduria to clear fungi from the urine. True infection of the urinary tract should be treated with a definitive course of an antifungal, usually fluconazole, in addition to catheter removal or exchange. Fluconazole can be administered intravenously in the ICU or via the nasogastric tube in the oral form, if the patient's gastrointestinal system is functioning. Infection with germ tube-negative *Candida* spp. (other than *C. albicans*) may require intravenous amphotericin B. There is no clear case for local intermittent or continuous bladder irrigation with amphotericin B; the procedure necessitates instrumentation of the urinary tract which might otherwise be unnecessary. Furthermore, although local irrigation with amphotericin B can achieve prompt clearance of funguria, the effect is short-lived compared with clearance rates achieved by fluconazole.

Finally, the candiduric patient who may have invasive fungal infection warrants more aggressive antifungal therapy. This must be based on careful assessment of combined clinical and laboratory findings. If *C. albicans* is isolated and the patient is stable, it is reasonable to treat with high-dose fluconazole for 2–6 weeks (400–800mg/day for a 70kg adult). However, if the same patient is clinically unstable or if the isolate is non-*albicans*, it would be prudent to treat with amphotericin B, as indicated in Table 96a.2 or with one of the newer agents such as voriconazole or caspofungin, although they have not yet been formally evaluated in this setting (see Chapter 208). The appropriate duration of therapy in this setting is unclear and must be determined according to the individual clinical situation and response to therapy.

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96.b Management of fever that relapses and remits

Nicholas Price

The patient who has recurrent febrile episodes commonly presents a particularly difficult diagnostic challenge. In addition to the classic criteria for fever of unknown origin (FUO; Chapter 82), Knockaert *et al.* have defined 'recurrent' FUO as repeated febrile episodes with fever-free intervals of at least 2 weeks. The number of relapses or overall duration was not specified but, in practice, the clinical course is typically protracted. One limitation of this definition is that several important infectious diseases that are characteristically associated with a recurrent fever pattern are excluded. However, a clear definition and strict adherence to defining criteria are helpful because they focus the diagnostic approach and are essential for meaningful comparative studies.

Causes

Infections, malignancy and multisystem inflammatory diseases are responsible for 60–70% of cases of classic FUO. If recurrent FUO is

TABLE 96.b-1 -- Causes of recurrent fever of unknown origin.*

CAUSES OF RECURRENT FEVER OF UNKNOWN ORIGIN
Infectious diseases
Focal bacterial infection (e.g. chronic prostatitis, subacute cholangitis)
Q fever endocarditis (*Coxiella burnetti*)
Rat-bite fever (*Spirillum minor, Streptobacillus moniliformis*)
Relapsing fever (*Borrelia recurrentis, Borrelia duttoni*)
Trypanosomiasis (*Trypanosoma gambiense, Trypanosoma rhodesiense*)
Whipple's disease (*Tropheryma whippelii*)
Yersiniosis (*Yersinia pseudotuberculosis, Yersinia enterocolitica*)
Multisystem diseases
Connective tissue diseases and vasculitides:
Churg-Strauss disease
Giant cell arteritis
Polymyalgia rheumatica
Mixed connective tissue disease
Polyarteritis nodosa
Systemic lupus erythematosus
Wegener's granulomatosis
Rheumatologic diseases:
Ankylosing spondylitis
Relapsing polychondritis
Rheumatoid disease
Still's disease
Inflammatory diseases:
Sarcoidosis
Neoplasia
Atrial myxoma
Colonic carcinoma
Lymphoma
Miscellaneous conditions
Castleman's disease
Cholesterol embolism
Crohn's disease
Cyclic neutropenia
Drug fever
Extrinsic allergic alveolitis
Factitious fever
Familial Mediterranean fever, familial Hibernian fever
Fume fever, hypersensitivity pneumonitis
Gaucher's disease, Fabry's disease
Hyper-IgD syndrome
Hypertriglyceridemia (type IV)
Mollaret's menigitis
Seizures ('thermal epilepsy')
Sweet's syndrome
The more common causes are indicated in bold type.

** Adapted from Knockaert et al., 1993.*

considered as a subset of classic FUO, these three causes account for only 20% of the cases; 50% go undiagnosed and a collection of diverse 'miscellaneous' conditions forms the largest subgroup (Fig. 14.6). Patients who have recurrent febrile episodes that persist for more than two years rarely have infections or malignant disorders.

Infectious causes

A silent focus of bacterial infection is the most common infectious cause that should be considered, rather than any of the limited number of specific infections listed (Table 96b.1). Other infections on the list of differential diagnoses that present with a relapsing and remitting fever pattern, which may not fulfill the stringent criteria for

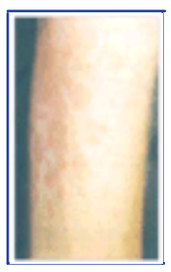

Figure 96.b-1 Characteristic evanescent rash of Still's disease.

recurrent FUO, include malaria, brucellosis, secondary syphilis, tuberculosis, trench fever (caused by *Bartonella quintana*), filariasis and visceral leishmaniasis. However, it is important to note that malaria does not always produce the classic 'quartan' or 'tertian' fever patterns since parasites may mature asynchronously.

Neoplastic causes

Many malignancies can cause FUO; however, those listed (Table 96b.1) have been specifically reported as causing recurrent FUO.

Multisystem disease

All vasculitides or connective tissue diseases can flare up suddenly and remit spontaneously, producing a recurrent fever. Still's disease is a seronegative arthritis of unknown etiology; it is essentially a diagnosis of exclusion. The diagnosis should be strongly suspected in a young adult who has the classic triad of high fever above 104°F (40°C), evanescent rash (Fig. 96b.1) and arthritis (particularly if pharyngitis is also reported).

Miscellaneous causes

Familial Mediterranean fever is a clinical diagnosis characterized by recurrent polyserositis but a molecular diagnosis may also be established by identifying mutations of the pyrin gene (*MEFV*), located on the short arm of chromosome 16. Similar to familial Mediterranean fever, 'hyper-IgD syndrome' is an autosomal recessive inherited disorder that has been described in European families and was recently linked to a locus on chromosome 12. Patients characteristically have elevated IgD levels, and cervical lymphadenopathy and abdominal pain are prominent clinical features. Tumor necrosis factor (TNF)-receptor-associated periodic syndrome (TRAPS) is another rare hereditary condition, first described in a large Irish family and originally called familial Hibernian fever. Affected individuals are thought to be unable to neutralize TNF because serum levels of soluble type 1 TNF receptor are typically low. Inheritance is autosomal dominant and common features are localized myalgia and conjunctivitis.

Crohn's disease is an important cause of recurrent FUO and may present with weight loss, fever and anemia without any gastrointestinal symptoms. Castleman's disease (angiofollicular lymph node hyperplasia) may present with focal mediastinal or generalized lymphadenopathy. The localized type occurs in young adults and is curable by surgery. The generalized form affects older patients, has a less benign prognosis and may undergo malignant transformation. Sweet's syndrome is characterized by a painful neutrophilic dermatosis and is

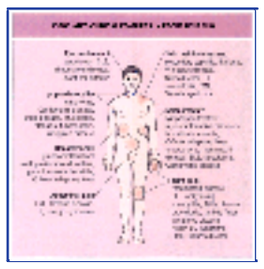

Figure 96.b-2 Clinical features associated with recurrent fever of unknown origin. SLE, systemic lupus erythematosus; FMF, familial Mediterranean fever.

associated with joint pains and malignancy. Very rarely, epileptic seizures may produce periodic febrile confusion.

'Idiopathic granulomatosis' and 'granulomatous hepatitis' should not be readily accepted as final diagnoses because it is likely that they encompass a variety of different underlying conditions, which may be revealed during careful long-term follow-up.

Clinical assessment

As for any FUO, a detailed history and thorough clinical examination are paramount and repeated assessments are often necessary. Associated clinical features are illustrated in Figure 96b.2 . In general, the pattern of the fever is seldom of diagnostic value but a notable exception is cyclic neutropenia, which has a classical 'periodic' fever pattern that reoccurs predictably after every 21 days. In addition, high fevers recurring at fixed intervals of 2–8 weeks are characteristic of the rare 'PFAPA' (*p*eriodic *f*ever, *a*phthous stomatitis, *p*haryngitis and cervical *a*denitis syndrome) that has recently been described in children.

Hereditary causes of recurrent FUO almost always present before 20 years of age, and febrile attacks start in the first year of life in hyper-IgD syndrome. In addition, a detailed family history and inquiry about racial background is particularly important in the evaluation of these inherited conditions. A thorough travel history should be obtained and in the tropical traveler the risk of disease transmission by insect vectors should be assessed by asking about visits to game parks, accommodation (camping, downmarket hotels, log cabins) and protective measures such as bed nets. A bite from a tsetse fly (vector in African trypanosomiasis) is often memorably painful and leaves an indurated lesion for days. In contrast, the bite in tick-borne relapsing fever is characteristically painless. Specific enquiry should also be made about consumption of unpasteurized milk (a cause of brucellosis), occupational exposure (e.g. fume fever), animal contact (e.g. rat-bite fever, Q fever, psittacosis), previous medical conditions (e.g. episodes of cholecystitis) and drug treatment (especially if taken intermittently). Repeated attacks of fever may also represent a relapse of a pre-existing infection, particularly if treatment fails or is discontinued. Treatment compliance or antimicrobial resistance may therefore need to be addressed.

Investigations

The investigative work up is the same as for classic FUO (Chapter 82). Because the range of potential underling causes is so diverse, no comprehensive diagnostic algorithms exist.

Hematologic indices occasionally provide useful clues but are not always reliable (e.g. eosinophilia may indicate lymphoma, Churg-Strauss disease, drug reaction or parasitic infection). In addition to malaria, the causative organisms in relapsing fever and trypanosomiasis may be visualized on a thick blood film taken during a febrile episode. A moderately elevated acute phase response is not remarkable in itself, although it does exclude factitious fever. However, in some conditions, inflammatory indices are exceptionally high: an erythrocyte sedimentation rate above 100mm/h is seen in drug fever, malignancy, giant-cell arteritis and Still's disease. Serum ferritin is nonspecifically elevated in inflammatory conditions, but extremely high levels (>1000mg/l) are also typical in Still's disease and a raised serum angiotensin converting

enzyme may indicate a granulomatous disorder. Although there may be a polyclonal increase in immunoglobulins, hyper-IgD syndrome is diagnosed by characteristic clinical findings and continuously high IgD levels (>100U/ml). Serologic and immunologic tests should be done as appropriate but, as in classic FUO, they are often unrewarding unless these are suspected to yield significant results beforehand.

Radioisotope-labeled white cell imaging may be useful in identifying occult foci of infection but often does not contribute more than computed tomography scanning of abdomen/ pelvis and chest. Magnetic resonance imaging is valuable when bone infection is suspected. There should be a low threshold for investigating the gastrointestinal tract (e.g. by endoscopy, small bowel transit study or barium enema) in order to look for inflammatory bowel disease and malignancy. In an elderly patient who has a very elevated erythrocyte sedimentation rate, temporal artery biopsy may be useful diagnostically where there is no prior localizing information.

General approach

Because the cause of recurrent FUO is generally not life threatening, if no clues are provided by diagnostic tests, a 'watch-and-wait' strategy can often be adopted. Periodic outpatient assessment is likely to reveal significant pathology in time and many of the undiagnosed cases may resolve spontaneously. It is useful to ask patients to record their own temperature using a digital thermometer and to keep a symptom diary. In order to make a more valuable clinical assessment it is often helpful to ask patients to come up to the hospital immediately when they become unwell or pyrexial.

In general, empiric trials of antibiotic or anti-inflammatory therapy as diagnostic tests are inadvisable. However, if empiric treatment is felt to be absolutely necessary, a full course should be prescribed so that complications arising from inadequate therapy are avoided. In the future, genetic studies will probably identify other rare causes of recurrent FUO that persist and are presently undiagnosed.

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96.c Infections associated with near drowning

Alastair Miller

It is thought that about 100 million North Americans use the marine environment for recreation each year. This leads to an estimated 8000 deaths from drowning per annum in the USA and at least 150,000 deaths worldwide. The epidemiology of 'near drowning' is less well known and estimates vary between two and 20 times the deaths from drowning. Drowning implies death due to cerebral hypoxia as a result of immersion in water. In the majority of cases water is aspirated into pulmonary air spaces. This produces a variety of pathologies depending on whether fresh or sea water is inhaled, but the end result is alveolar dysfunction, causing venous blood to be shunted into the systemic circulation past underventilated alveoli to cause hypoxemia. In a minority of cases hypoxemia can result from apnea caused by several different mechanisms. Although 'near drowning' by definition means that the victim survives the initial hypoxic insult, a number of complications may then ensue, including pulmonary edema, convulsions and infective problems such as pneumonia or sepsis.

Pathogenesis

The majority of people who have near drowning episodes have aspirated either sea water or fresh water. The resulting lung damage produces inflammation and edema, which damage alveolar defense mechanisms and enhance the risk of infection. The relatively anaerobic conditions may also favor infection. Infecting organisms may include those already colonizing the lungs or upper airways, which have been carried distally with the aspiration and have then taken advantage of improved conditions for growth. Alternatively, organisms in the aspirated water may give rise to infective problems. Finally, an ill patient who has lung damage may be admitted to hospital (and to an intensive care unit) and therefore be exposed to all the risks of nosocomial pneumonia.

Microbiology

The literature on the microbiology of near drowning consists mainly of single case reports rather than large-scale reviews but some common themes do emerge. Organisms that have been implicated are shown in Table 96c.1 , and these can be divided into those that are characteristically associated with pneumonia (either community-acquired or nosocomial) and those that are more specifically associated with immersion incidents. Gram-negative organisms predominate in the aquatic environment (both sea water and fresh water) but anaerobic organisms and *Staphylococcus* spp. can also be found. There may be some organisms that are more likely depending on whether immersion took place in sea water or fresh water and depending on whether the water was clean or contaminated. Certain organisms may be more common in particular geographic areas. For example, one might anticipate exposure to *Burkholderia pseudomallei* following a near-drowning episode in the paddy fields of South East Asia (Chapter 175).

Several cases of infection with *Aeromonas* spp. exist in the literature and these are associated with a high proportion of positive blood cultures and a high mortality. Fungal infections can also cause problems and there are reports of *Aspergillus* pneumonia and disseminated aspergillosis after immersion incidents. *Pseudallescheria boydii* is also reported. Infection is commonly polymicrobial.

Clinical features

The clinical features of infection after near drowning are similar to those seen when the particular infection arises from more conventional causes and depend on the site of infection. The main complication is pneumonia (as might be predicted from the portal of entry) but there is often an associated bacteremia, which may produce clinical features of sepsis. There have also been case reports of meningitis after near drowning.

TABLE 96.c-1 -- Micro-organisms implicated in pneumonia or sepsis after near drowning.

MICRO-ORGANISMS IMPLICATED IN PNEUMONIA OR SEPSIS AFTER NEAR DROWNING
Conventional respiratory pathogens (including atypical organisms and those associated with nosocomial pneumonias)
Staphylococcus aureus
Haemophilus influenzae
Streptococcus pneumoniae
Escherichia coli
Pseudomonas spp.
Moraxella spp.
Klebsiella spp.
Legionella spp.
Pathogens specifically related to immersion
Aeromonas spp.
Pseudomonas putrefaciens
Francisella philomiragia
Chromobacterium violaceum
Burkholderia pseudomallei
Vibrio spp.
Pseudallescheria boydii
Aspergillus spp.

Noninfective pulmonary edema is a common complication of near drowning and can progress to full adult respiratory distress syndrome. Pulmonary edema can be difficult to distinguish clinically and radiographically from pneumonia. In one series of 125 near drowning episodes, the incidence of pulmonary edema was 43% whereas the incidence of pneumonia was 14.7%. These figures are sensitive to changes in case definition, and clearly many patients who initially have pulmonary edema may subsequently go on to develop pneumonia, which tends to be a later complication.

Most patients who have pneumonia have fever (although recognition of this may be confounded if there is any residual hypothermia from the immersion). They may have clinical features of pulmonary consolidation or edema, or both.

Investigations

Near-drowning victims should have a chest radiograph on admission and this may well be clear or show nonspecific shadowing. They should also have a full blood count and arterial blood gas analysis. It is unlikely that an asymptomatic patient who has normal arterial blood gases and chest radiograph will develop any pulmonary complications. Leukocytosis is usual in patients who have pneumonia but is not specific for infection.

Pulmonary secretions must be examined microbiologically; these may include expectorated sputum or tracheal aspirates in intubated patients. There may be pus cells in the samples and it is common to find infecting micro-organisms by stain and by subsequent culture. Blood cultures must always be taken because there is a high rate of bacteremia. Empyema may develop later in the natural history, necessitating pleural aspiration.

Management

Patients who have survived a near drowning episode require emergency evaluation to determine whether they are at risk of subsequent delayed complications. If they are asymptomatic, with no abnormalities on physical examination and with a normal chest

radiograph, arterial blood gases and full blood count, they can be safely discharged because they are at low risk of pulmonary problems. However, any abnormality on this initial evaluation should prompt hospital admission for observation. The level of monitoring required depends on the clinical status and may include serial arterial blood gas analysis or oxygen saturation monitoring, serial full blood counts and chest radiographs in addition to frequent clinical evaluation.

If hypoxemia is present, supplemental oxygen should be given. If this does not correct the situation, it may be necessary to admit the patient to an intensive care unit for further respiratory support.

In common with many other intensive care situations, there used to be a widespread practice of administering glucocorticoids to patients who had undergone aspiration. There has never been evidence of benefit in near-drowning incidents and this practice is not recommended.

Antibiotics

Prophylactic antibiotics have been shown of no benefit in at least one study and their use is not recommended. However, there should be a low threshold for instituting antimicrobial therapy if there is any suspicion of developing pneumonia or sepsis (Table 96c.2). Features giving rise to concern include deteriorating arterial blood gases, new infiltrates on chest radiograph, hemodynamic disturbance or the development of fever or leukocytosis. It is likely that antibiotics will have to commence before any microbiologic information is available from the laboratory (although initial Gram stains may be helpful). Therefore, broad-spectrum empiric cover with good pulmonary penetration is indicated.

Numerous antibiotics have been used, including aminoglycosides, monobactams, carbapenems, cephalosporins and extended-spectrum penicillins (with and without ß-lactamase inhibitors). There are no large-scale trials to guide rational therapy. I suggest the use of clindamycin, which has good penetration and will provide good Gram-positive cover as well as treating anaerobic infection. This should be combined with ciprofloxacin to cover the Gram-negative organisms and also provide some cover against *Legionella* spp. Other reasonable combinations would be ticarcillin-clavulanate with gentamicin and ceftazidime with metronidazole, although neither of these two regimens offers cover against *Legionella* spp. Clearly the

TABLE 96.c-2 -- Antibiotic regimens for pneumonia and sepsis associated with near drowning.

ANTIBIOTIC REGIMENS FOR PNEUMONIA AND SEPSIS ASSOCIATED WITH NEAR DROWNING	
Dose for average adult patient	
Clindamycin	900mg q8h
Ciprofloxacin	400mg q12h
Ticarcillin-clavulanate	3g q6h
Gentamicin	5mg/kg/day
Ceftazidime	2g q8h
Metronidazole	500mg q8h
All these antibiotics are administered intravenously.	

initial regimen may need to be modified in the light of subsequent information from the microbiology laboratory, but it is important to remember that polymicrobial infection is common. If there is no adequate response, it may be necessary to consider the use of antifungal treatment.

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96.d Initial management of a suspected outbreak of smallpox

Andrew W Artenstein

Introduction

Smallpox, the human disease caused by infection with variola virus, was a worldwide scourge for thousands of years, recognized from the inception of recorded history. The disease accounted for more deaths than any other epidemic disease in history, and its impact on the course of human civilizations has been extensive and well documented. Following an intensive campaign from 1966–77 by the World Health Organization (WHO), smallpox was certified as 'eradicated' from the world in 1980, although viral stocks were officially deposited in the former Soviet Union and at the CDC in Atlanta. There have been persistent concerns about the availability of these stocks outside of their presumed secure internment.

The clinical occurrence of even a single case of smallpox would be pathognomonic for bioterrorism because natural disease no longer occurs and there is no known animal reservoir for the virus. The intentional re-introduction of variola virus would be an international public health crisis of massive proportions for the following reasons:

¦ case fatality rates were historically 25–30%;
¦ the virus is efficiently transmitted person to person among close contacts in an amplified fashion, with the potential for air-borne transmission over longer distances;
¦ most of the world's population are susceptible hosts, either because vaccination against smallpox ceased in most areas more than two decades ago or because of waning of previous vaccine-induced immunity; and
¦ vaccine supply is currently limited and there are no antivirals proven to be effective against this pathogen.

Clinical features

After an average incubation period of 10–12 days, a 2- or 3-day prodromal illness ensues characterized by the abrupt onset of high fevers, chills, malaise, prostration, headache, backache and vomiting. The temperature defervesces concurrent with the appearance of enanthema involving the oral mucous membranes and, a day later, by a macular rash that begins on the face and extremities and becomes papular over a 1–2-day period and subsequently vesicular over an additional 1–2 days, rapidly becoming generalized. All the lesions are generally present by day four of the eruption and evolve into umbilicated pustules over the next few days. The rash is typically centrifugal, not only in onset, but it remains denser peripherally than centrally (Fig. 96d.1) and involves the palms and soles Fig. 96d.2). Additionally, the lesions are typically synchronous (i.e. at similar stages of evolution and appearance). This distribution and appearance help to distinguish ordinary-type smallpox (variola major) from other eruptive illnesses (see Table 6.3 , Chapter 6).

The vesicles and pustules of smallpox are described as 'shotty', almost nodular, epidermal lesions. By the second week of the rash the lesions begin to crust and the scabs begin to separate, a process that is complete by day 21. The period of infectiousness

Figure 96.d-1 Typical centrifugal distribution of the rash in smallpox. *Courtesy CDC and Dr Paul B Dean.*

Figure 96.d-2 Patient with smallpox, Kosovo, Yugoslavia epidemic, March and April 1972. The scabs will eventually fall off leaving marks on the skin that will become pitted scars. The patient is contagious until all scabs have fallen off. *Courtesy CDC and Dr William Foege.*

extends from the onset of the enanthema to the complete separation of all scabs, although most transmission occurs during the first 7–10 days of illness, when virus is replicating to high titers in the oropharynx.

Variola major, 'ordinary' type of smallpox, traditionally accounted for nearly 90% of cases; the remaining cases during epidemics were generally distributed among a few different forms of the disease. 'Modified' type is a milder form more commonly noted in previously vaccinated individuals and less likely to be fatal. The malignant or 'flat' type of smallpox is characterized by the slow progression of flattened vesicular lesions that coalesce and is associated with death in the vast majority of cases. Historically, hemorrhagic smallpox accounted for less than 3% of cases but was associated with rapid progression to death in nearly 100%. Although pregnant women appear especially vulnerable to this form, it is likely that other forms of immune suppression may predispose to it. Both malignant and hemorrhagic smallpox pose difficult diagnostic dilemmas.

Diagnosis

The possible diagnosis of smallpox is suggested by clinical features and mandates the immediate institution of isolation procedures with contact and air-borne precautions and prompt notification of public health officials. The public health authorities will be essential in orchestrating an effective community-wide response to a smallpox outbreak. This will necessitate:

¦ coordination between hospitals and emergency personnel;
¦ prompt dissemination of vital health information for the public; and
¦ co-operation with military and law enforcement official investigations.

Epidemiologic information will be helpful in secondary cases or once a known outbreak is underway; however, early cases presenting before a bioterrorism event is recognized may be missed unless clinicians consider the diagnosis.

Recently immunized health care providers adhering to air-borne and contact precautions should obtain blood, aspirates from vesicular or pustular fluid and scrapings of crusts and skin lesions. This will generally require collaboration with public health officials because these specimens must be processed by designated laboratory facilities with high-level containment capabilities. Diagnostic assays for variola virus include electron microscopy, immunohistochemical analysis of viral antigens, or polymerase chain reaction for viral genetic sequences. Confirmation is obtained by viral isolation on chorioallantoic membranes.

Management

The initial management of a suspected case of smallpox involves immediate institution of appropriate infection control precautions, contact tracing, strategic deployment of pre- and post-exposure vaccine, and possibly the use of antiviral agents. A patient who has suspected smallpox must be placed in a negative-pressure respiratory isolation room. Contact and air-borne precautions are necessary; most transmission occurs between close contacts but in selected circumstances the virus is capable of longer distance dissemination via aerosol suspension. Standard N-95 masks (95% efficiency, small particle, filter masks used for prevention of

tuberculosis transmission) are widely available and useful to prevent transmission to health care workers.

If the number of suspected smallpox cases in an institution exceeds the number of negative-pressure rooms, cohorting may be necessary. In extraordinary circumstances portable high-efficiency particulate air filtration units with ultraviolet lights should be used. Overflow of patients in a massive outbreak will require coordination and assistance with the public health authorities.

Access to suspected smallpox cases should be limited. Clothing, linens and equipment in contact with the patient are considered to be contaminated and must be autoclaved or incinerated after use. Diagnostic specimens and body fluids must be collected and handled using rigorous biosafety precautions under the guidance of proper public health authorities.

Pre-exposure vaccination using live vaccinia virus is highly effective in inducing immunity against smallpox. Immunity after primary vaccination appears to wane after 5–10 years, a phenomenon also noted historically in those having experienced natural infection. There is, however, immunologic evidence that multiply revaccinated individuals maintain immunity for more than 30 years. In the event of confirmed or a highly suspected case(s) of smallpox, pre-exposure vaccination of healthcare and laboratory workers within an institution would be indicated.

Post-exposure immunization, using the ring vaccination and containment strategy, has shown proven effectiveness in controlling the spread of infection. Based on this strategy, persons exposed to an aerosol release of agent, those with face-to-face or household contact with an infected individual, or those caring for infected individuals should receive smallpox vaccine. Vaccination within 4 days of exposure may attenuate disease course, prevent death or prevent disease altogether.

Smallpox vaccination is generally contraindicated in immunocompromised individuals, pregnant women and those with eczema or other exfoliative skin disorders due to the high potential for complications. Additionally, vaccination is relatively contraindicated in close personal contacts of those in these risk groups. In the event of mass casualties related to bioterrorism the risks would need to be weighed against the potential benefits of vaccinating high-risk groups.

A number of serious complications have been described in association with smallpox vaccine. These include:

- postvaccinial encephalitis, a rare and potentially fatal neurologic syndrome;
- progressive vaccinia, a frequently fatal complication in immunocompromised recipients;
- generalized vaccinia, usually self-limited in primary vaccines;
- eczema vaccinatum, a severe dissemination of the vaccine virus in patients with active or previous eczema; and
- accidental infection, involving either auto-inoculation of virus from the skin lesion or transmission via close contact to household members.

A large, national survey reported at the end of the vaccine era in the USA noted 1254 complications and one fatality per million primary vaccines in 1968. These rates would likely be higher today given the increased prevalence of immunocompromising conditions and eczema. Vaccinia immune globulin (VIG), a preparation of pooled antibodies from hyperimmune individuals, is available in limited supply and may be beneficial in the management of selected vaccine complications.

Specific treatment of patients with smallpox involves:

- supportive care:
- the administration of fluids;
- adequate nutrition; and
- possibly systemic antimicrobial agents to treat secondary bacterial infections that may occur with the disruption in skin or mucosal integrity.

Recent data have demonstrated that cidofovir, currently licensed for the treatment of cytomegalovirus infections in humans, protects animals from lethal aerosol challenges with related orthopoxviruses. Despite the absence of direct efficacy data in humans, the use of this agent to treat smallpox should be considered an experimental option. VIG has no proven efficacy in the management of smallpox.

Conclusion

The initial management of a suspected outbreak of smallpox involves controlling the spread of the infection among susceptible hosts. Central to this is the early recognition of disease, followed by the expeditious institution of isolation procedures and the rapid deployment of contact tracing with ring vaccination. The general approach of integrating clinical observations, epidemiologic investigation, preventive actions and treatment strategies in the management of an outbreak is applicable to a wide variety of pathogens.

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96.e Prophylactic antibiotics for animal bites

Patricia Cristofaro

Annually, 1 to 2 million patients with animal bites are treated by US physicians; one out of every two people will be bitten in their lifetime. Wound infection is the most common complication. Other less frequent infectious complications include tenosynovitis, septic arthritis and osteomyelitis. Even more serious problems such as sepsis, meningitis, peritonitis and endophthalmitis result from severe penetrating and crushing injuries. Could any of these sequelae be prevented by prophylactic antibiotics and, if so, who should receive them, under what circumstances and which antibiotics? This practice point will discuss the use of prophylactic antibiotics in patients who have sustained cat, dog or human bites.

Given an accurate estimate of the potential for infection for each unique wound, one could calculate a risk:benefit ratio taking into account antibiotic toxicity/cost and setting a limit to treat when the risk of infection is greater than x%. Such precise data are not available. However, many experiential data and a number of prospective and randomized clinical studies as well as retrospective analyses have attempted to refine the concept of appropriate antibiotic use. Unfortunately, the use of antibiotics to prevent bite wound infection is so ingrained in current medical practice that this aspect of therapy can not be randomized. Wound decontamination, debridement and closure are also variables of clinical care that cannot be adequately controlled. Nonetheless, the following opinions represent a reasonable consensus of current information and practice.

Antibiotic prophylaxis is considered reasonable if the risk of infection is 5–10%. Dog bite wounds carry reported infection rates from 1.6% to 30%; cat bite wounds 15.6–50%; and human bite wounds 10–20% (although these infections may be quite severe due to location and microbiology). For comparison, simple lacerations seen in the emergency room carry an infection rate of 4.5–15.1%. Toxicity outside of frank allergy is unlikely to be significant from a 3–5 day course of oral antibiotics. That stated, how can these wounds be stratified so that those most likely to be problematic receive anticipatory treatment?

Type and depth of wounds; extent of contamination; proximity to tendon, joints and bones or prosthetic joints; potential for functional loss, especially of the dominant hand; and disfigurement are all variables that must be considered. Immunocompromised states of the host because of diabetes, renal or vascular insufficiency, cirrhosis, collagen vascular disease or its therapy, transplant-associated immunosuppression, age and frailty, as well as the potential for the complications of sepsis such as infectious endocarditis of a prosthetic valve, are all complicating factors. Obstruction of lymphatic or venous drainage of the involved body part predisposes to infection. In the absence of statistical data, clinical judgment would tend toward use of prophylactic antibiotics in these circumstances.

A 3–5 day course of antibiotics is now recommended for each of the following conditions:

- wounds seen less than 8 hours after infliction that are moderate or severe, with crush injury or edema;
- those that might involve bones or joints;
- hand wounds;
- cat bites;
- punctures, especially near a joint;
- wounds adjacent to a prosthetic joint; and
- wounds in those with co-morbid conditions that predispose them to serious infections.

Treatment of wounds in these situations should decrease the rate of wound infection from 15–20% down to approximately 5%. Wounds that are seen after 24 hours and are not infected are not likely to become infected.

Which antibiotics should be used? Cultures of the uninfected bite wound are likely to yield the mouth flora of the offending animal but are not predictive of which organism or organisms will cause infection, if any. Antibiotics are chosen on epidemiologic grounds. All bites deemed appropriate for therapy must be covered for *Staphylococcus aureus* and streptococcal species. Cat and dog bites must be covered for *Pasteurella multocida* (cats>dogs); human bites require coverage for *Eikenella corrodens*. Oral Gram-negative rods and anaerobes must also be considered.

TABLE 96.e-1 -- Types of wounds for which antibiotic prophylaxis should be considered (high risk).

TYPES OF WOUNDS FOR WHICH ANTIBIOTIC PROPHYLAXIS SHOULD BE CONSIDERED (HIGH RISK)	
Location	Hand, wrist or foot
	Scalp or face in infants
	Possibly involving bones or joints
	Near a prosthetic joint
Type of wound	Puncture (impossible to irrigate)
	Tissue crushing that cannot be debrided
	Edema less than 8 hours after infliction
Species	Domestic cat
	Human hand bite wounds

TABLE 96.e-2 -- Types of patients for whom prophylaxis should be considered (high risk).

TYPES OF PATIENTS FOR WHOM PROPHYLAXIS SHOULD BE CONSIDERED (HIGH RISK)
Diabetic
Renal insufficiency
Vascular insufficiency
Cirrhotic
Asplenic
Taking immunosuppressive drugs
Age <2 or >50 years
Drainage impairment to affected extremity (venous or lymphatic)
Valvular heart disease
Transplant

TABLE 96.e-3 -- Choice of agents.

CHOICE OF AGENTS

Allergy to penicillin	Preferred prophylactic antibiotic regimen
No	Amoxicillin-clavulanate 875/125mg po bid
Yes	Clindamycin 600mg po tid plus ciprofloxacin 500mg po bid (adults only) or Clindamycin plus TMP-SMX (adults or children)
	If feasible first dose may be given parenterally

Amoxicillin-clavulanate (875/125mg orally q12h) is the treatment of choice for both human and animal bites as it covers *E. corrodens* and most other major pathogens. For penicillin-allergic patients the choice is problematic. No single available agent is effective against all potential pathogens and a combination of agents must be used. Clindamycin plus trimethoprim-sulfa methoxazole (TMP-SMX) or clindamycin plus ciprofloxacin should provide adequate coverage in most situations. If erythromycin alone is used, then the patient will require closer follow-up. The first dose of antibiotic should be given parenterally if feasible, in order to ensure adequate tissue levels. Duration of therapy should be 3–5 days if the wound remains uninfected.

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96.f Management of a health care worker exposed to tuberculosis

E Jane Carter

Tuberculosis (TB) has been a recognized hazard for health care workers (HCW) since the discovery of the contagious nature of TB over a century ago. Patients with active pulmonary tuberculosis — both unsuspected and diagnosed — continue to be a risk to HCWs. Transmission of TB to HCWs has been documented in a variety of health care settings, most commonly in general medical wards but also in operating rooms, autopsy rooms, ICUs, renal transplant units and outpatient HIV clinics. The most common methods by which occupational exposure to TB occurs are either by exposure to the unsuspected case where effective chemotherapy for TB has not had the opportunity to reduce contagion risk or by failure of environmental controls, such as inadequate ventilation or lack of protective masks, to block transmission in identified cases with active TB. In developed countries the former risk is greater while in resource-poor countries, the latter risk often predominates. In low incidence regions of the world where TB is uncommon, the constant vigilance required to consider and actively pursue the diagnosis of TB has lessened over time. This increases the risk that an unsuspected case of TB will go unrecognized and expose HCWs to the possible risk of infection.

Typical case

A 40-year-old US-born patient with insulin-dependent diabetes and chronic renal failure is referred for persistent cough. One year earlier, she was diagnosed with asthma. Treatment with asthma medications resulted in disappearance of her symptoms. Six months later she developed increasing cough, unresponsive to intensification of her asthma regimen. A chest radiograph revealed a right mid-lung field cavity with surrounding infiltration. An outpatient bronchoscopy was performed without specific TB precautions. One day post bronchoscopy the patient experienced fever and dyspnea. She was admitted following a 10-hour wait in the emergency room (ER). Later on the day of admission, her bronchoscopy smear was reported as 4+ acid-fast bacilli (AFB) smear positive. Expectorated sputum was also 4 + AFB positive. Exposed HCWs included the admission clerks, the bronchoscopy nursing staff, the recovery room staff and the ER staff (nursing, physicians, respiratory therapy, maintenance and transport personnel).

Diagnosis/management options

Mycobacterium tuberculosis is spread through the air as droplet nuclei by a source case that aerosolizes the organism generally through cough, sneezing and respiration. The organism may stay suspended — and thus transmissible — in the air for as long as 6 hours. Exposure occurs to all individuals who share the air space with the source case and this exposure risk is delineated by history.

This case was not suspected so appropriate hospital infection control methods — placement in an isolation room, masking of individuals entering the room — were not performed. This is the most common clinical setting for HCW exposure. Risk of infection from exposure varies widely and is determined by multiple factors: contagiousness of the source case, time spent in the infected air space by the susceptible HCW, proximity to the index case, ventilation of the contaminated air space and the immune status of the susceptible host (Table 96f.1). Although a large number of individuals were exposed in this instance, their respective risks of actual infection are not equivalent.

The first step in evaluating the HCW's exposure risk is to evaluate the source case. How contagious is the patient? This evaluation is based on the expectorated sputum. In this instance, the source case was very infectious, with heavily positive smears. In addition, the source case was coughing; cough is an effective aerosolization method. For a proportion of the HCWs exposed, the issue was not just cough but rather a cough-inducing procedure — bronchoscopy. Although it is standard in the USA to mask HCWs for a bronchoscopy, masks are generally removed immediately following the procedure. HCWs remain in the room in which the patient is recovered. Patients are most

TABLE 96.f-1 -- Risk of infection to HCWs once exposed to tuberculosis.

RISK OF INFECTION TO HCWs ONCE EXPOSED TO TUBERCULOSIS	
Directly related to	**Inversely related to**
Contagion status of source case	Ventilation of the infected air space
Effectiveness in aerosolizing the organism (e.g. bronchoscopy vs random cough)	Time since institution of chemotherapy for the source case
Time spent in the infected air space	Distance between HCW and index case
Immune status of the exposed individual	Efficiency of room ventilation and efficiency of face mask

contagious in the hours after the bronchoscopy due to increased cough provoked by residual congestion following instillation of fluid in the form of lavage or washings.

The next step in evaluating the HCWs' exposure risk is to evaluate the time of exposure in the infected air space. The longer the time spent in the infected air space, the greater the risk of infection. Thus, the nurse who spent 45 minutes in the room will be at more risk than the dietary aide who was present for a few minutes only. Analysis of exposure time as well as the intensity of exposure leads to the development of a hierarchy of urgency in performing the subsequent evaluations.

The only test presently available for TB infection is the tuberculin skin test or purified protein derivative (PPD). In many countries, health care facilities are required to have TB screening programs in place. Therefore, HCWs should know their PPD status. Tuberculin skin testing is offered to exposed individuals to assess TB infection. Not every exposed HCW needs to be tested simultaneously; those with the most exposure — as determined by time exposed and/or presence at cough-inducing procedures — should undergo testing first.

It takes 2–10 weeks after infection for the tuberculin skin test to turn positive. Thus, the first or immediate test is to determine if the HCW has been infected by another, possibly unsuspected, exposure since last testing. Prior receipt of BCG immunization by the HCW can complicate the interpretation of the PPD. In general, BCG vaccination in childhood does not affect the interpretation of the PPD in adults, but the receipt of multiple BCG immunizations may cause positive PPDs, particularly at levels surrounding the 10mm cutoff. In these situations the risks and benefits of treatment for possible recent infection should be considered on an individual basis. Further discussion about BCG and its impact on PPDs is found in Chapter 37 and Chapter 233 . The follow-up skin test 10–12 weeks after the exposure tracks this exposure as the cause of resultant infection.

Circles of exposure, based on length of time and intensity of exposure, are performed until the percentage of positive skin tests within the circle meets the incidence of positive skin tests in the community at large. Thus if, in the circle of 2-hour exposure, there are no positive skin tests then it is reasonable to expect that individuals who were exposed to the source case for less than 2 hours are not at risk. If 25% of the individuals in the 2-hour circle show evidence of infection based on a positive skin test than further testing of individuals with lesser exposure must be initiated, e.g. those exposed for 1 hour only (Fig. 96f.1).

HCWs with latent TB infection (LTBI) should be evaluated for TB disease with a chest radiograph and a physical examination. Signs or symptoms of active TB or chest radiographic abnormalities are pursued. For those with a normal chest radiograph and no TB symptoms, treatment of LTBI should be offered. The first 2 years after TB infection is the highest risk period for development of disease; therefore, the risk: benefit ratio of treatment of LTBI is always in favor of treatment in a new infection. There are three LTBI treatment regimens approved in the United States: isoniazid for 6–9 months (9 months is the preferred length of therapy), rifampin/pyrazinamide for 2 months (60 doses) or rifampin for 4 months. The ultra-short course regimen of rifampin/pyrazinamide has been associated with 17 deaths due to hepatitis since its approval; patient selection for this regimen must involve screening for hepatitis risk and active blood surveillance throughout therapy. The choice of rifampin alone is not based on any clinical trial data, only expert opinion. Susceptibility testing of the source case must be checked to verify susceptibility to the drugs being used to treat contacts.

The rifampin or rifampin/pyrazinamide regimen is recommended when the index case is known to harbor an INH-resistant strain of *M. tuberculosis*. The treatment

options are much more limited in the event of exposure to multidrug-resistant (MDR) TB. MDR-TB is increasingly prevalent in eastern European countries and some regions of Asia and Africa. Latent infection with TB from INH and rifampin-resistant strains is generally managed with empirical chemoprevention therapies that include pyrazinamide with ethambutol or a fluoroquinolone such as levofloxacin or ofloxacin. None of these regimens have been shown to be clearly efficacious in controlled clinical trials.

Questions arise regarding recommendations for the immunocompromised HCW due to HIV infection or other medical problems. HIV-infected HCWs exposed to TB should be approached in the same manner as a young child. HIV-infected individuals, like children, may develop disease in an accelerated fashion so they should be quickly evaluated for active disease. If no disease is noted, primary prophylaxis is instituted until the issue of TB infection is determined. LTBI therapy is initiated even before any skin testing results can be obtained and is continued until both (baseline and follow-up) PPDs can be performed. If the HIV-infected HCW is in a circle in which the conversion rate of PPDs is high, consideration should be given to completion of INH therapy regardless of skin test results. If the contact evaluation is performed solely in the context of the work environment, knowledge of the medical conditions of the HCW may not be known; therefore, clear instructions regarding risk should be conveyed to the HCW for discussion with a personal physician in a confidential setting.

If a HCW is known to be PPD positive prior to exposure, treatment decisions are based on the prevalence of TB infection that occurs in the circle of exposure to which that HCW belongs. If the previously infected HCW is in a circle of exposure where the conversion rate is high, the HCW should be considered as reinfected and evaluation for retreatment pursued. If the rate of conversion is low, no further evaluation of the previously positive HCW need be performed.

Following completion of LTBI therapy, no further evaluation need be done. Surveillance chest radiographs for treated — or for untreated — individuals with LTBI are of low yield. Individuals should be counseled regarding the signs and symptoms of active TB; only symptom-driven chest radiographs should be considered.

HCWs cannot be barred from work for failure to participate in programs of screening or for refusing LTBI treatment. Only individuals with contagious TB can be barred from the workplace.

Conclusion

Health care workers are at risk for occupational exposure to TB, especially in low incidence areas where the awareness and necessary active surveillance for TB are difficult to maintain. Risk of exposure is related primarily to the unsuspected case of TB. Risk of infection is based on contagiousness of the source case, extent and length of exposure to the index case and immune status of the HCW. Skin test screening for exposure is based on a circle of contact approach; circles are determined by length and intensity of contact. Treatment of LTBI,

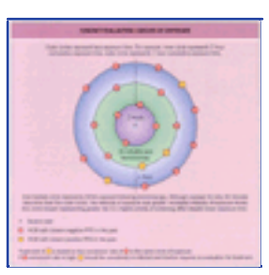

Figure 96.f-1 Contact evaluation: circles of exposure. Circles are constructed based on length of time of exposure and intensity of exposure. The innermost circle represents the highest risk; individuals in this circle are the first priority for screening. Circles of risk are constructed with each having less exposure — and thus less risk — until the PPD conversion rate in a circle is equivalent to the prevalence of LTBI in the local population.

if it occurs, should be offered and is effective in protecting against the development of TB disease.

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96.g Management of a health care worker with chickenpox and the subsequent infection control problem

Katherine N Ward

Definition of the problem

Chickenpox (varicella) is caused by primary infection with varicellazoster virus (VZV). After primary infection, the virus remains latent in sensory ganglia for life. Shingles (herpes-zoster) occurs when VZV reactivates.

In children the complications of chickenpox are sepsis, cerebellar ataxia and, rarely, encephalitis whereas in adults, especially pregnant women and those who smoke, the most common complication is pneumonia which may be fulminating. Neonates and the immunocompromised are at risk of severe disseminated disease. Varicella in pregnancy during the first two trimesters carries a small risk (probably <1%) of congenital varicella syndrome, which is characterized by one or more of the following: microcephaly, microphthalmia, mental retardation, chorioretinitis, Horner's syndrome, limb hypoplasia, contractures and skin scarring.

Chickenpox is very contagious and up to 90% of susceptible household contacts will develop the disease. Transmission is person to person by direct contact, droplet or air-borne spread of vesicle fluid or respiratory secretions. The usual incubation period for varicella is 14–16 days (range 10–21) and the infectious period about a week, i.e. from 2 days before the rash appears until the vesicles dry up. In shingles, VZV is shed from vesicle fluid but not respiratory secretions and is thus less transmissible, especially if covered by clothing. Virus transmission to antibody-negative contacts causing varicella occurs from the day of onset of the zoster rash until crusting of vesicles. In disseminated zoster the patient is infectious for longer, i.e. from 2 days before onset of rash. Finally, contagiousness may be prolonged in the immunocompromised with either varicella or zoster because of continuing virus replication.

Nosocomially acquired varicella is increasingly recognized as a problem. Sources of VZV infection include patients, visitors, especially children, and staff. For susceptible contacts, significant exposure includes sharing a hospital room with an infectious patient or prolonged, direct, face-to-face contact with an infectious person; in each case the type of infection, whether varicella or zoster, and the timing and closeness of exposure must be assessed. A common problem is that of the susceptible health care worker, inadvertently exposed to either chickenpox or zoster, who subsequently develops varicella and may transmit VZV to other staff and patients, e.g. in the antenatal clinic or hematology ward, with consequent morbidity and mortality.

Typical case

A medical student, attached to the hematology unit of a university teaching hospital during his elective, telephones his educational supervisor on a Friday to say that he has developed chickenpox. The rash appeared the day before when he briefly visited the sickle cell anemia outpatient clinic but had felt unwell and returned to his lodgings in the student hostel. On the previous 2 days, i.e. Tuesday and Wednesday, he had been clerking patients on the bone marrow transplant unit. His supervisor contacts the medical school's general practitioner who visits the student later that day and confirms varicella, adding that the patient smokes 20 cigarettes a day. The practitioner also reports that the student did not recall having chickenpox as a child but 2 weeks previously was present when samples were taken from a patient for the diagnosis of shingles.

Diagnostic methods

For the purposes of hospital infection control and timely patient management, laboratory tests should give a rapid result, preferably on the same working day.

Varicella and zoster can be reliably diagnosed clinically. However, where there is doubt, for example if only a few vesicles are present, the diagnosis can be confirmed with the help of the laboratory. The relevant samples are vesicle fluid and cells scraped from the base of the lesion and useful rapid tests are immunofluorescence to detect VZV antigen in cells from the lesion, electron microscopy to detect a herpesvirus in vesicle fluid and PCR to detect VZV DNA in either sample. Tests for VZV IgM or IgG antibody are of little help in diagnosis, although they may sometimes be used to detect the absence of IgG antibody in the acute illness to confirm chickenpox or to exclude zoster.

As regards immunity to VZV, more than 90% of adults are immune although rates of immunity may be lower for adults raised in certain tropical or subtropical areas. A reliable history of varicella is a valid indicator because the rash is distinctive. In contrast, a negative history of varicella is not a reliable test of lack of immunity since the infection may not be recalled or may have been subclinical. Moreover, in the immunosuppressed a past history of varicella does not guarantee the presence of antibodies and hence immunity. Therefore antibody tests are often required to determine susceptibility to infection with VZV. The criteria for assay selection include sensitivity, specificity and the length of time required to obtain results. In particular, the test must be capable of detecting low levels of antibody such as may be found many years after primary infection with VZV. Thus, enzyme-linked immunosorbent assay or latex agglutination tests are appropriate whereas complement fixation tests are not sensitive enough. Assays that are too labor intensive or time consuming include indirect immunofluorescence and neutralization.

Management options

The medical student with varicella

Valaciclovir or high-dose oral aciclovir is indicated, especially in a smoker, and should be given within 24 hours of the onset of rash to reduce the duration and severity of the illness. The student should be warned of the risk of varicella pneumonia and asked to report any respiratory symptoms, in which case he should be admitted to the hospital's infectious diseases ward for assessment and treatment with high-dose intravenous aciclovir. He should not otherwise return to the hospital until all his vesicles are dry and if at all possible he should move to alternative accommodation away from the hostel, e.g. be collected by his parents and taken home. In any case his contacts should be known to be immune to VZV (either by history or antibody positive). Finally, the question as to how this susceptible student was allowed to visit a patient with shingles and help to collect samples for diagnosis should be reviewed. No doubt there was a policy in place that only staff known to be immune should be exposed to VZV but it is all too easy to overlook the occasional visitor such as this elective student.

Susceptibility of contacts

A list should be made of patients and staff in the clinic and on the ward who had significant exposure to the student whilst infectious. Possible contacts in the student hostel should also be investigated. The lists should state whether each individual has had chickenpox or not and whether anyone was pregnant or immunosuppressed. Blood

should be taken for VZV antibody testing from persons with no history of chickenpox and from all immunosuppressed patients so as to determine susceptibility. Those with antibody can be regarded as immune and reassured.

In the event it was found that the student had been in the sickle cell anemia clinic before any patients arrived but had spent 15 minutes having tea and biscuits in the company of the receptionist who has no history of chickenpox and no antibody to VZV.

In contrast, the student had spent all of the previous 2 days on the hematology ward where there were 16 inpatients, all of whom had received a bone marrow transplant for various hematological malignancies. He had been on a ward round and had also visited and taken a history from several of the patients. All 16 patients were tested for VZV antibody and two were found to be seronegative. The student had also been in the ward kitchen for some time with the pregnant ward cleaner, who was not a permanent member of staff but had been supplied by an agency. She had lived in Bangalore, India, for the first 15 years of her life, her English was poor

and she gave an uncertain history of chickenpox. On antibody testing she was susceptible to varicella. Finally in the hostel, two students living in rooms on the same corridor as the student did not recall having chickenpox as children although both were in fact VZV seropositive and hence immune to varicella.

Management of susceptible health care workers

There were two susceptible health care workers, one of whom was pregnant and will be considered later. The other was the receptionist in the sickle cell anemia clinic who should be excluded from patient contact just before the incubation period for chickenpox has elapsed, i.e. 8–21 days after her exposure to VZV. This could either take the form of paid leave or, less realistically perhaps, reassignment to a location in the hospital well away from patients, such as the financial department. A less practical option is to screen daily for skin lesions, fever and systemic symptoms and exclude her from the clinic if she develops varicella. However, on some days of the week bone marrow transplant patients are reviewed in the clinic and this is therefore an especially high-risk strategy. Aciclovir prophylaxis is not recommended as it may not prevent varicella and may prolong the infectious period, but it might be appropriate if on enquiry the receptionist is a heavy smoker. Likewise, the use of human varicella-zoster immunoglobulin (VZIg) for prophylaxis is not an option as it does not necessarily prevent varicella and may prolong the incubation period for a week or more. VZIg is prepared from pooled plasma from blood donors with a history of recent chickenpox or zoster or from those who on screening are found to have suitably high titers of VZV antibody and is usually reserved for neonates, pregnant women and the immunosuppressed. Finally, if varicella develops valaciclovir or high-dose oral aciclovir is indicated.

Management of susceptible pregnant or immunosuppressed contacts

The pregnant ward cleaner and the two bone marrow transplant recipients should be given VZIg to prevent or modify varicella. During the likely infectious period, which may be longer than normal, the member of staff should be managed as described above except that aciclovir prophylaxis and treatment with valaciclovir are both contraindicated in pregnancy. As regards the two bone marrow transplant patients, they should be appropriately isolated, preferably on an infectious diseases unit, and only be cared for by immune staff. Both of these patients were receiving oral low-dose aciclovir to prevent herpes simplex virus infections but there are insufficient data to suggest that this could replace VZIg. If varicella develop high-dose intravenous aciclovir should be given.

Conclusion

The infection control measures described above may seem straightforward but in practice, tracing contacts of varicella and zoster and obtaining blood samples for VZV antibody testing is time consuming and expensive. Excluding potentially infectious health care workers from the hospital is also expensive as they must be replaced by agency staff. Moreover, contacts may be missed because people are unaware they have been in contact or ignorant of the risks. Secondary cases may then occur with further risk of transmission.

In summary, the control of varicella in hospitals will remain a considerable burden unless the pool of susceptibles is reduced. Fortunately varicella vaccine is now licensed in both the United States and the UK and vaccination of all health care workers identified as VZV antibody negative at the time of employment and before any possible exposure in hospital should become the preferred method for preventing nosocomial varicella outbreaks.

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Section 4 - INFECTIONS IN THE IMMUNOCOMPROMISED HOST

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Chapter 97 - Innate and Acquired Host Defenses against Infections

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INTRODUCTION

This chapter focuses on the immune response to infections, mainly concentrating on bacterial pathogens. As examples of different types of infection we primarily focus on three pathogens:

- *Listeria monocytogenes* as an example of an acute infection;
- *Mycobacterium tuberculosis* as a characteristic persisting chronic infection; and
- *Salmonella enterica* var *typhimurium* as a pathogen that falls somewhere between the two.

In such a broad review it is impossible to cover all aspects of immunity to even this limited range of pathogens, and therefore for further details additional reviews are recommended.[1] [2] [3] A functional immune response is paramount for the control of infections, as evidenced by the increased susceptibility to infection resulting from a range of immunodeficiency disorders. Some of these disorders will be referred to in the text of this chapter, and a more complete list is included in Table 97.1 .

INNATE IMMUNE RESPONSES

The innate immune response co-ordinates the initial response to pathogens in a rapid and non-specific way (Table 97.2). The recognition of microbial compounds by cells of the innate immune system generally leads to the production of cytokines and other effector molecules, which either activate cells for the initial control of the pathogen, or promote the development of the acquired immune response. The cells involved in this response include macrophages, dendritic cells and natural killer (NK) cells, which have the ability to recognize and respond to a wide variety of pathogens long before the development of antigen-specific acquired immunity. It is now clear, however, that these early events are critical in determining the nature of the adaptive immune response.

Innate immune mechanisms are critical for the control of pathogens encountered regularly and can be divided roughly into two stages. The initial immediate response occurs within the first 4 hours of the immune response and relies on preformed, non-specific components such as the activation of the complement cascade. Three distinct pathways of complement activation result in the generation of the key enzyme C3 convertase:

- the alternative pathway is activated by specific activation of complement on the pathogen surface;
- the classical pathway requires the formation of antigen-antibody complexes; and
- the mannose binding lectin pathway is activated via the binding of this lectin molecule to mannose residues on the pathogen surface.

Cleavage of C3 by its convertase can result in either direct lysis of the pathogen via the membrane attack complex or opsonization of the organism to facilitate phagocytosis.

A second, slightly delayed response (4–96 hours) has been termed the early induced response and relies on the recruitment of effector cells such as macrophages and neutrophils via the production of chemokines and the activation of these cells by cytokines, which will be discussed below.

Pathogen recognition

Pattern recognition receptors

In order to respond rapidly to the presence of a large variety of pathogens, innate immune recognition is based on the detection of conserved microbial products including bacterial peptidoglycan as well as lipopolysaccharide (LPS) specific for Gram-negative bacteria and lipoteichoic acid (LTA) of Gram-positive bacteria. A characteristic entity for fungi, zymosan, is used while double-stranded (ds) RNA and cognate structures of imidazoquinolines serve as characteristic markers for viral pathogens. These molecules are expressed exclusively by the pathogen and therefore signal the presence of infection. Although these compounds are not identical between all strains, they invariably contain a conserved molecular pattern, which is recognized by the immune system. This is exemplified by LPS, where it is the lipid A portion of the molecule rather than the strain-specific O antigen that activates the innate immune response. These conserved molecular patterns are termed pathogen-associated molecular patterns (PAMPs) and have two important characteristics:

- first, they are produced exclusively by the pathogen and have no host equivalent; and
- second, they are essential for microbial survival.

However, there are no unique differences in PAMPs between pathogenic and nonpathogenic strains of the same species, and how the innate immune system distinguishes this difference is unclear.

The receptors of the innate immune response are termed pattern recognition receptors (PRR), which are germline-encoded receptors including CD14, macrophage mannose receptor and the scavenger receptor of macrophages. Recently the Toll-like receptor (TLR) family have been shown to have an essential function in immunity.[4] [5]

Toll receptors were first identified to play a critical role in immune defense of *Drosophila* against bacteria and fungi, and it is now known that there are at least 10 human homologs, each with a distinct function. The TLRs are transmembrane receptors with a leucine-rich extracellular domain and an intracellular Toll/interleukin (IL)-1 receptor (TIR) domain. To date a wide variety of ligands have been identified for TLRs, but TLRs also exist for which no known ligands have been identified (Fig. 97.1).

It is now clear that TLRs are involved in the detection of and discrimination between an extensive variety of pathogens, and direct differential immune responses accordingly. Thus, TLR2 and TLR6 are recruited to the phagosome containing yeast and Gram-positive bacteria and initiate an inflammatory response, whereas the response to Gram-negative bacteria is mediated primarily via TLR4.[6] More recently, flagellated proteins from both Gram-positive and Gram-negative bacteria have been shown to be recognized by TLR5,[7] while unmethylated bacterial CpG sequences activate cells via TLR9.[8] The demonstration that dsRNA is recognized

TABLE 97-1 -- Diseases resulting from deficiencies in components of the immune system.

DISEASES RESULTING FROM DEFICIENCIES IN COMPONENTS OF THE IMMUNE SYSTEM			
Component	**Disease**	**Description**	**Infections**
Phagocytes	Chronic granulomatous disease	Abnormal NADPH oxidase	*Staphylococcus aureus*
	Chediak-Higashi disease	Deficient degranulation	*Pseudomonas* spp.
	Glucose-6-phosphate dehydrogenase deficiency	Deficient NADPH oxidase	*Escherichia coli*
			Aspergillus spp.
Complement	C1,C4,C2 deficiency	Deficiency of the classical pathway	Encapsulated bacteria
			Streptococcus pneumoniae
	C3, factor H, factor I	Deficiency	Pyogenic bacterial infections
	C3bi, properdin	Deficiency in the alternative pathway	Severe neisserial infections
	C5,6,7,8,9	Deficiency in the membrane attack complex	Meningococcal meningitis
Cytokines	IL-12	Mutation in ß1 chain of IL-12 receptor	*Salmonella* spp
			Mycobacteria spp
	IFN-?	Mutation in IFN-? receptor I	Atypical mycobacterial infections
	X-linked severe combined immunodeficiency (SCID) no T cell function	Mutation in common ? chain of IL-2, 4,7,9,13,15 receptors	Recurrent bacterial and viral infections with all opportunistic pathogens
MHC I/II	Bare lymphocyte syndrome	Defective cell-mediated immunity	Opportunistic pathogens (e.g. *Candida albicans*)
			Cryptococcus neoformans
			Intracellular bacteria
T cells	DiGeorge syndrome	Deletion on chromosome 2	Intracellular pathogens (e.g. mycobacteria)
		Failure in thymic development	
	AIDS	$CD4^+$ T-cell deficiency due to HIV infection	*Pneumocystis carinii*
			Invasive fungal infections (e.g. *Candida albicans*)
	Hyper IgM syndrome	Deficiency in CD40L signaling to B cells.	Pyogenic bacterial infections
		Isotype switching is blocked	*Cryptosporidium parvum*
B cells	X-linked agammaglobulinemia (Bruton's agammaglobulinemia)	Deletion of Bruton's tyrosine kinase	*Haemophilus influenzae*
		Block in pre-B cell maturation	*Streptococcus pneumoniae*
		No circulating B/plasma cells	
		Very limited antibody production	
T/B cell function	Adenosine deaminase deficiency	Accumulation of metabolites within T and B cells leading to impaired proliferation. Total lack of humoral and cellular function.	Recurrent infections with opportunistic pathogens (e.g. *Pneumocystis carinii,* mycobacteria)
	Wiskott-Aldrich syndrome	Deficiency in sialophorin	*Pneumocystis carinii*
			Herpes
			Pyogenic bacteria

by TLR3 and results in nuclear factor ?B (NF?B) activation suggests an additional role for TLR in the early response to viral antigens.[9] Furthermore, it is now apparent that co-operation between TLRs further extends their ability to direct a differential immune response to a variety of ligands. Toll-like receptor 2, in combination with TLR6, can recognize peptidoglycan, whereas TLR2 along with TLR1 interacts with lipopeptides. Interestingly, the cytoplasmic tail of TLR2 cannot induce tumor necrosis factor (TNF) production and requires co-operation with the signaling domains of TLR1 or TLR6 to do so.[10]

Evidence for the role of TLR expressed on cells of the innate immune system being critically involved in the orchestration of the specific immune response has been provided by studies using mice deficient in the signaling adaptor protein MyD88. The MyD88 signaling pathway is used by all TLRs, and a deficiency in this molecule results in a specific block of $CD4^+$ T helper (Th)1 responses; however, Th2 responses appear to remain intact.[11] Note that an additional MyD88-independent pathway exists.[12] In summary, TLR signaling fulfils two major functions:

- ¦ it induces an inflammatory response mediated by proinflammatory cytokines; and
- ¦ it induces expression of co-stimulatory molecules and immunoregulatory cytokines such as IL-12, thus promoting development of interferon (IFN)-? producing Th1 cells (see below).

Ligands for TLR that induce Th1 cells but no inflammation serve as regulatory molecules and are potential adjuvants.

Other receptors

Cells of the innate immune system express additional receptors that are important in the initial interaction with microbial pathogens. In particular the complement receptors (CRs), the receptors for immunoglobulin (FcR) and the fibronectin receptor are exploited by intracellular pathogens to gain access to their chosen host cell. The binding of bacteria to these receptors is mediated by serum components such as complement (C), immunoglobulins and fibronectin. The choice of receptor for entry into the cell can greatly influence the fate of the micro-organism. Thus, IgG opsonized *M. tuberculosis* induces antimicrobial defense mechanisms, whereas entry of complement-opsonized mycobacteria fails to do so. To this end, mycobacteria have evolved a mechanism to promote their uptake via CRs, by their ability to cleave C2 to become the C3 convertase, facilitating the fixation of C3b and entry via CR1 or CR3.[13]

TABLE 97-2 -- A comparison of the innate and acquired immune responses.

A COMPARISOn OF THE INNATE AND ACQUIRED IMMUNE RESPONSES		
	Innate	**Acquired**
Time (h)	0–4 (early)	>96
	4–96 (induced)	
Receptors	Not rearranged	Rearranged
Specificity	Broad specificity	Unique specificity
	Recognition of conserved molecular patterns	Recognition of specific epitopes
Cells	Macrophages	CD4 T cells
	Neutrophils	CD8 T cells
	NK cells	B cells
Soluble effectors	Complement	Cytokines (IFN-?, IL-4)
	Defensins	Antibodies
	Chemokines (IL-8, MIP, MIG)	
	Cytokines (TNF-a, IL-12, IL-6)	
	Interferons (IFN-a/ß)	
Effector mechanisms	Phagocytosis	Complement activation
	Cell recruitment	Cytotoxicity
	Macrophage activation	Pathogen destruction
	Pathogen destruction	

Innate effector functions

Chemokines

Recent interest has focused on a group of small molecular weight cytokines that regulate leukocyte migration. On the basis of a conserved cysteine (C) motif, four subgroups can be distinguished: CC chemokines (e.g. macrophage inflammatory protein (MIP)-1a,ß monocyte chemoattractant protein (MCP-1), RANTES); CXC chemokines (e.g. IL-8, monokine induced by interferon-? (MIG)); the CX_3 C chemokines (e.g. fractalkine); and the C chemokines (e.g. lymphotactin). Related members of these families are recognized by distinct chemokine receptors enabling further systematic grouping. Chemokines not only regulate physiologic leukocyte trafficking, but are also involved in the mobilization of leukocytes at sites of microbial invasion. Similarly, chemokines also attract members of the acquired immune response, namely T cells, to sites of inflammation. Hence, chemokines play a role at various stages throughout the infectious process.[14] [15] [16] [17]

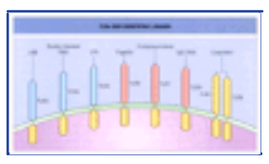

Figure 97-1 Toll-like receptors and identified ligands to date. The identified ligands that bind and signal via TLRs are shown here. Note that TLR2 and TLR6 combine to form a functional heterodimer to recognize peptidoglycan, and probably further combinations of TLRs will be identified, which will increase the diversity of ligands that are recognized. LAM, lipoarabinomannan.

Chemokines including IL-8 and MIP-1a also regulate selective migration of T-cell populations to the inflammatory foci. The chemokines involved bind to glycosaminoglycans on the endothelial surface, thereby attracting lymphocytes to the underlying tissues. Differential expression of chemokine receptors on Th1 and Th2 cells, as well as naive and memory T cells, allows the selective attraction of the required T-cell population.

Tumor necrosis factor-a

Tumor necrosis factor-a is produced by several cell types, including innate and acquired immune cells, such as monocytes, macrophages, neutrophils, mast cells and T cells. It has various pleiotropic effects on the immune system including leukocyte recruitment and macrophage activation. From experiments using mice deficient in TNF-a or its receptors, this cytokine has a proven role in host defense against acute infections such as listeriosis and salmonellosis, as well as in more chronic infections such as tuberculosis. One of its major functions in mycobacterial immunity is in the formation of granulomas, a critical process in which the bacilli are contained within a focus of infection and controlled by activated macrophages and T cells. Although the formation of granulomas is a highly complex process involving many cell types, chemokines and adhesion molecules,[18] the absence of TNF-a results in incomplete granuloma formation and/or disintegration. [19] [20] Treatment of rheumatoid arthritis patients with neutralizing monoclonal antibodies to TNF-a reactivates *M. tuberculosis* infection, illustrating the central role for this cytokine in containing mycobacteria in a dormant stage. [21] However, it must be noted that although TNF-a is required for effective host responses to a variety of pathogens, it is also responsible for many of the pathologic consequences of infection,[22] as discussed below. Its production must therefore be tightly regulated.

In addition to TNF-a, other proinflammatory cytokines are produced, including IL-1 and IL-6, which together with TNF-a induce the acute phase response in the liver. This results in the production of serum proteins such as C-reactive proteins and mannan binding lectin, which can directly, or indirectly via the activation of complement, facilitate phagocytosis. Additionally, these three cytokines act as endogenous pyrogens to raise the body temperature, which produces an unfavorable environment for the pathogen to grow in.

Interleukin-12/interleukin-18/interferon-?

One of the most important cytokine cascades in the early immune response is the production of IL-12 and IL-18 leading to the secretion of IFN-? by NK cells. These cytokines are induced by a variety

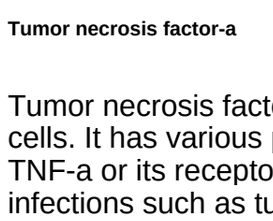

Figure 97-2 Pathways for the generation of the major antimicrobial effector mechanisms of macrophages. This shows the pathways for the generation of ROIs from NADPH oxidase, a process initiated by FcR cross-linking, or exposure of cells to IFN-?. Similar activation can also induce the production of RNIs from L-arginine, catalyzed by inducible nitric oxide synthase (iNOS). Also listed are various examples of bacterial genes or gene products that are known to counteract components of these pathways.

of bacterial products including LAM of mycobacteria, lipoteichoic acid (LTA) and oligonucleotides with an unmethylated CpG motif. The importance of this cytokine pathway has been well illustrated in patients with a mutation affecting IL-12 or IFN-? signalling, who are highly susceptible to infections with mycobacteria and *Salmonella* spp.[23] Interferon-? is the central cytokine required for the activation of macrophages for antimicrobial effector mechanisms outlined below, and the production of all three cytokines is the critical determinant for the development of a CD4 Th1 cell response, as discussed in the acquired immunity section.

Production of reactive nitrogen and oxygen radicals

Neutrophils and activated macrophages restrict the growth of or even kill pathogens that reside inside them. The primary mechanisms for this are the generation of highly toxic reactive oxygen and nitrogen intermediates (ROI and RNI, respectively), which act, alone or in synergy, on the pathogen to inactivate iron-sulfur containing enzymes or to damage DNA (Fig. 97.2).[24]

The production of ROIs is initiated by the activation of the NADPH oxidase following FcR ligation or exposure to IFN-? and LPS. Following further metabolic reactions, which are catalyzed by superoxide dismutase and require iron as a co-factor, oxygen radicals are generated. Reactive nitrogen intermediates are derived from L-arginine in a reaction catalyzed by nitric oxide synthase 2, also known as iNOS. In many cases these two processes are activated in tandem and their respective products synergize for maximum effect.

One interesting example of the requirement for both of these processes is in the control of experimental *Salmonella* spp. infections. Using mice deficient in iNOS and gp96 phox, a component of the NADPH oxidase complex, it was demonstrated that both these mechanisms are required to control infection, but the kinetics of their activation differ. During the first 2–4 days of infection the production of ROIs is critical, while the effects of RNIs are not observed until later in infection, after 7 days.[25]

In mycobacterial infections the roles for RNIs and ROIs have been controversial and differ according to the pathogen investigated. Whereas iNOS-deficient mice were highly susceptible to *M. tuberculosis* infection, they controlled replication of *M. avium* just as efficiently as wild-type mice. Moreover phox-deficient mice showed only a transient loss of resistance to *M. tuberculosis* infection,[26] perhaps reflecting again the distinct temporal requirement for the production of ROIs and RNIs. Although RNI production by human macrophages is low to absent, the critical enzyme, iNOS, has been identified in specimens from patients with tuberculosis. It is therefore likely that RNIs also play a role in human infection although their impact may be smaller.

Antimicrobial peptides

Host cells such as neutrophils and epithelial cells can produce small cationic peptides with antimicrobial activities. These are divided into two families — defensins[27] and cathelicidins [28] — and are induced by microbial components and cytokines such as TNF-a and IL-1ß. Both defensins and cathelicidins have direct microbicidal activity against a wide variety of pathogens including bacteria and fungi. The mechanism of action appears to be perturbation of cell wall membranes via depolarization or permeabilization. As defensins interact with negatively charged components such as LPS, which are not present in host cells, the host is protected from the deleterious effects. In addition to effects on the pathogen, defensins have been demonstrated to potentiate host-acquired immune mechanisms. Increased T-cell proliferation and cytokine production were observed in vitro by the addition of a defensin, while in vivo increased IgG1 and IgG2a levels were seen, as well as the chemoattraction of naive T cells and dendritic cells.[29] Antimicrobial peptides are synthesized as precursors, which must be cleaved before they are active, and therefore host cells must produce enzymes that can perform this task. Neutrophil elastase has been shown to be critical in the activation of cathelicidins and the absence of this molecule severely impairs the host's ability to clear bacterial infections.[30]

Pathogens have evolved ways to counteract the action of defensins. As these antimicrobial peptides are charged they require electrostatic interactions with components of the microbial cell wall such as LPS or LTA. *Staphylococcus aureus* can esterify its LTA, reducing the capacity of the defensin molecules to interact, and while defensins

are generally fairly resistant to destruction by proteases, the extracellular proteases of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* degrade proteoglycans to release dermatan sulfate, which inhibits the action of defensins.[31]

Restriction of nutrients

For intracellular pathogens in particular, the ability to acquire the appropriate nutrients inside the host cell is critical for their survival. Therefore, one potentially effective host defense strategy is to withhold essential compounds from the pathogen. Upon activation of macrophages by IFN-?, there is upregulation of the tryptophan degrading enzyme indoleamine 2,3 dioxygenase, which reduces the levels of tryptophan available within the cell. The absence of this amino acid potentiates the intracellular killing of pathogens including *Chlamydia* spp.[32] and *Bordetella pertussis*. [33]

Perhaps one of the most essential requirements for pathogens residing intracellularly is that of iron. As the host cell also requires iron as a cofactor for the production of ROIs and RNIs, competition for the available intracellular iron pool can markedly influence the outcome of infections with intracellular bacteria. The main pathway for iron acquisition in macrophages is via the transferrin receptor, which is internalized into early endosomal compartments after the binding of iron-loaded transferrin. Once internalized, the lowered pH facilitates the release of iron from its receptor. Most of the iron is transported to the mitochondria for use in the respiratory chain reaction, and excess iron is stored in the cytoplasm bound to ferritin. Limiting the supply of intracellular iron to pathogens is achieved by downregulating surface expression of the transferrin receptor following IFN-? activation, and for *Legionella* spp. this results in the death of the organism due to iron starvation. Therefore, to successfully compete for iron, many pathogens have specialized iron binding molecules called siderophores. For *M. tuberculosis* in particular, which establishes long-term residency within host cells, these molecules are critical, and a deficiency in this iron-uptake mechanism leads to attenuation of the bacteria. Indeed, in conditions of iron overload, either dietary or hereditary, the risk factor for tuberculosis increases significantly.[34] However, as the host also requires iron, it is not as simple as just removing iron from the pathogen. Recent experiments from our laboratory have indicated that chelation of iron exacerbates experimental infection with *Salmonella* spp. due to the inhibition of the NADPH-dependent respiratory burst,[35] illustrating that the availability of iron must be tightly controlled.

Neutrophil-mediated killing

In addition to the production of defensins and other toxic molecules contained within granules, it was assumed that neutrophils kill intracellular pathogens in much the same way as macrophages, that is via the generation of ROIs. However, recent evidence has demonstrated an additional mechanism by which neutrophils contribute to the innate host defense. Upon activation, there is a considerable potassium flux within the cell resulting in the activation of proteases such as elastase. In the absence of these proteases, microbicidal activity is reduced, despite the mechanisms for the generation of ROIs being intact.[36] Elastase in particular has been shown to specifically cleave the virulence proteins of *Shigella*, *Salmonella* and *Yersinia* spp., and an absence of this molecule results in the inability of neutrophils to contain the growth of these bacteria.[37]

ACQUIRED IMMUNE RESPONSES

In contrast to the rapid non-specific innate immune response outlined above, acquired or adaptive immunity relies on the recognition of specific foreign antigens (see Table 97.2). However, these two arms of the immune response are strongly inter-regulated, with events occurring early in infection dictating the direction of the adaptive response, while activation of acquired immune effector mechanisms has a direct feedback on cells of the innate system. The acquired immune response can be broadly divided into:

- T-cell activation and effector mechanisms, a so-called cell-mediated response; and
- the humoral response involving the maturation of B cells and the production of antibodies.

It must be stressed, however, that these two processes are highly interdependent, with B cells able to function as antigen-presenting cells (APCs) for T cells, and T-cell derived cytokines controlling isotype switching (Fig. 97.3).

Cell-mediated immune response

The T-cell response to infectious agents occurs in two consecutive stages. Initially the foreign antigen must be recognized, and this primarily occurs in the lymph nodes and spleen. Following presentation of antigen to naive T cells, they undergo a rapid and substantial proliferation and an increase in the expression of surface adhesion molecules such as selectins and integrins. During this process, the affinity of the adhesion molecules for their specific ligands within lymphoid organs decreases, facilitating the migration and homing of the activated T cells to the sites of infection and inflammation. The major subsets of conventional T cells, namely CD4 and CD8 T cells, undergo principally the same mechanisms of induction and activation, although their effector functions differ to a certain extent. We outline below the specific properties unique to each subset, as well as those for other, unconventional T-cell populations (Fig. 97.4).

CD4 T cells

CD4 T cells are critically important as effector T cells in the host defense against intracellular pathogens in particular. The induction of activated T cells occurs following the recognition of an antigenic peptide complexed to gene products of major histocompatibility complex (MHC) class II on the surface of APCs. Typically peptides are derived from exogenous protein, which are internalized by the APC and cleaved in endosomal/lysosomal compartments by intracellular proteases. Following this, the peptides are loaded onto MHC class II in a specialized MHC compartment and subsequently transported to the cell surface. Prior to the loading of the antigenic peptide, the MHC class II binding groove is occupied by a 23-amino acid peptide called class II-associated invariant chain peptide (CLIP), which is a cleavage product of the invariant chain. The antigenic peptides recognized by CD4 T cells are 15–22 amino acids in length and HLA-DM facilitates the exchange of CLIP for this peptide (Fig. 97.3).

Recognition of the MHC-peptide complex by the T-cell receptor (TCR) on CD4 T cells is itself not sufficient to result in T-cell activation. A second signal is required and this is provided by the interaction between co-stimulatory molecules and their ligands.[38] The major co-stimulation for naive T cells is the interaction between CD40 and CD40L, and the interaction between CD28 and members of the B7 family,[39] including the recently identified inducible co-stimulatory molecule (ICOS).[40] The primary APC for the generation of a CD4 T-cell response is the dendritic cell, which constitutively expresses high levels of MHC class II and co-stimulatory molecules. Macrophages are also efficient APCs, but require activation by IFN-? to reach full processing capacity because they express relatively low levels of MHC class II in the resting state. In such a complex process, there is always the potential that pathogens have developed strategies to interfere with the recognition of their antigens by the host immune system. Indeed, *M. tuberculosis*-infected cells have an impaired capacity to present exogenous antigens due to a downregulation of the synthesis of MHC class II molecules.[41] *Yersinia pseudotuberculosis* produces Yop H, an outer membrane protein that decreases the expression of B7-2 on APCs.[42]

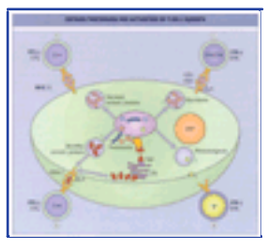

Figure 97-3 Pathways of antigen processing for activation of T-cell subsets. (a) CD4 T cells: within the APCs secreted or somatic antigens are digested by vacuolar proteases, which generate peptides of 15–22 amino acids in length — these peptides are loaded onto major histocompatibility complex (MHC) class II molecules in a specialized compartment before being transported to the cell surface. (b) CD8 T cells: endogenous antigens are cleaved in the cytoplasm by the proteasome to generate peptides of a final length of 8–9 amino acids — these are loaded onto MHC class I molecules in the endoplasmic reticulum (ER) and transported to the cell surface in association with ß$_2$ -microglobulin (ß$_2$ m). (c) CD1-restricted T cells: glycolipid antigens are presented by CD1 molecules on the surface of APC. Recent observations suggest that some processing events are required that involve components of the MHC class II pathway, but that antigen is loaded onto CD1 in the ER, similar to the MHC class I. (d) ?d T cells: to date no processing event or presentation molecule has been identified, but there is some suggestion that the nonpolymorphic Qa-1 molecule can function in antigen presentation. CTL, cytotoxic T lymphocyte; TAP, transporter associated with antigen processing.

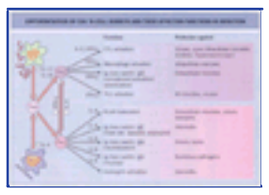

Figure 97-4 Differentiation of CD4 T-helper cell subsets and their effector functions in infection. Under the influence of cytokines produced by dendritic cells (DC) and NK cells, Th0 cells differentiate into Th1 cells (promoted by IL-12 and IL-18) or Th2 cells (promoted by IL-4). These differentiated T cells produce a characteristic pattern of cytokines, which perform various effector functions to eliminate pathogens. The major cytokine produced by Th1 cells is IFN-?, which promotes macrophage activation, critical in the elimination of intracellular pathogens. Th2 cells produce IL-4 and IL-5, which are critical for B-cell maturation and immunoglobulin class switching and hence are important in the control of helminths and extracellular pathogens. Note that via the production of these cytokines, each subset of Th cells can downregulate the differentiation of the other. DC1, myeloid dendritic cells derived from CD14$^+$ monocytes, which produce mainly IL-12; DC2, lymphoid dendritic cells derived from CD4$^+$ precursors, which produce mainly IL-4.

Following antigen recognition, CD4 T cells differentiate into Th cells that are capable of producing large amounts of cytokines (see Fig. 97.4). Murine Th cells were initially subdivided into Th1 and Th2 cells according to which cytokines they secreted. Thus, Th1 cells are characterized by the production of IFN-? and lymphotoxin-a, whereas Th2 cells secrete IL-4, IL-5, IL-6 and IL-13. In recent years other molecules including cytokine and chemokine receptors and transcription factors have provided additional markers to distinguish these cell types[43] and it is now clear that a similar dichotomy of CD4 T-cell subsets occurs in humans.[44]

The differentiation into polar subsets is determined by the initial encounter with the pathogen during the innate phase. Generation of Th1 cells is promoted by IL-12 and IL-18 produced by macrophages and dendritic cells in response to pathogens. These two cytokines are induced by a variety of microbial compounds, including LTA from Gram-positive bacteria such as *L. monocytogenes*, LPS from Gram-negative bacteria including *Salmonella* spp. and LAM from mycobacteria. In contrast, the central cytokine for the promotion of Th2 cell development is IL-4, although to date the precise cellular source for this cytokine is unclear. Once sufficient Th2 cells have

been generated, the autocrine IL-4 production can act as a growth factor and promote further differentiation. As described above, recognition of microbial PAMPs by TLRs are critical early events in the induction of immune responses to infectious agents driving the acquired immune response toward the Th1 pole. Consistent with this notion, mice deficient in MyD88 central to TLR signaling cannot mount Th1 responses to microbial pathogens.[45]

Such a polarization of the CD4 T-cell response obviously has an effect on the subsequent immune response. The production of IFN-? by Th1 cells is critical for the activation of macrophages for antibacterial effector mechanisms such as the generation of RNIs and ROIs and the downregulation of the transferrin receptor. Therefore, a Th1 response is considered to be optimal for resolution of infections with intracellular bacteria. In contrast, Th2 responses and the production of IL-4 and IL-5 promote the development of the humoral arm of the immune response, directing the differentiation of B cells into antibody-producing plasma cells secreting IgE and IgA. Consequently, a Th2 response predominates for the control of helminth infections and is critical for the production of antibodies that neutralize toxins. One of the best examples of the T-cell dichotomy in human disease is the host response to infection with *Mycobacterium leprae,* where Th1 responses characterize the tuberculoid form of the disease leading to the activation of macrophages, few detectable organisms and the formation of granulomas. In contrast, toward the lepromatous end of the disease spectrum, a Th2 cell response results in increased levels of IgE and IgG1, little granuloma formation and increased numbers of bacilli.

CD8 T cells

Like CD4 T cells, CD8 T cells also recognize peptide antigen in the context of MHC molecules on the surface of APCs. However, these are generally (with some exceptions discussed below) of cytoplasmic origin, either self-peptides or those generated from pathogens that reside in the cytoplasm of cells such as viruses or intracellular bacteria, such as *L. monocytogenes*. For CD8 T-cell activation, peptides are presented in the context of classical MHC class Ia molecules, which, in contrast to the relatively restricted expression of MHC class II, are expressed on almost every nucleated cell. Stable surface expression requires noncovalent association with ß$_2$ -microglobulin. Peptides for binding to MHC class Ia molecules are generated by the proteasome, a 28-subunit complex located in the cytoplasm (see Fig. 97.3). Peptides of 5–15 amino acids in length are generated, which are precursors of the final MHC class Ia binding peptides, which are eight to nine amino acids long. These are transported to the endoplasmic reticulum, where they bind to newly synthesized MHC class Ia molecules in a process involving molecular chaperones such as calnexin and gp96.

Regardless of the antigen-presenting molecule used, following antigen recognition and the appropriate co-stimulatory events, including cytokines produced by CD4 Th cells, CD8 T cells acquire two major effector functions:

- first, as for CD4 T cells they contribute to macrophage activation via the production of IFN-? and TNF; and
- second, CD8 T cells can directly lyse infected cells by one of two pathways — the perforin/granzyme pathway and a second pathway that induces apoptosis via the interaction of Fas and Fas ligand.

The perforin/granzyme pathway is the one now thought to be primarily responsible for the lysis of pathogen-infected cells. Perforin can effect lysis, but its primary role is to create channels in the membrane of the target cell through which cytolytic and bactericidal molecules such as granulysin can be introduced. Indeed, this particular

molecule has direct bactericidal activity against a variety of pathogens and is introduced directly into the bacterial phagosome.[46] The second pathway, which induces apoptosis via the interaction of Fas and Fas ligand, is now believed to be primarily responsible for the control of lymphocyte proliferation and activation.

Due to the nature of the antigens recognized by CD8 T cells, the activation of this T-cell population has been primarily considered to be involved in the control of viral infections and of pathogens that reside in the cytoplasm. Indeed, in the murine model of experimental listeriosis, mice deficient in components of the MHC class I processing machinery or in CD8 T cells themselves are highly susceptible to infection. Using MHC class I tetramers specific for immunodominant listerial antigens it has been shown that CD8 T cells dominate both the initial and the memory phase of infection.[47] The reason for this is the ability of *Listeria* to escape from their initial phagosomal location into the cytoplasm by means of the pore-forming enzyme listeriolysin. Once in the cytoplasm, listerial antigens are readily accessible to components of the MHC class I processing machinery.

There is now considerable evidence, however, that even pathogens that remain in a phagosomal compartment within the APC can induce a CD8 T-cell response. Thus, despite the obligate requirement for CD4 T cells in the control of *M. tuberculosis* infection described above, experimental evidence from mice lacking classical MHC class I has also supported a critical role for conventional CD8 T cells.[48] Additionally, CD8 T cells recognizing specific mycobacterial antigens in an MHC class I-restricted manner have been isolated from patients with tuberculosis.[49] Similarly, in *Salmonella* spp. infections, CD8 T cells contribute significantly to the control of infection.[50]

CD1-restricted T cells

The nonpolymorphic MHC-related CD1 molecules share some sequence homology with both MHC class I and MHC class II molecules and are expressed on the cell surface noncovalently bound to ß$_2$ -microglobulin. In contrast to protein-derived antigens presented by classical MHC molecules, CD1 present glycolipid antigens to T cells (see Fig. 97.3). [51] The CD1 molecules can be divided into two groups and are discussed separately.

Group I CD 1 molecules (CD1a, b, c) are present in humans but not in mice and are mainly expressed on professional APCs such as dendritic cells and B cells. To date a wide variety of glycolipid antigens, mainly derived from mycobacteria, have been described to bind to group I CD1 molecules for T-cell recognition and activation,[52] although antigens derived from *Haemophilus influenzae* as well as self-antigens can also be presented.[53] [54] In contrast to MHC I and II molecules, the processing requirements for antigens presented by group I CD1 have not been completely elucidated. The T cells that respond to CD1a-, b- and c-presented glycolipids express the a/ß TCR and are either CD8 positive or double negative. As with conventional CD8 T cells they are capable of both cytolytic activity and IFN-? secretion.[55]

Group II CD1 molecules consist of human CD1d and murine CD1.1 and the nonexpressed pseudogene CD1.2. The crystal structure of CD1.1 has been resolved and bears similarity to the MHC class I molecule in that it has a deep, highly hydrophobic antigen-binding groove. It is believed that ligands are bound by CD1 exclusively via hydrophobic interactions. Until recently, the only identified ligand for CD1d, a-galactosylceramide, was not of microbial origin but derived from a marine sponge. There have been controversial publications suggesting that glycosylphosphatidyl inositol moieties of parasites can bind to CD1d for T-cell activation. Recently, our laboratory has identified mycobacterial derived phosphatidyl-inositol-tetramannoside as the first bacterial ligand for CD1d. The T cells that recognize CD1d are termed NK T cells, and are characterized by a TCR consisting of the invariant a chain, Va14Ja281 in

mice and the homologous combination Va24JaQ in humans, together with the expression of the NK1 receptor. Upon activation, these cells rapidly produce large quantities of cytokines such as IL-4 and IFN-?. Bacterial infection rapidly shifts these T cells to Th1 promoting IFN-? production.[56]

?d T cells

In both mice and humans there is a minor population of cells that expresses the ?d TCR. The majority of human, but not murine, ?d T cells respond to bacteria-derived, nonprotein, phosphate-containing antigens.[57] In adult humans, 50% of these cells express a TCR comprising an invariant gamma chain (V?2) and delta chain (d2) and it is notable that, at least in vitro, all T cells expressing this TCR combination can be activated by mycobacterial phospholigands. Thus, if this were to hold true in vivo, then 1–2% of the entire T-cell population would have the potential to respond to infections with mycobacteria. Recent infection experiments with nonhuman primates revealed that profound activation of ?d T cells indeed takes place after *M. tuberculosis* infection.[58] To date no specific processing event or antigen-presenting molecule has been identified for ?d T-cell activation and it is assumed that there is direct activation of the TCR by the ligand. Murine ?d T cells recognize peptides rather than phospholigands, but again the mechanism of activation has not been elucidated. Functionally, ?d T cells produce cytokines such as IFN-? and IL-10 and these cells have been suggested to play primarily a regulatory role by downregulating conventional T-cell responses and limiting tissue damage. ?/d T cells and NK T cells express a marked TCR bias to few antigens of microbial origin, which allows their rapid activation. It is thus conceivable that these T cells perform regulatory functions at the very first encounter with microbial pathogens (Fig. 97.5).

HUMORAL IMMUNE RESPONSES

Humoral immune responses are considered to play a primary role in the counteraction of extracellular bacteria. As these bacteria replicate outside cells, their primary goal is to avoid internalization and killing by cells of the innate immune system. As a result of this the host relies on the activation of complement and the production of specific antibodies to counteract these pathogens. The absence of serum immunoglobulin from patients suffering from Bruton's agammaglobulinemia

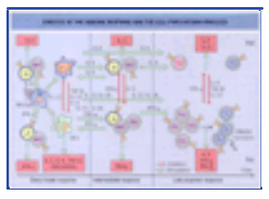

Figure 97-5 Kinetics of the immune response and the cell populations involved. Summary of the kinetics of the immune response to infection, from the recognition of microbial agents that initiates events, through to the generation of an effector T-cell response. The effector cytokines have been colored red for inhibitory and green for stimulatory.

renders them highly susceptible to infections with extracellular bacteria.

Like the induction of T-cell mediated immune responses, specific antibody responses are initiated via the interaction of antigen with a subset of mature B cells in the peripheral lymphoid organs. These B cells express either the IgM or the IgD receptor, and the antigen recognized can be divided into two groups, both of which are present in microbial pathogens.

T-dependent antigens

T-dependent antigens are proteins that are presented by B cells to activate CD4 Th cells. Following binding of the antigen to the membrane immunoglobulin of a specific B cell, it is internalized into endosomal compartments, processed and presented to CD4 T cells as previously described. Following activation, the resulting T-cell derived cytokines heavily influence the type of antibody produced due to their influence on isotype switching. Thus, the production of IFN-? will result in the production of IgG2a and IgG3. Both these isotypes activate the complement cascade to generate C3b and iC3b, and IgG2a can bind to the Fc?R1 receptor. Thus, under the influence of IFN-?, opsonization and phagocytosis of bacteria are promoted, consistent with the role of this Th1 cytokine in the control of many bacterial infections. The production of Th2 cytokines, such as IL-4 and IL-5, promotes the secretion of IgA and IgE. These isotypes cannot bind to FcR or CRs and consequently act primarily to block the adhesion of bacteria to host cells or to neutralize the harmful effects of toxins produced by extracellular bacteria. One example of this is the neutralization of the exotoxin of *Corynebacterium diphtheriae* by high-affinity IgA. These antibodies are also induced following vaccination against diphtheria where modified toxin molecules that retain the receptor binding site but lack the toxic activity are used.

T-independent antigens

T-independent antigens are nonproteins and stimulate antibody production by B cells in the absence of antigen-specific T-cell help.[59] The antibody class induced by these antigens is generally low-affinity IgM, and is the initial antibody produced against all bacterial infections. T-independent antigens are polymeric with repetitive epitopes, which facilitate the cross-linking of multiple immunoglobulin molecules

on the B cell surface. In particular the polysaccharides and glycolipids contained within the cell walls and capsules of many bacteria are particularly effective at inducing IgM responses.

Role of antibody in intracellular bacterial infections

Although it is clear that the major effector mechanisms against intracellular pathogens are those of the T-cell mediated response, there has been renewed interest in the protective role of antibody in such infections. For most intracellular bacteria, *L. monocytogenes* being a notable exception, there is a period of extracellular existence, either before initial internalization or as they are released from one cell to enter another. Thus, under such circumstances they are prone to complement-mediated lysis, and antibody may play a further role in facilitating phagocytosis via opsonization or in preventing entry of bacteria into cells. Experiments using B-cell deficient mice have revealed a role for humoral responses in the control of infections with *S. typhimurium*[60] and a role for Fc regulation of protective immunity in *Chlamydia trachomatis* infections have been proposed.[61] Even for *L. monocytogenes*, which spends relatively little time extracellularly due to its ability to spread directly from cell to cell, it has been shown that an antibody against listeriolysin is protective, and seems to function by neutralizing the activity of listeriolysin preventing the escape of *Listeria* into the cytoplasm.[62] Although such antibodies may be interesting vaccine candidates, apparently they do not contribute to protection during natural infection.

IMMUNOREGULATION

Despite the fact that the induction of an immune response is critical in preventing disease, it must be tightly regulated to prevent immunopathology. Most microbial infections induce an inflammatory response mediated by T cells and cytokines, which if not kept in check can result in tissue damage. The production of TNF-a is a classic example of such a problem — it is critical in the formation of granulomas for the control of intracellular bacteria such as *M. tuberculosis*, and yet is a key mediator of immunopathology when produced in excess, contributing for example to clinical conditions, such as severe sepsis and septic shock, or the adult respiratory distress syndrome that may result in fibrosis of lung tissue, which reduces lung function. Therefore, regulatory mechanisms must be in place to dampen down or switch off the ongoing immune response (see Fig. 97.5). This is mainly achieved by the production of cytokines such as IL-10 and transforming growth factor (TGF)-ß, which inhibit macrophage activation. However, in some cases T-cell activation is so overwhelming that these mechanisms fail and pathology results. One such case is the activation of T cells by so-called superantigens, which bind to the MHC molecule outside the peptide-binding site and activate a much larger population of T cells than processed protein antigen, resulting in the overproduction of T-cell derived cytokines. Thus, the fatal outcome of toxic shock is due to polyclonal T-cell activation by superantigenic exotoxins such as the toxic shock syndrome toxin 1 and enterotoxins of *S. aureus*.

T-cell-mediated immunopathology can also occur following recognition of cross-reactive epitopes between the microbial pathogen and the host — so-called autoimmunity. For example, the inflammatory damage to heart muscle in mice infected with *Chlamydia* spp. is caused by T cells that recognize peptides derived from the outer membrane protein of the pathogen, as well as a heart muscle-specific protein a-myosin.[63]

It is not only uncontrolled or cross-reactive T cells that can induce immunopathology, but also the induction of inappropriate effector mechanisms. This has been implicated in the pathology of leprosy discussed above, and also in infections with *Borrelia burgdorferi* where an inappropriate Th1 T-cell response leads to the development of arthritis. Recently, there has been a renewed interest in a subset of CD4T cells, namely the suppressor or regulatory T cells.[64] These cells have been described by many groups and have various characteristics including the expression of CD25 and the production of IL-10. It remains to be seen whether these T cells eventually turn out to be one population that develops in the absence of Th-cell-derived cytokines, and whose function is to downregulate an inappropriate Th1 or Th2 response.[65] Recent experiments identified CD4- and CD25-positive regulatory T cells in the listeriosis model. During secondary responses, CD8 T cells were markedly downregulated by these regulatory T cells. These findings suggest that pathologic sequelae of exaggerated T-cell responses are prevented by regulatory T cells once the pathogen has been eradicated.

In addition to dysregulated cell-mediated immune responses, immunopathology also commonly results following an uncontrolled humoral response. This is generally manifest as the deposition of immune complexes at tissue sites remote from the site of infection. Poststreptococcal glomerulonephritis can occur following the deposition of antigen-antibody complexes in the kidneys that activate complement and induce tissue damage. Like the situation described for T cells, cross-reactive antibodies can also occur, for example in infections with *Streptococcus pyogenes*. In this case, antibodies against the outer surface M protein of the bacteria cross-react with cardiac myosin proteins and so damage the heart valves. Poststreptococcal rheumatic fever occurs in up to 3% of people if the initial infection is left untreated.

IMMUNOLOGIC MEMORY

A major goal of the immune system is to develop long-lived immunologic protection, such that recovery from the initial encounter with an infectious agent provides an enhanced response that either resolves the infection completely or greatly reduces disease severity. To achieve this there are specialized populations of both T and B cells. Memory B cells and long-lived plasma cells secrete high-affinity neutralizing antibodies[66] whereas memory T cells (both CD4 and CD8) rapidly acquire effector functions such as the secretion of cytokines.

Memory T cells can be subdivided into two groups — central and effector memory T cells. This has been best characterized for the CD8 T-cell population but has parallels in the CD4 T-cell subset. These subsets are distinguished based on their expression of chemokine receptor 7 (CCR7) and L-selection (CD62L). Effector memory cells are located in the peripheral organs where they can rapidly respond to invading organisms. The maintenance of this cell population at these sites is achieved by low expression of CD62L and no expression of CCR7. These cells can rapidly produce effector cytokines such as IL-4 and IFN-?, but relatively little IL-2, and this population contains intracellular perforin. In contrast, central memory T cells home to the lymphoid organs as a result of the expression of CCR7, but produce IL-2 rather than IFN-? or IL-4. Thus, a model has been proposed that suggests that effector memory T cells are the direct progeny of effector T cells generated during the primary response. In contrast, central memory T cells do not acquire effector functions during the primary immune response, but persist in lymphoid tissues to form a reservoir to provide secondary effector T cells upon a second encounter with antigen.[67]

CONCLUSION

The central role of the immune system in the combat of infectious diseases finds increasing support from systematic analysis of the genetic basis for elevated susceptibility of immunodeficiency patients toward infection. Moreover, the realization that different infectious

diseases are preferentially associated with distinct types of immunodeficiency has provided support for the notions that:

- different pathogens have chosen distinct survival strategies in the host; and
- reciprocally that different immune mechanisms are responsible for the control of different infectious agents.

During the past decades, we have learned much from experimental infection studies with mouse mutants with distinct immunodeficiencies concerning the fine tuning of the anti-infective immune response. Transgene expression, knockin and knockout gene mutations — either constitutive or inducible — have all been developed in this species. Frequently, this hypothesis-driven research has proven the predicted role of distinct genes in the control of infection, such as the role of genes involved in IFN-? signaling in resistance to intracellular bacteria. In some cases unexpected results were obtained from infection experiments using mice with a gene deficiency thus far not associated with anti-infective control. Generally, however, meaningful predictions of functions of unknown genes were virtually impossible by this method.

The recent availability of the complete sequence of the human and murine genomes as well as of the genomes of all major pathogens has allowed a quantum leap. Using functional genomics, in particular transcriptome and proteome analyses, the cross-talk between host and pathogen can now be analyzed globally. In this way, the signature imprinted by the pathogen on the host can be elucidated. This will not only lead to the identification of novel genes that are expressed during infection and — by inference — can be considered as participants of the host response. It will also for the first time allow elucidation of co-ordinated gene expression profiles, which will lead to the identification of complete gene expression pathways relevant to susceptibility/resistance in the host. It goes without saying that the reciprocal analysis (i.e. the analysis of the global responses in the pathogen to the host) is equally possible. Although the plethora of data obtained through functional genomics is immense and can only be dealt with by computational bioinformatics, it will provide deep insights into the fine tuning of the host response to diverse pathogens and therefore provide the basis for novel intervention strategies against infectious diseases in normal as well as immunodeficient individuals. Such strategies include both manipulation of the host and the pathogen, such as immune replacement therapies or therapies with novel chemotherapeutic agents, respectively. With increasing incidences of multidrug-resistant strains, novel intervention strategies are urgently needed.

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Chapter 98 - Immunodeficiencies

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INTRODUCTION

White blood cells can be easily classified into lymphoid (T, B and natural killer (NK) cells) and myeloid (neutrophils, eosinophils, basophils and monocytes/macrophages) by virtue of their lineage-restricted progenitor's origin. Defects in these cells lead to defects in their respective pathways of host defense and specific and reproducible patterns of infection susceptibility. Therefore, the study of genetic immunodeficiencies is fundamentally about understanding the mechanisms of resistance to infection.

MYELOID CELLS AND DEFECTS

Mature neutrophils develop in the bone marrow from a myeloid stem cell over about 14 days. Mature neutrophils spend only 6–10 hours in the bloodstream before exiting by diapedesis to sites of inflammation.[1] Myeloid disorders can be generally divided into quantitative and functional disorders. Neutrophilia (>7500 neutrophils/ml in adults) is typically dependent on causes extrinsic to the neutrophils (e.g. acute or chronic infection, steroids, epinephrine (adrenaline)). On the other hand, neutropenia (mild: <1500 neutrophils/ml; moderate: 1500–1000 neutrophils/ml; severe: <500 neutrophils/µl) can be intrinsic or extrinsic to neutrophils or their progenitors. Although neutropenia can accompany many immunodeficiencies, the most common cause remains drug-induced (e.g. chemotherapy).

One should suspect a myeloid disorder in patients who have recurrent, severe bacterial or fungal infections. Unusual organisms (e.g. *Burkholderia cepacia, Chromobacterium violaceum*) or uncommon locations (e.g. liver abscess) should always prompt questions about neutrophil integrity (Table 98.1). Severe viral and parasitic infections are not typically increased in these patients and should direct attention to disorders involving lymphocytes or monocytes. Laboratory evaluation should consider the clinical presentation and where the defect is likely to be. Some assays, such as repeated white blood cell counts with differentials or microscopic evaluation of neutrophils, are relatively simple and can readily exclude certain disorders. Assays such as oxidative burst testing, phagocytosis, chemotaxis or flow cytometry are more difficult and few laboratories do them routinely.

SEVERE CONGENITAL NEUTROPENIA

Severe congenital neutropenia (SCN) or Kostmann syndrome (Online Mendeliain Inheritance in Man (OMIM)# 202700 see www.ncbi.nlm.nih.gov) comprises a heterogeneous group of disorders with variable inheritance patterns, that share the common characteristics of bone marrow granulocytic maturation arrest at the promyelocyte or myelocyte stage, severe chronic neutropenia (<200 neutrophils/µl) and increased susceptibility to acute myeloid leukemia. In 1956, Kostmann described a Swedish kindred who had severe congenital neutropenia inherited in an autosomal recessive pattern.[2] Recently, 22 out of 25 patients who have severe congenital neutropenia have been found to have heterozygous mutations in the gene encoding neutrophil elastase (*ELA2*, 19p13.3). [3] Interestingly, mutations in this same gene are also responsible for cyclic neutropenia. The clinical manifestations of this disease appear promptly after birth; 50% of affected infants are symptomatic before the first month of life and 90% within the first 6 months. Omphalitis, upper and lower respiratory tract infections, and skin and liver abscesses are the most frequent infections. Subcutaneous recombinant granulocyte colony- stimulating factor (G-CSF; 5µg/kg per day; range 1–120µg/kg depending on patient response) has dramatically changed the prognosis of these patients, leading to fewer infections and hospitalizations, and increased survival.[4]

Recently, an X-linked form of severe congenital neutropenia (XLN) due to discrete mutations in the Wiskott-Aldrich syndrome protein (WASP) has been identified.[5]

CYCLIC NEUTROPENIA/CYCLIC HEMATOPOIESIS

Although cyclic neutropenia (OMIM# 162800) is an autosomal dominant trait characterized by regular cyclic fluctuations in all hematopoietic lineages, it is only symptomatic because of variations in neutrophils. Neutrophil counts cycle about every 21 days (range 14–36 days), causing bouts of severe neutropenia (<200/µl), which last 3–10 days.[6] Heterozygous substitutions in *ELA2* (neutrophil elastase 2, 19p13.3) have been identified in all families studied.[6] Most patients have clinical manifestations of neutropenia in early childhood. Oral ulcers, gingivitis, lymphadenopathy, pharyngitis/tonsillitis and skin lesions are the most frequently reported findings. Early loss of permanent teeth due to chronic gingivitis and periapical abscesses is common. Bone marrow aspirates during neutropenia show maturation arrest at the myelocyte stage, or, less frequently, bone marrow hypoplasia. Granulocyte colony-stimulating factor lifts both the peak and nadir counts in cyclic neutropenia and dramatically improves quality of life and survival in these patients. [7] Interestingly, infections and hospitalizations appear to lessen naturally with age.

IMMUNE-MEDIATED NEUTROPENIAS

Alloimmune neonatal neutropenia

Alloimmune neonatal neutropenia (ANN) is caused by the transplacental transfer of maternal antibodies against NA1 and NA2, two isotypes of the immunoglobulin receptor Fc?RIIIb, leading to immune destruction of neonatal neutrophils.[8] This problem typically arises in otherwise normal children of apparently normal healthy mothers. The mothers do not express Fc?RIIIb on their own neutrophils, leading to the elaboration of antibodies against Fc?RIIIb expressed on fetal neutrophils to which the mother is sensitized during pregnancy. Antibody-coated neutrophils are phagocytosed and removed from the circulation, leading to neutropenia and infections. These antineutrophil antibodies can be detected in 1 in 500 live births and should be sought in all infants who have neutropenia. Omphalitis, cellulitis and pneumonia within the first 2 weeks of life may be the presenting infections. Detection of neutrophil-specific alloantibodies in maternal serum is diagnostic. Parenteral antibiotics

TABLE 98-1 -- Infecting agents and myeloid defects.

INFECTING AGENTS AND MYELOID DEFECTS		
Disease or syndrome	**Defect**	**Characteristic infections**
Chediak-Higashi syndrome	CHS1/LYST	*Staphylococcus aureus*
		Streptococcus pneumoniae
		Other streptococcal infections
		Haemophilus influenzae
		Gram-negative rods (skin and lung)

Specific granule deficiency	C/EBPepsilon/others	*S. aureus*
		S. pneumoniae
		Other streptococcal infections
		H. influenzae
		Gram-negative rods (skin and lung)
Myeloperoxidase deficiency	MPO	*Candida* spp. (when accompanied by DM)
Leukocyte adhesion deficiency 1	CD18	*S. aureus*
		Pseudomonas aeruginosa
		Gram-negative rods (skin and bowel)
Chronic granulomatous disease	NADPH oxidase	*S. aureus* (skin, liver, lymph nodes)
		Serratia marcescens (lung, skin, bone, sepsis)
		Burkholderia cepacia (lung, sepsis)
		Chromobacterium violaceum (skin, sepsis)
		Nocardia spp. (lung)
		Aspergillus spp. (other filamentous fungi; lung, bone)
Hyper-IgE syndrome (Job's)	Unknown	Primary pathogens:
		S. aureus (lung and skin)
		S. pneumoniae (lung)
		H. influenzae (lung)
		Pneumocystis carinii (lung)
		Secondary pathogens in lung cavities:
		Pseudomonas aeruginosa
		Aspergillus spp.
Interferon-?/interleukin-12 pathway	Multiple	Nontuberculous mycobacteria
		Salmonella spp.
		Mycobacterium tuberculosis
		Some DNA and RNA viruses

and G-CSF should be given; intravenous gammaglobulin may not be effective in reversing ANN. It resolves spontaneously with the waning of maternal antibody levels.

Primary autoimmune neutropenia

Primary autoimmune neutropenia (AIN) is the most common cause of chronic neutropenia (absolute neutrophil count <1500/µl lasting at least 6 months) in infancy and childhood.[9] It has a slight female preponderance and occurs in about 1:100,000 live births. Antibodies directed against different neutrophil antigens can be detected in almost all patients, almost 85% of which are IgG. Detection of granulocyte-specific antibodies may require repeated testing. Approximately one-third of these autoantibodies are directed against NA1 and NA2. Other antigens include CD11b/CD18 (Mac-1); CD32 (Fc?RII); and CD35 (C3b complement receptor).[10] The average age at diagnosis is 8 months. The majority of patients present with skin or upper respiratory infections but the diagnosis may be incidental, as patients may remain asymptomatic despite low neutrophil counts; severe infections are infrequent. Neutrophil counts are usually between 500 and 1500/µl at the time of diagnosis. The neutrophil count may transiently increase during severe infections, and bone marrow may be normal or hypercellular. The prognosis of primary AIN is good, since it is usually self-limited. The neutropenia remits spontaneously within 7–24 months in 95% of patients, preceded by the disappearance of autoantibodies. Antibiotics for infections are usually sufficient. In severe infections or emergency surgery G-CSF is used.[11]

Secondary autoimmune neutropenia

Secondary AIN can be seen at any age but is more common in adults and has a more variable clinical course. Systemic lupus erythematosus, Hodgkin's disease, large granular lymphocyte proliferation or leukemia, Epstein-Barr virus infection, cytomegalovirus infection, HIV infection and parvovirus B19 infection have been associated with secondary AIN. Antineutrophil antibodies typically have pan-Fc?RIII specificity, rather than to the Fc?RIII subunits, making the resulting neutropenia more severe. Anti-CD18/11b antibodies have been detected in a subset of patients. Secondary AIN responds best to therapy directed at the underlying cause.

DEFECTS OF GRANULE FORMATION AND CONTENT

Chediak-Higashi syndrome

Neutrophil granules house critical enzymes for bacterial and fungal killing, and are mobilized to the phagosome immediately after ingestion of an invader (Fig. 98.1). This intracellular trafficking requires molecular motors, which move granules inside the cell. The Chediak-Higashi syndrome is a rare and life-threatening autosomal recessive disease clinically characterized by oculocutaneous albinism, frequent pyogenic infections, neurologic abnormalities and a relatively late-onset lymphoma-like 'accelerated phase'. Affected patients show hypopigmentation of the skin, iris and hair. The latter is light brown to blonde, with a characteristic metallic silver-gray sheen. Under light microscopy, hair shafts in Chediak-Higashi syndrome show pathognomonic small aggregates of clumped pigment. Giant azurophil granules form from the fusion of multiple primary granules in neutrophils, eosinophils and basophils, but enlarged cytoplasmic granules are found in all granule-containing cells. Mild neutropenia is common as a result of intramedullary destruction of neutrophils.[12]

Chediak-Higashi syndrome is due to mutations in the lysosomal trafficking regulator gene, *LYST* or *CHS1* (1q42.1-q42.2; OMIM# 214500),[12] but the mechanism and pathophysiology of the syndrome are still elusive. Monocyte and neutrophil chemotaxis are diminished. Phagocytosis is normal or increased but bacterial killing is delayed, probably because of low levels of primary granule enzymes. Natural killer cells show very low cytotoxicity but neutrophil and monocyte antibody-dependent cellular cytotoxicity is intact. B cell function is usually unaffected. Progressive involvement of the peripheral and central nervous systems is common, with neuropathy of the legs, cranial nerve palsies, seizures, mental retardation and autonomic dysfunction.

The accelerated phase is one of the main causes of death in Chediak-Higashi syndrome and is clinically indistinguishable from other hemophagocytic syndromes. It is characterized by fever, hepatosplenomegaly, lymphadenopathy, cytopenias, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis and tissue lymphohistiocytic infiltration. Etoposide (VP16), steroids and intrathecal methotrexate (when the central nervous system is involved) have been effective. However, without successful bone marrow transplantation, the accelerated phase usually recurs.

Neutrophil-specific granule deficiency

The CCAAT/enhancer binding proteins (C/EBPs) are transcription factors that guide myelopoiesis and cellular differentiation.[13] Neutrophil-specific granule deficiency is a rare, heterogeneous, autosomal recessive disease characterized by the profound reduction

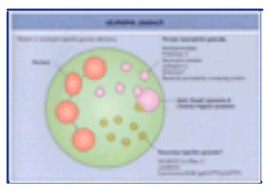

Figure 98-1 Neutrophil granules. Neutrophils contain primary, secondary and tertiary granules, each of which has specific contents that are produced at different points in myeloid ontogeny. The larger, azurophilic primary granules contain a host of proteins, only some of which are listed here. Note that secondary granule deficiency affects both all secondary granule contents as well as the primary granule defensins. Primary granules fuse in Chediak-Higashi syndrome, along with a smaller number of secondary granules, to give the characteristic cell inclusions.

or absence of neutrophil-specific granules and their contents, as well as the primary granule product, defensins (OMIM# 245480).[14] In several cases a homozygous, recessive mutation was found in C/EBPepsilon (14q11.2). However, other cases do not have mutations in C/EBPepsilon, suggesting genetic heterogeneity. These patients have markedly increased susceptibility to pyogenic infections of the skin, ears, lungs and lymph nodes. Neutrophils show bilobed nuclei (pseudo-Pelger-Huët anomaly). Electron microscopy shows absent peroxidase-negative granules in some patients and empty peroxidase-negative granules in others. Staphylococcidal activity may be reduced because of poor phagocytosis but candidacidal activity and superoxide production are normal. Hemostatic abnormalities, due to reduced levels of platelet-associated high-molecular-weight von Willebrand factor and platelet fibrinogen and fibronectin, may occur.

The diagnosis of specific granule deficiency is suggested by the peripheral smear and confirmed by electron microscopy and specific enzyme detection. Eosinophils may not be detectable on routine smears. Management is complicated by poor inflammatory responses. Gram-positive cocci infections are common. Aggressive diagnosis of infection, prolonged and intensive therapy, and early use of surgical excision and débridement are necessary.

DEFECTS OF OXIDATIVE METABOLISM

Chronic granulomatous disease

The nicotinamide-adenine dinucleotide diphosphate (NADPH) oxidase is the enzyme complex required for the generation of super-oxide and its metabolites hydrogen peroxide and bleach (Fig. 98.2). The nascent enzyme complex exists as two groups of components: a heterodimeric membrane-bound complex embedded in the walls of secondary granules, and four distinct cytosolic proteins.[15] The structural components are referred to as *phox* proteins, for phagocyte oxidase. The secondary granule membrane complex is cytochrome b_{558} , composed of a 91kDa glycosylated ß chain ($gp91^{phox}$) and a 22kDa nonglycosylated a chain ($p22^{phox}$), which binds heme and flavin. The cytosol contains the structural components $p47^{phox}$, $p67^{phox}$ and the regulatory components $p40^{phox}$ and Rac. On cellular activation the cytosolic components $p47^{phox}$ and $p67^{phox}$ are phosphorylated and bind tightly together. In association with $p40^{phox}$ and Rac, these

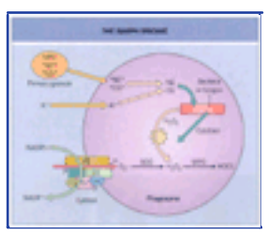

Figure 98-2 The NADPH oxidase. Cellular activation leads to assembly of the nascent NADPH oxidase by joining of secondary granule membrane and cytosolic components. The generation of an intravacuolar charge is rectified by potassium influx, which in turn liberates neutrophil elastase (NE) and cathepsin G (CG) from their associated matrix (**). As classically conceived, an ingested organism is shown degrading its own hydrogen peroxide, thus eliminating a supplement to the defective metabolic pathway in CGD. However, the pathologic relevance of the role of catalase in bacterial or fungal virulence is unclear.

proteins combine with the cytochrome complex ($gp91^{phox}$ and $p22^{phox}$) to form the intact NADPH oxidase. Following assembly, an electron is taken from NADPH and donated to molecular oxygen, leading to the formation of superoxide. In the presence of superoxide dismutase, this is converted to hydrogen peroxide, which, in the presence of myeloperoxidase and chlorine in the neutrophil phagosome, is converted to bleach. Reeves and co-workers[16] have recently shown that phagocyte production of reactive oxygen species facilitates activation of certain primary granule proteins inside the phagocytic

vacuole. This new paradigm for NADPH-oxidase-mediated microbial killing suggests that reactive oxidants are most critical as intracellular signaling molecules, leading to activation of other pathways.

Mutations in any of the structural components of the NADPH oxidase cause chronic granulomatous disease (CGD), a genetically heterogeneous disease characterized by recurrent life-threatening infections, due to catalase-positive bacteria and fungi, and exuberant granuloma formation (OMIM# 306400, 233690, 233700, 233710). The most common genotype involves mutations in the X-linked $gp91^{phox}$ and accounts for about two-thirds of cases.[15] The remainder of cases are autosomal recessive; there are no autosomal dominant cases of CGD. The frequency of CGD in the USA may be as high as 1:100,000. Clinically, CGD is quite variable but the majority of patients are diagnosed as toddlers and young children. Infections or granulomatous lesions are usually the first manifestations.[17]

The lung, skin, lymph nodes and liver are the most frequent sites of infection (see Table 98.1). The overwhelming majority of infections in CGD are due to only five organisms: *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia* spp. and *Aspergillus* spp. Trimethoprim-sulfamethoxazole (co-trimoxazole) prophylaxis has reduced the frequency of bacterial infections in general and staphylococcal infections in particular. On prophylaxis, staphylococcal infections are essentially confined to the liver and cervical lymph nodes. Staphylococcal liver abscesses encountered in CGD are dense, caseous and difficult to drain, requiring surgery in almost all cases.[17] With the great successes in antibacterial prophylaxis and therapy, fungal infections, typically those due to *Aspergillus* spp., are now the leading cause of mortality in CGD.[17] The recent introduction of itraconazole prophylaxis should further reduce the incidence of fungal infection.

The granulomatous manifestations of CGD are particularly troublesome, often involving the gastrointestinal and genitourinary tracts (Fig. 98.3). Esophageal, jejunal, ileal, cecal, rectal and perirectal involvement with granulomata mimic Crohn's disease. Gastric outlet obstruction is common and may be the initial presentation of CGD.[15] Bladder granulomata, ureteral obstruction and urinary tract infection are also common. Steroid therapy is quite effective and surprisingly well tolerated when used for treatment of obstructive lesions.[15] Prednisone

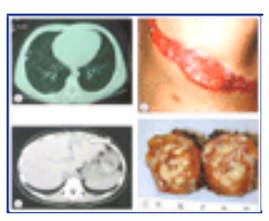

Figure 98-3 Manifestations of chronic granulomatous disease. (a) Pneumonia due to *Aspergillus* spp. can present subtly, both clinically and radiographically. This patient was asymptomatic but had multifocal pneumonia due to *Aspergillus fumigatus*. (b) Wound dehiscence typically presents 5–10 days postoperatively. It is an exuberant granulation tissue on biopsy and is best treated with short courses of corticosteroids. (c and d) Liver abscesses due to staphylococci are quite common in CGD ((c) arrow) and typically require *en bloc* resection because of their dense, granulomatous nature (d).

(prednisolone) given at about 1mg/kg for a brief initial period and then tapered to a low dose on alternate days is quite successful. The frequency of relapse/recurrence of gastrointestinal granulomatous disease is high, so prolonged low-dose maintenance is often necessary. Other therapies for severe granulomatous complications include cyclosporin A and colostomy for refractory rectal disease. Several cases have been treated with infliximab. The latter therapy must still be viewed with caution, as infliximab increases the rates of fungal infection, even in normal individuals.

The X-linked carriers of $gp91^{phox}$ have two populations of phagocytes, one that produces superoxide and one that does not, yielding a characteristic mosaic pattern on oxidative burst testing. Discoidlupus-erythematosus-like lesions, aphthous ulcers and photosensitive rashes have been seen in $gp91^{phox}$ carriers. Infections are not usually seen in these female carriers unless the normal neutrophils are below 5–10%; then, these carriers are at risk for CGD-type infections. [15] [17]

The diagnosis of CGD is made by a measure of superoxide production. Currently, we prefer the dihydrorhodamine (DHR) assay because of its relative ease of use, its ability to distinguish X-linked from autosomal patterns of CGD on flow cytometry and its sensitivity to even very low numbers of functional neutrophils. [15] Immunoblot and mutation analysis are required to identify the specific affected protein and genetic lesion respectively. Male sex, earlier age at presentation and relatively severe disease suggest X-linked disease, but these are only rough guides. Autosomal recessive forms of CGD (mostly $p47^{phox}$ -deficient) have a significantly better prognosis than X-linked disease. In a retrospective voluntary registry, mortality for the X-linked form of the disease was about 5% per year, as compared with 2% per year for the

autosomal recessive varieties.[17] The precise gene defect should be determined in all cases, as it is critical for genetic counseling and is prognostically significant.

Prophylactic trimethoprim-sulfamethoxazole (5mg/kg per day based on trimethoprim) reduces the frequency of major bacterial infections from about once every year to once every 3.5 years without increasing serious fungal infections in CGD. The greatest cause of mortality in CGD in developed countries remains *Aspergillus* pneumonia but itraconazole prophylaxis prevents fungal infection in CGD (100mg daily for patients under 13 years or 50kg; 200mg daily for those over

13 years or 50kg).[18] A large, multinational, multicenter, placebo-controlled study showed that interferon (IFN)-? reduced the number and severity of infections in CGD by 70% compared with placebo regardless of the inheritance pattern of CGD, sex or use of prophylactic antibiotics. Interestingly, no significant difference could be detected in terms of in-vitro superoxide generation, bactericidal activity or cytochrome b levels.[19] Therefore, our current recommendation is to use prophylaxis with trimethoprim-sulfamethoxazole, itraconazole and IFN-? (50µg/m^2) in CGD.

Since the differential diagnosis for a given process in these patients includes bacteria, fungi and granulomatous processes, a microbiologic or histopathologic diagnosis is critical. In severe infections, leukocyte transfusions are often used, although their efficacy is anecdotal. Bone marrow transplantation leading to stable chimerism has been successfully performed in patients who have CGD. Seger *et al.*[20] have reviewed the European experience with bone marrow transplantation for refractory infection, predominantly with *Aspergillus* spp. Horwitz *et al.*[21] performed low-intensity nonablative transplant from HLA-identical siblings into CGD patients. Success was greater in children than adults but transplant-related toxicities, such as graft-versus-host disease, remain problematic. Clinical trials of $p47^{phox}$ gene therapy have shown marking of cells in the periphery for several months but clinical benefit has not been shown, presumably because of the low numbers of corrected cells in the circulation (<0.01%).

Myeloperoxidase deficiency

Myeloperoxidase (MPO; 17q23) is synthesized in neutrophils and monocytes, packaged into primary granules and released either into the phagosome or the extracellular space, where it catalyzes the conversion of H_2O_2 to hypohalous acid (in neutrophils the halide is Cl^- and the acid is bleach). Myeloperoxidase deficiency is the most common primary phagocyte disorder; 1/4,000 individuals have complete MPO deficiency and 1/2,000 have a partial defect[22] (OMIM# 254600). It is an autosomal recessive trait with a variable range of expressivity. Despite in-vitro studies showing that MPO-deficient neutrophils are markedly less efficient than normal neutrophils in killing *Candida albicans* and hyphal forms of *Aspergillus fumigatus*, clinical infection in MPO deficiency is rare. Of the MPO-deficient patients who have had clinical findings, infections due to different *Candida* strains were the most common, and diabetes mellitus appears to be a critical cofactor. [22] Definitive diagnosis is established by neutrophil or monocyte peroxidase histochemical staining or

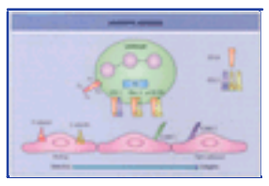

Figure 98-4 Leukocyte adhesion. Neutrophils sample the endothelium in the postcapillary venules through a series of receptors. Depicted here are the selectin and integrin pathways, which are critical for neutrophil adhesion (monocytes and eosinophils have other ligand and receptor options). CD15s binds to selectins on the endothelium with loose adhesion, allowing closer sampling of the endothelium for tight adhesion in the setting of endothelial activation, mediated through the integrins. Neutrophil integrins bind to intercellular adhesion molecules (ICAMs).

specific protein detection. There is no specific treatment for MPO deficiency.

THE LEUKOCYTE ADHESION DEFICIENCIES

For over a century, leukocyte movement from the bloodstream toward inflamed sites has been recognized as critical in preventing and fighting infections. Leukocyte adhesion to the endothelium, to other leukocytes and to bacteria is critical in the ability of leukocytes to travel, communicate, inflame and fight infection. Different families of adhesion molecules mediate these processes, critical among which are the integrins and selectins (Fig. 98.4).

Leukocyte adhesion deficiency type 1

The leukocyte $ß_2$ integrins are heterodimeric molecules on the surface of neutrophils, monocytes and lymphocytes that bind to intercellular adhesion molecules (ICAMs) on the endothelial surface in order to attach and exit the circulation.[23] ICAMs can also be expressed on other leukocytes, allowing for cell-cell adhesion. In addition, certain $ß_2$ integrins can bind directly to pathogens or to complement. Leukocyte integrins are composed of an a chain (CD11a, CD11b or CD11c), noncovalently linked to a common $ß_2$ subunit, CD18. The aß heterodimers of the $ß_2$ integrin family include CD11a/CD18 (lymphocyte-function-associated antigen 1, LFA-1), CD11b/CD18 (macrophage antigen 1, Mac-1, or complement receptor 3, CR3), and CD11c/CD18 (p150,95 or complement receptor 4, CR4). Since CD18 is required for normal expression of the aß heterodimers, recessive mutations in CD18 lead to either very low or no expression of CD11a, CD11b and/or CD11c, with resulting inability to bind to endothelium, each other, certain pathogens or complement opsonized particles (ITGB2, 21q22.3; OMIM# 116920). This disease is known as leukocyte adhesion deficiency type 1 (LAD1).[24]

The severe phenotype of LAD1 is caused by less than 1% of normal expression of CD18 on neutrophils, while the moderate phenotype can show up to 30% of normal. However, patients who have normal CD18 cell surface expression but no functional activity have been described, indicating that functional assays must be performed if the clinical suspicion of LAD1 is high.

Patients who have the severe phenotype of LAD1 characteristically have delayed umbilical stump separation and omphalitis, persistent leukocytosis (>15,000/µl) even in the absence of obvious active infection, and severe, destructive gingivitis and periodontitis

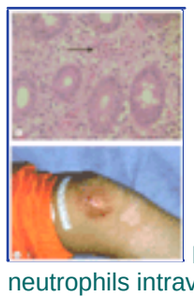

Figure 98-5 Examples of leukocyte adhesion deficiency type 1. (a) A biopsy from the bowel of a patient with extensive inflammation and intestinal ulceration. Note the abundance of neutrophils intravascularly (arrow) but the paucity of neutrophils in the parenchyma. (b) Characteristic dystrophic or 'cigarette paper' scarring following skin ulceration in a boy who has LAD1.

with associated loss of dentition and alveolar bone. Recurrent infections of the skin, lung, bowel and perirectal area are common and usually due to *S. aureus* or Gram-negative bacilli. Necrosis and ulceration without pus accumulation or neutrophil invasion of the infected site are common (Fig. 98.5). Impaired wound healing is characteristic of LAD1; scars tend to be dystrophic and have a 'cigarette-paper' appearance. In contrast to the severe phenotype, patients who have the moderate phenotype tend to be diagnosed later in life, have normal umbilical stump separation, have fewer life-threatening infections and live longer. However, leukocytosis, periodontal disease and delayed wound healing are still common. Aggressive medical management with antibiotics, and surgery when indicated, are requisite. Neutrophil transfusions may be helpful in severe cases but their use is anecdotal.

Complement-mediated phagocytosis is severely impaired because of absence of CD11b/CD18 (CR3/Mac-1) and antibody-dependent cell-mediated cytotoxicity is diminished. However, IgG-mediated phagocytosis is unaffected, as is superoxide production and primary and secondary granule release. At present, bone marrow transplantation is the only definitive corrective treatment. Gene therapy of LAD1 is not yet of clinical benefit in humans.

Other defects in leukocyte adhesion

Selectins are the molecules that mediate the loose, rolling adhesion of neutrophils along postcapillary venules.[23] Leukocyte adhesion deficiency type 2 is a metabolic defect now referred to as congenital disorder of glycosylation type IIc (CDG-IIc).[25] It is a very rare autosomal recessive inherited disease due to mutations in the GDP-fucose transporter (11p11-q11; OMIM# 266265)[26] leading to defective fucosylation of a variety of molecules, most notably the neutrophil ligand sialyl-Lewis^X . Patients have infections of the skin, lung and gums, leukocytosis and poor pus formation, as well as mental retardation, short stature, distinctive facies and the Bombay (hh) blood phenotype. In vitro, LAD2 cells show impaired neutrophil migration, aggregation and adherence to endothelial cells. Fucose supplementation may be helpful.

Defects in endothelial expression of E-selectin[27] and in the Rho GTPase RAC2 (RAC2, 22q12.13-q13.2; OMIM# 602049)[28] also lead to abnormalities in adhesion and increased infections.

INTERFERON-?/INTERLEUKIN-12 PATHWAY DEFECTS

The mononuclear phagocyte is critical for protection against intracellular infections. It mediates antigen presentation, lymphocyte stimulation and proliferation, and cytokine production and response. Mycobacteria infect macrophages, leading to production of interleukin (IL)-12, which in turn stimulates T cells and NK cells to produce IFN-?. Interferon-? increases production of tumor necrosis factor (TNF)-a, IL-12 and other cytokines, as well as mediating mycobacterial killing through unknown mechanisms. Interferon-? signaling depends on the signal transducer and activator of transcription 1 (STAT1), while TNF-a signaling depends on the nuclear factor (NF)?B essential modulator (NEMO; Fig. 98.6). Defects in several critical members of the pathway involving IFN-?, IL-12, TNF-a and their respective receptors and signaling molecules have been clearly identified at the functional and genetic levels as being responsible for infections with mycobacteria, salmonellae and certain viruses.[29] [30]

The IFN-? receptor is composed of ligand binding (IFN-?R1) and signal transducing (IFN-?R2) chains. Autosomal recessive mutations in either chain that lead to abolition of IFN-? signaling have severe infection phenotypes, predominantly with mycobacteria. Patients who have complete defects tend to present early in life, especially if they have received bacilla Calmette-Guérin (BCG) vaccination. They have poor or absent granuloma formation (but normal tuberculin skin tests) and typically develop repeated, disseminated, life-threatening infections due to mycobacteria, salmonellae and some viruses. Mortality is overwhelmingly due to mycobacterial disease. Treatment relies entirely on antibiotic and antiviral therapy. Rare recessive mutations with partial function have intermediate phenotypes and more curable infections.

The most common mutation in IFN-?R1 is due to a four-base deletion at or around base 818 (818del4), located just inside the intracellular domain of the molecule. This mutation allows the protein to remain stuck on the cell surface, where it interferes with the normal product and inhibits signaling[31] (Fig. 98.7). Patients who have this autosomal dominant mutation in IFN-?R1 usually present before age 7 with pulmonary nontuberculous mycobacterial infection but then often go on to develop recurrent multifocal nontuberculous osteomyelitis. Interferon-? signaling persists in this disease, but at a greatly reduced level compared to normal. Interferon-? therapy, sometimes at high dose, can be effective. Long-term prophylaxis against environmental mycobacteria with a macrolide seems prudent.

Mutations in IL-12p40, IL-12 receptor ß1 and STAT1 are typically not as severe as complete IFN-? receptor defects but they also

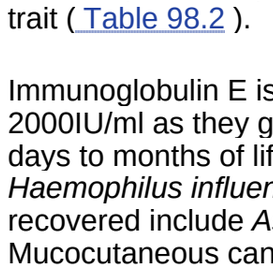

Figure 98-6 Critical cytokine pathways in the control of mycobacteria. Mycobacteria and salmonellae stimulate the elaboration of IL-12 by infected macrophages, leading to the production of IFN-? by T cells and NK cells. IFN-? in turn stimulates macrophages to produce TNF-a and IL-12. The critical signaling molecules STAT1 and NEMO are also indicated. IL-12-independent pathways for IFN-? production are also indicated (IL-15, IL-18), which work in concert with IL-12 for lymphocyte stimulation. Other, as yet undefined pathways are also suggested.

usually present with disseminated BCG, nontuberculous mycobacteria or *Salmonella* infections.[29] [30] Because these defects have preserved IFN-?R function, IFN-? can be used therapeutically, in addition to antimycobacterials.

Immune signaling, through the receptors for IL-1, IL-18, TNF-a, CD40 and the Toll-like receptors, and signaling for ectodermal (teeth, hair, sweat gland) formation converge at the activation of NF?B, a process dependent on the proper phosphorylation of its inhibitor, I?B. The X-linked gene for NEMO is necessary for this process. Defects in *NEMO* cause ectodermal dysplasia, along with a complex and overlapping set of immunodeficiencies with dysfunction in the innate (Toll-like receptors, TNF-aR, IL-1R) and acquired (CD40, IL-18) immune systems, due to disruption of their common signaling pathway. [32] [33] These patients typically require intravenous immune globulin, because of ineffective immunoglobulin class switching, as well as antibiotics. Macrolide prophylaxis to prevent the acquisition of environmental mycobacterial infection appears prudent.

HYPER-IgE RECURRENT INFECTION SYNDROME

Hyper-IgE recurrent infection syndrome (HIES; Job's syndrome) is a rare autosomal disorder characterized by recurrent infections, typically of the lower respiratory system and skin, eczema, extremely elevated levels of IgE, eosinophilia and abnormalities of the connective tissue, skeleton and dentition (Fig. 98.8). The majority of patients have characteristic facial abnormalities, including a broad, somewhat bulbous nose.[34] Failure of primary dental deciduation, leading either to failure of eruption of secondary dentition or retention of both sets of teeth, is common. Many patients also have abnormalities of bone formation and metabolism, which may result in fractures, scoliosis, kyphosis and osteoporosis. HIES occurs spontaneously in all racial and ethnic groups, and in many cases is transmitted as an autosomal dominant trait (Table 98.2).

Immunoglobulin E is greatly elevated at some point in the life of all patients who have HIES but about 20% have been observed to drop their IgE levels below 2000IU/ml as they get older while retaining their susceptibility to infection. The clinical manifestations of HIES are quite distinct. Eczema usually presents within the first days to months of life. Other early signs include mucocutaneous candidiasis and severe diaper rash. Sinus or pulmonary infections, predominantly with *S. aureus* or *Haemophilus influenzae*, are common, as are postinflammatory pneumatoceles. Otitis media and otitis externa are common. Other pathogens that have been recovered include *Aspergillus* spp., *P. aeruginosa, S. pneumoniae*, group A streptococci, *Cryptococcus neoformans, Pneumocystis carinii* and *C. albicans*. Mucocutaneous candidiasis involving the mouth, vagina, intertriginous areas, fingernails and toenails affects about 50% of HIES patients. Bony abnormalities are frequent, as are pathologic fractures.

A prolonged course of high-dose intravenous antibiotics is required for eradication of infection and to prevent bronchopleural fistula formation and bronchiectasis. Empiric acute coverage should consider *S. aureus, H. influenzae* and *S. pneumoniae*. The former two organisms account for the majority of acute infections. Infection of pneumatoceles and bronchiectatic lung with *P. aeruginosa* and *Aspergillus* spp. is common and can be especially problematic. Most experts use prophylactic antibiotics (e.g. a synthetic penicillin or trimethoprim-sulfamethoxazole) directed at coverage of *S. aureus*.

T AND B CELL IMMUNE DEFICIENCIES

T and B cell immune deficiencies comprise a heterogeneous group of disorders, all characterized by profound impairment in the development or function of the cellular and/or the humoral parts of the immune system. T cells not only directly mediate resistance to

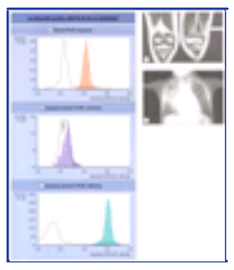

Figure 98-7 In-vitro and clinical aspects of IFN-?R1 deficiency. (a–c) Flow cytometry for the IFN-?R1 on peripheral blood monocytes. Dotted lines indicate the background fluorescence of the sample with an irrelevant antibody. (a) Normal IFN-?R1 fluorescence intensity. (b) Monocytes from a child who has complete IFN-?R1 deficiency. Note the lack of specific staining for IFN-?R1. (c) Increased intensity of IFN-?R1 staining on monocytes from a patient who has MAC osteomyelitis. (d) Chest radiograph from the same patient showing extensive right lung infection with MAC. (e) The corresponding magnetic resonance image of his distal femora (arrow indicates the infected lesion).

intracellular pathogens but also interact with B lymphocytes and antigen-presenting cells in the defense against extracellular pathogens. Consequently, defects in T cell development or function result in a combined immune deficiency with variable defects also seen in the B cell and/or NK cell compartments. Among combined immune deficiencies, the forms with most severe T cell depletion (also known as severe combined immune deficiency (SCID)) lead to increased susceptibility to severe infections from birth and must therefore be considered medical emergencies (Fig. 98.9). Failure to thrive, chronic diarrhea and interstitial pneumonia (often due to opportunistic organisms, such as *P. carinii, Candida* and *Aspergillus* spp., or viruses) are typical clinical features (Fig. 98.10). The overall frequency of combined immune deficiency is estimated to be 1 in 50,000 live births. Lymphopenia should raise suspicion. Quantification of the absolute $CD3^+$ cell count, analysis of human leukocyte antigen (HLA)-DR expression and T cell activation allow identification almost 98% of all SCID babies (Fig. 98.11).

Vaccination with live attenuated viruses or with BCG may lead to fatal disseminated infection (Fig. 98.12). Graft-versus-host disease, due to transplacental passage of alloreactive maternal T cells or transfusion of unirradiated blood products, can cause skin rash, diarrhea, hepatitis or bone marrow failure. If untreated, patients who have SCID die within the first few years of life. Prophylactic trimethoprim-sulfamethoxazole (to prevent *P. carinii* pneumonia) and aciclovir (for herpesviruses), immunoglobulin substitution therapy and aggressive treatment of any and all infectious episodes may prolong survival but only early allogenic bone marrow transplantation or (in selected cases) gene therapy or enzyme replacement therapy offer a chance to cure the disease.

In recent years molecular diagnosis has been achieved for most immune deficiencies (Fig. 98.13) and mutation databases have been established that are constantly updated and accessible via the Internet.[35] This has allowed precise and early diagnosis but has also demonstrated that defects in one gene may result in several different phenotypes (phenotypic heterogeneity) and that the same phenotype can be caused by different gene defects (genetic heterogeneity).

Severe combined immunodeficiency

In typical SCID, severe intestinal infections with malabsorption and failure to grow are prominent. The intestinal mucosa shows variable degrees of villus atrophy. The most frequently isolated pathogens include *Campylobacter, Salmonella, Shigella, Cryptosporidium parvum* and *Giardia lamblia*. The liver is often involved, ranging from hepatomegaly with elevated transaminases, to sclerosing

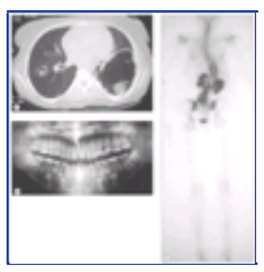

Figure 98-8 Clinical manifestations of hyper-IgE (Job's) syndrome. (a) Computerized tomogram of the chest showing characteristic postinflammatory pneumatocele formation. Note the development of bilateral aspergillomata with inflammation of the cavity walls. (b) A panoramic radiograph of the dentition of a 33-year-old woman who has HIES shows the characteristic retention of primary teeth due to failure of deciduation. (c) Extensive scoliosis is demonstrated in this radionuclide bone scan of a 25-year-old woman who has HIES. A list of some of the characteristic features of the syndrome is included in Table 98.2 .

TABLE 98-2 -- Features of Hyper-IgE syndrome.

FEATURES OF HYPER-IgE SYNDROME	
Feature	**%**
Eczema	100
Characteristic facies (> 16 years)	100
Skin boils	87
Pneumonias	87
Mucocutaneous candidiasis	83
Lung cysts	77
Scoliosis (> 16 years)	76
Delayed dental deciduation	72
Pathologic fractures	57

cholangitis due to *Cryptosporidium parvum*. Central nervous system involvement can also occur, mostly due to viral infections (herpes simplex virus (HSV), enterovirus, echovirus, adenovirus and poliovirus). Since SCID is typically classified by the presence or absence of T cells, B cells and NK cells, its nomenclature is complexly composed of cellular, genetic and molecular names.

$T\text{-}B^+$ severe combined immunodeficiency

X-linked severe combined immunodeficiency

The most common SCID phenotype is absence of mature T (and NK) cells in the blood and peripheral lymphoid organs, with a normal to increased number of B cells ($T\text{-}B^+$ SCID). Most commonly, this disease is inherited as an X-linked trait (SCID-X1, OMIM# 300400). SCID-X1 has an estimated incidence of between 1 in 150,000 and 1 in 200,000 live births and accounts for 35–40% of all cases of SCID. It is caused by mutations in the *IL-2RG* gene located at Xq13. The common gamma chain (?c) encoded by this gene is a cytokine receptor subunit, shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, involved in the maturation of T, B and NK cells[36] (Fig. 98.14). Lacking intrinsic catalytic activity, these cytokine receptors need an intracellular signal transducer, which for ?c is JAK3, a member of the Janus-associated kinase (JAK) family of protein tyrosine kinases. Therefore, absence of ?c leads to marked impairment of signaling for multiple cytokines.

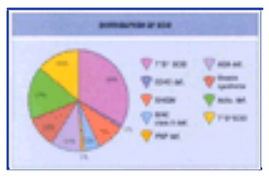

Figure 98-9 Distribution of severe combined immunodeficiency. Distribution of diagnoses in 100 consecutive SCID babies at the Children's University Hospital, Brescia,

Italy.

As in all forms of SCID, clinical onset of SCID-X1 is usually within the first few months of life with persistent diarrhea, pneumonia, failure to thrive and severe or persistent candidiasis. The diagnosis is usually suspected because of lymphopenia, with low to absent T and NK cell counts and normal to elevated B cell counts. Mitogen-induced proliferation is virtually absent and serum immunoglobulin levels are low to undetectable. Serum IgG may initially be normal because of persistence of transplacentally passed maternal antibodies. The thymus lacks a clear corticomedullary demarcation, Hassall's corpuscles are absent and in general there is a severe depletion of lymphoid tissues, with absent lymph nodes.

The diagnosis can be made by immunofluorescence analysis of ?c expression on the surface of lymphocytes or monocytes. However, some *IL2RG* gene mutations allow expression of nonfunctional ?c on the cell surface. Therefore, normal ?c expression does not rule out a diagnosis of SCID-X1. Further complicating matters, engraftment of maternal T cells (observed in as many as 50% of cases of SCID) may lead to an atypical immunologic phenotype, representing a diagnostic challenge. HLA typing and molecular analysis at highly

Figure 98-10 Typical severe combined immunodeficiency baby. Note the wasting and malnutrition.

polymorphic DNA loci can be used to identify the presence of maternal T cells. Other forms of SCID may present with the same cellular phenotype (T-B$^+$ SCID) and should be considered if ?c expression is normal. Detection of a mutation in *IL2RG*, most often inherited from the mother, confirms the diagnosis. Mutations found in different families are listed at http://www.nhgri.nih.gov/DIR/GMBB/SCID/IL2RGbase.html.

The standard treatment for SCID-X1 is allogeneic human stem cell (usually bone marrow) transplantation (BMT) was described in 1968.[37] It achieves success rates near 100% when an HLA-identical sibling is used as donor. Excellent results are also achieved with BMT from matched unrelated donors but, since early treatment is crucial and the search for a compatible donor often takes several months without a guarantee of finding one, haploidentical family donors have been increasingly used since the 1980s.[38] Prevention of graft-versus-host disease by elimination of T cells from the donor marrow or the

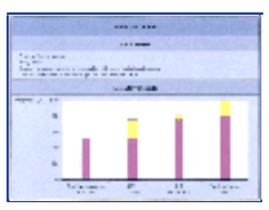

Figure 98-11 Diagnosis of severe combined immunodeficiency. Clinical and laboratory elements useful in the diagnosis of SCID.

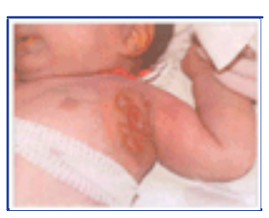

Figure 98-12 Axillary BCGitis in a T-B$^+$ severe combined immunodeficiency baby who has generalized BCG infection. Note the extensive cutaneous ulceration.

use of positively selected peripheral blood stem cells after mobilization is crucial to successful HLA-mismatched transplantation[39] and is now successful in as many as 70–80% of patients. Even in the absence of myeloablation, engraftment of donor T (and often NK) cells is easily achieved. In contrast, engraftment of donor-derived B cells is facilitated by chemotherapy or irradiation conditioning of the recipient. When functional B cell deficiency persists in patients without donor B cell engraftment, regular intravenous immunoglobulin substitution is required.[39] In-utero stem cell transplantation has been successfully performed in affected fetuses with good clinical and laboratory T cell reconstitution. However, this technique can only be applied after prenatal diagnosis.

Because functional ?c expression provides strong proliferative advantage to lymphocyte precursors, SCID-X1 is an ideal model for

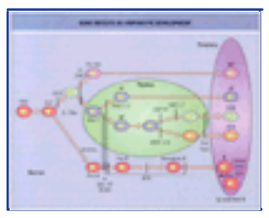

Figure 98-13 Gene defects and lymphocyte development. Model of lymphocyte development with the relative gene defects that may lead to immune deficiencies shown in red (for details see text).

gene therapy. Five patients have been successfully treated and have achieved normal T and NK cells and function after retrovirus-mediated ?c gene transfer into autologous CD34$^+$ bone marrow cells.[40] However, gene therapy is still quite new and may have unanticipated results, including leukemia in some recipients.

JAK3 deficiency

After the X-linked form, autosomal recessive *JAK3* mutations (19p12-13.1) are the second most common form of T-B$^+$ SCID (JAK3 deficiency, MIM#600173).[41] Because ?c and JAK3 are necessary components of the same signaling pathway (Fig. 98.15), JAK3 deficiency may be clinically and immunologically indistinguishable from SCID-X1. Diagnosis of JAK3 deficiency is established by immunoblot for JAK3 in patient lymphocytes or cell lines and/or demonstration of *JAK3* gene mutations. Since JAK3 deficiency is compatible with the presence of significant numbers of circulating, although poorly functioning, T cells, JAK3 deficiency should be considered in all undefined cases of combined immune deficiency, especially those presenting with a high proportion of peripheral B lymphocytes. Prenatal diagnosis, based on DNA analysis of chorionic villus biopsies, has been performed. As is the case for SCID-X1, definitive treatment of JAK3-deficient SCID is allogeneic BMT. *JAK3* gene transfer into hematopoietic stem cells in *jak3* knockout mice has been successful, indicating the possibility for *JAK3* gene therapy in humans. The mutations in *JAK3* are listed at http://www.uta.fi/imt/bioinfo/JAK3base.html.

IL-7Ra deficiency

Interleukin-7 is produced by stromal cells in the bone marrow and in the thymus and provides survival and proliferative signals to IL-7-receptor-bearing (IL-7R$^+$) cells. The IL-7 receptor consists of two subunits, the ?c chain and the IL-7Ra chain, which maps to chromosome 5p13. IL-7Ra is specific for the IL-7R, is expressed by lymphoid precursor cells and is essential for differentiation of early thymocytes. Consequently, mutations that impair expression of IL-7Ra result in an early block in T cell development, leading to SCID with absent T cells but normal (to elevated) B and NK cells.[42] The clinical picture is similar to that of other forms of SCID. Allogenic stem cell transplantation is the only curative treatment.

Figure 98-14 Receptors using the ? chain/JAK3 pathway. The six interleukin receptors that use the common ? chain/JAK3 pathway for signaling.

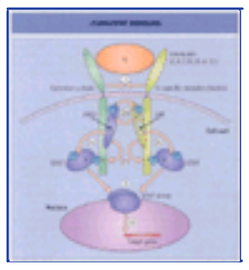

Figure 98-15 ?c/JAK3/STAT signaling. ?c/JAK3/STAT signaling is initiated by binding of the cytokine to its receptor. Heterodimerization (1) of the receptor chains (one IL-specific chain and the ?c) allows for reciprocal tyrosine phosphorylation (2a) of JAK3 and other JAK molecules, leading to their activation. Activated JAKs then phosphorylate (2b) tyrosine residues on both receptor subunits, creating docking sites for the STATs. These signaling elements are themselves tyrosine phosphorylated by the JAKs (2c), in order to dimerize and translocate to the nucleus (3), where they bind to consensus sequences within regulatory regions of cytokine-inducible genes and act as transcription factors driving transcription of the target genes.

IL-2Ra deficiency

IL-2Ra is another member of the IL-2 receptor complex required for IL-2-mediated signaling and peripheral T cell homeostasis. Consequently, mutations in the *IL2Ra* gene lead to an autosomal recessive immunodeficiency with reduced numbers of T cells and a normal number of B and NK cells. Autoimmunity and a progressive lymphoproliferative syndrome are typical, reflecting disturbed peripheral immune homeostasis.[43]

CD45 deficiency

CD45 modulates signaling through the T cell receptor (TCR)/CD3 complex. The immunologic phenotype is one of complete lack of T cells, with normal to increased B cell counts.[44]

T-B- severe combined immunodeficiency

Recombination of DNA in T cells and B cells is required for the generation of the immune diversity that is the hallmark of the mammalian immune system. This process requires specific gene products that cut and recombine DNA as well as specific sites to be acted upon. The genes that control these processes are responsible for phenotypes of SCID causing complete absence of both T and B lymphocytes in the periphery. The majority of these patients have a defect in the recombination process. [45] [46]

RAG1/RAG2 deficiency

To recognize foreign antigens, B and T cells use specialized receptors, namely the immunoglobulin (Ig) receptor and the TCR, respectively. These are characterized by highly polymorphic antigen-recognition sites, which are the coding products of variable (V), diversity (D) and joining (J) gene segments that undergo somatic rearrangement due to a mechanism known as V(D)J recombination. V(D)J recombination is crucial for the differentiation of T and B lymphocytes and is triggered by the lymphocyte-specific proteins recombinase activating gene (RAG)1 and RAG2. These gene products act together to recognize specific recombination signal sequences that flank each of the V, D and J gene elements in the Ig and TCR genes and break the DNA double strand there. Several ubiquitously expressed DNA repair proteins (including Ku70, Ku80, DNA-PKcs, XRCC4, DNA ligase I and IV, and Artemis — the latter involved in another form of SCID) then mediate the final steps of the V(D)J recombination process (Fig. 98.16).

The clinical presentation of RAG-deficient SCID (OMIM #601457) is very similar to that of the other autosomal recessive SCID forms (early onset, severe respiratory infections, chronic diarrhea leading to failure to thrive, persistent candidiasis). Frequent transplacental passage of maternal T cells leads to cutaneous manifestations suggestive of graft-versus-host disease. The immunologic phenotype is characterized by severe lymphopenia, with virtual absence of T and B lymphocytes. Almost all autologous circulating lymphocytes are NK cells. No specific immunoglobulins can be produced. Curative treatment is bone marrow transplantation.

Omenn syndrome

Omenn syndrome (OMIM #603554) affects infants of both sexes, who present with a prominent, generalized papular skin eruption or scaling exudative erythrodermia, alopecia, enlarged lymph nodes, hepatosplenomegaly, hypoproteinemia with edema, and eosinophilia,

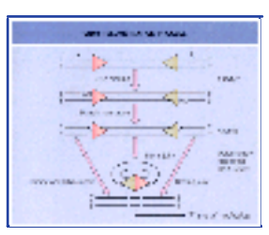

Figure 98-16 V(D)J recombination process. Diagram showing the V(D)J recombination process with the genes involved in T- B- SCID and Omenn syndrome.

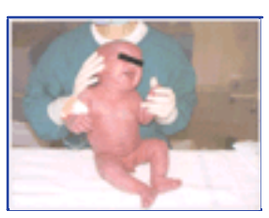

Figure 98-17 A baby who has Omenn syndrome. Note the diffuse erythema.

associated with severe respiratory infections, chronic diarrhea and failure to thrive [47] (Fig. 98.17). This clinical phenotype may mimic histiocytosis X of the Letterer-Siwe type, or graft-versus-host disease, and may in fact be occasionally seen in SCID infants with transplacental passage of alloreactive maternal T cells. While the latter condition is also referred to as Omenn-like syndrome, the term Omenn syndrome is reserved for cases in which the presence of alloreactive T cells has been ruled out.

The molecular pathogenesis of Omenn syndrome long remained a mystery. However, the demonstration of a variable number of autologous, oligoclonal, autoreactive, activated T cells in Omenn syndrome infants, and the simultaneous occurrence of Omenn syndrome and T- B- SCID in two siblings suggested a genetic relationship. This hypothesis was proven when mutations in *RAG1* and *RAG2* were demonstrated in Omenn syndrome patients. [48] In contrast to T- B- SCID, each patient carried at least one mutant allele that allowed expression of a partially functioning RAG protein, resulting in a leaky V(D)J rearrangement defect, with reduced, but not abolished, intrathymic T cell differentiation.

In contrast to other forms of SCID, Omenn syndrome may have leukocytosis with marked eosinophilia and variable (up to normal) T cell counts. A characteristic finding is the co-expression of activation/memory markers (HLA-DR, CD45R0, CD25, CD95, CD30) on the surface of T cells, which predominantly secrete T helper (Th)2-type cytokines such as IL-4 and IL-5. B lymphocytes are typically absent from peripheral blood and lymphoid tissues and immunoglobulin levels are markedly diminished, but IgE is usually elevated. Lymph nodes show lymphoid depletion and an increased proportion of interdigitating reticulum cells, eosinophils and histiocytes. Thymus also shows lymphoid depletion and lack of corticomedullary demarcation.

Omenn syndrome is diagnosed by its characteristic features after engraftment of allogenic T cells has been excluded. Mutation analysis of *RAG1* and *RAG2* confirms the diagnosis and can be used for prenatal diagnosis in affected families. Interferon-? may reduce the Th2-type T cell activity and ameliorate the clinical status but,

unless treated by BMT, Omenn syndrome is usually fatal within the first year of life. Steroids and cyclosporin A may be used for treatment of the graft-versus-host-disease-like skin reaction.

Radiation-sensitive severe combined immunodeficiency (Artemis deficiency)

Mutations in the RAG genes do not account for all V(D)J recombination defects in T^- B^- SCID patients. A subgroup of patients also show increased cellular radiosensitivity (even in nonlymphoid lineages), due to mutations in *Artemis*, a gene located on the short arm of chromosome 10 (OMIM #602450) that participates in the later phases of V(D)J recombination.[49] This latter form of T^- B^- SCID, radiation-sensitive SCID (Rs-SCID), is more common among Athabascan-speaking Native Americans, among whom the incidence is estimated to be approximately 1 in 2000 live births. The encoded protein seems to be involved in the opening of the hairpin necessary to join the selected V(D)J regions (see Fig. 98.16). To date, *Artemis* mutations have been severe, resulting in a complete T^- B^- SCID phenotype.

Adenosine deaminase deficiency

Adenosine deaminase (ADA) is an ubiquitously expressed intracellular enzyme responsible for the transformation of adenosine to inosine, and of deoxyadenosine to deoxyinosine. The *ADA* gene is on chromosome 20 and the disease is inherited as an autosomal recessive trait (OMIM *102700). Deficiency in ADA results in intracellular accumulation of deoxyadenosine and its phosphorylated metabolites, among which dATP is particularly toxic to lymphoid precursors.[50] Consequently, patients who have complete ADA deficiency present with a typical T^- B^- SCID phenotype with marked lymphopenia. Partial ADA defects may result in less severe clinical presentations with delayed (in infancy) or late (during adolescence or even adulthood) onset.[51] Since the enzyme is ubiquitously expressed, nonhematopoietic organs such as kidney, bone, cartilage and the central nervous system may also be involved.

Optimal treatment is BMT from an HLA-identical family donor. When this is not available, enzyme substitution treatment with pegy-lated bovine ADA (PEG-ADA) results in effective and sustained reduction of toxic metabolite levels and immune reconstitution.[52] *ADA* was the first SCID-causing gene to be cloned and ADA deficiency was the first human disease to be treated with gene therapy, although it was not curative.[53] Some 10 years after the first attempts, improvements in vector design, cell targeting and recipient preparation have resulted in improved outcomes of gene therapy for ADA deficiency.[54]

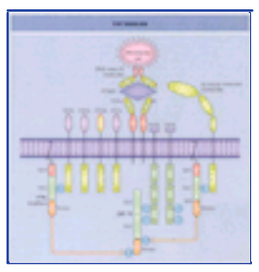

Figure 98-18 T cell receptor signaling. Stimulation of T cells through the CD3/TCR complex results in activation of p56lck, a src-tyrosine kinase, that mediates tyrosine-phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3-?, -d, -epsilon and -? chains. ZAP-70, an intracellular tyrosine kinase, is then recruited into the CD3/TCR complex through binding of its SH2 domains to phosphorylated ITAMs of the ? chain. ZAP-70 itself becomes phosphorylated by Src-family protein tyrosine kinases. Phosphorylation triggers ZAP-70 activation, allowing phosphorylation of downstream signaling molecules such as linker for activation of T cells (LAT) and SLP-76.

Purine nucleoside phosphorylase deficiency

Purine nucleoside phosphorylase (PNP) is another purine metabolism enzyme downstream of ADA that converts guanosine to guanine and deoxyguanosine to deoxyguanine. The *PNP* gene is on chromosome 14q13 and is inherited as an autosomal recessive trait. The accumulation of deoxyguanosine and its phosphorylated metabolites (dGTP in particular) inhibit ribonucleotide reductase, whose activity is essential to DNA synthesis. Although PNP is widely expressed, its deficiency is particularly deleterious to lymphoid development, especially T cells.[50] Consequently, patients who have PNP deficiency experience a dramatic and progressive T cell lymphopenia during the first years of life, leading to a SCID phenotype.[55] Toxicities of the metabolites also involve the central nervous system, with symptoms ranging from behavioral problems and low cognitive function to ataxia or tetraparesis. The only therapeutic option is BMT, since enzyme replacement therapy is not yet available.

Defects of the P56lck/ZAP-70 pathway

Stimulation of T cells through the CD3/TCR complex (Fig. 98.18) results in a complex series of activations focusing on p56lck, a Src-tyrosine kinase, immunoreceptor tyrosine-based activation motifs (ITAMs) and ZAP-70, an intracellular tyrosine kinase.[56] ZAP-70 itself becomes phosphorylated by Src-family protein tyrosine kinases. In humans, ZAP-70 is required for development of $CD8^+$ T cells in the thymus and peripheral blood T cell proliferation in response to mitogens and antigens.

Defective expression of p56lck leads to panhypogammaglobulinemia, lymphopenia with a reduced proportion of $CD4^+$ T cells and reduced in-vitro proliferation.[57] Defects in ZAP-70 result in impaired T cell development and function.[58] Infants who have ZAP-70 deficiency present with typical clinical features of SCID, virtual absence of $CD8^+$ T cells, and nonfunctional $CD4^+$ T cells. Both p56lck and ZAP-70 are autosomal recessive.

Major histocompatibility complex class II deficiency

T cells recognize foreign antigens in the context of self-MHC class II molecules expressed by antigen presenting cells. Major histocompatibility complex class II molecules are critical for numerous aspects of immune function, including antibody production, T-cell-mediated immunity, induction of tolerance and inflammatory responses. Therefore, MHC class II deficiency leads to an inability of T cells to recognize foreign antigens that would normally be presented by MHC class II and a combined humoral and cellular immune deficiency. This autosomal recessive immunodeficiency (OMIM #209920), also referred to as the bare lymphocyte syndrome type II (BLS II), is genetically heterogeneous and may be due to mutations in any of four components of transcription factors that control MHC class II gene expression:[59] class II transactivator (CIITA), RFXANK/RFX-B, RFX5 and RFXAP[60] (Fig. 98.19). The hallmark of MHC-II deficiency is the absence of HLA DR, DQ and DP molecules on B cells, monocytes and dendritic cells (which constitutively express HLA class II), and IFN-?-activated T cells. $CD4^+$ T cell counts are low, whereas $CD8^+$ T cells are normal to increased. In-vitro response to mitogens is often reduced and immunoglobulin levels are low. In the absence of allogenic BMT, patients generally die between the ages of 5 and 18 years.

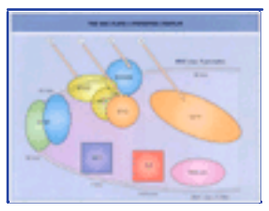

Figure 98-19 The major histocompatibility complex class II promoter complex. Mutations in these transcription factors account for the various complementation groups identified in MHC class II deficiency.

A moderate phenotype has also been described, with survival into adulthood.

TAP deficiency

Human leukocyte antigen class I molecules are polymorphic cell surface glycoproteins that play an essential role in presenting antigenic peptides to cytotoxic T lymphocytes and in modulating the activity of NK cells that bear HLA class I binding receptors. HLA class I molecules are composed of a polymorphic heavy chain, encoded by *HLA-A*, *HLA-B* and *HLA-C* genes, associated with the invariant ß$_2$ -microglobulin (ß$_2$ M). The assembly of HLA class I molecules occurs in the lumen of the endoplasmic reticulum, where they are loaded with peptides derived from the degradation of intracellular organisms or proteins. These peptides are transported into the endoplasmic reticulum via transporter-associated-with-antigen-presentation (TAP) proteins.[61] The TAP complex consists of two structurally related subunits (TAP1 and

TAP2), which interact to form a functional peptide transporter system. Defects in either TAP1 or TAP2 result in impaired peptide-HLA class I/ß$_2$ complex formation and reduced surface expression of HLA class I molecules.

Patients who have defective HLA class I molecule expression due to defects in either TAP1[61] or TAP2[62] have a far less severe clinical phenotype than that observed in MHC class II expression. They often have nasal polyposis and recurrent sinopulmonary infections caused by *H. influenzae*, *S. pneumoniae*, *S. aureus*, *Klebsiella* spp. and *P. aeruginosa*. Deep skin ulcers may also occur. The diagnosis is made by demonstration of low expression of HLA class I molecules on mononuclear cells.

IMMUNE DEFECTS WITH PREDOMINANT IMMUNOGLOBULIN DEFICIENCY

B cell differentiation is a multistage process regulated by transcription factors that leads to a complex scheme of gene activation resulting in specific intracytoplasmic and membrane-bound proteins. While B cell differentiation in the bone marrow is antigen-independent, later stages of B cell development in the periphery depend upon contact between the B cell and its cognate antigen (Fig. 98.20).

Immunodeficiency with predominant defects in antibody production

X-linked agammaglobulinemia

X-linked agammaglobulinemia (XLA), the prototype humoral immunodeficiency, was first described in 1952 by Bruton[63] (OMIM *300300). The most important clinical features are shown in Table 98.3 . A block in B cell differentiation at the pre-B-cell stage due to a mutated tyrosine kinase of the Src family (denominated Bruton tyrosine kinase, BTK) leads to a complete lack of circulating B lymphocytes and virtually no immunoglobulin production.[64] The disorder should be considered in males who have recurrent bacterial infections, low immunoglobulin levels and absent B cells. A positive family history on the maternal side is suggestive. T cell count and function are usually normal. Diagnosis can be confirmed by analysis of BTK protein expression (immunoblotting or flow cytometry) and DNA mutation analysis. No genotype-phenotype correlation has yet emerged. Therapy consists of lifelong immunoglobulin replacement, usually 400–500mg/kg every 3 weeks. Despite this, patients remain at risk for certain enteroviral infections of the central nervous system that can be especially devastating. The mutations are listed at http://www.uta.fi/laitokset/imt/bioinfo/BTKbase.

Autosomal recessive agammaglobulinemia

The recognition of females who had agammaglobulinemia, and the fact that in some kindreds boys and girls are equally affected, led to the search for autosomal recessive causes of agammaglobulinemia, which accounts for 5–10% of all patients with complete B cell deficiency.

The clinical picture is similar to that of XLA, with a somewhat earlier onset and perhaps a more severe progression. The disease is genetically heterogeneous, with defects identified in the genes that encode for the µ heavy chain,[65] the ?5 chain (part of the surrogate light chain),[66] the Iga chain of the membrane-expressed pre-B-cell receptor (BCR; involved in signal transduction),[67] and in BLNK,[68] an intracytoplasmic adapter that is phosphorylated by the tyrosine kinase Syk upon BCR signaling.

Figure 98-20 Blockage of B cell maturation. Model of B cell differentiation with the various proteins involved in the different stages shown below. The vertical red lines indicate a maturation block caused by the molecules indicated above.

TABLE 98-3 -- Characteristics of X-linked agammaglobulinemia.

CHARACTERISTICS OF X-LINKED AGAMMAGLOBULINEMIA
Onset after 6 months of life (when maternal antibodies wane)
Lack of tonsils and B lymphocytes (<2%)
Recurrent bacterial infections of the upper and lower respiratory tract
Risk of development of bronchiectasis
Gastrointestinal *Giardia lamblia*
Susceptibility to enteroviral encephalitis (echovirus, poliovirus)
Susceptibility to arthritis caused by enteroviruses and mycoplasmas

Selective IgA deficiency and common variable immune deficiency

Common variable immune deficiency (CVID) has an incidence of 1 in 10,000–100,000 live births and is characterized by low IgG and IgA levels associated with defective specific antibody production in the setting of a normal B cell count. In about 30% of patients, IgM levels are also low. The inability to produce specific antibodies puts patients at risk for frequent bacterial infections, predominantly in the respiratory and gastrointestinal tracts. The clinical picture may be complicated by the presence of autoimmune disorders such as hemolytic anemia, thrombocytopenia, inflammatory bowel disease and others. There are early-onset (usually between the ages of 1 and 5 years) and late-onset (during adolescence) clinical phenotypes, suggesting that there may be different molecular mechanisms underlying CVID.[69]

Selective IgA deficiency

Selective IgA deficiency (IgAD) is characterized by low IgA levels in the serum (<5mg/dl) and absent secretory IgA and occurs in 1/700 live births. IgG and IgM levels are normal, but defects in IgG2 and IgG4 have been described in a subgroup of patients. Circulating B cells are normal in number. In general, IgA deficiency is a benign disorder, with only 30% of affected patients being susceptible to bacterial infections, usually confined to the respiratory and gastrointestinal tract, or to autoimmune manifestations. A minor defect in IgA production (with levels <2SD), partial IgA deficiency, is characterized by a good prognosis, with a tendency of IgA levels to normalize with age. Little is known about the molecular bases of IgAD/CVID. However, given the fact that both may occur in the same family and that in some cases selective IgA deficiency has evolved into CVID, a common pathogenic mechanism for both forms of this humoral immune defect has been hypothesized. The best candidate gene seems to be located at position 6p21. There is also evidence that environmental factors, such as anti-inflammatory and antirheumatic drugs or perhaps viral infections may accelerate the development of IgAD and CVID.

The hyper-IgM syndromes

Four distinct genetic defects have been identified in hyper-IgM (HIGM). These syndromes are typically characterized by low IgG and IgA levels, normal to increased IgM and a normal number of circulating B cells. Both X-linked and autosomal forms of the disease are known. The clinical picture is variable, with most of the patients suffering from recurrent bacterial infections of the respiratory and gastrointestinal tracts.[70] In addition, some patients who have HIGM are susceptible to opportunistic pathogens including *P. carinii*, *Cryptosporidium* spp. and cytomegalovirus.

HIGM1

CD40 ligand (CD40L), encoded by the X-linked *TNFSF5*, is predominantly expressed by activated $CD4^+$ T cells.[71] Interaction of CD40L with CD40 on B cells, dendritic cells, monocytes and some activated epithelial cells is necessary for immunoglobulin isotype switching, high affinity immunoglobulin production, somatic hypermutation and the formation of germinal centers in secondary lymphoid organs. In addition, CD40L plays a crucial role in efficient interaction between T cells and

antigen-presenting cells, guiding the adaptive immune response to intracellular pathogens. HIGM1 (XHIM or CD40L deficiency, OMIM# 308230) is one of the two X-linked variants of HIGM. CD40L deficiency usually presents within the first year of life. Neutropenia is common. By the end of the first decade sclerosing cholangitis is common. Later in life, the incidence of gastrointestinal tumors is elevated.[72]

The diagnosis is made by demonstration of absent CD40L on activated T cells and confirmed by mutation analysis of the *TNFSF5* gene. Mutations are catalogued at http://www.uta.fi/imt/bioinfo/CD40Lbase. Despite regular administration of immunoglobulins, mortality is as high as 40% by age 25. Bone marrow transplantation should be pursued in appropriate cases. In murine models, unregulated CD40L expression has led to an increased incidence of autoimmune and lymphoproliferative diseases, making optimism for

human gene therapy without the authentic regulatory elements premature. A phase I/II trial with soluble trimeric CD40L is underway.

Hyper-IgM syndrome with hypohidrotic ectodermal dysplasia (ectodermal dysplasia with immunodeficiency)

This X-linked recessive immunodeficiency (NEMO deficiency; OMIM #300291) associates the HIGM phenotype with hypohidrotic ectodermal dysplasia, as mentioned above.[32] [33] Affected boys have defects in the sweat glands, relatively thin hair, dental abnormalities (conical or peg teeth) and suffer from a wide range of infections such as pneumonia, osteomyelitis and meningitis. Complete functional absence of NEMO in males leads to intrauterine death, whereas boys who have partial expression present as HIGM. In contrast, females heterozygous for severe mutations present with incontinentia pigmenti, a syndrome that may include various defects involving the skin, eyes and brain. Skewed X-inactivation in hematopoietic cells in females usually, but not always, avoids manifestation of the immune defect.

HIGM2

This autosomal recessive variant of HIGM, HIGM2 (activation-induced cytidine deaminase, AID, deficiency) is characterized by a pure B cell defect, with lack of immunoglobulin isotype switching, impaired somatic hypermutation and absence of high affinity antibodies (OMIM #606258).[73] Located at 12p13, AID encodes an mRNA editing protein that co-localizes with DNA-repair enzymes and is selectively expressed in activated B cells in germinal centers. Clinical symptoms include recurrent respiratory and gastrointestinal infections but systemic bacterial infections such as meningitis and sepsis are also common.[74] In contrast to the HIGM syndromes involving CD40-CD40L interactions, germinal centers are large, leading to hyperplasia of secondary lymphoid organs (tonsils). Surveillance for lymphoproliferative complications are indicated.

HIGM3

CD40 is the cognate receptor for CD40L. Consequently, lack of CD40 expression (CD40 deficiency, HIGM3; at 20q12, OMIM #606843) results in clinical and immunologic features very similar to those seen with CD40L deficiency, HIGM1. [75] Lack of isotype switching, impaired somatic hypermutation, defective memory B cell generation and lack of germinal center formation are typical features. The diagnosis is made by analysis of CD40 expression, and is confirmed by mutation analysis. Similarly to HIGM1, treatment requires regular administration of intravenous immunoglobulin, prophylactic anti-biotics and use of sterile/filtered water to prevent *Cryptosporidium* infection. Bone marrow transplantation should be considered in view of the poor long-term prognosis. Since CD40 is also expressed on some nonhematopoietic cells, unlike CD40L, patients who have defective expression of CD40 are not fully corrected by BMT. The clinical implications of this remain to be determined.

SYNDROME-ASSOCIATED IMMUNE DEFICIENCIES

Immune deficiency may also be part of a broader clinical syndrome. In some of these disorders, signs related to immune deficiency predominate and direct the clinical evolution of the disease; in other cases, immune deficiency is variable and does not represent the most important clinical problem. In spite of their complexity, most of these diseases are monogenic, with the affected gene encoding a protein with pleiotropic functions. Alternatively, as in DiGeorge syndrome, the disease may result from a chromosomal microdeletion.

Immune deficiencies due to DNA repair defects

In addition to Artemis deficiency, immunodeficiencies due to DNA repair include ataxia telangiectasia and several related disorders.[76] [77] Ataxia telangiectasia is due to mutations in the ataxia-telangiectasiamutated (*ATM*) gene (11q22-23; OMIM #208900; mutations catalogued at http://www.vmresearch.org/atm.htm). Ataxia telangiectasia is typically associated with increased chromosome radiosensitivity and defective DNA repair, leading to somatic translocations that involve the T cell receptor and immunoglobulin loci. Translocation between chromosomes 7 and 14 is particularly common. IgA, IgG2, IgG4 and IgE levels are low and a progressive decline in T cell number and function is typically observed. Most patients do not survive beyond young adulthood because of lymphomas and leukemia. Neurologic problems are a predominant sign of the disease.

The same clinical phenotype can be caused by mutations in the nearby gene *hMRE11*, which accounts for the so-called ataxia-telangiectasia-like syndrome (OMIM #600814).[78] *hMRE11* encodes a protein involved in the earmarking of DNA double-strand breaks. Mutations in *Nbs* (or nibrin) are the cause for the Nijmegen syndrome (OMIM #251260), another immunodeficiency characterized by growth failure, microcephaly and a characteristic bird-like facies. The DNA ligases are also involved in DNA repair, and mutations can cause immunodeficiency. [79] [80]

Di George syndrome; velocardiofacial syndrome

This syndrome (OMIM# 188400) has a wide range of clinical presentations, including thymus and parathyroid defects (hypocalcemia), overt facial abnormalities, growth failure, neurologic and neuro-psychiatric manifestations and heart defects, such as tetralogy of Fallot.[81] The predominant immunologic feature is a variably low T cell count predisposing to *Candida* and other infections. The molecular defect lies in a microdeletion in 22q11.21-23 (in 90% of the patients this occurs de novo). This defect is sometimes referred to as CATCH 22 (cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, hypocalcemia, chromosome 22).[82] However, this is a very common syndrome with quite variable expressivity. The frequency in the general population is thought to be 1 in 4000 live births and it can be transmitted as an autosomal dominant trait.

Wiskott-Aldrich syndrome

The Wiskott-Aldrich syndrome (WAS) protein, encoded by *WASP* at Xp11.2, is expressed selectively by cells of the hematopoietic lineages. It is involved in the activation-induced reorganization of the cytoskeleton, particularly actin polymerization. Eczema, thrombocytopenia and immunodeficiency are the three main features of Wiskott-Aldrich syndrome (OMIM #301000).[83] Microthrombocytes (low mean platelet volume) in a male neonate who has thrombocytopenia and eczema are highly suspicious, even before the immune deficiency characterized by recurrent bacterial airway infections is manifest. Immunoglobulin responses to polysaccharides are typically impaired and immune globulin replacement is often needed. Later in life there is an elevated incidence of leukemia, lymphoma and autoimmune diseases.

There is a relatively benign variant of WAS, X-linked thrombocytopenia, which occurs without eczema or immunodeficiency. Interestingly, most patients who have this variant show missense mutations in exons 1 or 2 of *WASP* that permit expression.[84] In some less severe cases, thrombocytopenia is intermittent, but mean platelet volume is consistently low. Interestingly, there is also one family reported that has a *WASP* mutation (L270P) leading to isolated X-linked neutropenia.[5] The diagnosis is confirmed by mutation analysis of the *WASP* gene. Given the recognized genotype-phenotype correlation, determination of specific mutations gives important prognostic information. Gene therapy may become another option, as there has been report of spontaneous mutation reversion in *WASP* leading to expansion of a 'normalized' lineage.[85]

X-linked lymphoproliferative syndrome

Patients who have X-linked lymphoproliferative syndrome (Duncan's syndrome, OMIM *308240) show an increased susceptibility to severe manifestations after Epstein-Barr virus (EBV) infections.[86] Hepatitis, lymphomas and/or hemophagocytic lymphohistiocytosis kill about 70% of affected males in their first decade. Those who survive, mostly affected by a milder variant, may present with hypogammaglobulinemia. The defective gene, *SH2D1A*, located at Xq25, encodes SLAM-associated

protein (SAP), an SH2-domain-containing protein expressed selectively in lymphocytes. SAP has a regulatory effect on cytokine production and is essential for the cytotoxic activity of NK cells. Other herpesviruses have also been shown to trigger XLP. The mutations are cumulated at http://www.uta.fi/imt/bioinfo/SH2D1Abase. Several mutational hot-spots have been identified, but so far no genotype-phenotype correlation has been recognized. BMT should be considered before EBV infection if an HLA-compatible donor is available.

Familial hemophagocytic lymphohistiocytosis

Similar to X-linked lymphoproliferative syndrome, familial hemophagocytic lymphohistiocytosis is an autosomal recessive inherited disease characterized by severe viral infections leading to hepatosplenomegaly, acute bone marrow failure with histiocytic activation, coagulopathy and hypertriglyceridemia. Onset is during the first few months of life; accelerated phases occur repeatedly, often with life-threatening hemorrhagic episodes.[87] The immunologic defect consists in virtually absent NK cytotoxicity due to a functionally deficient perforin (encoded by *PRF1* at 10q21-22), a protein normally released from the intracytoplasmic granules after the membranes of effector (NK or T cells) and target cells have fused.[88] The resulting prolonged contact between infected and 'intended' cytotoxic cell leads to increased cytokine (IFN-?) production that is thought to activate macrophages and histiocytes. [89] Forms of this disease that do not have clear genetic predispositions may be triggered by various infections or by drugs. Accelerated phases, which are true medical emergencies, may be brought into remission by chemotherapy

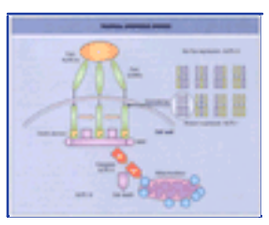

Figure 98-21 Fas/Fas ligand apoptosis system. The Fas/FasL apoptosis system and the various forms of ALPS caused by defects in it.

(etoposide) and maintained with immunosuppression, but the only curative treatment is BMT.

Autoimmune lymphoproliferative syndrome (Canale Smith syndrome)

At the core of normal development is the proper coordination of cell death. When cell death occurs in a programmed way (i.e. not through lysis by infecting agents or necrosis due to toxic events), it is referred to as apoptosis. Proper Fas/Fas ligand (FasL) interaction is critical for normal apoptosis (Fig. 98.21). The correct Fas/FasL interaction leads to the formation of the death inducing signaling complex (DISC), which acts through different caspases to induce mitochondrial damage and finally proteolytic cell death. Fas (CD95), encoded by *APT1* (*TNFRSF6*), is a transmembrane protein with an intracellular death domain. Death signaling requires assembly of homotrimers. The autoimmune lymphoproliferative syndrome type I (ALPS I) is caused by heterozygous mutations in Fas that lead to disruption of normal homo-oligomerization resulting in deranged lymphoid homeostasis due to defective apoptosis.[90] In this autosomal dominant disorder, only one in eight homotrimers will have three normal Fas molecules. However, the story remains quite complex, as the mutations in Fas have variable penetrance and expressivity. In contrast to these dominant mutations, those which abrogate protein expression are inherited as recessive traits. This complete null recessive form of the disease, ALPS O, may be especially severe with symptoms presenting during fetal life (hydrops). Autoimmune lymphoproliferative syndrome type 1 usually presents with lymphadenopathy, hepatosplenomegaly and hematologic symptoms of autoimmunity such as hemolytic anemia, autoimmune neutropenia, and immune thrombocytopenia. There is a markedly increased risk (up to 50 times) of lymphoma and leukemia.[91] Diagnostic findings include increased numbers of T cells that lack both CD4 and CD8 molecules, referred to as double-negative T cells.

Autoimmune lymphoproliferative syndrome type Ib is caused by mutations in the FasL (*TNFSF6*).[92] Defects in the caspases lead to a similar failure of lymphocyte apoptosis denominated ALPS II. ALPS

III is the name currently used for the ALPS phenotype for which genetic defects have not been identified.

IPEX

The clinical entity characterized by dysregulated immunity (anemia, lymphadenopathy), autoimmune **p**olyendocrinopathy (diabetes, thyroiditis), (inflammatory) **e**nteropathy of **X**-linked transmission is named IPEX (OMIM: #304930, #304790).[93] The molecular defect resides in the *FOXP3* gene, encoding scurfin, a protein expressed predominantly in the thymus and spleen. It appears to have a repressive role in T cell activation and cytokine expression.[94] Severely affected boys usually do not survive the first year of life. There is evidence for a somewhat milder phenotype associated with mutations sparing the coding sequence but affecting the polyadenylation signal. Opportunistic infections are not a part of IPEX, indicating that this is less a defect in protective immunity than a defect in regulation of cytokine production. Immunosuppression has proved beneficial in some patients who have IPEX, underlining the importance of *FOXP3* in the control of autoreactive cell clones.

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Chapter 99 - Immunodeficiencies Associated with Immunosuppressive Agents

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Giuseppe Pantaleo

INTRODUCTION

The goal of this chapter is to review the immunologic effects (e.g. the degree of immunodeficiency) associated with therapeutic interventions involving the use of immunosuppressive agents commonly used in transplantation and in the treatment of rheumatic and autoimmune diseases. The immunodeficiency caused by chemotherapeutic agents used in cancer therapy will not be the object of this chapter. Analyses of the mechanisms of action of the different immunosuppressive agents and of their immunologic target(s) show substantial qualitative and quantitative differences in the type of immunodeficiency caused by the therapeutic interventions. These differences can be explained by the ability of the immunosuppressive agents to target single or multiple components of the immune system. This translates into an impairment of the host immune defenses against bacteria, viruses, fungi and parasites. Furthermore, the intensity of the immunodeficiency is substantially influenced by the number of agents administered because immunosuppressive agents are mostly used in combination. Therefore, the clinical consequences associated with the administration of immunosuppressive agents result from their mechanisms of action and from the extent of perturbation of the fine regulatory immune mechanisms needed for the effective control of a variety of pathogens.

HOST DEFENSES — GENERAL CONCEPTS

The immune system represents the primary mechanism of defense against a variety of pathogens.[1] To understand the potential interference of immunosuppressive agents on the immune system, it is important to examine the different components of the immune system and the sites of action of these agents to predict the immunologic dysfunctions eventually caused by the treatment. Furthermore, on the basis of the functional impairment of one or more components of the immune system, it is possible to predetermine the pathologic conditions, mostly infectious, that can complicate the clinical picture during immunosuppressive treatment.

We also briefly review the effects of immunosuppressive agents on the natural mechanisms of defense such as the anatomic barriers.

Anatomic barriers

The natural mechanisms of defense are mediated by the anatomic barriers, primarily by the skin and mucosal (gastrointestinal and urinary tract) barriers. The ability of these barriers to protect against invading pathogens is dependent upon the intrinsic physical structure as well as a series of factors including the physicochemical features of the environment (e.g. the pH and temperature), the local bacterial microflora and soluble local mediators such as secretory IgA and a variety of proteins with enzymatic activity (e.g. lysozymes).

Immunosuppressive treatment may severely compromise the integrity of the mucosal barrier because the particularly high turnover of cell division of the mucosal epithelium is very sensitive to the antiproliferative activity of immunosuppressive agents. This in turn may cause lesions of the epithelium, and the clinical picture may be complicated by the emergence of mucositis of variable severity. As discussed below, this type of complication is relatively frequent in cancer immunosuppressive regimens but is rare in the fields of transplantation and immunologic diseases.

Innate immunity

Innate immunity is non-specific, and the rapidity of the response (e.g. previous antigen exposure is not required) and the lack of immunologic memory [2] represent the main characteristics of this immune response. The effector components of innate immunity include soluble and cellular mediators (see Chapter 97). Among the former, the complement (C) proteins play a critical role in the recruitment and activation of neutrophils (the C3a and C5a factors), the opsonization of bacteria (the C3b factor) and the destruction of certain micro-organisms through the C5–C9 factors. C-reactive protein (CRP) and lipopolysaccharide (LPS)-binding protein are additional soluble mediators of the innate immunity involved in the opsonization of bacteria or activation of C (e.g. CRP).

The cellular effector components of innate immunity include neutrophils, monocyte/macrophage cells and natural killer (NK) cells. These different types of cells, which are recruited at the site of infection or inflammation by the soluble mediators mentioned above, mediate either direct (e.g. phagocytosis and killing of bacteria) or indirect defense mechanisms (e.g. recruitment of other types of immune cells through the secretion of cytokines and chemokines).

Among the immunosuppressive agents, glucocorticoids (GCs) may markedly downregulate the innate immune response by causing both qualitative and quantitative defects. The pathologic conditions associated with this type of immunosuppression are characterized by an increased risk for bacterial infections caused by viridans streptococci, *Staphylococcus aureus,* coagulase-negative staphylococci, Gram-negative bacilli and certain fungi, mainly *Candida* and *Aspergillus* spp.

Adaptive immunity

Adaptive immunity[1] (see Chapter 97) is specific; it requires a certain time to become fully functional — the time is necessary for the generation, maturation and expansion of antigen-specific T and B cells — and is associated with the generation of immunologic memory following clearance of the pathogen.

T cells are a critical component of adaptive immunity. The mechanisms of antigen (Ag) recognition by T cells are extremely specific. Following appropriate processing and presentation by professional antigen-presenting cells (APCs), T cells recognize a complex formed by the major histocompatibility complex (MHC) products and the Ag peptide on the surface of APC. Two types of T cells (CD4 and CD8) are involved in the immune response against pathogens.

Based on their ability to produce different cytokines, CD4 T cells can generally be distinguished into two populations:

- T helper (T_H)1, which are mostly characterized by the production of interferon (IFN)-? and interleukin (IL)-2; and
- T_H2, which produce mostly IL-4, IL-5 and IL-10.

T_H1 CD4 T cells are necessary for the maturation, expansion and maintenance of the CD8T cell response, of NK cells and for the activation of macrophages; these latter cells are involved in the elimination of pathogens such as mycobacteria and fungi. Th2 CD4 T cells mediate the maturation and the activation of pathogen-specific B lymphocytes.

CD8 T cells are the primary effector component of adaptive cell-mediated immunity. They are able to lyse directly virus-infected cells and also tumor cells in particular.

It is therefore clear that immunosuppressive therapy, which is one of the primary causes of secondary immunodeficiency, may significantly impair T cell-mediated immunity and determine an increased susceptibility to a variety of pathogens including:

- viruses — herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV);
- bacteria — *Mycobacterium tuberculosis, Legionella* spp., *Listeria monocytogenes, Salmonella typhi*;

¦ fungi — *Candida* spp., *Apergillus* spp., *Histoplasma capsulatum*, *Cryptococcus neoformans*, and the closely related micro-organism *Pneumocystis carinii*; and
¦ parasites (*Toxoplasma gondii*, crytosporidia and *Leishmania* spp.

B lymphocytes mediate the immune response against pathogens through the production of immunoglobulins (Ig). Immunoglobulins may inhibit the entry of the pathogen into the target cells by binding to certain regions of the pathogen that are critical for the interaction with specific receptors on the surface of the target cells, a phenomenon known as neutralization. Furthermore, the primary role of Ig is the opsonization of bacteria and the activation of C and the different components of innate immunity that may lead to elimination of the pathogen.

The pathologic conditions associated with the absence or a quantitative defect of Ig are well known, particularly in the case of primary immunodeficiencies such as common variable immunodeficiency (CVID). A selective defect in the production of Ig characterizes CVID and is associated with recurrent infections of the upper and lower respiratory tract caused predominantly by *Streptococcus pneumoniae* and *Haemophilus influenzae*.

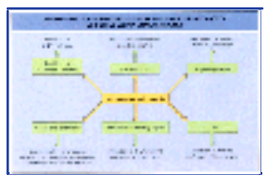

Figure 99-1 Mechanism of action of the different immunosuppressive agents currently in use in clinical practice.

IMMUNOLOGIC TARGETS OF AND CLINICAL ISSUES ASSOCIATED WITH IMMUNOSUPPRESSIVE THERAPY

In order to evaluate correctly the risk for infectious diseases associated with an impairment of immune function(s), it is essential to know the degree of the defect and most importantly the component(s) of the immune system targeted by the immunosuppressive agents. For these reasons, the mechanisms of action of the most frequently used immunosuppressive agents in the fields of transplantation, autoimmunity and rheumatology, and the clinical implications of the therapy are examined in detail.[3]

Glucocorticoids

Glucocorticoids (GC) are the cornerstone in the treatment of the majority of inflammatory diseases. They are also used in the treatment of asthma, acute rejection of transplanted organs and some hematologic malignancies. Their immunologic effects are extremely broad.

Mechanism of action

Glucocorticoids are highly lipid soluble and so they penetrate easily through the cell membrane. They bind in the cytoplasm to a specific receptor (glucocorticoid receptor, GR) present in all nucleated cells. This provides part of the explanation for why it is not possible to dissociate their anti-inflammatory effects from their metabolic effects. Following the interaction of GC with a GR, the GR migrates to the nucleus and there is a dimerization of the GC-GR complex. These dimers can bind to GC-response elements (GRE) of GC-responsive genes. Through the above mechanism GCs modulate gene transcription in a positive (e.g. transactivation) or negative (e.g. trans-repression) fashion.[4] [5] [6] [7]

Glucocorticoids block the transcriptional activity of certain transcriptional factors (Fig. 99.1) through direct inhibition of ubiquitous factors such as nuclear factor-kappaB (NF-?B) and activator protein-1 (AP1), or more specific elements such as NFAT (nuclear factor of activated T cells) or STATs (signal transducer and activator of transcription). They can also inhibit NF-?B through the induction of expression of its specific inhibitor (I?Ba). The transcription factors mentioned above are essential for the transcription of proinflammatory cytokine genes. Therefore, the broad effects of GCs result from

the suppression of ubiquitous and specific transcription factors. This explains why different cell types (e.g. macrophages, lymphocytes and neutrophils) involved in the inflammatory reaction and immune response are all targets of this class of drugs.

Anti-inflammatory effects

Glucocorticoids act on the early phase of the inflammatory reaction by inhibiting the production of chemotactic factors and proinflammatory cytokines such as IL-1, IL-6, tumor necrosis factor (TNF)-a and IL-8. This results in a reduced recruitment of monocytes/macrophages and neutrophils at the site of inflammation. Furthermore, they block the release of histamine and serotonin and the expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These effects result in a reduction of the capillary permeability and migration of cells with phagocyte activity through the endothelial barrier. Finally, GCs inhibit the synthesis of inflammatory mediators such as the eicosanoids (e.g. prostaglandins, leukotrienes and thromboxanes).

Immunosuppressive effects

The most important immunosuppressive effects of GCs include:

¦ inhibition of IL-2 receptor synthesis;
¦ suppression of antigen presentation by causing a reduction in the expression of MHC molecules on the surface of APCs;
¦ inhibition of lymphocyte proliferation by suppression of IL-2 production; and
¦ suppression of IFN-? production.

Apoptotic effects

Glucocorticoids may also mediate a cytotoxic effect through the induction of apoptosis. Two hypotheses have been proposed to explain this effect:

¦ apoptosis is triggered by the activation of death-inducing genes; and
¦ induction of apoptosis by inhibition of transcription of survival genes.

TABLE 99-1 -- Type of infection related to the immune system and immunosuppressive agents.

TYPE OF RISK INFECTION RELATED TO IMMUNE SYSTEM AND IMMUNOSUPPRESSIVE AGENTS		
Immunodeficiency	**Immunosuppressive agent or chemotherapy**	**Infectious risk**
Anatomic barriers (mucositis)	High-dose chemotherapy	Bacterial: Gram positive — staphylococci, viridans streptococci, enterocci; Gram-negative — Enterobacteriacae, *Pseudomonas* spp.
		Fungal: *Candida* spp.
		Viral: herpes simplex virus
Innate immunity (quantitative defects) (qualitative defects)	High-dose chemotherapy, radiotherapy	Bacterial: Gram positive — staphylococci, viridans streptococci, *Nocardia* spp.; Gram-negative — bacilli (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp.)
		Fungal: *Candida* spp., *Aspergillus* spp.
Adaptive immunity		

Cellular	High-dose chemotherapy, radiotherapy Immunosuppressive therapies (glucocorticoids, cyclophosphamide, ciclosporin A, tacrolimus, methotrexate, azathioprine, rapamycin, anti-lymphocyte serum, monoclonal antibodies)	Bacterial: *Legionella* spp., *Mycobacterium tuberculosis*, atypical mycobacteria, *Listeria monocytogenes*, *Salmonella* spp.
		Fungal: *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*
		Viral: cytomegalovirus, varicella-zoster virus, herpes simplex virus, Epstein-Barr virus, live viral vaccines (measles, mumps, rubella, poliovirus)
Humoral	High-dose glucocorticoids, cyclophosphamide, mycophenolate mofetil	Bacterial: Gram-positive — streptococci (S. pneumoniae, others); Gram-negative — *Haemophilus influenzae*, *Neisseria meningitidis*, *Capnocytophaga canimorsus*
		Viral: enterovirus
		Parasites: *Giardia lamblia*

Clinical effects

Due to their broad mechanisms of action, GCs may influence the function of a large number of different types of cells that are crucial for the regulation of the immune response and metabolic cascades. With regard to the immunosuppressive effects, they affect the function of cells of innate immunity (e.g. monocytes/macrophages and neutrophil) and adaptive immunity, particularly T cells. Humoral immunity (e.g. production of Ig) is affected only at high dosage.

The clinical picture of patients receiving treatment with GCs may be complicated by a variety of infections[8] caused by bacteria, fungi and viruses. The severity of these infectious complications depends upon the degree of immunosuppression resulting from the treatment (Table 99.1). Retrospective studies on large cohorts of patients treated with GCs have clearly demonstrated that the risk of infections (including lethal infections) in these patients is significantly increased compared to control groups. Furthermore, it is important to underscore that the GC dosage is strictly linked to the risk of infections. A dosage of prednisone of more than 20mg/day is associated with a risk of infection that is twice that of a control group, while patients receiving a dosage of less than 10mg/day do not show an increased risk of infection.

The type of infection complicating the clinical picture is related to the components of the immune system predominantly affected. The most frequent bacterial infections are those caused by Gram-positive (*S. aureus* or coagulase-negative staphylococci) and Gram-negative bacteria (*Escherichia coli*, *K. pneumoniae, Pseudomonas* spp. *or Salmonella* spp.).[8] Although less frequent, *Nocardia* spp. and anaerobic bacteria (e.g *Prevotella bivia*) may cause cerebral or joint infections. These infections result from the direct impairment of phagocytic function of neutrophils and monocytes/macrophages by GC treatment. Mycobacterial, fungal (*Aspergillus fumigatus* and *Candida* spp.) and viral (particularly enterovirus) infections are caused by the impairment of adaptive immunity, mostly cellular immunity.

The anatomic site most frequently affected by the infectious complications is the lung. The typical radiologic finding is the presence of

infiltrates. In this regard, it is important to mention that the presence of lung infiltrates is also typical of autoimmune and rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Wegener's granulomatosis. Therefore, in these pathologic conditions it is important to distinguish between a lung pathology caused by the underlying disease and the infection caused by GC treatment. The microbes most frequently isolated in the lungs in infections complicating GC treatment[8] include *Streptococcus pneumoniae, K. pneumoniae*, *Legionella* spp. and *P. carinii*.

Alkylating agents

Mechanism of action

The alkylation of DNA is responsible for the cytotoxic, mutagenic and chemotherapeutic properties of this class of immunosuppressive agents. Cyclophosphamide (CYC) is a very potent alkylant (nitrogen mustard-derived alkylating agent) that catalyzes the alkylation of purines within the DNA and RNA (see Fig. 99.1). DNA and RNA alkylation leads to an aberrant base pairing, ring cleavage and depurination. The ultimate result is cell death due to a loss of the ability to divide. Cyclophosphamide is effective on both 'resting' and dividing (e.g. activated) lymphocytes. Both T-cell (particularly T-helper) and B-cell functions are severely suppressed by CYC treatment.

Clinical effects

On the basis of the potent immunosuppressive effects on B and T cells, CYC is largely used in the treatment of autoimmune and rheumatic diseases. In particular, it is used as primary treatment in severe SLE, vasculitis[9] (anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis) and severe RA. [10] Cyclophosphamide treatment is generally very effective in obtaining remission of the underlying disease. However, hematologic toxicity and the oncogenicity due to its antiproliferative and mutagenic properties are major adverse effects. The hematologic toxicity is due to bone marrow suppression with associated leukopenia (particularly neutropenia and CD4 T-cell lymphopenia) of variable severity. The nadir of the leukopenia is generally observed 7–14 days after one pulse of intravenous administration. The toxicity of CYC is dependent upon the dosage and the duration of treatment (cumulative dose).

The infectious complications associated with CYC treatment (see Table 99.1) include bacterial (*S. aureus* (septic shock), *K. pneumoniae* pneumonia), fungal (*Candida albicans*) and viral (herpes zoster) infections.[11] The pathogens responsible for these infections are mostly typical opportunistic pathogens. It is important to underscore that CYC is generally used in association with GCs and therefore the risk of infectious complications is substantially increased, particularly if GC dosage is more than 0.5mg/kg per day in patients aged over 60 years and with a leukocyte nadir of less than 3×10^9 /l. In this regard, a recent study on the use of CYC in patients with autoimmune diseases has clearly demonstrated that age represents a crucial risk factor for infection. [11] The proportion of patients with infectious complications was significantly higher in patients aged over 65 years (34.2%) than in a control group aged less than 65 years (8.3%). To prevent infectious complications, patients treated with CYC require close hematologic and immunologic monitoring, and in certain cases the use of antimicrobial prophylaxis. Anti-pneumocystis prophylaxis with trimethoprim-sulfamethoxazole (co-trimoxazole) is recommended:[12]

- when there is continuous oral administration of CYC; and
- if the CD4T-cell count is less than 200/mm^3 .

Although no definitive demonstration is available, the general perception is that continuous oral administration of CYC carries an increased risk of infection compared with intravenous pulses.[13] [14]

As mentioned above, CYC also has oncogenic properties. In particular, CYC therapy was found to be associated with an increased risk for bladder carcinoma. [15] However, it should be underscored that this specific oncogenic effect of CYC is only in part due to the immunosuppression, but is mostly the result of the effect of acrolein,[16] a toxic metabolite of CYC. The relationship between acrolein and the risk for bladder carcinoma is supported by the fact that chlorambucil, another alkylating agent, does not exhibit similar toxicity. Although it is well known that patients with immunologic diseases have an increased risk for cancer, it is also clear that the risk is increased by the immunosuppressive treatment.[17] It seems that alkylating agents are more oncogenic than other immunosuppressive agents. In this regard, recent studies[18] that examined the risk for cancer in patients who received CYC treatment and were followed up for more than 24 years have shown that the risk for cancer correlates with the cumulative dosage of CYC and the duration of follow-up.

Immunophilin binding agents

Immunophilins are a large family of broadly expressed proteins that bind to certain immunosuppressive agents, such as cyclosporine A (CsA), tacrolimus (also known as FK-506) and rapamycin (also known as sirolimus). Due to their potent immunosuppressive effects that target predominantly T cells, these agents are fundamental components of the immunosuppressive regimens in both solid organ[19] [20] and bone marrow transplantation.[21]

Mechanism of action

Ciclosporin A is a lipophilic undecapeptide reversible inhibitor of T-cell activation that acts by interfering with calcineurin and thus IL-2 synthesis and release.[22] Ciclosporin A activity is lymphocyte specific and it blocks quiescent lymphocytes in G_0 –G_1 phase, thus reducing the number of cells that can be activated.

Physiologically, T-cell receptor (TCR) signaling induces an elevation in the concentration of Ca^{2+} in the cytoplasm and activates the transcription factor AP-1. Ca^{2+} binds to calcineurin, which in turn dephosphorylates the cytoplasmic form of NFAT. Once NFAT migrates into the nucleus, it forms a complex with AP-1, thus inducing the transcription of genes required for T-cell activation including IL-2. When CsA is present in the cytoplasm, it forms a complex with cyclophilin (Cyp). The CsA-Cyp complex can bind to calcineurin, blocking its ability to activate NFAT, and therefore inhibiting T-cell activation (Fig. 99.2). Furthermore, the other effects of CsA, such as renal toxicity and hypertension, can be explained on the basis of the increased activity of the endothelin-converting enzyme that causes increased production of endothelin-1 (ET-1), a vasoconstrictive and proinflammatory peptide. Endothelin-1 is potentially involved in transplant vasculopathy and chronic rejection.

Like CsA, tacrolimus binds to immunophilins, and in particular to the FK-506 binding proteins (FKBPs).[23] Although tacrolimus binds all FKBPs, FKBP 12 is the protein preferentially bound. Unlike CsA, tacrolimus is an antibiotic belonging to the macrolide class. Although CsA and tacrolimus bind to different proteins, their mechanism of action is very similar because tacrolimus also inhibits calcineurin. Tacrolimus, however, is 10- to 100-fold more potent than CsA and acts on multiple transcriptional factors such as NFAT and NF-?B, thus influencing the production of several cytokines, including IL-2, IL-3, IL-4, IL-5, IFN-?, granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF-a, and proto-oncogenes c-*myc* and c-*rel*. Like CsA, tacrolimus exerts a potent suppression of T cells (particularly Th1 cells). However, it also has a substantial inhibitory effect on Th2 cells, thus influencing the production of Ig and humoral immunity.

Rapamycin (sirolimus) is also a macrolide antibiotic. Discovered before tacrolimus, it was first used either as an antifungal or as antiproliferative agent in the treatment of certain tumors (prostate, lymphoma, glioblastoma, melanoma and small cell lung tumors).

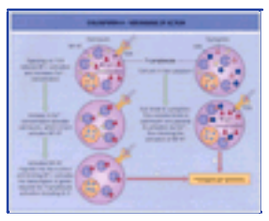

Figure 99-2 Mechanism of action of ciclosporin A. Ciclosporin A inhibits T-cell activation by interfering with calcineurin. It is a lipophilic undecapeptide reversible inhibitor of T-cell activation that acts by interfering with calcineurin. The T-cell receptor induces signaling through an elevation in concentration of Ca^{2+} in the cytoplasm. This activates the transcription factor AP-1. The Ca^{2+} binds to calcineurin, which in turn dephosphorylates the cytoplasmic form of NFAT. Then NFAT migrates into the nucleus and forms a complex with AP-1. This complex can induce the transcription of genes required for T-cell activation including IL-2. In the presence of CsA a complex forms with cyclophilin (Cyp). The CsA-Cyp complex can bind to calcineurin, blocking its ability to activate NFAT, and therefore inhibiting T-cell activation.

Sirolimus binds to the FKBP. However, the sirolimus-FKBP complex does not bind calcineurin, but binds to a protein called the mammalian target of sirolimus (mTOR), which regulates the translation of mRNA necessary for cell division. Therefore, sirolimus interferes with the transition from the G_1 to S phase of the cell cycle. The immunosuppressive potency of sirolimus is very similar to that of CsA and FK-506 but the toxicity profile is different. Finally, a derivative of sirolimus, everolimus, has recently been developed.[24] [25] [26] The clinical efficacy of this new agent, particularly in combination with CsA, is currently under investigation.

Clinical effects

Due to the selective immunosuppressive effects on T cells, the infectious complications associated with immunophilin binding agents are predominantly caused by viral and fungal infections (see Table 99.1). However, it should be emphasized that because these agents are often used in combination with other classes of immunosuppressive agents, it is difficult to identify infectious complications specific to a particular class of immunosuppressive agents. Since the immunophilin binding agents are predominantly used in the field of transplantation, the infectious complications that will be discussed are those encountered in transplanted patients. Two viral infections, namely CMV[27] [28] [29] [30] [31] and BK virus[32] [33] infection, are thought to be strictly related to the immunosuppressive treatment with CsA or tacrolimus. The risk of CMV disease also depends on the degree of immunosuppression, and it has been clearly shown that the concomitant use of other agents, such as monoclonal antibodies[34] (see below), increases the risk for CMV disease. Preliminary studies seem to indicate that everolimus, the sirolimus derivative, is associated with a lower frequency of CMV disease than CsA.[25]

The BK virus, a polyomavirus, is widely distributed in the healthy population. On the basis of its tropism for the urinary tract,[32] [33] BK virus causes pathologic conditions of variable severity such as asymptomatic hematuria, BK virus-induced interstitial nephropathy and BK virus-associated nephritis. At the present time, renal biopsy is the only valid diagnostic approach to confirm BK virus involvement when clinical signs of nephritis are present. The demonstration of viral inclusions in the proximal and distal tubules is typical of BK virus infection. Serology has no diagnostic value because of the large distribution of the virus in the healthy population.

Administration of immunophilin binding agents as well as other immunosuppressive agents increases the risk for cancer and particularly lymphomas. [27] The mechanisms responsible for the development of lymphomas or lymphoproliferative syndromes are directly related to EBV.[37] [38] In fact, in the case of transplantation in children, the incidence of post-transplant lymphoproliferative disease (PTLD) is up to 10% with a mortality rate of 60% and an elevated morbidity that may lead to loss of the transplant. PTLD is caused by an EBV primary infection and depends upon the intensity of the immunosuppressive treatment. There is spontaneous regression of PTLD following the decrease in intensity of immunosuppression, thus confirming the crucial role played by T cells, particularly CD8T cells, in the control of EBV. [39]

Finally, the toxicity profiles (e.g. renal toxicity, hypertension and neurotoxicity) of CsA and tacrolimus are very similar. Typical of CsA are hirsutism, coarsening of facial features and gingival hyperplasia, while diabetes and neurotoxicity are more frequently seen in patients treated with tacrolimus.

With regard to sirolimus, the infectious complications are similar to those of CsA and tacrolimus. However, the toxicity profile of sirolimus is quite different from that of CsA and tacrolimus. Nephrotoxicity, hypertension and neurotoxicity are rarely associated with sirolimus treatment. Side-effects associated with sirolimus include hyperlipidemia, leukopenia and thrombocytopenia. On the basis of the different toxic profile, sirolimus can be used in combination with CsA or tacrolimus.

Inhibitors of nucleotide synthesis

Azathioprine

Mechanism of action

Azathioprine (AZA) and its metabolite 6 mercaptopurine (6-MP) belong to the class of thiopurines. The targets of these drugs are the

enzymes involved in the de-novo synthesis of purines. In particular, AZA interferes with the de-novo synthesis of inosinic acid through a feedback inhibition of the 6-thioinosinic acid. Furthermore, it inhibits the conversion of puric bases, such as inosine, into adenosine or guanine ribonucleotides. Therefore, AZA inhibits DNA replication in dividing cells including lymphocytes (see Fig. 99.1). Activated T cells are the primary target of AZA. Azathioprine does not exert any cytotoxic effect on T cells before an antigen challenge, and the most potent immunosuppressive activity occurs following antigen stimulation. On the basis of this mechanism of action, AZA has no effect on memory T cells. In addition to activated T cells, AZA exerts potent immunosuppression on B cells. Due to its selective effect on dividing cells, AZA rarely causes severe lymphopenia and only prolonged treatment may be associated with a substantial reduction of circulating lymphocytes. Like other immunosuppressive agents, AZA also mediates an anti-inflammatory effect by inhibiting cell division of the different lineages of hematologic precursors.

Clinical effects

Originally largely used in the field of transplantation, AZA has been progressively replaced by more potent purine inhibitors such as mycophenolate mofetil (MMF, see below). However, it continues to be used in the treatment of autoimmune [40] and rheumatic diseases. Azathioprine has the advantage of being well tolerated and is

particularly effective against the hematologic, pulmonary and skin manifestations of autoimmune and rheumatic diseases. Furthermore, it may be safely administered during pregnancy and there is no evidence for an increased risk of malignancies in patients with SLE.

Three major side-effects characterize AZA administration:

- bone marrow suppression,
- gastrointestinal intolerance, and
- infections.

It is, however, important to underscore the moderate severity of the toxicity profile of AZA, which rarely requires the interruption of treatment and is rapidly reversed by the cessation of treatment. Severe bone marrow suppression has been reported in patients with concomitant treatment with allopurinol.

On the basis of the selective inhibition of actively dividing T and B cells following antigen-specific stimulation, the infectious complications

Figure 99-3 Mechanism of inhibition of purine synthesis by mycophenolic acid. MPA inhibits inosine monophosphate dehydrogenase (IMPDH) and leads to the depletion of guanosine nucleotides. PRPP, 5-phosphoribosyl-1(a)-pyrophosphate; ribose-5P, D-ribose-5'-phosphate.

associated with AZA treatment are predominantly caused by viruses (particularly herpesviridae) and to a lesser extent by fungi and parasites. The infectious complications are rarely observed in patients with autoimmune and rheumatic diseases. They have been mostly observed in the field of renal transplantation in patients receiving AZA as a part of a multiple immunosuppressive regimen. The most common infectious complication (see Table 99.1) was CMV infection, which was more frequent in the group receiving an immunosuppressive regimen including MMF compared with one including AZA.[41] As mentioned above for the other classes of immunosuppressive agents, the age of the patients (>60 years of age) represents an important risk factor for infectious complications during immunosuppressive therapy.[42]

The potential oncogenic properties of AZA are likely the result of the uptake of 6-thioguanosine triphosphate into cell DNA. An increase in the incidence of tumors, including non-Hodgkin's lymphoma (NHL), squamous skin cell carcinoma and others,[43] has been reported in nontransplanted patients receiving therapy with AZA, CYC or chlorambucil. In these studies the appearance of NHL occurred shortly after the initiation of the immunosuppressive treatment. However, several studies did not show an increased risk of tumors in patients with SLE, RA and inflammatory bowel disease treated with AZA.

Mycophenolate mofetil

Mechanism of action

Mycophenolate mofetil is the prodrug of mycophenolic acid (MPA). It is a highly selective noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH),[44] an enzyme involved in the denovo synthesis of guanosine nucleotides. In this regard, it is of note that purine synthesis may occur through two different pathways:

- de novo, and
- by the salvage pathway (Fig. 99.3).

Blood cells originating from the erythrocyte, megakaryocyte and myeloid lineages may use the salvage pathway if the de-novo pathway is inhibited. In contrast the salvage pathway is not functional in B and T cells. Therefore, the suppressive activity of MMF is quite selective for T and B cells, and particularly proliferating lymphocytes. The inhibition of IMPDH results in a depletion of

guanosine nucleotides that are critical for DNA synthesis and thus cell proliferation (see Fig. 99.1).

From an immunologic standpoint, MMF has no effect on resting B and T cells, but strongly suppresses proliferation of activated T cells by inducing apoptosis. It is a potent suppressor of primary and secondary T-cell responses. It also mediates potent inhibition of primary humoral immune response, but seems to be less effective in the inhibition of secondary humoral responses.[45] The dual effect on T and B cells is responsible for the infectious complications (particularly CMV infection, see below) associated with MMF therapy. Furthermore, due to the potent suppression of primary humoral immune responses, vaccination generally fails to induce humoral immune responses in patients under MMF treatment. Mycophenolate mofetil also reduces the synthesis of adhesion molecules, such as very late antigen 4 (VLA-4) and VCAM-1, which results in a decreased recruitment of lymphocytes in the transplanted organ.[44] In contrast to CsA and tacrolimus, MMF does not inhibit the synthesis of IL-2 and IL-2 receptor.

Clinical effects

Mycophenolate mofetil is predominantly used in the field of solid organ transplantation and has replaced AZA in combinations of immunosuppressive agents to prevent acute and chronic rejection. A series of studies have clearly demonstrated the superiority of MMF over AZA. Mycophenolate mofetil was initially used in renal [46] transplantation, but has also recently been used in heart and liver transplantation.

Mycophenolate mofetil is generally a well-tolerated drug that has the advantage of not being metabolized through cytochrome P450. The most common side-effects associated with MMF treatment are gastrointestinal (nausea, vomiting and diarrhea) and hematologic. These side-effects are quite important during the first 4 weeks of therapy and generally become less important after prolonged treatment. Serious adverse events are extremely rare and may include pancreatitis, hemorrhagic gastritis and cholestasis.

The infectious complications (see Table 99.1) are very similar to those described for AZA.[41] The frequency of CMV infection has been reported to be greater in patients receiving high (>3g/day) doses of MMF than in those receiving AZA or placebo. The increased incidence of CMV infection in patients treated with MMF has been confirmed in the fields of renal[41] and allogeneic stem cell[47] transplantation. The mechanism by which MMF treatment is associated with an increased risk for CMV infection is unclear. It has been proposed that the suppression of B in addition to T cells may play an important role.[48] It is, however, unclear whether CMV-specific humoral immunity has any protective role. In patients treated with MMF, but receiving aciclovir or ganciclovir for anti-CMV prophylaxis, the incidence of CMV infection was comparable to that in patients not treated with MMF. In contrast to CMV, treatment with MMF is not associated with an increased risk of infection with other herpesviruses. *Pneumocystis carinii* pneumonia (PCP) is also a typical infectious complication of transplanted patients.[49] The analysis of a large number of patients who underwent renal transplantation has shown an absence of PCP in the group of patients treated with MMF.[50] In this regard, it is noteworthy that it has been shown that MMF is extremely active against *P. carinii* in animal models.[51]

Like AZA, an increased risk for skin cancer and NHL has been associated with MMF treatment. Although some studies have shown a higher incidence of lymphoproliferative disorders in MMF-treated versus AZA-treated patients, other studies have failed to confirm a more potent oncogenic property for MMF. As already mentioned for other immunosuppressive agents, the oncogenicity is dependent upon the state of general immunosuppression caused by combination therapy rather than one particular immunosuppressive agent.

Methotrexate

Mechanism of action

Originally developed as an anticancer agent, methotrexate (MTX) is an antifolate[52] with a potent immunosuppressive action. Methotrexate has a structure similar to that of the folates and uses the same mechanisms of transport to cross the membrane. In particular, two mechanisms have been identified:[52]

- via a low affinity transporter, and
- via a folate-binding-protein (FBP) associated with the cell membrane.

In addition, MTX can also passively cross the membrane.

Methotrexate has numerous enzymatic targets. Critical for its cytotoxic activity and for the selectivity of its mechanisms of action is the fact that MTX, like folates, is subjected to a polyglutamation that increases the intracellular half-life.[53] The importance of the polyglutamation is demonstrated by the finding that cell lines defective in polyglutamyl synthetase, the enzyme that catalyzes the polyglutamation, are resistant to MTX treatment. It is indeed the polyglutamate form of MTX that is immunosuppressive. The primary target of MTX is the dihydrofolate reductase (DHFR) that blocks the formation of tetrahydrofolate (FH_4) from dihydrofolate (FH_2) within the metabolic cycle of the thymidylate (dTMP). The thymidylate synthase is the enzyme that catalyzes the reaction from uracyl-5'-monophosphate (dUMP) to thymidine-5'-monophosphate dTMP. The inhibition of DHFR is the primary mechanism of suppression of pyrimidine synthesis. Inhibition of pyrimidine synthesis by MTX also occurs through the suppression of another enzyme, thymidylate synthase. Furthermore, MTX inhibits the de-novo synthesis of purines by blocking the enzymatic activity of the 5-amino-imidazole-4-carboxamide-ribonucleotide (AICAR) transformylase. Therefore, the immunosuppressive effect of MTX is mostly the result of the suppression of pyrimidine and purine synthesis (see Fig. 99.1).

With regard to the anti-inflammatory effect, it has been shown that MTX reduces the chemotaxis of neutrophils, inhibits the synthesis of leukotriene B4 and reduces the synthesis of IL-1. In addition, the inhibition of the activity of AICAR transformylase induces an increase of adenosine, which is the most potent endogenous anti-inflammatory factor. In this regard, it is thought that the anti-inflammatory effect of MTX is mostly due to the increase in endogenous adenosine. Finally, it has been shown that MTX induces apoptosis of activated but not resting CD4 and CD8 T cells.[54]

Clinical effects

As mentioned above, MTX at higher dosage (100–1000mg/m^2 per cycle) has been extensively used in the field of oncology. At lower doses (5–25mg/week) it is essentially used as an anti-inflammatory agent and as an immunomodulator in the treatment of autoimmune diseases and in particular in patients with RA. Methotrexate as well as leflunomide (see below) are also known as disease-modifying anti-rheumatic drugs (DMARDs).[55] Furthermore, due to its anti-inflammatory and immunosuppressive effects, MTX together with CsA or tacrolimus is used in the treatment of graft versus host disease.[56]

A variety of side-effects have been described in patients treated with MTX. These include severe liver and lung (interstitial pneumonitis)[57] toxicity, and infectious complications (upper respiratory tract infections, urinary tract infections). In addition, the defect in folate concentration caused by MTX may be responsible for severe toxic effects such as myelosuppression, hepatotoxicity and diarrhea.[58] [59] These latter toxic effects can be in part prevented or controlled by the administration of folic or folinic acid, which compete with the activity of MTX.[60]

Leflunomide

Mechanism of action

Like MTX, leflunomide is an inhibitor of de-novo pyrimidine synthesis [61] (see Fig. 99.1). Leflunomide inhibits the mitochondrial enzyme dihydro-orotate dehydrogenase (DHODH), which is involved in the de-novo synthesis of ribonucleotide uridine monophosphate pyrimidine (rUMP). The active metabolite of leflunomide, A77 1726, inhibits DHODH. This inhibition is reversible, but prevents the denovo synthesis of pyrimidines. The inhibition of the de-novo synthesis of pyrimidines particularly affects lymphocytes as compared with other cell lineages. The inhibition of DHODH prevents the accumulation of sufficient levels of pyrimidine within lymphocytes in order to support DNA synthesis and thus proliferation.[62] In addition to the inhibition of DHODH, leflunomide suppresses TNF-a production by inhibiting NF-?B activity.[63]

Clinical effects

Leflunomide is used in the treatment of RA.[55] [62] The efficacy and toxicity of leflunomide are comparable to those of MTX.[58] [59] [64] These latter include particularly diarrhea, abdominal pain, nausea, vomiting and alteration of liver function parameters. The gastrointestinal effects are particularly important during the first 2 weeks of treatment and tend to decrease thereafter. In addition, allergic reactions have been reported following leflunomide treatment, and also reversible alopecia. Diarrhea has been more frequently associated with leflunomide than MTX treatment while oral ulcers are more frequent with MTX.

The most frequent infectious complications affect the upper respiratory tract.[59] These infectious complications are generally more frequent during the first year of treatment but only exceptionally cause an interruption of therapy.[58]

Monoclonal antibodies

Antilymphocyte antibodies are an important therapeutic tool in the field of immunosuppression and transplantation, and are among the first immunosuppressive agents used in liver transplantation.[20] A major limitation of these agents is their lack of specificity and their partial efficacy. In fact, the risk for acute or chronic graft rejection remains elevated. Another issue of serious concern is the toxicity profile, with an increased risk for opportunistic infections and malignancies.

The first antilymphocyte antibody preparations developed in the field of transplantation were polyclonal. As mentioned above, they are potent immunosuppressive agents that cause massive depletion of T cells. Although the clinical efficacy when used as an inductive immunosuppressive agent is controversial (the survival of the graft was improved[65] in certain studies but not in others[66]), they have been shown to be effective in the treatment of GC-resistant acute graft rejection.[66] Furthermore, the use of antilymphocyte antibodies is also difficult for a variety of reasons including:

- an increased risk for infectious complications and malignancies;
- intravenous administration in vessels of large caliber;
- side-effects such as thrombocytopenia;
- lack of specificity for the target;
- low proportion of antilymphocyte antibodies within the antibody preparations administered (about 2%); and
- neutralization of the therapeutic effect by the induction of anti-bodies directed against the antilymphocyte antibody preparations administered.[67]

On the basis of the limitations mentioned above, polyclonal antibodies have been progressively replaced by the development of a series of monoclonal antibodies.

Muromonab CD3

Muromonab CD3 is a highly specific monoclonal antibody directed against the surface antigen CD3 expressed by the majority of T cells. Although the use of muromonab CD3 is not associated with allergic reactions or serum sickness, its toxicity profile is characterized by the so called 'cytokine release syndrome'. [68] In fact, the CD3 surface antigen is physically linked to the TCR and the CD3 complex represents the signal transduction machinery for T cells. Therefore, the binding of anti-CD3 antibody to the CD3 surface antigen causes massive activation of T cells. The latter is responsible for cytokine release and the clinical picture may be complicated by a pharmacologic shock of variable severity. For these reasons, the use of muromonab CD3 has been abandoned.

Anti-interleukin-2 receptor antibodies

Interleukin-2 is a major T cell growth factor necessary for the expansion of T cells following antigen-specific stimulation, which represents a fundamental step in the generation of the T-cell-mediated immune response. Anti-IL-2 receptor (R) monoclonal antibody formulations for clinical use have been developed to interfere with the initial expansion of T cells. These antibodies are directed against the a chain of the IL-2R (anti-CD25 antibody).[69] The original preparations of anti-IL-2R antibodies were of murine origin. Recently, chimeric forms of antibodies have been generated. These contain the majority of sequences of human IgG with incorporated murine sequences in the hypervariable region specific for the achain of IL-2R.[69] Two preparations of IL-2R antibodies are currently available for clinical use: basiliximab [70] [71] and daclizumab. [72] [73] [74] Basiliximab is a chimeric antibody that contains less than 10% of murine sequences while daclizumab is a humanized antibody. The half-life of these antibody preparations is 6.5 and 11 days, respectively.

The primary goal of using anti-IL-2R antibody in the field of transplantation is to reduce the incidence of acute graft rejection[75] and eventually the dose of the other immunosuppressive agents (particularly the inhibitors of calcineurin). Combination of IL-2R antibody with other immunosuppressive agents has been shown to be effective in the prevention of acute rejection in renal and liver transplantation even if inhibitors of calcineurin are not part of the therapeutic combinations.

Therapy with IL-2R antibody is extremely safe and well tolerated. No increased risk for viral and bacterial infections has been reported.

Inhibitors of tumor necrosis factor-a

Tumor necrosis factor-a is a potent cytokine and a primary mediator of inflammatory reactions.[76] It plays a central role in many inflammatory diseases including inflammatory bowel and rheumatic diseases. Several studies have demonstrated the fundamental role of TNF-a in the pathogenesis of RA.[76] [77] Transgenic mice with a deregulation of the TNF-a gene develop a destructive arthritis similar to RA. In addition, TNF-a is clearly implicated in the pathogenesis of juvenile RA and psoriatic arthritis and Crohn's disease.[78]

Two specific inhibitors of the function of this cytokine have been developed:[77] infliximab and etanercept. Infliximab is a chimeric (human/mouse) monoclonal antibody directed against TNF-a. The Fc portion of infliximab is a human IgG1 while the antigen-binding variable domain that has a high affinity for the human TNF-a is of murine origin. Etanercept is the human recombinant form of the soluble TNF receptor. It is a dimerized fusion protein formed by an extracellular domain of the TNF type II receptor (p75) and by the Fc portion of a human IgG1. The mechanism of action of etanercept is the blocking of soluble and membrane-bound TNF and lymphotoxin a.

Both infliximab and etanercept are effective in blocking inflammation. [79] [80] The efficacy of etanercept has been extensively documented in clinical studies in patients with RA and even in the case of RA resistant to MTX treatment.[79] [80] In addition to the treatment of RA, juvenile RA and Crohn's disease, inhibitors of TNF activity can play an important role in the treatment of other pathologic conditions such as sarcoidosis, ankylosing spondyloarthritis and Wegener's granulomatosis.

With regard to the toxicity profile of TNF inhibitors, systemic (infliximab) and urticarial (etanercept) reactions never require discontinuation of treatment.[80] A major concern with treatment with TNF inhibitors is the increased risk for infectious complications. Infectious complications have been reported in 50 and 60% of patients treated with infliximab and etanercept, respectively. The increased risk for infections is the result of the dysfunction of the protective mechanisms against intracellular pathogens. Mycobacterial infections are those more commonly associated with the blocking of TNF-a activity. [81] Numerous cases of tuberculosis have been reported following infliximab administration. On the basis of these complications, the current guidelines for the therapeutic use of TNF inhibitors indicate that a purified protein derivative (tuberculin) test should be done before the initiation of treatment. In the case of a positive test without other signs of reactivation of the disease, treatment with isoniazid is advised during administration of infliximab.[82] The number of cases of reactivation of tuberculosis seems to be less important following treatment with etanercept. Other infections associated with TNF-inhibitor treatments include those caused by *P. carinii*, *Legionella* spp., *L. monocytogenes* and other fungi.[83] [84]

New immunosuppressive agents

FTY720

FTY720 is the synthetic derivative of myriocin, a potent immunosuppressive drug that was originally described in 1994.[85] This immunosuppressive agent has a unique mechanism of action. FTY720 induces an alteration of the homing and trafficking of lymphocytes through the modulation of the expression of cell surface molecules.[86] This results in a significant reduction in the number of lymphocytes. Neutrophils and monocytes are not affected by FTY720. The lymphopenia occurs 6–12 hours after administration and persists over time during treatment, but is reversed by an interruption in treatment. Apoptosis seems to be the mechanism responsible for the depletion of lymphocytes.

With regard to the clinical effects, the majority of data available have been generated in experimental animal models in the fields of transplantation and autoimmunity (autoimmune myocarditis and uveoretinitis or type I diabetes).[86] [87] [88] The results indicate that FTY720 is effective in prolonging the survival of the graft in renal, cardiac, liver and skin transplantation. Furthermore, as FTY720 is metabolized through cytochromes that differ from those involved in the metabolism of CsA, tacrolimus and rapamycin, the risk for pharmacologic interactions with other immunosuppressive agents is unlikely. Thus, FTY720 can potentially be used in combination with other immunosuppressive agents and this may allow the design of novel immunosuppressive therapeutic strategies for the prevention of the graft rejection.

Due to its selective effects on lymphocytes, FTY720 does not induce a global immunosuppression. The results obtained from animal models did not show the appearance of infectious complications even at high doses and after prolonged treatment. Similarly, there was no evidence for nephrotoxicity and mutagenicity.

CONCLUSION

Major advances have been made in the past two decades in our understanding of fundamental immunoregulatory mechanisms, in the development of novel immunosuppressive agents and in the prevention of the infectious complications associated with immunosuppressive therapy. Future goals in the field of immunosuppression should include an improvement in the management of the infectious complications and the development of novel and more selective agents. The generation of highly selective agents is the only valid strategy to maximize the immunosuppressive effects and minimize the immunodeficiency.

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Chapter 100 - Infections in the Neutropenic Cancer Patient

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Thierry Calandra

INTRODUCTION

Cancer occurs in one of every four people and is one of the leading causes of death in developed countries. Cancer can be subdivided in two main categories: solid tumors and hematological malignancies, which include leukemias, lymphomas and multiple myeloma. Solid tumors account for more than 90% of all new cancer cases and hematological malignancies for the remaining 5–10%.[1] Over the past decades, joint efforts of basic science and clinical research have resulted in substantial improvements of prevention, early detection and treatment of cancer. Indeed, overall 5-year survival rates in cancer patients have improved from 39% in the 1960s to 60% in the 1990s.[1] Solid tumors are frequently treated with combined treatment modalities including surgery, radiation therapy and chemotherapy. In contrast, chemotherapy is the cornerstone of the management of patients with hematological malignancies. New therapeutic options, such as immunotherapy and gene therapy, are being developed.

Infections frequently occur during treatment of cancer. Many factors contribute to increase the risk of infection: poor clinical and nutritional status, mechanical obstruction of natural passages, damage to anatomic barriers (surgery, use of prosthetic and intravascular devices) and defects of humoral and cell-mediated immunity that are either disease associated or secondary to radiotherapy or chemotherapy. Cytotoxic agents exert their effects on both malignant cells and normally replicating progenitor cells and thus also cause major toxicity on normal tissues with high turnover (i.e. bone marrow and mucous membranes), resulting in myelosuppression and alteration of physiological barriers. Historically, hemorrhage and infections have been major complications and leading causes of chemotherapy-related mortality (10–20% and 50–80%, respectively).[2] In the 1960s, both the severity and duration of granulocytopenia were identified as major determinants of infectious complications.[3] In the early 1970s, prompt empirical antibiotic treatment became the cornerstone of management of febrile neutropenic patients, resulting in drastic reduction of the mortality of bacterial infections.[4] Since then, major progress has been made in the understanding of the pathogenesis and treatment of infectious complications of cancer patients. Development of novel diagnostic and treatment strategies continues to improve the outcome of febrile neutropenic cancer patients.[5] [6] [7]

EPIDEMIOLOGY

The majority of infections in granulocytopenic cancer patients are caused by micro-organisms of the patient's endogenous flora.[5] [6] [7] However, exogenous air-borne and food-borne pathogens, acquired either in the community or in the health care system, can also cause infection.

Bacterial infections

Gram-positive and Gram-negative bacteria are the predominant pathogens in this clinical setting (Table 100.1). [5] In the past decades, most cancer centers have experienced major changes regarding the etiology of bacterial infections in the neutropenic host.[5] [6] [7] [8] While Gram-negative bacteria were predominant in the 1970s and early 1980s, the frequency of Gram-positive bacteria markedly increased in the late 1980s and early 1990s, when they became the prevalent pathogens in many institutions. However, this trend reversed in the late 1990s. Gram-negatives and Gram-positives now account for an equal proportion of infections in Europe (Fig. 100.1).[8]

Many factors are involved in these epidemiological shifts. The increasing incidence of infections due to coagulase-negative staphylococci and other Gram-positive skin colonizers has been associated with the increased use of intravascular access devices. The emergence of viridans streptococcal infections, sometimes associated with acute respiratory distress syndrome (ARDS) and septic shock, has been attributed to several factors including the toxicity of high-dose chemotherapy with cytosine arabinoside on oral mucous membranes, the reactivation of oral HSV infection and the use of fluoroquinolone prophylaxis.[9] Among Gram-negative bacteria, *Escherichia coli, Klebsiella* species and *Pseudomonas aeruginosa* are the most common bloodstream isolates. However, the incidence of *Pseudomonas aeruginosa* infections, a predominant cause of bacteremia in the 1960s and 1970s, has substantially declined over the last 30 years. The use of fluoroquinolone prophylaxis has undoubtedly played a major role in the decreasing incidence of Gram-negative infections observed in the late 1980s and early 1990s.[10] The recent re-emergence of Gram-negative infections is probably due to the reduced use of fluoroquinolone prophylaxis in many centers out of concern about increased resistance.[11]

Fungal infections

Fungal infections are a major threat to neutropenic cancer patients. Disseminated mycoses have been demonstrated in 10–40% of autopsies in patients with hematological malignancies, especially in patients who have been treated with broad-spectrum antibiotics and corticosteroids.[2] Classically occurring as secondary infections in patients with prolonged and profound neutropenia, fungal infections also account for approximately 5% of initial infections episodes. Mixed fungal and bacterial infections may occur and the fungal infection may manifest as persistent fever after eradication of the bacterial pathogen. Eighty to ninety percent of fungal infections are caused by *Candida* species (mainly *C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. pseudotropicalis*). *Aspergillus* (mainly *A. fumigatus* and *A. flavus*) and other emerging fungi including *Fusarium, Pseudallescheria boydii, Scedosporium, Rhizopus* and *Mucor* account for the remaining 10–20%.[2] Azole-resistant non-*albicans Candida* species (*C. krusei, C. glabrata*) have emerged in some cancer centers, usually in conjunction with several predisposing factors, including fluconazole prophylaxis.[12] [13]

Other pathogens

Reactivations of latent herpes simplex virus (HSV) and varicellazoster virus (VZV) infections are common in patients with hematological malignancies, especially after chemotherapy or treatment with corticosteroids.[5] [6] [7] In contrast to other immunocompromised

TABLE 100-1 -- Most common pathogens in neutropenic cancer patients.

MOST COMMON PATHOGENS IN NEUTROPENIC CANCER PATIENTS
Gram-positive aerobic bacteria
Coagulase-negative staphylococci
Viridans streptococci
Staphylococcus aureus
Other streptococci (*S. pneumoniae*, *S. pyogenes*)
Enterococcus spp.
Corynebacterium spp. (*C. jeikeium*)
Bacillus spp.

Listeria monocytogenes
Gram-negative aerobic bacteria
Escherichia coli
Klebsiella spp.
Pseudomonas spp.
Other Enterobacteriaceae (*Proteus*, *Enterobacter*, *Serratia*, *Citrobacter* spp.)
Other nonfermentative bacilli (e.g. *Stenotrophomonas maltophilia*)
Legionella spp.
Anaerobic bacteria
Bacteroides species
Clostridium species
Fusobacterium species
Propionibacterium species
Fungi
Candida species
Aspergillus species
Other molds (*Fusarium*, *Pseudallescheria boydii*, *Scedosporium*, *Rhizopus*, *Mucor*)
Viruses
Herpes simplex virus
Varicella-zoster virus
Respiratory viruses (influenza, respiratory syncitial virus)
Parasites
Strongyloides stercoralis
Other parasites in endemic areas (e.g. *Leishmania*)

patients, especially transplant recipients, cytomegalovirus (CMV) infections play a minor role in neutropenic cancer patients, as acquired immunity is less severely suppressed than innate immunity. Other viral infections such as respiratory viruses (influenza, respiratory syncytial virus) and parvovirus B19 occur occasionally. Primary

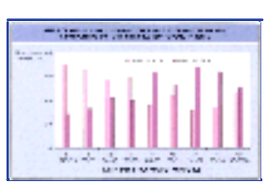

Figure 100-1 Single-organism bloodstream infections due to Gram-negative and Gram-positive bacteria in febrile neutropenic patients. European Organisation for Research and Treatment of Cancer — International Antimicrobial Therapy Group (EORTC-IATG) studies (1973–2000). *Adapted from reference* [8] .

parasitic infections as well as reactivations of latent infections, in particular those due to *Strongyloides* or *Leishmania*, only occur in patients who have lived in or visited endemic areas.

PATHOGENESIS

Underlying conditions

Microbial invasion and development of infection are facilitated by the presence of co-morbidities, immunosuppression and damage to anatomic barriers caused by the cancer itself or induced by chemotherapy.[5] [6] [7] Obstruction of the lumen of natural body passages (i.e. urinary, biliary, respiratory or digestive tract) by cancer impairs the flow of body fluids and secretions, creating conditions that promote microbial growth. Cytotoxic chemotherapy damages the epithelial tissue lining, resulting in loss of the integrity of the mucous membrane barrier. Development of mucositis therefore predisposes to infection by the patient's endogenous commensal flora and colonizing pathogens. Injury to the skin by venous puncture, presence of indwelling vascular access devices, bone marrow aspiration, lumbar puncture and other surgical interventions can also promote skin and soft tissue infections.

Defects of innate and acquired immunity

Neutropenia

Phagocytes (neutrophils, monocytes, macrophages and dendritic cells) are a critical component of the host innate immune defenses against infections. Thus, any alteration in function or number of these cells, especially neutrophils, will result in an increased risk of infection.[5] [6] [7] Neutropenia is defined as a neutrophil count <500 cells/mm^3 or <1000 cells/mm^3 with expected decrease to <500 cells/mm^3 within 48 hours. Studies performed in the 1960s have shown that there is an inverse relationship between the number of circulating neutrophils and the incidence of infections.[3] As the neutrophil count decreases to <1000 cells/mm^3 , the incidence of infections increases markedly (Fig. 100.2). The risk of severe infectious complications such as bloodstream infections is greatest when the neutrophil count drops below 100 cells/mm^3 .

The duration of neutropenia is also a major determinant of the risk of infection.[3] As shown in Figure 100.2 , the longer the duration of neutropenia, the greater the risk of infection. Profound and prolonged neutropenia (i.e. <500 cells/mm^3 for more than 10 days)

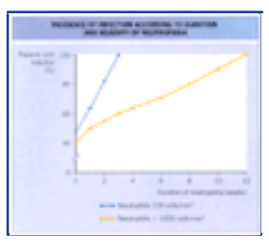

Figure 100-2 Incidence of infection according to duration and severity of neutropenia. *Adapted from reference* [5] .

is considered to be a major risk factor for both primary and secondary bacterial or fungal infections. Patients with neutropenia lasting less than 7–10 days are at low risk of complications.[5] Indeed, 95% of patients with neutropenia lasting less than 1 week respond to the initial empirical antibiotic therapy, while two-thirds of those with neutropenia for more than 2 weeks may require treatment modifications.[5] [14] Moreover, the risk of recurrence of fever and infection is also substantially lower (<1%) in patients with neutropenia of short duration than in those with neutropenia lasting more than 2 weeks (38%).[14]

Yet factors other than the severity and duration of neutropenia also help to identify who is at low or high risk of infectious complications (see Risk assessment, below).

Other immune defects

Neutropenia is the main immune defect of cancer patients. In general, defects of the humoral or cell-mediated components of acquired immunity are not predominant in these patients. However, specific immune defects directly associated with the underlying malignancy (e.g. hypogammaglobulinemia in chronic lymphatic leukemia or multiple myeloma) or its management (e.g. therapy with high-dose corticosteroids) may occur and further increase the risk of infections in conjunction with neutropenia.[5] [6] [7]

CLINICAL FEATURES

Fever

During neutropenia, fever develops in virtually all patients with hematological malignancies and in about half of those with solid tumors.[5] Although any temperature distinctly above baseline is indicative of fever, fever has been arbitrarily defined as a single temperature >101.3°F (38.5°C) or >100.4°F (38°C) on two or more occasions during a 12-hour period by the Consensus Expert Panel of the International Immunocompromised Host Society, or as a single temperature reading >100.9°F (38.3°C) or =100.4°F (38°C) during at least 1 hour by the Fever and Neutropenia Guidelines Panel of the Infectious Diseases Society of America.[5] [15] Temperature should be measured orally or by auditory canal probe. More than two-thirds of the febrile episodes are likely to be caused by infection, which may occur with or without focal symptoms or signs. Because of the impaired inflammatory response, the classic signs of infection (i.e. pain, heat, redness and swelling) are often reduced or may even be absent. Therefore, fever is generally the first and frequently the only sign of infection.[16]

Fever occurring in the context of neutropenia is considered to reflect ongoing infection unless proven otherwise. However, there are noninfectious causes of pyrexia, of which the most frequent are the underlying malignancy itself, cytotoxic chemotherapy, transfusion of blood products, antifungal and occasionally other antimicrobial agents, hematopoietic growth factors or allergic drug reactions. Uncommonly, infection may develop in the absence of fever because of the lack of inflammatory response such as during therapy with high-dose corticosteroids or when caused by certain micro-organisms (e.g. *Clostridium septicum*).

Types of infection

Classically, infections have been subdivided into three main categories. [5] [15]

- *Microbiologically documented infections* (MDI), subdivided into those with and without bloodstream infection. Bloodstream infections, caused predominantly by bacteria (bacteremia) and occasionally by fungi (fungemia), may be either primary (in the absence of a nonhematogenous focus of infection) or secondary to a proven focus of infection (e.g. pneumonia, cellulitis, catheter-related infection, urinary tract infection).
- *Clinically documented infections* (CDI) defined by the presence of a site of infection (e.g. pneumonia, cellulitis, oropharyngeal mucositis, enterocolitis, catheter exit site infection) without microbiological proof of the nature of infection.
- *Fever of unknown origin* (FUO) also designated as fever of undetermined origin, unexplained fever or pyrexia of unknown origin and defined as a febrile episode that is not accompanied by clinical or microbiological evidence of infection.

Figure 100.3 shows the proportions of MDI, CDI and FUO in febrile neutropenic patients with hematologic malignancies (mainly acute leukemia) or solid tumors in three consecutive multicenter studies conducted in the 1990s in Europe, the Middle East and North America.[17] [18] [19] [20] [21] Generally, MDI account for approximately 25–35% and 10–20%, CDI for 20–30% and 10–20% and FUO for 40–60% and 50–70% of the episodes of fever occurring in neutropenic patients with hematological malignancies or solid tumors, respectively.[17] [18] [19] [20] [21] Most episodes of MDI consist of bloodstream infections (Fig. 100.4), that occur almost exclusively in patients with profound neutropenia (<100 cells/mm^3). [3] [17] [18] [19] Although the etiology of fever remains by definition unclear in FUO, one may retrospectively assume that the fever was probably of infectious origin, if it resolved with antimicrobial therapy.

Sites of infection

The most frequent sites of infection in neutropenic cancer patients with hematological malignancies or solid tumors are, by decreasing order of frequency, the bloodstream, the oral cavity and nasopharynx, the skin and soft tissues, the respiratory tract, the gastrointestinal tract and the urinary tract (Fig. 100.4).[17] [18] [19]

Bloodstream

Bloodstream infections account for 80–90% of microbiologically documented infections (Fig. 100.3) and for half of the febrile episodes, for which a site of infection can be identified (Fig. 100.4). In primary bloodstream infections, the source remains unknown but disrupted physiological barriers (i.e. mucous membranes of the gastrointestinal tract and skin) are the most likely portals of entry. Bacteria are the most frequent blood isolates, accounting for over 90% of bloodstream

Figure 100-3 Causes of fever in neutropenic patients with hematological malignancies (n=1773) or solid tumors. *Data are derived from four consecutive EORTC-IATG studies conducted between 1991 and 2000 and from a North American study conducted between 1992 and 1997.*[17] [18] [19] [20] [21]

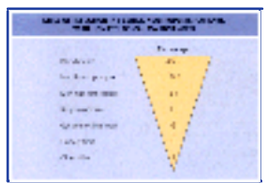

Figure 100-4 Sites of infection in febrile neutropenic patients with hematological malignancies. *Data are derived from three consecutive EORTC-IATG studies conducted between 1991 and 2000.*[17] [18] [19]

infections. A single micro-organism is implicated in the majority of the bloodstream infections, but polymicrobial infections occur in approximately 10% of the cases.

As previously mentioned, the epidemiology of single-organism bacteremia in neutropenic cancer patients has changed over the last 30 years (Fig. 100.1). Today, Gram-positive and Gram-negative bacteria cause an equal proportion of infections. The most common Gram positives are coagulase-negative staphylococci, viridans streptococci and *S. aureus*. Among Gram negatives, *E. coli, Klebsiella* spp. and other Enterobacteriaceae are predominant, while *P. aeruginosa* has declined progressively (Table 100.1). Resistant bacteria or fungi are classically isolated from blood in secondary febrile episodes occurring during antibiotic treatment. Bloodstream infections are potentially life threatening but with the prompt administration of empirical antibiotics at fever onset, severe complications such as severe sepsis and septic shock rarely occur today. Mortality rates between 1% and 3% have been reported in recent studies in febrile neutropenic cancer patients.

Mouth and pharynx

Maintenance of good oral hygiene and proper dental care are essential for the prevention of oral and systemic infections. Dental septic foci, such as braces and periodontitis, which may promote or facilitate the development of local and systemic infections, should be removed or treated prior to the initiation of chemotherapy. Indeed, infections of the oral cavity (e.g. mucositis, gingivitis, periodonitis) and pharynx occur in 15–25% of neutropenic cancer patients (Fig. 100.4). The frequency and severity of these infections are correlated with the degree of mucosal damage induced by cytotoxic chemotherapy and with the severity of neutropenia. Mucous

membranes of the mouth and pharynx are heavily colonized with viridans streptococci, Gram-positive rods and aerobic and anaerobic Gram-negative bacilli. Loss of integrity of the mucosal barrier is therefore a major portal of entry for infection. Neutropenic patients with no or minimal mucosal damage are at much lower risk of infection than patients with severe chemotherapy-induced mucositis. *Candida* also plays an important role in infections of the oropharynx, especially under the selective pressure of broad-spectrum antibiotics. Oral lesions caused by reactivation of HSV infection may

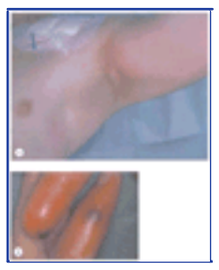

Figure 100-5 Cutaneous bacterial infections in neutropenic patients with acute leukemia. (a) Axillary *Pseudomonas aeruginosa* hydradenitis. (b) Ecthyma gangrenosum of fingers in a patient with *P. aeruginosa* sepsis.

mimic chemotherapy-induced mucositis and also serve as a portal of entry for bacterial infection.

Skin and soft tissues

Cutaneous infections are common in neutropenic cancer patients, causing about 10–20% of all septic episodes for which a source of infection is identified (Fig. 100.4). Primary skin and soft tissue infections often result from disruption of the integrity of the cutaneous barrier caused by needle punctures (venous or lumbar puncture, bone marrow biopsy) or by the presence of intravascular access devices. The anal and axillary areas are frequent foci of cutaneous infections as moisture, high bacterial colonization, relative skin frailty and microtrauma all facilitate the development of infection. Anal fissures are often complicated by perirectal cellulitis, classically caused by the fecal flora, including Gram-negative bacilli (Enterobacteriaceae, *P. aeruginosa*), enterococci and anaerobic Gram-negative bacilli. Axillary hydradenitis is a classic infection of neutropenic hosts caused by skin commensals and Gram-negative bacteria, including *P. aeruginosa* (Fig. 100.5a). Disseminated cutaneous infections may reflect the development of septic skin foci in patients with bacterial, fungal or viral bloodstream infections. Clinically, these skin lesions appear as papules or nodules (Fig. 100.6), which are usually associated with classic symptoms and signs of sepsis (i.e. fever, chills, headache, backache, myalgia and muscle tenderness). Ulcers, vesicles, hemorrhagic or crusted lesions that are either isolated (with or without dermatome distribution) or disseminated are typically associated with

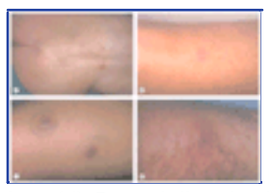

Figure 100-6 Cutaneous manifestations of disseminated fungal infections in leukemic patients. (a) *Aspergillus terreus*, back. (b) *Candida tropicalis*, arm. (c) *Fusarium*, leg. (d) *Pseudallescheria boydii*, leg.

herpetic skin infections, but may occasionally occur with staphylococcal or streptococcal infections.

A bacterial (e.g. *Pseudomonas* and *Aeromonas* species) or fungal etiology (*Fusarium, Aspergillus, Mucor* and *Rhizopus* species) should be suspected in cases of disseminated necrotic skin lesions. For example, ecthyma gangrenosum is a classic cutaneous complication in patients with *Pseudomonas* sepsis (Fig. 100.5b). Necrotizing fasciitis and metastatic musculoskeletal infections complicating bacteremia or fungemia have also been reported, albeit infrequently (Fig. 100.7a).

Intravascular access devices

Indwelling intravenous catheters, especially those inserted for prolonged periods of time (e.g. Broviac, Hickman, Port-a-cath), are a major source of infections, which most commonly arise at the exit site but may also occasionally affect the tunneled section of the catheter. Clinical manifestations include pain, erythema and tenderness with no or minimal swelling during marrow aplasia. Microbes of the skin flora (i.e. coagulase-negative staphylococci, *Propionibacterium* spp., *Corynebacterium* spp., *Bacillus* spp. and viridans streptococci), *Staph. aureus*, Gram-negative bacteria and *Candida* species are the most frequent pathogens. When skin necrosis occurs, infections due to *P. aeruginosa*, mycobacteria (especially *M. chelonae* and *M. fortuitum*) or molds (*Aspergillus, Fusarium* or *Mucor* species) should be suspected. Infection of intravascular access devices may be complicated by septic thrombophlebitis.

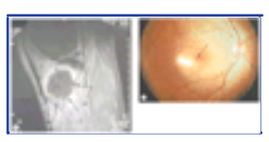

Figure 100-7 Disseminated *Staph. aureus* infection in a leukemic patient. (a) Muscular abscess, MRI. (b) Retinal infectious lesion with macular involvement and secondary bleeding (arrow), fundoscopy.

Respiratory tract

Respiratory infections account for 10–15% of the identified sites of infection in febrile neutropenic cancer patients (Fig. 100.4). Infections of the upper respiratory tract (sinusitis, otitis, epiglottitis, laryngitis and tracheitis) are uncommon, occurring in 1% of episodes. Sinusitis and otitis are mainly due to common community-acquired bacteria, but infections due to *Pseudomonas* species and anaerobes can occur. Fungal (*Candida, Aspergillus, Mucor, Rhizopus*) sinusitis is a severe and potentially life-threatening infection of patients with profound

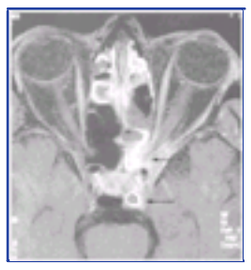

Figure 100-8 Ethmoidal sinusitis due to *Aspergillus fumigatus* in a leukemic patient. The MRI shows a bone destruction with invasion of the orbit, optical nerve (upper arrow) and central nervous system (lower arrow).

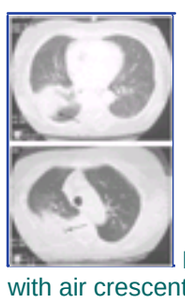

Figure 100-9 Invasive pulmonary aspergillosis in a patient with acute lymphoblastic leukemia. (a) Early stage with halo sign (arrow) during neutropenia, CT scan. (b) Late stage with air crescent sign (arrow) after bone marrow recovery, CT scan.

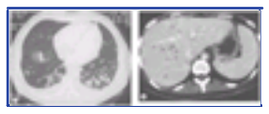

Figure 100-10 Disseminated candidiasis in a patient with acute myelogenous leukemia. (a) Multifocal nodular lung lesions, CT scan. (b) Multiple hepatosplenic abscesses, CT scan.

and prolonged neutropenia. It is often invasive and can infiltrate the nearby bone and skin and soft tissue structures of the orbit and extend into the central nervous system (Fig. 100.8).

Most frequently, lower respiratory tract infections (bronchopneumonia and pneumonia) are primary infections and account for about 10% of documented infections. However, they may occasionally be secondary to bloodstream infections. Due to the impaired inflammatory response, the classic symptoms and signs of pneumonia (such as fever, cough, dyspnea, chest pain, sputum and radiological infiltrates) are often attenuated or delayed, and present in only half to two-thirds of the patients.[16] Patients with profound neutropenia (<100 cells/mm^3) rarely produce sputum. The proportion of infections due to pneumonia tends to be lower in the first febrile episodes than in subsequent ones. Moreover, early-onset pneumonias are more likely to be of bacterial origin, while late-onset pneumonias are more likely to be due to fungi or other opportunistic pathogens

Figure 100-11 Pulmonary infections in neutropenic patients with hematological malignancies. (a) Interstitial pneumonia due to influenza virus. (b) Viridans streptococcal bacteremia with acute respiratory distress syndrome.

(Fig 100.9 , Fig 100.10a). Respiratory viruses (respiratory syncytial virus, influenza virus, parainfluenza virus, adenovirus) are rare (Fig. 100.11a).

Another classic pulmonary complication in febrile neutropenic cancer patients is the development of adult respiratory distress syndrome after viridans streptococcal bacteremia (Fig. 100.11b). This syndrome occurs despite adequate antibiotic therapy and is associated with important morbidity (respiratory failure requiring mechanical ventilation) and high mortality.[9]

Gastrointestinal tract and intra-abdominal organs

The gastrointestinal tract is the largest reservoir of micro-organisms of the human body and the endogenous gastrointestinal flora plays an important role in the pathogenesis of infections in neutropenic cancer patients. Extended chemotherapy-induced mucosal damage is therefore a major portal of entry of systemic infections. The

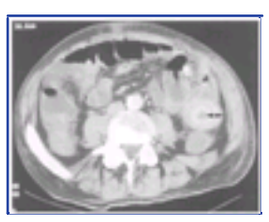

Figure 100-12 Neutropenic enterocolitis in a leukemic patient. Prominent thickening of a segment of ileum, CT scan (arrow).

gastrointestinal tract itself accounts for 4–8% of documented infections (Fig. 100.4). Esophagitis and enterocolitis are the two most frequent gastrointestinal infections in the neutropenic host. Esophagitis presents with severe retrosternal pain and dysphagia and is usually caused by the commensal bacterial flora, *Candida* species or reactivation of HSV. Symptoms and signs of enterocolitis include nausea, vomiting, bloating, abdominal discomfort, cramps, pain, constipation or diarrhea (often severe in the case of antibiotic-associated colitis). Typhlitis, a life-threatening necrotizing enterocolitis affecting the cecum or other bowel segments, typically occurs in patients with severe and prolonged neutropenia postchemotherapy for acute leukemia (Fig. 100.12). [22] The clinical manifestations are fever, abdominal pain (initially localized to the lower right abdominal quadrant, but becoming diffuse as the infection progresses) and diarrhea, that are often associated with a profound alteration of the patient's clinical condition. Typhlitis is caused by the endogenous gut aerobic and anaerobic bacterial flora, but yeasts and molds may also be implicated. Bleeding and perforation are the two major complications. Necrotizing fasciitis and septic shock can also occur, especially with clostridial infection, notably with *C. septicum*. Antibiotic-associated colitis due to *C. difficile* is a frequent cause of profuse diarrhea in neutropenic cancer patients exposed to multiple and prolonged antibiotic treatments.

Bacterial liver abscesses and viral hepatitis are rare in neutropenic cancer patients. Hepatosplenic candidiasis, a disseminated fungal infection mainly observed in patients with acute leukemia, classically becomes manifest at the time of marrow recovery after a prolonged period of neutropenia. Diagnosis should be suspected in patients with persistent fever, lack of appetite, abdominal discomfort or frank pain, hepatosplenomegaly and elevated alkaline phosphatase.[23] [24] Imaging techniques (ultrasonography, computed tomography (CT) scanning or magnetic resonance (MR)) show typical multiple target-like or bull's-eye lesions of the liver and spleen (Fig. 100.10b).

Urinary tract

Urinary tract infections are a rare cause of infection (1–3%) in neutropenic patients (Fig. 100.4) and are mainly due to common uropathogens, such as Enterobacteriaceae. They occur with minimal symptoms and dysuria is frequently absent, as is leukocyturia because of neutropenia. thus, diagnosis often relies on a positive urine culture in a febrile patient without evidence of another site of infection.

Other sites

Infections of the central nervous system, eye, heart, vasculature and bone are uncommon causes of sepsis in neutropenic patients with hematological malignancies and solid tumors (1% of all episodes; Fig. 100.4). Infections of the central nervous system comprise bacterial meningitis or brain abscesses. Acute primary bacterial meningitis occurs at a frequency similar to that of the general population. Bacterial meningitis also may occur in the context of bloodstream infections or as a complication of intrathecal chemotherapy. Infectious foci in the retina are observed in patients with disseminated bacterial and fungal infections (Fig. 100.7b). Endocarditis may occur even in the absence of risk factors, but is notably uncommon despite the frequency of bacteremia with organisms known to cause endocarditis. Clavicular osteomyelitis may be seen in patients with tunnel infections of subclavian intravascular access devices.

PREVENTION

Environmental measures

Preventive measures aimed at reducing the acquisition or transmission of nosocomial pathogens play an important part in reducing the risk of infection in neutropenic patients.[5] [6] [7] Special emphasis should be placed on careful handwashing by personnel, which can substantially diminish the risk of transmission of pathogens. Education of patients, family members, medical and nursing staff is essential. Contact with persons with overt respiratory or cutaneous infections must be avoided. Patients should receive well-cooked and low-microbial food and have access to safe water and ice supplies. For patients with severe and prolonged neutropenia, air ultrafiltration may be desirable in settings where *Aspergillus* infections are frequently observed. When an optimal protective environment is required, clean air is provided by means of constant positive-pressure air flow and/or high-energy particle air filtration. In addition, intensive disinfection measures are generally employed for high-risk patients. These include the use of antimicrobial mouthwash solutions, disinfectant soaps, creams and sprays and oral nonabsorbable antifungal agents. Proper oral and dental hygiene and careful attention to the anal region are essential. Insertion and manipulations of intravascular access devices and bone marrow aspiration should be performed with great care and under strict aseptic conditions. Whenever possible, the use of nasogastric tubing and urinary catheters should be

avoided.

Surveillance cultures of oropharynx, stools, urine and skin are of limited utility for the management of individual neutropenic patients, as they lack sensitivity and specificity for predicting and identifying the etiological agent of infection and are costly and time-consuming. However, these cultures might be useful for epidemiological studies and infection control purposes.

Antimicrobial prophylaxis

Prophylactic antimicrobial therapy has been used to suppress colonization by potential pathogens in high-risk neutropenic patients. Different strategies have been employed to prevent bacterial and fungal infections and reactivation of latent viral infections and have yielded mixed results. Thus, considerable controversy still srrounds the topic of antimicrobial prophylaxis for the prevention of infection in the neutropenic host.

Antibiotic prophylaxis

Oral nonabsorbable antibiotics have been used to achieve gut decontamination, since the bowel is the main reservoir of endogenous flora and an important source of infection in granulocytopenic cancer patients. However, the use of antibiotics such as vancomycin, gentamicin, polymyxin B, or colistin is associated with several problems. These antibiotics are nonpalatable and poorly tolerated, so that compliance

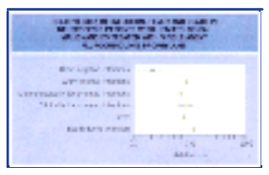

Figure 100-13 Relative risk of infection, fever and death in neutropenic patients with hematological malignancies treated with single-agent fluroquinolone prophylaxis. Yellow circles and black bars show relative risks with 95% confidence intervals in patients receiving fluoroquinolone prophylaxis compared to patients receiving placebo or other regimens. Adapted from reference [26] .

is poor. Clinical efficacy has not been clearly demonstrated. Furthermore, administration of prophylactic oral antibiotics may induce resistance among endogenous bacteria, such as resistance to vancomycin among enterococci. Thus, prophylaxis with nonabsorbable antimicrobial agents has been abandoned in most institutions.

Alternatively, attempts have been made to 'selectively' decontaminate the alimentary tract using antibiotics such as trimethoprim-sulfamethoxazole (TMP-SMX) to maintain the anaerobic flora, thereby preserving the 'colonization resistance' against aerobic bacteria and fungi. Results of initial studies suggested that TMP-SMX could decrease the incidence of infections, but this was not confirmed in subsequent studies. Furthermore, TMP-SMX is ineffective against *P. aeruginosa*, may prolong the duration of the bone marrow aplasia and promote the emergence of resistant organisms. Current guidelines recommend the use of TMP-SMX only in patients at high risk of *Pneumocystis carinii* infection.[5]

In contrast to all other antibiotics used for prophylaxis of bacterial infections, fluoroquinolones have been unequivocally shown to reduce the incidence of Gram-negative infections, but not of fever, microbiologically or clinically documented infections, or of infectious mortality (Fig. 100.13).[10] [25] [26] However, the use of fluoroquinolone prophylaxis was associated with an increased incidence of viridans streptococcal infections and emergence of resistance.[9] [11] Clinicians are divided on the utility and safety of antibiotic prophylaxis. While some consider that it should be strongly discouraged for the reasons mentioned above, others still favor the use of fluoroquinolones, especially in high-risk patients with hematological malignancies. However, because of a lack of compelling evidence of reduced morbidity and mortality, we do not use antibiotic prophylaxis in neutropenic cancer patients in our institution and therefore do not recommend this preventive measure.

Antifungal prophylaxis

Antifungal prophylaxis with fluconazole (400mg/d) has been shown to prevent infection with *C. albicans* and *C. tropicalis*, but not with *C. krusei*, in allogenic bone marrow transplant patients.[27] Its activity against *C. glabrata* is doubtful. In contrast, the place of antifungal prophylaxis in patients with hematological malignancies not undergoing bone marrow transplantation is uncertain. Prophylaxis with ketoconazole, amphotericin B, fluconazole or itraconazole was found to reduce the incidence of superficial infections, but no consistent effects were observed on the incidence of invasive fungal infections, empirical use of amphotericin B or mortality of invasive fungal infections.[28] [29] In some centers, the use of fluconazole prophylaxis was associated with a shift towards non-*albicans Candida* strains, that are either poorly susceptible (*C. glabrata*) or resistant (*C. krusei*) to azoles.[12] [13] Routine use of antifungal prophylaxis in all neutropenic patients is not recommended, but may be advisable in selected high-risk patients. The choice of the antifungal agent should be guided by local epidemiological data.[5] New azoles (e.g. voriconazole, posaconazole, ravuconazole) and a new class of antifungal agents, the echinocandins (e.g. caspofungin, micafungin, anidulafungin) are either available today or in clinical development. These new compounds, which are active against *Candida* and *Aspergillus*, may soon provide new prophylactic options against invasive mycoses in high-risk neutropenic patients.

Antifungal prophylaxis is not used routinely in neutropenic patients at our institution. However, we recommend the initiation of early empirical antifungal therapy in persistently febrile neutropenic patients at risk of invasive fungal infections (see Persistence of fever, below).

Antiviral prophylaxis

Viral serology tests should be performed at the outset of chemotherapy to determine whether patients with hematological malignancies have been previously infected with HSV, VZV or CMV, as these may reactivate during therapy. Reactivation of HSV infection occurs in 70–80% of patients with acute leukemia receiving chemotherapy. Aciclovir prophylaxis is therefore recommended for HSV-seropositive individuals.[30] Oral valaciclovir is an alternative to oral aciclovir. Patients unable to take oral medications should be switched to intravenous aciclovir. CMV-seronegative leukemic and hematopoietic stem cell or bone marrow transplant patients should receive leukocyte-depleted blood products to prevent CMV transmission. Those interested in further reading on antiviral prophylaxis should refer to Chapter 112 .

Immunoglobulins

Replacement therapy with intravenous immunoglobulins may be indicated for patients with low immunoglobulin levels, such as patients with chronic lymphatic leukemia or multiple myeloma or bone marrow transplant recipients in the post-engraftment period. Those interested in further reading on this topic should refer to Chapter 114 .

DIAGNOSIS

Evaluation of the febrile neutropenic cancer patient follows the universal principles guiding medical practice and includes a prompt and thorough medical history and physical examination and use of rigorous diagnostic measures. However, in contrast to the non-neutropenic patient, empirical broad-spectrum antibiotic therapy should be administered without delay after the onset of fever and before the results of the microbiological investigations become available.

Medical history

It is critical that cancer patients be thoroughly investigated at presentation with a special emphasis on the assessment of risk of infection. Medical history should include information on:

- travels to, or residence in, areas where infectious diseases (such as tuberculosis, fungal or parasitic infections, e.g. Leishmaniasis or Strongyloidiasis) are endemic, as these may reactivate during neutropenia;
- immunization history;
- and presence of vascular access devices.

Upon development of fever, the physician must obtain a meticulous history and be aware of the fact that symptoms may be modified because of the impaired inflammatory response.[5] [6] [7]

Physical examination

Physical examination should be particularly thorough and must be performed keeping in mind that the classic signs of infection are markedly attenuated (e.g. pain, redness and induration) or absent (pus) because of neutropenia.[5] [6] [7] The physician should look for subtle inflammatory signs at common sites of infection (e.g. oropharynx, esophagus, respiratory tract, skin, insertion site of intravascular catheters, gastrointestinal tract, perianal region). The mouth and pharynx should be examined for erythema, mucositis, gingivitis, white patches and pseudomembranes, vesicles and ulcerations. The anterior nares should be inspected for signs of congestion, rhinorrhea, crusts, bleeding and ulcers. The skin may present features of a localized (i.e. primary) infection or multiple lesions in distant areas suggestive of a disseminated infection (Fig. 100.5b , Fig. 100.6). Special attention should be paid to the presence of erythema, swelling and tenderness in the anal, perineal, axillary and periungual regions (Fig. 100.5a). Insertion sites of intravascular access devices and sites of cutaneous injury due to needle punctures or bone marrow aspiration should be thoroughly examined. Fundoscopy should be performed as it may reveal signs of systemic infection (Fig. 100.7b). Physical examination should be repeated frequently as clinical signs of infection may become manifest only a few days after the onset of fever.

Microbiology, radiology and histopathology

At least two sets of blood cultures should be obtained prior to the administration of antibiotics in the febrile neutropenic patient. It is recommended to perform blood cultures even in the absence of fever in any patient suspected of infection. Blood should be drawn from a peripheral vein and also from intravascular catheters, if present.[5] It is prudent to draw blood from every port of multilumen intravascular devices. Qualitative cultures are performed routinely, but quantitative cultures might be helpful for the diagnosis of catheter-related bloodstream infections.[31] Specimens (aspirate or biopsy) should be obtained from any site suspected of infection. The physician should carefully evaluate the benefits and risks (mainly bleeding in the thrombocytopenic patient) of initial (i.e. at the onset of fever) versus delayed (i.e. in those patients who are not responding to empirical therapy) invasive procedures to diagnose infections in poorly accessible sites. Exit sites of catheters should be cultured for bacterial, fungal and nontuberculous mycobacterial pathogens. Skin lesions should be aspirated or, whenever possible, biopsied for culture and/or histopathological examination. Lesions of the oral cavity, pharynx and paranasal sinuses can be brushed or biopsied. In the presence of severe dysphagia, endoscopy with brushing and biospy for cultures and histopathology should be considered. Stools from patients with diarrhea should be tested for *Clostridium difficile* toxins and cultured for bacteria (*Salmonella, Shigella, Campylobacter, Aeromonas* and *Yersinia*) and examined for protozoa (*Cryptosporidium*). Urinalysis and urine cultures should be performed routinely, even in the absence of urinary symptoms or of pyuria, a rare finding in neutropenic patients even when infection is present.

A chest radiograph is part of the standard investigation of febrile neutropenic patients. Whether and when to obtain additional radiological imaging (ultrasonography, CT, MRI) is guided by the patient's clinical condition and response to empirical broad-spectrum antibiotics. For example, severe abdominal complaints in neutropenic patients will prompt thorough investigations with standard radiography, ultrasonography, CT or MRI scans. Patients with lesions of the liver and spleen suggestive of hepatosplenic candidiasis should undergo CT-guided or laparoscopic liver biopsy. The presence of lung infiltrates has important implications for the management of neutropenic patients. Several studies have shown that CT scanning is superior to conventional radiography for revealing the presence of lesions indicative of invasive aspergillosis. That should prompt further investigations (bronchoalveolar lavage, transbronchial, transthoracic or open lung biopsy) to obtain samples for diagnosis.[32] Persistent or complicated sinusitis or otitis and suspected osteomyelitis or myositis are other indications for additional radiological imaging and tissue sampling. Once again, it should be remembered that radiological signs are attenuated and delayed in neutropenic patients and may become apparent only after bone marrow recovery.[16] Classic examples are hepatosplenic candidiasis and invasive pulmonary aspergillosis.[24] [33]

Baseline viral (HSV, VZV, CMV, EBV) and parasitic (*Toxoplasma gondii*) serology should be obtained in patients with hematological malignancies, but not in those with solid tumors as they are not at high risk of developing severe viral or parasitic infections.

Novel nonculture diagnostic tests including molecular and serological techniques have been described recently. These include the use of polymerase chain reaction (PCR) for the diagnosis of bacterial and fungal infections and the serological detection of circulating antigens (e.g. mannan for *Candida* species and galactomannan for *Aspergillus* species), metabolites (e.g. D-arabinitol for *Candida* species) and antibodies directed against fungal antigens (e.g. antimannan antibodies).[34] [35] [36] However, the utility of these diagnostic tools has not yet been fully validated and further studies are needed before they can be recommended.

Measurement of circulating biological markers of inflammation, such as C-reactive protein and proinflammatory cytokines (such as tumor necrosis factor-a, interleukin-1, 6 and 8), has not been found to be helpful for differentiating infectious from noninfectious causes of fever because of a lack of sensitivity and specificity. Procalcitonin, a circulating calcitonin precursor whose concentrations are markedly increased during sepsis and much less so in inflammatory diseases, looks promising and deserves further studies in febrile neutropenic cancer patients.

Other investigations

Hematology (complete blood count and differential) and chemistry tests (including electrolytes, tests of liver and kidney function) are an integral part of the monitoring of toxic reactions to cytotoxic and antimicrobial agents and should therefore be repeated at intervals. Moreover, a low threshold for measuring blood levels of antimicrobial agents should be maintained when renal or hepatic functions are altered, especially for agents with narrow therapeutic windows such as aminoglycosides or glycopeptides, but also when the patient does not respond to therapy despite demonstration of *in vitro* susceptibility.[5]

MANAGEMENT

Neutropenic patients with suspected infection, whether febrile or not, must receive prompt empirical antibiotic therapy with broad-spectrum antibiotics. The concept of treating these patients with empirical antibiotics as soon as they develop fever has radically changed the otherwise fulminant and almost uniformly fatal course of Gram-negative sepsis.[2] [4] This is the single most significant advance made over the last 30 years in the area of supportive care for cancer patients.

Risk assessment

Serious and potentially life-threatening complications may occur during the course of bone marrow aplasia, including hemorrhage,

TABLE 100-2 -- Factors associated with a low risk of severe complications in febrile neutropenic cancer patients.

Derived from references [5] [38] [39] .

FACTORS ASSOCIATED WITH A LOW RISK OF SEVERE COMPLICATIONS
Malignancy in remission
Absence of co-morbidities (chronic lung disease, diabetes mellitus, congestive heart failure, hemorrhage, liver disease, renal disease)
Absence of vomiting, diarrhea, dehydration
Outpatient status
Absolute neutrophil count =100 cells/mm^3
Absolute monocyte count =100 cells/mm^3
Anticipated duration of neutropenia <7–10 days
Normal chest radiograph
Peak temperature <102.2°F (39.0°C)
Absence of shivering
Does not appear ill
Absence of neurologic or mental alterations

Absence of abdominal pain
Absence of intravenous catheter site infection
Absence of septic complications (e.g. severe sepsis, septic shock, hypoxia, pneumonia or other deep organ infection)

disseminated intravascular coagulation, thrombosis, pulmonary embolism, organ dysfunction (such as congestive heart failure, renal insufficiency, respiratory failure) and septic complications (such as severe sepsis, septic shock, adult respiratory distress syndrome), that

Figure 100-14 Risk assessment and selection of oral or intravenous empirical antibiotic therapy in febrile neutropenic cancer patients. Severe sepsis and septic shock are defined according to reference[37] . *Adapted from reference[6] .*

may require admission to the intensive care unit.[37] [38] [39] Until recently, all febrile neutropenic cancer patients have been treated in a uniform fashion. Today, risk assessment is an important aspect of the evaluation of the febrile neutropenic patient. It is important to determine whether the patient is at low or high risk of serious infections and other medical complications because this will influence the treatment modalities and will have an impact on the patient's hospital course and length of stay. Several factors can be used to classify a given patient as low or high risk.

Factors associated with a *low risk* of complications are shown in Table 100.2 [5] [38] [39] Typically, a low-risk profile is defined by the following characteristics: cancer in remission, absence of severe comorbidities, outpatient status at onset of fever and neutropenia not likely to last for more than 7 days. Conversely, uncontrolled cancer, the presence of concomitant medical conditions (severe mucositis, hemorrhage, dehydration, renal, hepatic, respiratory, cardiac or circulatory failure, altered mental status), inpatient status and neutropenia likely to last for more than 10 days are factors likely to be associated with a *high risk* of complications. Different scoring systems have been proposed for risk stratification.[38] [39] The Multinational Association for Supportive Care in Cancer (MASCC) score is based on seven clinical factors derived and validated from prospective analyses of 756 and 383 episodes of febrile neutropenia, respectively.[39] A MASCC score greater than or equal to 21, on a maximum score of 26, was used to identify patients at low risk (<5%) of severe complications (sensitivity 71%, specificity 68%, positive predictive value 91%, negative predictive value 36%) and who might be candidates for outpatient empirical antimicrobial therapy. However, the experience with this and other scoring systems is still limited.[38] [39] Additional prospective studies are needed to more fully evaluate and finely tune the

use of these risk assessment systems. It will be important to determine whether patients identified as low risk may be safely treated on an outpatient basis with oral antibiotics (see Oral antibiotic therapy, below). An algorithm for risk assessment may be used to guide the choice of oral versus parenteral empirical antibiotic therapy (Fig. 100.14).

Empirical antibiotic therapy

Intravenous antibiotics

Over 90% of the first episodes of infection in neutropenic cancer patients are caused by Gram-positive or Gram-negative bacteria (see Fig. 100.1). Empirical antibiotic regimens must be broad spectrum and bactericidal, achieve high circulating and tissue levels and be nontoxic. For more than two decades, combinations of two or more intravenous antibiotics have been the 'gold standard' of empirical antibiotic therapy. Numerous combinations of antipseudomonal penicillins (e.g. mezlocillin, ticarcillin with or without clavulanic acid, azlocillin or piperacillin) or third- or fourth-generation cephalosporins (ceftazidime, ceftriaxone, cefpirome, cefepime) plus an aminoglycoside (e.g. gentamicin, tobramycin, netilmicin or amikacin) have been frequently utilized.[4] [17] [40] [41] But no particular one was shown to be superior to the others. However, aminoglycoside-containing regimens are associated with renal and auditory toxicity, especially in patients concomitantly receiving other toxic agents, and often require monitoring of drug levels.

Thus, new treatment approaches have been explored to avoid these complications, such as once-daily dosing of aminoglycoside or ß-lactam monotherapy. An international, multicenter trial by the EORTC-IATG showed that once-daily ceftriaxone and amikacin was as effective and at least no more toxic than a combination of ceftazidime and amikacin given thrice daily.[42] Monotherapy with broad-spectrum and highly bactericidal agents, such as third- or fourth-generation cephalosporins (ceftazidime, cefepime, cefpirome), carbapenems (i.e. imipenem and meropenem) or antipseudomonal penicillins combined with a ß-lactamase inhibitor (piperacillin/tazobactam), were found to be as effective as and less toxic than combinations of ß-lactam and aminoglycoside.[19] [43] [44] [45] [46] Choices of intravenous empirical antibiotic therapy for high-risk patients, typically those with hematological malignancies and long duration neutropenia, are shown in Figure 100.15 .

Choices should be guided by the patient's clinical condition and local epidemiological data. Consider using an aminoglycoside-containing

Figure 100-15 Choices of empirical intravenous antibiotics in high-risk febrile neutropenic cancer patients.

regimen in critically ill patients, such as those with severe sepsis or septic shock, when a *P. aeruginosa* infection is suspected or when resistant Gram-negative bacteria prevail.[5] [37] Consider using a glycopeptide antibiotic (i.e. vancomycin or teicoplanin) in patients with catheter-related infections, when penicillin-resistant streptococcal or methicillin-resistant staphylococcal infections are suspected or in critically ill patients (i.e. with severe sepsis or septic shock).[5] [37] However, outside these well-defined clinical circumstances encountered in a minority of patients, routine use of glycopeptide antibiotics is strongly discouraged. Empirical use of vancomycin has not been shown to improve patient outcome and is associated with increased costs, toxicity and emergence of resistance.

After proper investigations and microbiological cultures, it is reasonable to empirically use:

- an antifungal and/or antiviral agent in patients with esophagitis;
- a macrolide or 'respiratory' fluoroquinolone and/or TMP-SMX and/or an antifungal agent for coverage of opportunistic respiratory pathogens in patients with lung infiltrates;
- metronidazole in patients with an abdominal focus and/or severe diarrhea.

Oral antibiotic therapy

Several studies have examined the role of oral absorbable antibiotics as empirical therapy of fever and suspected infections in low-risk adult patients with solid tumors and neutropenia expected to be of short duration (less than 7 days). Oral antibiotic therapy is possible when the patient is compliant, can swallow tablets, has normal gastrointestinal motility and function and can be monitored for response to therapy, development of secondary infections and adverse reactions. Ofloxacin or ciprofloxacin given either alone or combined with amoxicillin/clavulanic acid have been studied in adult patients.[47] [48] However, ofloxacin and ciprofloxacin have suboptimal activities against Gram-positive bacteria and should not be used as monotherapy. New 'extended-spectrum' fluoroquinolones (i.e. levofloxacin, moxifloxacin and gatifloxacin) with improved activity against Gram-positive bacteria deserve further investigation in febrile neutropenic patients. In contrast, oral ciprofloxacin plus amoxicillin/clavulanic acid was found to be as efficacious and safe as standard parenteral treatment in two large studies of low-risk patients in an inpatient setting.[20] [21] However, it has not yet been shown that low-risk febrile patients can be safely managed on a fully outpatient basis. It is reasonable to discharge

compliant patients who have responded to therapy, despite persistence of neutropenia, provided that the patient is not alone at home, is under careful medical supervision and lives within a short distance of a hospital.

There are no data on upfront oral antibiotic therapy of fever in children with neutropenia. Limited information is available on early discharge of selected children on oral cefixime 48 hours after intravenous antibiotic therapy in a hospital setting.[49]

Reassessment of therapy

The appropriateness of the empirical antibiotic regimen should be reassessed within 24–72 hours based on the results of microbiological cultures and the patient's response to antibiotic therapy.

Therapeutic modifications based on culture results

If a pathogen has been isolated, antibiotic therapy is adapted based on *in vitro* susceptibility tests and clinical response (Fig. 100.16). Vancomycin (or teicoplanin, a glycopeptide antibiotic not approved by the US Food and Drug Administration and for which experience in neutropenic patients is limited) should be added if cultures grew methicillin-resistant staphylococci, penicillin-resistant streptococci or enterococci not covered by the empirical regimen. Two recently introduced antibiotics, quinupristin-dalfopristin and linezolid, an oxazolidinone, exhibit activities against methicillin-resistant staphylococci and vancomycin-resistant enterococci and might offer new

Figure 100-16 Algorithm for adjustment of empirical antimicrobial therapy based on results of microbiological cultures and clinical response.

therapeutic options for febrile neutropenic cancer patients. There is limited clinical experience with these antibiotics in this clinical setting. Moreover, linezolid may cause thrombocytopenia and anemia when used for more than 2–4 weeks.

Three studies have now clearly shown that there is no indication for empirical use of vancomycin in centers where resistance of Gram-positive bacteria to ß-lactam antibiotics is rare.[18] [50] [51] However, as mentioned previously, the empirical use of vancomycin is justified in critically ill patients with severe sepsis or septic shock, in presence of a catheter-related infection, and in centers with high levels of ß-lactam resistance among Gram-positive bacteria.[37] If suspicion of resistant Gram-positive infection is disproved by culture results, vancomycin should be rapidly discontinued. Likewise, empirical use of an aminoglycoside should be stopped if Gram-negative infection is ruled out.

Therapeutic modifications based on initial response to empirical antibiotic therapy

It is necessary to treat patients for 3–5 days before one can begin to evaluate the response to therapy with some degree of confidence. However, earlier treatment adjustments may be required if the patient's condition deteriorates. Treatment recommendations for patients who became afebrile and for those who remained febrile within 3–5 days after initiation of empirical therapy are presented in Figure 100.16 .

Resolution of fever

Intravenous (high risk) or oral (low risk) antibiotics should be continued in patients who have responded to therapy (Fig. 100.16). In low-risk patients receiving intravenous antibiotics, switching to oral antibiotics, such as ciprofloxacin and amoxicillin-clavulanic acid, should be considered. In the absence of microbiological proof and identified site of infection (i.e. FUO), it is usually not necessary to continue broad-spectrum antibiotics for longer than 7 days, if fever resolved quickly and the neutrophil count has returned to normal levels. Antibiotics can be stopped when the patient is afebrile for more than 48 hours and the neutrophil count is greater than 500 cells/mm^3 for two consecutive days.[5]

Opinions diverge regarding the optimal duration of therapy in patients with persistent neutropenia. Some physicians discontinue therapy in afebrile and clinically stable patients, while others prefer to continue broad-spectrum intravenous or oral antibiotics until recovery of neutrophils. Yet, the evidence supporting continuation of broad-spectrum antibiotics is not strong and is based on data obtained 20 years ago in a limited number of patients with FUO. In that study, nearly half of the patients with persistent neutropenia became febrile again within 3 days of stopping treatment and breakthrough infections and severe septic complications occurred. [52] Patients with microbiologically or clinically documented infections are usually treated for a total of 10–14 days (i.e. until cultures become sterile and clinical response is complete). However, one should keep in mind that prolonged antibiotic courses are costly and may be associated with toxicity and emergence of resistant bacterial or opportunistic fungal infections.

Persistence of fever

It is important to keep in mind that the median time to defervescence is 5 days in high-risk patients with hematological malignancies. Therefore, persistence of fever may not necessarily indicate that treatment is failing. If a specific causal organism or an infection site has been identified, treatment should be continued or modified, as appropriate.[5]

Considerable controversy surrounds the optimal management of a stable patient with unexplained fever after 3 days of empirical therapy. Persistence of fever may be due to a slow response, suboptimal levels of antibiotics, resistant pathogens, nonbacterial infection, development of a secondary infection or a noninfectious etiology (such as cancer, transfusion reaction or drug fever). A thorough clinical examination, follow-up microbiological cultures, extensive radiological imaging (including a CT scan of the chest and abdomen) and invasive investigations, whenever indicated, are an integral part of the investigation of the persistently febrile neutropenic patient. If the patient's clinical condition is deteriorating, the management depends on whether the patient has developed a clinically obvious focus of infection. If a focus of infection has been found, attempts should be made to identify the causative pathogen by culture or biopsy. The antimicrobial treatment should then be modified accordingly. Moreover, withdrawal of an infected intravascular catheter is indicated when the local signs of infection are extending, when the patient is clinically unstable, when a septic thrombophlebitis is documented or when infection is due to pathogens other than common skin colonizers.[5] In contrast, the utility of antibiotic lock solutions or rotation of antibiotic delivery via the different ports of multilumen catheters is controversial. If there is no obvious site of infection, the patient is not deteriorating and reassessment does not reveal a new focus of infection, the physician has the choice between several treatment options:

- continue the initial empirical antibiotics for 2 more days;
- change the antibiotics (change of broad-spectrum ß-lactam, addition of a glycopeptide or an aminoglycoside);
- empirical addition of an antifungal agent (classically intravenous amphotericin B, but other drugs including itraconazole, voriconazole or caspofungin may be considered).

Our approach in this situation is to continue the initial antibiotic regimen and initiate early empirical antifungal treatment, especially in patients with either:

- an expected long-lasting neutropenia (>10 days);
- recurrent episodes of fever during the same episode of neutropenia;
- prolonged therapy with broad-spectrum antibiotics;
- severe mucositis;

- *Candida* colonization;
- epidemiological data indicating a high incidence of mould infections.

Fungal infections due to *Candida* or *Aspergillus* are a frequent cause of persistent fever in this setting. Unfortunately, early diagnosis is difficult and infections were often diagnosed at autopsy in the past.[2] Empirical intravenous amphotericin B has been shown to promote the resolution of fever and reduces the morbidity and mortality due to invasive fungal infections. [53] [54] Treatment guidelines recommend to start empirical amphotericin B after 4–7 days of unexplained fever, but this is purely arbitrary as the optimal timing for starting therapy is unknown.[5] The potential advantage of an early start must be balanced against the risk of exposing many patients to unnecessary toxicity. Once started, it is recommended to continue empirical antifungal therapy until bone marrow recovery, at which time investigations should be repeated to confirm or rule out the presence of an until-then occult fungal infection. No further therapy is needed if an invasive fungal infection can be reasonably excluded. Conversely, any lesion suggestive of invasive mycosis, such as cavitating pulmonary or multiple hepatosplenic lesions, should be biopsied for culture and histopathology and treatment reassessed if a fungal pathogen is isolated. Prolonged antifungal therapy is often necessary when an invasive mycosis has been diagnosed, as the patient is likely to undergo multiple cycles of chemotherapy. On the other hand, surgical resection should be considered in between cycles of chemotherapy in the case of invasive pulmonary aspergillosis.[32]

For decades, amphotericin B deoxycholate, a fungicidal agent active against most species of *Candida* and *Aspergillus*, has been the standard treatment for fungal infections. However, renal and infusion-related toxicity often lead to suboptimal dosing or discontinuation of therapy and emergence of resistant fungi has been reported. Lipid forms of amphotericin B (lipid complex, colloidal dispersion and liposomal form) are better tolerated, but costly and thus mainly used as second-line treatment.[55] [56] Factors limiting the use of fluconazole as empirical antifungal treatment in persistently febrile neutropenic patients are fungistatic activity on *Candida*, lack of activity on *Aspergillus* and an increasing incidence of azole-resistant *Candida* species. Recent data suggest that itraconazole and voriconazole, a new azole antifungal, are treatment alternatives to amphotericin B for this indication.[57] [58] Moreover, higher than expected success rates for the treatment of invasive aspergillosis in immunocompromised hosts have been achieved with voriconazole.[59] Echinocandins, a new class of antifungal agents that inhibit fungal cell wall synthesis, show promising efficacy and toxicity profiles. Caspofungin has recently been licensed for salvage therapy of refractory invasive aspergillosis. A recent trial also showed that caspofungin was as effective and less toxic than amphotericin B for the treatment of invasive candidiasis.[60] Other new compounds, including ravuconazole, posaconazole, anidulafungin and micafungin, are in clinical development. Moreover, the efficacy and safety of combinations of antifungal agents of different classes are being intensively investigated.

Supportive care

Hematopoietic growth factors

The main goal pursued with the use of hematopoietic colony-stimulating factors (CSFs) is the acceleration of bone marrow recovery to reduce the severity and duration of neutropenia and thus the risk of infection. Several studies have examined the role of granulocyte colony-stimulating factor (G-CSF, filgrastim) and granulocytemonocyte colony-stimulating factor (GM-CSF, sargramostim) in the treatment of cancer patients not receiving bone marrow transplantation. Treatment with G-CSF or GM-CSF was found to moderately shorten the duration of neutropenia when given to patients with acute leukemia. Results were less striking in patients with solid tumors and other hematological malignancies. In some, but not all, of these studies, a shorter duration of neutropenia was associated with a reduction of infections, use of antibiotics and length of stay, but without clear-cut benefit on the incidence of severe infections and infection mortality. Moreover, no consistent effects on the duration of fever or antibiotic therapy, infection morbidity or mortality, or costs have been demonstrated using CSFs in febrile neutropenic patients. Recommendations for the use of CSFs have been published by the American Society for Clinical Oncology and by the European Society of Medical Oncology.[61] [62]

At the present time, the evidence supporting the use of CSFs in neutropenic patients not undergoing bone marrow transplantation is limited and expert opinions remain diverging.[5] [61] [62] Yet, high-risk patients with profound, long-lasting neutropenia and severe deep organ infections (e.g. pneumonia, typhlitis, invasive mycosis) not responding to appropriate antimicrobial therapy may benefit from an accelerated recovery of neutrophis. Although we occasionally use CSFs under such circumstances in our own institution, we acknowledge the fact that there is no definitive evidence to support the administration of CSFs in these clinical conditions. Intermittent administration of CSFs may be considered in patients with a myelodysplastic syndrome and recurrent infections due to chronic neutropenia.

Granulocyte transfusions

Some experts consider the transfusion of granulocytes in patients with profound neutropenia and life-threatening bacterial or fungal infections not responding to adequate antimicrobial treatment. However, the evidence of its efficacy is lacking and several problems are associated with this procedure, including transmission of CMV, alloimmunization, graft versus host reaction and damage to lung capillaries and risk of acute respiratory distress syndrome.

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Chapter 101 - Stem Cell Transplant Patients

Kieren A Marr

HEMATOPOIETIC STEM CELL TRANSPLANTATION: BACKGROUND AND CURRENT PRACTICES

Hematopoietic stem cell transplantation (HSCT) has been increasingly used to treat hematologic and nonhematologic malignancies and inherited immunodeficiencies. Multiple practices, including conditioning regimens, stem cell sources and supportive care strategies have changed since the first successful bone marrow transplantations, owed principally to an evolving understanding of how overall success is achieved, and to the development of new technologies. As these factors impact on the severity and duration of infectious risks, and sometimes even the clinical presentations and outcomes, clinicians involved in the care of HSCT patients benefit greatly from a baseline understanding of transplant practices.

In order to allow for donor hematopoiesis to establish in the recipient, a conditioning regimen must be applied before infusion of stem cells. The primary aim of the conditioning regimen is to suppress recipient T cells that could ultimately mediate rejection of the graft. It was also hypothesized that myeloablative conditioning regimens could effectively eliminate residual malignant disease. To accomplish these goals, conventional regimens have been highly myeloablative, employing total body irradiation (TBI) and high-dose chemotherapy (e.g. busulphan, cyclophosphamide). In this setting, severe and protracted neutropenia, and regimen-related organ toxicities can contribute to early infectious complications.

The type of conditioning therapy has been shown to impact on overall risks and timing of infectious complications. Recently, non-myeloablative or reduced-toxicity conditioning regimens have been explored for patients who are not eligible for conventional myeloablative HSCT. Success of this strategy relies on the therapeutic potential of graft versus host (GVH) effects, which are produced when donor-derived immune cells recognize and eventually destroy host cells and tissues.[1] Although graft versus host disease (GVHD) negatively impacts on overall survival by causing organ injury, necessitating the need for increasingly immunosuppressive therapies, GVH effects also induce the beneficial effects of 'graft versus tumor', sometimes to a degree that can result in complete remission.[2] A number of alternative conditioning regimens have been developed, which typically are associated with fewer organ toxicities (e.g. gastrointestinal tract mucositis) and short (if any) durations of neutropenia.[3] [4] [5] [6] [7]

The most common causes of death in allogeneic HSCT recipients are infection (frequently in the setting of GVHD) and relapsed malignancy. One method transplanters use to minimize the risks for relapse and GVHD is manipulation of the cells that compose the infused graft. Cellular composition is dependent on stem cell source and treatment of the stem cell product before infusion. Peripheral blood stem cells (PBSCs) can be used instead of bone marrow; these are harvested from peripheral blood of granulocyte-colony stimulating factor (G-CSF or filgastrim)-mobilized donors. Peripheral blood stem cell products often undergo ex-vivo manipulation in order to decrease donor T cells (T-cell depletion), thereby decreasing the risk of GVHD, or to select for specific stem cell precursors, such as $CD34^+$ cell selection. The latter has been shown to reduce the incidence of relapse of malignancy by decreasing the number of contaminating tumor cells.[8] [9] However, as expected, T-cell depletion through either method (removal or selection against cells not expressing CD34) impacts on overall immune reconstitution and increases the risks for infections.

New technologies have allowed for the performance of more high-risk HSCTs, enabling transplantation from unrelated, human leukocyte antigen (HLA)-mismatched, or even haploidentical donors. As these patients have relatively high rates of severe GVHD, the overall infectious risks and duration of susceptibility are expanded. Supportive care practices and the general approach to patients must then consider the primary risk periods (conditioning or ablation engraftment, GVH), specifically the pace of immune reconstitution and how that impacts on the risks of infections.

RISKS FOR INFECTIONS: IMPACT OF THE GRAFT, THE HOST, AND OTHER COMPLICATIONS

Historically, we have been tempted to consider patients' risks for fungal infections based solely on the presence (or absence) of neutropenia. Although T-cell immunodeficiency is understood to impact on risks for some infections, such as those caused by herpesviruses, the importance of cell-mediated immunity (CMI) in conferring risks for fungal infections post-HSCT has not been well appreciated. With increasing reports of invasive mold infections occurring late post-HSCT during GVHD,[10] [11] [12] [13] the weaknesses of the neutropenia-based risk paradigm have become increasingly evident. In fact, T cells contribute significantly to responses against both *Candida* and *Aspergillus* spp.[14] [15] [16] [17] It is now clear that risks for fungal infections hinge on multiple factors that impact on immune reconstitution and organ toxicities, including the type of transplant, the host and other post-therapy complications.

The graft and type of transplant

Donor and HLA-matching of the graft impact on overall risks for infections, primarily by dictating the likelihood and severity of GVHD and the need for and intensity of immunosuppressive therapy. The cellular composition of the graft (stem cell source and ex-vivo manipulation) dictates risks for infection through effects on immune reconstitution and the pace and severity of GVHD.

Transplantation with PBSCs may yield faster reconstitution of platelets, $CD4^+$ T cells, neutrophils and monocytes.[18] [19] [20] [21] [22] A large study compared immune reconstitution after myeloablation followed by either peripheral blood stem cell transplant (PBSCT) or bone marrow transplant, comparing co-incident infections. During the first year after transplantation, most lymphocyte subsets, especially $CD4^+$ T cells, were higher in PBSCT recipients. This was accompanied by fewer bacterial and fungal infections in patients who received PBSCT than in recipients of bone marrow transplants. [18] However, there is concern that the success of PBSCT, especially from unrelated or HLA-mismatched donors, may be limited by a more rapid onset of severe GVHD.[21] [22]

Use of cord blood is associated with delayed hematopoietic recovery. Slow engraftment and impaired neutrophil function may work

together to increase the risk of infection, particularly early after transplantation. [23] [24] [25] [26] Studies have reported increased risks for aspergillosis, candidiasis and infections due to adenovirus and human herpesvirus (HHV)-6 in cord blood transplant recipients.[13] [24] [27] On the other hand, the low rates of GVHD, even in patients who receive cord blood from unrelated donors, may confer a relative protection from infection late after transplantation.[28]

Although T-cell depletion decreases the pace and severity of GVHD, T-cell depletion itself is associated with delayed immune reconstitution and an increased risk for infections.[29] [30] [31] [32] Because $CD34^+$ selection results in removal of T cells, natural killer (NK) cells and monocytes, this practice may increase the risk for infections after allogeneic and autologous transplantation.[33] [34]

It appears that immune reconstitution is similar after nonmyeloablative and myeloablative HSCT, although few large comparative studies have been performed, and reconstitution depends on multiple host and therapeutic variables.[35] [36] At least one study suggested that nonmyeloablative HSCT may be associated with more rapid reconstitution of immune responses assessed in vitro.[36] Of course, the short durations of severe neutropenia are an important difference. One case-control study suggested that this is associated with fewer early bacterial and candidal infections.[3] [37] In this context factors other than conditioning therapy and stem cell source impact on overall infectious risks in both nonmyeloablative and myeloablative HSCT. These factors include host age, underlying disease and therapy to prevent or treat GVHD.[3] [38] Late-onset GVHD after nonmyeloablative HSCT poses increased risks for late cytomegalovirus (CMV) disease and aspergillosis.[3] [37] [38] Infections (e.g. CMV) may occur at increased frequency early after nonmyeloablative transplant in patients who receive certain conditioning regimens (e.g. the antibodies anti-CD52 or Campath-1H).[39]

Host factors that impact on infection risks

Underlying disease is one of the most important factors impacting on risks for infection post-HSCT. Underlying disease presumably impacts on the risks for post-HSCT infection by virtue of the immunologic defects associated with the hematologic condition itself and previous cytotoxic therapies. Patients who receive HSCT for a hematologic malignancy beyond first remission, for aplastic anemia and for myelodysplastic syndromes have higher risks for aspergillosis.[10] [11] [12] [13] [40] [41] Patients who have protracted courses of primary immunodeficiencies may also have increased risks, although few large studies have been performed.

Multiple studies have reported that older people have increased risks for infections after HSCT.[12] [13] [41] [42] Although it is not clear why older age predicts higher risks, factors that have been suggested include cumulative exposure to previous cytotoxic therapy, underlying disease, severity of GVHD, baseline organ dysfunction, previous microbial exposure and waning cellular immunity. On the other hand, youth may present increased risks for other infections; adenoviral complications occur at increased frequency in young transplant recipients. [43] [44] This may be explained by an increased likelihood of primary infection in the young, or even the eventual elimination of adenovirus infection with age.[43] [45]

Other genetic factors may play a role in modulating risks for infectious complications. Factors that control innate immune responses in the host and donor may impact risks for infections, and for GVHD. Studies to determine how host and donor defense polymorphisms impact on the risk for infection post-HSCT should provide novel therapeutic targets in the future.

Complications that impact on infection risks

Multiple complications that occur after HSCT alter the risks for invasive yeast and mold infections. These complications may cause organ dysfunction and immune modulation and the need for potentially toxic and immunosuppressive therapies.

The most obvious organ dysfunction that occurs immediately following conditioning therapy is mucositis involving the gastrointestinal (GI) tract. Breakdown of the mucosa of the gut is a primary mode of entry for bacteria and *Candida* spp. that colonize the GI tract. Other, subtle manifestations of organ toxicities may also lead to subsequent infection risks. Patients with renal or hepatic dysfunction may not tolerate typical doses of prophylactic or empiric antibiotics or antifungals. Other biologic variables such as iron overload or metal chelating therapy may have an effect, given their associations with bacterial and filamentous fungal infections. [46] [47] However, this remains largely unexplored in the transplant setting.

Graft versus host disease and corticosteroid-based therapies are perhaps the most important post-HSCT complications leading to infections. Risks for all infections — bacterial, fungal and viral — are increased in patients with severe GVHD. These risks increase yet further in patients who receive high doses of corticosteroids. Most likely, this represents the combined impact of the direct immunosuppressive effects of GHVD, as well as the corticosteroid-induced impairment in neutrophil and monocyte/macrophage immunity and CMI. Corticosteroids administered for other post-HSCT complications, such as bronchiolitis obliterans, and 'idiopathic' pulmonary syndromes may also lead to equivalent, high risks for infections. The negative impact of corticosteroid exposure is not limited to the above risks; cumulative exposure to high-dose corticosteroids is an important variable predicting persistent infection and poor prognosis of treatment for viral or fungal disease.[48] [49] [50]

It has been hypothesized that infection with herpesviruses, especially CMV, may directly impact on risks for other infections by modulating immune responses. Historically, the association between CMV disease and subsequent infections was largely attributed to the myelosuppressive effects of antiviral drugs administered for prophylaxis or therapy. However, high risks for subsequent bacterial and fungal infections have been noted in patients with active CMV or latent CMV disease, even after controlling for secondary neutropenia in multivariable models.[13] [40] [51] Cytomegalovirus-seronegative recipients of stem cells from seropositive donors (D+/R-) have increased risks for bacterial and fungal infections, even in the absence of CMV-specific therapy.[52] Donor or recipient seropositivity may have the most impact on survival in patients who receive HSCT from unrelated donors.[53] Prevention and treatment of viral infections, including CMV post SCT is discussed in detail in Chapter 112 .

FUNGAL INFECTIONS IN HSCT PATIENTS: EPIDEMIOLOGY, PATHOGENS AND MANIFESTATIONS

The risk periods for fungal infection are presented in Figure 101.1 . The overall risks are dependent primarily on the factors that modulate the pace of immune reconstitution, organ toxicities and microbial exposures.

Infections caused by *Candida* species

Although the yeasts that cause infection in HSCT patients include multiple other organisms, such as *Cryptococcus neoformans*, the vast majority of superficial and invasive yeast infections are caused by *Candida* spp.

Invasive *Candida* infections can be separated into two primary syndromes: acute candidiasis (bloodstream infection) and chronic candidiasis (hepatosplenic infection).

Bloodstream infections occur either through an indwelling intravascular catheter or through a damaged GI tract. Acute infection usually manifests as fever and signs of sepsis. Adequate therapy is

Figure 101-1 Primary risk periods for infections after hematopoietic stem cell transplantation. Typical risk periods for the most common infections after each type of HSCT are shown. Risks are based on typical prophylaxis strategies, which include trimethoprim-sulfamethoxazole (co-trimoxazole) for *Pneumocystis carinii*, screened or filtered blood products and ganciclovir for CMV, aciclovir for herpes simplex virus, and fluconazole for candidemia. *Adapted from Bowden.*[54]

essential, not only to cure the acute episode, but also to decrease the likelihood of embolic manifestations that may occur later (e.g. chorioretinitis, endocarditis). [55]

Chronic candidiasis occurs when *Candida* spp. (usually *C. albicans*) enter the portal vasculature, disseminating to liver and spleen. Although the infection develops during a neutropenic phase, it usually presents after resolution of neutropenia, with fever, abdominal pain and liver function abnormalities, and multiple hypodense granulomatous lesions in the liver and spleen.[56] [57] *Candida albicans* is considered to be the most pathogenic species, and this is attributed to multiple virulence factors, such as adhesion molecules, secreted proteases and phospholipases, and the unique ability to generate hyphae in tissues.[58] This organism is usually susceptible to the azole antifungals.[59] [60] [61]

The widespread use of fluconazole, which is supported by numerous randomized trials carried out in the early 1990s,[62] [63] has decreased the incidence and mortality rate attributable to both acute and chronic infections caused by *C. albicans*.[40] [64] [65] This practice has resulted in improved overall survival in allogeneic HSCT patients.[63] However, the decreased incidence of early candidiasis due to azole susceptible species (*C. albicans* and *C. tropicalis*) and improved survival late after HSCT with GVHD has allowed for an increase in infections due to fluconazole-resistant *Candida* spp., such as *C. glabrata* and *C. krusei*.[40] [66] [67] [68] There has also been a shift from *Candida* spp. to molds as the primary fungal pathogens (Fig. 101.2).

The appearance of azole-resistant organisms is not a failure of prophylaxis but a shift to resistant yeasts and molds as pathogens, reflecting the success of supportive care strategies. In patients who underwent allogeneic HSCT from unrelated donors for chronic myelogenous leukemia (CML), two of the most important variables predicting HSCT survival in the 1990s were use of ganciclovir-based strategies and fluconazole.[70]

Infections caused by filamentous fungi

Infections with filamentous fungi are usually acquired through the respiratory route. Although the respiratory tract is the most frequent route of infection, filamentous fungi can also invade through a damaged GI tract.[71] ,72 The most common cause of fungal infections in HSCT patients is currently *Aspergillus fumigatus*.[12] [69] The day of onset of aspergillosis has changed, from primarily the early neutropenic period to later after allogeneic transplant.[11] [12] [13] The risk factors for early and late

disease are different (Fig. 101.3). Risks for

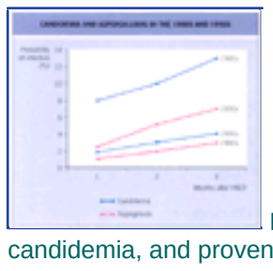

Figure 101-2 Probability of fungal infections in the late 1980s compared to late 1990s at the Fred Hutchinson Cancer Research Center. Probabilities of proven candidemia, and proven or probable aspergillosis are shown. *Data from the 1980s are provided by Dr Raleigh Bowden; data from the 1990s are abstracted from Marr et al.*[40] [69]

early disease are primarily those that impact on the pace of engraftment, such as the specific stem cell product. There is also some indication that a portion of patients who present with aspergillosis early after HSCT may have been exposed to the organism before conditioning therapy; this may explain some of the impact of 'host' variables, such as age and underlying diseases. Risks during the late period are largely those associated with GVHD and its therapies (see Fig. 101.3). Other infections, such as CMV disease, may pose both direct and indirect risks for subsequent disease. There has been an increase in invasive infections caused by Zygomycetes and *Fusarium* spp.[69] Patients who receive transplants from an unrelated or an HLA-mismatched donor, and patients with severe GVHD have particularly high risks for Zygomycetes infections. [69]

Filamentous fungi are usually acquired through the respiratory route. Conidia, which are present in the environment, are inhaled

Figure 101-3 Risk factors for aspergillosis after allogeneic hematopoietic stem cell transplantation. Specific risks are demonstrated according to primary risk period (shaded) and day of transplantation. [10] [13] [73] Day 0 represents day of receipt of stem cells. CML-CP, chronic myelogenous leukemia in chronic phase, LAF, laminar airflow.

into the lungs, and in the absence of an efficient phagocytic response, they can germinate into tissue-invasive hyphae. Although inhalation appears to be the most common mechanism of acquisition, with lungs the most common involved organ, these organisms can also invade through a damaged GI tract. Filamentous fungi, especially *Asperigillus* sp. and Zygomycetes, have been reported to cause neutropenic colitis and hepatosplenic disease in HSCT recipients[74] [75] .

SUMMARY

Infections remain a leading cause of death in HSCT patients, but the timing of onset and the spectrum of pathogens have evolved over the past two decades. Changes in transplantation practices, different hosts and the development of effective strategies to prevent early CMV disease and candidiasis have increased survival late after transplant with severe GVHD. This has resulted in the emergence of molds as a primary cause of death.

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Chapter 102 - Infection in Solid Organ Transplantation

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INTRODUCTION

About 40,000 transplants are performed throughout the world each year, with 1-year graft survival rates of 90% or more at many transplant centers, making organ transplantation the most practical means of rehabilitating patients with end-stage kidney, heart, liver and lung disease. However, infectious challenges must be overcome for clinical success.[1] [2] [3]

Treatable infection must be eradicated in the recipient before transplant. Immunosuppression exacerbates infections and increases the possibility of vascular suture line infections, pulmonary infections and others. Donors must be screened for HIV, hepatitis viruses and other viruses. Bloodstream infections in the donor threaten the anastamoses of the transplanted organ and must be avoided. Technical issues include not only the transplant operation itself, but also the management of vascular access devices, the endotracheal tube, drains and catheters. Immunosuppression is needed to prevent and treat rejection. In addition, an antimicrobial strategy is needed to make this immunosuppressive program safe. The specifics of this therapeutic prescription are linked to the immunosuppressive therapy.[4]

Rejection and infection remain the two greatest hurdles to successful transplantation. They are closely linked by immunosuppressive therapy and by the array of cytokines, chemokines and growth factors elaborated. The response to both processes is quite similar, such that these two processes influence one another and, in many cases, amplify the extent of tissue injury. If infection occurs, the prognosis is in part determined by how early the diagnosis is made and how soon treatment is initiated. The anti-inflammatory effects of immunosuppressive therapy, particularly corticosteroids, render symptoms, signs and radiologic findings less overt. Therefore, the clinician must be aggressive in diagnosis with intensive imaging and invasive testing, where indicated. Up to 75% of transplant patients have evidence of microbial invasion in the first year post-transplant.[2] [3] [4]

RISK OF INFECTION

The risk of infection, particularly opportunistic infection, in transplant patients is determined primarily by the interaction of three factors:

- technical and anatomic mishaps,
- environmental exposures, and
- the net state of immunosuppression.

Devitalized tissue, fluid collections and invasive devices compromise the primary mucocutaneous barriers (Table 102.1). Technical and anatomic abnormalities are the major cause of infection in the first month post-transplant, with their incidence being related to the complexity of the particular transplant operation. Thus, these problems are most common in liver transplantation, particularly with split livers transplanted into children. These problems are also common in lung transplantation, less common in heart transplantation and least common in kidney transplantation. Optimal therapy for infections in patients with technical or anatomic abnormalities combines appropriate antimicrobial therapy with correction of the abnormality.

Important environmental exposures occur in the community and in hospital (see Table 102.1). Major concerns include recent or remote exposures to *Mycobacterium tuberculosis*, the systemic mycoses (blastomycosis, coccidioidomycosis and histoplasmosis) and *Strongyloides stercoralis*. [2] [3] [4] *Strongyloides* can persist in the gastrointestinal tract for decades after acquisition, owing to a unique autoinoculation cycle in humans. With immunosuppression, infestation may be greatly accentuated, leading to hemorrhagic pneumonitis, enterocolitis and disseminated strongyloidiasis (in which the filariform larvae penetrate the gut and invade other tissues). This last syndrome is often accompanied by Gram-negative bacteremia or meningitis unresponsive to conventional therapy. Therapy requires treatment of both the bacteria and the strongyloidiasis. Even so, the mortality is 50–75% with optimal therapy. Accordingly, our policy is to screen the patient serologically before transplantation for strongyloidiasis and to treat any patient with a positive serology pre-emptively.[5]

Community-acquired respiratory virus infection (e.g. influenza, respiratory syncytial virus, parainfluenza) is a particular problem in transplant patients. Although vaccines and antiviral agents exist, they are only marginally effective in the transplant population. Therefore, avoidance of exposure to respiratory infections is the best strategy.[2] [3]

Gardening has been associated with the acquisition of *Aspergillus* and *Nocardia* infection following inhalation of organisms aerosolized by digging in the soil. Similarly, aerosols generated in the south-west and mid-west of the USA, and elsewhere, can lead to infection with *Coccidioides immitis* and *Histoplasma capsulatum*. Construction sites in the community should likewise be avoided. Inadequately cooked food or contaminated water can convey *Salmonella, Listeria* and *Giardia* infection.[2]

Nosocomial exposures can be divided into domiciliary and nondomiciliary. Domiciliary exposures occur when contaminated air or potable water is present in the hospital room or ward where the patient is housed. Cases of this type are usually clustered in time and space. Outbreaks due to *Aspergillus* spp., *Legionella* spp., *Pseudomonas aeruginosa* and other Gram-negative bacilli are well documented. Filtration of air by a high-efficiency particulate filter, surveillance and treatment of water, and barring flowers and plants from the hospital environment have significantly reduced this problem. Far more difficult to detect and control are nondomiciliary environmental hazards encountered in the rest of the hospital, such as radiology suites undergoing construction, operating rooms, the waiting areas outside cardiac catheterization suites and endoscopy suites. Because of the lack of obvious clustering in time and space, outbreaks of this type are more difficult to identify. The best clue to the presence of an undetected environmental hazard is the occurrence of opportunistic infection when the patient's net state of immunosuppression should not predispose to it.[2] [6] [7]

An additional source of nosocomial infection is person-to-person spread, largely from the hands of medical personnel. Organisms such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and azole-resistant *Candida* spp. are examples.

TABLE 102-1 -- Classification of infections occurring in transplant patient.

CLASSIFICATION OF INFECTIONS OCCURRING IN TRANSPLANT PATIENT

Infections related to technical complications*	Transplantation of a contaminated allograft
	Anastomotic leak or stenosis
	Wound hematoma
	Intravenous line contamination
	Iatrogenic damage to the skin
	Mismanagement of endotracheal tube leading to aspiration
	Infection related to biliary, urinary and drainage catheters
Infections related to excessive nosocomial hazard	*Aspergillus* spp.
	Legionella spp.
	Pseudomonas aeruginosa and other Gram-negative bacilli
	Nocardia asteroides
Infections related to particular exposures within the community	Systemic mycotic infections in certain geographic areas (*Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*)
	Stronglyloides stercoralis
	Community-acquired opportunistic infection resulting from ubiquitous saphrophytes in the environment† *(Cryptococcus neoformans*, *Aspergillus* spp., *Nocardia asteroides*, *Pneumocystis carinii*)
	Respiratory infections circulating in the community (*Mycobacterium tuberculosis*, influenza, adenoviruses, parainfluenza, respiratory syncytial virus)
	Infections acquired by the ingestion of contaminated food or water (*Salmonella* spp., *Listeria monocytogenes*)
Viral infections of particular importance in transplant patients	Herpes group viruses
	Hepatitis viruses
	Papillomavirus
	HIV
From Rubin[2]	

* All lead to infection with Gram-negative bacilli, *Staphylococcus* spp. or *Candida* spp.
† The incidence and severity of these infections and, to a lesser extent, the other infections listed, are related to the net state of immunosuppression present in a particular patient

The net state of immunosuppression (Table 102.2) is a complex function determined by a number of factors. Although the immunosuppressive therapy is the prime determinant, other factors are also important. Protein-calorie malnutrition, and presumably other metabolic derangements, contribute significantly to immunosuppression. If one stratifies patients by serum albumin level (<2.5g/dl or >2.5g/dl), there is a 10-fold increase in the incidence of life-threatening infection in the hypoalbuminemic. Approximately 90% of opportunistic infections occur along with one or more immunomodulating viruses. The 10% that are unassociated are usually due to previously unrecognized environmental hazards.[2] [3] [4] The immunogenetic makeup of the patient is probably important. For example, polymorphisms affecting the production of tumor necrosis factor (TNF)-a may be important, with low production of TNF-a being associated with an increased risk of infection. Platelets play an important role in controlling possible fungal infection. Given the

TABLE 102-2 -- Determinants of immune competence.

DETERMINANTS OF IMMUNE COMPETENCE	
Factor	**Examples**
Intensity of the immunosuppressive regimen	Monoclonal antibodies anti-TNF-a agents
	Calcineurin binding agents
	Corticosteroids
	Mycophenolate
Underlying immune dysfunction	Immunodeficiency
	Cytopenias
	Uremia
	Hyperglycemia
	Malnutrition
Breach of normal barriers	Intravenous catheters
	Urinary catheters
	Surgical drains
	Prolonged intubation
	Decubitus ulceration
Anatomic factors	Poor tissue perfusion
	Devitalized tissues
	Fluid collections
	Urinary retention
Immunomodulating infections	Cytomegalovirus
	Epstein-Barr virus
	HIV
	Hepatitis C virus
	Hepatitis B virus
Adapted from Fishman and Rubin[3]	

frequency of thrombocytopenia in liver transplant recipients, this may be important in these patients.[8] [9] [10] There is a semiquantitative relationship between environmental exposures and the net state of immunosuppression. The greater the exposure, the less immunosuppression is required to induce clinical infection. Conversely, the more profound the immunosuppression, the more trivial the exposure needed to induce disease.

TIMETABLE OF INFECTION IN THE ORGAN TRANSPLANT RECIPIENT

There is a definite temporal pattern to the types of infection that affect the transplant patient, which is useful in framing a differential diagnosis in a patient who presents with an infectious disease syndrome, in guiding cost-effective preventive strategies and as a tool for infection control (Table 102.3). Exceptions to the pattern usually

connote a previously unidentified environmental exposure. The basic timetable is identical for all solid organ transplant recipients, and can be divided into three time periods: the first month post-transplant, 1–6 months post-transplant, and more than 6 months post-transplant (Fig. 102.1).[2] [3] [4]

Infection in the first month post-transplant

Infections occurring in the first month post-transplant are usually due to infections conveyed with the allograft, infection that was present in the recipient before transplant that continues post-transplant, or infection caused by technical or anatomic difficulties. Active infection in the donor at the time of organ retrieval is a significant risk. HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) and other systemic viral infections (such as West Nile virus) are efficiently transmitted via the allograft. Bloodstream infection in the donor at the time of donation is not uncommon, given the aggressive care (e.g. multiple

TABLE 102-3 -- Likely etiologies of various clinical presentations of infection in transplant patients.

LIKELY ETIOLOGIES OF VARIOUS CLINICAL PRESENTATIONS OF INFECTION IN TRANSPLANT PATIENTS	
Clinical presentations	**Etiology and considerations**
Fever	Rejection
	Post-transplant lymphoproliferative disease
	Viral infection (cytomegalovirus, Epstein-Barr virus, human herpesvirus-6)
	Bacteremia
	Often more pronounced in children
Bacteremia or sepsis	Related to indwelling lines (coagulase-negative staphylococci, *Corynebacterium jeikeium*, enterococci, Gram-negative rods, *Candida* spp.)
Urinary tract infections	Related to urinary dysfunction or catheters (Gram negative rods, enterococci, *Candida* spp.)
Pulmonary infection	Community-acquired (*Streptococcus pneumoniae*, *Legionella* spp., *Mycoplasma* spp., *Chlamydia* spp., seasonal viral infections)
	Nosocomial (Gram-negative rods, *Staphylococcus aureus*)
	Opportunistic infections (*Pneumocystis carinii*, *Nocardia asteroides* (which may disseminate), *Mycobacterium tuberculosis*, filamentous fungi, endemic fungi, viral infections (cytomegalovirus, varicella-zoster virus, adenovirus))
	Drug reaction (sirolimus, methotrexate)
Hepatitis	Viral hepatitis (hepatitis B, hepatitis C)
	Drug related
Cutaneous	Cellulitis (staphylococci, streptococci, Gramnegative rods)
	Papules (*Candida* spp., fungi, *Nocardia* spp.)
	Exanthems (viral, drug reaction)
	Ulcers or vesicles (herpes simplex virus, varicellazoster virus, cytomegalovirus)
	Necrosis (embolic fungal infection)
Retrosternal pain	Esophagitis (herpes simplex virus, *Candida* spp.)
Abdominal pain (with or without diarrhea)	Diverticulitis
	Enteric infection (*Salmonella* spp., *Campylobacter* spp., *Listeria* spp. (which may be bacteremic), *Clostridium difficile* colitis, *Strongyloides stercoralis*, cytomegalovirus)
Central nervous system	Meningitis (*Listeria* spp., *Cryptococcus neoformans*, *Mycobacterium tuberculosis*, *Coccidioides immitis*, *Histoplasma capsulatum*)
	Focal cerebritis (bacterial, fungal, herpesviruses)
	Brain abscess (*Nocardia* spp., *Aspergillus* spp., *Toxoplasma gondii*, bacteria)
	Demyelination or dementia (JC virus (progressive multifocal leukencephalopathy))
	Drug related (fludarabine)

vascular access lines, bladder catheters, assisted ventilation) that many donors receive before brain death is declared. Although blood cultures that are positive for such commensal organisms as *Staphylococcus epidermidis*, diphtheroids and lactobacilli rarely cause a problem for the recipient, *Pseudomonas aeruginosa*, the Enterobacteriaceae and *S. aureus* may seed suture lines, resulting in mycotic aneurysms.[2] [3] [4] [11]

The first rule of transplant infectious disease is to eradicate all treatable infections before transplantation. Dialysis in patients undergoing renal transplantation provides the time and opportunity to accomplish this task. However, patients coming to transplantation of extrarenal organs may be quite debilitated and may require intubation, left ventricular assist devices or indwelling vascular catheters, all of which predispose to infection. Particular attention should be paid to lung injury, whether due to aspiration chemical injury, pulmonary infarction or bacterial infection. Transplantation in the setting of active pulmonary disease is highly associated with serial superinfections post-transplant, which carry an extremely high mortality.[2]

More than 95% of infections in the first month post-transplant are the same infections that occur in routine patients undergoing comparable surgery. Factors leading to deep wound infection include the need for re-exploration, fluid collections (a particular problem in liver transplantation) or bloodstream infection. [2] [3] [4] In the first month following transplantation the daily doses of immunosuppressives are at their highest, but opportunistic infections are extremely rare unless already present at the time of transplantation. Opportunistic infection during this period suggests an environmental hazard.[2] [3] [4]

Infection 1–6 months post-transplant

This is the period of the highest incidence of post-transplant infection resulting from the direct consequences of viral infection, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus (HHV)-6, HBV, HCV and HIV. Furthermore, there is a higher net immunosuppression caused by sustained immunosuppressive therapy in conjunction with immunomodulating viral infections, and opportunistic infections such as *Pneumocystis carinii*, *Listeria monocytogenes* and *Aspergillus* spp., with modest environmental exposure. Cytomegalovirus accounts for about two-thirds of all the febrile episodes during this period. Prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX, co-trimoxazole) prevents infection with *P. carinii*, *L. monocytogenes* and *Nocardia* spp. as well as toxoplasmosis and urinary tract infection. A single-strength tablet daily at bedtime is preferred, although alternate-day double-strength therapy is acceptable. Without such prophylaxis, the incidence of *Pneumocystis* infection is about 15% during this period. For patients intolerant of TMP-SMX, *Pneumocystis* prophylaxis should be atovaquone (1500mg/day orally), pentamadine (300mg/month intravenously), dapsone (100mg/day) or pyrimethamine-sulfadoxine weekly. Prophylaxis with TMP-SMX is strongly preferred because of its wide range of prophylactic benefits.

Infection more than 6 months post-transplant

Immunosuppressed patients with functioning allografts more than 6 months post-transplant fall into three categories. Approximately 80% will have good allograft function and minimal maintenance immunosuppression without chronic viral infection. Their biggest routine infectious disease risk is community-acquired respiratory virus infection and its sequelae. The most common opportunistic infection is an asymptomatic pulmonary nodule due to *Cryptococcus neoformans.* Approximately 15% will have a chronic viral infection with the hepatitis viruses, papillomavirus, HHV-8 or HIV. Without effective antiviral therapy, progressive liver disease, hepatocellular carcinoma, squamous cell carcinoma, Kaposi's sarcoma from HHV-8, or overt AIDS will occur. Approximately 10% will have a poor result with acute and chronic rejection, severe immunosuppression and, often, chronic immunomodulating viral infection. These patients are at highest risk of infection with *P. carinii, C. neoformans*,

Nocardia spp., *Aspergillus* spp., *L. monocytogenes* and other opportunistic organisms. Decreased immunosuppressive therapy, consideration of

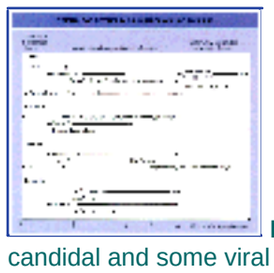

Figure 102-1 Timetable of infection in the organ transplant recipient. The typical infections are plotted by time following transplantation. Note the occurrence of bacterial and candidal and some viral infections in the first month. This is followed by predominantly viral infections between 1 and 6 months, and then fungal and chronic viral infections after 6 months, largely in patients who have chronic rejection and who are receiving more intensive immunosupression. Infections that deviate from this schema suggest a higher level of immunosuppression or a more intense environmental exposure. *Reproduced with permission from Fishman and Rubin.*[3] *CMV, cytomegalovirus; EBV, Epstein-Barr virus; PTLD, post-transplantation lymphoproliferative disease; VZV, varicella-zoster virus.*

re-transplantation, and lifetime prophylaxis with TMP-SMX and perhaps fluconazole are the important elements of management.[2] [3] [4]

ANTIMICROBIAL THERAPY IN THE TRANSPLANT RECIPIENT

Antimicrobial drugs in the transplant patient may interact with ciclosporin and tacrolimus, both of which are metabolized by hepatic cytochrome P450 enzymes, which are in turn modulated by certain antimicrobial agents. Rifampin (rifampicin), nafcillin and isoniazid upregulate ciclosporin and tacrolimus metabolism, resulting in lower blood levels and an increased risk of rejection. The macrolides (erythromycin more than clarithromycin and much more than azithromycin) and the azole antifungals (ketoconazole more than itraconazole more than voriconazole and much more than fluconazole) downregulate ciclosporin and tacrolimus metabolism, resulting in higher blood levels, an increased risk of nephrotoxicity and the possibility of excessive immunosuppression.

Synergistic nephrotoxicity (accelerated nephrotoxicity) can also develop. Renal injury commonly develops when nephrotoxic drugs (e.g. amphotericin deoxycholate, gentamicin) are added to calcineurin inhibitors. Renal toxicity can be observed after exposure to 100mg or less of amphotericin, just a few doses of aminoglycoside, or vancomycin. Single doses of amphotericin B, vancomycin, TMP-SMX and gentamicin can precipitate oliguric renal failure in the setting of calcineurin inhibitor therapy (idiosyncratic nephrotoxicity). A single strength TMP-SMX tablet is well tolerated in the face of calcineurin inhibitor therapy, but treatment doses for *Pneumocystis* infection are almost invariably associated with renal toxicity. Similarly, 250mg of ciprofloxacin twice daily is well tolerated in the face of ciclosporin or tacrolimus therapy, but 500mg twice daily is associated with a significant rate of renal injury (dose-related nephrotoxicity). It is not known whether these forms of renal injury have a similar pathogenesis.

Every effort should be made to avoid aminoglycosides, amphotericin and vancomycin, replacing them with compounds that are free of synergistic toxicity. The changes in calcineurin inhibitor metabolism induced by the drugs noted above can be managed by checking blood levels of ciclosporin and tacrolimus and making appropriate adjustments. The clinician must be alert for the need to adjust doses both at the initiation and cessation of antimicrobial therapy.

IMMUNOSUPPRESSIVE THERAPY AND INFECTION IN THE TRANSPLANT RECIPIENT

The standard immunosuppressive regimen employed today is a three-drug regimen consisting of cyclosporine or tacrolimus, azathioprine or mycophenolate, and prednisone. Antilymphocyte antibody therapy and monoclonal antibodies against the interleukin (IL)-2 receptor (daclizumab and basilixmab) are used as induction therapy in patients with oliguric renal injury in order to shorten the duration of the injury, or to treat rejection. Optimal use of these agents, as well as rapamycin (sirolimus), is still evolving. For detailed discussions of the mechanisms of action and toxicities of these agents, see Chapter 99 .

Corticosteroids have been important tools for immunosuppression since the early days of clinical transplantation. However, their extensive side-effect profile has driven the search for so-called corticosteroid-sparing therapies. Corticosteroids have anti-inflammatory and immunosuppressive effects. They inhibit proinflammatory cytokine production, decrease the accumulation of polymorphonuclear leukocytes at sites of inflammation, increase the ratio of B lymphocytes to T lymphocytes, increase the ratio of $CD8^+$ cells to $CD4^+$

cells, inhibit arachidonic acid metabolites, block vascular permeability in inflammatory processes and impede nitric oxide production. Therefore, by the time of clinical presentation, the microbial burden may be unusually large.[2] [3] [4]

Azathioprine is converted into 6-mercaptopurine after absorption. It inhibits both early stages in purine synthesis as well as steps in the purine salvage pathway, thus depleting purine stores and inhibiting nucleic acid synthesis. It is a potent inhibitor of actively dividing lymphocytes, with little impact on mature elements of antigenic memory. Its major toxicity is on rapidly dividing cells in the bone marrow. The key enzyme in its metabolism, thiopurine methyltransferase, exhibits considerable genetic heterogeneity resulting in fast and slow metabolizers with resulting effects on both immunosuppression and toxicity.

Mycophenolate mofetil (MMF) is a prodrug that is cleaved by plasma esterases to yield the immunosuppressive molecule mycophenolic acid, an inhibitor of inosine monophosphate dehydrogenase, which plays a key role in the de-novo biosynthesis of guanosine.[12]

Ciclosporin and its fellow calcineurin inhibitor, tacrolimus, have revolutionized organ transplantation, increasing 1-year cadaveric donor renal allograft survival from 50% to about 90%. Inhibition of IL-2 production is the dominant effect, resulting in a profound suppression of T-cell function and effect. In particular, a dose-related inhibition of microbial specific T-cell cytotoxic activity is of prime importance. Ciclosporin and tacrolimus amplify the level of replicating virus (i.e. viral load), which is of particular importance in CMV and EBV infection. Epstein-Barr virus-associated post-transplant lymphoproliferative disease occurs at an increased rate in patients achieving high levels of the calcineurin inhibitors. Therefore, antiviral therapy directed against the herpesviruses is necessary for any therapeutic prescription that includes ciclosporin or tacrolimus.[2] [3] [4] [13]

Tacrolimus shares a number of properties with ciclosporin. The immunophilins that bind to tacrolimus (FK506 binding proteins) are distinct from those binding ciclosporin. T-cell activation is blocked as a result of calcineurin inhibition. Tacrolimus and ciclosporin affect a similar array of cytokines, with IL-2 inhibition being the most important. The net result is inhibition of T-cell proliferation, inhibition of primary and secondary cytotoxic T-cell responses, and blockage of B-cell response to vaccine and other antigens. Secondary antibody responses, natural killer cell activity, and antibody-dependent cytotoxic cell functions are unaffected. Tacrolimus is 10–100 times more potent than ciclosporin.[4] [14]

Although rapamycin is similar in structure to tacrolimus, its mechanism of action and immunosuppressive effects are entirely different. Rapamycin enters the cell and forms a complex with FK506 binding proteins, but instead of targeting calcineurin and cytokine transcription, it binds to a protein kinase termed the 'target of rapamycin' (TOR), which results in a blockage in the translation of mRNAs encoding cell cycle regulators. In terms of infection, it decreases microbial specific T-cell function and should have similar effects to those of the calcineurin inhibitors. [4]

Polyclonal antilymphocyte preparations of either equine or rabbit origin are potent T-cell-depleting agents. OKT3, a murine monoclonal antibody directed against the CD3 T-cell receptor complex, is the most potent agent in the treatment of acute cellular rejection. The infectious disease impact of OKT3 is essentially identical to that of the polyclonal agents.[2] [3] [4]

The monoclonal antibodies daclizumab and basilixmab are both directed against the IL-2 receptor. These do not cause the massive release of proinflammatory cytokines associated with the other antibody preparations. These appear to be less potent in terms of predisposing to infection, while still having benefits in terms of preventing allograft rejection. However, optimal deployment of these agents is still being defined.[2] [3] [4]

IMPORTANT INFECTIONS IN ORGAN TRANSPLANT RECIPIENTS

Herpesviruses

Herpesviruses such as CMV, EBV, herpes simplex virus (HSV)-1, HSV-2, varicella-zoster virus (VZV), HHV-6, HHV-7 and HHV-8 are the most important class of pathogens affecting the transplant recipient. They demonstrate lifelong latency but can be reactivated in response to a variety of stimuli, particularly proinflammatory cytokines. In general, primary disease, as opposed to reactivation disease, is more severe.

Herpesviruses should be considered oncogenic until proven otherwise. This is exemplified by the relationship between EBV and post-transplant lymphoproliferative disease (PTLD) and between HHV-8 and Kaposi's sarcoma; direct effects of these viruses are critical to the pathogenesis of these neoplastic states. Cytomegalovirus disease increases the risk of subsequent PTLD by between 7 and 10 times.

Cytomegalovirus

Cytomegalovirus is the single most important pathogen affecting transplant patients, both directly as a cause of infectious disease syndromes and indirectly through cytokines and other mediators elaborated in response to viral replication and invasion. Acquisition of CMV can be through transfusion of a viable leukocyte-containing blood products from a CMV seropositive donor, transplantation of an organ from a CMV seropositive donor, reactivation of endogenous CMV post-transplant in a seropositive recipient, and, least often, acquisition of primary infection. The serologic status of the donor and recipient at the time of transplant and the immunosuppressive regimen determine the outcome of CMV infection.[2] [3] [4]

There are four key elements in the pathogenesis of CMV:

- reactivation;
- amplification and systemic dissemination;
- development of MHC-restricted, virus specific, cytotoxic T-cells; and
- elaboration of cytokines, chemokines and growth factors in response to the replicating virus.

Reactivation of virus from latency is largely accomplished by any processes that induce TNF-a. A 'second wave' phenomenon is seen when patients present with and are cured of bacterial sepsis, only to present 2–3 weeks later with active CMV infection. The stress catecholamines, epinephrine (adrenaline) and norepinephrine (noradrenaline), and proinflammatory prostaglandins are less important pathways in this regard. Inflammatory processes thus have a high probability of reactivating CMV from latency.[2] [3] [4] [15] [16]

Different immunosuppressive drugs have different effects on CMV. Ciclosporin, tacrolimus, prednisone and rapamycin have no ability to reactivate CMV from latency, but once replicating virus is present these drugs potentiate viral amplification.

Without antiviral prophylaxis, clinical CMV disease typically occurs 1–4 months post-transplant. An antiviral program may delay the onset of clinical disease for weeks to months, at which time the clinical effects are identical to those observed without prophylaxis. Most aspects of clinical CMV disease are identical in most forms of organ transplantation. However, the transplanted organ is usually far more affected by the virus than is the native organ. Thus, although transient abnormalities in liver function can be seen in all solid organ transplant patients, clinically, symptomatic hepatitis is only an issue in liver transplant patients; similarly, myocarditis is an issue in heart recipients and pancreatitis in pancreatic allograft recipients. The incidence of CMV pneumonia is far greater in lung transplant recipients than in other solid organ transplant patients.[17]

Cytomegalovirus infection can involve any part of the gut, causing enteritis, focal ulceration, or perforation. Functional abnormalities in

motility have been observed in the absence of gross abnormalities on endoscopy but with CMV clearly visible on biopsy. It is important to keep in mind that CMV enteritis can occur without demonstrated viremia.[2] [3] [4] Cytomegalovirus chorioretinitis is the major late complication of CMV (usually >6 months post-transplant), with the virus causing necrosis of the retina, but retinal detachment or anterior uveitis may occur.[2]

Cytomegalovirus infection may augment immunosuppression with potentiation of superinfection, such as *P. carinii,* invasive aspergillosis, Gram-negative infection (both colonization and subsequent invasion of the respiratory tract), bacteremia, listeriosis, candidemia and HCV increase.

In 1970, Richard Simmons first postulated that CMV infection could be linked to allograft injury.[18] Recently, trials of anti-CMV therapy have demonstrated a decrease in acute rejection and, probably, in chronic allograft injury, apparently substantiating this view.[19] [20]

The diagnosis of CMV has advanced significantly in the past decade (see Chapter 112 and Chapter 215). Viremia is the major determinant of prognosis. Serologic tests for CMV are most valuable pre-transplant, since they provide essential information for assessing risk of CMV disease and guide the appropriate preventive strategy. Serial blood samples assessing the patient's response to infection do not provide timely information for making therapeutic decisions, and determination of IgM anti-CMV antibody adds little.

The cornerstone of the treatment of CMV infection is ganciclovir. Valganciclovir should permit the successful oral treatment of CMV disease. Patients at risk of primary CMV infection (seronegative recipients with a seropositive donor) have 50% or higher incidence of clinical disease. In contrast, seropositive recipients treated with conventional immunosuppression have a less than 20% incidence of disease. Either prophylactic or pre-emptive regimens are acceptable.[2] [3] [4] Many centers use intravenous ganciclovir for the first several days of therapy, and then complete therapy with valganciclovir; optimal duration of therapy is unclear. Our approach is to employ full treatment doses until viremia is cleared, and then decrease the dose to prophylactic levels, continuing oral valganciclovir for an additional 2–3 months. If ganciclovir resistance develops, foscarnet is used, despite significant renal toxicity (see also Chapter 205).

Epstein-Barr virus

The natural reservoir for EBV is the epithelial cells of the upper respiratory tract. Long-lived B lymphocytes become infected as they travel through the lymphoid tissue of the oral cavity, which results in latent infection of these cells and subsequent immortalization. Antiviral therapy of these infections has little effect on the symptoms noted by the patient. It is likely that EBV produces clinical effects that are comparable to those produced by CMV (e.g. mononucleosis, hepatitis, fever). The major clinical effect of EBV that is recognized is its role in the pathogenesis of EBV-associated PTLD,[2] [3] [4] [21] [22] a number of pathologic entities that range from the benign to the frankly malignant (Table 102.4). Complicating the pathologic evaluation of biopsies of PTLD is the lack of uniformity within a particular lesion or in different lesions in the same patient. Thus, there can be sites of polyclonality, oligoclonality and even monoclonality in the same patient, making the staging of the process and evaluation of therapy difficult.

The incidence of PTLD is different in different forms of transplantation, at least in part related to the intensity of immunosuppression: 1–3% among kidney, heart and liver allograft recipients, but higher (7–33%) after lung, intestine and multivisceral transplantation. The pathogenesis of EBV-associated PTLD involves the number of B lymphocytes transformed by EBV (a function of viral load, which is dependent on the intensity of the immunosuppressive therapy) and the activity of EBV-specific cytotoxic T-cells, which is

TABLE 102-4 -- Society of Hematopathology classification of post-transplant lymphoproliferative disease (PTLD).

SOCIETY OF HEMATOPATHOLOGY CLASSIFICATION OF PTLD	
Lymphoid hyperplasia (early lesions)	Included in this category are plasma cell hyperplasia, lesions resembling infectious mononucleosis, and other forms of atypical lymphoid hyperplasia with preservation of the underlying architecture. These lesions are usually polyclonal and often regress with reduction of immunosuppression
Polymorphic PTLD	These are destructive lesions that infiltrate and destroy underlying tissue, with a wide range of B-cell maturation being present. Molecular studies suggest that virtually all of these tumors are monoclonal. Oncogene or tumor suppressor gene abnormalities are not a feature of these tumors. A lower percentage of these tumors respond to reduction of immunosuppression

Lymphomatous or monomorphic PTLD	Most such tumors are of the diffuse large B-cell lymphoma subtype, although Burkitt-like lymphomas and mucosa-associated lymphomas (MALT lymphomas) may also occur as variants of B-cell disease. Abnormalities in the ras or p53 genes of the diffuse B-cell lymphomas are not uncommon. These tumors are monoclonal, with only a minority responding to decreased immunosuppressive therapy. In addition, such uncommon forms of PTLD as T cell, null cell and NK cell lymphoma are placed in this category. A minority of these non-B-cell tumors are EBV-positive, and response to decreased immunosuppression is uncommon
Other forms of PTLD	Included in this category are such uncommon tumors as plasmacytoma, myeloma and T-cell-rich/Hodgkin's disease-like large B-cell lymphoma. These tumors may or may not be EBV-positive and tend to be clinically aggressive
In general, Epstein-Barr virus (EBV)-positive tumors occur in the first 6 months post-transplant; in contrast, although the EBV-negative PTLDs can occur as early as 6 months post-transplant, they occur at a mean time of 4 years post-transplant	

also affected by the immunosuppressive regimen. The cytokine milieu is an additional factor. Preceding CMV disease increases the incidence of PTLD 7- to 10-fold. Durandy used a monoclonal antibody against IL-6 in a group of 12 PTLD patients who had failed conventional therapy; six went into complete remission and six went into partial remission.[2] [3] [4] [23] [24]

Approximately 90% of adults harbor latent EBV and are EBV-seropositive; they develop PTLD as a consequence of EBV reactivation. Most children are EBV-seronegative before transplant; primary EBV infection is associated with higher viral loads than reactivation and hence with a higher incidence of PTLD (see Table 102.4).[2] [3] [4]

Post-transplant lymphoproliferative disease may present with unexplained fever, mononucleosis, gastrointestinal bleeding, abdominal pain, gut perforation or obstruction, hepatocellular dysfunction, acute cholecystitis, or focal disease in the brain. In pediatric transplant patients, a common presentation is with 'tonsillitis', which, on pathologic examination, is found to be lymphomatous. Post-transplant lymphoproliferative disease may behave like a B-cell lymphoma in the normal host, or it may be entirely extranodal. The allograft itself may be a privileged site for PTLD.[2]

Optimal management of PTLD has not yet been established. The first step is usually to stop immunosuppression or, if this is not possible,

to decrease it by 50–75%. About one-quarter of patients with PTLD, particularly children, will respond to this approach. Most centers use ganciclovir, acyclovir or foscarnet; antilymphoma chemotherapy, radiotherapy and surgery have not been particularly successful. Anti-B-cell monoclonal antibodies have a success rate of >60%.[2] [24]

Human herpesvirus-6

Human herpesvirus-6 infects and replicates within a variety of leukocyte populations, resulting in elaboration of a broad array of proinflammatory cytokines. Thus, HHV-6 may cause similar effects to those of CMV. Human herpesvirus-6 can also cause bone marrow suppression and encephalitis and has been reported to correlate both with prolonged hospital stay and with need for readmission.[25]

Replication of HHV-6 occurs in 30–50% of patients 1–6 months post-transplant, precisely when CMV is most active. The combination of two or more simultaneous herpesvirus infections (e.g. CMV plus HHV-6) may have worse consequences than a single infection. Human herpesvirus-6 infection is best diagnosed by polymerase chain reaction (PCR) assay on blood, and susceptibility to ganciclovir and foscarnet is similar to that of CMV. Clinical activity of these drugs has been reported in HHV-6 encephalitis.

Hepatitis viruses

The incidence of chronic hepatitis in solid organ transplant patients has remained largely unchanged for two decades, with 10–15% of transplant recipients infected, almost all of which are with HCV. Immunosuppression usually means that transplant patients will not clear HCV and HBV. The clinical impact of chronic hepatitis is far greater than in the normal host, and the time necessary to produce end-stage liver disease or hepatocellular carcinoma is shortened. The current antiviral therapies are less effective than in nonimmunosuppressed patients with similar infections. [2]

Hepatitis B virus

In immunosuppressed patients, reversion from HBV anticore antibody (anti-HBc) to HBV surface antigen (HbsAg) positivity has been documented, but is uncommon. A bigger concern is the impact of transplanting organs from an HbsAg-negative, anti-HBc-positive donor. Liver allografts from such donors carry a significant risk of transmitting HBV infection, with PCR on such livers often showing virus. In contrast, other organs transplanted from such donors carry a minimal risk of transmitting HBV infection.[26]

When HBV infection is already present, corticosteroid therapy promotes virus replication, with a marked increase in HBV DNA polymerase activity, HBV DNA, HBV e-antigen (HbeAg) and HbsAg. The calcineurin inhibitors also increase the probability of chronic, progressive infection. Kidney, heart and lung transplant recipients with active HBV infection do relatively well over the first 18–24 months post-transplant, but then begin to show evidence of chronic liver disease or hepatocellular carcinoma, or both. By 8–10 years post-transplant, patients with HBV-associated chronic liver disease have had increased death rates from hepatic failure, hepatocellular carcinoma and sepsis — rates above that seen with other forms of chronic liver disease.[2] Evidence of active viral replication at the time of transplantation has been associated with poor outcomes. Two approaches have markedly improved the outcome for these patients: indefinitely prolonged immunoprophylaxis with hepatitis B immunoglobulin at approximately monthly intervals, and prolonged therapy with lamivudine.[27] The combination of these two therapies has been proven effective, perhaps at a lower cost. Development of drug-resistant mutants usually becomes apparent after 12 or more months of lamivudine therapy (the incidence at 1 year being as high as 30% and at 2 years 38%). There is considerable debate about the optimal time for initiating therapy — before transplant, early post-transplant or later.[28] [29] Multidrug regimens akin to those used in HIV infection are likely to be needed.

Fibrosing cholestatic hepatitis may occur in liver transplant patients with either HBV or HCV infection, but it is more common with HBV. This presents as rapidly progressive hepatic failure, often in the first 6 months post-transplant, resulting in death within 4–6 weeks of presentation. Histology shows minimal inflammatory cells, ballooning of the hepatocytes, cholestasis and high HBV loads in hepatocytes and in the circulation. It is believed that this form of liver injury is caused by a direct cytopathic effect of the virus; lamivudine may be effective. [2] [30]

Hepatitis C virus

Hepatitis C virus accounts for >80% of the progressive liver disease that occurs post-transplant, and HCV liver disease is currently the most common indication for liver transplantation.[2] Risk factors that are associated with an increased morbidity and mortality from HCV include HCV genotype 1b, high viral load, intense immunosuppression, graft rejection, co-incident CMV viremia, iron overload, major histocompatibility complex (MHC) class II matching, and donor TNF-a promoter genotype.[2] [31] [32] [33] [34] Fibrosing cholestatic hepatitis, glomerulonephritis (both membranoproliferative glomerulonephritis, with and without cryoglobulinemia, and membranous glomerulonephritis), acute and chronic transplant glomerulopathy and thrombotic microangiopathy with anticardiolipin antibody are associated with HCV infection in the transplant patient.[2] [35] [36] [37]

The first step in therapy is to decrease immunosuppression. At present, IFN-a with ribavirin induces response in about 50% of cases (see Chapter 207).

Papovaviruses

The polyomaviruses BK virus and JC virus are normally acquired in childhood and reactivated post-transplant.

JC virus

JC virus is the cause of a subacute, progressive, demyelinating disease of the central nervous system (CNS) that is known as progressive multifocal leukoencephalopathy. Progressive multifocal leukoencephalopathy is characterized by the development of progressive motor and sensory deficits and dementia, with

death within 3–6 months. At present, there is no therapy except to decrease immunosuppression. [2] [38]

BK virus

BK virus infection causes a tubulointerstitial nephritis that mimics rejection in renal allografts. The increase in this condition in recent years has been linked to increased immunosuppression, particularly the use of tacrolimus with mycophenolate. BK virus nephritis adversely affects allograft function. Major reduction in immunosuppression is the only known way to ameliorate this process. [39] [40] [41]

Papillomaviruses

In post-transplant patients, papillomaviruses most commonly cause warts, which can be so extensive as to be disfiguring. The intensity of immunosuppression appears to be critical, with azathioprine and mycophenolate being particularly associated. These warts can undergo malignant transformation, particularly in sun exposed areas. Skin cancers in transplant patients are thought to occur through the interaction of papillomavirus infection, immunosuppression and ultraviolet irradiation.[42] A particular group of papillomaviruses, the epidermodysplasia verruciformis-associated types, appear to play a significant role in the pathogenesis of cutaneous and anogenital squamous cell carcinoma, which are more common in transplant

TABLE 102-5 -- Pulmonary radiographic presentations.

PULMONARY RADIOGRAPHIC PRESENTATIONS	
Acute infiltrates	Community-acquired pneumonias:
	Streptococcus pneumoniae
	Chlamydia pneumoniae
	Legionella spp.
	Gram-negative rods
	Respiratory viruses (e.g. respiratory syncytial virus, influenza virus)
Subacute or chronic infiltrates	*Nocardia* sp.
	Fungi (*Aspergillus* spp., *Mucorales* spp.)
	Pneumocystis carinii
	Mycobacterium tuberculosis
	Nontuberculous mycobacteria
	Mycoplasma pneumoniae
Diffuse infiltrates	*Pneumocystis carinii*
	Viruses (cytomegalovirus, influenza)
Miscellaneous	Sirolimus pulmonary toxicity
	Pulmonary embolism
	Pulmonary edema
	Atelectasis

patients. Similarly, cervical papillomavirus infection has been linked to cervical cancer.[2] [42] [43]

Fungal infections

Fungal infections have grown in importance in organ transplant recipients, as a result of endemic, geographically restricted systemic mycoses (blastomycosis, coccidiomycosis, histoplasmosis), opportunistic organisms (*Candida* spp., *Aspergillus* spp. and *C. neoformans*) and 'new and emerging' fungi (*Fusarium* spp., *Scedosporium* spp. and *Trichosporon* spp.) that cause opportunistic infection (see Table 102.5).

The endemic mycoses can present in a variety of ways in these patients:

- progressive primary infection with likely hematogenous dissemination,
- reactivation infection with secondary dissemination, or
- infection due to re-exposure after immunosuppression has attenuated previously acquired immunity.

Therapy of the endemic mycoses in the past has relied on amphotericin to gain control, followed by an azole to complete therapy. At present, itraconazole is the azole of choice, but this will probably change with the licensing of the newer azoles. [2] [44] [45] (see Chapter 208)

Candida has become one of the more important pathogens for transplant recipients. Although a great deal of candidal infection is clearly derived from endogenous sources (the gut and the female genital tract being the most common), person-to-person spread does occur, particularly on the hands of medical personnel. Candidal syndromes common in transplant patients include pharyngitis, esophagitis, wound infection and candidemia. In addition, candidiuria is of special concern because fungal balls can form at the ureterovesical junction, particularly in patients with poor bladder emptying, causing a form of obstructive uropathy that is difficult to eradicate. We advocate treatment of asymptomatic candidiuria, usually with fluconazole. A single blood culture positive for *Candida* requires systemic chemotherapy, to reduce the risk of presenting later with endophthalmitis or osteomyelitis. The isolation of *Candida* in the sputum does not indicate pneumonia, as primary candida pneumonia rarely, if ever, occurs.

Aspergillus infection is a therapeutic emergency. As with *Fusarium* and *Scedosporium*, *Aspergillus* is vasculotropic, and early blood vessel involvement leads to hemorrhage, infarct and spread. Fully 50% of patients have metastatic disease at the time of initial diagnosis, and mortality rates, particularly in lung transplant patients, have exceeded 50% despite treatment with amphotericin. Survival with voriconazole is better. Our approach has been to use voriconazole. In about 25% of transplant patients with cryptococcosis, skin lesions are the first manifestation of systemic infection. The most common presentation of the patient with cryptococcal involvement of the CNS is headache, fever or mild-moderate cognitive dysfunction. Signs of meningeal irritation are absent in many patients. Cerebrospinal fluid typically shows increased intracranial pressure, lymphocytic pleocytosis, elevated protein and depressed sugar, in addition to a positive cryptococcal antigen test. Immunosuppression (especially corticosteroids) should be decreased and induction therapy begun, typically a lipid amphotericin preparation with flucytosine. After control is achieved, consolidation with high-dose fluconazole (800–1200mg/day) is initiated. Therapy is continued until all evidence of disease is gone, with an additional 2–4 weeks of therapy as a 'buffer' added to the regimen.[2] [46]

Bacterial infections

Acute gastroenteritis syndromes in the transplant patient include the gut toxicity of mycophenolate, a variety of viruses and certain bacteria. *Clostridium difficile* appears to be more common, more symptomatic and more difficult to treat in the transplant patient, with a much greater propensity for relapse. Our policy is to treat it with metronidazole for 1 week after symptoms have resolved. Non-typhoidal *Salmonella* infections pose a particular hazard to the transplant patient. Whereas in the normal host *Salmonella* gastroenteritis is associated with bacteremia in <5% of cases, it occurs in more than 50% of transplant patients with gastroenteritis. Seeding of the urinary tract, with positive urine cultures, is common. Preexisting atherosclerotic lesions, aneurysms or fistulae can become infected. Therefore, *Salmonella* bacteremia is treated for an extended course, preferably with a fluoroquinolone. [2] [3] [4]

The portal of entry for *Listeria* is the gastrointestinal tract, with bacteremia sometimes preceded by a non-specific gastroenteritis. On occasion, the organism can be

isolated from the stool. Once bloodstream invasion occurs, bacteremia may cause endocarditis or CNS syndromes. Acute pyogenic meningitis typically presents with headache and fever, but overt meningeal signs are often absent. Focal or diffuse cerebritis may be manifestations of meningoencephalitis. Rhomboencephalitis involves the brainstem and produces a syndrome that resembles bulbar polio. Therapy is usually with high-dose intravenous ampicillin with or without gentamicin for 7–10 days, followed by ampicillin alone for a further 7–10 days. In patients who are allergic to ampicillin, high-dose TMP-SMX is used with success. In patients who allergic to both, we desensitize to the ampicillin and use the first regimen.[2] [3] [4] Prophylaxis with TMP-SMX protects against *L. monocytogenes*.

The manifestations of tuberculosis in transplant patients ranges from cavitary to miliary disease in the lung, from bowel to skeletal disease, and from skin to CNS disease. There appears to be a particularly high rate of skeletal involvement in transplant patients. Transmission of tuberculosis within an allograft has been documented. The usual approach to a positive tuberculin skin test is isoniazid prophylaxis. However, underlying liver function test abnormalities are common in these patients, and since the major toxicities of isoniazid, rifampin and pyrazinamide are hepatic, alternative

strategies have evolved. We have followed more than 125 patients with positive tuberculin tests but no other risk factors for more than 10 years without prophylaxis, without a single case of active disease. However, any of the following risk factors in addition to a positive tuberculin test should trigger therapy, often with nonclassical regimens. Risk factors requiring prophylaxis include:[2] [47]

- recent tuberculin skin test conversion;
- non-Caucasian ethnicity;
- other immunosuppressive conditions such as protein calorie malnutrition;
- a history of active tuberculosis, particularly if inadequately treated; and
- significant abnormalities on chest radiograph.

Transplant patients with active disease are initially tried on isoniazid, rifampin and pyrazinamide, with or without ethambutol. If this is not tolerated, we have used regimens such as ofloxacin and ethambutol for 2–3 years.

Nocardiosis is quite similar to the invasive molds in terms of pathogenesis and clinical effects. Nocardia typically has a subacute presentation caused by inhalation of organisms, which establish a pulmonary portal of entry followed by hematogenous dissemination to the skin, CNS and the skeletal system. Prophylaxis with low-dose TMP-SMX prevents this infection; high-dose TMP-SMX is used to treat it (see Table 102.5).[2] [47]

SUMMARY AND CONCLUSION

In organ transplantation the therapeutic prescription has two components:

- immunosuppression to prevent and treat rejection, and
- antimicrobial agents to make it safe.

Rejection and infection are closely linked. Infection is better prevented than treated, but if treatment is needed, the earlier such treatment is initiated, the better the outcome. Finally, the risk of infection is determined by the interaction of technical and anatomic factors, the net state of immunosuppression, and environmental exposures. These factors logically lead to a timetable of infections that helps to predict problems and solutions.

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Chapter 103 - Lung and Heart-Lung Transplant Patients

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INTRODUCTION

Lung and heart-lung transplantation has been clinically available for more than 20 years. Progress in candidate and donor selection, allograft preservation technique, recipient surgery and postoperative management have greatly improved the outcome of thoracic transplantation.[1] Despite these improvements, infectious complications remain an important cause of morbidity and mortality in patients undergoing these procedures.[2]

EPIDEMIOLOGY

Lung and heart-lung transplantation is offered to patients with chronic progressive pulmonary failure. Because of the high risk of rejection and infection, demanding postoperative care and severe shortage of grafts, these transplants are only considered when other treatment options are exhausted. Lung transplantation can be performed as an isolated procedure (double- or single-lung) or as a heart-lung transplant. Candidates for isolated lung transplantation are typically referred because of the presence of cystic fibrosis, interstitial lung disease, emphysema or pulmonary hypertension without important cardiac damage. The most common current underlying diagnoses of people undergoing heart-lung transplantation include complex congenital heart disease with secondary pulmonary hypertension, or pulmonary hypertension with significant cor pulmonale. Current 1-year survival for isolated lung and heart-lung transplantation are 74% and 62% respectively.[3]

PATHOGENESIS AND PATHOLOGY

Patients undergoing heart-lung or lung transplantation are at high risk for developing infectious complications. These account for approximately 40–50% of all deaths in these patients.[4] Unique among heart-lung and lung transplant recipients is the relationship between the presence of chronic rejection and bronchiolitis obliterans with chronic infection of the airway. This relationship may be explained in part by the fact that lung transplant recipients have altered lung immunity due to impaired ciliary clearance, poor cough reflex and abnormal lymphatic drainage, predisposing these patients to lower respiratory tract infections. Clinical manifestations of infection with these pathogens may be indistinguishable from rejection of the lung and may strongly resemble a classic 'pulmonary exacerbation' (increased cough and sputum production with a measurable decline on formal pulmonary function testing) seen with cystic fibrosis patients. The patient's underlying lung disease impacts on the presentation and the type of infection that occur after these procedures. This is illustrated by the increased risk of early and severe pneumonia with multiple antibiotic-resistant bacteria seen in cystic fibrosis patients undergoing heart-lung or lung transplantation. The high degree of immunosuppression required by heart-lung/lung transplant recipients also puts these patients at high risk of opportunistic pathogens, including cytomegalovirus (CMV), Epstein-Barr virus (EBV) and *Pneumocystis carinii* pneumonitis (PCP). The frequency and severity of disease due to these opportunistic pathogens exceeds that reported after most other types of organ transplantation.

Timing and patterns of infectious complications

The timing of infections follows the typical pattern observed after other solid organ transplant procedures and can be divided into early (<1 month), intermediate (1–6 months) and late periods (>6 months). Risk factors for infection after thoracic transplantation are described in Table 103.1 . During the early period, infections are related to the surgical site (including mediastinitis[4] [5]), presence of foreign bodies (e.g. central venous catheters) or nosocomial acquisition of infection.

A unique feature of cystic fibrosis patients who are undergoing lung/heart-lung transplantation is that they may develop bacteremia with organisms that normally inhabit their airway (*Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia*). Viral infections are infrequent in the early period, although herpes simplex virus (HSV) can reactivate during this period as a result of the stress of surgery and induction of immunosuppression. While uncommon, development of acute infection with adenovirus, influenza or respiratory syncytial virus (RSV) during this period can be severe.

The intermediate time period is characterized by reactivation of donor-transmitted disease from latent organisms such as CMV or

TABLE 103-1 -- Risk factors for infection after thoracic transplantation.

RISK FACTORS FOR INFECTION AFTER THORACIC TRANSPLANTATION
Recipient pretransplant factors
• Organ transplanted (heart-lung vs double-lung vs single lung)
• Underlying disease (cystic fibrosis)
• Severity of illness prior to transplant:
Malnutrition
Requirement for intensive care
Requirement for assist devices
• Age:
Immunologic immaturity
Primary exposure
Immunization history
Donor factors
• Latent infections
• Colonization of respiratory tract
Intraoperative factors
Post-transplant factors
• Technical problems
• Immunosuppression
• Indwelling cannulas
• Nosocomial exposure

EBV. Reactivation of latent infections from donor organs can also occur with histoplasmosis, tuberculosis and toxoplasmosis. The latter is more common in recipients of heart-lung transplants. PCP, which is more frequent after lung/heart-lung transplantation compared with other organ transplants, also most frequently presents in the intermediate period in the absence of chemoprophylaxis.

The pattern of infections seen during the late post-transplant time period is unique in lung/heart-lung transplantation compared to other organ recipients. The lung is the most important site of late infection in these patients and the presence of chronic rejection or bronchiolitis obliterans is the major risk factor for these infectious complications. Pathogens found in these late pulmonary infections can be the same as those recovered from cystic fibrosis patients, regardless of their underlying disease. They include *P. aeruginosa*, *A. xylosoxidans*, *S. maltophilia* and *Aspergillus fumigatus*. The presence of one or more of these potential pathogens may be associated with asymptomatic colonization or fatal disease. Opportunistic infection with *Nocardia* or *Cryptococcus* spp. can also occur late after transplantation.

TABLE 103-2 -- Prophylactic strategies in lung and heart-lung transplantation.

PROPHYLACTIC STRATEGIES IN LUNG AND HEART-LUNG TRANSPLANTATION				
Indication	**Prophylaxis**	**Dose and duration**		**Comments**
		Adults	**Children**	
Perioperative prophylaxis				
Not colonized prior to transplant and perioperative cultures from donor and recipient negative	Cefazolin	0.5–2g q8h iv	20–30mg/kg q8h iv	If all cultures negative, treat 48h; if any culture positive, treat 10–14d
Recipient with underlying colonization (e.g. cystic fibrosis)	Drugs tailored to previous sputum culture			
Pneumocystis carinii	TMP-SMX	80 or 160mg of TMP component daily	5mg/kg of TMP component 3 times a week or daily	May also give protection against toxoplasmosis
Aspergillus spp.	Liposomal amphotericin B	3mg/kg/day until perioperative cultures available from donor and recipient		If cultures negative: stop, if positive: continue 1–2 weeks, consider oral itraconazole
Candida spp.	Nystatin	5ml q6h po for 1 month	If <2 years old: 2ml q6h po for 3 months	
			If >2 years old: 5ml q6h po for 1 month	
Herpes simplex virus	Acyclovir	200mg po q8h for 3 months	5mg/kg q8h (max. 200mg) for 3 months	Used only for HSV-positive patients Begin once patient off ganciclovir
Cytomegalovirus				
Donor +, recipient -	Ganciclovir	5mg/kg iv q12h for 14d		Then 5mg/kg iv q24h for 7–14d if patient remains in hospital; then start valganciclovir to complete 90d of therapy (adults: 900mg po q24h; children: contact pediatric infectious disease specialist)
Donor ±, recipient +	Ganciclovir	5mg/kg/d iv q12h for 14d, then 5mg/kg iv q24h for 7–14d if patient remains in hospital		
Donor -, recipient -	No treatment			
Toxoplasma gondii				
High-risk: donor +, recipient -	Pyrimethamine	1mg/kg (max. 25mg) q24h for 2 weeks, then 3 times a week for 6 months	1 mg/kg (max. 25mg) q24h for 2 weeks, then 3 times a week for 6 months	*plus* folinic acid 5mg 3 times a week for 2 weeks then once a week
Low-risk: donor ±, recipient +	TMP-SMX	80 or 160mg of TMP component daily	5mg/kg of TMP component 3 times a week	Will also give protection against *P. carinii* pneumonia
SMX, sulfamethoxazole; TMP, trimethoprim				
These are the strategies used at the Children's Hospital of Pittsburgh.				

Regardless of the time period, differentiation between rejection and infection can often be difficult in thoracic transplant recipients. Accordingly, biopsy and cultures are often important to obtain to assist in appropriate management. Prophylactic strategies to prevent infection in thoracic transplantation patients used at the Children's Hospital of Pittsburgh are described in Table 103.2 .

INFECTIOUS SYNDROMES

Cytomegalovirus infection

Clinical presentation

Cytomegalovirus is the most common and important viral agent in thoracic transplantation patients. It can cause primary infection in a previously seronegative patient or secondary infection (with a different strain, or reactivation of their own CMV) in a previously seropositive person. Primary infection is usually transmitted via the donor graft or blood products. Infection with CMV typically presents between 1 and 6 months after transplantation as CMV syndrome

(with malaise, fever and bone marrow suppression), pneumonitis or invasive disease. [4] [6] Cytomegalovirus pneumonitis is particularly severe in high-risk patients — CMV-positive donor (D+)/CMV-negative recipient (R-) — but fatal pneumonitis has been less frequent since the availability of ganciclovir.[7] Invasive CMV disease involves the gastrointestinal tract, liver or lungs. Chorioretinitis due to CMV is infrequent after thoracic transplantation. Symptomatic CMV infection is associated with superinfection with bacteria and fungi as a consequence of viral alteration of cellular immunity. Interestingly, CMV infection has also been associated with an increased frequency of acute and chronic allograft rejection in lung/heart-lung transplant recipients.[8]

Diagnosis

The diagnosis of CMV infection is defined by the presence of symptoms attributable to CMV in a patient with a positive culture or histology in the absence of another pathogen explaining these symptoms. While the diagnosis of CMV should be made using strategies similar to those used for other organ transplant recipients, histologic stains and CMV cultures from bronchoalveolar lavage cells or lung biopsy material are useful in confirming the diagnosis in this patient population.[9]

Prevention

To combat the numerous deleterious effects of CMV, strategies of prevention, pre-emptive treatment and monitoring have been developed. Efforts at prophylaxis of CMV infection and disease have focused on the use of intravenous or oral ganciclovir for 1–3 months following transplant. While moderately effective in low-risk patients (D-/R+ or D+/R+), the use of long-term prophylactic ganciclovir delays the symptoms of CMV disease but has not prevented primary disease in the high-risk population (D+/R-). Accordingly, the use of viral load monitoring as an indicator for initiating pre-emptive ganciclovir therapy has also been used after lung and heart-lung transplantation. Unfortunately, specific cut-offs for different assays (e.g. pp65 antigenemia assay or CMV quantitative polymerase chain reaction (PCR)) as well as the route (oral ganciclovir or valganciclovir versus intravenous ganciclovir) and duration of pre-emptive therapy have not been well defined. In our hospital, we follow the prophylactic regimen described in Table 103.2 .

Treatment

Cytomegalovirus disease following heart-lung or lung transplantation should be managed similarly to strategies for other organ transplant recipients. Ganciclovir is the principal anti-CMV agent used. However, resistant strains have appeared and represented up to 10% of lung transplant recipients in one series.[10] Ganciclovir-resistant CMV infection has been associated with more frequent disease, earlier onset of bronchiolitis obliterans and shorter survival.[11] Foscarnet and cidofovir have been used as alternatives but resistance to foscarnet has also been reported and both drugs are associated with greater toxicities than ganciclovir (for dosage, see Table 103.3). These toxicities include nephrotoxicity, neutropenia, metabolic acidosis and electrolyte disturbances. Both drugs should be adjusted to renal function. New drugs may offer benefits for the near future. Duration of therapy has traditionally been 2–3 weeks. More recently, in many centers duration of therapy is titrated to clearance of CMV viral load.

Epstein-Barr virus infection

Clinical presentation

Disease due to EBV, including EBV-related post-transplantation lymphoproliferative disease (PTLD), is an important problem in lung/heart-lung transplant recipients. Epstein-Barr virus related PTLD is more common after primary EBV infection. The incidence of PTLD after pulmonary transplantation has been reported to be as high as 20% in children and 8% in adults.[12] [13] More than half of patients are symptomatic, with clinical signs ranging from a mononucleosis-like picture with lymph node swelling through isolated PTLD lesions in the lung or gastrointestinal tract to disseminated disease or lymphoma.

Diagnosis

When PTLD is suspected, computerized tomography of the neck, chest, abdomen and pelvis should be considered to identify occult lesions. Biopsies should be obtained from suspicious lesions and sent for histologic evaluation. The use of immunohistopathologic stains for the presence of EBV (e.g. Epstein-Bar-encoded RNA — EBER — in-situ hybridization) is necessary to distinguish EBV-infected cells from non-specific lymphocytic infiltrates. Patients with active EBV disease typically have an elevated EBV viral load in the peripheral blood. Elevated loads may be present prior to the onset of EBV disease and may remain persistently high even after resolution of PTLD.

Prevention

The high rates of morbidity and mortality attributed to PTLD have prompted efforts aimed at the prevention or pre-emptive treatment of EBV. Serial monitoring of the EBV viral load using quantitative PCR assays has been shown to predict occurrence of PTLD in other transplant populations[14] and is increasingly being used to guide initiation of pre-emptive therapy. However, specific target levels of load and specific site to sample (blood versus bronchoalveolar lavage), as well as therapeutic pre-emptive treatment regimens, vary from center to center and remain to be evaluated in prospective, comparative studies.

Treatment

The management of patients with PTLD is controversial. Reduction of immune suppression is the major therapeutic manipulation for transplant recipients with EBV-PTLD. This is felt to allow the body to develop a cytotoxic T lymphocyte response to control the infectious process. Concerns over the development of rejection can limit this approach. While frequently used, antiviral therapies (primarily nucleoside analogues — e.g. ganciclovir — and immunoglobulin) are probably of only limited benefit for the treatment of EBV-PTLD. The use of the new anti-CD20 monoclonal antibody (rituximab) appears very promising for these patients. The use of chemotherapy may be necessary for patients who fail to respond to reduction of immunosuppression or in whom the PTLD lesions are judged to be malignant.

Toxoplasma gondii infection

Clinical presentation

Toxoplasma gondii is most commonly found as a pathogen after cardiac or heart-lung transplantation.[15] Primary infection is associated with the transmission of latent *Toxoplasma* cysts via the donor organ.[16] The highest risk and most severe symptomatic disease occur in seronegative recipients of heart grafts from seropositive donors (D+/R-). Transmission in this scenario can be as high as 60%. Reactivation of the patient's own toxoplasmosis on immunosuppression is around 7% but does not generally result in significant disease. Severe toxoplasmosis due to community acquisition post-transplantation has not been reported but is theoretically possible. Donor-associated toxoplasmosis most commonly occurs within 1–6 months after transplantation. Clinical signs and symptoms occur in more than 10% of patients and range from asymptomatic seroconversion to severe disease with fever, chorioretinitis, myocarditis, neurologic abnormalities and interstitial pneumonia.

TABLE 103-3 -- Treatment strategies in lung and heart-lung transplantation.

TREATMENT STRATEGIES IN LUNG AND HEART-LUNG TRANSPLANTATION			
	Treatment	**Dose and duration**	**Comments**
Cytomegalovirus	Ganciclovir (first line therapy)	5mg/kg iv q12h for 14–21d	Maintenance therapy Smg/kg/d iv q24h or consider valganciclovir
	Foscarnet	180mg/kg/d divided q8h iv for 14–21d	Maintenance therapy: 90–120mg/kg/day iv
	Cidofovir	5mg/kg once weekly for 2 weeks iv	Continuing therapy: 5mg/kg iv once every 2 weeks as clinically needed
Toxoplasmosis: adults	Pyrimethamine	200mg q24h po	Then 75–100mg q24h po
			Total treatment 3–6 weeks
	Sulfadiazine	1–1.5g q6h po	
	Folinic acid	10–15mg q24h	
Toxoplasmosis: children	Pyrimethamine	0.5–1mg/kg/day po	(max 25mg)
			Total treatment 3–6 weeks
	Sulfadiazine	60–75mg/kg q12h po	
	Folinic acid	10–15mg q24h	

Aspergillus	Liposomal amphotericin B	5mg/kg/day iv q24h for 10–14d	Followed by itraconazole indefinitely:
			Adults: 100–200mg q12h po
			Children: 3–5mg/kg/d q24h po
			Alternative treatment: variconazole
Community-acquired viruses: RSV, adenovirus, parainfluenza virus (consider treatment in severe cases or early after transplantation)	Ribavirin	6 g/day in continuous aerosolization 12–18h daily if intubated	**Specific antiviral therapies are not proven to work but can be considered**
	Cidofovir	5mg/kg once weekly for 2 weeks iv	Continuing therapy: 5mg/kg once every 2 weeks as clinically needed
			Probenecid must be administered orally with each dose, as well as hyperhydration
			Alternative dosing strategies have been used
Influenza virus	Amantadine	Adults: 200mg/d	
		Children: 2.5mg/kg q12h po	Max. 200mg/d
	Rimantadine	Adults: 100mg q12h po	
		Children: 5mg/kg/d po	Max. 150mg/d
	Oseltamivir	Adults: 75mg q12h po	
		Children>1 year old: 2mg/kg q12h po	
	Zanamivir	Adults: 10mg q12h inhaled	Duration of therapy for influenza may depend on clearance
These are the strategies used at the Children's Hospital of Pittsburgh.			

Diagnosis

When suspected, toxoplasmosis should be looked for in bronchoalveolar lavage fluid, by histologic examination of biopsies from suspected tissues, or by PCR.

Prevention

Symptomatic toxoplasmosis is rarely seen outside of the high-risk (D+/R-) setting. Accordingly, preventive strategies have focused on these high-risk patients, who are treated with pyrimethamine for 6 months following heart-lung transplantation. *Toxoplasma gondii* serologies are monitored in order to identify those patients developing primary infection despite prophylaxis. Use of this preventative strategy in mismatched patients reduces their risk of acquiring *Toxoplasma* from over 50% to less than 15%. Daily prophylaxis with trimethoprim-sulfamethoxazole is an alternative and protects against PCP as well as toxoplasmosis.[17] [18] Corticosteroids given for rejection treatment increase the risk of toxoplasmosis. High-risk patients treated for acute rejection should therefore be monitored carefully.

Treatment

Treatment of toxoplasmosis following heart-lung transplantation is similar to that for other immunosuppressed patients. The combination of pyrimethamine and sulfadiazine is the mainstay of therapy (see Table 103.3).

Other organisms

Pneumocystis carinii pneumonia is a frequent pulmonary infection in immunocompromised hosts. It has a higher attack rate among lung and heart-lung transplant recipients than in other organ transplant patients.[19] Infections tend to occur within the first year after transplant[4] but have on occasion been seen later. Symptoms range from isolated fever to serious respiratory distress leading to death. Prophylaxis with trimethoprim-sulfamethoxazole given as a single daily dose or three times a week is usually successful in preventing *P. carinii* pneumonia.[17] Recurrent *P. carinii* colonization regardless of prophylaxis has been reported in heart-lung transplant recipients.[20]

Multiply-resistant organisms

Cystic fibrosis patients represent an important cohort of heart-lung and lung transplant recipients. These patients, as well as those with a prolonged intensive care stay just prior to transplantation, are at high risk of being colonized with unusual organisms and with multiply-resistant pathogens. These organisms can take advantage of the reduced immunity and diaphragmatic dysfunction after lung/heart-lung transplantation to cause infection. It is therefore important to have baseline cultures prior to transplant to identify potential pathogens. A 2-week course of appropriate antimicrobial

therapy is recommended for all patients with cystic fibrosis based upon these culture and susceptibility results. The use of reference laboratories for the performance of synergy studies for multiply-resistant organisms should be considered.

When lung-heart-lung transplant recipients are ill with pneumonic processes it is imperative to obtain cultures from the lower respiratory tract; empiric antibiotics should be initiated after obtaining these cultures. Bacteria to consider include methicillin-resistant *Staphylococcus aureus* and resistant *P. aeruginosa* as well as more common respiratory pathogens such as *Streptococcus pneumoniae*. [21] Patients with bronchiolitis obliterans are often colonized with *Pseudomonas*, *Stenotrophomonas* or *Alcaligenes* spp. Accordingly, initial antimicrobial regimens in these patients should consider these pathogens.

Fungal infections occur frequently in lung/heart-lung transplant recipients. Fungal pathogens may be newly acquired or reactivate from sites of latent infection within lung transplant recipients.[22] *Aspergillus* spp. can present as airway colonization (25% of the transplant recipients) or invasive disease (around 5%). Colonization at the time of transplantation can result in serious disease and may be associated with dehiscence of thoracic anastomoses. However, the presence of fungi preoperatively is not a contraindication to transplantation; we recommend a prophylactic course of liposomal amphotericin B (to avoid nephrotoxicity) intravenously or via the aerosol route post-transplantation for these colonized patients, followed by oral itraconazole (see Table 103.2).

More than half of all diagnoses are made in the first 6 months after transplantation. *Aspergillus* and other filamentous fungi have also been identified as causing colonization and disease in patients who develop bronchiolitis obliterans. Currently, we use itraconazole prophylaxis in patients with bronchiolitis obliterans to prevent this complication. Incidence of progression from airway colonization to invasive disease is less than 5%. Most of the patients with isolated tracheobronchitis respond to antifungal therapy and/or surgical debridement. However, the survival rate of invasive aspergillosis is less than 50%.[23]

Nocardia species

Nocardia infection is found in 2% of lung transplant recipients and has an attributable mortality rate of 30–40%. [24] Infection with *Nocardia* typically presents in lung/heart-lung transplant recipients more than 2 years after transplantation[4] and characteristically begins in the lungs as rounded nodular infiltrates, which can cavitate. Hematogenous spread can occur to the central nervous system, skin or, occasionally, other organs. Drainage of abscesses may be beneficial and long-term antibiotics are necessary.

Viruses other than CMV and EBV cause disease in up to one-third of all lung/heart-lung transplant recipients. An important feature of these infections is that they can

mimic rejection.[4] Herpes simplex virus can reactivate or cause primary pneumonitis, which can be fatal. Acyclovir prophylaxis (see Table 103.2) is effective in preventing this problem. The community respiratory viruses such as RSV, parainfluenza virus, influenza virus and adenovirus are also important causes of disease in this population. They often involve the lower respiratory tract and may be associated with significant morbidity and mortality.[25] In addition, the insult from these viruses (particularly adenovirus) can lead to chronic sequelae such as bronchiolitis obliterans.[26] The mainstay of treatment for severe disease from community-acquired viruses is decreasing immunosuppression and supportive care. Antiviral treatments are controversial and, while licensed therapies are available for RSV (ribavirin) and influenza (e.g. amantadine, rimantadine, oseltamivir, zanamivir), no proven therapies are available for parainfluenza or adenovirus (see Table 103.3). Controlled studies are needed to help guide in their use in the lung/heart-lung transplantation population.

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Chapter 104 - Heart Transplant Patients

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INTRODUCTION

According to the 2001 Registry of the International Society for Heart and Lung Transplantation, 57,818 heart transplants (HT) were performed in the year 2000.[1] The main indications for transplantation are coronary artery disease (46%) and cardiomyopathy (45%) in adults, and congenital heart disease (75%) in children.

Overall 1-year survival for HT is 80% and from the second year on, 4% of the patients die every year. Early mortality (first year) is mainly due to postsurgical problems (10%), primary graft failure and infection (30% of first-month deaths, 45% of deaths between 1 and 3 months and 9.7% thereafter). Late mortality (after the first year) is mainly caused by cardiac allograft vasculopathy (CAV), nonspecific graft failure and malignancy (8.8% at 5-year follow-up).[2] Patient median half-life is 9 years (11.6 after the first year) and approximately 40% of the patients are working at 5 years.

Infection is a very important cause of morbidity and mortality in HT recipients and it is a variable of special interest, since it is amenable to prevention and intervention. We will now briefly review some specific characteristics of infectious complications in this population.

EPIDEMIOLOGY AND SPECIFIC RISK FACTORS FOR INFECTION

The incidence of infection after a HT ranges from 30% to 60% (with a related mortality of 4–15%) and the rate of infectious episodes per patient was 1.73 in a recent series.[3] Infections are more frequent and severe than those occurring in renal transplant recipients, but less frequent than those occurring after liver or lung transplantation. Infection was found to be the leading cause of death occurring more than 30 days after transplantation (33%).[3] The most common agents responsible for infection, the expected chronology and the main forms of clinical presentation are summarized in Table 104.1 . Bacteria or viruses cause most infections. However, fungal pathogens, *P. carinii* and parasitic infections are also important.

The time of appearance of infection after transplantation is an essential component of the evaluation of the etiology of infection. Early infections occurring within the first month after transplantation are generally similar to nontransplant patients who have undergone major heart surgery. Intermediate infections (2–6 months) are usually caused by opportunistic micro-organisms, such as CMV, fungi and multiresistant bacteria. Finally, late infections (after 6 months) may be caused either by common community pathogens in healthy patients or by opportunistic micro-organisms in patients with chronic rejection.

Many different factors influence the incidence and type of infections in HT patients (Table 104.2). They may be classified into factors related to the patient (before and after transplantation) and those due to technical complications during the operative and perioperative periods. The need for ventricular assist devices is a specific risk factor for infection in HT recipients. In some series, up to one-third of the patients on biventricular assist devices will eventually die of sepsis; bacteremia (59%), driveline infection (28%) and pump infection (11%) are also common.

As in other solid organ transplant (SOT) patients, the degree of immunosuppression and epidemiological risk factors, such as the exposure to specific microbial pathogens, are key factors that will influence the etiology of infections occurring in the postoperative period.

SPECIFIC CLINICAL SYNDROMES

We will address only a few infectious problems of special interest in the heart transplant recipient. The remaining complications do not differ from what has been mentioned in the general chapter dealing with infection in all types of solid organ transplant patients (see Chapter 102).

Pneumonia

In HT recipients, both fatal and nonfatal infections predominantly involve the lung (28% of all infections), probably as a consequence of multiple prior episodes of congestive heart failure. The etiological agents of pneumonia are similar to those of other SOT patients: 60% are caused by opportunistic micro-organisms (mainly CMV), 25% by nosocomial pathogens and 15% by community-acquired bacteria and mycobacteria.[4] Pneumonia is one of the leading causes of death after HT. Mechanical ventilation is required in 37% of the cases of pulmonary infections and death occurs in 23–31% of the patients. This rate varies widely depending on the etiology of infection. *Aspergillus* pneumonia has the worst prognosis (mortality 50–62%), followed by nosocomial pneumonia (overall mortality 26%, and up to 50% for those patients on mechanical ventilation) and CMV pneumonia (mortality 13%).[4]

Nodular lesions may be detected in 10% of HT patients. They are mainly caused by *Aspergillus, Nocardia* and CMV. *Nocardia* nodules usually appear later (median 100 days, range 89–100) after transplantation and some clinical manifestations may suggest the etiology and may help determine empiric treatment in selected cases.[5]

Other pathogens such as *Rhodococcus equi* [6] and tuberculosis should also be considered. [8] *Pneumocystis carinii* was the third cause of pneumonia in our study (13% of the isolates). The incidence was 3.6% cases per 100 HTs (2–8% in other studies). Prophylactic trimethoprim-sulfamethoxazole reduces the incidence of *P. carinii* pneumonia to nearly zero. *Mycobacterium tuberculosis* deserves special attention. The incidence of tuberculosis in HT patients in Spain is 1.35 cases/100 heart transplant-years, more than 20-fold the national average.[8] On average, tuberculosis develops 76 days post transplantation and extrapulmonary disease is common. Reactivation may be triggered by antirejection therapy, although it may be acquired from the hospital environment or even from the allograft in heart-lung transplantation. Besides the potential difficulty in establishing a diagnosis, due to its atypical, paucisymptomatic presentation, the major problem of tuberculosis in this population concerns antituberculous therapy. It has been suggested that tuberculosis should be considered in HT patients with prolonged and culture-negative febrile episodes. If the patient's condition deteriorates,

TABLE 104-1 -- Etiology, incidence and timing of infections in heart transplant patients.

ETIOLOGY, INCIDENCE AND TIMING OF INFECTIONS IN HEART TRANSPLANT PATIENTS	
Etiology	**Incidence**
Bacteria	44–60%
Viruses	40–45%
Fungi	7–15%
P. carinii	2–8%

Parasites	0.5–2%

Chronology of infection	**Most common syndromes**
Early infection (1st month)	Pneumonia
	Surgical wound infection
	Mediastinitis
	Urinary tract infection
	Catheter-related infection
	Bloodstream infection
	Antibiotic-associated diarrhea
	Herpes simplex stomatitis
	Infections transmitted with the allograft
Intermediate infections (2–6 months)	Opportunistic infections (similar to other solid organ transplant; see Chapter 102)
Late infections (after 6th month)	Common community-acquired infections
	Respiratory tract infections
	Urinary tract infections
	Varicella-zoster infections
	Opportunistic micro-organisms

prompt specific therapy should be initiated after obtaining samples for culture. There is much controversy regarding what constitutes optimal antituberculous therapy. The benefit of rifampin must be balanced against the problem it causes by interfering with the metabolism of immunosuppressive drugs, especially cyclosporin. Rifampin results in a substantial decrease in the blood levels of cyclosporin, which has been associated with fatal allograft rejection. Although some authors have suggested that rifampin may be safely used as long as therapeutic cyclosporin levels are maintained with increased

TABLE 104-2 -- Risk factors for infections in heart transplant patients.

RISK FACTORS FOR INFECTIONS IN HEART TRANSPLANT PATIENTS		
Preoperative period	**Intraoperative period**	**Postoperative period**
Pulmonary hypertension not responsive to vasodilators	Prolonged operative time	Prolonged stay in ICU
Critically ill status and mechanically ventilated patients at time of transplantation	Complicated surgical procedure	Mediastinal complications and need for reintervention
Renal insufficiency	Need for large number of blood transfusions	Prolonged hospitalization
Cardiac cachexia Prior sternotomy	Need for ventricular assist devices	Prolonged antibiotic use
Donor's CMV positive serology	Presence of pathogens in the transplant allograft	Renal insufficiency
Older age		Induction therapy with OKT3
Repeated hospital admissions		Immunosuppressive drugs and treatment of allograft rejection
Lack of pathogen-specific immunity		Immunosuppression due to concomitant viral infections
Latent infections in the donor or recipient		Retransplantation

doses, we, and others, have found that it is very difficult to manage.[8] In the improbable case that the use of rifampin is mandatory, the cyclosporin dose should first be increased 3–5-fold, with the frequency of administration increased from twice to thrice daily. Cyclosporin levels should be monitored daily until levels are stable.

Postsurgical mediastinitis and sternum osteomyelitis

HT patients have a higher risk of postsurgical mediastinitis and sternal osteomyelitis than other heart surgical patients.[7] This complication may manifest as a bacteremia or sternum instability or dehiscence (Fig. 104.1). The main causes are bacterial pathogens (staphylococci, Gram-negative rods), but *Mycoplasma*, mycobacteria and other less common pathogens should be suspected in 'culture-negative' wound infections. A bacteremia of unknown origin during the first month after HT should always suggest the possibility of mediastinitis. Risk factors are prolonged hospitalization before surgery, early chest reexploration, low output syndrome in adults and the immature state of the immune response in infants. Therapy consists of surgical debridement and repair, and antimicrobial therapy given for 3–6 weeks.

Cardiovascular infections

Infective endocarditis is a relatively rare complication of HT (1.7–6%). Most of the cases are associated with previous nosocomial infections, mainly venous access devices and wound infections. Interestingly, 80% of SOT patients who developed endocarditis in one series had no previous history of valvular disease. As in other SOT patients, fungal etiology is a common cause of endocarditis. CMV, *Toxoplasma* and parvovirus B19 may cause myocarditis in this population.

Therapy of established infections is similar to that of other immunosuppressed patients. We will therefore focus our attention on some issues related to the prophylaxis of infection.

ANTIMICROBIAL PROPHYLAXIS

Viral infections

The most common viral infections are caused by HSV (24%), varicella-zoster (25%) and disseminated CMV (14%). Kaposi's sarcoma (KS) is caused by HHV-8 and its incidence after HT (0.75%) is higher than after kidney transplant (0.28%). KS usually

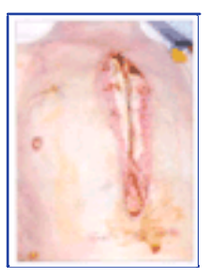

Figure 104-1 Postsurgical mediastinitis due to *aureus* in a HT recipient.

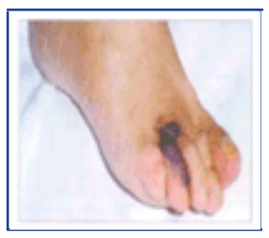

Figure 104-2 Kaposi's sarcoma in a HT recipient. Primary infection by HHV-8 was demonstrated.

develops a median of 24 months after SOT and the mortality is 28.5% (Fig. 104.2). Primary infection was found to be an important risk factor for KS in our experience.[16]

TABLE 104-3 -- Most commonly used antimicrobial prophylactic therapies in heart transplantation.

MOST COMMONLY USED ANTIMICROBIAL PROPHYLACTIC THERAPIES IN HEART TRANSPLANTATION				
Prophylaxis	**Indication**	**Dose and duration**	**Comments**	
Pre-transplant vaccination for *S. pneumoniae*, *H. influenzae*, hepatitis A and B, varicella zoster	As in other patients. *S. pneumoniae*, *H. influenzae* may be repeated after 5 years Annual influenza vaccine			
	Cefazolin	Perioperative prophylaxis	1–2g every 8h iv for 48h	Should be adjusted based on resistance patterns
Trimethoprim-sulfamethoxazole	*Pneumocystis carinii*	80 or 160mg of trimethoprim component po once daily or every 12h on weekend days, or three times a week	May protect against *Nocardia* spp., *Listeria* spp., *Toxoplasma* and other bacteria	
Ganciclovir	Cytomegalovirus	po: 1g tid iv: 5mg/kg bid plus hyperimmune globulins for mismatched cases	Used as prophylaxis or pre-emptive therapy; may protect against HHV-6 and HHV-7; valganciclovir may be used if available	
Isoniazid	*M. tuberculosis*	300mg/d for 6–12 months	Low risk of toxicity	
Pyrimethamine*	Toxoplasmosis (D+R-)	25mg/d for 6 weeks	With folinic acid	

* Trimethoprim-sulfamethoxazole three times a week is an effective protection for both PCP and toxoplasmosis

CMV is the most common etiological agent of infection in HT recipients. In this group of patients focal disease usually presents as pneumonia (27%) or gastrointestinal disease (19%). Depending upon the serological CMV status of both recipient and donor, without anti-CMV prophylaxis 30–90% of HT patients will show laboratory data of infection and 10–90% associated clinical manifestations (CMV disease). Recurrent CMV infections will occur in 10–25% of patients who developed CMV disease, often in the context of severe hypogammaglobulinemia.[8] Epidemiological data suggest that there might be a link between CMV and transplant atherosclerosis.[9]

Antiviral prophylaxis

The most important aspect of prophylaxis against viral infections is anti-CMV therapy. Mismatched recipients should receive prophylaxis immediately after transplantation. Currently, ganciclovir and gammaglobulins (4–6 weeks) seem to be the most effective combination (Table 107.3).[10] In seropositive recipients and in patients receiving OKT3 or antithymocyte globulins, prophylaxis against CMV may be performed with either intravenous or oral ganciclovir.[11]

Pre-emptive therapy is warranted in those with clinical or laboratory signs of CMV infection. Anti-CMV immunoglobulins plus ganciclovir seem to be more effective than ganciclovir alone for preventing the sequelae of CMV infection.[12] Valaciclovir was found to prevent CMV reactivation in HT patients.[13] Surveillance with antigenemia or PCR is recommended for patients not receiving prophylaxis.

Other aspects to consider regarding prophylaxis of viral infections in HT patients include the following.

¦ Annual immunization with influenza vaccine (recommended).
¦ Due to the relatively benign nature of most cases of herpes simplex infection, a majority of experts suggest that 'early therapy' rather than broad and long-term anti-HSV prophylaxis is the best approach.
¦ VZV vaccine should be given to seronegative patients before transplantation. Seronegative patients should be treated with hyperimmune globulin when exposed to patients with varicella-zoster infections. The benefit of VZV vaccination after HT is unknown.
¦ Transplantation of thoracic organs in HBsAg-positive and HBV-DNA-negative recipients is followed by HBV reactivation in a high percentage of cases. However, the clinical outcome and the availability of lamivudine suggest that these patients should not be excluded from transplantation. Vaccine against hepatitis B virus should be administered to susceptible transplant candidates.

Bacterial infections

Most bacterial infections occur early after transplantation and are often associated with invasive procedures. Micro-organisms involved are *Staphylococcus aureus, Pseudomonas aeruginosa* and some enterobacteria. In addition, infections caused by *Listeria monocytogenes* and *Rhodococcus equi* usually occur in severely immunocompromised patients.[6] Finally, some patients may develop late infections, such as pneumoccocal penumonia.

Antibacterial prophylaxis

Patient should receive antibacterial prophylaxis (2–3 doses of cefazolin) for surgery (Table 104.3). Surgery and postsurgical management should be carefully performed and mechanical ventilation, chest tubes and catheters withdrawn as soon as possible.

Perioperative selective bowel decontamination is not recommended in HT patients.

S. pneumoniae and *Haemophilus influenzae* vaccines should be offered to this population, if possible before transplantation. Pneumococcal vaccination may be repeated after 5 years.

Patients should be checked for previous exposure to tuberculosis (tuberculin test and chest X-ray). Antituberculous chemoprophylaxis is indicated in patients with tuberculin test conversion, with a clear-cut history of exposure to tuberculosis or with old tuberculous lesions on chest X-rays. Tuberculin-positive patients should submit samples for mycobacterial culture and receive chemoprophylaxis with isoniazid (Table 104.3).

Fungal infections

The most common fungal pathogen after HT is *Aspergillus*. The overall incidence of invasive aspergillosis in HT patients has decreased in recent years. It reached 25% before the cyclosporin era and decreased afterwards to 3.3–14%. Aspergillosis should be considered in the differential diagnosis of all pulmonary infections in a HT

patient (Fig. 104.3).

Prompt recognition of this fungal infection is essential for achieving a successful outcome with intensive antifungal therapy. However, both clinical symptoms and radiological manifestations may be nonspecific at early stages of the disease. The isolation of *Aspergillus* species from nonsterile respiratory samples may indicate invasive infection, colonization or laboratory contamination, and therefore make decisions about treatment of symptomatic transplant recipients difficult. Isolation of *Aspergillus* is not uncommon in the transplant population. We recovered it from 10.5% of the HT recipients in our institution and rates of 1.5–4.5% have been reported in liver and kidney recipients. Our data suggest that the isolation of any species of *Aspergillus* from any respiratory tract samples obtained in a HT recipient with suspicion of infection has a positive predictive value (PPV) of 60–70%. When analyzed by species, the PPV of recovering *A. fumigatus* was 78–91%. The PPV increased to 88–100% when *A. fumigatus* was recovered from a respiratory specimen other than sputum.[17]

Antifungal prophylaxis

Very few data are available on prophylaxis against *Aspergillus* in HT.[14] We have used oral itraconazole with good results and inhaled amphotericin is used at Stanford University.[3] The role of the new

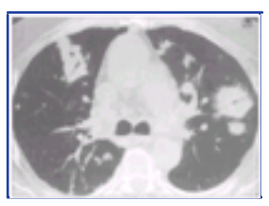

Figure 104-3 Bilateral invasive aspergillosis in a HT recipient.

antifungal drugs in *Aspergillus* prophylaxis in high-risk HT recipients remains to be determined. Anti-*Candida* prophylaxis is usually not necessary in HT patients.

Heart transplant recipients are at a substantial risk of *P. carinii* pneumonia which presents with an abrupt onset and a high mortality. Cyclosporin immunosuppression, older age, need for mechanical ventilation, low serum albumin and CMV co-infection have been reported as poor prognostic factors for *Pneumocystis* infections. Weekend TMP-SMX chemoprophylaxis has been very effective at our institution. [15]

Parasitic infections

The risk of primary toxoplasmosis is greater in HT patients (more than 50%) than either liver (20%) or kidney recipients (<1%). Patients with toxoplasmosis present with fever, altered mental status, focal neurological signs, myalgias, myocarditis or lung infiltrates. Infection transmitted by the transplanted organ is more often associated with acute disease (61%) than reactivation of latent infection (7%).

Chagas' disease is one of the principal indications for heart transplantation in some countries of South America.[16] Reactivation of the disease is a common problem that may be diagnosed by detection of *Trypanosoma cruzi* in blood or tissues, in association with symptoms or signs of infection. These episodes respond to treatment with benznidazole or allopurinol.

Antiparasitic prophylaxis

Toxoplasma-seronegative patients with positive donors are usually given 6 weeks of pyrimethamine with folinic acid. These patients may develop *Pneumocystis* infections despite standard 6-week prophylaxis against toxoplasmosis. We therefore recommend prescription of a double-strength tablet of TMP-SMX 3 days a week as prophylaxis for *Pneumocystis* infections.[17] This approach prevents both *Pneumocystis* infection and toxoplasmosis in high-risk HT patients.

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Chapter 105 - Liver Transplant Patients

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INTRODUCTION

The care of liver transplant recipients is a challenge considering the infectious complications that contribute to significant patient morbidity and mortality rates. Up to 70% develop bacterial, viral, fungal or parasitic infections following liver transplantation. Like other organ transplant recipients, the risk for infection among liver transplant recipients is influenced by the state of immunosuppression and the epidemiologic exposures of the recipient and the donor. Furthermore, the complexities of liver transplantation surgery with the potential break in areas of high microbial load (i.e. gastrointestinal tract), and the underlying medical and infectious indications for transplantation (e.g. fulminant hepatitis, chronic viral hepatitis) predispose liver transplant recipients to unique infectious complications that may not be commonly observed among other organ transplant recipients.

The detection of active infection in the liver transplant candidate may further complicate the decision of whether and when to perform liver transplantation. Infections related to underlying cirrhosis such as ascending cholangitis and spontaneous bacterial peritonitis are not uncommon before liver transplantation. In general, all infections identified before liver transplantation should be adequately controlled before, during and after the transplant procedure. Furthermore, an underlying infection does not absolutely contraindicate liver transplantation, unless it is systemic and uncontrolled. To illustrate, advances in the control of HIV infection, a condition once considered to be a contraindication to transplantation, have paved the way for the successful management of HIV-infected liver transplant recipients.

Thus, it is essential that liver transplant candidates undergo evaluation before transplantation so that they are adequately assessed for their risks of disease caused by, for example:

- human herpesviruses — cytomegalovirus (CMV), herpes simplex virus (HSV) and Epstein-Barr virus (EBV);
- HIV;
- hepatitis viruses (A–E);
- fungi (e.g. *Coccidioides immitis*, *Histoplasma capsulatum, Aspergillus fumigatus*);
- *Treponema pallidum*;
- *Mycobacterium tuberculosis*;
- *Strongyloides stercoralis*

Likewise, potential donors should be screened for infectious pathogens that could potentially be transmitted through allograft donation.[1] The recognition of these risks will guide the implementation of preventive, diagnostic and therapeutic measures following liver transplantation (Table 105.1).

This chapter emphasizes the risk factors and infectious syndromes that are more prevalent, if not unique, to liver transplant recipients, and the strategies for their prevention and treatment. Risk factors and strategies for prevention and treatment that are common to all organ transplant recipients are discussed elsewhere in this book.

RISK FACTORS

Risks related to epidemiologic exposures, underlying diseases and characteristics of the recipient and the donor

Unlike other solid-organ transplant populations, infection-related indications for liver transplantation are relatively common. Hepatitis C virus (HCV)-induced cirrhosis is the most common indication for liver transplantation.[2] Liver failure from hepatitis B virus (HBV) infection with or without a superimposed hepatitis D virus (HDV) infection or from acute fulminant hepatitis A or B infections are other infection-related indications for liver transplantation.[3] In the absence of effective therapy, the majority of patients transplanted for HBV- and HCV-induced cirrhosis will develop recurrence of the infection following liver transplantation.[2] [3] [4] [5] Fulminant hepatitis, regardless of etiology, also increases the risk for post-transplant viral and fungal infection.[6] Active or latent infection that involves the donor or recipient liver (e.g. *H. capsulatum*, tuberculosis) may be unrecognized before liver transplantation and may manifest clinically during the post-transplantation period.

Risks related to surgical factors

One major factor that influences the occurrence of infection relates to the liver transplantation procedure itself.[6] A prolonged and complicated surgical procedure and the amount of blood loss are directly related to the risk of infection following liver transplantation. The urgency of the procedure, as commonly observed during liver transplantation for fulminant hepatitis, could result in less time for optimal preparation of patients. Abdominal re-exploration, regardless of indication (e.g. need for re-transplantation, abdominal bleeding and other vascular complications), and the type of biliary duct anastomosis (e.g. choledochojejunostomy) further predispose the liver transplant recipient to bacterial and fungal infections.

Risks related to immunosuppression

Pharmacologic immunosuppression, for example with mycophenolate mofetil (MMF), prednisone or tacrolimus, to prevent acute rejection unfortunately place patients at high infectious risk. OKT3 monoclonal antibody and high-dose corticosteroids for induction or treatment of acute rejection is associated with CMV disease and other opportunistic infections such as human herpesvirus (HHV)-6, *Aspergillus* spp. and *Pneumocystis carinii* infection. Likewise, the type and degree of immunosuppression may influence HCV replication.[2] The combination of OKT3 and MMF has been reported to accelerate the course of post-transplant HCV infection.[2] Furthermore, the reactivation of immunomodulating viruses (e.g. HHV-6 and CMV) resulting from the use of immunosuppressive agents may paradoxically enhance the state of immunosuppression and influence the occurrence of superimposed bacterial and fungal opportunistic infections.[7] It has also been suggested that CMV and HHV-6 accelerate the progression of HCV infection following liver transplantation.[2]

TABLE 105-1 -- Risk factors for acquiring infection following liver transplantation.

RISK FACTORS FOR ACQUIRING INFECTION FOLLOWING LIVER TRANSPLANTATION		
Preoperative period	**Intraoperative period**	**Postoperative period**
Lack of pathogen-specific immunity	Presence of pathogens in the transplant allograft	Prolonged hospitalization
Severity of underlying clinical illness	Prolonged operative time	Prolonged duration of stay in intensive care unit
Fulminant hepatic failure	Complicated surgical procedure	Prolonged antibiotic use
Renal insufficiency	Profound blood loss and infusion of large volume of blood products	Renal insufficiency

Anemia	Choledochojejunostomy	Gastrointestinal and biliary complications
Previous fungal infection (i.e. endemic mycoses)		Vascular complications
		Corticosteroid use and treatment of allograft rejection
		Immunosuppressive drugs
		CMV and HHV-6 reactivation
		Re-operation within 1 month post-transplantation
		Re-transplantation

CLINICAL PRESENTATION

The natural history of infections following liver transplantation is influenced by various factors including the use of antimicrobial prophylaxis. Generally, these infections are predicted by the time elapsed following liver transplantation (Fig. 105.1). However, it should be emphasized that the use of antimicrobial prophylaxis, the selective use of immunosuppressive agents and improvements in surgical techniques have modified the epidemiology and clinical presentation of infectious complications following liver transplantation.[8]

First month

Like other solid-organ transplant recipients, the majority of infections during this period are related to the surgical procedure (see Fig. 105.1). Liver transplantation is distinct because the surgical manipulation involves parts of the body with high microbial content. The spillage of gastrointestinal contents during the actual liver transplantation procedure is a common source of abdominal infection. Abdominal abscesses and surgical site infections caused by bacteria

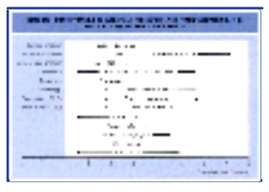

Figure 105-1 Natural history timeline of infections following liver transplantation in the absence of antimicrobial prophylaxis.

(e.g. *Staphylococcus aureus,* coagulase-negative staphylococci, enterococci, Gram-negative bacilli and anaerobic organisms) and fungi (e.g. *Candida albicans*) may disseminate to cause bacteremia and candidemia, respectively. The patients usually manifest with fever and a sepsis syndrome. Erythema, purulence and dehiscence of the surgical wound may be evident, and fluid collections may be demonstrated on radiographic studies. The risk of acquiring these infections is increased among patients who require abdominal re-exploration for any indication (i.e. hepatic artery thrombosis, portal vein thrombosis, biliary leakage or re-transplantation). Consequently, this results in prolonged hospitalization that will further increase predisposition to nosocomial pneumonia, urinary infections, bacteremia and antibiotic-related *Clostridium difficile* diarrhea. Herpes simplex virus reactivation has traditionally been the most common viral infection during the first month following liver transplantation. However, the widespread use of antiviral prophylaxis has significantly decreased the incidence of this viral infection. Among liver transplant recipients, the recurrence of HCV infection may become evident during this period and the degree of HCV replication during this period may be

associated with the recurrence rate and severity of HCV hepatitis and cirrhosis, and ultimately may influence the survival of the liver allograft.[2]

Second to the sixth month

The intense immunosuppression employed during the early months following liver transplantation when hepatic allograft rejection is usually observed and treatment with high-dose corticosteroids or OKT3 monoclonal antibody are administered increases the predisposition to opportunistic infections.

In the absence of antiviral prophylaxis, the ß-herpesviruses (CMV, HHV-6 and HHV-7) reactivate 2–6 weeks following liver transplantation with direct and indirect consequences.[7] However, the widespread use of antiviral prophylaxis has delayed the onset of CMV disease and may have diminished its severity among liver transplant recipients.[9] Several studies in liver transplant patients have suggested potential interactions among the ß-herpesviruses that enhance the severity of CMV disease.[10] Usually accompanied by a viral syndrome of fever and some degree of myelosuppression, organ invasion by CMV following liver transplantation manifests most commonly as CMV hepatitis with elevated serum bilirubin, alkaline phosphatase and transaminases. This is of particular concern because it is usually confused with acute cellular rejection. A liver biopsy is usually required to distinguish between infection, rejection and drug toxicity.

The occurrence of hepatic dysfunction may complicate the use of antimicrobial prophylaxis. Trimethoprim-sulfamethoxazole (cotrimoxazole) may be withheld in these patients and thus predispose them to *P. carinii* pneumonia. Trimethoprim-sulfamethoxazole also protects against bacterial infections such as *Nocardia* spp., which like *Listeria monocytogenes* may present with distinct clinical syndromes (e.g. *Nocardia* spp. brain abscess, *Listeria* spp. bacteremia and meningitis) at any time following liver transplantation, but most commonly during this period.

Invasive aspergillosis, mostly due to *A. fumigatus*, may occur among patients transplanted for fulminant hepatitis and those with epidemiologic exposure and profound immunosuppression.[6] The clinical illness reflects the vasculotrophic nature of *Aspergillus* spp. and causes abscesses in many organs including the liver and the brain. Infection with endemic fungi (e.g. *H. capsulatum*, *C. immitis*) and *Cryptococcus neoformans*, which have been transmitted occasionally through the transplanted liver allograft, may occur during this period.

Beyond the sixth month

Beyond the sixth month, the vast majority of liver transplant recipients have good hepatic allograft function and their level of immunosuppression may have been reduced. These patients are primarily at risk of infections similar to those observed in the non-immunocompromised population.

However, opportunistic infections similar to those observed during the first 6 months following liver transplantation may occur, primarily among patients with poor graft function (i.e. from chronic rejection) who remain on intense immunosuppression. Infection with endemic mycoses due to *H. capsulatum* and *C. immitis*, and the reactivation of varicella-zoster virus commonly occurs during this period. Epstein-Barr virus related post-transplant lymphoproliferative disorder may occur at any time following liver transplantation.

The majority of HCV-infected liver transplant patients demonstrate recurrence of infection with a significantly higher degree of viral replication following liver transplantation.[2] Likewise, HBV infection usually recurs in the absence of antiviral prophylaxis.[3] [4] During this period, an accelerated clinical course of HCV infection may occur among older recipients who have received organs from older donors and those who are receiving high-dose corticosteroids and MMF. [2] Cytomegalovirus infection may also accelerate HCV infection.[2] Among HCV-infected patients, the recurrence of HCV-associated allograft failure is the most common cause of death during the first 5 years following liver transplantation.

It must be re-emphasized that the natural history of infections following liver transplantation has changed and continues to change because of improved surgical techniques, more selective immunosuppression and the widespread use of prophylactic antimicrobial agents. In some cases, as exemplified by CMV infection, the onset of the infection may be delayed and the clinical presentation modified.[9] Thus, physicians must remain alert to the occurrence of opportunistic infections, even during this later period.

DIAGNOSIS

Knowledge of infection risks is essential in the proper implementation of diagnostic surveillance following liver transplantation. Clinical screening with history and physical examination is essential. Surveillance cultures or other sensitive methods such as polymerase chain reaction (PCR) to identify infection before its clinical

manifestation are employed.[11]

Routine surveillance using rectal swab and stool cultures are employed to identify colonization with drug-resistant bacteria (i.e. vancomycin-resistant enterococci) in an effort to interrupt transmission to other 'at risk' patients. In contrast, routine use of bacterial surveillance cultures of biliary drainage after liver transplantation is not recommended. Surveillance cultures for *Candida* spp. and *Aspergillus* spp. infections are not performed routinely, unless there are symptoms.[6] Environmental surveillance should be performed when outbreaks of environmental pathogens such as *Aspergillus* spp. occur.

Screening for CMV infection following liver transplantation is influenced by available diagnostic methods and antiviral drugs.[12] Because of poor sensitivity, virus culture should not be employed to guide pre-emptive therapy. Instead, highly sensitive PCR assays or CMV antigen pp65 detection should guide its implementation.[12] Otherwise, universal prophylaxis should be employed for the prevention of CMV disease, particularly among 'high-risk' individuals.

Surveillance for HBV with measurement of HBV DNA, hepatitis B e antigen and antibody, and hepatitis B surface antigen and antibody should be performed on all HBV-infected patients before and following liver transplantation to document the degree of viral replication (which influences the risk of recurrence) and to monitor effectiveness of viral suppressive therapy. Surveillance for HCV recurrence using molecular methods that measure degree of HCV replication are usually employed.

MANAGEMENT

Prophylaxis

The most important principle in the management of infection following liver transplantation is prevention (Table 105.2).[13] [14] Vaccines against hepatitis A and B viruses should be administered to susceptible liver transplant candidates to decrease the risk of HAV- and HBV-induced fulminant hepatitis following liver transplantation in naive patients.[15]

Oral selective bowel decontamination (consisting of colistin, gentamicin and nystatin) starting during the days immediately before and following transplantation up to the duration of hospitalization is used to apply selective pressure to the intestinal flora (i.e. to decrease colonization with Gram-negative bacilli and fungi, while sparing the anaerobic organisms) and decrease the incidence of bacterial and fungal infections following liver transplantation.[6] Patients

TABLE 105-2 -- Suggested prophylactic strategies in liver transplantation.

SUGGESTED PROPHYLACTIC STRATEGIES IN LIVER TRANSPLANTATION			
Prophylaxis	**Indication**	**Dose and duration**	**Comments**
Cefotaxime	Perioperative prophylaxis	1g q8h iv for 48 hours	Should be adjusted based on resistance patterns
Trimethoprim-sulfamethoxazole	*Pneumocystis carinii*	80 or 160mg of trimethoprim component po q24h	May protect against *Nocardia* spp., *Listeria* spp. and other bacteria
Aciclovir	Herpes simplex virus	200mg po q8h for 28 days	Should be withheld when ganciclovir is used; valaciclovir may be used if available
Ganciclovir	Cytomegalovirus	1g po q8h; duration variable	Used as prophylaxis or pre-emptive therapy; may protect against HHV-6 and HHV-7; valganciclovir may be used if available
Fluconazole	*Candida* spp.	400mg po daily for 28 days	Targeted to patients with complicated and prolonged surgery or profound blood loss
Oral bowel decontamination solution	Gram-negative bacilli and fungi	Variable	Selective pressure favoring anaerobic environment, with goal of decreasing risk of fungal and bacterial infection
Hepatitis B immunoglobulin	Hepatitis B virus	10,000IU/day for first week then every 4 weeks	Maintain serum hepatitis B immunoglobulin level >100IU; may be used in combination with lamivudine; role of other agents such as adefovir not yet defined
Amphotericin B	*Aspergillus* spp.	0.2mg/kg per day	Administered to patients with fulminant hepatic failure

should also receive intravenously administered antibacterial prophylaxis (e.g. cefotaxime) during the perioperative period to decrease surgery-related infections. Prophylaxis against *P. carinii* infection should be used, either trimethoprim-sulfamethoxazole or aerosolized or intravenous pentamidine as alternative regimens.

Antifungal prophylaxis, usually with relatively low-dose amphotericin B is given to patients with fulminant hepatitis.[6] Because of the risk of invasive fungal disease, oral fluconazole may be administered for up to 4 weeks to liver transplant recipients who require re-transplantation or re-operation, and to patients with significantly prolonged surgical time or who had profound blood loss during surgery.[6]

The prevention of HBV recurrence is accomplished with the administration of immunoglobulin with high titers against HBV (HBIg) with or without lamivudine. [4] [16] [17] The dose of HBIg is usually 10,000IU/day for the first week following liver transplantation and at 4-week intervals thereafter, with the goal of achieving serum HBIg levels above 500IU for the first year and over 100IU thereafter. This practice is very effective in reducing HBV recurrence but it is limited by its expense. Lamivudine may decrease HBV replication following liver transplantation and is now routinely employed following liver transplantation despite the lack of rigorous clinical trials showing efficacy. Strategies for decreasing the degree of HBV replication before liver transplantation with HBIg and lamivudine have also been tried. However, their prolonged use before transplantation is strongly discouraged because of the risk of selecting resistant mutants, and thus limiting their use during the post-transplant period. Prolonged use of HBIg is associated with the emergence of surface antigen mutants. Resistance to lamivudine is conferred by the YMDD mutation. Because of the risks of prolonged use and the inherent uncertainty and unpredictability of the timing of liver transplantation, HBIg and lamivudine are usually administered only as post-transplant prophylaxis.

There is currently no optimal strategy for preventing HCV recurrence following liver transplantation. The use of interferon-a and ribavirin has been shown in anecdotal studies to reduce HCV replication following liver transplantation. [18] Because of intolerance to adverse effects and high treatment failure rates, the current practice is not to give anti-HCV therapy unless histologic recurrence is demonstrated. Studies on the efficacy of pegylated interferon-a, which provides more sustained levels of circulating interferon-a, possibly with fewer side-effects, are eagerly anticipated.

Liver transplant recipients who are at risk for CMV infection or who have evidence of CMV infection are given ganciclovir (or valganciclovir) prophylaxis or pre-emptive therapy, respectively.[7] A targeted approach to CMV prevention should be administered to patients with fulminant hepatitis and those receiving OKT3 monoclonal antibody (Chapter 205).

Therapy of established infection

Early diagnosis and administration of specific and effective therapies are key to the optimal management of infections following liver transplantation. The use of antimicrobial therapy should be tailored to the micro-organism involved and should be guided by antimicrobial susceptibility when available.

Reduction of pharmacologic immunosuppression should complement the use of antimicrobial therapy as possible. In addition, drainage of infected fluid collections (e.g. infected hematoma and abdominal abscesses), debridement of surgical site infections and the removal of infected intravascular and urinary catheters are essential components of therapy.

CONCLUSION

Liver transplant recipients have unique characteristics that differentiate them from other transplant patient groups. The complexity of the surgical procedure places the liver transplant patient at higher risk of bacterial and fungal infections. The higher prevalence of infection-related indications for transplantation, such as HCV and HBV infection, among liver transplant recipients presents further challenges. Moreover, the diversity of the indications for liver transplantation requires a tailored and individualized approach to prophylaxis and surveillance.

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Chapter 106 - Pancreas Transplant Patients

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INTRODUCTION

Pancreas transplantation is currently a widely accepted modality for the treatment of insulin-dependent diabetes mellitus.[1] While most patients undergo pancreas transplantation in combination with renal transplantation, selected nonuremic patients may also be candidates for pancreas transplantation alone. Although rejection rates have decreased with better immunosuppressive therapy, infections remain a source of major morbidity and are one of the leading causes of death in this population. In contrast to infections after kidney transplantation, much of the infection-related morbidity in the pancreas allograft recipient is related to postsurgical intra-abdominal infections. Common postsurgical infectious complications include urine leak, abscess, infected pseudocyst, infected pancreatic or lymphatic ascites, leakage of pancreatic exocrine secretions or enteric contents, and, rarely, infected pseudoaneurysms.

To handle pancreatic exocrine secretions, drainage into the bladder (bladder drainage, BD) or the small intestine (enteric drainage, ED) are the two most commonly used techniques. Bladder drainage is associated with metabolic acidosis, intravascular volume depletion, reflux pancreatitis and urinary tract complications, including hematuria, urethritis, urethral strictures and urethral disruption, all of which may be compounded by infected urine, and recurrent urinary tract infections (UTIs). On the other hand, ED is associated with significantly fewer urologic and metabolic complications than BD, and ED has therefore become the preferred technique. However, in certain settings, such as solitary pancreas transplantation or severe diabetic enteropathy, BD may still be indicated.

Several factors contribute to the development of intra-abdominal infections after pancreas transplantation, including:

- intense immunosuppressive regimens;
- compromised defenses associated with diabetes;
- postreperfusion pancreatitis with local release of cytokines and digestive enzymes providing a favorable environment for micro-organisms;
- contamination of the operative field by micro-organisms present in the donor duodenum; and
- microbial translocation from the duodenum facilitated by postreperfusion edema, ischemia or rejection.

Infectious complications associated with pancreas transplantation include UTI, anastomotic leaks of the duodenal segment, mycotic aneurysms, infected pancreatic pseudocysts and infected abdominal ascites. In addition, cytomegalovirus may affect the pancreas allograft.

URINARY TRACT INFECTIONS

Urinary tract infections are a common problem in patients with primary BD of the duodenum segment in pancreas transplantation. Bladder-drainage pancreas transplants lead to significantly more UTIs than ED transplants do (63% vs 20% in the first postoperative year).[2] Urinary tract infections can occur either in the early or late post-transplant period, and they are frequently recurrent. The underlying pathophysiology includes impaired integrity of the bladder mucosa, alteration in urinary pH, urinary retention or stasis due to residual diabetic autonomic neuropathy, prolonged catheter drainage, microbial contamination by the contents of the donor duodenum, retained intravesical sutures and bladder stones. Activation of pancreatic enzymes in the bladder together with urinary alkalosis resulting from bicarbonate secretion appears to cause significant impairment of the mucosal defenses.[3]

The presentation of UTI in pancreas transplant recipients does not differ from the presentation in any other immunosuppressed patient. Symptoms of fever, urinary frequency, dysuria and urgency are common, although patients can be asymptomatic. The majority of UTIs are bacterial but fungal infections are also common. The most common bacteria encountered are *Escherichia coli* (27%) and other Gram-negative organisms, such as *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp.[4] Gram-positive organisms are present in approximately 20% of cases, primarily in males, and are probably related to the long-term use of indwelling catheters.[4] In BD pancreas transplants, 50–71% of patients developing a first UTI had a recurrence within the first 90 days. However, less than one-third of these recurrent infections were from the same pathogen as that responsible for the previous infection.[4] Occasionally, a simple lower UTI progresses to an upper UTI or pyelonephritis in the renal allograft, a process that may be accelerated by a diabetic neurogenic bladder and vesicoureteral reflux. Frequently, the donor duodenal segment is colonized with *Candida* spp., explaining the prevalence of fungal UTIs.

Most UTIs following pancreas transplantation can be treated successfully with appropriate antibiotics. Non-nephrotoxic antibiotics that concentrate in the urine, such as the fluoroquinolones, are first-line agents. Fungal UTIs can occasionally be successfully treated with fluconazole or amphotericin bladder irrigations; however, recurrence is common and systemic amphotericin B has been the mainstay of therapy. The role of newer potent antifungals is undefined. Recurrent UTIs merit further investigation, including cystoscopy to exclude lower urinary tract pathologies such as bladder calculi, foreign bodies or suture granulomata at the duodenum-bladder anastomosis, or a fungus ball (see also Chapter 96a). In the absence of lower tract abnormalities, the upper tracts is examined by retrograde pyelography and computerized tomography (CT) scans of the transplanted and native urinary systems. Recurrent, problematic, resistant infections in BD pancreas transplant recipients or those causing renal allograft dysfunction are generally considered indications for conversion to ED.[5]

Multiple recurrent UTIs are associated with poorer long-term renal transplant outcomes.[6] [7] Patients with chronic rejection have more UTIs per year, and an earlier onset of chronic rejection correlated with a higher incidence of UTI.[6] However, the pathophysiology uniting UTI with chronic rejection remains unclear. Potential mechanisms include direct injury, mediated by inflammatory cytokines and chemokines, or enhanced alloreactivity, stimulated by inflammation.

TABLE 106-1 -- Leaks in 747 consecutive simultaneous pancreas and kidney transplants at the University of Wisconsin from October 1983 to July 2000.

LEAKS IN 747 CONSECUTIVE SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANTS			
	Bladder drainage (n=446)	**Enteric drainage (n=301)**	***p* value**
Patients with leak (n)	84	19	0.0001
Patients with leak by 60 days	5.30%	5.50%	
Patients with leak by 1 year	16.1%	6.6%	
Patients with leak by 5 years	20.5%	6.6%	
Patients with leak by 10 years	22.9%	6.6%	
Graft salvage rate after leak	83/84 (98.8%)	6/19 (31.6%)	0.001
Candida-associated infection	15/84 (17.8%)	7/19 (38%)	0.01

Anastomotic duodenal segment leak: enteric drainage versus bladder drainage

Leakage of pancreatic exocrine secretions from the transplant results in severe life-threatening intra-abdominal infection requiring prompt surgical intervention. With greater experience and better immunosuppression, this surgical complication has become less common. A retrospective study at our center compared the incidence of pancreatic enzyme leaks with BD with the incidence with ED. In patients with ED of the duodenum segment the leak rate was 6.3% whereas in BD patients it was 18.8% (p=0.0001; Table 106.1). Most ED leaks occurred within the first 30 days after transplantation. In contrast, leaks in BD transplants may occur months to years after transplantation, suggesting different etiologies between the two groups. In both groups, early post-transplant leaks are largely technical, either related to ischemia of the duodenal segment or dehiscence of the anastomotic suture line. On the other hand, the precise etiology of late leaks in BD transplants is rarely identified and is probably multifactorial. Whereas early leaks are more commonly at the anastomotic suture lines, late leaks may occur anywhere in the duodenal segment and are possibly related to immunologic rejection, chronic distention, cytomegalovirus (CMV) disease or chronic urine irritation.

In addition to differing in their time of presentation, anastomotic duodenal segment leaks in ED and BD pancreas allografts differ in presenting signs and symptoms. Leaks in BD pancreatic transplants present with rapid-onset lower abdominal pain, fever and occasionally hematuria, reflecting sudden spillage of urine into the peritoneal cavity. A sentinel episode of gross hematuria is not uncommon. Concomitant elevation of serum creatinine and serum amylase are classic laboratory findings that usually improve steadily after insertion of an indwelling urinary catheter. In contrast, leaks in ED pancreatic transplants generally cause abdominal pain, fever and hyperamylasemia. An elevated serum creatinine is not part of the usual symptom complex, unless sepsis is severe.

In patients with BD transplants, the diagnosis is made by either a ^{99m} technetium (^{99m}Tc) voiding cystourethrogram (VCUG) or a CT cystogram. A ^{99m} Tc VCUG is performed by administering labeled diethylene-triamine-penta-acetic acid through an indwelling urinary catheter. Persistent radioisotope in the peritoneal cavity on delayed images indicates a leak (Fig. 106.1). In ED transplants the CT scan detects a fluid collection surrounding the pancreas allograft, frequently in proximity to the duodenal segment (see Fig. 106.1). The presence of air or contrast material in the fluid collection is the *sine qua non* for enteric leak. However, the absence of these findings does not exclude a leak and does not prove that the fluid collection is not infected. Determination of sterility depends on sampling the fluid, usually by percutaneous aspiration or laparoscopy. The presence of fluid that is rich in amylase and bilirubin

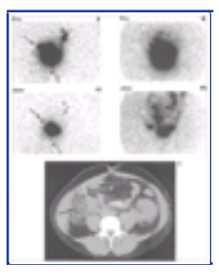

Figure 106-1 ^{99m} Technetium voiding cystourethrograms. (a) Filling and voiding phases of normal ^{99m} Tc DTPA VCUG. The large arrow represents the duodenal segment of the bladder-drained pancreas transplant. The small arrow represents retrograde flow of radioisotope into the pelvis of the transplanted kidney. (b) Abnormal ^{99m} Tc VCUG. Persistence of radioisotope in the peritoneal cavity is consistent with bladder leak (multiple white arrow heads). Although sensitive for diagnosing urine leak, a radioisotope VCUG does not localize the site of leak. A follow-up contrast VCUG may provide localization, if necessary. (c) Abdominal CT scan demonstrating an enteric leak. The pancreas (white arrow) and a peripancreatic fluid collection (black arrow) are seen. Air and contrast material in the fluid collection are diagnostic of an enteric leak. The absence of these findings, however, does not rule out the possibility of a leak.

confirms the diagnosis of enteric content leak. Polymicrobial infections with enteric organisms are common and indicate an enteric leak until proven otherwise. On the other hand, if amylase content is high and bilirubin content is low, then pancreatic parenchymal

injury causing leak of exocrine secretions is likely. This diagnosis is further supported if the fluid is sterile or contains only Gram-positive organisms.

Small leaks in BD transplants can occasionally be treated by Foley catheterization alone for 4–6 weeks. However, if symptoms do not resolve completely or if they recur after removal of the Foley catheter, patients may require surgical conversion to enteric drainage.[5] Leaks in ED transplants usually require urgent surgical intervention. In our experience, the most common organisms associated with leaks in ED transplants are *Candida* spp., including *Candida glabrata* or *Candida albicans* (38%), and vancomycin-resistant enterococci (14%). The mainstays of surgical treatment are debridement of devitalized tissue, tension-free re-closure of the duodenal segment, placement of continuous suction drains in conjunction with the use of appropriate antibiotics and construction of a Roux-en-Y intestinal diversion if this was not performed at the time of transplantation. However, despite these measures, the rate of re-leakage or failure to control sepsis is rather high even in experienced centers. These complications may ultimately necessitate pancreatectomy in a significant number of patients.

The impact of duodenal segment leaks and associated intra-bdominal infections on renal and pancreas allograft survival is significant. These infections and their antimicrobial treatment commonly contribute to renal allograft dysfunction. Furthermore, in the current immunosuppressive era, leak is a major cause of graft loss since ED transplants that develop a leak may require graft pancreatectomy.

INFECTED FALSE ANEURYSM OF THE ARTERIAL GRAFT

Mycotic aneurysms that occur in immunocompetent patients are often thought to be from septic emboli of cardiac origin. In contrast, the origin of mycotic aneurysms after pancreas transplantation is thought to be due to local infection around the iliac Y graft interacting with tissue digestion by activated pancreatic enzymes. A locally invasive infection promoted by enzymatic tissue digestion leads to disruption of the arterial anastomosis culminating in massive bleeding. *Candida* infection is commonly seen in these patients; however, other organisms, such as *Staphylococcus aureus*, *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Enterobacter* spp., can also be present individually or in mixed infections. Reduced cell-mediated immunity in transplant recipients, enzymatic digestion of tissues, and adhesiveness and invasion of *C. albicans*[8] may contribute to this infectious complication.

Patients with infected false aneurysms present in the immediate postoperative period with hypotension associated with intrabdominal bleeding and hemorrhagic shock. In some cases, gastrointestinal bleeding may also be present. A sentinel episode of mild intra-abdominal or gastrointestinal bleeding frequently presages a more life-threatening event. It is also occasionally preceded by a significant peripancreatic infection.

An infected false aneurysm of the transplanted pancreas is difficult to diagnose. In stable patients with peripancreatic infection and a sentinel bleeding episode, urgent iliac arteriography should be performed (Fig. 106.2). If unstable, an emergency laparotomy may be life saving. Ultrasound, CT scanning and magnetic resonance imaging are useful adjunctive tools to evaluate peripancreatic infection or bleeding, but only in the stable patient. They may show a dilated vessel in the region of the pancreatic arterial supply. Computerized tomographic angiography or conventional angiography is usually necessary however to confirm the diagnosis (see Fig. 106.2). Samples of the aneurysm wall and contents should be cultured for aerobic and anaerobic bacteria and fungi.

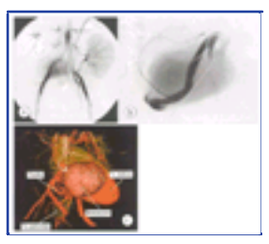

Figure 106-2 Infected false aneurysm of a pancreas transplant. (a), (b) Arteriogram of an infected false aneurysm originating from the ligated superior mesenteric artery stump of the pancreas transplant. (c) Triphasic CT reconstruction (90° rotation) demonstrating the feeding vessels of the false aneurysm in relation to the pancreas transplant.

Extent of active infection in the iliac artery or Y graft artery wall is difficult to determine at the time of surgery, and minimal intervention at the initial surgery is usually doomed to failure. Aggressive management is necessary in order to prevent death. Five general principles apply to the operative management of infected aneurysms:[9]

- control of hemorrhage;
- confirmation of the diagnosis by obtaining tissue specimens for culture;
- operative control of sepsis, including resection of the aneurysm and wide debridement of the infected tissue;
- prolonged antibiotic or antifungal therapy; and
- arterial reconstruction through uninfected tissue planes.

Therefore, the recommended procedure for an infected Y graft is complete excision of the donor and recipient iliac arteries, and transplant pancreatectomy followed by surgical bypass of the recipient common iliac artery in order to restore limb blood flow.[10] The conduit of choice is a blood-type compatible cadaver iliac graft from another donor or autogenous vein. Alternatively, an extra-anatomic femoral-femoral or axillary-femoral bypass with polytetrafluoroethylene is used. The identification of *Candida* spp. in the peripheral blood or blood clot removed from the Y graft indicates systemic fungal involvement. Broad-spectrum antibiotic and antifungal therapy should be initiated immediately until organism-specific antibiotic therapy can be instituted.

INFECTED PANCREATIC PSEUDOCYST

Pancreatic pseudocyst is rare after pancreas transplantation. It is defined as a peripancreatic inflammatory fluid collection rich in pancreatic enzymes but devoid of enteric contents that is localized by a nonepithelial fibrous wall. Patients typically present with nonspecific abdominal complaints including generalized malaise and weakness, nausea, abdominal pain, fevers and weight loss; associated elevations of serum amylase and lipase are common. In cases of BD, malodor of the urine and a UTI may co-exist. A CT scan should be

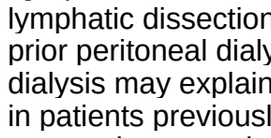

Figure 106-3 Pancreatic pseudocyst. (a) A CT scan demonstrating a pseudocyst of a pancreas transplant in the left pelvis (arrow). (b) Percutaneous drainage of a pancreas transplant pseudocyst. Injection of radiographic contrast material (black arrow) demonstrates the pancreatic duct (arrowhead) in communication with the pancreatic pseudocyst.

obtained. Pseudocyst formation in the pancreas transplant is thought to be similar in origin to that of the native pancreas — namely, inflammatory pancreatitis. Pancreatitis of the pancreas transplant is most commonly related to reflux and neurogenic bladder, alcohol or duct strictures. Biliary stone disease, while commonly contributing to native pancreatitis, is almost nonexistent in the transplant setting.

Loculated fluid encompassing the transplanted pancreas on CT scan suggests pancreatic pseudocyst (Fig. 106.3). A well-developed capsule of the pancreatic pseudocyst can sometimes be observed, as can a dilated pancreatic duct. Aspiration of the fluid collection is necessary to evaluate the presence of organisms as well as to determine the nature of the fluid. The absence of bilirubin and the presence of amylase in the aspirate is the *sine qua non* in the diagnosis of pancreatic pseudocyst.

Management mimics that for pseudocyst of the native pancreas. If the pancreatic pseudocyst is infected, external drainage and antibiotic therapy are key. Sterile pseudocysts that are smaller than 6cm in diameter in relatively asymptomatic patients can be managed expectantly. Persistent infection of the pancreatic pseudocyst can require prolonged antibiotic therapy (up to 6–8 weeks) and percutaneous drainage (Fig 106.3 and Fig 106.4). Persistent high amylase drainage despite conservative measures suggests a possible communication with the pancreatic duct. To confirm this, a fistulogram through the percutaneous drainage catheter is indicated. If a communication with the pancreatic duct is identified and the infection has been treated, a definitive internal drainage procedure is indicated. Internal drainage of a pancreas transplant pseudocyst is optimally achieved via a Roux-en-Y cysto-enterostomy.[11] In BD cases, the pancreatic pseudocyst may alternatively be drained into the bladder by performing a pseudocyst-vesicostomy through a combined transurethral and transabdominal approach. [12] The long-term outcome from these procedures should be excellent.

INFECTED ABDOMINAL ASCITES

Lymphatic ascites after pancreas and kidney transplantation in uremic patients occurs in up to 15–20% of cases. This complication is attributed to the extensive lymphatic dissection necessary for surgical exposure during the operative procedure. It is exacerbated by a poorly absorptive peritoneal membrane if there has been prior peritoneal dialysis complicated by peritonitis. In addition, impaired peritoneal macrophage function and accumulation of peritoneal effluent following peritoneal dialysis may explain the high incidence of deep wound infections in this population.[13] In a comparison study at our institution, the rate of postoperative fluid collections in patients previously on peritoneal dialysis was significantly greater than the rate in patients who had not previously received peritoneal dialysis (24% vs 13%, $p<0.01$; personal communication, Y. Becker). In many instances, fluid collections were associated with Gram-positive or Gram-negative bacterial or fungal contamination and required antibiotic and antifungal therapy. Peritoneal dialysis patients have a greater incidence of intrabdominal fluid collections infected with micro-organisms that colonize human skin.[14]

The initial diagnosis of ascites is based on physical examination. Abdominal radiographs will demonstrate a paucity of air or ground-glass appearance. Computerized tomography scanning or ultrasound confirms the diagnosis. The ascites fluid should be sampled for bacteria and fungi as well as to evaluate cell count, amylase, total protein and chylomicra. Elevated pancreatic enzymes and total protein are characteristic of pancreatic ascites.

Treatment of ascites is based on the use of therapeutic paracentesis, placement of a soft, small-caliber intraperitoneal, indwelling drainage catheter or judicious use of diuretic therapy, or both (see Fig. 106.4). For pancreatic ascites, cessation of oral feedings with the use of hyperalimentation and parenteral octreotide is usual. For infected ascites, percutaneous drainage and appropriate antibiotics usually result in complete resolution and excellent long-term outcomes. Polymicrobial or fungal infection suggests possible leak of enteric contents. Investigation for this entity should include injection of the percutaneous drain with contrast, a CT scan with oral contrast, or enteroclysis.

CYTOMEGALOVIRUS INFECTION OF THE PANCREAS ALLOGRAFT

Cytomegalovirus continues to be the most common viral pathogen affecting organ transplant recipients. The 1-year rate of CMV disease is 13–17% in prophylaxis-treated simultaneous pancreas-kidney transplant recipients.[15] [16] As in bone marrow and other solid organ transplants, the relative risk of CMV infection in pancreas transplantation varies according to the CMV antibody status of donor and recipient. Nearly 80% of cases of CMV occur in recipients in the high-risk donor-positive-recipient-negative cohort.[15] Symptomatic CMV infection and CMV disease increases the risk for subsequent renal graft rejection (relative risk, 2.11; $p=0.0032$) and non-CMV infections (relative risk, 2.22; $p=0.0011$). [16] Despite the high frequency of systemic CMV infection, CMV infection of the pancreas allograft is uncommon.[17]

The clinical presentation of CMV infection of the pancreas allograft is characterized by abdominal pain and fever, associated with elevation of serum amylase and lipase, and leukopenia. Distinguishing CMV allograft pancreatitis from acute rejection on clinical grounds alone is difficult.[17] Evaluation of allograft histology is essential in this setting. Cytomegalovirus pancreatitis demonstrates multifocal, predominantly acinar mononuclear inflammatory infiltrates associated with characteristic cytopathic changes. Marked cellular enlargement, intranuclear acidophilic inclusions with surrounding halos, and granular basophilic cytoplasmic inclusions are characteristic findings. Immunohistochemistry may be a useful adjunctive test in equivocal cases.[17]

Current CMV prophylaxis is based on the use of oral valganciclovir or intravenous ganciclovir for the first 10 days or while being treated with polyclonal antibody therapy. Doses are adjusted according to creatinine clearance. Long-term post-transplant prophylaxis (for 12 weeks) may be tailored to CMV antibody donor-recipient status, using valganciclovir or ganciclovir for high-risk patients, and aciclovir for those patients that have a low risk of CMV infection.

Treatment of tissue-invasive CMV disease, including allograft pancreatitis, includes lowering immunosuppression and using maximum antiviral treatment with either intravenous ganciclovir or oral

Figure 106-4 Algorithm for evaluating enterically drained pancreas transplant patients with abdominal pain.

valganciclovir. Persistent or recurrent cases may merit the addition of CMV immune globulin (starting dose of 100mg/kg intravenously every other day for 3 days, then 100mg/kg intravenously weekly as indicated.

In spite of adequate prophylaxis, CMV remains a problem following pancreas transplantation. Newer, more effective anti-CMV medications may help clinicians to achieve further reduction in symptomatic CMV infection and disease.

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Chapter 107 - Intestinal Transplant Patients

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Marian G Michaels
Michael DL Green

INTRODUCTION

Experimental models of intestinal transplantation in dogs were pioneered in the late 1950s; however, clinical trials in humans were initially unsuccessful because of graft rejection, sepsis and/or technical failure.[1] The availability of tacrolimus in 1989 facilitated better control of rejection and allowed intestinal transplantation to become feasible clinically.[2] [3] With more than 10 years of clinical experience, intestinal transplantation is now performed routinely by a select group of major transplant centers, although both the procedure and postoperative care remain complex.

EPIDEMIOLOGY

Intestinal transplantation is performed as treatment for intestinal failure, defined as a loss of function manifest by an inability to maintain a normal state of fluid and electrolyte balance, nutrition, growth and development. Intestinal failure may be present on the basis of congenital (e.g. intestinal atresia) or mechanical (e.g. pseudo-obstruction syndrome) problems or as a sequelae of an intestinal calamity (e.g. short gut syndrome following necrotizing enterocolitis or volvulus). While total parenteral nutrition (TPN) is available for patients experiencing intestinal failure, its use is associated with significant risks, including venous access complications, recurrent episodes of catheter-associated bloodstream infections and TPN-induced cholestatic liver disease.

Intestinal transplantation can be performed as an isolated procedure, concomitant with a liver allograft, or as a multivisceral (usually including stomach, duodenum, pancreas, liver and the small intestine) transplant procedure. The choice of procedure is individualized based on the underlying diagnosis associated with intestinal failure and the status of liver function in the presence of chronic TPN. Patient and graft survival vary according to which transplant procedure a patient receives; the best patient and graft survival are associated with isolated intestinal transplantation and the worst outcome is observed in recipients of multivisceral transplantation. Currently, 5-year survival for isolated intestinal transplantation, liver-intestine and multivisceral transplantation are 50%, 50% and 30% respectively. Without intestinal transplantation, the 2-year survival rate for patients with intestinal failure is approximately 40%,[4] depending on age and the presence or absence of liver disease. Given these poor results and the improved outcome of intestinal transplantation under tacrolimus-based immunosuppression regimens, this procedure has become a viable option for patients with intestinal failure. The optimal timing for performance of this procedure is unclear because the clinical course and life expectancy of patients with intestinal failure is variable.

Recipients of intestinal transplantation are on average younger than recipients of other types of organ transplantation; 66% of intestinal transplantation recipients are less than 20 years old and 50% are less than 5 years.[1] The young age of these patients reflects the relatively high prevalence of intestinal failure in children due to congenital malformations or intestinal calamity during infancy.

PATHOGENESIS AND PATHOLOGY

Patients undergoing intestinal transplantation are at high risk of developing infectious complications. Although the young age of many of the recipients of intestinal transplants may account for an increased risk of infection, this population's higher risk of infection is probably explained by the exposure of the transplanted organ to enteric bacteria colonizing the intestine, including the allograft. Unique among intestinal transplant recipients is the relationship that has been observed between the presence of rejection of the intestinal allograft and the development of bloodstream infection.[5] This relationship may be explained in part by the development of breaks in the protective barrier of the intestinal mucosa, which can be associated with rejection of the intestinal allograft. The overall incidence of rejection of the intestinal allograft is extremely high (90%); an average patient experiences between one and five episodes per graft.[6] Accordingly, the high rejection rate is one of the major factors accounting for the high frequency of bloodstream infections seen in intestinal transplant recipients.

Patients with rejection of the allograft can present with clinical signs and symptoms (including fever, abdominal pain or distension, nausea, vomiting and an increase in stomal output) that may be suggestive of infection. Because the presentation of intestinal rejection can mimic infection, it is critical to obtain endoscopic biopsy specimens to confirm the diagnosis. Empiric antibiotics (piperacillin-tazobactam or a third-generation cephalosporin with ampicillin, for example) should be considered in patients with severe rejection of the intestinal allograft until blood cultures are found to be negative.

Bloodstream infections are also associated with the presence of Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD) of the intestine in this patient population. This association is probably due in part to breaks in the protective barrier of the intestinal mucosa. Infections in intestinal transplant recipients can also be attributed to technical complications. An example of this might include an anastomotic leak resulting in bacterial peritonitis. The intense immunosuppression required by intestinal transplant recipients also puts these patients at high risk for opportunistic pathogens, including cytomegalovirus (CMV), EBV and *Pneumocystis carinii*, typically seen after organ transplantation. Finally, intestinal transplantation patients frequently require prolonged use of central venous catheters after transplantation, placing them at risk for line-associated bloodstream infections as long as the lines remain in place.

TIMING AND PATTERN OF INFECTIOUS COMPLICATIONS

In general, the timing of infectious complications after intestinal transplantation follows the classical pattern described for solid-organ transplantation (Table 107.1). However, some differences in the classic timetable of infection are seen and are probably related to prolonged and intense immunosuppression (see Table 107.1).

TABLE 107-1 -- Timing of infection in intestinal transplant patients.[§]

TIMING OF INFECTION IN INTESTINAL TRANSPLANT PATIENTS	
Type of infection	**Frequency of infection (%)**
Early (0–30 days after transplantation)	
Surgical site:	
• Intra-abdominal	10
• Superficial and deep wound	20–30
Catheter-associated infection:	
• Bloodstream[§]	25
• Urinary tract	10
Ventilator-associated pneumonia	10

Rejection-associated bacteremia	5–10
Intermediate (1–6 months)	
Catheter-associated infection:	
• Bloodstream§	10–20
Rejection associated bacteremia	10–15
CMV§	20–50*
EBV-PTLD§	10–33†
• Associated with bacteremia§	5
Late (more than 6 months)	
Catheter-associated infection:	
• Bloodstream§	5–10
Rejection associated bacteremia	10–15
CMV§	<10
EBV-PTLD§	<5
• Associated with bacteremia§	<5
Community-acquired infection	Varies‡

§ *Adapted from Fishman and Rubin.*[7]
§Indicates difference from solid-organ transplant recipients.
* Incidence higher in adult intestinal transplant recipients.
† Incidence higher in pediatric intestinal transplant recipients.
‡ Varies with age and community exposure.

The most important difference is that bloodstream infections occur at a higher rate and continue to occur for a prolonged (if not indefinite) period compared with other organ transplant recipients. A second difference is that intestinal transplant recipients appear to be at higher risk than other transplant recipients for morbidity and mortality from viral infections (Table 107.2), especially CMV and

TABLE 107-2 -- Laboratory evaluation of viral infections following intestinal transplantation.

LABORATORY EVALUATION OF VIRAL INFECTIONS FOLLOWING INTESTINAL TRANSPLANTATION			
Organism	**Frequency (%)**	**Diagnostic test**	**Follow-up**
HBV	<1	HBV serologies, HBV PCR, histology	HBV PCR, liver function tests
HCV	<1	HCV serologies	HCV viral load, liver function tests
RSV	Adults: <1	Antigen detection and culture in NP aspirate	None
	Children: 1–5		
Parainfluenza	Adults: <1	NP aspirate for antigen	None
Adenovirus	30–50	Viral culture, histology	None
Enterovirus	Adults: <1	Viral culture	None
	Children: 1–5		
Influenza	1–5	NP aspirate for antigen detection and culture	
HSV	1–5	Culture, Tzanck smear	Chronic aciclovir prophylaxis
CMV	>5	Culture, pp65 antigen, histology	Monitor pp65 antigen or quantitative CMV-PCR
EBV	>5	EBV PCR, histology, serology	Monitor EBV PCR, imaging studies
HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; NP, nasopharyngeal; RSV, respiratory syncytial virus.			

EBV disease. Cytomegalovirus may present much later in intestinal transplantation patients than in other organ recipients and they can develop chronic or recurrent CMV disease (particularly of their graft). Intestinal transplant recipients have an extremely high incidence of EBV disease and PTLD. In contrast to other organ recipients, EBV-PTLD occurs frequently in intestinal transplantation patients even in the face of pre-existing immunity to EBV at the time of transplant.

These differences between intestinal transplant recipients and patients undergoing other types of organ transplantation may be explained in part by the young age of intestinal transplant recipients and the accompanying increased likelihood that they will be seronegative for CMV and EBV at the time of transplant. Thus, they are at risk for developing primary infection, which tends to be more severe. The differences may also be explained by the large amount of lymphoid tissue in the intestinal allograft.

INFECTIOUS SYNDROMES

Bacteremia

Clinical presentation

Bacteremia, typically presenting as fever, alone or in combination with signs of sepsis, occurs most frequently during the first 4 months after transplantation. However, episodes can occur even years after intestinal transplantation. Sepsis has been identified as the major cause of death in more than half of cases reported to the International Intestinal Transplant Registry.[8] The rate of bacteremia in intestinal transplantation patients is much higher (about two episodes per patient)[9] [10] than that reported for patients undergoing other types of organ transplantation (0.14–0.28 episodes per patient for heart, kidney and liver recipients).[11]

The high incidence of bacteremia may be linked to prolonged use of central venous catheters as well as translocation of microbiologic flora from within the lumen of the intestinal allograft. In most studies, the obvious source of bacteremia is not identified[5] but abnormal histologic findings of the allograft (rejection, PTLD or both) are frequently present at the time of bloodstream infection. Enteric organisms (*Enterococcus* spp., *Escherichia coli, Enterobacter* spp., *Klebsiella pneumoniae,* for example) are the most frequently recovered pathogens and are commonly resistant to multiple antibiotic classes. A high correlation has been noted between bacteria

identified in the stool and those recovered from the blood. Bacterial overgrowth (defined as a bacterial count >10^9 cfu/ml of stools) has been postulated to facilitate the development of translocation and has been recognized in nearly 90% of bacteremic episodes in these patients.[12] Bacterial overgrowth may be attributed to surgical manipulation of the transplanted bowel, the absence of the protective ileocecal valve, abnormal gastrointestinal motility, lymphatic disruption, prolonged TPN and the use of antacid medication. In more than half the cases, the overgrowth includes both Gram-positive and Gram-negative bacteria.

Diagnosis

Blood cultures should be obtained from intestinal transplant recipients presenting with fever or other clinical signs suggestive of systemic infection. If enteric pathogens are recovered, endoscopy should be performed to look for underlying causes such as rejection or PTLD.

Treatment

The initial empiric therapy for suspected bacteremia should take into account previous isolates obtained from that patient and their antimicrobial resistance patterns. Final treatment should reflect cultures and antimicrobial susceptibility testing performed at the time of clinical presentation.

Cytomegalovirus

Clinical presentation

Disease from CMV occurs in almost 25% of intestinal transplant recipients and accounts for significant morbidity and mortality despite treatment with ganciclovir. As many as 90% of patients with CMV disease will have involvement of their allograft as well as their native gastrointestinal tract.[13] Similarly to other transplant recipients, CMV-positive donor (D+)/CMV-negative recipient (R-) patients present with disease that is more likely to be invasive and more difficult to treat. Historically, primary CMV disease initially presents in the second month after intestinal transplantation, while reactivation episodes present closer to a year post-transplantation.[13] Recurrent CMV disease occurs frequently (approximately 50%)[13] and should be treated promptly with antiviral agents.

Diagnosis

Cytomegalovirus disease is defined by the presence of symptoms attributable to CMV infection in a patient with a positive culture (or pp65 antigenemia) or histologic evidence of inclusions or positive

TABLE 107-3 -- Prophylactic strategies in small bowel transplantation.

PROPHYLACTIC STRATEGIES IN SMALL BOWEL TRANSPLANTATION			
Indication	**Prophylaxis**	**Dose and duration**	**Comments**
		Adults	
Cytomegalovirus			
Donor +, recipient -	Ganciclovir	5mg/kg iv q12h for 14d. Then 5mg/kg iv q24h for 7–14d if patient remains in hospital; start valganciclovir to complete 90–120d of therapy once there is evidence of good gut function and absorption (adults: 900mg po q24h; children: contact pediatric infectious disease specialist)	Adjust dose for renal function
	CMV-IVIG	150mg/kg within 72h of transplant and at 2, 4, 6 and 8 weeks post-transplant. 100mg/kg at 12 and 16 weeks post-transplant	
Donor ±, recipient +	Ganciclovir	5mg/kg/d iv q12h for 14d	Adjust dose for renal function
Donor -, recipient -	No treatment		
These are the strategies used at the Children's Hospital of Pittsburgh.			

immunofluorescent staining, in the absence of another pathogen to explain these symptoms. Since antigenemia and/or viremia are absent from 50% of proven cases of CMV enteritis, [14] histologic evaluation of biopsy specimens is often required.

Prevention

In general, CMV-seropositive donors should not be used for CMV-seronegative candidates awaiting isolated intestinal transplantation because of the increased risk of mortality and graft loss associated with primary CMV disease. Mismatches are often unavoidable in patients awaiting combined liver-intestine or multivisceral transplantation because the severity of illness makes it impossible to wait for the 'perfect' donor. For these patients, long-term (3–4 months) chemoprophylaxis using ganciclovir (or valganciclovir) as well as CMV intravenous immunoglobulin (IVIG) should be considered (Table 107.3). The potential role for viral load monitoring in combination with pre-emptive ganciclovir therapy has not been formally evaluated in this high risk population. Since CMV enteritis may not be associated with a positive viral load, this strategy is less likely to succeed after intestinal transplantation than after other organ transplant procedures.

Treatment

Cytomegalovirus disease following intestinal transplantation should be managed similarly to that in other organ transplant recipients. Serial measurement of the CMV viral load using the pp65 antigenemia assay or a quantitative CMV polymerase chain reaction (PCR) along with follow-up endoscopic evaluation should guide therapeutic interventions. Use of CMV-IVIG in combination with ganciclovir should be considered for patients with involvement of the gastrointestinal tract (native or allograft) or of the lungs (Table 107.4). The use of ganciclovir has benefited most patients. Children have experienced a better outcome than adults after appropriate anti-CMV treatment, with successful outcomes approaching 90%.[15] The median time to resolution (defined as the absence of inclusions on biopsy) on ganciclovir treatment is approximately 20 days.[13] Immunosuppression should be maintained at baseline levels to avoid rebound rejection after treatment of CMV disease. Recurrence of disease is common, especially in CMV D+/R- patients. Long-term suppression with oral ganciclovir or valganciclovir may be helpful in these patients. Persistence of disease or of elevated CMV viral load despite treatment suggests the presence of ganciclovir resistance. The use of foscarnet or cidofovir should be considered in these cases.

TABLE 107-4 -- Treatment strategies in small bowel transplantation.

TREATMENT STRATEGIES IN SMALL BOWEL TRANSPLANTATION		
Treatment	**Dose and duration**	**Comments**
Cytomegalovirus		
First line therapy		
Ganciclovir	5mg/kg q12h iv for 14–21d	Maintenance therapy 5mg/kg/d iv q24h or consider valganciclovir. Treat until pp65 or CMV PCR reverts to negative. Adjust dose for renal function
CMV IVIG	100mg/kg/dose q48h iv for 3 doses	May repeat every 2 weeks depending on results
Alternative therapy		
Foscarnet	180mg/kg/d divided q8h iv for 14–21d	Maintenance therapy: 90–120mg/kg/d iv
Cidofovir	5mg/kg iv once weekly for 2 weeks	Continuing therapy: 5mg/kg iv once every 2 weeks as clinically needed

Epstein-Barr virus		
Ganciclovir	5mg/kg q12h iv for 14–21d	Adjust for renal function. Clinical efficacy unproven
Aciclovir	500mg/m^2 q8h iv for 14–21d	Adjust for renal function. Clinical efficacy unproven
IVIG	100mg/kg/d q48h iv for 3 doses	May repeat every 2 weeks depending on results. Clinical efficacy unproven.
These are the strategies used at the Children's Hospital of Pittsburgh.		

Epstein-Barr virus

Clinical presentation

Compared with other organ transplant recipients, intestinal transplant recipients appear to be at the highest risk of developing EBV disease. It can present clinically as a febrile syndrome, mononucleosis, PTLD or malignant lymphoma. Non-specific symptoms of fever, weight loss and malaise are common. Lymphadenopathy, bloody diarrhea or ulcerated nodular tumors of the intestinal tract may be present in some cases. Likewise, disseminated disease can occur. Overall, 10% of intestinal transplantation patients develop PTLD but an incidence as high as 40% has been reported in children. [16] The most frequent site of involvement of EBV in intestinal transplant recipients is the intestinal tract. Since EBV frequently involves the allograft, it is important to note that EBV enteritis can be misdiagnosed as rejection. Accordingly, it is recommended that an Epstein-Barr-encoded RNA (EBER) stain be performed on bowel biopsies with presumed rejection to rule out the possibility of EBV infection before treating for rejection.[17] The mortality rate for patients with PTLD has been reported to be as high as 50% after intestinal transplantation; recent experience at our center demonstrates rates of patient and graft survival of approximately 75%.

There are three major differences between PTLD in intestinal transplant recipients and in other solid-organ recipients:

¦ EBV serostatus prior to intestinal transplantation has not been identified as an important risk for the development of PTLD;
¦ EBV-associated PTLD can be concurrent with rejection after intestinal transplantation. In other organ transplant recipients rejection tends to occur only after evidence of regression of the EBV disease; and
¦ as many as 30% of surviving intestinal transplantation patients will experience chronic and/or recurrent episodes of EBV disease, in contrast to a recurrence rate of about 5–10% after transplantation of other organs.[18]

This last difference may be explained by the difficulty in reducing immunosuppressive treatment in the presence of concomitant rejection, limiting the body's ability to generate a cytotoxic T lymphocyte response against EBV.

Diagnosis of Epstein-Barr virus post-transplant lymphoproliferative disease

When PTLD is suspected, computerized tomography of the neck, chest, abdomen and pelvis should be performed to identify occult lesions. Biopsies should be obtained from suspicious lesions and sent for histologic confirmation. The use of immunohistopathologic stains for the presence of EBV (e.g. EBER stain) is necessary to distinguish EBV-infected cells from non-specific lymphocytic infiltrates. Patients with active EBV disease will typically have an elevated EBV viral load in the peripheral blood. However, elevated loads may be present in otherwise asymptomatic individuals.

Prevention of Epstein-Barr virus post-transplant lymphoproliferative disease

The high rates of morbidity and mortality attributed to PTLD have prompted efforts aimed at the prevention or pre-emptive treatment of EBV in intestinal transplant recipients. Serial monitoring of the EBV viral load using quantitative PCR assays has been shown to predict the occurrence of PTLD. This is currently used to guide initiation of pre-emptive therapy at many centers.[19] However, specific target levels of viral load as well as therapeutic pre-emptive treatment regimens vary from center to center and remain to be evaluated in prospective, comparative studies.

Treatment of Epstein-Barr virus post-transplant lymphoproliferative disease

Reduction of immune suppression is the major therapeutic manipulation for transplant recipients with EBV PTLD. Reduction of immunosuppression is felt to allow the body to develop a cytotoxic T lymphocyte response and control the infectious process. Unfortunately, the high rates of rejection observed during attempts at immunomodulation of EBV limit this approach in intestinal transplant recipients. While frequently used, ganciclovir or aciclovir as well as immunoglobulin are probably of only limited benefit for the treatment

of EBV PTLD (see Table 107.4). The use of the new, anti-CD20 monoclonal antibody (rituximab) appears very promising for these patients.

Other pathogens

Like other organ transplant recipients, recipients of intestinal transplants are at risk for infection from a wide variety of pathogens. Infection may develop from nosocomial exposures or once the patient has returned to the ambulatory setting. Infectious pathogens of the intestinal tract that can mimic intestinal rejection include adenovirus, rotavirus, *Clostridium difficile*, herpes simplex virus and nontuberculous mycobacteria.[8] An effort should be made to search for these pathogens by culture, histology and/or immunoassay before treating the patient for rejection. Adenovirus in particular has been recovered at very high rates from intestinal transplant recipients. Interpretation of this finding (asymptomatic shedding versus clinical disease) must be done on a case-by-case basis.

Respiratory viruses (parainfluenza, respiratory syncytial virus, influenza) can be fatal in intestinal transplant recipients.[9] While the relative frequency of acquisition is greater in the intermediate to late time periods after transplantation (after exposure to community-acquired pathogens), occurrence of disease in the early post-transplant period is most dangerous. Additional risks for severe disease with respiratory viruses include age less than 1 year, pre-existing lung disease and exposure to augmented immunosuppression.

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Chapter 108 - Vasculitis and Other Immunologically Mediated Diseases

Jonathan Cohen

INTRODUCTION

This chapter is concerned with a diverse group of conditions that have in common the fact that their cause is thought to be related to disordered immune processes and that their treatment involves immunosuppressive therapy. In the past these diseases have been called 'autoimmune', 'autoallergic', 'collagen-vascular' or more frequently 'vasculitis', although as a group I think they are better referred to simply as immunologically mediated diseases (IMDs; Table 108.1). In addition, there is a large group of common disorders (e.g. asthma, eczema or inflammatory bowel disease) in which immunosuppressive therapy may be used, sometimes at high dose. Although not discussed in detail here, patients who have these conditions are at risk from the same type of opportunistic infections as other immunosuppressed patients.

EPIDEMIOLOGY

The importance of IMDs to the infectious diseases practitioner lies in the fact that affected patients frequently have severe multisystem disease and require high-dose immunosuppressive therapy, so the risk of opportunistic infection is high. Furthermore, they differ from other types of immunosuppression in that patients often receive several different modalities of immunosuppression (Table 108.2) and the duration of their treatment is much longer than for patients who have leukemia or cancer, in whom the neutropenic period is nowadays often not much more than 4 weeks (and sometimes much less). Infection is a major cause of morbidity and mortality in patients who have IMD[1] but the incidence of infection in these patients varies considerably depending on the stage of the disease and the intensity of the immunosuppression. In a study of 75 heavily immunosuppressed patients who had a variety of IMDs, we found a rate of 0.74 infections/patient/week.[2] In contrast, a study of 200 outpatients who had systemic lupus erythematosus (SLE), followed for 2 years, found that infections only occurred in a third of cases. Most were single, minor and associated with disease activity.[3]

Whereas organ or bone marrow transplant recipients are a homogeneous population, IMDs are not generally complicated by particular infections, although there are some associations of note. Salmonellosis frequently occurs as a complication of SLE, for instance, although the mechanism is not at all clear.[4] It is difficult to be certain whether the increased incidence of infection in patients who have IMD is attributable to the abnormal immune function of the underlying disease or simply a consequence of the immunosuppressive therapy. Probably both are implicated. As an illustration, Williams and colleagues studied 61 patients who had SLE or the antiphospholipid syndrome and required admission to an intensive care unit (ICU). [5] Infection was the single most common cause of admission, and infection on admission was associated with a significantly increased ICU mortality. However, cyclophosphamide administration, low white cell count and high severity of illness scores were all associated with reduced survival.

PATHOGENESIS AND PATHOLOGY

In some cases, there may be an etiologic association between an infection and the disease itself. A good example of this is the role of the hepatitis viruses in the pathogenesis of cryoglobulinemia, polyarteritis nodosa and other types of systemic vasculitis.[6] [7] Recently, both Epstein-Barr virus and parvovirus B19 have been associated with flares of SLE.[8] [9] There have also been intriguing reports suggesting that Wegener's granulomatosis may be caused by an abnormal response to an unknown infection, and that relapses of Wegener's granulomatosis can be prevented by chronic administration of trimethoprim-sulfamethoxazole. [10]

Although certain types of treatment are associated with particular defects in immune function,[11] patients who have IMD commonly receive combinations of drugs, and this makes predictions very difficult. Certainly, high-dose corticosteroid therapy is complicated by infections such as *Listeria*, herpesviruses and fungi, whereas patients who develop neutropenia as a consequence of cyclophosphamide, for instance, are susceptible to the same kinds of infection as neutropenic bone marrow transplant recipients. Plasma exchange (plasmapheresis) is a form of immunosuppression used particularly in these patients, and this has its own complications, in particular associated with intravenous access.[12] [13] It is generally true that the differential diagnosis of infection in patients who have IMD is considerably wider than in other kinds of immunosuppressed patients (see Clinical features and management, below).

It is not just the longer list of possible infections that makes assessment more difficult in these patients. It is frequently very hard to be sure whether the patient has infection or simply a relapse of the underlying disease. An acute flare-up of SLE involving the central nervous system can be indistinguishable from infective meningitis or encephalitis as a consequence of the immunosuppressive therapy. This leaves the clinician on the horns of an unpleasant dilemma; should the immunosuppression be reduced in order to allow antimicrobial therapy to be more effective, or should it be increased to bring the underlying disease back under control? It is helpful to ask whether the clinical features at this presentation are the same as on previous occasions when the presentation was known to be due to disease activity. Individual patients tend to be consistent in the form of disease they get when it is active.

A further complication is the phenomenon of 'infection provoked relapse'; in patients who have IMD an intercurrent infection can precipitate a relapse of the underlying disease.[14] The infection and the vasculitis need to be treated simultaneously.

PREVENTION

Patients who have IMD, like all immunosuppressed patients, are constantly at risk of a very wide range of infections but it is neither practicable nor desirable to try and prevent all of them. (For a detailed discussion of the approaches to infection prevention in immunocompromised patients see Chapter 100 and Chapter 102 .) Tuberculosis is a particular problem because its presentation may be

TABLE 108-1 -- Immunologically mediated diseases.

IMMUNOLOGICALLY MEDIATED DISEASES
Systemic lupus erythematosus
Polyarteritis nodosa
Wegener's granulomatosis
Lymphomatoid granulomatosis
Bronchocentric granulomatosis
Antiglomerular basement membrane disease (Goodpasture's syndrome)
Mixed essential cryoglobulinemia
Rheumatoid arthritis
Still's disease

Felty's syndrome
Mixed connective tissue disease
Progressive systemic sclerosis/scleroderma
Polymyositis/dermatomyositis
Relapsing polychondritis
Behçet's syndrome
Sjögren's syndrome
Churg-Strauss syndrome
Henoch-Schönlein purpura
Hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura
A list of IMDs in which high-dose immunosuppression is often used and major opportunistic infection is a common problem. The list excludes generally less severe diseases such as asthma; although such patients may occasionally need high-dose immunosuppression, it is much less common.

TABLE 108-2 -- **Immunosuppressive agents and procedures.**

IMMUNOSUPPRESSIVE AGENTS AND PROCEDURES
Corticosteroids
Thiopurines
6-mercaptopurine
Azathioprine
Alkylating agents
Mycophenolate mofetil
Cyclophosphamide
Monoclonal antibodies
Basiliximab
Daclizumab
Rituximab
Infliximab
Etanercept
Adalimumab
Ciclosporin and related drugs
FK506 (tacrolimus)
Sirolimus
Total lymphoid irradiation
Antilymphocyte globulin
Intravenous immunoglobulin
Plasma exchange
Types of immunosuppression typically used in patients who have IMD. It is common for several of these agents to be used in combination

atypical and extrapulmonary disease is common. Patients who are receiving more than 15mg/day of prednisone (prednisolone) for prolonged periods and who have a clinical history or radiologic evidence of past tuberculosis should be given prophylaxis with isoniazid 300mg/day plus pyridoxine 10mg/day.[15] If the risk is less clear, the complications of isoniazid must be considered (see Chapter 202), although my practice is to err on the side of advising prophylaxis.[16]

In my opinion, the routine use of trimethoprim-sulfamethoxazole to prevent *Pneumocystis carinii* pneumonia is not warranted in this population, even if patients are receiving corticosteroids, because of the low incidence of the infection. In marked contrast to patients who have AIDS, relapse of *Pneumocystis* pneumonia is most uncommon in this population and secondary prophylaxis is not indicated.

CLINICAL FEATURES AND MANAGEMENT

Immunologically mediated diseases can represent a very complex challenge to the infectious diseases physician. Factors that must be taken into account in assessing the patient include the nature of the

TABLE 108-3 -- **Causes of fever and pulmonary infiltrates in patients who have immunologically mediated disease.**

CAUSES OF FEVER AND PULMONARY INFILTRATES IN PATIENTS WHO HAVE IMD

Infective	Bacteria	Conventional respiratory pathogens*
		Mycobacteria
		Nocardia[18]
		'Atypical' bacteria (*Mycoplasma* spp., *Coxiella* spp.)
		Legionella spp.
	Fungi	*Aspergillus* spp.[19] *
		Candida spp.
		Cryptococcus neoformans
		Zygomycetes
		Primary systemic fungi (*Histoplasma* spp., *Blastomyces* spp., etc.)
		Other systemic fungi (rarely; e.g., *Sporothrix schenckii*)
		Pneumocystis spp.*
	Parasites	*Strongyloides stercoralis*
		Toxoplasma spp.
	Viruses	Cytomegalovirus*
		Herpes simplex virus
		Varicella-zoster virus
		Respiratory syncytial virus
		Adenovirus
		Influenza and parainfluenza virus
Noninfective		Edema*
		Hemorrhage[20] *
		Infarction
		Emboli
		Tumor
		Radiation
		Chemotherapy
		Vasculitis
		Leukoagglutinin reaction
The list is long but incomplete and, in practice, any organism isolated in pure or predominant culture from a bronchoalveolar lavage or open lung biopsy should be regarded as a pathogen until proved otherwise. Further details about some of the conditions are provided in the references, where indicated		

* Most common causes.

underlying disease and the particular type of immunosuppression used, its duration and its dose (see Chapter 99). Certain clinical syndromes merit particular attention.

Fever and pulmonary infiltrates

The development of fever and new pulmonary infiltrates is one of the most common and most difficult clinical syndromes that occurs in patients who have IMD. The differential diagnosis is extraordinarily wide (Table 108.3) and includes infective and noninfective conditions.[17] Details of specific infections may be found elsewhere in this book; here I consider some general principles that apply to the initial assessment and management of patients who have IMD and who develop fever and pneumonia.

¦ There may be very rapid deterioration, from low-grade fever and cough to severe hypoxia needing mechanical ventilation within 12 hours, particularly if the patient is liable to develop pulmonary hemorrhage.
¦ Radiologic appearances are very non-specific. It is rare to be able to 'guess' the diagnosis just on the basis of the radiograph, with the possible exception of *Pneumocystis* pneumonia.
¦ Multiple infections are common. Even if the physician correctly recognizes the clinical and radiologic features of *Pneumocystis*

pneumonia, the patient may be co-infected with an additional, equally treatable pathogen such as cytomegalovirus (CMV).
¦ Sputum microbiology can be confusing. The presence of *Candida* spp., for instance, may indicate nothing more than colonization of the nasopharynx, whereas important pathogens such as *Aspergillus* spp. or *Pneumocystis* spp. often do not appear in the sputum.

Consideration of the nature of the underlying disease, the type of immunosuppression and epidemiologic features in the history are all helpful in guiding therapy. The single most important factor is gauging the speed of progression of the condition. It cannot be overemphasized that in IMD patients a seemingly trivial community-acquired chest infection can proceed to life-threatening pneumonia and/or pulmonary hemorrhage within a frighteningly short period. Urgent evaluation and investigation is essential. All patients should have simple, basic laboratory investigations performed, including a full blood count, sputum and blood cultures, and measurement of blood gas concentrations and a chest radiograph.

Much has been written about the radiologic features of certain infections. It is perfectly true, for instance, that *Pneumocystis* pneumonia classically produces a bilateral 'ground glass' appearance, but so too does CMV infection and acute pulmonary hemorrhage in a patient who has pulmonary vasculitis. Combining the clinical and radiologic data results in a 'shortlist' of likely diagnoses, but too great a reliance on this is very hazardous. The main value of the chest radiograph is in indicating the extent and rate of progression of the process, not in guessing the pathogen. Empiric antibacterial therapy is usually indicated when the risk of a major opportunistic pathogen is judged to be low. Factors pointing toward this conclusion are:

¦ slow onset/development;
¦ modest immunosuppression (e.g. <10mg prednisone daily);
¦ absence of hypoxia; and
¦ clinical, epidemiologic or microbiologic evidence of a conventional pathogen (e.g. *Streptococcus pneumoniae*).

Pneumocystis pneumonia is said to often have a very characteristic presentation, and some advocate empiric therapy (see Chapter 241). However, this strategy is unwise in patients who have IMD because of the wide differential diagnosis and the possibility of co-infection with other pathogens.

In the majority of patients further specific investigations should be performed. The most useful is a bronchoscopy with a bronchoalveolar lavage. Close liaison with the laboratory is essential to ensure a rapid response and the maximum diagnostic yield. Two other tests deserve mention. Computerized tomography (CT) scans will undoubtedly give additional and sometimes useful information but in my experience are rarely diagnostic. In contrast, pulmonary function tests can be very valuable in this group of patients, in particular measurement of carbon monoxide uptake (the KCO) to detect intrapulmonary hemorrhage.[21]

What if the patient fails to respond to the initial treatment regimen? Infection with more than one organism is not uncommon; for example, in two studies of *Pneumocystis* pneumonia in non-AIDS patients, additional pathogens were found in 35% and 58% of cases.[22] [23] A repeat diagnostic procedure should be considered; the choice usually lies between a second bronchoscopy or an open lung biopsy. There are no published studies that adequately address this question. Comparisons between diagnostic procedures have been made, usually in the setting of solid tumors or hematologic malignancy, but these are not readily extrapolated to second procedures in a different patient population. Although open lung biopsy is more invasive and has a significant morbidity, it should be carefully considered. In a patient who is not responding to first-line therapy, the second procedure will usually be the last chance to make a diagnosis.

When a specific organism is identified the treatment follows conventional guidelines. 'Blind' empiric therapy is rarely advisable because of the wide differential diagnosis.

Acute neurologic problems

A wide differential diagnosis also exists for neurologic problems, and prompt evaluation, investigation and treatment are essential.[24] Knowledge of the nature of the immune deficit can narrow down the list of possibilities. This information can be linked to the clinical presentation to provide useful clues; thus, a patient who has a defect in cellular immunity as a result of high-dose steroid therapy and who develops a subacute meningitis is likely to have *Listeria*, cryptococcal or tuberculous meningitis, whereas in a neutropenic patient *Aspergillus* or a pyogenic bacterial infection is more common.[25] These mental exercises are intellectually challenging but in reality the clinician is faced with a patient in whom even the shortlist of likely causes all demand quite different treatment. Clearly, it is most important to make the diagnosis as quickly as possible.

The initial assessment should include a detailed clinical and epidemiologic history and a careful neurologic examination. Key areas are:

- exposure to family members or others as a potential source;
- relevant foreign travel (not forgetting malaria);
- previous episodes of neurologic manifestations associated with relapse of underlying disease; and
- speed of progression of the disease.

Immediate investigations include a blood film and full blood count, blood and urine cultures and a chest radiograph. If there are new skin lesions they should be biopsied for immediate smear and culture. If a CT scan is available it is invaluable, and a lumbar puncture should be performed provided there are no contraindications.

Meningitis

Meningitis (Table 108.4) can be caused by common bacteria (*S. pneumoniae*, *Neisseria meningitidis*), but in immunosuppressed patients *Listeria monocytogenes* is particularly important. Despite the name, the cerebrospinal fluid (CSF) usually contains neutrophils, not mononuclear cells.

Polymicrobial meningitis (particularly with aerobic Gram-negative bacteria) can be a clue to the presence of hyperinfection with strongyloidiasis, a complication of both corticosteroid therapy and also of infection with human T cell leukemia/lymphoma virus. Enteroviruses are common causes of meningitis in normal hosts; rather curiously, they also occur in patients who have hypogamma-globulinemia. [26] Tuberculosis, cryptococcosis and, much more rarely, *Nocardia* all manifest with a subacute picture and are recognized causes of meningitis in patients who have IMD.

The serum cryptococcal antigen test cannot be used as a surrogate for CSF examination, unlike in AIDS patients in whom the burden of infection is often very high.

Meningitis is rarely caused by relapse of the underlying disease, but it can occasionally be caused by drugs used in its treatment, notably nonsteroidal anti-inflammatory drugs (NSAIDs).[27]

Abscesses

In IMD patients, abscesses are usually nonbacterial (see Table 108.4). The commonest causes of focal neurologic lesions are fungi (especially *Aspergillus* spp.) and *Toxoplasma*. *Nocardia* spp. infections classically cause multiple focal abnormalities but occur less often. Tuberculoma and cryptococcoma are more common in textbooks than in patients. The diagnosis of single or multiple space-occupying lesions in IMD patients is particularly difficult. Neither the radiologic features nor the CSF findings are pathognomonic; it is rare for the causative organism to be identified from the CSF and, with the

TABLE 108-4 -- Causes of neurologic syndromes in patients who have immunologically mediated disease.

CAUSES OF NEUROLOGIC SYNDROMES IN PATIENTS WHO HAVE IMD		
Meningitis	**Abscesses**	**Encephalitis**
Common pyogenic bacteria	Toxoplasmosis	Toxoplasmosis
Mycobacterium tuberculosis	*Aspergillus* spp.	*Listeria* spp.
Listeria monocytogenes	*Mucor* spp.	Measles
Nocardia spp.	Tuberculoma	Progressive multifocal leukoencephalopathy (JC/BK virus)
Cryptococcus spp.	*Nocardia* spp.	Varicella-zoster virus
Candida spp.	Cryptococcoma	Herpes simplex virus
Echoviruses	Pyogenic bacteria and anaerobic bacteria	Cerebral vasculitis
Drugs (e.g. NSAIDs)	Cytomegalovirus	Corticosteroid-induced disease
	Cerebral lymphoma	
High-dose corticosteroids can cause acute neuropsychiatric symptoms		

exception of the cryptococcal latex agglutination test, serologic tests are unhelpful. Particular care is needed in making a presumptive diagnosis of toxoplasmosis. Whereas in other groups of immunosuppressed patients the appearance of multiple enhancing lesions on the CT scan will often be sufficient grounds to commence empiric therapy, in patients who have IMD the differential diagnosis is much wider and if at all possible a tissue diagnosis should be obtained. Furthermore, *Toxoplasma* infection in SLE can mimic lupus cerebritis.[28]

Encephalitis

Encephalitis can be caused by infective and noninfective processes (see Table 108.4). Listeriosis and toxoplasmosis can manifest with an encephalitic picture, as can measles. Cerebral vasculitis (typically in SLE) is a particularly important consideration. It can cause a florid and life-threatening illness that can be extremely difficult to distinguish from an opportunistic infection. Evaluation is complicated by the fact that high-dose corticosteroid therapy can itself cause neuropsychiatric manifestations. It is helpful if the patient is known to have a past history of cerebral vasculitis but sometimes the only course of action is to treat with immunosuppression and antimicrobial agents until the picture becomes clearer.

Two infections merit comment because of their rarity: herpes simplex encephalitis seems to be uncommon, despite the fact that local cutaneous reactivation often occurs; and likewise CMV encephalitis is very unusual in this population.

Gastrointestinal problems

Although immunosuppressed patients are susceptible to a wide range of bacterial, fungal, viral and protozoal infections of the gut,[29] it is largely those that are a feature of high-dose corticosteroid therapy that occur in patients who have IMD. The clinical features of common infections are often modified; herpetic stomatitis can be very severe, for instance (Fig. 108.1). Three infections merit comment.

Extrapulmonary tuberculosis is more common in immunosuppressed patients and even if suspected (e.g. because a patient comes from the Indian subcontinent) can sometimes be hard to prove. The use of polymerase chain reaction to detect mycobacterial DNA in ascitic fluid is becoming available and is invaluable; meanwhile peritoneal biopsy is often the only diagnostic procedure of use. Not infrequently the only option is empiric therapy.

Cytomegalovirus enteritis is perhaps underdiagnosed. It can affect any part of the gut but particularly the colon. Ganciclovir has been very effective. [30]

Strongyloides stercoralis can be present for many years without causing symptoms but after corticosteroid therapy it can cause subacute

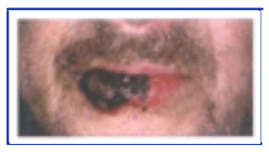

Figure 108-1 A large necrotizing lesion caused by herpes simplex type-1 in a patient who has a teratoma. Herpetic stomatitis is common in immunosuppressed patients and is often atypical; any ulcerating lesion in the perioral region should be considered to be herpetic until proved otherwise.

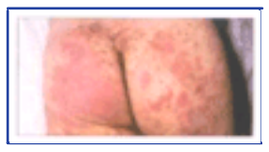

Figure 108-2 Extensive dermatophyte infection in a bone marrow transplant recipient. Many other infections (and graft-versus-host disease) can give a similar appearance but the diagnosis is quickly established by biopsy and microscopy. This condition is limited to the skin but nevertheless requires systemic antifungal therapy.

obstruction, pulmonary infiltrates and polymicrobial bacteremia or meningitis. Ivermectin is the drug of choice but it may need to be given for a longer period than in nonimmunosuppressed patients. Vasculitis (especially in SLE) can cause symptoms and signs indistinguishable from acute infection, including diarrhea, obstruction and perforation. Again, there are no diagnostic tests and management must depend on clinical evaluation and, if necessary, a therapeutic trial.

Skin, soft tissue and joints

Many organisms cause skin disease in immunosuppressed patients and the clinical manifestations are protean (Fig 108.2 and Fig 108.3). [31] In patients receiving plasma exchange, infections of the vascular access sites can be troublesome. Many noninfective causes of skin rash need to be remembered, including cutaneous vasculitis and drug eruptions. Soft tissue infections are unusual except that patients

Figure 108-3 Extensive skin lesions caused by *Mycobacterium chelonae* in a patient who had polyarteritis nodosa. The lesions were palpable but not especially painful.

who have hypogammaglobulinemia are susceptible to enterovirus polymyositis,[32] although myositis is much more likely to be caused by the underlying disease (dermatomyositis, polymyositis or polyarteritis nodosa).

Acute arthritis is always an indication for aspiration to exclude infection; *Staphylococcus aureus* is the most common isolate. Once again, relapse of the underlying disease is an important part of the differential diagnosis.

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Chapter 109 - Splenectomy and Splenic Dysfunction

Steven M Opal

INTRODUCTION

The spleen in postnatal life functions primarily as a specialized lymphatic organ. It clears particulate elements from the circulation and promotes a co-ordinated immune response to systemic antigens. The rapidly progressive and highly lethal syndrome of overwhelming postsplenectomy infection (OPSI) attests the critical importance of the spleen to host defense against disseminated infections in the systemic circulation.

EPIDEMIOLOGY

The incidence of fatal postsplenectomy sepsis has been estimated at approximately 1 per 300 patient-years in children and 1 per 800 patient-years in adults.[1] Serious infectious complications may also occur in patients who have splenic hypofunction found in a broad array of systemic disorders (Table 109.1). [2] [3] [4] [5] [6] [7]

Functional asplenia is suggested by the presence of Howell-Jolly bodies (nuclear remnants within red blood cells) and target cells in the peripheral blood smear, and decreased uptake of radioactivity by spleen scan. Splenic hypofunction is more frequent than is commonly appreciated. A recent laboratory based survey [8] analyzed over 100,000 blood smears per year and revealed that 0.5% of patient samples had Howell-Jolly bodies indicative of hyposplenism. This information was unknown to the majority of these patients and their physicians. The most common cause of functional asplenia is sickle-cell disease, which leads to repeated infarction of splenic tissue over the first few years of life. Infants born with congenital asplenia are at particularly high risk of death from systemic infection within the first year of life.[7] [9]

PATHOGENESIS AND PATHOLOGY

Structure-function relationships in the spleen

The spleen is organized to provide an optimal environment for particulate antigen clearance and immunologic surveillance within the systemic circulation. Although the spleen is a small structure that accounts for only 0.25% of body weight, it receives 5% of cardiac output and contains up to 25% of the total lymphocyte population within the body.[10] Blood enters the spleen through central arteries, which branch into penicillary arterioles (Fig. 109.1). These vessels are cuffed with T cells, forming a periarterial lymphocytic sheath. The white pulp of the spleen surrounds arterioles and consists of large populations of T cells with lesser numbers of B cells and natural killer (NK) cells. The marginal zone surrounds the white pulp and principally consists of large concentrations of B cells with lesser numbers of T cells and antigen-presenting cells. Memory cells of the B cell lineage are found primarily within the marginal zones of the spleen. The white pulp and marginal zone bring antigen-presenting cells, particulate antigens, T cells and B cells in close proximity. This microenvironment promotes a co-ordinated immune response to systemic antigens.[1] [2] [11]

The majority of the spleen consists of red pulp and venous sinuses. Before formed elements within the blood can reach the venous sinuses of the spleen, they must negotiate the red pulp with its tightly compact network of endothelial cells and macrophages (the cords of Billroth). This slow filtration process allows for careful immunologic surveillance and removal of damaged cellular elements and foreign particulate matter. The normal architecture of the spleen is depicted in Figure 109.1 .

Immunologic defects and factors predisposing for postsplenectomy sepsis

A number of immunologic defects have been described in the postsplenectomy state (Table 109.2).[1] [11] [12] [13] [14] [15] The work of Hosea and colleagues[16] has demonstrated that the principal immunologic defect associated with the postsplenectomy state is an impairment in clearance of poorly opsonized particulate antigens. Invasive, encapsulated bacterial pathogens possess an outer surface polysaccharide capsule that impedes opsonization by immunoglobulin or complement upon entry to the systemic circulation. The spleen is much more efficient than the liver in removing poorly opsonized bacterial pathogens. Following splenectomy, decreased clearance of these encapsulated organisms results in disseminated intravascular infection and the OPSI syndrome.

Predisposing factors to postsplenectomy infection

Multiple host factors determine the cumulative risk of OPSI. A number of the most important host determinants of infection following surgical or functional asplenia are listed in Table 109.3 . The principal determinant of risk of postsplenectomy sepsis is the age and immunologic experience of the patient before splenectomy.[7] [16] [17] In a recent review of over 12,000 patients who had undergone splenectomy, the incidence of serious postsplenectomy infections was found to be 15.7% in infants, 10.7% in children under the age of 5 years, 4.0% in children under 16 years and 0.9% in adults.[18]

The spleen is particularly important in the primary immunologic response to polysaccharide antigens. These IgM and IgG2 antibody responses are largely T-cell-independent B cell responses that require the spleen for an optimal immune response.[19] This occurs in the marginal zone of the spleen, where B cells are abundant for immunologic surveillance in the systemic circulation. This immune response is attenuated after splenectomy. T-cell-dependent B cell responses to protein antigens and cellular immune responses are reasonably well preserved after splenectomy.[20] [21] [22] The end result is that carbohydrate neoantigens are not recognized and processed efficiently in the postsplenectomy state. This results in delayed clearance of virulent microbial pathogens and their toxins[14] [15] as they first enter the systemic circulation.

If the growth rate of the pathogen exceeds the clearance rate of the host, overwhelming intravascular infection occurs with potentially lethal consequences. In experimental studies, macrophage synthesis of tumor necrosis factor (TNF)-a is upregulated after

TABLE 109-1 -- Conditions associated with functional asplenia.

CONDITIONS ASSOCIATED WITH FUNCTIONAL ASPLENIA	
Atrophic spleen	**Normal-sized or enlarged spleen**
Ulcerative colitis	Hemoglobinopathies other than sickle-cell
Celiac disease	Sarcoidosis
Graft-versus-host disease following bone marrow transplantation	Amyloidosis
Splenic irradiation	Systemic lupus erythematosus, rheumatoid arthritis
Thyrotoxicosis	Epstein-Barr virus infection
Dermatitis herpetiformis	Vasculitides with antineutrophil cytoplasmic antibodies
Idiopathic thrombocythemia	Liver disease — portal hypertension

Sickle-cell disease	AIDS, immunosuppressive agents

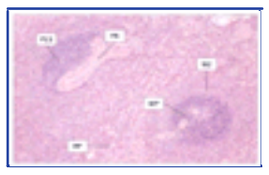

Figure 109-1 Normal splenic architecture in the adult human. PLS, periarterial lymphatic sheath; PA, penicillary arteriole; MZ, marginal zone (B lymphocytes predominate); WP, white pulp (T cells predominate); RP, red pulp (vascular cords and venous sinuses). Hematoxylin and eosin stain.

splenectomy. Enhanced TNF synthesis may increase the risk of systemic activation of the proinflammatory cytokines, and this may contribute to postsplenectomy sepsis. [23] The limited evidence available thus far indicates that patients who have HIV infection tolerate splenectomy reasonably well;[24] nonetheless, severe pneumococcal infections have occurred in splenectomized HIV-positive patients.

PREVENTION OF BABESIOSIS

Individuals living in areas where *Babesia* sp. is endemic should be advised to avoid areas where ticks are common or, if unavoidable, to check daily for the presence of ticks. If the tick is removed within 24 hours it appears to prevent transmission of *Babesia* spp. Babesiosis can be morbidly persistent in splenectomized adults as well as children, and in some cases has required exchange transfusion as well as treatment with clindamycin and quinine. Chills, fever, anemia, leukopenia and thrombocytopenia can all accompany babesiosis. The diagnosis is usually evident if suspected and sought for on the blood smear. Babesiosis can be confused with *Plasmodium falciparum* malaria.

TABLE 109-2 -- Immunologic defects found after splenectomy.

IMMUNOLOGIC DEFECTS FOUND AFTER SPLENECTOMY	
Defect	**Comment**
Decreased clearance of particulate antigens	Hepatic Kupffer cells will partially correct this defect
Diminished clearance of poorly opsonized bacterial antigens	The spleen is the most efficient organ for this purpose
Diminished primary humoral immune response to neoantigens	IgM levels and T-cell-independent antibody responses decrease
Diminished antibody response to polysaccharide antigens	Increased risk from encapsulated bacterial organisms
Decreased tuftsin levels and fibronectin levels	Diminished levels of this tetra peptide and serum protein reduce non-specific attachment and phagocytosis
Quantitative and qualitative defects in the alternative complement pathway	Functional defects in the alternative complement pathway interfere with opsonization

TABLE 109-3 -- Risk factors for postsplenectomy sepsis.

RISK FACTORS FOR POSTSPLENECTOMY SEPSIS	
Risk factors	**Comment**
Patient age	Immunologic experience (vaccines and naturally acquired infections) prior to splenectomy decreases subsequent risk
Absence of spleen at birth	Congenital asplenia results in serious bacterial infections in over 50% of patients in the first year of life
Time interval following splenectomy	Greatest risk of infection is within the first few years following splenectomy
Traumatic versus other indications for splenectomy	Splenosis (splenic implants within the peritoneum) following trauma offers some protection against infection
Immunocompromised states	Patients who have hematologic malignancies and continuing need for immunosuppressive medications have increased risk of postsplenectomy sepsis
Presplenectomy vaccine	Immune responses to polysaccharide antigens are better if administered before splenectomy

PREVENTION OF OVERWHELMING POSTSPLENECTOMY INFECTION

Splenic salvage method

The well recognized risk of OPSI indicates a need to prevent these infections if at all possible. The most direct approach is to minimize the frequency with which splenectomy is performed.[25] Recent evidence suggests that this has leveled off or decreased in North America,[8] [26] perhaps with the recognition of the infectious risks associated with splenectomy. Elective splenectomy for congenital hemolytic disorders should be delayed until after the first 5 years of life if possible (Table 109.4). Surgical repair of splenic hematomas, conservative management of splenic trauma without splenectomy, percutaneous drainage of splenic abscesses and a decreased use of splenic irradiation have led to fewer patients at risk of postsplenectomy infectious syndromes.[27] [28]

TABLE 109-4 -- Preventive measures in postsplenectomy patients.

PREVENTIVE MEASURES IN POSTSPLENECTOMY PATIENTS	
Method	**Comment**
Salvage splenic tissue (splenorrhaphy, autotransplants)	Reasonable, but unproven benefit
Delay elective splenectomy past childhood	Greatest risk of OPSI is in childhood; provide immunizations prior to splenectomy
Immunizations	Pneumococcal, meningococcal and *Haemophilus influenzae* vaccines provide partial protection
Antimicrobial prophylaxis	Indicated in childhood; efficacy in adults uncertain
Early empiric therapy	Unproven but rational approach to rapidly progressive syndrome
Medical alert bracelet	Reminder for patient and health care workers

Partial splenectomy and surgical repair of splenic injury have been used along with autotransplantation of splenic tissue in an attempt to limit the risk of OPSI.[29] [30] Enthusiasm for these splenic salvage maneuvers is tempered by the finding that these methods do not uniformly protect against sepsis in animal models[31] of splenectomy, and case reports exist of overwhelming infections despite these maneuvers. [29] [30] [31] Preservation of residual splenic tissue, if possible, may be preferable to total splenectomy in selected patients. One comparative study by Green and colleagues [32] found two episodes of sepsis in 18 children with splenectomy and no episodes in 16 children who had undergone partial splenectomy. The overall clinical applicability and practical value of splenic salvage techniques remain to be demonstrated in a large patient series.

Immunizations to prevent postsplenectomy sepsis

Immunization with the pneumococcal polysaccharide (PPS) vaccine is safe and offers significant protection in children who have sickle-cell disease. [33] The efficacy of the pneumococcal vaccine is also suggested in other patient groups after splenectomy.[22] [34] [35] To optimize antibody response against T-cell-independent immunogens, it is recommended that the pneumococcal vaccine be administered at least 2 weeks before an elective splenectomy. [16] Nonetheless, adequate antibody responses have been measured after splenectomy in non-immuno-compromised patients.[20] The current recommendations of the Adult Immunization Practices Task Force recommend repeat pneumococcal immunization every 5–6 years from the initial immunization. [36]

The role of newly introduced polysaccharide-protein conjugate pneumococcal vaccine (PCV) in asplenic patients has yet to be fully evaluated in large clinical trials but preliminary results appear promising.[19] [37] The conjugate protein linker allows for the development of T-cell-dependent B cell responses with T helper function amplifying the antibody response. Recent experimental studies[19] have verified that the conjugate vaccine produces peak antibody titers comparable to those in nonsplenectomized animals, albeit at a slower rate of increase. The secondary antibody response to subsequent doses of vaccine are well preserved with the PCV formulation. Attempts to mix unconjugated pneumococcal antigens with the protein conjugated antigens were unsuccessful in boosting antibody titers to nonconjugated pneumococcal antigens. The current PCV has only seven serotypes while the standard PPS vaccine contains 23 serotypes. Further studies are warranted with pneumococcal conjugate vaccines, which will be more efficacious than PPS vaccine.[38]

Haemophilus influenzae type b (Hib) conjugate vaccine is indicated in all children, including those who have functional or surgical asplenia. Immune responses to the conjugate vaccine are well preserved in the absence of splenic function.[38] [39] [40] It is unclear whether the Hib vaccine is useful in adults who have not been vaccinated before splenectomy. The risk-benefit ratio would argue that it is reasonable to immunize children and adults who undergo splenectomy if they have not been previously immunized.[40] The same rationale applies to meningococcal vaccine in children and young adults who undergo splenectomy.[17] [20] [41] The duration of protection and vaccine efficacy against *Haemophilus*, pneumococci and meningococci in the asplenic patient is speculative at the present time. Vaccination is limited by variable antibody responses of uncertain duration and incomplete coverage of important serogroups within *Streptococcus pneumoniae* and *Neisseria meningitidis* (i.e. serogroup B). Vaccine failures are well known in splenectomized patients and therefore additional preventive measures are needed.[19]

The efficacy of bacterial vaccines in the prevention of OPSI has not been adequately tested in large scale, randomized, controlled, clinical trials. The recommendations for these immunizations are largely based upon consensus opinion from expert committees and medical societies. Despite these recommendations, it is clear that the provision of adequate vaccine coverage for the asplenic host is woefully inadequate.[42] A recent survey in the UK found that only 31% of splenectomized patients had received the pneumococcal vaccine. [43] A more concerted effort will be necessary to provide adequate immunizations to these susceptible patients.

Antimicrobial prophylaxis

The efficacy of long-term penicillin prophylaxis to prevent pneumococcal infections has been studied in detail in the pediatric age group. Sufficient clinical evidence now exists to support the recommendation of penicillin prophylaxis in the first 5 years of life in asplenic children.[44] [45] Some authors suggest that prophylaxis be continued indefinitely in immunocompromised patients.[3] [16] [22] Amoxicillin may be preferable to penicillin in that it is better tolerated and has activity against most strains of *H. influenzae*.[8] [42]

The value of penicillin prophylaxis in adults is more controversial. The uncertain benefits of penicillin prophylaxis in the adult must be weighed against the potential risk of acquisition of penicillin-resistant strains of *S. pneumoniae* and infections by other organisms not susceptible to penicillin. [1] [11] [16] [20] I recommend penicillin prophylaxis for 2 years following splenectomy in adult patients at high risk for OPSI (hematologic malignancies, severe liver disease, immunocompromised states). Expectant management with early empiric therapy for symptoms suggestive of OPSI is recommended for non-immunocompromised asplenic adults.

An alternative strategy to continuous prophylaxis is to educate the patient to administer an initial dose of oral amoxicillin at the onset of symptoms compatible with systemic infection. Ideally, patients should have blood cultures taken before empiric antimicrobial therapy; however, this may not be feasible in all patients and treatment should not be delayed if symptoms compatible with OPSI exist.

A medical alert bracelet should be provided to patients following splenectomy. This bracelet serves to remind the patient as well as health care workers that the patient is at risk for OPSI and that urgent management for this potentially devastating syndrome may be life-saving. Patients have developed severe infections up to six decades after splenectomy.[11] [26] The alert bracelet should provide continued awareness of the risk of infection long after surgical removal of the spleen. A summary of preventive measures in the patient who has functional or surgical asplenia is provided in Table 109.4 .

TABLE 109-5 -- Microbial pathogens associated with postsplenectomy infections.

MICROBIAL PATHOGENS ASSOCIATED WITH POSTSPLENECTOMY INFECTIONS	
Micro-organism	**Comment**
Streptococcus pneumoniae	Most common, highly lethal, characteristic presentation
Haemophilus influenzae	Increased risk — especially in childhood
Neisseria meningitidis	Possible increased risk — typical features of primary meningococcemia
Salmonella spp.	Increased risk — especially sickle cell disease
Other streptococci, enterococci	Possible increased risk of infection
Capnocytophaga canimorsus	Gram-negative rod may cause infection following dog bites
Babesia microti	Tick-associated or blood transfusion-associated protozoan parasite; may cause severe infection in asplenic patients
Plasmodium spp.	Sporadic reports of activation of latent malaria and fulminant course with non-*falciparum* malaria species
Anaerobes, Gram-negative enteric organisms, *Pseudomonas*, *Burkholderia*, *Plesiomonas*, *Campylobacter* spp., fungal, parasitic and viral pathogens	Case reports; true association is unclear

CLINICAL FEATURES OF OVERWHELMING POSTSPLENECTOMY INFECTION

A large variety of bacterial, fungal, parasitic and viral pathogens have been reported to cause serious infection in patients who have splenic hypofunction or asplenia.[1] [3] [11] [17] [46] [47] *Streptococcus pneumoniae* continues to account for the majority of bacterial infections associated with OPSI. *Haemophilus influenzae* and *N. meningitidis* contribute approximately 25% of bacterial infections in the post-splenectomy state. The microbiology of systemic infection following splenectomy is provided in Table 109.5 .

The syndrome of overwhelming pneumococcal sepsis after splenectomy is one of the most dramatic and rapidly fatal infections in clinical medicine. Symptoms often begin with a vague sense of general malaise, sore throat, myalgia and gastrointestinal symptoms. Fever and true shaking chills are often seen during the prodromal phase of OPSI. While some patients note lower respiratory tract symptoms or symptoms of meningitis, the primary source of origin of the bacteremic infection is not localized in the majority of patients who have OPSI.

Within 24–48 hours of onset of symptoms patients rapidly deteriorate, with progressive hypotension, diffuse intravascular coagulation, purpuric lesions in the extremities, acute respiratory insufficiency, metabolic acidosis and coma. The rapidly progressive nature of the illness is suggestive of primary meningococcemia. The patient may develop refractory hypotension and die within hours of the onset of symptoms.[1] [14] [16] Long-term sequelae in survivors include gangrene of the extremities, bilateral adrenal hemorrhage, osteomyelitis from vascular insufficiency, endocarditis, meningitis and neurosensory hearing loss.[11] The polysaccharide capsular serotypes 12, 22 and 23 account for the majority of cases of OPSI from *S. pneumoniae*.[16] All three serotypes are found in the current 23 valent pneumococcal

polysaccharide vaccine.[36]

DIAGNOSIS OF OVERWHELMING POSTSPLENECTOMY INFECTION

The diagnosis of OPSI is often readily apparent upon clinical examination. It is important to remember that even a remote history of previous splenectomy should raise suspicion of possible OPSI. Supporting laboratory evidence includes findings compatible with a consumptive coagulopathy, lactic acidosis, hypoxemia and acute renal failure. Children are more likely to have concomitant bacterial pneumonia or meningitis than adults.[1] [11] As a consequence of high-grade bacteremia, micro-organisms can often be identified in the peripheral blood smear (Fig. 109.2). The blood smear should be reviewed for evidence of parasitemia from *Plasmodium* or *Babesia* spp. A Gram stain of the buffy coat may readily reveal organisms as the level of bacteremia may exceed 1 million cfu/ml.

Patients who have concomitant bacterial meningitis may have large numbers of organisms in the cerebrospinal fluid with minimal pleocytosis (Fig. 109.3). Blood cultures, bacterial cultures and results of antigen detection measures in the spinal fluid, sputum and urine will confirm the diagnosis.

MANAGEMENT OF OVERWHELMING POSTSPLENECTOMY INFECTION

This syndrome is a true medical emergency requiring immediate therapeutic administration of antimicrobial agents and intensive care support. High-dose intravenous penicillin has been the standard treatment for post-splenectomy pneumococcal sepsis. Vancomycin and ceftriaxone should be used empirically in areas where penicillin-resistant *S. pneumoniae* is prevalent, or in patients who have received prolonged penicillin prophylaxis. Moreover, broad-spectrum bactericidal antimicrobial agents such as an extended-spectrum cephalosporin or a carbapenem should be used along with an aminoglycoside in those cases in which the suspected micro-organism cannot be identified on Gram strain of the buffy coat or cerebrospinal fluid.

Passive immunotherapy with intravenous immunoglobulin has been shown to be of benefit in experimental models of postsplenectomy sepsis as has granulocyte-macrophage colony-stimulating factor (GM-CSF).[8] [9] The therapeutic efficacy of passive immunotherapy or GM-CSF in human OPSI is worthy of further clinical

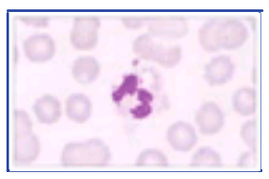

Figure 109-2 Peripheral blood smear of a patient who has pneumococcal sepsis and meningitis. Note polymorphonuclear leukocyte with several bacterial diplococci in the cytoplasm. Wright stain.

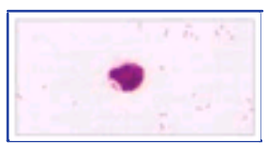

Figure 109-3 Gram stain of cerebrospinal fluid of a patient who has pneumococcal meningitis. Note numerous Gram-positive cocci in pairs and a single lymphocyte.

investigation. Fluid resuscitation, vasopressor agents, hematologic support, ventilatory support and expert acid-base and electrolyte management are essential to survival in these critically ill patients (see Chapter 56).

The mortality rate for OPSI caused by *S. pneumoniae* has been reported to be between 50% and 70%.[1] [2] [11] [12] [13] [14] [15] [16] With early recognition and treatment combined with skilled supportive care the mortality rate has decreased to as low as 10% in recent series.[26] [48]

During the post-OPSI convalescent period, it may be necessary to surgically debride necrotic tissues irreparably damaged by the prolonged hypotension and intravascular coagulation that often accompany this syndrome. Late complications such as adrenal insufficiency, osteomyelitis and endocarditis should be sought in patients who have persistent fever after an episode of OPSI. Patient education, vaccination and a medical alert bracelet should be offered to survivors to prevent recurrences.

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Chapter 110 - Practice Point

Infections in the renal transplant patient

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Renal transplantation has been extraordinarily successful in taking the transplant revolution forward, offering a significant improvement in both quality and quantity of life to recipients. During the evolution of the field there have been extraordinary advances in our understanding of basic and clinical immunology, as well as profound changes in the approaches to immune suppression. However, despite these changes, some aspects have perforce remained the same: the recipient has often been receiving either hemodialysis or peritoneal dialysis before transplantation; the renal allograft is surgically placed through an open abdominal incision; and the therapy required for maintenance of the graft is inherently immunosuppressive and occasionally nephrotoxic. Despite these factors, survival following renal transplantation is over 95% at 1 year and around 90% at 3–5 years. These rates continue to improve, with the improvement for the older recipient showing the greatest change (Briggs, 2001).

The causes of mortality in the renal transplant recipient have changed dramatically over the past several decades. Whereas during the period 1969–82, 40% of deaths were due to infection, in the period 1983–96 only 12% were, with 61% of deaths due to cardiovascular causes (Briggs, 2001). The rate of ischemic heart disease is five times higher in renal transplant recipients than in the general population, no doubt reflecting the myriad cardiovascular influences of underlying renal dysfunction as well as the consequences of transplantation and the drugs and immunologic changes it entails. As overall survival has improved, malignancy has become more common, and it remains a significant risk among long-term survivors. Up to 50% of renal transplant recipients may develop skin cancer of some sort by 20 years post-transplant in high-incidence regions such as Australia; up to 20% of recipients may develop solid organ cancer by 20 years post-transplant (Briggs, 2001). Therefore, management of the transplant patient is increasingly about good medical management, and anticipating other sequelae of long-term immunosuppression.

Renal transplantation has provided the critical workshop for the elucidation of the general principles of solid organ transplantation (see Chapter 102). Because renal transplantation has been practiced for decades and the numbers of recipients is so great in both the developed and the developing world, there is a large repository of data and experience that we can use to inform our understanding of solid organ transplantation in general as well as renal transplantation in particular (Table 110.1).

The factors that make solid organ transplant patients particularly complex and distinguish them from others are the underlying disease and its treatment (e.g. prolonged hemodialysis or peritoneal dialysis with their attendant complications); the surgery to place the allograft; and the dose, intensity and duration of the immunosuppression. This concatenation of events in each patient helps to frame the infectious issues as they arise and leads to the division of care of post-transplant patients into phases: the first month post-transplant, 1–6 months post-transplant and more than 6 months post-transplant. Of course the context in which this occurs is the pre-transplant condition of the patient, adding further disease- and patient-specific components.

The important infectious complications in the first month after renal transplantation are predominantly bacterial and involve the surgical site and the urinary tract. Surgical site infections are similar to those encountered in clean cases in general. Rates of infection in uncomplicated cases are less than 1% and can be further reduced with the use of perioperative antibiotic prophylaxis (e.g. cefazolin). Intraoperative and perioperative complications that necessitate reexploration (e.g. hematoma, anastomotic leak, lymphocele) increase the infection risk several-fold. Bladder catheterization represents a significant risk for bacterial urinary tract infection (UTI). In the absence of prophylaxis with either trimethoprim-sulfamethoxazole (TMP-SMX, co-trimoxazole) or a fluoroquinolone, rates of UTI in the 4 months following transplantation are about 35%, with half of affected patients developing pyelonephritis and 10% developing bacteremia. With prophylaxis, the rate of UTI in uncomplicated transplants is less than 5%. As in the general population, bladder dysfunction (e.g. because of neuropathy) or anatomic anomalies that lead to impaired emptying are important risk factors for UTI. Infections related to vascular access devices or prolonged intubation are also critical risks for bacterial infection in the immediate transplant period.

Viral infections predominate from 1 to 6 months post-transplant. These are related to pre-existing viral infections in the recipient that reactivate in the setting of profound immunosuppression, as well as viral infections transmitted from the donor for which the recipient has no prior or pharmacologically impaired immunity. The herpesviruses, particularly cytomegalovirus (CMV) and Epstein-Barr virus (EBV), receive the major infectious disease focus in this period, because of their involvement in a broad array of infectious disease and malignant presentations. In addition, and distinct from many other viral infections, successful therapeutic and prophylactic therapies and maneuvers exist for several of the herpesviruses. While the roles of these viruses in several clinical syndromes (e.g. fever, pneumonia, hepatitis) are clear, their hypothesized role in precipitating

TABLE 110-1 -- Special considerations in renal transplantation.

SPECIAL CONSIDERATIONS IN RENAL TRANSPLANTATION
Sirolimus may cause a diffuse pneumonitis
Ureteral stenting may predispose to infection
BK virus may mimic graft rejection
Synergistic toxicity of ciclosporin/tacrolimus and macrolides and azoles
Induction of metabolism of ciclosporin and tacrolimus by rifampin, isoniazid and nafcillin, among others

acute or chronic rejection or atherosclerosis is less well proven (Cainelli and Vento, 2002). The diagnosis and treatment of CMV is described in Chapter 112 .

General strategies include prophylaxis, pre-emptive therapy and secondary (post-treatment) prophylaxis. Since transplantation of an organ from a seropositive donor into a seronegative recipient carries a risk of clinical disease of >50%, prophylaxis with either valganciclovir or valaciclovir is indicated. Similarly, prophylaxis of primary EBV infection, varicella-zoster virus (VZV) infection or herpes simplex infection may be appropriate.

Pre-emptive therapy is triggered by a laboratory marker that indicates that clinical disease is likely. For example, CMV viremia suggests that clinical disease is imminent, and the higher the viral load, the greater the probability of symptomatic disease. This requires an infrastructure that can reliably deliver specimens appropriately and in a timely fashion. With many transplant centers serving patients over a wide geographic area, logistics are increasingly important. Although specimens for polymerase chain reaction can be sent by overnight mail, the antigenemia assay is rendered significantly less accurate with such handling. Virologic monitoring is not inexpensive, and when the cost of monitoring is included with the cost of the occasional patient who develops symptomatic CMV disease despite the monitoring effort, the cost saving can be minimal at best (Kusne *et al.*, 1999).

Another form of pre-emptive therapy uses identification of a predictive clinical or epidemiologic characteristic. For example, transplant recipients who are CMV seropositive have a 15–20% risk of clinical disease on standard three-drug immunosuppression; use of induction antilymphocyte antibody in addition leads to a doubling of the risk. If antilymphocyte antibody therapy is used to treat rejection, then 50–65% of CMV seropositive patients will become symptomatic. Ganciclovir (5mg/kg/day intravenously with dosage adjustment for renal function), administered for the duration of the antilymphocyte antibody course, reduces the incidence of clinical disease to about 20%; if 3 months of oral therapy (ganciclovir 2g/day or valganciclovir 900mg/day) is added, the incidence of clinical disease falls to zero.

Secondary prophylaxis is designed to prevent relapsing disease, which may occur during intense immunosuppression, in patients with a high viral load, after premature cessation of treatment doses of ganciclovir, with continuing viremia at ganciclovir cessation, and in the setting of an antigen mismatch between donor and recipient.

Oral valganciclovir prophylaxis for 2–3 months after treatment for clinical disease has been completed with clearance of the viremia may be helpful. Without antiviral agents the incidence of symptomatic herpes simplex disease post-transplant is 30–50%. Therefore, in patients who are not receiving antiviral therapy with ganciclovir, an aciclovir preparation or foscarnet, low-dose oral aciclovir prophylaxis (e.g. 300mg three times per day) is usually given to prevent reactivation disease due to herpes simplex. The primary acquisition of herpes simplex infection is uncommon in the transplant setting.

Development of post-transplant lymphoproliferative disease is correlated with EBV viral load. Cytomegalovirus disease has been shown to increase the incidence of post-transplant lymphoproliferative disease by seven to 10 times, but it is not yet proven whether prevention of CMV will reduce or modify EBV-related events.

Approximately 10% of transplant patients develop dermatomal zoster due to reactivation of VZV, but this rarely progresses to visceral disease. Primary VZV infection, in contrast, is a devastating illness, with dissemination and multiorgan involvement. Because of the high morbidity and mortality associated with primary VZV infection, we advocate pre-transplant VZV serology, with seronegative patients receiving the VZV vaccine and documentation of seroconversion. Seronegative transplant patients who have been exposed to this virus should receive zoster immune globulin immediately. This is about 80% effective in preventing clinical disease but it may not prevent visceral disease. Therefore, after zoster immune globulin, the clinician should remain alert for fever, abdominal pain or organ dysfunction, and initiate high dose aciclovir even without skin lesions. Human herpesvirus-6 can cause pneumonia, encephalitis and fever, and it may synergize with CMV in causing more severe disease. Human herpesvirus-8 has been linked to the pathogenesis of Kaposi's sarcoma.

Infections with BK virus pose a special problem in renal transplantation. Seroprevalence is 100% by age 9–10 years, but it wanes over time. Transplantation of grafts from seropositive donors into either seronegative or seropositive receipients leads to acquisition or reactivation of BK virus. BK virus can cause interstitial nephritis, which is difficult to distinguish from rejection on clinical and laboratory grounds alone; renal biopsy is necessary. Ureteral stenosis can also result from BK virus, also impeding graft function. Treatment for this virus is still quite elusive. Cidofovir has been reported to clear BK viruria in anecdotal series, but its use in renal transplant patients is limited by its own nephrotoxicity (Lin *et al.*, 2001).

There is still no universally accepted approach to vaccination and prophylaxis in the post-transplant patient. Although most centers use TMP-SMX as prophylaxis against *Pneumocystis* and UTI, the issues with regard to other potential pathogens are less clear (Batiuk *et al.*, 2002). There are also important regional differences to consider; for instance, the south-western USA has coccidioidomycosis. The role and value of post-transplant vaccination is also somewhat controversial, with most centers using influenza vaccination, but there is lingering concern about efficacy and the possible triggering of rejection, which limits the use of other vaccines (Batiuk *et al.*, 2002).

Our current approach to the renal transplant recipient is to use perioperative antibiotics; to maintain TMP-SMX prophylaxis against UTI, *Pneumocystis, Listeria, Nocardia*, and possibly toxoplasmosis; to use prophylaxis against CMV in patients at high risk (donor seropositive, recipient seronegative); and to treat all *Aspergillus* infections with antifungal agents (e.g. voriconazole, with careful attention to immunosuppressive levels), regardless of symptoms, because of the risk of invasive disease. Infections are actively sought and aggressively treated, but it must be kept in mind that acute rejection can mimic many of the systemic signs of infection, especially in children.

The spectrum of infectious complications following transplantation is also affected by socioeconomic status, as well as environmental exposures. For instance, 3.4% of Saudi Arabian renal transplant recipients developed *Salmonella* bacteremia. Almost 12% of Indian transplant recipients developed tuberculosis, as opposed to 0.01% of recipients in New York (Tan, 2000). This is especially problematic given the interactions of ciclosporin and rifampin in vivo. The Middle East has high levels of exposure to *Leishmania*, a point to be considered in view of the large number of North Americans and Europeans spending time there who may receive or donate organs in the future.

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Chapter 111 - Opportunistic Fungal Infections

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PATIENT POPULATIONS AT RISK FOR OPPORTUNISTIC FUNGAL INFECTIONS

Deficits in host defense that render persons susceptible to fungal infections are complex but can be broadly divided into the following categories:

- neutropenia;
- qualitative deficits in phagocyte function;
- deficits in cell-mediated immunity (CMI); and
- deficits in mucosal immunity.

Humoral immunity is important against *Cryptococcus neoformans* and probably against *Candida* spp. Patients often have multiple defects in immunity predisposing to opportunistic fungal infections (Table 111.1).

PRIMARY IMMUNODEFICIENCIES

Primary immune disorders are far less common than acquired (or iatrogenic) immunodeficiencies. Of the primary phagocytic disorders, patients with chronic granulomatous disease (CGD) are at highest risk for invasive filamentous fungal infections. Chronic granulomatous disease is an inherited disorder of the NADPH oxidase complex in which phagocytes are defective in generating the reactive oxidant superoxide anion and its metabolites, hydrogen peroxide, hydroxyl anion and hypohalous acid. Patients with CGD suffer from recurrent life-threatening bacterial and fungal infections. Aspergillosis is the most important cause of death in CGD.

The hyper-IgE syndrome (HIES; Job's syndrome) is a primary immunodeficiency characterized by eczema first manifesting during infancy, recurrent and severe soft tissue infections, pneumonias and markedly elevated serum IgE levels and several characteristic skeletal and craniofacial manifestations. Mucocutaneous candidiasis is commonly observed. Colonization of cavities (pneumatoceles) by *Aspergillus* spp. may lead to local invasion, abscess formation and hemoptysis.

The spectrum of opportunistic fungal infections in patients with primary T-cell deficiencies is similar to that for patients with AIDS, and includes *Candida* spp., *Pneumocystis carinii, C. neoformans,* dimorphic fungi and more rarely filamentous fungal infections (see Chapter 126).

CANCER

Intrinsic immune deficits in patients with cancer

Although the immune deficits associated with malignancies are overwhelmingly the result of antineoplastic chemotherapy, some intrinsic immune compromise may result from the malignancy itself. Neutropenia may develop independently of chemotherapy in certain patients with cancer. In acute leukemia, virtually the entire marrow may be replaced with malignant cells so that no normal circulating neutrophils exist in the periphery. Similarly, patients with premalignant hematologic disease, such as myelodysplastic syndrome, may have associated bone marrow failure.

Patients with untreated Hodgkin's disease have significant abnormalities in T-cell number and function, which often persist in long-term survivors. These findings may in part explain the predisposition of these patients to development of infections by *C. neoformans, P. carinii* and other opportunistic pathogens. Opportunistic infections occur most frequently in poorly controlled malignancy, when most patients receive corticosteroids, myelotoxic chemotherapy, or both.

Adrenal tumors and ectopic adrenal corticotropic hormone (ACTH)-secreting tumors resulting in high levels of cortisol are associated with defects in cellular immunity resulting in an increased risk of mucosal candidiasis, *P. carinii* infection and invasive aspergillosis.

Neutropenia

The risk of invasive filamentous fungal infection is strongly related to the duration and degree of neutropenia. In patients with aplastic anemia and refractory neutropenia, invasive aspergillosis is a major cause of death. In patients with acute leukemia, Gerson *et al.*[1] showed that aspergillosis was uncommon when neutropenia lasted for less than 14 days. However, after 14 days, the risk of aspergillosis increased in proportion to the duration of neutropenia. Multiple cycles of prolonged neutropenia, such as in the setting of refractory leukemia, further predispose to invasive filamentous fungal infection. Small foci of invasive fungal disease may be clinically and radiographically inapparent during the initial cycles of neutropenia, only to manifest during a later cycle. Concomitant therapy with systemic corticosteroids and other immunosuppresive agents also increases the risk of invasive filamentous fungal infection. Therefore, knowledge about current and prior cycles of chemotherapy is key in risk stratification for opportunistic fungal infections.

Antifungal prophylaxis and empiric antifungal therapy in patients with neutropenic fever unresponsive to standard antibacterial agents have led to a reduction in the morbidity and mortality rate due to candidal infections. However, infections by *Aspergillus* spp. and other filamentous fungi in high-risk patients with hematologic malignancies have become an increasing cause of death.

Lymphopenia

Little attention has been paid to the potential value of the absolute lymphocyte count and lymphocyte subsets in the assessment of infection risk in cancer patients. Among HIV-infected patients, a $CD4^+$ T-cell count less than 200cells/mm^3 is associated with an increased risk of opportunistic infections such *P. carinii* and mucosal candidiasis. Invasive aspergillosis, although relatively uncommon in AIDS, occurs in the setting of advanced HIV infection ($CD4^+$ T-cell count usually <100 cells/mm^3), and in patients with additional co-morbidities such as neutropenia or use of systemic corticosteroids (see Chapter 126).

Fludarabine is a fluorinated lymphotoxic analog of adenine that has been used in a variety of hematologic malignancies, including chronic lymphocytic leukemia, hairy cell leukemia and low-grade lymphoma, primarily affecting $CD4^+$ T cells. The combination of fludarabine and corticosteroids is more immunosuppressive than either agent alone, and results in a uniform depression of $CD4^+$ T cells that may persist for several months after completion of therapy. Patients with

TABLE 111-1 -- Principal risk factors for opportunistic fungal infections in immunocompromised patients.

PRINCIPAL RISK FACTORS FOR OPPORTUNISTIC FUNGAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS		
Patient population	**Principal risk factors**	**Fungal pathogens**

Hematologic malignancies		
Acute myelogenous leukemia	Neutropenia and mucous membrane toxicity	*Candida* spp. (mucosal and systemic infection), *Aspergillus* spp., other filamentous fungi*, *Trichosporon* spp.
Acute lymphocytic leukemia	Neutropenia, mucous membrane toxicity, T-cell depression (from corticosteroids)	*Candida* spp. (mucosal and systemic infection), *Aspergillus* spp., other filamentous fungi, *Pneumocystis carinii*, *Cryptococcus neoformans,* dimorphic fungi, *Trichosporon* spp.
Chronic lymphocytic leukemia and hairy cell leukemia	T-cell depression (from corticosteroids, fludarabine, other lymphotoxic agents)	*Candida* spp. (usually mucosal), *Pneumocystis carinii*, *Cryptococcus neoformans,* dimorphic fungi, filamentous fungi (less common than in acute leukemia)
Lymphomas	Neutropenia, mucous membrane toxicity and T-cell depression (from corticosteroids, fludarabine)	*Candida* spp. (mucosal and systemic infection), *Aspergillus* spp., other filamentous fungi, *Pneumocystis carinii, Cryptococcus neoformans*, dimorphic fungi
Multiple myeloma	Neutropenia and T-cell depression from corticosteroids	*Candida* spp. (usually mucosal), *Pneumocystis carinii*, *Cryptococcus neoformans,* dimorphic fungi, filamentous fungi (less common than in acute leukemia)
Hematopoietic transplantation		
First month	Neutropenia and mucous membrane toxicity	*Candida* spp. (mucosal and systemic infection), *Aspergillus* spp., other filamentous fungi, *Trichosporon* spp.
1–6 months	T-cell depression (from lack of donor-derived T-cell reconstitution and immunosuppressive agents), phagocyte dysfunction due mainly to corticosteroids, and depressed mucosal immunity due to GVHD	*Candida* spp. (mucosal and systemic infection), *Aspergillus* spp., other filamentous fungi, *Pneumocystis carinii, Cryptococcus neoformans*, dimorphic fungi, *Trichosporon* spp.
> 6 months	Similar to 1–6 months with GVHD requiring corticosteroids	
Solid organ transplantation		
First month	Postoperative infection, most common in liver and pancreatic/small bowel transplantation	*Candida* spp. (wound infection, peritonitis, cholangitis, candidemia)
1–12 months	T-cell and phagocyte dysfunction due to immunosuppressive agents	*Candida* spp. *Pneumocystis carinii*, *Cryptococcus neoformans,* dimorphic fungi, *Aspergillus* spp. (highest frequency in lung transplantation) and other filamentous fungi
>12 months		Similar to 1–12 months with organ rejection treated with intensive immunosuppressive agents
Collagen vascular diseases	T-cell and phagocyte dysfunction due to immunosuppressive agents	*Candida* spp. (mucosal), *Pneumocystis carinii,* dimorphic fungi, *Aspergillus* spp. and other filamentous fungi
Aplastic anemia	Neutropenia	*Aspergillus* spp. and other filamentous fungi
Primary immune disorders		
Chronic granulomatous disease	Defective phagocyte NADPH oxidase	*Aspergillus* spp. and other filamentous fungi
Job's syndrome	Defective phagocyte function	*Candida* spp. (mucosal and cutaneous), *Aspergillus* spp
AIDS and other T-cell deficiencies	Defective T-cell and macrophage function	*Candida* spp. (mucosal and cutaneous), *Pneumocystis carinii, Cryptococcus neoformans,* dimorphic fungi, dermatophyte infection (may be extensive), *Aspergillus* spp. and other filamentous fungi†

*Other filamentous fungi include zygomycetes, *Fusarium* spp., dark-walled molds, *Scedosporium* spp. and *Acremonium* spp.
†Invasive filamentous fungal infections in AIDS often occur in the setting of additional host defense deficits, such as neutropenia and use of corticosteroids.

hematologic malignancies are also being treated with novel monoclonal antibodies that cause a depletion of lymphocyte subsets, and may increase the risk of opportunistic fungal infections.

Mucosal immunity

The mucosal linings in the gastrointestinal, sinopulmonary and genitourinary tracts constitute the first line of host defense against a variety of pathogens. Chemotherapy and radiation therapy cause defects in mucosal immunity at several different levels. The physical protective barrier conferred by the epithelial lining is compromised, thus allowing access to colonizing microflora. Corticosteroids profoundly compromise mucosa-associated lymphoid tissue (MALT) by inducing apoptosis of M-cells and depleting lymphoid follicles of T and B cells.[2]

Mucosal epithelial cells secrete a variety of antimicrobial peptides, including lactoferrin (iron sequestration), lysozyme (hydrolysis of peptidoglycan of Gram-positive bacteria) and phospholipase A2 (cleavage of structural phospholipids of bacteria) and defensins. In patients receiving induction chemotherapy for newly diagnosed acute myelogenous leukemia, the risk of candidemia and hepatosplenic candidiasis is most strongly related to the degree of chemotherapy-induced damage to the intestinal epithelial surface.[3] Molecular studies examining candidemia support the gut as the predominant source of organisms.[4]

Corticosteroids

Corticosteroids have profound effects on the distribution and function of neutrophils, monocytes and lymphocytes (see Chapter 99). They reduce neutrophil adherence to the endothelium, thus inhibiting migration to inflammatory sites, inhibit neutrophil fungicidal activity and cause a monocytopenia that lasts 24 hours.[5] In addition, a number of monocyte functions are impaired, including chemotaxis, bactericidal activity and production of interleukin (IL)-1 and tumor necrosis factor (TNF)-a. Corticosteroids inhibit T-cell activation, leading to reduced proliferative responses and cytokine production, and induce redistribution of lymphocytes out of the circulation, resulting in peripheral lymphocytopenia. In addition to immunosuppression, corticosteroids directly stimulate the growth of *Aspergillus fumigatus* in vitro[6] possibly via sterol binding proteins in the fungus.

HEMATOPOIETIC TRANSPLANTATION

The spectrum of pathogens to which hematopoietic stem cell transplant (HSCT) recipients are most susceptible follows a time line corresponding to the predominant immune defects observed at different periods. In the early stage, neutropenia and mucosal toxicity from the conditioning regimens are the principal host defense defects. Patients are at risk for the same spectrum of fungal pathogens (principally *Candida* spp. and filamentous fungi) that afflict nontransplant patients with hematologic malignancies who have been treated with potent myelotoxic therapy.

Defects in CMI persist for several months, even in uncomplicated allogeneic transplant recipients, thus predisposing these patients to a variety of opportunistic infections. In addition to quantitative T-cell deficiencies, loss of T-cell receptor diversity is observed.[7] By 1 year after transplant, T-cell subsets and responses normalize if graft versus host disease (GVHD) has not occurred.

There is a predominance of invasive filamentous fungal infections in the post-engraftment period in allogeneic BMT/SCT recipients receiving the potent immunosuppressive regimens typically used to treat GVHD. Wald *et al.*[8] conducted a case-control analysis of 158 cases of proven or probable aspergillosis in BMT/SCT recipients. The onset of infection was bimodal, with the first peak occurring before or shortly after engraftment, and the second peak occurring at a mean of

96 days after transplant, commonly in the setting of acute GVHD. In autologous HSCT recipients, aspergillosis occurred most often during neutropenia, and was rare after engraftment. In contrast, aspergillosis was more likely to occur after the first 40 days in allogeneic transplant recipients. Marr *et al.*[9] noted an increase in non-*fumigatus Aspergillus* spp., zygomycetes, *Fusarium* and *Scedosporium* spp. in allogeneic HSCT recipients. Patients requiring multiple transplants were at particular risk for these 'emerging' amphotericin B-resistant pathogens. Aspergillosis also occurs in the post-engraftment period in allogeneic HSCT recipients, with GVHD being the principal risk factor. Cytomegalovirus viremia or disease may also be a risk factor for late-onset aspergillosis.[10]

SOLID ORGAN TRANSPLANTATION

Solid organ transplant (SOT) recipients are at increased risk for fungal infections during two periods. They are at risk for nosocomial candidemia and candidiasis early after transplantation. Reflecting the fact that the bowel is the principal host reservoir of *Candida*, liver recipients are at higher risk for postoperative candidemia and organ candidiasis. Pancreatic transplant recipients are also at high risk for intra-abdominal candidiasis. In renal transplant recipients, candiduria is a risk for an ascending *Candida* pyelonephritis and sepsis, particularly when there is obstruction at the ureteral anastomosis. Fluconazole prophylaxis reduces deep fungal infections and fungal infection-related deaths in liver transplant recipients.[11]

The second period of fungal infection risk is during the depression of immunity induced by agents used to prevent or treat graft rejection. The period of highest risk for opportunistic infections is generally within the first year of transplant. Intensification of immunosuppressive therapy to treat allograft rejection, which may include high-dose corticosteroids and anti-lymphocyte immunoglobulin, significantly increases the risk of opportunistic infections. In this setting, opportunistic fungal infections include candidiasis, *P. carinii, C. neoformans,* dimorphic fungi, *Aspergillus* spp. and other invasive filamentous fungal infections.

Among SOT recipients, lung transplant recipients are at the highest risk of invasive pulmonary aspergillosis. Anastamotic infections occur in approximately 5% of lung transplant recipients, principally due to *Candida* and *Aspergillus* spp. Infection typically responds to appropriate systemic antifungal therapy, and anastomotic dehiscence is uncommon. [12] Colonization of the native lung with *Aspergillus* spp. occurs commonly in end-stage lung disease, and is an important source of post-transplant aspergillosis in single lung transplants. Cytomegalovirus viremia is an additional risk factor for invasive aspergillosis in lung transplant recipients.[13]

Antifungal prophylaxis during the early post-transplant period [14] [15] and pre-emptive therapy in patients with *Aspergillus* airway colonization [16] have shown promising results in uncontrolled studies.

YEAST INFECTIONS

Candidiasis

Oropharyngeal and esophageal candidiasis

Oral mucosal candidiasis, thrush, usually reflects significant T-cell immunodeficiency. The diagnosis of oral candidiasis is usually made visually by identification of white adherent plaques on the palate, buccal mucosa, tongue or gingiva. In patients with cancer, the differential diagnosis includes chemotherapy-induced mucositis, bacterial infections and herpes simplex virus infection. A wet mount or Gram stain showing pseudohyphae establishes the diagnosis. A culture of the oral mucosa that grows *Candida* spp. is not by itself diagnostic because these species commonly colonize the mouth. Therapy for oropharyngeal candidiasis includes local treatments such as nystatin or clotrimazole troches, or fluconazole.

Esophageal candidiasis is a more severe mucosal disease that typically manifests with odynophagia. The differential diagnosis includes esophageal infection by herpes simplex virus, cytomegalovirus principally in allogeneic HSCT recipients, and bacterial infections. Systemic antifungal therapy is required. In fluconazole-resistant *Candida* infections, a second-generation triazole (cross-resistance with fluconazole may occur), amphotericin B preparation or an echinocandin are desirable.

Candidemia

Candida spp. are the fourth most common nosocomial blood culture isolates in the USA.[17] The crude mortality rate varies, but is generally between 30 and 60%. In a European surveillance study of candidemia in cancer patients, the overall 30-day mortality rate was 39%, with an increased mortality rate in older patients, in those with poorly controlled malignancy, and in cases in which *Candida* (*Torulopsis*) *glabrata* was isolated.[18] Bloodstream infection by non-*albicans Candida* spp. was associated with neutropenia in solid tumor and acute leukemia patients and with antifungal prophylaxis in hematology patients. Among hematology patients, additional factors associated with death were allogeneic HSCT, septic shock and lack of antifungal prophylaxis. In a retrospective study of 476 cases

of candidemia at the MD Anderson Cancer Center, the mortality rate was 52%. Neutropenia, a high APACHE score and disseminated candidiasis were associated with poorer outcomes. [19]

The isolation of non-*albicans Candida* spp., which account for approximately 50% of bloodstream isolates, has direct clinical significance. *Candida glabrata* isolates have a broad range of minimum inhibitory concentrations (MICs) to fluconazole, and *Candida krusei* is virtually always resistant. Antifungal susceptibility testing of bloodstream *Candida* isolates is becoming more common and gaining acceptance in clinical practice. Isolates with an intermediate MIC to fluconazole (16–32µg/ml) are referred to as 'susceptible dose-dependent', indicating that therapeutic serum levels are achievable with adequate fluconazole dosing.

Isolation of *Candida* spp. from blood remains unreliable even with modern blood culture isolation systems. Nonculture methods, such as amplification by polymerase chain reaction (PCR), antigen detection and detection of metabolites, are investigational, and may prove useful in complementing culture methods.[20] Molecular studies in the setting of persistent neutropenic fever may eventually guide the clinician in the application of antifungal therapy.[21]

All candidemic patients should receive systemic antifungal therapy. Early catheter removal may reduce the likelihood of late complications by eliminating a potential nidus of ongoing candidemia. Removal of intravenous catheters in candidemic patients reduces the time to blood sterilization in non-neutropenic patients when the catheter was the likely portal of entry.[22] The Infectious Diseases Society of America (IDSA) has advised removal of all intravascular catheters in patients with candidemia, but noted that this recommendation was stronger in non-neutropenic patients in whom the catheter is the most likely primary source of infection.[23] In patients who have received cytotoxic chemotherapy, candidemia is likely to arise from defects in the gut mucosa rather than the catheter. Nucci and Anaissie[24] noted the lack of association between early central venous catheter removal and improved survival, and questioned the routine practice of catheter removal in candidemia. If the catheter is not immediately removed, we advise rotating antifungal infusions through all ports to increase the likelihood of catheter sterilization. All intravenous catheters should be replaced in the setting of clinical instability, lack of resolution of fever within 2–3 days, or persistent candidemia after 1–2 days of appropriate antifungal therapy.

Chronic disseminated candidiasis

Chronic disseminated candidiasis (hepatosplenic candidiasis), typically manifests as a fever that persists after neutrophil recovery. Chronic disseminated candidiasis is a complication of highly mucotoxic chemotherapy regimens, such as those used as induction therapy for acute leukemia. During neutropenia, it is thought that the liver, spleen, kidneys, lungs, skin, bone and other sites become hematogenously seeded by *Candida*, which may go undetected by blood culture. With neutrophil recovery, numerous target lesions in the liver and spleen become apparent by computerized tomography (CT) scan, ultrasonography, or magnetic resonance imaging (MRI; Fig. 111.1). Serial ultrasound examination in patients for whom a high clinical suspicion exists may enhance the likelihood of detecting new or evolving lesions. [25] Liver biopsy is required for definitive diagnosis; but, because the lesions are discrete, a blind percutaneous biopsy may be falsely negative. Open or laporoscopic-guided liver biopsy is recommended if a percutaneous biopsy is not diagnostic.

Chronic disseminated candidiasis is not per se a contraindication for subsequent myelotoxic chemotherapy or hematopoietic transplantation.[26] Patients in whom fever and lesions have resolved with antifungal therapy can undergo further episodes of neutropenia without progression of the fungal infection if antifungal therapy is reinitiated during the neutropenic periods.[26]

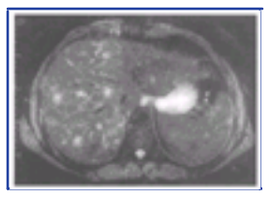

Figure 111-1 A T2-weighted MRI of the liver in a 24-year-old woman being treated for lymphoma who has chronic disseminated (hepatosplenic) candidiasis. The hepatic lesions are central densities surrounded by zones of lower signal intensity which create a 'bull's eye' appearance. Smaller lesions are also visible in the spleen, particularly in the subcapsular region.

Therapy for invasive candidiasis

A large randomized study comparing intravenous fluconazole (400mg/day) with amphotericin B (0.5mg/kg daily) as therapy for candidemia in non-neutropenic patients found the regimens to be equally effective, but fluconazole had less toxicity. [27] Due to the importance of *Candida* isolates with MICs to fluconazole in the susceptible dose-dependent range, a National Institute of Allergy and Infectious Diseases (NIAID) Bacteriology and Mycosis Study Group (BAMSG) trial compared fluconazole (800mg daily) plus amphotericin B (0.7mg/kg daily); (F+A) to fluconazole (800mg daily) plus placebo (F+P) in non-neutropenic patients with candidemia.[28] Despite a worse APACHE II score in the F+P arm at randomization, the survival was similar. The F+A arm had more rapid sterilization of blood, but more renal toxicity.

The echinocandins are a family of cyclic lipopeptides that are noncompetitive inhibitors of (1,3)-ß-D-glucan synthase, an enzyme complex that forms glucan polymers in fungal cell walls. Echinocandins have a broad spectrum of activity against *Candida* and *Aspergillus* spp., with activity against virtually all *Candida* clinical isolates, including those that are resistant to fluconazole or itraconazole. Echinocandins were highly effective in animal models of disseminated candidiasis. Three echinocandins are licensed or in major clinical trials: caspofungin, micafungin (FK463) and anidulafungin (LY303366) (see Chapter 208).

Echinocnadins are effective in mucosal candidiasis. Recently, a phase 3 randomized study comparing caspofungin with conventional amphotericin B in adult patients with invasive candidiasis was completed.[29] The study enrolled 239 patients; 185 met the prespecified criteria for evalution. In the evaluable-patient analysis, a favorable reaponse, defined as improvement or resolution of clinical symptoms and signs attributed to *Candida* infection plus eradication of *Candidan* infection, occurred in 81% of caspofungin recipients and 65% of amphotericin B recipients at the time of discontinuation of intravenous fungal therapy ($p < 0.05$). The modified intent-to-treat analysis showed a trend toward superiority in the caspofungin arm, but this did not reach statistical significnace. Caspofungin recipients had fewer drug-related toxicities. The overall survival and time to sterilization of blood was similar between the two groups. This study supports caspofungin as initial therapy for invasive candidiasis in adults (Table 111.2).

TABLE 111-2 -- **Antifungal agents.** (See also Chapter 208).

ANTIFUNGAL AGENTS	
Antifungal agents	**Comments**
Azoles	
Fluconazole	Acceptable alternative to amphotericin B for candidemia at dose of 400–800mg/day; broad range of MICs to *Candida glabrata* and resistant to *Candida krusei*; prophylaxis in high-risk patients (e.g. acute leukemia during neutropenia, hematopoietic transplantation, liver and pancreatic transplantation); maintenance therapy for cryptococcal meningitis; inactive against filamentous fungi
Itraconazole	Active against *Candida* spp., *Aspergillus* spp., dimorphic fungi, dark-walled molds. Cyclodextrin formulation has increased bioavailability compared with capsules and can be administered parenterally. Itraconazole solution approved for empiric therapy for neutropenic fever
Second-generation azoles	Second-generation antifungal triazoles (voriconazole, posaconazole and ravuconazole) have broad spectrum of activity, including *Candida* spp. (including most, but not all, fluconazole-resistant isolates), *Aspergillus* spp., dimorphic fungi, *Cryptococcus neoformans*, *Trichosporon* spp., *Fusarium* spp., *Pseudallescheria boydii* and dark-walled molds
Voriconazole	New standard of care as initial therapy for invasive aspergillosis; treatment of other filamentous fungi resistant to amphotericin B (*Fusarium* spp., *Pseudallescheria boydii* and dark-walled molds); poor activity against zygomycetes; acceptable alternative to amphotericin B as empiric therapy for neutropenic fever (may be particularly advantageous in relapsed acute leukemia and allogeneic hematopoietic transplantation)
Posaconazole*	Similar spectrum of activity to voriconazole, but active against zygomycetes; growing clinical database from compassionate use protocol for treatment of *Aspergillus* spp. and other refractory filamentous fungi (*Fusarium* spp., *Pseudallescheria boydii* and dark-walled molds); phase III study comparing posaconazole with fluconazole as prophylaxis in allogeneic hematopoietic transplant recipients with GVHD is ongoing
Ravuconazole*	Being evaluated in clinical trials
Polyenes	
Nystatin	Topical agent useful for mucosal candidiasis; parenteral liposomal nystatin is experimental
Amphotericin B deoxycholate (AMB-D)	Broad spectrum of antifungal activity, but with significant infusion-related and nephrotoxicity
Lipid formulations of amphotericin B	Equal to superior efficacy and lower toxicity compared with AMB-D; higher pharmacy acquisition cost
Liposomal amphotericin B (LAMB)	Reduced proven breakthrough fungal infections and less infusion- and nephrotoxicity vs AMB-D as empiric therapy for persistent neutropenic fever; less infusion- and nephrotoxicity vs amphotericin B lipid complex as empiric therapy
Amphotericin B lipid complex (ABLC)	Extensive compassionate use database for patients with refractory invasive fungal infections or intolerance to AMB-D; successfully used in hepatosplenic candidiasis in pediatric patients; high levels in the reticuloendothelial system
Amphotericin B colloidal dispersion	Reduced nephrotoxicity, but more infusion toxicity vs AMB-D as empiric therapy for persistent neutropenic fever
5-flucytosine (5-FC)	Randomized studies support combination of AMB-D and 5-FC for cryptococcal meningitis; pyrimidine analog with dose- and duration-dependent myelotoxicity and gastrointestinal toxicity; monitoring of serum levels and adjustment of dosing for azotemia required
Echinocandins	Class of antifungal peptides that inhibit synthesis of glucan, a fungal cell wall constituent; potently cidal against *Candida* spp., including fluconazole-resistant; fungistatic against *Aspergillus* spp., principally acting at growing hyphal tips; infrequent infusion-related events and not nephrotoxic
Caspofungin	Compassionate use study of patients with refractory invasive aspergillosis or intolerance to licensed antifungal agents showed 41% successful responses (superior to carefully matched historic controls) led to approval for this indication; recent randomized study showed comparable efficacy and less toxicity vs AMB-D for invasive candidiasis
Micafungin*	Phase III study comparing micafungin with fluconazole as prophylaxis in hematopoietic transplant recipients has completed enrollment
Anidulafungin*	

*Non-licensed compounds

Cryptococcus neoformans

Host defense against cryptococcal infection is principally dependent on T-cell immunity. Immunoglobulins directed against capsular epitopes and complement facilitate phagocytosis of the organism, and likely play a role in host defense. The principal portal of entry for C. *neoformans* is by inhalation, with subsequent spread to the

blood and central nervous system (CNS) with development of cryptococcal meningitis.

Although meningitis is the most common presentation of cryptococcal infection, pneumonia, fungemia and cutaneous and visceral dissemination also occur. Additional CNS complications include development of a mass lesion, obstructive hydrocephalus and visual loss. Visual abnormalities may be a consequence of endophthalmitis, a space occupying lesion in the visual pathway, direct invasion of the optic nerve (which may be rapidly progressive), or as a consequence of elevated intracranial pressure. In the pre-AIDS era, patients who died early during therapy were more likely to have rapidly progressive infection, cerebrospinal fluid (CSF) with a high opening pressure, low CSF glucose level, less than 20 leukocytes/µl of CSF, a positive India ink preaparation, culture of cryptococci from extraneural sites, and high titers of crytococcal antigen in serum and CSF.[30]

For AIDS-associated cryptococcal meningitis, the IDSA recommends amphotericin B (0.7–1.0mg/kg daily) plus 5-flucytosine (100mg/kg daily) for the first 2 weeks, followed by lifelong maintenance fluconazole therapy (see Chapter 126).[31] In the absence of recent randomized studies, the same induction regimen is recommended as in non-AIDS

associated cryptococcal meningitis, followed by fluconazole 400mg daily for at least 10 weeks.[31] In neutropenic patients, reduction of the dosage of 5-flucytosine may be considered to avoid delay in myeloid recovery. Interferon-? and passive immunization with monoclonal antibody (mAb 18B7) directed against the cryptococcal capsule[32] are newer therapies being evaluated.

Trichosporon spp.

Trichosporon spp. typically affect profoundly neutropenic patients and those receiving corticosteroid therapy. Acute disseminated trichosporonosis typically manifests with refractory fungemia, funguria, cutaneous lesions, renal failure, pulmonary lesions and chorioretinitis. Disseminated trichosporonosis may yield a false-positive cryptococcal latex antigen test because of cross-reactivity with the polysaccharide capsule of *C. neoformans*. This cross-reactivity may be clinically important because *C. neoformans* typically responds to amphotericin B therapy, while *Trichosporon* spp. are usually resistant.

Blastoschizomyces capitatus (formerly *Trichosporon capitatus*) usually presents as a chronic disseminated infection resembling chronic candidiasis. A CT scan may show lesions suggestive of hepatosplenic candidiasis, but definitive diagnosis requires either a positive culture from blood or biopsy.

INVASIVE FILAMENTOUS FUNGAL INFECTIONS

Aspergillosis

Aspergillus can involve virtually any organ in the immunocompromised host, but sinopulmonary disease is the most common. Alveolar macrophages constitute the first line of host defense against aerosolized conidia. Following germination, neutrophils are the dominant host defense arm against the hyphal stage. Invasive aspergillosis in the neutropenic host may present as fever, sinus pain or congestion, cough, pleuritic chest pain or hemoptysis. Erosion into the wall of a large central blood vessel can lead to massive pulmonary hemorrhage. The radiographic appearance of pulmonary aspergillosis includes bronchopneumonia, lobar consolidation, segmental pneumonia, nodular lesions resembling septic emboli, and cavities. Isolation of any *Aspergillus* sp. from a sputum or bronchoalveolar lavage specimen should be presumed to represent invasive disease in neutropenic patients. [8] [33] *Aspergillus fumigatus* followed by *Aspergillus flavus* are the most common species causing invasive disease. *Aspergillus terreus* is notable for being resistant to amphotericin B; voriconazole may be of value based on in-vitro sensitivity data.[34] See also Chapter 237 .

The CNS is a common target site for hematogenously disseminated aspergillosis. Gastrointestinal aspergillosis usually co-exists with pulmonary disease, but in rare instances it may be the sole organ involved. The manifestations include abdominal pain, gastrointestinal infarction with hemorrhage, perforation and polymicrobial sepsis. Early diagnosis of isolated gastrointestinal aspergillosis followed by resection of the involved bowel and systemic antifungal therapy may be life-saving. Other sites of disseminated aspergillosis include the skin, heart, eye, bone, kidney, liver and thyroid.

Early diagnosis of aspergillosis in profoundly immunocompromised patients remains difficult. Blood cultures are rarely positive; sputum and bronchoalveolar cultures have approximately 50% sensitivity in focal pulmonary lesions; definitive diagnosis often requires an invasive procedure and is usually only made when the disease is advanced. A CT scan of the chest may facilitate early detection by showing peripheral or subpleural nodules that are inapparent on plain chest radiographs (Fig. 111.2). The 'halo sign' is a characteristic chest CT feature of angioinvasion. The hazy alveolar infiltrates appear to correspond to regions of ischemia, and are highly suggestive of invasive aspergillosis. Early recognition followed by intensive antifungal therapy and surgical resection of localized disease was associated with an approved outcome in a retrospective series.[35]

Polymerase chain reaction and antigen-based detection of subclinical aspergillosis are promising tools for early diagnosis. A sensitive double sandwich enzyme-linked immunosorbent assay (ELISA) to detect the fungal cell wall constituent galactomannan has been developed. Maertens *et al.* [36] obtained serial serum galactomannan levels from neutropenic and HSCT patients at high risk for aspergillosis. The positive and negative predictive values for invasive aspergillosis were 87.5 and 98.4%, respectively. All proven cases of invasive aspergillosis, including 23 cases confirmed at autopsy only, had been detected by the ELISA before death, although serial sampling was necessary to maximize detection. Herbrecht *et al.*[37] evaluated the galactomannan antigenemia assay in four groups of patients: neutropenic fever of unknown etio-logy, suspected pulmonary infection, suspected extrapulmonary aspergillosis, and surveillance in HSCT recipients. The positive predictive value varied among the different patient groups reflecting the different frequencies of invasive aspergillosis. The rate was lowest (7.1%) in neutropenic fever. Clinical trials are required to delineate which of these diagnostic methods — or which combination — provides optimal positive and negative predictive value for invasive disease in high-risk patients.

In neutropenic patients and in allogeneic HSCT recipients, locally invasive disease such as sinusitis, primary cutaneous lesions, intravitreal disease, or bone lesions should receive combined surgery and systemic antifungal therapy because of the risk of dissemination.

Patients who recover from an episode of invasive aspergillosis are at risk for relapse during a subsequent course of myelotoxic chemotherapy. A large retrospective analysis on 48 patients with definite or probable aspergillosis who subsequently received bone marrow or stem cell transplantation (77% allogeneic) [38] found that the overall incidence of relapse of *Aspergillus* infection was 29% among patients receiving secondary prophylaxis but 57% among those who did not. Fourteen of 16 (88%) patients with relapse of infection died. Surgical resection does not obviate the need for secondary antifungal prophylaxis during subsequent chemotherapy, given the likelihood of residual foci of persistent inapparent disease.

Antifungal therapy for invasive aspergillosis

Until recently, standard therapy for invasive aspergillosis has been high-dose conventional amphotericin B (1–1.5mg/kg daily). Lipid formulations of amphotericin B may be more effective and certainly have less infusion-related toxicity than conventional amphotericin B, based on noncomparative and compassionate use studies.[39] [40] If a lipid formulation of amphotericin B is used, we suggest a dose of at least 5mg/kg daily. In a dose-escalation study of liposomal amphotericin B in patients with invasive fungal infection, dosing as high as 15mg/kg daily was well-tolerated. [41]

Voriconazole, posaconazole (SCH 56592) and ravuconazole (BMS 207147) are second-generation triazoles that are currently licensed or being evaluated in clinical trials. These agents are active against *Candida* spp. (including most fluconazole-resistant isolates), *Aspergillus* spp., dimorphic fungi, *C. neoformans* and several resistant fungi, including *Scedosporium* spp., dark-walled (dematiaceous) molds, *Trichosporon* spp. and *Fusarium* spp.

Voriconazole has been the most extensively studied of this group. In a noncomparative study of 116 patients with invasive aspergillosis in which voriconazole was given either as initial (52%) or salvage (48%) therapy, a complete or partial response occurred in 48% of patients, with a more favorable prognosis in the group that received voriconazole as initial therapy.[42] When voriconazole was compared with conventional amphotericin B (1.0–1.5mg/kg daily) as initial therapy in an open, randomized study of patients with invasive

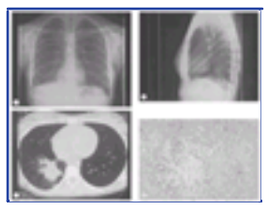
Figure 111-2 Aspergillosis. (a and b) A chest radiograph to evaluate persistent neutropenic fever in a patient with relapsed leukemia showed subtle increased markings in the right lower lung. (c) A chest CT scan obtained the same day showed a significant area of consolidation not apparent on the chest radiograph. Bronchoalveolar lavage grew *Aspergillus fumigatus*. This case illustrates the appropriate use of a chest CT scan in patients with persistent neutropenic fever at high risk for invasive mold infection. (d) Invasive pulmonary aspergillosis in another patient showing necrosis and invasive hyphae (Gomori methenamine silver).

aspergillosis,[43] voriconazole was more effective (53% with complete or partial response vs 32%, respectively) and had improved survival at 12 weeks (71 vs 58%, respectively). Voriconazole had a superior response rate in both neutropenic and non-neutropenic patients. The poorest prognosis was observed in extrapulmonary aspergillosis and in allogeneic HSCT recipients. This study establishes voriconazole as a more effective and less toxic standard of care for therapy of invasive aspergillosis (see Chapter 208).

Posaconazole was also an effective and well-tolerated salvage treatment for serious invasive fungal infections in immunocompromised patients.[44] It is currently being evaluated as prophylaxis in allogeneic HSCT recipients with GVHD. This study is important in view of the increasing frequency of invasive filamentous fungal infections in this setting and the poor prognosis they convey.[8]

The echinocandin caspofungin is approved for the treatment of invasive aspergillosis refractory to, and in patients intolerant of, conventional or lipid formulations of amphotericin B or itraconazole. This was based on a compassionate use study of 63 patients with invasive aspergillosis (53 refractory, 10 intolerant) in whom caspofungin led to a successful outcome (complete or partial response) in 41% of cases. This compared favorably with carefully matched historic controls. Echinocandins have not been evaluated as initial therapy for invasive filamentous fungal infections, but there is growing interest in evaluating echinocandins in combination with other classes of antifungal agents.

Zygomycosis

Risk factors for zygomycosis (also termed 'mucormycosis') include diabetic ketoacidosis, protein-calorie malnutrition, iron overload and prolonged neutropenia. Patients receiving potent myelotoxic chemotherapy for leukemia are at risk for locally invasive as well as disseminated disease. Zygomycosis typically manifests as rhinocerebral or pulmonary disease following inhalation of spores. In rhinocerebral disease, fever, facial pain and headache are common. Contiguous extension may lead to orbital involvement with proptosis and extraocular muscle paresis, involvement of the hard palate and spread to the brain. An eschar over the palate is suggestive of zygomycosis, but other filamentous fungi can produce similar findings in highly immunocompromised persons. Occasionally, isolated primary cutaneous disease may follow minor trauma. Injection drug

users may inadvertently inject spores directly into the bloodstream and present with isolated space-occupying lesions of the brain or other organs. Current therapy for zygomycosis involves high-dose amphotericin B (conventional or lipid formulations) plus early and aggressive surgical debridement. Voriconazole has poor activity against the zygomycetes, but posaconazole is active in vitro and merits further study.

Dark-walled molds

Dark-walled molds (pheohyphomycetes, dematiaceous molds) contain melanin in their cell walls which imparts a brown or olive-green pigment in culture. In immunocompromised patients, soft tissue infection, sinusitis, CNS infection, pneumonia, fungemia and disseminated disease are observed. Certain species have a strong predisposition for CNS disease.

Therapy in immunocompromised patients involves surgical excision of localized disease when feasible, and systemic antifungal therapy. Sensitivity to amphotericin B is variable and clinical failures have been reported. Itraconazole has been shown to be effective in infections by dark-walled molds refractory to amphotericin B.[45] Second-generation triazoles are also active against dark-walled molds.

Fusarium spp.

Fusarium spp. are soil saprophytes associated with soft tissue infection, onychomycosis and keratitis in immunocompetent hosts. With the widespread use of intensive antineoplastic therapy and BMT/SCT, more than 150 cases of invasive and disseminated fusariosis have been reported — most within the past 15 years.[46] Colonization of hospital water systems is a potential environmental reservoir.[47] The clinical findings and histologic appearance may be indistinguishable from those of aspergillosis. In the absence of a definitive culture diagnosis, the factors that suggest *Fusarium* spp. rather than *Aspergillus* are the presence of disseminated cutaneous lesions or the isolation of a mold from blood culture.

Boutati and Anaissie[46] made important observations about invasive and disseminated fusariosis in a retrospective review of 43 cases occurring in patients with hematologic malignancies. Most cases of disseminated fusariosis were diagnosed during neutropenia. Resolution of infection was only seen in patients who recovered from myelosuppression, and a high risk of relapse of infection was associated with subsequent myelosuppression. The skin was identified as an important portal of entry. Initial localized manifestations included onychomycosis, paronychia and cellulitis. Early identification of localized skin disease and surgical debridement may be life-saving. Inhalation of spores is another major portal of entry, leading to fungal sinusitis and pneumonia.

Survival from disseminated fusariosis is critically dependent on resolution of neutropenia. Susceptibility to amphotericin B is variable and itraconazole is inactive. For *Fusarium* spp. MICs to voriconazole range from 1–8µg/mL, which is higher than those for most pathogenic molds. The experience with voriconazole as therapy for fusariosis is limited, but appears to compare favorably with amphotericin.[48] In data presented to the Food and Drug Administration, 9 (43%) of 21 patients with fusariosis had a partial or complete response to voriconazole in a compassionate use protocol. Posaconazole has a similar in-vitro activity to voriconazole, and may be effective based on limited compassionate use data.[44] (Posaconazole is not yet licensed but is available under a compassionate use protocol through the manufacturer Schering-Plough.)

Scedosporium spp.

Scedosporium apiospermum (Pseudallescheria boydii) and *Scedosporium prolificans* are the principal pathogens in the genus *Scedosporium*. In neutropenic patients, *S. apiospermum* infection is virulent, and clinically and histologically resembles aspergillosis. Invasion of blood vessels leading to infarction is common. *Scedosporium apiospermum* causes sinopulmonary disease, endophthalmitis and dissemination to the CNS. The infection can also spread directly from the skin to bone and joint. Establishing a culture diagnosis of *S. apiospermum* is critical because of its frequent resistance to amphotericin B. Voriconazole is highly promising for *S. apiospermum* infections. Seventeen of 27 (63%) patients with *S. apiospermum* infections, in about one-third of whom there was dissemination or involvement of the CNS, responded to voriconazole salvage therapy.[49] Surgical resection of localized lesions is strongly advised. *Scedosporium prolificans* causes a similar spectrum of disease as *S. apiospermum* and is generally resistant to all antifungal agents.

Endemic dimorphic fungi

Endemic dimorphic fungi are so named because of their characteristic geographic distribution. These organisms include *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis* and *Penicillium marneffei*. *Penicillium marneffei* is endemic in South East Asia. These fungi are dimorphic, existing in nature in the mycelial stage, and convert to yeast stage at body temperature (see Chapter 39).

Endemic mycoses in the central USA include histoplasmosis and blastomycosis. In the immunocompetent host, inhalation of *Histoplasma* microconidia is typically asymptomatic, but may manifest with acute fever, pulmonary infiltrates and hypoxia. Immunocompromised patients have a higher risk of disseminated histoplasmosis involving the liver, spleen, lymph nodes, bone marrow, adrenal glands, mucocutaneous tissues, gastrointestinal tract and CNS. The chest radiograph may show a miliary reticulonudular appearance suggestive of tuberculosis. An acute sepsis syndrome with hypotension, disseminated intravascular coagulation, adrenal crisis and meningitis are potential lethal complications.

Rapid diagnosis of histoplasmosis can be made by Giemsa staining of peripheral blood smears or bone marrow aspirates demonstrating characteristic intracellular yeast forms. Lysis centrifugation is the preferred blood culture system. Antigen detection from blood and urine is sensitive and specific in disseminated disease.

Antibody detection may also be useful, but false-negative results may occur in immunocompromised patients. Biopsy specimens may show suggestive intracellular or narrow budding yeasts, which should be confirmed by culture. In patients with disseminated or life-threatening infection, histoplasmosis should be treated with high-dose amphotericin B (1–1.5mg/kg daily) or a lipid formulation. Prolonged therapy with itraconazole may be initiated after stabilization of disease, and should probably be continued for the duration of immunosuppression. Itraconazole is preferred over fluconazole because of the greater likelihood of emergence of resistance during fluconazole therapy leading to treatment failure.[50]

Coccidioides immitis is endemic in southwestern USA. In normal persons, infection is usually asymptomatic or self-limited. In patients with compromised cell mediated immunity, *C. immitis* is likely to be more virulent. Diagnosis is most easily established by serology or demonstration of pathognomonic spherules in sputum or tissue samples. Coccidioidomycosis can involve virtually any organ, but has a particular trophism for bone and the CNS. Therapy for nonmeningeal disseminated or extrapulmonary disease generally involves an azole. Amphotericin B is an alternative in rapidly progressive or severe infections. [51] In a randomized, double-blind study of progressive nonmeningeal coccidioidomycosis, oral itraconazole was associated with a trend toward increased efficacy compared with fluconazole (72 vs 57% response at 12 months, respectively). [52] Fluconazole is advised for meningitis, given its excellent CSF penetration. Some physicians initiate therapy with intracisternal amphotericin B in addition to systemic azole therapy.[51] In view of the potential life-threatening consequences of relapse, lifelong maintenance azole therapy is advised in patients with meningitis who have responded to initial azole therapy.[51] [53]

Pneumocystis carinii

Pneumocystis carinii is more appropriately classified as a fungus than a protozoan based on gene sequence data and cell wall constituents. Defective T-cell immunity is the principal risk factor for *P. carinii* infection (see Chapter 124). Sepkowitz *et al.*[54] reported that corticosteroid use was associated with 204 of 227 (90%) cases of *P. carinii* infection in patients without AIDS at Memorial Sloan-Kettering Cancer Center between 1963 and 1992. The median time that patients received corticosteroids was 2 months, although a minority of patients had received corticosteroids for less than 1 month. Approximately 60% of patients had hematologic malignancies, 25% had solid tumors, 10% were HSCT recipients and 5% were receiving relatively mild immunosuppressive regimens.

The risk of *P. carinii* infection increases with the intensity of the immunosuppressive regimen. In a study of pediatric patients with acute lymphocytic leukemia, the risk of *P. carinii* infection strikingly increased from less than 5 to 22% when cytosine arabinoside (ARA–C) was used.[55] Browne *et al.*[56] reported a 32% rate of *P. carinii* infection (probable plus definite) in patients with non-Hodgkin's lymphoma treated with an intensive regimen consisting of corticosteroids and multiple cytotoxic agents. There were no cases of *P. carinii* infection in patients who did not receive ARA–C and bleomycin. The diagnosis of *P. carinii* infection relies on visualization of the organism microscopically. Immunofluorescent staining using monoclonal antibodies is more sensitive than older staining methods, such as silver staining or Wright-Giemsa. [57]

Trimethoprim-sulfamethoxazole (co-trimoxazole; 15–20mg/kg daily divided into 3–4 doses) is the treatment of choice for *P. carinii* infection. Children with acute lymphoblastic leukemia and allogeneic HSCT recipients are known to be at high risk and should be offered prophylaxis. Adults with acute lymphoblastic leukemia, patients with CNS tumors receiving high-dose corticosteroid therapy and patients receiving combination corticosteroid therapy with either myelotoxic agents or fludarabine are also at high risk for *P. carinii* infection. In patients with collagen vascular disease, the risk of *P. carinii* infection is highest in those receiving concomitant high-dose corticosteroid and cytotoxic therapies, such as those with Wegener's granulomatosis or lupus nephritis. In these patients prophylaxis should be considered.[58]

FUNGAL INFECTIONS BY ANATOMIC SITE

Sinusitis

Invasive fungal sinusitis in immunocompromised patients often has devastating results. Infection by *Aspergillus* spp. is most common in patients with persistent neutropenia (e.g. aplastic anemia) and in BMT/SCT recipients. Zygomycetes are classically associated with rhinocerebral disease, leading to necrosis of the palate, and extension to surrounding structures. Sinusitis by 'emerging' fungal pathogens, including *Fusarium* spp., dark-walled molds and *Scedosporium* spp., are being recognized with increasing frequency. Symptoms and signs suggestive of fungal sinusitis include fever, nasal congestion, headache, maxillary tenderness and periorbital swelling. Sinus endoscopy may show necrotic material or ulceration. Hyphal invasion into blood vessels leads to tissue infarction and hemorrhage. Mental status changes or focal neurologic findings may indicate involvement of the brain.

Therapy for invasive fungal sinusitis often requires a combined medical and surgical approach. Initial therapy should include either high-dose lipid formulation of amphotericin B (=5mg/kg daily) or a second-generation triazole (voriconazole has poor activity against zygomycetes, posaconazole is active). When feasible, involved tissue should be surgically resected because medical therapy alone is unlikely to contain infection, especially in the setting of neutropenia or severe immunosuppression. Systemic antifungal therapy should be continued even if all necrotic tissue is fully debrided, given the likelihood of inapparent local and disseminated disease. The most important predictor of a successful outcome is usually resolution of neutropenia. Re-initiation of antifungal therapy during subsequent periods of neutropenia is important.[59]

Pneumonia

Aspergillus is the most common cause of fungal pneumonia in persistently neutropenic patients. Less common fungal pathogens include zygomycetes, *Trichosporon*, *Fusarium* and dark-walled molds. Isolation of *Candida* spp. from respiratory samples most likely represents upper airway colonization. *Candida* pneumonia occurs in unusual cases as a focal infiltrate or a miliary pattern reflecting hematogenous dissemination; biopsy is required to diagnose pulmonary candidiasis definitively.[23] *Aspergillus* spp. and other filamentous fungi are typically angioinvasive and may cause pulmonary hemorrhage, infarction, pleuritic chest pain or direct invasion of chest wall structures.

Bronchoalveolar lavage is relatively insensitive for diagnosing aspergillosis, detecting it in only approximately 50% of cases.[60] Percutaneous biopsy may increase the diagnostic yield, but in thrombocytopenic patients the risk of bleeding may be unacceptably high. Open lung biopsy allows for easier visualization and control of bleeding and therefore is the definitive diagnostic method in immunocompromised patients with pulmonary lesions. False-negative results occur in approximately 5% of open lung biopsies due to sampling error in the case of patchy lesions.

Central nervous system fungal infections

The CNS is the most common target organ for hematogenously disseminated aspergillosis. Manifestations of CNS aspergillosis include focal seizures, hemiparesis, cranial nerve palsies and hemorrhagic infarcts due to vascular invasion. Pulmonary infiltrates and focal neurologic deficits in an immunocompromised patient were significantly more predictive of CNS aspergillosis than CNS candidiasis or cryptococcosis in a multivariate discriminate analysis of autopsy-proven fungal CNS infections.[61] *Scedosporium* spp. and certain dark-walled molds also have a predilection for CNS infection.

Aspergillus brain abscesses are typically multiple, hypodense and non-enhancing with little mass effect. Computerized tomography scans with contrast initially may reveal no focal lesions, but later may demonstrate focal ring-enhancement or hemorrhagic changes. Magnetic resonance imaging may improve early detection. Biopsy of these CNS lesions shows vascular invasion and infarction similar to that seen in lung biopsy specimens.

Central nervous system aspergillosis has been almost universally fatal in highly immunocompromised persons. Voriconazole led to complete or partial response in three of 19 (16%) patients with CNS aspergillosis,[42] which compares favorably with the dismal historic experience using other antifungal agents.

Skin lesions and soft tissue infections

In the profoundly immunocompromised patient, skin lesions often arise and may be due to several different etiologies. The characteristic skin lesions of disseminated candidiasis are discrete raised erythematous papules, measuring about 0.5–1cm in diameter. The lesions are usually not tender. Concurrent myalgias raise the possibility of *Candida* myositis. The yeast is cultured from skin lesions in about half the cases, and blood cultures are typically positive. Biopsy and fungal staining of cutaneous lesions can provide an immediate diagnosis, prompting the early addition of antifungal therapy.

Trichosporon beigelii skin lesions cause 30% of disseminated fungal infections in some series, and are characterized by nontender erythematous nodules that may necrose. Histologically, budding

yeasts are present in the dermis, as distinct from *Candida* spp., which produce pseudohyphae. Disseminated *C. neoformans* presents as painless lesions that may appear as papules, pustules, plaques, ulcers, subcutaneous masses, or cellulitis. The discrete papular form may resemble molluscum contagiosum.

Localized cutaneous infection by dimorphic fungi may follow traumatic inoculation. The appearance of disseminated cutaneous infection varies, and is most commonly observed in patients with depressed cellular immunity. Disseminated cutaneous histoplasmosis typically presents with a papular or nodular rash and oral mucosal ulcerative lesions (*Histoplasma duboisii*, the agent of 'African histoplasmosis', typically produces large nodular and ulcerative cutaneous lesions). Large keratotic ulcers and subcutaneous draining abscesses may be observed in disseminated coccidioidomycosis. Erythema nodosum, a panniculitis presenting as tender, raised, red-to-violaceous lesions, typically over the extremities, may be a manifestation of histoplasmosis or coccidioidomycosis. *Penicillium marneffeii* causes disseminated infection resembling histoplasmosis in highly immunocompromised hosts.

Cutaneous infection by filamentous fungi may be primary or may represent systemic infection. Primary cutaneous infection with molds can occur in immunocompetent patients by traumatic inoculation. However, angioinvasion, infarction, extension to the soft tissue and dissemination occur only in the setting of profound immunosuppression. Localized cutaneous aspergillosis can be caused by intravenous arm boards. Surgical resection may be necessary, and has an excellent prognosis. In the neutropenic patient, the likelihood of subclinical systemic infection is high, and therefore systemic antifungal therapy is warranted. Hematogenously disseminated filamentous fungal infection may resemble ecthyma gangrenosum, but histologically hyphal elements are seen associated with angioinvasion and infarction.

EMPIRIC ANTIFUNGAL THERAPY FOR PERSISTENT FEVER IN NEUTROPENIA

The rationale for empiric antifungal therapy for persistent fever during neutropenia is that despite meticulous clinical examination and collection of cultures, our techniques are not sufficiently sensitive for early detection of fungal infections. Before the standard implementation of empiric antifungal therapy, there was a correlation between prolonged fever during neutropenia and death in patients with cancer; fungal infection was frequently found at autopsy. Two randomized prospective studies showed that empiric amphotericin B therapy was associated with a trend toward fewer serious fungal infections.[62] [63]

Because fungal infections are uncommonly encountered in the first 7 days of fever during neutropenia, empiric antifungal therapy is typically begun between days 4 and 7 of neutropenic fever. Empiric antifungal therapy should be continued for the duration of neutropenia. In a large, randomized study of patients with neutropenic fever unresponsive to standard antibacterial agents, liposomal amphotericin B (LAMB) was associated with fewer proven breakthrough fungal infections and less infusion-related and renal toxicity than conventional deoxycholate amphotericin B. [64]

Intravenous followed by oral itraconazole solution (this cyclodextrin formulation has much better bioavailability) was as effective as but less toxic than conventional amphotericin B as empiric therapy for fever during neutropenia,[65] leading to the approval of itraconazole solution for this indication. Previous use of prophylactic fluconazole was similar in both groups, an important consideration given the potential for cross-resistance of fungal pathogens to different classes of azoles. Fluconazole has been used successfully as empiric therapy for neutropenic fever.[66] However, given its lack of activity against filamentous fungi, fluconazole is more suitable as prophylaxis during neutropenia, rather than as empiric therapy in patients at high risk for invasive filamentous fungal infection (e.g. those with acute leukemia, allogeneic HSCT).

Echinocandins and second-generation triazoles are attractive candidates for antifungal prophylaxis and empiric therapy for fever during neutropenia. Voriconazole was compared with LAMB in a nonblinded, randomized study of empiric antifungal therapy in patients with persistent fever during neutropenia unresponsive to antibacterials. [67] The overall success rates were 26% with voriconazole and 31% with LAMB, but empiric voriconazole was associated with fewer breakthrough fungal infections (1.9 vs 5.0%). The greatest protective benefit occurred in high-risk patients, such as those with relapsed acute leukemia and allogeneic HSCT. Voriconazole caused fewer infusion-related and nephrotoxicities, but more transient visual changes and visual hallucinations. However, because of the absence of proof of 'non-inferiority' of voriconazole compared to LAMB, it was not approved by the US Food and Drug Administration for empiric therapy. Comparison of caspofungin with LAMB as empiric antifungal therapy has recently been completed.

Liposomal amphotericin B, the cyclodextrin formulation of itraconazole, and voriconazole are highly acceptable agents for empiric antifungal therapy. The selection of an empiric antifungal agent should be tailored to the individual patient and should broadly consider the risk of breakthrough fungal infection, toxicity and a pharmacoeconomic analysis, and not solely hospital or pharmacy acquisition costs.

ANTIFUNGAL PROPHYLAXIS DURING CHEMOTHERAPY-INDUCED NEUTROPENIA

In HSCT recipients, two double-blinded, placebo-controlled trials have shown that prophylactic fluconazole controlled yeast colonization and reduced the rate of mucosal candidiasis and invasive *Candida* infections.[68] [69] A reduction in mortality rate was noted in the study by Slavin *et al.*,[69] in which most of the patients were allograft recipients. Fluconazole use conferred significant long-term improvement in survival, possibly by reducing *Candida* antigen-induced gut GVHD.[70] Fluconazole is associated with colonization by azole-resistant *Candida* strains, which may be less intrinsically virulent than azole-sensitive *C. albicans*, based on their low rates of candidemia, invasive candidiasis and attributable death. [71]

Fluconazole prophylaxis has produced mixed results in patients with leukemia receiving chemotherapy. In one randomized study, fluconazole prophylaxis was associated with a reduction in skin and mucosal infection and a delay in empiric use of amphotericin B, but made no significant difference in invasive candidiasis or mortality rate compared to placebo.[72] In another study, fluconazole prophylaxis reduced fungal colonization, invasive infection and fungal infection-related mortality rate in chemotherapy patients with leukemia and in autologous transplant recipients.[73] The benefit of fluconazole prophylaxis was greatest in autologous transplant recipients who were not receiving colony growth factor support and in patients receiving mucotoxic regimens (cytarabine plus anthracyclines) — a finding that is consistent with the bowel being a principal portal of entry for *Candida* bloodstream infections. The emergence of resistant *Candida* spp. and the lack of activity against filamentous fungi remain major concerns for prophylactic use of fluconazole.

The erratic bioavailability of the capsular form of itraconazole mitigates its use in highly immunocompromised neutropenic patients. The cyclodextrin formulation of itraconazole allows for increased oral bioavailability, and the intravenous formulation allows for achievement of therapeutic levels within 3 days. The oral cyclodextrin formulation of itraconazole is, in general, safe and effective as prophylaxis during prolonged neutropenia as long as adequate

serum levels are maintained. A meta-analysis has shown that prophylaxis with itraconazole solution was effective in preventing invasive fungal infections in neutropenic patients and reduced the mortality rate due to fungal infections.[74]

IMMUNE AUGMENTATION

Colony growth factors

Normal myelopoiesis requires the establishment of myeloid stem cells. Under the influence of stem cell factor (SCF), IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), this gives rise to the colony forming unit granulocyte-macrophage (CFU-GM). Granulocyte colony stimulating factor (G-CSF) acts at a later stage in concert with other growth factors to specifically drive granulopoiesis. Prophylactic G-CSF has been evaluated in several prospective, randomized studies. The most consistent benefit has been a reduction in the neutropenic period. In some studies of patients receiving therapy for acute myelogenous leukemia, the acceleration of myeloid recovery was associated with a reduction in the duration of fever, use of antibiotics and hospitalization.

In one study, GM-CSF was only administered to patients who had a hypocellular or remission marrow on day 10 of induction chemotherapy.[75] Patients who achieved complete remission received the same study drug (GM-CSF or placebo) during consolidation chemotherapy. The median time to neutrophil recovery was significantly reduced in the GM-CSF group as was the frequency of fatal fungal infections and early infection-related deaths. There was a trend toward increased complete remission in the GM-CSF group. Most important, the 6-month mortality rate was significantly reduced in the GM-CSF arm. So far, this is the only study that has shown a survival advantage attributed to a colony stimulating factor. This study formed the basis for US Food and Drug Administration approval of GM-CSF for patients with acute myelogenous leukemia. Other randomized studies of prophylactic GM-CSF have not shown a protective benefit against fungal infections or impact on survival.

In addition to accelerating myelopoiesis, colony stimulating factors augment phagocyte function. G-CSF, GM-CSF and macrophage colony stimulating factor (M-CSF)

increase the fungicidal activity of phagocytes in vitro against *Candida* and *Aspergillus* spp. M-CSF increases phagocytosis, chemotaxis and secondary cytokine production in monocytes and macrophages.[76] GM-CSF stimulates various neutrophil effector functions and prolongs neutrophil survival in vitro, increases antibody-dependent cytotoxicity of eosinophils, accelerates the proliferation of the monocyte-macrophage system, and is a potent activator of monocytes and macrophages.[76] Thus GM-CSF may have a theoretic advantage against pathogens such as *Candida* and *Aspergillus* spp., in which host defense is dependent on both neutrophil and macrophage function.

Granulocyte transfusions

The impetus to re-evaluate granulocyte transfusion therapy stems from improvements made in the methods to mobilize large number of cells from donors and the feasibility of using non-human leukocyte antigen (HLA) matched community donors.[77] Recombinant G-CSF with or without corticosteroids is now routinely administered to donors approximately 12 hours before apheresis to increase granulocyte yield.

Successful outcomes using granulocyte transfusions have been described in patients with life-threatening fungal infections in small series and in case reports. In one non-randomized retrospective series, no benefit of granulocyte transfusions was noted in neutropenic BMT recipients with invasive mold infection.[78] A phase I/II trial using G-CSF-mobilized granulocyte transfusions in refractory fungal infections in neutropenic patients with hematologic malignancies reported favorable responses in 11 of 15 patients.[79] Peters *et al.*[80] evaluated granulocyte transfusions (G-CSF or prednisolone mobilized) in 30 patients with neutropenia and life-threatening, refractory infections. Infections cleared in 20 of 30 patients, including five of nine patients with invasive aspergillosis.

Price *et al.*[77] conducted a phase I/II study of granulocyte transfusions from unrelated, non-HLA-matched, community donors mobilized with G-CSF and dexamethasone for the treatment of hematopoietic transplant recipients with severe infections. The mean transfusion yield was 8.2×10^{10} neutrophils, and 17 of 19 patients had a transient restoration of a normal neutrophil count. Chills, fever and oxygen desaturation of 3% or more occurred in 7% of transfusions, but did not limit therapy. Eight of 11 patients with bacterial infections survived. However, none of 8 patients with invasive filamentous fungal infection survived.

We currently reserve granulocyte transfusions for patients with prolonged neutropenia and life-threatening infection refractory to conventional therapy. Filamentous fungi are likely to constitute the majority of such refractory infections. Infusions of amphotericin B should be separated by several hours from granulocyte transfusions to reduce the likelihood of pulmonary toxicity. In some highly alloimmunized patients, transfused granulocytes are rapidly consumed and are likely to have more toxicity than benefit. In cytomegalovirus-seronegative patients who are likely to be candidates for allogeneic HSCT, we suggest that only cytomegalovirus-seronegative granulocyte donors be used. A prospective randomized study is required to rigorously evaluate the benefits versus toxicity of granulocyte transfusions. In patients with impaired cellular immunity, it is crucial that the granulocytes are irradiated to prevent GVHD from the donor lymphocytes carried in the product.

Interferon-?

Interferon-? is a macrophage activating factor that is critical in host defense against intracellular infections such as *Leishmania* and *Mycobacteria* spp. Interferon-? (IFN-?) augments generation of microbicidal reactive oxidants in phagocytes, and is also a potent activator of oxidant-independent mechanisms, including augmentation of TNF-a production, tryptophan metabolism, granule protein synthesis and major histocompatibility complex surface expression. In several experimental fungal infections, host defense was enhanced by use of cytokines to augment CMI.

Prophylactic IFN-? has been shown to reduce the frequency of serious bacterial and fungal infections in patients with CGD, and is the standard of care in these patients.[81]

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Chapter 112 - Opportunistic Viral Infections

Pierre Reusser

INTRODUCTION

Patients who have had bone marrow or peripheral blood stem cell transplantation (SCT) and recipients of solid organ transplants are at increased risk for severe viral diseases, particularly during the period of marked immunosuppression early after transplantation. DNA viruses are generally a more common cause of infection than RNA viruses, which is related to their propensity to establish long-term latency after primary infection and to reactivate during subsequent periods of immunodeficiency.

During the past two decades, considerable progress has been achieved in the management of diseases due to several herpesviruses, including herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), and cytomegalovirus (CMV) in immunocompromised hosts, and promising treatment approaches have emerged for influenza A and B viruses. Advances were made possible primarily because of the introduction of rapid and sensitive diagnostic techniques, such as shell-vial cultures, antigen detection assays and the polymerase chain reaction (PCR), which permit the detection and treatment of viral infections at an early stage, and also because of the development of novel antiviral drugs.

HERPES SIMPLEX VIRUS INFECTION

EPIDEMIOLOGY

HSV-1 and HSV-2 are distributed worldwide and the seroprevalence in adults reaches approximately 80–95% for HSV-1 and 20–25% for HSV-2. Seropositivity is correlated with increasing age and lower socio-economic status. Without preventive antiviral drug treatment, HSV reactivation occurs in about 65–80% of seropositive patients receiving induction chemotherapy for acute leukemia or an allogeneic SCT, and is observed in one-third of HSV-seropositive kidney graft recipients. [1] [2]

PATHOGENESIS

Herpesviruses establish latency following primary infection. The mechanisms by which latent infection occurs remain to be elucidated. Latent HSV resides in the neuronal cells of sensory nerve ganglia and may reactivate and replicate upon external stimuli and during periods of immunosuppression.[3] HSV then spreads centrifugally via peripheral sensory nerves to the epithelial surface where it causes confined vesicular lesions. The vast majority of HSV infection and disease in adult cancer patients and organ transplant recipients is due to reactivation of latent virus rather than primary infection.[1] [4] HSV-specific cell-mediated immunity appears crucial in containing HSV infection.[1]

PREVENTION

Prevention of HSV disease in seropositive immunodeficient patients is primarily aimed at suppressing viral reactivation by the use of antiviral agents. An efficient prophylaxis of HSV disease was made possible by the introduction of aciclovir, valaciclovir and famciclovir into clinical use.[3] [5] Early studies of aciclovir prophylaxis among HSV-seropositive patients after allogeneic SCT or on chemotherapy for acute leukemia demonstrated a significant reduction of culture-proven HSV disease from about 70% with placebo to 0% with aciclovir.[1] A more recent placebo-controlled trial of valaciclovir for prevention of cytomegalovirus (CMV) disease in renal transplant recipients documented a significantly decreased risk of clinical HSV disease in both seronegative patients (hazard ratio 0.33) and seropositive patients (hazard ratio 0.16).[2] Recommended regimens for antiviral drug prophylaxis of HSV infection in immunocompromised hosts are summarized in Table 112.1 . While the prophylactic use of aciclovir is not justified in seronegative patients, it is recommended for HSV-seropositive patients receiving chemotherapy for hematologic malignancy or undergoing SCT.[6] [7] Furthermore, HSV-seronegative SCT recipients should be advised of behaviors that reduce the risk of HSV transmission by avoiding contact with potentially infectious secretions, such as saliva and cervical secretions.[7] Low-dose aciclovir has protective effects against HSV hepatitis in liver and kidney transplant recipients and is a recommended prophylactic regimen (Table 112.1).[4]

CLINICAL FEATURES AND DIAGNOSIS

HSV infection in immunocompromised hosts is usually associated with localized mucocutaneous disease which develops in the orofacial region in 85–90% of cases and the genital area in 10–15%.[1] [4] The diagnosis of mucocutaneous HSV lesions can often be made on clinical grounds and may be confirmed by virus cultures or PCR. Virus culture is the standard method for detection of HSV in clinical specimens and usually yields results within 48 hours of inoculation. Moreover, virus culture permits typing of isolates and testing for antiviral drug resistance. Antigen detection is more rapid, but the sensitivity of this assay diminishes with advanced stages of HSV lesions. The PCR is a highly sensitive and specific method for detection of HSV infection and has proved particularly useful in the diagnosis of HSV encephalitis.[3] In cancer patients with severe mucositis following chemotherapy or irradiation, the diagnosis of oropharyngeal HSV disease can be difficult and the identification of virus by culturing or PCR detection may be necessary (Fig. 112.1).[1] [6] In general, serological results are not helpful in confirming the diagnosis of active HSV infection in immunodeficient hosts but allow identification of those patients who might benefit from antiviral drug prophylaxis.

Immunosuppressed patients are at risk of developing disseminated mucocutaneous or visceral HSV infection which is associated with an increased morbidity and mortality.[1] [4] A frequent visceral manifestation of HSV infection in immunocompromised cancer patients is esophageal disease. In prospective endoscopic studies among patients with malignancy who had upper gastrointestinal symptoms, esophagitis associated with HSV was documented in about 10% of

TABLE 112-1 -- Antiviral drug prophylaxis and therapy of HSV infection and disease.[3] [5] [6]

ANTIVIRAL DRUG PROPHYLAXIS AND THERAPY OF HSV INFECTION AND DISEASE			
Indication	**Drug**	**Dose and route**	**Duration**
Prophylaxis			
HSV-seropositive patients	Aciclovir	250mg/m^2 or 5mg/kg every 12h iv	For the period of the most severe immunosuppression (usually 2–3 months)
		or	
		200–400mg 3 times daily to 800mg twice daily po	
	Valaciclovir	500–1000mg twice daily po	
	Famciclovir	500mg twice daily po	
Therapy			
Mucocutaneous or esophageal HSV disease	Aciclovir	250mg/m^2 or 5mg/kg every 8h iv	7–10 days
		or	
		200–400mg 5 times daily po	
	Valaciclovir	500–1000mg twice daily po	7 days
	Famciclovir	500mg twice daily or 250mg 3 times daily po	7 days
HSV encephalitis, pneumonia	Aciclovir	10–15mg/kg every 8h iv	14–21 days

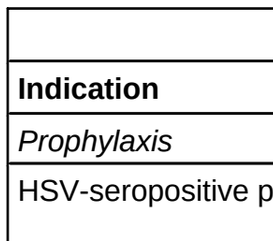

Figure 112-1 Hemorrhagic labial HSV lesions in a patient with leukemia and chemotherapy-induced thrombocytopenia. Diagnosis of HSV disease may be difficult and needs confirmation by virus culture or PCR.

cases.[1] Dysphagia and retrosternal pain with abrupt onset are the most common complaints and patients may also experience otherwise unexplained nausea and vomiting. The specific diagnosis of HSV esophagitis requires both virologic and histologic evidence of HSV infection. For endoscopic biopsy of suspected lesions in thrombocytopenic patients, a minimum platelet count of 50 × 10^9 /l is recommended. Infrequent but life-threatening presentations of HSV disease in immunodeficient hosts include hepatitis, pneumonia, encephalitis and disseminated infection.[1] [4] [6]

MANAGEMENT

Intravenous aciclovir is the therapy of choice for severe mucocutaneous or visceral HSV disease in immunocompromised hosts.[4] [5] [6] Intravenous aciclovir therapy administered for mucocutaneous HSV disease in various immunosuppressed patients significantly shortens the duration of virus shedding and lesion pain, and induces more rapid lesion healing. Oral aciclovir is also documented to be effective therapy of mucocutaneous HSV disease in allogeneic SCT recipients.

The newer antiviral compounds valaciclovir and famciclovir share a high oral bio-availability that is up to five-fold higher than that of

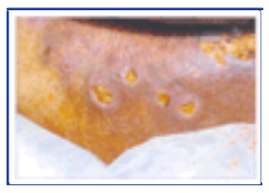

Figure 112-2 Aciclovir-resistant perineal ulcerative HSV lesions after allogeneic SCT.

oral aciclovir, allowing less frequent dosing during therapy of HSV disease. Both agents may be considered as therapeutic alternatives for less serious forms of HSV disease in immunodeficient hosts.[3] [5] Data on the therapy of life-threatening conditions, such as HSV pneumonia and HSV encephalitis in immunosuppressed patients, are limited but outcome may be more favorable with early initiation of high-dose intravenous aciclovir.[3] Table 112.1 lists recommended regimens for antiviral therapy of HSV disease in immunocompromised hosts.

With the introduction of antiviral drug treatment of HSV infection, the emergence of resistant HSV isolates that cause disease unresponsive to therapy has been reported with increasing frequency.[8] In a large retrospective cohort study, the rate of drug-resistant HSV infection among 148 immunodeficient patients previously exposed to aciclovir was 5% (14% after SCT, 8% after pancreas transplant, 5% after kidney transplant, 0% after liver or heart transplant, and 0% in nontransplant cancer patients).[9] Patients with aciclovir-resistant mucocutaneous HSV disease present well-demarcated ulcerative lesions that do not improve during treatment (Fig. 112.2). HSV resistance is observed almost exclusively among patients receiving therapeutic regimens, while

Figure 112-3 Mechanisms of HSV and VZV resistance to antiviral drugs. Most cases of HSV and VZV resistance are due to mutations in the gene of the virus-encoded thymidine kinase, which result in a reduced or abrogated conversion of aciclovir to aciclovir monophosphate. This leads to low or absent levels of aciclovir triphosphate, the active metabolite that inhibits the viral DNA polymerase and acts as DNA chain terminator. Viral replication may then occur despite aciclovir therapy. Foscarnet and cidofovir do not require viral thymidine kinase-dependent intracellular activation. Resistance to these two drugs may occur if HSV or VZV resistance is caused by mutations in the viral DNA polymerase gene *(adapted from[8])*.

prophylactic treatment does not appear to increase the risk for resistant virus strains. [8] [9]

The most common mechanism of HSV resistance is altered or deficient activity of the virus-encoded thymidine kinase which results in reduced or abrogated phosphorylation of aciclovir in HSV-infected cells (Fig. 112.3).[8] Less frequently, mutations in the gene encoding for the viral DNA polymerase lead to resistant HSV strains (Fig. 112.3). Aciclovir-resistant HSV isolates were shown to be susceptible to antiviral agents, such as foscarnet, that do not require intracellular activation. [8] Foscarnet was used successfully as alternative systemic therapy in several cases of aciclovir-unresponsive HSV disease in immunocompromised patients, but multiresistant HSV strains may occur when resistance is due to mutations in the viral DNA polymerase gene.[8] [10] In the case of treatment-refractory HSV disease, isolates should be tested for drug resistance. Therapeutic options in the case of drug-resistant HSV disease are shown in Table 112.2 . If aciclovir-unresponsive HSV lesions are accessible, systemic

TABLE 112-2 -- Alternative therapy for drug-resistant herpesvirus disease*

ALTERNATIVE THERAPY FOR DRUG-RESISTANT HERPESVIRUS DISEASE		
Virus	**Resistant to**	**Alternative therapy**
HSV	Aciclovir, valaciclovir, famciclovir	Foscarnet 60mg/kg twice daily or 40mg/kg 3 times daily iv for 7–21 days or until complete healing
		For accessible HSV lesions:
		• topical trifluridine 5% ophthalmic solution every 8h
		• topical cidofovir gel 0.3% or 1% once daily until complete healing
	Foscarnet	Cidofovir 5mg/kg iv once a week for 2 weeks, then once every 2 weeks until complete healing
VZV	Aciclovir, valaciclovir, famciclovir	Foscarnet 60mg/kg 2–3 times daily iv for 7–14 days or until complete healing
CMV	Ganciclovir	Foscarnet 60mg/kg 3 times daily or 90mg/kg twice daily iv for 2 weeks, then 90–120mg/kg once daily iv until complete recovery from CMV disease

* *(adapted from[6])*.

antiviral therapy may be replaced by topical trifluridine solution or cidofovir gel (Table 112.2).[8]

VARICELLA-ZOSTER VIRUS INFECTION

EPIDEMIOLOGY

Varicella (chickenpox)

Primary VZV infection causes varicella (chickenpox) and occurs in over 90% of cases in children under the age of 14 years. Following the introduction of a live attenuated varicella vaccine for persons 12 months of age or older, varicella cases have markedly declined in areas of extended vaccine coverage.[11] Varicella develops in 2–3% of children after allogeneic or autologous SCT for solid tumor or leukemia.[12] [13]

Herpes zoster (shingles)

The most common manifestation of VZV infection in immunocompromised hosts is herpes zoster which is due to reactivation of latent virus. Zoster is particularly frequent in adults with lymphoreticular malignancy or leukemia who have impaired cell-mediated immune responses. The incidence of zoster after SCT ranges from 14% to 32% and is highest in autograft recipients with Hodgkin's disease.[12] [13] [14] Median time to onset of zoster is 5 months after SCT and over 85% of cases occur within the first post-transplant year.[12] During the first 6 months after solid organ transplantation, zoster develops in 5–13% of patients and presents most often as localized dermatomal rash.[4]

PATHOGENESIS

Following primary infection, VZV establishes latency in dorsal root ganglia where it appears to reside in neurons and satellite cells.[15] In contrast to HSV, VZV reactivation yields extensive viral proliferation and cell-to-cell spread, resulting in numerous neurons infected and dermatomal vesicular rash; in this process, the cumulative damage to the ganglion and its neurons is substantial which explains the occurrence of neuralgia. Both humoral and cell-mediated immunity play a role in limiting the infectivity and replication of VZV.[16] Virus-specific cellular immunity appears to be particularly important in controlling

viral reactivation and dissemination.[15] [16] Immunocompromised children who fail to acquire a VZV-specific T-cell response during varicella are at risk for persistent viremia and life-threatening viral dissemination. Conditions leading to a deficient VZV-specific cellular immune response render VZV-seropositive patients susceptible to herpes zoster, which can disseminate and cause serious organ disease during profound immunosuppression.[16]

PREVENTION

Varicella

VZV is highly contagious and immunodeficient hosts susceptible to varicella should be isolated from infectious individuals. A critical issue is that patients with varicella may be contagious up to 2 days before the onset of mucocutaneous eruptions and transmission may occur before isolation precautions are taken. VZV-seronegative immunocompromised patients may benefit from intravenous VZV immune globulin infusions if administered within 96 hours of exposure.[15]

Active immunization with a live-attenuated VZV vaccine is safe and has protective effects in varicella-susceptible children with leukemia in remission or receiving a kidney graft. Seroconversion was documented in 98% of leukemic children after 1–2 doses of vaccine and was associated with a decreased attack rate of varicella after household exposure from 29% among seronegative children to 8%. [17] Vaccinated children and adolescents after renal transplantation developed a specific antibody response that remained detectable in 62% of cases at 1 year, and had a posttransplant incidence of varicella of 12% compared to 45% in unvaccinated patients with no prior history of varicella.[18]

In summary, the use of a live-attenuated VZV vaccine is recommended for immunization of VZV-seronegative leukemic (in remission) or renal transplant children (Table 112.3).

Herpes zoster

VZV vaccines reduce the risk of zoster in immunocompromised patients. Children with leukemia who were given a live-attenuated varicella vaccine had significantly lower rates of subsequent zoster (0.80 cases per 100 person-years) than those who had natural varicella infection (2.46 cases per 100 person-years).[19] Among VZV-seropositive patients who underwent autologous SCT for non-Hodgkin's or Hodgkin's lymphoma, boosting specific immunity by

TABLE 112-3 -- Prevention and therapy of VZV infection and disease.[5] [6]

PREVENTION AND THERAPY OF VZV INFECTION AND DISEASE			
Indication	**Drug**	**Dose and route**	**Other measures/comments**
Prevention			
Varicella	Not recommended		Strict isolation from infectious individuals
			Intravenous VZV immune globulins if within 96h of exposure
			Live-attenuated VZV vaccine in VZV-seronegative leukemic children in remission or before renal transplantation
Zoster	Not recommended		Inactivated VZV vaccine in VZV-seropositive SCT recipients
Therapy			
Varicella	Aciclovir	500mg/m^2 or 10mg/kg every 8h iv for 7–10 days	
Herpes zoster	Aciclovir	As above iv or 800mg 5 times daily po	For moderately immunosuppressed patients without visceral disease (7–10 days of therapy)
	Valaciclovir	1000mg 3 times daily po	
	Famciclovir	500mg 3 times daily po	

an inactivated varicella vaccine significantly decreased the incidence of zoster within the first 12 months after SCT (13% vs 30% in unvaccinated autograft recipients) and is therefore recommended (Table 112.3).[20] After renal transplantation, the rates of zoster were 7% in vaccinated patients compared to 13% in unvaccinated patients with a history of varicella and 38% in the VZV-naive patients who developed posttransplant varicella.[18]

The long-term use of oral aciclovir as another strategy for the prevention of zoster after allogeneic SCT has been assessed in placebo-controlled trials. Aciclovir prophylaxis given for 6 months after allogeneic SCT abrogates VZV reactivation during treatment. However, patients who receive aciclovir develop zoster at the usual rate following discontinuation of prophylaxis and the overall incidence 1 year after SCT was similar in aciclovir and placebo groups.[6] Thus, aciclovir prophylaxis for 6 months after SCT only delays VZV reactivation but does not reduce the overall incidence of zoster. Moreover, a concern regarding long-term aciclovir prophylaxis is the

potential of inducing resistant VZV strains, as observed in patients with AIDS.[8]

Recommended preventive measures for varicella and herpes zoster in immunosuppressed patients are summarized in Table 112.3 .

CLINICAL FEATURES AND DIAGNOSIS

Varicella in immunocompromised hosts is characterized by more numerous mucocutaneous lesions which may be hemorrhagic and which take about three times longer to heal than among individuals without impaired immunity (Fig. 112.4). In children with malignancy, varicella has a high risk of visceral dissemination in the absence of specific therapy. Among 127 children with untreated varicella, VZV pneumonia occurred in 32% of patients with acute leukemia and in 19% of those with other cancers, and was fatal in 7% of cases.[21] Although rare, primary VZV infection in solid organ transplant recipients can lead to hemorrhagic pneumonia, hepatitis, pancreatitis, encephalitis and disseminated intravascular coagulation which may be life threatening.[4] Figure 112.5 shows bilateral VZV pneumonia in a kidney graft recipient with varicella.

Herpes zoster in immunodeficient hosts tends to be more severe and of longer duration than in patients with normal immunity. In a series of 195 SCT recipients with zoster, cutaneous dissemination developed in 23% and visceral involvement in 13%, and the fatality

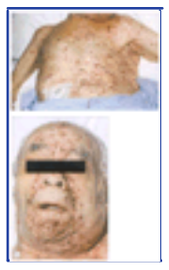

Figure 112-4 (a,b) **Disseminated cutaneous lesions in a patient with chronic lymphocytic leukemia who developed fatal varicella despite rapid initiation of intravenous aciclovir therapy.** Varicella lesions are numerous and hemorrhagic.

rate was 18%.[12] Risk factors for VZV infection after SCT as identified by multivariate analyses include age 10 years or older, the use of radiation in pretransplant conditioning, allografting, the occurrence of severe acute or chronic graft versus host disease (GVHD), and SCT for chronic myeloid leukemia. [12] [13]

Diagnosis of varicella and herpes zoster is usually made clinically based on history and the typical rash. During primary VZV infection, generalized vesicular lesions at varying stages are present on the skin and may also be seen on the oropharyngeal mucosa. Localized herpes zoster manifests as unilateral dermatomal eruptions (Fig. 112.6). Confirmation of VZV infection can be obtained by direct immunofluorescence detection of virus antigens which is a rapid and sensitive technique.[15] Virus culture can also be used but is positive in only 30–60% of cases.[15] However, isolation of virus by culture permits testing for antiviral susceptibility if drug resistance is suspected.[8] Visceral organ involvement requires documentation of VZV in biopsies by culture, immunohistology or PCR. For VZV pneumonia, identification of VZV in bronchoalveolar lavage

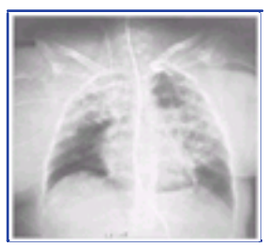

Figure 112-5 Varicella pneumonia in a renal transplant recipient with bilateral lung infiltrates on chest radiography.

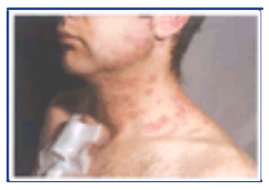

Figure 112-6 Herpes zoster in cervical dermatomes in a patient 5 months after allogeneic SCT for leukemia.

fluid may substitute for lung biopsy. PCR can also be used for detection of VZV infection of the central nervous system.[15] Results of serological assays are generally not helpful in diagnosing VZV infection in immunodeficient patients.

MANAGEMENT

A major goal of antiviral treatment of varicella or herpes zoster in immunocompromised hosts is the prevention and therapy of visceral dissemination which is associated with substantial morbidity and mortality.[12] [13] Aciclovir therapy was shown to be safe and to markedly decrease the risk for VZV dissemination and VZV disease-related mortality. [6] Intravenous aciclovir remains the treatment of choice for established varicella and zoster in profoundly immunodeficient patients (Table 112.3).[4] [5] [15] For moderately immunosuppressed patients without evidence of visceral VZV disease, high-dose oral aciclovir, valaciclovir or famciclovir are possible treatment alternatives (Table 112.3).[5] [15] [22]

Most drug-resistant VZV isolates have been recovered from patients with AIDS but have also been documented occasionally in SCT recipients.[8] [23] VZV resistance to aciclovir is due to altered or deficient thymidine kinase function (see Fig. 112.3).[8] Foscarnet does not require intracellular activation by the viral thymidine kinase, and may be used as alternative therapy when aciclovir-resistant VZV infection is suspected (see Table 112.2).[8] [23]

CYTOMEGALOVIRUS INFECTION

EPIDEMIOLOGY

CMV has a worldwide distribution and infects up to 50–70% of the population in industrialized countries. Immunocompromised hosts are at elevated risk of serious CMV disease following primary infection, reinfection or reactivation of latent virus. CMV is a leading infectious cause of morbidity and mortality in patients after SCT and solid organ transplantation, and is an emerging problem in adults with leukemia.[4] [6] [24] In the absence of preventive measures, CMV infection occurs in 60–70% of allogeneic SCT recipients when patient or donor are CMV seropositive, and is documented in about 50–60% of CMV-seropositive autograft recipients.[6] The strongest predictor of CMV infection after allogeneic SCT is pretransplant CMV seropositivity of the patient (fivefold risk increase).[25] Other predisposing factors include the occurrence of acute GVHD, HLA-mismatched transplant, and a positive CMV serology of the graft donor in seronegative patients.[25] The overall incidence of CMV infection after kidney, heart or liver transplantation is in the range of 44–85%, and the vast majority of CMV infections occur during the first 3–4 months after transplant when patients require intensive immunosuppressive regimens for prevention or therapy of graft rejection.[4]

PATHOGENESIS

Following primary infection, CMV remains in the host in a latent state and can reactivate intermittently, particularly during times of deficient cell-mediated immunity.[26] [27] The precise sites and mechanisms of latency remain to be elucidated but vascular endothelial cells, peripheral blood leukocytes, epithelial kidney cells and salivary glands are possible sites of latency or low-level replication. Transplant recipients may harbor latent CMV, but can also acquire the virus from seropositive organ or blood donors. CMV disease after SCT or solid organ transplantation correlates with the systemic viral load, as assessed by CMV antigenemia or quantitative PCR in peripheral blood.[27] [28] [29]

PREVENTION

In CMV-seronegative patients treated for leukemia or receiving an organ transplant from a seronegative donor, prevention of CMV infection is primarily based on avoiding the acquisition of exogenous virus. Primary CMV infection in these patients can efficiently be prevented by the exclusive use of CMV seronegative or leukocyte-depleted blood products.[30] Although this preventive strategy has only been validated in SCT recipients, it is also frequently used after solid organ transplantation.[30]

Passive immunization with intravenous immune globulins for prevention of CMV infection and disease after SCT remains controversial. Conflicting results between studies may be due to differences in the administration schedules, dosages, kinetics or antibody contents of the immune globulins used.[6] In view of the unclear benefit and the important costs of immune globulin infusions, CMV prophylaxis by passive immunization in SCT recipients cannot be advocated without further studies. By contrast, patients after solid organ transplantation may have some benefit from prophylactic immune globulin infusions. Except for CMV-seronegative patients with a seropositive organ donor, CMV immune globulins decrease the rate of severe CMV disease after liver transplantation.[31] In renal transplant recipients, however, CMV-seronegative patients with a seropositive donor who receive CMV immune globulin infusions have a reduced incidence of CMV syndrome (defined as illness having =2 of the features known to be associated with CMV, namely otherwise unexplained fever for =3 days plus pneumonitis without other cause, leukopenia, elevated serum alanine aminotransferase levels or atypical lymphocytosis) and a trend towards lower rates of CMV pneumonia.[32] Thus, the protective efficacy of CMV immune globulins may vary according to the type of solid organ transplantation.

Active immunization with a live-attenuated Towne strain of CMV is safe and decreases the severity but not the incidence of CMV disease among donor-seropositive/recipient-seronegative renal transplants.[4] Further immunization studies are being carried out with CMV subunit vaccines.

Antiviral drugs are used for suppression of CMV reactivation in CMV-seropositive patients or in organs transplanted from seropositive donors, and play an important role in the prevention of CMV disease. Valaciclovir, ganciclovir, valganciclovir, foscarnet and cidofovir are currently available systemic antiviral drugs for the management of CMV infection and disease, and will be discussed in more detail in the section on Management, below. Preventive measures for CMV infection and disease after allogeneic SCT are listed in Table 112.4 .

CLINICAL FEATURES AND DIAGNOSIS

The manifestations of CMV infection in immunodeficient hosts range from asymptomatic virus excretion to serious organ disease. In the past, various definitions of CMV infection and disease have been used, which renders the comparison of published data difficult. An effort was made to standardize the definitions of CMV infection and CMV disease in immunocompromised hosts at two international consensus conferences (Table 112.5). [33] [34]

Diagnosis of *CMV infection* is based on detection of CMV in clinical specimens by conventional tissue culture or rapid culture with confirmation by specific monoclonal antibodies, or by detection of the lower matrix pp65 CMV antigen in peripheral blood leukocytes (Fig. 112.7). Positive PCR is valid if the technique used was shown to correlate with CMV detection by culture methods. One exception is the demonstration of CMV by PCR in cerebrospinal fluid which is reliable, whereas virus cultures are insensitive. Histopathology, immunochemistry and in situ hybridization in biopsy materials are commonly used, but the sensitivity and specificity of these techniques are not well documented and need further clarification. Serological approaches are considered less reliable in immunocompromised patients, and are not recommended for the definition of CMV infection. Diagnosis of *CMV disease* requires documentation of CMV in tissue specimens or in bronchoalveolar fluid when CMV pneumonia is suspected, and detection of CMV must be associated with clinical symptoms and signs compatible with CMV organ disease.

The most severe manifestation of CMV disease after SCT is CMV pneumonia. Patients with CMV pneumonia usually present with fever, nonproductive cough, tachypnea, rales and uni- or bilateral interstitial lung infiltrates on chest X-rays (Fig. 112.8). Without pre-emptive therapy, CMV pneumonia develops in one-third of allograft recipients with active infection, and is fatal in 85% of cases if untreated.[25] [35] CMV pneumonia is less frequent after autologous SCT, but outcome is as serious as in allograft recipients.[36] CMV

TABLE 112-4 -- Prevention and therapy of CMV infection and disease after allogeneic SCT. [6] [38] [45]

PREVENTION AND THERAPY OF CMV INFECTION AND DISEASE AFTER ALLOGENEIC SCT			
Indication	**Drug**	**Dose and route**	**Other measures/comments**
Prevention			
Donor and patient CMV seronegative			CMV seronegative or leukocyte-depleted blood products
Donor and/or patient CMV positive	Ganciclovir	5mg/kg every 12h iv for 5 days, then once daily	From engraftment until day 100 after SCT
Pre-emptive therapy			
Positive CMV antigenemia or PCR in peripheral blood specimen	Ganciclovir	5mg/kg every 12h iv for 14 days, then 6mg/kg once daily iv for 5 days per week	Discontinuation of treatment after 14 days (or later) if CMV surveillance test negative
	Foscarnet	60 mg/kg every 12h iv for for 14 days, then 90mg/kg once daily iv for 5 days per week	Discontinuation of treatment after 14 days (or later) if CMV surveillance test negative
Therapy			

CMV pneumonia	Ganciclovir	5mg/kg every 12h iv for 14 days, then 5–6mg/kg once daily iv for additional 30 days or until complete recovery	
	plus		
	Intravenous immune globulins	500mg/kg every other day for 2 weeks, then 500mg/kg twice per week for additional 4 weeks	
Other CMV disease	Ganciclovir	As for CMV pneumonia	Ganciclovir alone probably adequate

TABLE 112-5 -- Definitions of CMV infection and CMV disease.[33] [34]

DEFINITIONS OF CMV INFECTION AND CMV DISEASE
CMV infection
Accepted diagnostic methods:
• conventional tissue culture
• shell-vial culture
• antigenemia (detection of pp65 lower-matrix protein in leukocytes)
• PCR in cerebrospinal fluid or in other specimens if PCR shown to correlate with culture results
Probably adequate diagnostic methods:
• histopathology
• immunochemistry
• *in-situ* hybridization
Inadequate for diagnosis of active infection:
• serological assays
CMV disease
• Documentation of CMV in tissue specimens or, in the case of CMV pneumonia, in bronchoalveolar lavage fluid
• Associated symptoms and signs of CMV organ disease

gastrointestinal disease may occur after SCT, whereas other organ diseases, such as CMV retinitis, are rare.[25] Detection of CMV in peripheral blood by culture, antigenemia or PCR is a major predictor for CMV disease. [25] [37] [38] Moreover, absence of a CMV-specific cytotoxic T-cell response in the first 3 months after allogeneic SCT is associated with a high risk for CMV pneumonia.[39]

Adults with leukemia who receive conventional chemotherapy are also at risk for CMV pneumonia. In a series of 2136 leukemic patient, 2.9% developed CMV pneumonia which was fatal in 57% of cases (case-fatality rate of 62% with antiviral therapy vs 50% without treatment).[24]

Without prophylaxis, CMV causes significant morbidity in all types of solid organ transplantation. Symptomatic CMV disease occurs in 10–65% of patients during the first 4 post-transplant months.[2] [27] [29] [40] [41] As in SCT recipients, CMV disease after solid

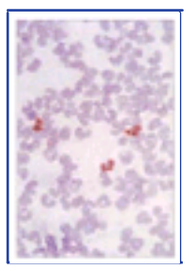

Figure 112-7 Positive CMV antigenemia assay. Three peripheral blood leukocytes containing the pp65 CMV antigen show positive immunoperoxidase staining.

organ transplantation has a propensity to develop in the lungs and in the gastrointestinal tract. Patients, however, have an additional susceptibility for CMV infection in the transplanted organ.[40] The lowest rates of CMV infection and disease are observed when patient and organ donor are both CMV seronegative. CMV-seropositive patients and seronegative patients with a seropositive organ donor show similar rates of CMV infection in most series, but primary CMV disease is generally more frequent and more severe than disease due to reactivation or reinfection. [2] [40] [41] Patients who require OKT3 or antilymphocyte globulins for therapy of graft rejection carry an additional risk for severe CMV disease.[42] Mortality from CMV disease in solid organ transplant recipients is primarily related to serious pneumonia with respiratory failure or to disseminated disease.

MANAGEMENT

Established CMV pneumonia in SCT recipients is a life-threatening condition which is generally treated by a combination of intravenous

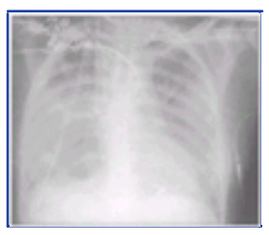

Figure 112-8 CMV pneumonia in an allogeneic SCT recipient with diffuse bilateral interstitial lung infiltrates on chest radiography.

ganciclovir and high-dose intravenous immune globulin infusions. However, the survival rate averages only about 57% early after completion of therapy, and ranges from 22–40% 6 months later.[6] CMV pneumonia was recently reported to also respond to cidofovir therapy in 56% of allograft recipients.[43] Nonleukemic deaths in patients who initially survive CMV pneumonia are often due to Gram-negative sepsis and invasive fungal infections which occur at a higher rate than among patients who never developed CMV pneumonia.[6] Because outcome of CMV pneumonia remains severe despite best available therapy, major emphasis must be placed on the prevention of CMV disease after SCT.

Following solid organ transplantation, single-agent therapy with intravenous ganciclovir is most widely used for therapy of CMV disease, and outcome appears to be favorable in most cases, independent of the site of CMV disease or the type of organ transplant.[4] In a series of various solid organ transplant recipients with tissue-invasive CMV disease, ganciclovir therapy resulted in resolution of symptoms and signs in 89% of patients, and no deaths were directly attributable to CMV disease.[44]

For CMV-seropositive transplant recipients or seronegative patients with a seropositive organ donor, two main strategies are currently available for prevention of CMV disease. Antiviral drug *prophylaxis* is aimed at suppressing CMV reactivation, and is given to all patients irrespective of the results of virologic monitoring.[2] [41] [45] [46] Alternatively, *pre-emptive therapy* consists of initiating antiviral drug treatment only when active CMV infection is demonstrated to prevent the development of CMV disease.[37] [38] [47] Thus, with pre-emptive therapy, the use of potentially toxic antiviral drugs is restricted to patients at the highest risk for CMV disease.

Following allogeneic SCT, intravenous ganciclovir prophylaxis reduces the rates of CMV disease, but has no impact on survival when compared to placebo.[45] [46] The

lack of survival advantage with ganciclovir prophylaxis may be related to a higher number of deaths from nonviral infections and to incomplete protection from fatal CMV pneumonia after SCT. By contrast, pre-emptive ganciclovir therapy based on positive CMV cultures substantially decreases the incidence of CMV disease, and is associated with significantly better survival than in controls receiving placebo. [47] A disadvantage of culture-based pre-emptive therapy, however, is that over 10% of patients screened are diagnosed with CMV disease without prior positive CMV cultures or with cultures becoming positive coincident with the onset of disease.[47] Newer diagnostic techniques, such as the CMV antigenemia assay or the PCR in peripheral blood specimens, are more sensitive and more rapid than viral cultures and permit the detection of CMV infection at an early stage when the systemic viral load is still low.[37] [38] In a trial comparing CMV PCR monitoring in blood specimens with virus cultures for initiation of pre-emptive ganciclovir therapy after SCT, CMV infection was detected a median of 17 days earlier by PCR, which resulted in significantly lower incidence of CMV disease and superior patient survival.[37] The introduction of antigenemia assays and PCR for detection of CMV in blood has thus greatly enhanced the efficacy of the pre-emptive therapy approach to the prevention of CMV disease.

Because of the important hematotoxicity observed with ganciclovir use after SCT, alternative drug treatment has been investigated. Prophylactic and pre-emptive intravenous foscarnet can safely be given after SCT and pre-emptive therapy with foscarnet shows similar efficacy as ganciclovir, but is associated with a lower rate of severe neutropenia and of patients who require discontinuation of antiviral therapy due to hematotoxicity.[38] Preliminary retrospective data on pre-emptive cidofovir treatment of CMV infection after allogeneic SCT are promising, but await confirmation by prospective trials.[43] Among allograft recipients who are able to take oral medication, oral valganciclovir might become an alternative to current intravenous treatment regimens if shown to be efficacious and safe.[48]

With the introduction of antiviral drug prophylaxis and pre-emptive therapy against CMV during the first 100 days after SCT, the occurrence of late CMV disease (beyond day 100 post transplant) has become an increasingly recognized problem. Late CMV disease after allogeneic SCT is now observed in 5–10% of patients and is associated with T-cell depletion, chronic GVHD, high viral load in plasma and prolonged (>4 weeks) prior antiviral drug treatment against CMV.[49] [50]

Following solid organ transplantation, the need for antiviral CMV prophylaxis is less clear than after SCT, since CMV disease appears to respond to ganciclovir therapy in most cases, and CMV-related mortality has become virtually nonexistent.[44] Nevertheless, morbidity due to CMV infection and disease after solid organ transplantation is significant in the absence of preventive measures, and may have an important impact on costs. CMV drug prophylaxis may furthermore have beneficial effects on organ outcomes, including rejection.[2] Prophylactic use of aciclovir, valaciclovir or ganciclovir decreases the incidence of CMV disease in various solid organ transplant settings.[2] [4] [41] As a result of its high oral bioavailability, valganciclovir may play a role in the future in replacing both intravenous and oral ganciclovir prophylaxis after solid organ transplantation.[48] [51]

Pre-emptive short-course antiviral treatment of CMV infection reduces drug exposure and is advocated as an alternative to prophylaxis after solid organ transplantation.[4] As observed in SCT recipients, patients after solid organ transplantation may develop CMV disease coincident with or without previous viral excretion. Pre-emptive antiviral treatment based on surveillance cultures might miss a substantial fraction of patients who will develop CMV disease and virologic surveillance by CMV antigenemia or PCR is therefore preferable.

Another concept in solid organ transplant recipients is the prophylactic use of antiviral drugs against CMV only during periods of more intensive immunosuppression irrespective of the results of viral monitoring. A significantly reduced rate of CMV disease was demonstrable among renal transplant recipients who received ganciclovir during antilymphocyte antibody therapy.[52]

Figure 112-9 Mechanisms of CMV resistance to antiviral drugs. Most cases of CMV resistance are due to mutations in the viral UL97 gene encoding for the phosphotransferase, which result in a reduced or abrogated conversion of ganciclovir to ganciclovir monophosphate. This leads to low or absent levels of ganciclovir triphosphate, the active metabolite that inhibits the viral DNA polymerase. Viral replication may then occur despite ganciclovir therapy. Foscarnet and cidofovir do not require viral phosphotransferase-dependent intracellular activation. Resistance to these two drugs may occur if CMV resistance is caused by mutations in the viral DNA polymerase gene *(adapted from[9]).*

CMV resistance to antiviral drugs is an increasing clinical problem in patients with AIDS and after solid organ transplantation and has infrequently been reported in SCT recipients.[8] [53] [54] Most CMV strains are resistant to ganciclovir, but CMV isolates may also be resistant to foscarnet or cidofovir.[53] [55] Drug resistance can be due to mutations in the UL97 phosphotransferase gene of CMV, to mutations in the UL54 gene encoding the viral DNA polymerase, or to both (Fig. 112.9).[55] Cases of ganciclovir-resistant CMV disease due to UL97 gene mutations generally improve or recover completely with intravenous foscarnet treatment (see Table 112.2).[8] Foscarnet, however, is not an alternative to ganciclovir when resistance is caused by mutation in the viral DNA polymerase gene (Table 112.2). Patients carrying multidrug-resistant CMV strains have been reported.[53] [55] The introduction of more rapid and efficient techniques for detection of resistant CMV isolates should allow us to better establish the incidence and clinical importance of CMV resistance in immunocompromised hosts, and alternative treatment options need to be developed and validated for (multi)drug-resistant CMV disease.

OTHER HERPESVIRUSES

EPSTEIN-BARR VIRUS INFECTION

Epstein-Barr virus (EBV) infection occurs in more than 90% of the normal population, and immunocompromised patients are predominantly at risk for disease due to EBV reactivation.[56] While EBV infection may cause fever, sore throat and malaise, EBV-induced posttransplantation lymphoproliferative disease (PTLD) can develop as a serious complication in solid organ transplant recipients and after allogeneic T cell-depleted SCT.[4] [56] [57] The incidence of PTLD varies with the organ transplanted and ranges from 1% for renal to 14% for small bowel transplant recipients, and is virtually always fatal in the absence of therapeutic measures.[4] [56] The clinical manifestations of EBV-associated PTLD may include fever, tonsillitis, lymphadenopathy, hepatosplenomegaly and symptoms and signs from other affected organs.

The pathogenesis of PTLD involves EBV-induced B-cell stimulation and transformation that occurs particularly after OKT3 or antilymphocyte globulin therapy, followed by cyclosporin treatment for suppression of graft rejection.[4] Insufficient immune control of EBV replication appears to be an important initial step in the pathogenesis of PTLD. EBV-related tumors tend to progress from polyclonal to monoclonal phenotypes, but can also present mixed phenotypes.[56]

Aciclovir, ganciclovir and foscarnet have inhibitory activity against EBV *in vitro* and antiviral drug therapy may contribute to improved outcome in early stages of EBV-induced B-cell transformation. In fully established monoclonal PTLD, antiviral agents are inefficient and marked reduction or complete withdrawal of immunosuppression is recommended, but outcome remains poor.[4] [56] A more promising approach to prevention and treatment of EBV-associated PTLD in transplant recipients is the restoration of specific T-cell immunity by infusion of donor-derived leukocytes or EBV-specific cytotoxic T-cell lines.[57] [58]

HUMAN HERPESVIRUS 6 AND 7 INFECTION

Primary infection of human herpesvirus 6 (HHV-6) causes roseola (exanthema subitum) in early childhood and human herpesvirus 7 (HHV-7) is also possibly involved in this syndrome. The seroprevalence of both HHV-6 and HHV-7 in healthy adults exceeds 80%. HHV-6 infection occurs in 38–60% of patients after autologous or allogeneic SCT and in 31–55% of solid organ transplant recipients and is most commonly detectable within 4 weeks of transplantation.[59] [60] The virus has two variants, A and B, and transplant recipients appear to be infected by variant B in most cases. Following SCT, HHV-6 infection is correlated with the severity of acute GVHD and risk factors for symptomatic HHV-6 infection include the use of a bone marrow graft, HHV-6 reactivation before engraftment and the presence of HHV-6 DNA in plasma.[60] HHV-6 infection in transplant recipients has been associated with bone marrow suppression, interstitial pneumonia and encephalitis, but the causative role of HHV-6 for these disease manifestations seems only established for encephalitis.[59] [61]

The clinical role of HHV-7 in immunocompromised hosts is less well defined. HHV-7 appears to be a co-factor in the development of CMV disease in renal and liver transplant recipients and may contribute to graft rejection.[62] [63]

The antiviral agents ganciclovir and foscarnet show inhibitory activity against HHV-6 *in vitro*, whereas aciclovir is less effective.[59] [64] Clinical data on the efficacy of antiviral drugs against HHV-6 are limited and no controlled therapeutic trials in immunodeficient hosts

have been reported to date. In the opinion of experts, intravenous ganciclovir or foscarnet therapy may be used for HHV-6 disease if active infection is documented by cell culture, shell-vial assay, PCR or immunohistochemistry.[59] [61] Because of a lack of data, no recommendations can be made regarding the clinical use of antiviral agents against HHV-7 at this time.

HUMAN HERPESVIRUS 8 INFECTION

Human herpesvirus 8 (HHV-8) is the causative agent of Kaposi's sarcoma and other rare disorders, including primary effusion B-cell lymphoma and multicentric Castleman's disease. The seroprevalence of HHV-8 among healthy adults varies by geographic location. It was reported to be 5% among US blood donors and up to 10% in the general US population, but is higher in areas with endemic forms of Kaposi's sarcoma, such as Mediterranean countries and Africa.[65] Following organ transplantation, the risk for HHV-8 infection due to primary acquisition of virus, reinfection or reactivation is increased. Transplant patients may acquire HHV-8 from the allograft, blood products, caregivers or from family members.[65] [66] Cutaneous or visceral Kaposi's sarcomas develop with a reported incidence of 0.5–5% in solid organ transplant recipients.[65] [66] The antiviral agents ganciclovir, foscarnet and cidofovir show inhibitory activity against HHV-8 *in vitro*, but data on their therapeutic efficacy in transplant recipients with HHV-8 infection are limited and nonconclusive.[64] [67]

OTHER VIRAL INFECTIONS

ADENOVIRUS INFECTION

Adenovirus infection is ubiquitous and the virus is usually acquired during early childhood. Like herpesviruses, adenovirus has the propensity to establish latency following primary infection, and to reactivate during immunosuppression. Over 40 serotypes of adenovirus have been identified. Adenovirus infection is observed in 5–21% of patients after allogeneic SCT and in 7–19% of solid organ transplant recipients.[4] [68] [69] Up to one-third of immunodeficient patients with active adenovirus infection develop disease, including hemorrhagic cystitis, gastroenteritis, colitis, hepatitis, pneumonia, encephalitis or disseminated organ disease.[4] [68] [69] [70] [71] [72] Risk factors for adenovirus disease that were identified in SCT recipients are the isolation of the virus from two or more sites and the occurrence of moderate to severe GVHD.[68] [69] After SCT, mortality related to adenovirus disease was reported to be between 50% and 54%.[68] [69]

There is currently no antiviral agent with proven efficacy for the therapy of adenovirus disease in immunocompromised hosts. Intravenous ribavirin and cidofovir were used therapeutically with mixed results in small series and single cases. In one report, intravenous ribavirin was given to five immunodeficient children, of whom three had pneumonia and two had hemorrhagic cystitis caused by adenovirus; one patient with pneumonia and one with cystitis had complete clinical recovery and viral clearance, whereas outcome was fatal in the other three.[72] Cidofovir therapy was administered to seven children after allogeneic unrelated SCT who had developed adenovirus disease; clinical improvement and clearance of virus were observed in five patients, but one child died from disseminated adenovirus disease and another from invasive aspergillosis.[71]

In the future, outcome of adenovirus disease might be improved by the introduction of diagnostic techniques predictive for severe adenovirus infection, such as the PCR in serum, which permits early initiation of antiviral drug treatment. [70]

COMMUNITY RESPIRATORY VIRUS INFECTIONS

Respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, rhinoviruses, coronaviruses and some serotypes of adenovirus cause respiratory infections, the clinical importance of which is increasingly recognized in immunocompromised hosts.[73] [74] [75] [76] Characteristics of community respiratory virus infections include seasonal outbreaks, acquisition in the community or in the hospital, rapid transmission from person to person with the respiratory tract as portal of entry, and a short incubation period. In a prospective study of adult SCT recipients hospitalized with acute respiratory disease, respiratory viruses were isolated by culture from 67 (31%) of 217 patients; 49% of these infections were due to RSV, 18% each to influenza viruses and to picornaviruses, 9% to parainfluenza viruses, and 6% were caused by adenovirus.[75] Of 87 prospectively studied leukemic adults with acute respiratory illness, nine (10%) had culture-proven RSV infection that was complicated by pneumonia in six, with a mortality rate of 83%.[74] Fatal respiratory failure was reported to occur in one-third of patients after SCT who developed parainfluenza virus infection involving the lower respiratory tract.[73] Community respiratory viruses also contribute to significant morbidity and possibly graft rejection after solid organ transplantation. Lung transplant recipients are at particularly high risk for serious infections due to these viruses.[76]

Thus, community respiratory viruses are responsible for important morbidity and mortality among immunocompromised hosts who develop acute respiratory disease, and RSV appears to be the most common cause of infection.

Immunocompromised patients with respiratory virus infection usually present with fever, coryza, cough, shortness of breath and wheezing, and may have radiographic sinus opacification or pulmonary infiltrates.[76] However, the clinical and radiological features are nonspecific and definite diagnosis requires documentation of virus by culture, antigen detection or PCR in bronchoalveolar lavage fluid or open lung biopsy specimens.

Although rapid detection assays are available for most community respiratory viruses, the therapeutic options remain limited. For therapy of RSV infection, ribavirin delivered by aerosol or administered intravenously is used, but is generally ineffective in patients with established RSV pneumonia and respiratory failure. Nevertheless, ribavirin might be beneficial if given early in the course of pneumonia or if administered pre-emptively when only the upper respiratory tract is involved.[74] [77] The combination of aerosolized ribavirin plus intravenous immune globulins containing high titers of RSV antibody could yield better results, and is being studied in immunosuppressed patients with RSV infection.[77]

For therapy of influenza, the recently introduced neuraminidase inhibitors zanamivir and oseltamivir have potent activity against both influenza A and B viruses, and are generally well tolerated. By contrast, the clinical effectiveness of amantadine and rimantadine is limited to influenza A virus infection, and these drugs are associated with gastrointestinal and neurological adverse events and with the rapid emergence of virus resistance, which limits their usefulness. The therapeutic efficacy of antiviral agents against influenza has been established in otherwise healthy individuals. Whether their prophylactic or therapeutic use is beneficial in severely immunocompromised hosts remains to be demonstrated.[6]

Picornaviruses may cause potentially life-threatening respiratory disease in immunocompromised patients. Pleconaril, a novel broad-spectrum antipicornaviral compound, has become available for clinical use. Following oral intake, pleconaril achieves serum concentrations superior to those required to inhibit 90% of clinical enterovirus and

TABLE 112-6 -- Infection control precautions for the prevention of nosocomial respiratory virus infections among immunocompromised patients at a cancer center*

INFECTION CONTROL PRECAUTIONS FOR THE PREVENTION OF NOSOCOMIAL RESPIRATORY VIRUS INFECTIONS
• Screening symptomatic patients by using rapid diagnostic tests for respiratory viruses
• If tests positive, cohorting of patients and initiation of therapy with aerosolized ribavirin
• Contact isolation of infected patients (gloves and gowns)
• Droplet precautions by wearing masks
• Personnel should wear masks and gloves if in close contact (<1m) with SCT recipients during the season of community outbreaks
• Strict enforcement of handwashing between patient visits
• Prohibiting symptomatic personnel from working on patient wards
• Screening visitors for upper respiratory symptoms
• Prohibiting children <12 years old from visiting patients
• Educational program for personnel

* *(adapted from[80])*.

rhinovirus isolates. Preliminary results of oral pleconaril therapy for acute picornaviral respiratory illness among both immunocompetent and immunocompromised patients are encouraging.[78] [79]

Because of the limited therapeutic possibilities, emphasis must be placed on the prevention of respiratory virus infections in immunocompromised hosts. Strict enforcement of infection control measures is of paramount importance to reduce the transmission of respiratory viruses by nosocomial spread or by acquisition from infected visitors of the community. Table 112.6 lists infection control precautions introduced at a cancer center, which resulted in significantly reduced rates of

nosocomial respiratory virus infection. [80] The mainstay of influenza prophylaxis remains active immunization with influenza virus vaccine, which should be offered to both patients at risk and hospital staff before the influenza season.[80] Other vaccines are under development and may help to further reduce the morbidity caused by community respiratory viruses in the future.

POLYOMAVIRUS INFECTIONS

The human polyomaviruses BK and JC are widespread, and their seroprevalence among adults ranges from 60% to 80%. Both viruses may establish a latent infection. BK virus is frequently reactivated and excreted in the urine of transplant recipients. [81] BK virus infection in these patients is often asymptomatic, but can cause ureteric stenosis, hemorrhagic cystitis and severe renal graft dysfunction.[4] [81] [82] Renal transplant recipients at risk for BK virus-related nephropathy may be identified by urinary shedding of 'decoy cells' (cells with viral inclusions) or by positive PCR in plasma specimens.[82] JC virus is the etiologic agent for progressive multifocal leukoencephalopathy (PML) and is occasionally isolated from urinary specimens in SCT and renal transplant recipients, but PML is very rare in these patients.[81] [83] To date, there is no antiviral therapy proven effective for diseases due to BK or JC virus in immunodeficient hosts, and treatment remains largely supportive. Recent data, however, suggest some activity of cidofovir in the treatment of PML among patients with AIDS, an observation that merits further investigation.[84]

OTHER VIRUSES

Several viruses that may cause diseases in SCT and solid organ transplant recipients are discussed in more detail elsewhere. Human papillomavirus infection is associated with anal warts and nonmelanoma skin cancers after renal transplantation (see Chapter 77). [85] [86] Hepatitis B and C viruses can complicate the posttransplant course in infected patients (see Chapter 214). Measles are best prevented by active immunization, but cases of serious disease have re-emerged among transplant recipients in areas with reduced vaccine coverage (see Chapter 212).

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Chapter 113 - Opportunistic Parasitic Infections

Jose G Montoya

TOXOPLASMA GONDII

GENERAL CONSIDERATIONS

Toxoplasma gondii is an obligate intracellular parasite that exists in nature in three forms: the oocyst (which releases sporozoites), the tissue cyst (which contains and may release bradyzoites) and the tachyzoite.

Oocysts are formed in the small intestine of members of the cat family and are excreted in their feces for periods varying from 7 to 21 days. Tachyzoites are crescent shaped and measure 4–8µm long and 2–4µm wide (Fig. 113.1). The presence of tachyzoites in human fluids or tissues is the hallmark of acute infection. Encystation and formation of tissue cysts usually occur following cell entry and replication of the tachyzoite form. The tissue cyst is formed within a host cell and may vary in size from those which contain only a few organisms (bradyzoites) to =200µm in size that contain several thousand bradyzoites (Fig. 113.2). The brain and eye as well as cardiac, skeletal and smooth muscles appear to be the most common sites for chronic latent infection. Because of this persistence in tissues, demonstration of cysts in histological sections does not necessarily imply that the infection was recently acquired or reactivated.

The term *T. gondii* infection is best reserved to describe the parasite's primary subclinical infection or its asymptomatic persistence in tissues (chronic or latent infection) and toxoplasmosis should be used to describe the parasite's clinical manifestations or pathological disease in its incidental hosts.[1]

Toxoplasmosis is primarily a disease of individuals with immature or severely impaired T cell-mediated immunity. *T. gondii* can have devastating consequences in the offspring of women who acquire their primary infection during or shortly before pregnancy; the premature or underdeveloped immune system of the fetus is the most likely reason for this potentially tragic outcome. In children and adults, toxoplasmosis most often occurs in those with defects in T cell-mediated immunity, such as hematological malignancies, bone marrow and solid organ transplants or AIDS. Biopsy-proven toxoplasmic myocarditis and polymyositis in the setting of acute toxoplasmosis have been reported in patients on corticosteroids. [2] In immunosuppressed patients, toxoplasmosis is characterized by protean and often subtle clinical manibestations with a high attributable mortality in untreated cases.

In organ transplant recipients, the disease may result from newly acquired infection, reactivation of a previous (latent) infection or infection via the allograft. Reactivation of latent infection is the most common mechanism by which *T. gondii* causes toxoplasmosis in patients with AIDS and bone marrow transplants, whereas the cause is infection via the donated organ in patients with heart and heart-lung transplants. Both mechanisms, reactivation of chronic infection and infection via the allograft, have been described in patients with kidney and liver transplants.

In the majority of otherwise healthy individuals, primary or chronic (latent) infection with *T. gondii* is asymptomatic; following the acute infection, a small percentage will suffer chorioretinitis, lymphadenitis, myocarditis or polymyositis.

TRANSMISSION AND EPIDEMIOLOGY

Toxoplasma gondii is transmitted primarily via the oral route and the definitive hosts are members of the cat family. Infection in humans most commonly occurs through ingestion of raw or undercooked meat that contains cysts, through water or food contaminated with oocysts or congenitally through transplacental transmission following infection during gestation.

Cat ownership should not be used as an epidemiological hint to establish whether the patient is at risk of acquiring infection with the parasite. Transmission of oocysts (shed by the small intestine of recently infected cats) occurs without knowledge of the patient and may be unrelated to direct exposure to a cat (e.g. transmission by contaminated vegetables or water). On the other hand, patients with an indoor cat that is fed only cooked food are not at risk of acquiring the infection from that cat. Serological investigation of a specific cat to determine whether it is a potential source of the infection should be discouraged. Seropositivity in a cat does not predict shedding of oocysts.

In humans, the incidence of *T. gondii* antibodies increases with increasing age. However, seropositivity tends to be lower in cold regions, in hot and arid areas and at high elevations. In general, the incidence of the infection varies with the population group and geographic locale.

In many areas of the world, such as in El Salvador and France, the prevalence of seropositivity is as high as 75% by the fourth decade of life. The overall age-adjusted seroprevalence of *T. gondii* infection in the United States has been reported to be 22.5%.[3] Age-adjusted seroprevalence was higher in the north eastern United States (29.2%) than in the south (22.8%), midwest (20.5%) or west (17.5%) ($p<0.05$). [3]

The incidence of toxoplasmosis in HIV-infected individuals is directly correlated with four factors: *T. gondii* seroprevalence among the general non HIV-infected population, the degree of immunosuppression estimated by the CD4 count, the use of active antiretroviral therapy, and the institution of effective prophylactic regimens against reactivation of the infection. It has been estimated that 30–50% of HIV and toxoplasma-seropositive individuals whose CD4 count is below 100 cells/mm^3 will ultimately develop toxoplasmic encephalitis (TE) if anti-*T. gondii* prophylaxis or antiretroviral therapy is not instituted.

CLINICAL FEATURES

In contrast to the relatively benign clinical course of toxoplasmosis in the vast majority of immunocompetent individuals, it is potentially life threatening in both AIDS and other immunocompromised patients.

The brain is the most commonly affected organ. The clinical presentation of TE varies from a gradual and subacute process evolving

Figure 113-1 Giemsa-stained smear of bronchoalveolar lavage from a bone marrow transplant patient with disseminated toxoplasmosis. Tachyzoite form is demonstrated (arrows).

over weeks to an acute confusional state, with or without focal neurologic defects, evolving over days. Clinical manifestations include mental status abnormalities, focal

motor deficits, sensory aberrances, seizures, cranial nerve disturbances, cerebellar signs, meningismus, movement disorders and neuropsychiatric findings. The most common focal neurologic findings are abnormalities of speech and hemiparesis.

Constitutional symptoms and signs, such as fever and malaise, may vary and may be the only initial clinical manifestations of disseminated toxoplasmosis. A high index of suspicion for toxoplasmosis should always be entertained in patients at high risk for the disease (i.e. bone marrow transplant patients with chronic toxoplasma infection or a toxoplasma-seronegative heart transplant patient who receives a heart from a seropositive donor).

Seizures, cerebral hemorrhage and diffuse TE can occur acutely and progress rapidly to death. Spinal cord toxoplasmosis in patients with organ transplants can present with motor or sensory disturbances of single or multiple extremities, or bladder or bowel dysfunction. Cervical and thoracic myelopathy and conus medullaris syndromes have also been reported.[4]

Toxoplasmosis in immunocompromised patients can also present as chorioretinitis, pneumonitis or multiorgan involvement, presenting with acute respiratory failure and hemodynamic abnormalities similar to septic shock.

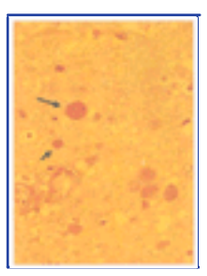

Figure 113-2 Hematoxylin-eosin stain of the cyst form of *T. gondii* in brain (arrows).

SPECIFIC SETTINGS OF IMMUNOSUPPRESSION

AIDS

The majority of AIDS patients with toxoplasmosis have evidence of prior *T. gondii* infection (as demonstrated by positive *T. gondii*-specific IgG in approximately 97% of patients with TE), a CD4 count less than 100 cells/mm^3 and are not taking appropriate toxoplasma-specific prophylaxis (for additional information see Chapter 127).

Clinical manifestations of toxoplasmosis in AIDS patients commonly reflect involvement of the brain (i.e. TE), the lung (pneumonitis) and the eye (chorioretinitis). TE is the most common presentation of toxoplasmosis in AIDS patients. Spinal cord involvement by *T. gondii* in AIDS patients manifests as motor or sensory disturbances of single or multiple limbs, bladder and/or bowel dysfunctions and local pain. Patients may present with a clinical syndrome resembling a spinal cord tumor.

Bone marrow transplantation

Toxoplasmosis mostly occurs by reactivation of latent disease within the first 6 months after marrow transplant. Infection develops in patients who are seropositive for *T. gondii* pretransplant, have received an allogeneic marrow and have severe graft versus host disease (GVHD). [5] [6]

The incidence of toxoplasmosis in bone marrow transplants has been estimated at 0.31 cases per 100 allogeneic transplants.[6] The occurrence of the disease in autologous marrow recipients is extremely rare. The median time of clinical presentation is usually day 60 post transplant; the attributable mortality has been estimated

at =40%. In autopsies of bone marrow transplant recipients, *T. gondii* tachyzoites have been found in brain, heart, lungs, liver, duodenum, bladder, lymph nodes and bone marrow.

Heart and heart-lung transplantation

In heart or heart-lung transplant recipients, toxoplasmosis usually occurs when a seronegative recipient (R-) receives a transplant from a seropositive donor (D+). Seropositive recipients frequently develop IgM and IgG antibody titer rises after transplantation without evidence of clinical disease.[7] [8] In the heart transplant recipient, toxoplasmosis may simulate organ rejection. In such cases, toxoplasmosis has been established by endomyocardial biopsy. In a recent study of infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center in California, serological testing for toxoplasma was available for 582 donors and 607 recipients, of whom 6% and 16.1% had *T. gondii*-specific IgG antibodies, respectively.[9] The higher incidence of previous *T. gondii* infection observed among recipients (16%) versus that among donors (6%) reflects the increasing seroprevalence with age.

Results of serological testing for toxoplasma were available for 575 D/R pairs; of these, 454 (79%) were D-/R-, 84 (14.6%) D-/R+, 32 (5.6%) D+/R- and five (0.8%) D+/R+. Of the 32 D+/R- patients, 16 received trimethoprim-sulfamethoxazole (TMP-SMX) and/or pyrimethamine prophylaxis and none developed toxoplasmosis; however, four (25%) of the 16 D+/R- patients who did not take either TMP-SMX or pyrimethamine developed toxoplasmosis, and all died of the infection. None of the 98 patients who were seropositive for *T. gondii* preoperatively developed clinical evidence of reactivation of the infection.[9] In this study, the highest mortality attributable to an infectious complication was observed among patients with toxoplasmosis and those with disseminated aspergillosis.

Kidney and liver transplantation

Toxoplasmosis in kidney transplant patients is more likely to occur as a result of primary infection in the setting of a toxoplasma-seropositive donor (D+) and a seronegative recipient (R-); it has also been described in D+/R+ and D-/R+ pairs.[10] Most affected patients develop clinically evident toxoplasmosis by 3 months post transplantation, with fever, neurological disturbances and pneumonia as the main clinical features.

Toxoplasmosis in liver transplant patients is more frequently reported as a result of reactivation of chronic infection[11] but it can also occur in the setting of primary infection in D+/R- pairs.[12] Toxoplasmosis usually develops within 3 months of the transplant. Fever, pneumonia and multiorgan failure are common.

DIAGNOSIS

The diagnosis of *T. gondii* infection or toxoplasmosis may be established by serological tests, polymerase chain reaction (PCR) in body fluids or tissues, histological methods (i.e. immunoperoxidase stain) or isolation of the parasite from body fluids or tissues.[13]

Because reactivation of the chronic infection is the most common cause of toxoplasmosis in patients with malignancies, recipients of organ transplants (particularly bone marrow and liver) or AIDS, initial assessment of these patients should routinely include *T. gondii* IgG antibodies. Those with a positive result are at risk of reactivation of the infection; those with a negative result should be instructed on how they can prevent becoming infected.

It should be kept in mind that in patients with heart and heart-lung transplants (and less frequently in patients with kidney, liver and liver-pancreas transplants), toxoplasmosis is most to likely occur in the previously seronegative patient who receives an allograft from a seropositive donor. Consequently, it is recommended that all candidates for solid transplantation and their potential donors be screened for *T. gondii*-specific IgG prior to transplantation. This strategy allows to identify those patients at risk of developing life-threatening disease, initiate appropriate prophylaxis and to prompt diagnostic tests and empiric treatment should the patient develop a syndrome consistent with the possibility of toxoplasmosis.

When reactivation of *T. gondii* infection is suspected in immunocompromised patients chronically infected with the parasite (those with documented positive *T. gondii*-specific IgG antibody prior to the immunosuppressive state), additional serological testing adds very little or may be misleading.[7] [8] In these patients, apparent reactivation (a rising IgG and IgM titer) may be present in the absence of clinically apparent infection. In addition, serological test results consistent with chronic infection may be seen in the presence of active toxoplasmosis. [8] [14] Thus, for immunocompromised patients in whom toxoplasmosis is suspected, additional diagnostic methods to attempt to establish the diagnosis are strongly recommended. These methods include PCR for the amplification of *T. gondii* DNA in blood or body fluids suspected of being infected (i.e. bronchoalveolar lavage fluid, cerebrospinal fluid, etc.), isolation of the parasite from blood or body fluids, and histological examination

of available tissues with *T. gondii*-specific stains such as immunoperoxidase (e.g. endomyocardial biopsy in heart transplant patients).

When clinical manifestations suggest involvement of the CNS and/or spinal cord, neuroimaging studies such as computed tomography (CT) or magnetic resonance imaging (MRI) are mandatory. Neuroimaging studies should be considered even without focal deficits on examination. Empiric anti-*T. gondii* therapy for patients with multiple ring-enhancing brain lesions (usually established by MRI), positive IgG antibody titers against *T. gondii* and advanced immunodeficiency is accepted practice (Fig. 113.3). Clinical and radiologic response to specific anti-*T. gondii* therapy is supportive of the diagnosis of CNS toxoplasmosis.

Brain biopsy should be considered in immunocompromised patients with presumed CNS toxoplasmosis if there is a single lesion on MRI, a negative IgG antibody test, inadequate clinical response to an optimal treatment regimen or in those who received and adhered to an effective prophylactic regimen against *T. gondii* (e.g. TMP-SMX).[15] If *T. gondii* serological and radiological studies do not support empiric treatment or are inconclusive, and if brain biopsy is not feasible, a lumbar puncture should be considered for PCR on the CSF. CSF can also be sent for isolation studies, although *T. gondii* has uncommonly been isolated from CSF in these patients. PCR examination of the CSF can also be used for detection of

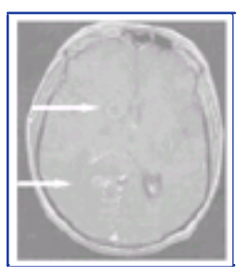

Figure 113-3 Magnetic resonance imaging of the brain in an autologous bone marrow transplant patient with toxoplasmic encephalitis (TE). Enhancing lesions are shown (arrows).

Epstein-Barr virus (EBV), JC virus or cytomegalovirus (CMV) DNA in patients in whom primary CNS lymphoma, progressive multifocal leukoencephalopathy (PML) or CMV ventriculitis, respectively, are included in the differential diagnosis.

TREATMENT

Monotherapy should not be used in the treatment of immunosuppressed patients with toxoplasmosis. The 'gold' standard' regimen for the treatment of toxoplasmosis is the combination of pyrimethamine (200mg loading dose followed by 50–75mg qd), sulfadiazine (2.0–4.0g loading dose initially followed by 1–1.5g q6h) and folinic (not folic) acid (10–50mg qd). Clindamycin (600mg q6h, up to 1200mg q6h) can be used instead of sulfadiazine in patients intolerant of sulfonamides. Duration of treatment should be 4–6 weeks after resolution of all signs and symptoms (often for several months or longer). Recently, TMP-SMX (at 10–15mg/kg/d of the trimethoprim component divided in four doses) has been found to be equivalent to pyrimethamine/sulfadiazine in patients with AIDS.[16]

Atovaquone has potent *in vitro* and *in vivo* activity against both cyst and tachyzoite forms in murine models of TE.[17] [18] In its tablet form, atovaquone has been used as a single agent for primary and maintenance therapy of TE in AIDS patients, with overall discouraging results. The bio-availability of atovaquone is highly dependent on formulation and diet.[19] An international, noncomparative study of atovaquone (as a more bio-available oral suspension) with either pyrimethamine or sulfadiazine for treatment of acute TE found that the atovaquone combination regimens were effective (=75% response rate), safe and had a frequency of adverse events leading to treatment discontinuation (28%) similar to standard treatment regimens (16–33%).[20] [21]

Atovaquone+pyrimethamine may be considered as an alternative treatment for patients intolerant of sulfonamides, and atovaquone+sulfadiazine for patients intolerant of pyrimethamine. The efficacy of atovaquone-based therapies has not been evaluated prospectively.

The roles of clarithromycin, azithromycin or dapsone in TE have not been well established. If used, they should always be combined with other drugs, preferably including pyrimethamine.

After treatment of the acute phase (primary or induction treatment) in patients with AIDS, maintenance treatment (secondary prophylaxis) should be instituted. This is usually accomplished with the same regimen used in the acute phase but at half doses. Currently, maintenance therapy is recommended to be continued for life or until the patient's CD4 count has been >200 cells/mm^3 and HIV PCR peripheral blood viral load nondetectable for at least 6 months. Other immunocompromised patients may require maintenance treatment for as long as their immunosuppressive regimens significantly affect their cell-mediated immunity, as is the case with bone marrow transplant patients.

PREVENTION

Prevention is most important in seronegative immunodeficient patients, and is most readily accomplished through education (Table 113.1).

The *T. gondii*-specific IgG test result should be documented in the medical records of all patients undergoing potent suppression of cell-mediated immunity. In addition, the toxoplasma IgG status of all organ donors should be documented.

In seronegative recipients of a heart, heart-lung, lung, liver or kidney from a seropositive donor, primary prophylaxis (pyrimethamine 25mg po qd for 6 weeks post transplantation) should be considered for patients not taking TMP/SMX for PCP prophylaxis. These

TABLE 113-1 -- Measures to prevent primary *T. gondii* infection in immunosuppressed patients.

MEASURES TO PREVENT PRIMARY *T. GONDII* INFECTION IN IMMUNOSUPPRESSED PATIENTS
Avoid contact with consumer goods potentially contaminated with cat feces, particularly cat litter and gardening.
Disinfect cat litter box with near boiling water for 5 minutes prior to handling.
Cook meat to 66°C or 'well done' or that is not pink in the middle (meat that is smoked or cured in brine may be infectious).
Wash hands thoroughly after contact with raw meat.
Kitchen surfaces and utensils that have come in contact with raw meat should be washed.
Avoid mucous membrane contact when handling raw meat.
Avoid ingestion of dried meat.
Wash fruits and vegetables prior to consumption.
Refrain from skinning animals.

patients now routinely receive TMP-SMX for PCP prophylaxis, and this regimen appears to be sufficient to also prevent toxoplasmosis. Prophylactic daily doses of TMP-SMX (one single-strength tablet a day) are probably sufficient to prevent toxoplasmosis and most likely do not require the addition of pyrimethamine.

Primary prophylaxis against *T. gondii* in AIDS patients at high risk for TE is effective. Potent antiretroviral therapy also has a profound impact in decreasing the incidence of TE in these patients. Primary prophylaxis is recommended for patients who are *T. gondii* seropositive whose lowest $CD4^+$ count has been below 100/mm^3 regardless of the HIV RNA viral load. Once the CD4 count increases above 200/mm^3 , primary prophylaxis can be discontinued particularly in patients with non-detectable or low HIV RNA viral load for at least 6 months. TMP-SMX (daily), dapsone (50mg/day) plus pyrimethamine (50mg/week), atovaquone (750mg qid) and fansidar have been reported to be effective regimens to prevent TE.

STRONGYLOIDES

GENERAL CONSIDERATIONS

Strongyloidiasis is caused by two species of the intestinal nematode Strongyloides, *Strongyloides stercoralis* and *S. fuelleborni. Strongyloides fuelleborni* has rarely been reported to cause infection in humans. *Strongyloides stercoralis* infects as many as 100–200 million people in 70 countries with defects in T cell-mediated immunity, (AIDS, hematological malignancies, bone marrow transplants and those taking relatively high doses of corticosteroids). The overall seroprevalence of *Strongyloides stercoralis* in the United States is between 0.4% and 4.0%.[22]

The unique ability of this nematode to replicate in the small intestine of humans permits cycles of autoinfection, leading to chronic asymptomatic disease that can last undetected for decades.

LIFE CYCLE

Strongyloides stercoralis exists in nature in two forms: filariform infective larvae and rhabditiform. The filariform larvae (infective stage) penetrate unbroken skin of humans that has been exposed to contaminated soil. The larvae pass through the circulation to reach the lungs, enter the airways and are swallowed into the gastrointestinal tract. At the small intestine they mature into adult egg-laying females. Female parasites produce eggs that hatch in the

intestine and the rhabditiform larvae are passed out in the feces. To complete the cycle in the soil, rhabditiform larvae develop into free-living nonparasitic adults (both females and males) and produce eggs that ultimately produce and develop into the rhabditiform stage (see Chapter 246). The rhabditiform larvae transform into the filariform infective larvae. In humans, the autoinfection cycle occurs when the intestinal rhabditiform larvae transform directly into the filariform infective larvae without leaving the gut or perianal areas. This cycle can be sustained in humans for decades after leaving endemic areas and explains the high parasite burden seen in the hyperinfection syndrome.

CLINICAL FEATURES

The vast majority of individuals infected with *S. stercoralis* are asymptomatic. A small minority of patients develop gastrointestinal, pulmonary and dermatological manifestations. Gastrointestinal symptoms of strongyloidiasis include nausea, anorexia, diarrhea, abdominal discomfort and abdominal bloating. Cutaneous manifestations include migratory, serpiginous, urticarial skin lesions on the buttocks, groin and trunk.

Pulmonary strongyloidiasis usually occurs in the setting of the hyperinfection syndrome with disseminated disease. Symptoms include cough, wheezing, sputum production, dyspnea, hemoptysis, tachypnea and pleuritic pain. Hyperinfection usually occurs in patients with chronic asymptomatic strongyloidiasis who become immunocompromised. It is usually a complication of impaired cell-mediated immunity and is seen in organ-transplant recipients, patients with lymphomas or patients receiving high-dose corticosteroids. It is characterized by intestinal obstruction/respiratory tract involvement, meningitis, skin rash or Gram-negative bacteremia. The high rate of Gram-negative bacteremia is attributed to the attachment of bacteria to the surface of the parasite as the latter penetrates the small intestine. A high parasite load is usually observed in tissues (particularly the lungs). It has been suggested that chronic intestinal infection and antigen stimulation may predispose to the development of small bowel lymphoma.

DIAGNOSIS

The diagnosis of the hyperinfection syndrome in immunosuppressed patients requires a high index of suspicion. Disseminated strongyloidiasis should be considered in potently immunosuppressed patients who have been in endemic areas who present with unexplained respiratory and gastrointestinal symptoms.

Finding the motile rhabditiform larvae in stool or duodenal aspirate establishes the diagnosis. Larvae can be found in respiratory secretions, cerebrospinal fluid, urine and ascites of patients with the hyperinfection syndrome. Eosinophilia is usually present in immunocompetent patients, but may be absent in the immunocompromised host. Most infected individuals have *S. stercoralis*-specific IgG. Serology and eosinophilia decrease after therapy and may be useful markers of treatment success.[23]

TREATMENT

Thiabendazole (25mg/kg bid) or ivermectin (200µg/kg/d) are the drugs of choice for the treatment of strongyloidiasis. For the hyperinfection syndrome, prolonged and repeated courses of therapy may be required.

AMEBAE OR RHIZOPODS

GENERAL CONSIDERATIONS

The majority of the amebae or rhizopods are free living and dwell in standing fresh water. Three genera, *Acanthamoeba* spp., *Balamuthia* spp. and *Naegleria* spp., have been reported to cause disease in humans. In normals *Naegleria fowleri* can cause life-threatening meningoencephalitis and *Acanthamoeba* spp. and *Balamuthia* spp. can cause keratitis associated with contact lenses. The latter species can cause subacute or chronic meningoencephalitis and disseminated disease in immunocompromised patients.

Several other genera of amebae, including *Entamoeba, Endolimax* and *Iodamoeba*, are obligate parasites of the human intestinal tract that are transmitted via the fecal-oral route and cannot survive as free-living organisms. Intestinal and extraintestinal amebiasis (mostly caused by *Entamoeba histolytica*) is quite common among homosexual men with or without HIV infection.

CLINICAL FEATURES

Human infections with *Naegleria fowleri* are rare. It enters through the nose and travels to the brain and spinal cord during swimming under water or diving. Infection is most common during dry, hot, summer months, when the ambient temperature is above 80°F and the water is warm. Fever, headache, stiff neck, nausea, vomiting, ataxia, seizures, mental status changes and hallucinations are common manifestations. *Naegleria fowleri* infection of the central nervous system is also called primary amebic meningoencephalitis (PAM) and usually results in death within 7–10 days.

Acanthamoeba spp. are found worldwide, most commonly in the soil and dust, in fresh-water lakes, rivers and hot springs and in hot tubs. *Acanthamoeba* may also be found in brackish and sea water. Heating, venting and air conditioner units (HVAC), humidifiers, dialysis units and contact lens equipment also support growth. Several species of *Acanthamoeba* have been found to infect humans: *A. culbertsoni, A. polyphaga, A. castellanii, A. healyi, (A. astronyxis), A. hatchetti, A. rhysodes* and possibly others. *Acanthamoeba* enter through a cut or wound or through the nostrils and reach the lungs and central nervous system. In immunocompromised patients, *Acanthamoeba* spp. can cause disseminated disease or chronic granulomatous amebic encephalitis (GAE).

Disseminated acanthamebiasis with widespread extracerebral disease is characterized by widespread cutaneous nodules with surrounding erythema which ulcerate with indurated borders. It is extremely rare and occurs mostly in AIDS patients or organ transplant recipients.

GAE is a subacute to chronic disease that results in death within one week to several months of the initial symptoms. It is characterized by weeks to months of headache, mental status changes, cranial nerve involvement, increased intracranial pressure and single or multiple space intracranial lesions. *Acanthamoeba* spp. as well as *Balamuthia* spp. (particularly *Balamuthia mandrillaris*) have been described as etiologic agents.

Entamoeba histolytica in humans usually causes diarrhea and cramping abdominal pain with or without dysentery. Extraintestinal manifestations include liver abscess which can rupture through the diaphragm into the lung or pericardium.

DIAGNOSIS

Demonstration of amebic trophozoites and cysts in tissues or growth in culture is diagnostic.[24] Skin and brain biopsy specimens are useful for culture and for histological examination (hematoxylin and eosin, Gomori's methenamine silver, periodic acid-Schiff stains). Immunohistological techniques such as indirect immunofluorescence assay or electron microscopy may be necessary to identify *Acanthamoeba* spp. or *Balamuthia mandrillaris* in tissue sections. Cerebrospinal fluid resembles the picture of aseptic meningitis with lymphocytic pleiocytosis, a normal to slightly low glucose and elevated protein. Serological tests can be helpful in patients with suspected GAE.

Diagnosis of *Entamoeba histolytica* relies upon the identification of the organism in stool. Extraintestinal amebiasis is more difficult to diagnose as the parasite is not usually visualized in liver aspirates. *Entamoeba histolytica*-specific serological tests can be helpful.

TREATMENT

The prognosis of disease caused by free-living amebae in humans is dismal and usually associated with a 100% attributable mortality. For *Naegleria fowleri* primary amebic meningoencephalitis, intravenous amphotericin B at 1mg/kg/d and intrathecal amphotericin B into the lateral ventricle is recommended. There are no standardized regimens for GAE. For *Acanthamoeba* spp. infections there are anecdotal reports on the use of pentamidine, itraconazole or ketoconazole, TMP-SMX and rifampin in different combinations. For *Balamuthia mandrillaris* infections, pentamidine, clarithromycin, fluconazole, sulfadiazine and flucytosine have been used.

The drug of choice for the treatment of diseases caused by *Entamoeba histolytica* continues to be metronidazole. Metronidazole treatment (500–750mg orally three times daily) should be followed by either paromomycin (500mg orally three times daily for 7 days) or iodoquinol (650mg orally three times daily for 20 days) to prevent relapses from intestinal cyst forms.

For cryptosporidiosis, microsporidiosis, isosporiasis and leishmaniasis see Chapter 127 .

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Chapter 114 - Practice Point

Infection prophylaxis for patients with common variable immunodeficiency

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Definition of the problem

The immunocompromised host is characterized by a state of immunodefiency, which affects the defense against micro-organisms and hence increases the susceptibility to infections. The type of immunodeficiency critically determines which kinds of infection may ensue, as is pointed out in Chapter 97 . Passive and active immunization are classic and effective methods of reinforcing host defense against pathogenic micro-organisms, not only in the normal host but also in the immunocompromised host. In the immunocompromised host, the nature of the host defense defect has a decisive influence on the success of active immunization; if the capacity to mount a specific and protective antibody response is defective (as in primary agammaglobulinemia), certain immunizations become futile. A similar situation is present when the protection conferred by the vaccine is dependent on T-cell defense and specific cellular immunity is of poor quality. An additional problem in the immunocompromised host is the risk of vaccines containing live (attenuated) micro-organisms. The host defense defect may be such that serious infection ensues. In this chapter these problems are discussed in practical terms. In addition, passive immunization (i.e. the administration of immunoglobulin preparations) is discussed in detail.

Typical case

A 48-year-old woman with a 12-year history of recurrent purulent infections of the respiratory tract and paranasal sinuses, caused by *S. pneumoniae* and *Haemophilus influenzae*, was diagnosed 7 years ago as suffering from common variable immunodeficiency. The serum concentrations of all immunoglobulin classes were extremely low. Immunoglobulin A was not detectable and therefore, the patient's blood was tested for antibodies against IgA. Her T cell subsets and function tests (lymphocyte transformation tests) were normal. Because of the presence of circulating anti-IgA, substitution was started with slow subcutaneous standard immunoglobulin 16% according to the protocol given in Table 114.1 . During this period of substitution the anti-IgA titer became negative.

After 3 months of weekly subcutaneous injections of immunoglobulin, she was transferred to substitution therapy with intravenous immunoglobulin (IvIg) preparations. From the start of the substitution therapy, her clinical condition was much improved. With a 4-weekly dose of 600mg/kg IvIg, IgG concentrations above 6g/l were attained. Her pulmonary function was stable. Occasionally, she still suffered from purulent sinusitis, which readily responded to amoxicillin therapy.

Two years ago she had a period of diarrhea. Stool examinations did not show *Giardia lamblia*, but cultures yielded *Campylobacter jejuni*. Despite a course of erythromycin, stool cultures remained positive for this pathogen. There were no episodes of fever.

Recently, she asked her physician which vaccinations she needs for a 3-week holiday in Tunisia, and what she should do about her immunoglobulin substitution therapy.

Management options

Active immunization issues

This patient with defective humoral immunity presents with a series of problems regarding active and passive immunization.

The first problem is the advice on vaccination for her travel. It is necessary to discuss with the patient whether traveling to Tunisia, a country with a high risk of infection with enteric pathogens even for healthy tourists, is a sensible thing to do for a hypogammaglobulinemic patient. It should be kept in mind that, despite adequate immunoglobulin substitution, mucosal immunity usually remains severely impaired in these patients, owing to the deficiency of mucosal immunoglobulins, especially secretory IgA. This could be assessed by measuring IgA in saliva or tears. The persistence of *C. jejuni* in her stools also points to impaired mucosal immunity, since persistence of this pathogen beyond 4 months does not occur in the normal host. Intravenous immunoglobulin substitution delivers only IgG and no other immunoglobulin classes; no measurable immunoglobulin substitution will be found at mucosal sites.

An additional concern is that many patients with common variable immunodeficiency suffer from hypochlorhydria because of abnormal function of the gastric antrum, and this enhances the susceptibility to enteric pathogens.

If the patient persists in her plan to go to Tunisia, the question arises as to which protective measures should be taken. A healthy traveler would receive the following advice:

¦ booster injection of diphtheria and tetanus toxoid and (in many countries) oral poliomyelitis vaccine (OPV), if there has been complete vaccination in the past and the last booster has been 10 years ago; in some countries, such as The Netherlands, OPV is not used at all, and inactivated poliomyelitis vaccine (IPV) is given instead;

¦ protection against hepatitis A if the history for this infection is negative (such protection may be given by administration of immunoglobulin 16% intramuscularly or, preferably, by hepatitis A vaccination); and
¦ protection against typhoid fever, which can be provided either by the oral vaccine containing live attenuated *Salmonella* Ty21a or by the intramuscular inactivated Vi polysaccharide vaccine.

TABLE 114-1 -- Procedure for immunoglobulin substitution in patients with anti-IgA antibodies or a history of serious reactions to intravenous immunoglobulins.

PROCEDURE FOR IMMUNOGLOBULIN SUBSTITUTION IN PATIENTS WITH ANTI-IgA ANTIBODIES OR A HISTORY OF SERIOUS REACTIONS TO INTRAVENOUS IMMUNOGLOBULINS	
Day 1	In the morning, under clinical observation, iv drip with saline; adrenaline (epinephrine) and hydrocortisone prepared for use; insert butterfly needle subcutaneously
	Start subcutaneous injection of IgG 16% at less than 0.5ml/h with infusion pump
	After 2 hours: increase to 1ml/h
	Stop after 4 hours
Day 2	Same conditions
	Start subcutaneous IgG 16% at a rate of 1ml/h
	Increase at 1ml/h to 3ml/h
	Infuse for 4 hours (total 9ml)

Day 3	3ml/h to total of 20ml
Day 7	3ml/h to total of 20ml

TABLE 114-2 -- Vaccines that are contraindicated in the immunocompromised host.

VACCINES THAT ARE CONTRAINDICATED IN THE IMMUNOCOMPROMISED HOST	
Vaccine	**Complication**
Oral poliomyelitis vaccine	Paralytic poliomyelitis
Measles*	Fever and rash
Mumps*	Parotitis, meningoencephalitis
Rubella*	Arthritis
Yellow fever vaccine	Encephalitis
Smallpox	Generalized vaccinia
Typhoid	Systemic response (?)
Bacille Calmette-Guérin	Local and disseminated infections
The designation of 'immunocompromised host' includes patients with cellular immunodeficiency disorders, agammaglobulinemia or hypogammaglobulinemia, HIV infection, leukaemia, lymphoma or immunosuppressive therapy	

* In HIV-infected children who are not severely immunocompromised measles-mumps-rubella is a recommended vaccine

In this immunodeficient patient, the advice would be very different. There are a series of considerations that are important if vaccination of a compromised host is considered.

First, live vaccines must be avoided in immunocompromised patients because infections with the vaccine strains may ensue. This holds for patients with hypogammaglobulinemia as well as for those with cellular immunodeficiency. Of the vaccines mentioned, OPV and oral *Salmonella* Ty21a are contraindicated. Inactivated poliomyelitis vaccine and the *Salmonella* Vi polysaccharide vaccine are safe. The live vaccines that are contraindicated in the immunocompromised host are listed in Table 114.2 .

Second, is it to be expected that the patient is able to mount a protective immune response to the vaccine? It is remarkable that we know very little about the relative contribution of antibodies and of cellular immunity (specific T cells) in the protective immune response. For most vaccines listed above, it is generally assumed that protection is conferred by the specific antibodies that develop after vaccination. An exception is probably the oral typhoid vaccine, which also elicits a cellular immune response that may contribute to protection (Table 114.3).

Third, assuming that the antibodies against the vaccine antigens are needed for protection, it is most likely that the IvIg substitution provides adequate concentrations antibodies against diphtheria, tetanus, poliomyelitis and hepatitis A. Antibodies against *Salmonella typhi* are not present in sufficient amounts in the IvIg preparations that are available.

Prevention of infection by enteric pathogens is important in this patient. Thus, thorough information on how to prevent alimentary infection in a country such as Tunisia should be provided to the patient, although it is known that there is generally poor compliance with such guidelines. In addition, it is good clinical practice to provide such an immunocompromised patient with a suitable antibiotic (e.g. levofloxacin) to be taken when fever or diarrhea occurs.

As noted above, responses to vaccines may differ according to the host defense defect (Table 114.4). For example, if the patient had systemic lupus erythematosus, rather than hypogammaglobulinemia, and was on low-dose prednisone and azathioprine, the situation would be different. A good boosting response to diphtheria and tetanus toxoid, as well as to IPV, would be expected, since neither the immune suppression nor the underlying disease would seriously hamper the B-cell response. Oral poliomyelitis vaccine would be considered dangerous, and the same holds for the live *Salmonella* vaccine. The *Salmonella* Vi polysaccharide vaccine would be safe and — since it is a T-cell-independent vaccine — would be as effective as in healthy volunteers. In Table 114.2 , the currently available vaccines are listed with regard to their effectiveness and safety in patients with different types of immune defects. The reader should realize that the data in the literature dealing with effectiveness and safety of vaccination in patients with various types of immunodeficiency are rather limited. Therefore, the data in Table 114.2 are not very robust but have often been obtained by prudent extrapolation.

Frequently asked questions address the effectiveness of vaccination in patients with diabetes mellitus, liver cirrhosis, end-stage renal disease, in patients on hemodialysis and in elderly patients. Generally, these patients are mildly immunocompromised and may have suboptimal antibody responses. In diabetic patients, there is no real concern about the response to influenza vaccine. In hemodialyzed patients, the response to hepatitis B vaccine is less than in a healthy population. Attempts have been made to augment the antibody response with concomitant administration of an adjuvant (such as monophosphoryl lipid A) or a cytokine (interleukin-2, interferon-?, granulocyte-macrophage colony stimulating factor) with variable success. In the elderly, the response to a vaccine such as influenza vaccine appears to be dependent on the scores attained in the activities of daily life (ADL) assessment — a high score correlates with a high immune response. It has been shown that stimulating physical activity in elderly patients leads to higher antibody titers.

Immunoglobulin substitution and passive immunization

Immunoglobulin substitution is the cornerstone of the treatment of patients with agammaglobulinemia or hypogammaglobulinemia. Nowadays, almost all patients who need such substitution receive the immunoglobulins by the intravenous route. The commercially available preparations are generally safe, although the quality in terms of antibody profiles and titers, functional capacity of the IgG molecules

TABLE 114-3 -- Effectiveness and safety of vaccines in the immunocompromised host.

EFFECTIVENESS AND SAFETY OF VACCINES IN THE IMMUNOCOMPROMISED HOST				
Vaccine	**Host defense defect**			
	Agammaglobulinemia or hypogammaglobulinemia		**Cellular immunodeficiency**	
	Effectiveness	**Safety**	**Effectiveness**	**Safety**
Bacterial				
Corynebacterium diphtheriae	-	+	+	+
Clostridium tetani	-	+	+	+
Bordetella pertussis whole cell	-	±	?	±
Bordetella pertussis subunit	-	+	?	+
Haemophilus influenzae type B conjugate	-	+	±	+
Neisseria meningitidis group C	-	+	±	+
Streptococcus pneumoniae polysaccharide	-	+	±	+

Streptococcus pneumoniae conjugate	-	+	+	+
Salmonella typhi ty21a	?	-	?	-
Salmonella typhi Vi	-	+	±	+
Bacillus anthracis	-	+	?	+
Bacillus Calmette-Guérin	+/±?	+/-?	-	-
Viral				
Oral poliomyelitis	-	-	±	-
Inactivated poliomyelitis	-	+	±	+
Measles virus		-		±
Mumps virus	-	-	±	±
Rubella virus	-	-	±	±
Hepatitis A virus	-	+	+	+
Hepatitis B virus	-	+	+	+
Influenza A virus	-	+	+	+
Rabies virus	-	+	+	+
Varicella-zoster virus				
Vaccinia virus				-
Yellow fever virus		-		-
Japanese B encephalitis		+		+
Frue sommer encephalitis	-	+	-	+
The designation of 'immunocompromised host' includes patients with cellular immunodeficiency disorders, agammaglobulinemia or hypogammaglobulinemia, HIV infection, leukaemia, lymphoma, or immunosuppressive therapy + effective/safe; - not effective/safe; ± moderately effective/safe; +/±?, +/-?, in common variable immunodeficiency with marked disturbance of cellular immunity, bacille Calmette-Guérin may not be safe and effective				

(especially the Fc part) and the side-effects differs. Most information about these differences have been obtained in vitro; a direct comparison of different preparations in vivo has not been performed.

There is a clear dose response for these preparations. Higher dosages (i.e. dosages of >600mg IgG/kg every 4 weeks) result in less infectious morbidity, and such higher dosages lead, in the long term, to better preservation of pulmonary function. The dosage regimen should be individualized to obtain optimal substitution; there is not a fixed trough level of IgG that is aimed at.

Because of the relatively long half-life of the infused IgG (most often 2–3 weeks), adequate substitution can be obtained with an interval of 3–4 weeks between infusions. Some patients, especially those with exudative enteropathy, need more frequent infusions, because of a shorter half-life of the IgG. With regard to the question of the present patient, we would aim at giving her IvIg shortly before the planned holiday.

It should be realized that IvIg preparations, although they contain mainly IgG and only trace amounts of the other immunoglobulin classes, are by no means pure preparations. They contain a variety of plasma proteins (e.g. prokallikrein, cytokines), which have biologic effects in the recipient. Recent studies have shown that IvIg infusion leads to a series of changes in the concentrations of inflammatory and anti-inflammatory molecules (e.g. adhesion molecules, cytokines inhibitors such as IL-1ra) and in the functional state of white blood cells. Further research is needed to establish whether such changes are benefical in terms of host defence and to what extent they are responsible for side-effects.

Serious side-effects may occur in patients with antibodies against IgA and sometimes other immunoglobulin classes. In particular, patients with common variable immunodeficiency that was preceded by complete IgA deficiency may have developed antibodies against IgA, which is a neoantigen for these patients. When these patients receive IvIg, they may respond with an anaphylactoid reaction, owing to traces of IgA in the IvIg preparations. During such a reaction, high concentrations of tumor necrosis factor may be found in the patient's blood. It is good clinical practice to assess the presence of anti-IgA in the blood before IvIg is given for the first time.

Patients with anti-IgA as well as those who have experienced side-effects to IvIg not due to anti-immunoglobulins can safely be given 16% immunoglobulin by slow subcutaneous infusion. In Table 114.3 , an example of a treatment protocol is given.

A special problem in the patient presented above is the persisting *C. jejuni* in her stools. This is not a rare occurrence in patients with agammaglobulinemia, and such carrier states may be complicated by recurrent febrile episodes caused by transient bouts of *C. jejuni* bacteremia. Occasionally, such episodes of bacteremia are accompanied by erysipelas-like skin lesions or even metastatic infection. In these patients, there is a bactericidal defect of the serum toward *C. jejuni*,

TABLE 114-4 -- Immunization strategies in immunocompromised hosts.

IMMUNIZATION STRATEGIES IN IMMUNOCOMPROMISED HOSTS											
Commonly used vaccines											
		Diphtheria toxoid	**Poliomyelitis**	**Pertussis**	**HiB conj**	**Hepatitis B**	**Pneumococcus**	**Meningococcus conj C**	**MMR**	**Influenza**	**BCG**
Primary ID	Complement deficiency	S	S	S	S	S	S	I	S	S	S
	Agammaglobulinemia	NE	NE	NE	NE	NE	NE	NE	NE	?	S
			OPV CI								
	Granulocyte defects	S	S	S	S	S	S	S	S	S	CI
	Macrophage defects	S	IPV	S	S	S	S	S	S	S	CI
	T cell defects	S	IPV	S	S	S	S	S	?	S	CI

Acquired ID	Secondary hypogammaglobulinemia (myeloma, chronic lymphoid leukaemia)	NE	NE	NE	NE	NE	NE	NE	S	?	S
			OPV CI								
	Myelosuppressed patients (acute leukemia)	S	IPV	S	S	S	S	S	S	S	CI
	Immunosuppressed patients (e.g. organ transplant recipients)	S	IPV	S	S	S	S	S	S	S	CI
	Lymphoma	S	IPV	S	S	S	S	S	S	S	CI
	BMT recipients	I	I, IPV	I	I	S	I	S	I	I	CI
	AIDS	S	IPV	S	S	S	I?	S	CI	I?	CI
	Postsplenectomy	S	S	S	I?	S	I	S	S	S	S

Vaccines for travel

		Hepatitis A	**Typhoid Ty21a**	**Typhoid Vi**	**Yellow fever**	**Hepatitis B**	**Meningococcus A/C**
Primary ID	Complement deficiency	S	S	S	S	S	I
	Agammaglobulinemia	NE	NE	NE	NE	NE	NE
			CI				
	Granulocyte defects	S	CI	S	S	S	S
	Macrophage defects	S	CI	S	S	S	S
	T cell defects	S	CI	S	CI	S	S
Acquired ID	Secondary hypogammaglobulinemia (myeloma, chronic lymphatic leukemia)	NE	NE	NE	NE	NE	
		CI	CI				
	Myelosuppressed patients (acute leukemia)	S	CI	S	S	S	S
	Immunosuppressed patients (e.g. organ transplant recipients)	S	CI	S	CI	S	S
	Lymphoma	S	CI	S	CI	S	S
	BMT recipients	S	CI	S	CI	S	S
	AIDS	S	CI	S	CI	S	S
	Postsplenectomy	S	S	S	S	S	S

The designation of 'immunocompromised host' includes patients with cellular immunodeficiency disorders, agammaglobulinemia or hypogammaglobulinemia, HIV infection, leukaemia, lymphoma, or immunosuppressive therapy I, indicated or commonly recommended for this condition. Note: specific recommendations or restrictions apply in some patient groups. Data indicate general principles and may not apply for individual patients; CI, contraindicated; S, standard indication as in normal hosts; NE, not effective; ?, unknown; OPV, oral poliomyelitis vaccine; IPV, inactivated poliomyelitis vaccine preferable; HiB, *Haemophilus influenzae* type b; MMR, mumps-measles-rubella; BCG, bacille Calmette-Guérin; conj, conjugated; ID, immunodeficiency; BMT, bone marrow transplant

and this defect is due to a deficiency of IgM, which should mediate the killing by complement. If antimicrobial treatment against *C. jejuni* fails, then IgM-containing immunoglobulin preparations or plasma infusions should be added to restore the serum bactericidal effect.

Special measures are also necessary in the rare agammaglobulinemic or hypogammaglobulinemic patients who suffer from persistent echovirus encephalitis and dermatomyositis-like syndrome and in those with disseminated refractory *Ureaplasma urealyticum* infection. In patients with persistent echovirus encephalitis, extremely high dosages of immunoglobulin preparations (preferably selected for high neutralization titers against the causative virus) are necessary for successful treatment. In patients with refractory *U. urealyticum* infection, specific antibodies have been raised by immunization of goats to obtain plasma for treatment of such patients.

So far, there are only a few examples of commercially available high-titer specific immunoglobulin preparations. Some of these have been developed for prevention or treatment in immunocompromised hosts. Immunoglobulin preparations with high antibody titers against cytomegalovirus (CMV) have been developed to prevent and treat CMV infections in bone marrow and organ transplant recipients. These preparations are used either alone or in combination with ganciclovir. Clinical trials in renal transplant recipients have demonstrated reductions not only in CMV-associated syndromes but also in secondary fungal and parasitic infections. In addition, increases in graft survival have been noted. Similar observations have been made in recipients of orthotopic liver transplant and probably apply to other transplant patients as well. The efficacy of these preparations in the prevention and treatment of CMV disease in bone marrow transplant recipients is much less clear.

Anti-respiratory syncytial virus (RSV) immunoglobulin has been recommended for prevention of RSV infection in patients at high risk, such as infants with chronic pulmonary disease. Alternatively, palivizumab, a humanized monoclonal antibody against the RSV F glycoprotein, is also effective in preventing RSV infection in high-risk patients. The use of either Anti-RSV immunoglobulin or palivizumab for the treatment of established RSV infection is controversial, and studies have failed to show beneficial effects of either drug.

Conclusion

It is clear from the above that the knowledge of active and passive immunization for the prevention of infections in immunocompromised patients is limited. With respect to active vaccination, we lack data on protective cellular and humoral mechanisms, on clinical efficacy and on risks to the patient. Although much knowledge has been assembled on immunoglobulin substitution in patients with primary antibody deficiency syndromes, it is less clear what should be done with secondary antibody deficiencies (such as occur in chronic lymphatic leukemia and myeloma). The nature and the effects of the nonimmunoglobulin components of the various IvIg preparations have not been studied sufficiently. With regard to specific antibody preparations, there are great opportunities to develop new preparations for the prevention of a variety of infections that occur in compromised hosts.

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Section 5 - HIV AND AIDS

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Chapter 115 - Epidemiology of HIV Infection

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INTRODUCTION

This chapter describes the distribution and transmission patterns of HIV infection. Although the biology and modes of transmission are broadly the same in the developing and industrialized world, there are some striking differences in the epidemiology, which are due to a variety of behavioral factors and socio-economic conditions. Furthermore, the AIDS problem is overwhelmingly concentrated in developing countries, where more than 90% of all people infected with HIV live.

In less than 15 years HIV has reached the level of a pandemic and AIDS has been reported in over 190 countries.[1] It is also increasingly clear that the HIV pandemic consists of several separate epidemics, each with its own distinct characteristics. On a world scale, the epidemic has evolved predominantly into a heterosexually transmitted disease in the developing world and, increasingly, of underprivileged and marginalized populations in the industrialized world.

FREQUENCY OF HIV INFECTION AND AIDS AND DEFINITION

Shortly after the first reports of AIDS in the USA in 1981 among homosexual men and injecting drug users, it became obvious that the disease was also present in Haitians living in North America and in Africans seen in Belgium for medical care at the end of 1983.[2] [3] Subsequently, surveys in Haiti and in central Africa confirmed the occurrence of several epidemic foci of HIV in these areas.[4] [5] [6] [7]

The identification of another variant of the virus, labeled HIV-2, among West African populations and then in other African countries with a Portuguese colonial history increased further the heterogeneity of what quickly emerged as a global pandemic. The routes of transmission and risk factors of HIV-2 and HIV-1 are similar, but it has become increasingly clear that the pathogenic effect of HIV-2 is lower than that of HIV-1.[8] In the rest of this chapter, HIV refers to HIV-1.

Although there are no doubts that the HIV epidemic has spread well beyond the most pessimistic predictions, its exact magnitude is difficult to assess. This is due to the largely silent nature of this infection and to limited surveillance in many countries. In addition, diagnostic facilities for HIV infection and its associated opportunistic diseases are still limited in many clinical settings. Another longstanding obstacle has been the absence of a simple definition for AIDS. Indeed, the early definition of AIDS of the Centers for Disease Control and Prevention required expanded laboratory diagnostic capabilities. The World Health Organization (WHO) has adopted a simplified definition of AIDS in adults (1985 Bangui definition), based on the recognition of at least two major clinical signs in combination with at least one minor sign. This is straightforward to use and allows identification of AIDS cases without the need to perform expensive tests. In early 1994, taking into account better access to laboratory diagnostic methods, a positive serologic test for HIV-1 and/or HIV-2 and a broader spectrum of clinical manifestations of HIV such as tuberculosis and pneumonia were added to this definition.[9]

GLOBAL CASES: REPORTS AND ESTIMATIONS

By the end of 2001, over 2.7 million cumulative AIDS cases had been reported to the WHO. However, because of under-reporting, underdiagnosis and delays in reporting, the joint United Nations Program on HIV/AIDS (UNAIDS) and WHO estimated that a total of more than 22 million cumulative AIDS cases in adults and children may have occurred worldwide. The developing world as a whole accounted for well over 80% of all AIDS cases. According to UNAIDS nearly 2.7 million cases are pediatric AIDS cases resulting from mother-to-child HIV transmission. During 2001 alone, HIV infection and AIDS-associated illnesses killed an estimated 3 million people, including 580,000 children; this represents 25% of all deaths since the start of this global epidemic, illustrating that on, a worldwide scale, mortality from HIV infection is accelerating, notwithstanding declining mortality from HIV infection in most industrialized countries.

A more complete picture of the extent of the epidemic is given by the number of people infected with HIV (Table 115.1).[1] By conservative estimates, well over 37.2 million adults and a further 2.7 million children under 15 years have been infected with HIV since the start of the pandemic. Figure 115.1 shows the estimated regional distribution of young people aged 15–24 who have HIV infection and AIDS. More than 13 million children under 15 years of age have lost their mothers or both parents as a result of AIDS-related death.

The incidence of HIV infection in the general population is not known but there have been several cohort studies or repeated surveys of the same population in some countries. The highest HIV incidence rates — 10–15% a year — have been found among sex workers and injecting drug users in various cities around the world. Based on HIV infection trends in different parts of the world, the number of new AIDS cases is expected to continue to increase, mostly in Africa, but also in Asia and eastern Europe.

Geographic distribution of HIV infection and AIDS

North America, Europe and Australia

The cumulative total of reported AIDS cases in the USA was nearly 717,000 at the end of 1999 and it was estimated that more than 940,000 adults and children are living with HIV/AIDS. The annual incidence of AIDS and deaths among people living with AIDS declined during 1996, reflecting the beneficial impact of new available therapies. Although this trend continued through 1998, data for 1999 suggest that the number of AIDS cases and deaths might be leveling out.[10] Early in the epidemic, most infections occurred in men who have sex with men, but the incidence in this group leveled off as early as 1985–7. However, HIV prevalence levels of 7–9% are still found among young adult homosexual and bisexual men in major cities such as San Francisco and New York. The largest decline in the proportion of reported AIDS cases in the USA has occurred among homosexual and bisexual men, whereas cases reported to be acquired by heterosexual transmission have increased, with highest rates in women. Each year approximately 7000 women with HIV

TABLE 115-1 -- Global estimates of HIV/AIDS worldwide at end of 2001.

GLOBAL ESTIMATES OF HIV/AIDS WORLDWIDE AT END OF 2001		
Number of people living with HIV/AIDS	Total	40 million
	Adults	37.2 million
	Women	17.6 million
	Children under 15 years	2.7 million
People newly infected with HIV in 2001	Total	5 million
	Adults	4.3 million
	Women	1.8 million
	Children under 15 years	800,000
AIDS deaths in 2001	Total	3 million
	Adults	2.4 million
	Women	1.1 million
	Children under 15 years	580,000

infection give birth, and without prophylactic treatment 1000–2000 of their infants would be infected with HIV. The prevalence of HIV among injecting drug users has been increasing steadily as well, but with large regional differences. Since the late 1980s on the west coast of the USA, about 90% of people who have AIDS are men who have sex with men, whereas on the northeastern coast the majority of newly diagnosed people with AIDS are injecting drug users. Young adults belonging to ethnic minorities (including men who have sex with men) face considerably greater risks of infection that they did 5 years ago. African-Americans, for instance, make up only 12% of the population of the USA but constituted 47% of AIDS cases reported in 2000 (Fig. 115.2).

Canada has a cumulative number of HIV infections estimated to be around 50,000. Women now represent 24% of new HIV infections, as compared with 8.5% in 1995. The estimated number of new infections has been slowly declining and was around 2000 in

Figure 115-1 Young women and men (aged 15–24 years) estimated to be living with HIV/AIDS, 2001. Total at end of 2001, 40 million.

2001. There was, however, concern about recent outbreaks in the injecting drug user community in Vancouver and an incidence of 5 new infections per 100 drug injectors per year in Montreal, one of the highest rates in North America.[11]

By the end of 1999, in western Europe as a whole, AIDS incidence appeared to have decreased and this seems mainly due to a decrease in the incidence of homosexually acquired AIDS and of injecting drug use (Fig. 115.3).[12] However, in Italy, Spain and Portugal, where the majority of the cases are acquired through injecting drug use, the AIDS incidence is still high. As a consequence of this shift, there is a marked increase in the proportion of reported female cases, from 12% in 1986 to 25% in 2000. Following the introduction of highly active antiretroviral therapy (HAART), the number of reports of deaths among AIDS cases also continued to decrease, at an average annual rate of -30% between 1997 and 2000.

In countries of eastern Europe, which are experiencing profound social change, HIV epidemics are much more recent but are increasing exponentially, mainly in relation to drug use. Ukraine recently reported a rise of HIV prevalence among injecting drug users in Nikolayev from 2% to 57% in 1996 within 1 years. Although fewer than 400 HIV infections were recorded between 1987 and 1994, a total of 1500 were recorded in 1995 and close to 40,000 in 2000. The Russian Federation and eastern European countries are experiencing a similar progression, especially among injecting drug users. Furthermore, in the former Soviet Union, syphilis incidence rates doubled in 1995 compared with those in 1994 to reach close to 200/100,000 population, illustrating increased unsafe sexual behavior. A particular problem has resulted from the nosocomial epidemics in children in Romania and the Russian Federation in the late 1980s, which are the cause of HIV infection in over 50% of children who have AIDS in central and eastern Europe today. With an estimated cumulative total of more than 113,000 HIV infections, the Russian Federation and Ukraine have a similar number of HIV infections to western Europe.

Figure 115-2 Percentage of newly reported AIDS cases by race/ethnicity, USA.

In Australia and New Zealand, just as in several countries of northern Europe and some parts of the USA and Canada, the vast majority of HIV infections have been acquired through sexual contacts between men, but the incidence reached a peak in the mid 1980s. Programs involving community members and allowing easy access to sterile injecting equipment and methadone treatment have permitted the prevalence in injecting drug users to remain very low, at around 1% in several major cities. Heterosexual transmission is rare. There is evidence that HIV infection rates have reached a plateau in Australia and are declining in New Zealand.

Sub-Saharan Africa

In the nations of sub-Saharan Africa, prevalence rates in the general population vary from less than 1% to 20% and more. Although the

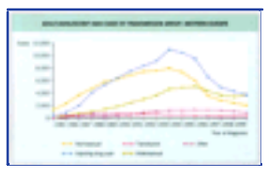

Figure 115-3 Adult/adolescent AIDS cases by transmission group, western Europe.

epidemic was first recognized in central and east Africa, where it remains at high levels, there is also a large epidemic in west Africa within and around the Ivory Coast. According to UNAIDS, at the end of 2000, 28.1 million adults and children had HIV infection in sub-Saharan Africa, of whom 50% were female. Of the 5.1 million estimated HIV-infected children born in the world since the beginning of the pandemic, over 90% have been born in Africa. In some cities in central and southern Africa, approximately 35% of women attending antenatal clinics have HIV.[1]

The prevalence of HIV continues to increase, mainly in the southern part of Africa and in the west. In major urban areas of Botswana, HIV prevalence levels in adults had reached more than 40% by 1997. HIV prevalence in pregnant women in South Africa has also increased dramatically; for example, between 1990 and 1998 HIV

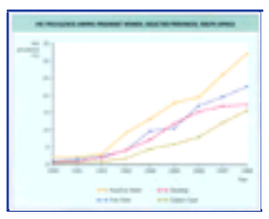

Figure 115-4 HIV prevalence among pregnant women, selected provinces, South Africa.

prevalence in pregnant women increased from 1% to 21% in the Free State, the North Cape, Mpumalanga and the northern and north west states (Fig 115.4).

National and city-based serosurveys carried out at the end of the 1980s have given age- and sex-specific seroprevalence rates that show a similar pattern everywhere, typical of heterosexual and perinatal transmission of HIV from mother to child. Rates usually show a bimodal curve with a first peak prevalence in children under 5 years of age, then very few infections, and a second peak at 20–30 years of age, with very few infections in people over 40. In Kinshasa, Zaire, but also in east Africa, young women under 25 years of age were three to five times more frequently infected with HIV than men of the same age. In contrast, after 35 years of age this ratio reverses and more men than women are infected. An explanation for these differences is probably found in a combination of biologic factors, such as a higher biologic susceptibility to HIV infection and other sexually transmitted diseases (STDs) in young women, and cultural patterns, such as the preference of men for female partners younger than themselves.[13] [14]

HIV-2 is primarily found in West Africa but has also been confirmed in other African countries. The highest prevalence of HIV-2 infection is found in Guinea Bissau, with prevalence rates as high as 9.5% among adults and an annual incidence of 0.9%. In contrast to the increasing spread of HIV-1, the prevalence of HIV-2 has remained rather stable in West Africa. This is probably the result of the higher transmissibility of HIV-1 compared with that of HIV-2.[15]

Asia and the Pacific

It is estimated that about 7.1 million people have HIV infection and AIDS in Asia and the Pacific and 90% of them live in India, Thailand and Myanmar. The spread of HIV in this region became detectable in the second half of the 1980s, at first among injecting drug users in Myanmar and Thailand. The HIV seroprevalence rate among injecting drug users varies substantially; rates of over 60% have been found in China's Yunnan Province, northern Thailand, Manipur state in India, Vietnam and Myanmar. The epidemic has quickly spread out of the injecting drug user and sex worker communities in some major cities of India and into the general population. In Mumbai, in less than 5 years (i.e. by 1996), HIV prevalence had reached 50% among sex workers, 33% in STD patients and 4.3% in pregnant women. Most HIV transmission in Asia and eastern Europe is due to the sharing of infected needles among injecting drug users (Fig. 115.5).

Estimates of the number of adults and children infected with HIV in India are close to 4 million and by the turn of the century there may have been more people in India who had AIDS than in any other country in the world. In most of Asia, heterosexual transmission is now by far the predominant mode of spread.

Surveillance data in China leads to an estimation of more than 1 million people living with HIV. Increasing evidence has emerged of serious epidemics in Henan province in central China, where many tens of thousands of rural villagers have become infected by selling their blood to collecting centers.

In Thailand, the epidemic has been particularly well studied. HIV has spread in overlapping waves through injecting drug users and sex workers, their clients and the female partners of clients. The prevalence among sex workers rose from 3% in 1989 to 30% in 1996 and that among STD clinic attenders rose from nearly zero to 9% over the same period. There is convincing evidence of a fall in risky behaviors following extensive programs to promote condom use in brothels and to discourage men from visiting them.[16] The number of new cases of STDs seen in clinics has fallen and the national prevalence of HIV among young military recruits dropped from 3.6% in 1993 to 2.5% in 1996. There are indications that transmission between spouses is now responsible for more than half of new infections.

An HIV epidemic has recently developed in Papua New Guinea, fueled mainly by heterosexual transmission.

Latin America and the Caribbean

As of the end of 2001, UNAIDS estimated that 1.4 million persons had HIV infection and AIDS in Latin America and the Caribbean, with 30% of all infections occurring in women. Approximately 200,000 adult and pediatric AIDS cases have been reported and more than 70% of these occurred in Brazil, Mexico, Argentina or Honduras.

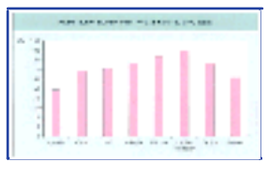

Figure 115-5 Proportion of new HIV infections in injecting drug users.

In the early years of the HIV epidemic in Latin America, over 50% of reported infections were among homosexual and bisexual men; heterosexual transmission contributed another 25%. However, since the mid-1980s, there has been an increase in heterosexual transmission, principally among bisexual men and their female sexual partners, and among sex workers and their clients. In Brazil, for example, the proportion of reported AIDS cases attributable to heterosexual transmission increased from 3% in 1985 to 31% in 1996. In Saõ Paulo, data suggest a different pattern of HIV spread between men and women; whereas HIV prevalence among male STD clinic attenders was stable between 1993 and 1994, rates may have increased more than 5-fold among female STD clinic attenders over the same period. It was estimated in 1999 in Brazil that, in addition to the 30,900 children orphaned as a result of HIV infection and AIDS, more than 100,000 children had mothers who were currently infected with HIV (Fig. 115.6). HIV is also reported to be spreading rapidly among injecting drug users in Brazil and Argentina and presents a growing problem.

Figure 115-6 Impact of the epidemic on children with HIV-infected mothers. *Model of Global Orphan Project, data from Brazil.*

Of the Central American countries, Honduras has been especially broadly affected by heterosexual transmission; nearly 60% of the AIDS cases reported in the subregion are in Honduras. In most of the Caribbean, heterosexual transmission has been the predominant mode of transmission for at least a decade. Prevalence rates among pregnant women have reached 4%, 10% and 6% in the Bahamas, Haiti and Trinidad-Tobago respectively. In contrast, infection rates remain relatively low in Jamaica but have risen in marginalized groups such as crack cocaine users and migrant laborers. In the Dominican Republic, HIV seroprevalence has reached levels of up to 11% among female sex workers, 3–4% among STD patients and 1.4% in pregnant women.

North Africa and the Middle East

By the end of 2001, approximately 440,000 persons in this region had HIV infection and AIDS, with a male:female ratio of 2.5:1. So far, the spread of the virus appears to be limited to homosexual men and injecting drug users in large cities. About 75% of the AIDS cases have been reported from Morocco, Sudan, Saudi Arabia, Tunisia and Djibouti. HIV seroprevalence levels among the general population show seropositivity far less than 1%. Djibouti seems to be the hardest hit country in the region, with prevalence levels of up to 9% in pregnant women. A rise in HIV prevalence among STD patients has recently been noticed in Sudan.

Dynamics of the HIV epidemic

Under circumstances that are not yet fully understood, epidemics may suddenly explode, with rates of infection increasing several-fold within only a few years. For example, estimation of HIV seroprevalence among injecting drug users seeking treatment in Bangkok increased from zero in 1985–6 to 16% in 1988 and 40–60% in 1992. Outside these vulnerable groups, contrasting situations have been found in the general population. Cambodia did not record its first diagnosis of HIV infection until 1991. By 1996, HIV prevalence among pregnant women was approaching 5% but was estimated to have declined to 2.3% at the end of 2000 through the implementation of 100% condom use in brothels.

In sub-Saharan Africa, most new HIV infections are now occurring among young people and particularly among young girls (Fig. 115.7). There is often a doubling of the HIV rate between the 15–19 age group and the 20–24 age group. In these relatively generalized epidemics, more women are infected with HIV than men both in their late teens and in their early 20s. The pattern is consistent

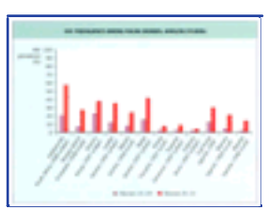

Figure 115-7 HIV prevalence among young women, African studies. Seropositivity among women aged 15–19 years and 20–24 years in selected community surveys in sub-Saharan Africa, 1995–2000.

Figure 115-8 Percentage of adolescents who had their first sexual experience before age 15 years.

regardless of the overall level of HIV prevalence, and regardless of whether the study is conducted in an urban or a rural area. On average, eight times more girls than boys are infected with HIV in their late teens, while among young people in their early 20s three times as many women are infected as men. Sexual behavior patterns and sexual networks are believed to play a critical role as well as an increased biologic susceptibility to HIV/STD associated with early age at first sexual intercourse (Fig 115.8).[17] Another factor influencing the efficiency of the transmission of HIV is the lack of male circumcision and the prevalence of other STDs in a community.

More attention is given now to the role of herpes simplex virus type 2 (HSV-2) as enhancing susceptibility to HIV per sexual act. Figure 115.9 shows the dramatic increase in HSV-2 seropositivity in young girls after a few sexual contacts in a mining town of South Africa.[18] A recent study in Thailand also found that the risk of female-to-male transmission of HIV is about 10 times higher in sex worker-client contact than in a stable sexual relationship, possibly because of the presence of other STDs, such as HSV-2, because of different sexual practices and because of new primary infections associated with a peak in infectiousness.[19] [20]

The possibility should not be excluded that different genetic subtypes of HIV-1 are associated with differences in the efficiency of spread of the virus in a population. Nine subtypes have already been identified in the dominant group M (A–H) and one outlier categorized as O. However, only scarce information exists on their distribution in HIV infected populations (Fig. 115.10).[21] Prospective studies and/or repeated studies in the same populations should allow control

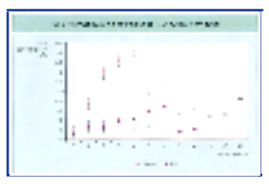

Figure 115-9 HSV-2 among young people in a South African town. Seroprevalence among male and female adolescents aged 15–24 years by number of lifetime sexual partners.

Figure 115-10 Estimated prevalence of HIV-1 *env* subtypes by region, 1998.

for the many confounders before concluding on differences in the transmissibility of HIV subtypes.[22] The recent observation that some sex workers who had been repeatedly exposed to HIV infections remained HIV-antibody-negative raised the hypothesis that some people have a weaker susceptibility to or even immunity against the virus or specific subtypes.[23] It has been suggested that factors such as certain HLA haplotypes and, more recently, polymorphisms of the chemokine receptor gene *CKR5* may explain such resistance but there is still no conclusive evidence.

The hope that the number of new infections is decreasing comes from studies in many countries in the developed world as well as from Thailand,[16] Senegal[24] and Uganda, a country with one of the older epidemics in Africa. A study of recent trends in HIV infection in young pregnant women in urban Uganda showed a 35% decline in HIV prevalence.[25] This may be explained by a variety of factors such as increased death rates among seropositive people, saturation of the most susceptible and decreased infectiousness of people with HIV over time until the occurrence of AIDS. However, a 2-year delay in the average age at first sexual intercourse, a slight reduction in the number of casual partners and a substantially increased use of condoms are the more likely explanations of the HIV decline among youth in Uganda. Figure 115.11 shows HIV prevalence levels over time in selected sites or nationally for Thailand.

MODES OF TRANSMISSION

Sexual transmission

In contrast to the industrialized world, heterosexual intercourse accounts for more than 85% of cases of HIV infection in developing countries. Early epidemiologic studies showed that risk factors associated with HIV infection were unprotected sexual intercourse with multiple partners or an infected partner and the presence of STDs or a history of STDs.[26] [27] [28] [29] [30] More recent studies have highlighted sex differences in patterns of HIV transmission; for many monogamous women the main risk factor for HIV may be the heterosexual and homosexual behavior of their steady partner. Although the probability of HIV transmission associated with unprotected vaginal sexual intercourse is not constant from one contact to another, estimates per episode — based on discordant couples — range from 0.0005 to 0.002 in the absence of co-factors.

A summary of biologic factors influencing the probabilities of HIV transmission from an infected person to a susceptible individual is given in Table 115.2 . The presence of STDs suggests a marked risk of concurrent HIV infection, for at least two reasons:

- the modes of transmission of HIV and other STDs are similar; and
- the role of STD-induced genital ulcers, including genital herpes, chancroid [31] and syphilis, as well as nonulcerative STD, facilitates transmission of HIV.

Studies among prostitutes in Kinshasa and Nairobi found gonorrhea, chlamydial infections and trichomoniasis to be independent risk factors for HIV acquisition, with

relative risks of 2.7–3.5[32] Lack of circumcision in males has been shown in most studies to be associated with the risk of acquiring STDs, especially genital ulcer and HIV.[33] Early diagnosis and treatment of STDs have been shown to reduce the incidence of HIV infection significantly in a controlled trial in Mwanza, Tanzania. In the intervention communities, a 42% decline in the rate of newly acquired HIV infections was observed.[34]

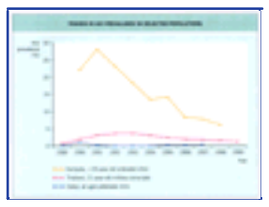

Figure 115-11 Trends in HIV prevalence in selected populations. HIV seroprevalence among pregnant women in Dakar and Kampala and among military conscripts in Thailand, 1989–1999.

TABLE 115-2 -- Biologic factors increasing the probability of sexual transmission of HIV.

BIOLOGIC FACTORS INCREASING THE PROBABILITY OF SEXUAL TRANSMISSION OF HIV	
Confirmed	Acute primary HIV infection
	Advanced clinical stage of HIV
	Sexually transmitted diseases
	Anal intercourse
	Menstruation
	HIV-1 versus HIV-2
Under study	Lack of male circumcision
	Hormonal contraception
	Cervical ectopy
	Genital trauma (use of vaginal products)
	Specific HIV-1 clades

Perinatal transmission

As a result of heterosexual transmission in women, the number of infants born with HIV infection is growing dramatically. Documented rates of transmission of HIV-1 from mother to child vary from 13% to 48%. HIV-2 is apparently very rarely transmitted perinatally. Mechanisms responsible for such variations in transmission rates of HIV are not yet well understood and probably involve multiple factors such as the immunologic status and viral load of the mother, maternal vitamin A deficiency, ingestion by the newborn at the time of delivery of the mother's HIV-infected blood or amniotic fluid, and ingestion of infected breast milk.

Although the virus has been isolated in breast milk, the risk attributable to breast-feeding and the timing of transmission has not yet been fully assessed and varied from 10% to 20% among mothers with HIV infection. A review of studies estimated that the risk of postnatal HIV transmission was about 30% when mothers seroconverted during the period of breast-feeding.[35] [36]

Blood products and contaminated equipment

Because of the rational use of blood, systematic HIV screening of blood donors and avoidance of donors self-reporting at higher risk, the risk of transmitting HIV infection through blood transfusion is estimated to be far less than 1/100,000 units of blood. In many developing countries, however, a significant proportion of blood donations remain unscreened for HIV and an estimated 5–10% of HIV infections may still occur through blood transfusion, with women and children at greater risk because of frequent anemia. Clinical indications for appropriate blood transfusions are often not met. Although the situation of contaminated blood is improving and most countries have national policies to screen blood that is to be used for transfusion, implementation is still not universal.

In many developing countries disposable needles and syringes are often not available and sterilization practices are not always adequate. The use of other skin piercing instruments, for instance for scarification and circumcision, also has some potential for HIV transmission. This results in potentially frequent parenteral exposure to HIV in populations where HIV prevalence is high, including among health care workers.

The probability of HIV infection due to puncture by a contaminated needle may be in the range of that estimated for a single episode of sexual intercourse with an infected partner or for a single episode of intravenous drug use with HIV-contaminated equipment (0.003–0.007). The potential for HIV transmission by unsterilized needles in medical settings is probably weak, but localized outbreaks in the Russian Federation, Romania and Libya among infants and young children have shown that it can occur in special circumstances. In Romania, over 90% of cases have occurred in children living in public institutions as a result of the re-use of contaminated and inadequately sterilized injection equipment or repeated micro-transfusions of contaminated blood from one child to another.

In 2001, it became widely known that, in a number of Chinese provinces but mostly in Henan in central China, paid blood donation had caused HIV infections, with estimates ranging from below 100,000 to several hundreds of thousands. Before 1996, poor rural

farmers had been selling blood and plasma to commercial blood processing companies to supplement their small income. Blood from many donors was collected and mixed. The red blood cells were separated from the pooled plasma and re-injected back into donors to reduce anemia. Infection rates between villages appear to be highly variable but might be more than 50% of the population in some villages. The risky practice of selling plasma to blood products companies has been illegal for several years now and should not be confused with blood donations for medical purposes, which are screened for HIV and other blood-borne diseases.

NATURAL HISTORY

Data from seroincident cohorts in industrialized countries suggest a median adult incubation period of about 10 years,[37] which increased to 10–12 years in the late 1990s with improvements in the use of antiviral therapy and will probably become even longer with the introduction of antiretroviral combination therapy.

Little information is available about the natural history of HIV infection in many developing countries. The literature suggests, nevertheless, that both the incubation and the symptomatic survival period may be shorter than in industrialized countries because of a combination of poor health care, the particularly high burden of other infections and faster progression to severe immunodeficiency. In African studies the reported median times to AIDS are 2–7.5 years. A recent cohort study in Uganda estimated the cumulative progression to AIDS in their incident group — 22% at 5 years — to be similar to that seen in cohorts of homosexual men.[38] Many previous studies have been of sex workers, in whom the disease may progress faster due to HIV infection with more than one HIV strain and/or immune depression linked with repeated infections by STDs. [39]

It has not been convincingly shown that other infections act as cofactors in the development of AIDS, except for tuberculosis. *Pneumocystis carinii* and Kaposi's sarcoma are less frequently associated with AIDS in developing countries than in industrialized countries.

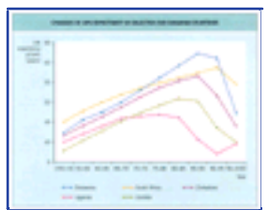

Figure 115-12 Changes in life expectancy in selected sub-Saharan countries. Projected life expectancy at birth, 1950–2000.

An unanticipated consequence of the AIDS epidemic has been a dramatic rise in the incidence of tuberculosis. In sub-Saharan Africa, it is estimated that the annual incidence of tuberculosis is 15 times higher among HIV-positive people than among those who are seronegative.[40] In addition, among people who have HIV infection, tuberculosis is now the most common and deadly opportunistic infection, accounting for 40% of all adult deaths, and it is likely that it increases immunodeficiency. Many countries have seen the number of tuberculosis cases doubling or tripling over the past 5 years. This new situation poses an unprecedented challenge to tuberculosis control programs in much of the developing world.

Demographic and social impact

In cities of western Europe such as Paris and Amsterdam, and in more than 66 cities in the Americas, AIDS was at one time the leading cause of death for young men aged 25–44 years and the third most common cause of death for young women. However, with the increased life-saving effect of antiretroviral therapy, deaths attributed to AIDS are declining rapidly. By 1989, in many cities of Africa, AIDS was the leading cause of death and of years of potential life lost in men over 15 years of age, and the second most important cause, after maternal mortality, among adult women. In a rural area of Uganda, with an HIV prevalence of 8%, 50% of all adult deaths and 89% of deaths in those aged 25–34 years were attributed to HIV.[41] Countries with such high HIV prevalence are showing national increases in adult mortality of the order of 300%. Recent projections suggest that life expectancy in sub-Saharan countries most affected by HIV will have been reduced as much as 15 years by the year 2000 when compared with projections without HIV (Fig. 115.12). [42] The long-term implications of AIDS mortality on the population pyramid for Botswana are illustrated in Figure 115.13 . In the absence of antiretroviral treatment for AIDS patients, it is projected that there will be more adults in their 60s and 70s in 20 years time than there will be adults in their 40s and 50s.

The HIV pandemic kills adults in their most productive years, when they are responsible for the support and care of dependants. It has been estimated that by the end of 2001 over 13 million children

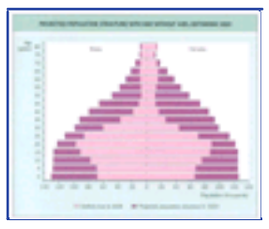

Figure 115-13 Projected population structure with and without AIDS, Botswana, 2020.

had lost their mothers or both parents because of AIDS.[1] Such orphanhood rates may cause traditional child fostering arrangements, which are common in many cultures, to break down.

AIDS has profoundly affected the health systems in industrialized and developing countries alike. In industrialized countries, AIDS has illustrated the need to strengthen some of the weakest components of health services, such as access to appropriate care, counseling and prevention activities and participation of the sick in treatment and medical decision making. In the developing world, HIV vulnerability has been fueled by rapid urbanization, increased migration, conflicts and wars and increased inequalities. In many countries of the developing world, the economic impact of AIDS is felt not only on health care costs but also on skilled labour forces and decreases in gross domestic product, which in turn has a negative impact on many social indicators such as education and health. In cities where HIV prevalence is higher than 10%, hospital wards are overloaded with patients who have AIDS and tuberculosis and require long, repeated hospitalizations, so consuming scarce resources. At the turn of this century, AIDS is posing a threat to economic growth and development in many regions. In June 2001, government and civil society representatives met for a Special Session of the General Assembly of the United Nations to consider an expanded response to HIV/AIDS. This policy forum endorsed the results of a study estimating that, by 2005, that response would require about US$9 billion annually, with half of these resources needed in sub-Saharan Africa. About half of the total amount is required for prevention and the other half is needed for palliative care, treatment and prophylaxis of opportunistic infections, support for orphans and antiretroviral therapy.[43]

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Chapter 116 - Prevention of HIV Transmission Through Behavioral and Biological Interventions

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Steven A Safren

HIV TRANSMISSION DYNAMICS

HIV transmission is a high-consequence but low-probability event with the majority of relevant exposures not resulting in new infections.[1] There are multiple co-factors that may affect HIV transmission, which is why there is a high level of variability in estimates of the relative risk of infection for specific exposures (Table 116.1). [2] [3] HIV may be transmitted as cell-free or cell-associated virus, and different factors may affect expression of virus concentrations in different body fluids (i.e. blood, semen or cervicovaginal secretions).[4] [5] [6] [7] Although lower blood concentrations of HIV are associated with lower rates of HIV transmission, [8] antiretroviral drugs do not necessarily make HIV-infected people noninfectious or incapable of transmitting the virus. In fact, the sexual transmission of multidrug-resistant HIV has been well documented,[9] [10] underscoring the need for providers to promote safer sex among their patients in their care, including those taking antiretroviral therapy.

BIOLOGIC ISSUES RELATED TO HIV TRANSMISSION

HIV is most often transmitted through intimate sexual contact by rapidly binding to cells that are present in the cervical, vaginal, penile, urethral and rectal mucosa.[11] The male foreskin contains abundant cells that can bind HIV; thus, being uncircumcised confers an additional risk for HIV seroconversion[12] [13] (Table 116.2). All the specific mechanisms responsible for the sexual transmission of HIV in humans are not fully understood, since HIV can be found either as cell-free or cell-associated virus in blood and genital secretions and can bind multiple cell types.[4] [14] [15] The cells that can bind HIV in the genital tract include T helper lymphocytes, monocyte/macrophage cells, Langerhans cells and follicular dendritic cells. These last cells may be particularly important because of their mobility, since they can bind HIV on their surface membrane and/or internalize it and migrate via draining lymphatics to distal sites, where propagation in submucosal lymphoid tissue can occur, resulting in subsequent viral dissemination through the bloodstream.

Factors associated with increased HIV infectiousness include sexually transmitted infections[16] and noninfectious factors that can result in genital tract inflammation, recruiting more white blood cells to genital mucosal surfaces.[17] Among the sexually transmitted diseases, ulcerative sexually transmitted diseases such as syphilis, chancroid and genital herpes simplex virus infection afford additional portals of entry through mucosal ulcerations but also recruit inflammatory cells that bind and propagate HIV infection.[18] Inflammatory sexually transmitted diseases, such as gonorrhea, *Chlamydia trachomatis* infection and trichomoniasis have also been associated with increased HIV susceptibility and infectiousness, and may act either by increasing the number of white blood cells in the genital tract or by elaborating cytokines and chemokines that upregulate HIV expression and increase the viral load in the genital tract.[19] [20] Other local genital factors associated with increased inflammation include douching and traumatic sexual intercourse, particularly after sexual assault.[21]

Different tissues in the genital tract have varying levels of susceptibility to being infected with HIV.[7] The vaginal epithelium is stratified and contains fewer cells with co-receptors that can bind HIV.[17] Thus, vaginal mucosa are less likely to become HIV-infected than the endocervix, which has a thinner layer, is highly vascular and contains a much higher concentration of HIV-binding cells. Any physiologic event that results in ectropion (i.e. increased exposure of the endocervix), such as the use of hormonal contraceptives or occult *C. trachomatis* infection, increases susceptibility to HIV.[22] The penile foreskin contains many cells that can readily bind and express HIV, resulting in increased HIV acquisition or transmission in uncircumcised males.[13] The oropharynx contains many fewer cells that can bind HIV, which may partially explain the relative inefficiency of oral exposure to HIV as a means of transmission. [23] Moreover, salivary secretions contain several compounds that have been found to inhibit HIV transmission in vitro, most notably secretory leukocyte protease inhibitor (SLPI).[24] However, rhesus macaques have been readily infected with simian immunodeficiency virus after oral challenge, with evidence of viral replication in tonsillar and adenoidal tissues.[25]

EPIDEMIOLOGIC ISSUES RELATED TO HIV TRANSMISSION

Because of the multiple co-factors that may alter the amount of virus in the blood and genital tract, the calculation of exact risk for infection for each type of HIV exposure is imprecise (see Table 116.1 and Table 116.2). Moreover, much of the data that has been obtained to generate per-contact risks has been based on cohort studies in which individuals recollect their level of risk during preceding time intervals (often every 3–6 months). Some of the participants in these studies may be worried that they have become newly infected or their sexual behavior may have been under the influence of drugs or alcohol, affecting precise recall. Thus, although many people want to have a precise calculation of risk associated with specific practices, it is very difficult to determine with any certainty the precise likelihood of transmission for each specific act. It is important when patients ask questions about the likelihood of risk after an exposure to reassure them that a one-time exposure to HIV is unlikely to result in transmission but that the reason why the epidemic has become so widespread is because of individuals engaging in recurrent risk-taking behavior.

Having noted the limitations of how risk calculations have been obtained, certain key principles have emerged. Direct intravenous exposure to HIV (e.g. blood transfusions) is the most efficient way of transmitting the virus, while percutaneous needle sticks are much less efficient in transmitting HIV.[26] Individuals who share needles who pull back on the syringe and leave substantial blood in the syringe when passing it to their partner are more likely to transmit HIV than in a common health care setting where an occupational needle stick occurs with a solid suture needle.[27] The range of risk for individuals who share intravenous drug paraphernalia ranges from 0.6% to 3% (see Table 116.2). This range overlaps with the level of

TABLE 116-1 -- Estimates of per-contact risk of HIV infection.

ESTIMATES OF PER-CONTACT RISK OF HIV INFECTION	
Type of contact	**Risk**
Needle-sharing	6/1000 to 3/100
Occupational needle stick	1/300
Receptive anal	8/1000 to 3/100
Receptive vaginal	8/1000 to 2/1000
Insertive anal or vaginal	3/10,000 to 1/1000
Receptive oral	Case reports/no denominator

TABLE 116-2 -- Modifiers of the efficiency of HIV transmission.

MODIFIERS OF THE EFFICIENCY OF HIV TRANSMISSION		
Modifier	**Infectiousness**	**Susceptibility**
Sexually transmitted diseases	?	?

Genital tract inflammation (e.g. traumatic sex, douching)	?	?
Circumcision	?	?
Cervical ectopy	?	?
Genetics*	?	?
HIV subtype†	?	NA
Monocytotropic strain	?	NA
Acute infection	?	NA
Advanced infection	?	NA
Antiretroviral therapy	??	NA

* CCR5 mutation.
† Subtype A or C compared with B.

risk for individuals who engage in unprotected receptive anal or vaginal intercourse. [28] One study suggested that, on average, receptive anal intercourse was more than seven times as efficient at transmitting HIV as insertive anal intercourse.[29]

For each type of exposure, many contextual variables may alter the risk of transmission. For example, variations in the prevalence of HIV in different communities may mean that a behavior carrying the same risk has a different likelihood of resulting in infection in two different communities. The amount of virus in the infected source plays a role in determining the risk of becoming HIV-infected after a contact. Co-factors, such as a source with a high plasma viral load[5] [8] [30] or concomitant sexually transmitted infection, can greatly increase the average per contact risk. [31] [32]

In the developed world, the epidemiologic data would suggest that men are more likely to transmit HIV to their female partners. However, in several studies of HIV serodiscordant couples in sub-Saharan Africa, the rates of male-to-female and female-to-male transmission were quite similar.[8] The reasons for the difference in the efficiency of female-to-male transmission in the developing world as compared with the developed world may include the decreased prevalence of male circumcision in the places where these studies were conducted, as well as the high co-prevalence of other sexually transmitted infections. For anal intercourse, the insertive partner is less likely to acquire HIV from an infected receptive partner than vice versa; but there are sufficient cells in the distal male urethra and the foreskin, and such an abundance of HIV-infected cells and mucus secretions containing virus in infected receptive partners, that an insertive partner is still at substantial risk of acquiring HIV from unprotected intercourse.

Figure 116-1 Approaches to preventing HIV transmission.

It is clear that receptive oral intercourse, either fellatio or cunnilingus, is a much less efficient way to acquire HIV, but case reports showing that oral exposure to ejaculate may result in HIV transmission have been well documented. The relative efficiency of oral exposure to HIV is below that of unprotected vaginal intercourse; thus, it may be in the realm of less than 1 per 1000 contacts. However, animal studies have demonstrated that HIV can readily be transmitted orally, since there is lymphoid tissue in the oropharynx that might acquire HIV from genital secretions. In counseling patients who have concerns about the risk of acquiring HIV through oral exposure, it is important to indicate that it is less efficient than unprotected anal or vaginal intercourse but that transmission can still occur. Thus, if an individual wants a zero-risk situation, it is preferable for them to avoid any oral exposure to semen or cervicovaginal secretions from a partner who is known to be HIV-infected or at risk. However, if oral sex is a substitute for unprotected anal or vaginal sex, the patient can be told that this is less risky. Although HIV has been found in very small concentrations in pre-ejaculatory secretions, there are no reliable case reports of HIV transmission through exposure to pre-ejaculate without semen. Thus, it is important to counsel patients that being able to negotiate with their partners about limiting exposure to semen may be a helpful means for 'harm reduction'.

PREVENTION OF HIV TRANSMISSION

HIV transmission may be decreased by biologic or behavioral means (Fig. 116.1). Treating sexually transmitted infections can decrease genital tract HIV load in co-infected patients and can decrease susceptibility in at-risk persons. Antiretroviral therapy can decrease genital as well as plasma viral load but the transmission of multidrug-resistant HIV suggests that these drugs may only have an impact on wider HIV transmission if coupled with programs that encourage safer sexual behavior and optimal drug therapy adherence. Antiretroviral drugs may be used to decrease the likelihood of maternal-child transmission or be used for postexposure prophylaxis. Other experimental approaches, such as microbicides and vaccines, will also be discussed elsewhere. However, for any biologically plausible intervention to work, attention will need to be paid to the behavioral context in which the intervention will be undertaken. For

example, a biologically effective microbicide may fail if the at-risk population refrains from lubricated sex. The following section reviews interventions designed to decrease HIV through sexually transmitted infection treatment and behavioral interventions.

TREATING SEXUALLY TRANSMITTED INFECTIONS

As noted above, there are several different ways that HIV transmission may be minimized; aggressively diagnosing and treating sexually transmitted infections is biologically plausible and cheaper than chronic antiretroviral therapy. There have been several studies conducted in sub-Saharan Africa to test this hypothesis. In a study in the Mwanza district of Uganda, specific syndromic management of sexually transmitted infections in an area where the epidemic was still in its early stages (i.e. 1% of the adult population were infected at the start of the study) resulted in decreasing HIV incidence in communities where the intervention was undertaken. [33] However, in a study in the Rakai district of Tanzania, periodic mass treatment of at-risk adults for sexually transmitted infections did not result in a decrease in HIV incidence.[31] In the latter study, the HIV epidemic was already much more advanced (i.e. 16% of the adult population were infected at the start of the study) and there was a high background rate of concomitant herpes simplex infection, which was not treated in the course of the study.

Thus, the lessons from these studies are that, if one is to decrease HIV spread through treating sexually transmitted diseases, the treatment should be specifically tailored to individual patients, focusing on aggressively diagnosing sexually transmitted diseases that are common in that community. In addition, the benefit of sexually transmitted disease control will be greatest in areas of lower HIV prevalence. In communities where the epidemic is already more widespread, the likelihood of encountering a new partner who is HIV infected may be substantial, so the benefit of modifying a cofactor will be more limited.

HIV SCREENING AS A PREVENTION MODALITY

Other approaches to the prevention of HIV in the developed world are so well established that they seem routine; (i.e. the routine screening of blood). [34] The use of more sensitive antibody screening and careful donor history have resulted in enhanced safety in the blood supply, such that the transmission of HIV via infected blood in the past decade and a half is exceedingly rare. In other parts of the world where the relative cost of blood screening is high, blood supplies may not be as safe.

Another routine practice that results in the prevention of HIV transmission is the guideline that pregnant women be universally offered HIV antibody testing before delivery.[35] Although this guideline is not commonly adhered to, the rate of new perinatally transmitted infections in the USA has decreased dramatically, with fewer than 100 new infections in the past year. The reasons why any perinatal transmission has occurred recently are generally the mother's refusal to be tested because she perceives that she is not at risk or the obstetrician/gynecologist's failure to offer the test, or to spend time with the patient to discuss the reasons for her resistance to being tested. At the present time, because of the great efficacy of antiretroviral medication in decreasing mother-to-child transmission from a rate of 1 in 3 to less

than 1 in 10, routine HIV screening for all pregnant woman is the standard of care.

BEHAVIORAL APPROACHES — OVERVIEW

Despite access to antiretroviral therapy, blood screening and treatment of sexually transmitted infections, the number of new HIV infections in the USA has remained at a plateau of approximately 40,000 new infections per year over most of the past decade. The decision to engage in HIV risk behavior is a complex one that may involve issues related to early life events (such as sexual abuse in childhood), low self-esteem, contextual issues in relationships, concomitant substance use and addiction to specific forms of sexual pleasure. Gender-related power dynamics (e.g. the role of women in many societies) may also limit opportunities to promote safer sex. Thus, no single behavioral approach will invariably lead to an adaptation of consistent safer sexual or drug using practices. Much like dieting, regular exercise and smoking cessation, no single program of HIV risk reduction will work for all at-risk individuals. However, several studies have now indicated that the provision of either individual counseling and/or small group sessions can be helpful in assisting at-risk individuals in moderating their risk.

Good elements of risk reduction programs include the ability of the counselor to approach the patient in a nonjudgmental manner to elicit a realistic assessment of the person's pattern of risk-taking behavior. Given the slow progress in the development of cheap, safe and effective vaccines, microbicides and other biologic approaches to the prevention of HIV transmission, the role of the primary care provider in patient education, discussion of risk-taking behavior, initiation of risk reduction counseling and triage to appropriate prevention services is an essential part of stopping further spread of the epidemic. In the next section, the elements of successful HIV risk reduction interventions are reviewed.

PSYCHOSOCIAL MODELS OF RISK BEHAVIOR UNDERLYING PREVENTION INTERVENTIONS

Knowledge is one aspect of HIV prevention but ongoing risk-taking is a function of many other complex psychosocial variables. The three most common models that have been employed to explain HIV risk-taking are the health belief model, the theory of reasoned action and social cognitive theory (i.e. self-efficacy models). [36] Health beliefs models emphasize the role of perceived benefits and barriers to condom use and perceived severity of and vulnerability to getting HIV.[37] In the theory of reasoned action, health behavior — condom use — is a function of intentions to use condoms and, in turn, intention to use condoms is a function of variables such as attitudes and norms regarding HIV and condom use.[38] Social cognitive models (i.e. self-efficacy) explain condom use as a function of an individual's knowledge about HIV, expectation about the outcomes of using condoms (i.e. pleasure reduction versus disease prevention) and self-efficacy — that the individual will be able to use a condom in different sexual situations.[39] [40] These psychosocial models of HIV prophylactic behavior have been tested both cross-sectionally and longitudinally in a variety of populations and are the basis of many behavioral interventions reviewed below.

Intervention models that address information, motivation and behavioral skills[41] typically take into account models of HIV risk prevention. One is the transtheoretical model of change,[42] which posits that an intervention needs to be adaptable to an individual's current readiness to change. For example, people who are currently at a 'precontemplative' level of readiness to change do not see that their behavior as problematic and do not see a reason to change; someone at a 'contemplative' level may be ambivalent about changing and someone at a 'determination or preparation' level is ready to make a commitment. Additional levels of change include 'action', 'maintenance' and 'relapse prevention', each with different suggested strategies to assist an individual in a counseling situation. Interventions based on the transtheoretical model try to move individuals to a more serious, higher level of readiness to change than where they are initially. Accordingly, an individual at an earlier level may benefit most from information and education, whereas someone

at a mid-level or higher might benefit from more intensive motivational support and skills training.

Many of the randomized controlled trials of interventions reviewed below use aspects of the transtheoretical model of behavioral change, other information-motivation-behavior skills interventions and/or variables relating to self-efficacy, health beliefs and attitudes in the risk-reduction interventions. HIV prevention studies typically collect sexual risk-taking data on HIV-negative individuals but sexually transmitted disease (STD) or HIV incidence may also be used as end points. To address the overarching public health significance of HIV, community randomized designs employ more of a wider-scale approach. These interventions are formulated to develop population-based HIV prevention approaches and compare communities that receive the intervention with another similar community that does not.

OUTCOME OF LARGE SCALE AND HIGH-IMPACT-FOR-HIV PREVENTION STUDIES IN THE USA

Trials primarily involving heterosexual individuals in STD and primary care clinics

Two different randomized controlled trials of HIV prevention interventions have examined the efficacy of risk-reduction counseling approaches in individuals at high risk for HIV infection, by studying heterosexual individuals attending STD or primary care clinics. The first study, the US National Institute of Mental Health's Multisite HIV Prevention Trial (Project Light), recruited 3,706 individuals from 37 inner-city community-based clinics in seven sites across the USA.[43] The three risk groups were:

- men presenting in an STD clinic;
- women presenting in an STD clinic; and
- women presenting in health service organizations.

All participants, at screening, reported engaging in unprotected vaginal or anal sex within 90 days. Approximately one-half were randomized to seven sessions of risk reduction counseling in a group format, and the other half to watch a 1-hour AIDS video followed by a question and answer session. Those in the intervention group reported fewer unprotected sexual acts and were more likely to use condoms consistently over the follow-up period. The intervention group also reported higher overall levels of condom use. With respect to STD infections, there were no overall differences in infection rates between intervention and control group; however, among the men who were recruited from STD clinics, there was a decreased incidence of gonorrhea.

The second large-scale study, Project RESPECT, recruited over 5,787 heterosexual HIV-negative patients who presented for care at STD clinics in across the USA.[44] Participants were randomized to a four-session ('enhanced counseling'), a two-session ('brief counseling') or a noninteractive ('didactic message') condition. The enhanced counseling was based on the theory of reasoned action and social cognitive theory, targeting variables such as self-efficacy, attitudes and perceived norms. At the various sessions, participants would set goals for a next step in risk reduction, and at the final session would interactively come up with a long-term plan. For the brief counseling, participants discussed differences between actual and perceived risk, and also worked on a next step behavioral plan. The didactic message group sought to mimic what is standard of care in most clinics. The counselor would deliver messages (noninteractively) regarding HIV prevention and the meaning of their test result. Those assigned four-session and two-session interactive interventions had fewer new HIV infections at both 6- and 12-month intervals than did those who received the noninteractive counseling. Additionally, self-reported 100% condom use was higher in both interactive counseling groups as compared with the didactic message control.

As HIV risk behavior is a necessary factor for HIV infection, the two studies taken together reveal that HIV risk reduction counseling can both increase condom use and decrease STD infections. They also provide data for the feasibility of adding risk reduction counseling to standard of care in STD and primary care clinics. However, it is not clear whether STD and primary care clinics are routinely implementing these approaches.

Trials including men who have sex with men

Individually randomized controlled trials

One of the earlier individually randomized intervention trials [45] compared an integrated cognitive-behavioral intervention to a waiting list control among 104 gay men in a metropolitan area. The integrated intervention included AIDS risk information as well as cognitive-behavioral self-management training, sexual assertion training and strategies for increasing positive social supports/relationship skills. The intervention was delivered in groups and consisted of 12 sessions. At the post-training assessment, the experimental group had fewer high-risk sexual practices and better behavioral skills for sexual coercive situations than the wait list control.

In a study of African-American men who have sex with men,[46] 318 individuals were randomized, who were recruited from bars, bathhouses, erotic bookstores, organizations for African-American men who have sex with men, street outreach and personal referrals of other participants. The study had three arms; two included cognitive-behavioral self-management training, assertion training and issues related to social identity and support. Of these two arms, one was a single session and the other was three sessions. The third arm was a wait-list control. Those in the triple session arm had strong reductions in risky behavior. Those in the single session

intervention had mild improvements with respect to frequency of unprotected anal intercourse compared with the control group.

To address issues such as attrition and dissemination of interventions, a study in London[47] examined the benefits of a 1-day workshop. Participants had an acute sexually transmitted infection and reported having unprotected anal intercourse at least once over the past year at entry. Both intervention and control participants received 20 minutes of one-to-one counseling about sexual risk behavior and could be referred for clinic-based community counseling services. At 6 and 12 months, the proportions of those who had unprotected intercourse were not different between groups. Also, those in intervention were slightly (borderline statistically significant) more likely to have a new STD.

Some have erroneously concluded, on the basis of this study, that behavioral interventions for HIV prevention do not work. Subsequent criticisms of this conclusion, however, highlight the fact that the intervention was only a one-off workshop, which is unlikely, in general, to have lasting effects.[48] [49] Given the complexity of the ontogeny of risk behavior, more than a single workshop would be necessary for high-risk individuals.

Community randomized controlled trials

One of the first community randomized controlled trials, the Mpowerment Project, studied a peer-outreach program for young gay men (aged 18–29 years), [50] which used a wait-list crossover design. The intervention involved peer outreach (training peers to spread prevention messages and to recruit more individuals to participate), small groups that focused on misperceptions of safer sex, eroticizing safer sex, verbal and nonverbal safer sex strategies, informal outreach and a publicity campaign. To assess outcome, a cohort of 300 individuals from two communities were surveyed. In the intervention community, the proportion of participants who reported unprotected anal intercourse with nonprimary partners decreased, as did the proportion of participants who reported unprotected

anal intercourse with their boyfriends. No significant changes occurred in the comparison community.

Another large-scale community randomized trial[51] involved delivering the intervention through opinion leaders (popular individuals) from the gay community in four US states. Each state randomly had both an intervention city and comparison city. In the intervention city, popular gay men were trained to spread behavior-change messages (to change norms), and in the comparison city pamphlets were placed at gay bars. The team identified 'popular' men with the assistance of bartenders in intervention city bars. The bartenders would observe their customers and record the names of individuals who were greeted most often, greeted others and seemed well-liked. Participants then completed surveys in the bars. Across all states, 1126 men completed baseline surveys and 1010 completed follow-up surveys. At 1-year follow-up, those in the intervention cities reported a significantly greater reduction in the frequency of unprotected anal sex during the previous 2 months and a significantly greater increase in condom use for anal sex compared with comparison cities. Consistent with this finding, more condoms were taken from bars in the intervention cities than in the comparison cities.

The idea of using community opinion leaders to promote HIV prevention among heterosexuals is currently being studied in five international sites — India, Russia, Zimbabwe, China and Peru. The community randomized trials among men who have sex with men validate the utility of providing HIV prevention, on a larger scale, to communities of men who have sex with men. From a public health perspective, raising awareness and changing norms regarding HIV prophylactic behavior can influence transmission rates on the community level. Although recruiting opinion leaders and/or providing an integrated prevention program involving peers, groups, workshops and outreach can be a complex undertaking, these two studies show that such efforts can be useful approaches to curtailing HIV risk-taking, which, in turn, would curtail HIV transmission.

Prevention trials for women

Individually randomized controlled trials

Several large-scale multisite studies have investigated risk reduction counseling among low-income and/or minority women. Kelly *et al.*[52] randomized 197 high-risk women from an urban primary health clinic to a cognitive-behavioral risk reduction intervention or comparison. The intervention consisted of five sessions, including skills training in condom use, sexual assertiveness, problem-solving risk, trigger self-management and peer support for change efforts. The comparison condition received three sessions covering health topics not specifically related to AIDS. Three months later, the intervention group evidenced better sexual communication and negotiation skills (assessed by role play and self-report) and less unprotected sexual intercourse. The comparison group had no changes on these measures.

A second study of HIV risk-reduction counseling among women[53] sought to adapt models of behavioral change to social and contextual variables relevant to 128 economically disadvantaged African American women between the ages of 18 and 29 years. Accordingly, the intervention used the theory of gender and power as a guide and was social skills based. It included the following components delivered in five 2-hour long sessions: ethnic and gender pride, risk reduction information, sexual assertiveness and communication training, condom use skills and norms, and cognitive coping skills, including sexual self-control. At the 3-month follow-up, women in the more intensive intervention showed increased consistent condom use, sexual self-control, sexual communication and sexual assertiveness, and partner's adoption of norms supporting consistent condom use than those in the delayed educational control.

A study of 206 pregnant inner city women randomized participants to an AIDS prevention group or one of two controls.[54] The AIDS prevention intervention consisted of four sessions, each delivered in group format, and included videos as well as group activities such as role play or discussions. Sessions also included cognitive rehearsal skills for behaviors such as using a condom during sex, aversive-conditioning segments that involved imagining a scene in which women practiced an unhealthy sexual behavior, and relapse prevention. After the intervention and after 6 month follow-up, the AIDS prevention group had increases in knowledge and safer sex behaviors in comparison with the two control groups.

Because of difficulties with retention and attrition of high-risk and hard-to-reach women in HIV prevention trials, Belcher *et al.* developed and tested the utility of a single session 2-hour one-on-one intervention using motivational interviewing and information-motivation-behavioral skills training.[55] No differences in HIV knowledge resulted but the group that received skills training and motivational interviewing showed higher levels of HIV protective behaviors than the control group, including higher rates of condom use during vaginal intercourse, demonstrating the efficacy of a brief, minimal intervention.

Carey and colleagues conducted two randomized controlled trials of an HIV risk reduction intervention using information, motivation enhancement and skills training intervention for low-income, primarily African-American women.[56] [57] In both studies, the intervention consisted of four 90-minute sessions that included personalized feedback about their HIV knowledge, risk perceptions and sexual behavior. It also included a motivational videotape, decisional balance motivational exercises (pros and cons of risky and safer sex) and the impact of HIV on other life issues. Finally, the intervention group worked on developing personalized plans for future behaviors, skills training and education. Women in the first study who received the intervention reported stronger intentions to practice safe sex and to communicate these intentions to partners, less unprotected intercourse and less substance use near the time of sexual activity; and these gains were maintained at the 3-month follow-up. In the second study, 102 women comprising a new sample were randomized. Overall, the results of the second study were corroborated.

Taken together, the series of randomized controlled trials of HIV prevention counseling for high-risk women demonstrate both the feasibility and the efficacy of such approaches. While many of these approaches are useful, some may be difficult to disseminate. The utility of the single-session intervention provides support for its further replication.[55] This and the others, however, require special training, and most require a significant expenditure of time by both the participants and the counselors. Future study of individually administered interventions should now focus on ways to implement and disseminate interventions in community-based settings.

Community randomized controlled trials

To attempt to address some of the limitations of clinic-based intervention approaches for women, two studies of community randomized trials have been undertaken. The first used nine low-income housing developments[56] and nine demographically matched control developments. The community-level intervention included workshops and community HIV prevention events implemented by popular opinion leaders within each community. The researchers identified opinion leaders by including questions in the baseline survey — asking each participant to name up to five women whom they liked and trusted most. The women in the housing developments (n = 690) were surveyed at baseline and 1 year later. This revealed that women in the intervention communities showed better decreases in unprotected sex (past 2 months) and frequency of unprotected acts.

A second community-based randomized control trial targeted low-income, primarily African-American women in four urban settings.[57] Four communities in

metropolitan areas were selected: two public housing communities, a low-income neighborhood and a group of inner-city neighborhoods. The intervention was specifically based on the transtheoretical model of change, attempting to reach women who would be at different levels of readiness to change. The intervention consisted of distribution of HIV prevention materials, developing a peer network of community organizers and businesses, and delivering prevention messages by outreach specialists, both individually and in groups. The intervention communities evidenced increases in talking with main partners about condoms and trying to get main partners to use condoms.

Prevention interventions specific to injection drug users

A recent review of 42 studies between 1989 and 1999 suggested that the majority found that needle-exchange programs prevent HIV risk behavior and seroconversion among injection drug users.[58]

Most of the other studies of HIV prevention interventions for drug users are observational or quasi-experimental evaluation studies, which show within-participant reductions in HIV risk behavior.[59]

The SAFE study was a randomized controlled trial of 117 HIV-negative injection drug users who had reported that they had used and shared injection drugs in the previous 6 months.[60] The innovative experimental condition was to have injection drug users bring in the members of their drug network whom they had previously listed in an interview. The index participant and his or her drug network members received a manualized intervention delivered by former heroin users who had stayed in contact with active drug users in their community. The intervention consisted of six sessions that involved recognizing personal risk, committing to practicing both individual and group vigilance toward risk reduction, making plans (and discussing previous plans), assertiveness skills and role play of real-life situations that would involve risk (e.g. one member wanting to share without cleaning). The comparison group received counseling and testing. At the 18-month outcome assessment, the experimental group had significantly less needle sharing and less sharing of cookers for the prior 6 months.

Although innovative and useful, this intervention was arguably hard to deliver. Some 22% of potential index participants did not return with members of their drug network and another 36% did bring in at least one other drug network member but did not ever start the sessions.

Other studies and less extensive interventions have revealed mixed results at best. Kwiatkowski and colleagues did not find differences in high-risk behaviors among 3357 injecting drug users, not in treatment, randomized to standard or enhanced interventions.[61] [62] In general, individuals in this study maintained risk behavior and the authors concluded that new and creative ways to target this population were needed. Another similar study of standard and enhanced interventions for out-of-treatment drug users found both interventions to be at least moderately effective in reducing risk but less so than with sexual risk.[63] Gibson and colleagues [64] studied 295 individuals who were in treatment for heroin detoxification. Participants were randomized to counseling or brochures. Although differences between groups did not emerge, self-reported decreases in injection-related and sexual risk behaviors were present in both groups 6 and 12 months later. However, it is notable that, in this study, participants were acutely presenting for treatment of drug abuse and may have been more motivated to change than those not already in treatment.

Among the studies of prevention interventions for injection drug users, methodologies differ and, consequently, so do the results. Sexual behavior, as shown in previous sections, is a difficult and complex behavior to change. When co-morbid with drug dependence or addiction, its complexity grows; intensive multimodal interventions currently show the most utility in this population.

SUMMARY

Behavioral interventions to decrease HIV transmission have been successful in a wide array of settings and with diverse populations. However, in most situations, interventions were needed to sustain behavior change. Moreover, there is limited experience with these interventions in parts of the world where the epidemic is spreading most rapidly and where the social construction of reality (e.g. disempowerment of women, limited health care infrastructure) may limit the effectiveness of programs developed in resource-rich settings. Clearly, additional work is needed to develop culturally specific behavioral interventions, while the development of more effective biologic prevention modalities (i.e. microbicides and vaccines) is underway.

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Chapter 117 - Preventing Occupational Infection with HIV in the Health Care Environment

David K Henderson

This chapter addresses strategies designed to prevent the transmission of HIV in the health care setting.

EPIDEMIOLOGY

Occupational risks, including risks for physical injuries, chemical exposures and infectious diseases, have long been prevalent in the health care workplace. The introduction of HIV infection into the health care workplace in the 1980s, however, focused the attention of health care providers, perhaps for the first time, on the issue of occupational risk. Ironically, HIV infection is only one of the blood-borne pathogen-associated risks in the health care setting. Blood-borne pathogens have been identified as occupational risks for health care workers since the epidemiology and routes of transmission of hepatitis B were delineated in the 1960s. For reasons incompletely understood at present, unlike occupational hepatitis B virus infection, occupational HIV infection remains relatively uncommon. Even with broad use of the hepatitis B vaccine, occupational hepatitis B virus infections continue to occur in US health care workers.

By June 2001, the US Public Health Service's Centers for Disease Control and Prevention (CDC) had recorded only 57 instances of documented occupational HIV infections and 138 instances of probable or possible occupational HIV infections among health care workers in the USA.[1] For each of these 57 cases, the health care worker sustained an occupational exposure to HIV, had a baseline serum sample drawn and evaluated by HIV serology and then, during follow-up, developed serologic evidence consistent with HIV infection. Fewer than 50 additional cases of documented occupational infections and approximately 60 instances of 'possible or probable' occupational infections have been reported from outside the USA. For the 138 'possible or probable' occupational infections reported to the CDC, the exposed health care workers either were unaware of the occurrence of an occupational exposure, did not report the exposure and/or did not have baseline serologic studies performed to document that they were not infected prior to the occupational exposure.

A comparison of the demographics of the 'possible/probable' and definite occupational infection cases reveals substantial differences. When one compares the demographics of these categories of infection with those of all health care workers in the USA, the likelihood that some of the 'possible/probable' cases have occurred as a result of community exposures seems quite high.[2]

A number of both general and specific factors, taken together, determine an individual practitioner's risk for occupational infection with HIV. First, the prevalence of HIV infection among the population of patients served is a major determinant of the overall risk. Second, the type of practice in which the provider engages (e.g. medical, emergency room, surgical) is associated with varying levels of risk for occupational exposure to blood-borne pathogens. Third, the types and frequencies of procedures performed in the practice, as well as the conditions under which the procedures are performed (i.e. emergent versus elective), also contribute to the risk equation. The extent to which the practitioner adheres to recommended infection control procedures and practices is also likely to be a determinant of risk for exposure and infection. Finally, the individual practitioner's technique and attention to detail are also likely contributors to the risk.

Several specific factors contribute to the risk for occupational HIV infection in the health care workplace. Table 117.1 and Table 117.2 list factors that have been demonstrated[3] or suggested in the literature to contribute to the risk for occupational infection with HIV.

Many of the reported cases share several features in common. Most are transcutaneous exposures and the majority of these are injection needlestick injuries. All of the clinical cases have occurred following exposure to blood or grossly blood-stained bodily fluids from HIV-infected patients. As yet, no cases have been documented following a needlestick injury with a solid surgical needle.

To attempt to identify specific factors associated with risk for occupational HIV infection, public health authorities from the USA, France and the UK conducted a retrospective case-control study, matching the known anecdotal case reports of occupational HIV infections with 'controls' from the public health surveillance studies of occupational exposures in each of these countries.[3] This study identified five specific risk factors for occupational infection; these five factors and the level of statistical significance assigned to each in the study are listed in Table 117.2 . The first four of these factors very likely relate directly to the inoculum effect. That an inoculum effect is present in this setting is supported by several pieces of information:

- transfusion of a unit of blood from an HIV-infected donor is associated with virtually 100% risk for infection;[4]
- the depth of a percutaneous exposure is an independent risk factor for occupational HIV infection;[3]
- the presence of visible blood on the device producing the injury was independently associated with risk for infection;[3]
- instruments that had been placed in source-patients' vascular channels were more likely to result in occupational infection;[3] and
- the fact that all of the needlestick exposures to blood have been caused by hollow-bore needles (i.e. injection, as compared with suturing needles).

Both in the CDC study, as well as in the majority of the anecdotal case reports, the source-patients for the exposures resulting in occupational HIV infections had advanced HIV disease. This finding is likely to be a surrogate marker for either the level of circulating viremia, the level of circulating 'free' virus, or both. Finally, most occupational infections have followed parenteral (as compared with mucosal or cutaneous) occupational exposures. These latter routes of exposure are associated with lower risks for occupational infection.

Percutaneous exposure

Several longitudinal studies have attempted to determine the magnitude of risk associated with different types of occupational exposures (summarized by Ippolito *et al.*[5] and Henderson[6] . Combining

TABLE 117-1 -- Factors contributing to the risk for occupational HIV infection.

FACTORS CONTRIBUTING TO THE RISK FOR OCCUPATIONAL HIV INFECTION
Exposure factors
1. Route of exposure (e.g. percutaneous,* mucous membrane, cutaneous)
2. Inoculum size
• Size of the device producing injury
• For needlestick exposures, type of needle (i.e. hollow-bore* vs solid)
• Extent of contamination (e.g. visible blood on device,† whether or not device had been placed in an artery or vein†)
• 'Depth/severity' of exposure* †
• Type of contamination (e.g. blood,* pleural fluid, etc.)
Source/'donor' factors
1. Extent of viremia (e.g. by polymerase chain reaction or branch-chain DNA assay)
2. Stage of illness (as a presumed surrogate for extent of viremia†)
3. Circulating free (as opposed to cell-associated) virus
4. Antiretroviral chemotherapy (presumably reducing level of viremia)

* Features shared by many, if not most, of the occupational infections reported in the literature
† Features identified as significantly associated with risk for occupational infection in the CDC case-control study[3]

the data from the available studies, health care workers have sustained more than 6800 percutaneous exposures to sharp devices contaminated with blood or other

blood-stained body fluids from patients known to be infected with HIV. Twenty-one occupational HIV infections have been documented in these studies, resulting in a risk of transmission per injury of 0.31% (Table 117.3). Thus, one in 324 parenteral exposures in these studies resulted in occupational HIV infection.

Although such a pooled risk estimate provides the best available data concerning the magnitude of risk for occupational HIV infection, this type of analysis has substantial limitations. For example, the longitudinal studies vary somewhat in experimental design and are therefore not directly comparable. Such an analysis implicitly assumes that all parenteral occupational exposures are associated with equal risk, an assumption that does not make intuitive sense and that has apparently been shown to be flawed by the CDC's case-control study cited above.[3] Similarly, all source-patients are also not likely to present the same level of risk for occupational infection, with patients with advanced disease (and high-grade viremia) more likely to transmit than are patients early in the course of HIV infection (see Table 117.2). Because of the large number of factors that influence the risk for occupational infection, these summary data cannot address the risk associated with a specific, discrete exposure in an individual health care worker.

Mucous membrane exposure

Occupational exposures other than parenteral exposures to blood present a lower level of risk for occupational infection. Anecdotal reports document, in rare circumstances, that mucous membrane or cutaneous exposures may produce occupational infections in health care workers.[7] Certain of the longitudinal studies cited above have also addressed occupational risks associated with mucous membrane exposure to blood from HIV-infected patients. To date, with more than 2700 exposures followed prospectively, only one study has reported a seroconversion following a mucous membrane exposure.[8] Thus, as a maximum estimate, (using the 'rule of three' as an approximation for a zero numerator),[9] the pooled risk estimate for infection associated with a mucous membrane exposure is 0.11%

TABLE 117-2 -- Risk factors for occupational HIV infection identified in a retrospective case-control study conducted in the USA, UK and France.[3]

RISK FACTORS FOR OCCUPATIONAL HIV INFECTION IDENTIFIED IN A RETROSPECTIVE CASE-CONTROL STUDY
The risk for occupational HIV infection was increased when:
• the occupational exposure was deep, as compared with superficial (p<0.0001)
• blood was visible on the device causing the occupational exposure (p=0.0014)
• the device causing the exposure had been placed in a source-patient's vein or artery (p=0.0028)
• the source-patient died within 60 days of the exposure (p=0.0011)
• the exposed health care worker did not take zidovudine postexposure chemoprophylaxis (p=0.0026)

TABLE 117-3 -- Occupational risks for HIV infection.

OCCUPATIONAL RISKS FOR HIV INFECTION		
	Percutaneous exposures	**Mucous membrane exposures**
Number of longitudinal studies	27	21
Number of exposures	6807	2768
Number of documented infections	21	0/1*
Infection rate per exposure	0.31%	0–0.11%†

* See text for discussion
† Using the rule of three[9]

per exposure (see Table 117.3). Again, such a pooled risk estimate provides only a framework for considering the risks associated with a discrete exposure.

Occupational exposures other than percutaneous and mucous membrane exposures

Occupational exposures other than percutaneous and mucous membrane exposures are even less likely to result in infection. Prospective studies,[10] [11] which include hundreds of person-years of follow-up, have not identified a single instance of transmission of HIV. Whereas exposures to other fluids are likely to be associated with some occupational risk, this risk is below currently measurable levels.

When one evaluates the occupational exposures that have produced infection, almost all of them result from exposure to blood from HIV-infected patients. Whereas other body fluids may ultimately be shown to represent a risk for occupational infection, the major risk in the health care setting has come from occupational exposures to blood from HIV-infected patients.

PATHOGENESIS

Although the retrospective case-control study of risk factors for occupational HIV infection cited above[3] provided some insight into factors associated with risk for occupational HIV infection, the precise pathogenetic mechanisms of the occupational infection event are, as yet, poorly understood. The major risk for occupational infection is associated with percutaneous injury with a needle or other sharp device that has been used on an HIV-infected patient. The risk is associated primarily with blood exposure. Precisely how the transmission event occurs in the skin, subcutaneous tissue or

underlying muscle remains unclear. Current interest in the pathogenesis of infection by this route focuses on the role of host defense and on the role of the dendritic cell.

The role of host defense in protection against occupational infection is poorly understood. Scientists working at the National Cancer Institute have demonstrated that 75% of health care workers exposed to blood from HIV-infected patients who do not become infected with HIV develop HIV-specific T-helper activity.[12] In a subsequent study these investigators demonstrated that 35% of uninfected health care workers who had sustained occupational exposure to blood from HIV-infected patients studied developed cytotoxic lymphocyte responses to HIV-related envelope antigens. [13] Among health care workers who had sustained occupational exposures to blood from patients who were not infected with HIV, none responded to HIV-associated envelope antigens. Whereas the precise role that cellular immunity plays in host defense against occupational HIV infection remains to be delineated, these data, when combined with results from animal studies, suggest that the role may be an important one. One recent case report also supports a significant role for cellular immunity in the defense against HIV infection.[14] In this case, a health care worker who sustained an HIV needlestick exposure had HIV DNA detected by nucleic acid sequence-based amplification during a course of three-drug antiretroviral postexposure chemoprophylaxis. Despite the detection of proviral DNA, this individual ultimately remained uninfected (as assessed by serial nucleic acid tests and antibody determinations). The health care worker did, however, develop a robust HIV-specific cellular immune response.

INTERVENTIONS DESIGNED TO DECREASE THE RISK FOR OCCUPATIONAL HIV INFECTION

Interventions designed to limit occupational exposures

Several approaches have been used to attempt to decrease risks for occupational exposure to (and therefore occupational infection with) blood-borne pathogens in the health care setting (Table 117.4). Such

TABLE 117-4 -- Prevention strategies for health care workers and institutions to decrease risks for occupational HIV infection.[2]

PREVENTION STRATEGIES FOR HEALTH CARE WORKERS AND INSTITUTIONS TO DECREASE RISKS FOR OCCUPATIONAL HIV INFECTION
1. Use of 'standard precautions' or other isolation procedures designed to place effective barriers between the health care worker and blood or other body fluids.

2. Educating new staff and retraining existing staff regarding occupational risks for blood-borne pathogen infection in the context of other occupational risks present and prevalent in the health care workplace; making certain staff are aware of these risks.
3. Including information about all occupational risks (including those associated with caring for patients who have blood-borne pathogen infections) in biomedical training schools' curricula.
4. Evaluating all procedures associated with occupational risk for exposure to blood-borne pathogens (particularly those presenting risks for transcutaneous exposures), with the intent of modifying the aspects of these procedures associated with risks for occupational exposures.
5. Aggressive use of newly developed engineered controls, including careful evaluation of 'safety devices' for safety, efficacy and cost-effectiveness; implementation of those devices that meet these tests.
6. Development of efficient, readily accessible, user-friendly institutional postexposure management systems, including the option for postexposure antiretroviral chemoprophylaxis for documented occupational HIV exposures.

interventions can be considered as primary prevention. Major categories of intervention include:

- education,
- work practice controls (e.g. adherence to infection control procedures designed to limit risk), and
- engineered controls.

Education and use of infection control procedures

Staff should be routinely informed about all occupational risks. Since the major risk for occupational HIV infection (and for infection with other blood-borne pathogens as well) is by parenteral inoculation, some critics of the use of infection control procedures for managing all patients as if they were potentially infected with blood-borne pathogens (e.g. universal precautions, body substance isolation, standard precautions (discussed below)) have suggested that these precautions will have little impact on the number of occupational infections. However, since these and later guidelines educate staff about the careful handling of needles and sharp objects, recommend against practices associated with a high risk for parenteral exposures (e.g. needle recapping, needle bending or needle clipping) and stress appropriate disposal of needles and other sharp objects,[15] [16] their implementation will probably be associated with a decreased parenteral exposure rate. Two centers have documented a significant decrease in such exposures,[17] [18] one in temporal association with training in, and implementation of, universal precautions. [17] Up to one third of parenteral occupational exposures may be preventable by following guidelines designed to minimize occupational exposures.[19] The use of appropriate barriers may actually reduce occupational risk; the act of piercing a latex glove with a needle covered with blood may reduce the blood inoculum by as much as 50%.[20] Making health care workers aware of the presence of occupational risks may, in itself, result in occupational behavior modification. In a survey of certified nurse-midwives, both knowledge of the routes of transmission of blood-borne pathogens and perception of risk for occupational infection were statistically associated with the appropriate use of precautions. However, risk perception and use of precautions were more closely linked, suggesting that knowledge, in itself, may be insufficient to produce behavior modification.[21]

Employers in the USA are governed by regulations issued by the Occupational Safety and Health Administration of the US Department of Labor in 1991. One regulation mandates that employers follow certain protocols when managing health care workers who have sustained occupational exposures to blood-borne pathogens. [22] This 'final rule' has become the subject of mandatory education for health care workers at every health care institution in the USA.

Work practice controls

In 1987, the CDC issued guidelines for the management of patients infected with blood-borne pathogens that have since provided the underpinnings for all subsequent guidelines in the USA.[15] These 'universal precautions' guidelines set out clearly the principle that health care workers should treat blood and blood-stained body fluids from all patients as potentially infectious, in order to prevent the transmission of blood-borne pathogens (in particular, HIV) from patients to health care workers. More recently, the CDC has issued revised isolation guidelines, called 'standard precautions', which focus on the bidirectional spread of organisms to and from patients and health care providers.[16] All of these sets of guidelines and precautions emphasize:

- that blood and other blood-containing body fluids represent risk to health care workers, and
- that health care workers should use barriers and take precautions to prevent occupational exposures to these materials.

Use of these kinds of precautions in health care settings has resulted in decreased risk for occupational exposure to blood and, presumably, decreased risks for occupational infections with blood-borne pathogens.

Based on self-reports of occupational exposures to blood, Fahey and co-workers at the National Institutes of Health estimated that, in the year prior to training the staff in universal precautions, staff members experienced an average of 36 cutaneous exposures to blood.[23] Eighteen months after the staff were trained in universal precautions, this number decreased to 18. For all categories of employees and information analyzed, exposures to blood were reduced by approximately 50%. These results suggest that changes in behavior occurred between the two surveys, and, although a causal relationship with training cannot be proved, such a relationship can reasonably be inferred.

Compliance with these precautions has been problematic. Gerberding and co-workers identified substantial noncompliance at San Francisco General Hospital. [24] Although Fahey identified substantial improvement in her staff's compliance with universal precautions, the reduction in blood exposures was only 50% (implying that 50% of such exposures continued to occur, despite implementation of these precautions).[23]

Although data regarding procedure-specific adverse exposure rates are limited, certain procedures and devices seem intrinsically associated with increased risk for occupational exposures. To the extent that such modifications are possible, inherently risky procedures should be modified. In some instances, risk modification can be achieved by practitioners modifying the procedure themselves. In other circumstances, new devices or engineering controls (discussed below) may be needed. Some work practice interventions have been shown to reduce the risks for blood exposures. For example, the use of 'double-gloving' in surgery reduced the risk for skin exposure to blood significantly.[25] [26]

Engineered controls

Whereas work practice controls can eliminate a substantial fraction of occupational exposures to blood-borne pathogens, modifying medical devices that, in their current formats, are intrinsically associated with exposure risks can reduce these risks further. Among authorities in the field, Jagger and co-workers detailed the importance of the design of medical devices in the prevention of occupational exposures to blood-borne pathogens.[27] [28]

In the past several years, a number of engineered controls (i.e. presumably 'safer' devices) have been introduced. Of these new safety devices, the following have been identified in at least one published study as being associated with decreased risks for cutaneous and/or percutaneous blood exposures:

- a surgical repair assist device,
- blunt surgical needles,
- surgical finger guards and glove liners,
- phlebotomy equipment,
- needleless intravenous administration systems, and
- modified (e.g. self-capping) intravenous catheters.

Despite their implementation, some exposures will still occur. Development of a process for the systematic, objective evaluation of these devices is crucial to effective risk reduction for all health care institutions.[29]

Interventions designed to decrease the risk for occupational infection once exposure to HIV has occurred

Immediate postexposure management

Despite the emphasis on the prevention of occupational exposures, institutions should also develop strategies for managing occupational exposures effectively. Important constituents of a postexposure management program are listed in Table 117.5 .

Health care workers must be educated about the importance of reporting occupational exposures. Institutional reporting procedures should be simple, straightforward and widely publicized. When an exposure occurs, first aid should be administered and the exposure site should be allowed to bleed freely. The wound should be cleansed and decontaminated as soon as patient safety permits. Wounds should be washed with soap and water and then irrigated with sterile saline, a disinfectant or other suitable solution. Mucosal exposures should be decontaminated by vigorously flushing with water. Eyes should be irrigated with clean water, saline or sterile eye irrigants.

Exposures should be reported promptly. A mechanism to facilitate reporting and provision of follow-up care should be both readily accessible and widely publicized. Reporting systems should offer access to expert consultants. Institutional occupational medical systems must protect the confidentiality of the exposed worker. If confidentiality is not preserved, institutional programs are doomed to failure.

At the time an exposure is reported, occupational medical personnel should draw baseline serologies and chemistries. For documented

TABLE 117-5 -- Components of postexposure management programs for health care workers exposed to HIV.

COMPONENTS OF POSTEXPOSURE MANAGEMENT PROGRAMS FOR HEALTH CARE WORKERS EXPOSED TO HIV
1. Institutions should develop thorough, thoughtful, aggressive, employee educational campaigns concerning the presence of occupational risks in the health care workplace, including the risks for blood-borne pathogen infection; these educational campaigns should emphasize risk prevalence, risk reduction strategies, the importance of reporting adverse occupational exposures and postexposure management protocols.
2. Postexposure management systems must include mechanisms to facilitate both exposure reporting and the provision of follow-up care; these systems should be readily accessible, widely publicized, convenient and user friendly; occupational medicine personnel should be instructed to provide immediate 'first aid' for staff sustaining adverse exposures to blood and body fluids.
3. Institutions should develop a system for categorizing occupational exposures that require differing management strategies and different types of follow-up; protocols should address 'source-unknown' and 'source refuses serologic testing' exposures.
4. Postexposure management protocols should include appropriate serologic testing (mindful of state and local laws regarding consent) of both the source-patient as well as the employee sustaining the occupational exposure.
5. Occupational medicine staff should be thoroughly trained in the counseling of staff sustaining occupational exposures to HIV; all exposed staff should be given appropriate counseling regarding risks for infection and prevention of secondary transmission; all staff should have access to further counseling, if needed; all exposed staff should be given access to experts in the areas of occupational risks and HIV infection.
6. Exposed employees should be counseled to return for appropriate clinical and serologic follow-up and should be instructed to return if signs and/or symptoms of the acute primary HIV infection syndrome should develop.
7. Postexposure management protocols should offer antiretroviral chemoprophylaxis, with appropriate follow-up, for health care workers sustaining occupational exposure to HIV.
8. Counseling should include attention to the known/expected toxicities of the selected regimen. Pre-emptive therapy of these symptoms/side-effects (e.g. prescriptions to treat nausea, diarrhea, etc.) may increase regimen adherence.
9. Postexposure management protocols must, at all costs, maintain the exposed health care worker's medical privacy and confidentiality.

occupational exposures to HIV, follow-up should occur at 6 weeks, 3 months, 6 months and 1 year following exposure. The value of the 1 year follow-up visit remains somewhat controversial; however, some case reports of late seroconversion (i.e. more than 6 months following exposure) have now appeared. More aggressive diagnostic evaluation, such as the use of polymerase chain reaction analysis to detect viral or proviral nucleic acids, is ordered if the health care worker develops symptoms suggestive of acute, primary HIV infection (i.e. the seroconversion illness). Institutions must develop policies regarding the testing of source-patients and employees that are consonant with local and state laws. Occupational medicine staff should counsel the exposed health care worker about the signs and symptoms of the seroconversion illness and should instruct the employee to return for evaluation should any illness consistent with this syndrome occur.

Postexposure chemoprophylaxis with antiretroviral agents

The use of antiretroviral agents as postexposure chemoprophylaxis for occupational exposures was controversial from its inception.[30] Data accumulated over the past 10 years, however, provide a firmer foundation for postexposure chemoprophylaxis programs. Recently, several animal studies of postexposure chemoprophylaxis (most of which use substantially lower viral inocula) have been able to demonstrate efficacy. Another piece of scientific evidence that indirectly supports its use comes from the success of antiretroviral agents administered in pregnancy in reducing maternal-fetal transmission of HIV[31] [32] (see Chapter 135). One of the factors identified in the collaborative retrospective case-control study as significantly associated with an increased risk for occupational HIV infection was 'not taking zidovudine chemoprophylaxis'.[3] In this study, administering zidovudine chemoprophylaxis to exposed health care workers was associated with an approximately 80% reduction in the risk for occupational infection following transcutaneous exposures to HIV.[3]

The US Public Health Service has published guidelines that recommended the use of postexposure antiretroviral chemoprophylaxis in some settings.[33] The current recommendations (summarized in Table 117.6) advocate the use of three agents (zidovudine, lamivudine and one of four additional agents (see Table 117.6)) for the most severe occupational exposures and the use of two of these agents (i.e. zidovudine plus lamivudine) or one of two other alternative two-drug regimens for lesser exposures. If the source-patient for an exposure is (or recently has been) receiving antiretroviral therapy, some authorities have recommended the use of alternative regimens

TABLE 117-6 -- Current US Public Health Service recommendations for chemoprophylaxis of occupational exposures to HIV.[33]

CURRENT US PUBLIC HEALTH SERVICE RECOMMENDATIONS FOR CHEMOPROPHYLAXIS OF OCCUPATIONAL EXPOSURES TO HIV		
HIV exposures with a recognized transmission risk	'Basic regimen'	Zidovudine (ZDV) plus lamivudine (3TC)
	Alternative	Stavudine (D4T) plus lamivudine
	'Basic regimen'	Stavudine plus didanosine (ddI)†
HIV exposures for which the nature of the exposure suggests an elevated transmission risk*	'Basic regimen' plus one of the following agents	Indinavir†
		Nelfinavir
		Abacavir
		Efavirenz†

† Agents not advisable for use in pregnancy and increasingly not recommended in practice
* Elevated risk is associated with 'larger' volume of blood and/or blood containing a high titer of HIV

comprising agents to which the source-patient's virus has not been exposed, tailoring the construction of the regimen in a fashion similar to the planning of 'salvage therapy' for active HIV infection.

This latter point underscores the importance of obtaining expert guidance from individuals knowledgeable about the use of anti-retroviral agents in tailoring a regimen

for a health care worker, especially in circumstances in which the source-patient is known or highly suspected to harbor a resistant virus. Clinicians who are skilled in providing care to HIV-infected patients are perhaps best situated to provide this kind of advice. If you cannot identify a local expert, the US Public Health Service sponsors a postexposure hotline that can provide this expertise (either over the telephone (888-448-4911) or via the World Wide Web at http://pepline.ucsf.edu/pepline). Recently, the use of nevirapine has been contraindicated because of the risk of severe liver toxicity, which can include hepatic failure.[34]

Another special circumstance worthy of additional consideration relates to the administration of postexposure prophylaxis to a health care worker who is (or thinks she may be) pregnant. Because of our extremely limited experience with the use of these agents in uninfected individuals, the risks associated with administering the drugs to pregnant women are essentially undefined. The only relevant clinical experience in humans comes from the administration of the drugs to HIV-infected pregnant women, a circumstance that is not exactly consonant with the postexposure prophylaxis setting. Based on the limited clinical data, as well as several relevant animal studies, general guidelines for this situation have been developed and are summarized in Table 117.7 . [33] [35]

Virtually all of the marketed antiretroviral agents have potential for carcinogenicity, teratogenicity and mutagenicity. Efavirenz has been shown to be teratogenic in cynomolgus monkeys at drug levels similar to those in humans. In addition, administration of the didanosine/stavudine combination in pregnancy has been associated with cases of severe pancreatitis and severe lactic acidosis among

TABLE 117-7 -- General principles for administering antiretroviral chemoprophylaxis to pregnant health care workers.[33] [35]

GENERAL PRINCIPLES FOR ADMINISTERING ANTIRETROVIRAL CHEMOPROPHYLAXIS TO PREGNANT HEALTH CARE WORKERS
1. A pregnant, exposed health care worker is the only person who can decide whether to take chemoprophylaxis, and she must be empowered to make this decision. No one should attempt to make this decision for her.
2. The practitioner providing care to a pregnant, HIV-exposed woman must provide up-to-date and accurate information about what is known (and not known) concerning:
• the magnitude of risk for infection associated with her exposure;
• the efficacy of postexposure prophylaxis;
• the safety of the treatment, including the potential for harm to the health care worker and her fetus; and
• the risk of the fetus becoming infected (and the possible interventions that could be taken to reduce this risk) should the health care worker become infected from the exposure.
3. The practitioner should select a regimen appropriate for the exposure (i.e. pregnancy per se should not dictate the regimen, but consideration should be given to agents for which an experience base exists (e.g. zidovudine and lamivudine)). Some agents/regimens (i.e. those with known toxicities in pregnancy) should be avoided whenever possible (e.g. didanosine plus stavudine; efavirenz, indinavir).
4. Pregnant workers electing to take postexposure chemoprophylaxis must be followed closely for signs of toxicity (both maternal and fetal); pregnancy represents another circumstance in which expert consultative advice is essential.

HIV-infected women, and both maternal and fetal deaths have been recorded.[36] Other issues that may be relevant to the administration of chemoprophylaxis to pregnant health care workers include data from France suggesting a risk for severe mitochondrial toxicity in uninfected infants born to mothers who had taken nucleoside analogues [37] (an experience that has not been detected in the USA) as well as the potential for hepatotoxicity and nephrolithiasis associated with indinavir use.

Despite the encouraging data concerning the use and efficacy of postexposure antiretroviral chemoprophylaxis, legitimate concern remains about the use of these agents in this setting. Antiretrovirals are not trivial agents in terms of potential toxicity. Toxicity data in healthy individuals, especially for the newer agents, are extremely limited; however, it is fair to emphasize that virtually all the studies of antiretroviral chemoprophylaxis for occupational HIV exposures in health care workers have demonstrated substantial side-effects, often limiting completion of the prescribed regimen.[38] Finally, several cases of zidovudine chemoprophylaxis failure (eight of which have direct clinical relevance) have appeared in the literature.

The US Public Health Service last revised its guidelines concerning postexposure prophylaxis in 2001[33] and intends to revise the guidelines whenever new information becomes available concerning the risks or benefits of postexposure chemoprophylaxis. In spite of these considerations, all institutions need to have a policy on postexposure prophylaxis. Offering chemoprophylaxis is seen as 'empowering' by exposed workers. Workers who have had these frightening occupational exposures appreciate the fact that their institutions are willing to offer these drugs.

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Chapter 118 - HIV Vaccines: Research and Development

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THE NEED FOR A VACCINE TO PREVENT HIV-1 INFECTION AND AIDS

Identification of HIV-1 as the cause of AIDS led to development of methods to diagnose the infection, quantify the virus in plasma and cells and document the evolution of the disease and treatments. Understanding the mode of transmission has led to some success in prevention, through interventions such as screening of the blood supply, drug treatment for pregnant women and promotion of barrier methods and safe sex. Despite these modest gains, the epidemic has moved with a speed that defies imagination. The hardest-hit countries, in sub-Saharan Africa, have experienced enormous losses, with up to half the young adults infected in some areas and the average lifespan decreased by as much as two decades.[1] The epidemic in Asia threatens to dwarf that in Africa. Meanwhile, in the USA, hard-won gains in education and behavioral prevention seem to be slipping away; infection rates are increasing in certain groups, particularly young gay men and minority women. [2] An effective vaccine is needed more than ever to stop the growth of this epidemic.

PROSPECTS FOR SUCCESS

HIV-1 is a formidable challenge for vaccine prevention programs, from the standpoint of science, development and eventual utilization of a safe and effective vaccine. Its immune evasion mechanisms include molecular tricks to avoid neutralization by antibody, down-regulation of immune functions, direct destruction of the CD4 T cells

TABLE 118-1 -- Evidence that an HIV-1 vaccine is possible.

EVIDENCE THAT AN HIV-1 VACCINE IS POSSIBLE			
Setting	**Type of evidence**	**Conclusion**	**Strength of evidence***
Human epidemiology	Studies of adults repeatedly exposed to HIV-1 by sex or injections and infants exposed during birth or nursing	Infection occurs infrequently after exposure; risk depends on quantity of virus	+++
	T-cell responses reported in some exposed but uninfected persons	T-cell responses are induced by exposure to HIV-1 (abortive infection?)	+
Natural history of HIV-1 infection in humans (and related viruses in animal models)	Virus concentration in blood is very high during acute infection, then drops as T-cell responses appear	T-cell responses induced by infection partially control viral replication, usually for years	+++
	Virus concentration in blood increases late in infection when T-cell levels are very low	T-cell responses control viral replication	+++
	Prompt postexposure drug treatment can prevent or abort infection	A small number of infected cells can be destroyed (by immune mechanisms)	++
Studies of active and passive immunization in animal models	Passive antibody transfer or monoclonal antibody infusion	Antibodies prevent infection (probably by neutralizing the virus)	++
	Vaccination with antigens inducing CD8 T cells or depletion of CD8 T cells in infected animals	T-cell responses limit viral replication after challenge	+++
		T-cell responses slow progression to disease	+++

* Evidence is strong when most or all of several studies lead to the same conclusion; evidence is weaker when there are few or conflicting studies. The evidence has recently been reviewed. [4]

that support both antibody and effector T-cell responses, and a very rapid mutation rate that gives rise to virus strains lacking the antigenic markers (epitopes) to which effective immune responses are directed. Nevertheless, there is evidence that immunity can prevent establishment of infection in exposed persons. The immune responses induced by acute infection clearly establish some control of HIV-1 replication. Vaccine-induced immunity could more frequently prevent or more effectively control virus replication. Limiting virus replication could, in turn, delay disease onset and reduce transmission of HIV-1 to sexual partners, infants or other contacts. Even a modest reduction in the susceptibility to infection or likelihood of transmission could have a significant impact on the HIV-1 pandemic.[3] A reduced replication rate might also diminish the ability of HIV-1 to develop mutations leading to drug resistance or escape from immune responses.

Three types of evidence suggest that vaccines will be effective in preventing HIV-1 infection and/or AIDS (Table 118.1). First, chronic infection with HIV-1 occurs after only a small fraction of all exposures, suggesting that a modest immune defense might be effective. [5] This reasoning is supported by the apparent resistance to infection of some persons who are repeatedly exposed to HIV-1.[6] Cell-mediated immune responses in exposed but uninfected individuals are thought to be induced by autologous infected cells, suggesting that, prior to the establishment of a chronic HIV-1 infection, there may be a brief phase in which the infection can be aborted, perhaps by immune destruction of the infected cells.[7] Second, the natural history of HIV-1 infection shows that, after an initial phase of rapid viral

replication, immune responses may control HIV-1 replication and slow its pathogenic effects for many years.[8] [9] Third, nonhuman primate models (e.g. SIV in macaques or the man-made chimeric SHIV in macaques) show that some vaccines given prior to challenge can prevent infection or slow viral replication and disease progression,[10] [11] [12] [13] an effect dependent at least in part on CD8 effector cells.[14] [15] Likewise, antibodies that are able to neutralize HIV-1 or SHIV can prevent infection in animal models.[16] [17] [18] [19] Immunization that induces mucosal responses is particularly effective against mucosal challenges.[20]

SCIENTIFIC BASIS FOR HIV VACCINE DEVELOPMENT

Immune targets in the HIV infection process

The HIV-1 replication cycle begins with the envelope protein binding to receptors on the host cell surface (CD4, a chemokine receptor molecule, or both) and fusing with the cell membrane, resulting in virus entry and introduction of the viral genetic material into the host cell nucleus. DNA transcribed from viral RNA integrates into the host genome and directs synthesis of viral RNA and proteins, which assemble to form progeny viral particles that bud from the surface of the infected cell. Shortly after infection of a cell, peptides derived from virus proteins (envelope, gag, polymerase and regulatory proteins) are displayed on the surface of the cell, in association with HLA molecules. These can activate T cells and serve as their targets. Some cells, however, can harbor HIV-1 without being 'visible' to T cells. [21] In the first days after infection, HIV-1 and related viruses rapidly spread from the portal of entry, usually at a mucosal surface, to blood, secondary lymphoid organs and lymphoid cells in tissues such as the gut. [22] [23]

Vaccines against other viral diseases may allow asymptomatic infection but prevent disease. Once it is established, HIV-1 infection may not be eradicable. The ideal HIV-1 vaccine will prevent the establishment of infection by inducing antibodies that neutralize HIV-1 virus by binding to envelope protein or by inducing effector T cells that can inhibit virus replication and eliminate the first few cells to become infected. Even if infection is not prevented, priming the immune response could lead to more rapid immune control of virus replication and delayed onset of disease. Immune responses that occur at mucosal sites may play a particularly important role, as most HIV-1 exposure occurs through mucosal routes and the gut lymphoid tissue is an important site for HIV-1 replication regardless of route of infection.[24] As in other viral diseases, it is likely that all components of the immune system will need to work synergistically: the nonadaptive component that offers initial protection and provides the impetus for adaptive responses,[25] [26] antigen-presenting cells of several types, B cells that make antibodies and a multiplicity of T cells, including effectors, that secrete antiviral cytokines and kill virus-infected cells or helpers that simply control the activities of effector T and B cells, and memory cells for each adaptive component will likely be involved in reducing the number of infectious virions, limiting viral replication and eliminating infected cells.[27]

Neutralizing antibodies

Neutralizing antibodies can block the virus from binding to the host cell membrane, thus interfering with envelope-receptor complex formation, and/or with membrane fusion. As a result, the virus cannot enter the cell and replicate.

Neutralizing antibodies are directed against either linear or conformational epitopes (antigenic portions) of the folded viral envelope glycoprotein. Several features of HIV-1 envelope (conformational flexibility, a complex and labile structure, the presence of variable loops and glycosylation of some antigenic regions) contribute to mechanisms by which HIV-1 can avoid inducing neutralizing antibodies or withstand their effects.[28] In addition, after infection, new HIV-1 'escape mutants' emerge that are no longer susceptible to neutralization by the host's antibodies.[29] [30]

Nevertheless, it is clear that neutralizing antibodies can be protective if they are present prior to infection. Neutralization of HIV-1 by passively infused antibodies protects chimpanzees[31] or SCID-hu mice (which lack their own immune system and have a transplanted human system[18] [32]) against infection with HIV-1, and monkeys from infection by the man-made chimeric viruses with the envelope of HIV-1 and other genes derived from the SIV.[19] [33]

When antibodies from individuals chronically infected with HIV-1 are tested for neutralization, the response is often weak. However, broadly cross-reactive neutralizing antibodies can be made by at least some humans; a few human monoclonal antibodies (mAbs) have been developed that efficiently neutralize a range of primary HIV-1 isolates.[34] [35] [36] Unfortunately, no vaccine has been identified that will induce high titers of broadly cross-reactive and durable neutralizing antibodies against strains of HIV-1 recently isolated from human peripheral blood mononuclear cells (PBMCs), often called primary isolates. This standard may be too stringent (it is not necessarily met by all successful vaccines against other viruses); only analysis of the immune responses from one or more efficacious vaccines will resolve the issue. Rational design approaches focusing on structure-function analyses of envelope-antibody binding, further elucidation of the structure of envelope, and library screening for identification of possible immunogens are currently under investigation and may lead to novel strategies for inducing broadly cross-reactive neutralizing antibodies.

Cell-mediated immunity

T cells may protect against HIV-1 infection by killing infected cells (cytotoxic lymphocytes (CTLs)), by producing soluble antiviral substances such as interferon-? or other inflammatory mediators or by 'helping' or amplifying the cytotoxic or antibody responses. T cells bearing the CD8 marker on their surface are effectors (cytotoxic or secrete cytokines), while T cells with CD4 on their surface frequently regulate antibody or cell-mediated responses or may have cytolytic activity. CD8 cells are thought to be most important in controlling virus infections, because they recognize peptides from endogenously produced antigens, such as those found on the surface of virus-infected cells.

T-cell responses are directed against HIV-1 proteins, regardless of their function (structural, enzymatic or regulatory).[37] Thus, T cells have a wider variety of targets and some of the targets are constrained in their genetic variability by the need to preserve functional structures. They can react with a variety of HIV-1 isolates, either because the epitopes are conserved between various strains of HIV-1 or because of immunologic cross-reactivity between similar epitopes.[38] [39] Nevertheless, T-cell epitopes may vary and when the predominant response to retrovirus infection is limited to one or a few specificities, viruses bearing a changed epitope may escape from immune control and cause disease progression.[40] [41] Additionally, due to its high mutation rate, HIV-1 may tend to evolve by losing epitopes to which a specific population responds well,[42] or it might lose epitopes contained in an initially effective vaccine.

Evaluation of vaccine-induced immune responses

Vaccine trials require robust, sensitive and reproducible methods to measure vaccine-induced immune responses. Most of the existing licensed vaccines were developed empirically with little knowledge of what immune responses mediate protection, in an era when only antibody responses could be measured accurately. It is likely that the

TABLE 118-2 -- Characteristics of HIV-1 neutralization assays.

CHARACTERISTICS OF HIV-1 NEUTRALIZATION ASSAYS	
Virus type	Strains adapted to long-term culture in T cells or recently isolated 'primary isolates' or genetically engineered viruses with HIV-1 envelope, some containing a reporter gene
Target cells	PBMCs stimulated with a mitogen to induce cell division, cell lines expressing appropriate cell surface markers (HIV-1 receptor and coreceptor) or genetically engineered cell lines containing a reporter gene
Indicator system	Cell death or cytopathic effect, viral protein production, molecular reporter gene (e.g. green fluorescent protein, luciferase, ß-galactosidase). Some are highly reproducible and capable of high throughput automation
Number of replication cycles	Single or multiple (affects assay duration and variability)

arms of the immune response work synergistically and with the nonadaptive innate immune system. However, the practical reality of vaccine development necessitates selection of one immune response as an indicator of vaccine potency. Manufacturers require highly reproducible and rapid potency assays. This immune response will be used to compare vaccine approaches, to decide which candidate vaccines progress to further testing, and eventually to monitor lot-to-lot consistency in a licensed vaccine or allow modifications in the vaccine without repeating full-scale, placebo-controlled efficacy trials. The ideal assay would be based on protective mechanisms (still unproven) and validated to meet the requirements of good laboratory practices.[43]

Antibody assays

Antibody assays measure binding to viral proteins or neutralization. Binding antibodies are detected by standard enzyme-linked immunosorbent assay (ELISA) colorimetry, Western blot or real-time binding assays.[44] Neutralization, though more difficult to measure, is thought to be more relevant to protection. Antibodies may neutralize HIV by binding to a specific neutralizing epitope[45] or by coating virions and interfering with viral envelope-host cell membrane fusion.[46]

Neutralization measures the reduction of infectivity of cell-free virus particles. Infection of cells by HIV-1 is measured in the presence and absence of serum or other fluids. Neutralizing activity often appears to be very weak, even in the serum of chronically infected individuals. However, the choice of HIV-1 strain(s) used in the neutralization assay will influence the measurements. It appears

TABLE 118-3 -- Characteristics of assays of T-cell responses to HIV-1 antigens.

CHARACTERISTICS OF ASSAYS OF T-CELL RESPONSES TO HIV-1 ANTIGENS					
	Chromium release	**Proliferation**	**ELISPOT**	**Intracellular CFC**	**Tetramer**
Measures	Cell killing	Cell division	Cytokine release	Cytokine production	MHC-peptide complex binding

Enumeration	Semi-quantitative	Semi-quantitative	Enumerates single cells	Enumerates single cells	Enumerates single cells
Cells	PBMCs expanded for 2 weeks in vitro	Separated PBMCs expanded in vitro for 4–7 days	Separated PBMCs, 1–2 day stimulation	Whole blood assay (6h stimulation)	Whole blood simple stain (2h)
Sensitivity (approximate)	1 in 1000	1 in 10,000	1 in 10–50,000	1 in 10–50,000	1 in 10–50,000
Read-out	Total cell killing	Total DNA synthesis	Cytokine-producing cells (or CD8 subset)	Multi-parametric	Multi-parametric

that most antibodies against HIV-1 have a very narrow spectrum of activity while HIV-1 can vary enormously, even within one infected person. Many methods have been developed for assessing neutralizing antibodies (Table 118.2). Conventional neutralizing assays mimic in vivo neutralization of HIV-1, using stimulated PBMCs as targets and measuring cell death[47] [48] [49] or the inhibition of viral protein production.[50] Newer assays may rely on viruses or cell lines that are genetically engineered to contain reporter genes[51] as a surrogate for productive infection, or on flow cytometry[52] to measure infection in individual cells. These assays must take into account the two different types of receptors utilized by HIV-1: CD4 and chemokine receptors. HIV-1 strains adapted to growth in cell lines are often much easier to neutralize than primary isolates. [53]

Assays of cell-mediated immunity

For all assays of cell-mediated immunity (Table 118.3), isolated mononuclear cells or whole-blood specimens are stimulated with antigens that may consist of proteins, peptides or virus-infected cells. The specimen is usually peripheral blood, although T cells can be cloned from secretions or tissue biopsies.[54] T cells are stimulated by peptides associated with HLA molecules on the surface of antigen-presenting cells such as dendritic cells.[55] The classic measurements of responses, killing of virus-infected or peptide-coated target cells (predominantly CD8 cells) and antigen-induced lymphocyte proliferation (CD4), are difficult to apply, especially in resource-poor settings. Assays that measure production of cytokines or chemokines (e.g. IFN-?, IL-2, TNF-a, MIP-1ß) by individual PBMCs in response to antigen or peptide are now widely used (Fig. 118.1). ELIspot enumerates individual cells that secrete particular cytokines, thus revealing the capabilities of the responding cells. Even more informative is intracellular cytokine flow cytometry, which identifies both the cytokine being produced and immune markers on the surface of a cell, such as CD4 or CD8, and molecules indicating activation or propensity to migrate to certain tissues. These assays are simpler, more robust and more informative and they can be validated to meet the requirements of good laboratory practices.[43] Assays on fresh blood, while more technically challenging, are more sensitive and reliable. Artificial fluorescent molecules that mimic antigens and bind to T-cell receptors can also be used (tetramer assay), but this assay is technically much more difficult because of genetic restrictions on T-cell receptor binding to epitopes.[56]

CLINICAL TRIALS

The process

Clinical trials for preventive HIV-1 vaccines are complex. Like all clinical studies, they must comply with ethical principles, applicable laws and good laboratory practices.[43] Volunteers must fully

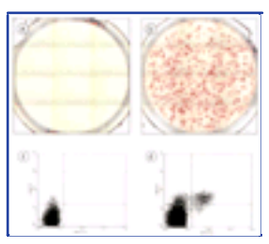

Figure 118-1 Two newer methods for studying responses to HIV vaccines. (a) and (b) are representative negative and positive wells (respectively) from an interferon-? (IFN-?) ELISPOT assay. Mononuclear cells are separated from blood, placed in wells coated with antibody against IFN-?, and stimulated overnight with (b) or without (a) peptides from the antigen of interest. On stimulation, antigen-specific T cells release IFN-?; the captured IFN-? is stained using a second antibody with an enzymatic tag to develop a color change, much like a conventional ELISA test. A colored spot appears for each cell producing IFN-?. The lower panels are representative of the result from a cytokine flow cytometry (CFC) assay. Whole blood from an HIV^+ patient is cultured without (c) or with (d) peptides from the HIV *gag* gene in the presence of an inhibitor of Golgi secretion. Newly synthesized cytokine within the cells, in addition to cell surface markers, are then stained using fluorescently labeled antibodies and analyzed on a fluorescence activated cell sorter (FACS). The cells in (c) and (d) have been labeled for CD3 (general T-cell marker), CD4 (helper class of T cells) and CD69 (activation marker); they are also permeabilized and stained to reveal intracellular IFN-?. The cells staining for CD3 but not CD4 (i.e. CD8 or effector T cells) were selected using automated software. The upper right quadrant indicates which of the CD8 cells are newly activated (CD69 positive) and producing IFN-?.

understand the trial, including the rationale for use of a placebo and the process of randomization.

Risk for HIV-1 infection can be reduced by behavior change. It is critical that volunteers understand the need to protect themselves against HIV-1 infection by standard methods (limiting partners, barriers and avoiding contaminated needles) and that they have the knowledge and means to do so; they need to know that an untested HIV-1 vaccine candidate is not a 'magic bullet'. Trial organizers must educate, counsel and provide protective devices such as condoms or clean needles and syringes to the extent that it is feasible and legally permissible. It is necessary to estimate the incidence of HIV-1 infection in the presence of such interventions in order to determine accurately the sample size for efficacy trials. Research suggests that, in US trials, risk-taking by volunteers has not increased during the trials. [57] This may differ depending on the social circumstances and education of volunteers. Many persons at risk from HIV-1 lack power over their own risks; they may be at risk, for example, through a spouse's risky behavior. These people are the most in need of an effective vaccine.

Certain vaccine candidates may induce antibodies that render a standard diagnostic HIV-1 test positive, even in the absence of HIV-1 infection, and each trial must offer accurate diagnostic testing for trial participants. Stigmatization or discrimination could occur as a result of false-positive testing or, more likely, simply based on trial participation.[58] [59]

Early trials (phase 1) enroll healthy volunteers who are usually not at high risk for HIV-1 infection, as indicated by a screening interview. Risk can change and occasionally such volunteers do encounter HIV-1 in the community and become infected. The clinical course of these infections has been scrutinized and it appears to be no different from acute HIV-1 infection in nonvaccinated individuals, [60] but the number of cases is small. Later stage (phase 2) trials and efficacy trials (phase 3) enroll participants at higher risk of HIV-1 infection, either through sexual contact or injection drug use. Infants at risk of infection through breast-feeding (after initial protection by perinatal drug therapy) are also at risk for infection and early-stage trials have shown that certain vaccine candidates are safe in neonates.[61]

The end points for clinical trials could include prevention of infection or, if infection occurs, modification of viral replication. Slower viral replication would be expected to result in slower disease progression and less risk of transmission to others. Data are insufficient to detect an effect of vaccination on disease progression at this time.[60] Nonhuman primate studies support both possible outcomes and a useful vaccine will likely have both effects.

Progress to date

Several HIV-1 vaccines have recently entered trials or are expected to do so soon.[62] A number of vaccines have been tested and, lacking credible immunogenicity data, abandoned. Table 118.4 shows the status of HIV-1 clinical trials at the time of writing, but the reader is encouraged to seek more current information through websites or newsletters (www.iavi.org/trialsdb; www.vrc.org; www.hvtn.org; http://www.iavi.org/iavireport/; and others).

One vaccine design, bivalent recombinant gp120 (envelope) protein adjuvanted with alum, has entered two phase 3 trials. One efficacy trial which enrolled primarily men who have sex with men, testing a bivalent vaccine in which both components are based on HIV-1 of the B subtype has failed to show overall efficacy in preventing HIV-1 infection (www.vaxgen.com). Post hoc analyses showed the possibility of benefit in certain subgroups, blacks and Asians. However, the numbers were too few to be conclusive and further studies will be needed. A trial in Thailand with an analogous vaccine containing one component derived from B subtype and one derived from E subtype is also underway, enrolling injection drug users. These vaccines induce antibodies that neutralize 'laboratory-adapted' HIV-1 strains but not freshly isolated HIV-1. A novel approach will be required to induce antibodies that can neutralize primary isolates.

A third efficacy trial is planned for Thailand, enrolling heterosexual men and women; this trial will combine a vaccine designed to induce cell-mediated immunity (primarily mediated by CD8 T cells). The vaccine, designed to induce cell-mediated immunity, is recombinant canarypox containing genes that encode HIV-1 envelope,

gag and protease, based on the B and E subtypes. The canarypox is an attenuated form of a virus that infects birds but not mammals. The virus enters mammalian cells, uncoats and releases its genetic material, which then directs the synthesis of endogenous antigens but does not replicate. This induces CD8 T cells that release antiviral substances such as interferon-? and/or kill HIV-infected host cells and CD4 (helper) T cells. In the Thai efficacy trial, the canarypox recombinant will be combined with gp120, in a 'prime-boost' regimen designed to induce both T-cell and antibody responses.[63]

Several novel vaccine candidates are now in clinical trials. Most of these are designed to induce CD8T cells and some may induce antibodies as well. Recently, lipopeptides have been tested in healthy volunteers;[64] these antigens are more effectively presented than the nonlipidated peptide vaccines tested some years ago.[65]

TABLE 118-4 -- Preventive HIV-1 vaccines currently in clinical trials (2003).

PREVENTIVE HIV-1 VACCINES CURRENTLY IN CLINICAL TRIALS (2003)					
Vaccine type		***Gene(s)*/protein(s)**	**Phase**	**HIV subtype**	**Countries**
Protein	Recombinant protein(s)*	gp 120	3	B	North America, Netherlands†
			3	B + E	Thailand
		tat/nef + gp120	1	B	USA
		tat	1§	B	Italy
Peptide	Lipopeptides‡	gag, nef, pol	1	B	France
Nucleic acid	DNA	*gag*	1	B	USA
	DNA	*gag plus epitopes from other genes*	1	A	UK, Kenya Uganda
	DNA	*envelope, gag, nef*	1	B	USA
	DNA	*gag, rt, envelope, tat, rev, vpu*	1	B	USA
	DNA	*nef*	1	B	Finland
	DNA	*gag, pol, envelope, nef*	1	A, B, C	US
	DNA	*CTL epitopes from: gag, pol, vpr, nef, rev, envelope*	1§	SHARED	US, Botswana
	DNA	*gag, pol, nef*	1§	B, C	Europe
Virus	ALVAC (Canarypox)	*envelope, gag, portions of enzymes (inactivated)*	2	B	North America, Caribbean, South America
	ALVAC (Canarypox)	*envelope, gag, portions of enzymes (inactivated)*	2	B + E	Thailand
	ALVAC plus gp 120	*Envelope, gag, portions of enzymes (inactivated)*	3§	B + E	Thailand
	Modified Vaccinia Ankara¶	*gag plus epitopes from other genes*	1	A	UK, Kenya, Uganda
	Adenovirus¶	*gag*	1	B	USA
	VEE	*gag*	1§	C	USA, South Africa

* gp 160 and p24 (gap component) have also been tested.
† Preliminary analysis available.
§ Scheduled for 2003
‡ Peptides without the lipid component have also been tested.
¶ Both recombinant MVA and recombinant adenovirus have been used as a 'boost' subsequent to 'priming' with the corresponding DNA vaccine.

DNA vaccines are directly injected into skin or muscle or applied topically. When taken up by host cells, the plasmids direct the synthesis of vaccine antigens. Early trials with DNA vaccines alone showed limited immunogenicity.[66] Therefore, several groups are studying DNA vaccines as a 'prime' followed by recombinant viral vectors as a boost. This is a different variation of 'prime-boost', in which the initial DNA vaccination is thought to focus the immune response, primarily mediated by T cells, on the products of the HIV-1 genes rather than on the gene products encoded by the virus vector itself,[67] while the recombinant vector induces more effector cells and/or antibodies. DNA priming followed by a recombinant, replication-defective vaccinia boost (MVA) is now being tested in the UK and Kenya[68] and DNA priming followed by a recombinant, replication-defective adenovirus is being tested in the USA.[10] In these trials, the current vaccine constructs are based on the *gag* gene; the groups plan to add new HIV-1 genes to the vaccines.

Which are the most important antigen(s) or gene(s) to include in a recombinant HIV-1 vaccine? The inherent variability of the gene within HIV-1 is likely to be important (clearly, a more conserved gene will be well matched to a larger spectrum of virus strains). For vaccines intended to induce neutralizing antibodies, envelope is the critical gene. However, the selection of the 'best' envelope sequence from among the myriad isolates is difficult. Vaccines could incorporate intact envelope proteins or modified versions that lack some of the features that modify immunogenicity such as variable 'loop' segments and heavy glycosylation.[69] For vaccines designed to induce T cells, the quantity of viral protein made in an infected cell may be important and targeting genes expressed early in infection could lead to destruction of infected cells before they release new virions. Even the optimal number of genes or gene products is debated; a larger number of targets seems desirable but some viral antigens may interfere with responses to others. This debate will be resolved finally by clinical trials.

Newer vaccines, not yet in clinical trials, may utilize different viral or bacterial vaccines as vectors. These vectors each have unique virtues. Some can be grown cheaply, amplifying the HIV-1 genes they carry along with their own. Some can be administered simply, such as bacterial vaccines that are effective when given orally. They may supply the stimulus to the innate immune system that will amplify immunity or deliver the antigen to dendritic cells in lymph nodes or to mucosal lymphoid tissues.

However, vectored vaccines also have real or theoretical drawbacks. For each novel vaccine, safety must be carefully evaluated in phase 1, 2 and 3 trials and then after marketing. They may confer long-lasting immunity; however, if immunity to the vector precludes effective boosting or interferes with the use of a different vaccine

employing the same vector, this may interfere with use on a worldwide, public health scale. Likewise, if naturally occurring immunity to the vector can interfere with immunization, the effects of a vaccine in different populations may be difficult to predict. These issues must be examined during vaccine development.

Older virus vaccines are almost all based on one of two designs: whole inactivated ('killed') or attenuated ('live') versions of the pathogenic virus. These have not been used in prophylactic HIV-1 vaccine trials. Whole killed HIV may be feasible, although inactivating without destroying envelope protein structure and proving that it has been entirely inactivated will be a challenge.[70] In animal studies, live attenuated SIV has proven to be an effective vaccine, but it is unsafe because of occasional reversion.[71] The seemingly insurmountable obstacle for a vaccine based on replication-competent HIV-1 is that there would be no clear way to prove its safety.[72] Such vaccines, however, may prove to be useful in experimental animal models, for understanding immunity to the retroviruses.

HIV-1 VACCINES AS THERAPY

Almost from the beginning of HIV-1 vaccine research, investigators have wondered whether an HIV-1 vaccine could improve the apparently inadequate and waning immune response of infected persons. A large, controlled trial of gp160 protein as a therapeutic vaccine showed clearly that the groups receiving vaccine and placebo had an identical clinical course.[73] Recent trials of whole killed vaccine as a therapy have failed to show convincing benefit. Measures of virus replication and perhaps clinical end points will be required in therapeutic vaccine trials. The best chance for a therapeutic vaccine would appear to be if it is given in the presence of highly

active antiretroviral therapy (HAART), otherwise immune activation of HIV-1-specific CD4 cells could render them more vulnerable to infection and destruction by HIV-1. Trials are now being undertaken to suppress virus with HAART, vaccinate and then interrupt therapy.[74] [75]

If vaccine therapy is successful, it will be encouraging for the field of preventive HIV-1 vaccine research, but not conclusive. If unsuccessful, it may not reflect the potential of that vaccine to prevent HIV-1 infection or AIDS if given prior to infection. After the initial days and weeks of virus replication, more cells may be infected, more genetic variation may have arisen and ineradicable reservoirs may exist in nonreplicating cells or inaccessible anatomic compartments in 'established' HIV-1 infection than the initial encounter with HIV-1.

FUTURE CHALLENGES

Conducting trials of HIV vaccines in the countries most affected by the epidemic is a challenge,[76] albeit one that has been successfully met in Thailand. When one or more HIV-1 vaccines is identified, the task will be just beginning. The next step will be ensuring access for populations where the need is the greatest. Currently, over 60 million people have become infected with HIV-1 and hundreds of millions are at risk. Many of these individuals have little access to medical care, no access to adult vaccinations and live in countries where the per capita expenditure on health care is a few dollars. Often, HIV-1 is ill understood and stigmatized. Diagnostic tests or algorithms may need to be altered. Education will be needed to convey not only the value but the anticipated limitations of the vaccines, so that other preventive strategies will be maintained. The role of community organizations in maintaining this balance will be critical (www.avac.org).

Regulatory approvals in numerous countries will be required. The issues in the USA are complex.[77] Internationally, there are greater challenges. If a vaccine has not been demonstrated to be universally effective against every HIV-1 subtype, the standard paradigm of approval in one or more industrialized countries followed years or even decades later by acceptance in and distribution to developing countries will not apply. Both the governments of countries affected by HIV-1 and the international regulatory and health authorities must plan ahead for the advent of an HIV-1 vaccine to avoid unnecessary delays.

HIV-1 vaccines might induce antibody responses that would completely prevent HIV-1 infection by strains of virus that are sufficiently well matched to the vaccine. Unfortunately, HIV-1 evolves continuously both within an individual (the source of infection) and within human populations. Therefore, a vaccinated person might be protected initially, but later encounter a virus against which he or she is not immune. Multivalent vaccines or repeated immunization with an updated vaccine may be required for continued protection. Thus, eventually, one might imagine licensure not of a specific HIV-1 vaccine but of a method for producing the vaccine and its updated versions, somewhat analogous to the annual updates of influenza vaccine.

The scientific challenges involved in discovery of a safe and effective vaccine against HIV-1, although daunting, are only the prelude. The task of developing and deploying a vaccine against HIV-1 will be enormous (i.e. gaining licensure and scaling up to produce vaccine for worldwide distribution, developing distribution methods and establishing purchase mechanisms will require an unprecedented co-operative effort by governments, international agencies, philanthropic organizations and the private sector).[78] Recent recognition of the importance of controlling HIV-1 for public health, economic and political stability has been encouraging and there has been substantial growth in academic, government, nonprofit and pharmaceutical industry vaccine research, but the fight is not over. Stopping the march of the pandemic will conserve economic and human resources needed to care for those already infected. Ending the HIV-1 epidemic is critical to allow economic development and promote political stability in the developing world and to reduce loss of life and suffering worldwide.

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Chapter 119 - Practice Point

Postexposure prophylaxis for nonoccupational HIV exposure

David K Henderson

Introduction

In spite of considerable investment in education and training of the public, community exposures to HIV continue to occur commonly and frequently require clinical intervention. The introduction of postexposure chemoprophylaxis for occupational exposures to HIV in the health care workplace quickly prompted questions about the potential use of these agents for nonoccupational exposures. Several issues related to the administration of postexposure chemoprophylaxis for community exposures are distinctly different from offering these agents for occupational exposures in the health care setting. Since none of the available agents has a specific indication for use in prophylaxis, all antiretroviral chemoprophylaxis (for both occupational and nonoccupational exposures) must be considered 'off-label' use. This Practice Point reviews the immediate management of nonoccupational exposures and discusses the issues that are uniquely relevant to providing chemoprophylaxis for sexual, needle-sharing and other nonoccupational exposures.

Efficacy of postexposure chemoprophylaxis

The recent San Francisco Post Exposure Prophylaxis study demonstrated the feasibility of developing and implementing a postexposure program for managing community/nonoccupational exposures to HIV. Much of what we know about the potential for efficacy of antiretroviral postexposure chemoprophylaxis for sexual or nonoccupational exposures is derived from our experience using these agents in health care workers. Despite differences in the route of exposure (and in other variables likely to influence, at least to some extent, the specific risks for transmission), the results from animal studies, studies of vertical transmission and clinical experience to date with postexposure chemoprophylaxis for occupational exposures would seem to be directly relevant to the use of these agents for chemoprophylaxis for sexual or nonoccupational exposures.

Risk for infection associated with nonoccupational exposures to HIV

The per-exposure risk for HIV infection associated with community exposures varies significantly by the type of exposure. Considering community-based exposures, the risks associated with needle sharing in intravenous drug abuse are perhaps the greatest (Table 119.1). Whereas reasonable estimates of the risks for transmission associated with various sexual encounters have been published, these data clearly represent estimates and the precise risks for infection associated with these exposures are not known (see also Chapter 116). In addition, such estimates are useful only to understand the general risk for events of a certain type, since a variety of additional factors influence the risk associated with a single exposure (e.g. blood exposure, partner's viral burden, presence of reproductive tract infections, viral strain-specific differences in infectivity, cervical ectopy, circumcision and a variety of other factors). With these limitations in mind, estimates for the risk of transmission associated with different kinds of HIV exposures are summarized in Table 119.1 .

Special problems

Chemoprophylaxis following consensual sexual exposure

Perhaps the most common circumstance for which postexposure prophylaxis is sought for community exposures to HIV is consensual sexual exposure. The details surrounding such exposures vary substantially and should be carefully considered before making the decision to initiate postexposure antiretroviral therapy. Circumstances of exposure and commitment to risk reduction/safe sex strategies should be an important determinant of the decision to administer prophylaxis. Several authorities have argued that postexposure prophylaxis is not appropriate for individuals who plan on continuing risk behavior. Conversely, offering postexposure prophylaxis to someone who is committed to risk reduction but had a temporary relapse of higher risk behavior makes implicit sense. From a practical perspective, determining an individual patient's commitment to risk reduction strategies may be extremely difficult, particularly in the context of a single patient-physician encounter. For this reason, most physicians decide to offer treatment unless they are convinced that the individual is not interested in risk reduction. Withholding treatment from someone who is uninterested in risk reduction seems cruel, but may actually be in the individual's best interest (particularly if the availability of postexposure prophylaxis is contributing to the individual's willingness to participate in risk behaviors). Offering repeated courses of prophylaxis may place the patient at increased risk and may be associated with societal risks as well (discussed below).

Rape/sexual abuse

A special set of problems arises with respect to the victims of sexual assault and/or sexual abuse. In most instances, the victim will not be aware of the HIV status of the assailant, and so these exposures represent the equivalent of 'source-unknown' exposures in the health care setting (see Chapter 117). Sexual assault, by its very nature, may be associated with increased risk for HIV transmission due to the increased likelihood of blood exposure, trauma and simultaneous exposure to other sexually transmitted diseases. In fact, several cases of HIV transmission have been directly linked to sexual assaults.

TABLE 119-1 -- Estimated HIV transmission risks associated with selected types of HIV exposures.

ESTIMATED HIV TRANSMISSION RISKS ASSOCIATED WITH SELECTED TYPES OF HIV EXPOSURES	
Route/type of exposure	**Risk for infection mean/range (%)**
Transfusion of contaminated blood	84–100
Intravenous drug use (needle sharing)	0.8
Receptive anal intercourse	0.3–0.8
Insertive anal intercourse	0.04–0.1*
Occupational needlestick exposure	0.28–0.33
Insertive vaginal intercourse	0.03–0.09
Receptive vaginal intercourse	0.005–0.02
Insertive oral intercourse	0.003–0.008*
Receptive oral intercourse	0.006–0.02*

** Estimates drawn from Varghese et al. (2002), Donegan et al. (1990), Henderson et al. (1986); Kaplan and Heimer (1994) and Royce et al. (1997).*

The Centers for Disease Control and Prevention has recommended the use of antiretroviral chemoprophylaxis for rape victims, but has stopped short of recommending

(either for or against) prophylaxis for consensual sexual exposures. The emotional trauma associated with a sexual assault is substantial and is compounded by the possibility of HIV transmission. For this reason, counseling must be an integral part of the postexposure management program. In many centers, follow-up for victims of sexual assault is extremely difficult, often because the victims do not give correct identification information and cannot be located for follow-up. In addition, many such victims do not return for follow-up appointments (even with prompting). Counseling of assault victims is crucial to the postexposure management process; such individuals must be made aware of the importance of follow-up, particularly if they embark on a postexposure prophylaxis regimen. Some authorities have suggested that offering postexposure prophylaxis may improve follow-up adherence.

Prophylaxis in children and adolescents who are the victims of sexual abuse

Children who are victims of sexual abuse may also be at risk for sexually transmitted diseases, including HIV infection. Experience administering antiretrovirals to healthy children is virtually nonexistent and the possibility exists that risks associated with the administration of these agents may be different, and perhaps higher, in children. Nonetheless, for documented sexual abuse exposures in children and adolescents, most authorities would recommend the administration of postexposure prophylaxis.

Parenteral exposures in the community

The most common source of parenteral exposures to HIV in the community setting is needle sharing in the process of intravenous drug use. If an individual shares needles with a partner known to be HIV infected, antiretroviral chemoprophylaxis is definitely indicated. For the more common set of circumstances (i.e. the HIV infection status of the needle-sharing partner is unknown), individualized decisions about postexposure prophylaxis should be made, based on the likelihood of HIV exposure in the community.

An unfortunately common circumstance in emergency rooms is the appearance of a child who has discovered a needle or syringe and has sustained a needlestick exposure. Quantitating the transmission risk in this setting is almost impossible, although some factors may be helpful in deciding whether to administer prophylaxis. Realistically, many such exposures present minimal risk for transmission of HIV; however, exclusion of risk may be almost impossible. The presence of blood in the syringe or on the needle would clearly identify increased risk. Each of these exposures must be assessed epidemiologically for circumstances that might be associated with increased risk and independent decisions must be made about each exposure.

Timing of prophylaxis in relationship to the exposure

Ideally, postexposure prophylaxis with antiretroviral agents should be administered as soon after an exposure as possible. In most of the more recent animal studies, if prophylaxis was begun within 24 hours of exposure the animals were protected. Clearly, delay of treatment is likely to be detrimental. In situations in which the picture of exposure is less than clear (e.g. an exposure to a source whose HIV status is uncertain), I would encourage the individual to initiate prophylaxis (to preserve the option) while the situation is being clarified. A special problem arises with respect to individuals who delay reporting of the exposure. As is the case for health care workers who, for a variety of reasons (e.g. denial, the urgencies of patient care, etc.), delay reporting infections, chemoprophylaxis should still be offered to exposed, susceptible individuals who have sustained community exposures to HIV. The practitioner should explain what is known about the importance of early administration of prophylaxis and should explain that, based on the animal models of retroviral infections, the chance for protection for individuals who delay reporting may be reduced and the risk:benefit ratio of prophylaxis may be altered. In fact, some authorities have recommended that prophylaxis should not be administered if more than 72 hours have elapsed from the time of the exposure. Nonetheless, in my view, withholding treatment in most instances is extremely difficult and I tend to err on the side of offering the treatment, despite a delay in reporting.

Postexposure management approach

As noted above, since an ongoing commitment to risk reduction by the exposed patient is an important determinant of the administration of prophylaxis, a thorough history is essential in the management of such exposures. Since these interactions often take place in a hectic emergency room, obtaining an appropriate history may be a challenge, but is essential to good management. Having trained counselors with experience in this field participate in the management (including history taking) is optimal. Important aspects of the history include (but are not limited to) the following: prior history of having taken courses of prophylaxis, prior history (especially recent history) of risk behaviors, assessment of current commitment to risk reduction, history (especially recent) of other sexually transmitted diseases, specific characteristics of the exposure for which prophylaxis is sought, and as much detailed information about the partner as can be gleaned.

Baseline laboratory studies should be obtained, including serologic testing for HIV infection. The so-called 'rapid' HIV tests are useful in that they are generally quite sensitive, but relatively nonspecific. As a screening test in the absence of risk behavior in the recent past, a negative test is quite useful. A positive test should be confirmed with traditional enzyme-linked immunosorbent assay (ELISA) testing, as well as by an additional confirmatory test, if the ELISA is positive. In addition, baseline chemistries and hematologic studies (in anticipation of prophylaxis administration) and a urinary pregnancy test for women should be undertaken, as well as microbiologic and serologic studies for other sexually transmitted diseases (e.g. gonorrhea, syphilis, chlamydia, hepatitis B and C). Depending on the patient's recent history of risk behavior, consideration should be given to assessing for circulating HIV nucleic acid using polymerase chain reaction (PCR) technology, if readily available. The HIV serologic tests should be repeated at 6 weeks, 3 months and 6 months following the exposure. If prophylaxis is administered, chemistries, including hepatic function studies, and hematologic

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studies should be conducted bi-weekly. Patients should be instructed to return immediately if they develop any of the signs and symptoms of the acute seroconversion illness or if any of the signs and symptoms of severe drug toxicity develop. Symptoms associated with treatment (e.g. nausea, vomiting, diarrhea, etc.) should be managed aggressively, and perhaps even pre-emptively, with medications addressing these symptoms.

Information about the source of the exposure is extremely useful. If the partner is known and can be tested, determining the partner's HIV antibody status, viral RNA, stage of illness, antiretroviral treatment history and presence of factors that may increase the risk for transmission (e.g. presence of other genitourinary infections, recent menstrual history, etc.) all can be of value. In instances in which the partner cannot be identified, the decision to offer prophylaxis should be based on the practitioner's best epidemiologic assessment of the likelihood of exposure (based on the patient's history, the prevalence of infection in the community, the type of encounter and the time from exposure to presentation), as well as the individual's commitment to risk reduction in the future.

The regimens for postexposure prophylaxis are summarized in Table 119.2 . These recommendations are based on experience with these agents for occupational exposures to HIV in the health care setting. The currently recommended duration of therapy is 4 weeks; however, the choice of 4 weeks is arbitrary and based on limited clinical experience, experience with animal models of retroviral infections and in vitro studies evaluating the prevention of retroviral infection of susceptible tissue culture cells.

No discussion of postexposure management would be complete without underscoring the importance of counseling in this setting. All patients presenting with possible nonoccupational exposures to HIV should be counseled in detail regarding risk behaviors and risk reduction strategies. Counseling should also focus on the specific circumstances of the individual's exposure and should offer constructive suggestions regarding risk reduction.

Managing exposures when the source patient is or might have been taking antiretrovirals

Frequently, a source/partner for an HIV exposure has been taking antiretrovirals as therapy. If the source/partner's HIV infection is not suppressed, the individual may harbor resistant isolates. Prophylaxis should be initiated immediately and then modified if additional information becomes available.

TABLE 119-2 -- Suggested basic and expanded postexposure prophylaxis regimens for chemoprophylaxis after nonoccupational exposures to HIV.‡

SUGGESTED BASIC AND EXPANDED POSTEXPOSURE PROPHYLAXIS REGIMENS FOR CHEMOPROPHYLAXIS AFTER NONOCCUPATIONAL EXPOSURES TO HIV		
HIV exposures with a recognized transmission risk	'Basic regimen'	Zidovudine (ZDV) plus lamivudine (3TC)
	Alternative 'basic regimens'	Stavudine (d4t) plus lamivudine
		d4t plus didanosine (ddl)‡

HIV exposures for which the nature of the exposure suggests an elevated transmission risk*	'Basic regimen' plus one of the following agents	Indinavir‡
		Nelfinavir
		Abacavir
		Efavirenz‡

‡ *Modeled after CDC (2001).*
† Agent(s)/regimens not advisable for use in pregnancy (see text)
* Elevated risk is associated with exposures associated with increased risks for transmission (e.g. sexual assault, trauma, blood exposure, concomitant sexually transmitted disease, source patient with high circulating viral burden, etc.; see text)

Influence on risk behavior — impact on primary prevention strategies and the relationship to societal risks

One significant concern frequently expressed is that the use of anti-retroviral agents for postexposure prophylaxis of nonoccupational exposures may actually increase risk behavior. Individuals may feel that they can take risks that can be abrogated by postexposure treatment and, in so doing, may ironically be putting themselves at increased risk for infection. Several studies have attempted to address this issue but failed to identify evidence that knowledge of the availability of prophylaxis was associated with increased sexual risk behavior. Conversely, the San Francisco prophylaxis study found that 12% of the individuals who participated in the trial returned for a second prophylaxis course within 6 months of the completion of the initial regimen. In spite of the 12% return rate in this study, the authors found that the majority of individuals who enrolled in the program following a sexual exposure to HIV had experienced a temporary lapse in risk reduction behavior that was not related to a consistent pattern of ongoing risk behavior.

Even authorities who are committed to working in this field have raised the concern that the availability of postexposure prophylaxis might undermine public health HIV prevention efforts. To avoid a negative public health impact, a postexposure prophylaxis program should be presented as a secondary prevention intervention, and primary prevention (i.e. avoiding unsafe behaviors, stressing condom use) should be underscored as far more effective, less toxic and less expensive interventions.

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Chapter 120 - The Immunopathogenesis of HIV-1 Infection

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Giuseppe Pantaleo

This chapter examines the immunologic and virologic mechanisms involved in the pathogenesis of HIV-1 infection and the interaction between the virus and the host. The recent availability of highly active antiretroviral combination therapy (HAART) has significantly influenced the natural history of the infection, delaying the progression to overt AIDS and prolonging survival. At the same time, increasing knowledge of the pathogenic mechanisms and of the limitations of HAART has made it clear that eradication of the virus with the available conventional antiviral drugs is not feasible. Recent advances in our understanding of the correlates of protective immunity have drawn attention to the development of immune-based interventions in order to achieve long-term control of HIV-1 disease.

THE NATURAL HISTORY OF HIV INFECTION

The typical course of HIV-1 infection is defined by different phases that generally occur during a period of between 8 and 12 years. Although the pattern and the course of the infection is highly variable among HIV-1-infected patients, three distinct phases can be identified (Fig. 120.1):[1]

- primary HIV-1 infection;
- chronic asymptomatic phase; and
- overt AIDS.

The three phases of the disease

Primary HIV-1 infection

Primary HIV-1 infection is a transient condition, revealed by a symptomatic illness of variable severity in 40–90% of patients, and is invariably accompanied by:

- an initial rapid rise in plasma viremia, often to levels in excess of 1,000,000 RNA copies/ml;
- a decrease in the blood $CD4^+$ T cell; and
- a large increase in the blood $CD8^+$ cell count.

The marked decline of plasma viremia generally coincides with the resolution of the clinical syndrome.[2] The decrease in the viral load correlates with the appearance of the virus-specific immune responses (see below), particularly HIV-1-specific cytotoxic T lymphocytes (CTLs), indicating that virus-specific immune responses certainly play a crucial role in the initial downregulation of virus replication.[3] [4] [5] [6]

The signs and symptoms of primary HIV-1 infection generally appear 2–4 weeks after virus exposure (Fig. 120.1).

The duration of the clinical syndrome ranges between a few days and more than 10 weeks but generally lasts less than 14 days. The clinical presentation of the primary HIV-1 infection may mimic acute mononucleosis (primary Epstein-Barr virus infection; see Chapter 122) as well as many other febrile acute illnesses, emphasizing the non-specific nature of these symptoms and the difficulty of obtaining an accurate early diagnosis.

Because the acute clinical syndrome associated with primary infection is not specific for HIV-1, the diagnosis is based on laboratory tests. In this regard, it is important to underscore the fact that anti-HIV-1 antibodies are usually negative during the acute phase of illness, as well as the Western blot (i.e. the laboratory assay used to confirm the diagnosis of HIV-1 infection), which evaluates the generation of specific antibodies against different HIV-1 proteins. The Western blot is considered to be positive when there are at least three specific bands and/or two bands but with antibody reactivity against HIV-1 *env* and *gag*. Early diagnosis, therefore, relies on a history of exposure, a positive p24 antigen enzyme-linked immunosorbent assay (ELISA) or the detection of plasma viral RNA (almost always more than 50,000 copies/ml of plasma).[2]

The chronic asymptomatic phase

The primary HIV-1 infection is followed by a long phase of clinical latency (median time of 10 years), during which neither signs nor symptoms of illness are present. Relatively stable levels of virus replication and of $CD4^+$ T cell counts for a variable period of time characterize this phase of infection. This 'stability' of measures of disease activity is apparent in the blood only. Virus replication and the accumulation of extracellular virus trapped in the follicular dendritic cell network are particularly active in the lymphoid tissue, where a progressive anatomic and functional deterioration occurs, impairing the ability to maintain effective specific immune responses over time.[7] [8] This is reflected by the rapid increase in the levels of viremia and by a drop in $CD4^+$ T cell counts, which may suddenly speed up the transition from this phase to the advanced stage of the disease.

The advanced stage of HIV-1 disease is marked by low $CD4^+$ T cell counts (below 200 cells/µl) and by the appearance of constitutional symptoms. It may be complicated by the development of AIDS-defining opportunistic infections.[1]

Overt AIDS

Overt AIDS defines the end stage of HIV-1 infection. In the absence of antiretroviral therapy, this phase leads to death in 2–3 years. The risk for death and opportunistic infections significantly increases with $CD4^+$ T cell counts below 50 cells/µl. Fortunately, the recent advent of HAART, including at present as many as 15 antiretroviral drugs administered in different combinations, is significantly decreasing the rate of progression, morbidity and mortality of HIV-1 infection.

Clinical course of the infection

The clinical course of HIV-1 infection is variable. In the majority (60–70%) of HIV-1-infected patients, the median time between infection and development of AIDS, in the absence of therapy, is 10–11 years. These HIV-1-infected persons are defined as typical progressers (Fig. 120.2), and the clinical course of the infection that they generally experience is the one described above.

However, about 10–20% of subjects progress rapidly, developing AIDS in less than 5 years of infection, and they are therefore called rapid progressers (see Fig. 120.2). In these patients, after the primary HIV-1 infection, plasma virus levels are often higher than 10^5 copies of HIV-1 RNA/ml and, in particular, $CD4^+$ T cell counts start to

Figure 120-1 Kinetics of viral load and immune response during the phases of HIV-1 infection. After HIV-1 exposure, initial virus replication and spread occur in the lymphoid organs, and systemic dissemination of HIV-1 is reflected by the peak of plasma viremia. A clinical syndrome of varying severity is associated with this phase of primary HIV-1 infection in up to 70% of HIV-1-infected persons. Downregulation of viremia during the transition from the primary to the early chronic phase coincides with the appearance of HIV-1-specific cytotoxic T cells and with the progressive resolution of the clinical syndrome. The long phase of clinical latency is associated with active virus replication, particularly in the lymphoid tissue. During the clinically latent period, $CD4^+$ T cell counts slowly decrease, as does the HIV-1-specific immune response. When $CD4^+$ T cell counts decrease below H 200 cells/µl (i.e. when overt AIDS occurs), the clinical picture is characterized by severe constitutional symptoms and by the possible development of opportunistic infections and/or neoplasms.

decrease much earlier and more rapidly during the chronic asymptomatic phase, leading to the eventual development of AIDS. Furthermore, both humoral and cell-mediated HIV-1-specific immune responses are either never detected or rapidly lost after the transition from the acute to the chronic phase of infection.

At the other extreme, it is estimated that 5–15% of HIV-1-infected people will remain free of AIDS for more than 15 years; these people are termed slow progressors (see Fig. 120.2). In this situation, $CD4^+$ T cell counts remain stable and they are frequently above 500 cells/µl, and plasma virus levels are usually below 10,000 HIV-1 RNA copies/ml.

Slow progressers include a further subgroup of HIV-1-infected people, so-called long-term nonprogressors (see Fig. 120.2). About 1% of HIV-1-infected subjects probably fall into this category. The definition of long-term nonprogressors should be limited to those who have had a documented infection for at least 8–10 years, are

naive to antiretroviral therapy and have no signs of disease progression (e.g. constant high counts of $CD4^+$ T cells and either low (500–1000 copies of RNA/ml) or very low (<50 copies/ml) plasma virus levels).[1] [9]

This wide variability of the natural course of the disease is evidence of the presence of different driving forces - genetic, immunologic and virologic factors - that determine the evolutionary pattern of HIV-1 infection in the individual patient.[10] It is therefore important, first, to identify the different determinants of the rate of disease progression and, second, to elucidate how these driving forces work together. Furthermore, the potential ability of HAART to restore some determinants of long-term control of the virus must be evaluated in depth. These arguments are discussed in detail below.

The variability of the natural course of HIV-1 infection also underlines the need for markers of disease progression (see below) that may identify as early as possible the patients who are at risk for a more rapid disease progression. This could warrant either the use of different, perhaps more aggressive, therapeutic strategies or the need to monitor these patients more closely, or both.

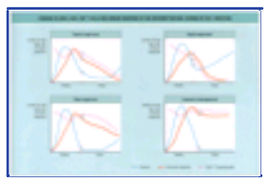

Figure 120-2 Changes in viral load, $CD4^+$ T cells and immune response in the different natural courses of HIV-1 infection. Typical progressors represent 60–70% of the total HIV-1-infected population, rapid progressors represent 10–20%, slow progressors represent 5–15% and long-term nonprogressors represent 1%.

SEQUENCE OF PATHOGENIC EVENTS LEADING TO THE ESTABLISHMENT OF HIV-1 INFECTION

HIV-1 can be transmitted by different routes: by sexual contact, through either genital-genital or genital-oral sex; by blood-blood contamination, via either transfusion of blood and infected blood-derived products or needle sharing among injection-drug users; and by maternal-fetal transmission. The most common route of infection is sexual transmission at the genital mucosa.[11]

Early pathogenic events after entry of HIV-1 into the body

The acute intravaginal infection of rhesus monkeys with the simian immunodeficiency virus (SIV) represents a very useful model for studying the sequence of cellular events that characterize the very early steps of infection after sexual transmission. In this model, tissue dendritic cells (i.e. Langerhans cells that reside in the lamina propria subjacent to the vaginal epithelium) are the first potential target cells of HIV-1 (Fig. 120.3). [12]

The dendritic cells constitute a complex and highly developed system of antigen-presenting cells that are able to prime naive T cells. Their potent antigen-presenting ability is associated with the high expression of major histocompatibility complex (MHC) class I and class II molecules and costimulatory molecules on the cell surface. The ability of dendritic cells to attract and prime naive T cells can be explained by the expression of a type II membrane protein with an external mannose binding, C-type lectin domain, named DC-SIGN.[13] [14] It has been suggested that interaction between DC-SIGN and intracellular adhesion molecule (ICAM)-3 is responsible for the initial contact between dendritic cells and resting T cells, which represents a critical step for the initiation of the T cell immune response. Furthermore, a major contribution to the ability of dendritic cells to initiate T cell immunity is also provided by the fact that dendritic cells express high levels of specific chemokines that target naive rather than memory T cells.[15] Therefore, dendritic cells play a key role, both in priming the initial virus-specific immune response and in serving as a carrier for the transport of HIV-1 to the nearest lymphoid station.

It is important, however, to mention the differences existing between the Langerhans cells (also called 'epidermal' or 'epithelial' dendritic cells) and the 'dermal' or 'subepithelial' dendritic cells.[13] Langerhans cells do not express DC-SIGN and, once they have encountered HIV-1 below the vaginal epithelium, they can either be infected or pick up HIV-1 virions. Epidermal, DC-SIGN-negative dendritic cells are thought to select for the macrophage (M)-tropic viruses that are the most frequently transmitted variants.[16] [17] The M-tropic HIV-1 carried by epidermal dendritic cells can bind to additional subepithelial DC-SIGN-positive dendritic cells. DC-SIGN can capture HIV-1 on the cell surface of dendritic cells without allowing viral entry. [18] It is thought that DC-SIGN-positive dendritic cells play the major role in the delivery of virus to T cells, thus greatly amplifying HIV-1 infection.[17] Subsequently, dendritic cells migrate to the internal iliac lymph nodes, where they target the T cell areas and then present

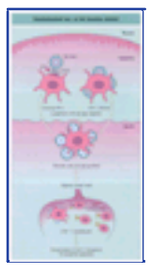

Figure 120-3 Transmission of HIV-1 at the mucosal surface. After entry at the mucosal epithelium, Langerhans cells, also named epidermal dendritic cells, can either be infected by R5 (macrophage (M)-tropic) strains of HIV-1 or pick up HIV-1 virions. Epidermal, DC-SIGN-negative dendritic cells are thought to select for the M-tropic viruses that are the most frequently transmitted variants. The M-tropic HIV-1 carried by epidermal dendritic cells can bind to additional subepithelial DC-SIGN-positive dendritic cells. DC-SIGN can capture HIV-1 on the cell surface of dendritic cells without allowing viral entry. It is thought that DC-SIGN-positive dendritic cells play the major role in the delivery of virus to T cells, thus greatly amplifying HIV-1 infection.

the viral antigens to activated virus-specific T cells. Dendritic cells can support viral replication only in the presence of activated T cells.[15]

Recent advances in our understanding of the mechanisms that modulate the infectivity of HIV-1 can explain why 95% of viruses transmitted are M-tropic. For more than 10 years, it has been known that human CD4 is sufficient for binding HIV-1 gp 120 to cells, but it is sufficient neither for fusion nor for penetration of the viral envelope into the host cell.[19] [20] [21] It is now clear that certain cell-surface receptors for chemokines function as co-receptors by co-operating with the CD4 molecule; moreover, different chemokine receptors, together with CD4, allow entry of HIV-1 strains with distinct cellular tropism. There are two classes of chemokine: CC, if the first two cysteines are adjacent; and CXC, if they are separated by a single amino acid. The CC-chemokine receptor (CCR)5, a seven-transmembrane G-protein-coupled receptor, is the major co-receptor for M-tropic or R4 strains of HIV-1, whereas CXCR4 is the one for T-cell-tropic or X4 strains of HIV-1. Langerhans cells or epithelial dendritic cells express CCR5 but they may not express CXCR4, giving a reason for the preferential transmission of R5 HIV-1 strains.[22]

To this extent, it is worth noting that subepithelial dendritic cells are able to capture the virus through DC-SIGN,[18] a mechanism that is independent of CD4 and co-receptors. However, although both R5 and X4 strains of HIV-1 are carried by dendritic cells to the nearest lymphoid station, only R5 HIV-1 envelopes have the unique ability to mediate an activation signal into $CD4^+$ T cells and to recruit them by chemotaxis.[23] Therefore, the combination of the two events (i.e. the differential expression of co-receptors on the initial target cells and the unique signaling ability of R5 envelopes of HIV-1) may explain why R5 HIV-1 variants are preferentially transmitted, ensuring the rapid recruitment of a large number of activated $CD4^+$ T cells and spread of HIV-1 in the lymphoid organs. It is important to emphasize that rapid recruitment and spreading of target cells (i.e. activated $CD4^+$ T cells) confers to HIV-1 a major advantage because these events occur before the appearance of effective virus-specific immune responses.

Within 2 days after infection, HIV-1 can be detected in the draining lymphoid tissue, and it rapidly disseminates throughout the lymphoid system. Afterwards, HIV-1 enters the bloodstream, where viral replication can be detected in plasma 5 days after infection (see Fig. 120.3). In humans, the time from mucosal infection and initial plasma viremia varies, ranging between 4 days and 11 days according to available estimates. It is of note that the risk of infection is increased by conditions that decrease the function of mucosal barriers, such as lesions caused by the presence of concomitant inflammatory or infectious diseases (e.g. cervicitis, urethritis, genital ulcers).

These same steps of infection can be described in the case of genital-oral HIV-1 transmission, because nasopharyngeal tonsils and adenoid tissue contain many cells of dendritic origin that can support viral replication more efficiently than Langerhans cells.[2]

The role of the lymph nodes in primary HIV-1 infection

The study of virologic events occurring during primary infection with either HIV-1 or SIV emphasizes the key role of lymphoid tissue in the establishment of infection. Longitudinal and cross-sectional analyses of HIV-1 or SIV distribution were performed on lymph node biopsies taken from rhesus monkeys or HIV-1-infected patients. In the SIV model of acute infection, virus can be detected in the lymph node as early as 5 days after infection. At this time, as shown by in-situ hybridization analysis for the detection of HIV-1/SIV RNA, virus is mostly present in the form of numerous individual cells expressing viral RNA, and the highest number of virus-expressing cells is observed 7 days after SIV inoculation. Interestingly, the occurrence of the peak number of virus-expressing cells in the lymph node occurs at the same time as the peak of plasma viremia, or shortly precedes it. The cross-sectional analysis of lymph nodes obtained from HIV-1-infected patients indicates that the kinetics of virus distribution in lymph nodes are consistent with those in the SIV model of acute infection. Altogether, these

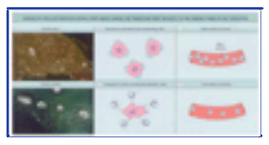

Figure 120-4 Changes in virus distribution within lymph nodes during the transition from the acute to the chronic phase of HIV-1 infection. (a) During the initial weeks of primary HIV-1 infection, the virus is detected in lymphoid tissue as individual virus-expressing cells. This is shown by in-situ hybridization for the detection of HIV-1 RNA (white dots indicate HIV-1 RNA-positive cells). (b) Numerous individual virus-expressing cells seen in the acute phase. (c) Elevated virion levels are found in the circulation in the acute phase. (d) After transition to the chronic phase of the disease, virions trapped in the follicular dendritic cell network become the dominant form of HIV-1, as shown by in-situ hybridization for the detection of HIV-1 RNA (diffuse white areas indicate virus trapped within follicular dendritic cells). (e) Binding of virions on the extracellular surface of follicular dendritic cells in the chronic phase. (f) The number of circulating virions is dramatically reduced in the chronic phase.

findings indicate that the lymph node is the primary anatomic site of infection. [8] [24]

During the transition from primary to chronic infection, a switch from individual virus-expressing cells to virus trapped by the follicular dendritic cell network of lymph node germinal centers occurs (Fig. 120.4). The trapped virus becomes the dominant form of virus present in lymph nodes, and this event is associated with a dramatic decrease in the number of individual cells expressing viral RNA.[8] [24]

At least in part, this is the result of the emergence of virus-specific CTLs that can be detected very early during primary infection and may mediate the elimination of HIV-1-producing cells. Furthermore, the trapping of HIV-1 virions in the follicular dendritic cell network is itself the result of the HIV-1-specific humoral response. In fact, HIV-1 virions are complexed with immunoglobulins and complement, and the binding of these complexes on the extracellular surface of follicular dendritic cells occurs through complement receptors expressed on follicular dendritic cells. In both SIV and HIV-1 infection, the transition from primary to early chronic infection is marked by a decrease of viral RNA in plasma and the resolution of the acute clinical syndrome. Therefore, it is clear that virus-specific immune responses are not only present very early during primary infection but may also significantly affect virus distribution occurring in the early phase of both SIV and HIV-1 infection. [25]

MECHANISMS RESPONSIBLE FOR VIRUS ESCAPE FROM IMMUNE RESPONSE AND ESTABLISHMENT OF CHRONIC INFECTION

Early in primary HIV-1 infection, vigorous virus-specific immune responses can be detected, and they may contribute to both control of the initial peak of virus replication and the reduction in plasma viremia.[3] [4] [5] [6] However, primary HIV-1 infection invariably results in the establishment of chronic disease in the host, inducing a progressive deterioration of the different components of the immune response.

HIV-1-specific immune responses lack the ability to control HIV-1 and to block the progression of the disease. Nevertheless, similar types of immune response are effective against other viruses, such as Epstein-Barr virus and cytomegalovirus (CMV). HIV-1 differs from these other viruses by being able to target very early (during the primary infection) a broad spectrum of effector components of the antiviral immune response and in being able to render these antiviral effector mechanisms ineffective or reshape them into self-defense mechanisms.[26]

Virologic mechanisms of HIV-1 escape from the immune response

HIV-1 possesses the ability to put in motion several mechanisms as the result of the interaction with the host. Some of these virologic mechanisms can be identified:

- formation of a stable pool of latently HIV-1-infected $CD4^+$ T cells containing virus that is capable of replicating;[27] [28] [29] [30] [31]
- the genetic variability of HIV-1;[32] and
- trapping of infectious virions on the surface of follicular dendritic cells. [33]

The rapid formation of a pool of latently HIV-1-infected $CD4^+$ T cells is a key event in the immunopathogenesis of HIV-1 infection for several reasons. First, this event occurs very early and most probably before the appearance of the host's virus-specific immune responses. Second, the pool of latently infected $CD4^+$ T cells contains replication-competent HIV-1 proviral DNA. The proviral DNA can be detected even in compliant HIV-1-infected persons who have been receiving

HAART for a long time (i.e. for more than 2 years); moreover, the virus is wild-type with respect to known drug-induced mutations in the genome. This emphasizes the fact that this pool of cells is a stable reservoir in which HIV-1 remains sheltered from the effects of host immune responses and HAART.[27] [28] [29] [30] [31] Furthermore, it is worth noting that initiation of HAART very early during primary HIV-1 infection does not appear to have a significant impact on the size of this pool of $CD4^+$ T cells. This indicates that this pool is created very rapidly after infection. Third, the decay of this pool of infected $CD4^+$ T lymphocytes is very slow, and the rate of decay is not much influenced by the effective suppression of virus replication obtained by HAART. This clearly represents a major obstacle to the goal of HIV-1 eradication (see below) and long-term control of virus replication.[29] [30] [31]

The high genetic variability of the virus is another efficient mechanism by which it escapes the host immune response.[32] [34] HIV-1 possesses the intrinsic ability to mutate very rapidly. Both during primary and established chronic infection, rapid mutations in the epitopes recognized by HIV-1-specific CTLs may occur. As a consequence, both humoral and cell-mediated virus-specific immune responses quickly lose their ability to control the virus efficiently.

An additional way by which HIV-1 is able to reshape antiviral mechanisms is the trapping of the virus on the surface of follicular dendritic cells in lymphoid tissue germinal centers. As already described, HIV-1 is trapped by the follicular dendritic cell network during the transition from primary to chronic infection and this becomes the dominant form of virus in lymphoid tissue in the chronic phase. Formation of immune complexes and their attachment to the follicular dendritic cell network are physiologic mechanisms that are generally devoted to the clearance of the pathogen and to the generation and maintenance of effective immune responses. In HIV-1 infection, however, these mechanisms lead to the formation of a stable reservoir of infectious virions, representing a continuous source for the infection of $CD4^+$ T cells, and to a chronic inflammatory reaction that ultimately results in the destruction of the lymphoid tissue.[7] [8]

Immunologic mechanisms of HIV-1 escape from the immune response

In addition to the virologic mechanisms described above, through which HIV-1 escapes and reshapes the host immune response, there are some immunologic mechanisms that can be identified:

- deletion of HIV-specific $CD4^+$ T cell clones;[35]
- deletion of HIV-specific cytotoxic $CD8^+$ T cell clones;[36]
- generation of virus escape mutants mediated by CTLs;[26]
- egress of CTLs from lymph nodes;[37]
- impairment of the function of antigen-presenting cells;[26] and
- interference with humoral neutralizing response.[26]

In the majority of HIV-1-infected patients who have established chronic infection, HIV-1-specific $CD4^+$ helper T cell responses cannot be detected, although it is

possible to find evidence of such HIV-1-specific $CD4^+$ T cell responses in long-term nonprogressors.[35] In contrast, HIV-1-specific effector $CD4^+$ T cell responses are consistently detected in patients who have chronic infection.[38] The persistence of the $CD4^+$ helper T cell response may represent one of the determinants of the course of the disease in long-term nonprogressors. In contrast, typical progressors do lose $CD4^+$ helper T cell responses very early in the natural history of the disease (during the primary HIV-1 infection) as a result of direct or indirect virus-induced cytopathology.[35] Furthermore, initiation of HAART during chronic infection and even during early HIV-1 infection is associated with the restoration of the HIV-1-specific $CD4^+$ helper T cell responses only in a subset (about 30–40%) of patients after prolonged treatment.[39] It is, however, possible to preserve HIV-1-specific $CD4^+$ T cell responses if HAART is initiated at the time of the peak of plasma viral RNA (i.e. very early during primary HIV-1-infection).[35]

It is also worth noting that T helper (Th)1 cells preferentially express CCR5 on the membrane surface, suggesting that HIV-1 R5 strains may preferentially and selectively infect Th1 cells during primary HIV infection, when R5 quasispecies predominate. In this regard, recent studies have demonstrated that the initial expansion of HIV-1-specific $CD4^+$ T cells with effector function is aborted during primary infection.[40] In contrast, the expansion of CMV-specific $CD4^+$ T cells in patients who experienced primary HIV-1 and CMV co-infection was not suppressed. These studies indicate a preferential infection of HIV-1-specific $CD4^+$ T cells. Furthermore, they provide evidence that HIV-1-specific $CD4^+$ T cell clones may be rapidly deleted very early during primary HIV-1 infection and that the possibility of rescuing these responses is strictly dependent upon the time of initiation of HAART.[35] [40]

Furthermore, the lack of HIV-1-specific $CD4^+$ helper T cell responses has two other important consequences. First, it can significantly affect the induction and persistence of HIV-1-specific $CD8^+$ CTL responses, because the latter require continuous cognate Th function provided by antigen-specific $CD4^+$ T cells. Therefore, generation and maintenance of vigorous virus-specific responses by CTLs can be compromised over time. Second, it can considerably affect the development of HIV-1-specific antibody responses, because the development of humoral responses is strictly dependent on $CD4^+$ antigen-specific Th cells. [26]

A rapid deletion of certain HIV-1-specific cytotoxic $CD8^+$ T cell clones also occurs during primary HIV-1 infection, in a manner that is analogous to the HIV-1-specific responses mediated by $CD4^+$ helper T cells.[36] These $CD8^+$ T cell clones undergo massive clonal expansion and may be deleted by a mechanism analogous to the clonal exhaustion that is observed in mice during acute lymphocytic choriomeningitis virus infection. This clonal exhaustion of HIV-1-specific $CD8^+$ CTLs causes the early impairment of the virus-specific responses by CTLs. The extent of this phenomenon may determine the varying ability to control virus replication efficiently and therefore it may significantly affect the rate of disease progression. In fact, it is now clear that the appearance of virus-specific responses by CTLs correlates with the decrease in plasma viremia. [6] [41] Furthermore, the higher the relative frequency of HIV-1-specific CTLs, the lower the levels of circulating viral RNA, and higher levels of CTL activity correlate with slower rates of disease progression.[6] [41] [42] However, the clonal exhaustion of virus-specific CTLs does not necessarily result in a complete loss of HIV-1-specific CTL activity, although it does provide additional evidence of how HIV-1 is able to target and impair host immune responses early in the course of the infection.

The detection of CTL-escape variants is common during the course of chronic HIV-1 infection; they may also, however, be found during primary HIV-1 infection. This event provides further evidence of the ability of HIV-1 to reshape some antiviral mechanisms of the immune response into self-defense mechanisms. In fact, although HIV-specific CTLs contribute to the control of both plasma viremia and disease progression,[6] [41] [42] the pressure exerted by CTLs can, at the same time, facilitate the selection of virus mutants that are able to escape the host immune response.[26] [36]

As described above, lymph nodes play a crucial role in the immunopathogenesis of HIV-1 infection. Very high levels of virus replication and spread occur in the lymph nodes during primary infection. Therefore, CTL responses specific for HIV-1 should be predominantly concentrated in the lymphoid tissue in order to achieve the most effective clearance of the virus. However, an early accumulation of HIV-1-specific CTLs in peripheral blood can be observed; CTLs therefore accumulate in a compartment where they cannot efficiently mediate their effector function (i.e. killing of productively HIV-infected cells). Egress of antigen-specific CTLs from lymphoid tissue into the circulation is likely to be a physiologic step that

occurs after the generation of the immune response in order to achieve a wide distribution of antigen-specific effector cells in different anatomic sites. In HIV-1 infection, however, this phenomenon also serves to redirect virus-specific CTLs away from the primary site of virus replication and spread. The observation that the number of HIV-1-expressing cells in lymph nodes does not significantly differ between primary and early chronic infection provides further support for the hypothesis of a defective control of HIV-1 during the early phases of infection.[8]

In addition to $CD4^+$ and $CD8^+$ HIV-1-specific T cells, HIV-1 may interfere with the function of antigen-presenting cells. These cells play a central role in the generation of an effective host immune responses. Cells of the monocyte-macrophage line and dendritic cells can function as specialized antigen-presenting cells and induce both humoral and cell-mediated immune responses. The effect of HIV-1 on these components of the immune system is produced by a quantitative depletion through direct cytopathogenesis or by interference with the formation of MHC-antigenic peptide complexes. To this extent, the expression of MHC class I molecules on antigen-presenting cells can be downregulated by HIV-1 Nef protein, thus affecting both generation of antigen-specific immune responses and recognition of virus-infected target cells by CTLs.[43]

Humoral response against HIV-1 can be detected early during primary HIV-1 infection. This response, however, comprises low-avidity Env-specific IgG that possesses little or no neutralizing activity. Although the reasons for the delay in the appearance of the neutralizing antibody response are poorly understood, such a response is detectable only either after the transition from primary HIV-1 infection to established early chronic HIV-1 infection, or even much later. The virologic and immunologic mechanisms described above significantly affect the global host immune response, within which the $CD4^+$ Th cell function and the interactions between T cells and B cells are profoundly altered. These events may ultimately interfere with circulating titers, avidity maturation and neutralizing activity of HIV-1-specific antibodies.

HOST AND VIROLOGIC FACTORS THAT INFLUENCE THE COURSE OF HIV-1 INFECTION

The events that result in the establishment of chronic HIV-1 infection emphasize the ability of the virus to target the host's antiviral immune response and reshape it into a self-defense mechanism. In this context, the mutual interactions between the virus and the host are major determinants of disease progression. Primary HIV-1 infection is a key phase in the immunopathogenesis of the infection, because all of the events that occur at this stage can determine both the pattern and rate of progression of the disease. In the transition from primary to early chronic HIV-1 infection, levels of plasma viral

TABLE 120-1 -- Host factors and virologic factors in HIV-1 infection.

HOST FACTORS AND VIROLOGIC FACTORS IN HIV-1 INFECTION		
Genetic host factors	**Immunologic host factors**	**Virologic factors**
HLA class I haplotype	Qualitative differences in the primary immune response	Extent of HIV-1 replication
Mutations in chemokine receptor or ligand genes:	Clonal deletion of HIV-1-specific cytotoxic T cells	Viral phenotype (syncytium-inducing or non-syncytium-inducing strains of HIV-1)
Ø32 in CCR5		
m303 in CCR5		
V641 in CCR2b	Persistence of HIV-1-specific $CD4^+$	Trapping of virions in follicular dendritic cell network
G801A in SDF	T cell responses	
Levels of ß-chemokines (RANTES, MIP-1a and MIP-1ß)	Chronic immune activation	Viral latency
		Size of inoculum

RNA tend to reach a virologic set point that is predictive of the rate of disease progression (see below).[44] [45] The virologic set point varies among HIV-1-infected patients and tends to remain stable in the same person during the chronic phase. The virologic set point that a person attains is determined both by the mechanisms involved in the establishment of chronic infection and by host factors that can modulate the course of HIV-1 disease.

Several factors play an important role in modulating both the antiviral host immune response and the susceptibility to HIV-1, and these factors can thus result in a

slower rate of disease progression. These factors are mainly genetic, immunologic and virologic (Table 120.1).[10] [26]

Genetic host factors

Patients infected with HIV-1 who experience a nonprogressive disease are more likely to possess certain HLA class I haplotypes than other members of the general population. Clusters of haplotypes rather than a single haplotype are involved; these haplotypes are more efficient than others at binding peptides that correspond to epitopes recognized by virus-specific CTLs. This suggests that persons who have inherited these clusters of haplotypes could generate HIV-1-specific CTL responses against multiple epitopes, thus resulting in a more efficient antiviral response, as a consequence of their HLA genetic background.[42]

The recent identification and study of the function of several a- and ß-chemokine receptors that can serve as HIV-1 co-receptors have provided evidence of varying chemokine receptor genetic polymorphisms that can influence susceptibility to HIV-1 infection and are associated with different rates of disease progression. Chemokines can be classified into two subfamilies, namely CXC or a-chemokines and CC or ß-chemokines, both of whose receptor molecules are part of the vast and functionally diverse family of seven-transmembrane G protein-coupled receptors. CXCR4, the first identified co-receptor, mediates T-cell-tropic viral fusion and entry (X4 HIV-1 strains), but it does not function as a co-receptor for the macrophage-tropic HIV-1 envelope. The major co-receptor for macrophage-tropic HIV-1 strains is CCR5, and other CC chemokine receptors, such as CCR2b, CCR3 and CCR8, can serve as co-receptors for R5 or dual-tropic (i.e. primary HIV-1 isolates) HIV-1 strains.[46] [47] [48] [49] [50] [51]

Some HIV-1-negative persons, despite being at high risk of exposure, appear to have an innate ability to 'resist' HIV-1 infection. Mapping of the CCR5 structural gene on the human chromosome 3p21 allowed the identification of a 32-base-pair deletion allele (?32CCR5) that encodes a truncated and nonfunctional molecule that fails to reach the cell surface. As a result, cells susceptible to HIV-1 are highly resistant to infection by R5 strains. Available prevalence estimates indicate that between 15% and 20% of Caucasians are heterozygous for the mutation, whereas 1% or fewer are homozygous.[52] The

study of large cohorts of HIV-1-negative persons, including those who have had multiple exposures to HIV-1, and HIV-1-infected patients has provided evidence that the homozygous genotype for the mutation (i.e. ?32CCR5-?32CCR5) confers high resistance to infection by HIV-1,[52] although a few HIV-1-infected patients possess the ?32CCR5-?32CCR5 homozygous genotype. However, the heterozygous genotype (CCR5-?32CCR5) does not prevent HIV-1 from infecting susceptible cells but is significantly associated with a slower rate of disease progression in HIV-1-infected patients and is found more commonly in long-term nonprogressors. [53] In heterozygotes there is a decreased expression of the CCR5 receptor, which can be explained by a transdominant inhibition of wild-type CCR5 receptor function owing to the concomitant intracellular presence of both normal and defective gene products withheld in the endoplasmic reticulum.[54] Another mutation (m303) in the *CCR5* gene prevents the expression of the receptor on the cell surface by introducing a premature stop codon. This mutation, when in *trans* with the ?32CCR5 defective gene, confers resistance because no expression of CCR5 receptor occurs. [55]

Furthermore, other genetic variants do not prevent infection but rather delay the progression of HIV-1 disease. However, their protective effect has not been confirmed in all studies, emphasizing the need to evaluate their role in larger cohorts. These genetic variants include a mutant CCR2b allele (V64I), which encodes a base mutation that replaces valine with isoleucine at position 64 in the transmembrane domain I of the CCR2b receptor;[56] [57] [58] [59] [60] and a guanosine-to-adenosine transition at position 801 (G801A) in the 3' untranslated region of the reference sequence in the gene of the stromal-derived factor (SDF)-1,[61] [62] which is the ligand for CXCR4.

Epidemiologically, the inheritance of the CCR2b mutant is less beneficial than the inheritance of the ?32CCR5 mutant in terms of reducing the risk of progression to AIDS; however, as in the case of CCR5-?32CCR5 heterozygosity, the V64I mutant is more commonly found in long-term nonprogressors.[58] Biologically, it is not clear how the V64I allele could interfere with the co-receptor function, because valine and isoleucine are chemically very similar. It is possible that the V64I allele is in linkage disequilibrium with other mutations,[56] [57] [59] as it has been described with a point mutation in the CCR5 regulatory region.[59]

As far as the SDF-1 G801A mutant allele is concerned, available data are somewhat puzzling. On the one hand, the status of homozygosity for the mutation has been associated with a lower risk of progression to AIDS.[61] A possible explanation for such a protective effect is that the presence of mutant alleles induces a higher than usual release of SDF-1, which inhibits X4 HIV-1 strains. X4 strains tend to emerge during the late phase of the disease and their appearance is generally associated with a more rapid progression. The SDF-1 at high levels could therefore interfere with the spreading of X4 HIV-1 strains, thus reducing the rate of disease progression. Homozygosity for the SDF-1 mutant has been linked to a higher risk of death, although the potential explanation is unclear.[62]

The initial studies that discovered the role of chemokine receptors as HIV-1 co-receptors originated from the observation that chemokines can potently modulate HIV-1 infectivity.[63] The ß-chemokines that are 'regulated upon activation, normal T expressed and secreted' (RANTES) — macrophage inflammatory protein (MIP)-1a and MIP-1ß — have been identified as major HIV-suppressive factors produced by $CD8^+$ T cells in vitro. Furthermore, endogenous levels of RANTES, MIP-1a and MIP-1ß expression in $CD4^+$ T cells were much elevated in some people who remained uninfected despite multiple sexual exposures to HIV-1-infected partners.[64] The binding of one chemokine to its cognate receptor essentially mediates the downregulation of surface chemokine receptor expression. Therefore, in addition to genetic control, chemokine levels themselves may influence infectivity and disease progression. In particular, there is evidence that very high levels of RANTES, MIP-1a and MIP-1ß are detectable in persons bearing the ?32CCR5-?32CCR5 homozygous genotype. [64] Similarly, unusually high levels of ß-chemokines have been found in people with hemophilia who have remained uninfected with HIV-1 despite repeated exposure to contaminated blood products before HIV-1 testing became available.[65] Furthermore, levels of MIP-1ß (which is the only suppressive CCR5-specific chemokine) in those who have overt AIDS can be significantly lower than those in HIV-1-infected patients who have chronic disease; moreover, higher levels of MIP-1ß are associated with a lower risk of disease progression.[66]

These observations suggest that suppressive ß-chemokines may play a role in the control of HIV-1. Production of chemokines by effector $CD4^+$ T cells occurs at the site of virus replication, and the chemokines may protect local target cells and activated effector cells by downregulating CCR5 in an autocrine manner. The varying extents to which these mechanisms are put in motion may explain the varying levels of protection against disease progression.

In summary, host genetic factors do play a role in modulating the course of HIV-1 infection. Furthermore, the potential role of chemokines in affecting infectivity and disease progression may further widen our therapeutic options along with HAART.

Immunologic factors

Importance of HIV-1-specific $CD4^+$ T cells

$CD4^+$ T cells play a fundamental role in the generation of antigen-specific immune responses. Studies performed in mice have clearly demonstrated that stimulation and expansion of antigen-specific $CD4^+$ T cells precedes that of $CD8^+$ T cells during acute virus infection in vivo.[67] Even although $CD4^+$ T cells do not seem to be critical for the generation of the primary virus-specific $CD8^+$ T cell response, the presence of virus-specific $CD4^+$ T cells is required for the maintenance of the $CD8^+$ T cell response over time during the phase of chronic infection.[68] [69]

The progressive depletion of $CD4^+$ T cells is the hallmark of HIV-1 infection.[70] However, even in the early stages of HIV-1 infection when the $CD4^+$ T cell count may still remain in the normal range, certain HIV-1-specific $CD4^+$ T cell functions, such as the ability to proliferate after stimulation with different virus protein, are already absent in the majority (80–90%) of HIV-1-infected patients.[71] [72] The antigen-specific proliferation ability is also known as T helper function. Therefore, the defect of HIV-1-specific $CD4^+$ Th cells occurs very early during HIV-1 infection. Despite the lack of virus-specific $CD4^+$ helper responses, HIV-1-specific $CD4^+$ T cells have been identified even in patients who have progressive disease on the basis of their ability to secrete interferon (IFN)-? following short (6h) stimulation with HIV-1 antigens. [38]

The discordance between the lack of HIV-1-specific CD4 proliferation and the detection of HIV-1-specific IFN-?-secreting $CD4^+$ T cells can be explained by the stimulation of different populations of antigen-specific $CD4^+$ T cells in the two assays. Stimulation of precursors of antigen-specific memory T cells occurs in the 6 days proliferation assay while the short-term (6h) stimulation IFN-? flow cytometry assay probably detects functionally differentiated antigen-specific T cells.

The hypothesis above is supported by a series of recent studies that have shed light on memory $CD4^+$ and $CD8^+$ T cells.[73] [74] These studies have shown that different populations of memory T cells can be distinguished upon the expression of different cell surface markers such as CD45RA and the chemokine receptor CCR7. Interestingly, these different populations of memory T cells are at different stages of differentiation and have different functions. Therefore, memory $CD4^+$ T cells with the proliferation function are contained within the precursor cell populations that are at earlier stages of differentiation while

memory T cells at late stages of differentiation acquire effector functions and are able to secrete proinflammatory cytokines such as IFN-? and tumor necrosis factor a. Therefore, in HIV-1 infection there is a selective defect of HIV-1-specific $CD4^+$ helper T cells while HIV-specific $CD4^+$ T cells secreting IFN-? are still present.[35] [38] [75]

How is it possible to explain the persistence of HIV-specific memory $CD4^+$ T cells secreting IFN-? in the absence of the memory cells, (e.g. T helper cells) that function as precursors? The likely explanation is that HIV-1-specific memory $CD4^+$ T cells secreting IFN-? belong to the population of long-lived memory T cells that may persist for several years even in the absence of the replenishment by the precursor cell populations.

$CD4^+$ T cell responses against other viruses such as CMV are detected in most HIV-1-infected patients but they are eventually lost in the advanced stages of disease.[76] In this regard, it is important to underscore the fact that both CMV-specific memory CD4 helper and effector T cell responses are consistently detected. Therefore, the defect of virus-specific memory $CD4^+$ T cells seems to be selective for HIV-1-specific and no for other virus-specific $CD4^+$ helper T cell responses. This suggests that the elimination of HIV-1-specific helper T cells does not occur randomly. It is likely that already at the time of primary HIV-1 infection, when there is massive virus replication and spreading and thus high antigen levels, the pool of precursors of memory HIV-1-specific T cells is rapidly eliminated in the process of responding to the infection.

In addition to the large number of studies in the field of basic immunology and in human virus infections such as CMV and hepatitis C virus,[76] [77] [78] there are also several observations in HIV-1 infection that link the presence of optimal $CD4^+$ T cell responses to lack of progression of HIV-1 disease and better control of virus replication. As mentioned above, a small percentage (1%) of HIV-1-infected patients (i.e. long-term nonprogressors) experience no signs of disease progression, no decline in $CD4^+$ T cell counts and low/absent levels of virus replication, even several (10–15) years after infection. HIV-1-specific $CD4^+$ helper and effector T cell responses are consistently found in long-term nonprogressers. Therefore, it is important to underscore that the detection of HIV-1-specific helper and effector T cell responses represents a constant feature of the effective immune response found in long-term nonprogressors.

Importance of HIV-1-specific $CD8^+$ T cells

There are at least three major lines of evidence for the central role played by CTLs in controlling HIV-1 replication. First, primary HIV-1 infection is associated with a very potent CTL response that generally coincides with the peak in viremia and precedes the neutralizing antibody response.[3] [4] [5] This response is associated with major oligoclonal expansion of HIV-1-specific $CD8^+$ T cells. In some patients a large percentage (up to 40%) of circulating $CD8^+$ T cells are specific for HIV-1.[5] The advent of CTL response is temporally associated with the downregulation of viremia.[4] [5] This observation indicates a role for CTLs, which are mostly contained in the T cell population characterized by the expression of the CD8 surface molecule, in the initial control of virus replication. However, direct demonstration of the important role played by $CD8^+$ CTLs comes from the SIV monkey model of HIV-1 infection. In fact, it has been demonstrated that the depletion of $CD8^+$ cells following infusion with a specific anti-CD8 monoclonal antibody resulted in a failure to control the early peak of viremia in the infected animals.[79] Along the same lines, the depletion of $CD8^+$ CTLs in SIV chronically infected monkeys was associated with transient rises in the viremia levels[80] and loss of immune control occurs following the emergence of virus mutants in vivo that are not recognized by CTLs.[81] [82] [83] [84] [85] [86]

Second, it has been shown that the HLA type may significantly influence the rate of HIV-1 disease progression.[87] In particular, HLA types such as HLA-B27 and HLA-B57 are associated with slow disease progression and HLA-B35 with faster disease progression. Since CTLs recognize virus peptides presented by HLA class I molecules, different HLA types may have different ability to present peptides and thus substantially influence the quality of the immune response elicited.

Third, HIV-1-specific $CD8^+$ T cell responses have been found in virus-exposed uninfected individuals[88] and/or animals.[89] [90] Therefore, the above observations, together with a large quantity of recently accumulated experimental evidence, indicate that $CD8^+$ T cells may potentially play a major role in the control of HIV-1 replication, and the development of a preventive HIV-1 vaccine based on the induction of CD8 CTLs may potentially confer partial and/or complete control of HIV-1 infection.

However, despite all this evidence in support of the fundamental role that $CD8^+$ T cells may exert in the control of HIV-1 replication and disease, this vigorous immune response is not able to eliminate HIV-1 at the time of primary infection and the control of HIV-1 replication after the transition to the chronic phase of infection is only partial. The partially effective control of HIV-1 replication does not fit very well with the magnitude of the HIV-1-specific immune response detected at the time of primary infection and its persistence for several years.[91] Therefore, the observations above indicate that the inability of the HIV-1-specific $CD8^+$ T cell immune response to control virus replication cannot be explained by a defect in the magnitude of the immune response. Even although it has been shown that certain HIV-1-specific $CD8^+$ T cell clones are rapidly deleted during primary infection,[44] this event does not seem to influence significantly the magnitude of the $CD8^+$ T cell response.

These latter observations indicate that the detection of large frequencies of HIV-specific CTLs does not necessarily reflect the effectiveness of the immune response in the control of HIV infection. $CD8^+$ T cells may mediate antiviral activity by the production of soluble factors such as the cytokine IFN-?,[92] [93] [94] the chemokines MIP-1a, MIP-1ß and RANTES,[95] [96] [97] and the partially characterized $CD8^+$ T cell antiviral factor CAF,[98] [99] and/or by lytic mechanisms. HIV-1-specific CTLs are able to produce the above antiviral factors. However, recent studies have strongly suggested that the HIV-1-specific $CD8^+$ T cells present in HIV-1-infected patients may have major abnormalities at both functional and maturational levels. In this regard, it has been shown that HIV-1-specific CTLs have a selective defect in their levels of intracellular perforin that may significantly affect their lytic capacity.[100] Therefore, although HIV-1-specific CTLs have been shown to lyse HIV-1-infected $CD4^+$ T cells following activation in vitro,[101] freshly isolated virus-specific T cells show poor lytic activity.[102] [103]

Furthermore, recent studies have also demonstrated that the pool of memory T cells is composed of several populations of memory $CD8^+$ T cells with different functional capacities and at different stages of maturation. [73] [74] Studies aimed at the functional characterization of the different populations of memory HIV-1-and CMV-specific $CD8^+$ T cells have allowed the development of a lineage differentiation pattern for memory $CD8^+$ T cells and have demonstrated major differences within the composition of the HIV-1- and CMV-specific memory $CD8^+$ T cell pools.[74] The HIV-1-specific memory $CD8^+$ T cell pool is predominantly composed of pre-terminally differentiated $CD8^+$ T cells, as compared with the CMV-specific memory $CD8^+$ T cell pool, which is mostly composed of terminally differentiated $CD8^+$ T cells.[74] The differences in the maturation between HIV-1-specific and CMV-specific $CD8^+$ T cells are probably associated with a different lytic capacity, since CMV-but not HIV-1-specific $CD8^+$ T cells have normal intracellular levels of perforin. [100] More importantly, the different composition in the

pool of memory $CD8^+$ T cells seems to translate into a different efficacy in the control of the two virus infections: effective control of CMV infection versus poor control of HIV-1 infection and progressive disease. Taken together, these observations indicate that the detection of large number of antigen-specific $CD8^+$ T cells by tetramer staining as well as a high frequency of IFN-? secreting cells is not necessarily an indicator of an effective immune response.

Virologic factors

As discussed above, interaction among different host factors helps to determine a certain virologic set point in each HIV-1-infected person after the transition from primary to chronic HIV-1 infection.[44] [45] This set point represents the level of plasma viral RNA that accurately predicts disease progression (i.e. the level of plasma viremia that corresponds to a risk of progression to either AIDS or death). In the past, many predictors have been identified, clinical, biologic and virologic. The $CD4^+$ T cell count is historically the most important and widely used predictor. However, the load of plasma RNA is nowadays the most accurate predictor, especially when used along with the $CD4^+$ T cell count.[45] Therefore, the higher the plasma viral RNA load, the greater the risk of rapid progression to AIDS and death. It is worth noting that the power of association between levels of plasma HIV-1 RNA and risk of progression does not significantly vary if viremia is measured after seroconversion (i.e. knowing the date and duration of HIV-1 infection) or during the established chronic asymptomatic phase (i.e. with no available information about the duration of the infection, as is often the case in clinical settings). [44] Viral load is therefore a very powerful predictor if measured during the chronic asymptomatic phase, once the virologic set point has been reached.

However, the level of plasma viral RNA lacks accuracy and reliability as a predictor if measured during primary HIV-1 infection.[104] In this phase, rapid disease progression is predicted by:

Figure 120-5 Three-phase decay model of virus replication. Viremia below 50 HIV-1 RNA copies/ml plasma does not correspond to complete suppression of virus replication. Residual viremia (5–10 HIV-1 RNA copies/ml plasma) may still be detected 48 weeks after initiation of HAART. However, it is still unclear whether HAART induces complete suppression of virus replication and, if it does, what duration of HAART is needed to achieve this goal.

- a retroviral syndrome lasting more than 14 days;
- the number and the intensity of clinical signs and symptoms;
- central nervous system involvement; and
- viral phenotype.

The viral phenotype is an important virologic factor that can contribute to the rate of disease progression. R5 HIV-1 strains are non-syncytium-inducing strains, whereas X4 HIV-1 strains are syncytium-inducing strains. Syncytium-inducing strains tend to emerge during the late phase of the disease and a shift in viral phenotype from non-syncytium-inducing strains to syncytium-inducing strains heralds disease progression. The viral phenotype of the non-syncytium-inducing strains is associated with prolonged AIDS-free survival and is more commonly found in long-term nonprogressors.[105]

ERADICATION OF HIV-1 INFECTION

An understanding of the immunologic and virologic events that occur during primary HIV-1 infection and that lead to the establishment of the chronic phase has been achieved. Also, the mechanisms that HIV-1 has evolved in order to escape the immune response, have been elucidated. These advances have challenged the most widely accepted theories about the feasibility of HIV eradication.

Decay of HIV-1-infected compartments after highly active antiretroviral combination therapy

The recent development of HAART has permitted the study of the decay of the different HIV-infected compartments after effective suppression of virus replication and, thus, the estimation of the turnover of both virions and cells supporting virus replication. These studies[106] [107] [108] have proposed a two-phase decay model of viral load (Fig. 120.5). By assessing the decay of plasma viral RNA after HAART, it has been estimated that about 10^{10} –10^{11} virions are produced

daily. Two factors determine the decay and the inclination of the slope of plasma viremia after HAART:

- the extent of clearance of virions, and
- the decrease in the number of cells actively producing virus as a result of the inhibition of new rounds of infection.

By means of mathematical models, it has been determined that free virions are eliminated with a half-life of 6 hours, whereas productively infected cells have a half-life of 1.6 days.[107] [108] [109] The combination of these two events explains the first rapid phase of decay of plasma viremia. The nature of this phenomenon is clarified by the mechanisms leading to productive HIV-1 infection. As discussed above, although HIV-1 can target different populations of cells — $CD4^+$ T cells, monocytes and macrophages, dendritic cells and others — virus replication is mostly supported by activated $CD4^+$ T cells with the memory phenotype. The preferential replication in $CD4^+$ T cells can be explained by the physiologic differences and by the differential anatomic distribution of the various cells targeted by HIV. No efficient virus replication can occur without activation of the target cell. Although HIV-1 is able to infect resting $CD4^+$ T cells, proviral DNA is not integrated and thus no active virus replication is achieved. The state of target cell activation is therefore a fundamental requirement for HIV-1 to replicate efficiently.

$CD4^+$ T cell activation may be caused either by antigen-specific stimulation or by the physiologic activation of the small number of cycling $CD4^+$ T cells. About 1% of $CD4^+$ and $CD8^+$ T cells are proliferating at any given time in healthy people. Monocytes, macrophages and dendritic cells turn over at a much lower rate than do T cells, and their activation is probably limited to sites of inflammation. Therefore, the activated pool of proliferating $CD4^+$ T cells can support efficient replication of HIV-1, whereas other cell types, although being susceptible to HIV-1 infection, are probably responsible for a negligible amount of virus production. In addition, cells that continuously recirculate from lymph nodes to the bloodstream are almost exclusively $CD4^+$ T cells. These observations explain not only why $CD4^+$ T cells are responsible for between 98% and 99% of the total virus produced, but also the fact that the use of HAART, which efficiently inhibits the virus so that it cannot complete its replication cycle, causes a rapid and steep decrease in the plasma viral load (first phase of decay).[108] [109]

The first phase of decay of plasma viremia is then followed by a much slower reduction of the levels of HIV-1 RNA in plasma (second phase of decay). Tissue macrophages and long-lived, latently infected T cells could support the residual virus replication, havinga half-life of 1–4 weeks and 0.5–2 weeks, respectively. More likely, HIV-1 virions are released from the deposits trapped in the follicular dendritic cell network in the germinal centers of the lymphoid tissues. In support of this hypothesis, HAART efficiently depletes these deposits over a period of 6–12 months.[110] Consistently, this second phase of decay is flatter and tends to last much longer than the first one.

On the basis of this two-phase decay model, eradication of HIV-1 was hypothesized as being achievable in a relatively short time — between 2 and 3 years.[109] However, this goal could be realized only if a complete suppression of virus replication had been sustained and if there were no pool of long-lived, latently infected cells to serve as a virus reservoir. [27] [28] [29] [30] [31]

The results obtained from HIV-1-infected patients after long-term HAART (2–3 years of treatment) have significantly challenged these theories. In this regard, a pool of latently HIV-1-infected resting memory $CD4^+$ T cells can be detected in HIV-1-infected patients who have adhered to HAART for up to 3 years.[27] [28] [29] [30] [31] This pool possesses a longer half-life than the original estimate of 1–4 weeks and is composed of quiescent memory $CD4^+$ T cells. More importantly, these cells contain replication-competent proviral DNA, and after appropriate activation they are able to support efficient viral replication.

Although the estimated extent of this pool is quantitatively limited, ranging between 50,000 and 5,000,000 resting memory $CD4^+$ T cells for the whole body,[27] [28] [29] [30] [31] this pool of cells has been estimated to have a half-life of 3–6 months,[109] which corresponds approximately to the half-life of uninfected resting memory $CD4^+$ T cells, thus dramatically changing the estimates of the potential time required for HIV-1 eradication.

As mentioned above, this pool probably originates from productively infected cells at the time of the primary HIV-1 infection. It is noteworthy that initiation of HAART as early as 10 days after the onset of symptoms of primary HIV-1 infection does not prevent the generation of this pool, despite the successful control of plasma viremia shortly after initiation of HAART. This emphasizes the rapidity with which viral reservoirs are established after initial infection, and HAART is probably not able to interfere with this immunopathogenic process. It is, therefore, clear that this pool of cells represents a major obstacle in the attempt to eradicate HIV-1 completely in infected people.

The immunopathogenic role of long-lived resting memory $CD4^+$ T cells in HIV-1 infection may reside not only in their function as a reservoir but also in the fact that they may possess the ability to support virus production in vivo, albeit at low levels.[111] Results from several clinical trials show that viral load decreases below detectable levels (i.e. below either 400 copies or 50 copies of HIV-1 RNA/ml plasma) in the majority of HIV-1-infected persons taking HAART for over 6 months. However, the recent availability of more sensitive tests that can detect viremia down to 5 copies of HIV-1 RNA/ml plasma[112] has significantly changed the interpretation of such results, indicating that viral load below 50 copies does not necessarily correspond to complete suppression of viral replication. Between 40% and 60% of patients have levels of viremia that are lower than 50 but higher than 5 HIV-1 RNA copies/ml plasma after 36–48 weeks of HAART. This indicates that the use of ultrasensitive tests measuring plasma viremia allows both detection and quantification of residual virus production.

These observations complicate the situation, in that persistence of very low residual levels of virus replication may be another mechanism through which HIV-1 is able to maintain itself in the host by renewing the pool of cells that can serve as a reservoir. In fact, long-lived infected $CD4^+$ T cells may be responsible for supporting the residual viral replication in vivo. The rapid rebound of plasma viremia to pretherapy levels that occurs within 2–4 weeks of stopping long-term HAART is consistent with this detection of viral persistence. [111]

However, another potential source of residual virus replication may be sanctuaries for the virus — cells and organs where the virus can be sheltered or where HAART does not achieve therapeutic concentrations of drug (Table 120.2). Tissue sanctuaries for the virus include the lymphoid tissue, the mucosa-associated lymphoid

tissue, the genital organs and the central nervous system, where the achievable

TABLE 120-2 -- Tissue and cellular sanctuaries for HIV-1.

TISSUE AND CELLULAR SANCTUARIES FOR HIV-1	
Tissue sanctuaries	**Cellular sanctuaries**
Lymphoid organs	Resting memory $CD4^+$ T cells
Mucosal-associated lymphoid tissue	Macrophages
Central nervous system	Microglial cells
Cerebrospinal fluid	Langerhans cells
Genital organs	

concentration of antiviral drugs, in particular of protease inhibitors, may be suboptimal. These sites may serve both as a potential source of low-level virus replication and as a reservoir of latently HIV-1-infected cells. Latently infected resting $CD4^+$ memory T cells, macrophages, microglial cells, dendritic cells and Langerhans cells may conceivably be cellular sanctuaries for HIV-1.

It appears, then, that cells with a prolonged turnover and a decreased extent of activation, which reduces the susceptibility of HAART, and structures where the bioavailability of antiviral drugs is limited further complicate the issue of HIV-1 eradication.

These observations may indicate the existence of residual virus replication (viremia), whose decay is difficult to evaluate, based on the limited sensitivity of the available laboratory assays.[111] Furthermore, it is difficult to envisage to what extent the population of long-lived latently HIV-infected resting $CD4^+$ T cells may affect the putative time needed to achieve eradication of HIV-1, because no conclusive data are available on the half-life of these cells. However, the time to eradicate HIV-1 has now been set up to 5–10 years, considering a half-life of 4 months for these cells and provided that effective and durable suppression of viral replication is achieved by HAART.

Production of $CD4^+$ T cells after highly active antiretroviral combination therapy

The issue of HIV-1 eradication is also linked to the extent of immune restoration that can be achieved with the prolonged use of HAART. In this context, the study of the kinetics of proliferating $CD4^+$ T cells carried out in both peripheral blood and lymph nodes of HIV-1-infected persons at an early stage of infection has yielded important insights into the extent of HAART-induced restoration of immunity.[113]

Estimates of the production of $CD4^+$ T cells can be obtained by assessing the proportion of cells that are positive for the Ki67 antigen, which is a nuclear antigen associated with proliferation. Using this approach, it is possible to define three phases in the kinetics of production of $CD4^+$ T cells after HAART. Shortly after the initiation of HAART, a rapid increase in peripheral $CD4^+$ T cell counts can be observed, which is not associated with an increase of proliferating $CD4^+$ T cells. The rise in the counts of $CD4^+$ T cells may be associated with a redistribution of T cells from lymphoid compartments, and therefore this first phase may be termed the 'phase of redistribution'. After this phase, there is a progressive and significant increase in the number of proliferating $CD4^+$ T cells. This occurs between 12 and 48 weeks of HAART. During this phase, the 'phase of production', a significant restoration in the $CD4^+$ T cell count occurs. Interestingly, in HIV-1-infected patients who are receiving HAART and who have peripheral $CD4^+$ T cell counts that return to normal levels and remain stable, the phase of production is replaced with a 'plateau phase', as the fraction of proliferating $CD4^+$ T cells tends either to stabilize or to decrease after 36–48 weeks of HAART.

These observations emphasize the fact that the regenerative capacity of $CD4^+$ T cells is present and functional during the early chronic phase of HIV-1 infection. Furthermore, the institution of HAART may play a crucial role in driving the restoration of $CD4^+$ T cells to normal levels.

HIGHLY ACTIVE ANTIRETROVIRAL COMBINATION THERAPY COMBINED WITH IMMUNE-BASED STRATEGIES

There are some important limitations associated with the use of HAART. First, eradication of HIV-1 after HAART cannot be achieved in the originally estimated time (2–3 years) and it probably cannot be eradicated in less than 50–60 years. As discussed above, the T cell reservoir of HIV-1, which is established very early in the natural history of the disease; the residual virus production that persists despite prolonged, effective HAART; and the presence of sanctuaries for the virus constitute major obstacles for the long-term control and eradication of the virus. At the same time, these facts emphasize that simply waiting for the natural extinction of long-lived, latently infected cells may not be a good option because it is unrealistic to maintain HAART over the estimated prolonged period of time needed to achieve HIV-1 eradication.

Second, the questions of when to initiate HAART during established chronic HIV-1 infection and which drugs to use in combination as first-choice therapy are important ones, owing to the observed virologic failure that occurs in about half the HIV-infected persons who take HAART.[114] [115] [116] Virologic failure, however, is more likely in persons who have previously received suboptimal antiviral therapy, such as monotherapy or dual therapy with reverse transcriptase inhibitors; moreover, virologic failure depends on both the clinical stage of HIV-1 disease and the adherence to treatment. On the other hand, the use of HAART in antiviral-therapy-naive asymptomatic HIV-1-infected persons at an early stage of the disease (i.e. when the $CD4^+$ T cell count is 300–400 cells/ml or more) is rarely associated with virologic failure, provided there is good adherence to therapy. Adherence is obviously related to the acceptability of antiviral combination therapy in terms of both daily number and schedule of pills and to drug toxicity. The acceptability of protease inhibitor-sparing regimens[117] is likely to be superior to that of protease inhibitor-containing regimens. However, the long-term virologic and immunologic responses to the former need to be investigated further.

Third, drug toxicity is a fundamental issue. Particular attention has recently been focused on the long-term effects of HAART on metabolism, which include glucose intolerance and altered levels of lipids and fat distribution;[118] however, from an intention-to-treat viewpoint, the benefits that HAART can provide outweigh the drug-toxicity-related risks.

Taken together, these observations emphasize that HAART used alone is unlikely to eradicate HIV-1 infection. However, HAART is undoubtedly the cornerstone of HIV-1 therapy, because it is able to induce effective and durable suppression of virus replication, to block disease progression and to provide some restoration of the immune response despite the presence of residual virus replication. Highly active antiretroviral combination therapy is at present the best available option to prepare the necessary background (i.e. the best achievable control of virus replication and the greatest possible immune restoration) for subsequent immune-based therapies aimed at long-term control or eradication of HIV-1.

There are several possible objectives of immune-based therapies to achieve both the induction of immune-mediated control of HIV-1 and the elimination of cellular sanctuaries for the virus (see Chapter 140).[111] These objectives include:

- the maintenance or enhancement of existing HIV-1-specific immune responses and the restoration or strengthening of nonspecific immune responses; therapies with interleukin-2 may be the best tool for achieving these goals, because they can improve $CD4^+$ helper T cell function and cell-mediated immunity;
- the induction of HIV-1-specific immune responses *de novo*, using therapeutic vaccine strategies with HIV-1-specific antigens; and
- contributing to the clearance of the pool of latently HIV-1-infected cells — strategies aimed at inducing massive activation of memory $CD4^+$ T cells are being developed because activating these cells may reactivate replication of latent HIV-1 and thus cause virus-mediated killing of the target cell, or virus eradication by HAART.

It is, however, worth noting that the accomplishment of these strategies must take into account both the time of initiation of HAART and the stage of the disease, because different immune-based therapies and the type of immune restoration vary according to these factors. Before the introduction of immune-based therapies it is necessary to suppress virus replication efficiently with HAART. Finally, the stage of HIV-1 disease may warrant different rationales of immune-based intervention.[118]

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Chapter 121 - Virology of HIV

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This chapter reviews the life cycle and genetic structure of HIV-1 in the context of understanding how viral and cellular regulatory factors may affect viral replication. Potential as well as current targets of antiviral chemotherapy are discussed. The synthesis of viral proteins and the manner in which viral assembly takes place is also considered.

GENERAL DESCRIPTION OF HIV-1

The virion

The HIV-1 virion forms an icosahedral sphere with projections consisting of the envelope (Env) glycoproteins gp120 and gp41. Gp120 is loosely and noncovalently associated with gp41 and the latter transverses the lipid bilayer. Figure 121.1 is a schematic representation of the mature HIV-1 virion. Under the lipid layer, the matrix (MA) protein (p17) covers the internal surface of the viral coat. The capsid (CA) protein (p24) constitutes the shell of the cone-shaped core, and the nucleocapsid (NC) protein (p7) forms part of a nucleoid structure that also consists of reverse transcriptase (RT), integrase (IN), and two copies of the single-stranded viral genomic RNA.[1]

Genomic organization of HIV-1

The HIV-1 provirus (i.e. the DNA form of the viral nucleic acid) is about 9.5kb in length and contains long terminal repeats (LTRs, 634bp) at each of the 5' and 3' ends.[2] Figure 121.2 is a schematic description of the HIV-1 genome and the known functions of its gene products. The LTRs consist of the U3, R, and U5 regions within which exist *cis*-acting elements essential for viral integration and transcription. RNA synthesis is initiated within the 5'-LTR at the junction between the U3 and R regions. HIV-1 harbors three structural genes (i.e. *gag*, *pol* and *env*) and six regulatory genes (i.e. *vif*, *vpr*, *tat*, *rev*, *vpu* and *nef*). The structural genes code for polyprotein precursors that are involved in virion construction. The open reading frames for the regulatory genes are positioned in the central portion of the genome and flank the *env* gene. The Vpr, Vif as well as Nef proteins are packaged into mature virions.[3] [4] [5]

Gag and Gag-Pol proteins

Pr55Gag and its products

The open reading frame of the *gag* gene (1536 nucleotides) is translated directly into a 55kDa Gag precursor (Pr55Gag; Fig. 121.3). This polyprotein is further cleaved by the viral protease (PR) to yield mature proteins, including MA, CA, NC and p6.[6] Although processing of Pr55 can be detected in the cytoplasm, it is generally believed that this event takes place mainly on the membrane of the host cell or inside the released viral particle. Processing of the precursor is accompanied by morphologic rearrangement of virus particles, and this can be visualized by electron microscopy. Notably, cleavage between p24 and p2 at the late stage of virus morphogenesis allows the formation of the cone-shaped core structure.[7] The final products include:

- p17 — MA protein, which comes from the N terminus of Pr55 and is myristylated at its N terminus;
- p24 — CA protein, which is derived from the central part of Pr55 and forms the cone-shaped core;
- p7 — NC protein, which is highly basic and tightly associated with viral genomic RNA;
- p6, which is part of the core structure; and
- p2 and p1 — spacer peptides, which regulate Pr55 processing.

The MA protein directs the intracellular transport and membrane association of the Gag polyprotein.[8] As part of the preintegration complex, MA is also critical for transporting the complex into the nucleus by virtue of a nuclear localization signal (NLS) at its N-terminus; this process is essential for productive infection of non-dividing cells and may be responsible for recruiting viral envelope proteins to the surface of host cells.[9]

The CA forms the core of the mature virion. It is believed that proteolytic liberation of Pro1 (i.e. the first amino acid in CA) allows the formation of a salt bridge between Pro1 and Asp51, which in turn triggers conformational rearrangement of CA and consequently the formation of the core structure.[10] CA contains a major homology region (MHR) at its C-terminus that plays crucial and yet ill-characterized roles in Gag aggregation. CA recruits the cellular factor cyclophilin A (Cyp A) into virus particles; Cyp A is needed for uncoating after virus entry into the host cells.[11]

NC serves as the interaction domain that mediates Gag-Gag interactions. This protein is highly basic and possesses two copies of zinc finger motifs (CCHC). NC binds to nucleic acid sequences and further modifies their structures to thermostable states; thus, this protein is defined as a nucleic acid chaperone. Multiple functional roles of NC protein have been identified and these are:[12]

- to stimulate reverse transcription;
- to assist in viral genomic RNA packaging; and
- to promote dimerization of viral RNA.

The protein p6 contains the late domain (P(T/S)AP) that is needed for successful budding of virus particles from the plasma membrane. p6 is also responsible for the incorporation of Vpr into virus particles.

Certain drugs have been identified that may antagonize the zinc finger regions of the NC proteins. These compounds may conceivably be tested in future clinical trials.

Pr160 and its products

The open reading frame of the *pol* gene (3045 nucleotides) is translated only as a Gag-Pol fusion protein, Pr160, by a translational frameshift mechanism as ribosomes read full-length genomic HIV-1 transcripts. In mature virions, the *gag* and *pol* gene products are found in a ratio of about 20:1 (see Fig. 121.3).[13] The Pol precursor is cleaved to produce PR (p10), RT (p66/51) and IN (p32).[14]

- PR is responsible for processing the Gag and Gag-Pol precursors — mutations in the catalytic region of PR are lethal to the virus;
- RT is responsible for catalyzing the conversion of viral RNA into DNA (reverse transcription); and
- IN plays a key role in inserting viral DNA into the host cell chromosome.

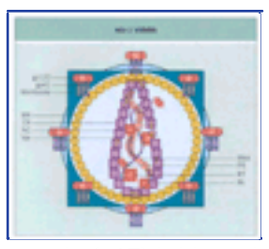

Figure 121-1 HIV-1 virion. The glycoprotein gp120 constitutes the outer envelope of the virus and is noncovalently linked to the transmembrane protein gp41. The matrix protein (p17) bridges the envelope protein with the cone-shaped structure formed by the capsid protein (p24). The viral genomic RNA and processed nucleocapsid (NC; p7) and Pol proteins, reverse transcriptase (RT) and integrase (IN), are located inside the capsid core. PR, protease.

To date, viral RT and PR have been the principal targets of antiviral chemotherapy. Viral IN represents another obvious target for such efforts, and development of drugs that target IN constitutes an important area of research.

Figure 121-2 Genetic organization of HIV-1 and known functions of gene products. The structural genes are *gag, gag-pol* and *env*. Catalytic proteins are encoded by the *pol* gene. Regulatory proteins are translated from fully spliced mRNA. Within the HIV-1 genome, there are additional open reading frames that flank the *env* gene and encode several regulatory proteins including Vif, Vpr, Tat, Rev, Vpu and Nef. LTR, long terminal repeat; PIC, pre-integration complex consisting of viral cDNA, IN, RT, Vpr, MA and NC.

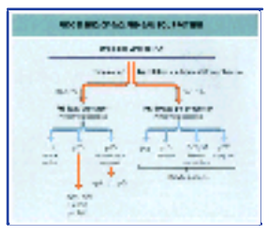

Figure 121-3 Processing of Gag and Gag-Pol proteins. The Gag proteins are initially translated as a 55kDa polyprecursor. Proteolytic processing of Pr55Gag generates several mature products. The catalytic proteins, including reverse transcriptase (RT), integrase (IN) and protease (PR), are first produced as a Gag-Pol precursor (Pr160Gag-Pol) through a translational frameshift mechanism; Pr160Gag-Pol is then cleaved to the smaller enzymatically active subunits.

The *env* gene and its products

The *env* gene is translated into a polyprotein of 160kDa. This precursor protein is folded and further glycosylated in the endoplasmic reticulum (ER) and Golgi apparatus before being transported

to the plasma membrane. A signal peptide (about 30 amino acid residues) at the N terminus of gp160 is removed and a subsequent proteolytic step in the Golgi compartment, mediated by a cellular enzyme, yields the N-terminal gp120 and the C-terminal gp41. [1] Although the external viral protein gp120 plays a key role in interacting with the CD4 receptor and/or co-receptor of susceptible cells,[15] the transmembrane protein gp41 is responsible for anchoring gp120 through noncovalent interactions and mediating the fusion process between viruses and target cells. Peptide inhibitors have been developed that specifically bind to gp41 and thus block virus entry.[16]

HIV-1 regulatory proteins

Tat protein, or viral transactivator

Tat belongs to a novel class of eukaryotic regulatory proteins that exert their effects on transcription through binding to RNA motifs. This protein is primarily located in the nucleus and nucleoli of infected cells and binds to a stem-loop structure, termed TAR (Tat-associated RNA), at the 5' end of nascent RNA (position 1–57). Interaction of Tat and RNA may:

- increase the stability of the RNA polymerase to allow more efficient synthesis of full-length transcripts;
- increase the frequency of RNA initiation; and
- increase the efficiency of translation of TAR-containing RNA.

Tat plays its roles in transcription activation through recruitment of cellular factors to the transcription complex that is assembled along the LTR promoter. Tat directly interacts with cellular factor cyclin T1 and the ternary complex thus assembled, together with TAR RNA, further recruits cyclin-T-dependent kinase (CDK)9, which, in turn, phosphorylates the C-terminal domain (CTD) of RNA polymerase II.[17] Tat can also regulate HIV-1 reverse transcription.[18] [19] In addition, Tat exerts a variety of effects on cell growth and proliferation and is likely to play a significant role in selective depletion of infected cells by triggering apoptosis.

Rev protein (regulator of virion protein expression)

Rev has a profound effect on the fate of primary RNA transcripts within the nucleus. Like Tat, Rev is located primarily in the nucleus and nucleoli of infected cells. The Rev protein binds to a complex stem-loop structure, the Rev-responsive region (RRE), within the HIV-1 *env* gene. The RRE is present in both full-length and singly spliced viral RNAs but is excluded by splicing from multiply spliced viral mRNAs. Consequently, in the absence of Rev, only multiply spliced RNAs, which encode regulatory proteins, are transported into the cytoplasm. In the presence of Rev, both unspliced and singly spliced viral mRNAs (for viral structural and catalytic proteins) are found in the cytoplasm. Rev shuttles between the nucleus and the cytoplasm. This activity is mediated by interactions between its nuclear export signal (NES) or NLS with cellular factors exportin-1 and importin-ß, respectively.[20] Although Rev has been found to be phosphorylated on serine residues, such modification is apparently not required for viral replication. Efforts are ongoing to develop drugs that can antagonize the function of Tat and Rev.

Vif, or the virion infectivity gene

Vif is made from a singly spliced mRNA that accumulates late in infection. Although Vif is dispensable for viral replication in some immortalized cell clones, it is required in peripheral blood mononuclear cells. This suggests that it may play an important role in infected hosts.[21] Vif is associated with viral RNA and may play a role in virus assembly. Because only traces of this protein are found in virions (amounts comparable with those of Pol proteins), its effects are assumed to be indirect.

Vpu protein

Vpu is made from the same singly spliced mRNA as the envelope glycoprotein. At least two functional roles have been identified:

- downmodulation of CD4 by stimulating degradation of CD4 in the ER; and
- enhancement of virion release by a yet to be defined mechanism.

Vpr regulatory protein

Vpr is packaged into mature virus particles through interactions with p6. It appears to be important in assisting the transport of the pre-integration complex from the cytoplasm into the nucleus. Vpr is also able to arrest infected cells at the G2 phase.

Nef protein

Nef is encoded by the extreme 3' end of the viral genome and accumulates even earlier than Tat and Rev in newly infected cells. Recent evidence suggests that Nef is involved in modulating CD4 expression by triggering the rapid endocytosis and lysosomal degradation of this main virus receptor. The Nef protein plays a major role in activation of quiescent T cells and thus facilitates viral replication.[22]

HIV-1 REPLICATION CYCLE

Entry of HIV-1

A schematic description of the HIV-1 life cycle is presented in Figure 121.4 . HIV-1 uses the CD4 receptor (a 58kDa transmembrane protein) to mediate initial attachment to cells through high-affinity interactions between the viral envelope glycoprotein (gp120) and a specific region of the CD4 molecule.[21] On the surface of both immature T cells and mature $CD4^+$ T helper cells, CD4 is present in abundance. It is present at lower concentrations on monocytes, macrophages and antigen-presenting dendritic cells.

A variety of co-receptors have now been identified on lymphocytes and monocytes that promote the entry of HIV-1 into target cells after the initial binding step between CD4 and viral gp120.[15] Two major co-receptors are CCR5 and CXCR4. While cells of monocyte/macrophage origin generally express only the former, many

lymphocyte populations can express both types of co-receptor. These differences in co-receptor expression help to explain why:

- HIV-1 variants may be either lymphocyte-tropic or macrophage-tropic, or both; and
- some viruses may be able to cause lymphocytes to fuse together into giant cells.

Certain host cell membrane proteins may also promote virus entry. One example is a C-type lectin (DC-SIGN) that is highly expressed on dendritic cells and binds to HIV-1 envelope glycoprotein gp120 (see Chapter 120).[23]

Efforts are underway to develop compounds that antagonize the entry of HIV-1 into susceptible cells by interfering with either the CD4 receptor or the various co-receptors on cells of different origins. Cell entry probably occurs by a fusion of viral and cell membranes, mediated by the viral transmembrane protein (gp41). Following fusion, the virion is uncoated by a proteolytic event that is most probably mediated by the virion-encoded protease.

Reverse transcription

After viral entry, the viral RNA is converted into DNA, which is then integrated into host cell chromosomal DNA. Reverse transcription initiates from a cellular $tRNA^{Lys.3}$ that is bound to a viral RNA fragment termed the primer binding site (PBS). This is followed by the first strand transfer, the priming of plus-strand DNA synthesis from the polypurine tract (PPT) and the second-strand transfer. The process of reverse transcription usually occurs within 4–6 hours of infection, takes place mainly in the cytoplasm

Figure 121-4 The HIV-1 life cycle. The diagram shows the various stages involved, including entry, uncoating, reverse transcription, integration, expression of proviral genome, viral assembly and particle release.

and is catalyzed by virion-encoded RT. Reverse transcriptase has at least three enzymatic functions:

- RNA-dependent DNA polymerase activity that copies viral RNA into viral cDNA;
- DNA-dependent DNA polymerase activity that copies (-) strand viral cDNA into (+) strand viral cDNA; and
- ribonuclease H activity (RNase H) that degrades the viral RNA template during synthesis of viral cDNA.

The final products of reverse transcription are double-stranded (ds) DNA molecules that are longer at each end than the viral RNA as a result of duplication of the LTR. It is likely that cellular factors are required for the completion of reverse transcription, as most reverse transcribed products in unstimulated quiescent peripheral blood lymphocytes consist of incomplete viral DNAs.[24] The nature of the host cell factors or cellular activation pathways involved is unknown.

Integration

As stated above, reverse transcription products are mainly generated in the cytoplasm of infected cells. These dsDNAs are then transported into the nucleus of the cell, where viral DNA is integrated into host cell chromosomal DNA. The pre-integration complex of HIV-1 consists of IN, RT, MA, NC, Vpr and reverse-transcribed DNA.

The integration reaction is catalyzed by the virion-encoded IN, which is found in the viral particle. After reverse transcription of genomic viral RNA, IN remains associated with viral DNA as a high-molecular-weight nucleoprotein pre-integration complex. The IN first removes two nucleotides from the 3' end of viral DNA and then cleaves target host DNA. This is followed by insertion of viral DNA into host cell DNA. The 5' gaps flanking the provirus as well as the two nonpaired nucleotides are presumably repaired or removed by a cellular enzyme. The final products (provirus) are flanked by 5-basepair (bp) direct target duplications, which have lost 2bp from each end. Once integrated, viral DNA remains permanently associated with the host genetic material for as long as the cell is alive.

Viral gene expression

HIV-1 exploits the host cell transcription and translation machineries to generate its own gene products. The primary RNA transcripts of the provirus are made by host cell RNA polymerase II. Cellular activation and proliferation signals result in the binding of transcription factors to the LTR and lead to increased rates of initiation of transcription. Tat and Rev are two key virion-encoded proteins that positively regulate viral gene expression and replication, whereas the accessory proteins, including Nef, Vif, Vpu and Vpr, are crucial determinants of HIV virulence. [25] The primary viral RNA transcripts are either transported into the cytoplasm to direct Gag/Gag-Pol synthesis and to serve as genomic RNA for packaging or are spliced to generate around 30 different species of RNAs for the production of additional viral proteins. Host cellular ribosomes translate proviral mRNA into viral proteins in either cap-dependent or cap-independent modes. The cap-independent mechanism involves an internal ribosomal entry site (IRES) present at the 5' end of viral RNA. All viral structural proteins are made as polyproteins. Regulatory proteins are made by translation of spliced mRNA.

Packaging and assembly

Gag proteins are the driving force for virus assembly. Three functional domains have been characterized within Gag; these are the membrane binding domain (M domain), interaction domain (I domain) and late budding domain (L domain). The M domain is located at the N terminus of MA, the I domain involves NC sequences, and the L domain consists of a P(T/S)AP sequence within p6. The Gag proteins play a central role in recruiting both viral proteins and host-cell-derived elements into mature viral particles. Env proteins are recruited into virus particles through interactions between MA and gp41. Two copies of full-length viral RNA are incorporated into each virus particle through interactions of their 5' end RNA sequences with Gag precursors. The replication primer $tRNA^{Lys.3}$ is most probably selected by the viral Gag-Pol protein, together with cellular enzyme $tRNA^{Lys.3}$ synthetase.[26] The L domain recruits a cellular factor, tumor susceptibility gene (TSG)101, to the virus assembly site on the plasma membrane and facilitates the 'pinch-off' of virus particles.[27] Virus budding may require ubiqitination of Gag proteins and cellular factors involved in protein transportation. HIV-1 particles assemble at microdomains of plasma membrane that are enriched in sphingolipids and cholesterol. HIV-1 also recruits other cellular factors, such as Cyp A, Staufen, translation elongation factor 1a and actin, that may assist to assemble infectious virus particles.

THERAPEUTIC CONSIDERATIONS

As stated above, the major targets of anti-HIV chemotherapy have been the viral RT and PR enzymes. The RT of HIV-1 is responsible for copying viral RNA into DNA. However, this enzyme has a high error rate (i.e. about one mutation/virus replication event). This means that mutants are constantly being generated that have the potential for drug resistance, which may be selected under treatment. Drug resistance to HIV will occur when these mutations result in altered forms of the viral RT and PR proteins that can still function yet are no longer efficiently inhibited by antiviral nucleoside and non-nucleoside inhibitors of RT and antagonists of the HIV PR enzyme. Since HIV-1 uses cellular machineries to complete its life cycle, it follows that reasonably conserved cellular components might also be potential targets for development of anti-HIV-1 compounds. The subject of HIV drug resistance is dealt with in detail elsewhere (see Chapter 137).

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Chapter 122 - Primary HIV Infection

Bernard Hirschel

INTRODUCTION

Although primary HIV infection (PHI) is a rarely diagnosed, self-limiting disease,[1] it is a topic of considerable interest, since the first encounter of HIV with the immune system sheds light on many aspects of pathogenesis. The severity of PHI predicts progression to immunodeficiency years later; therefore, it is reasonable to hope that treatment of PHI prevents or retards AIDS. From a public health perspective, a diagnosis of PHI is important because such patients are highly infectious;[2] [3] to miss a diagnosis of PHI is to miss an opportunity for prevention.[4]

Experience shows that the diagnosis is not difficult to make; education is the key.

EPIDEMIOLOGY

Primary HIV infection is often asymptomatic but sometimes it presents with spectacular manifestations requiring hospital admission. There is a spectrum between complete absence of symptoms during the time of seroconversion and severe disease; therefore, it is not surprising that opinions vary about the percentage of patients who have symptomatic PHI. A physician's previous experience with PHI and a high index of suspicion greatly increase the number of diagnoses. Retrospective analysis from the US armed forces showed that 33% of patients suffered from an identifiable disease between their last seronegative and first seropositive serum sample. At the other extreme, in Australia,[5] 93% of seroconverting persons reported having been 'sick' compared with 40% of controls; 12% of seroconverting patients were hospitalized.

It is not known what factors determine the severity of PHI. Theoretically, the size of the inoculum, the virulence of the infecting HIV strain (including such factors as cellular tropism and cytopathogenicity), and the patient's immune status could be involved, but evidence as to whether these factors are important is lacking. One case series of transfusion-associated cases found that symptomatic PHI was more frequent among those infected by people who had late-stage disease. There is little evidence that the frequency or severity of PHI differs between transmission categories or between men and women. Symptomatic PHI can occur with HIV-2 infection and in children, although almost all cases have been reported in adults infected with HIV-1. There are theoretic reasons to believe that co-infection with other viruses, particularly from the herpes group, might enhance the proliferation of HIV, and patients who are simultaneously co-infected with cytomegalovirus have had particularly severe symptoms.

Symptoms typically start 2–4 weeks after infection, with extremes of 5 and more than 90 days. The median duration of symptoms is difficult to quantify and is between 12 and 28 days.[6] Moderate and subjective symptoms such as fatigue may persist for months, although almost all patients eventually enter an asymptomatic phase lasting years.

PATHOGENESIS AND PATHOLOGY

Because PHI most often presents as a benign self-limiting disease, pathologic information is only available from easily biopsied tissues.

The skin rash is caused by a dermal perivascular lymphohistiocytic infiltrate around vessels of the superficial dermis; the epidermis is normal. The inflammatory cells are predominantly of the $CD4^+$ phenotype, and may represent a T-cell-mediated reaction to HIV and to the p24 antigen, which can be detected in the Langerhans cells.

Lymph node biopsies reveal abundant HIV, including the envelope proteins gp120 and gp160 in dendritic reticulum cells, as well as in lymphocytes. The structure of the germinal centers is relatively normal and quite unlike the follicular hyperplasia of established HIV-1 infection, but extrafollicular B lymphocytes are reduced in number and the follicles are infiltrated by $CD8^+$ T cells. [7]

Therapy has a pronounced effect on the quantity of HIV detectable in lymph nodes. There is a lag of more than 6 months between disappearance of the virus from plasma and disappearance from lymph nodes. However, even patients who are aviremic for several months while treated for PHI and whose lymph node biopsies are apparently free from HIV relapse after discontinuing medication, emphasizing the role of a virus reservoir such as memory T cells.[8]

CLINICAL FEATURES

During PHI, HIV floods the blood,[9] the central nervous system (CNS)[10] and the lymphatic system, and invades a number of other tissues. Therefore, it is not surprising that PHI is a disease with protean manifestations. Three main presentations have been described.

Cutaneous presentation

This is characterized by a maculopapular rash, 'roseola' and mucosal ulcerations (Fig. 122.1 , Fig. 122.2 , Fig. 122.3 , Fig. 122.4). The rash affects the face, neck and trunk more than the limbs, although the palms and soles may be involved. Individual lesions are usually less than 1cm in diameter and confluence is rare. Case reports have mentioned pustular eruptions, urticaria, erythema multiforme and, during the healing phase of PHI, alopecia and desquamation. Ulceration may occur on the genital and oral mucosa, including the esophagus, where differentiation from herpetic lesions or esophageal candidiasis is difficult.

Presentation resembling infectious mononucleosis

This is characterized by fever, pharyngitis, arthralgia, myalgia and lymphadenopathy. Although the expression 'mononucleosis-like illness' is firmly established, there are many differences from classic infectious mononucleosis, most notably the lack of prominent tonsillar involvement. In a large series (Table 122.1), only 20% of patients had a fever in combination with sore throat and enlarged cervical lymph nodes, whereas 10% did not have fever, sore throat or cervical lymphadenopathy.

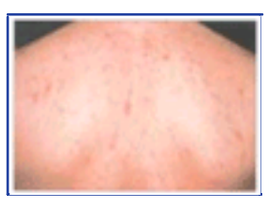

Figure 122-1 Maculopapular rash during primary HIV infection.

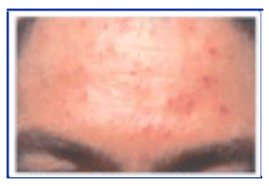

Figure 122-2 Acneiform lesions during primary HIV infection.

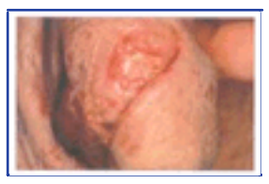

Figure 122-3 Penile ulcer during primary HIV infection.

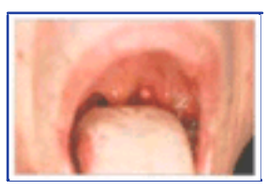

Figure 122-4 Mucosal ulcerations during primary HIV infection.

Meningoencephalitis

Meningoencephalitis is characterized by photophobia and neck stiffness, headaches and disordered consciousness. The headache is typically retro-orbital and exacerbated by eye movements. Depression and changes in mood are frequent and may reflect underlying encephalitis.

Other symptoms

Table 122.1 shows the frequencies of signs and recorded symptoms in the medical charts of more than 200 patients from Switzerland and Australia. Digestive manifestations have not been well recognized in the past, but they are quite common. In exceptional cases, esophageal candidiasis (an AIDS-defining disease) may occur with a transient decline in $CD4^+$ lymphocyte count.

TABLE 122-1 -- Signs and symptoms of primary HIV infection.

SIGNS AND SYMPTOMS OF PRIMARY HIV INFECTION		
	Symptom/sign	**%**
Reported by more than 50%	Fever	77
	Lethargy/fatigue	66
	Rash	56
	Myalgia	55
	Headache	51
Reported by 20–50%	Pharyngitis	44
	Cervical adenopathy	39
	Arthralgia	31
	Oral ulcer	29
	Pain on swallowing	28
	Axillary adenopathy	24
	Weight loss	24
	Nausea	24
	Diarrhea	23
	Night sweats	22
	Cough	22
	Anorexia	22
Reported by 5–20%	Abdominal pain	19
	Oral candidiasis	17
	Vomiting	12
	Photophobia	12
	Meningitis	12
	Genital ulcer	7
	Tonsillitis	7
	Depression	7
	Dizziness	6
These are the signs and symptoms reported by at least 5% of patients.[1] [2]		

Apart from these major groups of symptoms and signs, many unusual manifestations have been described during PHI, most notably:

- ¦ neurologic syndromes such as radiculopathy, peripheral facial neuropathy and Guillain-Barré syndrome, and severe encephalitis with prolonged coma and seizures; and
- ¦ pulmonary involvement, which may be more frequent in intravenous drug users where PHI can be associated with bacterial pneumonia; severe pneumonitis leading to intubation and *Pneumocystis carinii* pneumonia [11] (with $CD4^+$ lymphocyte counts of less than $100/mm^3$) is exceptional.

Differential diagnosis

Important differential diagnoses are listed in Table 122.2 ; PHI must be distinguished from Epstein-Barr virus infection (infectious mononucleosis) and enterovirus meningitis, and according to the local epidemiologic context, typhoid fever, rickettsial infections and many others.

DIAGNOSIS

Seroconversion (i.e. the appearance of HIV antibodies in the serum) occurs days after the beginning of the symptoms of PHI. Therefore, the usual antibody tests for HIV are not entirely reliable; they are expected to be negative during the first few days of PHI (Fig. 122.5). Assays differ in the duration of this 'seronegative period';

with the currently used sensitive tests it is usually less than 1 week.

The p24 antigen is positive when the antibody test is still negative during PHI, and the same is true of HIV viremia. Whereas both tests can be used to screen for PHI, the p24 antigen test is considerably cheaper.[12] Viremia levels reach extremely high values, often in excess of 10^6 viral genomes/ml[13] and high titers of infectious virus have

TABLE 122-2 -- Important differential diagnoses.

IMPORTANT DIFFERENTIAL DIAGNOSES		
Clinical feature	**Epstein-Barr virus infection**	**HIV infection**
Onset	Gradual	Abrupt
Tonsil involvement	+++	+
Pharyngeal exudate	+++	-
Rash	Rare except in patients treated with antibiotics	Frequent
Jaundice	10%	Never
Diarrhea	Rare	25%
Clinical feature	**Syphilis**	**HIV infection**
Serology	Always positive	At first negative
Chancre: timing	Before roseola	At the same time as rash
Chancre: pain	Painless	Painful
Clinical feature	**Enterovirus meningitis**	**HIV meningitis**
Population	Young adults	Young adults
Diarrhea	Rare	23%
Season	Summer–autumn	None in particular
Duration	<8 days	Often >20 days
Encephalitis	None	Frequent
Skin lesions	Rare	Frequent
Pain on swallowing	None	Frequent

Figure 122-5 Successive Western blots during primary HIV infection. Note that on September 30, 1986, when the patient presented with fever, rash, meningitis and subclinical hepatitis, the screening enzyme-linked immunosorbent assay (ELISA) test for HIV antibodies was negative, while the Western blot showed only a single weak band corresponding to the p24 antigen. CSF, cerebrospinal fluid; ASAT, aspartate transaminase; ALAT, alanine transaminase; H, hepatitis (A or B); ND, not done.

been isolated from many tissues, including seminal fluid, corroborating the epidemiologic evidence that patients who have PHI are highly infectious. Viremia decreases rapidly — at least 100-fold within days after seroconversion — but remains detectable in more than 95% of patients. Steady-state plasma viremia levels predict progression to advanced immunodeficiency and death.[14] Levels tend to remain higher in those who have symptomatic PHI.[15]

The occurrence of HIV infection without the presence of antibodies for many months ('seronegative HIV infection') has caused much controversy, fueled by conflicting results and the extreme sensitivity of the polymerase chain reaction, which makes it vulnerable

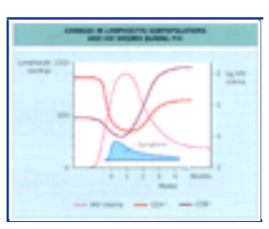

Figure 122-6 Changes in lymphocyte subpopulations and HIV viremia during primary HIV infection.

to contamination and false-positive results. Although patients with chronic seronegative infections may rarely occur, there is no evidence that these individuals are infective, and no patients with symptomatic PHI who have subsequently failed to seroconvert have been described. However, a seronegative patient who is in the process of seroconverting can transmit HIV, for instance by blood transfusion. The results of plasma viremia and p24 antigen tests as well as the clinical history and exposure must guide the interpretation of a negative serologic result.

Like viremia levels, lymphocyte subsets undergo rapid changes during PHI (Fig. 122.6). During the first 5–10 days, lymphopenia characteristically affects both $CD4^+$ and $CD8^+$ lymphocytes, with levels[11] that may be as low as those observed in AIDS. Although

opportunistic infections are rare, in-vitro tests of both B and T cells show immunosuppression. Within another 2–3 weeks there is a lymphocytosis. The $CD8^+$ lymphocyte count expands more than the $CD4^+$ lymphocyte count, leading to a $CD4^+$ /$CD8^+$ lymphocyte ratio of less than 1. This low ratio persists even in patients whose $CD4^+$ lymphocyte count subsequently rises to normal.

Many other laboratory values may be abnormal during PHI, reflecting the acute inflammatory response (e.g. high erythrocyte sedimentation rate, increase in C-reactive protein) and involvement of the bone marrow (thrombocytopenia), the liver (increase in hepatic transaminases) and the CNS (pleocytosis of the cerebrospinal fluid).

MANAGEMENT

Although PHI can be severe and prolonged, it is a self-limiting disease; the symptoms eventually abate and the patient becomes asymptomatic. Years later, immunosuppression may appear and AIDS may develop.

Features of primary HIV infection that may predict the subsequent course toward AIDS

A considerable body of evidence suggests that more severe and more prolonged PHI indicates a more unfavorable course toward AIDS.[16] [17] [18] For instance, in patients who were followed after seroconversion, 58% of those who had had symptomatic PHI had developed AIDS 7 years later compared with 28% of those who had asymptomatic PHI.[17] Another study suggested that the presence of neurologic signs at the time of PHI predicted accelerated immunosuppression,[18] although there was no specific relation to the neurologic signs of AIDS, such as AIDS-related dementia or opportunistic infections of the CNS.

Does treatment of primary HIV infection improve the long-term outcome?

This is a logical question that remains unanswered. Comparative studies between a treated group and an untreated control group face considerable practical obstacles, including the need for a large sample, extremely long follow-up and ethical issues.

Primary HIV infection is a self-limiting disease, usually of only slight or moderate severity. The balance has shifted back and forth between advocates and opponents of treatment.

Arguments in favor are:

- the association of symptomatic PHI with a worse prognosis;
- the limited heterogeneity of the viral population,[19] which should theoretically diminish the probability of emergence of resistance to antiviral drugs;
- the potential impact on infectivity and transmission;[2]
- the generalization of the HIV infection during PHI with invasion of the CNS[10] and lymphoid tissues; and
- the availability of more effective and better tolerated antiviral drugs. [20]

After pilot studies suggested that zidovudine was well tolerated and possibly effective in PHI, a prospective randomized trial in 77 patients compared placebo and zidovudine given for 6 months.[6] An effect on $CD4^+$ lymphocytes and a statistically significant decrease in minor opportunistic infections in patients treated with zidovudine were demonstrated.

Several uncontrolled trials have been conducted in PHI with combinations of antiviral drugs:

- with highly active antiretroviral treatment, including two inhibitors of reverse transcriptase and an inhibitor of the HIV protease, viremia decreases to undetectable levels in practically all compliant patients;[21] [22]
- progression to AIDS is less than in historical control groups;[23]
- the $CD4^+$ /$CD8^+$ lymphocyte ratio normalizes; and
- lymph node biopsies of some of the patients who have persistent suppression of viremia show disappearance of virus, and proviral DNA decays faster than in patients who start treatment during chronic HIV infection.[21] [24]

However, the hope of viral eradication remains unfulfilled, as discussed below.

Opponents of antiviral treatment for primary HIV infection point to the lack of studies showing clinical benefits, the high incidence of side effects, expense and problems with compliance. Some 6 months or so after having started treatment during PHI, patients often have high $CD4^+$ counts and would not otherwise qualify for antiretroviral treatment. Should they stop?

A small case series showed that viral load rebound after stopping was universal. However, even without treatment, viral loads often fell again to levels between 50 and 5000 copies/ml, possibly because of a vigorous immune response triggered by re-exposure to the virus. Many patients have now ceased therapy for more than 1 year and have done well.[25]

The present recommendations are to treat symptomatic primary infection as soon as diagnosed, particularly in patients who have a viral load greater than 100,000 copies/ml and a $CD4^+$ count less than 500 cells/µl. The optimal regimen would include one protease inhibitor (or a non-nucleoside reverse transcriptase inhibitor) and two nucleoside reverse transcriptase inhibitors. In certain communities, genotypic testing for resistance may be indicated because of a high prevalence of primary drug resistance (chapter 137). After a year or so of therapy, a trial of treatment interruption would appear reasonable.

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Chapter 123 - Prevention of Opportunistic Infections

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Stéphane de Wit

INTRODUCTION

One of the major clinical advances in the management of patients who have HIV infection has been the implementation in the mid-1980s of antimicrobial prophylaxis for patients with severe immune impairment. Together with the use of antiretroviral drugs, this has led to decreased morbidity and improved survival in patients with AIDS.

Such prophylaxis requires regular measurements of $CD4^+$ lymphocyte counts and compliance on the part of the patient, who must take many pills each day for the rest of his/her life. There are also issues of tolerance, drug interactions, emergence of resistance and cost.

Since the extensive use in Western countries of highly active antiretroviral therapy (HAART), a further marked decrease in the occurrence of AIDS-related opportunistic infections has been noted and the question of continuing or stopping prophylactic regimens has been raised in patients who have $CD4^+$ counts increasing beyond the critical level of 200 lymphocytes/mm^3 .[1] Figure 123.1 summarizes the evolution of the incidence of most opportunistic infections among a cohort of 2000 patients followed at CHU Saint-Pierre hospital in Brussels.

Although prophylactic regimens against most of the opportunistic infections that occur in patients who have HIV infection exist, the decision to use prophylaxis should consider factors such as:

- the incidence and prevalence of specific infections in HIV infected individuals;
- the potential severity of disease in terms of morbidity and mortality;
- the level of immunosuppression at which each disease is likely to occur;
- the feasibility and efficacy of preventive measures, and in particular their impact on quality of life and survival;
- the potential for emergence of organisms resistant to the agents used for prophylaxis;
- the risk of toxicities and drug interactions with antiretrovirals and other drugs used by HIV-infected patients;
- the issue of compliance; and
- the cost-effectiveness of prophylaxis.

INCIDENCE AND PREVALENCE OF OPPORTUNISTIC INFECTIONS

There is a wide geographic variability in the epidemiology of opportunistic infections. The probability of developing a given disease depends on the risk for exposure to potential pathogens, the virulence of the pathogens and the level of immunosuppression of the patient.

In the USA and Northern Europe, *Pneumocystis carinii* pneumonia (PCP), oropharyngeal or esophageal candidiasis, cytomegalovirus disease and infections caused by *Mycobacterium avium* complex (MAC) are common. The incidence of toxoplasmosis and tuberculosis is higher in central and southern Europe and in the developing countries, depending on the prevalence of latent infection in the general population. This geographic heterogenicity has important implications for prophylaxis. In the case of low incidence, prophylactic measures should be targeted to high-risk patients such as those who have a positive antitoxoplasma serology, a positive polymerase chain reaction for cytomegalovirus or a positive cutaneous tuberculin test for tuberculosis. In addition, for tuberculosis, epidemiologic assessment of risk should be used for some high-risk populations (intravenous drug users, migrants from an endemic area).

LEVEL OF IMMUNOSUPPRESSION

Blood $CD4^+$ lymphocyte levels is the best marker for immune status. It has been clearly established that the number of circulating $CD4^+$ lymphocytes is closely correlated with the risk of developing several opportunistic infections (Fig. 123.2). Once the $CD4^+$ lymphocyte count falls below 200 cells/µl, the cumulative risk for developing an AIDS-defining opportunistic infection is 33% by year 1 and 58% by 2 years. Therefore, $CD4^+$ lymphocyte counts remain an important parameter for monitoring patients who have HIV infection because of their predictive value for both opportunistic infections and mortality.

EFFICACY OF PROPHYLACTIC MEASURES AND IMPACT ON SURVIVAL

The survival benefit of prophylaxis for opportunistic infections has been demonstrated in a number of studies, particularly in patients who have severe immunosuppression. In the early 1980s, before the widespread use of antiretroviral therapy, it was demonstrated that trimethoprim-sulfamethoxazole (TMP-SMX) use for prevention of PCP did significantly prolong survival. A similar impact has been shown with MAC prophylaxis with clarithromycin or rifabutin.

It is clear that a prophylactic regimen that prolongs survival should be used in priority to one that does not. In this setting, the use of fluconazole for prophylaxis of systemic mycoses and oral ganciclovir for cytomegalovirus disease have failed to demonstrate a clear survival benefit and are not widely recommended.

EMERGENCE OF DRUG RESISTANCE

The development of resistance to the most commonly used agents is one of the major concerns with the use of long-term antimicrobial prophylaxis in patients who have HIV infection.

Resistance or cross-resistance has become increasingly common with prophylaxis for MAC, fungal infections and PCP. The major consequence is that, when the specific drugs are needed to treat acute infections, resistance hinders their use and alternative less effective or more toxic agents are the only option. [2]

This issue was well illustrated with clarithromycin as prophylaxis for MAC. Despite receiving prophylaxis, 58% of the patients developing MAC had clarithromycin-resistant isolates.[3]

Figure 123-1 Incidence of opportunistic infections among patients who have HIV infection. CHU Saint-Pierre, 1985–2001.

Figure 123-2 Association between opportunistic infections and CD4$^+$ cell count.

Prophylactic treatment of non-life-threatening infections, such as oral thrush with fluconazole, has probably contributed to the emergence and spreading of azole-resistant fungi.

Prophylactic regimens may also lead to the development of cross-resistance against more common pathogens. For example, rifabutin used for MAC prophylaxis may result in the emergence of rifampin-resistant strains of *M. tuberculosis*.[4] Likewise, the widespread use of broad-spectrum antibiotics, such as clarithromycin, azithromycin or TMP-SMX, may lead to the development of resistance among organisms such as pneumococci that were not the primary targets of prophylaxis. An increasing prevalence of *Streptococcus pneumoniae* resistant to TMP-SMX, for example, could decrease the effectiveness of this agent in preventing community-acquired pneumonia in advanced HIV patients and limit therapeutic options for treating common outpatient illnesses such as respiratory, skin and soft tissue infections.[2]

DRUG TOXICITY AND DRUG INTERACTIONS

Drug toxicity can be a major factor limiting the usefulness of widely used agents. The incidence of adverse drug reactions in patients who have HIV infection varies with the type of drug and dosages used, the interactions between drugs and the stage of HIV infection.[5]

HIV-related idiosyncratic factors, organ dysfunction in late stage disease and multiple drug therapy are the primary reasons for the increased risk of drug toxicities in HIV patients.

The issue of drug interactions has become particularly critical in the era of protease inhibitors. However, although the number of potential drug interactions is substantial, few require dosage modifications. Among these, clinicians should be vigilant when using concomitantly prophylaxis and treatment of mycobacterial diseases with rifamycin and macrolides and HIV protease inhibitors.

COMPLIANCE ISSUES

The use of combination therapy for prophylaxis against multiple opportunistic pathogens significantly increases the complexity of treatment of HIV patients. In particular, it may dramatically increase the number of pills that are necessary in combination antiretroviral regimens. Such increasingly complex regimens may lead to patient noncompliance and inability to tolerate other therapeutic regimens, inducing antiretrovirals.

COST-EFFECTIVENESS

To be most cost-effective prophylaxis should be directed at the most common infections in the patient population. However, an expensive prophylactic regimen may be cost-effective if it has a positive impact on quality and duration of life or if it reduces other costs. This is particularly true in severely immunocompromised HIV patients, in whom any intervention, even costly prophylaxis, that significantly reduces the incidence of hospitalization will have a favorable impact on the costs of caring. Studies have shown that prophylaxis for PCP and MAC is cost-effective, whereas prophylaxis for fungal disease and cytomegalovirus is not.[6]

DURATION OF PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS

Since HAART was introduced, it has become clear that prophylaxis against opportunistic infections need not necessarily be life long. As mentioned above, susceptibility to opportunistic infections can accurately be assessed by the CD4$^+$ T cell count.

It is thus logical to stop primary or secondary prophylaxis in patients whose immunity has improved as a consequence of HAART.

Data generated until now support this approach and recommendations concerning the safety of stopping primary or secondary prophylaxis can now be made for many pathogens.

By contrast, no data are available regarding the re-initiation of prophylaxis when the CD4$^+$ lymphocyte count decreases again to levels at which the risk for opportunistic infections exists. In particular, it is unknown whether it is better to use the threshold at which prophylaxis was stopped or the threshold below which initial prophylaxis is recommended.[7]

RECOMMENDATIONS FOR PROPHYLAXIS AGAINST OPPORTUNISTIC INFECTIONS

The US Public Health Services and the Infectious Diseases Society of America have established disease-specific recommendations for the prevention of opportunistic infections in individuals who have HIV infection, which were updated in November 2001. These recommendations include guidelines for preventing exposure to pathogens as well as on specific regimens for preventing initial episodes.[8]

Category I regimens are strongly recommended as standard of care. Category II should be strongly considered in eligible patients. Category III regimens are not routinely recommended but may be considered for use in selected patients (Table 123.1).

Recommendations on prophylactic regimens to prevent recurrence of opportunistic infections have also been updated (Table 123.2).

DISEASE-SPECIFIC CONSIDERATIONS

Pneumocystis carinii

Prophylaxis of PCP has been shown to be highly cost-effective.

A meta-analysis of 35 studies of PCP prophylaxis in 6583 patients showed that TMP-SMX was superior to dapsone or aerosolized pentamidine but there was no statistically significant survival advantage for TMP-SMX versus alternative agents. An advantage of TMP-SMX over alternative drugs is a significant reduction in bacterial infections. Side-effects are sufficiently severe to require discontinuation of the drug in 25–50% of TMP-SMX recipients compared to 25–40% of dapsone recipients and 2–4% of recipients of aerosolized pentamidine. There is good evidence that lower doses of TMP-SMX are better tolerated, and many advocate either the use of the lower dose regimens using one single-strength tablet daily or one double-strength tablet thrice weekly. Patients who have adverse reactions to TMP-SMX usually tolerate dapsone.

Primary and secondary prophylaxis against PCP should be discontinued in patients treated with HAART who show an increase in CD4$^+$ T cells to above 200/mm^3 for at least 3 months. Prophylaxis should be reintroduced if the CD4$^+$ T cell count decreases to less than 200/mm^3 .[9] [10]

Toxoplasma gondii

Toxoplasma seropositive patients who have a CD4$^+$ T cell count below 100/mm^3 or who have had a previous episode of toxoplasmic encephalitis should receive prophylaxis against toxoplasmic encephalitis.

The double-strength tablet daily dose of TMP-SMX is recommended. Alternative regimens in patients who cannot tolerate TMP-SMX include dapsone-pyrimethamine or atovaquone (with or without pyrimethamine). Prophylactic monotherapy with dapsone, clindamycin, pyrimethamine, azithromycin or clarithromycin are not

recommended.

Primary prophylaxis against toxoplasmic encephalitis should be discontinued in patients treated with HAART showing an increase in $CD4^+$ T cells to above 200/mm^3 for at least 3 months. Discontinuation of prophylaxis in patients where $CD4^+$ counts have increased to 100–200 cells/mm^3 has not been carefully evaluated. No firm recommendation can be made regarding discontinuation of secondary prophylaxis, but it appears reasonable to consider discontinuation in patients who have $CD4^+$ T cells above 200/mm^3 for at least 3 months. Prophylaxis should be reintroduced if the $CD4^+$ T cell count decreases to below 100–200/mm^3 .[10] [11]

Mycobacterium tuberculosis

Latent tuberculosis infection should be treated in all patients who have HIV infection and a positive tuberculin skin test, with no evidence of active tuberculosis and no history of treatment for active or latent tuberculosis.

TABLE 123-1 -- Prophylaxis of first episode of opportunistic infections in adults and adolescents with HIV infection.*

PROPHYLAXIS OF FIRST EPISODE OF OPPORTUNISTIC INFECTIONS IN ADULTS AND ADOLESCENTS WITH HIV INFECTION			
Pathogen	**Target population**	**First-choice regimen**	**Alternative regimens**
Category I: recommended as standard of care			
Pneumocystis carinii	$CD4^+$ lymphocyte count <200/mm^3 *and/or* oropharyngeal candidiasis	TMP-SMX 1 DS q24h or 1 SS q24h	Dapsone 100mg q24h or 50mg q12h
			Dapsone 50mg q24h plus pyrimethamine 50mg weekly plus leucovorin 25mg weekly
			Dapsone 200mg weekly plus pyrimethamine 75mg weekly plus leucovorin 25mg weekly
			Aerosolized pentamidine 300mg monthly by nebulizer
			Atovaquone 1500mg q24h
			TMP-SMX 1 DS three times a week
Toxoplasma gondii	$CD4^+$ lymphocyte count <100/mm^3 *and* positive anti-*Toxoplasma* IgG	TMP-SMX 1 DS q24h	TMP-SMX 1 SS q24h
			Dapsone 50mg q24h plus pyrimethamine 50mg weekly plus leucovorin 25mg weekly
			Dapsone 200mg weekly plus pyrimethamine 75mg weekly plus leucovorin 25mg weekly
			Atovaquone 1500mg q24h
Mycobacterium tuberculosis	Positive PPD (5mm) *and/or* previous positive PPD without treatment *and/or* contact with active case (regardless of PPD result)	Isoniazid 300mg q24h plus pyridoxine 50mg q24h for 12 months *or* isoniazid 900mg and pyridoxine 50mg twice weekly with directly observed therapy for 12 months	Rifampin (rifampicin) 600mg q24h for 12 months
			Rifampin 600mg q24h plus pyrazinamide 20mg/kg/day for 2 months
			Rifampin 450–600mg twice weekly plus pyrazinamide 1500–2500mg twice weekly for 2 months
			If isoniazid resistance: rifampin 600mg q24h for 12 months or rifabutin 600mg q24h for 12 months
			If multidrug resistance: choice of drugs depends on susceptibility of isolate from source patient
Mycobacterium avium complex	$CD4^+$ lymphocyte count <50 cells/mm^3	Clarithromycin 500mg q12h *or* azithromycin 1200mg weekly	Rifabutin 300mg q24h or azithromycin 1200mg weekly plus rifabutin 300mg q24h
Varicella-zoster virus (VZV)	Exposure to chickenpox or shingles *and* no history of either or negative serology	Varicella-zoster virus Ig, 5 vials im within 96 hours of exposure (preferably within 48h)	
Category II: generally recommended			
Streptococcus pneumoniae	$CD4^+$ lymphocyte count >200 cells/mm^3	23 valent polysaccharide vaccine 0.5ml im (single dose); repeat in 5 years time	
Hepatitis B virus	All anti-HBV-negative patients	Hepatitis B vaccine 10mg im (three doses)	
Influenza virus	All patients	Inactivated trivalent Influenza vaccine 0.5ml im each year in autumn	Oseltamivir 75mg q24h (influenza A or B)
			Amantadine 100mg q12h
			Rimantadine 100mg q12h (influenza A only)
Hepatitis A virus	All anti-HAV-negative patients at increased risk (illicit drug users, men who have sex with men, hemophiliacs) or with chronic liver disease, including chronic hepatitis B or C	Hepatitis A vaccine (two doses)	
Category III: not recommended for most patients. To be considered in selected patients only			
Cytomegalovirus	$CD4^+$ lymphocyte count < 50 cells/µl *and* positive cytomegalovirus antibodies	Oral ganciclovir 1g q8h	
Bacteria	Neutropenia	G-CSF 5–10µg/kg sc q24h for 2–4 weeks	
		or	
		GM-CSF 250µg/m^2 iv q24h for 2–4 weeks	

Cryptococcus neoformans	CD4$^+$ lymphocyte count <50 cells/mm^3	Fluconazole 100–200mg q24h *or* itraconazole 200mg q24h	
Histoplasmosis	CD4$^+$ lymphocyte count <100 cells/mm^3 *and* residence in endemic area	Itraconazole 200mg q24h	
DS, double strength; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; PPD, purified protein derivatives; SS, single strength; TMP-SMX, trimethoprim-sulfamethoxazole.			

** Adapted from USPHS/IDSA guidelines.*[6]

TABLE 123-2 -- Prophylaxis to prevent recurrence of opportunistic disease in adults and adolescents with HIV infection.*

PROPHYLAXIS TO PREVENT RECURRENCE OF OPPORTUNISTIC DISEASE IN ADULTS AND ADOLESCENTS WITH HIV INFECTION			
Pathogen	**Indication**	**First-choice regimen**	**Alternative regimens**
Category I: Recommended as standard of care			
Pneumocystis carinii	Previous PCP	TMP-SMX 1SS q24h *or* 1 DS q24h	Dapsone 50mg q12h or 100mg q24h
			Dapsone 50mg q24h plus pyrimethamine 50mg weekly *plus* leucovorin 25mg weekly
			Dapsone 200mg *plus* pyrimethamine 75mg *plus* leucovorin 25mg weekly
			Aerosolized pentamidine 300mg monthly by nebulizer
			Atovaquone 1500mg q24h
			TMP-SMX, 1 DS three times weekly
Toxoplasma gondii	Prior toxoplasmic encephalitis	Sulfadiazine 500–1000mg q6h *plus* pyrimethamine 25–50mg q24h *plus* leucovorin 10–25mg po q24h (confers protection against PCP)	Clindamycin 300–450mg q6–8h *plus* pyrimethamine 25–50mg q24h *plus* leucovorin 10–25mg q24h
			Atovaquone 750mg q6–12h *with or without* pyrimethamine 25mg q24h *plus* leucovorin 10mg q24h
Mycobacterium avium complex	Documented disseminated disease	Clarithromycin 500mg q12h *plus* ethambutol 15mg/kg q24h (*with or without* rifabutin 300mg q24h)	Azithromycin 500mg q24h *plus* ethambutol 15mg/kg q24h (*with or without* rifabutin 300mg q24h)
Cytomegalovirus	Previous end-organ disease	Ganciclovir 5–6mg/kg/day iv 5–7 days/week *or* 1000mg orally q8h *or* foscarnet 90–120mg/kg q24h *or* (for retinitis) ganciclovir sustained-release implant every 6–9 months *plus* ganciclovir 1.0–1.5g po q8h	Cidofovir 5mg/kg every 2 weeks (with probenecid)
			Fomivirsen 1 vial (330µg) injected into the vitreous, then repeated every 2–4 weeks
			Valganciclovir 900mg q24h
Cryptococcus neoformans	Documented disease	Fluconazole 200mg q24h	Amphotericin B 0.6–1.0mg/kg weekly
			Itraconazole 200mg q24h
Histoplasma capsulatum	Documented disease	Itraconazole 200mg q12h	Amphotericin B 1.0mg/kg weekly
Coccidioides immitis	Documented disease	Fluconazole 400mg q24h	Amphotericin B 1.0mg/kg weekly
			Itraconazole 200mg q12h
Salmonella spp. (non-*typhi)*	Bacteremia	Ciprofloxacin 500mg q12h for several months	Antibiotic chemoprophylaxis with another active agent
Category II: Recommended only if subsequent episodes are frequent or severe			
Herpes simplex virus	Frequent/severe recurrences	Aciclovir 200mg q8h *or* 400mg q12h *or* Famciclovir 250mg q12h	Valaciclovir 500mg q12h
Candida (oropharyngeal, vaginal or esophageal)	Frequent/severe recurrences	Fluconazole 100–200mg q24h	Itraconazole solution 200mg q24h
DS, double strength; SS, single strength.			

** Adapted from USPHS/IDSA guidelines.*[6]

Regimens include:

- daily or twice weekly isoniazid (plus pyridoxine) for 9 months;
- daily rifampin or rifabutin for 4 months; and
- daily rifampin (or rifabutin) plus pyrazinamide for 2 months (although severe liver injury has been associated with this combination).

In patients whose initial skin test is negative and whose CD4$^+$ T cell count has increased to above 200/mm^3 with HAART, a repeat tuberculin skin test should be considered.[12]

***Mycobacterium avium* complex**

Patients who have a CD4$^+$ T cell count below 50/mm^3 should receive clarithromycin (500mg q12h) or azithromycin (1200mg weekly). Combination with rifabutin is not recommended. Both macrolides confer protection against respiratory bacterial infections. Rifabutin is an alternative in patients who cannot tolerate macrolides.

Primary prophylaxis should be discontinued in patients treated with HAART who show an increase in CD4$^+$ T cells to above 100/mm^3 for at least 3 months and should be reintroduced if the CD4$^+$ T cell count decreases to less than 50–100/mm^3 .[13]

Patients who have disseminated MAC should receive lifelong therapy with clarithromycin (or azithromycin) and ethambutol with or without rifabutin. It is reasonable to consider discontinuation of treatment in patients who have completed a course of at least 12 months of therapy, have no symptoms and show a CD4$^+$ T cell count above 100/mm^3 following HAART for at least 6 months.[14]

Streptococcus pneumoniae

Patients who have a $CD4^+$ T cell count greater than 200/mm^3 should receive a single dose of 23-valent polysaccharide pneumococcal vaccine every 5 years. If the $CD4^+$ T cell count is below 200/mm^3 , vaccination should be considered (with revaccination when the count increases to above 200/mm^3).[15]

Cryptococcosis

Primary prophylaxis should not be used routinely. Patients who have had cryptococcosis should receive lifelong suppressive treatment with fluconazole unless they have a sustained (= 6 months) increase in the $CD4^+$ T cell count following HAART. Suppressive therapy should be reinitiated if the $CD4^+$ T cell count decreases to less than 100–200/mm^3 .[16]

Cytomegalovirus

Patients who have had active cytomegalovirus disease should receive lifelong maintenance therapy with any of the following regimens: parenteral or oral ganciclovir, parenteral foscarnet, combined parenteral ganciclovir and foscarnet, parenteral cidofovir or oral valganciclovir. Administration of ganciclovir via intraocular implant or repetitive intravitreous injections of fomivirsen may be used in patients who have retinitis only, and are generally combined with oral ganciclovir. Repetitive intravitreous injections of ganciclovir, foscarnet and cidofovir have been shown to be effective in uncontrolled case series.[17] [18]

Discontinuation of secondary prophylaxis should be considered in patients who have received HAART and show an increase in $CD4^+$ T cells to above 100–150/mm^3 for at least 6 months. All these patients should continue to undergo regular ophthalmologic examination. Secondary prophylaxis should be restarted when the $CD4^+$ T cell count falls to below 100–150/mm^3 . [19]

Varicella-zoster virus disease

HIV adults who have no history of chickenpox or are seronegative for varicella-zoster virus (VZV) should receive VZV immunoglobulin as soon as possible but within 96 hours after exposure to a patient who has chickenpox or shingles. The efficacy of aciclovir in this setting is unknown.

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Chapter 124 - Pneumocystis carinii Pneumonia

Pierre-Marie Girard

EPIDEMIOLOGY

Historically, the occurrence of *Pneumocystis carinii* pneumonia (PCP) in American homosexuals who had no previously known immune deficiency revealed the spread of the AIDS epidemic.[1] Although the implementation of prophylaxis (see Chapter 123) and the advances in effective antiretroviral therapy have markedly decreased its incidence, PCP remains frequent, especially in patients unaware of their HIV seropositivity or who have poor access to the health care system. The use of highly active antiretroviral therapy (HAART) has decreased the incidence of PCP as compared with other pulmonary diseases such as bacterial pneumonia and non-Hodgkin's lymphoma.[2] [3] [4] [5] Development of PCP is mainly due to reactivation of latent infection acquired during childhood or adolescence; however, genetic studies of *P. carinii* isolates suggest that some cases of PCP may be due to recent exposure to environmental strains.[6] In Africa, PCP, initially thought to be a rare complication of AIDS in this area, can actually account for as much as 30% of AIDS-defining diseases.[7] [8]

The best predictor of occurrence of PCP in HIV-infected patients is the $CD4^+$ lymphocyte blood count.[9] A significant risk of PCP exists when the $CD4^+$ lymphocyte count is less than 200/mm^3 or 15–20% of total lymphocytes, and clinical manifestations (e.g. thrush, herpes zoster, unexplained fever, weight loss) or a history of such manifestations are correlated with increased risk, independently of the $CD4^+$ lymphocyte count. Cytotoxic chemotherapies also increase the risk of PCP whatever the $CD4^+$ lymphocyte count. The rate of relapse after a first episode of PCP is high (approximately 60% incidence within 1 year) when neither specific prophylaxis nor HAART is initiated.

PATHOGENESIS AND PATHOLOGY

Development of *P. carinii* is restricted to the lung tissue in more than 95% of cases. The use of aerosolized pentamidine, which has negligible extrapulmonary deposition, explains the occurrence of disseminated infections in bone marrow, spleen, liver, retina and skin.[10]

Pneumocystis carinii pneumonia is characterized by a foamy eosinophilic exudate and the 'honeycomb' appearance of the lung tissue due to mild interstitial pneumonitis with proliferation of type II pneumocytes. *Pneumocystis carinii* cysts are visualized by methenamine-silver nitrate or toluidine blue O stains in the exudate, and trophozoite forms (visualized by Giemsa stains or its derivatives) proliferate and attach to type I pneumocytes. Extensive interstitial fibrosis is the natural process of untreated infections leading to respiratory distress. Mild fibrosis also occurs under treatment.

Other less common pathologic characteristics are seen in people who have AIDS. Diffuse alveolar damage may predominate without alveolar exudate. Cystic and cavitary lesions predominating in upper lobes may also develop. Pneumothorax is a frequent complication resulting from the rupture of cysts. Noncaseating granulomatous inflammation, sometimes with calcification, is occasionally seen.

Animal experiments indicate that $CD4^+$ lymphocytes play a critical role in the host's ability to resist and recover from *P. carinii* infection. In addition, macrophage function may be important for clearance of the micro-organisms and the macrophage mannose receptor seems important for binding and uptake of *P. carinii*. Among cytokines, tumor necrosis factor has been shown to be directly lethal to *P. carinii*.

CLINICAL FEATURES

The most common clinical presentation of PCP in AIDS is a progressive dyspnea with dry cough, fever (often mild) and weight loss. The mean duration of breathlessness is 3–4 weeks at presentation. On examination, fever and tachypnea are common, whereas lung auscultation may be normal or reveal only basal crepitations.

Chest radiography is an important step in the diagnosis procedure of lung diseases in AIDS. The chest radiograph is normal in less than 5% of cases. The most common pattern is a fine bilateral interstitial and then alveolointerstitial infiltrate progressing from the perihilar to the peripheral regions (Fig. 124.1). Without therapy, or even during the first days of treatment, the alveolar interstitial pattern worsens. In advanced cases, progressive consolidation with air bronchograms and complete opacification of the lungs may develop (Fig. 124.2).

Numerous atypical patterns of PCP may occur. They include localized infiltrates, notably of the upper lobes, in patients who fail to respond to aerosolized pentamidine prophylaxis, cavitary lesions, solitary lung nodules, spontaneous pneumothoraces and pleural effusions. Lymphadenopathies causing hilar or mediastinal enlargement are very rare and are usually linked to lymphoma and concomitant infections such as mycobacteriosis.

Parenchymal involvement is well assessed by computerized tomography (CT), which in typical cases exhibits bilateral ground-glass infiltrates that may not have been seen on chest radiography. The

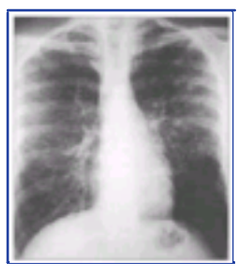

Figure 124-1 Mild *Pneumocystis carinii* pneumonia. There are bilateral micronodular lesions.

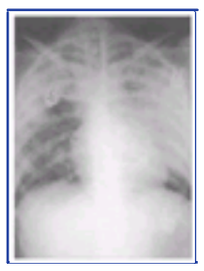

Figure 124-2 Severe *Pneumocystis carinii* pneumonia. This shows an extensive alveolar interstitial infiltrate with consolidation of the left lung and upper right lobe. *Courtesy of A Cabié.*

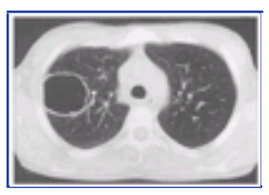

Figure 124-3 Six weeks after treatment of moderate *Pneumocystis carinii* pneumonia. Chest CT shows large, thin-walled bullae of the right lung.

infiltrates can be dense and homogeneous or patchy in both lungs. Parenchymal destruction, from bullae (Fig. 124.3) predominating in the apices to extensive emphysematous changes, may also be seen on CT. Small pulmonary cysts with thin walls throughout the lungs are common.

In extrapulmonary *P. carinii* infections, CT or ultrasonography demonstrates multiple abscess-like lesions varying from several millimeters to a few centimeters in size, mainly in the spleen (Fig. 124.4) and sometimes in the liver. The radiologic abnormalities are not characteristic of *Pneumocystis* infection.

DIFFERENTIAL DIAGNOSIS

A wide spectrum of lung diseases may occur during the course of AIDS, especially in patients who have severe immune deficiency, which is the usual situation for developing PCP.

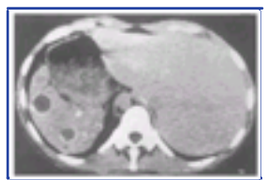

Figure 124-4 Splenic *Pneumocystis carinii* pneumonia. Abdominal CT shows multiple ring-enhancing abscess-like round formations. *Courtesy of C Bazin.*

Kaposi's sarcoma is the most common lung tumor and occurs almost exclusively in homosexual men. Clinical presentation may be a progressive dyspnea and the patient is usually afebrile. Cutaneous and/or palatal Kaposi's sarcoma is frequently diagnosed before lung localization. On chest radiography or CT, nodular lesions and peribronchovascular thickening are more frequent than interstitial abnormalities. In most cases, the macroscopic appearance of the tracheobronchial tract is characteristic. Other considerations include bacterial and fungal pneumonia, and tuberculosis.

Lymphocytic interstitial pneumonia is a rare disease due to lung infiltration by $CD8^+$ lymphocytes and occurs usually in patients who have a $CD4^+$ lymphocyte count above $200/mm^3$.

DIAGNOSIS

Establishing a diagnosis of PCP requires the morphologic demonstration of the micro-organism. Because the sampling procedures that combine both high sensitivity and specificity are invasive (i.e. fiberoptic bronchoscopy with bronchoalveolar lavage, or, less frequently, transbronchial biopsy), the need to make a definite diagnosis before starting anti-*Pneumocystis* therapy is still controversial. Diagnosis on pulmonary samples can be improved by the use of either monoclonal antibodies or polymerase chain reaction (PCR).

Arterial blood gases must be measured to assess the severity of the disease, but have no diagnostic value. Two functional tests that have been thoroughly evaluated — decreased diffusing capacity and increase of alveolar-arterial gradient with exercise — have yielded high sensitivity and low specificity. Increased serum lactate dehydrogenase (LDH) may confirm the lung disease in cases of subtle symptoms or radiologic abnormalities but has no specificity. The level of serum LDH and, more precisely, the slope of its decrease during the first days of treatment have prognostic value. These tests are therefore routinely included in the diagnostic algorithms used by some teams to select patients who will benefit from fiberoptic bronchoscopy. Because of their low specificity their use should not alter the time for bronchoscopy.

No specific biologic marker of *P. carinii* infection exists for individual diagnosis. Antibodies to *P. carinii* are commonly detected in the adult general population and may be less detectable in immunocompromised patients. Attempts to detect *P. carinii* antigens in serum have failed to establish a diagnosis of PCP. Even PCR detection of *P. carinii* genome in blood has been very disappointing. In fact PCR tests are sensitive enough only in the very rare cases of disseminated infection, although they may be useful for detecting *P. carinii* in lung

respiratory samples (bronchoalveolar lavage, induced sputum or possibly saliva). [11]

Identification of *Pneumocystis carinii*

Methods of sampling

Sputum examination is the least invasive procedure for collecting lower respiratory specimens. Because patients who have PCP generally do not have a productive cough, an induced sputum can be obtained after aerosolization of hypertonic saline, ideally under the supervision of a physiotherapist. In routine practice, the sensitivity of the test is approximately 50–60% and is slightly improved by using an immunofluorescence technique for visualization, with monoclonal antibodies directed to the cysts.

Fiberoptic bronchoscopy with bronchoalveolar lavage is the reference routine diagnostic procedure because its sensitivity is more than 95% and its specificity is 100%. When the diagnosis can not be obtained from bronchoalveolar lavage fluid, transbronchial lung biopsy may help, although it is more invasive and is associated with a risk of bleeding and pneumothorax (5–10%). This procedure has a higher sensitivity than bronchoalveolar lavage, especially in patients receiving prophylaxis by aerosolized pentamidine.

Methods of staining

It is recommended that two stains be used to visualize both cysts (methenamine silver or toluidine blue O for the cyst wall, Wright-Giemsa for nuclei) and trophozoites (Wright-Giemsa or Diff-Quik). Immunofluorescent stains are more expensive and are mostly valuable for less-than-optimal samples such as induced sputum, where their sensitivity is superior to conventional stains. The PCR methods are of little benefit on lung samples but can increase the diagnosis rate on induced sputum.

MANAGEMENT

The measurement of arterial gas is used routinely to delineate mild (Pa_{O_2} =70mmHg), moderate (Pa_{O_2} 50–70mmHg) and severe (Pa_{O_2} <50mmHg) PCP. In mild PCP, outpatient management with oral therapy is often possible providing no other disease is present, there is low risk of drug malabsorption and the patient has a good understanding of the management and is aware of the potential side effects of therapy. Hospitalization is required for moderate and severe PCP to ensure careful monitoring of treatment, which will be

TABLE 124-1 -- Factors associated with a poor prognosis of *Pneumocystis carinii* pneumonia.

FACTORS ASSOCIATED WITH A POOR PROGNOSIS OF PCP

At diagnosis	Prolonged history of respiratory symptoms
	Recurrent PCP
	Severe hypoxemia
	Marked radiographic abnormalities
	High plasma LDH level
	High plasma angiotensin-converting enzyme level
	Hypoalbuminemia
	Neutrophilia (>5%) in bronchoalveolar lavage fluid
During the course of the disease	Worsening hypoxemia
	Mechanical ventilation, especially when delayed after initiation of therapy
	Pneumothorax and multiple cavitary lesions or emphysema
	Bacterial co-infection of the respiratory tract
	Persisting high serum levels of LDH

administered intravenously, at least during the first days. Besides blood gas assessment, other factors listed in Table 124.1 are useful prognostic factors that can be taken into account for proposing hospitalization.

Anti-*Pneumocystis* drugs

Therapeutic progress, although quite laborious because of the difficulty encountered with in-vitro cultivation of *P. carinii*, has been made in four main fields:

- the optimal use of old drugs such as pentamidine and trimethoprim-sulfamethoxazole (TMP-SMX) has been defined through numerous trials;
- new combinations of old drugs such as dapsone plus trimethoprim, and clindamycin plus primaquine have been evaluated;
- two innovative compounds, atovaquone and trimetrexate, have been developed and are approved for treatment of PCP; and
- the indications of adjunctive corticosteroids have been carefully defined.

Last, but not least, despite much controversy, the real benefit of mechanical ventilation for a subset of patients has now been established.[12]

Trimethoprim-sulfamethoxazole is the drug of choice for PCP whatever its severity, and no other drug or combination of drugs has demonstrated improved efficacy in control trials. The classic dose of 20mg/kg/day trimethoprim with 100mg/kg/day sulfamethoxazole in three or four divided doses can be reduced to 15mg/kg/day trimethoprim with 80mg/kg/day sulfamethoxazole without loss of efficacy. The unexpectedly high rate of TMP-SMX side effects was noticed early in patients who have AIDS; these include rash, fever, pruritus, digestive disturbances and cytopenia.[13] Most side effects occur after 7–10 days of therapy. More recently, hyperkalemia has been reported. However, many of these side effects, whose mechanisms are still poorly understood, spontaneously resolve and in these cases treatment does not need to be discontinued. Approximately 10–20% of patients will need to be switched to another treatment because of TMP-SMX intolerance. It is possible that adjunctive treatment with corticosteroids decreases the incidence of the cutaneous side effects of TMP-SMX. Monitoring blood concentrations of trimethoprim (concentration maintained below 5–8µg/ml) in order to avoid hematologic toxicity is rarely necessary except in patients who have substantially impaired renal function.

Pentamidine (3–4mg/kg/day intravenously) an aromatic diamidine endowed with antiprotozoal activity, was the first drug successfully used for therapy of PCP in patients who did not have AIDS. Trials conducted in people who have AIDS have confirmed the risk of severe side effects but have not shown activity superior to that of TMP-SMX.[14] The side effects are severe and include hypotension, hypoglycemia, pancreatitis, diabetes, *torsades de pointes* and renal insufficiency. The indications for pentamidine have narrowed with the development of alternative oral combinations for mild PCP in TMP-SMX-intolerant patients and are now restricted to patients who have severe PCP but cannot tolerate or fail to respond to TMP-SMX.

Trimethoprim plus dapsone at 20mg/kg/day and 100mg/day is an oral alternative to TMP-SMX and has appeared to be as effective as TMP-SMX in several trials of mild PCP,[15] [16] although experience is limited. Side effects include rash, methemoglobinemia, hemolytic anemia and neutropenia.

Clindamycin (600–900mg/day) plus primaquine (15–30mg/day) is another oral alternative to TMP-SMX for therapy of mild PCP. These drugs have the advantage of structurally differing from sulfonamide or sulfone compounds, thus limiting the risk of cross-toxicity. This combination is as effective as the previously mentioned combinations in nonsevere PCP[16] but skin rashes, albeit often transient, and digestive complications due to clindamycin occur in 20–25% of patients.

Atovaquone (2250mg/day) is a hydroxynaphthoquinone compound initially developed for its antimalarial activity. Only an oral formulation, which must be taken with food, is available; it has a bioavailability of less than 10%. A new micronized formulation with improved bioavailability has been developed recently and offers better results.[17] Therefore, atovaquone is used as second-line therapy in patients with nonsevere PCP who are or have become intolerant to TMP-SMX and whose digestive absorption is not impaired. Administration of rifampin should be avoided.

Trimetrexate (45mg/m^2 of body surface area) is a lipophilic inhibitor of dihydrofolate reductase that is 1500 times more potent in vitro against *P. carinii* than trimethoprim. However, controlled trials have not shown it to be superior to conventional therapies such as TMP-SMX and intravenous pentamidine.[18] Serious treatment-limiting toxicity was significantly less frequent among patients receiving trimetrexate than TMP-SMX. Trimetrexate is currently used as a second-line therapy for patients requiring intravenous therapy who are refractory or fail to respond to other treatment. It must be administered with leucovorin acid (80mg/m^2 of body surface area) to prevent the development of cytopenia as a severe side effect.

Choice of therapy

The choice of therapy is guided by:

- severity of the disease (see Table 124.1);
- the likelihood of digestive drug absorption; and
- the history of drug intolerance.

The first two criteria will indicate whether an oral regimen is advisable. In case of drug intolerance, it is important to evaluate the severity of past toxicity. In practice, contraindication to reintroduction of TMP-SMX or to the use of a combination with potential cross-intolerance to sulfonamide is based on a history of anaphylaxis or exfoliative dermatitis or other life-threatening toxicity such as severe cytolytic hepatitis. Whenever possible, the first-line therapy is TMP-SMX. For cases of mild PCP and a history of nonsevere TMP-SMX intolerance, one can choose between the use of:

- TMP-SMX;
- dapsone plus trimethoprim; and
- clindamycin plus primaquine or atovaquone.

For cases of mild PCP and a high risk of severe intolerance to TMP-SMX, atovaquone is a reasonable alternative, providing its absorption will not be impaired by digestive malabsorption.

Mutations in *P. carinii* genes expressing genes targeted by current drugs (sulfonamides, sulfones and atovaquone) were reported in the late 1990s. Although several studies have reported an increasing frequency of mutations in the dihydropteroate synthase gene[19] [20] [21] and in cytochrome *b*,[22] the impact of these mutations on clinical outcome remains controversial.

In most cases, respiratory symptoms and oxygenation improve after 5–10 days. Early mild deterioration is not infrequent during the first days of treatment. Duration of

treatment is classically 21 days, although a shorter duration (2 weeks) might be sufficient but has not been adequately evaluated. When TMP-SMX is initiated intravenously, it can be switched to an oral formulation after a few days. There is no need for repeat fibroscopy, and persistence of *P. carinii*, the viability of which is unknown, is common. Secondary prophylaxis is required after treatment of the attack.

Mechanical ventilation should be considered in patients who fail to improve under non-invasive oxygenation methods. The decision is taken according to the patient's wishes and general condition. Anti-*P. carinii* therapy is administered intravenously and TMP-SMX remains the drug of choice, unless there is a history of severe intolerance. In this latter case, pentamidine or trimetrexate with folinic acid rescue are the two alternatives.

In addition to anti-*P. carinii* therapy, corticosteroids are administered as soon as possible in patients who have moderate to severe disease. The use of corticosteroids is recommended in patients whose *P*ao$_2$ is less than 65mmHg for improving oxygenation, reducing the risk of fibrosis, decreasing the need of mechanical ventilation and reducing the case fatality rate. The corticosteroid regimen used is as follows:

- on days 1–5, 40mg oral prednisone (prednisolone) q12h;
- on days 6–10, 40mg oral prednisone/day; and
- on days 11–21, 20mg oral prednisone/day.

When corticosteroid therapy is used one should be aware of possible reactivation of latent co-infections such as mycobacterial and herpes infections.

Management of deterioration on therapy

Patients who deteriorate under therapy need to be carefully evaluated for lung complications related to PCP and associated diseases. The value of changing the initial anti-PCP therapy for another one has not been well demonstrated, but this is common practice. Clindamycin-primaquine (the latter being not available in all countries) could be an attractive regimen in this setting, as suggested by a meta-analysis of 27 clinical trials.[23] Obviously, patients who deteriorate on oral therapy should receive intravenous therapy with either TMP-SMX, pentamidine or trimetrexate. Reasons for the deterioration are various and include:

- severe pulmonary lesions with alveolar and interstitial edema;
- pneumatoceles;
- bullae and cavities that can provoke pneumothorax; and
- emphysema.

In hypoxemic patients, cardiac failure is common and will require appropriate monitoring and treatment.

Associated diseases include bacterial pneumonia (community-acquired or, more frequently, nosocomial), fungal diseases and Kaposi's sarcoma. Repeat bronchoscopy with bronchoalveolar lavage and transbronchial biopsy should therefore be considered. Persistence of *P. carinii* is usual and does not indicate failure of anti-*P. carinii* therapy. In the past 15 years, the case fatality rate of PCP has markedly decreased and is now around 5%. This globally improved prognosis may be due to several factors:

- better awareness of the disease by physicians and patients leading to earlier diagnosis;
- aggressive therapy despite mild toxicity;
- the use of adjunctive corticosteroids; and
- better defined indications of intensive care.

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Chapter 125 - Viral Infection

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INTRODUCTION

Viral infections are an important cause of morbidity and mortality in patients who have HIV disease. Over the past two decades, substantial progress has been made in our understanding of the pathogenesis and natural history of viral infections in this patient population. The development of more sensitive diagnostic laboratory techniques and effective therapeutic agents has had a significant impact on the management of these conditions. The introduction of highly active antiretroviral therapy (HAART) for HIV disease has exerted a profound effect on the epidemiology, natural history, clinical manifestations and responses to treatment of opportunistic infections, including viral infections, in HIV-infected patients.[1] With recovery of immune function as a result of effective antiretroviral therapy, the incidence of opportunistic infections has fallen dramatically, chronic and refractory infections are more amenable to treatment, and lifelong treatment is no longer necessary in many instances.

Table 125.1 lists the spectrum of viral infections and associated diseases in HIV-infected people. Most clinically important viral infections in HIV disease are caused by DNA viruses, the majority belonging to the Herpesviridae family. However, improved life expectancy among HIV-infected patients has led to a change in the spectrum of infections influencing morbidity and mortality in this population. Hepatitis B and particularly hepatitis C have emerged as major contributors to morbidity and mortality in HIV-infected patients. This chapter examines the main clinical viral syndromes in HIV disease. The role of viral infections such as human herpes virus-8, Epstein-Barr virus and human papillomavirus in HIV-related neoplastic disorders is discussed elsewhere (see Chapter 130).

CYTOMEGALOVIRUS INFECTIONS

EPIDEMIOLOGY

Serologic evidence of cytomegalovirus (CMV) infection can be detected in approximately 60% of the adult population in the USA. The prevalence of infection is strongly influenced by socioeconomic status and sexual practices; up to 95% of homosexual men are seropositive for CMV.[2]

Before widespread use of HAART, the relative incidence of CMV disease had been increasing and CMV infection was the most common major opportunistic infection associated with AIDS, affecting as many as 45% of patients.[3] Retinitis is the commonest manifestation of CMV infection in patients who have HIV infection or AIDS, accounting for 85% of CMV disease, and is the leading cause of visual loss.[4] Other clinical syndromes include esophagitis, colitis, polyradiculopathy, ventriculoencephalitis, pneumonitis, adrenalitis and pancreatitis.

PATHOGENESIS AND PATHOLOGY

In patients who have AIDS, CMV disease usually results from reactivation of latent infection. Progressive loss of cell-mediated immunity in patients who have advanced HIV disease abrogates the immunologic suppression of CMV replication. Asymptomatic excretion of CMV in urine can be detected in approximately 50% of patients who have advanced HIV disease, and over half of the patients who have CMV viremia go on to develop clinical CMV disease within 8–12 months.[5] [6] Cytomegalovirus end-organ disease usually occurs when the $CD4^+$ lymphocyte count falls below 50 cells/mm^3 .[4] [7]

Cytomegalovirus infects many cell types and tissues. Infection results in tissue necrosis and non-specific inflammation. Microscopically the hallmark of CMV infection is a large (cytomegalic) cell containing a large basophilic intranuclear 'owl's eye' and intracytoplasmic inclusion bodies.

PREVENTION

Patients who have $CD4^+$ lymphocyte counts less than 50/mm^3 should have ophthalmologic screening performed every 3–6 months. Oral ganciclovir is approved for the primary prophylaxis of CMV retinitis in patients who have $CD4^+$ lymphocyte counts below 100/mm^3 . However, this approach is not universal. Results from two randomized controlled trials are conflicting,[8] [9] and the cost benefits of primary CMV prophylaxis are controversial. Newer viral quantitative measures such as quantitative polymerase chain reaction (PCR) and antigenemia assays may identify those patients who would benefit from 'targeted' prophylaxis or 'pre-emptive' therapy even in the absence of end-organ disease.

Patients on HAART experiencing sustained $CD4^+$ lymphocyte counts above 100/mm^3 have a greatly reduced risk of developing CMV disease and evidence suggests that primary and secondary prophylaxis can be discontinued safely in this situation.[1] [10] [11] However, CMV disease can recur after virological and immunological failure of HAART if $CD4^+$ lymphocyte counts fall below 50 cells/mm^3 .

CLINICAL FEATURES AND DIAGNOSIS

Cytomegalovirus retinitis

Cytomegalovirus causes a relentlessly progressive, necrotizing retinitis. Cytomegalovirus retinitis is usually unilateral in the first instance, progressing to affect the contralateral eye if untreated. Patients may be initially asymptomatic but may subsequently experience blurring of vision, floaters and painless progressive visual loss.

Diagnosis of CMV retinitis is made by systematic funduscopic examination by direct or indirect ophthalmoscopy. Characteristically, white, fluffy, or granular lesions with perivascular white exudates associated with retinal hemorrhages are seen (Fig. 125.1).

Cytomegalovirus lesions may be categorized as occurring in three arbitrarily defined anatomic zones. Retinitis located in the immediate vicinity of the macula or optic disc (zone 1) is sight-threatening and should prompt immediate initiation of treatment. Lesions outside the major vascular vessels (zones 2 and 3), commonly referred to as 'peripheral retinitis', are not immediately sight-threatening but will progress if left untreated.

TABLE 125-1 -- Viral infections and clinical syndromes in HIV-infected patients.

VIRAL INFECTIONS AND CLINICAL SYNDROMES IN HIV-INFECTED PATIENTS			
Family	**Subfamily**	**Genus and species**	**Clinical syndromes**

Herpesviridae (DNA)	Alphaherpesvirinae	Herpes simplex 1,2	Orolabial ulceration	
			Anogenital ulceration	
			Herpetic whitlow	
			Encephalitis	
		Varicella-zoster virus	Varicella	
			Herpes zoster	
			Disseminated VZV infection	
			Pneumonitis	
			Encephalitis	
			Hepatitis	
			Retinal necrosis	
	Betaherpesvirinae	Cytomegalovirus	CMV	viremia
				retinitis
				esophagitis
				enterocolitis
				pneumonitis
				encephalitis
				polyradiculopathy
				adrenalitis
		Human herpesvirus 6	Unknown	
			? Retinal disease	
			? Enhanced HIV replication	
	Gammaherpesvirinae	Epstein-Barr virus	Oral hairy leukoplakia	
			Lymphoma, Hodgkin's and non-Hodgkin's disease	
		Human herpesvirus 8	Kaposi's sarcoma	
			Primary effusion lymphoma	
			Multicentric Castleman's disease	
Papovaviridae (DNA)		Papillomavirus	Common and genital warts	
			Squamous intraepithelial neoplasia	
			Cervical carcinoma	
			Anal carcinoma	
		Polyomavirus, JC virus	Progressive multifocal leukoencephalopathy	
Hepadnaviridae (DNA)		Hepatitis B virus	Hepatitis, acute and chronic cirrhosis	
			Liver carcinoma	
Parvoviridae (DNA)		Human parvovirus B19	Aplastic anemia	
Poxviridae (DNA)		Molluscum contagiosum virus	Molluscum contagiosum	
Flaviviridae (RNA)		Hepatitis C virus	Hepatitis, acute and chronic cirrhosis	
			Liver carcinoma	

Gastrointestinal cytomegalovirus disease

The clinical manifestations of CMV infection in the gastrointestinal tract depend largely on the site of infection. In the upper gastrointestinal tract, CMV causes discrete esophageal ulcers, diffuse esophagitis, gastritis, gastric ulcers, duodenal ulcers and enteritis. Esophageal CMV infection usually presents with painful dysphagia. Endoscopic examination frequently reveals inflammation and ulceration, and diagnosis is established by finding characteristic pathologic features on tissue biopsy; it can be confirmed by immunohistochemical or CMV DNA in-situ hybridization techniques. Cytomegalovirus enterocolitis occurs in 5–10% of patients who have AIDS.[12] Lower gastrointestinal CMV disease usually presents with abdominal pain and persistent small-volume diarrhea. Endoscopic examination of the colon reveals plaque-like pseudomembranes, multiple erosions and ulcers, although a mucosa of grossly normal appearance is seen in approximately 10% of patients.

Cytomegalovirus disease of the central nervous system

The two major CMV neurologic syndromes associated with HIV disease are CMV polyradiculopathy and CMV ventriculoencephalitis.[12] [13] Polyradiculopathy is a devastating complication in patients who have advanced AIDS. Approximately 50% of patients have an associated myelitis. It is characterized by lower extremity pain, sensory deficits, weakness that can rapidly progress to flaccid paralysis, and bowel and bladder dysfunction. The most marked pathologic changes in CMV polyradiculomyelitis are found in the cauda equina and lumbosacral roots. In the appropriate clinical setting, findings on magnetic resonance imaging (MRI) of diffuse enhancement of the cauda equina and the surface of the conus strongly support the diagnosis. Cerebrospinal fluid findings include a polymorphonuclear pleocytosis, raised protein concentration and moderately low glucose concentration. Culture of cerebrospinal fluid may be negative

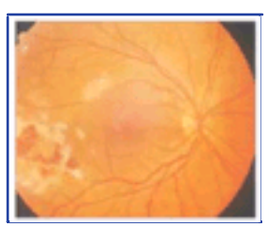

Figure 125-1 Cytomegalovirus retinitis, with characteristic perivascular hemorrhages and exudates.

but CMV antigen assays and PCR for CMV DNA are more sensitive techniques.

Ventriculoencephalitis usually occurs in the setting of a diagnosis of CMV disease elsewhere. Patients present with fever, lethargy and confusion. Characteristic neurologic findings include nystagmus and cranial nerve palsies. Magnetic resonance imaging with gadolinium enhancement may demonstrate periventricular enhancement. Cytomegalovirus DNA can often be detected in cerebrospinal fluid using PCR.

Pulmonary cytomegalovirus disease

There are no particular distinguishing clinical or radiologic features to differentiate CMV pneumonitis from other causes of pneumonitis in HIV disease. Patients present with shortness of breath, dyspnea on exertion and a dry, nonproductive cough. Chest radiographs shows diffuse interstitial infiltrates, and hypoxemia is usually present. Definitive diagnosis of pulmonary CMV disease in patients who have advanced HIV disease is difficult to establish because of a high incidence of asymptomatic CMV shedding — CMV can be isolated in approximately 50% of HIV-infected patients undergoing bronchoscopic examination.[14] However, the true incidence of CMV pneumonitis is less than 10% in patients who have diagnostic bronchoscopy for evaluation of pulmonary infiltrates of unknown origin. [15]

MANAGEMENT

The therapeutic options and strategies for the treatment and prevention of CMV retinitis are outlined in Table 125.2 . Similar treatment strategies are commonly employed in treating other end-organ CMV disease.[16] [17] Treatment options for CMV retinitis include systemic antiviral treatment and maintenance therapy, intraocular implant devices and intraocular injections of antiviral agents. However, the optimal use, combination and timing of each treatment remains to be determined. Important factors to consider include relative efficacies, potential toxicities, the use of concurrent medications and the need for permanent vascular access.

Five antiviral drugs are currently available for the treatment of CMV retinitis:

- ganciclovir, a nucleoside analogue;
- foscarnet, a pyrophosphate analogue;
- cidofovir, a nucleotide analogue;
- valganciclovir, a prodrug of ganciclovir; and
- fomivirsen, an antisense oligonucleotide.

For systemic treatment, each agent is initially given in high doses (induction) for 2–3 weeks, depending on clinical response, followed by lower maintenance doses. In the absence of HAART-mediated immune reconstitution, most patients eventually have reactivation of CMV infection despite long-term suppressive therapy and require further or re-induction therapy.

Ganciclovir, a nucleoside analogue, is phosphorylated in CMV-infected cells by viral-encoded thymidine kinase. In its active form, it inhibits viral DNA replication. It is available as a parenteral and oral preparation.

Intravenous foscarnet is a pyrophosphate analogue that inhibits DNA polymerase without the need for prior phosphorylation. It has a broad spectrum of activity against the herpesviruses.

In 80–90% of patients, intravenous administration of ganciclovir or foscarnet q12h for 14–21 days (induction) initially halts inflammation and retinal necrosis. In the only published trial directly comparing foscarnet with ganciclovir for the treatment of CMV retinitis, efficacy was similar for both agents. [18] However, there was a significant survival benefit for patients treated with foscarnet, probably owing to the intrinsic anti-HIV activity of foscarnet. Combination therapy with ganciclovir and foscarnet has been shown to be synergistic, or at least additive in vitro, and in one trial it was found to be superior to either drug alone for treatment of recurrent CMV retinitis.[19] This may be a useful strategy in patients who have immediately sight-threatening retinitis or repeated episodes of retinitis and in patients not responding to standard therapy. Both ganciclovir and foscarnet require permanent intravenous vascular access, such as a Hickman-Broviac catheter or a Port-a-Cath catheter, for maintenance therapy.

Oral ganciclovir is licensed for maintenance therapy of patients who have CMV retinitis. However, its bioavailability is poor and time to relapse of retinitis is shorter than with intravenous maintenance therapy.[20] It may be particularly useful for patients who do not have extensive or immediately sight-threatening disease and where long-term venous access is not feasible.

Valganciclovir, recently licensed for treating CMV retinitis, is a monovalyl ester oral prodrug that is rapidly hydrolyzed to ganciclovir. The bioavailability of ganciclovir from oral valganciclovir is 60%, with equivalent blood levels to standard systemic ganciclovir, and it is as effective as intravenous ganciclovir for the management of CMV retinitis in patients who have AIDS.[21]

Intravenous cidofovir is a nucleotide analogue of cytosine with potent in-vitro and in-vivo activity against a broad spectrum of herpesviruses including CMV, herpes simplex viruses 1 and 2, varicella-zoster virus and Epstein-Barr virus. [22] Unlike ganciclovir, which requires intracellular activation by viral-encoded enzyme, conversion of cidofovir to cidofovir diphosphate is by host cellular enzymes and therefore is independent of viral replication. The diphosphate form has a long intracellular half-life and a major advantage of cidofovir is the possibility for maintenance therapy to be given as one injection every 2 weeks, thus obviating the need for an indwelling venous catheter. However, renal toxicity is high and cidofovir therapy must be closely monitored.

Because of the toxicity associated with systemic therapy for CMV retinitis, local (intraocular) therapy has been developed. Intravitreal therapy is effective in controlling retinitis and may be particularly useful as salvage therapy in patients who are no longer tolerant of systemic therapy. Intravitreal injections of ganciclovir, foscarnet and cidofovir have been used. Fomivirsen, the first of a new class of highly selective and novel therapeutics, antisense oligonucleotides, has potent anti-CMV activity, and when it is administered intravitreously it is effective for peripheral retinitis and retinitis not controlled by other anti-CMV drugs.[23] [24] Another important approach has been the development of intravitreal devices or implants to deliver ganciclovir continuously over several months.[25] These provide better control of retinitis than systemic therapy and offer significant improvement in the quality of life. Specific complications include bleeding, infection, change in refraction and early retinal detachment. Although implants provide excellent local control of retinitis, the incidence of subsequent contralateral eye disease and systemic disease is high. For this reason, concomitant systemically active treatment (e.g. with oral valganciclovir) is recommended in patients who receive implants.

TABLE 125-2 -- Therapeutic regimens for management of cytomegalovirus retinitis in HIV-infected patients.

THERAPEUTIC REGIMENS FOR MANAGEMENT OF CMV RETINITIS IN HIV-INFECTED PATIENTS			
Drug	**Regimen**	**Adverse effects**	**Comments**
Ganciclovir (iv)	**Induction**	Neutropenia (15–40%)	Median time to progression 47–104 days
	5mg/kg q12h × 14–21d	Thrombocytopenia (5–10%)	Monitor hematology indices
	7.5mg/kg q12h × 14–21d for refractory disease	Anemia (5–10%)	Bone marrow suppression with AZT
	Maintenance	Nausea, vomiting	Administer G-CSF or GM-CSF for neutropenia (ANC <500/mm^3)
	5mg/kg q24h	Confusion, headaches	Drug of choice in patients with baseline renal insufficiency or taking nephrotoxic drugs
	range 5–10mg/kg q24h	Seizures (rare)	Requires indwelling venous catheter
Ganciclovir (po)	**Maintenance**	Nausea, dyspepsia (40–50%)	Median time to progression 29–56 days
	1g q8h	Diarrhea (50–60%)	8–10% bioavailability
		Neutropenia (25%)	? Primary prophylaxis
		Anemia (10%)	Less effective than maintenance iv ganciclovir
		Thrombocytopenia	Rarely used now because valganciclovir available
		Pancreatitis (rare)	

Ganciclovir intraocular implant	4.5mg implanted for 5–8 months	Retinal detachment (12%)	Median time to progression 196–226 days
		Hemorrhage, infection	Contralateral eye disease in 50% and extraocular CMV disease in more than 30% at 6 months
		Endophthalmitis	
Ganciclovir intraocular injections	400µg twice weekly	Hemorrhage, infection	
		Retinal detachment	
Foscarnet (iv)	**Induction**	Renal impairment (20–30%)	Median time to progression 53–93 days
	90mg/kg q12h × 14–21d	Hypocalcemia, hypomagnesemia, hypokalemia, hypophosphatemia (20–30%)	Monitor electrolytes and replete iv or oral hydration reduces adverse effects
	Maintenance	Nausea (25–40%)	Potential survival benefit over ganciclovir
	90–120mg/kg q24h	Confusion, agitation	Active against aciclovir-resistant herpes viruses
		Anemia	Requires indwelling venous catheter
		Genital ulceration	
Foscarnet intraocular injections	2400µg twice weekly	Hemorrhage, infection	
		Retinal detachment	
Cidofovir (iv)	**Induction**	Proteinuria (20%), renal insufficiency	Median time to progression 64–120 days iv hydration pre- and postadministration and pre- and post-probenecid reduces renal toxicity
	5mg/kg weekly × 14d	Fanconi syndrome (25%)	
	Maintenance	Nausea/vomiting	
	3–5mg/kg every 2 weeks	Neutropenia	
Valganciclovir (po)	**Induction**	Diarrhea 19%,	Median time to progression 160 days
	900mg q12h × 21d	Neutropenia 14%	Prodrug of ganciclovir with 60% bioavailability of ganciclovir
	Maintenance		
	900mg q24h		
Fomivirsen intravitreous injections	**Induction**	Anterior chamber inflammation	Median time to progression 71 days
	165µg once weekly × 21d	Increased intra-ocular pressure	Higher dose 330µg effective in relapsed disease or in infections failing to respond to alternative treatments
	Maintenance		
	165µg alternate weeks		

HERPES SIMPLEX VIRUS INFECTIONS

EPIDEMIOLOGY

Severe herpes simplex virus (HSV) infection was one of the initial clinical manifestations heralding the onset of the AIDS epidemic.[26] Herpes simplex virus 1 is normally acquired early in life, whereas HSV-2 is usually acquired through sexual contact, the risk of infection being correlated with the number of sexual partners.[27] [28] The highest prevalence of antibodies to HSV-2 in the USA is among female commercial workers and homosexual males. In HIV-infected people, seroprevalence rates up to 77% for HSV-2 have been reported, reflecting common risk factors for transmission. [29]

PATHOGENESIS AND PATHOLOGY

After initial or primary infection, which may be asymptomatic, HSV has the capacity to establish latent infection in the dorsal root or sensory ganglia. Viral reactivation occurs intermittently and leads to a recurrence of cutaneous or mucosal lesions. Immunosuppressed patients are at greater risk of both recurrent and disseminated HSV infections. Reactivation of HSV occurs frequently in patients who have advanced HIV disease, particularly in those who have low $CD4^+$ lymphocyte counts (<100 cells/mm^3). The histopathologic characteristics of primary or recurrent HSV infection reflect virus-mediated cellular death and associated inflammatory response.

PREVENTION

Frequent or severe recurrent HSV infections can be managed with suppressive oral aciclovir, valaciclovir and famciclovir therapy. The management of recurrent aciclovir-resistant HSV infections is not well established but may require maintenance therapy with foscarnet.

CLINICAL FEATURES AND DIAGNOSIS

The hallmark of herpetic lesions is painful vesicular formation at a mucocutaneous site, progressing rapidly to ulceration with an erythematous base, followed by eventual healing and re-epithelialization. Herpes simplex virus 1 most commonly causes orolabial lesions and HSV-2 generally infects the genital and perianal regions. However, there is considerable overlap in the epidemiology and clinical features of HSV-1 and HSV-2 infections. The clinical manifestations of HSV infections in HIV disease depend on:

- the subtype of HSV;
- the site of infection; and
- the degree of underlying immunosuppression.

As patients become more immunosuppressed, infections are characterized by prolonged viral shedding, more frequent episodes, and severe and persistent clinical disease. [26] [28] Diagnostic techniques available for the diagnosis of HSV infections include cytologic preparations (e.g. the Tzanck test) for multinucleated giant cells, fluorescein-conjugated monoclonal antibodies of scrapings from lesions, cell culture and PCR assays for HSV DNA.

Primary orolabial infection is more frequent in children who have AIDS, who are at risk of severe gingivostomatitis. In adults, orolabial infection is usually due to reactivation of latent infection. Recurrences may increase in frequency and severity as immunosuppression increases. Recurrent genital and perirectal ulcerative lesions are the most common manifestations of HSV infection in patients who have HIV disease and are usually due to reactivation of HSV-2. Lesions may be atypical and severe in patients who have advanced disease (Fig. 125.2). Prolonged new lesion formation, with continued tissue destruction, persistent viral shedding and severe local pain, is common.

Herpes simplex esophagitis may occur in the absence of herpetic lesions in the oropharynx and is difficult to distinguish from CMV esophagitis on clinical and radiologic grounds. Both cause retrosternal pain and dysphagia. Definitive diagnosis requires endoscopy and biopsy. Herpes encephalitis is a rare, life-threatening complication in HIV disease. It usually occurs as a complication of primary or reactivated orolabial HSV infection. Symptoms include headache, personality changes, meningismus, lethargy, confusion, focal neurologic deficits and fits. Computerized tomography (CT) scans or MRI may reveal temporal lobe abnormalities. Cerebrospinal fluid findings are usually non-specific, with a lymphocytosis and elevated protein. Detection of HSV DNA by PCR is highly sensitive and specific for HSV encephalitis.[30]

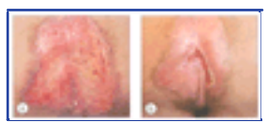

Figure 125-2 Severe perianal aciclovir-resistant herpes simplex virus 2 infection. (a) Untreated appearance. (b) Healing and re-epithelialization after treatment with foscarnet and institution of HAART.

MANAGEMENT

For over 20 years, aciclovir has been the drug of choice for treating HSV infections. Aciclovir undergoes selective phosphorylation by virus-induced thymidine kinase in HSV-infected cells. Aciclovir monophosphate is further phosphorylated by cellular kinases to the active triphosphate form, which selectively inhibits viral DNA polymerase. Bioavailability of oral aciclovir is approximately 10–20%. The optimum route of administration, dosage and duration of therapy will depend on the site and severity of the HSV infection (Table 125.3).

Valaciclovir and famciclovir are newer, effective and convenient alternatives agents for episodic and suppressive treatment of HSV infection. Oral valaciclovir is rapidly metabolized to aciclovir by the liver achieving a bioavailability of 3–5 times that of oral aciclovir. Famciclovir is a prodrug of penciclovir, an acyclic nucleoside similar to aciclovir but with a significantly longer intracellular half-life.

Infections with HSV that are resistant to aciclovir are increasingly recognized in patients who have advanced HIV disease.[31] Most aciclovir-resistant HSV isolates are deficient in thymidine kinase activity. Persistent ulcerative HSV lesions that fail to respond to aciclovir should alert the physician to the possibility of aciclovir resistance. The degree of immunosuppression and chronicity of mucocutaneous HSV infection are the strongest independent risk factors for the development of resistance in vitro. Foscarnet, which does not require viral-mediated phosphorylation for activity, is the treatment of choice for aciclovir-resistant HSV infections in HIV disease. Topical trifluorothymidine solution and cidofovir gel have also been shown to be effective against aciclovir-resistant HSV infections.

VARICELLA-ZOSTER VIRUS INFECTIONS

EPIDEMIOLOGY

Primary varicella or chickenpox is a common childhood infection. In the USA and Europe, most adults who have HIV disease have previously been infected with varicella-zoster virus (VZV). Among

TABLE 125-3 -- Treatment of herpes simplex virus and varicella-zoster virus infections in HIV-infected patients.

TREATMENT OF HSV AND VZV INFECTIONS IN HIV-INFECTED PATIENTS	
Type of infection	**Treatment**
Mucocutaneous HSV infection	Aciclovir 200–400mg po 5 times/d
	Famciclovir 500mg po q8h
	Valaciclovir 500mg po q12h
	Aciclovir 15–30mg/kg/day iv
Disseminated/visceral HSV infection	Aciclovir 30mg/kg/day iv
Recurrent mucocutaneous HSV infection	Aciclovir 400mg po bid to q6h
	Valaciclovir 500mg po q12h
	Famciclovir 500mg bid po q12h
Aciclovir-resistant HSV infection	Foscarnet 60mg/kg q12h iv
	Topical trifluorothymidine 2% q8h
Primary VZV infection (varicella)	Aciclovir 800mg po 5 times/day
	Aciclovir 10mg/kg/q8h iv
Herpes zoster	Aciclovir 800mg po 5 times/d
	Aciclovir 10mg/kg/q8h iv
	Valaciclovir 500mg po q12h
	Famciclovir 500mg po q8h
Disseminated/visceral VZV infection	Aciclovir 10mg/kg/q8h iv
Aciclovir-resistant VZV infection	Foscarnet 60mg/kg q12h iv
Treatment for HSV and VZV is usually for 5–10 days or until resolution of symptoms.	

HIV-infected children and adults, the manifestations of VZV infections include:

- uncomplicated primary varicella;
- disseminated varicella;
- localized and disseminated herpes zoster; and
- chronic varicella-zoster.

PATHOGENESIS AND PATHOLOGY

Primary VZV infection is usually acquired in susceptible hosts by contact with persons infected with chickenpox, either via the respiratory route or through contact with cutaneous lesions. It can also be acquired by contact with patients who have herpes zoster. Following initial replication, patients become viremic and develop the characteristic vesicular rash of varicella. Crops of new vesicles occur with successive episodes of viremia. Patients who have HIV disease can have prolonged viremia and an extended period of new lesion formation.

During primary infection, VZV enters cutaneous endings of sensory nerves and migrates to reach sensory nerve ganglia, where the virus establishes latency. With the waning of cellular immunity, the virus can reactivate, causing herpes zoster. The vesicular eruption of zoster typically remains confined to one dermatome or to several contiguous dermatomes, corresponding to the distribution of innervation of the affected sensory ganglion.

CLINICAL FEATURES AND DIAGNOSIS

Primary varicella zoster virus infection (Varicella)

The rash of primary VZV infection appears 10–21 days after infection. Lesions progress from small erythematous macules to papules and vesicles that ulcerate, dry and form crusts. A centripetal distribution, successive crops of lesions and lesions at all stages of development are characteristic of varicella. Owing to impaired cellular immunity, HIV-infected patients who have primary VZV are at risk of prolonged new lesion formation and are a higher risk of life-threatening visceral dissemination.

Herpes zoster

Herpes zoster may be the first indication of HIV disease and can occur at any stage of HIV infection. It usually appears as a localized or segmental painful erythematous maculopapular eruption along a single dermatome (Fig. 125.3). Lesions evolve over 1–3 days to form vesicles, pustules and crusts. In HIV-infected patients, zoster lesions may be particularly bullous, hemorrhagic or necrotic. Herpes zoster of the ophthalmic division of the trigeminal nerve can cause anterior uveitis and corneal scarring with visual loss. The diagnosis of herpes zoster is usually clinical but laboratory studies

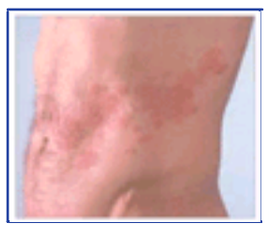

Figure 125-3 Herpes zoster in the T10 dermatome. *Courtesy of Professor Anthony J Pinching.*

may be required for confirmation. A Tzanck preparation from scrapings of lesions can demonstrate multinucleated giant cells but is not specific for herpes zoster. Fluorescein-conjugated monoclonal antibodies confirm the presence of VZV from scrapings from the base of lesions and their presence is a more rapid and reliable test than virus culture.

Patients infected with HIV are at risk of recurrent episodes of herpes zoster. A less frequent manifestation is persistent localized herpes zoster. These patients typically fail to clear lesions with aciclovir, or lesions recur rapidly after treatment is completed. Occasionally, widespread cutaneous and visceral dissemination may occur.

Visceral dissemination to the lungs, the liver and the central nervous system may cause life-threatening disease. The symptoms, signs and chest radiograph of VZV pneumonitis are non-specific. Encephalitis is a rare complication of VZV infection. Symptoms develop 1–2 weeks after the development of herpes zoster, and include headache and lethargy. Pathologically it is characterized by necrotic and demyelinating lesions, mostly in superficial white matter, the periventricular area and white-gray matter junctions. Cerebrospinal fluid findings may be non-specific, showing a mild mononuclear pleocytosis and elevated protein. Detection of VZV DNA by PCR analysis is diagnostic. Chronic disseminated herpes zoster may present as widespread erythematous ulcerative or hyperkeratotic verrucous lesions, particularly after prolonged treatment with aciclovir. Rapidly progressive herpetic retinal necrosis syndrome is a rare complication that is most often associated with VZV infection. Aggressive antiviral treatment is required to prevent loss of vision.[32]

MANAGEMENT

Primary VZV infection or herpes zoster in HIV-infected patients must be treated with specific antiviral therapy. Higher concentrations of aciclovir are required to inhibit replication of VZV than of HSV (see Table 125.3). Aciclovir significantly reduces pain and shortens the duration of viral shedding, the duration of new lesion formation, the time to crusting of lesions and the time to complete healing. High-dose oral aciclovir, 800mg five times daily, may be used for the treatment of localized herpes zoster in HIV-infected patients who do not require hospitalization. In severe or disseminated VZV infection, high-dose intravenous aciclovir (10mg/kg q8h) is indicated. Famciclovir and valaciclovir are licensed for use in non-immunocompromised patients who have herpes zoster.

Persistent disseminated VZV infection that fails to respond to aciclovir has been described in patients who have advanced HIV disease. In-vitro susceptibility studies have demonstrated isolates that are resistant to aciclovir, usually because of deficient or altered thymidine kinase activity. Intravenous foscarnet is the antiviral drug of choice for infection with aciclovir-resistant VZV.

For HIV infected patients who develop recurrent VZV lesions when aciclovir is discontinued, chronic suppressive aciclovir is indicated.

INFECTION WITH HEPATITIS C VIRUS

EPIDEMIOLOGY

With improved life expectancy among HIV-infected patients as a result of HAART, hepatitis C virus (HCV) infection is emerging as a leading cause of significant morbidity and death in this population. Because of shared risk factors, concomitant HCV and HIV infection is common. A recent study conducted in the USA estimated an overall prevalence of HCV 16% in a nationally distributed HIV cohort, rising to 73% in higher risk populations such as hemophiliacs and injection drug

users.[33] There are six known genotypes of HCV. In a recent study from the USA, type 1 was the most prevalent in co-infected patients and was found in 83%. Chronic HCV infection occurs in about 85% of patients, with cirrhosis and end-stage liver failure eventually developing in approximately 20% of infected individuals in two to three decades.[34] Co-infection with HIV accelerates HCV-related liver disease, and end-stage liver disease morbidity and mortality are greater in co-infected individuals.[35] Conversely, HCV may be independently associated with an increased risk of progression to AIDS and death.[36]

CLINICAL FEATURES AND DIAGNOSIS

Most patients who have acute HCV infection are symptom free, with a minority becoming jaundiced. It is usually with the development of chronic liver disease or extrahepatic manifestations that patients develop symptoms. Virtually all patients who have chronic HCV infection develop histologic features of chronic hepatitis, with as many as 20% progressing to cirrhosis over 20 years. Hepatitis C virus infection is readily diagnosed by enzyme-linked immunosorbent assay (ELISA) with specificity ensured using a recombinant immunoblot assay (RIBA). Signal amplification assays and PCR-based assays allow qualitative and quantitative detection of HCV RNA in plasma. Liver biopsy is an important tool to determine the degree of inflammation and fibrosis.

MANAGEMENT

In recent years significant advances have been made in the management of chronic HCV infection. Treatment is recommended for patients who have elevated alanine aminotransferase (ALT) levels, detectable serum HCV RNA and moderate to severe injury on liver biopsy. Table 125.4 outlines treatment options for HCV.

In 1998, two pivotal studies established the effectiveness of combination therapy with interferon-a-2b (3MU three times weekly) and ribavirin (1000–1200mg daily) for HCV infection in HIV-negative patients.[37] [38] Sustained virologic response rates were observed in 38–43% of patients at 48 weeks with improvement in liver inflammation on histologic examination. Genotypes 2 and 3 show the most favorable responses to treatment. Slow-release a-interferons, covalently bound to polyethylene glycol (PEG), a nontoxic

TABLE 125-4 -- Treatment options for chronic hepatitis B virus and hepatitis C virus infection in non-HIV-infected and HIV-infected patients.

TREATMENT OPTIONS FOR CHRONIC HBV AND HCV INFECTION IN NON-HIV-INFECTED AND HIV-INFECTED PATIENTS		
	Dosing schedule	**Response/comments**
Hepatitis B		
Interferon-a-2b	5MU C q24h or 10MU weekly for 4 months	HBeAg loss/seroconversion in 33%
Lamivudine	100mg po q24h for 1 year	HBeAg loss/seroconversion in 17%
		Prolonged use associated with genotypic-resistant variants
		Licensed for HIV infection at 300mg daily
Adefovir dipoxivil	10mg po q24h	Effective against lamivudine resistant HBV variants
		Mean drop in HBV DNA, -4.77$\log_{10}$, in HIV co-infected patients
Tenofovir	300mg po q24h	Licensed for HIV infection
Hepatitis C		
Interferon-a-2b and ribavirin	Interferon-a-2b 3MU 3 times weekly, ribavirin 1000–1200mg q24h for 6–12 months	SVR in 38–43%
		Higher response rates with genotypes 2 and 3
Pegylated interferon and ribavirin	Ribavirin 800–1200mg q24h; peg-IFN-a-2b (Peg-Intron) 1.5µg/kg or peg-IFN-a-2a (Pegasys) 180µg per week	SVR in 54–56%
		Higher response rates with genotypes 2 and 3
		SVR 44% in HIV/HCV co-infected patients (Pegasys plus ribavirin)
SVR, sustained virologic response.		

polymer, have been developed that allow for more convenient once-weekly injections. Pegylated interferon with ribavirin is now the standard of care in treating chronic HCV infection, with overall sustained virologic response rates of 54–56%.[39] [40]

Data are limited to date on treatment of HIV/HCV co-infected patients. Pegylated interferon-a-2a plus ribavirin has recently been reported to lead to significant treatment responses compared with conventional interferon-a-2a plus ribavirin in HIV/HCV co-infected patients.[41] In co-infected patients, progression of liver disease is associated with a $CD4^+$ lymphocyte count of less than 400cells/mm^3 and patients who have higher $CD4^+$ counts demonstrate greater response rates to HCV treatment.[42] Therefore, in co-infected patients treatment for HCV should be considered before significant decline in immune function occurs or, in those who have low $CD4^+$ counts, antiretroviral therapy should be a priority prior to HCV treatment.

INFECTION WITH HEPATITIS B VIRUS

EPIDEMIOLOGY

Hepatitis B virus (HBV) is a major world health problem and a common cause of cirrhosis and hepatocellular carcinoma. Because of shared risk factors, concomitant HBV and HIV infection is common, particularly in homosexual men. The outcome of acute HBV infection depends on the age of acquisition, the immune status of the host and the rate of replication of the virus. Less than 5% of adult-acquired infection progresses to chronic infection (chronic active hepatitis or asymptomatic carriers). In HIV-infected patients plasma HBV DNA levels are higher than in controls, [43] and recent data suggest that chronic HBV infection increases the risk of liver-related deaths in patients co-infected with HIV.[44]

CLINICAL FEATURES AND DIAGNOSIS

The clinical presentation of acute HBV ranges from subclinical hepatitis (70%) to icteric hepatitis (30%) with rare cases of fulminant hepatitis (0.1–0.5%). Chronic HBV ranges from the asymptomatic carrier through chronic active HBV to cirrhosis and hepatocellular carcinoma.

Serologic markers of HBV infection are well established. The persistence of hepatitis B surface antigen beyond 6 months suggests progression to chronic HBV infection. Hepatitis B e antigen (HBeAg) is a marker of active HBV replication and infectivity and is usually associated with active liver disease. The presence of serum HBV DNA (using signal amplification or PCR-based assays) is sensitive and specific for viral replication and is used to assess patients who have chronic HBV for treatment and to evaluate response.

MANAGEMENT

To date interferon-a, lamivudine and adefovir are the only agents approved for the treatment of chronic HBV (see Table 125.4). Viral clearance (HBeAg loss/seroconversion) occurs in 33% of those treated with interferon-a and 16–18% treated with lamivudine for 1 year. [45] [46] [47] Patients who have lower ALT levels or higher HBV DNA levels and immunosuppressed patients have a poorer response. Lamivudine profoundly suppresses viral replication and with prolonged use achieves an HBeAg seroconversion rate similar to interferon-a. It is also effective in patients not usually responsive to interferon. However, genotypic-resistant mutations emerge after 9–10 months of treatment with a frequency of 50% after 3 years. With both treatments, virologic responses are associated with biochemical and histologic improvement.

Adefovir dipivoxil, an adenine nucleotide analogue, has demonstrated encouraging preliminary results against HBV.[48] Moreover, it is active against lamivudine-resistant strains of HBV. Tenofovir, another nucleotide analogue, which is licensed to treat HIV, is being investigated for treatment of HBV and produces substantial reductions in HBV viral load in co-infected patients. Studies continue to define and refine optimal treatment regimens, and combination therapy studies with approved agents and experimental agents are now in progress.

OTHER VIRAL INFECTIONS

A variety of other viral infections and diseases occur in patients who have HIV disease (see Table 125.1). Those viruses implicated in neoplastic conditions, such as Epstein-Barr virus, human papillomavirus and human herpes virus 8, are discussed in detail in other sections (see Chapter 130). A brief summary of other viral conditions encountered in HIV-infected patients is outlined here.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

Progressive multifocal leukoencephalopathy (PML) is an opportunistic demyelinating infection caused by JC virus. JC virus is a ubiquitous DNA papovavirus and over 70% of adults are seropositive for it. Progressive multifocal leukoencephalopathy occurs in patients who have deficient cell-mediated immunity and is estimated to affect up to 4% of patients who have AIDS.[49] Mortality is high, and average reported survival in AIDS patients is 2–4 months. The symptoms and characteristic radiologic findings of PML are due to virus-induced lysis of oligodendrocytes, resulting in microscopic foci of myelin breakdown that coalesce to produce larger white matter

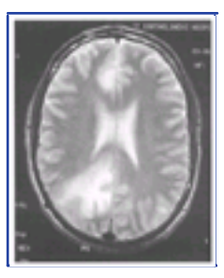

Figure 125-4 Progressive multifocal leukoencephalopathy. MRI scan showing frontal and occipital white matter lesions. *Courtesy of Dr Jane Anderson.*

lesions (Fig. 125.4). Definitive diagnosis requires tissue from brain biopsy but the identification of JC virus in the cerebrospinal fluid by PCR has a high specificity for active disease.

There is no definitive treatment for PML. Aciclovir, vidarabine and cytarabine have all been used to little effect. Cidofovir is active against polyomaviruses and several case reports have suggested therapeutic benefit in PML using dosing regimens recommended for CMV retinitis.[50] Immune reconstitution following antiretroviral therapy may influence the course of PML in HIV-infected patients, and there are several reports of regression of PML and prolonged survival in patients receiving HAART. Optimal retroviral therapy is now regarded as treatment of choice for PML.

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum is caused by a double stranded DNA virus, namely molluscum contagiosum virus (MCV). Molecular epidemiology studies have identified at least two major subtypes, MCV-1 and MCV-2. The majority of infections are caused by MCV-1. Cell-mediated immunity is important in the control of molluscum contagiosum and lesions occur in 5–18% of patients. Pathologic features consist of focal areas of hyperplastic and hypertrophied epidermis surrounding a core of keratin and epithelial debris. Ultrastructural studies have demonstrated MCV in all layers of the epidermis.

Lesions appear as small white or pink cutaneous papules. Larger lesions may be umbilicated. In HIV-infected patients, molluscum contagiosum may be extensive, large and disfiguring, and lesions are commonly localized around the head and neck.[51]

Diagnosis is usually made on the basis of the characteristic clinical features. Treatment involves direct disruption of lesions with enucleation, cauterization and cryotherapy. Treatment in HIV-infected patients is less satisfactory owing to the widespread nature of the lesions and the high rate of recurrence. Recalcitrant cases have been reported to respond to topical cidofovir cream 1–2%[52] and topical imiquimod 5% cream, an immune response modifier with antiviral properties. [53] Remission may occur after immune reconstitution with HAART.

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Chapter 126 - Fungal Infection

William G Powderly

Fungi are among the most ubiquitous pathogens seen in patients who have HIV disease but are not the most common causes of mortality. Virtually all major fungal pathogens cause disease in patients who have HIV infection.

CANDIDIASIS

EPIDEMIOLOGY

Candidal infection in AIDS is almost exclusively mucosal — systemic invasion is a rare and late event. Oropharyngeal candidiasis occurs in about three-quarters of all those who have HIV infection. In about one-third it tends to be recurrent and becomes progressively more severe with increasing immunodeficiency. Esophageal involvement occurs in 20–40% of all AIDS patients, predominantly in patients who have advanced disease and severe depletion of $CD4^+$ lymphocytes. Vulvovaginal candidiasis occurs in about 30–40% of women who have HIV infection; it appears that HIV infection per se is not an important risk factor for vaginal infection, although it may influence the severity and persistence of disease.

Most candidal disease, especially initial episodes, is associated with infection by *Candida albicans*. Recurrent disease is caused by the same strain of *Candida* in about 50% of cases; the remaining 50% are caused by new strains of *C. albicans* or new species.[1] Other species, notably *Candida glabrata*, *C. dubliniensis* and *C. parapsilosis*, tend to cause infection in patients who have very advanced disease and have had extensive previous exposure to antifungal agents (especially the azoles; see Chapter 237).

PATHOGENESIS AND PATHOLOGY

Most patients are readily colonized with *Canadia* spp., which appears as part of the mouth flora in over 80% of people who have HIV infection. The specific local immunologic defects that predispose to disease are unknown, although it is assumed that some deficiency in T cell immunity is important because disease is clearly more common as the $CD4^+$ lymphocyte count falls. Additional defects in local oral clearance mechanisms (e.g. epithelial barriers, salivary flow, lysozyme and lactoferrin release) have been described in people who have HIV infection and may be relevant to candidiasis. In addition, non-specific factors such as dental hygiene, smoking and the use of antibacterials for prophylaxis may predispose to candidiasis.

CLINICAL FEATURES

Most patients are symptomatic and complain of some oral discomfort. The classic presentation is of creamy-white plaques on an erythematous base — the pseudomembranous form of thrush (Fig. 126.1). Other manifestations include:

- an atrophic form that presents as erythema without plaques (often associated with patchy atrophic glossitis); and
- angular cheilitis, which appears as cracking, fissuring, ulceration or erythema at the corner of the mouth.

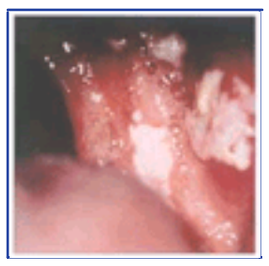

Figure 126-1 Pseudomembranous oral candidiasis ('thrush').

Most patients who have vaginal candidiasis present with vaginal itching, burning or pain, and usually complain of a vaginal discharge. Examination of the vaginal cavity usually reveals thrush, identical to that seen in the oropharynx.

Patients who have esophageal candidiasis develop ulcers and erosions of the esophagus and experience odynophagia or dysphagia. The combination of oral candidiasis and esophageal symptoms is both specific and sensitive in predicting esophageal involvement. Patients can be treated empirically with antifungal therapy. Endoscopy is reserved for those patients who fail to respond to evaluate for other diagnoses such as herpetic or cytomegalovirus esophagitis, idiopathic ulceration or resistant candidiasis.[2]

MANAGEMENT

The development of oral candidiasis in an HIV-positive patient should be taken as a sign of progressive immunodeficiency. Patients should have a $CD4^+$ count measured. If they are not currently receiving antiretroviral therapy, it should be initiated. If they are on antiretroviral therapy, it should be reassessed and, if necessary, changed.

A number of options — both local and systemic — are available for the treatment of oral candidiasis (Table 126.1). Initially, most patients respond well clinically to any form of antifungal therapy, although mycologic responses are less common. In general, topical therapy should be used initially and systemic therapy reserved for more difficult problems such as treatment failure or noncompliant patients. Of the local therapies, troches are generally used more effectively by patients than suspensions. Episodes of vulvovaginal candidiasis are also managed readily with topical therapy or short courses of systemic azoles. Esophageal disease requires systemic therapy. Fluconazole 200–400mg q24h orally for 2–3 weeks is probably the therapy of choice; [3] itraconazole 100–200mg q12h orally is also effective.

Relapses are common and at least one-third of patients develop recurrent mucosal candidiasis. One approach to management is to

TABLE 126-1 -- Therapeutic options for oral or esophageal candidiasis.

THERAPEUTIC OPTIONS FOR ORAL OR ESOPHAGEAL CANDIDIASIS		
Agent	**Formulation**	**Dosage**
Nystatin	Oral suspension	400–600 000 units (4–6ml) q6h
	Pastille	1–2 pastilles q6h
Glotrimazole	Troche	10mg (1 troche) 5 times daily
Ketoconazole	Tablet	200mg q24h
		400–600mg q24h (esophageal disease)
Fluconazole	Tablet	50–100mg q24h
		200–400mg q24h (esophageal disease)
	Oral suspension	50–100mg (5–10ml) q24h
Itraconazole	Capsule	200mg q24h
	Oral suspension	100mg (10ml) q12h

Amphotericin B	Oral suspension	500mg (5ml) q6h-q8h
	Lozenge	One q6h
	Intravenous infusion	0.3–0.5 mg/kg q24h
Not all formulations are widely available. Dosage is the usual dose for the typical patient — the average duration of therapy is 7–14 days and individual patients may require higher doses or more prolonged treatment.		

treat each episode as it occurs. However, in many patients, recurrent symptomatic disease may be sufficiently severe to warrant considering chronic suppression. Fluconazole 100–200mg q24h has proved highly successful in suppressing recurrent oropharyngeal disease and preventing esophagitis, and a dose of 100mg per week can prevent vaginal candidiasis. The major risk of this approach is the possibility of developing azole-resistant disease.

Approximately 5–7% of patients who have advanced HIV disease (usually resistant to available antiretroviral therapy) develop candidiasis that is refractory to standard fluconazole therapy. The major risk factors are:

- advanced immunodeficiency ($CD4^+$ lymphocyte counts less than 50 cells/mm^3 and often less than 10 cells/mm^3), and
- extensive previous exposure to fluconazole.[4]

Isolates also tend to be resistant in vitro, with minimum inhibitory concentrations of more than 64mg/ml to fluconazole. The clinical expression of disease is a progressive and symptomatic infection with frequent esophagitis.

Therapy for resistant candidal infection is unsatisfactory. Improving immune function (e.g. with antiretroviral therapy) is the best strategy, but options may be limited. Higher doses of fluconazole (up to 800mg) may be effective in patients who have infection caused by organisms with intermediate susceptibility but are usually not effective for truly resistant strains. The cyclodextrin oral suspension formulation of itraconazole has been reported to be effective in about 60% of cases but the benefit is short-lived if some form of chronic maintenance therapy is not given.[5] Many patients require parenteral amphotericin B or caspofungin for symptomatic control.

CRYPTOCOCCOSIS

EPIDEMIOLOGY

Virtually all HIV-associated infection is caused by *Cryptococcus neoformans* var. *neoformans*. The organism is found worldwide as a soil organism. About 5% of patients who have advanced HIV infection in the Western world develop disseminated cryptococcosis; the disease is more prevalent in sub-Saharan Africa and southern Asia. Most cases of infection occur in patients who have very low $CD4^+$ lymphocyte counts (<50,000/ml).

PATHOGENESIS AND PATHOLOGY

It is assumed that transmission occurs via inhalation of the basidiospores or unencapsulated forms, leading to colonization of the airways and subsequent respiratory infection. The absence of an intact cell-mediated response results in ineffective ingestion and killing of the organism, leading to dissemination and an increased cryptococcal burden. The polysaccharide capsule, composed mainly of glucuronoxylomannan, is thought to be the organism's primary virulence factor. It is unclear whether cryptococcal infection in patients who have AIDS represents an acute primary infection or reactivation of previously dormant disease.

CLINICAL FEATURES

Cryptococcosis most commonly presents as a subacute meningitis or meningoencephalitis with fever, malaise and headache.[6] Symptoms are usually present for 2–4 weeks before diagnosis. Classic meningeal symptoms and signs (such as neck stiffness or photophobia) occur in about one-third of patients. Some patients may present with encephalopathic symptoms such as lethargy, altered mentation, personality changes and memory loss. Analysis of the cerebrospinal fluid (CSF) usually shows:

- mildly elevated serum protein;
- normal or slightly low glucose;
- a few lymphocytes; and
- numerous organisms.

Cryptococcal antigen is almost invariably detectable in the CSF at high titer. The CSF opening pressure is elevated in 25% of patients, and this has important prognostic and therapeutic implications.

About one-half of patients have evidence of pulmonary involvement with cough or dyspnea and abnormal chest radiograms. Although most patients who have pneumonic involvement also have disseminated infection, isolated pulmonary involvement may be one of the earliest manifestations of cryptococcal infection. Most patients who have cryptococcal meningitis have positive blood cultures. Skin involvement is common and several types of skin lesion have been described; the most common form is that resembling molluscum contagiosum (Fig. 126.2).

DIAGNOSIS

The latex agglutination test for cryptococcal polysaccharide antigen in the serum is highly sensitive and specific for diagnosing of infection with *C. neoformans,* and a positive serum cryptococcal antigen titer

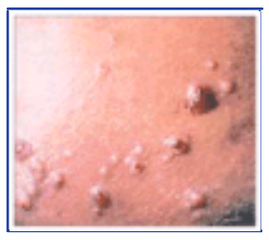

Figure 126-2 Cutaneous cryptococcosis. This lesion is typical of the skin lesions of most endemic mycoses that occur in patients who have AIDS, and such a lesion may therefore also be seen in an AIDS patient who has disseminated histoplasmosis or penicilliosis.

of more than 1:8 is presumptive evidence of cryptococcal infection. The serum cryptococcal antigen can therefore be used as a screening test for the presence of cryptococcal infection in febrile patients who have HIV infection. The finding of a positive cryptococcal antigen titer in this setting is sufficient to warrant antifungal therapy.

MANAGEMENT

An algorithm for the treatment of cryptococcal meningitis is shown in Figure 126.3 . Untreated, cryptococcal meningitis is fatal. Amphotericin B (0.7mg/kg intravenously) given for 2 weeks followed by fluconazole 400mg orally for a further 8 weeks is associated with the best outcome in prospective trials, with a mortality of less than 10% and a mycologic response of approximately 70%.[7] The addition of flucytosine 100mg/kg q24h to the amphotericin B phase does not improve immediate outcome but may decrease the risk of relapse. Initial azole therapy (itraconazole or fluconazole) is associated with a suboptimal 50% response rate. Early results with a combination of fluconazole 400–800mg q24h with flucytosine and the liposomal formulation of amphotericin B suggest that these may be options for patients who are unable to tolerate the usual formulation of amphotericin B.

Clinical deterioration may be due to cerebral edema, which may be diagnosed by a raised opening CSF pressure. Recent studies have linked elevated opening pressures (>200mmH_2 O) with an increased risk of early mortality and/or blindness.[8] The opening pressure of all patients who have cryptococcal meningitis should be measured when a lumbar puncture is performed, and strong consideration should be given to reducing such pressure (by repeated lumbar puncture,

Figure 126-3 Algorithm for the diagnosis and treatment of cryptococcal meningitis.

a lumbar drain or a shunt) if the opening pressure is high (>200mmH_2 O).

Cryptococcal meningitis requires lifelong suppressive therapy, unless there is improvement in immune function. Prospective trials have established the superiority of fluconazole 200mg q24h when compared with placebo, weekly amphotericin B or itraconazole.[9] Prospective trials have also established that fluconazole, in dosages ranging from 400mg per week to 200mg q24h, and itraconazole, 100mg q12h, are very effective in preventing invasive cryptococcal infections, especially in patients who have $CD4^+$ lymphocyte counts below 50–100 cells/mm^3 ,[10] and fluconazole use has probably resulted in the reduced incidence of cryptococcosis as a

complication of AIDS. Because of the relative infrequency of invasive fungal infections, antifungal prophylaxis is not associated with a survival advantage.

All patients who have cryptococcal infection should receive optimal antiretroviral therapy. An immune reconstitution syndrome has been described in some patients who initiate potent antiretroviral therapy after cryptococcal infection. Aseptic meningitis (with a prominent cellular reaction in the CSF) and lymphadenitis have been reported. Chronic suppressive therapy for cryptococcal infection may be discontinued in patients who have had at least 1 year of treatment and who have received successful antiretroviral therapy for at least 6 months. Such patients should have undetectable plasma HIV viral RNA and a rise in $CD4^+$ lymphocyte count to at least 100 cells/mm^3 .

HISTOPLASMOSIS AND PENICILLIOSIS

EPIDEMIOLOGY AND PATHOGENESIS

Histoplasmosis is caused by the dimorphic fungus *Histoplasma capsulatum,* which is endemic in the Mississippi and Ohio river valleys of North America as well as certain parts of Central and South America and the Caribbean (Fig. 126.4). Penicilliosis is caused by the dimorphic fungus *Penicillium marneffei*, which is endemic in South East Asia (especially northern Thailand and southern China; Fig. 126.5).

The mycelial form of histoplasmosis is found in the soil and is particularly associated with bird roosts and caves. The environmental niche for *P. marneffei* is unknown, although it is also assumed to be a soil organism. Both fungi cause disseminated infection in 20–30% of patients who have AIDS in endemic areas, as well as sporadic infection among HIV-positive migrants from and visitors to endemic areas.

Infection with both fungi results when spores are inhaled into the lung and these are then converted to the pathogenic yeast form at

Figure 126-4 North American Endemic areas for histoplasmosis and coccidioidomycosis.

Figure 126-5 Endemic area for penicilliosis.

TABLE 126-2 -- Characteristics of histoplasmosis and penicilliosis.

CHARACTERISTICS OF HISTOPLASMOSIS AND PENICILLIOSIS		
Characteristic	**Histoplasmosis**	**Penicilliosis**
Appearance of organism on biopsy	1–5µm round to oval	1–8µm pleomorphic, elongated
Method of duplication	Budding	Fission
Clinical features (% of cases)		
Fever	95	99
Weight loss	90	75
Anemia	70	75
Pulmonary disease	50	50
Lymphadenopathy	20	40–50
Skin lesions	5–10	70
Hepatosplenomegaly	25	50

body temperature. Usually, effective cell-mediated immunity limits acute infection to a mild respiratory illness. Patients who have HIV infection develop disseminated disease due to either reactivation of previously acquired infection or a progressive acute infection.

CLINICAL FEATURES

The characteristic features of histoplasmosis and penicilliosis are shown in Table 126.2 . The most common presentation of both infections is fever and weight loss, which occurs in about 75% of patients.[11] [12] Respiratory symptoms (cough, shortness of breath) occur in about 50% of cases. The chest radiogram is normal in 50% of cases and most of the remaining patients have diffuse nodular infiltrates. Local or generalized lymphadenopathy, hepatosplenomegaly, colonic lesions and skin and oral ulcers also occur. Skin involvement is more common in penicilliosis than histoplasmosis. Involvement of the gastrointestinal tract (usually as ulcers) may present with abdominal pain or gastrointestinal bleeding. Between 5% and 10% of patients have an acute septic-shock-like syndrome that includes hypotension and evidence of disseminated coagulopathy. This presentation carries a very poor prognosis. Laboratory findings may include anemia, neutropenia or thrombocytopenia (because of bone marrow involvement) and elevated hepatic enzymes.

DIAGNOSIS

The diagnosis is usually made by culture or by histopathologic examination of bone marrow aspirate or biopsy, lavage fluid or biopsy material from lung or skin lesions. A peripheral blood smear may show intracellular organisms in white cells in many patients. Blood cultures, especially when collected using the lysis centrifugation system, are positive in over 90% of patients. Anti-*H. capsulatum* antibodies are detected by immunodiffusion or complement fixation in about 70–80% and antigen detection in either urine or serum is an excellent method for diagnosing disseminated histoplasmosis. Serologic tests are not yet useful for diagnosing penicilliosis.

MANAGEMENT

The management of both infections appears to be similar. Patients should receive an initial period of treatment with amphotericin B (for 1–2 weeks) until there is clinical resolution (defervescence and improvement in skin lesions), followed by itraconazole 200mg orally q12h. A small study has suggested that initial treatment with liposomal amphotericin B (4 mg/kg q24h) was superior to amphotericin B deoxycholate for disseminated histoplasmosis suggesting this preparation should be considered for patients who have severe disease.[13] As with the other systemic mycoses, relapse is common and therefore long-term suppressive therapy with itraconazole 200mg q24h is warranted.[14] [15] Itraconazole can probably be used as sole therapy for mild disease.[16] Fluconazole is less effective in histoplasmosis than itraconazole. Itraconazole can also be considered as primary prophylaxis for histoplasmosis in patients residing in endemic areas, especially those with $CD4^+$ lymphocyte counts of less than 100 cells/mm^3 .

As with cryptococcal infection, all patients should receive optimal antiretroviral therapy. Immune reconstitution illness (with abdominal lymphadenopathy) has been described in histoplasmosis. Consideration can be given to stopping secondary prophylaxis in patients who have had immune recovery on potent antiretroviral

treatment.

COCCIDIOIDOMYCOSIS

EPIDEMIOLOGY AND PATHOGENESIS

Coccidioides immitis is a dimorphic fungus found in the soil in the desert around the south-western USA and northern Mexico (see Fig. 126.4), as well as focal areas of Central and South America, and primary infection is restricted to persons living in or visiting those areas (see Chapter 237). Infection follows inhalation of arthrospores, which form endospores and spherules within the lung. Mature spherules release new endospores, which perpetuate infection. Infection tends to occur in situations where soil is disturbed (e.g. construction sites, dry windy conditions), but is usually a self-limiting respiratory illness controlled by cell-mediated immunity. People who have HIV infection develop more severe, disseminated disease. Both

new primary infection and reactivation of previously acquired coccidioidomycosis can occur in patients who have HIV infection. Consequently, both individuals who have had previous coccidioidomycosis and those who have never been infected can develop progressive coccidioidal infections during the course of HIV infection. As with other endemic mycoses, migrants who have HIV infection and visitors to the endemic area are also at risk of infection.

CLINICAL FEATURES

Most patients, especially if their $CD4^+$ lymphocyte count is less than 250 cells/mm^3 , develop pneumonia, presenting with fever, weight loss, night sweats, cough and dyspnea with symptoms lasting from several weeks to several months before diagnosis.[17] Disseminated disease occurs in at least 30%, resulting in generalized lymphadenopathy, skin nodules or ulcers, peritonitis, liver abnormalities, and bone and joint involvement. Meningeal disease, with symptoms of lethargy, fever, headache, nausea, vomiting and/or confusion, occurs in about 10% of patients. Cerebrospinal fluid analysis typically reveals a lymphocytic pleocytosis with a lymphocyte count of more than 50/ml.

DIAGNOSIS

Diagnosis is made by culturing the organism from clinical specimens or by demonstrating the typical spherule on histopathologic examination. As *C. immitis* is a highly contagious organism and can infect laboratory workers handling specimens, the clinical laboratory should be warned of the possibility of positive cultures (see Chapter 90). Blood cultures are positive in a minority of patients. Coccidioidal serology is often positive, but may be negative in up to 25% of patients who have disseminated infection. Patients who present with fever and have positive coccidioidal serology without focal lesions are presumed to have coccidioidal infection.[18]

MANAGEMENT

Amphotericin B, 0.5–1.0mg/kg q24h, is the mainstay of therapy and should be used initially in patients who have diffuse pulmonary or disseminated disease. Some experts advocate combining amphotericin B with fluconazole initially. Fluconazole, 400–800mg q24h orally, may be an alternative for patients who have mild disease. Complete eradication is unlikely and chronic suppressive therapy with either fluconazole 200–400mg q24h or itraconazole 200mg q12h is needed. Successful treatment with itraconazole or fluconazole has been reported in approximately 80% patients who have *C. immitis* meningitis.

There is no evidence that chemoprophylaxis with any of the azole antifungals can prevent coccidioidomycosis. People living in or visiting endemic areas are advised to avoid activities that would increase their exposure to disturbed soil.

ASPERGILLOSIS

EPIDEMIOLOGY

Infection with *Aspergillus* spp. is increasingly seen in patients who have advanced HIV disease. Specific risk factors that have been identified include neutropenia, use of corticosteroids and broad-spectrum antibacterial therapy and previous pneumonia, especially *Pneumocystis carinii* pneumonia. There is also some evidence that aspergillosis may be a direct effect of advanced HIV disease and may occur in the absence of other predisposing factors. Typically, patients have extremely low $CD4^+$ lymphocyte counts and a history of other AIDS-defining opportunistic infections.

CLINICAL FEATURES, DIAGNOSIS AND MANAGEMENT

Two major syndromes predominate:

- respiratory tract disease, and
- central nervous system infection.[19] [20]

Patients often present with cough, shortness of breath and fever. Because aspergilli have a tendency to invade blood vessels and cause infarction, chest pain and hemoptysis are common. Nodular infiltrates, which may be localized or diffuse and commonly cavitate, are seen on chest radiography.

Diagnosis of invasive pulmonary aspergillosis may be difficult. A definitive diagnosis requires a biopsy demonstrating fungal invasion. A presumptive diagnosis of invasive aspergillosis may be made in patients who have pulmonary symptoms and new chest radiographic abnormalities and whose sputum or bronchial secretions grow aspergilli in culture. Additional respiratory syndromes of pulmonary aspergilloma and localized tracheobronchial aspergillosis have been reported occasionally in patients who have AIDS.

Patients who have central nervous system aspergillosis usually present with symptoms and signs of a mass lesion or with features of a stroke due to invasion of blood vessels. Therefore, seizures, hemiparesis and focal abnormalities are common. Computerized tomography or magnetic resonance imaging (MRI) of the head may show single or multiple lesions, usually nonenhancing, with surrounding edema. Bony invasion is common and, because the disease may have spread from involved sinuses, the sinuses may be abnormal. Patients who have fungal sinusitis usually have the classic features of sinusitis (fever, facial pain and swelling, nasal discharge and headache). Often, there is a history of previous sinus infection treated with broad-spectrum antibacterial therapy. As in other sites, aspergilli tend to invade locally. Computerized tomography of the sinuses will usually show bony erosion, and penetration into adjacent tissues such as the brain or orbit can occur.

The prognosis of aspergillosis is poor, in part because of the fungal infection itself and in part because it tends to occur in patients who have advanced AIDS and many other complications of end-stage HIV infection. There is a poorer response to therapy in patients who have AIDS than in other immunocompromised patients.[21] Therefore in most series the median time to death after a diagnosis of aspergillosis is only 2–4 months. Initial therapy should be with amphotericin B (1.0–1.5mg/kg/day of amphotericin B deoxycholate or 5–10mg/kg/day of liposomal amphotericin) or with voriconazole 200mg bid (see Chapter 208). A recent study[22] suggested voriconazole was superior to amphotericin B as initial therapy; however that study did not include many patients with AIDS.

OTHER FUNGAL INFECTIONS

The other endemic mycoses, blastomycosis and paracoccidioidomycosis, have been reported rarely in patients who have AIDS and do not appear to be major opportunists. Typically, there is disseminated disease.

There have also been case reports of disseminated sporotrichosis and localized mucormycosis in patients who have AIDS.

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Chapter 127 - Parasitic Infections

Christine Katlama

The use of highly active antiretroviral therapies has profoundly changed the epidemiology of the clinical manifestations of HIV disease. Overall, the incidence of opportunistic infections has decreased by 50–80%; however, toxoplasmosis remains frequent, often revealing AIDS and HIV infection in untreated patients; on the other hand cryptosporidiosis and microsporidiosis, which used to be common in the course of AIDS, are occurring much less frequently in patients with improved immune status because of potent antiretroviral therapy.

TOXOPLASMA GONDII

EPIDEMIOLOGY AND PATHOGENESIS

Toxoplasmosis, caused by *Toxoplasma gondii,* an obligate intracellular protozoan, is a common opportunistic infection in patients with AIDS. It has a prevalence ranging from 10–40% in Europe, the Caribbean area and Africa, to 5–10% in USA. This is due to the different prevalence of *T. gondii* infection in the general population of these areas (e.g. 50–70% in Europe, but 10–40% in the USA).[1] The widespread use of primary prophylaxis that is active against both *Pneumocystis carinii* and *T. gondii* in patients with HIV infection has decreased the incidence of these two most common opportunistic infections. The underlying immune cellular defect in HIV infection is the major cause of the reactivation of latent *T. gondii* infection that has persisted in the central nervous system (CNS) or extraneural tissues after earlier acute infection. Toxoplasmosis usually occurs late in HIV disease, when the $CD4^+$ lymphocyte count is less than $100/mm^3$. By now, given the impact of antiretroviral therapy in western countries and the use of systematic toxoplasmosis and *P. carinii* pneumonia prophylaxis as soon as CD4 cells count are below $200/mm^3$, most of the cases of toxoplasmosis are diagnosed in patients known to have HIV infection but without any medical follow-up (see also Chapter 245).

CLINICAL FEATURES

The CNS is by far the most common site of toxoplasmosis, representing approximately 80% of cases. The next most common site is the retina (5–10%); pneumonitis and myocarditis are less common manifestations.[2] Involvement of other organs, such as the liver and bladder, is usually a histologic or autopsy finding in the context of disseminated disease.

Encephalitis

Toxoplasmic encephalitis (TE) commonly manifests as single or multiple intracerebral abscesses, with focal neurologic signs and constitutional symptoms that progress over a few days or weeks.[3] [4] [5] Fever and headaches are present in 40–70% of cases, neurologic dysfunction including confusion and lethargy in 40% of cases, focal CNS deficits in 50–60% of cases, and seizures in 30–40% of cases, which frequently are the presenting symptom of TE.

The constellation of fever, headaches, mild neurologic deficit or any unexplained neurologic symptoms should suggest the diagnosis of TE (Fig. 127.1), and prompt computerized tomography (CT) scanning or magnetic resonance imaging (MRI) should be undertaken.

Retinitis

Toxoplasmic retinitis represents the third most common opportunistic infection of the retina in AIDS. Symptoms include decreased visual acuity, defects in the visual field, 'floaters' and loss of peripheral vision. Diagnosis relies on funduscopic examination (Fig. 127.2), which typically shows a thick, dense, opaque appearance of retinal lesions with very distinct borders, and an intense vitreal inflammation. There is usually little or no hemorrhage, which is the opposite of the situation in retinitis caused by cytomegalovirus.

Pneumonitis

Toxoplasmic pneumonitis occurs generally in a context of disseminated disease and presents as a bilateral interstitial pneumonia.[6] Clinical and radiologic symptoms are non-specific; they include fever, cough, dyspnea and interstitial radiologic abnormalities. Diagnosis is made by finding *T. gondii* cysts in bronchoalveolar fluid.

DIAGNOSIS

Because it occurs in AIDS as a result of reactivation of a latent preexisting infection caused by the immune suppression induced by HIV, a diagnosis of toxoplasmic disease should be suspected in patients with:

- a low $CD4^+$ lymphocyte count, usually less than $100/mm^3$;
- specific antitoxoplasmal antibodies, indicating past infection; and
- no specific current primary prophylaxis.

In patients with HIV infection and any neurologic symptoms, CNS imaging is the most urgent diagnostic procedure (see Fig. 127.1). Magnetic resonance imaging is more sensitive than CT scanning. Toxoplasmic abscesses are typically contrast-enhancing lesions surrounded by edema; there may be a mass effect, with displacement of the ventricles. Magnetic resonance imaging may also reveal hemorrhages, which are highly suggestive of toxoplasmic necrosis.

Although no neuroradiologic findings can be considered pathognomonic for TE, some findings, such as the presence of multiple lesions, a localization of lesions in the basal ganglia or at the corticomedullary junction, the presence of edema, a mass effect and hemorrhages, may help to distinguish TE from CNS lymphoma, which is the main differential diagnosis.

Antitoxoplasmic therapy leads to a decrease in number and size of the lesions within 10–15 days, and this may confirm the diagnosis. Mild radiologic sequelae with no clinical consequences may persist at the end of therapy. In the absence of any improvement, a cerebral biopsy should be performed to exclude a lymphoma or progressive multifocal leukoencephalopathy.

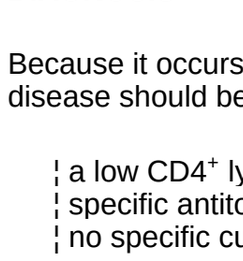

Figure 127-1 Diagnosis and management of cerebral toxoplasmosis in HIV-positive patients.

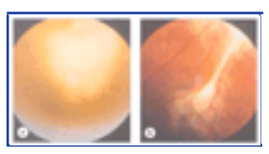

Figure 127-2 Toxoplasmic retinitis. (a) Diagnosis from fundoscopy. (b) Evolution after antitoxoplasma therapy.

The detection of *Toxoplasma* antibodies in patients with HIV infection has no significance other than the assessment of a previous exposure to the parasite, which indicates the potential risk of reactivation in case of severe immunodepression.

In most of cases of encephalitis or retinitis, the diagnosis of toxoplasmosis is presumptive, made on clinical-radiologic criteria and confirmed by the therapeutic response.

The identification of *T. gondii* in extraneural or extraretinal tissue or body fluid gives a definitive diagnosis of toxoplasmosis if a biopsy is performed. The characteristic

toxoplasmic lesions found on cerebral biopsy are necrotic abscesses surrounded by a prominent inflammatory infiltrate that contains extracellular *T. gondii* tachyzoites, edema, vasculitis and hemorrhages. Isolation of *T. gondii* from blood, cerebrospinal fluid, bronchoalveolar lavage fluid or by using polymerase chain reaction appears to be more frequent in disseminated disease than it is in localized disease.[7]

MANAGEMENT

Appropriate treatment is especially important, both because the response to therapy is the main criterion for diagnosis of TE, and because early initiation of therapy gives the best prognosis. Treatment consists of initial acute therapy over 3–6 weeks followed by lifelong maintenance therapy to prevent further reactivation (Table 127.1). Initiation of antiretroviral therapy should be preferentially delayed 3–4 weeks to avoid overlap in drug toxicities.

Sulfadiazine and pyrimethamine combination

The combination of pyrimethamine 50–75mg/day orally and sulfadiazine 4–6g/day is the first-line acute therapy.[3] [4] [5] These drugs act synergistically by blocking the folic acid pathway of tachyzoites, but have no effect on the cyst forms of the parasite. Folinic acid 25mg/day orally should be given as well to prevent hematotoxicity

Clinical improvement occurs within 5–10 days; the diagnosis of TE is confirmed by a decrease in both neuroradiologic and clinical abnormalities (Fig. 127.3). The duration of therapy is 3–6 weeks.

Fever and rash are the most common adverse events of pyrimethamine-sulfadiazine that may lead to discontinuation of therapy; they occur in 20–25% of cases. Other side-effects include hematotoxicity, crystalluria and transaminase elevation.

In the maintenance phase the recommended therapy is pyrimethamine 25mg/day orally plus sulfadiazine 2g/day orally lifelong or until a CD4 cell count of more than 200/mm^3 for more than 3 months is reached in cases of antiretroviral therapy. This combination is also effective as primary prophylaxis for *P. carinii* pneumonia.

Pyrimethamine plus clindamycin

The combination of pyrimethamine 50mg/day orally and clindamycin 2.4g/day orally or intravenously as acute therapy, followed by pyrimethamine 25mg/day orally plus clindamycin 1.2g/day orally as maintenance therapy[3] [4] is considered as second-line therapy for use in cases of intolerance to sulfadiazine, because it has a higher relapse rate (15%) than pyrimethamine-sulfadiazine (3%) in maintenance therapy.[4] [5] [6] Side-effects include rash (30%) and diarrhea (20%), and there is a risk of pseudomembranous colitis induced by clindamycin.

Atovaquone

Atovaquone 750mg q6h orally, a hydroxynaphthoquinone that is a potent in-vitro inhibitor of *T. gondii* and *P. carinii,*[8] [9] [10] may be used in acute or maintenance therapy for patients who are intolerant to the standard therapies mentioned above.[11] Although there have been no comparative controlled studies, the few available data suggest that atovaquone is less effective than standard therapies, with a 25% relapse rate that might be due to wide variations in plasma concentrations of the drug between patients and within the same patient. It is recommended that atovaquone should be combined with pyrimethamine.[10]

Macrolides

Clarithromycin 2g/day orally and azithromycin 500mg/day orally have been given in combination with pyrimethamine in small, open, noncomparative pilot studies. They have been found to have an efficacy rate of 50–70%.[11] However, these drugs are not considered to be major antitoxoplasma drugs.

PREVENTION

In patients with HIV infection who have negative toxoplasma serology, prevention measures to avoid contamination should be recommended (e.g. avoiding contacts with raw meat, cooking meat properly, washing vegetables). Control serology should be performed on a regular basis to warn that contamination might have occurred.

TABLE 127-1 -- Therapeutic management of toxoplasmosis.

THERAPEUTIC MANAGEMENT OF TOXOPLASMOSIS			
	Acute initial therapy	**Maintenance therapy**	**Notes**
First-choice treatment	Pyrimethamine 50mg/day plus sulfadiazine 4g/day po	Pyrimethamine 25mg/day plus sulfadiazine 2g/day po	Highest efficacy; also effective as prophylaxis for *P. carinii* pneumonia
Second-choice treatment	Pyrimethamine 50mg/day plus clindamycin 2.4g/day po or iv	Pyrimethamine 25mg/day plus clindamycin 1.2g/day po	Relapse rate is 25%; prophylaxis for *P. carinii* pneumonia should be added
Alternative treatment	Atovaquone 750mg q6h po	Atovaquone 750mg q6h po	Relapse rate is 25%; also effective as prophylaxis for *P. carinii* pneumonia
	Pyrimethamine-clarithromycin		

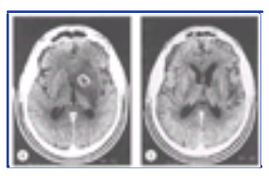

Figure 127-3 Toxoplasmic encephalitis. (a) CT scan at diagnosis. (b) Evolution of the disease after 42 days of treatment with pyrimethamine-sulfadiazine.

Primary prophylaxis is indicated in patients at high risk of infection with *T. gondii*. High-risk patients are defined as those who have a CD4$^+$ lymphocyte count of less than 200 cells/mm^3 and positive antitoxoplasmal serology.[12] The use of drugs having activity against both *P. carinii* and *T. gondii* should be a priority.

Trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole (co-trimoxazole) is effective in preventing both pneumocystosis and toxoplasmosis.[13] [14] The most commonly used regimens are trimethoprim 160mg and sulfamethoxazole 800mg every day orally or every second day, or trimethoprim 80mg and sulfamethoxazole 400mg every day. Main side-effects are rash and fever (in 5–20% of cases); toxicity appears to be dose-related. The superiority of trimethoprim-sulfamethoxazole over other prophylaxis for both pneumocystosis and toxoplasmosis justifies desensitization procedures in patients with moderate intolerance to these compounds; desensitization has a 50–60% success rate.

Dapsone-pyrimethamine

Recommended regimens are dapsone 50mg/day plus pyrimethamine 50mg per week.[15] A weekly regimen with dapsone 200mg orally plus pyrimethamine 75mg orally showed similar results.[16] However, side-effects are frequent, occurring in 25–70% of patients; rash, hematotoxicity and digestive intolerance are the most common. In clinical practice, dapsone-pyrimethamine offers almost no advantages over trimethoprim-sulfamethoxazole, and cross-intolerance between these two combinations is frequent.

Pyrimethamine

In cases of sulfadiazine intolerance, pyrimethamine alone 50mg three times per week orally may be used as primary prophylaxis in combination with folinic acid 25mg three times per week.[17] Pentamidine aerosol should be added, because pyrimethamine does not provide effective prophylaxis against *P. carinii* pneumonia.

Discontinuation of prophylactic regimen

Several observational and two randomized studies[18] [19] have shown that primary and secondary prophylaxis can be discontinued with minimal risk of developing TE in patients who have responded to highly active antiretroviral therapy (HAART) with an increase in $CD4^+$ T cells for at least 3 months. In case of changes in the immune state following failure or discontinuation of antiretroviral therapy that may lead to a decrease in CD4 cells, prophylaxis should be reintroduced if the $CD4^+$ T cell count decreases to less than 200 cells.

CRYPTOSPORIDIOSIS

EPIDEMIOLOGY

Cryptosporidiosis is a worldwide protozoan infection that is more prevalent in poorly developed countries. Cryptosporodiosis may affect patients with acute disease but more frequently patients with an immune deficit.[20] The prevalence of this infection in the context of HIV disease in Europe was 1–2% and in North America 3–4% before HAART, but the rate has fallen considerably given the generalized use of combined potent antiretroviral drugs in the year 2000; in Asia, Africa, and Central and South America it is 15–10%.[21] [22] Human cryptosporidiosis is generally caused by *Cryptosporidium parvum,* which is transmitted primarily by the fecal-oral route through person to person transmission or indirectly; outbreaks from municipal water, person to person transmission and animal to person transmission have been described. Healthy carriers of oocysts may play a major role in transmission.

CLINICAL FEATURES AND DIAGNOSIS

Diarrhea is the main clinical symptom, ranging in severity from mild diarrhea to cholera-like, watery diarrhea. Abdominal cramps, nausea, vomiting and anorexia usually accompany the diarrhea when it is moderate or severe. Fever is uncommon and may be due to other concurrent infections. The severity of the disease is more pronounced in patients with $CD4^+$ lymphocyte counts of less than 50/mm^3 . Cryptosporidiosis may involve the gallbladder, the biliary tract, the pancreatic ducts (leading to cholecystitis and pancreatitis), the bronchi and the lungs.

Diagnosis is based on the identification of the parasite in feces, in tissue specimens or in other fluids such as in bronchoalveolar lavage, using a modified acid-fast stain such as the Ziehl-Neelsen stain. Other techniques include Giemsa stain, fluorescent auramine-rhodamine, Sheather's sucrase flotation and indirect immunofluorescence.[22] Occasionally, cryptosporidiosis oocysts are associated with other pathogens, including *Entamoeba, Microsporidia* and *Giardia* spp. and cytomegalovirus.

MANAGEMENT AND PREVENTION

Treatment of cryptosporidiosis is essentially symptomatic. Efforts to find useful therapies have been impeded by the absence of an in-vitro model and the lack of a universally accepted animal model, and there is currently no agent that can reliably eradicate the organism. Among the various pharmaceutical agents that have been used, paromomycin,[23] azithromycin and nitazoxanide[24] have shown some efficacy in non-HIV related cryptosporidiasis. However, because the course of cryptosporidial disease depends mainly on immune status, the best option for therapy and prevention against chronic cryptosporidiosis is the restoration of immune function that has been observed as a consequence of potent antiretroviral therapy. Most of the devastating symptoms of cryptosporidiosis are observed in patients with severe immunosuppression and no antiviral therapy; initiation of antiretroviral therapy is the best option for treating cryptosporidiasis. This underlines clearly the role of the immune deficiency in the symptomatic disease. In the absence of specific effective treatment, the following preventive measures should be considered in patients whose $CD4^+$ lymphocyte count is less than 200/mm^3 :

- drinking boiled water or water filtered through a 1µm filter (bottled water may be an option);
- avoiding the consumption of raw fruits or vegetables that have been washed in unfiltered water; and
- avoiding contact with the feces of animals, or the wearing of gloves if such exposure cannot be avoided.

MICROSPORIDIOSIS

MICROBIOLOGY AND EPIDEMIOLOGY

Microsporidiosis is mainly seen in immunocompromised patients. [25] It is caused by an obligate intracellular protozoan parasite. Five genera have been reported in humans — *Enterocytozoon, Encephalitozoon* and *Septata* spp. are the most frequent; *Pleistophora* and *Nosema* spp. are uncommon. The source of human infection is unknown; contamination is thought to occur through ingestion of spores.

CLINICAL FEATURES AND DIAGNOSIS

The intestine is by far the most common site of microsporidiosis in patients with HIV infection, and 90% of cases are caused by *Enterocytozoon bieneusi*. Diarrhea of variable intensity between patients is the most frequent symptom; it may be accompanied by abdominal pain and cramps and nausea. Other localized forms of the disease include cholangitis and cholecystitis.[26] *Septata intestinalis* may cause a similar gastrointestinal syndrome or cholangitis, and it sometimes spreads to the urinary tract, when the organism may be be detectable in the urine.[27] *Nosema corneum* and *Encephalitozoon hellem* have been reported in corneal infection or keratoconjunctivitis.

Microsporidia can be identified in fluids (e.g. feces, urine, sinus mucus and bronchoalveolar lavage) using specific staining procedures such as modified trichrome, calcofluor or uvitex, and in tissue biopsies (e.g. intestinal and sinus biopsies) using staining procedures such as Giemsa, trichrome, periodic acid-Schiff or toluidine blue. Electron microscopy remains the reference diagnostic procedure that allows identification of the species of the organisms that are found (see Chapter 243).

MANAGEMENT

In a placebo controlled study, fumagillin (200mg q8h), an antiparasitic drug used in veterinary medicine, has been shown to be effective in clearing *E. bieneusi* in patients with HIV infection and chronic diarrhea related to this opportunistic infection. Bone marrow toxicity, mainly thrombocytopenia, is the principal side-effect of fumagillin.[28]

ISOSPORIASIS

Isosporiasis is a rare intestinal infection caused by *Isospora belli*. It is seen in immunocompetent patients in endemic areas — Central and South America, South East Asia and Africa — and in immunocompromised patients. Humans are the only known reservoir for *I. belli*. [29] The main route of transmission is thought to be oral absorption of food or water contaminated by *I. belli* oocysts. *Isospora belli* infection has been reported in less than 0.5% of AIDS patients in the USA, and in 15% of AIDS patients in Haiti and Africa. The generalized use of trimethoprim-sulfamethoxazole as prophylaxis against *P. carinii* pneumonia has lead to a large decrease in the prevalence of this infection in HIV-positive patients. Isosporiasis usually manifests in patients with HIV infection as chronic diarrhea that leads to malabsorption syndrome and weight loss.

Diagnosis is made by identification of *I. belli* cysts in specimens of stool; several stool samples must be examined (see Chapter 243).

Specific therapy of *I. belli* is trimethoprim-sulfamethoxazole (trimethoprim 160mg q12h, sulfamethoxazole 800mg q8h, for 10 days).[29] Long-term prophylaxis with trimethoprim-sulfamethoxazole (one double-strength tablet (trimethoprim 160mg and sulfamethoxazole 800mg) three times per week) is required to prevent relapse. Pyrimethamine (50–75mg/day) may be used in patients intolerant to sulfonamides.

LEISHMANIASIS

Visceral leishmaniasis (VL) in the course of HIV infection is mainly seen in southern Europe (Spain, Italy and the south of France) and south America (Venezuela and Brazil). It is estimated that 25–70% of adult VL cases in these areas are related to HIV infection and that 1.5–9% of AIDS patients develop newly acquired or reactivated VL. The majority of cases are due to *Leishmania infantum*. Visceral leishmaniasis usually occurs in late-stage HIV infection (when the $CD4^+$ lymphocyte count is less than $200/mm^3$), and it may be observed several years after the patient has left an endemic area.

The clinical manifestations are those of classic VL, with fever in 90% of cases. Other manifestations may include hepatomegaly and splenomegaly; digestive, pulmonary, or cutaneous disorders may be the only symptoms of VL. Biologic abnormalities include leukopenia and anemia, which may be at least partly due to the HIV infection.

Diagnosis relies on the identification of *Leishmania* spp. in a bone marrow aspirate; this test has a sensitivity of 95% for a first episode and 65% for relapses. Blood smears reveal the parasite in 50% of cases; this increases to 70% after culture of white blood cells. Serology has only limited diagnostic value, with only 35–50% positivity.

Acute therapy consists of meglumine antimoniate (20mg/kg body weight per day) for 28 days; this leads to a positive response in 80–90% of cases. Alternative drugs are pentamidine (4mg/kg/day on alternate days) for 4–12 weeks or amphotericin B (0.5–1mg/kg/day on alternate days) for 4–8 weeks.

The relapse rate is high (50% of cases relapse), justifying the need for long-term maintenance therapy. Different regimens have been suggested; pentavalent antimonials (850mg antimonials per month), pentamidine (2mg/kg every 2 weeks), or amphotericin B (1mg/kg every 2–4 weeks). In the absence of any comparative controlled trials, the best maintenance treatment remains uncertain.

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Chapter 128 - Bacterial Infections in HIV Disease

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Richard E Chaisson

Parasitic, fungal, mycobacterial and viral diseases are often viewed as the central opportunistic infections in HIV disease, because together they form the majority of AIDS-defining conditions. Bacterial infections as a group, however, are actually the most common complication of HIV disease. Patients with HIV infection are at greater risk for infections caused by common bacterial pathogens (e.g. *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella* spp.) than are HIV-seronegative persons. The relevance of bacterial infections was demonstrated by the addition, in 1993, of recurrent bacterial pneumonia to the Centers for Disease Control and Prevention definition of AIDS indicator conditions.[1] This chapter reviews the underlying immunologic changes that lead to the occurrence of bacterial infections in HIV-infected patients, the epidemiology of these infections and the clinical manifestations and management of these conditions, in the developed world.

EPIDEMIOLOGY

The epidemiology of bacterial infections as complications of HIV infection has been most carefully studied for bacterial pneumonia. The increased risk of bacterial pneumonia in HIV-infected injection drug users was demonstrated in a study that found the annual risk of community-acquired pneumonia was 9.7% in HIV-infected injection drug users without AIDS, compared with 2.1% in HIV-negative injection drug users and only 0.3% in the general population.[2] In another, prospective, study of over 4000 former injection drug users the incidence of community-acquired pneumonia was again markedly higher among HIV-infected patients (90.5/1000 person-years) than among HIV-negative persons (14.2/1000 person-years; Fig. 128.1).[3]

Other studies have examined the risk of bacterial pneumonia in populations that include other exposure categories as well as injection drug use. The epidemiology of bacterial pneumonia was described in the Pulmonary Complications of HIV Infection Study.[4] In this longitudinal study, the majority of participants were homosexual or bisexual men. Again, the rate of bacterial pneumonia was significantly higher in HIV-infected patients than in HIV-negative controls (5.5/100 person-years compared with 0.9/100 person-years). [4] Tobacco smoking was also found to be associated with a significantly increased risk of bacterial pneumonia in both HIV-infected and -uninfected patients.[4]

The introduction of highly active antiretroviral therapy (HAART) in the late 1990s has influenced the incidence of HIV-associated bacterial infections. There appears to have been a decline in the incidence of community-acquired and nosocomial bacterial pneumonia after potent antiretrovirals became widely used.[5] In contrast, the proportion of hospitalizations due to bacterial infections has increased as rates of *Pneumocystis carinii* and other more opportunistic infections have declined.[6] [7] Nevertheless, the increased rate of bacterial pneumonia in HIV-infected patients without AIDS, in comparison to HIV-negative persons, serves to emphasize that pyogenic bacterial infections may occur when immunity is relatively intact owing to the virulence of these organisms compared with other more opportunistic pathogens. Epidemiologic studies of other bacterial infections (e.g. bacteremia and infections caused by enteric pathogens) confirm the generally higher incidence of pyogenic infections in HIV-infected patients than in HIV-negative patients. Discussion of the increased frequency of specific diseases and the risk factors for disease development are found within the specific clinical sections of this chapter.

PATHOGENESIS AND PATHOLOGY

There are a variety of immunologic defects in HIV infection that may predispose patients to acquire bacterial infections. Among these immunologic abnormalities are deficits of mucosal immunity, cell-mediated immunity, humoral immunity and the complement system (Table 128.1). [8]

Defects of mucosal (or local) immunity are ascribed to decreased levels of IgA_2 at mucosal surfaces. It is postulated that this abnormality may increase the risk of invasive bacterial infections with *S. pneumoniae* and *Salmonella* spp. in particular. [8] [9]

A reduction in the $CD4^+$ lymphocyte count and a reversal of the CD4:CD8 ratio in HIV infection is implicated as a predisposing factor for bacterial infections in several ways. $CD4^+$ lymphocytes may also be involved in cell-mediated immunity as mediators of antibody-dependent and antibody-independent killing of enteric bacterial pathogens by mononuclear cells.[10] Changes in regulatory effects secondary to decreased levels of interleukin-2 and interferon-? production are also likely to contribute to an immunologic deficit. [11] In addition, abnormalities of $CD8^+$ lymphocyte activity may occur, leading to decreased cytotoxic function.[11]

Defects in humoral immunity are also present in HIV infection — although patients with HIV infection often have a polyclonal IgG gammopathy, the function of these antibodies is frequently abnormal and levels of specific IgG subclasses (e.g. IgG_2) may be low.[12] There is also evidence that some patients who have HIV infection have low levels of specific complement components or abnormalities of complement activation, or both. [13] [14] Deficits in hepatic and splenic clearance of opsonized organisms may occur. Children in particular are at an increased risk of developing infections caused by encapsulated bacteria (e.g. *S. pneumoniae, H. influenzae*), probably because of a lack of protective antibody in the absence of previous exposure to these organisms.

Neutropenia is a common complication of advanced HIV infection itself, as well as being a toxicity of many medications used in the management of HIV-infected patients. Neutropenia (absolute neutrophil count <1000 cells/mm^3) is an independent risk factor for the development of bacterial infections in patients with advanced HIV disease, and the risk rises as the absolute neutrophil count falls. [15] Low CD4 counts have also been shown to increase the risk of infection during neutropenia.[16] It should be noted, however, that infection rates in patients with HIV infection are substantially lower than in patients with neutropenia related to cancer chemotherapy. This may be because the nadir in the absolute neutrophil count in patients with HIV infection is usually higher than that seen in cancer patients.

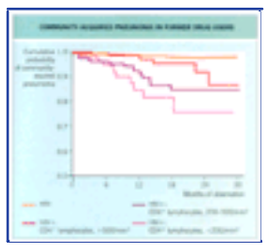

Figure 128-1 Community-acquired pneumonia in former drug users. Kaplan-Meier estimates of the cumulative probability of community-acquired pneumonia in HIV-negative and HIV-positive former drug users. *Adapted from Boschini et al.*[3]

TABLE 128-1 -- Factors associated with bacterial infections in HIV-infected patients.

FACTORS ASSOCIATED WITH BACTERIAL INFECTIONS IN HIV-INFECTED PATIENTS
Defects of mucosal immunity
Decreased levels of IgA_2 at mucosal surfaces
T-lymphocyte defects
Loss of $CD4^+$ lymphocytes
Impaired cytotoxic T-lymphocyte function
Decreased production of interleukin-2 and interferon-?
Defects of humoral immunity
Polyclonal gammopathy
Functional abnormalities of antibodies
Decreased levels of IgG subclasses
Granulocyte defects
HIV-related neutropenia
Drug-induced neutropenia
Complement defects
Low complement levels

Abnormal complement activation

PREVENTION

Prevention of bacterial infections may take several forms:

- avoidance of exposure,
- prophylactic antibiotics,
- vaccination,
- passive immunotherapy,
- the use of growth factors, and
- the use of HAART.

Avoidance of exposure

Measures involved in the avoidance of exposure range from avoiding the use of unsanitary water that may be associated with the transmission of enteric pathogens to the strict hygienic techniques recommended for patients with indwelling intravenous catheters. Patients should also avoid undercooked eggs and poultry and unpasteurized milk.[17]

Prophylactic antibiotics

The use of prophylactic antibiotics to prevent other opportunistic infections may have the added benefit of decreasing a patient's risk of bacterial infection. Trimethoprim-sulfamethoxazole (co-trimoxazole), a first-line agent for prophylaxis for *P. carinii*, may decrease the risk of bacterial infections as well. Studies have shown that its use is associated with a decrease in the number of confirmed episodes of bacterial pneumonia,[4] as well as bacterial infections in general.[18] Other studies, however, have failed to show a specific protective effect against pneumococcal infection;[19] this may be associated with selection of pneumococcal strains with decreased susceptibility to this agent. [20] In addition, trimethoprim-sulfamethoxazole has also been shown to be associated with a decline in the susceptibility of *S. pneumoniae* to penicillin.[21] The use of macrolide antibiotics (clarithromycin, azithromycin) as prophylaxis against the development of *Mycobacterium avium* infection has also been shown to be associated with a decreased risk of pyogenic bacterial infections. [22] [23] [24]

Thus, antibacterials being used for prophylaxis of *P. carinii*, toxoplasmosis of *M. avium* may decrease the risk of bacterial infections. Nevertheless, because of the risk of the development of resistance, antibiotics have not been recommended solely to prevent bacterial infections. Recent data from two placebo-controlled trials in Cote d'Ivoire suggest, however, that trimethoprim-sulmethoxazole prophylaxis may provide a health or survival benefit in developing countries where more costly therapies are not available. [25] [26]

Vaccination

Both the pneumococcal vaccine and the *H. influenzae* type b (Hib) vaccine have been evaluated in HIV-infected patients. Antibody responses to the 23-valent pneumococcal vaccine have been shown to be more effective in patients with higher $CD4^+$ lymphocyte counts (Fig. 128.2).[20] [27] There is also clinical evidence from observational studies indicating increased effectiveness when the vaccine is used earlier in the course of HIV infection (when the $CD4^+$ lymphocyte

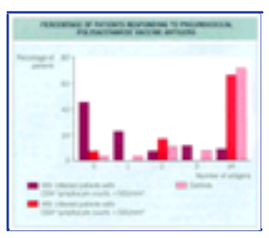

Figure 128-2 Percentage of patients responding to pneumococcal polysaccharide vaccine antigens. *Adapted from Rodriquez-Barradas et al.*[20]

count is greater than 200/mm^3).[19] In a placebo-controlled trial of pneumococcal vaccine in HIV-infected adults in Uganda, however, recipients of the 23-valent vaccine had a marginally higher rate of invasive pneumococcal infections and a significantly higher rate of all forms of pneumonia. [28] A clear rationale for why pneumococcal vaccination should increase the risk of disease is not apparent. Despite the findings of the Ugandan trial, the pneumococcal vaccine is currently recommended in the USA as a preventive measure for all patients with HIV infection and $CD4^+$ lymphocyte counts under 200/mm^3 , the vaccine is optional given the poor antibody and clinical response. Re-vaccination after 6 years is also recommended for patients with HIV infection, but a recent trial suggests that the risk of reactogenicity is considerable and the immunologic benefits may be limited.[29]

Vaccination against Hib has similarly been shown to elicit a better antibody response when used earlier in the course of disease.[30] The Hib polysaccharide vaccine is currently recommended for all children (regardless of HIV status).[31] The benefit of this vaccine in HIV-infected adults is less clear; although the incidence of *H. influenzae* is clearly increased in HIV-infected patients, the low absolute incidence of Hib infection mitigates against the routine use of the vaccine.[32]

Passive immunotherapy

Passive immunotherapy with high-dose intravenous immunoglobulin is often used to prevent recurrent bacterial infections in HIV-infected children. [33] [34] One controlled trial has shown that such therapy may be effective in HIV-infected adults as well; a reduction in the frequency of serious bacterial infections and hospitalizations in patients who were treated with intravenous immunoglobulin every 21 days was demonstrated. [35]

Use of growth factors

Measures to prevent or correct neutropenia must be tailored to the underlying cause of the cytopenia (e.g. treatment of infection causing bone marrow suppression, elimination of myelopsuppressive drugs). As an adjunct to these measures, when elimination of the underlying cause is not possible, or when there is no specific discernible cause of neutropenia, the use of granulocyte colony stimulating factor (G-CSF) should be considered. The use of G-CSF in patients with an absolute neutrophil count less than 1000/mm^3 is associated with a decline in bacterial infections, decreased duration of hospitalizations and improved survival.[36] [37] [38]

Use of highly active antiretroviral therapy

As noted above, HAART has been strongly associated with a decreased incidence of bacterial infections, particularly bacterial respiratory tract infections, in patients with HIV infection. The use of HAART is generally governed by guidelines from professional and governmental groups and is based on the potency and tolerability of specific regimens, the relative risk of disease progression based on clinical status and surrogate markers, and the long-term toxicities of treatment (see Chapter 139).

CLINICAL MANIFESTATIONS AND TREATMENT

The most common clinical manifestations of bacterial infection in HIV-infected patients are skin infections, respiratory infections, sinusitis, enterocolitis and bacteremia.

Skin infections

Bacterial skin infections in HIV-infected patients may vary from impetigo to folliculitis to cutaneous abscesses; cutaneous abscesses are often associated with injection drug use as well. Recurrences

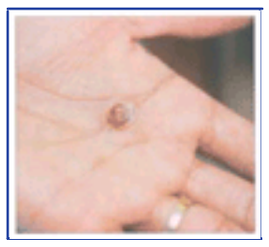

Figure 128-3 Typical appearance of bacillary angiomatosis. *Courtesy of Ciro Martins, MD.*

may be more frequent than in the HIV-negative population, but physical findings and responsible organisms (*Staphylcoccus aureus* and *Streptococcus* spp.) are similar. Treatment involves agents with good coverage of Gram-positive organisms, such as cephalexin or dicloxacillin.

Bacillary angiomatosis is a distinct skin infection that is specifically associated with HIV disease. The condition is caused either by *Bartonella henselae*, the organism that is also responsible for catscratch disease, or by *Bartonella quintana*. The clinical appearance is of very erythematous subcutaneous nodules that may occasionally resemble Kaposi's sarcoma (Fig. 128.3). Lymphadenopathy may be associated with these skin findings. Much less frequently, *B. henselae* has been identified as the cause of more deeply seated infections, including endocarditis, osteomyelitis and hepatic lesions (peliosis hepatis).[39] Diagnosis of the skin infection may be based on the characteristic appearance, but biopsy may be carried out to rule out other causes, and it is necessary to diagnose solid organ involvement. Histology shows vascular proliferation and mixed inflammatory cells. Warthin- Starry staining reveals the organism. Treatment is with a macrolide (erythromycin orally 250–500mg q6h) or a tetracycline (doxycycline orally 100mg q12h) for 1–2 months for skin disease, and longer for other sites of infection.

Sinusitis

Both acute and chronic sinusitis are common complications of HIV infection. Symptoms may include headache, fever, congestion and cough. However, many patients present with only headache or cough and without more typical symptoms of bacterial sinusitis (Table 128.2).

In one study, the organisms most commonly isolated as the cause of sinusitis were viridans streptococci, *S. pneumoniae* and *Pseudomonas aeruginosa*.[40] Other organisms that may be responsible include *H. influenzae*, other *Haemophilus* spp. and *Moraxella catarrhalis*.

The diagnosis is often made clinically, although sinus radiographs or computerized tomography (CT) scanning may be useful in demonstrating mucosal thickening, air-fluid levels or sinus opacification. Sinus CT is more sensitive for these findings than plain films.[40]

Treatment should include a decongestant in addition to antimicrobial therapy. The choice of antimicrobial is often made on empiric grounds with first-line options including amoxicillin-clavulanate, clindamycin, cefuroxime, clarithromycin or azithromycin. In patients who do not respond to such therapy, consideration should be given to sinus aspiration or the addition of antipseudomonal agents (see Chapter 32 and Chapter 229). In patients with $CD4^+$ lymphocyte counts less than 200/mm^3 , response to antibiotic treatment for acute sinusitis is often incomplete, leading to chronic sinusitis.[40]

TABLE 128-2 -- Signs and symptoms of sinusitis in HIV-infected patients.*

PRESENTING CLINICAL FEATURES OF SINUSITIS IN HIV-INFECTED PATIENTS		
		Percentage of patients
Symptoms	Fever	93
	Headache	89
	Nasal congestion	79
	Postnasal drainage	54
	Facial pain	36
	Watery discharge	21
	Purulent discharge	14
	Fever, headache	14
	Fever, headache, congestion	68
	Fever, headache, facial pain, discharge	14
Signs	Fever — temperature over 100.4°F (38°C)	79
	Facial tenderness	50
	Nasal discharge	31
	Facial swelling	21

** These data relate to 72 HIV-infected patients as reported by Godofsky et al.[40]*

Pneumonia

As described above, bacterial pneumonia is more common in patients with HIV infection than in HIV-seronegative comparison groups. The introduction of potent antiretroviral therapy, which has caused a decline in more opportunistic infections, has also led to bacterial pneumonia (and other bacterial infections) being responsible for proportionately more hospitalizations of HIV-infected patients. [6] [7] Risk factors for the development of bacterial pneumonia have been investigated in observational studies and include lower $CD4^+$ lymphocyte counts,[3] [4] [41] injection drug use,[4] cigarette smoking[4] and illicit drug use.[41] In addition, risk factors for pneumococcal infection specifically include any previous history of pneumonia, low serum albumin and a lack of receipt of the pneumococcal vaccination when the patient had a $CD4^+$ lymphocyte count greater than 200/mm^3 .[19] The specific amount of risk associated with declining immunity has been described; a rate of bacterial pneumonia of 2.3/100 person-years in patients with $CD4^+$ lymphocyte counts over 500/mm^3 was found.[4] This rate increased to 6.8/100 person-years in those with $CD4^+$ lymphocyte counts between 200 and 500/mm^3 , and 10.8/100 person-years in those with $CD4^+$ lymphocyte counts less than 200/mm^3 (Fig. 128.4).[4]

The clinical presentation of bacterial pneumonia in HIV-infected patients is indistinguishable from that in HIV-negative patients. The history usually reveals an acute onset of symptoms, including fever, productive cough, dyspnea and pleuritic chest pain. Physical examination commonly shows localized pulmonary findings, and laboratory evaluation may show leukocytosis and hypoxemia. Typical chest radiograph findings are of focal infiltrates, although diffuse disease may be seen. Sputum Gram stain will show multiple polymorphonuclear leukocytes, and sputum cultures are usually positive. These findings differ from the classic presentation of *P. carinii* pneumonia, the most common nonbacterial cause of pneumonia in HIV-infected patients, in which the presentation is more often subacute with a nonproductive cough, a paucity of physical findings and diffuse interstitial infiltrates on chest radiograph. Nevertheless, there are frequently cases in which induced sputum examination or bronchoscopy with bronchoalveolar lavage are necessary to obtain a microbiologic diagnosis.

The pathogens most commonly responsible for bacterial pneumonia are *S. pneumoniae, H. influenzae* and *Haemophilus* spp. other than

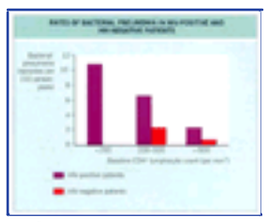

Figure 128-4 Rates of bacterial pneumonia in in HIV-positive and HIV-negative patients. The rates are shown by baseline $CD4^+$ lymphocyte count. *Data from Hirschtick et al.*[4]

H. influenzae.[42] Other organisms that may cause disease include *M. catarrhalis, Klebsiella pneumoniae* and *Staphylococcus aureus*. Empiric therapy usually includes a second- or third-generation cephalosporin. If there is a lack of clinical response, other bacteria that may not respond to such treatment must be considered as possible causes (e.g. *Chlamydia pneumoniae*,[3] *Nocardia* spp.[43] and *Legionella* spp.[44]). In addition, unlike HIV-negative patients, in whom *P. aeruginosa* is usually responsible for only nosocomial infections, in HIV-infected patients *Pseudomonas* pneumonia frequently occurs as a community-acquired infection.[45] [46] There are also data that suggest a shift in bacterial pathogens since the introduction of HAART. A decline in *P. aeruginosa* infections has been reported, and seems to be associated with both generally higher CD4 lymphocyte counts and a reduction in the use of trimethoprim-sulfamethoxazole prophylaxis.[47]

An unusual organism responsible for bacterial pneumonia in HIV-infected patients is *Rhodococcus equii*. This bacterium is a Gram-positive rod usually associated with infections of domestic animals.[48] In HIV-infected patients, the clinical presentation is often subacute and characterized by fever, cough, pleuritic chest pain, fatigue and weight loss.[49] Chest radiographs often show cavitating lesions.[48] [50] [51] Blood cultures may be more sensitive for diagnosis than sputum cultures — 83% sensitivity compared with 33% in one study.[51] Treatment is usually with vancomycin (intravenously 1g q12h) but ciprofloxacin (orally 750mg q12h) or imipenem (intravenously 500mg q6h) may also be used and duration of therapy is 2–4 weeks.

Bacteremia in the setting of pneumonia occurs more commonly in HIV-infected than in HIV-negative patients — increased rates of bacteremia with pneumococcal and *H. influenzae* pneumonia have been documented (Fig. 128.5). [52] [53] [54] [55] Bacteremia may also occur with other pathogens responsible for pulmonary infections (e.g. *P. aeruginosa*). Mortality rates from bacterial pneumonia may also be higher in HIV-infected patients, but there are conflicting data on this topic.[56] [57] [58] [59]

Enterocolitis

Many etiologic agents may cause diarrhea in HIV-infected patients. Among the bacterial pathogens commonly encountered are non-typhoidal *Salmonella* spp., *Shigella flexneri, Campylobacter jejuni* and *Clostridium difficile. Shigella* and *Campylobacter* infections manifest similarly — patients note severe diarrhea (which may be bloody) associated with abdominal cramping, nausea and fever.

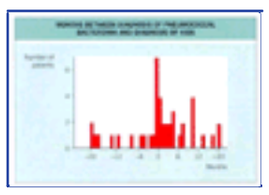

Figure 128-5 Months between diagnosis of pneumococcal bacteremia and diagnosis of AIDS. The data relate to 37 patients as reported by Redd *et al.*[53]

Diagnosis is by stool culture. These infections may both be treated with oral quinolones (see Chapter 43).

Nontyphoidal *Salmonella* spp. infections may present either with or without diarrhea and other symptoms of colitis. In patients without gastrointestinal symptoms, the infection may manifest solely as fever without any localizing findings. The occurrence of *Salmonella* bacteremia is more common in patients with HIV infection than in HIV-seronegative groups.[60] In addition, relapses of bacteremia are common.[61] Unlike the recommendations for patients who are not immunosuppressed, even *Salmonella* infections limited to the gastrointestinal tract should be treated in HIV-infected patients. Treatment is usually with ciprofloxacin (500mg q12h for 2–4 weeks), and suppressive therapy with ciprofloxacin is often used because of the risk of relapse.

Infection due to *C. difficile* also has characteristics in HIV-infected patients that differ from those seen in the HIV-negative population. Clinical symptoms may be more severe in HIV-infected patients, and these patients are more likely to have relapses and chronic symptoms. Antibiotic use and hospitalization are associated with the development of infection, just as they are in HIV-negative patients.[62] [63] *Clostridium difficile* may also occur as a community-acquired infection in HIV-infected patients.[64] Treatment is with oral metronidazole or vancomycin.

Bacteremia

As discussed above, bacteremia may frequently occur as a result of *Salmonella* spp. infections and pneumonia. Bacteremia may also be associated with soft tissue infections and urinary infections. Injection drug use is associated with the development of *S. aureus* bacteremia and endocarditis. In this setting, CD4+ lymphocyte count has been shown to have an inverse correlation with mortality rate.[65] In addition, bacteremia may be a complication of the use of long-term intravenous catheters, which may be needed in the management of HIV-infected patients (e.g. for the intravenous treatment of cytomegalovirus retinitis). In one study of HIV-infected patients admitted with bacteremia, 35% had an infection associated with an intravenous catheter.[66] In this and other studies, the causative organisms most frequently isolated were *S. aureus* and coagulase-negative staphylococci.[66] [67] Line infections caused by Gram-negative organisms are less frequent, but certainly not uncommon — *Escherichia coli, Proteus mirabilis, Serratia marcescens, P. aeruginosa*[46] [67] and other Gram-negative rods have all been isolated as the cause of infections related to an intraveneous catheter. Empiric therapy of line infections while culture results are awaited should therefore involve vancomycin and an aminoglycoside (or vancomycin and a ß-lactam with broad Gram-negative coverage). Removal of the intravenous line may not be necessary in treating *S. aureus* and coagulase-negative staphylococcal infections, but it is usually recommended for Gram-negative bacterial infections. Of interest, however, are data from one study indicating similar recurrence rates whether or not the catheter was removed.[66] As with other infections, the incidence of HIV-associated bacteremia declined after HAART became available. One study has shown that this decline has been associated with a reduction in the use of central venous catheters and in neutropenia.[68]

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Chapter 129 - Mycobacterial Infections in HIV-infected Patients

Alex Soriano
José M Gatell

EPIDEMIOLOGY

Mycobacterial infections are common AIDS-defining events in HIV-infected patients. *Mycobacterium tuberculosis* and *Mycobacterium avium* complex (MAC) are the most frequently found infections and have different distributions around the world. *Mycobacterium tuberculosis* has the highest incidence rate in Africa, Asia and southern Europe and MAC in the USA and northern Europe.

HIV infection, because of its immunosuppressive effect, is the most significant risk factor for the development of active tuberculosis.[1] The number of reported cases of tuberculosis increased dramatically during the 1980s and early 1990s and the World Health Organization estimated that, between the onset of the HIV pandemic and mid-1993, more than 5 million persons worldwide had been co-infected by both HIV and *M. tuberculosis*. More than 3.5 million of these are in sub-Saharan Africa (Fig. 129.1).

HIV infection has not only modified the incidence of tuberculosis during the last 15 years but has also altered the clinical presentation, with an increase in extrapulmonary forms of tuberculosis.[3] Furthermore, the mortality rate of tuberculosis before the introduction of new antiretroviral drugs was four to eight times higher in HIV-positive than in HIV-negative patients. [4] Since the introduction of

Figure 129-1 Estimated global distribution of adults co-infected with HIV and *Mycobacterium tuberculosis,* to mid-1993. *From the World Health Organization Tuberculosis Program. Redrawn with permission from Snider et al.*[2]

highly active antiretroviral therapy (HAART) the incidence of opportunistic infections and mortality has dramatically decreased. However, the decrease in the incidence of tuberculosis is less evident (Fig. 129.2) and it is still much higher among HIV-infected patients in all strata of $CD4^+$ lymphocytes than in the general population (Fig. 129.3).

Mycobacterium avium complex is ubiquitous in the environment and has been isolated from a variety of sources around the world, including soil, natural water, municipal water systems, food, house dust and domestic and wild animals. These isolates are thought to be the source of most human infections but there is no evidence of MAC transmission from person to person. In the USA the incidence of MAC disease has been studied prospectively in a number of cohorts; the cumulative probability of disseminated disease due to MAC in subjects who have $CD4^+$ lymphocyte counts below 50 cells/mm^3 is 30%, and 20% in those who have $CD4^+$ lymphocyte counts of 50–100 cells/mm^3 , while in Europe the rates are 20% and 11% respectively.[6] Furthermore, MAC was also infrequently isolated in AIDS patients from Africa. The differences in epidemiologic data between the USA and Europe or Africa have been attributed to a potential cross-immune protection between MAC and *M. tuberculosis* or MAC and bacille Calmette-Guérin (BCG) vaccine, which is much more frequently administered in Europe and Africa.

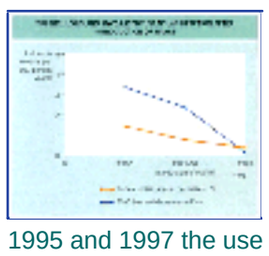

Figure 129-2 Tuberculosis and *Mycobacterium avium* infection after introduction of highly active antiretroviral therapy. Incidence among HIV-positive patients. Between 1995 and 1997 the use of HAART became general practice in Europe. *Redrawn with permission from Kirk et al.*[5]

The use of primary prophylaxis for MAC with clarithromycin, azithromycin or rifabutin and the introduction of HAART are the most likely explanations of the decreased incidence rate of MAC disease[5] (see Fig. 129.2).

PATHOGENESIS

Mycobacterium tuberculosis is acquired via inhalation. In the lung alveoli an immune response is established, mediated by a complex interaction between mononuclear phagocytes and different subsets of T cells. While the former cells act as the main effectors, the latter serve as the predominant inducers of protection. A co-ordinated

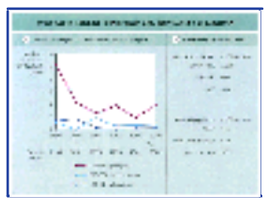

Figure 129-3 Tuberculosis incidence in recipients of highly active antiretroviral therapy, Hospital Clinic of Barcelona. (a) The incidence rate per 100 HIV-infected patients per year, separated into three different groups by $CD4^+$ lymphocyte level. (b) Tuberculosis incidence, 2000, expressed per 100,000 persons per year. It is notable that the incidence of tuberculosis is higher in HIV patients in all $CD4^+$ lymphocyte strata, as compared with the general population.

communication between both cells mediated by different cytokines is essential for optimum protection, especially cytokines such as interferon (INF)-? and tumor necrosis factor (TNF)-a. Such co-ordination' is best achieved in the granulomatous lesion. Frequently, if not always, full eradication of the pathogen is not achieved and the bacilli remain dormant or latent until the host defenses become impaired, as in HIV co-infected patients. This complex immune response is disrupted early in the evolution of HIV infection, which may explain why pulmonary tuberculosis is the first and most common AIDS-defining event in endemic areas. In addition, pulmonary tuberculosis develops in patients who have only mild immunosuppression in contrast to other opportunistic infections (Fig. 129.4).

The histologic patterns of tuberculosis reflect the degree of integrity of the cellular immune response of the patient. Different patterns have been identified and correlate well with the stage of HIV infection. Patients who have relatively intact cellular immunity develop a typical granulomatous response. Epithelioid macrophages and Langhans giant cells are abundant and numbers of acid-fast bacilli (AFB) are low. Patients who have moderate and advanced immunosuppression show a decrease in epithelioid macrophages, Langhans giant cells and $CD4^+$ T cells, which results in poor intracellular killing of mycobacteria. The granulomatous response is absent and there is a large number of AFB surrounded by tissue necrosis. [8]

Mycobacterium avium complex is acquired through inhalation or ingestion and adheres to specific receptors on the epithelial cells that allow the colonization and

invasion of the mucosa. It gains entry to macrophages by opsonic or complement-mediated pathways. Once into the phagosome, MAC inhibits lysosome fusion, allowing their intracellular survival and replication, enhanced by co-infection with HIV.

The immune response against MAC is mediated by $CD4^+$ lymphocytes, cytotoxic lymphocyte responses and local fluxes of the growth-enhancing (i.e. interleukin (IL)-6) and growth-inhibiting cytokines (i.e. INF-?, TNF-a, IL-12). The importance of $CD4^+$ cells is shown by the fact that the increase in $CD4^+$ T cells evoked by HAART considerably reduces the risk of disseminated MAC disease.

Figure 129-4 $CD4^+$ count and opportunistic infection. Relation between $CD4^+$ lymphocyte count and the main opportunistic infections in HIV-infected patients. *Data from Hospital Clinic AIDS unit, Barcelona. CAN, esophageal candidiasis; CMV, cytomegalovirus infection; LEIS, disseminated leishmaniasis; MAC, Mycobacterium avium complex infection; PCP, Pneumocystis carinii pneumonia; TBE, extrapulmonary tuberculosis; TBP, pulmonary tuberculosis; TOXO, central nervous system toxoplasmosis. Redrawn with permission from Miro et al.*[7]

Figure 129-5 Influence of HIV infection on tuberculosis epidemiology and strategies against spread. HIV^+ , HIV-infected patients; ICH, immunocompetent host; PPD, purified protein derivative; SP, smear-positive.

PREVENTION

The influence of HIV infection in the spread of tuberculosis is shown by the fact that HIV-infected patients who have latent tuberculosis (detected by a positive tuberculin skin test in immunocompetent hosts) have a substantially higher risk of developing active tuberculosis than those who are HIV-negative. Approximately 30–50% of people exposed to *M. tuberculosis* become infected. Immunocompetent people have an effective immune response that allows the successful containment of the infection and only have a 5–10% lifetime risk of developing active tuberculosis later. Conversely, 8% per year of HIV-positive intravenous drug abusers in a methadone program who had a positive tuberculin skin test developed active tuberculosis.[9] Another study demonstrated that 30% of HIV-positive patients who had a positive skin test followed up during 1 year and who did not receive prophylaxis developed active tuberculosis. [10] Therefore, in the HIV era it is very important to promote strategies to prevent the spread of tuberculosis such as investigation of the close contacts of tuberculosis patients, identification and treatment of latent tuberculosis infection, and early diagnosis and adequate therapy of active tuberculosis, including directly observed therapy when necessary (Fig. 129.5).

Vaccination with BCG has demonstrated a high protection rate against serious forms of tuberculosis in children (tuberculous meningitis and disseminated tuberculosis) but its protective efficacy against pulmonary tuberculosis shows enormous variability, ranging from 0% to over 75%. This wide range of protective efficacy is not well understood and, in countries with a low tuberculosis incidence, vaccination is not recommended so as to preserve the tuberculin skin test as a useful tool to diagnose latent tuberculosis infection.

Identification and treatment of latent tuberculosis infection

As $CD4^+$ lymphocyte count decreases, the percentage of patients who have latent infection whose tuberculin skin test is positive decreases. [11] Therefore, screening for latent tuberculosis should be performed as soon as HIV infection is diagnosed. HIV-infected patients who have had contact with persons who have contagious tuberculosis have a higher risk of being infected and of developing active disease; therefore, they must be evaluated for tuberculosis as soon as possible after exposure. For this reason, it is necessary to establish tuberculosis screening initiatives in settings where the prevalence of HIV infection and tuberculosis is high (prisons, drug abuse treatment programs, syringe exchange programs, residences for AIDS patients and homeless shelters). Because of the complexity of problems associated with active tuberculosis in HIV-infected patients, and as a part of the efforts to control and eliminate tuberculosis, all HIV-infected persons identified as latently infected with *M. tuberculosis* should complete a full recommended course of preventive therapy. In certain outpatient and institutional settings, directly observed preventive therapy should be implemented.

The tuberculin skin test with 5TU of purified protein derivative is used to diagnose latent tuberculosis infection. A reaction size of 5mm or more of induration is considered positive in HIV-infected patients. Persons who have less than 5mm but who have a history of exposure to tuberculosis should also be considered to be infected with *M. tuberculosis*. Unfortunately, false-negative tuberculin skin

test (anergy) is common in HIV-infected patients but there is no other test to identify latent tuberculosis infection. In anergic patients a course of preventive therapy with isoniazid does not reduce the incidence of tuberculosis, therefore it is not recommended. However, these patients should be tested with PPD after responding to HAART.[12]

Before starting preventive chemotherapy it is necessary to rule out active tuberculosis via medical history (fever, night sweats, cough, weigh loss or anorexia) and chest radiograph. Those patients who have clinical and/or radiologic abnormalities must collect three consecutive sputum samples for smear and culture to rule out active tuberculosis.

The therapy of choice for latent tuberculosis infection in HIV-infected patients is 9 months of daily isoniazid. It is possible to administer a 9-month regimen of isoniazid twice a week but direct observed therapy is always recommended. Recently it has been reported that 2 months of daily rifampin (rifampicin)—pyrazinamide in a cohort of HIV-infected patients is similar in safety and efficacy to a daily 12-month regimen of isoniazid.[13] However, the Centers for Disease Control and Prevention (CDC) have reported 21 non-HIV patients who developed fatal and severe liver injuries associated with a rifampin-pyrazinamide regimen for a latent tuberculosis infection and they recommend caution, especially in patients concurrently taking other medications associated with liver injury or/and alcoholism. Rifampin-pyrazinamide is not recommended for persons who have underlying liver disease or for those who have had isoniazid-associated liver injury. Therefore, close monitoring of patients receiving a rifampin-pyrazinamide regimen is necessary. Major indications are those patients likely to be infected with an isoniazid-resistant strain of tuberculosis and those who are under control only for brief periods of time (e.g. prisoners serving 2–4 months).

For patients who have pyrazinamide intolerance, a 4- to 6-month regimen of rifampin alone is recommended, although information on its effectiveness is still scarce. In addition, rifampin is contraindicated in HIV-infected patients on protease-inhibitor-containing regimens, except ritonavir, and taking non-nucleoside reverse transcriptase inhibitors, except efavirenz. Rifabutin can be used as an alternative for patients treated with indinavir, nelfinavir, amprenavir and nevirapine, adjusting the doses as described below (see Management).

Preventive treatment for patients who are likely to be infected with isoniazid-rifampin-resistant strains include the use of a combination of at least two antituberculous drugs (e.g. ethambutol, pyrazinamide or levofloxacin). The clinician should always review the drug susceptibility pattern of the *M. tuberculosis* strain isolated from the infecting source before choosing a preventive therapy regimen.

Mycobacterium avium complex prophylaxis

Prophylaxis for MAC is now recommended for all AIDS patients who have $CD4^+$ lymphocyte counts lower than 50 cells/mm^3 . This recommendation is based on the high incidence of MAC bacteremia in this population, the morbidity and mortality associated with disseminated MAC and the efficacy of the available prophylactic agents. In randomized, placebo-controlled trials, 6% of patients receiving clarithromycin 500mg/day developed MAC infection versus 16% of those assigned to receive placebo,[14] representing an estimated 69% reduction in risk for disseminated infection with MAC. In a similar study design using 1200mg of azithromycin once a week,

MAC bacteremia was reduced by 66%. Both regimens have proved to be superior to rifabutin for MAC prophylaxis. [15] It is of note that macrolide resistance has been identified in 29–58% of patients failing clarithromycin prophylaxis and in 16% on an azithromycin regimen. Not even one case of

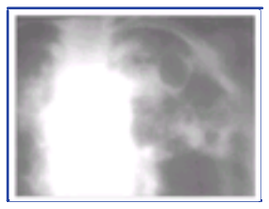

Figure 129-6 Characteristic upper lobe cavity on chest radiograph in an HIV patient who has tuberculosis.

macrolide resistance was reported during rifabutin prophylaxis. Before starting prophylaxis, MAC bacteremia and active tuberculosis should be ruled out.

Discontinuation of MAC prophylaxis is a reasonable option for patients who have a $CD4^+$ T lymphocyte count above 100 cells/mm^3 and low viral load for a sustained period of time[16] (e.g. more than 3–6 months).

CLINICAL FEATURES

Pulmonary manifestations of mycobacterial infections

Pulmonary tuberculosis is often the AIDS-defining event. The most common clinical symptoms are fever, weigh loss, anorexia, asthenia and non-specific cough. The diagnosis may be difficult because of atypical clinical and radiographic findings, especially in those patients who have a low $CD4^+$ lymphocyte count. Upper lobe disease and cavitation (Fig. 129.6) are associated with a high $CD4^+$ lymphocyte count, while miliary patterns and mediastinal and hilar adenopathy are associated with lower $CD4^+$ lymphocyte counts. Presence of adenopathies, pleural effusion and/or cavitation are useful to differentiate tuberculosis from *Pneumocystis carinii* pneumonia. In addition, a normal chest radiograph or diffuse infiltrates mimicking *P. carinii* pneumonia may occur. Acid-fast micro-organisms are found in the sputum smear in 40–67% of patients who have HIV-associated tuberculosis and sputum culture is usually positive in 74–95% of cases. The likelihood that a sputum sample will be smear-positive for AFB decreases with a decreasing $CD4^+$ lymphocyte count and when the chest radiograph is normal.

Pulmonary disease associated with MAC is rare and criteria for diagnosis are not well established. Patients should have a repeatedly positive culture in sputum, an infiltrate on chest radiograph, absence of other lung pathogens and preferably biopsy specimens showing AFB in abnormal lung tissue.

The most frequent nontuberculous mycobacterium isolated from sputum in HIV-infected patients is *Mycobacterium kansasii*. Patients who have *M. kansasii* infection tend to have a low $CD4^+$ lymphocyte count (less than 50 cells/mm^3) and the clinical and radiologic manifestations are not different from tuberculosis. The isolation of *M. kansasii* from sputum is always considered diagnostic of pulmonary disease, since colonization is uncommon.

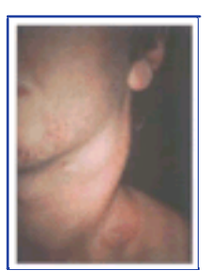

Figure 129-7 Laterocervical adenopathy in a HIV-infected patient. The needle aspiration demonstrated abundant AFB.

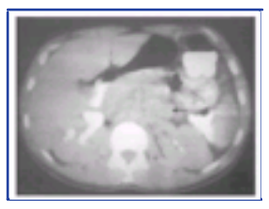

Figure 129-8 Abdominal CT scan of an HIV-infected patient who has tuberculosis. Multiple retroperitoneal lymph nodes (arrow) are typical findings.

Extrapulmonary manifestations of mycobacterial diseases

As the level of immunosuppression increases in HIV-infected patients, tuberculosis involving pulmonary and extrapulmonary locations and mycobacteremia become progressively more frequent. The disseminated disease, defined as having more than one focus or progressive hematogenous disease, has been reported in 38% of cases.[17] These forms show a rapidly and progressive evolution with diffuse pulmonary infiltrates, acute respiratory failure and high mortality rate.

Lymphadenitis is the most common extrapulmonary location (Fig. 129.7). This form is generally multifocal, also invading mediastinal and mesenteric lymph nodes, associated with general major systemic symptoms such as fever or weight loss. Low-density areas in the nodes visualized in computerized tomography (CT) are very characteristic (Fig. 129.8) and the material removed by fine-needle aspiration is usually positive on acid-fast stain (AFS).

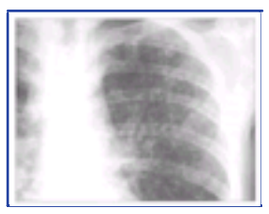

Figure 129-9 Chest radiograph of HIV-infected patient who has miliary tuberculosis.

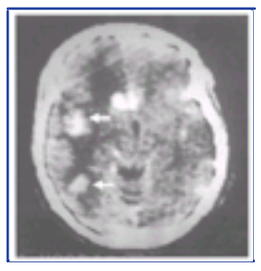

Figure 129-10 Multiple cerebral cortical densities (tuberculomas, arrows) on CT scan of a patient who has tuberculosis of the central nervous system.

Miliary tuberculosis in AIDS can be detected in 10% of pulmonary tuberculosis and in 38% of extrapulmonary tuberculosis cases (Fig. 129.9). The presence of major constitutional symptoms is characteristic. Only 10% of cases have a positive tuberculin skin test and the sputum smear is positive in only 25%, but cultures of other tissues may be positive (e.g. bone marrow, liver or peripheral blood).

The clinical picture of central nervous system tuberculosis is not altered by HIV infection, except for a higher frequency of nodular lesions (tuberculomas), which appear on CT as avascular masses with surrounding edema (Fig. 129.10). Medical therapy without surgery is the preferred approach. Other locations of tuberculosis (pleural, skeletal, genitourinary or gastrointestinal) have a similar clinical presentation to that in immunocompetent patients.

Disseminated MAC disease is characterized by fever, night sweats and weight loss. The gastrointestinal tract is frequently involved and clinical manifestations are nausea, vomiting, watery diarrhea and abdominal pain, which can be severe. At physical examination hepatomegaly, splenomegaly and lymphadenopathy are very common, and elevations of serum alkaline phosphatase, lactate dehydrogenase and anemia are the most frequent laboratory findings. Worsening anemia and an elevated alkaline phosphatase out of proportion to hepatic transaminase elevation should increase suspicion of disseminated MAC. Other unusual manifestations of MAC disease in AIDS patients include cutaneous disease, arthritis, sinusitis, orchitis, peritonitis, chylous ascites, appendicitis, endophthalmitis, choroiditis, pancreatitis, pericarditis and meningitis.[18] Other nontuberculous mycobacterias, including *Mycobacterium genavense, M. intracellulare, M. haemophilum, M. simiae, M. xenopi, M. scrofulaceum, M. marinum* and *M. fortuitum*, have also been described as a cause of disseminated infection in HIV-infected patients.

Paradoxic reactions

Paradoxic reactions during antimycobacterial therapy have been described in up to 36% of patients who began HAART early during antimycobacterial treatment.[19] In contrast, only 7% of patients who received antimycobacterial therapy but not antiretroviral therapy had paradoxic reactions. These reactions appear after clinical improvement and are characterized by fever, worsening chest infiltrates on radiograph and peripheral, mediastinal or abdominal regrowth of lymphadenopathy. However, these manifestations are not associated with changes in *M. tuberculosis* bacteriology (e.g. no change from negative to positive culture and smear) and patients generally feel well and have no signs of toxicity. These reactions are accompanied by a substantial reduction in the HIV burden and a marked increase in reactivity on tuberculin skin testing, which suggest that they are caused by inflammation from a stronger immune response to *M. tuberculosis* or MAC after antiretroviral therapy. In general, paradoxic reactions are self-limited in 10–40 days; however, some reactions are severe and may require a short course of treatment with corticosteroids.

DIAGNOSIS

The physician should always keep in mind the possibility of mycobacterial infection in HIV-positive patients and most especially in those AIDS patients who have fever of unknown origin, any kind of infiltrates on chest radiograph, multifocal lymphadenopathies and subacute lymphocytic meningitis.

The tuberculin skin test has a high proportion of false negatives in patients who have fewer than 400 cells/mm^3 $CD4^+$ lymphocytes/l. Therefore, a rapid diagnosis of tuberculosis in AIDS patients is based on AFS of sputum (instead of normal chest radiograph), urine, bone marrow, lymph node and other samples (e.g. ascites or cerebrospinal fluid). However, AFS is positive in only 50–75% of cases. In addition, a bronchoscopy in AIDS patients yields a rapid diagnosis (based on smears and histologic features) in only one-third of cases.[20] Therefore, it is necessary to start empiric antimycobacterial therapy in patients who have suspected and/or are at risk of tuberculosis even where AFS is negative until the definitive result of the Löwenstein culture is obtained (which requires from 15 days up to 2 months). New tests for rapid identification of *M. tuberculosis* complex based on polymerase chain reaction (PCR) have sensitivity and specificity higher than 95%. Polymerase chain reaction is not necessary in HIV-infected patients who have a positive AFS in whom the clinical suspicion of tuberculosis is high. However, when the AFS is positive and the suspicion of tuberculosis is intermediate or low, a positive PCR is useful to rule out other nontuberculous mycobacterias such as MAC or *M. kansasii* and it allows the use of therapy against tuberculosis only. Positive PCR when AFS is negative avoids invasive procedures (e.g. fiberoptic bronchoscopy) and allows an early diagnosis of tuberculosis. The PCR is also useful in epidemiologic investigations, especially since one study performed in San Francisco demonstrated that patients who have smear-negative and culture-positive tuberculosis were responsible for about 17% of tuberculosis transmission.[21] Restriction fragment length polymorphism (RFLP) analysis of *M. tuberculosis* isolates allows the identification of specific strains. This test has demonstrated that recent infection appears with a similar frequency to reactivation of infections acquired in the past[22] and that exogenous re-infection occurs after curative treatment, especially in areas with a high incidence of the disease.[23] Analysis using RFLP can also be helpful to determine cross-contamination in the laboratory.

The diagnosis of disseminated disease caused by MAC requires the isolation of the organism from a sterile site. A single blood culture has a high diagnostic yield, which is 90–95% sensitive. Currently there are a variety of culture systems that are useful. Liquid media is superior to conventional culture on Löwenstein-Jensen agar slants. In the Bactec radiometric system, the blood taken from a patient is inoculated into culture media containing radiolabeled substrate that is metabolized in the presence of mycobacteria to carbon dioxide and detected by radiorespirometric methods. A growth signal can usually be detected within 8–14 days. Once sufficient growth is achieved, the diagnosis of MAC can be made in few hours with the use of DNA probes. The diagnosis can also be made by identification of MAC from other sterile sites such as bone marrow, liver or lymph node biopsy.

Although colonization can occur in AIDS patients, the isolation of MAC in respiratory or gastrointestinal tract in those patients who have $CD4^+$ lymphocyte counts below 50 cells/mm^3 represents a high risk for the development of MAC bacteremia.[24] In this case prophylaxis or treatment should be considered.

MANAGEMENT

Treatment of drug-susceptible

Mycobacterium tuberculosis

Isoniazid is the most potent bactericidal drug and kills more than 90% of bacilli within 7 days by acting on the metabolically active ones. It is also quite effective at preventing the emergence of drug resistance. Rifampin and rifabutin are also good bactericidal drugs with a potent sterilizing activity and the ability to prevent drug resistance. Both drugs are active against dividing bacilli and those that remain inactive for long periods of time but have intermittent periods of active metabolism. Pyrazinamide is particularly effective at killing intracellular bacilli inside macrophages in an acid environment. Ethambutol and streptomycin are less potent drugs but are effective at preventing emergence of resistance to rifampin and isoniazid. A fourth drug (such as ethambutol or streptomycin) is necessary when the rate of isoniazid resistance in the community is known to be higher than 4%. These drugs can be withdrawn once test results indicate *M. tuberculosis* susceptibility to isoniazid and rifampin.

There is a debate on how long patients who have HIV disease with tuberculosis should be treated.[25] Current CDC and American Thoracic Society guidelines recommend a 6-month treatment regimen but suggest prolonged therapy (9 months or 4 months after culture conversion) for patients who have a delayed clinical and bacteriologic response to antituberculosis therapy. Therefore, sputum evaluation 2 months after initiation of treatment (induction phase) should be performed among persons infected with HIV.

The standard 6- or 9-month regimens based on rifampin or rifabutin and 9- or 12-month regimen without rifamycins are summarized in

TABLE 129-1 -- Treatment regimens for HIV-related tuberculosis.

TREATMENT REGIMENS FOR HIV-RELATED TUBERCULOSIS				
	Induction phase		**Continuation phase**	
	Drugs	**Interval and duration***	**Drugs**	**Interval and duration***
6–9† months rifamycin-based therapy	Isoniazid, rifampin or rifabutin, pyrazinamide, ethambutol‡	Daily for 2 months, or daily for 2 weeks and then twice a week for 6 weeks	Isoniazid, rifampin or rifabutin	Daily or twice a week for 4–7 months
Rifamycin free therapies				
12-month regimen	Isoniazid, pyrazinamide, ethambutol, streptomycin	Daily for 2 months, or daily for 2 weeks and then 2–3 times a week for 6 weeks	Isoniazid, ethambutol	Daily or 2–3 times a week for 10 months
9-month regimen§	Isoniazid, pyrazinamide, ethambutol, streptomycin	Daily for 2 months, or daily for 2 weeks and then 2–3 times a week for 6 weeks	Isoniazid, pyrazinamide, streptomycin	Daily or 2–3 times a week for 7 months

* All intermittent therapies should be directly observed.
† Duration of therapy should be prolonged for patients who have delayed response to therapy (see text).
‡ Ethambutol should be stopped if *Mycobacterium tuberculosis* is susceptible to isoniazid and rifampin.
§ We do not recommend this regimen, since a prolonged course of streptomycin is related to severe adverse events such as tubular necrosis and renal failure, deafness due to cochlear toxicity and

vertigo due to damage to vestibular organs.

Table 129.1 . The selection of a regimen in HIV-infected patients depends on consideration of a number of factors including:

- the high rate of adverse reactions to antituberculosis drugs;
- the drug interactions between antituberculosis and antiviral drugs; and
- the susceptibility pattern of the strain.

Adverse reactions to many drugs occur at high frequency among persons who have HIV infection. Several studies have compared HIV-infected and HIV-uninfected patients and have shown 20–40% adverse reactions in HIV-infected patients in comparison with 3–5% in persons not infected with HIV.[3] [26] The majority of adverse reactions occurred within the first 2 months after starting therapy. Rifampin adverse reactions are the most frequently observed. Generally, rifampin-free regimens are preferable, although there have been reports of successful desensitization to rifampin in HIV-uninfected persons.[27]

Drug interactions between antituberculosis drugs and antiretrovirals occur as a result of induction and inhibition of metabolic pathways. Rifamycins (rifampin and rifabutin) are potent inducers of isoenzyme CYP3A4 of the cytochrome P450 enzyme system and they lead to a reduction of the area under the curve (AUC) of those drugs metabolized by CYP3A4, such as protease inhibitors and non-nucleoside reverse transcriptase inhibitors[28] (interactions are summarized in Table 129.2). Nucleoside reverse transcriptase inhibitors are not metabolized via CYP3A4, but rifampin decreases the AUC of zidovudine by 30%. The clinical relevance of this change is unclear. Rifabutin does not alter the pharmacokinetics of nucleoside reverse transcriptase inhibitors.

Rifampin has the most potent CYP3A4 enzyme-inducing effect, with a resultant 80% decrease in the AUC of all protease inhibitors, with the exception of ritonavir, which is decreased by 35%. Therefore, the combination of rifampin with protease inhibitors is contraindicated (with the possible exception of ritonavir).

To overcome the problems associated with rifampin, the use of rifabutin has been recommended because of its lower enzyme-inducing effect. Rifabutin decreases the AUCs of indinavir and nelfinavir by 30%, amprenavir by 15% and lopinavir/ritonavir by 0%. When indinavir and rifabutin are co-administered, it is recommended that the dose of indinavir be increased to 1g q8h. Furthermore, protease inhibitors are inhibitors of CYP3A4, which can induce an increase in the plasma levels of rifabutin and thus increase its toxicity (leukopenia, uveitis and arthralgia). Therefore, it is necessary to reduce the doses of rifabutin (standard dose is 300mg q24h) to 150mg q24h when it is co-administered with indinavir, nelfinavir and amprenavir and to 150mg twice or three times weekly when administered with lopinavir/ritonavir (since ritonavir is the most potent inhibitor of CYP3A4). Rifabutin reduces the AUC of saquinavir by approximately 45%; therefore, this combination is contraindicated. Rifampin can be combined with ritonavir (at usual doses); however, the utility of this approach is limited because of the poor tolerability of full-dose ritonavir. It may be possible also to use rifampin with ritonavir 400mg and saquinavir 400mg q12h, although this combination has only been evaluated in a few patients.[29]

As in the case of protease inhibitors, rifampin induces a decrease in the AUCs of nevirapine, delavirdine and efavirenz by 37%, 96% and 25% respectively. Thus, co-administration of rifampin with delavirdine is contraindicated. It may be possible to use rifampin with nevirapine, but clinical experience is scarce. Pharmacokinetic studies have shown that levels of efavirenz at a dose of 800mg q24h plus rifampin are equal to 600mg efavirenz q24h without rifampin.[30] Rifabutin can be used with nevirapine without dosage adjustment. Data on the interaction between rifabutin 300mg and efavirenz 600mg showed no significant effect on the pharmacokinetics of efavirenz but a decrease in the AUC of rifabutin of about 30%. Therefore, it may be necessary to increase the dose of rifabutin to 450–600mg q24h without changing the dosage of efavirenz.

Taking into account this information, the most important priority for physicians managing tuberculosis in the HIV-positive patient is to treat tuberculosis, even more so in smear-positive cases. There are two well defined clinical situations: a patient who is naive to antiretroviral therapy and a patient who is already on therapy. In the former, HAART can be delayed in order to improve adherence to antituberculosis treatment and to avoid toxicity and paradoxic reactions. After 2 months of antituberculosis therapy, the recommendation for antiretroviral therapy should be determined on the basis of clinical factors, $CD4^+$ lymphocyte count, HIV viral load and patient commitment to therapy.

However, it is well known that the use of antiretroviral therapy leads to significant reductions in viral load, AIDS-defining illness and mortality, especially in those patients who have advanced HIV infection ($CD4^+$ <100 cells/mm^3). In such patients, delaying HAART is not warranted and it is recommended that HAART be

TABLE 129-2 -- Drug interactions with rifamycin.

DRUG INTERACTIONS WITH RIFAMYCIN						
Antiviral drug (AVD)	**Rifampin (RIF)**			**Rifabutin (RBT)**		
	RIF's effect on AVD	**AVD's effect on RIF**	**Comments**	**RBT's effect on AVD**	**AVD's effect on RBT**	**Comments**
Saquinavir	80% decrease	No data	Contraindicated	45% decrease	No data	Contraindicated
Ritonavir	35% decrease	Unchanged	No dosage adjustments required; not recommended*	No data	293% increase	Not recommended†
Indinavir	89% decrease	No data	Contraindicated	34% decrease	173% increase	The dose of indinavir should be increased to 1g q8h and RBT should be decreased to 150mg daily
Nelfinavir	82% decrease	No data	Contraindicated	32% decrease	200% increase	No dosage adjustments required; RBT should be decreased to 150mg daily
Amprenavir	81% decrease	Unchanged	Contraindicated	15% decrease	200% increase	The dose of RBT should be decreased to 150mg daily
Lopinavir/ritonavir	75% decrease	No data	Contraindicated	Unchanged	290% increase	Not recommended†
Nevirapine	37–68% decrease	Unchanged	No data	16% decrease	Unchanged	No dosage adjustments required
Delavirdine	96% decrease	Unchanged	Contraindicated	80% decrease	342% increase	Contraindicated
Efavirenz	25% decrease	Unchanged	The dose of efavirenz should be increased to 800mg daily	Unchanged	32% decrease	The dose of RBT should be increased to 450–600mg daily
The effects of rifamycin administration with protease inhibitors or non-nucleoside reverse transcriptase inhibitors on the plasma levels of each drug are expressed as a percentage change in the AUC of the concomitant treatment relative to the level for a single drug treatment.						

* Ritonavir is not well tolerated and could hinder the adherence to antituberculosis treatment.
† The association is possible using 150mg 2–3 times/week, however, it is not recommended due to the high risk of RBT toxicity (uveitis, leukopenia and arthralgia).

started early, at the same time as tuberculosis therapy.[31] The antiretroviral therapy we recommended is the combination of efavirenz plus two nucleoside reverse transcriptase inhibitors or, alternatively, three nucleoside reverse transcriptase inhibitors (including abacavir). Both regimens are simple, well tolerated and allow the use of a rifamycin-based regimen for tuberculosis therapy. The alternative therapy is the use of nelfinavir or indinavir plus two nucleoside reverse transcriptase inhibitors and the substitution of rifampin by rifabutin. Rifampin induction of the CYP3A system can persist for up to 2 weeks. Therefore, rifampin should be substituted by rifabutin for 2 weeks before antiretroviral therapy is initiated, with the required dosage adjustment (Fig. 129.11).

Patients already on treatment with a protease inhibitor regimen (indinavir, nelfinavir, amprenavir or lopinavir/ritonavir) and good $CD4^+$ and viral load response who develop tuberculosis could be treated with a rifabutin-based regimen. If the protease inhibitor is saquinavir, this should be switched to an alternative protease inhibitor. Rifampin therapy is possible if the protease inhibitor is ritonavir. In the case of non-nucleoside reverse transcriptase inhibitor regimens, rifampin or rifabutin could be used with efavirenz and nevirapine with appropriate dosage adjustment, while delavirdine should be changed to efavirenz or nevirapine. In all situations it is also possible to switch therapy to a nucleoside reverse transcriptase inhibitor regimen while tuberculosis treatment is ongoing. Non-rifamycin-containing regimens are only recommended for patients who have serious adverse effects with rifamycins or who are infected with a rifamycin-resistant isolate.

Treatment of drug-resistant tuberculosis

Resistance to isoniazid alone is the most common pattern of drug resistance. If the minimum inhibitory concentration (MIC) for isoniazid is more than 1mg/L, the association of rifampin (or rifabutin), pyrazinamide and ethambutol or streptomycin for the first 2 months followed by rifampin plus ethambutol for 10 months is the regimen recommended. It is possible to include isoniazid, when the MIC for isoniazid is more than 0.1mg/L but less than 1mg/L. Monoresistance to rifampin is more common in HIV-infected patients than in immunocompetent persons.[32] It is associated with nonadherence to therapy, the use of rifabutin as prophylaxis against MAC and the presence of diarrhea. The treatment regimen should consist of an initial 2-month phase of isoniazid, pyrazinamide and ethambutol or streptomycin and a second phase of isoniazid and ethambutol for 10 months. It is possible to reduce the duration of therapy using streptomycin associated with isoniazid and pyrazinamide for 9 months, but the toxicity and route of streptomycin administration makes this regimen less appropriate. For the treatment of multidrug-resistant tuberculosis (resistant to both isoniazid and rifampin), the regimen should include four or five drugs to which the organism is susceptible (aminoglycosides, fluoroquinolones, ethionamide, cycloserine, PAS, thiacetazone or clofazimine) for at least 18–24 months.[33]

Figure 129-11 Management strategies for patients who have HIV infection and tuberculosis. EFV, efavirenz; NRTI, nucleoside reverse transcriptase inhibitor; RBT, rifabutin; RIF, rifampin.

Therapy for *Mycobacterium avium* complex

Treatment regimens should include a macrolide (clarithromycin or azithromycin) plus ethambutol.[34] The addition of rifabutin should be considered, since triple therapy has been associated with a reduction in relapses and in the emergence of resistant strains.[35] Aminoglycosides and quinolones may be useful in macrolide-resistant cases. In the era of HAART, it is important to note that the association of clarithromycin and efavirenz is contraindicated since the AUC of clarithromycin is decreased by about 40%. In addition, ritonavir and lopinavir induce an increase of 100% in the AUC of clarithromycin; therefore, when creatinine clearance is less than 60ml/min, it is necessary to reduce the dose of clarithromycin by 50%.

In general, as with other opportunistic infections, therapy for disseminated MAC is for life. However, in situations where $CD4^+$ T cell counts increase to more than 100×10^6 cells/l after 6–12 months of HAART, patients are at low risk of recurrence of MAC and the treatment can be stopped. Although the number of patients who have been evaluated is small, there is increasing confidence that it is possible to discontinue maintenance therapy[36] in such patients.

Treatment of other nontuberculous mycobacterias

Mycobacterium kansasii is the second most frequent cause of pulmonary and disseminated nontuberculous mycobacterial disease. The treatment consists of a daily regimen of rifampin (or rifabutin), isoniazid and ethambutol for 12–18 months. Therapy of other nontuberculous mycobacterias is briefly described. *Mycobacterium genavense* and *M. haemophilum* are resistant to isoniazid, pyrazinamide and ethambutol. *Mycobacterium genavense* may be treated with a regimen similar to MAC and *M. haemophilum* with a combination of rifampin plus another active antituberculosis drug (ciprofloxacin, doxycycline, clarithromycin or amikacin). *Mycobacterium simiae* and *M. xenopi* are susceptible to isoniazid, rifampin, ethambutol and should be treated for 12 months. *Mycobacterium scrofulaceum* therapy requires surgery and isoniazid plus rifampin for 24 months with amikacin for 2–3 months. *Mycobacterium marinum* is resistant to isoniazid and pyrazinamide. Minocycline or clarithromycin or rifampin plus ethambutol for 3 months are possible therapies. *Mycobacterium fortuitum* therapy consists of amikacin plus cefoxitin plus ciprofloxacin for 1 month followed by quinolone plus clarithromycin for 3–6 months.

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Chapter 130 - Neoplastic Disease

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INTRODUCTION

Infection with human immunodeficiency virus (HIV) is associated with an increased risk of developing cancers, particularly Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL). The risk of KS and NHL is increased respectively 1000- and 100-fold among HIV-infected patients compared with the general population. [1] [2]

The widespread use of highly active antiretroviral therapy (HAART) has radically changed the clinical spectrum of HIV infection in industrialized countries since the mid-1990s. Incidence rates of opportunistic infections (OIs), KS, primary central nervous system lymphoma (PCNSL) and recently systemic NHL have significantly decreased in the HAART era.[3] [4] [5] [6] Kaposi's sarcoma, PCNSL, systemic intermediate/high-grade B-cell NHL and invasive cervical cancer have been designated as AIDS-defining illnesses, but other malignancies have been reported to be associated with HIV infection. These include Hodgkin's disease and high-grade anal epithelial lesions.[1] [2] [3] [7] Possible excesses of other types of cancer, such as nonmelanomatous skin cancer, lung and testicular carcinoma and myeloma, need to be confirmed.[1] [2] [3]

This chapter focuses on the epidemiology, pathology, clinical features and treatment of the two most common malignant tumors in HIV-infected patients: KS and NHL.

KAPOSI'S SARCOMA

EPIDEMIOLOGY

The epidemiology of KS among HIV-infected individuals has dramatically changed during the second decade of the AIDS epidemic. Kaposi's sarcoma incidence rates started to decline in the late 1980s and then more remarkably with the introduction of HAART in the mid-1990s.[1] [2] [3] [4] In a meta-analysis of data on 47,936 HIV-seropositive individuals from North America, Europe and Australia, the KS rate ratio for 1997–99 vs 1992–96 was 0.3.[3] The risk of KS among male homosexuals is greater than 100,000-fold that of persons with other HIV risk behaviors.[1]

In 1994, Chang *et al* discovered a new herpes virus, called KS-associated herpes virus or human herpes virus 8 (HHV-8), and subsequent studies showed that this was an essential causative agent for all forms of KS.[8] HHV-8 transmission correlates with a history of sexually transmitted diseases and number of male sexual partners. Co-infection with both HHV-8 and HIV increases the risk of developing KS as much as 10,000-fold as compared with HHV-8 infection alone. The 10-year probability of developing KS after co-infection with both HHV-8 and HIV approaches 50%.[8] [9]

PATHOLOGY AND PATHOGENESIS

Kaposi's sarcoma is an angioproliferative disease characterized by angiogenesis, endothelial spindle cell growth (KS cells), inflammatory cell infiltration and edema. The histological cell of origin of KS spindle cells remains uncertain, but is probably a mesenchymal progenitor cell of either endothelial or monocyte-macrophage lineage.

Kaposi's sarcoma lesions arise from a contest of immune dysregulation characterized by $CD8^+$ T-cell activation and production of Th1 type cytokines (i.e. interferon-?), interleukin (IL)-1ß, IL-6 and tumor necrosis factor-a) and angiogenic factors (i.e. basic fibroblastic growth factor and vascular endothelial growth factor), that induces a generalized activation of endothelial cells leading to adhesion and tissue extravasation of lymphomonocytes, spindle cell formation and angiogenesis. These phenomena are triggered or enhanced by HHV-8 infection that, in turn, is reactivated by the same cytokines. Productively infected circulation cells are recruited into 'activated' tissue sites where HHV-8 finds an optimal environment for establishing a persistent latent infection of KS spindle cells. Although early KS is a reactive process of polyclonal nature that can regress, in time it can progress into a true sarcoma. The progression of KS appears to be due to the deregulated expression of oncogenes and oncosuppressor genes and to the long-lasting expression of the HHV-8 latency genes and is promoted by proliferative and angiogenic effects of the HIV Tat protein.[10]

CLINICAL FEATURES

Kaposi's sarcoma ranges from an indolent to an aggressive disease with significant morbidity and mortality. Typically, the disease presents with disseminated skin lesions, often with lymph node and visceral involvement such as the gastrointestinal (GI) tract and lungs. Skin lesions arise as macular or papular eruptions, which progress to nodular plaques or lesions (Fig. 130.1); any area of the skin may be involved. Nodular KS does not usually cause necrosis of overlying skin and rarely invades underlying bone structure.

Lymphedema, particularly of the face, genitalia and lower extremities, may be out of proportion to the cutaneous disease and may be related not just to lymphatic obstruction, but also to the cytokines involved in the pathogenesis of KS. Lymphadenopathic KS primarily affects peripheral lymph nodes, sometimes causing massive nodal enlargement, and it may be present in the absence of mucocutaneous disease.

Oral cavity KS occurs in approximately 35% of patients and is the initial site of disease in about 15%. Intraoral lesions most commonly affect the palate and gingiva and may interfere with nutrition and speech.

Over 50% of patients with skin disease have GI lesions. Any segment of the GI tract may be involved, although the stomach and duodenum are most commonly affected. Gastrointestinal KS is seldom symptomatic, but may cause bowel malabsorption or obstruction and, rarely, bleeding.

Pulmonary involvement is also quite common and may be life threatening. In approximately 20% of cases it may occur in the absence of skin lesions. The symptoms, including shortness of breath, fever, cough, hemoptysis and chest pain, and radiologic appearance of pulmonary KS are indistinguishable from those of the more

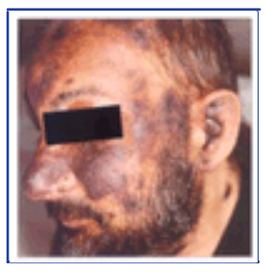

Figure 130-1 Kaposi's sarcoma. There are large confluent hyperpigmented patch-stage lesions with lymphedema.

common opportunistic infections. Radiographic findings vary greatly and can include nodular, interstitial and alveolar infiltrates, pleural effusion, hilar and mediastinal adenopathy, and even an isolated pulmonary nodule. The pleural effusions of KS are typically serosanguinous in nature and are associated with KS lesions on the visceral pleura.

DIAGNOSIS

Although a presumptive diagnosis of KS can often be readily made by a trained observer, a skin biopsy can confirm the diagnosis. It is especially important to biopsy lesions that are less typical of KS because other conditions, such as bacillary angiomatosis, may be confused with KS.

Lesions in the GI tract are often recognized easily on endoscopy; however, because the lesions tend to be submucosal, biopsies may not demonstrate KS.

Bronchoscopy is the procedure of choice for pulmonary KS, but gallium-thallium scanning may also be helpful in evaluating an abnormal radiograph. Kaposi's sarcoma is usually thallium avid and gallium negative, whereas infections are usually gallium avid and thallium negative.

The AIDS Clinical Trial Group (ACTG) classification groups patients according to extent of the tumor, immune status and severity of systemic illness (Table 130.1).[11]

MANAGEMENT

Kaposi's sarcoma is a heterogeneous disease and no specific therapy is curative. Therefore, treatment needs to be individualized, based on the patient's overall clinical and immunological status.

Localized KS cutaneous lesions are treated with radiation therapy, laser therapy, cryotherapy, intralesional injections of antineoplastic drugs and alitretinoin topical gel.[12]

Prolongation of time to treatment failure as well as clinical improvement of KS disease by HAART has been reported in the literature,[12] [13] and there are numerous

anecdotal reports of KS regression while on HAART alone. Anti-KS activity of HAART appears to be linked to immuno-reconstitution and to a lesser extent to suppression of HIV replication. Interestingly, a recent study indicates that protease inhibitors are also potent antiangiogenic molecules in *in vitro* and *in vivo* KS models.[14]

Cytotoxic chemotherapy is indicated for patients who do not respond to HAART and for patients with life-threatening or visceral disease. A wide variety of cytotoxic drugs, including vinca alkaloids (vincristine, vinblastine), bleomycin, doxorubicin individually

TABLE 130-1 -- Staging system for HIV-associated Kaposi's sarcoma.[*] [†]

STAGING SYSTEM FOR HIV ASSOCIATED KAPOSI'S SARCOMA		
	Good risk	**Poor risk**
Tumor	Confined to skin and/or lymph nodes and/or minimal oral disease (confined to palate)	Tumor-associated edema or ulceration; extensive oral Kaposi's sarcoma; gastrointestinal Kaposi's sarcoma; Kaposi's sarcoma in visceral organs
Immune system	$CD4^+$ lymphocytes =200/µl	$CD4^+$ lymphocytes <200/µl
Systemic illness	No history of opportunistic infection or thrush; no systemic 'B' symptoms*; Karnofsky performance status	History of opportunistic infection or thrush; systemic 'B' symptoms; Karnofsky performance status <70%; other HIV-related illness

* Systemic 'B' symptoms are fever, night sweats and/or weight loss >10% of normal body weight.
† *Adapted from reference.*[11]

and in combination, have produced tumor regression in 21–59% of patients, with lower rates for monochemotherapy. Randomized trials showed that liposomal anthracyclines are superior to conventional chemotherapy (BV or ABV regimens) in terms of response rate and toxicity profiles.[15] [16] Paclitaxel is the newest systemic chemotherapeutic agent approved for relapsed KS patients. Response rates range between 59% and 71% and the median duration response (10 months) is the longest of any chemotherapy trial reported thus far.[12]

New treatment modalities including al-transretinoic acid and angiostatic agents are currently under investigation.[12]

Corticosteroid therapy induces the development of KS and worsens pre-existing KS lesions.

PROGNOSIS

Prognostic factors for survival are the immune status of the patients (I) and, to a lesser extent, the initial stage (T) of the neoplasm. Median survival of patients with CD4 count =150/µl and early T stage disease (I_0 and T_0 according to the ACTG) is 35 months, while for patients with CD4 <150/µl (I_1) median survival is comparable for early (T_0) and advanced (T_1) T stage disease, being 13 and 12 months respectively. [12]

NON-HODGKIN'S LYMPHOMA

EPIDEMIOLOGY

The incidence of NHL among HIV-infected patients has decreased significantly since the introduction of HAART and the decline has been most pronounced for PCNSL. Systemic NHL incidence decreased less than KS and later than for the other AIDS-defining illnesses. Consequently, lymphoma has become the most common AIDS-associated cancer among patients receiving HAART. Non-Hodgkin's lymphoma constituted 16% and 8% of all AIDS-related diseased diagnosed in 1998, compared with 4% and 6% in 1994, in Western Europe and the United States, respectively.[1] [2] [3] [4] [5] [6]

Risk factors for HIV-related NHL are older age, degree and duration of immunosuppression, no prior HAART use or insufficient immunologic and virologic response to combined antiretroviral therapy.[7] Moreover, the risk of KS as well as NHL is decreased for people with the CCR5 ?32 polymorphism and NHL risk is increased with the stromal cell-derived factor 1 polymorphism. [1]

TABLE 130-2 -- Pathologic features of HIV lymphoproliferative diseases.

PATHOLOGIC FEATURES OF HIV-LYMPHOPROLIFERATIVE DISEASES	
Non-Hodgkin's lymphomas	
Body cavity-based lymphoma	Primary brain (immunoblastic)
Systemic	
Blastic cell lymphomas	Anaplastic' cell lymphomas
• large noncleaved cell (G-WF)	• anaplastic large-cell (CD30/Ki-I$^+$)
• immunoblastic (H-WF) with or without plasma cell differentiation	
• small noncleaved cell (J-WF) with or without plasma cell differentiation	Others (rare types)
• ?extramedullary plasmacytoma	
• blastic cell with 'intermediate' features	
Hodgkin's lymphoma	
Mixed cellularity	Lymphocyte depletion
Multicentric Castleman's disease	
WF, Working Formulation.	

Non-Hodgkin's lymphoma occurs among all population groups at risk for HIV infection, in all age groups and in different countries, with similar epidemiologic and clinicopathologic features.

HIV-related NHL are broadly divisible into three categories according to their anatomical site of origin: systemic NHL, PCNSL and body cavity-based lymphoma or primary effusion lymphoma (PEL). PELs are uncommon, accounting for approximately 3% or less of HIV-NHL. They exhibit a unique constellation of clinical, pathological and molecular characteristics and thus represent a distinct clinicopathological entity.

PATHOLOGY AND PATHOGENESIS

The pathological classification has been redefined at our institution (Table 130.2). The systemic NHLs are histologically heterogeneous, with 80–90% featuring three Working Formulation categories:

- ¦ large cell lymphoma (G group);
- ¦ large cell immunoblastic lymphoma (LCIBL, H group); and
- ¦ small noncleaved cell lymphoma (J group), the equivalent of Burkitt's-type lymphoma (BL).

Cases showing 'intermediate' histologic features may be detected and, in addition, B-cell CD30$^+$ anaplastic large cell lymphoma (ALCL), a heterogeneous group of high-grade lymphomas at the borderline between Hodgkin's disease and NHL, may be found. In contrast with the heterogeneity of systemic NHL, PCNSLs represent a more uniform group and in the vast majority of the cases share LCIBL histologic features. The PELs, typically growing as lymphomatous effusions, have morphologic and immunophenotypic characteristics similar to those of LCIBL or ALCL.

The pathogenesis of HIV-NHL is a multistep process involving factors provided by the host as well as alterations intrinsic to the tumor clone. The molecular pathways of viral infection and lesions of cancer-related genes associated with HIV-NHL vary substantially in different clinicopathological categories of the disease and highlight the marked degree of biological heterogeneity of these lymphomas.

At present, four major molecular pathways can be identified, each of which is associated with peculiar clinical features and restricted to a given NHL histological type. The first pathway associates with BL and is characterized by relatively mild immunodeficiency of the host and multiple genetic lesions of the tumor, including activation of c-*myc*, disruption of p53 and, less frequently, infection by Epstein-Barr virus (EBV). Typically, EBV-infected BL fail to express

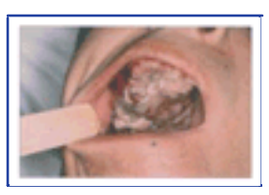

Figure 130-2 Non-Hodgkin's lymphoma. Bulky disease in the gingiva.

the viral transforming antigens latent membrane protein 1 (LMP-1) and Epstein-Barr virus nuclear antigen 2 (EBNA-2). Histogenetic studies have shown that BL derives from germinal center (GC) cells, of which the lymphoma closely mimics the phenotype.

Two distinct pathways associate with diffuse large cell lymphoma (DLCL), a type of NHL frequently characterized by a marked disruption of immunofunction. Whereas the majority of DLCL are EBV positive, only a fraction of cases express the viral antigen LMP-1. Expression of LMP-1 and BCL-6 segregate the two pathways associated with HIV-DLCL. On the other hand, LMP-1 positive HIV-DLCL fail to express the BCL-6 protein and display features consistent with immunoblastic-plasmacytoid differentiation, suggesting a derivation from post-GC cells. However, LMP-1 negative DLCL express BCL-6 and display a large noncleaved cell morphology, suggesting an origin from the GC.

Finally, the fourth pathway associates with PEL. This rare lymphoma type consistently harbors infection by HHV-8 and frequently also by EBV. All other genetic lesions commonly detected among HIV-NHL are consistently negative in HIV-PEL. Histogenetic studies have shown that PEL reflects a post-GC stage or differentiation close

to plasma cells.

The identification of the molecular and histogenetic heterogeneity of HIV-NHL is of potential clinical value, because the molecular and histogenetic features of the tumor have been shown to influence the prognosis of several B-cell disorders of immunocompetent hosts.[17]

CLINICAL FEATURES

One of the distinguishing features of NHL is the widespread extent of disease at initial presentation and the frequency of systemic 'B' symptoms, including fever, night sweats and weight loss of more than 10% of the normal body weight. At the time of diagnosis approximately 75% of patients have advanced disease with frequent involvement of extranodal sites, the most common being the central nervous system (CNS), bone marrow, GI tract and liver. Any site of the body, however, may be affected (Fig. 130.2).

Approximately 20–40% of patients have CNS meningeal infiltration at presentation, whereas 65% have brain infiltration at the time of autopsy examination. Leptomeningeal disease, identified during routine lumbar puncture as part of the initial staging evaluation, remains asymptomatic in approximately 20% of patients. Gastrointestinal tract involvement, sometimes at multiple sites, develops in 10–40% of the cases. Bulky disease can be observed in the anorectal region, particularly in homosexual men.

The PCNSL (i.e. intracranial parenchymal lymphoma limited to the CNS) is a manifestation of very advanced HIV disease. Usually the $CD4^+$ lymphocyte count at diagnosis is less than 50/µl. The lymphoma develops as single or multiple lesions in the deep regions of white matter, in the basal ganglia and in the cerebellum. The clinical presentation of PCNSL is not specific and approximately 50% of patients present with lethargy, confusion and personality change, whereas many others lack lateralizing neurologic signs.

The PEL grows exclusively or mainly within pleural, pericardial or peritoneal cavities as lymphomatous effusions, usually in the absence of a contiguous tumor mass. It usually remains strictly localized to the body cavity of origin and only infrequently spreads to local lymph nodes or distant sites.

In the setting of HIV disease, NHL can be difficult to diagnose because of its variable presentation. It can mask many conditions of both HIV disease itself and its associated opportunistic infections. For instance, systemic 'B' symptoms are frequently associated with both advanced HIV infection and opportunistic infections. These symptoms mandate a careful evaluation to exclude other causes, including the presence of *Mycobacterium avium-intracellulare*, cytomegalovirus or tuberculosis infection.

The PCNSL may be radiographically indistinguishable from cerebral toxoplasmosis or other CNS infections. It has been shown that detection of cerebrospinal fluid (CSF) EBV-DNA by polymerase chain reaction (PCR) in HIV-infected patients is reliably associated with PCNSL. By combining CSF EBV-DNA detection by PCR with ^{201} T1 single photon emission computed tomography, the presence of increased uptake and positive EBV-DNA had 100% sensitivity and 100% negative predictive value. Thus in patients with hyperactive lesions and positive EBV-DNA, brain biopsy may be avoided and patients could promptly undergo definitive therapy.[7]

DIAGNOSIS

A diagnosis of NHL should be made by histologic examination of the tissue obtained by incisional or excisional biopsy. It may be possible, however, to make an adequate diagnosis from needle aspiration cytology and this may be required if the patient's clinical condition is critical or deteriorating rapidly.

MANAGEMENT

Optimal therapy for HIV-NHL has not been defined and whether intensive or conservative chemotherapy regimens are indicated in these patients is still a matter of controversy. In fact, poor bone marrow reserve and underlying HIV immunodeficiency challenge the optimal management of systemic NHL. For PCNSL primary therapy consists of whole-brain radiation with intrathecal chemotherapy.

Therapeutic guidelines that should be considered in the management of systemic NHL are:

- first, as with other aggressive lymphomas, the use of combination chemotherapy is essential; and
- second, chemotherapy regimens must include intrathecal chemotherapy, as either a prophylactic or therapeutic modality.

Initially, intensive chemotherapy regimens have been associated with a significant risk of early death due to OIs, suggesting that less intensive treatment strategies should be explored. The use of a low-dose regimen of methotrexate-leucoverin rescue-bleomycin-doxorubicin-cyclophosphamide-vincristine and dexamethasone (m-BACOD) results in a complete response (CR) rate of 46–56%, but median survival is only 15 months for CR patients and 6.5 months for all patients. No significant differences are observed in response rate, response duration or survival when low-dose m-BACOD is randomly compared with standard-dose m-BACOD, in phase III trials. Only 27% of CR patients receiving low-dose therapy and 24% receiving standard-dose therapy survive more than 1 year.[18]

Studies conducted in the pre-HAART era demonstrated that a subset of patients with HIV-NHL is able to tolerate aggressive chemotherapy and appears to do reasonably well in terms of lymphoma-free survival.[7] The use of a continuous infusion of cyclophosphamide-doxorubicin and etoposide (CDE) plus didanosine results in a CR rate of 53% and a median survival of 18 months. However,

TABLE 130-3 -- HIV-associated non-Hodgkin's lymphoma.*

HIV-ASSOCIATED NON-HODGKIN'S LYMPHOMA	
Unfavorable prognostic factors for survival	
Major	$CD4^+$ lymphocyte count <100/µl*
	Previous AIDS diagnosis
	Low performance status
Minor	Bone marrow involvement
	Extranodal disease
	No response to therapy
	'B' symptoms
	Immunoblastic lymphoma
	Age =40 years
	Increased lactate dehydrogenase concentration

* <200/µl in some series.

in the pre-HAART era these encouraging results were associated with a significant and sustained reduction in the CD4 cell count and a twofold increase in the risk of OIs after chemotherapy.[19]

Preliminary studies show that combination therapy with cyclophosphamide-doxorubicin-vincristine and prednisone chemotherapy (CHOP) and HAART is feasible in HIV-NHL patients.[20] [21] Overall response rate as well as duration response and survival may be favorably affected by the use of HAART along with chemotherapy and future trials should be designed to address this issue.

In conclusion, our recommendations are to give standard-dose chemotherapy regimens (like CHOP or CDE) to low- or good-risk category patients and conservative chemotherapy regimens (i.e. single drug or low-dose CHOP-like regimens) to high- or poor-risk patients.

PROGNOSIS

NHL is significantly associated with a worse prognosis than many other complications of AIDS. Nevertheless, some prognostic factors for survival have been identified (Table 130.3). The classic prognostic criteria of the general population (i.e. age, performance status, stage, extranodal involvement) have to be supplemented by host prognostic criteria in the HIV setting, namely low $CD4^+$ lymphocyte count (<100/µl) and a previous AIDS diagnosis, both of which reflect the underlying immunodeficiency. Patients with a low $CD4^+$ lymphocyte count and a previous AIDS diagnosis have a median survival of 3 months, whereas patients without these adverse prognostic features have a median survival of 12 months.[7]

There is some evidence from single institution cohorts that median survival of HIV-NHL patients is improving and is at least three times longer in the HAART than in the pre-HAART era.[5] [6]

HUMAN PAPILLOMAVIRUS AS CAUSATIVE AGENT FOR CERVICAL/ANAL CANCER

An association between HIV infection and human papillomavirus (HPV)-related anogenital neoplasia has recently been recognized. The overall risk of all HPV-associated cancers and their in situ precursor lesions in both women and men is elevated across all HIV exposure risk categories.[1] [2] Risk factors include multiple sexual partners, cigarette smoking and sexually transmitted disease, particularly HPV.

Viral sequences are found in more than 99% of cervical squamous cell carcinomas and in most anal cancers, with HPV type 16 present in 50% and types 18, 31 and 45 in another 30%. High-grade

intraepithelial neoplasia precursor lesions have similarly high rates of the same HPV types. At the molecular level, HPV-associated oncogenesis appears to result from upregulated expression of viral-encoded transforming proteins, including E6 and E7. These proteins interact with and inactivate the products of host cell tumor suppressor genes, including retinoblastoma and p53. This process results in unregulated progression through the cell cycle, insufficient DNA repair and, eventually, transformation to a malignant phenotype.[22]

HIV appears to accelerate the pathogenesis of anogenital cancers at the molecular level although clinical evidence of disease progression is limited. HIV-infected lymphocytes, monocytes and macrophages can be detected in cervical and anal epithelium, and *in vitro* studies suggest the HIV-encoded Tat protein may enhance the expression of HPV E6 and E7 transforming proteins.[23]

The degree of HIV-related immunosuppression appears to be related to the occurrence and the severity of the anogenital neoplasia.

A direct effect of HAART on HPV infections and associated lesions seems unlikely.[23] Screening programs associated with local therapy of early lesions have dramatically reduced the incidence of cervical cancer among HIV-negative women and may have the potential to reduce anal cancer as well. Preventive and therapeutic approaches, i.e. HPV vaccines, need to be urgently evaluated by prospective studies in HIV-infected patients.

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Chapter 131 - HIV-associated Wasting and Nutrition

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George Griffin

EPIDEMIOLOGY

Before the advent of highly active antiretroviral therapy (HAART), wasting syndrome (defined as loss of more than 10% of body weight together with fever or diarrhea for more than 30 days) was present in about 10% of patients at the time of AIDS diagnosis[1] and occurred in the majority of patients at some stage before death. Although the introduction of HAART has decreased the incidence of opportunistic infections and associated wasting, wasting still remains a common problem in clinical practice.[2]

PATHOGENESIS AND PATHOLOGY

Basal metabolic rate is generally increased at all stages of HIV infection, particularly during opportunistic infections, but this is offset by reductions in physical activity and total energy requirements are therefore normal or reduced. [3] The key etiologic factor in wasting is therefore a reduction in energy intake. The numerous causes include nausea, taste disturbances, dysphagia, early satiety, depression and dementia. Profound anorexia mediated by cytokine release accompanies acute opportunistic infections and results in rapid weight loss (Fig. 131.1).

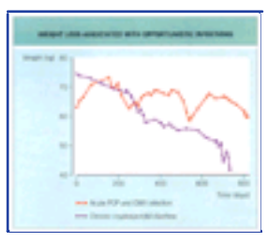

Figure 131-1 Weight loss associated with opportunistic infections. Weight chart of one patient who had episodes of rapid weight loss and partial recovery coinciding with episodes of acute opportunistic infection — *Pneumocystis carinii* pneumonia (PCP) and cytomegalovirus (CMV) — and one patient who had chronic progressive weight loss associated with cryptosporidial diarrhea. Reproduced with permission by the American Journal of Clinical Nutrition © Am L Clin Nutr. American Society for Clinical Nutrition.

Malabsorption is common in AIDS and can be idiopathic (HIV enteropathy) or secondary to gastrointestinal pathogens, especially protozoa. Although malabsorption alone rarely leads to significant weight loss, it may exacerbate the energy intake deficit when combined with other factors. Furthermore, the associated diarrhea may cause a voluntary reduction in food intake in an attempt to minimize symptoms. Chronic gastrointestinal disease often results in a pattern of progressive weight loss (see Fig. 131.1).

HIV infection is accompanied by disturbances in intermediary metabolism, but these are not necessarily of importance in determining the quantity or quality of tissue that is lost. However, hypogonadism is common and may result in preferential lean tissue depletion.

PREVENTION

Patients should be weighed regularly on the same set of scales and the results should be displayed graphically in the medical notes. Assessment at regular intervals by a dietitian is recommended for all patients who have HIV infection. Such reviews should reinforce the need to maintain adequate energy and protein intake to prevent wasting and may correct misconceptions about what constitutes a healthy diet. Regular physical exercise should be encouraged and will assist in maintaining muscle mass.

CLINICAL FEATURES

Clinical assessment of HIV-associated wasting is directed at confirming the diagnosis of malnutrition, estimating severity and also identifying any underlying cause.

Nutritional state can be assessed in several ways. Comparison of body weight with pre-illness weight or ideal weight (determined from standard tables) is the simplest method. A reduction of 10% indicates significant wasting; a loss of over 35% is associated with a grave prognosis. Calculation of body mass index (BMI) can also be used to grade the severity of malnutrition:

$$\text{BMI} = \frac{\text{weight in kg}}{\text{height in m}^2}$$

Severity of malnutrition is graded as follows:

- grade I, <18.5kg/m^2 ;
- grade II, <17kg/m^2 ; and
- grade III, <16kg/m^2 .

Grade III malnutrition is regarded as life-threatening. Measurement of mid-upper-arm circumference or estimation of body cell mass by bioelectrical impedance analysis (BIA) may be more sensitive than body weight or BMI in detecting early malnutrition and provide an additional estimate of severity in advanced wasting. Although single measurements of body weight, BMI, mid-upper-arm circumference and BIA are of some use, serial assessment gives a better indication of nutritional status and the urgency for treatment.

Complications of malnutrition include decubitus ulcers, hypothermia, amenorrhea in women and further propensity to develop

infections. Patients may die from severe wasting alone without other specific complications of HIV disease.[4]

As well as confirming the presence and estimating the severity of wasting, the history and examination should be directed at identifying potential causes. Specific inquiry should be made about anorexia, taste disturbances, dysphagia, odynophagia, nausea, vomiting, abdominal pain, diarrhea and symptoms of depression. Examination may help to distinguish HIV-associated wasting from the lipodystrophy syndrome that occurs as a complication of antiretroviral therapy. Loss of fat from

the face and limbs may initially suggest a diagnosis of HIV-associated wasting, but in the lipodystrophy syndrome muscle mass is preserved and truncal fat mass is usually maintained or increased, so that there is minimal change in overall body weight.

DIAGNOSIS

The purpose of laboratory investigations is to determine the cause of wasting. The choice of tests is influenced by the clinical features but investigations usually include:

- hematology — a full blood count, which may show anemia due to malabsorption;
- biochemistry — low serum albumin accompanies malnutrition and low serum testosterone indicates hypogonadism;
- microbiology — blood should be cultured and at least three stool samples should be assessed;
- virology — serial HIV viral load and $CD4^+$ lymphocyte count estimates may indicate accelerated viral replication;
- radiology — a plain chest radiogram may reveal lymphoma, tuberculosis or subclinical *Pneumocystis carinii* pneumonia, and an abdominal ultrasound may reveal lymphadenopathy (mycobacterial infection or lymphoma), hepatic lesions or abscess; and
- other investigations include an upper gastrointestinal endoscopy with small bowel biopsy or colonoscopy and biopsy.

MANAGEMENT

The aim is to increase lean body mass and so improve quality of life and physical functioning and increase survival. The initial step is to identify and remove any underlying causes for the wasting. Nausea and vomiting should be managed aggressively. Treatment and recovery from acute opportunistic infection is often accompanied by a repletion of body weight (see Fig. 131.1)[5] and lean tissue. Reduction of viral load by effective antiretroviral therapy may sometimes be followed by substantial weight gain, although lean tissue may not be restored fully. For patients who remain malnourished, dietary counseling, nutritional therapies and progressive resistance exercise should be tried in the first instance. Hypogonadal patients should have testosterone replacement. If these steps are unsuccessful, additional pharmacologic therapies may be indicated. Management should be tailored to the individual patient.

NUTRITIONAL THERAPIES AND EXERCISE

High-energy oral nutritional supplements

A valuable increase in total energy intake can be achieved by nutritional supplements, although some patients find them unpalatable or develop 'taste fatigue'.

Nasogastric feeding

Patients who have mechanical difficulties in swallowing or severe anorexia may benefit from feeding through a fine-bore nasogastric tube. This approach has the benefit of allowing nocturnal feeding. Although sometimes well tolerated for short periods of time, a nasogastric tube can be uncomfortable and distressing.

Percutaneous endoscopic gastrotomy tube feeding

Insertion of a percutaneous endoscopic gastrotomy tube is a straightforward procedure performed under local anesthetic. Percutaneous endoscopic gastrotomy feeding can produce dramatic increases in body weight in selected patients who have HIV disease, and serious complications are infrequent. However, pre-existing diarrhea may be exacerbated, and therefore an initial trial of nasogastric feeding is recommended.

Total parenteral nutrition

A controlled trial in malnourished AIDS patients demonstrated that total parenteral nutrition significantly increased body weight and lean tissue when compared with dietary counseling.[6] However, it is a complex and expensive treatment that should only be considered when enteral nutrition has failed. The duration of therapy should be clearly defined at the outset because the decision to stop is often difficult.

Progressive resistance exercise

A supervised training program of progressive resistance exercise can increase lean body mass and may have other health benefits such as increasing high-density lipoprotein (HDL) cholesterol.[7]

PHARMACOLOGIC THERAPIES

Growth hormone

Recombinant human growth hormone at a dose of 0.1mg (0.3 units)/kg q24h increases lean body mass and exercise capacity in patients who have HIV-associated wasting.[8] Although effective, widespread use of growth hormone is limited by its cost. A short course of growth hormone may prevent the loss of lean tissue accompanying opportunistic infection, although further data is needed on this approach and growth hormone should not be used in critically ill HIV patients.[9]

Testosterone

Hypogonadal HIV-positive men may benefit from physiologic testosterone replacement, which can be given by either intramuscular injection (e.g. testosterone enanthate 300mg every 3 weeks)[10] or a cutaneous patch. Supraphysiologic doses of testosterone administered for a few months increase lean body mass in eugonadal men but more safety data is needed before this approach can be advocated for longer term use.[7]

Megestrol acetate

At a dose of 800mg q24h, megestrol increases food intake and body weight in patients who have HIV infection, although the weight gain is predominantly fat.[11] This drug may have a deleterious antianabolic action mediated by decreases in testosterone levels. Although megestrol may be of use for the palliation of anorexia, increases in fat mass alone are unlikely to result in improved survival or physical functioning.

Others

Dronabinol, a synthetic cannabinoid, can stimulate appetite, although sedation and psychotropic symptoms are common. Anabolic steroids that have greater anabolic activity than testosterone are potentially useful in HIV disease, although there is insufficient data from controlled clinical trials to recommend their use and there are concerns over safety (especially in patients who have concomitant hepatic disease). Thalidomide can increase body weight, although drug tolerability (rash and fever) limit its use and treatment is associated with an increase in viral load. [12]

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Chapter 132 - Dermatologic Manifestations of HIV Infection

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Chris Bandel
Clay J Cockerell

INTRODUCTION

The skin is commonly affected in patients who have HIV infection and AIDS. Because it is the organ most readily evaluated by inspection it is essential that the clinicians become proficient in the recognition of skin disorders that herald the presence of HIV disease.[1] The prevalence of cutaneous involvement approaches 100% and in many cases is the motivating force the causes the patient to seek medical care.

To the alert physician, dermatologic manifestations of HIV infection may provide the initial diagnostic clues, not only of the presence of the infection per se but also in some cases the stage of involvement.[2] Early-stage disease (CD4 count 200–500/mm^3) can be associated with seborrheic dermatitis, oral hairy leukoplakia (OHL), Kaposi's Sarcoma (KS), oropharyngeal or recurrent vulvovaginal candidiasis, herpes zoster or recurrent herpes simplex virus (HSV) infection. With a CD4 count of 50–200/mm^3 , bacillary angiomatosis, esophageal candidiasis, disseminated deep fungal infections, molluscum contagiosum, chronic herpetic ulcers and eosinophilic folliculitis become more prominent. With advanced HIV disease (CD4 <50/mm^3), profound immunosuppression may lead to the occurrence of several co-existing infections and neoplasms. In general, skin infections and neoplastic conditions in patients who have AIDS are aggressive and difficult to treat. Early recognition of the different skin diseases will facilitate the use of a pertinent therapeutic approach.

With the introduction of highly active antiretroviral therapy (HAART) in the mid 1990s, changes in the dermatologic manifestations paralleled the stabilization and improvement in CD4 counts.[3] [4] Significant decreases in the incidence of KS, OHL, oral candidiasis, bacterial folliculitis, recurrent HSV and, to a lesser degree, seborrheic dermatitis, psoriasis, drug eruptions, dry skin and pruritus were noted. Conversely, conditions such as warts of all kinds, scabies, photosensitivity and eosinophilic folliculitis did not abate significantly and, in some cases, increased in incidence. Adverse drug reactions improved or worsened with different drugs and individuals. Some entities, such as molluscum contagiosum, became less prevalent in some series and more in others, reflecting varying influences such as immune restoration, altered cytokine patterns and improved lifestyle and increased exposure. As CD4 counts normalize with HAART therapy, many of the skin disorders affecting HIV-infected patients become similar to those seen in immunocompetent patients.

Modifying the influences of HAART therapy is the fact that fewer than 10% of the globally infected individuals have access to the therapy, which remains expensive. Resistant cases are also encountered. Thus, it behoves the clinician to remain knowledgeable about the skin conditions particular to HIV-infected patients. [5]

This chapter describes the most common dermatologic manifestations of HIV infection, including skin changes present early in infection and in advanced disease, such as neoplastic, infectious and noninfectious conditions, hair, nail and oral changes and drug reactions.

EARLY DERMATOLOGIC MANIFESTATIONS OF HIV INFECTION: ACUTE EXANTHEM OF HIV INFECTION

The acute exanthem, which is symptomatic in about 80% of patients, begins 1–5 weeks after exposure to the virus and is associated with prodromal symptoms such as lymphadenopathy, fatigue, fever and night sweats. The skin eruption consists of erythematous, round to oval macules and papules affecting the trunk, chest, back and upper back (Fig. 132.1). The syndrome generally lasts for 4–5 days and resolves with complete recovery. [6] Rarely, a severe form of acute HIV infection can evolve, characterized by recurrent viremia, rapid decline in $CD4^+$ cell numbers and an accelerated disease course. Pneumonitis, esophagitis, meningitis, abdominal pain and melena are the most common systemic manifestations and are often accompanied by urticaria, palatal ulceration, candidiasis and herpesvirus infections. The prognosis for patients who have the severe form of acute HIV infection is poorer than for those who are asymptomatic or have mild symptoms (see Chapter 122).[6]

NON-NEOPLASTIC INFECTIOUS DERMATOLOGIC CONDITIONS

Patients who have HIV infection may present with a broad array of infectious processes, some of which are characteristic of AIDS. Because of immunosuppression these infectious processes may exhibit more aggressive behavior than is usually expected in immunocompetent individuals.

Viral infections

Herpes simplex virus

Infections with HSV types 1 and 2 result in recurrent, severe painful grouped vesicles with an erythematous base localized mainly on the lips, genital and perianal areas. If untreated these lesions may enlarge and become confluent ulcerations that may persist for over 1 month, displaying slow healing and often becoming secondarily infected with bacteria. It is essential to establish the specific diagnosis by means of a biopsy and/or viral cultures. Sometimes, ulceration takes place without well-defined vesicles ever being noted.[7] While the immune system is intact, the course of the disease is similar to that in noninfected individuals. Once immunosuppression sets in, the lesions become persistent. Ulcers can expand and reach large size. Periungual infection is also a manifestation. Culture of tissue can be positive even if a swab is negative. An aggressive therapeutic approach should be used with these patients, as they may be poorly responsive to the standard treatment. Oral aciclovir in doses of up to 400mg five times a day for 10 days should be used. In severe cases hospitalization for administration of high-dose intravenous aciclovir may be required.[8] Foscarnet is recommended in patients in whom aciclovir resistance is suspected (see also Chapter 125).

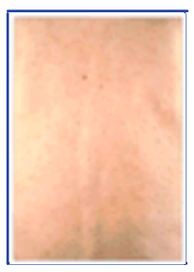

Figure 132-1 Acute exanthem of HIV infection. There is morbilliform eruption involving the trunk and extremities. The eruption is similar to a morbilliform drug eruption and to other viral exanthemata.

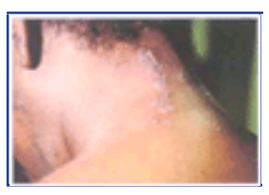

Figure 132-2 Herpes zoster. A painful linear-zosteriform eruption of vesicles on an erythematous base is characteristic of herpes zoster. The eruption may be persistent and

verrucous lesions are not uncommon.

Varicella-zoster virus

The development of recrudescent varicella-zoster virus (VZV) infection in a patient at risk of HIV infection may be a sign of the presence of HIV and should alert the clinician to screen the patient.[9] It usually occurs early in the course of the disease and precedes thrush and hairy leukoplakia by about a year. Varicellazoster virus exists in a dormant state in a dorsal root ganglion that becomes infected during prior varicella infection. With reactivation, the virus progresses downward through the nerve tracts of a solitary dermatome, leading to the characteristic zosteriform distribution of painful tense vesicles of skin (Fig. 132.2). In individuals who have HIV infection, the infection may be recurrent, severe, with more than one dermatome involved, and may run a protracted course associated with residual postherpetic neuralgia and scarring. Disseminated herpes-zoster is not as common but may be more common in HIV-infected individuals. Chronic lesions can be verrucous or ecthymatous. Chickenpox can develop in previously unexposed individuals with HIV infection. The infection can be

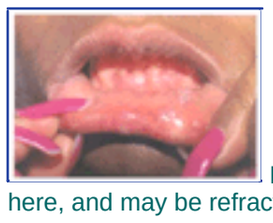

Figure 132-3 Human papillomavirus infection. Human papillomavirus infections are common in HIV-infected patients. They may have unusual features, as demonstrated here, and may be refractory to therapy.

more severe, cause visceral disease and be fatal. Large doses of aciclovir (up to 800mg q4h) are used to treat these patients and often systemic administration is necessary.

Cytomegalovirus

Cytomegalovirus (CMV) is the most common cause of serious opportunistic viral infection in patients who have AIDS.[10] However, cutaneous involvement is rare. In the skin, CMV has different clinical manifestations, including ulcerations, keratotic verrucous lesions and palpable purpuric papules. Because the mucocutaneous lesions caused by CMV do not have specific features, tissue biopsy of the lesions, as well as immunoglobulin titers (IgG and IgM) and viral cultures, are required to define the etiologic cause. The treatment of choice for CMV infection is intravenous ganciclovir. Foscarnet should be used if ganciclovir resistance is suspected.

Epstein-Barr virus

The majority of adults harbor the Epstein-Barr virus (EBV) in the latent phase. With advanced immunodeficiency seen in HIV-infected patients, EBV replication occurs, leading to OHL or EBV-associated large cell lymphoma. Oral hairy leukoplakia manifests as single or multiple white plaques on the lateral margins of the tongue, with a verrucous surface. The presence of oral leukoplakia correlates with moderate to advanced immunodeficiency and has also been correlated with progression from HIV infection to AIDS.[11] Oral hairy leukoplakia responds well to systemically administered aciclovir, although there is prompt recurrence after treatment. Good response to topical application of podophyllin has also been reported.[12] In some patients, OHL may regress with highly active antiviral therapy alone.

Human papillomavirus

Different types of human papillomavirus (HPV) tend to cause different clinical lesions, although there is significant overlap. Infections of the skin and mucous membranes by HPV can cause widespread warts in patients who have AIDS. Types of warts observed include filiform, flat and plantar. Warts can develop in unusual locations, such as on the lips, tongue and oral mucosa (Fig. 132.3). These lesions are often treated with cryotherapy, electrocauterization or topical treatment with caustic agents such as podophyllin. However, the majority of these lesions are resistant to treatment and should be treated repeatedly. In men, HPV affects the penis, urethra, scrotum, perianal, anal and rectal mucosa in the form of condylomata

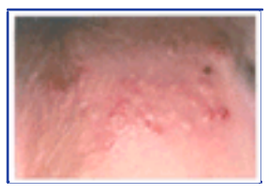

Figure 132-4 Molluscum contagiosum. These lesions are characteristically translucent, waxy papules with central umbilication.

acuminata that are usually recognized as soft sessile lesions with finger-like projections. In women, the spectrum of clinical disease induced by HPV is broad with vulvar, vaginal and cervical condylomata being observed. Other clinical presentations of HPV infection include bowenoid papulosis and epidermodysplasia verruciformis. The former manifests as small, brown, flat-topped papules affecting the perianal and genital areas in both sexes, but is more common in men. The latter consists of a widespread papular eruption of pink-red, flat, wart-like lesions distributed mostly on sun-exposed areas of the skin.[13]

Infection with HPV may cause development of carcinoma, especially in HIV-infected hosts.[14] The most common of these are cervical intraepithelial neoplasia in women and squamous cell carcinoma in men. As with zoster, whenever extensive warts develop in otherwise healthy patients known to be at risk of HIV infection, the patient should be screened for the infection.

Poxvirus

The most common disease caused by poxviruses is molluscum contagiosum, which develops in 10–20% of patients who have AIDS. Molluscum contagiosum is characterized by dome-shaped umbilicated translucent 2–4mm papules that develop in any part of the body but especially the face and genital areas (Fig. 132.4). In AIDS patients these lesions are widespread and may attain immense size.[15] Most patients who have extensive molluscum contagiosum associated with HIV infection have $CD4^+$ counts well below 250 cells/ml. In immunosuppressed patients the diagnosis of molluscum contagiosum should be confirmed by histologic examination in any case that is questionable because this may simulate more serious infections such as cutaneous pneumocystosis, histoplasmosis and *Penicillium marneffei* infection and cryptococcosis. Molluscum contagiosum is treated with cryotherapy, electrodesiccation, curettage or topical application of keratolytic preparations.

Other viral infections

Several viral infections have been reported to develop with increased frequency in patients who have HIV infection. Parvovirus B19, which causes erythema infectiosum, has been reported to produce an exanthem and polyarthralgia in HIV-positive patients. Coxsackie virus and enterovirus may also lead to morbilliform or vesicular eruptions. Measles occur sporadically in nonimmune patients who have AIDS. When associated with encephalitis and pneumonitis, measles can be fatal. Treatment with intravenous ribavirin and gammaglobulin has been used successfully in some patients.

Bacterial infections

Folliculitis

Bacterial folliculitis is common in HIV-infected patients, appearing as widely distributed acneiform papules and pustules. Lesions may be pruritic and become excoriated. Most cases are caused by *Staphylococcus aureus*,[16] but other organisms such as *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* may also cause folliculitis. Bacterial folliculitis in HIV-infected patients is often resistant to standard treatment and prolonged use of systemic antibiotics may be required.[17] Recurrent bacterial folliculitis may serve as a clue to screen a patient for possible HIV infection.

Impetigo, abscesses, cellulitis, lymphadenitis and necrotizing fasciitis

Impetigo, usually caused by *S. aureus*, is seen most commonly on the face, shoulders, axillary and inguinal areas. The infection begins with painful red macules that

become vesicles and pustules and contain purulent fluid; these soon rupture and give rise to the characteristic honey-colored crust. Soft tissue and deep-seated bacterial infections such as cellulitis, abscesses and necrotizing fasciitis may also develop in HIV-infected patients. These manifest as diffuse, red, warm, tender areas in the skin, associated with severe toxemia. Streptococcal axillary lymphadenitis is a diffuse, painful swelling of lymph nodes in the axilla that is usually bilateral. Aggressive antibiotic treatment is recommended for these processes.

Mycobacterial infections

Mycobacteria may produce a wide variety of skin lesions in HIV-infected individuals and infection by these organisms usually signifies severe disseminated systemic infection. Active infection is caused primarily by *Mycobacterium avium-intracellulare* and *M. tuberculosis* and less commonly by *M. kansaii, M. haemophilum, M. genavense, M. marinarum* and *M. leprae*. The infection can manifest as small papules and pustules resembling folliculitis, atopic dermatitis-like eruptions, cutaneous abscesses, lymphadenitis and ulcerations. Culture and tissue biopsy are required for specific diagnosis of the infection. *Mycobacterium haemophilum*, where the only manifestation of infection may be a single or multiple skin lesions, must be cultured at room temperature and with a source of iron in the media.

Bacillary angiomatosis

Bacillary angiomatosis (BA) is a pseudoneoplastic, infectious cutaneous vascular disorder[18] caused by bacteria of the genus *Bartonella*, including *Bartonella quintana* and *Bartonella henselae*.[19] There are a number of clinical manifestations of BA. The earliest and most common lesion appears as discrete pinpoint red-purple papules similar to pyogenic granulomata. These lesions may ulcerate and become crusted (Fig. 132.5). Another variant consists of subcutaneous nodules that may extend into the underlying skeletal muscle and bone. Patients who have BA may have systemic signs and symptoms, including fever, chills, night sweats and weight loss. In advanced cases, the liver and spleen may be involved. Bacillary angiomatosis occurs primarily in the context of the advanced stage of HIV infection, but may occur in patients who have other forms of immunosuppression or in a healthy host. Because the clinical presentation of this infection can easily be confused with pyogenic granuloma, biopsy should be performed. Bacillary angiomatosis responds to treatment with macrolide antibiotics such as erythromycin, clarithromycin and azithromycin or doxycycline. Recurrence is common.

Sexually transmitted disease

The accurate diagnosis of sexually transmitted diseases (STDs) is of exceptional importance in individuals at high risk of HIV infection,

Figure 132-5 Bacillary angiomatosis. There are elevated vascular papules of the glabrous skin. When incised, these lesions bleed profusely.

because their presence increases the risk of transmitting and acquiring HIV infection. Studies have demonstrated that, in some populations, the pattern of HIV acquisition parallels that of STD.[20]

Syphilis

A high prevalence of syphilis, active as well as latent, has been found among AIDS patients in the USA.[20] In HIV-infected patients, the primary infection with *Treponema pallidum* does not show major variations. Secondary syphilis may occur in a number of forms in patients who have HIV infection, ranging from the classic papulosquamous form with involvement of palms, soles and mucous membranes to unusual forms such as verrucous plaques, extensive oral ulcerations, keratoderma, deep cutaneous nodules and widespread gummata. The disease may progress faster from secondary to tertiary syphilis in HIV-seropositive patients than in HIV-seronegative individuals. Early central nervous system (CNS) relapse can also be more common in HIV-infected individuals. Syphilis is a strong indicator that an individual may be infected with HIV and all patients should undergo HIV serotesting. Serologic negativity may not rule out secondary syphilis in HIV-infected individuals as true negative serologic studies can be seen with both the FTA-ab and VDRL tests. Skin biopsies and demonstration of spirochetes may be necessary to establish the diagnosis.

Other sexually transmitted diseases

Although granuloma inguinale is a relatively uncommon STD in the USA and other developed countries, HIV-infected patients may develop this disease. In contrast, this condition is extremely common in Africa. It is caused by the Gram-negative rod *Calymmatobacterium granulomatis* and manifests clinically as vegetating lesions on the penis associated with pseudobuboes in the inguinal crease. Culture and skin biopsy for identification of the safety pin-shaped organisms (Donovan bodies) is required to establish the diagnosis. Lymphogranuloma venereum is uncommon in HIV-infected patients, but it manifests as generalized lymphadenopathy with vulvar or penile edema with ulcerations and erosions. Endemic in Africa, chancroid has become more common in the USA during the past few years. The causative organism is the Gram-negative coccobacillus *Haemophilus ducrei*. The disease manifests clinically as nonhealing ulcers in the genital organs and/or legs.[21]

Fungal infections

Candidiasis

Mucosal candidiasis is a very common finding in immunocompromised patients. Since the beginning of the AIDS epidemic a close

Figure 132-6 Cutaneous histoplasmosis. These lesions are characteristically quite nondescript and may simulate other infectious disorders and verrucous neoplastic conditions.

relationship between candidiasis and AIDS has been recognized.[21] The clinical presentation is that of a whitish exudate present on the tongue or buccal mucosa that can easily be scraped away. Candidiasis may involve the esophagus and can disseminate to produce candidal septicemia, brain abscesses and meningitis. Oral candidiasis may be the initial sign of HIV infection in many individuals and has been shown to be a predictor of progression from HIV infection to AIDS. Intertrigo as well as acute and chronic paronychia simulate those seen in non-HIV-infected individuals (see Chapter 126).

Dermatophytosis

Cutaneous dermatophytosis is common in HIV infection and can be extensive and severe. Usually feet, toenails and fingernails are affected. Tinea corporis may manifest as extensive widespread involvement of the trunk and extremities. In any individual with extensive tinea corporis the possibility of underlying HIV infection should be considered.

Systemic fungal infections

The most common opportunistic fungal infections to affect the skin in HIV-seropositive patients are histoplasmosis and cryptococcosis. These occur in patients with advanced HIV disease. Cutaneous involvement may be seen with disseminated disease. Blastomycosis and sporotrichosis have also been observed in HIV-seropositive patients but rarely. Mucocutaneous lesions associated with systemic fungal infections consist of pustules, ulcers, papules and nodules (Fig. 132.6). Less often, the infection manifests as patches, plaques and mucosal ulcerations. Mucocutaneous fungal infections in general may mimic other disorders such as HSV infection, cellulitis or molluscum contagiosum infection. For this reason, when the clinical diagnosis of possible fungal infection is considered, a tissue biopsy of the

lesion should be performed for histologic evaluation and microbiologic cultures (see also Chapter 126). [22]

Parasitic and ectoparasitic infections

HIV-seropositive individuals may present with a wide variety of parasitic and ectoparasitic infections, including scabies, demodicidosis, acanthamebiasis, leishmaniasis and toxoplasmosis. This group of infections manifest either as localized conditions or disseminated disease. The clinical presentation can be unusual and the use of cultures and skin biopsies is essential to render accurate diagnosis. [23]

Scabies

The causative agent of scabies is the mite *Sarcoptes scabei*. Scabies is one of the most frequent conditions encountered in HIV infection and is the most common ectoparasitic infection.[17] The clinical presentation can vary from discrete scattered pruritic papules and slight scale to a widespread papulosquamous eruption that resembles atopic dermatitis. A common clinical presentation is that of hyperkeratotic plaques present on the palms, soles, trunk and extremities. Patients complain of intense pruritus that is worse at night. Contacts are almost always infected.

Demodicidosis

The causative agent of demodicidosis is the mite *Demodex*. Demodicidosis has been reported only sporadically in patients who have AIDS. The clinical presentation is that of a persistent pruritic follicular eruption of the face, trunk and extremities.

Acanthamebiasis

Acanthamebiasis, caused by *Acanthamoeba castellani*, may be seen in AIDS patients and may have a very aggressive course because of immunosuppression. The clinical presentation is that of painful ulcerated nodules located on the trunk and extremities.

Strongyloidiasis

The helminth *Strongyloides stercoralis* can rarely cause skin disease in HIV patients. Clinically, lesions can manifest as urticaria, figurate erythema and livedo reticularis.[5]

Leishmaniasis

The skin lesions resemble those of kala-azar with scaly lichenified depigmented plaques with lichen simplex chronicus. Nonulcerated nodules on the extensor surfaces of the limbs overlying the joints have also been described. Given the prevalence of HIV infection in the Americas, the incidence of leishmaniasis in these patients is surprisingly low.

Pneumocystosis

Pneumocystis carinii may involve the skin, primarily in patients who use aerosolized pentamidine for prophylaxis of *P. carinii* pneumonia. Most common are friable reddish papules or nodules in the ear canal or nares. Small translucent molluscum contagiosum-like papules, bluish cellulitic plaques and deeply seated abscesses have also been observed. [5]

NONINFECTIOUS DERMATOSES

The noninfectious dermatoses associated with HIV infection are numerous and may occur in all stages of the disease. Although none of the disorders reported in this group has been linked directly to an infectious agent, these conditions may be in part caused by an abnormal host response to infectious agents. Noninfectious dermatoses in these patients may have atypical clinical presentations, greater severity and may fail to respond to routine treatment.[24] Because these dermatoses often have atypical presentations in these patients, biopsies and cultures are often required for diagnosis.

Acquired xerosis and ichthyosis

HIV-infected individuals commonly complain of increased dryness of skin. Typically, xerosis is most prominent on the anterior lower legs. In the winter it is more severe and may be associated with inflammation. Patients who have advanced AIDS may present with ichthyosis — dry, thick skin with plate-like scales. The severity of the ichthyosis correlates with the degree of wasting.

Seborrheic dermatitis

This is perhaps the best known dermatosis associated with HIV infection and is seen in up to 85% of all these individuals at some point during the course of the illness.[25] The etiology of seborrheic dermatitis is poorly understood but is thought to be multifactorial. Clinically, the disease is characterized by slightly indurated, diffuse or confluent pinkish-red plaques with yellowish greasy scales and crusting in typical locations, including malar and retro-auricular areas, nasolabial folds, eyebrows and scalp (Fig. 132.7). In severe cases, which are also more common in HIV-infected patients, it extends to the chest, neck and other parts of the body. Seborrheic dermatitis in patients who have HIV infection is often resistant to treatment, which should serve as a clue that a patient might be infected. With HAART therapy, the incidence of refractory cases appears to be diminishing, however.

Psoriasis

This develops in 5% of individuals with HIV infection[26] and may have a number of different manifestations. It may resemble the classic form found in immunocompetent hosts, which consists of reddish plaques with superficial gray to silver scales on the extensor surfaces and nail changes of onycholysis, pitting and subungual hyperkeratosis. Guttate psoriasis may also be seen, with or without classic psoriasis vulgaris. Severe forms may be encountered, such as erythroderma. Treatment of psoriasis in these patients may be difficult, although fortunately it is one of the inflammatory conditions that responds well to therapy, especially with systemic retinoids, either isotretinoin or etretinate. Psoriasis may undergo partial remission in response to zidovudine therapy, although recurrences are common. Other treatments that may be beneficial include topical keratolytic agents, systemic methotrexate and phototherapy.

Eosinophilic folliculitis

Eosinophilic folliculitis (EF), also known as eosinophilic pustular folliculitis, is an inflammatory process of the hair follicles, the etiology of which remains undetermined, although mites, Gram-negative bacteria and fungi have all been implicated as causative. The condition is more common in men and has a peak incidence in the third decade of life. Affected patients may develop marked eosinophilia and elevated levels of IgE. Virtually all patients who have EF have $CD4^+$ counts below 200 cells/mm^3 ; thus, it is an important cutaneous

Figure 132-7 Seborrheic dermatitis. Note the characteristic greasy scale and the erythematous plaques involving the face, especially the nasolabial folds and the eyebrows.

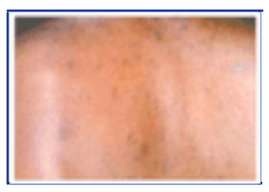
Figure 132-8 Eosinophilic folliculitis. There are follicular papules, many of which have been excoriated, involving the upper trunk and the face. Histologically, numerous eosinophils are present within the follicular ostia.

marker of advanced HIV infection.[27] Clinically, patients develop folliculocentric pruritic urticarial papules measuring 1–4mm in diameter on the upper trunk, face, neck and proximal extremities (Fig. 132.8). There may be coalescence of papules to form plaques and virtually all cases are associated with lichenification secondary to chronic rubbing and scratching. Therapy of EF includes exposure to ultraviolet B and natural sunlight, oral metronidazole, erythromycin and isotretinoin.

Papular dermatitis of AIDS

This is a non-specific chronic papular eruption that has been reported in HIV-seropositive patients.[28] The nosology of this condition has been the subject of debate and many experts consider this to represent a form of eosinophilic folliculitis. Clinically, skin-colored papules are present on the head, neck and upper trunk, many of which may be folliculocentric. The diagnosis is made only by exclusion; thus other eruptions with similar clinical presentation should be excluded, including EF, scabies, papular mucinosis, secondary syphilis, viral exanthem and drug eruption.

Prurigo nodularis

Prurigo nodularis refers to thickened, verrucous papules and nodules and is a reaction pattern of the skin that is associated with a number of pruritic conditions. Patients who have itchy skin chronically rub and scratch, resulting in thickening with formation of typical lesions of prurigo nodularis. Although many different disorders may lead to the condition, in many patients the preceding event has long since resolved and the condition is perpetuated by the so-called itch-scratch cycle. Treatment of the disorder is based on correcting the underlying pruritus by the use of systemic and topical agents to lessen itching, such as topical corticosteroids, topical menthol- and phenol-containing lotions, topical antihistamines and systemic antipruritic agents.

Atopic dermatitis

Atopic dermatitis is seen somewhat more commonly in children who have HIV infection, having been reported in up to 20%.[29] Patients who have atopic dermatitis antedating HIV infection experience increased severity and persistence of their disease. The pathogenesis of atopic dermatitis in these patients has been associated with elevated circulating IgE antibodies to HIV and *S. aureus*. The clinical presentation is similar to that in immunocompetent hosts, with erythematous patches and plaques with fine papulovesicles associated with scaling and crusting.

Granuloma annulare

There have been a number of sporadic case reports of granuloma annulare (GA) in HIV-seropositive patients.[30] A unique feature of GA in these patients is a tendency toward widespread distribution and photoexacerbation. The clinical presentation is that of violaceous, firm papules with annular arrangement distributed on the hands, feet, arms, legs and trunk. In some patients, there may be similarity to KS. The reason for the development of GA in these patients remains unknown.

Leukocytoclastic vasculitis

Patients who have HIV disease may develop leukocytoclastic vasculitis. [31] This condition is a manifestation of immune complex-mediated disease. The clinical presentation progresses through several stages, beginning as urticarial papules or small petechiae. In most cases, characteristic palpable purpuric papules develop. Lesions are distributed on the extremities, although any body site can be affected. In HIV-infected individuals, they are more numerous and more florid than in immunocompetent hosts. The treatment of leukocytoclastic vasculitis consists primarily of identifying the underlying cause and correcting the associated abnormalities. Administration of nonsteroidal anti-inflammatory agents may be beneficial, although colchicine, dapsone or systemic corticosteroids may be required in severe cases.

NEOPLASMS

A number of different neoplastic disorders may develop in these patients as well. Lymphoreticular and vascular neoplasms are commonly observed; however, epithelial, mesenchymal and melanocytic neoplasms have been also described. Among the vascular lesions, KS is the most common neoplasm and is also a hallmark of AIDS. The development of malignant neoplasms is of great significance because they are often sources of morbidity and mortality.[14]

Kaposi's sarcoma

Kaposi's sarcoma is a vascular neoplastic disorder that is divided into classic and epidemic forms. The classic form is an uncommon disorder that was first described in 1827 and is seen mainly in elderly men of Mediterranean origin, black equatorial Africans and patients who have lymphoma or with primary or iatrogenically induced immune deficiencies. The epidemic form of KS is associated with AIDS. This was one of the first indicators of the AIDS epidemic when it was noted in approximately 50% of male homosexual patients in San Francisco.[32] The pathogenesis of the epidemic KS has recently been associated with infection by a human herpesvirus type 8 (KS-associated herpesvirus) (see Chapter 215).[33] Today, of all patients who have HIV infection, 15% develop KS during their clinical course. The clinical presentation may be that of single or multiple skin lesions and there may be mucosal, visceral and/or lymphatic involvement, particularly in the gastrointestinal tract and pulmonary parenchyma. Clinically, KS has three stages, including macule or patch, plaque and nodule (Fig. 132.9). Regressions of KS have been seen in patients treated with antiretroviral combinations including protease inhibitors.

Macule or patch

These lesions are faint pink macules or patches oriented along skin cleavage lines. Initially lesions may be innocuous in appearance and are easily overlooked, being mistaken for bruises, purpura or nevi. The correct recognition of the early lesions can be accomplished by performing biopsies for histopathologic examination.

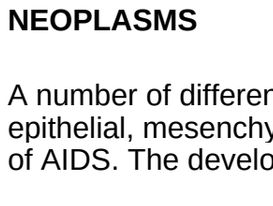
Figure 132-9 Kaposi's sarcoma, plaque stage. There is an erythematous plaque, which is linear in shape, arranged along skin cleavage lines.

Plaque

In time lesions darken and develop into raised firm indurated plaques. The color is purple to brownish because of the presence of abundant blood vessels, extravasated erythrocytes and siderophages. In some cases, lesions may ulcerate.

Nodule

Nodular lesions are dome-shaped, elevated lesions that are usually purple. On palpation they are firm and may be ulcerated. They may simulate bacillary angiomatosis

and pyogenic granulomata.

Treatment

Treatment of uncomplicated KS of the skin is performed for cosmetic purposes. Local destructive measures are generally the most effective. Liquid nitrogen cryotherapy, radiation and intralesional injections of vinblastine sulfate 2–4mg/ml and interferon-a-2b have all been used successfully. Systemic chemotherapy is effective but generally avoided as it further depresses the immune system unless the tumor has disseminated (see also Chapter 130).

Lymphomas and lymphoreticular neoplasms

A number of different lymphoreticular malignancies of both B and T cells may develop in HIV-positive patients. The majority of these are based in the lymph nodes and reticuloendothelial system, although the skin may be involved primarily or secondarily. Most are advanced at the time of diagnosis and are associated with a short medial survival time (see Chapter 130).

Non-Hodgkin's lymphoma with skin manifestations in patients who have AIDS is most commonly of B-lymphocyte origin and mostly high and intermediate grade. Cutaneous T-lymphocyte lymphoma (CTCL), Hodgkin's disease, lymphomatoid granulomatosis and adult T-lymphocyte leukemia/lymphoma also have been reported. The pathogenesis of lymphoreticular neoplasms in these patients is controversial. Chromosomal abnormalities have been encountered and a possible viral etiology has also been suggested.[34] Most cases involve visceral sites and, when the skin is affected, these are usually pink-purple papules or nodules with necrosis. Any site may be involved, including the head, neck, trunk and extremities. Deep-seated soft tissue involvement may expand superficially, forming dome-shaped nodules that often ulcerate. Hodgkin's lymphoma appears similar to non-Hodgkin's lymphoma, either as diffuse nodular lesions or as a panniculitis. CTCL may have the clinical appearance of mycosis fungoides with widespread erythematous scaly lesions distributed usually on the trunk and resembling 'eczema'. Lesions become red- brown ulcerated plaques and tumors during the more advanced stages of the disease.

The diagnosis of lymphoreticular neoplasms should be based on histopathologic examination of tissue biopsies. In many cases the use of gene rearrangement studies, flow cytometry and DNA analysis is necessary to characterize these neoplasms.

Treatment consists of the usual therapy for systemic lymphoma. CTCL may respond to psoralen and ultraviolet A therapy, total body electron beam or topical nitrogen mustard. The prognosis for HIV-positive patients who have lymphoma is poor; survival is in general between 5 and 10 months after diagnosis.

Other cancers

Several reports in the literature have described an increased incidence of intraepithelial and invasive carcinoma of the anus in patients who have AIDS-associated HPV infection. Although there are relatively few case reports, skin cancers may develop more rapidly and behave somewhat more aggressively. Among these malignancies, squamous cell carcinoma and basal cell carcinoma are the most frequently seen. Melanoma has been described, but only a few case reports have been published. There is no information on the incidence of melanomas in HIV patients compared with that in the general population.

Hair and nail changes

A number of abnormalities of hair and nails may be encountered in HIV-positive patients. Chronic inflammatory and noninflammatory alopecia has been observed. Alopecia universalis may also develop and is associated with decreased $CD4^+$ counts.[35] Other hair changes that have been observed include premature graying, thinning and diffuse hair loss. Hypertrichosis has also been associated with HIV infection; however, the cause is unknown. Nail changes, including yellow discoloration, hyperpigmentation, transverse and longitudinal ridging and decreased size or loss of the lunulae, have all been reported. Longitudinal hyperpigmentation is observed in association with treatment with zidovudine.

Oral manifestations

Oral manifestations are common in HIV disease and include novel presentations of previously known opportunistic diseases and some distinctive lesions. [36]

Oral candidiasis usually occurs when the CD4 counts are falling and may be manifest as a pseudomembranous, erythematous, hyperplastic form or as angular cheilitis (see Chapter 126).

Other opportunistic infections such as MAI, histoplasmosis and cryptococcosis may present as palatal masses or ulceration. Herpetic gingivostomatitis is more common in HIV-infected individuals and is painful and slow to heal. Herpes-zoster involving the oral cavity usually shows concurrent skin involvement. Oral HPV lesions are solitary or multiple and can be smooth-surfaced or papillomatous. The HPV types 7, 13 and 32 have been identified in these lesions.

Periodontal disease may occur in clean mouths in HIV-infected patients and present acutely with pain, rapid loss of bone and soft tissue. Response to treatment may be poor and recurrence common. Necrotizing periodontitis and linear gingival erythema are other manifestations.

Recurrent aphthous ulcers are usually idiopathic. They can be as simple as self-limited pinpoint lesions or progressively enlarging destructive lesions. There may be extensive hemorrhage and necrosis. They are frequently recurrent. Pain can be quite severe and esophageal ulceration is well described. Lesions should be cultured

for HSV and a biopsy may be needed to rule out malignancy (e.g. lymphoma) or infection. Smaller ulcers may be treated with a topical corticosteroid preparation. Thalidomide, 200mg/day orally, given for 4 weeks, has been shown to be effective for larger oral and esophageal lesions.[37] It is vital that precautions are taken to avoid exposure to thalidomide during pregnancy.

Oral hairy leukoplakia and intraoral KS may be the first clue to HIV infection. Oral hairy leukoplakia presents unilaterally or bilaterally as a whitish corrugated plaque on the lateral sides of the tongue and is caused by EBV. It is seen in 20% of HIV-infected patients and is a harbinger of progression to AIDS (30% over 3 years). *Candida* is often associated with it. Biopsy shows the characteristic acanthosis, marked parakeratosis and extensive ballooning degeneration of epithelial cells. Intraoral KS may appear alone or be associated with skin or disseminated lesions. It may be flat, raised, solitary or multiple and is red-blue or purple. A biopsy may be required to distinguish it from other vascular or pigmented lesions.

Salivary gland involvement with lymphoepithelial cyst formation can lead to xerostomia and salivary gland enlargement in HIV patients. Labial salivary glands may demonstrate lymphocytic infiltrates similar to Sjögren's syndrome although the infiltrate is composed predominantly of CD8 cells.

Complications of antiretroviral agents

With the advent of more effective antiretroviral agents and the use of HAART therapy, many of the cutaneous manifestations of HIV discussed previously in this chapter occur less frequently. However, a new subset of dermatologic conditions has arisen related to the use of these drugs, namely cutaneous side-effects of antiretroviral agents. Currently there are three classes of medications used for the treatment of HIV: protease inhibitors (PI), nucleoside analogue reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI).

A wide variety of side-effects occur with the potent antiretroviral therapy. Although each individual drug has its own cutaneous side-effects, therapy has been associated with lipodystrophy (LD). Lipodystrophy consists of the symmetric loss of fat from both the upper and lower extremities as well as the buttocks, causing the muscles and veins to appear more prominent, giving these patients a pseudoathletic appearance. The buccal, parotid and pre-auricular fat pads are also sites of lipoatrophy. Abnormal fat distribution may also be seen in patients with LD. Fat deposits may occur in the abdomen (crix belly), breasts, posterior neck (buffalo hump) and in the supraclavicular area. The concomitant occurrence of peripheral atrophy and abnormal fat deposition has been called the LD syndrome. Striae formation[38] and angiolipomas[39] have been reported in association with the LD syndrome. The simultaneous occurrence of lipoatrophy and fat accumulation is more common than either one occurring alone.[40] These fat abnormalities are often associated with metabolic derangements, including hypertriglyceridemia, hypercholesterolemia, insulin resistance, hyperglycemia and increased C-peptide levels (see also Chapter 139 and Chapter 141). [41] [42] [43]

Incidence of LD has been reported to be from 10% to 80%, but the largest reports put this number around 50%.[44] [45] This number is difficult to establish, in part

because there is no formal definition of LD and it has been measured differently in multiple studies. Signs of LD may begin as early as 2 months but no later than 1 year after initiation of combination therapy. Risk factors for the development of LD include female sex, nonintravenous drug user, increased age and longer duration of exposure to antiretroviral drugs.[46]

Although the LD syndrome has been connected to PI therapy, no known treatment for the LD syndrome exists. Metabolic abnormalities improve somewhat upon cessation of PIs or by switching to a different class of antiretrovirals, but the peripheral atrophy remains although some improvement on switching from thymidine analogues (e.g. stavudine) has been noted.[47] [48] [49]

Specific side-effects are associated with the individual PIs. Fixed drug eruptions have occurred as a result of therapy with saquinavir.[50] Indinavir has been associated with paronychia, most often seen on the great toes. Pyogenic granulomas may also occur in sulci of affected nails. Cessation of indinavir has led to partial or complete reversal of the paronychia over several weeks. Alopecia has also been caused by indinavir, usually on the lower extremities, but hair loss has also been seen in the pubic, axillary and thoracic regions as well as the scalp. Hair regrowth occurs within months of cessation of therapy. Additionally, indinavir has caused cheilitis and dry skin which, along with alopecia and paronychia, are side-effects of retinoid therapy.

Zidovudine, a NRTI, is the oldest drug approved for the treatment of HIV. Two well-known side effects of zidovudine are nail pigmentation and hypertrichosis. Nail pigmentation may occur on several[51] or all nails of the hands and feet.[52] The color of nail pigmentation ranges from yellow-brown to bluish. The discoloration may occur in longitudinal bands or transverse bands or it may affect the entire nail. Transverse bands occur presumably because zidovudine treatment was started and stopped over a short period of time in a patient who would have experienced entire nail pigmentary changes if zidovudine treatment had been prolonged. After cessation of zidovudine therapy, nail growth resumes normal coloration. Nail pigmentation may occur as a result of zidovudine stimulation or toxicity of the melanocytes in the nail matrix.

Hypertrichosis may also be seen with zidovudine. One patient who experienced nail discoloration also noticed increased length of hair on his dorsal forearm and darkening of his public hair.[53] HIV-induced alopecia has resolved in a female treated with zidovudine, then recurred upon cessation of therapy, and later resolved again when zidovudine was restarted.[54] Another patient complained of increased length of eyelashes after beginning treatment with zidovudine; this was the only place on the body affected.[55] A few other rare side-effects have been attributed to zidovudine therapy. An infant born to an HIV-positive mother who was perinatally exposed to zidovudine developed a severe paronychia secondary to *E. coli* and *C. albicans*.[56] The paronychia resolved after treatment with antifungal and antiseptic agents. Torres et al.[57] reported two patients who suffered from leukocytoclastic vasculitis thought to be caused by zidovudine. In both patients, the vasculitis started soon after initiation of zidovudine and resolved with withdrawal of the drug and then recurred when the patients were rechallenged with the drug. Increased sensitivity to mosquito bites has also been linked to zidovudine treatment. Within 3 months of starting zidovudine, three patients reported that following mosquito bites they experienced a heightened reaction to the bites, including immediate pruritic wheals that later developed into indurated areas and subcutaneous nodules lasting from 1 to 4 weeks. None of these patients had ever experienced this type of reaction to a mosquito bite before starting zidovudine therapy.[58]

Dideoxyinosine (DDI), a NRTI, has been reported to cause unusual side-effects in some HIV patients. One case of Ofuji papuloerythroderma, a disease in which the eruption of many confluent erythematous papules causes the appearance of erythroderma, has been reported in association with DDI use.[59] A small vessel vasculitis has been seen with DDI,[60] as has a morbilliform rash[61] (Fig. 132.10) and even the Stevens-Johnson syndrome.[62] Lamivudine, another NRTI, has also been associated with paronychia, mostly of the great toes, but also occasionally seen in fingernails.[63]

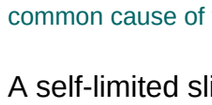

Figure 132-10 Morbilliform drug eruption. There is a diffuse eruption of fine pink macules and papules, which have coalesced, involving the trunk and the extremities. The most common cause of these eruptions is trimethoprim-sulfamethoxazole.

A self-limited slightly pruritic maculopapular eruption has occurred within the first few days of ritonavir (PI) use. The eruption faded within days despite continued use of ritonavir. Hypersensitivity reactions have also been reported with zidovudine and nevirapine. Hypersensitivity with nevirapine is fairly common and may often be prevented by using either a gradually escalating dose or by co-administration of prednisone or an antihistamine during the initial weeks of nevirapine therapy. [64] [65] Despite use of an escalating dose, the Stevens-Johnson reaction has been seen in patients on nevirapine therapy.[66] Abacavir, a NRTI, can cause a severe hypersensitivity reaction which may even be life threatening, especially if treatment is continued despite the reaction or if the patient is rechallenged with abacavir after resolution of the initial reaction.[67]

Acute generalized exanthematous pustulosis was seen in a patient on multiple antiretroviral drug therapy. Although the causative drug cannot be proven with certainty, there was a strong temporal relationship of onset and resolution of the reaction with the use of zidovudine, lamivudine and stavudine.[68]

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Chapter 133 - HIV/AIDS-related Problems in Developing Countries

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INTRODUCTION AND EPIDEMIOLOGY

Since the beginning of the HIV epidemic, approximately 60 million people have been infected.[1] As of December 2001, an estimated 40 million people (37.2 million adults and 2.7 million children) were infected with HIV and 25 million people had died. In 2001 alone, 5 million new HIV infections occurred worldwide. In the developing world, the majority of incident cases occur in young adults. People aged 15–24 years comprise about one-third of those living with HIV/AIDS. In 2001, illnesses associated with HIV/AIDS caused the deaths of approximately 3 million people, including an estimated 580,000 children younger than 15 years. More than 95% of these infections and deaths occurred in developing countries. Accompanying the morbidity and mortality borne by those infected with HIV is the dramatic alteration of the social structure attributable to the HIV epidemic. Because of the premature death of HIV-infected parents, 13 million children have been orphaned. The number of orphaned children is forecast to more than double by 2010.[2]

The contrast between the impact of HIV/AIDS in the developed world and in the developing world is striking. Figure 133.1 illustrates the discrepancy between the annual AIDS deaths in sub-Saharan Africa versus the USA. Whereas AIDS-related mortality is declining in the USA, western Europe and Australia, it is continuing to rise rapidly in sub-Saharan Africa, South East Asia and Latin America.[3] [4] [5] One in 200 adults aged 15–49 years is infected with HIV in the USA and Europe, but 10–40% of pregnant women in some parts of sub-Saharan Africa are infected with HIV. Although antiretroviral therapy is given to pregnant women and their newborn infants in developed countries to prevent transmission, most pregnant women who have HIV infection in developing countries receive little or no antiretroviral therapy, creating an enormous discrepancy in perinatal transmission of HIV in these two regions of the world. In addition, recent surveys have demonstrated that nearly 75% of people with HIV infection are aware of their serostatus in the USA and Europe, whereas 80–90% of infected people in developing countries have never been tested for HIV and remain unaware of their infection.

Since the initial recognition of the AIDS epidemic, much has been done to respond to HIV infection by way of prevention and behavioral modification. Government-supported efforts in Thailand, including HIV and AIDS surveillance systems and programs such as the '100% condom use' campaign for commercial sex, have contributed to decreased rates of HIV and sexually transmitted diseases in military recruits. [6] Similarly, in Cambodia the prevalence of HIV among pregnant women declined by almost a third between 1997 and 2000, to 2.3%. Widespread health education, increased used of condoms and voluntary HIV counseling and testing appear to have lowered the prevalence of HIV infection in some areas of sub-Saharan Africa. In rural Uganda, the most striking decline in HIV prevalence was seen among females aged 20–24. Age-specific prevalence decreased from 20.9% in 1989–90 to 13.8% in 1996–7.

However, in the setting of this mature epidemic, declines in HIV seroprevalence have been seen in the presence of stable and high incidence.[7] Death due to HIV disease, rather than a true decrease in the incidence of HIV infection, appears to have contributed most to the reduced HIV prevalence. Figure 133.2 models the lifetime risk of AIDS death for 15-year-old boys based on current HIV prevalence in adults aged 15–49 years. The upper line, based on current HIV prevalence, is compared with the lower line, which assumes that the risk for new HIV infection at each age decreases by half over the next 15 years. This indicates that, even with successful prevention campaigns, without access to treatment the proportion of young people who will die of AIDS is very high.[8]

Despite ongoing prevention efforts, the overwhelming burden of HIV disease is borne by the developing world. The factors responsible for the discrepancies between the developed and developing world in terms of the diagnosis and care of people with HIV infection and the magnitude of the HIV pandemic are multifactorial. Limited access to care, lack of diagnostic equipment and insufficient money to support either prevention or treatment programs are primarily responsible for the continuing rise in morbidity and mortality associated with HIV infection in developing countries. Underlying high prevalence rates of HIV infection and a combination of high-risk behavior and the widespread prevalence of sexually transmitted diseases act synergistically to propel the epidemic further in many areas of the developing world. Furthermore, the coexistence of other endemic diseases that are widely prevalent in developing countries, such as tuberculosis and gastrointestinal infections, complicate the care of people with HIV infection and pose additional problems for the medical personnel caring for them. This chapter reviews some of these aspects as they relate to the care of people with HIV infection living in developing countries.

CLINICAL FEATURES

The clinical manifestations of HIV infection in developing countries are diverse and reflect the wide variety of other endemic diseases within each region. [9] More than 100 pathogens, including viruses, bacteria, fungi, protozoa, helminths and arthropods, have been identified as having caused opportunistic disease in persons with HIV infection. relatively small number of these pathogens cause a majority of the infections, yet their impact on the health of persons with HIV infection is enormous. Although the reasons for the differences between the spectrum of opportunistic infections observed in developing countries and in developed countries are not completely understood, they are likely to include factors such as the prevalence of pathogens in the environment, social behaviors, ecologic factors that result in exposure to these pathogens, and other undefined factors.

Determining the spectrum of opportunistic infections in a given region requires surveillance systems and diagnostic services that may not be available in many developing countries. For example, opportunistic

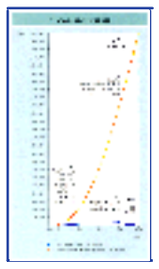

Figure 133-1 Annual AIDS deaths since 1982. Deaths in the USA are contrasted with those in sub-Saharan Africa.

infections that can be diagnosed with reasonable accuracy by physical examination (e.g., oral candidiasis) or by inexpensive laboratory techniques (e.g., Indian ink stain of cerebrospinal fluid for *Cryptococcus neoformans*) may be documented more frequently than opportunistic infections requiring more expensive diagnostic technologies (e.g., *Pneumocystis carinii* pneumonia, disseminated *Mycobacterium avium* complex (MAC) and cytomegalovirus disease). Because most clinical studies that document opportunistic infections are conducted in urban hospitals, those infected outside of urban centers are often not included in disease surveillance efforts. Furthermore, longitudinal cohort studies are costly to maintain in resource-poor settings. Biases in diagnosing and reporting opportunistic infections may be especially important among socially disadvantaged groups with limited access to diagnostic and health care services. Finally, differences in clinical definitions may make comparisons between published reports difficult. For these reasons and for others, much less is known about the frequency of different opportunistic infections in the developing world than in industrialized countries.

Although it is clear that HIV infection has a definite impact on a wide variety of microbial agents in developing countries, less is known about the impact of these diseases on HIV infection. Although it has been shown that HIV disease progresses more rapidly in developing countries, recent studies report similar rates of HIV disease progression when compared with the epidemiology of HIV disease in developed countries prior to the introduction of highly-active antiretroviral therapy (HAART).[10] Data quantifying the median time from seroconversion to AIDS is limited. Early studies suggested that time to AIDS was much shorter in sub-Saharan

Africa and South East Asia. Obstacles included pinpointing the time of seroconversion and reconciling different definitions of AIDS. Similarly, time from seroconversion to AIDS and death has been compared with that seen in the developed world early in the HIV epidemic. In a population-based study in Uganda, the median survival from AIDS to death was 9.3 months but varied according to AIDS-defining illness.[11] Median survival associated with wasting syndrome, Kaposi's sarcoma and esophageal candidiasis was less than 3.5 months, compared with survival longer than 20 months associated with cryptosporidial diarrhea, chronic herpes simplex virus (HSV) infection and extrapulmonary tuberculosis. The median $CD4^+$ lymphocyte count at the time of AIDS onset was 150 cells/mm^3 . Notably, the most common opportunistic infections in resource-poor settings, including tuberculosis and endemic bacterial infections, develop at $CD4^+$ lymphocyte counts higher than 150 cells/mm^3 . Without the benefit of prophylaxis against opportunistic infections and treatment with antiretroviral medications, those infected with HIV will ultimately suffer AIDS-related morbidity and death.

In addition to the clinical spectrum of disease defined by opportunistic infection, co-infection with endemic diseases and resulting immune activation may increase susceptibility to HIV infection. A person whose immune system is activated at the time of exposure to HIV may be more susceptible to infection with HIV. In this regard, the success of an aggressive sexually transmitted disease (STD) treatment program in Tanzania in decreasing the incidence of new HIV infections may be due in part to the removal of immune-activating factors. A similar community-based study conducted in Uganda and designed to evaluate the effect of STD treatment on the development of incident HIV infection found no difference.[12] The lack of a positive effect may be related to the effect of antihelminthic therapy provided to the control group. The eradication of helminths may have had an effect similar to the treatment of STDs. Furthermore, the trial did not treat for genital ulcerative disease due to HSV infection. Future clinical trials will evaluate the effect of treating genital HSV infection on HIV infection and transmission. A recent review outlines the documented associations between chronic immune activation and infections prevalent in the developing world that appear to enhance the pathogenesis of HIV.[13] Thus, both the rate of spread of the HIV epidemic as well as the clinical course in the developing world may be greatly influenced by the underlying state of heightened immune activation that exists in many people in these countries.

Tropical diseases may directly lead to an increased risk of infection with HIV. Treatment of severe anemia induced by malaria has led to HIV infection by transfusion. Female genital schistosomiasis, like other genital inflammatory conditions, may increase the efficiency of HIV transmission. Several drugs used in the treatment and prophylaxis of tropical diseases have an immunosuppressive effect and may thereby influence susceptibility to HIV. Specific interactions between HIV and infectious diseases in developing countries are listed briefly below.

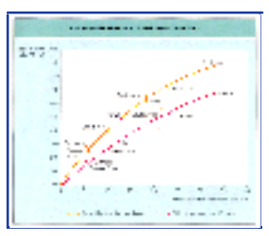

Figure 133-2 Risk of death from AIDS in developing countries. Lifetime risk of AIDS death among 15-year-old boys, assuming unchanged or halved risk of becoming infected with HIV, in selected countries.

Mycobacterial infections

Tuberculosis

Tuberculosis is the most important severe opportunistic infection observed among patients with HIV infection in developing countries because it occurs frequently, is transmissible to both people with HIV infection and uninfected persons, can be readily treated and can be prevented. Globally, tuberculosis is the leading cause of death among people with HIV infection, accounting for a third of deaths due to AIDS. The World Health Organization (WHO) estimates that the number of people infected with the tubercle bacillus is one-third of the world population; throughout the world a new tuberculosis infection occurs every second.[14] The greatest number of tuberculosis infections occur in people living in Asia, sub-Saharan Africa and Latin America, where about half of the adult population is infected. These are the same areas in which HIV continues to increase steadily. The WHO considers HIV as the single most important factor driving the increase in tuberculosis incidence in Africa over the past 10 years.

The increase in tuberculosis attributable to HIV has resulted in increased demand for already overburdened tuberculosis programs. During the initial stages of the HIV epidemic, when incidence rates increased dramatically, tuberculosis rates increased as well. For example, in the early stage of the HIV epidemic in northern Thailand from 1989–90, HIV seroprevalence increased exponentially from 5% to 40% and there was a 5–7% increase in tuberculosis between 1990 and 1992. In developing countries where young adults have high rates of infection with both HIV and tuberculosis, the risk of co-infection is correspondingly high. Worldwide, an estimated 640,000 incident cases of tuberculosis (8%) occur in the setting of HIV infection. Although the largest group of co-infected individuals lives in India, the burden of HIV infection per capita is highest in sub-Saharan Africa, with 32% of tuberculosis cases co-infected with HIV.

Infection with HIV and tuberculosis has become a particular problem in many African countries. A recent review of major causes of HIV-related diseases identified tuberculosis as the most frequently occurring opportunistic infection in hospital and clinic patients in Côte d'Ivoire, South Africa, Kenya and Ethiopia. In the central African countries of Zimbabwe and Malawi, the annual incidence of tuberculosis doubled in the 5 years 1985–90 and 20–44% of AIDS patients developed tuberculosis during the course of their infection. In contrast, only 4% of AIDS patients in the USA have tuberculosis. In a study of tuberculosis treatment in Uganda, a high prevalence (49%) of *Mycobacterium africanum* isolates was identified in patients with HIV infection who had pulmonary tuberculosis. In Uganda, the prevalence of HIV infection among tuberculosis patients was 5.9 times greater than that among patients who had inactive tuberculosis. In Lusaka, Zambia, as many as 37% of hospitalized children who had tuberculosis were infected with HIV, compared with 11% of nontuberculosis controls. Cohort studies have also shown that the risk of developing active tuberculosis among persons infected with *Mycobacterium tuberculosis* is much higher in persons with HIV infection than in HIV-seronegative persons. For someone who is co-infected with HIV and tuberculosis, the annual risk of developing active tuberculosis ranges from 5% to 15%, whereas the lifetime risk among people who have tuberculosis in the absence of HIV infection is only 5–10%.

During the period 1997–9, the estimated number of incident cases of tuberculosis increased from 8.0 million to 8.4 million. If the present trend continues, the WHO estimates an expected 10.2 million new cases by 2005. The progressive increase is largely due to the HIV/AIDS pandemic. In patients dying with HIV disease in Abidjan, Côte d'Ivoire, tuberculosis was present in more than half of those who had AIDS and was responsible for one-third of the deaths. The importance of tuberculosis was demonstrated in an autopsy study among patients dying of pulmonary disease in Abidjan; 40% of the HIV-positive patients died of tuberculosis, compared with only 4% of the HIV-negative patients. In Mexico, disseminated tuberculosis was found at autopsy in 25% of patients who had AIDS; this compares with 6% of patients in the USA and 5% of patients in Italy. This observation is consistent with the higher incidence of pulmonary tuberculosis in Latin America than in the USA, as well as with the

higher incidence of tuberculosis in the USA among foreign-born people and people of Latin American or Asian descent.

A prospective cohort study conducted in South Africa demonstrated that the increased mortality associated with tuberculosis was observed only in patients with a $CD4^+$ lymphocyte count greater than 200 cells/mm^3 and in those without AIDS at baseline.[15] The authors proposed that the immune activation due to tuberculosis increased HIV replication and accelerated HIV disease progression.

Diagnosis, treatment and prophylaxis of tuberculosis is discussed in Chapter 37 . Trials of isoniazid prophylaxis of HIV-positive populations with a positive tuberculin skin test have been conducted in Mexico, Haiti, Kenya, Zambia and Uganda. Meta-analyses of these studies showed a definite reduction in the development of active tuberculosis in the treated group compared with the placebo group during a period of 2–3 years.[16] [17] No significant protection could be demonstrated in the groups with skin test anergy who were included in the same trials. Thus, the benefit of prophylaxis for populations that are already anergic as a result of immunosuppression is more difficult to evaluate because they indicate either no previous exposure to tuberculosis or previous infection with loss of immunologic reactivity. As with many other diseases, the cost of medication needed to carry out effective treatment of tuberculosis is lacking in many developing countries, where the problem is greatest. Furthermore, patient compliance may be a limiting factor in treatment regiments of multiple drugs for periods of several months. Research priorities include the determination of the best method for detection of active tuberculosis in resource-poor settings, and optimum duration of preventive therapy.

In order to prevent active tuberculosis, up to 70% of the world's children are vaccinated with bacillus Calmette-Guérin (BCG). Unfortunately, the efficacy of BCG in HIV-infected populations is unclear. One study found no benefit of vaccination in HIV-seropositive children; another study found a benefit of childhood vaccination in

HIV-seropositive adults and protection from disease caused by *M. tuberculosis*. Recent data suggesting a beneficial effect of early BCG vaccination on mortality from all causes in children not infected with HIV indicate that measures of benefit in HIV-seropositive people need to be broader than mere prevention of tuberculosis. The WHO recommends that BCG should be given to people who have asymptomatic HIV infection in areas with a high risk of tuberculosis infection. In areas where the risk of tuberculosis is minimal, BCG is not recommended, particularly for those who are infected with HIV. Recombinant BCG vector-based vaccines are currently being evaluated in animal models for potential use in vaccination against HIV.

Mycobacterium avium *complex*

Although MAC is very common in advanced HIV infection in the USA and Europe, it has rarely been documented in developing countries. In one study in Uganda, none of 95 blood cultures from severely ill patients who had advanced AIDS were positive for *M. avium*; neither were any of 165 mycobacterial sputum cultures from HIV-seropositive and HIV-seronegative patients at the same hospital. In Côte d'Ivoire, none of 202 blood cultures from HIV-positive adult inpatients were positive from *M. avium*, whereas 4% grew *M. tuberculosis*. Intestinal biopsies from 98 Ugandan, Zairian and Zambian patients who had chronic HIV-related enteropathy yielded histology suggestive of *M. avium* infection in only one case. Similarly, autopsies on 78 HIV-seropositive children in Côte d'Ivoire revealed no evidence of *M. avium* infection. In Kenya and Mexico, *M. avium* has been documented in 6% of patients hospitalized with late-stage HIV disease; in Brazil it has been documented in 18% of 125 hospitalized patients. A recent study conducted in South Africa demonstrated a 10% prevalence of disseminated MAC in 10% of hospitalized patients with a $CD4^+$ lymphocyte count of less than 100 cells/mm^3 .[18]

The reasons for the low frequency of disseminated disease caused by MAC in the developing world are unclear, but there are many possibilities, including less exposure to MAC, exposure to different variants of MAC, differences in host susceptibility, greater acquired immunity to mycobacteria, earlier death from infection with more virulent pathogens, and diagnostic difficulties. There may be greater acquired immunity to mycobacterial disease through BCG vaccine or previous infection with *M. tuberculosis*, but the reported BCG coverage (50%) and purified protein derivative reactivity (82%) in Uganda seem unlikely to explain the lack of any disseminated MAC.

Mycobacterium leprae

To date, there is little evidence that HIV infection has a profound effect on the frequency of *M. leprae* infection (see Chapter 154). Several studies have examined serology for HIV in newly diagnosed leprosy. In one study in Uganda of 189 new cases of leprosy matched for age, sex and district of residence, no significant difference in overall rates of HIV seropositivity was found between the patients who had leprosy (12% HIV seropositive) and the controls (18% HIV seropositive). Interestingly, HIV seropositivity was more frequent among the multibacillary cases than the paucibacillary cases. An association between HIV and leprosy was seen in studies conducted in Zambia and Nigeria. Studies in Kenya and Nigeria both support the hypothesis that HIV infection favors the multibacillary form of leprosy. A different clinical association was noted in Zambian leprosy patients who had active neuritis. The study suggested that HIV-positive patients have poorer recovery of nerve function than controls after treatment with corticosteroids.

Thus, there appears to be no striking evidence that HIV infection has an adverse effect on the course of leprosy. Multibacillary disease could possibly develop under the influence of HIV infection but, because leprosy is chronic and slow in progression, it is difficult to discern the influence of HIV on leprosy directly.

Nonmycobacterial pulmonary infections

Bacterial pneumonia

Investigations of patients with HIV who have pulmonary disease demonstrate that bacterial pneumonia caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* occur as frequently in developing countries as they do in the developed world. In a study in Nairobi, Kenya, 79 episodes of invasive pneumococcal disease were seen in 587 HIV-positive women, whereas there was only one episode in 132 HIV-seronegative women.[19] Serotyping revealed that most recurrent events were related to re-infection. A wide spectrum of HIV-related pneumococcal disease was seen; 56% of cases were pneumonia, 30% were sinusitis and 11% were occult bacteremia. The mean $CD4^+$ lymphocyte count was 302 cells/mm^3 at first presentation and 171 cells/mm^3 for recurrent episodes. In this study *S. pneumoniae* caused more disease at an earlier stage of HIV immunosuppression than did *M. tuberculosis*.

The importance of pneumococcal disease in the host with HIV infection cannot be underestimated. Invasive pneumococcal disease has been shown to cause significant morbidity and mortality. The use of the pneumococcal polysaccharide vaccine to prevent infection in people with HIV infection has been evaluated and generally recommended by the Centers for Disease Control and Prevention. In light of the increased prevalence of antibiotic resistance and ease of administration, this vaccine would appear to be a prudent use of resources. However, studies have shown that the polysaccharide vaccine is not beneficial in individuals with HIV infection in African populations. Conjugate pneumococcal vaccines hold greater promise to reduce the incidence of invasive pneumococcal disease. Evaluations in pediatric population are underway in South Africa and the Gambia.

Pneumocystis carinii *pneumonia*

Several opportunistic infections that are common in developed countries are rarely identified in developing countries. For example, in Abidjan, *P. carinii* pneumonia accounts for only 8% of deaths from HIV-associated pulmonary disease and only 2% of all HIV-associated deaths. A recent report from Zimbabwe demonstrated that even after selecting patients who had abnormal chest radiographs that were consistent with *P. carinii* pneumonia, tuberculosis was still a more frequent diagnosis than *P. carinii* pneumonia.

Although *P. carinii* pneumonia appears to be infrequent in Africa and Asia, it is relatively common in Latin America and Caribbean countries, with rates similar to those documented in the USA. A retrospective case series in a clinic in London, UK, found a significantly higher rate of *P. carinii* pneumonia as a presenting diagnosis in non-Africans (34%) than in Africans (17%). Prevalence rates are also low in Haiti, with a case series incidence of less than 10%. Other reported rates include 7–25% in Thailand, 20–24% in Mexico and 32–45% in Brazil.

The reasons for lower rates of *P. carinii* pneumonia in parts of the developing world are unclear. Possible explanations include less environmental exposure to *P. carinii*, exposure to different strains of *P. carinii*, differences in host susceptibility, earlier deaths in patients in the tropics who have AIDS owing to exposure to more virulent organisms, and diagnostic difficulties. When it does occur, the clinical presentation of *P. carinii* pneumonia in developing countries appears to be similar to that in industrialized countries. Frequent co-infection with tuberculosis may obscure the diagnosis. The diagnosis and treatment of *P. carinii* pneumonia are discussed in Chapter 124 .

Diarrheal disease

In addition to tuberculosis, one of the more common clinical syndromes seen in persons with HIV infection in developing countries is a diarrhea-wasting syndrome frequently referred to as 'slim disease' (Chapter 131). Diarrhea lasting longer than 1 month occurs in up to 50% of patients in Africa who have AIDS, a rate that is more frequent than that observed in persons with HIV infection in industrialized countries. The diarrhea is usually intermittent and not associated with blood or mucus, and it is only rarely secretory in nature. In one-third to two-thirds of patients who have diarrhea in Uganda, Zaire and Zambia, no cause was found despite detailed examinations. In other studies, pathogens (including cryptosporidia, microsporidia, *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp.) have been identified with frequencies of 7–48%.

Other diagnoses are also common among patients who have such profound wasting. In an autopsy study in Côte d'Ivoire, 44% of patients dying with HIV wasting syndrome had disseminated tuberculosis, compared with 25% without the syndrome. A chronic fever syndrome is also frequently associated with tuberculosis and nontyphoid salmonellosis. Often there is very little that can be done for patients who have this syndrome except to provide nutritional support.

Protozoan infections

Toxoplasmosis

Toxoplasma gondii is a common opportunistic infection in both developed and developing countries, and the incidence is proportional to the prevalence of latent infection in the population at risk for HIV. A higher prevalence of cerebral toxoplasmosis has been documented among Latin American patients than in the USA, which is consistent with the higher underlying prevalence of toxoplasmosis in Latin America. Although there is a suggestion that up to 50% of seropositive AIDS patients in

some parts of the world may develop toxoplasmal encephalitis, its frequency in developing countries is unclear because of limited diagnostic capabilities. Autopsy series that have included examination of the brain have suggested disease prevalence rates in late-stage AIDS patients of 15% in Abidjan, 25% in Mexico City and 36% in Kampala. For more detailed information on toxoplasmosis, see Chapter 127 & Chapter 158 .

Visceral leishmaniasis

The overlap of visceral leishmaniasis and AIDS is increasing because of the spread of the AIDS pandemic to rural areas and the spread of visceral leishmaniasis to suburban areas. Consequently, cases of co-infection with *Leishmania* spp. and HIV are becoming more frequent, with important clinical, diagnostic, chemotherapeutic, epidemiologic and economic implications. Co-infection with *Leishmania* spp. and HIV is now considered an 'emerging disease', especially in southern Europe, where 25–75% of adult visceral leishmaniasis cases are related to HIV infection and 1.5–9% of patients who have AIDS suffer from newly acquired or reactivated visceral leishmaniasis. The WHO reviewed 692 retrospective cases that occurred between 1985 and 1996 in southern Europe and eastern Africa.[20] Of the cases of co-infection, 90% were observed in patients with $CD4^+$ lymphocyte counts of less than 200 cells/mm^3 . Bone marrow aspiration was the most frequently used technique for parasitologic diagnosis. In two-thirds of these cases, the diagnosis of HIV was made before that of leishmaniasis.

Multiple visceral locations outside of the reticuloendothelial system are frequent during co-infection; these locations include the blood, skin, gastrointestinal tract, lungs and central nervous system. Because of the high frequency of leishmaniasis in the peripheral blood of these patients, transmission via blood or needles, particularly among injecting drug users, is a major problem.

The *Leishmania* spp. frequently involved in HIV infection are those that cause visceral disease, such as *Leishmania donovani* and *L. infantum* in Asia, southern Europe and Africa, and *L. chagasi* in Latin America. Cutaneous leishmaniasis has a much wider geographic distribution than visceral disease but it is only rarely involved as a complication of HIV infection. Classic visceral leishmaniasis documented in patients with HIV infection is probably caused by reactivation of a latent infection, owing to increasing immunosuppression. In one study, the $CD4^+$ lymphocyte count was less than 200 cells/mm^3 in more than 75% of the patients with HIV infection in whom visceral leishmaniasis was diagnosed, and fewer than 5% of patients had counts of 500 cells/mm^3 or greater.

The clinical presentations of visceral leishmaniasis in HIV are quite similar to that described in HIV-negative patients; clinical features include weight loss, fever, pancytopenia and hepatosplenomegaly. Diagnosis is based on a high index of suspicion in a person who has resided in or traveled to an endemic area, and treatment is similar to that employed in patients without HIV infection who have leishmaniasis (see Chapter 127 and Chapter 172). In Ethiopia, a recent clinical trial evaluated the treatment of visceral leishmaniasis in patients with and without HIV infection. Those co-infected had a greater mortality (33.3% vs 3.6%) and relapse rate (16.7% vs 1.2%). The authors expressed the concern that HIV-positive patients with relapsing visceral leishmaniasis could serve as a reservoir of resistant organisms. Treatment guidelines support the use of two drug combination therapy.

Trypanosomiasis

Trypanosoma cruzi, the cause of Chagas' disease, infects millions of people in Latin America. Reports from Argentina, Brazil and Chile have described clinical and laboratory findings in about two dozen patients co-infected with HIV and *T. cruzi*.[21] These reports suggest that Chagas' disease may result from reactivation of latent *T. cruzi* infection and that clinical manifestations such as meningoencephalitis may be more frequent and more severe in those infected with HIV.

Activation of *T. cruzi* infection by HIV or AIDS usually presents with central nervous system manifestations, but in one review trypanosomes were demonstrated in the blood of five of six cases in whom the examination was done. In one pathologic study of 23 cases, acute myocarditis was frequently noted in those cases that were autopsied, but no information was presented as to whether clinical evidence of myocarditis was present during life.

Malaria

Multiple studies have examined the effect of HIV on the course of malaria, and in general they have found no changes in the severity, incidence or successful treatment of malaria (see Chapter 166). The failure of HIV to affect the course of malaria may be in part due to the complex immune response to malaria, which is not easily perturbed by a predominantly T-cell immunodeficiency. In one study of patients in Burkina Faso, investigators demonstrated a preservation of some components of the malaria-specific immune response in AIDS patients who were co-infected with *Plasmodium falciparum*. Similarly, studies have been unable to demonstrate a significant effect of HIV on malaria or vice versa in children, despite the fact that malarial infection is associated with increased levels of the proinflammatory cytokine tumor necrosis factor a, which would be expected to upregulate HIV replication.

Challenging the apparent lack of a significant interaction are several recent studies conducted in Uganda. A hospital-based case-control study, a rural population-based cohort study and an urban clinic-based cohort study all showed that HIV infection was associated with increased frequency and severity of clinical episodes of malaria parasitemia.[22] [23] [24] The effect increased with declining immunosuppression as indicated by decreasing $CD4^+$ lymphocyte counts. Two recent studies have shown that infant mortality is higher in babies born to mothers who are co-infected with placental malaria and HIV. It was also shown that HIV infection impairs the pregnant woman's ability to control *P. falciparum* infection. Thus, there is an interaction between HIV and malaria at the placenta. In addition, malaria can contribute to the increased spread of HIV, owing to the need for more frequent transfusions to treat the anemia of malaria.

Enteric parasitic infections

Enteric parasitic infections such as isosporiasis and cryptosporidiosis may be reported in as many as 5–10% of patients who have AIDS in the tropics, compared with 0.2% of patients who have AIDS in the USA. Reports have also shown that the risk of isosporiasis among residents of the USA with AIDS is higher among those born in Latin America and Haiti than among those born in the USA. In Zambia, evaluation of persistent diarrhea in patients with AIDS revealed cryptosporidiosis (7%), microsporidiosis (16%), isosporiasis (37%) and no etiology (40%); treatment with albendazole resulted in a complete or partial response in 60% of those shown to have enteric parasitic infection.[25]

Penicillium marneffei

A common fungal pathogen in AIDS patients in South East Asia is *Penicillium marneffei*.[26] It causes the third most common opportunistic infection in HIV disease in northern Thailand, after extrapulmonary tuberculosis and cryptococcal meningitis. *Penicillium marneffei* was first isolated in 1956 and infection was a rare event before the arrival of the AIDS pandemic in South East Asia. Since then hundreds of cases have been diagnosed, mainly in southern China, northern Thailand, Hong Kong, Vietnam, Singapore, Indonesia and Myanmar. The environmental reservoir of *P. marneffei* is unknown, but the organism has been isolated from the organs, feces and burrows of three species of bamboo rat. Exposure to soil appears to be a key factor in transmission.

Disseminated *P. marneffei* infection is characterized by fever, anemia, weight loss and papular skin lesions. Other frequent signs and symptoms include cough, generalized lymphadenopathy, hepatomegaly and diarrhea. The most common cutaneous manifestation is a generalized papular rash with a central umbilication that resembles the lesions of molluscum contagiosum. These are predominantly found on the face, scalp and upper extremities, but occur throughout the body. Chest radiographs are frequently abnormal, with diffused reticulonodular or localized alveolar infiltrates.

The mean duration of illness before presentation is 4 weeks. The incubation period of disseminated disease is unclear and the disease may be a result of reactivation of latent infection as opposed to new infection or re-infection. However, the development of clinically active disease within weeks of exposure in endemic areas, and the reports of children who have vertically transmitted HIV infection developing disease in the first months and years of life demonstrate that primary infection can quickly lead to disseminated disease. Finally, the pronounced seasonal variation in disease incidence implies an important role for exogenous re-infection and the expression of disease with *P. marneffei* in AIDS patients in endemic areas. In addition to endemic areas, travelers from regions where *P. marneffei* is not endemic have become infected with this pathogen while traveling in South East Asia. Diagnosis and treatment are discussed in Chapter 126 and Chapter 237 .

Other opportunistic infections

Other opportunistic infections, which may be similar in all areas of the world, include oral and esophageal candidiasis, cryptococcosis, cytomegalovirus infection and Kaposi's sarcoma. Herpes simplex virus infection, herpes zoster and cerebral toxoplasmosis also appear to be relatively common in most areas where diagnostic equipment is readily available. It should be noted that regional variations in frequencies of these diseases do exist within developing countries. Cryptococcosis

accounted for only 2% of AIDS deaths in Abidjan but is probably more common in central and southern Africa.

Mycobacterial infections apart from tuberculosis, such as *Mycobacterium kansasii*, have been long-standing health problems among miners in South Africa and may now be emerging as HIV-associated infections in those miners with HIV infection.

Endemic Kaposi's sarcoma has a striking geographic distribution, being most common in central Africa. Kaposi's sarcoma associated with HIV is likely to have a similar heterogeneous disease frequency, although the incidence of Kaposi's sarcoma has increased in all countries in which HIV disease occurs. Kaposi's sarcoma has recently been associated with a new human herpes virus, namely HHV-8, although the epidemiology of this virus and its distribution worldwide remains unknown (see Chapter 215).

HIV TESTING

Currently, HIV testing in the developing world is done mostly for purposes of surveillance. This involves very small population samples and is done anonymously. If people have little hope of treatment, they feel little incentive to be tested. In many countries there are no voluntary testing and counseling facilities because of lack of funds. In one study in South Africa, only 2% of people who were HIV-positive knew their status. In Kenya, only one of 63 randomly chosen women who tested HIV-positive was aware of her infection. In addition to cost, the fact that current testing procedures require at least two visits to a testing site further complicates access to testing. In rural South Africa, only 17% of people who asked to be tested came back for their results. Rapid test formats for HIV detection of antibody provide an alternative that has been successful in various countries, but the cost is high and the assay is not readily available in many

locations. In one study of these rapid tests, however, 96% chose to know the result and were informed before leaving the clinic.

Thus, it is apparent that over 90% of people with HIV infection in the developing world do not know their HIV status. At current estimates, this suggests that there are over 27 million people in the world today who have no idea that they are infected. There are important reasons for knowing one's HIV status. The earlier people know they are infected, the greater the opportunity for them to access treatment, some forms of which are not expensive, and to apply pressure on communities and governments for improved access to care. The earlier people are aware of their infection, the better able they are to make informed and responsible decisions about childbearing and avoiding transmission to spouses or partners, and to make plans for family welfare before they become ill or die. Furthermore, the one important benefit of self-knowledge of HIV status is that it helps unmask the invisible epidemic and permits a genuine community response. If people become aware of their infection early on, while they are still relatively healthy, this gives them time and energy to support one another as well as to alert the community to the epidemic.

However, these benefits to individuals, families and communities are realistically achievable only where people feel safe in finding out whether they are infected. Efforts by governments and civil society to combat rejection and discrimination directed at people who have HIV are vital (UNAIDS).

In 1997, WHO issued guidelines for the selection and use of HIV antibody tests applicable for use in developing countries.[27] The three main objectives for HIV antibody testing are:

- screening of blood and blood products;
- unlinked and anonymous testing for the purpose of monitoring prevalence and trends of HIV infection; and
- diagnosis of HIV infection among asymptomatic people and those with clinical signs and symptoms suggestive of HIV infection.

There are three strategies recommended by the WHO. In strategy 1, all serum and plasma is tested with one enzyme-linked immunosorbent assay (ELISA) or rapid assay. Serum that is reactive is considered HIV antibody positive. This strategy can be used for screening blood donors to protect the blood supply but it should not be used for notification of the blood donor unless strategy 2 or 3 is implemented. Strategy 1 can also be used for surveillance when screening anonymous samples.

Strategy 2 includes testing serum by one ELISA or rapid assay and, if it is reactive on the first assay, it is then retested with a second ELISA or rapid assay based on a different antigen preparation or a different test principle. Concordant results after repeat testing will indicate a positive or negative result. If the results of the two assays remain discordant, the specimen is considered indeterminate. Strategy 2 is recommended for surveillance, particularly when testing populations with a low prevalence of HIV. The additional test in strategy 2 compared with strategy 1 is necessary in order not to overestimate the HIV prevalence of such regions. Strategy 2 is also recommended for notification of the blood donor, particularly in developing countries, where strategy 2 is more cost effective than strategy 3.

Strategy 3 is similar to strategy 2, except that it requires a third test if the serum is found to be reactive on the second assay. The three tests in this strategy should be based on different antigen preparations or different test principles. A specimen is considered to be antibody-positive if it is reactive on all three assays. If the serum is discordant on any of the three assays, it is considered to be indeterminate. This strategy is recommended for diagnosis and patient notification.

In the selection of HIV antibody tests for use in strategies 2 and 3, the first test should have the highest sensitivity, whereas the second and third tests should have higher specificity than the first. The number of initial discordant or indeterminate results should not exceed 5%.

An HIV test kit bulk purchase program has been established by the WHO in collaboration with UNAIDS in order to provide national AIDS control programs that use tests giving the most accurate results at the lowest possible cost.

PREVENTION OF HIV INFECTION

Although the epidemic continues to spread throughout the developing world, it is also likely that increased preventive efforts could effectively limit the magnitude of the epidemic. For example, in those countries where the epidemic is still in an expansion phase but is not yet fully visible, the public health response is likely to have a decisive influence on its course. There is every reason to believe that the course of the pandemic could still be altered profoundly by the introduction of HIV prevention strategies that are within the technical reach of all countries. Several successes in slowing the HIV pandemic have provided encouragement and further stimulus for improved programs. Aggressive treatment of STDs coupled with a program of condom distribution has already been shown to be effective in decreasing HIV incidence rates in populations at high risk for HIV in sub-Saharan Africa and Asia. Needle-exchange programs have helped to stabilize and in some cases reduce HIV incidence among intravenous drug users in selected countries. HIV incidence rates can be reduced by an estimated 50% with adequately supported prevention programs. In Asia alone this could mean the prevention of several million AIDS deaths among young adults in their most productive years.

While we await the availability of a vaccine and the effect of therapeutic interventions, a primary prevention strategy must be focused on educational efforts to influence social, cultural and behavioral factors. To control the AIDS epidemic, countries will need not only to promote individual behavior change but also to address the related problems of social disruption associated with mounting unemployment, accelerated urbanization, commercial sex, rapid decline in health services and drug abuse. Fundamental social changes, such as improving the social status of women, will be required if AIDS control efforts are to succeed. In view of the rapid pace of HIV transmission in sub-Saharan Africa and Asia, implementation of these principles of AIDS prevention and care is needed urgently.

Despite the existing economic and logistic restraints of health services in some developing countries, good care for HIV/AIDS patients is possible. The value of symptomatic treatment combined with supportive psychological, pastoral and social services has been well documented in many localities, and establishment of services is a prerequisite for the acceptance of a preventive intervention within any comprehensive control program. Policy makers should invest limited resources in improving the infrastructure of the existing health services to enable them to cope with the increasing number of cases of tuberculosis, pneumonia and other opportunistic infections.

PREVENTION OF OPPORTUNISTIC INFECTIONS

Those opportunistic infections associated with the greatest degree of morbidity and mortality, as well as available preventive and therapeutic options, include tuberculosis and bacteremia due to non-*typhi Salmonella* spp. and *S. pneumoniae*.[28] Tuberculosis and other endemic bacterial infections regularly occur in patients with HIV infection but without profound immunosuppression. Primary preventive therapy against tuberculosis provides an important first line of

defense against the development of disease in earlier stages of HIV infection.

Recent studies have evaluated the role of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis in reduction of infections other than *P. carinii* pneumonia in patients with HIV infection. A randomized trial conducted in Côte d'Ivoire showed that events leading to hospitalization or death were 43% lower in African adults with early symptomatic HIV disease treated with TMP-SMX rather than with placebo. The beneficial effect was due to activity of TMP-SMX against bacterial infections, malaria and isosporosis. A recent observational study conducted by the Centers for Disease Control illustrated that TMP-SMX prophylaxis during intervals when patients had $CD4^+$ lymphocyte counts of less than 200 cells/mm^3 was associated with significant protection from toxoplasmosis, salmonellosis, infection with *Haemophilus* spp., invasive or any staphylococcal infection, and *P. carinii* pneumonia. In South Africa, TMP-SMX reduced mortality and the incidence of severe HIV-related illness in patients with advanced immunosuppression ($CD4^+$ lymphocyte count <200 cells/mm^3 or total lymphocyte count of 1250 cells/mm^3), despite the low incidence of *P. carinii* pneumonia in this population.[29] Concerns cited as limitations to the use of TMP-SMX include an anticipated increase in antimicrobial resistance in pathogens such as nontyphoidal salmonellas and the pneumococcus. Of additional concern is the potential for cross-resistance between pyrimethamine and trimethoprim, as sulfadoxine-pyrimethamine is one of the most widely used treatments against *P. falciparum*.

HIV TREATMENT IN DEVELOPING COUNTRIES

On April 22nd 2002, the WHO announced the first treatment guidelines for children and adults infected with HIV in the developing world.[30] This action has set the stage for a tremendous increase in access to care; WHO estimated that at least 3 million people needing treatment should have access to medicines by 2005. The treatment guidelines were accompanied by the expansion of the Essential Medicines List to include antiretrovirals in addition to nevirapine and zidovudine, which were previously listed for the prevention of mother-to-child HIV transmission. The WHO report presents a standardization and simplification of HAART. However, the complexity of providing care to millions of people in resource-poor areas extends well beyond access to medications.

In order to ensure the safe and appropriate use of antiretrovirals, resources for evaluating patients prior to the initiation of therapy and monitoring for response to therapy, as well as for the development of potential side effects, must also be available. The WHO recommends initiation of therapy based on clinical staging and $CD4^+$ lymphocyte count or total lymphocyte count.

- WHO stage I includes those individuals with HIV infection who are asymptomatic or manifest persistent generalized lymphadenopathy;
- stage II includes those with weight loss (<10% of body weight), minor mucocutaneous manifestations, herpes zoster and recurrent upper respiratory tract infections;
- stage III includes those with weight loss (>10% body weight), unexplained chronic diarrhea, unexplained prolonged fever, thrush, oral hairy leukoplakia, pulmonary tuberculosis, severe bacterial infections and bedridden for less than 50% of the day during the past month; and
- stage IV includes those with clinical syndromes consistent with AIDS and/or bedridden for more than 50% of the day during the past month.

The guidelines, shown in Table 133.1 , recommend starting HAART in those who have WHO stage IV disease irrespective of $CD4^+$ lymphocyte

TABLE 133-1 -- **World Health Organization recommendations for starting HIV/AIDS therapy.** Recommendations for initiating antiretroviral therapy in adults and adolescents with documented HIV infection.

WHO RECOMMENDATIONS FOR STARTING HIV/AIDS THERAPY.	
If CD4 testing is available	WHO stage IV disease irrespective of $CD4^+$ lymphocyte count
	WHO stage I, II, III with $CD4^+$ lymphocyte counts <200 cells/mm^3
If CD4 testing unavailable	WHO stage IV disease irrespective of total lymphocyte count
	WHO stage II or III disease with a total lymphocyte count <1000–1200 cells/mm^3

count and WHO stage I, II or III with $CD4^+$ lymphocyte count below 200 cells/mm^3 in areas where $CD4^+$ lymphocyte count is available. If $CD4^+$ lymphocyte count is unavailable, HAART is recommended in those with WHO stage II or III with total lymphocyte count below the range of 1000–1200 cells/mm^3 . Assessment of HIV viral load is not considered essential for determining the need for therapy.

In addition to the assessment of immunologic function as indicated by symptoms and lymphocyte count, further testing for the safe and effective use of HAART is divided into four categories listed in Table 133.2 : absolute minimum tests, basic tests, desirable tests and optional tests. Absolute minimum testing includes an HIV antibody test and hemoglobin or hematocrit level. Basic testing adds white blood cell count and differential, liver enzymes, serum creatinine and/or blood urea nitrogen, serum glucose and pregnancy tests for women. Desirable tests include bilirubin, amylase, lipid levels and $CD4^+$ lymphocyte count. Testing for HIV viral load is deemed to be optional.

The guidelines are based on rigorous evaluation conducted almost exclusively in developed countries. A matter for concern is whether guidelines created for the populations of developed nations are adaptable to HIV-infected populations worldwide. Specifics regarding the presence of different HIV subtypes, endemic infections such as tuberculosis, genetic determinants and environmental factors such as nutritional status may introduce factors that alter response to treatment.

Developing nations that have successfully implemented HAART include Brazil, Thailand, Senegal and Uganda. Studies are needed to examine responses to HAART and whether changes to the guidelines would better serve populations in different regions around the world.

TABLE 133-2 -- World Health Organization guidelines for laboratory monitoring of antiretroviral drug use.

WHO GUIDELINES FOR LABORATORY MONITORING OF ANTIRETROVIRAL DRUG USE	
Absolute minimum tests	HIV antibody test
	Hemoglobin or hematocrit
Basic tests	White blood cell count and differential
	Alanine or aspartate aminotransferase
	Creatinine and/or blood urea nitrogen
	Serum glucose
	Pregnancy test for women
Desirable tests	Bilirubin amylase
	Serum lipids
	$CD4^+$ lymphocyte count
Optional test	HIV viral load

With initiation of HAART on a population-wide scale, continuous surveillance of drug-resistant viruses will be needed to inform treatment guidelines. A recent study conducted in Gabon demonstrated resistance to antiretroviral therapy. Of great concern is that antiviral drug resistance due to suboptimal therapies could limit the potency of available treatments. In parallel with promulgation of the guidelines, the WHO, in collaboration with the International AIDS Society, is developing a global HIV drug resistance surveillance network.

Multiple studies conducted in developed nations have proved the tremendous benefit available to people with HIV infection by the initiation of HAART. Consequently, morbidity and mortality due to HIV have declined dramatically. In developed or developing nations, HAART provides the only hope of survival for those with HIV infection who are able to adhere to daily lifelong therapy. Moreover, the availability of HAART can reinforce prevention activities by offering an incentive to seek HIV testing, preventing mother-to-child transmission and decreasing the risk of sexual transmission.

IMPACT OF HIV AND AIDS ON HEALTH CARE SYSTEMS

As the number of people with HIV infection in developing countries continues to increase, HIV/AIDS will continue to make increasing demands on the health care system at all levels. UNAIDS has estimated that, by 2005, US$9.2 billion will be needed per year to support prevention ($4.8 billion) and treatment and support interventions ($4.4 billion).[31] The allocation of funds for prevention versus care and support varies according to region, with 66% and 32% of estimated expenditure needed for care and support in sub-Saharan Africa and Asia respectively. AIDS prevention activities include teacher training and peer education, condom promotion and distribution, treatment of STDs, voluntary testing and counseling, transfusion screening and prevention of mother-to-child HIV transmission. Included in care and support activities are diagnostic testing, palliative care, opportunistic infection treatment, drug costs and monitoring for HAART, as well as orphanage care and living assistance. Mobilization of such tremendous resources will require a considerable commitment from both domestic and donor sources.

Unfortunately, these demands come at a time of great financial vulnerability for health systems and at a stage, particularly in developing countries, when a great deal of work remains to be done to increase primary health care. Primary care is intended to be the interface of contact between communities and the national health care system, bringing health care as close as possible to where people live and work. Diagnosis and treatment to relieve symptoms and to prevent and treat opportunistic infections can ease suffering and prolong the productive lives of people who have HIV, sometimes at a low cost. As the patient's immune system collapses, however, available treatments become increasingly more expensive. An analysis by the World Bank of alternative treatment and care options concludes that community-initiated care provided at home, although often shifting costs from the national taxpayer to the local community, also greatly reduces the cost of care and thereby offers hope of affordability in improving the quality of the last years of life of people who have AIDS.

The epidemic will undoubtedly increase demand for medical care and reduce its supply at a given quality and price. As the number of people who have HIV infection increases, access to medical care will become more difficult and more expensive for everyone, including people not infected with HIV, and total health expenditure per capita will rise. Governments are under pressure to increase their share of health care spending and to provide special subsidies for the treatment of HIV infection. Unfortunately, because of the scarcity of resources and the inability or unwillingness of governments to increase public health spending enough to offset these pressures, either of these policies may exacerbate the impact of the epidemic on the health care sector.

Governments should ensure that patients who have HIV infection benefit from the same access to care as other patients who have comparable illnesses and similar ability to pay. Because of discrimination, people who have HIV are frequently denied treatment or face barriers to care that others do not encounter. Governments should also provide information about the efficacy of treatments for opportunistic illnesses, HIV infection and AIDS; subsidize the treatment of STDs and contagious opportunistic infections; subsidize the screening of the blood supply; and ensure access to health care for the poorest, regardless of HIV infection status.

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Chapter 134 - Pediatric HIV Infection

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EPIDEMIOLOGY

The vast majority of HIV infection in children is perinatally acquired, via transmission from mother to child. HIV in children less commonly occurs from blood transfusion or receipt of blood components (e.g. during treatment of hemophilia or other coagulation disorders). Transmission may also occur by sexual exposure, either sexual abuse in younger children, or consensual sex in older adolescents and young adults.

The first cases of AIDS in children were reported in 1982. The World Health Organization (WHO) estimated that by the end of 2001, for children under 15 years of age, there were 2.7 million children living with HIV/AIDS, 800,000 new infections, and 580,000 child deaths due to HIV/AIDS in that year. Most children with HIV infection live in sub-Saharan Africa, but all regions of the world are affected (Table 134.1).[1] Reported cases of pediatric AIDS make up 1–2% of the total number AIDS cases in developed countries, but 15–20% of the total number in some developing countries. [2] At least 10.4 million children under age 15 years have lost their mother or both parents to AIDS, and this number of orphans is expected to double by 2010.[3]

Because most children who have HIV infection acquire the virus by transmission from their mother, the epidemiology of perinatally acquired HIV infection closely parallels the epidemiology of HIV infection in women (see Chapter 115 and Chapter 135). In most regions of the world, heterosexual transmission is the most common cause of HIV infection in women (see Table 134.1). The greatest growth in numbers of children with HIV infection would be expected in regions where women make up a high percentage of the case total, and where heterosexual exposure is the most common mode of HIV transmission (see Chapter 133).

Half of all new cases of HIV infection are in children and young adults under 25 years of age.[3] Since younger women often have sex with older men, and since transmission from males to females is more likely to result in HIV transmission, HIV infection is more common in female teenagers, with rates in males increasing after the teenage years. HIV infection in adolescents can be associated with heterosexual activity, injection drug use and men having sex with other men (see Chapter 115).

PATHOGENESIS AND PATHOLOGY

In the absence of intervention, the risk of HIV transmission from mother to baby is about 25%, with estimates ranging from 13 to 43% in different regions of the world.[4] While maternal, obstetric, fetal, postnatal and genetic factors may modify perinatal transmission risk (Table 134.2), maternal virus load is critical in determining the risk of perinatal HIV transmission. Maternal-fetal HLA concordance increases perinatal transmission risk,[5] while CCR5 haplotype may be permissive or protective, depending on the specific mutation.[6]

Perinatal transmission of HIV occurs in utero by the virus entering the fetal circulation from maternal blood, by exposure to infected blood or secretions during labor and delivery, or by ingestion of breast milk. Children with HIV infection can be separated into groups of 'rapid progressors' (20–25% of cases) and 'slow progressors' (75–80% of cases), based on the timing of onset of clinical symptoms of HIV disease.[7] Some rapid progressors may have acquired infection in utero, and HIV-1 has been found in villous Hofbauer cells and in fetal tissue in the first trimester of pregnancy. In-utero infection is operationally defined as detection of HIV by culture or identification of HIV genome by polymerase chain reaction (PCR) in infant blood within 48 hours of birth.[8] Peripartum infection, operationally defined in children who were not breast-fed and who have negative HIV culture or tests for HIV DNA or RNA by PCR in the first week of life, followed by positive tests from days 7–90,[8] is associated with slower disease progression. Transmission occurs in utero 26–38% of the time, and in the peripartum period 65–74% of the time.[9] [10] Breast-feeding increases transmission risk by 14–16%.[11]

The immaturity of the neonatal immune response may be responsible for the more rapid progression of HIV infection in children than adults.[12] Neonatal natural killer cells have diminished antibody-mediated recognition and killing of HIV-infected cells. Neonatal T cells have diminished ability to produce cytokines. Responses of cytotoxic T cells ($CD8^+$) to HIV *gag* proteins are detected in a smaller proportion of infants (aged under 6 months) than adults.[13]

Genetic factors also play a role in the rate of disease progression in children. The CCR5?32 allele slows disease progression,[14] and the stromal cell derived factor 1 3'A mutation accelerates the rate of disease progression.[15]

Although the immunodeficiency of T helper ($CD4^+$) cells has great impact on the clinical manifestations of HIV infection in both adults and children, B-cell dysfunction has an important role in the clinical expression of illness in children. Hypergammaglobulinemia may precede $CD4^+$ T cell depletion in children. Many children have deficient antibody responses to vaccination, especially to polysaccharide antigens. B-cell immunodeficiency is frequently manifest in children as recurrent otitis media, sinusitis, or severe bacterial infections with *Streptococcus pneumoniae* or *Haemophilus influenzae* type b. Children also have impaired phagocytic cell oxidative capacity, which may further increase the frequency and severity of pyogenic bacterial infections.

PREVENTION

Treatment of the mother with zidovudine (ZDV) during pregnancy and labor, and treatment of the newborn for the first 6 weeks of life decreases mother to child transmission (MTCT) of HIV from 25 to 8% in the absence of breast-feeding (Table 134.3).[16] Zidovudine treatment begun before 28 weeks of gestation can interrupt in-utero transmission. [17] Other combinations of prepartum and postpartum therapy can reduce MTCT, including shorter courses of ZDV, with or without lamivudine, and a single prepartum and postpartum dose of nevirapine.[18] Treatment of the infant with ZDV within 48 hours after birth may diminish MTCT, but antiretroviral treatment efficacy is clearly better if started during pregnancy. For women being treated

TABLE 134-1 -- HIV/AIDS in children under 15 years of age and factors affecting the increase in HIV/AIDS in children by region, at year end 2001.*

HIV/AIDS IN CHILDREN UNDER 15 YEARS OF AGE AND FACTORS AFFECTING INCREASE IN HIV/AIDS IN CHILDREN BY REGION, AT YEAR END 2001					
Region	**HIV/AIDS in children under 15 years of age**			**Factors affecting Increase in HIV/AIDS in children**	
	Children living with HIV/AIDS at year end, 2001	**Children newly infected with HIV in 2001**	**Deaths from HIV/AIDS in children during 2001**	**% of HIV-infected adults who are women**	**Main modes of transmission for persons with HIV***
Sub-Saharan Africa	2,400,000	700,000	500,000	55	Hetero
North Africa and Middle East	20,000	12,000	6000	40	Hetero, IDU
South and South East Asia	200,000	65,000	40,000	35	Hetero, IDU
East Asia and Pacific	7000	3000	1500	20	IDU, Hetero, MSM

Latin America	40,000	10,000	8000	30	MSM, IDU, Hetero
Caribbean	20,000	6000	5000	50	Hetero, MSM
Eastern Europe and Central Asia	15,000	1000	<100	20	IDU
Western Europe	4000	<500	<100	25	MSM, IDU
North America	10,000	<500	<100	20	MSM, IDU, Hetero
Australia and New Zealand	<200	<100	<100	10	MSM
Total	2,700,000	800,000	580,000	48	

* *Adapted from World Health Organization.*[1]
* Hetero, heterosexual transmission; IDU, transmission through injecting drug use; MSM, sexual transmission among men who have sex with men

with three antiretroviral drugs whose viral load is below limits of quantitation on ultrasensitive assays, perinatal HIV transmission risk is less than 1%.[19] Some practitioners recommend elective cesarean section at 38 weeks be considered to reduce perinatal transmission because cesarean section performed before labor onset and before rupture of membranes reduces HIV transmission risk by 50% in women treated with ZDV alone.[20] Even for women with viral load less than 1000, antiretroviral therapy and elective cesarean delivery (before rupture of membranes and before labor onset) can reduce MTCT.[21] Cesarean delivery is not expected to further reduce the low risk of MTCT for women on triple therapy with undetectable virus load (see also Chapter 135).

Because breast-feeding increases the risk of perinatal transmission of HIV,[11] women with HIV infection should be advised not to breast-feed their infants in countries where safe alternatives to breast-feeding are readily available.[22] In developing countries, the risk of HIV infection from breast-feeding may be lower than the risk of death from other diseases that occur with high frequency in bottle-fed infants, and breast-feeding is still recommended for women in those areas, even those with HIV infection.[23]

Vaginal washes have not been successful in reducing vertical HIV transmission, and peripartum vaginal cleaning is not currently recommended for routine use. Information concerning MTCT of HIV is updated frequently by the WHO and UNAIDS, and is available at http://www.unaids.org/publications/documents/mtct/index.html. Issues of treatment of pregnant women with HIV infection are discussed more fully in Chapter 135 .

CLINICAL FEATURES

The Centers for Disease Control and Prevention (CDC) classification of adolescents[24] and children who have HIV infection (Table 134.4 , Table 134.6 and Table 134.7) [25] outlines clinical manifestations related either directly to HIV infection and the resulting immune response, or those related to the progressive loss of $CD4^+$ T cells and secondary (opportunistic) infections or cancers.

Compared with adults, children have more rapid disease progression, and have a different pattern of primary and secondary HIV-related manifestations (Table 134.5).[26] [27] Symptoms are most common in children under age 4 years, and in those with in-utero transmission, high virus load and rapid disease progression.[28] Growth delay (failure to thrive) and abnormalities of the central nervous system (CNS) with microcephaly and developmental delay are the most important primary manifestations of HIV infection in children. Cardiomyopathy, nephropathy and possibly enteropathy also represent complications primarily related to HIV infection, whereas swollen parotid glands, lymphoid interstitial pneumonitis, hepatomegaly, splenomegaly and lymphadenopathy may result from the immune response to HIV infection. Important opportunistic infections in children with HIV infection include *Pneumocystis carinii* pneumonia (PCP) and recurrent serious bacterial infections with organisms including *S. pneumoniae* and *H. influenzae* type b. Cryptococcosis and toxoplasma infections are much rarer in children than in adults with HIV infection. Cancers most commonly found in children with HIV infection include lymphomas, leiomyomas and Kaposi's sarcoma, but all cancers are much less common in children than in adults with HIV infection.

Because the normal range for the number of $CD4^+$ T cells changes with age, so does the number that defines immunodeficiency, and CD4% is often used (Table 134.6). Classification of children with HIV infection incorporates both clinical and immunologic information (Table 134.7). Before antiretroviral treatment was available, median survival for children with HIV infection in the USA was approximately 8 years (Table 134.8). [29] Survival is lower with lower $CD4^+$ T-cell counts and higher viral loads (Table 134.9).[30] Children diagnosed with symptomatic disease (CDC category B) or those with poor growth in the first 6 months of life, have more rapidly progressive disease and shorter survival.[31]

TABLE 134-2 -- Factors affecting vertical transmission of HIV.

FACTORS AFFECTING VERTICAL TRANSMISSION OF HIV	
Viral factors	• Increased transmission with high virus load
	• Syncytium-inducing phenotype (SI) does not increase transmission risk
	• Non-syncytium-inducing (NSI or macrophage tropic) increases transmission risk
	• HIV-1 is transmitted more readily than HIV-2
Maternal factors	• Advanced disease (low CD4, high CD8 or symptoms of AIDS) increases transmission risk
	• Primary infection is associated with increased transmission
	• Primiparous women may transmit HIV more frequently
	• Maternal Epstein-Barr virus shedding increases transmission risk
	• Maternal antiretroviral therapy reduces perinatal transmission risk
	• Vitamin A deficiency is associated with increased transmission risk
	• Maternal cigarette smoking may increase transmission risk
	• Other infections (condylomata or other sexually transmitted diseases) may increase transmission
	• Combined maternal infection with HIV-1 and HIV-2 may protect against transmission
Obstetric events	• First-born of twins has increased infection risk compared to second-born
	• Rupture of membranes >4 hours increases transmission risk, especially in preterm infants
	• Maternal bleeding during pregnancy increases transmission risk
	• Fetal scalp electrode increases risk of transmission
	• Elective cesarean section reduces perinatal transmission
Fetoplacental factors that increase transmission risk	• Chorioamnionitis
	• Placenta previa
	• Prematurity increases risk of peripartum transmission, but not in-utero transmission

Infant factors	• HLA type and maternal-infant HLA concordance
	• CCR5 chemokine receptor haplotype
	• Fetal immune response (responsiveness of cord blood leukocytes to HIV *env* determinants may be protective)
Postnatal factors important in transmission	• Breast-feeding increases transmission by 14–16% and breast-feeding longer than 15 months multiplies that risk
	• Maternal nipple lesions or mastitis increase transmission risk
	• Maternal seroconversion during breast-feeding increases transmission risk
	• Infant thrush at <6 months of age increases transmission in breast-feeding infants

With the availability of antiretroviral treatment, disease progression has been slowed considerably, with a change in the median age at death from 6 months to 3.6 years in a European study.[28] However, in areas of the world where treatment is not readily available, rapid disease progression and early death remain common. In Malawi, in a cohort of 190 children with perinatally acquired HIV infection, 89% were dead by 3 years, and only 1% were free of symptoms. [32] In South Africa, newborns co-infected with HIV and tuberculosis, syphilis or cytomegalovirus (CMV) had mean age at death of only 3.5 months, and 83% were dead by 9 months.[33]

At birth, infants who have HIV infection are on average 0.28kg lighter and 1.64cm shorter than those without HIV infection[34] born to women with HIV infection. Because both weight and length are affected, children with HIV infection appear symmetrically small, with average body mass index by 18 months of age.[34] Poor growth by 6 months of age is an indicator of rapid disease progression.[31] Growth failure is not usually secondary to neuroendocrine dysfunction, but may be at least partially related to reduced energy intake.

HIV-1 RNA has been found in cerebrospinal fluid of children with HIV infection, and clinical manifestations of this CNS involvement are common, with 9% of patients showing signs of progressive neurologic involvement by 12 months of age,[26] and 21% diagnosed with encephalopathy by 24 months of age.[35] A wide range of manifestations of neurologic involvement is possible, from developmental delay identified only by prospective testing, to profound cognitive deficits and motor disorders, including signs of pyramidal tract dysfunction, movement disorders and ataxia.[36] Some patients have isolated cognitive dysfunction, whereas others have primarily motor abnormalities and many have a mixed pattern of involvement. The clinical course of illness may be static, it may transiently plateau, or it may be progressive. Progression of cognitive and motor delays occurred by 30 months of age in 50% of 114 prospectively evaluated infants with perinatally acquired HIV infection, even in the absence of overt encephalopathy. In children who have HIV infection, small head circumference is common even without encephalopathy,[34] and microcephaly may occur in over half of the children with HIV-associated encephalopathy. Abnormalities on computerized tomography (CT) of the brain were found in 86% of 83 symptomatic children with HIV infection; these abnormalities included ventricular enlargement, cortical and cerebellar atrophy, and cerebral calcifications.[37] Basal ganglia calcifications associated with disruption of the blood-brain barrier and perivascular calcium deposits can be found in children with HIV encephalopathy.

Pulmonary lymphoid hyperplasia (PLH) and lymphoid interstitial pneumonitis (LIP) are focal (PLH) to diffuse (LIP) lymphocytic infiltratitve diseases of the lung. They begin as asymptomatic interstitial nodular pulmonary infiltrates with hilar and mediastinal lymphadenopathy on chest radiograph, and they may progress to an illness characterized by chronic cough and slowly or intermittently progressive hypoxemia associated with clubbing of the digits.

TABLE 134-3 -- Prevention of HIV transmission from pregnant women to their infants.[18]

PREVENTION OF HIV TRANSMISSION FROM PREGNANT WOMEN TO THEIR INFANTS[18]
1. Suggest the use of ZDV, antiretroviral therapy
a. ZDV, following the regimen from ACTG Protocol 076
Pregnant women
During pregnancy: ZDV 200mg/dose q8h or 300mg/dose q12h po, beginning after 14 weeks of gestation and continuing until labor begins
During labor: ZDV iv during labor: 2mg/kg load over 0.5–1 hour followed by 1 mg/kg per hour iv infusion. Add ZDV for infusion to 5% dextrose in water for concentration of =4mg/ml
Before scheduled cesarean section: iv ZDV regimen should be infused at least 3 hours before the cesarean section
Newborn infants
ZDV 2mg/kg/dose po q6h (ZDV 2.6mg/kg/dose po q8h being investigated. Prematures: 1.5mg/kg/dose po q12h 1st 2 weeks, then 2mg/kg/dose po q8h >2 weeks.) Start within 8–12 hours of age. Give 6 weeks of total therapy, if HIV DNA PCR negative. Adjust dose for weight gain as needed. Check hematocrit at 4 weeks. If HIV DNA PCR positive, repeat to confirm and refer to specialist for treatment.
b. Other combinations of prepartum and postpartum therapy can reduce perinatal transmission, including shorter courses of ZDV, with or without lamivudine, and a single prepartum and postpartum dose of nevirapine[18]
2. Avoid procedures that may increase the risk of exposure of the child to maternal blood and secretions (e.g. scalp electrode, scalp pH, etc.)
3. Avoid artificial rupture of membranes unless medically indicated. Rupture of membranes >4 hours increases the risk of MTCT of HIV
4. Cesarean section has been shown to decrease risk of vertical HIV transmission. Cesarean section may be considered if maternal HIV RNA PCR >1000
5. Wash the baby promptly
6. Advise against breast-feeding of the infant, and educate the mother about safe alternatives

PLH-LIP is frequently associated with generalized lymphadenopathy and salivary gland enlargement and, even though it is an AIDS-defining illness, it is associated with prolonged survival compared with survival in other patients with AIDS. Compared to children with PCP, those with PLH-LIP are older (usually over 1 year of age), have less tachypnea and fever, lower lactate dehydrogenase, higher total immunoglobulins and higher titers to Epstein-Barr virus (EBV) antigens.[38] PLH-LIP is pathogenically linked to infection with EBV as well as HIV, and it improves with immune system improvement with antiretroviral therapy. Corticosteroid therapy (e.g. oral prednisone) may be of benefit in selected patients.

Cardiac abnormalities are common. Children with HIV infection have faster heart rate, higher left ventricular mass and lower left ventricular function than uninfected children.[39] HIV genome has been identified in cardiac myocytes and in pericardial fluid in three infants with pericardial effusions and sudden death.[40] Children with rapid disease progression have higher resting heart rate and respiratory rate than other infants with perinatally acquired HIV, and chronic heart disease was found in 53% of 34 children with HIV who died before 5 years of age.[41] Of 81 children with HIV infection evaluated in one study, arrhythmias occurred in 35%, unexpected cardiac arrest in 9%, transient congestive heart failure in 10% and chronic congestive heart failure in 10%.[42] Patients who have encephalopathy may be at highest risk for chronic congestive heart failure and cardiac arrest.[42]

Nephropathy affects 3–40% of children with HIV infection, is more common in blacks, and occurs late in the course of disease. The most common pathologic findings are focal glomerulosclerosis and mesangial hyperplasia associated with lymphohistiocytic tubulointerstitial infiltrates.[43] Although renal disease may be associated with renal failure, it is not usually the cause of death in children with HIV infection.

The occurrence of opportunistic infections in children with HIV infection varies with age and $CD4^+$ T-cell count (Table 134.10).[44] *Pneumocystis carinii* pneumonia in adults occurs late in the course of HIV infection, when T-cell immunodeficiency has progressed enough for this chronic pulmonary colonizer to reactivate and cause acute hypoxemia. In children, PCP occurs at an early age, with peak age of onset at 3–6 months of age.[45] This probably represents primary infection with *P. carinii*, and 20–30% of cases are in children with $CD4^+$ T-cell counts of more than 1500/mm^3 .[46] [47] Compared with the chronic presentation of PLH-LIP in older children, PCP is characterized by acute onset of fever, tachypnea with rib retraction, significant hypoxemia with diminished breath sounds, wheezes and rhonchi.[38] Chest radiograph may be normal but it usually shows bilateral interstitial and alveolar infiltrates. Diagnosis can be made by using special stains to identify cysts or trophozoites in fluid from induced sputum, bronchoalveolar lavage or lung biopsy. Even though treatment with trimethoprim-sulfamethoxazole (co-trimoxazole) is better tolerated in children than adults, the case fatality rate from PCP in infancy is 33%, which is higher than that in adults.

Serious bacterial infections with organisms including *S. pneumoniae* and *H. influenzae* type b are common in children with HIV infection (see Table 134.5 and Table 134.10), and AIDS is defined by two or more episodes of sepsis, pneumonia, meningitis, bone or joint infection, or deep abscess in 2 years.[25] Otitis media, sinusitis and bronchitis are also common manifestations of the B-cell defect that accompanies HIV infection in children, and are caused by the usual childhood respiratory pathogens *S. pneumoniae*, *H. influenzae* and group A streptococci until late stages of immunosuppression, when *Staphylococcus aureus* and *Pseudomonas aeruginosa* may be more common. While intravenous immune globulin (IVIG) has been shown to decrease the number of bacterial infections in selected

TABLE 134-4 -- CDC classification for children (<13 years of age) with HIV infection: diagnostic criteria for clinical categories N, A, B and C.*

CDC CLASSIFICATION FOR CHILDREN (<13 YEARS OF AGE) WITH HIV INFECTION	
Clinical categories	**Diagnostic criteria**
N: Not symptomatic No signs or symptoms of HIV infection or only one of the conditions listed in category A	If <18 months of age two positive results on separate determinations from one or more of the following:
	(a) HIV culture,
	(b) HIV PCR or
	(c) HIV p24 antigen
	If =18 months HIV antibody positive by repeatedly reactive ELISA and confirmatory test (e.g. Western Blot or IFA)
A: Mildly symptomatic Two or more of the conditions listed, but none of the conditions listed in categories B or C	Lymphadenopathy (=0.5cm at more than two sites; bilateral at one site)
	Hepatomegaly
	Splenomegaly
	Dermatitis
	Parotitis
	Recurrent or persistent upper respiratory infection, sinusitis or otitis media
B: Moderately symptomatic Symptoms of HIV infection other than those listed for categories A or C. Examples include but are not limited to those listed	Anemia (<8), neutropenia (<1000) or thrombocytopenia (<100,000) persisting =30 days
	Bacterial meningitis, pneumonia or sepsis (single episode)
	Candidiasis, oropharyngeal thrush, persisting >2 months in children >6 months old
	Cardiomyopathy
	CMV infection, onset before 1 month of age
	Diarrhea, recurrent or chronic
	Hepatitis
	Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes within 1 year)
	HSV bronchitis, pneumonitis or esophagitis with onset before 1 month of age
	Herpes zoster (shingles) — two episodes or more than one dermatome
	Leiomyosarcoma
	Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia (AIDS defining, report to State)
	Nephropathy
	Nocardiosis
	Persistent fever (lasting >1 month)
	Toxoplasmosis, onset before 1 month of age
	Varicella, disseminated (complicated chickenpox)

C: Severely symptomatic Any condition listed in the 1987 surveillance case definition for AIDS with the exception of LIP	Serious bacterial infection; two in 2 years: sepsis, pneumonia, meningitis, bone or joint infection, abscess of organ or body cavity (excludes otitis media, skin or mucosal abscesses and indwelling catheter infections)
	Candidiasis, (esophageal, tracheal, bronchial, pulmonary)
	Coccidioidomycosis, disseminated or extrapulmonary
	Cryptococcosis, extrapulmonary
	Cryptosporidiosis or isosporiasis >1 month duration
	CMV disease (onset >1 month), other than liver, spleen or lymph nodes
	Encephalopathy: >1 finding for >2 months and no illness that explains:
	(a) failure to attain or loss of milestones or intellectual ability shown by neuropsychologic tests;
	(b) impaired brain growth or acquired microcephaly shown by OFC measurements or brain atrophy on CT scan or MRI (serial imaging needed if <2 years old);
	(c) acquired symmetric motor deficit with at least two of: paresis, pathologic reflexes, ataxia or gait disturbances
	Herpes simplex (ulcer >1 month duration or pneumonia or esophagitis >1 month old)
	Histoplasmosis, disseminated or extrapulmonary
	Kaposi's sarcoma
	Lymphoma, primary, in brain
	Lymphoma, B cell, non-Hodgkin's lymphoma
	Mycobacterium tuberculosis, disseminated or extrapulmonary
	Mycobacterium infection, noncutaneous, extrapulmonary or disseminated (except leprosy)
	Pneumocystis carinii pneumonia
	Progressive multifocal leukoencephalopathy
	Salmonella (nontyphoid) sepsis, recurrent
	Toxoplasmosis of the brain, onset >1 month old
	Wasting syndrome in absence of other illness that explains:
	(a) weight loss >10% of baseline or
	(b) downward crossing of =2 percentile lines on the weight chart in a child =1 year or
	(c) <5th percentile on weight for height on two consecutive measures =30 days apart plus
	(a) chronic diarrhea (=2 loose stools/day for =30 days) or
	(b) documented fever for =30 days, intermittent or constant

** Adapted from Centers for Disease Control and Prevention.[25]*

TABLE 134-5 -- Clinical findings in children with HIV infection.*

CLINICAL FINDINGS IN CHILDREN WITH HIV INFECTION				
Clinical finding	**By 6 months (%) (n=66)**	**By 9 months (%) (n=70)**	**By 12 months (%) (n=75)**	**Older children (%)**
Anemia	20	33	37	
Serious bacterial infection				55
— One	18	31	37	
— Two	5	9	15	
Persistent oral candidiasis	18	24	27	48
Generalized lymphadenopathy	17	31	39	90
Failure to thrive	17	30	30	62
Hepatomegaly	17	27	35	86
Splenomegaly	15	24	29	69
Thrombocytopenia	14	17	19	
Pneumocystis carinii pneumonia	9	16	15	
Persistent diarrhea	8	10	13	17
Hepatitis	6	9	13	
Other opportunistic infections	5	9	11	31
Progressive neurologic disease	5	7	9	34
Persistent fever	2	7	9	
Lymphoid interstitial pneumonitis	0	1	5	28
Parotitis	0	1	1	10
Cancers (lymphomas)	0	0	0	7
Asymptomatic	36	27	21	

** Adapted from Forsyth et al.[26] and Pahwa et al.[27]*

TABLE 134-6 -- CDC classification for children (<13 years of age) with HIV infection.[25]

CDC CLASSIFICATION FOR CHILDREN (<13 YEARS OF AGE) WITH HIV INFECTION

Immunologic categories	Age of child					
	<12 months		1–5 years		6–12 years	
	Cells/µl	(%)	Cells/µl	(%)	Cells/µl	(%)
1. No suppression	=1500	(=25)	=1000	(=25)	=500	(=25)
2. Moderate suppression	750–1499	(15–24)	500–999	(15–24)	200–499	(15–24)
3. Severe suppression	<750	(<15)	<500	(<15)	<200	(<15)
Immunologic categories based on age-specific $CD4^+$ T-cell counts and percent of total lymphocytes.						

TABLE 134-7 -- Summary of pediatric HIV classification.*

SUMMARY OF PEDIATRIC HIV CLASSIFICATION				
Immunologic categories (see Table 134.6)	Clinical categories			
	N: No signs/symptoms	A: Mild signs/symptoms	B^H : Moderate signs/symptoms	C^H : Severe signs/symptoms
1. No suppression	N1	A1	B1	C1
2. Moderate suppression	N2	A2	B2	C2
3. Severe suppression	N3	A3	B3	C3
Children whose HIV infection status is not confirmed are classified by placing the letter E (for perinatally exposed) before the classification code (e.g. EN1). H, both category C and lymphoid interstitial pneumonitis in category B are reportable to state health departments as AIDS.				

** Adapted from Centers for Disease Control and Prevention.[25]*

pediatric patients with HIV infection,[48] [49] in patients being treated with trimethoprim-sulfamethoxazole (co-trimoxazole) prophylaxis there is no added benefit of IVIG.[50] Children who may benefit from IVIG for prophylaxis of recurrent infections are those with hypogammaglobulinemia or bronchiectasis from recurrent pulmonary infections.

Although HIV is not itself oncogenic, children with HIV infection do have an increased risk of malignancy.[51] Kaposi's sarcoma, although rarer in children than in adults, is found in children with HIV infection.[52] Non-Hodgkin's lymphoma, predominantly of B-cell origin, is the most common malignancy reported in children with HIV infection, and lymphomas of T-cell origin also occur. Many lymphomas are

TABLE 134-8 -- Time in each stage and survival time from beginning of each stage in the absence of retroviral therapy.*

TIME IN EACH STAGE AND SURVIVAL TIME FROM BEGINNING OF EACH STAGE				
Stage	Mean time in stage (months)	Median survival (months)	Mean survival (months)	% Surviving 5 years
N	10	113	96	75
A	4	103	85	67
B	65	99	81	65
C	34	34	23	17

** Adapted from Barnhart et al.[29]*

TABLE 134-9 -- $CD4^+$ cell count, virus load and risk of death in children with HIV infection.*

$CD4^+$ CELL COUNT, VIRUS LOAD AND RISK OF DEATH IN CHILDREN WITH HIV INFECTION				
$CD4^+$ %	>15%	>15%	<15%	<15%
HIV RNA PCR	<100,000	>100,000	<100,000	>100,000
Number of patients	103	89	24	36
Mortality rate at 5.1 years (%)	14.6	36.0	62.5	80.6
Based on experience of 254 children enrolled in the NICHD IVIg Clinical Trial.[41] [42] Mean age at entry was 3.41 years, and mean follow-up time was 5.1 years. Organon NASBA assay was used to measure plasma HIV RNA. Relative risk of death was 2.75 for each 1 log increase in HIV RNA and 1.33 for each 5 point decrease in $CD4^+$ %. [62]				

** Adapted from Mofenson et al.[30]*

TABLE 134-10 -- Opportunistic infections (OIs) in 3331 children with HIV infection.*

OPPORTUNISTIC INFECTIONS IN 3331 CHILDREN WITH HIV INFECTION					
OI diagnosis	Number of events	Event rate/100 person years	Median age at OI diagnosis (years)	Median CD4% at OI diagnosis	% of patients with CD4 count <50 at time of OI diagnosis
Serious bacterial infection	879	15.1	3.5	17	23
Herpes zoster	199	2.9	7.6	13	27
Disseminated *Mycobacterium avium* complex	126	1.8	6.4	2	76
Pneumocystis carinii pneumonia	92	1.3	3.9	6	55
Candidiasis	87	1.2	4.8	4	60
Cryptosporidiosis	41	0.6	5.9	4	51
CMV retinitis	33	0.5	7.1	4	61
Tuberculosis	27	0.4	7.6	10	33
Other CMV disease	16	0.2	3.6	7	50
Fungal infection	8	0.1	12.3	3	65

Toxoplasmosis	4	0.06	11.5	4	100
Progressive multifocal leukoencephalopathy	4	0.06	10.8	2	100

** Adapted from Dankner et al. [44]*

associated with EBV infection. Smooth muscle tumors, including leiomyomas and leiomyosarcomas, are specifically associated with HIV infection in children,[53] and occur late in the course of illness when the $CD4^+$ T-cell count is low. Epstein-Barr virus genome has been found in the muscle cells of leiomyosarcomas from children with HIV infection, but not in such tumors from persons without HIV infection.[54]

DIAGNOSIS

Because many children with HIV infection remain asymptomatic for long periods of time, the clinical examination is not a sensitive test for the presence of infection. Infection with HIV in adults and children older than 18 months is presumptively diagnosed by using an enzyme-linked immunosorbent assay (ELISA) to screen blood for presence of antibody to HIV. A single positive ELISA is repeated, and if still positive, results are confirmed by Western blot for HIV-specific antibody. [55]

Children born to women with HIV infection passively acquire maternal IgG antibody to HIV, which can make the HIV IgG antibody ELISA reactive for 12–18 months after birth, even in infants not infected with HIV. Therefore, detection of virus or virus products is the preferred means of diagnosis because it can identify infected infants at a younger age.

Culture of HIV is the gold standard for diagnosis of HIV infection in infants under 18 months of age,[56] but DNA PCR is more widely available and has been shown to be equally sensitive. A single positive culture is not diagnostic of HIV infection in infants because 2–7% of children later found to be uninfected may have a positive culture within the first 6 months of life.[57] [58] [59] Therefore, at least two

positive cultures should be obtained to confirm the diagnosis of HIV infection in children.

The PCR for HIV DNA is the most rapid and accurate method for identification of HIV infection in children under 18 months of age.[9] [60] [61] Sensitivity is 38% on the day of birth, 93% by day 14 of life and 96% by day 28.[10] False-positive results are possible, owing to laboratory error,[60] but two positive tests together are 98.5% accurate in identifying infection status, and using three tests performed after 1 month of age achieves 100% accuracy. The first positive HIV DNA PCR after perinatal infection may appear as late at 183 days of life.[10]

Two positive HIV DNA PCR tests are needed to diagnose HIV infection in infants. Two negative PCR tests performed at more than 1 month of age, with one at more than 4 months of age, reasonably exclude HIV infection.[62] Because of the importance of a negative test, PCR testing should include controls to assess DNA sample adequacy. Samples with inadequate DNA should be rejected, and not reported as negative. In the child with two negative HIV DNA PCR tests at more than 1 month of age and 2–4 months of age, ELISA for IgG to HIV should be performed at 18 months to confirm absence of infection (seroreversion). Primers for PCR differ in their ability to identify nucleic acid from non-clade B HIV, and in some settings special testing may be required to identify or exclude such infection (see Chapter 136).[63]

Measurement of HIV p24 antigen in blood is not sensitive enough to be used for early diagnosis of HIV infection in children, even if immune complex dissociated methods are used for sample preparation.

MANAGEMENT

Issues of immediate importance in the care of children born to women with HIV infection are:

- continued intervention to prevent HIV infection if possible (see Table 134.3);
- identification of infected infants; and
- institution of prophylactic therapy for PCP.

Independent of specific HIV-associated problems, certain health-related procedures are necessary for all children with HIV infection. Height and weight are measured at regular intervals and plotted on appropriate growth charts. The head circumference should be measured at least every 3 months until 2 years of age, in conjunction with a careful neurologic assessment. Developmental screening is recommended every 6 months until 24 months of age and yearly thereafter.

Respiratory status should be monitored closely for the possible acute onset of PCP in younger patients or the more insidious onset of PLH-LIP in older children. Symptoms or signs of heart failure should prompt evaluation, including chest radiograph, pediatric cardiology evaluation, electrocardiography and possibly echocardiography. A

TABLE 134-11 -- Immunization of children with HIV infection.*

IMMUNIZATION OF CHILDREN WITH HIV INFECTION		
Vaccinate following routine for children without HIV Infection	**Special vaccinations to give children with HIV infection**	**Do not give these vaccines to children with HIV infection**
Polio: eIPV	Influenza	Polio: OPV
DTP/DTaP	Pneumococcus	
MMR	BCG	
Hepatitis B	Varicella	
Hib-conjugate		
See text for details. DtaP, diphtheria, tetanus and acellular pertussis vaccine.		

** Adapted from Centers for Disease Control and Prevention.[64]*

normal heart size on chest radiograph does not rule out cardiac dysfunction. Chest radiographs are indicated yearly for comparison with later films because of the frequency of pulmonary disease and the often subtle beginnings of PLH-LIP.

Ophthalmologic examinations are recommended annually for children treated with dideoxyinosine (ddI). For children who have CMV infection, more frequent eye examinations may be indicated.

Immunizations

Immunizations follow the schedule for infants and children recommended by the American Academy of Pediatrics (Table 134.11),[64] with the following caveats.

Polio

Enhanced-potency inactivated poliovirus vaccine (eIPV) should be given to infected children and to uninfected children when parents or siblings have HIV infection.

Oral polio vaccine (OPV) should not be used.

Measles, mumps and rubella

In spite of a small risk of immunizing immunosuppressed patients with live vaccine, measles, mumps and rubella vaccine (MMR) should be given because of the high case fatality rate of measles for HIV-infected children. It may be better to give measles vaccine at 12 months to improve the probability of seroconversion. Second dose may be given as soon as 28 days later Measles vaccination of an adult with an extremely low $CD4^+$ T-cell count has resulted in vaccine-associated pneumonitis, and measles vaccine is not recommended for children with CD4% less than 15%. Immunoglobulin prophylaxis is indicated for HIV-infected children exposed to measles, even if they have been previously vaccinated for measles.

Influenza

Influenza vaccine is indicated for children with symptomatic HIV infection, as well as asymptomatic or uninfected children if they are living with family members who have HIV infection and who are ill.

Pneumococcus

Children up to 5 years of age, infected with HIV, are vaccinated with 7-valent conjugate vaccine. After age 5 years, use 23-valent polysaccharide vaccine. For children 10 years of age or under, a single revaccination is recommended 3–5 years after the initial dose. For children over 10 years of age, a single re-vaccination is recommended if 5 or more years has elapsed since the previous dose

Haemophilus influenzae type b

Administration of *H. influenzae* type b (Hib) conjugate vaccine follows standard recommendations for infants and consideration should be given for its administration to newly diagnosed children who are over 60 months of age.

Bacille Calmette-Guérin

Although bacille Calmette-Guérin (BCG) is not recommended for use in the USA,[65] the WHO recommends its use in asymptomatic infants in regions where risk of tuberculosis is high.[66]

Varicella

Vaccination with varicella vaccine is indicated for children with HIV infection in CDC class N1 or A1, with CD4% of at least 25%. Give two doses with a 3-month interval between them.[67] Not addressed by the CDC recommendations is the patient with prior symptoms and prior low $CD4^+$ T-cell count (e.g. C3 in the past), now on triple antiretroviral therapy, asymptomatic, with CD4% over 25%. There are no data to assure the safety or efficacy of varicella vaccine under such circumstances. However, if such patients are clinically stable, with CD4% over 25% for more than 6 months, two-dose vaccination might be considered, with the understanding that safety and efficacy are not assured.

Laboratory monitoring

$CD4^+$ T-cell count and percentage and a full blood cell count with differential are indicated every 3 months to evaluate the integrity of the immune system. Immunoglobulin concentrations are measured once for patients under 6 months of age to identify those patients with hypogammaglobulinemia.

Virus load is measured as the plasma HIV RNA concentration at 3-month intervals to help in the decision to begin or alter antiretroviral therapy. Virus load testing is also performed 4–8 weeks after beginning or changing therapy, to assess the effectiveness of therapy.[68]

Patients with specific clinical problems may require extra laboratory monitoring.

Antiretroviral therapy

Antiretroviral therapy is indicated for children with symptomatic HIV infection, independent of $CD4^+$ T-cell count or virus load, but USA and European guidelines differ on the extent of symptoms that demand treatment (Table 134.12).[68] [69] Since $CD4^+$ T-cell count and virus load are predictive of disease progression and death in children with HIV infection (see Table 134.9) starting antiretrovirals in asymptomatic children with HIV infection may be based on either the $CD4^+$ T-cell count, or on the virus load (see Table 134.12). Before

TABLE 134-12 -- Clinical and immunologic characteristics that prompt initiation of antiretroviral therapy in children with HIV infection.*

CLINICAL AND IMMUNOLOGIC CHARACTERISTICS THAT PROMPT INITIATION OF ANTIRETROVIRAL THERAPY IN CHILDREN WITH HIV INFECTION			
Age group	**Characteristic**	**Treatment recommendations**	
		USA	**Europe**
Infant to 12 months	Any	Recommend for any infant <12 months of age	Consider for any infant <12 months of age
	Symptoms		Always start for CDC category C
	CD4 cell count		Always start for CD4 <20% or rapidly falling CD4
	Virus load		Always start if >1,000,000
Child > 12 months	Symptoms	Recommend for CDC category A, B or C	Always start for CDC category C. Consider for CDC category B. Defer for CDC category N or A
	CD4 cell count	Recommend for CD4 <25% or rapidly falling CD4	Always start for CD4 <15%. Consider for CD4 <20%. Defer for CD4 >20%
	Virus load	Recommend for high or increasing virus load	Consider if >100,000

** Adapted from Centers for Disease Control and Prevention[68] and Sharland et al.[69]*

starting therapy based on the $CD4^+$ T-cell count or virus load, persistent abnormality should be confirmed by repeating at least once, with at least 1 week between tests.

Plasma HIV RNA concentrations early in life are higher than in adults with primary HIV infection, and they decline slowly over an undetermined time period toward the values observed in adults. A 'stable baseline' plasma HIV RNA concentration may not be reached in perinatally infected children until they are 3–6 years of age. Adult guidelines for therapy based on plasma HIV RNA concentration might be applicable to children over 30 months of age, suggesting institution of antiretroviral therapy for children in this age group with plasma HIV RNA of more than 55,000 to 100,000 copies/ml, independent of $CD4^+$ T-cell count.[68] [70]

Some authorities suggest that antiretroviral therapy is indicated in all children with HIV infection who are less than 1 year of age,[68] hoping that early therapy will suppress HIV replication more efficiently by reducing viral quantity and diversity. The potential benefits of this approach are speculative and need to be weighed against

the risks of toxicity of therapy.[69] [71]

Single-agent antiretroviral therapy should not be used in the treatment of children or adolescents with HIV infection (Table 134.13).[71] A combination of three drugs is regarded as optimal therapy, most commonly two nucleoside analog reverse transcriptase inhibitors (NRTIs) plus a protease inhibitor.[68] [69] [70] Most experience in children is with ZDV-3TC or ZDV-ddI plus NFV, or RTV, or LPV-RTV. D4T-3TC, and either d4T or ZDV plus ABC are also reasonable combinations of NRTIs to which a protease inhibitor may be added. An alternative triple combination regimen of two NRTIs plus one non-NRTI (e.g. NVP or EFV) is acceptable. Triple NRTI regimens are possible (e.g. ZDV-3TC-ABC). Regimens of two NRTIs alone may produce initial clinical benefit but do not produce sustained viral suppression. Compared to adults, infants and children have poor absorption and more rapid metabolism of antiretroviral medications, especially non-NRTI and protease inhibitor, and higher doses may be needed for children compared to adults.

ZDV and d4T should not be used together because of antiviral antagonism and diminished clinical effectiveness. ddC should not be combined with ddI, d4T or 3TC because of the potential for overlapping toxicity. IDV should not be used with SQV.

Intolerance of antiretroviral therapy can lead to dosage modification or discontinuation of therapy. Clinical symptoms — including

TABLE 134-13 -- Dosage and administration of antiretrovirals for children.*

DOSAGE AND ADMINISTRATION OF ANTIRETROVIRALS FOR CHILDREN		
Drug name	**Recommended dosage**	**How supplied**
Nucleoside or nucleotide analog reverse transcriptase inhibitors (NRTIs)		
ZDV, zidovudine, azidothymidine, AZT, Retrovir	Premature infants	Syrup: 10mg/ml
	0–2 weeks: 1.5mg/kg/dose q12h po (1.0mg/kg/dose q12h iv)	Capsules: 100mg
	>2 weeks: 2.0mg/kg/dose q8h po (1.5mg/kg/dose q8h iv)	Tablets: 300mg
	Term infants	Combivir: ZDV 300mg plus 3TC 150mg in a single tablet
	0–6 weeks: 2mg/kg/dose q6h po (1.5mg/kg/dose q6h iv), 4mg/kg/dose q12h po (1.5mg/kg/dose q6h iv)	Trizivir: ZDV 300mg + 3TC 150mg + ABC 300mg
	4 weeks–13 years: 120–160mg/m^2 /dose q8h po, 180mg/m^2 /dose q12h po	Injection: 10mg/ml in 20ml vials
	=13 years: 200mg/dose q8h po or 300mg/dose q12h po	
ddI, dideoxyinosine, didanosine, Videx	<3 months: 50mg/m^2 /dose q12h po	Chewable tablets*: 25mg, 50mg, 100mg, 150mg, 200mg
	3 months to <13 years: 90–135mg/m^2 /dose q12h po or 240mg/m^2 /dose q24h po	Buffered powder packets: mix with water: 100mg, 167mg, 250mg
	=13 years, <60kg: tablets 125mg q12h po; powder 167mg q12h po; Videx EC 250mg q24h po	Enteric-coated delayed release tabs (Videx EC): 125mg, 200mg, 250mg, 400mg
	=13 years, >60kg: tablets 200mg q12h po, powder 250mg q12h po, Videx EC 400mg q24h po	Pediatric powder for oral solution mixed to final concentration of 10mg/ml
ddC, dideoxycytidine, zalcitabine, HIVID	<13 years: 0.01mg/kg/dose q8h po	Syrup: 0.1mg/ml (investigational)
	=13 years: 0.75mg q8h po	Tablets: 0.375mg, 0.75mg
d4T, stavudine, Zerit	<30kg: 1mg/kg/dose q12h po	Solution: 1mg/ml
	30–60kg: 30mg q12h po	Capsules: 15, 20, 30, 40mg
	>60kg: 40mg q12h po	Mix with apple sauce
3TC, lamivudine, Epivir	<1 month: 2mg/kg/dose q12h po	Oral solution: 10mg/ml
	<37.5kg: 4mg/kg/dose q12h po	Tablets: 150mg
	=37.5kg: 150mg/dose q12h po	Combivir: ZDV 300mg plus 3TC 150mg in a single tablet
		Trizivir: ZDV 300mg + 3TC 150mg + ABC 300mg
Abacavir, 1592U89, Ziagen, ABC	<37.5kg or 16 years: 8mg/kg/dose q12h po	Oral solution: 20mg/ml
	=37.5kg or 16 years: 300mg/dose q12h po	Tablets: 300mg
		Trizivir: ZDV 300mg + 3TC 150mg + ABC 300mg
TDF, tenofovir disoproxil	<13 years: 210mg/m^2 /dose q24h po (investigational)	Tablets: 300mg
	>13 years: 300mg q24h po	
Non-nucleoside analog reverse transcriptase inhibitors (NNRTIs)		
NVP, nevirapine, Viramune	<3 months: 5mg/kg/dose q24h × 2 weeks, then 120mg/m^2 /dose q12h × 2 weeks, then 200mg/m^2 /dose q12h po	Suspension: 10mg/ml
	<1m^2 : 120–200mg/m^2 /dose q12h po	Tablets: 200mg
	=1m^2 : 200mg q12h po (maximum dose)	
	Always start at half dose for 2 weeks, then to full dose if tolerated	
DLV, delavirdine, Rescriptor	<13 years: dosage not established	Tablets: 100mg
	=13 years: 400mg/dose q8h po	
EFV, DMP 266, efavirenz, Sustiva	<2 years: investigational	Capsules: 50, 100, 200, 600mg
	>2 years: 650mg/m^2 /dose q24h po	
	=28kg (0.95m^2) and adults: 600mg/dose q24h po	
Protease Inhibitors (PIs)		
SQV, saquinavir, Invirase (hard gel), Fortovase (soft gel)	<16 years: 50mg/kg/dose q8h po (investigational)	Hard gel capsules: 200mg
	= 16 years: 600mg/dose q8h po with fatty meal (Invirase: poorly absorbed: do not use), 1200mg/dose q8h po with fatty meal (Fortovase)	Soft gel capsules: 200mg
RTV, ritonavir, Norvir	3 months–13 years: 400–450mg/m^2 /dose q12h po	Oral solution: 80mg/ml
	=13 years: 600mg/dose q12h po	Gel caps: 100mg
		Store in original bottle

IDV, indinavir, Crixivan	3–13 years: 500mg/m^2 /dose q8h po	Capsules: 200 and 400mg. Must be stored in original bottle
	=13 years: 800mg q8h po	
NFV, nelfinavir, Viracept	1 month–13 years: 30–50mg/kg/dose q8h po, 55–60mg/kg/dose q12h po (max. 2000mg/dose; investigational)	Powder for oral suspension: 50mg/'level scoop', or 200mg/USA teaspoon (5ml)
	=13 years: 750–1250mg/dose q8h po, 1250mg/dose q12h po (adolescents may need higher dose than adults)	Tablet: 250mg
141W94, APV amprenavir, Agenerase	<3 years: not recommended	Oral solution: 15mg/ml
	3–12 years: 20mg/kg/dose q12h or 15mg/kg/dose q8h po (liquid: 85% bioavailable, so use 22.5mg/kg/dose q12h or 17mg/kg/dose q8h to maximum of 2800mg/day)	Tablets: 50mg, 150mg
	Adults: 1200mg/dose q12h po	
LPV/r, Abbott-378/r, lopinavir/ritonavir, Kaletra	Children: 300/75 (LPV-RTV)mg/m^2 /dose q12h po	Oral solution: 400mg/100mg LPV-RTV per 5ml (80mg/20mg LPV-RTV per ml). Can store at room temp for 2 months
	Adults: 400/100 (LPV-RTV)mg/dose q12h po, 533/133 (LPV-RTV)mg/dose q12h po if given with NVP	Capsules 133.3mg/33.3mg LPV-RTV per capsules
Combination therapy is recommended for treatment, and the preferred regimen is two NRTIs and one PI. Most experience in children is with ZDV/3TC or ZDV/DDI plus NFV or RTV. D4T/DDI, D4T/3TC and ZDV/ABC are also reasonable NRTI combinations to which a PI may be added. An alternative triple combination regimen of two NRTIs plus one NNRTI is acceptable but may be less likely to result in durable suppression of viral load. Regimens of two NRTIs alone may show initial clinical benefit but do not usually show sustained viral suppression. There is wide interperson variation in plasma concentration of antiretrovirals and therapeutic drug monitoring may be helpful to guide dose selection. Do not use monotherapy. Do not use the following combinations: ZDV/D4T; DDC/DDI; DDC/D4T; DDC/3TC; or IDV/SQV. Body surface area (BSA) = ([height (cm) × weight (kg)]/3600^2 .		

** Adapted from Centers for Disease Control and Prevention[68] Sharland et al.,[69] Havens et al.[71] and Centers for Disease Control and Prevention.[72]*

growth delay, new or worsening encephalopathy, or new or recurring opportunistic infections — are markers of progressive HIV disease, and may trigger a change in therapy. Rising plasma HIV RNA, or falling $CD4^+$ T-cell counts, are markers of disease progression that may be used to guide a change in antiretroviral therapy.

Prevention of opportunistic infections

Guidelines for the prevention of opportunistic infections in adults and adolescents who have HIV infection established by the US Public Health Service and the Infectious Diseases Society of America, and endorsed by the American Academy of Pediatrics, have been published. Recommendations in this section follow those guidelines except as noted.

Prophylaxis for PCP is indicated for all children with prior PCP, for all children with a percentage of $CD4^+$ T cells that is less than 15% and for all children with a $CD4^+$ T-cell count less than those indicated in Table 134.14 . Prophylaxis for PCP is indicated for all children born to women with HIV infection, and it should be started at 4–6 weeks of age, regardless of the child's $CD4^+$ T-cell count or HIV infection status.[62] [72] For children in whom HIV infection is reasonably excluded (usually by 4 months of age), PCP prophylaxis can be discontinued.

Children who have HIV infection should continue PCP prophylaxis until at least 12 months of age. Prophylaxis is discontinued at 12 months of age for children with HIV infection whose $CD4^+$ T-cell counts during the first year of life have been over 750/mm^3

TABLE 134-14 -- Normal $CD4^+$ cell counts and $CD4^+$ cell counts at which to initiate prophylaxis for *Pneumocystis carinii* pneumonia and *Mycobacterium avium* complex infections.†

NORMAL $CD4^+$ T CELL COUNTS AND $CD4^+$ T CELL COUNTS AT WHICH TO INITIATE PROPHYLAXIS FOR PCP AND MAC INFECTIONS			
Age	**Normal $CD4^+$ T cell count/mm^3 , median[64]**	**$CD4^+$ T cell count/mm^3 at which to start PCP prophylaxis[55]**	**$CD4^+$ T cell count/mm^3 at which to start MAC prophylaxis[63]**
1–11 months*	>3000	All*	<750
12–23 months	2600	750	<500
24–71 months	1700	500	<75
=6 years	1000	200	<50
Patients with prior PCP should receive prophylaxis. All patients should receive prophylaxis when the CD4% is less than 15%.			

† *Adapted from Centers for Disease Control and Prevention.*[62] [64] [72]
* Even patients for whom the diagnosis of HIV infection has not yet been confirmed should begin PCP prophylaxis at 4–6 weeks of age. For patients in whom HIV infection is reasonably excluded by at least two DNA PCR tests, PCP prophylaxis can be discontinued (usually at 4 months of age)[55]

TABLE 134-15 -- Prophylaxis of *Pneumocystis carinii* pneumonia in children.†

PROPHYLAXIS OF PCP IN CHILDREN		
Drug	**Dose**	**Comment**
Trimethoprim-sulfamethoxazole	150mg/m^2 /day ÷ q12h 3 days/week on consecutive days	Preferred regimen
	150mg/m^2 /day po q24h on consecutive days	More marrow toxicity
	150mg/m^2 /day ÷ q12h 7 days/week	More marrow toxicity
	150mg/m^2 /day ÷ q12h po for 3 days/week alternate days	
Dapsone*	2mg/kg/day, as a single dose (max. 100mg)	Absorption equal at high/low gastric pH
Atovaquone	1–3 months old: 30mg/kg/dose po q24h	
	4–24 months old: 45mg/kg/dose po q24h	
	>24 months old: 30mg/kg/does po q24h	
Pentamidine	300mg monthly by aerosol inhalation via Respirgard II nebulizer	>5 years old
	4mg/kg every 2–4 weeks iv	Of unproven benefit

† *Adapted from Havens et al.*[71]

* Testing for G6PD deficiency is indicated before starting therapy because dapsone may induce hemolysis in G6PD deficient patients

(15%) when regularly measured. Prophylaxis for PCP is continued after 12 months of age for children with HIV infection with any $CD4^+$ T-cell count that was less $750/mm^3$ (15%) during the first 12 months of life. For children between 12 and 24 months old who are not on prophylaxis, begin it if $CD4^+$ T-cell counts are less than $750/mm^3$ (15%). At 24 months, children with HIV infection who have been receiving PCP prophylaxis can discontinue treatment if $CD4^+$ T-cell counts have all been over $500/mm^3$ (15%) when regularly measured every 3 months. Prophylaxis for PCP is continued after 24 months of age for children with HIV infection with any $CD4^+$ T-cell count that was less than $500/mm^3$ (15%) during the first 24 months of life.

For HIV-infected children who are older than 24 months, PCP prophylaxis is begun when the $CD4^+$ T-cell count drops below the critical levels outlined in Table 134.14 . Usual PCP prophylaxis is trimethoprim-sulfamethoxazole $150mg/m^2$ per day (5–7mg/kg per day) divided into two daily doses and given on Monday, Tuesday and Wednesday every week. Alternative regimens are shown in Table 134.15 .

Children and adolescents who have HIV infection and a positive tuberculin skin test (more than 5mm induration) without evidence of active disease, prior antituberculous therapy or prior antituberculous prophylaxis should receive prophylactic isoniazid, 10–15mg/kg (to a maximum of 300mg per dose) once daily for 12 months. Such therapy should also be considered for skin test-negative children who are close contacts of persons with infectious tuberculosis, after active

tuberculous infection has been excluded. Rifampin (rifampicin), 10–20mg/kg (up to a maximum of 600mg per dose) given once daily is reasonable alternative prophylaxis if the strain to which the child was exposed is known to be resistant to isoniazid. [64]

Clarithromycin, 7.5mg/kg (up to a maximum of 500mg per dose) q12h is recommended for prevention of *Mycobacterium avium* complex (MAC) infections in children, based on age and $CD4^+$ T-cell count (see Table 134.14). Alternative regimens include azithromycin, 20mg/kg (up to a maximum of 1200mg per dose) once weekly; azithromycin 5mg/kg (up to a maximum of 250mg per dose) once daily; or rifabutin 5mg/kg (up to a maximum of 300mg per dose) once daily. Blood cultures for mycobacteria should be performed before beginning prophylaxis. Positive blood cultures prompt treatment with at least three drugs (e.g. clarithromycin, ethambutol and rifabutin). At the stage of infection when MAC prophylaxis is considered, patients may be on other medications that may interact with rifabutin. The advantages and disadvantages of beginning therapy should be carefully discussed with families.

Patients who have HIV infection and hypogammaglobulinemia may benefit from infusions of IVIG, 400mg once monthly to prevent serious bacterial infections. Intravenous immunoglobulin for prophylaxis of bacterial infections shows no additional benefit in children without hypogammaglobulinemia who are already receiving concurrent trimethoprim-sulfamethoxazole.

Varicella-zoster immune globulin is given to children who have HIV infection without prior varicella infection if they are exposed to chickenpox or zoster. Varicella-zoster immune globulin is not effective if given more than 96 hours after exposure. Prophylaxis with aciclovir is used for patients with frequently recurring zoster. The exact dose for this indication in children is unclear, but 5mg/kg q12h is a reasonable starting dose, increasing to as high as 10–20mg/kg q6-8h if breakthrough occurs.

Prophylaxis for CMV disease in children is not recommended at this time. Prophylaxis of herpes simplex virus disease and fungal infections in children follow guidelines for adults, with appropriate dose modifications.[64] Other prophylaxis may be indicated for patients with a variety of late complications of HIV infection.[64]

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Chapter 135 - Special Problems in Women who have HIV Disease

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EPIDEMIOLOGY

The number and proportion of women who have HIV-1 infection in the USA have been gradually increasing. Of 41,960 persons reported to the Centers for Disease Control and Prevention (CDC) who had AIDS in 2000, 10,459 (25%) were women.[1] This contrasts with 1985 statistics, when 7% (534/8153) of people reported to the CDC who had AIDS were women. Based on 1999 vital statistics data, HIV-1 infection is the fifth leading cause of death for women aged 25–44 years and the third leading cause of death for African-American and Hispanic women in this age group. The racial distribution of women who had AIDS in 2000 was 62.6% African-American (45.9/100,000), 18.1% white (2.2/100,000) and 17.7% Hispanic (13.8/100,000).

Heterosexual transmission has surpassed injection drug use as the primary route for women acquiring HIV-1 infection in the USA. Geographically, the north-eastern and southern USA have reported the greatest number of cases. Current estimates are that 40,000 new infections occur annually in the USA, of which 30% are in women. Of newly infected women, estimates are that 75% acquired HIV-1 through heterosexual sex and 25% through intravenous drug use.

Worldwide 2001 UNAIDS estimates are that, of the 37.2 million adults living with HIV-1/AIDS, 17.6 million (47%) are women.[2] More than 70% live in sub-Saharan Africa. One in every 100 adults worldwide aged 15–49 years is HIV-1-infected. In sub-Saharan Africa, 8.4% of all adults in this age group are HIV-1 infected. More than 80% of all adults worldwide acquired HIV-1 infection via heterosexual intercourse.

TRANSMISSION

Factors that are important in male-to-female transmission of HIV-1 infection include:

- advanced disease in the infected source partner;
- plasma HIV-1 RNA level (and virus shedding in genital secretions);
- anal receptive intercourse;
- presence of genital ulcers;
- absence of condom use; and
- absence of zidovudine use.[3] [4] [5] [6]

Factors associated with heterosexual female-to-male transmission of HIV-1 infection include:

- advanced disease in the infected source partner;
- plasma HIV-1 RNA level (and virus shedding in genital secretions);
- presence of genital ulcers;
- sexual intercourse during menses; and
- absence of condom use.

In developing countries, observational studies have also suggested an association of non-ulcerative sexually transmitted diseases with increased rates of HIV-1 transmission. For women, *Candida* vaginitis, bacterial vaginosis and use of depot medroxyprogesterone acetate have been associated with an increased incidence of HIV-1 infection. The efficiency of male-to-female transmission of HIV-1 has been estimated to be twice that of female-to-male transmission, although some studies have found equal rates of transmission between the sexes.[4] [5]

CLINICAL FEATURES

Disease manifestations and progression

Overall, there are few differences in the incidence of nongynecologic opportunistic diseases between men and women who have HIV-1 infection, with the exception of a higher incidence of esophageal candidiasis as an AIDS-defining condition and a lower incidence of Kaposi's sarcoma reported among women.[7] It has been postulated that women may have a higher incidence of candidiasis due to vaginal colonization with yeast or hormonal influences, although data supporting these hypotheses are lacking.

Several studies have now demonstrated that women have lower plasma HIV-1 RNA levels than do men after controlling for age, the interval from seroconversion and the $CD4^+$ lymphocyte count.[8] Another study found that this sex difference in plasma HIV-1 RNA levels disappeared 5–6 years after seroconversion. Although women may progress to AIDS with lower viral loads, the time to progression to AIDS appears to be similar for men and women. Similarly, for HIV-1-infected men and women who have adequate access to health care and treatment, survival appears to be equivalent.[9] [10]

Gynecologic manifestations

Infection

Gynecologic disorders are common in women who have HIV-1 infection. [11] Up to 50% of these women develop recurrent *Candida* vaginitis, which often precedes the development of oral or esophageal candidiasis. Several longitudinal cohorts have reported that 14–18% of women who have HIV-1 infection have or develop recurrent genital herpes simplex virus (HSV) infection, which can be more severe or refractory to treatment than among HIV-1-seronegative women.[10]

The relationship between HIV-1 and pelvic inflammatory disease (PID) in women is less clear. Several retrospective studies suggest that women presenting with PID have a high rate of co-infection with HIV-1 and that women who have HIV-1 infection with PID require surgical intervention more frequently because of abscess formation.[12] Prospective studies will be necessary to define this relationship and determine whether there are differences in response to therapy, microbiologic etiology and risk of recurrence or long-term sequelae.

Genital dysplasia

Cervicovaginal dysplasia is common in women who have HIV-1 infection. [11] [13] Abnormal Papanicolaou smears were found in 38.3% of 1713 HIV-1-infected women and 16.2% of 482 women uninfected with HIV-1 but at risk, in the Women's Interagency HIV-1 Study cohort.[13] In multivariate analyses, risk factors for abnormal cytology included HIV-1 infection, low $CD4^+$ lymphocyte counts,

high plasma HIV-1 RNA levels and detection of human papillomavirus (HPV). In follow-up of this cohort, HPV detection, $CD4^+$ lymphocyte count and plasma HIV-1 RNA levels predicted regression.[14] Rates of incidence, progression and regression of abnormal cytology did not differ between the HIV-1-uninfected controls and HIV-1-infected women with $CD4^+$ lymphocyte counts greater than 200 cells/ml and plasma HIV-1 RNA levels below 4,000 copies/ml. In another study, 20% (80/398) of HIV-1-seropositive women compared with 4% (15/357) of HIV-1-seronegative women had cervical intra-epithelial neoplasia (CIN) confirmed by colposcopy.[15] The presence of CIN was found to be independently associated with:

- HPV infection (odds ratio 9.8);
- HIV-1 infection (odds ratio 3.5);
- $CD4^+$ lymphocyte count less than 200/ml (odds ratio 2.7); and
- age greater than 34 years (odds ratio 2.0).

The rate of CIN in women who have HIV-1 infection with fewer than 200 $CD4^+$ lymphocytes/µl was 28% (27/95) compared with 19% (45/236) for those with higher $CD4^+$ lymphocyte counts.

Conflicting data exist regarding whether CIN and invasive cervical cancer have a more aggressive course or a less favorable response to therapy among women who have HIV-1 infection. In one study prior to the availability of highly active antiretroviral therapy (HAART), 62% of 127 women who had HIV-1 infection with CIN developed recurrent CIN within 36 months of treatment compared with 18% of HIV-1-seronegative controls.[16] During the 36-month follow-up period, progression to higher grade dysplasia, including one invasive cancer, occurred in 25% of women who had HIV-1 infection compared with 2% of controls. Recently, HIV-1-infected women in New York were reported to be at higher risk than HIV-1-seronegative women for other HPV-associated malignancies, including vulvar and anal cancers. Highly active antiretroviral therapy that reverses immunosuppression is likely to impact favorably on the rate of HPV detection and prevalence of genital tract and anal dysplasia.

The 2001 Consensus Guidelines for the management of women who have cervical cytologic abnormalities are detailed in Table 135.1 .[17] These guidelines are not specific to women with HIV-1 infection but reflect updated terminology for reporting cervical cytology results, recent availability of HPV DNA testing and further follow-up data on the natural history of atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesions that were not available for the February 2002 US Public Health Service (USPHS) guidelines for cervical dysplasia screening in women who have HIV-1 infection.[18]

TABLE 135-1 -- Guidelines for cervical dysplasia screening of women infected with HIV-1.*

GUIDELINES FOR CERVICAL DYSPLASIA SCREENING OF WOMEN INFECTED WITH HIV-1	
Initial gynecologic examination with Papanicolaou smear	If normal, repeat in 6 months
	If atypical squamous cells of uncertain significance (ASCUS) proceed to colposcopy
	If atypical glandular cells proceed to colposcopy
	If low grade squamous intraepithelial lesion proceed to colposcopy
	If high grade squamous intraepithelial lesion, proceed to colposcopy
If initial two Papanicolaou smears are normal	Repeat annually as long as smears remain normal

** These are based on the 2001 Consensus Guidelines and the United States Public Health Service Guidelines.[17] [18]*

Because *Candida* vaginitis, genital HSV disease, PID and cervical dysplasia are common in women who have HIV-1 infection, these conditions, along with other common sexually transmitted diseases such as gonorrhea, chlamydial infection and syphilis, should prompt a determination of HIV-1 risk factors and appropriate HIV-1 screening.

Menstrual cycle

The impact of HIV-1 infection on the menstrual cycle remains unknown. There are reports of increased rates of dysmenorrhea, oligomenorrhea, amenorrhea or menorrhagia, but these have been discounted by other studies.[19] Ongoing prospective observational studies of the epidemiology, manifestations and progression of HIV-1 disease in women should address these issues.

PREVENTION

Contraception

Condoms are important for preventing the transmission of HIV-1 and other sexually transmitted diseases. However, alone they may not be adequate to prevent pregnancy. Reported breakage rates for male condoms are less than 2% for vaginal and anal intercourse. An evaluation of the effectiveness of the female condom at preventing pregnancy in 147 women over a 6-month period demonstrated an annual failure rate of 26%. Among 86 women who reported using the condom consistently and correctly, the annual failure rate was still 11%.[20]

Hormonal contraceptives should be considered in addition to condoms for women who have HIV-1 infection, are sexually active and wish to avoid pregnancy. In view of the wide array of potential drugs available for antiretroviral therapy and prevention and treatment of opportunistic infections, the medications of women receiving hormonal agents should be critically reviewed to avoid drug-drug interactions such as:

- those that might lead to a reduction in the efficacy of hormonal agents; and
- those that may reduce the efficacy or increase the toxicity of other drugs in the presence of hormonal agents.

In particular, protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs) can affect the levels of hormonal agents. Table 135.2 details these interactions and recommendations for their concomitant use.[21]

Pregnancy

In the USA, in 1993, the rate of HIV-1 infection among women of childbearing age was 1.7 per 1,000 and there were between 1000 and 2000 perinatally infected infants born to 6000–7000 HIV-1-infected women. UNAIDS estimates for 2001 are that, worldwide, 4.3 million children under 15 years of age have died of AIDS; another 2.7 million are currently living with HIV-1/AIDS, of whom 800,000 were infected in 2001.[2]

Perinatal transmission can occur in utero and during labor and delivery, as well as postpartum, primarily through breast-feeding.[22] [23] Excluding postpartum transmission through breast-feeding, data suggest that 80% of maternal-infant transmission occurs late in gestation or during labor and delivery.[24] Several studies have found that breast-feeding may increase the rate of transmission by 7–22% and thus breast-feeding is not recommended when safe alternatives are available. [23] The majority of infections transmitted through breast milk occur during the first few weeks or months of life, suggesting that postpartum maternal and/or infant antiretroviral therapy could further reduce transmission in breast-feeding populations.[25] Risk factors associated with transmission via breast-feeding include level of virus in the breast milk, mastitis, breast abscesses and maternal seroconversion during lactation.

TABLE 135-2 -- Drug interactions between antiretrovirals and oral contraceptives.*

DRUG INTERACTIONS BETWEEN ANTIRETROVIRALS AND ORAL CONTRACEPTIVES

Agent	Effect on oral contraceptive	Recommendation		
		No dose adjustment	No data	Use alternative agent or second method
Indinavir	Norethindrone levels ? 26% ethinylestradiol levels ?24%	X		
Ritonavir	Ethinylestradiol levels ?40%			X
Saquinavir			X	
Nelfinavir	Norethindrone levels ?18% ethinylestradiol levels ?47%			X
Amprenavir	Potential for interaction		X	X
Lopinavir	Ethinylestradiol levels ?42%			X
Nevirapine	Ethinylestradiol levels ?20%			X
Delavirdine			X	
Efavirenz	Ethinylestradiol levels ?37% no data on norethindrone levels			X
Recommended adjustments are listed.				

** Data from CDC.*[21]

TABLE 135-3 -- Maternal-infant transmission of HIV-1.

MATERNAL-INFANT TRANSMISSION OF HIV-1	
Maternal factors	**Labor and delivery factors**
Advanced maternal HIV disease	Chorioamnionitis
Maternal p24 antigenemia	Prolonged rupture of membranes (>4h)
Low maternal $CD4^+$ lymphocyte counts	Premature delivery before 34 weeks gestation
High maternal plasma HIV-1 RNA levels	Use of fetal scalp electrodes
Acute maternal HIV infection during pregnancy	Non-elective cesarean section delivery
Genital inflammation or maternal sexually transmitted disease at the time of delivery	Lack of antiretroviral therapy
Episiotomy with severe lacerations	
Detectable genital tract HIV-1 RNA near delivery	
Breast-feeding	
Maternal, and labor and delivery factors that increase the risk of transmission.[22] [26]	

A number of maternal and delivery factors appear to influence the risk of transmission and are listed in Table 135.3 .[22] [26] In addition, the rate of maternal-infant transmission has been reported to be increased when maternal virus exhibits rapid or high titer replication in human peripheral blood mononuclear cells, T cell line tropism or resistance to neutralization by maternal serum. Among twin births, the firstborn twin is at greater risk of acquiring HIV-1 infection because of more prolonged exposure to maternal blood and secretions. Interventions currently recommended to reduce transmission include:

- avoidance of invasive monitoring whenever possible;
- avoidance of breast-feeding;
- the use of zidovudine with or without other antiretroviral therapy during pregnancy for the mother and in the peripartum and postpartum period for the infant;
- treatment of sexually transmitted disease or vaginitis; and
- elective cesarean section if plasma HIV-1 RNA level remains above 1,000 copies/ml near term.

Recently the USPHS published revised guidelines on the use of antiretroviral therapy in pregnant women.[27] Since the results of AIDS Clinical Trials Group (ACTG) 076, which demonstrated a 66% reduction in maternal-infant HIV-1 transmission with zidovudine use in the mother (100mg orally 5 times per day initiated at 14–34 weeks gestation), during labor and delivery (2mg/kg intravenous loading dose then 1mg/kg continuous infusion until delivery) and in the baby (2mg/kg orally q6h for the first 6 weeks of life) compared with placebo (7.6% vs 22.6% transmission, respectively), advancements have occurred in our understanding of the pathogenesis of HIV-1 disease and its treatment and management.[28] The precise mechanisms by which zidovudine diminishes maternal-infant transmission of HIV-1 remain unclear but they are probably multifactorial and include decreased maternal plasma HIV-1 RNA levels, resulting in decreased exposure of the fetus to the virus in utero, or of the infant at delivery, or both, thereby preventing infection becoming established in the fetus or infant. Additional analyses have now shown that transmission of HIV-1 occurs at all levels of $CD4^+$ lymphocyte counts and even in some women who have undetectable plasma HIV-1 RNA levels, although overall the risk of transmission is greater in women who have lower $CD4^+$ lymphocyte counts and higher plasma HIV-1 RNA levels, particularly at the time of delivery. [29] Detectable virus in the female genital tract at 38 weeks gestation has also been independently associated with maternal-infant transmission of HIV-1.[30]

It is important to stress that even intervening late in pregnancy can diminish the risk of transmission. Epidemiologic data from New York State showed decreased transmission to the infant when intravenous zidovudine was begun during labor followed by 6 weeks of oral zidovudine for the infant compared with no therapy (10% vs 27% transmission, respectively).[31] A study in Thailand randomized 397 HIV-1-infected pregnant women to receive placebo or zidovudine (300 mg q12h orally from 36 weeks gestation until onset of labor

and 300 mg q3h orally from onset of labor until delivery).[32] There was an 18.9% transmission risk for women receiving placebo compared with a 9.4% transmission risk for women receiving zidovudine. Thus, transmission in this non-breast-feeding cohort was decreased by 50% with the use of a shorter course of zidovudine, oral dosing during labor and no infant treatment compared with a 66% reduction with the more complicated ACTG 076 regimen.

In an African breast-feeding cohort, the PETRA study compared a combination regimen of zidovudine and epivir of four varying durations in the mother and infant: (1) starting at 36 weeks gestation, orally intrapartum and for 1 week postpartum to the woman and infant; (2) starting during labor and for 1 week postpartum to the woman and infant; (3) during labor only to the woman; and (4) placebo.[33] Arms 1 and 2 reduced transmission to the infant by approximately 50% and 38%, respectively, as compared with the placebo arm. Arm 3 provided no reduction in transmission compared with placebo (16% vs 17%, respectively), suggesting that any potential benefit was negated by breast-feeding.

More recently, in Uganda, a two-dose nevirapine regimen was compared with a short-course zidovudine schedule.[34] At the onset of labor, women received either oral nevirapine, 200mg as a single dose, or oral zidovudine, 600mg loading dose followed by 300mg q4h until delivery. Infants

TABLE 135-4 -- Reverse transcriptase inhibitors.‡

REVERSE TRANSCRIPTASE INHIBITORS					
Agent	**Transmission to fetus prevented***	**FDA approved**		**FDA pregnancy category†**	**Placental transfer (%)**
		Neonates	**Children**		

Zidovudine	Yes	Yes	Yes	C	85
Didanosine	No	Yes	Yes	B	50
Lamivudine	Yes	No	=3 months	C	100
Stavudine	No	No	=1 months	C	76 (rhesus monkeys)
Zalcitabine	No	No	No	C	30–50 (rhesus monkeys)
Abacavir	No	No	=3 months	C	Yes (rats)
Nevirapine	Yes	No	=2 months	C	100
Delavirdine	No	No	No	C	?
Efavirenz	No	No	=3 years	C	100 (rhesus monkeys)
Tenofovir	No	No	No	B	Yes (rat, monkey)

‡ *Data from CDC.*[27]
* Randomized trial showing benefit.
† A, adequate and well controlled studies of pregnant women fail to demonstrate a risk to the fetus during the first trimester and no evidence of risk during later trimesters; B, animal studies fail to demonstrate a risk to the fetus but no adequate studies exist in pregnant women; C, animal studies demonstrate risk or have not been conducted, and safety in human pregnancy has not yet been determined — however, the benefit of the drug may still outweigh the risk; D, positive evidence of human fetal risk exists based on adverse reaction data but benefits may still outweigh the risks.

TABLE 135-5 -- Protease inhibitors.‡

PROTEASE INHIBITORS					
Agent	**Transmission to fetus prevented***	**FDA approved**		**FDA pregnancy category†**	**Placental transfer**
		Neonates	**Children**		
Nelfinavir	No	No	=2 years	B	Minimal
Indinavir	No	No	No	C	Minimal
Ritonavir	No	No	=2 years	B	Minimal
Saquinavir	No	No	No	B	Minimal
Amprenavir	No	No	=4 years	C	?
Lopinavir/ritonavir	No	No	= 6 months	C	?

‡ *Data from CDC.* [27]
* Randomized trial showing benefit.
† A, adequate and well controlled studies of pregnant women fail to demonstrate a risk to the fetus during the first trimester and no evidence of risk during later trimesters; B, animal studies fail to demonstrate a risk to the fetus but no adequate studies exist in pregnant women; C, animal studies demonstrate risk or have not been conducted, and safety in human pregnancy has not yet been determined — however, the benefit of the drug may still outweigh the risk; D, positive evidence of human fetal risk exists based on adverse reaction data but benefits may still outweigh the risks.

Infants received matched drug to their mothers, either oral single dose nevirapine 2mg/kg within 72 hours of birth or oral zidovudine 4mg/kg q12h for 7 days. In this breast-feeding population, the risk of HIV-1 transmission at birth was 10.4% in the zidovudine group and 8.2% in the nevirapine group. By 6–8 weeks postpartum, 21.3% of infants had acquired HIV-1 infection in the zidovudine group versus 11.9% in the nevirapine group (p = 0.0027). These data demonstrated that the two-dose nevirapine regimen reduced the risk of maternal-infant transmission by 47% compared with a truncated zidovudine regimen. Follow-up data from this study, however, documented the occurrence of K103N mutations in virus recovered from up to 15% of women who were randomized to nevirapine, a mutation conferring cross-class resistance to NNRTIs.[35] These data may have implications for future treatment of women who received this regimen.

All pregnant women should be offered HIV-1 testing. In general, current recommendations are to approach the treatment of the HIV-1-infected pregnant woman as if she were not pregnant and strive for maximal suppression of viral replication. Nevertheless, knowledge that limited data exist regarding toxicity of the 16 Food and Drug Administration (FDA)-approved antiretroviral agents to the developing fetus and the infant must also be taken into account. Table 135.4 and Table 135.5 summarize the current information known about reverse

transcriptase inhibitors and protease inhibitors with regard to FDA approval status, placental transfer and carcinogenicity data. To date, neither pre-term delivery nor birth defects have been clearly associated with any antiretroviral agent; however, data are limited, particularly for infants exposed to combination therapy. Only efavirenz should be avoided in the first trimester, because of evidence of teratogenicity in rhesus macaques at human doses. A French group reported eight cases of mitochondrial dysfunction among 1754 uninfected infants who received zidovudine or the combination of zidovudine and epivir in utero and/or after birth. Two of these children developed progressive neurologic dysfunction and died. This syndrome has not been detected in over 20,000 children born to HIV-1-infected women in the USA.

On the basis of this information, the USPHS has drafted the following guidelines for the treatment of HIV-1-infected pregnant women.[27]

If the woman has had no prior antiretroviral therapy, treatment should be based on standard indications. However, at a minimum the three-part zidovudine regimen as outlined in ACTG 076 should be administered. In a meta-analysis, antenatal antiretroviral prophylaxis primarily with zidovudine alone was shown to reduce transmission for women who have plasma HIV-1 RNA levels to below 1000 copies/ml at or near delivery compared with no antenatal therapy (1% vs 9.8%, respectively).[36] Many experts feel comfortable substituting zidovudine 300mg q12h orally for the maternal component. Combination therapy is recommended for women who have a plasma HIV-1 RNA level over 1000 copies/ml. Consideration can be given to delaying the initiation of therapy until after 10–12 weeks gestation, which is thought to be the critical time for fetal organogenesis.

If the HIV-1-infected pregnant woman is already on antiretroviral therapy when pregnancy is diagnosed, then therapy should in general be continued, even in the first trimester. If the decision is made to stop therapy during the first trimester owing to concerns about teratogenicity, all drugs should be stopped and then restarted simultaneously in order to minimize the development of resistance.

Zidovudine should be incorporated into the regimen whenever feasible; however, if resistance or intolerance precludes its use, zidovudine should still be administered intravenously intrapartum and to the baby as outlined above. Additionally, zidovudine and stavudine should not be co-administered. Some experts would also recommend zidovudine in combination with other antiretroviral agents for infants born to mothers who have known or suspected zidovudine resistance.

For women receiving standard antenatal antiretroviral therapy, the addition of the two-dose nevirapine regimen did not further reduce transmission rates and, as previously discussed, resulted in the development of mutations conferring resistance to NNRTIs.[37] [38] Thus, for women who do not achieve adequate viral suppression near delivery, cesarean section is recommended and the addition of the two-dose nevirapine regimen is not.

If an HIV-1-infected woman presents in labor and no prior antiretroviral therapy has been given, four antiretroviral treatment options are recommended:

- single-dose nevirapine 200 mg orally to the mother as soon as possible and nevirapine 2mg/kg orally to the infant at 48 hours of life;
- oral zidovudine and epivir to the mother until delivery followed by 1 week of zidovudine and epivir to the infant;
- intrapartum intravenous zidovudine until delivery, and zidovudine to the newborn for 6 weeks; or
- the two-dose nevirapine regimen combined with intrapartum intravenous zidovudine and 6 weeks of zidovudine for the newborn.

Infants born to HIV-1-infected mothers who have received no anti-retroviral therapy during pregnancy or intrapartum should still receive zidovudine for the first 6 weeks of life. Consideration may also be given to treating the infant with additional antiretroviral medications. The mother's therapy should be re-evaluated after delivery in both these situations.

Resistance testing is available to guide antiretroviral therapy choices. The International AIDS Society-USA Panel and Euro-Guidelines Group for HIV-1 Resistance recommend that all pregnant women who have detectable viremia have resistance testing performed prior to initiating or changing antiretroviral therapy. The USPHS recommends resistance testing for HIV-1-infected pregnant women based on standard indications, which include the following settings: acute infection, failing a current regimen, or high likelihood of having resistant virus based on community resistance patterns or known drug resistance in the woman's source partner. While underlying resistance can affect the ability to achieve maximal viral suppression, it is not clear that the presence of mutations increases the likelihood of transmission to the infant. Resistant virus has been transmitted to infants; however, women who have zidovudine resistance mutations have not consistently transmitted infection to their infants at higher rates and, in some cases where transmission has occurred, wild-type virus was transmitted. When less than potent antiretroviral regimens (zidovudine monotherapy or dual nucleoside analogues) are administered to pregnant HIV-1-infected woman because antiretroviral therapy is not indicated for the women herself or the woman chooses to minimize exposure to antiretroviral therapy during pregnancy, development of resistance can occur and can potentially limit future treatment options.

A meta-analysis of 15 prospective cohort studies, conducted in an era when pregnant HIV-1-infected women received no antiretroviral therapy or zidovudine monotherapy, showed that elective cesarean section decreased the risk of maternal-infant transmission of HIV-1 compared with other modes of delivery.[39] For women on zidovudine, transmission was 2% with elective cesarean section and 7.3% with other modes of delivery. Because transmission rates are expected to be below 2% for women on potent antiretroviral therapy with controlled viremia, the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice recommends cesarean section before the onset of labor for women who have plasma HIV-1 RNA levels above 1,000 copies/ml near term. The cesarean section should be performed at 38 weeks gestation without amniocentesis to assess for fetal lung maturity. For a scheduled cesarean section, intravenous zidovudine should be started 3 hours before surgery. Cesarean section has greater morbidity than vaginal delivery but current data suggest that HIV-1 infected women have similar complications to those in uninfected women.

Duration of ruptured membranes is also associated with an increased risk of transmission. For the first 24 hours of membrane rupture, a meta-analysis of the same 15 studies used to assess the benefit of cesarean section showed a 2% increase in transmission for every additional hour of membrane rupture.[40] Cleansing the birth canal with a 0.25% chlorhexidine solution q4h until delivery did not reduce transmission in one study, except when membranes were ruptured more than 4 hours before delivery.[41] Whether or not non-elective cesarean section to shorten the duration of ruptured membranes or labor will further reduce transmission rates is currently unknown.

Safety monitoring guided by the pregnant woman's specific antiretroviral therapy should be performed. Routine hematologic and hepatic enzyme monitoring is recommended for women on zidovudine. Women receiving nucleoside reverse transcriptase inhibitors (NRTIs) should also be assessed for development of lactic acidosis and hepatic steatosis with frequent liver enzyme and electrolyte

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monitoring in the third trimester. Recently, cases of fatal lactic acidosis have been reported in pregnant women receiving prolonged courses of didanosine and stavudine. When other alternatives are available, this combination should be avoided during pregnancy. Women on protease inhibitors should be monitored for development of hyperglycemia. In general, the $CD4^+$ lymphocyte count and plasma HIV-1 RNA level should be monitored every 3–4 months. A plasma HIV-1 RNA level should be obtained at 34–36 weeks gestation to guide decisions on mode of delivery. A level II ultrasound is recommended to assess the fetus for women on combination antiretroviral therapy.

In the future, ongoing or planned studies will certainly provide more information regarding the use of the newer NRTIs, NNRTIs, nucleotide analogues, protease inhibitors and combination regimens for the treatment of pregnant women and the prevention of transmission to the fetus/infant. Studies are also ongoing to assess the feasibility of rapid HIV-1 testing for women who present in labor with unknown HIV-1 status. In the meantime, HIV-1-infected pregnant women should be referred for participation in clinical trials whenever possible and be reported to the appropriate agencies that collect safety and teratogenicity data. In the USA, the Antiretroviral Pregnancy Registry can be reached at www.APRegistry.com.

In general, pregnant women who have HIV-1 infection should receive prophylaxis for opportunistic infections appropriate for their stage of disease. [18] For *Pneumocystis carinii* pneumonia prophylaxis, some experts recommend avoiding trimethoprim-sulfamethoxazole and dapsone in the first trimester, and trimethoprim-sulfamethoxazole close to term to reduce the risk of kernicterus in the infant. Aerosolized pentamidine can be substituted during these time periods. While rifabutin and the macrolides have not been studied in pregnant women, those at high risk of disseminated *Mycobacterium avium* complex disease may benefit from the use of these medications after the first trimester. Azithromycin is favored owing to its safety profile because clarithromycin is teratogenic in animals. Pregnant women who have a positive purified protein derivative skin test for tuberculosis should also receive isoniazid prophylaxis after the first trimester. Pregnant women should also avoid eating raw or undercooked meat and avoid contact with cat feces to diminish the risk of toxoplasmosis. Good handwashing techniques should be employed for prevention of cytomegalovirus (CMV) disease, particularly in women who are health care workers or who have children in day care settings.[18] For CMV-seronegative women who require blood transfusions, only CMV-seronegative blood products should be used. [18]

Several studies have now demonstrated no adverse effect of pregnancy on the progression of HIV-1 disease for women who have $CD4^+$ lymphocyte counts above 200 cells/µl.[42] Unfortunately, women who have more advanced HIV-1 disease may not tolerate pregnancy as well and may have a higher rate of spontaneous abortion, prematurity, low birth weight infants and other complications of pregnancy.[43]

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Chapter 136 - Practice Point

Diagnosis of HIV in newborns

Peter L Havens

In the absence of intervention, children born to women who have HIV infection have about a 25% chance of being infected with HIV. Children born to women who have HIV infection need:

- therapy to prevent HIV infection;
- testing to identify infected infants; and
- institution of therapy to prevent *Pneumocystis carinii* pneumonia (PCP).

Pathogenesis

Perinatal transmission of HIV occurs in utero (about 25–30% of perinatal infections) and in the peripartum period (about 70%). Breast-feeding results in late postnatal HIV transmission in another 14–16% of infants.

Clinical features

Because many children who have HIV infection remain asymptomatic for long periods of time, the clinical examination is not a sensitive test for the presence of infection.

Diagnosis

In adults and children older than 18 months of age, HIV infection is diagnosed using an enzyme-linked immunosorbent assay (ELISA) to screen blood for the presence of antibody to HIV. A single positive ELISA should be repeated, and repeatedly positive tests should be confirmed by Western blot for HIV-specific IgG antibody.

Children born to women who have HIV infection passively acquire maternal IgG antibody to HIV, which can make the HIV IgG antibody ELISA and Western blot reactive for up to 18 months after birth, even in infants not infected with HIV. Therefore, positive tests for IgG antibody to HIV do not confirm the diagnosis of HIV infection in children less than 18 months of age but rather identify infants who are at risk of being infected. However, two or more negative HIV IgG antibody tests (ELISA) performed with an interval of at least 1 month in children over 6 months of age can be used to 'reasonably exclude' HIV infection among children without clinical evidence of HIV disease or previous positive laboratory evidence of HIV infection, such as HIV DNA polymerase chain reaction (PCR) or culture.

Immunoglobulin A does not cross the placenta as readily as IgG, and immunoassays for HIV-specific IgA have been developed to allow serologic diagnosis of children born to women who have HIV infection. Although these assays are 89–100% specific by 1–2 months of age, false-positive tests in the first week of life and low sensitivity as late as 6 months of age limit their usefulness in clinical practice.

Culture of HIV has been considered the gold standard for diagnosis of HIV infection in infants less than 18 months of age. Because up to 7% of children who are later found to be uninfected may have a positive culture within the first 6 months of life, at least two positive cultures are necessary to confirm the diagnosis of HIV infection in infants. Cultures are difficult and costly to perform, they can only be performed in special laboratories and they take a long time to produce a result; therefore, their clinical practicality is limited.

Polymerase chain reaction for HIV DNA is the most rapid and accurate method for identification of HIV infection in children less than 18 months of age. Sensitivity is 38% on the day of birth, 93% by day 14 of life and 96% by day 28. False-positive results are possible from laboratory error but two tests performed on separate samples are 98.5% accurate in identifying infection status and using three tests performed after 1 month of age achieves almost 100% accuracy.

Infants infected with HIV in utero have a positive HIV DNA PCR at birth and are at high risk of rapidly progressive symptomatic HIV infection. Infants infected in the peripartum period have negative HIV DNA PCR in the first few days of life, but in those infants HIV DNA PCR tests may be positive by as early as 2 weeks of age and 96% are positive by 1 month of age. Infants infected by breast-feeding may have negative HIV DNA PCR until after 6 months of age (Table 136.1).

Polymerase chain reaction for HIV RNA may be more sensitive than DNA in very young infants, and two positive HIV RNA PCR tests performed on separately collected blood samples are diagnostic of HIV infection in infants and children. HIV RNA PCR is not routinely recommended for screening at-risk infants because negative HIV RNA PCR testing does not exclude HIV infection. However, for infants infected with non-clade B HIV isolates, HIV RNA PCR using methods developed to include non-clade B RNA sequences may be preferable to standard HIV DNA PCR, which is less sensitive for identifying non-clade B virus.

Measurement of HIV p24 antigen in blood is not sensitive enough to be used for early diagnosis of HIV infection in children, even if immune complex dissociated methods are used for sample preparation. However, repeatedly positive HIV p24 antigen tests are diagnostic of HIV infection.

Management

The details of management of infants born to women who have HIV infection are shown in Table 136.2 .

TABLE 136-1 -- HIV infection and usual pattern of test results in newborns of women who have HIV infection.

HIV INFECTION AND USUAL PATTERN OF TEST RESULTS IN NEWBORNS OF WOMEN WHO HAVE HIV INFECTION							
HIV infection category		**Test**	**Infant's age at time testing performed and usual result of testing**				
			Day of birth	**1 month**	**4 months**	**>6 months**	**18 months**
Not infected	Seroreverter (nontransmitted maternal infection)	ELISA/Western blot	+	+	+	+/-	-
		HIV DNA PCR	-	-	-	-	-

Infection	In utero	ELISA/Western blot	+	+	+	+	+
		HIV DNA PCR	+	+	+	+	+
	Perinatal	ELISA/Western blot	+	+	+	+	+
		HIV DNA PCR	-	±	+	+	+
	Via breast milk	ELISA/Western blot	+	+	+	+	+
		HIV DNA PCR	-	-	±	+	+

TABLE 136-2 -- Laboratory monitoring and treatment of infants born to women who have HIV infection.

LABORATORY MONITORING AND TREATMENT OF INFANTS BORN TO WOMEN WHO HAVE HIV INFECTION		
Age	**Category of intervention**	**Test or treatment indicated**
Newborn	Evaluation	ELISA for antibody to HIV (and Western blot if ELISA is positive); a well documented history of HIV infection in mother can replace ELISA testing in the newborn
		HIV DNA PCR
		Complete blood count with differential, alanine transaminase, bilirubin and glucose if mother taking protease inhibitors during pregancy
	Treatment	Zidovudine for prophylaxis against vertical transmission (see Chapter 134)
		Advise against breast-feeding if safe alternatives are available
4 weeks	Evaluation	HIV DNA PCR
	Treatment	Continue zidovudine; hematocrit (optional; to check for anemia from azidothymidine)
6 weeks	Treatment	Stop zidovudine (assuming the HIV DNA PCR at birth and 4 weeks are negative)
		Begin treatment with trimethoprim-sulfamethoxazole for prophylaxis against Pneumocystis carinii pneumonia
4 months	Evaluation	HIV DNA PCR (if earlier testing negative)
	Treatment	Stop treatment with trimethoprim-sulfamethoxazole if the 4-month HIV DNA PCR returns and is negative, but continue if HIV DNA PCR test is positive
18 months	Evaluation	ELISA for antibody to HIV (Western blot confimation if ELISA positive); if negative, in the presence of previously negative HIV DNA PCR test and absence of symptoms of HIV infection, then the patient is a 'seroreverter', and HIV infection is definitively excluded at the present time; if positive, repeat
HIV culture may be used in place of HIV DNA PCR testing. Any positive culture or HIV DNA PCR is repeated as soon as possible to confirm the diagnosis. Patients diagnosed with HIV infection are treated as outlined in Chapter 134 .		

Prevention of perinatal transmission (see Chapter 135)

One regimen to prevent perinatal transmission of HIV includes 6 weeks of zidovudine therapy in the newborn infant. Breast-feeding increases the risk of perinatal transmission of HIV, so, in areas of the world where safe alternatives to breast-feeding are readily available, women who have HIV infection should be advised not to breast-feed their infants.

Prophylaxis of *Pneumocystis carinii* pneumonia

All children born to women who have HIV infection should be started on PCP prophylaxis at 4–6 weeks of age, regardless of $CD4^+$ T cell count or HIV infection status. The most common drug regimen for prophylaxis is trimethoprim-sulfamethoxazole, trimethoprim 150mg/m^2 /day and sulfamethoxazole 750mg/m^2 /day q12h and given 3 days weekly (see Chapter 134 , Fig. 134.14). For children in whom HIV infection is reasonably excluded, PCP prophylaxis can be discontinued. Prophylaxis against PCP should be continued until at least 12 months of age in children found to be infected with HIV, independent of their $CD4^+$ T cell count.

Follow-up visits

A baseline visit with the primary care provider is recommended. Follow-up visits at 2- to 3-month intervals to primary practitioners are adequate for most children, with timing determined in part by the immunization schedule in infancy. Diagnostic testing for HIV follows the schedule outlined in Table 136.2 . An office visit is not necessary each time laboratory testing is performed.

The uncertainty of an indeterminate infection status is difficult for families. An initial visit or discussion with personnel experienced in the care of children who have or who are at risk for HIV infection can give families an opportunity to learn about the illness and the follow-up

needed. Children who are found to be infected with HIV should follow the care recommendations outlined in Chapter 134 .

Further reading

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Chapter 137 - Diagnostic Tests for HIV Infection and Resistance Assays

Daniel R Kuritzkes

INTRODUCTION

Since the first human immunodeficiency virus (HIV-1) was isolated in 1983 and the first antibody detection tests were marketed in 1985, laboratory tests for the diagnosis and monitoring of HIV infection have evolved constantly. Technologic advances have led to the development of clinical assays that permit the precise quantification of plasma virus levels, detect the presence of key drug resistance mutations or generate recombinant viruses for phenotypic drug resistance testing. Indeed, few areas in medicine have witnessed such rapid and widespread adaptation of molecular biologic tools to everyday patient management.

DIAGNOSTIC TOOLS FOR HIV INFECTION

Serologic assays

Assays have been developed for detection of HIV antibodies in serum, whole blood, saliva, urine and dried blood collected on filter paper.

Enzyme-linked immunosorbent assay

Most laboratories screen for anti-HIV-1 and anti-HIV-2 antibodies by means of an enzyme-linked immunosorbent assay (EIA) based on antigens that consist of viral lysates or recombinant or synthetic proteins corresponding to the immunodominant epitopes from two HIV-1 subtype B variants (LAI and MN strains) and HIV-2 subtype A (ROD strain). These tests are therefore able to detect anti-HIV-1 and anti-HIV-2 antibodies. Current antigen sandwich EIAs have a sensitivity and specificity that approach 100%.[1] In contrast to earlier tests, which detected only IgG antibodies, third-generation HIV EIAs detect all classes of anti-HIV antibodies, considerably shortening the time to diagnosis following acute infection. However, these assays may fail to detect antibodies to the highly divergent HIV-1 subtypes from groups N and O.

Rapid tests

Diagnostic tests based on red cell or particle agglutination as well as dot blot assays have been developed that permit rapid diagnosis of HIV-1 infection. In laboratory comparisons, sensitivity and specificity are similar to those of the EIA, but performance may be somewhat lower in the field.[2] The simplicity and wide operating temperature of these tests make them ideally suited for use in resource-poor settings. Rapid tests are also useful for diagnosis of HIV infection in women during labor and delivery, and to establish the infection status of source patients following an occupational needlestick injury.

Home testing

'Home testing' for HIV infection in fact refers to home collection of a fingerstick blood sample, which is applied to a filter paper, dried and mailed directly to a central laboratory for analysis. Anonymity is preserved by the use of code numbers to identify specimens and their senders. Results and counseling are provided over the telephone. In the USA, fewer than 1% of samples submitted for testing during the first year of availability were positive, suggesting that these tests serve primarily to reassure the worried well.

Western blot

Despite a specificity of >99%, a reactive HIV EIA has a relatively low positive predictive value when applied to populations at low risk for HIV infection. Thus, a confirmatory test is essential to exclude false-positive results. The Western blot (WB) method is currently the most widely used. In a WB, viral proteins are separated by polyacrylamide gel electrophoresis and transferred by blotting onto a nitrocellulose strip. The strips are then reacted with the test serum to determine which, if any, viral proteins are recognized by patient antibodies. Figure 137.1 shows a typical WB positive for HIV-1, with the different reactive proteins. Alternatively, immunoblotting uses recombinant HIV-1 or HIV-2 proteins or synthetic peptides applied as individual strips or spots to a plastic support. Immunoblots are simpler to standardize than Western blots and are more sensitive in cases of recent seroconversion, but are more expensive. Because they use a limited number of synthetic proteins, immunoblots are less likely to detect antibodies to uncommon HIV-1 subtypes.

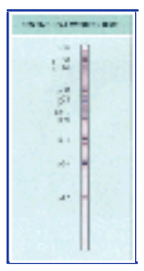

Figure 137-1 Positive HIV-1 Western blot. The binding of the patient's antibodies to viral antigens coated on the strip is revealed by an enzyme-labeled antihuman globulin. gp160, gp120 and gp41 are *env* gene products. p55, p24 and p17 are *gag* gene products. p68, p52 and p34 are *pol* gene products. MW, molecular weight of the viral proteins.

Strain serotyping

Differentiating between HIV-1 and HIV-2

Serologic differentiation is based on the detection of specific antibodies against the HIV-2 transmembrane protein (gp36) by Western blot or immunoblot assay. Monospecific EIAs for HIV-2 also are available for differentiation. Epitope differences between the immunodominant regions of HIV-1 and HIV-2 generally are sufficient for adequate serologic differentiation.

Serotypic differentiation of HIV-1 group M subtypes

The use of peptides corresponding to the V3 loop of the different group M subtypes can identify the infecting subtype with acceptable predictive value relative to genotyping. Alternatively, competitive peptide EIAs can be used. However, phylogenetic analysis of *pol* sequences obtained at the time of genotypic resistance testing (see below) offers a more convenient approach to subtype identification.

HIV isolation

HIV can be isolated from blood, plasma, cerebrospinal fluid (CSF), genital secretions or tissue by co-culture with lymphocytes from a seronegative donor that have

been stimulated with phytohemagglutinin and interleukin-2 before use. Viral replication is revealed by p24 antigen production in the culture supernatant. A positive culture provides direct evidence of HIV-1 infection, but virus culture is rarely necessary to establish a diagnosis. Morever, HIV culture has been supplanted for diagnostic purposes by polymerase chain reaction (PCR)-based assays; its use is limited primarily to specific research applications.

Virus isolation is successful in more than 95% of individuals infected with HIV-1, but sensitivity is lower in patients with higher $CD4^+$ cell counts. For a given $CD4^+$ cell count, the rate of HIV-2 isolation is significantly lower.[3] CD8 T cells that are present in peripheral blood mononuclear cells (PBMCs) exert an antiviral effect and may prevent outgrowth of virus in vitro. Therefore, it is often necessary to remove these cells in vitro in order to recover virus from patients on effective antiretroviral therapy.

Tropism

It has long been known that HIV-1 strains isolated from patients with AIDS can be more virulent in vitro than those isolated from asymptomatic persons. These strains, found in the later stages of immunodepression, are termed 'T lymphotropic' because they are able to multiply in T-lymphocyte lines and induce syncytium formation

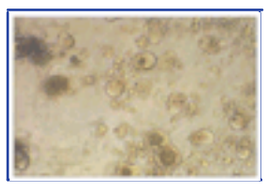

Figure 137-2 Syncytia formed by infection of MT-2 cells with an X4 (syncytium-inducing) isolate of HIV-1.

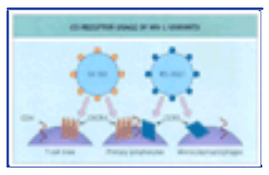

Figure 137-3 Relationship between co-receptor usage and cellular tropism of HIV-1.

in the MT2 cell line (Fig. 137.2). Syncytium-inducing strains are observed in up to 50% of patients in the later stages of infection. Emergence of SI variants is associated with an accelerated decline in $CD4^+$ lymphocyte counts.[4] Nearly all strains isolated during seroconversion usually are non-SI and macrophage tropic and are incapable of multiplying in T-cell lines.

A biologic basis for the switch in tropism associated with the SI phenotype was provided by discovery of the chemokine co-receptors for HIV. CCR5 is expressed by monocytes and primary T cells, but expression of this co-receptor is lost by T-cell lines. Conversely, CXCR4 is expressed by activated T cells and T-cell lines, but not by monocytes. Lymphotropic (SI) strains preferentially use the CXCR4 co-receptor, whereas macrophage-tropic (non-SI) strains use the CCR5 co-receptor (Fig. 137.3). Amino acid differences in certain hypervariable regions of gp120 determine co-receptor specificity. Consequently, SI strains now are referred to as X4 strains (because they use the CXCR4 co-receptor) and non-SI strains as R5 strains (because they use the CCR5 co-receptor). Occasionally duotropic (R5/X4) viruses are isolated. These viruses are able to use both CCR5 and CXCR4 co-receptors and can infect monocytes and $CD4^+$ lymphocytes with similar efficiency. It is thought that R5/X4 viruses represent intermediate forms in the evolution of SI viruses from non-SI strains.

At present, characterizing the co-receptor usage of patient isolates has little clinical utility and tests to distinguish R5 and X4 viruses are not routinely available. However, such knowledge might become important if HIV-1 entry inhibitors that specifically inhibit CCR5 or CXCR4 binding are developed for treatment of HIV-1 infection.

Viral antigen detection

Circulating HIV-1 capsid (p24) antigen can be detected by qualitative or quantitative antigen-capture EIA. High titers of p24 antigen are present during acute infection prior to seroconversion. Subsequently, p24 antigen is complexed with p24-specific antibodies and becomes undetectable in most asymptomatic patients. With advancing disease, p24 antibody titers fall and the antigen once again becomes detectable (a poor prognostic sign). Qualitative assays for p24 antigen are useful in diagnosing HIV-1 infection prior to seroconversion, but quantitative assays have been replaced by more sensitive viral RNA assays. A modified assay that dissociates p24 antigen-antibody complexes by heat treatment may provide an affordable alternative to RNA assays for quantifying plasma viremia in resource-poor countries.[5]

Viral nucleic acid detection

HIV-1 DNA polymerase chain reaction

Presence of integrated proviral HIV-1 DNA can be detected by qualitative PCR. Conserved sequences in *gag* or *pol* are amplified and the PCR product is detected by hybridization using an enzyme- or radiolabeled oligonucleotide probe. Under appropriate conditions, experienced laboratories can achieve a sensitivity and specificity of 100% in detecting subtype B virus.[6] However, sensitivity is lower in individuals with higher $CD4^+$ cell counts due to the lower titer of circulating infected PBMCs, and in patients with nonsubtype B infection. HIV-1 DNA PCR assays are used almost exclusively for early diagnosis of infection in neonates. These tests have limited utility in adults, but occasionally may be useful in resolving cases in which results of serologic tests are ambiguous.

Quantitative HIV-1 RNA assay

The development of sensitive and precise assays for quantifying HIV-1 RNA in plasma led to novel insights into HIV-1 pathogenesis and helped establish complete suppression of detectable virus replication as the appropriate goal of antiretroviral therapy. Use of these assays is now standard in the management of HIV-1-infected patients in the developed world.

Several different assay formats have been developed for HIV-1 RNA quantification. In PCR-based assays (Amplicor HIV-1 Monitor, Roche Molecular Systems), HIV-1 RNA is converted into DNA by reverse transcription followed by PCR amplification of the DNA. The PCR product is detected by hybridization to an enzyme-conjugated probe specific for HIV-1, and quantified by reacting bound probe with a substrate that undergoes a color change, as in an ELISA. The branched DNA assay (Quantiplex HIV-1 RNA, Bayer Nucleic Acid Diagnostics) uses nonenzymatic means to amplify the signal from HIV RNA. In this assay, viral RNA is 'captured' by hybridization to complementary oligonucleotides that are bound to the wells of a microtiter plate. The captured viral RNA target is then hybridized to branched oligonucleotides (hence the name 'branched' DNA assay), which in turn are hybridized to enzyme-conjugated oligonucleotides that can be quantified as above. The NASBA assay (HIV-1 RNA QT, Organon-Teknika) is similar in concept to the RT-PCR assay except that reactions occur at one temperature. A fourth assay, based on DNA hybridization and colorimetric detection (Digene Diagnostics), has been developed, but is not yet widely available.

Performance characteristics of the three commercially available assays are similar (Table 137.1), and studies have demonstrated the excellent correlation between plasma RNA titers in a given plasma sample tested by the three techniques. All three assays have a lower limit of quantification of approximately 50–80 copies/ml. Assay sensitivity can be extended by concentrating plasma virus from

TABLE 137-1 -- Performance characteristics of plasma HIV-1 RNA assays.

PERFORMANCE CHARACTERISTICS OF PLASMA HIV-1 RNA ASSAYS			
	RT-PCR (Roche)	**bDNA (Bayer)**	**NASBA (Organon Teknika)**
Linear range (copies/ml)	$400–8 \times 10^5$	$75–5 \times 10^5$	$80–4 \times 10^7$
Interassay variation (log_{10} SD)	0.12–0.22	0.05–0.17	0.038–0.261
Specimen volume	200µl	2ml	100µl–1ml
Preferred anticoagulant	EDTA/ACD	EDTA	EDTA/ACD/HEP

ACD, acid citrate dextran; EDTA, ethylenediaminetetra-acetic acid; HEP, heparin
The range of the 'ultrasensitive' RT-PCR assay is 50–50,000 copies/ml.

larger sample volumes. Although detection limits of 3–5 copies/ml are achievable by such means, these assays are much less precise at plasma HIV-1 RNA titers below 200 copies/ml.[7] Once infection becomes established, steady-state plasma virus levels are relatively stable, varying by 0.3–0.4 $\log_{10}$ copies/ml over weeks to months. Given these factors, changes of greater than 0.5–0.7 $\log_{10}$ (3- to 5-fold) are likely to reflect significant changes in HIV-1 replication.[8]

Although most strains of HIV-1 that circulate in North America belong to subtype B, more than 10 different subtypes are found around the world. The HIV-1 Monitor 1.0 (RT-PCR) assay is significantly less sensitive for detecting HIV-1 from subtypes A, E and F as compared with the Quantiplex version 3.0 (bDNA) assay.[9] Plasma HIV-1 RNA levels that appear to be lower than expected in a patient with advanced disease can be a clue to infection with a nonsubtype B strain. Incorporation of alternative primer sets in the new version of the HIV-1 Monitor assay (version 1.5) has improved the ability of this assay to detect diverse HIV-1 subtypes.

Drug resistance assays

A variety of assays are available for assessing drug resistance, including:

- genotypic assays, in which nucleotide sequencing of viral genetic material is used to detect the presence or absence of critical drug resistance mutations; and
- phenotypic assays, in which the concentration of drug necessary to inhibit virus replication in vitro is estimated in a drug susceptibility assay.

Each method has potential advantages and disadvantages. An important limitation of both approaches is that they provide a measure of the characteristics of the predominant viral species but do not indicate the presence of minor species that may emerge as resistant variants during subsequent treatment (Table 137.2).

Genotypic tests of HIV-1 drug resistance

Several approaches to genotyping are available, ranging from full-length sequencing of the target gene to point mutation assays, which focus only on a particular mutation of interest. The most commonly used genotypic assays rely on automated DNA sequencing. Using this technique, the nucleotide sequence of some or all of the gene of

TABLE 137-2 -- Comparison of genotypic and phenotypic drug resistance tests for HIV.

COMPARISON OF GENOTYPIC AND PHENOTYPIC DRUG RESISTANCE TESTS FOR HIV		
Type of assay	**Advantages**	**Disadvantages**
Genotypic assays	Rapid turn-around time	Genotype may not correlate with phenotype
	Appearance of resistance mutations may precede change in phenotype	Require expert interpretation
	Widely available	Fail to detect minor species
		Unable to assess mutational interactions
Phenotypic assays	Direct measure of viral drug susceptibility	Cost
		Longer turn-around time
	Assess net effect of mutational interactions and cross-resistance patterns	Fail to detect minor species
		Appropriate cut-offs not defined for all drugs

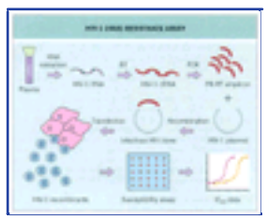

Figure 137-4 HIV-1 drug resistance assay. RT, reverse transcription reaction; cDNA, complementary DNA; PR-RT, protease and reverse transcriptase gene; IC_{50} , 50% inhibitory concentration.

interest (e.g. protease (PR) or reverse transcriptase (RT)) is obtained, then translated into the predicted amino acid sequence in order to determine whether specific mutations are present or absent. Automated sequencing offers the most complete data on viral genotype, but generates more information than is needed for most clinical purposes. For example, HIV-1 RT has 550 amino acids, but mutations at only a small number of these positions are implicated in drug resistance. Therefore, interpretation of the genotype is needed in order to help distinguish which changes are merely polymorphisms and which might be significantly associated with drug resistance.

In most commercially available genotypic tests, viral RNA is extracted from a sample of plasma and reverse transcribed into complementary DNA in the laboratory (Fig. 137.4). The PR- and RT-coding regions of the cDNA are then amplified by PCR, and the nucleotide sequence of the PCR product is determined on an automated DNA sequencer. Some laboratories use specific diagnostic kits that provide standardized reagents needed for the RT-PCR and DNA sequencing steps. Generally, these kits are part of an HIV genotyping system that includes equipment for running the sequencing assays and software for interpreting assay results. The TRUGENE HIV-1 genotyping kit and OpenGene DNA sequencing system and the ViroSeq HIV-1 Genotyping System are approved for clinical use by the US Food and Drug Administration (FDA) in conjunction with the appropriate FDA-cleared interpretation algorithm; approval of the PE/Applied Biosystems HIV-GT kit is pending. Other laboratories employ so-called 'home brew' assays using reagents, primers and interpretive algorithms developed individually by each laboratory.

Other types of genotypic resistance assays, such as the line probe assay (LiPA), differential probe hybridization assays or real-time PCR assays using selective PCR primers, are designed to provide more limited information by testing for the presence or absence of specific mutations at particular codons. These assays are faster than standard genotypic tests and may be more sensitive at detecting minor species. However, because these tests do not generate a comprehensive sequence, information needed to interpret complex genotypes might be missing. Furthermore, the tests must be reconfigured to include important new mutations as they are defined.

The frequency of false-positive and false-negative results is <1% for genotypic assays when assessed using samples that carry predominantly mutant or predominantly wild-type virus populations. However, sensitivity for detecting presence of a mutation is variable when both wild-type and mutant viruses are present as a mixture. In general, mutant species must constitute 10–20% of the population to be detected by standard sequencing methods. Some mutations may go undetected unless they are present as the majority species.

Phenotypic tests of HIV-1 drug resistance

Drug susceptibility tests with HIV-1 usually are performed using a recombinant virus assay, in which the viral genes of interest (e.g. PR and RT) are introduced into a plasmid that carries all of the other viral genes needed for replication in cell culture (see Fig. 137.4).[10] [11] Modification of the assay allows introduction of the integrase (IN) or envelope (ENV) genes in order to determine susceptibility to integrase inhibitors and entry inhibitors, respectively. Using these assays, small differences in susceptibility can be detected (~2- to 4-fold compared with control). Phenotypic assays are more complex and labor intensive than genotypic assays. Automation makes it possible to test many samples simultaneously, and allows for high throughput. However, the cost and complexity of the automation limit availability of these assays to a few reference laboratories.

Viral fitness and replication capacity assays

Accumulation of drug resistance mutations can decrease the replication capacity of the virus. In the absence of drug, resistant variants are significantly less fit than wild-type and are replaced by wild-type virus if antiretroviral therapy is interrupted.[12] Viral fitness can be assessed by growth competition assays, in which the relative replication of two or more viral species is tested in the same culture.[13] Alternatively, viral replication capacity can be measured by a modification of the phenotypic resistance assay. At present, the clinical utility of these assays remains undefined.

Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is used to maintain plasma levels of a drug within a defined therapeutic range in order to maximize efficacy and minimize toxicity. Plasma concentrations of antiretroviral drugs following administration of a standardized dose show considerable interpatient variation. In theory, TDM of antiretroviral agents could lead to dosage adjustments that would correct for this variation. Although measuring the plasma concentration of most antiretroviral drugs is relatively straightforward, establishing a therapeutic range is more difficult. Consequently, the data needed to guide dosage adjustment for currently available antiretroviral drugs given a particular plasma concentration are lacking. Moreover, in the case of nucleoside RT inhibitors, concentration-response relationships have not been demonstrated consistently. Nevertheless, TDM is likely to prove useful for non-nucleoside RT inhibitors and protease inhibitors, and efforts to generate the information needed to establish clinical utility of TDM are underway.

CLINICAL USE OF HIV DIAGNOSTIC TESTS

Diagnosing acute HIV-1 infection

After exposure to the virus, HIV RNA can be detected from day 12 and p24 antigen from days 14–16 (Fig. 137.5). Detection of HIV-1 p24 antigen has a sensitivity of approximately 90% and a specificity of 100% in the diagnosis of acute infection.[14] HIV-1 RNA assays have a sensitivity of 100% in diagnosing acute infection, but at the cost of lowered specificity (97%). Because of the potential social and legal ramifications of a false-positive HIV-1 test, and the need to perform additional testing to clarify a patient's HIV infection status, some experts prefer p24 antigen assays in this setting. However, false-positive HIV-1 RNA tests usually have titers less than 10,000 copies/ml, whereas HIV-1

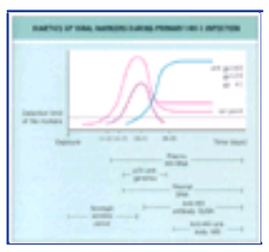

Figure 137-5 Kinetics of viral markers during primary HIV-1 infection. The first positive viral marker is plasma RNA 11–12 days after infection. p24 Antigenemia is detectable on day 14 or 15. The first anti-HIV antibodies are detectable by third-generation ELISAs on days 20–21. (Pink, plasma HIV RNA; purple, p24 antigenemia; blue, anti-HIV antibody).

TABLE 137-3 -- Diagnosis of acute and early HIV infection.

DIAGNOSIS OF ACUTE AND EARLY HIV INFECTION	
EIA negative	• p24 Antigen positive, or
	• Plasma HIV-1 RNA >10,000 copies/ml
EIA positive	• Evolving WB pattern, and/or
	• EIA-negative by 'detuned' assay

RNA levels generally exceed 100,000 copies/ml in primary infection. Thus, plasma HIV-1 RNA testing might be used in selected cases where a history of recent exposure and symptoms consistent with acute HIV-1 infection provide a high index of suspicion (Table 137.3).

The first antibodies are detectable on day 21. However, the kinetics of these markers can vary according to the patient and infecting strain; antibodies against non-B subtypes may be less well recognized early after infection.[15] It is generally agreed that beyond week 6 after infection antibodies are detectable in almost all patients. Reactivity by WB lags behind seroconversion by EIA. Therefore, a positive EIA and negative or evolving WB can provide evidence of recent infection, particularly if antibodies directed against gag proteins are present (Fig. 137.6). A fully reactive WB usually develops within 6 months.[16] Recent seroconverters can also be identified by use of a less sensitive ('detuned') third-generation EIA. Patients infected within 5–6 months prior to testing are reactive on the sensitive EIA but nonreactive on the detuned EIA. A two-step algorithm using a sensitive/less sensitive EIA testing strategy can correctly identify nearly all recent seroconverters.[15]

Diagnosing chronic HIV-1 infection

Testing for HIV antibodies is a two-stage process: samples that test positive by an initial screening assay are retested to exclude clerical

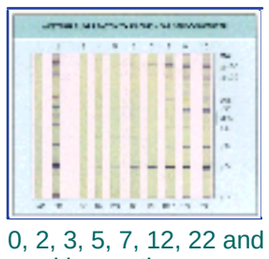

Figure 137-6 Western blot reactivity in one HIV-1 seroconverter. Lanes 1 and 2, negative (NC) and positive controls (PC). Lanes 3–10, serial samples collected on days (D) 0, 2, 3, 5, 7, 12, 22 and 30. Day 0 corresponds to the first collected sample. Anti-p24 was the first antibody detected, rapidly followed by anti-gp160, p55, p40 and gp120. Later, gp41 and p18 are weakly reactive.

or laboratory error and a confirmatory assay is performed on repeatedly reactive sera to verify that the antibodies are directed against HIV antigens. Screening in industrialized countries generally is based on the EIA and in most resource-poor countries on rapid kits. In some countries (France, Switzerland) two different kits must be combined for screening purposes to reduce the risks linked to human error and the possible failure of one test. The possibility of false positivity means that positive screening tests must always be confirmed with highly specific tests.

The WB is the most commonly used confirmatory test, although indirect immunofluorescence assays are used occasionally. Confirmation of a reactive EIA by positive WB establishes the diagnosis of HIV infection. A negative WB suggests a false-positive EIA or acute infection (see above). Criteria for interpretation of HIV-1 WBs have been developed by several groups, but no uniform standard has been adopted (Table 137.4).[17]

TABLE 137-4 -- Criteria for Western blot interpretation.

CRITERIA FOR WB INTERPRETATION	
Organization	**Criteria**
American Red Cross	At least one band from each structural gene product (*gag*, *pol* and *env*)
US Centers for Disease Control and Prevention and Association of State and Territorial Public Health Directors	Reactivity to any two of p24, gp41 or gp120/160
Directors	

Consortium for Retrovirus Serology Standardization	p24 or p31 and one of gp41 or gp120/160
Du Pont	p24 and p31 and gp41 or gp120/160
World Health Organization	Reactivity to two envelope glycoproteins

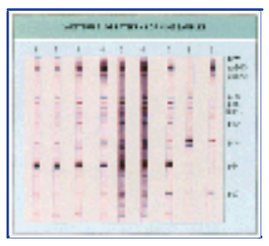

Figure 137-7 Different Western blot patterns of nine samples. Samples 1, 2 and 3 are from recent seroconverters infected by HIV-1 subtype B. Western blot is weakly reactive on gp41 and *pol* products. Patient 4 has a slight decrease in *gag* products by WB. Patients 5 and 6 are fully WB reactive. Patient 7 is a recent seroconverter infected by HIV-1 subtype A. gp120 and gp41 are weakly reactive. Patient 8 is infected with HIV-1 group O. The WB pattern is highly suggestive of infection by a variant, with no *env* reactivity and strong *pol* reactivity. Patient 9 is in the terminal phase of AIDS, with disappearance of *gag* reactivity.

Careful interpretation of the WB can be highly informative (Fig. 137.7).

- A complete pattern (all bands) with reactivity at least as strong as that of the positive control is suggestive of infection dating back more than 6 months.
- Absent or weak reactivity with proteins p68, p52 and p34 (*pol* gene products) is suggestive of recent primary infection (less than 6 months). Follow-up of the kinetics of appearance of these antibodies will confirm recent seroconversion.
- A fall in reactivity to the core proteins p55, p40 and p24 is found in patients infected many years previously.
- Weak reactivity with *env* gene products and strong reactivity with *pol* gene products can point to nonsubtype B infection.

Occasionally, individuals at low risk of infection are identified through screening as having repeatedly positive EIAs and persistent indeterminate WBs. This situation arises most often in patients with rheumatoid arthritis, systemic lupus erythematosuis or polyclonal gammopathy. Such individuals usually are not HIV infected; a negative HIV-1 DNA PCR test can provide additional reassurance.

Diagnosing HIV-1 infection in children

Transplacental passage of IgG leads to a reactive EIA and positive WB that decrease with time. Passively transmitted antibodies disappear by the age of 15 months; their persistence beyond this time is diagnostic of HIV-1 infection in the infant. Serologic screening of the mother should be offered routinely at the outset of all pregnancies. If the mother is HIV negative despite the existence of risk factors, the result should be checked before delivery, as primary infection is possible during pregnancy.

Early diagnosis of mother-to-child transmission (MTCT) is based on the detection of nucleic acids or the virus in several samples. Current guidelines recommend testing infants born to HIV-infected mothers at age 48 hours, 1–2 months and 3–6 months.[18] A positive

TABLE 137-5 -- Epidemiologic risk factors associated with HIV-2 infection.

EPIDEMIOLOGIC RISK FACTORS ASSOCIATED WITH HIV-2 INFECTION
• Sexual contact or needle sharing with HIV-2-infected individual
• Person originating from HIV-2-endemic region
• Receipt of blood transfusion in an HIV-2-endemic region
• Child born of an HIV-2-infected mother

test suggests the possibility of HIV-1 infection, and should be confirmed by a second test as soon as possible. DNA PCR tests are positive in 40% of infected neonates by 48 hours of age and in 93% of infected infants by day 14;[19] virus culture has similar sensitivity and specificity, but is more costly and time consuming. The sensitivity and specificity of RNA tests for diagnosis of HIV-1 infection in neonates have not been defined.

Diagnosing HIV-2 infection

Cross-reactivity of HIV-2 in HIV-1 EIAs ranges from 50% to 93%. [20] In the USA, blood banks use a combination HIV-1/HIV-2 antigen sandwich EIA to screen for HIV infection, but screening for HIV-2 is not routinely performed in other settings. Therefore, an HIV-2-specific EIA should be obtained when the epidemiologic setting suggests the possibility of HIV-2 infection (Table 137.5).

Virus load monitoring

Plasma HIV-1 RNA levels are correlated with disease stage. Patients who have symptomatic HIV infection or AIDS have significantly higher virus loads than do those with asymptomatic infection. The plasma HIV-1 RNA titer is also a powerful predictor of the risk of disease progression and death at all stages of disease. The change in plasma HIV-1 RNA level in response to treatment also provides important clinical information. Analyses from several large clinical trials show a significant correlation between the magnitude of plasma HIV-1 RNA reduction from baseline and the extent of clinical benefit.[21] Conversely, a rise in plasma HIV-1 RNA level suggests failure of a treatment regimen to suppress virus replication.

Analysis of the virologic response to therapy reveals the importance of the nadir achieved in plasma HIV-1 RNA levels as a marker for the duration of virus suppression.[22] Several studies also show that the early response to treatment is predictive of long-term outcome. Substantial increases in the CD4 cell count may be sustained even if plasma viremia remains detectable, so long as virus load remains significantly below the pretreatment baseline.

Samples should be collected in EDTA tubes and plasma separated and stored at -70°C until testing. Although HIV-1 RNA is stable in plasma at room temperature for up to 48 hours, samples should be processed within 6 hours if possible. Because clinical events that lead to immune activation can cause transient increases in virus load, plasma HIV-1 RNA testing should not be performed within 4 weeks of intercurrent infection or vaccination.

Treatment guidelines recommend obtaining two measurements of plasma HIV-1 RNA to determine the baseline or 'set-point' virus load.[23] Virus load testing should be performed immediately prior to initiating treatment and repeated within 2–8 weeks of starting treatment in order to assess the initial response to a regimen. In treatment-naive patients the plasma HIV-1 RNA level should drop by at least 1.0 $\log_{10}$ within 4–8 weeks of starting an initial antiretroviral regimen; by week 16 plasma virus should be undetectable (below 50 copies/ml) in most patients. More than 24 weeks may be required for plasma virus titers to fall below the limit of detection in patients with high pretreatment levels of viremia (above

Figure 137-8 Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance. Wild-type amino acids are shown above the bar, and mutant amino acids are shown below the bar. Numbers indicate amino acid position. Vertical bars indicate cross-resistance. Bold-face indicates major protease inhibitor resistance mutations. Complete explanation of figure and footnotes available at www.iasusa.org. *From D'Aquila et al.*[28] *, reprinted with permission.*

TABLE 137-6 -- Useful websites for interpreting HIV-1 genotypic resistance tests.

USEFUL WEBSITES FOR INTERPRETING HIV-1 GENOTYPIC RESISTANCE TESTS	
International AIDS Society-USA	www.iasusa.org
Stanford HIV RT and Protease	http://hivdb.stanford.edu
Sequence Database	
Los Alamos National Laboratory	http://hiv-web.lanl.gov
RetroGram	www.retrogram.com

100,000 copies/ml). Declines of 0.5 $\log_{10}$ or more should be expected within 8 weeks following a change in regimen due to treatment failure. Subsequently, plasma HIV-1 RNA levels should be repeated every 3–4 months in order to monitor the success of antiretroviral therapy.

CLINICAL USE OF DRUG RESISTANCE TESTING

Interpreting HIV-1 drug resistance tests

Various systems for interpreting HIV-1 genotypes have been developed. Most interpretations use a 'rules-based' approach in which a group of experts determine which mutations or combination of mutations are associated with resistance to specific drugs, and establish an algorithm for interpreting the genotype (Fig. 137.8). These algorithms are used as the basis of automated computer-generated reports, but require periodic updating as new information becomes available. Alternatively, clinicians may refer to one of a number of online websites that offer genotype interpretation (Table 137.6).

An alternative approach to interpreting genotypic resistance tests is the 'virtual' phenotype. This approach makes use of a large database (>30,000 samples) with paired genotypic and phenotypic data. Viruses with genotypes that are similar to the patient's virus are identified by searching the database and the average IC_{50} of these matching viruses is calculated. This information is then used to estimate the likely phenotype of the patient's virus. The actual and virtual phenotype show excellent correlation for most drugs. The strength of this approach is that it reduces complex genotypic data to simple phenotypic categories based on a rational, data-driven analysis of similar genotypes. However, the virtual phenotype only provides an estimate of the *probable* phenotype of the patient's virus. The confidence placed in the result depends on the number of matches and on picking the right codons to incorporate into the database search. Correlation between actual and virtual phenotype will be weaker for newer drugs or in cases where there are fewer matches due to unusual genotypes.

Interpreting phenotypic resistance tests requires appropriate definition of the 'cut-off' between susceptible and resistant viruses for each drug. Ideally, cut-offs would be defined as the fold-change in susceptibility that corresponds to a reduction in clinical activity of the drug in question. Such data are available only for a few drugs, however, including abacavir, tenofovir and lopinavir. Consequently, definitions of 'susceptible' and 'resistant' for other antiretroviral drugs are based on assay variation and/or population-based susceptibility data for wild-type viruses. Even when based on virologic response data, cut-offs can be misleading, as for most drugs there is a continuous relationship between susceptibility and antiviral activity. Thus, it might be more realistic to consider drugs as having greater or lesser activity against viruses that are more or less susceptible.

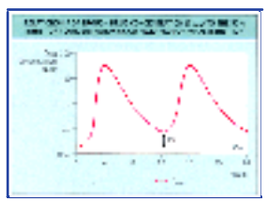

Figure 137-9 Relationship of trough drug concentration (C_{min}) to the 50% inhibitory concentration (IC_{50}) required for viral inhibition. IQ, inhibitory quotient (C_{min} :IC_{50}).

When combined with therapeutic drug monitoring, resistance tests can be interpreted by relating the observed IC_{50} of an isolate to the trough concentration (C_{min} of that drug in a particular patient). The relationship between drug exposure and drug susceptibility can be explored through the inhibitory quotient (IQ), which is the C_{min} :IC_{50} ratio (Fig. 137.9). A high IQ (significantly greater than 1.0) suggests the trough plasma concentration is greater than the amount of drug needed to inhibit the virus in question; a low IQ suggests inadequate drug levels or a highly resistant virus.

Clinical utility of drug resistance testing

Retrospective studies and randomized clinical trials demonstrate the clinical utility of drug resistance testing. A meta-analysis of studies that used either genotype or phenotype to characterize patient viruses found that the risk of virologic failure during salvage therapy was reduced by 30–50% for each drug in the new regimen that was predicted to have activity on the basis of the resistance test performed.[24] These studies also showed that drug resistance remained a significant independent predictor of the likelihood of treatment failure even after controlling for treatment history. Similar results have been obtained with the virtual phenotype.

In randomized clinical trials, virologic response to salvage therapy for patients failing a current treatment regimen generally was superior when therapy was chosen on the basis of genotypic or phenotypic drug resistance testing (Table 137.7). These studies also demonstrated the value of expert advice in selecting a salvage regimen and the necessity of achieving adequate drug levels in plasma. Overall, the benefit of genotyping is clearest in patients failing a first or second regimen, whereas the benefit of phenotyping is most evident in highly treatment-experienced patients. Given the high cost of antiretroviral therapy, using resistance testing to select the best salvage regimen is highly cost-effective.[25]

Recommended uses of drug resistance testing

Most experts recommend use of drug resistance testing to guide the selection of salvage therapy for patients in whom current therapy is failing, and in pregnant women.[26] Resistance testing should also be considered for patients with acute or recent HIV infection. Given the evidence that transmission of drug-resistant HIV-1 is on the rise,[27] testing of all patients prior to initiation of antiretroviral therapy is reasonable where affordable.

TABLE 137-7 -- Randomized trials of HIV-1 drug resistance testing.

RANDOMIZED TRIALS OF HIV-1 DRUG RESISTANCE TESTING			
	Study	**Result**	**Reference**

Genotyping	VIRADAPT	Genotyping superior to SOC	Durant *et al.*[29]
	GART	Genotyping + expert advice superior to SOC	Baxter *et al.*[30]
	ARGENTA	Genotyping superior to SOC in more adherent, less treatment-experienced patients	Cingolani *et al.*[31]
	NARVAL	Genotyping superior to SOC in patients with lower VL, less treatment experience	Maynard *et al.*[32]
	HAVANA	Genotyping and expert each superior to SOC, best in combination	Tural *et al.*[36]
	CERT	No benefit of genotyping	Wegner *et al.*[33]
Phenotyping	VIRA 3001	Phenotyping superior to SOC	Cohen *et al.*[34]
	NARVAL	No benefit of phenotyping	Maynard *et al.*[32]
	CCTG 575	Phenotyping superior to SOC in highly treatment-experienced patients	Haubrich *et al.*[35]
	CERT	Phenotyping superior to SOC in highly treatment-experienced and NNRTI-experienced patients	Wegner *et al.*[33]
SOC, standard of care; VL, virus load; NNRTI, nonnucleoside reverse transcriptase inhibitors			

For treatment-experienced patients, resistance testing will be most informative if samples are obtained while the patient continues on the failing regimen. Once a regimen is stopped, there is the possibility that residual wild-type virus will rapidly overgrow the less fit drug-resistant mutants, giving a potentially misleading test result. To ensure accurate results, testing should be reserved for patients with virus load above 1000 copies/ml. If resistance to a drug is identified, then that drug is likely to have little or no activity and should be avoided, if possible. Similarly, if resistance to a drug has ever been identified, it is safe to assume that resistant virus persists, even if not detected in a current sample. Absence of resistance in the context of treatment failure may be evidence of nonadherence or a pharmacologic barrier to drug activity.

Ultimately, the best choice of therapy for an individual patient should be determined by taking into account all the information available, including history, disease stage, virus load, CD4 count and patient preferences. Although resistance testing is a useful tool, it is not a substitute for sound clinical judgment.

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Chapter 138 - Principles of Management in the Developed World

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INTRODUCTION

The management of HIV infection continues to evolve as treatments, treatment strategies, laboratory investigations and our understanding of pathogenesis have changed. Not only is viral load testing now available but viral resistance assessments and pharmacokinetic measurements of antiretroviral agents are crossing over from the research laboratory to clinical practice. Improved longevity with better antiretroviral therapy has also been associated with increased incidence, awareness and understanding of various adverse effects of antiretroviral therapy. These include osteoporosis, lactic acidemia, disorders of fat accumulation and fat wasting (lipodystrophy), dyslipidemias, glucose intolerance, hypertension, hepatic toxicity and liver failure.

Traditionally, the $CD4^+$ lymphocyte count has been regarded as the key surrogate marker for prognostic staging and therapeutic monitoring of HIV-infected individuals. Then new molecular techniques became available to detect circulating virion-associated HIV RNA in plasma. The availability of HIV viral load testing led to a major revision of our understanding of the natural history of HIV disease.[1] [2] [3] [4] [5] [6] [7] The notion of a prolonged phase of virologic latency antedating the symptomatic phase of the disease has been replaced by one of continuous viral replication from the time of infection until the terminal phases of the illness. It became clear that an ongoing high viral turnover is directly responsible for the ultimate destruction of the immune system.[3] The rate of $CD4^+$ lymphocyte loss will ultimately be dependent on the balance between viral replication, hence $CD4^+$ lymphocyte destruction, and $CD4^+$ lymphocyte production (see Chapter 130).

Antiretroviral therapy has been shown to decrease AIDS-related death rates, opportunistic infections and hospitalizations.[8] [9] [10] [11] [12] The potency of the immunologic recovery seen with antiretroviral therapy has, in fact, been associated in some patients with recognition of previously subclinical infection and has been coined an 'immune reconstitution syndrome'.[13] [14] Immune reconstitution has also led to an ability to discontinue prophylaxis and even suppressive therapy for opportunistic infection in some settings.[15] [16]

In the past few years an awareness of the need to intervene for HIV with antiretroviral therapies at the correct moment has emerged. Long-term therapy has become associated with various adverse effects as well as incomplete adherence to medications — often leading to the development of drug-resistant strains of virus. Although the 'hit early, hit hard' philosophy has played a role in the management of HIV, current thinking has seen the pendulum swing back to a 'watchful waiting' scenario.[17] [18]

LABORATORY MARKERS OF DISEASE PROGRESSION

Three assays are currently available to quantify plasma HIV-1 viral load. These are commonly referred to as reverse transcriptase polymerase chain reaction (RT-PCR), branched chain DNA and nucleic acid sequence-based amplification. All three assays are generally comparable from a technical standpoint. They are also similar in their reproducibility and physiologic variability. The lower threshold of detection is in the range of 200–500 copies/ml, with the newer RT-PCR assays having a lower limit of detection of 50 copies/ml. The variability of the assays is approximately $0.3\log_{10}$ within the dynamic range of the test. As a result, a $0.5\log_{10}$ change in HIV RNA level is generally regarded as a significant viral load change in the context of antiretroviral therapy. It should be noted that intercurrent events (such as infections) or vaccinations can transiently but substantially increase plasma viral load.[19] [20] [21] [22]

Levels of viral replication appear to be set soon after primary infection.[23] Plasma RNA viral load has been correlated with disease progression and death in a seropositive cohort of untreated gay men using archival samples.[24] Patients were divided into four equal groups (quartiles) based on their viral load levels (Table 138.1). In contrast to the close relationship between baseline viral load and outcome, baseline $CD4^+$ lymphocyte count failed to show a similar gradient among quartiles for the risk of disease progression or death. Furthermore, among the three quartiles with the highest $CD4^+$ lymphocyte count, no differences in these outcomes were observed.

These data also provided conclusive evidence of the independence of viral load from $CD4^+$ lymphocyte counts with regard to prognosis. Among patients who had a $CD4^+$ lymphocyte count of more than $500/mm^3$, there was a significant difference in time to death according to whether the baseline viral load was above or below the median (10,190 copies/ml). The 10-year survival was 70% and 20%, for the low- and high-viral-load groups, respectively; both groups had a median $CD4^+$ lymphocyte count of approximately $780/mm^3$. Similarly, among those who had a baseline $CD4^+$ lymphocyte count below 500/ml, a significantly shorter survival was associated with a baseline HIV RNA greater than the median (17,320 copies/ml), despite similar baseline $CD4^+$ counts within the groups.

Despite the integral role of plasma viral load in HIV management, the $CD4^+$ count continues to be an important and useful test, helping to determine where a patient is on the continuum of HIV disease. In adults, a $CD4^+$ lymphocyte count range of approximately 400–1400 cells/mm^3 is considered normal. Counts below 200 cells/mm^3 are associated with increased risk of opportunistic infection.

TABLE 138-1 -- Relationship between viral load and outcome.*

RELATIONSHIP BETWEEN VIRAL LOAD AND OUTCOME				
Viral load (HIV RNA copies/ml)	**Progression to AIDS at 5 years (%)**	**Median time to AIDS (years)**	**Progression to death at 5 years (%)**	**Median estimated survival (years)**
=4530	8	>10	5	>10
4531–13,020	26	7.7	10	9.5
13,021–36,270	49	5.3	25	7.4
>36,270	62	3.5	49	5.1
These viral load thresholds specifically apply to the study group under the particular circumstances of the study. Caution should be exercised when extrapolating to specific clinical situations. The values obtained will vary depending on assay methodology. Also, samples run on a real-time basis are likely to be higher than those specified here.				

** Adapted from Mellors et al.[24]*

The $CD4^+$ lymphocyte count is usually reported as a fraction (or percentage) and an absolute count. Although the absolute $CD4^+$ lymphocyte count is usually sufficient to guide the clinical management of a given patient, it must be noted that under specific circumstances this may be misleading. For example, patients who have undergone splenectomy typically have a relatively high absolute $CD4^+$ count. In such patients the $CD4^+$ lymphocyte fraction is a more appropriate reflection of their immunologic status. It is therefore advisable to monitor the $CD4^+$ lymphocyte fraction in tandem with the $CD4^+$ count at all times to ensure that these are in general

agreement.

Substantial diurnal variation is shown by $CD4^+$ counts; they are lowest in the morning and highest in the evening. In normal individuals the evening $CD4^+$ lymphocyte count can be nearly double what it is in the morning. Although the normal physiologic variation may be reduced in HIV-infected patients, it is still recommended that specimens for $CD4^+$ counting in HIV-infected patients be collected in the morning. Patients should be advised to avoid alcohol, smoking and exercise before collection of the specimen. Other factors that may affect the count include acute infections such as common viral illnesses, certain pharmaceutical agents such as corticosteroids, vaccinations and stress. The results may also be influenced by differing laboratory methodologies. Short-term fluctuations in $CD4^+$ lymphocyte counts of up to 30% have been shown to occur in HIV-infected individuals who are clinically stable.[5] In addition, correlation between $CD4^+$ lymphocyte count and clinical status can be quite different from one patient to the next. Overall, it is important to monitor the trends in $CD4^+$ counts over time rather than placing too much emphasis on a single reading.[25] [26]

From a practical standpoint it is useful to consider the $CD4^+$ lymphocyte count as indicative of the level of immunosuppression or, better yet, 'the immunologic damage that has already occurred'. On the other hand, the plasma viral load better illustrates disease activity and therefore 'the damage that is about to occur'. The prognostic contribution of viral load determinations at any level of $CD4^+$ lymphocyte count is illustrated in Figure 138.1 . Clinical trials have demonstrated conclusively that a change in plasma HIV-1 RNA viral

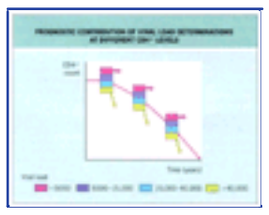

Figure 138-1 Prognostic contribution of viral load determinations at different $CD4^+$ levels. For a given $CD4^+$ lymphocyte count, individuals with high viral load will have a more rapid decline in $CD4^+$ count and therefore a worse clinical prognosis (demonstrated by the downward shift of the survival curve). The viral load is an independent predictor of rapidity of disease progression. A more aggressive virus phenotype — syncytium-inducing versus non-syncytium-inducing — may also adversely affect prognosis, as may viral strains that are resistant to antiretroviral medications.

load is associated with a change in the rate of disease progression.[27] [28] [29] In this context, a treatment-induced 10-fold ($1\log_{10}$) reduction in plasma HIV-1 RNA was associated with a decrease of approximately 50% in the relative risk of death.

On the basis of these data, $CD4^+$ lymphocyte counts and plasma viral load should be measured every 3–4 months in stable HIV-infected adults as part of their routine evaluation. More frequent determinations are warranted under special circumstances, such as when introducing antiretroviral therapy. It should be noted that the use of other surrogate markers such as $ß_2$ -microglobulin, C1q or immune complexes, erythrocyte sedimentation rate and neopterin are no longer recommended in clinical practice.

Advances in the research laboratory are becoming important in the clinical care of patients. Testing for resistance to antiretrovirals can now identify drugs that perhaps should be excluded from a new regimen (see Chapter 137).[30] However, the factors influencing successful therapy are multiple and include the patient's adherence to the regimen and serum or intracellular drug levels, in addition to many others — not only susceptibility testing. Therapeutic drug monitoring of patients receiving nelfinavir-based therapy was recently shown to improve virologic outcomes in a pilot study.[31] Further trials are currently underway aimed to characterize better the optimal use of therapeutic drug monitoring. Until these results are available, therapeutic drug monitoring should be regarded as an experimental tool to be used selectively in special cases, such as patients requiring dose adjustments due to unusual toxicities or those receiving poorly characterized drug combinations.

BASELINE EVALUATION

Baseline evaluation of an infected individual should include a complete history and physical examination. Counseling should be given regularly. Laboratory tests are also very important, particularly before the start of a treatment program. These should include those listed in Table 138.2 .

In terms of blood counts, it is worth noting that anemia, neutropenia and thrombocytopenia are common in patients who have HIV infection. Absolute lymphopenia should raise suspicion of advanced disease. The criterion for the tuberculin skin test is that an induration of 5mm or more in diameter after a 5 tuberculin units purified protein derivative (PPD) test should be regarded as a positive response in an HIV-infected individual; isoniazid prophylaxis

TABLE 138-2 -- Laboratory tests for baseline evaluation of HIV-infected patients.

LABORATORY TESTS FOR BASELINE EVALUATION OF HIV-INFECTED PATIENTS
• Plasma HIV RNA viral load
• $CD4^+$ lymphocyte count, absolute and percentage
• Complete blood count and differential and platelet count
• Liver (aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, bilirubin) and renal (blood urea nitrogen, creatinine) profiles
• Creatine phosphokinase, amylase, uric acid and triglycerides
• Hepatitis B, hepatitis C, syphilis, cytomegalovirus (CMV) and toxoplasmosis serologies
• Tuberculin skin test
• Cultures and smears for sexually transmitted diseases, as indicated by the history and physical examination
• Sputum cultures and smears for mycobacteriae, as indicated by history and physical examination
• Chest radiograph
• Urinalysis
• Pregnancy test
• Papanicolaou's smear test (if appropriate)
• Eye fundoscopy if the $CD4^+$ count is <100/ml

should therefore be considered. A negative response in persons who have advanced HIV disease does not rule out infection with *Mycobacterium tuberculosis*, as such individuals may be anergic.[32] [33]

FOLLOW-UP ASSESSMENT

The routine follow-up of a stable asymptomatic HIV-positive patient should include a history and physical examination as well as plasma viral load and $CD4^+$ lymphocyte count every 3–4 months. Counseling should take place at each visit. Patients should also have an opportunity to discuss the most recent trends in management and antiretroviral therapy.

Symptomatic patients and those who have AIDS require active drug therapy and as such they should be seen at least monthly. More frequent and additional laboratory investigations may be warranted for patients presenting with co-morbidities such as viral hepatitis (B or C) or psychiatric illness.

GENERAL APPROACH TO THE SYMPTOMATIC PATIENT

Not every illness in an HIV-infected patient is attributable, or unique, to HIV infection or AIDS. HIV-infected patients, like everyone else, are susceptible to all the usual

illnesses. In fact, as a result of the immune dysfunction that characterizes HIV infection even before the development of overt AIDS, patients typically present with often repeated bouts of common illnesses, such as seborrheic dermatitis, eczema, angular cheilitis, shingles, onychomycosis, sinusitis or community-acquired pneumonia. When signs and symptoms appear, management should be the same as in uninfected individuals.

When $CD4^+$ lymphocyte counts are within the normal range, immune function in adults is almost normal and opportunistic infections are unlikely. The work-up does not usually require any special initiatives. However, it should be noted that some conditions, such as tuberculosis, Kaposi's sarcoma or lymphomas, can occur at any level of $CD4^+$ lymphocyte count. One should have a higher index of suspicion for opportunistic infections as the $CD4^+$ lymphocyte count falls below normal, and particularly once it is below 200 cells/mm^3 or below a $CD4^+$ lymphocyte fraction of 15%.[34]

PROPHYLACTIC TREATMENTS AND VACCINATIONS

Prevention plays a major role in the management of HIV-infected individuals. The following interventions should be considered when a newly diagnosed patient is evaluated.

Tuberculin skin test

A tuberculin skin test should be performed. If there is induration of 5mm or more in diameter, then prophylaxis with isoniazid 5mg/kg daily (maximum 300mg/day) for 12 months should be initiated, along with pyridoxine to reduce the risk of isoniazid toxicity.[32] Prophylaxis may also be indicated in patients at high risk of tuberculosis who have cutaneous anergy or household contact with someone with active tuberculosis, patients who have chest radiograph findings suggestive of previous tuberculosis who do not have a history of adequate treatment, or patients who have not previously received isoniazid prophylaxis or tuberculosis treatment.[32] [33] [34] A positive tuberculin skin test should be interpreted with caution among immigrants, who may have received tuberculosis vaccine during childhood.

Vaccination against common pathogens

While antigen recognition is still intact, it is essential to boost humoral immunity against certain common pathogens. Recommended

TABLE 138-3 -- Vaccination against common travel-related pathogens.

VACCINATION AGAINST COMMON TRAVEL-RELATED PATHOGENS	
Vaccine	**Comments**
	In general, live vaccines should be avoided
Measles	Not recommended for travelers who are severely immunocompromised*
Typhoid	The inactivated parenteral typhoid vaccine should be given instead of the live, attenuated oral vaccine
Yellow fever	Live vaccine of uncertain safety in HIV†
Hepatitis A	A killed vaccine that should be used as for non-HIV-infected travelers‡
Diphtheria-tetanus	A killed vaccine that should be used as for non-HIV-infected travelers
Japanese encephalitis	A killed vaccine that should be used as for non-HIV-infected travelers
Rabies	A killed vaccine that should be used as for non-HIV-infected travelers

* Measles vaccine is recommended for nonimmune travelers. However, measles vaccine is not recommended for travelers who are severely immunocompromised; immune globulin should be considered for measles-susceptible, severely immunosuppressed travelers who are anticipating travel to measles endemic countries.
† Travelers with asymptomatic HIV infection who cannot avoid potential exposure to yellow fever should be offered the choice of vaccination. All travelers should avoid mosquito bites.
‡ In the setting of immunosuppression, these vaccines may not offer complete protection.

vaccines include polyvalent vaccine against *Streptococcus pneumoniae* (Pneumovax) given once, and influenza vaccine given annually in the autumn.[35] [36] The evidence in favor of the latter, however, remains controversial. Tetanus toxoid updates should be offered as necessary, and hepatitis B vaccination should be encouraged for any susceptible patient. Male homosexual HIV-positive men should also receive hepatitis A vaccine. The inactivated polio vaccination should be given to any patient who is traveling. Table 138.3 summarizes additional travel-related vaccine advice. Vaccines against *Haemophilus influenzae* type b and *Neisseria meningitidis* are at present not widely recommended.

Prophylaxis and treatment for opportunistic infections (see Chapter 123)

As shown in Table 138.4 , as HIV disease progresses prophylaxis and treatment of opportunistic infections become increasingly important. Recurrent genital herpes outbreaks may be dealt with using either intermittent treatment or regular suppressive therapy with oral acyclovir, depending on the frequency of the attacks.[37] Mucosal candidiasis may be treated with topical agents or systemic azoles as needed. Systemic azole therapy is usually needed as the immunodeficiency progresses. In some patients intermittent or even continued suppressive therapy with systemic azoles may be warranted to control frequent relapses.

More than 80% of untreated HIV infected individuals will have at least one episode of *Pneumocystis carinii* pneumonia (PCP) during their lifetime. Prophylaxis against PCP is indicated if the $CD4^+$ lymphocyte count is below 200/mm^3 , if the $CD4^+$ lymphocyte fraction is below 15%, if there is a history of recurrent *Candida* infections, or if there are chronic constitutional symptoms such as persistent unexplained fevers and weight loss. All patients should be offered PCP prophylaxis after an episode of PCP (secondary prophylaxis). Prophylaxis against PCP should also be offered to any patient who has had an AIDS-defining illness. The preferred regimen consists of one double-strength tablet of trimethoprim-sulfamethoxazole daily.[37] Alternatively, dapsone 100mg q24h, atovaquone 1500 mg q24h or aerosol pentamidine 300mg once a month can be used. Before starting prophylaxis, patients should be assessed to rule out active pulmonary

TABLE 138-4 -- Prophylaxis and treatment for opportunistic infections.

PROPHYLAXIS AND TREATMENT FOR OPPORTUNISTIC INFECTIONS	
$CD4^+$ lymphocyte count (no/ml)	**Management strategy**
>500	• General counseling (safer sex, nutrition, etc.)
	• History and physical examination every 3–6 months
	• Plasma viral load and $CD4^+$ count every 3–6 months
	• Pneumovax, annual influenza vaccinations
	• Tuberculin skin test and INH prophylaxis if indicated
	• Update diphtheria-pertussis-tetanus (tetanus toxoid for adults) and inactivated polio vaccinations
	• Hepatitis B vaccine if at risk
	• Syphilis serology

<500	• Antiretroviral therapy followed by plasma viral load 1 month later
	• Plasma viral load and $CD4^+$ count every 3–4 months
	• Herpes suppression if frequent recurrences (more than 4–6 outbreaks/year)
	• Relevant history, physical and laboratory investigations at least monthly if symptomatic, diagnosed with AIDS or on antiretroviral therapy
<200	• Start prophylaxis for PCP
<100	• Plasma viral load and $CD4^+$ count every 3–4 months
	• Start prophylaxis for toxoplasmosis if seropositive and not on trimethoprim-sulfamethoxazole
<75	• Consider MAC prophylaxis
<50	• Screening by an ophthalmologist for cytomegalovirus (CMV) retinitis, to be repeated at 3- to 6-monthly intervals; consider CMV prophylaxis

disease. In the context of continued use of highly active antiretroviral therapy (HAART), patients having adequate responses, such as HIV RNA levels below 50 copies/ml and a $CD4^+$ lymphocyte count recovery to 200 cells/mm^3 or more for at least 3 months should be encouraged to discontinue either primary or secondary PCP prophylaxis.[38] [39]

Prophylaxis for toxoplasmosis is indicated for those patients who have positive serum IgG for toxoplasmosis and $CD4^+$ lymphocyte count less than 100/mm^3 . It is worth noting that trimethoprim-sulfamethoxazole, as indicated for PCP prophylaxis, is also effective against toxoplasmosis.[37] As with PCP, prophylaxis may be discontinued for patients who experience HAART-induced immune reconstitution associated with an increase in the $CD4^+$ lymphocyte count to more than 200/mm^3 for at least 3 months. Toxoplasma prophylaxis should be restarted if $CD4^+$ lymphocyte counts fall to less than 100/mm^3 .

There are currently insufficient data for a recommendation regarding the advisability of discontinuing secondary prophylaxis although this is increasingly being done without ill effects once the $CD4^+$ lymphocyte count is above 200/mm^3 for at least 3 months with the use of effective HAART.

As disease progresses, prophylaxis for *Mycobacterium avium* complex (MAC) with intermittent azithromycin or daily clarithromycin may also be considered once the $CD4^+$ lymphocyte count is below 50/mm^3 . MAC prophylaxis can be safely discontinued in patients who have responded to HAART with increased $CD4^+$ counts to more than 100 cells/mm^3 for at least 3 months.[15] Prophylaxis should be restarted if the $CD4^+$ level falls to below 50 cells/mm^3 . Once the $CD4^+$ lymphocyte count is below 100/mm^3 , screening by an ophthalmologist for cytomegalovirus retinitis should be encouraged, and this should be repeated at 3- to 6-monthly intervals thereafter, while the $CD4^+$ count remains below 100/mm^3 .

ANTIRETROVIRAL THERAPY

The objective of antiretroviral therapy use is to prevent disease progression and prolong survival while maintaining quality of life. Long-term nonprogression will be achieved by reducing plasma viral load below 50 copies/ml on a long-term basis. The use of combinations of antiretrovirals with no overlapping toxicity and demonstrated antiviral additive to synergistic effect is recommended to maximize the duration of the antiviral response.[18]

Since 1996, triple drug combination antiretroviral therapy has been shown to decrease morbidity and mortality dramatically in symptomatic and asymptomatic HIV-1 infected individuals.[8] [9] [10] [24] [40] [41] [42] Recommendations for the initiation of therapy have been crafted based on thresholds of $CD4^+$ lymphocyte counts and plasma HIV-1 RNA that reflect the risk for disease progression in natural history and observational studies as well as randomized clinical trials. [30] [43] [44] [45] [46] [47] The optimal time for initiation of therapy, however, has not been defined. Recent work has characterized rates of disease progression to AIDS or death for $CD4^+$ and plasma viral load thresholds in treated patients.[17] [48] [49] [50] In an analysis of a population-based cohort of 1200 HIV-1 infected patients, Hogg *et al.*[17] demonstrated low rates of disease progression to AIDS and death (<3% at 12 months) among patients starting antiretroviral therapy with $CD4^+$ lymphocyte counts of 200 cells/mm^3 or more, independent of HIV-RNA levels. In their study, disease progression to AIDS and death clustered among patients starting therapy with $CD4^+$ lymphocyte counts below 200 cells/mm^3 . From these results, it appears that 200 cells/mm^3 represents a critical $CD4^+$ threshold below which the short-term clinical effectiveness of antiretroviral therapy is at least partially compromised. Currently, it is recommended that all symptomatic individuals, as well as those who have $CD4^+$ lymphocyte counts below 250/mm^3 , be treated with HAART. The risk-benefit ratio associated with earlier intervention (i.e. 250–350 or 350–500/mm^3) remains to be clarified. Patients who have 250–350/mm^3 $CD4^+$ lymphocyte counts should be monitored closely with repeated $CD4^+$ counts, given that rapid declines in $CD4^+$ lymphocytes can occur, particularly among those who have HIV RNA levels of more than 30,000 copies/ml. In such instances, monthly monitoring may be appropriate.

The effectiveness of antiretroviral therapy relies not only on the appropriate time of initiation of treatment but also on the degree to which patients are able to adhere to therapy. Incomplete adherence to antiretroviral treatment has been associated with premature virologic failure.[51] Intermittent adherence increases the risk of having suboptimal drug levels, which in turn may increase the likelihood of drug resistance.[52] [53] Moreover, resistance to one drug is frequently associated with cross-resistance to other members of the same class,[54] [55] thus limiting future treatment options (see Chapter 140). Furthermore, not only has incomplete adherence been associated with premature virologic failure but it has also been linked with increased risk for mortality.[56] Thus, the challenges of effectively treating HIV with antiretrovirals includes attention to both the optimal time for initiation of therapy and the means to maximize adherence.

Prior to initiating antiretroviral therapy, a long-term treatment strategy should be developed considering the possibility of intolerance and treatment failure due to resistance. Consideration must be given to enhancing compliance — with simpler regimens more likely to be effective. The current goal of therapy is to suppress plasma viral load below the level of quantitation of the assay (less than 50 copies/ml) on a long-term basis. If a patient experiences drug toxicity, brief cessation of all medications is recommended. Decreasing dosage or stopping only one medication is not to be encouraged as this will promote the development of resistance. If plasma viral load rebounds despite ongoing therapy, consider noncompliance and resistance as the most likely causes. A confirmed detectable plasma HIV RNA level

TABLE 138-5 -- Antiretroviral medications.

ANTIRETROVIRAL MEDICATIONS			
Drug	**Dosage**	**Comments**	**Cost**
Nucleoside reverse transcriptase inhibitors (NRTIs)			
Zidovudine (AZT)	400–600mg q24h in 2 divided doses	Most common adverse effects: nausea, headache, rash, anemia, leukopenia, elevated liver enzymes, elevated lactic acid and elevated CPK	$$
		Should not be combined with D4T	
Lamivudine (3TC)	150mg bid	Most common adverse effect is neutropenia (rare)	$$
Didanosine (ddI)	35–49kg: 100mg q12h	Main common adverse effects: gastrointestinal intolerance, pancreatitis, gout, reversible peripheral neuropathy	$
	over 50kg: 200mg q12h	Should not be combined with ddC	
	Full daily dose can be given once a day	Should be taken NPO	
DDI-EC	Over 50kg: 400mg q24h		
Zalcitabine (ddC)	0.75mg q8h	Most common adverse effects: reversible peripheral neuropathy, mouth ulcers, pancreatitis	$
		Should not be combined with d4T or ddI	
		Relatively weak risk-benefit ratio limits usefulness	

Stavudine (d4T)	40–60kg: 30mg q12h	Reversible peripheral neuropathy	$$
	Over 60kg: 40mg q12h	Lactic acid elevations (rarely fatal)	
		Should not be combined with AZT	
Tenofovir	300mg q24h	Most common adverse effect: gastrointestinal upset, low phosphate	$$$
Abacavir (ABC)	300mg q12h	Most common adverse effect is a hypersensitivity reaction, which may be characterized by fever, rash, myalgias, arthralgias, malaise. Reaction may be *fatal* if medication is continued or patient is rechallenged	$$$
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)			
Nevirapine (NVP)	200mg q24h for 2 weeks then increase to 200mg q12h	Most common adverse effects: rash, elevated liver enzymes	$$
	Full daily dose can be given once a day		
Delavirdine (DLV)	400mg q8h	Most common adverse effect is rash	$$
Efavirenz (EFV)	600mg q24h (or 300mg q12h)	Most common adverse effects: central nervous system toxicity ('hangover', drowsiness), rash	$$$
Protease inhibitors (PIs)*			
Saquinavir (INV — Invirase®)	Very poor bioavailability unless combined with RTV	Most common adverse effect is elevated liver enzymes	$$$$
	When given with RTV use INV/RTV 400mg/400mg q12h *or* 1000mg/100mg q12h *or* 1600mg/100mg q24h	Better tolerability (i.e. gastrointestinal) and similar PK to FTV when used with RTV boosting	
Saquinavir (FTV — Fortovase®)	1200mg q8h *or* 1000mg/100mg	Most common adverse effect is gastrointestinal toxicity and elevated liver enzymes	$$$$
	q12h *or* 1600mg/100mg q24h	Better bioavailability than INV in the absence of RTV	
Ritonavir (RTV)	600mg q12h	Most common adverse effects: gastrointestinal upset, diarrhea, circumoral paresthesias, elevated liver enzymes, hypertriglyceridemia	$$$$
		Most common use at present is as a PI booster at low doses (i.e. 100mg–400mg q24h)	
Indinavir (IDV)	800mg q8h	Most common adverse effects: elevated liver enzymes, nephrolithiasis, hypertension, ingrown toenails, benign hyperbilirubinemia	$$$$
	Can be given with RTV boosting: IDV 800mg/RTV 100mg q12h		
Lopinavir/ritonavir (LPV/RTV)	3 capsules q12h	Actually two drugs combined in one capsule	$$$$
	Dose should be increased to 4 capsules q12h if used with EFZ or NVP and in the presence of moderately to highly PI-resistant HIV	Most common adverse effects: gastrointestinal upset	
Amprenavir (APV)	1200mg q12h	Most common adverse effects: rash, gastrointestinal upset	$$$$$
	Can be used with RTV at a dose of 600mg APV/100mg RTV q12h		
Nelfinavir (NFV)	750mg q8h	Most common adverse effect is gastrointestinal upset, mostly diarrhea	$$$$

*PIs have multiple drug interactions and may be associated with various metabolic adverse effects such as diabetes mellitus, hyperlipidemias or lipodystrophy (limb and face wasting and accumulation of abnormal fat deposits)

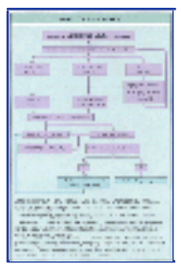

Figure 138-2 Approach to antiretroviral therapy. PI, protease inhibitors.

in an adherent patient indicates virologic failure. At such time the treatment should be re-evaluated and possibly changed to an alternative fully suppressive regimen to avoid development of resistance. Table 138.5 summarizes the currently available antiretroviral agents. For further discussion, refer to Chapter 139 and Chapter 204 . An approach to antiretroviral therapy is suggested in Figure 138.2 .

PRINCIPLES IN CHANGING THERAPY

Currently, drug failure is defined in virologic terms as the occurrence of a confirmed viral load rebound in the absence of other obvious explanation (e.g. treatment interruption, intercurrent illness or immunizations). A change in therapy in this setting will only be carried out after careful evaluation of prior drug exposure, prior response to therapies, prior tolerability and toxicity issues, as well as the results of resistance testing done on a real time basis and on stored samples. It is critically important to understand and correct the determinants of prior treatment failure in any given individual. This must be done before a change in therapy is implemented. Failure to address these issues effectively will invariably compromise the chances of success with the new regimen. Whenever possible, pharmacokinetic issues (past and present) should also be evaluated. Multiple variables are operational when changing regimens in the context of virologic failure in a given clinical case. As such, guidelines cannot replace expert advice in this setting. It is critical, therefore, that the decision of when to change and what to

change to be arrived at under the guidance of an experienced practitioner.

When a decision to change therapy for sustained virologic failure is made, the new regimen should be one with the highest probable effectiveness, as predicted by the patient's complete drug history and the resistance test result, as well as the highest likelihood of tolerability and adherence. New regimens should contain at least two, and if possible three, drugs deemed to be active. The viruses that replicate during treatment failure may not be resistant to all of the drugs in the failing regimen. However, latently infected lymphocytes may harbor archived viruses that are resistant to drugs used in the past but are not detected by routine resistance testing in the plasma. Frequently, shared-resistance mutations conferred by an individual drug lead to cross-resistance among drugs in the same class, complicating the choice of alternative regimens.

With the currently approved non-nucleoside reverse transcriptase inhibitors (NNRTIs), the risk of complete NNRTI-class cross-resistance is high when an NNRTI-containing regimen fails. With protease inhibitors, intraclass cross-resistance is not so predictable. Depending on the pattern of resistance, an alternative protease inhibitor (or a combination of protease inhibitors) can often be selected. With nucleoside reverse transcriptase inhibitors (NRTIs), the extent of class

cross-resistance is greater than anticipated previously, and the level of resistance to alternative drugs (e.g. to stavudine) is more difficult to infer from genotype or phenotype testing results. In rare circumstances, multidrug resistance to NRTIs may develop through a unique pathway of resistance. Alternative regimens must therefore be assessed on a case-by-case basis, based on predicted resistance patterns.

In the absence of virologic or immunologic failure, a regimen may pose problems due to adherence, intolerance or toxicity. Detailed discussion of the management of all possible antiretroviral-related toxicities is beyond the scope of this review. In general, as long as the antiviral potency of the regimen is preserved, exchanging an individual component of the regimen to deal with a toxicity problem is acceptable. The closer the agents are in terms of their potency and resistance profile, the easier and safer the change will be — for instance, replacing stavudine with zidovudine in a patient who has anemia, or zidovudine with stavudine in a patient who has neuropathy. Similarly, replacing nevirapine in a patient who has central nervous system toxicity with epavirenz would be generally safe and effective. Drug substitutions become more complicated as patients present with a history of prior drug exposure and/or failure to multiple regimens. In such cases, changes in therapy should only be performed under the guidance of an experienced physician.

SUMMARY

The overall management of the patient who has HIV infection is dictated by the level of disease activity, as indicated by the plasma viral load and by the degree of immunodeficiency. The latter is best characterized — in the absence of symptoms — by the $CD4^+$ lymphocyte count. As with any chronic disease, the primary care physician is best situated to co-ordinate care in an ongoing fashion in close collaboration with experienced specialists. Because of the multiple complexities associated with the use of currently available antiretroviral therapy regimens, it is best to reserve their use to those with an expertise in this rapidly evolving field. As signs and symptoms of specific diseases appear, referral for special investigation and treatment should be encouraged.

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Chapter 139 - Antiviral Therapy

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INTRODUCTION

HIV infection is a chronic disease in which, in the absence of appropriate treatment, high-level viral replication occurs continuously for years, even during the clinically latent phase and in the absence of symptoms and opportunistic infections. Symptoms and opportunistic infections typical of full-blown AIDS usually start occurring 10–12 years after HIV infection. Both viral and cellular turnover show fast kinetics, particularly during primary infection and, later, during the AIDS stage.[1] Viral replication drives the progression of HIV disease and viral load levels are highly predictive of the subsequent risk of disease progression and death.[2] Residual viral replication due to incomplete suppression in the presence of drugs is also responsible for the development of viral resistance to antiretroviral drugs, which further contributes to treatment failure and disease progression.[3] Antiviral treatment should therefore be aimed at obtaining maximal and sustained suppression of HIV replication. Indeed, suppression of HIV replication is associated with a significant delay in the progression to AIDS and increased survival, particularly in previously untreated patients.[4]

Plasma HIV-1 RNA levels and $CD4^+$ lymphocyte counts represent the main tools in the monitoring of antiretroviral treatment.[5] Previously untreated patients are expected to reach undetectable HIV plasma levels in no more than 4–6 months, with a concomitant rise in $CD4^+$ lymphocyte counts. Different drug combinations can produce this effect. It must, however, be stressed that 'undetectable' plasma HIV RNA level does not mean eradication of the virus at other sites (lymphoid tissues, central nervous system, semen). Studies on the effects of therapy in these compartments are ongoing,[6] but eradication of the virus is not considered a realistic goal for the moment.

Even with potent regimens, virologic failure of treatment (i.e. persistently elevated HIV plasma levels in the presence of treatment) is frequent. It may depend on different factors, which include low therapeutic adherence, presence of drug-resistant HIV strains, low drug levels in plasma or tissues and other still undetermined factors. Targeted strategies are being developed in order to improve treatment efficacy, including the development of new drugs with improved formulation and administration schedule, increased potency and reduced toxicity.

The availability of an increasing number of drugs and the rapidly evolving scientific information have made HIV treatment an extremely complex field. Treatment should be supervised by experts, and several national and international guidelines have been developed in order to help in the clinical management of HIV-infected adults and adolescents, pregnant women, infants and children, and uninfected individuals who have occupational exposure to HIV. Proper information for patients and their full involvement in each therapeutic decision are essential elements of a successful response to treatment.

This chapter also addresses the issue of resistance, mostly from the point of view of its clinical implications. Treatment in specific clinical settings such as primary/acute HIV infection and pediatric infection, and the prevention of HIV transmission in the occupational setting are discussed in more detail in Chapter 122 , Chapter 134 and Chapter 117 , respectively. Thus, this chapter focuses mainly on the treatment of established HIV infection. The following text summarizes the antiviral activity, toxicity profiles and clinical efficacy of drugs and combinations of drugs that are already available. Some information on drugs under initial clinical evaluation is also presented.

ANTIRETROVIRAL DRUGS

Until 1987, no drug was available for the treatment of HIV infection. In 1987, zidovudine was widely introduced into clinical practice and zidovudine monotherapy remained the basis of treatment for some years[7] until it became evident that its benefit was limited.[8] Subsequent experience of switching from zidovudine to another nucleoside reverse transcriptase inhibitor or adding a different nucleoside to a prolonged zidovudine regimen was limited in extent.[9] Development of resistance was common with all these drugs and was associated with clinical failure.

Nucleoside monotherapy was finally deemed to be a suboptimal treatment when clinical trials and viral load measurements clearly indicated that two-nucleoside regimens were able to induce a more obvious and prolonged effect on viral load and $CD4^+$ counts, and to delay progression and increase survival.[4] [10] In 1995 available protease inhibitors (PIs), in combination with reverse transcriptase inhibitors, produced a further reduction of HIV replication, often to undetectable levels, and a clinical benefit in intermediate and advanced disease.[11] Non-nucleoside reverse transcriptase inhibitors (NNRTIs), introduced after 1996, have also proved to be highly effective and have become widely used in clinical practice.

It is therefore now clear that the optimal treatment of HIV infection is a potent combination of drugs (termed highly active antiretroviral therapy, HAART), as already established for other important complex diseases such as cancer and tuberculosis. Most of the currently recommended potent regimens are based on a dual nucleoside 'backbone' plus a NNRTI or a PI (with the option of adding low-dose ritonavir to another PI for pharmacoenhancement). Three-nucleoside regimens including abacavir are also increasingly used because these regimens are easy to administer and are dual-class-sparing (i.e. allow deferred use of PIs and NNRTIs).

Many substances are active against HIV in vitro, and almost every step of the HIV replicative cycle is currently being analyzed to identify specific targets of antiviral treatment (Fig. 139.1).

At present, available antiretroviral drugs include six nucleoside reverse transcriptase inhibitors (zidovudine, didanosine, zalcitabine, stavudine, lamivudine and abacavir), one nucleotide reverse transcriptase inhibitor (tenofovir disoproxil fumarate), three NNRTIs (nevirapine, delavirdine and efavirenz) and six HIV PIs (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir; Table 139.1). Their mechanism of action and clinical characteristics are briefly summarized, together with those of some other drugs that are in an advanced phase of evaluation and could enter clinical practice in the next few months or years.


Figure 139-1 Possible sites of intervention in the inhibition of HIV replication.

REVERSE TRANSCRIPTASE INHIBITORS

Reverse transcriptase inhibitors act through at least two mechanisms. First, as 'chain terminators', they block the elongation of the DNA chain through blockage of further nucleosides. This mechanism is characteristic of the nucleoside and nucleotide analogues and depends on the intracellular phosphorylation of the drugs to the corresponding triphosphate. Second, they act by competition/binding of the reverse transcriptase in functionally essential sites; NNRTIs act only through this mechanism and not as 'chain terminators'. Nucleoside analogues have in general good oral bioavailability, are only minimally bound to plasma proteins and are excreted through the kidneys. Because of these metabolic characteristics, they have relatively few interactions with other drugs as compared with PIs. Cerebrospinal fluid (CSF) to plasma ratios may be variable, ranging between 10% and 80%. They are generally active on HIV-1 and HIV-2. Non-nucleoside reverse transcriptase inhibitors are characterized by an HIV-1-restricted antiviral activity and are generally metabolized by the liver; interactions with other drugs with hepatic metabolism may occur. Their binding to plasma protein can also be higher than that with nucleoside analogues, and binding site displacement effects are possible. Data on their

penetration into the central nervous system (CNS) are scarce.

Concerns were recently raised for the occurrence of mitochondrial toxicity due to inhibition of DNA ?-polymerase by nucleoside analogues. Although the clinical correlates of mitochondrial disfunction are poorly defined, a variety of clinical situations have been ascribed to this condition: chronic hyperlactatemia, with possible evolution into potentially fatal lactic acidosis with hepatomegaly and hepatic steatosis (three fatal cases reported in pregnant women receiving didanosine plus stavudine); pancreatitis, neuropathy, myopathy and cardiomyopathy; sporadic cases of severe and potentially fatal neurologic involvement in HIV-negative children exposed in utero to nucleoside analogues; and possibly, some of the features of the lipodystrophy syndrome (see below).[12] At present, lactic acidosis is considered to be the most serious condition associated with treatment with nucleoside analogues.

Zidovudine

Zidovudine (or azidothymidine; AZT) was the first drug approved for the treatment of HIV. It can be used in combination with most of the other antiretroviral drugs, with the exception of stavudine, and is available as dual co-formulation with lamivudine and as triple co-formulation with lamivudine and abacavir. The currently recommended dose is 600mg (200mg q8h or 300mg q12h). If tolerability is compromised by side effects, the dose of zidovudine is often reduced to 100mg q8h. It should be noted that, although currently available data suggest that 100mg q8h can have a favorable effect on surrogate markers, the clinical effectiveness of this dose has not been established. Pediatric dosages are about 180mg/m^2 q6h. Oral availability is approximately 60%. The drug concentrates in the semen, crosses the placenta and has good penetration into the CSF. Drugs inhibiting its glucuroconjugation, such as probenecid, valproic acid, naproxen, indomethacin and oxazepam, should be used with caution; methadone may increase zidovudine concentrations in serum.

The main adverse reactions include anemia, leukopenia, nausea, headache, insomnia and reversible myositis, with raised creatine phosphokinase (CPK) levels. Macrocytosis is a common hallmark of treatment with zidovudine.

Didanosine

Didanosine (ddI) is a nucleoside analogue to be administered in doses adjusted according to body weight: 200mg q12h or 400mg q24h in patients whose body weight is above 60kg, and 125mg q12h or 250mg q24h in patients whose body weight is below 60kg. Recommended dosage in children is on average 200mg/m^2 q24h (as oral solution). Didanosine is rapidly degraded at low pH and must

TABLE 139-1 -- Antiretroviral drugs available for highly active antiretroviral therapy combination regimens.

ANTIRETROVIRAL DRUGS AVAILABLE FOR HAART COMBINATION REGIMENS			
Class	**Drug**		**Dosage**
Nucleoside reverse transcriptase inhibitors	Zidovudine (AZT)	Retrovir®[*] [†]	200mg q8h or 300mg q12h
	Didanosine (ddI)	Videx®	>60kg: 200mg (tablets) or 250mg (powder) q12h, 400mg q24h (tablets or EC capsules)
			<60kg: 125mg (tablets) or 167mg (powder) q12h; 250mg q24h (tablets or EC capsules)
	Zalcitabine (ddC)	Hivid®	0.75mg q8h
	Lamivudine (3TC)	Epivir®[*] [†]	>50kg: 150mg q12h
			<50kg: 2mg/kg q12h
	Stavudine (d4T)	Zerit®	>60kg: 40mg q12h
			<60kg: 30mg q12h
	Abacavir (ABC)	Ziagen®[†]	300mg q12h
Nucleotide reverse transcriptase inhibitors	Tenofovir	Viread®	300mg q24h
Non-nucleoside reverse transcriptase inhibitors	Delavirdine (DLV)	Rescriptor®	400mg q8h
	Nevirapine (NVP)	Viramune®	200mg q24h for 2–4 weeks, then 200mg q12h
	Efavirenz (EFV)	Sustiva®	600mg q24h
Protease inhibitors	Saquinavir (SAQ)	Invirase® (HGC)[‡]	HGC: 400mg q12h, to be used only with ritonavir
		Fortovase® (SGC)[§]	SGC: 1200mg q8h
	Ritonavir (RTV)	Norvir®	300mg q12h, escalate to 600mg q12h in 2 weeks
	Indinavir (IDV)	Crixivan®	800mg q8h
	Nelfinavir (NFV)	Viracept®	750mg q8h or 1250mg q12h
	Amprenavir (APV)	Agenerase®	>50kg: 1200mg q12h
			<50kg: 20mg/kg q12h (max. 2400mg daily total)
	Lopinavir (LPV)	Kaletra®[¶]	400mg lopinavir + 100mg ritonavir q12h

* Also available (300mg AZT+ 150mg 3TC) as Combivir®.
† Also available (300mg AZT+ 150mg 3TC+ 300mg ABV) as Trizivir®.
‡ Hard-gel capsule.
§ Soft-gel capsule.
¶ Only available as Kaletra® (133.3mg lopinavir + 33.3mg ritonavir).

be taken on an empty stomach. The oral bioavailability of didanosine is about 35–40%, and lower penetration than with zidovudine into the CNS has been reported. The metabolism is likely to involve the same pathways as those responsible for the elimination of endogenous purines.

The main adverse effects associated with didanosine therapy include gastrointestinal intolerance, diarrhea, hyperamylasemia, pancreatitis, peripheral neuropathy, hyperuricemia, hypertriglyceridemia and, rarely, rhabdomyolysis and lactic acidosis. Side effects appear to be more frequent in patients who have advanced disease. Concomitant use of pancreotoxic or neurotoxic drugs may increase the risk of pancreatitis and peripheral neuropathy respectively.

Zalcitabine

Zalcitabine (ddC) is a nucleoside analogue generally used in combination with zidovudine. Dosage is 0.75mg q8h for a total of 2.25mg/day. In patients weighing less than 50kg, half dosing is recommended. It has good bioavailability (80%); when administered with food, adsorption is reduced by 14% and time to achieve peak plasma concentrations approximately doubles from 0.8 hours to 1.6 hours.

The main adverse effects associated with zalcitabine therapy include peripheral neuropathy and painful mouth and penile ulcers. Nausea, dysphagia, anorexia, diarrhea, headache and myalgias have also been observed. Less frequently, rash, hyperamylasemia and pancreatitis have occurred in the context of zalcitabine therapy. Because of the common risk of peripheral neuropathy and pancreatitis, concomitant administration of didanosine or stavudine and zalcitabine is not

recommended.

Lamivudine

Lamivudine (3TC), a nucleoside analogue inhibitor of the reverse transcriptase, is to be used in combination with zidovudine or stavudine. The recommended dose is 150mg q12h in adults (2 mg/kg q12h in patients below 50kg) and 8mg/kg/day in children. Because of the

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increased concentration of lamivudine in patients who have renal impairment, doses must be reduced in the presence of a creatinine clearance below 50ml/min. A dosage of 2mg/kg q12h is recommended in patients whose body weight is below 50kg. Lamivudine can be taken with food and its bioavailability is about 86% in adolescents and adults and 66% in children. Trimethoprim-sulfamethoxazole can increase the concentrations of the drug. Lamivudine can cross the blood-brain barrier, but to a limited extent, with CSF to plasma ratios of 6% in adults and 15% in children.

The main adverse effect associated with lamivudine therapy is reversible neutropenia. This can be exacerbated with zidovudine use and advanced HIV disease. Sporadic cases of pancreatitis and rash have also been reported. In pediatric patients receiving lamivudine, an increased frequency of pancreatitis has been reported, suggesting that particular caution is needed in children who have a previous history or at risk of pancreatitis. The drug has also shown in-vitro activity against hepatitis B virus (see Chapter 125).

Stavudine

The recommended dose of stavudine (d4T) is 40mg q12h (30mg q12h if weight is less than 60kg). The drug should be taken at least 1 hour before meals and has good bioavailability (80%); its terminal half-life is about 1.3 hours (3.5 hours intracellularly). It crosses the blood-brain barrier, although variable CSF to blood ratios have been reported. Elimination seems to occur by excretion in the urine (as unchanged drug) and by elimination through endogenous pathways. Pharmacokinetics in patients who have hepatic impairment are similar to those in patients who have normal hepatic function. Within the cell, zidovudine-5'-monophosphate may inhibit the production of stavudine-5'-monophosphate, suggesting that zidovudine and stavudine may be antagonistic and should therefore not be co-administered in combination.[13] This antagonism has been documented in vivo.

The main adverse effect associated with stavudine therapy is peripheral neuropathy, which is more frequent at higher doses (i.e. 40mg q12h), in advanced disease and in patients who have a previous history of neuropathy. The neuropathy is usually reversible at the cessation of treatment. A mild degree of bone marrow inhibition has also been reported, with moderate macrocytosis. Asymptomatic increases in liver function tests are also possible in about 10% of treated patients, and a small number of cases of pancreatitis have been observed. The risk of asymptomatic hyperlactatemia and lactic acidosis may be greater with stavudine than with other nucleoside analogues, particularly when used in combination with didanosine. Stavudine is also linked to the loss of peripheral fat (lipoatrophy).

Abacavir

Abacavir is the only guanosine analogue among the nucleoside reverse transcriptase inhibitors. After a unique phosphorylation pathway it is converted to carbovir triphosphate and subsequently metabolized by hepatic glycuroconjugation and renal excretion. The recommended dosage is 300mg q12h (with or without food). Central nervous system penetration (0.18 CSF:plasma ratio) and activity on viral load is probably superior to first-generation nucleoside analogues. It is increasingly being used in three-nucleoside combination regimens as a co-formulation with zidovudine and lamivudine. There is some degree of cross-resistance with other nucleoside analogues (didanosine, zalcitabine and lamivudine).

The toxicity profile is characterized, together with relatively nonspecific adverse events (diarrhea, nausea, abdominal pain, asthenia, headache, transaminase and creatinine increases) by a potentially serious hypersensitivity reaction (fever with nausea or vomiting, asthenia or flu-like symptoms, often accompanied by rash), which requires permanent discontinuation of the drug because of the risk of life-threatening reactions associated with drug rechallenge.[14]

Tenofovir

Tenofovir disoproxil fumarate is a prodrug that in vivo is converted to tenofovir, an acyclic nucleoside phosphonate, which represents a nucleotide analogue of adenosine 5'-monophosphate with inhibitory activity against HIV reverse transcriptase. The recommended dosage is 300mg q24h, to be taken with food in order to increase bioavailability. It is mainly excreted through the kidneys and the potential for interactions based on cytochrome P450 enzymes is considered to be low. Resistance profile of tenofovir seems to be unique among nucleoside reverse transcriptase inhibitors, with a limited degree of cross-resistance with other nucleoside analogues that needs to be further defined.

Information on toxicity profile is restricted to short-term side effects, mainly represented by gastrointestinal symptoms, asthenia and headache. Because of its renal metabolism, it is not currently recommended in patients who have low values of creatinine clearance. At present, clinical experience is more limited than with other nucleoside analogues, but preliminary results suggest that three-drug regimens (two nucleoside reverse transcriptase inhibitors plus one NNRTI) that include tenofovir may induce viral load reductions and $CD4^+$ lymphocyte increases comparable to those obtained with other commonly used three-drug regimens.

Nevirapine

Nevirapine (NVP) is the first compound to be approved among HIV NNRTIs. Recommended dosing is 200mg orally once daily for the initial 2 weeks, to be increased to 200mg q12h thereafter. Bioavailability is approximately 65%. Skin rash is the most common adverse event and may occur in up to 30% of nevirapine-treated patients by 24 weeks. In less than 5% of patients rash may be severe and, although rare, Stevens-Johnson syndrome has been described. Headache, diarrhea, nausea, fatigue, drowsiness, fever, chills, and joint and muscle aches are also reported. Hepatotoxicity usually occurs in the first 12 weeks of treatment, is more common in women and may be severe or fatal.

Delavirdine

Delavirdine (DLV) is another approved NNRTI. Recommended dosing is 400mg orally q8h. Adverse effects are headache, skin rash — generally maculopapular — fatigue, gastric intolerance, change in body temperature, increase in liver enzymes, faintness, dizziness or lightheadedness, changes in stools and leg cramps. Delavirdine inhibits cytochrome P450 enzymes, and various drug-drug interactions have been described when it is associated with PIs.

Efavirenz

Efavirenz is a NNRTI that can be administered once daily (600mg at bedtime, with or without food) because of a long plasma half-life. The main adverse effect associated with efavirenz is dizziness. Other CNS symptoms such as abnormal dreams, insomnia, hallucination and euphoria may also occur. Nausea, rash and hypersensitivity reactions have also been reported. Major congenital abnormalities have been observed in monkeys whose mothers were treated with efavirenz during pregnancy. Women on efavirenz treatment must therefore avoid pregnancy.

Like nevirapine, being an inducer of the P450 (CYP) 3A4 cytochrome isozyme, efavirenz can reduce the concentrations of drugs (such as indinavir, saquinavir or lopinavir) that are metabolized by this enzyme. It should not be administered with terfenadine, astemizole, cisapride, midazolam and triazolam because competition for the CYP3A4 isozyme may occur, leading to severe side effects. The resistance profile indicates cross-resistance with other NNRTIs.

PROTEASE INHIBITORS

Protease inhibitors act on the binding to the catalytic site of the HIV aspartic protease. This enzyme is critical in the post-translational processing of the polyprotein products of *gag* and *gag-pol* genes into the functional core proteins and viral enzymes, respectively. Its inhibition leads to the release of immature, noninfectious viral particles. Most of the PIs are compounds that mimic the part of the structure of Gag-Pol protein that is recognized by HIV protease.

Protease inhibitors, as nucleoside analogues, are active on HIV-1 and HIV-2, and have shown antiviral activity in primary human lymphoid and monocytic cell lines and against a variety of viral strains. However, unlike inhibitors of reverse transcriptase, which provide no protection in established in-vitro infection, PIs are active in chronically infected cells. Finally, PIs are active as the administered compound and do not need intracellular phosphorylation. Although these factors may imply a prolonged activity in a wider range of cells, many PIs are significantly bound to plasma proteins, with subsequent reduction in cellular uptake and, thus, intracellular levels. The possibility of achieving elevated blood levels is also affected by the bioavailability of these drugs, which is usually lower than that of reverse transcriptase inhibitors, mostly because of limited absorption and first-pass hepatic metabolism.

Protease inhibitors are generally dependent on the cytochrome P4503A hepatic isozyme for metabolism and can compete with other substrates of this enzyme. When the metabolism of other drugs that are dependent on the same enzyme is inhibited, the blood levels of these drugs can increase dramatically and toxic interactions may occur. This phenomenon has been evaluated for many of the compounds that are frequently used in patients who have HIV disease, and it has been shown that the combination of nucleoside analogues and PIs does not generally lead to untoward effects, although important pharmacokinetic interactions are possible for rifabutin, ketoconazole, rifampin (rifampicin), astemizole, terfenadine, cisapride and other drugs that are dependent on the P4503A hepatic isozyme for metabolism; therefore, attention should be paid before prescribing these drugs together with PIs. However, the concomitant administration of PIs and other drugs with hepatic metabolism may lead to decreased levels of these drugs (see Chapter 141).

Metabolic complications have recently emerged in patients treated with PIs. These include glucose metabolism abnormalities (hyperglycemia or diabetes), hyperlipidemia (mainly hypertriglyceridemia, with or without associated hypercholesterolemia), lipodystrophy or abnormal fat distribution (accumulation in the posterior neck, upper back and central abdomen). The pathogenesis of these abnormalities is still unclear and further studies are needed to define the role played by HIV infection, individual predisposing factors and treatment with specific drugs in the development of these complications.

Available data on the penetration of PIs into the CNS indicate that this is generally low, suggesting that, for optimal clinical use, these drugs should be combined with antiretrovirals that enter the CNS. The rapid development of resistance using reduced doses of PIs indicates that full dosage and adequate compliance should be maintained whenever possible. Protease inhibitors are generally used in combination with reverse transcriptase inhibitors or with another PI, taking advantage of a boosting effect on drug concentrations that occurs when PI are coadministered. The boosting effect, related to inhibitory activity on P450, is most pronounced with ritonavir, which is therefore commonly combined at low dosages (100mg q12h) with other PIs.

Saquinavir

Saquinavir (SQV) was the first PI to be approved in combination with other antiretrovirals for the treatment of HIV infection. A new formulation soft gel with enhanced bioavailability, to be administered at a dosage of 1200mg q8h or at lower dosages with ritonavir q12h, has actually replaced the previous formulation in clinical practice. Saquinavir has a low bioavailability (approximately 4% because of limited absorption and extensive first-pass metabolism). The drug should be taken with food, because drug bioavailability is significantly reduced in the fasting state. The drug is consistently tissue-bound, inactivation is rapid and elimination is predominantly nonrenal.

Diarrhea, abdominal discomfort or pain, headache, nausea, mouth ulcers, dizziness, numbness or tingling in limbs, rash, muscle aches and tiredness have been reported with increased frequency among patients taking saquinavir.

Ritonavir

Ritonavir (RTV) is a PI with potent antiviral activity in vivo. Recommended dosing is 600mg orally q12h. It should be taken with meals if possible. Bioavailability is about 60–70%, with minor differences in the blood levels between the nonfasted and fasted state. Ritonavir is approximately 98–99% plasma-protein-bound. After oral administration, 86% of the given radiolabeled dose was recovered in the feces and less than 4% in the urine. Ritonavir has a marked interference with hepatic metabolic enzymes, mainly represented by inhibition of the cytochrome P450 isoenzyme CYP3A, which can lead to remarkably increased blood levels of other drugs sharing the same metabolic process; these therefore must be avoided in patients receiving ritonavir. It is increasingly, and almost exclusively, used at low dosage as a pharmacokinetic enhancer of other PIs; ritonavir administration (100 or 200mg q12h) significantly increases the plasma levels of other PIs, allowing achievement of therapeutic plasma levels with lower doses and with more convenient schedules (q12h instead of q8h). Significant hepatic impairment is likely to induce a marked decrease in the metabolism and elimination of ritonavir.

The most common clinical side effects are circumoral paresthesias and diarrhea and/or vomiting. Headache, fever, numbness and tingling, muscle weakness and lightheadedness have also been described. Gastrointestinal side effects may be reduced by starting ritonavir alone and then adding the other drugs and gradually increasing ritonavir dosages over 7–10 days. The most frequently reported laboratory abnormalities have been liver enzyme elevations and elevations in blood lipids. Ritonavir should be used with caution among patients at high risk of bleeding, such as hemophiliacs.

Indinavir

Indinavir (IDV) is a PI of similar potency to ritonavir. Recommended dosing is 800mg q8h orally. Indinavir is rapidly absorbed in the fasted state, with a bioavailability of approximately 60%; a decrease in blood levels by approximately 80% is seen when the drug is administered with a high-fat meal, whereas lighter meals have no relevant effect on pharmacokinetics. Binding of indinavir to human plasma proteins is about 60% and metabolism is P450 dependent. After oral administration of a single 400mg dose, over 80% of radioactivity is detected in the feces, with a mean recovery in the urine of 19%; renal clearance studies suggest a secretory component in excretion, which may play a role, together with the observed supersaturation of the urine with indinavir dosages above 600mg, in the formation of indinavir urinary crystals.

Nephrolithiasis and microscopic hematuria can occur in 10–28% of treated patients, and a high fluid intake (at least 2 1/day) is therefore recommended to prevent this complication. Another laboratory side effect that has been frequently associated with indinavir sulfate is hyperbilirubinemia and, at times, jaundice. Other side effects include nausea, vomiting, diarrhea, rash, fatigue and headache.

Nelfinavir

Although introduced later than other PIs, nelfinavir (NFV) is widely used in clinical practice because of its relatively good tolerability/activity ratio. In clinical trials and observational studies, the use of nelfinavir together with nucleoside analogues has resulted in significant and sustained reductions in viral load and in marked increases in $CD4^+$ lymphocyte counts. It can be administered both q12h (1250mg per dose) or q8h (750mg per dose).

The tolerability profile is relatively good compared with other PIs, with diarrhea representing the most commonly observed adverse event and a main cause for drug discontinuation. Other PI-related adverse events may also occur, such as lipid and glucose abnormalities and possible increase in risk of bleeding in patients who have hemophilia. Resistance studies suggest that, if under treatment virus mutates along a specific mutation pathway (D30N), the efficacy of other PIs may be relatively preserved (see Chapter 137).

Lopinavir

Lopinavir is a very potent inhibitor of HIV-1 protease. Its bioavailability is greatly enhanced by coadministration of ritonavir and is therefore co-formulated at a 4:1 ratio with ritonavir (capsules: 133.3mg lopinavir/33.3mg lopinavir; oral solution 80mg lopinavir/20mg ritonavir per ml). Recommended dosage is 400/100mg q12h with food. Data on clinical progression are not yet available. Lopinavir has been used in patients in whom previous treatment has failed and in previously untreated patients. Available data indicate that the drug has high potency on viral load and $CD4^+$ lymphocyte counts, and efficacy both in salvage regimens and in previously untreated patients. Lopinavir is principally metabolized by the liver, and shares many of the drug interactions and contraindications common to other PIs.

The safety profile of the drug is not fully assessed because of the small number of patients as yet studied. The main adverse events observed in clinical trials were diarrhea and other gastrointestinal complaints. Hyperlipidemia is also relatively common. Rash was observed in some children. The high potency characteristics make this drug a potentially important element in the design of particularly potent first-line drug regimens or salvage strategies.

Amprenavir

Amprenavir (APV) is a PI approved for use in both adults and children. It is available both as capsules and as oral solution and can be administered q12h, without restrictions in terms of food timing. Coadministration of ritonavir, as for other PIs, improves amprenavir pharmacokinetic parameters and allows dosage reductions. There are limited data from controlled trials comparing amprenavir and other PIs in terms of efficacy.

The safety profile is also only partially assessed because of the relatively recent introduction of the drug. Preliminary data suggest that amprenavir is relatively well tolerated, with gastrointestinal disturbances and rash representing the main events leading to treatment discontinuation in early trials. Oral/perioral paresthesias have also been observed. The drug is metabolized by the P450 isozyme system and shares some of interactions and contraindications common to other PIs. Amprenavir should be avoided in patients who have sulfonamide allergy. Judging from the evidence of in vitro studies and on preliminary observations in vivo, the drug might also have, as compared with other PIs, a lower impact on metabolic status and on lipodystrophy. The resistance mutation profile is also potentially interesting, suggesting lower potential for cross-resistance with other drugs of this class. These specific tolerability and resistance characteristics, if confirmed by further evaluations, may prove useful in the design of sequential therapeutic strategies against HIV.

NEW INVESTIGATIONAL AGENTS (see also Chapter 204)

A number of novel nucleoside (nucleotide) reverse transcriptase inhibitors (NRTIs), NNRTIs and PIs are currently in development. Desirable characteristics of this new generation of drugs include easier administration schedule, perfected pharmacokinetics, potency, tolerability and the ability to inhibit HIV viruses that are resistant to the existing agents.

TMC125, a new NNRTI, showed high antiviral effect in preliminary studies based on small samples of patients. In treatment-experienced patients failing efavirenz- or nevirapine-containing therapy, TMC125 administered at a dose of 900mg q12h for 7 days decreased the median viral load by approximately 1$\log_{10}$ copies/ml compared with baseline.[15] Diarrhea and headache were the most frequent adverse effects reported by patients taking the drug.

Tipranavir, a new PI, administered together with a small dose of ritonavir in patients who were failing multiple PIs, showed an interesting resistance profile. After more than 1 year of tipranavir/ritonavir treatment, only 2% of the clinical isolates (5/41) exhibited a reduced susceptibility to tipranavir. Moreover, the number of mutations in the protease gene at baseline did not influence the virologic response to tipranavir/ritonavir, and tipranavir/ritonavir treatment did not affect the susceptibility of HIV to other PIs.[16] These data indicate that resistance to tipranavir is uncommon in HIV-positive patients who have failed multiple PI-containing regimens, either at baseline or after 1 year of treatment.

Atanazavir is a new once-daily PI in development that seems to be characterized by a favorable metabolic profile; the drug in antiretroviral-naive individuals had no negative impact on levels of total cholesterol, low-density lipoprotein or triglyceride.

Although current antiretroviral treatment is still based on reverse transcriptase and PIs, inhibitors of the entry/fusion process of HIV into the target cell (T20 and T1249) gave important results in preliminary clinical trials and are likely to join the therapeutic repertoire soon, mainly as components of salvage regimens. In addition, integrase inhibitors are also entering clinical evaluation.

The chemokine receptors CCR5 and CXCR4, which represent co-receptors used by HIV strains in addition to CD4 to enter target cells, are also potentially important therapeutic targets. Antiretroviral agents that inhibit HIV-1 entry by blocking these co-receptors are being developed and some of them recently entered phase I studies.

NEWLY RECOGNIZED ADVERSE EVENTS OBSERVED IN PATIENTS UNDERGOING POTENT ANTIRETROVIRAL THERAPY

In addition to drug-specific adverse events, the long-term use of potent antiretroviral therapy has highlighted a number of adverse events that were previously not recognized. Some of these events appear to be associated with a specific class of drugs, such as mitochondrial toxicity and lactic acidosis with NRTIs or hyperglycemia with PIs; in other cases the association with a single class of drugs is less evident and the pathogenesis of the adverse events is poorly understood (e.g. metabolic disturbances, fat maldistribution, bone abnormalities). All these events may seriously jeopardize adherence and continuation of antiretroviral treatment and therefore represent a major problem in clinical management.

Possible manifestations of NRTI-related mitochondrial toxicity include cardiomyopathy, myopathy, peripheral neuropathy, pancreatitis, proximal renal tubular dysfunction, hepatic steatosis and lactic acidosis. However, it needs to be considered that many effects associated with mitochondrial toxicity are difficult to distinguish from effects associated with HIV infection itself.

Fat distribution changes (also commonly described as lipodystrophy) represent a major event in terms of clinical relevance. This syndrome may present with atrophic changes (peripheral fat loss involving face and limbs), hypertrophic changes (visceral or dorsocervical fat accumulation, breast enlargement) or both. A dysmetabolic profile involving hyperglycemia, hyperlipidemia and hyperinsulinemia is frequently but not always associated with the lipodystrophy syndrome. A common case definition is still lacking, pathogenesis is uncertain and treatment strategies have proved unsatisfactory so far in terms of efficacy. The impact of metabolic changes on incidence of cardiovascular disease is also poorly defined. Although a number of biochemical and vascular abnormalities have been reported, observational studies have yielded conflicting results, with some studies showing association between potent antiretroviral therapy and increased risk of cardiovascular disease and others not indicating an increased number of cardiovascular events following introduction of potent antiretroviral therapy regimens. Bone abnormalities have also been reported, with no clear association with any specific class of antiretroviral drugs; the abnormalities observed include avascular osteonecrosis, osteopenia and osteoporosis, sometimes in association with metabolic changes.

Although lack of a standard case definition complicates characterization, diagnosis and tracking, the morphologic changes have a real and substantial impact on quality of life. Cardiovascular sequelae of dyslipidemia appear to be minimal in the short term but long-term outcome is unknown. So far, management approaches of fat redistribution syndromes and of metabolic toxicities are largely empirical. Only a few of them are based on short-term controlled studies: antiretroviral therapy switches, exercise and diet, anabolic steroids, recombinant human growth hormone (rhGH), testosterone, metformin, thiazolidinediones and plastic surgery for fat redistribution syndromes; lifestyle modification (diet, exercise), use of lipid-lowering agents and switching to non-PI regimens for dyslipidemic syndromes.

HIV RESISTANCE TO ANTIRETROVIRAL DRUGS

Definition and biologic basis of resistance

Emergence of HIV resistance to all classes of antiretroviral drugs used until now has been described. Although other factors may play a role in determining the loss of efficacy of antiretroviral treatment, drug resistance plays a major role in this phenomenon, and great attention is paid to possible strategies to prevent or delay it.

How treatment can affect resistance

Because resistant variants may exist before treatment and evolve under selective pressure, therapy can address viral resistance in three ways:

- by maximizing the suppression of viral replication;
- by using drugs where multiple mutations are required for resistance; and
- by forcing the emergence of variants that result in attenuated replication or decreased virulence (see Chapter 137).

Resistance to nucleoside analogues

HIV variants with decreased susceptibility were first reported in clinical isolates in 1989 for zidovudine, followed by reports of drug-resistance to the other nucleoside analogues and PIs. Advanced disease stage, baseline low $CD4^+$ lymphocyte count and high RNA plasma levels are strongly predictive of the development of resistance. With zidovudine (as with some PIs), resistance appears to be the consequence of a stepwise accumulation of mutations. For other drugs, such as didanosine and zalcitabine, the mechanisms and the molecular correlates of resistance are less clear, although a number of mutations responsible for reduced susceptibility have been identified.

Resistance to zidovudine is the most widely explored in its molecular and clinical aspects. It is related to the ordered emergence of HIV variants with mutations at reverse transcriptase codons 215, 70, 41, 67 and 219, with wild-type strains showing a generally narrow range of IC_{50} –IC_{90} . From a clinical point of view, zidovudine resistance has been shown to predict a more rapid progression of HIV disease, although the clinical significance of resistance to the dideoxynucleosides has not yet been completely defined. High-level resistance to didanosine or zalcitabine has not been reported to date and there is no clear explanation for these findings. Cross-resistance has been reported and multidrug resistance has been observed after combination therapy with nucleoside analogues.

Development of resistance may not necessarily represent an entirely negative event; under treatment with lamivudine, resistance occurs rapidly in vivo, associated with a substitution at codon 184. This codon change may antagonize the effect of zidovudine resistance mediated by the 215 (and 41) mutations, leading to a restored phenotypic sensitivity to zidovudine.[17] This mechanism, however, does not seem to be effective in all cases, with dual zidovudine/lamivudine resistance also observed.

Resistance to non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitor use in monotherapy is associated with a rapid development of resistance, which initially suggested a limited usefulness in clinical practice. However, when these drugs were used in triple combination regimens with nucleoside analogues, resistance did not occur in patients who had a sustained viral load suppression to undetectable levels, supporting the concept that resistance occurs as a direct consequence of viral replication. Many NNRTIs have a common pattern of resistance, and this limits sequential use of these drugs because of cross-resistance.

Resistance to protease inhibitors

As for the other classes of antiretrovirals, reduced sensitivity has been reported for all tested PIs, with some strains exhibiting cross-resistance to different PIs after in-vitro or in-vivo drug exposure.[18] The patterns of mutations, however, appear to be more complex than for reverse transcriptase inhibitors, with a higher number of sites involved and higher variability in the temporal patterns and combinations of mutations leading to 'phenotypic' resistance; this suggests that the protease can adapt differently and perhaps more easily than the reverse transcriptase under the genetic pressure induced by drugs. At present, although about 20 codons have been identified as possible mutation sites, several different genetic profiles can be identified and can therefore be employed in designing sequential treatment.

Resistance to entry/fusion inhibitors

Unfortunately, HIV resistance should be expected with any class of drugs used. The increasing clinical use of fusion inhibitors in HIV-infected patients has recently highlighted the occurrence of resistance in some patients receiving the fusion inhibitor enfuvirtide (T-20) in monotherapy.[19]

Clinical implications of drug resistance

Resistance may be a consequence of incomplete viral suppression (in the presence of antiretroviral drugs) and this what usually occurs during the virologic failure of first-line therapy. On the other hand, resistance may be a *cause* of incomplete viral suppression if mutations

pre-exist, which is the case with patients failing second- and third-line regimens.

In all cases, the presence of drug resistance, together with other factors (low antiviral potency of the regimen, poor adherence, pharmacokinetic interactions) may compromise the effectiveness of treatment. Information on resistance status is therefore considered useful both in instituting antiretroviral treatment in previously untreated patients and in managing therapeutic decisions in patients already on antiviral therapy. There is, however, uncertainty about the weight of resistance data in clinical decisions, because available resistance tests are as yet not fully validated and are based on different techniques. In the interpretation of results, absence of resistance (through genotypic or phenotypic testing) does not rule out the possibility of resistant virus in small quantities or in tissue reservoirs. Conversely, evidence of resistance, although indicating a potential reduction in the activity of the drug, should always prompt (before instituting treatment changes) a careful technical interpretation of the resistance test used, longitudinal evaluation of viral load and $CD4^+$ trends over time, an estimate of adherence to treatment and a review of concomitant treatments potentially interfering with drug efficacy. Further studies are needed to define which resistance test(s) will be more adequate in clinical management. Until their validation, changes in HIV RNA levels, together with treatment history, represent the dominant parameters that should guide treatment changes, and the additional information provided by resistance testing should at present be included in a global evaluation of the patient's response to treatment.

HIV TREATMENT STRATEGIES

Principles

The treatment of HIV infection is one of the most rapidly evolving fields in medicine. Advances in basic research, the development of new technologies for the monitoring of therapy, the continuous introduction of new drugs into clinical practice and the relentless dissemination of the results of recent trials contribute to create new expectations and to suggest new strategies for optimal management of the disease. The dynamics of HIV-1 replication in vivo strongly suggest that HIV should definitely be 'hit hard' with potent combinations of antiretroviral drugs to minimize the negative consequences of HIV replication and genetic evolution. Recent data, however, have indicated that replication-competent HIV may survive, even in the presence of highly active treatment, within latently infected cells in the blood or in tissue reservoirs and 'sanctuary sites' inaccessible to treatment in lymphoid tissues, bone marrow and other macrophagerich tissues and organs.

More studies are needed to define the size and distribution of the latent/infected cellular population, the impact of treatment on HIV load in latent reservoirs, and the extent to which immune reconstitution is possible in already immunocompromised patients.

A variety of therapeutic guidelines have been developed to keep clinical practice up to date, as far as possible, with the data emerging from basic and clinical research. The recommendations currently represent an authoritative reference in the field.[20] National guidelines have been published, in the USA[21] and in many other countries.

When to start treatment

Early initiation of therapy with a combination of antiretroviral drugs has been shown to produce more durable clinical benefit than the initiation of therapy in advanced disease. Therapy should be started before the development of symptoms and fall of $CD4^+$ lymphocyte count to low levels, using regimens able to induce and maintain maximal viral suppression and to prevent the emergence of resistant strains. Moreover, because even complete inhibition of viral replication may not be able, in the long term, to prevent the selection of resistance mutants if they exist at a high enough frequency before therapy, treating very early in the course of the infection (i.e. at or immediately before seroconversion), when the virus population is at its most homogenous, may be more beneficial. For these reasons, in the mid-late 1990s, the treatment philosophy for many HIV/AIDS physicians and patients was 'treat early, treat hard'.

However, more recently, the growing appreciation of the difficulties of taking antiretroviral therapy on a long-term basis, the adverse effects of many antiretroviral drugs and regimens, and their negative impact on quality of life has led to a re-evaluation of this early intervention strategy. With 'eradication' not on the horizon, the goal of therapy must now be redirected toward the long-term management of a chronic infection. The decision of when to initiate treatment is now based first on the patient's disease stage, because its clear that a symptomatic patient should always be treated. For an asymptomatic patient, the decision should be driven mainly by the $CD4^+$ lymphocyte count (which is an immediate predictor of progression) and the plasma HIV-1 RNA level (which is an indicator of the level of actual HIV replication and predicts the subsequent rate of loss of $CD4^+$ lymphocytes). Other elements should, however, be factored into the decision to start treatment: the patient's commitment to therapy and a knowledge of the limitations of current regimens.

Therefore, the decision to initiate therapy in asymptomatic patients should be based on prognosis as determined by the $CD4^+$ T cell count and RNA level. Despite the fact that serious complications of HIV infection do not frequently occur when $CD4^+$ lymphocytes are still above 200/mm^3 , a reasonable approach is to start treatment if $CD4^+$ lymphocytes decrease below 300–350/mm^3 and to treat independently of $CD4^+$ if viral load is particularly high (plasma HIV RNA >50,000–100,000 copies/ml). In any case, treatment should be started only when the patient has been educated regarding therapy and is committed to adherence.

A situation that may indicate treatment is primary/acute HIV infection; when viral load is generally remarkably high, the infection disseminates in several reservoirs and the interaction between HIV and the immune system establishes a 'set point' of viremia, which is predictive of the subsequent course of the disease. It now seems clear that this condition can be clinically identified in some patients, allowing a timely intervention that could potentially have positive consequences on the course of the

disease. Whether it is possible to discontinue therapy after a prolonged period of adequate suppression is the subject of ongoing trials (see Chapter 122).

What to start with

With combination therapy representing the key to an effective treatment, achieving a low or undetectable viral load is dependent on the availability of a sufficient number of drugs with as wide a specificity of action as possible, and by a rational design of combination regimens (Table 139.2). An optimal combination regimen should fulfill several requirements, the first of which is a high antiviral potency. Although viral suppression in vitro is indicative of the drug's efficacy, a better estimate is obtained by measuring the effect of the regimen on HIV viral load in vivo. Indeed, some of the new drugs and regimens have recently proved effective in reducing the number of plasma HIV-1 RNA copies by 3$\log_{10}$ from baseline, compared with the mean 0.5–1$\log_{10}$ reduction that is characteristic of most of the first-generation, monotherapy regimens, with a more durable response in terms of $CD4^+$ and HIV RNA (Fig. 139.2). Similarly, and perhaps more importantly, regimens characterized by a substantial proportion of patients achieving durably 'undetectable' viral load should be given priority in clinical practice.

TABLE 139-2 -- Criteria for the selection of combination regimens.

CRITERIA FOR THE SELECTION OF COMBINATION REGIMENS
• High and sustained antiviral activity in vivo (able to induce at least 2$\log_{10}$ reduction in viral load; able to achieve 'undetectable' HIV-1 RNA level in > 70% of patients)
• Simultaneous targeting of multiple viral enzymes
• Wide spectrum cell specificity (e.g. lymphocytes/macrophages, active/resting)
• Wide spectrum tissue activity (lymph nodes, other sanctuary sites, CNS)
• Absence of cross-toxicity patterns
• Induction of 'favorable' mutations (reduced replicative capacity, reversal of resistance, enhanced 'fidelity')
• Absence of cross-resistance (allowing a higher number of subsequent options)
• Favorable pharmacokinetic interactions (e.g. increase in plasma concentrations of one of the drugs used)
• Accessibility, tolerability, compliance and therapeutic index

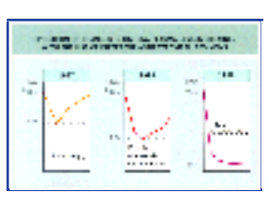

Figure 139-2 Evolution of changes in HIV RNA plasma levels obtained with the use of different antiretroviral regimens.

As for any combination therapy, the drugs selected should also show different toxicity and resistance profiles and be characterized by distinct tissue or cellular specificity to ensure a more generalized viral suppression in the body. Of particular relevance are:

- ¦ penetration into the CNS, because of its possible role in preventing HIV-associated neurologic disease;
- ¦ possible differences in antiviral activity among resting or activated cells, according to specific phosphorylation profiles of nucleoside analogues in different cells;[22] and
- ¦ wide cell and enzyme target profiles — two or more drugs 'convergent' against the same HIV enzyme can reduce the possibility of viral 'escape' of variants with functional mutations, whereas combining reverse transcriptase and PIs may allow activity on acutely and chronically infected cells, adding effectiveness to the regimen.

Another important tool in designing treatment is represented by information on the resistance profile of the drugs, the impact of drug-induced mutations on the replicative characteristics of the virus and sensitivity to other drugs that have been previously used or could be used sequentially. Pharmacokinetics also represent an important aspect in the design of the regimen because an adequate knowledge and rational use of drug interactions allows a simpler administration schedule and an increased potency regimen. Finally, it must always be considered that HIV infection is a long-term disease mostly affecting young people; in this condition, as for other chronic diseases necessitating continuous treatment, tolerability, compliance and accessibility are important factors that must carefully be considered in the design of treatment to ensure compliance and efficacy.

In conclusion, factors determining therapeutic efficacy are certainly linked to the quality of the antiretroviral agents (potent combinations, tolerability, convenient dosing, affordability) but also to the health care provider (who should be accessible in working with patients, give clear instructions and counseling, and use an individualized approach) and to patient commitment and motivation to treatment (conducive lifestyle, family support).

Different combinations of drugs belonging to the three classes have shown similar activity and efficacy in the short to mid term. Considerations in the selection of the most appropriate regimen for the individual patient may include potency; side effect profile; patient's predicted adherence, with potential effect on quality of life and pill burden; the potential for maintenance of future options in terms of drug class sparing and cross-resistance profile; the presence of co-morbid conditions and medications; and, finally, in certain clinical circumstances and geographic areas, the potential for primary acquisition of resistant viral strains.

The most widely used regimens include:

- ¦ PI (± low-dose ritonavir) + two NRTIs (NNRTI-sparing regimen);
- ¦ NNRTI + two NRTIs (PI-sparing regimen); and
- ¦ three NRTIs, including abacavir (PI/NNRTI-sparing regimen).

Other combinations under clinical investigation include a regimen with drugs belonging to all three classes (1–2 PIs + NNRTI + 1–2 NRTIs) and a combination that excludes NRTIs (PI/low-dose ritonavir + NNRTI).

All available regimens have both advantages and disadvantages. For PI-containing regimens the advantages include the solidity of available clinical data, the longest experience, high potency and a relatively high genetic barrier to resistance. Combinations of two PIs are increasingly being used instead of a single PI because they have pharmacokinetic advantages and possibly increase the PI regimen's potency while greatly improving adherence to therapy. Addition of a low dose of ritonavir (100 or 200mg q12h) to saquinavir, indinavir or amprenavir improves the pharmacokinetic profile, reduces pill burden, lowers the dose frequency, lowers the costs and obviates the need for administration of PIs on an empty stomach. A coformulation of lopinavir + ritonavir has recently been introduced. Disadvantages of PI-containing regimens are mainly represented by the high risk of metabolic complications, which, in the long term, may occur in a considerable proportion of patients (>40%).

Advantages of NNRTI-containing regimens, which appears to be equipotent to PI-containing regimens, include very low pill burden and convenience. The main disadvantage, together with some relevant although organ-focused toxicities, is the low genetic barrier to resistance, which limits the use of other members of this class.

Combinations composed only of NRTIs have the advantage of deferring the use of two classes of antiretroviral (the PIs and the NNRTIs). For patients who have high viral loads, more data are needed regarding the efficacy of triple NRTI regimens. The long-term consequences of NRTI additive toxicities are unknown. In addition, most available efficacy data refer to combinations including abacavir, with which serious hypersensitivity reactions may occur in about 5% of patients at institution of therapy.

Specific consideration is necessary for the selection of the most appropriate NRTI backbone combination. As initial treatment often fails, it is likely that a sequence of

NRTIs will be given during the lifetime of an HIV-infected patient. The best NRTI backbone to be used first to give the optimum chance of the second regimen being

successful is currently unknown and is under evaluation in strategic trials. In general we rarely use zalcitabine because of its significant potential for neurotoxicity and the need for q8h administration. Clinical data suggest that stavudine and zidovudine should not be given in combination. There is recent evidence of increased toxicity when stavudine and didanosine were used together and therefore we are less enthusiastic about this combination at the present time. We tend not to recommend abacavir and nevirapine together as initial therapy because there is evidence that the nevirapine rash may complicate the management of suspected abacavir hypersensitivity.

Most of the experience for initial therapy has been accumulated regarding zidovudine plus lamivudine, or stavudine plus lamivudine. Tenofovir plus lamivudine has also been shown in prospective trails to be effective and well tolerated. Zidovudine plus didanosine has been extensively studied in the past but issues of palatability relating to the didanosine formulation precluded widespread use of this combination. More recently, with the availability of an enteric-coated formulation of didanosine, this has been circumvented and the new formulations allow once-daily dosing, which is particularly attractive for certain patients. Despite the limited data available for abacavir in the initial regimen, this agent has worked well, particularly with lamivudine or as part of a zidovudine and lamivudine triple combination. However a recent study comparing zidovudine and lamivudine plus efavirenz to zidovudine and lamivudine plus abacavir suggested the three-nucleoside combination was inferior as initial therapy. Finally, the likelihood of potential toxicities will ultimately help us to decide what is the best backbone NRTI combination for a given patient.

Changing treatment

The success of antiretroviral treatment can be evaluated in different ways. From a laboratory point of view, sustained maximal viral load reduction is certainly proof of the activity of the selected regimen on HIV replication, together with a sustained rise in $CD4^+$ lymphocyte counts. However, we should not lose sight of the need for improved clinical outcome (in terms of opportunistic infections, symptomatic HIV disease and survival) and of the need to preserve the patient's quality of life.

Criteria for changing therapy (Table 139.3) include:

- a suboptimal reduction in plasma viremia after initiation of therapy;
- re-appearance of viremia after suppression to undetectable;
- significant increases in plasma viremia from the nadir of suppression; and
- declining numbers of $CD4^+$ T cells.

To exert a significant effect on the viral 'background' of the patient, changes should therefore be implemented before complete virologic failure, selecting at least two potent drugs not previously used by the patient with appropriate toxicity and resistance profiles. However, because of the lack of many options for changing treatment, one must not prematurely abandon a given regimen. In patients who have very high baseline RNA levels, maximal suppression may not be seen until after 12–24 weeks of potent therapy. Moreover, a careful assessment of adherence must also be made before deciding to switch the patient to a new therapeutic regimen.

Any discussions on salvage strategies requires a definition of treatment failure. For the adherent patient on an initial treatment regimen, confirmed detectable plasma HIV-1 RNA (>50 copies/ml) should be considered evidence of treatment failure. Continued treatment with the same regimen in this situation will eventually lead to development of high-level drug resistance and diminishes the likelihood that salvage regimens will be successful. Thus, for the patient who has clear treatment options, early switching could maximize the chances for therapeutic success of the next treatment regimen and preserve future options.

TABLE 139-3 -- Use of viral load in the management of antiretroviral therapy.

USE OF VIRAL LOAD IN THE MANAGEMENT OF ANTIRETROVIRAL THERAPY	
Parameter	**Recommendation**
Plasma HIV RNA levels that should lead to close monitoring and consideration of starting treatment, regardless of laboratory or clinical status	50,000–100,000 copies/ml or more
Target HIV RNA level after initiation of treatment	Undetectable
Suggested frequency of HIV RNA measurement	At baseline: two measurements, 3–4 weeks apart
	One measurement 4–6 weeks after starting therapy, to confirm antiviral activity of the regimen
	For patients starting at very high baseline RNA levels, maximal suppression may not be seen until 12–24 weeks, when HIV RNA should be remeasured
	Plasma HIV-RNA should thereafter be checked every 3–4 months, in conjunction with CD4 count (shorter intervals in proximity of critical decisions)
Change in HIV RNA suggesting treatment failure	Insufficient viral suppression after 4–6 months of starting treatment Significant viral rebound in patients who had previously undetectable viral load
	Definite trend toward pre-treatment levels in patients who have incomplete viral suppression

The situation differs for patients who are highly treatment experienced and for whom fewer options remain. In such cases, a more conservative approach may be warranted. Usually, virologic escape is followed by immunologic deterioration and eventually clinical evolution. However, the time lag between HIV RNA rebound and clinical failure varies from patient to patient, and it has become clear that $CD4^+$ lymphocyte count may remain high even in the presence of a clear rebound in HIV RNA.

Before making any decisions about changes in antiretroviral treatment, it is important to determine why the current regimen is failing, in order to avoid choosing an inappropriate remedy. In particular, factors such as drug resistance, inadequate drug exposure due to poor adherence, absorption and pharmacokinetics, and persistence of HIV in viral reservoirs all represent major causes of failure that should be investigated in depth before switching to a different regimen.

General criteria for the choice of second-line regimens

Unfortunately, because of the large degree of cross-resistance occurring within all antiretroviral drug classes, the options that actually exist are limited, especially for patients experiencing their second or third failure. In this heavily pre-treated population, both observational and prospective studies have shown quite disappointing results for any investigated salvage regimens.

Different studies have demonstrated that 90–95% adherence to a NRTI/PI regimen is required to achieve/maintain full suppression. In

addition to patient information and training, future opportunities to improve adherence may include once-daily NRTI dosing (didanosine, lamivudine, abacavir), once-daily PI dosing (saquinavir/ritonavir, lopinavir/ritonavir, indinavir/ritonavir), directly observed therapy, simplification after suppression, use of NRTI-class-sparing regimens and phosphokinase manipulation.

The path to be followed in the face of a confirmed treatment failure could be summarized as follows:

- review antiretroviral history;

- assess adherence and tolerability;
- distinguish first, second, multiple failures;
- consider newer agents through expanded access or clinical trials;
- consider phosphokinase enhancement; and
- perform resistance testing to identify susceptible drugs/drug classes.

Use of resistance testing

The most important issue in clinical practice is the relatively high level of cross-resistance among drugs in the same classes. Sequential utility in the case of resistance is almost nonexistent with NNRTIs and only moderate with PIs or NRTIs. The rationale for resistance testing in patient management includes demonstration of the correlation between drug resistance and virologic response to new regimen when prior therapy has failed; the fact that drug resistance is an independent risk factor for poor virologic response after controlling for HIV-1 RNA level, $CD4^+$ lymphocyte count and treatment history; and the fact that virologic failure is not inevitably accompanied by resistance to all drugs in the regimen.

Although representing a major advantage for the management of HIV patients, the routine use of resistance tests in the clinical setting has still to be validated. In general, whereas resistance is generally a good predictor of the probable failure of a drug, sus-ceptibility is no guarantee of success. It is becoming a common belief, therefore, that these assays have their major application in predicting which drugs not to use, rather than which are likely to be successful. Their best current application is therefore in the design of salvage regimens. Indeed, several retrospective and a few prospective studies have shown that, at least in the short term, a higher response rate is likely to occur when a salvage regimen is selected on the basis of genotype/phenotype testing results (see Chapter 137).[23] [24] [25] [26] [27]

Finally, transmission of drug-resistant virus at primary HIV infection is a growing phenomenon. Data from the USA show that the prevalence of high level resistance to NNRTI and PIs has increased in the last year compared with 1996-8. [28] Continuing surveillance is required and many suggest that drug resistance testing should be performed in patients who have primary HIV infection before initiating antiretroviral therapy.

FUTURE PERSPECTIVES

The development of new drugs within existing antiretroviral classes may provide patients with potent agents that have improved tolerability profiles and are more convenient to take than current drugs. However, as has happened in the recent history of antiretroviral therapy, a major impact has been made by the careful evaluation of new treatment strategies.

Treatment simplification strategies are necessary to improve patient adherence and hence outcome. A number of antiretroviral switch regimens have been proposed and/or studied in order to prevent or alleviate antiretroviral-associated toxicities, as well as to improve adherence. There are two main types of switching:

- PI switch regimen (i.e. from a PI-based regimen to a triple NRTI or one NNRTI/two NRTIs regimen); or
- NRTI-sparing switch (i.e. from one PI or NNRTI/two NRTIs regimen to one containing a PI and a NNRTI in order to minimize the risk of mitochondrial toxicity).

Many patients now being treated had marginal indications for therapy based on current guidelines and may be able to remain off therapy for a substantial period of time following a period of full viral suppression and $CD4^+$ gain. Structured or strategic treatment interruption has raised many practical and theoretical questions. This must be seen as a research tool and cannot be recommended apart from in controlled trials.

Despite impressive progress, much work remains to be done. In Western countries, increasing progress in antiretroviral therapy will result from new data obtained in clinical trials, a better knowledge of the pathophysiology of the disease, an increased awareness of the long-term toxicity of drugs and of viral cross-resistance, and the availability of new drugs.

Finally, we cannot forget that, on a global scale, the recent impressive therapeutic gains have only benefited 10% of those infected in the world. In the southern hemisphere the epidemic is reversing decades of development and widening the gap between rich and poor nations.

The dramatic inequalities between rich and poor nations in caring for persons living with HIV/AIDS highlight the moral imperative to develop strategies to increase access to life-saving treatments for the majority of infected people. The impact that AIDS is having on societies and communities makes it not only the most dramatic health emergency of our times but also the major developmental threat to populations and countries in the southern hemisphere. This is why — in addition to humanitarian and ethical reasons — there is an urgent need to start reversing this catastrophe and to bring HIV care and medicines to the developing world.

Although this may be seen as one of the most difficult scientific, social, political and economic challenges of our century, universal access to HIV treatment should be seen as the ultimate goal.

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Chapter 140 - Immunobased Therapies

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INTRODUCTION

Paralleling the development of more effective and better tolerated antiretroviral therapy for HIV infection has been the growing interest in developing alternative therapeutic approaches. Recent appreciation of the limitations and adverse effects of long-term antiretroviral therapy led to continued exploration of immune-based therapy for HIV infection. Both enhancement of HIV-specific immune responses and stimulation of general immune responses are potential strategies for immune-based therapies of HIV infection that are being developed. Table 140.1 outlines these approaches, which may be of particular value in the treatment of later stage patients, whose high levels of viremia and previous exposure to therapy place them at greatest risk of developing resistance to therapeutic agents and who also have the greatest need for a restoration of normal immune function. However, such interventions must be used with care to avoid compromising the effects of highly active antiretroviral therapy (HAART), which has significant antiviral and immunologic activity.

ENHANCEMENT OF HIV-SPECIFIC IMMUNITY

There are four approaches to the enhancement of HIV-specific response:

- ¦ structured treatment interruption or treatment cycling eventually resulting in viral rebound and rechallenge;
- ¦ administration of HIV-specific therapeutic and prophylactic vaccines (which are discussed in detail in Chapter 118);
- ¦ administration of passive immunotherapy, via either transfer of pooled immunoglobulin from people who have HIV infection or monoclonal antibodies to specific viral epitopes; and
- ¦ passive transfer of specific cell populations in an effort to enhance immune responsiveness to HIV.

A better understanding of host response to HIV infection, both by cellular immune responses and by the antibody response (particularly neutralizing antibody response), has led to refinement of the above strategies. However, enhancement of the HIV-specific immune response does not necessarily lead to improved clearance of the virus.

TABLE 140-1 -- Strategies of immunobased therapy.

STRATEGIES OF IMMUNOBASED THERAPY	
Enhancement of HIV-specific immunity	Therapeutic vaccine
	Transfer of HIV-specific cell populations
	Transfer of pooted immune sera
	Transfer of monoclonal antibody
General immune enhancement	Inhibition of proinflammatory cytokines
	Administration of immune modulators (i.e. interleukin (IL)-2, IL-12, interferon (IFN)-a)

Therapeutic vaccination

Although early attempts to enhance HIV-specific immunity by immunization with HIV-specific antigens in the era prior to effective antiretroviral therapy did result in enhanced lymphoproliferative response, these apparent immunologic benefits did not lead to improved clinical outcome with respect to disease progression or control of viral replication. Clinical trials of various gp120 vaccines in patients with early and later stage HIV infection did not show an effect on viral replication or rate of CD4 cell decline[1] despite immunologic response to immunization (particularly in the higher CD4 group). Another therapeutic vaccine tested extensively in clinical trials is the gp120 depleted Remune® product, which has been shown to induce LPA responses in several cohorts; although in a large multicenter US trial clinical benefit was not observed.[2] [3] However, the lack of benefit seen in the setting of continued viral replication, prior to treatment with effective antiretroviral therapy, may be due to the already maximal benefit of antigen-driven responses in these subjects and so the role of therapeutic vaccination in stimulating HIV-specific immunity and providing antigenic stimulation may be quite different in the setting of undetectable levels of circulating virus. Several approaches are being explored to induce immune response to envelope, internal coat and accessory protein epitopes; these include recombinant proteins, peptide, naked DNA, viral vectors and prime-boost combinations. In addition to exploring the types of vaccines that will prove effective in therapeutic vaccination, there is great interest in therapeutic vaccination in conjunction with other immune modulators, such as administration of interleukin-2 and the ALVAC vaccine, or vaccination in combination with treatment cycling.

Structured treatment interruption

Structured treatment interruption (STI) was first proposed as a strategy to boost HIV-specific responses capable of controlling viral replication after the report of a patient treated during primary HIV infection with effective antiretroviral therapy who was able to control viral replication for a prolonged period after cycling on and off therapy twice; this patient demonstrated T-cell-mediated immunity without detectable neutralizing antibodies to HIV.[4] Further study of STI in patients treated early in the course of HIV infection showed that, in many patients, control of viremia could be achieved by T- cell-mediated immune response.[5] [6] However, because the success of the autologous immunization during STI depends on a robust immune response, the promise of this strategy in the chronically HIV-infected patient is less clear, given the wider variation in immune response that can be induced in this patient population. The Swiss-Spanish Study is the largest clinical trial of STI in chronically infected patients. Subjects were treated for 2 weeks off followed by 8 weeks on HAART; this was repeated for four cycles, then therapy was interrupted until viral load rebounded to >5000 copies/ml. All but nine of 54 subjects reached this threshold within 12 weeks, including all subjects with baseline viral loads of >100,000 copies/ml, suggesting this approach has limited value in chronic

infection. Also, patients with highest viral loads and lowest CD4 counts at baseline were the most likely to be unable to achieve undetectable levels of viremia after resumption of therapy. The degree of virologic control and duration of suppression in these chronically infected patients seem to depend on the magnitude of cell-mediated responses. Although exposure to circulating virus results in mobilization of HIV-specific T-cell response, suppression is transient as these T-cell populations are eventually exhausted in the face of continued viral replication.[7] [8] [9]

HIV-specific cytotoxic T-lymphocyte responses

The interaction between HIV-1 and the cellular immune response is central to the immunopathogenesis of HIV infection, and delineation of this interaction may lead to interventions that can change the natural history of HIV infection.

Much attention has been focused on understanding the cellular immune response following primary infection as it is responsible in part for a several log drop in viral replication following the initial burst of viremia. Primary infection, with its high levels of viremia, is also the time when the virus begins to develop genetic diversity in

response to selective pressures exerted by the host immune response. This dynamic interaction at the initial phase of HIV infection sets the stage for the natural history of HIV infection.

Several studies have suggested that the cell-mediated immune response, rather than specific neutralizing antibody, is the main mechanism responsible for this initial control of viral replication.

The first piece of evidence is the finding that HIV-specific cytotoxic T lymphocytes (CTLs) can be found just as high levels of viremia begin to abate and before the rise in neutralizing antibody titers, suggesting that the CTL response is responsible for declines in initial viremia.[10] [11] In addition, the HIV-1-specific CTL response is demonstrable as early as 21 days following clinical presentation with primary infection and is directed at diverse epitopes within many of the HIV-1 gene products. [11]

Nonhuman primate models of primary infection with simian immunodeficiency virus (SIV) show a remarkably parallel sequence of events; within 4 weeks of detecting SIV p27 antigen in blood and SIV RNA in the extrafollicular and sinusoidal areas of lymphoid tissue, p27 antigen is cleared. Increased numbers of $CD8^+$ lymphocytes are detected in the periphery and in lymph nodes at the same time as this plasma viremia is declining. Humoral and cellular SIV-specific immune responses can be detected within 2 weeks of initial infection. Therefore, both human and simian data suggest that the remarkable containment of initial viremia is largely due to both humoral and cellular immune responses.[12]

Further study has shown that, although the CTL response during primary infection develops to multiple antigenic determinants, this repertoire is more limited than would be predicted. It is this limitation that perhaps allows the virus, with its genetic diversity, a chance to escape immune surveillance by mutating critical epitopes.[11] [13] Indeed, in a patient with early HIV infection, serial observations revealed an early CTL response aimed at a highly immunodominant epitope in gp160. However, with a mutation resulting in a single amino acid change, an escape mutant emerged that could not be recognized by epitope-specific CTLs. Therefore, the CTL response can exert selective pressure, which drives the emergence of resistant virus, just as is seen with antiretroviral therapy.[14] Implications of this finding include:

- vaccine strategies that result in CTL response to multiple codominant epitopes; and
- the importance of concurrent antiretroviral and immune-based approaches to therapy of HIV infection.

Data from natural history studies as well as study of long-term nonprogressors with HIV-1 infection suggest that the immunopathogenesis of HIV infection is influenced by the quality of the CTL response. For example, CTL activity is preserved in the long-term nonprogressors, which is in stark contrast to vertically infected children, who generally have little CTL response and rapid progression of clinical disease. [14] [15] Furthermore, longitudinal studies of CTL response over time show a steady decline in HIV-1-specific CTL precursor cells, paralleling the decline in the ability of $CD8^+$ lymphocytes to suppress HIV replication with progressive infection.[16]

Transfer of HIV-specific cell populations

Given the importance of cell-mediated immunity both in primary infection and in the asymptomatic nonprogressors, the ex vivo propagation and expansion of lymphocyte populations and reinfusion of expanded cell populations to people who have HIV infection is a potential treatment strategy that is currently being explored. One study examined the ex-vivo expansion of unfractionated lymphocytes from an HIV-negative identical twin and infusion of the expanded cell population to the HIV-infected twin. This appeared to be safe, and to result in increases in $CD4^+$ lymphocyte counts in the twin who had the infection.[17]

Dendritic cells, which are key in developing T-cell-dependent immunity by activating quiescent T cells, including naive T cells, and by inducing CD4 and CD8 killer T cells, are also being explored as an HIV-specific cell population that could help boost CTL response to HIV. Sensitized enriched populations of dendritic cells have been used to enhance CTL response in patients with lymphoma and melanoma, as well as other malignancies, with demonstrated immunologic and some clinical benefit. [18] [19] [20] [21] Clinical trials will employ this strategy comparing CTL responses in patients immunized with ALVAC alone versus those who receive subcutaneous reinfusion of populations of ALVAC-exposed dendritic cells.

Another approach is the adoptive transfer of $CD8^+$ lymphocytes following expansion. Preliminary reports suggest that these reinfused cells have little CTL activity, and that the majority are rapidly cleared from the circulation. The administration of $CD8^+$ lymphocytes expanded ex vivo with interleukin (IL)-2 has been investigated as a potential therapy for Kaposi's sarcoma.[22]

Another strategy under investigation is the administration of HIV-specific autologous CTL cell lines that have been selected in vitro and expanded for reinfusion; the safety and effects on immunologic parameters of this approach are currently under study. The finding that $CD4^+$ lymphocytes can be expanded in vitro in the presence of three antiretroviral drugs holds promise for autologous expanded $CD4^+$ lymphocytes as therapy as well.[23]

The limitations of these approaches are:

- the enormous investment in cell culture facilities;
- careful quality control; and
- the variable half-life of the reinfused cells, necessitating repeated administrations.

Transfer of pooled immune sera

The strategy of transferring pooled antibodies from donors who have HIV infection or of monoclonal antibodies directed against HIV is of interest not only as a potential prophylactic measure to prevent infection in exposed seronegative individuals, but also as an attempt to enhance antiviral activity in the serum of individuals who already have HIV infection. This could occur not only by neutralization of the virus, but perhaps also by enhancement of antibody-dependent cellular cytotoxicity and increased complement-mediated lysis. The long half-life of antibodies makes this approach particularly attractive, as intermittent therapy at bimonthly intervals may be practical. The drawbacks of this approach again relate to the genetic diversity of HIV and the potential for the development of mutants not recognized by the more specific antibody preparations. In addition, the vast amount of circulating viral antigen could potentially lead to the formation of immune complexes, resulting in adverse effects due to their

deposition in various tissues, although to date there have been no reported adverse events after these infusions that can be related to immune complex deposition.

Trials of polyclonal antisera

Two controlled trials evaluating the effect of HIV immune plasma on disease progression in HIV-positive individuals have been published. One reported no benefit;[24] in the second, in which patients received either immune plasma or plasma from negative donors every 2 weeks for 1 year, there was a significant decrease in the number of opportunistic infections in subjects receiving immune sera.[25] Others have reported preliminary results of similar strategies, using pooled hyperimmune plasma or a sterile filtered pooled high-titer anti-HIV plasma preparation (treated with ß-propiolactone to inactivate any HIV that may be present).[26] [27]

The role of hyperimmune anti-HIV intravenous immunoglobulin (IVIG) in preventing perinatal transmission has also been assessed. In a phase 1–2 double-blind controlled study by the pediatric AIDS Clinical Trials Group (ACTG), 28 maternal-infant pairs were randomized to receive either HIV hyperimmune IVIG or placebo (IVIG) beginning at between 20 and 30 weeks of gestation; all of the mothers enrolled were receiving zidovudine. Mothers received infusions every 28 days until delivery; infants received a single infusion within 12 hours of birth. All mothers and infants received zidovudine as per the ACTG 076 protocol. Sustained suppression of immune complex dissociated p24 antigenemia was seen in the treated women, although no change was seen in quantitative HIV RNA. Infants in the treatment group had higher levels of p24 antibody at birth, confirming trans-placental transfer of antibody from the HIV hyperimmune IVIG infusions.[28]

Further studies are necessary before the significance of these results and the potential for specific immunoglobulin as therapy are fully understood.

Various trials have also examined the efficacy of IVIG without specific anti-HIV activity and consistently show no effect on HIV-related virologic or immunologic parameters, although IVIG may have a role in reducing the frequency of bacterial infections.[29]

Transfer of monoclonal antibody

Another approach that has been considered is the administration of specific monoclonal antibodies to various epitopes of HIV. This has been limited by concerns that

the virus would acquire resistance to specific antibodies, although this may be overcome by administering antibody combinations that are directed at more than one neutralizing epitope. Another obstacle to clinical trials of specific monoclonal antibodies is the expense of the large-scale production that would be required.

The first step in developing monoclonal antibodies to be tested in clinical trials is to identify epitopes that will induce antibodies that will result in anti-HIV activity. Most attention to date has focused on antibodies to various surface epitopes of HIV, either to the V3 loop, C4 and gp41 or, in the case of F105 (a monoclonal antibody), directed against the CD4 binding site. Combinations of these antibodies in vitro show at least additive activity and, against some strains of HIV, synergistic activity.

The F105 antibody has been evaluated in a dose-escalating phase 1–2 ACTG trial; half-lives of the antibody were prolonged, but there was no change in HIV titers.[30]

GENERAL IMMUNE ENHANCEMENT

The availability of cytokine inhibitors that could potentially counteract the overproduction of cytokines seen in HIV infection and of general immune stimulators has led to interest in manipulating the immune system as a therapeutic tool.

Theoretically, the general immune stimulation or suppression that may result from these manipulations may hasten the rate of HIV progression, particularly as there is concern that stimulation of certain cell populations may enhance HIV replication. Therefore, active antiretroviral therapy has to be administered concurrently with any of these modulators of the immune system.

Inhibition of proinflammatory cytokines

The cytokine that has been most extensively studied is tumor necrosis factor (TNF)-a because:

- it is produced in large quantities in HIV infection; and
- it upregulates HIV expression in vitro.

Pentoxifylline, thalidomide and corticosteroids have all been studied as potential therapies because they inhibit TNF-a production.

Pentoxifylline

Studies of the use of pentoxifylline in people who have HIV infection have shown a decrease in TNF-a mRNA; however, in a controlled trial of pentoxifylline at the highest tolerable dosages, no significant antiviral effect was detected.[29] It seems that the benefit is seen mainly in patients who have high pretreatment levels of TNF-a. This observation led to the initiation of a placebo-controlled trial of pentoxifylline in people who have HIV infection with active pulmonary tuberculosis in Uganda, because they have very high levels of circulating TNF-a. The patients enrolled in this study had relatively early HIV infection, with a mean $CD4^+$ lymphocyte count of 380/µl, and did not receive other antiretroviral therapy. Patients in the pentoxifylline arm had lower levels of HIV RNA than the control arm and reduced levels of $ß_2$ -microglobulin; however, no difference was observed in the two groups with regard to new AIDS-defining illness or survival. [31] [32] [33] [34]

Thalidomide

Thalidomide is also known to inhibit production of TNF-a and has been shown to inhibit HIV-1 replication in vitro. A placebo-controlled trial has shown benefit in the treatment of HIV-associated oral and esophageal aphthous ulcers. Of note, patients in the thalidomide arm appeared to have increases in HIV-1 RNA, underscoring the importance of concurrent antiretroviral therapy.[35] [36]

Corticosteroids

The observation that corticosteroids appear to benefit patients with HIV-associated nephropathy has led to interest in them for interrupting cytokine activity, but there are insufficient controlled data to evaluate their use.

Ciclosporin

Ciclosporin has been proposed as an immunomodulatory agent with potential activity in HIV infection. A phase 2 trial of ciclosporin versus placebo in patients with early HIV infection ($CD4^+$ lymphocyte count >500/µl) has been completed; although the drug was well tolerated in this population, it had no effect on level of immune activation or CD4 count at the dose studied. However, Pantaleo and others reported a positive effect of a limited course of ciclosporin A when given in conjunction with HAART to patients during primary HIV infection as compared with HAART alone. The group that received ciclosporin showed acute benefit in terms of restoration of normal $CD4^+$ cell number and proportion, which was seen more than a year after the ciclosporin was stopped. It appears that dampening of T-cell activation during primary infection may have lasting immunologic benefit. [37]

Cyclophosphamide

Administration of cytotoxic therapy has been postulated as a way to reduce the cellular reservoir of HIV in chronically infected patients on HAART. A study, ACTG 380, was designed to address

this question: 10 treatment-naive individuals were randomized to receive either antiretroviral therapy alone or escalating doses of cyclophosphamide after they had achieved a viral load of <50 copies/ml. The drug was well tolerated, but no difference was seen between the two groups in terms of peripheral blood or lymphoid HIV DNA levels.[38]

Enhancement of immune response via cytokines and lymphokines: administration of immune modulators

Recent studies of administration of immune modulators have focused mainly on IL-2 and interferon (IFN)-a.

Interferon-a

Low-dose oral IFN-a has been studied with and without concurrent antiretroviral administration; there appears to be little benefit with regard to antiviral activity. Subcutaneous IFN-a in combination with other antiviral agents has also been evaluated. The most positive data regarding IFN-a to date are for its use in the treatment of HIV-associated thrombocytopenia. Patients in two uncontrolled studies showed benefit with regard to platelet levels at low doses of this agent.[39] [40] [41] [42] In general, systemic administration of IFN-a has resulted in unacceptable toxicity and marginal benefit in patients who have HIV infection, limiting its clinical usefulness, although the use of pegylated interferon as an antiviral agent for HIV infection is being tested in clinical trials.

Interleukin-2

Interleukin-2 has emerged as a potentially exciting agent for immune-based therapy of HIV infection. Results of a small trial of intermittent intravenous IL-2 (5-day continuous infusions every 8 weeks) are encouraging. Of patients who had baseline $CD4^+$ lymphocyte counts over 200/µl, 60% showed sustained increases in $CD4^+$ lymphocyte counts following treatment. This increase in $CD4^+$ lymphocyte count was seen over a 14-month follow-up of these patients, although no change was seen in plasma HIV RNA levels or levels of p24 antigenemia. In patients who had a baseline $CD4^+$ lymphocyte count of less than 100/µl, no benefit was seen in terms of $CD4^+$ lymphocyte count. However, the transient rise in viral load reported in preliminary studies of IL-2 use in patients who have later stage HIV infection was not seen in this study, presumably because of concurrent antiretroviral therapy.[43] [44]

A major drawback of this approach is the frequency of continuous intravenous therapy, and so trials of subcutaneous or intermittent intravenous IL-2 are in progress. ACTG 328 is a trial of intravenous IL-2 with HAART versus subcutaneous IL-2 with HAART versus HAART alone. Primary analysis at weeks 60 and 84 showed marked and sustained increases in $CD4^+$ cells counts. Furthermore, significant increases in naive and memory $CD4^+$ cells were seen along with increases in $CD25^+$, $CD28^+$

and CD95^{+} subpopulations in both treatment arms. Assessment of AIDS-defining events suggested a lower incidence of progression to AIDS in the two treatment arms compared with HAART alone. Two other long-term studies (SILCAT and ESPRIT) are underway examining the benefit of IL-2 in patients receiving HAART, but will require several years of follow-up to gather adequate clinical end-point data.

Interleukin-12

Interleukin-12 is currently in phase 1 clinical trials. It is known to increase natural killer lymphocyte and CTL activity in vitro and to be deficient in people who have HIV-1 infection. It is hoped that treatment will stimulate the development of T-helper type 1 lymphocytes, reverting the shift to T-helper type 2 predominance that is seen in HIV infection and may be a detrimental consequence of the host response to HIV-1 infection.[45] In a preliminary study, IL-12 was well tolerated in doses of up to 100ng/kg in patients with <50 CD4 cells/mm^3 , and up to 300ng/kg in a group with higher CD4 counts. Although IL-12 administration resulted in significant dose-related decreases in serum neopterin, no change in LPA response to HIV or *M. avium-intracellulare* was seen; nor was any change in serum IFN-? levels or HIV RNA levels seen. [46]

Granulocyte-macrophage colony-stimulating factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is another immune modulator that has been examined for its effect on HIV disease progression. ACTG 5041 was a two-step clinical trial with an initial randomized, placebo-controlled phase, followed by a open label phase, which looked at the effect of GM-CSF on CD4 counts and HIV viral loads in patients with stable, quantifiable viral loads. Initial analysis showed a trend toward higher viral load and higher CD4 counts in the treatment group at 16 weeks.[47]

CONCLUSION

In summary, both pharmacologic and immunologic approaches to new interventions for HIV infection have yielded a number of encouraging agents and potential combinations of agents. The challenge is to sort out which strategies are likely to result in long-term clinical benefit with minimum toxicity in the setting of planning therapy for a chronic disease such as HIV infection. In a rapidly changing environment of new antiretrovirals, the position of immunologic therapy will have to be carefully understood. Major developments in the suppression of HIV with significant improvements in immune function have been reported. However, whether immune function can be restored with antiretroviral therapy alone is unclear at present. It is likely that immune-based therapies will be an important adjuvant to antiretroviral therapy in some patient populations.

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Chapter 141 - Practice Points

141.a The role of resistance typing

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Introduction

The circulating plasma HIV-1 RNA level reflects the extremely high rate of virus replication that occurs in the lymphoid tissue, with billions of new virions being produced each day throughout all stages of HIV infection, including the period of clinical latency. Within the first year of primary HIV-1 infection, the level at which plasma HIV-1 RNA plateaus is strongly predictive of the rate of progression to HIV-associated disease and death. Furthermore, the degree to which HIV-1 replication can be inhibited by antiretroviral therapy in a sustained fashion, as determined by increasingly sensitive techniques to measure reductions in plasma HIV-1 RNA levels, is an important determinant of the clinical benefit of treatment. Both the nadir plasma HIV-1 RNA level that is reached and the time to reach the nadir determine the sustainability of the virologic response to treatment. Thus, the principal goal of modern-day antiretroviral therapy is to reduce virus replication as much as possible for as long as possible. Unfortunately, one of the factors that jeopardizes the achievement of this goal is the danger that viral drug resistance may develop.

Pathogenesis

The principal targets of currently available antiretroviral agents are the HIV-1 reverse transcriptase and protease enzymes. The viral genes that encode these two enzymes are prone to the development of mutations because the HIV-1 reverse transcriptase is highly error-prone when reverse transcribing viral RNA into proviral DNA, the characteristic step in the viral life cycle during each round of replication of a retrovirus, including HIV-1. Given the extremely high replication rate of HIV-1, mutations in the HIV-1 reverse transcriptase and protease-encoding genes, including those that confer drug resistance, can be expected to be generated on a daily basis and to be present in a patient in a minority of virus subpopulations, even in the absence of antiretroviral drug pressure. Thus, in the absence of sufficient drug pressure, such as the use of any current antiretroviral monotherapy or of insufficiently potent combination therapy, pre-existing drug-resistant viruses eventually show preferential growth that results in treatment failure (Fig. 141a.1). This forms the basis for the current standard of giving treatment with highly active antiretroviral therapy (HAART) using potent combinations of multiple antiretrovirals.

Clinical features

Clinically, a harbinger of the possible emergence of retroviral drug resistance is the repeated demonstration of levels of plasma HIV-1 RNA above the quantification limit of the assay being used, after HIV-1 RNA levels, following the institution of therapy, have become undetectable using the same assay. It is important to realize that this is not necessarily immediately accompanied by a decline in blood $CD4^+$ lymphocyte numbers or by the development of clinical symptoms. It is therefore crucial that patients being treated with HAART are monitored by regular determination of plasma HIV-1 RNA levels, so that the emergence of potential retroviral drug resistance can be noted in a timely manner.

Investigations

Although rebound of plasma HIV-1 RNA during HAART to detectable levels may indicate retroviral drug resistance, other causes for drug failure need to be considered to determine the subsequent approach to treatment in such patients. Certain of the combinations of antiretrovirals currently used for HAART dictate the use of large numbers of pills according to strict administration schedules, with different requirements for each drug in the combination with respect to timing, possibility of co-administration and concomitant consumption of food. Consequently, it is understandable why lack of continuous patient adherence to these often complicated treatment regimens is increasingly becoming apparent as an important determinant of HAART failure. Thus, taking the patient's history with respect to treatment adherence is crucial when evaluating 'drug' failure. Therapeutic drug monitoring by determining plasma levels, particularly of HIV protease and non-nucleoside reverse transcriptase inhibitors (NNRTIs), may yield additional information in judging patient compliance with therapy. Furthermore, such monitoring may identify patients who, despite proper compliance, have insufficient drug exposure because of variation between individuals in pharmacokinetics and/or pharmacodynamics of protease inhibitors or NNRTIs, or because of drug interactions that result from the use of certain concomitant medications.

Poor patient adherence leads to insufficient drug pressure and results in increased virus replication and emergence of virus resistant to those antiretroviral agents that are being continued (see Fig. 141a.1). This is

Figure 141.a-1 A way to explain troughs, mutants and staunch compliance. Relationship between lack of patient adherence to treatment, insufficient drug levels and emergence of resistance. *Adapted with permission from Mascolini M. The drugs we've got ... the drugs we're getting ... beyond blood: a three part look at the Fourth Conference on Retroviruses and Opportunistic Infections. J Int Assoc Physicians AIDS Care 1997;3:30.*

seen most rapidly and is most pronounced for those agents that are particularly vulnerable to resistance development, such as lamivudine and the currently available NNRTIs.

The measurement of retroviral drug resistance of circulating virus can be carried out with both genotypic and phenotypic assays (see also Chapter 137 and Chapter 204). Genotypic assays, which identify the presence of specific mutations in defined gene sequences (e.g. the HIV reverse transcriptase or protease genes), are relatively rapid to perform but only provide an indirect measure of resistance and do not necessarily correlate with phenotypic assays, a problem particularly pronounced for HIV protease inhibitors. Moreover, certain of these assays cannot detect the presence of minority populations of viruses (i.e. those that comprise <20% of total plasma viral RNA), which harbor potentially resistance-conferring mutations. Phenotypic assays, which measure the in-vitro growth capacity of a virus isolate in the presence of increasing concentrations of a drug, are a more direct measure of resistance but are labor-intensive, slower to perform and (like genotypic assays) may be insensitive to minority populations of virus. Finally, it is important to realize that the cut-off for determining phenotypic susceptibility or resistance of a virus to a particular drug should be judged in relation to the concentration of that drug that is achieved in vivo. Resistance to a drug that is present when dosed in its usual manner could be overcome by increasing the dose of the drug as long as increasing drug concentrations are not hampered by loss of tolerability. This demands the availability of drugs with a wider therapeutic margin than is currently the case.

Further standardization and validation of the currently available resistance assays in relation to the drug levels achieved in the patient will become increasingly important to enhance the usefulness of resistance testing in guiding therapeutic choices in individual patients.

Management

Several prospective randomized clinical trials have now demonstrated an improved short-term (6–12 months) virologic outcome for patients who had failed to maintain adequate virus suppression on their current treatment, when treatment was adjusted based on genotyping and/or phenotyping as compared with clinical judgment only.

In addition, these trials have demonstrated that the benefit of resistance testing is enhanced by providing the clinician not just with a result of resistance testing but also with expert advice on how to interpret and use this result. Clearly, given the significant degree of cross-resistance between drugs within each of the three currently available classes of antiretrovirals, the usefulness of resistance testing is hampered by the lack of available alternative agents. This is also reflected in the relatively small degree of improved virologic outcome in the above-mentioned trials. Whether to change therapy and which alternative drugs to use in patients who are failing treatment will always remain a multifactorial decision-making process. This will involve not only the use of resistance testing assisted by expert interpretation and advice but at the same time obtaining a detailed history of prior antiretroviral exposure, knowledge of patterns of cross-resistance, and information concerning patients' adherence and drug level monitoring. The increasing availability of rapid and reliable HIV drug-resistance assays is likely to help not only to guide the choice of therapy in patients who have become resistant to prior treatment, but also to select initial treatment in certain circumstances. Examples of these are the choice of initial treatment in regions that have a high prevalence of primary HIV infection with drug-resistant strains, in infants who have become perinatally infected despite the use of antiretroviral therapy by their mothers, and in the setting of drug prophylaxis following accidental or sexual exposure to HIV from a known source. In patients failing therapy with a history of exposure to multiple prior drugs and drug regimens, the usefulness of resistance testing will to a large extent remain dependent on the development and introduction of novel drugs that do not exhibit cross-resistance to the current armamentarium. (See also Chapter 137 , Chapter 138 and Chapter 204 .)

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141.b Drug interactions in HIV and AIDS

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Stephen C Piscitelli

Introduction

The recognition and management of pharmacokinetic and pharmacodynamic drug interactions is a major factor in choosing drugs and designing dosage regimens for the HIV-infected patient. Complex, multidrug regimens have become the standard of care and clinicians must be aware of the potential need for dosage alterations with combination therapy. Many of the antiretrovirals in current use can alter concentrations of concomitantly administered drugs or have their own concentrations altered by other agents. These interactions can be detrimental or beneficial to the patient.

Nucleoside reverse transcriptase inhibitors

The nucleoside reverse transcriptase inhibitors (NRTIs) are primarily eliminated by renal mechanisms and thus they are not generally involved in clinically significant pharmacokinetic interactions. These agents can be used with protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs) without the need for dosage alterations. Two NRTIs — zidovudine and abacavir — are eliminated in part by hepatic glucuronidation. Inducers of glucuronyl transferase activity could modestly lower plasma concentrations of these agents, although the clinical significance of this is uncertain since the active metabolites are intracellular nucleoside triphosphates.

Nucleoside reverse transcriptase inhibitors may interact pharmacodynamically with agents that have similar side-effect profiles. Increased bone marrow toxicity may be observed when zidovudine is used with other drugs capable of bone marrow suppression, such as ganciclovir and sulfamethoxazole. Didanosine and stavudine are associated with pancreatitis and should be used with caution in those receiving other agents with known pancreatic toxicity, such as pentamidine. Patients receiving combinations of didanosine, stavudine and zalcitabine should be counseled on the signs and symptoms of peripheral neuropathy and pancreatitis. Several NRTIs have been associated with lactic acidosis and should be used cautiously with other agents causing hyperlactatemia, such as metformin. The combination of didanosine and stavudine has been particularly associated with lactic acidosis and should be avoided.

The NRTI tenofovir increases the plasma levels of concomitantly administered didanosine and may increase the toxicity of didanosine if standard doses are used. A dose reduction to didanosine 250mg daily is recommended.

Non-nucleoside reverse transcriptase inhibitors

Nevirapine and efavirenz are moderate inducers of cytochrome P450 (CYP) 3A4. Both agents have been shown to decrease the area under the concentration-time curve (AUC) of amprenavir, indinavir and saquinavir (Table 141b.1). These agents should not be used with saquinavir, unless in combination with ritonavir, because of a large decrease in the saquinavir AUC.

TABLE 141.b-1 -- Consequences of selected cytochrome P450-mediated drug interactions.

CONSEQUENCES OF SELECTED CYP450-MEDIATED DRUG INTERACTIONS		
	Agents affected	**Consequence**
CYP450 inhibitors		
Amprenavir	Amprenavir, benzodiazepines, calcium channel antagonists, indinavir, lopinavir, rifabutin, saquinavir, terfenadine	Increased concentration of co-administered drug and possible increased therapeutic effect or toxicity
Cimetidine		
Clarithromycin		
Delavirdine		
Erythromycin		
Grapefruit juice		
Itraconazole		
Indinavir		
Ketoconazole		
Nelfinavir		
Ritonavir		
CYP450 inducers		
Carbamazepine	Amprenavir, calcium channel antagonists, indinavir, lopinavir, methadone, oral contraceptives, saquinavir	Decreased concentration of co-administered drug and possible decreased therapeutic effect
Efavirenz		
Nelfinavir		
Nevirapine		
Phenobarbital		
Phenytoin		
Rifabutin		
Rifampin (rifampicin)		
Rifapentine		
St John's wort		

Efavirenz affects other drug-metabolizing enzymes and may act as a P450 inhibitor in certain situations. It increases concentrations of ritonavir and nelfinavir by approximately 20% presumably through inhibition of CYP450 pathways. In general, no dosage adjustments are required with these combinations.

Delavirdine is a moderate inhibitor of the CYP 3A4 enzyme and can increase concentrations of saquinavir 5-fold and indinavir 3-fold (see Table 141b.1). Serious

toxicity could occur if delavirdine is used with certain 3A4 substrates, such as the nonsedating antihistamines astemizole and terfenadine (no longer marketed in the USA), cisapride (also no longer marketed), certain benzodiazepines or ergot derivatives (Table 141b.2). Absorption of delavirdine can be affected by changes in gastric pH and its administration should be separated from didanosine, antacids and H_2 -blockers.

Protease inhibitors

The protease inhibitors are metabolized by CYP450, mainly by 3A4, and can inhibit these enzymes. Thus, they possess the same contra-indications as noted above with delavirdine. Ritonavir has the greatest degree of inhibition and is associated with the largest number of observed and potential drug interactions. Amprenavir, indinavir and nelfinavir have a moderate potential to cause interactions and saquinavir has the fewest drug interactions described.

The potent inhibition of CYP 3A4 by ritonavir can be used to optimize the AUC of saquinavir, which previously required ingestion of up to 18 large capsules per day. Concomitant administration with ritonavir results in a 20-fold increase in steady-state saquinavir concentrations, allowing for a dosage reduction of saquinavir to as little as 400mg q12h, while maintaining high blood concentrations. Once-daily combinations of 1600mg saquinavir with 100mg of ritonavir are under investigation. This combination was successful at suppressing HIV replication in clinical trials in protease inhibitor-naive subjects. The combinations of ritonavir with amprenavir, indinavir or lopinavir show similar activity.

In addition to enzyme inhibition, ritonavir and nelfinavir possess moderate enzyme-inducing properties and decrease concentrations of a number of co-administered agents (see Table 141b.2). Ritonavir

TABLE 141.b-2 -- Specific contraindicated drugs with potential for serious drug interactions.

SPECIFIC CONTRAINDICATED DRUGS WITH POTENTIAL FOR SERIOUS DRUG INTERACTIONS		
Agent or drug class	**Interacting drugs**	**Consequence**
Terfenadine, astemizole and cisapride*	CYP450 inhibitors (macrolides, delavirdine, protease inhibitors, azole antifungals)	Decreased metabolism with increased concentrations and potential for cardiac toxicity
Long-acting benzodiazepines (alprazolam, midazolam)	CYP450 inhibitors	Decreased metabolism with potential for oversedation
Ergot derivatives	CYP450 inhibitors	Decreased metabolism with potential for cardiovascular toxicity
Protease inhibitors	CYP450 inducers (efavirenz, rifampin, St John's wort)	Increased metabolism with decreased concentrations and potential for resistance and treatment failure

* Removed from the US market.

and nelfinavir also increase glucuronyl transferase activity. Ethinyl estradiol and progesterone concentrations can be decreased by concomitant administration of these two protease inhibitors, necessitating alternative forms of birth control. Amprenavir appears to be an inducer of drug-metabolizing enzymes in some circumstances.

Combining protease inhibitors with ritonavir can offer additional advantages to patients. For example, indinavir requires three times daily administration on an empty stomach or with a light meal. When administered in combination with ritonavir, it can be dosed twice daily with food and still produce high plasma concentrations.

Certain enzyme-inducing agents can produce profound decreases in the concentrations of protease inhibitors. For example, rifampin (rifampicin) has been shown to decrease the saquinavir AUC by 80% and the indinavir AUC by 65%. The resulting low blood levels have the potential to promote drug resistance and treatment failure, so indinavir and saquinavir should be avoided in patients receiving rifampin. Other potent enzyme inducers, such as phenytoin, phenobarbital and carbamazepine, could produce similar reductions.

Management of drug interactions

A careful review of patient medication profiles is essential to managing drug interactions in the HIV-infected patient. This should include complementary and alternative medicines, since agents such as St John's wort induce CYP 3A4 and can reduce concentrations of indinavir and presumably other protease inhibitors. Clinicians need to have a general understanding of certain 'red flag' drugs that are potent inhibitors and inducers of CYP450 (i.e. ritonavir, efavirenz, etc.). In this era of complex regimens, a table of recommended dosages for each combination of protease inhibitors or NNRTIs should be immediately available.

Patient counseling is critically important when complex antiretroviral regimens are prescribed. Patients must be instructed on how to take their medications with regard to timing and content of meals. In some cases, a written daily calendar of medications and times of administration may be useful to the patient and may improve adherence. Proper separation of dosing of certain drugs (i.e. didanosine and indinavir) must be explained and can also be documented on a daily dosage planner.

The HIV clinician can use a variety of interventions to manage drug interactions. Selection of a drug with fewer interactions should be considered if warranted by the clinical situation. For example, azithromycin is not metabolized by CYP450 and has far fewer drug interactions compared with other macrolides. Also, drugs that can be administered once or twice daily may be useful to lessen food-related interactions or dosage separation problems. Finally, clinicians should be aware of and try to avoid drugs with overlapping toxicities. However such combinations are sometimes unavoidable and patients who receive these agents must be closely monitored for the development of toxicity.

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141.c How to manage the hepatitis C virus co-infected HIV patient

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Interactions between HIV and the hepatitis C virus (HCV) were widely studied before the introduction of highly active antiretroviral therapy (HAART). The latter has markedly modified the prognosis of HIV infection, resulting in a significant decrease in morbidity and mortality that may now reveal liver-related complications. Since the improvement in treating HCV infection paralleled the progress in treating HIV, the question is now how to manage HIV-HCV co-infected patients.

Why evaluate HIV-hepatitis C virus co-infection?

Consequences of HIV-hepatitis C virus co-infection

Anti-HCV antibodies are frequently detected in HIV-infected patients, especially in hemophiliacs and intravenous drug users (around 70–90%), and are usually associated with active infection as assessed by detectable HCV viremia. This prevalence may be underestimated in case of delayed seroconversion and seroreversions.

It has recently been suggested that genotype 1b HCV may worsen the natural history of HIV in hemophiliacs and drug users. Moreover, HCV infection (and active intravenous drug use) has been suggested as an important factor in the morbidity and mortality of HIV-1-infected patients. Liver disease associated with HCV is an important cause of morbidity (8.6% of admissions) and mortality (4.8%) in HIV-HCV co-infected patients.

On the other hand, HIV significantly modifies the natural history of HCV infection:

- ¦ by increasing levels of HCV viremia;
- ¦ by significantly increasing the risk of mother-to-child or sexual transmission (from a mean of 6% to 20% and from 0% to 3%, respectively);
- ¦ by increasing (2- to 5-fold) and accelerating the risk of cirrhosis: this translates into a significantly increased yearly progression of the fibrosis score in co-infected subjects depending on the CD4 cell count level (<200 cells/mm^3) and possibly chronic alcohol consumption; and
- ¦ by increasing the risk of fibrosing cholestatic hepatitis, related to direct cytotoxicity of HCV in case of high viremia leading to accumulation of viral proteins in the endoplasmic reticulum and hepatocyte death.

The pathophysiology of HCV-related chronic hepatitis is not fully understood. Immune-mediated mechanisms are mainly involved but a direct cytotoxicity of the high HCV viremia cannot be excluded. Infection with HIV may have a direct cytopathic effect on liver cells or may modify the pattern of cytokine production, leading to production of fibrogenic factors or to decrease in antifibrogenic factors.

Impact of highly active antiretroviral therapy

Late consequences of HCV-related chronic liver disease were probably overshadowed by the extrahepatic causes of deaths, related to severe immune deficiency. Given the increasing frequency of HCV-HIV co-infection, the significant increase of cirrhosis in immunocompromised patients and the significant improvement in survival with therapeutic progress in the field of anti-HIV therapy, the next few years may see the occurrence of symptomatic liver disease leading ultimately to hepatic carcinoma in HIV-HCV co-infected patients, underlining the need for early diagnostic procedures and therapeutic strategies.

Improvement in immunity could be deleterious in case of diseases involving immune-mediated mechanisms, such as chronic viral hepatitis. Antiretroviral therapy restores both CD4 and CD8 cells (directed against specific HCV antigens), which may participate in the pathologic deterioration. Several cases of liver deterioration paralleling immune restoration in HCV-HIV co-infected patients have been reported. Thus, a careful liver follow-up in HCV-HIV co-infected patients on triple combination antiretroviral therapy is required.

Antiretroviral therapy, by improving immune status, could decrease HCV viral load and thus the severity of HCV liver disease if direct viral cytotoxicity is, at least partially, responsible for the histopathologic lesions of chronic hepatitis. However, there is no significant variation in HCV RNA load after 3, 6 or 9 months of triple therapy in HCV-HIV co-infected subjects, as compared with baseline values, suggesting that HAART will not decrease HCV-related liver disease associated with HCV replication.

All antiviral drugs have potential toxicity. Nucleoside reverse transcriptase inhibitors cause rare but severe hepatitis, which is due to a mitochondrial cytopathy with severe microsteatosis and lethal lactic acidosis. Non-nucleoside reverse transcriptase inhibitors are responsible for clinical hepatitis in 2–5% of cases, which are sometimes severe (0.1% of cases) and are associated with signs of hypersensitivity in two-thirds of cases. Drug-related hepatitis in association with protease inhibitors is observed in 2–8.5% of treated patients, with a predilection for patients co-infected with HCV or HBV. In most cases of drug-related hepatitis, liver biochemical abnormalities resolve after discontinuation of the drug and do not relapse after changing of protease inhibitor. The rate of HAART withdrawal in relation to hepatotoxicity is around 2% without significant difference regarding the type of antiretroviral treatment.

Drug-related hepatitis may participate in the deterioration of liver histology, as well as hepatitis of immune function restoration, direct cytotoxicity of HCV and other usual causes of liver damage, including alcohol toxicity (Fig. 141c.1).

Figure 141.c-1 Involvement of putative liver lesions in HIV-HCV co-infection.

Therapeutic implications

Treatment of hepatitis C virus infection in co-infected patients

The increased risk of cirrhosis in HCV-HIV co-infected patients, the histologic deterioration under anti-HIV therapy related to immune restoration, direct viral cytotoxicity or drug toxicity, and the increase in survival of HIV-infected patients underline the need for reliable anti-HCV antiviral therapies. An important issue is to distinguish who among HIV-HCV co-infected patients should receive anti-HCV treatment with the aim of eradicating HCV.

In co-infected patients, interferon alone led to a low rate of viral eradication (around 15%) with significant side-effects but a low impact on the HIV status.

The standard treatment of HCV infection is now the combination of ribavirin and interferon. Ribavirin, a nucleoside analogue, has no clear effect as monotherapy on HCV or HIV and is now currently given in combination with interferon-a in treating hepatitis C in HIV-infected patients. The ribavirin-interferon-a combination results in a 40% rate of HCV eradication in the non-HIV population with a range from 15% to 80% according to the virologic (genotype and quantitative viremia) and pathologic (fibrosis) predictive factors and to the duration of therapy (6 or 12 months). Sustained viral eradication is usually associated with a long-lasting resolution and histologic improvement or normalization.

The results of the ribavirin-interferon combination in HIV co-infected patients have been reported. In-vitro inhibition of the phosphorylation by ribavirin of zidovudine, stavudine and zalcitabine has been reported but it does not seem to clearly modify the efficacy of HAART in vivo. By contrast, in vitro, ribavirin may positively interfere with phosphorylation of didanosine and the combination didanosine, ribavirin and interferon is highly synergistic and increases the side-effects of didanosine, especially lactic acidosis and pancreatitis.

The combination of interferon-a and ribavirin may lead, in naive HCV-HIV co-infected patients, to an overall 25% rate of sustained eradication, with a good tolerance, especially at the immunologic level. This low rate of sustained response is expected since cirrhosis, high pretreatment viremia and high genomic heterogeneity, predictors of poor response, are more frequent in co-infected patients. In summary, we recommend such a combination in HCV-HIV co-infected patients with close monitoring of CD4 and HIV viral load in those with a biopsy-proven fibrotic liver disease (Fig. 141c.2).

Recently, pegylated (long-lasting) interferon-a was shown to be more efficient than the usual interferon-a to obtain viral eradication with a better quality of life. In the non-HIV population, the combination of the pegylated interferon-a (1.0–1.5µg/kg/week subcutaneously) with ribavirin (800–1200mg/day, the recommended dose being >10.6mg/kg/day) for 48 weeks leads to an overall 55% rate of viral eradication, with a benefit mainly observed in patients with poor predictors of sustained response (genotype 1, high quantitative viremia). Preliminary results of studies of pegylated interferon-a plus ribavirin in HIV positive patients suggest it is superior to interferon-a plus ribavirin (see also Chapter 125).

Pragmatic management of hepatitis C virus co-infected patients

Pre-existing biochemical abnormalities do not contraindicate the use of antiretroviral drugs although nevirapine and ritonavir should be used cautiously. In some difficult situations, the measurement of the plasma levels of antiretroviral drugs may be valuable in order to avoid any overdoses that may lead to hepatotoxicity. In HCV-HIV co-infected patients treated with protease inhibitor, a monthly biochemical follow-up is warranted in the first 3 months after introduction of any new antiretroviral drug and then every 3 months in order

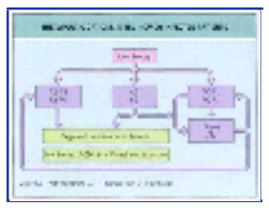

Figure 141.c-2 Therapeutic options in HIV-HCV co-infected patients.

to identify potential drug-related toxicity. An increase in ALT levels (>5-fold the upper normal value, but the rate of increase is more important than the absolute values) should lead to a switch of the protease inhibitor or to the introduction of a non-nucleoside reverse transcriptase inhibitor. Biochemical liver abnormalities occurring with non-nucleoside reverse transcriptase inhibitor should lead to treatment withdrawal, especially if cutaneous signs of hypersensitivity are associated. However, the interpretation of such biochemical liver abnormalities is difficult, raising the question of drug-related hepatitis but also of other toxic causes such as alcohol, cocaine, or metamphetamine.

An unsolved question is the respective place of anti-HCV and anti-HIV therapies. In clinical practice, most HIV patients are referred to the hepatologist while receiving HAART and the question is not relevant. In the future, physicians will have to consider the priority of each treatment according to liver histology and immune status. Given the chance of complete eradication of HCV-associated chronic hepatitis, anti-HCV therapy should be considered a priority and be treated first if the CD4 lymphocyte count is not less than 300 cells/mm^3 . A marked inflammatory activity or fibrosis (A2–A3 or F3–F4) indicates a need for anti-HCV treatment in order to avoid deleterious evolution; in contrast, a low inflammatory activity or fibrosis (A0–A1 or F0–F1) does not necessarily indicate anti-HCV treatment but a liver follow-up by biopsy and biochemical evaluation of fibrosis every 3 years. In intermediate situations (A2–F2), the choice to treat or not to treat should be based mainly on the predictive factors, including genotype and quantitative viremia (see Fig. 141c.2).

In conclusion, physicians should be aware of the potential risk of:

- symptomatic liver disease in HCV-HIV co-infected patients in the era of antiretroviral therapy;
- liver deterioration paralleling immune function restoration;
- lack of impact of active antiretroviral therapy on HCV load; and
- potential drug-related hepatitis that may modify the natural history of HCV-related liver disease.

Liver biopsies should be regularly performed to identify patients with severe liver disease who require early anti-HCV therapy (see Fig. 141c.2) under close monitoring of the immune status.

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141.d How to manage hyperlipidemia in the HIV patient

Judith Currier

Typical case

A 45-year-old woman with HIV infection presents to establish primary care. She was diagnosed with HIV during pregnancy 7 years ago. Initially her $CD4^+$ T cell count was 450 cells/mm^3 . She was treated with ZDV monotherapy for 8 months during her pregnancy and stopped all therapy 1 month postpartum. She restarted treatment 2 years ago when her $CD4^+$ T cell count was 225 cells/mm^3 and her HIV RNA is 65,000 copies/ml. She has maintained an undetectable HIV RNA on her current regimen of stavudine, lamivudine and nelfinavir. She has no family history of diabetes or heart disease. She has not had a menstrual period for 3 years. She smokes one pack of cigarettes per day. Her physical examination is normal and her blood pressure is 142/80mmHg. Fasting lipid values include triglycerides of 420mg/dl (4.75mmol/l), total cholesterol 260mg/dl (6.73mmol/l) and HDL cholesterol 28mg/dl (0.73mmol/l). Lipid values prior to starting treatment are not available. Does this patient have a clinically significant dyslipidemia and, if so, what are the options for management?

Dyslipidemia in HIV infection

The dyslipidemia associated with HIV infection may be a consequence of HIV infection per se, a direct effect of the antiretroviral agents used to treat HIV infection or it may be secondary to central adiposity and insulin resistance that can occur during treatment. Currently marketed protease inhibitors (PIs) differ slightly in their ability to cause dyslipidemia, whereas in general non-PI-containing regimens have been found to have less of an effect on lipids. Median increases in total cholesterol of 40–60mg/dl (1.04–1.55mmol/l) and triglyceride increases of 80–100mg/dl (0.90–1.13mmol/l) have been reported in studies examining currently marketed PIs. The magnitude of the effect on lipids may vary for different PI combinations. Patients with untreated advanced HIV infection have been noted to have a pattern of low HDL cholesterol and increased triglycerides.

Estimating cardiovascular risk

The long-term significance of dyslipidemia in HIV-infected individuals is not completely known. The patterns of lipid abnormalities most commonly observed in patients with HIV infection might be expected to result in increased cardiovascular morbidity. In many cases both antiretroviral therapies and HIV infection per se are thought to contribute. Large studies are currently underway to determine the incidence of cardiovascular events and atherosclerosis in HIV-infected individuals and the relative contribution of the identified risk factors. Preliminary recommendations for the management of dyslipidemia in HIV-infected individuals have been developed from existing guidelines applicable to the general population.

The National Cholesterol Education Program (NCEP) guidelines are a reasonable starting point for considering the need for intervention. These guidelines utilize threshold values of LDL cholesterol and consider the following as important risk factors for cardiovascular disease: current cigarette smoking, diabetes, hypertension, family history of coronary heart disease and HDL cholesterol. The first step in evaluating cardiovascular risk is to determine whether two or more risk factors are present. If so, then an estimate of the 10-year cardiovascular risk using the Framingham scoring system is suggested. In our example the patient has several classic risk factors for cardiovascular diseases; she is a smoker with untreated hypertension who is postmenopausal and she also has low HDL cholesterol and elevated total cholesterol. Using Table 141d.1 Table 141d.2 Table 141d.3 Table 141d.4 Table 141d.5 Table 141d.6 Table 141d.7 adapted from the NCEP ATP III guidelines, we can total her points for each of the risk factors to discover that she has a total of 23 points, which correlates with an estimated 10-year cardiovascular risk of over 20%. This is considered a high risk, similar to that of someone with established coronary heart disease.

The reliance on LDL cholesterol complicates the use of NCEP guidelines in the management of patients with HIV infection. When the value for triglycerides exceeds 400mg/dl (4.52mmol/l), calculation of LDL cholesterol from the total cholesterol and HDL is not accurate. Direct measurement of LDL is not available in many settings and hence it may be reasonable to base decisions for intervention on the values of non-HDL cholesterol. The value for non-HDL cholesterol is the total cholesterol minus HDL cholesterol. In our example, the value for non-HDL cholesterol would be 260 - 28 = 232mg/dl (6.0mmol/l). The NCEP ATP III guidelines include goals for non-HDL cholesterol in addition to LDL cholesterol and these are included in Table 141d.8 .

Management

Now that we have established that the patient in this example requires intervention for her dyslipidemia, we can consider the options for management. The first steps would include discussing her diet and lifestyle. The importance of smoking cessation and treatment of her hypertension should be addressed. These modifiable risk factors

TABLE 141.d-1 -- Calculating cardiovascular risk in women: age.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: AGE	
Age	**Points**
20–34	-7
35–39	-3
40–44	0
45–49	3
50–54	6
55–59	8
60–64	10
65–69	12
70–74	14
75–79	16

** (Adapted from Expert Panel 2001)*

TABLE 141.d-2 -- Calculating cardiovascular risk in women: total cholesterol.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: TOTAL CHOLESTEROL					
Total cholesterol	**Points**				
	Age 20–39	**Age 40–49**	**Age 50–59**	**Age 60–69**	**Age 70–79**
<160	0	0	0	0	0
160–199	4	3	2	1	1

200–239	8	em;6	4	2	1
240–279	11	8	5	3	2
=280	13	10	7	4	2

** (Adapted from Expert Panel 2001)*

TABLE 141.d-3 -- Calculating cardiovascular risk in women: smoking.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: SMOKING					
Smoking status	**Points**				
	Age 20–39	**Age 40–49**	**Age 50–59**	**Age 60–69**	**Age 70–79**
Nonsmoker	0	0	0	0	0
Smoker	9	7	4	2	1

** (Adapted from Expert Panel 2001)*

TABLE 141.d-4 -- Calculating cardiovascular risk in women: systolic blood pressure.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: SYSTOLIC BLOOD PRESSURE		
Systolic blood pressure (mmHg)	**Untreated**	**Treated**
<120	0	0
120–129	1	3
130–139	2	4
140–159	3	5
=160	4	6

** (Adapted from Expert Panel 2001)*

TABLE 141.d-5 -- Calculating cardiovascular risk in women: HDL cholesterol.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: HDL CHOLESTEROL	
HDL (mg/dl)	**Points**
=60	-1
50–59	0
40–49	1
<40	2

** (Adapted from Expert Panel 2001)*

TABLE 141.d-6 -- Calculating cardiovascular risk in women: total the points.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: TOTAL THE POINTS	
Risk factors	**Case example**
Age	3
Total cholesterol	8
Smoking	7
Systolic blood pressure	3
HDL cholesterol	2
Total	23

** (Adapted from Expert Panel 2001)*

TABLE 141.d-7 -- Calculating cardiovascular risk in women: point total and 10-year risk assessment.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: POINT TOTAL AND 10-YEAR RISK ASSESSMENT	
<9	**<1**
10–12	1
12–14	2
15	3
16	4
17	5
18	6
19	8
20	11
21	14
23	22
24	27
=25	=30

** (Adapted from Expert Panel 2001)*

TABLE 141.d-8 -- Calculating cardiovascular risk in women: NCEP III Risk Categories and Non-HDL Cholesterol Goals.*

CALCULATING CARDIOVASCULAR RISK IN WOMEN: NCEP III RISK CATEGORIES AND NON-HDL CHOLESTEROL GOALS		
Risk level	**LDL cholesterol**	**Non-HDL cholesterol**
CHD or risk equivalent	<100	<130
>2 Risk factors and <20% risk	<130	<160
0–1 Risk factor	<160	<190

** (Adapted from Expert Panel 2001)*

should be addressed prior to considering altering her currently successful antiretroviral regimen. Her diet should be reviewed and she should be given information on a low-fat (with reduced saturated fat), low-cholesterol diet. Given her calculated level of cardiovascular risk, additional measures to lower her non-HDL cholesterol are likely to be needed.

The additional measures for management of this patient's dyslipidemia include alteration in her antiretroviral regimen and the use of lipid-lowering agents. There are currently limited data to guide clinicians in this regard. Several studies have suggested a benefit of substituting a non-nucleoside analogue such as nevirapine or efavirenz as a reasonable option for patients who are well suppressed on a PI-containing regimen with dyslipidemia. The nucleoside analogue abacavir can also be substituted for the PI component of the regimen; however, in patients with a history of prior nucleoside analogue monotherapy the substitution of abacavir may be associated with an increased risk of virologic failure due to pre-existing nucleoside resistance mutations. The patient in this example received monotherapy with zidovudine for 8 months during an earlier pregnancy and this may increase her risk of failing an abacavir substitution. In our case, substitution of nelfinavir with nevirapine or efavirenz would be my preference for initial management of her dyslipidemia. If the patient is reluctant to change her therapy, the use of lipid-lowering agents should then be considered if changes in diet are not successful.

There are currently limited data on the efficacy of lipid-lowering therapy in patients with HIV infection. The HMG-CoA reductase inhibitors (statins) are generally the first-line intervention for lipid disorders in the general population. Important drug interactions between the statin agents lovastatin and simvastatin and the PIs have been reported and these agents are not currently recommended. The statin agent least likely to interact with PIs is pravastatin. Drug interactions are unlikely to occur with the use of fibric acid derivatives gemfibrozil and fenofibrate. Preliminary results of clinical trials evaluating the use of either gemfibrozil and statin agents have been reported. In the case above, with elevations in both triglycerides and non-HDL cholesterol, the use of gemfibrozil at a dose of 600mg po twice daily 30 minutes prior to meals in addition to dietary advice would be a reasonable first approach if substituting the nelfinavir with a non-nucleoside were not an option. If there was no response after 3 months consideration should be given to adding a statin agent-pravastatin at a dose of 20mg po qd. Close monitoring for hepatotoxicity and myopathy is warranted when statin and fibrate agents are used concurrently. Again, substituting the PI component of the regimen would be preferable to adding a second lipid-lowering agent.

As the treatment of HIV infection has evolved into the management of a chronic disease more emphasis is being placed on preventing long-term complications from both the disease and the therapies used to treat it. Currently, it is not known whether patients with HIV infection who sustain increases in lipids are at the same risk as would be expected if these lipid changes occurred spontaneously. It is important for clinicians to be aware of these issues and to screen patients for lipid disorders. Attention to modifiable cardiac risk factors such as smoking, diet and level of physical activity is critical and should not be overlooked. In settings where the underlying cardiovascular risk appears to be high, further interventions with lipid-lowering agents or modification of the antiretroviral regimen may be warranted.

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141.e Managing the patient with multidrug-resistant HIV

Pablo Tebas

Frequency of the problem

Unfortunately, because of difficulty with adherence to antiretroviral treatment or the sequential use of partially suppressive regimens when therapy was initiated, a very significant proportion of patients with HIV infection develop multidrug-resistant HIV. In a recent survey of the ongoing HIV Costs and Service Utilization Study (HCSUS), approximately 63% of the patients under care had a detectable viral load at the time of the last determination, 78% of those had resistance to at least one drug and more than 50% were resistant to two. These data suggest that more than 100,000 patients in the USA alone (or half of the total number of patients in care) are infected with viruses that are resistant to antiretroviral drugs. HIV disease is like other infectious diseases, such as tuberculosis or malaria, in which the development of resistance is a frequent clinical problem. The problem of drug resistance in HIV is more common among subjects receiving therapy and those who are in care than among patients who have never received treatment.

Primary resistance to HIV therapies is rising alarmingly among new and chronically infected individuals in the developed world. Unfortunately, this problem will also affect the developing world when antiretroviral therapy becomes more widely available. Primary resistance is more frequently seen with nucleoside reverse transcriptase inhibitors, since these are the drugs that have been used for the longest period of time. It is increasing among non-nucleoside reverse transcriptase inhibitors and protease inhibitors. The goal is to prevent this problem by the intelligent use of antiretroviral medications and resistance testing. Despite these efforts, the use of antiretroviral therapy will always be associated with the development of resistance. Clinicians will frequently face patients for whom no therapeutic options are readily available.

Therapeutic options for the patient who has multidrug-resistant HIV

When the number of treatment options is limited, the clinician must select between discontinuing a costly, and potentially ineffective toxic treatment that is increasing the risk of accumulating multiple resistance mutations, or maintaining a partially suppressive regimen in the hope that this approach will delay the appearance of immunologic and clinical failure.

Several considerations are important in this all too frequent situation. First, virologic failure is not equivalent to immunologic and clinical failure. A recent study indicates that the median period of time between virologic and immunologic failure is 36.4 months (3 years). The delay between virologic and immunologic failure seems to be associated with the decreased replicative fitness of viruses that harbor protease-inhibitor-associated resistance mutations. Viruses with multiple mutations are less cytopathic in vitro than wild-type isolates. Second, discontinuing therapy in the patient with multidrug-resistant virus is associated with the overgrowth of wild-type virus after approximately 12 weeks. This switch to wild type as the predominant quasispecies in the patient is associated with a significant decay in the CD4+ T cell count, rapid progression of HIV disease and, in some cases, serious opportunistic infections. Third, there is growing evidence that the rates of clinical progression are low in patients who continue antiretroviral therapy in the presence of virologic failure. These subjects maintain their CD4+ cell numbers, especially if the viral load is maintained below the natural set point of the patient (the HIV RNA load before the initiation of antiretroviral therapy).

Based on the above, most experienced clinicians would maintain a partially suppressive regimen in a patient with limited therapeutic options, especially if the disease is in an advanced stage. The goal of therapy in this situation shifts from complete suppression of viral replication to partial suppression to prevent immunologic and clinical decline.

Many clinicians also maintain lamivudine in these patients because the presence of the M184V mutation (usually associated with the use of lamivudine) may enhance susceptibility to zidovudine, stavudine or tenofovir. These drugs are frequently used individually as part of combination regimens in the patient with multidrug resistance. The presence of this mutation also stabilizes the reverse transcriptase of the virus and makes it less prone to make errors, which are associated with the development of resistance. This effect may be overcome by an accumulation of nucleoside associated mutations (also known as NAMs): M41L, E44D, D67N, K70R, V118I, L210W, T215Y/F and K219Q/E (see Chapter 137 and Chapter 204).

Other authors have suggested the used of multidrug combination regimens (six or more drugs), also called 'mega-HAART regimens', in this situation. Occasionally, significant antiviral activity has been observed; however, these regimens are complicated to use, especially in patients with adherence problems (as is usually the case in patients with multidrug resistance). They are also associated with significant side effects, drug-drug interactions and increased cost. The use of therapeutic drug monitoring (measuring antiretroviral drugs levels, and adjusting dosages accordingly) might be necessary in these cases.

Although some authors have suggested that transient discontinuation of antiretroviral treatment might 'resensitize' the virus and make it susceptible to previously used antiretroviral agents, this phenomenon is probably short-lived because of 'archived' resistance of the integrated HIV in the T cell memory reservoirs. This approach might work better in the patient who has some therapeutic options left, and should always be undertaken with extreme caution because of the high risk of significant clinical deterioration in the patient who has advanced disease.

In the patient with multidrug-resistant HIV, it is also critically important to maintain all prophylactic regimens that the subject should be taking at the recommended CD4+ T cell count thresholds. Thus, it is important to maintain *Pneumocystis carinii, Toxoplasma* and *Mycobacterium avium* complex prophylaxis if indicated. It is probably also a good idea to increase the frequency of the clinical visits to once every month or two, so opportunistic infections can be readily identified and treated.

New drug availability

Before the licensing agencies (e.g. the Food and Drug Administration in the USA) grant full approval, new drugs often become available as part of compassionate use and expanded access programs. The clinician frequently is pressed and tempted to add this 'single' new drug to the regimen that the patient is taking, hoping to obtain at least a partial virologic and clinical benefit in a patient with very limited options. The decision to do this should be based on the clinical situation of the patient and the types of drugs available. For some classes of drugs, such as non-nucleoside reverse transcriptase inhibitors and fusion inhibitors, resistance develops very quickly if those drugs are not used as part of fully suppressive regimens. With other classes of drugs, such as protease inhibitors or nucleoside/nucleotide analogues, resistance takes longer to develop and durable virologic and potentially clinical benefits might be obtained.

For compounds where the threshold for developing resistance is low, it might be reasonable to wait until more drugs become available and a combination regimen with a good chance of response can be developed. For drugs with a higher threshold for resistance, immediate use might be advisable, especially in the patient who is clinically deteriorating. For example, enfuvirtide (also known as T-20) is a peptide fusion inhibitor that binds to the HR-1 domain of gp41. Its use has been associated with significant decreases in viral load when used as monotherapy, and improvements in the viral load response of otherwise optimized rescue regimens. HIV becomes resistant to this drug very quickly if it is not used as part of a fully suppressive antiretroviral regimen. Resistance mutations in the gp41 envelope gene have been identified primarily at positions 36 to 45 of the first heptad repeat (HR1) region. On the other hand, tenofovir, a recently approved nucleotide, was evaluated as an 'intensification' (the addition of a single drug to a failing antiretroviral regimen) in Gilead study 907. The virologic benefits (approximately half a log decrease in HIV viral load) were still present after 48 weeks of treatment.

If the patient is clinically failing antiretroviral therapy or the addition of a single drug is being considered, it is important to maximize the benefits of the background regimen that the patient is taking. Phenotypic resistance testing might have an edge over genotypic or 'virtual phenotype' testing (a genotype test with an automatic interpretation linked to a large database of geno-phenotypes) in this very complicated situation (see Chapter 137). It might better identify which drugs maintain a residual activity in vitro in a patient who has limited therapeutic options. It also might provide a measurement of 'replicative capacity' of the patient's virus.

The use of the replication capacity assay

Replicative fitness is the ability of a species or strain of virus to compete against others in a defined environment; for example, a wild-type virus is more 'fit' than a virus with multidrug resistance in a environment where there is no drug, but the reverse is true in the presence of antiretroviral therapy. To evaluate replicative fitness, it is necessary to conduct very cumbersome assays in which the two different strains to be tested compete against each other. Recently a 'replication capacity assay' has been provided as part of the phenotypic assay (see Chapter 137). In this assay patient-derived HIV reverse transcriptase or protease undergoes a single round of replication. The vector contains a luciferase gene that permits quantitation of replication, which is then compared to the level of replication of a wild-type HIV reference strain. The result is provided as the ratio of the patient strain replication to the wild-type (reference) strain replication. Normally, multidrug-resistant viruses have replication capacity values less than 1. Theoretically, the lower the number the less fit the virus is.

The number of studies using this assay is limited. Changes in the results obtained with this assay predicted the change in viral load when therapy was discontinued in patients with evidence of multidrug resistance. Based on those results and the results of small-cohort studies, some clinicians are using this assay to guide therapy in the patient with multidrug resistance by selecting combination regimens that lower the replication capacity of the predominant quasispecies of the patient. However, clinical validation of the utility of this assay is still lacking. Until then this approach might lead to unnecessary changes of otherwise well-tolerated regimens. Ensuring that the viral load is kept as low as possible, preferably below the natural set point of the patient, might be another approach to handle the patient who has limited therapeutic options.

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Section 6 - GEOGRAPHIC AND TRAVEL MEDICINE

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Chapter 142 - Geography of Infectious Diseases

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INTRODUCTION

Infectious diseases vary by geographic region and population, and they change over time.[1] Increasingly, humans are moving from one region to another, thereby becoming exposed to a variety of potential pathogens and also serving as part of the global dispersal process.[2] Microbes picked up at one time and in one place may manifest in disease far away in time and place. Because many microbes have the capacity of persisting in the human host for months, years or even decades, the relevant time frame for study of exposures becomes a lifetime. Furthermore, microbes also move and change and reach humans via multiple channels.

Caring for patients in today's world requires an understanding of the basic factors that underlie the geography of human diseases and events that cause shifts in the distribution and burden of specific diseases. Current technology contributes to massive population movements and rapid shifts in diseases and their distributions, but it also provides communication channels that can aid clinicians who care for patients with complicated medical problems. This chapter reviews the factors that shape the global distribution of infectious diseases and the forces that are expected to shift distributions in the future. Several examples are used to illustrate the broad range of factors that affect the distribution and expression of infectious diseases.[3]

Many authors have traced the origins and spread of specific infectious diseases through human history. A century and a half ago, John Snow noted that epidemics of cholera followed major routes of commerce and appeared first at seaports when entering a new region.[4] Yersinia pestis, the cause of plague, accompanied trade caravans and moved across oceans with rats on ships. Exploration of the New World by Europeans introduced a range of human pathogens that killed one-third or more of the local populations in some areas of the Americas. The plants and animals introduced as a result of this exploration have also had profound and long-lasting consequences for the ecology and economics of the new environment. [5] The speed, reach and volume of today's travel are unprecedented in human history and offer multiple potential routes to move biologic species around the globe. Pathogens of animals and plants are being transported as well and this can affect global food security.[6] This chapter focuses only on pathogens that directly affect human health and on their sources (Table 142.1). When thinking about geography of human infections, it is useful to consider both the origin of the organism and the conveyor or immediate source for the human (Fig. 142.1).

This chapter addresses three key issues:

- factors influencing geographic distribution: why are some infectious diseases found only in focal geographic regions or in isolated populations?
- factors influencing the burden of disease: why does the impact from widely distributed infections vary markedly from one region or one population to another? and
- factors influencing emergence of disease: what allows or facilitates the introduction, persistence and spread of an infection in a new region and what makes a region or population resistant to the introduction of an infection?

FACTORS INFLUENCING GEOGRAPHIC DISTRIBUTION

In past centuries, lack of interaction with the outside world could allow an infection to remain geographically isolated. Today, most infections that are found only in focal areas have biologic or geoclimatic constraints that prevent them from being introduced into other geographic regions. For example, the fungus *Coccidioides immitis*, which causes coccidioidomycosis, thrives in surface soil in arid and semiarid areas with alkaline soil, hot summers and short, moist winters; it is endemic in parts of south-western USA, Mexico and Central and South America. People become infected when they inhale arthroconidia from soil. An unusual wind storm in 1977 lifted soil from the endemic region and deposited it in northern California, outside the usual endemic region.[7] In general, infection is associated with residence in or travel through the endemic region. However, because the fungus can persist in the human host for years, even decades, after initial infection (which may be mild and unrecognized), disease may be diagnosed far from the endemic regions.

Vectors

Many microbes require a particular arthropod vector or animal host and hence inhabit circumscribed regions and may be unable to survive in other habitats. Malaria is a vector-borne infection that cannot persist in a region without a competent vector. The presence of a competent vector is a necessary but not sufficient condition for human infection. The mosquito must have a source of malarial parasites (gametocytemic human who may be asymptomatic), an appropriate incubation period to allow development of the parasite to a form that is infective via a bite, and access to other humans. Prevailing temperature and humidity must allow the mosquito to survive long enough for the malarial parasite to undergo maturation to reach an infective state for humans. Competent vectors exist in many areas without malaria transmission, because the other conditions are not met. These areas are at risk of the introduction of malaria, as illustrated by several recent examples in the USA and elsewhere.[8] [9]

Malaria was endemic in many parts of the USA into the 20th century (Fig. 142.2), with estimates of more than 600,000 cases in 1914.[9] Even before extensive mosquito control programs were instituted, transmission declined. Demographic factors (population shifts from rural to urban areas), improved housing with screened doors and windows and the availability of treatment were among the factors that may have contributed to this decrease.

The flavivirus dengue is transmitted primarily by the widely distributed mosquito, *Aedes aegypti*, which is well adapted to human habitats. This viral infection has now become a major and growing problem in many tropical and subtropical regions throughout the world (see below).

The distribution of onchocerciasis in Africa is notable for its association with rivers.[10] The reason becomes clear by understanding that the vector of this filarial parasite, the black fly (genus *Simulium*), lays her eggs on vegetation and rocks of rapidly flowing rivers and

TABLE 142-1 -- Origins and conveyors of human pathogens.

ORIGINS AND CONVEYORS OF HUMAN PATHOGENS		
Origin or carrier	**Conveyor or immediate source**	**Examples of disease**
Humans	Humans	HIV, syphilis, hepatitis B
Humans	Humans (air-borne pathogen)	Measles, tuberculosis
Soil	Soil, air-borne	Coccidioidomycosis
Soil	Food	Botulism
Animals	Water	Leptospirosis
Humans	Mosquitoes	Malaria, dengue
Humans	Soil	Hookworm, strongyloidiasis
Animals	Ticks	Lyme disease
Animals, humans	Sand flies	Leishmaniasis
Animals	Animals	Rabies
Rodents	Rodent excreta	Hantaviruses
Humans	Water, marine life	Cholera
Humans or animals (with snails as essential intermediate host)	Water	Schistosomiasis
Humans	Food	Typhoid fever
Animals	Water	Cryptosporidiosis, giardiasis
Some pathogens have multiple potential sources.		

usually inhabits a region within 5–10km on either side of a river. Another name for onchocerciasis, river blindness, describes the epidemiology as well as one consequence of infection.

Some pathogens have a complex cycle of development that requires one or more intermediate hosts. Distribution may remain relatively fixed, even when infected humans travel widely, if other regions do not supply the right combination and geographic proximity of hosts (Fig. 142.3). Although persons with schistosomiasis visit many regions of the world, the parasite cannot be introduced into a new region unless an appropriate snail host is present, excreted eggs (in urine or feces) are released into water where they reach the snail hosts and humans subsequently have contact with the untreated water.[11]

Many hantaviruses exist worldwide with distributions that are still being defined. Each hantavirus seems to have its specific rodent reservoir with which it has evolved. As with many zoonoses, humans are incidental to the survival of the virus in rodents, yet humans can develop severe and sometimes fatal disease if they happen to enter an environment where they are exposed to the virus. Undoubtedly, other rodent-associated viruses and other pathogens (as well as pathogens associated with other animals or insects) with the capacity to infect humans will be identified as humans enter unexplored environments in the future.

Lassa and Ebola viruses are other viruses that have focal distributions, although the reservoir host for Ebola virus has not yet been defined. Because these infections can be spread from person to person, secondary household and nosocomial spread in several instances has amplified what began as an isolated event. Lack of adequate resources in hospitals in many developing regions contributes to the spread of infections within hospitals and to persons receiving outpatient care, such as those receiving injections.

Figure 142-1 Life cycles of some important pathogens.

Cultural practices can lead to unusual infections in isolated areas. Residents of the highlands of Papua New Guinea developed kuru after ingestion (or percutaneous inoculation) of human tissue during the preparation of the tissues of dead relatives.

Thus, the presence of a pathogen in a region may reflect the biologic properties of the organism, its need for a certain physicochemical environment or its dependence on specific arthropods, plants or animals to provide the milieu where it can sustain its lifecycle (Table 142.2). The presence of a pathogen in a region does not necessarily equate with human disease, because mechanisms must exist for the pathogen to reach a susceptible human host for human disease to occur. Sometimes it is only with exploration of new regions or changes in land use that humans place themselves in an environment where they come into contact with microbes that were

Figure 142-2 Areas of the USA thought to be endemic for malaria during the years 1882–1912.

Figure 142-3 Worldwide distribution of schistosomiasis.

previously unrecognized as human pathogens. Preferences for specific foods, certain preparation techniques or cultural traditions may place one population at a unique risk for infection.

TABLE 142-2 -- Biologic attributes of organisms that influence their epidemiology.

BIOLOGIC ATTRIBUTES OF ORGANISMS THAT INFLUENCE THEIR EPIDEMIOLOGY
• Host range
• Duration of survival in host
• Route of exit from host
• Route of entry into human

• Virulence
• Capacity to survive outside host
• Resistance to antimicrobials and chemicals

TABLE 142-3 -- Modes of transmission for major global infectious diseases.[*]

MODES OF TRANSMISSION FOR MAJOR GLOBAL INFECTIOUS DISEASES	
Mode of transmission	**% of total**
Person-to-person	65
Food-borne, water-borne or soil-borne	22
Insect-borne	13
Animal-borne	0.3

** The figures are based on an estimated 17.3 million deaths due to infectious diseases in 1995, as reported by the World Health Organization.[12]*

FACTORS INFLUENCING THE BURDEN OF DISEASE

Among the infectious diseases that impose the greatest burden of death globally, most are widely distributed: respiratory tract infections (e.g. influenza, *Streptococcus pneumoniae* and others), diarrheal infections, tuberculosis, measles, AIDS and hepatitis B.[12] Most of these infections are spread from person to person. The World Health Organization estimated that about 65% of infectious diseases deaths globally in 1995 were due to infections transmitted from person to person (Table 142.3).[12]

Burden from these diseases is unevenly distributed across populations and among different countries. Poor sanitation, lack of clean water, crowded living conditions and lack of vaccination contribute to the disproportionate burden from many of these infections in developing regions of the world. In industrialized countries, pockets of high risk persist. Disadvantaged populations have higher rates of tuberculosis, HIV and many other infectious and noninfectious diseases. Rates of reported cases of tuberculosis vary widely by region (Table 142.4).[13] Variation also exists within countries. Figure 142.4 shows the effect of crowded living conditions on rates of tuberculosis in England and Wales in 1992.[14] Among welfare applicants and recipients addicted to drugs or alcohol in New York City, the rate of tuberculosis was 744 per 100,000 person years or more than 70 times the overall rate for the USA. [15] The impact of an infection derives not only from the risk of exposure but also from the access to effective therapy. For example, treatment of a patient with active tuberculosis can cure the individual and eliminate a source of infection for others in the community.

Neonatal tetanus, which was estimated to kill 459,000 persons (mostly neonates) in 1995,[12] is caused by a widely distributed bacterium, but the disease predominates in areas where pregnant women and their infants are not protected by immunization with tetanus toxoid. Diphtheria, controlled in many parts of the world,

TABLE 142-4 -- Rates of reported cases of tuberculosis worldwide by region (2000).[†]

RATES OF REPORTED CASES OF TUBERCULOSIS WORLDWIDE BY REGION (2000)		
Region	**Rate per 100,000 population**	**Range of rates[*]**
Africa	115	17–635
Americas	28	0–151
Eastern Mediterranean	28	4–628
Europe	42	0–160
South East Asia	91	32–153
Western Pacific	48	0–253

† *Source: WHO Report. Global tuberculosis control. Geneva: WHO; 2002.*
* Highest and lowest rates reported by countries in region.

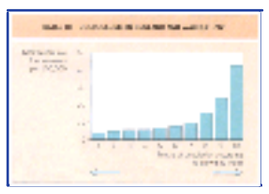

Figure 142-4 Rates of tuberculosis in England and Wales by crowding index (1992). *Adapted from Bhatti et al.*[14]

resurged in new independent states of the former Soviet Union in the 1990s, a reminder of the tenuous control over many infectious diseases. Populations in other countries also felt the impact as cases related to exposures in the Russian Federation were reported in Poland, Finland, Germany and the USA. Serologic studies in America and Europe suggest that up to 60% of adults may be susceptible to diphtheria.

Travelers to tropical and developing regions of the world can pick up geographically focal, often vector- or animal-associated infections (such as malaria and dengue), but travelers most often acquire infections with a worldwide distribution that are especially common in areas lacking good sanitation. Food- and water-borne infections are common and lead to traveler's diarrhea, which is caused by multiple agents (including *Escherichia coli, Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and others), typhoid fever and hepatitis A. Respiratory tract infections may be acquired from other travelers from all over the globe during the crowding that occurs in travel (e.g. in buses, airplanes, terminals and on cruise ships) as well as from persons in the local environment. Table 142.5 and Table 142.6 note factors that influence the types and abundance of microbes in a community and the probability of exposure to pathogens.

Hepatitis A virus remains a common cause of infection in developing regions of the world although it is not considered a major cause

TABLE 142-5 -- Factors that influence the types and abundance of microbes in a community.

FACTORS THAT INFLUENCE THE TYPES AND ABUNDANCE OF MICROBES IN A COMMUNITY
• Geoclimatic conditions
• Socio-economic conditions
• Public health infrastructure
• Urban versus rural environment
• Density and mobility of population

• Season of the year

TABLE 142-6 -- Factors that influence the probability of exposure to pathogens.

FACTORS THAT INFLUENCE THE PROBABILITY OF EXPOSURE TO PATHOGENS
• Living accommodation
• Level of sanitation
• Occupational and recreational activities
• Food preparation and preferences
• Sexual activities and other behavior
• Contact with pets and other animals
• Time spent in the area

of morbidity or mortality in those regions where most persons are infected at a young age and become immune for life. The presence and severity of symptoms are related to the age at which a person becomes infected. Infection in young children is typically mild or inapparent. Persons living in areas of high transmission may be unaware of the presence of high levels of transmission, although nonimmune, older people (such as travelers) who enter the environment may develop severe, and occasionally fatal, infection. Some countries with an improving standard of living have noted a paradoxic increase in the incidence of disease from hepatitis A virus as the likelihood of exposure at a young age decreases, shifting upward the age of infection to a time when jaundice and other symptoms are more likely to occur.

Travelers may also contribute to the spread of infectious diseases and influence the global burden of these diseases. *Neisseria meningitidis*, a global pathogen, occurs in seasonal epidemics in parts of Africa: the so-called meningitis belt (Fig. 142.5).[16] Irritation of the throat by the dry, dusty air probably contributes to invasion by colonizing bacteria. Pilgrims carried an epidemic strain of group A *N. meningitidis* from southern Asia to Mecca in 1987. Other pilgrims who became colonized with the epidemic strain introduced it into sub-Saharan Africa, where it caused a wave of epidemics in 1988 and 1989. Using molecular markers, investigators were able to trace the spread of the epidemic clone to several other countries.[17] In 1996 in Africa, major outbreaks of meningococcal meningitis occurred (>185,000 reported cases with a case fatality rate of ~10%) caused by *N. meningitidis* serogroup A, clone III-1.[18] A virulent group C, ET-15 strain of *N. meningitidis* spread in Canada and was associated with an increased case fatality rate and a higher proportion of cases in persons over the age of 5 years.[19] In these examples, the virulence of the microbe and travel and trade acted synergistically to change the epidemiology and burden of disease. In the spring of 2000 serogroup W135 *N. meningitidis* caused an outbreak of infection in pilgrims to the Hajj and subsequently spread to their contacts and others around the world. Studies using serotyping, multilocus sequence typing, multilocus

Figure 142-5 Meningitis belt in Africa.

DNA fingerprints and other techniques found identical W135 isolates in multiple countries. Pilgrims are required to receive a meningococcal vaccine but before this outbreak, pilgrims from many countries received a vaccine that protected against serotype A but not W135. The vaccine reduces risk of disease but does not prevent oropharyngeal carriage of *N. meningitidis*.[20]

FACTORS INFLUENCING EMERGENCE OF DISEASE

Regular, rapid movement of persons from tropical regions to major urban areas throughout the world raises concerns that unusual infections could be introduced into an environment where they could spread to large populations. How likely is this? Microbes are repeatedly carried around the globe, yet endemic regions for many infections remain relatively fixed. Most examples of the appearance of a microbe or disease in a region are not followed by sustained spread.

In order to assess the potential for a pathogen to be introduced into a new population, information is required about the biologic properties of the organism, the region and population being considered and the mechanisms of transmission (see Table 142.1). The key factor that determines whether a pathogen can survive and spread in a new environment is its basic reproductive rate, which is the average number of successful offspring a pathogen can produce. To become established in a new host population, a parasitic species must have a basic reproductive rate that exceeds one offspring per pathogen. [21] The concept is simple but invasion and persistence are affected by a range of biologic, social and environmental factors.

Certain factors restrict the introduction and spread or persistence of infection in a region (Table 142.7); many of these are discussed above. Before measles vaccine was introduced, the epidemiology of measles exhibited marked periodicity in large populations, with peaks typically occurring every 2–3 years.[22] In general, a community size of about 250,000 is necessary to provide a sufficient number of susceptible people to sustain the virus. In small island communities

TABLE 142-7 -- Factors that restrict the introduction and spread of infections.

FACTORS THAT RESTRICT INTRODUCTION AND SPREAD OF INFECTIONS
• Geoclimatic factors that cannot support vector or intermediate host
• Genetics of human population, making it genetically resistant or relatively resistant
• Immunity of human population, making it not susceptible because of past infection with same or related microbe or via vaccination
• Demographic factors (e.g. size and density of population will not support sustained transmission of diseases such as measles)
• Social and behavioral factors (absence of activities such as iv drug use and unprotected sex with multiple partners)
• Food preparation habits and local traditions (e.g. certain dishes not eaten, food always well cooked)
• High-quality housing, sanitation, public health infrastructure
• High standard of living, good nutrition, lack of crowding

(or other isolated populations), outbreaks typically occur only after periodic introductions from outside. Size and density of a population thus influence the epidemiology of some infections. It has been suggested that measles as it has been known in the 20th century could not have established itself much before 3000 BC because before that time human populations had not achieved sufficient size to sustain the virus. Measles could not have persisted in nomadic, hunting communities.

Examples of emerging pathogens

It is instructive to look at examples of infections that have recently undergone major shifts in distribution and to review the key factors that have influenced their geographic spread. They are a reminder of the complexity of the interactions among host, microbe and the environment. A recurring theme is the movement of humans who introduce pathogens into a new region (see also Chapter 4).

Human immunodeficiency virus and other pathogens carried by humans

Organisms that survive primarily or entirely in the human host and are spread from person to person (e.g. by sexual or other close contact or by droplet nuclei) can be

carried to any part of the world. The spread of HIV in the past two decades to all parts of the world is a reminder of the rapid and broad reach of travel networks. Although the infection has also spread via blood and shared needles, it has been the human host engaging in sex and reproduction who has been the origin for the majority of the infections worldwide. Person-to-person spread accounted for the rapid world-wide distribution of SARS (severe acute respiratory syndrome), a coronavirus infection, in the spring of 2003.

Drugs or vaccines injected by reused inadequately sterilized needles and syringes have been and continue to be an important means of spread of blood-borne infections, such as hepatitis C, hepatitis B and HIV, in some parts of the world. [23]

A large outbreak of antibiotic-resistant shigellosis involving more than 50% of the estimated 12,700 persons at a mass gathering in North Carolina, USA, was followed by nationwide dissemination of the organism and outbreaks in at least three other states.[24] Using molecular techniques coupled with epidemiologic data, investigators were able to show the emergence of multiple drug-resistant tuberculosis clones in New York and their dissemination in New York and to at least four other cities in the USA.[25] It is not only the pathogens carried by humans that are relevant. Humans also carry resistance and virulence factors that can be transferred to and exchanged with other microbes.[26]

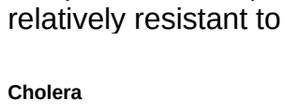

Figure 142-6 Areas reporting dengue fever and areas with a competent vector (2002). Many areas with a competent vector do not report dengue epidemic activity. *Data from the Centers for Disease Control and Prevention.*[16]

Dengue fever

Dengue fever is a mosquito-borne viral infection that has now spread to most tropical and subtropical regions of the world and threatens to continue to increase in incidence and severity. Viremic humans regularly enter regions infested with *Aedes aegypti*, the principal vector of dengue, transporting the virus for new outbreaks. Infection can spread rapidly and outbreaks are sometimes massive, involving >30% of the population. Because four serotypes of dengue virus exist and infection with one serotype does not confer lasting immunity against other serotypes, a person can be infected more than once. The risk of developing severe dengue (e.g. dengue shock syndrome or dengue hemorrhagic fever, DSS or DHF) after repeat infection is 82–103 times greater than after primary infection. [27] In an outbreak in Cuba, 98.5% of cases of DSS or DHF were in persons with a prior dengue infection. The rate of DSS or DHF was 4.2% in persons with prior dengue infection who became infected with a new serotype. [28] Geographic regions where multiple serotypes are circulating have continued to expand, setting the stage for more severe consequences of infection. Factors that have aided the spread of dengue include increasing (and rapid) travel to and from tropical regions; expansion of the regions infested with *Aedes aegypti*; increasing urbanization, especially in tropical areas, which has provided large, dense populations; the use of nonbiodegradeable and other containers that make ideal breeding sites for the mosquito; and lack of support for vector control programs.

Most of the world population growth is occurring in tropical and developing regions. The expectation is that more urban areas in tropical regions will reach the critical population size, perhaps somewhere between 150,000 and 1 million people, to permit sustained transmission of dengue and to increase the risk of the severe forms of infection, dengue hemorrhagic fever and dengue shock syndrome. [29] But travelers are also at risk and the 90 laboratory-diagnosed infections in travelers returning to the USA in 1998 represented a 70% increase from 1997.[30]

It is instructive to ask not only where dengue occurs but also where it does not. Although large dengue epidemics occurred in the USA earlier in this century, only a handful of cases have been acquired in the USA in recent years, despite the presence of epidemic disease in adjacent areas of Mexico and the presence of a competent vector (*Aedes aegypti*) in south-eastern USA (Fig. 142.6).[16] It is possible that the presence of screened dwellings and air conditioning may make an area relatively resistant to the introduction of infection, even if a competent vector infests a region.

Cholera

Cholera is an ancient disease that is continuing to spread and to change. In 1991 it entered Latin America for the first time in more than a century and spread rapidly from Peru to infect persons throughout the region. By 1996, more than 1.4 million cases had been reported to cause more than 10,000 deaths.[31] In 1992, a novel strain of cholera, classified as *Vibrio cholerae* 0139 Bengal, emerged in India and Bangladesh, causing major epidemics in India, Bangladesh and other Asian countries. Travelers carried infections back to Europe, the USA and Japan. This new strain was unusual in that it was the first non-01 strain that could cause epidemics. Persons in cholera-endemic regions who had immunity to the well-known cholera strains were susceptible to this new strain. The already licensed vaccines did not protect against *V. cholerae* 0139.

Cholera illustrates the complex interactions between microbe, environment and host.[32] Epidemics are seasonal in endemic regions. *V. cholerae* lives in close association with marine life, binding to chitin in crustacean shells and colonizing surfaces of algae, phytoplankton, zooplankton and water plants. *V. cholerae* can persist within the aquatic environment for months or years, often in a viable but dormant state, nonculturable by usual techniques. Environmental factors, including temperature, salinity, pH and sea-water nutrients, affect the persistence, abundance and viability of the organisms, and hence have a striking influence on human epidemics.

Under conditions of population crowding, poor sanitation and lack of clean water, cholera can have a devastating impact, as was shown by the massive outbreak of El Tor cholera in Rwandan refugees in Goma, Zaire, which caused 12,000 deaths in July 1994.[33]

The organism can be carried by humans, who sometimes have few or no symptoms, and introduced into new regions. Trade probably also plays a critical role. Ballast water, picked up by boats in multiple locations and discharged at another time and place, carries a wide range of species, including many that have no direct impact on human health.[34] [35] [36] In studies of the ballast and bilge of cargo ships in the USA Gulf of Mexico, researchers were able to identify *V. cholerae* identical to the strains causing epidemic disease in Latin America. [37]

Food-borne disease

The globalization of the food market means pathogens from one region can appear in another; some are common pathogens with a worldwide distribution but others are not. An outbreak of cholera in Maryland, USA, was traced to imported, contaminated commercial frozen coconut milk.[38] Alfalfa sprouts grown from contaminated seed sent to a Dutch shipper caused outbreaks of infections with *Salmonella* spp. on two continents, in at least Arizona and Michigan in the USA and in Finland.[39] Commercial movement of fruits and vegetables redistributes resistance factors along with the microbes. Tracing the source after an infection has been diagnosed can be convoluted and often is not carried out unless disease is severe, lethal or epidemic or involves a highly visible person or population.

Travel and trade are key features in the epidemiology of the infection *Cyclospora*, a cause of gastroenteritis. Recognized for many years in multiple regions of the world, cases were often associated with living in or travel to areas where sanitary facilities were poor. Most of the experience in the USA with the disease was in overseas travelers. In the summer of 1996, a large outbreak occurred in persons who had not traveled. Over a period of a few months, 1465 cases of cyclosporiasis were reported from 20 states. The outbreak was linked to eating raspberries imported from Guatemala.[40] During some seasons of the year up to 70% of selected fruits and vegetables sold in the USA come from developing countries.

Schistosomiasis

Seemingly unrelated events can profoundly alter the epidemiology of infectious diseases in humans. Changes in the way land is used is an important one. In Egypt, prevalence of *Schistosoma mansoni* infection increased from 21.7% in 1985 to 42.1% in 1992 among settlers in a region where recent irrigation projects had reclaimed land from the desert. The irrigation water came from the Nile River or its irrigation dams, which made it likely that snail vectors would be introduced into the new regions. Many settlers to the area were already infected and so contributed to the contamination of the waters because sanitary treatment of human excreta was generally unavailable. New settlers came from other regions and had little or no pre-existing immunity to schistosomiasis, making them especially vulnerable to the

consequences of infection.[41]

In Senegal, extensive agricultural development and the building of two large dams led to the introduction of *Schistosoma mansoni* into the Senegal river basin.[42] The first case was discovered in 1988. Infection spread rapidly and the prevalence of schistosomiasis reached 45–70% by 1990.

Visceral leishmaniasis

In the past, visceral leishmaniasis in Brazil was primarily a rural disease. Recently, however, several cities have reported large outbreaks of visceral leishmaniasis.[43] Reasons for the change in epidemiology include geoclimatic and economic factors (drought, lack of farm land, famine) leading to migration of large numbers of persons, who settle in periurban areas where they live in densely crowded shanties, lacking basic sanitation. The presence of domestic animals, such as dogs, chickens and horses, in and adjacent to human dwellings provides ample sources of blood meals for the sand fly, the vector of leishmaniasis. Outbreaks have occurred in many cities in Brazil, including Teresina, São Luis and Natal. Children and young people have been most affected. Malnutrition also can contribute to the severity of the disease.

Disease-disease interactions can also alter the epidemiology of infections. Visceral leishmaniasis has become an important infection in HIV-infected people in Spain and other areas where the two diseases co-exist.[44] The presence of HIV leads to increased risk of progression of infection; late appearance of disease can occur years to decades after exposure in an endemic region, leading to the appearance of cases of leishmaniasis in regions distant from endemic areas. A common consequence is missed or delayed diagnosis.

Movement of vectors and other species

Movement today involves all forms of life and the movement of nonhuman species can affect infections in humans. *Aedes albopictus* introduced into the USA via used tyres shipped from Asia[45] has since become established in at least 21 contiguous states of the USA and in Hawaii. *Aedes albopictus* can transmit dengue and is a competent laboratory vector of La Crosse, yellow fever and other viruses. It is also hardier than many other mosquito species and therefore may spread widely and be extremely difficult to eradicate. Multiple strains of eastern equine encephalitis virus have been isolated from *Aedes albopictus* in Florida.

An example from the past illustrates the potential consequences of the introduction of a mosquito vector into a new region. In March 1930, an entomologist in Natal, Brazil, came upon *Anopheles gambiae* larvae in a small, wet, grassy field between a railway and a river.[46] He was surprised, because the usual habitat for this mosquito was Africa. Investigation revealed that the probable route of entry into South America was via boats that made mail runs between Dakar in Senegal and Natal in Brazil, covering the 3300km in less than 100 hours. In Dakar the boats were anchored a distance from the shore within easy flight range of *A. gambiae*. In Brazil, over the ensuing years, the mosquito spread along the coastal region and inland. Natal, as an ocean port, terminus of two railway lines and the hub of truck, car and river transportation, was well suited for dissemination of *A. gambiae* into the region. Although malaria already existed in the region, the local mosquitoes were not efficient vectors. *Anopheles gambiae*, in contrast, lived in close proximity to humans, entered houses, sought human blood and was an efficient biter. In 1938 and 1939, devastating outbreaks of malaria killed more than 20,000 persons. In this instance, the simple introduction of a new vector into a region led to severe problems. Fortunately, an intensive (and expensive) eradication campaign was effective.

Current transportation systems regularly carry all forms of life, including potential vectors, along with people and cargo.[47] In an experiment carried out several years ago, mosquitoes, house flies and beetles in special cages were placed in wheel bays of 747 aircraft and carried on flights lasting up to 7 hours. Temperatures were as low as -62°F (-52°C) outside and ranged from 46 to 77°F (8–25°C) in the wheel bays. Survival rates were greater than 99% for the beetles, 84% for the mosquitoes and 93% for the flies. [48] Occasional cases of so-called airport malaria — cases of malaria near airports in temperate regions — attest to the occasional transport and survival of a commuter mosquito long enough to take at least one blood meal in the new environment.[49]

In the USA, transportation of racoons in the late 1970s from Florida to the area between Virginia and West Virginia (in order to stock hunting clubs) unintentionally introduced a rabies virus variant into the animals of the region. From there, the rabies enzootic spread for hundreds of miles, reaching racoons in suburban and densely

Figure 142-7 Worldwide distribution of malaria (2001). *Data from the Centers for Disease Control and Prevention.* [16]

populated regions of the north-east USA. Spill-over of the rabies virus variant into cats, dogs and other animal populations and direct racoon-human interactions have had extremely costly and unpleasant consequences.[50]

GEOGRAPHIC INFLUENCES ON DIFFERENTIAL DIAGNOSIS

Geographic exposures influence how one thinks about probable diagnoses in a given patient. In Mexico, for example, more than 50% of patients with late-onset seizures have CT evidence of the parasitic infection, neurocysticercosis.[51] In Peru, 29% of persons born outside Lima who had onset of seizures after age 20 years had serologic evidence of cysticercosis.[52] In northern Thailand, melioidosis is a common cause of sepsis, accounting for 40% of all deaths from community-acquired sepsis.[53]

In considering the consequences of exposures in other geographic regions, relevant data in assessing the probability of various infections include the duration of visit, activities and living conditions during the stay and the time lapsed since the visit. Among British travelers to West Africa, the relative risk of malaria was 80.3 times higher for persons staying for 6–12 months than among those staying 1 week.[54] In Malawi, the risk of schistosome infection increased directly with duration of stay. Seroprevalence was 11% for those present for 1 year or less, but this increased to 48% among those present for 4 years or longer.[55] In a study of persons with cysticercosis, the average time between acquisition of infection and onset of symptoms was about 7 years.[56]

For malaria, it is necessary to know not only whether infection can be acquired in a specific location but also the types of parasites present and the patterns of resistance to antimalarial agents. As chloroquine resistance has spread, maps now typically highlight the few remaining areas of chloroquine sensitivity. Because the resistance to antimalarial agents is a dynamic process, with levels of resistance generally increasing over time (involving *Plasmodium vivax* in some areas as well as *P. falciparum*), it is essential to base decisions about chemoprophylaxis and treatment on up-to-date information. Figure 142.7 shows the distribution of malaria and resistance patterns globally as of 2001.

Expression of disease may vary depending on age of first exposure, immunologic status of the host, genetic factors and the number and timing of subsequent exposures. Temporary residents of endemic regions have different patterns of response to a number of helminths from those of long-term residents. In cases of loiasis, temporary residents have immunologic hyperresponsiveness, high-grade eosinophilia and severe symptoms that are not seen in long-term residents of the same area.[57] Genetic factors can affect susceptibility to infection or expression of disease. Some persons, for example, are genetically resistant to infection with parvovirus because they lack appropriate receptors on their erythrocytes.[58] Persons lacking Duffy factor cannot be infected with the malarial parasite, *P. vivax*.

CONCLUSION

Knowledge about the geographic distribution of diseases is essential for informed evaluation and care of patients, who increasingly have had exposures in multiple geographic regions. Infectious diseases are dynamic and will continue to change in distribution. Changes in virulence and shifts in resistance patterns will also require ongoing surveillance and communication to health care providers. Multiple factors favor even more rapid change, perhaps in unexpected ways, in the future: rapidity and volume of travel, increasing urbanization (especially in developing regions), the globalization of trade, multiple technologic changes that favor mass processing and broad dispersal and the backdrop of ongoing microbial adaptation and change, which may be hastened by alterations in the physicochemical environment.

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Chapter 143 - Pretravel Advice and Immunization

David R Hill

The pretravel care of the international traveler is entirely preventive medicine.[1] The first step in providing this care is to assess the health risk of a particular trip. This is done by determining a traveler's itinerary (not only the country of destination, but also the areas within the country that will be visited) and the types of accommodation. For example, a prolonged research expedition to the shores of Kenya's Lake Victoria will expose the traveler to more health risks than a short business trip to Nairobi. The epidemiology of infectious disease health risks can be found in several publications[2] [3] [4] and Internet sites (See Sources of Information and Keystone *et al* [5]) (Fig. 143.1). The duration of travel is also important. With longer trips there will be a cumulative risk of disease as well as the possibility that the traveler will become increasingly lax in preventive measures such as malaria chemoprophylaxis. [6] Finally, the purpose of the trip needs to be determined.

The next step is to assess the traveler's current health. Although more than 25% of individuals travel with chronic medical conditions, these generally do not interfere with the traveler's plans. Nevertheless, consideration of specific vaccines or prophylactic medications will need to be matched against the traveler's health. Once assessed, the traveler should be given vaccines, medications and preventive advice. Education about illness avoidance may be the most cost-effective measure, but it is difficult to ensure compliance. Finally, each traveler should be informed how to access medical care during travel and on return. With constantly changing epidemiology of disease and patterns of microbial resistance, complexity of travel itineraries and newly released vaccines and preventive medications, most travelers should be cared for in specialized travel clinics that have trained personnel, carry all vaccines and provide accurate preventive advice.[7]

IMMUNIZATIONS

It is helpful to divide immunizations into three categories (Table 143.1):

- recommended as part of routine health maintenance irrespective of international travel;
- may be required for entry into a country; and
- recommended because of risk during travel.

It is imperative that adequate records are kept for immunizations. This includes the type and dose of vaccine, date of administration, manufacturer and lot number, site of administration and administrator's signature. Adverse reactions to vaccines should be reported to the appropriate monitoring agency. Prior to administration of any vaccine patients should undergo informed consent procedures. The use of vaccine information sheets will help to explain to travelers the benefits and risk of each vaccine. These are often available from vaccine manufacturers or can be downloaded from the Centers for Disease Control and Prevention (CDC): www.cdc.gov/nip/publications/vis.

Most vaccines may be administered simultaneously at different sites. Patient tolerance, therefore, usually dictates how many may be given at any one time. A few rules do apply. Live viral vaccines should be either given together or separated by at least 1 month and immunoglobulin should not be given less than 5 months before or less than 2 weeks after measles, mumps or rubella vaccines and 3 weeks after varicella vaccine.[8] Other specific conditions are discussed later. The major travel vaccines, their administration schedule and side effects are listed in Table 143.2 . Full manufacturer's prescribing information should be consulted before administration of each vaccine, as schedules, doses and products often differ between countries.

Immunizations for routine health maintenance

The pretravel visit is an ideal time to update routinely recommended immunizations. In most areas of the world this includes tetanus-diphtheria, pertussis, measles, mumps and rubella, polio, pneumococcal, influenza and, increasingly, hepatitis B vaccines. Many older adults have never been adequately immunized against tetanus and diphtheria or have waning serologic evidence of protection because of failure to receive regular boosters. In the USA this has translated into tetanus cases, most of which occur in adults over the age of 50 years. Diphtheria has occurred in regions where vaccine coverage has declined secondary to population migration or failure of the infrastructure for vaccination. These regions include some countries in Latin America, the Caribbean, Africa, Asia and Eastern Europe. All travelers should have completed a primary series against tetanus-diphtheria and then received boosters on a 10-yearly basis. Because a tetanus-prone wound requires a booster if more than 5 years have elapsed since immunization, some travelers may benefit from vaccination at this interval, particularly if it would be difficult to obtain vaccination overseas.

Although many countries only require a single dose of live measles vaccination in childhood, in 1989 the USA adopted a two-dose policy in response to an increase in measles cases. This means

TABLE 143-1 -- Three categories of immunization.

THREE CATEGORIES OF IMMUNIZATION		
Routine health care	**Required**	**Recommended because of exposure**
Tetanus-diphtheria	Yellow fever	Hepatitis A
Varicella	(Cholera)	Typhoid
Measles (mumps, rubella)		Meningococcal
Haemophilus influenzae type b		Rabies
Polio		Japanese B encephalitis
Pertussis		Tick-borne encephalitis
Influenza		
Pneumococcal		
Hepatitis B		
Note that cholera vaccination is no longer required as a condition of entry to any country.		

TABLE 143-2 -- Immunizations for foreign travel.

IMMUNIZATIONS FOR FOREIGN TRAVEL						
Vaccine	**Type**	**Route**	**Schedule**	**Indications**	**Precautions and contraindications**	**Side-effects**
Toxoids						
Tetanus-diphtheria	Adsorbed toxoids	im	Primary: 3 doses, first 2, 4–8 weeks apart; 3rd dose 6–12 months later	All adults	First trimester of pregnancy	Local reactions
			Booster: every 10 years		Hypersensitivity or neurologic reaction to previous doses	Occasional fever, systemic symptoms
					Severe local reaction	Arthus-like reactions if history of multiple boosters
						Rare: systemic allergy
Inactivated bacterial vaccines						
Cholera	Phenol-killed *Vibrio cholera* (4 × 10^9 /ml)	im, sc or id	Primary: 2 doses 1 week to 1 month apart, =6 days before travel	Oral vaccines preferred	Safety in pregnancy not known	Local reaction of pain, erythema and induration lasting 1–2 days
			Booster: every 6 months	No longer required by individual countries	Previous severe local or systemic reaction	Occasional fever, malaise
				No longer available in USA	No protection against *V. cholerae* 0139	

Cholera	Killed, whole cell *Vibrio cholerae* with recombinant B subunit of cholera toxin	Oral	Primary: 2 doses at 0 and =7 days	No longer required for international travel	Hypersensitivity to previous dose	Mild gastrointestinal side-effects
			Booster: 6 months to 2 years	May give some protection against enterotoxigenic *E. coli* (traveler's diarrhea)		
				Not available in USA		
Streptococcus pneumoniae	Polysaccharide containing 23 serotypes	sc or im	Primary: single dose	=5 years old and at increased risk of pneumococcal disease and its complications	Safety in pregnancy not known	Mild erythema and pain at injection site in c.50%
	7-valent conjugate vaccine available (see text)		Booster: recommended for high-risk patients after 5 years	Healthy adults 65 years or older	Previous pneumococcal vaccination (relative)	Systemic reaction in <1%
						Arthus-like reaction with booster doses
Neisseria meningitidis	Polysaccharide containing serotypes (A, C, Y, W135) (serotype C conjugate vaccine available in some countries)	sc	Primary: 1 dose	Travel to areas with epidemic meningococcal disease	Safety in pregnancy is not known	Infrequent, mild local reactions
			Booster (after 5 years): not officially recommended. May be given after 3–5 years	Asplenia or certain complement deficiency states		
Typhoid	Heat-phenol-inactivated	sc	Primary: 2 doses given =4 weeks apart	Risk of exposure to typhoid fever	Previous severe local or systemic reaction	Frequent local reaction of pain; swelling and induration lasting 1–2 days
	Salmonella typhi (10^9 /ml)		Booster: every 3 years	No longer available in USA	Acetone-killed vaccines should not be given id	Occasional systemic reaction, can be severe
					Pregnancy	
Typhoid	Vi polysaccharide	im	Primary: 1 dose	Risk of exposure to typhoid fever	Safety in pregnancy not known	Local pain and induration in 10–20%
			Booster: every 2 years		Hypersensitivity to vaccine components	Systemic reaction in <5%
Attenuated live bacterial vaccine						
Typhoid	Attenuated Ty21a strain of *Salmonella typhi*	po	Primary: 1 capsule every other day for 4 doses	Risk of exposure to typhoid fever	Safety in pregnancy not known	Infrequent gastrointestinal upset, rash
			Booster: every 5 years		Immunocompromised host	
					Children <6 years	
					Acute febrile or gastrointestinal illness	
					Antibiotics or mefloquine (separate the doses by =24h)	
					Capsules must be refrigerated	
Cholera	Live, attenuated CVD 103-HgR strain of *V. cholerae*	Oral	Primary: single dose	No longer required for international travel	Safety in pregnancy is not known	Mild nausea, cramping and diarrhea in about 2%
			Booster: 6 months	Not available in USA	Immunocompromised host	
					Antibiotics (separate dose by =24h)	
					Complete vaccination at least 1 week before malaria chemoprophylaxis	
Attenuated live virus vaccines						
Measles	Attenuated live virus: monovalent form or combined with rubella (MR) ± mumps (MMR)	sc	Primary: 2 doses, first at 12–15 months, 2nd at 4–12 years of age.	People born after 1956 who have not had documented measles or received 2 doses of live vaccine	Pregnancy	Temperature =39.4°C, 5–21 days after vaccination in 5–15%
			For adults, 2 doses separated by =1 month		Immunocompromised host; can be considered for asymptomatic HIV-infected persons (see text)	Transient rash in 5%
			Booster: none		History of anaphylaxis to eggs or neomycin	Local reaction if previously immunized with killed vaccine (1963–67): 4–55%
					Recent (<5 months) administration lg	
Mumps	Attenuated live viral	sc	Primary: 1 dose (usually given as part of MMR vaccine)	People born after 1956 who have not had documented mumps	Pregnancy	Mild allergic reactions uncommon
			Booster: none		Immunocompromised host	Rare: parotitis
					History of anaphylaxis to eggs or neomycin	
					Recent (<5 months) administration of lg	

Poliomyelitis	Attenuated live virus, trivalent	po	Primary: 3 doses, the first 2 given at a 6–8 week interval, the third 8–12 months later	<18 years old	Immunocompromised host or immunocompromised contacts of recipients	Rare: paralysis
			Booster: 1 oral dose	Boost previously immunized people; complete series in partially immunized adults		Occasional outbreaks
				No longer used for vaccination in USA		
Rubella	Attenuated live virus	sc	Primary: 1 dose (usually given as part of MR or MMR)	All people, particularly women of childbearing age, without documented illness or receipt of live vaccine at =12 months of age	Pregnancy	Post pubertal women: up to 40% have joint pains, transient arthritis, beginning 3–25 days after vaccination, persisting 1–11 days
			Booster: none		Immunocompromised host	Frank arthritis in <2%
					History of anaphylaxis to neomycin	
					Recent (<5 months) administration of Ig	
Varicella	Attenuated live virus	sc	Primary: children of 1–12 years, 1 dose; >12 years, 2 doses at a 4–8 week interval	=12 months old and no history of varicella	Pregnancy	Local pain and induration in 20%
					Immunocompromised host	Fever in 15%
					Potential for rare transmission of vaccine virus to susceptible hosts	Localized or systemic mild varicella rash in 6%
					Recent (<5 months) administration of Ig	
Yellow fever	Attenuated live virus	sc	Primary: 1 dose, 10 days to 10 years before travel	As required by individual countries	Avoid in pregnant women, unless high-risk travel	Mild headache, myalgia, fever, 5–10 days after vaccination in 2–5%
			Booster: every 10 years		Infants <9 months	Rare: immediate hypersensitivity, multiorgan system failure (see text)
					Immunocompromised host	
					Hypersensitivity to eggs	
Inactivated virus vaccines						
Hepatitis A	Inactivated	im	Primary: 2 doses, 2nd dose after 6–24 months provides long-term (=10 years) protection	Travel to developing countries	Safety in pregnancy is not known	Local reaction of pain and tenderness in <20%
			Booster: not currently recommended	Used in routine immunization of children in some regions of USA (see text)		Occasional fever in <5%
				Some travelers may benefit from pre-vaccine hepatitis A testing		
Hepatitis B	Yeast-derived recombinant hepatitis B surface antigen	im	Primary: 3 doses at 0, 1 and 6 months. Can accelerate vaccine schedule (see text)	Health care workers in contact with blood	Pregnancy is not a contraindication in high-risk persons	Mild local reactions in 10–20%
			Booster: not routinely recommended	Residence or sexually active in areas of high endemicity for HBsAg	Hypersensitivity to vaccine components	Occasional fever, headache, fatigue and nausea
				Contact with blood, body fluids, or blood-contaminated medical or dental instruments		
Hepatitis A and B antigens combined	Inactivated virus (A) plus recombinant hepatitis B surface antigen	im	Primary: 3 doses at 0, 1 and 6 months	Travelers at risk for both hepatitis A and B; lower age limit of vaccination varies between countries	Safety in pregnancy is not known	Local reaction in ~35%
			Booster: not currently recommended	Give at least 2 doses of vaccine before departure to protect against hepatitis A	Hypersensitivity to vaccine components	Systemic symptoms of headache and fatigue, similar to single antigen preparations
Poliomyelitis	Killed poliomyelitis virus, trivalent enhanced potency	sc	Primary: 3 doses, first 2 at a 4–8 week interval; 3rd 6–12 months after 2nd	Travel to polio-endemic countries	Safety in pregnancy not known	Mild local reaction
			Booster: 1 lifetime dose		Anaphylactic reactions to streptomycin or neomycin	

Influenza	Inactivated whole and split influenza A and B virus	sc	Annual vaccination with current vaccine	=6 months old and at increased risk of complications from influenza	First trimester of pregnancy is a relative contraindication	Mild local reactions in <33%
				Healthy adults >50 years old	Anaphylaxis to eggs	Occasional systemic reaction of malaise and myalgia: begins 6–12h after vaccination; lasts 1–2 days
				Medical care personnel		Rare: allergic reaction
				Travelers at risk		
Japanese B encephalitis	Inactivated virus	sc	Primary: 3 doses at weekly intervals	Travel to areas of risk with rural exposure or prolonged residence	Pregnancy	Local mild reactions lasting 1–3 days in 20%
			Booster: 1 dose at 3-year intervals		Allergy to mice or rodents	Systemic symptoms of fever, myalgia, headache or GI upset, in 10%
					History of anaphylaxis or urticaria	Severe reactions with urticaria, rash, angioedema, or respiratory distress (0.01–1%)
Rabies	Inactivated virus	im or id	Pre-exposure: 3 doses at days 0, 7, and 21 or 28	Itinerary and activities that place traveler at risk of rabies	Allergy to previous doses	Local reactions in ~30%
			Booster: depends upon risk category and is based upon serologic testing at specified intervals		May be given in pregnancy if indicated	Mild systemic reactions: headache, nausea, aches, dizziness in ~20%
					id route should be completed =30 days before travel	Rare: neurologic illness
					id route should not be used with concurrent chloroquine or mefloquine	Immune-complex reactions with booster doses of human diploid cell vaccine occurring 2–21 days after vaccination, in 6%
					id dosing no longer available in USA	
Tick-borne encephalitis	Inactivated virus	im	Primary: 3 doses at 0, 1 and 9–12 months	Hiking, camping in areas of risk; exercise tick avoidance	Safety in pregnancy is not known	Occasional local reactions of swelling, redness or swollen regional lymph nodes
			Booster: 3 years		Hypersensitivity to previous doses	Infrequent fever, headache
						Rare neuritis
Passive prophylaxis						
Immunoglobulin	Fractionated Ig (primarily IgG)	im	Travel <3 months: 0.02ml/kg	For prevention of hepatitis A	Not to be given less than 2 weeks after (3 weeks for varicella) or 5 months before, measles mumps and rubella, or varicella vaccines	Transient local discomfort
			Travel >3 months: 0.06ml/kg every 4–6 months	Some travelers may benefit from pretravel hepatitis A antibody testing		Rare systemic reaction

Manufacturer's full prescribing information should be consulted because vaccines, doses and schedules may differ among countries. Only major precautions, contraindications and side-effects are listed. Indications are discussed in more detail in the text. Immunocompromised host refers to persons immunocompromised because of immunodeficiency disease, leukemia, lymphoma, generalized malignancy or AIDS, or immunosuppressed from therapy with corticosteroids, alkylating agents, antimetabolites or radiation.

TABLE 143-3 -- Accelerated immunization of children under 2 years of age.

ACCELERATED IMMUNIZATION OF CHILDREN UNDER 2 YEARS OF AGE			
Vaccine	**No. of doses after which protection may be achieved**	**Earliest age at which dose may be given**	**Interval (weeks)**
Diphtheria-tetanus-pertussis	3	6 weeks	=4
Measles	1	6 months	
Polio (oral)	3	6 weeks	=6
Polio (inactivated)	3	6 weeks	=4
Haemophilus influenzae type b	2	6 weeks	=4
Hepatitis B	2	newborn	=4
The data shown are for children who will travel to developing areas and require protection faster than by the routine schedule. Protection conferred by these schedules may not be complete. Children vaccinated with measles at under 12 months of age should be revaccinated at 12–15 months. In polio-endemic countries, the first oral polio dose may be given in the newborn period but three additional doses should be given, the first at 6 weeks of age and then at 4-week intervals.			

that adults born in 1957 and after, a time before which it is assumed that measles infection was universal, should have received two doses of live vaccine. Measles vaccine coverage may be limited in developing countries, particularly those in the African and Eastern Mediterranean regions, so it is important to ensure adequate protection in travelers.[9] It may be helpful in some travelers to perform serologic testing for immunity to measles and rubella.

Pneumococcal and influenza vaccines are routine for healthy, older adults (=65 years), who make up nearly 15% of all travelers. In addition to the older adult, those with chronic illness who would be adversely affected by pneumococcal pneumonia or influenza, such as persons who have HIV or AIDS, diabetes or chronic pulmonary, renal, hepatic or cardiac disease, should be vaccinated. Pneumococcal vaccine should also be administered to those with hemoglobinopathies and

functional or surgical asplenia, and influenza vaccine to health care workers, children up to the age of 18 years on chronic aspirin therapy, pregnant women in their second and third trimesters, and to healthy adults and others if they desire it. Influenza strains included in the vaccine are selected on the basis of worldwide influenza activity during the season preceding vaccine manufacture. The risk of influenza is year round in tropical areas and between April and September in countries in the southern hemisphere. Outbreaks can occur out of season when persons from diverse regions of the world congregate in close quarters, such as on cruise ships.

Routine vaccination of children is important. Children should receive vaccines that are age recommended; however, the schedule may be advanced if a child is traveling before they would have received a scheduled vaccine and the risk during their trip is sufficient (Table 143.3).[3] In the USA vaccines recommended in childhood are measles, mumps and rubella, polio, *Haemophilus influenzae* type b, diphtheria-pertussis-tetanus (DPT), hepatitis B, varicella and pneumococcal. An acellular pertussis vaccine has been incorporated into the DPT vaccine to decrease the risk of febrile reactions from the pertussis component. A 7-valent conjugate vaccine against *Streptococcus pneumoniae* has been introduced for children aged 2–23 months (and in high-risk older children), and in some countries a conjugate *Neisseria meningitidis* type C is administered.[10] Other countries routinely administer BCG vaccine for the prevention of tuberculosis.

Required immunizations

The only vaccine that may be required for international travel is yellow fever vaccine. Yellow fever is prevalent throughout the Amazon basin of South America and sub-Saharan Africa between 15° north and 10° south of the equator. It is one of the diseases that is re-emerging and expanding into new regions and in recent years has caused the deaths of unvaccinated short-term tourists to infected areas.[11] Travelers to rural regions in the endemic zone for yellow fever (areas

TABLE 143-4 -- Mosquito avoidance.

MOSQUITO AVOIDANCE	
Repellents	DEET-containing products (=35%)
	Apply to exposed areas of skin
Protective clothing	Long sleeves and trousers may be impregnated with permethrin-containing sprays or solutions
Screens and netting	May be impregnated with permethrin-containing sprays or solutions
Pyrethroid insecticide sprays and coils	May be sprayed or burned in enclosed areas
For avoidance of *Anopheles* malaria mosquitoes, precautions should be exercised from dusk to dawn. *Aedes* spp. mosquitoes, which transmit dengue and yellow fever, are active in the daytime hours.	

with the appropriate ecology for transmission, but without cases) and to areas actually infected with yellow fever should receive vaccine. Infected areas are listed in the CDC publication *Summary of health information for international travel* which can be accessed on-line at the CDC travel website: www.cdc.gov/ travel/bluesheet.htm. Other countries that have no reported cases of yellow fever and are not in the endemic zone may require vaccination of travelers arriving from yellow fever countries. The specific regulations governing this may be found in the respective CDC and World Health Organization (WHO) publications, *Health information for international travel*[3] and *International travel and health*. *Vaccination requirements and health advice.*[4] Yellow fever vaccination has to be recorded in the International Certificate of Vaccination (see Sources of Information). In addition to vaccine, travelers should protect themselves against the daytime-biting *Aedes* mosquito (Table 143.4).

There have been recent reports of multiorgan system failure in apparently normal hosts following yellow fever vaccine.[12] There was increased risk with advancing age of the vaccine recipient, particularly in those over the age of 75 years. Considering that these cases are rare, there have been no changes made to vaccine recommendations. Health care providers should ensure that vaccine is only administered to persons traveling to infected or endemic areas.[12A]

Cholera vaccine is no longer required for international travel, although some local authorities may act outside international health regulations and request documentation. The removal of cholera vaccination for travelers is likely because of the very low risk of disease in travelers, the variable efficacy of vaccines against cholera and the lack of a clear role of vaccination in the control of cholera in nonepidemic settings.[13] In addition, the only vaccine which was available for US travelers (a whole-cell, inactivated vaccine which was poorly

tolerated and had a limited duration of efficacy) is no longer being produced. Cholera is endemic throughout Latin America, Asia and Africa. *Vibrio cholerae* 01 circulates in all areas and *V. cholerae* 0139 in Asia. More than 85% of cholera cases are reported from Africa.[14]

Two oral vaccines are now available in many countries outside the US.[13] [15] One is live attenuated (Mutachol®, Berna) and the other is inactivated (Dukoral®, SBL Vaccine AB). These vaccines are well tolerated and provide improved protection (60–80% protective efficacy depending upon age of the recipient) compared with the parenteral, inactivated vaccine. They may be considered for individuals, often health care or relief workers, living in a highly endemic area under poor sanitary conditions.

Smallpox vaccine has not been required for international travel since 1982 following the global eradication of smallpox in 1977. There has been renewed interest in smallpox vaccine with concerns about a potential use of smallpox in a bioterrorist attack. New guidelines for the existing vaccinia virus vaccine are being developed.[16]

Immunizations recommended because of risk

The following vaccines are recommended because there is a risk of exposure during travel to particular regions of the world or during travel under certain conditions such as poor sanitation. There are three vaccine-preventable diseases transmitted because of poor food and beverage hygiene: hepatitis A, typhoid fever and polio (for information on cholera see above).

Hepatitis A is the most common vaccine-preventable infection with a risk of 1–10 cases per 1000 travelers (Fig. 143.1), and can be acquired even during 'first-class' trips.[17] [18] For this reason most travelers should receive protection. The first step is counseling about safe food and liquids and then providing either passive protection with immunoglobulin or active protection with one of the inactivated vaccines. Immunoglobulin provides immediate protection with antibodies that are circulating for 2–6 months. Because of its shorter duration of protection and a decreased acceptance by both provider and traveler because it is a blood product, most protection against hepatitis A is now done with inactivated vaccines.

There are several inactivated vaccines marketed throughout the world.[19] Two of the most completely studied are Havrix® (GlaxoSmithKline) and Vaqta® (Merck). These vaccines provide long-term protection with efficacy rates exceeding 90%. On the basis of mathematical models, two doses of vaccine should provide protection for at least 10–20 years. The use of inactivated vaccines in other risk groups such as raw seafood eaters, sewer workers, men who have sex with men, illegal drug users, persons with chronic liver disease and health care workers is being debated. In the USA vaccine is administered as part of routine childhood immunization in regions with high endemic rates of hepatitis A, e.g. the south-west USA.[20]

The first dose of an inactivated hepatitis A vaccine should be administered at least 2 weeks before departure, but for immediate protection immunoglobulin plus vaccine may be given simultaneously in separate sites. However, indirect information suggests that protection begins immediately following immunization with inactivated vaccines.[21] [22] Travelers with a high likelihood of previous hepatitis A infection may benefit from hepatitis A antibody testing to avoid unnecessary vaccination if they are positive. These include those born before 1945, those born and raised in developing countries and those with a history of jaundice.[17] [20]

Protection against both hepatitis A and B may be achieved with the use of a combined antigen vaccine, Twinrix® (GlaxoSmithKline), in a three-dose schedule. Two doses of vaccine should be given before departure to ensure protection against hepatitis A since a lower concentration of antigen is used in this preparation compared with the single antigen hepatitis A vaccines.

Figure 143-1 Estimated monthly incidence of health problems for travelers to tropical areas. *Adapted from reference* [18] .

Hepatitis E is enterically transmitted in the developing world, particularly during periods of high rainfall. In travelers, most cases have originated from India. Pregnant women have a high mortality. Immunoglobulin and the current hepatitis vaccines do not prevent hepatitis E so food and liquid hygiene is the best prevention (see Chapter 214).

Most cases of imported typhoid in the USA have been acquired in Mexico, although travel to the Indian subcontinent represents the highest risk with 1–4 cases per 10,000 travelers.[23] Multidrug-resistant *Salmonella typhi* is also common. There are three vaccines for protection (see Table 143.2) but production of the whole-cell inactivated vaccine was discontinued in the USA in 2000. These

vaccines have similar efficacy rates (60–70%) but they differ widely in method of administration and side-effects.[24] [25] The whole-cell inactivated vaccine may have uncomfortable local and systemic side-effects. Although it is inexpensive, its main place is in providing protection for children aged 6–24 months, an age range when the other vaccines are not effective. The oral, live-attenuated vaccine (Ty21a) is well tolerated, effective in children older than 4 years and provides protection for 5 years. If a traveler is on mefloquine for malaria prophylaxis or taking antibiotics, they should wait at least 24 hours before taking the Ty21a vaccine so that its replication will not be inhibited. A polysaccharide vaccine that uses the Vi antigen of *S. typhi* is given in a single intramuscular dose and is effective for 2–3 years. For persons who cannot take the oral vaccine because of time or compliance issues or for children between the ages of 2 and 4 years, this vaccine may be the one of choice. Conjugate vaccines in development should provide higher levels of protection.[26]

All travelers should have completed a primary series against poliomyelitis. Because the risk of polio during travel is low, a polio booster is not given routinely. Also, efforts at global eradication of polio have dramatically decreased the risk associated with travel to many areas of the world;[27] the western hemisphere was declared polio free in September 1994, the Western Pacific region in October 2000 and the European region in June 2002. The Indian subcontinent and Africa account for most polio cases each year.

At-risk travelers who have completed a primary series of vaccine should receive a one-time adult booster with the enhanced-potency inactivated polio vaccine (eIPV). Production of the oral vaccine was discontinued in the USA in January 2000 to eliminate the rare risk of flaccid paralysis from this vaccine. Those who have never completed a primary series should complete one.

Immunizations recommended because of exposure during certain activities or risk behavior include those against hepatitis B, *Neisseria meningitidis*, rabies, Japanese B encephalitis, tick-borne encephalitis and plague.

In an effort to control hepatitis B infection in the USA, vaccination is now included during routine childhood immunization. Unimmunized health care workers who will reside in endemic regions should receive the hepatitis B vaccine. Protection against both hepatitis A and B is now available in a combination vaccine (see section on hepatitis A). The vaccination schedule for hepatitis B (Engerix® only, GlaxoSmithKline) may be accelerated to 0, 1 and 2 months with a booster at 6–12 months. It can be further accelerated by giving doses at 0, 7 and 21 days; 65% will seroconvert at 28 days.[28]

Meningococcal vaccine is recommended for travelers to areas with high risk of meningococcal disease such as the meningitis belt of sub-Saharan Africa (particularly during the months from December through June).[29] Saudi Arabia requires it for religious pilgrims during the Hajj. Neither the bivalent (A, C) or quadrivalent vaccine (A, C, Y, W135) contains serogroup B, but this serogroup is less frequently a cause of meningitis in endemic regions. A conjugate group C vaccine is being used in some European countries and Canada for protection of children. [10] In the USA it is recommended that first-year university students consider vaccination.

All travelers should be counseled about rabies. In much of the developing world rabies is transmitted through the bite of a dog (see Chapter 153), although other mammals (e.g. bats, cats, foxes) may transmit the virus. Rabies-free countries may be determined by consulting the CDC[3] or going on-line to the Rabnet site of the WHO (oms2.b3e.jussieu.fr/rabnet/). All bites should be thoroughly cleansed with soap and water; postexposure rabies prophylaxis should then be obtained. Regimens may differ throughout the world, but if prophylaxis is administered properly with rabies antiserum (either a human or equine product) plus vaccine, the traveler should be protected.[30] If there is any question as to the potency of vaccine a traveler may have received, they should have serology checked upon return and postexposure treatment initiated while awaiting serologic evidence of protection (see Chapter 219).

Pre-exposure protection against rabies is considered for those traveling to endemic areas for 1 month or more, for persons with high-risk travel over a shorter period or for persons who will have difficulty obtaining safe and effective postexposure rabies biologics. Pre-exposure prophylaxis eliminates the need for rabies immune globulin which can be difficult to obtain in many areas of the world. Most pre-exposure vaccination is now administered intramuscularly to ensure adequate development of immunity.

Japanese B encephalitis is a viral encephalitis in Asia transmitted by the *Culex* spp. mosquito. The complete listing of risk areas and seasons of transmission may be found in *Health information for international travel*.[3] It is recommended that persons receive vaccine if they will have prolonged residence in endemic areas or will engage in high-risk activities such as camping, bicycling or field work. Rural Asia, particularly where rice and pigs are farmed, is the highest risk area; the pigs act as a reservoir for the virus and the rice fields as a breeding ground for the mosquito vector. The vaccine would probably receive wider use but for the serious, rare (approximately 0.1–5 per 1000) hypersensitivity reactions. About 20% of vaccinees have mild local and systemic reactions but serious allergic reactions, including anaphylaxis, urticaria, angio-edema and respiratory distress, have occurred at intervals ranging from minutes to as long as 1 week after vaccination. [31] Reactions can occur after any of the three doses. The traveler should remain in the waiting room for 30 minutes after receipt of vaccine and not travel within 10 days of completing the series in case a reaction occurs during flight or on arrival in the country of destination. Patients who have a history of allergies or urticaria may be at a slightly higher risk of severe reaction, so they should be vaccinated only after careful consideration.

Plague (see Chapter 176) is a rare disease for international travelers. Seven countries have reported cases each year from 1995 through 1999: Madagascar, Tanzania, Peru, USA, China, Mongolia and Vietnam. The vaccine is of uncertain efficacy and is generally not recommended. The rare adult traveler with exceptionally high exposure can take tetracycline or doxycycline chemoprophylaxis and children can take sulfonamides.

Tick-borne encephalitis is a viral meningoencephalitis spread by *Ixodes* ticks throughout forested areas of Eastern and Central Europe, and Siberia in the spring and summer months. Unpasteurized dairy products in endemic areas may also transmit the virus. There are two inactivated vaccines (Encepur®, Chiron, and FSME-Immun®, Baxter AG), but they are not available in the USA and require three doses over a year to achieve full protection, which is not practical or possible for most travelers. Travelers to these areas should exercise precautions against ticks by the use of protective clothing, repellents and insecticides. These measures will help to prevent Lyme disease, which is also transmitted throughout Europe and the USA by the bite of *Ixodes* ticks. Production of a Lyme disease vaccine in the USA was recently discontinued.

Tuberculosis is endemic in many parts of the world. The incidence of infection can be as high as eight cases per 1000 person-months; this rate was seen in PPD-negative health care personnel working in tuberculosis-endemic regions. [32] Most children reared outside the USA have received the BCG vaccine in childhood. BCG vaccine is not advocated for travel except for children who will have unavoidable, close exposure to persons with untreated tuberculosis. [33] Long-term travelers should receive pretravel and post-travel tuberculin (purified protein derivative) skin testing to check for conversion and, therefore, infection. The post-travel skin test should be administered 1 month or more after return.

TABLE 143-5 -- Immunization of the pregnant traveler.*

IMMUNIZATION OF THE PREGNANT TRAVELER

Vaccine		Is the vaccine safe?	Notes
Bacterial	Tetanus-diphtheria	Yes	Should ideally wait until after first trimester of pregnancy
	Pneumococcal	Yes	
	Meningococcal	Yes	Probably safe but has not been studied conclusively
	Typhoid		
	Killed	No	
	Live-attenuated	Not known	Vaccination should generally be avoided
	Vi polysaccharide	Not known	May be given with high-risk exposure; vaccine of choice in pregnancy
	Cholera		
	killed	No	
	oral	Not known	
	BCG	No	
Viral	Poliomyelitis		
	Inactivated	Yes	
	Live-attenuated	Yes	
	Yellow fever	Yes	Should generally be avoided, but may be given with high-risk exposure
	Measles, mumps, rubella	No	
	Influenza	Yes	Should ideally wait until after first trimester of pregnancy
	Rabies	Yes	
	Japanese B encephalitis	Not known	Should generally be avoided, but may be given with high-risk exposure
	Hepatitis B	Yes	
	Hepatitis A		
	Immunoglobulin	Yes	
	Inactivated	Not known	Should generally be avoided, but may be given with high-risk exposure
	Tick-borne encephalitis	Not known	Should generally be avoided, but may be given with high-risk exposure
	Varicella	No	

** Adapted from references [3] and [4] .*

Immunization in special groups

Two major groups of travelers require special consideration before immunization — pregnant women and immunocompromised hosts, particularly those who have HIV or AIDS. For pregnant women, any vaccine should have a clear indication to avoid potential adverse fetal effects (Table 143.5).[3] [4] [34] Although many inactivated vaccines may be given safely, those that have the potential for major systemic side effects, such as whole-cell typhoid vaccine, should be avoided. Measles, mumps and rubella, and the varicella vaccine should not be given, although data have not clearly demonstrated adverse outcomes when women have received rubella vaccine. Although yellow fever vaccine strain virus may be transmitted to the unborn child, this has not been associated with fetal abnormalities.[35] Other vaccines are likely to be safe, but there is insufficient experience to make a clear recommendation.

HIV-infected patients are another group to consider separately (Table 143.6).[36] [37] All travelers should be asked about HIV risk factors before vaccination. Then the safety, immunogenicity and efficacy of the vaccine need to be balanced against the risk of the disease. It is generally agreed that immunogenicity decreases with advanced disease; a $CD4^+$ lymphocyte count of <200–400 cells/ml or <25% by age-specific percentages correlates with decreased immunogenicity. Although it has not been clearly studied, this may also be a cut-off point for an increased risk of adverse consequences of live viral vaccines. If assurance of immunity is needed, then post-vaccination serology should be obtained.

In addition to vaccination, HIV-infected travelers should consider the health risks associated with travel to developing regions. Many enteric infections, such as *Salmonella, Cyclospora* and *Cryptosporidium*, and systemic infections such as leishmaniasis and tuberculosis

TABLE 143-6 -- Immunization in HIV infection.*

IMMUNIZATION IN HIV INFECTION			
Vaccine		**Is the vaccine safe?**	**Notes**
Bacterial	Tetanus-diphtheria	Yes	
	Pneumococcal	Yes	
	Meningococcal	Yes	
	Typhoid		
	Killed	Yes	
	Live-attenuated	Not known	Safety is not known and vaccination should be avoided
	Vi polysaccharide	Yes	
	Cholera		
	Parenteral	Yes	
	Oral, attenuated	No	
	Oral, killed	Not known	
	BCG	No	
	Tick-borne encephalitis	Not known	Safety is not known and vaccination should be avoided

Viral	Poliomyelitis		
	Inactivated	Yes	
	Live-attenuated	No	
	Yellow fever		Probably safe in persons without immunosuppression (e.g. CD4 count =200 cells/mm^3), but has not been conclusively studied
	Measles		Can be given to persons without severe immunosuppression (e.g. CD4 count =200 cells/mm^3)
	Influenza	Yes	
	Rabies	Yes	
	Japanese B encephalitis	Not known	Safety is not known and vaccination should be avoided
	Hepatitis B	Yes	
	Hepatitis A		
	Immunoglobulin	Yes	
	Inactivated	Yes	
	Varicella		Can be considered for children without severe immunosuppression (e.g. CD4% of >25%)

** Data from references [3] and [4] .*

are more prevalent and can be prolonged and difficult to treat in HIV-infected individuals. The ability to access sophisticated medical care may also be an issue.

TRAVELER'S DIARRHEA

Traveler's diarrhea is the most common illness in developing areas of the world and affects 30–50% of travelers.[38] Illness usually begins in the first week after arrival and is typically mild, characterized by three or more loose to watery stools with nausea, abdominal cramping and malaise. Fever is usually less than 101°F (38°C) and vomiting is unusual. In most cases, illness is self-limiting over 3–5 days. Dysentery with tenesmus and bloody stools occurs in less than 10% of patients. Although most individuals can continue with their activities, 20–30% will need to alter plans. Enterotoxigenic *Escherichia coli* accounts for about 50% of the known causes, and *Shigella, Salmonella* and *Campylobacter* spp. for a large proportion of the other bacteria (see Chapter 144). Viruses cause 10–20% of cases and protozoa cause 5–10%. New etiologic agents are being described continually; some of the most recent ones have been *Cryptosporidium* and *Cyclospora* spp. and new types of *E. coli*, such as enteroaggregative types. The incidence of diarrhea does not seem to decline with increasing time of residence in developing areas.

Prevention

A full description of the prevention and treatment of traveler's diarrhea can be found in Chapter 43 . The best prevention is care in the selection of food and liquids. Although most travelers understand the importance of being careful, many still make errors soon after arrival overseas. Foods and liquids that are likely to be contaminated are ground-grown greens and vegetables, incompletely cooked or poorly stored meats and seafood, untreated water and ice cubes, unpasteurized milk products and food from street vendors. Thus, travelers should restrict themselves to commercially prepared or heated beverages, recently and thoroughly cooked meats and greens, and fruits that can be peeled by the traveler. Water may be purified by bringing

it to the boil or by halogenating (iodine or chlorine preparations) and then filtering it with a filter of pore size "1µm.[39] The cysts of *Cryptosporidium* spp. and *Cyclospora* (and eggs of helminths) are likely to be halogen resistant, so water potentially contaminated with these parasites should be filtered or boiled.

Several nonantimicrobial agents have been used to prevent diarrhea. Bismuth subsalicylate is modestly effective either in tablet (2 tablets (252mg/tablet), q6h) or liquid form (2 oz qid), decreasing the incidence of diarrhea by about 65%. It should not be taken by individuals who are allergic to salicylates, who are taking large doses of them for other reasons or are on anticoagulant therapy. It can decrease the absorption of doxycycline. Ingestion of prophylactic *Lactobacillus* spp. does not confer significant protection and antimotility agents such as loperamide and diphenoxylate should not be taken preventively.

Antibiotics are effective for prophylaxis but they may be associated with side effects, contribute to bacterial resistance and are not practical for travelers going for more than 2–3 weeks. There are also many areas of the world that have sulfonamide-resistant and tetracycline-resistant bacteria, making these agents less effective. Therefore, most persons should not be given prophylaxis and it should be reserved for travelers in whom an episode of diarrhea would have extreme consequences.

Treatment

If the traveler becomes ill, prompt treatment should be initiated with hydration (see Chapter 161 for a full discussion of rehydration). Commercial rehydration packets combining electrolytes, sugar and bicarbonate are easy to use and, for small children, are probably safer than home-made preparations. They are widely available throughout the developing world or can be purchased before travel. Infants who are breast-feeding should continue nursing. As diarrhea improves, the diet can be increased by adding bland foods (breads and cereals, potatoes, soups, bananas, fish and chicken) in frequent small meals.

Mild disease may be treated with bismuth subsalicylate. This reduces the number of loose stools by about 50%, but does not begin to work until about 4 hours after taking it. Antimotility agents such as loperamide rapidly decrease cramping and loose stools. They should be avoided if there is blood in the stool or a fever >101.5°F (38.5°C). Because most episodes of diarrhea are self-limiting, symptomatic therapy alone may be sufficient.

A short course of antibiotics will often improve diarrhea within 1 day. Antibiotics combined with loperamide may control symptoms within hours, but there has been variable success with this approach depending upon the etiologic agent. [40] [41] The wide prevalence of sulfonamide and tetracycline resistance in *E. coli, Salmonella, Shigella* and *Campylobacter* spp. has made the fluoroquinolones such as ciprofloxacin, norfloxacin, ofloxacin and levofloxacin the most commonly recommended antibiotics in treatment. In Asia where *Campylobacter* may be resistant to the fluoroquinolones, azithromycin may be used. This agent can also be safely given to children. Antibiotics are prescribed for up to 3 days, but single-dose therapy may be sufficient.[42] Medical care should be sought by persons with dysentery if self-treatment does not result in improvement within 24 hours, or in cases of severe dehydration. Diarrhea that persists after return should be evaluated (see Chapter 144 for a full discussion of persistent diarrhea) for causes ranging from functional bowel disease to infection with *Giardia* or *Cyclospora* spp. to tropical sprue.

MALARIA PREVENTION

Malaria is one of the most important diseases to prevent as it can be fatal. The type of malaria and risk of acquisition vary by destination and reason for travel, but worldwide there are approximately 10,000 cases in returned travelers. Over 80% of cases of the most severe form of malaria, *Plasmodium falciparum*, are acquired by travelers on trips to Africa, where resistance to chloroquine is widespread and transmission may occur in urban and rural areas. Deaths in travelers can almost always be prevented by adherence to mosquito avoidance, compliance with appropriate chemoprophylaxis and prompt recognition of malaria symptoms and consequent initiation of treatment.[43]

Malaria is transmitted by the *Anopheles* mosquito, which is most active during the nighttime hours from dusk to dawn. During these times travelers should wear loose-fitting cotton clothing which covers their arms and legs, apply repellents to exposed areas of skin and sleep in enclosed areas behind screens or under netting (see Table 143.4). The most effective repellents are those that contain *N,N*-diethyl-3-methylbenzamide (DEET).[44] There is no need to exceed a concentration of 20–35%. DEET-containing repellents are safe to use in children and pregnant women, but should be used sparingly to avoid systemic absorption and rare neurologic toxicity. Travelers should not apply repellents to mucous membranes and irritated skin and should wash them off when coming indoors. Residual insecticide preparations (e.g. permethrin-containing compounds) can be applied to clothing and netting to kill insects rather than only repel them. Mosquito coils and sprays containing pyrethroids may be used in enclosed sleeping areas.

Chemoprophylaxis needs to be taken on a regular basis during travel and for a period of time after return that depends on which medications were taken (Table 143.7). Many cases of malaria occur not because of drug resistance but because of poor compliance. Fifty to sixty per cent of short-term travelers will be completely compliant and less than this number of long-term travelers. The choice of a chemoprophylactic regimen should be based on risk of exposure, types of parasites prevalent in the travel destination and health status of the traveler. Up-to-date sources should be consulted before prescribing any antimalarial.[3] [4]

Chloroquine as a single agent is effective only in areas where *P. falciparum* is not present or remains sensitive: Mexico, Central America west and north of the Panama Canal, the Dominican Republic and Haiti, Egypt, most areas of the Middle East and parts of China. Travelers to other risk areas in Africa, Asia and Latin America will need to take mefloquine, a new combination medication called Malarone® (atovaquone/proguanil, GlaxoSmithKline), doxycycline or add proguanil to chloroquine.

In the USA, mefloquine and Malarone® are the drugs of choice for travel to areas with chloroquine-resistant *P. falciparum*. [3] Mefloquine is highly efficacious in preventing malaria but concern has been expressed by both health care providers and travelers as to the drug's potential for side effects. When it is taken in prophylactic doses, minor GI and neuropsychiatric events occur in 5–30% of users.[45] The neuropsychiatric side effects may include sleep disturbance, vivid dreams, mood changes, anxiety, headache and dizziness. Serious adverse events such as psychosis are rare with an occurrence of about one case per 13,000 users.[46]

Contraindications to mefloquine are a known hypersensitivity to the drug, a history of seizures or psychiatric disorder, an underlying cardiac conduction abnormality, but not the use of ß-blockers for blood pressure control. It is likely to be safe in pregnancy, but should be avoided if possible during the first trimester. Some travel health consultants prescribe mefloquine 2–3 weeks before departure to assess patient tolerance and allow a switch to other agents if there is a problem.[4] Seventy per cent of adverse reactions occur during the first three doses. Loading doses of the drug are usually not advocated.

Malarone® is a fixed combination antimalarial containing atovaquone and proguanil.[47] [48] It is available in the US, Canada and

TABLE 143-7 -- Prophylaxis of malaria.*

PROPHYLAXIS OF MALARIA		
Drug	**Adult dose**	**Pediatric dose**
Chloroquine	300mg base (500mg salt) orally, once/week, beginning 1 week before travels, weekly whilst traveling and for 4 weeks after travel	5mg/kg base (8.3mg/kg salt), once per week (not to exceed adult dose)
Mefloquine	250mg salt orally, once/week	<15kg: 5mg of salt/kg/week
		15–19kg: 1/4 tablet/week
		20–30kg: 1/2 tablet/week
		31–45kg: 3/4 tablet/week
		>45kg: 1 tablet/week
Atovaquone/proguanil (A/P) (Malarone®)	250mg A/100mg P	62.5mg A/25mg P
	1 tablet daily, beginning 1–2 days before travel and for 7 days after travel	11–20kg: 1 tablet daily
		21–30kg: 2 tablets daily
		31–40kg: 3 tablets daily
		>40kg: adult dosing
Doxycycline	100mg orally, once/day, beginning 1–2 days before travel and for 4 weeks after travel	>8 years of age: 2mg/kg orally, once/day, not to exceed adult dose
Proguanil	200mg orally, once/day	<2 years: 50mg/day
	Used in combination with chloroquine	2–6 years: 100mg/day
		7–10 years: 150mg/day
		>10 years: 200mg/day
Primaquine	15mg base (26.3mg salt) orally, once/day for 14 days	0.3mg/kg base (0.5mg/kg salt) Orally, once/day for 14 days
	Used to eradicate extraerythrocytic stage	
Full manufacturer's prescribing instructions should be consulted on dosing guidelines as they may vary between countries.		

* *Information from reference[3] .*

many European countries and comes in both adult and pediatric formulations. It is effective in treatment and prophylaxis of all malaria species, although experience with non-falciparum species is limited. It is started the day before exposure to malaria, continued daily and discontinued 7 days after leaving the malarious area. It has causal prophylactic effect (kills developing hepatic-stage parasites but not the hypnoziotes of *P. virax* or *P. ovale*) which allows the shortened period of time post travel. It is ideally targeted for short-term travelers to areas of risk.

Rural, forested, border areas of Thailand with Myanmar (formerly Burma) and Cambodia have multidrug-resistant *P. falciparum* malaria; the few travelers to these areas should take daily doxycycline for prophylaxis. Malarone may also be effective in these areas. Doxycycline is also an alternative medication for those intolerant of mefloquine, although it cannot be given to children under the age of 8 and to pregnant women. Doxycycline should be swallowed with a large volume of liquid to prevent esophageal irritation. It may predispose to vaginal yeast infection and act as a photosensitizer.

The combination of chloroquine plus proguanil has been recommended for areas in which there is chloroquine-resistant *P. falciparum* malaria but only a small risk of acquisition, such as India.[4] It is in these limited risk areas that US and European recommendations differ; the CDC no longer recommends this combination.[3] Health practitioners should consult the appropriate source for their country.

All travelers need to be told that no antimalarial is 100% protective and that they can develop malaria in spite of being compliant with prophylaxis. If they develop a fever or flu-like illness overseas that could be malaria, they should seek medical care. Their evaluation needs to include a blood smear performed by a competent laboratory, because the sensitivity of symptoms or physical findings alone is low. The use of self-administered diagnostic kits (dipstick tests) is controversial as travelers can have difficulty in using and interpreting these tests.[49] If medical care cannot be obtained within 24 hours the traveler can consider self-treatment. The combination drug sulfadoxine/pyrimethamine (Fansidar, three tablets at one time) may be taken in many areas of the world except where there is resistant disease such as in South East Asia.[4] There, quinine alone or with doxycycline or tetracycline can be used. Malarone is another option for standby treatment. Fansidar should not be taken by those with a sulfonamide allergy. Halofantrine has excellent activity against all species of malaria but it should not be used as standby therapy because of potentially fatal cardiac side effects in predisposed individuals.[50]

Travelers who have had prolonged exposure to malaria in areas of *P. vivax* and *P. ovale* activity can consider primaquine to eradicate hepatic-stage parasites. Before taking primaquine a glucose-6-phosphate dehydrogenase (G-6-PD) test should be obtained. Primaquine cannot be given to pregnant women because the G-6-PD status of the fetus cannot be determined. Chloroquine-resistant *P. vivax* has been described primarily from South East Asia (Papua New Guinea and Irian Jaya) with sporadic cases from Myanmar, India and Guyana and Brazil, but is unusual. Primaquine resistance may also occur.

Pregnant women should not travel to malarious areas unless absolutely necessary, because of the added risk of complications of malaria during pregnancy. Chloroquine is safe. Mefloquine can be taken after the first trimester (and may be safe in the first trimester), although the US Food and Drug Administration has not

approved mefloquine use in pregnancy. Doxycycline and primaquine are contraindicated and there is insufficient data on Malarone®.

ENVIRONMENTAL RISK

Travel to the tropics is associated with increased heat and humidity; the traveler will need to take into account the effects these changes may have on their health. These can range from a feeling of malaise

and tiredness to increased loss of salt and water with resultant dehydration. Travelers should maintain hydration, limit exercise and sleep in a cool environment, particularly if they are elderly or have chronic medical problems. Excessive sun exposure should be avoided by wearing loose-fitting cotton clothing to cover exposed skin, wearing hats and using sunscreens with a sun protection factor of at least 15. Water insolubility may extend the life of the sunscreen. If the patient is taking doxycycline for malaria prophylaxis, it is particularly important to limit sun exposure. In addition to protecting the skin against sunburn, it should be kept dry and clean to avoid cellulitis and dermatophyte infection.

Travelers to altitudes above 2500–3000 meters may experience acute mountain sickness (AMS) or the more severe high-altitude pulmonary edema, retinal hemorrhage and cerebral edema.[51] AMS is characterized by headache, nausea, vomiting, insomnia and lassitude and may affect up to 50% of persons. The risk of illness can be lessened by acclimatization: spending a few days at intermediate altitudes of 1500–2200 meters and gradually ascending, sleeping at elevations no more than 300–500 meters higher each night. Acetazolamide, a carbonic anhydrase inhibitor, may be taken to assist acclimatization. It is given at a dose of 125–250mg orally twice daily, starting 2 days before being at altitude and for several days at altitude. It has also been used to treat mild symptoms of AMS. Dexamethasone may be used to treat AMS but in severe illness the safest course is always to descend.[51] Acetazolamide is contraindicated in persons with sulfonamide allergy.

Jet lag is a common problem, particularly when more than five time zones are crossed. It is easier to travel west and lengthen the day than to travel east and shorten the day. In order to help with jet lag, several methods have been proposed. Exposure to bright light after arrival may help. Taking a short to intermediate-acting benzodiazepine or a pyrazolopyrimidine can help travelers to fall asleep and maintain sleep, which decreases the contribution of exhaustion to the effects of time zone adjustment. Melatonin, which is secreted during the night hours, has also been studied.[52] A dose of 5–8mg taken at night for the first few nights may be helpful but the purity and effectiveness of over-the-counter preparations have not been documented and the effectiveness of this approach is controversial.

The risk of deep venous thrombosis (DVT) and pulmonary embolism has received recent attention. DVTs can occur in as many as 5% of persons flying for 10–15 hours and who have cardiovascular risk factors. Some of these will go on to have pulmonary embolism.[53] At-risk travelers should maintain their hydration, exercise at regular intervals and consider wearing below-the-knee support stockings to decrease the risk.

BEHAVIORAL RISK

Although health care providers and travelers tend to focus on infectious and medical illness, the most important contributor to severe morbidity and mortality, particularly in young adults, is accidents and injuries.[18] [54] To prevent assault and theft, travelers should not wear jewellery and ostentatious clothing and they should travel in groups, avoiding high-risk urban areas, particularly at night. The US State Department posts travel advisory and safety information at: http://travel.state.gov/. Motor vehicle safety can be enhanced by riding in vehicles with seat belts, avoiding excessive speed and not driving at night. One should avoid riding in the back of open bed trucks and overcrowded buses. When swimming, travelers should be aware of undercurrents and never dive into unknown waters.

Sexually transmitted diseases, including HIV, gonorrhea, syphilis and chancroid, are prevalent. In some countries in sub-Saharan Africa nearly 50% of sexually active adults may be HIV positive. In parts of Latin America, South East Asia and China, HIV has increased exponentially.[55] Although condoms and spermicides may help to prevent transmission, the safest course is abstinence. In spite of these statistics, many travelers continue to engage in high-risk sexual behavior, often without the protection of condoms.[56] In all situations, alcohol contributes to increased risk behavior.

OTHER DISEASES AND CONSIDERATIONS

Dengue fever, a viral disease (see Chapter 184) transmitted by *Aedes* mosquitoes, has seen a resurgence throughout Asia, sub-Saharan Africa, the Caribbean basin and Latin America and has become a theoretical risk in the south-eastern USA.[57] Recent outbreaks have also occurred in Hawaii. Dengue is characterized by the sudden onset of fever, headache, myalgias and arthralgias, abdominal discomfort, rash and mild liver abnormalities. Severe disease can progress to a hemorrhagic shock syndrome. There is no vaccine currently available for prevention, so travelers need to exercise precaution against this daytime feeding mosquito. Complying with the measures outlined in Table 143.4 will help to prevent not only dengue and malaria, but also the other less common insect-transmitted diseases such as leishmaniasis, trypanosomiasis, filariasis and rickettsial infection. East African trypanosomiasis has recently been seen in several travelers to Tanzanian game parks.[58]

Schistosoma spp. (see Chapter 167) can infect travelers who swim in fresh water in endemic areas of the Caribbean, South America, Africa and Asia. Travelers to these areas should avoid all fresh-water swimming unless it is in a chlorinated pool. Letting water stand for 48 hours or warming it to 50°C for 5 minutes will render it safe from the *Schistosoma* parasites. Fresh-water swimming, particularly after periods of flooding, can be a risk for acquisition of leptospirosis.

Although the viral hemorrhagic fevers — Ebola, Lassa and Marburg (see Chapter 183) — garner a great deal of media attention, they are generally not a risk for travelers. Current outbreaks of disease can be followed by subscribing to the listserv ProMED or by checking the disease outbreak sites of the WHO and CDC web pages (see Sources of Information). Any returning traveler who is suspected of having a viral hemorrhagic fever should be managed according to WHO guidelines.[59]

Access to medical care overseas can be accomplished in several ways. Travelers can purchase a travel health insurance package that should include the following: help in locating medical care, paying for the care upfront and, if necessary, providing for emergency evacuation. There are several air ambulance and insurance companies (www.travel.state.gov/medical.html). Embassies or consulates may provide names of physicians and mission hospitals can be a source of care. Tattooing, injections and dental instruments should be avoided to decrease the risk of acquiring blood-borne pathogens such as hepatitis B and C and, less likely, HIV. The International Association of Medical Assistance to Travelers (www.iamat.org) will provide a list of English-speaking physicians throughout the world. A small first-aid kit that contains analgesics, bandages, a thermometer and any over-the-counter medications frequently used is helpful as it is often difficult to find even the simplest medicines overseas.

Finally, it is important to alert travelers to recognize major problems, such as fever, persistent diarrhea or rash, that may occur after return. Although routine posttravel follow-up for short-term travelers is usually not necessary, anyone who experienced major illness overseas or new-onset illness after return should be evaluated.[60] After a history, physical examination and targeted laboratory testing, a predominant syndrome can be described and a differential diagnosis generated. Each potential diagnosis can be matched against its incubation period, geographic area of risk, frequency of occurrence

and the traveler's preventive measures. The appropriate evaluation and interventions should then be pursued. A more detailed discussion of individual syndromes and diseases is provided in other chapters.

SOURCES OF INFORMATION

There are a number of helpful sources of information in travel medicine. Major ones are listed.

United States resources

Centers for Disease Control and Prevention (USA)

- *Health information for international travel*, published annually. Atlanta, GA: DHHS. This can be purchased in hard copy (+1 877-252-1200), ordered on-line at http://bookstore.phf.org, or downloaded from the CDC travel medicine home page.
- Travel medicine home page: www.cdc.gov/travel/index.htm

¦ Morbidity and Mortality Weekly Report: www.cdc.gov/mmwr

US State Department

¦ Travel advisories: http://travel.state.gov
¦ Medical Information for Americans Traveling Abroad (has information on travel medical insurance and air ambulance companies): www.travel.state.gov/medical.html

World Health Organization

¦ *International travel and health. Vaccination requirements and health advice.* Geneva: World Health Organization, published annually. This can be purchased in hard copy (+41 22 791 24 76), ordered on-line at bookorders.who.int:8080/newaccess/anglais/home1.jsp or viewed at www.who.int/ith
¦ Home page: www.who.int/home-page
¦ Emerging infections: www.who.int/csr/don/en
¦ Weekly Epidemiologic Record: www.who.int/wer
¦ Rabnet (rabies epidemiology and biologics availability): http://oms2.b3e.jussieu.fr/rabnet

Canadian resources

¦ Health Canada — Travel Medicine: www.travelhealth.gc.ca
¦ Committee to Advise on Tropical Medicine and Travel: www.hcsc.gc.ca/pphb-dgspsp/tmp-pmv/catmat-ccmtmv/index.html

United Kingdom resources

¦ Communicable Disease Report: www.phls.co.uk/publications/cdr/index.html
¦ Department of Health: www.doh.gov.uk/traveladvice/index.htm
¦ Fit for Travel — Scotland: www.fitfortravel.scot.nhs.uk/

European Surveillance website: www.eurosurveillance.org

International Association of Medical Assistance to Travelers

¦ IAMAT, 417 Center Street, Lewiston, NY 14092 (+1 716 754-4883), email: info@iamat.org
¦ Home page: www.iamat.org

International Society of Travel Medicine

¦ PO Box 871089, Stone Mountain, GA 30087-0025, USA (+1 770 736-7060); email: istm@istm.org
¦ *Journal of Travel Medicine.* BC Decker Inc, Hamilton, Ontario, Canada (+1 905 522-7017).
¦ Home page: www.istm.org
¦ Travel clinic directories: www.istm.org/disclinics.html

American Society of Tropical Medicine and Hygiene

¦ 60 Revere Street, Suite 500, Northbrook, IL 60062, USA (+1 847 480-9592); email: astmh@astmh.org.
¦ Homepage: www.astmh.org
¦ Travel clinic directories: www.astmh.org/scripts/clinindex.html

Royal Society of Tropical Medicine and Hygiene

¦ Manson House, 26 Portland Place, London W1B 1EY, UK (+44 (0)20 7580-2127).
¦ Home page: www.rstmh.org

ProMed Electronic Network

¦ This is a communication system to monitor emerging infectious diseases (sometimes unverified). To subscribe send an email message to majordomo@promedmail.org and in your message type 'subscribe promed' and your name.

International certificate of vaccination

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¦ Also available through the WHO: call +41 22 791 24 76 or order on-line at bookorders.who.int:8080/newaccess/anglais/home1.jsp

Commercial travel medicine databases

¦ Travax EnCompass (Shoreland Inc, US): www.shoreland.com
¦ Travax (Travel Medicine — Scotland): www.travax.scot.nhs.uk
¦ MASTA (England): www.masta.org
¦ Exodus Software Ltd (Ireland): www.exodus.ie
¦ Edisan (Médecine des Voyages — France): www.edisan.fr
¦ Tropimed® (Switzerland, Germany, USA): www.tropimed.com

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¦ Keystone JS, Kozarsky PE, Nothdurft HD, *et al*, eds. Travel medicine. London: Harcourt; 2003.
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Chapter 144 - Diarrhea and Food-borne Illness

Andrew T Pavia

INTRODUCTION

It has been said that 'travel broadens the mind and loosens the bowels'. Although diarrhea is not the most serious travel-related condition, it is by far the most common illness among travelers and expatriates from developed countries who visit less developed nations.[1] In the first 2 weeks abroad, 20–50% of travelers will develop diarrhea. A large proportion will still have symptoms when they return home. In one study, 12% first developed symptoms after returning home. Travelers' diarrhea is usually defined as the passage of three or more unformed stools with associated symptoms, including nausea, vomiting, abdominal pain or cramps, tenesmus or passage of mucus or blood. The median duration of symptoms is 2–3 days, but 10–15% have symptoms lasting more than 1 week. In two large studies, 0.9% of Swiss travelers[1] and 1.7% of Peace Corps volunteers[2] developed persistent diarrhea lasting more than 1 month.

The risk of developing diarrhea is related to destination, the age and mode of travel and the care taken in selecting food and drink (Fig. 144.1). The highest incidence of diarrhea is associated with travel to or residence in Africa, Asia, the Middle East and Latin America. Intermediate risk areas include central and southern Europe and some Caribbean islands. The incidence is highest among children younger than 2 years old[3] and among young adults (age 15–24).[4] [5] Eating in private homes is generally safer than eating in restaurants and, not surprisingly, eating food from street vendors is associated with markedly increased risk. The prevalence of diarrhea episodes peaks in the first few weeks and declines with prolonged residence in an endemic region.

The range of causative pathogens and the clinical spectrum of diarrheal illness among travelers parallel diarrheal illness among children living in the country. Intensive studies of the etiology of travelers' diarrhea over the past 30 years have shown a wide range of causative agents. The most important pathogens among travelers are, in decreasing order of frequency, enterotoxigenic *Escherichia coli* (ETEC), *Shigella* spp., *Campylobacter jejuni*, *Salmonella* spp., *Plesiomonas shigelloides*, noncholera *Vibrio* spp. and *Aeromonas* spp. (Table 144.1).[6] [7] [8] [9] More than one pathogen is isolated in 10–20%. Rotavirus has been detected in 0–24% of travelers with diarrhea, but generally is found with similar frequency among asymptomatic travelers. *Cyclospora* has been recently described as a cause of travelers' diarrhea,[10] as have microsporidia sp.[11] Enteroadherent *E. coli* which demonstrate local, diffuse or aggregative adherence to HEp-2 cells have been implicated in travelers' diarrhea.[12] [13] Recent data demonstrate that these organisms are a frequent cause of illness, perhaps second only to ETEC.[14]

PRESENTING SIGNS AND SYMPTOMS THAT POINT TO A DIAGNOSIS

Acute diarrhea

It is difficult to make an etiologic diagnosis for most episodes of diarrhea in the returning traveler. Most illness is of moderate severity and resolves spontaneously; empiric antimicrobial therapy is highly effective. ETEC is the most common pathogen, yet it cannot be detected in most clinical laboratories. Because of the wide range of potential pathogens, an unfocused laboratory evaluation will be far reaching and expensive. Therefore, a stepwise focused approach is recommended. There is substantial overlap in the clinical manifestations of different pathogens and it is impossible to determine reliably the etiology of travelers' diarrhea based on the presentation.[15] [16] Nonetheless, clinical and epidemiologic clues can point to a diagnosis and are a valuable guide to therapy and diagnostic approach (Table 144.2).

Vomiting

Illnesses that manifest as vomiting alone are generally short-lived and less likely to be seen after the traveler returns. Toxin-mediated food-borne illness caused by *Staphylococcus aureus* or *Bacillus cereus* may occur among travelers because of exposure to foods that were not adequately refrigerated. Vomiting may be the only symptom among adults with viral gastroenteritis. Vomiting and intense abdominal pain that persists may suggest anisakiasis. This syndrome is caused by invasion of the gastric or intestinal mucosa by larvae of *Anisakis* spp. or *Pseudoterranova* spp. after eating raw fish, including herring, salmon, cod, halibut, pollack, greenling and mackerel.

Watery diarrhea

Acute watery diarrhea is the most common presentation of travelers' diarrhea; ETEC is the most frequent cause of this syndrome. Vomiting is present in 10–20% of patients. Vomiting may suggest gastroenteritis caused by Norwalk-like viruses (norovirus), astrovirus or calicivirus rather than ETEC infection.[17] The sudden onset of vomiting after a short incubation period suggests toxin-mediated food-borne illness caused by *Staph. aureus* or *B. cereus*. Explosive watery diarrhea that is short-lived may be caused by preformed toxin from *Clostridium perfringens* or *B. cereus*. Outbreaks of food-borne illness caused by preformed toxin, Norwalk virus, ETEC and *Shigella* spp. have been common on cruise ships. Airline meals have been implicated in outbreaks of *Shigella* and cholera.

A history of seafood ingestion suggests infection with *Vibrio parahaemolyticus, Vibrio cholerae* non-O1, other *Vibrio* spp. or *Aeromonas* spp. Profuse dehydrating diarrhea may occasionally be caused by toxigenic *V. cholerae* O1 or O139. The diarrhea caused by *Cryptosporidia* and *Cyclospora* spp. is most often watery, profuse and prolonged; fever is uncommon.[10] [18]

Although most acute diarrhea among travelers will not be associated with a larger outbreak, physicians should be alert to this possibility. Prompt reporting of cases (along with the travel history) to local or national public health authorities may lead to identification of widely dispersed outbreaks.

Inflammatory or bloody diarrhea

Symptoms of inflammatory diarrhea include the passage of bloody or mucoid stools, high fever, abdominal pain and tenesmus; white

Figure 144-1 Food-borne transmission probably accounts for the majority of diarrheal illness in travelers and expatriates. The high rates of shigellosis and campylobacteriosis compared with cholera among travelers in cholera-endemic areas suggest that they may be better able to avoid exposure to contaminated water than to contaminated food.

blood cells are often present in the stool. Approximately 10–20% of episodes of diarrhea will be bloody. Pathogens associated with inflammatory or bloody diarrhea include *Shigella* spp., *C. jejuni*, nontyphoidal *Salmonella* spp., *V. parahaemolyticus, Entamoeba histolytica, P. shigelloides, Clostridium difficile* and enteroinvasive *E. coli* (EIEC). Of these, *Shigella* spp. and *Campylobacter* spp. are the most common in most settings. *Vibrio parahaemolyticus* is common among Japanese travelers and should be suspected if there is a history of seafood consumption. *Clostridium difficile* should be considered in a patient who has previously been treated with antimicrobials. The presence of fecal leukocytes suggests infection with an invasive organism, most often *Shigella* spp., *Campylobacter* spp. or *Salmonella* spp.

Leukocytes are not invariably present in these

TABLE 144-1 -- Etiology of diarrhea in travelers by region.[*]

ETIOLOGY OF DIARRHEA IN TRAVELERS BY REGION					
Organism	**Nepal (%)**	**South East Asia (%)**	**India (%)**	**Latin America (%)**	**Africa (%)**
Enterotoxigenic *E. coli*	20–28	6–30	24	26–72	25–75
Enteroadherent *E. coli*	13–18	3–8	19	15	-
Enteroinvasive *E. coli*	0–3	0–3	-	2	0
Shigella	10–23	2–7	10	0–22	0–15
Campylobacter	4–28	15–58	3	2–15	1–5
Salmonella	3–4	3–17	10	0–16	0–5
Yersinia	0–2	1–3	-	-	0
Vibrio spp.	0–1	5–13	5	-	3
Plesiomonas	4	2–13	7	-	2–7
Aeromonas			3	-	2
Rotavirus	3–11	8	5	0–24	0–6
Giardia	9–16	0–2	2	0–36	0
Entamoeba histolytica	3	-	5	-	0
Cryptosporidium	4–5	1–2	2	-	0–2
Cyclospora	11	-	-	-	-
No pathogen	40–53	25–42	45	22–50	29–64
Results are compiled from studies of varying populations ranging from short-term tourists to military personnel and Peace Corps volunteers. Not all pathogens were sought in all studies.					

** Adapted from references*[6] [7] [8] [9] [29] .

infections; assays for fecal lactoferrin have greater sensitivity and specificity than microscopy but are not widely used.[19] In one study, fecal leukocytes were detected by microscopy in 67% of patients who have *Shigella* infection,[20] 24% of those with *Campylobacter* and 27% of those with *Salmonella*. Fecal leukocytes are strikingly absent in amebic dysentery caused by *E. histolytica.*

Patients who have typhoid fever may present with diarrhea and abdominal pain, and diarrhea is a more common manifestation of typhoid among children. In some instances, diarrhea may persist for 1–2 weeks; fecal leukocytes are usually present. The stepwise increase in fever along with associated symptoms, such as headache, confusion, weakness, cough or myalgia, should raise the suspicion of typhoid. A febrile patient who has a relatively slow pulse should be suspected of having typhoid.

Among sexually active homosexual men, proctitis caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis* can potentially be mistaken for inflammatory diarrhea if a sexual history is not obtained. Another diagnostic pitfall is the failure to consider malaria in a person with high fevers, headaches and myalgias, because patients who have acute malaria may complain of diarrhea or vomiting.

Diarrhea with neurologic symptoms

Several types of food-borne illness that have distinctive neurologic manifestations may occur in travelers.[21] Nausea, vomiting, diarrhea and abdominal cramps followed by paresthesias, myalgias, arthralgias, reversal of hot and cold sensation and a sensation of loose teeth are symptoms of ciguatera poisoning. Ciguatera follows ingestion of large predatory reef fish, usually barracuda, grouper, amberjack and snapper, which contain high concentrations of toxins produced by dinoflagellates. Symptoms may persist for anything from several days to months. Shellfish that contain toxins from the toxin-producing planktonic dinoflagellates (*Gonyaulax catenella* and *Gymnodinium breve*) cause paralytic and neurotoxic shellfish poisoning. Paralytic shellfish poisoning manifests after an incubation period of between 5 minutes and 4 hours, with symptoms of paresthesias of the mouth, lips, face and fingers, followed by dysarthria, dysphonia, muscle weakness and respiratory compromise. The symptoms of neurotoxic shellfish poisoning are similar but less severe.

Because of the relatively long incubation period of food-borne botulism (median 24 hours, range 12 hours to 7 days) and the potential subtlety of symptoms, travelers with this disease might not

TABLE 144-2 -- Characteristics of selected food-borne and diarrheal illnesses of importance to travelers, arranged by incubation period.

CHARACTERISTICS OF SELECTED FOOD-BORNE ILLNESSES						
Organism	**Incubation period in hours median (range)**	**Vomiting**	**Diarrhea**	**Fever**	**Other symptoms**	**Common vehicles**
Histamine fish poisoning (scombroid)	5 min to 1 hour	+	+++	-	Headache, flushing, urticaria	Tuna, mackerel, bonito, mahi-mahi, bluefish
Staphylococcus aureus	3 (1–6)	+++	++	-		Ham, poultry, cream-filled pastries, potato and egg salad
Bacillus cereus (emetic syndrome)	2 (1–6)	+++	+	-		Fried rice
Ciguatera	2 (1–6)	+	++	-	Paresthesias, myalgias, headache, arthralgia	Barracuda, snapper, grouper, amberjack
Bacillus cereus (diarrheal syndrome)	9 (6–16)	+	+++	-	Abdominal cramps	Beef, pork, chicken
Clostridium perfringens	12 (6–24)	+	+++	-	Abdominal cramps	Beef, poultry, gravy
Vibrio cholerae non-O1	11 (5–96)	+	+++	+++	Abdominal cramps, bloody diarrhea (25%)	Fish, shellfish
Vibrio parahaemolyticus	15 (4–96)	++	+++	++	Abdominal cramps, headache, bloody diarrhea (rare)	Fish, shellfish
Norwalk virus	24 (12–48)	+++	+++	++	Headache, myalgias	Water, ice, shellfish, salads
Shigella spp.	24 (7–168)	+	+++	+++	Abdominal cramps, bloody diarrhea	Lettuce, street food
Enterotoxigenic *Escherichia coli*	36 (16–72)	+	+++	+	Abdominal cramps, headache, myalgias	Ice, water, produce

Vibrio cholerae O1	48 (6–120)	++	+++	+	Dehydration	Shellfish
Salmonella spp.	36 (12–72)	+	+++	++	Abdominal cramps, headache, myalgias	Beef, poultry, pork, eggs, dairy products, vegetables, fruit
Campylobacter jejuni	48 (24–168)	+	+++	+++	Abdominal cramps, bloody diarrhea, myalgias	Poultry, milk
Clostridium botulinum	18 (6–240)	++	++	-	Dysarthria, diplopia, dry mouth, paralysis	Canned food, fermented seafood, garlic under oil, dried salted fish
-, rare symptom (<10%); +, infrequent symptom (11–33%); ++, frequent symptom (33–66%); +++, classic symptom.						

present until after their return home. Symptoms include dysphagia, dysphonia, blurred vision, dry mouth and diplopia; up to 50% will initially have nausea, vomiting and diarrhea. Home canning and unregulated commercial canning pose a risk of food-borne botulism. Type E botulism is associated with foods of marine origin, and travelers with a taste for exotic food might be at risk. Vehicles of type E botulism include fermented seal and whale blubber, and dried salted whitefish.

Persistent diarrhea

Persistent diarrhea is usually defined as diarrhea that lasts for 14 days or more. Although only about 3% of travelers will have diarrhea lasting at least 2 weeks and perhaps 1% will have symptoms for more than 1 month, these patients account for many physician visits and a substantial amount of time lost from work. [1] The etiology of persistent diarrhea in travelers (Table 144.3) has not been systematically investigated and is not well understood.[22]

Protozoal agents are relatively uncommon causes of acute travelers' diarrhea, but are much more likely to be responsible for protracted illness.[11] *Giardia lamblia* has long been recognized as a cause of prolonged diarrhea, often associated with bloating, malabsorption and offensive flatus. The incubation period is 1–2 weeks. *Cryptosporidium parvum* infections cause prolonged illness even in immunocompetent hosts. In one study of Finnish travelers and in several outbreaks in the USA, the median duration of diarrhea ranged from 9 to 12 days. *Cyclospora cayetanensis* is a recently described cause of diarrhea (originally referred to as blue-green algae, cyanobacteria-like organism and coccidia-like), which has been identified in North, South and Central America, Africa, Europe and Asia. In studies in Nepal, and in produce-associated outbreaks in North America, the illness was intermittent, lasted a median of 7 weeks and was associated with anorexia, weight loss and pronounced fatigue.[10] [18] *Isospora belli* can also cause persistent diarrhea; it was identified in 1.4% of military personnel with persistent diarrhea in Vietnam. *Entamoeba histolytica* and *Dientamoeba fragilis* are less common protozoal causes in travelers. Helminths may occasionally cause persistent diarrhea, including *Strongyloides stercoralis, Schistosoma mansoni, Trichuris trichiura* and *Capillaria philippinensis*. Schistosomiasis should be considered if there is fever, hepatosplenomegaly, eosinophilia and a history of immersion in fresh water in an endemic region. Eosinophilia strongly suggests helminthic infection but is unusual in protozoal disease. The exception to this rule is isosporiasis, in which eosinophilia is common. However, eosinophilia may be an unrelated finding, caused by coexistent helminthic infection with agents such as hookworm, which is not responsible for the diarrhea.

Bacterial agents are also important causes of persistent diarrhea. ETEC, *Shigella* spp., *Campylobacter* spp., *P. shigelloides* and *Aeromonas hydrophila* may cause prolonged diarrhea. These infections may be associated with passage of blood and mucus. Enteroadherent *E. coli* strains are important causes of persistent diarrhea in children in tropical countries [23] [24] and they may be important in travelers as well.[14] [25] Three classes are currently recognized by adherence pattern to HEp-2 cells. Locally adherent *E. coli* include most classic EPEC

TABLE 144-3 -- Causes of persistent diarrhea in travelers and expatriates.

CAUSES OF PERSISTENT DIARRHEA IN TRAVELERS AND EXPATRIATES		
Infectious		
Persistent bacterial infection	**Persistent protozoal infection**	**Helminth infections**
Salmonella spp.	*Giardia lamblia*	*Strongyloides stercoralis*
Campylobacter spp.	*Cryptosporidium parvum*	*Schistosoma* spp.
Yersinia spp.	*Cyclospora cayetanensis*	*Capillaria philippinensis*
Enteroadherent *E. coli* (3 subgroups)	*Entamoeba histolytica*	
Clostridium difficile	*Isospora belli*	
Aeromonas spp.	*Dientamoeba fragilis*	
Plesiomonas spp.	*Balantidium coli*	
Noninfectious		
Dietary	**Gastrointestinal pathology**	
Lactose intolerance	'Postdysenteric irritable bowel syndrome'	
Osmotic diarrhea	Crohn's disease	
	Ulcerative colitis	
	Bacterial overgrowth	
	Celiac disease	
	Collagenous colitis	
Unclassified (likely infectious)		
Tropical sprue		
Chronic idiopathic ('Brainerd') diarrhea		
Within each category, the causes are listed in roughly the order of frequency. Enteroadherent *Escherichia coli* includes three subgroups of organisms, grouped on the basis of patterns of adherence to HEp-2 cells: enteroaggregative, locally adherent (EPEC) and diffusely adherent.		

strains. Diffusely adherent *E. coli* are currently of uncertain significance. Enteroaggregative *E. coli* (EAggEC) display a unique 'stacked brick' adherence and may elaborate a unique enterotoxin. Studies in adult volunteers show that some strains cause prolonged illness in normal hosts but suggest that there is substantial heterogeneity.[22]

In many patients who have persistent diarrhea the infectious etiology will not be identifiable despite intensive investigation. In some patients, lactose intolerance may be present. Small bowel overgrowth with aerobic and anaerobic flora may follow acute enteric infection and lead to chronic non-specific symptoms. Gastrointestinal problems such as inflammatory bowel disease and carcinoma of the colon may occur coincidentally with travel or perhaps be exacerbated or unmasked by acute travelers' diarrhea. A syndrome of intermittent diarrhea and abdominal pain after acute enteric infection in patients who have no demonstrable infections, structural abnormality or evidence of malabsorption has been termed postdysenteric irritable bowel syndrome, for lack of a better term. These patients usually improve over time and appear to respond to fiber supplement and dietary modification.

Tropical sprue is a rare but serious syndrome of persistent diarrhea, usually seen in persons who have resided overseas for prolonged periods. It is suggested by symptoms of chronic malabsorption, including bulky, greasy stools, weight loss, anemia and neuropathy. Some travelers with persistent diarrhea appear to have a disease resembling 'Brainerd diarrhea', named after a town in Minnesota, USA, where a large outbreak of chronic diarrhea occurred as a result of consumption of unpasteurized milk.[26] This illness is characterized by 10–20 daily bouts of painless, urgent watery diarrhea, and lasts 1–2 years. Water-borne outbreaks of this illness have occurred in Illinois, USA, and among travelers to the Galapagos Islands.

DIFFERENTIAL DIAGNOSIS BY GEOGRAPHICAL AREA

Studies of travelers' diarrhea from different geographic areas are summarized in Table 144.1 . Varying microbiologic techniques limit direct comparison of studies. However, ETEC is the most common organism in virtually all regions. *Campylobacter* infections have been identified more commonly in studies of travelers, expatriates and military personnel from Nepal and South East Asia. *Vibrio* and *Aeromonas* infections have been primarily reported among travelers to South East Asia. *Giardia* and *Cryptosporidium* infections have been prominent among travelers to St Petersburg, Russia. *Capillaria philippinensis* has only been reported in the Philippines region. Certain regions appear to be at high risk for tropical sprue: Puerto Rico, Haiti, the Dominican Republic, India, Nepal, Myanmar (formerly Burma) and the Philippines.

Season may also provide a clue to the likely etiology: ETEC infections are most common during the wetter and warmer season in several regions. Studies among travelers to Mexico and Morocco found that *Campylobacter* infections are more common in the winter months.

INVESTIGATIONS TO CONFIRM THE DIAGNOSIS

The evaluation of the returned traveler with diarrhea should begin with a careful travel history and details of the onset, duration and character of the symptoms. The presence of signs of inflammatory diarrhea (fever, bloody or mucoid stools) raises the likelihood of infection with an invasive pathogen and usually should lead to obtaining cultures for *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp. Fecal lactoferrin is a more sensitive assay to identify polymorphonuclear cells, but staining a fresh stool specimen with methylene blue is quick and inexpensive and can be used in resource-poor settings to identify patients most likely to benefit from antimicrobial therapy. An additional benefit is that *Giardia* trophozoites may be identified occasionally. Seafood consumption will raise the likelihood of *Vibrio* or *Plesiomonas* infection. The incubation period, if known, is helpful. Incubation periods of less than 18 hours suggest toxin-mediated food-borne illness and further diagnostic testing is not helpful. Incubation periods of more than 5 days suggest diarrhea caused by protozoa or helminths.

Acute diarrhea

In patients who have milder illness of less than a few days' duration, rehydration and an antidiarrheal medication (loperamide or bismuch

Figure 144-2 A suggested approach to the evaluation and management of acute diarrhea in the returned traveler. The most likely pathogens for each scenario are given.

subsalicylate) alone may suffice (Fig. 144.2). For patients who have moderate to severe acute watery diarrhea, many physicians favor an initial empiric course of antimicrobial therapy (see below). ETEC is the most likely organism in this setting. If watery diarrhea persists despite empiric antimicrobial therapy, *Giardia, Cryptosporidium* and *Cyclospora* infection should be considered. Antigen detection assays for *Giardia* spp. and *Cryptosporidium* spp. are more sensitive than routine examination of stools for ova and parasites and can be performed initially.[11] If symptoms continue, three liquid stool samples should be examined for ova and parasites. In settings in which resources are limited, an empiric trial of metronidazole (250mg q8h) can be used as a therapeutic trial to treat *Giardia* infection.

For patients who have inflammatory diarrhea or dysentery and those who have failed antimicrobial therapy, stool cultures for *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp. and up to three ova and parasite examinations to look for *E. histolytica* are indicated. In patients who have taken antibiotics, assays for *C. difficile* toxin should be performed.

Persistent diarrhea

Persistent diarrhea warrants a more complete evaluation. The evaluation can be time consuming, costly and frustrating. The approach should be stepwise (Fig. 144.3). At least one specimen should be examined for bacterial agents. Selective media such as thiosulfate citrate bile salts sucrose agar may enhance recovery from *Vibrio* spp., but many strains can be identified from conventional media such as McConkey agar if the laboratory is alerted to look for oxidase-positive organisms. A careful parasitologic examination of three liquid stools should include concentration techniques and staining to improve the identification of *Isospora* spp. and *Cyclospora* spp., because both respond well to treatment with trimethoprim-sulfamethoxazole (co-trimoxazole). If a potential pathogen is identified, it does not prove causation, but a trial of specific therapy is warranted. Identification of enteroinvasive, enterotoxigenic and enteroadherent *E. coli* strains requires carefully standardized adherence assays and DNA probes, which are only available in research laboratories. Because diagnosing these infections is impractical, a trial of 5–7 days of a fluoroquinolone may be reasonable. When diarrhea persists, especially if there is weight loss, bloody stools or symptoms of malabsorption, a complete evaluation for malabsorption and endoscopy with biopsies should be considered. HIV infection should always be considered in unexplained chronic diarrhea.

The management of persistent diarrhea is difficult if diagnostic resources are limited. A trial of antimicrobial therapy, preferably with a fluoroquinolone, can be given. If this fails, and symptoms suggest *Cyclospora* or *Isospora* infection, a 2-week trial of trimethoprim-sulfamethoxazole (co-trimoxazole) can be considered. Empiric therapy with metronidazole for persistent diarrhea may treat giardiasis, *Balantidium coli* and *C. difficile*.

IMMEDIATE MANAGEMENT

All patients who have diarrheal illness need careful attention to hydration. In adults, oral rehydration with mineral water, weak tea or dilute fruit juice along with a source of sodium chloride (salty crackers, dilute broth) is usually sufficient. Oral rehydration

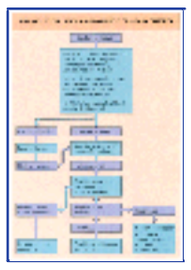

Figure 144-3 A suggested approach to the evaluation and management of chronic diarrhea in the returned traveler. The presence of significant weight loss or evidence of malabsorption should influence the pace and aggressiveness of the evaluation. This approach is designed for use in a Western setting.

solutions such as the WHO/UNICEF oral rehydration solution or commercial products such as Pedialyte or Ricelyte take advantage of glucose-coupled transport and provide more physiologic replacement of fluid, sodium, bicarbonate and potassium (see Chapter 161 and Chapter 222). They should be used for young children and persons with significant dehydration. Popular remedies such as tea, soft drinks and sports drinks are very low in sodium and should not be used alone. Bismuth subsalicylate has been clearly shown to provide symptomatic relief.[27] Antimotility agents such as loperamide (4mg initially followed by 2mg after each loose stool) provide prompt decrease in the number of stools. Short-term use of loperamide has been proven safe in studies of travelers' diarrhea and can be used in addition to antimicrobials. Because of the risk of toxic megacolon, loperamide should be avoided in patients who have dysentery and in young children. Patients who have diarrhea may develop secondary lactose intolerance and should avoid diary products until the diarrhea is resolved. Foods with high fat content and spicy foods may also aggravate symptoms. Although there are no scientific data evaluating dietary modification, some experts advise beginning with plain carbohydrates, such as rice, pasta or breads, then adding protein and finally fats as the symptoms resolve.

The preferred agents for empiric treatment of diarrhea in travelers are trimethoprim-sulfamethoxazole, 1 double-strength tablet twice daily for 3 days, or a

fluoroquinolone (ciprofloxacin 250–500mg q12h, ofloxacin 200–300mg q12h, or norfloxacin 200mg q12h).[28] For severe travelers' diarrhea, a 3-day course is generally recommended. Single-dose therapy with ofloxacin and ciprofloxacin has shown equivalent efficacy compared to 3-day courses in clinical trials, and clearly reduces the cost of treatment. In inland Mexico during the summer, trimethoprim-sulfamethoxazole remains effective but in many regions, resistance to this agent among ETEC and *Shigella* is common and recently, increasing resistance to fluoroquinolones has been reported. Fluoroquinolones should not be used in pregnant women and should be used with great caution in prepubertal children. Azithromycin has been successfully used in regions where quinolone-resistant *Campylobacter* spp. are prevalent.[29] Rifaxamin, a poorly absorbed oral antimicrobial, is under investigation but appears equivalent to fluoroquinolones. [30]

Specific treatment of protozoal and helminthic infections is covered in Chapter 242 , Chapter 243 , Chapter 244 , Chapter 245 , Chapter 246). The management of tropical sprue is discussed in Chapter 163 . For further discussion of the management of bacterial gastrointestinal infections, see Chapter 43 and Chapter 93 .

COMPLICATIONS

Most cases of diarrhea in travelers are self-limiting. Serious electrolyte abnormalities are uncommon in adults, with the exception of those with toxigenic *V. cholerae*. Inappropriate rehydration solutions may lead to hyponatremia or hypokalemia, especially in children and those on diuretics. Hypoglycemia and seizures are less common complications.

Rectal complaints are common in patients who have chronic diarrhea. Hemorrhoids can result in rectal bleeding, which may be confused with bloody diarrhea. Weight loss, skin changes, anemia and neuropathy caused by folate and vitamin B12 deficiency are complications of malabsorption caused by chronic diarrhea resulting from giardiasis, strongyloidiasis, tropical malabsorption and sprue. Extraintestinal complications of amebiasis are unusual but can include liver and brain abscesses.

Although diarrheal illnesses remain among the leading killers of children, the greatest impact of diarrhea on adults is economic. In addition to the effect on tourist income in developing countries and the direct medical costs, diarrhea among travelers results in a substantial amount of lost productivity.[1] [31] Despite recent advances, improved strategies for prevention, treatment and diagnosis are still needed.

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Chapter 145 - Fever

Anthony D Harries

INTRODUCTION

There has been a marked increase in international travel in the past 20 years and it is estimated that over 50 million people from industrialized countries visit the developing world each year.[1] Infectious diseases cause considerable morbidity among travelers and fever in returning travelers, including children, has become a relatively common clinical problem.[2] [3] [4] [5] [6] [7] Fever is an elevation of body temperature above the peak normal range and is normally considered to be present if the oral temperature is above 100.2°F (37.8°C).

As a result of the high prevalence of infections and parasitic diseases in the tropics, febrile illnesses are more common after a visit to tropical countries than after a visit to more developed countries. In one report, about 3% of 8000 German-speaking Swiss tourists reported a high fever or chills after a short-term visit to a tropical country compared with 1% of tourists who visited Greek or Canary Islands.[8] Among returned travelers who are managed in specialist tropical disease centers, fever tends to be a common clinical problem. For example, of 1084 patients admitted to the Hospital for Tropical Diseases, London, UK, during a 12-month period, nearly 50% presented with 'fever'.[9]

Travelers are liable not only to the usual causes of fever seen in temperate climates, but also to more exotic infections such as malaria, dengue fever and typhus. Because some tropical infections, such as malaria, may be rapidly fatal if undiagnosed or poorly treated and others, such as typhoid fever, can be a public health risk, this chapter focuses on the diagnosis and management of fever in travelers returned from tropical countries.

CLINICAL FEATURES

The common final diagnoses in febrile patients returned from the tropics and admitted to centers specializing in tropical medicine are malaria, typhoid fever, viral hepatitis and dengue fever (Table 145.1). In an appreciable proportion of patients the cause of fever is one that occurs worldwide such as an upper respiratory tract infection, community-acquired pneumonia and urinary tract infection. In addition, many fevers are undiagnosed and settle spontaneously and presumably are due to a non-specific viral infection.

History

A detailed history allows an appropriate differential diagnosis to be made and is useful in guiding initial investigations. Specific presenting symptoms and a full medical history (including a drug history) must be documented. Although complaints such as headache, lassitude and sweating have no useful diagnostic or localizing value, other symptoms such as cough and pleuritic chest pain (suggesting pneumonia) or dysuria and loin pain (suggesting acute pyelonephritis) may provide a clue to the anatomic site of disease. Several important travel-related topics also must be addressed.

Travel history

Arrival and departure dates, countries visited, duration of stay in each country, places visited within each country and activities should be precisely determined. Most tropical infections are transmitted more easily in rural than in urban areas. The backpacker or volunteer who travels in such areas and has closer contact with the local population therefore has a greater risk of acquiring a tropical infection than the person who stays in first-class hotels in the capital city. However, the affluent traveler who stays in air-conditioned hotels is sometimes at risk of contracting enteric bacterial infections and respiratory infections such as Legionnaires' disease.

A knowledge of incubation periods will help to eliminate the possibility of several potential infections and to narrow the differential diagnosis (Table 145.2). For example, malaria would not be considered in a febrile patient whose potential exposure was less than 7 days earlier. Likewise, dengue fever would be unlikely in a patient who had returned from an endemic area more than 10 days previously. Some tropical infections such as malaria due to *Plasmodium vivax* or *Plasmodium ovale*, African trypanosomiasis and visceral leishmaniasis may present weeks, months or even years after departure from the tropics (see Chapter 157 , Chapter 166 and Chapter 172).

Vaccination and prophylaxis

A history of vaccination against yellow fever, hepatitis A and hepatitis B virtually rules out these infections. If there is doubt about vaccination status, it is important to check vaccination certificates as patients may be confused about what inoculations they have received.[10] Oral and injectable typhoid vaccines are from 70% to 90% effective and prophylactic intramuscular human immunoglobulin against hepatitis A is effective for up to 6 months, with the protective efficacy declining with increasing time after administration. Childhood vaccinations against polio, measles and diphtheria may not be protective if boosters have not been given (see Chapter 143).

With the progressive spread of drug-resistant *Plasmodium falciparum* malaria and the recent emergence of chloroquine-resistant *P. vivax*, no chemoprophylactic antimalarial regimen is 100% protective even if compliance is excellent.[1] [11] Poor compliance with chemoprophylaxis, both during and after travel, increases the risk of breakthrough malaria. Antimosquito measures (screened rooms for sleeping, pyrethroid-impregnated bed nets and insect repellents) provide useful additional protection, and if they are used during travel in endemic areas they will decrease the risk of contracting malaria.

Specific exposures

Sometimes there are unique exposures that point to a specific diagnosis (Table 145.3). [2] [6] African tick typhus is strongly suspected if there is a history of a tick bite followed by fever, rash, regional lymphadenopathy and skin eschar. Unprotected sexual intercourse with commercial sex workers in high HIV seroprevalence areas in sub-Saharan Africa or parts of Asia may point to a diagnosis of acute HIV syndrome in a patient with a 'glandular fever'-like illness. Leptospirosis should be considered as a cause of fever in adventure travelers who have freshwater contact (such as rafting) in South East Asia.[12] It is also useful to find out whether the patient traveled in a party and if so whether other members have also been ill. There have been several outbreaks of acute schistosomiasis

TABLE 145-1 -- Common final diagnoses in the returned traveler with 'fever'.

COMMON FINAL DIAGNOSES IN THE RETURNED TRAVELER WITH 'FEVER'			
	Hospital for Tropical Diseases, London [9]	**McGill Centre for Tropical Disease, Montreal [4]**	**Hospital for Tropical Disease, London [5]**
Number of patients	523	587	195
Percentage with:			
Malaria	44	32	42
Hepatitis	11	6	3

Dengue fever	–	2	6
Typhoid fever	5	2	2
Respiratory infection	5	11	4
Urinary tract infection	2	4	3
Diarrheal illness	3	5	7
No diagnosis	7	25	25

TABLE 145-2 -- Usual incubation periods for selected infections.

USUAL INCUBATION PERIODS FOR SELECTED INFECTIONS	
Short (about 1 week or less)	• Arboviral infections (including dengue fever)
	• Enteric bacterial infections
	• Legionnaires' disease
	• Relapsing fever (*Borrelia* spp.)
	• Crimean-Congo hemorrhagic fever
	• Plaque
Intermediate (1–3 weeks)	• Malaria
	• Typhoid fever
	• Typhus
	• African trypanosomiasis (*Trypanosoma brucei rhodesiense*)
	• Viral hemorrhagic fevers (e.g. Lassa, Marburg, Ebola)
	• Brucellosis
	• Leptospirosis
Long (>3 weeks)	• Malaria
	• Viral hepatitis
	• Acute schistosomiasis
	• HIV infection
	• Miscellaneous infections (e.g. amebic liver abscess, brucellosis, melioidosis, visceral leishmaniasis, African trypanosomiasis (*Trypanosma brucei gambiense*), filarial lymphangitis)

('Katayama fever') among parties of nonimmune travelers after exposure to infected fresh water in sub-Saharan Africa.[13] A 'swimmers itch' may occur at the time of schistosomal cercarial penetration, followed several weeks later by a febrile illness associated with eosinophilia. In the spring of 2003, an acute febrile respiratory illness with sometimes severe pneumonia was described initially in southern China and Vietnam. The syndrome, severe acute respiratory syndrome (SARS) spread to all continents due to air travel and is associated with considerable morbidity and mortality. It is presumably of viral origin with the leading candidate being a member of the corona virus family.

Physical examination

Temperature patterns

The temperature and pulse rate must be measured initially and regularly thereafter. Some patients who are being investigated for fever are in fact afebrile or the fever may settle spontaneously

TABLE 145-3 -- Specific exposures that assist in the diagnosis of infections.

SPECIFIC EXPOSURES THAT ASSIST IN THE DIAGNOSIS OF INFECTIONS	
Exposure	**Infection or disease**
Raw or uncooked foods	Enteric bacterial infections, viral hepatitis
Untreated water	Enteric bacterial infections, viral hepatitis
Unpasteurized milk	Brucellosis, salmonellosis, abdominal tuberculosis
Fresh water swimming	Schistosomiasis, leptospirosis
Promiscuous sexual contact	HIV infection, viral hepatitis B, syphilis, gonococcal bacteremia
Mosquito bite	Malaria, dengue fever, filarial lymphangitis
Tick bite	Typhus, borreliosis, Crimean-Congo hemorrhagic fever, babesiosis
Louse bite	Typhus, borreliosis
Tsetse fly bite	African trypanosomiasis
Reduviid bug bite	Chagas' disease
Sand fly bite	Visceral leishmaniasis, arboviruses
Animal contact	Q fever, brucellosis, anthrax, viral hemorrhagic fevers, histoplasmosis, rabies, plague
Infected person contact	Viral hemorrhagic fevers (Lassa, Ebola, Marburg, Crimean-Congo), viral hepatitis, typhoid fever, meningococcal disease

before a diagnosis is reached. Such patients should be reassured and discharged because it is usually unrewarding to pursue investigations retrospectively.[9] For patients with a documented fever, the height of the temperature curve may assist in establishing a diagnosis. High temperatures above 106.8°F (41.5°C) are uncommon and may be caused by infections such as malaria, bacteremia, meningoencephalitis and typhoid fever or by noninfectious disease such as heat stroke, intracerebral hemorrhage and drugs.

Although fever patterns are not pathognomonic for particular infections, they can occasionally provide diagnostic clues:

¦ a pattern of short paroxysms of high fever accompanied by rigors and separated by periods when the temperature is normal (intermittent fever) is often a feature of malaria, pyogenic abscesses, miliary tuberculosis and bacteremia;
¦ paroxysms occurring every third or every fourth day occur typically with *P. vivax* and *P. ovale* (benign tertian malaria) and *P. malariae* (benign quartan malaria), respectively;

¦ a temperature that remains elevated throughout the day with minor fluctuations (continuous fever) may be caused by typhoid fever or typhus;
¦ dengue fever typically presents with 'saddle back' fever (i.e. two febrile periods separated by an afebrile interval of 1–3 days' duration, as shown in Fig. 145.1);

¦ several episodes of fever alternating with periods of normal temperature may occur in relapsing fever due to *Borrelia* spp.

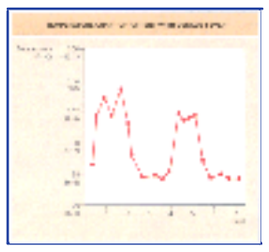

Figure 145-1 Temperature pattern in a patient with dengue fever.

For every 1.8°F (1°C) that the temperature rises above normal, the pulse rate rises by about 10–15 beats/minute. A slower pulse rate than is predicted from the temperature chart (relative bradycardia) is sometimes seen in typhoid fever, meningitis, brucellosis and Legionnaires' disease; it may also indicate factitious fever.

Physical signs

Physical findings in many tropical infections are non-specific and often overlap, although it can be rewarding to carefully examine the skin (Table 145.4), the liver, spleen and lymph nodes, and any other organ system associated with symptoms. Generalized lymphadenopathy is a feature of many infections, although a localized enlarged tender lymphadenopathy (bubo) suggests plague and tenderness over lymphatic vessels suggests filarial lymphangitis. Hepatosplenomegaly occurs in several tropical infections such as malaria, viral hepatitis and typhoid fever. Tender hepatomegaly suggests amebic liver abscess and splenomegaly of more than 10cm below the left costal margin suggests visceral leishmaniasis.

The absence of physical signs can be helpful. Malaria is an unlikely diagnosis in the febrile patient who has lymphadenopathy or a maculopapular rash.

Differential diagnosis by geographic area

Several infections have a worldwide distribution among tropical countries. These include malaria, dengue fever, hepatitis, typhoid fever, tuberculosis, HIV infection and amebic liver abscess. However, the risks of infection can be higher in certain geographic regions (see Chapter 142):

¦ among travelers from Europe and North America, malaria due to *P. falciparum* occurs most often after travel to sub-Sahara Africa, whereas malaria due to *P. vivax* is more common after travel to India and South East Asia;[14] [15]
¦ travelers to Central and South America are particularly at risk of dengue fever due to the growing incidence of the infection in this region;[16]
¦ although schistosomiasis occurs widely, Katayama fever in travelers is usually a result of exposure in sub-Saharan Africa;[17] and
¦ high HIV infection rates are found in commercial sex workers in sub-Saharan Africa and increasingly in India, Thailand and Cambodia;
¦ SARS in travellers to South East Asia.

TABLE 145-4 -- Skin lesions that provide diagnostic clues.

SKIN LESIONS THAT PROVIDE DIAGNOSTIC CLUES	
Skin lesion	**Infection**
Eschar	Tick typhus, scrub typhus, anthrax
Chancre	African trypanosomiasis
Rose spots on trunk	Typhoid fever
Urticaria	Acute schistosomiasis
Orbital edema (bilateral)	Trichinosis
Orbital edema (unilateral)	Chagas' disease
Maculopapular rash	Dengue fever, other arbovirus infections, viral hemorrhagic fever, typhus, leptospirosis, meningococcal bacteremia, syphilis, African trypanosomiasis
Petechiae and hemorrhage	Viral hemorrhagic fever, meningococcal bacteremia, louse-borne relapsing fever, louse-borne typhus

TABLE 145-5 -- Tropical infections with limited geographic distribution.

TROPICAL INFECTIONS WITH LIMITED GEOGRAPHIC DISTRIBUTION	
Region	**Infection**
Sub-Saharan Africa	• African trypanosomiasis
	• Ebola, Lassa and Marburg viral hemorrhagic fever
	• Specific arbovirus infections (e.g. Rift Valley fever)
	• African tick typhus
Asia and South East Asia	• Scrub typhus
	• Japanese encephalitis
South and Central America	• Chagas' disease
	• Specific arbovirus infections (e.g. Argentine hemorrhagic fever)

Some infections have limited geographic distribution (Table 145.5). In sub-Saharan Africa, trypanosomiasis has a sporadic distribution in rural savanna areas and game forests, so the safari enthusiast has the highest risk of acquiring this disease. African tick typhus due to *Rickettsia africae*[18] and Asian scrub typhus due to *R. tsutsugamushi*[19] are contracted by walking in grass or scrubby vegetation in the countryside.

DIAGNOSIS

Initial investigations include:

¦ thick and thin blood films for malaria parasites plus a simple diagnostic strip test (*Para*Sight F test) for *P. falciparum*,
¦ full blood count with differential white cell count,
¦ urinalysis, and
¦ chest radiograph (if indicated).

Thick and thin blood films plus simple diagnostic strip test for *Plasmodium falciparum*

Malaria is a great 'imitator' of other diseases and blood films should always be examined for malaria parasites if the patient has visited an endemic area. Thick films have greater sensitivity for identifying malaria parasites, whereas thin films are better for differentiating the species and, in *P. falciparum* infection, for estimating the degree of parasitemia. The films should be examined by someone who has relevant experience to avoid missing a low-grade parasitemia. Sometimes no parasites are

found in blood films as a result of ongoing malaria chemoprophylaxis, partial antimalarial treatment or sequestration of parasitized red cells in deep capillaries in the case of *P. falciparum* infection. A simple diagnostic strip test for *P. falciparum* malaria (*Para*Sight F test, Becton Dickinson Advanced Diagnostics), which detects a water-soluble antigen produced by the blood stages of *P. falciparum*, has high sensitivity and specificity for the initial assessment of *P. falciparum* in returning travelers.[20] The test does not remove the need for blood film examination but has a useful role in screening, especially where laboratory staff are not experienced in diagnosing malaria. If malaria is still suspected despite negative initial screening, blood films and a *Para*Sight F test should be repeated and smears of blood-stained tissue from an intradermal puncture may help in difficult cases (see Chapter 166). Hemolytic jaundice and thrombocytopenia are valuable pointers to malaria, although thrombocytopenia may also be due to an arbovirus infection or bacteremia.

Blood films are essential in the diagnosis of relapsing fever (*Borrelia* spp.) and acute infection with trypanosomiasis (*Trypanosoma brucei rhodesiense*). Spirochetes of *Borrelia* spp. are plentiful and easily recognized, whereas several blood films may need to be examined to find trypanosomes.

Differential white blood cell count

This simple investigation can be rapidly performed and the results help to focus the differential diagnosis and further investigations (Table 145.6).[21]

In patients who have a neutrophil leukocytosis, the additional findings of anemia, raised serum alkaline phosphatase and elevated right hemidiaphragm on chest radiography strongly suggest amebic liver abscess. Liver ultrasound may initially be negative, but the

TABLE 145-6 -- Differential white blood cell (WBC) count and diagnostic possibilities.

DIFFERENTIAL WBC COUNT AND DIAGNOSTIC POSSIBILITIES		
WBC differential	**Diagnostic possibilities**	**Further investigations**
Neutrophil leukocytosis	• Bacteremia	Blood culture
	• Deep sepsis	Ultrasound, CT scan
	• Amebic liver abscess	Ultrasound, serology
	• Relapsing fever	Blood films
	• Leptospirosis	Serology, culture of body fluids
Eosinophilia	• Schistosomiasis	Serology, microscopy of stool, urine, rectal snips
	• Fascioliasis	Serology
	• Trichinosis	Creatinine phosphokinase, serology
	• Lymphatic filariasis	Nocturnal blood film, serology
Leukopenia	• Brucellosis	Serology, blood and bone marrow culture
	• Disseminated tuberculosis	Biopsies (multiple sites), CT scans
	• Visceral leishmaniasis	Serology, bone marrow or spleen aspirate for amastigotes
Normal WBC count and differential	• Arbovirus infection	Serology
	• Typhus	Serology
	• Typhoid fever	Blood and stool culture

amebic immunofluorescent antibody test is very sensitive and usually positive in proven cases.

At the onset of acute schistosomiasis (Katayama fever), microscopy for ova may be negative and the eosinophilia may be mild, so a high index of suspicion may be required to make the diagnosis.

Tuberculosis is rarely contracted by short-term travelers. However, the risk increases with the duration of stay and the diagnosis must be strongly considered and aggressively investigated in immigrants and foreign students. Diagnosis can be difficult and in some patients with disseminated tuberculosis, the chest radiograph may remain normal until after treatment has started.

Blood cultures are often positive in the first week of illness in typhoid fever and in the second and subsequent weeks the stool cultures become positive. Previous treatment with antibiotics decreases the positive yield of blood cultures; in these cases, bone marrow culture may prove more sensitive.

Other investigations

If the initial screening tests prove nondiagnostic, further investigations will be needed and noninfectious diseases such as connective tissue disease, granulomatous disease and malignancy must be considered. In viral hepatitis, the liver function tests usually show a marked elevation of serum transaminases and these may also be mildly to moderately raised in malaria, typhoid fever and typhus. It is advisable to store an 'acute' serum specimen for antibody detection that can be used with a paired convalescent specimen at a later date.

MANAGEMENT

Recognition and treatment of malaria

The first step in management is to ensure that malaria due to *P. falciparum* is diagnosed and correctly treated (Table 145.7). Falciparum malaria in the returning traveler can be regarded as severe or complicated if:

- the patient is unable to take oral medication,
- there is any sign of specific organ failure,
- parasitemia is greater than 2%, or
- there is hypoglycemia.[22] [23]

If falciparum malaria is suspected in an ill patient but no malaria parasites are found, it is reasonable to treat the patient empirically

TABLE 145-7 -- Immediate management for the returned traveler who was fever.

IMMEDIATE MANAGEMENT FOR THE RETURNED TRAVELER WITH FEVER
Step 1: Blood films for malaria parasites (if from endemic area)
Step 2: Malaria parasites identified
• Uncomplicated falciparum malaria — treat with quinine sulfate and pyrimethamine-sulfadoxine **or** mefloquine **or** halofantrine **or** artemether
• Non-falciparum malaria — treat with chloroquine
• Complicated falciparum malaria — treat with parenteral quinine or quinidine together with pyrimethamine-sulfadoxine **or** artesunate **or** artemether
Step 3: Malaria suspected, patient ill, no parasites identified
• Treat as for complicated falciparum malaria — *Para*Sight F test useful for diagnosis of falciparum malaria and serology useful for retrospective diagnosis

• Blood cultures followed by antibiotics as for suspected bacteremia
Step 4: Possibility of viral hemorrhagic fever (VHF)
• Obtain expert advice
• If possibility of VHF is high, no further blood specimens or body secretions sent to the routine laboratory until VHF excluded
• If VHF confirmed, transfer patient to special isolation facility and perform all investigations in a high-security laboratory
Step 5: Clinical diagnosis made but unconfirmed by tests
• Perform appropriate investigations first and commence empiric treatment
Step 6: No clinical diagnosis and patient not seriously ill
• Avoid empiric treatment and perform appropriate investigations
Step 7: Exotic tropical infection identified
• Consider transfer to hospital facility specializing in tropical medicine

with parenteral quinine. Several sets of blood cultures should also be taken and antibiotics started as treatment for a possible bacteremia. Blood films for malaria parasites should be examined daily during treatment and other causes of fever rigorously pursued (see Chapter 166) .

Exclusion of viral hemorrhagic fever

The rare possibility of viral hemorrhagic fever should be considered in all febrile patients who have been in endemic areas during the 3 weeks before the onset of illness. Lassa, Ebola, Marburg and Crimean-Congo hemorrhagic fever viruses can all be transmitted from person to person through close contact with infected blood or other body secretions, so placing health and laboratory personnel at high risk. These infections are serious and cause a high mortality, although ribavirin (tribavirin) is useful in treating Lassa fever and Crimean-Congo hemorrhagic fever. [24] Clinical diagnosis is difficult as the differential diagnosis is wide (see also Chapter 183 and Chapter 186a).

Particular risk factors for travelers to endemic areas include:

- camping in the bush,
- staying in rural farming areas,
- contact with sick animals, and
- tick bites.

If viral hemorrhagic fever is suspected, it is vital to seek expert advice promptly. [25] Many such patients in fact have falciparum malaria, which in its own right requires rapid diagnosis and treatment.

Empiric treatment

If a clinical diagnosis has been made with or without initial screening tests, empiric treatment can be commenced while awaiting results of specific investigations. For example, in suspected typhoid fever, treatment can be commenced with a quinolone antibiotic after three sets of blood cultures and a stool culture have been obtained. African tick typhus can be diagnosed on clinical grounds and treated with doxycycline.

If no diagnosis has been made after the initial screening procedures and the patient is not seriously ill, empiric treatment is best avoided and investigations are continued as appropriate. For a deteriorating patient who has suspected disseminated tuberculosis, it is sometimes justified to start (and observe the response to) empiric therapy with specific antituberculosis drugs such as isoniazid, pyrazinamide and ethambutol while awaiting results of mycobacterial cultures.

Exotic tropical infections

Exotic tropical infections, such as African trypanosomiasis or visceral leishmaniasis, are uncommon and cases are best referred to specialist tropical centers because treatment is potentially toxic, of long duration and requires expert parasitologic monitoring.

Infection control procedures

Many tropical infections, such as malaria, dengue fever and typhus, can be managed without special isolation facilities. Typhoid fever, some enteric bacterial infections, viral hepatitis, HIV infection and plague can be transmitted from person to person, and appropriate measures must be instituted to prevent transmission. The rare viral hemorrhagic fevers are extremely dangerous and suspected cases should be managed in a high-security isolation facility (see Chapter 186a).

COMPLICATIONS

Many of the common infections in returned travelers do not cause long-term sequelae and are not transmitted to immediate family members. However, some arbovirus infections (including dengue fever) are associated with postviral fatigue syndromes, hepatitis B and hepatitis C can cause chronic active hepatitis and liver cirrhosis, and almost all patients infected with HIV will eventually develop features of AIDS.

If *P. vivax* or *P. ovale* malaria is not treated with an appropriate course of primaquine, relapsing malaria can occur for several years after leaving a malaria-endemic area, and trypanosomiasis with central nervous system involvement may relapse after inadequate treatment with melarsoprol B.

Some pathogens may be transmitted to family members either by the enteric route (e.g. *Salmonella typhi* in the chronic typhoid carrier, hepatitis A and E), by sexual intercourse (HIV, hepatitis B and C) and by nasopharyngeal spread (*Neisseria meningitidis*). In such cases, family members must be counseled and offered appropriate vaccination (hepatitis B) or chemoprophylaxis (*N. meningitidis*).

Viral infections are usually associated with prolonged immunity to the particular virus serotype, but most bacterial or parasitic infections in the short-term traveler confer no useful immunity for the future. Malaria acquired during last year's visit to Africa will not protect against malaria for next year's African safari!

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Chapter 146 - Coma and Confusion

Geoffrey Pasvol

INTRODUCTION

Although infrequent, the management of returning travelers with signs of confusion and/or any level of impaired consciousness, ranging from disorientation to coma, presents a particular challenge to the clinician.[1] [2] The management of any comatose patient is difficult because of the wide number of possible diagnoses (Table 146.1)[3] and because the urgency for diagnosis and specific treatment constitutes a medical emergency.[4] In addition to all the common non-infection-related etiologies, such as those associated with trauma (e.g. subdural hematoma), hyperpyrexia, alcohol poisoning, drug overdose, cerebrovascular lesions and diabetes mellitus, the differential diagnosis will also include more exotic and less well-known causes of coma that could result from the patient's travels. Travel can also be associated with many factors that could exaggerate and complicate an acute confusional state, such as alcohol intoxication or withdrawal, psychologic stress, sleep deprivation, jet lag and sensory overload. Confusion in elderly travelers is especially common, even in the presence of non-neurologic conditions such as pneumonia and urinary or biliary tract infections. The combination of air travel, dehydration, alcohol ingestion and relative hypoxia can be enough to induce confusion in elderly travelers.

Patients from tropical destinations may also present with multiple pathologies, for example an HIV-positive patient with *Salmonella* sepsis or a patient who has cerebral malaria and meningitis.

The most common consideration for any traveler returning from an area where malaria is endemic and presenting with a decreased level of consciousness is the possibility of malaria, although equally important diagnostic possibilities are meningitis and encephalitis. In any patient presenting with an alteration in conscious state, it is imperative to exclude malaria either as a cause or complicating factor by means of a thick and thin blood film.

CLINICAL FEATURES

For patients with confusion or coma it is important to obtain a history from accompanying travelers or attending ambulance or aircraft cabin crew (Table 146.2). It is important to establish whether the onset of confusion or coma was rapid or gradual, part of an ongoing known disease process, unpredictable in someone with a known underlying condition (e.g. epilepsy) or entirely unexpected. The patient may present with rambling speech, abnormally aggressive behavior, disorientation, impairment of memory, hallucinations or any decrease in level of consciousness, including coma. Fever or the history of fever suggest the presence of an infection.

Examination needs to be careful and thorough. The level of consciousness may be best monitored serially using the Glasgow Coma Scale.[5] Since the reticular activating system in the brain stem plays such an important role in consciousness level, elucidation of signs of brain stem involvement is essential. This requires careful attention to pupillary size and response to light. Any change might suggest uncal herniation as might occur in conditions that cause unevenly distributed changes in intracranial pressure. The oculocephalic ('doll's eye') and oculovestibular (elicited by instilling ice cold water into the external auditory meatus) responses may be tested to indicate whether the brain stem is intact. The corneal response is usually retained until coma is deep. Brain stem involvement may also manifest as an abnormality in respiratory pattern with tachypnea, long-cycle periodic breathing or Cheyne-Stokes respiration. This should not be confused with the deep sighing breathing of metabolic acidosis (Kussmaul's breathing) or that due to a respiratory cause.

Examination of the fundi is important, with special attention to the appearances of papilledema, hemorrhage and the changes of hypertensive or diabetic retinopathy.

Patients who have **malaria** have a history of travel to an endemic area, often with inadequate or no prophylaxis. They may present with complications, other than cerebral malaria, that may impair consciousness (e.g. repeated convulsions or even status epilepticus, metabolic acidosis, hypoglycemia, sepsis, respiratory involvement with hypoxia, renal failure and severe anemia; see Chapter 166).[6] A history of mosquito bites neither suggests nor refutes the diagnosis. The shortest possible incubation period for falciparum malaria is about 7 days and patients who have progressed to confusion or coma have usually been ill for a number of days, often with a flu-like illness.

In children it is important to distinguish cerebral malaria from transient postictal coma, which may last anything up to an hour after a fit. It is also important in cases of malaria to determine that the decreased level of consciousness is not due to sedative drugs (e.g. benzodiazepines) or hypoglycemia. In cerebral malaria the gag reflex is usually preserved. Grinding of the teeth (bruxism) has been observed in some patients. Examination of the fundus may reveal retinal hemorrhages, extramacular whitening and vessel changes in which the vessels turn white in isolated segments, especially at branch points.

Typhoid is suggested by travel to a highly endemic area, particularly the Indian subcontinent, and a compatible food history. Symptoms are predominantly abdominal, with a fever and a dry cough. The symptomatology is generally not abrupt and has an onset over a few days with increasing fever. The so-called characteristic features of typhoid, such as rose spots (Fig. 146.1), relative bradycardia, pea soup stools or constipation, may be absent. The early neurologic manifestations of typhoid are often subtle, with mild disorientation and a 'glazed', vacant look.

A wide range of **viral infections**, especially arboviral, may present with neurologic manifestations, including dengue fever, which is the most frequent in travelers. Patients with dengue may have a characteristic blanching erythematous rash, which is often confused with sunburn. In more severe infections there may be petechiae and even evidence of frank hemorrhage into the skin. West Nile virus more commonly causes fever, arthralgia and a rash rather than alterations in consciousness. Japanese B encephalitis is more dramatic and can produce a severe meningoencephalitis with altered sensorium. It can also cause a newly recognized polio-like acute flaccid paralysis syndrome.

TABLE 146-1 -- Causes of confusion and coma in a traveler.

CAUSES OF CONFUSION AND COMA IN A TRAVELER	
Infections	**Other**
Cerebral malaria	Drug-induced
Bacterial meningitis	Alcohol abuse
Viral meningitis	Cerebrovascular accidents, including subarachnoid hemorrhage
Encephalitis including arboviral such as:	
Dengue	
Japanese B	Head trauma
West Nile	Dehydration

Viral hemorrhagic fevers	Hepatic encephalopathy (e.g. hepatitis)
Lassa	
Marburg	Renal failure
Ebola	Hypoglycemia
Typhoid	Diabetic ketoacidosis
AIDS-related infections	Epilepsy and postepileptic states
Toxoplasmosis	Space-occupying lesions (especially abscess or tumors)
Cryptococcal meningitis	
Tuberculous meningitis	
AIDS dementia	
Protozoan infections	
Trypanosomiasis	
Toxoplasmosis	
Naegleria	
Acanthamoeba	
Relapsing fevers (Louse and tick-borne)	
Other spirochetal infections	
Leptospirosis	
Lyme disease	
Neurosyphilis	
Helminthic infections	
Schistosomiasis	
Cysticercosis	
Hydatid disease	
Strongyloidiasis	
Paragonimiasis	
Angiostrongylus	
Rabies	
Any severe infection, especially septicemia, endocarditis, pneumonia (especially Legionnaires' disease), pyelonephritis or cholecystitis	

Figure 146-1 Rose spots. In *Salmonella typhi* and *S. paratyphi* infections (enteric fever) classic rose spots of 1–3mm diameter can be found, especially on the abdominal wall, lower thorax and back of the trunk. These are small erythematous macular lesions, which tend to come and go during infection. *Courtesy of Anthony Bryceson.*

Incubation of the **viral hemorrhagic fevers** (which include Lassa, Ebola, Marburg and Crimean-Congo hemorrhagic fevers) ranges from 3 to 21 days. Symptoms include sustained fever, malaise and headache as well as muscle and joint pains. Nausea, diarrhea and vomiting may occur. In the case of Lassa fever there is often a history of travel to west Africa, contact with infectious patients and a characteristic illness beginning with pharyngitis and circumoral pallor. Ebola and Marburg diseases may produce a measles-like rash after 4–7 days. Overt bleeding is a late or terminal event in these infections.[7]

In **trypanosomiasis** there may be a history of travel to game parks in east Africa and a painful tsetse fly bite followed by a local chancre, a rash (Fig. 146.2) and lymphadenopathy.

Leptospirosis occurs worldwide, particularly in those participating in recreational water sports or working with animals. It is usually the patients with jaundice, conjunctival hemorrhage (Fig. 146.3) and renal failure that present with confusion.

Lyme disease due to *Borrelia burgdorferi* more commonly leads to fatigue and personality disorders than alterations in conscious level.

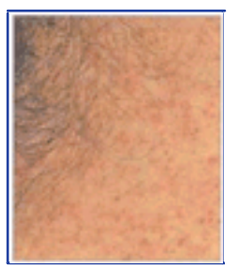

Figure 146-2 Trypanosomal rash. In fair-skinned individuals, each peak of fever may be accompanied by a remarkable skin eruption in the form of annular patches of erythema. In other cases, the rash may be more generalized, as seen here on the sixth day of an infection with *Trypanosoma brucei rhodesiense*. *Courtesy of Anthony Bryceson.*

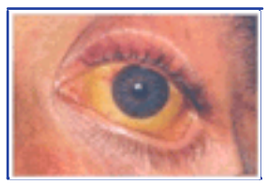

Figure 146-3 Subconjunctival hemorrhages and jaundice in leptospirosis. Asymptomatic or atypical infection probably occurs in 90% of cases and, in some tropical areas, leptospirosis may account for up to 15% of all patients with undiagnosed pyrexia. While this form can be mild, the infection may develop into a generalized septic form with confusion within 1–2 weeks. This is characterized by fever, myalgia and often subconjunctival hemorrhages. The patient illustrated was in the second week following the onset of symptoms. The most dangerous form (Weil's disease) may be very severe and can involve several organs, with jaundice, renal failure, haemorrhagia, vascular collapse and obtundation.

TABLE 146-2 -- Features suggesting the cause of confusion and coma in a traveler.

FEATURES SUGGESTING THE CAUSE OF CONFUSION AND COMA IN A TRAVELER	
Symptom or sign	**Possible diagnosis**
General	

Fever	Malaria
	Meningitis/encephalitis
Jaundice	Malaria
	Hepatic encephalopathy, especially hepatitis
Lymphadenopathy, oral thrush, shingles scar	HIV infection associated with cerebral toxoplasmosis, cryptococcal meningitis or tuberculous meningitis
Venepuncture marks	Injection drug abuse
Dehydration	Diabetic coma
Acidotic breathing	Severe malaria
	Diabetic ketoacidosis
	Renal failure
Sweating	Malaria
	Hypoglycemia
Rash	Meningococcal infection
	Dengue fever
	Louse-borne typhus
	Erythema migrans of Lyme disease
Game park visit and tsetse fly bite	Trypanosomiasis
Hyperpigmentation	Addisonian crisis (tuberculosis)
Drug history	Mefloquine or chloroquine
	Isoniazid
	Cannabis
	Other drug abuse (e.g. opiates, cocaine, barbiturates, amphetamines)
Recent head injury	Concussion
	Contusion
	Laceration
	Subdural hematoma
Fetor	Alcohol intoxication or withdrawal
	Diabetic ketoacidosis
Water contact	Leptospirosis
	Cerebral schistosomiasis
	Cysticercosis
	Cerebral hydatid
Cardiovascular	
Bradycardia (relative)	Typhoid
	Yellow fever
Irregular pulse, significant murmur	Cerebral embolus, endocarditis
Raised blood pressure	Hypertensive encephalopathy
Low blood pressure, postural drop	Hypoadrenalism
Respiratory	
Cyanosis, hypoxia	Respiratory failure
Respiratory distress	Severe pneumonia
	Severe malaria
Consolidation	Meningitis (including tuberculous)
	Cerebral abscess
Abdominal	
Pain, constipation, diarrhea	Typhoid
Jaundice	Hepatitis
	Leptospirosis
	Severe malaria
Neurologic	
Cuts, abrasions, fractures	Trauma
Neck stiffness, positive Kernig's or Brudzinski's sign	Meningitis
	Meningoencephalitis
	Subarachnoid hemorrhage
Seizures	Malaria
	Idiopathic epilepsy
	Focal neurologic lesions
Constricted pupils	Opiate drug abuse
	Organophosphate poisoning
Retinal hemorrhage	Cerebral malaria
Subhyaloid hemorrhage	Subarachnoid hemorrhage
Decerebrate/decorticate rigidity	Cerebral malaria
Focal neurologic deficits as suggested by hemiplegia, unequal pupil size, external ophthalmoplegia, asymmetric reflexes	Cerebral abscess
	Cerebral tumor (e.g. pituitary adenoma)

Relapsing fevers. In louse-borne relapsing fevers due to *Borrelia recurrentis* and tick-borne fevers due to *Borrelia duttonii* there may be a history of a visit to an endemic area and evidence of a bite that develops into an eschar associated with a more generalized rash. Tick-borne relapsing fever is usually milder than the louse-borne form.

Neurosyphilis patients can present with a history of a gradual onset of confusion.

Helminthic infections, which sometimes involve the central nervous system, need to be considered especially when the clinical findings suggest a focal lesion. These infections more commonly present with a fit than with confusion. They include neurocysticercosis (*Taenia solium*), hydatid disease due to *Echinococcus granulosis* or *Echinococcus multilocularis* and schistosomiasis. Less likely are *Strongyloides stercoralis*, *Trichinella spiralis* from undercooked

Figure 146-4 Meningococcal septicemia. Meningococcal infections are common in the tropics, most notably in the relatively dry 'meningococcal belt' of sub-Saharan Africa, stretching from Senegal and the Gambia in the west to Ethiopia in the east. The nonblanching skin rash and the petechiae and purpura are often difficult to see in individuals with pigmented skin, as is demonstrated in this patient.

meat (usually pork) and *Paragonimiasis* spp. from freshwater crustaceans.

Cerebral abscesses of whatever cause are another important differential in focal neurologic presentations. It is important to ensure that these do not relate to infective endocarditis.

Meningitis and encephalitis. If acquired when traveling, these infections do not necessarily differ from meningitis and encephalitis occurring elsewhere. The meningococcal rash is sometimes difficult to see in individuals with pigmented skin (Fig. 146.4). The specific cause of an encephalitis may be suggested by the region of travel (see below) and this applies especially to some of the less common causes of viral meningitides and encephalitides. Eosinophilic meningitis due to *Angiostrongylus cantonensis* (rat lungworm) is found in South East Asia, the Pacific Basin and more recently in the Caribbean. While most cases occur sporadically and are self-limited, outbreaks have been reported and neurologic sequelae and sometimes death do occur. Headache with raised intracranial pressure associated with paresthesias or hyperesthesias, and cerebrospinal fluid (CSF) pleocytosis, with or without eosinophilia, in a traveler should alert clinicians to the possibility of eosinophilic meningitis (Fig. 146.5). Amebic infection due to *Naegleria fowleri* (usually from freshwater) and *Acanthamoeba* spp. from brackish warm water can lead to a meningoencephalitis.

While **rabies** and **tetanus** usually occur in the setting of an individual who is conscious, brain stem rabies is an exception and can present with profound obtundation.

Drug (heroin, cocaine, barbiturate or amphetamine) **and alcohol abuse** are important considerations in the differential diagnosis in returning travelers who are confused. A history of personality change and self-neglect, together with sleep disturbance and poor appetite, are suggestive factors. Needle tracks, superficial thrombophlebitis or bruising are telltale signs. **Insecticide poisoning** is another possibility, which is particularly common in India and Sri Lanka. Occasionally **antimalarials**, most notably mefloquine and chloroquine, can lead to neuropsychiatric symptoms and manifest as confusion in a traveler.

It is important to determine whether the unconsciousness developed suddenly or gradually. The former might suggest a subarachnoid hemorrhage, whereas the onset of coma due to trypanosomiasis or an intracranial tumor is gradual except when there is hemorrhage into a tumor. The onset of coma in malaria may be heralded by a grand mal seizure.

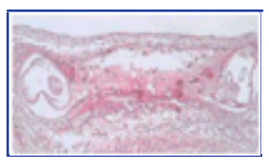

Figure 146-5 Section of *Angiostrongylus cantonensis* larvae in meninges of human brain. Humans may be infested by ingesting third-stage larvae in raw or inadequately cooked intermediate hosts such as snails, prawns, frogs or fish, or in contaminated salads. The larvae migrate to the brain, where they cause eosinophilic meningitis or meningoencephalitis. The diagnosis is aided by the serologic examination of paired specimens, using a specific antigen from adult worms. *Courtesy of Colonel JC Crook, RAMC.*

Symptoms and signs that may suggest the cause of confusion and coma in a traveler are given in Table 146.2 .

If there are any indications in the history of risk factors for HIV infection or any findings on clinical examination suggesting HIV infection, such as oral candidiasis, generalized lymphadenopathy or shingles, an HIV test should be done. This is important as the causes of coma, and therefore the clinical approach, are quite different in the HIV and non-HIV setting.[8] In an HIV-positive individual, diagnoses such as toxoplasmosis, tuberculosis, cryptococcal and coccidioidal meningitis, as well as HIV dementia, become important. A simple algorithm for distinguishing between some of the important diagnoses in the traveler with confusion or coma is shown in Figure 146.6 .

Differential diagnosis by geographic area

In attempting to distinguish the different causes of confusion and coma in the traveler, a clear history of the exact details of travel can be helpful and sometimes diagnostic.

The majority of cases of falciparum malaria are acquired in sub-Saharan Africa and the Indian subcontinent. A few cases are acquired in Papua New Guinea and Vanuatu and occasionally in South East Asia and South America. Knowledge of the rainy season in a particular area and the altitude of the places visited can also assist in the assessment of the risk of malaria.

Typhoid occurs worldwide, but especially in the Indian subcontinent.

The major endemic areas for louse-borne relapsing fever are the highlands of Ethiopia and Burundi. Tick-borne disease has a much wider distribution in both the Old and New Worlds.

Dengue fever is most frequent in travelers who have visited the Caribbean, Central and South America and most of Asia, but is also acquired in west Africa.

Lassa fever is endemic in rural west Africa. Most cases have been acquired by health care workers in the rural areas of Nigeria, Liberia and Sierra Leone.

Ebola virus disease has occurred in central (Zaire, Congo, Uganda, Sudan and Gabon) and west Africa (Ivory Coast).

Japanese B encephalitis is most commonly found in rural areas of Asia, tick-borne encephalitis virus in central Europe and Murray Valley encephalitis virus in Australia. West Nile virus is found across much of Africa, southern Europe and the Middle East. Recent outbreaks of West Nile encephalitis have occurred in several parts of the USA and in Israel.

Figure 146-6 Management of a confused or comatose traveler who has suspected viral hemorrhagic fever, malaria or an HIV-related problem.

Marburg virus disease has occurred in laboratory workers handling the African green monkey from Uganda. Crimean-Congo hemorrhagic fever has occurred in sporadic outbreaks in the areas indicated by its name (e.g. Greece, Turkey and Albania) and in east and west Africa, central Asia and the former USSR. Transmission is by tick bite.

Lyme disease occurs in the north east, mid-west and western USA and many parts of Europe, including Scandanavia.[9]

DIAGNOSIS

All patients who are in a coma require blood tests for glucose (and lactate), electrolytes, urea and creatinine, liver function tests, a full blood count, prothrombin time, a screen for drug abuse if suspected and, in certain cases, arterial blood gas measurement.

Malaria must be excluded for all patients with a fever (or a history of fever) who have visited a malaria-endemic area (see Chapter 166). The cornerstone in the diagnosis of malaria is provided by thick and thin blood films, which may need to be repeated if negative or if there is any doubt. Malaria can be responsible for coma even after the parasites have cleared, especially in the context of renal failure (Fig. 146.7).

In typhoid the most important investigations are blood, bone marrow and stool cultures.

For suspected trypanosomiasis, lymph node aspiration and examination of the blood and CSF for the parasite may be required.

Leptospirosis and Lyme disease are usually clinical diagnoses and are confirmed serologically.

Suspicion of the very many other causes of confusion and coma in a returning traveler will dictate specific further investigations.

Figure 146-7 Peripheral blood film of a patient with past malaria. The patient had returned 5 days previously from Malawi, having been treated there for malaria. At this stage no parasites were visible on the film. She was confused and delirious on admission and was found to have a profound acidosis (pH 6.98) and acute renal failure, thought to be due to the malaria. She was hypotensive, which suggested a secondary bacterial infection, and was found to have a *Salmonella* sepsis. Secondary bacterial infections are not uncommon in severe malaria associated with immunosuppression. The only evidence on blood film for malaria was the presence of malarial pigment (hemozoin) in many of the neutrophils, one of which is demonstrated here (arrow).

Computerized tomography

A contrast-enhanced computerized tomography (CT) scan of the brain should be carried out if there is any suspicion of raised intracranial pressure, as suggested by focal neurologic signs, which include abnormal patterns of respiration, loss of oculocephalic (doll's

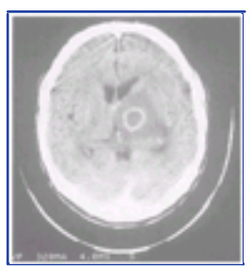

Figure 146-8 Toxoplasmosis. This patient presented to the hospital confused, with a right-sided stroke. An astute doctor requested an HIV test, which was positive. The CT scan carried out with injected contrast shows a typical ring-enhancing lesion in the left internal capsule with considerable surrounding edema. Further lesions were noted on different sections. The *Toxoplasma* IgG was positive and the patient made a full response to anti-*Toxoplasma* therapy.

eye) or oculovestibular (caloric) reflexes, or papilledema. A cerebral abscess, cerebrovascular event, subdural hematoma, subarachnoid hemorrhage or significant cerebral edema may be diagnosed in this way. If the patient has HIV infec-tion, features suggestive of toxoplasmosis (Fig. 146.8), a tuberculous lesion (Fig. 146.9), cryptococcal meningitis or cerebral atrophy may be detected. Space-occupying lesions such as those due to helminth infections can also be recognized by CT (e.g. cysticercosis (Fig. 146.10), schistosomiasis, hydatid disease, paragonimiasis, etc.), as can those due to abscesses, tumors or hemorrhage.

Lumbar puncture

For patients with fever and stable neurologic signs it is important to proceed with a lumbar puncture to exclude meningitis, encephalitis or hemorrhage into the subarachnoid space, which can be missed on CT. The CSF in malaria is usually normal apart from showing a raised lactate concentration. An India ink stain, or preferably an antigen agglutination test, is necessary to diagnose cryptococcal meningitis. In trypanosomiasis examination of the CSF is essential to determine whether there is central nervous system involvement with the presence of trypanosomes, leukocytes above 4/µl and/or protein above 0.4g/l. The CSF must also be examined to determine the extent of neurologic involvement in syphilis.

Magnetic resonance imaging

This is of particular value for a suspected demyelinating illness such as a viral encephalitis, for which the sensitivity of CT is limited, and in situations of brain stem or cerebellar involvement.

MANAGEMENT

Coma is a life-threatening condition until vital functions are stabilized and the underlying cause is identified and corrected. The first priority for a comatose patient is to preserve vital functions. Resuscitation may be necessary, paying special attention to airway protection, support of respiration and circulation, and the urgent

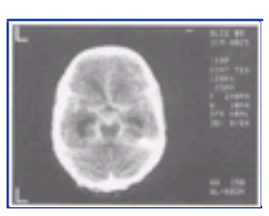

Figure 146-9 Tuberculous meningitis. A CT scan of the brain showing increased uptake of contrast around the vessels at the base of the brain in the circle of Willis in a patient with tuberculous meningitis. Obtundation in a patient presenting with tuberculous meningitis is an indication for the use of steroids.

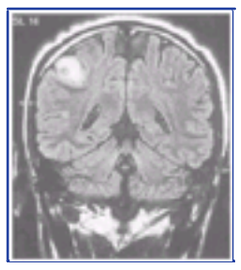

Figure 146-10 Coronal section of brain in cerebral cysticercosis. This magnetic resonance imaging scan shows a cyst with a surrounding white area of edema. While this 22-year-old man presented with focal convulsions of his left arm, edematous lesions placed more centrally, especially within the brain stem, can lead to both confusion and coma.

treatment of hypoglycemia after blood has been taken for various biochemical, hematologic, microbiologic and toxicologic tests. Other metabolic causes of coma other than hypoglycemia, such as acidosis, electrolyte abnormalities and drug intoxication, may need urgent attention. Once the patient is stable the next priority is to establish a diagnosis, which is critical if specific therapy is to be instituted.

All patients who have confusion or are comatose should be managed using the highest level of care appropriate for the particular health care setting. Unconscious patients may need to be moved to the intensive therapy unit. The risk of infection to others needs to be reviewed initially for all travelers, especially if there is any suspicion that the patient has a viral hemorrhagic fever or if he or she is to be moved to a ward area rather than to an isolation unit (see Chapter 186a). Until a diagnosis is made, drugs should be used sparingly. Haloperidol (5–30mg) or chlorpromazine (25–50mg) given intramuscularly may be used for restless or confused patients at risk of injuring themselves or attending staff and to allow diagnostic procedures such as imaging (CT or magnetic resonance imaging) or lumbar puncture. Haloperidol has fewer sedative and hypotensive effects and can be administered by different routes. Travelers with confusion will be in unfamiliar surroundings and are disoriented with regard to time, place and person. It is important that, during recovery, simple but firm communication as to the location and date, a visible clock and the presence of a relative are considered in the overall management of such patients.

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Chapter 147 - Skin Rashes and Ulcers

Roderick J Hay

INTRODUCTION

In most developing and tropical countries skin disease is the second or third most common reason for presentation in primary health care. The majority of the diseases that are seen are infective and easily treatable. It is therefore not surprising that people who have visited tropical areas should return with skin disease and are often alarmed that this may have been acquired during their stay overseas. It is important to consider the possible predisposing factors that may have affected the acquisition of skin disease.

PREDISPOSING FACTORS THAT POINT TO A DIAGNOSIS

Diseases endemic to specific geographic areas

As with other diseases, some skin conditions are confined to specific endemic areas. Most of these are infectious diseases and geographic localization depends on climate or the presence of an appropriate vector or host. Examples include onchocerciasis and cutaneous leishmaniasis, in which climatic factors and the appropriate vectors are both specific to certain regions. Endemic dermatoses are not necessarily restricted to the tropics — other infections show similar geographic restrictions. For example, erythema chronicum migrans, an annular erythema caused by primary infection with *Borrelia burgdorferi*, may be acquired during a visit to an area in the USA, Scandinavia or Central Europe, where Lyme disease is endemic.[1]

Activities that predispose to skin disease

Certain activities expose travelers to unusual environments that may affect the development of skin disease. These vary from overland trekking through desert or rainforest to sunbathing on the beach. The beach, in particular, is a potential source of skin diseases, including those due to sun exposure (e.g. discoid lupus erythematosus, polymorphic light eruption) as well as the acquisition of larva migrans from sitting in contaminated sand. Trekking exposes the traveler to other noninfectious hazards, such as contact with plants that may cause allergic or irritant contact dermatitis. Under this heading, reactions to drugs, such as antimalarials, that have been taken during the trip should also be considered.

Climate

Climatic conditions also affect the development of skin disease. Factors identified as important include increased ambient temperature or high levels of atmospheric humidity, both of which have an effect on bacterial growth on the epidermis. Infections due to *Staphylococcus aureus* are much more common in hot and humid climates than in colder climates. Some other infections, such as pityriasis versicolor, are also more frequent in hot and humid environments.

Table 147.1 shows a list of additional questions that are important in taking a history from a patient returning from a trip overseas.

MAKING A DIAGNOSIS

It is important to differentiate between:

- skin diseases in travelers that were already present before the patient left home and skin diseases to which the patient is susceptible and that may have been exacerbated during travel;
- skin diseases that have been caused by exposure to a different climate or environment; and
- newly acquired skin diseases that are normally confined to the area visited.

Skin disease seen in travelers can therefore be considered under the following three main headings.

Conditions made worse by travel

Almost any pre-existing skin condition can be affected by travel, although certain conditions are more likely to present for treatment on return. These include acne vulgaris, which is often considerably worsened in a tropical environment with rapid spread of new pustular lesions. Pre-existing photosensitivity (which includes polymorphic light eruption, discoid lupus erythematosus and solar urticaria) is also exacerbated by sun exposure. Atopic eczema behaves differently in different environmental conditions. For instance, it may be improved by exposure to mild sunlight and sea-bathing, but it is usually made worse by very hot and humid conditions. It is also exacerbated by travel to very cold areas, because this increases skin dryness.

Some forms of dermatophytosis are also more common in the tropics and patients who have pre-existing tinea pedis, for instance, often have an exacerbation when visiting a hot climate; the infection may spread beyond the initial site to affect other areas such as the groin or trunk.

Conditions acquired by exposure to different environmental conditions

Although it is always possible for patients to acquire new skin diseases under different environmental conditions (e.g. light-exacerbated psoriasis), the common presentations are very specific to travel.

Pityriasis versicolor is a common superficial fungal infection that is caused by *Malassezia* yeasts, which are normal commensals on the skin surface.[2] They become pathogenic under certain conditions, one of which is climatic change and sun exposure. The condition presents with widespread scaly hypopigmented or hyperpigmented macules, which tend to become confluent on the trunk and neck (Fig. 147.1). It is not itchy and discoloration is the usual reason for seeking medical help, often a few weeks after return from overseas. Although the definitive diagnosis is best made by microscopic examination of skin scrapings, a simple measure is to demonstrate the presence of fine scales by scratching the lesions. The treatment is ketoconazole shampoo, topical azole antifungal creams (e.g. clotrimazole, miconazole) or oral ketoconazole (200mg daily for 3–5 days) or itraconazole (200mg daily for 5 days). There is no reliable way of preventing this infection, although some habitual sufferers treat themselves

TABLE 147-1 -- Additional useful questions in taking a history from travelers with skin lesions.

ADDITIONAL USEFUL QUESTIONS IN TAKING A HISTORY FROM TRAVELERS WITH SKIN LESIONS
What countries have you visited during your vacation or trip?

Try to find out where they have been within these countries and any potential exposures (e.g. camping by a fast-flowing river in an area endemic for onchocerciasis). Also, it is important to find out whether they were traveling rough or using luxury hotels — there is sometimes a difference in disease exposure.
Before you went overseas did you have any skin problems?
Find out about potential reactions to sunlight and atopic disease, acne, etc.
What sort of things did you do on your trip — bathing in the sea or lake, trekking, sunbathing on a beach?
Were you taking any medications during your stay overseas?

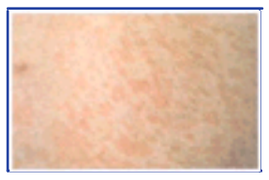

Figure 147-1 Pityriasis versicolor. The rash shows confluent scaly macules.

during their vacations with a single application of selenium sulfide or ketoconazole shampoo.

A related condition, often misdiagnosed as acne, is *Malassezia* folliculitis, which characteristically presents with small itchy pustules on the shoulders and upper chest after sun exposure.[3] The distribution and the itching, as well as the absence of comedones, distinguish this condition from acne. It too responds best to oral itraconazole or ketoconazole.

Infections acquired specifically overseas

Although the common infections seen in tropical communities are scabies, bacterial pyoderma, pediculosis and fungal infections (Table 147.2), they are not as common in travelers as in local people. They are usually acquired as a result of close contact (rather than casual contact) with infected people and they are particularly associated with overcrowding in houses. Likewise, tropical ulcer (see below), which is very common in certain areas of the tropics, is seldom seen in visitors unless they stay for long periods and share the living conditions of the local inhabitants.

Specific examples of conditions acquired overseas are discussed below.

INSECT BITE REACTIONS

Most people become tolerant of the biting insect fauna of their local area; for example, reactions to mosquito bites decline with increasing age owing to sensitization to mosquito salivary antigens.[4] (There

TABLE 147-2 -- Skin symptoms and signs in travelers and potential infective causes.

SKIN SYMPTOMS AND SIGNS IN TRAVELERS AND POTENTIAL INFECTIVE CAUSES
Generalized itching
Scabies
Schistosomiasis
Localized itching
Onchocerciasis
Abscesses
Staphylococcus aureus infection
Melioidosis
Nodules
Rations to insect bites
Larva migrans
Dirofilariasis
Onchocerciasis
Tungiasis
Leishmaniasis
Urticaria
Strongyloidiasis
Schistosomiasis
Ulcers
Insect bites
Leishmaniasis
Streptococcal ecthyma
Corynebacterium diphtheria
Tungiasis
Tick-bite fever
African trypanosomiasis
Scaly rashes
Dermatophytosis (e.g. tinea imbricata)
Annular erythema
Borreliosis

are exceptions to this in people in whom severe hypersensitivity persists.) However, exposure to a new set of antigens from mosquitoes has been associated with an increased level of sensitivity and it is therefore not surprising that insect bites are a very common cause of complaint in travelers. The range of clinical manifestations of bite reactions includes small papules, blisters, dermal nodules and small ulcers. In particular, it is not uncommon for these to become secondarily infected by *Streptococcus* spp. or *Staphylococcus aureus*, which make lesions become more indurated and weepy.

Clues to the diagnosis of prolonged bite reactions are the presence of multiple itchy lesions, in some cases clustering on exposed sites, and a history of evolution over a few days. It is important, though, to exclude secondary infection and treat with appropriate antibiotics. In some cases, cutaneous leishmaniasis may present with indurated itchy papules and nodules that may mimic bites where, of course, they originated. If in doubt, a skin biopsy is often helpful, because a dense polymorphic

cellular infiltrate in the debris with large numbers of eosinophils is highly suggestive of a bite reaction.

Other common bite or sting reactions that may be found in travelers, apart from scorpion sting and snake bite, which require more immediate attention, include jellyfish stings and sea urchin granulomas.

Jellyfish stings[5] usually present with a linear rash on an exposed area. The rash typically consists of multiple papules or small bullae. However, patients usually know what has caused this reaction because the symptoms are virtually instantaneous. Application of cool packs or vinegar may provide immediate relief. In severe cases, such as those due to the Portuguese man-of-war jellyfish, stings may be accompanied by systemic symptoms such as fever, diarrhea and cardiovascular shock.

The spines of sea urchins cause localized clusters of small dermal granulomas. The presence of spines can, if necessary, be confirmed by radiograph. However, in addition, sarcoid-like granulomas that do not contain spines may form in the area of the puncture wound; these persist for months. Recent studies suggest that a small proportion of such wounds may be infected with *Mycobacterium marinum*.[6] Once again, patients know the cause of a sea urchin injury but may delay consultation until their return from overseas. The main treatment is removal of spines if these are still present.

This may be difficult if much time has elapsed after the injury, and broad-spectrum antibiotic cover is also advisable.

Tunga penetrans (the jigger flea) may cause a localized tender nodule on the feet and toes. It may ulcerate and is usually itchy. It is usually covered with a small crust or pustule but where this is carefully removed the presence of a dark posterior segment can be seen. Treatment is by covering the lesion with Vaseline and once the larva appears it can be removed by forceps; alternatively excision after exploration is usually curative. Myasis or infestation with fly larva can also occur and although this affects other sites, the presentation, a tender nodule or ulcer, is similar.

BACTERIAL INFECTIONS

These are not common in travelers except when they are secondary to some pre-existing skin lesions such as insect bites or varicella.[7] Cutaneous diphtheria is still possible in patients who have been traveling rough in some areas such as the Saharan region, and it should be considered in those presenting with ulcers that are covered with a grayish slough. Tropical ulcer, a synergistic bacterial infection, is rare in travelers.[8] One condition that is seen regularly in those working or vacationing in some parts of the Mediterranean region or Africa is the initial ulcer of tick bite or boutonneuse fever. This is caused by *Rickettsia conori*, which is endemic in eastern Africa and some Mediterranean countries.[9] The presence of a necrotic localized ulcer, and often a fine macular rash, in a patient who has an undiagnosed fever is highly suggestive; patients can sometimes describe the tick that caused the reaction. The treatment is with doxycycline (200mg daily for 7 days).

FUNGAL INFECTIONS

Most of the unusual endemic fungus infections are exceptionally rare in travelers, although existing infections may be exacerbated.[10] However, two fungal infections that may occur are *Scytalidium* infections and tinea imbricata.

Scytalidium dimidiatum, a plant pathogen, and *S. hyalinum* are both found in a wide range of tropical environments and both cause superficial infections that mimic those caused by the dermatophyte *Trichophyton rubrum*. Although they are not common in travelers, they occasionally cause nail infections which in Caucasians may present with patchy melanonychia (black discoloration of the nail-plate; Fig. 147.2). [11] Treatment is difficult, because neither of these infections responds to conventional antifungal agents.

Trichophyton concentricum is a dermatophyte that causes the infection tinea imbricata in remote areas of the Western Pacific and in Central and South America. It may occur in travelers, particularly if they have been traveling rough. It presents with very typical

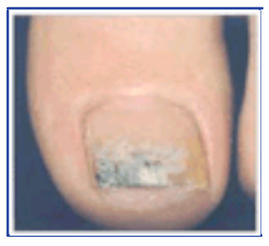

Figure 147-2 Onychomycosis caused by *Scytalidium dimidiatum* in a traveler.

lesions with concentric rings of scales.[12] It responds best to oral terbinafine (250mg daily) or itraconazole (200mg daily).

PARASITIC INFECTIONS

The common diseases of the developing world, such as scabies and pediculosis, are also seen in travelers. A variety of parasitic diseases are seen in those spending short periods in the tropics.

Cutaneous leishmaniasis

This is discussed in detail elsewhere (see Chapter 172). Leishmaniasis can affect travelers who have visited a wide range of countries in the Middle East, Africa and Central and South America. It is also seen in certain areas of Europe around the Mediterranean such as northern Greece, Turkey, Spain and the Balearic Islands, although it is not common in these areas. [13] [14]

Patients present with either single or multiple lesions. These range in morphology from indurated nodules to ulcers. Occasionally they form a chain of nodules along a lymphatic vessel, as in sporotrichosis (see Chapter 13). The typical history is that they appear like an insect bite that persists after return home. It is important to take a biopsy of such lesions.

Onchocerciasis

Onchocerciasis (see Chapter 170 and Chapter 174) may appear in those traveling to endemic areas of Africa, Central and South America and the Yemen.[15] The usual skin presentation of onchocerciasis in the traveler is acute papular onchodermatitis[16] with localized areas of small itchy papules that are often confined unilaterally to a limb or the waist or shoulder region. There is also a form of dermal edema that resembles *peau d'orange* (Fig. 147.3). The diagnosis is made by demonstrating microfilariae in skin snips. The skin changes in travelers are often very subtle and the presence of localized itching and a history of travel, often involving camping by a river, are helpful clues.

Cutaneous larva migrans

Cutaneous larva migrans (creeping eruption; see Chapter 174) is not uncommon in travelers, particularly those who have visited tropical beaches, and is caused by skin infection with animal hookworm larvae such as *Ancyclostoma brasiliense, A. caninum* and *Uncinaria stenocephala*. Often, patients have returned from a vacation during which they have sat on the beach. Lesions present with an itchy

Figure 147-3 Acute papular onchodermatitis. There is a combination of dermal edema and a papular rash.

cluster of lesions on an exposed site such as the legs or buttocks. The morphology of the lesions varies. In some cases there is a typical sinuous track, but in other cases the lesions are nodular but grouped in a specific area. In cases where the lesion is clearly worm-like, diagnosis is easy; otherwise it may be necessary to biopsy lesions. Treatment with albendazole (400mg daily for 3 days) is usually curative. [17]

Other parasites

Other tropical parasites very occasionally cause imported infections in travelers.

Cercarial dermatitis

Cercarial dermatitis[18] presents as an itchy rash that often occurs after swimming. Patients describe intense itching after exposure to water, sometimes accompanied by papules or small weals. Although it is usually a harmless symptom caused by contact with avian cercaria in areas that are endemic for schistosomiasis, it can be an early symptom of exposure to pathogenic schistosomes.

Katayama fever

In patients returning from areas that are endemic for schistosomiasis, the development of urticaria, joint pains and fever should be regarded with suspicion, because it is an early sign of sensitization in the early stages of schistosomiasis. [19] These symptoms usually occur several weeks after exposure.

Dirofilariasis

This occurs in several regions — Africa, the Far East, South America and the southern USA. The cutaneous infection is due to *Dirofilaria* species such as *D. tenuis* or *D. ursi*. Patients present with a localized itchy papule or nodule, which is often near the eye, elsewhere on the face or on the chest.[20] The diagnosis is made by biopsy.

Other important parasitic infections such as dracunculiasis, loiasis and lymphatic filariasis are uncommon in travelers, but have all been reported occasionally.

DRUG REACTIONS

Many patients take medications during trips overseas, including antimalarial agents, antidiarrheal agents and antihistamines. All may produce skin lesions; some drugs (e.g. tetracyclines) may cause photodermatitis. It is important to take an adequate drug history in those returning from overseas. The most common cause of drug rashes in this group are the antimalarial agents:

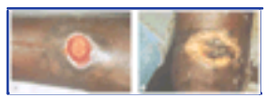

Figure 147-4 Tropical ulcer. (a) Acute. (b) Chronic.

- chloroquine can cause a range of different rashes, but the main reactions are lichenoid (lichen planus-like) reactions and pruritus;
- mepacrine causes a diffuse yellowish pigmentation of the skin;
- mefloquine can cause pruritus and urticaria;
- pyrimethamine combined with sulfodoxine can cause urticaria, erythema multiforme and toxic epidermal necrolysis — it is thought that the skin reactions are largely due to the sulfonamide component; and
- quinine can cause erythema and flushing and pruritus.

It is important to obtain a drug history to ensure that all medications, including antimalarial agents, that were taken during the trip are considered in making the diagnosis of any skin rash.

ULCERS

Ulceration of the skin is a common and important physical sign. Diagnosis of ulcers depends on the underlying clinical situation, the evolution of the condition, the area affected and the appearance. Many of the conditions discussed above may develop into small ulcers. These include leishmaniasis, insect bites and boutonneuse fever. However, there are others that are important not to miss.

Tropical ulcer

Tropical ulcer is a common condition in remote parts of the tropics mainly seen in children and teenagers and affecting the lower limbs.[8] The lesion usually starts with mild discomfort and overlying hyperpigmentation on the skin that progresses over a few days until the skin breaks down and sloughs revealing an underlying ulcer. The ulcer, therefore, is often described as occurring rapidly.

Tropical ulcer is mainly seen in Africa, India, the West Pacific and part of Indonesia and the Philippines. The disease is due to a combined infection by a number of different bacteria together with a fusiform bacterium, *Fusobacterium ulcerans*, and an as yet unidentified spirochete. The disease is associated with poor living conditions and exposure to stagnant water and mud. *Fusobacterium ulcerans* has been isolated from mud in endemic areas.

The lesion is often clean on first presentation, round, with smooth edges (Fig. 147.4a). It generally starts on the lower leg or ankle and in about 10% of cases it progresses to become an irregular, enlarged and chronic ulcer (Fig. 147.4b). The condition heals well in most patients with simple cleansing and treatment with penicillin. However, early grafting may be necessary where healing is delayed.

The differential diagnosis of tropical ulcer includes yaws, diphtheritic ulcer and leishmaniasis.

- The primary lesion of yaws is usually more exophytic and appears to stand out from the skin. It may then ulcerate before the development of secondary lesions. Although it was once controlled, yaws has resurfaced in some of the previous endemic areas of West Africa and the West Pacific, areas where tropical ulcer may also occur.
- Diphtheritic ulcer is uncommon but the lesion is more irregular and the base is covered with a gray to yellow slough. It is more often seen in desert or semidesert areas.
- Leishmaniasis can be distinguished by its slower speed of onset with a skin nodule often with surrounding plaque breaking down to produce ulceration. Inflammation around the ulcer is more intense.

Overall tropical ulcer is unlikely to occur in the traveler unless the area visited is remote and the patient has lived under local conditions.

Buruli ulcer

This condition is not often seen in travelers although it is sometimes considered.[21] Once again, it occurs in the remote parts of Africa, particularly Central and West Africa, and the West Pacific. Cases have been reported from Australia. It is caused by *Mycobacterium ulcerans* and usually affects children and young adults. Like tropical ulcer, there is an association with exposure to water. The ulceration starts as a small raised nodule that breaks down to develop into an extensive undermined and irregular ulcer that may extend for 10 cm or more. Treatment is largely surgical. The undermining is characteristic.

Other ulcers seen in travellers

Mycobacterium marinum causes ulceration of the skin preceded by the development of a nodule. In many cases it causes a characteristic form of spread involving local lymphatics (sporotrichoid or lymphangitic). The primary ulcer is followed by secondary nodules that may break down and discharge. While typically this is an infection seen in those handling tropical fish at home, it can be acquired naturally by swimming in shallow coastal waters. *Mycobacterium marinum* is a natural pathogen of fish.

Streptococcal ecthyma is also seen frequently in the tropics. This is a more severe form of impetigo where the whole epidermis and part of the dermis is involved in necrosis to give 1–2cm punched-out ulcers. It is often a secondary infection (e.g. on an insect bite or following chickenpox). It responds slowly to penicillin. It may be seen in returned travelers at the site of scratches or bites.

There is no reason why travelers cannot develop the same types of ulceration abroad as at home and the differential diagnosis should include:

- stasis, arterial or diabetic ulcers;
- ulcers due to vasculitis such as polyarteritis nodosa; and
- hemoglobinopathy-associated ulcers (e.g. sickle cell disease).

Taking a thorough history of the time course of onset of ulceration and any relevant background information is very important. Where necessary, it may also be useful to biopsy the edge of ulcers as this may provide further diagnostic information. Histology from the central area is only rarely useful.

CONCLUSION

Skin disease is common and patients not infrequently present with lesions that appear to have been acquired during the course of foreign travel. In making the diagnosis, it is important to remember that the majority of such cases are due to skin conditions that are just as likely to occur at home as overseas. However, in some patients, the condition is a genuine imported infection that has followed exposure during the course of travel.

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Chapter 148 - Sexually Transmitted Diseases

Mark W Tyndall

INTRODUCTION

The incidence of sexually transmitted diseases (STDs) in the returned traveler is not known as reporting systems are sporadic, diagnosis is difficult and self-treatment is common. The risk of STD is directly related to the frequency of sexual contact, choice of sexual partner and type of exposure. Despite considerable risk, surveys conducted among travelers show that up to 25% have new sexual partners, contact with commercial sex workers is common, condom use is inconsistent and perceived risk of infection is underestimated.[1] [2] [3] [4] [5] [6]

Increased international travel through tourism, business and migration has dramatically changed the patterns of STD transmission.[7] [8] [9] [10] Many of the most popular travel destinations in Africa, Asia and the Caribbean are regions with high STD prevalence, high rates of prostitution and little capacity to implement effective STD control. The AIDS epidemic tragically illustrates how the spread of sexually transmitted pathogens transcends international borders and frustrates control efforts.[11] [12] [13] [14]

PREVENTION

Prevention of STDs among travelers is of the highest priority and should be discussed prior to departure. Contracting an STD pathogen while traveling may have serious short-term and long-term implications. Counseling should include the promotion of responsible sexual behavior, including abstinence, condom use and avoiding commercial sex. Prevention efforts to date have been inadequate as shown in surveys of both departing and returning travelers.[3] [4] [5]

CLINICAL FEATURES

Sexually transmitted disease pathogens can broadly be divided into those that cause nonulcerative lesions and those that cause ulcerative lesions. For clinical management, the nonulcerative pathogens can be further divided into anatomic sites. The most common presentation for men is urethritis, followed by epididymitis, prostatitis and proctitis. Infection involving the lower genital tract is the most common presentation in women and includes cystitis, vulvitis, vaginitis, urethritis and cervicitis. HIV infection, multiple concurrent STDs or other infectious complications, however, may alter this simplified clinical approach.

Urethritis in men

The etiology of urethritis in men has traditionally been divided into gonococcal and nongonococcal infections. This classification was developed due to the characteristic presentation of *Neisseria gonorrhoeae*, which is generally symptomatic and involves profuse, purulent, urethral discharge, occurring 2–5 days following sexual contact. The less severe clinical presentation of mucoid discharge and/or dysuria has been associated with nongonococcal infections, which include *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma genitalium* and *Trichomonas vaginalis*. These infections have longer incubation periods of 1–6 weeks. Nongonococcal urethritis is more often asymptomatic (10–30%) and may therefore go undiagnosed. Where available, etiologic diagnostic testing should be performed due to the poor sensitivity and specificity of a clinical diagnosis.

Mucopurulent cervicitis

Most serious infections of the lower genital tract in women involve *N. gonorrhoeae* and *C. trachomatis*, either of which may result in ascending infections with severe consequences. These organisms are clinically indistinguishable and cause urethritis and/or cervicitis. Vaginal discharge and dysuria are the most common clinical presentations, but are extremely variable and the conditions are easily confused with vaginitis. Asymptomatic infections are common (<50%) and may only be detected when complications occur. Herpes simplex virus is also associated with inflammation of the urethra and cervix, but does not result in ascending infections.

Other causes of vaginal discharge

The major causes of vaginitis are *Trichomonas vaginalis, Candida albicans* and bacterial vaginosis. These are difficult to distinguish clinically and may occur together. Vulvitis and cystitis also present with lower genitourinary tract symptoms and must be distinguished from vaginal infections.

- ¦ Trichomoniasis typically presents with profuse, yellow-green, frothy vaginal discharge with erythema of the vaginal wall.
- ¦ Candidiasis presents with pruritus and white curd-like discharge. It is extremely common in women during child-bearing years and the role of sexual transmission remains controversial.
- ¦ Bacterial vaginosis presents with malodorous white vaginal discharge and is characterized by a change in normal vaginal flora. This process is likely multifactorial and, despite being a common diagnosis at STD clinics, it is not considered to be sexually transmitted.
- ¦ Vulvitis may be due to *C. albicans*, herpes simplex virus (HSV) or human papillomavirus (HPV). Pruritus, burning discomfort, edema and erythema are the common manifestations. In cases of HSV and HPV infection, characteristic lesions may be seen on close examination.
- ¦ Cystitis, which may be related to sexual activity, can usually be excluded from sexually transmitted infections by history and urine culture.

Other nonulcerative sexually transmitted diseases

Other nonulcerative STDs that may be seen in the returned traveler include:

- ¦ genital warts, caused by HPV types 6 and 11, which affect genital and anal regions and can range from asymptomatic lesions to large confluent masses;
- ¦ molluscum contagiosum, which is a benign, self-limited, papular condition affecting the skin and mucous membranes, with lesions characterized by central umbilication;

- ¦ public lice, caused by *Phthirus pubis*, which present with itching and inflammation in the pubic area. The lice can usually be seen by the naked eye and infestation is usually self-limited; and
- ¦ scabies, caused by *Sarcoptes scabiei*, is commonly sexually transmitted and may lead to severe inflammation and excoriation in the genital region, mimicking other infections.

Genital ulceration

Genital ulcer disease (GUD) is most commonly caused by HSV, syphilis or chancroid (Fig. 148.1), although in some regions donovanosis and lymphogranuloma venereum should be considered. Occasionally scabies, trichomoniasis and nonsyphilis spirochetes may present as genital ulcers. The differential diagnosis also includes

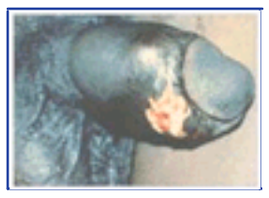

Figure 148-1 Chancroid. Characteristic purulent lesion with ragged borders, friable base and surrounding inflammation.

TABLE 148-1 -- Clinical features of genital ulcer disease.

CLINICAL FEATURES OF GUD		
Infection (pathogen)	**Incubation period**	**Clinical features and natural history**
Syphilis (*Treponema pallidum*)	Primary (14±28 days)	Single, rounded, well-defined borders, nontender, indurated base; may occur in extragenital sites; resolves spontaneously in 3±6 weeks
	Secondary (1±12 weeks following primary lesion)	Systemic features; papular rash classically affecting palms and soles; condylomata lata in moist areas
	Tertiary (uncommon) (years)	Neurosyphilis; cardiovascular syphilis; late benign syphilis
Chancroid (*Haemophilus ducreyi*)	4±10 days	Single or multiple, deep, painful, ragged borders; inguinal swelling (bubo) occurs in 30% and may suppurate; progressive and destructive
Herpes (HSV-2)	2±7 days	Multiple vesicular ulcers, which break down; superficial and painful; resolve spontaneously in 1±3 weeks, but may recur
Granuloma inguinale or donovanosis (*Calymmatobacterium granulomatis*)	1±10 weeks	Single or multiple elevated, painless, beefy red; progressive verrucous lesions; systemic illness
Lymphogranuloma venereum (*Chlamydia trachomatis* serovars L1, L2, L3)	Primary disease (3±12 days)	Penile and urethral which resolves spontaneously in days; urethritis
	Secondary disease (10±30 days)	Lymph node disease; persistent and destructive; inguinal swelling (groove sign); proctitis, lymphangitis; sinus formation; systemic illness

noninfectious causes such as a fixed drug eruption, Behçet's syndrome, Reiter's syndrome, trauma and malignancy. Table 148.1 describes the classic clinical presentation of the major infectious etiologies but, as with other STDs, there is considerable variation in presentation and mixed infections are common.[15] [16]

SEXUALLY TRANSMITTED DISEASES WITH THE GREATEST GLOBAL SIGNIFICANCE

The persistent viral STDs, which include HIV-1, HIV-2, human T-lymphocyte leukemia/lymphoma virus-1 (HTLV-1), cytomegalovirus, HSV, HPV and hepatitis B and hepatitis C viruses, represent the most serious long-term health risks for the sexually active traveler. Ironically, most of these infections do not present with genital symptoms. Table 148.2 shows the estimated sexual transmission efficiency and clinical manifestations of these viral agents. There is convincing evidence that the transmission of these viral pathogens is facilitated by other genital infections, both ulcerative and nonulcerative. [17] [18] [19]

Historically, the complications of STDs have primarily affected women, manifesting with severe local disease, pelvic inflammatory disease, infertility and complications in pregnancy. Although this remains true, the persistent viral infections pose serious long-term risks for both men and women:

- HIV infection continues as a global pandemic with heterosexual transmission being the predominant route of infection;
- HPV is widespread and has resulted in high rates of cervical cancer, especially among women in developing countries;
- hepatitis B produces chronic liver disease and liver cancer and the role of sexual transmission is important;
- sexual transmission of hepatitis C virus appears to be less efficient than that of hepatitis B virus, but does occur;
- herpesviruses type 1 and type 2, and cytomegalovirus are now widespread global infections with major implications in the immunocompromised; and
- the implications of retroviruses (e.g. HTLV-1), other than HIV, lead to a range of illnesses and sexual contact may play a role in transmission.

TABLE 148-2 -- Persistent viral sexually transmitted diseases: transmission efficiency, clinical manifestations and epidemiology.

PERSISTENT VIRAL STDs: TRANSMISSION EFFICIENCY, CLINICAL MANIFESTATIONS AND EPIDEMIOLOGY			
Virus	**Estimated efficiency of sexual transmission**	**Clinical manifestations**	**Endemic areas**
HIV-1	Low-moderate	Immunosuppression/AIDS	Sub-Saharan Africa, India, South East Asia
HIV-2	Low-moderate	Immunosuppression/AIDS	West Africa
HTLV-1	Unknown	Adult T-lymphocyte leukemia/lymphoma, HTLV-1-associated myelopathy or tropical spastic paraparesis	Japan, Caribbean
Cytomegalovirus	Moderate-high	Systemic disease in the immunosuppressed	South East Asia, Africa
HSV-1 and HSV-2	Moderate-high	Recurrent genital ulcers	Worldwide
HPV	Moderate-high	Cervical cancer in women, anal cancer in men, recurrent genital warts	Worldwide
Hepatitis B virus	Moderate-high	Chronic hepatitis, cirrhosis, hepatic failure, carcinoma	South East Asia, Africa
Hepatitis C virus	Low	Chronic hepatitis, cirrhosis, hepatic failure, carcinoma	Worldwide

DIAGNOSIS

Laboratory diagnosis

The diagnostic work-up for STDs in the returned traveler does not differ significantly from the approach used for locally acquired infections, although knowledge of the geographic distribution of infection

TABLE 148-3 -- Diagnostic testing for pathogens causing nonulcerative and ulcerative infections in men and women.

DIAGNOSTIC TESTING FOR PATHOGENS CAUSING NONULCERATIVE AND ULCERATIVE INFECTIONS		
Infection (pathogen)	**Specimen collection**	**Diagnostic tests**
Gonorrhea (*Neisseria gonorrhoeae*)	Men: meatal swab/urine; women: cervical swab/urine	Gram stain (Gram-negative diplococci) or media culture or nucleic acid amplification (LCR/PCR)
Chlamydiosis (*Chlamydia trachomatis*)	Men: endourethral swab/urine; women: cervical swab/urine	Tissue culture or ELISA or nucleic acid amplification (LCR/PCR)
Trichomoniasis (*Trichomonas vaginalis*)	Men: endourethral swab/urine; women: vaginal fluid	Microscopy (motile trichomonads) or media culture

Candidiasis (*Candida albicans*)	Women: vaginal fluid	Microscopy (wet mount or potassium hydroxide preparation showing yeast and mycella); media culture
Bacterial vaginosis (*Gardnerella vaginalis*, mycoplasmas, anaerobic bacteria)	Women: vaginal fluid	Microscopy (clue cells), vaginal pH <4.5, potassium hydroxide test, white discharge
Syphilis (*Treponema pallidum*)	Serum	Nontreponemal serology: RPR, VDRL; treponernal serology: MHA-TP, FTA-ABS
	Ulcer scrapings	Dark-field microscopy
Chancroid (*Haemophilus ducreyi*)	Ulcer swab or bubo aspirate	Media culture, nucleic acid amplification (PCR)
Herpes (HSV-2)	Ulcer swab	Tissue culture, nucleic acid amplification (PCR)
		Type-specific HSV-2 antibody serology
Donovanosis (*Calymmatobacterium granulomatis*)	Ulcer tissue or scrapings	Giemsa or Wright staining (identify Donovan bodies)
Lymphogranuloma venereum (*Chlamydia trachomatis* serovars L1,2,3)	Serum	Serology (complement fixation or immunofluorescent antibody tests)
	Ulcer scrapings	Tissue culture
FTA-ABS, fluorescent treponemal antibody-absorbed test; LCR, ligase chain reaction; MHA-TP, microhemagglutination-*Treponema pallidum*; RpR, rapid plasma reagin; VDRL, Venereal Disease Reference Laboratories test.		

may assist with the differential diagnosis. Although an efficient and rational approach to diagnosis should be taken, it may be necessary to order several diagnostic tests simultaneously because of the poor sensitivity and specificity of clinical examination, the propensity of STD pathogens to occur together and the variability in test performance. In addition, the potential complications of infection and the risk of further transmission demand that the infection is identified and treated expeditiously.

Test availability, patient circumstances and controversies in test performance preclude rigid diagnostic algorithms. In general, first-line tests should include microscopy, which can be conducted in a clinic setting and may provide a rapid diagnosis. Media-based cultures and serology require 48–72 hours, but are inexpensive and should be used as first-line tests when available. Tissue culture and amplification procedures are the most expensive tests and should be used with restraint. It is anticipated that with the development, commercial availability and affordability of sensitive and specific amplification procedures, many of the current culture-based methods will be replaced. Table 148.3 outlines the specimen collection and diagnostic testing available for the most common nonulcerative and ulcerative pathogens.

The risk of HIV infection and other viral STDs introduces a special problem in the approach to patient care. Presentation with a bacterial STD is essentially a marker for potential exposure to viral STDs. Among viral STDs the focus initially is on HIV infection, which arguably has the most serious consequences, and early treatment may alter disease progression. Most HIV screening currently tests for both HIV-1 and HIV-2, although the transmission of HIV-2 remains unusual outside West Africa. Screening for other persistent viral infections should be evaluated on an individual basis. For other viruses, the risk is not immediate and there are no specific interventions to block or delay chronic infection. The implications for current and future sex partners, however, are important and screening may be warranted.

Diagnosis by geographic region

A comprehensive picture of the global epidemiology of STDs is not available. Much of what is known about the geographic prevalence of infection is based on ad hoc surveys in selected populations.[6] The highest prevalence of STDs is found in resource-poor countries, which have the least capacity for monitoring and recording information. Sporadic cases and outbreaks of STDs are common and can occur in any region.

Prevalence figures for HIV-1 infection, which are more complete, generally indicate that it is common in regions in which other STDs are prevalent such as sub-Saharan Africa and South and South East Asia. Sexually transmitted diseases are prevalent in any geographic area that is characterized by poverty, prostitution and social disruption.

Genital ulcer diseases are one group of STDs for which knowledge of the geographic distribution may be useful. They appear to occupy specific geographic niches, knowledge of which may aid in the differential diagnosis. Table 148.4 indicates regions where etiologic-specific GUDs are endemic.

The persistent viral STDs have spread globally, although endemic regions are well described (see Table 148.2). Although HIV-1 infection has been the most lethal of these viral pathogens, cervical cancer caused by HPV and hepatic diseases caused by hepatitis B virus have serious consequences and are much more prevalent globally.

Knowledge of antibiotic resistance patterns in various geographic locations may be useful for empiric therapy, but as with STD prevalence figures, the data are sparse and the patterns change rapidly. In high-prevalence regions, the capacity to monitor antibiotic resistance and disseminate this information is inadequate. It is the returned traveler who fails to respond to recommended antimicrobials who

TABLE 148-4 -- Geographic distribution of genital ulcer disease.

GEOGRAPHIC DISTRIBUTION OF GUD	
GUD	**Endemic areas**
Syphilis	Sub-Saharan Africa, India, eastern Europe
Chancroid	Sub-Saharan Africa, South East Asia, central America
Herpes	Worldwide
Granuloma inguinale (donovanosis)	South-east India, New Guinea, Caribbean, Brazil, Vietnam, Japan, central Australia, Zambia, South Africa
Lymphogranuloma venereum	East Africa, West Africa, South East Asia, South America, Caribbean

may indicate emerging resistance. In fact, penicillinase-producing *N. gonorrhoeae*, which is now found globally, was first isolated from an American traveler returning from South East Asia.[19] The overuse and misuse of antimicrobials has promoted the emergence of *N. gonorrhoeae* resistance to penicillin, tetracycline and fluoroquinolones in many parts of the world.[20] Although the recommended empiric treatments should be available and effective for most returning travelers, ongoing surveillance for emerging resistance is a priority for reference laboratories.

MANAGEMENT

An STD in a returned traveler has major implications beyond diagnosis and treatment. The psychologic stress can range from minimal to debilitating. Issues of confidentiality, reporting and contact tracing may result in serious social disruption. Infection with a persistent viral infection may have long-term health consequences. An experienced health care team that can request appropriate diagnostic testing, monitor response to treatment, provide counseling, organize contact tracing and arrange longer term follow-up is essential.

The immediate management will be guided by a detailed history, including frequency of sexual exposure, type of exposure (e.g. oral, anal, vaginal), selection of partner (e.g. commercial sex worker, traveling companion), use of condoms and previous treatment (e.g. local, self-administered). It is not uncommon for travelers to have sought treatment abroad and often these individuals do not know what they received.

A thorough genital examination is important, despite the limitations of establishing an accurate etiologic diagnosis. This is especially true for women, for whom complications of infection are common and multiple anatomic sites might be involved. If an STD is suspected but rapid diagnostic testing is unavailable or inconclusive, empiric treatment is required, due to the risk of:

- developing complications,

¦ transmission to other partners, and
¦ potential loss to follow-up.

Table 148.5 summarizes the recommended treatments for the major nonulcerative and ulcerative pathogens. It is recommended that the treatment for gonorrhea always include treatment for chlamydia and that the treatment for chancroid always include treatment for syphilis. Beyond this, the choice of empiric treatment must be based on:

¦ severity of illness,
¦ knowledge of local infections,
¦ availability of diagnostic tests, and
¦ opportunity for follow-up.

Contact tracing in the returned traveler is generally concerned with informing and screening any sexual partners since returning. This is usually carried out by public health authorities who respond to

TABLE 148-5 -- Recommended treatment for nonulcerative and ulcerative STDs.

RECOMMENDED TREATMENT FOR NONULCERATIVE AND ULCERATIVE STDS		
Infection		**Empiric treatment**
Nonulcerative	Gonorrhea	Cefixime 400mg po single dose or ciprofloxacin 500mg po single dose or ceftriaxone 125mg im single dose
	Chlamydiosis	Azithromycin 1 g po single dose, doxycycline 100mg po q12h for 7 days
	Trichomoniasis	Metronidazole 2g po single dose
	Ureaplasma infection	Doxycycline 100mg po q12h for 7 days
	Bacterial vaginosis	Metronidazole 2g po single dose
Ulcerative	Syphilis	Benzathine penicillin G 2.4 million units im single dose
	Chancroid	Azithromycin 1g po single dose or
		Ceftriaxone 250mg im single dose or
		Ciprofloxacin 500mg po q12h for 3 days or
		Erythromycin 500mg po q8h for 7 days
	Herpes	Aciclovir 400mg po q8h for 7–10 days or
		Aciclovir 200mg po five times a day for 7–10 days or
		Famciclovir 250mg q8h po for 7–10 days or
		Valaciclovir 1g po q12h for 7–10 days
	Lymphogranuloma venereum	Doxycycline 100mg po q12h for 21 days or erythromycin 500mg po q6h for 21 days
	Donovanosis	Doxycycline 100mg po q12h for at least 21 days or
		Trimethoprim-sulfamethoxazole one DS q12h for at least 21 days

reports of STDs in the community. With the variability in symptoms and delays in seeking treatment, it is not uncommon to transmit infection and many imported STDs are diagnosed only after symptoms appear in secondary contacts.

HIV-1 screening

Perhaps the most difficult issue for the returned traveler is whether to screen for persistent viral infections. This may be a concern of returned travelers and should be discussed by the health care worker if it is not. The decision for HIV-1 screening must be made after an informed discussion and a careful evaluation of the risk. Testing for HIV-1 should be strongly recommended for those who:

¦ have engaged in unprotected sex with a commercial sex worker;
¦ present with any GUD (especially chancroid);
¦ have failed standard treatment;
¦ have developed a flu-like illness associated with the STD (which may indicate a seroconversion illness); or
¦ are pregnant.

Testing should also be offered to anyone contracting an STD while traveling in high-prevalence areas, including those who are worried but well.

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Chapter 149 - Jaundice

Hilton C Whittle

INTRODUCTION

The common afflictions experienced by the returned traveler are diarrhea and malaise.[1] However, jaundice in the returned traveler excites fears of highly infectious and serious exotic infections. In fact, since the advent of vaccines for hepatitis A virus and hepatitis B virus, jaundice in returned travelers is unusual and is equally likely to be due to mundane causes that are common in the developed world.

Jaundice is caused by the accumulation of bilirubin, resulting in yellow discoloration of the sclera and the skin. It is exacerbated when an infection causing hemolysis or hepatic damage occurs in a person who has pre-existing liver damage or in a person who has a congenital tendency to hemolysis.[2] The discussion in this chapter is limited to frank jaundice that is due to infection in a traveler recently returned from a developing country or a nonendemic zone or in a repatriated person who has worked or resided in such a place.

HISTORY

A precise and thorough history is the key to diagnosis. In the excitement and drama of the onset of jaundice, especially if it is associated with mental confusion, the vital question of the recent whereabouts of the patient may be overlooked.

Figure 149.1 and Figure 149.2 show the distribution of some exotic viral infections that can cause jaundice. The duration of stay, mode of travel and lifestyle at home and abroad, including eating, drinking and sexual and drug habits, affect the risk of infection during travel. For example, a young student adventurer intent on meeting and living like the locals is obviously in danger of being infected through contaminated food, water or insects. Pot-holing, caving and fresh water sports represent a risk for leptospirosis. Did this young traveler attend a local health center to receive injections or a blood transfusion? On the other hand, a businessman frequenting luxury hotels and nightclubs is in less danger of endemic infections but is at higher risk of alcoholic hepatitis and sexually transmitted hepatitis B. Drug abuse is common among some travelers, who share cheap drugs and dirty needles with their local counterparts, thus placing themselves at high risk of viral hepatitis and other infections. An expatriate doctor, nurse or researcher working with contaminated blood in a rural hospital is in obvious danger of needle-stick injuries, which can transmit viral and other infections that may cause hepatitis and jaundice. Other obvious questions, which are often forgotten, relate to illness in fellow travelers, outbreaks of infection with or without jaundice in local communities, and exposure to toxins such as methyl alcohol in local potions or the ingestion of raw shellfish or other inadequately cooked food. Behind all these questions is the crucial one; is this person likely to have been nonimmune before exposure to infection during travel in an endemic or epidemic area?

Past medical history

The past medical history contains essential information. Was the traveler immunized against hepatitis A virus, hepatitis B virus, yellow fever and typhoid? What antimalarials did the traveler take? Does the traveler use recreational drugs such as ecstasy or cocaine or take other medications, such as isoniazid, dapsone or methyldopa, that may cause hepatitis? Was local food eaten and local water drunk? Was any attempt made to prevent mosquito bites? A previous history of chronic liver disease due to alcohol or viral hepatitis or a tendency to hemolysis (as in some hemoglobinopathies or enzyme deficiencies, such as glucose-6-phosphate dehydrogenase deficiency) may explain exacerbation of jaundice associated with infections of the liver.

SIGNS, SYMPTOMS AND DIFFERENTIAL DIAGNOSIS

A simple classification of jaundice and a list of common infectious causes is found in Table 149.1 , and their likely geographic origins are shown in Figure 149.1 and Figure 149.2 . Other noninfectious causes of jaundice must also be kept in mind (e.g. alcoholic and other causes of cirrhosis, autoimmune hepatitis, gallstones or metastases blocking the bile ducts, drugs such as isoniazid or chlorpromazine, and poisons such as aflatoxin).

A full and thorough clinical examination is obviously mandatory and this must include a good examination of cerebral function. If cerebral function is disturbed, jaundice may be due to hypoglycemia, which is found in severe malaria, especially in children, or it may be due to liver damage itself; headache and meningeal irritation are common in leptospirosis. Signs of chronic liver disease should be looked for because chronic liver disease is sometimes the backdrop to a fulminant infection.

Hemorrhage in skin or mucosa suggests viral hemorrhagic fever, relapsing fever, leptospirosis, severe bacteremia (e.g. meningococcemia) or fulminant hepatitis itself. Assess the size of the liver. If it is small and hard, cirrhosis may be the cause; if it is large and severely tender, ascending cholangitis, flukes or gallstones may be the cause; if there is localized tenderness between the ribs, or inflamed localized swelling, an amebic abscess or another type of abscess may be the cause. The chest should be examined carefully and radiography performed if possible. Severe pneumonia can cause jaundice; a raised diaphragm on the right suggests liver abscess.

Other diagnostic clues

The incubation period is of obvious importance, whether a patient presents with an infection while traveling or after returning. Thus, hepatitis B virus, which has an incubation period of up to 6 months, may cause jaundice long after the holiday is forgotten,[3] whereas the acute hemorrhagic fevers present within 3–6 days of infection, often precipitating emergency removal from the area of travel.[4] The incubation period of leptospirosis is of intermediate duration, being 7–12 days,[5] similar to that of malaria, although the incubation of malaria may be prolonged as a result of inadequate use of prophylactic or curative drugs.[6]

Blood transfusion

In tropical or developing countries blood transfusion is fraught with danger because screening procedures are often not as rigorous

Figure 149-1 Distribution of human malaria.

Figure 149-2 Distribution of some viral hemorrhagic fevers that cause jaundice.

TABLE 149-1 -- Differential diagnosis of jaundice due to infection in the returned traveler.

DIFFERENTIAL DIAGNOSIS OF JAUNDICE DUE TO INFECTION IN THE RETURNED TRAVELER				
Type of jaundice		**Cause**	**Diagnostic test**	**Comments**
Prehepatic	Hemolysis	Malaria; *Bartonella* spp.	Thick and thin blood film	Hemolysis is severe in 'blackwater' fever; *Bartonella* spp. are found in Columbia, Peru, Ecuador
Hepatic	Viruses	Hepatitis viruses A–E, hemorrhagic fevers (yellow fever, Rift Valley fever, Crimean-Congo hemorrhagic fever)	Serum IgM antibody, antigen detection tests, polymerase chain reaction	Was patient transfused? Any local outbreak of jaundice? Jaundice is uncommon in most hemorraghic fevers
	Bacteria, spirochetes	Any severe bacteremia, leptospirosis	Blood culture, micro-agglutination test	Any contact with infected soil or water?
	Protozoa	Amebic or pyogenic liver abscess	Amebic antibodies	East coast of Africa, Mexico and South East Asia are notorious 'hot' spots
Posthepatic	Bile duct obstruction	*Ascaris*, liver flukes, clonorchiasis opisthorchiasis, fascioliasis	Examination of stool for eggs	Unusual in schistosomiasis. Has traveler been eating raw fish?

as in the Western world. Malaria, hepatitis B, C and D viruses, Epstein-Barr virus and cytomegalovirus are possible blood-borne agents that may cause jaundice, especially in subjects who have underlying immunodeficiencies or who are pregnant. Travelers in developing countries should avoid blood transfusions if possible.

Malaria

Malaria is probably the most common cause of fever in the returned traveler. Jaundice caused by massive hemolysis and a degree of hepatocellular necrosis may be manifest during severe *Plasmodium falciparum* malaria in adults, and milder forms are apparent in *Plasmodium vivax* and *Plasmodium ovale* malaria. Underlying liver disease or red cell enzyme deficiencies exacerbate this tendency, and primaquine used to treat *P. vivax* and *P. ovale* may precipitate hemolytic jaundice in some patients who have glucose-6-phosphate dehydrogenase deficiency (see Chapter 166).

Hepatitis viruses

Hepatitis viruses are the next most common cause of fever in the returned traveler, although a minority of those infected develop jaundice.[7] The incubation periods of hepatitis A and E virus are around 15–50 days after contact with infected food or water. Pregnant women are particularly prone to severe hepatitis E virus infection.[8] Hepatitis B, which has a much longer incubation period of 3–6 months, may be transmitted in contaminated blood or needles, as may hepatitis C, which has an incubation period of 2 months. Fever accompanied by general malaise is seldom ascribed to these viruses (see Chapter 48).[9]

Yellow fever and other hemorrhagic fevers

Yellow fever causes jaundice in 1 in 10 infections; Rift valley fever and Crimean-Congo hemorrhagic fever are less common causes. The other hemorrhagic fevers — Lassa, Junin, Ebola, Marburg, Hanta or Dengue — are not noted to cause jaundice, except occasionally in very severe cases (see Chapter 183). All cause hemorrhage and some degree of hepatic and renal damage, the latter being manifest by uremia and proteinuria.

Yellow fever is characterized by the acute onset of fever, chills, muscle aches, nausea and vomiting, followed by jaundice. The pulse may be slower than expected from the degree of fever. Leukopenia is common. After a brief remission of a day or more, a toxic state may develop, with liver and renal failure. Serologic diagnosis is achieved by demonstrating antigen or viral DNA in serum or liver or by IgM antibody.

There are two types of transmission cycle, a sylvatic or jungle cycle involving *Aedes* spp. of mosquitoes and nonhuman primates and an urban cycle involving humans and *Aedes aegypti*. Endemic areas now include parts of Africa and Latin America (see Chapter 222).

Leptospirosis

Weil's disease, a severe form of leptospirosis, is characterized by jaundice, fever, rashes, which may be hemorrhagic, and renal failure.[5] Severe headache, conjunctivitis and myalgia are characteristic, along with a history of working in an environment infested by rats. The organism, which lurks in contaminated food, soil and water, enters through abrasions in the skin or through intact mucous membranes (see Chapter 181).

Relapsing fever

The louse-borne type of relapsing fever is particularly likely to cause jaundice and petechiae in its severe form.[10] The more common, tick-borne form of relapsing fever seldom causes jaundice (see Chapter 182).

Bacterial infections

Severe bacterial infections caused by *Salmonella typhi*, *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Neisseria meningitidis* can cause mild jaundice, especially in patients who have cirrhosis or hemoglobinopathies. *Bartonella bacilliformis*, which is endemic in the western Andes, infects red blood cells and causes hemolytic anemia and jaundice.

Differential diagnosis by geographic area

Knowledge of the geography of infections causing jaundice is essential when weighing the relative risk of each. However, the risk is also related to the level of endemicity and, in the case of malaria, to the level of drug resistance; these are variable within countries and often poorly documented. Thus, the following notes are offered only as a rough guide and should be interpreted with caution.

Malaria

The worldwide distribution of malaria is shown in Figure 149.1 .[6]

Africa

Sub-Saharan tropical Africa is the major focus of *P. falciparum* malaria. *Plasmodium malariae* is widespread, but *P. vivax* is uncommon. Low-level transmission of *P. vivax* occurs in northern Africa. Chloroquine resistance is now widespread in Africa.

Asia

Plasmodium vivax is common in India, Pakistan, Sri Lanka and New Guinea, whereas multidrug-resistant *P. falciparum* occurs in many parts of South East Asia, New Guinea and the islands of the Pacific.

Americas

Plasmodium vivax is the common cause of malaria in central America, whereas *P. falciparum* predominates in Haiti and both infections are found in the low-lying areas of South America.

Hepatitis viruses

Hepatitis A and B viruses are very widespread.[7] The level of endemicity is low in northern Europe and North America, intermediate in southern Europe and high in Africa, India, Asia and parts of South America. Thus, if the risk of hepatitis A virus infection to the unvaccinated British person traveling in France and Scandinavia is considered to be 1, the relative risk in Spain is 5, in eastern Europe 20, in the Middle East 85, in sub-Saharan Africa 235, in South America and the Caribbean 243 and in the Indian subcontinent a massive 1835.[11]

Hepatitis E virus infection, which occurs in large outbreaks transmitted mainly to young adults by the fecal-oral route, has been described in Afghanistan, India, Pakistan, South East Asia, Ethiopia, Yemen, Mexico and in a colossal outbreak in the Xinjiang region of China.[8] Sporadic disease is also a major problem in India and, no doubt, in other parts of the developing world where it is yet to be diagnosed. Even more hepatitis viruses await discovery.

Viral hemorrhagic fevers

The distribution of the viral hemorrhagic fevers that are most likely to cause jaundice are shown in Figure 149.2 .[12]

During outbreaks, yellow fever is transmitted by mosquitoes from monkeys to humans and from human to human. Hunters, expatriate or otherwise, may be exposed to mosquitoes infected from monkeys.

Rift Valley fever virus, a bunyavirus, is mainly a disease of sheep and goats but it can be transmitted to humans by mosquitoes or by direct contamination from infected animals.

Crimean-Congo hemorrhagic fever is transmitted mainly by ticks but air-borne infections occur in both hospital and laboratory environments.

Spirochetes

Leptospirosis occurs worldwide.[5] Infected rodents that associate with infected domestic animals, such as cattle, horses, pigs and dogs, are the chief source of infection. Farmers and other people working with or in contact with these animals are infected by their urine. Louse-borne relapsing fever is endemic in the highlands of Ethiopia but outbreaks occur in Sudan, Somalia, western Africa and Vietnam.

Protozoa

Entamoeba histolytica is very widespread.[13] It can cause colitis and hepatic abscess, which is occasionally associated with jaundice. The invasive form is prevalent in the forest zones of western Africa and on the east coast of Africa, in the whole of South East Asia and in Mexico and the west coast of South America.

Metazoa

Ascaris lumbricoides, which is very widespread, is the most common metazoic cause of obstructive jaundice. Relapsing cholangitis caused by *Opisthorchis* sp. occurs in north and north-eastern Thailand, Laos and Cambodia, where humans may eat infected raw fish, which are the second intermediate hosts. The parasite usually cycles between its definitive hosts (cats, dogs and fish-eating mammals). Clonorchasis, caused by *Clonorchis sinensis*, is associated with eating raw fish in Japan, Korea, China, Taiwan and Vietnam, and occasionally causes an obstructive cholangitis. Fascioliasis is common in sheep in many parts of the world. The common causative organism, *Fasciola hepatica*, is cycled through snails. Infected water cress grown in snail-infested waters is the common source of infection in humans. Extensive outbreaks of fascioliasis have occurred in France and Cuba but infections are also found in the USA, including Hawaii, the Middle East and Asia.[14]

DIAGNOSIS

First-line tests

Simple dipstick tests for both blood and urine are available to measure levels of glucose, bilirubin and urobilinogen. Conjugated bilirubin in the urine indicates hepatocellular or obstructive jaundice; the froth after the urine is shaken is yellow and the urine is dark brown. Urobilinogen, which can be detected by dipstick or Ehrlich's reagent, indicates hemolysis. Biochemical tests for serum conjugated and unconjugated bilirubin, serum aspartate, alanine aminotransferase and alkaline phosphatase are useful in differentiating the two types of jaundice. Viral hemorrhagic fevers are distinguished from viral hepatitis by aspartate aminotransferase concentrations being disproportionally high compared with the alanine aminotransferase concentrations. Blood glucose and urinary protein should be measured if the patient's level of consciousness is altered. Thick and thin blood films may reveal malaria parasites or spirochetes; leukocytosis with a predominance of polymorphs suggests bacteremia, leptospirosis, amebic abscess or cholangitis. Stool microscopy may show ova of *Ascaris*, *Opisthorchis*, *Clonorchis* or *Fasciola* organisms.

TABLE 149-2 -- Basis of treatment of common infections that cause jaundice.

BASIS OF TREATMENT OF COMMON INFECTIONS THAT CAUSE JAUNDICE		
Infection	**Therapeutic agent**	**Comment**
Severe malaria	Quinine or artemether	Quinine resistance increasing in South East Asia
Viruses	Ribavirin	Ebola and possibly Crimean-Congo hemorrhagic fever virus[15]
Leptospirosis	Penicillin or tetracycline	Jarisch-Herxheimer reaction in severe infection
Relapsing fever	Tetracycline, or erythromycin for pregnant women and children	Jarisch-Herxheimer reaction common in severe louse-borne type; relapse common in tick-borne type
Amebic liver abscess	Metronidazole or tinidazole	Aspirate if large
Ascaris	Mebendazole	Surgery often required for obstructive jaundice
Clonorchis, Opisthorchis	Praziquantel	Only one dose necessary
Fascioliasis	Triclabendazole or bithionol	Multiple courses may be required

Specialized tests

These are outlined in Table 149.1 but are usually available only at centers specializing in tropical diseases or at large public health laboratories. Serum IgM antibody tests have the advantage of early diagnosis using a single specimen. Antigen detection tests are also becoming available for malaria and may prove very useful because microscopy, although simple in concept, requires experience and skill if mistakes are to be avoided. Polymerase chain reaction tests are now available for

most human viral infections. They are rapid, reliable and safe for samples that are inactivated during the extraction procedure.

MANAGEMENT

General measures

Acute hepatitis is often poorly managed, especially in its severe form, which is characterized by deep jaundice, liver flap of the hands and confusion or coma. Signs of previous chronic liver disease, such as spider angioma, a small liver, ascites and a history of alcohol abuse, bode ill, as does the presence of renal failure. Full details of the complexities of the management of severe liver failure and severe infections can be found in standard texts.

Specific treatment

The primary issue at stake is what the likely cause of the jaundice is and whether this is treatable (Table 149.2). Good clinical skills and simple diagnostic tests, available even in resource-poor countries, allow a rational decision, which often hinges on the following questions.

- Is the jaundice due to underlying liver disease?
- Is it due to a virus that is not treatable?
- Is it due to an infection that is treatable?

Other considerations

Important and agonizing decisions hinge on whether the patient should be treated locally or referred to a specialist hospital or, in extreme cases, to a high-containment facility. The decision is doubly difficult if the patient presents in a developing or resource-poor country because transport by air is expensive and may endanger the patient and, if the cause of the jaundice is highly infectious, the vehicle and other people in it may be contaminated.

In this respect the viral hemorrhagic fevers are greatly feared, although in these cases the most likely routes of human-human transmission are by direct contact, blood and contaminated syringes rather than by aerosol droplets. [4] However, the patient may be confused, bleeding and severely infected by high titers of virus and thus should be isolated when the diagnosis of viral hemorrhagic fever is suspected, and attendants should be protected by gowns, mask and gloves.[16]

Patients who have yellow fever are usually viremic only during the first 4 days of illness. By the time hepatitis occurs they pose little risk except from residual virus in viscera. The exact duration of viral excretion and level of infectivity of convalescent patients who have other hemorrhagic fevers is variable, but there is no evidence of long-term chronic carriage.

The proper disposal of excreta after inactivation by disinfectants is mandatory even in small hospitals or clinics. Laboratory specimens should be kept in sealed, plastic containers and handled and stored with care. They may be highly infectious (see Chapter 186a). [4]

In summary, when in a developing country do not transfer the patient unless there is a strong reason to think that this will be of real benefit to the patient. This caveat applies to any patient who has acute viral hepatitis; it is simpler, cheaper and easier to send specimens. In a developed country, most patients will be sent to a specialist hospital, particularly if the exact cause of viral hepatitis is not known. Other infections are more likely to be treated locally.

PREVENTION AND PROPHYLAXIS

Sensible behavior should be the order of the day; travelers should eat well-cooked food and drink boiled water, sleep under a mosquito net, preferably one treated with insecticide, and bring their own sterile needle and syringe if they need an injection.[17] Immunization status should be checked well before traveling because optimal regimens are available for vaccines against yellow fever, hepatitis A or B virus, rabies and meningococcal disease. These regimens should be used before entering an endemic or epidemic area, especially if the traveler intends to stay there. Booster polio and tetanus vaccines are also available.

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Chapter 150 - Eosinophilia in the Returned Traveler

Peter F Weller

INTRODUCTION

Eosinophilia represents increased numbers of eosinophils in the blood and/or tissues and is defined as when eosinophils exceed 450/µl blood. Eosinophilia can be a feature of a diverse array of infectious, allergic, neoplastic and other diseases (Table 150.1). [1]

Of the infectious etiologies, helminthic parasites are the predominant agents associated with eosinophilia. Therefore, for travelers returning from brief or prolonged stays in regions where parasitic infections are endemic, infections with various helminths are likely causes of eosinophilia.[2] Analyses of the frequency or risks for developing eosinophilia in travelers are not available. One retrospective study of asymptomatic expatriates who had returned from the tropics indicated that the sensitivity of eosinophil counts as a screening test for acquired infections due to filariasis, schistosomiasis or strongyloidiasis was 38%, with a positive predictive value of 9%. It concluded that in this population eosinophil counts contributed little to detecting these three parasitic infections if asymptomatic patients had full stool examinations and specific serologic testing. [3] Nevertheless, eosinophilia provides a valuable clue to the presence of many helminthic infections.

Travelers who have eosinophilia differ from other eosinophilic patients:

- first, as a consequence of their travel, they may have had recent exposures to infectious agents or medications that cause eosinophilia;
- second, the duration of travel, whether brief or prolonged, constitutes a definable time period for the onset of potential infection — the limited exposure period of travelers is important because they may return with infections in early stages of evolution; and
- third, travelers do not usually have any previous exposure to helminthic parasites, and so their response to helminths may differ from that of residents in endemic areas who have had lifelong exposure to helminthic infections — several helminthic infections result in more pronounced immune reactions and eosinophilia in travelers or temporary residents than in long-term inhabitants.

Evaluation of eosinophilia in a traveler is therefore largely targeted at helminthic infections, although other etiologies for eosinophilia must also be considered.

ETIOLOGIES OF EOSINOPHILIA IN TRAVELERS

Bacterial and viral infections

Acute bacterial or viral infections characteristically produce eosinopenia. The development of a bacterial, viral or protozoan (i.e. malaria) infection in patients who have eosinophilia due to helminthic or allergic diseases suppresses the blood eosinophilia.

Eosinophilia may accompany HIV infection for several reasons:

- leukopenia may lead to an increased eosinophil percentage in the absence of a true eosinophilia;
- reactions to medications may elicit eosinophilia;
- eosinophilia may arise from adrenal insufficiency resulting from cytomegalovirus and other infections in patients who have AIDS;
- a modest and rarely marked eosinophilia is observed in some patients who have HIV infection; and
- eosinophilia accompanies eosinophilic folliculitis in HIV infection.

Eosinophilia is also seen with human T-cell lymphotropic virus-1 (HTLV-1) infections.

Fungal infections

Two fungal diseases are associated with eosinophilia:

- aspergillosis, in the form of allergic bronchopulmonary aspergillosis; and
- coccidioidomycosis.

Eosinophilia is a feature of primary coccidioidal infection and at times disseminated coccidioidomycosis. In travelers to the south western USA, where *Coccidioides immitis* is endemic, eosinophilia may reflect infection with this organism.

Protozoan infections

Infections with single-celled protozoan parasites do not elicit blood eosinophilia. This is true of all intestinal-, blood- and tissue-infecting protozoa with two exceptions: *Dientamoeba fragilis* and *Isospora belli*.

Helminthic infections

Infections with many helminthic parasites elicit eosinophilia (Table 150.2).[4] Although eosinophilia can be a hematologic indicator of helminthic infections, neither the absence of blood eosinophilia nor the presence of only low-grade or episodic eosinophilia excludes such infections. The eosinophilic response to helminths is determined both by the host's immune response and by the parasite, including its distribution, extent of tissue migration and development within the infected host.

For several types of helminth infections, the migration of infecting larvae or subsequent developmental stages through the tissues is greatest early in infections and at these times the eosinophilia will be most marked (Table 150.3). For a detailed description of the parasitology see Chapter 174 & Chapter 246 .

Eosinophilia may be absent in established infections when the parasites are antigenically sequestered within tissues (e.g. intact echinococcal cysts) or present only in the intestinal lumen (e.g. adult *Ascaris*, tapeworms).

For some established infections, there may be episodic blood eosinophilia. Intermittent leakage of fluids from echinococcal cysts can elicit episodic increases in blood eosinophilia and allergic (urticaria, bronchospasm) reactions. For tissue-dwelling helminths, the eosinophilia may increase during the migration of adult parasites, as in loiasis and gnathostomiasis.

Intestinal nematodes

Ascaris lumbricoides infections

These are acquired by ingesting fecally derived eggs, which may contaminate agricultural products or foods. Larvae derived from the

TABLE 150-1 -- Diseases associated with eosinophilia.

DISEASES ASSOCIATED WITH EOSINOPHILIA	
'Allergic' diseases	Atopic and related diseases
	Medication-related eosinophilias
Infectious diseases	Parasitic infections, mostly helminth infections (see Table 150.2)
	Specific fungal infections: allergic bronchopulmonary aspergillosis; coccidioidomycosis
	Other infections — infrequent, including HIV-1 and HTLV-1 infections
Hematologic and neoplastic disorders	Hypereosinophilic syndrome
	Leukemia
	Lymphomas, including nodular sclerosing
	Hodgkin's disease
	Tumors
	Mastocytosis
Diseases with specific organ involvement	Skin and subcutaneous diseases, including urticaria, bullous pemphigoid, eosinophilic cellulitis (Well's syndrome), episodic angioedema with eosinophilia
	Pulmonary diseases, including acute or chronic eosinophilic pneumonia, allergic bronchopulmonary aspergillosis
	Gastrointestinal diseases, including eosinophilic gastroenteritis
	Neurologic diseases (e.g. eosinophilic meningitis)
	Rheumatologic diseases, especially Churg-Strauss vasculitis; also eosinophilic fasciitis
	Cardiac diseases (e.g. endomyocardial fibrosis)
	Renal diseases, including drug-induced interstitial nephritis, eosinophilic cystitis; dialysis
Immunologic reactions	Specific immune deficiency diseases: hyper-IgE syndrome, Omenn's syndrome
	Transplant rejection: lung, kidney, liver
Endocrine	Hypoadrenalism: Addison's disease, adrenal hemorrhage
Other	Atheroembolic disease
	Irritation of serosal surfaces, including peritoneal dialysis
	Inherited, sarcoidosis, inflammatory bowel disease

eggs pass hematogenously to the liver and lungs. About 1–2 weeks after infection, larvae in the lungs penetrate from the capillaries into the alveoli and mature into third-stage larvae. These ascend the tracheobronchial tree and are swallowed to enter the intestine. In the intestine, the larvae mature into adult male and female worms, which begin producing eggs 2–3 months after the initial infection.

The first manifestations of *Ascaris* infections during the transpulmonary passage of larvae produce a syndrome of eosinophilic pulmonary infiltrates (Loeffler's syndrome).[5] [6] Symptoms develop when the larvae are within the lungs, about 9–12 days after ingesting the *Ascaris* eggs. A nonproductive cough, burning substernal discomfort, low-grade fever and wheezing are common. Eosinophilia increases after several days of symptoms and resolves slowly over many weeks. Chest radiographs reveal round or oval infiltrates up to several centimeters in size, which clear over many weeks.

A diagnosis of early-phase pneumonic ascariasis is made with certainty by detecting *Ascaris* larvae in respiratory secretions or gastric aspirates. At least 40 days must elapse before the intrapulmonary larvae responsible for *Ascaris* pneumonia will have matured to produce eggs detectable on stool examinations. Negative stool

TABLE 150-2 -- Helminthic parasitic diseases associated with eosinophilia.

HELMINTHIC PARASITIC DISEASES ASSOCIATED WITH EOSINOPHILIA
Ancylostoma caninum
Angiostrongylus cantonensis
Angiostrongylus costaricensis
Anisakiasis
Ascariasis
Capillaria philippinensis
Clonorchiasis
Coenurosis
Cysticercosis
Dicroceliasis
Dirofilariasis
Dracunculiasis
Echinococcosis
Echinostomiasis
Fascioliasis
Fasciolopsiasis
Filariases: lymphatic (*Wuchereria* spp., *Brugia* spp.), loiasis, mansonelliasis, onchocerciasis, tropical pulmonary eosinophilia
Gnathostomiasis
Heterophyiasis
Hookworm (*Ancylostoma* spp., *Necator* spp.)
Hymenolepsiasis
Metagoniamiasis
Nanophyetus salminocola
Opisthorchiasis
Paragonimiasis

Schistosomiasis: schistosome dermatitis, *Schistosoma mansoni, Schistosoma haematobium, Schistosoma japonicum, Schistosoma intercalatum*
Sparganosis
Strongyloidiasis
Trichinosis
Trichostrongyloidiasis
Trichuriasis
Visceral larva migrans: *Toxocara canis, Bayllascaris* spp

TABLE 150-3 -- Helminthic parasitic diseases causing marked eosinophilia (>3000/mm^3). [5]

HELMINTHIC PARASITIC DISEASES CAUSING MARKED EOSINOPHILIA	
Disease	**Notes**
Angiostrongylus costaricensis	
Ascariasis	Early transpulmonary larval migration, often absent when mature
Hookworm infection	Early transpulmonary larval migration, often mild when mature
Strongyloidiasis	
Trichinosis	
Visceral larva migrans	Primarily pediatric
Gnathostomiasis	
Filariasis: tropical pulmonary eosinophilia	
Filariasis: loiasis	Especially in expatriates
Filariasis: onchocerciasis	
Flukes: schistosomiasis	During early infection in people who are not immune (Katayama fever)
Flukes: fascioliasis	During early infection
Flukes: clonorchiasis	During early infection
Flukes: paragonimiasis	During early infection
Flukes: fasciolopsiasis	During early infection

examinations during or soon after acute infection do not exclude *Ascaris* as an etiology. Finding that the stools are free of eggs during pneumonic involvement but contain *Ascaris* eggs 2–3 months later

supports the role of *Ascaris* as the etiologic agent of acute eosinophilia and pneumonitis.

Hookworm

Infections with hookworms (*Necator americanus* or *Ancylostoma duodenale*) are acquired when larvae in fecally contaminated soil penetrate the skin. Even brief exposures of travelers to contaminated soil may be sufficient because as few as three larvae can produce infection. Larval penetration of the skin often produces a pruritic maculopapular eruption, and in those who have been previously infected there are serpiginous tracks of larval migration, as in cutaneous larva migrans.

In experimental infections pulmonary symptoms have not developed (although larvae penetrate through to the lungs), but gastrointestinal symptoms, including nausea, diarrhea, vomiting and abdominal pain, are common. Blood eosinophilia increases after 2–3 weeks and peaks after 5–9 weeks of infection.

In untreated hookworm infections the eosinophilia slowly diminishes, but the eosinophil count can remain elevated for several years due to the attachment of adult worms to the intestinal mucosa. Eggs are detectable in feces about 6–8 weeks after infection with *N. americanus*. The larvae of *A. duodenale* may persist within the tissues before returning to the intestine so egg laying can be delayed. Cutaneous larva migrans from other animal hookworm species is commonly associated with eosinophilia when humans become infected as accidental hosts.

Tissue and intravascular nematodes

Filariasis

Infection occurs following the introduction of infective larvae by biting insect vectors (see Chapter 170). Early infections with lymphatic-dwelling (*Brugia* and *Wuchereria* spp.) filariae can cause lymphadenitis, lymphangitis and eosinophilia, usually without detectable microfilaremia. Infections with *Loa loa* in long-term residents of endemic areas in equatorial West and Central Africa are manifested by microfilaremia, episodic angioedema (Calabar swellings), transocular migration of adult worms and modest eosinophilia. In contrast, among those who acquire loiasis after temporary residence, microfilaremia is less common, episodic angioedema is more severe and elevations in serum IgE, antifilarial antibody titer and eosinophilia are more pronounced. Eosinophilia and inflammatory reactions are more prominent in acute filariasis among previously nonimmune patients who exhibit immunologic hyper-responsiveness to infection than among long-term residents of endemic regions who develop partial immunity to infections.

Trichinosis

Trichinosis is acquired by consuming the meat of carnivores that contains viable encysted larvae of *Trichinella* spp. The muscle phase of trichinosis begins about 1 week after infection when the larvae from the intestine disseminate hematogenously and begin to encyst in striatal muscle. Patients may experience subconjunctival, retinal and subungual splinter hemorrhages, and periorbital and facial edema. As the larvae encyst in muscle, myalgias, fatigue, elevated muscle enzymes and eosinophilia develop. Eosinophilia is present in over 90% of those who have symptomatic trichinosis and abates slowly over months. As *Trichinella* spp. are globally distributed, travelers are at risk of acquiring trichinosis if they ingest undercooked meats from domestic and wild pigs, wart hogs, boars, bears, walruses or horses containing *Trichinella* larvae.

Visceral larva migrans

The ingestion of eggs of the dog ascarid, *Toxocara canis*, produces a syndrome of visceral larva migrans. This is most common in young children who ingest soil contaminated with fecally derived eggs and is less frequently seen in adults, when it is potentially acquired by ingesting foods contaminated with *Toxocara canis* eggs. Most infections are subclinical and marked only by blood eosinophilia. Eosinophilia is also prominent with heavier infections.

Trematodes

Acute schistosomiasis

Schistosomiasis (see Chapter 167) is acquired by exposure of the skin to fresh water containing cercariae of *Schistosoma* spp. A distinct syndrome of acute schistosomiasis (Katayama fever) develops principally in previously unexposed individuals who have heavy infections. Patients develop fever, chills, anorexia, weight loss, abdominal pain, diarrhea, urticaria, myalgias, a dry cough and bronchospasm 2–8 weeks after infection. Blood eosinophilia is very prominent. In the later stage of

this syndrome, antischistosomal antibodies develop and egg laying begins and schistosome eggs become detectable in the stools, urine and rectal mucosa. The syndrome is self-limiting and resolves over 1–2 months, but can be shortened by treatment with corticosteroids and antischistosomal therapy.

Paragonimiasis

Paragonimiasis (see Chapter 168) results from infection with the lung fluke (*Paragonimus* spp.). *Paragonimus westermani* is endemic in Asia, whereas other *Paragonimus* spp. cause infections in other regions of the world. Infection is acquired by ingesting freshwater crabs or crayfish that harbor metacercariae. After ingestion, the metacercariae excyst in the duodenum, penetrate the gastrointestinal wall and migrate within the peritoneal cavity. Most young flukes penetrate the diaphragm to migrate within the pulmonary parenchyma, where they become surrounded by an inflammatory infiltrate and later a fibrous capsule. After 7–8 weeks of infection mature flukes begin egg production within the capsule, which enlarges and ruptures, often into a bronchiole.

Eosinophilia is most pronounced in the early phase of paragonimiasis. In this phase, larval migration into the pleural cavity may result in a pleurisy and exudative eosinophil-rich pleural effusions. Chest radiographs reveal transient migratory pulmonary infiltrates and eosinophilia is prominent.

Diagnosis of early-phase paragonimiasis before egg production has been initiated is difficult and often based presumptively on compatible clinical findings in a patient who has eosinophilia and a history of exposure in an area where paragonimiasis is endemic.

Fascioliasis

Infection with *Fasciola hepatica*, the liver fluke of sheep and cattle, is acquired by ingesting cysts attached to aquatic plants (e.g., wild watercress). In early-stage fascioliasis marked eosinophilia is common as the parasites burrow into the liver and enter the bile ducts. The symptoms may be minimal or include fever, abdominal pain, malaise, pruritus, urticaria and coughing. Hepatomegaly and cholestatic liver function test abnormalities develop. Because there are no eggs in the stool or biliary or duodenal fluid samples until about 3 months after infection, the diagnosis of acute fascioliasis will be suggested by the triad of fever, marked eosinophilia and hepatomegaly.

Intestinal flukes

Eosinophilia is prominent in the early stages of infections with several intestinal flukes. *Fasciolopsis buski* is acquired by ingesting metacercariae on water plants such as water chestnuts, *Metagonimus yokogawai* or *Heterophyes heterophyes* by ingesting metacercariae in raw or undercooked fish, and *Nanophyetus salminocola* by ingesting salmon.

Chronic helminthic infections

One hallmark of helminthic infections in addition to their characteristic elicitation of eosinophilia is their ability to survive within infected human hosts for prolonged periods of time. Some can cause eosinophilia lasting for years (e.g. hookworm, strongyloidiasis, filariasis, hepatic trematodes). In contrast to their early phases, the diagnostic stages of the helminthic parasites will have formed in mature infections.

Other travel-related causes of eosinophilia

Ectoparasites (e.g. scabies) may be associated with eosinophilia. Eosinophilia can also accompany adverse drug reactions and can occur with numerous travel-related medications.

EVALUATION OF EOSINOPHILIA IN TRAVELERS

History

Although many helminths are widely distributed, some have more discrete geographic distributions.[7] The parasite causing clonorchiasis is found in Asia. *Angiostrongylus cantonensis*, a cause of eosinophilic meningitis, is principally but not exclusively found in the Pacific Basin. Of filarial parasites, *Loa loa* is limited to Central and West Africa and *Onchocerca volvulus* is found in equatorial Africa and elevated regions in Central America.

Dietary history is pertinent for several helminthic infections, including anisakiasis (raw fish), fish tapeworm (fish), *N. salminocola* (salmon), *Taenia solium* (pork), *Taenia saginata* (beef), fascioliasis (watercress), fasciolopsiasis (horse chestnut), gnathostomiasis (freshwater fish, eels, frogs, snakes and poultry and pigs fed on fish), *A. cantonensis* (land snails or slugs, freshwater shrimps, crabs, some marine fish) and trichinosis (pork, boar, bear meat, horse meat, walrus, wart hog). Exposure in sheep-rearing areas is pertinent for *Echinococcus granulosus*.

A history of swimming in or having contact with fresh water is relevant in areas where there is snail-borne schistosomiasis. Contact with fresh or salt water followed by a rash on water-exposed skin and eosinophilia suggests schistosome dermatitis, which can be caused by avian schistosome species. Skin contact with soil potentially contaminated with human or dog feces, as may occur by walking barefoot or occupational exposure, is relevant for the acquisition of cutaneous larva migrans, hookworm or *Strongyloides* infections.

Clinical features

Because diagnostic-stage parasites are not detectable in early helminthic infections, the clinical findings are especially important:

- trichinosis is suggested by eosinophilia in a patient who has myositis, periorbital edema and subungual splinter hemorrhages;
- in stronglyloidiasis, a migratory serpiginous linear urticarial eruption ('larva currens') due to larvae migrating in the skin is a pathognomonic manifestation experienced by some patients;
- urticaria can be a feature of several helminthic infections, including acute ascariasis, strongyloidiasis, acute schistosomiasis, acute fascioliasis and echinococcosis;
- angioedematous subcutaneous (Calabar) swellings and subconjunctival migrations of adult worms are cardinal manifestations of *Loa loa* infections; and
- gnathostomiasis causes subcutaneous swellings with eosinophilia.

Investigations

Although stool examinations help in the identification of enteric helminths, many helminths capable of eliciting eosinophilia, either tissue- or blood-dwelling helminths or those causing early-phase enteric infections, cannot be identified by fecal examination. Among the established helminthic infections capable of inducing eosinophilia that are not diagnosable from stool examinations are trichinosis, filariasis, anisakiasis, gnathostomiasis, visceral larva migrans and echinococcosis. Even some intestinal helminths, notably hookworm and *Strongyloides*, may not be readily detectable on routine stool examinations.

Other diagnostic tests include examination of respiratory secretions (sputum, bronchoalveolar lavage fluid) for larvae of *Strongyloides*, hookworm and ascaris, or for eggs of *Paragonimus* spp.

For a diagnosis of filariasis, blood should be examined for microfilariae by blood filtration. Blood-borne microfilariae include those of:

- lymphatic-dwelling *Wuchereria bancrofti* and *Brugia* spp. — generally the yield is greatest from night-time blood samples;
- *Los loa* — yield is greatest from morning samples; and
- *Mansonella perstans* and *Mansonella ozzardi*.

Skin snips should be obtained to detect the microfilariae of *Onchocerca volvulus, M. ozzardi* and *M. streptocerca*. Urine is examined for the eggs of *Schistosoma haematobium*. Tissue biopsies may help in diagnosing trichinosis (muscle biopsy), schistosomiasis (rectal biopsy, liver biopsy) and loaisis or gnathostomiasis (involved soft tissues).

Serologic testing can be valuable for several helminthic parasites. For *Strongyloides stercoralis*, enzyme-linked immunosorbent assay serology has proved useful, even when stool examinations are unrevealing. Because of the difficulty in detecting this parasite and its potential for persisting and causing later serious infections, serologic evaluation for strongyloidiasis is indicated for a patient who has eosinophilia. Serologic tests are the most expedient way for diagnosing visceral larva migrans. In trichinosis diagnostic serologic titers may not rise until the third week of infection. Serologic tests are also helpful in diagnosing schistosomal and filarial infections, cysticercosis and echinococcosis. In echinococcosis, the serology may be negative, even when there are obvious lesions; the tests are positive in only 50% of patients who have isolated pulmonary lesions and in 85–90% of those who have hepatic hydatid cysts (Chapter 169).

Management

Treatment should be directed at identified parasites. Although some advocate presumptive therapy with mebendazole or thiabendazole for undiagnosed eosinophilias in travelers, it is preferable to pursue an etiologic diagnosis for eosinophilia and to exclude the presence of undetected intestinal or extraintestinal helminths. This is especially important because *Strongyloides stercoralis*, with its potential for long-term persistence, may cause prolonged eosinophilia.

Following the initiation of anthelmintic therapy, blood eosinophilia may increase for several weeks before subsiding. The magnitude of the post-treatment eosinophilia correlates with the number of parasites present before treatment and is an immune reaction to killed parasites. Resolution of eosinophilia can be monitored as one measure of the efficacy of anthelmintic therapy, with hematologic studies being repeated in the months after therapy.

Complications

Sustained blood eosinophilia (the idiopathic hypereosinophilic syndrome) can lead to cardiac complications, including the development of intraventricular thrombi and endomyocardial fibrosis with secondary mitral or tricuspid regurgitation. [8] Most patients who have eosinophilia develop no endomyocardial damage, but such damage has been noted occasionally in Americans and Europeans who have eosinophilia due to loiasis and acute trichinosis. Patients who have sustained eosinophilia should be monitored by echocardiography.

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Chapter 151 - Cough and Respiratory Tract Infections

Thomas C Jones

EPIDEMIOLOGY

Acute respiratory illness (ARI) is common throughout the world. As a result, respiratory illness is common among travelers.[1] There are over 4 million childhood deaths due to ARI each year in the developing world.[2] Especially common causes of these deaths are:

- respiratory syncytial virus (15–20%),
- pneumococcal pneumonia (20%),
- hemophilus pneumonia (10–20%),
- postmeasles pneumonia (15%),
- pertussis (10%), and
- parainfluenza virus (7–10%).

These statistics show that the traveler is highly exposed to respiratory pathogens. It has been reported that 2.2% of travelers return with symptoms of rhinitis and 1–2% have acute respiratory tract infection with fever.[1] These infections are primarily due to respiratory viruses but mycoplasma, legionella and other less common organisms also contribute.

Atypical causes of respiratory illness can occur in the traveler because of exposure to unusual microbes. Although these illnesses are rare, they are very important for the physician caring for a traveler with respiratory illness because they are potentially serious, transmissible and treatable. Although pneumonia due to *Streptococcus pneumoniae, Mycoplasma pneumoniae, Mycobacterium tuberculosis* or influenza makes up less than 0.001% of diseases in travelers, it can account for up to 10% of cases of febrile disease involving the respiratory tract presenting to a physician. These patients must be diagnosed and managed in the same way as nontraveling patients with community-acquired pneumonia.[3] [4] Hidden in this group of travelers with pneumonia are a few patients whose diseases are specifically a result of exposure to agents or environmental conditions associated with their travel. These diseases may be misdiagnosed because of their rarity.

This chapter is directed at the microbial causes of respiratory illness, and of cough in particular. Nonmicrobial causes can also affect the traveler and require carefully managed medical intervention.

PATHOGENESIS AND PATHOLOGY

The physician caring for the traveler with cough must be aware of the potential causes, both common and rare, of respiratory disease throughout the world. Toxins, allergens, viruses, mycoplasma, chlamydia, rickettsia, bacteria, fungi, protozoa and helminths may each be responsible for respiratory signs and symptoms.

Toxins or allergens

A cause of cough in travelers is exposure to respiratory toxins or allergens that are not present in his or her home environment. Some of these, along with their associated pulmonary illnesses, are listed in Table 151.1 . High levels of air pollution in large cities can cause respiratory tract irritation and exacerbate cough. A new environment may also expose an allergic traveler to grasses, pollens and dust in much higher concentrations than normal. This is particularly a problem for the young traveler, who may not have developed full immunotolerance for potential allergy-producing substances. For example, among Cambodian refugees on the Thailand border, the most common respiratory illness, 'Khao-I-Dang lung', was due primarily to dust inhalation.

Of special relevance to the traveler is exposure to unusual concentrations of respiratory allergens such as inhaled fungi. The best example of this is bronchopulmonary aspergillosis, caused by inhalation of *Aspergillus* spp. (see Chapter 237). In 'farmer's lung', the likely allergenic organisms are various molds that accumulate in silos.

Viral, mycoplasmal, chlamydial and rickettsial causes

The microbial agents in these groups that cause respiratory symptoms are listed in Table 151.2 , along with their most common related illnesses. As in the home environment, respiratory viruses cause a significant number of the pulmonary signs and symptoms that affect travelers.[1] [3] The traveler enters an environment that is foreign to his or her immune system and is likely to develop illness due to the different rhinoviruses, adenoviruses and other respiratory viruses that are prevalent in the communities visited.

The chances of contracting a viral illness during air travel are enhanced by crowding, spread of respiratory droplets and by rapid turnover of dry cool air, which reduces airway defenses. Alcohol and fatigue further increase the likelihood of an overt respiratory illness shortly after returning home.

TABLE 151-1 -- Toxins and allergens as causes of respiratory signs and symptoms in the traveler.

TOXINS AND ALLERGENS AS CAUSES OF RESPIRATORY SIGNS AND SYMPTOMS IN THE TRAVELER		
Causative agent		**Main pulmonary disease**
Toxins	Smoke fumes	Rhinitis, bronchitis, pneumonia
	Carbon monoxide, sulfur dioxide, ozone	Bronchitis
	Halides, phosgene, pesticides	Bronchitis, pneumonia
Environment	Hypobaric conditions during air travel	Stress bronchoconstriction, hypoxia
	Mountain sickness	Pulmonary edema
Allergens	Dust and mites	Rhinitis, conjunctivitis, bronchitis, asthma
	Pollens and grasses	Rhinitis, conjunctivitis, bronchitis, asthma
	Aspergillus spp. (allergic bronchopulmonary aspergillosis)	Asthma, eosinophilic pneumonia
	Molds (farmer's lung)	Eosinophilic pneumonia

TABLE 151-2 -- Viral, mycoplasmal, chlamydial and rickettsial causes of cough in travelers.

VIRAL, MYCOPLASMAL, CHLAMYDIAL AND RICKETTSIAL CAUSES OF COUGH IN TRAVELERS

	Condition/microbial agent	Sources of infection	Main illness
Viruses	Influenza	Worldwide, varied seasons, epidemics, pandemics	Fever, bronchitis, pneumonia
	Rhinovirus, adenovirus and other respiratory viruses	Worldwide, varied seasons	Rhinitis, pharyngitis, bronchitis
	Hantavirus	Worldwide	Respiratory distress syndrome
	Chickenpox, measles	Contact with infected patient	Fever, pneumonia, rash
	Coronavirus	Contact with infected patient	Fever, severe pneumonia, SARS
Mycoplasmas	Atypical pneumonia: *Mycoplasma pneumoniae*	Worldwide, crowding	Acute pneumonia
Chlamydiae	*Chlamydia pneumoniae*	Worldwide	Acute pneumonia
	Chlamydia trachomatis	Worldwide, children	Paroxysmal cough, pneumonia
	Psittacosis: *Chlamydia psittaci*	Contact with psittacine birds	Acute pneumonia
Rickettsiae	Q fever: *Coxiella burnetii*	Contact with sheep, cattle, goats, ticks	Acute pneumonia

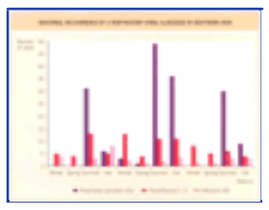

Figure 151-1 Respiratory illness in southern Asia (February 1985 to December 1987). *Data modified from reference* [5] .

Seasonal patterns related to the spread of viruses differ in various parts of the world. For example, many respiratory viruses that are spread from December to April in the northern hemisphere are spread from May to September in the southern hemisphere, and most often during the rainy season in tropical countries. Figure 151.1 shows the frequency of three groups of viruses in southern India during different seasons over a 3-year period. The highest prevalence occurred in the summer and fall, rather than the winter as recorded in temperate regions in the north.[5]

The most important and best-documented virus in travelers is influenza. For example, during the pandemic of 1957, influenza reached the USA from Asia, beginning at a conference in the midwest and spreading throughout the country as the participants returned home.[6] Another example comes from the war in Vietnam, when military personnel in the Philippines often had holidays in Hong Kong. In 1968, thousands of cases of influenza occurred among these people and their contacts in the Philippines owing to the new antigenically shifted virus (H3N2) that had emerged that year in Asia. A recently recognized epidemic due to a Coronavirus has emerged from Asia. This new virus causes the illness now termed Severe Acute Respiratory Syndrome (SARS).[7] [8] [9]

A newly recognized cause of respiratory illness is hantavirus, which is transmitted as aerosolized virus from rodents. It has caused localized epidemics in many parts of the world including the USA, Europe, South America and Asia.

In the past, careful attention has been given to the possibility that a traveler with the first respiratory signs of smallpox might infect a large number of people on the airplane or at the airport before the tell-tale signs of cutaneous eruption had appeared. Smallpox remains a threat as an agent of bioterrorism and patients with chickenpox or measles could have only cough and fever at the beginning of the illness. It is important for the physician caring for a traveler with cough to document any potential exposure to these diseases.

TABLE 151-3 -- Bacterial and fungal causes of cough in travelers.

BACTERIAL AND FUNGAL CAUSES OF COUGH IN TRAVELERS			
	Condition/infecting agent	**Geographic distribution and sources of infection**	**Main illness**
Bacteria	Pneumococcal pneumonia	Worldwide	Acute pneumonia
	Tuberculosis	Worldwide, infected patients	Chronic pneumonia
	Legionellosis	Aerosolized water, cooling systems	Acute pneumonia
	Bordetella pertussis	Worldwide	Acute glottitis and chronic cough
	Actinomycosis	Worldwide, soil	Draining sinuses, chronic pneumonia
	Plague: *Yersinia pestis*	Infected patients or rodents, fleas	Lymphadenitis, pneumonia
	Meningococcal pneumonia	Worldwide epidemics, central Africa	Pharyngeal carriage, pneumonia, meningitis
	Tularemia	Contact with infected small mammals	Lymphadenitis, pneumonia
	Bacillus anthracis	Animal hides, bioterrorism	Skin lesions, pneumonia
	Melioidosis	South East Asia	Pneumonia, systemic illness
Fungi	Histoplasmosis	Worldwide, decayed vegetation, bat caves	Erythema nodosum, acute or chronic pneumonia, hilar adenopathy
	Coccidioidomycosis	Aerosolized soil, south-west USA	Acute or chronic pneumonia
	Paracoccidioidomycosis	South America	Acute or chronic pneumonia
	Blastomycosis	Southern USA	Pharyngeal lesion, pneumonia
	Cryptococcosis	Contact with feces from pigeons	Pneumonia, meningitis
	Penicilliosis	Northem Thailand	Skin lesions, pneumonia
	Pneumocystis pneumonia	Worldwide, pneumocystis	Acute pneumonia in those who are immunodeficient (i.e. HIV)
	Aspergillosis	Worldwide	Aspergilloma: pneumonia in those who are immunodeficient

Mycoplasmal pneumonia can cause cough in travelers. *M. pneumoniae* is transmitted in crowded conditions, as has been demonstrated among military recruits. There are three important chlamydial causes of pneumonias:

- *Chlamydia pneumoniae*, an increasing cause of community-acquired pneumonia,
- *Chlamydia trachomatis*, particularly in children, and
- *Chlamydia psittaci*, the cause of psittacosis.

Psittacosis is transmitted by contact with either psittacine birds such as parrots or with people who have psittacosis. Although rare in wild birds, psittacosis occurs when birds are housed together.

Q fever is a cause of pneumonia in those exposed to rickettsia aerosolized from or in the secretions of infected farm animals. Small epidemics have occurred in many parts of the world, and have been well studied in Australia, California, Israel and Switzerland. A detailed history of possible exposure to these microbes is the key to

correct diagnosis.

Bacterial and fungal causes

Table 151.3 lists bacterial and fungal causes of cough in travelers. The most common cause of bacterial pneumonia among travelers is the same as among nontravelers — *Strep. pneumoniae*. This must always be considered in a recently returned traveler who has acute symptoms of fever, cough, purulent sputum and chest pain.

Legionellosis has recently been identified as an important disease among travelers because of the presence of the organism in the air conditioning systems and spas of hotels and ships.[10] [11]

M. tuberculosis is a major cause of lung disease worldwide. Recently, spread of the infection among airline passengers has been reported.[12] Figure 151.2 shows a diagram of the rear section of the airplane and the evidence that six passengers and one flight crew member were exposed to tuberculosis during the 11-hour flight. Tuberculosis is particularly noteworthy at present because it is increasing in frequency after being brought under partial control early in the 20th century and as it now demonstrates increased multiantibiotic resistance.

Pneumonia caused by the plague bacillus *Yersinia pestis* must be considered in the differential diagnosis of pneumonia for a patient who has been in countries where plague may occur near ports or

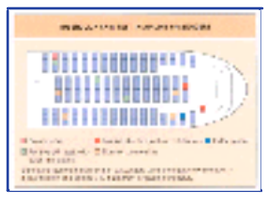

Figure 151-2 Significant tuberculin skin tests in passengers and one flight crew member in the rear section of a Boeing 747 flight from Chicago to Honolulu.

among wild rodent populations.[13] The pulmonary disease occurs in an environment where the more common from of the disease (bubonic plague) is being reported. Epidemic spread of this organism to the patient's contacts and health care workers may occur and is a true medical emergency.

Tularemia is another potential cause of pneumonia in those exposed to small wild animals or those who have been bitten by infected flies or ticks. Tularemia is geographically restricted and outbreaks have occurred in North America, Europe, the former Soviet Union and Japan (*Francisella tularensis*). Anthrax can be acquired by exposure to animals during slaughter, or to their hides or fur, and if the organism, *Bacillus anthracis*, is used as an agent of bioterrorism.

Pertussis, caused by the organism *Bordetella pertussis*, has been controlled by vaccine in most countries but because of incomplete immunization of children in many regions of the world, and waning immunity in adults, travelers are at risk. Recent outbreaks have occurred in the former Soviet Union and among adults in Europe.[14] It

TABLE 151-4 -- Protozoal and helminthic causes of cough in a traveler.

PROTOZOAL AND HELMINTHIC CAUSES OF COUGH IN A TRAVELER			
	Condition/infecting agent	**Sources of infection**	**Main illness**
Protozoa	Malaria	Mosquitoes in Africa, Asia, South America	Fever, hemolysis, hepatitis, 'flu syndrome'
	Amebiasis	Tropics and subtropics	Fever, liver abscess, secondary pneumonia
Helminths	Filariasis	Worldwide, mainly tropics, particularly India	Eosinophilic pneumonia
	Strongyloidiasis	Contact with fecally contaminated soil	Transient pneumonia, diarrhea, severe pneumonia in those who are immunodeficient
	Ascariasis	Ingestion of fecally contaminated food or water	Transient pneumonia, intestinal obstruction
	Paragonimiasis	Ingestion of raw crabs	Chronic pneumonia
	Echinococcosis: *Echinococcus granulosus*	Contact with dog feces in cattle or sheep regions	Hepatic or lung cyst
	Echinococcus multilocularis	Contact with feces of wild carnivores in the northern hemisphere	Chronic invasive lung lesion
	Acute schistosomiasis	Contact with cercariae while swimming	Fever, dry cough

can be the cause of both acute upper respiratory illness and prolonged paroxysmal cough. The illness is underdiagnosed because physicians have become unfamiliar with the characteristic 'gasping' cough and because special PCR or serologic tests are required for diagnosis.

Other infrequent causes of either an acute or chronic cough in the traveler include melioidosis, brucellosis, actinomycosis and pulmonary meningococcosis. The likelihood of each of these is increased by relevant exposure, for example to:

- soil in South East Asia (melioidosis);
- animals or animal products (brucellosis); and
- epidemic meningococcal disease.

The most important fungal disease acquired by the traveler is histoplasmosis. This is particularly common in travelers who have explored bat caves or areas where dead and decaying trees allow aerosolization of the organism *Histoplasma capsulatum*.[15]

Cryptococcus neoformans can be aerosolized from pigeon droppings and lead to respiratory illness, although the illness most commonly associated with this organism is meningitis.

Aspergillus fumigatus can cause pulmonary pathology other than allergic alveolitis. It is more likely to cause symptomatic disease in a traveler with previously damaged lungs.

Travelers to south-west USA may acquire an acute or chronic respiratory illness caused by *Coccidioides immitis*,[16] which is inhaled with contaminated soil. Diagnosis requires the use of appropriate serologic tests and application of skin test antigens. In South America a similar disease is caused by *Paracoccidioides brasiliensis*. Blastomycosis is endemic in the southern USA. Penicilliosis, caused by *Penicillium marneffei*, has recently been recognized in Thailand. It causes skin lesions and pulmonary symptoms.

Protozoal and helminthic causes

Table 151.4 lists important protozoa and helminths that can cause cough in a traveler. The most important protozoal cause of respiratory illness is malaria, not because the lung usually exhibits the pathology of malaria but because early in the infection malaria is often misdiagnosed as 'flu syndrome'.[17]

Entamoeba histolytica infection (amebiasis) must be considered in travelers with cough. Although the lung may be involved in severe amebiasis, cough is more commonly caused by an amebic liver abscess elevating the right diaphragm; this results in segmental atelectasis of the lung and the patient presents with a secondary bacterial pneumonia. Even without this complication, the patient may present with cough due to diaphragmatic irritation and chest pain due to the enlarged inflamed

liver.

Among the most interesting patients are travelers with eosinophilia and cough (see Chapter 150). After evaluating the patient for hypersensitivity reactions that can cause cough, allergic aspergillosis and asthma, helminthic causes need to be considered. Three diseases are due to the migration phase of helminths:

- *Ascaris lumbricoides* is acquired by ingesting the eggs in fecally contaminated food or water;
- *Schistosoma* spp. are acquired by exposure to lakes or rivers containing infected cercariae; and
- *Strongyloides stercoralis* is acquired by walking near fecal deposits.

These helminths cause transient, usually undetected, febrile pulmonary illnesses unless, as in the case of strongyloidiasis, the traveler is immunosuppressed, in which case a serious and potentially fatal pneumonia may develop.

Tropical pulmonary eosinophilia (Weingarten's syndrome) is usually due to *Wuchereria bancrofti* or *Brugia malayi* and is found mainly in southern Indians and in Indonesians. Symptoms are characterized by cough, wheezing, fever with eosinophilia and fluffy infiltrates on chest X-ray. *Dirofilaria* can cause similar symptoms but usually present as a 'coin lesion' on chest X-ray.[18]

Paragonimiasis, caused by *Paragonimus* spp., most commonly *P. westermani*, is characterized by chronic cough and pulmonary symptoms very similar to those of tuberculosis. It is acquired by ingesting uncooked fresh-water crabs that contain the intermediate stage of the organism. The diagnosis is made by identifying the characteristic operculated eggs in sputum or stool.

Echinococcus multilocularis causes disease in wild animals in the northern parts of the world. Exposure to the feces of the primary hosts (wild wolves, dogs and other carnivores) primarily through eating contaminated berries or plants can lead to infection, which is manifest as progressive invasion and damage to the liver and lung. *Echinococcus granulosus* can be transmitted by the feces of dogs in domestic farms. This helminth occasionally causes lung cysts, although usually the abdominal viscera are involved.

PREVENTION

Physicians should advise appropriate vaccinations for travelers visiting potentially epidemic regions (e.g. influenza, pertussis, measles and even pneumococcal vaccine[19] and BCG[20] in some situations). Of

course, influenza strains and pneumococcal serotypes may differ in the visited areas from those contained in the vaccines. Physicians should warn travelers of any epidemics in the regions to be visited. Care regarding exposure to new agents (such as SARS) includes wearing face masks and protection against spread by finger/hand contact on contaminated surfaces.

CLINICAL FEATURES

The time and character of the onset of cough must be carefully recorded because these will be closely related to its cause and so to the differential diagnosis and treatment. For example, respiratory symptoms caused by toxins or allergens occur within hours of exposure, viral illnesses occur 3–5 days after exposure and slowly progressive diseases such as tuberculosis may bring the patient to the physician weeks or months after exposure.

The nature of the patient's illness — fever, malaise and chest pain, the rapidity of onset and the degree of associated general toxicity — can distinguish acute illnesses (e.g. influenza, malaria, pneumococcal pneumonia, legionellosis, plague, psittacosis) from more chronic diseases (e.g. tuberculosis, melioidosis, paragonimiasis, tropical pulmonary eosinophilia).

The type of cough may be helpful; for example, the paroxysmal cough of chlamydial infection or pertussis can be distinguished from the rhinitis and cough of viral infections caused by rhinoviruses.

Signs and symptoms in organs other than the lung may provide important clues to the etiology, for example:

- fever and splenomegaly in malaria;
- lymphatic buboes and marked toxicity in plague;
- hepatic tenderness in amebiasis; and
- meningitis in cryptococcosis and meningococcal disease.

In many conditions, symptoms are most common in patients with pre-existing pulmonary disease. For example, on exposure to allergens or viruses asthmatic patients may wheeze and be short of breath or may present with cough and increased respiratory secretions, whereas patients with pre-existing chronic pulmonary disease may simply show a deterioration in pulmonary function demonstrated by dyspnea.

DIAGNOSIS

There are a number of questions that should be asked of the traveler with cough (Table 151.5). Once answered, the physician can begin to focus on whether special tests need to be done for unusual diseases. A flow chart for the diagnosis and treatment of cough in the traveler is shown in Figure 151.3

Immediate tests should include:

- a complete blood count and differential;
- chest radiography;
- sputum and blood culture;
- sputum Gram stain and acid-fast stain; and
- as appropriate on the basis of history of exposure, serologic or polymerase chain reaction (PCR) tests for mycoplasma, influenza, legionella, psittacosis, pertussis and the specific tests for the more unusual diseases listed above.

Patients with fever, malaise and cough who have returned from an endemic malaria area should have a malaria smear. If the patient has eosinophilia, stool and sputum examination for helminth eggs or larvae should be considered. If the patient has a leukocytosis, investigations should include serologic testing for amebiasis in addition to appropriate cultures for bacteria and fungi.

TABLE 151-5 -- Questions to ask a patient who has traveled.

QUESTIONS TO ASK A PATIENT WHO HAS TRAVELED
Where/when/how long?
• Which countries did you visit?
• What time of year was your trip?
• How long was the trip and when did you return?
Medical History
• Do you have a history of allergy; if so to what?
• Do you have a history of lung disease such as bronchitis, emphysema, tuberculosis, bronchiectasis?
Special activities
• Did you visit caves or assist in activities such as clearing trees or debris?

• Did you experience dry, dusty or moist environmental conditions?
• Did you eat uncooked vegetables, berries or fish, or drink untreated water?
Special contacts
• Did you inhale dust, smoke or toxic fumes?
• Did you have contact with domestic or wild animals?
• Did you have contact with ill people? What illness?
• Were you bitten by mosquitoes, ticks or other arthropods?

Differential diagnosis

To determine whether a patient has a common, easily managed infection or one that is difficult to diagnose and manage is a major task for the physician. A key decision to be made is whether hospitalization and full work-up are indicated (Fig. 151.3). Malaria smears or sputum examinations for paragonimus eggs on each traveler would be inappropriate. However, viewing every illness as 'flu syndrome' is inviting disaster. The correct diagnosis is arrived at by a balanced review of the detailed history, the type and severity of the signs and symptoms, and the initial laboratory tests.

Sometimes the differential diagnostic process must include the response to initial treatment. This will be helpful, for example, in distinguishing pneumococcal pneumonia, in which a response generally occurs within a few days, from other bacterial pneumonias. A diagnosis of tropical eosinophilia may not be confirmed until the patient has responded to diethylcarbamazine.

Most importantly, the physician must imagine being in the environment the traveler has been in. He or she will then take the right steps towards the correct diagnosis and treatment.

MANAGEMENT

The appropriate treatment will become clear as the correct diagnosis is made. Empiric therapy with antibiotics is seldom necessary; however, hospitalization followed by appropriate cultures and therapy for the most likely serious diagnoses is appropriate for an acutely ill patient with pneumonia. Depending on the history, in some patients with respiratory infections not requiring hospitalization, macrolide antibiotic therapy is appropriate.

Patients with respiratory symptoms after exposure to chickenpox or measles should be advised that a rash may follow and that they should avoid putting others at risk of acquiring the infection. It should be determined whether the patient has an immunodeficiency that warrants the use of immunoglobulins. The possibility of contagious disease in a traveler should be immediately reported to local public health authorities and the Centers for Disease Control in the USA.

Figure 151-3 Diagnostic/treatment flow chart for cough in travelers.

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Chapter 152 - Lymphadenopathy, Splenomegaly and Anemia

Tom Doherty

INTRODUCTION

Lymphadenopathy is a common feature of many infectious diseases. It may be generalized or localized to one group of lymph nodes. Usually, the cause is obvious; it is commonly the result of local sepsis. Both non-specific viral infections and bacterial skin infections are common among travelers. Occasionally, however, lymphadenopathy may be due to something more exotic, particularly in travelers returning from tropical parts of the world.

Splenomegaly implies an underlying systemic illness. Anemia, unless it is very mild and has an obvious cause, requires further evaluation. Both splenomegaly and anemia are uncommon among returned travelers but both should be sufficient reason for further investigations.

'Travelers' are a heterogeneous group of people — young people in search of a bit of excitement, members of the international business community, people returning from developed countries to their original country of origin, aid workers, volunteers, missionaries and, increasingly, holidaymakers. With such an ill-defined group, it is difficult to make precise recommendations that apply to everyone. Travelers may well suffer from 'exotic' conditions such as brucellosis or Katayama fever; however, it is worth emphasizing that some preexisting or relatively mundane disease may often explain their symptoms. For example, 9% of travelers returning from the tropics who required admission to hospital with a febrile illness were found to have a urinary tract infection, community-acquired pneumonia or a streptococcal sore throat, and another 25% were assumed to have a non-specific viral infection that settled spontaneously.

LYMPHADENOPATHY

A diagnosis of lymphadenopathy is usually a clinical one; it is therefore subjective and not uncommonly wrong. Other conditions to bear in mind include:

- abscesses, particularly in the early stages before they have become fluctuant;
- cystic lesions (in the context of tropical diseases, cysticerci and *Trichinella* deserve special mention);
- lipoma and other similar lesions such as dermoid tumors;
- aneurysms, particularly if they are full of clotted blood and therefore less likely to be pulsatile;
- migratory helminthic infections, particularly gnathostomiasis, the Calabar swellings associated with loaiasis, and onchocercal nodules;
- pyomyositis, which can develop spontaneously; and
- myiasis or 'tumbu fly'.

A list of those conditions that are associated with lymphadenopathy and of conditions that do not usually give rise to lymphadenopathy is shown in Table 152.1 .

Presenting signs and symptoms that point to a diagnosis

As with any clinical problem, the diagnosis should be based on a full and detailed history and thorough general examination. In the case of returned travelers, it is essential to determine not only where they went and when they returned but precisely what they did when they were away. One useful maxim is to try to answer four questions: why did this person, from this place, develop these symptoms at this time?

A point that may be useful and is often overlooked is a person's occupation. For example, people traveling on business and staying in international hotels are unlikely to contract trypanosomiasis but agriculturists working in rural areas are more likely to do so. Tsetse flies, the vector for African trypanosomiasis, exist only in rural parts of Africa, and their bites are extremely painful and unlikely to be forgotten. A full sexual history is important. People traveling alone may be at higher risk of acquiring sexually transmitted diseases. A dietary history is often overlooked and may provide a clue to a possible diagnosis of brucellosis (from unpasteurized milk or cheese, and more often from camel's milk than cow's milk) or gnathostomiasis (from raw fish or crustaceans). The combination of alcohol intolerance, pruritus and eosinophilia combined with lymphadenopathy is suggestive of Hodgkin's lymphoma. HIV seroconversion illness can exactly mimic infection with Epstein-Barr virus (EBV). Kikuchi's disease, which causes painful, usually unilateral, cervical lymphadenopathy and a fever, is most often seen in young Japanese women.

The size of palpable lymph nodes is worth noting. In general, palpable lymph nodes less than 0.5cm in diameter are unlikely to have clinical significance, particularly in thin people. The site of the palpable lymph nodes is also important — local sepsis is more likely to result in localized lymphadenopathy than in lymphadenopathy that is generalized. Epitrochlear lymphadenopathy is of particular significance as it suggests an underlying systemic disease, specifically secondary syphilis, HIV, or a reticulosis. Remember the anatomy — lesions of the perineum drain to the inguinal group of nodes while lesions of the testes, for example, drain to the para-aortic nodes and, ultimately, to the neck. Pre-auricular nodes usually react to a lesion of the eye or to pathogens that invade through the conjunctiva. Do not forget to examine the teeth, particularly in the context of cervical lymphadenopathy.

The consistency of enlarged lymph nodes is often claimed to be of diagnostic importance. Lymphadenopathy that is due to an infectious process is often tender and asymmetric. Lymph nodes that are enlarged as a result of lymphoma are usually described as matted and 'rubbery', whereas fixed, hard, or 'craggy' nodes are more suggestive of a metastatic lesion. In practice, in most cases, such information is subjective and unreliable, and other associated findings are of greater significance. The combination of lymphadenopathy with either hepatomegaly or splenomegaly suggests a generalized process rather than local sepsis, as does the presence of anemia.

Differential diagnosis by geographic area

Many infectious diseases that give rise to lymphadenopathy are ubiquitous, particularly viral infections such as EBV, sexually transmitted diseases such as syphilis or HIV, and tuberculosis. Others

TABLE 152-1 -- Causes of lymphadenopathy (continued over page).

CAUSES OF LYMPHADENOPATHY		
	Causes	**Notes**
Common	Local sepsis	Cause is usually obvious
	Tonsillitis	Particularly streptococcal
	EBV	Palatal petechiae, splenomegaly, atypical lymphocytes
	Non-specific viral infection	

Less common but important	HIV seroconversion illness	Transient lymphadenopathy
	HIV (established disease)	Permanent lymphadenopathy
	Kaposi's sarcoma	
	Tuberculosis	
	Trypanosomiasis	Localized lymphadenopathy in early infection with chancre; generalized lymphadenopathy in late infection
	Toxoplasmosis	
	Secondary syphilis	
	Dengue fever	Lymphadenopathy occurs in 50% of cases; usually mild
	Chancroid	Usually inguinal lymphadenopathy
	Lymphoproliferative disorder	
	Neoplasia	Usually metastatic lymphadenopathy
	Sarcoidosis	Particularly hilar lymphadenopathy
	Leishmaniasis	Cutaneous and visceral
	Typhus	Usually painful eschar
Uncommon	*Wuchereria bancrofti*	
	Brugia malayi	
	Parvovirus	
	Q fever	
	Lymphogranuloma venereum	
	Castleman's disease	
	Rheumatoid arthritis	
	Systemic lupus erythematosus	
	Leptospirosis	
	Still's disease	
	Pityriasis rosea	
	Cat-scratch fever	
Rare	Lepromatous leprosy	
	Erythema nodosum leprosum	
	Bartonellosis	Often tender
	Tularemia	
	Anthrax	
	Diphtheria	
	Plague	Extremely tender 'buboes'
	Kikuchi's disease	
	Ehrlichiosis	
	Behçet's syndrome	
	Wegener's granulomatosis	
	Midline granuloma	
	Whipple's disease	
	Weber-Christian disease	
	Podoconiosis	
Infections usually not associated with lymphadenopathy	Brucellosis	
	Loaiasis	
	Onchocerciasis	Secondary infection of scratched skin may result in lymphadenopathy
	Malaria	
	Hepatitis	
	Typhoid	Lymphadenopathy has been reported in chronic salmonellosis
	Tuberculoid leprosy	
	Schistosomiasis	May occur in acute disease
	Flaviviruses	
	South American trypanosomiasis (chronic form)	
	Intestinal helminths	
	Strongyloides spp.	
	Hydatid disease	
	Cholera	
	Tetanus	
	Melioidosis	
	Cysticercosis	
	Amebiasis	
	Fascioliasis	

have relatively well-defined areas of transmission. For example, leishmaniasis is uncommon in western and southern Africa but appears to be spreading from countries bordering the Mediterranean south into Sudan and northern Kenya, and it also occurs in Central and South America and throughout much of the Indian subcontinent. Bartonellosis is confined to the Americas. Dengue is particularly common in South East Asia, West Africa and the Caribbean and often causes epidemics.

Investigations to confirm the diagnosis

In most cases, the cause of regional lymphadenopathy is obvious and requires little further investigation, if any. A throat swab may confirm a clinical suspicion of a streptococcal sore throat (and rule out the possibility of diphtheria) and a skin swab may be useful in determining appropriate antibiotic therapy for skin sepsis.

In those cases where the lymphadenopathy is more generalized, and particularly when there are systemic features, further investigations may be warranted. A normal full blood count with differential white blood cell count is reassuring. A blood film, looking specifically for atypical lymphocytes, is suggestive of infection with EBV, although atypical lymphocytes also are a feature of other viral infections and toxoplasmosis. Both the Paul-Bunnell test and monospot test for EBV may be negative in patients with the infection, and EBV serology is a more sensitive investigation.

Serologic markers for toxoplasmosis may provide useful information, particularly if facilities are available for measuring IgM as well

as IgG. A negative Venereal Disease Research Laboratory (VDRL) test virtually excludes secondary syphilis. HIV antibody tests may be negative during a seroconversion illness, and measurement of p24 antigenemia or HIV RNA may be more informative. Serologic markers exist for cytomegalovirus and parvovirus infections, although their clinical relevance is questionable. *Leishmania* serology is nearly always negative in patients with cutaneous disease but almost always positive in those with visceral disease, as long as they are not also infected with HIV. Most patients with both HIV and visceral leishmaniasis do not mount a serologic response.

For patients with a history of systemic upset or chronic disease, markers of inflammation such as the erythrocyte sedimentation rate, C-reactive protein and serum albumin concentration may be helpful. However, the erythrocyte sedimentation rate may be greatly elevated in residents of tropical countries, particularly in Africa, as a result of non-specific hyperglobulinemia. A Mantoux test is usually positive in patients with tuberculous lymphadenopathy, unless they have miliary disease or hypoalbuminemia.

Imaging may also be useful. A chest radiograph may provide evidence of hilar lymphadenopathy and suggest sarcoidosis or tuberculosis. Abdominal ultrasonography with or without computerized tomography (CT) scanning may not only confirm the presence of intra-abdominal lymphadenopathy but also enable accurate biopsy material to be obtained. Sometimes, isotope scanning with either indium or gallium may define an unexpected local source of infection.

In certain cases, where the diagnosis is in doubt (and particularly in the context of systemic illness), more invasive investigations are warranted. Fine-needle aspiration of enlarged lymph nodes may provide a diagnosis in case of tuberculosis, trypanosomiasis and neoplastic disease. In most sites, open biopsy of enlarged lymph nodes can be performed under local anesthesia and, although it is helpful to cut across the excised node to discover tuberculous caseation, histologic examination of the tissue obtained may be necessary to provide the answer. In general terms it is advisable not to choose inguinal nodes for biopsy — those in the neck are more likely to generate useful information.

Bone marrow aspiration and liver biopsy specimens may occasionally be required to confirm or refute a diagnosis of tuberculosis or a lymphoproliferative disorder. Liver biopsy is of particular significance in patients with lymphomas as it provides information regarding the staging of the disease. To reduce the risk of iatrogenic complications, it may be appropriate to obtain biopsy material under direct imaging, either ultrasound or CT, where such facilities are available. Beware of taking biopsies from inguinal nodes in patients with suspected lymphatic filariasis — both lymphatic leakage and secondary infections occur not uncommonly.

Complications

Lymphadenopathy is unlikely to lead to any serious complications. Tuberculous nodes, especially *Mycobacterium bovis* infections in the neck, not uncommonly break down and suppurate — and the addition of oral steroids to antituberculous therapy may reduce the degree of scarring should this occur. Similarly, metastatic lymph nodes may ulcerate, but usually in the late stages of the disease. Very large intrathoracic lymph nodes can compress the major airways and other structures. The treatment depends on the nature of the underlying condition.

SPLENOMEGALY

Presenting signs and symptoms that point to a diagnosis

Splenomegaly may be part of an acute febrile illness or of a more chronic underlying process. Commonly, the differential diagnosis for these two situations differs, although there is some overlap. A list of the causes of splenomegaly is provided in Table 152.2 .

Malaria

In a traveler recently returned from the tropics with a febrile illness, the most important diagnosis is *Plasmodium falciparum* malaria; this must be excluded. In acute malaria, the spleen is often enlarged, although not greatly so; however, the absence of clinical splenomegaly is of little value in excluding the diagnosis. In a series of 482 cases of malaria admitted to a single hospital in Canada over a 12-year period, only 24% had detectable splenomegaly at the time of admission.

TABLE 152-2 -- Causes of splenomegaly.

CAUSES OF SPLENOMEGALY

Mild splenomegaly	*Malaria*
	EBV
	Hepatitis
	Typhoid and other salmonelloses
	Tuberculosis
	Dengue fever
	Katayama fever
	Toxoplasmosis
	Cytomegalovirus
	HIV
	Leptospirosis
	Brucellosis
	Sepsis
	Trypanosomiasis
	Histoplasmosis
	Rheumatoid arthritis
	Systemic lupus erythematosus
	Hemaglobinopathies
	Sarcoidosis
	Lepromatous leprosy and erythema nodosum leprosum
	Amyloidosis
Moderate splenomegaly	Lymphoproliferative disorder
	Subacute bacterial endocarditis
	Splenic abscess
	Portal hypertension due to *chronic schistosomiasis*
Marked splenomegaly	*Visceral leishmaniasis*
	Hyperreactive malarious splenomegaly
	Myelofibrosis
	Chronic myeloid leukemia
	Glycogen storage diseases
Each section reflects the importance and/or frequency of the various conditions. The italic type shows the conditions that are more likely to occur in travelers.	

Tropical splenomegaly syndrome, recently reclassified as hyper-reactive malarial splenomegaly, is a poorly understood condition. It is uncommon in travelers but does occur. Several diagnostic criteria have been proposed, including hyperglobulinemia, pancytopenia, strongly positive malaria serology (particularly IgM), a negative blood film for malaria parasites and perisinusoidal lymphocytosis on liver biopsy specimens. The condition represents an inappropriate immunologic response to malaria. Usually the splenomegaly responds to long-term treatment with antimalarial drugs, either weekly chloroquine, daily proguanil or both. Those cases that prove refractory usually turn out to be misdiagnosed cases of lymphoma.

Dengue fever

Dengue fever commonly mimics malaria and is increasing both in frequency and in geographic distribution. The 'classical' rash of dengue fever is petechial, but in practice it is more often morbilliform in the early stages, at least in travelers. A generalized erythematous reaction that blanches with pressure is suggestive of the diagnosis, but both these signs are difficult to see, particularly on dark skin (Fig. 152.1). The combination of severe low back pain, pain in the long bones and retro-orbital pain, particularly when this is accentuated by extreme lateral gaze, is very suggestive of a diagnosis of dengue. Although splenomegaly is unusual among children with the disease in endemic areas, most travelers with dengue have some degree of splenomegaly.

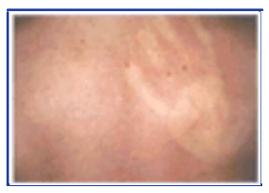

Figure 152-1 Early rash of dengue fever.

Typhoid

Splenomegaly is a common feature of patients with typhoid fever, particularly in the second week of the illness, and it also occurs in patients with nontyphoid salmonelloses and in patients with *Shigella* infections. Several features suggest a diagnosis of typhoid. Usually, patients with the disease are clinically extremely unwell; cough, abdominal pain with either constipation or diarrhea, and neurologic symptoms may all occur. Although the fever of malaria may spike and return to normal values, patients with typhoid usually have fevers that fluctuate to some extent but rarely return to baseline. The value of features such as a relative bradycardia has been overemphasized. Rose spots (pale pink papules on the upper trunk extending into the axillae) are difficult to see, even on Caucasian skin, and occur at least as commonly with nontyphoid salmonelloses as with typhoid. Splenomegaly is common, but again its absence does not rule out the diagnosis.

Epstein-Barr virus infection

Epstein-Barr virus (EBV) infection is common in tropical countries, particularly in Africa. It causes an acute febrile illness, often associated with a sense of misery, lethargy and profound malaise. Splenomegaly occurs in most cases; usually there is associated lymphadenopathy with or without palatal petechiae, both of which are uncommon in malaria or typhoid. An idiosyncratic morbilliform rash after ampicillin is an avoidable but characteristic sign. Although EBV infection is more common among children and young adults, it can cause severe disease even in late middle age, and has been associated with acute splenic rupture.

Visceral leishmaniasis

Kala-azar, or visceral leishmaniasis, is widely distributed in India and is currently epidemic in southern Sudan and northern Kenya. In addition, its frequency is increasing and its epidemiology is changing in response to the immunosuppression associated with HIV. Visceral leishmaniasis is found in as many as 17% of HIV-positive symptomatic people in southern Mediterranean countries.

Classically, it presents as a chronic febrile illness associated with considerable systemic upset and associated wasting. The spleen is usually firm and grossly enlarged. Pancytopenia is common. *Leishmania* serology is invariably positive in immunocompetent people but in only about 40% of patients co-infected with HIV. The parasite is often identifiable in biopsies of the reticuloendothelial system, including bone marrow, spleen, lymph nodes and liver tissue.

Schistosomiasis

Katayama fever is a poorly understood immunologic condition that is apparently triggered by the onset of ovulation by maturing schistosomulae, which usually occurs 4–6 weeks after exposure to infected water. It occurs most commonly in people who have not previously been exposed to schistosomiasis and is less common with *Schistosoma haematobium* than with either *Schistosoma mansoni* or *Schistosoma japonicum*. Only a small minority of people who become infected with schistosomiasis develop the syndrome — probably fewer than 1%.

A diagnosis of Katayama fever can only be made on clinical grounds, as there is no definitive investigation. As it occurs in response to ovulation, examination of stool or urine for schistosome ova is usually negative and serologic responses only become positive once the acute symptoms have resolved. Splenomegaly occurs in approximately 25% of affected people, usually in combination with a brisk rise in the total eosinophil count and non-specific changes in liver function tests. Urticarial reactions and hepatomegaly may also occur.

Splenomegaly is also a feature of established schistosomiasis, commonly the result of portal hypertension resulting from the periportal hepatic fibrosis associated with *S. mansoni*. However, although long-term sequelae of this kind may be common in endemic areas, they are very rare among travelers. As the liver damage that results from schistosomiasis is the result of fibrosis induced by a granulomatous response to egg antigens (with a characteristic pattern on liver biopsy), hepatic function is usually preserved when assessed with conventional liver function tests. Schistosomal serology is usually, but not always, positive. Often the adult worms have died of old age by the time the diagnosis is made, and therefore ova are not commonly found in stool or urine or in rectal snips. Similarly, eosinophilia, associated with early infection, is uncommon in established disease.

HIV

The seroconversion illness of HIV infection mimics that of EBV infection, and transient splenomegaly, lymphadenopathy, atypical lymphocytosis and a morbilliform rash may all occur. As the acute illness resolves, the spleen returns to normal. However, as the HIV syndrome continues, both lymphadenopathy and splenomegaly may recur as part of the AIDS-related complex.

Other causes of splenomegaly

Patients with acute hepatitis may have splenomegaly but the diagnosis is relatively easy to confirm. Leptospirosis is uncommon among travelers but has been reported, particularly in military personnel; exposure may occur during white-water rafting and pot-holing. Tuberculosis, brucellosis, trypanosomiasis and histoplasmosis are all uncommon among travelers but each does occur. Infectious endocarditis is well recognized as a cause of splenomegaly but is no more common among travelers than among the indigenous population. Equally, noninfectious causes of splenomegaly, such as autoimmune disease and lymphoproliferative disorders, may need to be excluded. Adult Still's disease is an uncommon condition and one that is difficult to diagnose; it is therefore easily missed, but the clinical picture includes fever and splenomegaly.

ANEMIA

Anemia is uncommon among travelers. When it does occur, the differential diagnosis is very similar to that among the indigenous population. Broadly speaking, anemia results from one or a combination of: a deficiency of iron, folate or vitamin B12; chronic blood loss; decreased red cell survival; chronic disease; or bone marrow aplasia.

Any patient with significant anemia for which there is no immediately obvious cause should ideally be admitted to hospital for investigation. The basic work up for any such patient should follow conventional lines, including a full history and clinical examination. Questions regarding blood loss, menorrhagia, systemic upset, nutrition, use of nonsteroidal anti-inflammatory drugs and pre-existing disorders such as abnormal red cell phenotypes are obviously important. Examination should focus particularly on the mucous membranes (for evidence of bleeding), the spleen and lymph nodes (for evidence of an underlying inflammatory process), a rectal and vaginal exam-ination where appropriate (for evidence of blood loss or malabsorption), and some assessment of nutritional status.

A few particular diagnoses deserve special mention. Chronic malaria, especially due to infection with *Plasmodium vivax*, can present with marked anemia and splenomegaly, but anemia is relatively uncommon in travelers with acute *P. falciparum* infection. Visceral leishmaniasis invariably results in some degree of anemia, and the anemia may be profound. Tropical sprue appears to be decreasing in frequency; although it has been reported most commonly from the Indian subcontinent, sporadic cases do occur among travelers to sub-Saharan Africa, and often these patients have a macrocytic anemia. Intestinal helminthic infections are often cited as a cause of anemia; in practice, this is very unlikely in returning travelers. Of the common intestinal helminths, only hookworm actually consume blood from the host. Each worm only consumes 0.6–1.0ml of blood per day and, in common with other helminthiases (except *Strongyloides* sp.), hookworms are unable to multiply within the gastrointestinal tract. Although people who live in tropical areas, particularly children, may have very high worm burdens as a result of repeated infection, and often survive on a poor diet with the added burden of repeated malaria attacks — all factors that make anemia very likely — such factors are uncommon in adult travelers.

INVESTIGATIONS TO CONFIRM THE DIAGNOSIS OF SPLENOMEGALY AND/OR ANEMIA

A suggested schedule for the investigation of travelers with either splenomegaly or anemia is shown in (Table 152.3). A few points are worth emphasizing. Malaria can only be excluded after repeated blood film examinations, particularly in those patients with low parasitemia and in those taking chemoprophylactic agents. Mefloquine, in particular, has a long half-life and may delay the time taken for symptoms to develop. Alternative strategies — for example, the use of dipstick techniques such as the ICT test — may replace microscopy in the future, particularly in settings where staff have relatively little experience in identifying low-density infections.

Aspiration of the spleen to confirm a diagnosis of visceral leishmaniasis is a relatively safe procedure; however, it should only be performed by experienced practitioners and ideally under ultrasound control. Any aspirate should be cultured as well as subjected to microscopy; the polymerase chain reaction is now the method of choice for identifying the infecting strain of *Leishmania*. As with liver biopsy, the procedure should only be undertaken once clotting indices and a platelet count have been measured and a supply of compatible and safe blood identified.

Fiberoptic endoscopy to biopsy the mucosa of the small intestine is the investigation of choice in cases of suspected sprue. Endoscopy has the added advantage of enabling aspiration of jejunal fluid, which can be examined for the presence of *Giardia* or *Strongyloides* spp. in particular. It is worth remembering that celiac disease is not confined to children; it can present even in late middle age. Measurement of anti-endomysial antibodies may be useful.

Other serologic tests may be useful but several measurements may be necessary to confirm a diagnosis and a change in titer may be

TABLE 152-3 -- Suggested reasons for splenomegaly and anemia in a patient recently returned from abroad.

SUGGESTED REASONS FOR SPLENOMEGALY AND ANEMIA IN A PATIENT RECENTLY RETURNED FROM ABROAD	
Initial investigations	Thick and thin blood films for malaria (repeated several times)
	Blood cultures (repeated several times)
	Full blood count (red cell indices, white cell count, evidence of hypersplenism)
	Blood film examination (atypical lymphocytes; red cell morphology; evidence of hemolysis)

If anemia predominates	Serum iron
	Transferrin, total iron binding capacity
	Red cell folate
	Vitamin B12
	Fecal occult blood (repeated several times)
	Hemoglobin electrophoresis
	Also consider: bone marrow aspirate endoscopy
Serologic investigations	EBV
	Toxoplasmosis
	Cytomegalovirus
	Malaria immunofluorescent antibody test
	Schistosomal ELISA
	Brucella
	Leishmania
	Widal agglutination test
	HIV
	Histoplasmosis
	Acute and convalescent sera for dengue fever and other flaviviruses
Imaging	Abdominal ultrasound and CT
	Chest radiography
	Indium-labeled white blood cell scan
	Labeled red blood cell survival scan
Tissue diagnosis	Liver biopsy
	Splenic aspiration
	ELISA, enzyme-linked immunosorbent assay.

more valuable than a single reading. Imaging techniques are very useful — sarcoidosis, for example, gives a characteristic appearance on chest radiograph. Tissue biopsy of bone marrow, liver or spleen may well be indicated, particularly in more complicated cases, and biopsies that are taken under either ultrasound or CT guidance are rather more likely to provide useful material.

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Chapter 153 - Animal Bites and Rabies

Charles E Rupprecht

INTRODUCTION

An estimated 4.4 million persons sustain animal bites annually in the USA.[1] These bites range in severity from insignificant to fatal maulings. Sequelae include disfigurement, dismemberment, envenomation, localized infection of soft tissues and deep structures, as well as rabies and other systemic infections.[2]

Rabies is a viral zoonosis transmitted via the saliva of infected mammals. The virus spreads to the central nervous system, causing an encephalomyelitis which progresses to coma and death. On average, 1–2 cases of human rabies and 5000–10,000 cases of animal rabies are reported in the USA each year, 90% diagnosed in wild animals.[3] Worldwide, an estimated 50,000 cases of human rabies occur per year according to the World Health Organization. Between 1960 and 1979, eight (22%) of the 37 cases of human rabies diagnosed in the USA were acquired abroad, whereas 13 (33%) of the 39 cases of human rabies that occurred since 1980 were acquired outside the USA.[3] [4]

PRESENTING SIGNS AND SYMPTOMS THAT POINT TO THE DIAGNOSIS

Patients either present for first aid immediately after a bite or considerably later with signs and symptoms of a bite-related infection.

It is important to determine the extent of the injury and the structures involved in the bite. Feline teeth can penetrate deeply into tendons, bones and joints, in contrast to dog bites, which are more likely to devitalize large areas of soft tissue by crushing injury.[5] Besides the common organisms that are isolated from bite wounds, which include *Staphylococcus, Streptococcus* and *Corynebacterium* spp., the type of animal involved can also help to implicate other specific pathogens that are known to cause well-defined syndromes (Table 153.1). When a patient presents with cellulitis 12–24 hours after cat bite, the likely agent is *Pasteurella multocida* rather than the patient's skin flora, which usually requires an incubation period of 24 hours or longer. Although specific case histories have documented etiologic agents associated with animal bites other than those listed in Table 153.1 (e.g. *Francisella* spp., *Yersinia* spp., nonoxidative Gram-negative rods, etc.), these other instances appear rather uncommon.

Rabies virus is transmitted in the saliva of mammals, almost always by a bite. Rabies in humans has an incubation period of 1–3 months, but occasionally periods may be as short as a week or last as long as several years. Early symptoms include a nonspecific flu-like prodromal illness with headache, fatigue and fever associated with paresthesiae at the site of the bite or proximal to it. These symptoms are followed by the onset of an acute neurological phase with anxiety, irritability and other behavioral changes. Some patients develop classic 'furious' rabies, characterized by periods of hyperactivity, disorientation, hallucinations and other bizarre behaviors alternating with periods of calm. Additional signs and symptoms can include autonomic instability manifested as hyperthermia, tachycardia and hypersalivation, as well as hydrophobia and aerophobia. This phase of disease rapidly progresses to seizures, coma and death, usually within 10 days of the first onset. Occasionally, patients present with 'dumb' rabies and develop flu-like symptoms, paralysis and finally disorientation, coma and death.

DIFFERENTIAL DIAGNOSIS BY GEOGRAPHIC AREA

Most people who sustain an animal bite are able to provide an adequate history of the type of animal, the location and the circumstances that may have provoked the event. Thus, the differential diagnosis is not directly related to the type of trauma that caused the injury, but rather to the organisms that are likely to result in infection due to the bite. Comprehensive lists of bacterial flora that have been cultured from the mouths and bite wounds of various animals have been published elsewhere, together with case reports of rare exotic pathogens that have been transmitted by a bite at least once. In general, the organisms that most often cause bite-related infections are the Gram-positive skin flora of the victim and the bacteria that reside in the animal's mouth, which are predominantly Gramnegative rods and anaerobes.[5] There is no known variation to this generalization by major geographic regions.

In rabies, however, there is significant geographic variability in both the incidence and the species that are likely to transmit the virus. In the early 1940s in the USA, dogs were the predominant reservoir for rabies.[3] With the utilization of rabies vaccines for domesticated dogs and stray animal control laws, the total number of cases of canine rabies decreased substantially and wildlife rabies cases increased. Today, on the eastern coast of the USA, the predominant reservoir for rabies is the racoon. In California and the upper and lower mid-west, skunks account for the majority of animal rabies cases. Rabid bats have been diagnosed in all of the continental states of the USA.[3] A case of rabies occured recently in a bat-handler in Scotland. Outside the USA, regions of South East Asia, Africa and Latin America have large populations of stray dogs that are unvaccinated, and in these regions an unprovoked dog bite should always lead to active consideration of rabies. Figure 153.1 illustrates the predominant types of rabid animals in different regions. The World Health Organization maintains a list of countries that are supposedly rabies free or that have achieved secondary elimination (internet access: http://www.rabnet.who.int). Knowledge of this situation can help in deciding whether patients should receive postexposure prophylaxis (PEP).

INVESTIGATIONS TO CONFIRM THE DIAGNOSIS

Specific diagnostic tests may have limited value in the initial evaluation of animal bites. Radiographs can be used if there is concern about fractures or embedded foreign bodies. Cultures of fresh bite wounds are likely to produce positive results, but not all patients will develop infection. If infection results, the recovered organisms may not be the cause. Patients presenting later with established infections should be evaluated according to standard procedures for skin and soft tissue infections. Such evaluations should include a white blood

TABLE 153-1 -- Pathogens associated with bites from specific animals.

PATHOGENS ASSOCIATED WITH BITES FROM SPECIFIC ANIMALS		
Animal	**Pathogen**	**Comments**
Dog	*Capnocytophaga canimorsus*	Causes a sepsis syndrome in asplenic patients
Cat	*Bartonella henselae*	The agent of cat-scratch disease
	Pasteurella multocida	Causes an aggressive soft-tissue infection, usually within 24 hours of inoculation
Macaque	Herpesvirus simiae (B virus)	Macaques can be asymptomatic carriers, whereas the disease is fatal in other monkeys; postexposure prophylaxis not routinely recommended; documented infections can be treated with aciclovir[6]
Rat	*Streptobacilus moniliformis*	Cause of rat-bite fever; occurs 7–10 days after a bite with fever, chills, headache, rash on palms and soles, and arthritis
	Spirillum minus	Cause of rat-bite fever; longer incubation period with fever and regional adenopathy; arthritis and rash less prominent

Fresh-water species	*Aeromonas hydrophila*	Snake and leech bites have also resulted in *Aeromonas* infections
Salt-water species	*Vibrio* spp.	Can present with an ulcerating lesion at the site of inoculation and bacteremia
Mammal	Rabies	All mammals are capable of transmitting rabies by biting, but carnivores and bats predominate

Figure 153-1 Animal rabies cases by geographic region for 1998. A total of 32,342 cases are displayed here. According to WHO sources in the 34th World Survey (Via Rabnet document, 2000, WHO/CDS/CSR/APH/99.6), based upon data from 110 countries reporting out of 193 members, wildlife rabies predominates in some regions, such as the USA and Canada, whereas dogs remain a significant reservoir in many other countries. Values shown are percentages. (Note: Rabies has been diagnosed among bats in Australia but these do not appear in the above report.)

cell count and appropriate cultures that can be used later to adjust ad hoc antimicrobial therapy.

Before the onset of symptoms, there are no diagnostic tests for assessing exposure to rabies virus. Rather, to rule out the likelihood that a patient has been exposed to the virus, the biting animal should be captured and either put into quarantine for observation over a sufficient time frame or euthanized so that appropriate samples of brain tissue can be examined for the presence of rabies virus antigen, using the direct fluorescent antibody test http://www.cdc.gov/ncidod/dvrd/rabies/professional/publications/DFA_diagnosis/DFA_protocolb.htm). The length of time a domestic animal needs to be under observation to determine if it was rabid at the time of the bite

TABLE 153-2 -- Recommendation for rabies postexposure prophylaxis.[§]

RECOMMENDATION FOR RABIES POSTEXPOSURE PROPHYLAXIS			
Geographic area	**Mammals**[*]	**Prophylaxis recommendations**[†] [‡]	
		Bite	**Non-bite**
Group 1: Rabies enzootic or suspected in species involved in the exposure	Bat, racoon, skunk, fox, coyote; mongoose in Puerto Rico; stray dogs and cats along border with Mexico	Treat	Treat
Group 2: Rabies not enzootic in species involved in the exposure but reported in other animals in region (e.g. most of continental USA)	Most wild carnivores (wolf, cougar, bobcat, bear, etc.)	Treat	Treat or consult
	Domestic dogs, cats and ferrets	Observe or consult	Observe or consult
	Wild rodents and lagomorphs, except groundhogs	Consult or do not treat	Do not treat
	Livestock	Consult or do not treat	Do not treat
Group 3: Rabies not enzootic in species involved in the exposure and only sporadic reports in region (e.g. Pacific North-West)	Dogs, cats, other domestic animals and many wild terrestrial animals	Consult or do not treat	Consult or do not treat
Group 4: Rabies not reported in region (e.g. Hawaii, Guam, Samoa, Virgin Islands, etc.)	Any terrestrial mammal	Do not treat	Do not treat
The regimens are applicable for all age groups, including children. The deltoid area is an acceptable site of vaccination in adults and older children; the outer aspect of the thigh may be used in younger children. Vaccine should never be administered in the gluteal area. People who are previously vaccinated are those with a history of pre-exposure vaccination with HDCV, PCEC or RVA, prior postexposure prophylaxis with HDCV, PCEC or RVA, or previous vaccination with any other type of rabies vaccine and documented history of antibody response to the prior vaccination. HDCV, human diploid cell vaccine; RVA, rabies vaccine absorbed; PCEC, purifed chick embryo cell.			

§ *Information from Centers for Disease Control and Prevention, 1999.*[6]
* All mammals are susceptible to rabies, but reservoirs include bats and certain carnivores. Small rodent bites are common, but almost never require prophylaxis.
† 'Consult' means consultation with a knowledgeable state or local health department, especially if the animal is not available for testing or observation. If the risk of rabies in the species involved in the exposure is considered low and the animal's brain is available for testing, prophylaxis may sometimes be delayed for up to 48 hours, pending the results of laboratory diagnosis. A healthy domestic dog, cat or ferret that bites a person should be confined and observed for 10 days. Any illness in the animal should be evaluated by a qualified veterinarian and reported immediately to the local health unit. If signs suggestive of rabies develop, human prophylaxis is begun immediately. If the suspected animal is determined to be a stray dog, cat or ferret, rather than confined, it may be euthanized, the head removed and the package shipped, under refrigeration, for examination by a qualified diagnostic laboratory.
‡ Rabies may develop in ~<1 to >70% of untreated humans bitten by a rabid animal (depending in part upon species, route, dose, severity, etc.) and in ~>0.1 to 1–2% of those untreated after direct exposure to a rabid animal but not bitten (e.g. licked on an open wound or mucous membrane, or scratched).

depends on the species of animal. In practice, only domestic dogs, cats and ferrets undergo a 10-day observation period. Other animals are usually euthanized because the length of the viral shedding period has not been determined.[7] Rabies should be included in the differential diagnosis of any suspected acute progressive viral encephalitis, regardless of a history of animal bite.[8] Once a patient develops symptomatic rabies, available diagnostic tests include assays for viral antibodies in the serum or cerebrospinal fluid (CSF); viral isolation from CSF or saliva; viral antigen detection in biopsies of skin, corneal impressions or brain tissue; and reverse transcription polymerase chain reaction of saliva, CSF or related tissues (such as salivary glands or brain tissue).[3] [4] [8] [9]

IMMEDIATE MANAGEMENT

One of the most important steps in the initial management of animal bites to decrease the incidence of infection, including rabies, is aggressive cleansing of the wounds with copious irrigation and debridement of devitalized tissue. Issues that remain controversial are whether to apply primary sutures to the wound and whether to prescribe prophylactic antibiotics.

Most bites that receive medical attention within a few hours can be cleaned and sutured immediately, especially if rabies is not a concern, so as to avoid the opportunity of viral contamination to deeper tissues. Otherwise, wounds are packed, observed and either sutured later or allowed to heal by secondary intention. Prophylactic antibiotics may be indicated for some high-risk events, such as facial bites and involvement of deep structures such as tendons and joints.[5] In addition, patients should be up to date with tetanus vaccination.

A thorough history of the type of animal, the location and the circumstances surrounding the incident should be noted. Many animal bites are provoked. [5] Unprovoked attacks by animals exhibiting unusual behavior are more likely to be rabies exposures.[9] If the bite was caused by an animal that is likely to have rabies in that particular geographic location, that animal should be confined or euthanized.[9] If the animal tests positive or if it is unavailable for testing, the patient should receive rabies PEP (Table 153.2). Regardless of the length of the interval between true rabies exposure and presentation of the healthy patient, PEP should be initiated as soon as possible after the bite. Although rabies is uniformly fatal once symptoms have started, with proper intervention the disease is essentially completely preventable.

COMPLICATIONS

Figure 153.2 illustrates the case of a person from the USA who was bitten by a dog while traveling abroad.[10] There were multiple opportunities to receive PEP, yet it did not occur. Patients traveling to rabies-endemic regions who are likely to be more than 24–48 hours away from appropriate medical care are candidates for

pre-exposure vaccination.[9] They should be counseled that, despite pre-exposure vaccination, boosters would be required if they were to be bitten.

Recommendations for reducing the frequency of animal bites include both education and public policy initiatives. Potential owners of animals should be discouraged from trying to tame or handle wildlife, such as racoons or bats, and should consider more docile breeds of dog. Behavioral training should be directed at both dogs and children to ensure that animals are properly socialized and that children are taught proper conduct around animals. Responsible pet ownership should be promoted and all stray and wildlife should be avoided.[7] [9]

Figure 153-2 A preventable case of rabies in a returned traveler. The patient had been traveling for 6 months. She did not receive pre-exposure rabies vaccine and despite multiple opportunities, did not receive postexposure prophylaxis.[10]

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Chapter 154 - Leprosy

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EPIDEMIOLOGY

Leprosy is a chronic infection of the skin and nerves with *Mycobacterium leprae* which, although rarely fatal, is a significant cause of disability. Over the past decade there have been dramatic changes in the prevalence of leprosy since the introduction of multidrug therapy (MDT).[1] [2] As a result of the shorter duration of therapy and more intensive control programs, the number of registered leprosy patients receiving chemotherapy has fallen from 10–12 million to 600,000 in 2000.[3] The annual case detection rate, however, has remained unchanged at approximately 700,000 new patients/year. This contributes to the pool of 2–3 million patients with permanent nerve impairment as a consequence of leprosy.

Leprosy is widely distributed in tropical and warm temperate countries and 1.3 billion people live in regions where there is active transmission of *M. leprae*. There has been a reduction in the number of endemic countries (prevalence rate >1/10,000) from 122 in 1985 to 15 in 2000,[3] mainly in Africa, Asia and Latin America. Currently 83% of registered cases occur in only six countries:

- India (accounting for 64% of all registered cases); and
- Brazil, Myanmar, Madagascar, Nepal and Mozambique, in order.[3]

Because of the long incubation period of leprosy, an individual from an endemic country may develop leprosy years after migration elsewhere. Delay in diagnosis is usually longer in nonendemic than endemic regions and therefore leprosy should be considered as a diagnostic possibility in any person who is from an endemic country and who has chronic lesions of the skin or peripheral nerves.

Subclinical infection with *M. leprae* is far more common than overt disease.[4] Analysis of *M. leprae*-specific immune responses[5] has demonstrated that *M. leprae* infection is common after exposure, but the majority of individuals control the infection.

The major mechanism of transmission of *M. leprae* is through nasal secretions, particularly from lepromatous patients. [4] Organisms probably enter through the mucosa of respiratory tracts and, if not controlled, they disseminate to the skin and peripheral nerves. Other possible modes of transmission include breast milk from mothers with untreated lepromatous disease and rare cases of cutaneous inoculation. Although infection with *M. leprae* has been documented in wild armadilloes and some primates, zoonotic transmission does not contribute to human disease.

Proximity to leprosy patients is important in transmission, and the relative risk for disease for household contacts is 8- to 10-fold greater for lepromatous cases and 2- to 4-fold greater for tuberculoid cases.[4] Nevertheless, the majority of leprosy cases are sporadic.

Genetic factors influence both the development of leprosy and the pattern of disease. Whole genome screening has identified susceptibility loci on chromosome 10p13, close to the gene for the mannose receptor C type 1,[6] and the HLA region with linkage to both the HLA class II and tumor necrosis factor genes,[7] in Indian and Brazilian patients respectively. The HLA locus also affects the pattern of disease; HLA-DR2 and DR3 are associated with tuberculoid disease and HLA-DQ1 with lepromatous leprosy.[5] Racial and geographic factors also influence the type of leprosy, with lepromatous leprosy being less common in Africans than Indians and most common in Chinese and Caucasians.

The incidence of leprosy peaks in two age groups (10–15 and 30–60 years of age) and there is a male predominance in most regions of about 2:1.[4] The incubation period varies widely from months to over 30 years, but is usually prolonged, averaging 4 years for tuberculoid and 10 years for lepromatous leprosy. In contrast to tuberculosis, there is no definite evidence for an association between HIV infection and clinical leprosy,[8] although one study suggested an increase in HIV prevalence in leprosy patients in Tanzania.[9]

PATHOGENESIS AND PATHOLOGY

Although *M. leprae* was the second bacterium to be associated with a human disease, it still cannot be cultivated in vitro. The organism is capable of limited multiplication in mouse footpad, with a doubling time of 11–13 days, and this has permitted drug sensitivity studies. [10] *Mycobacterium leprae* is an acid-fast, Gram-positive bacillus and is an obligate intracellular parasite with tropism for macrophages and Schwann cells. The bacilli show preference for growth in cooler regions of the body. The unique characteristic of *M. leprae* is its predilection to infect Schwann cells. The receptor complex on the Schwann cell is the G-domain of the laminin a2 chain in the basal lamina of Schwann cells and the laminin receptor, a-dystroglycan.[11] A number of ligands on the surface of *M. leprae* bind to this complex, including the specific trisaccharide of phenolic glycolipid (PGL)-I and a 21kDa surface protein.

Genetic and structural analyses have confirmed that *M. leprae* is a member of the family Mycobacteriacae. A major advance has been the sequencing of genome of an Indian isolate of *M. leprae*. [12] The genome contains 1605 genes encoding proteins and 50 genes for stable RNA molecules. Remarkably, half of the functional genes in the *M. tuberculosis* genome are absent, being replaced by many inactivated or pseudogenes. This gene decay has removed entire metabolic pathways and regulatory genes, particularly those involved in catabolism. This may render the leprosy bacillus dependent on host metabolic products and may explain its long generation time and inability to grow in culture.[12] The availability of the *M. leprae* and other mycobacterial genomes has major implications for the development of new antimycobacterial drugs [13] and *M. leprae*-specific diagnostic reagents. The complex cell wall contains important targets of the immune response, including a species-specific PGL-I and immunomodulatory lipoarabinomannan. The cell wall biosynthetic pathways are relatively intact in *M. leprae*, despite the loss of other genes, indicating that these represent the essential genes for the formation of a minimal mycobacterial cell wall.[14] *Mycobacterium leprae* is relatively inert and the host immune response is responsible for most of the tissue damage.

The manifestations of leprosy form a wide clinical spectrum determined by immunopathologic responses to the organism (Fig. 154.1).[15] Patients who have the polar forms of tuberculoid

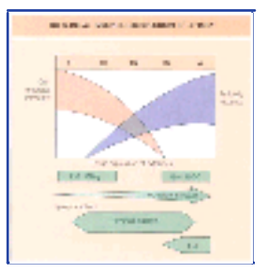

Figure 154-1 The clinical-immunologic spectrum of leprosy. This reflects the underlying host immunity as measured by the T-cell and antibody responses to *M. leprae*. Spontaneous fluctuations in the immune response are responsible for reversal reactions and erythema nodosum leprosum (ENL). TT, tuberculoid leprosy; BT, borderline tuberculoid; BB, mid-borderline leprosy; BL, borderline lepromatous leprosy; LL, lepromatous leprosy; IFN, interferon; IL, interleukin.

(TT) and lepromatous leprosy (LL) are immunologically stable, but those who have the intermediate types of borderline-tuberculoid (BT), mid-borderline and

borderline-lepromatous (BL) leprosy are immunologically unstable and subject to either a gradual decline toward the lepromatous pole or upgrading reversal reactions (RRs; see Fig. 154.1). In TT a vigorous cellular response to *M. leprae* limits the disease to a few well-defined skin patches or nerve trunks.[15] The lesions are infiltrated by interferon-? secreting $CD4^+$ T cells, which form well-demarcated granulomas containing epithelioid and multinucleate giant cells around dermal nerves.[5] Few, if any, bacilli are demonstrable. Cellular immunity may be confirmed by in-vitro lymphocyte responses to *M. leprae* antigens or skin test reactivity. Intradermal injection of heat-killed *M. leprae* causes a transient swelling at 48 hours in a sensitized subject (Fernandez reaction), followed by the development of a granulomatous nodule at 3–4 weeks (Mitsuda reaction).[5] [15] The latter confirms an individual's capacity to mount a T-cell response to *M. leprae*. Antibody responses to *M. leprae* are absent or low titer.

The hallmark of LL is the absence of *M. leprae*-specific cellular immunity and this results in uncontrolled proliferation of the bacilli with extensive infiltration of the skin and nerves and numerous lesions.[5] Histologically, the dermis contains foamy macrophages filled with multiple bacilli and a scattering of $CD4^+$ and $CD8^+$ lymphocytes, but no organized granulomas.[15] There are high titers of antibodies to *M. leprae*-specific PGL and protein antigens. In borderline cases a progressive reduction in cellular responses is associated with a greater bacillary load, more frequent skin and nerve lesions and increasing antibody levels.

The dynamic nature of the immune response to *M. leprae* is responsible for spontaneous fluctuations in the clinical pattern, termed leprosy reactions:[16]

- a type 1 reaction is usually an 'upgrading' RR caused by increased cellular reactivity to mycobacterial products, results in edema and acute inflammation of skin lesions and nerves, is most common in borderline patients and is a major cause of nerve damage; and
- a type 2 reaction or erythema nodosum leprosum (ENL) is a systemic inflammatory response to the deposition of extravascular immune complexes, leading to neutrophil infiltration and activation of complement in multiple organs,[9] and is accompanied by high circulating levels of tumor necrosis factor a and systemic toxicity.

PREVENTION

The chief means of preventing leprosy is the interruption of transmission by treating those with infectious leprosy early. Multidrug therapy was introduced because of the increasing spread of primary and secondary dapsone resistance worldwide. [1] Its advantages are its proven efficacy[2] and improved compliance, which is related to the limited duration of therapy and its monthly observed component (see Management, below). Furthermore, early treatment before the onset of nerve damage reduces the long-term disability associated with leprosy.[3] [17] The effectiveness of MDT has prompted a World Health Organization (WHO) co-ordinated campaign to implement MDT in all endemic countries, with the aim of reducing the prevalence rate of leprosy to less than 1/10,000.[3] This has been successful, with 15 countries left to attain this goal. Importantly, however, the case detection rate has not yet fallen, probably because of the prolonged incubation period of clinical leprosy, indicating that control programs must be sustained.

The response to immunization with *M. bovis* BCG has been variable, but in a major trial in Malawi BCG induced 50% protective efficacy against clinical leprosy, both tuberculoid and lepromatous forms.[18] Reimmunization enhanced the protective effect by a further 50%. The benefit of BCG has been confirmed in subsequent case-control studies. Extensive BCG immunization of children in endemic countries has probably made a significant contribution to the decline of leprosy. The addition of heat-killed *M. leprae* to BCG did not increase the observed protective efficacy of BCG in two trials.[18] Other experimental vaccines are being tested against leprosy infection at present.

Leprosy is commonly associated with poverty and overcrowding, and improved socioeconomic conditions have contributed to the decline of leprosy in Europe and some Asian countries.

CLINICAL FEATURES

Types of leprosy

Indeterminate leprosy

This is the earliest form and occurs as a single slightly hypopigmented, ill-defined macule in children, who are often contacts of leprosy patients.[19] The majority of these lesions are self-limiting and resolve without therapy. A minority (<25%) develop into defined lesions within the clinical spectrum.

Tuberculoid leprosy

These lesions occur as 1–3 large asymmetric macules or plaques with sharply defined borders and hypopigmented anesthetic centers (Fig. 154.2).[19] [20] Although leprosy lesions are usually hypopigmented, in light skins the macules may appear erythematous or dyschromic. Involvement of sweat glands and hair follicles results in dryness and loss of hair. Enlarged cutaneous nerves may be palpable at the edge of the lesion, but nerve trunk involvement is minimal.

Figure 154-2 Tuberculoid leprosy. Single hypopigmented anesthetic plaque with raised border and dry surface.

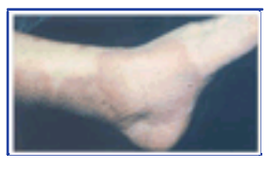

Figure 154-3 Borderline tuberculoid leprosy. Three large well-defined erythematous patches with reduced sensation, spreading borders and satellite lesions.

Borderline tuberculoid leprosy

This is the commonest form of leprosy. The skin lesions resemble those in TT leprosy, but are more frequent and variable in appearance and their borders are less well demarcated (Fig. 154.3). The outline may be irregular with adjacent 'satellite' lesions suggesting local spread. Occasionally, large patches of BT leprosy may involve a whole limb. Asymmetric enlargement of several peripheral nerves is usual and patients may present with muscle weakness or trauma secondary to sensory impairment. Progressive nerve damage is common.

Mid-borderline leprosy

This is the most immunologically unstable form with the propensity to shift rapidly toward BT leprosy during a reversal reaction or to downgrade toward BL leprosy. The skin lesions are numerous and vary in size, shape and distribution. They may be hypopigmented or erythematous. The characteristic 'target' lesion has a broad, erythematous border with a vague outer edge and 'punched-out' pale center with sensory impairment (Fig. 154.4).

Borderline lepromatous leprosy

In borderline lepromatous leprosy there are numerous small erythematous macules, which initially may be limited in distribution, but become progressively more symmetric.[19] Papules, nodules and succulent plaques may develop and, in contrast to tuberculoid leprosy, the lesions have normal sensation. The intervening skin is normal. Widespread nerve involvement is typical, especially if the patient has downgraded from BT leprosy.

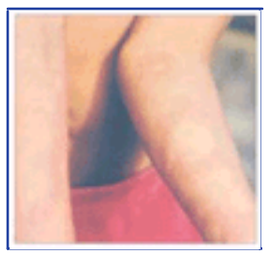

Figure 154-4 Mid-borderline leprosy. Characteristic target lesion with raised erythematous annular border and 'punched-out' central area with impaired sensation.

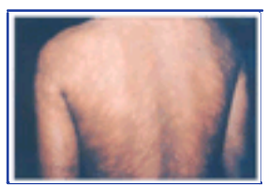

Figure 154-5 Lepromatous leprosy. Multiple, small, slightly erythematous macules with intact sensation and symmetric distribution. The skin smears of both the lesions and intervening skin are positive for acid-fast bacilli.

Lepromatous leprosy

This is a systemic disease with a generalized bacteremia leading to widespread involvement of the skin and other organs.[19] The first manifestation may be a diffuse infiltration of the dermis, causing a smooth shiny appearance of the skin. More typically, there are numerous symmetrically distributed macules, papules or nodules (Fig 154.5 and Fig 154.6). Progressive thickening of the skin results in coarsening of the facial features and nodular thickening of the ear lobes. With time the eyebrows and eyelashes become thinned.

Bacillary infiltration is responsible for gradual tissue damage in the involved organs. The nasal mucosa is infiltrated at an early stage, resulting in discharge and obstruction. Erosion of the cartilage and nasomaxillary bones results in perforation of the nasal septum, collapse of the nose and saddle-nose deformity. Laryngeal involvement produces hoarseness and stridor. Direct bacillary involvement of the eye causes keratitis and iritis.

Infiltration of the dermal nerves results in a peripheral sensory loss similar to that of a 'glove and stocking' neuropathy,[19] which leaves the skin susceptible to ulceration and secondary infection. Reactional episodes cause edema of the feet, shins and hands. Dactylitis develops in the hands and feet and, together with trauma and osteomyelitis, results in phalangeal erosion.

Both testicular infiltration and orchitis contribute to testicular atrophy and secondary gynecomastia. Glomerulonephritis may occur and is usually associated with ENL. Secondary amyloidosis is a consequence of recurrent ENL reactions.

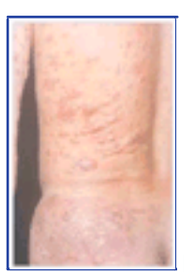

Figure 154-6 Nodular lepromatous leprosy. Diffuse infiltration of the skin by multiple nodules of varying size, each teeming with bacilli.

Peripheral nerve involvement

The nerves of predilection occur at superficial sites where the nerve trunks are cooler, more readily traumatized and often anatomically constricted. [19] These include the:

- ulnar nerve at the medial epicondyle of the humerus,
- median nerve at the wrist,
- lateral popliteal nerve at the neck of the fibula,
- posterior tibial nerve behind and inferior to the medial maleolus, and
- radial nerve in the humeral groove posterior to the deltoid insertion.

Easily palpated superficial cutaneous nerves include the:

- superficial radial nerve at the wrist,
- greater auricular nerves,
- supraorbital nerve, and
- sural nerves.

These nerves should be examined for enlargement and associated weakness and sensory loss. The resulting muscle imbalance leads to the characteristic deformities of clawhand, footdrop, clawtoes and wristdrop. Autonomic nerve dysfunction contributes to impaired sweating and dry skin, which is subject to cracking, infection and poor healing. The combination of insensitive feet and clawtoes leads to recurrent plantar ulceration, a major cause of disability.

In pure neural (PN) leprosy the nerve trunks are affected without any skin lesions. On biopsy the neural lesions tend to be 'lepromatous' in appearance and PN leprosy involving more than one nerve should be treated as multibacillary (MB).

Before and during therapy the function of the commonly involved nerves should be assessed at regular intervals by voluntary muscle and sensory testing (preferably with nylon monofilaments) to determine whether there is ongoing nerve function impairment. This may presage the onset of a reversal reaction before nerve pain or typical skin lesions develop. Nerve function impairment may develop or worsen despite effective chemotherapy, and early recognition and therapy prevent permanent nerve damage.[16] [17] Patients with pre-existing nerve damage at diagnosis and MB patients are at greatest risk for new nerve function impairment and should be carefully monitored.[17]

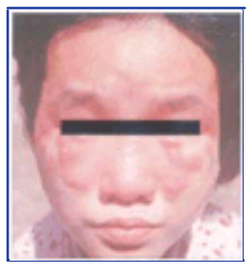

Figure 154-7 Reversal reaction. Erythema and edema in the facial lesions of a patient who has borderline-tuberculoid leprosy undergoing an upgrading reversal reaction.

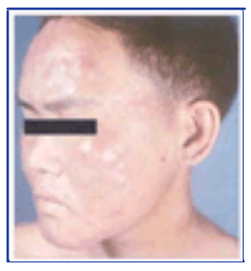

Figure 154-8 Erythema nodosum leprosum. Tender papules associated with fever, arthralgia and acute neuritis in a patient who has lepromatous leprosy.

Leprosy reactions

Reversal reactions

These develop in about one-third of patients who have BT-BL leprosy, usually within the first 6 months of treatment.[16] They present with:

- increased inflammation in established BT-BL skin lesions or new swollen lesions in BL and subpolar LL patients (Fig. 154.7),
- acute neuritis with pain or tenderness in the involved nerve and loss of function, and
- recent (<6 months) or progressive nerve function impairment in the absence of painful nerves.

Silent neuritis responds to therapy for the RR.[21] Patients who have a particular risk of developing a RR are those who have facial patches, more extensive disease involving more than two body areas or *M. leprae*-specific IgM anti-PGL antibodies.

Erythema nodosum leprosum

This once affected 30–50% of BL and LL patients, but the frequency and severity of ENL have reduced since the regular use of clofazimine in MDT.[2] It may develop at any stage of therapy, but usually within the first year, and is often recurrent. An episode begins with fever and malaise and the rapid emergence of painful erythematous nodules, typically over the extensor surfaces of the limbs.[20] In severe cases widespread nodules may form pustules and ulcerate (Fig. 154.8). Painful neuritis is the most common complication. Erythema nodosum leprosum has features of widespread immune complex deposition and these may include small vessel vasculitis, iridocyclitis, polyarthritis, orchitis, lymphadenitis and glomerulonephritis.

Recurrent or uncontrolled ENL reactions can result in the development of secondary amyloidosis (amyloid A protein) within 3 months.

Eye involvement

Involvement of branches of the facial and trigeminal nerves results in lagophthalmos and corneal anesthesia, respectively, and if combined there is a considerable risk of ulceration and infection of the exposed insensitive cornea. [22]

In 25–30% of patients who have LL, infiltration of the anterior segment of the eye causes a superficial punctate keratitis and iridocyclitis, which may be painless and only recognized by slit-lamp examination. Iridocyclitis is exacerbated during episodes of ENL, but can occur independently of overt reactions. The iritis may be complicated by glaucoma or cataract, both of which contribute to leprosy-associated blindness (see Chapter 20).

DIAGNOSIS

A diagnosis of leprosy is usually straightforward if it is suspected as a cause of any skin or peripheral nerve lesion in a person from an endemic country. The cardinal signs of leprosy[19] [20] are:

- skin patch with sensory loss,
- nerve enlargement, and
- acid-fast bacilli (AFB) in the skin.

The presence of one or more of these features establishes the diagnosis, which should be confirmed with a full-thickness skin biopsy. Approximately 70% of all leprosy patients can be diagnosed by the single sign of a skin patch with sensory loss, but 30% of patients, including many MB patients, may not present with this sign, indicating the importance of nerve enlargement as an additional sign and the importance of clinical suspicion for the diagnosis.

Acid-fast bacilli are best demonstrated in slit-skin smears, which should be taken from the edges of at least two lesions and both ear lobes.[23] If these are not available a skin biopsy should be stained for AFB with a modified Wade-Fite stain. The extent of the bacillary load can be quantitated as a bacterial index[10] on a logarithmic scale of 1+ to 6+. The percentage of solid staining AFB in smears, the morphologic index,[10] is an indirect measure of the viability of leprosy bacilli. In PN leprosy a biopsy from a sensory nerve such as the superficial radial nerve may be diagnostic. Polymerase chain reaction (PCR) can be used to identify *M. leprae* DNA and, together with PCR-based detection of rifampin (rifampicin)-resistant strains, it is a valuble tool for epidemiologic studies.[24]

Lepromin testing and serology may be used for accurate classification of patients in research studies. Antibodies to PGL and other *M. leprae*-specific protein antigens are present in MB patients and their titer falls with effective therapy.[5] In patients who have BL and LL, evidence of chronic inflammation includes anemia, hypergammaglobulinemia, elevated serum amyloid A protein and positive antinuclear and anticardiolipin autoantibodies.

Other skin diseases can be differentiated from tuberculoid leprosy by the absence of anesthesia in the lesions and the presence of nerve involvement elsewhere.[20] [23] Lepromatous skin lesions are not anesthetic and biopsy may be necessary to distinguish these from those due to other systemic infections such as leishmaniasis and secondary syphilis and other nodular or infiltrative skin conditions. Other causes of nerve enlargement such as primary amyloidosis and familial polyneuropathy are excluded by biopsy and family history.

MANAGEMENT

Successful management of leprosy requires prolonged drug treatment and careful monitoring for complications and it is essential to enlist the patient as an ally in this process. The patient should be educated about:

- the importance of compliance,
- the first symptoms of a reaction, and
- the elements of self-care needed to prevent secondary tissue damage if there is sensory nerve impairment.

The most important step in disability prevention is the early initiation of bactericidal drug therapy.

Antileprosy drugs

Dapsone

This is an important antileprosy drug because of its bactericidal effect at full dosage, low cost and low toxicity.[2] When used alone stepwise dapsone resistance emerges as a major problem,[1] but this is prevented by combination therapy. Mild hemolytic anemia is common, but is only severe in the presence of glucose-6-phosphate dehydrogenase deficiency, which should be excluded where possible. Occasionally dapsone allergy, and rarely agranulocytosis, may develop after 2–6 weeks.

Rifampin

Rifampin is a key component of MDT because it is the most effective bactericidal drug against *M. leprae* when given either daily or monthly.[2] [10] Toxicity is low with monthly dosage, although thrombocytopenia, hepatitis and a flu-like syndrome occasionally occur. It must be used with at least one other effective drug to prevent rifampin resistance. Tuberculosis should always be excluded before monthly rifampin is started (see Chapter 37 and Chapter 202). Rifapentine is a long-acting rifamycin-derivative that is more bactericidal than rifampin in mice and is currently being tested in humans.

Clofazimine

This is a fat-soluble dye that is deposited within the skin, fat stores and macrophages. It has similar bactericidal activity to that of dapsone, [2] [15] and also a significant anti-inflammatory effect. It is relatively nontoxic and its only disadvantage is the associated development of a reddish skin pigmentation, which resolves after the drug has been discontinued. When used in high doses for prolonged periods, clofazimine is deposited in the small intestinal wall and can cause diarrhea and pain.

Other drugs

Three additional drugs have proved effective against *M. leprae* in human and mouse studies:[2] [23]

- ¦ the fluoroquinolones ofloxacin and moxifloxacin have moderate bactericidal activity and infrequent side-effects involving the gastrointestinal tract and central nervous system;
- ¦ minocycline, the only fat-soluble tetracycline, has moderate anti-*M. leprae* activity. It has proved effective in patients with LL and has low toxicity in adults when used as long-term therapy for acne and so is a useful alternative drug for leprosy; and
- ¦ clarithromycin has modest bactericidal activity.

Multidrug therapy

The principle underlying MDT is the use of three drugs when the bacterial load is high in MB leprosy to treat and prevent the emergence of dapsone-resistant strains. Two drugs are sufficient for paucibacillary disease. Since its introduction in 1982,[1] MDT has proved highly effective and over 10 million patients have been treated with few treatment failures and remarkably low relapse rates of about 0.1/100 patient-years.[2] [3] [25]

Multibacillary multidrug therapy

This is recommended for adult patients with BB, BL, LL, smear-positive BT and PN leprosy. In leprosy control programs in endemic

countries, a simplified form of classification is employed, based on the number of patches, so that MB leprosy >5 patches and paucibacillary (PB) "5 patches. Multibacillary MDT comprises:

- ¦ rifampin, 600mg once a month, supervised administration;
- ¦ dapsone, 100mg/day, self-administered; and
- ¦ clofazimine, 300mg once a month, supervised administration; 50mg/day, self-administered.

Originally, MB-MDT was continued for at least 2 years and then until the skin smears became negative.[1] In subsequent field trials a fixed duration of MB-MDT for 2 years was as effective[2] and this was utilized in control programs with very low rates of relapse.[25] Patients who have MB leprosy and a skin smear bacterial index of 4+ or over may require a longer duration of therapy.[2] More recently in 1998, the WHO recommended that MB-MDT of 12 months duration may be sufficient for use in control programs.[25] Although the results of continuing clinical trials comparing 12 and 24 month duration MB-MDT are not available as yet, 12 month MB-MDT is currently used in leprosy control programs in endemic countries.

Some authorities in developed countries prefer to use more frequent doses of rifampin and continue to treat until the smears are negative, which can take 5–8 years. One approach is to double the dose of rifampin to 600mg daily for two consecutive days each month,[23] while others use daily rifampin 450–600mg for 2–3 years with dapsone and clofazimine. There is no evidence, however, that daily rifampin is more effective than when given once monthly.

If clofazimine is unacceptable because of pigmentation or if dapsone hypersensitivity occurs, minocycline (100mg daily) or ofloxacin (400mg daily) may be substituted.[25] Patients who have rifampin intolerance require two new drugs, minocycline and ofloxacin, along with clofazimine (50mg/day) for 6 months and then either drug with clofazimine for another 18 months.

Paucibacillary multidrug therapy

This is recommended for indeterminate, TT and smear-negative BT leprosy[2] and in the control programs for patients with "5 patches. PB-MDT comprises:

- ¦ rifampin, 600mg once a month, supervised administration; and
- ¦ dapsone, 100mg/day, self-administered.

This is continued for 6 months. If the skin smear is positive at any site, the patient is given MB-MDT. If a RR develops after completion of chemotherapy, then MDT should be recommenced while on prednisone.[23]

In some countries patients with solitary leprosy skin lesions are common. A large field study in India established that the combination of rifampin (600mg), ofloxacin (400mg) and minocycline (100mg) was almost as effective as PB-MDT in the treatment of patients with single smear-negative skin lesions and no nerve involvement, although the follow-up period was only for 18 months.[26] This regimen is helpful for treating carefully selected patients in endemic countries with a high proportion of single lesion cases, but should be reserved for this setting.

Treatment for reactions

Patients who have RRs, including silent nerve function impairment, require high-dose corticosteroids for a prolonged duration to permit nerve function recovery.[16] Prednisone is started at 40mg/day and increased to 60mg/day if there is no response, and then to 120mg/day if necessary. Once there is evidence of improvement on serial voluntary muscle and sensory testing, the dose is reduced over 6 weeks to 20mg/day and this is continued for some months before gradual removal. Therapy is usually required for 4–6 months, but often for longer durations in MB leprosy.[23] It is important to maintain treatment with antimycobacterial drugs to reduce the bacillary load. Adequate analgesia is essential along with physical support during the period of active neuritis. This therapy can be successfully administered without admission if other infections and medical problems are excluded. The expected recovery rate for nerve function is 60–70%,[16] but may be up to 88% in patients with no nerve damage at diagnosis who develop acute neuropathy during MDT.[27] Recovery is less in those with pre-existing nerve function impairment or with chronic or recurrent reactions.

Mild ENL responds to aspirin or nonsteroidal anti-inflammatory drugs, increased clofazimine dosage and rest. Moderate or severe episodes and those with neuritis require prednisone, usually starting at 40–60mg/day.[16] The response is rapid, but as ENL is liable to become corticosteroid dependent the prednisone should be withdrawn over 2–3 months. Clofazimine at a higher daily dose of 300mg suppresses ENL after 4–6 weeks and can be used to prevent further episodes.

If the ENL is poorly controlled or recurs, it usually responds to thalidomide, 400mg/day for 2–3 weeks, and then 100–200mg/day as maintenance. [16] Thalidomide inhibits the release of tumor necrosis factor from macrophages and results in prompt relief. Its use, however, is severely limited by its teratogenicity and should be restricted to male and postmenopausal patients under strict supervision. Thalidomide may cause a peripheral neuropathy, but this has not been reported in leprosy patients.

Eye involvement is common and iritis requires local treatment with corticosteroid and atropine drops.[22]

Other therapies

Prevention of disability is an important component of care.[28] Regular monitoring of nerve function will reveal early reversible RRs. Patients with irreversible nerve function impairment must learn to care for insensitive hands and feet and be provided with appropriate footwear. Plantar ulceration requires prolonged rest for healing. Physiotherapy and reconstructive surgery for clawhands and clawfeet, footdrop and lagophthalmos may prevent further tissue damage and restore appearance, and facial deformity can be corrected by plastic surgery.[28] Community-based rehabilitation is proving effective in assisting patients with persistent nerve impairment to return to full participation in their own communities.[29]

Websites

http://genolist.pasteur.fr/Leproma. Web-based tool for extracting information about annotations from the *M. leprae* genome database. http://www.who.int/lcp/. Provides access to WHO documents on the epidemiology and treatment of leprosy, including the 7th Report of WHO Expert Committee on Leprosy, and information on MDT and leprosy elimination campaigns.

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Chapter 155 - Ectoparasites

Wallace Peters

EPIDEMIOLOGY

Definition and nomenclature

In the strictest sense, an ectoparasite is defined as a parasite that derives its nourishment from the skin, as distinct from an endoparasite, which lives inside the body of the host. For this chapter, the definition has been adjusted to include both parasites that derive their nourishment from the skin and parasites that live within the skin and subcutaneous tissues. Not included here are skin-invading protozoa of the genus *Leishmania* that are relatively common in returning travelers from endemic areas (see Chapter 172). Purely hematophagous parasites, such as mosquitoes and ticks (see, for example, Chapter 11 , Fig. 11.3 and Chapter 166 and Chapter 179) are of major public health importance, especially as vectors of viral and protozoal diseases, and many groups of these invertebrates are more or less cosmopolitan. However, the parasites themselves are most unlikely to be found in direct association with travelers returning to temperate climates from endemic tropical and subtropical areas. The reader is referred, therefore, to Chapter 14 , Chapter 166 and Chapter 179 & Chapter 247 and other publications for information on these parasites.[1] [2] [3] This chapter particularly considers the various species of arthropods that may invade and may become apparent in the returned traveler (Table 155.1).

Geographic distribution

The arthropods referred to below, some of which have relatively well-defined geographic distributions in warm regions (see Table 155.1), are often found in returning travelers. Current infestations with mites and ticks that have been acquired abroad are rarely seen, although their sequelae may still be apparent. However, myiasis such as that caused by *Cordylobia anthropophaga*, also called the Tumbu or mango fly, is common in parts of sub-Saharan Africa and that caused by *Dermatobia hominis*, the human botfly, is not infrequently acquired by travelers to rural areas of Central and South America. The Tumbu fly lays batches of 200–300 eggs on dry soil in shaded areas. Domestic dogs are most frequently infested by the first instar larvae, but humans are also commonly infected. *Dermatobia hominis* infests many species of domestic and wild animals and is a serious pest of cattle in some endemic areas of the New World.

The sand, chigoe or jigger flea, *Tunga penetrans*, has a wide distribution within Central and South America, west and east Africa as well as some parts of the Indian subcontinent. The larvae are found mainly in dry, sandy soil where they mature into very small adults that actively seek a mammalian or avian host. Pigs are commonly infested in or around houses, where humans are also readily attacked.

Infestation with other arthropods, such as the New World screw-worm *Cochliomyia hominivorax*, formerly a very serious, destructive pest of cattle and occasionally of humans, as well as the Congo floor maggot *Auchmeromyia senegalensis*, are now rare and unlikely to be acquired by travelers.[1] [4] Myiasis caused by other species of flies (see Table 155.1) is uncommon and not likely to be found in the returning traveler. Any suspicious object recovered from such indi-viduals should, however, be referred for specialist identification.

Mites such as *Sarcoptes scabiei* and harvest mites of various species are cosmopolitan.[5] [6] Scabies or pediculosis caused by body, head or public lice (Fig 155.1 and Fig 155.2) may of course be acquired during travel anywhere through contact with infected people and may be diagnosed only after return home. The bites of anopheline mosquitoes may result in malaria that becomes manifest only after some time, as may certain viral infections transmitted by mites, ticks, mosquitoes or other hematophagous ectoparasites[1] [3] [7] [8] (see also Chapter 166 Chapter 179 & Chapter 247 , Fig 247.1 , Fig 247.2 , Fig 247.3 , Fig 247.4 , Fig 247.5 , Fig 247.6 , Fig 247.7 , Fig 247.8 , Fig 247.9 , Fig 247.10 , Fig 247.11 , Fig 247.12).

PATHOGENESIS AND PATHOLOGY

Immunologic responses of the skin

The skin is a complex organ with complicated immune regulatory and effector functions, which involve both cellular and humoral responses to invasion by ectoparasites.[9] In order to survive within the skin and subcutaneous tissues, the parasites have evolved a number of ways in which they, in turn, modulate the host's immune response. Invading larvae of myiasis-causing flies produce proteases that facilitate their penetration of the dermal tissues as well as providing nutrition. The host response to the foreign proteins is intense and comprises a massive cellular response to both the foreign protein and the bulky, growing organism, with eosinophils and macrophages being predominant.[5] Although much research has been conducted in recent years into the parasite-host relationship of invasive arthropods, it has been directed mainly at those of veterinary importance and little detail is known about the parasites that affect humans. In areas endemic for Tumbu flies, for example, a marked level of cellular immunity, first localized but later more general, develops after prolonged and repeated exposure. [5]

Pathology

The pustular skin lesions associated with invasive larvae of myiasiscausing flies present a similar histologic picture of a fistulous track leading to a dermal cavity. This is densely infiltrated by macrophages, eosinophils and plasma cells. As the larva grows, it extends its posterior end toward the surface, through which its respiratory tubes project in the form of a pair of spiracles in a chitinous plate. The general appearance is of an acutely inflamed furuncle. As such larvae mature they push outward and are eventually extruded to the exterior, where they fall to the ground and pupate.

The pathology of *T. penetrans* is different since it is the adult female that actively burrows its way into the skin of the feet or under the toenails until her head reaches the papillary dermis. The posterior end of the insect, which bears the respiratory spiracles and sexual organs, remains at the surface. As the flea feeds and grows, its eggs develop until its abdomen becomes a pea-sized sac from which several hundred eggs are released to the exterior, a few at a time. The flea becomes encased in an inflammatory, foreign body granuloma, which may be surrounded by microhemorrhages.

TABLE 155-1 -- Classification, common names and geographic distribution of the major ectoparasites that affect humans.

CLASSIFICATION, COMMON NAMES AND GEOGRAPHIC DISTRIBUTION OF THE MAJOR ECTOPARASITES THAT AFFECT HUMANS					
Class	**Order/suborder**	**Genera and species**	**Common name**	**Geographic distribution**	**Notes**
Arachnida	Acari (mites)	*Trombicula* spp.	Scrub mites	Cosmopolitan	Include vectors of viruses, rickettsias
		Sarcoptes scabei		Cosmopolitan	Cause of scabies
	Acari (ticks)	Ixodidae and Argasidae	Hard and soft ticks	Cosmopolitan	Include many vectors of viruses, rickettsias, *Borrelia, Babesia, Ehrlichia* spp.

Insecta	Diptera	Many genera and species	Including biting and myiasis flies		
	Nematocera	Numerous genera and spp.	Mosquitoes, *Simulium*, etc.	Cosmopolitan	Major vectors of malaria, viruses, helminths
	Brachycera	*Chrysomyia bezziana*	Old World screw-worm	Widespread in tropical Africa, South East Asia and the south west Pacific	Common cause of cutaneous myiasis in South East Asia
		Cochliomyia hominivorax	New World screw-worm	Central and South America	Causes massively destructive myiasis
		Lucilia spp.	Greenbottles	Cosmopolitan	Facultative wound myiasis
		Calliphora spp.	Bluebottles	Cosmopolitan	Facultative wound myiasis
		Auchmeromyia senegalensis	Congo floor maggot	Sub-Saharan Africa	Sucks blood but no myiasis
		Cordylobia anthropophaga	Tumbu, mango fly	Sub-Saharan Africa	Causes furuncular myiasis
		Wohlfahrtia spp.	Flesh flies	Mainly in the South	Can cause tissue destruction
				Palearctic region	
		Dermatobia hominis	Human botfly	Central and South America	Causes furuncular myiasis
		Oestrus ovis	Sheep nasal botfly	Cosmopolitan	Larvae may invade nose, eye
		Hypoderma spp.	Warble, botflies	Cosmopolitan	Causes 'creeping eruption'
	Siphonaptera	Numerous genera and spp.	Fleas	Cosmopolitan	Vectors of plague, rickettsias, helminths
		Tunga penetrans	Chigger, jigger flea, chigoe	Central and South America, tropical Africa, India	Female invades dermis (especially of the foot) to develop and lay eggs
	Phthiraptera	Numerous genera and spp.	Biting and sucking lice	Cosmopolitan	
		Pediculus humanus	Body and head louse	Cosmopolitan	Vector of rickettsias, *Borrelia* spp.
		Phthirus pubis	Pubic louse	Cosmopolitan	
Parasites that derive their nourishment from the skin and parasites that live within the skin and subcutaneous tissues are included.					

Figure 155-1 Adult *Pediculus humanus corporis* (the body louse) feeding. These insects are not only a source of considerable skin irritation but also the vectors of epidemic typhus and trench fever. *Courtesy of Dr med H Lieske. With permission from Peters and Pasvol.*[7]

Figure 155-2 The 'crab louse', *Phthirus pubis.* The crab louse, which is commonly acquired during sexual intercourse, infests not only the public region but also other sites, including the eyelashes. *Courtesy of Dr med H Lieske. With permission from Peters and Pasvol.*[7]

PREVENTION

The newly hatched larvae of *C. anthropophaga* live on the ground where they are attracted to warm skin, which they can rapidly penetrate with the aid of oral hooks and backward pointing body spines (Fig. 155.3). They are also attracted to clothing, for example underwear bearing human body odors. Prevention of infection involves not lying directly on potentially contaminated ground as well as not laying washing on the ground to dry, a common practice in many tropical areas.

Dermatobia hominis (Fig. 155.4) has a more complex life cycle. The female lays its eggs individually on various species of biting flies. When these take a blood meal the eggs hatch and the emerging larvae rapidly invade the mammalian host's skin, in which they begin to develop. No rational method of prevention is therefore available. The avoidance of sites that are known to be frequented by *T. penetrans*, for example chicken houses and farm buildings, and especially the wearing of solid footwear help to prevent infection with this flea. It is also important not to disperse eggs when removing the adult females (see below).

CLINICAL FEATURES

The clinical features of furuncular myiasis are similar for all species. The infection site is inconspicuous at first but a small, reddened papule develops within about 24 hours. The papule itches intermittently but especially at night. As the larva grows within the dermal cavity over the next 2–3 weeks a dome-like swelling develops on the skin surface

Figure 155-3 Third instar larva of *Cordylobia anthropophaga*, the Tumbu fly. The powerful mouth hooks, with which the larva feeds, are seen as long, dark bars. *With permission from Peters.*[1]

Figure 155-4 Adult female *Dermatobia hominis.* The fly lays eggs on blood-sucking insects such as mosquitoes or on ticks. After about 1 week larvae hatch from the eggs to infest the skin of a human or other warm-blooded host, which is fed on by the phoretic host. *Courtesy of Dr AJ Shelley. With permission from Peters.*[1]

with a surrounding area of inflammatory edema. Itching may become intense and a throbbing pain may be present. At the center of the dome a hole develops, through which the posterior tip of the larva bearing its spiracles emerges, together with a purulent, bloody exudate. As the larva grows it moults twice; this process is often accompanied by severe pain. The clinical course runs for 14–16 days in infestation with *C. anthropophaga* but up to 1 month with *D. hominis*. Lymphangitis and regional lymphadenopathy are common from about weeks 2–4 of infestation. During this time the larva repeatedly protrudes its posterior end through the hole in the skin but rapidly withdraws if contacted (Fig. 155.5). Eventually the mature larva forces its way out of the skin, causing little pain, and drops to the ground to pupate. The skin lesion usually heals rapidly and only a pigmented scar marks the site of the lesion (Fig. 155.6).

Infection with *T. penetrans* initially causes itching and sometimes pain; multiple lesions are common. The sites most commonly attacked are the skin of the feet and under the toenails. As the insect grows it produces a firm nodule, the surface of which becomes hyperkeratotic with a central, black spot. This is the site of the projecting spiracles. Secondary infection with pyogenic bacteria is common and may be associated with lymphangitis and regional lymphadenopathy. Infection with tetanus bacilli is a particular hazard.

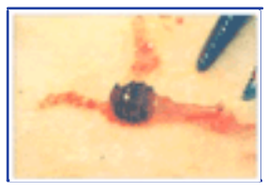

Figure 155-5 Second instar larva of *Dermatobia hominis* after surgical removal. The characteristic rows of dark spines are seen clearly. *Courtesy of Dr RP Lane. With permission from Peters and Pasvol.*[1]

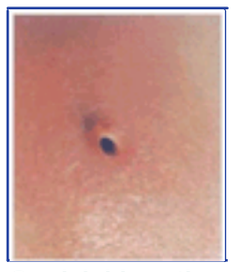

Figure 155-6 Cavity left in the skin of the back of a woman who returned to Europe from an African holiday with multiple furuncular lesions caused by larvae of *Cordylobia anthropophaga.* Note the marked surrounding inflammation. *Courtesy of Professor T Rufli. With permission from Peters.*[1]

DIAGNOSIS

Any pustule of several days' or weeks' duration in a returning traveler should lead to a presumptive diagnosis of myiasis, and the travel history will immediately give a clue to the possible species involved. The presence of a central dark point in the pustule containing a spiracular plate or even a protruding larval extremity confirms the diagnosis but a specialist examination of any larva removed from the lesion may be required to confirm the species involved. Similarly, the presence of tender nodules on the feet or under the toenails should give rise to a possible diagnosis of tungiasis. In this case, too, the posterior end of the flea may be observed in the center of the skin overlying the nodule.

The differential diagnosis of myiasis includes infection with pyogenic bacteria or an embedded foreign body with secondary infection. Lesions of tungiasis may resemble plantar or subungual warts or acute infective paronychia. Multiple, serpiginous, subcutaneous lesions, especially on the feet (Fig. 155.7), buttocks or hands should give rise to a suspicion of skin invasion by ground-dwelling, infective larvae of dog hookworms ('creeping eruption', see Chapter 174 and Chapter 246).

MANAGEMENT

Attempts to express the embedded larvae of flies such as *C. anthropophaga* or *D. hominis* by squeezing the pustules are likely to do more harm than good. If the surface pore through which the spiracles of *C. anthropophaga* protrude is covered with paraffin for some hours, the larva can sometimes be pulled out with forceps (Fig. 155.8) but this is rarely successful with *D. hominis*.

In endemic areas of Central and South America, it is claimed that the larva can be tempted to emerge if the lesion is covered with a piece of pork fat for 24 hours or so, but this is not a method that one would suggest for use in returning travelers! In most cases it is best to

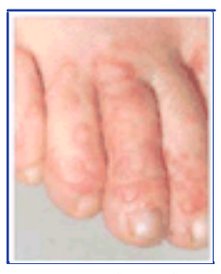

Figure 155-7 Cutaneous larva migrans ('creeping eruption') due to invasion of infective larvae of the dog hookworm *Ancylostoma caninum.* This condition may be mistaken for infestation with the larvae of ectoparasitic arthropods. The lesions respond rapidly to treatment with albendazole or ivermectin. *With permission from Peters and Pasvol.*[7]

remove the larva surgically after infiltrating round the lesion with an appropriate local anesthetic (see Fig. 155.5). This also facilitates removal of the larva because it is anesthetized. If necessary, the cavity can be curetted and, if it is large, it can be stitched, after which it can be left to granulate. The wound usually heals in about 1 week, leaving a pigmented scar.

Gravid *T. penetrans* (Fig. 155.9) should also be removed surgically. Inhabitants of endemic areas often extract the flea with a sharp sliver of bamboo or a razor blade, frequently inadvertently breaking open the insect's abdomen in the process and releasing the eggs to continue the insect's life cycle. The residual cavity in the skin, which must be kept clean to minimize secondary bacterial contamination, usually heals rapidly but secondary infection may require appropriate antibiotic therapy.

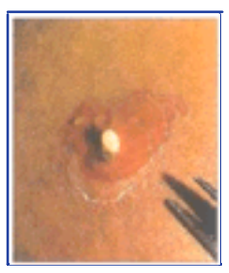

Figure 155-8 Extracting a larva of *Cordylobia anthropophaga* after covering it with paraffin. The pair of black spiracles can just be seen in the center of the posterior tip of the larva. *Courtesy of Professor A Bryceson. With permission from Peters and Pasvol.*[7]

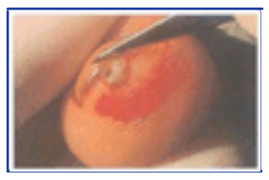

Figure 155-9 Surgical extraction of a gravid female *Tunga penetrans.* Care must be taken not to disrupt the abdomen and release the eggs. *Courtesy of Professor C Curtis. With permission from Peters.*[1]

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Chapter 156 - Endemic Treponematoses

André Z Meheus
Om P Arya

The endemic treponematoses include yaws (also known as buba, framboesia, parangi and pian), endemic syphilis (also known as bejel, dichuchwa and sklerjevo) and pinta (also known as azul, carate and mal de pinto), all of which are chronic bacterial infections. The causative organisms (*Treponema pallidum* subsp. *pertenue, T. pallidum* subsp. *endemicum* and *Treponema carateum*, respectively) are morphologically and serologically indistinguishable from *T. pallidum* subsp. *pallidum*, which is the causative organism of venereal syphilis.

Small genetic differences, (a single base pair change) have been identified between the organisms of the venereal and nonvenereal (endemic) treponematoses. [1] [2] [3] However, none of these genetic variations identified so far can differentiate one subspecies from another and it remains unresolved whether they are all the same or different organisms. Nevertheless, there are significant clinical and epidemiologic differences.[4]

EPIDEMIOLOGY

Historic perspective

The endemic treponematoses, because of the disfigurement and disability they cause, were a major public health problem in the preantibiotic era. In 1948, the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) sponsored a global control program. This involved 46 countries and brought these diseases under control with the help of long-acting penicillin. Unfortunately, the diseases were not eradicated and the lack of continuing vigilance allowed persistence of endemic foci in some countries, resulting in resurgence of these disease in the 1980s.

Geographic distribution

The endemic treponematoses are now largely confined to communities in remote rural areas living in poor, overcrowded and unhygienic conditions. Yaws occurs mainly in the warm, humid areas of Africa, South East Asia and the Pacific, the Caribbean and Central and South America. Endemic syphilis occurs mainly in the arid areas of sub-Saharan Africa and among the nomadic people of the Arabian peninsula. Pinta occurs mainly in Central and South America (among Indian tribes in the Amazon region and adjacent areas). Cases of imported yaws and endemic syphilis are sometimes encountered in the countries of the northern hemisphere, where clinicians may need to include them in the differential diagnosis.

INCIDENCE AND PREVALENCE

Accurate incidence and prevalence data are unavailable. The most recent estimate by WHO in 1995 yields a global prevalence of 2.5 million cases, including 460,000 infectious cases.

Age and sex

Yaws and endemic syphilis usually occur in children aged 2–14 years. For pinta the range is 10–30 years. The sexes are probably equally affected.

Mode of transmission

Yaws and pinta are transmitted by direct skin-to-skin contact with infectious lesions, transmission being facilitated by a breach in the skin of the recipient. The role of nonbiting flies in the transmission is uncertain. In the case of endemic syphilis, because the initial lesions are often in or around the mouth, the infection spreads by direct contact (e.g. older children kissing their younger siblings) and by indirect contact through infected communal eating or drinking utensils.

PATHOLOGY

Yaws and endemic syphilis affect skin and bones, whereas pinta is confined to the skin.

The basic pathology in endemic treponematoses is the same as in venereal syphilis. However, the vascular changes in the endemic treponematoses are less marked. A recent study of skin biopsies from yaws patients showed numerous plasma cells but few T and B cells; the treponemes were found mostly in the epidermis, whereas in venereal syphilis they were demonstrated mainly in the dermis and dermal-epidermal junction.[5] Because these differences are relative, they cannot be used to differentiate yaws from venereal syphilis.

In pinta, there is loss of melanin in basal cells, the presence of many melanophages in the dermis and the absence of inflammatory cells and treponemes in the achromic lesions.[6]

Contrary to occasional reports, endemic treponematoses are not believed to be associated with congenital transmission or with involvement of the cardiovascular and nervous systems.

PREVENTION

Essential in prevention of the endemic treponematoses is not only to identify and treat clinical cases but also to recognize that the presence of clinical cases in a community necessitates an immediate search for further clinical and latent cases, which also must be treated.

The preventive measures include:

- strengthening and improving accessibility of the primary health care facilities;
- training of clinicians to detect and treat patients and to examine and treat household and other obvious contacts;
- health education of the population;
- improvement in the standard of living and in personal and environmental hygiene; and
- provision of soap and water and clothing to children.

CLINICAL FEATURES

The incubation period is 9–90 days (mean 21 days).

Yaws

Early stage

The initial or primary lesion ('mother yaw') appears at the site of infection on an exposed part of the body. It may be a localized

maculopapular eruption or a papule, which may develop into a large papilloma 2–5cm in diameter. It is painless but itchy and may ulcerate as a result of scratching. It may heal in 3–6 months with or without scarring. Secondary lesions, which are the result of lymphatic and hematogenous spread of organisms, appear a few weeks to 2 years after the primary lesion. They may consist of multiple excrescences, often resembling the initial papilloma. The papillomas may ulcerate and the exudate may dry to form a yellow crust which, when removed, gives the lesion an appearance of a raspberry (hence the name 'framboesia'). The lesions may be irregular, crescentic or discoid in shape and on moist areas may mimic condylomata lata of venereal syphilis. They are rather florid and become more numerous in the rainy season (Fig. 156.1). They may last up to 6 months and heal with or without scarring. Infectious relapses may occur for 5 years and, rarely, for 10 years.

Other manifestations include:

- regional lymphadenopathy;
- palmar and plantar lesions, which may be painful, resulting in a crab-like gait; and
- osteoperiostitis of the proximal phalanges of the fingers (dactylitis) or of long bones, causing nocturnal bone pains.

The patient may at any time enter latency, with only serologic evidence of the infection.

Late stage

About 10% of patients develop late lesions after 5 years or more of untreated infection. The late stage is characterized by gummatous lesions of skin, bones and overlying tissues. The manifestations, some of which also occur in early stage but are now more destructive, include:

- ¦ hyperkeratosis of palms and soles with deep fissuring;
- ¦ juxta-articular subcutaneous nodules;
- ¦ more extensive osteoperiostitis of long bones (e.g. sabre tibia);
- ¦ hyperostosis of the nasal processes of the maxillae ('goundou'); and
- ¦ ulceration of the palate and nasopharynx (rhinopharyngitis mutilans; Fig. 156.2) with secondary infection resulting in foul-smelling discharge ('gangosa').

Figure 156-1 Mixed early yaws lesions: papillomata, ulceropapillomatous lesions and squamous macules. *Courtesy of WHO, Geneva.*

Endemic syphilis

The primary lesion is seldom seen. The early manifestations include mucous patches (i.e. shallow painless ulcers on the lips and in the oropharynx) and other mucocutaneous and bone lesions resembling those of venereal syphilis and yaws. Papilloma favor warm and moist areas and occur as split papules or angular stomatitis at the labial commissures (Fig. 156.3). Later, the patient may enter the latent phase, which may be prolonged; after this some patients develop late lesions, which are similar to those seen in yaws.

Pinta

Pinta is confined to the skin and is the mildest of all treponematoses. The primary lesion, appearing at the site of entry of *T. carateum* on an exposed part of the body, is an itchy, red, scaly

Figure 156-2 Gangosa in late stage of yaws (rhinopharyngitis mutilans; occurs also in endemic syphilis). *Courtesy of WHO, Geneva.*

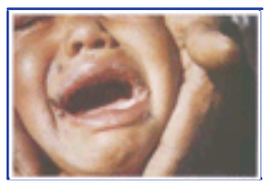

Figure 156-3 Angular stomatitis (also called split papules) of endemic syphilis; these lesions are also found in early yaws. *Courtesy of Dr GM Antal.*

papule, sometimes associated with satellite lesions and regional lymphadenopathy. The secondary stage develops several months later, in other areas, with the appearance of pintids, which are similar to the initial lesions. These are also itchy. In due course, they undergo a variety of color changes from red to copper-colored, gray and bluish-black. The lesions remain infectious for many years.

The late lesions are characterized by varying degrees of hypochromia, discoloration, atrophy and achromia. Sometimes these features are seen in the same area.

Attenuated endemic treponematoses

In areas of reduced transmission, the clinical expression of endemic treponematoses can be much milder (a few or even a single papilloma) or many of the infected subjects can be asymptomatic.[4] In the Gambia, 9.3% of pregnant women were seropositive for a treponemal infection; children of seropositive mothers showed no signs of congenital syphilis and there was no increase in perinatal, neonatal or child deaths. No clinical signs of endemic treponematoses were found, indicating the asymptomatic nature of the infection.[7] [8]

HIV infection and endemic treponematoses

As yet, no information is available on any interaction between HIV infection and endemic treponematoses.

DIFFERENTIAL DIAGNOSIS

In endemic areas, an accurate clinical diagnosis can be made in the presence of classic lesions. This will, however, necessitate appropriate training of clinicians, especially in view of the rather milder forms being encountered. The difficulties arise when there are no clinical lesions (i.e. latent cases), when venereal syphilis is also locally prevalent and when the patient is an immigrant from an endemic area presenting at a clinic in a nonendemic country. Differentiation from venereal syphilis is important because of social stigma implications. A careful and detailed history (including that of mother, father and siblings when appropriate) and thorough physical examination are always essential.

Apart from venereal syphilis, the conditions to be considered for differential diagnosis include:

- ¦ skin sepsis, scabies, fungal infection, lichen planus, plantar warts, psoriasis and tungiasis in a patient who has early skin lesions;
- ¦ tropical ulcer, cutaneous leishmaniasis, mycotic lesions and leprosy in a patient who has gummatous ulceration; and
- ¦ tuberculosis and sickle cell disease in a patient who has dactylitis. Pinta may need to be differentiated from pityriasis versicolor, tinea corporis, vitiligo, leprosy and chloasma.

DIAGNOSIS

There is no test that can differentiate the treponematoses (including venereal syphilis) from one another. The diagnosis of treponemal infection is confirmed by the demonstration of treponemes (but beware of nonpathogenic commensals) in a wet preparation of the material from early lesions by dark-field microscopy or in the biopsy material stained by the silver impregnation technique.

Serologic tests (rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) nontreponemal tests; *T. pallidum* hemagglutination assay (TPHA) or

fluorescent treponemal antibody absorption (FTA-ABS) treponemal (i.e. specific) tests) should be carried out in all cases, but their interpretation requires expertise. The treponemal tests are particularly useful to confirm a reactive nontreponemal test (exclusion of false positives). A reactive treponemal test may indicate a current infection or a past infection ('serologic scar'). Radiologic evidence of osteoperiostitis may assist in the diagnosis.

If the differentiation from venereal syphilis is difficult, the patient should be managed as for venereal syphilis.

MANAGEMENT

Penicillin remains the drug of choice. Long-acting benzathine penicillin G, given intramuscularly in a single session, is preferred. The dose is 600,0000 units (0.45g) for children under the age of 6 years; 1.2 million units (0.9g) for those aged 6–15 years; and 2.4 million units (1.8g) for those over 15 years. The dose may be divided, half to be given into each buttock. While it is recognized that treponematoses have remained exquisitely sensitive to penicillin, there is a recent report of penicillin treatment failures of yaws in Papua New Guinea. [9] Three penicillin treatment failures have also been observed in Ecuador.[10] The distinction between relapse, re-infection or true resistance is difficult to make but these clinical failures are worrisome and should be further researched.

Although there is little information on the use of drugs other than penicillin to treat these conditions, oral erythromycin or tetracycline 500mg q6h for 15 days or oral doxycycline 100mg q12h for 15 days are likely to be effective for those allergic to penicillin. Children between 12 and 15 years of age should receive half that dose. Tetracyclines (including doxycycline) are not recommended for pregnant and breast-feeding women and for children under the age of 12 years.

Contacts

Arrangements should be made to examine and, if appropriate and after proper explanation, to treat the household contacts and other close contacts.

Prognosis and follow-up

The lesions become noninfectious within 24 hours after the injection of penicillin. Whereas treatment in early stages should result in cure in almost 100% of patients, it will not reverse any destructive change in late stages. Rapid plasma reagin (or VDRL) titers should decline within 6–12 months, becoming negative in about 2 years. However, in a small proportion of cases, especially if treated in late stages, the RPR (or VDRL) may remain positive, albeit in low titer (i.e. below 1:8). The specific tests (i.e. TPHA, FTA-ABS) will remain positive throughout life.

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Chapter 157 - African Trypanosomiasis

David G Lalloo

EPIDEMIOLOGY

African trypanosomiasis (sleeping sickness) causes considerable mortality and morbidity across much of the African continent. Approximately 45,000 cases were reported to the World Health Organization (WHO) in 1999, but the true burden of disease is thought to be much higher, with estimates of between 300,000 and 500,000 individuals being infected.[1] [2] Intensive control activities in the 1950s and 1960s were extremely successful in reducing the incidence of disease but a lack of resources and civil conflict in many of the most heavily affected countries has led to re-emergence of the disease as a major health problem.

African trypanosomiasis occurs in two distinct clinical forms: an acute form, caused by *Trypanosoma brucei rhodesiense*, transmitted in endemic situations by savanna *Glossina morsitans* group flies and in epidemic situations by a 'riverine' species, namely *Glossina fuscipes*; and a more chronic form caused by *Trypanosoma brucei gambiense*, transmitted by the riverine species *Glossina palpalis*, *Glossina tachinoides* and *G. fuscipes* (Fig. 157.1). [3] [4] *Trypanosoma b. rhodesiense* is distributed in east and central Africa between Ethiopia and Botswana. It is a zoonosis with several game animal reservoirs; in the epidemic cycle, cattle are an important reservoir. *Trypanosoma b. gambiense* is found from Uganda west to Senegal and south through Zaire to Angola; humans are the prime reservoir of infection (Fig. 157.2 , Table 157.1). [2]

PATHOGENESIS

Members of the genus *Trypanosoma* are parasitic in the blood and tissues of vertebrates and are transmitted by blood-sucking insects. A developmental cycle occurs in the gut and sometimes in the salivary glands of the insect vectors with the production of infective metacyclic trypanosomes; mammals acquire the infection by the bite of the tsetse fly vector. Trypanosomes are microscopic, varying in length from 15µm to 35µm, and are highly active when observed under the microscope.[4]

Metacyclic trypanosomes inoculated during tsetse feeding multiply locally in extracellular spaces and induce the typical 'chancre' (Fig. 157.3), with a marked local tissue response characterized by vasculitis, perivascular mononuclear cell infiltration, edema and local tissue damage. Trypanosomes enter the lymphatics and multiply within lymph nodes, leading to parasitemia 5–12 days after infection. Antigenic variation of the surface glycoproteins causes successive waves of parasitemia as the parasite evades the host immune response.[5]

Parasites enter the central nervous system (CNS) via the choroid plexus or by transcytosis across endothelial cells to cause a lymphocytic meningoencephalitis, which particularly affects the brain stem, although cortical areas and the cerebellum are also involved.[6] Perivascular infiltration with lymphocytes, plasma cells, macrophages and characteristic morular cells occurs; microglia and astrocytes proliferate and there is neuronal destruction and demyelination. Similar lesions also occur in the heart, serous membranes and endocrine organs.

Polyclonal activation of B lymphocytes leads to elevation of IgM concentrations. Heterophile antibodies, rheumatoid factor, immune complexes and autoantibody production may occur. Some neuropsychiatric manifestations may be biochemically induced; elevated prostaglandin D_2 concentrations have been found in advanced *T. b. gambiense* infection, which may be responsible for the circadian sleep disorders.[7]

PREVENTION

There are two major components of sleeping sickness control: detection and treatment of cases, and vector control. In *T. b. rhodesiense* areas, patients who present with symptoms of early parasitemia (passive surveillance) can be treated at local rural centers: in epidemics, rapid deployment of active surveillance using blood film screening and the establishment of effective local treatment centers is important (Fig. 157.4). In *T. b. gambiense* areas, limited clinical symptoms in the early stages require active surveillance. Individuals can be screened using gland aspiration or rapid antigen tests (e.g. the card agglutination test for trypanosomes, CATT). Although prophylactic measures such as 6-monthly intramuscular injections of pentamidine have been suggested for populations most at risk, concerns about development of drug resistance and the masking of second-stage infections means that this is no longer advocated.[2]

Vector control using insecticide-impregnated traps and targets has been demonstrated to be effective in epidemics of *T. b. gambiense* in the Congo[8] and in epidemics of *T. b. rhodesiense* transmitted by *G. fuscipes* in Uganda.[9] Sterile insect release methods may also be useful in reducing vector populations.[10] Residual insecticide application to *Glossina* resting sites, insecticide spraying and the clearing of riverine habitat have been used in the past but resource and environmental considerations means that these can no longer be considered. In epidemic situations, treatment of the cattle reservoir by cattle trypanocides may also be a strategy for prevention of human sleeping sickness.[11]

CLINICAL FEATURES

A local skin lesion, the chancre, may develop at the site of inoculation (see Fig. 157.2). This is a raised, tender, edematous papule that rapidly increases in size with surrounding local edema, erythema and local lymphadenopathy. Chancres resolve after 2–3 weeks; they are common in *T. b. rhodesiense* infections, but rare in *T. b. gambiense* infections. Trypanosomes subsequently invade lymphatics and blood, leading to the hemolymphatic stage (stage I) and may invade the central nervous system and cerebrospinal fluid (CSF), leading to meningoencephalitis (stage II). *Trypanosoma b. gambiense* causes a mild but protracted illness over months or years, which is followed by the late development of meningoencephalitis. In contrast, *T. b. rhodesiense* causes a severe, acute, febrile disease, with rapid progression to meningoencephalitis and death within months.[2] [12] [13]

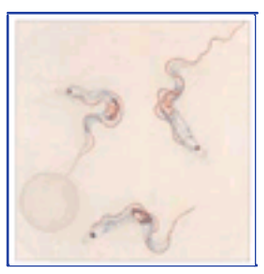

Figure 157-1 The causative organisms of sleeping sickness in humans. Reproduction of Dutton's original drawing of *Trypanosoma brucei gambiense* from the blood of a man. The organisms possess nucleus kinetoplast and flagella, and their relative size can be assessed from the red blood cell diameter of 7µm.

Figure 157-2 Endemic areas for trypanosomiasis.

Figure 157-3 Typical chancre of a patient infected with *Trypanosoma brucei rhodesiense.*. The chancre develops at the site of the infecting fly bite.

TABLE 157-1 -- Epidemiology and distribution of the human trypanosomiases.

EPIDEMIOLOGY AND DISTRIBUTION OF THE HUMAN TRYPANOSOMIASES		
	Trypanosoma brucei gambiense	***Trypanosoma brucei rhodesiense***
Distribution	Uganda to Senegal and Angola	Ethiopia south to Botswana and east of Rift Valley
Vector	*Glossina palpalis, tachinoides, fuscipes*; feed on any available host	*Glossina morsitans, pallidipes, swynnertoni*; host is game or cattle
Acquisition	Sites of high human-vector contact: river crossings, sacred groves, streams; end of dry season in Guinea savannas; peridomestic transmission in derived humid savanna; plantations of coffee and cocoa	Human penetration into savanna woodland; associated occupations (e.g. hunting, fishing, gathering of firewood and honey)
Epidemic characteristics	Lack of control (surveillance, diagnosis, treatment and vector control) as a result of expansion of human reservoir	Changes of habitat; intense human-fly-cattle contact for *G. fuscipes* transmission; encroachment on human habitation for *G. morsitans* transmission
Animal reservoirs	Pigs; to a lesser extent other domestic animals, rarely game animals	Game animals in endemic situation (bushbuck and hartebeest); cattle in epidemic situation

Hemolymphatic trypanosomiasis (stage I)

The characteristic clinical features of hemolymphatic trypanosomiasis comprise:

- episodes of fever, often accompanied by chills and rigors, malaise and prostration;
- headache;
- joint pains; and
- loss of weight.

In *T. b. gambiense* infections the disease slowly increases in severity as it progresses. Lymphadenopathy occurs in *T. b. gambiense* infections, and enlargement of the posterior cervical glands is characteristic

Figure 157-4 Emergency treatment center Uganda. This center was established to provide facilities for the care and treatment of patients with sleeping sickness during the epidemic in Busoga.

(Winterbottom's sign). Lymph nodes are moderately enlarged, firm and discrete. Splenomegaly occurs in around one-third of cases, and may be associated with hepatomegaly. A fleeting erythematous rash ('circinate erythema') on the trunk or upper extremities is evident in light skinned people. There are focal areas of edema, facial puffiness, dependent edema and serous effusions including ascites, pleural effusions and pericardial effusions. Myocarditis occurs especially in *T. b. rhodesiense* infections.[12] [13]

Meningoencephalitic trypanosomiasis (stage II)

In *T. b. rhodesiense* infections, cerebral involvement occurs within weeks of the onset of infection, whereas in *T. b. gambiense* infections meningoencephalitis may be delayed several years after initial infection. Headache becomes more severe and protracted. Personality changes may occur early, especially in *T. b. gambiense* infections, with apathy, lack of attention, loss of appetite, antisocial behavior and paranoid or delusional states. Abnormalities of sleep are characteristic and result from disturbance of normal circadian rhythms. Diurnal and inappropriate somnolence is often associated with nocturnal insomnia.

Neurologic features include tremors, muscle fasciculation, increased muscle tone and rigidity, choreic and athetotic movements. The gait is affected early with progressive ataxia. Speech is slow, slurred, or incoherent. Convulsions and hemiplegia can occur. Pyramidal tract and cranial nerve lesions are less common. Kerandel's sign refers to deep hyperesthesia, often with a delayed response to painful stimuli. Intractable pruritus is a feature of *T. b. gambiense*, especially in the later stages. Ocular manifestations can include iritis, chorioretinitis and papilledema.

The course of the disease is a relentless deterioration to a stuporose state, with cachexia, wasting and progressive malnutrition (Fig. 157.5). Patients become increasingly difficult to rouse and pass into deepening coma and death. This is relatively rapid in *T. b. rhodesiense* but may be protracted over months or years in *T. b. gambiense*. Intercurrent infections, especially bronchopneumonia, are common in the late stages of the disease. In *T. b. gambiense*, amenorrhea and impotence are common.[12] [13] [14]

Sleeping sickness in special groups

Although sleeping sickness is less common in children than adults, the clinical course of infection in children may be more severe and, with *T. b. rhodesiense* infection, progression to meningoencephalitis may be even more rapid than in adults.[15] Congenital infection has been described in *T. b. gambiense*.

Figure 157-5 Comatose terminal stage patient with sleeping sickness. Note the degree of cachexia.

Trypanosomiasis caused by *T. b. rhodesiense* or *T. b. gambiense* may occur in tourists and visitors to endemic areas. Severe early infections with high peripheral parasitemia are most common in travelers, characterized by a severe systemic illness with fever, anemia, biochemical abnormalities and circulating immune complexes. Thrombocytopenia is common and there may be evidence of a coagulopathy and disseminated intravascular coagulation. Most travelers do not develop CNS involvement, although delayed diagnosis may be a problem outside endemic areas, especially if no chancre is present.[16]

DIAGNOSIS

Routine laboratory tests demonstrate normal white cell counts, raised erythrocyte sedimentation rate, anemia, thrombocytopenia, low serum albumin and elevated serum IgM.

Parasitologic diagnosis

Diagnosis of sleeping sickness is dependent on finding trypanosomes in the chancre, blood, lymph gland juice or CSF — either in unfixed or unstained preparations, when moving parasites are observed, or in stained preparations. Blood films are usually positive in *T. b. rhodesiense* infection but parasitologic diagnosis may be difficult in chronic *T. b. gambiense* disease; high levels of parasites are often only found during a fever. Repeated examination and concentration techniques such as microhematocrit centrifugation may improve diagnostic sensitivity. *Trypanosoma b. gambiense* may also be diagnosed by finding parasites in aspirates from enlarged cervical lymph glands.

Diagnosis of second stage (CNS) disease requires examination of CSF. Before lumbar puncture, a single dose of suramin eliminates trypanosomes from the blood, avoiding the risk of contamination of CSF with trypanosoma.[2] [12] Parasites may be found in the centrifuged deposit, which should be examined within 15 minutes of the lumbar puncture. A raised CSF cell count (>5/mm^3) or raised CSF protein are also suggestive of CNS infection.

Immunologic diagnosis

A number of serologic tests have been developed; CATT is particularly useful for rapid preliminary screening of large populations for *T. b. gambiense* infection. A more recent approach to indirect detection of parasites is the use of an antigen detection test (CIATT) to detect *T. b. rhodesiense* in serum and CSF. This may be useful for following the response to therapy.[17]

Differential diagnosis

In endemic areas, *T. b. rhodesiense* is most common in certain occupational groups such as hunters, game wardens and fishermen, although all members of the community may be affected. Transmission of *T. b. gambiense* occurs at places of contact between humans and water, and both sexes and all age groups are affected. Outside endemic areas, trypanosomiasis caused by *T. b. rhodesiense* should be considered in travelers from eastern and southern Africa (especially those who have visited game parks) who present with an acute severe febrile illness. *Trypanosoma b. gambiense* infections may present months or years after exposure, with a febrile illness or neuropsychiatric features.

Chancres may be confused with insect bites, skin infections, an eschar or cutaneous anthrax. Early hemolymphatic disease mimics a wide range of febrile illnesses, especially malaria, relapsing fever, typhoid, brucellosis and arboviral infections. Occasionally, myocarditis may dominate the clinical picture. The differential diagnosis of meningoencephalitis includes a wide range of inflammatory cerebral infections; in particular, cryptococcal or tuberculous meningitis complicating HIV infection needs to be excluded. The neuropsychiatric presentations of trypanosomiasis may be confused with a variety of psychiatric syndromes, especially when personality change or psychotic behavior predominate. Late-stage disease may also mimic focal neurologic disorders, parkinsonism and space-occupying cerebral lesions.

MANAGEMENT

Treatment of sleeping sickness depends upon the stage of disease; a lumbar puncture is therefore crucial to determine whether there is evidence of CNS involvement. At least one dose of suramin or pentamidine should be given to clear blood parasites before a lumbar puncture is performed to prevent inoculation of parasites into CSF at the time of lumbar puncture. There have been few recent advances in the chemotherapy of trypanosomiasis and the number of available drugs is limited (Table 157.2). [18] Most treatment regimens are relatively toxic and it is therefore important to diagnose and treat nutritional deficiencies or intercurrent infections; routine anthelmintic and antimalarial treatment is often given. Hemolymphatic trypanosomiasis is treated with suramin or pentamidine; the latter is only effective in *T. b. gambiense* infection. Meningoencephalitis requires treatment with drugs that cross the blood-brain barrier in trypanocidal concentrations. The mainstays of therapy are arsenical

TABLE 157-2 -- Drugs currently used for the treatment of sleeping sickness.

DRUGS CURRENTLY USED FOR THE TREATMENT OF SLEEPING SICKNESS					
Drug	**Species**	**Indication**	**Route**	**Typical dosage regimen**	**Side-effects**
Suramin	*Trypanosoma brucei rhodesiense (Trypanosoma brucei gambiense)*	Stage 1	Slow iv infusion	Day 1: 5mg/kg test dose	Anaphylaxis
				Day 3: 10mg/kg	Cutaneous reactions
				Days 5, 11, 23, 30: 20mg/kg	
Pentamidine	*T. b. gambiense*	Stage 1	im/iv	7–10 doses of 4mg/kg daily (or alternate days)	Local reactions (im)
					Hypotension
					Hypoglycemia
Melarsoprol	*T. b. rhodesiense*	Stage 2	iv infusion	Three series of four daily doses of 1.2–3.6mg/kg, each separated by 7–10 days	Arsenical encephalopathy
	T. b. gambiense				Peripheral neuropathy
					Dermatitis
Eflornithine	*T. b. gambiense*	Stage 2	iv infusion (po)	400mg/kg daily in four divided doses for 7–14 days	Diarrhea
					Pancytopenia
					Convulsions
Nifurtimox (under evaluation)	*T. b. gambiense*	Stage 2	po	15–20mg/kg daily in three divided doses for 14 days	Anemia
	?T. b. rhodesiense				Neurologic side-effects

drugs, particularly melarsoprol, used since the beginning of the 20th century. Eflornithine and nifurtimox are also used for the treatment of late-stage *T. b. gambiense* infections. In most individuals, drugs rapidly clear parasitemia or CNS parasites, but relapses occur and 2-year cure rates are the best estimate of drug efficacy. Treatment responses vary according to the drug, severity of illness and geographic location.

Hemolymphatic trypanosomiasis

Suramin is given intravenously as a 10% solution. Fever, nausea, vomiting and urticaria are common but the most severe side-effect is an idiosyncratic anaphylactic reaction, which is infrequent (1 in 2000–4000) but may be more common in the presence of onchocerciasis. Proteinuria and renal failure may also occur. Intramuscular or intravenous pentamidine isethionate or pentamidine methanesulfonate are alternatives to suramin in *T. b. gambiense* infection and are usually well tolerated. Side-effects include a histamine-like reaction with hypotension and circulatory collapse, hypoglycemia and, after prolonged administration, hyperglycemias and diabetes; adrenaline (epinephrine) and glucose should be available when this drug is administered.

Meningoencephalitic trypanosomiasis

Melarsoprol is a trivalent arsenical compound that crosses the blood-brain barrier. A number of treatment schedules have been used, most lasting for 4–5 weeks. However, shorter 10-day treatment regimens have recently been shown to be effective in *T. b. gambiense* infection. [19] Thrombophlebitis and cellulitis following extravascular leakage are common (Fig. 157.6). The most important side-effect is reactive arsenic encephalopathy, which occurs in 2–10% of patients, usually after the third or fourth dose. Presentation is usually as an acute deterioration in conscious level often heralded by convulsions. There is an associated case fatality rate of 10–50%. Prophylactic prednisone (prednisolone) reduces the incidence of reactive arsenic encephalopathy in *T. b. gambiense* (but not *T. b. rhodesiense*) infection.[20]

Other toxicity is also common — a peripheral neuropathy occurs in up to 10% of patients and skin reactions, hepatic and renal toxicity are also seen; hemolysis may occur in glucose-6-phosphate dehydrogenase deficiency. Treatment with melarsoprol normally leads to a striking improvement in the mental and physical condition of patients with sleeping sickness. However, recent data suggest that rates of relapse following melarsoprol therapy are increasing.[18]

Figure 157-6 Treatment of a patient with an intravenous injection of melarsoprol. It is vital to adhere to the schedules of treatment and to have a scrupulous technique of injection in order to avoid destruction of local tissue as a result of leakages of the melarsoprol suspended in propylene glycol.

Eflornithine (difluoromethyl-ornithine, DFMO) is an ornithine decarboxylase inhibitor that is effective in the treatment of stage 1 and stage 2 *T. b. gambiense* infection but is poorly effective against *T. b. rhodesiense*. Most current regimens use intravenous administration, although oral preparations are being evaluated. The drug is less toxic than melarsoprol. Standard courses last 14 days; 7-day courses have been advocated but appear to be less effective in new cases of sleeping sickness. [21] Eflornithine may be particularly useful for the treatment of late-stage *T. b. gambiense* infection when the organism has become resistant to melarsoprol.

Nifurtimox is a drug that has occasionally been used in the treatment of arsenic-refractory *T. b. gambiense*, with reported response rates that vary from 50% to 80%. Its role is still being evaluated.[18]

Post-treatment follow-up

Following treatment, patients should be reviewed every 3 months for 6 months and then 6-monthly for 2 years to identify episodes of relapse, which may occur after both early and late-stage disease. In late-stage infection, the CSF abnormalities improve slowly after treatment and values usually return to normal within 1–2 years; CSF examination should be repeated before discharge and routinely at follow-up to detect parasites or a rising cell count. Relapse in *T. b. gambiense* following treatment with suramin or pentamidine is often treated with melarsoprol; eflornithine can also be used. Relapse in *T. b. rhodesiense* is usually treated with a second course of melarsoprol; nifurtimox may be effective if further relapse occurs but more data are needed.[2] Combination therapy has also been used in the treatment of relapse but there are inadequate data to determine its role at present.

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Chapter 158 - Other Parasitic Infections of the Central Nervous System

Euan M Scrimgeour

This chapter reviews parasitic infections of the central nervous system (CNS), with brief reference to trypanosomiasis and cerebral malaria (see Chapter 146 , Chapter 157 , Chapter 166 and Chapter 173).

EPIDEMIOLOGY

Numerous species of protozoa and helminths parasitize man, especially in tropical and developing countries where environmental conditions promote mass transmission. Many sources of infection exist (Table 158.1). Table 158.2 lists parasites that can invade the CNS as adults, larvae or ova, depending upon the species, and gives their principal geographic distribution. It excludes parasitic Diptera, whose maggots occasionally invade the brain from the eye, nose or ear, and ticks that cause tick bite paralysis.

Many parasitic diseases are zoonoses. Examples, identifying the mammalian hosts, include:

- toxoplasmosis (cats and rodents),
- South American trypanosomiasis (domestic and wild animals),
- Rhodesian trypanosomiasis (antelopes, cattle),
- angiostrongyliasis (rodents),
- gnathostomiasis (fish-eating mammals),
- trichinosis (pigs and rats),
- toxocariasis (dogs and cats),
- schistosomiasis japonica (domestic animals and rodents), and
- echinococcosis (dogs and herbivores).

Gambian trypanosomiasis, schistosomiasis mansoni and schistosomiasis haematobium are essentially anthroponotic. In taeniasis solium (*T. solium*) humans are the definitive host but the larval cysticerci are found in pigs.

Relatively few parasites commonly involve the CNS. In some (e.g. *Plasmodium falciparum, Trypanosoma* spp., *Toxoplasma gondii, Angiostrongylus cantonensis* and *Taenia solium* (cysticercosis)), it is part of the life cycle. In others, it is accidental (e.g. eosinophilic meningoencephalitis caused by the racoon ascarid *Bayliscaris procyonis*).

Large populations are exposed to the risk of CNS parasitism. Worldwide, toxoplasmosis is probably the most prevalent neurotropic parasitosis. Schistosomiasis affects 200 million people, with prevalence rates of 10–80% in endemic areas. About 50 million individuals have African trypanosomiasis (prevalence 50–70/10,000 in endemic areas) and some 20 million — including thousands of migrants from South and Central America living in North America — have trypanosomiasis cruzi (Chagas' disease). In South East Asia and Oceania, angiostrongyliasis is common although diagnosis is infrequent.[1] Because CNS involvement is often underdiagnosed and silent, it is difficult to estimate prevalence and incidence rates. However, autopsy studies of patients with cysticercosis in Peru, Mexico, India and Zimbabwe have shown CNS infection rates of 0.5–3%, usually without clinical sequelae; in Mexico, 80% were asymptomatic.[2] Schistosomiasis japonica affects 70 million people and 2–5% of cases develop CNS complications.[3] Clinical evidence of CNS involvement is uncommon in infections with S. mansoni and S. haematobium, but autopsy studies in Zimbabwe, Nigeria, Egypt and Brazil have revealed ova in the brain in 3–28% and in the spinal cord in 0.3–2% of cases.[4] Worldwide, cerebral malaria is the most commonly diagnosed manifestation of CNS parasitism; in African children with severe falciparum malaria, 30–70% have this complication.[5]

PATHOGENESIS AND PATHOLOGY

Parasites invade the CNS as adults, larvae or ova, through the systemic circulation or retrogradely via the vertebral venous system (Fig. 158.1). Clinical sequelae depend upon the nature and number of parasites and the immune response. Immune evasion is exhibited by many parasites (e.g. *Toxoplasma gondii, Trypanosoma* spp.), with minimal tissue reaction.

In falciparum malaria, maturation of the trophozoite to the schizont requires sequestration of the parasitized erythrocyte by cytoadherence in capillaries, including those of the CNS. This is usually without clinical sequelae in immune subjects, but in cerebral malaria massive sequestration obstructs capillaries, the most plausible explanation for coma.[5] In acute African trypanosomiasis trypomastigotes rapidly invade the brain. They may become dormant but later, in stage II of the disease, especially in Gambian trypanosomiasis, they cause nonsuppurative encephalomyelitis (see Chapter 157).

Migrating larvae of the nematodes *Angiostrongylus cantonensis, Gnathostoma spinigerum, Toxocara canis* and *Toxocara cati, Trichinella spiralis*, occasionally *Loa loa, Mansonella perstans, Ascaris lumbricoides* and *Dirofilaria immitis* (the dog heartworm) produce transient focal cerebral lesions or eosinophilic meningoencephalitis.[6] [7] *Bayliscaris procyonis* and the saprophytic soil nematode *Micronema deletrix* have also been incriminated. *Angiostrongylus cantonensis*, and others that cannot complete their life cycle in humans, die after a few weeks.

Certain trematode ova and larvae can involve the CNS. In early schistosomiasis, anomalous migration of worms to the CNS is followed by a cell-mediated response to ova deposition to form a periovular granuloma.[8] When the subsequent humoral response to adult worms and egg antigens is excessive, Katayama fever occurs, especially in *Schistosoma japonicum* infection, with fever, eosinophilia and self-limiting encephalopathy or myelopathy. In chronic schistosomiasis, retrograde passage of ova and occasionally worms, through Batson's vertebral venous plexus, may result in myelopathy or cerebral lesions. This also occurs in *S. haematobium* infection with obstructive uropathy. When hepatosplenic schistosomiasis develops, ova pass through portopulmonary anastomoses to the lungs and reach the systemic circulation through arteriovenous shunts or by pulmonary veins (see Fig. 158.1). Schistosomal cor pulmonale also promotes cerebral embolization of ova. Ova in the CNS may incite little histologic reaction, but the development of a granuloma, focal vasculitis, localized infarction or rarely subarachnoid or cerebral hemorrhage can result.[4] In paragonimiasis, flukes migrate from lung to brain through the soft tissues of the neck.

TABLE 158-1 -- Sources of parasite infection and resulting disease.

SOURCES OF PARASITE INFECTION AND RESULTING DISEASE		
Source of infection		**Disease**
Food	Salads	Amebiasis, angiostrongyliasis, ascariasis, cysticercosis
	Raw aquatic plants	Fascioliasis
	Uncooked vegetables	Angiostrongyliasis, echinococcosis, cysticercosis
	Uncooked pork	Trichinosis, taeniasis solium, sparganosis
	Uncooked beef	Toxoplasmosis
	Uncooked freshwater fish	Gnathostomiasis, diphyllobothriasis, heterophyiasis, metagonimiasis
	Uncooked freshwater crayfish or crabs	Paragonimiasis, angiostrongyliasis
	Uncooked snakes, frogs*	Sparganosis
	Uncooked land molluscs (e.g. *Achatina fulica* snails)	Angiostrongyliasis
Fresh water	Skin contact	Schistosomiasis
	Nasal contact	*Naegleria fowleri* meningoencephalitis
	Consumption	Sparganosis, dracontiasis, amebiasis

Other environmental sources	Geophagy	Toxoplasmosis, toxocariasis, ascariasis, hydatidosis
	Soil	Strongyloidiasis
	Airborne	*Acanthamoeba castellani* meningoencephalitis, ascariasis
Arthropods	Mosquito (*Anopheles* spp.)	Falciparum malaria
	Mosquito (*Culex* spp.)	Bancroftian filariasis, dirofilariasis
	Midge (*Culicoides* spp.)	Mansonellosis
	Tsetse fly (*Glossina* spp.)	Rhodesian and Gambian trypanosomiasis
	Blackfly (*Simulium* spp.)	Onchocerciasis
	Chrysops flies	Loiasis
	Reduviid bugs (e.g., *Triatoma* spp.)	American trypanosomiasis (Chagas' disease)
Other sources	Blood transfusion/contaminated syringes and needles	Falciparum malaria, American trypanosomiasis (Chagas' disease) toxoplasmosis
	Cardiac, renal transplant	Toxoplasmosis
	Transplacental	American trypanosomiasis (Chagas' disease), toxoplasmosis
	Autoinfection‡	Cysticercosis, strongyloidiasis

* In South East Asia, frogs applied as a poultice may transmit sparganosis
† Patient with taeniasis solium inadvertently consumes eggs he or she has passed in feces

In cysticercosis, the larval oncospheres reach cerebral and meningeal capillaries and mature to cysticerci in the gray matter and meninges. The spinal cord is largely spared. Cysticerci survive silently for 2–10 years. Intense inflammation follows their death and antibodies appear in the cerebrospinal fluid (CSF). Healing, with fibrosis and calcification, follows. If the oncosphere enters the subarachnoid space or ventricles, then chronic meningitis with hydrocephalus may result.[2] Rarely, parasites produce large cystic lesions (e.g. in paragonimiasis, hydatidosis and coenurosis). In dracontiasis an extradural abscess containing an adult worm may compress the spinal cord, and in diphyllobothriasis an adult tapeworm competes with the host for vitamin B12 and can produce myelopathy (see Chapter 168).

Immunodeficiency and central nervous system parasitism

Immunodeficient patients are susceptible to the same parasites as the immunocompetent. Falciparum malaria is not more common in patients with HIV/AIDS but the parasite count may be increased. In toxoplasmosis, following acute infection, cysts containing the resting phase bradyzoites remain dormant in muscle, heart, brain or choroid for decades. (The risk of a nonimmune subject developing toxoplasmosis following organ transplant from a seropositive donor is 50% for cardiac and 20% for renal transplant.) In immunodeficiency states (e.g. AIDS, leukemia and lymphomas, inherited immunodeficiency syndromes, systemic lupus erythematosus, immunosuppressive drug therapy, radiotherapy, etc.), reactivation of bradyzoites produces multiplying tachyzoites, with cerebral abscess formation (see Chapter 127). Reactivation results from impaired interferon-? dependent cell-mediated and humoral immunity.[10] In AIDS, this occurs when the CD4 lymphocyte count falls below 350 cells/mm^3 . Trypanosomiasis cruzi spares the brain in the immunocompetent (except children), but in immunodeficiency (in AIDS, CD4 cell count <200/mm^3), often after symptom-free decades, reactivation produces cardiomyopathy and acute, multifocal, necrotizing meningoencephalitis or a cerebral granuloma.[10] Other opportunistic parasites that invade the CNS include free-living amoebae (eg. *Acanthomoeba culbertsoni* and *Balamuthia mandrillaris*), which produce chronic granulomatous meningoencephalitis, [11] and the intestinal microsporidium *Encephalitozoon cuniculi*, which disseminates to produce encephalopathy.[12] Some *Strongyloides stercoralis* larvae normally hatch in the jejunum, penetrate the intestinal mucosa, migrate through the lungs and return to the jejunum as adults. In immunodeficiency, especially in human T-cell lymphoma virus 1 (HTLV-1) infection, *Strongyloides* hyperinfection may develop with massive tissue invasion, encephalitis and complicating *Escherichia coli* meningitis.

PREVENTION

Control and prevention of parasite infections are based on health education for exposed populations and visitors to endemic regions (see Table 158.1) and implementation of public health measures. Vector control includes destruction of breeding habitats, spraying with insecticides and controlling freshwater snails. As a rule, it is impossible to control zoonoses effectively.

Important personal precautions include (see also Chapter 143):

TABLE 158-2 -- Parasites that cause generalized or focal or space-occupying lesions and their principal geographic distribution.

PARASITES THAT CAUSE GENERALIZED OR FOCAL OR SPACE-OCCUPYING LESIONS AND THEIR PRINCIPAL GEOGRAPHIC DISTRIBUTION			
Parasite		**CNS disease**	**Geographic distribution**
Protozoa	*Acanthamoeba castellani*[*]	Meningoencephalitis	Worldwide
	Balamuthia mandrillaris	Meningoencephalitis	Worldwide
	Encephalitozoon cuniculi	Encephalitis	Worldwide
	Entamoeba histolytica[*]	Meningoencephalitis	Tropics, subtropics
	Naegleria fowleri[*]	Meningoencephalitis	Worldwide
	Plasmodium falciparum	Cerebral malaria	Tropics, subtropics
	Toxoplasma gondii	Encephalitis, SOL brain	Worldwide
	Trypanosoma brucei gambiense	Encephalitis	West Africa eastward to Rift Valley
	Trypanosoma brucei rhodesiense	Encephalitis	Central and East Africa
	Trypanosoma cruzi	Meningoencephalitis	Central and South America
Helminths			

Nematodes	*Angiostrongylus cantonensis*	Meningoencephalitis	South East Asia, Oceania
	Ascaris lumbricoides[*]	SOL brain	Worldwide
	Bayliscaris procyonis[*]	Meningoencephalitis	North America
	Mansonella perstans[*]	Meningoencephalitis	Tropical Africa
	Dirofilaria immitis[*]	Meningitis	Worldwide
	Dracunculus medinensis[*]	SOL spinal cord	Tropical Africa, Asia and Brazil
	Loa loa[*]	Meningoencephalitis	Central and West Africa
	Gnathostoma spinigerum	Meningoencephalitis	Far East
	Micronema deletrix[*]	Meningoencephalitis	North America
	Onchocerca volvulus[*]	SOL brain	West Africa, South America, Yemen
	Strongyloides stercoralis[*]	Meningoencephalitis	Tropics, subtropics
	Trichinella spiralis	Meningoencephalitis	Worldwide
	Toxocara canis, Toxocara cati	SOL brain	Worldwide
	Wuchereria bancrofti[*]	SOL brain	Tropics, subtropics
Trematodes	*Fasciola hepatica*[*]	SOL brain	Worldwide, sheep-farming countries
	Heterophyes heterophyes[*]	SOL brain and cord	Far East, Middle East
	Metagonimus yokogawi[*]	SOL brain and cord	Far East, Europe
	Paragonimus westermani	SOL brain and cord	Far East, tropical Africa, South America
	Schistosoma japonicum	SOL brain and cord	Far East, Philippines, Indonesia (Sulawesi)
	Schistosoma mansoni	SOL brain and cord	Africa, Middle East, South America
	Schistosoma haematobium	SOL brain and cord	Africa, Middle East
	Schistosoma intercalatum	Myelopathy	Sâo Tomé e Príncipe
Cestodes	*Diphyllobothrium latum*[*]	Myelopathy	Russia, Canada and subartic Europe
	Echinococcus granulosus (hydatidosis)	SOL brain and cord	Worldwide
	Echinococcus multilocularis[*]	SOL brain and cord	Northern Europe, Canada, Japan
	Spirometra spp. (sparganosis)[*]	SOL brain and cord	South East Asia, North America
	Taenia multiceps (coenurosis)[*]	SOL brain and cord	Worldwide
	Taenia solium (cysticercosis)	SOL brain and cord	Worldwide, South America
SOL, space-occupying lesion.			
Parasites of the order Diptera, whose maggots may invade the CNS, and ticks that cause tick bite paralysis are excluded. Note that related species of some parasites listed produce similar symptomology (e.g. *Angiostrongylus mackerrasae* in Queensland, Australia, *Angiostrongylus malayensis* in Malayasia and Indonesia, and numerous *Paragonimus* spp. in different parts of the world).			

* CNS disease is infrequent or rare.

- avoidance of drinking water or eating salads or uncooked food (vegetables, meat, freshwater fish, crustaceans or terrestrial molluscs) in regions where contamination by various protozoa and helminths is probable;
- efforts to discourage children from geophagy (risk of toxoplasmosis and toxocariasis from cat and dog excreta, respectively);
- regular anthelmintic treatment of pet dogs and cats;
- prevention of skin exposure to fresh water in regions endemic for schistosomiasis;
- prevention of arthropod bites (protective clothing, use of insect repellent creams and mosquito nets);
- effective prophylaxis for falciparum malaria;
- screening patients who have been exposed to strongyloidiasis prior to immunosuppressive therapy; and
- trimethoprim-sulfamethoxazole prophylaxis for toxoplasmosis in AIDS when the CD4 count falls below 350/mm^3 .

CLINICAL FEATURES

Most parasitic CNS infections lack specific diagnostic features. Diagnosis depends on suspecting a parasitic etiology and obtaining a history of residence in an endemic area (at any time from recent to remote, depending upon the parasite suspected), when exposure to infection may have occurred. The latter often requires a searching inquiry because patients are usually ignorant of the ways in which infection is contracted. Clinicians unfamiliar with this complex field must refer to a differential diagnosis checklist, remembering that multiple parasite infections are common in the tropics.

Major neurologic syndromes

Cerebral malaria

Fever, coma, absence of meningitis or focal neurologic signs (in the early stages) and presence of falciparum trophozoites in the blood

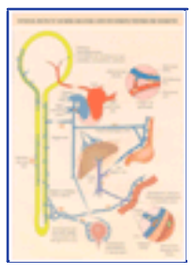

Figure 158-1 Potential routes to the brain and spinal cord for parasitic protozoa and helminths. Collateral circulation (e.g. in hepatosplenic schistosomiasis with portal hypertension) allows ova to embolize via portopulmonary anastomoses to the lung and thence to the systemic circulation. Batson's vertebral venous plexus allows retrograde access to the spinal cord and brain by parasites and/or ova.

suggest cerebral malaria. Alternative causes in a person who coincidentally has falciparum parasitemia include encephalitides, various systemic and toxic infections, and heat stroke.[5]

Trypanosomiasis

In stage II of African trypanosomiasis (sleeping sickness), chronic encephalomyelitis develops. This presents with change of personality, apathy, extrapyramidal signs

including tremor, chorea, expressionless facies and reversal of sleep rhythm (see Chapter 157). Acute American trypanosomiasis causes meningoencephalitis in children.

Meningitis or meningoencephalitis

A specific helminthic cause is suggested by periorbital edema and generalized myositis (trichinosis) or subcutaneous migratory swellings followed by CNS complications (gnathostomiasis).[6] In angiostrongyliasis (history of eating raw *Achatina* snails), signs may fluctuate markedly; the patient may have headache, confusion, severe generalized dysesthesia, neck stiffness and various focal neurologic signs but later the same day the patient may be almost symptom free. Fever is often absent.[1] [7] Other migrating larval nematodes can produce similar variable features.

Focal or space-occupying lesions

Parasitic space-occupying lesions in the CNS are usually without diagnostic features (see Table 158.2). However, cysts in the third ventricle (e.g. cysticercus, hydatid or coenurus cysts) cause intermittent internal hydrocephalus with periodic headache and loss of consciousness.

Toxoplasmosis

Immunodeficient patients developing reactivation toxoplasmosis, with abscess formation usually in the basal ganglia, present with low-grade fever, headache, seizures, raised intracranial pressure and hemiparesis. Diffuse encephalitis is less frequent.

Cysticercosis

Acute infection presents with headache, diffuse hyperesthesia and myalgia. The first manifestation may be seizures, developing months or years after exposure to infection. Careful examination of the whole skin surface may reveal firm, painless, pea-sized, subcutaneous nodules in 50% or more of patients.[2] Differentiation from other causes of seizures is required.

Schistosomiasis

In *S. japonica* infection, and less frequently in *S. mansoni* and *S. haematobium* infections, ova in the CNS may cause seizures or present as a space-occupying lesion. There may be no other evidence of schistosomiasis.

Myelopathy

Myelopathy is uncommon in parasitic infection, except in schistosomiasis. [14] Asymptomatic deposition of ova in the spinal cord is frequent in *S. haematobium* infection but *S. mansoni* is the usual cause of myelopathy; it is uncommon in *S. japonicum* infection. *S. intercalatum* was incriminated in two cases from Sâo Tomé e Príncipe. The usual presentation is acute, flaccid, areflexic paraparesis caused by a granuloma in the conus medullaris but spasticity is present in higher lesions. Acute massive necrosis of the lower cord, presumably immunologically mediated, has been described in Brazil. The differential diagnosis includes tuberculosis, neoplasia and HTLV-1 infection. In diphyllobothriasis, megaloblastic anemia is present in association with posterior column degeneration.

Ophthalmic involvement by parasites

Reports of direct or immunologic involvement of the eye by parasites are extensive and include the following.

- Iritis: *Toxoplasma gondii, Trypanosoma gambienese* and *rhodesiense, Leishmania donovani, Onchocerca volvulus, Wuchereria bancrofti, Dirofilaria immitis, Toxocara* spp., *Gnathostoma spinigerum* and cysticerci.
- Choroidoretinitis: *T. gondii* (bilateral, congenital; unilateral, acquired), *O. volvulus, Angiostrongylus cantonensis, Toxocara* spp., *Loa loa, Schistosoma mansoni*, cysticerci and *Armillifer armillatus* (pentastomid larvae).
- Optic neuritis: *T. gondii, Trypanosoma gambiense* and *rhodesiense, O. volvulus, A. cantonensis* and *Paragonimus westermani*.
- Orbital myositis: *Trypanosoma cruzi*.
- Orbital or retro-orbital mass or lesion: *Trichinella spiralis, G. spinigerum, S. mansoni, Fasciola hepatica, P. westermani, Echinococcus granularis* cyst, cysticerci and *Taenia brauni* coenurus (in tropical Africa).

The maggots of various Diptera can invade the eye (e.g. *Oestrus ovis*), ear or nose (e.g. *Cochliomyia hominivorax*), rarely extending to the brain.

Tick bite paralysis

In Australia, Africa, America and south east Europe, various hard (ixodid) and soft (argasid) ticks possess salivary neurotoxins. Between 1 and 6 days after it starts feeding, symmetric, ascending, flaccid paralysis appears, reaching the facial and bulbar muscles. Pain, fever and sensory abnormalities are absent. Death may result from respiratory failure. Recovery follows removal of the tick.

DIAGNOSIS

Cerebral malaria

The demonstration of ring forms of *Plasmodium falciparum* in the blood and exclusion of other causes of coma (including normal CSF) are the basic diagnostic criteria.

African trypanosomiasis: sleeping sickness

Trypanosomes may be present in blood films. The enzyme-linked immunosorbent assay (ELISA) IgM is positive in 90%. Suramin is given prior to lumbar puncture (LP) to destroy blood tryptomastigotes that might otherwise enter the CSF. The CSF contains lymphocytic pleocytosis, raised protein and normal glucose levels and occasionally motile tryptomastigotes. Polymerase chain reaction (PCR) may detect *Trypanosoma* DNA in CSF. Computerized tomography (CT) and magnetic resonance imaging (MRI) scans show brain edema (see also Chapter 157).

Meningitis or meningoencephalitis

Examination of the CSF is essential. This is often deferred if papilledema or CT scan evidence of raised intracranial pressure is present, but if an early diagnosis is critical (e.g. to detect tuberculous meningitis) cisternal or LP to aspirate a small sample of CSF is justified. In protozoal infections, lymphocytosis, normal glucose and mildly raised protein levels are typical. Similar findings are present in helminthic meningoencephalitis, but eosinophilia is usually present. Nonparasitic causes of eosinophilic meningitis include Hodgkin's disease, polyarteritis nodosa and occasionally bacterial or viral meningitis. Micro-organisms observed include amebae in primary amebic meningoencephalitis, tryptomastigotes in African trypanosomiasis and in *T. cruzi* infections in children (and immunodeficient adults), and occasionally larval worms in angiostrongyliasis and in disseminated strongyloidiasis.

Other investigations may point to the diagnosis. Stool may contain larvae in strongyloidiasis and sputum ova or larval worms in many helminthic infections (see Chapter 165 and Chapter 246). In filarial diseases, microfilariae may appear in the peripheral blood at night (Bancroftian filariasis), at noon (loiasis) or at any time (mansonellosis) or in a skin snip (onchocerciasis). In trichinosis, biopsy of a tender muscle may reveal larvae.

Focal or space-occupying lesions

Basic parasitologic investigations may explain focal or space-occupying lesions. The stool may contain ova in *S. mansoni* and *S. japonicum* infections, paragonimiasis, fascioliasis, heterophyiasis, metagonimiasis and diphyllobothriasis. The terminal drops of urine may contain ova of *S. haematobium*. Multiple rectal snips may reveal

schistosome ova of all species. In paragonimiasis, and occasionally schistosomiasis, the sputum contains ova.

The CSF in toxoplasmosis and cysticercosis reveals a lymphocytic pleocytosis and raised protein and occasionally reduced glucose levels. In schistosomiasis, a modest lymphocytosis is typical and protein and glucose levels are usually normal, whereas in paragonimiasis, eosinophilia is accompanied by raised protein levels. Eosinophilia is present in only 25% of cases of cerebral hydatidosis.

Immunodiagnostic tests for protozoa and helminths (see Chapter 245 and Chapter 246).

When organisms cannot be detected, antibody or antigen detection tests in serum and/or CSF confirm exposure or support diagnosis.[14] Investigations include immunoblot, monoclonal antibody tests and PCR for DNA detection. Immunodiagnosis is especially useful in the following: toxoplasmosis (ELISA, PCR for antigens); trypanosomiasis; neurocysticercosis (ELISA is 90% specific in blood, and almost 100% in CSF; immunoblot may be negative if few cysticerci are present); echinococcosis (IHAT positive in 60% of sera); and schistosomal myelopathy (ELISA in the CSF is positive in >75% of cases). To confirm active infection with *T. cruzi*,[15] xenodiagnosis is performed utilizing laboratory-raised vector reduviid bugs to feed on the patient (see Chapter 173).

Ultrasonography of the liver or CT or MRI scans of the abdomen detect amebic and hydatid hepatic cysts (the latter calcify), *F. hepatica* flukes in bile ducts or periportal fibrosis (pathognomonic of hepatosplenic schistosomiasis). Chest radiography and CT scans may reveal pulmonary paragonimiasis, hydatid cyst (non-calcifying) or schistosomal cor pulmonale. In schistosomal myelopathy, myelography typically reveals an intramedullary lesion of the conus with a complete block between T12 and L1. Computerized tomography or MRI may identify a granuloma; occasionally diffuse cord edema is observed, as in the Katayama syndrome.

Computerized tomography and MRI scans assist diagnosis of cerebral parasitic space-occupying lesions. Toxoplasmosis presents as one or more low-density lesions, usually ring enhancing (Fig. 158.2). In early cysticercosis, small nonenhancing hypodense lesions are present. Later, a hyperdense center (the scolex) develops (Fig. 158.3), with ring enhancement when it dies (Fig. 158.4). Finally, calcification supervenes.[2] Plain radiographs may show multiple calcified cysticerci in muscle. In hydatid cyst, the CT scan is the most definitive investigation; dead cysts calcify. The CT/MRI scan in paragonimiasis reveals cystic calcified lesions containing flukes, in schistosomiasis a contrast-enhancing granuloma, and in sparganosis a granuloma containing a worm. Other investigations (e.g. gallium-67 scans and positron emission tomography) are useful but non-specific for identifying inflammatory foci.

Finally, whenever the diagnosis is in doubt, brain or spinal cord biopsy under CT guidance is required.

Immunodeficient patients

Meningitis in immunodeficiency (e.g. AIDS) is more likely to be caused by cryptococcosis or tuberculosis than parasites. In meningoencephalitis in South or Central American patients, Chagas' disease should be considered. Rarer causes include chronic granulomatous meningoencephalitis (caused by *Acanthomoeba culbertsoni* or *Balamuthia mandrillaris*) diagnosed by brain biopsy. Encephalopathy in disseminated microsporidiosis is diagnosed by identifying *Encephalitozoon cuniculi* cysts in the urine. Cerebral space-occupying lesions are most likely to be caused by toxoplasmosis (10% of cases in North America, up to 50% in Africa) or tuberculoma. In North America, 2% are due to primary CNS lymphomas; the specific investigation is thallium-201 single photon emission CT. In South America, a 'chagoma' caused by *T. cruzi* should be considered (Fig. 158.5). Many other opportunistic infections can produce space-occupying lesions (e.g. nocardiosis, cryptococcoma, aspergilloma and syphilitic gumma). Myelopathy is most likely to be caused by tuberculosis but many other causes, including toxoplasmosis and HTLV-1 infection, must be considered.

MANAGEMENT

Specific treatment of parasite infections listed in Table 158.1 is discussed in the relevant chapters. Here, the management of the major CNS parasitic disease syndromes is summarized.

Cerebral malaria

Any comatose patient who has suspected or diagnosed falciparum malaria should be treated at once with intravenous quinine (after assessing blood glucose), pending further evaluation (see Chapter 166). Corticosteroid drugs are not helpful.

African trypanosomiasis: sleeping sickness

Suramin is given to clear trypanosomes from the blood. After 1 week, melarsoporol is administered. Eflornithine is effective in Gambian trypanosomiasis only (see Chapter 157 and Chapter 209).

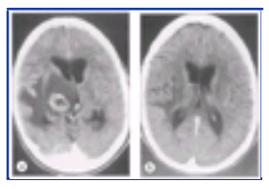

Figure 158-2 Toxoplasma abscess. (a) This CT brain scan shows a toxoplasma abscess in the left internal capsule, compressing the lateral ventricles. Contrast demonstrates a typical ring-enhancing effect. (b) Same patient after 17 days of treatment with pyrimethamine and sulfonamide showing resolving abscess.

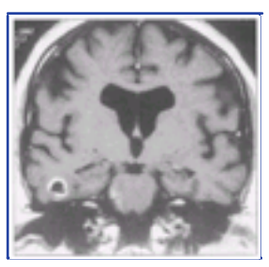

Figure 158-3 Coronal MRI of brain showing living cysticercus, with the scolex appearing as a hyperintense center ('pea in the pod' appearance). There is no visible inflammatory reaction.

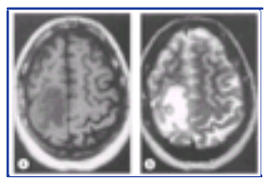

Figure 158-4 Cerebral cysticercus. (a) This MRI of brain shows a dying cysticercus surrounded by intense inflammation. (b) Post-contrast MRI T2-weighted image demonstrating the isointense wall of the cyst and surrounding hyperintense edema.

Meningitis and meningoencephalitis

When a parasitic cause is diagnosed, corticosteroid treatment (prednisolone 1–2mg/kg/day) should be considered if there is raised intracranial pressure or if the patient is seriously ill. If

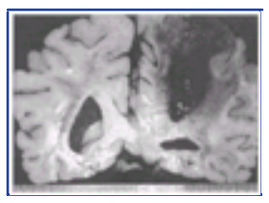

Figure 158-5 Extensive necro-hemorrhagic lesions of the right cerebral hemisphere in an AIDS patient with reactivated acute Chagas' disease. *Courtesy of Professor L Chimelli.*

specific treatment is lacking (e.g. in angiostrongyliasis, trichinosis, gnathostomiasis and toxocariasis), it may be the only option other than repeated LP to reduce pressure. Diethylcarbamazine and thiabendazole have been tried in toxocariasis and albendazole in gnathostomiasis (together with corticosteroids) with some evidence of benefit.

Focal or space-occupying lesions

In cystericercosis, when living cysticerci are present, praziquantel (without corticosteroids, which reduce its efficacy) 10–20mg/kg q8h for 21 days, sometimes increased to 25mg/kg q8h for up to 30 days,[2] is advocated. Albendazole (with preceding corticosteroid therapy) 15mg/kg/day for 1 week has replaced praziquantel in some centers. Seizures are controlled by anticonvulsants. Despite the apparent benefit of these drugs, if untreated, the majority of cysts disappear without sequelae within 1 year. Surgery should be avoided unless the cysticercus is blocking the third ventricle.

In schistosomiasis, praziquantel 40mg/kg is routinely given as a single dose; 60mg daily for 3 days may achieve more complete eradication of worms. Suspected schistosomal myelopathy should be treated conservatively with praziquantel. When this develops acutely in the Katayama syndrome, with edema of the spinal cord (Fig. 158.6), adjunctive, high-dose corticosteriod treatment is essential. Schistosomal myelopathy may improve even after several months of paraplegia. Surgery is required if there is deterioration despite treatment. If schistosomal myelopathy is encountered unexpectedly at operation, only a biopsy should be obtained.[4] It is difficult to know when schistosomiasis has been cured; antibody titers decline slowly and antigen detection may be negative although some worms persist (see Chapter 167).

Cerebral paragonimiasis is treated conservatively with praziquantel or bithionol. Hydatid cysts are completely excised, first administering praziquantel or albendazole. Surgical resection is required for a sparganum and for a coenurus (with adjunctive praziquantel).

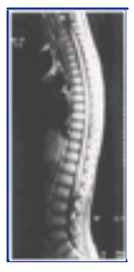

Figure 158-6 MRI of the spinal cord of a 9-year-old Omani boy with acute schistosomiasis mansoni, who presented with transverse myelitis (vertical scale in cms). Extensive cord edema from the first thoracic vertebral level to the conus medullaris is present (arrows). He recovered and was ambulant after 2 weeks treatment with prednisone and praziquantel.

Treatment of central nervous system parasitism in immunodeficiency

Standard treatment applies for most parasitic infections. The immune status should be improved if possible (e.g. highly active antiretroviral treatment in AIDS). If brain biopsy is not possible, empiric treatment for suspected toxoplasmosis in an IgG-seropositive patient (IgM remains negative) is pyrimethamine and sulfadiazine or high-dose trimethoprim-sulfamethoxazole. A convincing response within a week to 10 days supports the diagnosis. Lifelong trimethoprim-sulfamethoxazole prophylaxis follows recovery. When *Toxoplasma* IgG is negative, an intracerebral space-occupying lesion is treated either as a tuberculoma or a primary CNS lymphoma. In South America, early treatment of Chagas' meningoencephalitis (which may coexist with toxoplasmosis) with benznidazole is imperative. No effective treatment for chronic granulomatous meningitis exists (see Chapter 173 and Chapter 244). Cerebral microsporidiosis (*Encephalitozoon cuniculi*) is almost invariably fatal, but has responded temporarily to albendazole (Chapter 209 and Chapter 243). Hyperinfection with strongyloidiasis responds to ivermectin and broad-spectrum antibiotics for the usual coexistent *E. coli* meningitis.

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Chapter 159 - Epidemic Bacterial Meningitis

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INTRODUCTION

Bacterial meningitis remains a major cause of morbidity and mortality throughout the world. *Neisseria meningitidis* is unique among major causes of bacterial meningitis for its ability to cause large epidemics, especially in sub-Saharan Africa. This chapter focuses on *N. meningitidis* (the meningococcus) and epidemic bacterial meningitis.

EPIDEMIOLOGY

Although acute bacterial meningitis can be caused by a variety of organisms, until recently three-quarters of all cases were caused by *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* and *N. meningitidis*.[1] The introduction of effective Hib conjugate vaccines in industrialized countries has led to a dramatic decline in the incidence of Hib meningitis and other invasive Hib disease.[2] The Hib conjugate vaccines are progressively being introduced into routine childhood vaccination programs in developing countries with the support of the Global Alliance for Vaccines and Immunizations (GAVI), the World Health Organization (WHO) and other partners (http://www.vaccinealliance.com). Routine use of a new 7-valent pneumococcal conjugate vaccine in infants has recently been adopted in the USA.[3] Additional vaccine and disease burden data are needed to inform decision-making for the introduction of pneumococcal conjugate vaccines in developing countries. Serogroup C meningococcal conjugate vaccines have been developed and have been introduced in the UK, other European countries and Canada.[4] Other meningococcal conjugate vaccines are under development.[5]

Haemophilus influenzae type b, pneumococci and meningococci can each cause sporadic disease and case clusters, but *N. meningitidis* is the major agent of epidemic bacterial meningitis. Endemic meningococcal disease is estimated to cause 120,000 cases per year worldwide; during epidemics, attack rates can reach 1000 times baseline rates. When epidemic meningococcal disease occurs, it commands public health attention and calls for a large-scale public health response.[6] The case-fatality rate of meningococcal disease is 5–25% and, among survivors, there is also considerable morbidity, including persistent neurologic sequelae.

Humans are the only natural host of meningococci. The organisms are spread through close contact with nasopharyngeal secretions. Only a fraction of those who are exposed to meningococci develop clinical infections; 10% of individuals carry the organism asymptomatically in the nasopharynx and can transmit it to others.

The African meningitis belt (Fig. 159.1), originally characterized by Lapeysonnie in 1963,[7] is a broad region that extends from Ethiopia in the east to Senegal in the west and includes portions of 10–15 countries.[7] [8] The following three consistent epidemiologic features characterize epidemics in the African meningitis belt:

- an increase in the rate of meningococcal disease compared with historic trends;
- a shift in age-specific incidence rates, with a higher proportion of disease in older children and young adults; and
- clonality of the organism causing epidemics.

In this region, high rates of sporadic infections (1–20 cases per 100,000 population) occur in annual cycles with large-scale epidemics (usually caused by serogroup A but occasionally by serogroup C and more recently by serogroup W-135) superimposed periodically. In the countries of the African meningitis belt, epidemics with incidence rates as high as 1000 cases per 100,000 population have occurred every 8–12 years over at least the past 50 years. In addition, major epidemics have occurred in adjacent countries not usually considered part of the African meningitis belt (e.g. Kenya, Tanzania).

In periods of endemic disease the highest attack rates occur among young children but during some epidemic situations a higher proportion of cases occur in older children and young adults.[9] Small local epidemics, often unrecognized at a national level, may precede major epidemics. Typically, once an epidemic begins to expand, it does so rapidly, reaching a peak within a few weeks and, in the absence of extensive vaccination, lasting for several months. A countrywide epidemic may consist of a series of smaller epidemics, centered at the village or province level.

Neisseria meningitidis can be classified into serogroups based on the structural and antigenic differences in the capsular polysaccharide expressed. The epidemic potential varies among the serogroups. Among the nine serogroups that cause invasive disease, serogroups A, B, C and, more recently, W-135 have been associated with epidemic disease. Less common serogroups, including Y and X, have also recently been associated with disease clusters in some parts of the world.[10]

Serogroup A

Although epidemics of serogroup A meningococcal disease were common in industrialized countries early in the 20th century, they have been rare or absent in these countries since the end of the Second World War. In the past 20 years, limited epidemics concentrated among groups of low socio-economic status have occurred in Finland and New Zealand, with attack rates below 15 cases per 100,000 people in the population. In contrast, in the developing world, attack rates can exceed 1% of the population. In the past 20 years, intense group A epidemics have occurred in Brazil, Mongolia, Nepal and various regions in sub-Saharan Africa.

Between 1996 and 1998, the largest epidemic ever reported (over 350,000 cases — probably a substantial underestimate) occurred in the African meningitis belt, with more than 10 countries recording large increases in the number of cases of serogroup A meningococcal disease. Although rapid detection and early response to epidemics can reduce illness and deaths through prompt vaccination campaigns, the region was not adequately prepared to implement comprehensive control efforts. The medical and economic impact of this epidemic was substantial. Essential services and personnel were diverted and limited health budgets strained to cope with the epidemic.

The potential for global dissemination of an epidemic-associated serogroup A strain is evident from an epidemic caused by one clonal group, identified by multilocus enzyme electrophoresis (MLEE) as

Figure 159-1 The African meningitis belt.

ET-III-1 complex. Epidemics of group A meningococcal disease caused by this clonal group occurred in Nepal in 1983 and 1984, and in Pakistan and New Delhi, India, in 1985, and may have caused earlier epidemics in China. In 1987, an epidemic of group A meningococcal disease caused by ET-III-1 strains occurred in association with the annual Moslem pilgrimage (Hajj) to Mecca.[11] The epidemic started among persons from south Asian countries, including Nepal. As the pilgrims returned to their home countries, meningococci belonging to this complex were carried throughout the world, causing secondary epidemics among pilgrims and their contacts and eventually in their immediate communities. Isolated secondary cases did occur in industrialized countries but, for reasons not entirely clear, did not spread to the general community.[12] In 1987, strains belonging to the ET-III-1 complex then caused widespread epidemics in the meningitis belt of sub-Saharan Africa, including countries traditionally considered outside the meningitis belt, such as Kenya (1989), Tanzania (1990) and Burundi (1992). During 1996, ET-III-1 strains were again responsible for the large epidemics that occurred in the African meningitis belt. Other large-scale movement or displacement of populations, such as refugees, may pose similar risks.

Serogroup B

Serogroup B meningococcal disease, a major cause of sporadic disease in industrialized countries, has been associated with epidemics with attack rates of between 10 and 50 per 100,000 population, which are generally lower than in the major serogroup A epidemics. In the late 1970s, a serogroup B strain belonging to a clonal group known as ET-5 emerged in north western Europe and caused epidemics in Norway (1974–5), the UK (1974–6), Iceland (1976) and Denmark (1981). [13] Intercontinental spread of clones from this group has been documented and was probably responsible for subsequent epidemics in Cuba (1980), Chile (1985) and Brazil (1987). Starting in 1989, an epidemic of serogroup B meningococcal disease occurred in Oregon and Washington, USA, due to the ET-5 clone, with attack rates peaking in 1996 that were seven times higher than the US national average;[14] since 2001 these rates have decreased. An ongoing serogroup B epidemic has been affecting New Zealand since 1991 with annual rates of 16.9 cases per 100,000 population; highest incidence rates are found among Maori and Pacific Island children.[15]

Serogroup C

Serogroup C meningococcus, like serogroup A, can cause endemic disease, small clusters and major epidemics, such as those that occurred in Brazil (1972) and Vietnam (1977). Although attack rates in serogroup C epidemics are usually lower than in serogroup A epidemics, they can reach high levels. During a serogroup C epidemic in Burkina Faso in 1979, attack rates exceeded 500 cases per 100,000 population. Serogroup C strains identified as a single enzyme complex, ET-37, have been found to be responsible for a number of recent epidemics and a large proportion of cases of endemic disease in the USA, Canada, Europe and Latin America.

Serogroup Y

Serogroup Y meningococcal disease has been recognized as a cause of endemic disease in some populations in the USA since the 1970s. Beginning in 1988, the proportion of meningococcal disease due to serogroup Y increased in the USA from 2% (in 1988–91) to 26% (in 1996–8). From 1999–2001, about one-third of meningococcal disease in the USA was due to serogroup Y.[16] Smaller increases have been reported in Canada, Sweden and Israel. Serogroup Y is often associated with pneumonia and disease among older age groups, as compared with serogroups B and C.

Serogroup W-135

Serogroup W-135 meningococcus has recently emerged as a cause of epidemic disease. This serogroup is responsible for only 2–5% of endemic disease worldwide. However, an epidemic of serogroup W-135 meningococcal disease occurred concurrently with a serogroup A epidemic during the 2000 Hajj in Saudi Arabia, with a serogroup W-135 attack rate of 9 cases per 100,000 population. The serogroup W-135 isolates causing the epidemic belonged to the ET-37 complex as defined by MLEE and were designated as the (W)ET-37 clone.[17] In 2000 and 2001, following this epidemic, cases of serogroup W-135 meningococcal disease were identified in Europe, North America, the Middle East, Asia and Africa, associated with returning Hajj participants or their contacts. Carriage of serogroup W-135 meningococci was observed among returning Hajj pilgrims.[18] [19]

In 2002, the first major W-135 meningococcal disease epidemic occurred in Burkina Faso. More than 13,000 suspected meningitis cases and 1,400 deaths were reported, with an overall attack rate of 104 cases per 100,000 population (Ministry of Health/WHO/Centers for Disease Control and Prevention (CDC), unpublished data).[20] Serogroup W-135 isolates found in Burkina Faso in 2002 belong to the ET-37 complex and were closely related to the Hajj 2000 isolates (Ministry of Health/WHO/CDC, unpublished data). These strains were also closely related to serogroup W-135 ET-37 complex strains found in sporadic cases or carriage in other parts of the world since the 1970s, suggesting expansion of an existing clonal group rather than introduction of a new one.[17]

The potential for dissemination of serogroup W-135 meningococcal disease epidemics in African meningitis belt countries is of great concern, given the clonal association between these two recent epidemics and the fact that historically serogroup A meningococcal disease epidemics caused by the ET-III-1 complex were first seen during the Hajj in 1987 and subsequently caused epidemics in multiple regions of sub-Saharan Africa.

RISK FACTORS

Individual host factors that predispose to invasive meningococcal infections include functional or anatomic asplenia, properdin deficiency, congenital and acquired immunoglobulin deficiencies and terminal or C3 complement deficiency. [21] In addition, genetic polymorphisms such as tumor necrosis factor (TNF) promoter region polymorphisms, mannose-binding protein abnormalities, plasminogen activator and inhibitor expression and cytokine induction have been linked with severity of meningococcal disease. People infected with HIV may be at increased risk of meningococcal disease, although the risk is probably lower than that for pneumococcal infections.

In the African meningitis belt, both sporadic and epidemic disease are characterized by seasonality, with increased rates during the dry season (December to May) and rapid decline with the onset of the annual rains. Low humidity and blowing dust may damage the mucosal membranes or inhibit mucosal defenses, leading to an increase in the proportion of those who acquire the meningococcus and develop invasive disease. Poor and crowded living conditions and smoke exposure are associated with meningococcal disease. These environmental and economic factors may result from people clustering in poorly ventilated dwellings and may facilitate the spread and risk of disease. Concurrent or preceding upper respiratory infections have also been implicated in several meningococcal disease epidemics and may contribute to their seasonality.[22] These viral or mycoplasmal 'cofactors' may increase transmission and susceptibility by damaging mucosal membranes, by causing transient immune suppression and by enhancing coughing and sneezing.

Other risk factors for meningococcal disease have been difficult to characterize because they may vary between industrialized and developing countries, between endemic and epidemic disease and among illnesses caused by the different serogroups. Differences between strains may lead to variability in exposure to the organism, in acquisition of carriage and in progression to invasive disease. Furthermore, factors that place an individual person at risk of sporadic disease may differ from risk factors for an epidemic.

Meningococcal disease epidemics are usually caused by strains that are clonal, as noted by the intercontinental spread of the serogroup A III-1 clone and serogroup W135 ET-37. In contrast, the overall population structure of meningococci is panmetric. These invasive strains appear to possess particular virulence factors that confer the capacity to be transmitted more effectively, cause increased invasive disease or have antigenic characteristics not recognized by the population, leading to enhanced susceptibility. Waxing and waning of the immunologic susceptibility of a population may contribute to the apparent periodicity of meningococcal epidemics. The striking phenomenon of meningococcal epidemics results from a complex interaction that involves strain characteristics, population characteristics and cofactors such as smoking and upper respiratory tract infections.

PATHOGENESIS AND PATHOLOGY

The human nasopharynx is the natural reservoir of *N. meningitidis* and the site from which meningococci are transmitted by aerosol or secretions to others, spread to adjacent mucosal surfaces (e.g. in the lower respiratory tract) and invade and gain access to the bloodstream to produce systemic disease. [21] Meningococci overcome clearance (e.g. mucus and ciliary activity) and other local host defenses in order to attach to and multiply on this mucosal surface (i.e. colonization). The meningococcus is carried asymptomatically in the nasopharynx by 5–10% of adults in nonepidemic periods. In closed populations (e.g. among military recruits) nasopharyngeal carriage rates can be greater than 50%. Nasopharyngeal colonization is an important immunizing process that may protect against future systemic illness.

The nasopharynx also appears to be a major site of mucosal invasion of meningococci and other meningitis pathogens. Crossing of the nasopharyngeal epithelium by meningococci allows access to subepithelial tissues and blood vessels. Meningococci may also penetrate nasopharyngeal epithelium damaged by environmental

factors such as smoking or by viral or mycoplasmal co-infections.[22] In meningococcal disease, systemic illness usually follows acquisition of the organism in the nasopharynx within 2–10 days. Successful penetration through this epithelial barrier of even a few organisms that are capable of surviving in the bloodstream may be sufficient to cause systemic disease.

Survival of meningococci after invasion into the bloodstream is determined by both organism-specific and host-specific factors. Encapsulation, outer membrane proteins, lipo-oligosaccharide, inhibition or defects in serum bactericidal activity (both bactericidal antibody and complement) and, to a lesser extent, opsonophagocytic activity are involved. Levels of bacteremia correlate with the release of inflammatory cytokines (interleukins 1 and 6 and TNF-a), which are important in the pathogenesis of meningococcemia.

The mechanism by which meningococci cross the blood-brain barrier and enter the cerebrospinal fluid is not well characterized. Endothelial cell invasion, which is important in meningococcemia, may also be involved in the invasion of the central nervous system. Meningitis results from bacterial survival and multiplication in the cerebrospinal fluid, the release of inflammatory cytokines and other host factors (e.g. nitric oxide), leukocyte infiltration across the blood-brain barrier and breakdown of the blood-brain barrier with edema, coagulation and ischemia.[23]

PREVENTION

Epidemic bacterial meningitis are primarily caused by serogroups A, C and W-135. The persistence of large serogroup A epidemics of meningococcal disease in developing countries and their virtual disappearance in industrialized countries suggests that continued improvements in the standard of living in developing countries may decrease the occurrence of epidemic meningococcal disease. For now, however, prevention of serogroup A meningococcal disease epidemics focuses on vaccination. The recent serogroup W-135 epidemics in Saudi Arabia and Burkina Faso and the possible spread of this clone to other parts of the world indicates the need for broadly based meningococcal vaccines, including the development of nonserogroup-specific vaccines.

Vaccination

Meningococcal A, C, Y and W-135 polysaccharide vaccines are composed of purified capsular polymers. In adults and in children over 2 years of age, antibodies develop rapidly after vaccination and protection is achieved within 7–10 days. These vaccines have high efficacy in adults; however, antibody levels decline over 2–3 years. Following the occurrence of epidemics in military populations, military recruits in some countries have been routinely vaccinated at the start of their military service. Because these vaccines are generally poorly immunogenic in young children and have limited duration of efficacy, meningococcal A, C, Y and W-135 polysaccharide vaccines have not widely been used in routine childhood immunization programs.

Meningococcal capsular polysaccharide vaccines are distributed in freeze-dried form, injectable by intramuscular route and available either as a bivalent A/C vaccine or as a quadrivalent A/C/Y/W-135 vaccine, containing 50µg of each antigen per dose. They are generally well tolerated, with the most common side-effects being pain and redness at the injection site.

The polysaccharide vaccines can be useful in control of epidemics of meningococcal disease. During the 1996 epidemic in northern

Ghana, vaccination campaigns reduced the number of cases by an estimated 23%.[24] Data collected during the epidemic of meningococcal disease in northern Ghana in 1996–7 was used to assess the potential effect of different vaccination strategies. A strategy of using disease incidence thresholds to trigger vaccination campaigns after the epidemic was started would have prevented 61% of cases. A similar proportion of cases (61%) could have been prevented if routine childhood and adult immunization had been used, assuming that a high vaccine coverage rate had been achieved and maintained before the epidemic.[24]

The decision about when to initiate a vaccination campaign and who to vaccinate is complicated. Vaccination campaigns are expensive and logistically difficult, involving a large commitment of resources. Vaccinating large numbers of people on the basis of only a few cases of meningococcal disease may result in wasted efforts if the epidemic fails to materialize. The WHO has published guidelines for detecting and responding to meningococcal disease epidemics; these guidelines include recommendations about approaches to surveillance, case management, and formation of epidemic response teams[25] as well as recently revised recommendations on the use of threshold incidence rates to predict epidemics and trigger early decisions to implement campaigns.[26]

Following the meningococcal disease epidemics in 1996–8 in Africa, the WHO and other international agencies created the International Coordination Group (ICG) with the objective of better assuring international co-ordination to epidemic response.[27] The ICG manages a security stock of the bivalent A/C polysaccharide vaccine, specific treatment and injection material for use in epidemic emergencies. Since 1997 a stock of approximately 7 million doses of bivalent A/C vaccine has been maintained. Every year countries experiencing epidemics have used the ICG mechanism to rapidly obtain quantities of high-quality vaccine and injection materials at preferential prices.

Meningococcal A/C/Y/W-135 polysaccharide vaccines are not assured by the ICG and are produced in limited quantities worldwide. The serogroup W-135 meningococcal disease epidemic in Burkina Faso in 2002 has represented a major challenge to the ICG and international community, given the major shortage of meningococcal A/C/Y/W-135 vaccines for use in Africa. Early in 2003, a serogroup A/C/W-135 meningococcal polysaccharide vaccine (trivalent vaccine) has been produced in limited quantities for use in African countries with the prospect of increased production in the following years.

Conjugate serogroup A and A/C/Y/W-135 vaccines are currently being developed, using methods similar to those used for Hib conjugate vaccines, in which capsular polysaccharides are covalently linked to carrier proteins to convert the T-cell-independent polysaccharide to a T-cell-dependent antigen.[28] The first clinical trials with a meningococcal serogroup A and C conjugate vaccine were conducted in the Philippines and the USA. In the late 1990s, the safety and immunogenicity of a meningococcal A/C conjugate vaccine was evaluated in infants from Niger. This and other studies have shown that meningococcal conjugate vaccines are safe, improve immune response in infants, prime immunologic memory and lead to a booster response to subsequent doses.[29] [30]

At the end of 1999, conjugate C meningococcal vaccines were introduced in the UK among children and young adults under 18 years of age. Rates of meningococcal disease in the UK were approximately 2 per 100,000 population, and 30–40% of cases were caused by serogroup C.[31] Age-specific vaccine efficacy is estimated to be 89% in children under 12 months, 89% in children aged 12–24 months and 94% in adolescents aged 15–17 years.[32] [33] Although serogroup carriage rates are low, preliminary data on the effect of a serogroup C meningococcal conjugate vaccine on carriage suggest that the conjugate vaccine may reduce carriage of serogroup C meningococci, which implies that it may decrease transmission, leading to herd inmmunity.[33] No significant increases have occurred so far in carriage of meningococci expressing other disease-associated serogroups.[33] Serogroup C conjugate vaccines have also recently been introduced in several European countries and Canada.

The newly created Meningitis Vaccine Project (funded by the Bill and Melinda Gates Foundation) seeks to promote and facilitate development of meningococcal serogroup A and serogroup A/C/Y/W-135 conjugate vaccines at a reasonable price for use in Africa.[34] Production of vaccines will be followed by introduction into some of the poorest nonindustrialized countries in the African meningitis belt. As has been demonstrated for the Hib vaccine, meningococcal conjugate vaccines could have a major impact on prevention of meningococcal disease. If these conjugate vaccines prove to be capable of providing a durable antibody response, particularly in infants and young children, then integrating them into routine childhood immunization, especially in hyperendemic areas of sub-Saharan Africa, would appear to be warranted.

Because the capsule of serogroup B meningococci is identical to human cell antigens (N-CAM) and is poorly immunogenic in humans, vaccine development has largely focused on outer membrane protein vaccines. The immunogenicity and protective efficacy of vaccines against several serogroup B outer membrane proteins have been recently evaluated; results have been encouraging, although questions remain about their effectiveness against heterologous strains and in young children.[35] [36]

Chemoprophylaxis

Most people become infected after contact with asymptomatic carriers rather than with persons who have meningococcal disease, but close contacts of patients (e.g. those living in the same home) have a 500–800 times increased risk of developing disease.[37] Antimicrobial chemoprophylaxis of close contacts of patients who have meningococcal disease remains the primary preventive measure after the occurrence of sporadic cases. Systemic antibiotics that eliminate nasopharyngeal carriage include rifampin (rifampicin), ciprofloxacin and ceftriaxone. Nasopharyngeal cultures are not useful in determining who should receive chemoprophylaxis. Mass chemoprophylaxis is not currently recommended to control meningococcal disease epidemics, given that meningococcal vaccines constitute a better epidemic control strategy.

Travelers

Since the 1987 outbreak associated with the Hajj, pilgrims to Mecca have been required to show proof of vaccination against meningococcal disease on entry to Saudi Arabia. Because of the serogroup W-135 meningococcal disease outbreak in 2000, pilgrims are now required to show proof of vaccination with quadrivalent (A/C/Y/W-135) polysaccharide vaccine when entering Saudi Arabia. For other travelers, guidelines for immunization against meningococcal disease vary. Vaccination with the quadrivalent A/C/Y/W-135 polysaccharide vaccine may benefit persons traveling to or residing in countries that are undergoing an epidemic or have hyperendemic disease, particularly if contact with the local population will be prolonged.[38]

CLINICAL FEATURES

The clinical presentation of epidemic meningococcal meningitis is similar to that of other forms of acute purulent meningitis (see Chapter 22), with sudden onset of headache, fever, nausea, vomiting, photophobia, neck stiffness and alteration in mental state. Some patients who have meningococcal meningitis, but not all, have an assotension,

Figure 159-2 Meningococcal septicemia with purpura fulminans. (a) In an infant and (b) in an adult.

purpuric or petechial rash. This may be less common in adults and is difficult to recognize in dark-skinned people. Meningococcal sepsis is characterized by abrupt onset of fever and the characteristic petechial or purpuric rash, which is often severe (purpura fulminans; Fig. 159.2) and may be associated with the rapid onset of hypotension,

TABLE 159-1 -- Antibiotic treatment, chemoprophylaxis and vaccination for epidemic meningococcal meningitis.

ANTIBIOTIC TREATMENT, CHEMOPROPHYLAXIS AND VACCINATION FOR EPIDEMIC MENINGOCOCCAL MENINGITIS	
Treatment in an outbreak setting in developing countries[22]	Long-acting chloramphenicol (e.g. tifomycin) in oil suspension, single dose: Adults, 3.0g (6ml); children 1–15 years, 100mg/kg; children <1 year, 50mg/kg
Other treatment options	Penicillin G 18–24 megaunits/day iv in divided doses q4h (250,000 units/kg/day)
	Ceftriaxone 1–2g iv q12h (100mg/kg/day)
	If penicillin-allergic: chloramphenicol 100mg/kg/day im in divided doses q6h
Vaccination (generally limited to epidemics or to travelers to endemic areas)	A/C/Y/W-135 vaccine or A/C vaccine given as single 0.5ml sc injection
	Decreased efficacy in children <2 years of age; vaccine efficacy wanes after 3–5 years
Chemoprophylaxis (recommended for close contacts of cases)	Rifampin (rifampicin): Adults, 600mg po q12h for 2 days; children >1 month, 10mg/kg po q12h for 2 days; children <1 month, 5mg/kg po q12h for 2 days
	Ciprofloxacin: Adults, 500mg po (single dose)
	Ceftriaxone: Adults, 250mg im (single dose); children <15 years, 125mg im (single dose)

acute adrenal hemorrhage (Waterhouse-Friderichsen syndrome) and multiple organ failure. In infants under 1 year of age, the presentation may be atypical with slow onset, absence of neck stiffness and the presence of bulging fontanelle.

DIAGNOSIS

Diagnosis is strongly suspected based on clinical findings of headache, fever, stiff neck and a petechial or purpuric rash. Meningococcal meningitis is confirmed by a lumbar puncture, which classically shows purulent or turbid cerebrospinal fluid, elevated white blood cell count, elevated protein level and decreased glucose, and a Gram stain showing Gram-negative diplococci (often intracellular). Meningococci can be grown on Mueller-Hinton or chocolate agar. Rapid antigen detection, by latex agglutination or countercurrent immunoelectrophoresis, can identify specific serogroup capsular antigens in cerebrospinal fluid. Blood cultures are commonly positive. If a rash is present, a skin biopsy can be examined for Gram-negative diplococci. Serogroup specific polymerase chain reaction (PCR) techniques have been used, primarily in industrialized countries, for the diagnosis of meningococcal disease; rapid PCR techniques for use in Africa are under development.

In the midst of an epidemic in a developing country, the flood of patients may overwhelm the health facilities and laboratory capacity. As soon as a meningococcal epidemic has been confirmed, rapid initiation of treatment and initiation of prevention measures take priority. Laboratory confirmation of cases throughout the epidemic is encouraged, mainly for epidemics of serogroup W-135 meningococcal disease.

MANAGEMENT

Many antimicrobial agents, including penicillin, remain active against meningococci (Table 159.1), but choice of antibiotic is increasingly threatened by drug resistance, lack of central nervous system penetration and cost. Since the emergence of sulfa-resistant meningococcal strains in the 1960s, sulfa drugs are no longer considered suitable as initial therapy. Third-generation cephalosporins, such as ceftriaxone, are excellent but currently expensive alternatives in developing countries. Recently, meningococcal isolates with increased resistance to penicillin have been recognized in the USA, Spain and Canada

and isolates with increased resistance to chloramphenicol have been recognized in Vietnam and France;[39] [40] [41] however, the clinical significance and potential impact on treatment is unclear.

The care of a large number of patients during an epidemic in a developing country makes repeated injections with crystalline penicillin or even ceftriaxone impractical. A single intramuscular dose of an oily suspension of chloramphenicol has been shown to be as effective as a 5-day course of crystalline penicillin in the treatment of meningococcal meningitis[42] and it is a useful first-line therapy during epidemic periods in developing countries. If the patient fails to improve within 48 hours, a second dose should be administered and other causes of bacterial meningitis should be considered. Management of sporadic cases consists of intravenous penicillin or ceftriaxone or, if the patient is allergic to penicillin, chloramphenicol.

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Chapter 160 - Eye Infections in the Tropics

Robin Bailey

This chapter discusses the contribution of infection to the major blinding diseases of the tropics and the ocular features associated with common tropical infections. In tropical practice, visual prognosis in both major blinding infections and simple trauma is often worsened by late presentation, secondary infection and inappropriate use of traditional eye medicines.

MAJOR BLINDING INFECTIONS OF THE TROPICS

Cataract, vitamin A deficiency, trachoma and onchocerciasis are classically considered to be associated with blindness in developing countries, although community-based studies of the causes of blindness have only been conducted in a few countries.[1] [2] In two of these diseases, trachoma and onchocerciasis, and in two other important tropical infectious diseases, leprosy and measles, infection and the host response to it play prominent and contrasting roles in the pathogenesis of blindness and other ocular complications. These are discussed below.

TRACHOMA

Trachoma is a chronic follicular keratoconjunctivitis caused by infection with *Chlamydia trachomatis*, almost exclusively of serotypes A, B, Ba and C. (The 'genital' *C. trachomatis* serotypes D–K may cause disease that is indistinguishable from trachoma, but this is rare.) Trachoma is characterized by scarring sequelae of the conjunctiva after repeated infections (see Chapter 18 and Chapter 236).

Epidemiology

Trachoma is one of the most common infectious diseases; it is estimated that 500 million people are exposed to infection, and in 1995 the World Health Organization estimated that 6 million people have been blinded by trachoma. [1] As a result of demographic trends, 12 million further cases of blindness are expected within 30 years.[3] It is a disease of poverty, associated with poor personal and environmental hygiene, and it was common in much of Europe and North America during the 19th century. The map (Fig. 160.1) shows the current distribution of active trachoma based on reports reaching the WHO. 'Endemic' disease is considered to be present if there is more than 10% prevalence in school-aged children. Trachoma is found in northern and sub-Saharan Africa, the Middle East and the Indian subcontinent. There are also foci in parts of Central and South America, Australia and the Pacific.

In trachoma-endemic communities, the main reservoir of infection is the eyes of affected children; active trachoma is unusual among adults, and there is evidence that most transmission of trachoma occurs within the family[4] [5] as a result of close contact between young children and their mothers and other caregivers. Transmission is favored by poor environmental and personal hygiene, lack of water for washing, inadequate sleeping space, inadequate disposal of rubbish or sewage, and the proximity of domestic animals. It is considered to take place via fingers and fomites. Recent evidence implicates the bazaar fly *Musca sorbens*, which prefers to breed in fresh human feces on the ground, as a mechanical vector of trachoma in conditions of poor sanitation. [6] [7] The relative importance of these means of transmission probably varies from one community to another.

Pathogenesis

There is evidence that the pathologic features of trachoma are not the result of direct tissue damage but are immunologically mediated. A single infection with *C. trachomatis* usually leads to a self-limiting follicular conjunctivitis, and repeated episodes appear to be necessary for the development of intense inflammation and of scarring sequelae. The most characteristic histologic finding in active trachoma is the presence of follicles, which resemble germinal centers, in the superior tarsal conjunctiva. Subsequently subconjunctival scarring occurs and contraction of the scars causes distortion of the tarsal plate, entropion and trichiasis (inturned eyelashes).

Evidence from animal models suggests that cellular immune mechanisms are of primary importance in limiting and clearing chlamydial infection. There is evidence that scarred subjects have a reduced capacity for clearance of chlamydial infection, which occurs by way of specific T helper-1 lymphocyte responses[8] and possibly by way of cytotoxic lymphocyte activity.[9] Tumor necrosis factor-a has pro-inflammatory and antichlamydial activity and appears to participate in the process by which repeated episodes of intense disease lead to scarring.[10] Transforming growth factor-ß, a cytokine with established fibrogenic properties, is expressed to a greater extent in the conjunctivae of scarred subjects[11] and is also directly implicated in the scarring process. Antibodies to a chlamydial heat shock protein, HSP 60, are also associated with scarring in both the eye and the genital tract, but it is uncertain whether these reflect a causal role for this antigen or an epiphenomenon.

Prevention

The goal of prevention is to reduce transmission to a level at which exposure to reinfection does not occur often enough to cause blinding trachoma. Measures focused on improving personal and community hygiene, such as adequate water supplies and control of *Musca sorbens* through pit latrine provision, are likely to reduce the incidence of trachoma. A study of community education targeted at face washing showed that, with intense effort, reductions in trachoma prevalence are possible but that they were not well sustained.[12] Treatment of active cases with topical antibiotics may be effective temporarily, but such a strategy usually results in rapid reinfection either from an extraocular reservoir or from members of the household or community who are subclinically infected or incubating disease. In order to produce an effective reduction in the infectious reservoir in a community, whole families, households or communities may need systemic treatment. There is no vaccine at present.

Clinical features

In endemic communities, *C. trachomatis* infection is usually acquired early in childhood and the progressive scarring and distortion of the

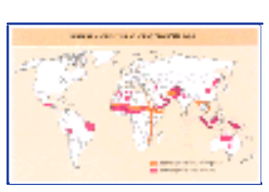

Figure 160-1 Current distribution of active trachoma (WHO).

eyelid may lead to corneal scarring and blindness, usually in late middle age. In severely affected communities signs of trachoma can be found in over 90% of children aged between 1 and 2 years, and blindness rates may approach 25% of those over 60 years. In most endemic areas, there is no sex difference in prevalence or incidence until after adolescence, but both active trachoma and its scarring sequelae are more common in women, probably reflecting their closer contact with children.

Subjects who have trachoma typically have few symptoms until the final stages, when inturned eyelashes (trichiasis) develop. Secondary bacterial infection may play a role in the mucopurulent conjunctivitis, nasal discharge and chronic otitis media seen in some patients.

The most prominent sign of active trachoma is the presence of lymphoid follicles, which are usually found on the superior tarsal conjunctiva and can be easily visualized by everting the upper eyelid, although they may also occasionally be found at the corneoscleral junction (limbal follicles). The presence of five or more of these pale yellow or white spots with a diameter <0.5mm is needed in the central area of the superior tarsal conjunctiva to meet the accepted definition of trachomatous inflammation of follicular grade (TF; Fig. 160.2). Active trachoma is also associated with capillary congestion of the conjunctiva, visible either as small red dots (papillae)

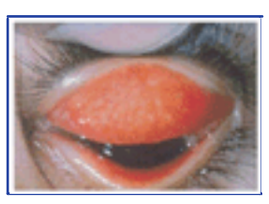

Figure 160-2 Everted eyelid showing follicular trachoma (TF). *Courtesy of the WHO Program for the Prevention of Blindness.*

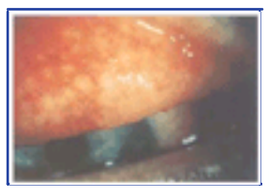

Figure 160-3 Everted eyelid showing intense inflammatory trachoma. Follicles are also present.

or as obscuration of the normally visible tarsal blood vessels. If the blood vessels are obscured in more than half of the central area over the tarsal plate, trachomatous inflammation of intense grade (TI) is said to be present (Fig. 160.3). Neovascularization of the cornea or 'pannus' is associated with active inflammatory disease and in trachoma typically involves the superior corneal margin.

Conjunctival scars (which, if clearly visible, would be graded trachomatous scarring (TS)) are initially small and stellate, but eventually become broad and confluent (Fig. 160.4). The scars contract, causing distortion of the tarsal plate and loss of its normal protective functions and resulting in inturning of the lashes (trichiasis, which is graded TT if any lash is deviated towards the eyeball), which rub on the cornea (Fig. 160.5). This can lead to corneal opacity and ultimately to blindness from abrasion of the cornea. Limbal follicles resolve to leave small depressions at the limbus known as 'Herbert's pits' (see Fig. 160.4).

Trachoma may be confused with other conditions producing a follicular conjunctivitis (Table 160.1). With the exception of viral conjunctivitis, which is acute and self-limiting, the other conditions in Table 160.1 are never endemic in a community; however, limbal follicles or Herbert's pits (Fig. 160.4) are the only clinical sign unique to trachoma[13] and these do not occur even in a majority of cases.

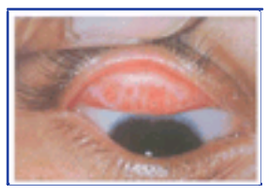

Figure 160-4 Everted eyelid showing trachomatous scarring (TS). There are also Herbert's pits visible at the corneoscleral junction. *Courtesy of the WHO Program for the Prevention of Blindness.*

TABLE 160-1 -- Causes of follicular conjunctivitis.

CAUSES OF FOLLICULAR CONJUNCTIVITIS	
Cause	**Comments**
Folliculosis	Follicles are few and occur in the inferior fornix without inflammation or hyperemia; a common finding
Viral infections	Acute, self-limiting with signs of resolution in 2 weeks
Trachoma	
'Inclusion conjunctivitis' and other ocular chlamydial infections	Also caused by ocular chlamydial infection, often with genital serotypes; frequently unilateral
'Toxic' follicular conjunctivitis:	
Molluscum contagiosum	Caused by spillage of contents of molluscum lesions on eyelids
Drug induced	Follows use of eye medications for months or years
Eye cosmetics	Granules of cosmetic seen in follicles
Bacterial infections: *Moraxella* spp. and others	Angular blepharitis with *Moraxella lacunata*; seen in adolescent girls who share eye make-up
Axenfeld's chronic follicular conjunctivitis	Reported in institutionalized children and native Americans; probably a mild form of trachoma[11]
Chronic follicular conjunctivitis of Thygeson	Outbreak in a Californian high school that contained trachoma cases; features compatible with mild active trachoma[11]
Parinaud's oculoglandular syndrome	Associated with pathogens invading through the conjunctivae; associated with systemic malaise and gross pre-auricular lymphadenopathy; some cases associated with exposure to cats and may be due to feline strains of *Chlamydia psittaci*; many other causes (e.g. syphilis, lymphogranuloma venereum, tuberculosis, tularemia)
Vernal catarrh	Occurs in atopic subjects; characteristic appearance with giant 'cobblestone' papillae

Diagnosis

Trachoma is usually diagnosed on clinical grounds. The simplified grading scheme including TF, TI, TS and TT has been developed by the World Health Organization for public health purposes.[14] Several laboratory tests can be used to confirm a diagnosis of trachoma, but these are rarely available in endemic areas. Chlamydial infection is characterized by blue intracytoplasmic inclusions in Giemsa-stained epithelial cell scrapings. The organism can be cultured in cell monolayers, visualized in smears using direct fluorescent antibody methods, or detected by enzyme immunoassay. Deoxyribose nucleic acid amplification by polymerase chain reaction and ligase chain reaction based on target sequences in the common plasmid

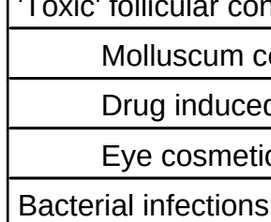

Figure 160-5 Trachomatous trichiasis (TT) and secondary corneal opacity. *Courtesy of the WHO Program for the Prevention of Blindness.*

pCT1 of *C. trachomatis* are the most sensitive methods described for demonstrating ocular chlamydial infection.[15]

Management

Individual sporadic cases are normally treated with 1% tetracycline ointment topically q12h for 6 weeks. A single oral dose of azithromycin (20mg/kg) is also effective.[16] Treatment of patients in endemic areas is usually compromised by rapid reinfection, as discussed above, and mass treatment or systemic treatment may need to be considered. Trichiasis can be treated by epilation, but this normally provides only temporary relief and lid surgery is usually needed to prevent blindness. Tarsal rotation[17] is the operation of choice. The

International Trachoma Initiative currently supports azithromycin donation to trachoma control programs as a component of the SAFE (Surgery Antibiotic treatment

Facial cleanliness and Environmental improvement) strategy endorsed by the WHO.

ONCHOCERCIASIS

Pathogenesis

In the eye, as in the rest of the body, pathology is due to an inflammatory reaction to dead microfilariae of *Onchocerca volvulus*. As these can be found in the cornea, the anterior chamber, the iris, the lens, the retina and the choroid, onchocercal lesions may involve all these sites. In the conjunctiva and iris, an immunohistochemical study has found that chronic ocular onchocerciasis is associated with predominant infiltration by $CD8^+$ lymphocytes and is associated with major histocompatibility complex class II antigen expression in resident cell populations, suggesting activation (see Chapter 18 and Chapter 246). [18]

Clinical features

Eye involvement in onchocerciasis is usually bilateral and affects men more commonly than women.

Punctate or 'snowflake' keratitis occurs as inflammatory cells accumulate around dead microfilariae, and it may respond to topical corticosteroids. Sclerosing keratitis, in which neovascularization and scarring develop nasally and temporally in the cornea and then extend inwards from the inferior limbal margin to involve the whole surface to produce a total corneal scar, has been a common cause of blindness, particularly in savannah regions. Microfilariae may be visible in the anterior chamber with a slit lamp, often as movement that is closely associated with the posterior corneal surface. Inflammatory reactions may produce iritis, and cataract may also contribute to reduced vision. In the posterior segment of the eye, choroidoretinal atrophy, with clumping and breaking up of the retinal pigment epithelium, and associated optic atrophy may follow onchocercal chorioretinitis (leading to the 'Hissette-Ridley' fundus). These changes in the posterior segment contribute further to visual loss and there is no specific treatment.

MEASLES

Pathogenesis

Measles virus infection and its consequences are a major risk to sight in tropical practice. In Africa they cause about 50% of childhood blindness, usually from corneal scarring.[19] Although measles virus infects the corneal epithelium and the conjunctiva, its devastating effects on the cornea are the result of secondary processes, which include infection, acute vitamin A deficiency, exposure and the effects of traditional eye medicines. Measles virus-associated immunosuppression appears to be responsible for reactivation of herpes simplex virus, which has been found in corneal ulcers after measles,[20] and gut involvement appears to precipitate acute vitamin A deficiency in those with marginal reserves.

Clinical features

The direct effects of measles on the eye are a punctate keratitis and sometimes conjunctival lesions that are analogous to Koplik's spots, which normally resolve without sequelae. Corneal ulceration and keratomalacia with liquefaction of the cornea and the whole eye may supervene in acute vitamin A deficiency. Secondary ulceration due to herpes simplex virus may be typically dendritic or modified by other factors as discussed below. If subjects are too sick or dehydrated to close their eyes, corneal dryness and exposure ulceration may result, and in tropical practice, traditional medicines are frequently applied and may contribute to secondary infection and a worse prognosis. Subjects who have ocular complications of measles should be treated with vitamin A, topical antibiotics and measures to avoid corneal exposure.

LEPROSY

Pathogenesis

In leprosy three main mechanisms operate in the eye to cause pathology. Overwhelming bacterial infection in lepromatous leprosy may lead to atrophy of the involved tissues. The eye may be involved in type I reactions (reversal reactions), in which motor and sensory nerve loss is prominent, and in type II reactions, in which inflammation within the eye is prominent. As elsewhere in the body, it is the reactions that cause most damage, manifesting as visual loss and blindness in the case of the eye.

Clinical features

Lepromatous leprosy may be characterized by limbal lepromata, painless yellow or pink nodules at the corneoscleral junction. Chalky deposits may be associated with corneal invasion by *Mycobacterium leprae*. The iris may become thin and atrophic, and pathognomonic 'iris pearls', which are calcified foci of dead leprosy bacilli, appear as white nodules on the surface of the iris.[20] Type I reactions involving the fifth cranial nerve can result in corneal anesthesia and lagophthalmos (inability to close the eyes) may occur if the seventh cranial nerve is involved. Together these produce a cornea that is both anesthetic and exposed, and therefore requires protection. Eye health education, blinking exercises, protective spectacles and surgical procedures such as tarsorrhaphy may all be needed.[20] Type II reactions may cause acute or chronic iritis, which requires treatment with topical corticosteroids and mydriatics, or scleritis, which may require systemic treatment with corticosteroids and clofazimine (see Chapter 154).

CLINICAL ASPECTS OF EYE INVOLVEMENT IN COMMON TROPICAL INFECTIONS

BACTERIAL INFECTIONS

Tuberculosis

Primary tuberculosis may affect the conjunctiva with nodular lesions and associated chronic conjunctivitis that is not responsive to standard topical treatment. In miliary disease, tubercles may be found in the conjunctiva, the iris or the choroid. Phlyctenular conjunctivitis and granulomatous uveitis may be associated with tuberculosis but are not specific for it. Optic neuritis may complicate tuberculous basal meningitis.

Sexually transmitted diseases

Ocular gonococcal infection, usually as a result of autoinoculation from the genital tract, causes an acute purulent conjunctivitis that may progress rapidly to corneal ulceration and perforation in the absence of appropriate treatment.

Gonococcal ophthalmia neonatorum, acquired by an infant passing through an infected birth canal, is similarly threatening to sight and usually presents in the first week of life as a bilateral purulent conjunctivitis.[21]

Ocular autoinoculation from genital *C. trachomatis* infection causes a clinical picture identical to trachoma, except that it is commonly unilateral. Among infants born to infected mothers, 30% develop chlamydial ophthalmia neonatorum, which is usually

a self-limiting bilateral mucopurulent conjunctivitis presenting within 2 weeks of delivery. In a small proportion of cases, pneumonia and permanent lung sequelae follow. Thus chlamydial and gonococcal ophthalmia neonatorum both require systemic and topical therapy.

Iritis, retinal vasculitis, optic neuritis and disseminated chorioretinitis may be features of secondary acquired syphilis (see Chapter 75).

Other bacterial infections

Petechiae may be seen at the conjunctiva in meningococcal meningitis; conjunctivitis, anterior uveitis and even panophthalmitis may complicate meningococcemia. Diphtheria may present with a membranous conjunctivitis, lid edema and local effects of exotoxin. Cholera with rapid dehydration has been associated with the acute development of cataracts. Rose spots in typhoid fever may involve the conjunctiva. The clinical picture of Parinaud's syndrome (follicular or granulomatous conjunctivitis, pre-auricular lymphadenopathy, often with systemic malaise) is associated with pathogen invasion via the conjunctiva and may be seen with tuberculosis, syphilis, tularemia and lymphogranuloma venereum. Brucellosis has been associated with chronic granulomatous uveitis. Dilatation of the conjunctival vessels and subconjunctival hemorrhages may be presenting features of leptospirosis or typhus.

PARASITIC INFECTIONS

Retinal hemorrhages may be seen in malaria and may be the earliest sign of cerebral involvement. Unilateral edema of the eyelid (Romaña's sign) may be seen in American trypanosomiasis if the inoculation site is in the region of the eye. Chorioretinitis is the most common ocular manifestation of toxoplasmosis. An ocular larva migrans syndrome (larvae migrating within the eye) may be seen with *Toxocara* spp. and *Gnathostoma* spp., and *Loa loa* typically migrates under the conjunctiva. Egg granulomas may occur in the conjunctiva or choroid in schistosomiasis. Cysticerci of *Taenia solium* may occur within the eye, often subretinally.

VIRAL INFECTIONS

Herpes simplex virus, which has a worldwide distribution, can have devastating effects on the eye. In many tropical environments this is the most common cause of corneal ulceration, often occurring as a complication of measles or causes of high fever such as malaria. A narrow, branching, dendritic ulcer that is best seen with fluorescein staining is typical (Fig. 160.6), but in tropical practice the time to presentation and inappropriate use of traditional eye medicines often modify this, leading to larger ameboid ulcers. If available, idoxuridine

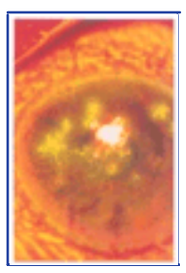

Figure 160-6 Typical dendritic ulcer caused by herpes simplex virus, visualized with fluorescein staining.

or aciclovir drops should be given very frequently until epithelial healing takes place.

Infection with HIV may itself cause a retinopathy with cotton wool spots, indicating ischemic damage to the retina. Cytomegalovirus retinopathy does not seem to be common in tropical practice, but syphilis, tuberculosis and herpes simplex virus infections and their associated ocular manifestations are more common among HIV patients. Kaposi's sarcoma may involve the eyelid or conjunctiva.

FUNGAL INFECTION AND SUPPURATIVE KERATITIS

Corneal ulceration, usually arising from mismanaged traumatic corneal abrasion, is prone to secondary colonization and infection with both bacteria and filamentous fungi. This results in a suppurative keratitis: an infected corneal ulcer with or without pus in the anterior chamber (hypopyon). The management of this condition depends on demonstrating a causative pathogen on culture or corneal scraping or on a local knowledge of the patterns of infection commonly encountered. A recent study in Ghana and India found that infected ulcers were commonly colonized with bacteria, *Streptococcus* spp. (India) or *Pseudomonas* spp. (Ghana), or fungi, *Fusarium* or *Aspergillus* spp.[22]

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Chapter 161 - Secretory Diarrheas: Cholera and Enterotoxigenic *Escherichia coli*

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INTRODUCTION

Cholera, the most severe of the secretory diarrheas, can cause dehydration and death within hours of onset in a severely purging individual. Although less likely to lead to dehydration than cholera, a more common cause of secretory diarrhea is enterotoxigenic *Escherichia coli* (ETEC); see Chapter 144). Although morbidity and mortality from cholera tend to be greatest in epidemic settings, ETEC infections occur more commonly as sporadic cases, especially in travelers to or children living in developing regions of the world.

Treatment programs that emphasize oral rehydration therapy have greatly reduced mortality from cholera and other watery dehydrating diarrheas worldwide, although diarrhea still remains the second most important cause of death in children in developing countries. Diarrheal disease is also a major cause of morbidity in the USA, where physicians are consulted for more than eight million episodes annually [1] and there is a yearly reported average of approximately 300 diarrheal deaths for children under 5 years of age, with most deaths related to dehydration.[2]

EPIDEMIOLOGY

Vibrio cholerae

Although the Bangladesh region has been the traditional home of cholera, the current pandemic — the seventh recorded in modern times — began in Indonesia in 1961 before traveling to the Indian subcontinent, the Middle East, the Soviet Union, Sub-Saharan Africa and finally to South America in 1991. The organism associated with the current pandemic is an El Tor biotype. The South American epidemic that began in Peru in January 1991 caused over a million cases in the first 3 years. The disease occurred in all age strata since the population had no protective antibody. Unboiled drinking water, unwashed fruits or vegetables and food or water from street vendors were implicated in this explosive outbreak. [3]

A newly described toxigenic non-01 strain, now designated *Vibrio cholerae* 0139 Bengal, was first identified in 1992 in southern India and Bangladesh.[4]

Cholera occurs sporadically along the Gulf Coast of the USA, mainly in Texas and Louisiana.[5] Among the millions of American travelers to endemic areas in foreign countries, only 42 imported cases of cholera were reported in the USA in the period 1965–1991.[6]

Humans are the only host for *V. cholerae* and carriers (about 5% following exposure) are seen only with the El Tor biotype. Contaminated water is the primary vehicle for spread, but certain foods have been implicated in some epidemics, especially if washed with contaminated water or, in the case of seafood (especially crustaceans and bivalves), harvested from water containing *V. cholerae*.[3] Person-to-person transmission is uncommon and health personnel rarely acquire cholera. Reduced gastric acidity, whether idiopathic or caused by drugs (e.g. H_2 -blockers, proton pump inhibitors), or previous gastrectomy increases susceptibility to infection. The inoculum of *V. cholerae* leading to natural infection is not known, but samples of water sources epidemiologically linked to outbreaks rarely contain more than 10^3 organisms/ml. *Vibrio cholerae* 01 can enter into a viable nonculturable state but still retain its pathogenicity. The bacterium can also survive on aquatic vegetation (i.e. water hyacinth) and in the hindgut mucosa of zooplankton. Both of these factors might account for the low vibrio counts in contaminated water and explain where *V. cholerae* resides in the noncholera season.[7]

Enterotoxigenic *Escherichia coli*

These infections are also acquired from other humans as animal strains of ETEC are host specific. Like cholera, the major vehicles of infection are contaminated food and beverages.

Infection occurs primarily in children, with the highest incidence in the tropics, where the reported annual incidence of ETEC-related diarrhea in children varies from 15% to 50%.[8] Reports of ETEC infection in the USA have generally demonstrated a low incidence in most cities. However, ETEC is the most common cause of diarrhea in those traveling from North America or northern Europe to areas of the developing world where diarrheal disease is prevalent.

PATHOGENESIS AND PATHOLOGY

Acute bacterial diarrhea can be classified into:

- toxigenic types, in which an enterotoxin is the major if not exclusive pathogenic mechanism; and
- invasive types, in which the organism penetrates the mucosal surface as the primary event, but enterotoxin may be produced as well.

Diarrheal toxins can be grouped broadly into two categories:

- cytotonic, producing fluid secretion by activation of intracellular enzymes such as adenylate cyclase without causing any damage to the epithelial surface; and
- cytotoxic, causing injury to the mucosal cell as well as inducing fluid secretion but not primarily by activation of cyclic nucleotides.

Both *V. cholerae* and ETEC produce cytotonic enterotoxins.

There have been several exciting developments in the area of pathogenesis during recent years, including the finding that the structural genes for the cholera toxin are encoded by a filamentous bacteriophage.[9]

Vibrio cholerae

Vibrio cholerae is an aerobic, motile, Gram-negative rod that is shaped like a comma. Toxigenic *V. cholerae* that agglutinate in 01 antiserum are the main cause of epidemic cholera. The two major biotypes of *V. cholerae* 01, classic and El Tor, can both produce identical severe watery diarrhea. Although the percentage of mild and moderate cases is higher with El Tor infections, there are more cases of El Tor and thus more severe illness is caused by this biotype. The major serotypes associated with clinical disease are Inaba and Ogawa. The clinical features of illness caused by different sero- and biotypes of *V. cholerae* 01 are virtually indistinguishable.

All wild strains of *V. cholerae*, including 0139, elaborate the same enterotoxin: a protein molecule composed of two subunits, A and B.

The B subunit is responsible for binding to the receptor on the mucosa, whereas the A subunit is responsible for binding and activation of adenylate cyclase located on

the inner cellular membrane. *Vibrio cholerae* requires both the cholera enterotoxin and toxin-coregulated pili (required for intestinal colonization) for full virulence.

The secretory diarrhea seen in cholera is caused by the action of the toxin on the epithelial cells of the small intestine. There appears to be a differential action on the mucosal cells characterized by:

- a direct secretory effect on the crypt cells; and
- an antiabsorptive effect on the villous cells.

Fluid loss originates in the duodenum and upper jejunum; the ileum is less affected. The colon is usually in a state of absorption because it is relatively insensitive to the toxin. The large volume of fluid produced in the upper intestine overwhelms the capacity of the lower bowel to absorb it.

The electrolyte composition of the stool is isotonic with plasma and the effluent has a low protein concentration. No inflammatory cells are visible on microscopic examination. Stool of heavily purging patients has very little odor except for a slightly 'fishy' smell.

Enterotoxigenic *Escherichia coli*

Inspired by the discoveries in cholera, investigators focused on *E. coli* as a possible cause of acute toxigenic diarrheal disease and found that some strains of *E. coli* elaborated an enterotoxin similar to the toxin of *V. cholerae*. There are two types of enterotoxins produced by ETEC with some species producing both or one:[10]

- the heat-labile toxin (LT) is a protein that is destroyed by heat and acid, acts like cholera toxin by activating adenylate cyclase, thereby causing secretion of fluid and electrolytes into the intestinal lumen, and shares antigenic components with cholera toxin; and
- the heat-stable toxin (ST) has no biochemical similarity to cholera toxin, is able to withstand heating to 212°F (100°C) and activates guanylate cyclase.

As is the case with *V. cholerae*, ETEC must elaborate both adherence factors and enterotoxin in order to be pathogenic.

PREVENTION

The four possible approaches to preventing cholera and ETEC infection are:

- avoidance of potentially contaminated water and food;
- prophylactic antibiotics;
- other prophylactic medications; and
- immunization.

Food and water precautions

These are necessary to prevent diarrhea and other diseases transmitted by the fecal-oral route. In endemic areas, if the quality of water is in doubt, bottled or boiled water should be used. Carbonated beverages are safer than noncarbonated ones as the organisms are very sensitive to the lower pH (4.0–5.0). Tea and coffee prepared with boiling water are generally safe. Food should be well cooked and eaten hot if possible. Snacks prepared by street vendors carry a higher risk, especially if the food is not well cooked. In coastal areas of Louisiana and Texas, seafood, especially crustaceans, should be well cooked.[6]

Prophylactic antibiotics and other prophylactic medications

Prophylactic antibiotics should not be used for cholera, especially on a mass scale, as resistance may develop. Antimicrobials have been extensively studied for the prevention of diarrhea in travelers.[10] Studies employing trimethoprim-sulfamethoxazole have shown protection rates of 71–95%. Similar rates have been observed with norfloxacin and ciprofloxacin. The prophylactic use of antibiotics should generally be discouraged, however, as it increases the risk of adverse reactions to the agent used and may enhance antimicrobial resistance.

Bismuth subsalicylate has been used for prevention, based on its antimicrobial and antisecretory properties, but provides only modest protection and must be taken daily at high doses, creating side-effects (black tongue and stool, tinnitus) that many find unacceptable.

Immunization

People infected with ETEC develop antibodies against the enterotoxin and colonization factors. Those living in high-risk areas appear to develop immunity as the attack rate for symptomatic infections for resident adults is much less than for visitors. At present there are no commercial vaccines for ETEC. An experimental ETEC oral killed vaccine has proven to be safe and immunogenic and field tests are now under way.[11]

Exposure to vibrios, whether by actual infection or asymptomatic carriage, causes an elevation of vibriocidal antibody. Protection is related to, but not guaranteed by, the presence of vibriocidal antibody titer. In endemic areas, vibriocidal activity increases with age, as acute cholera in endemic areas is a disease of young children. Antitoxin titers rise slowly after acute infection and remain elevated for months. Natural immunity is short term (3–6 months), biotype and serotype specific, and primarily vibriocidal, with some antitoxin component. The susceptibility of adults in cholera-endemic areas to *V. cholerae* 0139 Bengal strain indicated that these populations were immunologically naive and that previous exposure to *V. cholerae* 01 and its toxin provided incomplete cross-protection.

The current commercially available cholera vaccine is a parenteral killed whole-cell preparation that provides approximately 50% protection from disease for 3–6 months. Cholera vaccine has no protective value in epidemics as it requires two doses and takes weeks to be effective. As the risk of contacting cholera is small and the vaccine has limited effectiveness, the World Health Organization (WHO) recommends that a vaccine not be required for routine international travel and most countries no longer require a cholera vaccination for entry.

Limited immunity to the toxin can be achieved by injecting the B subunit of cholera toxin. By combining the B subunit with a killed *V. cholerae* vaccine, an oral vaccine with a protective efficacy of about 85% has been evaluated in field trials.[12] [13] Several genetically engineered vaccines for both *V. cholerae* O1 and O139 have been developed and have provided some promising results.[14]

CLINICAL FEATURES

For cholera and ETEC, like many other infectious diseases, there is a spectrum of clinical manifestations, from an asymptomatic carrier state to the person with one loose stool to a desperately ill patient with severe dehydration.

Cholera

After an incubation period of 16–72 hours, the initial stage of cholera is characterized by a feeling of fullness. This is soon followed by diarrhea, which accelerates over the next few hours to frequent purging. There may be nausea and vomiting. As the purging increases in frequency and volume, the stool becomes liquid and the color changes from brown to green to yellow and finally to 'rice water' (so named because it looks like water left over after boiling a pot of rice).

All of the clinical signs and symptoms of cholera can be ascribed to fluid and electrolyte losses. The stool is isotonic with plasma,

TABLE 161-1 -- Electrolyte composition of infectious diarrhea and fluid therapies.

ELECTROLYTE COMPOSITION OF INFECTIOUS DIARRHEA AND FLUID THERAPIES	
	Electrolyte concentrations (mmol/l)

	Sodium	Potassium	Chloride	Bicarbonate
Stool				
Cholera, adult	124	16	90	48
Cholera, child	101	27	92	32
Non-specific diarrhea, child	56	25	55	14
Intravenous therapy				
Lactated Ringer's solution	130	4	109	28*
Dhaka solution	133	13	98	48
Oral rehydration therapy				
WHO formula	90	20	80	30
Pedialyte	45	20	35	30†
Ceralyte	70	20	60	30†

* Equivalent from lactate conversion
† Equivalent base in the form of citrate

although there is an inordinate loss of potassium and bicarbonate (Table 161.1). With increasing fluid loss the external signs of dehydration are:

- the pulse progresses from rapid to thready;
- skin turgor decreases (often difficult to assess in infants and the elderly);
- the patient may develop 'washerwoman's hands';
- urine specific gravity increases; and
- urine volume decreases.

Breathing becomes deep and rapid as factors leading to respiratory alkalosis attempt to compensate for the metabolic acidosis brought on by the profound loss of bicarbonate in the stool. Metabolic acidosis may mask hypokalemia, which occurs in severely malnourished children or patients on diuretics. Severely dehydrated patients may present in hypovolemic shock which, if not immediately treated, can lead to cardiovascular collapse and, uncommonly, to renal failure. Mild fever may be a feature, but there are no signs of sepsis. Diarrhea can persist for up to 5 days in the untreated patient.

Enterotoxigenic *Escherichia coli* infection

There is nothing distinctive about the clinical presentation of ETEC infection. Following an incubation period of 24–48 hours, the patient develops a sense of intestinal distress (cramping is uncommon), followed shortly thereafter by watery diarrhea. As in cholera, the illness ranges from mild to quite severe, although it rarely leads to the profound dehydration seen in cholera. Although most people have 3–5 loose stools/day, about 20% of cases have 6–15 watery bowel movements/day. The average duration of illness is 3–5 days. Strains that produce only ST cause a milder attack of diarrhea than LT-producing strains, but cause more vomiting and constitutional symptoms.[15]

DIAGNOSIS

Cholera

Cholera should be suspected in patients with watery diarrhea that has little odor (especially during the periods of heavy purging), and which on microscopic examination fails to reveal formed cellular elements such as erythrocytes and leukocytes. If fresh unstained cholera stool is examined under a dark-field microscope, spiral shaped organisms will be seen to have a 'shooting star' pattern of motility; they are immobilized by antisera. If cholera is suspected, fresh stool should be cultured on thiosulfate citrate bile salts (TCBS) sucrose medium. A colorimetric test that uses monoclonal antibodies can be used to identify *V. cholerae* rapidly in clinical specimens with a high degree of sensitivity and specificity. [16]

Enterotoxigenic *Escherichia coli* infection

Diagnosis of ETEC in a routine bacteriologic laboratory is difficult and generally impractical; ETEC cannot be differentiated from nonpathogenic strains of *E. coli* on routine culture on MacConkey agar and special tests such as enzyme immunoassays, DNA probes or polymerase chain reaction are required to determine virulence factors at either the genotypic or phenotypic level.

MANAGEMENT

The major goal of treatment is replacement of fluid and electrolytes lost in the diarrheal stool. The traditional route of fluid administration has been intravenous, but oral rehydration solutions (ORSs) have proved equally effective and safe for all but the most severely dehydrated, heavily purging patients.[17] The mortality from even severe cholera should be less than 1% with proper fluid therapy (in contrast to as much as 50% if untreated). The composition of intravenous solutions and ORS is directly related to the electrolyte composition of secretory diarrhea (see Table 161.1).

Oral rehydration therapy is based on the physiologic principle that glucose enhances sodium absorption in the small intestine, even in the presence of secretory losses caused by bacterial toxins. From practical, economic and logistic perspectives, ORS is the preferred treatment in developing countries. It is also the treatment of choice for mild-to-moderate diarrhea in both children and adults in the USA and it can be used for maintenance therapy in patients with severe diarrhea after initial parenteral fluid replacement.[18]

The volume and rate of fluid replacement are based on the patient's degree of dehydration (Table 161.2). [18] The patient is thirsty when mildly dehydrated and begins to show the physical signs of dehydration without vascular collapse when fluid loss is moderate. Clinical signs of dehydration become evident after 4–5% of body weight has been lost. The level of dehydration may be difficult to determine in the elderly and often goes unrecognized. A changing

TABLE 161-2 -- **Assessment of dehydration.** Physical findings highlighted in bold type are important signs in the assessment of dehydration.*

ASSESSMENT OF DEHYDRATION			
Physical finding or symptom	**No signs of dehydration (loss of <2.5% body weight)**	**Some dehydration (loss of 2.5–10% body weight)**	**Severe dehydration (loss of >10% body weight)**
Mental status	Alert, appears well	**Irritable, restless**	**Lethargic or unconscious, floppy infant**
Eyes	Normal	Sunken	Very sunken and dry
Tears	Present	Absent	Absent
Oral mucosa, tongue	Moist	Dry	Dry
Thirst	Absent, drinks normally	**Thirsty, drinks eagerly**	**Drinks poorly or is unable to drink**
Fontanelle	Normal	Sunken	Very sunken
Skin pinch	Goes back rapidly	**Goes back slowly**	**Goes back very slowly**

* *Adapted from Tacket et al.*[14]

mental status may be an early sign. After rehydration has been completed, the amount given should be at least equal to that lost in the stool. The most common reason for treatment failure with either intravenous fluids or ORS is that insufficient amounts are given. Vomiting is not a contraindication to the use of ORS; rather, ORS should be given in smaller, more frequent sips until emesis stops, which is usually within the first 4 hours of therapy. Figure 161.1 Figure 161.2 Figure 161.3 illustrate the dramatic effect that ORS fluid replacement has on the rehydration of an adult cholera patient.

The ORS formula recommended by the WHO has been used effectively in hundreds of millions of cases. Recent studies have suggested that an ORS formula that has a lower content of sodium chloride and glucose may be better absorbed in children, therefore resulting in a need for less ORS.[18] Others have suggested, however, that the change in formula provided only marginal improvement in stool output that is more than offset by a negative net salt balance and its consequences, especially in the adult cholera patient.[19] An inexpensive alternative to glucose-based ORS is a solution in which starch derived from rice or other cereals is substituted for glucose. The taste of cereal-based solutions is more appealing to some, but additional preparation time is required and heating (and fuel use) may be necessary.

Eating during an acute episode of watery diarrhea is an important aspect of effective treatment. The traditional approach of dietary abstinence, which restricts the intake of necessary calories, has no place in the treatment. Diarrhea may be prolonged if a child is starved during the illness, and so it is particularly important to

Figure 161-1 Severe dehydration from cholera. Decreased skin turgor in a severely dehydrated cholera patient. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

Figure 161-2 Oral rehydration. The patient is immediately given ORS to correct her dehydration. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

Figure 161-3 Complete recovery from cholera after rehydration. The patient 24 hours later is completely rehydrated on ORS alone. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

restart feeding as soon as the child is able to accept food. Breast-feeding should not be discontinued. Full-strength dairy products may increase diarrhea in some individuals as a result of lactose intolerance, and so should be avoided during the episode of acute diarrhea in those not dependent upon milk as the principal source of caloric intake. Alcohol and beverages that contain caffeine or methylxanthine such as coffee or tea may increase intestinal motility, so they should be avoided.

Antimicrobial agents can be useful in the treatment of cholera as the duration of an episode, volume of stool output and period of *V. cholerae* excretion can be reduced with effective therapy (Table 161.3).[20] A 3-day course of tetracycline is effective; there is no proven value in lengthening the duration of therapy to 4 or more days. A single-dose ciprofloxacin (1g in adults) is also effective in the treatment of *V. cholerae* O1 or O139.[21]

Antimotility drugs such as loperamide have little place in the treatment of watery diarrhea in children in developing countries. The same can be said for various absorbing agents such as kaolin or pectin. Literally hundreds of antidiarrheal remedies can be found in pharmacies and assorted medical establishments throughout the world. Many products contain a combination of drugs, most of them therapeutically ineffective and others potentially dangerous.

Although few studies have evaluated the treatment of ETEC infections (as a distinct group) with antibiotics, short courses of therapy with trimethoprim-sulfamethoxazole or fluoroquinolones such as ciprofloxacin will reduce the mean duration of diarrhea in travelers who frequently have ETEC as the underlying enteropathogen.[10] Results with single-dose therapy of acute traveler's diarrhea with fluoroquinolones are also encouraging.

Travelers to areas with high rates of diarrhea would be advised to take two or more 1-liter ORS packets and, if possible, a collapsible 1-liter container for accurate measurement of water.

TABLE 161-3 -- Antimicrobial therapy of cholera.

ANTIMICROBIAL THERAPY OF CHOLERA		
	First-line therapy	**Alternatives***
Adults	Tetracycline 500mg po q6h for 3 days	Single dose of ciprofloxacin 1000mg po
	Single dose of doxycycline 300mg po	Erythromycin 250mg po q6h for 3 days
		Trimethoprim-sulfamethoxazole 160mg/800mg po q12h for 3 days
		Furazolidone 100mg po q6h for 3 days
Children	Tetracycline 12.5mg/kg po q6h for 3 days	Erythromycin 10mg/kg po q8h for 3 days
	Single dose of doxycycline 6mg/kg po†	Trimethoprim-sulfamethoxazole (5mg/kg trimethoprim plus 25mg/kg sulfamethoxazole) po q12h for 3 days
		Furazolidone 1.25mg/kg po q6h for 3 days

* For use when resistant organisms are suspected or documented present or if the patient has a history of an allergic reaction to the first-line therapy
† Not recommended for use in children under 8 years of age as it may lead to permanent discoloration of teeth

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Chapter 162 - Tropical Malabsorption and Sprue

Gerald T Keusch

INTRODUCTION

Tropical malabsorption and sprue are clinical syndromes of still uncertain pathogenesis, although many possibilities have been proposed over the years. These syndromes are expressed as a continuous spectrum of manifestations from asymptomatic malabsorption with mild histologic changes of the small bowel (tropical enteropathy) to persistent diarrhea with laboratory evidence of malabsorption (tropical malabsorption), extending to an overt clinical malabsorptive disease, tropical sprue, in which severe villous atrophy is associated with steatorrhea, chronic diarrhea and a wasting syndrome. Some of these episodes can be proven to be infectious in nature, and household or community outbreaks have been described. The term 'post-infectious tropical malabsorption' is used by a number of authorities for such illnesses.[1] In other patients, the onset of malabsorption is insidious, and no specific intestinal infection is ever documented. However, there is no evidence that tropical enteropathy is a precursor lesion for overt tropical malabsorption or sprue. As a result, tropical malabsorption and sprue have become paradigms of illnesses acquired in the tropics by native and long-term expatriate residents alike, in which infection, malabsorption and malnutrition interact to a varying extent in individual patients to cause an illness with similar overlapping manifestations.

EPIDEMIOLOGY

Definition and nomenclature

Because there is no specific diagnostic test or universally agreed definitions for this syndrome, tropical malabsorption and sprue are defined by a constellation of clinical and laboratory findings, often including the response to therapy.

Tropical enteropathy is a biochemical and histologic process occurring in residents or visitors to the tropics, in which D-xylose malabsorption[2] is associated with alteration of the small bowel villus architecture from the normal finger-like villus to a shorter, broader, tongue-like structure, with lymphocyte infiltration of the mucosa. There may be no symptoms or mild diarrhea and minimal weight loss. Tropical malabsorption is defined as clinical malabsorption associated with an identified infection, whereas in tropical sprue no etiology is found. Both are more severe than tropical enteropathy; that is, they are associated with more profound malabsorption of two or more unrelated nutrients (including carbohydrate, fat or vitamin B12), more severe villus atrophy and more intense clinical symptoms, which if not treated last for months. The associated clinical conditions include megaloblastic anemia and, on occasion, neurologic complications of vitamin B12 deficiency.

Typically, tropical enteropathy and malabsorption or sprue develop during a period of residence of months to years in the tropics, especially in the Caribbean and the Indian subcontinent, although cases are also reported from Africa [3] and other areas of Asia[4] (Fig. 162.1). Neither condition is commonly contracted by short-term visitors to the tropics.

In southern India, epidemic waves of tropical malabsorption were documented over years, typically associated with fever, leading to the speculation that a specific infectious agent was responsible.[5] In some cases, a 'corona virus-like' structure was observed in biopsy specimens of small bowel, although a specific enteric virus was not isolated.[6] Preparations for a prospective field study to isolate and identify the agent were made, but epidemic sprue subsequently disappeared and the investigators have been unable to do the study since.

PATHOGENESIS AND PATHOLOGY

Asymptomatic tropical enteropathy

With the development of safe and simple instruments for biopsy of the small bowel approximately 40 years ago, a new entity, tropical enteropathy, was defined. Studies in Bangladesh[7] and Thailand [8] in the mid-1960s reported that apparently healthy residents of these countries had shortened villi, deepened crypts and lymphocyte infiltration of the lamina propria of the jejunum compared with apparently healthy residents of industrialized nations. When simple intestinal function studies were carried out, these subjects were generally lactase deficient and malabsorbed xylose but not fat or vitamin B12. Studies of Peace Corps volunteers living in the community in Thailand demonstrated that they acquired the same histopathologic lesion and intestinal functional abnormalities during the first year in the country, and some progressed to overt tropical sprue, whereas US military personnel in the same country living in more sanitary conditions and generally eating on a military base retained a more normal mucosa and normal function.[8] Studies of Bangladeshi subjects and former American residents of Pakistan living in the USA demonstrated that the lesions of tropical enteropathy regress after a few years. [9] [10] These studies suggest that living in a fecally contaminated environment with poor sanitation in which water and food commonly contain large numbers of enteric micro-organisms is the basis for the structural and functional intestinal lesions of tropical enteropathy.

Pathologic and pathophysiologic changes

The essential features of tropical enteropathy develop in early childhood, when the finger-like villi of the small bowel of neonates become shorter and broader, with increased crypt thickness, reduced ratio of villus length to crypt length, more frequent mitotic figures and increased cellularity of the lamina propria with lymphocytes and plasma cells.[11] The enterocyte turnover rate is increased, suggesting enterocyte injury with compensatory increase in cell generation in the crypts. Studies in acute epidemic tropical sprue in India have shown focal degeneration of crypt cells at a time when villus cells are intact, which argues for a primary insult to the crypt cells. With the reduced mature villus absorptive surface, carbohydrate absorption, commonly measured by the xylose tolerance test, is diminished as well. This is associated with the failure to absorb as much as 5% of the energy content of the diet in some studies. Abnormal gut permeability as measured by the lactulose-mannitol test has been shown in such subjects.

Figure 162-1 Global distribution of tropical malabsorption and sprue. Tropical malabsorption and sprue in central equatorial Africa remains largely unexplored or unreported.

With the development of tropical sprue, greater damage of villus cells is noted, including disturbed brush border, dilated rough endoplasmic reticulum, loss of mitochondrial cristae and an increase in lysosomes. This is associated with extrusion of damaged villus cells, and an increase in cells with pyknotic nuclei, surrounded by lymphocytes and plasma cells. Fat droplets are present in the cytoplasm, in the thickened basement membrane and in the lamina propria. These changes reverse with prolonged residence in industrialized countries.[10]

It is difficult to document the sequence of physiologic changes that occur in tropical malabsorption and sprue. However, delayed small intestinal transit time may be an early and common event. Cook[1] postulates that the process is initiated by epithelial cell injury, probably postinfectious, leading to the release of enteroglucagon, which in turn results in intestinal stasis, secondary bacterial overgrowth and malabsorption. Cultures of small bowel fluid obtained by aspiration in sprue patients reveals high numbers of bacteria, including a variety of Gram-negative rods (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia*

marcescens and others). Enterocyte injury is reflected in the increased permeability of the intestine as measured by increased urinary lactulose excretion following an oral dose of lactulose. Colonic function is often abnormal, with increased losses of water and electrolytes; this correlates with the level of free unsaturated fatty acids in stool.

If the diagnosis is restricted to adults without HIV infection who have persistent diarrhea, clinical malabsorption and wasting, the number of patients meeting the case definition of tropical malabsorption and sprue has diminished remarkably over the past 20 years throughout the world. The reasons for this remain speculative, although some authorities believe the increased use of antibiotics for acute diarrhea, prompt attention to dehydration and nutritional status during and after acute diarrhea, and improved environmental hygiene in many developing countries may account for the observations. With more aggressive nutritional and antimicrobial therapy, mortality rates have dropped dramatically in the non-HIV-infected population.

PREVENTION AND RISK FACTORS

Other than residence in the tropics and acute or recurrent diarrhea, no specific risk factors have been identified. Although genetic factors have been suspected for a long time, and some HLA associations are reported,[12] no specific genes mediating susceptibility have been found. Presumably eating foods that are prepared in a sanitary environment and, therefore, are likely to be less contaminated may be useful in preventing the illness.

CLINICAL FEATURES

There are generally no clinical manifestations of tropical enteropathy in indigenous populations of the tropics; expatriates may experience a moderate increase in stool number, with softer stools and modest weight loss. Acquired lactase deficiency can result in clinical lactose intolerance, presenting as the acute onset of cramps and diarrhea after ingestion of lactose-containing dairy products. With the progression to tropical malabsorption and sprue, stool bulk usually increases and the classic manifestations of steatorrhea develop, including foul-smelling, greasy stools that float. This may be accompanied by bloating, belching, borborygmi and flatus. Continuing malabsorption is manifested by anorexia and progressive weight loss, which can be profound. Progressive vitamin deficiency (e.g. of vitamin B12 and folate) leads to megaloblastic anemia, with fatigue and limited exercise tolerance and, rarely, severe neurologic complications of vitamin B12 deficiency (subacute combined degeneration of the spinal cord).

DIAGNOSIS

The requirement for a diagnosis of tropical malabsorption and sprue is the intestinal lesion, for which small bowel biopsy is necessary. Biopsy has become much simpler than it used to be with the advent of endoscopy, which can also be used to diagnose giardiasis and strongyloidiasis and to obtain small bowel fluid for culture. There is, however, no reason to biopsy patients for suspected tropical enteropathy if they have no clinical manifestations. Malabsorption of multiple nutrients should be documented; the classic tests are carbohydrate tolerance tests (xylose or lactose), 3-day fecal fat excretion and the Schilling test for vitamin B12 absorption. Breath tests for carbohydrate absorption, small bowel overgrowth, bile salt deconjugation and intestinal transit time, as well as intestinal permeability as evaluated by the lactulose-mannitol test, are useful adjuncts to help to characterize the pathophysiologic lesion more fully. A simple full blood count and examination of the cells can document megaloblastic anemia, which suggests the value of measuring vitamin B12 and folate levels. Small bowel overgrowth is a common finding in tropical sprue, but culture of the small bowel contents is not necessary. It is, however, mandatory to look for specific treatable causes of the

TABLE 162-1 -- Differential diagnosis of tropical malabsorption and sprue.

DIFFERENTIAL DIAGNOSIS OF TROPICAL MALABSORPTION AND SPRUE	
Category	**Cause**
Malabsorption syndromes	Tropical malabsorption or sprue
	Celiac sprue
	Short bowel syndromes (trauma, surgery)
	Blind loop syndromes
	Venous or lymphatic obstruction (constrictive pericarditis, idiopathic tropical cardiomyopathy, filariasis)
Digestive conditions	Chronic calcific pancreatitis
	Bite salt deficiency (chronic liver disease, ileal tuberculosis)
Infectious diseases	Parasitic infections (*Giardia lamblia*, *Cryptosporidium parvum*, *isospora belli*, *Strongyloides stercoralis*, *Capillaria philippinensis*, leishmaniasis and others)
	Viral infections (HIV, possibly Burkitt's lymphoma)
	Bacterial infections (enteroaggregative *Escherichia coli*, possibly others)

malabsorption syndrome by culture and examination of stool for enteric bacteria and parasites, often supplemented by a string test for *Giardia* spp. and *Strongyloides* spp. In endemic areas such as the Indian subcontinent, a dye contrast study or endoscopy may be indicated to rule out intestinal tuberculosis.

Differential diagnosis

Giardia lamblia, once cause of acute travelers' diarrhea, may progress to a malabsorption syndrome with steatorrhea that mimics tropical sprue. It seems likely that one of the earliest described cases of tropical sprue in Barbados was, in reality, giardiasis.[13] Persistent diarrhea (symptoms continuing for a minimum of 2 weeks) is a hallmark of tropical sprue; however, most patients who have this complaint do not have this syndrome. Many are infected with a limited list of infectious agents, including *G. lamblia*, *Cyclospora cayetanensis* and *Cryptosporidium parvum*, and on occasion a bacterium such as enteroaggregative *Escherichia coli*, *Campylobacter jejuni* or *Aeromonas hydrophila*. Such patients respond to specific treatment for the infection (see Chapter 144).

Other illnesses overlap the clinical manifestations of tropical malabsorption and sprue (Table 162.1). [14] Since the mid 1980s, persistent diarrhea and malabsorption have been common manifestations of AIDS, especially in the tropics. These episodes are often due to opportunistic infections with *Cryptosporidium parvum*, *Isospora belli* or a *Microsporidium* spp. Other causes of persistent diarrhea and malabsorption in the tropics include parasitic hyperinfection syndromes with *Strongyloides stercoralis* or *Capillaria philippinensis*, or persistent enteritis with *Salmonella typhimurium*, *Shigella* spp. or *Campylobacter jejuni*. HIV itself appears to be capable of infecting enterocytes, resulting in a chronic diarrhea-malabsorption syndrome known as AIDS enteropathy.[15] By convention, such specific infections and AIDS enteropathy in patients with HIV infection are not classified as tropical malabsorption or sprue, even though the pathophysiology may be similar if not identical.

Several other specific causes of malabsorption may present in the tropics and are distinct from tropical malabsorption and sprue. These include gluten-induced enteropathy, intestinal tuberculosis, Burkitt's lymphoma, kala-azar and even pellagra in maize-eating cultures. Gluten sensitivity can appear for the first time during residence in the tropics, and this can be readily confused with tropical sprue, as can the less common Whipple's disease.

Persistent diarrhea in infants in the tropics, whether or not the infant is HIV seropositive, is usually accompanied by carbohydrate malabsorption and failure to thrive; this is not classified as tropical malabsorption or sprue. This is considered to be a postinfectious malabsorption syndrome, whether or not the etiology is identified.

MANAGEMENT

Treatment for specific causes of infectious malabsorption syndromes in the tropics does not differ from treatment in industrialized countries. Thus, the drugs of choice are now:

- metronidazole (2g/day for 3 days) or tinidazole (a single dose of 2g) for *G. lamblia*;
- albendazole (400mg q12h for 7 days) or thiabendazole (25mg/kg q12h for 3 days) for *S. stercoralis*; and

¦ albendazole (400mg q12h for 7 days) for *C. philippinensis*.

Patients who have AIDS and have *C. parvum* infections and malabsorption are optimally managed when combination antiretroviral therapy is given, but this is not possible in many tropical countries because of the cost of the drugs. *Isospora belli* responds to trimethoprim-sulfamethoxazole (co-trimoxazole) 160mg/800mg q6h for 10–14 days, even without antiretroviral therapy, but relapse is common when therapy stops; therefore chronic suppressive therapy is recommended (160mg/800mg three times weekly). When no specific etiology is discovered, therapy is directed toward the elimination of small bowel overgrowth with an antibiotic. Tetracycline is commonly used for this, and doses as low as 250mg q8h for 4 weeks are usually successful. Metronidazole is an alternative (500mg/day) because the anaerobic flora may be the primary culprits. The antibiotic regimen is usually supplemented with oral folic acid (5mg q12h) for 3 months; however, folic acid alone may be sufficient to reverse all manifestations of the illness.

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Chapter 163 - Typhoid Fever

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EPIDEMIOLOGY

Typhoid fever is a special form of salmonellosis that is confined to humans and characterized by prominent systemic symptoms. It is often lumped with the closely related paratyphoid illnesses under the term 'enteric fever'. Typhoid has been virtually eliminated from more affluent countries, but high levels of transmission occur notably in the Indian subcontinent, in Indonesia, in much of sub-Saharan Africa and in parts of South and Central America. Point-source outbreaks of typhoid occasionally occur in industrialized countries when contaminated foods are imported or when measures to protect the community from excreting carriers break down. Apart from this, the majority of cases of typhoid seen in industrialized countries occur in travelers returning from endemic areas.

Typhoid has been estimated to cause 33 million infections per year.[1] Community-based studies of typhoid transmission in areas of high endemicity have shown annual incidences reaching 1200 per 100,000 of the population. Transmission rates can be high both in dry weather, when access to water is poor and personal hygiene declines, and with the onset of rains, when contaminated matter is washed into water catchment areas.

PATHOGENESIS AND PATHOLOGY

At the macroscopic level, the main effects of typhoid infection are to be seen in the lymphoid tissue of the ileum known as Peyer's patches, which display an initial hypertrophy and subsequent ischemia and necrosis (Fig. 163.1). At the microscopic level, systemic *Salmonella* infections such as typhoid are characterized by the location of organisms predominantly within macrophages, where their virulence is closely linked to an ability to survive within 'spacious' phagosomes[2] and to trigger an acute inflammatory response capable of producing harmful local ischemia and necrosis. At the moleculer level, recently identified triggers of the acute inflamatory response include a caspase-1 dependent necrosis,[3] the release of interleukin (IL)-1ß and IL-18, and the release of bacterial lipopolysaccharide.

Following ingestion, typhoid bacilli multiply within the bowel lumen. They then pass through epithelial cells to reach the lamina propria and are conveyed from there to the mesenteric lymph nodes. From these nodes, organisms reach the bloodstream via the thoracic duct and, in the course of a transient primary bacteremia, the infection seeds to other reticuloendothelial sites. During the incubation period of 1–2 weeks, organisms multiply silently within sites to which they have been seeded by the primary bacteremia. The onset of symptoms correlates with the spill-over into the blood of large numbers of organisms from primarily infected sites. Infection now disseminates widely throughout the liver, spleen and bone marrow. The gallbladder is an important reservoir of infection that constantly delivers more bacilli to the intestine and Peyer's patches via the bile. It has recently been suggested this re-exposure of Peyer's patches to antigen produces a reaction analagous to the Schwartzman reaction and Koch phenomenon resulting in necrosis (Fig. 163.2).[4]

As the infection progresses, non-specific changes associated with systemic sepsis appear in many organs, such as the heart, brain and kidneys, culminating in circulatory collapse in the most severely affected patients. The local effects of ischemia and necrosis of typhoid nodules in Peyer's patches may lead to perforating intestinal ulcers. The mortality of untreated typhoid used to be about 10%. The majority of patients mount an effective immune response involving a mix of cell-mediated, humoral and mucosal elements.

PREVENTION

There are three broad strategies that can be used to interrupt the transmission of typhoid. The first is to identify and eradicate *Salmonella typhi* from infected people (patients or carriers), primarily through the use of antibiotic therapy. This will include both the management of typhoid patients and measures to detect and treat asymptomatic carriers.

The second strategy is to take measures to prevent ongoing transmission by people who are known to be infected but in whom treatment measures have failed or have not yet produced results. This includes such measures as safe disposal of infected urine and feces from patients, prevention of nosocomial transmission and legislation to exclude chronic carriers in whom eradication measures have failed from food-handling professions.

The third strategy involves protective measures that can be taken by people who may be exposed to risk of infection. These measures include avoidance of unsafe water and food in areas of typhoid transmission and typhoid vaccination.

The identification and treatment of carriers are dealt with in the section on Management, below. In areas of typhoid endemicity, tap water is unsafe when water supplies are contaminated by raw effluent, and foodstuffs (such as fresh vegetables) are unsafe when washed with such water. *Salmonella typhi* thrives in a wide range of foods. Notable outbreaks have occurred in association with dairy products, including ice cream, processed meats and shellfish, which efficiently concentrate *Salmonella* spp. from sea water that has been contaminated by untreated effluent.[5]

A range of vaccines have been developed for typhoid.[1] None of them is wholly effective and protection may be overcome by heavy inocula of bacteria. Because efficacy data on typhoid vaccines come principally from studies that have been conducted in endemic areas, where higher levels of naturally acquired immunity are present, the degree of protection for previously unexposed people tends to be overestimated.

Vaccines that are currently available do not offer concomitant protection against paratyphoid strains. A recent meta-analysis of published studies of vaccines against typhoid reported 3-year cumulative efficiencies of 73% for two doses of the parenteral whole-cell vaccine, 55% for a single dose of the parenteral Vi vaccine and 51% for three doses of the oral Ty21a vaccine.[6] Local and systemic reactions are common with whole-cell vaccines and abdominal symptoms may occur with the oral vaccine.

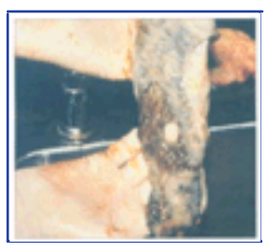

Figure 163-1 Perforating typhoid ulcer of the terminal ileum. This large, necrotic ulcer covered in dark brown slough is on the antimesenteric side of the distal ileum. A gloved fingertip is seen within the large perforating ulcer that caused the patient's death. *Reproduced with permission.*[22]

CLINICAL FEATURES

Typhoid affects the sexes equally. In endemic areas it shows a predilection for children and young adults.

The majority of patients with typhoid seen in affluent countries present with the triad of persistent fever, headache and abdominal symptoms (mainly abdominal pain

and diarrhea or constipation). Their symptoms rarely progress beyond this stage. In developing countries, patients tend to present later and are more likely to be seen with the vast array of less common symptoms and complications that are associated with typhoid.

The fever of typhoid has special characteristics that help to differentiate it from other fevers. The onset is typically gradual and in the early part of the infection a persistent high fever with relatively little

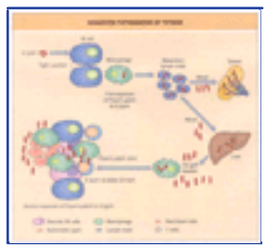

Figure 163-2 Suggested pathogenesis of typhoid. A Schwartzman-type reaction occurs when Peyer's patches are re-exposed to Salmonella typhi following initial uptake, hematogenous dissemination and return to small intestine via gallbladder.[4]

diurnal variation is common. Rigors are not typical of early typhoid but they may be observed in the second and third weeks of untreated infection. Prominent temperature-pulse dissociation is a well-known feature of typhoid but it is not invariably present, nor is it unique to typhoid.

Headache is a prominent feature of typhoid and its absence should cast considerable doubt on the diagnosis.

The primary focus of typhoid within the small bowel gives rise to a variety of abdominal symptoms. Gastroenteritic symptoms (diarrhea, abdominal pain, nausea, vomiting) are prominent in some patients (more so in children), but constipation is equally well known. Severe diarrhea has been described in AIDS patients who have typhoid. The absence of abdominal symptoms does not exclude typhoid and in some patients the systemic symptoms very much dominate the clinical picture. A dry cough is a common feature of typhoid and scattered wheezes may be audible on auscultation.

As the infection progresses, typhoid patients may display a variety of neuropsychiatric manifestations.[7] A depressed, apathetic appearance may occur first and progress later to an agitated, twitching delirium before the onset of coma. Typhoid presenting with psychotic symptoms is well known in Africa and India. Such neuropsychiatric manifestations carry important prognostic significance, the mortality in a study from Indonesia being 50% in patients with marked confusion.[8]

Findings on examination

When examining patients with suspected typhoid, a search should be made for the classic rose spots that develop in 60% of Caucasian patients at about the 10th day. Lesions are less common and also less easy to discern on dark skins. Rose spots tend to be quite few in number, are most likely to be found on the skin of the abdomen (Fig. 163.3) and can extend to the chest, back and upper arms.

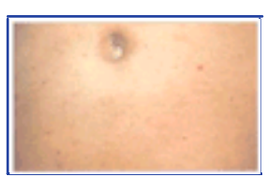

Figure 163-3 Typhoid rose spots. The abdomen is the best place to look for these lesions, which appear at the end of the first week and are usually quite sparse. They usually take the form of pink macules that blanch when the skin is stretched, but they may take on a more purpuric, nonblanching character, as in this patient. *Reproduced with permission.*[22]

Typically, they appear as small pink macules that blanch with pressure. Purpuric lesions may also be found. More florid versions of the same rash are associated with paratyphoid.

Abdominal examination may reveal enlargement of the liver and spleen in some cases and a diffuse abdominal tenderness is often present. In more serious cases gaseous abdominal distention occurs and this may herald the onset of the acute abdomen that accompanies ileal perforation.

Clinical assessment of patients should also include an assessment of the mental state and hemodynamic status, because hypotension and mental abnormalities carry important prognostic significance.

Complications

The important complications of typhoid are:

- bleeding and perforation of ileal ulcers,
- circulatory collapse in severely ill patients,
- relapse following treatment, and
- long-term carriage of infection.

A comprehensive list of the complications of typhoid, most of which are rare and seen mainly in patients in whom there has been a delay in diagnosis and treatment, is presented in Table 163.1 .

DIAGNOSIS

The clinical features of typhoid are often non-specific and many other causes of fever may have to be entertained. The most dangerous condition that requires exclusion is usually falciparum malaria. Vasculitic conditions, notably temporal arteritis, can masquerade as typhoid. Complicated cases of typhoid may present with pneumonia, nephritis, meningitis or psychosis.

The definitive diagnosis of typhoid requires isolation of *S. typhi*. Less definitive but sometimes more rapid evidence of infection can be obtained by demonstrating the presence of *S. typhi* antigens in body fluids or of antibodies to those antigens.

Culture

Salmonella typhi can be grown from feces, urine, blood, bone marrow, bile or rose spots. The highest yield is obtained from bone marrow, which can continue to yield positive cultures in patients who have been exposed to effective antibiotic therapy before culture attempts. Fine-needle aspiration methods can be used to minimize patient discomfort.

TABLE 163-1 -- Complications of typhoid.

COMPLICATIONS OF TYPHOID.
Abdominal complications
Intestinal perforation
Intestinal hemorrhage
Hepatitis
Cholecystitis

Spontaneous splenic rupture
Rupture and hemorrhage of mesenteric nodes
Pancreatitis
Genitourinary complications
Urinary retention
Glomerulonephritis
Pyelonephritis
Cystitis
Orchitis
Cardiovascular complications
Myocarditis
Pericarditis
Endocarditis
Electrocardiographic abnormalities
Phlebitis and arteritis
Deep venous thrombosis
Gangrene
Shock
Sudden death
Respiratory complications
Bronchitis
Pneumonia
Laryngeal ulceration
Glottal edema
Neuropsychiatric complications
Delirium
Psychosis
Depression
Deafness
Meningitis
Encephalomyelitis
Transverse myelitis
Upper motor neuron signs
Extrapyramidal disorders
Impairment of co-ordination
Optic neuritis
Peripheral and cranial neuropathy
Guillain-Barré syndrome
Pseudotumor cerebri
Hematologic complications
Anemia
Disseminated intravascular coagulation
Hemoloysis
Hemolytic-uremic syndrome
Focal infections
Abscesses of brain, liver, spleen, breast, thyroid, muscle, lymph nodes
Parotitis
Pharyngitis
Osteitis
Arthritis
Other complications
Myopathy
Hypercalcemia
Decubitus ulceration
Abortion
Development of chronic carrier state
Relapse
Most of these complications occur rarely and are seen mainly in patients in whom there has been a delay in diagnosis and treatment.

Serology and antigen detection

The tube agglutination tests originally developed by Widal and others to demonstrate a serologic response to *S. typhi* continue to enjoy widespread use, but the interpretation of such test has many pitfalls. Preferably, they should be used in conjunction with more reliable methods. A 4-fold rise of antibodies to *S. typhi* O, H or Vi antigens provides support for a diagnosis of typhoid in culture-negative patients. High levels of antibody are found in some healthy people in endemic areas and after vaccination. Some patients fail to mount a response to specific antigens. A modification of the Widal test carried out as a slide agglutination using commercially

available antigens offers a useful way of obtaining rapid support for a diagnosis of typhoid. Detection of *S. typhi* antigens in urine can also be used for rapid diagnosis of typhoid.[9]

Hematologic and biochemical findings

Patients with typhoid commonly have leukopenia. Hemoglobin and platelet counts may show a modest reduction.

Biochemical findings commonly include a modest degree of hyponatremia and hypokalemia and modest elevation of transaminase levels. Occasionally, a typhoid hepatitis develops with more pronounced derangements of liver function tests.

Imaging

Chest radiography may be prompted in typhoid patients by the presence of cough and abnormal auscultatory findings. Usually, the appearance of the chest radiograph is normal, but occasionally typhoid presents with, or is complicated by, a secondary pneumonia. Abdominal sonography may demonstrate enlargement of the liver and spleen, prominent mesenteric lymph nodes and, in the event of perforation, abdominal fluid collections.

MANAGEMENT

A decision about initiating therapy must be made according to the patient's condition and the likelihood of typhoid. Initiation of fluorinated quinolone therapy will bring rapid relief of symptoms to typhoid patients but delay in initiating therapy carries few hazards for

TABLE 163-2 -- Treatment options for patients with typhoid.

TREATMENT OPTIONS FOR PATIENTS WITH TYPHOID						
Drug	**Daily dose**	**Route**	**Doses/day**	**Duration**	**Comments**	**Key trials**
Fluorinated quinolones						
Ciprofloxacin	0.5–1g	po/iv	2	7–14 days	Rapid fever clearance and low relapse rates. Short courses (as little as 3 days) work well for those living in endemic areas. Longer duration may be required for nonimmune travelers	Arnold *et al.*[16]
Fleroxacin	400mg	po/iv	1			
Ofloxacin	800mg	po/iv	2			
Pefloxacin	800mg	po/iv	2			
Extended-spectrum cephalosporins						
Cefixime	20mg/kg	po	1	7–14 days	Slower response rates and higher treatment failure than with quinolones. Short course therapy more likely to fail. Less concern about toxicity in pregnancy and children	Cao *et al.*[17]
Ceftriaxone	50–60mg/kg	im/iv	2			
Azalides						
Azithromycin	500mg	po	1	7 days	Compares favorably with quinolone therapy. Useful in areas with quinolone resistance. Not validated in severe typhoid	Chinh *et al.*[18]
These treatments are suitable for most patients infected with multiple-resistant strains of *Salmonella typhi*.						

mild cases. At least one trial has reported no difference in outcome for children with uncomplicated typhoid treated with and without antibiotics[10] and management without antibiotics is well validated for other forms of uncomplicated salmonella diarrhea.[11]

Antibiotics for typhoid

Chloramphenicol was shown to be effective in the treatment of typhoid in 1950 and for many years it was considered the treatment of choice. Later studies showed equally good results from treatment with amoxicillin and trimethoprim-sulfamethoxazole.

The management of typhoid has been complicated over the past 30 years by the emergence all over the world of strains that are multiply resistant to these antibiotics. In the search for newer antibiotics with good activity against multiple-resistant strains of *S. typhi*, most of the attention has been on fluorinated quinolones, extended-spectrum cephalosporins and, more recently, azithromycin. Randomized comparisons of quinolones and cephalosporins suggest that the quinolones tend to give higher cure rates and more rapid defervescence, [12] whilst azithromycin is proving useful in areas with emerging quinolone resistance.

The incidence of long-term carriage and relapse after treatment with quinolones also appears to compare favorably with other antibiotics. An important concern about quinolone use has been its suitability for pregnant women and children, but there is now an increasing amount of evidence that quinolones are superior to alternative agents and that children can be treated safely with these drugs.[13] Many different fluorinated quinolones have been tried in the treatment of typhoid and most appear to be effective. Fleroxacin[14] [15] has the advantage of once-daily oral treatment and recent trials have indicated that as little as 3 days treatment are sufficient for children with uncomplicated typhoid in Vietnam. To summarize, most authorities would prefer quinolones over cephalosporins for the treatment of multiple-resistant typhoid in all age groups.[12] The optimal duration of treatment for typhoid is not clearly established. Short courses of quinolone therapy often work well for those resident in endemic areas but may be less adequate for travelers. Treatment recommendations for typhoid are listed in Table 163.2 . Quinolone resistance is being reported increasingly from Asian countries. A UK reference

laboratory has reported quinolone resistance in 23% of 179 infections investigated in 1999.[19] Such cases may respond to prolonged maximum dose quinolone therapy, a cephalosporin or azithromycin.

Management of severe typhoid

Trials of quinolones in typhoid have mostly been conducted in patients with mild disease. So far there are few data on the impact of quinolones and the optimal duration of therapy in patients with severe typhoid. Work conducted in Indonesia in the 1980s defined a group of patients, on the basis of circulatory and mental findings, who were at greatly increased risk of a fatal outcome.[8] The criteria for 'severe' typhoid were mental confusion (or more profound alterations of conscious level) or shock, defined as systolic blood pressure of below 12kPa (90mmHg) in adults and 10.67kPa (80mmHg) in children under 12 years, associated with evidence of decreased organ perfusion — peripheral shutdown, oliguria after rehydration (<0.3ml/kg urine output per hour) or an abnormal state of consciousness. In a randomized controlled trial involving patients who met these criteria, the administration of high-dose dexamethasone (3mg/kg infused intravenously over 30 minutes, followed by eight further doses of 1mg/kg q6h) resulted in a case fatality rate of 10.0% compared with one of 55.6% in controls not treated with corticosteroids.[8]

Ileal perforation and hemorrhage

In a small number of patients, one or more of the ileal Peyer's patch ulcers progresses to full perforation, resulting in an acute abdomen. The onset of symptoms can be gradual with perforation occurring against a steadily increasing background level of abdominal pain. Gas under the diaphragm may be demonstrable radiographically and ultrasound can be used to locate feculent fluid collections. The outcome of patients with perforation is greatly improved by prompt diagnosis, full preoperative resuscitation, early surgery and the addition of metronidazole to the antibiotic regimen. Conservative management of perforation, although once in vogue, is no longer advocated[20] because available data suggest poorer outcomes, but no controlled trials have ever been undertaken. Hemorrhage from ileal ulcers may accompany perforation or it may occur in isolation. A minority of bleeds require transfusion; life-threatening hemorrhage is rare.

Relapse

Typhoid shows a natural tendency to relapse in about 10% of patients. Relapse occurs about 2 weeks after recovery from the initial episode and usually takes the pattern of the initial episode in milder and shorter form. *Salmonella typhi* isolates from relapsing patients show the same antibiotic susceptibilities as isolates from the

initial episode and thus treatment with the same drugs is feasible. Lower relapse rates have been reported since the introduction of fluorinated quinolones in typhoid management.

Typhoid carriers

Typhoid carriers play a central role in typhoid transmission and the detection and management of carriers are central to the control of epidemics. Excretion of organisms may occur during acute illness, during the convalescent phase and, in a small proportion of those infected, it continues for life. After treatment of typhoid, a series of stool and urine specimens should be cultured for *S. typhi*. In the first 3 months following infection, most carriers identified excrete on a temporary basis only and the only intervention called for its strict personal hygiene and safe disposal of excreta. Anyone still excreting *S. typhi* at 3 months is likely to become a long-term carrier. Detection of persistently high Vi antibody offers an additional way of screening for long-term carriers. Successful eradication of long-term carriage can be achieved using ciprofloxacin 750mg q12h or norfloxacin 400mg q12h orally for 4 weeks.[21]

Chronic typhoid carriage is more likely in the presence of chronic diseases of the liver (opisthorchiasis), gallbladder (cholelithiasis) or urinary tract (nephrolithiasis or schistosomiasis), and treatment of these associated conditions facilitates antibiotic eradication of *S. typhi*.

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Chapter 164 - Amebiasis and Other Protozoan Infections

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INTRODUCTION

In terms of its morbidity and mortality, invasive amebiasis is the most important protozoan infection of the gastrointestinal tract. Most of this chapter is therefore devoted to this disease. Giardiasis and cryptosporidiosis are also frequently encountered in geographic and travel medicine and are briefly discussed.

Amebiasis is the infection of the human gastrointestinal tract by the protozoan parasite *Entamoeba histolytica*. The motile form of the parasite, the trophozoite, lives in the lumen of the large intestine, where it multiplies and differentiates into the cyst, the resistant form responsible for transmitting the infection. Trophozoites can invade the colonic mucosa and produce dysentery and through blood-borne spread give rise to extraintestinal lesions, mainly liver abscesses. The course of dysentery is usually self-limiting, but amebic liver abscess is potentially fatal unless diagnosed promptly and treated appropriately.

During the past 20 years there has been growing evidence in the fields of biochemistry, immunology, molecular biology, clinical medicine and epidemiology that many asymptomatic intestinal infections formerly attributed to 'nonpathogenic' strains of *E. histolytica* are caused by a different species of ameba, namely *E. dispar*. This species is morphologically identical to *E. histolytica*, but has a non-invasive nature, as suggested by Brumpt in 1925 (see Chapter 242).[1]

AMEBIASIS

EPIDEMIOLOGY

Invasive amebiasis is a major health and social problem in certain areas of Africa, Asia and Latin America. In most industrialized countries, however, severe amebiasis is much less common, but knowledge of the disease is still important in these areas because failure to identify an amebic infection can result in a lethal outcome (e.g. intestinal amebiasis may be treated as chronic ulcerative colitis). In addition, high infection rates can exist among certain immigrant groups and epidemic outbreaks can occur in institutions such as schools or psychiatric hospitals. A major increase in intestinal amebic infections has been detected in male homosexual populations in several large cities of the USA, Canada and England with point prevalence rates varying from 20% to 31%. In these populations most reported cases are asymptomatic, probably because many of the infections are caused by *E. dispar*. In Japan, however, invasive amebiasis due to *E. histolytica* is not uncommon among sexually active male homosexuals.

In areas of high prevalence invasive amebiasis is characteristically endemic. In 1984 it was estimated that 40 million people worldwide developed disabling colitis or liver abscesses. At least 40,000 deaths that year were attributable to amebiasis, mostly as a consequence of liver abscesses.[2] Therefore, on a global scale, amebiasis is the third most common parasitic cause of death, behind only malaria and schistosomiasis. More recent data on the morbidity and mortality of amebiasis are lacking.

Symptomatic intestinal amebiasis occurs in all age groups, whereas liver abscesses are mostly seen in adult males. The people who pose the greatest risk of transmitting the infection are those who pass cysts of *E. histolytica*, especially if they are involved in food preparation and handling. In endemic areas a variety of conditions, including poor education, poverty, overcrowding, inadequate and contaminated water supplies and poor sanitation, favor fecal-oral transmission from one person to another.

PATHOGENESIS AND PATHOLOGY

The lytic and invasive characteristics of *E. histolytica* are related to multifactorial mechanisms that include:

- striking trophozoite motility and phagocytic capacity; and
- release of membrane pore-forming peptides and proteases that produce contact-dependent lysis of target cells and degradation of extracellular matrix components.[3]

Invasion of the colonic and cecal mucosa by *E. histolytica* begins in the interglandular epithelium. Cell infiltration around invading amebae leads to rapid lysis of inflammatory cells and tissue necrosis. Acute inflammatory cells are therefore seldom found in biopsy samples or in scrapings of rectal mucosal lesions. The ulcerations may deepen and progress under the mucosa to form typical 'flask ulcers', which extend into the submucosa, producing abundant microhemorrhages (Fig. 164.1). This explains the finding of hematophagous amebae in stool specimens and rectal scrapings, which are the best indication of the amebic nature of a case of dysentery or bloody diarrhea. Macroscopically, the ulcers are initially superficial, with hyperemic borders, a necrotic base and normal mucosa between the sites of invasion. Progression of the lesions may result in a loss of the mucosa and submucosa covering the muscle layers and eventually lead to rupture of the serosa. Recent advances in the study of amebic pathogenicity have started to unravel the molecular basis of the pathologic findings in invasive amebiasis.[4]

Complications of intestinal amebiasis include perforation, direct extension to the skin and dissemination, mainly to the liver. Amebae probably spread from the intestine to the liver through the portal circulation. The extent of liver involvement bears no relationship to the degree of intestinal amebiasis, and the two conditions do not necessarily coincide. The early stages of hepatic amebic invasion have not been studied in humans. In experimental animals, inoculation of *E. histolytica* trophozoites into the portal vein produces multiple foci of neutrophil accumulation around the parasites, followed by focal necrosis and granulomatous infiltration (Fig. 164.2). As the lesions increase in size, the granulomas are gradually replaced by necrosis until the lesions coalesce and necrotic tissue progressively occupies an increasing proportion of the liver. Hepatocytes close to the early lesions show degenerative changes, which lead to necrosis, but direct contact between liver cells and amebae is very rarely observed.[5] Human liver abscesses consist of areas in which the parenchyma has been completely replaced by a semisolid or liquid

material (Fig. 164.3) composed of necrotic material and a few cells. Neutrophils are generally absent and amebae tend to be located at the periphery of the abscess. Liver abscesses may heal, rupture or disseminate.

If appropriately treated, invasive amebic lesions almost invariably heal without the formation of scar tissue whether localized in the large intestine, liver or skin. The absence of fibrotic tissue following regeneration is particularly striking in the liver.

PREVENTION

The main reservoir of *E. histolytica* is humans. The infection has a prepatent period ranging from 2 days to 4 months. The transmissibility period of untreated intestinal infections is variable as cysts have been demonstrated in feces for as long as 2 years. The cysts may remain viable and infective for a few days in feces. Because they are killed by desiccation, cyst-laden dust is not infective. Cysts are also killed by temperatures higher than 154.4°F (68°C), and so boiled water is safe to use. The amount of chlorine usually used to purify water is insufficient to kill cysts; higher concentrations of chlorine are effective, but the water must be dechlorinated before use.[6]

Invasive amebiasis could be controlled through improvements in living standards and the establishment of adequate sanitary conditions

Figure 164-1 Pathology specimen from a fatal case of human amebic colitis. Deep ulcerations into the submucosa have produced abundant hemorrhages. *Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaria de Salud.*

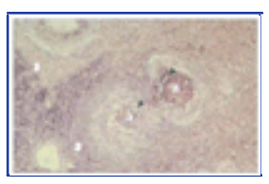

Figure 164-2 Experimental amebic liver abscess. Two characteristic granulomas can be observed with several trophozoites (arrowheads) around its necrotic center (N) and epithelioid cells limiting the lesion, surrounded by an area of fibrosis (F).

in countries where the disease is prevalent. Strategies should be aimed at:

- the community, through the improvement of environmental sanitation including water supply, food safety and health education to prevent fecal-oral transmission; and
- the individual, through early detection and treatment of cases of infection and/or disease.[7]

Cases of invasive amebiasis require prompt chemotherapy and asymptomatic carriers should be treated if infected with *E. histolytica*. Mass chemotherapy of high-risk populations has been attempted with only partially successful results. Individual or collective chemoprophylaxis is not indicated. [8]

CLINICAL FEATURES

Intestinal amebiasis

The clinical spectrum of intestinal *E. histolytica* infection ranges from the asymptomatic carrier state and acute colitis to fulminant colitis with perforation, depending upon the host's nutritional status and susceptibility, including age and, probably, differences in the virulence of amebic strains.

Invasive intestinal amebiasis usually manifests as an acute rectocolitis. Most patients present with a nontoxic dysenteric syndrome and constitutional symptoms are not as prominent as in *Shigella* dysentery. The onset of acute rectocolitis is gradual and 85% of patients have intense abdominal pain. Initially there are loose watery stools, but these rapidly become blood-stained and contain mucus. Tenesmus occurs in 50% of patients and is always associated with rectosigmoidal involvement. Watery diarrhea or loose stools without blood may be present for a few days, particularly if the distal colon is involved.[9]

Amebic liver abscess

This is the most common extraintestinal form of invasive amebiasis. Amebic abscesses occur in all age groups, but are 10 times more frequent in adults than in children and are more common in males than in females. Although liver abscess develops after intestinal infection, patients rarely have associated amebic rectocolitis, but the large intestine is colonized with *E. histolytica* in more than 70% of cases. Lesions are usually single and localized to the right lobe of the liver in the posterior, external and superior portions.

Figure 164-3 Human amebic liver abscess. Multiple abscesses, one cavitated, can be observed occupying virtually all lobes of the liver parenchyma, which is replaced by a semisolid material. *Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaría de Salud.*

In most patients, mainly those under 30 years of age and children, the clinical presentation and course of the disease are typical (Table 164.1). The onset is abrupt, with pain in the upper abdomen and high fever. The pain is intense and constant, radiating to the scapular region and right shoulder; it increases with coughing, deep breathing or when the patient rests on the right side. When the abscess is located in the left lobe, the pain tends to be felt in the epigastrium and may radiate to the left shoulder. Fever is present in most cases; it varies between 100.4°F (38°C) and 104°F (40°C), frequently in spikes, but is sometimes constant over several days, with rigors and profuse sweating. There is anorexia and rapid weight loss and approximately one-third of patients have nonproductive cough. Nausea and vomiting may occur and in some cases there may be diarrhea or dysentery. Physical examination reveals a pale wasted patient who has an enlarged tender liver. Digital pressure in the right lower intercostal spaces produces intense pain and there is often marked tenderness on percussion over the right lower ribs in the posterior region. Movement of the right side of the chest and diaphragm is greatly restricted, as is the intensity of respiratory sounds. Older patients may present with a chronic and milder nonspecific febrile illness, hepatomegaly, anemia and abnormal liver function tests.[9]

Complications

Amebic liver abscesses commonly produce thoracic complications, particularly pleurisy with a nonpurulent pleural effusion, rupture into the bronchial tree and, less commonly, rupture into the pleural cavity or amebic pericarditis. Rupture into the abdomen occurs in approximately 8% of patients who have amebic liver abscess; only rarely do abscesses rupture into the gallbladder, stomach, duodenum, colon or inferior vena cava. Occasionally, an abscess may erode through the abdominal wall and reach the skin. Secondary infection of amebic liver abscesses is an uncommon complication, which can be suspected when the patient presents with a severe toxic state and there is lack of response to antiamebic chemotherapy.[10]

TABLE 164-1 -- Clinical features of amebic liver abscess.*

CLINICAL FEATURES OF AMEBIC LIVER ABSCESS	
Clinical feature	**Proportion of cases (%)**
Symptoms for <2 weeks	37–66
Symptoms for 2–4 weeks	20–40
Symptoms for 4–12 weeks	16–42
Symptoms for >12 weeks	5–11
Fever	71–98
Abdominal pain	62–98
Diarrhea	14–66
Cough	10–32
Weight loss	33–53
Tender liver	80–95
Hepatomegaly	43–93
Epigastric tenderness	22
Rales, rhonchi	8–47
Jaundice	10–25
White cell count >10,000/mm^3 (10×10^9 /1)	63–94
Hemoglobin <2g/dl (20g/l)	25–90
Elevated transaminases	26–50
Elevated alkaline phosphatase	38–84
Elevated bilirubin	10–25
Increased erythrocyte sedimentation rate	81

* *Data from Martínez-Palomo and Ruiz-Palacios.*[3]

DIAGNOSIS

Intestinal amebiasis

Rectosigmoidoscopy and colonoscopy of benign cases show small ulcerations with linear or oval contours, 3–5mm in diameter and covered by a yellowish exudate containing many trophozoites. In the great majority of cases rectosigmoidoscopy and immediate microscopic examination of rectal smears for the presence of motile

hematophagous trophozoites of *E. histolytica* are the most important diagnostic procedures. The microscopic examination of amebae has several drawbacks, including the requirement for a skilled technician and the need to perform the examination on fresh clinical specimens. Cyst detection usually requires concentration methods including flotation or sedimentation procedures. In cases of colonic invasive amebiasis serologic detection of antiamebic antibodies is positive in approximately 75% of cases. Reliable and sensitive assays such as immunoassay or hybridization using gene probes to distinguish *E. histolytica* from *E. dispar* infections are already in the market, but still unavailable to clinical laboratories in developing countries due to high costs. However, initial pilot studies have produced excellent results using specific diagnostic reagents.[11]

Amebic liver abscess

This condition should be suspected, particularly in endemic areas or if there is a history of travel to those countries, in patients who present with a spiking fever, weight loss and abdominal pain in the upper right quadrant or epigastrium and tenderness in the liver area. Other signs include leukocytosis, an elevated alkaline phosphatase and an elevated right diaphragm in chest radiographs. Liver imaging with sonography or computerized tomography will demonstrate a space-occupying lesion in 75–95% of cases depending upon the procedure used and course of the illness. This should be followed by testing for antiamebic antibodies, which are elevated in more than 90% of cases. The tests currently used are indirect hemagglutination, counterimmunoelectrophoresis and enzyme immunoassays. The antibody response is directly related to the duration of the illness. It may be negative during the first week after onset and titers reach a peak by the second or third month, decreasing to lower but still detectable levels by 9 months.[3]

Differential diagnosis

The differential diagnosis of amebic liver abscess should include pyogenic abscess and neoplasm (see also Chapter 49). Pyogenic abscess is more common in older patients who have a previous history of hepatobiliary diseases, abdominal sepsis, appendicitis, diverticulitis or abdominal surgery. These patients are more likely to present with jaundice, pruritus and septic shock. Hepatomegaly and an elevated diaphragm in the chest radiographs are uncommon and amebic serology is negative. Aspiration is indicated for microscopy and culture if there is a space-occupying lesion and negative serology.

Liver neoplasm is a differential diagnosis when the patient is febrile and wasted and has vague abdominal discomfort. Neoplasms produce distinct images, particularly on CT scanning, and testing for tumor markers such as alpha fetoprotein or carcinoembryonic antigen is useful.

Stool microscopy for the identification of trophozoites or cysts of *E. histolytica* is of value for the diagnosis of amebic liver abscess because, as mentioned above, many patients have associated asymptomatic intestinal amebiasis.

MANAGEMENT

Metronidazole and related nitroimidazole compounds have contributed greatly to decreasing the morbidity and mortality rate of

amebiasis. They are reasonably well tolerated and despite their reported carcinogenic effect in rodents and their mutagenic potential in bacteria, no such effects have been reported in humans. Emetine hydrochloride, dehydroemetine and chloroquine, which have activity against the organism, are seldom used now.

Amebic liver abscess should be treated with chemotherapy; surgery is rarely indicated. The recommended oral dosage for metronidazole is 1g q12h for 5–10 days for an adult and 30–50mg/kg/day in three divided doses for 10 days for children. The intravenous route is highly effective for patients who have a complicated hepatic abscess; the recommended dosage in those cases is 500mg q6h for 5–10 days. In many cases of amebic hepatic abscess, a favorable response is obtained following the third day of treatment, but administration of the drug for 10 days increases the rate of cure to nearly 95%. Other nitroimidazole derivatives may be effective within 1–3 days but in view of the serious nature of amebic liver abscess as a disease, there is little reason to shorten the duration of therapy. Despite some isolated reports of failure in the treatment of amebic liver abscess with metronidazole, in-vitro studies and experiments with animal models of liver abscess have not demonstrated the existence of metronidazole-resistant strains of ameba. In 85% of cases liver imaging reveals resolution of amebic abscesses within 6 months of treatment; the remaining 15% of cases still show imaging defects 3 years after treatment.[3]

Oral administration of metronidazole is occasionally accompanied by symptoms of gastrointestinal upset such as abdominal pain, nausea and vomiting. Additionally, patients often report a metallic taste and a brownish discoloration of the urine. Undesirable reactions are observed on ingesting alcoholic beverages, probably due to inhibition of alcohol dehydrogenase. Metronidazole and its derivatives should not be administered during the first trimester of pregnancy and should only be prescribed under strict supervision during the second and third trimesters because of their ability to cross the placental barrier and rapidly enter the fetal circulation. The effect of these drugs on fetal development is unknown. Similarly, because of their elimination in breast milk, they are not recommended for nursing mothers; breast-feeding should be suspended if metronidazole is prescribed. To prevent recurrences and transmission, patients who have amebic liver abscess treated with metronidazole should also be treated with a luminal amebicide because up to two-thirds of them have asymptomatic intestinal colonization with *E. histolytica*. The most frequently used amebicides with luminal action are diloxanide furoate, di-iodohydroxyquin and paromomycin.

Current indications for percutaneous drainage of an amebic liver abscess are:

- imminent rupture of a large abscess;
- as a complementary therapy to shorten the course of the disease when the response to chemotherapy has been slow; and
- when pyogenic or mixed infection is suspected.

Drainage should be carried out under ultrasound or CT guidance. Catheters should not be left in for drainage and should be rapidly removed to avoid contaminating the track and skin.

Indications for surgical drainage include:

- imminent rupture of an inaccessible liver abscess, especially of the left lobe;
- a risk of peritoneal leakage of necrotic fluid after aspiration; and
- rupture of a liver abscess.

Monitoring the patient's condition

A prompt diagnosis and adequate chemotherapy will control most cases of liver abscess produced by *E. histolytica*. In general, a full clinical recovery and disappearance of the liver lesions (as confirmed by CT scanning) can be expected for uncomplicated cases. The prognosis is favorable in the absence of severe malnutrition or alcoholism, age over 50 years, multiple lesions, signs of peritonitis, evidence of toxemia or a history of operative treatment for amebiasis. A poor prognosis is associated with ascites or coma, especially if in a patient over 50 years or if the patient has severe jaundice, signs of peritonitis or toxemia.

GIARDIASIS

Giardiasis is a common infection of the human small intestine by the protozoan parasite *Giardia lamblia*. Most patients are asymptomatic, but an unspecified percentage of those infected develop acute or chronic symptoms. Acute manifestations include a sudden onset of explosive, watery, foul diarrhea with flatulence, cramps and abdominal distention and absence of blood, mucus or cellular exudate in the stools. Subacute or chronic infections may be accompanied by flatulence, mushy foul stools, cramps and abdominal distention. Spontaneous resolution of the infection seems to be common. Diagnosis is carried out by finding trophozoites or cysts on microscopic examination of the stools. Treatment is usually effective with metronidazole, tinidazole or furazolidone. Metronidazole is the drug of choice.

Extraintestinal complications of giardiasis are rare and include chronic cholecystitis with pain in the right upper quadrant of the abdomen, perhaps as a consequence of the presence of trophozoites in the gallbladder. In addition, granulomatous hepatitis and cholangitis have been reported in association with chronic diarrhea, weight loss, fever, hypoalbuminemia and anemia. In all of these reports, eradication of the parasite with specific chemotherapy has produced a rapid improvement of symptoms and a resolution of the histologic changes when liver biopsy has been performed. For a fuller description of the clinical and laboratory aspects of giardiasis see Chapter 46 and Chapter 242 .

CRYPTOSPORIDIOSIS

Cryptosporidium are intestinal protozoan parasites of domestic and wild animals and have recently been found to be an uncommon cause of debilitating diarrhea in humans. In immunocompetent patients the infection is usually self-limiting and the symptoms include diarrhea, abdominal pain, nausea, vomiting and anorexia. In contrast, in immunocompromised patients, particularly those who have AIDS, the symptoms may last for several months and produce profound weight loss. The diagnosis is based on the microscopic finding of *Cryptosporidium* in stool samples using acid-fast stains to differentiate the parasite from yeasts (see also Chapter 46 , Chapter 127 and Chapter 243).

An effective treatment for cryptosporidiosis in humans is not yet available. Paromomycin may have modest activity, but has not proved to be efficacious in controlled trials.

Cryptosporidium infection of the gallbladder and biliary tract has been found in 10–26% of patients who have AIDS and results in acalculous cholecystitis, extrahepatic bile duct stenosis and sclerosing cholangitis. Sonographic or CT imaging show an enlarged gallbladder with a thickened wall, dilated or irregular intra- and extrahepatic biliary ducts and a normal or stenotic distal common bile duct. Diagnosis is made histologically after cholecystectomy or ampullary biopsy or by examination of the bile for oocysts. Among patients who have HIV infection who are exposed to *Cryptosporidium*, those who have a $CD4^+$ T cell count less than 50 cells/mm^3 have an increased risk of developing biliary symptoms and of death within 1 year after the infection. Paromomycin treatment decreases stool frequency and oocyst excretion, but biliary disease progresses despite long-term therapy. Operative treatment includes cholecystectomy and sphincterotomy, with variable therapeutic success.[12]

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Chapter 165 - Ova, Cysts and Parasites in the Stool

Peter L Chiodini

INTRODUCTION

The objective in the diagnosis of parasitic disease is to find the parasite and, for most infections, microscopy remains the 'gold standard'. If the specimen is examined by a competent microscopist, then a rapid, highly specific diagnosis can be obtained. However, the very need for experienced laboratory staff is also the weakness of microscopy as a diagnostic method, because well-trained pathologists and laboratory technologists are in short supply, especially in those areas of the world where parasitic disease is most prevalent. New methods for detection of parasite antigens by enzyme-linked immunosorbent assay (ELISA), 'dipstick' technology or detection of parasite DNA are coming into routine use. Optimum diagnosis requires:

- an understanding of the life cycles of the parasites and of their geographic distribution;
- examination of freshly collected samples, preserved where appropriate; and
- the use of the correct parasite isolation method and stain for those parasites potentially present in a given specimen.

When helminth eggs or larvae are found in the stool, the adult worms are usually situated in the bowel. Exceptions to this rule are:

- *Fasciola* spp., *Clonorchis* spp. (Fig. 165.1) and *Opisthorchis* spp., the adults of which live in the bile ducts;
- *Schistosoma* spp., paired adult worms of which live in the mesenteric or perivesical veins; and
- *Paragonimus* spp., the adults of which reside in the lungs.

The protozoa whose cysts or trophozoites are detected in stool samples are all primarily parasites of the gastrointestinal tract, although extraintestinal spread occurs in *Entamoeba histolytica* infection and in *Cryptosporidium parvum* or microsporidial infections in immunocompromised hosts (see Chapter 46).

Geographic distribution

The geographic distribution of the common parasites that can be diagnosed by stool examination is shown in Table 165.1 .[1]

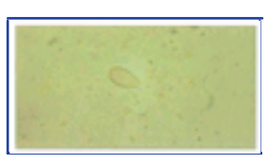

Figure 165-1 Ovum of *Clonorchis.* Large, operculated ova in fecal specimen.

INVESTIGATIONS TO CONFIRM THE DIAGNOSIS

Relevant information

The test request form should state the patient's clinical symptoms and signs, where the patient is normally resident and whether there has been any recent overseas travel, stating the particular location visited if so. If the patient is immunocompromised, this should also be stated because it should prompt the diagnostic laboratory to perform tests for parasites such as microsporidia, which might otherwise be omitted.

Sample collection

Unless the fecal sample is properly collected and taken care of, it will be of no value for diagnosis. A clean, dry container is essential; urine and water destroy protozoal trophozoites and dirt renders identification more difficult. Approximately 100g of feces is required. Ideally, the sample should be brought to the laboratory as soon as it is passed, to avoid deterioration of protozoal trophozoites. Diarrheal specimens and specimens containing blood or mucus should be examined promptly after receipt in the laboratory because such samples may contain motile amebic or flagellate trophozoites, which may become rounded and non-motile and be missed if examination is delayed. Where amebic dysentery is suspected, the laboratory should be advised that a 'hot' stool is being submitted, to permit its examination within 20 minutes of being passed. (It should be noted that the term 'hot stool' refers to the urgency of the request. The stool should not be heated but merely prevented from cooling significantly by prompt delivery to the laboratory.) With the exception of 'hot' stools, samples that cannot be examined on receipt should be stored at 39°F (4°C).

Rectal scrapes for amebae should be placed directly on to a microscope slide by the clinician performing the proctoscopy. A drop of 0.9% sodium chloride solution kept at 98.6°F (37°C) is added and a coverslip is applied. The scrapes are then taken directly to the laboratory, which should be informed in advance so that the specimen can be examined on receipt.

Rectal snips for schistosome eggs should be placed directly onto a microscope slide by the clinician taking them, a drop of 0.9% sodium chloride added and then a coverslip applied. The snips need to be examined promptly in the laboratory in order to prevent them from drying up.

Many laboratories routinely advise preservation of fecal samples as soon as possible, either immediately after collection or on receipt by the laboratory. Examples of preservation fluids include formalin, merthiolate-iodine-formalin and sodium acetate-acetic acid-formalin. The choice of preserving fluid is influenced by the subsequent technique to be used. For example, formalin is not a suitable fixative if permanently stained fecal smears are to be prepared.

Visual observation of the fecal sample

The macroscopic appearance of the fecal sample can sometimes give a clue to the underlying pathology; for example, the pale fatty

TABLE 165-1 -- Geographic distribution of the common parasites that can be diagnosed by stool examination.*

GEOGRAPHIC DISTRIBUTION OF THE COMMON PARASITES THAT CAN BE DIAGNOSED BY STOOL EXAMINATION	
Widespread, especially in areas of poor sanitation	**Organism**

Protozoa		*Giardia intestinalis (lamblia)*
		Entamoeba histolytica and
		Entamoeba dispar
		Cryptosporidium parvum
		Isospora belli
		Cyclospora cayetanensis
		Dientamoeba fragilis
		Blastocystis hominis
		Microsporidia (various species)
Helminths	Nematodes	*Strongyloides stercoralis*
		Hookworm
		Ascaris lumbricoides
		Trichuris trichiura
		Enterobius vermicularis
	Cestodes	*Hymenolepis nana*
		Hymenolepis diminuta
		Taenia saginata

Focal distribution		Geographic areas
Helminths		
Nematodes	*Capillaria philippinensis*	Philippines, Thailand, Egypt, Indonesia
Cestodes	*Taenia solium*	Central and South America, southern Asia, China, parts of Africa
	Diphyllobothrium latum	Russia, parts of North and South America, potential for transmission in eastern Scandinavia and eastern Europe
Trematodes	*Schistosoma haematobium* (ova occasionally seen in stool rather than urine)	Africa, eastern Mediterranean, Indian Ocean Islands, western Asia
	Schistosoma mansoni	Arabian peninsula, Africa, parts of South America, some Caribbean islands
	Schistosoma japonicum	China, Indonesia, Philippines, Thailand
	Paragonimus spp.	Asia, Africa, Central and South America (including China, Taiwan, Thailand, Cambodia, Nigeria, Peru, Ecuador)
	Clonorchis spp. and *Opisthorchis* spp.	Far East, eastern Europe, former Soviet Union
	Fasciola gigantica	Southern and South East Asia, Africa
	Fasciola hepatica	Parts of Europe, the Middle East, Africa, Central and South America.
	Fasciolopsis buski	Asia
	Heterophyids (including *Heterophyes* spp. and *Metagonimus*)	Asia, Siberia, Turkey, the Balkans

** Data from the World Health Organization.*[1]

stool of malabsorption may suggest giardiasis. The presence or absence of blood, mucus and exudate are noted. Adult nematodes (e.g. *Ascaris*, *Enterobius*) or tapeworm proglottids may also be seen in the specimen.

Preparation for microscopic examination

A formalin-ether or formalin-ethyl acetate concentration method should be performed on all fecal samples examined for parasites. This increases the numbers of ova, cysts and larvae per given volume by approximately 20-fold but does not improve the yield of trophozoites, which are usually destroyed by this method. Direct microscopy should be done on all unformed and liquid samples. This permits detection of amebic or flagellate trophozoites and provides information on the presence of any exudate in the stool (e.g. fecal leukocytes). All fecal specimens should be examined for the presence of *Cryptosporidium*. If laboratories are unable to do this, a minimum requirement is that all specimens from children up to and including 15-year-olds be tested.[2] Ideally, a permanently stained direct fecal smear should be prepared from all stool samples. Where resources are limited, its application may be restricted to bloody, liquid or semiformed stools. Permanently stained smears can reveal the presence of intestinal parasites that are either destroyed or missed by the formalin-ether or ethyl acetate concentration method. A plan for testing stool specimens is given in Figure 165.2 .

Reporting criteria

Ideally, the presence of all parasites should be reported, especially in the case of protozoan cysts, whether or not they are considered to be pathogens. If the practice of the laboratory is to report all parasites, the report should then state whether they are regarded as pathogens. The stage of the parasite should also be stated:

- for protozoa, whether cysts or trophozoites were seen; and
- for helminths, whether adult worms or their eggs were detected and the developmental stage of any larva found.

Morphology

The microscopic appearances of common protozoan cysts and trophozoites are summarized in Table 165.2 and Table 165.3 .

Particular care must be taken to distinguish oocysts of *Cyclospora* spp. from those of *Cryptosporidium* spp. because both are acid-fast in fecal smears stained with modified Ziehl-Neelsen stain (see Table 165.3).[3] [4]

It is important to note that cysts of *E. histolytica* and *Entamoeba dispar* cannot be distinguished from each other morphologically but, if amebic trophozoites are evident in a fresh stool preparation, the presence of ingested erythrocytes within them is diagnostic of *E. histolytica*. However, macrophages can also ingest erythrocytes, which can lead to confusion with amebae. Permanently stained preparations may be required to resolve the diagnosis.

Microsporidial spores can be visualized in a thin fecal smear by non-specific fluorescence[5] using calcofluor or uvitex 2B stains, but the presence of microsporidial spores must be confirmed using a modified trichrome stain[6] as small fungi and some artefacts may also fluoresce.[7] With the modified trichrome stain, the spores are seen as oval, pinkish structures with a polar vacuole, often with a diagonal line across the spore. Size varies according to species, with an approximate range of 1.5–3µm for the microsporidia found in human feces.

A key to identifying eggs of the common intestinal helminths is given in Figure 165.3 .[1]

Nematode larvae in fresh fecal specimens are usually the result of *Strongyloides* spp. infection. However, if an unfixed stool sample more than 12–24 hours old is received, hookworm eggs (Fig. 165.4) may hatch to release larvae, which then need to be distinguished from those of *Strongyloides*. When seen in fecal concentrates, rhabditiform larvae of hookworm are 100–150µm × 15–17µm in size, with a long (15µm) buccal cavity. The esophagus occupies one-third of the body

length and has two swellings. The hookworm larva has a small (7µm) genital primordium and the anal pore is 80µm from the

Figure 165-2 Laboratory examination of fecal specimens.

TABLE 165-2 -- Morphology of common protozoal cysts and trophozoites in fecal samples.

MORPHOLOGY OF COMMON PROTOZOAL CYSTS AND TROPHOZOITES IN FECAL SAMPLES					
Species	**Cyst**				**Trophozoite**
	Size (µm)	**Number of nuclei**	**Chromidial bar**	**Glycogen inclusion**	
Entamoeba hartmanni	7–9	1–4	Blunt, round end-young cyst	Diffuse	Clear pseudopodia
Entamoeba histolytica/dispar	9–14.5	1–4	Blunt, round end-young cyst	Diffuse	Ingested RBC (*E. histolytica* only), clear pseudopodia
Entamoeba coli	14–30	1–8	Seldom seen, sharp, splintered	Diffuse	Blunt pseudopodia, sluggish movement
Iodamoeba butschlii	9–15	1	-	One compact mass	Rarely seen
Endolimax nana	6–9	4 (pin-point)	-	-	-
Dientamoeba fragilis	No cyst stage				Small, angular, two nuclei
Refractile inclusion					
Giardia intestinalis (lamblia)	8–12	4 (not obvious)	Clear axostyle	-	Pear shaped, two nuclei, undulating flagella, little motility
Chilomastix mesnili	5–6	1	Axostyle	-	Size similar to *Giardia*, pointed tail, rapid motility
Trichomonas hominis	5–6	1	-	-	Undulating membrane
Enteromonas hominis	No cyst stage				Small, three flagellae

TABLE 165-3 -- Comparison of microscopic appearances of *Cyclospora*, *Isospora* and *Cryptosporidium*.

COMPARISON OF MICROSCOPIC APPEARANCES OF *CYCLOSPORA, ISOSPORA* AND *CRYPTOSPORIDIUM*			
	Cyclospora	***Isospora***	***Cryptosporidium***
Size range (µm)	8–10	20–33 × 10–19	4–6
Appearance in formalin-ether concentrate	Spherical refractile, greenish central morula. Unsporulated when passed in feces	Oval. Usually unsporulated when passed in feces	Not usually seen
Sporulated occyst	Two oval sporocysts, each containing two sporozoites	Two spherical sporocysts, each containing four sporozoites	Spherical or slightly ovoid; four sporozoites
Appearance in modified Ziehl-Neelsen stain	Irregular staining	Stains well, often cyst wall only	Stains well with pale center
Appearance under ultraviolet light	Bright blue autofluorescence	No effect	No effect
Fluorescence with auramine	Poor	Variable	Good; bright yellow discs, often with erythrocyte-shaped pattern
Fluorescence with monoclonal antibody to *Cryptosporidium*	Absent	Absent	Good; often shows line on surface of oocyst

posterior end. Rhabditiform larvae of *Strongyloides* (Fig. 165.5) are 200–300µm × 15–18µm in size, with a short (4µm) buccal cavity. The esophagus occupies one-third of the body length and has two swellings. The genital primordium is large (22µm) and the anal pore is 50µm from the posterior end.

Incubation of fecal samples in a charcoal-based culture system to yield filariform larvae is more sensitive than concentration methods for the diagnosis of *Strongyloides*. Again, any larvae isolated must be distinguished from those of hookworm. Filariform larvae of *Strongyloides* are 500µm × 14–20µm in size, unsheathed and have a forked tail. The esophagus occupies half the body length. Filariform larvae of hookworm are 500µm × 14–20µm in size, with a sheath and a tapered tail. The esophagus occupies one-third of the body length.

Serodiagnosis by ELISA provides a useful screening method for the diagnosis of *Strongyloides* but it exhibits some cross-reactivity with other nematode infections, which can render interpretation difficult.

Alternative methods to classical microscopy for the diagnosis of cysts, ova and larvae in the stool

In recent years there has been increasing interest in the development of new methods to detect parasites in fecal samples.

Cryptosporidium parvum

Cryptosporidial infection in humans is most commonly due to *C. parvum*, but *Cryptosporidium felis*, *Cryptosporidium muris* and *Cryptosporidium meleagridis* have been identified in immunocompromised individuals. *Cryptosporidium parvum* has two genotypes, type 1 (human-derived) and type 2 (animal- and human-derived), and it has been suggested that they may represent two distinct species. [8]

Oocysts of *C. parvum* can be detected with improved specificity by fluorescence microscopy using *Cryptosporidium*-specific monoclonal antibody. It is often possible to demonstrate a 'suture' line on the oocyst surface using this method. Cryptosporidial infection can also be diagnosed by an antigen-capture ELISA[9] for the detection of oocyst antigen in stool supernatant. The test has a reported sensitivity of 94% and specificity of 99% compared with microscopy. Using fluorescence-labeled probes for real-time detection of *Cryptosporidium* and melting curve analysis of the polymerase chain reaction (PCR) products to differentiate species and genotypes, the genetic polymorphism in the small subunit ribosomal RNA of *Cryptosporidium* can be exploited to detect and speciate cryptosporidia infecting humans. This provides a rapid tool for the investigation of water-borne outbreaks.[10] Detection of *Cryptosporidium* in water supplies also provides a powerful tool for outbreak investigation. Filtration and purification techniques such as immuno-magnetic separation and flow cytometry with fluorescent activated cell sorting provide efficient oocyst capture and

separation from noncryptosporidial debris. Polymerase chain reaction or reverse transcription (RT)-PCR methods permit speciation and genotyping of the parasite.[11]

Giardia intestinalis (Giardia lamblia)

Monoclonal antibody-based immunofluorescence can be used to detect *Giardia* sp. in a fecal smear. An ELISA is available for the detection of *Giardia*-specific antigen (GSA)-65, a glycoprotein of molecular weight 65kDa, in aqueous extracts of feces.[12] The assay has a reported sensitivity of 98–100% compared with conventional fecal microscopy and has the substantial advantage of analyzing many samples simultaneously without the subjectivity of microscopy.

Entamoeba histolytica and Entamoeba dispar

Understanding of human amebiasis has been transformed by the demonstration that the organism formerly known as *E. histolytica* is, in fact, two separate species, *Entamoeba histolytica* and *E. dispar*, the cysts of which are morphologically identical.[13] *Entamoeba histolytica* is pathogenic to humans; *E. dispar* is not. If cysts of *E. histolytica* or *E. dispar* are found in a fecal sample, further evaluation is required in order to determine whether *E. histolytica* is present. Amebic serology, if positive, supports the diagnosis of *E. histolytica* but, especially in areas where amebiasis is common, seropositivity may be related to past infection rather than to current infection. Furthermore, sensitivity of serology is low in asymptomatic amebic cyst passage.

Entamoeba histolytica infection can be diagnosed by an ELISA that detects antigen by using antibodies to the galactose adhesin of *E. histolytica*.[14] Reported sensitivity and specificity for identification of *E. histolytica* antigen are 92.6% and 96.7% respectively, compared with positive specimens in which *E. histolytica* could be identified by zymodeme analysis. Correlation between zymodeme analysis and the antigen detection ELISA is 94.7%.

Detection of *E. histolytica* and *E. dispar* in fecal samples can be undertaken by PCR-solution hybridization enzyme-linked immunoassay (PCR-SHELA).[15] [16] The PCR assay is based on primers specific for a 145bp DNA sequence in *E. histolytica* and a 133bp DNA sequence in *E. dispar*. These are based on highly repeated sequences in

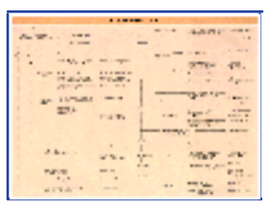

Figure 165-3 Key to the identification of helminth eggs. *Adapted from the World Health Organization.*[1]

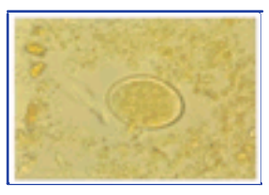

Figure 165-4 Ovum of *Diphyllobothrium latum* in a fecal specimen.

ribosomal DNA episomes, several hundred of which are present in each amebic trophozoite. Hybridization of PCR product to probes labeled with 5' digoxigenin (for *E. histolytica*) and 5' fluorescein (for *E. dispar*) is followed by colorimetric detection using peroxidase-labeled antidigoxigenin and peroxidase-labeled antifluorescein. The method is reported to identify 10^{-1} *E. histolytica* and 1–10 *E. dispar* trophozoites per gram of feces when they are present separately, and 10 *E. histolytica* and 100 *E. dispar* trophozoites per gram of feces in the presence of 10^6 trophozoites per gram of feces of the other species. When this assay was applied to 18 clinical specimens from which *E. histolytica* or *E. dispar* had been cultured and identified by isoenzyme analysis, the PCR-SHELA gave the correct result in each case.

Microsporidia

Microscopy for these organisms requires a high level of expertise and is time-consuming. Diagnosis can be aided by use of species-specific

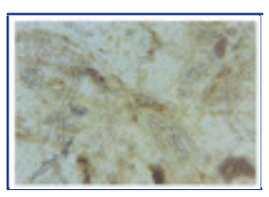

Figure 165-5 Numerous rhabditiform larvae of *Strongyloides* in a duodenal juice specimen.

immunofluorescence of microsporidial spores,[17] when sensitivity comparable to that achieved by PCR has been reported,[18] and by PCR.[19] One study comparing light microscopy and PCR for the detection of microsporidia in fecal specimens[20] recorded 89% sensitivity and 98% specificity for PCR, compared with 80% sensitivity and 95% specificity for light microscopy. Neither species-specific immunofluorescence nor PCR for these emerging pathogens is widely available at present.

TREATMENT AND COMPLICATIONS

Provided samples have been examined by a competent microscopist, identification of named parasitic ova, cysts or parasites in a fecal sample is diagnostic, and so specific treatment can be given. Empiric treatment is, therefore, rarely required.

The treatment and complications of these infections are discussed in Chapter 46 and Chapter 209 .

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Chapter 166 - Malaria

Geoffrey Pasvol

INTRODUCTION

Malaria is an infection caused by the coccidian protozoan parasite of the genus *Plasmodium* carried by female *Anopheles* spp. mosquitoes. The clinical disease in humans may vary widely according to the species of parasite — *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* or *Plasmodium malariae* — and the genetics, immune status and age of the host. These variables have a major influence on all aspects of the disease, including epidemiology, pathogenesis, clinical features and management.

EPIDEMIOLOGY

Geographic distribution

Wherever temperatures are favorable and humans and mosquitoes co-exist, there is the potential for malarial transmission. Malaria certainly existed until the mid-20th century in Europe, especially in Italy, as well as in northern parts of Asia adjoining the former USSR. Almost 2 billion people are at risk of malaria in endemic areas and each year it is estimated that up to 250 million clinical cases occur and over 1 million die, largely among infants and young children in Africa. [1] [2]

Malaria occurs throughout the tropics and subtropics, especially where the temperature exceeds the 60.8°F (16°C) isotherm (see Chapter 245 and Fig. 166.1). The four species of malaria parasites that affect humans differ in their geographic distributions:

- *P. falciparum* is most common in sub-Saharan Africa and Melanesia (Papua New Guinea and the Solomon Islands);
- *P. vivax* is found mainly in Central and South America, North Africa, the Middle East and within the Indian subcontinent;
- *P. ovale* is found predominantly in West Africa but also in Asia; and
- *P. malariae* occurs worldwide, although most cases occur in Africa.

With modern air travel, individuals with malaria can be rapidly transported within hours to any part of the world and malaria is the single most common imported infection occurring in travelers.

There have been occasional reports of 'airport malaria' where infected mosquitoes have been imported on board aircraft into a nonendemic area of the world where they infect local inhabitants who have not traveled.

A few outbreaks have also been documented in nonendemic areas where environmental conditions have become optimal for the transmission of disease by local susceptible mosquitoes becoming infected after biting individuals who have obtained their infection elsewhere (e.g. as occurred in New York in the summer of 1993). In addition, there is now the threat of a global climate change. If the predictions of a 3.6°F (2°C) rise by the year 2100 become a reality, this might lead to the spread of malaria back into areas previously affected by malaria.

Malaria can also be transmitted by blood and blood products.

The epidemiology of malaria depends upon a complex interplay between the:

- host (humans),
- vector (mosquito), and
- malarial parasite.

Population density and prevalence of infection among children are important factors because children tend to have both high parasitemias and rates of carriage of the sexual forms of the parasite (gametocytes), which are necessary for transmission of the infection. Paradoxically, in the 2 weeks after effective treatment of *P. falciparum* malaria, the numbers of gametocytes in the blood rises, so that while the patient improves clinically, mosquitoes biting the patient during this time are more likely to transmit infection.

The longevity of the mosquito is also crucial because it needs to be of sufficient duration to allow for full development of the parasite. Ambient temperatures have a major impact, because higher temperatures significantly shorten this period of maturation in the mosquito (the extrinsic incubation period) and increase transmission. Seasonal rainfall dramatically increases the breeding of mosquitoes.

Where malaria prospers, human societies prosper the least and there is a striking correlation between malaria and poverty. The effects of malaria are felt on diverse areas including fertility, population growth, savings and investment, worker productivity, absenteeism, premature mortality and medical costs.

PATHOGENESIS AND PATHOLOGY

Malaria is one of the few infective agents of humans that invades red cells. [3] [4] All four species of malarial parasites that infect humans have a similar life cycle that alternates between human and mosquito (see Chapter 245). The clinical symptoms and signs are produced by the asexual forms of the parasite, which invade and destroy red cells, localize in critical organs and tissues in the body, and induce the release of many proinflammatory cytokines (see Fig. 166.2), of which tumor necrosis factor (TNF)-a is thought to be the most important. The sporozoites injected by the bite of the infected mosquito, the exoerythrocytic parasites, which subsequently develop in the liver, and the sexual forms of the parasite (macro- and microgametocytes), which arise from the asexual forms do not cause clinical disease.

Invasion of red cells

Merozoites in the peripheral blood invade red cells (and occasionally platelets) and the rate and degree to which the parasite multiplies appear to relate to disease severity in nonimmune individuals. Invasion is a highly specific, ordered and sequential process in which the invasive form, the merozoite, attaches to a susceptible red cell, reorients itself so that its apical end is apposed to the red cell membrane, and then slowly moves into a localized invagination. [5] The entire process of invasion is completed within 30 seconds. In falciparum malaria the erythrocyte binding antigen (EBA 175) and/or merozoite surface proteins (MSP-1 and MSP-2) appear to interact with the red cell sialoglycoproteins (glycophorins), whereas in *P. vivax* infection, the red cell Duffy antigen on the uninfected red cell is involved (see Table 166.1). There is surprising redundancy in the

Figure 166-1 Distribution map of malaria. Despite intensive control measures over the past 50 years, malaria is still widely distributed in the tropics and subtropics. The breakdown of large-scale vector control operations and the emergence of multidrug-resistant parasites have even led to an increase in the incidence of malaria in some regions. O, areas where malaria has disappeared, been eradicated or never existed; +, areas with limited risk; + +, areas where malaria transmission occurs. *(Adapted from WHO 1999, Map No. WHO 99419 EF.) Courtesy of Dr C Lavaissiere.*

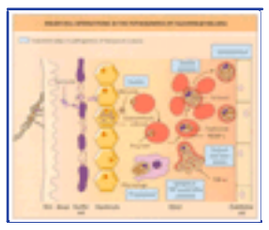

Figure 166-2 Major cell interactions in the pathogenesis of falciparum malaria. The injected sporozoites invade hepatocytes. Merozoites released from rupturing liver schizonts invade red cells. The parasite matures via the ring to the trophozoite to the erythrocytic schizont stage. Such schizonts can bind to uninfected red cells (rosette formation) or to the endothelial cells lining the postcapillary venules (cytoadherence). When the mature schizont ruptures, 'toxin'-like molecules are released which induce the release of proinflammatory cytokines such as TNF-a.

invasion pathways of *P. falciparum* and a number of sialoglycoprotein and nonsialoglycoprotein pathways have been identified.

Attachment and orientation is followed by interiorization accompanied by deformation of the red cell membrane. Although each merozoite of *P. falciparum* can theoretically produce from 16 to 32 new merozoites every 48 hours, a more realistic figure *in vivo* is between three and 10. It is only recently (largely because of technical difficulties) that parasite multiplicative ability within red cells (i.e. the ability to invade) has been shown to relate to disease severity.

TABLE 166-1 -- Major interactions of red cell invasion, rosetting, cytoadherence and TNF induction and some of the molecules involved in the pathogenesis of falciparum malaria.

MAJOR INTERACTIONS OF RED CELL INVASION, ROSETTING, CYTOADHERENCE AND TNF INDUCTION AND SOME OF THE MOLECULES INVOLVED IN THE PATHOGENESIS OF FALCIPARUM MALARIA				
	Invasion	**Rosetting**	**Cytoadherence**	**TNF Induction**
Parasite-induced molecules	Erythrocyte-binding antigen (EBA-175)	*Plasmodium falciparum* erythrocyte membrane-1 (PfEMP-1)	PfEMP-1	Glycosylphosphatidylinositol (GPI)-anchored molecules
	Merozoite surface protein-1 (MSP-1)	Rosettins	PfEMP-3	Phospholipid
	MSP-2/4/5		Histidine-rich protein-1 (HRP-1)	Hemazoin
	Apical membrane antigen (AMA)		Ring surface protein-1 + 2 (RSP1/2)	
	Rhoptry-associated protein (RAP-1)		Modified band-3 (Pfalhesin)	
	RAP-2		Cytoadherence-linked asexual gene protein (CLAG)	
	RAP-3			
Molecules of host cell origin	Glycophorin A (Gp-A)	CD36	CD36	CD 36 on monocyte/macrophages
	Gp-B	Rosettins	Thrombospondin	Red cell membrane
	Gp-C	Blood group A	Intercellular adhesion molecule (ICAM-1) (ICAM-2)	
	Sialic acid a2–3 linkage	Complement receptor-1 (CR1)	Vascular cellular adhesion molecule (VCAM-1)	
	Sialic acid independent pathways-as yet unspecified		Platelet endothelial cellular adhesion molecule (PECAM-1)	
			E-selectin	
			P-selectin	
			Chondroitin sulfate A (CSA)	
			Hyaluronic acid (HA)	
			Heparan sulfate-like glycosaminoglycans (GAG)	
			a_v ß$_3$ integrin	
Molecules in the host serum/plasma	Immunoglobulin	Nonimmune IgM	Immune immunoglobulin	Unknown
		Immune immunoglobulin		
		Other undefined factors		

Cytoadherence

Cytoadherence, the process whereby mature infected cells specifically bind to endothelial cells in postcapillary venules, appears to play a central role in the pathogenesis of falciparum malaria, possibly by localizing mature forms of the parasite in critical organs such as the brain.[6] In addition, cytoadherence of mature parasites:

- prevents their passage through the spleen, a major site of parasite destruction;
- localizes maturing parasites at sites of reduced oxygen tension, which favors parasite growth; and
- may facilitate the invasion of uninfected red cells.

However, despite these effects many studies have failed to find an association between cytoadherence and severe disease.[7] Cytoadherent parasites presumably lead to microvascular obstruction, although the role and extent of this obstruction remain unclear. Cytoadherence may also serve to localize the effect of parasite toxins, which lead to endothelial cell activation and damage as a result of cytokine release.

The molecular interactions that occur in cytoadherence have been studied in some detail. During parasite maturation a number of regular, symmetrically arranged 'knobs' appear on the surface of the infected cell. These knobs are thought to be the sites at which the parasitized red cell attaches to the endothelial cell. A number of high-molecular weight parasite proteins protrude from these knobs, of which the best known is the *P. falciparum* erythrocyte membrane protein (PfEMP)-1 coded for by the *var* (variant) genes. A single parasite may be capable of expressing up to 50 variants of PfEMP-1. Other possible adhesive parasite antigens on the infected red

cell surface include a molecule called sequestrin and a modified form of band 3 (the main anion transporter of the red cell) called pfalhesin.

In turn PfEMP-1 may bind to a number of potential receptors on the surface of endothelial cell. These include:

- the adhesion molecule CD36;
- the bridging molecule thrombospondin (a major component of the platelet a granule);
- two members of the immunoglobulin superfamily — intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule;
- E-selectin; and

- the recently described glycosaminoglycan, chondroitin sulfate A (see Table 166.1).

Whether PfEMP-1 is the molecule that binds to all of the above molecules has yet to be established. It appears that ICAM-1 and CD36 are the major ligands;[8] ICAM-1 acts as a rolling receptor, whereas CD36 and thrombospondin seem to be involved in more stable interactions. Cytokines, especially TNF-a, can upregulate the expression of ICAM-1. How cytoadherence leads to severe pathology remains an unresolved issue.

Rosetting

In rosetting, red cells containing the more mature stages of parasite bind uninfected red cells to their surface. The mechanisms by which rosetting leads to disease remain obscure, but may involve microcirculatory obstruction. Rosetting falciparum parasites have been associated with severe disease,[9] but both *P. vivax* and *P. ovale* are capable of rosetting without causing severe disease. The specific molecules involved in rosetting on the infected and uninfected red cell have not been fully characterized, although PfEMP-1 and small (between 20 and 40kDa) molecular weight 'rosettins' on the parasitized cell and CD36 and the ABO blood group molecules on the uninfected cell have been implicated (see Table 166.1). Most recently the complement receptor CR1, which is present at low levels (approximately 250 copies per cell) on uninfected cells, has been implicated as binding to PfEMP-1 and playing a role in rosetting.[10]

Parasite toxins and cytokines

The paroxysmal increase of many cytokines, notably TNF-a and interferon-?, during a febrile episode and coinciding with rupture of schizont-infected red cells suggests the release of a toxin. Attempts to identify a definitive malarial 'toxin' remain unproductive. Parasite molecules anchored in the red cell membrane by a glycosyl phosphotidylinositol (GPI) structure are favored candidates, but other molecules have been proposed, including an as yet undefined phospholipoprotein molecule and protease-sensitive components associated with malarial pigment (hemozoin). Antibodies to GPI anchors are associated with a lack of disease in adults. However, even products from lysed uninfected red cells are capable of inducing cytokine release from macrophages.

Different falciparum parasite lines vary in their capacity to stimulate TNF-a from macrophages. It is not known whether this observation is due to a quantitative or qualitative attribute of the toxin or why infection by *P. vivax*, sometimes leading to TNF levels comparable with falciparum malaria, results in relatively benign disease. This may be because *P. vivax* does not exhibit cytoadherence which would localize effects of the parasite in critical organs such as the brain.

It is proposed that such a malarial toxin or toxins lead to the release of the cytokines TNF-a interferon-? and interleukin (IL)-1 among others. There is a good correlation between high levels of TNF-a and the outcome of falciparum malaria. Production and release of TNF-a could account for the fever, leukocytosis, enhanced sequestration, hypoglycemia, acidosis and dyserythropoiesis, and possibly even the impaired consciousness observed in malaria. In addition, TNF-a may upregulate the expression of adhesion molecules such as ICAM-1 and other receptors that bind the parasitized red cell. Other cytokines may be synergistic.

Pathophysiologic events leading to cerebral malaria

There is a debate as to whether the major mechanism in the pathogenesis of malaria is:

- ischemia due to obstruction of the microcirculation by sequestering parasites; or
- release of mediators induced by the parasite toxin(s).

A model of the pathogenesis of cerebral malaria due to *P. falciparum* needs to take into account the delicate interplay between both factors, which may prove to be equally relevant. For example, sequestration, the result of rosetting and cytoadherence of infected cells, would not only lead to microvascular obstruction, but could also localize the effect of parasite toxins when released by rupturing schizonts.

In cerebral malaria there appears to be no reduction in total cerebral blood flow, although blood flow is low relative to the cerebral arterial oxygen content.[11] Cerebral malaria can occur in the absence of a localized inflammatory cell response, direct tissue invasion, a breakdown in the blood-brain barrier, cerebral edema, disseminated intravascular coagulation and hypoglycemia. The cerebrospinal fluid in cerebral malaria shows no increase in cell number or protein concentration and may only show a raised lactate concentration, and in some cases, especially in children, a raised opening pressure. [12] A raised cell count or protein concentration should cause one to think of an alternative or additional diagnosis.

Raised intracranial pressure has been invoked in the pathogenesis of cerebral malaria in children in Africa, but its role in adult disease has yet to be determined, although the volume of the brain is increased, probably as a result of sequestration and compensatory vasodilation of the cerebral vasculature.[13]

Tissue infarction is not a major feature of cerebral malaria. In addition, the majority of patients who recover from coma due to malaria appear to have few, if any, neurologic sequelae, in contrast to those resulting from other neurologic infections of equal severity.

Small microhemorrhages may occur around capillaries and venules, but there are few platelets or microthrombi.

The syndrome of cerebral malaria appears to be related to the tight packing of schizonts in the small capillaries of the brain.[14] This process is brought about by sequestration, which in turn results from cytoadherence, rosetting or the decreased deformability of infected cells or a combination of these factors.

PREVENTION

There are a number of points in the malaria parasite's life cycle where the infection can be interrupted. This mainly involves reduced mosquito contact and the use of antimalarial chemoprophylaxis. Vaccination against malaria is currently not a reality.

Antimosquito measures

In endemic areas those at risk should:

- sleep in properly screened rooms;
- use mosquito nets without holes and impregnated with permethrin and tucked in carefully under the mattress before nightfall;
- wear long-sleeved clothing and long trousers when outdoors after sunset; and
- use other adjuncts — insect spray (usually containing permethrin) and mosquito coils or repellents such as diethyltoluamide, DEET or citronella.

For those living in highly endemic areas, the use of permethrin-impregnated bednets has been found to reduce both malarial morbidity and mortality. However, problems remain regarding cost, state of repair of the net, regular impregnation and how the bednets might change the rate of acquisition of immunity and consequently the pattern of disease, especially relating to severity. By protecting the very young against the severe manifestations of malaria, it is argued that severe complications may be deferred until they are older, but this remains a theoretic possibility only.

Malarial chemoprophylaxis

The spread of drug-resistant *P. falciparum* malaria and awareness that some of the more effective combination drugs, such as pyrime-thamine with sulfadoxine

(Fansidar) and pyrimethamine with dapsone

TABLE 166-2 -- **Brief guidelines for the chemoprophylaxis of malaria.**

BRIEF GUIDELINES FOR THE CHEMOPROPHYLAXIS OF MALARIA		
Chemoprophylaxis	**Area to be visited**	**Dose/comments**
None	North Africa (Morocco, Algeria, Tunisia, Libya, tourist areas of Egypt)	Antimosquito measures should still be applied. Risk of other vector-borne diseases still possible
	Tourist areas of South East Asia (Thailand, Philippines, Hong Kong, Singapore, Bali, China)	
Chloroquine	Middle East (including summer months in rural Egypt and Turkey)	300mg base (2 tablets) once per week
or	Central America	
proguanil (Paludrine)	Rural Mauritius	200mg (2 tablets) once per day
Chloroquine and proguanil	Indian subcontinent	Doses as above. Also indicated:
	Afghanistan and Iran	• in pregnancy (safe)
	South America	• in children (liquid formulations)
		• when other antimalarials cannot be tolerated
Mefloquine (Lariam), doxycycline or Malarone (each tablet contains atovaquone 250mg and proguanil 100mg)	Sub-Saharan Africa, e.g.	*Mefloquine*: 250mg (1 tablet) per week.
	Cameroon, Kenya, Malawi, Tanzania, Uganda, Zaire, Zambia	Use especially in areas of high risk. Use up to 1 year
	All rural areas of SE Asia, Papua New Guinea, Solomon Islands and Vanuatu	Contraindicated in epilepsy and psychiatric disorders
		Doxycycline: 100mg per day. Beware light sensitization
		Malarone: Start 1 day before entry, during and 1 week after exit from a malarious area
Doxycycline	As above and mefloquine-resistant parts of South East Asia	100mg daily
		Can be used as an alternative to mefloquine, Malarone or chloroquine and proguanil where these are not tolerated
Specialist advice should be sought for details.		

(Maloprim) and amodiaquine (Camoquin), may rarely have severe and sometimes fatal side-effects[15] have complicated malarial chemoprophylaxis. The risk of contracting malaria in any given country or situation needs to be weighed constantly against the risk of a serious adverse reaction to any drug used. In the absence of adequate data, this becomes difficult. Compliance is of extreme importance because, although those who comply poorly have a similar attack rate, their risk of death is much greater than that of individuals on no prophylaxis.

A brief guide to antimalarial chemoprophylaxis is shown in Table 166.2 . If there is any doubt, specialist advice should be sought (see also Chapter 143). Chemoprophylaxis should start 1 week before entering an endemic area (to ensure adequate blood levels and to evaluate any potential side-effects), and continue while within such an area and for 4 weeks after return except in the use of Malarone which can be commenced on the day before entry into a malarious area and continue for a week after leaving. Chloroquine, two tablets (300mg base) once a week, together with proguanil, two tablets (200mg) daily, is one of the safest and most inexpensive regimens, but is of diminishing efficacy. These drugs have only minor side-effects, the commonest being difficulty in visual accommodation in the case of chloroquine and mouth ulcers with proguanil. There is increasing use of mefloquine one tablet (250mg) weekly, doxycycline one tablet (100mg) daily and Malarone one tablet daily, by travelers to sub-Saharan Africa, Papua New Guinea and the Solomon Islands because of chloroquine resistance.[15] The main side effects of mefloquine are neuropsychiatric and are of varying severity. Doxycycline can lead to light sensitization and Malarone can cause gastroenterological upset.

More detailed and specialist advice should be sought in specialized circumstances, including for:

- long-term visitors,
- children under 12 years of age,
- individuals who have drug allergies, immunosuppression because of disease or therapy, or epilepsy, and
- women who are pregnant.

In individuals born and living in an endemic area chemoprophylaxis should be made available for those with sickle cell disease and pregnant women.

Vaccination

An effective and safe malarial vaccine is still not available. A live attenuated whole sporozoite vaccine has been shown to work, but only on a very small scale and is impractical for widespread use. Results with the SPf66 vaccine, a synthetic peptide, have proved disappointing. Most recently a vaccine using part of the circumsporozoite protein linked to hepatitis B surface antigen and administered in a formulation with a novel adjuvant has shown preliminary promise but protection appears short-lived. A DNA-based vaccine has also been tested.[16]

CLINICAL FEATURES

The most frequent presentation of malaria is that of a pronounced febrile illness with rigors. However, the clinical features of malaria can be extremely diverse because the parasitized red cell circulates to every organ and tissue within the body and therefore has the

TABLE 166-3 -- Manifestations of severe malaria requiring special management.

MANIFESTATIONS OF SEVERE MALARIA REQUIRING SPECIAL MANAGEMENT	
Manifestation	**Comment**
Cerebral malaria	Coma with peripheral parasitemia and other causes of encephalopathy excluded
Severe anemia	Normocytic anemia with hemoglobin <50g/l (5gm/dl) (<15% hematocrit) in presence of parasitemia >10,000/µl
Respiratory distress	Pulmonary edema or adult respiratory distress syndrome
Renal failure	Urine output of less than 400ml/24h (or less than 12ml/kg in children) and a serum creatinine >3.0mg/dl (265µmol/l)

Hypoglycemia	Whole blood glucose <40mg/dl (2.2mmol/l)
Circulatory collapse (shock)	Systolic blood pressure less than 70mmHg or core skin temperature difference >18°F (10°C)
Coagulation failure	Spontaneous bleeding or laboratory evidence of disseminated intravascular coagulation
Impaired consciousness of any degree, prostration, jaundice, intractable vomiting, parasitemia =2%	In nonimmune individuals should be managed as severe malaria (i.e. with parenteral antimalarials)

potential for producing a wide variety of pathology. In endemic areas the manifestations of severe disease in children are mainly those of cerebral malaria, often with convulsions, respiratory distress and severe anemia (Table 166.3), whereas adults are more likely to develop multiorgan failure (e.g. renal failure) and are less likely to have convulsions or severe anemia. Complications of prolonged malarial infection such as hyperactive malarial splenomegaly (HMS) and nephrotic syndrome due to *P. malariae* infection are rare in travelers.

Mild malaria

The incubation period for malaria is variable, but under optimal conditions may be as short as 7 days and in exceptional cases up to 20 years, as in the case of *P. malariae* infections. The majority (>90%) of *P. falciparum* infections in travelers occur within 6 weeks of leaving an endemic area.

The clinical presentation of mild malaria with rigors is well known. There is usually a history of travel to or residence within an endemic area. A history of even the best compliance with the most effective antimalarial chemoprophylaxis cannot exclude the diagnosis. There may be a prodromal period of tiredness and aching. The features of a classic paroxysm are:

- an abrupt onset of an initial 'cold stage' associated with dramatic rigors in which the patient visibly shakes;
- an ensuing 'hot stage' during which the patient may have a temperature of well over 104°F (40°C), may be restless and excitable, and may vomit or convulse; and
- finally, the sweating stage, during which the patient defervesces and may fall asleep.

Such a paroxysm may last 6–10 hours and a prolonged asymptomatic period may follow and last 38–42 hours in the case of *P. vivax* and *P. ovale* infections and 62–66 hours in *P. malariae* infections. In *P. falciparum* infections the periodicity of fever tends to be less predictable and the fever may be continuous. There may be an accompanying headache, cough, myalgia (flu-like symptoms), diarrhea and mild jaundice.

Malaria is rarely, if ever, the cause of lymphadenopathy, pharyngitis or a rash, and alternative explanations need to be considered for these specific symptoms.

Severe malaria

Definitions of the clinical manifestations of severe falciparum malaria are included in Table 166.3 . [17] However, many of these definitions are for study purposes in order to compare data from different parts of the world, especially for the standardization of clinical trials, and must be taken in context. For example, any degree of impairment of consciousness, prostration, jaundice or evidence of renal impairment, especially in a nonimmune individual, should be taken seriously. Furthermore, parenteral therapy is regarded by many as necessary for a parasitemia of 2% or above in a nonimmune patient and in the presence of vomiting.

Cerebral malaria

Cerebral malaria in which the patient passes from drowsiness into coma may develop insidiously over a few days or abruptly within 1–2 hours and is often heralded by a convulsion. The majority of patients have no focal neurologic signs, but there may be a wide variety of neurologic manifestations such as a cranial nerve palsy, monoplegia or hemiplegia, extensor posturing, decerebrate or decorticate rigidity, conjugate or even dysconjugate eye movements, grinding of the teeth (bruxism) or hiccoughs. Some patients have retinal hemorrhages (Fig. 166.3), sometimes with extramacular whitening of

Figure 166-3 Retinal hemorrhage in severe *falciparum* malaria. Examination of the fundus is important in the physical examination of a patient with severe *falciparum* malaria as it can give some indication as to prognosis. In this case the hemorrhage is near the macula. Such hemorrhages have been found in as many as 18–30% of patients with cerebral malaria. In children, additional changes of extramacular whitening and changes in which the vessels turn white in isolated segments, often at branch points, occur.

Figure 166-4 Massive hepatosplenomegaly in a patient with severe malarial anemia due to *P. falciparum.* This CT scan of the abdomen was taken in a traveler from West Africa who, after a prolonged history of fevers, presented with a hemoglobin concentration of less than 50g/l. The scan shows a massively enlarged liver, the left lobe of which is encircling an equally enlarged spleen.

the fundus and retinal vessel changes where they turn white in isolated segments, particularly at branch points. Coma in malaria may not only be due to primary neurologic involvement, but may also be part of a prolonged postictal state, status epilepticus or a severe metabolic disorder such as acidosis or hypoglycemia. Thus drowsiness and coma may be the result of a number of different pathological processes.

Anemia

The anemia of falciparum malaria is both complex and multifactorial. [18] [19] The fall in hemoglobin is often far in excess of what can be accounted for by the loss of infected red blood cells alone. The major mechanisms in the pathogenesis of anemia are those of:

- red cell destruction because of rupture of infected cells, removal of uninfected cells due to antibody sensitization or other physicochemical changes, and increased reticuloendothelial activity, particularly in organs such as the spleen; and
- decreased red cell production due to marrow hypoplasia as seen in acute infections and dyserythropoiesis, a morphologic appearance that in functional terms results in ineffective erythropoiesis.

Two clinical presentations of anemia predominate which represent the ends of a clinical spectrum.

- Severe acute malaria in which anemia supervenes but only after a few days of severe illness such as cerebral malaria. There may be respiratory distress with acidosis, cardiac failure (often difficult to diagnose in children), poor tissue perfusion and death. There is shortened red cell survival (hemolysis) and evidence of bone marrow suppression despite the progressive fall in hematocrit.
- Severe anemia in patients in whom the illness has developed insidiously over a period of days or sometimes weeks (Fig. 166.4). These patients are often very young children living in endemic areas and are anemic when first seen. They have splenomegaly of varying degree and the peripheral blood film may show only scant asexual parasitemia. The bone marrow often shows a picture of dyserythropoiesis, although in this setting erythropoietin levels may be raised but not necessarily appropriate for the degree of anemia. In many cases gametocytes and malarial pigment are seen in phagocytic cells. Because the development of this

type of anemia is slow, there is adaptation to the low hematocrit and the clinical condition may often conceal the severity of the underlying anemia.

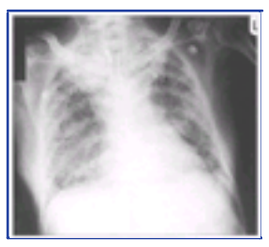

Figure 166-5 Chest radiograph of a patient with acute respiratory distress sydrome (ARDS) due to *falciparum* malaria. This X-ray shows new, bilateral, diffuse, homogeneous pulmonary infiltrates without cardiac failure, fluid overload, chest infection or chronic lung disease in an adult with severe *falciparum* malaria. The prognosis is poor. This condition is rare in children.

Respiratory distress

Respiratory distress is manifest by rapid labored breathing and sometimes abnormal rhythms of respiration.[20] In children there may be intercostal recession, use of the accessory muscles of respiration and flaring of the alar nasae, making it difficult to differentiate from an acute respiratory infection.

Respiratory distress in patients with malaria may be the result of a number of pathologies:

- ¦ respiratory compensation for a profound metabolic acidosis in the majority of cases;
- ¦ a direct effect of the parasite or raised intracranial pressure on the respiratory center in the brainstem;
- ¦ secondary lung infection as a consequence of immunosuppression;
- ¦ air hunger as a result of severe anemia; and
- ¦ pulmonary edema as a consequence of hypoalbuminemia, iatrogenic fluid overload or direct alveolar capillary damage by parasites and neutrophils leading to the acute respiratory distress syndrome (Fig. 166.5);
- ¦ overuse of anticonvulsants, particularly phenobarbitone which depresses the respiratory center drive.

Identification of these different causes of respiratory distress is important as they each require different modalities of management.

Acidosis

Acidosis (base excess =12) or acidemia (pH <7.3) in malaria indicates a poor prognosis and can be due to a number of causes:[21]

- ¦ poor tissue perfusion, in some cases due to hypovolemia, leading to reduced oxygen delivery;
- ¦ lactate production by the parasite;
- ¦ lactate generation as a result of cytokine activity, especially TNF, in the acute phase response;

- ¦ reduced hepatic blood flow and therefore lactate clearance;
- ¦ impaired renal function and therefore acid excretion; and
- ¦ exogenous acids due to aspirin (salicylate) administration.[22]

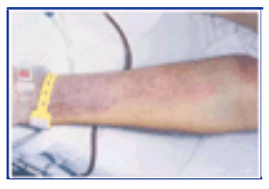

Figure 166-6 Disseminated intravascular coagulation in *falciparum* malaria. Bleeding into the skin seen in a patient with a thrombocytopenia, a prolonged prothrombin time, increased fibrinogen degradation products and hypofibrinogenemia. The patient had no signs of cerebral malaria.

Hypoglycemia

The characteristic clinical manifestations of hypoglycemia may not be evident in malaria, often because the patient is already unconscious. Suspicion of this important complication is often circumstantial: on admission in children and during quinine therapy in adults. The cause of hypoglycemia is multifactorial: [23]

- ¦ depletion of glucose stores because of starvation or malnutrition;
- ¦ malabsorption of glucose due to decreased splanchnic blood flow;
- ¦ increased tissue metabolism of glucose;
- ¦ parasite utilization of glucose;
- ¦ cytokine-induced impairment of gluconeogenesis; and
- ¦ hyperinsulinemia due to quinine therapy.

Anaerobic metabolism of glucose leads to acidemia and the production of lactate. Acidemia (blood pH <7.3) and hyperlactatemia are important prognostic factors in severe malaria. Acidemia is associated with respiratory rhythm abnormalities (especially a slow respiratory rhythm) and death.

Shock

Unlike the sepsis syndrome, shock is relatively rare in severe malaria. In most cases the blood pressure of patients with malaria is at the lower end of the normal range, probably due to vasodilatation. Marked hypotension in a few cases may be the result of dehydration, but is more commonly due to concomitant sepsis. Care should be taken to look for signs of sepsis, especially septicaemia and respiratory and urinary tract infection.

Bleeding

Bleeding due to the commonly occurring thrombocytopenia in malaria is rare. Bleeding is more likely to occur in the setting of disseminated intravascular coagulation (Fig. 166.6). However, more often there is only subtle activation of the coagulation cascade with a reduction in antithrombin III concentration, an increase in

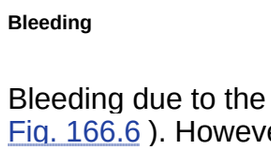

Figure 166-7 Blackwater fever. Urine specimen on admission (left) and days 2, 3 and 4 in a cross-Africa traveler with *falciparum* malaria on quinine treatment, showing the characteristic dark urine of blackwater fever, which showed gradual clearing. The same patient presented with a fever 1 week later and when treated presumptively for malaria with quinine, developed dark urine once again. Renal function was only mildly impaired.

thrombin-antithrombin III complexes and a reduction in factor XII and prekallikrein activities, which do not appear to be clinically significant.[24]

Renal involvement and blackwater fever

A degree of renal impairment, often due to hypovolemia but not always clinically evident, almost always occurs in severe malaria. Acute renal failure, which is less common, may occur in malaria both during the acute parasitemic phase, but also after parasite clearance.[25] In addition, the acute renal failure of malaria may be nonoliguric. Although the urinary manifestation of blackwater fever may be dramatic (Fig. 166.7), occurring in the setting of glucose-6-phosphate dehydrogenase (G6PD) deficiency or a semi-immune patient given quinine, it does not invariably lead to renal failure and appears to be considerably more benign than the classically

described syndrome.[26]

Differential diagnosis

Malaria may have little to distinguish it from other febrile illnesses. In the absence of a travel history, malaria can be transmitted in

'airport malaria' where infected mosquitoes are brought from endemic areas on planes or due to autochthonous spread during a hot summer in a nonendemic area where infected individuals pass the infection on to the local mosquito population. Malaria must also be considered in patients with a fever after blood transfusion, organ transplantation or needlestick injury. A critical step in the diagnosis of malaria, especially outside endemic areas, is consideration of the possibility of this diagnosis. A travel history should now be a routine part of any clinical consultation, especially in patients with a fever. Malaria needs to be excluded in any febrile patient in or returning from an endemic country whether or not they have been taking antimalarials. No antimalarial at present can guarantee absolute protection.

Malaria is a great mimic and must enter the differential diagnosis of a number of clinical presentations.

- In the acute presentation fever due to malaria needs to be differentiated from typhoid, viral illnesses such as dengue fever and influenza, brucellosis and respiratory and urinary tract infections. Less common causes of tropical fevers include leishmaniasis, trypanosomiasis, rickettsial infections and relapsing fevers.
- The coma of cerebral malaria need to be differentiated from meningitis (including tuberculous meningitis), encephalitis, enteric fevers, trypanosomiasis, brain abscess and other causes of coma (see Chapter 153).
- The anemia of malaria can be confused with other common causes of hemolytic anemia in the tropics such as that due to the hemoglobinopathies (e.g. sickle-cell disease, thalassemia), G6PD deficiency, and the South East Asian form of ovalocytosis. The anemia of malaria must be differentiated from that of iron, folate or vitamin B12 deficiency.
- The renal failure of malaria must be distinguished from renal impairment due to massive intravascular hemolysis seen in G6PD deficiency, sickle-cell disease, leptospirosis, snake envenomation, use of traditional herbal medicines and chronic renal disease resulting from glomerulonephritis and hypertension.
- The jaundice and hepatomegaly of malaria must be distinguished from that of viral hepatitis (A, B and E, cytomegalovirus and Epstein-Barr virus infections), leptospirosis, yellow fever, biliary disease and drug-induced disease, including alcohol.

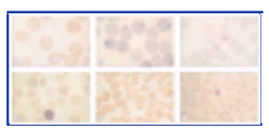

Figure 166-8 Thin blood films from patients with malaria. (a) Delicate small ring forms of *Plasmodium falciparum* showing multiply infected red cells and a characteristic 'appliqué' form in the uppermost parasite in the central red cell where the parasite appears as if it is applied to the surface, rather than within the red cell. (b) Ring forms of *P. falciparum* in a heavy infection and where the pH of the stain is 7.2 rather than 6.7 showing the irregular, basophilic Maurer's clefts in the cytoplasm of infected cells characteristic of *P. falciparum.* (c) Very early trophozoites of *P. falciparum* in the peripheral blood film of a patient with severe disease. The relative size and presence of pigment indicate the greater maturity of the parasite and may indicate a poorer prognosis. (d) Peripheral blood film from a patient with *vivax* malaria showing mixed ring and schizont forms. The ring forms are far more fleshy and ameboid and the cytoplasm of the infected cell shows the characteristic regular and eosinophilic Schüffner's dots, which help in diagnosis. (e) Peripheral blood film from a patient with *ovale* malaria showing a small ring form on the left, which could quite easily be mistaken for *P. falciparum*. The larger central parasite has enlarged the cell into an oval shape and has also formed a fimbriated fringe at the upper pole of the cell. (f) Peripheral blood film from a patient with *malariae* malaria showing the characteristic rosette schizont with daughter merozoites (usually eight) around a central piece of pigment (*hemazoin*). The ring forms of this species characteristically form a band stretching across the width of the red cell.

Clinical diagnosis on its own is notoriously inaccurate and may be incorrect in up to 50% of cases.

DIAGNOSIS

The definitive diagnosis of malaria is made by prompt microscopic examination of thick and thin blood films. There is no need to wait for a fever peak before carrying out a blood film as parasites are often present throughout the red cell cycle. Malarial chemoprophylaxis should be withheld during investigation for malaria as antimalarials can suppress peripheral parasitemia.

The most common abnormality on full blood count is thrombocytopenia, especially in the nonimmune. This is thought to be largely splenic pooling of platelets but also platelet activation.[34] The total white count is usually in the normal range but often there is a lymphopenia on presentation due to lymphocyte redistribution and more recently, apoptosis of lymphocytes has been identified in falciparum malaria.[35]

Thick blood films

One or two drops of blood from a fingerprick are stirred in a circle on a glass slide, allowed to air dry and then stained with Giemsa or Field's

stain. With this method, the red cells lyse whereas the white cells and parasites remain intact. Parasites are identified by recognizing both the eosinophilic nucleus and the basophilic cytoplasm of the malarial parasite. Parasite density can be related to the number of white cells present. This method has far greater sensitivity than the thin blood film.

Thin blood films

A thin film is produced by spreading a small drop of blood across a slide using the edge of a second slide, thereby producing a monolayer of red cells. Fixation is usually with methanol and the staining technique is as for the thick blood film (optimally at pH 7.2). The red cells remain intact. The thin blood film allows accurate speciation of the parasite and quantitation, in which the number of parasites is related to the number of red cells present. It is important that parasites are accurately recognized, as platelets or debris can often be mistaken for parasites. The size, shape and stippling of the red cell cytoplasm help in the speciation of the parasite. Examples of the four species are shown in Figure 166.8 .

Other valuable information can be obtained from the blood film, especially in severe disease. Careful staging of the parasites in the peripheral blood can indicate disease severity as the presence of more mature parasites may reflect a greater proportion of sequestered parasites and indicate more severe disease (Fig. 166.8).[27] The presence of malarial pigment in more than 5% of neutrophils provides some indication of the total parasite load and is associated with a poor prognosis.[28]

Other methods

Malaria can be diagnosed using other methods, but each has its own drawbacks with regard to time, cost or being nonquantitative or nonspecific.

- The polymerase chain reaction is useful for making an accurate species diagnosis and detecting low level parasitemias, but its expense, the time taken and requirement for specialized equipment make it impractical. This methodology is currently more frequently used in epidemiological and pharmacological studies.
- The QBC (quantitative buffy coat) method involves taking blood into a small capillary tube containing a float and an acridine orange stain, which stains the nuclear material of parasites and increases the sensitivity of detection, but its expense and inability to speciate or quantitate parasites accurately are limiting factors. Rapid dipstick methods have in many cases replaced this methodology.
- The ParaSight F and the Malaria PF antigen capture tests use a monoclonal antibody to the histidine-rich protein 2 of *P. falciparum* and are very useful tests in those who have not had malaria before, requiring minimal expertise. However, these tests are expensive, not quantitative and can only detect the presence of *P. falciparum*.
- The OptiMAL test detects parasite lactate dehydrogenase (pLDH) which can be distinguished from human LDH. This test can also distinguish *falciparum* from *vivax* infections.
- Autopsy diagnosis.

MANAGEMENT

Once a definitive diagnosis of malaria has been made treatment with specific antimalarial drugs can be initiated.[29]

Non-falciparum malaria

Malaria due to *P. vivax*, *P. ovale* or *P. malariae* requires a standard course of treatment with chloroquine, which usually leads to defervescence (Table 166.4). Chloroquine-resistant asexual forms of *P. vivax* have recently been documented and may require quinine treatment. In the case of *P. vivax* and *P. ovale* malaria treatment with an 8-aminoquinoline (primaquine) is given to eradicate the exoerythrocytic forms, especially the hypnozoites responsible for relapses. Levels of G6PD should be measured in all patients before they are given primaquine, an oxidant drug which can lead to major hemolysis in G6PD-deficient individuals. Treatment with primaquine should be delayed until after delivery and/or breastfeeding in pregnant women. Primaquine-resistant *vivax* hypnozoites have been identified which require more prolonged (often 3 weeks) and higher dose (22.5mg/day) therapy.

Falciparum malaria

Mild falciparum malaria

In endemic areas the treatment of malaria in children involves the use of drugs that are locally affordable and appropriate. For this reason mild *falciparum* malaria in many parts of Africa is still treated with chloroquine as for non-*falciparum* malaria and recrudescences are treated with pyrimethamine/sulfadoxine (Fansidar). For travelers and in areas where there is resistance to chloroquine and pyrimethamine/sulfadoxine, the mainstay of treatment is oral quinine sulfate used as shown in Table 166.4 , followed by the use of pyrimethamine/sulfadoxine or doxycycline to eradicate remaining asexual forms of the parasite. Mefloquine may be used and more recently drug combinations such as atovaquone with proguanil (Malarone) and artemether/lumefantrine (Coartemether) have been successfully used. Whichever drug is used, parasitemia may paradoxically rise in the first 24–36 hours and is not generally indicative of treatment failure.

Severe falciparum malaria

The management of severe *falciparum* malaria constitutes a medical emergency.[29] The diagnosis needs to be confirmed microscopically and intravenous access obtained as soon as possible. Depending upon the clinical manifestations, the investigations detailed in Table 166.5 should be carried out.

Patients with severe malaria should be transferred to the highest possible level of clinical care (e.g. a high-dependency or intensive therapy unit). Measurement of glucose and where possible lactate and arterial blood gases should be performed in the initial assessment. An effective antimalarial, at present quinine in most cases, should be given intravenously by slow infusion. Meticulous care must be given to fluid balance as both dehydration and overhydration can occur as a result of the disease or treatment. Convulsions should be treated with intravenous diazepam and attention paid to hypoglycemia and hyponatremia. The routine use of prophylactic anticonvulsants is unwarranted.

Blood should be taken for cross-matching and coagulation studies. A baseline electrocardiogram should be obtained with careful observation of the rhythm and QT interval in elderly patients, particularly those with underlying heart disease, and where possible a cardiac monitor should be set up.

In endemic areas a loading dose of quinine (20mg/kg) should be given to young children and fit young adults. Care is necessary in the administration of quinine to the elderly, especially where there is underlying cardiovascular disease because of the risks of arrhythmias. Quinidine can be safely and effectively substituted for intravenous quinine (intravenous quinine is not available in the USA). Cardiac dysrhythmias and hypotension may occur and therefore quinidine should be administered in an ICU setting.

Recent evidence in childhood malaria has shown that blood transfusion may be of benefit in patients who have respiratory distress and metabolic acidosis. [30] In units with appropriate facilities complicated hyperparasitemia may be treated with exchange transfusion. The use of exchange transfusion is controversial,[31] but should be considered where safe blood is available for all patients in whom the parasitemia exceeds an arbitrary 30% and for those in whom parasitemia is lower, but who:

TABLE 166-4 -- Summary of the drug treatment of malaria.

SUMMARY OF THE DRUG TREATMENT OF MALARIA			
Type of malaria	**Drug**	**Dose**	**Comments**
Non-*falciparum* malaria	Chloroquine phosphate or sulfate (each tablet contains 150mg base)	Loading dose 600mg, 300mg 6 hours later, then 300mg daily for 2 days (i.e. 10 tablets)	Chloroquine and primaquine resistance now documented for *vivax* malaria
	Followed in *vivax* and *ovale* malaria by primaquine	15mg daily for 14 days	Not given in G6PD deficiency or 45mg weekly for 6 weeks with monitoring for hemolysis. Not given in pregnancy
***Falciparum* malaria**			
Mild			
(A range of treatments can be used depending on availability of drugs and choice of local practice)	Quinine sulfate	10mg (salt)/kg (usually 600mg) q8h po for 3–7 days (at a practical level when parasite clearance has been achieved for 24 hours)	Almost all patients develop cinchonism (ringing in the ears, deafness, nausea, vomiting, etc.) and especially if they have liver or renal impairment. Reduce dose to q12h if the parasite count is falling
	Followed by: doxycycline or pyrimethamine/sulfadoxine (Fansidar)	200mg loading dose, then 100mg daily for 6 days Single dose of three tablets (each tablet contains 500mg sulfadoxine and 25mg pyrimethamine)	Not for children or in pregnancy Mainly for malaria from West Africa; doxycycline is preferred with increasing resistance to Fansidar in Africa
	Atovaquone and proguanil (Malarone)	Four tablets daily for 3 days (each tablet contains atovaquone 250mg and proguanil 100mg)	Fewer side-effects than quinine — mainly gastrointestinal. Caution as newly approved drug
	Artemether and lumefantrine (Riamet)	Four tablets q12h for six doses (each tablet contains artemether 20mg and lumefantrine 120mg)	Relatively few side-effects. Caution as newly approved drug
	Mefloquine	750mg as a single dose, repeated after 6 hours	Contraindicated in early pregnancy and in patients who have a neuropsychiatric history
Severe	Quinine dihydrochloride	10mg (salt) per kg, q8h until parasites cleared, then doxycycline or Fansidar as above when the patient can take medication orally	Can induce hypoglycemia and cardiac arrhythmias; a loading dose 20mg/kg can be given to young otherwise healthy patients when hyperparasitemia cannot be treated by exchange transfusion

Severe — newer regimens	Artemether (a qinghaosu (artemesinin) derivative)	3.2mg/kg iv followed by 1.6mg/kg daily (usual adult dose 160mg followed by doses of 80mg)	An alternative to quinine given im. Doxycycline usually required as recrudescences are common
	Artesunate	2.4mg/kg iv followed by 1.2mg/kg at 12 and 24h; then 1.2mg/kg daily (usual adult dose 120mg followed by doses of 60mg)	Can be given intravenously as it is water soluble Also requires doxycycline as recrudescences are common
	Quinidine gluconate	7.5mg/kg q8h over 4h until patient can swallow	For emergencies and in the USA where intravenous quinine is not available. Requires ECG monitoring
G6PD, glucose-6-phosphate dehydrogenase.			

- have manifestations of severe complicated malaria;
- have underlying medical complaints, such as diabetes mellitus and ischemic heart disease;
- are elderly; or
- are pregnant.

During the course of treatment useful parameters for monitoring progress should include twice-daily parasite counts, regular pH and blood gas measurements and, where appropriate, measurement of glucose and lactate concentrations and renal function.

Each patient needs to be assessed individually. During an infusion of quinine it is essential to monitor blood glucose carefully.

Elective ventilation needs to be considered where facilities are available, especially if there is severe acidosis, clear evidence of raised intracranial pressure or respiratory failure of any cause. Further details of investigations and management are given in Table 166.5 .

Cerebral malaria

Antimalarials form the mainstay of treatment for cerebral malaria. A number of adjuvant therapies such as corticosteroids and heparin have been tried, but have not been shown to be effective. Some children and a few adults show evidence of raised intracranial pressure,

TABLE 166-5 -- Investigations in the management of malaria.

INVESTIGATIONS IN THE MANAGEMENT OF MALARIA		
Investigation	**Relevance**	**Management**
Full blood count		
Hemoglobin	Often not anemic on presentation; an indicator of duration of infection	Generally threshold for transfusion is high (e.g. <7.5g/dl (75g/l) in adults, <5g/dl (50g/l) with respiratory distress in children in an endemic area); self-recovery is generally rapid once the parasites have been removed
White blood cells	Normal in uncomplicated cases; often lymphopenic; in severe malaria a neutrophil leukocytosis is common	Generally none; secondary bacterial infection is common in severe cases and will require antibiotics
Platelets	Often low	Bleeding in the absence of DIC is uncommon
Blood film and parasite count	Essential for diagnosis and continuing management if high; more mature forms or pigment in =5% neutrophils indicates a poor prognosis (see text)	Depending upon setting and severity (see text) exchange transfusion might be required
Electrolytes		
Sodium	Often low; some cases are due to the syndrome of inappropriate antidiuretic hormone secretion, others due to an inability to secrete free water	Self-correcting with treatment
Potassium	Normal unless high due to acute renal failure	Dialysis may be necessary
Creatinine	Normal or high	Dialysis may be necessary
Calcium	Often low in severe cases	May need replacement especially if the QT interval is prolonged on the electrocardiogram
Magnesium	Can be low	May need replacement especially if the QT interval is prolonged on the electrocardiogram
Glucose and lactate		
Glucose	Often low in severe cases in children and also during quinine administration in adults; often there is an absence of classical symptoms and signs of hypoglycemia	Regular monitoring of glucose in severe cases; immediate administration of 50ml 50% glucose (0.5–1.0gm/kg in children)
Lactate	Raised in severe cases; good prognostic and progress marker from hour to hour; important to measure in spinal fluid if lumbar puncture performed	Important to ensure good tissue perfusion, especially by correction of any hypovolemia
Coagulation		
Including prothrombin time, thrombin time, D-dimers (or fibrinogen degradation products) and platelets	Activated in almost all cases of malaria to some degree	Fresh frozen plasma and platelets might be required if there is clinical evidence of bleeding
Liver function tests		
Albumin	Often low in acute infection	Does not require correction unless clinically relevant; danger of fluid overload and pulmonary edema
Transaminases	Can be moderately raised; if very high consider other concomitant infections (e.g. hepatitis)	Quinine dosage may need modification (e.g. reduction to q12h regimen)
Alkaline phosphatase	Not raised in malaria	If raised think of other causes
C-reactive protein	Raised in acute attack	Useful for daily monitoring in severe cases
Blood gases		
pH	Acidosis important in the prognosis of severe cases	Requires adequate fluid replacement, possible blood transfusion in anemic cases and avoidance (if possible) of epinephrine if inotropes are required [33]

Partial pressure of oxygen	Hypoxia uncommon unless there is pulmonary edema or infection	Oxygen
Partial pressure of carbon dioxide	Can be low in acidosis	
Bicarbonate	Low in acidosis	Replacement unlikely to help in acidemia
Other investigations		
Quinine levels	Free rather than total quinine levels are relevant to efficacy and toxicity. (a_1 -acid glycoprotein (a_1 -AGP) is the main quinine binding plasma protein)	Not generally helpful in management; maintain at 10–15mg/l according to parasite sensitivity and for quinidine 4–6mg/l
Electrocardiograph monitoring (corrected QT (QTc) interval)	Interval can be prolonged in nonimmune patients, especially if there is an underlying cardiac disorder	Quinine dosage may be need to be reduced
Blood and urine culture	Patients often acquire a secondary infection (most commonly respiratory, renal tract or sepsis) due to immunosuppression	May require systemic antibiotics
Lumbar puncture (only when patient is stable)	Relevant in very young and elderly and when other causes of encephalopathy, especially meningitis, must be excluded	Appropriate antimicrobial chemotherapy
The feasibility of these investigations and their management will depend upon the severity of disease and availability of facilities. DIC, disseminated intravascular coagulation.		

in which case a therapeutic trial of an osmotic agent such as mannitol may be attempted.

Acute renal failure

Dialysis or hemofiltration may be required. The indications are similar to those for any other form of renal failure. Nonoliguric renal failure may be managed conservatively.

Acidosis

Adequate fluid replacement avoiding fluid overload is essential. Sodium bicarbonate has not been shown to be of any benefit and may worsen acidosis. Transfusion of anemic patients has been shown to improve severe acidosis and reduce lactate concentration in young children.[30] Early hemodifiltration and ventilation may be used according to availability. The inotrope epinephrine (adrenaline) should be avoided unless absolutely necessary as it may worsen the acidosis, unlike dopamine, dobutamine and norepinephrine (noradrenaline). [32] Aspirin can also exacerbate metabolic acidosis.

Bacterial superinfection

Bacterial superinfection is common in malaria and must be suspected, particularly if the fever remains high despite antimalarial treatment or if there is evidence of septicemia or focal sepsis (e.g. pneumonia or urinary tract infection).

Adjunctive therapies

Many adjunctive therapies have been tried in malaria but few, if any, have been shown to be of benefit. The use of anti-TNF antibodies has been disappointing, leading only to a decrease in fever but no difference in clinical outcome. Corticosteroids are clearly not indicated in the treatment of acute cerebral malaria. The role of iron chelators and heparin remains unresolved, as does the use of an anti-TNF agent, pentoxifylline.

The role of mannitol in patients who have evidence of raised intracranial pressure, dichloroacetate in patients who have hyperlactatemia and the free radical scavenger, desferrioxamine, remain unclear.

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Chapter 167 - Schistosomiasis

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INTRODUCTION

Schistosomiasis is the most significant helminthic infection in humans because of its global prevalence, the protean nature of its associated disease manifestations and the remarkable difficulties encountered in attempts to control its spread.[1] Humans may be infected with one of five species: *Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi* and *Schistosoma intercalatum*.

EPIDEMIOLOGY

The geographic distribution of schistosomiasis in endemic areas is dependent on the availability of a considerable reservoir of infection in humans or, in the case of *S. japonicum*, in domestic animals and specific snail intermediate hosts. Transmission necessitates a set of cultural, social and health habits that facilitate the spread of infection. In addition, this infection is extending its geographic distribution to new areas because of irrigation projects[2] or massive population movement. The main areas of endemicity of the five schistosome species responsible for the bulk of human infection are given in Table 167.1 .[3] Attention should always be given to the nonuniform distribution of infection in any specific locality; endemicity, therefore, must be precisely mapped out. Detailed information on geographic distribution is unfortunately missing in most circumstances.

The schistosomes, similar to most other helminthic infections of humans, have a unique biologic characteristic in that they do not replicate within their definitive host. With the availability of procedures to quantify infection, it is therefore possible to appreciate several unique epidemiologic features of schistosomiasis. In endemic countries children encounter infection in their early years of life (4–6 years). Prevalence of infection increases with age and peaks in the age group 15–20 years. This age-dependent prevalence of infection is a constant finding in all endemic areas, irrespective of the peak percentage of infected individuals.[4] Intensity of infection, which is a measure of the number of eggs excreted in urine or feces and consequently is an estimate of worm load, follows a similar pattern to prevalence in the age group 5–20 years. Infection intensity increases with age to peak at age 15–20 years; thereafter the curves of prevalence and intensity diverge. Although prevalence remains stable, intensity of infection decreases remarkably, so that by age 30 years or older the number of eggs quantified in urine or stools is significantly lower than peaks achieved in persons a decade younger. The age-specific dependence of intensity of infection may be explained by acquisition of immunity or change in water exposure patterns of individuals living in endemic areas.[5] [6]

Another of the remarkable epidemiologic features of schistosomiasis in the populations of endemic areas is its overdispersed distribution.[7] This means that among infected individuals most harbor low worm burden and only a minority (5–15%) are heavily infected. What determines the susceptibility to heavy infection is not known, but age (the young acquire heavier infection) and/or genetic susceptibility[8] are among the better recognized factors.

Expression of disease manifestations caused by schistosome infection is a multifactorial process. It depends on the species of the parasite and where ova are trapped in host tissues. Within the different species and perhaps geographic strains, a heterogeneity of disease manifestations is appreciated. For example, a considerable percentage of children infected with *S. haematobium* will complain of dysuria and hematuria, and objective evaluation of blood in urine demonstrates positive results in up to 80% of examined populations. In contrast, in children and young adults infected with any of the intestinal schistosomes, no more than 10–20% will complain of gastrointestinal symptoms or show signs of hepatosplenomegaly. Although the high prevalence of symptoms in *S. haematobium*-infected individuals may be related to the anatomic localization of worms and ova, the paucity of specific clinical features in intestinal schistosomiasis raises the possibility of additional pathogenetic factors such as age, genetic make-up and degree of immune responsiveness.[9]

PATHOGENESIS AND PATHOLOGY

Disease manifestations caused by schistosome infection are multiple, not only because of the several species that infect humans but also because of the multiple stages of the pathogen within the human host and the myriad responses it elicits. A summary of the main pathogenetic mechanisms in acute and chronic schistosomiasis is given in Table 167.2 . Acute schistosomiasis is seen more commonly in infections with either *S. mansoni* or *S. japonicum*. It may manifest either as cercarial dermatitis, which is caused by the development of humoral and cellular immune responses to invading cercariae or as Katayama fever, which is probably a serum sickness-like illness caused by antigen-antibody complex deposition and proinflammatory cytokines in response to the maturing worms and the shower of egg antigens that follow. [10]

In contrast to the general immunologic nature of pathogenesis in acute schistosomiasis, the causes of disease in the chronic stages are mainly localized in specific sites within infected individuals. Central to the pathology of chronic infection is egg deposition and the subsequent host granulomatous response. Mature worms begin oviposition 6–9 weeks after cercarial invasion. Egg deposition occurs intravascularly in the small venous tributaries of the measenteric or vesical plexuses. Through the secretion of enzymes, and perhaps aided by urinary tract or intestinal movement, the parasite eggs find their way to the lumen of these viscera where they are carried to the outside via urine or stools. A proportion of parasite eggs, however, fail to traverse from the venous lumen to visceral cavities and are either retained locally in host tissues or carried via venous blood to distant organs such as liver, lung, etc. At these sites, the effector arms of the host immune system mount a granulomatous response to enclose the parasite ova (Fig. 167.1). The response is initiated by cell-mediated mechanisms and is regulated by multiple humoral and cytokine cascades. The result is a space-occupying lesion that may obstruct portal blood flow through the liver, leading to portal hypertension, or may obstruct urine flow through the ureters, leading to

TABLE 167-1 -- Geographic distribution of human schistosomes.

GEOGRAPHIC DISTRIBUTION OF HUMAN SCHISTOSOMES					
Species	**Africa**	**Middle East**	**Asia**	**Americas**	**Caribbean**
S. haematobium	+	+	+	+	+
S. mansoni	+	+	+	-	-
S. japonicum	-	-	+	-	-
S. mekongi	-	-	+	-	-
S. intercalatum	+	-	-	-	-

TABLE 167-2 -- Pathogenetic mechanisms in human schistosomiasis.

PATHOGENETIC MECHANISMS IN HUMAN SCHISTOSOMIASIS			
Syndrome	**Parasite stage**	**Immunologic mechanisms**	
		Induction	**Regulation**

Swimmer's itch	Cercarial	Humoral and cellular	Undefined
Acute stage	Adults and eggs	Antigen-antibody complexes	Undefined
Chronic stage			
Granuloma	Eggs	Cell mediated	Antigens, cells, cytokines
Fibrosis	Egg antigens	Cell mediated	Cytokines

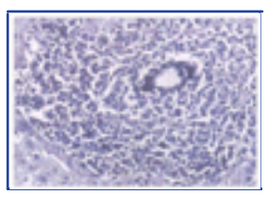

Figure 167-1 The basic pathologic lesions of intestinal schistosomiasis. A liver biopsy is depicted with egg deposition, granuloma formation and fibrosis in the periportal areas. *Courtesy of Professor MA Madwar.*

hydroureters and hydronephrosis. The nidus for these granulomatous lesions is the parasite eggs with their multiple antigens that are recognized by the host. The miracidia within the eggs, however, survive for only 6–8 weeks and then die, thus eliminating the continuous antigenic stimulation. The result is a decrease in granuloma size, but more significant is tissue healing through fibrosis. Such mechanisms lead to the permanence of schistosomal tissue injury and hemodynamic effects.

PREVENTION

For individuals traveling to areas endemic for schistosomiasis, prevention simply means no contact with infected bodies of fresh water. Because the distribution of infection in most endemic areas is not clearly demarcated, avoidance of all fresh-water sources is most prudent. Of particular importance is the unreliability of equating fast-running water with less chance of infection. Anticercarial preparations may be applied under special circumstances, but their widespread use has not been tested. In contrast, prevention in people living in endemic areas is complex and multifaceted. It reflects the degree of education, economic growth and cultural and recreational practices. Four strategies have been used and are still in use: mollusciciding; provision of sanitary water and sewage disposal; chemotherapy delivered to infected individuals as mass chemotherapy or targeting treatment to specific segments of infected populations; and economic and social development in general. Each of these strategies has its limitations and none has achieved widespread and sustained control of infection or disease in endemic areas. Significant effort is given to discovery of antischistosome vaccines. Although such a goal is important, understanding of the complexity of the organisms and the host immune responses has not resulted to date in a practical vaccination strategy.[11]

CLINICAL FEATURES

The clinical manifestations of disease caused by schistosomiasis are related not only to the species of the parasite but also to intensity of infection, genetic make-up of the host and other interactions with infectious and nutritional conditions. It is, therefore, essential to use the clinical descriptions that follow with a full understanding that the phenotypic expression of disease caused by schistosomiasis is multifactorial in nature.

The first major set of clinical manifestations of schistosomal infection occurs during its acute phase and results in varying degrees of morbidity in association with the five species responsible for the bulk of human infection. Swimmer's itch or cercarial dermatitis is a maculopapular itchy rash that usually occurs 1–2 days after exposure. The rash is related to cercarial invasion and, therefore, it usually occurs in areas of the skin that are exposed to infected waters. Cercarial dermatitis has been reported more often in newly exposed persons in endemic areas. The lesions are usually self-limited and require little medical attention. A more severe form of cercarial dermatitis occurs after human exposure to avian schistosomes. These helminths are endemic in many parts of the world, including North America. Exposure usually occurs in spring when transmission of the parasites in their natural habitat begins. The maculopapular eruptions are more severe and recur with each exposure.

A few weeks after schistosome infection occurs (4–8 weeks) some individuals exhibit systemic manifestations of acute schistosomiasis.[12] This is the more common manifestation seen in travelers who return after exposure to infection in endemic areas. The symptoms include fever, malaise, general aches and, upon examination, lymphadenopathy and hepatomegaly may be detected. Most of these individuals will show moderate peripheral blood eosinophilia, positive serology for schistosomiasis and schistosome eggs may be demonstrated in their excreta. Acute schistosomiasis is more prevalent in individuals newly exposed to the helminth, particularly those experiencing heavy exposure. The condition has resulted in a few reported fatalities, but the majority survive.

Chronic schistosomiasis and its sequalae and interactions with other clinical conditions occur within several months to several years after infection. Symptoms, signs and pathologic lesions are

species specific. Clinical features are described according to the parasite species below.

Urinary schistosomiasis

Symptoms and signs related to *S. haematobium* infection are seen in a considerable proportion of infected individuals, significantly more often than in those infected with any of the other species of schistosome. Furthermore, these features are seen earlier in the course of infection. In endemic areas children infected with *S. haemotobium* will complain of hematuria, which may be terminal, dysuria and frequency. Urine examination may show evidence of hematuria, proteinuria and cellular markers of inflammation. [13] The prevalence of these symptoms varies from 40% to 80% of infected individuals. Ultrasonographic examination of the urinary tract, which is the current suggested investigative modality, demonstrates thickening of urinary bladder wall, granulomas, hydronephrosis and sometimes evidence of bladder and ureteric calcification. At this stage of disease, the functional abnormality in the urinary tract is bladder neck obstruction. Later the course of disease may be complicated by frequent urinary tract bacterial infection, bladder or ureteric stone formation, renal functional abnormalities and ultimately kidney failure. In several endemic areas there is a strong epidemiologic association with squamous cell carcinoma of the bladder, which occurs characteristically in the age group 30–50 years.[14]

Intestinal/hepatic schistosomiasis

Clinical features that are specifically linked to any of the four intestinal species of schistosomes (*S. mansoni, S. japonicum, S. mekongi* and *S. intercalatum*) are seen in a relatively small proportion of infected individuals. Most of the clinical descriptions available report on either *S. mansoni* or *S. japonicum* infection. Established infection with either of these two species results in intestinal and/or hepatic disease.[15] The intestinal presentations are characterized by vague abdominal pains and bloody diarrhea; the latter feature is usually mild, not dysenteric in nature and variable in course. Liver disease manifests originally as hepatomegaly that is subsequently associated with evidence of portal hypertension, including splenomegaly and development of portosystemic collaterals, particularly at the esophagogastric junction and anterior abdominal wall (Fig. 167.2). Liver function tests at this stage are preserved because of the slow rate of development of the portal hypertension, because of the fibrotic and not cirrhotic pathologic changes in the liver, and because of arterialization of the hepatic blood supply that preserves parenchymal cell perfusion and oxygenation. The late stages of liver disease are associated with fibrosis and shrinking of organ size (Fig. 167.3),

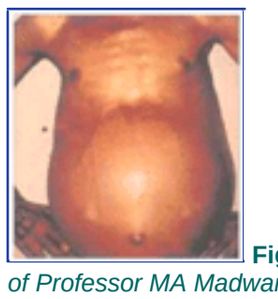

Figure 167-2 Late manifestations of disease caused by *Schistosoma mansoni* infection. Note marked ascites and collateral circulation on anterior abdominal wall. *Courtesy of Professor MA Madwar.*

Figure 167-3 Advanced liver fibrosis in portal tracts (clay pipe-stem). *Courtesy of Professor MA Madwar.*

extensive splenomegaly, ascites, repeated episodes of hematemesis and finally hepatic failure. The phenotypic expression of hepatic disease in schistosomiasis may not only relate to intensity of infection but also to intercurrent infections, such as viral hepatidites,[16] [17] and the genetic constitution of the host.

Schistosomal disease may affect other organs in the body;[1] examples include cor pulmonale, cerebral or transverse myelitis, and cutaneous and genital manifestations. In general, these are less frequent than the previously described features and are usually seen in populations of endemic areas.

DIAGNOSIS

Infection with any of the schistosome species should be suspected in a variety of clinical presentations, as indicated above. A high index of suspicion, obtaining accurate geographic history and enquiry about possible exposure to infected water bodies are, therefore, elementary but essential steps in approaching the individual who possibly has schistosomal infection. Epidemiologic evaluation of infection in travelers necessitates similar examination of others who were exposed. Diagnosis is based on demonstrating the presence of parasite eggs or on serologic evidence (see Chapter 246).[18] Schistosome eggs may be detected in urine samples (for *S. haematobium*). Urine should preferably be collected between 1100h and 1300h, when egg passage is maximal. Sedimentation or filtration allows more sensitive diagnosis and quantification. The usefulness of detecting hematuria with reagent strip and correlating its presence with *S. haematobium* infection has been demonstrated in several studies in endemic areas. This practice, however, is not recommended for the returning occasional traveler in the developed world because of the multiple etiologies of hematuria. For intestinal schistosomes stool examination is performed by any of several concentration techniques, the Kato being the easiest and providing quantitative data. Assessing the parasite burden is important in appreciating the degree of tissue damage and in following up success of therapy. Diagnostic methods that are based on egg identification in urine or stool samples may also be combined with ova hatching to determine their viability. Serologic testing gives evidence for current or past infection by demonstrating antigen-specific antibodies; in the USA these tests are available in some state laboratories or at the Centers for Disease Control and Prevention. Serology for schistosomiasis detects antibodies against several stages of the parasite. It usually converts within 1–2 weeks after exposure and remains positive for a long period, even after chemotherapeutic cure. The test as performed has no useful quantitative value and does not differentiate present from past infection. Antigen detection techniques are also available that allow examination of serum or urine and may be helpful in determining the viability of the worms and their numbers.[19] The decision to work up a person with positive history depends on specific exposure and on the symptoms and signs related to schistosomiasis.

Other diagnostic tests include tissue examination for parasite eggs. Samples may be obtained from rectal mucosa, bladder and liver

biopsies, or from other tissues. Care should be taken when examining tissue for schistosome eggs to identify the parasite species, the viability of the miracidia contained within the egg shells and any evidence of calcification. Clinical diagnosis of schistosomiasis based on symptoms or signs without laboratory or serologic evidence should not be used, except in rare circumstances. These include peripheral blood eosinophilia in those suspected of having acute schistosomiasis, the characteristic liver or urinary tract pathology seen upon ultrasonographic examination or the rare central nervous system and other ectopic disease manifestations.

MANAGEMENT

Praziquantel is the current drug of choice for treating active infection with any of the five schistosome species.[20] The drug is administered orally 40mg/kg body weight in one dose for *S. haematobium, S. mansoni* or *S. intercalatum* infection, and 60mg/kg body weight in three divided doses over 1 day for *S. japonicum* or *S. mekongi* infection. Praziquantel administration is usually not associated with significant side-effects; however, in those with heavy burdens, abdominal pain, nausea and vomiting may occur. The drug is effective in producing parasitologic cure in approximately 80% of treated individuals and achieves over 90% reduction of egg counts in the remaining individuals. Follow-up urine or stool examination within 3 months is advised to assess efficacy of therapy. Praziquantel therapy in early infection also will result in reversal of pathologies such as hepatomegaly, bladder wall thickening or hydroureters. In established pathologic conditions, such as portal hypertension or extensive hydronephrosis, no reversal of pathology may be accomplished.

Treatment of the chronic sequelae of schistosomiasis may necessitate measures other than specific chemotherapy. For example, management of individuals with end-stage liver or kidney disease should be handled along general medical practices. Corrective surgical procedures for portal hypertension or urinary tract anatomic alterations also may be necessary in some cases.

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Chapter 168 - Cestode and Trematode Infections

Guy Baily

INTRODUCTION

Cestode and trematode worms are highly specialized, ubiquitous flatworm parasites infecting both vertebrate and invertebrate animals. Typically, they have a complex life cycle that involves more than one host species harboring stages of the parasite that differ markedly in morphology. Most of these parasites are restricted in their range of host species, particularly in the definitive host of the adult reproductive worms. From among the enormous number of known parasitic flatworms, humans are the preferred host of only a handful, with rather more being capable of incidental or paratenic human infection.

Despite this variety, the life cycles of the parasites that infect humans have certain features in common. The strategy of the trematode parasites is to use aquatic snails as an amplification host. Snails are infected by a first larval stage hatched from eggs that have entered the aquatic environment in the waste products (most often feces) of a definitive host. For the blood flukes that cause schistosomiasis, the larvae (known as cercaria) released by the snails invade directly through skin or mucous membranes in contact with infected water. Other human trematode parasites cause infection through ingestion and rely on their cercaria 'hitching a lift' by encysting on an item of human diet present in the aquatic environment. Adult cestodes are tapeworms that live in the gut of their definitive hosts. Intermediate hosts are infected by ingesting ova, which develop into a sessile larval form, often with a cystic structure, in their tissues. The life cycle is completed when these infected tissues are eaten by a suitable definitive host. The larval forms of cestode parasites often have serious consequences for the function of the intermediate host and are among the most grave human helminthic infections. (Schistosomiasis is discussed in Chapter 167 and hydatid disease in Chapter 169 .)

CYSTICERCOSIS

Epidemiology and prevention

Cysticercosis is infection with the larval or metacestode stage of the pork tapeworm *Taenia solium*. It is acquired as a feco-oral infection from human tapeworm carriers and is therefore a disease of poor sanitation. Historically it occurred worldwide but it has now been largely eliminated from affluent countries through sanitation and meat inspection. The prevalence is strongly influenced by local customs in both diet and animal husbandry. It remains common in Central and South America, south Asia and China, and locally in Africa. A postmortem prevalence of nearly 2% has been reported from Mexico City. Individuals can protect themselves through the ordinary hygienic precautions employed against other feco-oral infections. Mass treatment campaigns with praziquantel can reduce cysticercosis by eliminating tapeworm infections; this may be a useful additional benefit of praziquantel use in schistosomiasis control in Africa.

Pathogenesis and pathology

Ingested *T. solium* ova are activated by exposure to the gastric and duodenal environments into invasive larval forms, termed onco-spheres. These migrate into the tissues, where they develop into a sessile cyst about 1cm across and surrounded by a host-derived capsule. Almost any tissue may be infected but skeletal muscle and the central nervous system, where cysts may be a little larger, are the preferred sites. Significant clinical sequelae are related to neurologic involvement (Fig. 168.1). Unless they are degenerating, cysts elicit very little immune response.

Clinical features

Most people who have cysticerci in their brain have no attributable symptoms. Much the commonest consequence is epilepsy, which is often focal. Where cysticercosis is prevalent, it may account for more than one-third of cases of adult-onset epilepsy.[1] Other neurologic manifestations are very varied. Focal neurologic syndromes may occur, including paralysis, extrapyramidal movement disorders and spinal cord syndromes. When there are many cysts, raised intracranial pressure may be the dominant problem. If untreated, this may lead to permanent loss of cerebral function with dementia and cortical blindness.

In children, who tend to mount a more substantial local immune response, a subacute encephalitis syndrome, with fitting and global changes in cerebral function, may occur.[2]

A minor abnormality of cerebrospinal fluid, mild pleocytosis or slightly elevated protein, can be found in around half of cases of neurocysticercosis; this is a reflection of how commonly cysts have some contact with the meninges. Much less commonly, cysticerci in the larger subarachnoid spaces, especially the cisterna magna, give rise to a clinical presentation of chronic meningitis with headache and global deterioration in cerebral function associated with markedly abnormal cerebrospinal fluid.[3] This may progress to hydrocephalus through obstruction of the ventricular foramina.

Cysticerci in the eye are not uncommon. Usually they are sited in the vitreous or under the retina, where they give rise to visual scotomata. Any significant inflammatory response is likely to result in loss of vision. Serious clinical consequences from cysts at other extraneurologic sites are very rare.

Diagnosis

A clinical diagnosis of neurocysticercosis is seldom possible because the neurologic presentation is never specific and could reflect a number of disease processes. For this reason it may be consierably underdiagnosed in the resource-poor regions where it is most prevalent. Occasionally, evidence of extracranial cysticercosis provides a clue to the origin of neurologic disease (e.g. palpable subcutaneous cysticerci or spindle-shaped calcification of muscle cysts seen on radiography). Eosinophilia is seldom present in established infection. Serology for cysticercosis is available in a number of centers and, although it is not completely sensitive and despite some cross-reactivity among other cestode infections, it provides a very valuable

Figure 168-1 Cysticerci in the brain.

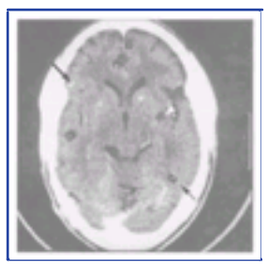

Figure 168-2 Computerized tomography appearances in neurocysticercosis. Viable cysts appear as radiolucent defects (arrowhead). The central protoscolex appears as a radiodense spot in about 50% (small arrow). Cysts that show ring enhancement are probably degenerating. Calcified cysts (large arrow) are dead and will not benefit from specific therapy.

screening tool for neurology patients in endemic countries.[4] Cysticerci can usually be visualized with intracranial imaging (Fig. 168.2). Magnetic resonance imaging is superior to computerized tomography (CT) for intraventricular cysticerci, cysticerci in the posterior fossa and spinal disease.

Management

Anthelmintic drug therapy is now widely used for neurocysticercosis, although its exact role remains controversial. Much of this controversy results from the complexity of the natural history of the disease. Cysts that enhance on CT are inflamed and likely to be degenerating. Prospective studies have shown that they will almost always disappear within a year without specific treatment.[5] This is the usual sequence of events in children unless they are heavily infected. Nonenhancing cysts, often seen in adults, are likely to persist. Praziquantel in large doses (40mg/kg daily orally for 15 days) has been shown to reduce the size and number of nonenhancing parenchymal brain lesions and to lessen fitting.[6] Albendazole (15mg/kg daily orally for 1 month) appears to be at least as effective. [7]

The value of anthelmintics for other clinical presentations is not well documented. On balance, children who have enhancing lesions can reasonably be managed with corticosteroids, symptomatic treatment such as fit control and close follow-up. Nonenhancing parenchymal cysts should be treated with anthelmintics.

The principal complication of both praziquantel and albendazole treatment is acute raised intracranial pressure, which may occasionally be life-threatening. It can be largely prevented with corticosteroids, although this may reduce the efficacy of praziquantel. Practice varies, but a favored opinion is that anthelmintic treatment for cysticercosis should always be accompanied by corticosteroids (e.g. dexamethasone 2mg q12h for 3 days).

Shorter courses of both albendazole and praziquantel may improve CT appearances but are not yet sufficiently established to be generally recommended. [8] Finally, surgery retains a place, particularly in the management of ventricular cysts and hydrocephalus.

OTHER LARVAL CESTODE INFECTIONS

Coenurosis

Humans are occasionally infected by the metacestode larvae of *Taenia multiceps*, which is normally maintained between dogs and sheep. The metacestode is a coenurus; it is a large cyst, more than 2cm in diameter, with multiple invaginated protoscolices, most often localizing in the brain. It is a rare illness but one with a wide geographic distribution.[9] Biopsy is necessary for diagnosis once a large cyst has been identified by imaging. Surgical excision has been advocated; the value of anthelmintics is unknown.

Sparganosis

Sparganosis is infection with the migratory plerocercoid larvae of cestodes of the genus *Spirometra*. These have an aquatic life cycle, similar to *Diphyllobothrium* spp. The larvae ascend the aquatic food chain from invertebrates such as *Cyclops* to reptiles and amphibians. Humans may become infected from drinking water that contains infected *Cyclops* spp., from eating inadequately cooked frog or snake meat, or from using the skin of these animals to dress wounds and sore eyes, as is the custom in some parts of eastern Asia. It is an uncommon but widespread disease in warm climates.

Clinically, there is a localized subcutaneous inflammatory swelling, which migrates rather slowly and contains a single worm-like sparganum (Fig. 168.3). The eye may be damaged by adjacent lesions; entry of the worm into the brain is rare but may be devastating. Excision of the worm is the only reliably effective treatment.

TAPEWORM INFECTIONS

Taeniasis

Humans are the only definitive host for two *Taenia* spp: *T. saginata*, the beef tapeworm; and *T. solium*, the pork tapeworm (Fig. 168.4). Infection is acquired by eating undercooked infected beef or pork, respectively. The clinical consequences of taeniasis are generally trivial and are limited to minor abdominal symptoms, such as occur in irritable bowel syndrome. *Taenia saginata* generates highly motile proglottids (pale, rectangular tapeworm segments) that may cause distress by emerging spontaneously from the anus. *Taenia* spp. do not appear to have any significant nutritional effect on their host. The principal concern in taeniasis is that *T. solium* may give rise to cysticercosis in the same host through feco-oral autoinfection.[10]

Figure 168-3 A sparganum worm dissected from an inguinal mass.

Figure 168-4 *Taenia saginata.* A mature worm may be over 33 feet (10m) long.

Diphyllobothriasis

Diphyllobothrium spp. are a large group of tapeworms that include a complex aquatic phase in their life cycle, ascending the food chain in fish. The species adapted to humans is *Diphyllobothrium latum*. Most human infection now occurs in Russia. Humans acquire infection by eating inadequately cooked or preserved fish. A large tapeworm, up to 33 feet (10m) long, develops in the small bowel; multiple infections are common. Clinical consequences, as with other tapeworm infections, are minor. Tapeworm anemia, described principally from Finland, was an association between diphyllobothriasis and deficiency of vitamin B12. It has not been recorded for several decades and is of historic interest only.[11] A variety of other *Diphyllobothrium* spp. may infect humans, principally in the sub-Arctic and the northern Pacific areas. Prevalences of up to 30% have been recorded in Canadian Inuit communities for *Diphyllobothrium dendriticum*. Clinical consequences of these infections are not well documented, but they are also thought to be minor.

Hymenolepis

The dwarf tapeworm, *Hymenolepis nana*, has the unique distinction among cestodes of being able to complete its life cycle within a single human host. Ova ingested from the environment encyst within a villus of the small bowel epithelium and, after a few days, evaginate a protoscolex. This develops into a new tapeworm only 1.6 inches (4cm) long in the gut lumen. The worm is thus transmitted directly between humans as a feco-oral infection. It is strongly associated with poor sanitation and is common in warm countries, especially among children, in whom prevalences may exceed 10%. In addition to abdominal symptoms, some systemic features such as headache and irritability occur, and eosinophilia is common. There is evidence that heavy infections may contribute to growth retardation. [12]

Zoonotic tapeworms

Various tapeworms that are not primarily adapted to humans may nevertheless occasionally cause human infection. *Hymenolepis diminuta* is a parasite of rats and mice, using their fleas as an intermediate host. Similarly, *Dipylidium caninum* passes between dogs and their fleas. Both are occasional sources of human infection worldwide, principally in children, through the accidental ingestion of fleas. There are no known serious clinical consequences.

Diagnosis and management of tapeworm infections

The diagnosis of tapeworm infection is by direct microscopy of feces for the detection of ova which, within the genera *Taenia* and *Diphyllobothrium*, cannot be readily speciated. Entire proglottids or larger worm portions may sometimes be seen. Treatment with a single dose of praziquantel at 10mg/kg is effective for all the human tapeworms except *H. nana*, for which at least 20mg/kg should be given.

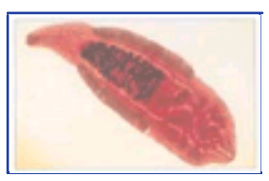

Figure 168-5 An adult liver fluke (*Clonorchis sinensis*). The worm is typically about 0.75 inches (2cm) in length.

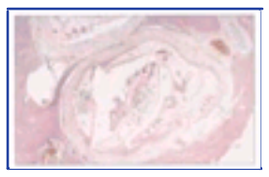

Figure 168-6 Low-power section of a human bile duct containing adult *Clonorchis sinensis* flukes.

LIVER FLUKE INFECTIONS

Epidemiology and prevention

There are three closely related trematodes, *Clonorchis sinensis, Opisthorchis viverrini* and *Opisthorchis felineus*, which for clinical purposes can be considered as one entity; the human liver flukes (Fig. 168.5). Aquatic snails are infected when human feces containing ova contaminate their environment. Cercaria released from the snails encyst under the scales of freshwater fish and will infect a new human host if eaten raw. Thus, either the sanitary disposal of feces or the thorough cooking of fish will interrupt transmission. The liver flukes are almost entirely restricted to eastern Asia and some foci in Siberia. Local prevalences may reach 35%.[13]

Pathogenesis and pathology

Ingested larvae migrate from the duodenum through the ampulla into the biliary tract, developing into adult flukes within small bile ducts in the liver, where they may live for many years. Pathologic consequences arise from the constant abrasion and inflammation of the bile duct in contact with the rough tegument of the flukes (Fig. 168.6). With time the bile duct walls become fibrotic and thickened and the liver becomes enlarged. The gallbladder is frequently abnormal, with distension, irregular thickening, or stones.

Clinical features

Light infections are likely to be asymptomatic. Heavier infection may be associated with right hypochondrial pain and other abdominal symptoms.[14] Significant complications include biliary obstruction and acute cholangitis. There is a strong association between liver fluke infections, especially with *O. viverrini*, and cholangiocarcinoma, which may be among the leading causes of mortality in highly endemic areas.[15]

Diagnosis and management

Liver fluke infection can be sensitively detected by stool microscopy with appropriate concentration technique — the fecal egg burden is a guide to the worm burden, which is in turn proportional to the severity of the pathology. A single dose of praziquantel 40mg/kg is usually effective, although more prolonged treatment may be preferable in heavier infections.

FASCIOLIASIS

Humans are also occasionally infected with liver flukes that are primarily adapted to animals. Principal among these are two species of *Fasciola*: the sheep liver fluke, *F. hepatica*; and the cattle fluke, *F. gigantica*. The cercaria of *Fasciola* spp. encyst on the leaves of aquatic plants, hoping to be eaten by their herbivorous definitive host. Humans are most often infected through wild watercress. Fascioliasis is uncommon but widely distributed. There is typically an acute illness as larvae migrate to the liver; this stage is characterized by fever, eosinophilia and painful hepatomegaly. Even in established infection, ova are difficult to find in the stool, so the diagnosis depends on serology. This is the only human flatworm infection that is unresponsive to praziquantel. Corticosteroids will alleviate much of the immune-mediated acute illness. Bithionol is the established specific treatment, but few physicians have much experience with this drug.[16]

LUNG FLUKE INFECTIONS

Epidemiology

Several trematodes in the genus *Paragonimus* cause human infection. Cases have been reported from most tropical areas but the principal foci are in eastern Asia and central and western Africa. *Paragonimus* cercaria encyst in the gills and other organs of fresh water crustaceans such as crabs and crayfish. Where these are customarily eaten raw or lightly cooked, human paragonimiasis is likely to occur.

Pathogenesis and pathology

Ingested larvae migrate through the intestinal wall and cross the diaphragm to enter the lungs, where they mature, inhabiting a host-derived fibrous capsule typically in the upper zones of the lungs. Pathologic consequences may result from the inflammatory response to larval migration or from the local effects of established adult worms. Occasionally worms develop in ectopic sites, most significantly in the brain, where the inflammatory response is likely to be very destructive.

Clinical features

The pattern of disease differs between *Paragonimus* spp. Mature flukes give rise to a chronic, low-grade, cavitating pulmonary disease. There is chronic sputum production and often hemoptysis. In the African disease, extrapulmonary manifestations are not a feature, but Asian parasites may give rise to migratory subcutaneous inflammatory

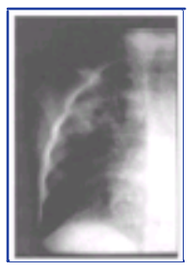

Figure 168-7 Shadowing in the right upper zone with cavitation in a 6-year-old-boy with an African lung fluke infection (*Paragonimus uterobilateralis*).

swellings and more seriously to an eosinophilic meningitis with a variety of focal or global neurologic features.[17]

Diagnosis and management

Diagnosis is by the detection of characteristic ova in the sputum or stool (which they reach via the larynx and esophagus). Serologic tests are also available. The chest radiograph is often abnormal but not diagnostic. Typical appearances are of a cavitating disease in the upper zones; this is easily confused with tuberculosis (Fig. 168.7). Eosinophilia is common. Praziquantel at a dose 25mg/kg q8h for 3 days is very effective.[18]

INTESTINAL FLUKE INFECTIONS

There are a variety of trematodes in eastern Asia that can inhabit the human intestine, most notably *Fasciolopsis buski*, which is principally a parasite of pigs. Symptoms are generally absent or mild but heavy *Fasciolopsis* infections can result in significant malabsorption. Intestinal flukes are almost always eliminated by a single dose of praziquantel at 15mg/kg.

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Chapter 169 - Hydatid Disease

Bruno Gottstein

Echinococcus spp. are cestode parasites commonly known as small tapeworms of carnivorous animals. Their medical importance lies in the infection of humans by the larval stage of the parasites, predominantly including two species:

- *Echinococcus granulosus*, which is the causative agent of cystic hydatid disease (or cystic echinococcosis, CE); and
- *Echinococcus multilocularis*, which causes alveolar echinococcosis (AE).[1] [2]

Two other species, namely *Echinococcus vogeli* and *Echinococcus oligarthrus*, are extremely rarely found in humans and are therefore not covered in this chapter.

EPIDEMIOLOGY

Echinococcus granulosus

Echinococcus granulosus lives as a small intestinal tapeworm of dogs and occasionally other carnivores. The shedding of gravid proglottids or eggs in the feces occurs within 4–6 weeks after infection of the definitive host (Fig. 169.1). Ingestion of eggs by intermediate host animals or humans results in the release of an oncosphere into the gastrointestinal tract, which then migrates to primary target organs such as liver and lungs, and less frequently to other organs (Fig. 169.2). Usually, the fully mature metacestode (i.e. hydatid cyst) develops within several months or years.

Infections with *E. granulosus* occur worldwide, predominantly in countries of South and Central America, the European and African part of the Mediterranean area, the Middle East and some sub-Saharan countries, Russia and China. The annual incidence rates of diagnosed human cases/100,000 inhabitants vary widely, for example 13 in Greece, 143 in some provinces of Argentina, 197 in the Hinjang province of China and 220 in the Turkana district of Kenya. Most cases observed in Europe and the USA are associated with immigrants from highly endemic areas. Various strains of *E. granulosus* have been described, and differ especially in their infectivity for intermediate hosts such as humans. The most important strains for human infection include sheep and cattle as intermediate hosts.

Echinococcus multilocularis

The natural life cycle of *E. multilocularis* involves predominantly red and arctic foxes as definitive hosts (Fig. 169.3), but domestic dogs or house cats can also become infected and represent an important infection source for humans in highly endemic areas.[3] In the definitive host, egg production starts as early as 28 days after infection. After egg ingestion by a rodent or a human, larval maturation will occur within the liver tissue in more than 98% of the cases (see Fig. 169.2); subsequent metastases may occur in adjacent or distant tissues. Proliferation occurs by exogenous budding of metacestode tissue with a progressive tumor-like growth.

The geographic distribution of *E. multilocularis* is restricted to the northern hemisphere. In North America, the cestode is present in the subarctic regions of Alaska and Canada. The parasite has been discovered in its wildlife cycle in several other states, therefore indicating an apparent expansion of distribution within the North-Central American continent.

In Europe, relatively frequent reports of AE in humans occur in central and eastern France, Switzerland, Austria and Germany. The Asian areas where *E. multilocularis* occurs include the whole zone of tundra, from the White Sea eastward to the Bering Strait, covering large parts of the Soviet Union, China and northern Japan.

Worldwide there are scant data on the overall prevalence of human AE. Some well-documented studies demonstrate a generally low prevalence among affected human populations. The annual mean incidence of new cases in different areas including Switzerland, France, Germany and Japan has therefore been reported to vary between 0.1 and 1.2/100,000 inhabitants.[1] [2]

PATHOGENESIS AND PATHOLOGY

Echinococcus granulosus

Cystic echinococcosis (cystic hydatid disease) is clinically related to the presence of one or more well-delineated spherical primary cysts, most frequently formed in the liver, and then in the lungs and other organs such as kidney, spleen, brain, heart and bone (see Fig. 169.2). Tissue damage and organ dysfunction result mainly from this gradual process of space-occupying displacement of vital host tissue, vessels or parts of organs. Consequently, clinical manifestations are primarily determined by the site, size and number of the cysts, and are therefore highly variable. Accidental rupture of the cysts can be followed by a massive release of cyst fluid and hematogenous or other dissemination of protoscolices. Occasionally, this results in anaphylactic reactions and multiple secondary cystic echinococcosis (as protoscolices can develop into secondary cysts within the intermediate host).

The histology of a typical hydatid cyst exhibits the germinal layer as the primary site of parasite development (see Fig. 169.1). It is surrounded by a parasite-derived thick laminated layer, which is rich in aminocarbohydrates, as shown by periodic acid-Schiff positivity. The germinal layer forms protoscolices and brood capsules within the cyst lumen. Granulae, calcareous corpuscules and occasionally free daughter cysts are often observed. The parasite evokes an immune response, which is involved in the formation of a host-derived adventitious capsule. This often calcifies uniquely in the periphery of the cyst, one of the typical features found in imaging procedures. In the liver there may be cholestasis. Commonly, there is pressure atrophy of the surrounding parenchyma. Immunologically, the coexistence of elevated quantities of interferon (IFN)-?, interleukin (IL)-4, IL-5, IL-6 and IL-10 observed in most of hydatid patients supports Th1 and Th2 cell activation in CE. In particular, Th1 cell activation seemed to be more related to protective immunity, whereas Th2 cell activation was related to susceptibility to disease.[4]

Echinococcus multilocularis

In infected humans the *E. multilocularis* metacestode (larva) develops primarily in the liver (see Fig. 169.2). Occasionally, secondary lesions form metastases in the lungs, brain and other organs. The

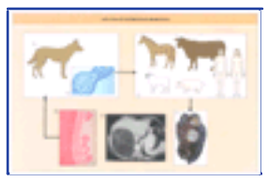

Figure 169-1 Life cycle of *Echinococcus granulosus.* Adult tapeworms parasitize the small intestine of definitive hosts, mainly dogs (1). Parasite proglottids and eggs are shed with the feces (2), such eggs being infectious for intermediate hosts including humans (3). Hydatid cyst formation occurs predominantly in the liver (4), but also in lungs and other organs. Imaging techniques such as CT (5) demonstrate well-delineated, fluid-filled, usually unilocular bladder-like lesions. Internal daughter cysts may be visible in larger cysts as septated segments within the primary cyst. Histologically, the cyst by itself consists of a very thin inner germinal and nucleated layer with a predominantly syncytial structure (6). The germinal layer is externally protected by an acellular laminated layer of variable thickness. The endogenous formation of brood capsules and protoscolices is a prerequisite for completion of the life cycle (6), which occurs when definitive hosts ingest protoscolex-containing hydatid cysts.

typical lesion appears macroscopically as a dispersed mass of fibrous tissue with a conglomerate of scattered cavities with diameters ranging from a few millimeters to centimeters in size. In advanced chronic cases, a central necrotic cavity containing a viscous fluid may form, and rarely there is a bacterial superinfection. The lesion often contains focal zones of calcification, typically within the metacestode tissue.

Histologically, the hepatic lesion is characterized by a conglomerate of small vesicles and cysts demarcated by a thin laminated layer with or without an inner germinative layer (see Fig. 169.3). Parasite proliferation is usually accompanied by a granulomatous host reaction, including vigorous synthesis of fibrous and germinative tissue in the periphery of the metacestode, but also necrotic changes centrally. In contrast to lesions in susceptible rodent hosts, lesions from infected human patients rarely show protoscolex formation within vesicles and cysts.

Genetic and immunologic host factors are responsible for the resistance shown by some patients in whom there is an early 'dying out' or 'abortion' of the metacestode.[5] Therefore, not everyone infected with *E. multilocularis* is susceptible to unlimited metacestode proliferation and develops symptoms 5–15 years after infection. [2] [3] The host mechanisms modulating the course of infection are most likely of an immunologic nature, including primarily T cell interactions. Thus, the periparasitic granuloma, mainly composed of macrophages, myofibroblasts and T cells, contains a large number of $CD4^+$ T cells in patients with abortive or died-out lesions, whereas in patients with active metacestodes the number of $CD8^+$ T cells is increased. An immunosuppressive process is assumed to downregulate the lymphoid macrophage system. Conversely, the status of cured AE is generally reflected by a high in-vitro lymphoproliferative response. The cytokine mRNA levels following *E. multilocularis* antigen stimulation of lymphocytes show an enhanced production of Th2-cell cytokine transcripts IL-3, IL-4 and IL-10 in patients, including a significant IL-5 mRNA expression in patients and not in healthy control donors.[6] The phenomenon of immunologic or constitutional resistance may be dependent upon

a potential immunogenetic predisposition associated with HLA-DR.[7] [8] Conversely, lack of Th cell activity such as in advanced AIDS is associated with a rapid and unlimited growth and dissemination of the parasite in AE.[9]

PREVENTION

Prevention of both CE and AE focuses primarily on veterinary interventions to control the extent and intensity of infection in definitive host populations, which may indirectly be approached by controlling the prevalence in animal intermediate hosts also. The first includes

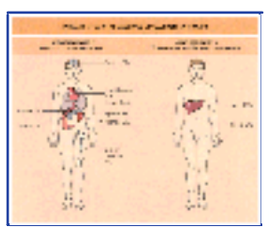

Figure 169-2 Primary sites of metacestode development in humans. Organ distribution of the primary sites of metacestode development for *Echinococcus granulosus* (cystic echinococcosis) and *Echinococcus multilocularis* (alveolar echinococcosis) in human disease.

regular pharmacologic treatment and taking sanitary precautions for handling pets or meat to prevent infection and egg excretion, respectively.[10] For the second, a vaccine for ruminant intermediate hosts is in evaluation.[11] Prevention of human infection is strategically very difficult.

CLINICAL FEATURES

The initial phase of primary infection is always asymptomatic. The infection may then remain asymptomatic for years or even decades depending upon the size and site of the developing cyst or metacestode mass. After a highly variable incubation period, the infection may become symptomatic due to a range of different events.

Cystic echinococcosis

Depending on the size and the site of the developing hydatid cyst, the infection can remain asymptomatic for months, years or even longer. After a highly variable incubation period, the infestation may become symptomatic due to a range of different events.

¦ The growing cyst exerts pressure on or induces dysformation of adjacent tissues, thus inducing dysfunction of the affected organ or vascular compromise. In the case of hepatic CE, signs and symptoms may include hepatomegaly with or without a palpable mass in the right upper quadrant, right epigastric pain, nausea, vomiting and occasionally cholestatic jaundice. In inoperable cases, hepatic compromise may lead to biliary cirrhosis and the Budd-Chiari syndrome.
Infestation of the lungs may present with chronic cough, hemoptysis, bilioptysis, pneumothorax, pleuritis, lung abscess and parasitic lung embolism.
Rare but often catastrophic infestations can affect the heart or the brain. In the heart this can present as tumor, pericardial effusion up to tamponade, complete heart block and sudden death. In the spine and brain presentation is as a tumor with neurologic symptoms. Hydatid disease should be considered as a cause of stroke in young patients.
¦ A cyst may rupture and spill its content into the adjacent site. Rupture into the biliary tree will mimic biliary colic or result in cholestatic jaundice and cholangitis or pancreatitis. This is the presenting symptom in 5–25% of patients. Ruptures in the liver but also in the lungs and other organs may result in acute anaphylactic shock reactions which usually represent the initial and life-threatening manifestation.
¦ The cyst can become superinfected; in hepatic hydatid disease this occurs in about 9% of patients and is an indication for rapid surgical intervention.[12]

The majority of patients with CE have single organ involvement with solitary cysts. Simultaneous involvement of two or more organs is observed in 10–15% of patients, depending on the geographic origin of the patient and the strain of the parasite. In hepatic CE, the right lobe is more frequently affected than the left lobe. Cyst size varies usually between 1 and 15cm in diameter. Cyst growth ranges between a size increase of a few millimeters (1/3 of the patients) to approximately 10mm (most of the patients); 1/10 of the patients exhibit a rapid increase with an annual average of 30mm. In Europe, the average age of patients at diagnosis is 36 years. Approximately 10% of the CE cases occur in children, and the rate of lung involvement is significantly increased among this group of young patients. Pulmonary cysts occasionally become superinfected and this is best detected by computerized tomography (CT) scanning. The ratio of males to females may vary dependent on the geographic area but is statistically not significant overall.

Hepatic alveolar echinococcosis

In patients with hepatic AE, the size of the liver lesion will range between a few millimeters up to 50cm or more when patients present for diagnosis and initial treatment.[2] Typical calcifications occur in 70% of cases and central or peripheral necrotic cavities are also found in approximately 70% of cases. Clinical signs at diagnosis

Figure 169-3 Life cycle of *Echinococcus multilocularis.* This involves predominantly foxes as definitive hosts (1) and occasionally other carnivores such as domestic dogs or house cats. Egg production by the tapeworm starts as early as 28 days after infection (2). Eggs must be ingested by a suitable intermediate host (3), including humans and various rodent species (4). As a result, the parasite metacestode primarily becomes established in the liver. Macroscopically, the typical lesion is characterized by a dispersed mass of fibrous tissue with a multitude of interconnected vesicles ranging from a few millimeters to centimeters in size (5). The lesion often contains focal necrotic zones with scattered calcifications, as demonstrated by CT (6). Histologically, the hepatic lesion consists of a conglomerate of small vesicles and cysts demarcated by a thin laminated layer with or without an inner germinal layer and, predominantly in the rodent intermediate host, protoscolex formation (7). Oral ingestion of protoscolex-containing metacestodes by definitive hosts completes the life cycle.

include hepatomegaly-cholestasis-jaundice, secondary biliary cirrhosis, liver abscess, portal hypertension and Budd-Chiari syndrome. The disease starts frequently with non-specific symptoms such as epigastric pain or cholestatic jaundice. In complicated cases, evidence of secondary biliary cirrhosis and/or cholangitis will be found. Evidence of cholestasis is frequently present, while transaminases are only rarely and moderately elevated, in particular when there is central necrosis. One of the most feared complications is infection of a necrotic cavity and/or obstructed bile ducts, which are associated with very high mortality due to development of septic shock. Distant metastases can occur late in the disease; these have been described in brain, spine, lung and bone. Metastatic disease occurs in approximately 10–20% of patients.

The growth rate of the metacestode tissue is usually slow in immunocompetent patients. Analysis by CT scans indicated an average volume increase of 15ml/year for progressive forms of AE. In Europe, the average age of AE patients at diagnosis is 55 years.[2] Young children rarely develop AE, unless the cellular immune system is compromised.[9] The ratio of males to females varies geographically, but any variation is not statistically significant.

DIAGNOSIS

Echinococcus granulosus

In most cases, imaging procedures together with serology will yield the diagnosis. Sonography is the primary diagnostic procedure of choice for hepatic cases, although false positives occur in up to 10% of cases due to the presence of nonechinococcal serous cysts, abscesses or tumors.[2] The main diagnostic features of hydatid disease include:

¦ separation of the membrane from the wall,

¦ daughter cysts, and
¦ ruptured cysts.

Computerized tomography is the best investigation for detecting extrahepatic disease and volumetric follow-up assessment; magnetic resonance imaging (MRI) assists in the diagnosis by identifying changes in the intra- and extrahepatic venous systems. Ultrasonography is also helpful in following up treated patients as successfully treated cysts become hyperechogenic. Calcification of variable degree occurs in about 10% of the cysts.

Aspiration cytology appears to be particularly helpful in the detection of pulmonary, renal and other nonhepatic lesions for which

imaging techniques and serology do not provide appropriate diagnostic support. The viability of aspirated protoscolices can be determined by microscopic demonstration of flame cell activity and trypan blue dye exclusion. Anti-Ag5 monoclonal antibody has been used for the detection of the respective antigen in diagnostic fine-needle aspiration biopsies (FNABs) from patients with suspected CE.[13] Immunodiagnostic tests to detect serum antibodies or circulating antigens are used to support the clinical diagnosis of CE.[14] The indirect hemagglutination tests and the enzyme-linked immunosorbent assay using *E. granulosus* hydatid fluid antigen are diagnostically relatively sensitive for hepatic cases (85–98%). For pulmonary cysts the diagnostic sensitivity is markedly lower (50–60%) and for multiple organ involvement it is very high (90–100%). These tests are usually used for primary serologic screening. Specificity is low for other cestode infections. To increase specificity, primary seropositive sera are retested using a confirmation test such as immunoblotting for a relatively specific 8kDa/12kDa hydatid fluid polypeptide antigen.[14]

Serologic studies to follow-up patients with CE postoperatively have emphasized the detection of circulating immune complexes or antigens. The detection of circulating parasite antigens proved useful for monitoring the course of disease and for assessing the extent of surgical removal of parasite lesions.[14]

Besides skin tests and basophil degranulation tests, diagnostic cellular immunodiagnosis has focused on in-vitro lymphoproliferative responses to *E. granulosus* antigens. The diagnostic sensitivity of cell-mediated immunodiagnosis is 75%, including the finding of seronegative patients with a positive proliferation test.[14]

Echinococcus multilocularis

Among the imaging procedures, ultrasonography, CT and MRI are of greatest diagnostic value, none of those being uniquely superior.[15] Irregularly dispersed clusters of calcifications on plain abdominal radiographs may give the first clue as to the etiology of the disease; the percentage of calcifications within the lesions increases from 30% to nearly 100% as the disease progresses. Hyperechogenic and hypoechogenic zones characterize the lesions. The cystic appearance may reflect central necrotic cavities. Similar findings can be found on CT and the lesions are typically not enhanced with contrast medium. The lesions are heterogeneous hypodense masses with irregular contours and lacking well-delineated walls. Hilar involvement can lead to liver atrophy, which is easily visualized by CT. Ultrasonography is the preferred imaging procedure for mass screening programs. Magnetic resonance imaging adds to diagnosis, in particular in cases with appropriate organ localization such as brain and bone, and to visualize pathologically altered microstructures in certain affected organs. Thus, MRI can give a precise analysis of the different components of the parasitic lesions such as necrosis and fibrosis.[15] However, in contrast to CT microcalcifications are not visualized by MRI. Assessing the parasite viability in vitro following therapeutic interventions may be of tremendous advantage when compared with the invasive analysis of resected or biopsied samples. Such alternatives may be offered by magnetic resonance spectrometry or positron emission tomography. The latter technique has recently been used for assessing the efficacy of chemotherapy in AE.[16]

Immunodiagnosis represents a valuable secondary diagnostic tool complementary to imaging procedures and is useful for confirming the nature of the etiologic agent.[14] Serologic tests are more reliable in the diagnosis of AE than CE. The use of purified *E. multilocularis* antigens such as the Em2 antigen or recombinant antigens II/3–10 (identical to EM10 and Em18) exhibits diagnostic sensitivities ranging between 91% and 100%, with overall specificities of 98–100%.[14] These antigens allow discrimination between the alveolar and the cystic forms of disease with a reliability of 95%. Seroepidemiologic studies reveal asymptomatic preclinical cases of human AE as well as cases in which the metacestode has died at an apparently early stage of infection (see above).[3] Serologic tests are, however, of limited value for assessing the efficacy of treatment and chemotherapy. The best respective information is provided by the detection of anti-II/3–10 (and Em18) antibodies, a status reflecting the presence of viable metacestode lesions. Cellular immune tests show that the in-vitro lymphoproliferative response to *E. multilocularis* antigen stimulation is high in cured patients who have had radical surgery and in patients with dead lesions, and is significantly lower in patients who have had partial or no surgical resection.

Histopathologic and immunohistochemic procedures to analyze surgically resected samples or biopsies obtained by FNAB include the use of species-specific MAbs such as MAbG11[17] or molecular techniques such as polymerase chain reaction.[18]

MANAGEMENT

The management of CE and AE follows the strategy recommended in the manual on echinococcosis published in 2001 by the Office International des Epizooties and the World Health Organisation.[10]

Echinococcus granulosus

Surgery remains the mainstay in the treatment of hepatic hydatid disease. Cystectomy and pericystectomy offer a good chance for cure and should be undertaken wherever possible. Occasionally, formal hepatic resection will be required. Radical surgery — either pericystectomy or resection — is possible in 50–85% of cases. In the absence of complications this can be achieved with little mortality and an acceptable morbidity. Recently, laparoscopic pericystectomy has been demonstrated to be as safe and effective as open laparotomy in selected cases with hepatic and/or splenic involvement.[19]

If surgical removal of the cyst is contraindicated, treatment of CE has several alternatives such as PAIR (Puncture, Aspiration, Injection of an heminthicide and Reaspiration), chemotherapy or 'wait and observe' approach.

Basically, indications for hepatic surgery include large liver cysts with putatively multiple daughter cysts; single liver cysts, situated superficially, which may rupture spontaneously or as a result of trauma; bacterially superinfected cysts; cysts communicating with biliary tree and/or exerting pressure on adjacent vital organs; brain, heart and kidney cysts; and spinal and bone cysts. Relative contraindications are inoperable cases as defined for surgical procedures in general; patients with cysts difficult to access; and abortive cysts either partly or totally calcified.

A direct communication between the hydatid cyst and the biliary tree may contraindicate the use of protoscolicidal solutions, which can cause chemical cholangitis leading to sclerosing cholangitis. Formalin should not be used for this reason. Effective protoscolicides with a relatively low risk of toxicity are 70–95% ethanol or 15–20% hypertonic saline solution.

Preoperative chemotherapy with albendazole or mebendazole is indicated for reducing the risk of secondary echinococcosis after operation and should begin at least 4 days before surgery and be continued for at least 1 or preferably more months. Diagnostic puncture of hydatid cysts harbors the risk of cyst rupture and dissemination of protoscolices and is therefore not recommended.

PAIR has become well justified in selected cases but it still needs to be practiced by experienced specialists.[20] Indications for PAIR are patients refusing surgery; infected cysts not communicating with the biliary vessel system; inoperable patients (see contraindications for surgery, above); pregnant patients; children >3 years; anechoic lesion =5cm in diameter; cysts with a regular double laminated

layer; cysts with more than five septal divisions; multiple cysts (=5cm in diameter) in different liver segments; relapse after surgery; and failure to respond to chemotherapy. Relative contraindications for PAIR are inaccessible or risky location of the cyst in the liver; multiple septal divisions; cysts with echogenic lesions; inactive cysts or calcified lesions; communicating cysts; cysts located in the lung and bones; and some others. It should not be performed when exophytic cysts or dilated bile ducts are observed on preoperative imaging.

Treatment with benzimidazoles (preferably albendazole) is highly recommended for 4 days prior to intervention. After successful instillation of protoscolicides and re-aspiration, benzimidazoles should be given for 3 months.

Treatment of nonresected cysts with benzimidazoles (albendazole or mebendazole) results in cyst disappearance in 30% of cases; in 30–50% of patients there is cyst degeneration or a significant reduction in cyst size and in 20–40% of patients the cysts show no morphologic change.[10] Indications for chemotherapy include inoperable patients as listed above.

The formerly conventional dosage of albendazole (10–15mg/kg/day in several 1-monthly courses with 14-day intervals) included three courses at minimum, and more than six courses were usually not necessary. This strategy is more commonly replaced by continuous treatment, which demonstrated equal or improved efficacy without increased adverse effects when compared with cyclic treatment.[21] For mebendazole, the usual dosage is 40–50mg/kg/day for at least 3–6 months. Praziquantel has been proposed as an additional antiprotoscolicidal drug to be given once a week in a dose of 40mg/kg along with benzimidazoles. It is also recommended before and after surgery/PAIR when there is a risk of cyst rupture and release of protoscolices.

Echinococcus multilocularis

The following strategies are commonly accepted for treatment of AE:

- the first choice of treatment is radical surgical resection of the entire parasitic lesion from the liver and other affected organs in all operable cases, with excision of the parasitic lesion following the rules of radical tumor surgery;
- concomitant chemotherapy for all cases after radical surgery or after nonsurgical interventional procedures;[21] and
- long-term chemotherapy for inoperable or only partially resectable cases and all patients after liver transplantation.[10]

Presurgical chemotherapy is not indicated for AE. The daily dosage for albendazole and mebendazole treatment is the same as for CE. For albendazole, continuous treatment is well tolerated for a duration up to 6 years, and is replaced by the former discontinuous scheme (see above) only in cases with side-effects related to medication. For mebendazole, plasma drug levels should be over 74ng/ml (250nmol/l). Generally, the duration of treatment is at least 2 years after radical surgery or continuously for many years for inoperable cases or if resection is incomplete.

As an ultimate goal liver transplantation has been proposed for a selected group of patients who have inoperable AE and chronic liver failure. However, the indications are limited and focus on cases with extensive lesions restricted to the liver and secondary liver disease leading to chronic liver failure;[22] relapse is frequent and caused by extrahepatic metacestodes, which rapidly proliferate under immunosuppressed conditions.

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Chapter 170 - Filariasis

Eric A Ottesen

INTRODUCTION

Lymphatic filariasis, onchocerciasis and loiasis are the three most important filarial infections of humans (Table 170.1) and for two of these three diseases (lymphatic filariasis and onchocerciasis) there are now major public health initiatives to control or eliminate them completely. [1] [2] [3] [4] All of these infections are caused by parasites transmitted by biting arthropods (mosquitoes or flies). Each goes through a complex life cycle that includes a slow maturation (often 3–12 months) from the infective larval stage, carried by the insects, to the adult worm that resides either in the lymph nodes and adjacent lymphatics or in the subcutaneous tissue. The offspring of the adults, the microfilariae, are 200–350µm in length and either circulate in the blood (lymphatic filariasis and loiasis) or migrate through the skin (onchocerciasis), awaiting ingestion by the insect vectors in which they develop infectivity for humans to continue this complex life cycle (see Chapter 246).

EPIDEMIOLOGY

Lymphatic filariasis

There are 120 million people in at least 80 countries of the world who are infected with lymphatic filarial parasites, and it is estimated that 1000 million people (20% of the world's population) are at risk of acquiring infection. [1] [3] Of these infections, 90% are caused by *Wuchereria bancrofti*, whose only host is humans, and most of the remainder are caused by *Brugia malayi*. The major vectors for *W. bancrofti* are culicine mosquitoes in most urban and semiurban areas, anopheline mosquitoes in the more rural areas of Africa and elsewhere, and *Aedes* spp. in many of the endemic Pacific islands. For the *Brugia* parasites, *Mansonia* spp. serve as the major vector, but in some areas anopheline mosquitoes are responsible for transmitting the infection. *Brugia* parasites are confined to areas of eastern and southern Asia, especially India, Malaysia, Indonesia, the Philippines and China.

Onchocerciasis

There are 17 million people in 35 endemic countries who are infected with *Onchocerca volvulus*; 95% of these people live in sub-Saharan Africa.[2] [4] Humans are the exclusive host for *O. volvulus*; there is no nonhuman reservoir of infection. Blackflies of several *Simulium* spp. are the vectors of infection, and because the larvae of these flies require fast-flowing water for their development, transmission is limited to those areas within the flight range of the flies that breed in such rivers. Thus, the distribution of onchocerciasis ('river blindness') is very much determined by the river systems and tributaries in Africa, Yemen and the Americas, where the infection is endemic.

Loiasis

Loa loa infects approximately 12 million people in the rainforest belt of western and central Africa and equatorial Sudan, where the *Chrysops* vector flies can easily find breeding spots. Although *L. loa* can be found in a number of nonhuman African primates, there is little or no cross-infection between the cycles of *L. loa* in humans and in nonhuman primates.

PATHOGENESIS AND PATHOLOGY

The pathology associated with filariases results from a complex interplay of the pathogenic potential of the parasite, the immune response of the host and external ('complicating') bacterial and fungal infections.[5] [6]

Lymphatic filariasis

Although genital abnormalities (especially hydrocele) and lymphedema or elephantiasis are the most recognizable clinical entities associated with lymphatic filarial infections (Fig. 170.1), earlier changes can be detected when lymphoscintigraphy[4] or ultrasound techniques are used,[5] including lymphatic dilatation with abnormal lymphatic function. These can occur with and without an inflammatory response. Secondary host inflammatory responses, including responses to bacterial and fungal superinfections of tissues with compromised lymphatic function, cause most of the progression and physical destruction associated with elephantiasis.

The pathology in lymphatic filariasis that is immune mediated most commonly derives from the lymphatic obstructive consequences of the responses to dead or dying worms in the lymphatics, but in the syndrome of tropical pulmonary eosinophilia, the pathogenesis is distinctly different[7] and results from immunologic hyperresponsiveness to the microfilaria-stage parasites.

Onchocerciasis

Most of the significant pathology in onchocerciasis affects the skin (Fig. 170.2) or the eyes. There are both noninflammatory and inflammatory routes to tissue damage.[6] In 'steady-state', chronic *O. volvulus* infections, between 10,000 and 500,000 microfilariae are produced each day. These microfilariae migrate through the skin, inducing little inflammatory response; however, the elaboration of collagenases, elastases and other enzymatic molecules leads to hyperpigmentation, atrophy and thinning of the skin (see Fig. 170.2). Superimposed inflammatory reactions to dying microfilariae result in episodic papular dermatitis. In the eyes, these inflammatory responses result either in a characteristic punctate keratitis and, ultimately, anterior segment blindness or in uveitis and retinal lesions that can lead to posterior segment blindness. The possibility that a bacterium (*Wolbachia* spp.) that is an endosymbiont living within *O. volvulus* has a role in the pathogenesis of onchocerciasis has recently been advanced.[8]

Loiasis

'Calabar' swellings, the angio-edematous lesions characteristic of loiasis, have been less well studied. Presumably, these are immune-mediated inflammatory responses to migrating subcutaneous adult worms (Fig. 170.3) and are more common in the immunologically

TABLE 170-1 -- Principal filarial diseases.

PRINCIPAL FILARIAL DISEASES							
Disease	**Number of people infected worldwide**	**Parasite**	**Vector**	**Principal clinical manifestations**	**Distribution**	**Location of microfilariae within the body**	**Periodicity**

Lymphatic filariasis	120 million	*Wuchereria bancrofti*	Mosquitoes	Lymphedema, elephantiasis, genital pathology (hydrocele)	Tropics worldwide	Blood	Nocturnal (95%), 'subperiodic' (5%)
		Brugia malayi	Mosquitoes	Lymphedema, elephantiasis	Asia, India, Philippines	Blood	Nocturnal (75%), 'subperiodic' (25%)
Onchocerciasis	17 million	*Onchocerca volvulus*	Blackflies	Dermatitis, blindness	Africa (95%), Americas, Yemen	Skin	Minimal
Loiasis	12 million	*Loa loa*	Deer flies	Angio-edema, 'eyeworm'	Africa	Blood	Diumal

Figure 170-1 Elephantiasis. (a) Already advanced elephantiasis in a 14-year-old Indian girl who has bancroftian filariasis. Although such clinical expression of filarial disease is more commonly seen in adults, infection in endemic areas is usually established in early childhood. (b) Scrotal elephantiasis in an adult man who has bancroftian filariasis.

naive. Not common in untreated patients, but increasingly problematic in populations receiving ivermectin for co-endemic onchocerciasis, is a central nervous system (CNS) depression syndrome leading to coma or even death.[9] Its pathogenesis is suspected to involve inflammatory responses to dying microfilariae in cerebral vessels, but many details remain uncertain.

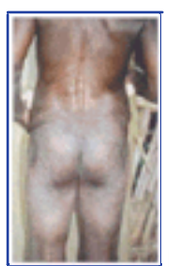

Figure 170-2 Onchocerciasis. Evidence of excoriation caused by the patient's trying to relieve the maddening pruritus caused by onchocerciasis. Note also the marked dermal atrophy associated with chronic infection.

PREVENTION

Filarial infections can be acquired only from vector-borne infective larvae. Therefore, prevention of infection can be achieved either by decreasing contact between humans and vectors, generally through vector control efforts, or by decreasing the amount of infection the vector can acquire, through treating the human host.

Lymphatic filariasis

Population-based prevention

Efforts to decrease filariasis in populations through mosquito-vector control have usually proved ineffective owing to high cost and the long lifespan of the parasite (4–8 years). More recently, especially with the advent of extremely effective single-dose, once-yearly, two-drug regimens (albendazole 400mg plus either ivermectin 200µg/kg or diethylcarbamazine (DEC) 6mg/kg), the alternative approach of decreasing microfilariae in the population has been preferred.[10]

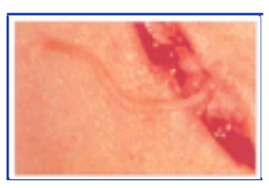

Figure 170-3 *Loa loa* adult worm. The worm has been teased from the subcutaneous tissue after incision was made through a small pruritic papule (0.5cm in diameter) in an expatriate patient who had loiasis. Such papules can occur spontaneously or after treatment with DEC.

Indeed, it is this strategy that forms the basis of the new Global Program to Eliminate Lymphatic Filariasis undertaken by the World Health Organization (WHO) and a global alliance of public and private sector partners.[3]

Individual-based prevention

Contact with infected mosquitoes can be decreased through the use of personal insect repellents, bednets or insecticide-impregnated materials. Alternatively, suggestive evidence from animal models and some limited experience in human populations indicate that a prophylactic regimen of DEC (6mg/kg/day for 2 days each month) could provide effective protection against infection.

Onchocerciasis

Population-based prevention

A program to prevent onchocerciasis in 11 West African countries has been undertaken by the WHO, the United Nations Development Programme and the World Bank. It was initially based on eliminating the blackfly vectors of the infection for a period that needed to exceed the lifespan of all adult *O. volvulus* worms (12–15 years) in that area. This 'Onchocerciasis Control Program' has now run for more than 25 years and been extraordinarily successful in 'reclaiming' both land and lives of people otherwise severely compromised by onchocercal disease.[4] However, because this approach is both expensive and difficult, a new strategy for treatment and consequent prevention of infection through the use of once-yearly ivermectin in affected populations has been undertaken in all of the remaining African countries where onchocerciasis is endemic. [4]

Individual-based prevention

Decreased contact with infected blackflies through protective clothing and repellents is helpful in preventing infection. Although no prophylactic treatment regimen has yet been defined, recent studies employing monthly doses of ivermectin in cattle exposed to the related parasite *Onchocerca ochengi* show a dramatic prophylactic effect of ivermectin, but studies have not yet been undertaken to see whether similar prophylactic efficacy can be shown for ivermectin in humans as well.

Loiasis

Population-based prevention

No specific prevention efforts in populations have been undertaken.

Individual-based prevention

There are good data that repeated use of DEC (300mg weekly or 6mg/kg/day for 2 days each month in adults) is effective prophylaxis against acquisition of *L. loa* infection.[11]

CLINICAL FEATURES

Lymphatic filariasis

Chronic manifestations

Hydrocele, even though it is found only with *W. bancrofti* infections (and not *Brugia* infection), is the most common clinical manifestation of lymphatic filariasis. Uncommon in childhood, it is seen more frequently after puberty and there is a progressive increase in prevalence with age.[12] In many endemic communities, 40–60% of all adult males have hydrocele. It often develops in the absence of overt inflammatory reactions and, indeed, many patients who have hydrocele also have microfilariae circulating in the blood. The localization of adult worms in the lymphatics of the spermatic cord leads to a thickening of the cord so that the cord is palpable on physical examination of most patients. Hydroceles can become massive but still occur without the development of lymphedema or elephantiasis in the penis and scrotum[13] (see Fig. 170.1).

Although lymphedema can also develop in the absence of overt inflammatory reactions and in the early stages be associated with microfilaremia, the development of elephantiasis (either of the limbs or the genitals) is most frequently associated with a history of recurrent inflammatory episodes. Patients who have chronic lymphedema or elephantiasis are rarely microfilaremic. Very important in the progression of these lesions is the fact that the redundant skin folds, cracks and fissures of the skin provide havens for bacteria and fungi to thrive and intermittently penetrate the epidermis, leading to either local or systemic infections.

Chyluria, another of the chronic filarial syndromes, is caused by the intermittent flow of intestinal lymph (chyle) through ruptured lymphatics into the renal pelvis and subsequently into the urine. The mechanisms underlying this have not been well defined and the clinical course is known to be intermittent. Nutritional compromise can, however, be severe in patients who have chronic chyluria; special diets (low-fat and high-protein, supplemented with fluids) can often be helpful. [13]

Acute manifestations

There are four distinct acute manifestations of lymphatic filariasis, each with a different set of causative mechanisms and pathogenic implications.

The first and most important is acute inflammation of the limbs or scrotum that is related to bacterial or fungal superinfection of tissues with already compromised lymphatic function.[7]

Another type of 'filarial fever' was confused with this picture in the past. In this second type, the inflammation is initiated in the lymph node (commonly the inguinal node) with 'retrograde' extension down the lymphatic tract and an accompanying 'cold' edema. Here the inflammation appears to be immune mediated and less frequent (10–20% of cases) than the episodes of inflammation initiated by dermal infection.[13]

A third acute filarial syndrome is tropical pulmonary eosinophilia, a distinctly different syndrome caused by an immunologic hyperresponsiveness to filarial infection.[7] It is characterized by:

- extremely high levels of peripheral blood eosinophilia;
- asthma-like symptoms;
- restrictive (and often obstructive) lung disease;
- very high levels of specific antifilarial antibodies; and
- an excellent therapeutic response to appropriate antifilarial treatment with DEC.

It occurs with a frequency of less than 1% of all filariasis cases, but it is a severe condition that can lead to chronic interstitial fibrosis and pulmonary failure.

The fourth (and least commonly recognized) form of acute inflammatory reaction is that seen early after infection, particularly in expatriates who are exposed to and acquire filarial infection for the first time. Lymphangitis occurs around developing larval and early adult stages in these patients, associated with acute eosinophilic inflammation.[7]

Asymptomatic presentations

Of all the patients who have lymphatic filariasis, at least half appear clinically asymptomatic, although they have microfilariae circulating in their blood and essentially all have hidden damage to their lymphatic or renal systems. [14]

A second asymptomatic 'presentation' exists in people who do not have demonstrable microfilaremia but who do have parasite antigen in the blood (which will disappear after appropriate treatment). The clinical features and long-term sequelae of infection in this group remain to be defined.

A variety of other syndromes co-existing with filariasis are found in filarial-endemic regions and because they show some evidence of therapeutic response to DEC they have been regarded as possible manifestations of lymphatic filariasis. These include arthritis (typically monoarticular), endomyocardial fibrosis, tenosynovitis, thrombophlebitis, lateral popliteal nerve palsy and others. Although future studies may strengthen an etiologic relationship with filariasis, such presentations cannot now be confidently attributed to filarial infection.

Onchocerciasis

Chronic presentations

Most damage from onchocerciasis occurs in the skin and eye. Subcutaneous nodules (generally 1–6cm in diameter) can be palpated superficially. However, most of the skin activity is a waxing and waning of maculopapular rashes, which are essentially always accompanied by itching (see Fig. 170.1),[2] presumably because of allergic responses to dying microfilariae. During the long course of infection, the skin becomes extensively damaged, losing much of its elasticity and even pigmentation. Indeed, when the skin over the inguinal nodes (which often are enlarged owing to their continual stimulation by dying microfilariae) becomes so atrophic that it cannot support the underlying lymph nodes, the clinical presentation of 'hanging groin' occurs.

In the eye, acute changes are those associated with dying microfilariae and the local inflammatory reactions that they induce.[2] In the cornea, 'fluffy opacities' (inflammatory cells associated with the dying microfilariae) can lead to punctate keratitis, but in prolonged and heavy infection, inflammation in the cornea results in sclerosing keratitis, whereas inflammatory responses located elsewhere in the eye lead to iridocyclitis, choroidoretinitis or optic atrophy. Complications of these inflammatory eye processes also include glaucoma and cataract.

Loiasis

The two most characteristic clinical features of loiasis are the passage of an adult filarial worm across the eye ('eye worm'), often in an otherwise asymptomatic person, and Calabar swellings. Calabar swellings are localized areas of erythema and angio-edema that may be 5–10cm or more in size. Often they occur in the extremities and last for several days before regressing spontaneously. If the inflammatory reaction extends to nearby joints or peripheral nerves, corresponding symptoms may develop. Routine radiographs of people in endemic areas may reveal calcified dead worms lying between the metacarpals.

Expatriate syndrome

Recently, this 'new' filarial syndrome has been recognized as one of clinical and immunologic hyperresponsiveness that is found in expatriate visitors to regions endemic for loiasis *and other filariases*. These people manifest prominent signs and symptoms of inflammatory reactions (including allergic reactions) to the mature or maturing parasites. In loiasis, these manifestations have included primarily Calabar swellings, hives, rashes and occasionally asthma. In bancroftian filariasis (when military personnel or other migrants to endemic areas have acquired these infections), the manifestations have usually been lymphangitis, lymphadenitis and genital pain (from inflammation of the associated lymphatics), with hives, rashes and other 'allergic-like' manifestations, including blood eosinophilia. [7]

DIAGNOSIS

Except for *W. bancrofti* infections, diagnosis of filarial infections depends on the direct demonstration of the parasite (almost always microfilariae) in blood or skin specimens using relatively cumbersome techniques and having to take into account the periodicity (nocturnal or diurnal) of microfilariae in blood (see Table 170.1). Most alternative methods based on detection of antibodies by immunodiagnostic tests have not proved to be satisfactory because of their failure to distinguish between active and past infections and their problems with specificity. There is good evidence, however, that recombinant antigens will greatly improve the value of such antibody-based immunodiagnostics in the future.

Lymphatic filariasis

Antigen detection

Circulating filarial antigen detection with almost complete specificity and high sensitivity should now be regarded as the 'gold standard' for diagnosing *W. bancrofti* infections.[15] Two commercial forms of this assay are available. One is based on the methodology of an enzyme-linked immunosorbent assay and yields semiquantitative results; the other is based on a simple card (immunochromatographic) test and yields only qualitative (positive or negative) answers. No such test is currently available for brugian filariasis.

Microfilaria detection

Before the development of the circulating filarial antigen assay, detection of microfilariae in blood was the standard approach to diagnosing lymphatic filarial infection, and it is the one still required today for both brugian filariasis and those situations where the antigen detection test is not available for bancroftian filariasis. Such assessments must take into account the possible nocturnal periodicity of the parasites when the the optimal time for drawing blood is chosen; this is between 10.00pm and 2.00am for most brugian filariasis and bancroftian infections.[1] [13] The simplest technique for examining blood or other fluids (e.g. hydrocele fluid or articular effusions) is to spread 20µl evenly over a clean slide that is dried and then stained with Giemsa or a similar stain. A wet smear may also be made by diluting 20–40µl of anticoagulated blood with water or 2% saponin, which will lyse the red blood cells but allow the microfilariae to remain motile and thus more readily identifiable. The larger the blood volumes examined, the greater will be the likelihood of detecting low levels of parasitemia. Other concentration techniques are also available.[13]

Clinical diagnosis

Many lymphatic filariasis patients are amicrofilaremic and therefore the diagnosis of these infections must be made 'clinically'.[16] For amicrofilaremic syndromes other than tropical eosinophilia syndrome (see Chapter 150), serologic findings based on detecting IgG_4 antibodies have proved helpful, because this subclass of IgG has

greater diagnostic specificity and is stimulated by the presence of active infection. Such antibody analyses also are particularly helpful in diagnosing the 'expatriate syndrome', in which background (i.e. pre-exposure) levels of IgG and especially of IgG_4 antibodies to filarial antigens are very low, so that elevated levels have significant diagnostic implications in association with the clinical presentation. [16]

Eosinophilia is a frequent concomitant of all filarial syndromes but is diagnostically helpful only when the eosinophil levels are extremely high (as in tropical eosinophilia or the expatriate syndrome).

Onchocerciasis

Parasitologic techniques are most commonly used to diagnose onchocerciasis. [2] Microfilariae can be visualized directly in the anterior chamber fluid of the eye by slit-lamp examination of patients who have been 'prepared' by remaining for 2 minutes in a head-down position to allow microfilariae to drift forward into the anterior chamber. Skin microfilariae can be visualized after a skin snip has removed the most superficial layers of skin with either a corneoscleral punch or a small needle and disposable razor blade to obtain approximately 1mg of a bloodless piece of skin. This sample is then placed in saline or water for examination for the emergence of microfilariae after 30 minutes to 24 hours. Alternative tests include a polymerase chain reaction on skin-snip specimens or a patch test, in which DEC is incorporated into a cream that is placed on the skin, covered by gauze and the area later observed for development of papular dermatitis resulting from the death of the microfilarial parasites. Also, subcutaneous or deep nodules (generally 1–6cm in size) can be detected by palpation or ultrasound, and the adult worms they contain can be identified histologically in specimens that have been removed surgically.

Loiasis

Diagnosis of loiasis remains dependent on direct parasitologic identification (most frequently microfilariae in the blood) or indirect serologic approaches in association with a compatible clinical presentation and exposure history. [16] *Loa loa* microfilarial periodicity in the blood means that blood sampling must be done near midday (usually between 12.00pm and 2.00pm). Treatment with DEC of some amicrofilaremic patients who have suspected loiasis can induce an inflammatory nodule which, on biopsy, frequently discloses an adult worm surrounded by acute inflammatory cells. Eosinophilia and antifilarial antibodies are important diagnostic tools in expatriates with extensive exposure to infection.[16] Radiographic assessment is of little diagnostic value in these patients, but because hyper-eosinophilia resulting from loiasis has been associated with endomyocardial disease, echocardiography is of value in establishing whether cardiac damage has occurred.

MANAGEMENT

Lymphatic filariasis

Treatment of the infection

Remarkable advances in treating lymphatic filarial infection have recently been achieved, but most of these have focused not on individual patients but on the community. Community reduction of microfilaremia through once-yearly treatment of parasites has been described already. Few clinical trials, however, have focused on optimizing treatment of the individual patient, so there is still insufficient data to permit recommendation for a change from the older treatment regimens (DEC 6mg/kg/day for 12 days in bancroftian filariasis and for 6 days in brugian filariasis).[1] These regimens can be repeated at intervals of 1–6 months if necessary but, interestingly, essentially the entire effectiveness of a 'course' of DEC results from the first dose.[17] Ivermectin, although very effective in decreasing levels of microfilaremia, appears not to kill adult worms and thus it cannot be expected to cure infection completely. Albendazole, on the other hand, appears to kill the adult worms after prolonged courses (2–3 weeks) and to inhibit production of microfilariae after single doses (400mg)[18] but optimization of its usage is just now beginning.

Therefore, for treating infection in individual patients, single or repeated courses of DEC are still recommended. However, because the use of DEC in patients who have either onchocerciasis or loiasis can be unsafe (see below), it is important that individual patients who have bancroftian filariasis who live in areas endemic for these other infections should be examined for co-infection with these parasites before being treated with DEC.

Both diethylcarbamazine and, ivermectin, given separately at the doses necessary to treat microfilaremic patients, have minimal or no-side-effects per se. However, their rapid killing of the microfilariae releases enough antigen to overwhelm the modulating effects of the host's immune system and to induce a variety of side reactions.[19] These occur in proportion to the microfilarial levels before treatment and include headaches, fever, myalgia, lymphadenopathy and occasionally rash, itching and other symptoms. Although the most severely affected patients can also experience postural hypotension, generally these reactions are well managed through the use of antipyretics, antihistamines or, in the most severe instances, corticosteroids. In the tropical eosinophilia syndrome, as there are no microfilariae in

the blood, there is no exacerbation of symptoms, but rather a steady improvement over the 2–4 weeks during which DEC is administered.

Treating the disease

Although it is important to try to cure the infection itself, management of the consequences of that infection (particularly the lymphedema, elephantiasis and genital pathology) is what is often of greatest concern to the patient. For early disease manifestations, it has been shown repeatedly that community treatment of infection with either intermittent (monthly, 6-monthly or yearly) drug administration or the steady use of DEC-fortified table or cooking salt leads to clinical improvement, with decreases in hydrocele size and prevalence and in regression of early lymphedema.

In more chronic states, patients with hydroceles or related urogenital pathology must be subjected to surgical procedures in order to obtain relief.[20]

The most dramatic change in managing patients with lymphatic filariasis has come from the recent recognition that bacterial and fungal superinfections of tissues with compromised lymphatic function play a prominent and progressively exacerbating role in disease development, so that careful attention to these infections can dramatically improve the outcome.[13] Rigorous hygiene in the affected limbs removes much of the excess stress on the lymphatic system and allows it (although still functionally compromised) to handle much more of the extracellular fluid. Management regimens should include the following:

- twice-daily washing of the affected parts with soap and water;
- raising the affected limb at night;
- regular exercise of the affected limb to promote lymph flow;
- keeping the nails clean;
- wearing shoes; and
- the use of antiseptic or antibiotic creams to treat small wounds or abrasions.

The addition of elastic bandages and other adjunctive measures can further improve results.

These same intensive local hygiene efforts and antibiotic ointments can also decrease the frequency of recurrent infection episodes in patients who have elephantiasis of the penis or scrotum but, unfortunately, specific guidelines for management have not yet

been developed for successfully reversing such anatomic distortions caused by the infection.

Noninvasive management of chyluria relies on nutritional support, especially replacement of fat-rich diets with high-protein, high-fluid diets supplemented where possible with medium-chain triglycerides.[13] Surgery, the sclerosing effects of lymphangiography or, often, time alone can also lead to the cessation of the lymphatic leakage into the renal pelvis, collecting system and urine.

Onchocerciasis

Complete cure of *O. volvulus* infections is difficult to achieve because the only drug available that kills the adult worms is intravenous suramin, which is highly toxic, difficult to administer and probably not even indicated.[2]

Rather, for most patients, because the pathology is generally associated with the microfilarial stage of the parasite, treating to kill the microfilariae both rids the patients of existing symptoms and protects them from development of further eye lesions. The safest, most effective microfilaricide is ivermectin at the recommended dosage of 150–200µg/kg; in various settings it has been repeated at 12-, 6- or even 3-monthly intervals. For individual patients, the frequency of treatment can best be determined by the rate at which symptoms (primarily itching and rash) recur. For individual patients, optimal management requires a thorough eye examination before initial treatment to ensure that no microfilariae are present, since it is the inflammatory complications of treatment with microfilaricides that must be avoided, especially in the eye. If there are microfilariae in the eye, the most conservative approach to treatment would include administration of prednisone 1 day before the dose of ivermectin is given and for 2 days after it. Short courses of corticosteroids have little negative effect on the microfilaricidal activity of ivermectin, and they are clearly effective in diminishing the side reactions caused by killing the microfilariae.

The side reactions that follow treatment of onchocerciasis with ivermectin (or, earlier, with DEC) have been termed the Mazzotti reaction. They consist primarily of headache, fever, pruritus, adenopathy, rash and, occasionally, postural hypotension.[21] Although pronounced after DEC, they are much milder after ivermectin and are self-limiting (beginning within hours of treatment and persist-ing as long as 4–5 days); they can be managed satisfactorily with antipyretics, analgesics, antihistamines and, if necessary, systemic corticosteroids.

Because adult worms, which are not killed by either ivermectin or DEC, continue to shed microfilariae for up to 12–15 years, symptoms may recur and require additional treatment over an extended period of time.

Loiasis

The approach to treatment of loiasis depends on the clinical presentation. In patients who do not have microfilaremia, DEC 6–8mg/kg/day for 3 weeks is the optimal treatment and results in cure of approximately half of the patients. Repeated courses of the drug are indicated when patients become symptomatic again and each repeated treatment results in additional patient cures.[22]

For microfilaremic patients, the approach to treatment is more difficult because the side reactions induced by the dying microfilariae can include CNS effects and even death. Such severe reactions rarely, if ever, occur in patients who have blood microfilaria counts of less than 2000/ml of blood (drawn at the time of day for peak parasitemia). However, even in a very controlled, hospital setting, when highly microfilaremic loiasis patients were treated with DEC (initially at very low dosages — 0.25mg/kg — and then increased progressively), there were still some patients in whom there was development of a post-treatment encephalopathy and death. This was not prevented, even when corticosteroids were co-administered. [23] When ivermectin is used instead of DEC, the clearance of microfilaremia from the blood is very much slower and not so complete. While it is very much safer than DEC, both in terms of the systemic side reactions that it elicits (which are similar to those of the Mazzotti reaction) and in terms of avoiding the catastrophic neurologic complications in patients with extremely high levels of microfilaremia (15,000–100,000/ml), ivermectin has still led to instances of CNS deterioration, coma and death. With optimal clinical care, the transient CNS compromise of such ivermectin-treated patients can be managed successfully and catastrophic results minimized. However, the treatment of loa-endemic populations with ivermectin (usually as part of national programs linked to the African Program for Onchocerciasis Control[4] or the Global Program to Eliminate Lymphatic Filariasis[3]) often is rendered in remote areas without access to optimal medical management. Therefore, this potential complication of treatment provides a major challenge that must be overcome before these massive public health initiatives can be successful in the loa-endemic regions of Africa.

If patients experience an adult *L. loa* crossing the eye below the conjunctiva, such worms can be removed through simple surgical incision of the conjunctiva, but because usually there are multiple parasites within the patient, a single procedure may not be curative.[24]

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Chapter 171 - Infections in Sickle Cell Disease

Graham R Serjeant

DEFINITION

Sickle cell disease is a 'generic' term that embraces a group of genotypes characterized by pathology associated with the presence of sickle hemoglobin (HbS). This abnormal hemoglobin (Hb) results from a single amino acid substitution of valine for glutamic acid at position 6 in the ß-chain. Inheritance of this abnormal gene from one parent and a normal gene for HbA from the other results in the harmless carrier state, the sickle cell trait. Sickle cell trait (AS genotype) is excluded from the definition of sickle cell disease because it causes no clinical problems in the great majority of subjects unless they are exposed to hypoxic environments such as high altitude or respiratory depression. The principal genotypes of sickle cell disease include:

- homozygous sickle cell (SS) disease, in which the abnormal HbS gene is inherited from both parents;
- sickle cell HbC (SC) disease, in which the gene for HbS is inherited from one parent and the gene for HbC from the other. HbC is the second most common abnormal Hb among people of West African origin; and
- inheritance of the sickle cell gene with one of the genes for ß-thalassemia.

The ß-thalassemia genes reduce the synthesis of ß-chains; the degree of ß-chains produced determines the amount of HbA. This inhibits sickling, and influences the hematology and clinical course. Several molecular mutations causing ß-thalassemia have been described in association with HbS. In sickle cell $ß^{O}$ -thalassemia there is no HbA and a generally severe course, whereas a variety of sickle cell $ß^{+}$ -thalassemia syndromes result in variable amounts of HbA and variable clinical courses. The most common form of sickle cell $ß^{+}$ -thalassemia among peoples of African origin results in 20–30% of HbA and a very mild clinical course.

DISTRIBUTION AND PREVALENCE

In equatorial Africa, the prevalence of the sickle cell trait commonly reaches 20–30%. Contrary to the common belief that the sickle cell gene is confined to peoples of African ancestry, the gene is widespread in populations around the Mediterranean (Sicily, northern Greece, southern Turkey, the Levant and northern Africa), eastern Saudi Arabia and central India. HbC is a marker of west African ancestry, reaching prevalences of 20% in parts of Ghana and Burkina Faso, falling to 3–5% in Nigeria, and not occurring in central or east Africa, Saudi Arabia, Greece or central India.

In Nigeria, where approximately 25% have the sickle cell trait, it is estimated that SS disease occurs once in every 50 births, or in 100,000 births annually. The prevalence of SS disease at later ages is determined by the mortality of this genotype, which in Africa is often very high with infrequent survival to adult life. In Jamaica, the relative frequencies of the four major genotypes at birth (Table 171.1) are also influenced at later ages by their relative mortality, which is greater in SS disease and $Sß^{O}$ -thalassemia. The prevalence of these four major genotypes among African-Americans and among the UK population of Afro-Caribbean origin are similar to those in Jamaica (because of similar gene frequencies), although the increasing population of direct African origin has markedly increased trait and disease frequencies in the UK. In all populations, the prevalence of sickle cell disease at birth is determined by the gene frequency but at later ages is influenced by the relative survival of affected patients; in hospital-based populations frequency is influenced by the relative severity, which determines presentation.

PATHOGENESIS AND PATHOLOGY

On deoxygenation, HbS molecules form rigid linear structures (polymers), which increase the intracellular viscosity and deform red cells into an abnormal 'sickled' shape. This can be reversed on oxygenation but after several sickle-unsickle cycles, these cells may become permanently deformed. These less deformable red cells have difficulty negotiating the capillary beds where normal red cells with an average diameter of 7µm must bend and fold to traverse a capillary measuring 3µm. As a result, these abnormal red cells become prematurely destroyed (hemolysis) and may also block blood flow (vaso-occlusion).

Accelerated hemolysis results in anemia, jaundice, an increased prevalence of pigment gallstones and marked bone marrow expansion with high metabolic demands. The consequences of vaso-occlusion are determined by the site of the occlusion, but may include strokes, retinal ischemia, acute chest syndrome, a variety of splenic pathologies and chronic leg ulcers. Some manifestations, such as bone marrow necrosis, are influenced by both the hemolytic and vaso-occlusive components giving rise to dactylitis, the painful crisis, and avascular necrosis of long bones and femoral head. In these conditions, the pathology is confined to areas of active bone marrow activity, where the metabolic demands are believed to exceed the supply, which has been diminished either by vaso-occlusion or, more likely, by shunting of blood away from the active marrow.

The pathologies of particular relevance to infections in sickle cell disease are the accelerated bone marrow activity, the prevalence of leg ulcers, acute chest syndrome and — most important of all — the early loss of splenic function. These processes are most marked in SS disease and sickle cell $ß^{O}$ -thalassemia, and less marked in the generally mild conditions of SC disease and sickle cell $ß^{+}$ -thalassemia.

The spleen in sickle cell disease

The spleen acts like a filter in the circulation, removing damaged red cells and bacteria from the bloodstream. It achieves this function by requiring red cells to squeeze between endothelial cells (Fig. 171.1) as the blood traverses from the cordal tissue to the splenic sinuses before returning to the circulation. In addition to the filtering mechanism, the spleen also represents a large mass of reticuloendothelial tissue that is in intimate contact with the circulation and is important in the production of specific antibodies. These are particularly important if the liver is to participate actively in the removal of blood-borne antigens. Splenectomy removes these protective mechanisms and, in patients

TABLE 171-1 -- Relative frequency of principal genotypes of sickle cell disease in Jamaica.

RELATIVE FREQUENCY OF PRINCIPAL GENOTYPES OF SICKLE CELL DISEASE IN JAMAICA	
Genotype	**Frequency at birth**
Homozygous sickle cell (SS) disease	1 in 300
Sickle cell-hemoglobin C (SC) disease	1 in 500
Sickle cell $ß^{+}$ -thalassemia	1 in 3000
Sickle cell $ß^{0}$ -thalassemia	1 in 7000
In these data, sickle cell $ß^{+}$ -thalassemia refers to the common Jamaican form with 20–25% HbA.	

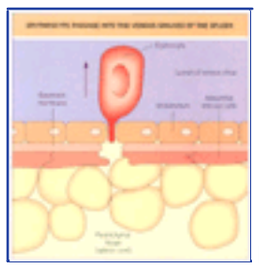

Figure 171-1 Erythrocyte passage into the venous sinuses of the spleen. A red cell passing between endothelial cells (arrow shows direction of movement) from the cordal tissue to the vascular sinus. Sickle cells do not have this deformability, so they accumulate in the spleen. Modified from Weiss.[1]

without sickle cell disease, splenectomy has been calculated to increase the risk of sepsis 50- to 60-fold (see Chapter 109).

In sickle cell disease, the abnormal red cells damage splenic function early in life. Even when the spleen is clinically enlarged, splenic uptake of ^{99m} technetium sulfur colloid is abnormal;[2] and elevated pitted red cell counts, which suggest abnormal splenic function, may occur as early as 6 months of age, and are seen in 20% of SS children by 1 year of age and in 40% by 2 years of age.[3] This loss of splenic function appears to be directly related to the susceptibility to infection [4] and may be reversed by chronic transfusion in young children (Fig. 171.2). [5] It is also delayed in SS patients with high levels of fetal Hb (HbF), which inhibit sickling and allow persistence of splenic function in SS patients in eastern Saudi Arabia.[6]

CLINICAL FEATURES

Bacterial infection Streptococcus pneumoniae

The susceptibility to pneumococcal sepsis in SS disease is well substantiated and was first reported in 1928. The relative risk has been

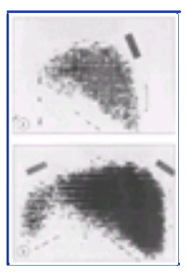

Figure 171-2 ^{99m} Technetium sulfur colloid scans (posterior view) in a 2-year old child with SS disease and splenomegaly. (a) The scan shows hepatic uptake but no splenic uptake. (b) A repeat scan 6 days after a blood transfusion shows restoration of splenic uptake of colloid. From Pearson *et al.*[5]

calculated to be at least 20 times that in the general population, and age-specific incidence rates are highest before the age of 2 years and fall sharply after 5 years. [7] Infection is closely linked to the appearance of clinical splenomegaly before 6 months of age.[8]

Prophylactic penicillin markedly reduces this risk, whether given orally[9] or by depot monthly intramuscular injection, [10] and has now become routine for children with severe genotypes (SS disease, Sß^{O} -thalassemia). Although there is some evidence of an increased risk in SC disease, the risk is not generally considered of sufficient magnitude to justify routine prophylaxis in the milder genotypes (SC disease, Sß$^{+}$ -thalassemia). The maximum risk from pneumococcal sepsis in Jamaica is in the first 3 years of life (Fig. 171.3), and depot injections of penicillin during that period prevent infection. It is unclear when to stop but the current Jamaican protocol provides intramuscular penicillin monthly from 4 months to 4 years with a single dose of 23-valent pneumococcal vaccine at the time of the last penicillin injection. Intramuscular injections are preferred in Jamaica to avoid the problems of compliance, which may compromise the twice-daily oral penicillin generally favored in the USA. Twice-daily erythromycin may be used in children who are allergic to penicillin. The nonconjugated capsular polysaccharide pneumococcal vaccine does not confer adequate protection in young children, but its immunogenicity improves with the age of the patient, and preliminary data suggest that protective levels against many of the serotypes are achieved by the vaccine given at 4 years of age.

Two recent factors may cause these policies to be reassessed: the increasing prevalence of penicillin-resistant pneumococci, which may account for 20–50% of isolations in children with SS disease in the USA; and the development of a conjugate pneumococcal vaccine, which may be effective given at 2, 4 and 6 months, and is currently under assessment. Preliminary data suggest this vaccine may generate what are believed to be protective levels of antibody but potential disadvantages are the high cost and the less comprehensive coverage (7- or 9-valent) compared with the standard 23-valent nonconjugate vaccine.

This susceptibility to the pneumococcus has not been shown in all sickle cell populations, and several studies on the bacteriology of

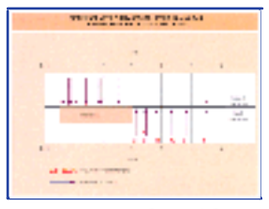

Figure 171-3 Experience with pneumococcal sepsis in Jamaican children from birth to 6 years of age. Pre-trial experience shows that sepsis episodes commenced at 6 months and 9 or 10 episodes occurred before 3 years. During penicillin prophylaxis from 6 months to 3 years, no episodes occurred but seven episcodes occurred after cessation of penicillin. The numbers by the arrows refer to pneumococcal serotypes.

septic children with SS disease in Nigeria[11] and Uganda revealed the most common agents to be *Klebsiella* spp., staphylococci, *Salmonella* spp. and *Escherichia coli*, with a paucity of *S. pneumoniae*. Possible interpretations of this infrequency of pneumococci include the early use of over-the-counter penicillin, the rapid demise of septicemic patients so that they do not reach hospital, the environmental dominance of Gram-negative organisms, or the intriguing postulate that malaria-induced splenomegaly may allow persistence of splenic function.[11] The role of the pneumococcus in SS disease in equatorial Africa must be clarified if governments are to be advised to spend limited resources on pneumococcal prophylaxis programs in sickle cell disease.

Haemophilus influenzae *type b*

It seems likely that children with SS disease are also more prone to *Haemophilus influenzae* type b (Hib) infection, which has been increasing in importance with the advent of effective pneumococcal prophylaxis.[12] Extensive data from the US Cooperative Study of Sickle Cell Disease showed an incidence rate of 0.45 per 100 patient-years under 6 years of age, not significantly different from the incidence in the normal black population;[6] however, a susceptibility of 20–160 times that in the normal population has been proposed from other studies. [13] Although the risks of Hib infection in SS disease are unclear, prophylaxis appears justified and may be effected by conjugated Hib vaccines given between 2 and 6 months of age.[14]

Salmonella

There is a long-standing and well-recognized susceptibility to *Salmonella* osteomyelitis in SS disease, and a less well-recognized association with *Salmonella* sepsis. In a Jamaican study, half of the *Salmonella* isolations from blood occurred without obvious bone involvement and were associated with a 22% mortality because the potential significance of *Salmonella* spp. in a septic patient often went unrecognized.[15] On the other hand, none of the patients with *Salmonella* isolations associated with bone involvement died because the increased awareness of the association led to the early use of specific therapy against *Salmonella* spp.

Salmonella osteomyelitis is believed to be a secondary infection of avascular bone marrow, and its distribution reflects that of the underlying bone marrow necrosis. The complication may become superimposed upon dactylitis in young children and should be suspected if the swelling is marked or there is high fever, in which case a surgical opinion should be sought regarding drainage; infection may be followed by a premature epiphysial fusion and a permanent shortening of affected small bones. At later ages, osteomyelitis may follow bone marrow necrosis in the shafts of the long bones, ribs or sternum, pelvis and vertebrae. Infection of the avascular femoral head may be particularly difficult to diagnose, but this can lead to very rapid dissolution and extensive bone damage. Diagnosis depends on positive blood culture, and the diagnostic yield may be increased by invasive procedures such as bone marrow aspirate or trephine biopsies. The differential diagnosis of sterile avascular necrosis and osteomyelitis is a difficult clinical challenge and even bone scanning techniques have generally been unhelpful, the diagnosis resting on clinical judgment.

Treatment is by specific antibiotics against *Salmonella* spp., such as chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole (co-trimoxazole), or third-generation cephalosporins. Surgical drainage and the removal of sequestra may be necessary for complete healing and, even after apparent recovery, patients are prone to recurrence, sometimes years later, suggesting that the organisms remain dormant or loculated.

The source of *Salmonella* organisms in SS disease remains unknown, but common speculations include microinfarction of the gut wall in patients carrying *Salmonella*, or gallbladder colonization associated with gallstones and an abnormal gallbladder wall. However, a recent study showed no association with gallstones or with indices of vaso-occlusion, and *Salmonella* isolation from stools is only occasionally reported in patients with *Salmonella* osteomyelitis. The high prevalence of *Salmonella enteritidis*, which accounted for one-third of all isolations in the Jamaican series, suggests a dietary source; an intriguing possibility is carriage of *Salmonella* spp. by white cells, which would have the ironic effect of introducing the organism to the site of initially sterile avascular necrosis. More work is needed on the method of acquisition of *Salmonella* spp. in SS disease.

Escherichia coli

The extent of the increased risk of *Escherichia coli* infections in SS disease is unknown, but a significant excess of the SS genotype has been noted among black children admitted to hospital with serious infection and diagnosed as having *E. coli* sepsis. [16] *Escherichia coli* sepsis has also been associated with osteomyelitis and stroke. Urinary tract infections appear more common in SS disease and are a likely origin of *E. coli* sepsis.

Splenectomy and infection

Splenectomy, which may avoid morbidity and potential deaths in acute or chronic red cell sequestration, may be deferred because of concerns over loss of the splenic contribution to immune competence. However, the spleen in such patients is already immune compromised and no increase in severe infections or deaths occurred in 130 splenectomized SS patients when compared with 130 age-and sex-matched SS controls over the same period.[17] If indicated, splenectomy in SS disease should not be deferred for fear of losing persistent splenic immune function.

Malaria

There is a special relationship between malaria and the HbS gene, which was initially noted because malaria endemicity tended to coincide with high frequencies of the sickle cell trait in Africa. This has been the basis of many studies, which have reached general agreement that during a critical period in early childhood (between the loss of passively acquired maternal immunity and the development of active immunity), the presence of the sickle cell trait confers some protection against malaria. The mechanism remains controversial and may be multifactorial, but increased sickling of parasitized red cells has been demonstrated and may serve to identify the host cell to the spleen and brings about its more effective removal. The maintenance of high frequencies of the sickle cell gene in areas of falciparum malaria led to the hypothesis of balanced polymorphism, proposing that a survival advantage in the sickle cell trait was balanced by the disadvantage and presumed loss of two genes in the early deaths occurring in SS disease. For patients with SS disease, malaria is believed to be a major cause of morbidity and mortality; further hemolysis being superimposed upon that already present. Some African workers suggest that such patients do not die of malaria but succumb to symptoms that are related to sickle cell disease but are precipitated by malaria.

Viral infection

Patients with SS disease are not intrinsically more prone to viral infections. Antibody responses to viral vaccines appear normal and infection rates by human parvovirus are similar in SS disease and AA controls. The greater exposure to blood transfusion may render patients more prone to transfusion-acquired viral infections such as hepatitis C virus and HIV.

Human parvovirus infection appears to be the cause of aplastic crisis in SS disease, but this reflects a difference in response rather than an increased susceptibility. Human parvovirus infection colonizes and destroys red cell precursors in the bone marrow, but the virus becomes neutralized by specific antibody, and bone marrow function returns after 7–10 days. In SS disease, the red cell survival may be as short as 7–10 days and, unless oxygen delivery is maintained by transfusion, the aplastic crisis may result in death. Human parvovirus displays the general characteristics of a viral infection — most affected patients are under 15 years of age, epidemics occur at 3- to 4-year intervals, and there is high infectivity between siblings. The risk of aplasia among susceptible siblings of an affected patient is 50% within 3 weeks. A human parvovirus vaccine has been developed but it is still awaiting clinical trial.

Infection with atypical organisms

Mycoplasma pneumoniae and *Chlamydia pneumoniae* have been associated with the acute chest syndrome in patients with SS disease, especially in the autumn. The clinical course of these infections may be severe but it is unknown whether SS patients are more prone to develop these infection. The increased rate of hospital admission among SS patients also exposes them to an increased chance of hospital-acquired infections.

Other mechanisms of infection

Gallstones

The rapid hemolysis and consequent high excretion of bilirubin leads to increased gallstone formation, which occurs in 50% of unselected SS patients by the age of 25 years. Jamaican experience[18] suggests that most are asymptomatic, but acute or chronic cholecystitis may occur. The organism involved is usually *E. coli*, although anaerobes may also occur.

Leg ulceration

Chronic leg ulceration, a feature of SS disease, affects approximately 70% of Jamaican SS patients; they occur most commonly for the first time between 15 and 20 years of age and run a healing-relapsing course. The ulcer surface is commonly colonized by *Staphylococcus aureus, Pseudomonas aeruginosa* and ß-hemolytic streptococci, but these ulcers are rarely associated with evidence of systemic infection. A possible association between ulcer-borne ß-hemolytic streptococci and glomerular disease with proteinuria has been suggested, similar to the association between skin carriage of this organism and acute glomerulonephritis described from Trinidad, but subsequent analysis has indicated that, although both leg ulceration and proteinuria increased with age, there was no relationship between the two after correction for age. Leg ulcers occasionally act as a portal of entry for tetanus.

The lack of evidence for systemic infection in leg ulceration suggests a limited role for antibiotic therapy, although infection with *P. aeruginosa* may occasionally be associated with ulcer deterioration and poor healing.

Acute chest syndrome

The acute chest syndrome is a pneumonia-like pathology with elements of infection, infarction, pulmonary sequestration and fat embolism. It is a major cause of morbidity and the most common single cause of mortality at all ages after 2 years. The contribution of primary infection is controversial, and, although early studies reported pathogens in approximately half the cases in children aged under 3 years, recent studies have found evidence of bacterial infection in only 4–14% of episodes. Furthermore, the poor response to antibiotics and the striking improvement in many cases following transfusion has favored a vascular pathology rather than an infective one. Infections with the atypical agents *M. pneumoniae* and *C. pneumoniae* have been mentioned above.

CONCLUSION

Patients with sickle cell disease are susceptible to some but not all infections. However, any infective illness coinciding with sickle cell disease may precipitate sickle-related complications, such as painful crisis, by inducing fever and possibly dehydration from vomiting and diarrhea.

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Chapter 172 - Leishmaniasis

Robert N Davidson

EPIDEMIOLOGY

Protozoa of the genus *Leishmania* can cause cutaneous (CL), mucocutaneous (MCL) and visceral (VL, kala-azar) leishmaniasis. The distribution of the leishmaniases is shown in Figure 172.1 and Figure 172.2 . About 10 million cases of CL and 400,000 cases of VL occur annually.[1] A country by country review has been published by the World Health Organization (WHO).[2] Phlebotomine sandflies transmit leishmaniasis, either to humans from a wide range of infected animals as a zoonosis, or from human to human. Transmission varies geographically depending upon climate, habitat, season and opportunities for sandfly contact. However, the numbers of all forms of leishmaniasis are increasing in many areas. For example, in Brazil, the increase in VL and CL is due to deforestation, which brings humans into close contact with animal reservoirs and forest vectors of *Leishmania braziliensis* and other species. In north Africa and the Middle East, irrigation projects have resulted in increased numbers of gerbils and construction of new townships in these areas has led to marked increases in endemic CL caused by *Leishmania major*. Breakdown of the infrastructure in Afghanistan has caused outbreaks of urban CL due to *Leishmania tropica*.

Visceral leishmaniasis

This is caused by *Leishmania donovani*, *Leishmania infantum* and *Leishmania chagasi* (see Fig. 172.1); the latter two species are indistinguishable. A reduction of dichlorodiphenyltrichloroethane (DDT) spraying against malaria vectors in India and Bangladesh has been blamed for the present epidemic of VL (due to *L. donovani*), which affects hundreds of thousands annually. From 1984 to 1999 there was a major epidemic of VL (*L. donovani*) in southern and then eastern Sudan brought on by population movement, famine, civil war and ecologic change. [3] [4] In Europe prior to widespread use of effective antiretrovirals, 20–70% of cases of VL (due to *L. infantum*) were co-infected with HIV, and 1.5–9% of AIDS patients had VL.[5] Co-infections of HIV and *Leishmania* are increasingly reported from Africa, India and South America.

Serologic and leishmanin skin test surveys suggest that subclinical self-healing infection occurs more frequently than clinical VL, particularly where *L. infantum* or *L. chagasi* is involved.[6] In epidemics involving *L. donovani*, however, most infections are symptomatic, and the mortality rate is high.[7]

Cutaneous leishmaniasis

In the Old World, *L. tropica* causes anthroponotic CL in villages, towns and cities; *L. major* causes zoonotic CL in those living or working near gerbil burrows. Smaller numbers of CL cases are caused by *L. infantum* in Europe and *Leishmania aethiopica* in Ethiopia and parts of Kenya. In the New World CL is mainly caused by members of the *Leishmania mexicana* complex (*L. mexicana mexicana*, *L. m. amazonensis*, *L. m. venezuelensis*) and the *Leishmania braziliensis* complex (*L. braziliensis braziliensis*, *L. b. panamensis*, *L. b. guyanensis*, *L. b. peruviana*; see Fig. 172.2).[8] [9]

PATHOGENESIS AND PATHOLOGY

Infected macrophages rely mainly on nitric oxide production as an innate mechanism for killing *Leishmania* spp.; this is specifically inhibited by the parasite, which is able to multiply in the parasitophorous vacuole. Eventually infected macrophages rupture and amastigotes are taken up by new phagocytic cells. Macrophages and dendritic cells present *Leishmania* antigens to T cells and this results in either:

- an effective cellular immune response — a T-helper (Th)1 pattern; or
- an ineffective humoral response — a Th2 pattern).

In the Th1 response, T cells activate macrophages by releasing the cytokines interferon (IFN)-? and interleukin (IL)-2. In the Th2 response, T cells release cytokines IL-4, IL-5, IL-10 and transforming growth factor (TGF)-ß which inhibit macrophages from killing *Leishmania* spp. (see Chapter 2). Although each *Leishmania* sp. produces a typical pattern of disease, host cellular immunity will determine whether:

- a clinical or subclinical infection results;
- the disease is visceral, cutaneous or mucocutaneous;
- lesions are few or diffuse; and
- response to treatment is complete or partial.[10]

PREVENTION

Helpful measures for individual protection are wearing long sleeves and trousers, using insect repellents and impregnating mosquito nets and clothing with permethrin.[11]

In the community, known animal reservoirs can be controlled, for example by bulldozing gerbil burrows, destroying infected dogs, or providing the dog population with deltamethrin-impregnated collars. Active case finding and treatment of patients who have VL and post-kala-azar dermal leishmaniasis (PKDL) caused by *L. donovani* and CL caused by *L. tropica* should reduce human-to-human transmission. Early case finding has been helped by the use of serologic tests that are suitable for field use, mainly the direct agglutination (DAT) test and rapid test strips using a recombinant antigen, rK39.[12] [13] Sandflies remain susceptible to residual insecticides and spraying of homes or fogging of streets will reduce the density of peridomestic sandflies.

Two doses of a vaccine, combining killed *Leishmania* promastigotes and live bacillus Calmette-Guérin (BCG), were more than 70% protective against CL in Ecuador;[14] in Iran one dose of a similar vaccine was ineffective[15] and in Sudan a similar vaccine did not prove effective against VL.[16]

CLINICAL FEATURES

Visceral leishmaniasis

In VL, amastigotes disseminate throughout the reticuloendothelial system. After an incubation period of 2–8 months (range 10 days to

Figure 172-1 Global distribution of visceral leishmaniasis. More than 90% of VL cases occur in India/Nepal/Bangladesh, Sudan/Ethiopia and Brazil.

Figure 172-2 Global distribution of cutaneous leishmaniasis. More than 90% of CL cases occur in the regions of Brazil/Peru, Algeria, Saudi Arabia and Syria/Iraq/Iran/Afghanistan.

over 2 years), the patient develops pyrexia, wasting and hepatosplenomegaly, which may become massive (Fig. 172.3). Males and females are equally affected in Sudan but outdoor activities make males more frequently affected in Kenya, Uganda, India and some other areas. Most cases in Europe are seen in children.

The onset can be ill-defined, and months elapse before the patient presents with fever, discomfort from an enlarged spleen, abdominal swelling, weight loss, cough or diarrhea. In some patients, such as those who are infected during an epidemic, the disease has an abrupt onset with high fever and rapid progression resulting in prostration, weakness, dyspnea and acute anemia.

The physical signs (Table 172.1) depend upon the duration of the disease, the nutritional state of the patient and the presence of complications. Patients who present late are thin, with wasted muscles. Hair changes and pedal edema may accompany hypoalbuminemia, but ascites is rare. Hyperpigmentation of the face, hands, feet and abdomen is characteristic of VL in India (kala-azar means 'black sickness'). The

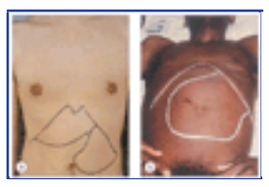

Figure 172-3 Visceral leishmaniasis. (a) Hepatosplenomegaly and pallor in a 29-year old Italian man. (b) Splenomegaly and pallor in a 23-year old Angolan. Both complained of weight loss, fatigue and fever of several weeks' duration.

TABLE 172-1 -- Features of visceral leishmaniasis *(Leishmania donovani)*.

FEATURES OF VISCERAL LEISHMANIASIS *(LEISHMANIA DONOVANI)*	
Clinical feature	**Proportion affected (%)**
Age <9 years	22 (*L. infantum* and *L. chagasi* more commonly affect children and infants)
Age <15 years	44
Fever	83–100
Wasting	70–100
Loss of appetite	62–74
Uncomfortable spleen	81–88
Cough	72–83
Epistaxis	44–55
Diarrhea	25–55
Vomiting	2–37
Splenomegaly	93 (adults), 98 (children)
Hepatomegaly	55–65
Lymphadenopathy	55–86 (uncommon outside Africa)
Jaundice	2–7
Edema	2–7
Laboratory findings	**Proportion (%)**
Globulin >30g/l	98
Albumin <30g/l	88
Anemia	61–92
Leukopenia	84
Thrombocytopenia	73
Elevated bilirubin	17
Elevated liver transaminases	22
Elevated alkaline phosphatase	40
Positive *Leishmania* serology	95
Parasitologically proven	96
The duration of symptoms is 2–4 months but is shorter in children.	

spleen is massively enlarged, often reaching the left or even right iliac fossa. It is smooth and nontender unless there has been a recent infarct. The liver is moderately enlarged in one-third of cases. Lymphadenopathy is common only in African patients, in whom unimpressive, peanut-sized lymph nodes are often palpable in the groins. Jaundice, mucosal and retinal hemorrhage, and episcleritis

are occasional features. After several weeks to months of illness, approximately 90% of patients who have VL will die, often as a result of uncontrolled bleeding, secondary bacterial pneumonia, tuberculosis or dysentery, or other infections such as cancrum oris.

Leishmaniasis in patients who are immunosuppressed or have HIV infection

Visceral leishmaniasis caused by *L. infantum* occurs as an opportunistic infection among patients co-infected with HIV (typically $CD4^+$ lymphocytes <200 cells/mm^3),[6] patients on corticosteroid treatment and patients who have undergone organ transplantation or thymectomy. Travel to an endemic area may have been years previously. The clinical features are often atypical; the symptoms may be vague, the laboratory abnormalities may be less severe and hepatosplenomegaly may be

absent or unimpressive. Amastigotes may be found unexpectedly in bone marrow aspirates or skin biopsies of febrile HIV-positive patients. Amastigotes may be found in unusual cells (e.g. circulating neutrophils). Gastrointestinal symptoms may predominate and amastigotes of *Leishmania* spp. found in rectal or duodenal biopsies. *Leishmania* serology is negative in one-third of immunosuppressed patients. Such patients may respond well to antileishmanial treatment only to relapse 2–12 months later. Alternatively, the response to treatment may be incomplete or the patient may be completely nonresponsive or experience exaggerated drug toxicity. In Ethiopia, *L. donovani* infection in HIV-infected patients is clinically indistinguishable from VL in HIV-negative patients. However, those who have HIV co-infection have around 30% mortality during treatment, rising to about 50% by 6 months, and have higher rates of relapse and PKDL.[17]

Cutaneous leishmaniasis has been reported in patients who have HIV infection in Africa and South America. The lesions often resemble those of diffuse cutaneous leishmaniasis (DCL, see below).

Viscerotropic Leishmania tropica

Nine US soldiers who served in the Persian Gulf area in 1990–1 were found to have systemic infection caused by viscerotropic *L. tropica*.[18] They experienced a non-specific febrile illness with fatigue, arthralgia and diarrhea. Some soldiers recovered spontaneously, whereas others progressed and developed a chronic condition with adenopathy or splenomegaly. Most responded to treatment with sodium stibogluconate.

Post-kala-azar dermal leishmaniasis

After successful treatment for VL due to *L. donovani* (but not *L. chagasi* or *L. infantum*), patients may develop a rash called PKDL.

In Africa, PKDL occurs toward the end of apparently successful treatment or within a few weeks or months later. In Sudan, PKDL occurs in approximately 55% of patients who have VL, including those who have had subclinical VL.[19] In India, PKDL is less common, and occurs months to years after VL. Occasionally, PKDL is acute and severe, resulting in desquamation of skin and mucosae. More commonly, it is characterized by the development of hypopigmented patches, nodules and plaques. Parasites are infrequent or absent from the biopsies. People who have PKDL may act as a reservoir of *L. donovani* between outbreaks.

Cutaneous leishmaniasis

In CL (Fig. 172.4) amastigotes multiply in dermal macrophages near the site of inoculation, typically on the arms, legs, face or ears. The lesions may be:

- nodular or ulcerative; and
- single or with multiple satellite nodules or lymphangitic spread.

The most typical lesion of CL is a chronic ulcer with a diameter of 2–5cm and indurated margins. The ulcer may be covered by a

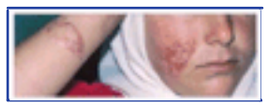

Figure 172-4 Cutaneous leishmaniasis. *Leishmania tropica* recidivans leishmaniasis lesions on the face and forearm of a Syrian girl. These had been present for 4 years with slow healing in the center and multiple recurrences despite courses of intralesional meglumine antimonate.

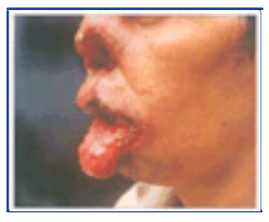

Figure 172-5 Mucocutaneous leishmaniasis. A young man from Peru who had a 2-year history of slow enlargement of the lips and ulceration of the nostrils. *Courtesy of Professor Luis Valda Rodriguez.*

fibrinous crust or there may be an exudate. It is painful if large or secondarily infected.

The histologic picture is of an intense lymphoid and monocytic infiltrate with granulomas. A 'tissue-paper' scar remains after healing. The spontaneous healing rate differs for each species and is typically:

- less than 5 months for *L. major*;
- less than 8 months for *L mexicana*; and
- approximately 1 year for *L. tropica* and *L. braziliensis*.[8]

There are two chronic forms of CL. Difuse cutaneous leishmaniasis is rare but disfiguring. Widespread plaques containing huge numbers of amastigotes persist for decades. People who have DCL are anergic to leishmanin, but do not have visceral dissemination or systemic symptoms. It is caused mainly by *L. aethiopica* in Africa and *L. amazonensis* in South and Central America. Leishmaniasis recidivans is a chronic, nonhealing or relapsing cutaneous infection, seen mainly with *L. tropica* infection in the Middle East. These patients are hypersensitive to leishmanin and organisms are rarely identified (see Fig. 172.4).

Mucocutaneous leishmaniasis

Mucocutaneous leishmaniasis (MCL; Fig. 172.5) occurs in approximately 3–10% of cases of CL due to *L. b. braziliensis*; it is commonest in Peru and Bolivia. The mucosal lesions usually manifest months to years after the cutaneous sores have healed, but cases of simultaneous CL and MCL occur, as do cases that have no history of CL. Usually the tip of the nose, nasal cartilage or upper lip are involved first with a painless induration or ulceration. The condition may remain static or there may be extension over months to years into the nasopharynx, palate, uvula, larynx and upper airways. The nose may be destroyed.

Biopsies show a chronic inflammatory and granulomatous infiltrate with very few amastigotes. Cultures of biopsies are usually positive for *L. braziliensis* but this may require repeated attempts. Less

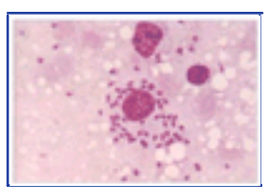

Figure 172-6 Amastigotes (Leishman-Donovan bodies) in bone marrow aspirate from a patient who had *Leishmania infantum* visceral leishmaniasis and AIDS. The nucleus and kinetoplast stain deeply with Giemsa and give the organism its characteristic appearance. *Histoplasma* spp. are the main source of mistaken identification in bone marrow smears, but lack these structures. Amastigotes measure 2–3μm in length and are found within macrophages in tissue sections, but usually lie free in smears because infected macrophages burst as they are smeared.

severe oral or nasal mucosal involvement rarely occurs with other species, such as *L. infantum*, and this often indicates an underlying immune defect.

DIAGNOSIS

Parasitologic diagnosis

Leishmaniasis is suggested by clinical features and supported by serologic or skin tests but should be confirmed by finding or culturing the parasite. *Leishmania* spp. may be isolated from material taken from reticuloendothelial tissue or from biopsies of skin or mucosal lesions. Some of the sample is smeared onto glass slides stained with Giemsa stain, and examined for amastigotes (Fig. 172.6). The rest of the sample is inoculated into suitable media and cultured at 78.8–82.4°F (26–28°C)

and a positive culture will produce microscopically visible motile promastigotes within 2 weeks.

In VL, positive yields from smears of aspirates are of the following order: spleen, more than 95%; bone marrow or liver, 70–85%; lymph node (Africa), 58–65%; and buffy coat of blood up to 70%.[12] Cultures yield about another 10% in good hands. The technique of splenic aspirate is shown in Figure 172.7 .

In CL, DCL, PKDL and MCL, slit skin smears are taken from the raised edge of the CL ulcer or center of the nodule (Fig. 172.8 ; see Chapter 154). Amastigotes are most abundant in fresh CL lesions and are very numerous in DCL. Conversely, they are infrequent in old CL lesions, in MCL and in PKDL.

Immunologic diagnosis

In VL, 95% of cases have positive serology for *Leishmania* with high titers using DAT, the immunofluorescent antibody test (IFAT) or enzyme-linked immunosorbent assay.[12] The leishmanin skin test is invariably negative, indicat-ing antigen-specific anergy and an absence of Th1 cell-mediated immunity.

In CL, *Leishmania* serology may be weakly positive and the leishmanin skin test is usually positive. In MCL and PKDL, both serology and the leishmanin skin test are usually positive. In DCL, there is anergy and both serology and the leishmanin skin test are negative.

Figure 172-7 Splenic aspiration. The picture shows a splenic aspirate being performed under field conditions on a child suffering from *Leishmania donovani* kala-azar in south Sudan. The procedure is simple, painless and safe if the prothrombin time is normal and the platelet count is above 40×10^9 /l. Palpate the spleen and mark its outline. Using a 1.25 inch (30mm) long 21-gauge needle attached to a 5ml syringe, penetrate the skin over the spleen. Withdraw the plunger 1 ml and plunge the needle into the spleen upwards at an angle of 45° and withdraw immediately, maintaining suction. The tiny amount of material obtained is sufficient for culture and smear. *Courtesy of Drs Robert Wilkinson and Jill Seaman.*

Figure 172-8 Slit skin smear. The picture shows a slit skin smear being taken from the edge of a chronic *Leishmania infantum* ulcer obtained in Malta. Smears are taken from the raised edge of the ulcer or center of the nodule, where amastigotes are most abundant. The skin is cleaned and then firmly pinched throughout the procedure to squeeze away blood. A 5mm-long and 3mm-deep incision is made and then the scalpel is turned through 90° and the blade is used to scrape the edge of the slit. A line of tissue scrapings is gently streaked on to a slide and the process is repeated until two or three lines of scrapings are present on at least two slides. Further scrapings and fluid oozing from the pinched slit are put into culture medium.

MANAGEMENT

First-line agents

Pentavalent antimonials

Pentavalent antimonials (Sb^V) have been used for millions of cases since the 1940s.[8] [9] [20] Sodium stibogluconate contains Sb^V 100mg/ml; meglumine antimonate contains 85mg/ml. In the systemic treatment of VL, CL and MCL, a single daily dose of Sb^V of 20mg/kg is used for 28 days. Intravenous injections are less painful than intramuscular injections. Courses of up to 3 months are used for PKDL. Primary resistance to Sb^V is seen in approximately 1% of cases in Africa and

up to 60% in parts of India. Relapse rates should be less than 5%, but secondary Sb^v resistance is likely to develop in patients who relapse unless they are retreated very thoroughly.

Toxicity

This relates to the daily and cumulative dose of Sb^v , as well as to unknown factors; for example, toxicity is almost never seen in patients treated for PKDL, whereas patients who have VL regularly have symptoms suggestive of toxicity and occasional sudden deaths occur in VL patients that might be due to arrhythmias. Children tolerate Sb^v better than adults and may be given higher doses per unit of body surface area. Before starting treatment, ideally a full blood count, biochemistry profile and electrocardiograph should be obtained. Patients should be hospitalized during systemic Sb^v therapy and, where possible, blood tests and electrocardiographs should be repeated twice weekly. Hospital-based Sb^v treatment is usually impossible in endemic countries, where Sb^v is administered by a nurse to outpatients without the facilities for monitoring toxicity. Nonetheless, serious adverse events are rare and deaths due to Sb^v very rare, even in severely debilitated VL patients.[9] [20]

The toxicity is reversible and includes an elevation of serum amylase and liver enzymes, arthralgia and myalgia, thrombocytopenia, leukopenia, anorexia and thrombophlebitis. Patients may complain of lethargy, headache, nausea, vomiting, a metallic taste or pruritus. The most common electrocardiograph changes are ST-segment and T-wave changes; prolongation of the corrected QT interval to more than 0.5s is an indication to temporarily discontinue therapy.[21] Toxicity can usually be managed by stopping Sb^v treatment for 1–2 days. If toxicity recurs the daily dose should be reduced. Acute renal failure, thrombocytopenia, arthritis, tremors and exfoliative dermatitis occur occasionally.

Pancreatic toxicity is a common complication; asymptomatic hyperamylasemia is very common, and symptomatic[22] and even fatal [23] Sb^v -associated pancreatitis have been reported.

Intralesional administration

When used intralesionally, approximately 1ml of undiluted Sb^v is infiltrated into the base and edges of a CL lesion. The injections are repeated every 2–3 days for up to 2–3 weeks. There are no systemic side-effects, but the injections are painful.

Amphotericin B

Amphotericin B deoxycholate is a very powerful antileishmanial and is a first-line drug in India. Amphotericin B is remarkably nontoxic in the regimens used for Indian VL. The optimal regimen is 20 doses of 1mg/kg on alternate days.[24]

Amphotericin B is the drug of choice for advanced MCL, for which Sb^v treatment is often ineffective, and total doses of 30mg/kg are used.

Amphotericin B has not been systematically assessed for CL or PKDL.

Lipid-associated amphotericin B

These compounds are all taken up by macrophages and therefore target amphotericin B to the site of infection, achieving very high levels in liver and spleen. All have lower toxicity than amphotericin B but are more expensive.

Liposomal amphotericin B (AmBisome®) is rapidly effective and nontoxic for VL in Europe[25] and is of value for VL in Sudan[26] and India.[27] The usual regimen is a total dose of 20–30mg/kg, given as at least five daily doses of 3–4mg/kg over a period of 10–21 days. Very short courses of liposomal amphotericin B 1mg/kg daily for 5 days, or 5mg/kg as a single dose, have a high cure rate in India[27] but are unlikely to be effective in Sudan.[26] A few complicated cases of CL have been successfully

treated with long courses of liposomal amphotericin B.

Amphotericin B cholesterol dispersion (Amphocil®) has been used for Brazilian VL at a dosage of 2mg/kg/day for 7 or 10 days.[28] Amphotericin B lipid complex (Abelcet®) has been used successfully for Indian VL in a regimen of 3mg/kg on consecutive or alternate days for five doses.[29]

Second-line drugs

Miltefosine

Miltefosine is the first oral drug with demonstrated efficacy against kala-azar, the dose in adults who have VL being 50mg twice daily for 28 days. [30] It is teratogenic, so cannot be given to pregnant women or women who could become pregnant within 6 months after treatment. Experience thus far is limited to India, where it has been licensed.

Paromomycin (aminosidine)

Paromomycin may be synergistic with Sb^v , a suitable regimen in VL being paromomycin 15–17mg/kg/day plus Sb^v 20mg/kg/day, given together for 17 days.[31] Paromomycin as a single agent is safe and effective at doses of up to 16–20mg/kg/day for 30 days, the optimal regimen being 15mg/kg/day for 21 days.[32]

Pentamidine

Pentamidine is too weak to be routinely used for VL, although short courses — seven doses of 2mg/kg on alternate days or four doses of 3mg/kg on alternate days — are effective for New World CL.[33]

Immunotherapy

Interferon-? added to Sb^v improves the cure rates for relapsed or Sb^v -unresponsive VL and MCL[34] but toxicity and expense exclude it from routine use.

Granulocyte-macrophage colony-stimulating factor combined with Sb^v in the treatment of VL induces a more rapid increase in leukocyte count and fewer secondary infections[35] but cannot be recommended for routine use.

Second-line oral agents

Ketoconazole is effective for CL caused by *L. major* and *L. mexicana*, but less effective against *L. tropica, L. aethiopica* and *L. braziliensis*. Fluconazole[36] or itraconazole have similar efficacy and are better tolerated. Imidazoles cannot reliably cure VL or PKDL and there are no studies of their use in the treatment of MCL.

Allopurinol has been assessed in the treatment of all forms of leishmaniasis but has not shown consistent benefit.

Topical treatment

Topical aminosidine 15% in methylbenzethonium chloride applied twice daily for 10–30 days is effective in the treatment of *L. major* CL.[37] Preparations without methylbenzethonium chloride are of little value.

Monitoring response to treatment

Visceral leishmaniasis

Intercurrent infections such as malaria, tuberculosis and dysentery must be treated, and good hydration and nutritional supplements should be provided.

If responding, the patient will be afebrile within 1 week and clinical and laboratory abnormalities will improve within 2 weeks.

After successful treatment, amastigotes will be absent from aspirates and culture will be negative, and these should be confirmed before treatment is stopped. The patient should be reviewed during 6–12 months after treatment. Slight splenomegaly may persist for several months. Most relapses occur within 6 months. Body weight, spleen size, full blood count, serum albumin concentration and

erythrocyte sedimentation rate are all sensitive markers of recurrent VL. A relapse rate of less than 5% is expected for immunocompetent patients but more than 80% for patients who also have HIV infection. Maintenance with intravenous pentamidine every 2–4 weeks or amphotericin B once or twice weekly may be useful to prevent or delay relapse for patients who have HIV infection but its efficacy is unproven.

A second course of Sb^v may be used successfully to treat a relapse of VL but a different drug such as amphotericin B is probably more effective.

For patients who have HIV co-infection, relapses may be less severe than the first attack and accompanied by vague, minor or atypical clinical features. For such patients, the benefit to be gained from any treatment must be weighed against the adverse effects of prolonged or repeated courses of toxic drugs.

Cutaneous leishmaniasis

Treatment is necessary if the lesions are large, multiple, disfiguring or overlie a joint. Intralesional Sb^v is cheap and usually effective but CL due to *L. braziliensis* should be treated systemically to reduce the risk of subsequent MCL. Most relapses of CL will occur within 12 months.

Mucocutaneous leishmaniasis

Untreated MCL will slowly progress to produce extensive mutilating lesions. Early lesions respond better to treatment[38] but the response is slow and relapses are common. Corticosteroids should be added if the larynx or airways are involved, to prevent edema complicating the start of treatment. Relapse may occur up to several years after treatment, so prolonged clinical follow-up is necessary.

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Chapter 173 - Chagas' Disease (American Trypanosomiasis)

Michael A Miles

EPIDEMIOLOGY

Chagas' disease was first described by the Brazilian scientist Carlos Chagas in 1907.[1] The causative agent, *Trypanosoma cruzi*, is a kinetoplastid protozoan parasite. It is transmitted to mammals by blood-sucking triatomine bugs (order Hemiptera, family Reduviidae, subfamily Triatominae) not by their bite but by contamination of the host with *T. cruzi*-infected bug feces. Secondary routes of transmission include:

- blood transfusion;
- organ transplant;
- transplacental transmission; and
- orally by consumption of food contaminated with triatomine bug feces or of uncooked meat from infected mammals.

Infective forms (metacyclic trypomastigotes) of *T. cruzi* gain entry to the mammalian host from triatomine feces by penetrating mucous membranes or abraded skin. Trypomastigotes can then enter nonphagocytic or phagocytic cells, in which they transform to amastigotes and divide by binary fission to produce a pseudocyst. Before rupture of the pseudocyst amastigotes transform to trypomastigotes, which upon release re-enter cells or circulate in the blood (Fig. 173.1) from where they are picked up when the host is again attacked by bugs.

Multiplication within the insect vector occurs by binary fission as the epimastigote stage in the intestinal tract. Triatomines acquire *T. cruzi* infection only by feeding on an infected host and not by transovarial transmission. They are highly susceptible to infection and once infected usually remain infective for life.

The trypomastigote, amastigote and epimastigote life cycle stages are distinguished by the position of a discrete organelle, the kinetoplast, in relation to the nucleus and by the presence or absence of a free flagellum (see Diagnosis, below). Organisms at all stages of the life cycle can be grown in culture.[2]

The majority of triatomine bug species[3] are found in the New World and *T. cruzi* is restricted to the Americas. A few bug species cause widespread and abundant household infestation, principally:

- *Triatoma infestans* (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay);
- *Rhodnius prolixus* and *Triatoma dimidiata* (northern South America and Central America);
- *Panstrongylus megistus* (central and eastern Brazil); and
- *Triatoma brasiliensis* (north eastern Brazil).

The natural habitats of triatomines are trees, burrows and rocks, where they feed on mammals, birds and reptiles. All mammals, but not birds or reptiles, are considered to be susceptible to *T. cruzi* infection and the organism has been reported in more than 150 mammal species of 24 families. Domestic dogs, guinea pigs, cats, rats and mice living in houses may therefore be important domestic reservoir hosts. In addition, chickens, although not infected, are a significant factor because they can sustain large bug infestations. The most common sylvatic reservoir host of *T. cruzi* is the opossum, *Didelphis*. Sylvatic cycles of *T. cruzi* transmission are found from southern Argentina and Chile (latitude 46° south) to northern California (latitude 42° north), although human infection is rare in the USA and sporadic in the Amazon basin because local vectors have not adapted to colonize houses.[4]

Serologic surveys indicate that more than 10 million people carry *T. cruzi* in the Americas, with prevalence rates of more than 70% in some communities. In some endemic localities seropositivity rates among blood donors may reach 20%. Transmission cycles have been described as:

- enzootic, with rare human infections but sympatric sylvatic transmission (as in USA; Amazon basin);
- discontinuous, with separate domestic and sylvatic transmission cycles involving different triatomine vector species (as in Southern Cone countries, see below); and
- continuous, with overlapping domestic and sylvatic transmission cycles involving the same triatomine vector species (as in parts of Venezuela).[5]

The initial, acute phase of *T. cruzi* infection may be asymptomatic (see Clinical features, below) and is not commonly reported. Although 10% of acute phase infections in children may be fatal, the epidemiologic importance of Chagas' disease also arises from chronic infection. Once *T. cruzi* infection has been acquired, in the absence of treatment it is usually retained for life.

Trypanosoma cruzi frequently invades heart muscle and smooth muscle of the alimentary tract. Up to 30% of chronic infections have been reported to lead to chagasic cardiomyopathy, electrocardiograph (ECG) abnormalities and sudden death or progressive heart disease. Associated chronic phase syndromes are mega-esophagus and megacolon (see below).

Biochemical studies and genotyping have demonstrated that *T. cruzi* is genetically diverse. Two principal subspecific groups have been identified and named *T. cruzi* I and *T. cruzi* II, the latter with five subgroups (a–e). *Trypanosoma cruzi* I predominates in the Amazon basin and in endemic countries north of the Amazon; *T. cruzi* II is the predominant cause of Chagas' disease throughout the Southern Cone countries of South America.[6] The disparate distribution of *T. cruzi* strain groups has been circumstantially linked to regional differences in the severity of chronic Chagas' disease.[6] *Trypanosoma cruzi* I hybrids have been generated in the laboratory from clonal parental genotypes.[7] Phylogenetic evidence indicates that genetic exchange may have contributed significantly to the evolution of *T. cruzi*.[7] [8] Genetic exchange may facilitate the spread of virulent and drug-resistant strains, or the extension of host range.[7]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of Chagas' disease is still somewhat enigmatic. Many infected individuals who survive the acute phase show no progression to chronic disease. Pathogenesis has been described as involving an inflammatory response, focal neurologic damage and, in some cases, resurgence of a chronic inflammatory response that may be triggered by autoimmunity. An immunocompromised state may lead to reactivation of infection, producing symptoms typical of the acute phase.

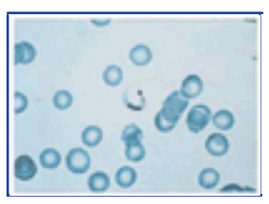

Figure 173-1 *Trypanosoma cruzi* C-shaped trypomastigote in Giemsa-stained thin blood film.

TABLE 173-1 -- Phases of Chagas' disease.

PHASES OF CHAGAS' DISEASE		
Acute phase		
Inflammatory response to ruptured pseudocysts		
Mononuclear infiltrate (macrophages, lymphocytes)		
Trypanosoma cruzi antigen, immunoglobin and complement in situ		
Spreading lymphocytic infiltration, some destruction of nonparasitized tissue		
Focal destruction of conducting tissue (cardiac failure)		
Inflammatory and degenerative changes subside		
Chronic phase		
Indeterminate	'Asymptomatic' chronic infection	
	Refined techniques may detect some abnormalities (electrocardiograms, septal endomyocardial biopsy)	
Chronic disease	Neurogenic	Minimal active inflammatory lesions
		Focal fibrosis
		Pronounced neuron loss (heart, esophagus, colon)
		Apical aneurysm
		No progressive congestive heart failure
		Sudden death
	Myogenic	Moderate to intense diffuse active progressive myocarditis (macrophages, lymphoid cells, fibrosis) in the absence of parasites
		Progressive congestive heart failure

Local multiplication at the portal of entry of *T. cruzi* may lead to a skin lesion or conjunctivitis (see Clinical features, below) with a local inflammatory response. Pseudocyst rupture in the heart or other organs generates an inflammatory response with infiltration of lymphocytes, monocytes and/or polymorphonuclear cells (Table 173.1). In those surviving acute phase infection, intracellular multiplication and parasitemia in the blood subside, although trypomastigotes may still be detectable by sensitive methods (see Diagnosis, below).

Focal lesions in the conducting system of the heart are associated, both clinically and experimentally, with corresponding ECG abnormalities. The

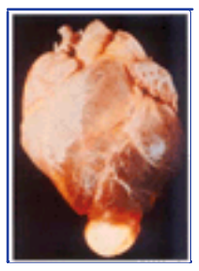

Figure 173-2 Apical aneurysm of the left ventricle in chronic Chagas' disease. *Courtesy of Dr JS Oliveira.*

Figure 173-3 Mega-esophagus on radiograph. *Courtesy of Dr JS Oliveira.*

pathogenesis of this 'neurogenic' form of chronic Chagas' disease is thought to depend upon irreversible neuron loss in the acute phase, exacerbated by further loss with age, such that a threshold is reached beyond which organ function is perturbed. Electrocardiogram abnormalities or aperistalsis of the alimentary tract then ensue, with organ enlargement, or mega syndromes. Gross pathology of the heart consists of megacardia and focal thinning of the myocardium, especially at the apex of the left ventricle, which may lead to apical aneurysm formation, which is considered to be pathognomonic of chronic chagasic cardiomyopathy (Fig. 173.2). Apical aneurysm can be produced experimentally without *T. cruzi* infection by inoculating catecholamines, suggesting that it may be associated with sympathetic dominance. Chagasic mega-esophagus (Fig. 173.3) is more common than chagasic megacolon (Fig. 173.4) and either or both may be associated with chagasic cardiomyopathy. [9]

Figure 173-4 Megacolon. *Courtesy of Dr JS Oliveira.*

Some patients who have chronic 'myogenic' Chagas' disease present with a renewed inflammatory response and a progressive diffuse myocarditis associated with a slow decline in cardiac function. It has been proposed that this pathology can be explained by an autoimmune pathogenesis. It is known that antigens released from ruptured pseudocysts may spread from the immediate site of infection, and be adsorbed to uninfected cells. This may lead to an extension of focal damage and the release of normally sequestered host antigens, which could precipitate autoimmunity. Candidate cross-reactive epitopes between *T. cruzi* and mammalian tissues have also been described, including the C-terminus of the *T. cruzi* ribosomal P protein, and myosin epitopes. It is not clear whether autoantibodies are markers of pathology or have a causal role.[10]

A tentative overall explanation of the pathogenesis of Chagas' disease is that:

- direct and indirect focal neuronal damage in the acute phase may with time culminate in ECG abnormalities and sudden death or mega syndromes; and
- in a proportion of patients there is autoimmune reactivation of the inflammatory response and progressive myocarditis.

The Pan American Health Organization has produced a review of Chagas' disease and the nervous system.[11]

PREVENTION

There is no vaccine for Chagas' disease. Crude or fractionated antigens can protect experimental animals against a normally fatal challenge infection. Prospects for vaccine development are remote because of the alleged involvement of autoimmunity in the pathogenesis of Chagas' disease and the impracticality of vaccine trials.

Immunotherapy has been proposed but not devised, and is likely to be of limited use and not cost-effective.

Chagas' disease is maintained by poverty and poor housing, which prevent families and communities from controlling domestic triatomine populations. Prevention of new cases of vector-borne *T. cruzi* infection relies on insecticide spraying, health education and improved housing. Screening or treatment of donor blood, and of organ donors and recipients are also essential measures.

Pyrethroids, which have low toxicity and high residual activity, are the insecticides of choice for killing triatomines. Control campaigns are organized and run in three phases — preparatory, attack and vigilance:

- during the preparatory phase the distribution of dwellings is mapped, the number of infested houses assessed and the second and third phases are costed and planned;
- in the attack phase all houses and peridomestic buildings are sprayed, irrespective of the known presence of bug infestation; and
- in the vigilance phase a community surveillance system reports residual or new triatomine bug infestations, eliciting a rapid respraying response for those houses affected.[12]

Blood for transfusion and organ donors or recipients can be screened by one of several serologic methods. In highly endemic areas, if serology cannot be performed, blood may be treated with crystal violet (at 250mg/l) and stored at 39.2°F (4°C) for a minimum of 24 hours, which will kill *T. cruzi* trypomastigotes.

Serology is also crucial for monitoring the success of control programs. Children born after control campaigns are initiated should be serologically negative, except for some of those under 9 months of age who will retain transplacentally transferred IgG from seropositive mothers (see Diagnosis, below). Seropositivity in the relevant age group will pinpoint residual triatomine bug infestation and vector-borne transmission or sporadic cases of congenital transmission of infection from mother to child (see Diagnosis and Clinical features, below). To monitor control campaigns serology can be used economically to screen:

- entire populations of countries or endemic regions; and
- selected populations in areas of high seroprevalence or on the edges of endemic areas where new epidemic outbreaks might occur.

These well-established control principles have lead to a Southern Cone initiative to eliminate *Triatoma infestans* from the southern countries of South America (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru and Uruguay).[13] The cost of prevention in this way is a small proportion of the economic burden of the diagnosis, management and treatment of acute and chronic Chagas' disease. The Southern Cone program has stimulated ministries of health to invest in triatomine control and has led to a dramatic reduction in domestic infestation over wide areas. The success of the program is assisted by the fact that *T. infestans* is thought to be restricted to domestic habitats throughout its range, except in Bolivia where it is also found in feral guinea pig colonies. Surveillance programs are being planned to protect the Amazon basin from immigration by domestic triatomine species and to report adaptation of local sylvatic bugs to colonization of houses.[4] [14]

The prospects for vector control in northern South America and Central America are less straightforward, as in some regions domestic bugs may be continuously replenished from nearby sylvatic foci. Morphologic similarities between *Rhodnius* spp.[15] may, however, have led to an overestimation of the degree of movement between sylvatic and domestic bug populations. Andean and Central American initiatives to control Chagas' disease have been launched in an effort to mimic the success of the Southern Cone program.[16]

CLINICAL FEATURES

Acute phase *T. cruzi* infections are most common in children. If bug feces contaminate the eye, metacyclic trypomastigotes may penetrate the conjunctiva, leading to unilateral conjunctivitis and periophthalmic edema known as Romaña's sign (Fig. 173.5). If the portal of entry is the skin, a cutaneous lesion (chagoma) may result. Occasionally multiple chagomas may be seen in acute phase infections in infants. With both sites there may be regional lymphadenopathy and local infiltration of lymphocytes and monocytes. Further clinical signs during the acute phase may include fever, hepatosplenomegaly, generalized lymphadenopathy, facial or generalized edema, rash, vomiting, diarrhea and anorexia. There may be early ECG abnormalities, including sinus tachycardia, increased PR interval, T-wave changes and low QRS voltage. The incubation period between exposure to infection and the appearance of symptoms may be as short as 2 weeks but can be as long as several months if infection results from blood transfusion. Shorter incubation times in vector-borne infections may be due to the adaptation of metacyclic organisms to rapid invasion of cells, whereas trypomastigotes in contaminated transfusion blood may less efficiently invade the heart or other organs.

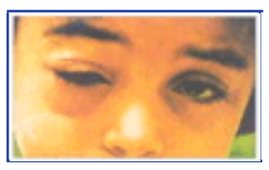

Figure 173-5 Romaña's sign.

General lymphadenopathy and splenomegaly are common in patients who have acquired infection by blood transfusion.[17]

Signs of congenital infection may include fever, edema, metastatic chagomas and neurologic signs such as convulsions, tremors and weak reflexes, and apnea. Hepatosplenomegaly is also common in congenital infections. The ECG picture in congenital cases is usually normal but there may be low-voltage complexes, decreased T-wave height and increased atrioventricular (AV) conduction time.[17]

Meningoencephalitis is infrequent in adults but more common in infants and carries a poor prognosis. Meningoencephalitis is also common in those immunocompromised by AIDS, as the organism frequently traverses the blood-brain barrier in these patients.[18]

Individuals who recover from the acute phase may lead entirely normal lives without any further signs. Indeed, one of the first cases described by Carlos Chagas, a young girl called Berenice, lived into her eighth decade with no associated illness, even though *T. cruzi* was isolated from her on several occasions and late into life. After a symptom-free indeterminate phase of unpredictable duration, ECG abnormalities typical of chronic Chagas' disease arise in up to 30% of patients who recover from the acute phase. Cardiac signs include dysrhythmias, palpitations, chest pain, edema, dizziness, syncope and dyspnea. The most typical reported ECG changes are right bundle branch block and left anterior hemiblock, but there may also be AV conduction abnormalities, including complete AV block. Many different types of dysrhythmia may occur, including sinus bradycardia, sinoatrial block, ventricular tachycardia, primary T-wave changes and abnormal Q waves. The severity of heart disease is graded according to the extent of the disturbance. Radiography of the thorax is a useful aid for detecting cardiac enlargement (megacardia). [17]

Signs of mega-esophagus include loss of peristalsis, regurgitation and dysphagia. Megacolon may be associated with failure of defecation and severe constipation. In both cases there may be progressive dilatation of the organs, which is clinically graded to describe severity.[9] [19]

Differential diagnosis includes distinction from all other types of heart disease and ECG abnormalities, but changes such as right bundle branch block and left anterior hemiblock associated with a history of exposure to *T. cruzi* infection (see Diagnosis, below) are indicative.[20] Megacolon due to Hirschsprung's disease is usually recognizable, in part because of its rarity in adults.[9]

DIAGNOSIS

During the acute phase of infection circulating trypomastigotes may be detectable by direct microscopy of unstained wet-blood preparations. Several methods can be attempted to improve the sensitivity of parasitologic diagnosis. These include microscopy of:

- Giemsa-stained thick blood films;
- the buffy coat layer after centrifugation of hematocrit capillaries (with care to avoid exposure to infection);
- centrifugation sediment from recently separated serum (Strout's method); and
- centrifugation sediment after lysis of red blood cells with 0.87% ammonium chloride.

All these methods may fail, even in the acute phase of infection, if the parasitemia is low. A more sensitive method of parasitologic diagnosis is the process known as xenodiagnosis, in which triatomine bugs from laboratory colonies maintained on birds are fed on the suspect patient. The fed bugs are then dissected about 20–25

days later and the hind gut contents are examined for the presence of *T. cruzi* epimastigotes. The hind gut and rectum are drawn out into a drop of sterile physiologic saline, mixed with a blunt instrument such as a microspatula, and examined for the typical motile epimastigotes and trypomastigotes. Care must be taken to avoid infection during this procedure and bugs are usually dissected behind a small Perspex screen or, if available, in a microbiologic safety cabinet. It is also necessary to ensure that colony bugs are not infected with the monoxenous flagellate *Blastocrithidia triatomae*, which may be found in *T. infestans*.

Culture of venous blood on to a blood agar base medium may be used as an alternative to xenodiagnosis[21] but demands better laboratory facilities, is difficult to perform under field conditions and seldom achieves the sensitivity of xenodiagnosis.

In the chronic phase of Chagas' disease xenodiagnosis is still the parasitologic method of choice throughout most of Latin America.

Detection of DNA by the polymerase chain reaction (PCR) may be an adjunct to parasitologic diagnosis for research purposes but is not a routine clinical procedure.[22] In areas where *Rhodnius prolixus* is a vector of *T. cruzi*, xenodiagnosis may yield the nonpathogenic human trypanosome *Trypanosoma rangeli*. Frequently, *T. rangeli* infections in *Rhodnius* spp. can be identified by the presence of long, slender (up to 80µm) epimastigotes, by the smaller kinetoplast and the capacity to invade the bug salivary glands (*T. rangeli* is transmitted by inoculation not by contamination).

Serum antibody is usually detectable within a few days of *T. cruzi* infection and usually persists for life unless the infection is elim-inated by chemotherapy. Rarely, serologic reversion may occur without treatment. There is an initial IgM response and a sustained IgG response, which is detectable by a variety of assays. Commonly used tests are the indirect fluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) and the indirect hemagglutination test. Washed organisms (IFAT) or lysates of epimastigotes grown in vitro (ELISA) are used as antigens. Around 50% of seropositive cases may yield a positive parasitologic result by careful xenodiagnosis.

No universal specific, highly sensitive, recombinant antigen is yet available.[23] [24] Diagnostic assays must be standardized adequately to determine cut-off titers, with positive and negative control sera, and it is advisable to establish reference laboratories for checking assay reproducibility. Cross-reactions may occur, especially with visceral and cutaneous leishmaniasis, which may be sympatric (co-exist in the same geographic area) with Chagas' disease. Infants born of seropositive mothers may be seropositive until up to 9 months of age due to transplacental transfer of IgG. Seropositivity in such infants using an IgM-specific conjugate suggests congenital infection.

Short-term visitors to endemic areas are extremely unlikely to acquire Chagas' disease and, if infection is suspected through exposure to triatomine bug bites or blood transfusion, serologic status may be used to exclude more likely causes of heart disease (Fig. 173.6).

Strain groups of *T. cruzi* may be identified using enzyme electrophoresis or DNA amplification of kinetoplast minicircle DNA, or ribosomal and mini-exon gene targets, but the identification of infecting strain is not yet a proven prognostic indicator. Antibody recognition of the *T. cruzi* II specific epitope of a mucin-like protein is reported to be associated with confirmed Chagas' disease in patients from Argentina, Brazil and Chile.[25] This is not surprising, because *T. cruzi* II predominates in human populations in this region, whereas *T. cruzi* I predominates in endemic countries north of the Amazon

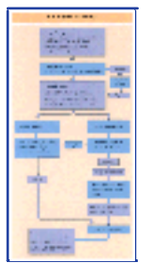

Figure 173-6 Diagnosis of Chagas' disease. ELISA, enzyme-linked immunosorbent assay; IFAT, indirect fluorescent antibody test; IHAT, indirect hemagglutination test.

basin. Chagasic myocardiopathy occurs in *T. cruzi* I endemic regions of northern South America and Central America, even though chagasic megacolon and mega-esophagus appear to be rare or absent.

MANAGEMENT

The oral synthetic nitrofuran nifurtimox has been used for treatment of Chagas' disease but is no longer readily available. The sole drug to treat *T. cruzi* infection is now benznidazole, which is an orally delivered nitroimidazole. The drug is given at 5–7mg/kg/day orally for

Figure 173-7 Modified Duhamel-Haddad procedure for surgical correction of megacolon.[9] [19] [27]

adults, in two divided doses, for 60 days using 100mg tablets. Children tolerate higher doses of 10mg/kg/day given in two divided doses. Side-effects include rashes, fever, nausea, peripheral polyneuritis and leukopenia, and rarely agranulocytosis. Adverse effects may lead to interruption of treatment.

Chemotherapy is recommended during the acute phase of infection as it suppresses the parasitemia and may be life-saving, and for chronic cases in children, who are less susceptible to side-effects.[26] Elimination of infection is not guaranteed. Chemotherapy for chronic Chagas' disease in adults is more controversial as the pathogenesis might be largely attributable to acute phase damage. Adult chronic cases are thus not always treated because:

- the contribution of continued low-level infection to pathogenesis is uncertain [10] ;
- side-effects may cause interruption of treatment;
- treatment often fails to eliminate the organism; and
- cure is difficult to prove (negative parasitology is not sufficiently sensitive to prove absence of infection and reversion of serology may take decades).

Immunocompromised patients must be treated, and double or even higher dose rates, if tolerated, may be recommended to treat meningoencephalitis. Similarly, congenital cases also demand treatment (10mg/kg/day for up to 60 days).

Supportive chemotherapy is often required (e.g. for fever, vomiting, diarrhea and convulsions).[17] Sodium intake is restricted if there is acute-phase heart failure and diuretics and digitalis may be indicated. Management of acute meningoencephalitis may require anticonvulsants, sedatives and intravenous mannitol. Vasodilatation (angiotensin-converting enzyme inhibitors) and maintenance of normal serum potassium may be required initially for chronic chagasic heart disease; digitalis is advisable only as a last resort because it may aggravate dysrhythmias.

Bradycardia that does not respond to atropine, atrial fibrillation with a slow ventricular response, or complete AV block may necessitate a pacemaker.

Amiodarone has been suggested as the most useful drug to treat dysrhythmias, but may produce cutaneous side-effects, such as photosensitivity. Lidocaine (lignocaine), mexiletine, propafenone, flecainide and ß-adrenoreceptor antagonists are effective treatment for ventricular extrasystoles.

Management of chronic chagasic heart disease may therefore be a balancing act between patient management, drug administration and use of a pacemaker. In emergencies lidocaine may be used intravenously. Surgical resection of dysrhythmic endocardial regions and of ventricular aneurysms has been suggested. Detailed expert reports, such as that by the World Health Organization (WHO),[17] and physicians experienced in the management of chagasic cardiomyopathy should be consulted directly to assist in prolonging patient life expectancy and optimizing prognosis.

Surgical treatments have been developed in Brazil for megaesophagus and megacolon. The modified Duhamel-Haddad operation is recommended for surgical correction of megacolon.[9] [19] [27] This procedure involves resection of the sigmoid loop, closure of the rectal stump and bringing the descending colon through the rear wall of the rectum as an initial perineal colostomy (Fig. 173.7). The stump of the colon is subsequently sectioned into anterior and posterior halves, with peridural anesthesia, and the anterior wall of the colon and posterior wall of the rectum sutured in an inverted V to widen the junction. Sigmoidostomy as a separate operation and recovery before Duhamel-Haddad surgery may allow more of the colon to be retained.

Mega-esophagus may improve with dietary control, or respond to dilatation of the cardiac sphincter using probes, air or hydrostatic pressure. The Heller-Vasconcelos surgical procedure for alleviating megaesophagus involves selective removal of a portion of muscle at the junction between the esophagus and stomach. [27] More severe mega-esophagus may demand replacement of the distal esophagus with another part of the alimentary tract such as the jejunum.

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Chapter 174 - Migrating Worms

Stephen H Gillespie

INTRODUCTION

Somatic migration of larvae is a normal part of the life cycle of many nematode pathogens, for example *Ascaris lumbricoides*. However, some nematodes are able to invade the human host but are unable to complete their development into adults. In this circumstance the somatic migration stage is prolonged and gives rise to the condition of visceral larva migrans. The most common cause of this syndrome is the canine ascarid *Toxocara canis*, although more rarely *Gnathostoma* larvae and *Angiostrongylus* spp. and the dog hookworms are implicated.

TOXOCARA CANIS

LIFE CYCLE

Toxocara canis is a pathogen of canids, including the domestic dog and feral fox. Ingested eggs hatch in the intestine and the larvae invade the wall and migrate through the liver and lungs. Passing through four larval moults, mature larvae are coughed up and adults develop in the small intestine. This classic ascarid migration occurs mainly in dogs less than 6 months old. In older dogs larval maturation is halted at the second larval stage (L_2), and the larvae migrate and persist in the tissues. Larvae reactivate in pregnant bitches during week 6 of gestation, cross the placenta and are excreted in milk to infect puppies. This mechanism of transmission is very efficient, so that almost all dogs are infected. In dogs, adult worms continue to lay eggs until they are expelled, usually when the dog is about 6 months old. The fertilized eggs must mature in the soil for 2–4 weeks before they are infectious (Fig 174.1 and Fig 174.2). In humans, *T. canis* eggs hatch and larvae invade in the same way but are unable to develop beyond the L_2 stage and continue to migrate through the body for a prolonged period.[1] Humans, along with many other animals, are paratenic hosts. Infection can follow ingestion of fertilized embryonated eggs or uncooked tissue of another paratenic host.

EPIDEMIOLOGY

Serologic studies among adults show that between 2% and 8% of the population has evidence of previous infection. Seroprevalence in children is higher, up to 35% in warm, moist tropical areas with poor sanitary conditions. Symptomatic disease typically occurs in children, with a male predominance. The peak incidence of visceral larva migrans occurs between 3 and 7 years of age and the peak incidence of ocular disease occurs between 7 and 10 years of age. Infection is commonly associated with dog ownership and pica. Several occupational groups that have an excess contact with dogs (e.g. kennel workers) have increased risk of infection, but symptomatic disease is rare in adults. Fecal-oral transmission of infection by ingestion of fertilized mature eggs is the main route of acquisition of *T. canis*. Public parks are thought to be an important source of infection. There have been a few reports of outbreaks of *T. canis* infection associated with the ingestion of raw meat containing L_2 larvae. Examples include undercooked chicken and a Lebanese dish that includes raw sheep liver.[2]

PATHOGENESIS

The L_2 larvae of *T. canis* excrete a complex mixture of glycoproteins, the excretory antigens (*Toxocara* excretory secretory antigens, TES or TEX) from the larval surface. These antigens activate complement, stimulate cytokine production and have potent elastase, acetylcholinesterase and superoxide dismutase activities.[3] As larvae migrate through the tissue there is an intense inflammatory response to TES. The symptoms and signs of toxocariasis are a consequence of this immune response. When the larval load is high there is an intense systemic response, leading to the syndrome of visceral larva migrans (see below). If a larva is trapped in the retina, the inflammatory response is localized, leading to ocular complications such as endophthalmitis or uveitis. Healing of the lesion may be followed by fibrosis with the potential for retinal traction and detachment.[4]

CLINICAL FEATURES

Visceral larva migrans

This syndrome was clearly described in Paul Beaver's initial report as consisting of prolonged fever, cough, wheeze, hepatosplenomegaly and eosinophilia. [5] Since then, more subtle clinical symptoms and signs have been associated with infection, including failure to thrive, urticaria and abdominal pain, and clinicians may include toxocariasis in the differential diagnosis of children with asthmatic symptoms, chronic non-specific abdominal pain, failure to thrive and anemia in addition to the classic visceral larva migrans syndrome (Table 174.1 and Table 174.2). [6] [7] [8] [9] Rarely, heavy infections are associated with myocarditis and a fatal outcome. Associations between toxocariasis and asthma and between toxocariasis and seizures have been made, but a clear etiologic role for *T. canis* has not been identified.

Ocular infection

The pattern and severity of ocular disease depend on the age of the child, the location of the lesion and the immune response. In younger children early lesions may go unrecognized and untreated, so that they present only in routine medical examinations, by which time treatment may not be effective. Ocular toxocariasis may present with endophthalmitis or uveitis. Vision may be reduced by the presence of inflammatory cells in the ocular medium or damage may be caused by a retinal granuloma. Macular lesions can cause a complete loss of vision. Ocular toxocariasis is usually unilateral, but bilateral disease has been reported in up to 3% of series.[4]

Diagnosis

As *T. canis* does not complete its life cycle in humans, adult worms do not develop and eggs cannot be found in the stool. The diagnosis

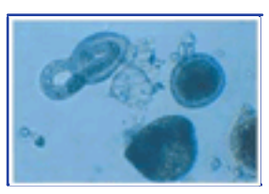

Figure 174-1 Fully embryonated egg of *Toxocara canis* hatching. To the right are two unfertilized eggs.

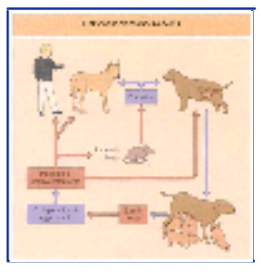

Figure 174-2 Life cycle of *Toxocara canis.* This demonstrates the importance of transplacental transmission in maintaining canine infection, and the role of young dogs in transmitting infection to humans.

of toxocariasis depends on serologic methods and hematologic parameters. An antibody capture enzyme immunoassay, based on TES antigens, is used worldwide. This test has proved sensitive and specific, with few cross-reactions with other helminth parasites. [10] Alternative diagnostic approaches have included IgE-specific antibody testing and detection of TES antigens, but none of these techniques has proved sufficiently robust for routine clinical diagnosis.[11] There is evidence that IgE detection, although less sensitive, does make the diagnosis of cases of eosinophilia where the serum IgG test is negative. Also serum IgE becomes negative after treatment and has been proposed as a method of following patients who have been given anthelmintic agents.[12]

TREATMENT

The natural history of visceral larva migrans tends toward resolution over a period of weeks or months. Treatment should be considered when the severity or prolonged nature of the symptoms makes it necessary. A single comparative trial showed no enhanced clinical benefit for albendazole over thiabendazole.[13] Albendazole 400mg for 7 days is probably the treatment of choice, although diethyl carbamazine and thiabendazole are alternatives.

TABLE 174-1 -- Clinical symptoms of toxocariasis: comparison of studies.

CLINICAL SYMPTOMS OF TOXOCARIASIS: COMPARISON OF STUDIES				
Feature	**No of patients (%)**			
	Harrison-Synder [6]	**Huntley *et al.*** [7]	**Taylor *et al.*** [8]	**Gillespie** [9]
Pica	100	90	NR	10
Fever	55	80	33	39
Cough	20	80	68	46
Wheeze	20	63	51	28
Abdominal pain	0	0	63	32
Failure to thrive	NR	39	NR	4
Anemia	40	NR	NR	4
No symptoms	0	0	NR	12
Male sex	75	66	NR	60
Eosinophilia	100	100	NR	77
NR, none recorded.				

TABLE 174-2 -- Signs of clinical toxocariasis: comparison of studies.

SIGNS OF CLINICAL TOXOCARIASIS: COMPARISON OF STUDIES				
Feature	**No of patients (%)**			
	Harrison-Synder [6]	**Huntley *et al.*** [7]	**Taylor *et al.*** [8]	**Gillespie** [9]
Hepatomegaly	85	65	26	15
Splenomegaly	40	NR	8	11
Lympadenopathy	NR	8	62	21
Bronchospasm	NR	43	NR	17
Skin lesions	NR	22	5	5
NR, none recorded.				

In ocular disease therapy is directed toward reducing the severity of the inflammatory response. There is no controlled trial evidence to suggest whether specific anthelmintic therapy is beneficial. There are several reports that support the value of therapy with both albendazole and corticosteroids.[14] [15] Vitrectomy and surgical correction of retinal detachment is beneficial in approximately half of patients with retinal traction. [16] Laser treatment of retinal granuloma has been described but its benefit has not been systematically assessed in comparison with other therapy.

PREVENTION AND CONTROL

Control measures are directed toward reducing contact between children and infective eggs. Because eggs require a period of embryonation in soil, contamination of soil in public parks is an important target for control measures. Dog owners should be encouraged to worm their dogs regularly and to clean up after their pets have defecated. This may be supported by local legislation punishing promiscuous canine defecation in public areas with fines. It is especially important that children's play areas should be made dog proof and children should be encouraged to wash their hands thoroughly after playing in a park.

ANGIOSTRONGYLUS CANTONENSIS

Angiostrongylus cantonensis is a nematode parasite closely related to hookworm. The life cycle involves two hosts, a vertebrate and a snail in which the infective larvae develop. Humans become infected

when they eat slugs or snails or fruits and vegetables on which larvae have been shed in slime. Humans are abnormal hosts for *A. cantonensis* and severe symptoms may result: eosinophilic meningitis, eosinophilic meningoencephalitis and eosinophilic radiculomyeloencephalitis.

A closely related species, *Angiostronglus costaricensis*, causes abdominal symptoms that may mimic acute appendicitis or ileitis and is found in Central America.[17] Treatment with benzimidazoles may be beneficial.

Infection with *A. cantonensis* principally occurs in South East Asia, Oceania, India, Madagascar, Côte d'Ivoire and Egypt, but a new focus of infection has been described in the Caribbean.[18] Treatment may exacerbate symptoms by releasing parasite antigens, and management with corticosteroids may be beneficial, although experience is limited.

GNATHOSTOMIASIS

Gnathostomia spinigerum is the commonest species associated with human gnathostomiasis. The organism has a complex life cycle involving an adult in the intestinal wall of the cat and eggs in the feces, which are ingested by a water-dwelling cyclops and are subsequently ingested by fish, snakes and amphibians. Human infection arises by eating raw or undercooked fish by handling ducks or chickens that have eaten intermediate hosts. The larvae are unable to complete their life cycle in humans and migrate through the tissues for prolonged periods. The disease principally presents as migratory cutaneous swellings but, more seriously, invasion of the central nervous system or ocular disease may develop, causing eosinophilic meningitis, which may involve the spinal cord. Meningitis in gnathostomiasis is frequently fatal. In the eye, the parasite can produce severe inflammation and hemorrhagic lesions. Treatment is unsatisfactory although some have recommended the use of benzimidazoles for treatment.

ANCYLOSTOMA CANINUM AND *ANCYLOSTOMA BRAZILIENSE* INFECTIONS

Dog hookworms cannot complete their life cycle in noncanine hosts and may cause cutaneous larva migrans.[19] Cutaneous larva migrans does not occur after the first exposure to *Ancylostoma caninum* and *A. braziliense* larva, suggesting that the disease is due to hypersensitivity to larval secretions. The lower extremities are more often affected, with eruptions on the feet making up almost two-thirds of all cases. Infection is commonly reported in patients returning from tropical travel. Lesions may be found in the upper legs, urogenital region and on the arms and trunk. Lesions on the head are extremely rare but have been described.[19] The lesions are intensely itchy, red and edematous and show a worm-like migratory pathway under the skin. However, recent studies indicate that *A. caninum* can achieve a wider migration and is implicated in the condition of eosinophilic enteritis.[20] Eosinophilic enteritis is characterized by abdominal pain that is often colicky moving to the periumbilical region or right of the iliac fossa. It usually lasts up to 1 month. It is associated with anorexia, nausea and diarrhea, and some patients can be sufficiently ill to present with an acute abdominal condition that may mimic acute appendicitis or intestinal obstruction.[21]

The diagnosis of cutaneous larva migrans is made on the basis of the characteristic clinical features. The laboratory has no role to play in diagnosis. Eosinophilia is only a feature of a minority of cases. The total serum IgE is usually normal and other serologic tests for helminth infections are unhelpful. In eosinophilic enteritis the patient has significant eosinophilia and a high total IgE level but these laboratory features may be absent in some patients. The diagnosis is made histologically using tissue biopsies obtained during colonoscopy.[20] Aphthous ulcers can be seen in the cecum and terminal ileum on colonoscopy. Laparotomy, when performed for a suspected diagnosis of appendicitis, often reveals an inflamed ileum with intense serositis and enlarged mesenteric lymph nodes. Antibodies to the excretory-secretory antigens of adult *A. caninum* patients can be found in more than 85% of patients with eosinophilic enteritis by antibody capture enzyme-linked immunosorbent assay.

Cutaneous larva migrans is readily treated by application of 10% thiabendazole paste and an occlusive dressing for 24 hours. In severe cases systemic treatment with albendazole or ivermectin may also be used. Eosinophilic enteritis is readily treated with 200mg mebendazole. Failure to respond within 24 hours would suggest an alternative diagnosis.

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Chapter 175 - Melioidosis

David AB Dance

INTRODUCTION

The term 'melioidosis' refers to infections caused by the Gramnegative bacillus *Burkholderia* (formerly *Pseudomonas*) *pseudomallei*, which is recognized increasingly as a public health problem in some tropical regions.[1] The considerable recent progress in our understanding of the pathogenesis of this infection and the sequencing of the bacterial genome contrast starkly with our poor knowledge of the epidemiology and distribution of the disease worldwide.[2]

EPIDEMIOLOGY

The distribution of melioidosis is shown in Figure 175.1 . Most cases are diagnosed in South East Asia and northern Australia. The disease is probably under-recognized elsewhere (e.g. half the recent cases diagnosed in the UK originated from the Indian subcontinent). Imported infection is seen mainly in immigrants or soldiers serving in endemic areas, but occasionally occurs in tourists.

Burkholderia pseudomallei is a saprophyte found in soil and surface water, particularly rice paddies, in endemic areas. A closely related, arabinose-assimilating, avirulent organism, *Burkholderia thailandensis*, is similarly distributed, and may give rise to confusion, particularly in serologic tests. Humans and a wide range of other animals acquire infection from contact with soil (e.g. through rice farming), probably by inoculation or inhalation.[3] Recent clusters of infection in Australia have also been associated with contamination of potable water supplies. [2] Most cases present during the rainy season, when people are maximally exposed to the organisms in the environment.[3] [4] Animal-to-human and person-to-person spread, iatrogenic infection and laboratory-acquired infection have all been reported occasionally. All ages may be affected, with a peak from 40–60 years. Males are more often affected than females.[3] [4]

PATHOGENESIS AND PATHOLOGY

The outcome of infection with *B. pseudomallei* depends on a balance between the virulence of the organism, the size of inoculum and the resistance of the host. Between 50% and 70% of patients who have melioidosis have predisposing underlying diseases, especially diabetes mellitus but also chronic renal disease, malignancy, immunosuppressive treatment (e.g. corticosteroids), liver disease, alcohol or drug abuse, pregnancy and cystic fibrosis.[3] [4] Interferon (IFN)-? appears to play a key role in controlling the infection in experimental animals. Recent studies have shown that capsular polysaccharide is a major virulence determinant in *B. pseudomallei*, possibly by reducing intracellular killing.[5] Other potential virulence factors include lipopolysaccharide, a lethal exotoxin, various enzymes (lecithinase, lipase, proteases and acid phosphatase), type III secretion systems and a siderophore. Several of these appear to be encoded on 'pathogenicity islands', possibly acquired by horizontal transfer.[2] Intracellular survival of *B. pseudomallei* probably contributes to the recalcitrant nature of melioidosis.

Burkholderia pseudomallei causes localized abscesses or granulomas at the site of primary infection, depending on the duration of the lesion. Invasion of the bloodstream leads to sepsis, which may in turn result in metastatic foci of infection in other tissues. The host response may also contribute to pathogenesis, because serum levels of several cytokines, including IFN-?, tumor necrosis factor, interleukin (IL)-6 and IL-8, are also correlated with mortality.

PREVENTION

Because *B. pseudomallei* is ubiquitous in the environment in endemic areas, avoidance of the organism is virtually impossible. No *B. pseudomallei* vaccine has been developed for human use, although several experimental vaccines are under development and some have been used on animals. The organism should be handled in containment level 3 facilities in the laboratory. Patients should ideally be cared for in standard isolation, although person-to-person spread is very rare.

CLINICAL FEATURES

None of the clinical classifications of melioidosis is entirely satisfactory. Infections may be acute or chronic and localized or disseminated, but one form of the disease may progress to another and individual patients are often difficult to categorize. Several reviews of the clinical features of melioidosis have been published.[4] [6] The manifestations of the disease in Australia are similar to those in Thailand, although prostatic abscesses and neurologic involvement are more frequently described and parotid abscesses less often.[4]

Mild and subclinical infections

Antibodies to *B. pseudomallei* are very common yet disease is rare, so the majority of infections are presumably mild or asymptomatic. A flu-like illness associated with seroconversion has been reported.

Latent infections

Unusually for a bacterial infection, long periods of latency (up to 29 years) may occur before the disease becomes apparent, which usually happens during intercurrent stress (hence the name 'the Vietnamese time bomb').

Septic melioidosis

Positive blood cultures are found in 46–60% of cases of culture-positive melioidosis.[4] [6] Most of these cases present with a picture of fulminant sepsis syndrome with a short history of high fever and rigors, although some patients have a less acute, typhoidal picture with a remittent fever. Only half have evidence of a primary focus of infection, usually in the lung or skin and subcutaneous tissues. Confusion and stupor, jaundice and diarrhea may also be prominent features. Initial investigations usually reveal:

- anemia;
- a neutrophil leukocytosis;
- coagulopathy; and
- evidence of renal and hepatic impairment.

Figure 175-1 World distribution of *Burkholderia pseudomallei* and *Burkholderia pseudomallei*-like organisms. The boundaries are political and are not intended to define

the true limits of distribution. *With permission from Dance.*[1]

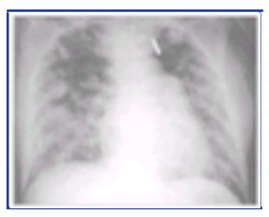

Figure 175-2 Chest radiograph of patient who has septic melioidosis. Note the multiple areas of consolidation scattered throughout both lung fields (blood-borne pneumonia). *With permission from Prof N J White.*

Patients often deteriorate rapidly, developing widespread metastatic foci, metabolic acidosis with Kussmaul's breathing, and shock, and many die within 48 hours of hospital admission. Poor prognostic features include absence of fever, leukopenia, azotemia and abnormal liver function tests.[6]

If the patient survives the acute phase, the manifestations of metastatic septic foci become prominent. An abnormal chest radiograph is found in 60–80% of patients, the most common pattern being widespread, nodular shadowing (Fig. 175.2). Multiple liver and splenic abscesses are common.[7] Cutaneous pustules or subcutaneous abscesses occur in 10–20% of cases.[6] Other common sites for secondary lesions include the urinary tract (kidneys and prostate gland), bones and joints. Involvement of the central nervous system may comprise cerebral abscesses or a syndrome of peripheral motor weakness, brain stem encephalitis, aseptic meningitis and respiratory failure.[4]

Localized melioidosis

Localized melioidosis is most common in the lung, where it usually causes a subacute cavitating pneumonia accompanied by profound weight loss. This may be confused with tuberculosis, although relative sparing of the apices and the infrequency of hilar adenopathy may help to distinguish the two. Any lung zone may be affected, although there is a predilection for the upper lobes. Complications include pneumothorax, empyema, purulent pericarditis and ultimately progression to sepsis. Acute suppurative parotitis (Fig. 175.3) is a characteristic manifestation of melioidosis in children in Thailand, although it is rarely reported elsewhere. Localized *B. pseudomallei* infection may affect any other tissue or organ (e.g. cutaneous and subcutaneous abscesses, lymphadenitis, osteomyelitis and septic arthritis, liver or splenic abscesses, cystitis, pyelonephritis, prostatic abscesses, epididymo-orchitis, keratitis, brain abscesses and mycotic aneurysms).

DIAGNOSIS

Melioidosis should be considered in any patient who has sepsis or abscesses who has ever visited an endemic area, particularly if the patient has an underlying disease such as diabetes mellitus. Microscopy of a Gram-stained smear of pus or sputum is neither specific nor sensitive but immunofluorescent microscopy, which is only available in a few centers, offers the best opportunity of making a rapid diagnosis. Definitive diagnosis depends on isolation and identification of *B. pseudomallei* from cultures of blood or clinically affected sites (e.g. pus, sputum). It is important to alert the laboratory to the suspicion of melioidosis. Preliminary culture results should be available within 48 hours, although identification may be delayed in nonendemic areas because microbiologists are not familiar with the organism. Several rapid diagnostic techniques for the detection of *B. pseudomallei* antigens or nucleic acids have been developed, but these are not yet sufficiently sensitive or specific to be widely used.[9]

The serologic test most widely used in endemic areas is an indirect hemagglutination (IHA) test, although other assays that detect IgG antibodies give similar results. There is a need for internationally standardized serologic tests. High background seropositivity means that false-positive reactions are common in people from endemic areas,[6] but a single high IHA titer (>1:40) in someone

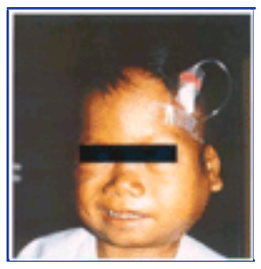

Figure 175-3 Acute suppurative parotitis. This form of melioidosis accounts for around one third of pediatric cases in north east Thailand, but has rarely been reported elsewhere. *With permission from Dance et al.*[6]

from a nonendemic area, or a rising titer, may be diagnostically useful. Tests for specific IgM (e.g. indirect immunofluorescence, enzyme-linked immunosorbent assay) correlate better with disease activity and, along with measurement of C-reactive protein, they may be useful during follow-up of patients on treatment. Imaging, including the use of labeled white cell scans, is also useful in determining the initial extent of dissemination and monitoring the response to treatment.

MANAGEMENT

Supportive treatment

Patients who have septic melioidosis usually require aggressive supportive treatment, including correction of volume depletion and septic shock, respiratory and renal failure, and hyperglycemia or ketoacidosis. Abscesses should be drained whenever possible.

Specific treatment

Burkholderia pseudomallei is intrinsically resistant to many antibiotics, including aminoglycosides and early ß-lactams, and a failure to respond to these agents is characteristic of melioidosis.[6] Several recent studies have shown that the mortality of acute severe melioidosis can be substantially reduced by newer ß-lactam agents such as ceftazidime, imipenem, amoxicillin-clavulanic acid and cefoperazone-sulbactam, with or without trimethoprim-sulfamethoxazole.[10] Very encouraging results have also been reported from Australia using meropenem plus trimethoprim-sulfamethoxazole.[4] The role of granulocyte colony-stimulating factor, which has also been used as adjunctive treatment in Australia, remains to be determined. Ceftazidime or a carbapenem are currently the treatments of choice and should be given in full doses (ceftazidime 120mg/kg/day, imipenem and meropenem 60mg/kg/day, or a dose appropriately adjusted for renal function) for 2–4 weeks according to the clinical response.

Following parenteral treatment, prolonged oral antibiotics are needed to prevent relapse, which occurs in up to 23% of patients and is more common in patients who have more severe disease. The proportion of patients who relapse can be reduced to less than 10% if antibiotics are given for 20 weeks.[10] The combination of chloramphenicol (40mg/kg/day), doxycycline (4mg/kg/day) and trimethoprim-sulfamethoxazole (10mg/kg trimethoprim plus 50mg/kg sulfamethoxazole per day) has been associated with a lower relapse rate than amoxicillin-clavulanic acid (60mg/kg amoxicillin plus 15mg/kg clavulanic acid per day). Lately the chloramphenicol has been omitted from this regimen without apparent detriment[11] and trimethoprim-sulfamethoxazole alone has been used in Australia,[4] although doxycycline alone and fluoroquinolones are inadequate. Amoxicillin-clavulanic acid is preferable in children and pregnant or lactating women. In patients who have mild localized disease, any of the oral regimens described above may be used.

OUTCOME AND FOLLOW-UP

Even with optimal treatment, the mortality from acute severe melioidosis is high (30–47% in Thailand, 19% in Australia).[4] [9] In patients who survive, there is often chronic morbidity resulting both from the disease itself and from the underlying conditions. Patients require long-term follow-up to detect relapse. Susceptibility tests should be carried out on isolates obtained during or after treatment, because resistance may emerge in 5–10% of cases.

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Chapter 176 - Plague

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Kenneth L Gage

Plague is an acute, life-threatening zoonosis caused by the bacterium *Yersinia pestis*. The disease is best known for three devastating pandemics, including the Black Death of the Middle Ages. Plague is primarily a disease of rodents, and humans typically acquire the disease as a result of being bitten by rodent fleas, less commonly by handling infected animals, and rarely by inhaling infectious respiratory particles. Urban, rat-borne plague outbreaks have historically been responsible for most human plague cases; in recent decades, however, plague has occurred typically in remote, rural populations. The three principal clinical forms of plague are bubonic plague, septic plague and pneumonic plague. The most common of these is bubonic plague, an acute illness characterized by fever and one or more enlarged tender lymph nodes (buboes) that usually appear in the groin or the axillary or cervical regions proximal to the site of an infective inoculation. Bacteremia and sepsis occur when lymphatic defenses have been breached, and plague pneumonia can arise secondarily as a result of blood-borne seeding of the lungs. Occasionally, pneumonic plague spreads from person to person, most often in crowded, substandard living conditions.

Plague is often fatal when not diagnosed and treated with appropriate antibiotics early in the course of infection. The aminoglycosides, tetracyclines and chloramphenicol are the antibiotics most commonly used to treat plague. Pneumonic plague patients should be isolated under respiratory droplet precautions until no longer infectious, and it is recommended that persons who have been in close contact with pneumonic plague patients be placed on antimicrobial prophylaxis and monitored for fever. Prevention and control of plague relies on environmental sanitation, human and animal disease surveillance, flea and rodent control, and early detection, treatment and isolation of cases, as indicated. Plague is considered to be an important potential weapon of bioterrorism.

EPIDEMIOLOGY

Agent

Yersinia pestis is a Gram-negative, microaerophilic coccobacillus belonging to the family Enterobacteriacae (see Chapter 228). The entire genome of *Y. pestis* has been decoded, showing that the organism recently evolved from *Yersinia pseudotuberculosis*, a gut pathogen.[1] It is nonmotile and nonsporulating, does not ferment lactose and exhibits bipolar staining with Wayson's, Giemsa's or Wright's stains. Growth occurs in a variety of media at a wide range of temperatures (39–104°F (4–44°C); optimal 82–86°F (28–30°C)) and pH values (5.0–9.6; optimal 7.2–7.6). It is a facultative intracellular pathogen that normally grows in extracellular environments; importantly, it invades, multiplies within and is transported by phagocytes during the initial phases of infection.[2]

Genetic factors that enable *Y. pestis* to survive in its mammalian hosts and flea vectors and be transmitted between them are outlined in Table 176.1 .[2] [3] [4] [5] [6] Some factors are expressed selectively at temperatures and environments encountered in fleas or mammals. For example, hemin storage locus (*hms*) products are expressed in the low temperature environment of the flea, apparently enabling the bacteria to form the gut blockages necessary for their survival and efficient transmission.[2] [6]

Three biotypes of *Y. pestis*, which are classified according to their ability to ferment glycerol and reduce nitrate, have been correlated with the three principal plague pandemics. The biotype that spread around the world during the third pandemic beginning in the late 19th century is termed the orientalis biotype; it occurs alone in South East Asia, portions of Africa, Madagascar and in the Western Hemisphere. The antiqua biotype occurs in parts of Africa, southeastern Russia and in Central Asia. The mediaevalis biotype, thought to have been responsible for the Black Death, occurs in natural foci around the Caspian Sea. Results of typing by restriction fragment length polymorphism analysis of rRNA genes (ribotyping) supports these distinctions and has shown chromosomal rearrangements in the orientalis biotype after its spread around the world about 100 years ago.[7]

Life cycle

Understanding the epidemiology of plague requires a working knowledge of the ecology of *Y. pestis* and its transmission cycles. In general, *Y. pestis* is maintained in both enzootic and epizootic cycles involving various sylvatic and commensal rodent species and their fleas (Fig. 176.1).[8] Historically, the commensal black or roof rat, *Rattus rattus*, the sewer rat, *Rattus norvegicus*, and their fleas (especially the oriental rat flea, *Xenopsylla cheopis*) have been the principal sources of epidemics of plague and its pandemic spread.

During interepizootic periods the plague bacterium is maintained in 'silent' enzootic cycles involving populations of wild rodents that exhibit variable responses to *Y. pestis* infection. The most susceptible members of an enzootic host population are likely to develop high bacteremias and serve as suitable sources for infecting feeding fleas. Other members of the same population of rodents develop little or no bacteremia and infect few fleas. These more resistant animals will, however, survive to reproduce and are likely to have offspring that vary in their susceptibility to plague, a factor that promotes the survival of enzootic host populations and yet allows the transmission cycle to be maintained. Most enzootic host species also have relatively high reproductive rates, which further promotes the ongoing introduction of susceptible, nonimmune animals into the population.[9] Under certain conditions, plague is likely to spread from enzootic hosts to more susceptible rodent species (epizootic hosts), causing explosive epizootics and massive die-offs. Commensal rats and certain burrowing rodents, such as prairie dogs, marmots and various ground squirrels, are among the most important epizootic hosts.

Humans and other incidental hosts of *Y. pestis* are not directly involved in maintaining its natural cycle. *Yersinia pestis* can, however,

TABLE 176-1 -- Proposed virulence and transmission factors for *Yersinia pestis*.

PROPOSED VIRULENCE AND TRANSMISSION FACTORS FOR *YERSINIA PESTIS*		
Genomic element	**Virulence or transmission factor**	**Proposed role in virulence or transmission**
9.5kb plasmid (pesticin plasmid; a 19kb dimer of this plasmid also exists)	Pesticin sensitivity (*pst*)	Loss of sensitivity to pesticin (a bacteriocin) is associated with reduced siderophore binding capability (affects iron uptake)
	Plasminogen activator (*pla*)	Fibrinolytic activity (important for dissemination)
70–75kb plasmid (low calcium response plasmid)	*Yersinia* outer proteins (Yops — genes found in the Yop virulon, a type III secretion system; includes *lcrV* or V antigen)	Proposed functions vary among Yops and include: translocation of other Yops (effectors) across cell membranes; disturbance of phagocyte cytoskeleton dynamics (interferes with phagocytosis); blocking production of proinflammatory cytokines and interfering with ability of B and T cells to be activated by means of antigen receptors (immunosuppression); or binding thrombin (interferes with thrombin-platelet aggregation)
100–110kb plasmid	Murine toxin (*ymt*)	Required for survival in fleas; also has ß-adrenergic antagonist activity in rats and mice but not guinea pigs, rabbits, dogs or nonhuman primates
	F1 'capsular' antigen (*caf1*)	Resistance to phagocytosis by monocytes
Chromosomal	Pigmentation (*pgm* locus, includes genes of *hms* locus, high pathogenicity island and *ybt* operon)	Pigment-positive strains bind hemin and appear pigmented on culture media containing Congo Red; *pgm* locus contains genes of the high pathogenicity island (HPI), which is found in other Yersiniae and certain related enteric bacteria; the HPI contains the *ybt* (yersiniabactin) operon, which encodes genes of a siderophore-based iron uptake system; the *pgm* locus also contains the *hms* locus, which must be functional for 'blocking' to occur in the flea vector (blocking is required for efficient transmission of plague by fleas)
	Endotoxin (lipopolysaccharide)	Lipopolysaccharide release responsible for major pathogenic effects of plague sepsis, systemic inflammatory response syndrome and associated adult respiratory distress syndrome, cytokine activation, complement cascade, DIC, bleeding, unresponsive shock and organ failure
	Serum resistance (lipopolysaccharide in part)	Resistance to complement-mediated lysis; proposed to be related in part to lipopolysaccharide structure
	pH 6 antigen (*psa*)	Entry into naive macrophages; assists in delivery of Yops into phagocytic cells

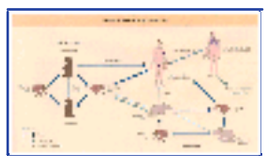

Figure 176-1 Transmission cycles of *Yersinia pestis*.

Figure 176-2 Global distribution of plague. Compiled from sources of the WHO, the Centers for Disease Control and Prevention, and the individual countries.

be directly transmitted from one person to another by respiratory secretions, causing primary plague pneumonia in the recipient (see Fig. 176.1).

Geographic distribution

Plague foci are widely distributed throughout the world, and human cases are typically reported from 10 or so countries each year.[10] Countries reporting human or animal plague at some time in the past 15 years are shown in Figure 176.2 .

Populations affected

Plague is mostly a public health problem of impoverished populations living under substandard conditions. It typically affects inhabitants of rural villages in the developing world that are heavily infested with susceptible rodent hosts and their fleas. Urban rat-borne plague is now unusual around the world, occurring most recently in Madagascar in the 1990s. In the USA, most persons acquire plague from exposures to infection around rural residential properties, which often have poorly maintained woodpiles, abandoned vehicles, dilapidated buildings and other debris that provide favorable harbor for rodents. Cases also occasionally arise among campers, hikers, hunters and others exposed to plague in natural settings.

In the absence of control measures, plague has the potential to spread from rural areas to population centers, including major cities and ports, either through unintended transport of infected rats and fleas or through direct exchange of infection between contiguous rodent populations.[8] Occasionally, persons incubate plague while traveling (peripatetic plague), develop plague pneumonia and transmit plague to others along the way or at their destination. The risk of plague to persons visiting endemic areas for business or tourism is, in general, extremely low.

Disease incidence

The International Health Regulations of the World Health Organization (WHO) require prompt reporting of human plague cases to the WHO.[11] Twenty-four countries reported to the WHO a total of 33,948 cases (mean of 2263 cases per year) and 2653 (8% fatality rate) deaths during the years 1985–99 (Table 176.2).[10] Countries in eastern and southern Africa, and the adjacent island of Madagascar, reported 77% of these cases, with the remaining cases occurring in Asia (17%) and the Americas (6%). Madagascar alone reported 28% of the cases reported to WHO from 1985 to 1999 and

TABLE 176-2 -- Reported cases of plague in humans by country, 1985–99.

REPORTED CASES OF PLAGUE IN HUMANS BY COUNTRY (1985–99)			
Region	**Country**	**Number of cases**	**Number of deaths**
Africa	Botswana	173	12
	Congo	3008	607
	Kenya	44	8
	Madagascar	9650	795
	Malawi	665	15
	Mozambique	1787	28
	Namibia	2865	110
	Tanzania	6646	478
	Uganda	556	61
	Zambia	320	27
	Zimbabwe	418	35
	Total	**26,132**	**2176**
Americas	Bolivia	135	22
	Brazil	293	6
	Ecuador	17	16
	Peru	1436	74
	USA	144	14
	Total	**2025**	**132**
Asia	China	361	53
	India	876	54
	Indonesia	6	0
	Kazakhstan	18	6
	Laos	10	0
	Mongolia	82	30
	Myanmar	815	6
	Vietnam	3623	196
	Total	**5791**	**345**
World totals		**33,948**	**2653**

Tanzania accounted for almost another 20% of cases. The recent reemergence of plague, both urban and rural, in southeastern Africa

and Madagascar has been striking.[12] [13] The rise in the proportion of cases reported from Africa and Madagascar was particularly pronounced in the 1990s, and the number of cases occurring in these countries has remained high. During 1985–99, Vietnam (11% of world total) and Peru (4% of world total) reported the most cases from Asia and the Americas, respectively.

Plague was introduced into the USA in 1900 and is considered an emerging disease there.[13] More than 400 cases of plague have been reported in the USA since 1950. Although enzootic and epizootic plague occurs in rural areas of 17 of the contiguous western states of the USA, extending from the Pacific coastal states to the Great Plains states and eastern Texas, 80% of human cases occur in the southwestern states of New Mexico, Arizona and Colorado, and approximately 10% in California.[13] [14]

Sources of infection and risks for humans

Flea bites are the most common source of *Y. pestis* infection. The risk of plague for humans increases greatly during rodent epizootics when large numbers of *Y. pestis*-infected fleas seek new hosts to replace those killed by plague. Human plague can also be a direct result of handling tissues or body fluids of infected animals. This is an important source of plague among persons who hunt and skin marmots (*Marmota* spp.) in Central Asia and northern China, and occasionally this is the source of plague in the USA in persons who handle the carcasses of infected prairie dogs, rabbits and carnivores.[8] Pet owners and veterinary staff may become infected with *Y. pestis* while caring for domestic cats that develop oropharyngeal or pneumonic plague from having ingested infected rodents.[15] Outbreaks of plague have occurred in Saudi Arabia, Libya and Jordan as a result of persons handling and consuming infected camel and goat meat.[16]

Primary pneumonic plague occurs when persons inhale infectious respiratory secretions. This usually arises in the setting of an outbreak of bubonic plague in which some persons who develop secondary pulmonary infection spread infection to others through infectious respiratory droplets, starting a chain of respiratory transmission. Persons living in the same quarters as a pneumonic plague patient and persons attending the sick, such as family members and health care personnel, are especially at risk. *Yersinia pestis* is classified as a category A agent of potential danger as a weapon of bioterrorism because it could be aerosolized and cause outbreaks of severe or fatal illness, create panic, and requires special actions for medical and public health preparedness (see Chapter 6).[17] [18] The release of an aerosol of *Y. pestis* in a biologic terrorism event would be expected to result in an outbreak of respiratory plague with potential for person to person spread. Under natural conditions, primary pneumonic plague usually comprises only a small fraction of the total number of cases in any plague-endemic region (less than 2% in the USA); nevertheless, this form of plague is extremely dangerous because of a high case fatality rate and the risk of epidemic spread.[19]

Seasonality

Flea-borne cases of human plague in the temperate Northern Hemisphere are most likely to occur between late spring and the end of summer when epizootic transmission peaks.[8] In tropical and semitropical plague foci, transmission may vary between a wet, relatively cool season and another season of hotter, drier weather, with the numbers of cases being lowest during the latter period.[20] Although flea-borne cases of human plague are occasionally reported during 'off-season' months in various plague foci, particularly those in tropical or semitropical regions, they are rare in other areas such as the USA. Most 'off-season' (winter) cases in the temperature Northern Hemisphere occur among hunters or trappers handling infected animals. In the USA, these animals include rabbits, hares and carnivores, such as coyotes and wild and domesticated cats.

PATHOGENESIS AND PATHOLOGY

Yersinia pestis is among the most pathogenic bacteria known. Both chromosomal and plasmid-encoded gene products are associated with adaptability to its various hosts and to virulence (Table 176.1).[2] [3] [4] [5] [6] The virulence of the organisms is expressed in a wide range of severe disease.[21] [22] [23] [24] [25]

Yersinia pestis organisms inoculated through the skin or mucous membranes travel to and multiply within regional lymph nodes. In the early stages of infection, affected nodes are found to be edematous and congested and to have minimal inflammatory infiltrates and vascular injury. Fully developed buboes, however, contain large numbers of infectious plague organisms and show vascular damage, hemorrhagic necrosis and infiltration of neutrophilic leukocytes. The affected nodes are usually surrounded by a collection of serous fluid. When several adjacent lymph nodes are involved, a boggy edematous mass can result. In later stages, abscess formation and spontaneous rupture of lymph glands may infrequently occur.

Plague sepsis in the absence of signs of localized infection, such as a bubo, is termed primary septic plague. It can result from direct entry of *Y. pestis* through broken skin or mucous membranes or from the bite of an infective flea. Secondary septic plague can occur in the course of bubonic or pneumonic plague when lymphatic or pulmonary defenses are breached and the plague bacillus enters and multiplies within the bloodstream. Bacteremia is common in all forms of plague; septicemia is less common and immediately life-threatening.

Yersinia pestis can invade and cause disease in almost any organ, and untreated infection usually results in widespread and massive tissue destruction. Diffuse interstitial myocarditis with cardiac dilatation, multifocal necrosis of the liver, diffuse hemorrhagic splenic necrosis and fibrin thrombi in renal glomeruli, are commonly found in fatal cases.[19] [21] [22] [24] If disseminated intravascular coagulation (DIC) occurs, it results in thrombosis within the microvasculature, necrosis and bleeding, with widespread cutaneous, mucosal and serosal petechiae and ecchymoses. Gangrene of acral parts, such as fingers and toes, may occur in the late stages of this process (Fig. 176.4).

Primary plague pneumonia, which results from inhalation of infective respiratory particles, usually begins as a lobular process and then extends by confluence, becoming lobar and then multilobar. Typically, plague organisms are numerous in the alveoli and in pulmonary secretions. Secondary plague pneumonia arising from hematogenous seeding of the lungs typically begins more diffusely as an interstitial process, with plague bacilli most numerous in the interstitial spaces. In untreated cases of both primary and secondary plague pneumonia, the usual pathologic findings are diffuse pulmonary congestion, edema, hemorrhagic necrosis and scant neutrophilic infiltration.[19] [24]

PREVENTION AND CONTROL

Plague prevention and control is best accomplished by a combination of:

- environmental sanitation;
- awareness campaigns to promote avoidance of potential infective exposures; and
- early detection of human and animal plague to focus remedial environmental actions and to institute early treatment of cases, infection control procedures and prophylaxis of exposed persons, as indicated.

In endemic areas, public health services must provide a continuing system of human and animal plague surveillance, epidemiologic investigations and control actions.

The principal environmental remediation measures during outbreaks of human plague or dangerous epizootics are insecticidal flea control, rodent control and sanitation. Flea control should be carried out before or in conjunction with the killing of rodents to reduce the chances that infected fleas will feed on humans. [8] [26]

A killed, whole-cell plague vaccine has limited availability and usefulness. It was available in the USA until the late 1990s, but is no longer being manufactured there. Use of this vaccine was limited primarily to certain groups that were thought to be at high risk, including research laboratory workers, biologists working with susceptible animal populations and some military personnel. The efficacy of this vaccine was never evaluated in clinical trials, and evidence for vaccine protection was based on animal experiments, immunogenicity studies in humans and observations on its use in USA servicemen during the Vietnam conflict. It was thought to be protective against flea-borne exposures but to be only partially protective, if at all, against respiratory exposures. Primary immunization consisted of a series of three injections followed by boosters at intervals of 6 months or more.[27] Research is underway to develop improved plague vaccines that are likely to be protective against airborne routes of exposure, but it is unknown when these vaccines will be approved and commercially available. At present, the most promising candidates are recombinant subunit vaccines that express both the F1 and V antigens of *Y. pestis*.[28] Interest in developing an effective plague vaccine has increased greatly in recent years because of concerns about bioterrorism and biowarfare.

In the event of an outbreak of plague, measures should be taken to rapidly control spread, as described in international regulations and manuals of plague control.[11] [26] These measures include:

- determining the source;
- defining the geographic limits of activity;
- establishing active surveillance;
- laboratory confirmation of cases, and isolation of pneumonic cases;
- rapid treatment of cases and others at risk of infection, including close contacts of symptomatic pneumonic plague cases; and
- control of fleas and rodents in plague-infected areas, in port facilities and on ships and other conveyances.

CLINICAL FEATURES

Bubonic plague

Bubonic plague has a usual incubation period of 2–6 days, occasionally longer. Typically, the patient experiences the acute onset of chills, fever that rises within hours to 100.4°F (38°C) or higher, myalgias, arthralgias, headache and a profound lethargy. Soon, usually within

Figure 176-3 Left inguinal and femoral buboes, demonstrating surrounding edema and overlying desquamation.

24 hours, tenderness and pain occur in one or more regional lymph nodes proximal to the site of inoculation of the plague bacillus. The femoral and inguinal groups of nodes are most commonly involved, axillary and cervical nodes less so, varying with the site of inoculation. The enlarging bubo or buboes become progressively swollen, painful and tender, sometimes exquisitely so. Typically, the patient guards against palpation and limits movement, pressure and stretching around the bubo. The surrounding tissue often becomes edematous, sometimes markedly, and the overlying skin may be reddened, warm and tense (Fig. 176.3). Inspection of the skin surrounding the bubo or distal to it may reveal the site of a flea bite marked by a small papule, pustule, scab or ulcer (phlyctenule). Larger furuncular lesions, sometimes with eschars that are similar to those caused by tularemia, occur rarely. The bubo of plague differs from lymphadenitis of most other causes by its rapid onset, extreme tenderness, surrounding edema, accompanying signs of toxemia and absence of cellulitis or obvious ascending lymphangitis.

If treated in the uncomplicated state with an appropriate antimicrobial agent, bubonic plague usually responds quickly, with defervescence and resolution of other systemic manifestations over a 2- to 5-day period. Buboes often remain enlarged and tender for a week or more after treatment has begun and infrequently become fluctuant. Without effective antimicrobial treatment, typical bubonic plague patients manifest an increasingly toxic state of fever, tachycardia, lethargy leading to prostration, agitation and confusion and, occasionally, convulsions and delirium. Mild forms of bubonic plague, called pestis minor, have been described in South America and elsewhere; in these cases, the patients are ambulatory and only mildly febrile and have subacute buboes.

Differential diagnostic possibilities for bubonic plague include streptococcal or staphylococcal adenitis, tularemia, cat-scratch disease, mycobacterial infection, acute filarial lyphadenitis, chancroid and strangulated inguinal hernia.

Septic plague

Septic plague is manifest as a rapidly progressive, overwhelming endotoxemia.[21] [22] [23] [24] [25] Primary sepsis occurs in the absence of regional lymphadenitis, and the diagnosis of plague is often not suspected until results of blood culture are reported by the laboratory. Furthermore, patients who have septic plague often present with gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain, making misdiagnosis even more likely. If it is not treated early with appropriate antibiotics and aggressive supportive care, septic plague is usually fulminant and fatal. Petechiae, ecchymoses, bleeding from puncture wounds and orifices, and ischemia of acral parts are manifestations of DIC (Fig. 176.4). Refractory hypotension, renal shutdown, obtundation and other signs of shock are pre-terminal events. Acute respiratory distress syndrome, which

Figure 176-4 Septic plague patient who demonstrated disseminated intravascular coagulation, bleeding into the skin and acral gangrene as a late manifestation.

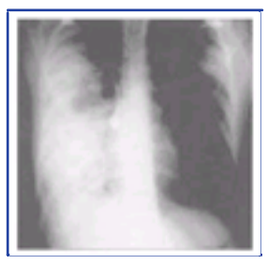

Figure 176-5 Chest radiograph of a patient who has primary plague pneumonia, showing extensive infiltrates in the right middle and lower lung fields.

can occur at any stage of septic plague, may be confused with other conditions such as hantavirus pulmonary syndrome.

Differential diagnostic possibilities include septicemia caused by other bacterial infections, including other Gram-negative bacteria, meningococcemia, bacterial endocarditis and tularemia.

Pneumonic plague

Pneumonic plague is the most rapidly developing and fatal form of plague.[19] [21] [22] [24] The incubation period for primary pneumonic plague is usually 3–5 days (range 1–6 days). The onset is most often sudden, with chills, fever, headache, body pains, weakness, dizziness and chest discomfort. Cough, sputum production, increasing chest pain, tachypnea and dyspnea typically predominate on day 2 of the illness, and these features may be accompanied by hemoptysis, increasing respiratory distress, cardiopulmonary insufficiency and circulatory collapse. In primary plague pneumonia, the sputum is most often watery or mucoid, frothy and blood-tinged, but it may become frankly bloody. Chest signs in primary plague pneumonia may indicate localized pulmonary involvement in the early stage; a rapidly developing segmental consolidation may be seen before bronchopneumonia occurs in other segments and lobes of the same and opposite lung (Fig. 176.5). Liquefaction necrosis and cavitation may develop at sites of consolidation and leave significant residual scarring.

Plague pneumonia arising from metastatic spread (secondary pneumonic plague) typically manifests first as a diffuse interstitial pneumonitis in which sputum production is scant, and is more likely to be inspissated and tenacious in character than the sputum found in primary pneumonic plague.

Differential diagnostic possibilities include other bacterial conditions such as tularemia, community-acquired bacterial pneumonias such as mycoplasma pneumonia, Legionnaires' disease and staphylococcal or streptococcal pneumonia. Viral pneumonias to be differentiated include influenzal pneumonitis, hantaviral pulmonary syndrome and pneumonia caused by respiratory syncitial virus or cytomegalovirus infection. Q fever may need to be considered.

Other manifestations

Meningitis is an unusual manifestation of plague. In the USA there were 12 (3%) cases of meningitis among the total 390 cases of plague reported in the period from 1947 to 1996.[14] All these cases were complications of treated bubonic plague and all the patients survived. Plague occasionally presents as pharyngitis accompanied by fever, sore throat and cervical lymphadenitis. In its early stages, this may be clinically indistinguishable from more common infectious causes of pharyngitis. Plague pharyngitis can arise in primary form from respiratory exposures or from ingestion of undercooked tissues of infected animals, and it is usually associated with marked cervical glandular enlargement. Inoculation of *Y. pestis* through the conjunctiva can result in oculoglandular plague.

DIAGNOSIS

Except in outbreak situations, a high index of clinical suspicion and a careful clinical and epidemiologic history and physical examination are required to make a timely diagnosis of plague. A delayed or missed diagnosis is associated with a high case fatality rate,[14] and infected travelers who seek medical care after they have left

endemic areas are especially at risk. Laboratory tests for plague are highly reliable when conducted by persons experienced with *Y. pestis*, but such expertise is usually limited to selected reference laboratories.

When plague is suspected, clinical specimens should be obtained promptly for microbiologic studies, chest radiographs taken and specific antimicrobial therapy initiated pending confirmation of diagnosis. Blood and other clinical materials such as bubo aspirates, sputum, tracheal washes, swabs of skin lesions or pharyngeal mucosa and cerebrospinal fluid, as indicated, should be inoculated onto suitable media (e.g. brain-heart infusion broth, sheep blood agar, chocolate agar or MacConkey agar).[29] Bubo aspirates typically yield only small amounts of serosanguinous fluid and 1–2ml of saline may need to be injected first to obtain adequate material for diagnosis. Smears of each specimen should be stained with Gram's, Wayson's or Giemsa's stain. Direct fluorescent antibody testing is a useful presumptive diagnostic procedure available at some reference laboratories. An acute-phase serum specimen should be collected for *Y. pestis* antibody testing, followed by a convalescent-phase specimen collected 3–4 weeks later. For diagnosis in fatal cases, tissues, including buboes and samples of liver, spleen, lungs and bone marrow, should be collected at autopsy for culture, fluorescent antibody testing and histologic studies, including possible immunohistochemical staining. Cary Blair medium or a similar holding medium can be used to transport *Y. pestis*-infected tissues. Presumptive identification of *Y. pestis* can be made by polymerase chain reaction or antigen capture enzyme-linked immunosorbent assay. A recently developed rapid immunogold dipstick assay designed to detect *Y. pestis* antigens in patient samples also appears highly promising.[30]

Laboratory confirmation of plague depends on isolation of *Y. pestis* from body fluids or tissues. When the patient's condition allows, several blood cultures taken over a 45-minute period before treatment will usually result in successful isolation of the bacterium. *Yersinia pestis* strains are readily distinguished from other Gram-negative bacteria by polychromatic and immunofluorescence staining properties, characteristics of growth on microbiologic media, biochemical profiles and confirmatory lysis by the *Y. pestis*-specific bacteriophage. Laboratory mice and hamsters are susceptible to *Y. pestis* and are used in specialized laboratories to make isolations from contaminated materials and for virulence testing.

In the absence of a cultural isolation, a diagnosis of plague can be made by the demonstration of a 4-fold or greater change in serum antibodies to *Y. pestis* antigen using passive hemagglutination testing. A serum antibody titer of 128 or greater in a single serum sample from a patient who has a compatible illness and who has not received plague vaccine is also diagnostic. A few plague patients will develop detectable antibodies as soon as 5 days after the onset of illness, most seroconvert 1–2 weeks after onset, a few seroconvert 3 or more weeks after onset and a few (<5%) fail to seroconvert. Early specific antibiotic treatment may delay seroconversion by several weeks. After seroconversion, positive serologic titers diminish gradually over months to years. Enzyme-linked immunosorbent assays for detecting IgM and IgG antibodies to *Y. pestis* have been found to be useful in identifying antibodies in early infection and in differentiating them from antibodies developed in response to previous vaccination.

TABLE 176-3 -- Treatment guidelines for plague.

TREATMENT GUIDELINES FOR PLAGUE			
Drug		**Dosage**	**Route of administration**
Streptomycin	Adults	1g q12h	im
	Children	15mg/kg q12h*	im
Gentamicin	Adults	1–1.5mg/kg q8h†	im or iv
	Children	2.0–2.5mg/kg q8h	im or iv
	Infants/neonates	2.5mg/kg q8h	im or iv
Tetracycline	Adults	0.5g q6h	po
	Children >8 years old	6.25–12.5mg/kg q6h	po
Doxycycline	Adults	100mg q12h	po or iv
	Children >8 years old and >45kg	100mg q12h	po or iv
	Children >8 years old and <45kg	2.2mg/kg q12h	po or iv
Chloramphenicol	Adults	12.5mg/kg q6h‡	po or iv
	Children >1 year old	12.5mg/kg q6h‡	po or iv
Hematologic values should be monitored closely			

* Not to exceed 2g/day
† Daily dose should be reduced to 3mg/kg as soon as clinically indicated
‡ Up to 100mg/kg per day initially. Dosage should be adjusted to maintain plasma concentrations at 5–20µg/ml.

Plague patients typically have white blood cell counts of 10,000–25,000/mm^3 with a predominance of early stage polymorphonuclear leukocytes. Leukemoid reactions with white cell counts as high as 50,000/mm^3 or more can occur.

MANAGEMENT

Untreated, plague is fatal in over 50% of patients who have bubonic disease and in nearly all patients who have septic or pneumonic plague. The overall mortality rate in plague cases in the USA in the past 25 years has been approximately 15%.[14] Fatalities are almost always due to delays in seeking treatment, misdiagnosis and delayed or incorrect treatment. Rapid diagnosis and appropriate antimicrobial therapy (Table 176.3) are essential.[31]

Streptomycin has long been considered the drug of choice for treating plague, but gentamicin is increasingly being used in its place because of its wider availability and ease of administration, and it is currently recommended for use in managing patients in a bioterrorism attack.[18] Tetracyclines or chloramphenicol are effective alternatives to the aminoglycosides. Chloramphenicol is indicated for conditions in which high tissue penetration is important, such as plague meningitis, pleuritis, endophthalmitis or myocarditis. It may be used separately or in combination with an aminoglycoside. Although doxycycline has, because of its ease of administration and rapid action, become the tetracycline of choice for treating plague, clinical trials of its efficacy have not been performed. Trimethoprim-sulfamethoxazole (co-trimoxazole) has been used successfully to treat bubonic plague, but it is not considered a first-line choice. *Yersinia pestis* is highly sensitive to several fluoroquinolones, and ciprofloxacin has been recommended as an alternative antimicrobial for treating plague cases in the event of a bioterrorism attack.[18] Penicillins, cephalosporins and macrolides have a suboptimal effect and should not be used to treat plague. In general, antimicrobial treatment should be continued for 7–10 days or for at least 3 days after the patient has become afebrile and has made a clinical recovery. Patients begun on intravenous antibiotics may be switched to oral regimens as indicated by clinical response. Improvement is usually evident 2–3 days from the start of treatment, even though fever may continue for several more days.

Complications of delayed treatment of plague include DIC, adult respiratory distress syndrome and other consequences of bacterial sepsis. Patients who have these disorders require intensive monitoring and close physiologic support. Buboes may require surgical drainage if they threaten to rupture spontaneously. Abscessed nodes can be a cause of recurrent fever in patients who have otherwise made satisfactory recovery; the cause may be occult if intrathoracic or intra-abdominal nodes are involved. Viable *Y. pestis* organisms have been isolated from affected nodes 1–2 weeks after clinical recovery from acute disease. Strains of *Y. pestis* that are resistant to antimicrobials have only rarely been isolated from humans. Such resistant strains have usually involved partial resistance to a single agent only and have not been associated with treatment failure. Recently, however, a multidrug-resistant strain of *Y. pestis* was isolated from a bubonic plague patient in Madagascar.[32] This isolate was resistant at high levels to all first-line antibiotics recommended for treating plague (including tetracycline, streptomycin and chloramphenicol). Resistance was plasmid-mediated and transferable to other strains of *Y. pestis* and to *Escherichia coli*. Fortunately, surveillance for multidrug-resistant *Y. pestis* in Madagascar and elsewhere has not disclosed any other such resistant strains. Isolated instances of strains resistant to streptomycin or tetracycline are occasionally reported.

Antimicrobials are recommended in some situations as prophylaxis against plague.[27] [31] Postexposure treatment for 7 days with a tetracycline, chloramphenicol or

trimethoprim-sulfamethoxazole is recommended for persons who have been in close contact with a pneumonic plague patient in the previous 7 days. Prophylaxis with doxycycline or ciprofloxacin has been recommended in the event of a bioterrorism attack.[18] Short courses of antimicrobial prophylaxis are sometimes recommended for household members of bubonic plague patients because of possible rodent flea exposures. Prophylaxis is only rarely warranted for people who visit or reside in an area where plague is occurring. Isolation and respiratory droplet precautions are recommended for managing patients with pneumonic plague, including the use of masks for persons caring for these patients while they are infectious. The use of masks may be an important measure for interrupting person to person transmission in the event of a pneumonic plague outbreak. Respiratory plague patients are generally considered to be non-contagious following 48 hours of antibiotic treatment.

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Chapter 177 - Tularemia

David T Dennis

Tularemia is an uncommon, potentially severe bacterial zoonosis caused by *Francisella tularensis*. The natural cycle of the causative organism involves maintenance of infection in a wide diversity of animal hosts and in certain hard ticks. Transmission of *F. tularensis* to humans, which are incidental hosts, occurs by several modes, including bites by infective ticks and other arthropods, direct inoculation of *F. tularensis* through skin or mucous membranes from handling infectious materials, ingestion of contaminated water or food, or by inhalation of contaminated aerosols or dusts. The agent of tularemia is widely distributed in temperate and subarctic regions of North America and Eurasia. Human infection results in various clinical presentations of varying severity depending on the route of inoculation, the dose and virulence of the infecting strain, and the host defenses. The most common clinical form, ulceroglandular tularemia, presents as an illness with fever, an ulcer at the site of inoculation and regional lymphadenitis. Several other forms occur involving various organ systems. Tularemia is considered to be an important potential weapon of bioterrorism (see Chapter 6).

EPIDEMIOLOGY

Agent

Francisella tularensis (formerly *Pasteurella tularensis*) is a small, facultatively intracellular, Gram-negative coccobacillus. The organism has a lipidated envelope and is able to survive under favorable conditions for several weeks in water, moist soil and decaying animal carcasses. *Francisella tularensis* strains may be divided into two main groups by virulence testing, biochemical reactions and epidemiologic features. Formerly, it was thought that strains of the more virulent type, termed Jellison type A (*F. tularensis* subsp. *tularensis*), were restricted to North America. Recent molecular studies have shown that some *F. tularensis* strains found in Central Asia and in Japan share significant genetic homology with *F. tularensis* subsp. *tularensis*. [1] The less virulent Jellison type B strains (*F. tularensis* subsp. *holarctica*) are found throughout Eurasia, and they are also found widely in North America. A closely related subspecies, *Francisella tularensis* subsp. *novicida*, has been associated with febrile illness in a small number of patients in North America. *Francisella tularensis* is considered to be a potential agent of bioterrorism because it can be weaponized as an aerosol, could result in large numbers of casualties, and because it requires special actions for medical and public health preparedness.[2]

Life cycle

Francisella tularensis is widespread in nature and has been recovered from more than 100 species of wild mammals, at least nine species of domestic animals (including cats, dogs and cattle), numerous species of birds, some amphibians and fish, and more than 50 species of arthropods.[3] [4] The principal natural cycles of the agent involve maintenance of infection in wild mammalian hosts, such as lagomorphs (wild hares and rabbits), terrestrial rodents (especially voles and meadow mice) and aquatic rodents (water rats, muskrats, beaver). Certain species of hard ticks are able to maintain infection from one developmental stage to another. Transmission among animals is accomplished by the bites of blood feeding arthropods or by direct exposures to contaminated materials in the environment (Fig. 177.1).[3] [4] Predation and cannibalism may also contribute to the natural cycle.

Humans become infected:

- when they intrude into the arthropod-borne cycle and are bitten by ticks, which are true biologic vectors, or by blood-feeding flies or mosquitoes that have contaminated mouthparts;
- by handling or ingesting infectious animal tissues or fluids;
- by ingestion of contaminated water or food; or
- by inhalation of infective aerosols or dusts.

Occasional cases occur following infective bites or scratches by cats,[5] or other carnivores or predators with contaminated mouths or claws. Although the agent is highly infectious, requiring only 10–50 organisms to regularly cause experimental infections of humans, person-person transmission has not been documented.

Geographic distribution

Tularemia is endemic throughout much of the Nearctic and Palaearctic regions between latitudes 30°N and 71°N. This includes all of North America from the Arctic Circle to northern Mexico, much of Eurasia and some states of northern Africa along the Mediterranean coast.[3] [4] In North America, the highest incidence of tularemia in humans occurs in south-central, south-eastern, Great Plains and Rocky Mountain regions of the USA (Fig. 177.2),[6] but cases have been reported throughout the continental USA, across Canada, and in Mexico as far south as Guadalajara. In Eurasia, the disease occurs most frequently in Scandinavia and in states of the former Soviet Union. Tularemia also occurs sporadically throughout most of Europe, in some areas of the Near East and Middle East, in Central Asia and in Mongolia. It has not been documented in Central or South America, Australia or Africa outside the Mediterranean littoral.

Populations affected

Tularemia is a rural disease. It affects persons of all ages and both sexes. Groups at highest risk include:

- hunters and trappers, wildlife specialists, animal skinners and dressers, butchers and others who handle potentially infective animal carcasses;
- rural residents, especially farmers, who are exposed to water, soils and dusts contaminated by infected wild animals, such as meadow voles, lagomorphs and aquatic mammals; and
- persons exposed in enzootic areas to bites by certain hard ticks, tabanid flies or mosquitoes.[7] [8] [9] [10]

Disease incidence

Global incidence figures are not available. In Eurasia, recent outbreaks involving hundreds of cases each have been reported from Scandinavia,[10] Kosovo[11] and Spain,[12] and outbreaks involving

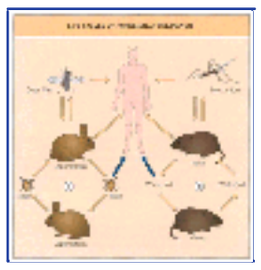

Figure 177-1 Life cycles of *Francisella tularensis.* The two major life cycles in nature are shown. In cycle (a), which is dominant in North America, *F. tularensis* is maintained predominantly among lagomorphs and hard ticks. In cycle (b), which is dominant in Eurasia, *F. tularensis* is principally maintained among cricetine rodents, especially field voles and mice, water voles and other aquatic rodents. Humans are incidental hosts that are infected by tick vectors and by the bites of flies or mosquitoes that have contaminated mouthparts, by direct contact with infected animal carcasses or other contaminated materials, by ingestion of contaminated matter or by inhalation of infectious aerosols or dusts.

Figure 177-2 Reported cases of tularemia per 100,000 population in the USA, 1990–2000.

thousands of persons have been reported in the past from the former Soviet Union. Tularemia incidence is relatively stable in the USA, where the disease has been in steady decline since 1945.[6] In the period 1990–2000, a total of 1368 cases were reported from 44 states, averaging 124 cases (range 86–193) per year. Four states accounted for 56% of all reported cases: Arkansas (315 cases), Missouri (265 cases), South Dakota (96 cases) and Oklahoma (90 cases). The age distribution was bimodal, with highest incidence rates in the age groups 5–9 years and 75 years of age and older. Males predominated in all age groups. Disease onsets peaked in the period May–August (70% of all cases), although cases were reported from all months of the year.[6]

Sources of human infection

Eurasia

Cricetine rodents (especially, meadow voles, lemmings, water voles and muskrats), water and soil contaminated by these animals, hares (*Lepus* spp.) and bites by contaminated mosquitoes (especially *Aedes cinereus* and *Aedes excruscians*) are the principal sources of human tularemia in Eurasia.[3] [4] Mosquito-borne infection occurs in forested and marshy Scandinavian and Baltic regions. Sporadic cases also result in Eurasia from bites by infected ticks and by blood-feeding flies. Outbreaks of tularemia among farmers have been described in Europe following respiratory exposure to dusts from contaminated stored and fresh mown hay,[13] [14] and among workers in agricultural processing plants exposed to contaminated water sprays. Ingestion of water and food contaminated by infected rodents or hares has also resulted in outbreaks in the region, such as recently reported from Kosovo and Turkey.[11] [15] In Japan, the disease has historically been associated with the trapping, handling and eating of wild hares.

North America

The principal animal sources of infection in North America are the cottontail rabbit (*Sylvilagus* spp.), wild hares and rodents (muskrats, beaver, voles, ground squirrels).[3] [4] [7] The agent is vectored by certain species of hard ticks, especially the dog tick, *Dermacentor variabilis*, the lone star tick, *Amblyomma americanum*, and the Rocky Mountain wood tick, *Dermacentor andersoni*. [3] [4] Biting tabanid flies, especially deer flies (*Chrysops* spp.), mechanically transmit the infection.[9] The epidemiology of tularemia in North America has changed significantly since the 1930s and 1940s, when the disease most commonly called 'rabbit fever' had a much higher incidence and when cases were more likely to be linked to the hunting, dressing and butchering of wild rabbits and hares than to arthropod bites.[16] [17]

Seasonality

Mosquito-borne transmission in Eurasia peaks in the summer months. In North America, a peak of tularemia cases in the spring and summer months is associated mostly with bites by ticks and blood-feeding flies, and a second peak in the late autumn and winter is associated with handling infected animals, especially among hunters and trappers.[17]

PATHOGENESIS AND PATHOLOGY

The principal pathologic changes in localized disease occur at the cutaneous site of inoculation and in the regional lymph nodes draining the site; when the disease is disseminated, the lungs, spleen, lymph nodes, liver and skin are most often involved.[18] [19] [20] The primary skin lesion begins as a papule several days following inoculation. The papule rapidly progresses to a vesicle that erodes and develops into an ulcer, which is typically 2–3cm in diameter with an irregular slightly raised and erythematous border. The base is necrotic, and frequently covered with a thick dark scab that can mimic the eschar of cutaneous anthrax (Fig 177.3). Affected lymph nodes show hemorrhagic necrosis and may suppurate. Secondary skin lesions have also been described in tularemia, including papular and papulovesicular lesions, erythema nodosum and erythema multiforme.

Francisella tularensis is a facultative intracellular organism, and the response to infection has a prominent component of cell-mediated immunopathology.[21] Histologically, the early disease is characterized by focal suppurative necrosis. The central area of necrosis is at first composed primarily of polymorphonuclear leukocytes and macrophages, which may be replaced by epithelioid cells in more advanced lesions. A wall of fibroblasts may surround the acute inflammatory reaction. Later, smaller lesions may be indistinguishable from miliary tubercles. A frequent finding on pathologic examination of affected lungs is small (3–12mm), yellowish, necrotic subpleural nodules. Patchy interstitial infiltrates are common in pneumonic tularemia; bronchopneumonia is found in about 30% of cases, and lobar pneumonia with consolidation of an entire lobe in about 15% of pneumonic cases. Lung abscesses occasionally occur. Hilar lymph nodes may be inflamed and enlarged.

Prevention

Persons exposed in endemic areas to ticks, biting flies or mosquitoes should, when feasible, wear protective clothing, tuck their trouser legs into their socks and apply repellents containing diethyltoluamide (DEET) to skin and clothing as directed by the manufacturer. Permethrin-based products can be applied to clothing to kill ticks and biting flies on contact. Frequent examinations should be made to identify and remove ticks on clothing and skin. Persons should always avoid direct contact with sick or dead animals, and hunters, trappers, dressers and butchers should wear impervious gloves when skinning and handling wild animal carcasses. Recently, an outbreak of pneumonic tularemia occurred in Massachusetts, USA, among landscapers using power tools that typically raise environmental dusts,[22] and the use of fine particle masks while engaged in these activities has been suggested as a possible means of reducing infective inhalation exposures.

Live attenuated vaccines have been used to protect laboratory personnel who routinely work with *F. tularensis*. Vaccines have also been used in an attempt to reduce the incidence of disease among rural residents of highly endemic areas of the former Soviet Union. A live attenuated tularemia vaccine was until recently available in the USA under investigational new drug (IND) protocol; its manufacture is currently under Food and Drug Administration review. Persons exposed to a laboratory accident possibly resulting in aerosolization or inoculation of *F. tularensis* should be considered for prophylactic antibiotic administration or placed on fever watch and closely monitored for early signs of illness.

CLINICAL FEATURES

The primary forms of tularemia include:[18] [19] [20]

- ulceroglandular tularemia (45–85% of cases),
- glandular tularemia (10–25% of cases),
- oculoglandular tularemia (<5% of cases),
- typhoidal (septic) tularemia (<5% of cases),
- oropharyngeal tularemia (<5% of cases), and
- pneumonic (inhalation) tularemia (<5% of cases).

The incubation period is usually 2–5 days (range 1–14 days). Onset is sudden; typically, the patient has fever of 100–104°F (38–40°C) and a constellation of non-specific manifestations including chills, headache, generalized body aches (often prominent in the lumbosacral region), nausea, weakness, cough and chest pain.[18] [19] [20] Without treatment, nonspecific symptoms usually persist for several weeks. Sweats, chills, progressive weakness and weight loss characterize the continuing illness. Any of the principal forms of tularemia may be complicated by bacteremic spread that may lead to secondary sepsis, tularemic pneumonia, meningitis or other metastatic infection.

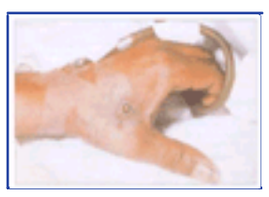

Figure 177-3 Tularemic ulcer with eschar formation after percutaneous inoculation of *Francisella tularensis*.

Before antibiotics became available, the overall mortality rate from infections with the more severe type A strains was in the range of 5–10%; however, a considerably higher fatality rate was reported for typhoidal and pneumonic forms of disease. Untreated, infections with type B strain have been associated with a fatality rate of only 1–3%. In the USA, the fatality rate for all forms in recent years has been less than 2%.[23]

Ulceroglandular tularemia

A local papule appears at the site of inoculation at the time of, or shortly after, the onset of fever and other generalized symptoms. This becomes vesiculated and pustular, and then ulcerates within a few days of its first appearance. Typically, the ulcer is tender, has an indolent character and may be covered by a scab (Fig. 177.3). By the time of ulceration, painful lymphadenitis occurs in one or more adjacent nodes in the afferent pathway. In persons infected by handling contaminated materials, the epitrochlear nodes (8%) and the axillary nodes (65%) are the most commonly affected. In persons infected by arthropod bites, the femoral-inguinal nodes (64%), the axillary nodes (24%) and the cervical nodes (6%) are commonly involved.[20] In some cases, an abscessed node may suppurate, create a sinus tract and

discharge purulent material to the outside.

Oculoglandular tularemia

Oculoglandular tularemia (Parinaud's syndrome) follows contamination of the conjunctival sac. Ulceration may occur on the conjunctiva, which becomes severely inflamed, with marked edema and vasculitis. Characteristically, there is painful swelling of nodes draining the periorbital tissues, such as the preauricular, submandibular and cervical chain nodes.

Glandular tularemia

Glandular tularemia differs from the ulceroglandular type only in not having the local cutaneous ulceration. It is more likely to follow arthropod-borne inoculation than direct percutaneous inoculation of the hands and fingers of persons handling infected animal tissues.

Typhoidal tularemia

Typhoidal tularemia presents as an acute illness without localizing signs.[20] The diagnosis is most often made by the identification of *F. tularensis* in cultures of the blood. Abdominal pain, diarrhea and vomiting may be prominent in the early illness. Sepsis may occur and the systemic inflammatory response syndrome may ensue, rarely

accompanied by complications such as disseminated intravascular coagulation and bleeding, acute respiratory distress syndrome, shock and organ failure. Typhoidal tularemia may result from inapparent inhalation exposures, that then progresses to pneumonia in more than 50% of cases; infection of the kidneys and the meninges may also occur. In some cases, the upper gastrointestinal tract may be the principal target organ in typhoidal tularemia.

Oropharyngeal tularemia

Oropharyngeal tularemia is acquired by ingesting inadequately cooked game, or contaminated water or food. The patient may develop a painful exudative pharyngitis or tonsillitis, or a stomatitis, sometimes with ulceration, and tender cervical lymphadenopathy. Suppuration, fistula formation and drainage of cervical nodes may occur.[11]

Pneumonic tularemia

Pneumonic tularemia is a common secondary complication of other forms of tularemia. Infrequently, primary pneumonia arises from inhalation of an infective aerosol or dust. In addition to fever, chills, fatigue and other generalized symptoms of infection, pulmonic manifestations include cough (usually with minimal sputum production), chest discomfort, sometimes with pleuritic pain, dyspnea, tachypnea and occasionally mild hemoptysis. *Francisella tularensis* was weaponized for aerosol delivery by biowarfare programs during and after the Second World War, and it is assumed that this would be the most likely mode of delivery in a potential terrorism attack. The expected result would be an outbreak of pneumonic tularemia and non-specific febrile illness (typhoidal tularemia) beginning 3–5 days after exposure; this might at first be difficult to distinguish from the many usual causes of community-acquired infection. It is possible that an aerosol exposure could also result in cases of oropharyngeal and oculoglandular tularemia. Since naturally acquired tularemia is almost entirely a rural disease, bioterrorism should quickly be suspected should a cluster of cases occur in an urban population. The recognition, and medical and public health management of tularemia as a weapon of bioterrorism has recently been outlined.[2]

DIAGNOSIS

The presumptive diagnosis of tularemia is made by clinical examination combined with information on potentially infective exposures. Differential diagnostic possibilities are many, as follows:

- in persons who have glandular or ulceroglandular disease they include plague, sporotrichosis, cat-scratch fever, lymphogranuloma venereum, streptococcal or staphylococcal lymphadenitis, toxoplasmosis, mycobacterial infection, chancre and chancroid;
- in persons who have oropharyngeal tularemia, other bacterial and viral causes of stomatitis, pharyngitis and cervical adenitis must be considered, such as streptococcal infection, infectious mononucleosis, mycobacterial infection, adenoviral infection and diphtheria;
- in persons who have pneumonia, they include mycoplasmal pneumonia, chlamydial pneumonia, Legionnaires' disease, staphylococcal or streptococcal pneumonitis, *Haemophilus influenzae* pneumonia, plague, histoplasmosis and tuberculosis; and
- in persons who have typhoidal tularemia, they include bacterial endocarditis, disseminated mycobacterial or fungal infection, typhoid fever, brucellosis, listeriosis, leptospirosis, Q fever, plague and other causes of sepsis.

The clinical diagnosis of tularemia is confirmed by cultural isolation of *F. tularensis* or diagnostic rises in serologic titers.[24] The organism can be grown in routine culture systems; however, clinical suspicion of tularemia is critical in directing selection of the correct (cysteineenriched) culture media that favors growth of *Francisella* spp. In addition to culture, materials other than blood should be streaked on glass slides for presumptive diagnosis by direct fluorescent antibody testing. The agglutination reaction for combined IgM and IgG immunoglobulins is the routine serological procedure in use in most laboratories. Reference laboratories use microagglutination methods that are more sensitive than tube agglutination procedures. Antibody titers usually do not rise before 10 days or more of illness onset. A 4-fold rise in titer between acute and convalescent serum specimens, or a single titer of 1:160 or greater is considered diagnostic for *F. tularensis* infection. Potentially useful diagnostic procedures include enzyme-linked immunoassay, immunoblotting for IgM antibodies, polymerase chain reaction assays immunochromatographic handheld assays, and DNA probes, but these are still in the experimental stages of development. [25] Many routine diagnostic laboratories have policies that exclude work on *F. tularensis* because it readily aerosolizes from culture and is notorious as a cause of laboratoryacquired infections. [26] Biosafety level 2 precautions are essential for routine procedures, and biosafety level 3 precautions are required for safe manipulation of cultures and for animal studies.[27]

MANAGEMENT

Patients are best managed under hospital care until a full diagnostic evaluation and satisfactory treatment response has occurred. Streptomycin, which is bactericidal, is the drug of choice based on experience and efficacy. It is given to adults intramuscularly in a dosage of 0.5–1.0g q12h for 10 days. Gentamicin, an acceptable alternative, is given parenterally in an adult dosage of 3–5mg/kg per day, once daily or in equal divided doses at 8-hour intervals[28] for 10 days. A tetracycline (most commonly, doxycycline) or chloramphenicol may be used in place of an aminoglycoside, especially in less severely ill patients, but use of these bacteriostatic agents occasionally results in primary treatment failures, and dosage schedules of at least 14 days are recommended to prevent relapses. Oral or parenterally administered ciprofloxacin has been used to treat adults and children with good success in standard doses for 10 days.[29] Patients begun on parenterally administered antimicrobials can switch to oral administration when clinically indicated. *Francisella tularensis* organisms routinely produce ß-lactamase and are resistant to ß-lactam antibiotics and azithromycin, but they are generally highly susceptible to aminoglycosides, tetracyclines, chloramphenicol and quinolones.[30] [31] Penicillins and cephalosporins are not effective and should not be used. Typically, fever and general symptoms of acute infection begin to regress within 24–48 hours of initiation of appropriate antibiotic administration. Factors associated with a poor outcome include delays in seeking medical care, or delays in diagnosis and treatment, and underlying medical disorders, such as diabetes or alcoholism. [32] Standard (universal) precautions only are required for purposes of hospital infection control.[2] Postexposure prophylactic antibiotic treatment of close contacts is not recommended because human to human transmission is not known to occur.

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Chapter 178 - Diphtheria

Androulla Efstratiou

EPIDEMIOLOGY

Mass immunization with diphtheria toxoid during the 1940s and 1950s resulted in the virtual elimination of diphtheria in many countries by the 1970s as a result of the introduction of the Expanded Program on Immunization by the World Health Organization (WHO). However, an all-time low of 623 reported cases in the WHO European Region was reached in 1980, which is in contrast to the total global figure of 97,811. Since 1989, major resurgence was observed in Europe, centered mostly in the newly independent states (NIS) of the former Soviet Union. The epidemic within the NIS commenced in 1990 and affected all 15 countries of the NIS by the end of 1994. In 1990, the number of reported cases was 1481 and peaked at 50,449 in 1995. In 1995, cases in the NIS accounted for 88% of reported cases globally (56,966). As a result of vigorous action taken in the NIS and collaboration between the epidemic countries and various international agencies, the incidence began to decline in 1996 (Fig. 178.1). The decreasing trend has continued; the number of cases reported in 1999 and 2000 was approximately 1500.[1] There is therefore evidence of progress toward control of the epidemic in the NIS since 1996. Diphtheria control is still urgently required in Georgia, Kyrghyzstan, Latvia, the Russian Federation and Tajikistan. During 2001–2, Latvia had the highest rate of diphtheria in Europe and the reasons for the resurgence in Latvia are unclear.[2] The overall situation within the European Region is still considered to be of high priority by the WHO.

A major contribution toward the control of these epidemics has been the formation of a specific microbiologic and epidemiologic global network for diphtheria. Initially, the European Laboratory Working Group on Diphtheria (ELWGD) was formed in 1993 at the request of WHO, with participation from primarily European countries.[3] During the past 7 years the network has expanded significantly and now comprises participants from 40 countries worldwide. The Diphtheria Surveillance Network (DIPNET) continues its collaborative and co-ordinated approach to support countries to improve diphtheria surveillance for early detection of cases and contacts by accurate microbiologic surveillance and the establishment of a network of national and international laboratories (http://www.phls.org.uk/inter/diphtheria/menu.htm).

Small epidemics are also occurring in other parts of the world, including South East Asia, India, Pakistan, Bangladesh and South America.[4] All these outbreaks and epidemics strongly emphasize the need for maintaining full immunization coverage and clearly demonstrate that, when immunization programs are disrupted by social, political or other changes, the disease may return. Vaccine-induced immunity does not last for life and various serologic studies in developed countries have shown that many adults are potentially susceptible, particularly when immunization courses are incomplete.

Increasing international travel and migration from areas where the disease is prevalent clearly indicate the need for maintaining clinical awareness of the disease, particularly among travelers from these areas and their contacts. All these epidemics have been classically associated with the causative agent of diphtheria, namely toxin-producing *Corynebacterium diphtheriae*. However, more recently within Europe, there have also been increasing reports of diphtheria caused by toxigenic *Corynebacterium ulcerans*. The latter is a known veterinary pathogen and causes mastitis in cattle and other domestic and wild animals. Toxigenic strains of *C. ulcerans* have been associated with classical diphtheria as well as milder symptoms. At least one death in the UK has recently been attributed to such an infection.[5]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of the disease is complex. Virulence is thought to be associated with the production of an exotoxin by the causative organisms, potentially toxigenic corynebacteria, *C. diphtheriae* or *C. ulcerans*. The *C. diphtheriae* genome has now been sequenced and the complete genome is finished and is currently undergoing annotation and analysis. The annotation has revealed a number of interesting features that should provide an insight into the life and pathogenicity of the organism (sequence data: http://www.sanger.ac.uk/Projects/C_diphtheriae/).

Humans are the only known reservoir for *C. diphtheriae*. The mode of transmission is usually by direct contact with a patient or a carrier by aerosol transmission. More rarely, contact with articles contaminated with discharges from lesions or infected material can also occur.

Corynebacterium ulcerans, however, has always been regarded as a zoonosis and has always traditionally been associated with the ingestion of unpasteurized dairy products. In the UK, there has been recent documentation of toxigenic *C. ulcerans* in domestic cats with chronic rhinitis.[6] There had previously been no reports in the literature of infection among small domestic animals with *C. ulcerans*, so the implications are unclear. The risk to humans is greatest in household contacts of the cats and other very close human contacts who may examine them, such as veterinary surgeons. Overall, the bacteria do not actively invade deep tissues or the blood but tend to multiply locally and produce diphtheria toxin. Despite the proven role of the toxin, toxigenicity does not appear to be synonymous with pathogenicity because strains that do not produce toxin can survive for long periods in the upper respiratory tract and are also able to cause disease, ranging from relatively mild pharyngitis to severe tonsillitis and, in rare instances, systemic disease such as endocarditis.

PREVENTION

Diphtheria is notifiable in all countries of Europe and North America. It is imperative that all cases be rapidly identified and properly investigated according the WHO guidelines.[7] Cases are usually classified as suspected, probable or confirmed and are definitively described in the WHO manual. Local European guidelines are also available, in particular within the UK, where clinical recommendations have also been established for the management of toxigenic *C. ulcerans* cases. The advice is similar to that given for

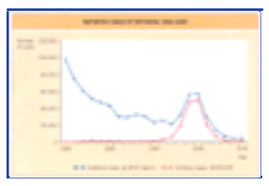

Figure 178-1 Reported cases of diphtheria 1980–2000. Cases reported to the WHO worldwide and within the WHO European Region.

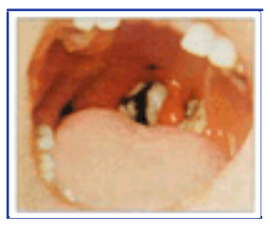

Figure 178-2 Characteristic diphtheria pseudomembrane in a child. *Courtesy of Dr Norman Begg.*

toxigenic *C. diphtheriae* with management of close contacts as well as index cases.[5] The main aim of prevention and control of diphtheria is to eradicate toxigenic corynebacteria from the community and to maintain adequate levels of protective immunity by active immunization. Published data have indicated that, for example, approximately 38% of adult UK blood donors are susceptible to diphtheria. A significant trend of decreasing immunity with increasing age is apparent from many recent serologic studies.[8] [9] The recommendations and guidelines for immunization strategies are given by many health authorities and consist of primary immunization (e.g. within the UK three doses for children aged 2–4 months with an interval of 1 month between each dose) and reinforcing immunization (preschool booster dose and

another booster just before leaving school for all teenagers). However, in any country where an epidemic situation exists or is imminent, aggressive mass immunization of high-risk groups must be implemented. Low levels of immunity in adults and a gradual increase in the percentage of children not immunized with at least three doses of adsorbed diphtheria—tetanus—pertussis vaccine pose a danger for recurrence of diphtheria, as seen in eastern Europe.[10] [11]

CLINICAL FEATURES

Diphtheria is usually classified according to its site of manifestation; there are two major 'forms' of disease the classic respiratory condition and nonrespiratory (cutaneous) diphtheria. The clinical manifestations vary from place to place and from time to time depending

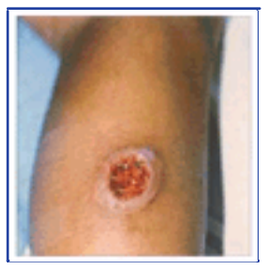

Figure 178-3 Characteristic diphtheria lesion of the lower limb, showing the classic rolled, 'crater-like' edge and eschar.

on a range of host and environmental factors that are not fully understood. The most important factor is individual immunity to the toxin, which is mediated either by neutralizing antibody induced by toxoid or by natural immunity.

The incubation period for the disease is 2–5 days, occasionally longer. The first symptoms are malaise, low-grade fever, sore throat and loss of appetite. A pseudomembrane forms in the throat and may extend into the lungs (Fig. 178.2). The disease is usually subdivided into three stages: early, late and severe. Each stage is associated with the isolation of the causative organism and the presence of the pseudomembrane. The pseudomembrane adheres to underlying tissue and the tissue bleeds when attempts are made to remove it. This feature is useful for diagnosing diphtheria because pseudomembranes caused by other infectious agents are not adherent.

In the early stage of disease, manifestations are localized in the upper respiratory tract or in skin lesions, leading to the severe stage resulting in toxic circulatory collapse, severe edema of the neck, submucosal or skin petechial hemorrhages and acute renal insufficiency. The late stage is associated with neurologic and cardiologic features owing to the dissemination of the toxin to the major organs. This results in extensive cardiac and neurologic damage: myocarditis, blurred vision and paralysis of the soft palate, diaphragm and limbs. Between 10% and 25% of patients who have

clinical respiratory diphtheria develop some form of myocardial damage and up to 75% develop a neuropathy. Symptoms may range from pharyngitis with low-grade fever in faucial diphtheria to partial or even complete respiratory obstruction due to the formation of the pseudomembrane in laryngeal or tracheobraonchial diphtheria.

Cutaneous diphtheria is a chronic condition associated with the tropics; it is prevalent within South East Asia. The characteristic lesions are difficult to treat and they often take months even years to heal. They are quite marked in their appearance with a characteristic rolled, 'crater-like' edge (Fig. 178.3). The lesions are covered with an eschar, a hard bluish-gray membranous scar that is slightly raised. The lesion, therefore, acts as a potential reservoir for transmission and spread of the pharyngeal form of the disease. Cutaneous diphtheria lesions proved to be the 'vehicles' of transmission of the organism in the outbreak that occurred in the USA in the late 1970s among the Skid Row alcoholics.[12]

DIAGNOSIS

The presumptive clinical diagnosis of respiratory diphtheria is based on the presence of the pseudomembrane. However, in many instances, particularly in countries where the disease is uncommon, diagnosis is often difficult and diphtheria may be misdiagnosed or confused with other diseases such as severe streptococcal sore throat, glandular fever or even Vincent's angina. This thus highlights the importance of the microbiologic diagnosis of the disease, and isolation and confirmation of the causative organism, toxigenic *C. diphtheriae* or *C. ulcerans* (see Chapter 226). However, bacteriologic diagnosis should be complementary to the clinical diagnosis and should not delay immediate and specific treatment of the patient. A throat swab and, if possible, samples of the membrane should be collected and cultured. The most important test is, of course, the test to detect diphtheria toxin. Guidelines for the microbiologic diagnosis of diphtheria are described in the WHO manual for the laboratory diagnosis of the disease.[13] Guidelines that incorporate the laboratory and clinical diagnosis of *C. ulcerans* are described further in the UK guidelines.[14] Current approaches to laboratory diagnosis in Europe and beyond have recently been updated.[15]

Close (household and kissing) contacts may be asymptomatic carriers or at risk of developing the disease and they should also be sampled by taking throat swabs. Local public health authorities must be alerted if a suspected case has been identified; the appropriate measures for swabbing, contact tracing and management (including immunization and antibiotic prophylaxis) should be initiated. Guidelines for the control, treatment and management of the disease, cases and contacts have been outlined by the WHO.[7] A travel, medical and immunization history of the suspected case and close contacts should be ascertained.

MANAGEMENT

Because patients who have suspected respiratory diphtheria may deteriorate rapidly, treatment should not be delayed and should not depend solely upon the result from the microbiology laboratory. In suspected cases, treatment should commence with diphtheria antitoxin, antibiotics and strict isolation. The three main areas of therapy are essentially:

- administration of diphtheria antitoxin;
- administration of antibiotics; and
- strict isolation procedures.

Diphtheria antitoxin

Administration of diphtheria antitoxin (hyperimmune horse serum) aims at neutralizing circulating toxin that has not yet bound to tissue. The dose of antitoxin depends on the severity of the disease and the site and extent of the pseudomembrane. Before administration of antitoxin the patient must be tested for sensitivity to horse serum and, if necessary, desensitized. Usually, in cases of tonsillar or pharyngeal diphtheria, the dose is 15,000–40,000 units of antitoxin by intramuscular or intravenous injection; in severe forms, doses as high as 80,000–100,000 units are given.[7]

Antibiotics

Antibiotics are essential for eradicating the organism and eliminating its spread but they are not a substitute for antitoxin treatment. Penicillin (0.5–1.0g q6h orally) or erythromycin (500mg q6h) is recommended. Antibiotic therapy should be continued for 14 days. Antibiotics are also important for eradicating colonization in contacts and for postexposure prophylaxis.[7] At present, there is no significant, relevant antimicrobial resistance among *C. diphtheriae*; however, erythromycin resistance has been reported from South East Asia.[16] [17]

Isolation procedures

Patients should be cared for in strict isolation and attended by staff whose immunization history is documented. Before patients are discharged they should be confirmed as 'culture-negative' for *C. diphtheriae* or *C. ulcerans* by collection of three swabs at 24-hour intervals and also given a booster dose of diphtheria toxoid, because natural infection does not necessarily confer immunity. It is important also to monitor the cardiac status closely with early intervention if appropriate (e.g. pacing for cardiac conduction disturbances and therapy for arrhythmias).

Cutaneous diphtheria

For cutaneous diphtheria, vigorous cleaning of the wound with soap and water is recommended in addition to antibiotic therapy. It is also advisable to take respiratory

tract swabs from both the index case and close household contacts. Some authorities also advocate the use of antitoxin therapy because toxic sequelae have been documented among patients who have cutaneous disease.[7]

SUMMARY

In summary, it is important to consider the following practical points:

- the diagnosis of diphtheria should be considered in travelers returning from endemic and epidemic areas or from areas where recent cases have occurred;
- a history of the immunization status of the patient does not necessarily exclude the diagnosis of diphtheria;
- microbiologic confirmation is essential for the definitive diagnosis but treatment must not be delayed pending confirmation; and
- expert opinion should be sought early and contacts must be actively followed up.

Acknowledgements

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Chapter 179 - Scrub Typhus and Other Tropical Rickettsioses

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INTRODUCTION

Rickettsioses (also called typhus) are infectious diseases caused by obligate intracellular bacteria formerly grouped in the order Rickettsiales. These organisms were first described as short, Gram-negative rods that retained basic fuchsin when stained by the method of Gimenez.[1] Recent developments in molecular taxonomic methods have resulted in the reclassification within the Rickettsiales.[1] However, four groups of diseases are still usually called rickettsioses. These include:

- scrub typhus due to *Orientia tsutsugamushi*;
- diseases due to bacteria of the genus *Rickettsia* (including the spotted fever group and the typhus group);
- ehrlichioses due to bacteria within the family Anaplasmataceae (ehrlichioses have not, however, been properly demonstrated to occur in the tropics); and
- Q-fever, which is due to *Coxiella burnetii*.

DIFFERENTIAL DIAGNOSIS BASED ON REGION

The agents of rickettsioses are associated with arthropods including ticks, mites, fleas and lice, which may act as vectors, reservoirs and/or amplifiers of the organisms. Most of these vectors favor specific optimal environmental conditions, biotopes and hosts. These factors determine the geographic distribution of the vector and consequently the risk area for the rickettsioses. This is particularly true when vectors are also reservoirs of pathogens, as seen in the case of ticks for most spotted fever group rickettsioses.[1] [2] Thus, although some rickettsioses are distributed worldwide (i.e. Q-fever, murine typhus), a specific area is usually associated with specific diseases. Therefore, most rickettsioses are geographic diseases. Table 179.1 presents the rickettsioses occurring in tropical areas of Africa, Asia, America and Australia.[1] [2]

EXPOSURE

Most rickettsioses are transmitted to humans by arthropods. Thus, exposure to the disease is closely linked to exposure to the arthropod vectors. Furthermore, although *C. burnetii*, the agent of Q-fever, has been found to infect more than 40 species of ticks throughout the world, the role of ticks in human infections is not confirmed. In fact, Q-fever is usually acquired by the ingestion or inhalation of virulent organisms from infected mammals, mostly goats, sheep and cats, and their products.[2]

Chiggers

'Chiggers' is the commonly used name of several species of larval-stage, trombiculid mites, which are the vectors (and reservoirs) of scrub typhus due to *Orientia tsutsugamushi*. Risk areas range from typical tropical secondary growth (scrub) vegetation in the Asia-Pacific region to temperate zones and even the Himalayas. Although scrub typhus is essentially an occupational disease among rural residents engaged in agricultural or gathering activities, travelers or soldiers in the field may be infected when entering the biotope of the vectors. [3]

Ticks

Ticks are obligate hematophagous acarins that parasitize every class of vertebrate throughout the world and may bite people. Ticks are not only vectors but also reservoirs of most of the currently known spotted fever group rickettsiae. Ecologic characteristics of the tick are keys for the epidemiology of tick-borne diseases. For example, the brown dog tick *Rhipicephalus sanguineus*, which is the vector of *Rickettsia conorii*, lives in dog environments (kennels and human houses) and has a low affinity for people. Cases of Mediterranean spotted fever are sporadic in endemic areas and most cases are encountered in urban areas. In contrast, *Amblyomma hebraeum*, which are vectors of *Rickettsia africae* in southern Africa, emerge from their habitats and actively attack animals, particularly nearby ruminants. They also feed readily on people that enter their biotopes. Furthermore, numerous ticks can attack a host at the same time. These ticks are highly infected by rickettsiae. Thus, cases of African tick-bite fever often occur as grouped cases among subjects entering the bush (e.g. during a safari) and people can suffer several tick bites simultaneously.[2] [4]

House mouse mite

The house mouse mite (*Liponyssoides sanguineus*) has been described as a vector of *R. akari*, the agent of rickettsialpox. These hematophagous arthropods maintain *R. akari* among house mice (*Mus musculus*) and may transmit the disease when biting people. Exposure to the mite is linked to contact with house mice. However, *Rickettsia akari* has been also identified in Korean voles (*Microtus fortis pelliceus*).

Fleas

Fleas are hematophagous insects. The rat flea *Xenopsylla cheopis* is the main vector of murine typhus due to *Rickettsia typhi*, whereas rodents, mainly *Rattus norvegicus* and *Rattus rattus*, act as reservoirs. It is generally accepted that most people become infected when flea feces containing *R. typhi* contaminate disrupted skin or are inhaled into the respiratory tract. [1] Infections may result from flea bites as well. Exposure to rat fleas is linked to exposure to rats. The diseases are urban as well as rural. Fleas are also suspected to be the vectors of the emerging infection due to *Rickettsia felis*. Cat fleas (*Ctenocephalides felis*), dog fleas (*C. canis*) and human fleas (*Pulex irritans*) are all possible vectors, but the disease cycle has not yet been described.

Lice

Human body lice (*Pediculus humanus corporis*) are insects that live in human clothing. They thrive during periods of cold weather, particularly in conditions or areas of reduced hygiene maintenance, poverty and wars. The body louse is the vector of *Bartonella quintana* (the agent of trench fever), *Borrelia recurrentis* (agent of relapsing fever) and *Rickettsia prowazekii* (the agent of epidemic typhus). Until recently, epidemic typhus was considered to be endemic only in Ethiopia. However, an outbreak of typhus in approximately

TABLE 179-1 -- Tropical rickettsioses throughout the world.

TROPICAL RICKETTSIOSES THROUGHOUT THE WORLD					
Location by continent	**Vectors**	**Disease**	**Agent**	**Specific areas**	**Risk of exposure**

Africa	**Ticks**				
	Rhipicephalus sanguineus	Mediterranean spotted fever	*Rickettsia conorii*	Mediterranean area (Algeria, Tunisia, Morocco, Libya, Egypt) Kenya, Somalia, Central African Republic, Zimbabwe and South Africa	Urban (2/3) and rural (1/3)
	Amblyomma sp.	African tick-bite fever	*R. africae*	Sub-Saharan Africa	Rural area. Safari
	Hyalomma marginatum	Unnamed	*R. aeschlimmanii*	Morocco, Zimbabwe, South Africa	
	H truncatumyalomma[*]	Unnamed	*'R. mongolotimonae'*	Niger[*]	
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous. High prevalence in coastal areas	Contact with rats and rat fleas
	Pulex irritans (human flea)[*]	Flea-borne spotted fever	*R. felis*	Ethiopia[*]	Lack of hygiene
	Lice				
	Pediculus humanus corporis	Epidemic typhus	*R. prowazekii*	Ethiopia, Burundi, Rwanda, Uganda, Algeria	Civil war, refugee camps, lack of hygiene in cold or mountainous areas
Americas	**Ticks**				
	Amblyomma cajennense	Rocky Mountain spotted fever	*R. rickettsii*	Central America (Mexico, Panama, Costa Rica), South America (Brazil, Colombia)	Rural areas
	Amblyomma spp.	African tick-bite fever	*R. africae*	West Indies	Rural areas
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous	Contact with rats and rat fleas
	Ctenocephalides felis (cat flea)[*]	Flea-borne spotted fever	*R. felis*	Texas, California, Mexico, Brazil, Peru	
	Lice				
	Pediculus humanus corporis	Epidemic typhus	*R. prowazekii*	Peru and Andean area	Lack of hygiene in mountainous area
Asia	**Ticks**				
	Rhipicephalus sanguineus	Indian tick typhus	*R. conorii* Indian	India. Suspected in Thailand	
	Ixodes granulatus[*]	Flinders Island spotted fever	*R. honei*	Thailand[*]	
	Ixodes ovatus	Oriental or Japanese spotted fever	*R. japonica*	Japan[†]	Agricultural activities, bamboo cutting
	Dermacentor taiwanensis				
	Haemaphysalis longicornis				
	Haemaphysalis flava				
	Ixodes ovatus,[*] *I. persulcatus*,[*] *I. monospinus*[*]	Unnamed	*R. helvetica*	Japan.[†] Suspected in Thailand	
	Haemaphysalis asiaticum[*]	Unnamed	*'R. mongolotimonae'*	China (Inner Mongolia)[*] ,[†]	
	Dermacentor nuttalli, *D. marginatus*	North-Asian tick typhus	*R. sibirica*	Northern China, former USSR (Asian republics, Siberia), Armenia, Pakistan	
	Haemaphysalis concinna				
	Dermacentor silvarum	Unnamed	*'R. heilongjiangii'*	North-eastern China	
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous	
	Ctenocephalides felis (cat flea)[*]	Flea-borne spotted fever	*R. felis*	Thailand	
	Trombiculid acarins				
	Leptothrombidium spp.	Scrub typhus	*Orientia tsutsugamushi*	Asia-Pacific region from Korea to Papua New Guinea and Queensland, Australia, and from Japan to India and Afghanistan	Rural activities Agricultural activities Soldiers in the field
	Lice				
	Pediculus humanus corporis	Epidemic typhus	*R. prowazekii*	China. India (Kashmir)	Civil war, refugee camps, lack of hygiene in cold or mountainous areas
	House mouse mite				
	Liponyssoides sanguineus	Rickettsialpox	*R. akari*	Korea[‡]	

Australia	**Ticks**				
	Unknown	Flinders Island spotted fever	*R. honei*	Flinders Island, north-eastern Australia	
	Ixodes holocyclus	Queensland tick typhus	*R. australis*	North-eastern Australia	
	Fleas				
	Xenopsylla cheopis (rat flea)	Murine typhus	*R. typhi*	Ubiquitous	Contact with rats and rat fleas
	Trombiculid acarins				
	Leptothrombidium spp.	Scrub typhus	*Orientia tsutsugamushi*	Northern Territory and Western Australia, Queensland, Australia	Rural activities
					Agricultural activities
					Soldiers in the field
Note that Q-fever due to *C. burnetii* is distributed worldwide (except in New Zealand).					

*Suspected by detection of the pathogen in the relevant arthropod
†Although not included in tropical areas, Japan and China are mentioned regarding the aspects of travel medicine
‡Isolated from voles (*Microtus fortis pelliceus*)

100,000 people was reported during the civil war in Burundi in 1997, in addition to cases that were reported in Peru, Russia, the USA, Algeria and France in the 1990s.[5] Epidemic typhus, then, must still be considered a potential major health risk in tropical countries; this is thought to be particularly true of refugee camps in the cooler mountainous areas. Infected body lice always die within 1–2 weeks (red louse disease). People, therefore, are considered to be the major reservoirs of this disease. *Rickettsia prowazekii* is transmitted to people by infected louse feces (in which *R. prowazekii* survives for weeks), through aerosols (thought to be the main route of infection for health workers attending patients) or by skin autoinoculation, following scratching.

CLINICAL FEATURES

Scrub typhus

Symptoms occur usually 7–10 days after the chigger's bite. A papule at the bite site that later ulcerates, forming a black crust of eschar (Fig 179.1 and Fig 179.2), is typically associated with fever, regional lymphadenopathy, a macular or maculopapular rash, headache and myalgia. However, eschar and rash may be absent or unnoticed.[3]

Spotted fevers

These diseases include tick-borne rickettsioses, rickettsialpox due to *R. akari*, and the emerging flea-borne infection due to *R. felis*. Generally, the clinical symptoms of spotted fever group rickettsioses begin 6–10 days after the arthropod bite and typically include fever, headache, muscle pain, rash, local lymphadenopathy and a characteristic inoculation eschar ('tache noire') at the bite site.[1] However, the main clinical signs vary depending on the rickettsial species involved and therefore may allow us to distinguish between the diseases. For example, African tick-bite fever is characterized by the occurrence of multiple inoculation eschars and grouped cases, due to the fact that numerous highly infected *Amblyomma* may attack and bite many people in several places at the same time.[4] In contrast, in cases of Mediterranean spotted fever due to *R. conorii*, a single eschar is usual because of the low likelihood of the tick biting people and a low rate of infection of the ticks. Details of each pathogen and clinical pictures are presented elsewhere (see Chapter 14).

Murine typhus

Murine typhus is a mild disease with non-specific signs. The incubation period is 7–14 days and at presentation the classic triad of fever,

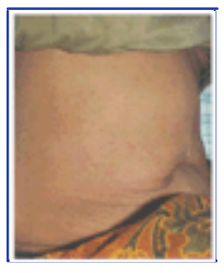

Figure 179-1 Macular rash in a patient with scrub typhus.

headache and skin rash is observed in less than 15% of cases. Later in the disease progression, fever and headache occur more frequently than rash. Rash is present in less than 50% of patients and is often transient or difficult to observe. Nausea, vomiting, abdominal pain, diarrhea, jaundice, confusion and seizures have also been reported.[1]

Epidemic typhus

The incubation period is about 10–14 days. Patients develop malaise and vague symptoms before the abrupt onset of signs including fever (100%), headache (100%) and myalgia (70–100%). Other common

Figure 179-2 Eschar at the bite site, a hallmark of rickettsial diseases.

signs include nausea or vomiting, coughing and neurologic involvement ranging from confusion to stupor and coma. Diarrhea, pulmonary involvement, myocarditis, splenomegaly and conjunctivitis may also occur. Most patients (20–80%) develop a skin rash that classically begins on the trunk of the body and spreads to the limbs. It may be macular, maculopapular or petechial and may be difficult to detect on darker skin tones. Epidemic typhus may be confused with typhoid. Recrudescence of epidemic typhus, also called Brill-Zinsser disease, can appear many years after the acute disease and has milder symptoms.[5]

Acute Q-fever

This disease is usually mild, with up to half of the infected people being asymptomatic. A self-limited febrile syndrome or 'pseudo-flu' occurs most frequently in symptomatic patients, but in more serious cases there might be hepatitis, pneumonitis and prolonged fever[2] (see Chapter 235).

INVESTIGATIONS

Routine laboratory investigation

Common non-specific laboratory abnormalities in rickettsioses include leukocyte count abnormalities, anemia and thrombocytopenia. Hyponatremia, hypoalbuminemia and hepatic and renal abnormalities may also occur.[3]

Serology

Serological tests are the most valuable tools in the diagnosis of rickettsioses.[1] The Weil-Felix test, the oldest serologic assay for rickettsioses, is based on the detection of antibodies to various *Proteus* antigens that cross-react with rickettsiae (*P. vulgaris* OX2 with spotted fever rickettsiae, *P. vulgaris* OX19 with typhus-group rickettsiae and *P. mirabilis* OXK with *O. tsutsugamushi*). Although it lacks both specificity and sensitivity, it is still used in many tropical countries. However, immunofluorescence is currently considered the reference method for the diagnosis of spotted fever group and typhus group. Cross-absorption of sera and Western blotting can be used to differentiate infections within rickettsial antigens when cross-reactions occur. For scrub typhus, the major surface protein antigen is the 56kDa protein including group-specific and strain-specific epitopes. However, several major serotypes (Karp, Kato, Gilliam, Kawasaki and Boryon) have been shown to present sufficient cross-reactivity with antigen from other strains to be used for serologic diagnosis. Immunofluorescence and immunoperoxidase assays are the most reliable serologic tools, but dot blot immunoassay and enzyme-linked immunosorbent assay tests have been developed and are commercially available. For Q-fever, the antigenic variation of *C. burnetii* is extremely useful to differentiate between the acute and chronic forms of the disease. Indeed, when isolated from animals or humans, *C. burnetii* expresses phase I antigen and is highly infectious. After subculture in the laboratory, there is an antigenic variation of *C. burnetii* to phase II form, which is less infectious. In acute Q-fever, antibodies to phase II antigens predominate and their titer is higher than the phase I antibody titer. On the other hand, in chronic forms of the disease, elevated antiphase I antibodies are uniformly detected.

Culture

The growth of rickettsiae in reference laboratories requires living host cells (animal mouse models, embryonated eggs) or cell cultures (VERO, L929, HEL or MRC5 cells), as well as a P3 safety level laboratory. The centrifugation shell-vial technique using HEL fibroblasts is the reference method. Rickettsiae can be seen in tissue by Giemsa or Gimenez staining or by immunodetection methods. [1]

Molecular tools

Polymerase chain reaction and sequencing methods are useful, sensitive and rapid tools to detect and identify rickettsiae in blood and skin biopsies (the inoculation eschar being the most useful specimen).[1] [2] [4] Primers amplifying sequences of several genes can be used for typhus group and spotted fever group rickettsiae, including *OmpA, OmpB, gltA* and gene D. Polymerase chain reaction amplification and sequencing of the 56kDa protein gene of *O. tsutsugamushi* have also been developed. Q-fever can also be diagnosed by molecular tools. Arthropods may also be used as epidemiologic tools. For example, *R. prowazekii* was detected in lice collected from refugees in Burundi after having been sent to our laboratory in Marseille to confirm the presence of epidemic typhus.

COMPLICATIONS

Spotted fever group rickettsioses range from mild to severe and fatal diseases. For example, to date no mortalities or severe complications have been reported in patients with African tick-bite fever, whereas the mortality rate may be as high as 2.5% for Mediterranean spotted fever.[1] [2] [4]

Murine typhus is usually a mild disease, although severe forms requiring hospitalization have been reported. Signs in untreated patients last for 7–14 days after which there is usually a rapid return to health.

Epidemic typhus is a potentially fatal disease. Without treatment, mortality rates are 10–30% depending on the presence of other underlying conditions and the patient's nutritional state.[1]

Scrub typhus is a public health problem in Asia; about 1 million cases occur each year and 1 billion people may be exposed. The severity of the disease varies from asymptomatic to fatal (up to 30%), depending on the susceptibility of the host and/or the virulence of the strain.[3]

Chronic Q-fever represents the development of the acute disease in predisposed hosts. It may present as endocarditis in patients with underlying heart valve lesions or, more rarely, as vascular aneurysms, graft infections, chronic bone infections or pseudotumors of the lung.

MANAGEMENT

Empiric treatment of rickettsioses is started before laboratory confirmation of the diagnosis.

Unless contraindicated, doxycycline is the preferred treatment of scrub typhus and the usual adult oral dose is 100mg twice daily for 7 days. Tetracycline 500mg q6h for 7 days may also be used. Chloramphenicol is the common alternative to the tetracyclines. The usual adult dosage is 500mg q6h for 7 days or 50–75mg/kg/day in children. Based on preliminary studies, rifampin (1-week 600–900mg/day oral treatment) and azithromycin (500mg on the first day followed by 250mg daily for 2–4 more days) may be proposed as alternatives to doxycycline and chloramphenicol.

The treatment of choice for spotted fever rickettsioses is 200mg doxycycline/day for 1–7 days depending on the severity of the

disease. In children and pregnant women, macrolides including josamycin (50mg/kg/day in children or 3g/day in adults) and roxythromycin, but not erythromycin, can be used for 8 days.

The treatment of choice for murine and epidemic typhus is a single 200mg dose of oral doxycycline usually leading to defervescence within 48–72 hours.

PREVENTION

There are no vaccines currently available for travelers against scrub typhus and other tropical rickettsioses. Thus prevention is mainly based on avoiding the arthropod bite.

Currently, the best method to avoid tick, flea and chigger bites comprises two components: topical DEET (N,N-diethyl-m-toluamide) repellent applied to exposed skin and treatment of clothing with permethrin, which kills arthropods on contact. These products are commercially available in a wide variety of formulas. Bites may also be limited by wearing long trousers that are tucked into boots. People staying in infested area should be advised to check their bodies routinely for the presence of arthropods. Any tick found attached should be removed immediately using blunt, rounded forceps.

In the case of epidemic typhus, louse eradication (e.g. in refugee camps) is the most important preventive measure and is essential in the control of outbreaks. Since body lice live only in clothing, the simplest method of delousing is to remove and then destroy or wash and boil all clothing. Dusting of all clothing with 10% DDT, 1% malathion or 1% permethrin is also a rapid and effective method of killing body lice and reduces the risk of re-infestation.

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Chapter 180 - Brucellosis

Finn T Black

EPIDEMIOLOGY

Brucellosis, also known as undulant fever or Malta fever, is a zoonosis caused by bacteria of the genus *Brucella*. The disease exists worldwide, with the highest prevalence in the Mediterranean countries, Asia, Africa and Central and South America. Around 500,000 new cases are reported annually worldwide, of which fewer than 100 are in the USA,[1] but brucellosis is probably underreported.

Brucellosis is transmitted to humans by direct contact with infected animals, by ingestion of unpasteurized milk or milk products, through cuts and abrasions or by inhalation of aerosols. In many European countries and in the USA it is mainly an occupational disease occuring in abattoir workers, butchers and farmers. Veterinary surgeons may become infected by accidental inoculation of live attenuated *Brucella* vaccine. Person-person transmission is extremely rare.

Four *Brucella* spp. can cause infection in humans:

- *Brucella melitensis*, which is found in goats, sheep and camels, is the most widespread and is the most virulent;
- *Brucella abortus*, which is found in cattle and camels, is less virulent;
- *Brucella suis*, which is found in pigs, is also less virulent; and
- *Brucella canis*, which is found in dogs, is the least common.

Other animals, including wildlife, may provide a reservoir for brucellae.[2]

PATHOGENESIS

Brucellae are facultative intracellular bacteria that are able to survive and multiply within mononuclear phagocytes. The mechanism is poorly understood but seems to include the suppression of degranulation of myeloperoxidase-containing granules, suppression of phagosome-lysosome fusion and production of protective enzymes.

The host reaction to brucellae is the formation of granulomas. *Brucella melitensis* and *B. suis* cause the most severe disease with caseating granulomas. Granulomas eventually heal with fibrosis and calcification.

Humoral antibodies seem to play some role in protection against reinfection. The control of the infection, however, depends on cell-mediated immunity. [2]

PREVENTION

The prevention of human brucellosis is dependent on the elimination of brucellosis in domestic animals. The use of veterinary vaccines for *B. abortus* and *B. melitensis* together with pasteurization of milk has resulted in a dramatic decrease in the incidence of human brucellosis. There is no effective vaccine available for *B. suis*. People at high risk of infection, such as veterinary surgeons and abattoir workers, should wear protective clothing. Laboratory-acquired brucellosis can be prevented by adherence to biosafety level 3 precautions. No effective vaccine is available for human use. Travelers to high endemic areas should be advised not to drink unpasteurized milk.

CLINICAL FEATURES

Brucellosis is a systemic infection that can involve any organ or organ system. The incubation period is normally between 2 and 4 weeks, but it may be months. The onset of clinical disease can be acute or insidious. Subclinical infection has been observed. Brucellosis is characterized by numerous somatic complaints in contrast to the few abnormal physical findings. Hepatosplenomegaly is present in 20–60%, depending on the species of *Brucella*, and mild lymphadenopathy is present in 10–20%. The non-specific symptoms (e.g. fever, sweats, anorexia, fatigue, myalgia, malaise, headache and depression) are common and may mimic diseases such as tuberculosis, toxoplasmosis, mononucleosis, hepatitis, systemic lupus erythematosus, typhoid and many others.

When symptoms related to a single organ or organ system are dominant it is often referred to as localized disease. The most common complications are listed in Table 180.1 . The term 'chronic brucellosis' should be reserved for patients who have complaints of ill health for more than 12 months.[2] This includes patients who have relapsing illness or persisting focal infection and patients complaining of weakness, fatigue and depression, but with no objective signs of infection and no elevation of IgG antibody titer. This last group is believed to suffer from a psychoneurosis or a syndrome akin to chronic fatigue syndrome (see Chapter 94).

Complications

Osteoarticular complications

Osteoarticular complications affect 20–40% of patients. Sacroiliitis is the most common reported complication, especially when *B. melitensis* predominates.[3] The characteristic radiographic findings are blurring of articular margins and widening of the sacroiliac space. The clinical presentation is systemic symptoms and pain.

Spondylitis is most often seen in the lumbosacral region in elderly men, probably reflecting pre-existing anomalies in the spine, and it may be complicated by paraspinal abscesses (Fig. 180.1). The main symptoms are fever and vertebral pain. The typical radiographic findings are epiphysitis of vertebrae and narrowing of the intervertebral disc (Fig. 180.2). Bone scans and computerized tomography scans may detect infection earlier than radiography.[4]

Differential diagnosis includes tuberculosis, fungal and pyogenic osteomyelitis, multiple myeloma and metastatic carcinoma.

Arthritis especially involves the hips, knees and ankles.

Gastrointestinal complications

Up to 70% of patients have intestinal complaints such as anorexia, nausea, vomiting, abdominal pain, diarrhea or constipation. The liver is probably always involved, but liver function tests are usually only mildly abnormal. Cirrhosis does not seem to follow *Brucella* infection.

Pulmonary complications

Respiratory symptoms are reported in 15–25% of patients. They range from flu-like symptoms to bronchitis, interstitial pneumonitis, lung abscesses, hilar lympadenopathy and lung effusions.

TABLE 180-1 -- Common complications of brucellosis

COMMON COMPLICATIONS OF BRUCELLOSIS	
Organ system	**Patients (%)**
Cardiovascular	1–2
Endocarditis	0–2
Cutaneous	5–10
Gastrointestinal	50–70
Genitourinary	1–5
Orchitis	1–4
Neurologic	2–4
Osteoarticular	20–40
Sacroiliitis	10–15
Spondylitis	8–10
Pulmonary	15–25

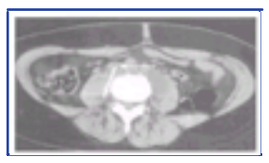

Figure 180-1 CT scan of fine needle aspiration of paraspinal abscess (arrow) in a patient with brucellosis.

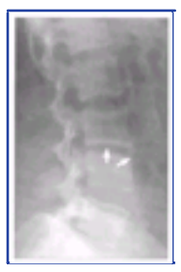

Figure 180-2 Radiograph of the lumbar spine in a patient who has discitis and spondylitis of $L_{3–4}$ caused by brucellosis. Note the reduced disc space and the destruction of the upper articular margins of L_4 (arrows).

Genitourinary complications

Complications from the genitourinary tract are rare. Acute orchitis or epididymo-orchitis with signs of systemic infection do occur and interstitial nephritis, glomerulonephritis and pyelonephritis resembling tuberculosis have been described. Brucellosis during pregnancy is rare but it can result in abortion like any other systemic infection.

Neurologic complications

Depression is a common complaint, but invasion of the central nervous system occurs in only 2–4% of cases. It usually presents as acute or chronic meningitis. Encephalitis, polyradiculopathy, psychosis and meningovascular complications have also been described.[5] Analysis of cerebrospinal fluid reveals elevated protein, lymphocytic pleocytosis, low to normal glucose and most often intraspinally produced specific antibodies. Brucellae are isolated from cerebrospinal fluid in less than 30% of patients.

Cardiovascular complications

Endocarditis, although rare, is the main cause of death related to brucellosis.[6] The aortic valve is involved more often than the mitral valve. Other complications include mycotic aneurysms, myocarditis and pericarditis.

Cutaneous involvement

Cutaneous manifestations of brucellosis consist mainly of transient non-specific lesions including erythema nodosum, petechiae, vasculitis, papules and rashes.

DIAGNOSIS

Because the symptoms of brucellosis are non-specific, it is crucial that the attending physician anticipate the probability of the disease. A certain diagnosis of brucellosis is made when brucellae are isolated from blood, bone marrow or other body fluids or tissues. Most laboratories employ rapid isolation methods for blood cultures. However, these cultures need to be maintained for up to 30–40 days to successfully isolate *Brucella* spp. Bone marrow cultures are more sensitive than blood cultures in acute brucellosis and tend to remain positive later in the course of the infection, even during antimicrobial treatment.

The serum agglutination test is the simplest, best standardized and most widely used test.[8] It measures both IgG and IgM antibodies; IgM antibodies are removed by pretreating the serum with 2-mercaptoethanol. Antibodies to *Vibrio cholerae*, *Francisella tularensis* and *Yersinia enterocolitica* can give false-positive reactions. False-negative reactions due to blocking antibodies are seen and dilutions of 1:640 should be made. A titer >1:160 is normally considered positive, as is a 4-fold or greater rise in titer.

Most patients who have acute infection develop IgM and IgG antibodies. The IgG antibodies persist as long as the infection is active and they increase with relapse and decrease with cure. The enzyme-linked immunosorbent assay appears to be more sensitive than and as specific as the serum agglutination test. It is rapid, easy to perform and can be automated.[9] The polymerase chain reaction has also been shown to be a very sensitive and specific rapid diagnostic test.[7]

MANAGEMENT

Doxycycline is the most effective single drug for treatment of brucellosis because of its excellent activity against brucellae, its penetration into cells and its passage over the blood-brain barrier.[10] Because of the high relapse rate (5–40%) with single drug therapy, a combination of two or three drugs is usually recommended. Uncomplicated brucellosis is treated with oral doxycycline 200mg q24h plus oral rifampin (rifampicin) 600–900mg q24h for 3–6 weeks. An alterna-tive is oral doxycycline for 3–6 weeks plus an intramuscular amino-glycoside (e.g. streptomycin 1g q12h or gentamicin 240mg q24h) for 2–3 weeks. Children less than 8 years of age and pregnant women should not be treated with doxycycline.

Instead, oral trimethoprim-sulfamethoxazole can be used (20mg/kg sulfamethoxazole and 4mg/kg trimethoprim q12h in children for 3–6 weeks, 800mg sulfamethoxazole and 160mg trimethoprim q12h in adults for 3–6 weeks) plus intramuscular gentamicin (5mg/kg q24h in children for 1 week and 240mg q24h in adults for 1 week). Gentamicin can be replaced by oral rifampin (10–20mg/kg q24h in children for 3–6 weeks, 600mg q24h in adults for 3–6 weeks).

Complications

Complications of brucellosis such as meningitis and endocarditis require longer courses of therapy, directed by the response.[2] In severe cases, a combination of three agents is often recommended (e.g. doxycycline and aminoglycoside plus rifampin or trimethoprim-sulfamethoxazole and aminoglycoside plus rifampin). Endocarditis often requires additional surgical intervention.[6]

Many other antimicrobial agents have shown in-vitro activity against *Brucella* spp., including fluoroquinolones, third-generation cephalosporins and azithromycin. However, when these drugs are administered alone the relapse rates are unacceptable and they should be kept as second-line drugs until further studies have been carried out.

With chemotherapy the overall mortality rate is less than 2%.

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Chapter 181 - Leptospirosis

Charles N Edwards

EPIDEMIOLOGY

Leptospirosis is caused by pathogenic serovars of *Leptospira*, of which 17 species are now recognized, defined by DNA-DNA hybridization. The disease is maintained in nature by chronic renal infection of mammals and is probably the most widespread zoonosis.[1] Human infection follows exposure to infected animals, either directly or indirectly through contaminated soil and water. Leptospires survive for days or weeks in warm, damp, slightly alkaline conditions, especially in still or slowly moving fresh water in the temperate summer and in damp soil and water in the tropics, especially in the rainy season.

Over 230 serovars of leptospires are recognized — the most ubiquitous serovars and their reservoir are *icterohaemorrhagiae*, usually derived from rats (*Rattus norvegicus*), and a number of serovars associated with domestic livestock animals, such as *hardjo* (cattle) and *pomona* (pigs). Other serovars are more restricted in their distribution, such as *lai*, which causes most cases of human infection in China and the Korean peninsula.

In temperate climates, leptospirosis is acquired mainly through recreational or occupational exposure, but in tropical regions exposure through avocational activities is more widespread.[2] Leptospirosis is an important cause of febrile illness in tourists returning from the tropics, particularly those involved in adventure tourism.[3] The reported incidence of leptospirosis in developed countries is declining and the disease is no longer reportable in the USA. The burden of disease is greatly underestimated in the tropical developing world.

PATHOGENESIS AND PATHOLOGY

Leptospires gain access to the circulation through penetration of abraded skin or intact mucous membranes, disseminate and ultimately penetrate various tissues. This action results in a systemic illness with a wide spectrum of clinical features. A systemic vasculitis with endothelial injury is the basic microscopic finding in the disease. Damaged endothelial cells usually show varying degrees of swelling, denudation and necrosis. Leptospires have been documented in large- and medium-sized blood vessels and capillaries in various organs. The major affected organs are:

- the kidneys, with a diffuse tubulointerstitial inflammation and tubular necrosis;
- the lungs, usually congested, with focal or massive intra-alveolar hemorrhage; and
- the liver, which shows cholestasis associated with mild degenerative changes in hepatocytes.

Whether a direct toxic effect of the leptospires or immune complex deposition causes the vascular injury is unclear. Production of humoral antibodies (IgM in the second and third weeks, IgG later) produces inflammatory responses such as meningoencephalitis and anterior uveitis. During recovery leptospires continue to be excreted in the urine for some days.

PREVENTION

Prevention measures are based upon an awareness of the epidemiology of disease occurring in a region.

Rodent control should be attempted where appropriate and feasible. Occupational protective clothing is effective in groups at risk. In some high-risk groups (e.g. soldiers on jungle maneuvers), oral chemoprophylaxis with doxycycline 200mg weekly is effective,[4] but there have been few attempts to use this approach on a large scale, such as after massive flooding.

Vaccines for human use are licensed in France, China, Japan and Cuba.[5] The large number of serovars makes general protection of human populations by immunization almost impossible. Vaccines for use in dogs and livestock animals historically have been unable to prevent renal infection and thus the reservoir state, and generally have not generated lasting immunity to acute infection. A new generation of vaccines for use in cattle appears to stimulate a cell-mediated immune response and overcomes the limitations of earlier vaccines.[6]

CLINICAL FEATURES

Humans become ill 7–12 days after exposure to leptospires. The majority of patients (90%) experience a mild febrile illness while a minority (10%) have a severe illness called Weil's syndrome. A biphasic course of illness can be seen in all patients. The first or septicemic phase is characterized by a sudden onset of fever, retro-orbital headache, chills, myalgias classically involving the paraspinal, calf and abdominal muscles, conjunctival suffusion (Fig. 181.1), vomiting, prostration and a skin rash, which may be maculopapular or purpuric. Defervescence of fever occurs after 5–7 days in this phase.

The second or immune phase is characterized by the appearance of IgM antibodies. Symptoms recur and signs of meningitis may develop in up to 50% of cases. In severe cases, fever may persist and be associated with renal insufficiency or failure, pulmonary hemorrhage, varying levels of jaundice and myocarditis. Death, occurring in 10–15% of these severe cases, is thought to be due to pulmonary hemorrhage or cardiac failure and arrhythmias secondary to myocarditis. Chest radiographs may reveal numerous abnormalities including segmental opacities or a diffuse pneumonitis.

Clinical laboratory investigations reveal red and white cells in the urine with albuminuria. An elevated white cell count, thrombocytopenia and a high creatinine phosphokinase are commonly observed. When the bilirubin is elevated, the transaminases are only mildly elevated.

The differential diagnosis of leptospirosis includes dengue fever, malaria, influenza and several other acute febrile illnesses depending on the geographic location.

DIAGNOSIS

Because of the broad spectrum of symptoms and the wide differential diagnosis, a high index of clinical suspicion is required if appropriate diagnostic tests are to be made.[7] During the first week of

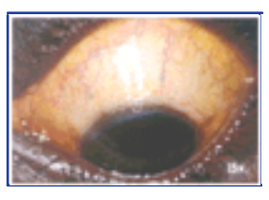

Figure 181-1 Conjunctival suffusion and jaundice.

illness leptospiremia occurs, while in the second week leptospires are excreted in urine and are found in the cerebrospinal fluid of patients with meningitis. Isolation of

the organism requires special media and may take several weeks of incubation, and thus does not contribute to individual patient diagnosis. Detection of leptospiral DNA by polymerase chain reaction is more sensitive than culture, but has yet to be optimized.

A strong IgM antibody response, which appears about 5–7 days after onset of symptoms, may be detected using several commercial assays. The microscopic agglutination test is a complex assay that detects antibodies against live antigen suspensions and is performed only in reference laboratories. Diagnosis using this assay requires paired acute and convalescent sera. This test yields information about the presumptive infecting serogroup and thus has epidemiologic value. In endemic areas, single elevated titers must be interpreted with caution because antibodies persist for years after acute infection.

Direct microscopic examination of clinical samples is of little value, but immunohistochemical staining of autopsy specimens is a valuable diagnostic tool.[8]

MANAGEMENT

Patients with mild or anicteric disease usually get better without treatment. Doxycyline 100mg daily has been shown to shorten the duration of the illness.[9] Hospitalization is recommended for severe cases. Excellent supportive care with particular attention to fluid and electrolyte balance and pulmonary and cardiac function is critical. Renal failure should be treated by peritoneal or hemodialysis. Until the efficacy of antibiotics in this severe illness is resolved, patients should be treated with intravenous penicillin G (benzylpenicillin)[10] or cefotaxime.

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Chapter 182 - Relapsing Fever

David A Warrell
Eldryd HO Parry

INTRODUCTION

Repeated abrupt episodes of fever, separated by afebrile periods, give relapsing fever its name. There are two forms:

- tick-borne relapsing fever, caused by various *Borrelia* spp.; and
- louse-borne relapsing fever, caused by *Borrelia recurrentis*.

The two diseases differ in their epidemiology, clinical features and management (see Chapter 230).

EPIDEMIOLOGY

The epidemiology of the relapsing fevers has been considered in a number of studies.[1]

Tick-borne relapsing fever

Borrelia—*tick complex*

Different species of soft ticks of the genus *Ornithodoros* (Argasidae) and of *Borrelia* spirochetes are involved in different parts of the world:

- in central and western USA and Mexico, *Ornithodoros hermsi*, *Ornithodoros parkeri* and *Ornithodoros turicata* with *Borrelia hermsi, Borrelia parkeri* and *Borrelia turicatae*;
- in Central and South America (chiefly in Colombia and Venezuela, with a focus around northern Argentina, Bolivia and Paraguay), *Ornithodoros rudis* (*O. venezuelensis*) with *Borrelia venezuelensis*;
- in east, central and southern Africa, *Borrelia duttonii* with the *Ornithodoros moubata* complex;
- in Senegal and some other parts of north and east Africa and the Middle East, *Borrelia crocidurae* with *Ornithodoros sonrae* (*O. erraticus* small form);
- in the Middle East, Iran and a belt stretching eastward through Uzbekistan and other countries of the former Soviet Union to western China, *Borrelia persica* with *Ornithodoros tholozani* (*O. pappilipes*); and
- in north Africa and the Iberian peninsula, *Borrelia hispanica* with *Ornithodoros erraticus*.

The soft tick vectors are found in dry savanna areas and scrub, particularly rodent burrows, caves, piles of timber and dead trees, or in the roof spaces and beneath the floors of log cabins — anywhere that small rodents can establish their nests. Unlike louse-borne relapsing fever, tick-borne relapsing fever is a zoonosis, except for *B. duttonii* infection, which is transmitted only between humans. The vertebrate reservoir, which varies with the ecology of the area, may be various species of rodent (rats, gerbils, mice, squirrels, chipmunks) or even dogs and some birds. Ticks become infected when they feed on an infected animal (or human) and in turn they infect the next host either by a bite, when infected saliva is injected, or perhaps when infected coxal fluid contaminates mucosal membranes.

Humans are accidentally infected when they are in contact with infected ticks. In eastern Africa, humans have displaced rodents and have become the reservoir for the *O. moubata - B. duttonii* complex. This also occurs in Senegal.[2] A tick remains infected for life and can survive prolonged starvation. The female can transmit the spirochete transovarially to its offspring, so that the infection is enzootic among ticks and awaits the reservoir animal or human to infect. Borrelias are not found in tick feces.

Tick-borne relapsing fever is endemic in most continents except Australasia and the Pacific region. In western Senegal, a recent survey revealed a prevalence of 1% among children.[2] At one health center in Rwanda, 1650 proven cases are treated each year (6% of all patients) and the disease is prevalent in the Dodoma region of Tanzania.

Louse-borne relapsing fever

This classic epidemic disease of armies, refugees and immigrants remains endemic in the highlands of Ethiopia and adjacent countries, hilly areas of Yemen and some parts of the Peruvian and Bolivian Andes.[3] The human body louse, *Pediculus humanus*,[4] and head louse, *Pediculus capitis*, are obligate blood-sucking ectoparasites that ingest borrelias when they feed on humans and then transmit the organism when they are crushed against broken skin or rubbed on a mucous membrane such as the conjunctiva, so that spirochetes in their celomic fluid enter the person's blood. Unlike ticks, lice cannot infect their progeny. Humans are the only reservoirs of this infection.

Louse-borne relapsing fever thrives where people are crowded and poor, where a cold, wet climate (as in the highlands of Ethiopia) encourages them to wear clothes that harbor lice, where water for washing may be scarce and where agents to kill lice are unavailable. The clothes of an infected person teem with lice, particularly around the waist and the buttocks, and lice move from person to person. In the highlands of Ethiopia, the disease flares up in the rainy season because people are crowded and cold. The prevalence of lice is higher during the rainy season but the prevalence of lice in the communities where louse-borne relapsing fever is endemic is high and constant, and it is linearly related to the intensity of infection in an individual person. Rarely, relapsing fevers are diagnosed in travelers who have returned from endemic areas, in intravenous drug users and in recipients of blood transfusions.

PATHOGENESIS

Relapses are due to fresh antigenic variants of borrelias. Extra chromosomal DNA in linear plasmids recombines to activate the genes that control variable major protein synthesis.[5] Experimentally, spirochetes persist in the brain or eye in immunodeficient animals even though they are apparently cleared from the peripheral blood.[6]

PREVENTION

Tick-borne relapsing fever can be prevented if ticks are suppressed, if rodents are controlled and if travelers avoid sleeping in places where ticks or their rodent reservoir could be abundant, such as poorly maintained log cabins. Rodent-proofing of such cabins is feasible in some places, such as the North Rim of the Grand Canyon.[7] There are sporadic cases in travelers in the Middle East, Africa and Europe, and small outbreaks in the USA (e.g. Browne Mountain, the Grand

Canyon) during the vacation season or among those who have worked or stayed in caves, but the risk of transmission of borrelias from an infected tick to humans is not high. Ticks can be eliminated with pyrethroids, benzene hexachloride, malathion or dichlorodiphenyltrichloroethane (DDT).

Prevention of louse-borne relapsing fever depends on breaking transmission from louse to the susceptible population, and this can only be achieved by eradicating the body louse. Infected clothing should be washed and treated with chlorine bleach and an insecticide. The lice are abundant in hair, which must be shaved off or washed and treated too. This is better than giving tetracycline to people at risk of relapsing fever.[8]

CLINICAL FEATURES

Tick-borne relapsing fever

The bite of the tick is painless and produces no eschar. Therefore, it may not be noticed. An incubation period of 3–18 days follows, long enough that potential exposure may be forgotten.

The first symptoms are explosive: fever, headache, muscle and joint pains, extreme fatigue with prostration and drenching episodes of sweating. [9] Epistaxis, abdominal pain, diarrhea and cough may follow. In 5–10% of patients neurologic symptoms and signs, which are the most important clinical problem, manifest as a wide range of focal deficits and are typically transient. They include paresthesias, cranial nerve palsies (especially VII) and visual symptoms, and hemiparesis or paraparesis. Meningeal symptoms, which are usually accompanied by a lymphocytic pleocytosis, are rare. Various erythematous rashes and petechiae may also be seen. The density of spirochetemia governs the clinical severity. Symptoms abate after a few days — the duration depends on the severity of the initial episode — only to recur about 7–15 days later. As many as eight relapses may occur, but they become steadily less severe. Few cases remain undiagnosed for so long. Pregnancy is interrupted in up to a third of cases.

Louse-borne relapsing fever

There is a very wide range of clinical features, from a mild and insignificant fever to a disease that affects many systems in a critically ill person. [1] Typically, after an incubation period of 4–17 (average 7) days, the first symptoms are fever, chills, headache, muscle and body aches, fatigue, dizziness, anorexia and nightmares. Then, in at least 50% of patients, there is evidence of bleeding, commonly manifest as epistaxis, subconjunctival hemorrhage (Fig. 182.1) and petechial hemorrhages, especially on the trunk. An enlarged and tender liver, often with an enlarged spleen, is palpable in as many as 50% of cases in some outbreaks, and about half of these are jaundiced. The respiratory system is affected in about 15% of cases. Cough may indicate pneumonia but it may also precede clinical pulmonary edema. Rarely, there are manifestations of adult respiratory distress syndrome.

Transient myocardial damage may lead to pulmonary edema, which is an additional hazard during treatment. Neurologic and

Figure 182-1 Ethiopian patient who has louse-borne relapsing fever 4 days after the start of febrile symptoms, showing subconjunctival hemorrhages and jaundice.

meningeal signs, transient focal deficits and, ominously, a confusional state and coma are less common than in tick-borne relapsing fever. Fetal loss is very common in pregnant women.

Spontaneous crisis and Jarisch-Herxheimer reaction

The clinical course of relapsing fever, especially louse-borne relapsing fever, is usually terminated either by a mild 'spontaneous crisis' on about day 5 of the untreated illness or by a Jarisch-Herxheimer reaction. This much more severe reaction is induced by, and follows 1–3 hours after, antimicrobial treatment. [1] [10] The patient becomes restless 1–3 hours after treatment. Frank rigors may develop and the temperature, respiratory rate, pulse rate and blood pressure increase rapidly. There may be associated vomiting, diarrhea, coughing, musculoskeletal pains and delirium, and some patients die of hyperpyrexia at the height of the fever. During the ensuing flush phase of this endotoxin-like reaction, there is profuse sweating, intense vasodilatation with a fall in mean arterial pressure and a slow decline in temperature over the next 6–12 hours. Fatalities during this phase are due to hypovolemic shock or acute pulmonary edema resulting from myocarditis. The incidence of Jarisch-Herxheimer reactions varies from 30% to almost 100% in different published reports.

A borrelial pyrogen, an outer membrane variable major lipoprotein [11] [12] released by the action of antimicrobial agents, stimulates an explosive release of cytokines from macrophages through NF-?B,[13] principally tumor necrosis factor (TNF)-a, interleukin (IL)-6, IL-8 and IL-1ß, just before the start of the clinical manifestations of the Jarisch-Herxheimer reaction (Fig. 182.2).[14] The reaction is unaffected by corticosteroids, is delayed by meptazinol (an opiate agonist-antagonist) and can be prevented by a polyclonal antibody against TNF if this is given just before antimicrobial treatment.[15]

LABORATORY FINDINGS

Spirochete density in peripheral blood may exceed 500,000/mm^3 . There is a peripheral neutrophil leukocytosis but, during the

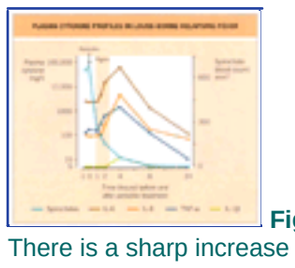

Figure 182-2 Plasma cytokine profiles in louse-borne relapsing fever. These profiles are from an Ethiopian patient treated with penicillin (at time 0 on the horizontal axis). There is a sharp increase in (TNF)-a, IL-6 and IL-8 concentrations at the start of the phase of violent rigors.

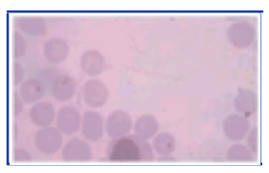

Figure 182-3 Thin blood smear from an Ethiopian patient who has louse-borne relapsing fever (Giemsa stain), showing numerous spirochetes.

Jarisch-Herxheimer reaction or spontaneous crisis, there is a transient profound fall in leukocyte count. Thrombocytopenia is common and there is a coagulopathy attributable partly to hepatic dysfunction and partly to disseminated intravascular coagulation with increased fibrinolytic activity. Biochemical evidence of hepatocellular damage is found in most patients.[1] A few patients show transient, mild renal impairment. A mild neutrophil-lymphocyte pleocytosis has been described.

DIAGNOSIS

If the patient has an acute fever and has traveled in the tropics, it is essential to do a blood film to look for malarial parasites. This may also reveal the spirochetes of tick-borne relapsing fever, which will be recognized in a thin or thick blood film (Fig. 182.3). In suspected tick-borne relapsing fever, the spirochetes must be searched for repeatedly; in louse-borne relapsing fever the higher and more persistent spirochetemia is more easily detected. In suspected cases of tick-borne relapsing fever, blood must be taken at the height of a relapse because it is unlikely to reveal borrelias between relapses.

The serologic response in tick-borne relapsing fever may be helpful but *Borrelia burgdorferi*, the organism responsible for Lyme disease, leads to production of antibodies, mainly to 41kDa and 60kDa antigens, which cross-react with the antiborrelial immunoglobulins of tick-borne relapsing fever. This is due to conserved antigenic epitopes expressed by borrelias.[16] [17] Both *B. duttonii* and *B. recurrentis* have been cultivated in vitro. [18]

In the clinical diagnosis of a traveler at risk, two less common causes of episodic recurrent fever are possible: trench fever (caused by *Bartonella quintana*) if the traveler might have been in contact with the body louse, or rat-bite fever (caused by *Spirillum minus*).

The differential diagnosis of a febrile patient with jaundice, petechial rash, spontaneous systemic bleeding and hepatosplenomegaly includes, apart from relapsing fevers, falciparum malaria, yellow fever and other viral hemorrhagic fevers (such as Rift Valley fever in the Horn of Africa), viral hepatitis, rickettsial infections, especially louse-borne typhus, which has the same epidemiologic predispositions as louse-borne relapsing fever, and leptospirosis. Secondary infections, known to complicate louse-borne relapsing fever, include bacillary dysentery, salmonellosis, typhoid, typhus, malaria and tuberculosis.

PROGNOSIS

Reported case fatalities during epidemics have exceeded 40% but this can be reduced to less than 5% with antimicrobial treatment, provided that appropriate ancillary treatment is given during the life-threatening Jarisch-Herxheimer reaction. Deaths during relapses are most unusual and occur only in tick-borne relapsing fever.

MANAGEMENT

The principles of treatment are:

- to eliminate spirochetemia and prevent relapses, using antibiotics;
- to monitor the patient very carefully through the Jarisch-Herxheimer reaction; and
- to restore and maintain circulating volume during the 24 hours after starting antibiotic treatment.

Antibiotic agents

The choice of agents is based on clinical experience.[19]

Tick-borne relapsing fever

Doxycycline 100mg per day for 10 days is recommended for adults. For pregnant women and young children, erythromycin can be used.

Louse-borne relapsing fever

A single 500mg oral dose of tetracycline or erythromycin stearate is effective. In severely ill patients who are likely to vomit, effective parenteral treatment consists of either a single intravenous dose of tetracycline hydrochloride (250mg)[20] or, for pregnant women and children, a single intravenous dose of erythromycin lactobionate (300mg for adults, 10mg/kg for children). In mixed epidemics of louse-borne relapsing fever and louse-borne typhus, a single oral dose of doxycycline 100mg has proved effective. Penicillins and chloramphenicol are also effective.[20]

Supportive treatment

Postural hypotension and cardiac arrhythmias are prevented by nursing the patient flat in bed for 24 hours after antibiotic treatment. Hyperpyrexia and hypovolemia must be prevented. Acute heart failure with pulmonary edema responds to intravenous furosemide (frusemide) and digoxin. Bleeding and clotting problems are treated with vitamin K, platelets and clotting factor concentrates. Complicating infections, notably typhoid, salmonellosis, bacillary dysentery, tuberculosis, malaria and typhus in some endemic situations, must be treated appropriately.

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Chapter 183 - Viral Hemorrhagic Fevers

Joseph B McCormick

INTRODUCTION

Viral hemorrhagic fevers (VHFs) are endemic in every continent with the possible exception of Australia. The diseases are characterized by an acute onset and high fever, and in some cases, a high mortality rate. The bleeding by which they are known is a complication of severe disease, but the underlying pathology is a leaky capillary syndrome with prominent pulmonary edema. Death, when it occurs, is usually due to hypovolemic shock with or without adult respiratory distress syndrome (ARDS).

VIROLOGY AND NATURAL HISTORY

These diseases, almost all zoonoses, are caused by a range of enveloped RNA viruses. Humans are not part of the natural history of these viruses, and therefore infection of humans is an accident, usually the consequence of intrusion into the ecologic niche of the virus. The viruses belong to four major families — Bunyaviridae, Arenaviridae, Filoviridae and Flaviviridae (Table 183.1).

The bunyaviruses Crimean-Congo hemorrhagic fever virus (CCHFV), hantaviruses and Rift Valley fever virus (RVFV) cause VHF in humans. Crimean-Congo hemorrhagic fever virus is spread by ticks and occurs widely across Africa, south-eastern Europe, the Middle East and Asia. Hantaviruses are found throughout the world as natural silent infections in many rodents. A hantavirus in the USA, a pathogen of deer mice, causes the hantavirus pulmonary syndrome (HPS). Rift Valley Fever virus is mosquito borne and causes an acute illness in livestock and wild animals as well as humans.

Arenaviruses also infect rodents, the most important being Lassa virus, which is confined to West Africa, and the South American hemorrhagic fever viruses, of which four are known and cause VHF. The filoviruses, Ebola and Marburg viruses, are a unique family of filamentous viruses known as Filoviridae, from Africa. African filovirus infections have a high mortality rate, but recently described Asian filoviruses have not, so far, been seen to be pathogenic for humans.

The Flaviviridae include yellow fever virus (see Chapter 222) and dengue virus (see Chapter 184), which are both spread by mosquitoes.

Other viruses can cause hemorrhagic fever, such as those causing Kyasanur Forest disease and Omsk hemorrhagic fever, but these are confined to very local areas and are not discussed in detail here. Neither are viruses such as West Nile virus, which, although common and causing increasing numbers of cases in a widening geographic area, rarely cause hemorrhagic disease (and is covered in Chapter 222).

EPIDEMIOLOGY

These infections are primarily rural diseases in developing communities occurring in areas where there is substantial contact with rodents, ticks or mosquitoes and usually with poor facilities for medical care. They are often undiagnosed, particularly single sporadic cases. Much of our knowledge of these diseases is the result of outbreak investigation, and rarely based on experience with endemic sporadic infections. Most of these infections are predominant in the poor. Some of these infections are also transmissible from person to person, such as Lassa virus, Ebola virus and CCHFV. These are particularly notorious for causing nosocomial outbreaks. Only dengue and occasionally yellow fever are seen in the cities where large populations of humans form effective reservoirs along with the abundant mosquitoes that act as both reservoirs and transmitters of the viruses. However, as a result of the increasing mobility of populations everywhere, infected patients can and do appear almost anywhere in the world. Missionaries and medical staff working in remote areas are at risk, but often more likely to reach tertiary care facilities than locals. Large epidemics of VHF can kill thousands of people, such as with yellow fever. Some viruses, particularly filoviruses, have only ever emerged in small epidemics, but their very high mortality rates have given them a notoriety perhaps disproportionate to the number of people actually infected. Monkeys and chimps appear to be as sensitive (or even more so) to the filoviruses as are humans. As a result, there have been many cases of monkey infection now documented, including their onward transmission to humans. There is no evidence, however, that chimps or monkeys are the natural reservoir.

DIAGNOSIS AND CLINICAL FEATURES

The most critical element in the clinical diagnosis in nonendemic areas is to take a thorough history covering the incubation period (3 to a maximum of 4 weeks before the onset of fever). The element that alerts the physician to VHF (and usually indicates which one it is likely to be) is the contact the patient has had with known ecologic niches (see Table 183.1). The history must include:

- a thorough travel history, particularly in Africa, and contact with known severely ill febrile individuals;
- any possible contact with ticks, fresh animal blood, rodent urine or blood, wild animals or mosquitoes and other insects;
- any recent camping in exotic and potentially endemic areas;
- any entry into bat caves; and
- attendance at ceremonial funerals.

Usually these risks occur in rural and remote areas. A medical care provider or other worker who might have had contact with blood from a primary case should also alert the physician to possible VHF.

The essential clinical features (Table 183.2) are a short history of fever, which is usually high and of sudden or rapid onset. Severe body pains and headache are prominent and may be excruciating. Other features may include severe pharyngitis, nausea and vomiting, petechiae, oozing from the gums and bradycardia. Proteinuria is common. Peripheral white blood cell counts are often low very early in disease, but may go up dramatically later, and therefore the presence of neutrophilia may falsely indicate a bacterial disease and be misleading. Thrombocytopenia is common and platelet function is often impaired, even in the presence of low normal platelet counts. Partial thromboplastin times may be prolonged, but prothrombin times are relatively unaffected. Disseminated intravascular coagulation

TABLE 183-1 -- Epidemiologic characteristics of the most important viral hemorrhagic fevers.

EPIDEMIOLOGIC CHARACTERISTICS OF THE MOST IMPORTANT VHFs								
	Arenaviruses*		**Filoviruses**		**Bunyaviruses**			**Flaviviruses**
	Lassa virus	**Junin, Machupo, Guanarito, Sabia**	**Ebola**	**Marburg**	**Hantavirus**	**Crimean-Congo**	**Rift Valley fever**	**Yellow fever**
Geography	West Africa	South America, North America	Central and West Africa	East and Central Africa	Worldwide	Europe, Asia, Africa	Africa	South America and Africa

Primary source of infection	Rodent (*Mastomys natalensis*)	Rodent (*Calomys, Zygodontomys* sp.)	Unknown; possibly bats		Rodents; species depends on location	Tick-borne, (>27 species)	Mosquito	Mosquito-borne (*Aedes* and *Hemogogus* spp.)
Transmission	Nosocomial Rodent to human/person to person	Rodent to human	Nosocomial; possibly reservoir to human; person to person; also infected chimps and possibly monkeys can transmit to humans		Rodent to human	Nosocomial tick to human; possibly animal to human; possibly person to person	Mosquito to human; possibly animal to human via blood	Mosquito to human (human reservoir in outbreaks)
Risk factors	Close contact *Mastomys* rural West Africa; close contact infected persons	Contact rodents in circumscribed agricultural areas South America, possibly North America	Close contact infected blood or secretions from infected persons; environmental risk unknown (possibly caves for Marburg)		Contact with rodent or rodent urine in dust; laboratory	Tick bites; contact with infected livestock; contact with blood or secretions from infected persons	Mosquito bites rural Africa	Mosquito bites rural West Africa and South American rainforests
Treatment	Responds to ribavirin when treated early	Junin responds to immune plasma given early; all may respond to ribavirin	No present treatment; does not respond to ribavirin		Ribavirin may be beneficial early; trial and current use in China; open-label trial in USA for acute pulmonary disease was inconclusive	Sensitive to ribavirin; several published reports of successful case treatment	Sensitive to ribavirin; limited data in monkeys; no human data	No therapy; does not respond to ribavirin
Vaccine	Vaccinia vectored glycoprotein vaccine protects primates Development of human vaccine possible	Attenuated vaccine to Junin virus has greatly reduced cases in Argentina; no vaccine available for others	Protection in small animal studies. Protection of primates not clear	Nothing available	M gene DNA vaccine expressing glycoproteins 1 and 2 protects small animals from challenge and elicits neutralizing antibody in Rhesus monkeys; also formalin-inactivated vaccine but no trial data	Killed vaccine available in Eastern Europe and China, but no published trials	Attenuated vaccine for animals, none for humans	Live attenuated vaccine since 1940s; one of the most effective vaccines ever

* Arenaviruses have been isolated from wood rats in the south-western USA — their capacity to cause human infection is uncertain

TABLE 183-2 -- Key clinical features of viral hemorrhagic fevers.

KEY CLINICAL FEATURES OF VHFs										
	Arenavirus		**Filovirus**			**Bunyavirus**				**Flavivirus**
	Lassa virus	**South American**	**Ebola****	**Marburg**	**Reston****	**Old World hantavirus**	**New World hantavirus**	**Crimean-Congo**	**Rift Valley fever**	**Yellow fever**
Untreated case fatality (%)	16–20	16	50–90	6–50	0	1–15	45–50	10–>50	1–2	20–50
Cardiovascular system										
Thrombocytopenia	+	+++	+++	+++	-	++	+	+++	++	++
Oozing	++	++	+++	+++	-	++	-	+++	++	+++
Petechiae	-	+++	+	+	-	+++		+		
Ecchymoses			++	++	-		-	++		
Circulatory shock	+++	+++	+++	+++	-	+++	+++	+++	+++	+++
Tissue edema	++		+	+	-	+	-			
Major hemorrhage	+	+	++	++	-	+	-	++	+	++
Central nervous system										
Encephalopathy	+++	+	+	+	-	-	-	+		
Ataxia	+	++				+				
Deafness	++									
Blindness			+							
Mood alteration	+	+	+	+				++		
Intracranial bleeding						+				
Other major systems										
Renal	-	-	+/-	+/-	-	+++	+			+
Pulmonary (ARDS)	+++	+	++	+	-	+	+++			+
Hepatic	+	+	+	+	-	+	+	+	+	+++
+ Denotes present to differing degrees indicated by number of marks										
- Denotes absence										
No mark indicates no reliable data										

** Viruses from West and Central Africa appear to vary with those in West and North Central Africa; may be less virulent and with lower case fatality than Central Africa
* Reston is not a human pathogen

is not a feature of VHF except as a complication of the general deterioration of patients in the terminal phase.

As the disease progresses hypovolemic shock, pulmonary edema and frank bleeding ensue. Aspartate aminotransferase (AST) is usually raised and virtually all VHFs distinguish themselves from viral hepatitis because the AST is disproportionately high compared with alanine aminotransferase (ALT). Ratios of AST to ALT may be as

high as 11 to 1, and the level of AST also reflects prognosis. Patients are rarely jaundiced (except in yellow fever) and the bilirubin is usually normal.

The viruses are pantropic, primarily targeting the reticuloendothelial system. There are usually high titers of virus in the blood and tissues. The central nervous system is relatively spared, but encephalopathy and neurologic sequelae such as ataxia and deafness can occur, particularly in the early convalescent phase. Viruses are rarely recovered from the cerebrospinal fluid.

Care must be taken in collecting, handling and transporting specimens, and consultation with the laboratory is essential. Gloves must be worn at all times and the specimens clearly labeled as hazardous. Blood samples should preferably be drawn into a vacuum tube system. Specimens for transport should be transferred to a leakproof plastic container and double-wrapped in leakproof containers in which they can be transported to a suitable reference laboratory (see Chapter 222).

Laboratory diagnosis may be provided by several methods, depending on the virus in question by:

- presence of virus-specific IgM in the serum;
- presence of viral RNA, usually in serum or white cells, demonstrated by a reverse transcriptase polymerase chain reaction (PCR);
- presence of viral antigen through enzyme-linked immunosorbent assay (ELISA) for specific viral antigens in serum or blood;
- isolating the virus from serum; or
- demonstrating a 4-fold rise in antibody titer;

Sera may be inactivated for serology by gamma irradiation or, if this is unavailable, heating at 140°F (60°C) for 30 minutes. Immunofluorescence assays and ELISAs detect both virus antibody and antigen. More recently, molecular techniques such as PCR performed directly on serum or tissues to detect viral RNA have been found to be rapid, reliable and safe, and while they are more widely available, they are not always available in the areas endemic for VHFs. No truly successful diagnostic system has yet been developed that can be easily and uniformly applied in the remote areas where these diseases occur. New advances in PCR offer the most likely method.

MANAGEMENT

Viral hemorrhagic fevers are self-limiting diseases, and if the patient can be brought through the acute crisis recovery is rapid and complete, although fatigue and general weakness may persist well after the acute disease. The main challenge of acute disease is careful management of fluid balance. Patients often present with a high hematocrit due to dehydration. Despite this, pulmonary edema is a real risk and patients should be infused with caution. Blood and platelet replacement may be necessary. Full intensive care support may be required including mechanical ventilation, monitoring of central venous pressure and dialysis. Seizures and arrhythmias will need to be controlled. Any necessary operation (e.g. obstetric intervention) should be carried out. Pregnant patients are a major challenge. They often present with absent fetal movements and the survival of the mother in Lassa fever has been shown to depend upon the presence of aggressive obstetric intervention to remove the dead fetus.

Lassa virus and CCHFV are highly treatable using the antiviral agent ribavirin, provided therapy is instituted as early as possible in the course of the disease (see Table 183.1). Therapy with immune plasma has also been advocated, but efficacy has never been demonstrated except for Argentine hemorrhagic fever. Disseminated intravascular coagulation is not an underlying feature and heparin is contraindicated. Early accurate diagnosis and good intensive care support are the most important underlying principles for the physician.

Patients who have VHF do not travel well because their cardiovascular system is often unstable and trauma is likely to induce bleeding. It is therefore advised that moving a suspected patient is avoided wherever possible. Moving the patient also exposes a greater number of people to secondary infection. Patients may be managed quite successfully in standard hospital isolation rooms with rigorous barrier nursing because these diseases do not transmit from person to person by aerosol.

PREVENTION

The fearsome reputation of some of these viruses comes from their ability to spread to medical staff and patients in facilities where poor training and inadequate materials for barrier nursing lead to blood-to-blood contact with the virus, for example as a result of a needlestick injury, blood spill on unprotected damaged skin or mouth- to-mouth resuscitation. In some countries, the re-use of needles and syringes has produced devastating nosocomial outbreaks. There are also reports of outbreaks among surgical teams who have unwisely performed laparotomies on infected patients. In these circumstances, the mortality rate has been high.

The key to the prevention of nosocomial transmission in both endemic and nonendemic areas has consistently been good hospital and laboratory practice, with strict isolation of febrile patients and rigorous use of gloves and disinfection.

A small number of named personnel should undertake direct care and be kept fully informed about the nature of the virus and the precautions to be taken. Intensive care, operative interventions and air evacuation in the absence of any local hospital facility should not be denied because they do not pose substantial threats to a well-trained staff accustomed to simple barrier nursing techniques. Aerosol spread in hospitals has not been documented; indeed, there is much published evidence showing that this is not a major hazard. Past recommendations for strict isolation of patients in a plastic isolator have been abandoned in favor of simple strict barrier nursing. This practice presents no excess risk to hospital personnel and allows substantially better care of the patient.

The major factor in nosocomial transmission is the combination of unawareness of the possibility of the disease by a worker who is also inattentive to the requirements of effective barrier nursing. Contacts should be carefully assessed and monitored. Postexposure prophylaxis may be offered where appropriate. Once the diagnosis has been considered and appropriate precautions instituted, the risk of nosocomial transmission is very small (<1%).

A high risk of infection is associated with direct percutaneous or mucosal contact with blood or body fluids and prophylaxis with ribavirin should be offered after exposure to CCHFV and arenaviruses. Other contacts (includes most unprotected contact with blood or body fluids) may safely be observed daily for the development of a persistent high fever for 3 weeks from the last date of contact. The practice of following up airline passengers who do not have any direct physical contact and other low-risk contacts with Lassa fever virus has been discontinued (see Practice Point 186a).

The 1988 USA Centers for Disease Control and Prevention Guidelines for the Management of Patients with VHFs therefore recommend routine patient isolation in a single room, preferably but not necessarily with a negative air pressure gradient from the hallway through an anteroom to the patient room. Staff education, use of gloves, gowns and masks, and rigorous disinfection with fresh liquids are mandatory. The recommendations issued for patient management

and handling of clinical specimens from patients who have AIDS are adequate for the containment of VHFs. The major event to be avoided is direct contact with the blood or other fluid or excretions from an acutely infected patient.

Lassa and Ebola viruses in particular are robust and even withstand some drying. Blood from severely ill patients may contain as much as 10^9 infectious units/ml. However, all the viruses can be inactivated by heat, detergents, chlorine, formalin and ultraviolet radiation (including sunshine). Disinfection can be accomplished by washing with 0.5% phenol in detergent, 0.5% hypochlorite solution, formaldehyde, glutaraldehyde or paracetic acid. Care should be taken to ensure solutions are freshly and correctly made up and time allowed for disinfectant to work on spills.

Finally, a major hazard of VHFs, particularly viruses such as Ebola virus, is the fear and press attention they receive. A single case can be quite traumatic for an institution unless the situation is carefully handled. A measured and informed approach from a collaborative team of doctors, nurses, administrators and others is needed. Careful education of all medical staff, emphasizing the real risks and the ways to avoid them and allaying unnecessary fears, and avoiding panic will result in appropriate management of the patient, and avoid secondary infections. Press attention can be quite disruptive and is best managed by the sharing of accurate and regular information.

Details of appropriate management and containment facilities, contact handling, surveillance, laboratory procedures and resource laboratories have been published.

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Chapter 184 - Dengue Fever/ Dengue Hemorrhagic Fever

Scott B Halstead

The dengue viruses (types 1, 2, 3 and 4) are enveloped ssRNA viruses of the Flaviviridae family. Transmission from human to human is by the mosquito *Aedes aegypti*, which bites in the daytime, is adapted to human habitats and has a strong preference for human blood meals. It breeds in relatively clean water stored for drinking or washing purposes in a variety of containers, and in rainwater that collects in manmade containers (e.g. tires, plastic containers, bottles, pails, tanks, cisterns, shallow wells).

The population explosion since World War II and subsequent migration from rural to urban areas have resulted in large cities, deteriorating urban environments and the spread of *A. aegypti* to almost all tropical countries.[1] [2] All four dengue virus types are now endemic around the globe. Of the 2.5 billion inhabitants of these areas, it is estimated that about 50–100 million individuals become infected by dengue viruses each year. Since the end of World War II, a syndrome first recognized in South East Asia — dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) — has spread globally (see Fig. 184.1), resulting in hundreds of thousands of hospitalizations and thousands of deaths each year.[2] Travelers to most tropical areas are at risk of dengue infection. In 2001, Hawaii experienced a 6-month epidemic of dengue type 1 transmitted by *Aedes albopictus*.

PATHOGENESIS

Dengue hemorrhagic fever/DSS is an immunopathologic syndrome that occurs in:

- infants infected for the first time who have acquired maternal dengue antibody in utero;[3] and
- children and, less commonly, adults during a second dengue virus infection. [4] [5]

Enhancing antibodies appear to be responsible for DHF/DSS. From protective levels at birth, maternal dengue antibodies degrade from neutralizing to enhancing concentrations over a period of 2–10 months.[3] Non-neutralizing antibodies, even in very small numbers, form infectious immune complexes with dengue viruses.[6] These attach efficiently to the Fc receptors of mononuclear phagocytes (macrophages, monocytes, Kupffer cells). The virus then attaches to receptors and fuses through adjacent plasma membrane, extruding viral RNA into the cytosol. The net effect is enhanced infection — more cells infected more rapidly than without enhancing antibody, a phenomenon now documented in humans.[7] Sequential infection in children over 1 year of age accounts for 85% of cases of DHF/DSS. In this setting, enhanced infections occur when antibody from a first infection fails to cross-neutralize a second dengue virus type.[8]

Only viruses of South East Asian origin appear to produce DHF/DSS. [9] [10] In the American genotype dengue 2 virus, the occurrence of dengue 1-like epitopes results in significant cross-reaction and some level of protection in persons immune to dengue 1 virus.[11]

The mediator of DSS has not yet been identified. Recent data suggest that IL-2, interferon-? and tumor necrosis factor released as a result of interactions between activated T cells and infected macrophages may damage postcapillary endothelial junctions. Dengue hemorrhagic fever/DSS is more severe in:

- whites and Asians (versus black people);[12] [13]
- females (versus males); and
- well-nourished (versus malnourished) children.[2]

PREVENTION

Aedes aegypti is a furtive black-and-white striped mosquito that often bites shadowed areas of the skin on the back of the neck, arms and legs. Most dengue infections are acquired at home. Prevention consists of scrupulous destruction and control of breeding sites. All unwanted containers should be discarded, buried or filled with sand.[2] Salt prevents eggs from hatching in water-filled ant traps and water coolers. Standing water that needs to be conserved can be treated with a 1% sand granule formulation of Abate, 1ppm [0,0'-(thiodi-*p*-phenylene) 0,0,0,0'-tetramethylphosphorothioate].

Larvivorous small fish can provide reliable control of mosquito larvae. Adult mosquitoes can be destroyed by pyrethrin knock-down sprays or organophosphate sprays delivered in microdroplets. However, source reduction is always the best method of preventing dengue infections.

For travelers to tropical countries, the only practical method of prevention is to avoid mosquito bites. Topical repellents are effective, but prevention comprises avoiding daytime visits to high-risk areas.

A tetravalent live-attenuated dengue vaccine is in the final stages of development.

CLINICAL FEATURES

Most primary dengue infections in children and many in adults are silent. Dengue infection presents clinically as three overlapping syndromes: undifferentiated fever, dengue fever syndrome and DHF/DSS.[2] The early signs and symptoms of overt dengue infections are common to many acute viral, bacterial and parasitic infections. The pathophysiologic presentation of classic DSS (history of recent high fever, thrombocytopenia, elevated hematocrit and hypotension or narrow pulse pressure) is unique in infectious diseases. Presumptive diagnosis of dengue fever or DHF requires a careful travel history to establish possible exposure to dengue infection. The differential diagnoses for the dengue fever and viral hemorrhagic fever syndromes are shown in Table 184.1 .

Undifferentiated fever

This occurs in young children and is a mild febrile illness lasting 1–3 days, often with upper respiratory signs.

Dengue fever syndrome

This occurs in adolescents and adults. After an infective mosquito bite, there is an incubation period of 3–8 days, followed by a sudden onset of fever with severe headache, pain behind the eyes, backache, chills, lack of appetite, gastrointestinal disturbances and generalized

Figure 184-1 Geographic distribution of dengue.

TABLE 184-1 -- Differential diagnosis of dengue fever and dengue hemorrhagic fever.

DIFFERENTIAL DIAGNOSIS OF DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER
Dengue fever syndrome
• *Mosquito-transmitted febrile exanthems*
- chikungunya (Africa, Asia)
- o'nyong nyong (Africa)
- West Nile (US, Europe, Africa, Asia)
• *Insect transmitted — no rash*
- sandfly fever (Europe, Middle East)
- Rift Valley fever (Africa, Egypt)
- Ross River fever (Australia, Pacific Islands)
- Oropouche (Amazon basin)
• *Febrile diseases in dengue-endemic areas*
- most acute viral and bacterial infections

- malaria
- yellow fever
- leptospirosis
- scrub typhus
- viral hepatitis
Hemorrhagic fevers
• *Ubiquitous*
- meningococcemia
• *Viral hemorrhagic fevers*
- mosquito-borne: Rift Valley fever, yellow fever
- tick-borne: Crimean-Congo HF, Omsk HF, Kyasanur Forest disease
- infectious secretions/tissues: Argentine HF, Bolivian HF, Venezuelan HF, Lassa fever, Marburg disease, Ebola HF, hemorrhagic fever with renal syndrome

pains in the muscles and bones ('breakbone fever'). A maculopapular rash usually appears on the trunk between the third and fifth day of the illness, spreading to the face and extremities. Fever is accompanied by leukopenia, relative lymphocytosis and moderate thrombocytopenia. Dengue fever may be complicated by bleeding, particularly in menstruating women or adults who have peptic ulcer disease.[14] The illness usually lasts for about 4–10 days. Convalescence may be accompanied by prostration and depression.

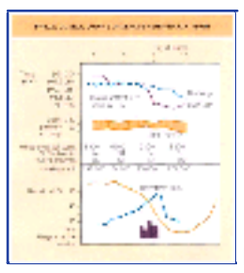
Figure 184-2 Typical clinical course of dengue hemorrhagic fever.

DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME

This is an acute vascular permeability syndrome accompanied by abnormal hemostasis. Although both adults and children develop the syndrome, children are inherently more susceptible due to greater capillary fragility,[5] [15] [16] with a characteristic illness progression (Fig. 184.2). A relatively mild first phase with abrupt onset of fever, malaise, vomiting, headache, anorexia and cough may be followed after 2–5 days by a rapid deterioration and physical collapse due to hypovolemia secondary to increased vascular permeability.[17]

In this second phase, as the temperature becomes normal the patient may have cold clammy extremities, a warm trunk, flushed face, circumoral cyanosis, diaphoresis, restlessness, irritability and midepigastric pain. Respirations and pulse are rapid. Blood pressure may exhibit narrow pulse pressure (20mmHg) or low systolic and diastolic pressures. Scattered petechiae may be seen on the forehead and extremities along with spontaneous ecchymoses, easy bruising and bleeding at venepuncture sites.

As the disease progresses, the liver is usually palpable two or three fingerbreadths below the costal margin, firm and nontender. Chest radiography shows unilateral (right) or bilateral pleural effusions. Sonograms show ascites and perivesicular edema. Approximately 10% of patients have gross ecchymoses or gastrointestinal bleeding. Laboratory abnormalities include thrombocytopenia, elevated hematocrit and abnormal levels of liver enzymes. Convalescence is fairly rapid for children who recover after a 24–36-hour period of crisis.

In addition to classic DSS, adults may have a stormy, often fatal course characterized by elevated liver enzymes, hemostatic abnormalities and gastrointestinal bleeding.

MANAGEMENT

Dengue fever

Dengue fever should be treated supportively. Aspirin is avoided because it may exacerbate the bleeding tendency. Patients who go into shock with normal or falling hematocrit levels should be investigated for gastrointestinal bleeding. [14]

Dengue hemorrhagic fever/dengue shock syndrome

Dengue hemorrhagic fever/dengue shock syndrome is life-threatening and requires immediate evaluation of vital signs, hemoconcentration, dehydration, urine output and electrolyte imbalance (Fig. 184.3). Close monitoring for at least 48 hours is essential because shock may occur or recur precipitously early in the disease. Patients who are cyanotic or have labored breathing should be given oxygen.

Rapid intravenous replacement of fluid and electrolytes using normal saline can often sustain patients until they have a spontaneous recovery. [2] [17] Colloid preparations, such as dextran 70, should be given if the pulse pressure is 10mmHg or less or the hematocrit remains elevated after fluid replacement.[18] Care should be taken to


Figure 184-3 Dengue hemorrhagic fever/dengue shock syndrome. Case definition, clinical staging and treatment strategies for DHF/DSS.

avoid overhydration, which is heralded by a fall in hematocrit and a wide pulse pressure. Diuretics may be necessary. Fresh frozen plasma, whole blood, platelets or heparin (if there is laboratory evidence of severe consumptive coagulopathy), together with rigorous replacement of fluid and protein with colloids, may be required if bleeding complicating DHF/DSS is sufficiently severe. Bleeding is thought to be due to one or more platelet abnormalities.[19]

Chloral hydrate or diazepam may be necessary to manage agitated children.

Corticosteroids, vasopressors, a-adrenergic blocking agents and aldosterone have no role in treatment. Salicylates are contraindicated.

The etiology can be established by recovering the virus from the acute-phase serum, usually on or before the fifth day after the onset of fever. An IgM antibody-capture enzyme-linked immunosorbent assay on serum obtained between 7 days and 2 months after the onset of the fever enables identification of a recent dengue infection. Increases in antibody titer can be detected by hemagglutination inhibition or neutralization tests on paired sera (optimally separated by 2 weeks). [20]

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Chapter 185 - Anthrax

Mehmet Doganay

INTRODUCTION

Anthrax is an ancient disease that was rarely seen outside certain well-defined geographic areas, except as an occasional occupational hazard, but which has recently assumed greater importance as a result of its potential use as an agent of bioterrorism.

EPIDEMIOLOGY

Anthrax is usually a disease of herbivores and only incidentally infects humans. This infection still persists in arid and semiarid regions of the Middle East, in Africa, Asia, South America and Haiti. Humans almost invariably acquire anthrax directly or indirectly from infected animals. The main route of transmission is contact with or inhalation of *Bacillus anthracis* spores.[1] [2] Human cases may occur in an agricultural or an industrial environment.[1] [3]

Agricultural cases have occurred in individuals who came into contact with sick or dead animals in rural areas. In certain impoverished communities, livestock owners are forced to slaughter animals at the first sign of infection in order to salvage the meat, hair and hides because of economic problems. Farmers, butchers, knackers, shepherds and veterinarians are therefore the most frequently infected. Anthrax is also reported in women spinning wool with hand spindles and in carpet weavers.[1] [3] [4] Another route of infection is by ingestion of raw or undercooked meat from an infected carcass.[5] Travelers should be aware that, in certain societies, some traditional meals are made of raw meat and are consumed without any cooking or preservation methods. In our series of 113 cases of anthrax, 89 patients gave a history of handling of, or contact with infected dead animals or their products. In four cases, there was a history of consumption of meat from the carcass of a diseased cow or sheep. The source of infection in the remaining 20 cases could not be determined but they were clearly agricultural in origin.[6]

There is also an infection risk after contact with a commercial product prepared from inadequately treated wool or leather. For example, a human case of cutaneous anthrax was acquired from contact with imported souvenir drums with drumheads made of goatskin and another case was most probably acquired from a purchased wool coat. A fatal case of inhalation anthrax was also recorded in a home weaver as a result of contact with imported yarn from Pakistan that contained animal fibers.[1] [3]

Insect vectors, such as horseflies, have been reported to transmit *B. anthracis* from an infected animal to a second animal. They could also theoretically infect humans by mechanical transfer but this has not been well documented.[1] [3]

Industrial anthrax occurs as a result of the inhalation of spore-laden dust or other aerosols or contact with spores. In the industrial environment, spores in dust clouds created from the handling of dry hides, skins, sheep wool, goat hair, bone meal and the like are inhaled or spread through contact with the skin of workers. Most cases in industrialized countries are associated with exposure to animal products, particularly goat hair, imported from countries in which anthrax is endemic.[1] [3] [7]

Records of person-to-person spread are very rare; an unqualified nursing orderly in Zimbabwe acquired an anthrax lesion on his finger as a result of removing dressings from a patient who had cutaneous anthrax, and communal loofahs were found to be responsible for, spreading infection from person to person in the Gambia. Laboratory-acquired infections occur occasionally.[1] [3] In 2001 there was an outbreak of anthrax in the USA that apparently resulted from the deliberate distribution of spores in the postal system, resulting in a number of cases of both cutaneous and inhalational anthrax.[2]

CLINICAL FEATURES

The disease occurs primarily in three forms: cutaneous, respiratory and gastrointestinal. Sepsis and meningitis can rarely develop after the lymphohematogenous spread of *B. anthracis* from a primary lesion (cutaneous, gastrointestinal or pulmonary).

Cutaneous anthrax

Cutaneous anthrax accounts for 95% of human cases. The spore is introduced to the skin via a cut, abrasion or insect bite. The incubation period ranges from 1 to 19 days, usually 2–7 days. The lesion begins as a pruritic papule. The papule enlarges and a ring of vesicles develops around the papule at day 2–4 of the disease. Vesicular fluid may be a hemorrhagic exudate (Fig. 185.1). This area is surrounded by a small ring of erythema and marked edema develops. Unless there is secondary infection, there is no pus and the lesion is not painful, although painful lymphadenitis may occur in the regional lymph nodes. Eventually, the vesicle or vesicular ring ruptures, discharging a clear fluid, and a central depressed black necrotic lesion known as an eschar is formed (Fig. 185.2). Edema extends some distance from the lesion. The eschar begins to resolve about 10 days after the appearance of the initial papule. Resolution is slow (2–6 weeks), regardless of treatment (Fig. 185.3).[1] [7] [8] [9]

The lesion is usually 1–3cm diameter and remains round and regular. Rarely, a lesion may be larger and irregularly shaped. Systemic symptoms, including low-grade fever, malaise and headache, may be present. The cutaneous reaction may be severe in some patients and is characterized by significant local and spreading edema associated with blebs, bullae, induration, chills and fever (see Fig. 185.2b). Clinical symptoms may be more severe if the lesion is located in the face, neck or chest. In these more severe forms, clinical findings are high fever, toxemia, regional painful adenomegaly and extensive edema; shock and death may ensure (Fig. 185.4). [6] [7] [8] [9] [10] [11]

More than 90% of the lesions occur in exposed areas such as the face, neck, arms or hand. The site of infection often reflects the occupation of the patient. Workers who carry hides or carcasses on their shoulders are prone to infection on the back of the neck. Handlers of contaminated animal products tend to be infected on the arms, wrists and hands. The patients generally have a single cutaneous lesion but sometimes they have two or more. For example, if the

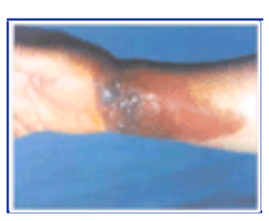

Figure 185-1 A cutaneous anthrax lesion with extensive erythema and hemorrhagic bullae on the wrist.

infection has been acquired by skinning an infected dead animal with hands and arms, multiple lesions can be seen on hands, wrists and arms. Atypical localization can also be seen.[1] [3] [4] [6] [7] The distribution of lesions in 114 cases of anthrax treated in our clinic is shown in Table 185.1 . A skin lesion was seen on the hand and fingers in the majority of cases. Lesions localized to other anatomic sites were less frequently observed.

Gastrointestinal anthrax

Ingestion of *B. anthracis* in contaminated food or drink can cause gastrointestinal anthrax. The incubation period is commonly 3–7 days. There are two clinical forms of gastrointestinal anthrax: intestinal and oropharyngeal.[1] [5] [7] [8]

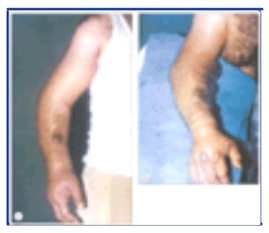

Figure 185-2 Cutaneous anthrax. (a) A well developed lesion on the right forearm (third day of disease). (b) The extension of the skin lesion in the same patient on the sixth day. Extensive edema, induration and bullous changes have occurred over the last 3 days despite antibiotic therapy. Antibiotic therapy does not prevent inflammatory reactions.

The symptoms of intestinal anthrax are initially non-specific and include nausea, vomiting, anorexia and fever. With progression of the illness, abdominal pain, hematemesis, bloody diarrhea and massive ascites occur, and signs suggestive of acute abdomen appear. Then toxemia and shock develop, followed by death. The lesions occur most commonly on the wall of the terminal ileum or cecum. The stomach, duodenum, upper ileum and large bowel are occasionally affected.[1] [7]

Oropharyngeal anthrax is less common than the gastrointestinal form. The lesion is generally localized in the oral cavity, especially on the buccal mucosa or tongue, or the tonsils, and the posterior wall of the pharynx. In some cases, the lesion may be present in two or more places in the gastrointestinal system, oropharynx and intestine. The oral lesion is generally 2–3cm in diameter and covered with a gray pseudomembrane surrounded by extensive edema. When infection is localized on the tonsils, the affected tonsil is also intensely edematous and covered with pseudomembrane. The main clinical features are sore throat, dysphagia, fever and painful regional lymphadenopathy in the neck. The illness progresses rapidly and edema develops around the lymph node and may extend to the upper anterior chest wall. Bacteremia may develop. The infection leads to toxemia and acute respiratory distress syndrome. Shock and coma ensue. In some cases, toxemia leads to sudden death. Despite intensive medical therapy, the mortality is about 50%.[5]

Inhalation anthrax

This natural form of anthrax was previously almost always caused by industrial exposure to spores; however, the most serious outbreaks of anthrax in 1979 in Sverdlovsk and in October 2001 in America are new and notable exceptions. [2] [12]

Inhalation anthrax shows a biphasic clinic pattern with a mild initial phase followed by an acute and severe second phase. After an incubation period of 1–6 days (up to 43 days in the event at Sverdlovsk), the illness begins with mild fever, fatigue, malaise, myalgia, nonproductive cough and some chest or abdominal pain.

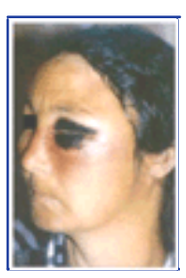

Figure 185-3 A dried black anthrax eschar on the eyelids on the 15th day of therapy (third week of the disease). The lesion healed leaving a deep scar. *Courtesy of Professor O Ural, Konya, Turkey.*

TABLE 185-1 -- Distribution of lesions in 114 cases of anthrax.

DISTRIBUTION OF LESIONS IN 114 CASES OF ANTHRAX		
Site of lesion	**No. of cases**	**No. of deaths**
Cutaneous	**107**	**0**
Hands and fingers	79	
Wrist and arms	10	
Eyelid and face	11	
Neck	2	
Foot and leg	5	
Oropharyngeal	**6**	**3**
Tonsil	5	
Tongue	1	
Meningitis	**1**	1

The disease progresses to the second phase within 2–3 days. The second phase is characterized by high fever, toxemia, dyspnea and cyanosis. Hypothermia and shock develop, resulting in death. In up to half of patients, meningitis develops as a complication.[7] [13]

Anthrax meningitis

The meningeal form of anthrax is very rare. The world's literature contains approximately 100 cases of anthrax meningitis, with a mortality rate of over 90%.[8] [14]

Meningitis may be a complication of the three forms of primary anthrax. The most common portal of entry is skin (52%) and then the lungs (22.9%). Anthrax meningitis also occurs in cases of gastrointestinal anthrax. The organisms can spread to the central nervous system by hematogenous or lymphatic routes. The primary focus of infection can not be determined in about 10% of cases and it is called primary anthrax meningitis. Blood cultures are positive for *B. anthracis* in 70% of patients who have meningitis.[14]

The clinical presentation includes sudden onset of fever, fatigue, myalgia, headache, nausea, vomiting, agitation, seizures, delirium and meningeal symptoms. The initial signs are followed by rapid neurologic deterioration and death. The cerebrospinal fluid is often bloody and contains many Gram-positive bacilli. [7] [8] [14]

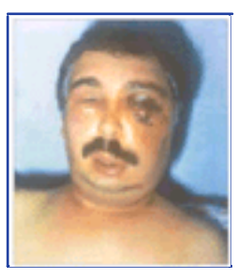

Figure 185-4 An anthrax lesion of the eyelids surrounded by erythema and massive edema extending from the left eye to the right and down to and beyond the neck. Such extensive edema is characteristic of anthrax. This lesion healed with therapy and left a deep scar.

Anthrax sepsis

Sepsis may occur by spreading of *B. anthracis* via lymphohematogenous route from a primary lesion. Sepsis is rarely seen in patients who have cutaneous anthrax; it is more commonly seen in patients who have inhalation and gastrointestinal anthrax. Clinical features include fever, respiratory distress and changing mental status. Severe toxemia and shock may lead to death in a short time.[5] [8]

COMPLICATIONS

Some 10–20% of untreated cases of cutaneous anthrax might be expected to result in death. With treatment, the mortality rate is less than 1%. Toxemic shock due to massive edema, airway obstruction by compression on the trachea from edematous swelling around the neck, deep scar tissue, deep tissue necrosis and secondary infection are all recorded as complications in cases of cutaneous anthrax. [1] [7] [8] In our 107 cases of cutaneous anthrax, toxemic shock occurred in two cases, airway obstruction in two, eyelid deformity in two, temporal artery inflammation in one, and secondary infection and deep tissue necrosis in two.[6] [10] [11]

Serious complications such as sepsis and meningitis can be seen in inhalation anthrax and gastrointestinal anthrax. These complications are less frequently seen in cutaneous anthrax. The mortality rate in industrial-related inhalation anthrax is over 80%, despite treatment. Gastrointestinal anthrax is also a potentially fatal disease. Mortality is greater than 50%. If an early diagnosis is made and an appropriate treatment is given, the disease can be cured.[1] [5] [7] [8] [13]

DIFFERENTIAL DIAGNOSIS

A history of exposure to contaminated animal materials, occupational exposure and living in an endemic area are all important clues for the suspicion of anthrax.[3] [8]

Cutaneous anthrax should be suspected when the patient describes a painless, pruritic papule, surrounding vesicles and edema, usually on an exposed part of the body. The ulcerative eschar of cutaneous anthrax must be differentiated from other papular and ulcerative lesions that present with regional lymphadenopathy. If regional lymphadenopathy together with a purulent lesion is present, a cutaneous anthrax lesion may be superinfected with pyogenic bacteria such as staphylococci. The differential diagnosis should include ecthyma gangrenosum, rat-bite fever, ulceroglandular tularemia, plague, glanders, orf, rickettsialpox, erysipelas, staphylococcal skin and lymph node infection, syphilitic chancre and cutaneous tuberculosis.[7] [8] [9] [13] Occasionally, the cutaneous reaction may be severe. A severe

cutaneous anthrax lesion involving the face, neck and anterior chest wall must be differentiated from orbital cellulitis, dacryocystitis and deep tissue infection of the neck. Necrotizing soft tissue infections — particularly group A streptococcal infections and gas gangrene — and severe cellulitis due to staphylococci should also be considered in the differential diagnosis of severe forms of cutaneous anthrax. Gas and abscess formation are not observed in patients who have cutaneous anthrax. [7] [8] [9]

Intestinal anthrax mimics food poisoning (in the early stages), acute abdomen of other causes and hemorrhagic gastroenteritis, particularly necrotizing enteritis due to *Clostridium perfringens*.[7] [8] In the differential diagnosis of oropharyngeal anthrax, streptococcal pharyngitis, Vincent's angina, Ludwig's angina, parapharyngeal abscess and deep tissue infection of the neck should be considered.[5]

The clinical picture of anthrax meningitis is acute hemorrhagic meningitis. Differential diagnosis should include acute meningitis of other bacterial etiology and subarachnoid hemorrhage. In the differential diagnosis of anthrax sepsis, sepsis due to other bacteria should be considered.[7] [8]

The initial symptoms of inhalation anthrax are non-specific and clinical presentation is similar to those of atypical pneumonia from other causes and cardiovascular collapse with noninfectious causes.[7] [13] More details on inhalation anthrax are given in Chapter 231 .

INVESTIGATIONS

The investigation of a potential exposure to the infectious agent is very important for suspicion of anthrax. However, the source of infection cannot be determined in some cases.

The well-developed lesion of cutaneous anthrax is readily recognized by its central eschar, ring of vesicles and accompanying edema. Swabs are appropriate for collecting vesicular exudates for microscopy and bacterial culture. In a well formed eschar, in which vesicular exudate is absent, the edge of the eschar can be lifted up with forceps and fluid obtained by a capillary tube. A smear is made from the material and is stained with polychrome methylene blue and examined microscopically for the presence of the pink-staining encapsulated bacilli (McFadyean reaction). The samples are also inoculated on blood agar.[1] [8]

For the isolation of *B. anthracis* in patients who have suspected gastrointestinal anthrax, swabs from oropharyngeal lesion, vomit, fecal specimens, blood and ascites samples are obtained. Specimens likely to be contaminated with commensal flora should be cultured on polymyxin-lysozyme-EDTA-thallous acetate (PLET) agar as a selective medium for *B. anthracis*. [1] [8]

Radiographic examination of the chest usually reveals widening of the mediastinum in inhalation anthrax. Parenchymal infiltration and pleural effusion can also be seen. Direct examination of a smear of pleural fluid or blood, stained with polychrome methylene blue or Gram stain, may show encapsulated bacilli. *Bacillus anthracis* can be isolated from the cultures of these specimens. Culture of nasal swabs has been used for the determination of inhalation exposure to *B. anthracis*.[2] [7] [13]

Blood or cerebrospinal fluid smear stained with polychrome methylene blue should be examined for the encapsulated bacilli and blood or/and cerebrospinal fluid cultures are taken for the isolation of *B. anthracis* in cases of sepsis or meningitis.[7] [8] [14]

Serologic tests are also useful for diagnosis of anthrax. For routine confirmation of anthrax infection or in monitoring the response to the anthrax vaccine, a determination of antibodies against protective antigen alone appears to be satisfactory. Diagnosis may be confirmed serologically by demonstrating an increase in antibody titers; two or more serum samples taken 2–4 weeks apart will give greater diagnostic reliability. If only one serum sample is collected, it will be of greater diagnostic value if collected more than a week after the onset of symptoms. [15]

New diagnostic techniques include immunohistochemical testing of clinical specimens by using *B. anthracis* capsule and cell wall antibody and, most recently, by *B. anthracis*-specific polymerase chain reaction. These new rapid methods may become useful in early diagnosis and in culture-negative patients.[2] [7]

MANAGEMENT

Viable *B. anthracis* organisms disappear from the lesions of cutaneous anthrax within a few hours of the initiation of treatment with parenteral penicillin G. Given the severity of disease, however, patients who have suspected anthrax should receive immediate empiric therapy pending definitive diagnosis. Penicillin G has been the drug of choice, an alternate being doxycycline (although recently ciprofloxacin has been added as a first line agent — see below). Treatment is usually continued for 7–10 days. Naturally occurring strains have also been sensitive to erythromycin, cefazolin, tetracycline, chloramphenicol, gentamicin and ciprofloxacin (see also Chapter 226).

The recommendations for antibiotic use in the setting of a bioterrorism attack are based upon a small series of cases in humans, studies in experimental animals and the knowledge that strains may have been engineered for antibiotic resistance. [16] Because of the rapid course of bioterrorism-related symptomatic inhalation anthrax, early antibiotic administration is essential. Limited early information from the 2001 anthrax attacks suggest that those treated with two or more intravenous antibiotics active for *B. anthracis* had a greater chance of survival.[2] Although small numbers make these observations statistically inconclusive, given the severity of the illness, this seems prudent. The treatment of choice has been ciprofloxacin, with doxycycline as an alternative. An aminoglycoside is often added. Once clinically stable, patients are switched to oral regimens but, because of the risk of delayed germination of spores, it has been suggested that antibiotic therapy should be continued for at least 60 days.

PREVENTION

The mainstay of prevention of anthrax is the avoidance of contaminated animals or animal products. An inactivated acellular vaccine exists and is used mostly in

occupational and military settings. This vaccine is not recommended for normal travelers unless there is likely to be occupational exposure. A live attenuated vaccine has been produced and used in the former Soviet Union. Accelerated development is underway to produce safer and more effective vaccines.

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Chapter 186 - Practice Points

186.a Management of a patient who has suspected viral hemorrhagic fever

Steven M Opal

Introduction

Continued expansion of human populations into tropical rain forests, economic pressures and changing ecologic conditions around equatorial regions of the world have increased the risk of exposure to a variety of tropical viral diseases. Global markets and expanded international trade in combination with improved access to remote areas by expanded international airline transportation makes it feasible that these viral illnesses could spread worldwide. Physicians in endemic areas must be aware of the potential threat of viral hemorrhagic illnesses. Moreover, physicians in nonendemic regions must recognize the potential risk of hemorrhagic viral illness in international travelers from tropical regions. Animal handlers in primate research laboratories throughout the world are also at risk. Regrettably, these viral agents are potentially exploitable as a bioterrorist weapon as well. It is essential that health care facilities develop a plan to manage viral hemorrhagic fever (VHF) even if the chances of seeing such patients seem remote.

It is important that physicians are aware of the potential risk of tropical VHFs for several reasons:

- to ensure appropriate diagnosis and management of index cases;
- to provide advice, counseling and possible prophylaxis to close contacts;
- to minimize the risk of nosocomial transmission among health care workers (HCWs) caring for such patients; and
- to contact public health authorities immediately in the event of a possible outbreak of VHF.

Strict adherence to basic infection control techniques and some advance planning will minimize the risk to HCWs and allow rapid, compassionate and safe care of affected patients.

Microbiology and pathogenesis

Hemorrhagic viruses in which person-to-person transmission has been documented include representatives of the arenavirus, bunyavirus and the filovirus groups. The most important examples are Lassa fever, Ebola virus and Marburg virus, and Crimean-Congo hemorrhagic fever, caused by a tick-transmitted bunyavirus (see Chapter 222). Lassa, Ebola, Marburg and Crimean-Congo hemorrhagic fever viruses are particularly important to recognize because nosocomial transmission to HCWs is a real possibility. Although the animal reservoir and mode of transmission to humans is reasonably well understood for Lassa and Crimean-Congo hemorrhagic fevers, the method of transmission of Ebola virus and Marburg virus remain an unsolved mystery.

These viral syndromes share many overlapping clinical features in humans. After an incubation period of between 3 and 21 days, patients develop the abrupt onset of fever, headache, myalgia, sore throat, respiratory symptoms, abdominal pain, nausea, vomiting, diarrhea and conjunctivitis with associated pharyngitis and cervical lymphadenitis. A macular skin eruption may occur in infections with Ebola and Marburg virus; this is less common in Lassa and Crimean-Congo hemorrhagic fever. Various degrees of mucosal and cutaneous hemorrhage occur associated with thrombocytopenia and disseminated intravascular coagulation. The geographic location or travel history of the patient is most useful in distinguishing between the different types of VHFs before virologic confirmation.

The viruses share rapid growth potential and the ability to invade a variety of cell types, resulting in high-grade viremia. The patient's blood and body fluids become potentially contagious to others who come in direct contact with them. Transmission may also occur through handling of bodies during burial rituals, as demonstrated in recent Ebola outbreaks in central Africa. Although many other febrile illnesses, such as malaria, typhoid fever, meningococcemia, arboviral infections and leptospirosis, may present in a similar fashion, infection control measures must be instituted to guard against potential transmission of VHFs until the diagnosis is established.

Diagnosis and management

Recent improvements in the serologic diagnosis of VHFs now make it possible to make a specific diagnosis in the majority of acutely ill patients. An antigen-capture enzyme-linked immunosorbent assay (ELISA) has been developed for Ebola virus and may allow rapid diagnosis in acutely ill patients. Specific IgM and IgG capture ELISA antibody studies are available for serologic diagnosis in convalescent samples. Unfortunately, diseases such as Ebola virus infection, in

TABLE 186.a-1 -- Infection control methods for suspected viral hemorrhagic fevers.

INFECTION CONTROL METHODS FOR SUSPECTED VHFs	
Isolation method	**Comments**
Isolation room	In the past, the negative pressure room was the ideal and still may be used where available. However, the patient can be managed through strict contact isolation, universal blood and body substance precautions and enhanced prevention measures in the handling of blood and body fluids
Personnel and visitors	Traffic flow into the patient's room should be restricted. A daily record of those who enter and leave the patient's room should be kept. Only essential personnel should be exposed to the patient and the patient's body fluids
Personal protection	Fluid-impervious gowns, gloves, face shields or surgical masks with eye protection (goggles); if cough, vomiting or extensive hemorrhage, respirators with filters (high-efficiency particulate air respirators) and leg and shoe coverings should be worn
Clinical samples	Clinical samples should be placed in plastic sealed bags and transported in a leak proof container without contaminating the external surfaces. Samples should be handled in a biologic safety cabinet (biosafety level III). Serum should be pretreated with a polyethylene glycol phenolic for 1 hour before handling. Automated analyzers should be disinfected with 1:100 dilution of bleach after use. Fixation of blood smears and tissue samples will inactivate the virus and can be handled in a routine manner
Decontamination of the environment and of linen	Contaminated environmental surfaces should be disinfected using a registered hospital disinfectant or 1:100 dilution of bleach. Soiled linens can either be decontaminated by use of an autoclave or incineration. Hot cycle laundering with bleach may be acceptable.
Human excrement and blood and body fluids	As an added precaution, human excreta, blood and body fluids should be decontaminated by 1:100 dilution of bleach for at least 5 minutes before disposal
Surgical procedures and autopsy	If a surgical procedure or autopsy is essential, extreme precautions must be used to avoid blood contamination. Double gloves, full face shields with high-efficiency particulate air filtration, water-impervious gowns and shoe covers should be worn. Every effort should be taken to avoid generation of an aerosol. Deceased persons should not be embalmed. The body should be placed in leakproof, sealed material and cremated or buried in a sealed casket

which mortality rates exceed 75%, do not often allow the opportunity to study convalescent samples. Virus isolation from the blood and body secretions of acutely ill patients is the definitive diagnostic method. This is, of course, a severe biohazard and it should only be attempted in biosafety level IV facilities. Reverse transcription and polymerase chain reaction for specific viral RNA is also a useful diagnostic method in patients who have viral hemorrhagic illnesses. Many of these methodologies

are unavailable in regions of the world where these diseases are endemic. For this reason, the recent development of an immunochemical staining method for skin biopsy samples is particularly valuable. This method allows for fixation of tissues at the site of diagnosis and eliminates the biohazard of transportation of infected human tissues.

Routine diagnostic methods to evaluate other common febrile illnesses should not be delayed because of suspected VHF. In particular, care must be taken to exclude falciparum malaria, which may be fatal if unrecognized and left untreated. In practice, most cases of suspected VHF turn out to be malaria. Universal precautions when handling blood and body secretions should suffice to protect HCWs from hemorrhagic viruses (see Chapter 183).

Infection control methods

The viruses are transmitted through direct contact with the patient or the patient's secretions. There is a remote risk of airborne transmission based upon studies with nonhuman primates, and one potential transmission by respiratory aerosol in a patient who had Lassa fever with extensive pulmonary involvement has been reported. Therefore, the primary infection control strategy is strict contact isolation, universal blood and body substance precautions, and enhanced preventive measures in the handling of blood and body fluids. Body substances are contagious during the acute febrile illness, but there is no evidence of transmission during the incubation phase of the illness. The guidelines listed in Table 186a.1 should be instituted in patients who have suspected VHF.

Postexposure prophylaxis

The arenavirus that causes Lassa fever is susceptible to ribavirin, and this antiviral agent may be of some value to HCWs exposed to blood or body fluids (e.g. by percutaneous needlestick accident). Passive immunotherapy with plasma from surviving patients with high-titer antibody has been shown to be of limited benefit in the prevention of VHF.

Reporting

It is essential that patients who have suspected VHF are reported to public health authorities as soon as possible. This allows a coordinated response to a potential epidemic situation and ensures that diagnostic and therapeutic efforts will be handled appropriately. Expert international assistance may be necessary should a VHF occur in an international traveler.

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186.b Follow-up of the traveler who has swum in Lake Malawi

Nick J Beeching

Introduction

Lake Malawi is a huge freshwater resource 630km long and 50km wide in Central Africa, providing essential food and income for a large proportion of Malawians and people of the other nations that border its shores (Fig. 186b.1). Over the past two decades it has become a major attraction for both 'local' tourists and for backpackers and overlanders from outside Africa, with the associated development of hotels and facilities for watersports such as scuba diving and windsurfing. These are especially found in the south around Cape Maclear and Monkey Bay, where the shores are relatively shallow and the snail vectors for *Schistosoma haematobium* have become established. Further north and centrally, the ecology of the lake differs, with deep water close to the land. Bilharzia was recognized by early European visitors to Malawi and was noted in 50% of lakeshore inhabitants in the early 1900s. It has become more widely

Figure 186.b-1 Lake Malawi.

established since then, particularly with the development of irrigation schemes. The predominant species is *Schistosoma haematobium*, but small pockets of *Schistosoma mansoni* also exist.

Schistosomiasis has become a common diagnosis in expatriates and short-term tourists in Malawi. Similar risks are already well-recognized for visitors to the Dogon area of Mali or to the waters of the Kariba Dam and the Zambesi, but Lake Malawi has overtaken these sites as the main source of the many cases of schistosomiasis imported annually from Africa to the UK and elsewhere. A large case-control study showed that 33% of foreigners in Malawi had serologic evidence of exposure to infection, the highest risk being associated with repeated exposure to the lake, especially around Cape Maclear. Our own experience of screening groups of returned travelers is that 75% of those who spend a week scuba diving at Cape Maclear will have clinical or asymptomatic laboratory evidence of infection. People are at risk from showers and swimming pools that are fed directly with unchlorinated lake water as well as from paddling and swimming. Brief exposure is sufficient; we recently screened students from the same school who had spent 48 hours only at two locations around the lake. Of those camping near Cape Maclear 90% (19/21) had been infected, compared with none of 17 students camping on Likoma Island, another popular tourist spot.

General approach to the traveler who has swum in Lake Malawi

The general approach to the traveler who has swum in Lake Malawi is the same as that for any other traveler, including the need to exclude malaria in febrile patients. A detailed travel and exposure history must be taken, including the precise timing, frequency, type and locations of any freshwater contact. Previous travel and possible schistosomal risk activity should be checked along with pre-existing illnesses including atopy and urologic or gastrointestinal problems. Enquiries should routinely review adherence to antimalarial chemoprophylaxis and mosquito avoidance measures, and the use of measures that might reduce schistosomal load such as vigorous rubbing of the skin immediately after immersion, or pre-exposure use of antischistosomal soaps or permethrin on the skin.

Only a minority of people will remember experiencing 'swimmer's itch', lasting from a few to 48 hours after swimming, caused by penetration of the skin by cercariae. This does not reliably predict later symptoms, which first appear in a substantial minority of patients 3–8 weeks after exposure, as the 'Katayama syndrome,' which is related to an immunologic reaction to final migration of schistosomules around the body and the onset of oviposition by the maturing flukes (see Chapter 167). Typical symptoms include fever, headache, malaise, wheezing, dry cough and dyspnea. Transient urticarial rashes are common (Fig. 186b.2), and lymphadenopathy and hepatosplenomegaly may be found on examination. Usually this is a diagnosis of exclusion and the supportive laboratory finding of eosinophilia exceeding 2×19^9 /l is not always present. We have seen cases misdiagnosed as glandular fever due to coincident lymphocytosis, false-positive slide tests for infectious mononucleosis and mild disturbance of liver function tests. Transient shadows may be seen on chest radiographs.

Whether or not patients have experienced earlier symptoms, continued oviposition from 3 to 6 months after exposure may then cause symptoms related to the organs involved. *Schistosoma haematobium* principally affects bladder, prostate and seminal vesicles and typical complaints are of terminal hematuria, perineal discomfort and, in males, alteration in the consistency (thin or lumpy) or color (yellow

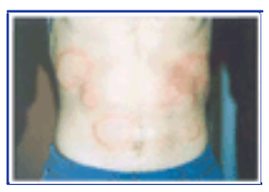

Figure 186.b-2 Giant urticaria associated with Katayama syndrome after swimming in Lake Malawi. *Courtesy of Dr ME Jones, Edinburgh.*

or frank blood) of semen. Women occasionally notice a wart-like genital granuloma. *Schistosoma mansoni* primarily affects the large bowel, leading to blood in feces and alteration in bowel habit, but the anatomic location of both species has considerable overlap. Symptoms are more common with *S. haematobium* infections and may be associated with non-specific fatigue.

The long-term outcome of untreated, relatively light infections of visitors is unknown, but is likely to be benign and not to lead to the complications caused by chronic and repeated infections of inhabitants of endemic areas, such as bladder cancer and portal or pulmonary hypertension (see Chapter 167). However, a small minority of travelers develop central nervous system complications such as epilepsy or spinal cord damage due to ectopic deposition of ova, and the general expert consensus is that all exposed travelers should be screened and treated.

Screening

The minimum investigations, whether or not symptoms are present, should include an absolute eosinophil count, testing of urine for blood, and microscopy for ova in feces and on a filter of a 4-hour mid-day urine specimen. 'Routine' urine microscopy is insufficiently sensitive. Semen microscopy may become positive earlier than urine or feces, and males can be asked to provide a sample for microscopy, particularly if being screened in the first few months after water exposure (Fig. 186b.3). If the index of clinical suspicion remains high and other tests are negative, a squash preparation of a fresh rectal biopsy can be examined for ova. Viability of ova, hence current infection, can be inferred from the observation of active flame cells within the miracidium developing in the ovum. Eosinophilia is only found in about 50% of patients in the chronic phase of infection.

The rather crude serologic tests available from European reference centers include an enzyme-linked immunosorbent assay (ELISA) using circumoval protein as antigen to detect circulating antischistosomal antibodies. These become positive from about the time of oviposition, but seroconversion may take up to 6 months and late follow-up screening is essential for those who are asymptomatic and who have negative tests before this time. The serologic tests available from the US Centers

for Disease Control and Prevention become positive soon after exposure to infection, and discriminate between *S. haematobium* and other species. Once positive, serology remains positive for years and titers may even increase during the first year after treatment. Follow-up serologic tests after treatment are not, therefore, useful as a test of cure or even for distinguishing reinfection

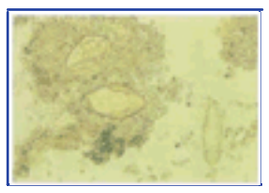
Figure 186.b-3 Empty ovum case and hatched miracidium of *Schistosoma haematobium* in semen. Note oligospermia, which usually resolves after treatment.

after subsequent re-exposure of the patient to infected water. The disappearance of any symptoms or other positive tests, including eosinophilia, is more reliable for follow-up after treatment.

Treatment

Treatment is now relatively easy and harmless, using oral praziquantel in a dose of 40mg/kg, sometimes split into two doses 6 hours apart to reduce any associated nausea. Many scuba divers and other visitors treat themselves with locally purchased praziquantel immediately after diving, but this is useless as postexposure prophylaxis. Praziquantel has little effect on migrating schistosomules, and so patients treated at the stage of Katayama syndrome symptomatology need to be re-treated 3 months later. Patients with moderate-to-severe Katayama syndrome probably also benefit from a short course of prednisone (e.g. 40mg daily for several days), preferably started a few hours before anthelmintic treatment. However, this anecdotal recommendation merits a prospective randomized trial to establish both short-term benefit and longer term benefit in reducing chronic fatigue, which is quite common after Katayama symptomatology. Coticosteroids reduce the efficacy of praziquantel, so increased dosing of praziquantel is therefore recommended by some.

Patients who present later, with symptoms such as hematuria or positive laboratory tests, should also be treated and should be followed up 6 months later to establish disappearance of any previous positive findings. Asymptomatic travelers with negative screening tests 6 months or more after lake exposure do not require treatment or further follow-up. It is debatable whether every patient who presents with hematuria and ova in the urine should have further investigations such as ultrasonography or cystoscopy to exclude coincident tumors or other problems. We have a low threshold for urologic referral, which is essential if local symptoms do not resolve after antischistosomal drugs.

Summary

Overall, the risk of acquiring schistosomiasis from swimming in the south of Lake Malawi is so high that screening of returned expatriates, travelers and immigrants is essential and is unlikely to yield false-positive results. Many expatriates regard the risk as so common that they bypass the screening process and treat themselves and their families as a routine every 6 months. We suggest that this approach is inappropriate for the occasional traveler who has swum in Lake Malawi (or similar freshwater bodies), who should be fully assessed

before possible treatment with an anthelmintic that is only available on a named-patient basis outside the tropics.

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186.c Indications for exchange transfusion in severe malaria

Robin Bailey

Introduction

Patients with high proportions of their red cells parasitized by *P. falciparum* are at increased risk of developing all the complications of severe malaria. It has been suggested that this risk is proportional to the parasitemia and thus that exchange transfusion might benefit some patients with very high parasite counts. The rationale for exchange transfusion is that physical removal of infected red blood cells from the circulation and their replacement by healthy and unparasitized red cells will:

- lower the parasite burden more quickly than chemotherapy alone;
- reduce the antigenic load and its concomitant burdens of parasite-derived toxins and metabolites and the host responses to them;
- correct anemia and improve the oxygen-carrying capacity and microcirculatory properties of the blood.

With these theoretical benefits come dangers of the procedure, including hypocalcemia, hemodynamic disturbance, transfusion reactions and infection.

The use of exchange transfusion has been reported in over 200 patients since 1974. A recent meta-analysis of eight studies involving 279 subjects with severe malaria found no evidence for a survival benefit and concluded that the evidence base supporting its use was inadequate, with publication bias and a tendency for more severely ill patients to receive exchange transfusion being particular difficulties in the interpretation of published studies. No adequately powered randomized controlled trial has been conducted and it is doubtful whether such a trial could be conducted given the logistic difficulties and the number of centers that would need to be involved. However, the balance of expert opinion favors its use as an adjunct to optimal chemotherapy in extreme situations.

When should exchange transfusion be considered?

Exchange transfusion only becomes an option if large amounts of adequately screened, pathogen-free and properly cross-matched blood are available, there are adequate monitoring facilities in a high-care environment, and the exchange itself can be carried out safely. The benefits may be greatest where nonimmune patients have high parasitemias and have not responded to optimal chemotherapy.

The WHO suggests the following indications for exchange transfusion in patients receiving optimal chemotherapy.

- Parasitemia of greater than 30%.
- Parasitemia of greater than 10% in the presence of severe or complicated disease, especially cerebral malaria, acute renal failure, adult respiratory distress syndrome or jaundice, or severe anemia.
- Parasitemia of greater than 10% not responding to optimal chemotherapy after 12–24 hours.
- Parasitemia of greater than 10% and poor prognostic features (for example, elderly patients or late-stage schizonts in the peripheral blood).

There are some problems in basing decisions on the parasitemia. Although high parasite densities imply severity, the reverse is not always true, particularly in nonimmune persons where there may be wide differences between the proportions of the burden of parasitized cells circulating in the peripheral blood as opposed to sequestered in the microvasculature. Serial blood films at 6–12 hour intervals may reveal rapid changes in parasitemia, especially in synchronous infections, even with optimal treatment. In particular, the relation between the peripheral blood and total body parasite burden depends on the stage of the parasite development. If early *P. falciparum* trophozoites are predominant the peripheral parasitemia is likely to be more representative of the parasitized cell burden in the body and, presumably, more easily removed by exchanging. The later pre-schizont and schizont stages sequester in the microvasculature, a phenomenon believed to be responsible for the cerebral and renal manifestations of severe malaria. Thus although the presence of late-stage schizonts on the peripheral blood film may indicate that another cycle of replication is imminent, a greater proportion of the parasitized red cells are sequestered and thus not accessible to removal during exchange transfusion.

Methods of exchange transfusion

Traditionally double-lumen catheters or venesection/transfusion through separate lines have been used to perform manual exchanges, usually of six units of blood, one unit at a time. This is time consuming and inevitably causes some hemodynamic disturbance. The

use of cell separator hardware and software to remove only the red cell fraction and replace it with donor red cells in a single automated isovolemic procedure of 'erythrocytapheresis' is preferable as the method of choice where the expertise and facilities are available, for example in hematology departments in developed country settings.

Conclusion

Exchange transfusion as an adjunct to optimal chemotherapy in severe malaria is recommended by many centers, especially for high parasitemias in nonimmune patients. This cannot be considered an evidence-based recommendation, but it is supported by expert opinion for the indications listed above. A definitive randomized controlled trial is needed, but would be difficult to carry out.

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186.d What are the treatment options for a pregnant patient with malaria?

Edgar Dorman
Caroline Shulman

Definition of the problem

Falciparum malaria is responsible for massive maternal and perinatal morbidity and mortality globally. The clinical features in pregnancy depend to a large extent on the immune status of the woman, which in turn is determined by her previous exposure and continued exposure to malaria.

In pregnant women with little or no pre-existing immunity, such as travelers or women from nonendemic areas, infection is associated with extremely high risks of both maternal and perinatal mortality. Women of all parities are affected and are at 2–3 times greater risk of developing severe disease than nonpregnant women. They are also at approximately three times greater risk of dying if they do develop severe disease. Severe disease in pregnant women has been associated with 20–30% maternal mortality and at least a 60% risk of miscarriage, premature delivery or neonatal death. Particular dangers are hyperpyrexia, hypoglycemia, severe hemolytic anemia, cerebral malaria and pulmonary edema. The hyperpyrexia can precipitate miscarriage or premature labor. Hypoglycemia may be severe and refractory and may be associated with fetal heart rate abnormalities. It may be present prior to commencement of treatment but is particularly common in patients treated with quinine, due to quinine-induced hyperinsulinism. Although hypoglycemia usually presents as an alteration in the woman's conscious level or as abnormal behavior, often with sweating and an increased respiratory rate or dyspnea, it may be asymptomatic. Pulmonary edema associated with malaria in pregnancy is usually due to abnormal capillary permeability and can occur without positive fluid balance, as acute respiratory distress syndrome.

In areas of moderate or high transmission (holo- or hyperendemic), including large parts of sub-Saharan Africa, adults usually have a high level of immunity to malaria, maintained by continued exposure to infection. During pregnancy, this immunity is altered and pregnant women are at greater risk of infection than nonpregnant women. Primigravidae are affected most, with the risk decreasing in each successive pregnancy. In women with substantial pre-existing immunity, severe disease is uncommon and infection is frequently asymptomatic. However, even when asymptomatic, placental parasitization is common, whereby infected red cells sequester in the placenta in the intervillous space and malaria in pregnancy is associated with the development of severe maternal anemia and low birth-weight delivery. The low birth weight is mediated through a combination of intrauterine growth restriction (IUGR) and prematurity. Because it is asymptomatic, malaria may go unsuspected and undetected, particularly as the peripheral film may be negative despite placental infection (Fig. 186d.1).

The other species of malaria are not associated with severe disease and usually present with fever, although infection with *Plasmodium vivax* during pregnancy is associated with mild anemia and low birth-weight delivery.

Clinical cases

Case 1 — nonimmune

A 37-year-old West African woman presented to a London maternity department at 29 weeks' gestation in her first pregnancy, with a 5-day history of fever. She had recently returned to London, her home for the past 2 years. On admission her temperature was 103.1°F (39.5°C), pulse 130 beats/min (bpm) and, blood pressure 108/56mmHg. She was conscious and responsive. Her urine contained protein ++ on ward testing and was sent for culture (subsequently negative). Her uterus was soft and fetal size was appropriate for 29 weeks. There was a fetal tachycardia (180 bpm) with a suspicious pattern: a flat trace with reduced short-term variability (<5 bpm). Her initial investigation results were as follows: hemoglobin (Hb) 8.2g/dl, white blood cell (WBC) count 18 × 10^9 /1 and platelets 66 × 10^9 /1. Apart from her thrombocytopenia, her coagulation screen was normal. Two hours after admission her malaria film was reported to be positive with a 10% *Plasmodium falciparum* parasitemia. Her blood sugar was subsequently found to be 2.6mmol/l.

The patient was transferred to the intensive care unit and commenced on an infusion of 10% dextrose after a bolus of 50%. She received a loading dose of intravenous quinine 20mg/kg over 4 hours. In view of the hyperparasitemia, she was transferred to a tertiary referral unit for consideration of exchange transfusion. She required two top-up transfusions over the subsequent 3 days but her parasitemia

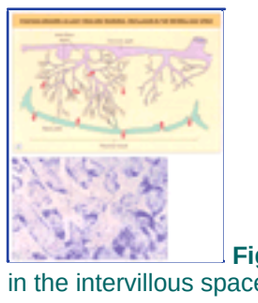

Figure 186.d-1 Heavy placental sequestration of malaria parasites is a common feature of malaria in pregnancy. (a) Placenta showing villous trees and maternal circulation in the intervillous spaces (broad arrows). (b) Photomicrograph showing trophozoites in maternal red blood cells in the intervillous spaces.

decreased and was negative after day 5. The patient remained well thereafter.

Ultrasound assessment on day 2 revealed an appropriately grown fetus with normal amniotic fluid volume. There was bilateral notching of the uterine artery Doppler waveforms with raised resistance indices, suggesting a degree of placental dysfunction. The umbilical artery and fetal arterial Doppler studies were normal. Over the subsequent 5 days the uterine artery Doppler waveforms normalized and remained normal throughout the rest of the pregnancy. However, subsequent serial scans for growth demonstrated IUGR, despite normal Dopplers.

HIV testing was undertaken during antenatal follow-up and the patient was found to be HIV positive. For this reason and because of the IUGR, she was delivered by cesarian section at 37 weeks' gestation.

Case 2 — semi-immune

A 26-year-old woman from East Africa presented at 24 weeks' gestation for antenatal booking. She was an asylum seeker who had arrived in the UK 2 weeks previously. She reported no specific symptoms other than tiredness and lethargy. Routine booking investigations were carried out. All results were unremarkable apart from the hemoglobin (6.2g/dl with iron-deficient indices). The patient was recalled to clinic and was subsequently admitted for transfusion of four units of packed cells. She was followed up in the clinic and 4 weeks later was severely anemic again (Hb 6.5g/dl). On this occasion stool was sent for microscopy and a malaria film was requested. Further transfusion of packed cells was arranged. The stool microscopy and malaria film were reported to be negative. However, the patient complained of flu-like symptoms over the ensuing days and a further malaria film was positive (*P. falciparum* <1% parasites seen). After treatment with oral quinine for 5 days followed by sulfadoxine-pyrimethamine (SP), the patient recovered. She had no further episodes of anemia during the antenatal period.

Diagnosis

The investigation of a patient with suspected falciparum malaria in pregnancy should be treated with great urgency.

Thick and thin blood films, stained with Giemsa, should be examined by an experienced microscopist. If the film is negative, it should be repeated if the patient remains symptomatic. Rapid dipstick tests (such as the ICT card test or OPTIMAL) are available as an aid to diagnosis in addition to microscopy. However, they are not as

sensitive as microscopy in the hands of an experienced microscopist.

Blood and urine should be cultured to exclude other infections and in the comatose patient, lumbar puncture should be considered to exclude meningitis, providing there is no evidence of raised intracranial pressure.

TABLE 186.d-1 -- Drugs that can be used for treatment of clinical malaria in pregnancy.

DRUGS THAT CAN BE USED FOR TREATMENT OF CLINICAL MALARIA IN PREGNANCY	
In any trimester	• Quinine
	• Chloroquine: though widespread drug resistance has now made this redundant for the majority of cases of falciparum malaria.
	• Sulfadoxine-pyrimethamine: theoretical risk of teratogenicity when used early in the first trimester and of kernicterus when used late in the third trimester. However, evidence suggests these risks are very low, so provided there is not likely to be drug-resistant disease, it can be used at any gestation for symptomatic disease, as the risks will be outweighed by benefits.
In the second and third trimesters only	• Artemisin derivatives
	• Amodiaquine
	• Mefloquine: to be used only if no other effective drugs are available
Drugs that are contraindicated in pregnancy	• Halofantrine
	• Primaquine
	• Doxycycline
NB: No drug is safe enough for treating malaria in pregnancy if there is a high level of resistance to the drug in the population. The most effective drug available should be used	

In a woman from an endemic country with severe anemia and suspected asymptomatic disease, presumptive treatment should be given even if a blood slide is negative, as illustrated by the second case history.

Management

Nonimmune pregnant women with malaria are more ill, more hypoglycemic and deteriorate more rapidly than nonpregnant patients and should be managed jointly by a physician and an obstetrician with an interest in materno-fetal medicine. As well as monitoring the parasitemia, regular hemoglobin, platelet counts and blood sugar measurements should continue during the course of the infection. The priority is to treat the mother. Delivery should not be contemplated until her condition is stabilized. Evidence of fetal compromise may resolve during the course of treatment, as in the first case history.

Unless there is multidrug resistance, quinine is the first-line drug for all symptomatic falciparum malaria infections in pregnancy. It is safe for use at any gestation, although it may have some weak oxytocic activity. In clinical practice, quinine use is not associated with premature labor and the greatest risk factor for premature labor is fever in the inadequately treated patient. If there is doubt about the malaria species, treatment with quinine should be commenced, pending confirmation from a reference laboratory.

In nonsevere disease, oral therapy with quinine should be given at a dose of 10mg salt/kg (maximum 600mg) q8h. If severe tinnitus or deafness occurs, dosage should be reduced to q12h. After 5 days or after clearance of parasitemia, whichever is the longer, a single treatment dose of SP may be given and quinine discontinued. Where possible, SP should be avoided both in the first trimester, due to the theoretical risk of teratogenicity, and near to term, due to the theoretical risk of kernicterus in the newborn. Both of these risks appear extremely low, however, and it can be used if alternatives are not available.

In severe disease, intravenous therapy should be instituted, converting to oral treatment as above as soon as the patient can swallow. A loading dose of quinine dihydrochloride 20mg/kg (maximum 1400mg) should be infused over 4 hours. Eight hours after the start of treatment, maintenance therapy with 10mg/kg over 4 hours should be started and repeated every 8 hours.

Malaria imported from parts of Thailand, Papua New Guinea and elsewhere in South East Asia may be resistant to quinine, SP and other drugs. Mefloquine may remain effective for these infections but it should be used with caution in pregnancy. The other effective alternative for treatment failures, alone or in combination with quinine, are the Qinghausu derivatives (artemether or artesunate), which appear safe in pregnancy and which the World Health Organization recommends for treatment of quinine-resistant severe malaria.

Careful attention should be paid to fluid balance in any patient with falciparum malaria, specifically avoiding the use of fluid challenges in response to oliguria, as this may precipitate pulmonary edema.

Transfusion with packed cells should be given slowly in any severely anemic patient. Patients with high parasitemia should be transfused earlier, as their hemoglobin will continue to fall due to hemolysis, despite treatment. In patients with parasitemia >10%, exchange transfusion allows physical clearance of parasites from the circulation. Exchange transfusion may also be considered at lower parasitemia levels in the presence of pre-rupture schizonts in the peripheral film, as this indicates an escalating parasitemia.

Conclusion

In nonimmune women, malaria in pregnancy is a dangerous condition associated with a high risk of maternal and perinatal mortality. Particular dangers are hyperpyrexia, hypoglycemia, severe hemolytic anemia, cerebral malaria and pulmonary edema. Even in immune women, falciparum malaria in pregnancy is associated with severe maternal anemia and low birth-weight delivery.

Malaria should be considered likely in any febrile patient with a travel history in the past year and treatment commenced for falciparum malaria if there is any doubt about the diagnosis. Severe or complicated malaria should be managed in an ITU setting and should involve early liaison between obstetricians and experts in tropical medicine.

Pregnant women should be advised against travel to malaria-endemic areas and if travel is unavoidable, advice on personal protection and chemoprophylaxis must be given.

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186.e Treatment of dysentery in a pregnant woman

Lucia Larson

Introduction

Encountering dysentery or debilitating diarrhea in a pregnant woman sets the physician on a challenging path. Fetal health is inextricable from maternal health, making concerns about morbidity doubly important. Physiologic stresses of severe diarrhea may be well tolerated by a young woman but they may not be when she is pregnant. Furthermore, her unborn child relies for its survival on maternal volume status by way of placental blood flow. The dysenteric pregnant woman should be hospitalized immediately if at all possible. The clinician must work quickly to ensure hydration, to establish a diagnosis and to select a rational management plan.

Pathogenesis

Etiologic considerations are the same for pregnant and nonpregnant patients but there are a few specific organisms to which a pregnant woman may be particularly predisposed. Noninfectious causes of dysentery should also be considered, particularly inflammatory bowel disease.

For example, physiologic changes of pregnancy may place the patient at risk for contracting or manifesting certain conditions. Cellular immunity is suppressed, predisposing the woman to intracellular pathogens, such as *Listeria* spp. The decreased gastrointestinal motility of pregnancy may allow for higher concentrations of enteric pathogens to accumulate in the bowel lumen, leading to more severe enteric illness. This, in combination with increased mucosal vascularity, may increase systemic access of enteric pathogens.

The normal physiology of pregnancy can obscure diagnosis in the dysenteric patient. Leukocytosis is common. Uterine enlargement complicates the abdominal examination. Common symptoms of pregnancy can delay recognition of disease or lead to interventions that place the patient at risk of more significant dysenteric disease. For example, decreased bowel motility and laxity of the gastroesophageal sphincter, both caused by elevated estrogen levels, can cause severe gastroesophageal reflux and intestinal bloating and cramping. These symptoms may obscure the early recognition of enteric infections in pregnant women.

Microbiology

The classic bacterial organisms associated with dysenteric disease are *Salmonella* spp. and *Shigella* spp., with the former more commonly associated with bacteremia and a carrier state. Enterohemorrhagic strains of *Escherichia coli* are an increasingly recognized cause of bloody diarrhea and may cause severe dysentery in pregnant women (Chapter 43). In addition, a number of cases of postpartum hemolytic uremic syndrome have had evidence of Shiga toxin-producing *Escherichia coli* infection.

Listeria monocytogenes infection typically causes a febrile diarrheal illness in pregnancy. Pregnancy is the most common independent risk factor for infection with *Listeria* spp., which are invasive, intracellular bacterial pathogens. Most reported cases of listeriosis in otherwise immunocompetent hosts are in pregnant women, particularly in the third trimester. Transplacental infection of the fetus and amnion, with severe outcomes, is well described even with mild maternal illness, making aggressive detection and treatment essential. The patient who has listeriosis may have additional signs and symptoms, including myalgia, pharyngitis or meningitis. She may, unfortunately, present only with a mild febrile diarrheal illness and intrauterine fetal death, which should focus the clinician immediately on *Listeria* spp. (Chapter 63).

Campylobacter spp. are increasingly common agents of diarrheal illness that can afflict patients by multiple pathogenic mechanisms (Chapter 43). Symptoms are often suggestive of appendicitis, bowel perforation or inflammatory bowel disease. *Campylobacter* spp. have been described as a cause of abortion, chorioamnionitis and perinatal sepsis. *Salmonella* spp. have also been documented to cause transplacental infection causing fetal loss, neonatal sepsis and even episiotomy site infection. These pathogens should be vigorously pursued in pregnant women who have diarrheal illness.

Parasitic infections deserve consideration in the pregnant patient who has diarrhea. *Entamoeba histolytica* may cause severe amebic colitis without typical symptoms of amebic dysentery in pregnant women. *Giardia lamblia*, although not a classic cause of dysentery, is problematic in pregnancy. Achlorhydria and any form of immunodeficiency each enhance risk of infection. Although pregnancy has never been identified as an independent risk factor for giardiasis, the disease may easily be missed in the pregnant patient who has intermittent bouts of gastrointestinal distress. Misdiagnosis can range from psychosomatic illness to hyperemesis gravidarum (Chapter 242). Giardia has not been known to cause intrauterine or fetal infection.

Clinical features

What is most striking about pregnant women with any major illness is how quickly they can become severely ill, making early diagnosis and treatment essential. Pregnant women have at least a 20% greater plasma volume than nonpregnant women, with corresponding requirements for fluid intake. They and their fetuses tolerate fluid losses poorly, whether from diarrhea, fever or the decreased fluid intake that often accompanies enteric illness. Decreased peripheral vascular tone with vasodilatation contributes to a tendency for

postural hypotension or presyncopal symptoms that may develop rapidly.

In the course of any significant infectious illness, particularly one with tissue invasion or associated Gram-negative bacteremia, the previously healthy pregnant woman is at substantial risk of developing pulmonary edema owing to the lowered colloid oncotic pressure of the gravid state. This is true even before rigorous hydration. A high index of suspicion for pulmonary edema is warranted in assessing and managing the pregnant patient with any infection. Her fetus will not tolerate hypoxemia as well as she does.

Many features of dysentery in pregnancy are no different from those in the nonpregnant patient; these include fever, abdominal cramping and bloody diarrhea. In *Shigella*, *Salmonella* and *Campylobacter* enteritis, the abdomen may be so tender that peritonitis is suspected. Peritoneal signs may be difficult to distinguish from chorioamnionitis in pregnancy. Vigilance and serial clinical examinations are essential to ensure prompt and proper diagnosis.

As with any infectious illness in a pregnant patient, infectious diarrhea or dysentery may present as preterm labor. In addition, the fetus may show signs of distress because of decreased uterine blood flow resulting from maternal volume depletion, hypoxemia or other physiologic alterations.

Investigations

When evaluating the pregnant woman who has a dysentery-like illness, it is important to account for normal physiologic changes of pregnancy (Table 186e.1).

Blood cultures may provide the definitive diagnosis in diarrhea caused by *Salmonella* spp. or *Listeria* spp. If the patient is febrile, it is appropriate to culture all potentially infected body fluids, such as urine, stool and even amniotic fluid. Where there is a question of peritonitis

TABLE 186.e-1 -- Normal laboratory findings in pregnancy.

NORMAL LABORATORY FINDINGS IN PREGNANCY				
Test	**Increased**	**Unchanged**	**Decreased**	**Comment**
Hemoglobin and hematocrit			?	Red cell indices unchanged

Leukocyte count	?			
Platelet count		?		
Blood urea nitrogen			?	
Serum creatinine			?	Usually less than 0.6mg/dl (<53µmol/l)
Sodium		?		
Potassium			?	
Chloride		?		
Bicarbonate			?	
Alanine aminotransferase		?		
Aspartate aminotransferase		?		
Bilirubin		?		
Alkaline phosphatase	?			
Albumin			?	
Creatinine clearance	?			1.5–2 times baseline

or chorioamnionitis as well, amniocentesis is appropriate and is best performed by a qualified obstetrician.

A Gram stain of a stool specimen to look for evidence of mononuclear or polymorphonuclear white blood cells can quickly focus further investigations. Most importantly, bacterial isolates of enteric pathogens should undergo antibiotic susceptibility testing to avoid repeated trials of ineffective multiple antibiotics that lead to unnecessary and potentially injurious drug exposures in pregnant patients.

Ulcerative colitis or colonic Crohn's disease is often diagnosed by a flexible sigmoidoscope examination and biopsy, strategies with no excess risk in pregnancy. Occasionally, radiographic imaging of the abdomen is warranted. Fetal risks of radiation exposure must be weighed carefully against the risk of delayed or inaccurate diagnosis. However, fetal well-being depends on maternal well-being. A pregnant woman should not be denied a potentially life-saving diagnostic intervention, nor should it be delayed on account of her pregnancy.

Management

After an appropriate diagnostic evaluation (Table 186e.2) empiric antimicrobial agents need to be considered. Further, if there is any suspicion of an infection that may be life threatening to the mother or fetus, such as listeriosis, then empiric therapy should be instituted. Precautions should be taken to prevent neonatal infection should a delivery occur at the time of an active maternal infection.

Numerous sources of information are available on the safety of antibiotics in pregnant women. Most readily available is the manufacturer's package insert, which usually includes a 'pregnancy category'. These categories are not always consistently applied. It is therefore wise to seek more detailed information from other sources, such as the online resources that are widely available in medical libraries and hospital pharmacies.

The number of commonly recommended antibiotics for diarrheal diseases is small. None of them are known teratogens. Ampicillin and other ß-lactam antibiotics (with the potential exception of ticarcillin) have a strong record of safety in pregnancy and are best administered in doses at the upper end of the therapeutic range for women in the second and third trimesters owing to altered pharmacokinetics.

Metronidazole is teratogenic in experimental animals and its use in pregnancy is controversial in some settings. Its use is probably warranted when the patient has a severe illness, such as symptomatic amebiasis. Fluoroquinolones are more controversial. As human data are scant, this class of agents is best avoided in pregnant women owing to the risk of arthropathy in the developing fetus, which has been well characterized in animal models. For all potential causes of dysenteric illness where fluoroquinolones are considered first-line therapy, alternative agents exist that have better characterized safety risk profiles in pregnancy. Tetracyclines (which carry the risk of hepatotoxicity and staining of permanent teeth), chloramphenicol (which suppresses bone marrow), and prolonged courses of aminoglycosides (which are ototoxic) are best avoided in pregnancy if possible. Macrolides are generally safe, but even

TABLE 186.e-2 -- Management of the pregnant woman who has dysentery.

MANAGEMENT OF THE PREGNANT WOMAN WHO HAS DYSENTERY
1. Hospitalization
2. Fluid and electrolyte replacement
3. Oxygen monitoring
4. Fetal evaluation by an obstetrician
5. Begin diagnostic evaluation
6. Empiric therapy if indicated

erythromycin has been occasionally associated with hypertrophic pyloric stenosis in infancy. Sulfa drugs should be avoided in the third trimester because of the potential increased risk of kernicterus. Trimethoprim-sulfamethoxazole (co-trimoxazole) has been associated in some studies with cleft palate when administered to rats at high doses, and it has the potential for interference with folate metabolism, raising concern about potential neural tube defects in the developing fetus. However, these have not been shown to be significant risks for humans.

The clinician unaccustomed to the uncertainties of prescribing in pregnancy may find little reassurance in such data. Absolute risk from a given agent in a given situation may be minimal, but the anxiety created by the unknown can be substantial. It must be remembered that delay of needed therapy may offer far more risk to the fetus than the therapy itself. Thus, as in the early stages of patient evaluation, the clinician must resist being paralyzed by indecision and the unknown regarding therapies. Rather, it is appropriate and wise to seek additional information. Most important, open and honest discussion of uncertainties with the patient and her obstetrician will serve all parties well.

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Section 7 - ANTI-INFECTIVE THERAPY

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Chapter 187 - Principles of Anti-infective Therapy

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The science of antimicrobial chemotherapy began in the last century, during which time we have witnessed a dramatic reduction in morbidity and mortality due to infections caused by common bacterial agents, only to be presently threatened by the worldwide emergence of antibiotic-resistant bacteria. Antimicrobial resistance limits the usefulness of antimicrobial agents, and careful attention to appropriate antimicrobial use might mitigate the extinction of these life-saving drugs. The past century also saw the introduction of effective chemotherapy directed against fungi and viruses as well as protozoa and parasitic organisms. This chapter focuses on the principles that guide appropriate use as well as an understanding of the mechanisms of action, pharmacokinetics and pharmacodynamics, indications and clinical selection of antimicrobial agents.

ANTIBIOTICS

General principles of antibiotic selection

The selection of appropriate antibiotic therapy depends on a number of important factors. First, antibiotics are useful only for the treatment of bacterial infections, and so general confirmation of the presence of such an infection is critical. The clinical presentation of an obvious pyogenic infection with such features as fever, purulent cough or exudate, shaking chills, tachycardia, diaphoresis and a localized inflammatory site in the chest, abdomen, urinary tract, joint or meninges, or on the skin should prompt appropriate diagnostic tests. These usually include blood cultures and smears and cultures from the clinically obvious infected site. Samples of pus, purulent exudate, cerebrospinal fluid, synovial fluid, urine or other likely infected material should be examined microscopically with a Gram-stained smear. If a predominating pathogen is strongly suggested on this examination, then the choice of antimicrobial agent is facilitated. Appropriate use of bacteriologic cultures and new molecular techniques such as DNA probes and polymerase chain reaction will help in selecting the most appropriate antibiotic and limit antibiotic resistance resulting from the selective pressure of unbridled antibiotic use.

Often, the presence of a bacterial infection is suspected simply on clinical grounds and there is no direct source of pus or other material for culture. In office practice of medicine, many patient encounters are via the telephone and antibiotic selection is often made in the absence of a physical or laboratory examination. Although the majority of these encounters are for viral infections, in those cases where bacterial infection is more probable, a reasonable choice of antimicrobial can be made based on the usual pathogens responsible for the symptoms in question. For example, purulent tonsillitis with fever and tender adenopathy is likely to be caused by *Streptococcus pyogenes*, as is uncomplicated cellulitis on an extremity. A carbuncle or furuncle is often caused by *Staphylococcus aureus* and uncomplicated cystitis with fever or pyelonephritis is usually due to infection with *Escherichia coli* or other enteric Gram-negative bacteria. Other clinical presentations may suggest the presence of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae* or *Streptococcus pneumoniae*, among others. In these situations it might be possible to select appropriate antibiotics directed against these specific pathogens (Table 187.1). [1] When empiric therapy is prescribed in these situations, the provider must be aware of local susceptibility patterns. Antibiotics that might have sufficed a decade or more ago now may be less effective as a result of increasing antibiotic resistance.

As a general rule, whenever possible appropriate clinical specimens should be obtained to attempt a culture-proven diagnosis so that the most effective antibiotic can be selected for targeted therapy and for the correct duration. Cultures are also important to isolate the infecting organism and determine the presence of antibiotic resistance. Unnecessary use of antibiotics for prophylaxis and for probable nonbacterial infections, as well as excessively prolonged courses for uncomplicated bacterial infections, should be avoided.

Bacteriostasis and bactericidal effects

Antibiotics exert an antibacterial effect that results either in inhibition of bacterial growth (bacteriostasis) or in bacterial killing (bactericidal effect). While not always specific for a given antibiotic and a particular bacterial pathogen, bacteriostatic antibiotics include tetracyclines, sulfonamides, clindamycin and chloramphenicol as examples. However, chloramphenicol is bactericidal against pneumococci, meningococci and *Haemophilus influenzae*. Aminoglycosides, ß-lactams and fluoroquinolones are bactericidal against most susceptible bacterial pathogens. Macrolide and azalide antibiotics may be inhibitory or bactericidal depending on drug concentrations, bacterial inocula and growth rates. [2] Some organisms, notably *Enterococcus* spp., require two agents to effect bacterial killing — a cell-wall-active agent such as a penicillin or glycopeptide plus an aminoglycoside are required for bactericidal activity, although either drug alone might induce an inhibitory or bacteriostatic effect.[3]

Some infections, such as uncomplicated bacterial cystitis, may be adequately treated with antibiotics that inhibit bacterial growth, such as sulfonamides. If bacterial multiplication is arrested, then normal host defenses including micturition and dilution with uninfected urine from the upper urinary tract will help to eradicate the infection. More invasive bacterial infections such as meningitis, bacterial endocarditis, peritonitis and bacteremia are best treated with bactericidal agents. Similarly, host factors might determine the choice of bactericidal versus bacteriostatic agents. In general, in neutropenic patients or patients who have other immune defects, bacterial infections are best treated with bactericidal drugs.[4]

Bactericidal antibiotics differ in the time course of bacterial killing. Some agents, such as cephalosporins, penicillins and penems, show time-dependent killing and produce maximal effect approximately 6–8 hours after exposure. Higher concentrations of antibiotic do not produce a greater bactericidal effect. The percentage of time during a given dosing interval that antibiotic concentration is above the minimal inhibitory concentration for a given organism (time above MIC) is the pharmacodynamic parameter that predicts the effect of time-dependent antibiotics (see below). Other antibiotics such as aminoglycosides and fluoroquinolones produce concentration-dependent killing. With these antibiotics

TABLE 187-1 -- Common clinical sites for bacterial infections in adults, frequently encountered organisms and appropriate antibiotics.

COMMON CLINICAL SITES FOR BACTERIAL INFECTIONS IN ADULTS, FREQUENTLY ENCOUNTERED ORGANISMS AND APPROPRIATE ANTIBIOTICS		
Infection site	**Common bacterial etiology**	**Appropriate antibiotics***
Oropharynx, tonsil	*Streptococcus pyogenes*	Penicillin V ×10 days, azithromycin, second-generation cephalosporin, clindamycin, erythromycin, clarithromycin
Acute sinusitis	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis,* group A streptococcus	Amoxicillin, ampicillin-clavulanate, cefpodoxime, cefuroxime axetil, cefdinir, fluoroquinolone
Acute exacerbation of chronic bronchitis	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*	Mild: amoxicillin, doxycycline, trimethoprim-sulfamethoxazole, oral cephalosporin
		Severe: ampicillin-clavulanate, azithromycin, oral cephalosporin, levofloxacin, gatifloxacin, moxifloxacin
Pneumonia, community-acquired, smoker	*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*	Outpatient: azithromycin, fluoroquinolone, second-generation cephalosporin
Pneumonia, community-acquired, non-smoker	*M. pneumoniae*, *Chlamydia pneumoniae*, *S. pneumoniae*	As above
Pneumonia, community-acquired	As above	Inpatient: third-generation cephalosporin, plus erythromycin or azithromycin, fluoroquinolone
Urinary tract infection, uncomplicated cystitis	Enterobacteriaceae	Trimethoprim-sulfamethoxazole, trimethoprim, fluoroquinolone
Pyelonephritis	Enterobacteriaceae, *Enterococcus* sp.	Fluoroquinolone, ampicillin plus gentamicin, third-generation cephalosporin, ticarcillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam
Urethritis, gonococcal	*Neisseria gonorrhoeae*	Ceftriaxone, cefixime, ciprofloxacin, ofloxacin
Urethritis, non-gonococcal	*Chlamydia* sp., *Mycoplasma hominis*, *Ureaplasma* sp.	Doxycycline, azithromycin
Pelvic inflammatory disease	*N. gonorrhoeae*, *Chlamydia* sp., *Bacteroides* sp., Enterobacteriaceae, *Streptococcus* sp.	Ofloxacin or levofloxacin plus metronidazole, ceftriaxone plus doxycycline, cefotetan or cefoxitin plus doxycycline, ampicillin-sulbactam plus doxycycline

Prostatitis, <35 years old	*N. gonorrhoeae*, *Chlamydia* sp.	Ofloxacin, ceftriaxone plus doxycycline
Prostatitis, >35 years old	Enterobacteriaceae	Fluoroquinolone, trimethoprim-sulfamethoxazole
Gastroenteritis	*Shigella* sp. *Salmonella* sp., *Campylobacter* sp., *Escherichia coli* 0157H7	Fluoroquinolone
Cholecystitis	Enterobacteriaceae, enterococci, anaerobes	Ampicillin-sulbactam, piperacillin-tazobactam, imipenem, meropenem
Diverticulitis	Enterobacteriaceae, anaerobes, enterococci	Ampicillin-sulbactam, piperacillin-tazobactam, imipenem, meropenem, metronidazole plus fluoroquinolone
Spontaneous bacterial peritonitis	Enterobacteriaceae, *S. pneumoniae*	Cefotaxime, ceftriaxone, ticarcillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam
Cellulitis	Group A streptococcus, *Staphylococcus aureus*	Nafcillin, oxacillin, first-generation cephalosporin, erythromycin, ampicillin-clavulanate
Septic arthritis, monoarticular, sexually active	*N. gonorrhoeae*	Ceftriaxone, cefotaxime, ceftizoxime
Septic arthritis, monoarticular, not sexually active	*S. aureus*, *Streptococcus* sp., Gram-negative rod	Nafcillin or oxacillin plus third-generation cephalosporin or ciprofloxacin
Septic arthritis, prosthetic	*Staphylococcus epidermidis*, *S. aureus*, Enterobacteriaceae, *Pseudomonas* sp.	Vancomycin plus ciprofloxacin or aztreonam, or ceftazidime or cefepime
Osteomyelitis	*S. aureus*	Nafcillin, oxacillin, first-generation cephalosporin, vancomycin
Meningitis	*S. pneumoniae*, *N. meningitidis*	Ceftriaxone ± vancomycin
Endocarditis, native valve	*Viridans* streptococcus, other streptococcal species, enterococci, staphylococci	Penicillin or ampicillin, plus nafcillin-oxacillin, plus gentamicin; vancomycin plus gentamicin
Endocarditis, prosthetic valve	*S. epidermidis*, *S. aureus*	Vancomycin plus gentamicin ± rifampin
This table is not intended to be all-inclusive. Specific infections are considered in detail in other chapters.		

* This is a general overview, the choice of antibiotics must consider the resistance pattern in any given geographic area (e.g., penicillin and macrolide resistance in *S. pneumoniae*; ampicillin and trimethoprim-sulfamethoxazole resistance among *E. coli*), as well as adjustments based on the identified etiologic agent and susceptibility testing.

higher concentrations produce more rapid and more complete bacterial killing. The ratio of maximal drug concentration to MIC (C_{max} /MIC) and the ratio of the area under the concentration-time curve (AUC) to MIC (AUC/MIC) are pharmacodynamic predictors of the effect of concentration-dependent antibiotics. The clinical significance of these differences is not clear, primarily because clinical trials are usually designed to test different doses rather than different dosing intervals.[5]

Postantibiotic effects

Bacterial growth may be inhibited following exposure to an antibiotic even after the drug concentration has fallen far below the MIC. This is

known as the postantibiotic effect (PAE) and is determined in vitro by observing bacterial growth after the drug has been removed. Animal models have been described to measure PAE in vivo.[6] Postantibiotic effects vary with different drugs and micro-organisms. For example, prolonged PAEs have been reported after aminoglycoside or fluoroquinolone exposure to Gram-negative rods, whereas most ß-lactam antibiotics exhibit shorter PAEs. It has been reported that PAEs in animal models may be longer than those measured in vitro.[6] Postantibiotic leukocyte enhancement (PALE) considers enhanced white blood cell killing of organisms that have just been exposed to antibiotic. It can add to the inherent PAE of a given drug-organism pair. Both PAE and PALE contribute to the dosing interval; drugs exhibiting prolonged PAEs may be dosed less frequently.

Antibiotic resistance

Bacteria have evolved complex mechanisms to resist the action of antibiotics. The mechanisms are discussed in detail in Chapter 189 . Although some organisms (e.g. *Enterococcus gallinarum*) are inherently resistant to some antibiotics, much of the impetus for bacterial resistance is believed to relate to excessive antibiotic use. It is remarkable that such mechanisms exist because widespread antibiotic use has been available for only 60 years. Bacteria may exhibit antibiotic resistance based on the elaboration of an enzyme that renders the antibiotic ineffective. There are a large number of ß-lactamases produced by many different bacteria that hydrolyze the ß-lactam ring of penicillins, cephalosporins and penems.[7] Examples of common ß-lactamases include a penicillinase of *S. aureus*, which is responsible for penicillin-resistant staphylococci, and TEM-1 ß-lactamase in *E. coli*, which mediates ampicillin resistance. Some of these enzymes are responsible for inactivation of broad-spectrum cephalosporins (extended spectrum ß-lactamases (ESBLs) such as OXA-11, OXA-14 in *Pseudomonas aeruginosa*, SHV-2 in *Klebsiella pneumoniae* and TEM 3–29, also in *K. pneumoniae* and other Enterobacteriaceae). ß-Lactamase genes may be found on plasmids and transposons or may be chromosomally mediated. Aminoglycoside antibiotics may be inactivated by bacterial enzymes, which result in phosphorylation, acetylation or nucleotidylation. Transposons or plasmids may transfer these modifying enzymes.

Bacteria can increase the elimination of antibiotics by upregulating efflux mechanisms, as is seen with macrolide antibiotics and Grampositive cocci carrying the *mef* gene.[8] Other bacteria resist antibiotics by altering their cell wall structure to reduce permeability through outer or internal membranes. Alterations in the shape or number of porin channels make it difficult for antibiotics to transfer from the external milieu to the ribosomal targets within the organism. These mechanisms have been found in imipenem resistant *P. aeruginosa* and other Gram-negative rods.[9]

Still other bacteria can modify the target site of antibiotic action rendering the usual binding impossible. Mutations in *gyrA* or *gyrB* genes responsible for DNA gyrase production alter the binding sites for fluoroquinolone antibiotics. Vancomycin resistance in enterococci is mediated by *vanA* or *vanB* genes that encode cell-wall proteins that have altered affinity for the antibiotic. [10] Ribosomal binding sites might be modified by methylation in macrolide-resistant bacteria and mutations in the DNA-dependent RNA polymerase mediate resistance to rifampin (rifampicin). Rifampin resistance may develop rapidly during therapy as a result of one-step bacterial mutations. For this reason, rifampin is rarely used alone except in the four-dose regimen used for meningococcal prophylaxis.

There is growing evidence to suggest that increasing antibiotic use results in increased rates of antibiotic resistance.[11] [12] Global travel, worldwide food distribution, antibiotics in animal feed and other products, as well as poor adherence to infection control techniques enhance the spread of antibiotic resistance.[13] Within hospitals or hospital units, it has been possible to show decreased resistance associated with decreased use of a given antibiotic. However, it has been more difficult to show clearly a reduction in resistance in the community associated with such usage changes.[14] Since antibiotics are found in foods, animal feeds, battleship paints, household products and other sources, this difficulty is not surprising. Common sense dictates that limited and appropriate use of these resources will limit the emergence of resistance. Patients who exhibit no evidence of bacterial infections should be encouraged to accept other therapeutic agents for symptom relief and to decrease their demand for unnecessary antibiotics. Several novel population interventions are in progress to test new approaches to the control of antibiotic resistance in the community.[15] Maximizing infection control efforts within the hospital and global education of physicians and patients will help to reduce the emergence of resistance. Optimizing dosing using pharmacokinetic/pharmacodynamic principles also might be successful. [5]

Although the classic teaching has been to select appropriate older antibiotics to which organisms are susceptible and use the lowest successful dose, in order to minimize the selection of resistant organisms perhaps the use of the most active or most potent antibiotics might be preferred. In the early part of the last century Ehrlich wrote, '*frapper fort et frapper vite*' ('hit hard and hit fast').[16] This might translate today into the use of rapidly bactericidal drugs that achieve concentrations at the infection site high enough to eradicate all the infecting organisms and thus minimize the opportunity for the development of resistance. Whether this approach will result in less antibiotic resistance remains to be tested clinically.

Determinants of the antimicrobial effect

In-vitro and in-vivo techniques

The activity of an antibiotic against an isolated pathogenic bacterium may be expressed using a number of techniques. The most frequently used of these methods are disc diffusion tests (such as the Kirby-Bauer method in North America, or Stokes or the BSAC method in Europe), and the determination of the MIC of the antibiotic. Disc susceptibility testing involves the agar inoculation of an approximated number of bacteria as a 'lawn', with the overlaying of antibiotic-impregnated discs. After overnight incubation, zones of inhibition appear around the discs impregnated with antibiotics to which the organism is susceptible (Fig 187.1a). MIC testing used to be done by agar or broth dilution, but now most laboratories use an ingenious antibiotic-impregnated strip, the E-test (Fig. 187.1b) or other automated techniques. In the USA, the National Committee for Clinical Laboratory Standards (NCCLS) sets 'breakpoints' for the MIC based on the integration of MICs with achievable antibiotic levels in serum and tissues, clinical pharmacology and data from in-vitro and animal models.[17] Typically these results are presented to the clinician as a report of 'sensitive', 'intermediate' or 'resistant'. Determinations of MIC are rough guides to the susceptibility of an isolated pathogen and these values form the usual basis for antibiotic selection. In clinical situations, bacterial concentrations might be larger than those used in the in-vitro determination. Pharmacokinetic considerations are not included in the MIC determination, and pharmacokinetic and pharmacodynamic parameters are important in predicting outcome (see below).

Determinations of MIC might not provide the most complete assessment or optimal prediction of the likely outcome of antibiotic therapy. Minimal inhibitory concentration values correlate with the ability of an antibiotic to inhibit a given bacterium under specified laboratory conditions. In some clinical situations, such as bacterial endocarditis, it is useful to know the ability of an antibiotic to kill the infecting organism. The minimal bactericidal concentration (MBC) can be determined using microtiter plate well or tube dilutions of antibiotic inoculated with a known concentration of the isolated infecting organism. After overnight incubation and subsequent quantitative subculture of the non-turbid wells or tubes, the concentration of drug that produces a

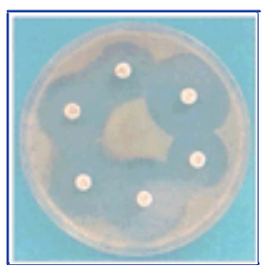

Figure 187-1a A disc diffusion sensitivity plate showing a fully sensitive coliform tested against a typical range of first-line antibiotics. *Courtesy of Dr M Cubbon, Brighton, UK.*

Figure 187-1b An E-test showing a methicillin-sensitive S. *aureus*. The MIC of oxacillin for this strain is 0.25 mg/L, and is obtained by noting the point at which the zone of inhibition intersects with the test strip. *Courtesy of Dr M Cubbon, Brighton, UK.*

99% or 99.9% reduction in the starting inoculum determines the MBC. For research purposes bacterial killing curves are studied using known bacterial inocula and antibiotic concentrations that approximate achievable serum levels. The time course of bacterial killing is determined by sampling and bacterial quantitation. In some clinical situations it is useful to determine the ability of various dilutions of serum sampled after an antibiotic dose to kill a standardized inoculum of the patient's infecting organism. The serum bactericidal assay is performed infrequently today but it has been used to estimate the adequacy of an antibiotic regimen in patients who have bacterial endocarditis or in bacteremic neutropenic patients.[18] [19]

The effects of antibiotic combinations also can be studied in vitro. Various techniques have been introduced to apply increasing concentrations of two antibiotics to known concentrations of bacteria. These include antibiotic-impregnated disc approximations, tube dilution tests, microtiter well methods, replicator plating techniques and bacterial killing concentrations. Experimental animal models have also been used to study the effects of antibiotic combinations.

Antibiotic combinations may be judged as additive when their activities can be summed, synergistic when the effect is greater than the

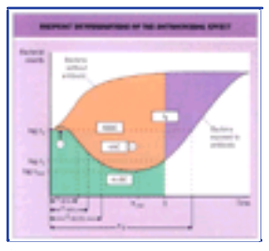

Figure 187-2 End-point determinations of the antimicrobial effect. See text for details. *Redrawn with permission of the American Society for Microbiology from Firsov et al.*[22]

sum of each drug's activity (i.e. the MIC of each drug in the presence of the other is reduced significantly) or antagonistic when the effect of the drugs in combination is less than the sum of each alone. Although several techniques have been described, [20] the study of antibiotic combinations is not routinely performed in clinical microbiology laboratories and this information is rarely available to the clinician.

In-vitro pharmacodynamic models have been introduced in the past two decades to incorporate pharmacokinetic and pharmacodynamic parameters into in-vitro predictions of the antimicrobial effect.[21] These models mimic human antibiotic dosing and can be used to study the relationship of pharmacodynamics to the antimicrobial effect (see below). These models have been used to describe additional end-point determinations of the antimicrobial effect. In the presence of changing concentrations of antibiotic following a simulated dose, several bacterial end points can be used[22] (Fig. 187.2). These include the time to reduction of the starting inoculum by 90%, 99% or 99.9% (t_{90} , t_{99} , $t_{99.9}$); the difference between the starting inoculum and the number of bacteria at the nadir of the killing and regrowth curve (n_0 -n_{min}); the time to the nadir of the kill curve (t_{min}), n_{min} itself; the viable count at the end of the usual dosing interval, t (n_t); and several integral end points that reflect areas above or below the bacterial time curve. The integral end points may be related to the dosing interval, t, such as the area under the bacterial curve (AUBC), the area above the curve (AAC) or the area between the curves in the presence and absence of antibiotic (ABBC); or the area may extend beyond the dosing interval and reflect the area between the control growth curve and the curve in the presence of a simulated antibiotic dose (intensity of the effect, I_E).[23] Of these, I_E has been shown most comprehensively to reflect the antimicrobial effect in in-vitro dynamic models.

Animal models

A variety of animal models have been developed and used to study optimal dosing and scheduling of antibiotics as well as antimicrobial pharmacodynamics. Animal and human pharmacokinetics differ significantly, especially in small animals. Although these models are rarely used clinically, they are quite important in the preclinical evaluation of antimicrobials. Specific models might be particularly useful in developing appropriate therapeutic regimens for bacterial endocarditis or meningitis, for example. In the endocarditis model, New Zealand rabbits are used and an intravascular catheter is inserted to cross the heart valve.[24] The catheter remains in place and organisms are introduced intravenously to establish typical bacterial vegetations similar to those that occur in clinical endocarditis. Meningitis models have been described in many animal systems and usually involve a stereotactic injection of bacteria directly into the

cerebrospinal fluid.[25] Other models have been developed for peritonitis and intra-abdominal abscess, pyelonephritis, pneumonia and osteomyelitis. The neutropenic

mouse thigh infection model of Craig and colleagues has been particularly helpful in correlating pharmacodynamic parameters with outcome. [26]

Antibiotic pharmacology

As with all drugs, pharmacologic considerations aid in the appropriate selection of antibiotics. Antibiotics may be administered orally or intravenously (and less often via the intramuscular route). Oral antibiotics must be absorbed via the gastrointestinal tract. Bioavailability, *F*, refers to 'the fraction of the administered dose that is absorbed intact'. [27] The bioavailability of intravenously administered antibiotics is 100%. The bioavailability of orally administered antibiotics is dependent on absorption across the gastrointestinal tract and ranges from 20% for sulfasalazine to up to 100% for sulfadiazine, ciprofloxacin, nitrofurantoin and cefaclor, for example. To be effective at the site of infection, antibiotics must be well distributed in the tissues. In general, lipophilic agents (e.g. chloramphenicol) and uncharged or nonpolar drugs (e.g. fluoroquinolones) are able to cross biologic membranes and achieve effective concentrations in tissues. The apparent volume of distribution of an antibiotic, V_d, is calculated by dose/plasma concentration and may reflect the ability of the drug to enter inflammatory cells and tissues. Distribution is affected by the protein binding of the drug in plasma and, in general, very highly bound drugs (e.g. >95%) may not cross biologic membranes as well as less highly bound drugs. However, as most of this binding to albumin and other plasma proteins is in reversible equilibrium, the clinical significance of protein binding remains confusing. In general, very highly protein bound antibiotics can be expected to penetrate into cerebrospinal fluid and abscesses less than drugs with lower degrees of protein binding. [28]

TABLE 187-2 -- Pharmacokinetic and pharmacodynamic parameters that affect antibiotic therapy.

PHARMACOKINETIC AND PHARMACODYNAMIC PARAMETERS THAT AFFECT ANTIBIOTIC THERAPY		
Parameters		**Details**
Pharmacokinetic	*F*	Bioavailability, fraction of the administered dose absorbed intact; intravenous drugs have 100% bioavailability; oral drugs vary with absorption and are usually less bioavailable than intravenous forms
	C_{max}	Maximal serum concentration after single or multiple doses
	t_{max}	Time after drug administration to reach C_{max}
	$t_{½\ elim}$	Elimination half-life; time to reduce peak serum concentration by 50%
	AUC	Area under the concentration-time curve (relates to total drug exposure following a dose)
Pharmacodynamic	C_{max} /MIC, Peak/MIC	Ratio of the maximum serum concentration to the MIC (predicts activity of concentration dependent bactericidal antibiotics)
	AUC/MIC	Ratio of the area under the concentration-time curve to the MIC (also predicts activity of concentration dependent bactericidal antibiotics)
	t>MIC, t_{eff}	Time above the MIC; the duration of time during a dosing interval that serum concentration remains above the MIC (predicts activity of time-dependent bactericidal antibiotics)

Metabolism is another important consideration in the selection of antibiotics. A rapidly metabolized drug that is excreted by the kidney in an inactive form would not be an appropriate choice for the treatment of urinary tract infections, for example. Some drugs are metabolized in the liver by the cytochrome P450 system and as such may interact with other drugs metabolized via this system. Metabolism is a critical determinant of drug interactions and these must be understood and appreciated when patients receive multiple pharmaceutical interventions.

A clear understanding of the elimination half-life and excretion of antibiotics also influences selection. Drugs with a short half-life are rapidly eliminated and need more frequent administration to produce high levels of the antibiotic at the site of infection. Drugs with a longer half-life are often more convenient because single daily dosing is usually effective. Drugs that are primarily excreted via the kidney often need dose adjustment in the face of renal insufficiency; drugs primarily excreted via the hepatic and biliary system might accumulate inappropriately in patients who have hepatic insufficiency. Although antibiotics are not usually thought to be highly toxic drugs, some concentration-related adverse effects are likely to be more prevalent in the face of dysfunctional organs of excretion.

The pharmacokinetic variables that are most useful in antibiotic chemotherapy include bioavailability (*F*), maximal serum concentration (C_{max}), time to reach C_{max} (t_{max}), elimination half-life ($t_{½}$) and area under the drug concentration curve following a dose (AUC; Table 187.2).

Recently, pharmacodynamics has been popularized in the antibiotic field. Antibiotic pharmacodynamics can be described as the interrelations between pharmacokinetics (drug concentrations) and the antibacterial effects that result from these concentrations in, for instance, serum, tissues and body fluids. The most useful pharmacodynamic variables (see Table 187.2) include the ratio of the area under the 24-hour concentration-time curve to the MIC (AUC_{24} /MIC), the ratio of C_{max} to MIC (Peak/MIC) and the time during a given dosing interval that the serum concentration remains above the MIC (time above MIC, *t*>MIC, t_{eff}).

Pharmacokinetic/pharmacodynamic (PK/PD) variables are of increasing interest as possible predictors of the antimicrobial effect. Such parameters may be exploited to develop optimal dosing regimens, although there are relatively few clinical studies to test the ability of these parameters to predict outcome. One such clinical study suggested that C_{max} /MIC was useful in predicting outcome of levofloxacin treatment for several different infections. [29]

Other in-vitro and clinical studies suggest that PK/PD parameters might be exploited to minimize the emergence of resistant organisms during exposure or therapy. It remains to be determined whether PK/PD-based antibiotic prescribing and dosing can reduce adverse effects and costs while providing an optimal antibacterial effect.

Antibiotics in general are very safe drugs. However, adverse effects of antibiotics do occur and include gastrointestinal events (nausea, vomiting, diarrhea, antibiotic-associated colitis, pseudomembranous colitis), cutaneous reactions (rash, urticaria, Stevens-Johnson Syndrome), neurologic symptoms (agitation, insomnia, seizures), hepatic dysfunction, renal insufficiency, anemia, agranulocytosis and thrombocytopenia, among others. Some adverse events associated with antibiotics are idiosyncratic or hypersensitivity reactions and some are related to their concentration in serum and tissues. For example, prolongation of the QT_c interval with macrolide and other antibiotics might increase at higher drug concentrations as a result of either increased dose or decreased elimination. [30] Some hepatic toxicities are also related to dose and duration, and toxicity in general is dose-limiting for many antibiotics. Careful attention to appropriate use and dosing should reduce antibiotic-associated toxicity.

Choice of antibiotics for empiric therapy

Combinations: monotherapy versus multiple antibiotics

A single antibiotic is usually sufficient for the treatment of most bacterial infections. In some situations more than one antibiotic is prescribed. Most commonly two or more antibiotics are administered because the diagnosis is not obvious or not clearly established. Antibiotics are often combined in presumed mixed bacterial infections such as intra-abdominal abscesses or peritonitis, where antibiotics with activity against facultative Gram-negative rods, such as ampicillin plus gentamicin or a quinolone or third-generation cephalosporin, are combined with an antianaerobic agent such as metronidazole or clindamycin. For some specified infections such as pelvic inflammatory disease, multiple organisms, including *C. trachomatis*, *N. gonorrhoeae* and facultative and strict anaerobic Gram-negative rods, are assumed to be present and in fact might not be cultured. Antibiotic combinations are often prescribed for this infection.

Early studies of carbenicillin plus aminoglycosides in severe *Pseudomonas aeruginosa* infections suggested that these two antibiotics were more effective than either alone; this has led to the routine use of two antibiotics for infections caused by this organism. This concept has recently been challenged, but the importance of high concentrations of bactericidal antibiotics remains cogent. [31] Optimal results for pseudomonal infections might require two active agents. Even more clearly, bacteremic infections caused by *Enterococcus faecalis* are best treated with a combination of a cell-wall-active penicillin or glycopeptide plus an aminoglycoside (assuming that high-level resistance to one or both agents is not present). [32] Some studies of bacterial endocarditis caused by viridans streptococci and bacteremia caused by *Staphylococcus aureus* suggest better outcomes with combination therapy.

Antibiotics also might be combined to minimize or prevent the selection of resistant organisms. This is most clearly demonstrated with *Mycobacterium tuberculosis*, where use of two or more anti-mycobacterial agents (depending on the likely inoculum size) reduces the likelihood of selecting for resistant bacteria. Whether this also applies to pyogenic bacteria is not definitively proven.

Antibiotic combinations (e.g. an aminoglycoside and an anti-pseudomonal ß-lactam) were initially recommended for the treatment of presumed bacterial infections in febrile, neutropenic patients. Prolonged and profound granulocytopenia places these immunocompromised patients at particular risk for Gram-negative rod bacteremia. Although combination therapy is still considered acceptable for these patients, several potent antibiotics (such as imipenem, meropenem, cefepime and ceftazidime) are currently acceptable for single-agent empiric therapy.[4]

Duration of therapy

For most acute bacterial infections in the respiratory or urinary tracts, the duration of therapy can be short (e.g. from 3 to 7 or 10 days). In the early antibiotic era, patients who had pneumococcal pneumonia were successfully treated with courses of antibiotics that lasted for 3–5 days after the patient became afebrile. Modern therapy duration may be inappropriately based on standardized clinical trials needed for drug registration purposes. There is a trend now for shorter courses of treatment for some respiratory infections such as acute bacterial exacerbations of chronic bronchitis. Rigid adherence to 10- to 14-day treatment regimens for many acute infections is probably not necessary and may contribute to excess antibiotic use, increased adverse effects and complications of therapy, as well as the emergence of resistant organisms.

Subacute and more chronic infections such as endocarditis and osteomyelitis are treated for prescribed lengths of time. Six weeks of intravenous therapy is often required for bacterial osteomyelitis and 4 weeks is usually prescribed for patients who have bacterial endocarditis (although 2-week treatment courses are clearly effective for certain organisms, e.g. viridans streptococci; see Chapter 52 & Chapter 59).

Route of administration

The intravenous administration of antibiotics is preferred for patients who are critically ill or bacteremic, or in whom gastrointestinal absorption cannot be guaranteed. For most acute bacterial infections (e.g. pneumonia, pyelonephritis) it is possible to switch to oral therapy with the same or comparable agents when the patient has stabilized and vital signs are returning toward normal.[33] Fluoroquinolone antibiotics are particularly useful in these situations, assuming that the infecting organism is susceptible. Home administration of intravenous antibiotics is now entirely possible and preferable for long-term treatment with these drugs for infections such as endocarditis and osteomyelitis, where high concentrations at the site of infection are desired.

Topical use of antibiotics is limited to specific infections. [34] Although minor cutaneous infections might respond to topical therapy, some evidence suggests that sensitization via this route is frequent for some agents. Excessive use of topical antibiotics may lead to increased bacterial resistance. Topical administration may be employed to treat bacterial conjunctivitis with ciprofloxacin, trachoma with tetracycline, and acne with clindamycin, tetracycline or erythromycin, for example. Topical aminoglycosides have been useful in the prophylaxis and treatment of burns and burn wound infection (see Chapter 85). Topical mupirocin (its only formulation) is used to treat impetigo and nasal carriage of *S. aureus*. Polymyxin and neomycin are used in topical therapy of minor wound infections. Adverse effects seen with intravenous or oral administration of the same drug can certainly occur following topical therapy.

Cost, restricted use policy and formulary constraints

Antibiotics comprise a significant portion of the pharmacy budget for most hospitals and managed care plans. The appropriate use of first-line agents for proven or strongly suggested bacterial infections should help to control these costs. The cost to pharmaceutical companies to develop a new antibiotic may be as high as US$800,000,000. Increasing regulatory requirements and safety concerns clearly contribute to these costs. Incentives for continued development of antibiotics in the face of rising rates of bacterial resistance are clear societal needs, and these factors also contribute to the high cost of these drugs.

Some hospitals adopt policies to restrict use of certain antibiotics to infectious disease clinicians or other designated experts. Although these schemes have been shown to reduce costs and improve appropriate antibiotic use, their success is tied to continual monitoring and control of antibiotic prescribing.[35] Most successful programs include physician education to sustain their impact. Many managed care organizations limit their formularies to less expensive and older drugs. The impact of these policies on clinical outcome and resistance development is under study.[15]

Antibiotic use in special populations

Special considerations modify the use of antibiotics at the extremes of age, in the presence of renal or hepatic insufficiency, in pregnancy and in the presence of foreign bodies. In neonates and infants less than 1 year of age, dosing is often based on the body mass index. In elderly patients the fever and leukocytosis associated with bacterial infections may be blunted or absent, making diagnosis more problematic. Antibiotic dosing must consider the aging-related slight decline in gastrointestinal absorption and decreases in renal function associated with nephron loss. Drug interactions are particularly worrisome in the elderly because of the large number of drugs that such patients may be taking. QT_c prolongation might be enhanced when some antibiotics are combined with other classes of drugs known to increase the QT_c interval. Metronidazole may interact with warfarin,

resulting in increased effect of the anticoagulant. Loop diuretics may increase aminoglycoside-related ototoxicity. Adherence to drug regimens should be stressed as elderly patients may find the addition of any new agent confusing, even for a short course of therapy. Side effects are also more common in elderly patients. [36]

The dosing of some antibiotics, notably those primarily excreted by the kidney, must be reduced in the presence of renal insufficiency. The more severe the renal failure the more the dose or its interval must be altered. Patients on hemodialysis or peritoneal dialysis need special dosing modifications to supplement antibiotics (usually those of low molecular weight) that are removed during the procedures. For some drugs, such as aminoglycosides, the dosing interval can be extended and/or the dose itself reduced according to the estimated creatinine clearance. Some adverse effects of frequently used antibiotics are increased in patients who have renal insufficiency. For example, seizures may be seen with usual dosing of imipenem or quinolones, and hearing loss may occur with erythromycin. Several tables have been published to guide the appropriate dosing of antibiotics in patients who have renal insufficiency. [1] [37]

In the face of hepatic failure additional considerations affect the choice or dose of antibiotic. Drugs excreted by the liver such as metronidazole, tetracycline and clindamycin often need dose adjustment in hepatic failure. In the presence of large-volume ascites, some antibiotics might need to be administered in a larger dose to ensure appropriate concentrations at the site of infection.

Antibiotic selection in pregnancy is determined by the specific infections under treatment. Penicillins and cephalosporins are generally considered safe in pregnant women, and aminoglycosides also can be used if needed. Trimethoprim-sulfamethoxazole and other sulfonamides may be used in pregnancy but not in the last few months because of their effect on bilirubin conjugation and the risk of kernicterus in neonates. Fluoroquinolones, tetracyclines and chloramphenicol should not be used in pregnancy.

The treatment of infections in the presence of foreign bodies such as intravascular or bladder catheters and orthopedic prostheses is covered elsewhere (see Chapters 53). As foreign bodies may be coated with a microbiologic biofilm, agents that reduce bacterial mucus, slime or biofilm production, even if they are not bacteriostatic or bactericidal for the infecting organisms, might be useful in conjunction with other antibacterial agents. Macrolides and fluoroquinolones have been shown to reduce bacterial mucoid production.[38] In most cases, infected foreign bodies need to be removed to resolve the infection completely.

Prophylaxis

Surgical prophylaxis is discussed in Chapter 190 . Medical prophylaxis against bacterial infections is limited to specific indications, such as the prevention of *S. pyogenes* infection in patients who have known rheumatic fever or to reduce the possibility of bacterial endocarditis in patients who have known valvular cardiac disease and are undergoing dental, gastrointestinal or genitourinary procedures. Prophylaxis against traveler's diarrhea is not usually recommended (but empiric therapy is preferred, see Chapter 143). Inappropriate or excessive use of prophylactic antibiotics is likely to contribute to the increased incidence of antibiotic resistance.

New targets for antibiotics

With increasing antibiotic resistance and few new antibiotics in the pharmaceutical pipeline, it is urgent to begin to identify new bacterial targets and novel approaches to antimicrobial chemotherapy. Several lines of work have begun to find new inhibitors of bacterial efflux pumps, biofilm production, essential bacterial protein secretion, membrane proteins, signaling systems, DNA replication and bacterial cell division. Identification of bacterial genomes also has revealed important potential targets and functional genomics should allow opportunities for new drug development. [39] Recent work on quorum sensing by bacteria also might provide new chemotherapy targets.[40]

ANTIFUNGAL THERAPY

Health care providers are faced with increasing numbers of patients who are susceptible to severe, invasive fungal infections. Multiple factors play a role, including chemotherapy-induced neutropenia, immunosuppression secondary to chronic infections such as HIV, and immunosuppression related to organ transplantation, as well as exposure to lengthy courses of broad-spectrum antibiotics. These agents are used to treat documented infections and increasingly for prophylaxis of invasive fungal infection in these at-risk patients. Prior to the late 1970s only amphotericin B and flucytosine were available for the treatment of serious fungal infections. In the past two decades many new agents have been developed and approved, and new classes of antifungals are now entering clinical trials. Treatment of specific fungal infections is covered in detail in Chapter 111 , Chapter 126 and Chapter 237 Chapter 238 Chapter 239 Chapter 240 Chapter 241 ; here the classes of antifungal agents, mechanisms of action and the emerging topics of antifungal pharmacodynamics and resistance are outlined briefly.

Amphotericin B, a polyene, and its newer formulations remain the mainstay of antifungal therapy for severe infection. The mechanisms of action primarily include binding to ergosterol, the principal sterol in the fungal cell membrane, leading to permeability changes and cell death. Secondary actions include the generation of oxidative metabolites and free radicals, as well as stimulation of host macrophages. The newer formulations (lipid complex, colloidal dispersion, cholesteryl complex and liposomal amphotericin B) offer some reduced toxicity and the ability to deliver higher drug concentrations. Amphotericin B is active against a wide variety of fungi; it is fungicidal against many of these but fungistatic against others. Some fungi (*Pseudallescheria boydii*, *Fusarium* spp., *Trichosporon* spp. and some *Candida* spp.) demonstrate reduced amphotericin B susceptibility, while others have developed frank resistance.[41]

Nystatin, a tetraene-diene, was the first antifungal polyene. It has broad antifungal activity similar to that of amphotericin B. Nontopical formulations are presently being investigated and may prove to be efficacious, given this agent's fungicidal properties.

Flucytosine, a low-molecular-weight, synthetic pyrimidine analogue, is taken up by the fungal cell wall and, after enzymatic modification, causes RNA miscoding and inhibition of DNA synthesis. Flucytosine has activity against *Candida* spp., *Cryptococcus neoformans*, *Saccharomyces cerevisiae* and some dematiaceous models. At clinically achievable doses it is fungistatic. When flucytosine is used as monotherapy, resistance emerges rapidly and thus it is usually used in combination with amphotericin B or fluconazole. Resistance can emerge from mutations that affect production of uridine monophosphate pyrophosphorylase, cytosine permease or cytosine deaminase, or increased pyrimidine production. Toxicity includes bone marrow suppression, which is most evident when used in combination with amphotericin B. This occurs as a consequence of the high rate of renal impairment with amphotericin B and subsequent high flucytosine levels. Recent pharmacokinetic studies have suggested that flucytosine may be safer and more effective at lower and less frequent dosing.[42]

As a class the azoles act by inhibiting fungal cytochrome-P450-dependent conversion of lanosterol to ergosterol, ultimately leading to altered cell membrane properties and inhibition of cell growth. The imidazoles clotrimazole, ketoconazole and miconazole have two nitrogens in the five-member ring whereas the triazoles have three. For many years ketoconazole was the only available oral agent for the treatment of systemic fungal infections. Because of difficulty with absorption, substantial toxicity and disappointing treatment

TABLE 187-3 -- Antiviral drugs: non-HIV.

ANTIVIRAL DRUGS: NON-HIV				
Agent	**Mechanism of action**	**Antiviral activity**	**Mechanism of resistance**	**Toxicity/side effects**
Aciclovir	Inhibits DNA polymerase, chain terminator. Requires viral thymidine kinase and cellular enzymes	HSV-1, HSV-2, VZV, CMV (much less activity), EBV (in vitro)	Mutations in thymidine kinase (more common) and mutations in DNA polymerase	Intravenous: phlebitis, crystalline nephropathy. Confusion, delirium, lethargy, tremors, nausea, vomiting, lightheadedness, diaphoresis, rash
Adefovir	Nucleotide analogue	HBV	As of 2000, no mutations	Renal impairment at >30mg q24h
Amantadine	Inhibits transmembrane protein M2, reduced uncoating of viral genome	Influenza A	Point mutation in gene encoding transmembrane domain M2 protein	Nervousness, anxiety, lightheadedness, confusion, insomnia
Cidofovir	Acyclic nucleoside phosphonate (does not require a virus-specific thymidine kinase)	HSV-1, HSV-2, VZV, EBV, CMV	Mutations in DNA polymerase	Severe nephrotoxicity, neutropenia, ocular hypotony, metabolic acidosis. Carcinogenic, teratogenic
Famciclovir	Prodrug to penciclovir	See penciclovir	See penciclovir	Headache, nausea, diarrhea, vomiting, pruritus, LFT abnormalities
Fomivirsen (intravitreal)	Antisense oligonucleotide	CMV	In-vivo resistance not seen	Iritis, vitreitis, increased ocular pressures, visual changes
Foscarnet	Noncompetitive inhibitor of viral DNA polymerase (does not require thymidine kinase)	HSV-1, HSV-2, VZV, CMV, EBV, influenza A, influenza B, HBV, HIV	In CMV, single mutation in conserved region of DNA polymerase	Renal impairment, electrolyte disturbances, seizures, anemia, neutropenia, fever, nausea, vomiting, diarrhea, headache
Ganciclovir	Inhibitor of DNA polymerase, also competitive inhibitor of deoxyguanosine triphosphate (monophosphorylation by infection-induced kinases in HSV and VZV, and viral-encoded phosphotransferase in CMV-infected cells)	HSV-1, HSV-2, VZV, CMV, EBV, HHV-6	One or more point mutations in UL97, mutations in CMV DNA polymerase	Bone marrow suppression, fever, rash, increased LFTs, nausea, vomiting, eosinophilia, seizures, confusion, encephalopathy

Interferon-a	Induces changes in infected/exposed cells to promote resistance to infecting virus. Produces proteins that inhibit RNA synthesis, cleaves cellular and viral DNA, inhibits messenger RNA, alters cell membranes, inhibits release of replicated virions	Papillomavirus, HCV, HBV, HDV, HIV	Fever, headache, chills, arthralgias, myalgias, fatigue, dizziness, neutropenia, thrombocytopenia, somnolence, depression, cognitive changes, suicidal ideation, increased LFTs, altered thyroid function, nausea, vomiting, diarrhea	
Lamivudine	Competitively inhibits viral reverse transcriptase, terminates proviral DNA chain extension	HBV, HIV	Mutations at YMDD locus (conserved domain reverse transcriptase)	Low-dose equivalent to placebo. High dose: headache, fatigue, insomnia, myalgias, arthralgias, diarrhea, rash, lactic acidosis, hepatomegaly
Lobucavir	Guanosine analogue	HBV	Mild anorexia, dizziness, abdominal pain. Clinical testing halted with concerns for carcinogenesis	
Oseltamivir	Neuraminidase inhibitor	Influenza A, influenza B	Mutation in viral neuraminidase and viral hemagglutinin	Nausea, vomiting
Penciclovir (topical)	Incorporated into DNA molecule	HSV-1, HSV-2, VZV, EBV (less so), CMV (less so), HBV (in vitro)	Mutations in thymidine kinase and mutations in DNA polymerase	Topical same as placebo
Pleconaril	Capsid binding compound prevents viral attachment to cells and/or release of viral RNA from the capsid	Picornaviruses (enterovirus, rhinovirus)	Observed in vitro but clinical significance not clear	Crystalluria
Ribavirin	Guanosine analogue, three possible mechanisms: competitive inhibition of host enzymes, inhibition of viral RNA polymerase complex, inhibition of messenger RNA formation	RSV, HCV (clinically); but also influenza A, influenza B, mumps, measles, parainfluenza, herpesviruses, togavirus, bunyavirus, adenovirus, Coxsackie virus, hemorrhagic fever virus, HAV, HBC, Lassa fever virus, Hantaan virus, ?Hantavirus	Anemia, hyperbilirubinemia, elevated uric acid, nausea, headache, lethargy. Teratogenic, mutagenic, embryotoxic, gonadotoxic	
Rimantadine	Inhibits transmembrane protein M2, reduced uncoating of viral genome	Influenza A	Point mutation in gene encoding transmembrane domain M2 protein	Nervousness, anxiety, lightheadedness, confusion, insomnia (much less so than amantadine)
Trifluridine (topical)	Pyrimidine nucleoside	HSV-1, HSV-2, CMV, vaccinia, some adenoviruses		
Valganciclovir	Metabolized to ganciclovir	See ganciclovir	See ganciclovir	Bone marrow suppression, fever, nausea, headache, vomiting, insomnia, abdominal pain, peripheral neuropathy, paresthesias, potential carcinogen
Zanamivir (aerosolized/intranasal)	Neuraminidase inhibitor	Influenza A, influenza B	Mutations in viral neuraminidase and viral hemagglutinin	Nasal, throat discomfort, bronchospasm in asthmatics
CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HHV, human herpesvirus; HSV, herpes simplex virus; LFT, liver function test; RSV, respiratory syncytial virus; VZV, varicella-zoster virus.				

results in immunocompromised patients, it has largely been replaced by the triazoles.

The triazoles are less susceptible to degradation and have greater target specificity, increased potency and an expanded activity spectrum. The first generation triazoles fluconazole and itraconazole are active against dermatophytes, *Candida albicans* and some non-*albicans* candidal species; in addition, itraconazole has activity against *Aspergillus* spp. and some dematiaceous molds. Fluconazole is generally fungistatic and itraconazole is both fungicidal and fungistatic depending on the fungal strain. Second-generation triazoles include posaconazole, ravuconazole and the FDA-approved voriconazole. These drugs have enhanced target activity especially against *Aspergillus* spp., specificity and a wide spectrum of activity. Available data show that voriconazole is well tolerated, with rash, fever and visual disturbances reported most frequently. Like the triazoles, these agents also appear to be both fungicidal and fungistatic depending on the specific organism. As a group they have potential for substantial drug-drug interactions with agents metabolized by the cytochrome system.[43]

The echinocandins inhibit the synthesis of 1,3-ß-D-glucan, a polysaccharide in the cell wall of many pathogenic fungi. Glucan fibrils are involved in the maintenance of osmotic integrity of the cell wall as well as playing a role in cell division and growth. Caspofungin, micafungin and anidulafungin all possess potent, broad antifungal activity against *Candida* and *Aspergillus* spp. They are not metabolized through the cytochrome P450 system. In-vitro models have demonstrated both fungicidal and fungistatic properties. Caspofungin has recently been approved by the US Food and Drug Administration (FDA) for the treatment of candidemia and invasive aspergillosis in patients refractory to or intolerant of other therapies.

Sordarins exert their antifungal effect by selective inhibition of fungal protein synthesis by interacting with translocation elongation factor 2 and the large ribosomal subunit stalk rpP0. In vitro, they have shown fungicidal activity against *C. albicans*, some non-*albicans* species, *C. neoformans*, as well as some other yeast-like fungi and endemic molds.

There is little doubt that severe invasive fungal infections will continue to be clinically challenging, especially in immunocompromised patients. In addition to minimizing the use of broad-spectrum antibiotics, limiting immunosuppression to the lowest safe doses and protecting HIV-infected patients by judicious use of antiretrovirals, there will still be a need for newer and safer antifungal agents. In addition we need more studies investigating the pharmacodynamics and pharmacokinetics of already available agents in order to offer patients safer and equally, if not more, efficacious treatment options. Readily available and clinically relevant resistance testing would be a major advance in the therapy of these infections.

ANTIVIRAL THERAPY

Specific therapy for viral infections has become possible only recently. The discovery of many potent and effective antiviral drugs as well as marked improvements in diagnostic techniques that allow more rapid identification of viral infections have made effective therapy possible. Although most viral infections are usually self-limiting, others are overwhelming and devastating, with significant morbidity and mortality. Antiviral therapy is now available for herpesviruses, hepatitis C virus (HCV) and hepatitis B virus (HBV), papillomavirus, influenza and HIV, among others. Antiviral drugs share the common principle of being virustatic; they are only active against replicating viruses and do not affect latent virus. Therapeutic approaches to viral infections share very little other common ground. Some infections require monotherapy for very brief periods of time (aciclovir for herpes simplex virus), others require dual therapy for prolonged periods of time (a-interferon/ribavirin for HCV), while others require multiple drug therapy for indefinite periods of time (HIV).[44] Table 187.3 briefly summarizes the antiviral activity, resistance mechanisms and more

common toxicities of the non-HIV antivirals.[44] [45] Chapter 205 to Chapter 207 address specific antiviral agents.

The approaches to treatment of HIV infection are in constant flux. Like other infections, cure is the goal; however, like other viral infections, cure is not possible at this time, although suppression of viral replication and preservation of the immune system are short-term goals of therapy. In less than two decades antiretroviral therapy has emerged as a great success but it remains an enormous challenge.

TABLE 187-4 -- Antiviral drugs: HIV.

ANTIVIRAL DRUGS: HIV		
Agent	**Mechanism of action**	**Toxicity/side effects**
Abacavir	Nucleoside reverse transcriptase inhibitor	Hypersensitivity syndrome, rash, fever, nausea, vomiting, diarrhea, abdominal pain, elevated LFTs
Amprenavir	Protease inhibitor	Nausea, vomiting, diarrhea, rash, oral paresthesias, dysgeusia, mood disorder
Atazanavir	Protease inhibitor	Increased unconjugated bilirubin, gastrointestinal symptoms
DAPD	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, abdominal pain, hepatitis
Delavirdine	Non-nucleoside reverse transcriptase inhibitor	Rash, headache, Stevens-Johnson syndrome
Didanosine	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, abdominal pain, peripheral neuropathy, pancreatitis
Efavirenz	Non-nucleoside reverse transcriptase inhibitor	Dizziness, difficulty concentrating, nausea, vomiting, diarrhea, rash, flu-like symptoms
Emtricitabine	Nucleoside reverse transcriptase inhibitor	Nausea, diarrhea, headache
Enfurvirtide	Fusion inhibitor	Injection site inflammation
Hydroxyurea	Potentiates didanosine, may facilitate immune reconstitution	Myelosuppression, stomatitis, leg ulcers
Indinavir	Protease inhibitor	Nephrolithiasis, nausea, dysgeusia, benign hyperbilirubinemia
Interleukin-2	Peripheral expansion of existing $CD4^+$ lymphocytes	Nausea, vomiting, diarrhea, fever, asthenia, pruritus
Lamivudine	Nucleoside reverse transcriptase inhibitor	No significant toxicity, peripheral neuropathy, pancreatitis
Lopinavir	Protease inhibitor	Nausea, asthenia, diarrhea
Nelfinavir	Protease inhibitor	Diarrhea
Nevirapine	Non-nucleoside reverse transcriptase inhibitor	Dizziness, rash, difficulty concentrating, nausea, vomiting, diarrhea, flu-like symptoms
Ritonavir	Protease inhibitor	Dysgeusia, nausea, vomiting, diarrhea, circumoral paresthesias, increased triglycerides, LFTs, CPK and uric acid
Saquinavir	Protease inhibitor	Nausea, vomiting, diarrhea, headache
Stavudine	Nucleoside reverse transcriptase inhibitor	Peripheral neuropathy
Tenofovir	Nucleoside reverse transcriptase inhibitor	Nausea, vomiting, diarrhea, headache, elevated LFTs and CPK
Tipranovir	Protease inhibitor	Nausea, vomiting, diarrhea
Zalcitabine	Nucleoside reverse transcriptase inhibitor	Oral ulcers, peripheral neuropathy, pancreatitis
Zidovudine	Nucleoside reverse transcriptase inhibitor	Anemia, neutropenia, nausea, vomiting, myositis, neuropathy
CPK, creatine phosphokinase; LFT, liver function test		

There is no doubt that antiretroviral therapy has saved lives; however, this has occurred at considerable expense, including morbidity due to immediate side effects and long-term metabolic toxicities, as well as recognition of serious drug-drug interactions and the emergence of highly resistant virus. Much attention has been focused on combination studies, including older work that revealed potential antagonistic antiretroviral combinations. More recent pharmacokinetic and pharmacodynamic studies have revealed drug-drug combinations with reduced toxicity and improved efficacy. Newer agents and new formulations have allowed for simpler therapeutic regimens, which hopefully will add the benefit of improved adherence and consequently decreased resistance. These agents are no longer used only for the treatment of chronically infected patients but also to treat acutely infected patients, to prevent maternal-child transmission and as prophylaxis against infection following sexual exposure or exposure in a health care setting. Table 187.4 briefly outlines the agents used in the treatment of HIV infection. Chapter 139 and Chapter 204 address antiretroviral agents and the therapy of HIV infection in detail.

The list of available agents to combat both HIV and non-HIV viral infections is expanding rapidly. In addition to new agents, the agents already available are being manipulated by using them in combination at lower, less toxic doses against viruses for which they were not initially investigated and stretching their pharmacology to optimize efficacy and minimize toxicity. As with antibiotics, increased use of antiviral agents will lead to resistance, a major impediment that warrants constant attention.

ANTIPARASITIC THERAPY

Parasitic infections are major causes of significant morbidity and mortality worldwide. In developing countries, lack of resources for many basic services has allowed vector-borne illnesses to persist and propagate. In developed countries health care providers are more frequently encountering parasitic infections as a consequence of increased international travel and a growing number of patients immunocompromised by HIV, antineoplastic therapies and chronic medication-induced immunosuppression.

Although new agents have been introduced in recent years, advances in antiparasitic therapy have lagged behind antibacterials, antivirals and antifungals. The reasons for this are multifactorial and include lack of in-depth knowledge of the life cycle and potential targets for many of these organisms, lack of financial incentives for pharmaceutical companies to invest in drug development for infections not frequently encountered in the developed world, and hesitancy to invest the monies needed to pursue FDA approval for drugs already developed.

Antiparasitic drugs can loosely be characterized as antiprotozoal or anthelmintic, although some agents do have activity against both. They are used in the treatment of acute infections and also in chronic infections. Prophylactic use is increasing in international travelers. For many agents the mechanisms of action are incompletely understood, and this is especially true for many antimalarials. Anthelmintic agents can be characterized as inhibitors of metabolic

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pathways, inhibitors of neuromuscular function or drugs that disrupt reproduction and larval development.[46] [47]

Resistance to antiparasitic therapy is most clearly recognized in the antimalarials. Recent studies have suggested potential resistance mechanisms (p-glycoprotein involved in efflux of the antimalarial) but these explanations are just beginning.[48] Antiparasitic agents are discussed in detail in Chapter 209 , and parasites and the treatment of parasitic infections are covered throughout the text under organ-specific infections, Infections in the Immunocompromised Host, HIV and AIDS, Geographic and Travel Medicine, and Clinical Microbiology.

SUMMARY

Antimicrobial therapy is one of the great advances of the last century. Currently, there are many classes of antimicrobials with activity against most agents of infection, including bacteria and mycobacteria, rickettsiae, fungi, viruses and parasites. Appropriate use of these drugs should include applications of their pharmacology to specifically diagnosed or suspected infections. Doses should be adequate to ensure eradication of the pathogens when possible and the duration of treatment should follow standard regimens based on carefully performed clinical trials. Physicians should be encouraged to use these agents sparingly and only for their proven indications. Excessively long courses, inappropriate prophylactic use, unnecessary combinations and failure to consider the impact of each prescription on the bacterial ecology will ultimately limit the lifespan and usefulness of these life-saving drugs. Careful and thoughtful diagnosis should precede the use of antimicrobial agents and knowledge of their adverse event profiles and potential for drug interactions are prerequisites for their appropriate use. Appropriate antimicrobial use will not only reduce selection of resistant organisms but is likely to improve patient care by reducing adverse effects, toxicities, drug-drug interactions and cost.

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Chapter 188 - Mechanisms of Action

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ANTIBIOTICS THAT ACT ON THE CELL WALL

The basis of the bacterial cell wall is peptidoglycan, a polymer that contains alternating residues of glucosamine and muramic acid in ß-1?4 linkage. The carboxyl groups of muramyl residues are substituted by short peptides (usually pentapeptides such as L-Ala-D-Glu-D-Asp-D-Ala-D-Ala, L-Ser-D-Glu-D-Asp-D-Ala-D-Ala or Gly-D-Glu-D-Asp-D-Ala-D-Ala). Cell-wall-active antibiotics act by inhibiting the activity of enzymes involved in the synthesis of the precursors or in the reticulation of peptidoglycan.

ß-lactams

The ß-lactam nucleus is the basic building block of an exceptionally large class of antibiotics, all of which share a common mode of action but have quite distinct properties in terms of spectrum, pharmacokinetics and activity against resistant strains (see Chapter 193).

Chemical structure

All antibiotics in this class contain a cyclic amide called ß-lactam, but different classes have been described according to the nature of the cycle or of the heteroatom included in the cycle. The main classes are (Fig. 188.1):

- penams — ß-lactams with a five-membered ring containing a sulfur atom (penicillins);
- clavams — ß-lactamase inhibitors that contain a five-membered ring with an oxygen as heteroatom (e.g. clavulanic acid; some sulfur analogs have also been reported);
- carbapenems — five-membered rings with a double bond (e.g. thienamycin, imipenem);
- penems — five-membered unsaturated ring with a sulfur atom (faropenem);
- cephems — six-membered unsaturated rings with a sulfur atom (cephalosporins);
- oxacephems — the oxygen analogs of cephems (latamoxef); and
- monobactams — cyclic amides in a four-membered ring (azetidine) with a methylcarboxylate function in the case of nocardicins and a sulfonate in the case of the other monobactams (e.g. aztreonam).

Other representatives members of the ß-lactams are thiacephems, dethiacephems, dethiacephams, heterocephems and cephams, as well as diverse bicyclic systems.

Some non-ß-lactam analogs have been also reported but seem to be of little interest.

Mode of action

ß-lactams act primarily as inhibitors of the synthesis of the cell wall, by blocking the action of transpeptidases (Fig. 188.2).[1]

Specialized acyl serine transferases or transpeptidases are involved in the assembly of the bacterial cell wall. The structural properties of ß-lactams mimic the D-Ala-D-Ala sequence in that the distance between the carboxylate and the cyclic amide is similar. Thus, these antibiotics act as a false substrate for D-alanyl-D-alanyl transpeptidases. The carboxylate or the sulfonate of the ß-lactams react with a serine residue of the transpeptidases [also called penicillin-binding proteins (PBPs)] to give an acyl enzyme, with the formation of a covalent bond (Fig. 188.3).[2] The acylated enzyme is inactive. Such a mechanism is called suicide inhibition or mechanism-based enzyme inactivation. Transpeptidases are located in the perisplamic space, which is directly accessible in Gram-positive bacteria. In Gram-negative bacteria, ß-lactams have to cross the outer membrane of the bacteria either through the membrane (by passive diffusion) or via porin channels.

The perturbations induced by the ß-lactams in cell wall formation explain the inhibition of the growth of the bacteria, but the bactericidal effect results from indirect mechanisms (mostly the activation of autolytic enzymes). ß-lactams are active only against rapidly dividing bacteria.

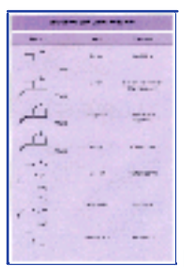

Figure 188-1 Diversity of ß-lactam antibiotics: main ring structures, names and representative antibiotics.

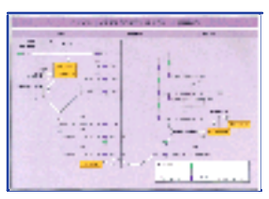

Figure 188-2 Site of action of antibiotics that perturb the synthesis of peptidoglycan. The peptidoglycan unit is formed in the cytosol of bacteria by the binding to uridine diphosphate (UDP)-*N*-acetylmuramic acid of a short peptide (the nature of which differs between bacteria). This precursor is then attached to a lipidic carrier and added to *N*-acetylglucosamine before crossing the bacterial membrane. At the cell surface peptidoglycan units are reticulated by the action of transglycosylases (catalyzing the polymerization between sugars) and of transpeptidases (catalyzing the polymerization between peptidic chains). The antibiotics act as follows: fosfomycin is an analog of phosphoenolpyruvate, the substrate of the *N*-acetylglucosamine-3-*o*-enolpyruvyl transferase synthesizing *N*-acetylmuramic acid from *N*-acetylglucosamine and phosphoenolpyruvate; cycloserine is an analog of D-Ala and blocks the action of D-Ala racemase and D-Ala:D-Ala ligase; bacitracin inhibits the transmembrane transport of the precursor; vancomycin binds to D-Ala-D-Ala termini and thus inhibits the action of transglycosylases and transpeptidases; and ß-lactams are analogs of D-Ala-D-Ala and suicide substrates for transpeptidases.

Resistance

Resistance of ß-lactams may occur at four different levels (see Chapter 189):

¦ first, access to the PBPs in Gram-negative bacteria might be abolished by an alteration of porin channels — this phenomenon predominantly affects highly water-soluble ß-lactams;
¦ second, the antibiotic concentration in the periplasmic space of Gram-negative organisms such as *Pseudomonas aeruginosa* or *Escherichia coli* can be reduced by active efflux mechanisms[3] — the corresponding pumps are characterized by a large substrate specificity, conferring cross-resistance to antibiotics from unrelated classes and by an ill-explained selectivity for some ß-lactams (e.g. meropenem is a better substrate than imipenem);
¦ modification of PBPs can also be observed, in particular, for the PBP2, which is indeed an essential protein involved in the 'shaping' of the bacteria — resistant strains (methicillin-resistant staphylococci), produce a PBP2 protein with a very low affinity for ß-lactams and other PBPs can also show the same decreased affinity;
¦ the fourth and most abundant mechanism is the production of hydrolyzing enzymes called ß-lactamases[2] [4] [5] [6] [7] — these enzymes are serine proteases enzymes that cleave the ß-lactam ring by opening the amide bond and the corresponding genes may either be carried on chromosomes (and their expression may be constitutive or inducible) or on plasmids — this system of resistance is very efficient because these enzymes are secreted out of the cell wall in Gram-positive bacteria and in the perisplamic space in Gram-negative bacteria, and the affinity for ß-lactams is greater than that for PBPs.

Most ß-lactamases open the ß-lactam ring in exactly the same way as transpeptidases, but the major difference is that the hydrolysis rate is far quicker in the case of ß-lactamase than in the case of PBP (see Fig. 188.3). In other words, the speed of hydrolysis of the acyl enzyme is higher and explains the high efficiency of ß-lactamases. The turnover of PBPs and ß-lactamases is indeed very different (1 ß-lactam per hour and 1000 ß-lactams per second respectively). Analytic data and genetic studies of ß-lactamases show a high level of structural homology, which suggests that both derive from a common ancestor. A number of ß-lactams have been

Figure 188-3 ß-Lactam antibiotics as substrates for transpeptidases and ß-lactamases. The left part of the illustration shows how a ß-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one ß-lactam per hour), making the enzyme inactive. The right part of the illustration shows that the same reaction occurs in the case of a ß-lactamase. Hydrolysis of the acylated enzyme is, however, very rapid (1000 ß-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

made resistant to ß-lactamases by appropriate steric hindrance or change in conformation (Fig. 188.4), giving rise to the large number of successive generations of penicillins and cephalosporins. ß-lactamases, however, have an extraordinary plasticity and inevitably develop activity against all new derivatives at a fast pace (Table 188.1).

Thanks to their specific structure, clavams are poor antibiotics but bind tightly to ß-lactamases. Given in combination with ß-lactams, they provide protection unless the bacteria produces ß-lactamases. Some ß-lactamases can also hydrolyze the clavams.

Pharmacodynamics

ß-lactams are relatively slow-acting antibiotics and show only limited post-antibiotic effects. They must therefore be present at a concentration above the minimum inhibitory concentration (MIC) as long as possible (from 40% in moderately severe infections to probably much more and perhaps up to 100% in severe, life-threatening infections). Conversely,

Figure 188-4 Structural modifications of ß-lactam antibiotics that overcome ß-lactamase degradation. A first strategy, applied in penicillins, cephalosporins, oxacephems and monobactams consists of the introduction of a large side chain on the nucleus, possibly containing a substituted imine or alkene. A second strategy, applied in oxacephems and cefoxitin consists of the introduction of a methoxy group on the ß-lactam ring.

concentrations higher than 4–5 times the MIC provides little gain in activity so frequent dosing is more appropriate than infrequent administration of large doses (administration by continuous infusion is being developed, but may encounter difficulties due to the intrinsically fragile character of the ß-lactam ring, making the molecules potentially unstable in aqueous media).[8]

Glycopeptides

Chemical structure

Glycopeptide antibiotics (vancomycin, teicoplanin) contain two sugars and an aglycone moiety made of a relatively highly conserved heptapeptide core, in which two amino acids bear a chloride substituent. The aglycone fraction is responsible for the pharmacologic activity of the molecule, whereas the sugars are thought to modulate its hydrophilicity and its propensity to form dimers (see below). As a result of their large size, glycopeptides are not only unable to cross the outer membrane of Gram-negative bacteria (which explains their inactivity against these organisms), but are also unable to penetrate inside bateria, which limits them to an extracellular target.

Mode of action

Glycopeptide antibiotics inhibit the late stages of cell wall peptidoglycan synthesis (see Fig. 188.2). Glycopeptides bind to D-Ala-D-Ala terminals of the pentapeptide-ending precursors localized at the outer surface of the cytoplasmic membrane. At the molecular level, glycopeptides form a high affinity complex with D-Ala-D-Ala by establishing hydrogen bounds via their aglycone moiety.[9] The strength of this binding is, however, greatly enhanced either by:

¦ dimerization of the glycopeptide molecules (mediated by their sugars and the chloride atoms substituents on the aglycone — as observed in vancomycin); or
¦ anchoring of glycopeptide molecules in the membrane by a fatty acyl chain substituent (as observed in teicoplanin).[10]

The subsequent steric hindrance around the pentapeptide terminals blocks the reticulation of peptidoglycan by inhibiting the activity of transglycosylases (responsible for the new dissacharide-pentapeptide subunit on the nascent peptidoglycan) and of transpeptidases (catalyzing the formation of interpeptide bridges).[9]

Resistance

Resistance to glycopeptides results from substituting a D-lactic acid in place of terminal D-Ala of the pentapeptide. Although this does not prevent the action of the transpeptidase, it prevents the binding of the glycopeptides because of the loss of one crucial hydrogen bound.[9]

Pharmacodynamics

Glycopeptide antibiotics show a very slow bactericidal activity, which is not very dose-dependent, for reasons that are unclear. It has been proposed that their inhibition of cell wall synthesis blocks the growth of bacteria and therefore the synthesis of DNA, RNA and proteins, whereas the autolytic enzymes could continue to function. As their activity is time-dependent, glycopeptides need repeated

TABLE 188-1 -- Functional classification of ß-lactamases.[§]

FUNCTIONAL CLASSIFICATION OF ß-LACTAMASES				
Group	**Molecular class**	**Preferred substrates**	**Active ß-lactams**	**Typical examples**
Group 1: serine cephalosporinases not inhibited by clavulanic acid	C	Cephalosporins I and II (» cephalosporins III, monobactams, penicillins)	Carbapenems	AmpC from Gram-negative; variable upon the species
			Temocillin (cephalosporins III and IV, variable upon level of expression)	
Group 2: serine ß-lactamases				
2a: penicillinases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin » carbenicillin » oxacillins)	Amoxicillin + clavulanic acid	Penicillinases from Gram-positive
			Cephalosporins	
			Carbapenems	
2b: broad-spectum ß-lactamases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin » carbenicillin » oxacillins)	Cephalosporins III and IV,	TEM-1, TEM-2, SHV-1 from Enterobacteriacea, *Haemophilus* spp.
			Monobactams[*]	
		Cephalosporins I and II	Carbapenems	*Neisseria gonorrhoeae*
			Amoxicillin + clavulanic acid	
2be: extended-spectrum ß-actamases inhibited by clavulanic acid (ESBL)	A	Penicillins	Carbapenems	TEM-3 to -26 from Enterobacteriacea
		Cephalosporins I II III (IV)	Temocillin	SHV-2 to -6 from *Klebsiella* spp.
		Monobactams		K1-OXY from *Klebsiella oxytoca*
2br: broad-spectrum ß-lactamases with reduced binding to clavulanic acid	A	Penicillins	Most cephalosporins	TEM-30 to -41 (=IRT-1 to IRT-12) from *Escherichia coli*
			Monobactams[*]	
			Carbapenems	
2c: carbenicillin-hydrolyzing ß-lactamases generally inhibited by clavulanic acid	A	Penicillins	Piperacillin + tazobactam	PSE-1, PSE-3, PSE-4 from *Pseudomonas aeruginosa*
		Carbenicillin	Cephalosporins III and IV	
		(Cephalosporins I and II)	Monobactams[*]	
			Carbapenems	
2d: cloxacillin-hydrolyzing ß-lactamaxes generally inhibited by clavulanic acid	D	Penicillins	Carbapenems	OXA-1 to -11, PSE-2 from Enterobacteriacea and *P. aeruginosa*
		Cloxacillin	Cephalosporins III	
		Cephalosporins I and II	Monobactams[*]	
			Piperacillin + tazobactam	
2e: cephalosporinases inhibited by clavulanic acid	A	Cephalosporins I and II	Cephalosporins III and IV	FPM-1 from *Proteus vulgaris*
			Monobactams[*]	Cep-A from *Bacteroides fragilis*[†]
			Penems	
2f: carbapenem-nonmetallo-hydrolyzing ß-lactamases	A	Penicillins	(Cephalosporins III and IV)	NMC-A, IMI-1 from *Enterobacter cloacae*
		Cephalosporins	(Monobactams[*])	Sme-1 from *Serratia marcescens*
		Carbapenems		
Group 3: Metallo ß-lactamases inhibited by EDTA	B	Most ß-lactams, including carbapenems	Monobactams[*] [‡]	L-1, XM-A from *Stenotrophomonas maltophilia*
				CcrA from *Bacteroides fragillis*
				A2h, CphA from *Aeromonas hydrophila*
				IMP-1 in *Pseudomonas* spp. and *Serratia* spp.
Group 4: Penicillinases not inhibited by clavulanic acid		Penicillins, including carbenicillin and oxacillin	Monobactams[*] [†] [‡] and generally carbapenems	SAR-2 from *Burkholderia cepacia*
The number of enzymes as well as their spectrum of activity is continually evolving.				

§ *Data from Bush et al.*[6]

* Monobactams are not active on Gram-positive bacteria
† Penems are the only molecules active in this case
‡ Remain active for most of the rare published studies

administration, yet, they show a moderate (2-hour) postantibiotic effect, which combined with their long half-life (6 hours for vancomycin, and more than 24 hours for teicoplanin), makes continuous infusion of less interest than for ß-lactams.

Glycopeptides show, at least *in vitro,* a synergistic effect with aminoglycosides, probably by facilitating the penetration of these polar molecules into bacteria.

Future developments

New derivatives with a hydrophobic substituent (e.g. oritavancin) act against vancomycin-resistant strains and show a very fast and highly concentration-dependent bactericidal effect, which suggests a distinct mode of action that could involve drug dimerization and membrane destabilization.[11]

Other agents that act on cell wall synthesis

D-cycloserine is a broad-spectrum antibiotic active through its similarity with D-Ala (see Fig. 188.2 ; Fig. 188.5), inhibiting the conversion of L-Ala into D-Ala (reaction

catalyzed by a racemase) and the dimerization of D-Ala (reaction catalyzed by the D-Ala:D-Ala ligase). [12]

Fosfomycin, which bears structural similarities to phospho-*enol*-pyruvate, inhibits a very early stage of peptidoglycan synthesis by impairing the formation of uridine diphosphate (UDP)-*N*-acetylglucosamine-*enol*-pyruvate, a precursor of UDP-*N*-acetylmuramic acid (see Fig 188.2 , Fig 188.5). [13]

Bacitracin is a polypeptide of complex structure. It acts as an inhibitor of peptidoglycan synthesis at the level of translocation of the precursor across the bacterial membrane (see Fig. 188.2).[14]

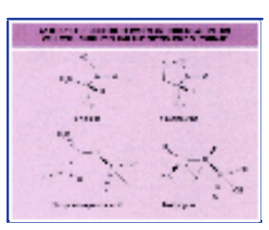

Figure 188-5 Analogy of structure between antibiotics acting on cell wall synthesis and the physiologic substrate. The two antibiotics act as analogs of the corresponding substrate.

ANTIBIOTICS THAT ACT ON PROTEIN SYNTHESIS

Bacterial ribosomes comprise:

- a 30S subunit, which binds mRNA and initiates the protein synthesis; and
- a 50S subunit, which binds aminoacyl tRNA, catalyzes the peptide bond formation and controls the elongation process.

The main sites identified in the ribosome are the donor peptidyl site (P-site), where the growing peptide chain is fixed, and the acceptor aminoacyl site (A-site), where peptide bond formation occurs.

Aminoglycosides

Chemical structure

Streptomycin was discovered in 1944, but this compound had a relatively limited spectrum of activity. Several other compounds, with a broader spectrum of activity, especially towards aerobic and facultative Gram-negative bacilli, were extracted from bacteria or semisynthesized over the subsequent 20 years (aminoglycosides from kanamycin or gentamicin families). In the 1970s, netilmicin and amikacin demonstrated the possibility of developing compounds active against strains resistant to earlier aminoglycosides.

Aminoglycosides are made of several aminated sugars joined by glycosidic linkages to a dibasic cyclitol.[15] The latter is streptidine in streptomycin and derivatives, fortamine in the fortimicin series, and two-deoxystreptamine in most aminoglycosides used clinically. The two-deoxystreptamine moiety links to cyclic sugars either at positions 4 and 5 (neomycin and paromomycin) or 4 and 6 (kanamycin, tobramycin, amikacin and dibekacin in the kanamycin family; gentamicin C_1 , C_{1a} , C_2 , and isepamicin in the gentamicin family, sisomicin and netilmicin; Fig. 188.6). All compounds are positively charged at physiologic pH.

Bacterial targeting

Aminoglycosides selectively disturb the protein synthesis of bacteria because they bind to the 30S subunit of bacterial ribosomes, which does not exist in eukaryotic cells. However, molecules that display a hydroxyl function in C6' in place of an amino function affect also protein synthesis in cultured mammalian cells, as do high doses of gentamicin, probably through nonspecific binding to ribosomes or nucleic acids.

Mode of action

As a result of their highly polar character, aminoglycosides are unable to diffuse through membranes, and therefore require specific mechanisms of transport. Their passage across the outer membrane of Gram-negative bacteria occurs by a process that is not energy dependent and involves the drug-induced disruption of Mg^{2+} bridges between adjacent lipopolysaccharide molecules. By contrast, their transport across the cytoplasmic (inner) membrane is dependent upon electron transport, and is therefore termed energy-dependent phase I (EDP-I). The greater the transmembrane electrical potential, the greater the antibacterial effect of the aminoglycoside. In an anaerobic environment, at low external pH and in high osmolar culture media, this transmembrane electrical potential is decreased, which explains the low activity against anaerobes as well as in purulent collections.

Once in the bacterial cytosol, aminoglycosides bind to the aminoacyl site of the 30S subunit of ribosomes[17] (and, to a lesser extent, to specific sites of the 50S subunit), again through an energy-dependent process (EDP-II), disturbing the elongation of the nascent peptide. Their mechanism of action is complex, involving inhibition of the transfer of the peptidyl tRNA from the A-site to the P-site and impairment of the proofreading process that controls translational accuracy. The latter action leads to misreading or premature termination in protein synthesis. The final effects vary somewhat from one compound to another, which possibly explains differences in the killing rates. The aberrant proteins may be inserted into the cell membrane, which results in altered permeability and further stimulation of aminoglycoside transport.

Resistance

Resistance occurs mostly by the production of enzymes that inactivate the functions responsible for activity of the natural aminoglycosides (Fig. 188.7 , see Chapter 189). Semisynthetic derivatives (e.g. netilmicin, amikacin, isepamicin) were therefore made specifically to afford protection against these enzymes. However, whereas previously resistant bacteria harbored only one of a very few types of enzymes, the simultaneous production of several enzyme types is increasingly more common, causing multiple resistance. It is believed that most of these enzymes have physiologic effects on natural substrates and acted on aminoglycosides only opportunistically in the initial introduction of these antibotics. However, point mutations and selection may have quickly increased their specificity and efficacy.[18] [19]

A second mechanism of resistance is membrane impermeability, which confers resistance to all aminoglycosides. Its molecular mechanism is unclear.

Pharmacodynamics

Aminoglycosides demonstrate a rapid, concentration-dependent bactericidal effect and an important postantibiotic effect (probably because of a largely irreversible binding to the ribosomes). A once-a-day regimen is therefore the optimal mode of administration for these antibiotics, allowing elevated serum peak concentrations (over eight times the MIC, thereby maximizing efficacy while minimizing toxicity) to be reached.

Aminoglycosides show a synergistic activity with antibiotics that act on cell wall synthesis, because they facilitate the penetration of aminoglycosides into the bacteria. In contrast, their activity is antagonized by bacteriostatic agents such as chloramphenicol and tetracyclines, probably by inhibition of their energy-dependent uptake and by interference with the movement of the ribosome along mRNA.

Future developments

Efforts are being undertaken in two directions:

- to increase the binding affinity while retaining binding selectivity; and

¦ to develop new aminoglycoside derivatives resistant to these enzymes.

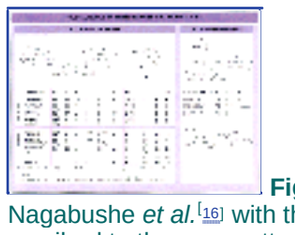
Figure 188-6 Structural formulae of the 2-deoxystreptamine-containing aminoglycosides. The numbering of the atoms shown here follows the recommendations from Nagabushe *et al.*[16] with the primed numbers (') being ascribed to the sugar attached to C4 of the 2-deoxystreptamine (as this C is of the R configuration) and the doubly primed numbers ('') being ascribed to the sugar attached to either the C6 (S configuration) for the 4,6-disubstituted 2-deoxystreptamine or the C5 (R configuration) for the 4,5-disubstituted 2-deoxystreptamine. Molecules indicated in bold denote the aminoglycosides in widespread clinical use.

Although some derivatives have been made by pharmacochemical approaches, little success has been obtained. A more innovative approach could be to use our understanding of the aminoglycoside-inactivating enzymes to produce enzyme inhibitors or to design totally new aminoglycoside derivatives that would be intrinsically resistant to these enzymes.

Tetracyclines

Chemical structure

The first tetracyclines discovered were isolates from *Streptomyces* spp. (tetracycline, oxytetracycline), whereas more recent long-acting compounds (doxycycline, minocycline) are semisynthetic. All such molecules, contain four hydrophobic fused rings, which are diversely substituted, but principally by oxygenated hydrophilic groups (see Chapter 200).

Bacterial targeting

Tetracyclines penetrate the outer membrane of Gram-negative organisms through porins. Accumulation inside the bacteria depends on the pH gradient between the cytosol and the external medium, but it is unclear whether transmembrane transport occurs by diffusion or via a proton-driven carrier. The main argument in favor of the latter is that it could explain the selective action of tetracyclines by preferential transport in bacterial cells (Fig. 188.8).

Mode of action

Tetracyclines interfere with the initiation step of protein synthesis (see Fig. 188.8). More precisely, they inhibit the binding of aminoacyl tRNA to the A-site of the ribosome. The 7S protein and the 16S RNA show the best affinity for tetracyclines, and are therefore the main targets involved pharmacologically.[20] This binding inhibits the fixation of a new aminoacyl tRNA on the ribosome. At higher concentrations, tetracyclines also bind to the 23S RNA, which is part of the peptidyl transferase region of the ribosome. However, the enzymatic activity of this site does not seem not to be disturbed by tetracyclines. Additional actions on ribosomal functions have been proposed:[21]

¦ tetracyclines bind, or at least protrude, in the P-site, thanks to the change in ribosome conformation in the post-translocational state; and
¦ tetracyclines modify the ribosome conformation at the head of the 30S subunit and at the interfacial side of the 50S subunit.

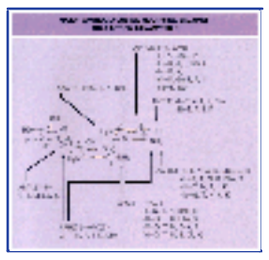
Figure 188-7 Major aminoglycoside-modifying enzymes that act on kanamycin C. This aminoglycoside is susceptible to the largest number of enzymes. The *N*-acetyltransferases (AACs) affect amino functions and the *o*-nucleotidyltransferases affect hydroxyl functions. Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (Roman numerals) with different substrate specificities (phenotypic classification). At least one enzyme is bifunctional and affects both positions 2'' (*o*-phosphorylation) and 6' (*N*-acetylation). The main aminoglycosides used clinically on which these enzymes act are amikacin (A), dibekacin (DbK), commercial gentamicin (G), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T). The drug abbreviations that appear in parentheses are those for which resistance was detectable *in vitro* although clinical resistance was not conferred. *Data from Shaw et al.*[19]

More recently, it has been shown that tetracyclines have chondroprotective effects in inflammatory arthritis models, an action related to their ability to inhibit the expression of nitric oxide synthases induced by inflammatory conditions.[22] Clinical evaluation is, however, needed to document further the potential usefulness of tetracyclines as modulators of the inflammatory response. A possible application would be the treatment of arthritis of bacterial origin (e.g. Lyme disease).

Resistance

Resistance to tetracyclines is now widespread, and is related to a decrease in the bacterial drug content caused by an active drug efflux (see Fig. 188.8).[23] Efflux mechanisms appear more important and are somehow largely, but not entirely unrelated to the drug structure (see fluoroquinolones and macrolides below; see also Chapter 189).

Pharmacodynamics

Tetracyclines are essentially bacteriostatic, but demonstrate persistent effects. They need to be administered at intervals short enough, in terms of the drug half-life, to maintain their serum level above the infecting organism's MIC for as long as possible. The total dose administered also appears to be important.

Future developments

Glycylcyclines are tetracyclines derivatives that bear a glycyl substituent and are able to bind to the tetracycline binding site on the ribosome. [24] Their main advantage is that they conserve their activity against strains with acquired resistance to conventional tetracyclines by the production of efflux pumps or by a mechanism of ribosomal

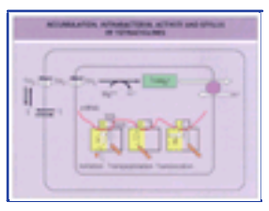
Figure 188-8 Accumulation, intrabacterial activity and efflux of tetracyclines. Tetracyclines diffuse freely through the extracellular membrane of Gram-negative bacteria. Penetration inside bacteria is an energy-dependent process depending on the pH and Mg^{2+} gradient between the extracellular medium of Gram-positive bacteria or the periplasmic medium of Gram-negative bacteria and the intracellular medium. Only the protonated form is highly diffusible, so accumulation is favored by lowering of the extracellular pH. Once inside the cytosol the tetracycline molecule forms a nondiffusible complex with Mg^{2+} . This type of complex with a bivalent cation is also the substrate of the efflux pumps present in the membrane of resistant bacteria and acting as H^{+} antiports (pink circle). The antibacterial action of the teracyclines (T) is due to the binding to the 30S subunit of the ribosomes. In the pretranslocational state, tetracyclines inhibit the binding of aminoacyl tRNA (arrow 1) to the A-site (yellow part of the ribosome). In the post-translocational state, tetracyclines protrude in the P-site (white part of the ribosome) and inhibit the binding of the

peptidyl tRNA (arrow 2). *Data from Geigenmüller and Nierhaus*[21] *and Yamaguchi et al.*[23]

protection (acquisition of a gene that encodes a Tet protein, (i.e. an elongation factor able to displace the tetracycline bond on the ribosome). However, mutants resistant to glycylcyclines have already been selected *in vitro*, with the possibility that such mutants may emerge in clinical strains.

Fusidic acid

Chemical structure

Fusidic acid is a steroid-like structure and a member of the fusidane class. It is used in its sodium salt form.

Mode of action

Fusidic acid prevents the dissociation of the complex formed by guanosine diphosphate, the elongation factor 2 and the ribosome. It thereby inhibits the translocation step of the peptidyl tRNA from the P-site to the A-site of the ribosome and, therefore, the elongation of the nascent polypeptide chain.

Pharmacodynamics

Fusidic acid is bacteriostatic, but may be bactericidal at high concentrations.

Mupirocin

Chemical structure

Mupirocin contains a short fatty acid side chain (9-hydroxynonanoic acid) linked to monic acid by an ester linkage. Mupirocin is also called pseudomonic acid because its major metabolite is derived from submerged fermentation by *Pseudomonas fluorescens*. Pseudomonic acid A is responsible for most of the antibacterial activity;

three other minor metabolites of similar chemical structure and antimicrobial spectrum are called pseudomonic acids B, C, and D.

Mode of action

Mupirocin inhibits bacterial RNA and protein synthesis by binding to bacterial isoleucyl tRNA synthetase, which catalyzes the formation of isoleucyl tRNA from isoleucine and tRNA. This prevents incorporation of isoleucine into protein chains, and so halts protein synthesis. This unique mechanism of action results in no cross-resistance between mupirocin and other antimicrobial agents.[25]

Pharmacodynamics

Mupirocin is bacteriostatic at low concentrations, but becomes bactericidal at concentrations achieved locally by topical administration. *In vitro* antibacterial activity is greatest at acidic pH, which is advantageous in the treatment of cutaneous infections because of the low pH of the skin.

Future developments

The recognition of the peculiar mode of action of mupirocin has triggered a large genomic-based research towards similar targets at the level of the other amino acids.[26]

Macrolides

Chemical structure

The main active macrolides are 14-, 15- or 16-membered lactone rings, substituted by two sugars, of which one bears an aminated function. Erythromycin, the first clinically developed macrolide, is a natural product. Most of the molecules developed in the mid 1980s are semisynthetic derivatives, and have been designed to be stable in acidic milieu. They are therefore essentially characterized by an improved oral bioavailability. 16-membered macrolides are intrinsically acid stable. In 15-membered macrolides (azithromycin), an additional aminated function is inserted in the lactone ring, conferring to this subclass of molecule the name of 'azalides'. They are acid stable and characterized by an exceptionally large volume of distribution and prolonged half-life. Ketolides are 14-membered macrolides in which the cladinose is replaced by a keto function and which possess in their macrocycle a carbamate linked to an alkyl-aryl extension (Fig. 188.9).[27] They are also intrinsically acid stable. Moreover, they remain active against most of the strains resistant to other macrolides.

Bacterial targeting

Macrolides specifically bind to the 50S subunit of the ribosomes (more precisely, to the 23S rRNA), which does not exist in eukaryotic cells.

Mode of action

Macrolides reversibly bind to the peptidyl transferase center, located at the 50S surface, which results in multiple alterations of the 50S subunit functions.[28] [29] While macrolides bind to the domain V of the 23S rRNA, ketolides have a dual anchoring to the ribosome. They not only bind to domain V, like other macrolides, but also bind to domain II of 23S rRNA (see Fig. 188.9).[30] [31] This additional binding involves the carbamate extension, which is absent in conventional macrolides.[27] Because of their double interaction, ketolides are characterized by a higher affinity for their target and therefore by an improved efficacy. Macrolides are classically thought to block the peptide bond formation or the peptidyl tRNA translocation from the A- to the P-site. However, additional consequences of macrolides binding to ribosomes have been reported. A proposal is that they could also favor the premature dissociation of peptidyl tRNA from the ribosome during the elongation process, leading to the synthesis of incomplete peptides.[28] A further suggestion is that erythromycin prevents the assembly of the 50S subunit, but this does not appear to be applicable to other macrolides.

Resistance

Clinically meaningful resistance occurs primarily by modification of the bacterial target and therefore affects all macrolides (and will also affect lincosamides and streptogramins). This resistance may be inducible or constitutive. Ketolides (due to their lack of cladinose) and 16-membered macrolides are not inducers and therefore show activity on a subset of resistant strains.[27] Moreover, as a result of their double anchoring to the ribosome, ketolides remain able to bind to domain II of the 23S ribosomal RNA of strains resistant by methylation of the domain V.[30] [31] [32] They can therefore maintain antibiotic activity against strains resistant to other macrolides.

Efflux mechanisms are also now being observed and, again, 16-membered macrolides are spared this effect. The frequency of strains susceptible to 16-membered macrolides and resistant to 14- and 15-membered macrolides remains small, however.

Pharmacodynamics

Macrolides are essentially bacteriostatic antibiotics, except at high concentrations. Thus, their concentration at the infected site needs to be consistently maintained above the MIC of the pathogen.[33]

As their mode of action is similar, macrolides, streptogramins, lincosamides and chloramphenicol have antagonistic pharmacologic activity. Moreover, the common

binding site to ribosomes of macrolides, streptogramins, and lincosamides shows that a mutation of the target causes cross-resistance to these three classes of antibiotics.

Future developments

Efforts are still being made to discover macrolide derivatives active against bacteria resistant to the macrolides used at the present time in the clinics. Research in the field of ketolides is still active. Erythromycylamines modified at their cladinose moiety show activity against inducible resistant strains and also against strains resistant through the production of efflux pumps.

Lincosamides

Chemical structure

Lincomycin and its 7-chloro-7-deoxy derivative, clindamycin, comprise a propylhygrinic acid linked to an aminosugar.

Mode of action

Lincosamides bind to the 50S ribosomal subunit and have a mode of action similar to that of macrolides.[29] They inhibit early chain elongation by interfering with the transpeptidase reaction.

Resistance

The main mechanism of resistance to lincosamides is similar to that found in resistance to macrolides and streptogramins, and consists of alteration of the 50S subunit. Rare cases of enzymatic inactivation of the antibiotic have also been described for clindamycin (adenylation reaction).

Pharmacodynamics

Lincosamides are bacteriostatic, and are antagonists of macrolides and streptogramins, which bind at the same site on the ribosomes.

Streptogramins

Chemical structure

Streptogramins are antibiotics that comprise a pair of synergistic constituents, namely a depsipeptide (group I) and a lactone macrocycle (group II). The combination of quinupristin and dalfopristin is used in the clinic.[34]

Mode of action

Streptogramins bind to the 50S subunit of bacterial ribosomes and interfere with the protein synthesis by a double mechanism, involving an inhibition of the incorporation of the aminoacyl tRNA in the ribosomes

Figure 188-9 Chemical structure of the macrolides. The upper panel shows the degradation of erythromycin in the gastric milieu (substituents responsible for the instability of the miolecule are shown in gray). 16-membered macrolides and ketolides are intrinsically stable. The structural modifications conferring stability in acidic milieu to 14- and 15-membered macrolides are highlighted in gray in the middle panel. The lower panel compares the binding of macrolides and ketolides to the petidyl transferase site of the 50S subunit of ribosomes. Macrolides are characterized by a single anchoring point and ketolides by a double anchoring point, which increases the affinity of ketolides for wild type and methylated ribosomes.

and of the translation of the mRNA. The synergy between the two components could be due to a modification of the conformation of the ribosome caused by the binding of the group I component, which exposes a site of fixation for the group II component. [28]

Resistance

Resistance by mutation of the ribosomal target will also result in resistance to macrolides and lincosamides. Resistance to streptogramins alone is rare and occurs by enzymatic inactivation (involving an hydrolase and an acetylase).

Pharmacodynamics

Streptogramin constituents are highly synergistic and show a dose-dependent bactericidal activity if given together.[33] In addition, they increase the antibiotic activity of aminoglycosides and rifamycins.

Streptogramins also exhibit prolonged bacteriostasis, which consists of a delay of regrowth when the antibiotic concentration falls under its MIC. This could be interpreted as a consequence of the persistent binding of the drug to its target.

Future developments

Streptogramins are not largely used today. However, their potential role for bacteria resistant to other antibiotics (MRSA, vancomycin-resistant enterococci) may reactivate research in this area.

Chloramphenicol and thiamphenicol

Chemical structure

Chloramphenicol and thiamphenicol are based on dichloroacetamide bearing a diversely substituted phenyl group (see Chapter 200).

Bacterial targeting

Chloramphenicol acts principally by binding to the 50S subunit of the bacterial ribosomes. However, it can also interact with mitochondrial ribosomes of eukaryotic cells,

which results in its toxicity.

Mode of action

Chloramphenicol enters the bacteria by an energy-dependent process. Its antibiotic activity results from competitive inhibition of aminoacyl tRNA binding to the peptidyl transferase domain of the 50S subunit. This induces conformational changes of this part of the ribosomes, which slows or even inhibits (at high enough concentrations) the incorporation of the aminoacyl tRNA and, therefore, the transpeptidase reaction.[35]

Resistance

Resistance to chloramphenicol derives mainly from the production of a specific acetyl transferase that inactivates the antibiotic.[36] The gene encoding the transferase is often located on plasmids that also confer resistance to other antibiotic classes. Another mechanism of resistance is reduced entry of the drug into the bacteria.

Pharmacodynamics

Chloramphenicol is bacteriostatic. It competes in binding to the ribosomes with macrolides and lincosamides, making its combination with these drugs useless.

Oxazolidinones

Chemical structure

Oxazolidinones are totally synthetic molecules. The first derivatives endowed with antimicrobial activity were described at the end of the 1970s. Structures were then refined on the basis of structure-activity relationships[37] to give rise so far to linezolid, the first clinically available molecule (Fig. 188.10). The 5-(S)-configuration of the oxazolidinone ring is essential for activity, which is further improved by its substitution by an N-fluorinated aryl group and a C5 acylaminomethyl group.

Mode of action

Oxazolidinones inhibit protein synthesis at an earlier step that other antibiotics acting on the ribosome. Their binding site is located in the vicinity of the peptidyl transferase center of the 50S subunit.[38] This interaction prevents the formation of the initiation ternary complex which associates $tRNA^{met}$, mRNA, and the 50S subunit of the ribosome,[39] and therefore the binding to the ribosome as well as the synthesis of peptide bonds, and the translocation of $tRNA^{met}$ into the P-site.[40]

Resistance

Because of the unique mode of action of oxazolidinones, there is no cross-resistance with other antibiotics acting on protein synthesis. The introduction of linezolid in clinics is too recent to draw any conclusion concerning the incidence and mechanisms of resistance. Case reports of resistant clinical isolates emerging during therapy have, however, been published.[41] Mutation of the 23S rRNA *in vitro* confers resistance to linezolid.[38]

Pharmacodynamics

Oxazolidinones are bacteriostatic, time-dependent antibiotics, with a short post-antibiotic effect.[41] They can compete for binding to the 50S subunit of the ribosome with other antibiotics (e.g. lincosamides, chloramphenicol).

Future developments

Other molecules are currently under investigation. Efforts are essentially directed towards broadening the spectrum of activity and increasing intrinsic activity.

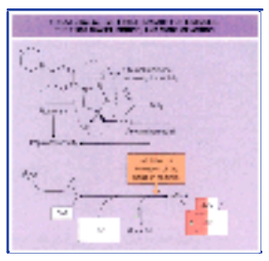

Figure 188-10 Structural activity relationship for linezolid, the first oxazolidinone, and mode of action. The drug prevents the formation of the ternary complex between mRNA, ribosome subunits and $tRNA^{met}$ necessary for protein synthesis.

DRUGS THAT AFFECT NUCLEIC ACIDS

Fluoroquinolones

Chemical structure

Fluoroquinolones are totally synthetic products originally derived from nalidixic acid (see Chapter 198). All current compounds have a dual ring structure, with nitrogen at C1, a free carboxylate at C3 and a carbonyl at C4. A fluorine substituent at C6 usually greatly enhances activity, whereas the substituents at C7, C8 and N1 modulate the spectrum, pharmacokinetics and side-effects of the drugs (Fig. 188.11).[42] In this respect, new molecules (among which moxifloxacin and gatifloxacin are now used in the clinic) have been designed to better cover Gram-positive organisms, keep activity against Gram-negative organisms and also be, to some extent, active against anaerobes.[43] They all present a small hydrophobic substituent on N1 and a diaminated small-sized ring substituent in 7.

Bacterial targeting

Fluoroquinolones cross the outer membrane of Gram-negative bacteria via porins. Their affinity for the bacterial target is 1000 times greater than that of the corresponding eukaryotic enzyme, which ensures their specificity.

Mode of action

Fluoroquinolones inhibit the activity of topoisomerases, which are enzymes responsible for the supercoiling of the DNA (DNA gyrase) and relaxation of supercoiled DNA (topoisomerase IV). Both enzymes have a similar mode of action, which implies:

- binding of DNA to the enzyme;
- cleavage of the DNA;
- passage of the DNA segment through the DNA gate;
- resealing of the DNA break and the release from the enzyme.

Gyrase and topoisomerase IV are tetramers made of two types of subunits, namely two GyrA or ParC that catalyze DNA cutting and resealing, and two GyrB or ParE responsible for the transduction and binding of adenosine triphosphate. The main target of fluoroquinolones

Figure 188-11 Structure-activity, structure-pharmacokinetics and structure-toxicity relationships of the fluoroquinolones. These considerations form the basis of the rational development of the new molecules of this class, which have a very extended spectrum (including Gram-positive bacteria and anaerobes), a long half-life and minimal phototoxicity and metabolic interactions.

is DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria. [44]

Fluoroquinolones form a ternary complex with DNA and the enzyme (Fig. 188.12).[45] This binding site for fluoroquinolones is formed during the gate-opening step of the double-stranded DNA. Cooperatively, four fluoroquinolone molecules are fixed to single-stranded DNA. Their stacking is favored by the presence of coplanar aromatic rings in their structure and by the tail-to-tail interactions between the substituents at N1. Interaction with DNA occurs by hydrogen bonds or via Mg^{2+} bridges established with carbonyl and carboxylate groups. Interaction with the enzyme is mediated by the fluorine at C6 and substituents at C7. The binding of the fluoroquinolones stabilizes the cleavable complex (formed by the cut DNA and the enzyme) and leads to the dissociation of the enzyme subunits. The latter action is observed only for potent molecules or at higher concentrations.

Quinolones have other effects on bacterial cells, such as induction of the DNA repair response, which involves three proteins (RecA, LexA and RecBCD). Induced RecA cleaves the repressor part of the SOS regulon (LexA), stimulating repair of damage caused by fluoroquinolones to DNA. Induced RecBCD binds to the chromosome at the double-strand break created by the ternary complex of topoisomerase-DNA-quinolone, and results in mutagenesis as well as increased cell survival in the presence of quinolones. This system therefore protects against the antibacterial activity of fluoroquinolones.[42]

Resistance

Resistance occurs mostly by mutation of the topoisomerases (reducing drug-binding ability), by porin impermeability or by efflux. These mechanisms affect all fluoroquinolones and result in progressive slight increases in the MIC. New fluoroquinolones may remain active against resistant strains based upon higher intrinsic activity, which is a structure-related property. In particular, the presence of a methoxy substituent in position 8 reduces the potential for selecting resistant mutants (see Fig. 188.11). [46]

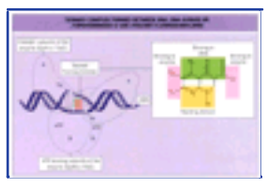

Figure 188-12 Ternary complex formed between DNA, DNA-gyrase or -topoisomerase IV and stacked fluoroquinolones. Subunits A form covalent bonds via Tyr122 with the 5' end of the DNA chain. The binding site for fluoroquinolones is located in the bubble formed during the local opening of the DNA molecule. The right panel shows the parts of the antibiotic molecules interacting with DNA, with the enzyme or favoring the stacking of the fluoroquinolone molecules. *Adapted from Shen et al.*[45]

Pharmacodynamics

The mechanism described above requires RNA and protein synthesis as well as cell division for bactericidal action. The latter probably results from cutting of the DNA and the subsequent creation of a barrier for its transcription. Alternative mechanisms, however, confer bactericidal activity to certain molecules either in the absence of protein and RNA synthesis or without bacterial multiplication.

The activity of fluoroquinolones is largely concentration dependent, but these drugs show also persistent effects. Accordingly both the peak/MIC and the 24-hour area under the serum concentration curve (AUC)/MIC ratios are important for activity (pharmacodynamic studies have shown that effective doses often need to be considerably higher than was originally tought; breakpoints should be revised to lower values, and based on pharmacodynamic considerations, should be <1µg/ml; [47] recent studies also suggest that the a peak/MIC ratio >10 protects or retards the emergence of resistance). Fluoroquinolones also show a postantibiotic effect, the duration of which varies according to the pathogen, drug concentration and period of exposure.

Future developments

New molecules with extended spectrum and high intrinsic activity are still in development, among which des-fluoroquinolones (i.e. molecules lacking the F substituent in position 6).[48] When applied to molecules of high intrinsic activity, this structural change was shown to not affect activity while maintaining the mode of action of fluoroquinolones. These molecules have also a low potential for selecting resistance.

Nitroimidazoles and nitrofurans

Chemical structure

The nitroheterocyclic drugs include nitrofuran and nitroimidazole compounds (Fig. 188.13).

Mode and spectrum of action

The activity of nitroheterocyclic drugs requires activation of the nitrogroup attached to the imidazole or furan ring, which must undergo single- or two-electron enzymatic reduction in the bacteria.[49] [50] [51] Single-electron reduction of nitroaromatics is most frequently catalyzed by flavoenzyme dehydrogenase electrotransferases and bacterial oxygen-sensitive nitroreductases. Under aerobic conditions, the single electron reduction of nitroaromatics to give their anion radicals results in their reoxidation by oxygen with formation of superoxide and other activated oxygen species that damage proteins, nucleic acids, and lipids. Under hypoxic conditions, enzymes that transfer single electrons reduce nitroaromatics to amines or, less frequently, to hydroxylamines. Two-electron reduction of nitroaromatics to nitroso compounds and, subsequently, to hydroxylamines is catalyzed by bacterial oxygen-insensitive nitroreductases and mammalian DT-diaphorase NADPH:quinone reductase.

Although the nitro radicals generated by reduction of the parent drugs are similar for the nitroimidazoles and the nitrofurans, these drugs differ by their reduction potential, and, therefore in their effects on bacteria and their spectrums of activity. Thus, the reduction of nitroimidazoles causes depletion in the intracellular stock of reduced coenzymes. Moreover, reduced forms of these antibiotics are highly reactive and may damage the DNA molecule. Reduced nitrofurans also inhibit the activity of enzymes involved in the degradation of glucose and pyruvate. In addition, they covalently bind to proteins and DNA by an alkylation reaction.

Future developments

The variety of substitutions that can be attached to the ring structures may allow for a large amount of flexibility. The major interest in these drugs is the use of 2-nitroimidazole probes as radiosensitizers of hypoxic cells on a cell-to-cell basis and for noninvasive detection.

Ansamycins

Chemical structure

Ansamycins, which are macrocyclic antibiotics, are lipophilic and therefore easily diffuse through membranes. They comprise two aromatic rings (containing a quinone),

connected by a long chain (or 'ansa' — hence the name given to this class of antibiotics), which confers a rigid character to the whole molecule.

Mode of action

Ansamycins inhibit the initiation of DNA transcription to mRNA and therefore the subsequent protein synthesis.[52] The RNA polymerase contains five subunits (a_2 ßß's):

- a subunits establish contact with transcription factors;
- ß' subunit is a basic polypeptide that binds DNA;

- ß subunit is an acidic polypeptide and is part of the active site;
- s-subunit initiates the transcription and then leaves the polymerase nucleus.

Figure 188-13 Modes of action of nitrofurans and nitroimidazoles. The modes of action include passage through the cell membrane, reduction to highly reactive products, interaction with intracellular targets and release of inactive end products.

The core polymerase (a_2 ßß') therefore retains the ability to synthesize RNA but is defective in its ability to bind and initiate DNA transcription.

Inhibition by rifamycins follows binding of the antibiotic to the ß subunit of the RNA polymerase or, to a lesser extent, of the DNA-RNA complex. This binding is mediated by hydrophobic interactions between the aliphatic ansa chain and the ß subunit. The precise site of binding has been identified only partly, by studying mutants in RNA polymerase that have acquired resistance to rifampin (rifampicin). All the mutations that affect drug binding belong to three clusters of amino acids in the central domain of the ß subunit.

Inhibition of transcription caused by rifamycins is essentially noncompetitive. A model has been proposed in which rifamycins block the translocation event during transcription initiation, without hindering the synthesis of the first phosphodiester bridge between the two first nucleotide triphosphates of the mRNA molecule.[53] Specificity of action depends on the fact that ansamycins alter mammelian cell metabolism only at concentrations 10,000 times those necessary to cause bacterial cell death (Fig. 188.14).

Pharmacodynamics

Rifamycins are bactericidal — an effect that results either from the high stability of the complex formed between rifampin and the enzyme or from the formation of superoxide ions on the quinone ring of the antibiotic molecule. As their action is to hinder bacteria multiplication, they are, at least *in vitro*, antagonists to antibiotics that require active bacterial growth to exert their activity (ß-lactams) or other antibiotics that act on protein synthesis (macrolides and aminoglycosides). This antagonism is, however, not observed *in vivo*, because of the different distribution of these antibiotics (intracellular for rifamycins; extracellular for ß-lactams and aminoglycosides). Their postantibiotic effect is longlasting because of the irreversible

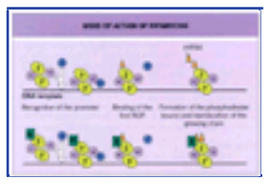

Figure 188-14 Mode of action of rifamycins. Synthesis of mRNA by RNA polymerase is shown in the upper panel and inhibition by rifamycins (R in the green squares) is shown in the lower panel. The RNA polymerase core is made up of four subunits, of which the ß' subunit binds to the DNA template and the ß subunit binds the ribonucleotide diphosphate (NDP; triangle). The s factor only participates to the initiation step by allowing for the recognition by the enzyme core of promoter sequences on the DNA template. Rifamycins bind to the ß subunit. They do not interfere with the binding of the nucleotide diphosphate, but rather inhibit the transcription initiation either by impairing the formation of the first phosphodiester bond or the translocation reaction of the newly synthesized dinucleotide.

nature of their binding. The efficient cell penetration of ansamycins gives them excellent activity against sensitive intracellular organisms.

Future developments

Benzoxazinorifamycins constitute a new group of semisynthetic molecules that show greatly enhanced activity against *Mycobacteria* spp., the main clinical target of this class of antibiotics.[54]

ANTIMETABOLITES

Sulfonamides and diaminopyrimidines

Prontosil (sulfamidochrysoidine) was one of first synthetic compounds with antibacterial activity found by Domagk in 1932. In fact, this product was a prodrug and elucidation of its metabolism led to the development of the sulfonamides. With diaminopyridines, they inhibit the folate pathway in bacteria.

Chemical structure

Sulfonamides are derived from *p*-aminobenzenesulfonamide which is a structural analog of *p*-aminobenzoic acid, a factor required by bacteria for folic acid synthesis. A free amino group at C4 and a sulfonamide group at C1 are required for antibacterial activity. Heterocyclic or aromatic rings substituting the sulfonamide enhance this activity by modifying absorption and gastrointestinal tolerance.

Diaminopyrimidines, such as trimethoprim and pyrimethamine, are pyrimidines substituted at C5 by an aromatic group. Pyrimethamine has an additional substituent at C6.

Mode of action

Sulfonamides inhibit tetrahydrofolic acid synthesis.[55] [56] Briefly, this synthesis requires successive enzymatic reactions, among which are:

- formation of pteroic acid from *p*-aminobenzoic acid and dihydropteridin catalyzed by the dihydropteroate synthetase, and
- reduction of dihydrofolic acid in tetrahydrofolic acid (the active form of folic acid) catalyzed by the dihydrofolate reductase.

Sulfonamides act via a double mechanism. First, as analogs as *p*-aminobenzoic acid, they are competitive inhibitors of dihydropteorate synthetase. Second, they can also function as alternative substrates for the synthetase and become incorporated into a product with pteridine.

Diaminopyrimidines are specific inhibitors of bacterial dihydrofolate reductase[55] [56] and act as competitive inhibitors of this enzyme. Even though dihydrofolate

reductase is present in bacteria as well as in eukaryotic cells, action selectivity occurs; this might be explained by the different conformation formed in the cavity of the bacterial enzyme compared with the conformation in the eukaryotic enzyme [radiographic cocrystallization data (trimethoprim-enzyme) suggest that trimethoprim in bacterial enzymes establishes more binding interactions than in the eukaryotic enzymes]. In addition, the NADPH cofactor may stabilize the enzyme-trimethoprim complex in the bacteria.

Resistance

For sulfonamides, resistance mainly occurs by hyperproduction of *p*-aminobenzoic acid or by reduction of the affinity of the dihydrofolate reductase for the antibiotic, which causes resistance to the whole class. For diaminopyrimidines, resistance mostly occurs by enzyme mutations that prevent binding [a single point mutation (e.g. Phe98?Tyr) is sufficient to prevent any binding of trimethoprim to the enzyme, because of the loss of a critical hydrogen bound].[57]

Pharmacodynamics

Sulfonamides are only bacteriostatic. Their combination with diaminopyrimidines confers to them a bactericidal activity because of synergism.

ANTIBIOTICS ACTING ON THE MEMBRANE

Cyclic polypeptides (polymyxins/colistins)

Chemical structure

These are a collection of cyclic, branched polypeptides (molecular masses about 1000Da) containing both cationic and hydrophobic aminoacids. Some of these are the D configuration or are non-DNA coded, which confers resistance to mammalian peptide-degrading enzymes (60–90% of a parenterally-administered dosis is excreted intact in the urine). Polymyxins are obtained from *Bacillus polymyxa* and colistins from *Aerobacillus colistinus*. Only polymyxin B and colistin A (identical to polymyxin E) are used in clinical practice.

Mode of action

Because of their amphipathic character, polymyxins and colistins act as detergents and alter the permeability of the cytoplasmic membrane.[58] They, therefore, affect bacteria at all stages of development. However, they cannot easily diffuse through the thick peptidoglycan layer of Gram-positive bacteria. In contrast, they easily bind to the phospholipids of the outer membrane of Gram-negative bacteria from where they reach the cytoplasmic membrane through polar as well as nonpolar channels. These properties explain both their strong and fast bactericidal activity through major perturbation of the inner membrane permeability properties (but recent findings have challenged this mechanism) and their narrow spectrum, which is essentially limited to Gram-negative organisms.

Resistance

Acquired resistance to polymyxins and colistins is chromosomal and results from a decreased permeability of the outer membrane secondary to changes in its biochemical composition. Bacteria with decreased sensitivity are indeed characterized by a decreased phospholipid/lipid ratio and a higher content in divalent cations (Ca^{2+} , Mg^{2+}). Protein H1 from *P. aeruginosa* (presently known as OprH) prevents binding of polymyxins and colistins to lipopolysaccharide and its overproduction has been correlated with less sensitivity [this change is, however, not sufficient *per se* and must be combined with other modifications of the membrane; two genes downstream to OprH (PhoP and PhoQ) coregulate OprH and polymyxin B resistance]. Although still exceptional, resistance to polymyxins and colistins has now been described in strains exhibiting multiple resistance to ß-lactams and aminoglycosides.[59]

Pharmacodynamics

Colistin A and polymyxin B show concentration-dependent activity but no or little post-antibiotic effect (rapid regrowth after the concentration falls below the MIC), justifying the administration of repeated daily doses.[60]

Nonantibiotic pharmacologic and toxicologic properties related to chemical structure

As membrane-disrupting and lipid-binding agents, polymyxins and colistins display a number of non-antibiotic effects, some of which are potentially useful [inactivation of endotoxins (immobilized polymyxin B is currently used to remove endoxins from protein solutions), and synergy with serum bactericidal activities], but many others are highly detrimental to the host [activation of the alternate pathway of complement, mast cell degranulation with histamine release, decreased production of cytokines (but increased TNF-a release), increase in membrane conductance in epithelia, apoptosis].

Future developments

Because of the widespread emergence of resistance to other antimicrobials, polymyxins are being re-evaluated for chronic, difficult-to-treat infections (e.g. pulmonary infections in cystic fibrosis) and new, potentially less toxic derivatives are therefore being synthesized and evaluated.[61] The use of polymyxin B as an anti-endotoxin agent is also being investigated.[62]

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Chapter 189 - Mechanisms of Antibacterial Resistance

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INTRODUCTION

Although treatment of infections is often initiated empirically, the determination of bacterial susceptibility to an antimicrobial agent is an essential test in clinical microbiology because of widespread resistance to all classes of antimicrobial agents. Standardized methods for the determination of susceptibility and resistance have been formulated.[1] Bacterial isolates that are considered resistant to an antibiotic by these methods usually cannot be treated by this antibiotic, although the successful clinical outcome for isolates deemed susceptible is not guaranteed.

There is a strong correlation between the presence of some determinants of bacterial resistance and the outcome of antimicrobial therapy. The presence of a ß-lactamase in *Neisseria gonorrhoeae* strongly correlates with penicillin treatment failure. The presence of the *mecA* gene in *Staphylococcus aureus* is highly predictive for treatment failure with oxacillin, and in fact oxacillin-resistant *S. aureus* (usually called methicillin-resistant *S. aureus* (MRSA) because of their resistance to the oxacillin analog methicillin) are by definition also considered resistant to all other ß-lactam antibiotics.

However, the presence of a resistance gene is not equivalent to treatment failure. The gene should also be expressed in sufficient levels to lead to phenotypic resistance, and expression may differ depending on culture conditions or site of infection. For example ß-lactamase production is common among Enterobacteriaceae but resistance to penicillins depends on the mode and amount of expression.

Antibiotic resistance can be divided into six basic groups depending on the mechanism involved:

- the presence of an enzyme that inactivates the antibiotic;
- the presence of an alternative enzyme for that inhibited by the antibiotic;
- mutation in the target, which reduces binding of the antibiotic to the target;
- modification of the target, which reduces binding of the antibiotic to the target;
- reduced uptake of the antibiotic; and
- active efflux of the antibiotic.

The genetic determinants for resistance against antimicrobial agents can be located on the bacterial chromosome or on plasmids. The analysis of resistance genes and their distribution through the use of modern DNA techniques has provided new insights into the mechanisms of resistance and the spread of resistance genes through hospitals and the community.

RESISTANCE TO ß-LACTAM ANTIBIOTICS

Penicillin is the oldest ß-lactam antibiotic; since its introduction we have witnessed the development of a whole array of ß-lactam-based antibiotics, such as the first-generation cephalosporins through to the fourth-generation cephalosporins, carbapenems and monobactams. Almost immediately after the introduction of penicillin, resistance was observed in staphylococci. The ß-lactam antibiotics interfere with cell wall synthesis by binding to the enzymes involved in the process. These enzymes are called penicillin-binding proteins (PBPs). Resistance to ß-lactams is mainly caused by either the presence of ß-lactamases, which destroy the lactam ring, or the presence of altered PBPs, which are not inhibited by these antibiotics. In Gram-positive bacteria the ß-lactamase is excreted into the environment, and in Gram-negative bacteria the ß-lactamase is excreted in the periplasm. Whether the ß-lactam antibiotic is effective against the bacterium depends on a number of factors (Fig. 189.1), including:[2]

- the concentration of the antibiotic in the environment;
- the rate of entry through the outer membrane (in the case of Gram-negative bacteria);
- the amount of ß-lactamase;
- the hydrolysis rate for the antibiotic by the ß-lactamase; and
- the affinity of the PBPs for the antibiotic.

The number of ß-lactamases has steadily risen since the introduction of penicillin. The ß-lactamases have been classified according to their functional aspects. [3] This system is based on hydrolysis rates for a number of substrates and the level of inhibition by clavulanic acid, but simple point mutations may alter the classification. They have also been classified according to the nucleotide sequences that encode ß-lactamase.[4] Classes A, B and D have a serine at their active site, whereas class B has a zinc atom at the active site. Class A enzymes are encoded mostly on plasmids, whereas class C enzymes are generally chromosomally encoded. Class A enzymes are generally constitutively expressed. Class C enzyme genes are present in almost all Gram-negative bacilli, except *Salmonella* spp., but their presence (e.g. in *Escherichia coli*) does not necessarily lead to resistance. Class C enzymes are usually inducible and a total of four genes are required for expression of ß-lactamase activity. *Escherichia coli* possesses the *ampC, ampD* and *ampG* genes, but lacks the *ampR* gene. Why *E. coli* possesses three of these genes and lacks the fourth is unclear. Class D is a limited group of enzymes able to hydrolyze oxacillin; they are related to class C enzymes. Class B is of increasing importance because many act as carbapenemases. The ß-lactamases range between 30 and 40kDa in size.

The commonest ß-lactamases in Enterobactericeae are TEM-1, TEM-2 and SHV-1 (TEM are the first three letters of the patient from which the isolate came that harbored the first TEM ß-lactamase; SHV stands for sulfhydryl variable). These are simple penicillinases and their activity can be inhibited by compounds such as clavulanic acid and tazobactam, thereby rendering penicillin derivatives active again. However, TEM and SHV enzymes can easily obtain a broader spectrum through mutations, which may lead to resistance against third-generation cephalosporins. Inactivation of aztreonam, ceftazidime, cefotaxime or ceftriaxone is considered an indication for the presence of such an extended-spectrum ß-lactamase (ESBL). However, these antibiotics can also be inactivated by the overproduction of ampC. True ESBLs are determined by their inhibition with clavulanic acid.

Resistance to third-generation cephalosporins was first described in 1983 and was mediated by a plasmid encoding for a TEM-related

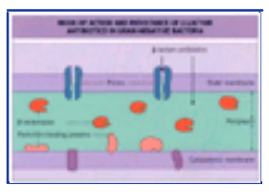

Figure 189-1 Mode of action and resistance of ß-lactam antibiotics in Gram-negative bacteria.

ß-lactamase. The majority of ESBLs are found in *Klebsiella pneumoniae* isolates. Extended spectrum ß-lactamases are encoded by plasmids, and these are highly transmissible. More than 60 TEM-type ESBLs have been described (Table 189.1). [5] In addition, more than 30 SHV-type ESBLs are known. Besides these common ESBL-types, some ESBLs have been described that do not belong to these two classes. Some plasmid-encoded cephalosporinases, which are also called

cephamycinases, have properties similar to ampC but are produced constitutively. Spread of these genes through the hospital or the community may further endanger the use of cephalosporins.

Another group is formed by the carbapenemases. These enzymes are encoded chromosomally and inactivate the highly active carbapenems, imipenem and meropenem, but resistance is still rare.

Altered PBPs are also a major reason for resistance against ß-lactam antibiotics. An altered PBP is involved in methicillin resistance in staphylococci. Both MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are important causes of nosocomial infections. These infections are difficult to treat because both MRSA and MRSE are generally multiresistant and are susceptible to only a limited number of antibiotics.

This PBP2a is encoded by the *mecA* gene. Regulation of methicillin is complex. Expression can be heterogeneous and only a few cells express the phenotype, although all cells are genotypically identical and possess the *mecA* gene. Through mutation the resistance phenotype may become homogeneous. Expression is also influenced by the plasmid-encoded ß-lactamase regulatory (blaR) systems blaR1 and blaI inducer-repressor system, which interacts with the mec-associated mecR1 and mecI system.[6] The *mec* determinant appears to originate in coagulase-negative staphylococci, which have a much higher prevalence of the gene, and horizontal transfer appears to take place on a regular basis. The *mecA* gene appears to be located on a transposon, but the genetic environment of the *mecA* gene may vary considerably among different strains of staphylococci, although there is a core region and some specific genetic determinants are frequently associated with the presence of *mecA* (Fig. 189.2).[7] Besides the presence of *mecA*, some methicillin-resistant strains are overproducers of ß-lactamases.[8] [9]

Resistance to penicillin in *Streptococcus pneumoniae* is also due to the presence of altered PBPs, and this mechanism can be responsible for chromosomally mediated penicillin resistance in *N. gonorrhoeae*.

RESISTANCE TO AMINOGLYCOSIDES

The first clinically effective aminoglycoside was streptomycin, which was isolated from *Streptomyces griseus* and first described in 1944. Numerous aminoglycosides have been isolated from species belonging to the genera *Streptomyces*, *Micromonospora*, *Bacillus* and *Pseudomonas*. Also, synthetic derivatives were produced, such as amikacin. Clinically, the most important aminoglycoside antibiotics are gentamicin, tobramycin, amikacin and streptomycin. They have a broad antimicrobial spectrum and are effective against both Gram-positive and Gram-negative organisms. However, they are not effective against anaerobes. Aminoglycosides bind to the ribosomes and thus interfere with protein synthesis (see Chapter 188).

Aminoglycosides enter the bacterial cell in several phases. In the first phase the aminoglycosides bind to anionic sites on the cell and, after binding, diffuse through outer membrane proteins. In *Pseudomonas aeruginosa* the entry is enhanced; the lipopolysaccharides of *P. aeruginosa* are rich in phosphate groups to which the aminoglycosides bind. This binding displaces magnesium ions, thus allowing entry of the aminoglycoside. The second and third phases are energy dependent and transport the aminoglycoside molecules across the cytoplasmic membrane. The lack of activity of aminoglycosides against anaerobes is explained by the fact that the transport of aminoglycosides across the cytoplasmic membrane depends on aerobic respiration.

Inactivation of aminoglycosides is the major mechanism of resistance against these antibiotics, but ribosomal modification and reduced permeability may also lead to resistance. The enzymes that are responsible for inactivation belong to three classes, depending on the type of modification that causes inactivation: phosphotransferases (APH), acetyltransferases (AAC) and nucleotidyltransferases (ANT). Each class is subdivided on the basis of the site of modification on the substrate and substrate specificity (Table 189.2). Often these enzymes are able to modify several closely related antibiotics, which is not surprising in view of their chemical similarity. The enzymes have to inactivate their targets before they reach the ribosomes and they appear to be either located inside the cell or associated with the inside or the outside of the cytoplasmic membrane.[10]

Resistance to aminoglycoside antibiotics is widespread and of clinical importance. It is observed in both Gram-negative and Gram-positive bacteria. Studies concerning the evolution of resistance using nucleotide sequencing clearly show that, at least within enzyme classes, the genes are related. For example, immunologic and hybridization tests have shown that the APH(3') enzymes from streptococci and staphylococci are closely related, as are those enzymes on the Gram-negative transposons (Tn), Tn*5* and Tn*903*. However, there is far less homology between enzymes from Gram-negative bacteria compared to enzymes from Gram-positive bacteria. Nevertheless, the genes from Gram-negative bacteria appear to be more closely related to a gene of *Streptomyces fradiae*. In fact it was concluded that it is likely that gene transfer had taken place between these species. Aminoglycoside resistance genes are believed to have originated from genes involved in the production of aminoglycosides in aminoglycoside-producing species.[11]

Besides the transfer of genes between bacteria and aminoglycoside-producing species, transfer between Gram-positive and Gram-negative species has been documented. [12] It should be noted, however, that transfer of a functional resistance gene from one species or even within one species does not necessarily lead to the expression of resistance.

Aminoglycoside resistance in staphylococci is well documented. [13] Up to six genes have been identified. Often more than one resistance gene is present. One of the most remarkable aminoglycoside enzymes is the bifunctional AAC(6')APH(2") enzyme, which is found on Tn*4001* of *S. aureus* and in *Enterococcus faecalis* isolates. Nucleotide sequencing data suggest that the enzyme arose through the fusion of two genes, each encoding one of the partners. The epidemiology of Tn*4001* is well studied and illustrates that substitutions, insertions

TABLE 189-1 -- Characterization of the first 52 TEM ß-lactamases.

CHARACTERIZATION OF TEM ß-LACTAMASES																	
ß-lactamase	**Amino acid at position**																
	21	**39**	**42**	**69**	**104**	**153**	**164**	**165**	**182**	**237**	**238**	**240**	**244**	**265**	**268**	**275**	**276**
TEM-1	L	Q	A	M	E	H	R	W	M	A	G	E	R	T	S	R	N
TEM-2		K															
TEM-3		K			K						S						
TEM-4	F				K						S			M			
TEM-5							S			T		K					
TEM-6					K		H										
TEM-7		K					S										
TEM-8		K			K		S				S						
TEM-9	F				K		S							M			
TEM-10							S					K					
TEM-11		K					H										
TEM-12							S										
TEM-13		K												M			
TEM-15					K						S						
TEM-16		K			K		H										
TEM-19											S						
TEM-20									T		S						
TEM-21		K			K	R					S						

TEM-22		K			K					G	S						
TEM-24		K			K		S			T		K					
TEM-25	F										S			M			
TEM-26					K		S										
TEM-27							H					K		M			
TEM-28							H					K					
TEM-29							H										
TEM-30													S				
TEM-31													C				
TEM-32				I					T								
TEM-33				L													
TEM-34				V													
TEM-35				L													D
TEM-36				V													D
TEM-37				I													D
TEM-38				V												L	
TEM-39				L				R									D
TEM-40				I													
TEM-41													T				
TEM-42		K	V								S	K		M			
TEM-43					K		H		T								
TEM-44		K											S				
TEM-45				L												Q	
TEM-46		K			K		S					K					
TEM-47											S	K		M			
TEM-48		F									S	K		M			
TEM-49		F									S	K		M	G		
TEM-50				L	K						S						D
TEM-51													H				
TEM-52					K				T		S						

A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, Leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan.

Figure 189-2 *mecA* region of different staphylococcal strains. IS, insertin sequence; mecA, methicillin-resistant gene; R1, regulatory element; Tn, transposon; vertical lines, position on restriction enzyme cleavage sites.

and deletions play an important role in the adaptation of these elements to new hosts.

Hybridization studies in Australia showed that Tn*4001* could be present on the bacterial chromosome as well as on a number of structurally related plasmids, and this was linked to the rapid spread of gentamicin- and tobramycin-resistant *S. aureus* in Australia. The same transposon was also observed in the USA but with shorter inverted repeats and insertion sequences flanking the resistance gene.

The presence of multiple genes is also well known for other species, especially Gram-negative organisms, but the myriad aminoglycoside resistance genes makes it difficult to study the extent of this problem.

Initially, only resistance against the naturally occuring aminoglycosides was observed and only APH(3')-II and APH(3')-III were capable of modifying amikacin in vitro, but the introduction of this antibiotic quickly changed this. In the San Juan Veterans Administration Medical Center, the prevalence of amikacin resistance increased from 0.2 to 3.6% among aerobic Gram-negative bacilli in 4 years through the use of amikacin as a first-line antibiotic. Analysis of plasmids obtained from amikacin-resistant *Serratia marcescens* and *K. pneumoniae* showed that these were almost identical. This suggested that the plasmid disseminated through the hospital and that this dissemination occurred in a relatively short time span.[14]

Resistance to streptomycin is often caused by mutation of the S12 protein in the small ribosomal subunit (30S subunit).

RESISTANCE TO MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS

Macrolide, lincosamide and streptogramin (MLS) antibiotics are chemically distinct inhibitors of bacterial protein synthesis. Macrolides are composed of a minimum of two amino sugars or neutral sugars attached to a lactone ring of variable size. Macrolides can be subdivided according the chemical structure of their lactone ring (into 14-membered, 15-membered or 16-membered lactone ring macrolides). These classes differ in their pharmacokinetic properties and in their responses to bacterial resistance mechanisms. Lincosamides are alkyl derivatives of proline and are devoid of a lactone ring. Streptogramin antibiotics are mixtures of naturally occurring cyclic peptide compounds. They are composed of two factors, A and B (e.g. pristinamycin II and I, virginiamycin M and S), with synergistic inhibitory and bactericidal activities[15] [16] (see Chapter 194).

Intrinsic resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in Gram-negative bacilli is due to low permeability of the outer membrane to these hydrophobic compounds.

Three different mechanisms of acquired MLS resistance have been described in Gram-positive bacteria. First, target modification alters a site in 23S rRNA that is common to the binding of MLS_B antibiotics. Modification of the ribosomal target confers cross-resistance to MLS_B antibiotics (MLS_B -resistant phenotype) and remains the most frequent mechanism of resistance, although enzymatic modification of the antibiotics and active efflux appear to be increasingly prevalent. [15] [17] [18]

Target modifications

Macrolide, lincosamide and streptogramin antibiotics bind to 50S ribosomal subunits and inhibit elongation of peptide chains. Resistance to MLS_B antibiotics is mostly

due to acquisition of erythromycin resistance methylase (*erm*) genes that encode enzymes that N^6 -dimethylate an adenine residue of 23S rRNA. The precise site of methylation has been located in a highly conserved region of the

TABLE 189-2 -- Characteristics of aminoglycoside-modifying enzymes.

CHARACTERISITICS OF AMINOGLYCOSIDE-MODIFYING ENZYMES			
Enzyme subclass	**Number of different genes described that encode enzymes capable of the same aminoglycoside modification**	**Modification**	**Distribution**
AAC(1)	1		
AAC(3)-I	2	Gentamicin	Gram-negative bacteria
AAC(3)-II	3	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-III	3	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-IV	1	Gentamicin, tobramycin	Gram-negative bacteria
AAC(3)-VI	1	Gentamicin	Gram-negative bacteria
AAC(3)-VII	1		Fungi
AAC(3)-VIII	1		Fungi
AAC(3)-IX	1		Fungi
AAC(3)-X	1		Fungi
AAC(6')-I	9	Amikacin	Gram-negative and Gram-positive bacteria
AAC(6')-II	2	Gentamicin, tobramycin	Gram-negative bacteria
AAC(6')-III	1		Gram-negative bacteria
AAC(6')-APH(2")	1	Amikacin, gentamicin, tobramycin	Gram-positive bacteria
AAC(2')I	1	Gentamicin, tobramycin	Gram-negative bacteria
ANT(2")-I	3	Gentamicin, tobramycin	Gram-negative bacteria
ANT(3")-I	1	Streptomycin	Gram-negative bacteria
ANT(4')-I	1	Amikacin, tobramycin	Gram-positive bacteria
ANT(4")-I	1	Amikacin, tobramycin	Gram-negative bacteria
ANT(6)-I	1	Streptomycin	Gram-positive bacteria
ANT(9)-I	1		Gram-positive bacteria
APH(3')-I	3		Gram-negative bacteria
APH(3')-II	1		Gram-negative bacteria
APH(3')-III	1	Amikacin	Gram-positive bacteria
APH(3')-IV	1		Gram-positive bacteria
APH(3')-V	3		Fungi
APH(3')-VI	2	Amikacin	Gram-negative bacteria
APH(3')-VII	1		Gram-negative bacteria
APH(3")-I	2	Streptomycin	Fungi
APH(6)-I	4	Streptomycin	Fungi
APH(4)-I	2		Gram-negative bacteria, fungi
Only the clinically relevant antibiotics are listed. AAC, acetyltransferase; ANT, nucleotidyltansferase; APH, phosphotransferase.			

rRNA. Nucleotide alterations in 23S rRNA, both mutational and post-transcriptional, cluster in the peptidyltransferase region in 23S rRNS domain V, providing a physical basis and a common location for MLS_B antibiotic sites of action. Methylation of rRNA probably leads to a conformational change in the ribosome that results in decreased affinity and leads to co-resistance to all MLS_B antibiotics. This suggests that the binding sites for these drugs overlap or at least functionally interact. Streptogramin A-type antibiotics are unaffected, and synergy between the two components of streptogramin against MLS-resistant strains is maintained.

A sequence comparison of *erm* genes from various bacterial species and results of hybridization experiments under stringent conditions led to the recognition of at least nine classes of resistance determinants (Table 189.3). Because a number of these genes cross-hybridize, clinical isolates can be assigned to one of four hybridization classes: *ermA, ermC, ermAC* and *ermF*. This gene distribution is relatively species specific. The amino acid sequences of the methylases encoded by these determinants are related, indicating that the *erm* genes are derived from a common ancestor, possibly belonging to an antibiotic producer. However, various degrees of similarity among the enzymes can be observed.

Expression of MLS_B resistance can be constitutive or inducible. The character of resistance is not related to the class of *erm* determinant but, rather, depends on the sequence of the regulatory region upstream from the structural gene for the methylase. Regulation by these regions occurs by a translational attenuation mechanism in which mRNA secondary structure influences the level of translation. In laboratory mutants and clinical isolates, single nucleotide changes, deletions or duplications in the regulatory region convert inducibly resistant strains to constitutively resistant ones that are cross-resistant to MLS_B antibiotics.

Expression of MLS resistance in staphylococci may be constitutive or inducible. When expression is constitutive, the strains are resistant to all MLS_B antibiotics. Streptogramin A-type antibiotics escape

TABLE 189-3 -- Distribution of *erm* genes in clinically important bacterial species.*

DISTRIBUTION OF *erm* GENES IN CLINICALLY IMPORTANT BACTERIAL SPECIES		
Hybridization class	**Gene**	**Host**
ermA	*ermA*	*Staphylococcus aureus*
		Coagulase-negative staphylococci

ermAM	*ermP*	*Clostridium perfringens*
	ermZ	*Clostridium difficile*
		Enterococcus faecalis
	ermBC	*Escherichia coli*
		Lactobacillus reuteri
	ermAM	*Streptococcus sanguis*
		Streptococcus pneumoniae
		Streptococcus agalactiae
		Streptococcus pyogenes
ermC	*ermB*	*Staphylococcus aureus*
		Bacillus subtilis
		Lactobacillus spp.
	ermC	*Staphylococcus aureus*
		Coagulase-negative staphylococci
	ermM	*Staphylococcus epidermidis*
ermF	*ermF*	*Bacteroides fragilis*
		Bacteroides ovatus

** Data from Leclercq and Courvalin.*[15]

resistance, and synergy with streptogramin B-type antibiotics is retained. When expression is inducible, the strains are resistant to 14- and 15-membered macrolides only. The 16-membered macrolides, the commercially available lincosamides and the streptogramin antibiotics remain active. This dissociated resistance is due to differences in the inducing abilities of MLS antibiotics; only 14- and 15-membered macrolides are effective inducers of methylase synthesis in staphylococci.

Resistance to MLS antibiotics in streptococci can also be expressed constitutively or inducibly. However, unlike the situation with staphylococci, various macrolides or lincosamides may act as inducers to various degrees. Thus, in streptococci, whether inducible or constitutive, ribosomal methylation leads to cross-resistance among macrolides, lincosamides and streptogramin B antibiotics.

In addition, alterations in ribosomal protein L4 account for resistance in pneumococcal strains selected in vitro by macrolide passage. The presence of alterations in the L4 ribosomal protein is consistent with the interpretation that this protein is in contact with or near the peptidyltransferase region in domain V of 23S rRNA. Thus, this alteration may act indirectly to alter 23S rRNA confirmation. [19] In some cases, these modifications also reduce the in-vitro activities of ketolides, derivatives of macrolides, which were designed in order to act against macrolide-resistant micro-organisms.

Antibiotic inactivation

Unlike target modification, which causes resistance to structurally distinct antibiotics, enzymatic inactivation confers resistance only to structurally related drugs.[15] [17] [18] [19]

Enzymes (ErmA and ErmB) that hydrolyze the lactone ring of the macrocyclic nucleus and phosphotransferases (type I (*mphA*) and type II) that inactivate macrolides by introducing a phosphate on the 2'-hydroxyl group of the amino sugar have been reported in members of the family Enterobacteriaceae and in *S. aureus*. The gene *linA* mediates resistance to lincosamides. The product of *linA* has been partially purified and demonstrated to act as a lincosamide O-nucleotidyl transferase. Lactonases that are capable of cleaving the macrocyclic lactone ring structure of type B streptogramins have been identified in staphylococci (*vgb* gene). Two staphylococcal-related determinants, *vat* and vat_B , encoding an acetyltransferase that inactivates type A streptogramins, have been characterized. The *vat* and *vgb* genes are adjacent to each other on plasmid pIP630. This vat-vgb region is flanked by inverted copies of the insertion sequence IS257, suggesting a role for this element in dissemination of these determinants.

Active efflux

The presence of multicomponent macrolide efflux pumps in staphylococci (msrA, msrB) and *N. gonorrhoeae* (mtr), as well as an efflux system in streptococci (mefA, mefE), has also been documented.[20] [21] [22] [23] *msr* genes confer resistance only to 14- and 15-membered ring macrolides. Recent epidemiologic surveys have shown that some erythromycin-resistant strains of pneumococci and group A streptococci have been shown to have the M phenotype, namely resistance to macrolides but susceptibility to lincosamide and streptogramin B antibiotics. These strains contain the *mefA* or *mefE* gene coding for an efflux pump for 14- and 15-membered macrolides. The presence of a plasmid-mediated gene, *vga*, encoding for a putative ATP-binding protein, has been associated with an active efflux of streptogramin A group compounds. An overview on macrolide resistance genes was recently published.[19]

RESISTANCE TO FLUOROQUINOLONES

Fluoroquinolone antibiotics exert their antibacterial effects by inhibition of certain bacterial topoisomerase enzymes, namely DNA gyrase (bacterial topoisomerase II) and topoisomerase IV.[13] [23] [24] [25] [26] These essential bacterial enzymes alter the topology of double-stranded (ds) DNA within the cell. In most bacteria, the chromosome exists as a single circle of dsDNA, which is maintained in a highly negatively supercoiled state. This energetically activated form is required for critical cellular processes such as replication and transcription.

Deoxyribonucleic acid gyrase and topoisomerase IV are heterotetrameric proteins composed of two subunits, designated A and B. The genes encoding the A and B subunits are referred to as *gyrA* and *gyrB* (DNA gyrase) or *parC* and *parE* (DNA topoisomerase IV (*grlA* and *grlB* in *S. aureus*)).

Deoxyribonucleic acid gyrase is the only enzyme that can effect supercoiling of DNA. Inhibition of this activity by fluoroquinolones is associated with rapid killing of the bacterial cell. Topoisomerase IV also modifies the topology of dsDNA, but whereas DNA gyrase seems to be important for maintenance of supercoiling, topoisomerase IV is predominantly responsible for separation of daughter DNA strands during cell division.

In Gram-negative organisms, DNA gyrase is the primary target for quinolones, whereas topoisomerase IV appears to be the primary target in *Staphylococcus aureus* and *Streptococcus pneumoniae*. In Gram-positive species, mutations in genes encoding topoisomerase IV appear to precede mutations in DNA gyrase. Nevertheless, in *S. pneumoniae* it has been shown that different quinolones can have different primary targets in the same bacterial species (i.e. quinolone structure determines the mode of antibacterial action). Thus, the primary target seems to be dependent on the bacterial species as well as on the quinolone structure.

Target modification

Alterations of the target enzymes appear to be the most dominant factors in expression of resistance to quinolones.[13] [23] [24] [25] [26] [27] Many Gramnegative fluoroquinolone-resistant organisms contain a *gyrA* mutation, resulting in inhibition of supercoiling of DNA and elevated minimum inhibitory concentrations (MICs; Table 189.4). The first molecular characterization of a quinolone resistance mutation in *gyrA* was reported

TABLE 189-4 -- Alterations in DNA gyrase subunit A conferring quinolone resistance.*

ALTERATIONS IN DNA GYRASE SUBUNIT A CONFERRING QUINOLONE RESISTANCE	
Organism	**Amino acid substitution**
Acinetobacter baumanni	Gly 81 ? Val
	Ser 83 ? Leu
Aeromonas salmonicida	Ser 83 ? Ile
	Ser 83 ? Ile
	Ala 67 ? Gly
Coxiella burnetti	Glu 87 ? Gly
Campylobacter jejuni	Ala 70 ? Thr
Campylobacter lari	Thr 86 ? Ile
	Asp 90 ? Ala, Asn
	Ser 83 ? Arg
	Glu 87 ? Lys, Gly
	Thr 86 ? Ile
	Pro 104 ? Ser
Enterobacter cloacae	Ser 83 ? Leu
Escherichia coli	Ala 67 ? Ser
	Gly 81 ? Cys, Asp
	Ser 83 ? Leu, Trp, Ala
	Ala 84 ? Pro
	Asp 87 ? Asn, Val, Thr, Gly, His
Enterococcus faecalis	Ser 83 ? Ile
	Gln 106 ? His, Arg
Helicobacter pylori	Asn 87 ? Lys
	Ala 88 ? Val
	Asp 91 ? Gly, Asn, Tyr
	Asp 91 ? Asn
	Ala 97 ? Val
Mycobacterium avium	Ala 90 ? Val
Mycobacterium smegmatis	Ala 90 ? Val
	Asp 94 ? Gly
Mycobacterium tuberculosis	Gly 88 ? Cys
	Ala 90 ? Val
	Ala 90 ? Val
	Ser 91 ? Pro
	Asp 94 ? Asn, His, Gly, Tyr, Ala
Neisseria gonorrhoeae	Ser 83 ? Phe
	Ser 83 ? Phe
	Asp 87 ? Asn
Pseudomonas aeruginosa	Thr 83 ? Ile
	Asp 87 ? Tyr, Asn, Gly, His
Shigella dysenteriae	Ser 83 ? Leu
Salmonella typhi	Ser 83 ? Phe
Salmonella typhimurium	Ser 83 ? Phe, Tyr
	Asp 87 ? Gly, Tyr, Asn
	Ala 119 ? Glu
	Ala 67 ? Pro
	Gly 81 ? Ser
	Ser 83 ? Ala
	Asp 87 ? Asn
Staphylococcus aureus	Ser 84 ? Leu, Ala, Phe
	Ser 85 ? Pro
	Glu 88 ? Lys, Gly
Staphylococcus epidermidis	Ser 84 ? Phe
The Ser 83 to Phe substitution for *Neisseria gonorrhoeae* is based on *Escherichia coli* sequence.	

** Adapted from Everett and Piddock.*[24]

in 1988 from *E. coli*. The mutations found were situated in a relatively hydrophilic region of the polypeptide and close to a tyrosine residue at amino acid 122 at the active site, which has been shown to be the site covalently bound to the DNA. The small region from codon 67 to 106 was designated the quinolone resistance determining region (QRDR). In almost all instances, amino acid substitutions within the QRDR involve the replacement of a hydroxl group with a bulky hydrophobic residue. This suggests that mutations in *gyrA* induce changes in the binding site conformation or charge (or both) that may be important for interactions between quinolones and DNA gyrase.

Although quinolones are thought to interact primarily with the A subunit of DNA gyrase, there are mutations in the B subunit that also confer quinolone resistance in some species, such as *E. coli*. However, the frequency of *gyrB* mutations has been shown to be relatively low compared with the frequency of *gyrA* mutations in clinical isolates of *E. coli* and other Gram-negative organisms. Until now, no *gyrB* mutations have been reported as resulting in cross-resistance between quinolones and the B subunit inhibitors coumermycin and novobiocin. This is consistent with evidence that suggests that the GyrB protein comprises two distinct domains: an *N*-terminal domain containing the sites for hydroloysis of adenosine triphosphate and binding of coumermycin, and a *C*-terminal domain containing the QRDR of GyrB.

Topoisomerase IV is a secondary target for fluoroquinolone action in *E. coli* in the absence of a sensitive DNA gyrase. Mutations in *parC* result in further decreased susceptibility. These mutations in *parC* have been shown to occur at Ser80 and Glu84, which are analogous to codons Ser83 and Asp87 of *E. coli gyrA*, and to be common in fluoroquinolone-resistant clinical isolates of *E. coli*. A mutation has also been reported in the *parE* gene that results in decreased fluoroquinolone susceptibility. However, as in the case with *gyrB*, such mutations appear to be rare in clinical isolates.

In *Staphylococcus aureus*, topoisomerase IV is the primary target of fluoroquinolones. Strains with mutations in *gyrA* and *gyrB* without *grlA* mutations resulting in high-level fluoroquinolone resistance can be isolated by single-step selection with fluoroquinolones in *E. coli* but not in *S. aureus*. Previously, 116 clonally unrelated *S. aureus* isolates originating from nine different countries were screened for mutations in the *gyr* and *grl* gene loci.[27] In correlating the characterized mutations to the resulting MIC of ciprofloxacin, it is clear that all studied isolates without the *grlA* mutation at position Ser80 were susceptible to ciprofloxacin. All ciprofloxacin-resistant isolates had the *grlA* mutation Ser80 in combination with either a Ser84 mutation or a Glu88 mutation within the *gyrA* gene. In two isolates a Ser80 to Phe mutation was combined with no mutations in the *gyrA* gene, resulting in a MIC value for ciprofloxacin of 2µg/ml, which, although elevated from a wild-type level, is still below the breakpoint for resistance. These data support the finding that in *S. aureus*, *grlA* mutations precede *gyrA* mutations in developing resistance to ciprofloxacin. Combinations of single point mutations within the *gyrA* gene from various species have been shown to be associated with higher MIC values for ciprofloxacin than single point mutations. Similarly, two combinations of single point mutations within *grlA*, of a Glu84 to Val mutation or a Ala48 to Thr mutation in combination with a Ser80 to Phe mutation, were associated with relatively higher ciprofloxacin MIC values (64–256µg/ml) than only a single Ser80 to Phe mutation (8–64µg/ml). Sequence data show that some *grlA* and *gyrA* mutations are conserved in both MRSA and methicillin-sensitive *S. aureus* from unrelated clones of *S. aureus* isolated from different countries.

Topoisomerase IV mutations have now been characterized in several other organisms. In *Streptococcus pneumoniae* topoisomerase IV also appears to be the primary target for fluoroquinolone action. Ciprofloxacin-resistant mutants of *S. pneumoniae* were

generated by stepwise selection at increasing drug concentrations. First-step mutants exhibiting low-level resistance had no detectable changes in their topoisomerases QRDR, suggesting altered permeation or another novel resistance mechanism. Second step mutants exhibited an alteration in ParC at Ser79 to Tyr or Ser79 to Phe or at Ala84 to Thr. Third and fourth step mutants displaying high-level ciprofloxacin resistance were found to have, in addition to ParC alteration, a change in GyrA at residues equivalent to *E. coli* GyrA resistance hot spots Ser83 and Asp87 or in GyrB at Asp435 to Asn, equivalent to *E. coli* Asp426. ParC mutations preceded those in GyrA, suggesting that topoisomerase IV is the primary topoisomerase target and gyrase the secondary target for ciprofloxacin in *S. pneumoniae*. Additionally, it has been shown that in *S. pneumoniae* different quinolones can have different primary targets. The targeting of DNA gyrase by sparfloxacin in *S. pneumoniae* but of topoisomerase IV by ciprofloxacin indicates that target preference can be altered by change in quinolone structure.

Decreased uptake

Deoxyribose nucleic acid gyrase and topoisomerase IV are both located in the cytoplasm of the bacterial cell. In order to reach their targets, fluoroquinolone antibiotics must traverse the cell envelope. In Gram-positive bacteria this consists of the cell wall and a single membrane, whereas in Gram-negative bacteria the fluoroquinolone must first cross the outer membrane. Changes in the cell envelope of Gram-negative bacteria, particularly in the outer membrane, have been associated with decreased uptake and increased resistance to fluoroquinolones.[23] [24] [25] [26] Some of these changes may be due to the effect of quinolones or *gyrA* mutations, or both, on differential expression of outer membrane proteins, because it has been shown that *gyrA*-mediated changes in supercoiling of DNA can affect the expression of porin genes. In contrast, decreased uptake has not been demonstrated to be a mechanism of resistance in Gram-positive bacteria.

Active efflux

Increased efflux as a mechanism of fluoroquinolone resistance has been reported in fluoroquinolone-resistant *Staphylococcus aureus*. The *norA* gene encodes the multidrug efflux pump NorA.[28] The NorA protein has a hydropathic amino acid profile consistent with a location in the cytoplasmic membrane and it exhibits a low level of fluoroquinolone efflux, with a preference for hydrophilic fluoroquinolones. Efflux is an active process and can be inhibited by protonophores. NorA-mediated fluoroquinolone resistance is due to overexpression of the wild-type gene *norA*. In *P. aeruginosa*, resistance to fluoroquinolones as well as to a number of other antimicrobial agents has often been associated with decreased accumulation and increased expression of outer membrane proteins, often with concomitant increase in cytoplasmic membrane proteins. Resistance is due to overexpression of one or more efflux systems (i.e. OprK) capable of removing fluoroquinolones and other antibiotic compounds. *Escherichia coli* has also been shown to possess efflux systems, notably EmrAB and AcrAB.[24]

MAR operon

Escherichia coli and a number of other organisms possess mechanisms that provide intrinsic protection against a wide range of chemically unrelated toxic substances, including quinolone antibiotics. Multiple antibiotic resistance (MAR) in *E. coli* has been shown to be the result of mutations in the *mar* locus of the *E. coli* chromosome. A homolog of the *mar* locus exists in other members of the Enterobacteriaceae as well as in other bacteria. In *E. coli*, the *mar* locus consists of two divergently expressed operons, marC and marRAB, both of which are required for full expression of the MAR phenotype. Expression of the MAR phenotype, whether by induction or mutation, protects the cells

TABLE 189-5 -- Location of the tetracycline-resistance determinants.*

LOCATION OF THE TETRACYCLINE-RESISTANCE DETERMINANTS	
Plasmid	**Chromosome**
TetA–E	TetB (rare)
TetX	-
TetG, TetH	-
TetK	TetK
TetL	TetL (rare)
TetM (rare)	TetM
TetO	TetO
TetP	-
-	TetQ
TetS	-
-	OtrA–C
Tetk can be associated with an integrated plasmid. Tet, tetracycline-resistance determinant; Otr, oxytetracycline-resistance determinant.	

** Data from Roberts.* [30]

from fluoroquinolone killing at up to four times the MIC. This may be more clinically important than the relatively modest increases in MIC associated with MAR because cells that escape death would have the potential to mutate to higher levels of fluoroquinolone resistance.[23] [24] [29]

RESISTANCE TO TETRACYCLINES

Tetracyclines probably penetrate bacterial cells by passive diffusion. Tetracycline acts by reducing the affinity of the A and P sites of the 30S ribosomal subunit for aminoacyl transfer RNA, resulting in the inhibition of protein synthesis. [13] [23] [30] [31] [32] [33]

A growing number of bacterial species are acquiring resistance to the bacteriostatic activity of tetracycline. Until now, at least 16 tetracycline-resistance (Tet)

determinants and three oxytetracycline-resistance (Otr) determinants, first found in oxytetracyline-producing *Streptomyces* spp., have been described and characterized, with new Tet determinants being identified continually. Of these determinants, at least 13 are frequently associated with plasmids, whereas others are on the chromosome (Table 189.5). Resistance to tetracyclines is primarily due to acquisition of Tet determinants rather than to mutation of existing chromosomal genes.

The two widespread mechanisms of bacterial resistance do not destroy tetracycline: one is mediated by energy-dependent efflux pumps; and the other involves an elongation-factor G-like protein that confers ribosome protection. Both mechanisms are widespread among Gram-negative and Gram-positive bacteria (Table 189.6 and Table 189.7). Oxidative destruction of tetracycline has been found in a few species. Nevertheless, the enzymatic inactivation of the antibiotic is not thought to be important in nature. The classification of Tet determinants according to their mechanism of resistance is shown in Table 189.8 .

Reduced intracellular concentration of tetracycline

Because the ribosome is the target, antibiotic activity of tetracycline depends on the presence of the drug in the cytoplasm. A reduced tetracycline concentration in the cytoplasm can be achieved by two means:[13] [23] [30] [31] [32] [33]

- the permeability of the cell envelope may be lowered; or
- tetracycline may be pumped out of the cytoplasm in an energy-dependent fashion.

Bacteria differ in their cell wall composition, causing differences in permeability and hence insensitivity to antibiotics. The peptidoglycan

TABLE 189-6 -- Distribution of tetracycline-resistance determinants among Gram-negative bacteria.*

DISTRIBUTION OF TETRACYCLINE-RESISTANCE DETERMINANTS AMONG GRAM-NEGATIVE BACTERIA			
Efflux		**Ribosomal protection and/or efflux**	
Genus	**Tet determinant**	**Genus**	**Tet determinant**
Actinobacillus	TetB	*Bacteroides* (anaerobic spp.)	TetM, Q, X
Aeromonas	TetA, B, D, E	*Campylobacter*	TetO
Citrobacter	TetA, B, C, D	*Eikenella*	TetM
Edwardsiella	TetA, D	*Fusobacterium* (anaerobic spp.)	TetM
Enterobacter	TetB, C, D	*Haemophilus*	TetB, M
Escherichia	TetA, B, C, D, E	*Kingella*	TetM
Klebsiella	TetA, D	*Neisseria*	TetM
Moraxella	TetB	*Prevotella* (anaerobic spp.)	TetQ
Pasteurella	TetB, D, H	*Veillonella* (anaerobic spp.)	TetM
Plesiomonas	TetA, B, D		
Proteus	TetA, B, C		
Pseudomonas	TetA, C		
Salmonella	TetA, B, C, D, E		
Serratia	TetA, B, C		
Shigella	TetA, B, C, D		
Vibrio	TetA, B, C, D, E, G		
Yersinia	TetB		
Ribosomal-protection encoding genes have not yet been found in enteric genera, and when these genes are cloned into *Escherichia coli* the level of resistance to tetracycline conferred is relatively low. Tet, tetracycline-resistance determinant.			

* *Data from Roberts.*[30]

layer surrounding most Gram-positive bacteria does not reduce cytoplasmic accumulation of low molecular weight antibiotics such as tetracycline. In contrast, the outer membrane of Gram-negative bacteria is an effective permeability barrier for hydrophobic compounds. The effects of permeability barriers are usually supported by additional resistance mechanisms to achieve high-level resistance. Energy-dependent efflux of tetracycline causes high-level resistance in bacteria by itself. Two different types of efflux pumps are involved in tetracycline resistance: multidrug-resistance pumps and tetracycline-specific transporters. A multidrug-resistance pump belonging to the Acr family is responsible for the reduced tetracycline accumulation in *P. aeruginosa*. Furthermore, *E. coli* has a chromosomal multidrug resistance efflux system that is associated with the *mar* locus. Multidrug efflux pumps transport their substrate straight out of the cell into the surrounding medium. In contrast to the broad substrate range of multidrug transporters, many of the efflux pumps identified in Gram-positive and Gram-negative bacteria specifically transport tetracycline (see Table 189.8). The efflux proteins exchange a proton for a tetracycline-cation complex and are antiporter systems. Efflux determinants from Gram-negative bacteria (TetA-E, TetG-H) share a common genetic organization, which is different from the one in Gram-positive bacteria. Both Gram-negative and Gram-positive contain a structural and a repressor gene that are expressed in opposite directions from overlapping operator regions. The Gram-positive *tetK* and *tetL* genes encoding tetracycline-efflux proteins are regulated by mRNA attenuation in a similar way to that described for Gram-positive *erm* genes encoding rRNA methylase and *cat* genes encoding chloramphenical acetyltransferases.

Tetracycline-specific exporters pump their substrate into the periplasm and not across the outer membrane, as found for the multidrug efflux pumps.

Protection of the ribosome

Protection of the ribosome from the action of tetracycline as a mechanism of tetracycline resistance was discovered in streptococci. Tetracycline resistance can result from production of a protein that interacts with the ribosome such that protein synthesis is unaffected by the presence of the antibiotic. To date, six classes of Tet determinants that confer tetracycline resistance on the level of protein synthesis have been identified (see Table 189.8). Most of the work on the mechanism of ribosomal protection has been done on TetM. The ribosomal protection proteins encoded by the other classes have an amino acid sequence similarity of at least 40% to TetM. Therefore, the mechanism of action may be similar for all ribosomal protection proteins. TetM ribosomal protection protein resembles elongation factors (EFs) in three properties:

- it has amino acid sequence similarity to EF-G (which translocates the peptidyl transfer RNA during protein synthesis) and EF-Tu;
- it has a ribosome-dependent guanosine triphosphatase activity; and
- it seems to confer resistance by reversible binding to the ribosome.

However, to date, the biochemical basis of tetracycline resistance mediated by TetM remains unclear. One possibility is that TetM stabilizes the ribosome-transfer RNA interaction in the presence of tetracycline.[13] [23] [30] [31] [32] [33]

RESISTANCE TO CHLORAMPHENICOL

Chloramphenicol is a bacteriostatic antibiotic that binds to the 50S ribosomal subunit and inhibits the peptidyltransferase step in protein synthesis. Resistance to chloramphenicol is mostly due to inactivation of the antibiotic by a chloramphenicol acetyltransferase (CAT) enzyme that acetylates the antibiotic. Chloramphenicol resistance most commonly results from the acquisition of plasmids that encode CAT, but in certain Gram-negative bacteria decreased outer membrane permeability can confer resistance to chloramphenicol and structurally unrelated compounds.[13] [23]

Enzyme inactivation

Chloramphenicol contains two hydroxyl groups that are acetylated in a reaction catalyzed by CAT. Monoacetylated and diacetylated derivatives are unable to bind to the 50S ribosomal subunit to inhibit prokaryotic peptidyltransferase. Expression of the *cat* genes in *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecalis* is typically inducible, and expression appears to be regulated by translational attenuation in a similar manner to the *erm* genes

TABLE 189-7 -- Distribution of tetracycline-resistance determinants among Gram-positive bacteria.*

DISTRIBUTION OF TETRACYCLINE-RESISTANCE DETERMINANTS AMONG GRAM-POSITIVE BACTERIA	
Genus	**Tet determinant**
Actinomyces	TetL
Aerococcus	TetM, O
Bacillus	TetK, L
Clostridium (anaerobic spp.)	TetK, L, M, P
Corynebacterium	TetM
Enterococcus	TetK, L, M, O
Eubacterium	TetK, M
Gardnerella	TetM
Gernella	TetM
Lactobacillus	TetO
Listeria	TetK, L, M, S
Mobiluncus (anaerobic spp.)	TetO
Mycobacterium (acid-fast bacteria)	TetK, L, OtrA, B
Mycoplasma (cell-wall-free bacteria with a Gram-positive metabolism)	TetM
Peptostreptococcus (anaerobic spp.)	TetK, L, M, O
Staphylococcus	TetK, L, M, O
Streptococcus	TetK, L, M, O
Streptomyces (multicellular bacteria)	TetK, L, OtrA, B, C
Ureaplasma (cell-wall-free bacteria with a Gram-positive metabolism)	TetM
Tet, tetracycline-resistance determinant; Otr, oxytetracycline-resistance determinant.	

** Data from Roberts.[30]*

TABLE 189-8 -- Classification of tetracycline-resistance determinants according to their mechanism of resistance.*

CLASSIFICATION OF TETRACYCLINE-RESISTANCE DETERMINANTS ACCORDING TO THEIR MECHANISM OF RESISTANCE			
Efflux	**Ribosomal**	**Enzymatic**	**Unknown**
TetA–E	TetM	TetX	OtrC
TetG–H	TetO		
TetK	TetS		
TetL	TetQ		
TetA (P)	TetB (P)		
OtrB	OtrA		
Tet, tetracycline-resistance determinant; Otr, oxytetracycline-resistance determinant.			

** Data from Roberts.[30]*

conferring resistance to macrolides. The *cat* gene is preceded by a nine-amino acid leader peptide, and the leader mRNA can form a stable stem-loop structure, which masks the ribosome binding site of the *cat* gene. Chloramphenicol appears to cause the ribosome to stall on the leader sequence, opening the stem-loop structure, thereby exposing the *cat* ribosome binding site and allowing *cat* expression. In Gram-negative bacteria, resistance to chloramphenicol is usually mediated by plasmid-mediated or transposon-mediated genes that are generally expressed constitutively.

Decreased permeability

In Gram-negative bacteria, resistance may also be due to chromosomal mutations that result in decreased outer membrane permeability. In *P. aeruginosa*, nonenzymatic chloramphenicol resistance is associated with the presence of the *clmA* gene. The ClmA protein appears to result in reduced expression of the outer membrane porins OmpA and OmpC and decreased chloramphenicol uptake. In *E. coli*, resistance to chloramphenicol and structurally unrelated antibiotics is part of the MAR phenotype.[34]

RESISTANCE TO GLYCOPEPTIDES

The glycopeptide antibiotics vancomycin and teicoplanin inhibit cell wall synthesis in Gram-positive bacteria by interacting with the terminal D-alanyl-D-alanine (D-Ala-D-Ala) group of the pentapeptide side-chains of peptidoglycan precursors. This interaction prevents the transglycosylation and transpeptidation reactions required for polymerization of peptidoglycan. Almost all bacteria synthesize peptidoglycan terminating in D-Ala-D-Ala, but the exclusion limits of the porin proteins of Gram-negative outer membranes prevent transport of the glycopeptides, and so only Gram-positive species are susceptible to clinically achievable concentrations of this class of antibiotics.

Glycopeptide resistance in enterococci

The vancomycin enterococci can be divided into six different phenotypic groups: A, B, C, D, E and H (Table 189.9).[13] [23] [35] [36] [37] [38] [39] [40]

The origin of the *van* genes in enterococci is not known. The *vanA* gene has 52% amino acid sequence identity to the D-Ala-D-Ala ligase of *Salmonella* spp. and can complement a temperature-sensitive ligase mutant of *E. coli*. The D-Ala-D-Ala ligase is responsible for the production of the D-Ala-D-Ala dipeptide, which in Gram-positive bacteria is the target for glycopeptide antibiotics. The *vanB* and *vanC* genes are also both highly comparable in sequence to D-Ala ligases.

VanA resistance phenotype

The *vanA* gene is carried within a transposon together with several other genes, many but not all of which are required for the expression of resistance to vancomycin. The VanA product is a D-Ala-D-lactate (D-Lac) ligase. The *vanH* gene apparently encodes an enzyme that catalyzes the conversion of pyruvate to D-lactic acid. The VanA ligase uses this as a substrate to form the depsipeptide D-Ala-D-Lac, which is than incorporated into an alternative, vancomycin-resistant peptidoglycan precursor (Fig. 189.3). The VanX protein appears to cleave the D-Ala-D-Ala dipeptide, decreasing the amount of substrate that is available for the formation of the normal pentapeptide. The VanX protein does not hydrolyze the D-Ala-D-lactate pentapeptide or pentadepsipeptide.

Most vancomycin-resistant strains of enterococci also produce a carboxypeptidase. The structural gene for this carboxypeptidase in VanA-harboring strains is *vanY*. The carboxypeptidase may reduce the levels of the normal precursor so that the alternative precursor predominates. The *vanY* gene, however, is not required for resistance to cyclic glycopeptides. The inducible nature of glycopeptide resistance in most VanA enterococci suggests that expression is regulated at the genetic level. The genes *vanR* and *vanS* are involved in the regulation of VanA resistance, and the analysis of the amino acid sequences of the gene products has indicated similarity with two-component signal transducing regulatory systems that sense and respond to environmental stimuli. VanR seems to act as a transcriptional activator and seems to be stimulated by VanS. The phosphorylated VanR peptide acts on a promoter that lies between *vanS* and *vanH* and from which the *vanH*, *vanA* and *vanX* genes are co-transcribed. The environmental stimulus that triggers the initial phosphorylation of VanS has not been identified, but it is probably related to the presence of vancomycin and its interaction with the D-Ala-D-Ala target site, which inhibits transglycosylation. In addition to the high-level glycopeptide resistance mediated by the *vanH*, *vanA*, *vanX* and *vanY* genes, a second mechanism of resistance

TABLE 189-9 -- Resistance to enterococcal glycopeptides.*

GLYCOPEPTIDE RESISTANCE IN ENTEROCOCCI						
Resistance	**Acquired**					**Intrinsic**
Phenotype	VanA	VanB	VanD	VanG	VanE	VanC
MIC (mg/l)						
Vancomycin	64–1000	4–1000	64–128	8–16	16	2–32
Teicoplanin	16–512	0.5–1	4–64	0.5	0.5	0.5–1
Expression	Inducible		Constitutive	?	Inducible	Constitutive Inducible
Location	Plasmid Chromosome		Chromosome	?	Chromosome	Chromosome
Modified target	D-Ala-D-Lac			D-Ala-D-Ser		

* *Adapted from Shales and Rice.*[35]

Figure 189-3 Peptidoglycan biosynthesis. ATP, adenosine triphosphate, Lac, lactate; UDP, uridine diphosphate. *Adapted from Shlaes and Rice.*[35]

exists. The *vanZ* gene mediates resistance to teicoplanin while vancomycin MICs are unaffected. The mechanism by which the VanZ peptide confers this low-level teicoplanin resistance has yet to be established.

VanB and VanC resistance phenotypes

The drug resistance of VanB-harboring strains appears to be similar to that of VanA-harboring strains, except that the VanB-harboring strains originally described remained susceptible to teicoplanin. However, it has become clear that glycopeptide-resistant enterococci containing the *vanB* gene are phenotypically diverse, exhibiting a wide range of vancomycin MICs, including high-level resistance. In addition, the emergence of mutants that express *vanB* constitutively has been described. Resistance mediated by *vanB* may also be transferable, with the gene located either on the chromosome or on plasmids. The *vanC* resistance determinants are present on the chromosome in *Enterococcus casseliflavus* and *Enterococcus gallinarum* and are intrinsic characteristics of these species. VanC-harboring enterococci have low-level resistance to vancomycin and remain susceptible to teicoplanin. The pentapeptide that results from the action of the VanC ligase terminates in D-Ala-D-Ser. This substitution probably reduces vancomycin binding, albeit not to the same degree as the depsipeptide found in VanA and VanB enterococci. Insertional inactivation of *vanC* caused reversion to vancomycin susceptibility, suggesting the existence of a second chromosomal ligase that synthesizes vancomycin-susceptible precursors.

VanC-harboring strains with high-level resistance to glycopeptides as a result of the acquisition of the *vanA* gene cluster have also been isolated. The biochemical basis for the VanC phenotype displayed by most isolates of *E. casseliflavus* and *Enteroccocus flavescens* remains to be clarified in detail. Two genes, designed *vanC-2* and *vanC-3*, have been identified in these species. There is extensive similarity between the *vanC-2* or *vanC-3* gene and the *vanC* gene, now designated *vanC-1*, from *E. gallinarum*, although they do not cross-hybridize.

Glycopeptide resistance in staphylococci

Resistance to glycopeptides among staphylococci is phenotypically diverse[40] *S. aureus* strains resistant to vancomycin have been obtained in vitro either by selection of resistant mutants or by the conjugational transfer of *vanA* from enterococci.[41] Recently, the first vancomycin-resistant *S. aureus* isolate has been detected in the

USA. This strain contained the *vanA* gene, probably originating from a vancomycin-resistant *E. faecium* isolate. One of the enterococcal plasmids containing the *vanA* gene was found to be able to transfer to a strain of *S. aureus* and to be able to express resistance to vancomycin in an inducible fashion, and the resultant staphylococcal strain appears to have stably inherited the resistance. [41] Probably the same mechanism has appeared now in vivo. Teicoplanin-resistant derivatives of teicoplanin-susceptible *S. aureus* strains have also been obtained in vitro.

Recently, *S. aureus* isolates with reduced vancomycin susceptibility were described worldwide (GISA isolates — isolates with reduced susceptibility to glycopeptides).[40] As far as has been studied, all of these isolates contained thickened cell walls. Furthermore, all of them with the exception of the Illinois isolate

showed a reduced cross-linking when compared with isogenic revertants. Interestingly, only some of them (Michigan, New Jersey, Duesseldorf) showed a reduction in D-glutamic acid amidation.[40] The data described so far on GISA strains seem to indicate that, depending on the strain studied, several independent mutations have been accumulated in these strains, which in various combinations lead to the observed resistance phenotype.

Probably the following mechanisms are associated with the appearance of GISA strain:[40]

- ¦ accelerated cell wall synthesis, which leads to a thickened cell wall capable of affinity trapping large amounts of vancomycin and shielding the membrane-associated lipid II target molecules;
- ¦ most probably caused by the accelerated cell wall synthesis — in addition, cross-linking will be reduced due to the fact that nonamidated precursors are poor substrates for the staphylococcal transpeptidation reaction; and
- ¦ since lower nonamidation and lower cross-linking both lead to even higher consumption of vancomycin per unit cell wall weight, they also contribute positively to the resistance phenotype.

Glycopeptide resistance in other Gram-positive species

Glycopeptide resistance is intrinsic in some Gram-positive species such as *Lactobacillus* spp., *Leuconostoc* spp., *Pedicoccus* spp. and *Erysipelothrix rhusiopathiae*. The exact resistance mechanism is these species has not yet been clarified.

RESISTANCE TO TRIMETHOPRIM AND SULFONAMIDES

Trimethoprim and sulfonamides are synthetic agents that affect the biosynthesis of tetrahydrofolic acid, an essential derivative used in amino acid and nucleotide synthesis.[13] [23] [42] [43] [44] Sulfonamides are analogs of *p*-aminobenzoic acid. They competitively inhibit the enzyme dihydropteroate synthase (DHPS), which catalyzes the condensation of dihydropteridine with *p*-aminobenzoic acid synthesis. Trimethoprim is an analog of dihydrofolic acid. It competitively inhibits the enzyme dihydrofolate reductase (DHFR). Dihydrofolate reductase catalyzes the reduction of dihydrofolic acid to tetrahydrofolic acid, the final step in tetrahydrofolic acid synthesis. Trimethoprim-sulfamethoxazole (co-trimoxazole) is a combination of trimethoprim with a sulfonamide.

Intrinsic resistance to trimethoprim and sulfonamides

Outer membrane impermeability results in trimethoprim and sulfonamide resistance in *P. aeruginosa*. Intrinsic resistance to trimethoprim in a number of species is due to host DHFR enzymes with low affinity for the drug. Folate auxotrophs such as *Enterococcus* spp. and *Lactobacillus* spp. that are able to use exogenous pre-formed folates exhibit reduced susceptibilities to sulfonamides and trimethoprim.

Resistance to trimethoprim

Both high- and low-level resistance has been reported in several species. In some cases, chromosomally encoded trimethoprim resistance may be due to

- ¦ overproduction of the host DHFR;
- ¦ mutations in the DHFR structural gene *folA*; or
- ¦ mutations that inactivate thymidylate synthetase, an enzyme that converts deoxyuridylate to thymidylate.

These *thy* mutants require exogenous thymine or thymidine for DNA synthesis and are thus resistant to folate pathway antagonists.

High-level resistance to trimethoprim in enterobacteria is almost always caused by the acquisition of DNA that specifies a trimethoprim-resistant DHFR with an altered active site. At least 11 modified DHFRs have been characterized in Gram-negative organisms. In staphylococci, trimethoprim resistance is encoded by the *dfrA* gene, which encodes a trimethoprim-resistant type S1 DHFR. The transposon encoded *dfrA* gene appears to be responsible for both high- and low-level trimethoprim resistance in *S. aureus* and coagulase-negative staphylococci. The differences in resistance level correlate with differences in transcription caused by deletions adjacent to a copy of IS*257* in Tn*4003*, which affects the promoter used by *dfrA*. The *S. epidermidis* trimethoprim-sensitive chromosomal DHFR gene *dfrC* differs from *dfrA* by only four base pairs, strongly suggesting that *dfrA* originated from the *S. epidermidis* chromosomal gene. Site-directed mutagenesis of *dfrA* and kinetic analyses of the purified DHFRs indicate that a single alteration (Phe98 to Tyr) is responsible for the trimethoprim resistance of the type S1 DHFR.

Resistance to sulfonamides

Chromosomally encoded sulfonamide resistance has been described and resistance seems to be due to increased production of *p*-aminobenzoic acid. Furthermore, alterations of DHPS could lead to low affinity for sulfonamides. Acquired sulfonamide resistance can result from the acquisition of plasmids that encode a drug-resistant DHPS.

RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

Mycobacterial resistance to first-line antimicrobial agents is a considerable concern to health care. The slow growth rate of *M. tuberculosis* and the serious consequences of inappropriate therapy make the study of the mechanisms of antibiotic resistance of particular importance. The main antibiotics for the treatment of mycobacterial infections are isoniazid and rifampin (rifampicin). Resistance was reported soon after the introduction of isoniazid in 1952. Isoniazid acts by inhibiting an oxygen-sensitive pathway in the mycolic acid biosynthesis of the cell wall. The mechanism of resistance appears to multifactorial, and genetic modifications in a number of genes may have an effect. These genes include the *katG* gene-encoded catalase, the isoniazid target encoding *inhA* gene, the *oxyR* and neighboring *aphC* genes and their intergenic region. [45]

The molecular basis of rifampin resistance is understood better. Rifampin interferes with RNA synthesis. At least eight amino acid substitutions in the rpoB subunit of RNA polymerase have been described as conferring resistance. [46]

MULTIPLE RESISTANCE

Bacteria are often resistant to more than one antimicrobial agent. Multiple resistance is conferred by three mechanisms:

- ¦ reduced permeability,
- ¦ active efflux, and
- ¦ multiple resistance genes.

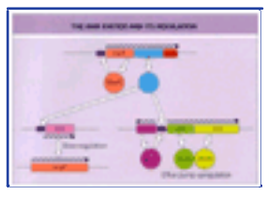

Figure 189-4 The MAR system and its regulation.

Reduced permeability is generally caused by alteration in the cell wall of the bacterium, especially the reduced expression of porins. The best studied is the outer membrane protein (omp) ompF, from *E. coli*.

Multidrug resistance pumps may play an important role in antibiotic resistance. Several families of these efflux pumps have been described in both Gram-positive and Gram-negative bacteria. Some of these pumps not only recognize diverse classes of antibiotics, but also a number of disinfectants such as chlorhexidine.[47] In the socalled MAR system in *E. coli*, reduced uptake and active efflux are combined in a single regulatory system. The MAR system was discovered by Levy and coworkers, who observed that resistant mutants were obtained at a frequency of 10^{-7} when *E. coli* was plated on agar media containing either tetracycline or

chloramphenicol. Usually, mutants obtained with one antibiotic were also resistant to the other, but cross-resistance to ß-lactams, puromycin, rifampin and nalidixic acid was also observed. Genetic mapping studies revealed that a three-gene operon, containing the *marRAB*, was involved (Fig. 189.4).

Sequencing studies revealed that resistant mutants had either mutations in the putative operator-promoter region of the operon or in the *marR* gene. The MarR product acts as a negative regulator for the mar operon. The MarA product is required for resistance. The MarA product is supposed to act on at least two different promoter regions. The first is involved in the expression of OmpF protein. Expression of OmpF is regulated by the *micF* gene. Transcription of this gene leads to the production of an antisense RNA for the ompF mRNA. Stimulation of micF RNA production by the MarA product causes reduced translation of the OmpF mRNA owing to its blockage by the micF antisense RNA and thereby reduced permeability through OmpF. The MarA product also interacts with the acr operon. This operon encodes two subunits of an efflux pump. Inactivation of this efflux system results in hypersensitivity to a wide variety of antimicrobial agents. The expression of the acr operon appears to regulated by AcrR, the product of the third gene of this operon. MarA apparently interacts with the binding of AcrR to its operator, resulting in increased expression of the two subunits of the efflux pump. However, the expression of the pump is also increased by a variety of environmental stimuli, such as ethanol and high concentrations of salt, but these stimuli apparently do not operate through either MarA or AcrR. Despite the detailed molecular knowledge about this mechanism of multiple resistance, its clinical impact is not clear.[48]

Multiple resistance caused by the presence of a number of different resistance genes can originate from the sequential acquisition of mutations or resistance genes, but often these genes are transferred as complete units. These units are located either on transposons or

Figure 189-5 Class I integrons. Open arrows point in the direction of transcription from each promotor site. orf, open reading frame.

multiresistance plasmids that have acquired these genes over time. An example of plasmid-borne multiple resistance comes from a study into eight strains of *Enterococcus* [49] that were resistant to chloramphenicol, erythromycin, minocycline and tetracycline. Genetic analysis showed that all these genes were transferred on a conjugative plasmid, except in the case of three strains. In these strains the chloramphenicol and erythromycin resistance was located either on a nonconjugative plasmid or on the chromosome.

Transposons, easily mobilizable genetic elements that range in size from a few kilobases to more than 150 kilobases, are an important source for the spread of antibiotic resistance. Numerous different transposons have been described and many of them carry one or more antibiotic resistance determinants. Resistance to any class of antimicrobial agent may be encoded on a transposon. Transposons may integrate either in plasmids or the bacterial chromosome and may be present in multiple copies, thereby enhancing their effectiveness in the expression of resistance. A number of transposons have been well studied. These include Tn*5*, Tn*7*, Tn*10* and Tn*21* from Gram-negative bacteria and Tn*554*, Tn*916* and Tn*4001* from Gram-positive bacteria.[50] Transposition may be very efficient and the spread of Tn*4001* described above is an example. Tn*916*, found in *E. faecalis*, was one of the first conjugative transposons discovered. It encodes resistance against tetracycline and chloramphenicol. Tn*916* is a member of a large family of related conjugative transposons, but other families have also been described. Conjugative transposons are not limited to Gram-positive bacteria; they are also present in Gram-negative bacteria. Tn*916* has also been found in *N. gonorrhoeae* for example. Conjugative transposons have a somewhat different mechanism of transfer from the more common transposons such as Tn*5* and Tn*10*, but they are well equipped for dissemination between species, although transfer is regulated. For some conjugative transposons, this transfer may be upregulated (by up to 10,000 times) by antibiotics, which enhances the dissemination of antibiotic resistance determinants. In addition, these conjugative transposons may mobilize co-resident plasmids, which also may carry antibiotic resistance determinants, resulting in the transfer of multiple antibiotic resistance.[51]

Integrons are a special group of genetic elements. The most common are class I integrons, but two other classes are known as well. The class I integrons are characterized by two conserved sequences (CSs). The 5'-CS contains the *int* gene, which encodes a protein homologous to other members of the integrase family. The 3'-CS consists of the *qacE?1*[52] and *sulI*[53] genes and an open reading frame, orf5.[54] The *qacE?1* and *sulI* genes define resistance against quaternary ammonium compounds and sulfonamide, respectively (Fig. 189.5). Integration of gene cassettes by the integrase takes places between the conserved segments. Cassettes can also be excised by the integrase and cassettes can exist as free circular DNA molecules. This process can also lead to the rearrangement of the

TABLE 189-10 -- The first gene cassettes.

GENE CASSETTES					
Gene cassettes			**Protein**	**Length of cassette (base pairs)**	**59-base element (base pairs)**
Resistance to ß-lactams	Class A ß-lactamases	*blaP1*	PSE-1/CARB-2	1044	111
		blaP2		1044	111
		blaP3	CARB-4	>1023	>92
	Class B ß-lactamase	bla_{IMP}	IMP-1	880	127
	Class D ß-lactamases	*oxa1*	OXA-1	1004	90
		oxa2	OXA-2	876	70
		oxa3	OXA-3	>861	>56
		oxa5	OXA-5	915	106
		oxa7	OXA-7	874	65
		oxa9	OXA-9	957	69
		oxa10	OXA-10 (PSE-2)	920	111
Resistance to aminoglycosides	Aminoglycoside adenylyltransferases	*aadA1a*	AAD(3")	856	60
		aadA1b	AAD(3")	856	60
		aadA2	AAD(3")	856	60
		aadB	AAD(2")	591	60
	Aminoglycoside acetyltransferases	*aacA1*	AAC(6')-Ia	>778	
		aacA4	AAC(6')-Ib	637	70
		aacA (orfB)	AAC(6')-Id	526	72
		aacA7	AAC(6')-II	591	112
		aacA	AAC(6')-IIa	628	60
		aacA	AAC(6')-IIb	653	97
		aacC1	AAC(3)-Ia	577	109
		aacC	AAC(3)-Ib	>498	>34

Resistance to chloramphenicol	Chloramphenicol acetyltransferases	*catB2*	CATB2	739	72
		catB3	CATB3	715	60
		catB5	CATB5	>677	>25
	Chloramphenicol exporter	*cmlA*	CmlA	1549	70
Resistance to trimethoprim	Class A dihydrofolate reductases	*dfrA1*	DHFRIa	577	95
		dfrA5	DHFRV	568	87
		dfrA7	DHFRVII	617	134
		dfrA12	DHFRXII	584	90
		dfrA14	DHFRIb	>523	>43
	Class 8 dihydrofolate reductases	*dfrB1*	DHFRIIa	485	57
		dfrB2	DHFRIIb	384	57
		dfrB3	DHFRIIc	408	57
Resistance to streptothricin	Streptothricin acetyltransferase	*sat*	SAT-2	584	60
Resistance to antiseptics and disinfectants	Quaternary ammonium compound exporter	*qacE*	QacE	587	141
Unidentified orfs		orfA		501	69
		orfC		507	60
		orfD		320	60
		orfE		262	60
		orfF		320	60

order of cassettes in an integron. Each gene cassette has an imperfect inverted repeat element. This so-called 59-base pair element, which may vary in length between 57 and 141 base pairs, is unique for each gene cassette.[55] At least 42 gene cassettes have been described, including genes defining resistance against ß-lactam antibiotics, aminoglycosides, trimethoprim, chloramphenicol and antiseptics and disinfectants (Table 189.10).[56] Generally the cassettes do not have promoters, but transcription occurs from one of two promoter sequences present in the 5'-CS. Integrons are widespread in Enterobacteriaceae but are also found in pseudomonads. Isolates may carry more than one integron.[57] Remarkably, the 59-base pair elements show a close relationship with *Vibrio cholerae* repetitive sequences; these are 123–126 base pairs in length and there may be up to 100 copies. The role of these sequences is unknown, but if they are part of gene cassettes, then integration of gene cassettes may play a significant role in bacterial evolution.[55]

CONCLUSION

Worldwide antibiotic resistance is widespread and increasing. The best-known examples are MRSA, vancomycin-resistant enterococci, penicillin-resistant *Streptococcus pneumoniae* and ESBL-carrying Enterobacteriaceae. Studies into the molecular mechanisms of antimicrobial resistance help us to understand the problem and to monitor outbreaks, but other measures are required to quell the spread of resistance genes. Local, national and international antimicrobial surveillance studies are required to gain insight into trends in antimicrobial resistance on which empiric treatment of patients can

be based. However, only the prudent use of antibiotics and infection prevention measures will limit or even prevent the spread of antibiotic resistance.

It is not only the use of antimicrobial agents for the treatment of humans that plays a role in the spread of resistance; their use for the treatment of animals also plays a role. The practice in animal husbandry of using antibiotics in subtherapeutic concentrations as growth enhancers is a particular cause for concern. This practice started in the 1950s. In the 1960s it became controversial and experts questioned the wisdom of adding antibiotics to feed owing to the emergence of multidrug-resistant Enterobacteriaceae. (In fact, the first multidrug-resistant Enterobactericeae had been observed in the 1950s.) In 1966 a multi-resistant *Salmonella* strain ingested via food caused an outbreak that resulted in six deaths. Many studies were issued, but on a political level little action has been taken and the controvesy continues today. An example is avoparcin, which gives rise to cross-resistance to vancomycin. Evidence was recently provided that transfer of vancomycin-resistant enterococci from animals to humans may be possible.[58] Although transfer of resistant strains from animals to humans has been demonstrated, it is often contended that strains of bacteria living in animals are not able to survive in humans because they are not well adapted. However, animal strains of at least some multiresistant strains are able to survive for weeks in humans. They may not cause disease directly, but they provide a reservoir of resistance determinants, which can spread easily between strains and species.[59]

Interestingly, in Europe the use of avoparcin has been high and the use of vancomycin in hospitals low, but the levels of vancomycin-resistant enterococci causing infections in patients are low. In the USA avoparcin was not used in feed and the amount of vancomycin used in hospitals was high and vancomycin-resistant enterococci are often isolated from patients. This suggests that the use of antibiotics in hospitals may pose a greater threat for the spread of resistance than use of subtherapeutic concentrations of antibiotics in feed.

Therefore, inappropriate use of antibiotics in both veterinary and medical practice contributes to the spread of antibiotic resistance, a situation that leads to potentially untreatable common infections.

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Chapter 190 - Antibiotic Prophylaxis in Surgery

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INTRODUCTION

The prevention of surgical site infection (SSI) remains a focus of attention because wound infections continue to be a major source of expense, morbidity and even death. The US Centers for Disease Control and Prevention (CDC) refers to postoperative wound infections as 'surgical site infection' and divides these into superficial (involving skin and subcutaneous tissue) and deep (involving the fascia and muscle) incisional infections, and organ/space infections.

A patient who develops a wound infection while hospitalized has an approximately 60% greater risk of being admitted to the intensive care unit, and an attributable extra hospital stay of 6.5 days, at an extra direct cost of $3000. Risk of re-admission within 30 days is five times more likely for infected patients, at a cost of more than $5000.[1] [2]

The epidemiologic data testifying to the significance of SSI are overwhelming. Surgical site infections are the third most frequently reported nosocomial infection, accounting for 14–16% of nosocomial infections in hospitalized patients. Approximately 40% of nosocomial infections occurring among surgical patients are SSIs, two-thirds of which affect the incision and one-third involve organ/space infection. Three-quarters of deaths of surgical patients with SSI are attributed to that infection, nearly all of which are organ/space infections.[3]

Because of the importance of these infections following operation, considerable effort has been expended to identify other potentially controllable variables that influenced infection rates. A major review of this subject and an extensive list of recommendations for preoperative patient preparation and operating room environment has recently been published by the Hospital Infection Control Practices Advisory Committee (HICPAC) of the CDC.[4]

An early finding of surveillance research was that there were variations in infection rates by surgeon. In an extension of the Hawthorne effect, in which the act of studying a human process improves results, it was then shown that the existence of a wound surveillance system and the reporting of the results normalized surgeon-specific infection rates.[5] This information supported the development of hospital-based surgical wound surveillance programs as a quality monitoring and improvement activity.[6] The trend to more rapid hospital discharge has, however, significantly decreased the accuracy of these programs, which are dependent upon in-hospital examination of wounds and reporting, and no generally applicable technique has replaced it. Surgeon and patient questionnaires have been employed, as well as computerized screens for physician visits and antibiotic prescribing. None have been found as reliable as wound inspection.[7] [8] [9] [10] So, we are now flying blind and an appreciation for the fundamental mechanisms involved in preventing wound infection gains in importance.

This chapter describes current notions of risk factors for SSIs and discusses problems relating to knowing what our infection rates really are. The chapter will then provide recommendations for practices and describe the data supporting these practices. Guidelines published by several expert groups have created a near uniform approach to antibiotic usage for prophylaxis. Nonetheless, it is important to note that administration of systemic anti-infectives is only part of a broad program of infection control involving adequate operating room ventilation, sterilization, barrier usage and delicate surgical technique.[11]

RISK FACTORS FOR SURGICAL SITE INFECTION

Information on appropriateness of antimicrobial prophylaxis is of considerable significance because of the cost of infection that might have been prevented had prophylaxis been given and, conversely, the cost of providing antimicrobial therapy to a very large number of patients if the yield is only the prevention of a relatively small number of infections or even the prevention of no infection. The costs of providing therapy extend far beyond the acquisition and administration charges. They include costs of treating adverse reactions and the more ominous potential cost of dealing in future times with drug-resistant bacteria. Therefore, enormous effort has been expended to identify factors that increase the risk of infection and would, at least potentially, suggest providing antimicrobial prophylaxis.

Whether surgical prophylaxis has any substantial impact on bacterial resistance patterns is unknown but unlikely. In comparison to the raw tonnage of antibiotics prescribed in the community for upper respiratory infections, the amount provided to surgical patients for prophylaxis is quite small. Furthermore, within the hospital, antimicrobial resistance is principally engendered in the intensive care units. The intensive care unit is home to patients at great risk of infection by virtue of acute and chronic disease and by the insertion of a range of monitoring and infusion catheters. These elements lower the inoculum needed to initiate infection and provide portals of entry.

HISTORICAL ASPECTS

Administration of antibiotics to decrease the incidence of postoperative wound infection is a surprisingly recent strategy. The investigational background for the use of anti-infectives for this purpose was developed only in the 1950s and 1960s, considerably later than the initial availability of anti-infectives.[12] In fact, early studies of anti-infective prophylaxis, performed in the 1950s, reported either no decrease in infection rates or even higher rates than control. These results are explained by the fact that anti-infectives were begun only in the postoperative period. During the late 1950s and 1960s, important developments were made to rationalize antimicrobial prophylaxis. The most fundamental was definition of the decisive period, the time following wound contamination that antibiotics would still reduce the incidence of infection.

WOUND CLASSIFICATION SYSTEMS FOR IDENTIFYING RISK OF INFECTION

It is assumed that at least three categories of variables serve as predictors of SSI risk:

- those that estimate the intrinsic degree of microbial contamination of the surgical site;
- those that measure the duration of the operation and other less easily quantifiable elements of the procedure; and
- those that serve as markers for host susceptibility.

In 1964, the National Research Council sponsored an examination of the efficacy of ultraviolet irradiation, and that provided the data to validate a wound classification scheme describing risk of infection in relation to the extent of wound contamination. [13] That document is a landmark in this area, and the classification scheme has remained useful to the present day. This classification is presented in Table 190.1 . A clear connection between the contaminating flora at various surgical sites and subsequent infecting pathogens was established. This microbiologic correlation included the recognition of the role of anaerobes in postoperative wound infection and abscess formation.

Two subsequent CDC efforts, the SENIC project (Study of the Efficacy of Nosocomial Infection Control) and NNIS (National Nosocomial Infection Surveillance), sought to examine these other variables as predictors of infection.[3] [14] These showed that even within the category of clean wounds, the SSI risk varied from 1.1 to 15.8% (SENIC) and from 1.0 to 5.4% (NNIS), depending on the presence of other risk factors.

The size of these studies is truly phenomenal. Information was collected on 58,498 patients undergoing operations in 1970 to develop a simple multivariate risk index. Analyzing 10 risk factors with stepwise multiple logistic regression techniques, they developed a model that combined information on four of the risk factors to predict a

patient's probability of getting a wound infection. Information was then collected on another sample of 59,352 surgical patients seen in 1975–6 to validate the proposed index.

TABLE 190-1 -- Surgical wound classification.[13]

SURGICAL WOUND CLASSIFICATION		
Class	**Description**	**Definition**
I	Clean	An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria
II	Clean-contaminated	An operative wound in which the respiratory, alimentary, genital or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered
III	Contaminated	Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g. open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute nonpurulent inflammation is encountered are included in this category
IV	Dirty-infected	Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation

The variables that were significantly and independently associated with subsequent SSI included:

- an abdominal operation;
- an operation lasting over 2 hours,
- a surgical site with a wound classification of either contaminated or dirty/infected and
- an operation performed on a patient having at least three discharge diagnoses.

Each of these variables contributes one point when present, and the risk index varies from 0 to 4. This means that each variable has the same significance as any other. Using this index predicted SSIs about twice as well as relying on wound classification. With the simplified index, a subgroup, consisting of half the surgical patients, can be identified in whom 90% of the surgical wound infections will develop. By the inclusion of factors measuring the risk due to the patient's susceptibility as well as that due to the level of wound contamination, the simplified index predicts surgical wound infection risk about twice as well as the traditional classification of wound contamination.

The problem with this system is that it is not operation specific and depends on variables collected after the operation (at discharge). To further refine the risk scoring system, a second study was then performed through the NNIS System from 44 hospitals from January 1987 through December 1990.[14] A risk index was developed to predict a surgical patient's risk of acquiring a surgical wound infection. The risk index score, ranging from 0 to 3, is the number of risk factors present from among the following:

- a patient with an American Society of Anesthesiologists preoperative assessment score of 3, 4 or 5;
- an operation classified as contaminated or dirty-infected; and
- an operation lasting over T hours, where T depends upon the operative procedure being performed.

The surgical wound infection rates for patients with scores of 0, 1, 2 and 3 were 1.5, 2.9, 6.8 and 13.0, respectively. The risk index is a significantly better predictor of surgical wound infection risk than the traditional wound classification system and performs well across a broad range of operative procedures.

It is important to note that this system provides little insight into risk of infection in clean or clean-contaminated wounds, other than identifying a correlation with length of operation.

SURVEILLANCE TECHNIQUES FOR IDENTIFYING SURGICAL SITE INFECTIONS: WHAT YOU GET IS WHAT YOU LOOK FOR

Given the clinical and economic importance of SSIs, all hospitals are required to have a program to monitor the incidence of post-operative infections. The methods for monitoring such infections were developed at a point in time when most surgical procedures were occurring in the hospital and patients were generally hospitalized for the procedure and remained in hospital for several days post-operatively. One of the weak points, in fact, of the SENIC and NNIS data presented above is that they by and large relied on in-hospital patient monitoring. Identification and reporting schemes for infections occurring outside the hospital were not well developed or tested. This means that the available data primarily address major surgical procedures, primarily done for intra-abdominal or intrathoracic pathology, for which patients were confined in hospital.

It is known that approximately half of SSIs occur post-discharge, with most occurring within 21 days after operation.[10] Although SSIs occurring after hospital discharge cause substantial morbidity, their epidemiology is not well understood, and methods for routine post-discharge surveillance have not been validated. A post-discharge surveillance program including self-reporting of infections by patients

and return of questionnaires by patients and surgeons is labor and resource intensive. A variety of techniques have been tested, including physician questionnaires, direct patient contacts and computer screens of pharmacy, outpatient, microbiologic and re-admission databases. None has been found superior to others, and it is likely that as more and more elements of patients' medical care are computerized, automated surveillance systems will become increasingly effective.

ACCEPTED INDICATIONS FOR ANTI-INFECTIVE PROPHYLAXIS

There is a wide consensus on specific procedures that warrant antimicrobial prophylaxis. Consensus statements by the Surgical Infection Society, the Infectious Diseases Society of America, the American Society of Hospital Pharmacists, the Canadian Infectious Diseases Society and the French Society of Anesthesia and Intensive Care all agree on a number of indications (Table 190.2).[15] [16] [17] [18] [19] There is also considerable agreement as to which procedures do not warrant prophylaxis.

Controlled trials of antimicrobial prophylaxis in minimally invasive procedures have recently been reported. In low risk laparoscopic

TABLE 190-2 -- Pathogens causing surgical site infections and antimicrobial drugs of choice for prophylaxis.

PATHOGENS CAUSING SSIs AND ANTIMICROBIAL DRUGS OF CHOICE FOR PROPHYLAXIS			
Procedure	**Likely pathogen(s)**	**Drug/dosing**	**For history of anaphylactoid reactions**
Clean procedures for which prophylaxis is accepted	*Staphylococcus aureus* and *Staphylococcus epidermidis*	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g
Head and neck procedures entering the oropharynx; esophageal procedures	Streptococci; oropharyngeal anaerobes (e.g. peptostreptococci)	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g
High-risk gastroduodenal and biliary	Enterobacteriaceae and streptococci	Cefazolin 1g preoperatively	Quinolone selected for low cost[†]
Placement of all grafts, prostheses or implants	*S. aureus*; coagulase-negative staphylococci	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g

Cardiac	*S. aureus*; coagulase-negative staphylococci	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g
Neurosurgery	*S. aureus*; coagulase-negative staphylococci	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g
Breast	*S. aureus*; coagulase-negative staphylococci	Cefazolin 1g preoperatively	Clindamycin 600mg or vancomycin 1g
Orthopedic — total joint replacement, closed fractures/use of nails, bone plates, other internal fixation devices, functional repair without implant/device, trauma	*S. aureus*; coagulase-negative staphylococci; Gram-negative bacilli	Cefazolin 1g q8h × 3	Gentamicin 2mg/kg + clindamycin 600mg q1 2h × 2
Noncardiac thoracic — thoracic (lobectomy, pneumonectomy, wedge resection, other noncardiac mediastinal procedures), closed tube thoracostomy	*S. aureus*; coagulase-negative staphylococci; *Streptococcus pneumoniae*; Gram-negative bacilli	Cefazolin 1g × 1	Clindamycin 600mg
Vascular	*S. aureus*; coagulase-negative staphylococci	Cefazolin 1g × 1	Clindamycin 600mg
Appendectomy*	Gram-negative bacilli; anaerobes	Cefazolin 1g + metronidazole 500mg q8h × 3 or cefotetan 1g × 1 or cefoxitin 1g × 4	Quinolone selected for low cost† + metronidazole 500mg q1 2h × 2
Colorectal	Gram-negative bacilli; anaerobes	Cefazolin 1g + metronidazole 500mg preoperatively or cefotetan 1g preoperatively or cefoxitin 1g†	Quinolone selected for low cost† + metronidazole 500mg preoperatively
Obstetric and gynecologic	Gram-negative bacilli; enterococci; group B streptococci; anaerobes	Cefazolin 1g preoperatively	Quinolone selected for low cost† + metronidazole 500mg preoperatively
Urologic (may not be beneficial if urine is sterile)	Gram-negative bacilli	Cefazolin 1g preoperatively	Quinolone selected for low cost preoperatively†

†† Ciprofloxacin, levofloxacin, gatifloxacin or moxifloxacin
* For nonperforated appendicitis. If perforated, treatment is therapeutic
† Re-dose if procedure lasts >4 hours

cholecystectomy and arthroscopic surgery, routine prophylaxis is not indicated.[20] In contaminated laparoscopic procedures, such as high-risk cholecystectomy and bowel surgery, it is best to apply the standards for similar open procedures.

In many areas of antibiotic administration sufficient numbers of studies have been carried out to allow synthesis of the data.[21] [22] [23] [24] [25] While there is some skepticism regarding this process, termed meta-analysis, there is no doubt that it is useful in selected situations where the primary literature is of good quality, heterogeneity in the response to treatment is small and well-understood, and there is a specific, critical parameter of outcome. Prophylaxis lends itself well to this, in that much of the literature is of good quality, the response to therapy is uniform, and the outcome parameter (SSI) is a specific and well-defined event.

It is worthwhile to note that one benefit of meta-analysis is the identification of benefit early in the evolution of a practice concept, thereby sparing many patients either the extra risk that their procedure might carry were prophylaxis not given or the extra risk of an adverse event from receiving a medication that would not benefit them. This is perhaps best illustrated with regards to antibiotic prophylaxis for elective colon surgery.

CHOICE OF ANTI-INFECTIVES FOR PROPHYLAXIS

It is certainly not necessary to cover the entire spectrum of contaminants of a surgical wound. The anticipated pathogens from various operative sites are detailed in Table 190.2 .

Little investigational work has been done on appropriate dosing. In general, doses of the selected agent that would be used for the treatment of established infection are recommended. The more important issue for prophylaxis concerns the need to maintain effective antibiotic levels throughout the procedure. This is typically accomplished by providing repetitive dosing for lengthy procedures. This is in part a function of the half-life of the agent selected, and is an additional argument in favor of agents such as cefazolin that have half-lives approaching 2 hours. A current recommendation is to re-dose the patient at intervals of twice the half-life of the agent provided. It is important to note that increasing the dose of an agent provides less benefit than shortening the dosing interval because drug clearance is logarithmic.

A large number of studies now document effective prophylaxis with no further dosing after the patient leaves the operating room.[26]

Gastroduodenal procedures

Prophylaxis is recommended for most gastrointestinal procedures. The density of organisms and proportion of anaerobic organisms progressively increase along the gastrointestinal tract, so the recommendation depends on the segment of gastrointestinal tract entered during the procedure. The intrinsic risk of infection associated with procedures entering the stomach, duodenum and proximal small bowel is quite low and does not support a routine recommendation for prophylaxis. However, any disease or therapeutic intervention that decreases gastric acidity causes a marked increase in the number of bacteria and the risk of wound infection. Therefore, previous use of antacids, histamine blockers or a proton pump inhibitor qualifies the patient for prophylaxis. Prophylaxis is also indicated for procedures treating upper gastrointestinal bleeding. Stasis also leads to an increase in bacterial counts, and so prophylaxis is warranted in procedures to correct obstruction. In addition, the intrinsic risk of infection in patients with morbid obesity and advanced malignancy is sufficiently high to warrant prophylaxis in these cases. Although the local flora is altered in these patients, cefazolin provides adequate prophylaxis and is the recommended agent.

Generally, elective surgery on the stomach or duodenum for ulcer disease is often not included in those procedures requiring prophylaxis. The highly acidic environment results in a very low endogeneous bacterial density, and rates of postoperative infection without prophylaxis are low. High-risk gastroduodenal procedures include operations for cancer, gastric ulcer, bleeding, obstruction and perforation, as well as operation in the presence of acid-reducing medical or surgical therapy. Prophylaxis is also recommended for gastric procedures for morbid obesity.

Colorectal procedures

Colorectal procedures have a very high intrinsic risk of infection and warrant a strong recommendation for prophylaxis. Several studies have demonstrated efficacy with rates of infection decreasing from over 50% to less than 9%. Antibiotics are directed at Gram-negative aerobes and anaerobic bacteria.

Mechanical cleansing

Commonly used colon preparation routines have changed substantially in that most patients self-administer these regimens at home and are admitted to hospital the morning of surgery. All prophylactic regimens begin with a mechanical bowel preparation, intended to greatly reduce the amount of feces present. Most commonly, polyethylene glycol (PEG) regimens are used. It is worth noting that the true value of these preparative activities is primarily to facilitate the operative procedure. Several trials have recently documented that mechanical cleansing does not alter wound infection rates if systemic antibiotic prophylaxis is used.

A current standard is a 4-liter PEG preparation. Bowel preparation with bisacodyl and 2 liters of PEG is reportedly more acceptable to patients than a 4-liter regimen and is equally effective in cleansing the colon.

It is important to be aware of the fluid losses that occur following PEG preparations. Compared with patients who receive inpatient preparation, patients receiving outpatient preparation require significantly more intraoperative fluid and colloid administration, greater amounts of fluid in the first 24 hours postoperatively and significantly more postoperative fluid challenges. Patients with multiple medical problems may not tolerate extensive fluid shifts; therefore, other preoperative arrangements, such as inpatient or outpatient intravenous fluid therapy, need to be considered to minimize complications that may outweigh potential cost savings.

Another alternative is 90ml of sodium phosphate (NaP) and bisacodyl. This is available in kit form. In one study comparing the two, patient tolerance to NaP was superior to PEG with less trouble drinking the preparation, less abdominal pain, less bloating and less fatigue. The preparations clear the colon equally well. Patients undergoing afternoon surgery may take their preparation early in the morning so that they have nothing by mouth for 6 hours before operation.

These regimens decrease fecal bulk but do not decrease the concentration of bacteria in the stool. In fact, the risk of infection with mechanical preparation alone is still 25–30%. The gastrointestinal side-effects of the osmotic mechanical preparations now used complicate the oral administration of antibiotics.

Antibiotics

In the USA, it is common to use a regimen of erythromycin base and neomycin given at 1 p.m., 2 p.m. and 11 p.m. (1g of each drug per dose) the day before a colorectal procedure scheduled for 8 a.m. Times of administration are shifted according to the anticipated time of starting the procedure, with the first dose given 19 hours before operation. Metronidazole can be substituted for erythromycin. If this regimen is used, there is no advantage to also providing parenteral prophylaxis.

Outside the USA, however, oral nonabsorbable antibiotic preparation have largely been abandoned in favor of parenteral treatment. A major systematic review has recently been reported for colorectal prophylaxis.[27] [28] This review examined trials published between 1984 and 1995, and some 147 trials were suitable for analysis. These included over 23,0000 patients and 70 different regimens were tested. The results confirmed that the use of antimicrobial prophylaxis is effective for the prevention of surgical wound infection after colorectal surgery. There was no significant difference in the rate of surgical wound infections between many different regimens. However, certain regimens were found to be inadequate. Inadequate regimens included metronidazole alone (which lacks activity again facultative and aerobic Gram-negative organisms), doxycycline alone, piperacillin alone (which lacks activity against anaerobes), and oral neomycin plus erythromycin on the day before operation. The addition of an effective parenteral agent reduced infection rates seen with neomycin-erythromycin to the same level as that seen with the parenteral agent alone. Several trials showed extra benefit of oral antibiotics if inadequate parenteral antibiotics such as metronidazole alone or piperacillin alone were employed. These authors found that a single dose administered immediately before the operation (or short-term use) is as effective as long-term postoperative antimicrobial prophylaxis.

This study also found no evidence to suggest that the new-generation cephalosporins are more effective than first-generation cephalosporins. Antibiotics selected for prophylaxis in colorectal surgery should be active against both aerobic and anaerobic bacteria. No additional benefit was observed in six trials that compared parenteral anti-infectives alone with parenteral plus topical.

Oral or topical application of antibiotics in addition to the parenteral administration of appropriate anti-infectives is of no benefit. Antibiotics selected for prophylaxis in colorectal surgery should be active against both aerobic and anaerobic bacteria. Administration should be timed to make sure that the tissue concentration of antibiotics around the wound area is sufficiently high when bacterial contamination occurs. Guidelines should be developed locally to achieve a more cost-effective use of antimicrobial prophylaxis in colorectal surgery.

Prophylaxis is also recommended for appendectomy. Although the intrinsic risk of infection is low for uncomplicated appendicitis, the preoperative status of the patient's appendix is typically not known. Cefotetan or cefoxitin are acceptable agents, although a high rate of *Bacteroides fragilis* resistance to cefotetan has recently been identified.[29]

Metronidazole combined with a quinolone is also an acceptable regimen. For uncomplicated appendicitis, coverage need not be extended to the postoperative period. Complicated appendicitis (e.g. with accompanying perforation or gangrene) is an indication for antibiotic therapy, thereby rendering any consideration of prophylaxis irrelevant.

Biliary tract procedures

The recommendations for antibiotic prophylaxis for procedures of the biliary tract depend on the presence of specific risk factors. In general, prophylaxis for elective open cholecystectomy (either open or laparoscopic) may be regarded as optional. Risk factors associated with an increased incidence of bacteria in bile and thus of increased risk for postoperative infection include age over 60 years, disease of the common duct, diagnosis of cholecystitis, presence of jaundice and previous history of biliary tract surgery. Only one factor is necessary to establish the patient as high risk. In most cases of symptomatic cholelithiasis meeting high-risk criteria, cefazolin is an acceptable agent. Agents with theoretically superior antimicrobial activity have not been shown to produce a lower postoperative infection rate.

Neurosurgical procedures

Studies evaluating the efficacy of antibiotic prophylaxis in neurosurgical procedures have shown variable results. Nonetheless, prophylaxis is currently recommended for craniotomy, laminectomy and shunt procedures. Coverage targets *Staphylococcus aureus* or *Staphylococcus epidermidis*.

Head and neck procedures

For procedures entailing entry into the oropharynx or esophagus, coverage of aerobic cocci is indicated. Prophylaxis has been shown to reduce the incidence of severe wound infection by approximately 50%. Either penicillin or cephalosporin-based prophylaxis is effective. Cefazolin is commonly used. Prophylaxis is not indicated for dentoalveolar procedures, although prophylaxis is warranted in immunocompromised patients undergoing these procedures.

General thoracic procedures

Prophylaxis is routinely used for nearly all thoracic procedures. Antimicrobial prophylaxis is particularly important when there is high likelihood of encountering high numbers of micro-organisms during the procedure. Pulmonary resection in cases of partial or complete obstruction of an airway is a procedure in which prophylaxis is clearly warranted. Likewise, prophylaxis is strongly recommended for procedures entailing entry into the esophagus. Although the range of micro-organisms encountered in thoracic procedures is extensive, most are sensitive to cefazolin, which is the recommended agent.

Cardiac procedures

Prophylaxis against *S. aureus* and S. *epidermidis* is indicated for patients undergoing cardiac procedures. Although the risk of infection is low, the morbidity of mediastinitis or a sternal wound infection is great. Numerous studies have evaluated antibiotic regimens based on penicillin, first-generation cephalosporins, second-generation cephalosporins or vancomycin. Cardiopulmonary bypass reduces the elimination of drugs, and so additional intraoperative doses typically are not necessary.

Antistaphylococcal penicillins and first-generation cephalosporins have traditionally been the prophylactic antibiotics of choice for patients undergoing cardiothoracic operations. Recently published studies have claimed improved outcomes with respect to postoperative wound infection when second-generation cephalosporins were used for prophylaxis.

A meta-analysis of placebo-controlled trials of cardiothoracic prophylaxis demonstrated a consistent benefit from the administration of antibiotic prophylaxis, with an approximate 5-fold reduction in wound infection rate.[22] The second-generation cephalosporins, cefamandole and cefuroxime, performed better than cefazolin, with an approximate 1.5-fold reduction in wound infection rate. Administration of prophylaxis beyond 48 hours was not associated with improved infectious outcomes.[22]

Obstetric and gynecologic procedures

Prophylaxis is indicated for cesarean section and abdominal and vaginal hysterectomy. Numerous clinical trials have demonstrated a reduction in risk of wound

infection or endometritis by as much as 70% in patients undergoing cesarean section. For cesarean section, the antibiotic is administered immediately after the cord is clamped to avoid exposing the newborn to antibiotics. Despite the theoretic need to cover Gram-negative and anaerobic organisms, studies have not demonstrated a superior result with broad-spectrum antibiotics compared with cefazolin. Therefore, cefazolin is the recommended agent.

When 25 randomized controlled trials of antibiotic prophylaxis that used rigorous protocols were analyzed,[25] overall 21.1% (373 of 1768) of the patients who did not receive antibiotic prophylaxis had serious infections after abdominal hysterectomy. Among patients who received any antibiotics, 9.0% (166/1836) had serious postoperative infections. Cefazolin was evaluated in 615 patients. The differences in the prevalence of infection between women who received prophylaxis and women who did not receive prophylaxis were statistically significant (any antibiotics, p=0.00001; cefazolin, p=0.00021) The authors concluded that preoperative antibiotics are highly effective in the prevention of serious infections associated with total abdominal hysterectomy, and that they should be used routinely. They also noted that the use of controls who receive no treatment is no longer justified in trials of antibiotic prophylaxis for total abdominal hysterectomy.

Urologic procedures

The range of potential urologic procedures and intrinsic risk of infection vary widely. In general, it is recommended that preoperative sterilization of the urine be achieved if clinically feasible. For procedures entailing the creation of urinary conduits, recommendations are similar to those for procedures pertaining to the specific segment of the intestinal tract being used for the conduit. Procedures not requiring entry into the intestinal tract and performed in the context

of sterile urine are regarded as clean procedures. It should be recognized, however, that prophylaxis for specific urologic procedures has not been fully evaluated.

Orthopedic procedures

Antibiotic prophylaxis is clearly recommended for certain orthopedic procedures. These include the insertion of a prosthetic joint, ankle fusion, revision of a prosthetic joint, reduction of hip fractures, reduction of high-energy closed fractures and reduction of open fractures. Such procedures are associated with a risk of infection of 5–15%, but this is reduced to less than 3% by the use of prophylactic anti-biotics.[3] *Staphylococcus aureus* and *S. epidermidis* predominate in wound or joint infections. Cefazolin provides adequate coverage. The additional use of aminoglycosides and extension of coverage beyond the operative period is common but lacks supportive evidence.

Noncardiac vascular procedures

Available data support the recommendation for coverage of procedures using synthetic material, those requiring groin incisions and those affecting the aorta. Cefazolin is the recommended agent because most infections are caused by *S. aureus* or *S. epidermidis*. Prophylaxis is not recommended for patients undergoing carotid endarterectomy.

Anti-infective prophylaxis for clean procedures

The biggest controversy regarding antibiotic prophylaxis centers around prophylaxis for clean surgery. Prophylaxis has prevented postoperative wound infection after clean surgery in a majority of clinical trials with sufficient power to identify a 50% reduction in risk. The low control rates of infection means that very large studies must be carried out to see a significant effect; studies of more than 1000 procedures are needed to detect such reductions reliably.

The major study on this subject was a randomized, double-blind trial of 1218 patients undergoing herniorrhaphy or surgery involving the breast, including excision of a breast mass, mastectomy, reduction mammoplasty and axillary-node dissection.[30] The prophylactic regimen was a single dose of cefonicid (1g intravenously) administered approximately half an hour before surgery. The patients were followed up for 4–6 weeks after surgery.

The patients who received prophylaxis had 48% fewer probable or definite infections than those who did not. For patients undergoing a procedure involving the breast, infection occurred in 6.6% of the cefonicid recipients (20 of 303) and 12.2% of the placebo recipients (37 of 303); for those undergoing herniorrhaphy, infection occurred in 2.3% of the cefonicid recipients (7 of 301) and 4.2% of the placebo recipients (13 of 311). There were comparable reductions in the numbers of definite wound infections, wounds that drained pus and those infected with *S. aureus*. There were comparable reductions in the need for postoperative antibiotic therapy, nonroutine visits to a physician for problems involving wound healing, incision and drainage procedures, and re-admission because of problems with wound healing.

An observational study was then carried out on the effects of antibiotic prophylaxis on definite wound infections:[31] 3202 patients undergoing herniorrhaphy or selected breast surgery procedures were identified preoperatively and monitored for 4 or more weeks; 34% of patients received prophylaxis at the discretion of the surgeon; 86 definite wound infections (2.7%) were identified. Prophylaxis recipients were at higher risk for infection, with a higher proportion of mastectomies, longer procedures and other factors. Patients who received prophylaxis experienced 41% fewer definite wound infections and 65% fewer definite wound infections requiring parenteral antibiotic therapy after adjustment for duration of surgery and type of procedure. Additional adjustment for age, body mass index, the presence of drains, diabetes and exposure to corticosteroids did not change the magnitude of this effect. The effect of prophylaxis was similar for all procedures studied.

The argument then is not whether such therapy lowers infection rates but rather whether it is worth the cost. Additionally, the control infection rate is so low that physicians will not be aware of a decreased infection rate unless very careful surveillance is performed, and then only for patients from several practices. Comparing one effective regimen with another, as has been done with colorectal surgical prophylaxis, is simply not going to happen. Effective regimens are effective against *S. aureus* and other pathogens that may be carried in the nares or on the skin. In addition, relatively long half-life in the serum and low cost are important considerations. Cefazolin is a good prophylaxis agent for many clean surgical procedures.

To justify the use of prophylaxis for clean procedures at a single institution, an accurate assessment of infection rates must be available. This requires a considered effort at post-discharge follow-up. When these data are available, the risk/benefit ratio can be more knowledgeably assessed. Without accurate information on infection rates by procedure, known risk factors described above may serve as guides. Extremes of age, poor nutritional status, diabetes and obesity are recognized as significant additional risk factors.

The use of systemic prophylaxis for hernia repairs entailing the insertion of mesh is considered desirable because the morbidity of infected mesh in the groin is substantial. However, no prospective trials demonstrate the effectiveness or necessity of this practice. Modified radical mastectomy and axillary node dissection also warrant prophylaxis because wounds near or in the axilla have an intrinsic risk of infection. If prophylaxis is desired or indicated for any of these procedures, cefazolin is the agent of choice.

Laparoscopic and thoracoscopic procedures

Specific data supporting a recommendation of antibiotic prophylaxis for laparoscopic or thoracoscopic procedures are lacking. Therefore, pending the availability of new data, recommendations for the same procedure performed using the 'open technique' should be followed.

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Chapter 191 - Home Therapy with Antibiotics

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INTRODUCTION

Most antibiotics given at home are administered orally. However, over the past 20 years, home intravenous antimicrobial therapy has developed as an important component of health care delivery. Over a quarter of a million patients are treated in the USA annually in this manner.[1] The use of home intravenous antimicrobial therapy was first reported in 1974 for children who had cystic-fibrosis-associated pneumonia and subsequently for patients who had osteomyelitis.[2] [3] In addition to the USA, home intravenous antimicrobial therapy is now a common treatment modality in many regions, including Europe and Australia.

This chapter focuses on the home antibiotic treatment of patients who, because of the serious nature of their infections, would otherwise require in-hospital therapy. The use of intravenous antimicrobial therapy outside the hospital has been termed outpatient parenteral antimicrobial therapy (OPAT) in the USA, and hospital-in-the-home (HITH) in some other parts of the world.[1] [4] These programs are useful both for patients who have infections requiring prolonged intravenous antibiotic therapy (e.g. osteomyelitis or endocarditis) and for patients who have common infections such as cellulitis, in whom in-hospital admission may be avoided entirely. The potential advantages and disadvantages of OPAT are summarized in Table 191.1 , but a crucial component for successful OPAT is that patients are clinically stable and have appropriate home circumstances. Although there have been very few randomized trials of OPAT, those that have been done, as well as the many published case series, have reported good treatment outcomes.[5] [6]

MODELS FOR HOME INTRAVENOUS ANTIMICROBIAL THERAPY

The delivery of high-quality, safe home therapy is best achieved by an OPAT team consisting of physicians, nurses and pharmacists who use clearly delineated treatment protocols.[6] [7] Physicians should be experienced in the treatment of infectious diseases and have a good understanding of antimicrobial pharmacokinetics to allow appropriate decisions regarding the selection and duration of therapy, as well as drug monitoring. Since nursing staff administer therapy, they have regular contact with the patient and carer(s) and are often the initial contact when problems arise. Outpatient parenteral antimicrobial therapy pharmacists assist in the choice and mode of therapy, drug supply and compounding.

Many OPAT units have an infusion center (generally located within a hospital or clinic) where patients can be medically reviewed and receive directly observed therapy. outpatient parenteral antimicrobial therapy can be administered either by a nurse visiting the patient at home, the patient receiving treatment in an infusion center, or patients (or their relatives) self-administering therapy. Self-administration requires a well-motivated patient and carer who are capable of being educated regarding safe drug administration. It can be particularly useful for patients requiring prolonged or multidose therapy, or for those who require repeated courses of intravenous therapy (e.g. patients who have cystic fibrosis). The keys to a successful OPAT program include:

- a well structured OPAT team;
- appropriate patient selection based on medical need and suitability for treatment at home;
- informed patient and carer consent;
- careful monitoring of patients for response to therapy and adverse events; and
- 24 hour access to OPAT staff, particularly for emergencies.

The decision to accept a patient for home intravenous antimicrobial therapy should be based on medical need and appropriateness and should not be driven by bureaucratic or economic factors.

TECHNOLOGY USED IN OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Recent advances in medical technology have allowed development of new venous access devices and drug delivery systems that have improved the safety of home intravenous antibiotic administration.

Venous access devices

The optimal choice of vascular access is generally based on a number of factors, including the proposed treatment duration, the medication to be infused and the type of delivery system to be used.

Peripherally inserted central catheters

Peripherally inserted central catheters (PICCs) are a convenient form of intravenous access for OPAT therapy. They are made of flexible silicone, are introduced into the cubital vein and advanced into the superior vena cava, and are easily held in position with an adhesive dressing. Advantages of PICCs include the fact that they can be inserted and removed in the outpatient setting, are very durable, can be kept patent with an infrequent saline flush and have a relatively low infection rate.[8] Because of their central positioning, they are suitable for administration of concentrated antibiotic solutions such as used in continuous-infusion dosing.

Peripheral intravenous cannulae

Peripheral intravenous cannulae are generally used for short-duration therapy, but to minimize the risk of phlebitis they should be changed every 2–3 days. Thus, nursing staff need to be skilled in cannula insertion.

Long-term central venous catheters

These catheters (e.g. Hickman's, Port-A-Cath) are occasionally used in patients who have few other options for intravenous access, or who require them for administration of parenteral nutrition or cancer chemotherapy. In-hospital admission and anesthesia are generally required for insertion; however, they have a low infection rate and provide effective access for patients who require prolonged or repeated intravenous therapy.

TABLE 191-1 -- Potential advantages and disadvantages of outpatient parenteral antimicrobial therapy.*

POTENTIAL ADVANTAGES AND DISADVANTAGES OF OPAT	
Potential advantages	**Potential disadvantages**
Patient at home with family	Disruption to home environment

Continue work, school	Increased patient/family stress
Decreased nosocomial infections	Non-adherence with therapy
Fewer cannula-associated infections	Misuse of intravenous access
Improved utilization of hospital beds	Decreased supervision
Patient sense of empowerment	Feeling of abandonment
Reduced health care costs (possible)	Inappropriate antibiotic selection
	Non-adherence to bed rest, leg elevation
	Potential for unnecessarily prolonged duration of OPAT because of less medical incentive to stop treatment

** Adapted from Howden and Grayson.* [6]

Drug delivery systems

Like the choice of intravenous access, the optimal OPAT drug delivery system is influenced by the agent to be delivered and the proposed treatment duration. [6] [9]

Direct push

Intravenous injection over 5–10 minutes is useful for antibiotics such as cephalosporins and penicillins. Spring-loaded devices are available that can deliver an intravenous push using small (e.g. 10–20ml) syringes.

Gravity

Drug administration by gravity is usually used for agents that require dilution in larger volume solutions (e.g. 100–1000ml) before infusion, or where infusions require administration over an extended period of time (e.g. vancomycin, amphotericin B).

Controlled-rate infusion devices

A number of compact, battery-operated, computerized infusion pumps are available that can be programmed to deliver antibiotic by either continuous infusion or intermittent bolus. They can be readily carried in a small bag around the waist or neck, and allow the patient to continue with normal activities while receiving therapy. These pumps are generally expensive to purchase but are reusable and most models will alarm if the intravenous line becomes blocked or develops in-line air bubbles. Nonprogrammable continuous-infusion devices are also available that are either spring-loaded or elastomeric — in these the tension in either the spring or the elastomeric 'bladder' propel the infusion. Although these pumps are cheaper, they are generally not reusable and will not alarm if the infusion is interrupted. Both devices are ideal for the continuous infusion of antimicrobials that are stable in solution over a 24-hour period and that have optimal activity when stable high serum concentrations are maintained (e.g. antistaphylococcal penicillins).[10] [11] [12]

INTRAVENOUS ANTIBIOTIC REGIMENS

Although there may be a tendency for the practicalities of home antibiotic administration to influence the choice of antibiotic used (e.g. once-daily agents), the principles used for appropriate antibiotic prescribing should be similar to those applied to patients managed in hospital. Appropriate antimicrobial agent(s) include those with the narrowest antibacterial spectrum appropriate for the responsible pathogen, most practical dosing regimen and lowest purchase and delivery costs. Patient-specific factors are also important, such as avoiding aminoglycosides in patients who have significant renal impairment. Antibiotics that can be administered by continuous infusion or that have a long serum half-life (and therefore require infrequent dosing) are most appropriate for OPAT. Agents, such as ceftriaxone or glycopeptides, that are simple to use because they require only once-daily dosing but often have an antibacterial spectrum that is broader than necessary for many indications should be used only cautiously. Antibiotics that would be considered optimal for in-hospital use should, where possible, be used for OPAT, although in some cases these may require innovative delivery methods.

Beta-lactams

The clinical efficacy of ß-lactams against many pathogens is related to the proportion of the dosing interval during which the serum drug concentrations are maintained above the minimum inhibitory concentration (MIC) of the infecting pathogen(s).[10] Thus, ß-lactams with a short half-life (e.g. penicillin, ampicillin, antistaphylococcal penicillins) should either be dosed frequently (e.g. q4-6h, which is generally impractical for OPAT) or administered by continuous infusion. There is increasing experience with the successful use of continuous-infusion antistaphylococcal penicillins (e.g. flucloxacillin, oxacillin) and some cephalosporins (e.g. ceftazidime) for the treatment of a range of conditions, including osteomyelitis, endocarditis and pneumonia.[11] [12] [13] Limiting factors with continuous-infusion administration include the availability and cost of accurate drug delivery devices and the instability in solution of some agents (e.g. ampicillin) after compounding (Table 191.2).

Cephalosporins such as ceftriaxone, which have a sufficiently long serum half-life to allow once-daily dosing, can be extremely useful for OPAT. Similarly, recent studies in which oral probenecid was used to prolong the half-life of the first-generation cephalosporin cefazolin have established that this combination given once-daily is effective in the treatment of conditions such as cellulitis. [14] [15]

Aminoglycosides

A number of clinical studies suggest that administration of aminoglycosides (e.g. 4–5mg/kg/d gentamicin) as a once-daily dose rather than as two or three divided doses is associated with similar efficacy and probably reduced toxicity as compared with multidosing regimens when treating Gram-negative infections.[23] Once-daily gentamicin is now the preferred regimen when treating infections such as pyelonephritis, cholangitis and moderate-severe Gram-negative pneumonia. However, data regarding the efficacy of once-daily aminoglycoside therapy is limited or lacking in some settings, including pregnancy, neonates, burns patients, cystic fibrosis and some cases of endocarditis; in these situations, the use of once-daily aminoglycosides may not be appropriate.

Glycopeptides

Glycopeptides (e.g. vancomycin, teicoplanin) are effective against many Gram-positive pathogens. Vancomycin generally needs to be administered twice daily over at least 1–2 hours, while teicoplanin, after initial loading, may be given rapidly once daily. Although glycopeptides have been used in OPAT because of their infrequent dosing requirements, they are usually only an appropriate choice when used to treat resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), or patients who are anaphylactic to ß-lactams. The efficacy of teicoplanin in some situations has been questioned and the emergence of resistant pathogens such as vancomycin-resistant enterococci reinforce the view that glycopeptides should only be used when clearly indicated.[6]

TABLE 191-2 -- Outpatient parenteral antimicrobial therapy treatment regimens, monitoring and potential complications. £

OPAT TREATMENT REGIMENS, MONITORING AND POTENTIAL COMPLICATIONS					
Condition	**Intravenous regimen**	**Oral alternative available**	**Monitoring** *	**Complications and side effects**	**Comments and references**

Cellulitis	**Recommended:**	Yes[†]	Clinical response	Nausea and drug interactions a potential problem with probenecid	Cefazolin has narrower antimicrobial spectrum than ceftriaxone[14] [15] [16] [17]
	cefazolin 2g iv q12h *or* 2g q24h + probenecid 1g po q24h				
	Alternative:				
	ceftriaxone (1g iv q24h)				
Osteomyelitis and septic arthritis (MSSA)	**Recommended:**	Usually none	Clinical response	Nausea, vomiting, liver dysfunction from antistapylococcal penicillin	Vancomycin generally reserved for patients who have ß-lactam allergy[11] [12]
	antistaphylococcal penicillin[‡] 8–12g/d by continuous infusion for 4–6 weeks		Inflammatory markers		
	Alternative:		Drug levels		
	vancomycin 1g iv q12h for 4–6 weeks				
Endocarditis (*viridans* streptococci, uncomplicated)[§]	**Recommended:**	None	Clinical response	Cardiac decompensation	Most authorities recommend 2–3 doses of gentamicin per day for endocarditis
	ceftriaxone 2g iv q24h for 4 weeks		Echocardiogram	Emboli	Published reports regarding continuous infusion penicillin are limited, although this regimen is recommended by some authors[18] [19] [20]
	Alternative:		Gentamicin levels	Renal or vestibular damage from aminoglycosides	
	ceftriaxone 2g iv q24h + gentamicin 3mg/kg q24h for 2 weeks *or* penicillin 8.4g/d by continuous infusion for 4 weeks		Audiometry		
			Renal function		
Endocarditis (MSSA)	**Recommended:**	None	Clinical response	Nausea, vomiting, liver dysfunction from antistaphylococcal penicillin	Uncomplicated disease and in-hospital stabilization crucial prior to OPAT[11] [12]
	antistaphylococcal penicillin[‡] 8–12g/d by continuous infusion for 6 weeks		Echocardiogram		
			Drug levels		
Pyelonephritis	**Recommended:**	Yes[†]	Clinical response	Renal or vestibular damage from aminoglycosides	Complete 14 days therapy with oral agents
	gentamicin 4–6mg/kg/d iv *or* ceftriaxone 1g iv q24h		Renal function		Exclude prostatitis
			Aminoglycoside levels		
			Urine microscopy and culture		
Pneumonia (moderate severity — pneumonia severity index III)	**Recommended:**	Yes[†]	Clinical response	Respiratory failure	Careful patient selection[7] [21]
	ceftriaxone 1g iv q24h		Chest radiography		
Cystic fibrosis (infective exacerbation)	**Recommended:**	None	Clinical response		Treatment guided by results of sputum culture
	cefepime 2g iv q12h + tobramycin 4–6mg/kg q24h iv		Chest radiography		
Meningitis	**Recommended:**	None	Clinical response	Seizures	Tice *et al.*[22]
	ceftriaxone (2g iv q24h-q12h)				
Cytomegalovirus (CMV) disease (induction therapy)	**Recommended:**	Yes[†]	Clinical response	Neutropenia	May follow with long-term suppressive therapy
	ganciclovir 5mg/kg iv q12h for 2–3 weeks		CMV antigenemia or viral load		
Invasive fungal infection (e.g. cryptococcal meningitis)	**Recommended:**	Yes[†]	Clinical response	Renal impairment	
	amphotericin B 0.6–1.5mg/kg daily or 3 times per week		Cerebrospinal fluid glucose, antigen titer	Hypokalemia	
			Renal function, electrolytes	Nausea, chills	
MSSA, methicillin-susceptible *Staphylococcus aureus*					

£ Adapted from Howden and Grayson.[6]
* Generally, all patients receiving OPAT should have routine hematology and biochemistry monitored weekly.
† See Table 191.3 .
‡ Includes nafcillin, oxacillin, flucloxacillin.
§ Native valve, no complications, penicillin MIC of organism <0.1mg/ml.

Antiviral agents

Ganciclovir is effective for both acute treatment and long-term suppression of serious cytomegalovirus disease and is administered once or twice daily. Such regimens are suitable for OPAT administration, although the availability of oral ganciclovir and the recent availability of the highly bioavailable valganciclovir have reduced the need for long-term intravenous suppressive therapy and may avoid the need for intravenous induction therapy in some patients.[24] Other intravenous antiviral agents (e.g. foscarnet) may occasionally be used in OPAT.

INDICATIONS FOR HOME INTRAVENOUS ANTIMICROBIAL THERAPY

A wide variety of infections can be safely and conveniently treated with OPAT, should such treatment be appropriate (see Table 191.2).[4] [6] [25] Although this latter point may seem rather obvious, there is a growing body of data to support the use of some highly bioavailable oral antibiotic regimens for conditions such as osteomyelitis and pyelonephritis — conditions that previously were thought to require intensive parenteral therapy (Table 191.3). Infections that are relatively common and generally

require only brief OPAT include cellulitis, pyelonephritis, pneumonia, bacterial

TABLE 191-3 -- Oral antibiotics with excellent bioavailability that may be an alternative to intravenous therapy.[†]

ORAL ANTIBIOTICS WITH EXCELLENT BIOAVAILABILITY THAT MAY BE AN ALTERNATIVE TO INTRAVENOUS THERAPY				
Antibiotic	**Adult dose**	**Peak serum concentration (µg/ml)**	**Key indications**	**Notable side effects**
Fluoroquinolones:				
Ciprofloxacin	500–750mg q12h	1.8–2.8	UTI, OM, GI, STI	GI symptoms, rash, dizziness, headache
Ofloxacin	400mg q12h	4.0–6.0	UTI, RTI, OM, GI, STI	Seizures in elderly patients
Levofloxacin	500mg q24h	5.7	UTI, RTI	
Gatifloxacin	400mg q24h	4.2–4.6	RTI, STI	QT prolongation, drug interactions, rash, occasional hypoglycemia, dizziness, GI symptoms
Moxifloxacin	400mg q24h	4.5	RTI, STI	
Macrolides:				
Azithromycin	500mg q24h	0.4	RTI, STI, Myc	GI symptoms, rash, drug interactions
Clarithromycin	250–500mg q12h	0.8–3.0	RTI, Myc, HPY	GI symptoms, rash, drug interactions
Metronidazole	200–500mg q8h	6.2–25	ANA, C diff	GI symptoms, peripheral neuropathy
Clindamycin	150–600mg q6h	2.5–8.0	SKN, ANA	GI symptoms, pseudomembranous colitis, rash
Cephalosporins:				
Cefixime	400mg q24h	3–5	RTI, UTI, STI	Rash, GI symptoms
Cefpodoxime	100–200mg q12h	2.9	RTI, STI	Rash, GI symptoms
TMP-SMX	160/800mg tabs (1q12h to 2 q6h)	1–2/40–60 (1 tab)	UTI, RTI, PCP, *Nocardia*	Rash, GI symptoms, neutropenia, thrombocytopenia, renal dysfunction
Doxycycline	100mg q24h-q12h	1.5–2.1	RTI, STI, malaria	GI symptoms, photosensitivity, fetal effects
Oxazolidinones:				
Linezolid	600mg q12h	15–20	SKN, RTI (resistant organisms)	GI symptoms, rash, thrombocytopenia
Antivirals:				
Valganciclovir	900mg q24h-q12h	4.0–8.8 (ganciclovir)	CMV disease	Neutropenia, diarrhea
Valacyclovir	500mg q24h to 1g q6h	5.0 (1g q6h)	HSV, VZV	Headache, nausea, diarrhea
Famciclovir	125–500mg q8h	2.8–4.0 (500mg)	HSV, VZV	Headache, nausea
Triazoles:				
Fluconazole	100–400mg q24h	20–30 (400mg)	Cryptoc, *Candida*	Rash, GI symptoms, elevated liver enzymes, drug interactions
Itraconazole	100–400mg q24h	0.4–2.0[*]	Dermatophytes, *Candida*, molds, dimorphic fungi	Rash, GI symptoms, elevated liver enzymes, drug interactions

ANA, anaerobic infection; C diff, *Clostridium-difficile*-associated diarrhea; CMV, cytomegalovirus; Cryptoc, *Cryptococcus neoformans.*; GI, infective diarrhea; HPY, *Helicobacter pylori*; HSV, herpes simplex virus; Myc, mycobacterial infection; OM, osteomyelitis; PCP, *Pneumocystis carinii* pneumonia; RTI, respiratory infection; SKN, skin and soft tissue infection; STI, some sexually transmitted infections; TMP-SMX, trimethoprim-sulfamethoxazole; UTI, urinary tract infection; VZV, varicella-zoster virus.

† *Data on peak serum concentration from Kucers et al.*[26] *and Gilbert et al.*[27]
* Use of the itraconazole formulated in cyclodextrin leads to improved oral absorption and higher serum levels.

meningitis and infective exacerbations associated with chronic lung disease. Such patients do not generally need long-term venous access and ideally can be treated with agents that require only infrequent dosing (e.g. once or twice daily). Serious diseases that are often suitable for OPAT include endocarditis, osteomyelitis, septic arthritis, deep abscesses (e.g. brain, psoas, liver — generally after initial drainage), and invasive fungal and cytomegalovirus disease in transplant recipients and HIV-infected patients. Since these conditions usually need prolonged intravenous antibiotic therapy, long-term venous access (e.g. PICC) is often required and innovative treatment regimens (e.g. continuous-infusion agents) may be appropriate, depending on the responsible pathogen(s). Although these conditions are not common, they are often large consumers of in-hospital bed days; hence, OPAT may substantially improve in-hospital bed utilization by treating a relatively small number of patients.

Patient selection

Appropriate patient selection is a crucial component in ensuring a safe and successful OPAT program. Both patient-specific and disease-specific factors are important in the decision to accept a patient for OPAT (Fig. 191.1). In particular, special care should be taken when assessing for OPAT, elderly or isolated patients, who often do not cope well with medical illness, and patients who have serious

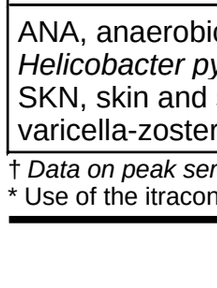
Figure 191-1 Clinical pathway for selection of patients for outpatient parenteral antimicrobial treatment of infections.

diseases such as endocarditis, where potentially catastrophic disease-related complications can occur.

Patient-specific factors

Factors that may be potential contraindications to OPAT include:

- patients who live alone or in isolated areas, or who do not have a telephone or other means of rapid communication;
- active substance abuse;
- aggressive patients, relatives or pets — these generally argue against patient suitability for OPAT care, since OPAT nurse safety is crucial; and
- the presence of a language barrier between patient and staff that cannot be overcome with the assistance of interpreters or family members — this suggests that

safety at home cannot be assured and that in-hospital therapy may therefore be more appropriate.

Disease-specific factors

A clearly defined diagnosis is important before embarking on OPAT. Patients who have common, less serious, conditions such as cellulitis can often be transferred directly from the emergency department to the OPAT program, avoiding in-hospital admission. Patients who have more serious conditions such as endocarditis, osteomyelitis and meningitis generally require a period of inpatient assessment, treatment and stabilization before transfer to an OPAT program to complete their treatment course.

Disease-specific indications

General recommendations for disease-specific therapy are outlined in Table 191.2 . However, further discussion regarding some common conditions is warranted.

Cellulitis

Cellulitis is usually the most common indication for OPAT. Since *Streptococcus pyogenes* and *Staphylococcus aureus* are the most frequent responsible pathogens, treatment with a first-generation cephalosporin or antistaphylococcal penicillin is often appropriate. Numerous US studies have demonstrated the efficacy of once-daily ceftriaxone 1–2g for cellulitis, although the appropriateness of this relatively broad-spectrum agent for this indication has been questioned. The first-generation cephalosporin cefazolin, when given 2g twice daily, or 2g once daily together with oral probenecid 1g once daily, is effective. Both regimens have comparable clinical efficacy to that of once-daily ceftriaxone and represent practical, appropriate OPAT treatment options.[14] [15] [16] Subsequent switching to oral agents such as

dicloxacillin (500mg q6h), cephalexin (500mg q6h) or clindamycin (300mg q6h) after initial improvement usually results in cure.

Pyelonephritis

Gentamicin (4–5mg/kg intravenously q24h), ceftriaxone (1g intravenously q24h) or ciprofloxacin (500–750mg orally q12h) are appropriate empiric single agents for pyelonephritis, since the usual pathogens are often Gram-negative bacilli. Ampicillin or penicillin may also be given empirically to treat possible enterococcal infections, although this is an uncommon pathogen in young patients. Antibiotic selection should be reviewed once the results of urine and blood cultures are available. Recent studies have demonstrated that oral fluoroquinolones are highly effective for treatment of many cases of pyelonephritis, and they may be considered as an alternative to parenteral therapy for patients in whom adherence is assured. [28] Although ciprofloxacin would not usually be the first-line choice for in-hospital care of pyelonephritis, the fact that its use may avoid the need for intravenous access offers a significant practical advantage.

Community-acquired pneumonia

Ceftriaxone 1–2g once daily is the agent most commonly used for the home intravenous treatment of pneumonia. However, in many regions ceftriaxone would not be the drug of first choice for in-hospital care of community-acquired pneumonia and the drug's broad spectrum of activity may be unnecessary. There are currently few data regarding the OPAT use of continuous-infusion penicillin for community-acquired pneumonia.

Endocarditis

The treatment of endocarditis currently accounts for about 5% of US OPAT treatment courses but almost certainly a higher proportion of treatment days.[29] Endocarditis can be successfully treated with OPAT but poses a particular problem because of the risk of life-threatening complications.[7] [11] [12] Outpatient parenteral antimicrobial therapy selection criteria for patients who have endocarditis have been proposed.[30] They suggest that most patients who have endocarditis should generally be managed in hospital for the initial 2 weeks. One exception may be patients with uncomplicated viridans streptococcal endocarditis who have rapidly become afebrile and cleared their bacteremia; such patients may be suitable for transfer home after 1 week. Patients who have complicated endocarditis (heart failure, conduction abnormality, perivalvular abscess), aortic valve disease, prosthetic valve endocarditis, acute endocarditis or infection caused by virulent organisms such as *S. aureus* should generally be managed primarily as inpatients.

Various OPAT options for common pathogens are shown in Table 191.2 . Ceftriaxone (2g/d) has been most commonly used for the home treatment of uncomplicated viridans streptococcal endocarditis; it appears to be effective when given either alone for 4 weeks or together with an aminoglycoside for 2 weeks. [18] [19] [20] Monitoring of renal and auditory function is particularly important if aminoglycosides are being used for prolonged periods. Although there are some case reports of treatment with intermittent-dose (and occasionally continuous-infusion) penicillin via a computerized pump for viridans streptococcal endocarditis, this is not currently a common OPAT regimen.

There are a number of reports of successful OPAT for staphylococcal endocarditis. Treatment options include the use of antistaphylococcal penicillins given by computerized pump as a continuous infusion or by intermittent bolus.[11] [12] [13] Vancomycin may be used in ß-lactam-allergic patients but appears to be less effective than ß-lactams for susceptible staphylococcal strains.[31] There are very limited OPAT data for endocarditis caused by other organisms such as enterococci, HACEK organisms, and fungi. Outpatient parenteral antimicrobial therapy for endocarditis generally requires central venous access (e.g. PICC) and close weekly clinical and drug monitoring.

Osteomyelitis

Most forms of osteomyelitis require 4–6 weeks of parenteral antimicrobial therapy, although the treatment duration and need for surgery may differ depending on the responsible pathogen(s), host factors and the bone involved. *Staphylococcus aureus* is the most common cause of osteomyelitis, but other pathogens such as coagulase-negative staphylococci, *Pseudomonas* spp. and Enterobacteriaceae may be involved when osteomyelitis is nosocomial in origin or associated with foreign bodies or intravenous drug abuse.[32] Thus, various treatment options may need to be considered (see Table 191.2). Long-term intravenous access is generally required unless oral fluoroquinolones are considered appropriate.[32] [33]

STEP-DOWN AND ORAL THERAPY

So-called 'step-down' therapy is a relatively recent term that can be applied to either the transfer from in-hospital to OPAT management or to the switch from parenteral to oral therapy. Used in this latter context, 'step-down' is simply a fashionable means of describing a routine component of managing many infectious diseases.

The decision to switch a patient from intravenous to oral therapy is dependent on a number of factors, including the nature of the disease and the bioavailability of effective oral agents. For some infections such as endocarditis or meningitis, oral antibiotics rarely play a significant role in therapy as, once intravenous therapy is completed, antimicrobials can usually be stopped. For a number of other infectious diseases (e.g. osteomyelitis or cytomegalovirus disease) intravenous therapy has, until recently, been the mainstay of treatment. However, the availability of more potent oral therapy (e.g. fluoroquinolones, valganciclovir) may allow an early switch to oral therapy, or possibly even the avoidance of intravenous therapy altogether. For the common conditions such as cellulitis and pyelonephritis the timing of switching to oral therapy is dependent on the severity of illness and the response to initial intravenous therapy. There are no strict criteria for the duration of intravenous treatment in these conditions.

A number of antimicrobials with excellent oral bioavailability are now available (see Table 191.3). As clinical trials evidence accumulates, treatment with some of these agents is likely to be considered a reasonable alternative to intravenous therapy for a range of serious infections; the use of ciprofloxacin for Gram-negative pyelonephritis is one such example. Such oral agents allow simple home therapy while avoiding the need for intravenous access devices. However, ensuring such agents have an appropriate spectrum of activity (not too broad) and that patients adhere to these oral regimens will be crucial to avoid the emergence of resistance and treatment failures.

MONITORING PATIENTS RECEIVING OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Patients require careful monitoring while receiving both OPAT and oral therapy. Although the home environment has many advantages for the patient, the careful regular monitoring that generally occurs in hospital is not present at home. In general, patients should be reviewed by the OPAT physician at least once a week, usually

in the outpatient department or office. Specific factors to assess on review include:

- patient's reaction to OPAT;
- response to therapy;
- drug side effects; and
- other complications (e.g. venous cannula infection).

Routine hematology and biochemistry, as well as serum drug levels, are also often monitored weekly.

Since in some situations more frequent reviews, or even emergency assessments, may be necessary, all good OPAT programs should have a system to manage these.

SUMMARY

Given recent trends worldwide, an increasing proportion of medical care that previously would have been administered in hospital is likely to be delivered at home. To be successful, patient selection should be based primarily on medical suitability rather than economic considerations. Ensuring appropriate antibiotic selection, safe delivery systems and continuity of care will be key challenges.

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Chapter 192 - Short-course Antibiotic Therapy

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INTRODUCTION

The length of antibiotic course of therapy has been rarely studied in conventional infections while in tuberculosis, malaria and, interestingly, in venereal diseases it has been investigated thoroughly. The reasons for this discrepancy may be that in out-of-hospital studies, where compliance has always been a problem, investigators attempted to formalize the shortest possible course, to increase patient adherence and reduce the medical workload and costs. In the hospital until 15 years ago, the questions of compliance and cost were not as vital as they are today. In addition, the issue of bacterial resistance was 20 years ago of far less importance and wide implication as it is today. The introduction of new bactericidal agents with a rapid onset of action as well as better definition of patient population and deeper understanding of pharmacodynamics have also contributed to the shortening of antibiotic therapy courses. The best possible examples are the single-dose therapy for gonorrhea with ceftriaxone, cefixime, ciprofloxacin, ofloxacin, azithromycin or doxycycline. The reduction of the very long courses of tuberculosis treatment from around 18–24 months to much shorter periods (4–6 months in immunocompetent hosts[1] due in part to the introduction of rifampin, a highly bactericidal agent) and the introduction of directly observed therapy (DOT), which assured better compliance and therefore lower rates of resistance development, have lead to shorter therapy courses. The ability to treat meningococcal meningitis in children with 4 days of ceftriaxone therapy or even a single administration of long-acting penicillin,[2] or in adults for 2 days with ceftriaxone or for 4 days with penicillin G also demonstrates the changes occurring in our understanding of the necessary length of therapeutic periods. In hospitals also, the reduction of presurgical prophylaxis to a single dose,[5] with no untoward effect on wound infection, but with a significant reduction in resistant Gramnegative wound isolates, demonstrates the utility of short and ultrashort therapy (and prophylaxis) courses.

A course of therapy is conventionally defined as the time period during which an antimicrobial is administered. Due to the presence of counterfeit agents with decreased activity, irregular absorption and other uncontrolled parameters, a better definition may be the period during which therapeutic concentrations are maintained at the site of infection.

The advantage of a short therapy (or prophylaxis) period is that patient compliance improves with the reduction of the number of dosages to be administered and the time period necessary for a full treatment course.[3] A short-term therapy course is conventionally less expensive than prolonged courses because of the decreased amounts of drugs used and decreased cost of physicians, medical personnel and laboratory tests associated with more prolonged courses. In short-course therapy, if the full course is interrupted, there is less waste of unused antibiotics. Obviously when fewer antibiotics are prescribed the risk of adverse events and drug-drug interaction would be less. There is less risk of bacterial resistance development of the treated pathogen as well as of commensals if therapy is short. This has been shown to be true in the community,[4] where therapy of URTI in children for >7 days increased the risk of selection of penicillin-resistant *Strep. pneumoniae* (PRSp; OR = 3.5, 95% CI 1.3–9.8) compared to shorter treatment courses, as well as in the hospital, where prophylaxis of >48 hours increased the risk of acquired antibiotic resistance of Enterobacteriaceae and enterococci (OR 1,6, CI 1.1–2.6) compared to prophylaxis of <48 hours.[5] There is potentially less harm done to the environment in the immediate vicinity of the patient and less risk to his family members of becoming carriers of resistant bacteria, if fewer antibiotics are excreted and secreted from the patient into his immediate surroundings.

Evidently there is a lower limit under which short-course therapy becomes ineffective. Thus, for example, it was shown that for uncomplicated cystitis in women short therapy of 3 days was as effective as 5 days and as 7 days therapy with ß-lactam antibiotics, trimethoprim-sulfamethoxazole and fluoroquinolones. Nevertheless, reduction of the therapy course to <3 days was associated with an increasing rate of relapses and is thus not recommended.[6] In endocarditis also, reducing the duration of therapy to less than 28 days in aortic valve endocarditis was associated with unsatisfactory results compared with conventional duration of therapy. Another example is the observation that treating staphylococcal bacteremia for <10 days was associated with a high relapse rate.[7] Similarly, in catheter-related *Staph. aureus* bacteremia, antistaphylococcal therapy for >14 days was associated (albeit not significantly) with favorable outcome, fewer complications and less attributable death.[8] Other investigators also confirmed the need for 10–15 days, therapy for catheter-associated bacteremia.

A successful abbreviated treatment course depends on several mandatory factors on the part of the patient, pathogen, infection and therapeutic agent, as detailed in Table 192.1 .

The patient needs to be fully immunocompetent to be able to be cured with short courses of therapy and needs the full number of active leukocytes and macrophages. He needs to be able to produce adequate antibodies and to mount a satisfactory cell-mediated immune response. He must have adequate concentration of albumin to carry the antibiotic in the circulation and to have adequate distribution of intra- and extracellular water to allow the agent to penetrate or diffuse to the sites of infection.

The pathogen involved must be highly susceptible to the administered agent and not have the tendency to develop resistance to the agent used (low spontaneous mutation rate). The pathogen should preferably be extracellular and should be able to divide frequently enough to allow for prolonged antibiotic-vulnerable periods. The pathogen, at the site of infection, should preferably be in a planktonic form and not adherent to solid phases like bone and cartilage.

The infection should be at a site that is easily accessible to antibiotics; thus infections in sanctuaries like the brain, the prostate and eye are not good candidates for abbreviated therapy courses. The infection should not be life threatening and should not be localized in or around foreign bodies to which the pathogen is adherent and at which it forms a biofilm. It should be caused by a single pathogen and should not be an abscess, empyema, granuloma, etc., that would

TABLE 192-1 -- Features necessary for successful abbreviated therapy courses.

FEATURES NECESSARY FOR SUCCESSFUL ABBREVIATED THERAPY COURSES			
Patient factors	**Pathogen factors**	**Infection factors**	**Antibiotic factors**
Immunocompetence	Susceptible to antibiotics	At an easily accessible site	Bactericidal
Adequate WBC	Low spontaneous mutation rate	Not as a biofilm	Rapid onset of action
Normal albumin level	Extracellular	Lack of foreign body	Lack of propensity to induce mutations
Adequate hydration	Rapid multiplication rate	Not life-threatening	Easy penetration to tissues
Adequate compliance		Caused by a single pathogen	Active against nondividing bacteria
		Not a closed space infection	Not affected by adverse conditions
		Lack of adverse environmental factors	
		Early infectious state	

not allow antibiotics to penetrate or that have conditions of low pH, WBC debris and other factors that inhibit antibiotic action. Experience has shown that infections on mucosal surfaces (like upper and lower respiratory tract infections, intestinal infections and genitourinary infections) are best suited for abbreviated courses of therapy

while infections in bones, joints, the brain or intracellular infections are not suited for such treatment modality.

In order for the agent to be successful in abbreviated mode therapy, it has to be bactericidal and act rapidly, be associated with the lowest rate of resistance induction, be able to penetrate easily to tissues and body fluids (that is, having a low molecular weight and being lipophilic) and be present at the site of infection in sufficiently high concentrations for those agents with concentration-dependent killing (e.g. aminoglycosides, fluoroquinolones and imidazoles) and in concentrations above the MIC for long enough periods for agents that possess time-dependent bacterial killing (ß-lactams, macrolides, glycopeptides, etc.).[9]

GROUP A STREPTOCOCCAL PHARYNGITIS

Therapy of group A streptococcal (GAS) pharyngitis is intended to prevent both suppurative and nonsuppurative complications (rheumatic fever and perhaps poststreptococcal glomerulonephritis).

The treatment currently recommended by the American Heart Association, Infectious Disease Society of America and other organizations is 10 days of penicillin. [10] Prevention of acute rheumatic fever is believed to require eradication of the infecting streptococci from the pharynx, an effect that depends on prolonged rather than high-dose penicillin therapy.

The efficacy of 10-day treatment with penicillin was first documented in the early 1950s. By 1953 the American Heart Association recommended treatment of GAS pharyngitis with oral penicillin for 10 days.[11] In 1981 Schwartz *et al* re-evaluated the duration of treatment in a study which compared patients with proven GAS infection treated for 7 or 10 days with penicillin V in 8 hourly regimens.[12] They concluded that the 10-day regimen was more effective than a 7-day regimen in eradicating GAS, but also concluded that persistence of GAS after adequate therapy may be common. It is now accepted that approximately 15% of patients continue to harbor the original infecting GAS serotype in their pharynx after completing a course of oral penicillin.[10]

Gerber *et al*[13] compared 5 versus 10 days of penicillin V treatment in a randomized controlled trial. Patients in the two treatment groups were comparable with respect to clinical findings, compliance and serologic response to GAS. The same serotype of GAS was present in the follow-up in 18% of the 73 patients treated for 5 days versus 6% of 99 patients treated for 10 days. Thus, the need for 10 days of penicillin V treatment was confirmed.

Macrolides are an alternative choice, especially for penicillin-allergic patients. Several studies have examined short courses of various macrolides as an optional treatment for GAS pharyngitis. McCarty *et al* [14] compared clarithromycin to penicillin V and demonstrated comparable rates of clinical success and a higher eradication rate with clarithromycin (94% vs 78%). Boccazzi *et al*[15] compared a 3-day azithromycin regimen to 5-day cefibuten. They showed a somewhat higher eradication rate after cefibuten than after azithromycin treatment. Yet, the widespread use of macrolides has been associated with the development of resistance by GAS and this should limit the use of macrolides for GAS pharyngitis only to penicillin-allergic patients.

Oral cephalosporins are also highly effective in treating streptococcal pharyngitis. A meta-analysis of 19 studies suggested that streptococcal eradication rates and clinical cure rates attained with these agents are even slightly higher than those achieved with penicillin.[16]

Since the mid-1990s several randomized controlled studies have been carried out to compare shorter therapeutic courses (4–5 days of treatment) of cephalosporins with either the standard 10-day penicillin V regimen or with 10-day treatment with cephalosporins. These studies demonstrated that the shorter treatments were equivalent or superior in bacteriological eradication and clinical response. However, none of these studies evaluated the incidence of post-streptococcal sequelae. The general concern that shorter treatment courses might lead to an increased incidence of post-streptococcal sequelae was increased by clusters of rheumatic fever which occurred in the USA in the late 1980s.

Two recent large-scale European studies were performed by Adam *et al*,[17] to compare short-course treatments with the standard 10-day penicillin treatment. They evaluated 4782 culture-proven cases of GAS pharyngitis and also measured the incidence of post-streptococcal sequelae for a follow-up of 1 year. They examined 5-day regimens with six antibiotics that have been shown effective in previous trials: amoxicillin/clavulanate, ceftibuten, cefuroxime axetil, loracarbef, clarithromycin and erythromycin estolate. The 5-day regimens were as effective as the 10-day penicillin treatment. Both bacteriologic eradication and clinical success rates were equivalent. However, among the patients treated with the short course

there were four cases of poststreptococcal late sequelae (three cases of rheumatic fever and one glomerulonephritis), while only one patient in the 10-day penicillin group developed glomerulonephritis. The authors claim that the poststreptococcal sequelae in these cases could not be definitely related to the streptococcal episode treated in the study according to their histories. A separate evaluation for cefuroxime axetil (5-day) versus penicillin (10-day) showed equivalence with no streptococcal sequelae.

In summary, abbreviated courses (less than 10 days) of penicillin V were unsuccessful. Short courses of macrolide drugs have led to equivalent clinical and bacterial eradication results, but the concern of resistance development in GAS should limit these drugs to treating penicillin-allergic patients. Short courses of cephalosporins were equivalent or slightly better in clinical and bacterial eradication, but it is difficult to prove that poststreptococcal sequelae will not increase with these treatments and the concern about antibiotic resistance should probably limit these drugs to treating special cases. In addition, most of these agents are more expensive than penicillin, even when administered for short courses. Therefore, the best choice is probably still penicillin.

OTITIS MEDIA

Acute otitis media (AOM) remains one of the most common bacterial infections in childhood and the leading indication for antimicrobial use in this population. The objective of treating AOM is achieving a rapid clinical relief and preventing complications such as mastoiditis, meningitis, jugular vein thrombosis, etc. Failure to eradicate the causative pathogens in the middle ear was shown to lead to a higher risk of relapse and long-term sequelae. Thus, most expert panels have recommended treating children with AOM. However, other studies demonstrated that even among bacterial infections, the majority will resolve spontaneously, with only a minor advantage of antibiotic treatment over nontreatment, and thus have recommended withholding antimicrobial treatment entirely in some or all cases of AOM unless symptoms persist or worsen.[18]

Most clinical guidelines and expert panels still recommend 10 days of ß-lactams (first drug of choice being amoxicillin) for most patients, some limiting this treatment to children younger than 2 years, and allowing a shorter course of treatment (5 days) for older children.[19]

Recently, interest in shortening the course of antibiotic therapy from the traditional 10 days to 5 days has emerged for the reasons previously mentioned. Cefpodoxime, cefdinir and azithromycin are the only oral antibiotics currently approved by the US FDA for 5-day short-course therapy of AOM.

A meta-analysis[20] of randomized, controlled trials of shortened antibiotic therapy in AOM suggested that a 5-day course of a short-acting antibiotic was an effective treatment. However, patient subgroup sizes were too small to provide a reliable estimate of the risk of treatment failure in children younger than 2 years. Yet it is precisely children in this age group who gain the greatest benefit from treatment and who have the highest risk of treatment failure.

When evaluating the results of AOM studies it is particularly important to critically review the criteria used for diagnosis and for assessing outcome. In some of the previous trials, a combination of middle ear effusion and one or more nonspecific signs or symptoms has been considered sufficient. Outcomes in some of the studies have been based on symptomatic response alone, without regard to specific tympanic membrane findings, thus permitting inclusion of patients who do not actually have AOM but have otitis media with effusion (OME), a condition that is self-limiting and with minor symptoms.

In summary, abbreviated 5-day courses have been shown to be equivalent to standard therapies in children older than 2 and may be equivalent in those younger than 2 years who do not attend a daycare center. Children younger than 2 years of age who attend a daycare center should continue to receive the standard 10-day treatment in order to achieve good clinical success and prevent relapses.

ACUTE BACTERIAL SINUSITIS

Acute bacterial sinusitis is a common upper respiratory tract infection, with an estimated 20 million cases reported in the US annually. There are no specific clinical features to distinguish between bacterial and viral etiologies. It is, however, accepted that patients who have symptoms of rhinosinusitis (purulent nasal secretions and maxillary facial pain) for less than 7 days are unlikely to have bacterial infection. The presence of symptoms for more than 7 days is a sensitive but nonspecific predictor of bacterial sinusitis. Two recent meta-analyses[21] [22] have concluded that antibiotics are statistically more efficacious than placebo in reducing symptoms,

although their benefit is relatively small. It is generally recommended that treatment should be initiated with narrow-spectrum agents, e.g. amoxicillin, doxycycline or trimethoprim-sulfamethoxazole (TMP-SMX); however, these recommendations do not consider the increasing incidence of penicillin-resistant *Strep. pneumoniae* as a causative pathogen. The Sinus and Allergy Health Partnership guidelines for treatment of bacterial rhinosinusitis recommend stratifying patients according to severity of disease, rate of progression, recent antibiotic exposure and local resistance data.[23] Recommendations for initial therapy for adults with mild disease who have not received antibiotics include the following choices: amoxicillin/clavulanate, amoxicillin (1.5–3g/day), cefpodoxime proxetil or cefuroxime axetil. However, the duration of therapy is not indicated in these guidelines.

As the optimal duration of antimicrobial treatment for acute sinusitis has not been adequately studied until recently, the duration of treatment was not well defined and was therefore generally based on physician preference. The standard duration of antibiotic treatment for acute bacterial sinusitis ranges between 7 and 14 days. All the controlled studies published between 1990 and 2002 that compared the efficacy of short-course treatment (fewer than 7 days) with that of a long course (more than 7 days) showed equivalent clinical and bacteriologic efficacy results for 3–5 days, compared with 8–14 days of therapy with all agents studied. A single randomized, double-blind, placebo-controlled study[24] has compared amoxicillin/clavulanate administrated for 5 days with a 10-day course. Risk factors for failure in the abbreviated course were a history of more than four bouts of sinusitis during the 2 years prior to therapy or a history of surgical drainage. Thus, longer treatment courses might be indicated for certain risk groups. Further studies are needed to resolve this question.

Azithromycin has a prolonged half-life ranging between 2 and 5 days and a slow release from tissues. These properties suggest that a short course of azithromycin could be comparable to prolonged antimicrobial therapy. Several studies demonstrated high efficacy of azithromycin in acute sinusitis when administrated for 3–5 days. The response rates are comparable with those of prolonged therapy.

In summary, several studies have demonstrated similar efficacy of a short antimicrobial course compared with 8–14 days of antimicrobial treatment. Nevertheless, most studies included patients with maxillary sinusitis and the results cannot be extrapolated to patients with frontal, ethmoidal and sphenoidal or pan-sinusitis. It is important to emphasize that most studies did not verify the bacterial etiology of sinusitis by the use of sinus punctures. As most cases of acute rhinosinusitis are caused by viruses and less than 2% are complicated by bacterial infection and since there are no specific clinical features to distinguish between bacterial and viral etiologies, the true efficacy of prolonged antibiotic therapy compared to short-course therapy is difficult to assess.

COMMUNITY-ACQUIRED PNEUMONIA

There are no controlled trials that compare short antimicrobial courses in community-acquired pneumonia (CAP) with long courses (more than 7 days). The Infectious Disease Society of America[25] recommends treating pneumonia caused by *Strep. pneumoniae* until the patient is afebrile for 72 hours. Pneumonia caused by bacteria that can cause necrosis of pulmonary parenchyma (e.g. *Staph. aureus* or *P. aeruginosa*) should be treated for longer than 2 weeks, and pneumonia caused by atypical pathogens (e.g. *M. pneumoniae* or *C. pneumoniae*) should be treated for at least 2 weeks. However, these recommendations are not based on randomized clinical trials and are supported by expert opinion only.

ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Approximately 5% of Americans in their middle and late years have chronic bronchitis, which predisposes them to frequent episodes of illness. Acute infectious exacerbation of chronic obstructive pulmonary disease (AE-COPD) contributes considerably to the morbidity and mortality of this population. The benefit of antibiotics in AE-COPD in reducing mortality and morbidity has been demonstrated in several randomized placebo-controlled studies. A meta-analysis has demonstrated a small but statistically significant improvement due to antibiotic therapy in acute exacerbations of COPD. [26]

The administration of therapy for 10–14 days has been the standard treatment. A large number of studies have been published during the last few years which support the use of short-course antimicrobial therapy in AE-COPD. Most studies used different antibiotic regimens that compared the efficacy of a short course (fewer than 7 days) with that of a long course (more than 7 days). All trials enrolled patients with a clinical diagnosis of AE-COPD. Across studies involving 6629 patients published between 1988 and 2001, clinical and bacteriologic efficacy of various classes of antibiotics were equivalent for 3–5 days compared with 8–14 days of therapy.

The new fluoroquinolones have a wide antimicrobial activity (including penicillin-resistant *Strep. pneumoniae, H. influenzae* and atypical pathogens), favorable pharmacokinetics and pharmacodynamics, including high bio-availability, extensive distribution into respiratory fluids, long elimination half-life and potent and rapid killing. Based on their pharmacokinetic and pharmacodynamic profile, a shorter course of fluoroquinolones could have equivalent efficacy to the traditional 10–14 days therapy for AE-COPD. Several randomized trials have demonstrated high efficacy of the new fluoroquinolones in the treatment of AE-COPD. Abbreviated courses of various fluoroquinolones of 5 days' duration had similar efficacy compared to standard duration therapy.

In summary, most studies have demonstrated similar efficacy of a short course of antimicrobial therapy in AE-COPD compared with standard duration therapy. Therefore, abbreviated therapy should be considered for AE-COPD.

URINARY TRACT INFECTIONS

When considering treatment for UTI the prognosis of the untreated infection and the long-term results to be expected from therapy should be measured and compared to the side-effects, cost and inconvenience of different therapeutic regimens. As the prognosis of UTI in nonpregnant adult women is excellent and reinfection is common, therapy probably makes little contribution to the patient's well-being other than alleviating the symptoms. Hundreds of patients have been followed for years with persistent or recurrent infections without documenting progression of renal disease caused by the infection.

Bacteriuria in the elderly is associated with degenerative and debilitating diseases, but does not seem to aggravate the underlying condition. Routine treatment of asymptomatic bacteriuria in the elderly is thus not recommended by most experts.

Asymptomatic as well as symptomatic bacteriuria in preschool children with vesicourethral reflux can result in stunted growth of the kidney, scar formation and, rarely, renal failure. Bacteriuria in pregnancy may also have serious implications — for example, preterm deliveries, low birth weight, etc. Thus, treatment of children and pregnant women is most likely to be beneficial. Symptomatic patients, regardless of age, should be adequately treated, even when infection is likely to recur.

In the past, 7–10 days of therapy were routinely recommended for patients with lower urinary tract symptoms. However, in recent years it has become apparent that most women with lower UTI have only a superficial mucosal infection that can be cured with much shorter courses of therapy, even with a single dose of an antimicrobial agent.

Short-course therapy for UTI with sulfonamides has been intensively studied and more than 70 reports have been published. A meta-analysis of these trials revealed that a single-dose therapy was less effective than longer duration regimens (87% eradication vs 94%), but 3-day regimens were equivalent to longer duration treatments and were associated with lower rates of adverse events.[6] Several trials have demonstrated the efficacy of a single-dose of ciprofloxacin; however, this drug, like most agents studied, appeared to be more effective when given as a 3-day regimen.[27]

Since then, several randomized controlled studies have confirmed that low-dose, short courses (3 days) of fluoroquinolones and short-course TMP-SMX are superior to 7-day treatments of many commonly used drugs (fluoroquinolones, TMP-SMX and nitrofurantoin), mainly due to the lower rate of adverse events.[28] [29] [30]

ß-Lactam short-course studies revealed that all ß-lactam regimens (amoxicillin, pivamecillinam, cefadroxil, etc.) were less effective when given in a shorter than 7 day regimen, demonstrating higher recurrence and lower eradication rates with the short courses.

To summarize: ß-lactam and nitrofurantoin short-course regimens have failed and are less effective than the standard 7-day regimens. Three-day regimens of TMP-SMX are equivalent to longer duration treatments and may be the drug of choice in areas in which resistance to this drug is low. Three-day regimens of fluoroquinolones are equivalent to longer duration regimens and may be more effective than 3-day regimens of TMP-SMX in areas where resistance to the latter is high.

INTESTINAL INFECTIONS

These infections would be the most amenable to abbreviated courses of therapy as in most instances the pathogen is located on the epithelial surface or has penetrated the intestinal epithelial cell but does not proceed to invade other tissues. The pathogen is classically a rapidly dividing bacteria, there is no foreign body and

the peristalsis of the intestine would inhibit biofilm formation. The major obstacles to abbreviated therapy are the penetration of the antibiotic into the intestinal lumen and into the epithelial cells, the adverse effect of fecal material on the antibacterial activity of the agent (binding to fecal material), and the inactivation of the antimicrobial agent by neighboring bacteria that may produce extracellular enzymes, e.g. ß-lactamases, aminoglycoside-inactivating enzymes, etc.

In shigellosis, a randomized double-blind controlled trial compared 5 days of therapy with ciprofloxacin or azithromycin in 70 hospitalized adults men in Bangladesh.[31] Clinical success (determined as disappearance of loose stools on day 5 and temperature <37.8° on day 3) and bacteriological eradication rates were equal in both groups. A study in 135 Vietnamese children with bacillary

dysentery, in a region with a high rate of multidrug-resistant shigellosis (of 63 isolates, 62% were MDR), compared the conventional therapy of nalidixic acid (55mg/kg for 5 days) with two doses of ofloxacin (total 15mg/kg). Resolution times for fever and diarrhea were similar, excretion time for stool pathogens was longer in the nalidixic acid group, there were 25% treatment failures in the nalidixic acid group compared to 10% in the ofloxacin group (p>0.1). The authors concluded that the two regimens were equally effective and thus a preference for the shorter therapy was obvious.[32]

TYPHOID FEVER

Typhoid fever has traditionally been treated with 10–14 days of therapy with chloramphenicol, ceftriaxone, etc. Attempts have been made to shorten this treatment period from 10–14 days to 5–7 days but the long-term relapse rate remained undefined.[33] The current practice is to administer systemic antibiotic until the patient defervesces and to continue with an oral cephalosporin for a total of 10–14 days. The fluoroquinolones, because of their *in vitro* activity against Salmonellae, intracellular penetration and transepithelial intestinal elimination reaching high intraepithelial concentrations, were considered attractive for shorter treatments of typhoid fever and other systemic salmonellosis.[34] Seven days of treatment with ciprofloxacin 500mg twice daily were compared with azithromycin 1g initial oral dose followed by 500mg once daily for 6 additional days in 123 Egyptian patients. Cure was similar in the two groups with similar time to defervescence in both groups with no relapse in either group.[35] When ofloxacin 5-day course (200mg twice daily) was compared with ceftriaxone 3g once daily for 3 days, ofloxacin cured 100% of patients (22/22) while ceftriaxone cured 18/25 patients (72%) (p=0.01) with defervescence in the ofloxacin group occurring far earlier than in the ceftriaxone group.[36] In a study that compared ofloxacin 3 days to 5 days in MDR typhoid fever in 438 patients in Vietnam, both regimens were equally effective.[37] For fluoroquinolone-resistant and nalidixic acid-resistant *S. typhi* (NARST) treated with ofloxacin, the time for blood clearance was double (156h) that for susceptible strains and a third of the NARST patients required retreatment (compared to 0.4% of the susceptible strains). NARST-infected patients were, however, effectively treated with short-course (5 days) azithromycin therapy with rapid defervescence, sterilization of stool cultures and no relapses.[38]

ENDOCARDITIS

In 1971, Paul Beeson wrote that in cases where signs and symptoms were favorable, 2- or 3-week therapy was sufficient whereas in abacteremic patients, in those with long-standing disease, those with large vegetations and those with relatively resistant organisms, it is wiser to continue therapy for 6–10 weeks. Today infections with penicillin-susceptible streptococci and *Streptococcus bovis* (with a penicillin MIC of =0.1µg/ml) require a 2-week regimen of ceftriaxone or penicillin G combined with gentamicin as long as the patient does not have extracardiac foci of infections, myocardial abscesses or prosthetic valve endocarditis. When the pathogen is relatively resistant to penicillin (MIC =0.1 but =0.5µg/ml) penicillin (or ceftriaxone) for 4 weeks are required accompanied in the first 2 weeks by gentamicin. In right-sided staphylococcal endocarditis in intravenous drug abusers, 2-week therapy with methicillin (or nafcillin) combined with gentamicin is effective.[39] [40] The reason for shortening the therapy duration may be greater awareness of the possibility of endocarditis that is now supported by echocardiography, allowing for earlier diagnosis, and a deeper understanding of the pharmacokinetics and pharmacodynamics of the antibiotics used for treating this infection.

NOSOCOMIAL INFECTIONS

The widespread use of antibiotics in hospitals has substantial implications for the cost of care, side effects and spread of resistant micro-organisms. Generally, nosocomial infections are treated for 10–14 days. However, there are no randomized clinical studies evaluating the optimal duration of antimicrobial treatment in most nosocomial infections. As the majority of the hospitalized patients who develop infections are critical patients, immunocompromised or patients who have undergone surgery, or elderly and those with foreign bodies, an abbreviated antimicrobial course could lead to therapeutic failure or relapse. On the other hand, prolonged antimicrobial therapy exposes these patients to drug toxicity and the emergence of resistant micro-organisms at an individual level and also influences the hospital or unit ecology.

Nosocomial pneumonia

Respiratory tract infections account for approximately 50% of all antibiotics prescribed in intensive care units. The American Thoracic Society recommends that the duration should be adapted to the severity of the disease, the time to clinical response and the micro-organism involved.[41] Prolonged antimicrobial treatment is recommended for the following situations: multilobar involvement, cavitation and isolation of *P. aeruginosa* or *Acinetobacter* spp. and pneumonia associated with bacteremia. A short antimicrobial treatment (7–10 days) is recommended for *Staph. aureus* and *H. influenzae* pneumonia.

A prospective nonrandomized clinical study in ventilator-associated pneumonia (VAP) evaluated the efficacy of a clinical guideline restricting the total duration of antimicrobial therapy to 7 days.[42] The mean duration of treatment in the first period was 14.8 days compared to 8.6 days. No significant differences in hospital mortality and hospital lengths of stay were found between the two study groups. However, patients in the before-evaluation group were more likely to develop a second episode of VAP compared with those in the after-evaluation group (24.0% vs 7.7%, p = 0.030).

Currently there are no randomized clinical studies comparing the efficacy of shorter antimicrobial treatment courses in severe VAP. However, in many cases, antibiotic treatment is prescribed for pulmonary infiltrates without pneumonia. Because VAP in the ICU has a high attributable mortality, empiric antimicrobial treatment is prescribed when new pulmonary infiltrates appear, despite a low likelihood of infection. In a randomized clinical study,[43] patients with low likelihood of nosocomial pneumonia (CPIS <6) were randomized to receive ciprofloxacin (experimental group) for 3 days or standard therapy (choice and duration of antibiotics at the physician's discretion). Antibiotics were continued beyond 3 days in 90% of patients in the standard therapy compared with 28% in the ciprofloxacin group. The duration of antibiotics in patients with CPIS <6 and no other documented infection was 3 days compared to 9.8 days in the standard therapy group. Mortality and length of hospital stay did not differ between the two groups. Nevertheless, antimicrobial resistance or superinfection was documented in 15% of patients in the ciprofloxacin group compared with 35% in the control group. Antimicrobial cost was significantly lower in the ciprofloxacin group. Such an approach, in patients with mild-moderate nosocomial pneumonia, including VAP, may lead to significantly lower antimicrobial therapy costs, antimicrobial resistance rates and superinfections. A recent French study[44] compared 8 vs. 15 days of antibiotic therapy in 401 VAP patients. No excess motility or pulmonary infections were detected on day 28 in the short treatment group, favoring short duration therapy for patients with ICU-VAP.

Catheter-related bloodstream infections

Currently, there are no randomized controlled clinical trials evaluating the optimal duration of therapy for bloodstream infection.

Patients with catheter-related bacteremia are separated into those with complicated infections (e.g. endocarditis, septic thrombosis) and those with uncomplicated infections. Patients with an uncomplicated infection should receive 10–14 days of antimicrobial therapy. Patients with complicated bacteremia should receive a prolonged course of antimicrobial therapy (4–6 weeks). There are no randomized trials evaluating the efficacy of abbreviated courses.

Nosocomial *Staph. aureus* bacteremia is a serious and common disease often associated with serious complications including septic thrombosis, infective endocarditis, osteomyelitis and metastatic abscesses. Despite several studies, the optimal duration of antimicrobial treatment for catheter-related *Staph. aureus* bacteremia (CRSAB) remains unknown. In the past, CRSAB has been treated for 4–6 weeks, but recent studies have reported a low complication rate when the duration of treatment was 10–14 days. In several retrospective studies among patients with CRSAB and no early complications, a 10–15-day course of parenteral antibiotics was equivalent to longer courses of therapy.[45] However, treatment with antimicrobial agents for less than 10 days appeared to be inadequate due to a high rate of relapse. Delayed removal of the catheter is associated with a high rate of relapse. A meta-analysis of studies reporting outcome for patients with *Staph. aureus* bacteremia treated with short-course therapy (=2 weeks) has identified 11 studies. [46] Late complication rates ranged from 0% to 29% and the relapse rate was 6.1%. However, most of the studies were uncontrolled.

No controlled trials have assessed the optimal duration of antibiotic treatment of catheter-related bloodstream infection due to Gram-negative bacilli. Randomized trials are necessary to determine the optimal duration of treatment for bloodstream infection caused by various micro-organisms.

CONCLUSION

In summary, there is good evidence for shortening antibiotic therapy provided the correct treatment is applied to the appropriate patient in a well-defined patient population for a clearly defined infection caused by a known pathogen with known antibiotic susceptibilities. Most effort in this field was dedicated to infections that can be treated on an outpatient basis; for severe infections, length of therapy still needs additional data.

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Chapter 193 - ß-Lactam Antibiotics

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INTRODUCTION

In 1928, Alexander Fleming observed that a mold of the genus *Penicillium* inhibited the growth of bacteria in culture.[1] Over a decade later in 1941, Florey, Chain and Abraham used penicillin for the first time in patients with staphylococcal and streptococcal infections.[2] [3] More than a half century later, the ß-lactam antibiotics remain the mainstay of treatment for a variety of bacterial infections (Table 193.1) and now include:

- penicillins (natural penicillins, penicillinase-resistant penicillins, aminopenicillins, carboxypenicillins and ureidopenicillins);
- cephalosporins (first-, second-, third- and fourth-generation);
- monobactams;
- carbapenems; and
- ß-lactamase inhibitor combinations.

PENICILLINS

The natural penicillins are used primarily for the treatment of selected Gram-positive and anaerobic infections as well as selected Gram-negative infections. The penicillinase-resistant penicillins are primarily used for the treatment of infections due to staphylococci, but are also active against other Gram-positive organisms. The aminopenicillins have a similar spectrum of activity as the natural penicillins, but have additional coverage of Gram-negative organisms including many Enterobacteriaceae. When used in conjunction with ß-lactamase inhibitors, they have extended coverage against Gram-positive, Gram-negative and anaerobic organisms that produce ß-lactamases, which normally hydrolyze these agents. The carboxypenicillins and ureidopenicillins cover Gram-negative bacilli that are resistant to the aminopenicillins, in particular *Pseudomonas aeruginosa*. The carboxypenicillins and ureidopenicillins can also be used in conjunction with ß-lactamase inhibitors for extended activity against ß-lactamase-producing organisms.

Cephalosporins

The first-generation cephalosporins have excellent activity against Gram-positive cocci, but can also treat some community-acquired Gram-negative infections. The second-generation cephalosporins have improved Gram-negative coverage compared with that of the first-generation cephalosporins, and selected agents (i.e. cefoxitin and cefotetan) have excellent activity against anaerobes. Third-generation cephalosporins have further improved Gram-negative coverage; ceftazidime is used for *P. aeruginosa* infections, but has limited Gram-positive coverage. Ceftriaxone, a third-generation cephalosporin, not only has excellent Gram-negative activity but also provides excellent coverage of *Streptococcus pneumoniae* and other viridans streptococci. The fourth-generation cephalosporins (cefepime is the only drug available in the USA at this time) have excellent Gram-positive and Gram-negative coverage. No cephalosporin has any activity against *Enterococcus* spp.

Monobactams

The monobactams (aztreonam is the only available agent in this class) are effective only against aerobic Gram-negative organisms and have no activity against Gram-positive organisms or anaerobes.

Carbapenems

The carbapenems (imipenem, meropenem and ertapenem) have the broadest bacterial coverage of the ß-lactam antibiotics, treating most infections with Gram-positive, Gram-negative and anaerobic bacteria.

Mechanism of action

ß-Lactam antibiotics are bactericidal. Their mechanism of action involves interference with bacterial cell wall synthesis. More specifically, they attach to penicillin-binding proteins on the inner surface of the bacterial cell membrane, thereby interrupting the transpeptidation process that cross-links the amino acids of the individual petidoglycan components of the forming bacterial cell wall. Ultimately, loss of viability and, in some bacteria, lysis occurs as the result of the activation of autolytic enzymes through a poorly understood mechanism. [4] Of all ß-lactam agents, only the carbapenems possess an extended inhibitory effect on bacterial growth after levels of these agents are below inhibitory levels (postantibiotic effect). In fact, only the carbapenem class of ß-lactam antibiotics exhibits concentration-dependent killing — maximal bactericidal activity occurs at 4–5 times the minimum inhibitory concentration (MIC) of the organism, whereas all other ß-lactam classes (penicillins, cephalosporins, monobactams) exhibit time-dependent killing pharmacodynamics (activity is greatest only during a period when the concentration is above the MIC) and there is no postantibiotic effect. In addition, regrowth of bacteria occurs rapidly after withdrawal of cephalosporins.

Bacterial resistance

Three major mechanisms lead to bacterial resistance to ß-lactam antibiotics:

- failure of the antibiotic to penetrate the bacterial cell membrane;
- alterations in the penicillin-binding proteins that reduce the binding affinities of the ß-lactams (intrinsic resistance);[5] and
- bacterial production of ß-lactamases, which hydrolyze the ß-lactam ring and render it inactive.

The most important and most common cause of resistance is the production of ß-lactamases. [6] Strategies to combat resistance, including the use of ß-lactamase inhibitors, are discussed later in this chapter.

PHARMACOKINETICS AND DISTRIBUTION

Absorption

The ß-lactams have variable absorption from the gastrointestinal tract. Some agents, such as the antipseudomonal penicillins and methicillin, are acid-labile and cannot be taken orally. The absorption characteristics and pharmacokinetics of the ß-lactams are shown in Table 193.2 . Of note, amoxicillin is almost totally absorbed when administered orally whereas ampicillin is only partially absorbed.

TABLE 193-1 -- The different classes of ß-lactam antibiotics and sample indications for selected agents.

THE DIFFERENT CLASSES OF ß-LACTAM ANTIBIOTICS AND SAMPLE INDICATIONS FOR SELECTED AGENTS			
Class of ß-lactam	**Example**	**Route**	**Sample indication**
Penicillins			
Natural penicillin	Penicillin V (phenoxymethyl penicillin)	po	Streptococcal pharyngitis
	Penicillin G (benzylpenicillin)	iv	Neurosyphilis
Penicillinase-resistant penicillin	Flucloxacillin	po	Cellulitis
	Nafcillin	iv	*Staphylococcus aureus* endocarditis
Aminopenicillin	Amoxicillin	po	Endocarditis prophylaxis
	Ampicillin	iv	*Listeria monocytogenes meningitis*
Amidinopenicillin	Piv-mecillinam	po	Urinary tract infection
Carboxypenicillin	Ticarcillin	iv	*Pseudomonas aeruginosa* pneumonia
Ureidopenicillin	Piperacillin	iv	Cholangitis
Cephalosporins			
First-generation	Cephalexin	po	Cellulitis
	Cefazolin	iv	Prophylaxis before surgery
Second-generation	Cefuroxime	po	Sinusitis
	Cefaxitin	iv	Intra-abdominal infection
Third-generation	Cefixime	po	*Escherichia coli* urinary tract infection (cystitis)
	Ceftriaxone	iv	Pneumococcal meningitis
	Ceftazidime	iv	*P. aeruginosa* pneumonia
Fourth-generation	Cefepime	iv	Septicemia secondary to Enterobacteriaceae resistant to other agents
Monobactam	Aztreonam	iv	Gram-negative septicemia
Carbapenems	Imipenem	iv	Monotherapy for intra-abdominal infections
	Meropenem	iv	Monotherapy for ultra-abdominal infections
	Ertapenem	iv	Complicated skin and soft tissue infections
ß-Lactamase inhibitors	Clavulanic acid (+ amoxicillin)	po	Animal bite
	Clavulanic acid (+ ticarcillin)	iv	Neutropenic sepsis
	Sulbactam (+ ampicillin)	iv	Head and neck infection
	Tazobactam (+ piperacillin)	iv	Nosocomial sepsis

The presence of food in the stomach can delay absorption and can lower the peak serum concentration attainable for some ß-lactams, such as ampicillin, cefaclor, cefixime and ceftibuten. In contrast, food can increase the absorption of cefuroxime and cefpodoxime.

Distribution

Following absorption, ß-lactams are variably bound to serum proteins, mostly albumin. Protein-bound drug does not exert antimicrobial activity, but binding is reversible. In general, the degree to which a ß-lactam antibiotic is protein bound does not influence the decision to use the antibiotic and the effect of protein binding on drug efficacy is not clear.[14] Excretion of the ß-lactams is primarily renal (glomerular filtration and tubular secretion) and, in general, the serum half-life of these drugs is short.

Procaine penicillin G and benzathine penicillin G are intramuscular preparations that are absorbed slowly, allowing for longer dosing intervals, but the half-life of the drug is the same. Nafcillin, the ureidopenicillins (20–30%), cefoperazone (70%), ceftriaxone (40%) and cefotetan (20%) have significant excretion in bile.[10]

Imipenem is inactivated by an enzyme present on the renal brush border, dehydropeptidase I. Cilastatin is a dehydropeptidase inhibitor administered along with imipenem to prevent subtherapeutic levels of the antibiotic in urine. Cilastatin does not possess any antimicrobial activity nor does it alter the pharmacokinetics of other drugs.[15]

The ß-lactam antibiotics achieve therapeutic concentrations in most tissues such as lung, kidney, bone, muscle and liver, and in secretions such as synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid and bile. The microenvironment that may be found in an abscess, including a low pH, the presence of neutrophils and associated proteins, and low oxygen tension, does not inhibit the function of ß-lactam antibiotics. However, ß-lactams do not penetrate host cells and are therefore ineffective against intracellular organisms. Low concentrations of ß-lactams are found in prostatic secretions, brain tissue, intraocular fluid and cerebrospinal fluid (CSF; Fig. 193.1). In the presence of inflammation, however, concentrations in the CSF are much higher, accounting for the efficacy of some ß-lactams in the treatment of meningitis.[16] The penicillins and cephalosporins can penetrate the aqueous humor of the eye, but do not reach therapeutic levels in the posterior chamber.

ROUTE OF ADMINISTRATION AND DOSAGE

ß-Lactams are available for oral, intravenous and intramuscular use. Generic and trade names, routes of administration and standard dosages for adult and pediatric patients with normal renal function are listed in Table 193.3 . In dosing the ß-lactam antibiotics, it is important to remember that:

- food can have an effect on oral absorption (e.g. food decreases absorption of ampicillin); and
- absorption of both cefuroxime and cefpodoxime are decreased by H_2 -blockers or nonabsorbable antacids.[17]

In general, high doses of a ß-lactam antibiotic should be used in patients who are neutropenic and for severe infections such as bacteremia, meningitis and otitis media in children. Dosages must be reduced in patients with renal failure (with the exception of nafcillin, cefoperazone and ceftriaxone) and in neonates, in whom renal function is not yet fully developed. Dosages of nafcillin, the ureidopenicillins and cefoperazone must be reduced in patients with severe liver disease (see Dosage in special circumstances, below).

INDICATIONS

The ß-lactam antibiotics can be effectively used for the treatment of a variety of infections. These agents are widely distributed following administration and are routinely used in the treatment of sinusitis, otitis, pharyngitis, epiglottitis, dental infections, bronchitis, pneumonia, meningitis, infections of the genitourinary tract (including cervicitis and urethritis caused by *Neisseria gonorrhoeae*), peritonitis, biliary and gastrointestinal infections, skin and soft tissue infections, osteomyelitis, septic arthritis and infection of prosthetic devices, including venous access catheters. The choice of antibiotic and recommended duration of therapy for these infections is discussed in the chapters on the specific diseases. The remainder of this section focuses on the use of the ß-lactam antibiotics in special circumstances. Table 193.4 summarizes the relative susceptibilities of various micro-organisms to the ß-lactam antibiotics.

TABLE 193-2 -- Pharmacokinetics for selected ß-lactam antibiotics. [7] [8] [9] [10] [11] [12] [13]

PHARMACOKINETICS FOR SELECTED ß-LACTAM ANTIBIOTICS					
Generic name	**Oral absorption (%)**	**Effect of food on absorption**	**Protein binding (%)**	**Serum half-life (h)**	**Biliary excretion (%)**
Penicillins					
Amoxicillin/clavutanic acid	75/-	-/Increases	20/9–25	1.0/1.0	
Ampicillin/sulbactam	40/-	Decreases/-	20/38	0.8–1.5/1.0	3/-
Azlocillin	0		30	0.8–1.5	20–30
Bacampicillin		None	20	1.1	
Carbenicillin	30				0
Cloxacillin	50	Decreases			
Dicloxacillin	50	Decreases	95	0.7	
Methicillin			35–60	0.5–1.0	
Mezlocillin	0		16–42	1.0	20–30
Nafcillin	Erratic	Decreases	85	0.5	60–70
Oxacillin	30	Decreases	93	0.5	
Penicillin G	20	Decreases	60	0.5	
Penicillin V	60	None	80	0.6	
Piperacillin/tazobactam	0		16–30/30	0.6–1.2/1.0	20–30/-
Ticarcillin/clavulanic acid	0/-		45/9–25	1.2/1.0	4/-
Cephalosporins					
First-generation					
Cefadroxil		None	20	1.4	
Cefazolin			80	1.4–2.0	
Cephalexin	95	None	20	0.7–1.1	
Cephalothin			65	0.5–1.0	
Cephapirin			45–60	0.4	
Cephadrine	90	None	10	0.7–1.3	
Second-generation					
Cefaclor	52–95	Decreases	25	0.6–0.9	
Cefamandole			75	0.5–1.0	
Cefdinir	16–25	-	60–70	1.7	-
Cefmetazole			75	1.2	
Cefonicid			>90	4.5	
Cefotetan			88	3–4.6	20
Cefoxitin			41–75	0.7–1.1	
Cefprozil	95	None	36	1.3	
Ceftibuten		Decreases		2–2.4	
Cefuroxime	30–52	Increases	50	1.2–1.9	
Loracarbef	90	Decreases	25	1.0	
Third-generation					
Cefditoren	14	Increased with fatty meal	88	1.6	-
Cefixime	40–50	Delays peak	65	3–4	
Cefoperazone			82–93	2.0	70
Cefotaxime			37	1.0	8
Cefpodoxime	30–50	Increases	22–33	2.1–2.8	
Ceftazidime			<10	1.9	<1
Ceftizoxime			30	1.7	<1
Ceftriaxone			85–95	5.8–8.7	40
Fourth-generation					
Cefepime			20	2	
Monobactam					
Aztreonam			55	1.5–2.0	10
Carbapenems					
Ertapenem	-	-	85–95	4	-
Imipenem/cilastatin			20/40	1–3/1.0	
Meropenem			2	1.0	
Absence of information is due to either lack of information or no effect.					

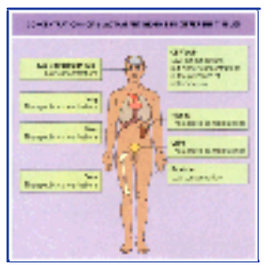

Figure 193-1 Concentrations of ß-lactam antibiotics in different tissues.

Prophylaxis

Antimicrobial prophylaxis in surgery (see also Chapter 190)

ß-Lactam antibiotics are commonly used to decrease the incidence of infection for selected surgical procedures.[18] A single dose of cefazolin has been shown to decrease the incidence of wound infection for selected 'clean' procedures and is used commonly for cardiac, noncardiac thoracic, vascular, orthopedic, ophthalmic and neurosurgical procedures. For 'clean-contaminated' procedures in which colonized mucosa is violated, such as head and neck surgery, abdominal surgery and gynecologic surgery, antibiotic prophylaxis may also be used. Cefazolin or ampicillin-sulbactam can be used before head and neck surgery. Patients who are obese, who have reduced intestinal motility or who have decreased gastric acidity may be at high risk for infection following abdominal surgery and may also be candidates for prophylaxis with cefazolin.

Similarly, patients undergoing biliary tract surgery who are at high risk of infection due to advanced age, acute cholecystitis, a nonfunctioning gallbladder, obstructive jaundice, or choledocholithiasis may benefit from preoperative cefazolin. In the setting of acute appendicitis, cefoxitin or cefotetan have been shown to decrease the incidence of infection postoperatively. Women undergoing vaginal or abdominal hysterectomy, emergency cesarean section or first trimester abortion may be candidates for antibiotic prophylaxis with cefazolin or other agents.

Antibiotics should be used not only as prophylaxis but also as treatment for 'dirty' surgical procedures in which the surgical site is obviously contaminated by bacteria (e.g. a perforated viscus). Antimicrobial prophylaxis in surgery is summarized periodically in the publication *The Medical Letter*.[18]

Prophylaxis is not routinely recommended for patients undergoing cardiac catheterization, gastrointestinal endoscopy, herniorrhaphy, varicose vein surgery, most plastic surgery, arterial puncture, thoracentesis, paracentesis, repair of simple lacerations, outpatient treatment of burns, dental extractions or root canal therapy. Antimicrobial prophylaxis before breast surgery is controversial. A recent study suggests that antibiotic prophylaxis for endoscopic retrograde cholangiopancreatography does not prevent cholangitis.[19]

Endocarditis prophylaxis

Patients who have underlying cardiac or congenital valvular abnormalities are candidates for antibiotic prophylaxis when they undergo procedures that can cause transient bacteremia. Cardiac conditions that place a patient at increased risk of endocarditis include prosthetic valves, a previous history of endocarditis, most congenital cardiac abnormalities (except an isolated secundum atrial septal defect), rheumatic and other acquired valvular dysfunction, hypertrophic cardiomyopathy and mitral valve prolapse when accompanied by regurgitation.

Procedures that can cause transient bacteremia and may place a patient at risk of endocarditis include:

- dental procedures (including professional cleaning),
- tonsillectomy and/or adenoidectomy,
- surgical procedures involving intestinal or respiratory mucosa,
- rigid bronchoscopy,
- sclerotherapy for esophageal varices,
- esophageal dilatation,
- gallbladder surgery,
- cystoscopy,
- urethral dilatation,
- urethral catheterization and/or urinary tract surgery if there is infection,
- prostatic surgery, and
- incision and drainage of infected tissue.

The antibiotic of choice for prophylaxis for dental, oral or upper respiratory tract manipulations is a single dose of amoxicillin 2g taken orally 1 hour before the procedure. For high-risk patients undergoing genitourinary or gastrointestinal procedures, ampicillin 2g intravenously with gentamicin 1.5mg/kg intravenously should be given within 30 minutes of starting the procedure and then ampicillin or amoxicillin 1g orally should be given 6 hours later. Alternatives exist for patients who are allergic to penicillins.[20]

Rheumatic fever prophylaxis

Because patients who have had acute rheumatic fever are at risk of recurrent attacks if they have group A streptococcal infections, the American Heart Association recommends prophylaxis with penicillin for these patients. The dose is either a single injection of benzathine penicillin G 1.2 million U intramuscularly every 4 weeks or penicillin V 250mg orally q12h. It seems that prophylaxis can be safely discontinued in patients with a history of carditis after 10 years or at age 25. In patients without a history of carditis, prophylaxis can be stopped after 5 years or at age 18. The decision to stop prophylaxis, however, must be individualized because a patient who is at continued risk of streptococcal infection (e.g. teacher or pediatrician) may benefit from continued antibiotic prophylaxis (see also Chapter 60).[21]

Pneumococcal infections

Penicillin is the antibiotic of choice for infections (such as pneumonia, bacteremia or meningitis) caused by susceptible strains of *S. pneumoniae*. However, an increasing proportion of isolates of this pathogen are resistant to penicillin. Intermediate resistance is defined as a strain with an MIC of 0.1–1µg/ml and high-level resistance is defined by an MIC greater than or equal to 2µg/ml. The E test (an antibiotic strip with graded concentrations) is a convenient and reliable method for the detection of penicillin or cephalosporin resistance in pneumococci.[22] In the early 1990s, 4–5% of clinical isolates in the USA were found to be either intermediately or highly resistant to penicillin.[23] [24] In a study that examined isolates collected from outpatients at different sites in the USA between 1994 and 1995, 14.1%

TABLE 193-3 -- ß-Lactams — spectrum of activity, trade names, routes of administration and dosage in patients with normal renal function. [9] *

ß-LACTAMS — SPECTRUM OF ACTIVITY, TRADE NAMES, ROUTES OF ADMINISTRATION AND DOSAGE IN PATIENTS WITH NORMAL RENAL FUNCTION					
Class	**Antimicrobial spectrum**	**Generic name**	**Trade name**	**Route**	**Adult dose (pediatric dose) for normal renal function**
Penicillins					

Natural penicillins	Gram-positives, anaerobes, selected Gram-negatives	Penicillin V	Betapen, Ledercillin, Pen-Vee, Robicillin, S-K penicillin, V-cillin, Veetids	po	250–500mg q6h (25–50mg/kg/day divided q6h)
		Penicillin G, benzathine	Bicillin, Permapen	im	600,000–1.2 million U every 1–4 weeks (300,000–600,000U every 1–4 weeks for weight <27kg; 900,000–1.2 million U every 1–4 weeks for weight >27kg)
		Penicillin G, procaine	Crysticillin, Duracillin, Wycillin	im	600,000–1.2 million U q12-24h
		Penicillin G, sodium or potassium		iv	1–4 million U q4h
Penicillinase-resistant penicillins	Penicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Also active against streptococci	Cloxacillin	Tegopen	po	250–500mg q6h
		Dicloxacillin	Dynapen, Pathocil, Veracillin	po	125–500mg q6h (12–25mg/kg/day divided q6h)
		Flucloxacillin*	Floxapen	po	500mg q6h
				im/iv	1–2g q6h
		Methicillin	Celbenin, Staphcillin	im/iv	1–2g q4-6h
		Nafcillin	Unipen	im/iv	1–2g q4-6h (50mg/kg/day divided q4-6h)
				po	250–500mg q4-6h
		Oxacillin	Bactocill, Prostaphlin	im/iv	1–2g q4-6h (50–100mg/kg/day divided q4-6h)
				po	250–500mg q4-6h
Aminopenicillins	Same as penicillin G plus added Gramnegative cocci and Enterobacteriaceae	Amoxicillin	Amoxil, Larotid, Polymax	po	250–500mg q8h (20–40mg/kg/day divided q8h)
		Ampicillin	Alpen, Amcil, Omipen, Penbritin, Polycillin, Principen, Probampcin, Totacillin	po	250–500mg q6h
				im/iv	1–2g q4-6h (50–200mg/kg/day divided q4-6h)
		Bacampicillin		po	400–800mg q12h (25mg/kg/day divided q12h)
	ß-Lactamase inhibitors expand Gram-positive, Gram-negative and anaerobic coverage	Amoxicillin-clavulanic acid	Augmentin	po	250–500mg q8-12h or 875mg q12h (20–40mg/kg/day divided q8-12h)
		Ampicillin-sulbactam	Unasyn	im/iv	1.5–3.0g q6h
Carboxypenicillins	Gram-negative aerobic rods resistant to ampicillin, including *Pseudomonas aeruginosa*	Ticarcillin	Ticar	im/iv	3–4g q4-6h (200–300mg/kg/day divided q4-6h)
		Ticarcillin-clavulanic acid	Timentin	iv	3.1g q4-6h (200–300mg/kg/day ticarcillin divided q4-6h)
Ureidopenicillins	Similar to carboxypenicillins	Azlocillin	Azlin	im/iv	3–4g q4-6h (90–120mg/kg/day divided q6-8h)
		Mezlocillin	Mezlin	im/iv	3–4g q4-6h (300mg/kg/day divided q4h)
		Piperacillin	Pipral, Pipracil	im/iv	3–4g q4-6h
		Piperacillin-tazobactam	Zosyn	iv	3.375g q4-6h
Cephalosporins					
First-generation	Gram-positive cocci and some community-acquired Gram-negative bacilli	Cefadroxil	Duricef, Ultracef	po	1–2g/day q24h or q12h (30mg/kg/day divided q12h)
		Cefazolin	Ancef, Kefzol	im/iv	0.5–1.5g q6-8h (25–100mg/kg/day divided q6-8h)
		Cephalexin	Biocef, Keflex, Keftab	po	250–500mg q6h (25–100mg/kg/day divided q6h)
		Cephalothin	Keffin, Seffin	im/iv	0.5–2g q4-6h (100mg/kg/day divided q4-6h)
		Cephapirin	Cefadyl	im/iv	0.5–2g q4-6h
		Cephradine	Anspor, Velosef	po	1–2g/day divided q6-12h
				im/iv	1g q6h

Second-generation	Improved Gram-negative coverage as compared with first-generation; some agents have activity against anaerobes	Cefaclor	Ceclor	po	250–500mg q8h (20–40mg/kg/day divided q8h)
		Cefamandole	Mandol	im/iv	0.5–1.0g q4-8h (50–100mg/kg/day divided q4-8h)
		Cefdinir	Omnicef	po	300mg po q12h or 600mg po q24h (7mg/kg po q12h or 14mg/kg po q24h)
		Cefmetazole	Zefazone	im/iv	2g q6-12h
		Cefonicid	Monocid	im/iv	1–2g q24h
		Cefotetan	Cefotan	im/iv	1–2g q12h
		Cefoxitin	Mefoxin	im/iv	1–2g q6-8h (80–160mg/kg/day divided q6h)
		Cefprozil	Cefzil	po	250–500mg q24h or divided q12h (7.5–15mg/kg/day divided q12h)
		Ceftibuten	Cedax	po	400mg q24h (9mg/kg/day)
		Cefuroxime	Ceftin	po	250–500mg q12h (20–30mg/kg/day divided q12h)
			Kefurox, Zinacef	im/iv	0.75–1.5g q8h (50–240mg/kg/day divided q8h)
		Loracarbef	Lorabid	po	200–400mg q12h (15–30mg/kg/day divided q12h)
Third-generation	Improved Gram-negative coverage; excellent *Streptococcus pneumoniae* coverage (ceftriaxone, cefotaxime), modest staphylococcal coverage, excellent *P. aeruginosa* coverage (ceftazidime, cefoperazone)	Cefditoren	Spectracef	po	200–400mg po q12h
		Cefixime	Suprax	po	400mg/day q24h or divided q12h (8mg/kg/day q24h or divided q12h)
		Cefoperazone	Cefobid	im/iv	2–4g q12h
		Cefotaxime	Claforan	im/iv	1–2g q4-12h (50–180mg/kg/day divided q4-6h)
		Cefpodoxime	Vantin	po	200mg q12h (10mg/kg/day q24h or divided q12h)
		Ceftazidime	Fortaz, Tazicef, Tazidime	im/iv	1–2g q8-12h (90–150mg/kg/day divided q8h)
		Ceftizoxime	Cefizox	im/iv	0.5–4g q8-12h (150–200mg/kg/day divided q6-8h)
		Ceftriaxone	Rocephin	im/iv	1–2g q24h (50–100mg/kg/day divided q12-24h)
Fourth-generation	Excellent Gram-positive and Gram-negative coverage (including *P. aeruginosa*)	Cefepime	Maxipime	im/iv	0.5–2g q12h
Monobactam	Gram-negatives including *P. aeruginosa*, but no Gram-positives and no anaerobes	Aztreonam	Azactam	im/iv	0.5–2g q6-12h
Carbapenems	Excellent activity against Gram-positives, Gram-negatives (including *P. aeruginosa*) and anaerobes	Ertapenem	Invanz	im/iv	1g q24h
		Imipenem-cilastatin	Primaxin	im/iv	0.25–1g q6-8h
		Meropenem	Merrem	iv	1–2g q8h (60–120mg/kg/day divided q8h)

* Not available in the USA. Note that the trade name may differ in other parts of the world.

had intermediate resistance and 9.5% were highly resistant.[25] Because of the emergence of resistance, some suggest that suspected cases of pneumococcal pneumonia and meningitis should be treated with vancomycin and/or a third-generation cephalosporin such as ceftriaxone until susceptibilities are known (see Chapter 34). There have been reports of failure of third-generation cephalosporins in the treatment of penicillin-resistant pneumococcal meningitis, again suggesting that vancomycin should be included until susceptibilities are known.[26]

Staphylococcal infections

Soon after the introduction of penicillin for the treatment of staphylococcal infections, penicillinase-producing strains became so common that it was no longer effective. Penicillinase-resistant penicillins (nafcillin, oxacillin, methicillin, dicloxacillin, cloxacillin and flucloxacillin) are now the agents of choice for susceptible strains of *Staphylococcus aureus*. Other ß-lactam antibiotics that are effective in the treatment of staphylococcal infections are:

- the aminopenicillins in combination with a ß-lactamase inhibitor (ampicillin-sulbactam or amoxicillin-clavulanate);
- the antipseudomonal penicillins in combination with a ß-lactamase inhibitor (ticarcillin-clavulanate, piperacillin-tazobactam); and
- the carbapenems (ertapenem, imipenem and meropenem).

The first-generation cephalosporins, which are as effective as the penicillinase-resistant penicillins in the treatment of staphylococcal

TABLE 193-4 -- Relative susceptibilities to ß-lactam antibiotics.[* †]

SUSCEPTIBILITIES OF MICRO-ORGANISMS TO ß-LACTAM ANTIBIOTICS						
Generic name	**Streptococci**	**Penicillinase-producing *S. aureus****	**Enterococci**	**Enteric Gram-negative bacilli†**	***Pseudomonas aeruginosa***	**Anaerobes**
Penicillins						
Amoxicillin	+++	0	+++	+	0	+
Amoxicillin-clavulanate	+++	++	+++	+++	0	+++
Ampicillin	+++	0	+++	+	0	+
Ampicillin-sulbactam	+++	++	+++	+++	0	+++
Azlocillin	++	0	+	++	+++	++
Bacampicillin	+++	0	+++	+	0	+
Carbenicillin	++	0	+	++	++	+

Cloxacillin	++	+++	0	0	0	0
Dicloxacillin	++	+++	0	0	0	0
Flucloxacillin	++	+++	0	0	0	0
Methicillin	++	+++	0	0	0	0
Mezlocillin	++	0	++	++	+++	++
Nafcillin	++	+++	0	0	0	0
Oxacillin	++	+++	0	0	0	0
Penicillin	+++	0	+++	0	0	+
Piperacillin	+++	0	++	++	+++	+
Piperacillin-tazobactam	+++	++	++	+++	+++	+++
Ticarcillin	+++	+	+	++	+++	++
Ticarcillin-clavulanate	+++	++	+	+++	+++	+++
Cephalosporins						
Cefaclor	++	++	0	++	0	+
Cefadroxil	++	++	0	++	0	+
Cefamandole	++	++	0	++	0	+
Cefazolin	+++	+++	0	+	0	+
Cefditoren	+++	++	0	++	0	+
Cefdinir	+++	++	0	++	0	0
Cefepime	+++	++	0	+++	+++	+
Cefixime[1]	+++	0	0	+++	0	+
Cefmetazole	++	+	0	++	0	++
Cefonicid	++	++	0	++	0	+
Cefoperazone	+	+	0	++	++	+
Cefotaxime	++	++	0	+++	+	+
Cefotetan	++	+	0	++	0	+++
Cefoxitin	++	+	0	++	0	+++
Cefpodoxime	+++	++	0	++	0	++
Cefprozil	++	+	0	++	0	+
Ceftazidime	+	+	0	+++	+++	+
Ceftibuten	++	0	0	+	0	0
Ceftizoxime	++	+	0	++	+	++
Ceftriaxone	+++	+	0	+++	+	+
Cefuroxime	+++	++	0	++	0	+
Cephalexin	+++	+++	0	+	0	+
Cephalothin	+++	+++	0	+	0	+
Cephapirin	++	+	0	++	0	+
Cephradine	++	+	0	++	0	+
Loracarbef	+++	+++	0	++	0	+
Monobactams						
Aztreonam	0	0	0	+++	+++	0
Carbapenems						
Ertapenem	+++	+++	+	+++	0	+++
Imipenem	+++	+++	++	+++	+++	+++
Meropenem	+++	+++	++	+++	+++	+++

* Methicillin-sensitive *Staphylococcus aureus* and *S. epidermidis*. All methicillin-resistant *S. aureus* and *S. epidermidis* are resistant to all ß-lactams.
†Primarily *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Proteus mirabilis*.
1. Discontinued in the USA. MMWR 2002;51:1052.

infections, require less frequent dosing and may be used in patients with a history of mild penicillin allergy.

It is extremely important to remember that isolates of *S. aureus* or *Staphylococcus epidermidis* that are resistant to methicillin should be considered to be resistant to all other ß-lactam antibiotics, including the cephalosporins and carbapenems, even if in-vitro testing suggests otherwise.[27]

Gram-positive bacilli

Penicillin G is the treatment of choice for:

- infections (oral-cervicofacial, thoracic, abdominal) due to actinomycosis;
- elimination of the carrier state of diphtheria;
- infections (pulmonary, cutaneous, gastrointestinal) due to anthrax;
- gas gangrene caused by species of *Clostridium* spp.; and
- erysipeloid caused by *Erysipelothrix rhusiopathiae*.

Either penicillin G or ampicillin may be used for infections caused by *Listeria monocytogenes*. No cephalosporin has any activity against *L. monocytogenes*.

Infections caused by Gram-negative organisms including *Pseudomonas aeruginosa*

The ß-lactam antibiotics that have activity against *P. aeruginosa* are ticarcillin, carbenicillin, azlocillin, mezlocillin, piperacillin, ceftazidime, cefoperazone, cefepime, aztreonam, imipenem and meropenem. During treatment of pseudomonal infections, resistance to all ß-lactam agents used as sole therapy has been observed.[28] For this reason, a suitable ß-lactam antibiotic is generally used in conjunction with an aminoglycoside.

Antipseudomonal penicillins are often used in conjunction with a ß-lactamase inhibitor — for example ticarcillin-clavulanic acid or piperacillin-tazobactam — to extend their spectrum. The ß-lactamase inhibitor does not usually confer activity against *Pseudomonas* spp. that are resistant to the ß-lactam because the mechanism of resistance is not due to ß-lactamase production.[29] Treatment failures of nosocomial pneumonia caused by *P. aeruginosa* have occurred when piperacillin-tazobactam was used in the dose recommended by the manufacturer (3.375g intravenously q6h), and so this agent should be used in higher doses and in conjunction with an aminoglycoside when treating infections caused by or thought to be caused by *P. aeruginosa*.

The development of drug-resistant isolates of *P. aeruginosa* and other Gram-negative bacilli has become a problem in nosocomial infections. These micro-organisms are commonly found in intensive care units where patients can be on broad-spectrum antibiotics for prolonged periods of time. Many organisms produce inducible and extended-spectrum ß-lactamases. In fact, certain bacteria (*Citrobacter freundii* and *Serratia* spp., *Proteus, Providencia, Pseudomonas, Enterobacter* and *Acinetobacter*) have developed resistance to cephalosporins during therapy.[30] [31] Many recommend the concurrent use of an aminoglycoside in conjunction with a cephalosporin for the treatment of infections caused by these bacteria to prevent therapeutic failures.

Because of its broad antibiotic spectrum of activity against Gramnegative organisms resistant to other antibiotics, imipenem is frequently used in the treatment of nosocomial infections. Alternatives to the use of imipenem for resistant Gram-negative infections are now available and are discussed in the following two paragraphs.

Cefepime is a fourth-generation cephalosporin that is effective in the treatment of severe infections of the lower respiratory and urinary tracts, the skin and soft tissue, the female reproductive tract and neutropenic patients with fever. It has been shown to be more effective than ceftazidime in the treatment of pneumonia in patients with cystic fibrosis where *P. aeruginosa* is a common pathogen.[32] In addition to having activity against strains of *P. aeruginosa* resistant to ceftazidime, cefepime has also shown activity against *Enterobacter* spp. that are resistant to other ß-lactam antibiotics.[33] It has a low potential for inducing bacterial resistance, excellent activity against nonenterococcal streptococci and activity against staphylococci similar to that of cefotaxime. It has little or no activity against *Bacteroides fragilis* and other anaerobes.

Meropenem is a carbapenem antibiotic that has a very broad spectrum of activity, similar to that of imipenem. Imipenem has more activity than meropenem against staphylococci and enterococci, but meropenem provides better coverage of *Pseudomonas*, *Enterobacter, Klebsiella, Morganella, Providencia, Alcaligenes, Aeromonas, Moraxella, Kingella, Actinobacillus, Pasteurella* and *Haemophilus* spp.[34] [35] In one in-vitro study many isolates of *P. aeruginosa* resistant to imipenem were found to be sensitive to meropenem. [36] Meropenem has been effective in abdominal infections, meningitis in children and adults, community-acquired and nosocomial pneumonia, and neutropenic fever.[37]

Anaerobic infections

Anaerobic bacteria may play a significant role in brain abscess, dental infection, sinusitis, lung abscess, intra-abdominal abscess and bone and soft tissue infection. ß-Lactam antibiotics have been used extensively in the treatment of anaerobic infections, but there is a trend for an increased resistance of anaerobes to some ß-lactam antibiotics. As with the aerobes, the most common mechanism of resistance is the production of ß-lactamase.[38]

Most *Clostridium* strains (with the exception of some strains of *C. ramosum, C. clostridiforme* and *C. innocuum*) remain susceptible to penicillin. Penicillin resistance is increasingly seen in the genus *Fusobacterium*, most commonly in *F. varium* and *F. mortiferum,* and although generally still sensitive to penicillin, the MICs for *F. nucleatum* have increased. Penicillin resistance is a major problem encountered in the treatment of infections caused by *B. fragilis* and other *Bacteroides* spp.

Penicillin is more effective than nafcillin against anaerobes. Ticarcillin, mezlocillin and piperacillin also have excellent activity against anaerobes, although there has been an increase in *B. fragilis* strains resistant to ticarcillin. Of the ß-lactamase stable cephalosporins, cefoxitin, cefotetan, cefmetazole and ceftizoxime all show activity against anaerobes. Cefoxitin remains the most active cephalosporin against *B. fragilis*. Resistance to these cephalosporins is seen with some species of *Clostridium*, *Fusobacterium* and non-spore-forming Gram-positive rods. The first-generation cephalosporins such as cefazolin and cephalothin have poor activity against the Gram-negative anaerobes whereas the third-generation cephalosporins cefotaxime, cefoperazone and ceftriaxone have only modest activity (resistance seen in 30–60% of strains) and are therefore not the agents of choice for the empiric treatment of anaerobic infections. Cefotaxime has a desacetyl metabolite that works synergistically with the parent compound in the treatment of some anaerobic species in vitro, but is still not a primary agent for anaerobic infections in vivo. Ceftazidime has poor activity against both Gram-positive and Gram-negative anaerobes.

ß-Lactamases are responsible for most resistance to ß-lactam antibiotics in anaerobes. The ß-lactamases in the *B. fragilis* group are typically cephalosporinases, whereas those in non-fragilis *Bacteroides* spp., *Clostridium* spp. and *F. nucleatum* are penicillinases. Almost all *B. fragilis* isolates produce ß-lactamases. ß-Lactamase production has not been reported in strains of *Clostridium perfringens*.

The addition of a ß-lactamase inhibitor increases the activity of some of the ß-lactams against ß-lactamase producing anaerobes, in particular *Bacteroides* spp., and so ticarcillin-clavulanate, piperacillin-tazobactam, amoxicillin-clavulanate and ampicillin-sulbactam

are effective. The most active ß-lactam agents against anaerobic isolates in the USA are imipenem, meropenem and ertapenem. Interestingly, in Japan, anaerobe resistance to imipenem is becoming a clinical problem, but this has not yet occurred in the USA. Aztreonam has no activity against anaerobes and must be used with other agents when treating mixed aerobic and anaerobic infections.[39]

Central nervous system infections (meningitis)

Certain ß-lactam antibiotics are able to penetrate inflamed meninges and are commonly used to treat meningitis (e.g. penicillin G, ampicillin, nafcillin, oxacillin, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime and meropenem). The most common pathogens in a series of adult patients with meningitis in descending order were *S. pneumoniae*, *Neisseria meningitidis* and *L. monocytogenes*.[40] In the pediatric population, *Haemophilus influenzae* also plays a role, but disease caused by this organism is less frequent as a result of the *H. influenzae* B vaccine. Penicillin G at a dose of 20–24 million U/day intravenously q4h for 14 days is the treatment of choice for susceptible strains of *S. pneumoniae*. The existence of resistance may necessitate the use of ceftriaxone and/or vancomycin until sensitivities are known. In an animal model, a combination of ceftriaxone and vancomycin was found to be synergistic against penicillin-resistant pneumococci. [41]

Penicillin or ampicillin are usually sufficient to treat *N. meningitidis* meningitis; however, a few ß-lactamase producing strains have been seen and ceftriaxone has been used effectively. ß-Lactams are not sufficient to eliminate the carrier state of *N. meningitidis*, and so rifampin (rifampicin) must be given at the completion of therapy. The agent of choice for the treatment of meningitis due to *L. monocytogenes* is ampicillin or penicillin alone or in combination with gentamicin; of note, the cephalosporins have no activity against this organism.

In children, the preferred agents for the treatment of *H. influenzae* meningitis are the third-generation cephalosporins cefotaxime or ceftriaxone.[42] There are several case reports that document the failure of cefuroxime to treat meningitis caused by *H. influenzae*[43] and there are instances where patients on cefuroxime have developed *H. influenzae* meningitis while being treated with cefuroxime for a non-meningeal infection caused by this organism. These failures are thought to occur as a result of resistant organisms or as a result of the inoculum effect whereby an increase in the inoculum of bacteria can significantly increase the apparent MIC/minimum bactericidal concentration (MBC) of the antibiotic to above attainable levels. A randomized trial found that ceftriaxone resulted in less hearing impairment and sterilized the CSF earlier than cefuroxime when used as treatment for meningitis in children.[44]

Patients with staphylococcal meningitis (which is usually seen after trauma or neurosurgical procedures) are best treated with high doses of nafcillin or oxacillin if the organism is susceptible. *Pseudomonas aeruginosa* meningitis has been effectively managed with ceftazidime, and meropenem may prove to be an alternative.[37]

Corticosteroids have been shown to reduce the neurologic sequelae of meningitis. In an animal model, corticosteroid treatment diminished the penetration of ceftriaxone into the CSF and markedly diminished the penetration of vancomycin. [45] Levels of rifampin in the CSF were not affected and the use of ceftriaxone with rifampin was successful in the treatment of meningitis in this model, whether or not the animal received corticosteroids. However, a recent prospective, randomized, double-blind trial demonstrated that the use of dexomethasone early in the treatment of patients with bacterial meningitis resulted in improved morbidity and mortality,

particularly for patients with pneumococcal meningitis. [45A]

Biliary system infections (cholangitis)

Infection of the biliary tract generally occurs if there is an abnormality such as gallstones, strictures or a stent. Infection rarely complicates malignant obstruction of the biliary tree. In the obstructed biliary tract, there is very little excretion of any antibiotic. For example, biliary excretion of cefoperazone is responsible for 70% of the excretion of this compound, yet no levels are detectable in the biliary tract when there is an obstruction. However, after an obstruction in the biliary tree is relieved, therapeutic levels of antibiotics can be achieved within 24 hours.[46] The ß-lactams that achieve significantly higher biliary than serum levels are nafcillin, mezlocillin, piperacillin, cefamandole, cefmetazole, cefoperazone and ceftriaxone. Ampicillin and carbenicillin achieve concentrations in the bile equal to or greater than those in serum. Interestingly, biliary levels are higher after oral amoxicillin or ampicillin than they are after intravenous administration. Biliary concentrations of ticarcillin, cefazolin, cefotaxime, ceftazidime and cefuroxime are all less than serum concentrations.[47]

For this reason, the ureidopenicillins mezlocillin and piperacillin are commonly used in biliary tract infections. Cefoperazone has been successful in the treatment of biliary infections. Cefoxitin, cefuroxime and ceftriaxone are also commonly used in conjunction with an aminoglycoside in patients with cholangitis.

In patients undergoing biliary surgery, adequate serum levels of antibiotic have been shown to be more important than biliary levels when the goal is to reduce postoperative infection.

Intra-abdominal infections (see Chapter 47)

Intra-abdominal infections, such as acute appendicitis, penetrating abdominal trauma and bowel perforation, are generally polymicrobial in nature and caused by a combination of aerobic, anaerobic and facultative anaerobic organisms. Historically, a regimen consisting of clindamycin and an aminoglycoside was the first to demonstrate superior efficacy in treating patients with penetrating abdominal trauma.[48] Since then, a number of studies have confirmed the efficacy of the ß-lactam antibiotics alone or in combination with other agents for various intra-abdominal infections.[49] Cefoxitin, imipenem, cefotetan, piperacillin and ticarcillin-clavulanic acid have all been shown to be effective in treating intra-abdominal infections when used as monotherapy. Meropenem has been shown to have efficacy similar to that of imipenem for the treatment of intra-abdominal sepsis.[50] [51] Ertapenem has similar efficacy in treating intra-abdominal infections as piperacillin-tazobactam.[52] [53] [54] The combination of clindamycin with either ceftazidime or aztreonam has been successful in the treatment of intra-abdominal infections. Failures of ampicillin-sulbactam have occurred when pseudomonal infections occur.

Although enterococci are commonly isolated from intra-abdominal infections (14–33%), many physicians do not include anti-enterococcal therapy in the initial treatment of these infections. There is a high incidence of enterococcal superinfections when moxalactam and ceftazidime are used; this phenomenon is not seen with cefotaxime, ceftizoxime or imipenem. 'Breakthrough' enterococcal infections occur in patients who have been hospitalized for long periods of time with persistent or recurrent intra-abdominal sepsis or who are immunosuppressed.[55]

Spontaneous bacterial peritonitis

Few studies have evaluated the efficacies of different antibiotics in the treatment of spontaneous bacterial peritonitis (SBP). The organisms that typically cause SBP are the Gram-negative bacilli (especially *Escherichia coli* and *Klebsiella* spp.), Gram-positive cocci (including pneumococci, other streptococci, enterococci and staphylococci) and anaerobes. When used in conjunction with an aminoglycoside, ampicillin had a cure rate of 76% in one study. Cefotaxime was shown to be more effective (cure rate 85%) than ampicillin and tobramycin (cure rate 56%) in another study of severe infections in patients with cirrhosis of which approximately 75% were SBP. In

another uncontrolled study, amoxicillin and clavulanic acid had a cure rate of 80% for SBP. Aztreonam monotherapy has been associated with Gram-positive superinfection. Therefore, if aztreonam is to be used for SBP, then an additional antibiotic providing Gram-positive coverage is needed.[56] [57]

Pancreatitis and its complications (see Chapter 47)

The prophylactic use of antibiotics in uncomplicated acute pancreatitis is controversial. Early studies that used ampicillin, an antibiotic that does not achieve therapeutic levels in pancreatic tissue, showed no benefit. However, several recent studies have shown a potential benefit. Patients with acute necrotizing pancreatitis treated with imipenem for 14 days had a lower incidence of pancreatic sepsis than those not treated; however, a trend toward a decreased mortality rate was not statistically significant.[58] A study that used cefuroxime in patients with acute necrotizing pancreatitis found that rates of bacteremia and mortality were both lower than those of controls.[59]

It is clear that ß-lactam antibiotics have a role in the management of infectious complications of pancreatitis such as abscess or infected pseudocyst. The agents commonly used in addition to cefuroxime and imipenem include ticarcillin-clavulanic acid, piperacillin-tazobactam, ampicillin-sulbactam and meropenem.

Endovascular infections (endocarditis) (see Chapter 59)

A recent review provides guidelines for the treatment of endocarditis in adults.[60] The drug of choice for the treatment of endocarditis caused by the viridans streptococci is penicillin G. Depending upon the drug susceptibility of the organism, gentamicin can be added for part or all of the course and the duration of treatment can be 2–6 weeks. Alternatively, a 4-week course of ceftriaxone can be used.[61]

Enterococcal endocarditis is best treated with ampicillin or penicillin in combination with an aminoglycoside for 4–6 weeks.

The treatment of choice for native-valve endocarditis caused by *Staphylococcus aureus* is nafcillin or oxacillin for 4–6 weeks. Gentamicin has been used for the first 3–5 days to decrease the number of days of bacteremia, but has not been shown to change the outcome.[62]

Intravenous drug users with right-sided staphylococcal endocarditis have been successfully treated with 2 weeks of nafcillin and tobramycin.[63] Prosthetic valve endocarditis with coagulase-negative staphylococci is optimally treated with nafcillin or oxacillin (if the organism is sensitive) in combination with rifampin and gentamicin (for the first 2 weeks) for at least 6 weeks.

Endocarditis caused by the slow-growing fastidious Gram-negative organisms *Haemophilus parainfluenzae, H. aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae* (the HACEK group) can be treated with ampicillin and gentamicin for 4 weeks or ceftriaxone alone for 4 weeks.

Neutropenic fever

ß-Lactam antibiotics (for those who are not allergic to them) are the agents of choice for the management of fever in patients with cancer and treatment-induced neutropenia. Because life-threatening infections can occur with Gram-negative rods, including *P. aeruginosa*, ticarcillin-clavulanic acid is used in conjunction with an aminoglycoside for the treatment of neutropenic fever in our institution. However, monotherapy with agents such as ceftazidime or imipenem has also been shown to be effective.[64] A combination of an extended-spectrum ß-lactam antibiotic with a third-generation cephalosporin (a double ß-lactam combination) has also been found to be effective. [65] In patients allergic to the penicillins, aztreonam provides excellent coverage of the Gram-negative bacilli and is an acceptable alternative, along with Gram-positive coverage, such as vancomycin (see Chapter 100).

Lyme disease

Early infection caused by *Borrelia burgdorferi* can be managed with either amoxicillin or doxycycline. Since co-infection with *Ehrlichia* spp. is known to occur in many areas, many clinicians now prefer to use doxycycline. For later manifestations of Lyme disease, however, the ß-lactams are the agents of choice. Lyme carditis can be successfully treated with either a 2-week course of ceftriaxone or intravenous penicillin G. Lyme meningitis and Lyme arthritis can also be treated with ceftriaxone or penicillin G, but for 2–4 weeks. A 30-day treatment course of amoxicillin and probenecid has been used for the treatment of Lyme arthritis. In pregnant women, doxycycline cannot be used, amoxicillin is used in early Lyme disease and intravenous penicillin G is used for disseminated early Lyme disease or any manifestation of

late disease (see Chapter 54).[66] [67]

Syphilis

Parenteral penicillin G is the preferred agent for treating all stages of syphilis and is the only therapy that has proved effective for neurosyphilis, syphilis in pregnancy and congenital syphilis.[68] Primary and secondary syphilis can be treated with a single dose of benzathine penicillin G (2.4 million U in adults, 50,000U/kg intramuscularly in children up to the adult dose). Late latent syphilis is treated with benzathine penicillin G 2.4 million U intramuscularly every week for 3 weeks. Procaine penicillin can be used where benzathine penicillin in unavailable, and there are alternatives for patients who are allergic to penicillin (see Chapter 75).

It is important to remember that patients being treated for any of the spirochete diseases — syphilis, Lyme disease or borreliosis — may develop a Jarisch-Herxheimer reaction, which may produce fever, tachycardia, chills, headaches, sore throat, malaise, myalgias, arthralgias, rash and, rarely, hypotension. This reaction has been observed in approximately 50% of patients treated for primary syphilis and 75% of patients with secondary syphilis.[69] Generally, it occurs a few hours after the first dose of penicillin, lasts for only a few hours and does not occur with subsequent doses of the antibiotic. Pre-treatment of patients with louse-borne relapsing fever (caused by *Borrelia recurrentis*) with hydrocortisone or acetaminophen does not prevent the Jarisch-Herxheimer reaction.[70] It is important to distinguish the reaction from penicillin allergy so that appropriate treatment is not discontinued.

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment

The majority of the ß-lactam antibiotics are excreted almost entirely via the renal route, and so dose adjustments are necessary in the presence of kidney disease. Failure to reduce the dose of penicillin in uremic patients has resulted in toxicity, most notably encephalopathy.[71] As biliary secretion plays a major role in the excretion of ceftriaxone, cefoperazone, nafcillin and oxacillin, the doses of these antibiotics do not need to be adjusted in renal failure. Because biliary secretion plays a lesser, although significant role in the excretion of the ureidopenicillins, the dosages of these drugs do not have to be reduced as much as for the other penicillins. Some ß-lactams must be re-dosed after peritoneal dialysis, which removes variable amounts of the drug. With the exception of ceftriaxone, cefoperazone, cefonicid, mezlocillin, methicillin, nafcillin, cloxacillin, dicloxacillin and oxacillin, the ß-lactams need must re-dosed following hemodialysis. Specific dose adjustments are needed for patients with renal impairment and for patients on hemodialysis or peritoneal dialysis (Table 193.5).

Hepatic impairment

The dosages of some ß-lactams must be adjusted in patients with severe hepatic disease. As a result of reduced desacetylation in

TABLE 193-5 -- Drug dosages in patients with renal failure. [7] [11] [72] [73] [74] [75]

DRUG DOSAGES IN PATIENTS WITH RENAL FAILURE									
Generic name	**Dose in normal renal function**	**Max. daily dose with normal renal function**	**Adjustment in dose (D) or interval (I)**	**GFR (ml/min) >50**	**GFR (ml/min) = 10–50**	**GFR (ml/min) <10**	**Supplement after HD**	**Supplement with PD**	**CVVHD**
Penicillins									
Amoxicillin	250–500mg q8h	1.5g/day	I	q8h	q8-12h	q24h	Yes	250mg q12h	
Amoxicillin-clavulanate	250–500mg q8h		I	q8h	q8-12h	q24h	Yes		
Ampicillin	0.25–2g q4-6h	12g/day	I	q6h	q6-12h	q12-24h	Yes	250mg q12h	
Ampicillin-sulbactam	1.5–3g q6h		I	q6-8h	q12-24h	q24h			1.5–3g q8-12h
Azlocillin	2–3g q4h	18g/day	I	q4-6h	q6-8h	q8h	Yes	Dose for GFR<10	
Dicloxacillin	250–500mg q6h	2g/day	D	100%	100%	100%	No	No	
Methicillin	1–2g q4h	12g/day	I	q4-6h	q6-8h	q8-12h	No	No	
Mezlocillin	1.5–4g q4-6h	24g/day	I	q4-6h	q6-8h	q8h	No	No	
Nafcillin	1–2g q4-6h	12g/day		100%	100%	100%	No	No	1–2g q4h
Oxacillin	1–2g q4-6h	12g/day		100%	100%	100%	No	No	
Penicillin G	0.5–4MU q4-6h	24MU/day	D	100%	75%	20–50%	Yes	Dose for GFR <10	4 million U q6-8h
Penicillin V	250–500mg q6h	2g/day	D	100%	100%	100%	Yes	Dose for GFR <10	
Piperacillin	3–4g q4h	24g/day	I	q4-6h	q6-8h	q8-12h	Yes	Dose for GFR <10	3–4g q8h
Piperacillin-tazobactam	3.375g q4-6h		D&I	3.375g q6h	2.25g q6h	2.25g q8h	Yes — 0.75g	Dose for GFR <10	2.25–3.375g q8h
Ticarcillin	3g q4h	24g/day	D&I	1–3g q4h	1–2g q8h	1–2g q12-24h	Yes — 3g	Dose for GFR <10	
Ticarcillin-clavulanate	3.1g q4-6h		D&I	3.1g q4-6h	2g q4-8h	2g q12h	Yes — 3.1g	3.1g q12h	
Cephalosporins									
First-generation									
Cefadroxil	0.5–1g q12h	2g/day	I	q12h	q12-24h	q36h	Yes — 0.5–1g	250mg q8-12h	
Cefazolin	0.5–2g q8h	12g/day	I	q8h	q12h	q24-48h	Yes — 0.5–1g	0.5g q12h	1g q8h
Cephalexin	250–500mg q6h	4g/day	I	q8h	q12h	q12h	Yes	Dose for GFR <10	
Cephalothin	0.5–2g q6h	8g/day	I	q6h	q6-8h	q12h	Yes	1g q12h	
Cephapirin	0.5–2g q6h	8g/day	I	q6h	q6-8h	q12h	Yes	1g q12h	
Cephradine	0.25–2g q6h	8g/day	D	100%	50%	25%	Yes	Dose for GFR <10	
Second-generation									

Cefaclor	250–500mg q8h	1.5g/day	D	100%	50–100%	50%	Yes — 250mg	250mg q8-12h	
Cefamandole	0.5–2g q4-8h	12g/day	I	q6h	q6-8h	q12h	Yes — 0.5–1g	0.5–1g q12h	
Cefdinir	300mg po q12h	600mg/day	I	q12h	q24h (GFR <30)	q24h	Yes 300mg and then 300mg every other day		
Cefmetazole	2g q6-12h	8g/day	I	q12-16h	q24h	q48h	Yes	Dose for GFR <10	
Cefonicid	1–2g q24h	2g/day	D&I	0.5g q24h	0.1–0.5g q24-48h	0.1g/1–5 days	No	No	
Cefotetan	1–2g q12h	6g/day	I	q12h	q12-24h	q48h	Yes — 1g	1g/day	
Cefoxitin	1–2g q6-8h	12g/day	I	q8h	q8-12h	q24-48h	Yes — 1g	1g/day	
Cefprozil	250–500mg q12h	1g/day	D&I	250–500mg q12h	250mg q12-16h	250mg q24h	Yes — 250mg	Dose for GFR <10	
Ceftibuten	400mg/day		D	100%	25–50%	25%			
Cefuroxime — po	250–500mg q12h	1g/day		100%	100%	100%	Yes	Dose for GFR <10	
Cefuroxime — iv	0.75–1.5g q8h	6g/day	I	q8h	q8-12h	q24h	Yes	Dose for GFR <10	1.5g q8h
Loracarbef	200–400mg q12h	800mg/day	I	q12	q24h	every 3–5 days	Yes		
Third-generation									
Cefditoren	200–400mg po q12h	800mg/day	D&I	200–400mg q12h	200mg q12h	200mg q24h (GFR < 30)			
Cefixime	250mg q12h	500mg/day	D	100%	75%	50%	Yes — 300mg	200mg/day	
Cefoperazone	1–2g q12h	12g/day	D	100%	100%	100%	Yes — 1g	No	1g q12h
Cefotaxime	1–2g q4-12h	12g/day	I	q6h	q8-12h	q24h	Yes — 1g	1g/day	1g q8-12h
Cefpodoxime	200mg q12h	400mg/day	I	q12h	q16h	q24-48h	Yes — 200mg	Dose for GFR <10	
Ceftazidime	1–2g q8h	6g/day	I	q8-12h	q24-48h	q48h	Yes — 1g	0.5g/day	1g q12h
Ceftizoxime	0.5–2g q8-12h	12g/day	I	q8-12h	q12-24h	q24h	Yes — 1g	0.5–1g/day	
Ceftriaxone	1–2g q24h	4g/day		100%	100%	100%	Yes	750mg q12h	1–2g q24h
Fourth-generation									
Cefepime	0.25–2g q12h	4g/day	I	q12h	q16-24h	q24-48h	Yes — 1g	Dose for GFR <10	1g q12h
Monobactam									
Aztreonam	1–2g q8-12h	8g/day	D	100%	50–75%	25%	Yes — 0.5g	Dose for GFR <10	1g q8-12h
Carbapenems									
Ertapenem	1g iv q24h	1g/day	D	q24h	0.5g q24h (GFR <30)	0.5g q24h	150mg Supplement if dosed within 6 hours prior to HD	Dose for GFR <10	
Imipenem-cilastatin	0.25–1g q6h	4g/day	D&I	250–500mg q6-8h	250mg q6-12h	125–250mg q12h	Yes	Dose for GFR <10	250mg q6-12h
Meropenem	0.5–2g q8h	6g/day	D&I	1g q8h	0.5–1g q12h	250–500mg q24h	Yes	Dose for GFR <10	1g q12h
CVVHD, continuous venovenous hemodialysis. GFR, glomerular filtration rate; HD, hemodialysis; PD, peritoneal dialysis.									

patients with liver disease, the half-life of cefotaxime may increase slightly, but the half-life of cefoperazone may increase significantly and dosage reductions are required. Although biliary excretion plays a role in the excretion of ceftriaxone, no dose adjustment is needed in patients with liver disease.

Extremes of age

Dose reductions of the ß-lactam antibiotics should be made in the elderly in the presence of renal dysfunction (see Table 193.5). Otherwise, elderly patients tolerate standard doses of the ß-lactam antibiotics.

Because neonates do not have fully developed renal function, special modifications in dosage are necessary. In addition, because children have a high risk of cholestatic complications with ceftriaxone, another agent should be used when possible (see Adverse reactions and interactions, below).

ß-Lactams in pregnancy

The penicillins, the ß-lactamase inhibitors and the cephalosporins, aztreonam and meropenem (as of May, 1997) are considered category B in pregnancy (Table 193.6). This means that animal studies have shown no risk to the fetus, but adequate human studies have not been performed, or that animal studies have shown risk and human studies have shown no risk. When they are indicated, these antibiotics are commonly used in clinical practice in pregnant women.

Imipenem-cilastatin and moxalactam are pregnancy category C, meaning that animal studies show toxicity to the fetus and human studies are inadequate. However, the benefit of using these drugs may exceed the risk of not treating a serious infection in a pregnant woman when no alternatives exist.[76] Meropenem, category B, may be a suitable alternative to imipenem, category C, for infections with resistant Gram-negative aerobic organisms.

ß-Lactam antibiotics that are not protein bound are transported across the placenta and reach the drug levels that are present in maternal serum. ß-Lactams that are highly protein-bound reach only low concentrations in amniotic fluid and the fetus.[77]

As a general principle, the ß-lactam antibiotics have accelerated elimination and lowered plasma concentrations in pregnant women as compared with nonpregnant women. As a result, the dose or frequency of administration should be increased in pregnant women.[76]

ADVERSE REACTIONS AND INTERACTIONS

Adverse reactions that occur with the ß-lactam antibiotics are summarized in Table 193.7 .

Allergic reactions

The most common adverse event associated with the use of ß-lactam antibiotics is an allergic reaction. The reported frequency of allergic reaction to penicillin varies from 0.7 to 10%, and anaphylaxis, the most feared reaction, occurs in 0.004–0.015% of patients.[78] A maculopapular rash occurs late in the treatment course of 2–3% of patients receiving a course of penicillin. Ampicillin and amoxicillin induce rashes in a higher percentage than other ß-lactams of patients treated (5.2–9.5%) and almost invariably cause a rash when given during acute infectious mononucleosis (Epstein-Barr virus) or cytomegalovirus, and rarely when given to patients with acute lymphocytic leukemia. Such patients may tolerate ß-lactam antibiotics when re-challenged after the acute illness has resolved. Reactions to penicillins are characterized according to the time of onset following administration of the drug:

- immediate reactions occur in the first hour;
- accelerated reactions occur 1–72 hours after drug administration; and
- late reactions occur 72 hours or more after starting a course of the antibiotic.

TABLE 193-6 -- **Pregnancy categories of the ß-lactam antibiotics (as of May 1997).**

PREGNANCY CATEGORIES OF THE ß-LACTAM ANTIBIOTICS	
Class of ß-lactam	**Pregnancy category**
Penicillins — all	B
ß-Lactamase inhibitors — all	B
Cephalosporins — all	B
Moxalactam	C
Aztreonam	B
Imipenem-cilastatin	C
Meropenem	B
Ertapenem	B
Category B means that animal studies have shown no risk to the fetus, but adequate human studies have not been performed, or animal studies have shown risk and human studies have shown no risk. Category C means that animal studies show toxicity to the fetus and human studies are inadequate.	

TABLE 193-7 -- Adverse reactions with ß-lactam antibiotics.

ADVERSE REACTIONS WITH ß-LACTAM ANTIBIOTICS	
Reaction	**Examples**
Local	Pain, induration, tenderness at sile of im injection; burning during iv administration, phlebitis
Hypersensitivity	Rash, pruritus, urticaria, fever, chills, Stevens-Johnson syndrome, anaphylaxis
Gastrointestinal	Diarrhea, nausea, vomiting, abdominal pain, *Clostridium difficile* diarrhea
Hematologic	Eosinophilia, leukopenia, anemia, positive Coombs' test, hemolytic anemia, neutropenia, lymphopenia; thrombocytosis, thrombocytopenia, elevated prothrombin time, bleeding, abnormal clotting time, abnormal platelet aggregation
Hepatic	Elevated transaminases (aspartate transaminase, alanine transaminase), hepatitis, elevated alkaline phosphatase, elevated bilirubin
Renal	Elevated blood urea nitrogen and creatinine, falsely elevated creatinine, casts in urine
Central nervous system	Headache, dizziness, somnolence, confusion, tremor, myoclonus, seizures, encephatopathy
Genitourinary	Vaginitis
Superinfection	Thrush, vaginal candidiasis, infection with resistant bacteria

Both immediate and accelerated reactions may result in urticaria and anaphylaxis.

Previous exposure to penicillin does not seem to increase the risk of penicillin allergy. However, it is clear that people who have had allergic reactions to penicillin have a higher risk of allergic reactions than people who have tolerated therapy in the past. In patients with a history of penicillin allergy, re-challenge with penicillin results in acute reactions in an estimated 65% of patients, anaphylaxis in 5–10% and fatal anaphylaxis in 0.2–0.5%.

Skin testing is a useful technique in the evaluation of patients with a history of penicillin allergy, but is not useful as a screening test for

the general population because many skin test positive patients without a history of penicillin allergy can tolerate penicillin therapy. Skin testing is not useful for identifying non-IgE-mediated adverse drug reactions such as drug fever.

Although fatalities have occurred as a result of the skin test itself, the procedure is generally regarded as safe. A wide range in the incidence of positive skin tests in patients with a previous history of penicillin allergy has been noted (8.75–63%), and therefore a significant proportion of patients who give a history of penicillin allergy can tolerate the drug.[79] In one large study, penicillin skin testing allowed the safe use of penicillin in 90% of patients who gave a history of penicillin allergy.[80] The incidence of positive skin test results in patients who have tolerated penicillin in the past range from 4 to 7%.[81] When penicillin is administered to patients with a previous history of penicillin allergy, but with a negative skin test, the overall reaction rate (early and accelerated) is low and similar to the rate of allergy reported in the general population. In addition, the reactions are generally mild and self-limiting. In one study, only 1.2% of patients who had a history of penicillin allergy and a negative skin test had a possible IgE-mediated reaction.[82] Anaphylaxis has occurred in patients with negative skin tests, but is extremely rare. Skin test reactivity declines with time in patients with a history of penicillin allergy. People with dermatitis and allergic rhinitis do not have an increased risk of penicillin allergy, but the risk may be increased for atopic individuals.[83]

Patients with a history of penicillin allergy are four times as likely to have a reaction to first-generation cephalosporins than patients without a history of allergy (8.1 vs 1.9%). Second- and third-generation cephalosporins have an incidence of skin reaction (rash) ranging from 1 to 3%, similar to the incidence of rash with penicillin. Anaphylaxis, however, is uncommon with cephalosporins. There seems to be a lower incidence of allergy to second- and third-generation cephalosporins in patients with a history of penicillin allergy. Patients with allergy to penicillins should be considered allergic to the carbapenems, but there seems to be no cross-reactivity with aztreonam. No major adverse reactions to aztreonam have been reported, but rarely patients will develop a rash. There is more allergic cross-reactivity among penicillin derivatives than among cephalosporin derivatives. However, allergic cross-reactivity between cephalosporin derivatives is greater than cross-reactivity between cephalosporins and penicillins.[84] For example, a patient who is known to be allergic to ceftriaxone is more likely to be allergic to ceftazidime than a patient who is known to be allergic only to penicillin.[85]

At times, it may be necessary to administer penicillin to patients with a previous severe reaction to the drug. For instance, penicillin is the only acceptable treatment for a pregnant woman with syphilis. Effective methods of desensitization have been described,[86] but adverse reactions are common and the patient should be in an intensive care unit for close monitoring.

Hematologic effects

Hematologic toxicity is rare, but leukopenia (occurring in 0.2% of patients on mezlocillin in one study) has been observed when the penicillins or cephalosporins are used at high doses,[87] and also rarely with imipenem. Counts return when the drug is discontinued, and lower dosages can often be tolerated without neutropenia. Isolated eosinophilia can occur in patients on cephalosporins (1–7%). A Coombs' positive hemolytic anemia is rarely observed with the penicillins[88] and cephalosporins.[89]

Dose-dependent defects of platelet aggregation and a prolongation of the bleeding time can be seen with carbenicillin and ticarcillin and can occur with all of the penicillins at high doses. Clinically significant bleeding can occur but is uncommon.[90] Hypoprothrombinemia has occurred frequently with cephalosporins that possess a methylthiotetrazole (MTT) group (cefamandole, cefoperazone, cefotetan, moxalactam, cefmetazole, cefmonoxime), which may interfere with the activation of factors II, VII, IX and X, and may also prevent the activation of vitamin K.[84] [90] [91] Patients with renal failure, malnutrition, intra-abdominal infection or recent gastrointestinal surgery seem to be at the highest risk and may benefit from weekly prophylaxis with vitamin K when being given one of these antibiotics.[89] The frequent occurrence of bleeding complications with moxalactam has led to minimal use of this antibiotic. [92] No coagulation abnormalities have been associated with imipenem.

Isolated thrombocytopenia rarely complicates the use of the ß-lactam antibiotics. An immune mechanism has been documented.[93] Thrombocytopenia can occur as soon as 5 days after the initiation of the antibiotic and generally resolves when the agent is withdrawn.[94] [95]

Renal effects

Interstitial nephritis, characterized by fever, rash, eosinophilia, proteinuria, hematuria, eosinophiluria and occasionally renal insufficiency can be seen with the penicillins, most commonly methicillin.[96] ß-Lactam antibiotics that exist as sodium salts, particularly carbenicillin and ticarcillin, can induce hypokalemia.[97] Cephalothin can cause renal damage that histopathologically resembles that of nafcillin.[98] The concurrent use of aminoglycosides may add to the nephrotoxicity of cephalosporins [99] such as cephalothin. About 1% of patients on ceftazidime have elevated blood urea nitrogen or creatinine, but these are generally not clinically significant.[100] The sodium load of some penicillins can be high, most notably with ticarcillin (4.7mEq/g), but also with ampicillin, methicillin, penicillin G, azlocillin, mezlocillin and pipercillin, thereby posing a problem for patients with cognestive heart failure.

Neurologic effects

Many of the ß-lactams can cause neurotoxicity and, in particular, seizures. Seizures have been reported following the use of penicillin,[70] ampicillin, amoxicillin, oxacillin, nafcillin, carbenicillin, ticarcillin, piperacillin, cefazolin, cefonicid, cephalexin, ceftazidime and imipenem. Benzylpenicillin, cefazolin and imipenem have the highest neurotoxic potential of the ß-lactam antibiotics. In fact, seizures occur in 0.4–1.5% of patients taking imipenem.

Several risk factors that may predispose a patient to neurotoxicity have been identified, for example:

- high doses;
- renal insufficiency;
- disruption of the blood-brain barrier,
- pre-existing central nervous system (CNS) disease;
- advanced age;
- concurrent administration of nephrotoxic drugs;
- concurrent drugs that may reduce the seizure threshold; and
- concurrent administration of other ß-lactam antibiotics.[101]

Neurotoxicity of penicillins is clearly related to elevated CSF antibiotic levels, such as may occur when high doses are being used in patients with impaired renal function. Penicillin levels in CSF should not exceed 5mg/l. Seizures have occurred in patients receiving meropenem only if they have underlying CNS abnormalities.

Gastrointestinal effects

Gastrointestinal upset and diarrhea are common side-effects of the ß-lactams. Enterocolitis caused by *Clostridium difficile* may result from use of any of the ß-lactams, but particularly ampicillin.

Hepatitis is a rare side-effect of carbenicillin,[102] mezlocillin and nafcillin, and resolves after discontinuation of therapy. Hepatitis as a result of intravenous oxacillin can occur as early as 2 days into treatment,

is thought to result from a hypersensitivity reaction, does not appear to be dose-related, and is reversible on discontinuation of the drug.[103] [104] Mild elevations in transaminases and alkaline phosphatase also occur with the cephalosporins and carbapenems, but the drug can usually be continued.[105] Serum transaminases become elevated in 2–4% of patients receiving aztreonam.

Gallbladder sludge formation[106] [107] and cholelithiasis[108] have occurred in patients on ceftriaxone. Children, patients receiving prolonged or high doses, and patients on total parenteral nutrition appear to be at risk of this complication.

A disulfuram-like reaction has been associated with the cephalosporins with an MTT group.[109] [110] Patients taking these agents and then ingesting alcohol have developed flushing, tachycardia, diaphoresis, headache, nausea, vomiting and dizziness.

Other reactions

Local side-effects of the ß-lactam antibiotics are not uncommon. At the intramuscular injection site, patients may experience pain, tenderness and edema. Thrombophlebitis can occur in up to 5% of patients receiving parenteral therapy with some agents.

Other reactions to penicillin are less common. Serum sickness, consisting of fever, urticaria, joint pains and angioneurotic edema, can occur and, rarely, exfoliative dermatitis, the Stevens-Johnson syndrome and allergic vasculitis. Late-onset morbilliform rashes can develop as a result of penicillin therapy and may disappear, even if the penicillin is continued, but desquamation can occur.

Drug interactions

The most clinically important drug interaction with the ß-lactam antibiotics occurs with probenecid, a uricosuric and renal tubular blocking agent. Probenecid causes a 2- to 4-fold increase in the peak serum concentration of the ß-lactam antibiotics. It also prolongs serum levels for these antibiotics. It is used most often with penicillin (e.g. in the treatment of gonococcal infections), but can also be used with ampicillin, methicillin, oxacillin, cloxacillin and nafcillin. The recommended dose in adults is 2g/day in divided doses, and in children a 25mg/kg initial dose is followed by 40mg/kg/day in four divided doses (adult dose used for children who weigh over 50kg). The mechanism of action involves not only inhibition of renal tubular secretion of the ß-lactams, but also a decrease in the apparent volume of distribution of the drug.[111] Probenecid has little effect on the serum levels of imipenem and aztreonam and has no effect on drug levels of ceftazidime. Adverse reactions, including anaphylaxis, can occur with probenecid and the clinician must also be aware that toxicity can result from supratherapeutic levels of the ß-lactam antibiotics when used with probenecid.

As cephalosporins with the MTT side-chain can interfere with hemostasis, care must be taken when using these antibiotics in patients taking warfarin.

Synergistic activity against various bacteria occurs when the penicillins, cephalosporins, carbapenems and monobactams are used in conjunction with aminoglycosides.[112] [113] [114] However, using two ß-lactam antibiotics together may result in either synergy or antagonism.

The bactericidal effect of ampicillin may be reduced when other antibiotics (chloramphenicol, erythromycin, sulfa drugs and tetracycline) are used simultaneously. The

clinical significance of this is unclear.

When ampicillin is used in patients who are taking oral contraceptive agents, breakthrough bleeding may occur and the contraceptive may be less effective.

Piperacillin and ticarcillin must be used cautiously in any patient on vecuronium because the neuromuscular blockade can be further prolonged. Piperacillin can also lower serum levels of tobramycin if the drugs are used together.

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Chapter 194 - Macrolides, Ketolides, Lincosamides and Streptogramins

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INTRODUCTION

Macrolides, lincosamides and streptogramins are chemically unrelated compounds but they possess closely related properties, such as mechanisms of action, antibacterial spectrum, pharmacokinetics and pharmacodynamics, and clinical use. They are considered in parallel in this chapter.

Macrolides

Erythromycin is considered as the reference macrolide antibiotic. First reports on this compound appeared in 1952. Other natural macrolides were launched soon after this. In the past few decades, efforts have been made to generate new semisynthetic compounds with improved chemical, biologic and pharmacokinetic properties and fewer side effects. The recent modifications of the macrolides, leading to the new class of ketolides, seem to be promising because they allow persistent activity against some micro-organisms that are otherwise resistant to the macrolides.

Lincosamides

Two compounds represent the lincosamide group:

- lincomycin, which is currently of limited clinical use, and
- clindamycin, which is still currently used in the treatment of anaerobic and some parasitic infections.

Streptogramins

Streptogramins are a group of cyclic peptides produced by various *Streptomyces* spp. Pristinamycin and virginiamycin are water-insoluble mixtures of naturally occurring compounds. More recently, the synthesis of water-soluble derivatives has allowed the development of an injectable streptogramin, namely Synercid (RP 59500). A common pattern to streptogramins is that they are composed of at least two structurally unrelated molecules (group A and group B), which act synergistically against most susceptible bacteria.

STRUCTURE

Macrolides and ketolides

The chemical structure of the macrolides is characterized by a large lactone ring containing between 12 and 16 atoms of carbon to which are attached one or more sugars, mainly desosamine and cladinose, by way of glycosidic bonds. Simplified classifications of the macrolides divide the compounds according to the size of the lactone ring (i.e. 12-, 14-, 15- or 16-membered rings; Fig. 194.1).[1] Within each of these groups, compounds are classified according to their natural or semisynthetic origin. The lactone ring is modified by a hydroxyl or alkyl group, one ketone at C7 in the 12-membered macrolides, at C9 in the 14-membered macrolides, and one aldehyde in the 16-membered compounds. The only compound with a 15-membered ring, the azalide azithromycin, contains a tertiary amino group. Neutral or basic sugars can substitute one, two or three hydroxyl groups of the lactone ring, conferring a more or less basic character to the molecule. The most basic compounds are the most active.[2]

The 12-membered macrolides have never become important in clinical practice. Numerous derivatives have been synthesized from the 14-membered macrolides derivatives of erythromycin A.[3] Efforts at extending the biochemical modifications of the 16-membered compounds have been less productive. The objective of this research was to retain the antibacterial activity of erythromycin while improving acid stability and thereby enhancing bioavailability.

In order to overcome the problem of bacterial resistance to macrolides, attempts have been made to modify the basic structure of 14-membered compounds. This has led to new derivatives, called ketolides because a ketone group replaces the L-cladinose of erythromycin A in position 3, a sugar long considered as essential for the antibacterial activity of macrolides. [4] Several additional modifications have allowed the isolation of several molecules. One of them, telithromycin, is already marketed in a number of countries; the others are currently under preclinical or clinical investigation. The telithromycin molecule has a second site of modification at positions C11,12 of the lactone ring, where an alkylaryl extension has been added to a carbamate group.

Lincosamides

Lincomycin was isolated from *Streptomyces lincolnensis*. It is an alkyl derivative of proline that is composed of an amino acid linked to an amino sugar; it is devoid of a lactone ring. Clindamycin is closely related; a hydroxyl group was substituted by a chlorine atom in position 7.

Streptogramins

Streptogramins, as mentioned above, are composed of two unrelated molecules. Group A streptogramins are polyunsaturated macrolactones. Group B streptogramins are cyclic hexadepsipeptides. Table 194.1 presents a summary of streptogramins and lists the compounds available for clinical use.[5]

MODE OF ACTION

Macrolides, ketolides lincosamides and streptogramins, although chemically unrelated, have similar modes of action against bacteria. The hydrophobicity of these molecules explains why they penetrate poorly through the external membrane of Gram-negative bacilli, thus inducing a limited effect against these bacteria. Macrolides and lincosamides exhibit a slow bactericidal effect against susceptible pathogens. Both components A and B of streptogramins demonstrate a synergistic effect that is responsible for a more rapid bactericidal action against some bacteria in their spectrum of activity.

Macrolides and lincosamides inhibit protein synthesis by binding to the 50S subunit of prokaryotic ribosomes, especially to the peptidyl transferase domain of 23S ribosomal RNA, close to the *P* site. Sites of fixation are different for the different classes of drugs. However, they partially overlap. The key sites of interaction for

Figure 194-1 Classification of macrolides. *Adapted from Bryskier et al.*[1]

TABLE 194-1 -- Streptogramin compounds.*

STREPTOGRAMIN COMPOUNDS
Natural mixtures
Pristinamycin (produced by *Streptomyces pristinaespiralis*) is a mixture of several molecules
Virginiamycin (produced by *Streptomyces virginiae*)
Chemically defined natural molecules
Group A streptogramins
Pristinamycin II_A (synonym — streptogramin A)
Pristinamycin II_B
Group B streptogramins
Pristinamycin I_A (synonym — streptogramin B)
Pristinamycin I_C
Semisynthetic derivatives
Synercid, for parenteral use, is a 30:70 mixture of quinupristin (derived from natural pristinamycin I_A) and dalfopristin (derived from natural pristinamycin II_B)
RPP 106972, for oral use, is under clinical evaluation

** Data from Pechère.*[5]

those drugs are at nucleotides A2058 and A2059 within domain V of the 23S RNA, A752 within domain II, and parts of ribosomal proteins L4 and L22, which together form a single drug-binding pocket. Telithromycin binds to wild-type ribosomes with 10-fold greater affinity than erythromycin A.[6] As a result of this binding, a blockade of the peptide bond formation has been reported, with an inhibition of peptide elongation during synthesis.[7] It has also been suggested that the binding could block the peptidyl-transfer (t)RNA translocation from the *A* to the *P* site of the ribosome.[8] Because some mutations can confer resistance simultaneously to macrolides and lincosamides, it is possible that both classes inhibit protein synthesis by stimulating peptidyl-tRNA dissociation from ribosomes.

The relevant target of macrolides and parent compounds against protozoan parasites such as *Toxoplasma gondii* remains unknown. Even very high concentrations have no effect on intracellular parasite survival in the parasitophorous vacuole, extracellular survival or invasion into the subsequent host cell. Replication with the second host cell is inhibited immediately upon entry, suggesting that a key event could be the establishment of the new parasitophorous vacuole.

Both group A and group B streptogramins bind to the ribosome and inhibit the translation of mRNA during the elongation step (Fig. 194.2).[5] Group A compounds interfere with the function of the peptidyl transferase. They block two steps of the peptide chain elongation process. Group B compounds interfere with the correct positioning of peptidyl-tRNA at the *P* site. They inhibit peptide bond formation, resulting in the release of incomplete peptide chains (see Chapter 188).

MECHANISMS OF RESISTANCE

Intrinsic resistance

As mentioned above, Gram-negative bacilli, in particular members of the family Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp., are resistant to macrolides, lincosamides and streptogramins owing to the relative impermeability of their cellular outer membrane to these hydrophobic compounds. However, concentrations of erythromycin far above those achievable in serum have proven efficacy against Gram-negative bacilli in the intestinal tract and have been used for intestinal selective decontamination. Furthermore, high intra-cellular levels achieved by the newest macrolides can inhibit some Gram-negative bacteria. Enterococci are resistant to lincosamides and *Enterococcus faecalis* is resistant to streptogramins. *Mycobacterium tuberculosis* is not susceptible.

Acquired resistance

Three mechanisms account for acquired resistance to the macrolides: [9] [10] [11]

- modification of the target of the antibiotics, by methylation or mutation;
- inactivation of the antibiotics; and
- active efflux.

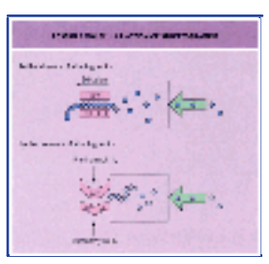

Figure 194-2 Possible molecular action of streptogramins. In the absence of streptogramins, the exit channel for peptide chains is free. In the presence of streptogramins, type A may induce a conformational change of L10 and L11, leading to an increase in the association of type B streptogramins for L24 and to a constriction of the exit channel. L10, L11 and L24 are the main proteins of the exit channel of peptide chains. *Adapted from Pechère.*[5]

In the first type of resistance, a single alteration in 23S rRNA confers cross-resistance to macrolides, lincosamides and group B streptogramins: the so-called MLS_B phenotype. The other two types confer resistance to structurally related compounds only.

Resistance by target methylation

Resistant strains produce an enzyme that demethylates an adenine residue in the 23S rRNA. This methylation leads to a conformational change in the ribosome, which in turn leads to co-resistance to MLS_B -type antibiotics. Streptogramin-A-type compounds are unaffected, and synergy between the two components of streptogramins against MLS_B -resistant strains is maintained. This enzyme production is under the control of erythromycin ribosome methylation (*erm*) genes. The distribution of *erm* genes among clinically important bacterial species is shown in Table 194.2 . These genes may be located on chromosomes but are mainly present on plasmids or transposons. The existence of cross-transfer of genetic material from Gram-positive to Gram-negative bacteria under natural conditions has been demonstrated. In Gram-positive cocci, the expression of MLS resistance may be constitutive and the strains are therefore resistant to all macrolides, lincosamides and streptogramin-B-type compounds. Streptogramin-A-type antibiotics escape resistance, and the combination of group A and group B compounds still exhibits a synergistic bacteriostatic effect. However, depending on the level of resistance to the group B component, the in-vivo bactericidal activity of the combination can be limited, especially against *Staphylococcus aureus*. Resistance to macrolides, lincosamides and group B streptogramins can be divided into constitutive resistance, when the methylating enzyme is produced continuously, and inducible resistance, when the presence of an inducing antibiotic is required for enzyme production. When

the expression of resistance is inducible, staphylococci are resistant to

TABLE 194-2 -- Distribution of *erm* genes in clinically important bacterial species. *

DISTRIBUTION OF *ERM* GENES IN CLINICALLY IMPORTANT BACTERIAL SPECIES		
Hydridization class	**Gene**	**Host**
*erm*A	*erm*A	*Staphylococcus aureus*
		Coagulase-negative staphylococci
*erm*AM	*erm*P	*Clostridium perfringens*
	*erm*Z	*Clostridium difficile*
		Enterococcus faecalis
	*erm*BC	*Escherichia coli*
		Lactobacillus reuteri
	*erm*AM	*Streptococcus sanguis*
		Streptococcus pneumoniae
		Streptococcus agalactiae and
		Streptococcus pyogenes
*erm*C	*erm*B	*Staphylococcus aureus*
		Bacillus subtilis
		Lactobacillus spp.
	*erm*C	*Staphylococcus aureus*
		Coagulase-negative staphylococci
	*erm*M	*Staphylococcus epidermidis*
*erm*F	*erm*F	*Bacteroides fragilis*
		Bacteroides ovatus

** Data from Leclercq and Courvalin.*[9] [10]

14-membered and 15-membered macrolides. The 16-membered antibiotics, the lincosamides and the streptogramin antibiotics remain active, because only 14- and 15-membered macrolides are inducers of methylase synthesis. Ketolides are not inducers. In the case of streptococci, various macrolides and lincosamides can be inducers. Thus, among streptococci, there is a cross-resistance in any case, whether the expression of resistance is constitutive or inducible. As telithromycin is not an inducer of methylase production, it is effective against bacterial subpopulations harboring this type of resistance, but constitutively resistant bacteria are often resistant to telithromycin.[6]

Resistance among anaerobes (*Bacteroides* spp., *Clostridium* spp.) is mainly of the MLS_B constitutive phenotype, although inducibility has been reported. This resistance is either plasmid- or chromosome-mediated. Among *Campylobacter* spp., *Mycoplasma pneumoniae* and *Corynebacterium diphtheriae,* the resistance profile is of the MLS_B phenotype.

Resistance by target mutation

The clinical importance of this mechanism was only recently recognized, with identification of mutations at either A2058 or A2059 domain V of rRNA. A2058 and A2059 confer MLS_B resistance and ML resistance respectively. This mechanism has been identified in *Mycobacterium avium, Helicobacter pylori, Treponema pallidum* and *Streptococcus pneumoniae*. Mutations in ribosomal proteins L4 and L22 have been identified in *S. pneumoniae*.

Resistance by antibiotic modification

Various enzymes are responsible for this type of resistance by inactivation. This mechanism confers resistance to structurally related antibiotics only. Its clinical impact seems rather limited. Phosphotransferases encoded by *mph* (C) have been shown in *S. aureus.* Lincosamide nucleotidyltransferases encoded by *lnu*(A) and *lnu*(B) genes isolated in staphylococci and *Enterococcus faecium* inactivate

TABLE 194-3 -- Phenotypic and genotypic resistance to macrolides resulting from different mechanisms. *

PHENOTYPIC AND GENOTYPIC RESISTANCE TO MACROLIDES RESULTING FROM DIFFERENT MECHANISMS						
Organism	**Mechanism**	**Gene**	**Phenotype designation**	**Resistance phenotype**		
				14–15M	**16M**	**Cli**
Staphylococci	Ribosomal methylation	*erm*	MLS_B inducible	R	s	s
			MLS_B constitutive	R	R	R
	Efflux	*msr*A	MS_B	R	S	S
	Lincosamide inactivation	*Lin*A	L	S	S	S
Streptococci and enterococci	Ribosomal inactivation	*erm*	MLS_B inducible	R or I	R or I	R or I
			MLS_B constitutive	R	R	R
	Efflux	*mef*A	M	R or I	S	S
Enterococcus faecium	Lincosamide inactivation	*Inu*B	L	S	S	s
14/15/16M, 14-, 15- or 16-membered macrolides; I, intermediate susceptibility; R, resistant; S, susceptible; s, reduced *in-vivo* susceptibility.						

** Adapted from Leclercq.*[11]

TABLE 194-4 -- Staphylococcal resistance to macrolides, lincosamides and streptogramins. *

STAPHYLOCOCCAL RESISTANCE TO MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS

Mechanism	Genotype	Erythromycin	Oleandomycin	16-membered macrolides	Lincomycin	Clindamycin	Streptogramin B-type antibiotics*	Streptogramin A-type antibiotics†	Streptogramin antibiotics
Target modification	*erm* (inducible)	R	S or R	S	S	S	S	S	S
	erm (constitutive)	R	R	R	R	R	R	R	S
Drug inactivation	*lin*A	S	S	S	R	S	S	S	S
	Isa	S	S	S	I	I	S	R	I
	saa-sbh	S	S	S	S or I	S or I	R	R	R
Active efflux	*erp*A (detected in coagulase-negative staphylococci only)	R	R	S	S	S	S	S	S
	*msr*A	R	R	S	S	S	R (after induction by erthromycin)	S	Not determined
I, intermediate resistance; R, resistance; S, susceptible.									

* *Adapted from Leclercq and Courvalin P.*[9]
*Pristinamycin factor I, virginiamycin factor S.
†Pristinamycin factor II, virginiamycin factor M.

lincomycin and, to a lesser degree, clindamycin, which loses any bactericidal effect.

Resistance by active efflux

Two types of resistance by active efflux have been identified first in coagulase-negative staphylococci. The different types of resistance shown by bacteria are summarized in Table 194.3 & Table 194.4 . Resistance through active efflux is increasingly recognized in *Streptococcus pyogenes* and *S. pneumoniae*. The protein encoded by the macrolide efflux (*mef*)A or *mef*E gene causes resistance to 14- and 15-membered macrolides but not to other macrolides and lincosamides. This phenotype is called the M phenotype. Telithromycin does not appear to fit into this efflux pump and thus remains largely unaffected by *mef*.

Adequate in-vitro testing by combining disks of different MLS compounds may allow the interpretation of the phenotype of resistance and therefore help in choosing the best compound to use. The antibacterial effect of macrolides in vitro varies essentially according to the pH of the medium used; it decreases in acidic conditions and increases in alkaline conditions (see Chapter 188).

CLINICALLY RELEVANT SPECTRUM OF ACTIVITY

Macrolides

The clinical spectrum of macrolides (Table 194.5) is a function of several parameters:

- intrinsic activity;
- spread of resistance mechanisms;
- actual concentrations of active drug achieved in extracellular fluids; and
- intracellular concentrations able to reach intracellular pathogens.

TABLE 194-5 -- Clinically relevant antimicrobial spectrum of the macrolides.*

CLINICALLY RELEVANT ANTIMICROBIAL SPECTRUM OF THE MACROLIDES				
Type of infection	**Extracellular bacteria**	**Intracellular bacteria**	**Mycobacteria**	**Parasites**
Upper or lower respiratory tract infections*	*Streptococcus pneumoniae*	*Chlamydia pneumoniae*		
	Haemophilus influenzae	*Mycoplasma pneumoniae*		
	Streptococcus pyogenes	*Legionella* spp.		
	Moraxella catarrhalis			
	Corynebacterium diphtheriae			
	Bordetella spp.			
Sexually transmitted diseases	*Treponema pallidum*	*Chlamydia trachomatis*		
	Neisseria gonorrhoeae			
Skin or soft tissue infections	*Streptococcus pyogenes*			
	Peptococcus spp.			
	Peptostreptococcus spp.			
Gastrointestinal infections	*Helicobacter pylori*			*Cryptosporidium* spp.
	Campylobacter spp.			
Systemic infections	*Borrelia burgdorferi*	*Rickettsia* spp.	*Mycobacterium avium-intracellulare*	*Toxoplasma* sp.
			Mycobacterium leprae	
			Mycobacterium fortuitum	

*; spectrum of clinical activity of telithromycin.

As discussed below in the sections on pharmacokinetics and pharmacodynamics, predicting in-vivo efficacy on the basis solely of extracellular levels of free drug is difficult with MLS compounds. Therefore, the clinically relevant spectrum can be better established on the basis of animal experiments and clinical trials than from in-vitro results and pharmacokinetic data alone.

The prevalence of resistance to macrolides such as erythromycin is highly variable among different countries.[12] Resistance of *S. aureus* to erythromycin ranges from 1% to 50%, with marked differences between community isolates (usually 70–90% susceptible) and hospital isolates, where less than 25% of methicillin-resistant *S. aureus* isolates remain susceptible. Generally more than 75% of methicillin-resistant *Staphylococcus epidermidis* are resistant to macrolides. An increase in the frequency of erythromycin resistance was noted in many hospitals when erythromycin was extensively used.[13] A similar phenomenon has been noted in Finland regarding the influence of macrolide prescription on the development of resistance of group A streptococci to erythromycin. In Finland, after a nationwide reduction in

the use of macrolides for outpatient therapy, a significant decline in the frequency of erythromycin resistance among group A streptococci was observed.[12]

Resistance of *S. pneumoniae* to macrolides is also highly variable: 34% worldwide, with rates ranging from 77% in Asia to 7% in Australia. It is especially frequent in penicillin-resistant isolates. The efflux mechanism seems to be more frequent in the USA than in Europe, the *erm*-mediated mechanism being more frequent in Europe.[12] Telithromycin is active against 97.6% of erythromycin-resistant strains.

Enterococcal resistance has increased over the years and is probably a source of resistance in streptococcal species and staphylococci. Macrolide activity against *Haemophilus influenzae* is variable since the systemic levels of macrolides are similar to the minimum inhibitory concentration (MIC). Some strains of *H. influenzae* are resistant to clinically achievable blood levels of the macrolides. Some Gram-negative organisms, mainly *Campylobacter jejuni* and *H. pylori*, are major therapeutic targets of macrolides.

Some differences in terms of in-vitro activity and demonstration of in-vivo efficacy have been reported between erythromycin and the newest macrolide compounds (see Indications). However, it should be emphasized again that these compounds share the main resistance patterns with erythromycin.

Ketolides are active against Gram-positive cocci that are resistant to erythromycin by an efflux mechanism or by an inducible MLS_B mechanism.

Lincosamides

Lincosamides offer advantages of efficacy against anaerobes, including *Bacteroides* spp. (mainly *B. fragilis*), *Fusobacterium* spp., anaerobic cocci, *Clostridium perfringens* and other clostridia, certain non-spore-forming Gram-positive rods and *Capnocytophaga* spp. Resistance has been described with an increased incidence among anaerobes isolated from patients previously treated with clindamycin. The antiparasitic spectrum of clindamycin includes *Plasmodium* spp. and *T. gondii*.

Streptogramins

Streptogramins have a broad spectrum of antibacterial activity in vitro, roughly the same as that of the macrolides. However, interesting differences can be pointed out; the parenteral compound Synercid® is effective against staphylococci with the inducible type of resistance and against some of the constitutively resistant ones. Macrolide-resistant *S. pneumoniae* remain susceptible to streptogramins. These compounds are also active against constitutively macrolide-resistant strains of streptococci. Multiple-resistant *Enterococcus faecium* are susceptible in vitro to Synercid®.[14]

PHARMACOLOGY

Pharmacokinetics

Macrolides

The major drawback of earlier macrolides was their poor intestinal absorption (with large variations both within and between subjects), short half-life, high degree of binding to serum proteins (mainly the a-1 acid glycoprotein) and poor gastrointestinal tolerance. The main kinetic properties of macrolides are listed in Table 194.6 .[15] It is important to note wide variations in peak serum concentrations, elimination half-lives and areas under the serum concentration curve (AUC). Furthermore, differences in terms of C_{max} (peak blood level) have been described for the different erythromycin preparations. Generally, the bioavailability of macrolides is low to moderate. Various factors may affect their absorption: the nature of the salt

TABLE 194-6 -- Pharmacokinetics of macrolides and azalides.

PHARMACOKINETICS OF MACROLIDES AND AZALIDES						
Antibiotic	**Oral dose (mg)**	**C_{max} (mg/l)**	**t_{max} (hours)**	**Half-life (hours)**	**Area under serum concentration curve (mg/l hour)**	**Protein binding (%)**
Erythromycin base	500	2.00	3.7	2.0	7.7	74
Roxithromycin	300	10.8	1.6	11.9	116.9	95
Clarithromycin	400	2.1	1.7	4.7	17	70
Azithromycin	500	0.4	2.0	14	4.5	50
Dirithromycin	600	0.32	4.2	28	1.4	15–30
Spiramycin	6 MU	3.3	1	8	8.5	12 (albumin); 6 (a-1 acid glycoprotein)
Josamycin	500	1.2	1	2	7.9	10
C_{max} , peak blood level; t_{max} , time to maximum blood level.						

that is administered (as mentioned above for erythromycin) and the presence of food. Therefore, specific conditions of oral administration should be checked for each compound (Table 194.7). The free fraction of macrolides diffuses into most tissues. Penetration into the cerebrospinal fluid is poor.

All macrolides exhibit intracellular accumulation mainly in polymorphonuclear leukocytes and macrophages; some of them have prolonged persistence of high intracellular levels. This phenomenon has also been observed in nonphagocytic cells and it has been proposed as the reason for the sustained bioactivity of macrolides against intracellular pathogens. Important variations in intracellular levels (with average intracellular to extracellular concentration ratios of more than 10) and half-lives have been described. The entry of macrolides into cells is rapid and almost complete within 15 minutes. The highest intracellular concentrations are currently achieved by azithromycin, which also has the longest intracellular half-life. The uptake rates of the compounds correspond to their lipid solubility, and this process is not saturable. The mechanisms underlying macrolide cellular uptake are still poorly understood, apart from the role of lipid solubility. One possible mechanism involves active transport systems, especially those used by nucleosides. A likely mechanism for the accumulation of macrolides involves their weak basic nature and the possibility of trapping by protonation within acidic cellular compartments, lysosomes and polymorphonuclear neutrophil granules. [16]

Efflux of macrolides from the cells into a drug-free medium is rapid but highly variable from one drug to another. It is therefore possible to distinguish three types of macrolides by comparing them with erythromycin:

- azithromycin, with low extracellular levels, high intracellular penetration, and long extracellular and intracellular elimination half-lives;
- roxithromycin, with relatively high serum levels and shorter half-life than azithromycin; and
- clarithromycin, which has properties of both compounds; some older compounds such as spiramycin and josamycin are also in this intermediate position.

Obviously, the high intracellular concentrations go a long way to explaining the high tissue concentrations that are observed following the administration of macrolides. The therapeutic relevance of these high intracellular levels is less clear. Indeed, high intracellular levels are a prerequisite for the cure of intracellular organisms. However, the location of the offending agent within the cell and the acidic intracellular pH are not always compatible with the expected effect. Furthermore, the concept of the transport of the drug to the infected focus by the means of phagocytic cells remain rather theoretic in the absence of direct proof that the release of the drug from the cell is able to maintain extracellular levels that are sufficient to inhibit extracellular bacteria.

The primary site of metabolism of macrolides is the liver, with the metabolites excreted into the bile. The cytochrome P450 enzymes play a key role. A lesser degree of metabolism occurs in kidneys and lungs. The known metabolic pathways exhibit some features that are common to many members of the class and others that are unique to specific compounds.[17] Within the 14-membered compounds, *N*-demethylation of desosamine and hydrolysis of cladinose are commonly observed. Some specific events can occur, such as the hydroxylation at C14 of clarithromycin, leading to a 14-hydroxy derivative, which acts synergistically with the mother compound

on *H. influenzae*. Azithromycin, a 15-membered macrolide, is not highly metabolized; the unaltered parent compound accounts for 75% of drug-related substances excreted in humans. Within the family of 16-membered macrolides, cleavage of the 4''-O-acyl group on the terminal neutral sugar by liver esterases commonly occurs. In addition, oxidations at either the ß-carbon of the 4''-ester or at the C14 of the lactone ring are frequently observed. Interactions of macrolides with hepatic enzymes, especially cytochrome P450-dependent mono-oxygenases, is very important. Several drug interactions involve this system, which controls the oxidative metabolism and elimination of medicinal compounds. Erythromycin and troleandomycin (a macrolide that is no longer available in most countries) exert a marked disturbance of liver cytochromes P450 of the 3A subfamily in humans. This is the consequence of three concomitant phenomena:

- the induction of a cytochrome P450 3A;
- its inactivation by the formation of an iron-metabolite complex caused by the strong binding of a nitrosoalkane metabolite derived from the macrolide to P450 iron(II); and
- the accumulation of this complexed P450 through its stability in the presence of degrading enzymes.

Three structural factors are important in the formation of inhibitory P450-iron-metabolite complexes during macrolide oxidation:

- the presence of an $N(CH_3)^2$ amine function;
- the accessibility of this amine function, which is required for the strong binding of the nitrosoalkane metabolite to heme; and
- the hydrophobicity of the molecule.

All macrolides that are prone to forming these complexes are good inducers of P450 3A. Metabolism can be affected by liver function abnormalities. Binding of a-1 acid-glycoprotein can be decreased by a reduction in the production of this protein, with a subsequent

TABLE 194-7 -- Mode of administration of macrolides and modification of dosage in specific conditions.

MODE OF ADMINISTRATION OF MACROLIDES AND MODIFICATION OF DOSAGE IN SPECIFIC CONDITIONS					
Macrolides	**Presentation**	**Route of administration**	**Dosage**	**Effect of food**	**Modification of dosage in specific conditions**
Azithromycin	Capsules (250mg)	po	500mg on day 1, then 250mg q24h	50% reduction in AUC	If creatinine clearance >30ml/minute, no modification
	Pediatric suspension				
	or				If creatinine clearance <30ml/minute, situation uncertain
	Granules	po	10mg/kg/day then 5mg/kg q24h		
					Caution in severe hepatic failure
Clarithromycin	Tablets (250mg)	po	250–1000mg q12h	No effect	50% reduction in dose if creatinine clearance <30ml/minute
	Tablets (500mg modified release)				No data in hepatic failure
Dirithromycin	Tablets (250mg)	po	500mg q24h	Increased bioavailability	50% reduction in dose if creatinine clearance <5ml/minute and in those aged over 80 years
Erythromycin lactobionate	Vials (500mg and 1000mg)	iv	2000mg/day by continuous infusion or 500mg q6h (infused over 1 hour) in adults; 30–40mg/kg/day in children	No effect	50% reduction in dose in case of renal failure
					Caution in the elderly
Erythromycin ethylsuccinate	Pediatric suspension or power (125mg and 250mg)	po	30–50mg/kg/day in children	No effect	Avoid in hepatic failure
	Tablets (500mg and 1000mg)		2000–3000mg/day in adults		No modification in renal failure
Erythromycin propionate	Tablets (500mg)	po	500mg q12h	Decreased bioavailability	Avoid in hepatic failure
					Reduce dose in severe renal failure
Dihydrated erythromycin	Capsules (250mg)	po	500mg q12h	Decreased bioavailability	Avoid in hepatic failure
					Reduce dose in severe renal failure
Midecamycin diacetate	Tablets (400mg)	po	800mg q12h	No effect	Avoid in hepatic failure
					No modification in renal failure
Josamycin	Sachets (1000mg)	po	500–1000mg q12h	No effect	Avoid in hepatic failure
	Tablets (500mg)				No modification in renal failure
	Pediatric suspension				
	Granules				
Roxithromycin	Tablets (100mg or 150mg)	po	150mg q12h	No effect	Avoid in case of hepatic failure
	Pediatric suspension		3mg/kg q12h		No modification in renal failure
Spiramycin	Tablets (1.5×10^6 units or 3×10^6 units)	po	3×10^6 units q8-12h	No effect	No modification in renal failure
	Granules or suspension (1.5×10^6 units)	iv			

increase in the free fraction of the macrolide. In normal conditions, the main route of elimination of macrolides is the liver. Renal elimination contributes little to total clearance. Changes in macrolide pharmacokinetics due to hepatic or renal impairment depend on the molecule considered, the route of administration and the extent of disturbance in renal or hepatic functions. Table 194.7 indicates the changes to be considered in the dosages in the case of renal or hepatic functional disturbances.

The route of administration of macrolides is usually oral, except for erythromycin and, in some countries, azithromycin and spiramycin, which have a parenteral form. The number of daily doses (usually two) depends on the elimination half-life of the drug and the severity of infection (see Table 194.7).

Ketolides

Telithromycin is administered orally at a 800mg od daily dose. (Max is 2.3mg/L, AUC 0.24h:12.5µg.h/mL; elimination half-life 9.8h.) The compound is 70% bound to serum proteins. Hepatic metabolism (through CYP 3 A4) represents 37% of the elimination process.

Lincosamides

Orally administered clindamycin has good bioavailability (about 90%), and food does not interfere with its absorption. Protein binding varies from 60% to 95%. Serum C_{max} is around 4.5mg/l after a 300mg dose. The elimination half-life is about 3 hours. Penetration into the cerebrospinal fluid is limited. Lincosamides are actively transported into phagocytic cells. Approximately 95% of administered clindamycin is excreted unchanged or is metabolized through the liver; 5% is excreted unchanged into the urine. Active metabolites represent 20% of the total amount excreted. Lincomycin can be administered orally or intravenously. The common doses for adults are 300mg q8h orally or 600mg q8h intravenously, and for children 10–30mg/kg/day (divided into three doses). No dose adjustment is necessary for patients who have renal insufficiency but a 50% reduction in the dose may be needed for patients who have severe hepatic impairment.

Streptogramins

The data on the pharmacokinetics of pristinamycin are very limited, owing to the complexity of the components of the commercial preparation, the number of metabolites and the great instability of the drug in biologic fluids. In some countries, pristinamycin is available for oral administration, 2–3g/day (50–100mg/kg/day in children), to be given as two to four doses. No dosage modification is needed in renal or hepatic failure.

The pharmacokinetic properties of the two components of Synercid® given intravenously in a 30:70 ratio (quinapristin:dalfopristin) at doses

of 5, 10 and 15mg/kg have been studied in healthy human volunteers.[18] Mean maximal concentrations (mg/ml) were 1.3, 2.4 and 3.3 for quinupristin and 5.1, 7.1 and 8.5 for dalfopristin. Elimination half-lives ranged between 0.6 hours and 1 hour for quinupristin and between 0.3 hours and 0.4 hours for dalfopristin. Plasma clearance was high for both compounds: 1l/h/kg for quinupristin and 0.8l/h/kg for dalfopristin. The plasma levels of the active metabolite of dalfopristin were 20–45% those of the parent drug, showing a trend to increase with the dose. Synercid® must be administered by intravenous infusion using a central vein. The recommended doses are 7.5mg/kg q8-12h, depending on the organism responsible for infection and the severity of the infection.

Pharmacodynamics

Antibacterial mechanisms

Macrolides

The efficacy of macrolides against extracellular pathogens depends on the extracellular concentrations of free drug and the level of susceptibility of the organisms.[19] Macrolides exhibit a time-dependent bactericidal effect against most bacteria of their clinical spectrum, particularly against streptococci. They are slowly bactericidal and increasing the concentration has little influence on the rate of killing. The size of the inoculum affects the antistreptococcal effect and also antistaphylococcal activity. In the presence of serum, the MIC of some macrolides (e.g. roxithromycin, rokitamycin) against *S. pyogenes* increases 1- to 4-fold. In contrast, the MIC for *S. pneumoniae* does not change. The optimal effect is observed at pH 8, with a significant decrease in efficacy at pH less than 6. Against extracellular pathogens, the time during which the concentration of free extracellular drug is above the MIC is the major determinant of the efficacy of macrolides.

Ketolides

Telithromycin exhibits a concentration-dependent effect against *S. pneumoniae*, the AUC to MIC ratio being the best predictive factor of bacterial outcome both in animal and human studies.[20]

Streptogramins

The two components of Synercid® are bacteriostatic against MLS_B -susceptible and methicillin MLS_B inducibly resistant *Staphylococcus aureus*, whereas the MLS_B constitutively resistant strains are resistant to quinupristin. Synergy between antibiotics in vitro is a phenomenon found at concentrations close to the MIC, except for strains with constitutive resistance. High-level resistance to quinupristin does not significantly reduce the activity of the combination, and generally the MIC index is found to be less than 0.5. The bactericidal effect of Synercid® against *S. aureus* appears to be time-dependent but not concentration-dependent, because neither quinupristin nor dalfopristin alone is bactericidal. Synercid® is more rapidly bactericidal against *S. pneumoniae* (1h) than against *S. aureus* (6h); it is slowly active against *E. faecium*.

Postantibiotic effect

The presence of a postantibiotic effect (PAE) is a feature common to all macrolides (see Chapter 188). Several factors may affect the in-vitro PAE of these drugs.[21] The duration of this effect is longer with Gram-positive cocci than with *H. influenzae*. Increasing macrolide concentrations and exposure time prolongs the duration of PAE to a point of maximum response.

The PAE of Synercid® has been evaluated against a variety of bacteria, mainly staphylococci, pneumococci and streptococci. The PAE is constantly observed at concentrations above the MIC.[21]

Nonantibacterial pharmacologic effects

Erythromycin and 14-membered macrolides exhibit agonistic activity to motilin receptors and are therefore able to accelerate gastric emptying.

In relation to their high and prolonged intracellular concentrations in phagocytic and nonphagocytic cells, macrolides show an effect on some cellular functions (e.g. they enhance phagocytosis, bacterial killing and chemotaxis). These effects are observed mainly with very high extracellular levels. The macrolides with antibacterial effect have very few immunosuppressive effects, if any. Some molecules derived from this class of macrocyclic antibiotics, such as FK506, are used as immunosuppressive agents.

INDICATIONS

Macrolides in adult patients

Macrolides have a number of uses in both immunocompetent and immunocompromised patients (Table 194.8).[22]

In respiratory tract infections, the use of a macrolide as a first-line monotherapy is, in some areas, made difficult by a high rate of resistance of *S. pneumoniae* to erythromycin. In community-acquired pneumonia, the use of a macrolide in patients who are aged less than 60 years and who have no risk factors or signs of severe pneumonia has been proposed.[23] However, this point is considered controversial by some authors, who argue that the role of intracellular pathogens as etiologic agents of community-acquired pneumonia is limited in this subgroup of patients. As far as *Legionella pneumophila* or *Chlamydia pneumoniae* can be recognized or highly suspected as responsible for the infection, a macrolide is considered a useful therapy.[24] Finally, in cases of severe infection, the prescription of a combination of a ß-lactam with a macrolide is recommended (see Chapter 34).[23]

Macrolides are used to treat some sexually transmitted diseases. [25] Azithromycin, given as a single dose of 1g, has been documented as effective in cervicitis and urethritis caused by *Chlamydia trachomatis*.[26] Conversely, such a short course of therapy is not effective in gonococcal infections, which require longer administration. For gonococcal infections and syphilis, macrolides are considered a second-choice treatment, even in cases of allergy to ß-lactams, because more efficient alternatives exist. Activity of macrolides has been documented in donovanosis and lymphogranuloma venereum (see Chapter 74 & Chapter 78).

Given their limited activity against staphylococci, macrolides are not considered major agents in the therapy of skin and soft tissue infections, except when streptococci are highly suspected (impetigo) or documented, and in cases of allergy to ß-lactams.

Azithromycin and clarithromycin have been evaluated as major components of the combinations that are active against *H. pylori*, including metronidazole and an

inhibitor of the proton pump. The selection of strains with high-level resistance after the use of macrolide has been demonstrated as the major drawback of macrolide monotherapy. *Campylobacter jejuni* is highly susceptible in vivo to macrolides. However, the use of macrolides in acute gastroenteritis supposes a precise bacteriologic documentation (see Chapter 43). Azithromycin has been shown to be effective in typhoid fever.[27] The clinical experience remains limited and this drug is considered a second-choice alternative therapy.

The C16 macrolide spiramycin is marketed in a limited number of countries. Very few well-designed clinical studies have been performed. Some studies indicatethat its activity is equivalent to that of other macrolides in the major indications allowing the oral route. In some countries, spiramycin is considered as a drug of choice for the treatment of toxoplasmosis occuring during prenancy. Data on the efficacy of the compound in such an indication are of limited scientific value. Tolerance of spiramycin is good with very limited drug-drug interactions.

The impact of a large use of C16 macrolide in a community on the level of resistance of streptococci and pneumococci, actually depends on the respective prevalence of the different genotypes of resistance to macrolides amongst those organisms.

TABLE 194-8 -- Potential uses of macrolides in adult patients.

POTENTIAL USES OF MACROLIDES IN ADULT PATIENTS		
	Condition	**Comments**
Respiratory tract infections	Acute sinusitis	Not first-line agents
	Otitis media	Not first-line agents
	Pharyngitis	In cases of allergy to penicillins
	Acute exacerbation of chronic bronchitis	In areas with low rate of pneumococcal resistance to macrolides
	Community-acquired pneumonia	First-line drug in adults aged over 60 years and without risk factors, signs of severe pneumonia or evidence of pneumococcal infection; alternatives in cases of allergy to ß-lactams; evidence of infection with *Legionella* spp. or *Chlamydia* spp.; combine with a ß-lactam in severe cases
Sexually transmitted diseases	Chlamydial urethritis or cervicitis	Single dose (1g) or azithromycin
	Donovanosis or lymphogranuloma venereum	Demonstrated efficacy
	Syphilis	Alternatives to ß-lactams (limited activity)
	Gonococcal infection	Alternatives to ß-lactams (limited activity)
Skin and soft tissue infections	Streptococcal infections	Limited activity versus staphylococcal infections
Gastrointestinal infections	*Helicobacter pylori*	Azithromycin or clarithromycin in triple combinations
	Campylobacter jejuni	See text
	Typhoid fever	Azithromycin is an alternative to other therapies (limited experience)
Specific organisms	*Borrelia burgdorferi*	See text
	Rickettsia typhus group	See text
	Bartonella spp.	Azithromycin can be used in cat-scratch disease (limited experience)
Immunocompromised host	Mycobacteria (*Mycobacterium leprae, M. chelonae, M. fortuitum*)	See text
	Mycobacterial disseminated infections (*M. avium*)	In combination with other agents
	Toxoplasmal encephalitis	Minor alternative to other regimens (in combination)
	Cryptosporidial intestinal infection	Azithromycin has a limited activity at high doses
	Rhodococcus equi infection	Limited experience
	Bacillary angiomatosis	Activity has been demonstrated
Nonantibacterial use	Gastric paresis	Erythromycin has documented activity
Possible future uses	Atherosclerosis	Possible role in control of coronary restenosis and prevention

The use of azithromycin or clarithromycin in the treatment of Lyme disease remains speculative and some studies indicate that amoxicillin might be superior (see Chapter 54). Macrolides are active against *Rickettsia* spp. of the typhus group, and josamycin may be considered a safe alternative to treat Mediterranean spotted fever.[28] Both *Bartonella henselae* and *Bartonella quintana* are susceptible to macrolides. Azithromycin has been demonstrated to be of potential use in the treatment of cat-scratch disease (Chapter 91).[29] The clinical experience with macrolides is still limited in the treatment of other forms of infections caused by *Bartonella* spp. in immunocompetent patients.

Monthly regimens that include clarithromycin and minocycline have demonstrated a significant clinical efficacy in the treatment of lepromatous leprosy. Further investigations are needed to determine the proper dosage of both drugs. [30] Infections due to *Mycobacterium chelonae* and *M. fortuitum* may be susceptible to azithromycin or clarithromycin (see Chapter 3 and Chapter 233). [31]

Erythromycin is used for its effects on gastric and intestinal motility to treat gastric paresis or gastrointestinal disturbances in diabetic or postsurgical patients.

In immunocompromised patients, mainly those infected by HIV, the newest macrolides are considered helpful agents in the treatment of disseminated *M. avium* infections. Monotherapy with azithromycin or clarithromycin causes the rapid selection of resistant variants, and therefore a multiple combination is required. Neither azithromycin nor clarithromycin is very helpful in the treatment of encephalitis caused by *T. gondii* because they need to be given in high doses, often intravenously and in combination with other compounds. Azithromycin, even at high doses, has shown limited activity against diarrhea caused by *Cryptosporidium parvum.* Bacillary angiomatosis and some forms of *Rhodococcus equi* infections are susceptible to macrolides.

Macrolides have a number of potential prophylactic uses (Table 194.9). In surgical prophylaxis, erythromycin can be used in combination with neomycin and enemas for the preparation of the large bowel before surgery. Macrolides are included in the recommendations for the prevention of bacterial endocarditis in low-risk patients undergoing dental procedures who are intolerant of ß-lactams. [32] There is a still limited experience in the use of azithromycin for the prevention of malaria.[33]

In immunocompromised hosts, macrolides are mainly used in the prevention of disseminated *M. avium* complex infection (see Chapter 129).[34] Azithromycin has been shown to be effective in primary prevention when given once weekly as has daily clarithromycin.[35] A combination of clarithromycin (or azithromycin) and another drug can be considered as an effective choice for the prevention of recurrences.

TABLE 194-9 -- Potential prophylactic uses of macrolides in adult patients.

POTENTIAL PROPHYLACTIC USES OF MACROLIDES IN ADULT PATIENTS		
	Indication	**Comments**
Immunocompetent host	Surgical prophylaxis (large bowel preparation)	Oral therapy in combination with neomycin and enema
	Bacterial endocarditis	Alternatives to ß-lactams in allergic patients
	Malaria	Limited experience with azithromycin
Immunocompromised host	Primary prevention of *Mycobacterium avium* complex infections	Weekly administration of azithromycin or daily clarithromycin has documented activity
	Prophylaxis of recurrences of *Mycobacterium avium* complex infections	Clarithromycin or azithromycin combined with another agent

TABLE 194-10 -- **Specific uses for macrolides in pediatric patients.**[*]

SPECIFIC USES FOR MACROLIDES IN PEDIATRIC PATIENTS		
	Indication	**Comments**
Upper respiratory tract infections	Acute otitis media	Not first-line choice
		Combine with sulfonamides
	Sinusitis	Not first-line choice
	Tonsillitis	Alternative to penicillin G and penicillin V in allergic patients
	Diphtheria	Eradication of acute and chronic carrier states
	Acute bronchitis	Antibiotic therapy has questionable efficacy
	Pneumonia	Not first-line choice as single agents
		Combination with ß-lactam in severe cases if infections caused by *Legionella* spp. and *Chlamydia* spp. a concern
	Whooping cough	Shortens the duration of disease
		Eliminates carrier state
Skin and soft tissue infections	*Campylobacter jejuni* enteritis	Treatment of choice
	Ureaplasmal or chlamydial infections in neonates	Treatment of choice
Local uses	Acne	See text
	Conjunctivitis	Alternatives as prophylaxis of ophthalmia neonatorum
Prophylactic uses	Endocarditis	Alternatives to ß-lactams in case of allergy
	Recurrences of rheumatic fever	Alternatives to ß-lactams in case of allergy

** Data from Guay.*[36]

Ketolides

Telithromycin has demonstrated equivalent activities to those of the comparators in the following indications: mild to moderate community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute maxillary sinusitis and streptococcal pharyngitis, in adult patients at a single daily dose of 800mg. In most of those indications, a duration of treatment of 5 days has been shown to be acceptable.[20] Its use as a first-line choice for the treatment of community-acquired pneumonia is considered in many countries.

Use of macrolides in special circumstances

Some indications, more or less specific to children, are presented in Table 194.10 .[36] Macrolides are no longer considered to be first-choice drugs in the treatment of otitis media, owing to major changes in the susceptibility of pneumococci to erythromycin. [37] The combination of a macrolide and a sulfonamide has been shown to increase the effect against *H. influenzae*. The use of macrolides in tonsillitis should be considered in patients who are intolerant to ß-lactams in areas where group A streptococci have a low rate of resistance to erythromycin. The newest macrolides allow a reduction in the duration of treatment from 7–10 days to 4–5 days.[38] Topical preparations are used in the treatment of acne and for the prevention of ophthalmitis in neonates.

Table 194.11 gives some points to consider when choosing a particular compound once it has been established that the use of a macrolide is indicated. Efficacy for the indication should be the first point to be taken into consideration, together with the risk of side effects, drug interactions and poor patient compliance.

Table 194.7 mentions the modifications to be made in dosing in case of renal failure for the macrolides, and cautions around the use of those drugs in case of hepatic failure. In elderly patients, the degree of deterioration of the renal function should be taken into consideration to adapt the dose and dosing regimen of the intravenous forms of the macrolides. Also, as indicated in Table 194.12 , special attention should be paid to drugs currently taken by the patients at the moment when a macrolide is prescribed. The same type of measures are to be envisaged with telithro-mycin. For this latter drug, no specific adaptation of the dose is needed in patients who have renal failure or in elderly patients. Similar reasoning is acceptable for lincosamides and synergistins. Macrolides and lincosamides are not contraindicated during pregnancy. There are no data regarding Synercid® in that

TABLE 194-11 -- Advantages and disadvantages of specific macrolides.

ADVANTAGES AND DISADVANTAGES OF SPECIFIC MACROLIDES		
Agent	**Advantages**	**Disadvantages**
Erythromycin	Long clinical experience	Poor bioavaliability
	Good documentation of efficacy	Poor tolerance 2–4 doses per day
	Low cost	Drug-drug interactions
		Acquired resistance
Roxithromycin	Increased absorption	Drug-drug interactions
	Decreased side effects	Same resistance profile as erythromycin
	Adequate extracellular levels	
	Improved activity against mycobacteria than erythromycin	

Clarithromycin	Improved activity against some Gram-negative bacteria and some mycobacteria	Drug-drug interactions
	Increased absorption	Same resistance profile as erythromycin
Azithromycin	High intracellular levels	Poor extracellular levels
	Improved activity against some Gram-negative bacteria and mycobacteria than erythromycin	Poor documentation in bacteremic pneumococcal diseases
	Limited drug-drug interactions	Same resistance profile as erythromycin
	Once-daily administration	
	Short course of therapy	
	Single dose in *Chlamydia trachomatis* infection	
Dirithromycin	Improved biovailability than erythromycin	Same resistance profile as erythromycin
	Once-daily administration	
	Few drug-drug interactions	
Spiramycin	Improved bioavailability than erythromycin	Limited clinical documentation
	Good tolerance	Same resistance profile as erythromycin
	Limited drug interactions	
Josamycin	Good tolerance	Same resistance profile as erythromycin
		Drug-drug interactions

TABLE 194-12 -- Macrolide drug interactions.[*]

MACROLIDE DRUG INTERACTIONS	
Erythromycin	Avoid concomitant use of theophylline, ergotamine, bromocriptine, warfarin, carbamazepine, triazolam, midazolam, alfentamil, cyclosporin, terfenadine, cisapride and astemizole
	Caution in the use of digoxin (increased bioavailability — check serum levels)
Josamycin	Avoid concomitant use of astemizole, cisapride, ergotamine, bromocriptine, terfenadine, triazolam
	Caution in the use of theophylline and cyclosporin
Roxithromycin	Avoid concomitant use of triazolam, midazolam, cisapride, astemizole and cyclosporin
Clarithromycin	Avoid concomitant use of terfenadine, carbamazepine, ergotamine and cisapride
	Take zidovudine 2 hours apart from macrolide
	Caution in the use of theophylline
Midecamycin	Avoid concomitant use of ergotamine, cisapride and bromocriptine
	Caution in the use of cyclosporin and warfarin
Dirithromycin	Caution in the use of cyclopsorin and cisapride (?)
Spiramycin	Caution in the use of levodopa and cisapride (?)
Azithromycin	Avoid concomitant use of ergotamine, cisapride and bromocriptine
	Caution with use of cyclosporin

** Data from Guay.*[36]

situation. However, the risks related to the infection caused by multiresistant organisms should be considered if Synercid® represents the only therapeutic option.

Future developments in macrolide use

The potential use of the newest compounds in the treatment of atherosclerosis, because of their activity against *C. pneumoniae*, one of the micro-organisms potentially involved in the pathogenesis of atherosclerosis, is the most attractive future development in the use of macrolides.[39] On the basis of controversial results, investigations are in progress to delineate their role in the therapy and possibly the prevention of the disease. Other areas of potential interest could be the effects of macrolides on the expression of bacterial virulence factors, the formation of bacterial biofilms and their use as immunomodulators.

Synergistins

Oral pristinamycin is marketed in a limited number of countries. It is used in minor or moderately severe infections such as community-acquired pneumonia and community-acquired staphylococcal infections. The development of the parenteral streptogramin Synercid® is focused on methicillin-resistant *S. aureus, E. faecium* resistant to glycopeptides, macrolide- or penicillin-resistant pneumococci, and the treatment of Gram-positive cocci infections in patients not able to tolerate a more conventional agent.[40]

Clindamycin

Clindamycin is used in polymicrobial infections outside the central nervous system, including intra-abdominal, gynecologic, bronchopulmonary, and skin and soft tissue infections.[41] Its use may be compromised by the development of resistance among staphylococci and some anaerobes such as *B. fragilis*. Clindamycin has been shown to improve the efficacy of doxycycline in the treatment of severe malaria caused by *Plasmodium falciparum* and that of quinine in the treatment of babesiosis.

Topically, clindamycin is used for the treatment of acne and bacterial vaginosis.

It is used in combination with pyrimethamine as an alternative for the treatment or prevention of *T. gondii* encephalitis in HIV-infected patients.[34]

ADVERSE REACTIONS AND INTERACTIONS

Macrolides

Adverse reactions

In general, the macrolides have a high degree of safety.[36] The main adverse effects are referable to the gastrointestinal tract. Those effects are partly related to the dose-dependent prokinetic effects of the macrolides, with a higher incidence in young adults than in people over 70 years old. The 14-membered macrolides exhibit the most important effect on intestinal motility; the 16-membered have no such effects. These effects include nausea, vomiting, diarrhea and abdominal discomfort. Erythromycin is responsible for the highest percentages of gastrointestinal side effects, with values around 30% and a rate of discontinuation of therapy around 5%. These effects are significantly lower with the newest compounds, partly as a result of better bioavailability allowing lower oral doses (gastrointestinal side effects in

between 3% and 10% of patients and discontinuation of therapy in 1–4%).

Reversible cholestatic hepatitis has been reported with erythromycin estolate, mainly in children, in about one case in 1000. A hypersensitivity mechanism has been suggested in view of the rapid development of cholestatic hepatitis in patients who have previous drug-induced hepatitis. Erythromycin estolate is contraindicated in pregnancy. The frequency of hepatitis with other erythromycin preparations, mainly ethylsuccinate, is much lower. In patients who have had a previous episode of hepatitis induced by erythromycin estolate, the other macrolides should be used with caution or even considered as contraindicated. No cases of hepatitis have been reported with the newest macrolides. Transient elevation of hepatic enzymes have been rarely reported; this has been observed in about 10% of patients receiving high doses of clarithromycin (2g/day or more).

Erythromycin can cause a dose-dependent, reversible hearing loss, usually in patients who have renal or hepatic impairment. Hearing loss has rarely been reported with high doses of clarithromycin.

Hypersensitivity reactions can be observed with any macrolide.

Prolongation of the QT interval on electrocardiography leading in some cases to recurrent *torsades de pointes* may rarely occur following erythromycin lactobionate administration. Cases of *torsades de pointes* have also been described in patients receiving clarithromycin and azithromycin. Telithromycin and presumably other ketolides have also the potential to cause a prolongation of the QT_c interval, especially in elderly patients who have predisposing conditions or those who are concurrently receiving drugs that are substrates for CYP2D6 and 3A4.[41] Macrolides should be used with caution in patients at risk of these disorders, such as elderly female patients, patients who have inborn prolonged QT_c interval, patients who have congestive heart failure, patients who have hypomagnesemia or hypokalemia, those on diuretic therapy and patients receiving concomitant drugs able to potentiate the toxic effects of the macrolide (e.g. antiarrhythmic agents from class II, amiodarone, certain antihistaminics, certain fluoroquinolones). Disturbances of the intestinal microbial flora, with selection of staphylococci, enterococci or *Candida* spp., have been reported. Like many other oral antimicrobial agents, macrolides can induce *Clostridium difficile* colitis.

Ketolides

The ketolides have a similar spectrum of adverse events as the macrolides. In clinical trials in which a daily dose of 800mg was used the adverse events rate and profile were similar to the comparator agent, with diarrhea (13.3%) and nausea (8.1%) being the most common; other less frequent adverse events were vomiting, abdominal pains and elevation of liver enzymes.[42] From more than 1.5 million patient treated with telithromycin, no unexpected adverse event was reported. QT_c prolongation was not reported. In 0.6% of the cases, blurred vision related to a transient, fully reversable, trouble with accomodation was reported.

Drug interactions

Apart from the interaction of macrolides with digoxin, which is related to the destruction by the macrolide of enteric flora that are partly responsible for digoxin metabolism, most of the interactions between macrolides and other drugs involve inhibition of drug metabolism via cytochrome P450 microsomal enzymes (see Pharmacology). Erythromycin and troleandomycin are the macrolides that are most involved in these interactions. Azithromycin, rokitamycin and spiramycin neither activate the cytochrome P450 nor form complexes with it, and therefore they are unable to modify the pharmacokinetics and metabolism of other drugs.[15] Clarithromycin, roxithromycin, josamycin and midecamycin induce cytochrome P450 isoenzymes but, unlike erythromycin, they do not form complexes with the glucocorticoid-inducible isoenzymes.

Some of these interactions suggest that the concomitant use of a macrolide and the other drug in question should be avoided. If the combination cannot be avoided, then serum concentrations of the concurrently administered drug should be monitored and patients observed for signs of toxicity. Table 194.12 summarizes the main macrolide drug-drug interactions and their clinical relevance.[36] [42]

Streptogramins

Local pain and erythema in the vein used for infusion of this antibiotic has been frequent and dose-dependent. In 5% of patients this tolerance leads to discontinuation of the therapy and in more than 6% a change in the infusion site is required. Venous tolerability is improved by dissolving quinupristin-dalfopristin in larger quantities (over 250ml) of 5% dextrose and by using a central vein. Moderate and transient elevation of liver enzymes has also been reported in doses exceeding 10mg/kg. Itching, burning and erythema of the front of the neck and upper torso have also been reported in a few patients.[40] Arthralgia, myalgia, nausea and a rash are also common and elevation of liver enzymes are not uncommon. Synercid is a potent inhibitor of CYP3A4 and should be used with caution in patients taking drugs that are substrates of 3A4. Elevation of serum levels of nifedipine, midazolam and cyclosporin have been observed. Synercid has also the potential to prolong the QT_c interval when administered with drugs metabolized by 3A4 (e.g. cisapride).

Clindamycin

Adverse reactions include a morbilliform-rash, urticaria and, rarely, anaphylactoid reactions. Liver injury has also been reported rarely. Clindamycin-related diarrhea is reported in 2–30% of patients. It is usually a self-limiting problem, disappearing once clindamycin therapy is stopped. A small number of patients develop *C. difficile* colitis with various degrees of severity. [41]

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Chapter 195 - Oxazolidinones

Franklin D Lowy

The oxazolidinones are a relatively new family of bacteriostatic antimicrobials that are protein synthesis inhibitors. Because this new family works at the early stage of protein synthesis involving formation of the 70S initiation complex, there does not appear to be cross-resistance with other protein synthesis inhibitors. Originally developed as an agent for the treatment of bacterial and fungal infections of plants, the agents were subsequently found to have activity against Gram-positive bacteria. Upjohn originally developed two oxazolidinones, eperezolid and linezolid. Based on its more advantageous pharmacologic profile, linezolid was selected for further investigation. At present linezolid is the sole oxazolidinone available. The discussion below is therefore limited to this product. A number of comprehensive reviews of oxazolidinones and linezolid have recently been published.[1] [2] [3] [3A]

PHARMACOKINETICS AND DISTRIBUTION

Linezolid has similar pharmacokinetics whether administered parenterally or orally. It is completely absorbed following oral administration achieving a bioavailability of 100%. Food causes a slight decrease in the rate of absorption but not in the overall amount absorbed. In normal volunteer studies peak plasma concentrations were achieved in 1–2 hours. Steady state concentrations were approximately 12 and 18mg/l following oral doses of 375 and 625mg twice daily respectively. Following intravenous administration of 625mg twice daily to volunteers for 7.5, days the steady state level was 3.8mg/l.[4] [5] The half-lives of oral and intravenously administered linezolid are 5.4 and 4.8 hours, respectively. The drug is excreted by both renal and non-renal routes; 90% of circulating drug is not metabolized, but there are two inactive metabolites that are the result of oxidation of the morpholine ring. The P450 system does not appear to be involved in the metabolism of linezolid thus limiting the number of possible drug-drug interactions.

Linezolid is 31% bound to plasma proteins. It has a relatively large volume of distribution of 40–50l. Information on tissue penetration is still incomplete, however linezolid appears to penetrate well into tissue blister fluid, pulmonary alveolar macrophages, and sweat.[2] [6] Preliminary studies suggest reasonable penetration of bone, fat and muscle. Mean ratios of linezolid in tissue fluid/plasma were 0.55, 1.2 and 0.71 for sweat, saliva and cerebrospinal fluid, respectively. There is little additional information at present on the tissue distribution of linezolid in humans.

ROUTE AND DOSAGE

As noted, linezolid can be administered orally or intravenously. Adjustment of dosage is not necessary when switching from one route to the other. The recommended dosage for serious infections such as nosocomial pneumonia or complicated skin and soft tissue infections is 600mg q12h. For uncomplicated infections including community acquired pneumonias or uncomplicated cutaneous infections 400mg q12h is adequate.

In patients with mild-to-moderate renal impairment (creatinine clearance 10–79ml/min) dosage adjustment is not necessary. However with more severe forms of renal disease dosage adjustment may be necessary.

The pharmacokinetics of linezolid appears unaffected by age. Dosage adjustment for the elderly is unnecessary. The clearance of linezolid is more rapid in children than in adults. As a result, the dose recommended for infants and children is 10mg/kg q8–12h. The only dosage schedule investigated to date in children has been q12h.

INDICATIONS

The oxazolidinones are primarily indicated for the treatment of bacterial infections caused by the Gram-positive staphylococci, streptococci and pneumococci, although their spectrum of antibacterial activity extends beyond these species. Linezolid has excellent *in-vitro* activity against staphylococcal species including methicillin-susceptible and resistant strains. There is little difference in the average minimal inhibitory concentrations (MICs) for methicillin-resistant and susceptible staphylococcal isolates. Linezolid is also active against the recently described *Staphylococcus aureus* isolates that are intermediate in susceptibility to glycopeptides — *Staph. aureus* with intermediate susceptibility to vancomycin (VISA) or *Staph. aureus* with intermediate susceptibility to glycopeptides (GISA) isolates.

In contrast with the protein synthesis inhibitor combination dalfopristin/quinupristin, linezolid is active against all enterococci, including *Enterococcus faecalis* and *Enterococcus faecium*, as well as those enterococcal strains that are vancomycin-resistant. It is also active against penicillin-susceptible and resistant *Streptococcus pneumoniae*, again with comparable MIC values (Table 195.1). In addition to these common Gram-positive pathogens, linezolid also has activity (MIC =4µg/ml) against some of the less frequently encountered Gram-positive organisms such as *Corynebacterium spp.*, *Bacillus spp.*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae* and *Rhodococcus equi*.

Linezolid is moderately active against some anaerobes including *Clostridium spp.*, *Peptococcus spp.*, *Bacteroides fragilis* and *Fusobacterium nucleatum* and *F. meningosepticum*. It has limited activity against some Gram-negative bacteria such as *Moraxella*, *Bordetella* and *Haemophilus* spp. and has no activity against Enterobacteriaceae or *Pseudomonas* species. Of considerable interest for future development, is linezolid's *in-vitro* activity against *Mycobacterium tuberculosis* and *M. avium* complex.[7] [8] This activity includes several multidrug resistant *M. tuberculosis* isolates. Chemical modification of linezolid is currently being explored to develop new drugs within the oxazolidinone class with enhanced antimycobacterial activity.

In animal studies linezolid has shown activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *E. faecium* in the endocarditis model.[9] [10] In a rabbit model of penicillin-resistant and susceptible pneumococcal meningitis, meningeal penetration of linezolid was good (38% of serum levels), however overall it was less effective than ceftriaxone.[11]

TABLE 195-1 -- **In-vitro antimicrobial susceptibility for linezolid against common Gram-positive pathogens.**†

IN-VITRO ANTIMICROBIAL SUSCEPTIBILITY FOR LINEZOLID AGAINST COMMON GRAM-POSITIVE PATHOGENS			
Organism	**MIC (µg/ml) 50%***	**MIC(µg/ml) 90%**	**Overall (µg/ml)**
***Staph. aureus* (n**=2256)			
Oxacillin susceptible	1–4	1–4	0.5–8
Oxacillin resistant	1–4	1–4	0.5–8
Coagulase-negative staphylococci (n=48)			
Oxacillin susceptible	0.5–2	1–4	0.25–4
Oxacillin resistant	0.5–2	1–2	0.5–4
ß-haemolytic streptococci (n=47)	1–2	2–4	1–4
***Strep. pneumoniae* (n**=454)			
Penicillin susceptible	0.5	1	<0.016–1
Penicillin resistant	0.5–1	1	0.06–4
***Enterococcus* spp. (n**=980)			
Vancomycin susceptible	1–4	1–4	0.5–4
Vancomycin resistant	2–4	2–4	1–4
Source: adapted from ref 16 with original data in refs 26 and 31–36.			

† *With permission from Diekema DJ, Jones RN, 2001.*[1]
*Minimum concentration at which 50% of strains are inhibited.

TABLE 195-2 -- Potential clinical indications for the use of linezolid.

POTENTIAL CLINICAL INDICATIONS FOR THE USE OF LINEZOLID	
FDA-approved Indications Nature of infection	**Potential pathogens**
Skin & soft tissue (complicated)	MSSA, MRSA, *Streptococcus pyogenes*, *Streptococcus agalactiae*
Skin & soft tissue (uncomplicated)	MSSA, *Streptococcus pyogenes*
Infection with bacteremia	*Enterococcus faecium*
Nosocomial pneumonia	MRSA, MSSA, *Streptococcus pneumoniae* (penicillin susceptible)
Community-acquired pneumonia	*Streptococcus pneumoniae* (penicillin susceptible), MSSA
Potential future indications for linezolid	
Serious vancomycin-resistant *Enterococcus faecium* and *faecalis* infections or vancomycin-susceptible infections that are poorly responsive to therapy	
Serious MRSA, VISA (*Staphylococcus aureus* isolates that are intermediate in susceptibility to vancomycin), VRSA (vancomycin-resistant *Staphylococcus aureus*) infections, or infections that are poorly responsive to vancomycin therapy	
Complicated infections requiring long-term oral therapy with an antistaphylococcal or enterococcal agent where ß-lactams cannot be used (e.g. chronic osteomyelitis)	
Treatment of infections (e.g. nosocomial pneumonia, endocarditis, meningitis) caused by highly resistant (but linezolid susceptible) Gram-positive bacteria where alternative agents are not available or are contraindicated	
Combination therapy for antimycobacterial infections where first- and second-line agents cannot be used	
MRSA, methicillin-resistant *Staphylococcus aureus;* MSSA, methicillin-susceptible *Staph. aureus*	
VISA, *Staphylococcus aureus* with intermediate susceptibility to vancomycin	

At present linezolid is approved for the treatment of infections caused by vancomycin-resistant *E. faecium* with an associated bacteremia, nosocomial infections caused by both MRSA and methicillin-susceptible *Staph. aureus* (MSSA) as well as penicillin-susceptible *Staph. pneumoniae*, complicated skin and soft tissue infections caused by MRSA or MSSA, *Streptococcus pyogenes* and *Streptococcus agalactiae* and uncomplicated skin/soft tissue infections caused by MSSA and *Strep. pyogenes*. [12] [13] It is also approved by the FDA for the treatment of community-acquired pneumonia due to *Staph. pneumoniae* (penicillin-susceptible) and MSSA (Table 195.2). Stevens *et al.*[14] recently reported that linezolid was comparable to vancomycin in the treatment of MRSA infections in a randomized open-label trial.

There is additional clinical and experimental experience with linezolid suggesting that it may ultimately have a broader therapeutic role although these other indications have not been FDA approved. These reports include the successful treatment of enterococcal endocarditis and meningitis (the latter a case of vancomycin-resistant *E. faecium*), MRSA prosthetic hip infections and the elimination of nasal carriage with *Staph. aureus*. [15] [16] [17] It is at present not clear how effective linezolid will be in the treatment of infections, such as endocarditis, that require bactericidal activity.

Linezolid is an important addition to the Gram-positive armamentarium. It is an alternative agent for the treatment of resistant Gram-positive infections (see Table 195.2). It has an antibacterial spectrum that is similar to vancomycin with advantageous pharmacokinetics. The availability of an oral preparation allows completion of therapy with the same therapeutic agent and therefore may help reduce the duration of hospital stays.[18]

Dosage in special circumstances

As noted above adjustment of dosage for moderate degrees of renal failure is not necessary. Because linezolid (as well as the linezolid metabolites) are cleared during hemodialysis, it is recommended that patients receive a supplemental dose following dialysis. For moderate degrees of hepatic disease dosage adjustment is not necessary.

ADVERSE REACTIONS AND INTERACTIONS

Linezolid has, in general, been well tolerated. The most common adverse events in the comparator-controlled trials were diarrhea (2.8–11%), nausea (3.4–9.6%) and headaches (0.5–11.3%).

Potentially, the most serious adverse event, thrombocytopenia, was seen in 2.4% of patients. It was seen most often during prolonged therapy (longer than 2 weeks) and resolved upon completion of therapy. Others have reported a higher incidence of thrombocytopenia.[19] [20] As a result, hematologic monitoring of these parameters is recommended during prolonged therapy, especially for subjects who are already immunocompromised.

The oxazolidinones are monoamine oxidase (MAO) inhibitors. Linezolid appears to be a relatively weak MAO inhibitor. No evidence of adverse events related to this potential interaction has been reported to date. However, it is recommended that patients taking linezolid avoid tyramine-containing foods. There is also the potential for interaction with both serotonergic and adrenergic compounds.

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Chapter 196 - Aminoglycosides

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INTRODUCTION

Despite predictions that aminogylcosides would in time become obsolete, or at best, of limited usage because of their ototoxic and nephrotoxic potential, they have increased in popularity and clinical importance. With continued usage since the 1940s, it has become apparent that their toxicity is modest as long as the dose is adjusted for renal function, and they are not administered for extended periods. In addition, with once-daily aminoglycoside dosing rather than the traditional intermittent dosing method, their toxicity has been reduced further.

A major reason for their increased use has been the increasing number of nosocomial infections by bacteria that are either initially resistant or develop resistance to ß-lactam and the fluoroquinolone antibiotics. Many of these organisms have, however, not only retained their susceptibility to aminoglycosides, but seldom develop resistance during therapy. Unlike ß-lactam antibiotics, which are often less active in the presence of high concentrations of bacteria, the aminoglycosides are not subject to this inoculum effect. In contrast to penicillins and cephalosporins, aminoglycosides continue to suppress regrowth of aerobic Gram-negative bacteria for hours after the blood levels fall below the minimum inhibitory concentrations (MICs) for these organisms.

Another important attribute of aminoglycosides is their ability to achieve an additive or synergistic effect against most aerobic Gram-negative bacilli and Gram-positive cocci when combined with ß-lactam antibiotics and a similar effect against Gram-positive cocci when combined with vancomycin or teicoplanin.

BACKGROUND

The aminoglycosides were discovered following systematic screening of soil actinomycetes for the production of substances with antimicrobial activity. These compounds exhibited particular activity against aerobic Gram-negative bacilli and Gram-positive cocci. Streptomycin, the first clinically useful aminoglycoside, was isolated from *Streptomyces griseus* in 1944. Neomycin, kanamycin, tobramycin and paromomycin are also natural compounds that were subsequently isolated from various species of streptomyces. Gentamicin and sisomicin (not marketed in the USA) are natural products produced by *Micromonospora* spp. Amikacin and netilmicin are semisynthetic aminoglycosides derived from kanamycin and sisomicin, respectively. The suffix 'mycin' and 'micin' indicates that the compound was isolated directly or indirectly from *Streptomyces* spp. or *Micromonospora* spp., respectively.

CHEMISTRY

The chemical structures of the aminoglycosides are shown in Figure 196.1 . All aminoglycosides include a central six-membered ring containing amino groups termed an aminocyclitol, which is linked to two or more amino- or non-amino-containing sugars by glycosidic bonds. Spectinomycin is a pure aminocyclitol and is often considered with the aminoglycosides, but is not strictly speaking an aminoglycoside because it contains neither aminosugars nor glycosidic bonds. For this reason, the complete group of compounds is more accurately referred to as aminoglycoside-aminocyclitol antibiotics.

Microbiologic activity

When evaluating the microbiologic activity of the aminoglycosides, it is useful to divide them into two groups:

- one that includes gentamicin, tobramycin, netilmicin and amikacin; and
- the other including streptomycin, neomycin, kanamycin, spectinomycin and paromomycin.

The former group of agents are closely similar in their microbiologic activity and clinical usage, whereas the latter group of drugs have more limited clinical indications.

Owing to the impressive broad-spectrum microbiologic activity of gentamicin, tobramycin, netilmicin and amikacin, they have gained wide popularity in the empiric treatment of patients in whom the suspected pathogens can be multiple (Table 196.1).

Although aminoglycosides alone do not inhibit enterococci and streptococci, they are often used in the therapy of serious infections caused by these organisms because of their frequent additive or synergistic effect when combined with other antibiotics.

In contrast to the cephalosporins and penicillins, the microbiologic effect of aminoglycosides is the same at low or high inocula of organisms.[1] This observation has particular relevance in the treatment of intra-abdominal infections where the density of bacteria may be high. In addition, aminoglycosides show a significant postantibiotic effect (PAE), which is the persistent suppression of bacterial growth after exposure to an antibiotic, against both Gram-negative and Gram-positive bacteria. [2] Except for the carbapenems such as imipenem and meropenem, ß-lactams show no significant PAE against Gram-negative bacteria. The duration of the PAE with aminoglycosides that have activity against *P. aeruginosa* and Enterobacteriaceae is about 1–3 hours and 0.9–2 hours, respectively.[3] These PAEs are even longer if the once-daily aminoglycoside dosing technique discussed later in this chapter is used.

Absorption

Because of their highly charged nature, there is minimal absorption of aminoglycosides when given by mouth, topical application or rectal instillation. [4] Nevertheless, in patients with hepatic encephalopathy and renal impairment, large and frequent doses of neomycin have been associated with sufficient absorption into the systemic circulation to produce deafness.[5] Similarly, detectable blood levels of aminoglycosides can be found if they are used topically in burn patients with large areas of denuded skin. Because aminoglycosides penetrate extremely well into body spaces with large serosal surfaces (e.g. pleural space, peritoneal cavity, pericardial space, synovial fluid), it is unwise to instill them directly into these sites, and there have been reports of neuromuscular blockade (see Adverse reactions, below) associated with large doses injected into the peritoneal

Figure 196-1 Chemical structures of the aminoglycosides. All aminoglycosides include an aminocyclitol (a central six-membered ring containing amino groups), which is linked to two or more amino- or non-amino-containing sugars by glycosidic bonds. For streptomycin, the aminocyclitol ring is a streptidine, whereas for the remainder of the clinically available aminoglycosides it is 2-deoxystreptamine. Neomycin contains approximately equal amounts of neomycin B (R_1 =H; R_2 =CH_2 NH_2) and neomycin C (R_1 =CH_2 NH_2 ; R_2 =H). Kanamycin is principally

kanamycin A, as shown. Gentamicin is gentamicin C complex with roughly equal amounts of C_1 (R_1 =R_2 =CH_3), C_{1a} (R_1 =R_2 =H) and C_2 (R_1 =CH_3 ; R_2 =H).

cavity.[6] Following endotracheal administration or aerosolization of aminoglycosides, systemic absorption is usually modest. However, significant concentrations can be achieved via aerosolized generators that use high pressure and small droplets (1–3mm).[7]

Distribution

Because aminoglycosides demonstrate linear pharmacokinetics, there is a direct proportionality between dose and area under the plasma concentration curve (AUC).[8] Although following an intravenous dose they exhibit a three-compartment model — initial distribution (alpha) phase, a rapid elimination (beta) phase and a slow elimination (gamma) phase — a one-compartment model can be used clinically for establishing dosage regimens.

After an intravenous or intramuscular dose of an aminoglycoside, peak serum concentrations occur in 30 and 45 minutes, respectively. In patients such as those with diabetes mellitus who have poor vascular perfusion into soft tissue structures, there may be a delay in the time to peak concentration. Because aminoglycosides are highly water soluble, their volume of distribution (Vd) is similar to that of the extravascular compartment. As predicted, the mean aminoglycoside concentration in interstitial fluid approximates the mean plasma concentration. Studies performed in normal adult volunteers have found a Vd of 0.2–0.3l/kg.[8] In patients with excess fluid in the extravascular space, as in patients with ascites or burns, the Vd is increased, whereas in markedly obese patients it is decreased.

The low serum protein binding (10%) of aminoglycosides increases the ease with which they are distributed into the extravascular space. In the unobstructed biliary tract, aminoglycoside levels are about 30% of the serum concentration; however, as with ß-lactam antibiotics, bile levels are exceedingly low in the presence of biliary tract obstruction.

Aminoglycosides penetrate poorly into human cells because of the large size of the molecules, their low lipid solubility, and their high polycationic charge. High concentrations are observed only in specialized cells such as the tubular cells of the renal cortex and the hair cells of the ear, which have an active transport mechanism for aminoglycosides, and levels in these cells can even exceed those of plasma or interstitial fluid. Aminoglycoside levels can remain above therapeutic concentrations for extended periods of time (48–200 hours) as a result of renal tubular cell absorption and the prolonged release of aminoglycosides into urine. [9]

Aminoglycosides penetrate poorly into the cornea and the aqueous and vitreous humors of the eye.[10] In patients with serious eye infections such as bacterial endophthalmitis, direct intravitreal injections are usually needed. Subconjunctival injections achieve high aqueous humor levels. The penetration of aminoglycosides into cerebrospinal

TABLE 196-1 -- Microbiologic activity of gentamicin, tobramycin, netilmicin and amikacin.

MICROBIOLOGICAL ACTIVITY OF GENTAMICIN, TOBRAMYCIN, NETILMICIN AND AMIKACIN	
Susceptible	**Resistant**
Escherichia coli	Streptococci (e.g. *Streptococcus pneumoniae, Streptococcus pyogenes*)
Klebsiella spp.	
Proteus mirabilis, Proteus vulgaris, Proteus penneri	*Enterococci*
	Methicillin-resistant *Staph. aureus*
Providencia stuartii, Providencia alcalifaciens	Anaerobes
	Stenotrophomonas maltophilia
Enterobacter spp.	*Burkholderia cepacia*
Morganella morganii	*Flavobacterium* spp.
Salmonella spp.	*Mycobacterium kansasii, Mycobacterium avium-intracellulare*
Shigella spp.	
Serratia marcescens	*Burkholderia cepacia*
Citrobacter spp.	*Mycoplasma* spp.
Aeromonas spp.	*Rickettsiae*
Pseudomonas aeruginosa	Fungi
Acinetobacter baumannii, Acinetobacter Iwoffi	Viruses
Methicillin-susceptible *Staph. aureus*, non-Staph. *aureus* spp. (e.g. *Staphylococcus epidermidis*)	
Yersinia pestis	
Francisella tularensis	
Brucella spp.	
Haemophilus influenzae	
Mycobacterium tuberculosis, selective atypical mycobacteria (e.g. *Mycobacterum fortuitum*)	
Neisseria meningitidis, N. gonorrhoeae	
Moraxella catarrhalis	
Legionella spp.	

fluid (CSF) is poor, both in the presence and absence of meningeal inflammation. [11] Penetration of gentamicin into CSF was studied in 26 patients aged 6–20 years with mumps meningoencephalitis after administration of a single intramuscular dose of 1.2–4.0mg/kg. Activity was found in only five of the 26 patients, with the highest level being 0.19µg/ml.[12]

Elimination

After a rapid distributive phase of 15–30 minutes, the elimination phase begins with 99% of the drug excreted unchanged in the urine and a half-life of 1.5–3.5 hours.[13] In patients with increased extravascular fluid, the Vd increases, resulting in a longer half-life. Because of this type of elimination, any impairment in renal function can result in considerable prolongation of the serum aminoglycoside half-life. There is active reabsorption of aminoglycoside into the proximal renal tubular cells, as indicated by the observation that renal clearance of these agents is somewhat less than that of simultaneous creatinine clearance. In neonates less than 1 week of age or in small premature babies, the half-life is typically prolonged to 8–11 hours.[14]

Following the elimination phase, there is a final extremely slow terminal elimination phase of 30–700 hours secondary to prolonged release from the proximal renal tubules back into the urine.[15] Because the amount of aminoglycoside eliminated is so low, it has no effect on dosing. Less than 1% of aminoglycoside is eliminated into feces, bile and saliva. Aminoglycosides are not metabolized.

Pharmacodynamic concepts

In the past 5 years, much has been learnt from animal models of infection, *in-vitro* pharmacodynamic studies, volunteer experiments and clinical trials that enable us to

establish the best mode of antibiotic administration to maximize bacterial killing and minimize toxicity. From this information, we know that the higher the aminoglycoside concentration, the faster the rate of bacterial eradication.[16] As a result, this type of killing is designated concentration- or dose-dependent killing. For the aminoglycosides, the rate of bacterial eradication increases with increasing concentration up to approximately 10–12 times above their MICs.[17] Thereafter, increasing the concentration does not improve the rate of bacterial killing. If this favorable peak to MIC ratio is obtained, most bacteria die within a short period of time, and as a result the effect of the drug exposure time is minimal and can even be ignored.

In neutropenic and non-neutropenic animal models of infection, significantly more animals survive a potentially lethal challenge of bacteria if the animals are treated with a single large dose of an aminoglycoside than with the same dose divided on an 8-hour schedule.[18] [19] Compared with intermittent dosing, once-daily dosing results in a larger probability that aminoglycoside concentrations will exceed the MIC by a factor of 10–12 times, and also result in a longer PAE with a lower chance for the emergence of aminoglycoside-resistant pathogens.[20]

ROUTE OF ADMINISTRATION AND DOSAGE

Once-daily dosing in adults

Recently, considerable attention has been given to using these pharmacodynamic concepts by giving the entire dose of the aminoglycoside on a once-daily basis in order to maximize bacterial killing and minimize toxicity. Different approaches have been used to monitor and adjust the dose in patients on once-daily aminoglycoside therapy with normal and diminished renal function to ensure adequate treatment and to minimize toxicity. In the USA, the most popular dosing method used by hospitals has been modelled after the one developed at Hartford Hospital, Hartford, Connecticut. [21] To optimize the serum peak to MIC ratio against *Pseudomonas aeruginosa*, the organism that most often warrants aminoglycoside therapy, a 7mg/kg dose is given. Through computer simulation of gentamicin and tobramycin concentrations versus time profile for once-daily dosing, 7mg/kg and 5mg/kg, respectively, would be the best dose to optimize the serum peak to MIC ratio (=10) against *P. aeruginosa*, an organism that is typically inhibited by 2µg/ml of these agents. This dose, when diluted in 50ml of a compatible intravenous solution and given over 1 hour attains the target peak serum concentration of 20µg/ml (Fig. 196.2).

In this simulation, a one-compartment intravenous model was used with a fixed apparent Vd of the aminoglycoside of 0.3l/kg. In patients with normal renal function, the serum concentrations typically fall below 0.5µg/ml within 12 hours and then remain essentially undetectable for the remainder of the day (Fig. 196.3). This drug-free interval is crucial for reduced toxicity (see Adverse Reactions, below), for it allows a greater amount of aminoglycoside to egress from the renal tubular and inner ear cells than with the conventional intermittent dosing method.

In patients receiving the intermittent dosing approach of gentamicin or tobramycin (1.5mg/kg q8h), peak serum levels of only about 4–5µg/ml are obtained with a trough level still slightly above

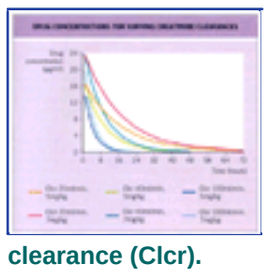

Figure 196-2 Simulated concentration versus time profiles for once-daily 7mg/kg and 5mg/kg gentamicin regimens in patients with varying degrees of creatinine clearance (Clcr).

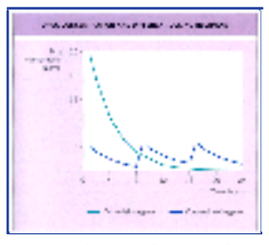

Figure 196-3 Simulated concentration versus time profiles for once-daily (7mg/kg q24h) and conventional (1.5mg/kg q8h) gentamicin regimens in patients with normal renal function.

0.5µg/ml (see Fig. 196.3). Therefore, the absence of any prolonged drug-free period with intermittent dosing slows the egress of aminoglycosides from these cells, resulting in greater tissue accumulation. It is this lower accumulation of aminoglycosides with once-daily dosing than with intermittent dosing that results in less nephrotoxicity and ototoxicity (see below).

Although a dosing weight may be individualized for each patient, dosing is usually based on actual body weight unless the patient is obese [i.e. greater than 20% over ideal body weight (IBW)]. Calculation of IBW is accomplished by the following formulas:

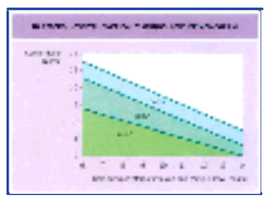

Figure 196-4 Hartford Hospital once-daily aminoglycoside nomogram for gentamicin and tobramycin using the 7mg/kg dose.

- IBWmale = 50kg + 2.3kg for every inch (2.54cm) over 5 feet (1.524m);
- IBWfemale = 45.5kg + 2.3kg for every inch (2.54cm) over 5 feet (1.524m).

For the obese patient, a dosing weight can be calculated using the following: obese dosing weight = IBW + 0.4 (actual body weight — IBW).

Monitoring of patients on once-daily aminoglycoside therapy involves measuring a single random aminoglycoside serum concentration 6–14 hours following a 60-minute infusion of the 7mg/kg dose of gentamicin or tobramycin. Depending upon where the concentration falls on a nomogram (Fig. 196.4), the patient is then given the same 7mg/kg dose every 24, 36 or 48 hours. If the concentration falls on the line of the nomogram that separates the dosing intervals, then the longer interval is chosen. If the random level falls off the nomogram, then the dosing interval should be based on more frequent serum concentration determinations to decide on the most appropriate time for the next dose.

Although it is unnecessary to draw two serum samples in most patients, it may be necessary to obtain additional levels in some patients with rapidly changing creatinine clearance. If once-daily aminoglycoside therapy is continued beyond 4 days, a random concentration should be obtained on the fifth day, and then weekly thereafter. Serum creatinine should be measured every 2–3 days.

In certain patients the risk of toxicity is low and it is even unnecessary to obtain a random determination of the aminoglycoside level. This subset of patients includes adults under 60 years of age who have a normal serum creatinine concentration who are not receiving concurrent nephrotoxic agents (e.g. amphotericin B, ciclosporin, vancomycin) or contrast media, and are neither quadriplegic nor amputees. Even in this subset of patients, however, determinations of serum creatinine should be performed at 2- or 3-day intervals, and for those patients on once-daily therapy for longer than 5 days, a random serum aminoglycoside concentration should be obtained on the fifth day and weekly thereafter. Because of insufficient clinical or pharmacokinetic data or both, once-daily aminoglycoside dosing is not recommended for:

- those on chronic peritoneal dialysis or hemodialysis.
- pregnant women; and
- those with major burns (>20%), ascites or enterococcal endocarditis.

In hospitals where it is difficult to measure serum concentrations of gentamicin or tobramycin, the appropriate dosing interval can be

selected based on the patient's creatinine clearance, which can be easily calculated by using the Cockcroft-Gault equation.[22] With this method, the creatinine clearance can be determined in males by multiplying (140-age) with (weight in kg) and then dividing this value by (serum creatinine concentration × 2), and in females, by using the same equation, but the calculated creatinine clearance is multiplied by 0.85. For those patients who have a creatinine clearance of =60, 40–60, and 20–40ml/min, the aminoglycoside should be given every 24, 36 and 48 hours, respectively.

In the USA, gentamicin and tobramycin are by far the most commonly used aminoglycosides. Nevertheless, a once-daily dose of amikacin could also be employed by administering a single dose of 15mg/kg with the dosing interval determined by the estimated creatinine clearance. In addition, because aminoglycosides exhibit linear pharmacokinetics, the amikacin dosing interval can also be determined by applying the same nomogram used for gentamicin and tobramycin by merely halving the random amikacin level obtained 6–14 hours after the infusion of a 15mg/kg dose.

Results from a number of studies using both neutropenic and non-neutropenic animal models of sepsis and clinical trials have shown that high peak concentrations and long dosing intervals of aminoglycosides improve efficacy and reduce toxicity. [23] [24] In a recent meta-analysis comparing once-daily aminoglycoside with intermittent dosing in immunocompetent adults, once-daily dosing was equivalent with regard to bacteriologic cure, but showed a trend towards reduced mortality rates and reduced toxicity.[25]

In the largest outcome study in adults (2184 patients) treated with once-daily gentamicin or tobramycin, nephrotoxicity (as defined as an increase in serum creatinine concentration to 0.5mg/dl or more over baseline during aminoglycoside therapy) was detected in only 1.2% (27 patients) and ototoxicity in 0.1% (three patients). [21] The incidence of nephrotoxicity was significantly lower than the 3–5% observed from the same hospital when aminoglycosides were given in the conventional dosing technique. Furthermore, in the 27 patients referred to above, there were other possible explanations for nephrotoxicity (e.g. concomitant nephrotoxic agents, volume-related renal dysfunction). Of these patients 94% received gentamicin, 5% tobramycin and less than 1% amikacin. In the three patients who developed vestibular dysfunction, it was transient in two and permanent in the other, but this patient had received over 5 weeks of aminoglycoside therapy. The major exclusion criterion for once-daily aminoglycoside therapy was any patient who had enterococcal endocarditis.

The same investigators performed a detailed pharmacoeconomic analysis of their conversion program from intermittent to once-daily aminoglycoside and noted the movement to once-daily aminoglycoside dosing saved the hospital US$128,000 due to reductions in preparation, administration, monitoring and nephrotoxicity costs. [26] In a recent review of the economic impact of nephrotoxicity at six Philadelphia hospitals involving 1756 patients, it was determined that the mean total additional cost of an episode of aminoglycoside nephrotoxicity in a patient was US$2501. These costs were mainly related to a prolongation of hospital stay, additional consultations and increased laboratory tests and ancillary services. [27]

Once-daily dosing in children

Although the clinical outcome data on once-daily aminoglycoside dosing in children are much more limited, there is no reason why the same pharmacodynamic concepts should not apply. Although children have slightly higher Vd and elimination rates for drugs than adults, it is unlikely that these differences are of a significant magnitude to necessitate major changes in the once-daily aminoglycoside dosing method outlined above for adults. In fact, aminoglycoside dosing in children may require higher dosing. In children 6–12 months of age and those older than 1 year given a single intravenous dose of 20mg/kg dose of amikacin, the Vds were 0.5l/kg and 0.33l/kg, respectively.[28] Therefore, the Vd in children under 1 year of age is slightly higher than that of an adult, whereas that in children over 1 year of age is similar to that in adults. In addition, in this same study, the half-life in the younger children was longer than that in the older children and in adults, yet the peak and trough levels were similar. These observations suggest that children under 1 year of age require a higher single dose (20mg/kg) of amikacin than the dose (15mg/kg) in older children and in adults. Other studies using a 20mg/kg once-daily dose of amikacin in children undergoing bone marrow transplantation or with serious Gram-negative bacterial sepsis have shown no differences in efficacy or toxicity compared with standard intermittent dosing.[29] [30]

Similarly, netilmicin given as a single 2mg/kg dose q8h, or gentamicin given as a single 6mg/kg/day dose or divided into two or three daily doses for treatment of infections in children showed no difference in clinical efficacy, ototoxicity or nephrotoxicity.[31] Identical results were also obtained in the treatment of 20 full-term neonates who received a single 4mg/kg dose of gentamicin given either once or in divided doses.[32]

Intermittent or traditional dosing

In the intermittent dosing technique, a loading dose is given and followed by a maintenance dose.[33] Because the loading dose is independent of renal function, it is the same in patients with abnormal or normal renal function. The loading dose should be calculated according to IBW as discussed above in the once-daily dosing method. The peak serum concentration is usually obtained 30–60 minutes after the infusion of the initial dose or after the first maintenance dose. The usual loading dose is:

- 2mg/kg for gentamicin, tobramycin and netilmicin; and
- 7.5mg/kg for amikacin and streptomycin.

In patients who have a creatinine clearance =90ml/min, the usual maintenance doses are:

- gentamicin 1.7mg/kg q8h;
- tobramycin 1.7mg/kg q8h;
- netilmicin 2.0mg/kg q8h;
- amikacin 7.5mg/kg q12h; and
- streptomycin 7.5mg/kg q12h.

The desired peak serum and trough concentrations are:

- 4–10µg/ml and 1–2µg/ml, respectively, for gentamicin, tobramycin and netilmicin; and
- 15–30µg/ml and 5–10µg/ml, respectively, for amikacin and streptomycin.

In patients with renal impairment, there are two ways to modify the dose, of which one is to lengthen the dosing interval and the other is to reduce the dose. Of these two methods, increasing the dosing interval is preferable because it provides the best peak to MIC ratios and thereby maximizes concentration-dependent bacterial killing. In this method the dose remains the same, but the dosing interval changes based on the patient's estimated creatinine clearance using the Cockcroft-Gault equation. [22]

For instance, in patients with an estimated creatinine clearance (ml/min) of 80–90, 50–80, 10–50 and <10, the dosing intervals become every 12h, 12–24h, 24–48h and 48–72h, respectively. The serum creatinine concentration should be measured every 3–5 days, and if it remains stable there is no reason to perform repeat measurements of peak and trough concentrations. If there is, however, a significant increase in serum creatinine concentration, then a new dosage is recalculated.

INDICATIONS

Use in patients with fever and neutropenia, sepsis syndrome and nosocomial infections

Gentamicin, tobramycin, netilmicin and amikacin have assumed a major clinical role for decades in the empiric treatment of the febrile neutropenic patient (see Chapter 100) and in patients with serious hospital-acquired infections (see Chapter 56) because of their broad spectrum of bactericidal activity against common and unusual Enterobacteriaceae, *P. aeruginosa*, and staphylococci. In addition, these agents usually exhibit synergy against these bacteria in combination with ß-lactam antibiotics and against enterococci in combination with penicillins or vancomycin or teicoplanin. With the increasing usage of carbapenems such as imipenem and meropenem, and fluoroquinolones such as ciprofloxacin and ofloxacin, there has been a significant increase in the emergence of multiantibiotic resistant bacteria (e.g. *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, enterococci), which often require treatment with an aminoglycoside alone or in combination with other antibiotics.

Although physicians often try to avoid aminoglycosides because of concerns for toxicity, these adverse events are actually infrequent, as discussed below, especially if one uses once-daily administration and avoids prolonged administration. Their low acquisition costs also make them attractive choices in the need for fiscal restraints in the health care system of all countries. In the USA, 100mg of gentamicin costs under US$1.

Once gentamicin and the carboxypenicillins (e.g. carbenacillin, ticarcillin), agents with antipseudomonal activity, became available the usage of both kanamycin and polymyxin as part of a 'fever regimen' for neutropenic patients became negligible. As a result, the preferred empiric approach for febrile neutropenic patients or those considered to have a serious bacteremia became gentamicin and carbenicillin. With time, the empiric approach to patients such as these has remained quite similar in that the traditional approach remains an aminoglycoside in combination with an antipseudomonal penicillin (e.g. ticarcillin, piperacillin, mezlocillin, ticarcillin-clavulanate, piperacillin-tazobactam) or cephalosporin (e.g. ceftazadime, cefepime).

The choice of the antipseudomonal penicillin has changed to a greater use of the newer carboxypenicillin ticarcillin, or the ureidopenicillins mezlocillin or piperacillin. Because neither ticarcillin nor piperacillin has significant antistaphylococcal activity and the intrinsic activity of these drugs by themselves against anaerobes is moderate, these two agents are often given in their new formulations in which they are combined with a ß-lactamase inhibitor such as clavulanate or tazobactam. In combination with these inhibitors, ticarcillin-clavulanate and piperacillin-tazobactam, are active against almost all anaerobes with MICs typically below 2µg/ml. The MICs of these penicillins by themselves against anaerobes such as *Bacteroides* spp. is about 32–64µg/ml.

Although the aminoglycosides are often used clinically in combination with ß-lactams, the combination may lead to inactivation of both drugs. The reaction, which is time and concentration dependent, occurs by nucleophilic opening of the ß-lactam ring and acylation of an amino group of the aminoglycoside resulting in a biologically inactive amide.[34] [35] Gentamicin and tobramycin appear to be more susceptible to inactivation than amikacin or netilmicin. This phenomenon is probably clinically meaningful only in patients with significant renal failure in whom ß-lactams accumulate to very high concentrations. [36] [37] [38] [39] On account of this interaction aminoglycosides should not be mixed with penicillins before infusion and serum samples used for aminoglycoside assay should be run immediately or frozen until used.

Gentamicin, tobramycin and amikacin usually exhibit the same activity against Enterobacteriaceae, although *Serratia marcescens* is somewhat more susceptible to gentamicin, whereas tobramycin is more active than the other aminoglycosides against *P. aeruginosa*. Some gentamicin-resistant *P. aeruginosa* remain susceptible to tobramycin. Because these enzymes are a poor substrate for amikacin, and occasionally netilmicin, these two aminoglycosides may be effective against organisms that are resistant to both gentamicin and tobramycin. However, with the use of once-daily aminoglycoside dosing, these modest differences in microbiologic activity appear to have little, if any, clinical relevance, except for isolated patients.

Vancomycin or teicoplanin (not available in the USA) is often added to the ß-lactam-aminoglycoside combination in the initial treatment of the febrile neutropenic patient if one or more of the following findings are present:

- clinically suspected intravenous catheter-related infections;
- known colonization with ß-lactam-resistant pneumococci or staphylococci (methicillin-resistant *Staphylococcus aureus* — MRSA);
- positive blood cultures for Gram-positive bacteria;
- evidence of cardiovascular impairment;
- patient on previous prophylaxis with a fluoroquinolone;
- abrupt increase in temperature to =104°F (40°C).

Therapy of enterococcal infections

Aminoglycosides are used in the therapy of systemic enterococcal infection because no single antibiotic by itself exhibits bactericidal activity against these bacteria. Usually the aminoglycoside, either gentamicin or streptomycin, is combined with either a penicillin or vancomycin to obtain a bactericidal effect, and this is crucial in the therapy of endocarditis. Although all enterococci are resistant to aminoglycosides based on achievable serum concentrations, synergistic activity with penicillins (usually ampicillin) or vancomycin results in effective therapy.

Therapy of enterococcal infection is, however, becoming more difficult owing to the increasing frequency of enterococcal isolates that have high-level resistance to aminoglycosides (MIC >2000µg/ml), penicillins and even vancomycin. Enterococci with high-level resistance to gentamicin contain the bifunctional aminoglycoside-modifying enzyme acetyltransferase (AAC) (6')-phosphotransferase (APH) (2'), which abolishes synergy with all aminoglycosides except streptomycin. It is important to note that, although the bifunctional enzyme modifies amikacin and abolishes synergy with ß-lactams and vancomycin, it does not lead to a resistant phenotype. For these reasons, amikacin should not be used against strains with high-level resistance to gentamicin even if the strain is reported to be susceptible. Streptomycin remains the only alternative for strains with high-level resistance to gentamicin. In the case of high-level resistance to streptomycin, this aminoglycoside should also not be used.

Treatment of other infections

Streptomycin

The major uses of streptomycin have been as part of combination therapy for enterococcal and mycobacteria infections and monotherapy for tularemia, brucellosis and plague (see Chapter 176 , Chapter 177 and Chapter 180). The clinical role of streptomycin as well as the other aminoglycosides in the treatment of mycobacterial infections is discussed in detail in Chapter 37 and Chapter 38 .

Paromomycin

Because paromomycin is too toxic following intravenous administration and is not absorbed following oral administration, its usage has been restricted to the treatment of intestinal infections, particularly cryptosporidiosis in patients who have AIDS.[40] The usual oral dose to

treat cryptosporidiosis is 1g q12h for 1 month. For the treatment of intestinal amebiasis, the usual dose in adults and children is 25–35mg/kg/day q8h with meals for 5–10 days. Paromomycin has been used for treatment or prevention of traveler's diarrhea because it is also active against *Escherichia coli* and *Salmonella* spp.

Unlabeled uses of paramomycin include treatment of other parasitic infections such as:

- *Dientamoeba fragilis* (25–30mg/kg/day q8h for 7 days);
- *Diphyllobothrium latum*, *Taenia saginata*, *Taenia solium*, *Dipylidium caninum* (adults — 1g every 15 minutes for four doses; pediatric — 11mg/kg every 15 minutes for four doses); and
- *Hymenolepis nana* (45mg/kg/day for 5–7 days).

Like neomycin, paromomycin has been used to treat hepatic coma where the usual adult dose of neomycin is 4g/day in divided doses administered at regular intervals for 5–6 days. The activity of paromomycin closely parallels that of neomycin and kanamycin and there is complete cross-resistance between these agents.

Neomycin

Like paromomycin, neomycin is too toxic for systemic use. It has mainly been used by mouth as a prophylactic agent along with erythromycin in colonic surgery and in hepatic coma to reduce the number of aerobic enteric organisms. When it is used for preoperative prophylaxis for elective colorectal surgery, it usually given as a 1g oral dose along with a 1g oral dose of erythromycin, at 1p.m., 2p.m. and 11p.m. on the day before surgery. Metronidazole can be used in place of erythromycin and is usually given at 7p.m. and 11p.m. on the day before surgery.

In the treatment of hepatic coma, the usual dose in adults is 4–12g/day in divided doses, whereas in children the recommended dose is 50–100mg/kg/day in divided doses. Treatment is usually continued for 5–6 days. In patients with chronic hepatic insufficiency, neomycin may be required (in adults up to 4g/day) indefinitely.

The use of neomycin topically has been mainly for the treatment of external otitis media, usually with other antibiotics in the formulation.

Infections by Listeria *spp.,* Neisseria *spp. and* Haemophilus *spp.*

An infrequent use of gentamicin is to combine it with a penicillin, usually ampicillin, in the therapy of *Listeria monocytogenes* meningitis or endocarditis to achieve more rapid killing of the organism. In patients allergic to penicillin, trimethoprim-sulfamethoxazole (co-trimoxazole) can be used either alone or in combination with the gentamicin.

Although all aminoglycosides exhibit activity against *Neisseria* spp., only the aminocyclitol spectinomycin is used for gonococcal infection; this is, however, very uncommon because most gonococci are susceptible to third-generation cephalosporins and the fluoroquinolones such as ofloxacin and ciprofloxacin.

Haemophilus and *Legionella* spp. are often susceptible to aminoglycosides. Although their use as single agents for infections by these organisms has never been studied, it is useful to be aware that if a patient is on an aminoglycoside for some other infection, these organisms should be covered.

DOSING IN PATIENTS ON CHRONIC HEMODIALYSIS OR CHRONIC AMBULATORY PERITONEAL DIALYSIS

Because aminoglycosides are dialyzable, a supplemental dose should be given after each hemodialysis. The usual supplemental doses are:

- 1–2mg/kg for tobramycin and gentamicin;
- 2mg/kg for netilmicin; and
- 5–7mg/kg for amikacin.[41]

For patients on chronic ambulatory peritoneal dialysis (CAPD) who develop peritonitis without evidence of systemic infection, the aminoglycoside can be added directly to the dialysis fluid by one of two methods.[42] Most patients on CAPD have four 2l-exchanges/day with a dwell time of 6 hours:

- in the first method, 'therapeutic' concentrations (e.g. 4–8mg/l gentamicin, tobramycin or netilmicin; 6–12mg/l amikacin) are added to each bag of dialysis fluid; and
- in the second method, high concentrations (e.g. 20mg/l gentamicin, tobramycin or netilmicin; 60mg/l amikacin) are added to only one of the usual four exchanges of dialysis fluid.

ADVERSE REACTIONS

General

The major adverse effects associated with aminoglycosides include:

- neuromuscular blockade;
- nephrotoxicity; and
- ototoxicity (auditory and vestibular).

Aminoglycosides seldom produce hypersensitivity reactions, hematologic dyscrasias, hepatitis or drug fevers. Because they do not produce inflammatory reactions, they seldom produce phlebitis on intravenous injection, pain on intramuscular injection, or irritation of serosal surfaces on direct instillation into pleural space, joint space or the peritoneal cavity, or when incorporated into methylmethacrylate prosthetic joint cement. In addition, they are extremely well tolerated when injected into the CSF and have not been associated with epileptogenic reactions.

Neuromuscular blockade

The potential for neuromuscular blockade can be avoided if aminoglycosides are not given by bolus administration or instilled in large concentrations into the peritoneal cavity. Intravenous aminoglycosides should be administered over at least 20–30 minutes, especially if once-daily aminoglycoside therapy is used. The risk of neuromuscular blockade is increased in patients who are concomitantly receiving D-tubocurarine or succinylcholine, or possibly calcium channel blockers.[43] [44] Infant botulism, myasthenia gravis, hypocalcemia and hypomagnesemia have also been associated with an increased risk for this adverse reaction.[45]

The classic manifestations of neuromuscular blockade include weakness of the respiratory musculature, flaccid paralysis and dilated pupils. Blockade results from the ability of aminoglycoside to prevent internalization of calcium into the presynaptic region of the axon, which is essential for the release of acetylcholine. When the neuromuscular blockade occurs, it can be rapidly reversed by the administration of calcium gluconate. With supportive care alone, in time, the blockade will resolve. Neomycin is the most likely aminoglycoside to cause this adverse reaction.

Nephrotoxicity

Except for spectinomycin, all aminoglycosides are capable of producing nephrotoxicity by interfering directly with renal tubular function and indirectly with glomerular filtration. Among the commonly prescribed aminoglycosides, such as gentamicin, tobramycin, netilmicin and amikacin, there appear to be no clinically relevant differences in toxicity. Using a definition of nephrotoxicity as a rise in serum creatinine concentration of 0.5mg/dl above the baseline, approximately 3–5% of patients on these agents develop this adverse reaction using standard dosing regimens.

Neomycin is the most nephrotoxic aminoglycoside and streptomycin the least. The most important molecular basis for nephrotoxicity appears to be the number of amino groups (NH_3). Neomycin, gentamicin, tobramycin, netilmicin, amikacin and streptomycin

contain six, five, five, four, four and three amino groups, respectively.[46] The magnitude of the toxicity has been shown to be the greatest when the daily dose is divided into multiple small doses rather than as the same dose given once.[47] This observation has been the basis for the recent popularization of once-daily aminogycoside dosing (see Once-daily dosing, above). Loop diuretics such as ethacrynic acid aggravate aminoglycoside renal toxicity through volume depletion or hypokalemia or by increasing renal tubular uptake of aminoglycosides.

The concomitant use of aminoglycosides with vancomycin, *cis*-platinum, foscarnet, amphotericin B, methoxyflurane and intravenous radiocontrast agents has also been shown to accentuate nephrotoxicity.[48] Although elderly patients are traditionally considered to be at a higher risk for aminoglycoside nephrotoxicity, they may actually not be at an increased risk if one adjusts for an age-related decrease in glomerular filtration rate. High serum levels of aminoglycosides are not considered to be a risk factor for toxicity because there is a saturable movement of aminoglycosides into the renal tubular cell. Certain agents (e.g. potassium supplements, thyroid hormone, high-dose calcium, extended-spectrum penicillins) may reduce aminoglycoside toxicity, but there have been no controlled attempts to evaluate them.

Aminoglycosides bind to specific receptors on the proximal convoluted renal tubule causing an increased excretion of brush border enzymes, magnesium and calcium into the urine. Although there was considerable interest in using enzymuria as a marker of renal toxicity, it never became clinically useful because significant amounts can be detected in the urine even after a single dose. After binding to the renal cell, aminoglycosides are rapidly internalized into the cell by pinocytosis. Once inside the cell, they interfere with ribosomal-mediated protein synthesis and mitochondrial respiration, resulting in cell damage and necrosis.[49] Interference with liposomal enzyme production also occurs, resulting in a deposition of a material that resembles myelin by electron microscopy (myeloid bodies).[49] These myeloid bodies can be detected in the urine. Injury to the lysosome also results in an increased excretion of phospholipids into the urine (lysosome phospholipidosis).

The renal tubular cell is relatively resistant to the toxic effect of aminoglycosides as it takes several days of drug administration to produce functional or anatomic evidence of toxicity.[50] This is important since it reinforces the observation that the best way to prevent renal toxicity is to avoid prolonged administration of these agents. Even when renal tubular necrosis occurs, it is reversible, and surprisingly the renal tubule can even regenerate despite continued administration of the aminoglycoside.

Of equal clinical relevance is the observation that the aminoglycoside enzyme transfer system has a finite capacity for internalizing all aminoglycosides within the renal tubular cell.[51] This saturable transport system means that the amount of aminoglycoside that enters the cell is the same over 24 hours whether the dose is given in the traditional divided fashion or as a single dose. Because of the low or undetectable levels of aminoglycosides for 10–12 hours during the 24-hour period with once-daily dosing, most of the previously internalized drug gets transported out of the cell, resulting in less accumulation.

How damage of the renal tubular cell results in diminished glomerular filtration as indicated by an increase in serum creatinine concentration and the use of creatinine clearance remains controversial. The most frequent explanations mentioned include a release of vasoconstrictive hormones affecting the afferent arterioles, cellular debris obstructing nephrons, and a change in glomerular fenestrae.[48] After discontinuing aminoglycoside therapy, evidence of nephrotoxocity usually disappears within several days in the absence of other causes of nephrotoxicity.

The major clinical manifestations of distal nephron damage by aminoglycosides include:

- decreased urine concentrating ability; and
- polyuria.

Because of excessive magnesium losses in the urine, hypomagnesemia may occur, which in turn can lead to secondary hypocalcemia and hypokalemia.[52] Progression to anuric renal failure as a result of aminoglycoside nephrotoxicity is rare.

Ototoxicity

Auditory

Auditory toxicity occurs as a result of the accumulation of aminoglycosides in the perilymph of the inner ear with subsequent damage of the sensory cells of the organ of Corti. A reduction in the number of cochlear ganglion cells has also been reported as an additional cause of this adverse reaction.[53] Penetration of aminoglycosides into this space is facilitated by elevated trough levels, which impairs their back-diffusion into the plasma.

The exact mechanism by which aminoglycosides destroy hair cells is unknown, but possible explanations include saturation of the detoxification capabilities of the hair cells, binding of aminoglycosides to polyphosphinositides, mitochondrial dysfunction and inhibition of decarboxylase.[54]

Unlike patients with serious nephrotoxicity which is generally reversible, severe cochlear damage is usually permanent because cochlear hair cells do not regenerate. Interestingly, there may be a hereditary component to hearing loss caused by aminoglycosides due to a mutation of mitochondrial DNA.[55]

Symptoms of auditory toxicity include:

- hearing loss;
- tinnitus; and
- a sensation of fullness in the ear.

Although they may develop unilaterally or bilaterally, most patients experience symptoms in both ears. Both auditory and vestibular toxicity can occur in the same patient. The onset of symptoms may occur during or after cessation of treatment.

Cochlear toxicity is usually assessed by pure-tone audiometric testing of air and bone conduction by increasing the frequency from 0.5 to 8kHz. In general, an increase in threshold from the baseline of at least 15dB at any of two or more frequencies is considered to be a significant hearing loss.[56] The earliest signs of cochlear toxicity are usually detected at frequencies above 8kHz. Because perception of human speech occurs in the 0.3–3kHz range, significant cochlear damage can occur before the patient becomes aware of it. Many audiometers do not test for frequencies above 8kHz.

Clinical studies show that the incidence of cochlear toxicity due to aminoglycoside use varies from 5 to 15% with conventional intermittent dosing of aminoglycosides. The frequency varies and depends upon the method of establishing toxicity and whether the investigators include high-frequency loss. The frequency of ototoxicity may be less with once-daily aminoglycoside dosing, presumably because less drug accumulates in the perilymph.

In a guinea pig model evaluating the ototoxicity of amikacin, the investigators found that it was related to total perilymph accumulation rather than to peak concentrations.[57] Therefore, as in the renal tubular cell, there appears to be a saturable transport system into the sensory hair cells.

There is considerable controversy regarding the comparative potential for cochlear damage among the aminoglycosides, but it appears that there is little, if any, clinically relevant difference between gentamicin, tobramycin, amikacin and netilmicin. Compared with these agents, neomycin more often has a greater association with cochlear damage.

Risk factors for cochlear damage include:

- age (= 60 years);
- elevated plasma trough levels (i.e. drug accumulation);
- pre-existing ear disease;
- prolonged therapy;
- repeated treatment with aminoglycoside; and
- concomitant use of loop diuretics (e.g. ethacrynic acid) and other ototoxic drugs.[58]

Of these risk factors, duration of therapy remains the most important. When aminoglycosides are given for less than 10 days, toxicity is seldom a problem. In fact, if ototoxicity or nephrotoxicity occur during the first week of aminoglycoside usage, it is wise to search for another cause.

The potential for ototoxicity in humans with topical preparations remains controversial, but animal studies have shown sensorineural hearing loss with the administration of neomycin and gentamicin.[59] In a study of 44 children with chronic suppurative otitis media given topical preparations containing five different aminoglycosides (four neomycin; one gentamicin), there was no evidence of ototoxicity.[60]

Vestibular

As with cochlear toxicity, vestibular toxicity results from excessive accumulation of aminoglycoside in the perilymph of the inner ear, but the targets for damage differs and are the sensory hair cells of the vestibular epithelia located at the summit of the ampullar cristae.[61] Serious damage to these cells typically results in a permanent deficit because these sensory hair cells, like those of the organ of Corti, do not regenerate.

The risk factors for vestibular toxicity are identical to those mentioned above for auditory toxicity. As with cochlear damage, vestibular toxicity can occur during or after therapy and be unilateral or bilateral. Occasionally it occurs together with auditory toxicity.

Symptoms of vestibular toxicity include:

- nausea;
- vomiting;
- vertigo;
- nystagmus;
- difficulty with gait; and

¦ difficulty fixating on objects.

Difficulty with gait is especially prominent in the dark because of the loss of sight, which compensates for vestibular dysfunction.

The frequency of vestibular toxicity is difficult to establish because vestibular function testing is poorly standardized. The usual method is to record the response to caloric stimulation with water or air on an electronystagmogram. It is speculated that the frequency of vestibular toxicity is comparable to that of cochlear damage. Streptomycin more often has a much greater association with vestibular toxicity than neomycin, gentamicin, tobramycin and amikacin. This differential toxicity is the basis for the choice of streptomycin to obliterate vestibular function in patients with Ménière's disease.

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Chapter 197 - Folate Inhibitors

S Ragnar Norrby

INTRODUCTION

Sulfonamides

Sulfonamides are competitive inhibitors of para-aminobenzoic acid (PABA), which is essential for folic acid synthesis in most bacteria, some protozoa and *Pneumocystis carinii* (Fig. 197.1).[1] A consequence of the mode of action is that sulfonamides lack activity against organisms for which PABA is not an essential metabolite (e.g. *Enterococcus* spp.). The eukaryotic cell does not use PABA and sulfonamides do not interfere with human folic acid synthesis.

Trimethoprim

Trimethoprim is a diaminopyrimidine that competitively inhibits dihydrofolate reductase.[1] [2] [3] Compared with many other diaminopyrimidines with antimicrobial activity (e.g. pyrimethamine), trimethoprim has a higher affinity for bacterial dihydrofolate reductase than for the human enzyme, thus reducing the risk of folic acid deficiency in the treated patient.[4] Pyrimethamine is also a competitive dihydrofolate reductase inhibitor. It has a high affinity for protozoal enzyme.

In addition to the above modes of action, it has been proposed that trimethoprim may inhibit the adhesion of bacteria to human mucosal cells.[5]

Combinations of sulfonamides and trimethoprim or pyrimethamine

Combinations of sulfonamides and trimethoprim or pyrimethamine interfere with two consecutive steps in the same metabolic chain in the micro-organism. This may lead to synergistic antimicrobial activity. The rationale for a fixed combination of trimethoprim and sulfamethoxazole is that, although both antibiotics alone are bacteriostatic, the combination may be bactericidal. The optimal trimethoprim-sulfamethoxazole (TMP-SMX) ratio for synergism is 1:20 and is obtained systemically with a 1:5 dosage combination.[6]

The clinical relevance of the synergism is difficult to prove in experimental infections and even more so in clinical trials. This has led to questioning of the clinical usefulness of the combination in comparison with trimethoprim alone for the treatment of bacterial infections.[7] An argument in favor of the combination is the possibility of the reduced risk of resistance; organisms initially susceptible to both sulfonamides and trimethoprim are less likely to develop resistance to combinations than to single drugs.

Folate inhibitor combinations are used for treating bacterial as well as fungal and protozoal infections. Because of the emergence of bacterial resistance and risks for adverse reactions, the sulfonamides have most of their usefulness as single agents. Emphasis will be put on combinations of sulfonamides and trimethoprim, especially the most widely used, namely TMP-SMX and pyrimethamine-sulfadoxine.

PHARMACOKINETICS

The sulfonamides are classically subdivided on the basis of their elimination time into short-acting (plasma half-life, $t_{½}$ <8h), medium-acting ($t_{½}$ =8–16h), long-acting ($t_{½}$ =17–48h) and ultra-long-acting ($t_{½}$ >48h). Sulfonamides used as single agents today are short- or medium-acting.

Plasma kinetics

The sulfonamides used today, as well as trimethoprim and pyrimethamine, are well absorbed after oral administration and have high bioavailability. Following an oral dose of 160mg of trimethoprim and 800mg of sulfamethoxazole, maximal plasma concentrations of 1.6–1.9mg/l and 26–41mg/l, respectively, are achieved. [8] After intravenous administration of 240mg trimethoprim and 1200mg sulfamethoxazole q12h, peak plasma concentrations in the steady state are about 6mg/l for trimethoprim and 180mg/l for sulfamethoxazole.[9] The protein binding of the sulfonamides varies from less than 50% for sulfadiazine to more than 90% for sulfadoxine. Importantly, sulfonamides bind firmly to albumin and may displace other compounds (e.g. bilirubin). In newborns this may lead to toxic levels of unbound bilirubin with a subsequent risk of 'kernicterus' (see Central nervous system reactions, below).

Distribution

Trimethoprim is lipid-soluble at physiologic pH and has a large volume of distribution (100–120l), whereas sulfamethoxazole is a weak acid with poor lipid solubility at pH values above 7, leading to a volume of distribution corresponding to that of the extracellular space (i.e. 12–18l). In tissues concentrations similar to or higher than those in plasma are achieved with trimethoprim, whereas considerably lower levels of sulfamethoxazole reach peripheral compartments. Concentration above the minimum inhibitory concentrations (MICs) of trimethoprim-susceptible strains are achieved in most tissues and tissue fluids. With sulfamethoxazole, the peripheral concentrations are sometimes so low that it should be questioned whether therapeutic levels are reached. All of the sulfonamides as well as trimethoprim achieve high urine concentrations.

Elimination

The main routes of elimination of sulfonamides, trimethoprim and pyrimethamine are via liver metabolism and renal excretion.[10] In patients who have normal renal function, half-life varies from less than 6 hours for sulfisoxazole and sulfamethizole to 11–17 hours for sulfamethoxazole and sulfadiazine and more than 200 hours for sulfadoxine. Trimethoprim has a half-life of about 15 hours and pyrimethamine is eliminated slowly, with a half-life of about 100 hours.

Kinetics in children

The kinetics of both sulfamethoxazole and trimethoprim differ between children and adults (Table 197.1). Elimination is faster in children, who must be given higher doses than adults.

ROUTE OF ADMINISTRATION AND DOSAGE

Most sulfonamides, trimethoprim and pyrimethamine are available for oral use. Trimethoprim, sulfamethoxazole and sulfadiazine are

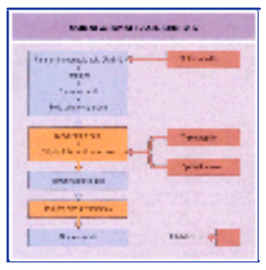

Figure 197-1 Mode of action of folate inhibitors.

TABLE 197-1 -- Comparative kinetics of trimethoprim (T) and sulfamethoxazole (S) in children and adults.

COMPARATIVE KINETICS OF TRIMETHOPRIM AND SULFAMETHOXAZOLE IN CHILDREN AND ADULTS				
Parameter	**Age (years)**			
	<1	**1–9**	**10–19**	**20–63**
T dose (mg/kg)	32	20	14	13
S dose (mg/kg)	160	100	70	65
Peak T concentration (mg/l)	6.5	7.2	6.7	8.7
Peak S concentration (mg/l)	146	176	126	176
T volume of distribution (l/kg)	2.0	1.6	1.5	1.4
S volume of distribution (l/kg)	0.5	0.5	0.4	0.4
T plasma half-life (h)	11	5.6	10	16
S plasma half-life (h)	7.5	9.8	10	15

also used intravenously; intravenous use of TMP-SMX is recommended when patients are unable to take the drug orally. Dosages for some of the sulfonamides and for combinations of sulfonamides and dihydrofolic acid inhibitors are given in Table 197.2 .

INDICATIONS

Table 197.3 summarizes the current antibacterial spectrum of TMP-SMX; little information is available on the current activity of the individual components. The activity of TMP-SMX against enterococci is controversial. There are reports in the literature of enterococcal bacteremia during treatment with TMP-SMX despite full in-vitro sensitivity pre-therapy, and of high failure rates and rapid emergence of resistance in enterococcal urinary tract infections (UTIs).[11] [12]

Reduced susceptibility or resistance to penicillin G by *Streptococcus pneumoniae* seems to be coupled to resistance to TMP-SMX

TABLE 197-2 -- Adult dosages of some folate inhibitors.

ADULT DOSAGES OF SOME FOLATE INHIBITORS	
Drug	**Indication and recommended dose for adults**
Pyrimethamine	Malaria prophylaxis (with sulfadoxine) 25mg once weekly; malaria therapy (with sulfadoxine; Fansidar) 50–75mg as single dose; toxoplasmosis therapy (with sulfadiazine) 75–200mg loading dose followed by 25–100mg q24h for 3–6 weeks followed by 25–50mg q24h (maintenance therapy in AIDS)
Sulfadiazine	UTI (with trimethoprim, not licensed in USA) 410mg q12h; toxoplasmosis therapy (with pyrimethamine) 0.5–1.5g q6h
Sulfadoxine	Malaria prophylaxis 500mg once weekly (with pyrimethamine; Fansidar); malaria therapy 1–1.5g (with pyrimethamine; Fansidar) as single dose
Sulfamethizole	UTI 500mg q6h
Sulfamethoxazole (with trimethoprim)	UTI 1.6g single dose or 400–800mg q12h; systemic bacterial infections 800mg q12h; pneumocystis pneumonia treatment 18.75–25mg/kg q6h; pneumocystis pneumonia prophylaxis 800mg thrice weekly or daily
Sulfisoxazole	UTI 500mg q6h
Trimethoprim	UTI 100mg q12h or 200mg q24h; UTI (with sulfadiazine, not licensed in USA) 90mg q12h; UTI (with sulfamethoxazole) 320mg single dose or 80–160mg q12h; systemic bacterial infections (with sulfamethoxazole) 160mg q12h; pneumocystis pneumonia treatment (with sulfamethoxazole) 3.75–5mg/kg q6h; pneumocystis pneumonia prophylaxis (with sulfamethoxazole) 160mg thrice weekly or daily
For pediatric doses, see the manufacturers' recommendations.	

in a very high percentage of strains studied (Table 197.4).[13] [14] [15] [16] Overall, resistance to TMP-SMX in pneumococci is a rapidly increasing problem.[17]

Streptococcus pyogenes is normally sensitive to TMP-SMX, but resistance has been reported in macrolide-resistant isolates. [18] Of other Gram-positive organisms, *Listeria monocytogenes* is susceptible to the combination.[19]

In *Escherichia coli* there is a very marked variation of susceptibility to TMP-SMX, not only between but also within countries. However, there is a clear trend towards increasing frequencies of resistance and in most countries 12% or more of *E. coli* isolates are resistant to trimethoprim and TMP-SMX.[20] [21] [22]

Shigella and *Salmonella* spp. also show varying sensitivity to TMP-SMX, with frequencies of resistance ranging from less than 5% to over 50%.

Several studies have indicated a relatively high frequency of selection of resistance to TMP-SMX in Enterobacteriaceae when the antibiotic is used therapeutically or, in particular, prophylactically.[23]

In *Haemophilus influenzae* also, reduced rates of susceptibility to TMP-SMX have been reported.[17]

The spectrum of the folate inhibitors also includes micro-organisms other than bacteria. The treatment of choice for *Toxoplasma gondii* remains a combination of pyrimethamine and sulfadiazine. The combination of sulfadoxine and pyrimethamine is used for treatment but less often for prevention of falciparum malaria in areas

TABLE 197-3 -- Susceptibility of common bacterial pathogens to trimethoprim-sulfamethoxazole.

SUSCEPTIBILITY OF COMMON BACTERIAL PATHOGENS TO TMP-SMX

Generally susceptible species (>90% susceptible)	*Streptococcus pyogenes*
	Staphylococcus saprophyticus
	Listeria monocytogenes
	Bordetella pertussis
	Yersinia enterocolitica
	Aeromonas spp.
	Burkholderia pseudomallei
	Burkholderia cepacia
	Stenotrophomonas maltophilia
Varying susceptibility	*Streptococcus pneumoniae*
	Staphylococcus aureus
	Coagulase-negative staphylococci
	Enterococcus spp.
	Escherichia coli
	Enterobacter spp.
	Klebsiella spp.
	Salmonella spp.
	Shigella spp.
	Campylobacter spp.
	Haemophilus influenzae
	Moraxella catarrhalis
Resistance common	*Mycobacterium tuberculosis*
	Pseudomonas aeruginosa
	Treponema pallidum
	Mycoplasma spp.

TABLE 197-4 -- **Correlation between reduced susceptibility to penicillin and resistance to trimethoprim-sulfamethoxazole in streptococcal pneumonia.**

CORRELATION BETWEEN REDUCED SUSCEPTIBILITY TO PENICILLIN AND RESISTANCE TO TMP-SMX IN STREPTOCOCCAL PNEUMONIA			
Reference	**Sensitivity[*] to TMP-SMX**		
	Penicillin S[†]	**Penicillin R[‡]**	**All**
Jorgensen[13]	439/467 (94%)	11/19 (58%)	450/486 (93%)
Shlbl[14]	ND	ND	204/358 (57%)[‡]
Liñares[15]	ND	1/68 (1.5%)	ND
Marton[16]	151/273 (55%)	1/77 (1.3%)	152/350 (43%)
Lehtonen[18]	39/56 (70%)	25/79 (32%)	64/135 (47%)
ND, no data.			

* MIC of trimethoprim of <2mg/l with a 1:19 TMP-SMX ratio.
† MIC <0.1mg/l.
‡ MIC >0.1mg/l.

with chloroquine resistance. However, unlike *T. gondii,* for which no resistance against pyrimethamine-sulfadiazine has yet been reported, resistance against pyrimethamine-sulfadoxine is not uncommon in *Plasmodium falciparum* and might become a problem also in *Pneumocystis. carinii.*[24] [25]

Use of sulfonamides alone

Sulfonamides for systemic use are recommended only for the treatment of uncomplicated UTIs. There are several arguments for reducing or abandoning such use:

- resistance to sulfonamides is relatively common in *E. coli* and other urinary pathogens;
- short-term treatment is poorly documented; and
- treatment times of 5 days or longer are associated with a risk of serious adverse reactions (see below).

TABLE 197-5 -- Clinical use of trimethoprim-sulfamethoxazole.

CLINICAL USE OF TMP-SMX	
Type of infection	**Limitations**
Uncomplicated UTI	None for short-term therapy (single dose or 3 days)
Other types of UTI	Resistance and safety
Shigellosis	Resistance
Salmonellosis	Resistance
Enteric fever	Resistance
Travelers' diarrhea	Safety and resistance
Otitis media	Resistance
Community-acquired pneumonia	Resistance, safety
Melioidosis	Resistance, efficacy
Prophylaxis in immunocompromised patients	Safety, resistance, efficacy
Pneumocystis carinii pneumonia	None

Use of trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole has lost some of its usefulness through the emergence of resistance and increased awareness of the risk of adverse reactions, which may be serious or life-threatening.

Urogenital infections

Urogenital infections are the most common indications for TMP-SMX (Table 197.5). High frequencies of clinical and bacteriologic cure have been documented in women who have uncomplicated cystitis as well as in patients who have pyelonephritis, complicated UTIs or prostatitis.

Importantly, the use of TMP-SMX is well documented for single-dose or short-term treatment of uncomplicated cystitis in women.[26] It is equally effective if given for 3 days compared with treatment for 5–10 days, and a single dose is only slightly less effective than 3-day treatment. For other types of UTI, longer treatment times are required. In countries where resistant uropathogens are uncommon, single-dose or short-term TMP-SMX is an inexpensive and effective treatment of cystitis in women. For other types of infection, where longer treatment times are required, the relatively high frequency of potentially serious adverse reactions must be taken into account, and in adults a fluoroquinolone would probably be preferred in most patients.

Prostatitis is commonly treated with TMP-SMX and the few studies evaluating its use for this infection indicate a high degree of efficacy.[27]

For uncomplicated gonorrhea a single-dose of 640mg trimethoprim and 3200mg sulfamethoxazole was shown to be effective for 96% of 1069 patients and did not mask concurrent infections with *Treponema pallidum*. [28]

For *Haemophilus ducreyi* infections 160mg of trimethoprim plus 800mg sulfamethoxazole q12h for 3 days has given high cure rates, whereas shorter treatment times seem ineffective.[29] [30]

Infections caused by *Chlamydia trachomatis* respond poorly to TMP-SMX.[30]

Enteric infections

These have been extensively treated with TMP-SMX because of its activity against *Salmonella* spp. (including *Salmonella typhi*), *Shigella* spp., *Vibrio cholerae* and enterotoxigenic *E. coli*. In a well-controlled trial in patients who had enteritis of verified etiology, the

causative pathogens were eliminated on treatment day 2 in 41% of patients treated with TMP-SMX compared with 23% of those receiving placebo and 91% of patients on norfloxacin.[31]

A review of patients who had shigellosis showed that 97% of 149 patients treated with TMP-SMX responded clinically and 90% bacteriologically.[32] With the comparators (ampicillin, furazolidone or sulfadimidine) the clinical success rate was 78%. However, TMP-SMX may have lost some of its usefulness in shigellosis through the emergence of resistance.

Trimethoprim-sulfamethoxazole has been shown to be as effective as chloramphenicol for the treatment of enteric fever caused by susceptible strains of *S. typhi*.[33] [34] However, a study comparing TMP-SMX with pefloxacin for this indication demonstrated a shorter time to defervescence with pefloxacin. [34]

In salmonellosis TMP-SMX is effective for treatment of invasive infections caused by sensitive strains, but, like other antibiotics, it seems less effective in eliminating the carrier state of *Salmonella* spp.[35]

Treatment of cholera should be aimed mainly at rehydration of the patients. However, antibiotics may reduce symptoms and shorten the duration of the carrier state of *V. cholerae*, thereby reducing the risk of transmission. Trimethoprim-sulfamethoxazole has proved effective in patients who have cholera and one study showed that it was better than tetracycline or sulfamethoxazole alone.[36]

In several studies TMP-SMX has been shown to be effective in preventing travelers' diarrhea.[37] [38] [39] However, it has also been found to select for resistance.[40] This aberration, plus the fact that serious adverse reactions are not uncommon, makes this type of prophylaxis of doubtful value.

Respiratory tract infections

Considering the etiology of such infections and the fact that the most important pathogen is *S. pneumoniae*, which at present is often resistant to TMP-SMX, the antibiotic has lost much of its usefulness in the treatment of community-acquired pneumonia. Also, its role in the treatment of acute exacerbations of chronic bronchitis should be questioned, although invasive pneumococcal infections are less common in this category of patients.

Trimethoprim-sulfamethoxazole has become a common alternative to ß-lactam antibiotics for acute otitis media in children who have failed to respond to other antibiotics. This is probably due to its activity against *H. influenzae*. However, again the problem of resistant pneumococci limits its usefulness and TMP-SMX can not be recommended as empiric treatment.

In pneumonia caused by *Burkholderia pseudomallei*, melioidosis, TMP-SMX remains a choice for oral long-term treatment after standard treatment with ceftazidime, although some doubt exists about its effectiveness.[41]

Trimethoprim-sulfamethoxazole is the drug of choice for the treatment and prevention of *P. carinii* pneumonia.[42]

Other infections

Both trimethoprim and sulfamethoxazole penetrate the blood-cerebrospinal fluid barrier and the combination has been found to be effective in experimental bacterial meningitis.[43] Trimethoprim-sulfamethoxazole has therefore been used as a second-line drug for the treatment of bacterial meningitis. Favorable clinical results have been reported in the treatment of meningitis caused by *L. monocytogenes* as well as other types of meningitis. [44] [45]

In patients who have brucellosis TMP-SMX can be considered a second-line drug.

Several studies have shown excellent clinical results with TMP-SMX, alone or in combination with aminoglycosides, in the treatment of actinomycosis or nocardiosis.[46]

A controversial field for the use of TMP-SMX is prophylaxis in neutropenic patients. Early studies showed significant protection against bacterial infections but others have shown the emergence of resistance, superinfections and high frequencies of side effects.[47] A reason for reassessing this indication is the possibility that some of these patients (e.g. those with megaloblastic leukemia) may be prone to develop serious hematologic side effects. Another argument against the use of TMP-SMX for prophylaxis in neutropenic patients is the possibility of reduced resistance to colonization with fecal flora. [4]

Pyrimethamine-sulfadiazine and pyrimethamine-sulfadoxine

Pyrimethamine-sulfadiazine remains the first-line drug combination for the treatment of toxoplasmosis.[48]

Pyrimethamine-sulfadoxine is an alternative for the treatment of *P. falciparum* malaria in areas with chloroquine resistance.[49] However, as pointed out above, resistance to pyrimethamine-sulfadoxine is not uncommon and safety aspects reduce its usefulness for prophylaxis.

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment results in prolonged elimination times. Table 197.6 gives dosages of sulfamethoxazole and trimethoprim in patients who had decreased renal function. This includes patients of advanced age. As pointed out, the above doses used in children should be higher than those in adults because of different kinetic profiles (see Table 197.1). All sulfonamides should be avoided in patients aged less than 6 weeks because of the risk of cerebral accumulation of free bilirubin (kernicterus). There are no recommendations for reduced dosage of folate inhibitors in patients who have hepatic disease. Most of the folate inhibitors pass to breast milk but at concentrations that make effects on the child unlikely. During pregnancy, trimethoprim and pyrimethamine should be avoided because of the possible risk of altered folate metabolism in the fetus. Sulfonamides should not be given during the last trimester of the pregnancy because of the risk of kernicterus.

ADVERSE REACTIONS AND INTERACTIONS

A summary of the potential adverse effects of folate inhibitors is given in Table 197.7 .

TABLE 197-6 -- Effect of renal function on dosage of trimethoprim and sulfamethoxazole.

EFFECT OF RENAL FUNCTION ON DOSAGE OF TRIMETHOPRIM AND SULFAMETHOXAZOLE		
Creatinine clearance	**Serum creatinine**	**Dosage**
>25ml/min	<320µmol/l	Normal dose
15–25ml/min	320–405µmol/l	160mg trimethoprim + 800mg sulfamethoxazole q12h for 2 days and then every day until the serum concentration of sulfamethoxazole reaches >600µmol/l
<15ml/min	>405µmol/l	160mg trimethoprim + 800mg sulfamethoxazole q12h until the serum concentration of sulfamethoxazole reaches >600µmol/l

TABLE 197-7 -- Adverse actions of folate inhibitors in humans.

ADVERSE ACTIONS OF FOLATE INHIBITORS IN HUMANS		
Body system	**Sulfonamides**	**Trimethoprim/pyrimethamine**
Central nervous system	'Kernicterus' in newborns	Aseptic meningitis, especially in patients who have collagen diseases
Liver	Toxic hepatitis	Probably none
Lung	None	None
Kidney	Crystalluria	Increased serum creatinine (inhibition of creatinine excretion)
Prostate/genitourinary	None	None

General safety profile

Several studies have shown a correlation between the treatment time and the risk of adverse reactions to TMP-SMX when used for uncomplicated UTIs. [26] As no differences have been found in the efficacy of short-term treatment and treatment for 5 days or longer, the use of TMP-SMX for more than 3 days for uncomplicated cystitis is discouraged.

Hematologic reactions

The mode of action of trimethoprim has caused concerns over possible bone marrow toxicity. Studies in patients treated for 1 month or more with TMP-SMX have shown moderate folate deficiency.[50] [51] [52] The possibility of immune reactions causing hematologic adverse effects has been proposed.[53]

Serious and even fatal hematologic adverse reactions to TMP-SMX have been reported. In a Swedish study of about 50 million daily doses an approximate frequency of fatal reactions to TMP-SMX was calculated to be 3.7/million treatments (data from SWEDIS, Medical Products Agency, Uppsala, Sweden). It was noteworthy that the mean age of the patients who died was 78 years (range 41–96 years) and that only three of 18 patients were below the age of 70. Taking into consideration the effect of aging on renal function, the doses of TMP-SMX were high. In addition, the treatment time was long (range 3–73 days, mean 17 days, median 12 days).

Pyrimethamine hematologic toxicity is less well described. Many use folinic acid to avoid folic acid deficiency. Support is lacking and hematologic reactions may very well be due to other mechanisms.

Skin, mucocutaneous and allergic reactions

These reactions may in some cases be serious, for example Stevens-Johnson or Lyell syndromes.[54] [55] Such reactions seem to be related to the sulfonamide component rather than to trimethoprim.

It is worth noting that, in most reports on the safety of TMP-SMX or sulfonamides, skin reactions are only rarely reported in children. Possible explanations for this are the reduced risk for overdosing in children due to efficient elimination and less risk of sensitization to trimethoprim or sulfamethoxazole from previous exposures.

High numbers of serious cutaneous reactions have been reported following treatment with pyrimethamine-sulfadoxine.[56] Between 1974 and 1989, 126 cases of mucocutaneous syndromes were reported, giving an estimated risk of about 1.1/million treatments. This risk, which is most probably related to the sulfadoxine component, is considered to be high enough to discourage routine use of the combination for malaria prophylaxis.

Patients who have AIDS and *P. carinii* pneumonia and are treated with high doses of TMP-SMX have high frequencies of cutaneous reactions as well as other adverse reactions.[57] [58] [59] These reactions seem to be related to dose and treatment time, and many patients who have AIDS who have developed skin reactions later tolerate low-dose TMP-SMX prophylaxis against *P. carinii*.

Hepatic side effects

Cases of severe hepatic reactions to TMP-SMX have been reported and are most likely to be caused by the sulfonamide component.[60]

Gastrointestinal adverse reactions

Like many other orally administered antibiotics, TMP-SMX causes upper gastrointestinal adverse effects in some patients. Because of its low activity on the intestinal anaerobic flora it causes diarrhea only infrequently.

Renal safety

Sulfonamides with poor solubility can cause crystalluria. With sulfamethoxazole this does not seem to be a problem but, with sulfadiazine in high doses, crystalluria has been reported in AIDS patients who had toxoplasmal encephalitis. [61]

Increased serum creatinine in patients treated with TMP-SMX has been reported, but in most cases seems to be related to competitive inhibition of the renal excretion

of creatinine by trimethoprim.[62]

Central nervous system reactions

Aseptic meningitis is related to trimethoprim therapy. Several cases have been reported in the literature with some over-representation of patients who have collagen vascular diseases (e.g. Sjögren's syndrome).[63] [64] The pathogenesis remains obscure but seems to be of an allergic nature, with rapid onset and relapses after provocation.

Sulfonamides can cause central nervous system toxicity in newborns (kernicterus) because of displacement of bilirubin from albumin, resulting in toxic bilirubin concentrations in the brain.

TABLE 197-8 -- Interactions between trimethoprim-sulfamethoxazole and other drugs.

INTERACTIONS BETWEEN TMP-SMX AND OTHER DRUGS	
Drug	**Interaction**
Sulfonylureas	Reduced clearance of tolbutamide; possible hypoglycemia
Dicoumarol	Reduced metabolism of dicoumarol
Warfarin	Reduced metabolism of warfarin
Digoxin	Reduced tubular secretion of digoxin
Procainamide	Reduced clearance of procainamide
Methotrexate	Possible increased risk of hematologic side effects
Ciclosporin A	Reversible decrease of renal function; risk of accumulation
Phenytoin	Reduced metabolism of phenytoin
Amantadine	Possible reduced excretion of amantadine
Zidovudine	Reduced excretion of TMP-SMX
Ritonavir	Reduced metabolism of sulfamethoxazole

Drug-drug interactions

Considering the liver metabolism of trimethoprim, pyrimethamine and many of the sulfonamides, there is surprisingly little published on drug-drug interactions involving these drugs.[65] [66] Table 197.8 lists possible interactions. The field needs further systematic evaluation, especially because TMP-SMX is likely to be used increasingly by patients who also receive other drugs (e.g. those who have AIDS and are treated for fungal and viral infections).

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Chapter 198 - Quinolones

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INTRODUCTION

The quinolones are a heterogeneous group of synthetic antimicrobial agents. Originally deriving from 1,8-naphthyridine compounds (e.g. nalidixic acid), modern quinolones have evolved as shown in Figure 198.1 to give compounds initially with improved activity against Gram-negative bacteria (e.g. ciprofloxacin, ofloxacin) and more recently with greater activity against Gram positives (e.g. gatifloxacin, moxifloxacin). A number of broader spectrum agents have been developed (e.g. clinafloxacin, trovafloxacin) but have had to be withdrawn due to problems with toxicity.

Quinolones have excellent tissue and tissue fluid penetration so that they are suitable for infections in a wide range of organ systems. Adverse reactions are uncommon in marketed agents and relate mainly to the skin, the gastrointestinal system and central nervous system (CNS) and rarely warrant cessation of therapy. However, there are a number of potentially more serious adverse effects such as arthropathy, cardiotoxicity and phototoxicity. These occur as a class effect (although to different extents in different compounds) and have been a problem in drug development.

Modern fluoroquinolones are available in both intravenous and oral formulations. One of their major advantages has proved to be the ability to treat many serious infections with oral or intravenous-oral switch regimens, for example in the management of enteric fever, Gram-negative pyelonephritis, osteomyelitis, nosocomial pneumonia, severe exacerbations of both chronic bronchitis and cystic fibrosis. Many of the above previously demanded lengthy therapy with intravenous ß-lactams, aminoglycosides or their combinations.

The activity of fluoroquinolones such as ciprofloxacin and ofloxacin in Gram-positive infections, notably those caused by pneumococci, has been disputed. Newer compounds, such as gatifloxacin and moxifloxacin, have markedly improved activity against Gram-positive pathogens and may find a place in the management of infections caused, for example, by penicillin-resistant pneumococci.

ANTIBACTERIAL SPECTRUM AND POTENCY

The antibacterial spectrum of quinolones is shown in Table 198.1 . Quinolones are notable for the considerable knowledge that has been gained regarding structure-activity relationships.[1] The activity of the original naphthyridine and quinolone compounds (e.g. nalidixic acid) was limited to Gram-negative pathogens, primarily the Enterobacteriaceae, including Shigellae and Salmonellae. A major step forward in the development of the class was the addition of a fluorine at position 6, giving rise to the fluoroquinolones (Fig. 198.2). These agents are 10–100 times more active than their precursors against Gram-negative pathogens, including *Pseudomonas aeruginosa*, and have gained activity against the organisms causing atypical pneumonia. Potency, spectrum of activity and adverse effects/drug interactions are largely determined by substitutions at positions 1, 5, 6, 7 and 8:

- substitutions at position 1 (e.g. trovafloxacin) can alter potency (particularly against anaerobes) but may also affect interactions with theophyllines;
- substitutions at position 5 (e.g. grepafloxacin) can increase potency but may cause increased cardiotoxicity;
- substitutions at position 7 (e.g. moxifloxacin, gemifloxacin, garenoxacin) can increase activity against Gram-positive organisms and increase the plasma half-life;
- substitutions at position 8 (moxifloxacin, garenoxacin) can increase potency and reduce the rate selection of resistant mutants but can be associated with increased phototoxicity (sparfloxacin).

Early representatives such as ciprofloxacin only have borderline activity against Gram-positives pathogens. However, developments such as the addition at position 7 of a five-membered ring (gemifloxacin) or an azabicyclo group (moxifloxacin, garenoxacin) have brought increased Gram-positive activity. Unfortunately, this has been partly at the expense of some activity against *P. aeruginosa*. Agents with good activity against both Gram-positive and Gram-negative bacteria have been developed (e.g. clinafloxacin) but have been withdrawn due to toxicity problems.

Fluoroquinolones have good activity *in vitro* against many intracellular pathogens such as *Legionella* spp., *Mycoplasma* spp., *Ureaplasma urealyticum, Chlamydia* spp., *Brucella* spp., *Salmonella typhi* and *Coxiella burnettii*. This may be enhanced by the concentration of fluoroquinolones within cells (see below). As shown in Table 198.1 , *Mycobacterium tuberculosis* is susceptible to most of the fluoroquinolones with greater activity displayed by most of the newer agents. Of the other Mycobacteria, *M. kansasii, M. marinum* and *M. fortuitum* tend to be fluoroquinolone susceptible, whereas *M. avium* complex, *M. chelonae* and *M. scrofulaceum* are more resistant.[2]

The quinolones are rapidly bactericidal against most susceptible species in a concentration-dependent manner and have a postantibiotic effect (PAE) of 2–4 hours. The pharmacodynamic determinants of efficacy are C_{max} /MIC (ratio of the maximum plasma concentration to MIC) and AUC_{0-24} /MIC (ratio of the area under the 24h drug concentration curve to MIC). Various groups have attempted to define the AUC_{0-24} /MIC ratio that would predict a successful outcome. It appears that the optimal ratio varies for different organisms so that a ratio of >125 has been proposed for infection caused by Gram-negative enteric pathogens and *P. aeruginosa*, but a much lower ratio of >34 is proposed for pneumococcal lower respiratory tract infections. [3] [4]

MODE OF ACTION

Quinolones act by the rapid inhibition of bacterial DNA synthesis, leading to cell death. The primary targets are DNA gyrase and topoisomerase IV which are involved in the maintenance of the superhelical structure of DNA. Both enzymes are composed of two subunits that are homologous: DNA gyrase subunits encoded by *gyrA* and *gyrB*; topoisomerase IV encoded by *parC* (*grlA* in *Staphylococcus aureus*) and *parE* (*grlB* in *Staph. aureus*). Although inhibition of these enzymes is the most important determinant of antibacterial activity it appears that secondary activities may affect bactericidality.

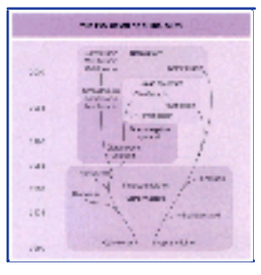

Figure 198-1 The evolution of quinolones.

The addition of RNA and protein synthesis inhibitors or the use of high quinolone concentrations (which also inhibit RNA synthesis) can lead to a diminution in the cidality of some quinolones, suggesting that synthesis of some gene products contributes to the killing effect.

BACTERIAL RESISTANCE

The major mechanism for acquired resistance to quinolones is by mutational modification of the antimicrobial target site. Mutations around the active site of *gyrA* have been identified in many strains of *E. coli* and many other Gram-negative bacilli, giving rise to greater resistance to nalidixic acid than the fluoroquinolones. Alterations in *gyrB* are less common and cause lower levels of resistance.[5] The main site for resistance mutations in Gram positives such as *Staph. aureus* and *Streptococcus. pneumoniae* is the *parC* gene although mutations in *parE* have been described. In both Gram-negative and Gram-positive pathogens resistance develops in a stepwise fashion as mutations arise in one and then both targets. Following an initial mutation, the susceptibility to a quinolone will depend on the specificity of the agent for the alternative target. For example, in clinical practice it has been shown that an isolated *gyrA* mutation in *E. coli* will confer high-level resistance to nalidixic acid but only reduced susceptibility to ciprofloxacin. The acquisition of an additional *parC* mutation confers high-level resistance to ciprofloxacin.[6] For bacteria such as *P. aeruginosa* that inherently have less susceptibility to fluoroquinolones, a single mutation can give rise to clinically significant resistance.

Resistance to quinolones can also be achieved by active efflux of the drug from the bacterial cell. This has been best described in *P. aeruginosa* in which quinolone resistance has been associated with increased expression of the MexAB-OprM, MexCD-oprJ or MexEF-oprN efflux pumps.[7] In *E. coli* the pump is the acrAB-tolC system. Among Gram-positive pathogens, the *norA* pump has been described in *Staph. aureus* and the PmrA pump in *Strep. pneumoniae*.[8] [9] On their own, efflux pumps will generally only cause low-level resistance and therefore may not be clinically important in inherently highly susceptible pathogens such as *E. coli*. However, the overexpression of efflux pumps becomes more significant in less susceptible organisms such as *P. aeruginosa*.

Resistance rates to quinolones have increased over the last decade. Ciprofloxacin resistance in the UK among *E. coli* increased from 0.8% to 3.7% between 1990 and 1999 when resistance was seen in 8.1% of *P. aeruginosa*.[10] Although methicillin-sensitive *Staph. aureus* is usually sensitive to fluoroquinolones, some clones of MRSA (e.g. EMRSA-16 seen in the UK) are resistant. Resistance among pneumococci remains uncommon although in some areas there is evidence that resistance is more common among penicillin-resistant pneumococci.[11]

Cross-resistance between fluoroquinolones is almost complete and minor differences in activity are not usually clinically exploitable. Cross-resistance to unrelated antimicrobials only occurs with over-expression of efflux pumps. In *P. aeruginosa*, for example, this leads to a low-level increase in resistance to chloramphenicol, tetracycline and macrolides.

PHARMACOKINETICS AND DISTRIBUTION

The quinolones are generally well absorbed and are widely distributed in body tissues and fluids, including the intracellular environment. They are excreted either by glomerular filtration or hepatic biotransformation or a combination of these routes, and by biliary or transintestinal elimination. Bio-availability is high and protein binding usually low to intermediate. Fluoroquinolone kinetics are summarized in Table 198.2 .

Absorption

Fluoroquinolones are well and rapidly absorbed after oral administration and exhibit linear absorption kinetics so that doubling the dose produces twice the plasma level.[12] Peak plasma concentrations are usually present 1–2 hours after an oral dose. Absorption may be delayed by food and is impaired by co-administration of antacids and ferrous iron, and possibly by zinc in multivitamin preparations.

Distribution

The fluoroquinolones are extensively distributed to the tissues as can be seen in Table 198.3 . Apparent volumes of distribution are usually 2–31/kg although values for precursor compounds are lower (e.g. 0.51/kg). Protein binding varies from 15–40% with norfloxacin, ofloxacin and ciprofloxacin[12] to 65% for gemifloxacin and higher still for garenoxacin and trovafloxacin (>80%).

Fluoroquinolones are concentrated approximately 10 times in polymorphoneutrophils (PMNs). Although it has been suggested that this may increase their *in vivo* efficacy against intracellular pathogens, there is evidence that the intracellular activity of different fluoroquinolones is variable, possibly related to where they are concentrated within the cell.[13] An additional result of the intracellular concentration of fluoroquinolones is that they may be transported by PMNs to a site of infection and then released.[14]

Elimination

Elimination half-lives vary from 1–2 hours for nalidixic acid to 3–5 hours for ciprofloxacin and 7–14 hours for newer agents.

Excretion of fluoroquinolones is primarily by renal glomerular filtration, hepatic metabolism and transintestinal elimination. The relative importance of glomerular filtration varies between agents and some compounds such as ofloxacin, levofloxacin and gatifloxacin exhibit minimal metabolism and are excreted largely unchanged in the urine. For these agents renal clearance almost equals total clearance and dose modification is required in renal

TABLE 198-1 -- Activity of quinolones against common pathogenic bacteria: MIC_{90} (mg/l).

ACTIVITY OF QUINOLONES AGAINST COMMON PATHOGENIC BACTERIA										
Pathogen	**Nalidixic acid**	**Norfloxacin**	**Ciprofloxacin**	**Ofloxacin**	**Levofloxacin**	**Grepafloxacin**	**Gemifloxacin**	**Gatifloxacin**	**Moxifloxacin**	**Garenoxacin**
Streptococcus pneumoniae	>64	2–16	1–4	2–4	2	0.25	0.06	0.25	0.12	0.12
Staphylococcus aureus	>64	2	0.5–2	0.5–2	0.25	0.12	0.03	0.12	0.06	0.03
Enterococcus spp.	>64	8–16	1–8	2–8	2–8	1–16	0.25–>16	1–>16	0.5–4	0.5–8
ß-Hemolytic streptococci	>64	4–8	2	4	1	0.25	0.03	0.5	0.25	0.12
Listeria spp.	NA	NA	1	2	1	NA	0.12	0.5	0.5	0.5
Haemophilus influenzae	1	0.25	0.03	0.03	0.03	0.016	0.015	0.03	0.06	0.03
Moraxella catarrhalis	4	0.25	0.06–0.25	0.12	0.06	0.015	0.03	0.12	0.12	0.03
Neisseria spp.	0.5	0.03	0.03	0.06	0.015	0.008	0.008	0.03	0.03	0.008
Escherichia coli	4–8	0.12–2	0.06–0.25	0.12–0.25	0.06–0.25	0.03	0.015	0.06–0.25	0.06	0.06–0.5
Klebsiella spp.	8–16	0.25–1	0.12–0.25	0.25–1	0.06–0.5	0.25–0.5	0.25	0.06–0.5	0.12–0.5	0.25–1
Enterobacter spp.	8–16	0.12–0.5	0.12–0.5	0.25–1	0.12–2	0.5–2	0.25–1	0.12–1	0.25	0.25–4
Salmonella spp.	2–4	0.25	0.12	0.25	0.25	0.015	0.06	0.06	0.25	0.12
Shigella spp.	8	0.25	0.12	0.25	0.03	0.008	0.008	0.03	0.06	0.03
Campylobacter spp.	8	0.25	0.12	0.25	0.12	NA	NA	0.12	0.06	0.12
Pseudomonas aeruginosa	>64	0.5–2	0.5–2	0.5–4	4	16	8	>4	8	16
Acinetobacter spp.	>64	>16	1–2	1–2	0.25–8	0.5–>16	0.5–>16	0.5–>16	0.25–16	0.12–8

Stenotrophomonas maltophilia	>64	NA	8	8	2–8	4	4	4	1	4
Bacteroides fragilis group	>64	16–32	4–16	8	2	8	1	1	0.5	0.5
Mycoplasma spp.	NA	4–16	1–2	1–2	0.5	0.12	0.12	0.12	0.12	0.06
Chlamydia spp.	NA	4–16	1–4	0.25–1	0.5	0.06	0.25	0.12	0.06	0.015
Legionella pneumophila	1	2	0.06	0.1	0.03	0.015	0.015	0.03	0.06	0.06
Mycobacterium tuberculosis	NA	2–8	1–4	0.5–2	1	NA	>4	0.25	0.12	2
NA, not available.										

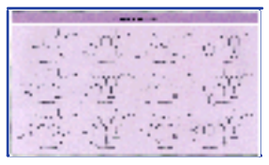

Figure 198-2 Structure of quinolones.

TABLE 198-2 -- Basic pharmacokinetic parameters of quinolones.

BASIC PHARMACOKINETIC PARAMETERS OF QUINOLONES								
Agent	**Dose (g)**		**C_{max} (mg/l)**	**AUC_{0-24} (mg/lh)**	**t ½ (h)**	**Protein binding %**	**% dose excreted unchanged in urine**	**Route**
Nalidixic acid	1	QDS	V	V	1.5	90	<1	po
Norfloxacin	0.4	BD	2	12.5	3	15	25–40	po
Ciprofloxacin	0.75	BD	3	30	4	40	30–50	po/iv
Ofloxacin	0.4	BD	6	80	7	30	70–85	po/iv
Levofloxacin	0.5	OD	6.4	54	7	40	85–90	po/iv
Grepafloxacin	0.4	OD	2.2	14	14	50	<10	po
Gatifloxacin	0.4	OD	4.6	37	9	20	70–80	po/iv
Gemifloxacin	0.32	OD	1.8	9	7	65	30	po
Moxifloxacin	0.4	OD	4.5	44	13	50	20	po/iv
Garenoxacin	0.4	OD	6.5	84	12.5	80	40	po/iv
V, variable.								

impairment.[12] [15] Others, such as ciprofloxacin and moxifloxacin, have moderately extensive hepatic biotransformation (to oxo-, desethyl- and sulfo- derivatives — subsequently partly eliminated as inactive glucuronides in the bile). For these compounds renal clearance is half of the total clearance and dosage modification may not be required in renal impairment as long as other routes of elimination are intact.[16]

In hepatic impairment, the dosage of agents primarily cleared by the kidney (ciprofloxacin, ofloxacin and levofloxacin) rarely requires modification. However, for extensively metabolized drugs such as grepafloxacin, dose modification is necessary in patients with cirrhosis.

Fluoroquinolones that are not primarily eliminated by the kidney are present in significant quantities in the stool, partly by biliary excretion and, notably with ciprofloxacin, by transintestinal elimination. The majority is bound to ligands in the stool.

ROUTE OF ADMINISTRATION AND DOSAGE

Most agents are available in both oral and intravenous formulations. The high oral bio-availability of fluoroquinolones means that oral administration is adequate in most situations unless this route is unavailable. The manufacturer's dosage recommendations for quinolones are given in Table 198.4 .

TABLE 198-3 -- Tissue distribution of fluoroquinolones.

TISSUE DISTRIBUTION OF FLUOROQUINOLONES	
Tissue	**Tissue: plasma ratio**
Lung	
Bronchial mucosa	1.6
Epithelial lining fluid	2.1–8.7
Alveolar macrophages	11.8–21
CSF	
Uninflamed meninges	<0.1
Inflamed meninges	0.3–0.5
Brain tissue	0.9
Skin/soft tissue	
Skin	1.8
Muscle	3.3
Subcutaneous tissue	~1
Blister fluid	~1
Sweat	2.5
Prostate	
Prostatic tissue	2.1–5.7

Prostatic fluid	0.25
Seminal fluid	6–8
Eye	
Aqueous humor	0.5
Kidney	
Kidney tissue	~6
Urine	~100
Liver	
Hepatic tissue	4
Bile	6
Heart	
Myocardium	2–4
Heart valves	~1

INDICATIONS

Early quinolones such as nalidixic acid were largely used for Gram-negative UTI and shigellosis. The development and evolution of fluoroquinolones have led to a number of agents with differences in spectrum of activity and therefore indications. Some, such as norfloxacin, are used almost exclusively for UTI. Agents such as ciprofloxacin and ofloxacin have been used for a broad range of infective syndromes. Newer compounds, such as levofloxacin, grepafloxacin, gatifloxacin and moxifloxacin, have improved activity against Gram-positive pathogens and may be more appropriate for respiratory tract infections.

Genitourinary tract infections

Uncomplicated lower urinary tract infection

Oral fluoroquinolone therapy is highly effective but to limit selection pressure for resistance should be used only when bacterial resistance precludes the use of other agents. Fluoroquinolones eradicate bowel reservoirs of uropathogenic *E. coli* and may reduce the incidence of early recurrence. Long-term suppression with low-dose norfloxacin or ciprofloxacin has been shown to be effective in preventing recurrent UTI in selected patients.[17] [18]

Complicated ascending urinary tract infection

Fluoroquinolones given for 1–2 weeks are the recommended agents for the treatment of ascending or complicated UTI.[19] Oral ciprofloxacin has proved as efficacious as an intravenous regimen for initial empiric therapy.[20]

Prostatitis

Fluoroquinolones are concentrated in prostatic tissue and are recommended therapy for both acute and chronic bacterial prostatitis.[21] Ciprofloxacin for 28 days can give a clinical response of 98% in chronic bacterial prostatitis although relapse may occur in up to 40% of patients.[22]

Gonorrhoea

Single-dose oral therapy with ciprofloxacin or ofloxacin is equivalent to other parenteral single-dose regimens and is therefore a recommended choice in the management of uncomplicated urethral gonorrhea.[23] Pharyngeal and rectal disease also respond. However, quinolone resistance is emerging, particularly in South East Asia where high-level resistance can be found in up to 50% of isolates.[24]

Nongonococcal urethritis/cervicitis

The antichlamydial activity of fluoroquinolones varies and ofloxacin is the most potent of the established agents. A 7-day course of ofloxacin is as effective as doxycycline therapy.[25] Newer compounds such as moxifloxacin have excellent *in vitro* activity and may have a role in therapy.

Chancroid

A 3-day course of ciprofloxacin gives excellent cure rates, equivalent to standard ß-lactam or trimethoprim-sulfamethoxazole (cotrimoxazole) therapy. [26]

Pelvic inflammatory disease

The ideal antimicrobial treatment for acute pelvic inflammatory disease has not been established by randomized clinical trials. However, ofloxacin is active against many of the potentially causal pathogens and is included in recommended treatment regimes.[23]

Respiratory tract infections

The fluoroquinolones ciprofloxacin and ofloxacin have been used extensively for upper and lower respiratory tract infections. However, there have been concerns regarding their activity against *Strep. pneumoniae*. Newer agents, such as gatifloxacin, gemifloxacin, moxifloxacin and garenoxacin, have improved activity against pneumococci, including macrolide- and penicillin-resistant strains, and are often termed 'respiratory quinolones'.

Sinusitis

Oral fluoroquinolones have comparable efficacy to macrolides or cephalosporins and give cure rates of >85% in acute sinusitis.[27] [28] [29]

Ear infections

Topical preparations of ofloxacin or ciprofloxacin are effective for the treatment of acute otitis media in children with tympanostomy tubes and for chronic suppurative otitis media.[30] Clinical cure rates of >85% for otitis externa can be obtained with the topical preparations. Malignant otitis externa, which is usually caused by *P. aeruginosa*, can be treated with oral ciprofloxacin. A prolonged course is required (3 months) and gives cure rates in excess of 90%.[31]

Acute exacerbations of chronic bronchitis

Fluoroquinolones are among the agents of choice for the management of moderate to severe exacerbations of chronic bronchitis. They have equivalent efficacy to macrolides or ß-lactam/ß-lactamase inhibitor combinations and achieve cure rates of >90%.[32] [33]

Community-acquired pneumonia

Older quinolones are not indicated for pneumococcal pneumonia when alternative antibiotics are available. However, results with

TABLE 198-4 -- **Dosing recommendations for quinolones** (from manufacturers' data sheets).

DOSING RECOMMENDATIONS FOR QUINOLONES								
	Nalidixic acid	**Norfloxacin**	**Ofloxacin**	**Ciprofloxacin**		**Levofloxacin**	**Gatifloxacin**	**Moxifloxacin**
	po	**po**	**po/iv**	**po**	**iv**	**po/iv**	**po/iv**	**po/iv**
Urinary tract infection	500–1000mg qds	400mg bd (3–21 days)	200mg bd (3–10 days)	100–500mg bd (3–14 days)	200–400mg bd (7–14 days)	250mg od (3–10days)	200–400mg od (3–10 days)	
Chronic bacterial prostatitis		400mg bd (28 days)	300mg bd (6 weeks)	500mg bd (28 days)	400mg bd (28 days)			
Acute sinusitis				500mg bd (10 days)	400mg bd (10 days)	500mg od (10–14 days)	400mg od (10 days)	400mg od (10 days)
Acute bacterial exacerbation of chronic bronchitis			400mg bd (10 days)	500–750mg bd (7–14 days)	400mg bd-tds (7–14 days)	500mg od (7 days)	400mg od (5 days)	400mg od (5 days)
Community-acquired pneumonia			400mg bd (10 days)			500mg od (7–14 days)	400mg od (7–14 days)	400mg od (7–14 days)
Skin and skin structure infection			400mg bd (10 days)	500–750mg bd (7–14 days)	400mg bd-tds (7–14 days)	500–750mg od (7–14 days)		400mg od (7 days)
Bone and joint infection				500–750mg bd (=4–6 weeks)	400mg bd-tds (=4–6 weeks)			
Intra-abdominal infection				500mg bd (7–14 days)	400mg bd (7–14 days)			
Infectious diarrhea				500mg bd (5–10 days)				
Uncomplicated urethral and cervical gonorrhea		800mg single dose	400mg single dose	250mg single dose			400mg single dose	
Nongonococcal cervicitis/urethritis			300mg bd (7 days)					
Pelvic inflammatory disease			400mg bd (10–14 days)					
Inhalational anthrax (postexposure)				500mg bd (60 days)	400mg bd (60 days)			

ciprofloxacin and ofloxacin suggest clinical response and bacterial eradication rates of 90% or greater and with levofloxacin, equivalence or superiority to ceftriaxone.[34] However, concerns have been raised regarding the efficacy of ciprofloxacin in severe pneumococcal pneumonia following reports of clinical failures.[35] Failures with levofloxacin have also been reported and in Europe it is suggested that it should be given at an increased dose of 500mg BD or in combination with benzyl penicillin in cases of severe pneumonia.[36] [37] Newer agents such as gatifloxacin, gemifloxacin and moxifloxacin, which have improved activity against pneumococci and also atypical pathogens, show promising results in clinical trials with clinical cure rates in excess of 90%.[38] Although there are few specific data regarding infections with penicillin-resistant pneumococci, trovafloxacin gives clinical success rates of over 95%. Legionellosis can be successfully treated with quinolones such as ciprofloxacin, ofloxacin or levofloxacin. There are few clinical data to show whether or not they are superior to macrolides and often they are given in combination with a macrolide or rifampicin.[39]

Nosocomial pneumonia

A large-scale study of ciprofloxacin showed equivalence with imipenem in moderately to severely ill patients, most of whom required ventilation and treatment in an intensive care unit.[40] In the 20–25% with infection caused by *P. aeruginosa*, the results with both regimens were less satisfactory, underlining the need for combination therapy. Newer fluoroquinolones such as gemifloxacin, gatifloxacin and moxifloxacin have reduced *in vitro* potency against *P. aeruginosa* and will probably not have a role in the management of hospital-acquired pneumonia where *Pseudomonas* is a likely etiological agent.

Cystic fibrosis

Oral ciprofloxacin is effective for exacerbations caused by *P. aeruginosa*, producing results equivalent to those of standard ß-lactam and aminoglycoside therapy. In the UK a 3-week course of ciprofloxacin combined with colistin is recommended for the treatment of early pseudomonal infection.[41]

Mycobacterial infections

Ofloxacin and ciprofloxacin have moderate activity against *Mycobacterium tuberculosis* and are bactericidal *in vivo*. [42] Their role in therapy is currently limited to use in combination regimens for the treatment of multiply drug-resistant *Mycobacterium tuberculosis* infection.[43] Newer agents such as moxifloxacin have enhanced anti-mycobacterial activity and animal studies suggest they may have a future role in antituberculous chemotherapy.[44]

As noted above, the susceptibility of nontuberculous mycobacteria to fluoroquinolones is variable. *Mycobacterium avium* complex is relatively resistant to quinolones. Nevertheless, the addition of ciprofloxacin to standard therapeutic combinations has been shown to be of benefit in HIV patients with disseminated disease.[45]

Skin and soft tissue infections

The fluoroquinolones give excellent results when compared with cephalosporins for the treatment of both uncomplicated and complicated skin and soft tissue infections.[46] [47] However, more effective agents are routinely available for Gram-positive infections and usefulness in MRSA infections is limited by high rates of quinolone resistance.

Skeletal infections

Oral fluoroquinolones are highly effective for Gram-negative mixed acute (or chronic) contiguous osteomyelitis, giving cure rates of 80–90% after 3–6-month courses. They are also effective for post-surgical cases, salmonella osteitis and in some cases of chronic *P. aeruginosa* osteomyelitis (ciprofloxacin), although resistance may emerge causing a failure of treatment or relapse.[48] In patients with orthopedic prostheses infected with staphylococci, ciprofloxacin or ofloxacin in combination with rifampicin have been successfully used for conservative management (i.e. preserving the prosthesis).[49] [50]

Gastrointestinal infections

Typhoid and paratyphoid fevers

Ciprofloxacin or ofloxacin are the agents of choice for typhoid and paratyphoid fevers.[51] Convalescent excretion states and long-term fecal carriage are rare after fluoroquinolone therapy, thereby reducing the human reservoir and possibly leading to a fall in incidence. Carriage states persisting after other antibiotic therapy may

also respond to fluoroquinolones.

Decreased quinolone susceptibility has emerged in Asia over the last 10 years. Strains are typically resistant to nalidixic acid and have raised MICs of ciprofloxacin of 0.5–1mg/l. These strains are ciprofloxacin susceptible by NCCLS or BSAC criteria but there is evidence that infection by such strains responds less well to ciprofloxacin and longer courses or alternative agents are recommended. [52]

Salmonellosis

A 5–7-day course of oral fluoroquinolone is effective in reducing the duration and severity of severe salmonellosis.

Cholera

Three-day courses of oral fluoroquinolones are equal to standard trimethoprim-sulfamethoxazole or tetracycline regimens. A cure rate of >90% can be achieved with a single 1g dose of ciprofloxacin.[53]

Shigellosis

Fluoroquinolones are drugs of choice for invasive shigellosis. A single oral dose (ciprofloxacin 1g) is effective in adults.

Campylobacter

Fluoroquinolones have been used for gastrointestinal *Campylobacter* infections. However, resistance levels are increasing and may be as high as 50% in some areas of the world.[54]

Travelers' diarrhea

Ciprofloxacin or norfloxacin in full oral dosage for 5 days is effective for 80% of unprotected subjects who develop profuse diarrhea (>3–5 watery stools/day).

Other treatment indications

Ocular infections

Topical fluoroquinolones are effective for the treatment of bacterial conjunctivitis and keratitis. Penetration of systemic quinolones into the vitreous is relatively good but may not exceed the MICs of all likely pathogens. Intravitreal ciprofloxacin has been used in the treatment of endophthalmitis.[55]

Infections associated with chronic ambulatory peritoneal dialysis

Ciprofloxacin and ofloxacin have been used with success both orally and intraperitoneally. However, the emergence of resistant staphylococcal infection has limited their usefulness as monotherapy.

Q fever

Fluoroquinolones are active against *Coxiella burnetii in vitro* and a combination of a fluoroquinolone (ofloxacin) with doxycycline has been suggested for Q fever endocarditis.[56]

Anthrax

A 60-day course of ciprofloxacin is recommended for postexposure prophylaxis against anthrax.[57] In patients with inhalational anthrax a combination of ciprofloxacin plus another active agent (e.g. doxycycline) is recommended.[58]

Meningitis

Fluoroquinolones have been successfully used for Gram-negative meningitis.[59] Newer agents such as moxifloxacin show promising results in animal models of pneumococcal meningitis.[60] Trovafloxacin had comparable efficacy to ceftriaxone in a trial of pediatric meningitis. [61]

Chemoprophylaxis

Meningococcal infection

Single-dose (500mg) ciprofloxacin is effective in eradicating nasopharyngeal carriage in over 95% of subjects.[62]

Neutropenic patients

Norfloxacin, ofloxacin and ciprofloxacin have been widely used in the prophylaxis of opportunistic infection among neutropenic patients. Although prophylaxis has been shown to prevent febrile episodes of an infectious nature, current recommendations do not suggest their use due to concerns regarding the emergence and spread of antimicrobial resistance.[63]

Travelers' diarrhea

Once-daily prophylactic use of a fluoroquinolone (e.g. norfloxacin 400mg or ciprofloxacin 500mg) for the duration of potential exposure gives 75–90% protection from travelers' diarrhea caused by enterotoxigenic *E. coli* and other bacterial enteropathogens.

Surgical infections

Fluoroquinolones have been used effectively for the prevention of infection following transurethral prostatectomy and biliary surgery.

Pediatric use of fluoroquinolones

Pediatric use of fluoroquinolones has been limited by concerns regarding arthropathy observed in weight-bearing diarthrodial joints in juvenile dogs after prolonged high-dose administration. Nevertheless, accumulated experience has established some situations when the benefits of fluoroquinolones outweigh potential risks. These include typhoid fever, cholera and shigellosis, complicated UTI due to multiresistant pathogens, chronic suppurative otitis media caused by *P. aeruginosa*, multiresistant Gram-negative sepsis (including osteomyelitis), prophylaxis of meningococcemia (single-dose) and infection in neutropenia.

Treatment of pseudomonal infections in patients with cystic fibrosis is one of the commonest indications for the use of fluoroquinolones in children. Prolonged courses are often given but there has been little evidence of related arthropathy and fluoroquinolones continue to be widely used.

TABLE 198-5 -- Manufacturers' dosage recommendations for patients with renal impairment.

MANUFACTURERS' DOSAGE RECOMMENDATIONS FOR PATIENTS WITH RENAL IMPAIRMENT			
	Renal impairment		**Hemodialysis/CAPD**
	Mild	**Moderate/severe**	
Ciprofloxacin (iv)		200–400mg 18–24 hourly (CC = 5–29ml/min)	
Ciprofloxacin (po)	250–500mg bd (CC = 30–50ml/min)	250–500mg 18 hourly (CC = 5–29ml/min)	250–500mg od after dialysis
Ofloxacin	400mg od (CC = 20–50ml/min)	200mg od (CC <20ml/min)	
Levofloxacin	250mg od* (CC = 20–50ml/min)	250mg 48 hourly* (CC = 10–19ml/min)	250mg 48 hourly* (CC = 10–19ml/min)
Norfloxacin		400mg od (CC <30ml/min)	
Gatifloxacin		200mg od* (CC <40ml/min)	200mg od* (CC <40ml/min)
Moxifloxacin	No adjustment required		
CC, creatinine clearance			

* Initial loading dose of 500mg
** Short courses up to 3 days do not require dosage alteration

DOSAGE IN SPECIAL CIRCUMSTANCES

Renal impairment

The extent to which the dosage requires modification is dependent on the degree of renal elimination. Table 198.5 shows the manufacturer's recommendations for selected quinolones. Essentially, agents such as ofloxacin and levofloxacin that are extensively renally excreted have the dose reduced to one-quarter of the normal daily dose in severe renal impairment and most other agents have the dose halved. However, as noted above, there is evidence that ciprofloxacin may not require dose modification as long as alternative routes of elimination are intact.[16] Moxifloxacin, which has only 20% renal excretion, does not require dose modification.

Hepatic impairment

Apart for extensively metabolized quinolones, such as pefloxacin, dose modification is not necessary in patients with hepatic impairment. However, experience with newer agents such as moxifloxacin in patients with severe liver failure (Child Pugh Class C) is limited.

Elderly patients

No specific changes in dosage are required for the elderly provided appropriate changes are made for reduced renal clearance.

Pediatrics

Optimal pediatric doses have not been established. Suggested doses of ciprofloxacin are 7.5–40mg/kg/day (oral) or 5–10mg/kg/day (intravenous) administered on a 8–12-hourly basis.

Pregnancy and lactation

Quinolones are not approved for use in pregnancy or during lactation.

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

Fluoroquinolones are generally well tolerated although there are a number of potentially serious adverse effects that have been seen in some agents. [64] When adverse effects are reported, they are usually gastrointestinal (2–20%), dermatologic (0.5–3%) and CNS (0.5–2%) reactions which rarely necessitate withdrawal of therapy (1–3%). In most cases there are no specific age or racially related effects, but adverse drug reactions are more common in neutropenic patients and possibly in people who have AIDS.

Most fluoroquinolone adverse drug reactions are class effects, but incidence varies between compounds and can often be related to the specific structure of different agents. Certain group members have specific effects or more serious class effects that have led to restrictions on use or withdrawal. For example, the phototoxicity of sparfloxacin has restricted licensing by some registration authorities and temafloxacin, which caused hemolytic uremic syndrome and hypoglycemia, was withdrawn in 1992.

Gastrointestinal reactions

The usual reported symptoms are nausea, anorexia and dyspepsia. While diarrhea, abdominal pain and vomiting are less frequent, they are more likely to result in discontinuation of treatment. Antibiotic-associated diarrhea caused by *Clostridium difficile* is uncommon following fluoroquinolone therapy. Liver enzyme abnormalities occur in 2–3% of patients receiving fluoroquinolones and are usually mild and reversible. However, more severe liver abnormalities have been seen with some agents which, in the case of trovafloxacin, led to its withdrawal.

Dermatologic reactions

Although non-specific skin rashes, pruritus and urticaria have been reported, it is phototoxicity that has received most attention. This is a class effect and is thought to be related to the photodegradation of the fluoroquinolone and its ability to induce free radicals. The incidence and severity of phototoxicity differ between agents. Structurally, a fluorine moeity at position 8 causes more phototoxicity and sparfloxacin, which has this moiety, has caused a higher rate of phototoxicity. Phototoxic reactions are rare with ciprofloxacin, ofloxacin and levofloxacin. Gemifloxacin can cause a nonphototoxic rash which is seen particularly in female patients between the ages of 20 and 40 years.

Central nervous system reactions

These occur in less than 2% of patients with most fluoroquinolones and usually manifest as headache, dizziness, mild tremor or drowsiness.

Convulsions occur rarely both as a primary effect and as a result of interactions with theophylline or nonsteroidal anti-inflammatory drugs (NSAIDs). Although the mechanism of quinolone toxicity has not been fully elucidated, it is believed to be due to inhibition of $GABA_A$ receptors.

Musculoskeletal effects

Fluoroquinolones as a class produce destructive arthropathy in weight-bearing, diarthrodial joints of juvenile animals, notably dogs, by production of cartilage erosions

after prolonged high dosage. Some agents, notably precursors such as nalidixic acid, are considerably more likely to induce arthropathy. This effect has never been observed in human children and MRI follow-up and autopsy studies in children receiving both nalidixic acid and modern fluoroquinolones have revealed no evidence of joint damage.[65] Experience with ciprofloxacin in 1500 children noted reversible arthralgia in 3.2% of patients treated for pulmonary exacerbations of cystic fibrosis.[66]

Tendinitis occurs rarely as a class effect although it may be more common with concomitant corticosteroid therapy. The Achilles tendon is most commonly affected and patients are usually >50 years of age. MRI can be useful for early detection of damage and discontinuation is recommended at the first sign of tendon pain or inflammation.

Cardiovascular effects

Cardiotoxicity is manifest as prolongation of the QT interval with the potential to cause ventricular arrhythmias. The significance of this effect varies between agents and appears to be affected by substitutions at position 5 (see Fig. 198.2).[67] Adverse effects due to cardiotoxicity are rare although recently grepafloxacin was withdrawn voluntarily following reports of seven cardiac-related fatalities.

Other (rare) effects

Hypersensitivity occurs at a frequency of ~1%. Crystalluria and secondary interstitial nephritis are rare and relate to pH-associated solubility of fluoroquinolones in urine.

Interactions with other drugs

Interactions largely occur as a result of interference with fluoroquinolone absorption or by inhibition of biotransformation of unrelated drugs by the hepatic cytochrome P450 isoenzyme system. Central nervous system interactions due to GABA receptor inhibition occur with NSAIDs, notably fenbufen, and convulsions may follow, as reported with enoxacin.

Interactions affecting absorption of fluoroquinolones

The absorption of fluoroquinolones is reduced by up to 80% by co-administration of aluminium and magnesium-containing antacids, probably by the formation of insoluble complexes and, to a lesser extent, by calcium antacids, sucralfate and ferrous iron preparations. H_2 -antagonists have no effect.

Interactions affecting drug metabolism

Fluoroquinolones reduce the hepatic clearance of xanthines via the P450 cytochrome system. The effect is most marked with enoxacin and grepafloxacin, but ciprofloxacin and pefloxacin also reduce clearance of theophylline by 30% and co-administration may result in theophylline toxicity, usually nausea but possibly convulsions. Dosage of theophylline should be interrupted or reduced and serum levels monitored if enoxacin, pefloxacin or ciprofloxacin is to be administered.

A similar effect, induced by the same fluoroquinolones, is responsible for inhibition of caffeine metabolism and resultant insomnia. Metabolism of warfarin, cimetidine and cyclosporin is affected much less by P450 cytochrome inhibition and interaction may not be clinically significant.

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Chapter 199 - Glycopeptides

Jihad Slim
Leon Smith

VANCOMYCIN

Vancomycin is a tricyclic glycopeptide antibiotic obtained from *Amycolaptosis orientalis*, which is found in the soil of Borneo and India. This antibiotic-producing bacterium was also known in the past as *Streptomyces orientalis* and *Nocardia orientalis*. Vancomycin has been used clinically since 1956 but recent improvements in its manufacture have increased its purity and reduced its toxicity. It is an extremely valuable and relatively safe antibiotic against the major Gram-positive organisms. In the past few years the emergence of resistance in enterococci, and especially in staphylococci, has become an important clinical issue.[1]

PHARMACOKINETICS AND DISTRIBUTION

Vancomycin is bactericidal and appears to exert its effect by binding to the precursor units of peptidoglycan synthesis (D-alanyle-D-alanine units) inhibiting the transpeptidase reaction. This step is necessary for cross-linking newly synthesized peptidoglycan precursors into a complete structure. The net result is an alteration of bacterial cell wall permeability. In addition, RNA synthesis is inhibited. Perhaps because of this dual mechanism of action, resistance to vancomycin is uncommon, although it has been reported in strains of enterococci, coagulase-negative staphylococci and a few strains of *Staphylococcus aureus*. Gram-negative organisms are not sensitive to vancomycin, perhaps because porin channels in the cell wall of the Gram-negative organism do not accommodate the large, bulky vancomycin molecule. Vancomycin exhibits concentration-independent (or time-dependent) bactericidal action against susceptible bacteria. It kills better in aerobic conditions than in anaerobic conditions.

Concomitant use with streptomycin or gentamicin is usually synergistic against susceptible pathogens, especially enterococci and *viridans* streptococci, while rifampin (rifampicin) exhibits synergism with vancomycin against most coagulase-negative staphylococci but not against *Staph. aureus*.

TABLE 199-1 -- Concentration of vancomycin in body tissues and fluids.*

CONCENTRATION OF VANCOMYCIN IN BODY TISSUES AND FLUIDS				
Body fluid or tissue	**Dose**	**Route of administration**	**Sampling time (hours after administration of vancomycin dose)**	**Mean vancomycin concentration in sample (µg/ml)**
Pericardial fluid	500mg	Intravenous	1.5–5.5	0.6–5.5
Lung	1g	Intravenous	6	1.3
Synovial fluid	500mg	Intravenous	1	5.7
Ascitic fluid	500mg	Intravenous	1.5–5.2	3.6
Bile	500mg	Intravenous	1	3.1
Urine	500mg	Intravenous	1	30–90
Stools	500mg	Oral	6	100–350

** Data from Cunha and Klein.[6]*

Generally, vancomycin is only administered intravenously, although oral administration is important in treatment of pseudomembranous colitis; the oral bioavailability of vancomycin is too low to treat systemic infections. Patients who have colitis, however, develop detectable serum levels after oral administration, especially if they have renal impairment.

A two- or a three-compartment model best explains the pharmacokinetics of vancomycin. A 500mg dose of vancomycin hydrochloride results in a mean peak serum concentration of approximately 30µg/ml immediately after infusion. Concentrations after 1 hour are approximately 6µg/ml, and after 6 hours they are approximately 3µg/ml. After a slow intravenous infusion of 1g vancomycin, peak serum levels are approximately 48µg/ml, with trough concentrations of 2µg/ml at 12 hours. Ideally, peak concentrations of approximately 30µg/ml and trough concentrations of 10µg/ml or less are desirable. After 24 hours, concentrations are less than 1µg/ml.[2] [3] [4] [5]

Vancomycin is distributed into most body tissues and fluids, including pericardial, pleural, ascitic and synovial fluids (Table 199.1). Unless the meninges are inflamed, there is little diffusion into cerebrospinal fluid. Vancomycin is about 55% bound to serum protein. It is not known whether any metabolism takes place. Excretion is mainly by glomerular filtration, with about 80% of the drug excreted in 24 hours in the urine and only small amounts excreted in the feces. Owing to poor bioavailability, oral doses are excreted mainly in the feces. Vancomycin given by the oral route concentrates intraluminally in the distal gastrointestinal tract, resulting in high intraluminal vancomycin concentrations. There is approximately 1000µg/ml in the stool after a dose of 2g/day. Low serum concentrations may occur in patients who have a damaged intestinal mucosa. [3] [4] [7] [8] [9] [10]

The kidneys eliminate vancomycin through glomerular filtration. Urine concentrations are approximately 300µg/ml after a 500mg intravenous dose, and tubular reabsorption is not important in the renal handling of vancomycin. Because vancomycin is eliminated via the renal route, it accumulates in the presence of renal insufficiency. The serum half-life of vancomycin in anuric patients is approximately 7 days, in contrast to 4–6 hours in adults who have normal

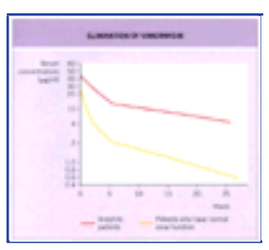

Figure 199-1 Elimination of vancomycin by anephric patients and by patients who have normal renal function. *Adapted with permission from Cunha et al.[11]*

renal function (Fig. 199.1). If F60 or F80 polysulfone filters are used, significant amounts of vancomycin can be removed during hemodialysis.

ROUTE OF ADMINISTRATION AND DOSAGE

Adults and children who have normal renal function

The usual dose is 2g/day divided into two or four doses and administered intravenously at a rate no faster than 10mg/minute or over a total of at least 60 minutes.

Oral vancomycin is effective for pseudomembranous enterocolitis or staphylococcal enterocolitis, and the dose is 500–2000mg/day in three or four daily doses for 7–10 days.

For children the usual dosage is 10mg/kg q6h.

Vancomycin serum level is readily available in most laboratories and should be measured to monitor efficacy and prevent toxicity, especially in patients who have fluctuating renal function.

INDICATIONS

Most strains of *Staph. aureus* and *Staphylococcus epidermidis* are susceptible to vancomycin, as are streptococci (including enterococci), *Corynebacterium* spp. and *Clostridium* spp. (Table 199.2). Vancomycin is particularly useful against methicillin-resistant *Staphylococcus aureus* (MRSA) infections and for treating Gram-positive infections in patients who are allergic to penicillin. Some strains of Gram-positive bacteria — *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp. and *Erysipelothrix* spp. — possess inherent resistance to vancomycin. Gram-negative bacteria, fungi, viruses and mycobacteria are resistant to vancomycin.

Synergistic bactericidal effects can be achieved when vancomycin is combined with aminoglycosides against enterococci and MRSA, but this increases possible renal toxicity. Vancomycin is useful against a wide variety of clinical infections caused by these organisms (Table 199.3), although it should not be used in patients who have meningitis unless absolutely necessary because of its poor penetration

TABLE 199-2 -- Organisms treatable by vancomycin.

ORGANISMS TREATABLE BY VANCOMYCIN
Actinomyces spp.
Bacillus cereus and *Bacillus subtilis*
Clostridium difficile and other *Clostridium* spp.
Corynebacterium jeikeium and other *Corynebacterium* spp.
Enterococcus faecalis and *Enterococcus faecium*
Listeria monocytogenes
Staphylococcus spp. including *Staphylococcus aureus, Staphylococcus epidermidis* and methicillin-resistant staphylococci
Streptococcus spp. including *Streptococcus agalactiae* (group B streptococci), *Streptococcus bovis, Streptococcus pneumoniae, Streptococcus pyogenes* and *viridans* streptococci
Resistant strains of enterococci and staphylococci may occur.

TABLE 199-3 -- Conditions treatable by vancomycin.

CONDITIONS TREATABLE BY VANCOMYCIN	
Diabetic foot ulcers	Skin and soft tissue infections
Endocarditis	Urinary tract infections
Osteomyelitis	Lower respiratory tract infections
Peritonitis	Intra-abdominal infections
Pneumonia	Bone and joint infections
Pseudomembranous colitis	Some forms of meningitis
Sepsis	
These conditions are treatable by vancomycin if they involve the organisms listed in Table 199.2 .	

into the cerebrospinal fluid. Oral vancomycin is used for severe colitis caused by *Clostridium difficile*. Metronidazole should be used as the first-line agent for most cases of *C. difficile* related diarrhea. This limits the risk of overuse of vancomycin with its attendant risk of selection of vancomycin-resistant bacteria.

Susceptible organisms are usually sensitive to concentrations of 1–5µg/ml.

DOSAGES IN SPECIAL CIRCUMSTANCES

Vancomycin should be used with caution in patients who have renal failure because it can accumulate. High serum concentrations increase the possibility of developing ototoxicity and nephrotoxicity (see below). Dose adjustments are necessary and lower doses of the drug are recommended for patients who have renal dysfunction or in patients receiving other ototoxic or nephrotoxic drugs. Renal function tests should be performed routinely during therapy.

In patients who have impaired renal function, the patient can be given a loading dose of 15mg/kg with a daily maintenance dose in milligrams of 150 plus 15 times the creatinine clearance, which provides a steady state concentration of 20µg/ml.[12] Alternatively, a nomogram can be used.

Hemodialysis usually removes little or no vancomycin, and 1g (15mg/kg as a pediatric dose or for a small adult (<50kg)) every 7–10 days usually provides adequate serum concentrations in functionally anephric adults.

Patients on continuous ambulatory peritoneal dialysis can be given vancomycin intravenously with a loading dose of 23mg/kg and a maintenance dose of 17mg/kg every 7 days,[13] or 1.5mg/kg q6h.[14] Monitoring of serum concentrations is recommended.

Vancomycin dosage for neonates is 15mg/kg bolus followed by 10mg/kg q12h in the first week of life, q8h up to 1 month of age. For children it is administered at 10mg/kg q6h.

In premature infants and elderly patients, vancomycin dosage needs to be adjusted to their clearance of creatine.

Vancomycin serum concentrations must be monitored carefully in patients who have severe hepatic impairment since accumulation can occur.

Serum levels above 40µg/ml can be associated with nephrotoxicity, and ototoxicity.

ADVERSE REACTIONS

Too rapid an infusion of vancomycin can trigger histamine release, which may cause anaphylactoid reactions including fever, chills, sinus tachycardia, pruritus, paresthesiae, flushing, rash or redness in the face, neck, upper body, arms or back, and muscular spasms. It is seen infrequently now following the introduction of improved pharmacological formulations. In some cases, hypotension occurs. This histamine release reaction is often referred to as the 'red man' syndrome. Slowing the infusion rates, administering a histamine-1 blocker or lowering the size of the dose may reduce the incidence or severity of the reaction. These reactions usually resolve within 20 minutes but may persist for several hours. In animal studies, hypotension and bradycardia occurred in animals given large doses of vancomycin at high concentrations and rates. Such events are infrequent if vancomycin is given by slow infusion over 60 minutes. In studies of normal volunteers, infusion-related events did not occur when vancomycin was administered at a rate of 10µg/min or less.

Rarely, vancomycin causes nephrotoxicity. Renal failure is principally manifested by increased serum creatinine or blood urea nitrogen concentrations, especially in patients who have been given large doses of vancomycin. Rare cases of interstitial nephritis have also been reported. Most of these have occurred in patients who were given concomitant aminoglycosides or who had pre-existing kidney dysfunction. When vancomycin was discontinued, azotemia resolved in most patients. Generally the nephrotoxicity risk is minimized if trough serum concentrations are kept below 10µg/ml.

A few dozen cases of hearing loss associated with vancomycin have been reported; these occurred at serum concentrations of about 60–80µg/ml. Most of these patients had kidney dysfunction or a pre-existing hearing loss, were receiving concomitant treatment with an ototoxic drug, were dehydrated, or were bacteremic. Vancomycin-induced hearing loss can be manifested as either cochlear toxicity (causing tinnitus or hearing loss or both) or vestibular toxicity (causing ataxia, vertigo, nausea, vomiting and nystagmus). Reducing the serum levels may reverse the ototoxicity.

Orally administered vancomycin should be used with caution with cholestyramine or colestipol. These anion-exchange resins can bind vancomycin and reduce its effectiveness. If patients must take both drugs, doses should be administered several hours apart.

Concomitant use of parenteral vancomycin with other nephrotoxic drugs (e.g. aminoglycoside, cidofovir, foscavir, amphotericin B) can lead to additive nephrotoxicity.

RESISTANCE

There has been increasing concern about the emergence of vancomycin-resistant bacteria, especially enterococci. High-level resistance is mediated by a gene complex found on plasmids known as the VanA genotype. The phenotypic expression of vancomycin resistance (inducible and associated with teicoplanin resistance) is known as the VanA phenotype (see Chapter 189). The VanA phenotype is carried on a transposon designated as transposon (Tn) 1546, which carries clusters of seven genes that code for vancomycin resistance, five of which are required for the expression of the VanA phenotype. Intermediate-level resistance is mediated by the VanB gene cluster. These genes are transferable by conjugal plasmids and mediate the VanB resistance phenotype. Teicoplanin will not induce the expression of VanB resistance. Transfer of these genes between organisms can spread resistance.

This has led to recommendations by several governmental agencies to prevent vancomycin resistance. The guidelines from the Centers for Disease for Disease Control and Prevention in the USA state that vancomycin is not recommended for:

- routine surgical prophylaxis;
- treatment of single positive blood culture of coagulase-negative *Staphylococcus* spp.;
- empiric treatment of a febrile neutropenic patient in whom no evidence of Gram-positive infection exists;
- continued empiric therapy;
- selective decontamination of the gut;
- colonization with MRSA;
- primary treatment of pseudomembranous colitis;
- topical application or irrigation;
- treatment of methicillin-sensitive *Staphylococcus aureus* in dialysis patients;
- prophylaxis in continuous ambulatory peritoneal dialysis;
- systemic or local prophylaxis for indwelling central or local catheters; or
- Lyme disease.

TEICOPLANIN

Teicoplanin (formerly called teichomycin A) is a complex of five closely related glycopeptides that have the same heptapeptide base and an aglycone that contains aromatic amino acids, with D-mannose and *N*-acetyl-D-glucosamine as sugars, with a molecular weight of 1562–1891Da. Teicoplanin is structurally similar to vancomycin. It is produced from the actinomycete *Actinoplanes teichomyceticus*.

Teicoplanin is similar but not identical to vancomycin in its spectrum of activity. Minimum inhibitory concentrations (MICs) for most Gram-positive bacteria and anaerobes are comparable, but teicoplanin is less active against some strains of *Staphylococcus haemolyticus* (MIC 16–64mg/l compared to =4mg/l for vancomycin). Its ease of administration and its low toxicity potential make it a potential alternative to vancomycin for the treatment of Gram-positive aerobic and anaerobic bacteria, in both the immunocompetent and the immunocompromised host. In-vitro activity against most Gram-positive organisms is equal to or greater than that of vancomycin.

In both open and comparative clinical trials, teicoplanin has been well tolerated, and adverse reactions have rarely prompted discontinuation of treatment. Nephrotoxicity caused by teicoplanin is uncommon, even when it is used concomitantly with aminoglycosides or cyclosporin A. Favorable pharmacokinetics allow for intramuscular administration as well as intravenous bolus dosing and, after appropriate loading doses, maintenance therapy may be given on a once-daily basis. The combination of all of these factors makes teicoplanin an effective, safe alternative to vancomycin in the treatment of Gram-positive infections. Although it is widely used in Europe, it is still yet to be approved by the Food and Drug Administration in the USA.

PHARMACOKINETICS AND DISTRIBUTION

Teicoplanin binds to the terminal D-alanine-D-alanine sequence of peptides that form the bacterial cell wall and, by sterically hindering the transpeptidase and transglycosylation reaction, inhibits the formation of peptidoglycan. Teicoplanin is a large polar molecule and, as

it cannot penetrate the lipid membrane of Gram-negative bacteria, they are resistant to it (see Chapter 188). Enterococci expressing VanA (high-level) vancomycin resistance are also resistant to teicoplanin. Teicoplanin is not significantly absorbed from the gastrointestinal tract but it can be administered intravenously or intramuscularly, in a once-daily dosing schedule. It has a long half-life of approximately 47 hours, allowing for once-daily dosing once therapeutic serum levels are attained. In a study in which healthy volunteers were given intravenous injections of teicoplanin, doses of 3mg/kg gave average peak plasma concentrations of 53.5µg/ml and doses of 6mg/kg gave average peak plasma concentrations of 111.8µg/ml.[15] Bioavailability after intramuscular injection of teicoplanin is 90%, with peak plasma concentration occurring 2 hours after injection.[15]

Teicoplanin is approximately 90% protein-bound. It is more lipophilic than vancomycin and has excellent penetration into tissues and tissue fluids with a large volume of distribution after intravenous administration. High concentrations are achieved in peritoneal and blister fluid, bile, liver, pancreas, mucosa and bone. [16] Penetration into cerebrospinal fluid across uninflamed meninges is poor.

Teicoplanin does not undergo extensive metabolism and is excreted almost entirely by the kidneys.[17] As with vancomycin, its half-life is prolonged by renal failure.[18] Neither hemodialysis nor peritoneal dialysis significantly affects the clearance of teicoplanin.

ADMINISTRATION AND DOSAGE

Dosing recommendations for adults who have normal renal function have been based both on pharmacokinetic properties and on results of open and comparative clinical trials. A single loading dose of 400mg on the first day followed by maintenance doses of 200mg/day (3mg/kg/day for pediatric dose) appears adequate for the treatment of urinary tract infections, skin and soft tissue infections and lower respiratory tract infections. For serious infections, including endocarditis, osteomyelitis and sepsis, it is necessary to maintain serum concentrations of teicoplanin at 10µg/ml or more.[19]

Treatment of endocarditis caused by *Staph. aureus* has been difficult with teicoplanin, especially when used as monotherapy. [19] [20] In these cases, it is recommended that an aminoglycoside should be added and that teicoplanin trough levels should be maintained in the range of 20–60µg/ml.

INDICATIONS

The indications for the use of teicoplanin are similar to those for vancomycin (Table 199.4). They include Gram-positive infections caused by strains that are resistant to penicillin, cephalosporin or methicillin, or Gram-positive infections in patients who are allergic to penicillin. In addition, teicoplanin may be used for subacute bacterial endocarditis or surgical prophylaxis, in patients who are allergic to ß-lactam drugs and as an alternative to vancomycin or metronidazole in the treatment of pseudomembranous colitis caused by *C. difficile* (approved in Europe).

Teicoplanin, alone or in combination with other antibiotics, has proved effective in the treatment of various Gram-positive infections, including sepsis, endocarditis, skin and soft tissue infections, osteomyelitis and lower respiratory infections. It has also been found to be effective in both prophylaxis and treatment of Hickman catheter infections in immunocompromised patients.[21] [22] Specially prepared catheters loaded with teicoplanin have been developed and tested *in vitro* and have been shown to prevent bacterial colonization for at least 48 hours, thus showing promise for inhibiting early-onset catheter infections.[23] Teicoplanin has also been used along with other

TABLE 199-4 -- Indications for the use of teicoplanin.

INDICATIONS FOR THE USE OF TEICOPLANIN	
Infections with:	*Staphylococcus* spp., including methicillin-resistant strains
	Streptococcus spp., *Streptococcus agalactiae, Streptococcus bovis, Streptococcus pneumoniae, Streptococcus pyogenes* and *viridans* streptococci
	Enterococci
	Clostridium spp., including *Clostridium difficile*
	Corynebacterium spp., including *Corynebacterium jeikeium*
The following types of infection with the above organisms:	Sepsis
	Endocarditis
	Skin and soft tissue infections
	Osteomyelitis
	Lower respiratory tract infections
	Diarrhea associated with *Clostridium difficile*
	Nosocomial intravascular catheter infections (prophylaxis and treatment)

antibiotics for empiric treatment of febrile neutropenic patients and in the treatment of documented Gram-positive infections in neutropenic patients.[24] [25]

Oral teicoplanin is as effective as vancomycin in the treatment of diarrhea associated with *C. difficile*. A 10-day course of regimen of 100mg oral teicoplanin q12h was found to be as effective as 500mg oral vancomycin q6h.[26] Again, the risk of glycopeptide resistance development argues for the use of metronidazole for routine

therapy for *C. difficile* associated diarrhea (see Chapter 44).

DOSAGE IN SPECIAL CIRCUMSTANCES

A dosage nomogram for teicoplanin has been designed,[27] based on the relationship between teicoplanin clearance and creatinine clearance and an average desired steady-state concentration of 20µg/ml. Although intravenous teicoplanin penetrates well into peritoneal fluid in normal patients, it does not penetrate well into the effluent of continuous ambulatory peritoneal dialysis patients and is not recommended for the treatment of peritonitis in these patients. Intraperitoneal teicoplanin has been effective in continuous ambulatory peritoneal dialysis patients who have Gram-positive peritonitis, however.

Another regimen for renal impaired patients includes a normal loading dose followed by doubling the dosage interval for patients who have a creatinine clearance of 30–80ml/minute and tripling the dosage interval for patients who have severe renal impairment (creatinine clearance <10ml/minute).

ADVERSE REACTIONS AND INTERACTIONS

Teicoplanin is generally well tolerated at therapeutic dosages, with side effects occurring in approximately 6–13% of the patients (Table 199.5).

Side effects (including nephrotoxicity) with teicoplanin are consistently less common than with vancomycin, even when teicoplanin is used concomitantly with aminoglycosides.[22] [24] The incidence of red man syndrome or anaphylactoid reactions caused by teicoplanin administration is exceptionally low.

The commonest side effects were injection site intolerance, skin rash, bronchospasm and eosinophilia. Nephrotoxicity and ototoxicity are uncommon.

TABLE 199-5 -- Adverse reactions to teicoplanin.

ADVERSE REACTIONS TO TEICOPLANIN	
Hypersensitivity	Skin rash
	Bronchospasm
	Anaphylaxis
Biochemical abnormalities	Increased liver function tests
Hematologic abnormalities	Eosinophilia
Local intolerance	Redness, pain (after intramuscular administration), phlebitis (after intravenous administration)
Non-specific reactions	Nausea
	Diarrhea
	Dizziness
	Tremor

Like vancomycin, teicoplanin is bound and inactivated by bile binding resins such as cholestyramine.[28]

DAPTOMYCIN

Daptomycin is a ovel cyclic lipopeptide antibiotic derived from *Streptomyces roseosporus*. Its mechanism of action is not very well elucidated yet, but it is different from that of vancomycin and teicoplanin. It exerts bactericidal activity against Gram-positive organisms by binding to cell membranes in the presence of free ionized calcium.

Daptomycin has a relatively long plasma half-life, approximately 8.5 hours, which allow for once-daily administration; it is highly protein-bound.

The in-vitro spectrum of activity includes most aerobic and anaerobic Gram-positive bacteria, including vancomycin-resistant *Enterococcus*, vancomycin-intermediate *Staph. aureus*, methicillin-resistant *Staph. aureus* and *Staph. epidermidis*, penicillin-resistant pneumococcus, *C. difficile*, *Clostridium perfringens* and *Bacillus anthracis*.[29]

Resistance in naturally occurring *S. aureus* is unusual.

As of February 2002, phase III studies documented daptomycin's good tolerability and efficacy compared with vancomycin and antistaphylococcal penicillin. The adverse event profile of 4mg/kg/day was similar to that of the standard dose of vancomycin.

Daptomycin may have an advantage over vancomycin, by virtue of its faster killing of *Staph. aureus* in vitro.[30]

ORITAVANCIN

Another glycopeptide in phase III studies, oritavancin differs from vancomycin by the addition of a lipophilic side chain (*N*3'-chlorobiphenyl) and of an aminated sugar (22-*O*-4-epivancosamine). This results in strongly amphiphilic molecule, which improves its ability to penetrate eucaryotic cells (e.g. macrophage) for better intracellular bactericidal activity.

Oritavancin is highly bound to human plasma protein (86–90%), and has a long terminal half-life, offering the potential of shorter treatment duration. [31] It is active against a broad range of Gram-positive organisms, including MRSA and vancomycin-resistant *Enterococcus*.

A double-blind controlled study in 517 patients who had complicated skin and skin-structure infection compared oritavancin 1.5mg/kg or 3 mg/kg for 3–7 days followed by oral placebo, versus vancomycin for 3–7 days followed by oral cephalexin, for a total therapy course of 10–14 days. At the conclusion of the study the three arms were comparable in efficacy and tolerability.[32]

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Chapter 200 - Tetracyclines and Chloramphenicol

Kjell Alestig

TETRACYCLINES

INTRODUCTION

In 1948, Benjamin Duggar at the Lederle Laboratories isolated the first tetracycline, chlortetracycline, from a drop of Missouri mud containing a fungus producing a golden pigment. The fungus was therefore called *Streptomyces aureofaciens* and the antibiotic aureomycin.

In 1950 oxytetracycline was isolated from a strain of *Streptomyces rimosus* by workers at Charles Pfizer & Co. and in 1953 tetracycline was produced semisynthetically from chlortetracycline. Demeclocycline, derived from a mutant strain of *S. aureofaciens*, was introduced in 1957 and in the following years the semisynthetic derivatives rolitetracycline and methacycline were also introduced. A so-called second generation of long-acting tetracyclines, doxycycline and minocycline, were synthesized in 1966 and 1972 respectively.

Chemically, the tetracyclines have the structure of a hydronaphthacene nucleus containing four fused rings. The specific analogues are obtained by substitutions on the fifth, sixth or seventh position of the basic structure (Fig. 200.1).

Figure 200-1 Molecular structure of some tetracyclines.

Tetracyclines are mainly bacteriostatic and they act by binding to the 30S subunits of the ribosomes in susceptible micro-organisms, thereby inhibiting protein synthesis.

There are few reasons to use any of the older tetracyclines; doxycycline and to some extent minocycline should be preferred because they are better absorbed and distributed than the older drugs. However, one exception may be that dermatologists often seem to prefer tetracycline or another first-generation drug for the treatment of acne.

ANTIMICROBIAL SPECTRUM

When they were introduced the tetracyclines were effective against a variety of Gram-positive bacteria and Gram-negative organisms within the Enterobacteriaceae group. Development of resistance has, however, led to restricted use of the drugs for infections caused by streptococci, staphylococci, *Escherichia coli* or *Proteus* spp. More important now is the tetracyclines' good activity against *Mycoplasma*, *Chlamydia* and *Rickettsia* spp., *Borrelia* spp. (especially *Borrelia burgdorferi*) and *Propionibacterium acnes* (Table 200.1).[1]

Doxycycline and minocycline often have a better activity in vitro than the other tetracyclines[2] [3] but the differences are probably of little practical importance for clinical treatment.

TABLE 200-1 -- Resistance against tetracyclines.

RESISTANCE AGAINST TETRACYCLINES	
Generally susceptible species	*Mycoplasma pneumoniae*
	Ureaplasma urealyticum
	Chlamydia spp.
	Rickettsia spp.
	Brucella spp.
	Francisella tularensis
	Propionibacterium acnes
	Borrelia burgdorferi
	Yersinia spp.
Resistance common (great variations between countries)	Streptococci
	Staphylococci
	Enterobacteriaceae
	Haemophilus influenzae
	Meningococci
	Gonococci
	Legionella pneumophila
Resistance usually found	Enterococci
	Proteus spp.
	Pseudomonas spp.
	Serratia spp.
	Bacteroides spp.

RESISTANCE

Oral therapy with tetracyclines has a marked influence on the bowel flora and resistant strains may be quickly selected. This risk is somewhat reduced with doxycycline,[4] which is nearly completely absorbed.[5] Extensive use of tetracyclines leads to plasmid-mediated multiresistance, but chromosomal alterations may also occur.

Tetracyclines have been widely used in the veterinary field, not only to cure infections but also as a food additive to promote the growth of newborn animals. Penicillin and tetracyclines are not now permitted as growth stimulators within the European Union but are still used in the USA. In pigs, antibiotics promote an increase in weight of 8% and pigs are ready for slaughter 3 weeks earlier than animals that have not received antibiotics — an important economic advantage. This misuse of antibiotics is probably one important reason for the worldwide spread of tetracycline resistance within the Enterobacteriaceae group.[6] [7] [8] However, restrictions on the veterinary use of antibiotics have now been introduced in some countries. In Sweden the use of any antibiotic to promote the growth of animals was prohibited as early as in 1986, and similar legislation for the whole European Union is expected within the next few years.

Resistance to tetracyclines is now common among bacteria causing respiratory infections such as pneumococci, *Haemophilus influenzae* and *Moraxella catarrhalis*.[9]

PHARMACOKINETICS AND DISTRIBUTION

Clinical pharmacokinetics

After oral administration tetracyclines are absorbed from the stomach and the small intestine. Absorption is usually highest in the fasting state, but doxycycline and minocycline are also well absorbed with food. The degree of absorption and other pharmacokinetic parameters for some of the derivatives are shown in Table 200.2 . The usually nearly complete absorption of doxycycline salts is reduced if the gastric pH is increased, as can occur in people who have atrophic gastritis or be caused by acid-reducing drugs. In some countries doxycycline is available in tablets bound to a polysaccharide, carragenate, which has been shown to increase absorption at higher pH.[10] There are also gelatin capsules available containing coated pellets of doxycycline hydrochloride that are resistant to gastric acid so that absorption will occur in the duodenum.[11]

Distribution

The tissue distribution of tetracyclines is clearly related to their different lipid solubility, which is higher for doxycycline than for

TABLE 200-2 -- Pharmacokinetics of some tetracyclines.

PHARMACOKINETICS OF SOME TETRACYCLINES			
	Tetracycline	**Doxycycline**	**Minocycline**
Oral absorption in fasting state (%)	80	90–93	100
Serum half-life (h)	6–12	18–22	13
Serum protein binding (%)	24–65	80–90	55–75
Lipid solubility in comparison with tetracycline	1	5	10
Excretion in urine (%) after oral administration	20	35–40	4–9

all older tetracyclines. Doxycycline concentrations are therefore sufficient for treatment in the respiratory tract and lung tissue, the bile and the genital tract of both sexes.[12] [13] Levels achieved in the central nervous system (CNS) are increased in chronic meningeal inflammation,[14] enabling treatment of neuroborreliosis. [15] [16]
Tetracyclines cross the placenta and bind to metal ions in fetal bone and teeth. They are also excreted in human milk.

Minocycline is even more lipid-soluble than doxycycline. This may not be an advantage because side effects such as vertigo and other CNS symptoms may be caused by increased drug concentrations in the cerebrosides of the brain. [17]

Elimination

Tetracyclines are metabolized in the liver in small amounts only, chlortetracycline being an exception with rapid metabolism. However, inducers of liver enzymes such as diphenylhydantoin may cause some metabolism of doxycycline. There is biliary excretion of tetracyclines to a varying degree and possibly enterohepatic circulation.

Tetracyclines are partly excreted by glomerular filtration. For minocycline this excretion is less than 10% but it is more than 50% for tetracycline.

Incomplete absorption will contribute to high concentrations in feces for the older tetracyclines. Only a small fraction of doxycycline is found in active form in feces, the larger part being bound as chelate.

Doxycycline may be given in normal doses to patients who have renal insufficiency and to those undergoing hemodialysis as the reduced renal excretion is compensated for by intestinal excretion of bound substance.[18] For minocycline, caution is recommended because of a larger risk of side effects.

ROUTE OF ADMINISTRATION AND DOSAGE

Peak serum levels after an oral dose of 500mg tetracycline or 200mg of doxycycline or minocycline are usually 3–5mg/l after 2 hours. Half-life in serum is longest for doxycycline (16–18 hours), followed by minocycline (11–13 hours) and tetracycline (8 hours). Doxycycline and minocycline can therefore be given once daily. A higher starting dose on day 1 is recommended in order to achieve a steady state level of the drug as early as possible. Dosages for adults are given in Table 200.3 .

Doxycycline is also available for intravenous infusions but there is little difference in serum levels compared with oral administration.

DOSAGE IN SPECIAL CIRCUMSTANCES

In patients who have renal insufficiency or disease, doxycycline is the only tetracycline that can be used safely without risk of accumulation and toxicity.

TABLE 200-3 -- Usual adult dosages for some tetracyclines.

USUAL ADULT DOSAGES FOR SOME TETRACYCLINES		
General name	**Oral preparations**	
	First dose	**Common dosage**
Tetracycline	500mg	500mg q6h
Oxytetracycline	500mg	500mg q6h
Doxycycline	200mg	100mg q24h
Minocycline	200mg	100mg q12h
Doxycycline and minocycline may be given intravenously in the same doses. Higher doses of doxycycline are often used for sexually transmitted diseases and Lyme disease.		

In patients who have hepatic disease tetracyclines should generally be avoided. If treatment is important, liver tests should be performed repeatedly during treatment. However, toxicity has mostly been observed when older tetracyclines have been used at high doses or given during pregnancy.

During pregnancy tetracyclines should be avoided because of the depressive effect on the skeleton of the child. In lactating patients a small amount of a tetracycline is excreted in the milk but is harmless to the baby.

In elderly patients kidney function is reduced according to age and the daily dose of a tetracycline should be reduced unless doxycycline is used.

INDICATIONS

Respiratory infections

Tetracyclines were first used for many types of respiratory tract infection. Because of increasing resistance in pneumococci and *H. influenzae*, they have been replaced in many areas by other antibiotics, mainly ß-lactams.

Exacerbation of chronic bronchitis has been a classic indication for tetracyclines. Doxycycline has been found to be as effective as ampicillin. The advantage of one single dose per day may increase compliance in patients who have chronic respiratory diseases and many other medications (see Chapter 33).

Doxycycline is distributed to maxillary sinuses and can be used as a second-line drug in patients who have sinusitis and are allergic to ß-lactams or in whom treatment with such drugs has failed.

Tetracyclines have good activity in pneumonias caused by *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia psittaci* and *Coxiella burnetii*. Macrolides probably have an equal effect in infections caused by *M. pneumoniae* but clinical experience with the other infections is far greater with tetracyclines.

Sexually transmitted diseases

Tetracyclines are effective therapy for nongonococcal urethritis caused by *Chlamydia trachomatis* or *Ureaplasma urealyticum*. In an open evaluation of doxycycline in the treatment of urethritis and cervicitis caused by *C. trachomatis* the symptoms disappeared in 76%.[18] Treatment should usually be given for 10 days and concurrent treatment of sexual partners is recommended (see Chapter 74).

Tetracyclines are usually effective for the treatment of lymphogranuloma venereum and granuloma inguinale. Non-penicillinase-producing strains of gonococci are sensitive, which is an advantage in mixed infections with *C. trachomatis*.

Tetracyclines can be used as alternative therapy for syphilis in penicillin-allergic patients. Treatment time is 15 days for early disease and 30 days for later stages of the disease.

Lyme disease and ehrlichiosis

Doxycycline can be used in penicillin-allergic patients to treat erythema migrans and is as effective as penicillin in a dose of 200mg daily for 10 days. In erythema migrans with signs of dissemination such as multiple erythema and fever, doxycycline is often recommended as the primary drug. Treatment of neuroborreliosis with doxycycline 200mg daily for 2 weeks has given similar results to those achieved with penicillin G (see Chapter 54). [19]

Doxycycline is also the preferred drug for infections due to *Ehrlichia* spp (see Chapter 14).[20]

Other indications

Tetracyclines are very effective drugs for rickettsial infections (see Chapter 14). Doxycycline has been used for single-dose treatment of louse-borne typhus, but for other infections 7–10 days of treatment is usually needed. These infections can only be treated with tetracyclines or chloramphenicol. In children tetracyclines can often be given with minimal risk of staining the teeth if repeated treatments are avoided and the dose is kept as low as possible.

Doxycycline is also effective in a single dose for infections with *Borrelia recurrentis*. Tetracyclines are usually used in combination with other antibiotics such as streptomycin or rifampin (rifampicin) for brucellosis and tularemia.

For cholera in adults, tetracycline 500mg q6h for 5 days or a single dose of 300mg doxycycline have been recommended.[21]

Most tetracyclines have been used for oral treatment of chronic acne. The drugs have an antibacterial effect on *P. acnes* but also a general anti-inflammatory effect, which is probably of importance.[22] However, resistance of *P. acnes* to tetracyclines, including doxycyclines, has been reported from England and the USA. Cross-resistance does not include minocycline, which may be used clinically.[23]

Doxycycline may also be used for malaria prophylaxis in areas where *Plasmodium falciparum* is resistant to other antimalarial drugs.[24]

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

All oral preparations of tetracyclines can cause nausea and epigastric discomfort. It is usually an advantage if the drugs can be taken with food without decreasing absorption, as with doxycycline and minocycline. Diarrhea is less common but may occur, especially when tetracyclines with low absorption are used.

Phototoxic reactions can occur with all tetracyclines.

Tetracyclines should not be used in children aged 8 years or less because of the risk of enamel hypoplasia and tooth discoloration. They should also not be used during pregnancy. Tetracyclines, except doxycycline, are contraindicated in patients who have renal impairment, because inhibition of protein synthesis increases azotemia from amino acid metabolism.

Vertigo and dizziness are CNS symptoms that occur with minocycline only and must be considered a major disadvantage of that antibiotic.

Hepatotoxicity and other severe organ reactions have occurred, mainly after parenteral therapy — often with high doses — and also when the drugs have had to be used during pregnancy.

Drug-drug interactions

Tetracyclines form chelate complexes with many drugs containing metal ions (Table 200.4). When combined with diuretics the risk of accumulation of urea increases, with the exception of doxycycline. Some drugs seem to stimulate liver enzymes, so increasing doxycycline metabolism and shortening its half-life.

TABLE 200-4 -- Drug interactions with tetracyclines.

DRUG INTERACTIONS WITH TETRACYCLINES	
Interacting drug	**Effect**

Antacids with metal ions, calcium, zinc, iron, didanosine	Chelate formation and impaired absorption
Diuretics	Risk of increased serum urea concentration — not with doxycycline
Rifampin, phenobarbital, phenytoin, carbamazepine	Half-life of doxycycline shortened

CHLORAMPHENICOL

INTRODUCTION

Chloramphenicol was first isolated in 1947 from a sample of soil from Venezuela and the actinomycete was called *Streptomyces venezuelae*.

MODE OF ACTION AND SPECTRUM

Chloramphenicol inhibits protein synthesis by binding to the larger 50S subunit of the 70S ribosome. It is mainly a bacteriostatic agent but a bactericidal effect on some bacteria, such as *H. influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, has been reported.

Chloramphenicol has a very broad spectrum, similar to that of tetracyclines, and it includes aerobic and anaerobic bacteria, spirochetes, rickettsias, chlamydias and mycoplasmas. It is very active against most anaerobic bacteria of clinical interest, including *Bacteroides fragilis*.

Chloramphenicol resistance can occur and is mediated by a bacterial enzyme, acetyltransferase, which inactivates the drug. This mechanism is R-factor-mediated, and epidemics of chloramphenicol-resistant typhoid fever and *Shigella* infections have occurred.

Unrestricted use of chloramphenicol seems to result in a resistance problem very similar to that observed with tetracyclines.

ROUTE OF ADMINISTRATION AND DOSAGE

Chloramphenicol is conjugated with glucuronic acid and is then excreted in active form by the kidneys. The metabolites are not toxic and dose reduction is not needed in renal insufficiency.

Chloramphenicol is a rather small lipophilic molecule and is well distributed in the body. The serum protein binding is about 44%. It reaches the CNS better than most other antibiotics and its concentration in the cerebrospinal fluid is often 30–50% of the serum concentration. Chloramphenicol also crosses the placenta and is found in breast milk.

Chloramphenicol is well absorbed (over 90%) after oral administration. Chloramphenicol 1g gives a serum concentration of 10mg/l and the half-life is 3–4 hours. It may also be given intravenously as a succinate ester but intramuscular injections should be avoided as absorption is unreliable.

INDICATIONS

Chloramphenicol is toxic and therefore it should be used carefully in systemic infections, when other alternatives are lacking. It can be used instead of tetracyclines for the treatment of rickettsial infections and for bacterial meningitis in the few patients who have an allergy to ß-lactam drugs that includes third-generation cephalosporins and meropenem. It may also have a place as an oral alternative for CNS infections, especially brain abscesses.

Topical administration of chloramphenicol in drops or ointments is widely used for superficial bacterial infections of the eyes. Such treatment is still effective in comparison with the newer drugs such as quinolones or fusidic acid, which are also used locally for eye infections.[25] [26]

ADVERSE REACTIONS AND INTERACTIONS

Adverse drug reactions

Neonates have a diminished ability to conjugate chloramphenicol and to excrete the active form in the urine. A dose of 25mg/kg/day should not be exceeded[27] otherwise the 'gray baby syndrome' may develop, with severe cyanosis and circulatory collapse.

Dose-related reversible bone marrow depression can occur in adults given high doses of more than 4g/day. The daily dose should not exceed 3g, and when the accumulated dose exceeds 25g reticulocytes should be checked regularly (e.g. twice weekly) until treatment is stopped.

A very severe reaction is aplastic anemia, which occurs with a frequency of 1/25,000–40,000 treatment courses.[28] No clear correlation to dose or duration of treatment has been observed and no route of administration is exempt from causing this catastrophic complication. There are also reports to indicate that the use of chloramphenicol may increase the risk of leukemia in children.[29]

Drug-drug interactions

As chloramphenicol is almost completely metabolized in the liver by cytochrome P450 enzymes, there is a possible risk of interactions with other drugs if they are metabolized by the same enzyme system. Chloramphenicol will decrease the rate of metabolism of tolbutamide, phenytoin, cyclophosphamide and warfarin. Rifampin may lower chloramphenicol concentrations by induced metabolism.

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Chapter 201 - Nitroimidazoles: Metronidazole, Ornidazole and Tinidazole

S Ragnar Norrby

INTRODUCTION

The nitroimidazoles were developed as antimicrobial agents against protozoa, initially *Trichomonas vaginalis* and subsequently *Entamoeba histolytica* and *Giardia lamblia*. During the 1970s it was recognized that they are also highly active against strictly anaerobic bacteria, including difficult-to-treat organisms such as *Bacteroides fragilis* and *Clostridium difficile*. The mechanism(s) by which nitroimidazoles exert their antiprotozoan and antibacterial activities are not known in detail. It seems clear, however, that, after anaerobic reduction, the various derivatives interact with DNA and possibly other metabolic processes in bacteria and protozoa.[1] [2] Although they are still used against protozoa, anaerobic infections and, recently, treatment of gastric ulcer caused by *Helicobacter pylori* have become the main indications for the nitroimidazoles. In addition they have also been used as radiosensitizing agents in patients who have solid tumors.

The nitroimidazoles that are available are metronidazole, tinidazole and ornidazole, but tinidazole and ornidazole are not available in the USA. In this chapter, the emphasis will be on metronidazole.

PHARMACOKINETICS

Following oral administration all nitroimidazoles are almost completely absorbed.[3] After rectal administration of metronidazole the absorption is estimated to be about 60%, with considerable variability between individuals. When given vaginally the bioavailability of metronidazole is 20% or less. Following a 400mg oral dose of metronidazole or tinidazole, peak plasma concentrations of about 10mg/l are achieved after 3–5 hours. Dose proportional kinetics have been seen for doses up to 2g. The concentrations after normal oral doses are well above the minimum inhibitory concentrations (MICs) for anaerobes but are borderline for *Gardnerella vaginalis*.

The nitroimidazoles are well distributed to peripheral compartments, including brain tissue and cerebrospinal fluid.[3] However, there are low concentrations (15% or less of concurrent serum levels) in subcutaneous fat.[4]

Nitroimidazoles are eliminated mainly via liver metabolism. The plasma half-life is about 8 hours for metronidazole and 12–13 hours for tinidazole. Metronidazole is partly metabolized to hydroxymetronidazole, which has a longer half-life (10–13 hours). Metronidazole elimination is prolonged in newborns and infants, and also in adults who have serious liver impairment (e.g. cirrhosis). Decreased renal function does not affect the half-life. The drugs are partly eliminated by hemodialysis. These antibiotics have no reported effects per se on the central nervous system, liver, lungs, kidneys, prostate or genitourinary system.

ROUTE OF ADMINISTRATION AND DOSAGE

The two most frequently used nitroimidazoles, metronidazole and tinidazole, are available for parenteral and oral use and also as suppositories and for vaginal administration. A metronidazole gel is used

TABLE 201-1 -- Dosages of metronidazole.

DOSAGES OF METRONIDAZOLE			
Type of infection	**Adult dose**	**Child dose**	**Duration of treatment (days)**
Trichomoniasis	2g	Not applicable	Single dose
Giardiasis	600mg q12h	15mg/kg q12h	6–7 days
Amebic dysentery	800mg q8h	20mg/kg q12h	5–10 days
Amebic abscess	800mg q8h	20mg/kg q12h	10 days
Vaginosis	400mg q12h	Not applicable	7 days
Helicobacter infection	400mg q8h	Not applicable	7–14 days
Clostridium difficile enteritis	800mg q8h	7.5mg/kg q12h	7–10 days
Anaerobic infections (treatment)	800mg q8h	7.5mg/kg q12h	7–14 days
Anaerobic infections (prophylaxis)	800mg	7.5mg/kg	Single dose
Child dose is for children aged 8 weeks or more.			

for periodontitis. Dosages of metronidazole are given in Table 201.1 . Those of tinidazole and ornidazole are similar but, because their half-lives are longer, these drugs can be given q24h or q12h instead of q12h or q8h. Recommendations in the USA are normally for shorter dose intervals (i.e. q6h) for metronidazole than are indicated in Table 201.1 . Considering the half-life, dose intervals shorter than q8h should not be needed and in most cases q12h regimens should be optimal.

In elderly patients the same doses should be used as in younger adults. Doses should be reduced in patients who have reduced liver function but full doses can be given irrespective of renal function. Metronidazole passes to breast milk but the concentrations achieved are unlikely to affect a child. There is no documentation on the use of metronidazole during pregnancy.

INDICATIONS

Bacterial infections

The spectrum of activity of nitroimidazoles against strictly anaerobic bacteria is summarized in Table 201.2 . No clinically important differences exist between the antianaerobic activities of the various derivatives in the group.[7] Nitroimidazoles are the most active antibiotics for the treatment and prevention of anaerobic infections and resistance is rare but has been reported.[8] However, their disadvantage is their lack of activity against aerobes. As anaerobic infections, with few exceptions, are mixed aerobic/anaerobic infections, nitroimidazoles are routinely combined with other antibiotics. The most common combinations are with cephalosporins or aminoglycosides. Anaerobic infections for which nitroimidazole use is well documented are brain

TABLE 201-2 -- Activity of metronidazole against anaerobic bacteria.[*]

ACTIVITY OF METRONIDAZOLE AGAINST ANAEROBIC BACTERIA		
Organism	**Metronidazole MIC (mg/l) for 90% of isolates**	**Percent sensitive (NCCLS)**

Bacteroides fragilis	1–4	100
Prevotella spp.	4	100
Fusobacterium nucleatum	0.25–2	100
Fusobacterium spp.	0.25–4	100
Peptostreptococcus spp.	0.5–4	100
Propionibacterium acnes	>16	0
Clostridium difficile	0.5–4	100
Clostridium spp.	2–16	95
NCCLS, National Committee for Clinical Laboratory Standards.		

** Data from Wexler et al.[5] and Spangler et al.[6]*

abscesses, intra-abdominal infections, gynecologic infections and antibiotic-associated diarrhea or colitis caused by *C. difficile*. For the latter infection, metronidazole should be preferred over oral vancomycin, which might increase the risk of selection of resistant Gram-positive aerobic bacteria (e.g. enterococci) in the lower intestinal tract.

Two bacterial species that are not obligate anaerobes have also been found to be sensitive to metronidazole treatment:

- *G. vaginalis*, implicated in the etiology of vaginosis, is susceptible to nitroimidazoles with MIC values higher (8–16mg/l) than for anaerobes;[9] and
- *H. pylori*, which causes gastric ulcers and has been correlated to gastric cancer, is often susceptible to nitroimidazoles. Metronidazole is one of several antibiotics that, in combination with other antibiotics, can be used for treatment of gastric ulcers caused by *H. pylori* despite the fact that resistance to metronidazole is not uncommon.[10]

Protozoal infections

The nitroimidazoles seem to be uniformly active against protozoa. However, resistance has been reported in *T. vaginalis* and in some cases the organisms have been resistant to metronidazole but more sensitive to tinidazole.[11] Nitroimidazoles are first-line treatment for giardiasis, amebiasis and trichomoniasis. For these infections there are few alternatives outside this group.[12] This is also the case for bacterial vaginosis.[13]

ADVERSE REACTIONS AND INTERACTIONS

Although nitroimidazoles have been found to be mutagenic and carcinogenic in animal studies, they are generally well tolerated in humans. One meta-analysis of more than 1300 pregnant women who had received metronidazole during their pregnancies showed no indication of teratogenicity.[14] The most common side effect is a metallic taste, especially when high doses are used. Reversible neuralgia has been reported in patients receiving high doses for prolonged periods. If combined with alcohol, metronidazole may cause an Antabuse-like reaction. Severe psychotic reactions have been reported in patients receiving metronidazole and disulfiram together, and so nitroimidazoles should not be given with disulfiram.

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Chapter 202 - Antituberculosis Agents

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INTRODUCTION

Tuberculosis is at least as old as recorded human history, and a major preoccupation of the medical profession over the millennia has been the search for a cure for this 'Captain of all of these Men of Death', as the evangelist John Bunyan termed it. Numerous remedies have been described; many, such as bleeding, purging and John of Gaddeston's prescription of a mixture of pigeon's dung and weasel's blood were undoubtedly worse than useless. In 1782, Sir William Buchan remarked that, apart from a trip to the West Indies, milk was probably as effective as the entire pharmacopeia. In the Indian ayurvedic medical system, the malabar nut (*Adhatoda vasica*) was advocated and several British military doctors of the 19th century were impressed with its efficacy. In Europe, cod liver oil was widely used after its introduction by Percival in 1770; indeed, it was the most widely prescribed remedy at the Brompton Hospital for Consumptives, London, for several decades after its foundation in the mid-19th century. Owing to its high vitamin D content, it may well have had a positive effect because calcitriol, the active metabolite of this vitamin, is involved in the activation of human macrophages.

The discovery of the tubercle bacillus raised serious hopes that an effective remedy would soon be found and many workers attempted to develop immunotherapeutic agents. The best known of these attempts was Robert Koch's development of old tuberculin, but the British bacteriologist Sir Almroth Wright also conducted extensive studies, which are immortalized in George Bernard Shaw's play *The Doctor's Dilemma*.

It was, however, the discovery of streptomycin in 1944 by Albert Schatz and Selman Waksman in the USA that opened the door to effective therapy and led many health workers to believe that the disease would soon be conquered. Early jubilation turned to disappointment when it was found that patients treated with streptomycin often made an initial improvement but soon relapsed with disease because their tubercle bacilli became resistant to this agent. Fortunately, other active antituberculosis agents were soon discovered and, as a result of extensive trials initiated by Sir John Crofton in the UK, multidrug regimens that cured patients and prevented the emergence of drug resistance were developed.[1]

Therapy of tuberculosis with these early drug regimens, usually consisting of streptomycin, isoniazid and *para*-aminosalicylic acid, was beset with problems. Streptomycin had to be given by injection and *para*-aminosalicylic acid caused such severe gastrointestinal effects that patients often failed to comply with therapy. In addition, it was necessary to treat patients for 18–24 months in order to achieve a cure.

The second therapeutic revolution came in the early 1970s when regimens containing rifampin (rifampicin) were developed. The introduction of this drug had three major effects on the treatment of tuberculosis. First, the duration of therapy could be reduced to only 6 months so that the era of 'short course' therapy had arrived. Secondly, regimens could be entirely oral ones and thirdly, as a consequence, hospitalization could often be avoided.

Modern short course therapy, properly used, can achieve a cure in around 98% of patients and is among the most effective and cost-effective of all therapeutic interventions for a chronic disease.[2] Far from being conquered, however, tuberculosis remains one of the most prevalent causes of mortality and morbidity; it is responsible for one in seven deaths among young adults and it was declared a global emergency by the World Health Organization (WHO) in 1993. The problem is currently fueled by the HIV pandemic and the increasing prevalence of multidrug-resistant tuberculosis (MDRTB), and there is accordingly a very urgent need to develop new therapies and to use the available therapies in a much more responsible manner.

CLASSIFICATION

Antituberculous agents can be classified in several ways (Table 202.1). First, they can be divided into those that are synthetic molecules and those that are antibiotics or semisynthetic antibiotic derivatives. Second, they can be divided into agents with a broad spectrum of activity and use and those only active against mycobacteria or, specifically, members of the *Mycobacterium tuberculosis* complex. Third, they can be divided into the first-line drugs that form the basis of the modern short-course regimens advocated by the WHO and the second-line drugs, which are used in cases of drug resistance and where toxic reactions prevent the use of one or more first-line drugs. Finally, they can be divided into bacteriostatic and bactericidal agents, with the latter being further divided into those that are bactericidal in vitro and those that are able to sterilize lesions of tubercle bacilli in vivo (Table 202.2). This distinction is a clinically important one.

MODE OF ACTION AND PHARMACOKINETICS

Previously poorly understood, there have been considerable advances in recent years in our understanding of the genetic basis of action of the drugs used specifically for treating mycobacterial disease as a result of the successful sequencing of the genome of *M. tuberculosis*.[3]

The targets of streptomycin and other aminoglycosides, rifampin and the fluoroquinolones are the same in mycobacteria as in *Escherichia coli*, and resistance is due to single amino acid substitutions in the target proteins. Some other drugs, notably isoniazid, pyrazinamide, ethambutol, ethionamide and prothionamide, target specific components of the complex and lipid-rich mycobacterial cell wall. The mode of action and target genes are discussed under the individual drug headings below and are summarized in Table 202.3 ; the targets are shown in Figure 202.1 . Most of the antituberculosis agents are readily absorbed from the gastrointestinal tract. Exceptions are streptomycin and other aminoglycosides, capreomycin and viomycin, which must therefore be given parenterally. Binding to serum proteins varies from agent to agent, as does entry into the cerebrospinal fluid (CSF). Agents that enter into the CSF poorly in health often pass the inflamed meninges so that therapeutically useful levels are achieved in cases of tuberculous meningitis. The pharmacokinetics of the conventional antimycobacterial drugs, their principal

TABLE 202-1 -- Spectrum of activity, class of compound and cross-resistances of the antituberculosis agents.

SPECTRUM OF ACTIVITY, CLASS OF COMPOUND AND CROSS-RESISTANCES OF THE ANTITUBERCULOSIS AGENTS			
Agent	**Class of compound**	**Spectrum of activity**	**Cross resistance to other antituberculosis agents**
First-line agents			
Rifampin	Antibiotic	Broad	Other rifamycins
Isoniazid	Synthetic	Tubercle bacilli	None
Pyrazinamide	Synthetic	Tubercle bacilli	None
Ethambutol	Synthetic	Tubercle bacilli	None
Streptomycin	Antibiotic	Broad	Other aminoglycosides, viomycin, capreomycin
Second-line agents			
Thiacetazone	Synthetic	Tubercle bacilli	Ethionamide and prothionamide

para-aminosalicylic acid	Synthetic	Tubercle bacilli	None
Ethionamide and prothionamide	Synthetic	Tubercle bacilli	Thiacetazone
Capreomycin	Antibiotic	Tubercle bacilli	Aminoglycosides, viomycin
Viomycin	Antibiotic	Tubercle bacilli	Aminoglycosides, capreomycin
Cycloserine	Synthetic	Broad	None
Ofloxacin	Antibiotic	Broad	None
Agents that are active against tubercle bacilli (*Mycobacterium tuberculosis* complex) may also show activity against some other species of mycobacteria. Strains of *Mycobacterium bovis* are naturally resistant to pyrazinamide. There are only limited data on other activities of capreomycin and viomycin.			

TABLE 202-2 -- **Efficacy of antituberculosis agents.**[*]

EFFICACY OF ANTITUBERCULOSIS AGENTS			
Agent	**Early bactericidal activity**	**Sterilizing activity**	**Prevention of emergence of drug resistance**
Rifampin	?	??	??
Pyrazinamide	x	??	x
Isoniazid	??	?	??
Ethambutol	?	x	?
Streptomycin	x	x	?
Thiacetazone	x	x	x
In sterilizing lesions, reducing viable bacterial population rapidly and preventing the emergence of drug resistance. ??, good; ?, fair; x, poor.			

** Data from Mitchison.*

TABLE 202-3 -- Antituberculosis agents: targets and genes for resistance.

ANTITUBERCULOSIS AGENTS: TARGETS AND GENES FOR RESISTANCE		
Agent	**Target**	**Gene(s) encoding target(s) or those in which mutations conferring resistance occur**
Isoniazid	Mycolic acid synthesis	*inhA, katG, KasA, oxyR-ahpC*
Rifampin	DNA-dependent RNA polymerase	*rpoB*
Pyrazinamide	Fatty acid synthetase-1	*pncA*
Ethambutol	Arabinosyl transferase, involved in cell wall arabinogalactan synthesis	*embA, embB and embC*
Streptomycin	30S ribosomal subunit	*rspL* (encodes for ribosomal protein S12)
Other aminoglycosides	30S ribosomal subunit	genes encoding 16S-rRNA (and possibly *aac(2')* encoding aminoglycoside acetyltransferase)
Thiacetazone	Mycolic acid synthesis	Unknown
para-aminosalicylic acid	Mycobactin synthesis (?)	Unknown
Ethionamide and prothionamide	Mycolic acid synthesis	*inhA*
Macrolides	50S ribosomal subunit	Gene encoding peptidyl transferase region in 23S rRNA
Capreomycin and viomycin	50S or 30S ribosomal subunit	*vicA* (50S) or *vicB* (30S)
Clofazimine	Unknown; possibly RNA polymerase	-
Cycloserine	Peptidoglycan	*alrA*
Fluoroquinolones	DNA gyrase (topoisomerase)	*gyrA*

metabolites and routes of excretion are summarized in Table 202.4 and Table 202.5 and are reviewed in depth elsewhere.[4]

DRUG TOXICITY

Although all antituberculosis drugs have some untoward side effects, drug toxicity is, in general, not a serious problem in modern short-course chemotherapy based on the first-line agents and is a small price to pay for the very real curative benefits. The major side effects are hepatotoxicity, peripheral neuropathy, mental disturbances, skin reactions (Fig 202.2 Fig 202.3 Fig 202.4) and fevers. Side effects are particularly likely to occur in HIV-positive patients and, of these, skin reactions due to thiacetazone are particularly serious and may be fatal.

The three principal drugs used in modern short-course regimens — isoniazid, rifampin and pyrazinamide — are all potentially hepatotoxic,

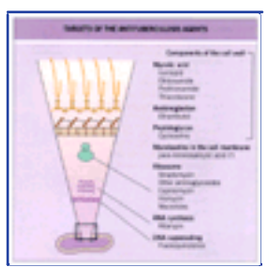

Figure 202-1 Targets of the antituberculosis agents.

but this is seldom a problem in clinical practice. Some physicians, however, take a more cautious view and advocate regular liver function tests during therapy.[5] The adverse effects of the various drugs are discussed under the individual headings below and are summarized in Table 202.6 .

FIRST-LINE DRUGS

Isoniazid (isonicotinic acid hydrazide)

The most widely used of all antituberculosis drugs, this is included in all modern regimens. It is also used as preventive

TABLE 202-4 -- Pharmacokinetics of the antituberculosis agents.

PHARMACOKINETICS OF THE ANTITUBERCULOSIS AGENTS			
Agent	**Binding to serum proteins**	**Absorption from gastrointestinal tract (time to reach peak serum level)**	**Entry into CSF (with healthy meninges)**
Isoniazid	Very low	Very rapid (30–60 minutes)	Good
Rifampin	High (up to 95%)	Rapid (2 hours)	Poor
Pyrazinamide	Very low	Rapid (1–2 hours)	Good
Ethambutol	Binds to erythrocytes	Rapid (2 hours); 80% of dose absorbed	Poor
Streptomycin	Moderate (30–35%)	Not absorbed	Poor
Thiacetazone	Not bound	Rapid (2 hours)	Limited data
para-aminosalicylic acid	High (60–65%)	Very rapid	Poor
Ethionamide and prothionamide	Limited data	Very rapid (30 minutes)	Good
Capreomycin	Limited data	Not absorbed	Poor
Viomycin	Limited data	Not absorbed	Poor
Clofazimine	Limited data	Slow (8–12 hours)	Limited data
Cycloserine	Not bound	Rapid (3 hours)	Good
Ofloxacin	Low	Rapid (1–1.5 hours)	Moderate

monotherapy for infected (tuberculin-positive) persons, particularly in the USA.

It has a powerful bactericidal action against actively replicating tubercle bacilli and thus rapidly reduces infectiousness by reducing the number of viable bacilli in cavities. It has little or no activity against slowly replicating bacilli but is included in the continuation phase of modern short-course therapy to kill any rifampin-resistant mutants that commence replication. It inhibits the synthesis of mycolic acids — long-chain fatty acids that form an important part of the mycobacterial cell wall. Although mycolic acids are common to all mycobacteria, and similar molecules occur in the genera *Nocardia* and *Corynebacterium*, susceptibility to isoniazid is virtually restricted to the *M. tuberculosis* complex, although some strains of *Mycobacterium xenopi* and *Mycobacterium kansasii* are susceptible. Some, but not all, isoniazid-resistant strains of *M. tuberculosis* lack catalase-peroxidase activity because of point mutations in, or deletion of, the *katG* gene that encodes this enzyme. Conversely, many strains from south India have either weak catalase-peroxidase activity or none at all but are fully susceptible to isoniazid. Other mutations associated with isoniazid resistance occur in the *inhA* gene, which encodes for long-chain enoyl-acyl carrier protein reductase required for synthesis of mycolic acids, and the *oxyR-ahpC* genes that encode for antioxidant proteins.[6] Thus, several mutational changes induce isoniazid resistance and the predominant mutation(s) show geographic variations in their distribution.

Isoniazid is readily absorbed from the gastrointestinal tract and is converted to inactive metabolites, principally by acetylation, the rate of which is genetically determined. Thus, patients can be divided into rapid acetylators and slow acetylators, in whom the elimination half-lives of the drug are 0.5–1.5 hours and 2–4 hours respectively. About half of Caucasian and black patients but over 80% of Chinese and Japanese patients are rapid acetylators. If administered regularly, response to therapy is unaffected by acetylator status but drug interactions (see below) are more likely to occur in slow acetylators.

Owing to its widespread use since the 1950s, resistance to isoniazid is common and many strains that are resistant to other antituberculosis drugs, particularly to rifampin, are also resistant to isoniazid.

Adverse events are usually mild and are more likely to occur in slow acetylators. They include several neurologic effects, including

TABLE 202-5 -- Principal metabolic products and excretion of the antituberculosis agents.

PRINCIPAL METABOLIC PRODUCTS AND EXCRETION OF THE ANTITUBERCULOSIS AGENTS		
Agent	**Principal metabolic products**	**Excretion**
Isoniazid	Acetyl derivatives: rate of acetylation is genetically controlled	Unchanged and as acetyl derivatives in urine (ratio depends on rate of acetylation)
Rifampin	Desacetyl derivative	As desacetylrifampin in bile
Pyrazinamide	Pyrazinoic acid	Mostly as pyrazinoic acid in urine
Ethambutol	Oxidation products and aldehydes	Mostly unchanged in urine; about 15% as metabolites
Streptomycin	None	Unchanged in urine
Other aminoglycosides	None	Unchanged in urine
Thiacetazone	Unknown	20% eliminated in urine, fate of remainder unknown
para-aminosalicylic acid	Acetylation products and glycine conjugates	About 80% in the urine, mostly in the acetylated form
Ethionamide and prothionamide	Sulfoxide (biologically active) and methyl derivatives	Less than 1% unchanged in urine
Capreomycin	None	Unchanged in urine
Viomycin	None	Unchanged in urine
Clofazimine	Very small amounts of unidentified metabolites	Unchanged in urine and feces
Cycloserine	Up to 35% converted to unidentified metabolites	Varying amounts unchanged in urine
Ofloxacin	5% metabolized to oxides and dimethyl derivatives	70–95% unchanged in urine, small amounts in bile

Figure 202-2 Severe dermal reaction to isoniazid. *Courtesy of Dr P Mwaba, Zambia.*

insomnia, restlessness, peripheral neuropathy, optic neuritis and various, but usually mild, psychiatric disturbances. More serious, but less common, neurologic effects include severe psychiatric disturbance and encephalopathy. The latter is particularly likely to occur in renal dialysis patients.[7]

Other adverse effects include hepatitis, particularly in patients aged over 35 years, arthralgia, fever and skin rashes. Very rare complications include hyperglycemia and agranulocytosis.

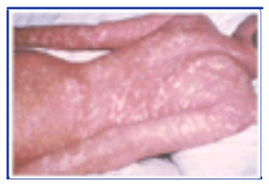

Figure 202-3 Erythema multiforme reaction to rifampin. *Courtesy of Dr P Mwaba, Zambia.*

Adverse effects, particularly neurologic ones, are usually preventable by administration of pyridoxine (vitamin B_6) 10mg daily. In particular, pyridoxine should be given to patients who have liver disease, pregnant women, alcoholics, renal dialysis patients, HIV-positive patients, the malnourished and the elderly. Encephalopathy in renal dialysis patients may not respond to pyridoxine but usually resolves when isoniazid is withdrawn.[7]

Rifampin

This is one of the rifamycins, semisynthetic derivatives of rifamycin S, a fermentation product of *Amycolatopsis (Streptomyces) mediterranei*. Rifampin inhibits protein synthesis by a very specific inhibition of bacterial DNA-dependent RNA polymerase, thereby blocking the synthesis of mRNA. The corresponding mammalian enzyme is

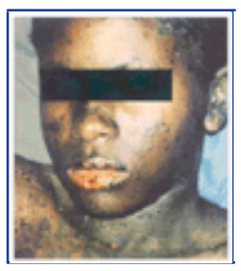

Figure 202-4 Stevens-Johnson syndrome induced by thiacetazone. *Courtesy of Dr P Mwaba, Zambia.*

inhibited only by very high concentrations of rifampin. Resistance is due to single amino acid mutational changes in the *rpoB* gene, which encodes for the ß subunit of the polymerase. Rifampin is the most effective of the antituberculosis drugs because it kills both rapidly dividing bacilli and those that exhibit only occasional short bursts of metabolism. Therefore, when cost considerations allow, it is given throughout the course of therapy. It is also used in the treatment of leprosy and for some other mycobacterial diseases.

Rifampin is rapidly absorbed from the gastrointestinal tract, although absorption is delayed if it is given with food, and it is widely distributed in the internal organs. Only small amounts enter the CSF in health but much more enters when the meninges are inflamed. Rifampin enters cells and is therefore active against intracellular mycobacteria. It is metabolized by hepatic microsomal enzymes to the desacetyl derivative, which is excreted in the bile. As this enzymatic activity is inducible, the rate of plasma clearance of rifampin increases as treatment proceeds. Although principally excreted in the bile, some rifampin and the desacetyl derivative enter the urine and impart an orange-red color to it. It also enters saliva and lachrymal secretions and may cause pink staining of soft contact lenses. The induction of microsomal enzymes may have clinically significant effects on the metabolism of several other drugs (see below).

Rifampin may cause an influenza-like syndrome, which, paradoxically, occurs less often if the drug is given daily rather than intermittently. It causes transient abnormalities in liver function and, occasionally, clinically evident hepatitis, although this is usually mild. Other adverse effects include gastrointestinal disturbances, skin rashes and antibody-mediated thrombocytopenia.

Acute renal failure is a rare complication, although in some regions it is more frequent; in one center in India it accounted for 11 of 607 (1.8%) of admissions for acute renal failure.[8] The renal prognosis is usually favorable. It typically occurs after reintroduction of rifampin and intermittent therapy is a risk factor.

Although the evidence that rifampin is teratogenic is very limited, it is best avoided if possible during the first 3 months of pregnancy. For the same reason, women receiving rifampin should avoid becoming pregnant. In this respect it is important to note that this drug interferes with the action of oral contraceptives.

Pyrazinamide (pyrazinoic acid amide)

This is regularly included in the initial intensive phase of short-course chemotherapy because it has the important property of killing intracellular tubercle bacilli and, possibly, extracellular bacilli in anoxic, acidic inflamed lesions. It is inactive in neutral or alkaline microenvironments. Its target is the fatty acid synthetase (FAS)-1 enzyme.[9] Pyrazinamide first requires conversion to pyrazinoic acid by mycobacterial pyrazinamidase enzymes encoded for by the *pncA* gene. Resistance is usually associated with mutations in this gene, which are detectable by a polymerase chain reaction (PCR)-based system,[10] and the enzymatic activity is not detectable in most pyrazinamide-resistant mutants of *M. tuberculosis* or in strains of *Mycobacterium bovis*, which are naturally resistant to this agent. A few pyrazinamide-resistant strains, however, lack mutations in the *pncA* gene, suggesting alternative mechanisms for resistance to this agent.[11]

It is readily absorbed from the gastrointestinal tract and freely enters the CSF, in which levels similar to those in plasma are found. It is metabolized in the liver; the metabolites, mostly pyrazinoic acid, are excreted in the urine.

Adverse effects are uncommon. It causes raised serum transaminase levels but overt hepatotoxicity, despite earlier reports, is uncommon. It should, however, be used with caution in alcoholics and in patients who have pre-existing hepatic disease, who should have regular liver function tests. Other adverse effects include anorexia, nausea, photosensitization of the skin,[12] arthralgia and gout caused by the inhibition of the excretion of uric acid by pyrazinoic acid.

Ethambutol

Ethambutol (*S*,*S*'-2,2'-(ethylenediimino)di-1-butanol) is now frequently used in short course therapy of tuberculosis as a fourth drug in the intensive phase of therapy. It is also included in therapeutic regimens for disease caused by other slowly growing mycobacteria, particularly *Mycobacterium avium* complex (MAC), *M. kansasii*, *M. xenopi* and *Mycobacterium malmoense*. In addition to its own activity, there is evidence that ethambutol may enhance the activity of some of the other drugs by affecting cell-wall permeability, particularly in the MAC but possibly also in multidrug-resistant strains of *M. tuberculosis*. [13]

Ethambutol inhibits the synthesis of the polysaccharide arabinogalactan, a macromolecule essential for the structural integrity of the mycobacterial cell wall, by inhibiting the enzyme arabinosyl transferase. Resistance is associated with mutations in the *embA*, *embB* and *embC* cluster of genes (principally *embB*), which encode for this enzyme.[14]

The drug is given orally and about 80% of the dose is absorbed from the gastrointestinal tract. Absorption is inhibited by antacids containing aluminum hydroxide. It does not cross the healthy meninges but up to 40% of the plasma level is found in the CSF in cases of tuberculous meningitis. It is mostly excreted unchanged in the urine but up to 15% is excreted as metabolites.

The principal side effect is optic neuritis, which may have an irreversible effect on vision. This complication is rare if the drug is given for no longer than 2 months at a daily dose of 25mg/kg body weight, or for longer at a dose not exceeding 15mg/kg. The 15mg/kg dose is used throughout therapy in some regimens. Nevertheless, care should be observed in the use of this drug, its recommended dose and duration of therapy should never be exceeded, and the patient should be informed of the risk of visual impairment and advised to discontinue the drug if such impairment occurs. Loss of color discrimination is the first sign of visual toxicity. Where facilities are

available, visual acuity should be assessed before therapy and at intervals during it.

Most guidelines recommend that the drug should not given to children under the age of 5 years because their visual acuity cannot be readily assessed, even though ocular complications in such young children are extremely rare.

TABLE 202-6 -- Adverse reactions to the antituberculosis agents.

ADVERSE REACTIONS TO THE ANTITUBERCULOSIS AGENTS	
Agent	**Adverse reactions**
Isoniazid	
Uncommon reactions	Hepatitis, cutaneous hypersensitivity reactions including erythema multiforme, peripheral neuropathy
Rare reactions	Vertigo; convulsions; optic neuritis and atrophy; psychiatric disturbance; hemolytic anemia; aplastic anemia; dermal reactions including pellagra, purpura and lupoid syndrome; gynecomastia, hyperglycemia, arthralgia
Rifampin	
Uncommon reactions	Hepatitis, flushing, itching with or without a rash, gastrointestinal upsets, 'flu-like syndrome', headache
Rare reactions (usually associated with intermittent therapy)	Dyspnea, hypotension with or without shock, Addisonian crisis, hemolytic anemia, acute renal failure, thrombocytopenia with or without purpura, transient leucopenia or eosinophilia, menstrual disturbances, muscular weakness, pseudomembranous colitis
Pyrazinamide	
Common reactions	Anorexia
Uncommon reactions	Hepatitis, nausea and vomiting, urticaria, nausea, arthralgia
Rare reactions	Sideroblastic anemia, photosensitization, gout, dysuria, aggravation of peptic ulcer
Ethambutol	
Uncommon reactions	Optic neuritis, arthralgia
Rare reactions	Hepatitis, cutaneous hypersensitivity including pruritis and urticaria, photosensitive lichenoid eruptions, parasthesia of the extremities, interstitial nephritis
Streptomycin	
Uncommon reactions	Vertigo, ataxia, deafness, tinnitus, cutaneous hypersensitivity
Rare reactions	Renal damage, aplastic anemia, agranulocytosis, peripheral neuropathy, optic neuritis with scotoma, severe bleeding due to antagonism of factor V, neuromuscular blockade in patients receiving muscle relaxants and in those with myesthenia gravis
Other aminoglycosides	
Uncommon reactions	Cutaneous hypersensitivity, vertigo, deafness
Rare reactions	Renal damage, hypoglycemia, hypokalemia
Thiacetazone	
Common reactions	Gastrointestinal upsets, cutaneous hypersensitivity, vertigo, conjunctivitis
Uncommon reactions	Hepatitis, erythema multiforme, exfoliative dermatitis, hemolytic anemia
Rare reactions	Agranulocytosis
***para*-aminosalicylic acid**	
Common reactions	Gastrointestinal upsets
Uncommon reactions	Cutaneous hypersensitivity, hepatitis, hypokalemia
Rare reactions	Acute renal failure, hemolytic anemia, thrombocytopenia, hypothyroidism
Ethionamide and prothionamide	
Common reactions	Gastrointestinal upsets, salivation, metallic taste
Uncommon reactions	Cutaneous hypersensitivity, hepatitis
Rare reactions	Alopecia, convulsions, deafness, diplopia, gynecomastia, hypotension, impotence, psychiatric disturbance, menstrual irregularity, hypoglycemia, peripheral neuropathy
Capreomycin and viomycin	
Common reactions	Eosinophilia (with capreomycin), pain and induration at injection site
Uncommon reactions	Loss of hearing, vertigo, tinnitus, electrolyte disturbances including hypokalemia, leukopenia or leukocytosis
Rare reactions	Renal impairment, hepatitis, thrombocytopenia
Clofazimine	
Common reactions	Discoloration of skin and body fluids, nausea, vomiting, abdominal pain, diarrhea
Uncommon reactions	Dryness of skin, ichthyosis, photosensitivity
Rare reactions	Intestinal obstruction
Cycloserine	
Common reactions (especially with daily doses exceeding 500mg)	Convulsions, drowsiness, sleep disturbance, headache, tremor, vertigo, confusion, irritability, aggression and other personality changes, psychosis (sometimes with suicidal tendencies)
Uncommon reactions	Cutaneous hypersensitivity, hepatitis, megaloblastic anemia
Rare reactions	Congestive heart failure
Ofloxacin	
Uncommon reactions	Gastrointestinal upsets, headache, dizziness, insomnia, cutaneous hypersensitivity reactions
Rare reactions	Restlessness; convulsions; psychiatric disturbances including psychotic reactions and hallucinations; edema of face, tongue and epiglottis; disturbance of taste and smell; anaphylactoid reactions

Other side effects of ethambutol include skin rashes, arthralgia, peripheral neuritis, hyperuricemia and, rarely, jaundice and thrombocytopenia.

Streptomycin

This was the first of the antituberculosis drugs to be discovered and it still has an important role in the treatment of tuberculosis. It inhibits

protein synthesis by binding to the 30S subunit of the bacterial ribosome. It is active in neutral or alkaline environments such as the cavity wall but not in the more acidic environment of the closed, inflammatory foci and is therefore not a good sterilizing drug. It is very poorly absorbed from the gastrointestinal tract and must be given parenterally.

Streptomycin is toxic for the eighth cranial nerve, including that of the fetus, and its use should therefore be avoided in pregnancy. Other adverse reactions include impairment of renal function and hypersensitivity reactions — usually mild skin rashes or fever but occasionally anaphylactic reactions or exfoliative dermatitis.

SECOND-LINE DRUGS

Aminoglycosides

In addition to streptomycin, the aminoglycosides kanamycin, amikacin and aminosidine (paromomycin) have activity against *M. tuberculosis*. Cross-resistance with streptomycin is usual. Kanamycin is included in some regimens for the treatment of MDRTB and amikacin in some regimens for the treatment of disease due to the MAC, particularly in HIV-positive patients. In common with streptomycin, these aminoglycosides are not absorbed from the gastrointestinal tract and must therefore be given parenterally.

Para-aminosalicylic acid

The mode of action of this bacteriostatic drug is not fully understood although there is some evidence that it inhibits the salicylate-dependent synthesis of the mycobactins — a class of iron-chelating lipids unique to the mycobacteria. It is readily absorbed from the intestine and rapidly acetylated in the liver. About 80% is excreted in the urine, mostly in the acetylated form.

It is rarely used as adverse effects are common. Gastrointestinal effects including nausea, abdominal pain and diarrhea occur in up to 30% of patients. Other adverse effects include thyroid dysfunction, crystalluria, blood dyscrasias and, rarely, Löffler syndrome and encephalitis.

Capreomycin and viomycin

These structurally closely related cyclic polypeptides are very rarely used and are seldom available. In common with the aminoglycosides, they inhibit protein synthesis by blocking ribosomal function. They are mutually completely cross-resistant and high-level resistance shows cross-resistance with the aminoglycosides. They are supplied as a water-soluble sulfate and, because they are not absorbed from the intestine, they are given by intramuscular injection. They do not readily enter cells or the CSF and are mostly excreted unchanged in the urine. Adverse effects include ototoxicity, nephrotoxicity and pain, bleeding and induration at the injection site.

Clofazimine

This is one of a group of iminophenazines originally developed for treatment of tuberculosis but now used principally for leprosy. It is occasionally used for treatment of MDRTB but there is only anecdotal evidence of efficacy. It was included in regimens for treatment of AIDS-related MAC infection but is now rarely used for this purpose on account of its toxicity in such patients.

Its mode of action is unclear. Some reports suggest that it potentiates intracellular killing by enhancing the generation of free oxygen radicals, whereas others suggest an interference with RNA polymerase activity. It is absorbed from the intestine; it has a very long half life — around 70 days — and is excreted in the urine and feces. Adverse effects include nausea, abdominal pain and diarrhea and, rarely, edema of the wall of the small intestine leading to subacute obstruction. It also causes skin discoloration, which may be a stigmatizing feature leading to nonadherence to therapy.

Ethionamide and prothionamide

Ethionamide (ethylthioisonicotinamide) and prothionamide (propylthioisonicotinamide) are closely related drugs that are structurally similar to isoniazid; in common with isoniazid they inhibit the synthesis of mycobacterial mycolic acids. Also in common with isoniazid, resistance is associated with mutations in the *inhA* gene encoding for long-chain enoyl-acyl carrier protein reductase but, surprisingly, cross-resistance to isoniazid does not develop. This may, in part, be explained by differences in the activation pathways of the two agents.[15] They are degraded into several metabolites in the liver and only a very small amount, less than 1%, is excreted unchanged in the urine.

The common occurrence of gastrointestinal irritation with these agents, even when they are given as enteric-coated tablets, limits their use in the treatment of tuberculosis. Prothionamide is slightly better tolerated and is used in some regimens for leprosy. Other adverse effects include skin reactions, hepatitis, impotence and gynecomastia in male patients, menstrual irregularities and various neurologic complications such as convulsions, mental disturbance and peripheral neuropathy.

Thiacetazone (acetylaminobenzaldehyde thiosemicarbazone)

In common with isoniazid, thiacetazone inhibits the synthesis of mycolic acid, but by a poorly understood mechanism. Resistance develops readily and is common in developing nations where, on account of its low cost, thiacetazone has been widely used. It is readily absorbed from the gastrointestinal tract. About 20% is eliminated in the urine but it is not known what happens to the remainder.

Adverse effects are common. Skin rashes frequently occur, particularly in patients of Chinese ethnic origin. A very high incidence of severe, sometimes fatal, skin reactions — exfoliative dermatitis and Stevens-Johnson syndrome — in HIV-positive patients has raised serious doubts as to the advisability of using this drug in regions where HIV-related tuberculosis is common. Less frequent adverse effects include gastrointestinal upsets, hepatitis, hemolytic anemia and, rarely, agranulocytosis.

Rifabutin and rifapentine

These are closely related to rifampin, being semisynthetic derivatives of rifamycin S. Although rifabutin (ansamycin) is considerably more active than rifampin in vitro, its in-vivo action against *M. tuberculosis* is similar to that of rifampin. Cross-resistance between the rifamycins is usual, so the place for rifabutin in the treatment of MDRTB is limited. Its principal use is for the prevention and treatment of HIV-related disease due to the MAC. Rifabutin, rifapentine and a new rifamycin derivative, benzoxazinorifamycin (KRM-1648), have long plasma half-lives and raise the possibility, currently under investigation, of once-weekly dosage for preventive therapy and during the continuation phase of therapy of active tuberculosis.[16]

Fluoroquinolones

Fluoroquinolones inhibit the enzyme DNA gyrase, which is responsible for the supercoiling of DNA. They have bactericidal activity against *M. tuberculosis* at clinically achievable levels in vitro, and there is increasing evidence that several of them, including ciprofloxacin, ofloxacin, sparfloxacin, levofloxacin and lomefloxacin, have a valuable place in the therapy of MDRTB.[17] Thus, for example, there is preliminary evidence that a combination of sparfloxacin, kanamycin and ethionamide is both safe and effective in the therapy of MDRTB.[18] [19] Fluoroquinolones are readily absorbed from the gastrointestinal tract and enter tissues and fluids, including the CSF. Although metabolized to some extent by the liver, they are

largely excreted unchanged in the urine. Doses therefore require modification in patients who have renal failure. Adverse effects include nausea and abdominal pain and various neurologic abnormalities including headache, vertigo, insomnia, restlessness, epileptiform attacks and psychiatric disturbances. They should be used with care in epileptic patients.

Cycloserine

This D-alanine analog inhibits synthesis of peptidoglycan. It is bacteriostatic and thus of limited efficacy, although it is used in some cases of MDRTB. Psychiatric symptoms, including psychotic episodes, occur commonly and further limit the usefulness of this drug, although the risk may to some extent be reduced by giving pyridoxine. Allergic skin rashes are rare. In-vitro synergy with ß-chloro-D-alanine, another peptidoglycan inhibitor, has been demonstrated and suggests that therapy with a greatly reduced dose of cycloserine may be possible.[20]

Macrolides

These are broad-spectrum antibiotics that inhibit protein synthesis by binding to the ribosomal 50S subunit. Erythromycin is active against some mycobacteria in vitro and there are anecdotal reports of its efficacy in the treatment of post-BCG (bacillus Calmette-Guérin) abscesses and disease due to *M. kansasii* and *M. xenopi*. The newer macrolides, clarithromycin, azithromycin and roxithromycin, are more active than erythromycin against MAC, and clarithromycin is used to treat HIV-related disease due to MAC. They have limited in-vitro activity against *M. tuberculosis* but there is evidence that they act in synergy with rifampin and isoniazid against this species, particularly against intracellular bacilli.[21] Macrolides are well absorbed from the gastrointestinal tract and are excreted in urine. Adverse effects include gastrointestinal upsets with occasional cases of pseudomembranous colitis and various psychiatric disorders, including acute mania.

EXPERIMENTAL AGENTS

Research into novel therapy for tuberculosis is based on new use of old drugs, new delivery of old drugs, new drugs within old classes and new classes of drugs. A number of new rifamycins, macrolides, pyrazinamide analogues, nitroimidazoles and isonicotinoylhydrazones are being evaluated.[22] [23] [24] Synergy between antituberculosis agents and cell-wall assembly inhibitors is also a promising approach, which may overcome drug resistance and permit lower concentrations of toxic drugs to be used.[20] [25]

Attempts to treat MDRTB by enhancing or modifying immune defense mechanisms have been made, either by the use of individual cytokines or bacterial adjuvants, and more clinical studies are indicated.[23] [26]

Certain agents usually used for other infections may have useful activity against *M. tuberculosis*. For example, a combination of amoxicillin and clavulanic acid has in-vitro activity against *M. tuberculosis* and there is anecdotal evidence of a beneficial effect against MDRTB.[27] It has been suggested that metronidazole might kill dormant *M. tuberculosis* in anaerobic situations. Although one study indicates that addition of metronidazole to short-course regimens hastens the clinical improvement in cases of advanced pulmonary tuberculosis, information from studies on murine models of dormancy is conflicting.[28]

Dynamics of action of antituberculosis drugs in vivo and the design of therapeutic regimens

The aims of modern chemotherapeutic regimens are:

- to cure the patient;
- to reduce infectivity as rapidly as possible; and
- to prevent the emergence of drug resistance.

In order to cure patients, it is necessary to destroy all the tubercle bacilli in the tissues; if even a few survive, there is a high chance of relapse. In this respect, drugs that are bactericidal in vitro may not be able to effectively sterilize the tissues in vivo.[29] This difference occurs because tubercle bacilli in vivo are in a number of different physiologic states or 'compartments'. These physiologic 'compartments' are:

- freely dividing extracellular bacilli, found mainly in the cavity walls;
- slowly dividing bacilli, found within macrophages and in acidic, inflammatory lesions; and
- dormant and near-dormant bacilli, within cells and in firm caseous material.

The antituberculosis drugs vary in their ability to destroy bacilli in these compartments and in preventing the emergence of resistance to a second drug.

During chemotherapy with modern short-course regimens, the freely replicating bacilli in the walls of the cavities are rapidly killed; this is termed the early bactericidal effect. Subsequently, the slowly replicating and near-dormant bacilli are destroyed, but at a much slower rate.

Isoniazid plays a key role in achieving the early bactericidal effect because it is particularly effective in destroying the freely multiplying extracellular bacilli, particularly those in the walls of cavities. It has little or no effect on near-dormant bacilli and is therefore not a good sterilizing drug. Rifampin also contributes to the early bactericidal effect.

Ethambutol has bactericidal activity in the early stage of therapy but is not a sterilizing drug. Streptomycin is bactericidal in the slightly alkaline cavity walls but is likewise not a sterilizing agent because it is ineffective in the acidic environment within cells and caseous lesions. By contrast, pyrazinamide is effective within macrophages and acidic, anoxic inflammatory lesions, but not in the neutral or alkaline environment.

Thus, modern regimens commence with an intensive phase of therapy, usually lasting for 2 months, to optimize the early bactericidal effect, thereby eliminating most of the bacilli and rendering the patient noninfectious. The principal drugs used are:

- isoniazid (active against bacilli in the cavity walls);
- pyrazinamide (active against bacilli in acidic closed lesions); and
- rifampin (active against both).

In view of the widespread and increasing prevalence of drug resistance, many regimens now include a fourth agent, usually ethambutol but sometimes streptomycin. The daily drug doses are listed in Table 202.7 and the intermittent doses in Table 202.8 .

The intensive phase is followed by a continuation phase, usually lasting 4 months, in which any remaining dormant or near-dormant bacilli are destroyed. For this purpose, rifampin is the most powerful sterilizing drug. Although isoniazid is not a sterilizing drug, it is, by its potent activity against replicating bacilli, very good at preventing the emergence of rifampin-resistant mutants. It is thus given together with rifampin throughout the regimen.

Accordingly, the most effective modern short-course regimens are based on a 2-month phase of rifampin, isoniazid, pyrazinamide and either ethambutol or streptomycin, followed by a 4-month phase of rifampin and isoniazid. The WHO has issued clear recommendations on drug regimens for the four categories of tuberculosis seen in clinical practice (Table 202.9).

In most regimens drugs are given daily but, provided that therapy is closely supervised so that all doses are taken, they may be given three times weekly during the continuation phase or, in some regimens, throughout. Such intermittent therapy renders the direct administration of the drugs less of a burden for both patients and supervisors.

There is general agreement, supported by clinical trials, that the modern chemotherapeutic regimens discussed above are suitable for the treatment of all types of tuberculosis, both pulmonary and non-pulmonary,

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TABLE 202-7 -- Daily doses of the antituberculosis agents.

DAILY DOSES OF THE ANTITUBERCULOSIS AGENTS		
	Daily dose	
Agent	**Adults**	**Children**
Rifampin	450mg if body weight <50kg	10mg/kg to maximum of 600mg
	600mg if body weight =50kg	
Isoniazid	200–300mg	5mg/kg
Pyrazinamide	1.5g if body weight <50kg	25mg/kg
	2.0g if body weight =50kg	
Ethambutol	15mg/kg	
Streptomycin	750mg if body weight <50kg	As for adult
	1g if body weight =50kg	15mg/kg to maximum of 0.75g
	750mg if age =40 years	
	500mg if age =60 years	
Thiacetazone	150mg	50mg
para-aminosalicylic acid	10–12g	300mg/kg
Ethionamide and prothionamide	500mg if body weight <50kg	15–20mg/kg
	750mg if body weight =50kg	
Capreomycin	1g	Avoid
Viomycin	1g	Avoid
Cycloserine	500mg if body weight <50kg	Avoid
	750mg if body weight =50kg	
Ofloxacin	800mg	Avoid

TABLE 202-8 -- Doses of the first-line antituberculosis agents in three times weekly intermittent therapy.

DOSES OF THE FIRST-LINE ANTITUBERCULOSIS AGENTS IN THREE TIMES WEEKLY INTERMITTENT THERAPY		
Agent	**Dose (mg/kg (adults and children))**	**Maximum dose**
Isoniazid	15	750mg
Rifampin	15	600mg
Pyrazinamide	50	2.0g if body weight <50kg
		2.5g if body weight =50kg
Ethambutol	30	1.8g
Streptomycin	15–20	750mg if body weight <50kg
		1g if body weight =50kg

TABLE 202-9 -- Principal antituberculosis regimens recommended by the World Health Organization.

PRINCIPAL ANTITUBERCULOSIS REGIMENS RECOMMENDED BY WHO		
Intensive phase	**Continuation phase**	**Category of patient**
HRZE (HRZS) for 2 months	HR daily for 4 months	New patients with smear-positive pulmonary tuberculosis; extensive smear-negative pulmonary tuberculosis; severe nonpulmonary tuberculosis
HRZE (HRZS) for 2 months	HR three times weekly for 4 months	
HRZE (HRZS) for 2 months	HE daily for 6 months	
HRZES for 2 months + HRZE for 1 month	HRE daily for 5 months	Cases of relapse, treatment failure or recommencing treatment after interruption
HRZES for 2 months + HRZE for 1 month	HRE three times weekly for 5 months	
HRZ for 2 months	HR daily for 4 months	New smear-negative pulmonary tuberculosis (other than those in the first category); less severe nonpulmonary tuberculosis
HRZ for 2 months	HR three times weekly for 4 months	
HRZ for 2 months	HE daily for 6 months	
Treat as though drug-resistant		Case still positive after supervised retreatment
E, ethambutol; H, isoniazid; R, rifampin; S, streptomycin; Z, pyrazinamide.		

and even for life-threatening forms such as tuberculous meningitis. There is less agreement over the duration of therapy for non-pulmonary forms of tuberculosis; some physicians continue therapy for up to 12 months or even longer, particularly in the case of tuberculous meningitis, in which a relapse would be particularly devastating.

DRUG-RESISTANT TUBERCULOSIS

Resistance to any given anti-infective agent occurs by mutation at a low but constant rate, so that treatment with a single drug, however powerful, will inevitably lead to selection of resistant mutants.[5] The problem of treatment failure caused by the emergence of drug resistance became apparent soon after the introduction of antituberculosis chemotherapy and led to the universal advocacy of multiple-drug

regimens. Under ideal conditions and in the absence of drug resistance, relapses after completion of a modern short-course chemotherapeutic regimen are uncommon and are mostly due to drug-susceptible bacilli.[24] Unfortunately, ideal treatment conditions are the exception rather than the rule and many deficiencies in the use of multiple-drug regimens has led to the increasing emergence of strains resistant to one or more drugs.[30]

Although resistance to isoniazid or streptomycin, or to both, is common, patients whose disease is caused by such resistant strains usually respond to short-course chemotherapy. Resistance to rifampin is much more serious, in view of the unique ability of this drug to eliminate near-dormant persisting bacilli. Many strains resistant to rifampin are also resistant to isoniazid and, because these drugs are the principal components of modern regimens, this combination renders such regimens ineffective. Thus, the term multidrug resistance has been adopted by the WHO to refer to strains that are resistant to these two drugs, with or without resistance to

additional drugs.[31]

Two forms of drug resistance are encountered: acquired and primary (or initial). Acquired resistance is the result of suboptimal therapy that encourages the selective growth of mutants resistant to one or more drugs. Primary resistance is due to infection from a source case who has drug-resistant disease. In practice, it is often difficult to be sure that a patient who has apparent primary resistant tuberculosis has, in fact, not received any antituberculosis therapy, and some workers therefore prefer the term initial resistance. The division of resistance into acquired and primary forms is of epidemiologic value because an increasing incidence of the former indicates that drug regimens or the supervision of therapy are suboptimal, whereas the continuing occurrence of primary resistance indicates that the transmission of the disease in the community is not being adequately controlled.

The development of drug resistance is due to many avoidable failures in the management of the disease (Table 202.10).

The traditional explanation for treatment failure and emergence of drug resistance is noncompliance or nonadherence to therapy by the patient, thereby attempting to exonerate the health care services. While acknowledging that there will always be a small number of patients who will default on treatment in any situation, there is ample evidence that it is more often the health services than the patient that are at fault. In order to enhance effective tuberculosis control, the WHO has widely advocated the strategy of directly

TABLE 202-10 -- Factors leading to suboptimal therapy and the emergence of drug and multidrug resistance.

FACTORS LEADING TO SUBOPTIMAL THERAPY AND THE EMERGENCE OF DRUG AND MULTIDRUG RESISTANCE
Intermittent drug supplies
Use of time-expired drugs
Unavailability of combination preparations
Use of poorly formulated combination preparations
Prescription of inappropriate drug regimens
Unregulated over-the-counter sale of drugs, including cough mixtures containing isoniazid
Addition of single drugs to failing regimens in the absence of bacteriologic control
Poor supervision of therapy
Unacceptably high cost to patient in respect of the drugs, travel to the clinic and time off work

observed therapy, short course (DOTS). This is a six-point strategy incorporating:

- government commitment to tuberculosis control;
- provision of a regular supply of good-quality drugs free at the point of delivery;
- passive case finding by sputum microscopy;
- directly observed therapy;
- training and ongoing support of staff; and
- regular evaluation of the efficacy of the control program.

Combination drug preparations have been used to prevent patients from receiving monotherapy, but irregular and intermittent use of such preparations has led to drug and multidrug resistance.[30] In addition, the use of poorly formulated combination preparations has led to reduced bioavailability of the constituent agents and the risk of development of drug resistance.

There is no doubt that the blind addition of drugs to a failing regimen is very likely to generate multidrug resistance. Unfortunately, the determination of drug resistance is far from easy and most parts of the world lack the facilities for conducting drug susceptibility tests under good quality control. Errors are not uncommon even in the most sophisticated centers in the developed world and may be noticed only when laboratory reports are considered in the light of clinical data.

A combined initiative by the WHO and the International Union Against Tuberculosis and Lung Disease was launched in 1994 to perform a global survey of resistance to first-line antituberculosis drugs. The first report, published in 1997 and covering 35 countries, showed that resistance to these drugs was more widespread than was previously recognized and revealed certain areas with a very high incidence (Fig. 202.5).[32] These 'hotspots' included Estonia, Latvia, Russia, Argentina, China, the Dominican Republic and Côte d'Ivoire. The second report, published in 2000 and covering 72 countries, confirmed the widespread occurrence of MDRTB.[33] While, overall, the median prevalence of MDRTB in new cases was low, only 1% of all cases, a very high prevalence was found in the Henan province of China (35%), Ivanovo Oblast (Russian Federation; 32.4%), and Latvia (29.9%). Estonia showed 8.5% resistance to all four antituberculosis frontline drugs. On the other hand, no MDRTB was detected among new cases in Cuba, Finland, France and New Caledonia.

The situation in Russia is of particular concern.[34] Under the communist regime, tuberculosis in that country had been in decline for several decades, reaching an annual incidence of 34 cases per 100,000 population in 1991. Since the end of communism in 1991, however, the incidence of tuberculosis rose steadily to 85/100,000 by 1998. The cause of this upsurge appears to be a combination of poverty, malnutrition, poor housing, conflict and a fragmentation of health services. In addition, tuberculosis has flourished in overcrowded Russian prisons where there may be as many as 1 million prisoners, of whom 100,000 have active tuberculosis. The incidence of MDRTB is high; in one surveyed prison population it occurred in 24.6% of tuberculosis patients, rising to 92% among nonresponding cases. Despite implementation of a strict DOTS program in the prisons, and the use of WHO retreatment regimens for all new cases, there was a treatment failure rate of 35% in a tuberculosis referral prison in western Siberia.

The presence of HIV infection does not *per se* lead to an upsurge of MDRTB. Despite the increase in HIV-associated tuberculosis seen in most African countries, such an upsurge has not been documented. Exceptions include Mozambique and Côte d'Ivoire, where more than 3% of new cases are multidrug-resistant and in Yaonde, Cameroon, where the corresponding figure rises to 27.6%.

The problem of drug resistance is not encountered only in the developing nations. Several well documented epidemics of MDRTB have occurred in the USA, notably in New York City.[35] As mentioned above,

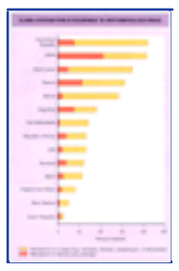

Figure 202-5 Global distribution of resistance to antituberculosis drugs. The resistance is given as a percentage of all isolates. *Data from the World Health Organization.*[32]

HIV infection *per se* does not generate multidrug resistance and although such an association has been found in New York and some other regions it has not been found in others. The reason for the association, where it occurs, is that HIV infection facilitates outbreaks of tuberculosis in hospitals, prisons or common lodging facilities where such immunocompromised persons are crowded together. Thus, if the source case has MDRTB, a mini-epidemic of such disease will ensue. The first such outbreak in the UK occurred in 1995; four patients who presented with tuberculosis in mid-June had been exposed to the source case in April in a six-bed ward in an HIV unit in London.[36]

Ethnic minority communities, originating in countries where drug resistance is common, often have higher levels of drug resistance than the majority populations.[37]

The incidence of single-drug-resistant tuberculosis in a community can be reduced by establishment of good disease control programs.[38] This raises the question of whether the implementation of the WHO DOTS strategy would also reduce the incidence of MDRTB in a region. According to one mathematical model,[39] it would have

such an effect, but the model assumes that multidrug-resistant strains of *M. tuberculosis* are less virulent than their drug-susceptible counterparts, an assumption for which there is no clear

TABLE 202-11 -- Principles for management of multidrug-resistant tuberculosis.

PRINCIPLES FOR MANAGEMENT OF MULTIDRUG-RESISTANT TUBERCULOSIS
Single agents should never be blindly added to failing regimens
Before drug susceptibility tests become available, patients should be started on three agents that they have never received before
All therapy should be fully supervised (use of an injectable agent enhances adherence to therapy)
Therapy should last at least 24 months and should be continued for at least 18 months after bacteriologic conversion
Drug susceptibility tests should be repeated if cultures remain positive after 3 months of therapy

supporting evidence. Thus, further field studies are required to answer this important question.

Therapy of multidrug-resistant tuberculosis

Tuberculosis due to bacilli resistant to isoniazid alone usually responds to short-course drug regimens based on four drugs during the intensive phase but, by contrast, resistance to both isoniazid and rifampin (i.e. multidrug resistance) requires prolonged treatment with drugs that are much more costly, less effective and more toxic. The cost of such therapy is high; in the USA it can exceed $US250,000, compared with the cost of $US2000 for treating a patient who has drug-susceptible disease. The prognosis for patients who have MDRTB has improved considerably and, provided that the patient is diagnosed before severe lung damage has occurred and that the best supervised therapy and laboratory support is available, the outlook is good in the majority of cases. Under these optimal conditions, cure rates of 96% have been achieved.[40]

The successful management of MDRTB requires laboratory support and a team of dedicated supervisors of therapy, the so-called 'DOTS-plus' strategy. [41] Ideally regimens should be designed for each patient on the basis of in-vitro susceptibility. Various regimens have been used and there have been few comparisons between them. Currently used regimens are usually based on a fluoroquinolone with at least two other drugs to which the strain is susceptible such as kanamycin and ethionamide (or prothionamide).[18] Other agents include rifabutin, the new macrolides, amikacin, capreomycin, clofazimine, cycloserine and *para*-aminosalicylic acid. Great care and dedication is required for the successful management of MDRTB (Table 202.11).

In many parts of the world, neither these drugs nor the requisite laboratory support are available, and MDRTB is thus often fatal. Alternative forms of treatment such as immunotherapy are therefore urgently required.

TREATMENT OF PATIENTS IN SPECIAL CIRCUMSTANCES

Patients who have renal or hepatic disease

Modification of drug regimens and dosages may be required when there is substantial liver disease or renal impairment. The first-line drugs (rifampin, isoniazid, pyrazinamide, ethionamide) and also ethionamide and prothionamide are either completely metabolized or eliminated in the bile. They may therefore be used safely at the normal doses in patients who have renal impairment. Isoniazid occasionally causes encephalopathy in patients who have renal failure and in those on dialysis but the risk is reduced, although not eliminated, by administering pyridoxine.[7] Although ethambutol is mainly

eliminated by the kidney it can be used in reduced doses in patients who have impaired renal function. Streptomycin and other aminoglycosides are eliminated entirely by the kidney and are potentially nephrotoxic and special care must be taken.

In severe renal failure the dose of isoniazid should be reduced to 200mg once daily (ensuring that pyridoxine is given to prevent peripheral neuropathy). Streptomycin and ethambutol are excreted by the kidney and adjustment to doses is necessary in renal failure. Streptomycin levels must be monitored and doses and spacing be adjusted to achieve a level of 4mg/ml to avoid toxicity. For patients on dialysis, streptomycin should be given 8 hours before commencing dialysis. Ethambutol dosages are dependent on creatinine clearances. For patients who have creatinine clearances between 50ml/min and 100ml/min, the dose is 25mg/kg three times weekly; at 30–50ml/min, the dose is 25mg/kg twice a week; and at 10–25ml/min the dose is 15mg/kg at 2-day intervals. Patients on hemodialysis may be given 25mg/kg ethambutol 6 hours before the procedure.

There is no clear evidence that the potentially hepatotoxic drugs rifampin and pyrazinamide are any more toxic in patients who have impaired hepatic function. Nevertheless, if they are used, hepatic function should be carefully and regularly monitored during therapy. Some physicians avoid them and treat such patients with isoniazid and ethambutol for 1 year, with the addition of streptomycin for the first 2–3 months. An alternative is to use a fluoroquinolone such as ofloxacin instead of rifampin.[42]

If rifampin is used, it should be used with caution; doses should be reduced in patients who have bilirubin concentrations exceeding 50mmol/l. Liver function should be regularly monitored, where possible, in alcoholics, the elderly, malnourished children and children under 2 years of age.

If jaundice develops during antituberculosis therapy, treatment should bm stopped until the jaundice resolves. In many cases resumption of treatment does not cause a recurrence of the jaundice. If the patient is seriously ill with tuberculosis, he or she may be treated with streptomycin and ethambutol even in the presence of jaundice.

HIV-positive patients who have tuberculosis

The treatment of tuberculosis in HIV-positive patients (see also Chapter 129) follows the same well-established principles used in the treatment of non-HIV infected patients. [43] Despite a good bacteriologic response to treatment, patients who have HIV-related tuberculosis in Africa are almost four times as likely to die within 13 months of diagnosis than HIV-negative patients, with most deaths occurring during the first month of treatment.[44] This is largely due to other opportunistic infections but may also, in part, be due to an apparent synergistic immunosuppressive action of HIV infection and active tuberculosis. For this reason, prevention of tuberculosis is preferable to cure in HIV-positive persons (see below). Drug reactions tend to be more severe in HIV-positive patients than in HIV-negative patients. In particular, thiacetazone often causes severe dermal reactions, with some patients developing fulminant and potentially fatal exfoliative dermatitis. Drug interactions are also a particular problem in HIV-infected patients (see Chapter 129).

Pregnancy and the postpartum period

There is general agreement that the management of tuberculosis in pregnancy and in the postpartum period should be similar to that in other patients, although some advocate avoiding pyrazinamide. Short-course regimens seem to have a minimal risk of causing fetal abnormalities, and side effects in the pregnant woman are no higher than in those who are not pregnant.[45] Opinions concerning the safety of pyrazinamide differ because there are limited experimental data on its effect on the fetus but, notwithstanding this, it is often used, particularly in regions where drug resistance is common. Streptomycin is avoided owing to its ototoxic properties. The treatment of drug-resistant tuberculosis, especially MDRTB, during pregnancy and the management of the neonate requires careful consideration, and experience is very limited.[46] Expert clinical and laboratory guidance, if available, is required.

An increased incidence of isoniazid-related epileptiform attacks and other neurologic symptoms has been reported in pregnant women but these are preventable by the prescription of pyridoxine 10mg daily. Mothers taking antituberculosis drugs at the time of birth can care for their infants with little risk, unless the mother's disease is drug-resistant or not responding to therapy. Likewise, although some of the drugs enter the milk in small concentrations, breast-feeding has no adverse effects on the infant.[47]

Adjunct corticosteroid therapy in tuberculosis

It has been postulated that corticosteroids, by reducing the host's immune response, would allow dormant bacilli to replicate freely and thereby facilitate killing by the drugs. There is little evidence to support this, and their use has not been shown to affect the outcome of modern short-course chemotherapy.[48] On the other hand, in

some forms of tuberculosis corticosteroids aid recovery and reduce long-term sequelae by suppressing inflammatory reactions and limiting subsequent scar formation. For details see Chapter 37 and Chapter 40.d and a review by Alzeer and FitzGerald.[49]

CHEMOPROPHYLAXIS AND PREVENTIVE THERAPY OF TUBERCULOSIS

Chemoprophylaxis is defined as the prescription of antituberculosis drugs for uninfected persons who are exposed to a risk of infection; preventive therapy refers to the treatment of persons who have already been infected with tubercle bacilli (as indicated by tuberculin testing) but who show no clinical or radiologic evidence of active disease. Chemoprophylaxis is principally used to protect children who are at risk of infections, particularly those under the age of 3 years, who are prone to develop serious extrapulmonary forms of tuberculosis, including meningitis.

Preventive drug therapy for those infected by *M. tuberculosis* (i.e. tuberculin reactors) is used in some countries, notably the USA, where tuberculosis is uncommon and where BCG is not used. Although highly efficacious, it is not without its problems. Isoniazid monotherapy, for up to 1 year, is the most widely used form of preventive therapy, although 6-month regimens are becoming more common. Although there is a theoretic risk of generating isoniazid resistance, this does not seem to happen in practice, probably because there are so few bacilli present. Hepatic toxicity has given cause for concern, particularly in older adults, and so such preventive therapy is not recommended for those aged over 35 years.[50]

Policies for the use of chemoprophylaxis vary from country to country. National guidelines should be consulted for indications for chemoprophylaxis and for the recommended drug regimens. In general, chemoprophylaxis in tuberculosis control in regions with a high incidence of tuberculosis has, in view of problems of compliance and organization, not played a major role in tuberculosis control programs.

Transplant recipients receiving corticosteroids and other immunosuppressive drugs are at risk of developing tuberculosis. It has been suggested that such patients should be given isoniazid 300mg, and pyridoxine 25–50mg daily if they have one or more of the following:[51]

- a history of inadequately treated tuberculosis;
- an abnormal chest radiograph;
- a positive tuberculin test of more than 10mm in diameter; and
- recent contact with a case of active tuberculosis.

Prophylaxis of tuberculosis in HIV-infected patients

In view of the very high risk of a co-infected person developing active tuberculosis, and the adverse effect of this disease on the immune status and survival of the patient, there is a very good theoretical case for provision of prophylactic treatment for those at risk. Several placebo-controlled studies of isoniazid monotherapy in patients co-infected with *M. tuberculosis* and HIV have shown that such chemoprophylaxis is effective.[52] In practice, serious problems have been encountered in diagnosing dual infection, ruling out active tuberculosis and ensuring compliance with therapy without breach of confidence or enhancement of stigma. Studies of varying design from Haiti, Zambia and Uganda have shown that chemoprophylaxis in HIV-infected adults significantly reduced the incidence of tuberculosis. The questions of how long the protection lasts, whether prophylactic treatment is safe and whether such therapy can lead to the emergence of drug-resistant strains of tuberculosis require attention.

Although prophylaxis leads to a reduction of the risk of tuberculosis by around 60% in tuberculin-skin-test-positive adults who have HIV infection, identifying HIV-infected individuals is difficult in resource-poor settings. A major problem is ensuring compliance with therapy.[53] The development of voluntary counseling and testing centres was seen as an effective tool to promote safer sex and to offer those who have HIV infection interventions such as preventive therapy for tuberculosis.

It is very important to ensure that HIV-positive persons receiving chemoprophylaxis do not have active tuberculosis or there is a strong risk of masking the disease and encouraging the emergence of drug resistance. It is also necessary to supervise the therapy, and this adds another burden to stretched tuberculosis control services.

Initially, 12-month courses of isoniazid monotherapy were evaluated but, subsequently, shorter combination regimens were also shown to be effective. These include a 3-month course of a rifamycin (rifampin or rifabutin) plus isoniazid, and a 2-month course of a rifamycin plus pyrazinamide, but the only clear advantage over isoniazid monotherapy is the shorter duration of treatment. A study in Zambia revealed that the 2-month combination regimens or 6 months of isoniazid, administered twice weekly, reduced the incidence of tuberculosis by about 40% compared with a placebo group, although the overall mortality due to all causes was not reduced. [54]

The relatively short-term benefit of preventive therapy is a further problem. By 18 months, the incidence of tuberculosis in those who receive prophylactic therapy is similar to that in those not receiving such therapy, indicating the need to consider repeated courses or, perhaps, lifelong prophylactic treatment.[55] As a general rule, prevention is more effective in those who have relatively limited immunosuppression (positive tuberculin tests, high lymphocyte counts and high hemoglobin levels). This, together with the difficulty in detecting co-infection, has led to the current recommendation to restrict preventive therapy to tuberculin positive, HIV-positive persons.

DRUG INTERACTIONS

Clinically significant interactions between the first-line antituberculosis drugs themselves are uncommon but such reactions could well occur when more complex regimens are used to treat MDRTB. Antituberculosis drugs may interact with drugs used to treat unrelated conditions (Table 202.12). Rifampin is the most important in this respect because it is a potent inducer of cytochrome isoenzymes involved in the metabolism of many drugs. The increased metabolism and clearance of these drugs may lead to therapeutic failure unless levels are adjusted and then readjusted when rifampin therapy ceases. Patients on oral contraceptives should be advised to use alternative forms of birth control.

TABLE 202-12 -- Clinically significant drug interactions with antituberculosis agents.

CLINICALLY SIGNIFICANT DRUG INTERACTIONS WITH ANTITUBERCULOSIS AGENTS	
Effects opposed by rifampin	
Antiretroviral agents	Opioids
Azathioprine	Oral contraceptives
Corticosteroids	Phenytoin
Cyclosporin	Propranolol
Diazepam	Quinidine
Digoxin	Theophylline
Haloperidol	Tolbutamide
Imidazoles	Warfarin
Potentiates the effects of rifampin	
Trimethoprim-sulfamethoxazole	
Effects potentiated by isoniazid	
Phenytoin	
Carbamezapine	
Potentiates the effects of isoniazid	
Insulin	

Effects opposed by isoniazid
Enflurane
Opposes the effects of isoniazid
Prednisone
Antacids (inhibit absorbtion)
Effects potentiated by streptomycin
Neuromuscular blocking agents
Effects potentiated by quinolones
Aminophylline and theophylline
Potentiates the effects of quinolones
Cimetidine
Opposes the effects of quinolones
Antacids, iron preparations, sucralfate, didanosine (all inhibit absorbtion)

Rifampin reduces the plasma concentrations and half-lives of the imidazole and triazole antifungals and these agents reduce plasma levels of rifampin. Because some patients, notably those who are HIV-positive, may also require antifungal therapy, these interactions, which may lead to treatment failure, are of increasing importance.[56] Patients who are HIV-positive may also be receiving trimethoprim-sulfamethoxazole for prevention or treatment of *Pneumocystis carinii* infection. This agent significantly increases the serum levels and half-life of rifampin, leading to an increased incidence of adverse effects, including hepatotoxicity.[57]

Drug interactions with isoniazid are more pronounced in slow acetylators. The effects of isoniazid are potentiated by insulin and opposed by prednisone (prednisolone); its absorption from the intestine, and that of ethambutol and the quinolones, is inhibited by antacids containing aluminum hydroxide. The effects of carbamazepine and phenytoin are potentiated by isoniazid and those of enflurane are opposed.

A limited number of drug interactions with other antituberculosis agents have been described and reviewed.[58]

Drug interactions with antiretroviral agents

Protease inhibitors such as saquinavir, ritonavir, indinavir and nelfinavir all interact with rifampin.[58] [59] Rifampin accelerates the metabolism of protease inhibitors (through induction of hepatic P450 cytochrome), resulting in subtherapeutic levels of the protease inhibitors and thereby increasing the risk of the development of viral resistance. In addition, protease inhibitors retard the metabolism of rifampin, resulting in increased serum levels and the likelihood of increased drug toxicity (see Chapter 129).

When prescribing antiretrovirals with antituberculosis drugs, it is important to refer to the latest guidelines on the subject since these are updated frequently. As new antiretroviral agents are being discovered and used, new interactions with antituberculosis drugs are being discovered. Updates on these periodically appear on the US Centers for Disease Control and Prevention website — http://www.cdc.gov/epo/mmwr/mmwr_rr.html.

Two pharmacokinetic issues complicate treatment: the possibility of malabsorption of drugs and the complex drug-drug interactions between antiretroviral and antituberculosis drugs described above. While HIV-infected patients who have tuberculosis commonly experience adverse drug interactions, current recommendations are that highly active antiretroviral therapy (HAART) is commenced early in patients who have advanced HIV disease ($CD4^+$ counts <100 cells/mm^3). [60] In clinically stable patients who have $CD4^+$ cells in excess of 100×10^6 cells/mm^3 /l, HAART should be deferred until the continuation phase of tuberculosis treatment (i.e. after 2 months of antituberculosis therapy). The current recommendation in this case is to replace rifampin by rifabutin, a much less powerful inducer of cytochrome enzymes, and to commence or continue with the antiretroviral drugs.[61]

DRUG SUSCEPTIBILITY TESTING

The purpose of drug susceptibility testing is not to detect small numbers of drug-resistant mutants, which will inevitably be present in every patient who has tuberculosis and in every culture, but rather to determine whether the great majority of the bacilli are susceptible to levels of the drugs that are achieved clinically. In the developed nations with the requisite facilities, and particularly where MDRTB is common, susceptibility testing of all clinical isolates is definitely indicated.[62] In most developing countries, facilities for conducting drug susceptibility tests are very limited.

TABLE 202-13 -- Techniques used to determine susceptibility to antituberculosis agents in vitro.

TECHNIQUES USED TO DETERMINE SUSCEPTIBILITY TO ANTITUBERCULOSIS AGENTS IN VITRO		
Technique	**Where technique is used**	**Description of technique**
Proportion method	USA and some European countries	Drug-free and drug-containing media are inoculated with test strains and the colony counts are compared; strains are reported as resistant if the colony count on the drug-containing medium is over 1% of that on the drug-free medium
Absolute concentration method	Some parts of Europe	Based on growth on media containing doubling dilutions of a known concentration of drug, so that the minimal bactericidal concentrations of drugs may be determined
Resistance ratio method	UK and those countries influenced by British bacteriologists	Similar to the absolute concentration method except that results are expressed as the ratio of the drug concentration inhibiting the test and drug susceptible control strains, rather than as the actual inhibiting concentration
Disk diffusion method	Rarely used	Similar to absolute concentration method and resistance ratio method but technically simpler, as disks containing the drugs are placed on the solid media, thereby avoiding the need to prepare batches of media containing the various drugs

The epidemiologic importance of susceptibility testing has recently been emphasized by the need to monitor the global incidence and distribution of acquired and primary drug resistance. Unfortunately, susceptibility testing is expensive and time-consuming and requires good laboratory facilities, a high level of technical expertise and rigid quality-control procedures. There is no point in doing such testing unless high standards of accuracy can be maintained because much harm may be done by modifying regimens to include less effective and more toxic drugs on the basis of false reports of resistance.

Global surveys on drug resistance have been compromised by the variety of methods used for surveillance and for drug susceptibility testing and the lack of standardization of the methods. The WHO has therefore prepared guidelines for standardized surveillance techniques and has established a network of supranational reference laboratories to co-ordinate surveillance and to provide technical guidance and assistance.[32] [63]

Methods of susceptibility testing

Methods for drug susceptibility testing can be divided into:

- those that are based on inhibition of bacterial growth on drug-containing standard media;
- those that detect growth inhibition by automated radiometric and related systems;
- those that use biologic indicators of bacterial viability, such as enzyme activity and bacteriophage replication; and
- those that use nucleic-acid-based technology to detect mutations in genes determining susceptibility to drugs.

Conventional techniques

Methods for drug susceptibility testing based on growth on conventional media are well established but have the great disadvantage that there is a long delay before results are available. Four methods are currently in use (Table 202.13).

In an (unpublished) investigation carried out by members of the European Society of Mycobacteriologists, there were only minor discrepancies between results obtained by different workers using the first three of the methods listed in Table 202.13 . All these methods may be used either for direct susceptibility tests on smear-positive sputum or for indirect susceptibility tests on cultures. The relative merits and usefulness of these methods in differing circumstances are reviewed in several places.[64] [65]

Conventional tests for susceptibility to pyrazinamide pose particular problems because the drug acts only in acidic environments in which bacterial growth is poor. Thus, the tests require careful standardization and interpretation.

Rapid techniques

The radiometric technique has been widely used in the industrially developed countries.[66] It is more costly than the conventional methods but the rapidity of the results justifies the extra cost, especially where multidrug resistance is common. Susceptibility to all antituberculosis drugs, including pyrazinamide, can be determined by this method. Automated, nonradiometric systems for performing rapid drug susceptibility tests are increasingly used.[67] The latter systems are based on the unquenching of a fluorescent dye when oxygen is consumed by mycobacterial metabolism or on color changes in dyes when carbon dioxide is liberated from nutrients in the medium.

Several rapid methods for the detection of mutations in the *rpoB* gene conferring resistance to rifampin have been described; one of these, based on a number of DNA probes for wild-type and mutated regions of the gene (line hybridization assay), is available in a commercially available kit form. The sites of mutations responsible for resistance to some other antituberculosis drugs, including streptomycin, pyrazinamide, isoniazid and ethionamide, are also known, and so commercially available kits for rapid detection of resistance to these and other drugs may soon be available. [68]

Bacteriophages have been used to detect bacterial viability in the presence of antituberculosis agents.[69] Enzyme activity has been used for the same purpose and one rapid and inexpensive method is based on detection of nitrate reductase activity.[70]

CONCLUSIONS

Chemotherapy is the mainstay of tuberculosis control and modern short-course regimens are among the most effective and cost-effective of all therapeutic interventions for any human disease. Sadly, this potent intervention has been so badly used that tuberculosis remains the leading infectious cause of death worldwide and control of the disease is now seriously threatened by the emergence of multidrug resistance. Although recent advances in immunology and molecular biology may eventually yield novel preventive and therapeutic agents, the overwhelming need at the present time is ensure that the available disease control tools are universally deployed and used in the most effective ways possible.

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Chapter 203 - Miscellaneous Agents: Fusidic Acid, Nitrofurantoin and Spectinomycin

S Ragnar Norrby

This chapter deals with three antibiotics that are chemically different and have different antibacterial spectra and clinical uses.

FUSIDIC ACID

Fusidic acid has a steroidal chemical structure. It inhibits protein synthesis in Gram-positive bacteria.

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Fusidic acid is available for intravenous, oral or topical administration. The bioavailability after oral administration is 75–90% with tablets but only about 23% in children given a suspension.[1] After an oral dose of 500mg given as tablets, the initial plasma concentration is approximately 30mg/l and in steady state the concentration is about 100mg/l after a dosage of 500mg q8h. The protein binding is about 97%.

Fusidic acid is lipid-soluble and is efficiently distributed to peripheral compartments, including brain tissue, but excluding cerebrospinal fluid. [2] The elimination half-life is about 9 hours. Fusidic acid is eliminated mainly by conjugation to glucuronide in the liver and subsequent biliary excretion. Fusidic acid *per se* has no known effects on the central nervous system, lung, liver, kidney or prostate/genitourinary system.

The dosage of fusidic acid is:

- 500mg q8h orally or intravenously for adults; and
- 15–20mg/kg q12h for children.

Doses should be reduced in patients who have hepatic diseases, particularly biliary obstruction. Full doses can be given to patients who have renal insufficiency and to elderly. Because of the high protein binding, administration of fusidic acid should be avoided during the last trimester of pregnancy and to newborn children (risk for accumulation of bilirubin in the central nervous system — 'kernicterus').

INDICATIONS

The antibacterial spectrum of fusidic acid includes mainly *Staphylococcus aureus* but also coagulase-negative staphylococci and Gram-positive anaerobes, including *Clostridium difficile*.[3] In addition, fusidic acid has been found to be active against *Mycobacterium kansasii* and *Mycobacterium leprae*.[4] [5] The antistaphylococcal spectrum includes methicillin-resistant strains, and sensitive strains typically have minimum inhibitory concentrations (MICs) of 0.25mg/l or less.[3]

Additive, synergistic or antagonistic antibacterial activity may result when fusidic acid is combined with other antibiotics.[3] [6] The lack of predictive interaction between fusidic acid and other antibiotics is a problem because fusidic acid should be combined when used in systemic infections to avoid the possible emergence of resistance. In experimental staphylococcal endocarditis it was shown that, when fusidic acid was used as a single agent, resistance emerged in five of 12 animals, whereas no resistance was seen in animals treated with vancomycin plus fusidic acid.[7]

The spectrum of fusidic acid makes it one of the few antibiotics that can be used for the oral treatment of methicillin-resistant staphylococci. It should then preferably be combined with another antibiotic to avoid the emergence of resistance. Candidates for combinations with fusidic acid are clindamycin, rifampin (rifampicin), and possibly linezolid. Fusidic acid is widely used for the treatment of bone and joint infections due to *S. aureus*, usually combined with flucloxacillin, although there are no formal studies showing that the combination is better than flucloxacillin alone. Topical fusidic acid has been used to eliminate carriage of methicillin-resistant *S. aureus*.

ADVERSE REACTIONS AND INTERACTIONS

Intravenous fusidic acid causes local irritation and thrombophlebitis in about 15% of patients treated, and hemolysis may occur following rapid infusion (normal infusion time is 2 hours or more). In newborns fusidic acid may cause kernicterus. Fusidic acid may interact with coumarin derivatives and oral contraceptives, reducing the bioavailability of these drugs through interference with the fecal flora.

NITROFURANTOIN

Nitrofurantoin interacts with bacterial protein synthesis in aerobic bacteria.

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Nitrofurantoin is administered orally as a microcrystalline or macrocrystalline formulation, of which the latter has a slower absorption rate. Absorption is almost complete, with 2–4% of the dose being recovered from the feces.[8] Serum concentrations are not measurable, except in patients who have severe renal failure. This is because of destruction of nitrofurantoin in the tissues and, in particular, a very rapid renal elimination by glomerular filtration (20%) and tubular secretion, resulting in a serum half-life of only 20 minutes in patients who have normal renal function.[8] Excretion is complete within 6 hours after intake and urine concentrations achieved are 200–400mg/l after a dose of 100mg q8h. In patients who have renal failure — who should not be given nitrofurantoin — there are measurable but still very low serum and urine concentrations.[9] Nitrofurantoin has no effects on the central nervous system and does not affect the kidneys, the prostate or the genitourinary system. It may cause toxic hepatitis or allergic lung reactions (see below).

Therapeutic doses of nitrofurantoin are 50–100mg q8h or q6h for adults and 3mg/kg/day q12h or q8h for children. Prophylactically, the adult dose is 50–100mg at bedtime, and the pediatric dose is 1–2mg/kg. The duration of treatment when nitrofurantoin is used therapeutically should be 5–7 days. Dosages are not affected by liver function. Nitrofurantoin can be used during pregnancy and lactation.

INDICATIONS

Nitrofurantoin is active against aerobic Gram-negative and Grampositive bacteria, including enterococci but excluding *Pseudomonas aeruginosa*. [10] Resistance is still rare in *Escherichia coli* but is frequently seen in *Klebsiella* spp.[11] Because nitrofurantoin loses most of its antibacterial activity in alkaline pH, it is not active against *Proteus, Morganella* and *Providencia* spp., even if susceptibility testing shows sensitivity. Nitrofurantoin should only be used for the treatment and prevention of urinary tract infections — complicated and uncomplicated bacterial cystitis.

In a study comparing 3-day treatment regimens of trimethoprim-sulfamethoxazole 160–800mg q12h, cefadroxil 500mg q12h, amoxicillin 500mg q8h and nitrofurantoin 100mg q6h for the treatment of uncomplicated cystitis in women, significantly better results were obtained with trimethoprim-sulfamethoxazole than with the other three regimens, which did not differ from each other.[12] Possible reasons for the lower activities of nitrofurantoin and the ß-lactams are lack of activity on the fecal and vaginal flora and the short duration of treatment. Despite these findings, nitrofurantoin has a place in the therapy of cystitis, especially for pregnant women and children. However, the duration of treatment should be at least 5 days; similar efficacy results have been reported for trimethoprim-sulfamethoxazole and nitrofurantoin when they are used for 7 days.[13]

The use of a single dose of nitrofurantoin at night to prevent cystitis is documented, albeit with some study design deficiencies.[14]

ADVERSE REACTIONS

Table 203.1 lists the most important adverse reactions to nitrofurantoin. They occur at low frequencies (<0.5%).[15] The risk for these reactions can be markedly reduced by:

- avoiding long-term (>7 days) treatment, especially in the elderly;
- avoiding daily doses higher than 300mg in adults; and
- reducing the dosage for elderly patients and patients who have renal impairment.

Nitrofurantoin should not be used for patients who have renal failure because the urine levels are too low and there is an increased risk of adverse reactions. Upper gastrointestinal adverse reactions (nausea, vomiting, anorexia) may occur and seem to be more common with the old microcrystalline formulation than with the macrocrystalline one that is now used.[13]

TABLE 203-1 -- Serious adverse reactions to nitrofurantoin.

SERIOUS ADVERSE REACTIONS TO NITROFURANTOIN	
Adverse reaction	**Risk factor**
Eosinophilic lung infiltrates, fever	Prolonged treatment time, high doses
Pulmonary fibrosis	Elderly female patients, high doses
Polyneuropathy	High dose relative to renal function
Hepatitis	Long treatment time
Hemolytic anemia	Hereditary glucose-6-phosphate dehydrogenase deficiency

SPECTINOMYCIN

Spectinomycin is an aminocyclitol antibiotic with a chemical structure similar to that of the aminoglycosides. Another similarity to the aminoglycosides is that spectinomycin acts by inhibiting bacterial protein synthesis at the 30S ribosomal level.[16]

PHARMACOKINETICS, ROUTE OF ADMINISTRATION AND DOSAGE

Spectinomycin is always given intramuscularly. It is rapidly and completely absorbed and a concentration of about 80mg/l is achieved after a dose of 2g.[17] Its protein binding is low (<5%) and it has an apparent volume of distribution of 0.3l/kg. The elimination is renal, with a half-life of about 1 hour. It has no reported effects *per se* on any organ system.

Spectinomycin is given as a single intramuscular dose of 2g for gonococcal urethritis and at a dose of 2g q12h for 3 days for disseminated gonorrhea. Dose reductions are not necessary in any patient category.

INDICATIONS AND ADVERSE REACTIONS

Spectinomycin was developed to act against the increasing number of strains of *Neisseria gonorrhoeae* resistant to ß-lactam antibiotics and other drugs used to treat gonorrhea. Resistance to spectinomycin is uncommon in gonococci.[18] The only indication for spectinomycin is gonococcal urethritis. It is not usually a first-line choice but should be considered as an alternative to other, and often more effective, regimens, for instance in pregnancy when the patient is allergic to cephalosporins.[19]

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Chapter 204 - Antiretroviral Agents

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INTRODUCTION

The field of antiretroviral therapy is approaching its 20th anniversary. It began shortly after the discovery of HIV-1 in 1983, following which in-vitro cultivation of the virus permitted the screening of agents for antiviral activity. The availability of viral-specific targets (Fig. 204.1) has facilitated high throughput screening and rational drug design efforts, which resulted in the availability of 19 US Food and Drug Administration (FDA)-approved agents by mid-2003 (Table 204.1).

The availability of potent combination therapy has led to dramatic reductions in morbidity and mortality in the developed world.[1] Despite these advances, the challenges of adherence, toxicities and drug resistance have placed recognizable limits on the currently available agents. Thus, continued drug development is a necessity if further progress is to be made and, in this respect, cautious optimism is justified. The developmental horizon includes the potential for several new agents within existing drug classes and promising agents in the novel drug classes. The clinical use of antiretroviral agents involves a pathogenesis-based, combination treatment approach. The principles and approach to antiretroviral therapy and clinical monitoring tools are addressed in Chapter 138 , Chapter 139 , Chapter 140 . The reader is also referred to published antiretroviral therapy guidelines from the Department of Health and Human Services[2] and the International AIDS Society—USA.[3] This chapter will review the characteristics of both approved and selected investigational agents. Although they will be discussed individually, it is critical to remember that these drugs must be used in appropriate combinations to provide sufficiently potent therapy to realize durable clinical benefits.

NUCLEOSIDE ANALOG REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside analog reverse transcriptase inhibitors (NRTIs) were the first class of antiretroviral agents developed (Fig. 204.2). These drugs share a common mechanism of action. As purine or pyrimidine analogs, they require intracellular anabolic phosphorylation to triphosphate forms to be active inhibitors of the HIV reverse transcriptase. The NRTI triphosphates act as competitive inhibitors of the normal nucleoside triphosphates, are incorporated into the growing proviral DNA chain, and act as chain terminators.

Zidovudine

Description

Zidovudine — 3'-azido-,3'-deoxythymidine (AZT, ZDV; see Fig. 204.2) —is converted to ZDV triphosphate sequentially by cellular thymidine kinase, thymidylate kinase and nucleoside diphosphate kinase. Zidovudine triphosphate possesses a 100-fold greater selectivity for the HIV-1 reverse transcriptase than for the cellular DNA polymerase alpha, thus accounting for its viral specificity. The drug is active against HIV-1, HIV-2 and human T-cell lymphotropic virus type 1 (HTLV-1).

Pharmacokinetics and distribution

Zidovudine is rapidly and well absorbed following oral administration, but exhibits a mean systemic bioavailability of about 64% due to significant first-pass metabolism. Peak plasma concentrations of intravenous and orally administered ZDV range from 1.5 to 18µmol/l with single and multiple doses of 1–10mg/kg. [4] Zidovudine is highly lipophilic and widely distributed throughout the body.[5] Concentrations in the cerebrospinal fluid (CSF) in adults have ranged from 15 to 135% of plasma concentrations. Zidovudine is approximately 25% protein bound, primarily to albumin, and is primarily metabolized by hepatic 5'-glucuronidation forming a glucuronidated metabolite that is renally excreted.[5] [6] The serum half-life of ZDV is approximately 1 hour.

Route and dosage

Zidovudine is available as capsule, tablet, oral liquid and intravenous preparations. The latter is used nearly exclusively intrapartum to prevent maternal-fetal HIV-1 transmission. Oral formulations are available as ZDV alone or in fixed dose combinations with lamivudine (Combivir) or with lamivudine and abacavir (Trizivir). The usual adult therapeutic dosage of ZDV is 300mg q12h.

Indications

Zidovudine was the first approved antiretroviral agent in the USA and has been a cornerstone of therapy throughout the nucleoside monotherapy, dual nucleoside therapy and multiple drug combination therapy eras. It is indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents. Typically, it is paired with another NRTI to form a dual nucleoside component of a three- or four-drug regimen. The second NRTI is most commonly lamivudine or didanosine but should never be stavudine because of demonstrated antagonism.[7] In antiretroviral naive patients, these dual nucleoside components need to be prescribed with a protease inhibitor (PI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) or the potent NRTI abacavir, to form a combination regimen capable of suppressing plasma HIV-1 RNA to less than 50 copies/ml. The use of ZDV in treatment-experienced patients should be guided by the previous treatment history and the results of drug resistance testing. The presence of NRTI class cross-resistance often limits the efficacy of ZDV in second- and third-line regimens.

Zidovudine plays a role in the prevention of HIV-1 acquisition in the following circumstances:

- as a cornerstone of regimens to prevent maternal-fetal transmission following the landmark findings of the AIDS Clinical Trials Group, Study 076, in which transmission was reduced by two-thirds;[8] (see Chapter 135)
- following accidental needle-stick exposure in health care workers; and
- following unprotected sexual exposure, as in cases of rape.

In the setting of the prevention of maternal-fetal transmission, published guidelines suggest including ZDV as part of the regimen irrespective of the treatment history of the mother. In the setting of

accidental exposure, the risk of drug resistance in the index case should be factored into the choice of the appropriate prophylactic regimen.

Figure 204-1 Life cycle of HIV-1 and major targets of antiretroviral agents.

TABLE 204-1 -- US Food and Drug Administration approved agents.

FDA APPROVED AGENTS			
Nucleoside/nucleotide reverse transcriptase inhibitors	**Non-nucleoside reverse transcriptase inhibitors**	**Protease inhibitors**	**Entry inhibitors**
Zidovudine (Retrovir)	Nevirapine (Virammune)	Saquinavir (Invirase, Fortovase)	Enfuvirtide (Fuzeon)
Didanosine (Videx, Videx EC)	Delavirdine (Rescriptor)	Indinavir (Crixivan)	
Zalcitabine (Hivid)	Efavirenz (Sustiva)	Ritonavir (Norvir)	
Stavudine (Zerit)		Nelfinavir (Viracept)	
Lamivudine (Epivir)		Amprenavir (Agenerase)	
Abacavir (Ziagen)		Lopinavir/ritonavir (Kaletra)	
Tenofovir disoproxil fumarate (Viread)		Atazanavir (Reyataz)	
Emtricitabine (Emtriva)			

Resistance

Zidovudine, being the first antiretroviral agent in widespread use, became the first drug to which the development of drug resistance was described in 1989.[9] The mechanism of resistance is thought to be mediated by pyrophosphorolysis, which facilitates the removal of ZDV after its incorporation into the proviral DNA chain. Resistance to ZDV is mediated primarily by six mutations, which include M41L, D67N, K70R, L210W, T215F/Y and K219Q/E. High level ZDV resistance requires the accumulation of three to four mutations. These ZDV-associated mutations are now referred to as nucleoside analog-associated mutations (NAMs) because of the increasing recognition of their role in cross-resistance to other members of the NRTI and nucleotide RTI classes (Fig. 204.3). Resistance to ZDV can also be mediated by two multinucleoside resistance complexes: the Q151M complex (A62V, V75I, F116Y, Q151M) and a T69S 6bp insertion in the presence of NAMs (see Fig. 204.3).

Dosage in special circumstances

Dosage adjustment is necessary in patients with severe renal disease (Table 204.2). In patients with hepatic dysfunction, ZDV clearance is reduced and dosage modification is recommended. Limited data are available regarding specific dosage recommendations for patients with liver disease. Based on limited pharmacokinetic data in 14 patients with liver cirrhosis, a ZDV dose reduction by 50% or a doubling of the interval has been recommended. [6]

Zidovudine crosses the placenta with concentrations in the fetal circulation approximately 85% of maternal plasma concentrations. Teratogenic effects with ZDV have only been reported in rodents when exposed to lethal maternal doses (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicities of ZDV include nausea, headache, anemia, neutropenia and myopathy (Table 204.3). Nucleoside reverse transcriptase inhibitor class toxicities are listed in Table 204.4 . Clinically significant drug interactions are minimal because ZDV is predominantly renally excreted.

Didanosine

Description

Didanosine — 2',3'-dideoxyinosine (ddI; see Fig. 204.2) — is sequentially converted intracellularly to 2',3'-dideoxyinosine monophosphate by 5'-nucleotidase, and to 2',3'-dideoxyadenosine monophosphate by adenylosuccinate synthetase and adenylosuccinate lyase. It is then converted to the ddA-triphosphate, which is the active form of the drug, possessing activity against HIV-1 and HIV-2.

Pharmacokinetics and distribution

Didanosine bioavailability varies from 21 to 54% following oral administration in adults. It is recommended that didanosine be

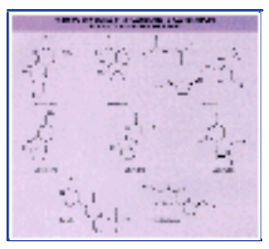

Figure 204-2 Chemical structures of approved nucleoside analog reverse transcriptase inhibitors.

administered on an empty stomach to increase absorption. Administration of didanosine tablets and delayed release capsules with food decreases the didanosine area-under-the-curve (AUC) by approximately 55 and 19%, respectively. Didanosine exhibits linear pharmacokinetics with peak plasma concentrations ranging from 0.52 to 2.79mg/l after oral doses of 125–375mg q12h.[11] Results of pharmacokinetic studies suggest similar AUC values comparing the standard twice daily regimen to the same total daily dose administered once daily.[12] Didanosine is less than 5% protein bound. Concentrations in the CSF have been reported to be approximately 21% of those in plasma.[11] The plasma half-life of didanosine is short (<2 hours), but the in-vitro intracellular half-life of the triphosphate appears prolonged (>25 hours).[13] Didanosine is partially metabolized to ddATP or uric acid, or enters the purine metabolic pool.

Route and dosage

Didanosine is given orally and exists as enteric-coated capsules, chewable tablets (buffered), and powder for oral solution formulations. Dosage is weight based. The enteric-coated preparation improves tolerance and is administered once daily. The other formulations may be administered q24h or q12h. In adults, the usual dosage is 400mg q24h for those weighing over 60kg and 250mg q24h for those under 60kg.

Indications

Didanosine is indicated for the treatment of HIV infection in combination with other antiretroviral agents. It has proven efficacious throughout the monotherapy, dual therapy and potent combination therapy eras. Like ZDV, it is typically prescribed as part of the dual nucleoside component of three- to four-drug combination regimens. It is most commonly paired with ZDV or stavudine, although the toxicity of stavudine-didanosine has raised concerns about this combination (see below).

Didanosine-containing dual NRTI components must be used with a PI and/or an NNRTI to provide a potent combination regimen. The drug can be used in both treatment naive and experienced patients. Its use in the latter situation should be driven by the treatment history and the drug resistance profile.

Hydroxyurea increases the intracellular activity of dideoxyadenosine triphosphate and has been studied in combination with didanosine or stavudine-didanosine. Although antiviral activity has been demonstrated, the blunting of CD4 cell responses and toxicity have dampened the enthusiasm for this adjunct to didanosine therapy.

Resistance

Low-fold changes in susceptibility to didanosine are sufficient to compromise the response to the drug and this must be recognized to properly interpret phenotypic resistance results. Genotypically, the signature mutation conferring didanosine resistance is L74V but K65R and M184V have also been associated with low-fold changes in susceptibility. The Q151M complex, the T69S insertions, and multiple NAMs also confer diminished susceptibility to didanosine (see Fig. 204.3).

Dosage in special circumstances

Didanosine clearance is significantly reduced in patients with renal disease and dosage modification is necessary (see Table 204.2). No dosage adjustment is recommended in patients with liver disease.

Didanosine crosses the placenta. Studies evaluating long-term carcinogenicity and teratogenicity have produced negative results (pregnancy category B).[10]

Adverse reactions and drug interactions

The major toxicities of didanosine include pancreatitis, peripheral neuropathy and diarrhea (see Table 204.3). The drug can also cause hepatotoxicity and has been associated with hepatotoxicity and

Figure 204-3 HIV drug resistance mutations. For each amino acid residue the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. HR1, first heptad repeat. *Courtesy of International AIDS Society — USA (for full details and footnotes see www.iasusa.org).*

TABLE 204-2 -- Nucleoside and nucleotide reverse transcriptase inhibitor dosage modifications in patients with renal dysfunction and with dialysis.

NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITOR DOSAGE MODIFICATIONS IN PATIENTS WITH RENAL DYSFUNCTION AND WITH DIALYSIS							
Drug	**Usual adult dose CrCl >50ml/min**	**CrCl 30–50ml/min**	**CrCl 10–30ml/min**	**CrCl <10ml/min**	**Hemodialysis***	**Peritoneal dialysis**	**Continuous renal replacement therapy**
Abacavir (po)	300mg q12h	300mg q12h	300mg q12h	300mg q12h	300mg q12h	300mg q12h	300mg q12h
Didanosine (po) =60kg	200mg q12h	100mg q12h	150mg q24h	100mg q24h	100mg q24h	100mg q24h	100mg q24h
<60kg	125mg q12h	75mg q12h	100mg q24h	75mg q24h	50–75mg q24h	75mg q24h	75mg q24h
Lamivudine (po)	150mg q12h	150mg q24h	100mg q24h	25–50mg q24h	25–50mg q24h	25–50mg q24h	50–150mg q24h
Stavudine (po) =60kg	40mg q12h	20mg q12h	20mg q24h	20mg q24h	20mg q24h	20mg q24h	N/A
<60kg	30mg q12h	15mg q12h	15mg q24h	15mg q24h	15mg q24h	15mg q24h	N/A
Tenofovir DF (po)	300mg q24h	Use not currently recommended in patients with CrCl <60ml/min	Use not currently recommended in patients with CrCl <60ml/min	Use not currently recommended in patients with CrCl <60ml/min	N/A	N/A	N/A
Zalcitabine (po)	0.75mg q8h	0.75mg q12h	0.75mg q12h	0.75mg q24h	0.75mg q24h	0.75mg q24h	N/A
Zidovudine (po)	300mg q12h	300mg q12h	100mg q8h	100mg q8h	100mg q8h	100mg q8h	100mg q8h
CrCl, creatinine clearance; N/A, specific dosing recommendations not available							

* Drug should be administered after the hemodialysis session.

TABLE 204-3 -- Nucleoside and nucleotide reverse transcriptase inhibitor specific toxicities.

NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITOR SPECIFIC TOXICITIES						
Zidovudine	**Didanosine**	**Zalcitabine**	**Stavudine**	**Lamivudine**	**Abacavir**	**Tenofovir DF**
Bone marrow suppression	Pancreatitis	Peripheral neuropathy	Pancreatitis	Headache	Hypersensitivity reaction*	Asthenia
Anemia or neutropenia	Peripheral neuropathy	Stomatitis	Peripheral neuropathy	Nausea		Headache
Gastrointestinal intolerance		Pancreatitis		Diarrhea		Diarrhea
Headache	Nausea			Insomnia		Nausea
Insomnia	Diarrhea			Pancreatitis		Vomiting
				Neuropathy		

* Hypersensitivity reaction can be fatal: symptoms may include fever, rash, nausea, vomiting, malaise, fatigue, loss of appetite, and respiratory symptoms such as pharyngitis, dyspnea, or cough

TABLE 204-4 -- Nucleoside reverse transcriptase inhibitor and protease inhibitor class toxicities.

NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR AND PROTEASE INHIBITOR CLASS TOXICITIES	
Nucleoside reverse transcriptase inhibitors	**Protease inhibitors**
Mitochondrial toxicity	Gastrointestinal intolerance
Lactic acidosis	Fat redistribution
Hepatomegaly with steatosis	Lipid abnormalities
Lipoatrophy	Insulin resistance and hyperglycemia
	Hepatitis
	Possible ? bleeding episodes in patients with hemophilia

lactic acidosis when used in combination with stavudine in pregnant women. For NRTI class toxicities, see Table 204.4 .

The didanosine buffered tablets have the potential for drug interactions with agents affected by concomitant antacid administration and with any agents that require gastric acidity for absorption. Clinically significant drug interactions have been reported between didanosine formulations and tenofovir disoproxil fumarate (tenofovir DF), delavirdine and indinavir. Administration of once daily didanosine enteric-coated capsules (Videx EC) 2 hours before tenofovir DF administered with a light meal resulted in an approximate 46% increase in didanosine exposure. Co-administration with a light meal resulted in an approximate 60% increase in didanosine exposure. Co-administration of the didanosine buffered tablets with tenofovir DF in the fasting state resulted in an approximate 44% increase in didanosine exposure. There appears to be no effect of either didanosine formulation on the levels of tenofovir DF. If didanosine and tenofovir are used concomitantly, the dosage of didanosine should be reduced to 250mg q24h. Simultaneous administration of didanosine buffered tablets with delavirdine and indinavir resulted in significant decreases in AUC by 20 and 84%, respectively. Consequently, delavirdine or indinavir should be administered 1 hour before didanosine buffered tablets.

Zalcitabine

Description

Zalcitabine — 2',3'—dideoxycytidine (ddC; see Fig. 204.2) — is converted to its active, triphosphorylated form by cellular kinases. The drug is active against both HIV-1 and HIV-2.

Pharmacokinetics and distribution

Zalcitabine exhibits linear pharmacokinetics. Oral bioavailability exceeds 80%. Administration of zalcitabine with food decreases the rate and extent of absorption. With food, the peak concentration

decreases 39%, bioavailability decreases 14% and the time to reach the peak concentration doubles.[14] [15] Concentrations of zalcitabine in CSF have been reported as 14% of plasma levels. [16] Zalcitabine is not significantly metabolized and elimination is more dependent on renal mechanisms because approximately 75% of unchanged drug is recovered in the urine. The half-life of zalcitabine is less than 2 hours.

Route and dosage

Zalcitabine is available as a tablet formulation. It is administered orally with the usual adult dosage being 0.75mg q8h.

Indications

Major clinical end-point trials of zalcitabine reported in the mid-1990s in combination with ZDV demonstrated a clinical benefit to this combination over ZDV monotherapy in treatment naive patients.[17] [18] Despite these findings, the drug has a very limited, if any, role in current antiretroviral therapy. Its limited potency, ineffectiveness in treatment-experienced patients and neurotoxicity contribute to the drug's current position as a low-priority agent.

Resistance

The genotypic patterns that confer reduced susceptibility to zalcitabine are similar to those reported for didanosine. They include K65R, L74V, M184V, multiple NAMs, the Q151M complex and the T69S insertions; T69D also confers zalcitabine resistance (see Fig. 204.3).

Dosage in special circumstances

Dosage adjustment is necessary in patients with renal disease (see Table 204.2). No dosage adjustment is necessary with hepatic impairment.

Zalcitabine crosses the placenta. Zalcitabine has been shown to be carcinogenic in rodents and teratogenic in mice and rats at high doses (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicities of zalcitabine include peripheral neuropathy and pancreatitis (see Table 204.3). For NRTI class toxicities, see Table 204.4 . Clinically significant drug interactions are minimal.

Stavudine

Description

Stavudine — 2',3'-didehydro-3'-deoxythymidine (d4T; see Fig. 204.2) — is a thymidine analog that is converted to the active form, stavudine triphosphate, by a series of cellular kinases; the initial phosphorylation is the rate limiting step. The drug has activity against both HIV-1 and HIV-2.

Pharmacokinetics and distribution

Stavudine is rapidly absorbed and exhibits linear pharmacokinetics. Peak concentrations of about 0.9mg/l are achieved within 2 hours. The oral bioavailability is 82–86%. The mean serum half-life is short and ranges between 1 and 1.67 hours, with an intracellular half-life of about 3–4 hours. Protein binding is minimal. Stavudine penetrates into the CSF achieving levels approximately 40% of plasma levels.[19] Stavudine is excreted by renal and non-renal routes with approximately 50% of a dose excreted unchanged in the urine.[20]

Route and dosage

Stavudine is administered orally with the dosage weight adjusted. It is available in capsule and oral solution formulations. For adults weighing over 60kg, the dose is 40mg q12h; for those under 60kg, the dose is 30mg q12h. For the extended-release preparation of stavudine that is currently under investigation, the dose for persons

over 60kg is 100mg q24h.

Indications

Like ZDV, stavudine is commonly used as part of dual NRTI components with lamivudine or didanosine. These are then typically combined with a PI, NNRTI or abacavir to form potent combination regimens. Stavudine can be used in treatment naive patients or as an alternative to ZDV in those patients who exhibit intolerance to ZDV in the first few weeks after initiation of therapy. The association of stavudine with lipoatrophy, perhaps more than with other NRTIs, has led to greater circumspection about using this agent in initial regimens. However, more data concerning the relative risks of the various NRTIs for peripheral lipoatrophy are needed. Its use in treatment-experienced persons should be dictated by the treatment history and the results of drug resistance testing.

Resistance

Low-fold changes in susceptibility can impair the response to stavudine in vivo and this is important to keep in mind in the interpretation of phenotypic resistance testing. Genotypically, the V75T has been thought to be a signature mutation for stavudine resistance but this mutation is only rarely seen in clinical isolates. Recent data suggest that stavudine and stavudine/didanosine can select for ZDV-associated mutations (NAMs) and the Q151M complex. Zidovudine and stavudine share cross-resistance at both the virion and enzyme levels. Zidovudine-resistant isolates should be considered resistant to stavudine (see Fig. 204.3).

Dosage in special circumstances

Stavudine requires dose adjustment in patients with renal disease (see Table 204.2). No dosage modification is recommended in patients with liver disease.

Stavudine crosses the placenta and teratogenicity studies in rodents were negative with a decrease in sternal calcium noted at high doses (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicity of stavudine is peripheral neuropathy and the drug has been strongly implicated in peripheral lipoatrophy and mitochondrial dysfunction syndromes (see Table 204.3). For NRTI class toxicities, see Table 204.4 . Clinically significant drug interactions are minimal.

Lamivudine

Description

Lamivudine — (-)2',3'-dideoxy-3'-thiacytidine (3TC; see Fig. 204.2) — is the (-) enantiomer of a sulfur containing cytidine analog. This enantiomer was chosen on the basis of its potency and cytotoxicity profile. It is phosphorylated to its active form, lamivudine triphosphate, by cellular kinases. The drug is active against HIV-1 and HIV-2, and hepatitis B virus.

Pharmacokinetics and distribution

Lamivudine is well absorbed following oral administration with a mean bioavailability of over 80% in adults. Systemic drug exposure is not influenced by administration with food. Peak and trough concentrations of approximately 2µg/ml and 0.33µg/ml, respectively, have been achieved following oral administration of 150mg q12h.[21] The mean serum half-life is approximately 4–6 hours, with the intracellular half-life ranging from 10.5 to 15.5 hours.[22] Lamivudine is less than 36% protein bound. It penetrates the CSF, but the CSF to serum ratio is lower than that of other nucleoside analogs. Lamivudine is not significantly metabolized and is eliminated primarily unchanged via the kidney.

Route and dosage

Lamivudine is administered orally and is available in tablet and liquid formulations. The usual adult dosage is 150mg q12h but 300mg q24h dosing is being explored in clinical trials. The drug is also available as part of a fixed dose combination with ZDV (Combivir) and ZDV plus abacavir (Trizivir).

Indications

Lamivudine is one of the cornerstones of current antiretroviral therapeutics given its potency and excellent tolerability. It is commonly prescribed as part of the nucleoside component of initial regimens, typically paired with ZDV or stavudine. Recent data also suggest that it can be successfully paired with tenofovir DF. As noted previously, these dual NRTI components must be prescribed with a PI, an NNRTI or abacavir to create potent combination regimens. When lamivudine is not part of an initial regimen, it is useful in treatment-experienced patients if the key resistance mutation, M184V, is not present.

Lamivudine is widely used as part of maternal therapies and as part of maternal-fetal transmission interruption regimens. Concerns raised about potential fetal toxicity in a French study have not been borne out by larger reviews of experience in the USA.

Resistance

High-level phenotypic resistance (>500-fold change in susceptibility) quickly and nearly uniformly develops in patients treated with partially suppressive regimens containing lamivudine (e.g. dual nucleoside regimens). This is mediated through the lamivudine signature mutation, M184V. The latter has also been reported to increase the fidelity of the HIV reverse transcriptase and to decrease replicative fitness. The M184V mutation can delay the emergence of ZDV resistance and reverse ZDV resistance when the T215F/Y mutation is present. However, high-level ZDV/lamivudine co-resistance can develop when multiple ZDV associated mutations (NAMs) and the M184V are present.

Other genotypic correlates of resistance to lamivudine are the E44D and V118I mutations which, in the presence of NAMs, can reduce susceptibility to lamivudine in vitro; the clinical significance of these mutations, however, needs further confirmation. The Q151M complex and the T69S insertions also confer lamivudine resistance. Multiple NAMs alone, however, do not reduce susceptibility to lamivudine. This distinguishes lamivudine from the other approved NRTIs (see Fig. 204.3).

Dosage in special circumstances

Dosage adjustment is required in patients with renal disease (see Table 204.2). No dosage adjustment is necessary in patients with liver disease.

Lamivudine crosses the placenta. No carcinogenicity or teratogenicity has been observed in long-term animal studies (pregnancy category C).

Adverse reactions and drug interactions

Lamivudine is generally very well tolerated. Insomnia, headache, pancreatitis and peripheral neuropathy can occur (see Table 204.3). For NRTI class toxicities, see Table 204.4 . Clinically significant drug interactions are minimal. Pancreatitis has been described in children.

Abacavir

Description

Abacavir sulfate — (1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclo pentene-1-methanol (ABC; see Fig. 204.2) — is converted to carbovir intracellularly. Adenosine phosphotransferase catalyzes the first phosphorylation step. A cytosolic 5'-nucleotidase then converts abacavir monophosphate to carbovir

monophosphate. Cellular kinases then complete the di- and triphosphorylation steps. Carbovir triphosphate is active against HIV-1 and HIV-2.

Pharmacokinetics and distribution

Abacavir is rapidly and well absorbed with a reported absolute oral bioavailability of approximately 83%. Administration with food does not significantly affect the oral bioavailability. Peak concentrations achieved following multiple dose administration of 300mg q12h were reported to be approximately 2.2mg/l.[23] The mean plasma half-life of abacavir is less than 2 hours. Abacavir is 50% protein bound. Its high lipophilicity facilitates its distribution and penetration into the CSF, where concentrations have been reported to be approximately 30% of plasma levels.[24] Abacavir undergoes extensive hepatic metabolism by alcohol dehydrogenase and glucuronyl transferase.

Route and dosage

Abacavir is administered orally and is available in tablet and oral solution formulations. The usual adult dosage is 300mg q12h but single daily dosing is being explored in clinical trials. As noted previously, abacavir is available in a fixed dose combination formulation with ZDV and lamivudine (Trizivir).

Indications

Abacavir's potency and efficacy has permitted a new option in antiretroviral naive patients — the triple NRTI regimen option. Data from two clinical trials comparing ZDV-lamivudine-abacavir with ZDV-lamivudine-indinavir have shown general comparability of these two combinations in intent-to-treat analyses. [25] More long-term efficacy data are needed in patients with high viral loads (e.g. >100,000 plasma HIV RNA copies/ml) and low CD4 cell counts (e.g. <50/mm^3) to provide clinicians with confidence about the triple NRTI option in this circumstance. Patients must be antiretroviral naive and harbor no NRTI-associated mutations to avoid a higher risk of virologic failure on this regimen.

Abacavir does not have to be solely reserved for the triple NRTI option and can be paired with any other NRTI as part of a PI- or NNRTI-based regimen. In previously naive individuals suppressed on a PI-containing regimen and harboring no NRTI-associated mutations, a switch of the PI to abacavir is virologically safe and can improve serum lipid abnormalities.[26] In treatment-experienced patients, abacavir's usefulness depends on the degree of cross-resistance that may have been conferred by previous NRTI therapy (see below).

Resistance

Changes in susceptibility of 8-fold or greater compromise the clinical efficacy of abacavir. Genotypically, a number of mutations confer resistance to abacavir. The M184V mutation alone confers a 2-fold change in abacavir susceptibility and the drug should still be useful in this situation. However, the M184V mutation in the presence of multiple NAMs and/or L74V and K65R will confer higher level abacavir resistance and compromise the drug's efficacy. The Q151M complex and the T69S insertion also confer abacavir resistance (see Fig. 204.3).

Dosage in special circumstances

Dosage adjustment is not necessary in patients with renal disease. No specific dosage modification is recommended in patients with liver disease.

Abacavir crosses the placenta. Developmental toxicity secondary to abacavir has been observed in rats (pregnancy category C).[24]

Adverse reactions and drug interactions

The major abacavir toxicity of concern is a hypersensitivity reaction, which has a 3–5% incidence and can be fatal (see Table 204.3). Abacavir hypersensitivity has been linked to the HLA B*5701 genotype.[27] For NRTI class toxicities, see Table 204.4 . Clinically significant drug interactions are minimal.

Emtricitabine

Emtricitabine — 5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC, Coviracil) — is a cytidine analog with activity against HIV and hepatitis B. It is more potent than lamivudine in vitro and can be administered q24h. It has proven efficacy in phase III trials. Resistance is mediated by M184V, the same mutation, which confers resistance to lamivudine. The drug has generally been well tolerated.

Selected investigational nucleoside analog reverse transcriptase inhibitors

A number of NRTIs are currently under development with the objectives of improving pharmacokinetics, avoiding toxicities and/or targeting drug-resistant virus.

Amdoxovir

Amdoxovir — (-)-B-D-2,6-diaminopurane dioxolane (DAPD) — is a prodrug of dioxolane guanosine (DXG), an agent with both anti-HIV and hepatitis B activity. Dioxolane guanosine is active against NRTI-resistant strains except for those bearing the Q151M multi-nucleoside complex. Resistance to DAPD/DXG is mediated by K65R and L74V in vitro. The drug has shown dose-dependent antiviral activity in both naive and experienced patients in a short-term phase I trial.

Others

ACH-126, 443 (ß-L-F-d4C) is a once daily NRTI with activity against NRTI-resistant strains and the potential for diminished mitochondrial toxicity given its L-nucleoside configuration. SPD-754 is the (-) enantiomer of dOTC (development of which was halted due to animal toxicity). SPD-754 is active against lamivudine and ZDV-resistant isolates in vitro and may have decreased potential for inducing mitochondrial toxicity. In vitro, K65R and V75I mutations can be selected. Clinical trial results with these two NRTIs are awaited.

NUCLEOTIDE ANALOG REVERSE TRANSCRIPTASE INHIBITOR

Tenofovir disoproxil fumarate

Description

Tenofovir DF — (R)-9-(2-phosphonomethoxypropyl)adenine (TDF; see Fig. 204.2) — is a prodrug of the nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA). Tenofovir DF represents a new class of antiretroviral agents, the nucleotide reverse transcriptase inhibitors (NtRTIs). These drugs differ from the NRTIs by having a phosphate group in the parent molecule. They thus require only diphosphorylation to be converted to their active compounds. Tenofovir DF is converted to tenofovir by serum esterases. Tenofovir is converted to its active diphosphate form serially by adenylate kinase and nucleotide diphosphate kinase. The drug is active against HIV-1, HIV-2 and hepatitis B virus.

Pharmacokinetics and distribution

Tenofovir is administered orally as a prodrug, tenofovir DF. The oral bioavailability of tenofovir is approximately 25 and 40% in the fasting and fed state, respectively, as compared with 1mg/kg intravenous dosing. Following oral administration of tenofovir DF 300mg q24h, mean steady state peak concentrations were reported as 303ng/ml with an estimated half-life of approximately 14 hours. [28] The intracellular half-life of tenofovir diphosphate ranges from 12 to 50 hours.[29] Tenofovir is primarily eliminated renally (70–80%) via a combination of glomerular filtration and active tubular secretion.

Route and dosage

Tenofovir DF is administered orally and is available in a tablet formulation. The usual adult dosage is 300mg q24h.

Indications

Tenofovir DF is approved for the treatment of HIV infection in combination with other antiretroviral agents. Its approval was based upon two randomized trials in treatment-experienced persons in which tenofovir DF was added to previous therapy. Plasma HIV-1 RNA declines of 0.6 $\log_{10}$ and modest CD4 cell rises were seen.[30] In antiretroviral-naive persons, the combination of tenofovir DF-lamivudine-efavirenz was comparable to a regimen of stavudine-lamivudine-efavirenz with respect to virologic suppression and CD4 cell increases.

Resistance

A phenotypic change in susceptibility of over 4-fold compromises the virologic response to tenofovir DF. The K65R is a signature mutation for tenofovir DF but its appearance in patients treated with tenofovir DF is infrequent. Four or more NAMs (especially M41L and L210W) and the T69S insertion also confer resistance to tenofovir DF. Interestingly, the M184V mutation enhances susceptibility to tenofovir DF, but the clinical significance of this finding is unclear. Specifically, the advisability of continuing lamivudine or abacavir to place selective pressure on the M184V mutation (to enhance susceptibility to tenofovir DF) in the setting of resistance to these drugs must be demonstrated in clinical trials before a formal recommendation can be made (see Fig. 204.3).

Dosage in special circumstances

Dosage adjustment and monitoring for drug toxicity are necessary in patients with renal disease. The pharmacokinetics of tenofovir have not been evaluated in patients with creatinine clearances less than 60ml/min and use in this patient population is not recommended until more information becomes available. The presence of hepatic insufficiency is likely to have a limited effect on tenofovir pharmacokinetics and no specific dosage modifications are recommended in this population.

Tenofovir is classified as pregnancy category B. In rats and rabbits, studies have found no evidence of impaired fertility or teratogenicity.

Adverse reactions and drug interactions

Neutropenia, headache, fatigue, pancreatitis, elevated creatinine and hypophosphatemia have been reported (see Table 204.3). As mentioned previously, concomitant use of tenofovir DF with didanosine increases exposure to didanosine and increases the potential for drug toxicity.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

The NNRTI class of antiretroviral agents is a chemically heterogeneous group of compounds that share a common mechanism of action. These agents differ from the NRTIs in that the parent compound is active and no intracellular metabolism is necessary. The drugs in this class allosterically bind in a noncompetitive fashion to a hydrophobic pocket near the active site of the reverse transcriptase and 'lock' the enzyme into an inactive state. The agents in this class also differ from NRTIs in that they are active against HIV-1 except for subtype O and are inactive against HIV-2 strains.

Nevirapine

Description

Nevirapine — 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido [3,2-b:2',3'-][1,4]diazepin-6-one (NVP; see Fig. 204.4) — is the lead

compound in this class and was the first NNRTI approved in the USA.

Pharmacokinetics and distribution

Nevirapine is well absorbed with an oral bioavailability of 90%. Absorption does not appear to be affected by co-administration with food or antacids. Maximum concentrations are achieved approximately 2 hours after an oral dose with a second peak occurring approximately 14 hours after a dose, presumably due to enterohepatic recycling.[31] Nevirapine exhibits linear pharmacokinetics. Following administration of a single 200mg and 400mg dose, a peak concentration of 7.5µmol/l and 12.8 µmol/l is achieved, respectively. Average steady state plasma concentrations are peak concentrations of 27.1µmol/l and trough concentrations of 15–17µmol/l following 200mg daily. Nevirapine is very lipophilic and widely distributed throughout the body.[32] It is approximately 60% protein bound. Concentrations in the CSF are approximately 45% of those achieved in plasma.[32] Nevirapine is primarily metabolized by the CYP3A4 and CYP2B6 isoenzymes to hydroxy-nevirapine metabolites and induces both these enzyme systems.[33] Auto-induction of its own metabolism has been demonstrated. The half-life of nevirapine is approximately 25–30 hours.

Route and dosage

Nevirapine is administered orally and is available as tablet and oral suspension formulations. The usual adult dosage is 200mg q24h for 14 days followed by 200mg q12h. This dose escalation regimen is recommended to reduce the incidence and severity of rash during treatment initiation.

Indications

Nevirapine is indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents. Due to the vulnerability of nevirapine to single-step, high-level resistance, this drug needs to be used in potent combination regimens designed to suppress plasma HIV-1 RNA levels to less than 50 copies/ml. Partially suppressive regimens or poor drug adherence carry a high risk of engendering nevirapine resistance.

Combination regimens of NNRTI/dual NRTI are now among the recommended first-line therapies for treatment-naive patients. In individuals intolerant to the central nervous system (CNS) side-effects of efavirenz or in women of child-bearing age for whom access to effective contraception is problematic, nevirapine is an appropriate alternative to efavirenz. In previously naive persons who are virologically suppressed on a PI-containing regimen, a switch of the PI to nevirapine can maintain virologic suppression and improve serum lipid abnormalities.

Drug class cross-resistance among currently approved NNRTIs (nevirapine, delavirdine, efavirenz) severely limits the use of NNRTIs as alternative agents in NNRTI-experienced persons with virologic failure. However, in NNRTI-naive persons failing a PI- or triple NRTI-based regimen, this class of agents is critical to the ability to successfully construct a salvage regimen.[34] It is important, however, to be able to support the NNRTI component with at least two other active agents in the alternative regimen to try to avoid the rapid emergence of NNRTI resistance.

Nevirapine has assumed an important role in the prevention of maternal-fetal HIV transmission in the developing world.[35] A single dose of nevirapine to the mother and the infant can reduce HIV transmission by 50%. When added to combination therapy that the mother may be receiving, single-dose nevirapine has not been shown to further reduce HIV transmission in the developed world.[36]

In the prophylaxis of accidental needle-stick exposure in health care workers, the use of nevirapine should be limited given the reports of severe hepatotoxicity when used in this setting.

Resistance

Low-level changes in susceptibility to nevirapine (and other NNRTIs), of the order of 2.5- to 10-fold, are the result of natural polymorphisms in wild-type strains and do not affect the response to these agents. Higher level resistance compromises or eliminates the virologic response to nevirapine. Genotypically, the signature mutation for nevirapine is Y181C, but other nevirapine-associated mutations include L100I, K103N, V106A, V108I, Y188C/I/H and G190A (see Fig. 204.3).

Dosage in special circumstances

Dosage modification is not required for patients with renal dysfunction. Limited data are available in patients with hepatic impairment and no specific dosage modification is currently recommended.

Nevirapine is not associated with teratogenicity in rabbits or rats (pregnancy category C).[10] It rapidly crosses the placenta.

Adverse reactions and drug interactions

The major toxicities associated with nevirapine are rash and hepatotoxicity (which can be fatal; Table 204.5). Nevirapine is a moderate inducer of CYP3A4. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.7 and Table 204.8 .

Delavirdine

Description

Delavirdine — 1-(5-methanesulfonoamido-1H-indol-2-ylcarbonyl)-4-[3-(1-methylethylamino)pyridinyl]piperazine (DLV; see Fig. 204.4) — was the second approved NNRTI approved in the USA. Its use has been limited by the associated high pill burden.

Pharmacokinetics and distribution

Delavirdine is rapidly absorbed with peak concentrations occurring 1–2 hours after administration with an oral bioavailability of about 85%. Administration with food does not significantly affect steady state AUC within a dosage interval, trough plasma concentrations or time to peak concentrations, but reduces peak concentrations approximately 22%.[37] Absorption may be reduced in patients with gastric hypoacidity and with concomitant antacid administration. Delavirdine exhibits nonlinear pharmacokinetics. Mean C_{min} and C_{max} concentrations following delavirdine 400mg q8h are approximately 15µmol/l and 35µmol/l, respectively.[37] Delavirdine is approximately 98% protein bound. Penetration into the CSF is poor, with concentrations only 0.4% of plasma levels.[37] Delavirdine undergoes extensive metabolism into inactive metabolites primarily by CYP3A4 with less than 5% excreted unchanged in the urine. The mean half-life of delavirdine is approximately 6 hours.

Route and dosage

Delavirdine is administered orally and is available as a tablet formulation. The usual adult dosage is 400mg q8h.

TABLE 204-5 -- Non-nucleoside reverse transcriptase inhibitor specific toxicities.

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR SPECIFIC TOXICITIES		
Nevirapine	**Delavirdine**	**Efavirenz**
Rash	Rash	CNS symptoms*
Hepatotoxicity	Fatigue	Rash
	Nausea	Hepatotoxicity
	Diarrhea	

* CNS symptoms may include dizziness, insomnia, impaired concentration, somnolence, abnormal dreams, euphoria, confusion, agitation and hallucinations

TABLE 204-6 -- Drug interactions between non-nucleoside reverse transcriptase inhibitors and protease inhibitors.

DRUG INTERACTIONS BETWEEN NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AND PROTEASE INHIBITORS									
Drug affected	**Nevirapine (NVP)**	**Delavirdine (DLV)**	**Efavirenz (EFZ)**	**Saquinavir (SQV)**	**Indinavir (IDV)**	**Ritonavir (RTV)**	**Nelfinavir (NFV)**	**Amprenavir (APV)**	**Lopinavir/ritonavir (LPV/r)**
Nevirapine (NVP)						NVP AUC ?			
Delavirdine (DLV)							DLV levels ? 50%		
Efavirenz (EFZ)	EFZ levels ? 22%			EFZ levels ? 12%; co-administration not recommended when SQV only PI		EFZ levels ? 21%			
Saquinavir (SQV)	SQV levels ? 25%	SQV levels ? 5-fold; consider Fortovase 800mg q8h + standard DLV dose	SQV levels ? 62%; combination not recommended when SQV only PI		SQV levels ? 4- to 7-fold	SQV levels ? 20-fold; consider Invirase or Fortovase 400mg q12h + RTV 400mg q12h	SQV levels ? 3- to 5-fold; consider Fortovase 800mg q8h or 1200mg q12h with standard NFV dose	SQV levels ? 19%	SQV levels ? consider SQV 800mg q12h with standard LPV/r dose
Indinavir (IDV)	IDV levels ? 28%; consider IDV 1000mg q8h + standard NVP dose	IDV levels ? >40%; consider IDV 600mg q8h + standard DLV dose	IDV levels ? 31%; consider IDV 1000mg q8h + standard EFZ dose			IDV levels ? 2- to 5-fold; dose IDV 400mg q12h + RTV 400mg q12h or IDV 800mg q12h + RTV 100 or 200mg q12h	IDV levels ? approximately 50%; consider IDV 1200mg q12h + NFV 1250mg q12h (limited data)	IDV levels ? 38%	IDV levels ? consider IDV 600mg q12h + standard LPV/r dose

Ritonavir (RTV)	RTV levels ? 11%	RTV levels ? 70%	RTV levels ? 18%; consider RTV 600mg q12h + standard EFZ dose						(Co-formulated with ritonavir)
Nelfinavir (NFV)	NFV levels ? 10%	NFV levels ? 2-fold	NFV levels ? 20%	NFV levels ? 20%	NFV levels ? 80%; consider 1200mg IDV q12h + NFV 1250mg q12h (limited data)	NFV levels ? 1.5 times; consider NFV 500–750mg q12h + RTV 400mg q12h			
Amprenavir (APV)			APV levels ? 36%; consider APV 1200mg q8h *or* APV 1200mg q12h + RTV 200mg q12h	APV levels ? 32%	APV levels ? 33%	APV levels ? 2.5-fold; consider APV 600mg q12h + RTV 100mg q12h *or* APV 1200mg q24h + RTV 200mg q24h	APV levels ? 1.5-fold		APV levels ?; consider APV 600–750mg q12h + standard LPV/r dose
Lopinavir/ritonavir (LPV/r)	LPV minimum concentration ? 55%; consider LPV/r 533/133mg q12h with standard NVP dose		LPV levels ? 40%; consider LPV/r 533/133mg q12h with standard EFZ dose						
AUC, area under the curve									

TABLE 204-7 -- Drugs that are not recommended for use with non-nucleoside reverse transcriptase inhibitors.

DRUGS THAT ARE NOT RECOMMENDED FOR USE WITH NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS			
Drug class	**Nevirapine**	**Delavirdine**	**Efavirenz**
Anticonvulsants		Phenytoin, phenobarbital, carbamazepine	
Antihistamines		Astemizole, terfenadine	Astemizole, terfenadine
Antimycobacterials	(Insufficient data)	Rifampin (rifampicin), rifabutin	Rifampin, rifabutin*
Ergot derivatives		Dihydroergotamine, ergonovine, ergotamine, methylergonavine	Dihydroergotamine, ergonovine, ergotamine, methylergonavine
Gastrointestinal drugs		Cisapride	Cisapride
Herbal products	St John's wort†	St John's wort†	St John's wort†
Lipid lowering agents		Simvastatin, lovastatin	
Neuroleptics		Pimozide	
Oral contraceptives	All oral contraceptives		Ethinyl estradiol and all oral contraceptives
Sedatives/hypnotics		Alprazolam, midazolam, triazolam	Midazolam, triazolam

* Increase daily dose of rifabutin 50%. Consider doubling rifabutin dose in regimens where rifabutin administered two or three times a week
† Co-administration of NNRTIs with St John's Wort is expected to substantially decrease NNRTI concentrations

Indications

Delavirdine is indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents. Its use in clinical practice has been very restricted because of the high pill burden, the reluctance to use this agent if a severe reaction to nevirapine or efavirenz has occurred and the cross-resistance within this class of agents. One advantage the drug does have is its ability to raise the levels of co-administered PIs.

Resistance

Resistance to delavirdine is conferred primarily by the K103N, Y181C and Y188L mutations. The P236L mutation, which was described as a unique delavirdine-associated mutation in vitro, is only rarely seen in clinical isolates (see Fig. 204.3).

Dosage in special circumstances

Delavirdine does not require dosage adjustment in patients with renal dysfunction. No specific recommendations are available for patients with hepatic disease.

Delavirdine crosses the placenta. Carcinogenesis studies are incomplete, with teratogenicity (ventricular septal defects) shown in rats at doses equivalent to human therapeutic exposure (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicities of delavirdine are rash, nausea, fatigue and diarrhea (see Table 204.5). Delavirdine is a potent inhibitor of CYP3A4 and has the potential for serious drug interactions and toxicity with selected agents. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.7 and Table 204.8 .

Efavirenz

Description

Efavirenz — (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3, 1-benzoxazin-2-one (EFZ; see Fig. 204.4) — is one of the most widely prescribed NNRTIs because of its potency, q24h administration and lower incidence of rash compared to nevirapine.

Pharmacokinetics and distribution

Efavirenz is well absorbed with peak concentrations achieved 5 hours after oral administration. Absorption appears unaffected by administration with meals containing a moderate fat content. When administered with high-fat meals, a mean increase in AUC of 50% has been shown, and concomitant administration with high-fat meals is not recommended. Efavirenz exhibits linear pharmacokinetics. Average steady state plasma C_{min} and C_{max} following oral administration of 600mg daily are approximately 6µmol/l and 13µmol/l, respectively. [32] Efavirenz is over 99% bound to plasma proteins, predominantly albumin, and crosses the blood-brain barrier, with CSF concentrations on average 0.69% of total plasma concentrations.[38] Efavirenz is metabolized in the liver, predominantly to inactive metabolites by CYP3A4 and CYP2B6. After multiple-dose oral administration, the half-life of efavirenz is approximately 40–55 hours. Efavirenz induces CYP3A4 in vivo, but has also been shown to inhibit CYP3A4, CYP2C9 and CYP2C19 in vitro.[39]

Route and dosage

Efavirenz is administered orally. The usual adult dosage is 600mg q24h, which is now available in a single tablet formulation.

Indications

Efavirenz is indicated for the treatment of HIV-1 infection in combination with other antiretroviral agents. Clinical trials have demonstrated the comparability of efavirenz-based regimens (i.e. combined with two NRTIs) with indinavir-based regimens in patients with both high and low viral loads. The drug now plays a major role in the initial treatment of antiretroviral-naive patients. Clinical trials have also demonstrated the value of efavirenz in patients with virologic failure, but the rapid emergence of NNRTI resistance can occur if the overall regimen potency is compromised by cross-resistance to the other components of the regimen. [34]

The drug should be avoided in patients with a history of significant psychiatric illness because of its CNS side-effect profile. It is also contraindicated in pregnancy because of demonstrated teratogenicity in primates.

Resistance

Resistance to efavirenz during in-vitro passage is mediated by mutations at the following positions: Y179D, Y181C, L100I, K103N and V108I. The drug maintains some degree of activity against viruses containing only the Y181C mutation, but virologic failure rates

TABLE 204-8 -- Significant drug interactions between non-nucleoside reverse transcriptase inhibitors and other drugs.

SIGNIFICANT DRUG INTERACTIONS BETWEEN NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AND OTHER DRUGS				
		Nevirapine (NVP)	**Delavirdine (DLV)**	**Efavirenz (EFZ)**
Antiarrhythmics/cardiac	Bepridil		? Bepridil; use with caution	
	Amiodarone, lidocaine, quinidine, flecainide, propafenone		? Antiarrhythmics (concentration monitoring recommended); use with caution	
	Dihydropyridine calcium channel blockers*		? Calcium channel blocker; use with caution	
Anticoagulant	Warfarin		? Warfarin; monitor international normalized ratio (INR)	Potential to ? or ? warfarin; monitor INR
Anticonvulsants	Phenobarbital, phenytoin, carbamazepine	Use with caution; monitor anticonvulsant levels	May ? DLV levels	Potential to ? anticonvulsant and/or ? EFZ; monitor anticonvulsant levels
Antifungals	Ketoconazole	? Ketoconazole AUC 63%; ? NVP levels approximately 15–30%; not recommended		Potential to ? antifungal
Antimycobacterials	Rifampin	? NVP C_{min} approximately 37%; not recommended	? DLV AUC approximately 97%; not recommended	? EFZ AUC approximately 26%; not recommended
	Rifabutin	? NVP C_{min} approximately 16%	? Rifabutin AUC 230%, ? DLV AUC 82%; not recommended	? Rifabutin AUC approximately 38%; consider rifabutin ? 50% or doubling of rifabutin when given two or three times a week
	Clarithromycin	? NVP approximately 26%; ? clarithromycin 30%	? Clarithromycin AUC 100%; reduce clarithromycin dose in patients with renal dysfunction	? Clarithromycin AUC approximately 39%; consider use of azithromycin
Corticosteroids	Dexamethasone		? DLV; use with caution	
Immunosuppressants	Ciclosporin		? Immunosuppressant; monitor immunosuppressant levels	
	Tacrolimus			
	Rapamycin			
Lipid lowering agents	Atorvastatin, Fluvastatin		? Statin levels; use with caution or consider pravastatin	
Narcotic analgesics	Methadone	? Methadone; monitor for withdrawal	? Methadone	? Methadone AUC approximately 52%; monitor for withdrawal
Oral contraceptives	Ethinyl estradiol	? Ethinyl estradiol approximately 20%; consider alternative method of contraception	? Ethinyl estradiol	? Ethinyl estradiol approximately 37%; not well characterized; consider alternative method of contraception
Miscellaneous	Sildenafil		? Sildenafil; do not exceed 25mg sildenafil in 48-hour period	

* Dihydropyridine calcium channel blockers: amlodopine, felodipine, isradipine, nifedipine, nicardipine, nimodipine, nisoldipine

are high when this mutation is present at baseline.[40] The most common mutation encountered clinically is K103N, which confers cross-resistance to efavirenz and delavirdine. Other clinically relevant mutations are L100I, V108I, Y188L, G190S/A and P225H. High-level resistance is seen with the double mutations K103N-V108I and L100I-K103N (see Fig. 204.3).

Dosage in special circumstances

No dosage adjustment is required in patients with renal disease. Following a single-dose study in patients with chronic liver disease, efavirenz C_{max} was reduced and the half-life increased with no significant change in AUC compared to healthy volunteers.[32] Administration of the standard dose with close monitoring for toxicity is recommended in patients with liver disease.

Efavirenz crosses the placenta. Teratogenicity has been noted in primates (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicities associated with efavirenz are CNS related (e.g. impaired concentration, abnormal dreams, euphoria, anxiety and depression) and rash (see Table 204.5). As above, the drug is teratogenic in primates. Efavirenz acts as an inducer or inhibitor of CYP3A4 depending on the concomitantly administered drug. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.7 and Table 204.8 .

Selected investigational non-nucleoside reverse transcriptase inhibitors

Capravirine

Capravirine — 5-(3,5-dichlorophenyl)thio-4-isopropyl-1-(4-pyridyl) methyl-1H-imidazol-2-ylmethylcarbamate (CPV) — is active against a range of NNRTI-resistant isolates, including those bearing the K103N mutation. The drug has shown substantial antiviral activity in

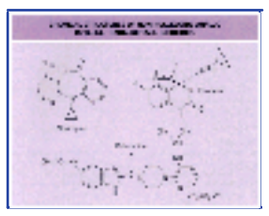

Figure 204-4 Chemical structures of approved non-nucleoside reverse transcriptase inhibitors.

a phase I trial in naive patients. Development was temporarily halted because of animal toxicity but is now continuing.

TMC 125

TMC 125 is a diarylpyrimidine compound that has potency in the nanomolar range against a broad range of NNRTI-resistant isolates. The flexibility of the molecule and its high binding affinity likely account for these favorable characteristics. The drug has demonstrated substantial antiviral activity in short-term, phase I trials in naive and experienced subjects.

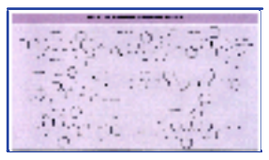

Figure 204-5 Chemical structures of approved protease inhibitors.

PROTEASE INHIBITORS

Mature HIV virions are produced as the virus buds off the cell surface and *gag* and *gag-pol* polyprotein precursors are cleaved by a virally encoded aspartyl protease. Successful inhibition of this enzyme marked a revolution in antiretroviral therapy starting in 1996. Enthusiasm for inclusion of this class of agents in initial regimens has waned with the growing awareness of the associated metabolic complications, but the value of this potent class of agents should not be forgotten. The six currently approved PIs are all peptidomimetic compounds, which bind to the active site of the enzyme and inhibit both HIV-1 and HIV-2.

Saquinavir

Description

Saquinavir — *N*-tert-butyldecahydro-2-[2(R)-hydroxy-4-phenyl-3-(S)-[[*N*-(2-quinolylcarbonyl)-L-asparaginyl]-amino]butyl](4aS,8aS)-isoquinoline-3(S)-carboxamide (SQV; Fig. 204.5) — was the first PI approved in the USA.

Pharmacokinetics and distribution

Saquinavir-hard gel capsule (hgc) is poorly bioavailable with the mean absolute bioavailability of a 600mg oral dose administered with food averaging 4%. This is presumed to be due to limited absorption and extensive first-pass metabolism. [41] The relative bioavailability of saquinavir-soft gel capsule (sgc) is estimated at over 3-fold higher than the hgc formulation. Absorption is improved upon administration with food or up to 2 hours after a meal. The mean C_{max} following oral administration of saquinavir-sgc 1200mg three times daily was 2477ng/ml. Saquinavir is approximately 97% bound to plasma proteins and is extensively hepatically metabolized to mono- and di-hydroxylated inactive compounds, primarily by CYP3A4 (>90%).[41] The half-life following intravenous administration is approximately 7 hours.

Route and dosage

Saquinavir is administered orally and is available as hard-gel and soft-gel capsule formulations. In the absence of pharmacoenhancement, the approved dose of saquinavir-hgc in adults is 600mg three times daily but the drug should not be used in this fashion. Given its poor oral bioavailability, the hgc formulation should only be prescribed with low dose ritonavir enhancement. The dose of saquinavir-sgc is 1200mg three times daily. Low-dose ritonavir is also commonly used to decrease the pill burden associated with the sgc formulation. Under investigation are the following saquinavir-ritonavir dosage regimens: 1000mg/100mg q12h and 1600mg/200mg q24h.

Indications

Saquinavir is indicated for the treatment of HIV infection in combination with other antiretroviral agents. The drug is most commonly used with low-dose ritonavir enhancement and for initial therapy is typically combined with two NRTIs. For the management of treatment-experienced patients with virologic failure, saquinavir as part of single or dual PI ritonavir-enhanced regimens can prove useful depending upon the previous regimen, the results of drug resistance testing and the number of other active agents in the regimen.

Resistance

Resistance to saquinavir is mediated principally by the L90M and to a lesser extent the G48V mutation. Other codon alterations that can contribute to saquinavir resistance include L10I, I54L, A71V/T, G73S, V77I, V82A and I84V (see Fig. 204.3). L90M is one of the major PI mutations associated with drug class cross-resistance.

Dosage in special circumstances

Saquinavir does not require dosage adjustment in patients with renal disease. The pharmacokinetics of saquinavir have not been studied in patients with liver disease. No specific dosage recommendations are available in this patient population.

TABLE 204-9 -- Drugs that are not recommended for use with protease inhibitors.

DRUGS THAT ARE NOT RECOMMENDED FOR USE WITH PROTEASE INHIBITORS						
Drug class	**Saquinavir**	**Indinavir**	**Ritonavir**	**Nelfinavir**	**Amprenavir**	**Lopinavir/r**
Anticonvulsants						
Antihistamines	Astemizole, terfenadine	Astemizole, terfenadine	Astemizole, terfenadine	Astemizole, terfenadine	Astemizole, terfenadine	Astemizole, terfenadine
Antimycobacterials	Rifampin*	Rifampin*	Rifampin*	Rifampin*	Rifampin*	Rifampin*
Cardiac			Bepridil, amiodarone, flecainide, propafenone, quinidine	Amiodarone, quinidine	Bepridil	Flecainide, propafenone
Ergot derivatives	Dihydroergotamine, ergotamine	Dihydroergotamine, ergotamine	Dihydroergotamine, ergotamine	Dihydroergotamine, ergotamine	Dihydroergotamine, ergotamine	Dihydroergotamine, ergotamine
Gastrointestinal drugs	Cisapride	Cisapride	Cisapride	Cisapride	Cisapride	Cisapride
Herbal products	St John's wort	St John's wort	St John's wort	St John's wort	St John's wort	St John's wort
Lipid lowering agents	Simvastatin, lovastatin	Simvastatin, lovastatin	Simvastatin, lovastatin	Simvastatin, lovastatin	Simvastatin, lovastatin	Simvastatin, lovastatin
Neuroleptics		Pimozide	Pimozide		Pimozide	Pimozide
Oral contraceptives					Ethinyl estradiol/norethindrone	
Sedatives/hypnotics	Midazolam, triazolam	Midazolam, triazolam	Midazolam, triazolam	Midazolam, triazolam	Midazolam, triazolam	Midazolam, triazolam

* Rifampin decreases levels of protease inhibitors. Alternative antimycobacterial agents such as rifabutin (with dosage modification) should be considered.

Saquinavir only minimally crosses the placenta. Animal studies have shown no mutagenicity or teratogenicity at 40–50% of AUC values achieved in humans (pregnancy catgory B).[10]

Adverse reactions and drug interactions

Clinically significant drug interactions are summarized in Table 204.6 , Table 204.9 and Table 204.10 .

The major toxicity associated with saquinavir is gastrointestinal symptomatology (Table 204.11). For PI class toxicities, see Table 204.4 . Saquinavir is a weak inhibitor of CYP3A4.

Ritonavir

Description

Ritonavir — 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic-acid, 5-thiazolyl-methyl ester [5S-(5R*, 8R*, 10R*, 11R*)] (RTV; see Fig. 204.5) — was the second PI approved in the USA. Its intolerability at full therapeutic doses and its potent CYP3A4 inhibitory activity have combined to position this drug largely, if not exclusively, as a pharmacoenhancer of other PIs, including saquinavir, indinavir, amprenavir and lopinavir.

Pharmacokinetics and distribution

Ritonavir's oral bioavailability is estimated to range from 60 to 80%.[42] Relative to the fasting state, the AUC of ritonavir from the capsule formulation is approximately 15% higher when administered with food. For the oral solution, the AUC is decreased 7% when administered with food.[42] Following oral administration of ritonavir 600mg q12h, the C_{max} and C_{min} were reported as 11mg/l and 4mg/l, respectively. Ritonavir is greater than 98% protein bound, both to albumin and a_1 -acid glycoprotein. Because of the high degree of protein binding, CSF concentrations are low and reported to be less than 0.05mg/l.[42] Ritonavir is extensively metabolized, primarily by CYP3A4 isoenzymes, with the CYP2D6 isoenzyme also contributing

to the production of the isopropylthiazolyl oxidation metabolite.[42] The half-life of ritonavir ranges from 3 to 5 hours.

Route and dosage

Ritonavir is administered orally and is available in capsule and oral solution formulations. It is also available as a co-formulation with lopinavir (Kaletra). When administered as the sole PI, the adult dose of ritonavir is 600mg q12h. As noted, however, ritonavir's major role is as a pharmacoenhancer of other PIs, given the gastrointestinal intolerance conferred by full doses of this agent. Pharmacoenhancement doses depend upon the co-administered PI(s) and whether an inducer of CYP3A4, such as efavirenz or nevirapine, is also included in the regimen. Most typically, ritonavir doses of 100–200mg q12h are used in pharmacoenhanced regimens.

Indications

Ritonavir in full dose carries an indication for the treatment of HIV infection in combination with other antiretroviral agents. As a pharmacoenhancer, it is approved as a co-formulation with lopinavir. It is also commonly used in combination with saquinavir, indinavir and amprenavir.

Resistance

The major mutations conferring resistance to ritonavir are V82A/F/T/S and I84V. Other important mutations include L10F/I/R/V, K20M/R, V32I, L33F, M36I, M46I/L, I54V/L, A71V/T, V77I and L90M (see Fig. 204.3). Cross-resistance between indinavir and ritonavir is nearly complete. When used in low dose as a pharmacoenhancer of a second PI, the pattern of mutations that emerges with virologic failure may be influenced by the presence of ritonavir. The ability to boost the levels of other PIs has brought into focus the importance of pharmacodynamics in the treatment of HIV infection. The relationship of achievable drug concentrations to the 50% inhibitory concentration (IC_{50}) of the individual patient's virus has reinvigorated the concept of the inhibitory quotient in treating microbial pathogens. This has resulted in an attempt to define phenotypic susceptibility cut-offs that are clinically relevant and the consideration of whether therapeutic drug level monitoring has a role in the management of patients with drug-resistant virus.

Dosage in special circumstances

Renal disease is expected to have little effect on ritonavir pharmacokinetics and no dosage modification is necessary. In patients with mild-to-moderate hepatic insufficiency, the ritonavir pharmacokinetics varied little compared to patients with normal hepatic function when the dosage was reduced by 20%.[42] In addition, the elimination half-life increased from 4.6 hours in patients with normal hepatic function to 6.3 hours in patients with moderate hepatic disease. No specific dosage recommendations are available in patients with liver disease.

Less than 10% of ritonavir appears to cross the placenta. Ritonavir was not mutagenic in bacteria or mammalian cells and teratogenicity has only been seen in rats at maternally toxic doses (pregnancy category B).[10]

Adverse reactions and drug interactions

The major toxicities associated with ritonavir are headache, diarrhea, altered taste, circumoral and peripheral paresthesias and hyperlipidemia (see Table 204.11). For PI class toxicities, see Table 204.4 . Ritonavir is the most potent inhibitor of the cytochrome P450 system of all the PIs. Ritonavir inhibits CYP3A4 and CYP2D6 and also increases glucuronosyltransferase activity. Ritonavir also induces CYP3A4 activity and has been shown to induce its own metabolism. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.9 and Table 204.10 .

Indinavir

Description

Indinavir — *N*-(2(R)-hydroxy-1(S)-indanyl)-2(R)-(phenylmethyl)-4(S)-hydroxy-5-[1-[4-(3-pyridylmethyl)-2(S)-(*N*-tert-butylcarbamoyl)piperazinyl]]pentanamide (IDV; see Fig. 204.5) — was the third PI approved in the USA and contributed substantially to ushering in the modern era of potent antiretroviral chemotherapy.

Pharmacokinetics and distribution

Indinavir is rapidly absorbed with peak concentrations occurring within 1 hour. The oral bioavailability is approximately 70%.[43] Administration of indinavir with meals containing high fat, carbohydrate, or protein significantly reduces the AUC by approximately 35–70% compared to the fasting state.[44] Food has little effect on the pharmacokinetics of indinavir when administered concomitantly with low-dose ritonavir. Steady-state peak and trough concentrations were 12.6µmol/l and 0.25µmol/l, respectively, after oral administration of 800mg q8h.[43] Indinavir is approximately 60% protein bound. Studies have shown that indinavir concentrations in the CSF are 2 and 6% of plasma concentrations 2 and 3.75 hours after administration, respectively.[45] A more recent study of indinavir pharmacokinetics in the CSF of eight adults infected with HIV found the free indinavir concentrations in the CSF to be approximately 15% of plasma levels.[46] Indinavir is extensively metabolized by CYP3A4 isoenzymes. The half-life of indinavir is approximately 1.8 hours.

Route and dosage

Indinavir is administered orally and is available as a capsule formulation. The dosage in adults is 800mg q8h in the absence of ritonavir enhancement or the concomitant use of efavirenz or nevirapine. Ritonavir reduces variation in the pharmacokinetic profile of indinavir, eliminates the food effect and converts indinavir to a twice daily agent. Indinavir/ritonavir combinations of 800mg/100mg, 800mg/200mg, 400mg/400mg and 400mg/100mg, respectively, have all been studied and are used clinically. The dose of indinavir should be increased to 1000mg q8h when used with the NNRTIs efavirenz or nevirapine because of the CYP3A4-inducing effect of these drugs (see Table 204.6). This effect can be blocked, however, by the concomitant use of ritonavir. Adequate hydration (approximately 1l of water per day) needs to be maintained to try to prevent nephrolithiasis.

Indications

Indinavir is indicated for the treatment of HIV infection in combination with other antiretroviral agents. There is a considerable published experience with indinavir establishing its clinical and long-term virologic efficacy.[47] [48] It is one of the more commonly prescribed PIs for initial therapy, particularly in patients presenting with advanced disease. It is also useful in the management of antiretroviral failure if the patient's virus isolate remains susceptible and other active drugs remain available for inclusion in the regimen.

Resistance

The major PI mutations conferring resistance to indinavir are M46I/L, V82A/F/T and I84V. Other important mutations include L10I/R/V, K20M/R, L24I, V32I, M36I, I54V, A71V/T, G73S/A, V77I and L90M (see Fig. 204.3). Alterations at three or more codons are necessary before substantial changes in phenotypic susceptibility can be detected. Indinavir and ritonavir cross-resistance is nearly complete.

Dosage in special circumstances

Indinavir's pharmacokinetics are likely little affected by renal disease. In patients with mild-to-moderate hepatic insufficiency and cirrhosis, indinavir AUC was 60% higher following a single 400mg dose and the half-life was increased to 2.8 hours. It is recommended that the

TABLE 204-10 -- Significant drug interactions between protease inhibitors and other drugs.

SIGNIFICANT DRUG INTERACTIONS BETWEEN PROTEASE INHIBITORS AND OTHER DRUGS							
		Saquinavir (SQV)	**Indinavir (IDV)**	**Ritonavir (RTV)**	**Nelfinavir (NFV)**	**Amprenavir (APV)**	**Lopinavir/r (LPV; LPV/r)**
Antiarrhythmics/cardiac	Amiodarone Lidocaine, quinidine, flecainide, propafenone, bepridil			May ? antiarrhythmic levels; use with caution			May ? antiarrhythmic levels; use with caution
	Dihydropyridine calcium channel blockers*		May ? calcium channel blocker levels; use with caution	May ? calcium channel blocker levels; use with caution			May ? calcium channel blocker levels; use with caution
Anticoagulant	Warfarin			May ? warfarin levels; use with caution			May affect warfarin levels; use with caution

Anticonvulsants	Phenobarbital, phenytoin, carbamazepine	May ? SQV levels; use with caution	May ? IDV levels; use with caution	May ? carbamazepine levels; may ? phenytoin levels; monitor anticonvulsant levels; use with caution	May ? NFV levels; monitor anticonvulsant levels; use with caution	May ? APV levels; monitor anticonvulsant levels; use with caution	May ? LPV levels; use with caution
Antifungals	Ketoconazole itraconazole	? SQV levels	Ketoconazole ? IDV AUC approximately 68%; itraconazole ? IDV levels; use with caution	? Ketoconazole AUC approximately 3.4-fold; ? RTV AUC approximately 18%	? NFV AUC approximately 35%	? APV AUC approximately 31%; ? ketoconazole AUC approximately 44%	? Ketoconazole AUC approximately 3-fold; ? itraconazole levels; use with caution
Antimycobacterials	Rifampin	? SQV AUC approximately 84%; not recommended unless using SQV + RTV	? IDV levels approximately 89%; not recommended	? RTV AUC approximately 35%; not recommended	? NFV AUC approximately 82%; not recommended	? APV AUC approximately 82%; not recommended	? LPV AUC approximately 75%
	Rifabutin	? SQV AUC approximately 43%; consider rifabutin 150mg 3 times per week when using SQV + RTV	? IDV AUC approximately 32%; ? rifabutin AUC approximately 204%; use with caution	? Rifabutin AUC 4-fold; consider rifabutin dose reduction to 150mg every other day or 3 times per week	? Rifabutin AUC approximately 207%; ? NFV AUC approximately 32%; consider rifabutin dose decrease by 50%	? APV AUC approximately 15%; ? rifabutin AUC approximately 193%; consider rifabutin dose decrease to 150mg daily or 300mg 3 times per week	? Rifabutin AUC approximately 3-fold; consider rifabutin dose reduction to 150mg every other day or 3 times per week
	Clarithromycin	? Clarithromycin AUC approximately 45%; ? SQV AUC approximately 177%	? Clarithromycin AUC approximately 53%; ? IDV AUC approximately 29%	? Clarithromycin AUC 77%; ? RTV AUC approximately 12%; ? clarithromycin dose in patients with renal dysfunction		? APV AUC approximately 18%	? Clarithromycin levels; ? clarithromycin dose in patients with renal dysfunction
Corticosteroid	Dexamethasone	May ? SQV levels; use with caution		May ? dexamethasone levels; use with caution			May ? LPV levels; use with caution
Immunosuppressants	Ciclosporin tacrolimus, rapamycin			May ? immunosuppressant levels; monitor levels; use with caution	May ? ciclosporin and tacrolimus levels; monitor levels		May ? immunosuppressant levels; monitor levels; use with caution
Lipid lowering agents	Atorvastatin, fluvastatin	May ? statin levels; use with caution	May ? statin levels; use with caution	May ? statin levels; use with caution	? Atorvastatin levels approximately 74%; use with caution	May ? statin levels; use with caution	? Atorvastatin AUC approximately 5.8-fold; use with caution
Narcotic analgesics	Methadone			? Methadone AUC approximately 36%	May ? methadone levels	? Methadone AUC approximately 35%	? Methadone AUC approximately 53%
	Meperidine			? Meperidine AUC approximately 62% and ? normeperidine AUC approximately 47%;			
Neuroleptics	Perphenazine, risperidone, thioridazine			Potential for ? neuroleptic levels; use with caution			
Oral contraceptives	Ethinyl estradiol		? Ethinyl estradiol AUC approximately 24%	? Ethinyl estradiol AUC approximately 40%; consider alternative method of contraception	? Ethinyl estradiol AUC approximately 47%; consider alternative method of contraception	? APV AUC approximately 22%; not recommended	? Ethinyl estradiol AUC approximately 42%; consider alternative method of contraception
Sedative/hypnotics	Clorazepate, diazepam, estazolam, flurazepam, zolpidem			Potential for ? sedative/hypnotics levels; use with caution			
Miscellaneous	Sildenafil	? Sildenafil AUC approximately 210%; do not exceed 25 mg sildenafil in 48-hour period	? Sildenafil AUC approximately 340%; do not exceed 25mg sildenafil in 48-hour period	? Sildenafil AUC approximately 11-fold; do not exceed 25mg sildenafil in 48-hour period	Potential for ? sildenafil levels; do not exceed 25mg sildenafil in 48-hour period	Potential for ? sildenafil levels; do not exceed 25mg sildenafil in 48-hour period	Potential for ? sildenafil levels; do not exceed 25mg sildenafil in 48-hour period
	Desipramine			? Desipramine AUC 145%; consider ? dosage of desipramine			
	Theophylline			? Theophylline AUC approximately 43%			

* Dihydropyridine calcium channel blockers: amlodopine, felodipine, isradipine, nifedipine, nicardipine, nimodipine, nisoldipine

TABLE 204-11 -- List of protease inhibitor specific toxicities.

LIST OF PROTEASE INHIBITOR SPECIFIC TOXICITIES					
Saquinavir	**Indinavir**	**Ritonavir**	**Nelfinavir**	**Amprenavir**	**Lopinavir/r**
Nausea	Nephrolithiasis	Nausea	Diarrhea	Nausea	Nausea
Diarrhea	Nausea	Vomiting		Vomiting	Vomiting
Abdominal pain	Headache	Diarrhea		Diarrhea	Diarrhea
Dyspepsia	Asthenia	Paresthesias (circumoral and extremities)		Rash	Asthenia
Headache	Dizziness	Hepatitis		Oral paresthesias	
	Rash	Pancreatitis			
		Asthenia			
		Taste perversion			
		? Uric acid			
		? Creatine phosphokinase			

indinavir dose be reduced to 600mg q8h in patients with mild-to-moderate hepatic insufficiency due to cirrhosis.[43]

Indinavir crosses the placenta. In rats, carcinogenicity (an increased incidence of thyroid adenomas) and teratogenicity (increased incidence of supernumerary ribs and unilateral anophthalmia) have been shown (pregnancy category C).[10]

Adverse reactions and drug interactions

The major toxicities associated with indinavir include asymptomatic rises in indirect bilirubin and nephrolithiasis (see Table 204.11). For PI class toxicities, see Table 204.4 . Indinavir is a moderate inhibitor of CYP3A4. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.9 and Table 204.10 .

Nelfinavir

Description

Nelfinavir — [3S-(3R*, 4aR*, 8aR*, 2'S*, 3'S*)]-2-[2'-hydroxy-3'-phenylthiomethyl-4'-aza-5'-oxo-5'-(2''-methyl-3''-hydroxyphenyl)pentyl[decahydroisoquinoline-3-*N*-t-butyl-carboxamide (NFV; see Fig. 204.5) — was the fourth PI approved in the USA.

Pharmacokinetics and distribution

Following administration of nelfinavir 1250mg q12h, peak and trough plasma concentrations were reported as 4mg/l and 1.3–2.2mg/l, respectively. When administered in the fasting state, the AUC of nelfinavir is reduced 27–50%. [49] Nelfinavir should be administered with food. It is 98% bound to plasma proteins. Nelfinavir is hepatically metabolized by CYP450 isoenzymes, primarily CYP3A4 followed by CYP2C19, CYP2D6 and CYP2C9, to two active metabolites with the major oxidative metabolite (M8) exhibiting comparable in-vitro antiviral activity.[49] [50] The half-life of nelfinavir is approximately 3.5–5 hours.

Route and dosage

Nelfinavir is administered orally and is available in tablet and oral powder formulations. The two approved dosage regimens in adults are 750mg q8h and 1250mg q12h.

Indications

Nelfinavir is indicated for the treatment of HIV infection in combination with other antiretroviral agents. It has been a mainstay of potent antiretroviral regimens for several years and has more commonly been used in the treatment of antiretroviral-naive patients (combined with two NRTIs) than as part of salvage regimens. It also has an established record of safety in pregnant women. Comparative clinical trials suggest that nelfinavir combined with two NRTIs is a less potent regimen than lopinavir-ritonavir or efavirenz-based regimens.[51]

Resistance

Resistance to nelfinavir may evolve along one of two pathways — either the D30N or the L90M. The factors that determine which pathway is chosen are not completely defined, but baseline polymorphisms and viral subtype may play a role. The D30N mutation by itself does not confer resistance to the other PIs and therefore successful alternative regimens in the face of virologic failure can often be constructed. When the L90M pathway is chosen, drug class cross-resistance may result. Additional relevant mutations include L10F/I, M36I, M46I/L, A71V/T, V77I, V82A/F/T/S, I84V and N88D/S (see Fig. 204.3).

Dosage in special circumstances

Dosage modification is not necessary in patients with renal disease. No dosage recommendations are available for patients with hepatic disease.

Nelfinavir concentrations in cord blood are low or undetectable compared to maternal concentrations (pregnancy category B). In animal studies, nelfinavir has not been found to be carcinogenic or teratogenic.[10]

Adverse reactions and drug interactions

The principal toxicity associated with nelfinavir is diarrhea (see Table 204.11). For PI class toxicities, see Table 204.4 . Nelfinavir is a moderate inhibitor of CYP3A4. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.9 and Table 204.10 .

Amprenavir

Description

Amprenavir — (3S)-tetrahydro-3-furyl-*N*-[(1S, 2R)-3-(4-amino-*N*-isobutylbenzenesulfonamido)-1-benzyl-2-hydroxypropyl] carbamate (APV; see Fig. 204.5) — was the fifth PI approved in the USA. An amprenavir prodrug, GW 433908, designed to reduce pill size and burden, is under investigation.

Pharmacokinetics and distribution

Amprenavir is rapidly absorbed following oral administration. Peak concentrations of approximately 7.66µg/ml are achieved within 1 to

2 hours following administration of 1200mg q12h. The relative bioavailability of amprenavir oral solution is 14% less than amprenavir oral capsules. Effects of food on amprenavir pharmacokinetics (decreased AUC 23%) are not clinically significant except with high-fat meals, which should be avoided.[52] Amprenavir is approximately 90% protein bound, predominantly to a_1 -acid glycoprotein. Amprenavir is hepatically metabolized by CYP3A4. The plasma half-life of amprenavir ranges from 7.1 to 10.6 hours.

Route and dosage

Amprenavir is administered orally and is available as capsule and oral solution formulations. The dosage in adults is 1200mg q12h when administered without ritonavir enhancement (which increases amprenavir levels) or concomitant efavirenz (which diminishes amprenavir levels). The size and number of amprenavir pills at standard dosing poses a problem for tolerance and drug adherence. Therefore, amprenavir is mostly used with low-dose ritonavir enhancement at a dose of 600mg/100mg q12h, respectively. The dose to use when combined with efavirenz is uncertain but some advise increasing the amprenavir/ritonavir doses to 750mg/200mg q12h, respectively, to ensure adequate amprenavir levels in the face of drug-resistant virus.

Indications

Amprenavir is indicated for the treatment of HIV infection in combination with other antiretroviral agents. Although efficacy for naive patients has been established, the pill burden has generally restricted the drug to the management of treatment-experienced persons. Clinical trials have demonstrated the efficacy of amprenavir in this circumstance[34] and it is now commonly used as part of dual ritonavir-enhanced regimens with lopinavir.

Resistance

Viral isolates resistant to the other approved PIs may remain susceptible to amprenavir. The major mutations conferring amprenavir resistance are I50V (a signature mutation) and I84V. Other important mutations include L10F/I/R/V, V32I, M46I/L, I47V, I54L/V/M, G73S and L90M (see Fig. 204.3).

Dosage in special circumstances

The effects of renal disease on amprenavir pharmacokinetics are limited and no dosage modification is necessary. The AUC of amprenavir in patients with moderate and severe cirrhosis is significantly greater than in patients with normal hepatic function. It is recommended that patients with a Child-Pugh score of 5–8 receive a reduced amprenavir dosage of 450mg q12h, and patients with Child-Pugh score of 9 to 12 receive a reduced amprenavir dosage of 300mg q12h. The oral solution of amprenavir contains propylene glycol (55%) and is contraindicated in patients with renal failure or hepatic failure, in pregnant women and in patients receiving disulfiram or metronidazole.

Amprenavir is classified as pregnancy category C. In rabbits and rats, an increased incidence of abortions (rabbits) and ossification defects (rabbits and rats) have been shown.[10]

Adverse reactions and drug interactions

The major toxicities associated with amprenavir include gastrointestinal symptomatology and rash (see Table 204.11). For PI class toxicities, see Table 204.4 . Amprenavir is a moderate inhibitor of CYP3A4. Clinically significant drug interactions are summarized in Table 204.6 , Table 204.9 and Table 204.10 .

Lopinavir (co-formulated with ritonavir)

Description

Lopinavir — [1S-[1R*, (R*), 3R*, 4R*]]-*N*-[4-[[2,6-dimethylphenoxy) acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl] tetrahydro-alpha-(1-methylethyl)-2-oxo-1(2H)-pyrimidine-acetamide (LPV; see Fig. 204.5) — was the sixth PI approved in the USA. It is co-formulated with ritonavir (lopinavir/r).

Pharmacokinetics and distribution

Lopinavir is poorly bioavailable because it is rapidly metabolized by NADPH and cytochrome P450 3A4/5-dependent enzyme systems. As such, lopinavir is co-formulated with ritonavir. Ritonavir inhibits the metabolism of lopinavir such that the AUC for lopinavir is increased over 100-fold when co-administered with ritonavir.[53] Administration of lopinavir/r with food increased the AUC by 48 and 80% for the capsule and liquid formulations, respectively. Lopinavir is approximately 98–99% protein bound, both to albumin and a_1 -acid glycoprotein. At steady state, lopinavir peak and trough concentrations were reported as 9.6mg/l and 5.5mg/l following twice daily lopinavir/r 400/100mg.[53] Lopinavir undergoes extensive oxidative metabolism via CYP3A. The half-life of lopinavir/r has been reported to be approximately 6 hours.

Route and dosage

Lopinavir/r is administered orally and is available in capsule and oral solution formulations. Lopinavir/r capsules contain 133.3mg of lopinavir and 33.3mg of ritonavir. The usual adult dosage is 400mg/100mg q12h, respectively. When administered with efavirenz, the dose should be increased to 533mg/133mg q12h of lopinavir/r, respectively (see Table 204.6).

Indications

Lopinavir/r is indicated for the treatment of HIV infection in combination with other antiretroviral agents. In antiretroviral-naive patients, lopinavir/r in combination with two NRTIs has shown superior antiviral activity to nelfinavir plus two NRTIs.[51] Lopinavir/r has also shown substantial virologic efficacy in the treatment of NNRTI-naive subjects with both single and multiple PI experience. Lopinavir/r is also used in dual PI-enhanced regimens with amprenavir or saquinavir in the management of patients with drug-resistant virus. Formal studies of the efficacy of this latter approach are underway.

Resistance

The major mutations conferring lopinavir resistance are L10F/I/R/V, K20M/R, L24I, V32I, L33F, M46I/L, I47V, I50V, F53L, I54V/L, L63P, A71V/T, G73S, V82A/F/T/S, I84V and L90M (see Fig. 204.3). The pharmacoenhancement of lopinavir by ritonavir permits the drug to be active against viruses with up to 10- and possibly 40-fold changes in susceptibility to lopinavir.

Dosage in special circumstances

Dosage adjustment is not necessary in patients with renal disease. Close monitoring is advised in patients with liver disease. No specific dosage recommendations are available for this patient population.

Lopinavir has been shown to cross the placenta in rats. Developmental toxicities (skeletal variations and delayed ossification) have been shown in rats at maternally toxic doses (pregnancy category C).[10]

Adverse reactions and drug interactions

The principal toxicities associated with lopinavir/r include gastrointestinal symptomatology, hyperlipidemia and liver enzyme abnormalities (see Table 204.11). For PI class toxicities, see Table 204.4 . Lopinavir is a moderate inhibitor of CYP3A4 and the combination of lopinavir with ritonavir (a potent CYP3A4 inhibitor) is likely to have drug interactions similar to those of full-dose ritonavir alone, but potentially to a lesser degree. Clinically significant drug interactions are summarized in Table

204.6 , Table 204.9 and Table 204.10

Atazanavir

Atazanavir sulfate — dimethyl (3S, 8S, 9S, 12S)-9-benzyl-3,12,di-tert-butyl-8-hydroxy-4,11-dioxo-6-(p-2pyridylbenzyl)-2,5,6,10,13-pentaazatetradecanedioate sulfate (ATV, Zrivada) — is an azapeptide PI whose advantage is q24h administration and the lack of induction of hyperlipidemia. Clinical trials reported to date in antiretroviralnaive patients have suggested that atazanavir sulfate in combination with two NRTIs has comparable efficacy to nelfinavir and efavirenz-based regimens, although in the latter study the efavirenz arm appeared to perform less well than in previously reported trials.[55] The use of atazanavir sulfate in treatment-experienced persons is currently under study. The drug, when administered without ritonavir pharmacoenhancement, has been reported to engender a signature mutation, I50L, in the setting of virologic failure. This mutation confers diminished susceptibility to atazanavir sulfate and appears to induce sensitization to other PIs. This characteristic may be exploitable in future treatment strategies. Other relevant mutations include V32I, M46I, I54L, A71V, V82A, I84V, N88S and L90M (see Fig. 204.3). The drug, in general, has been well tolerated, with an absence of hyperlipidemia a notable feature. The major toxicity noted thus far has been an asymptomatic rise in indirect bilirubinemia, not dissimilar to that seen with indinavir. PR and QT prolongation have also been seen at higher doses of the drug, but the clinical significance of this remains to be clarified.

Selected investigational protease inhibitors

Tipranavir

Tipranavir disodium — [R-(R*, R*)]-N-[3-[1-[5,6-dihydro-4-hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H-pyran-3-yl]propyl]phenyl]5-(trifluoromethyl)-2-pyridinesulfonamide disodium salt (TPV) — is a dihydropyrone, non-peptidic PI whose molecular advantage is the flexibility it can demonstrate in binding to the active site of the HIV protease. It is highly active against viral strains with diminished susceptibility to the approved PIs.[54] Tipranavir disodium is being developed for co-administration with low-dose ritonavir to diminish the pill burden and permit q12h dosing. The major toxicity seen thus far has been gastrointestinal-associated, which appears to be dose related. Clinical trial results reported to date have been promising with phase III studies pending. Its role will likely be in the management of patients with PI-resistant virus.

TMC 114

TMC 114 is a highly potent PI with in-vitro anti-HIV activity in the nanomolar range. The drug is a flexible molecule that binds tightly into the active site of the HIV protease. These qualities help to confer its potency against PI-resistant variants. Phase I trials of this agent are underway.

ENTRY INHIBITORS

Remarkable advances have been made in the past few years in our understanding of the HIV entry process. Specifically, the identification of HIV co-receptors (e.g. CCR5 and CXCR4) and the understanding of the events involved in fusion of the viral envelope with the cell membrane have created new therapeutic targets. Entry inhibitors can be divided into three subcategories: attachment inhibitors, chemokine receptor antagonists and fusion inhibitors.[56] [57] Of these, the fusion inhibitors have demonstrated proven clinical efficacy and have come the furthest in development.

Enfuvirtide

Description

Enfuvirtide — C_{204} H_{301} N_{51} O_{64} , T-20 — is a 36-amino acid peptide derived from the HR2 region of HIV-1_{LAI} , which binds to the HR1 region of the HIV gp41 fusion peptide and prevents the coil-coil zipping reaction, which leads to six-helix bundle formation and eventual viral-host membrane fusion (Fig. 204.6). Enfuvirtide is active against both R5 and X4 viral strains with susceptibility influenced by the time that the gp41 HR1 target is exposed to the drug as the viral entry process proceeds. Co-receptor density and affinity may influence the susceptibility of HIV strains to enfuvirtide.

Pharmacokinetics and distribution

Enfuvirtide is rapidly digested by peptidases in the gastrointestinal tract and consequently is not orally bioavailable. Following subcutaneous dosing of enfuvirtide, the mean C_{max} and C_{min} at steady state were reported as 2626ng/ml and 972ng/ml, respectively, at 50mg q12h, and 4725ng/ml and 1774ng/ml, respectively, at 100mg q12h.[58] The time to maximal concentrations was approximately 4 hours. The serum half-life of enfuvirtide after intravenous administration has been reported as approximately 2 hours, but more sustained concentrations throughout the 12-hour dosing interval have been reported following subcutaneous administration.

Route and dosage

Enfuvirtide is administered by subcutaneous injection. The adult dosage is 90mg q12h.

Indications

Enfuvirtide is indicated for the treatment of HIV infection in combination with other antiretroviral agents. Given the parenteral nature of the drug and its activity against drug-resistant virus, its role lies in the management of patients with treatment failure in whom other options are constrained. It is important to try to have at least two (and preferably more) other active drugs to administer with enfuvirtide so that enfuvirtide-resistant virus does not quickly emerge. Two large phase III trials have demonstrated the efficacy of enfuvirtide when combined with background therapy optimized with the assistance of drug resistance testing. The enfuvirtide groups in both studies averaged a 0.9–1.0 $\log_{10}$ greater drop in plasma HIV-1 RNA than the control groups at 24 weeks.[59]

Resistance

Resistance to enfuvirtide has been documented to occur in vivo with most mutations mapping to positions 36–45 of the amino terminal (HR1 region) of gp41. The most commonly described mutations are G36D/S, I37V, V38A/M, Q39R, N42T and N43D (see Fig. 204.3). Interestingly, enfuvirtide-resistant viruses may be less fit than wild-type isolates. Thus an immunologic (and presumably clinical) benefit may persist beyond the point of virologic failure, similar to what has been described for PIs.

Dosage in special circumstances

The dose of the drug should not be influenced by renal or hepatic dysfunction given its peptide nature.

Adverse reactions and drug interactions

The major toxicity of enfuvirtide is injection site reaction, which occurs in a large proportion of patients to varying degrees. Bacterial infection at the injection sites can occur and has resulted in occasional bacteremia. A hypersensitivity syndrome has also been described.

Selected investigational entry inhibitors

Fusion inhibitors

T-1249 is a 39-amino acid peptide that binds to an overlapping but not identical region on the HR1 region of the HIV gp41 fusion peptide as enfuvirtide. It is somewhat more potent than enfuvirtide,

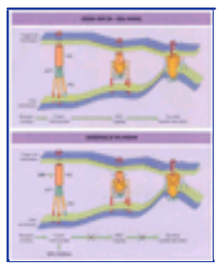

Figure 204-6 Mechanism of HIV fusion with host cell membrane and its inhibition by enfuvirtide (ENF, T-20).

can be administered subcutaneously q24h and is active against enfuvirtide-resistant viruses in vitro. In a phase I study, dose-dependent decreases in plasma HIV-1 RNA have been reported.

Attachment inhibitors

Attachment inhibitors bind to HIV gp120 and prevent virion attachment to the cell surface receptors. Two compounds in development are PRO 542 and BMS 806. PRO 542 is a tetravalent CD4-IgG2 fusion protein that binds to gp120. Proof of principle has been established in a single-dose, phase I study.[60] BMS 806 is an orally bioavailable molecule that inhibits gp120/CD4 interactions by direct binding to gp120.[61] Resistance can be selected in vitro and is mediated by mutations in the binding site on gp120. Clinical trials of BMS 806 are planned.

Chemokine receptor antagonists

Chemokine receptor antagonists targeting both CCR5 and CXCR4 are under development. Approaches include a monoclonal antibody to CCR5 (PRO 140) and small molecule inhibitors of CCR5 (SCH-C, SCH-D, UK 427857, TAK compounds) and CXCR4 (AMD 3100, AMD 070).[62] [63] [64] [65] Proof of principle in phase I human studies has been reported for SCH-C and AMD 3100. Development of the latter has been halted, however, due to limited potency.

INTEGRASE INHIBITORS

HIV integrase is essential for viral replication and has been a recognized target for several years. However, only recently have the characteristics of true integrase inhibitors been described and effective in-vitro screening approaches defined.[66] [67] Two compounds, S-1360, a diketo acid, and L-870810, a naphthyridine compound, are currently in phase I trials. L-870810 has shown substantial antiviral activity in the SHIV rhesus macaque model.

FUTURE APPROACHES

Although entry and integrase inhibition are likely to represent the next major breakthroughs in antiretroviral chemotherapy, a number of other novel approaches are being intensively investigated and bear watching over the next few years. These include inhibition of nucleocapsid zinc fingers, alpha-defensins, interference with the HIV gag chaperone protein, TSG 101, and the exploding area of RNA interference (RNAi).[68] [69] [70] [71]

TABLE 204-12 -- List of selected investigational agents.

LIST OF SELECTED INVESTIGATIONAL AGENTS				
Nucleoside reverse transcriptase inhibitors	**Non-nucleoside reverse transcriptase inhibitors**	**Protease inhibitors**	**Entry inhibitors**	**Integrase inhibitors**
Amdoxovir	Capravirine	GW 433908	T-1249	S-1360
ACH-126, 443	TMC 125	Tipranavir	PRO 542	L-870810
SPD-754		TMC 114	BMS 806	
			PRO 140	
			SCH-C	
			SCH-D	
			UK 427857	
			AMD 070	

CONCLUSION

The field of antiretroviral therapy has shown dramatic growth over the past 17 years with five drug classes now available to clinicians. On the near horizon, clinicians and patients are likely to have more choices within these existing drug classes as well as one additional class (i.e. integrase inhibitors) available (Table 204.12). Along with this will be the challenge of investigating and applying new drug regimens such as combinations of entry inhibitors and entry and integrase inhibitors, together with existing agents to formulate new strategies of therapy. On the more distant horizon, new molecular approaches hold promise for continued fundamental improvements in the treatment of those with HIV infection.

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Chapter 205 - Drugs for Herpesvirus Infections

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INTRODUCTION

Over the last 25 years, the armamentarium of antiviral compounds has expanded from a handful of drugs with low potency and substantial toxicity to over two dozen drugs now in widespread clinical use. Initial attempts at antiviral drug development were thwarted by the complex interactions between viral and host cellular metabolism. Compounds that blocked viral replication also frequently caused unacceptable host cell toxicity. Many of the early drugs evaluated as antiviral therapeutics were serendipitously discovered through screening of compounds for anticancer activity. More recently, better understanding of molecular virology has permitted rational design of drugs specifically targeted for unique steps in the viral replication cycle. While most of the first-generation drugs were developed for treatment of infections caused by herpesviruses, new compounds are now entering clinical use for treatment of a variety of viruses, including respiratory viruses, enteroviruses and hepatitis viruses. Another emerging trend is the use of combinations of drugs for therapy of serious viral infections, pioneered in the field of HIV therapy.

DRUGS FOR TREATMENT OF HSV AND VZV INFECTIONS

Aciclovir and valaciclovir

Mechanism of action and in vitro activity

Aciclovir, 9-(2-hydroxyethoxymethyl) guanine, an acyclic analogue of guanosine, is a selective inhibitor of the replication of herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV).[1] Valaciclovir is an orally administered prodrug of aciclovir with improved pharmacokinetic properties. Aciclovir is converted to its monophosphate derivative by virus-encoded thymidine kinase (TK), a reaction that does not occur to any significant extent in uninfected cells (Fig. 205.1). Subsequent diphosphorylation and triphosphorylation steps are catalyzed by cellular kinases, producing high concentrations of aciclovir triphosphate within HSV- or VZV-infected cells. Aciclovir triphosphate inhibits viral DNA synthesis by competing with deoxyguanosine triphosphate as a substrate for viral DNA polymerase. Since aciclovir triphosphate lacks the 3'-hydroxyl group required for further DNA chain elongation, incorporation into viral DNA results in obligate chain termination. Viral DNA polymerase has much higher affinity for aciclovir triphosphate than does cellular DNA polymerase, resulting in little incorporation of aciclovir into cellular DNA. Aciclovir exhibits good *in vitro* activity against HSV-1, HSV-2 and VZV, with median inhibitory concentrations necessary to reduce plaque counts by 50% (IC_{50}) of 0.04, 0.10 and 0.50µg/ml, respectively. Human cytomegalovirus (CMV) is not inhibited by aciclovir at clinically achievable concentrations.

Pharmacokinetics and distribution

Following oral administration, aciclovir is slowly and incompletely absorbed, with bio-availability of about 15–30%. After oral administration of multiple doses of 200 or 800mg of aciclovir, mean plasma peak concentrations at steady state are about 0.6 and 1.6µg/ml, respectively. Steady-state peak plasma aciclovir concentrations after intravenous doses of 5 or 10mg/kg of body weight every 8 hours are about 10 and 20µg/ml, respectively. Plasma protein binding is less than 20%. Aciclovir penetrates well into most tissues, including the central nervous system (Table 205.1). Relative to plasma, aciclovir concentrations in cerebrospinal fluid, aqueous humor and breast milk are about 50%, 37% and 300%, respectively. Aciclovir is minimally metabolized and about 85% of an administered dose is excreted unchanged in the urine via glomerular filtration and renal tubular secretion. The terminal plasma half-life of aciclovir is 2–3 hours in adults and 3–4 hours in neonates with normal renal function, but is extended to about 20 hours in anuric subjects.

Valaciclovir is an orally administered prodrug of aciclovir designed to overcome the problem of poor oral bio-availability.[2] Valaciclovir, the L-valine ester of aciclovir, is well absorbed from the gastrointestinal tract via a stereospecific transporter and undergoes essentially complete first- pass conversion in the gut and liver to yield aciclovir and L-valine. With this prodrug formulation, bio-availability of aciclovir is about 54%, yielding peak plasma aciclovir levels that are 3–5-fold higher than those achieved with oral administration of the parent compound. Oral doses of 500mg or 1000mg of valaciclovir produce peak plasma aciclovir concentrations of 3.3 and 5–6µg/ml, respectively. After administration of valaciclovir at a dose of 2gm orally four times daily, plasma aciclovir area under the curve (AUC) values approximate those produced by aciclovir given intravenously at a dose of 10mg/kg every 8 hours. Following enzymatic conversion of valaciclovir to aciclovir, the antiviral spectrum of activity, pharmacokinetic properties and excretion are the same as those described above.

Route of administration and dosage

Aciclovir is available in topical, oral and intravenous formulations. Outside the United States, aciclovir is also available as a 3% preparation for ophthalmologic use. The dermatologic preparation consists of 5% aciclovir in a polyethylene glycol ointment base. Topical aciclovir is intended for treatment of minor mucocutaneous or genital HSV infections. Oral aciclovir preparations include a 200mg capsule, 400 and 800mg tablets, and a liquid suspension (200mg/5ml). Aciclovir sodium for intravenous infusion is supplied as a sterile water-soluble powder that must be reconstituted and diluted to a concentration of 50mg/ml.

The recommended dose of aciclovir will vary with the specific indication (see Table 205.2). Because of the greater intrinsic resistance of VZV to aciclovir, the doses required for treating VZV infections are higher than those used for HSV infections. In adults with normal renal function, oral aciclovir is given at a dose of 200mg (for HSV) to 800mg (for VZV) five times daily. The recommended dose of intravenous aciclovir is 5mg/kg every 8 hours for HSV infections or 10mg/kg every 8 hours for VZV infections, although higher doses

Figure 205-1 Activation of aciclovir is dependent on monophosphorylation via viral thymidine kinase (TK). Aciclovir triphosphate inhibits the activity of viral DNA polymerase, thus blocking viral replication. Penciclovir and ganciclovir are activated by similar mechanisms.

(12–15mg/kg) are sometimes used for life-threatening infections, especially in immunocompromised patients.

Valaciclovir is available as 500mg and 1000mg tablets. The recommended doses are 500mg twice daily for episodic treatment of recurrent

TABLE 205-1 -- Tissue distribution of aciclovir.*

TISSUE DISTRIBUTION OF ACICLOVIR*							
	CSF/CNS	Lung	Liver	Kidney	Breast milk	Amniotic fluid	Heart

Aciclovir concentration (µg/ml)	10	26.7	26.7	206	66.7	<0.1–2.6	26.7

* Based on a steady-state plasma concentration (20.6µg/ml) after intravenous aciclovir dosing of 15mg/kg every 8 hours.[38]

TABLE 205-2 -- Indications for aciclovir therapy.

INDICATIONS FOR ACICLOVIR THERAPY	
Infection	**Route and dosage**†
Genital HSV	
Initial episode	200mg po 5 times/d (or 400mg tid) × 10 days
Initial episode with complications	5mg/kg iv q8h × 5–7 days
Recurrent episodes	200mg po 5 times/d (or 400mg tid) × 5 days
Suppression	400mg po bid daily
Mucocutaneous HSV in immunocompromised patient	400mg po 5 times/d × 10–14 days* ; or 5mg/kg iv q8h × 10 days
Disseminated or visceral HSV (including encephalitis)	10–15mg/kg iv q8h × 14–21 days
Neonatal HSV	10–15mg/kg iv q8h × 14–21 days*
Varicella (chickenpox)	
Normal host	20mg/kg (max. 800mg) po 4–5 times/d × 5 days
Immunocompromised patient	10–15mg/kg iv q8h × 7–10 days
Herpes zoster (shingles)	
Normal host	800mg po 5 times/d × 7–10 days
Immunocompromised patient (disseminated or visceral VZV)	10–15mg/kg iv q8h × 7–10 days

† Given doses are indicated for patients with normal renal function
* This indication is not approved by the US Food and Drug Administration

genital HSV infections and 1000mg three times daily for treatment of herpes zoster. A suspension preparation is not available.

Indications

HSV infections

Initial episodes of genital HSV infection can be treated with topical, oral or intravenous aciclovir. Intravenous aciclovir is the most effective treatment for initial genital herpes, but is not usually practical and should be reserved for patients with severe local disease or systemic complications. Oral aciclovir (200mg five times daily) is nearly as effective as intravenous therapy and significantly reduces the duration of symptoms, virus shedding and time to healing. Topical aciclovir is less effective than oral or intravenous therapy for initial genital herpes. Aciclovir treatment of acute HSV infection does not alter the risk of subsequent recurrences. Lesions associated with recurrent genital herpes are less severe than those seen in primary infection and the benefit from episodic therapy with oral aciclovir is relatively modest. A more effective approach in patients with frequent recurrences of genital herpes is to give aciclovir daily to prevent HSV reactivation. Daily administration of aciclovir will reduce the frequency of genital HSV recurrences in 90% of patients and a significant proportion will have no subsequent recurrences while taking suppressive medication. Titration of the dose of aciclovir (beginning with 400mg twice daily) may be required to establish the most effective dose. Periodic interruption of suppression to reassess the need for continued prophylaxis is recommended. There is no evidence of cumulative toxicity or emergence of drug-resistant

HSV in immunocompetent patients even after years of suppressive therapy.[3] Aciclovir suppression will also significantly reduce (but not eliminate) the frequency of asymptomatic viral shedding.[4]

In a study comparing valaciclovir (1000mg twice daily) with aciclovir (200mg five times daily) for treatment of first-episode genital herpes, both drugs were well tolerated and equivalent in efficacy.[5] For episodic treatment of recurrent genital herpes, valaciclovir (500mg twice daily for 3 days) reduced the duration of viral shedding and accelerated pain resolution and lesion healing.[6] For suppression of genital herpes, valaciclovir doses ranging from 250mg to 1000mg once daily and from 250mg to 500mg twice daily have been evaluated in clinical trials. A dose — response relationship is evident across the various valaciclovir regimens, with better suppression at higher doses, although all were significantly more effective than placebo. The recommended starting dose of valaciclovir for genital herpes suppression is 500–1000mg daily; patients who have breakthrough recurrences on this regimen may require 500mg twice daily for effective suppression. In general, aciclovir and valaciclovir are equally potent for suppression of genital herpes, although the once-daily dosing regimen for valaciclovir may be more convenient.

Oral aciclovir therapy can reduce the duration of symptoms for children with acute HSV gingivostomatitis. Topical aciclovir is relatively ineffective for recurrent herpes labialis. Treatment of herpes labialis with oral aciclovir (400mg five times daily for 5 days) offers some clinical benefit, if initiated during the prodromal or erythematous stage of lesion evolution. Oral aciclovir can be used for short-term prophylaxis of recurrent herpes labialis in situations where exposure to a known stimulus such as ultraviolet light is anticipated (e.g. a snow skiing or beach holiday). Patients with frequent recurrences of herpes labialis may benefit from long-term suppressive therapy with oral aciclovir (400mg twice daily), which can reduce the frequency of clinical recurrences by about 50%. A high-dose, short-course regimen of valaciclovir (e.g. 2g bid for 1 day) for treatment of herpes labialis has recently been approved in the United States.

Aciclovir prophylaxis of HSV infections is highly effective in severely immunocompromised patients, particularly those undergoing induction chemotherapy or organ transplantation. In bone marrow transplant recipients, aciclovir reduces the incidence of symptomatic HSV infection from approximately 70% to 10% and is especially valuable during the first 30 days following transplantation. A sequential regimen of intravenous followed by oral aciclovir (at doses ranging from 200mg three times daily to 800mg twice daily) for 3–6 months can virtually eliminate symptomatic HSV infections in organ transplant recipients. In immunocompromised patients with established mucocutaneous HSV infections, intravenous (5–10mg/kg every 8 hours) or oral aciclovir (400mg five times daily) can significantly reduce the duration of pain and accelerate lesion healing. Clinical experience suggests that intravenous aciclovir (10mg/kg every 8 hours) is the treatment of choice for disseminated or visceral HSV infection (e.g. pneumonitis, hepatitis, esophagitis, etc.) in immunocompromised patients, although data from controlled clinical trials are lacking.

HSV infection of the central nervous system is associated with substantial morbidity and mortality despite the use of antiviral therapy. Aciclovir was proven superior to vidarabine for HSV encephalitis and should be given intravenously at a dose of 10–15mg/kg for 14–21 days. Even with aggressive aciclovir therapy, the mortality rate at 6 months is 19% for patients with HSV encephalitis.

Neonatal herpes is a potentially devastating infection that can develop in infants born to mothers who are actively shedding HSV from the genital tract at the time of delivery. Intravenous aciclovir therapy can significantly reduce both morbidity and mortality among these infants. Studies are under way to establish the value of long-term aciclovir suppression for preventing progressive neurologic deterioration among surviving infants. Another promising approach currently under evaluation is the use of oral aciclovir or valaciclovir suppression during the third trimester in pregnant women with genital herpes. Data suggest that prophylaxis can significantly reduce the requirement for cesarian section due to active genital HSV lesions present at the onset of labor.[7]

VZV infections

Oral aciclovir therapy for immunocompetent children with chickenpox will reduce both the duration of fever and total lesion count if treatment is begun within 24 hours of disease onset. However, the benefits of aciclovir administration are relatively modest and many pediatricians consider antiviral treatment of chickenpox to be optional.

In immunocompetent children, the dosage of oral aciclovir for chickenpox is 20mg/kg (up to a maximum of 800mg) four times daily. Varicella can be a more severe disease in adolescents and adults, with higher lesion counts and a greater risk of complications, especially pneumonitis. For this reason, aciclovir therapy (800mg orally five times daily for 7 days) is recommended for adolescents and adults who present within 24–48 hours of disease onset. Valaciclovir is also likely to be effective in this setting, but data from controlled clinical trials are lacking. Because of the high frequency of visceral involvement in immunocompromised children (or adults) with chickenpox, aggressive therapy with intravenous aciclovir (10mg/kg or 500mg/m^2 every 8 hours for 7–10 days) is warranted. Controlled trials of intravenous aciclovir in immunocompromised patients with varicella clearly demonstrated a significant reduction in the frequency of progression to VZV pneumonitis. Although no data from controlled trials are available, clinical experience suggests that intravenous aciclovir is the treatment of choice for patients with VZV infections complicated by visceral involvement (e.g. pneumonitis, encephalitis, etc.).

For treatment of herpes zoster in immunocompetent adults, oral aciclovir (800mg five times daily for 7 days) accelerates cutaneous healing and reduces the severity of acute neuritis. Benefits are maximized when treatment is initiated within 48 hours of appearance of lesions. Aciclovir does not alter the incidence of postherpetic neuralgia, but can accelerate the resolution of pain.[8] Aciclovir is especially beneficial for preventing ocular complications in patients with herpes zoster ophthalmicus. Severely immunocompromised patients (e.g. bone marrow transplant, cancer chemotherapy, etc.) with herpes zoster are at high risk for disseminated VZV infection and should be treated with intravenous aciclovir (10mg/kg every 8 hours). AIDS patients with herpes zoster can usually be treated effectively with oral therapy.

Valaciclovir (1000mg three times daily for 7 days) was compared with aciclovir (800mg five times daily for 7 days) in over 1000 immunocompetent patients (>50 years of age) with herpes zoster.[9] The progression of cutaneous healing was similar in the two treatment groups. Patients in the valaciclovir treatment group had a slightly shorter duration of zoster-associated pain (38 days versus 51 days). Extending valaciclovir therapy to 14 days did not result in any additional benefit. Both aciclovir and valaciclovir are effective for treatment of localized herpes zoster in immunocompetent patients if therapy is initiated within 72 hours of rash onset. Valaciclovir has the advantage of a simpler dosing regimen.

Other viral infections

While aciclovir is ineffective for established CMV infections, high-dose oral aciclovir or valaciclovir may have value for CMV prophylaxis in high-risk populations such as AIDS patients and organ transplant recipients.[10] Administration of aciclovir does not alter the course of infectious mononucleosis, but can induce regression

TABLE 205-3 -- Aciclovir dosage modification for renal impairment.

ACICLOVIR DOSAGE MODIFICATION FOR RENAL IMPAIRMENT			
Normal dosage regimen	**CrCl (ml/min/1.73m^2)**	**Adjusted dosage regimen**	
		Dose	**Dosing interval (h)**
Aciclovir 200mg po q4h	>10	200mg	4 (5 × /d)
	0–10	200mg	12
Aciclovir 400mg po q12h	>10	400mg	12
	0–10	200mg	12
Aciclovir 800mg po q4h	>25	800mg	4 (5 × /d)
	10–25	800mg	8
	0–10	800mg	12
Aciclovir 5mg/kg iv q8h	>50	5mg/kg	8
	25–50	5mg/kg	12
	10–25	5mg/kg	24
	0–10	2.5mg/kg	24
Aciclovir 10mg/kg iv q8h	>50	10mg/kg	8
	25–50	10mg/kg	12
	10–25	10mg/kg	24
	0–10	5mg/kg	24
CrCl, creatinine clearance			

TABLE 205-4 -- Valaciclovir dosage modification for renal impairment.

VALACICLOVIR DOSAGE MODIFICATION FOR RENAL IMPAIRMENT			
Normal dosage regimen	**CrCl (ml/min)**	**Adjusted dosage regimen**	
		Dose (mg)	**Dosing interval (h)**
Valaciclovir 1000mg q8h	>50	1000	8
	30–49	1000	12
	10–29	1000	24
	<10	500	24
Valaciclovir 1000mg q12h	>30	1000	12
	10–29	1000	24
	<10	500	24
Valaciclovir 500mg q12h	>30	500	12
	<30	500	24
Valaciclovir 1000mg q24h	>30	1000	24
	<30	500	24
Valaciclovir 500mg q24h	>30	500	24
	<30	500	48
CrCl, creatinine clearance			

of EBV-induced oral hairy leukoplakia in HIV-infected patients. Aciclovir is not effective for treatment of chronic fatigue syndrome. It is considered the drug of choice for therapy of rare human infections caused by cercopithecine herpesvirus-1 (B virus). Aciclovir is not active against HIV; studies of the survival benefit of aciclovir therapy in AIDS patients have reached varying conclusions.

Dosage in special circumstances

Aciclovir is cleared primarily by renal mechanisms and dosage modification of aciclovir and valaciclovir is required for patients with significant renal dysfunction (see

Table 205.3 , Table 205.4). The mean elimination half-life of aciclovir after a single 1g dose of valaciclovir is about 14 hours in patients with end-stage renal disease.[2] No specific dosage modification is required for patients with hepatic impairment. Aciclovir and valaciclovir are not approved for use in pregnancy, but have been widely used to treat serious HSV and VZV infections in pregnant women without evidence of maternal or fetal toxicity.[11]

Aciclovir AUC values after oral valaciclovir dosing are slightly higher in elderly individuals when compared with younger control groups, presumably due to declines in creatinine clearance associated with aging. Because no liquid valaciclovir preparation is available, experience with that drug in young children is limited.

Adverse reactions

Aciclovir is an extremely well-tolerated drug with very few significant adverse effects. With intravenous aciclovir therapy, inflammation and phlebitis may occasionally occur following localized drug extravasation. Renal dysfunction resulting from accumulation of aciclovir crystals in the kidney has been observed following administration of large doses of aciclovir by rapid intravenous infusion, but is uncommon and usually reversible. The risk of nephrotoxicity can be minimized by administering aciclovir by slow infusion (over 1 hour) and ensuring adequate hydration. A few reports have linked administration of aciclovir with CNS disturbances, including agitation, hallucination,

disorientation, tremors and mild clonus. Neurotoxicity has most often been recognized in elderly patients with underlying CNS abnormalities and renal insufficiency. Patients receiving oral aciclovir therapy occasionally complain of nausea, diarrhea, rash or headache, but these possible adverse effects have not differed significantly between aciclovir and placebo recipients in large-scale clinical trials. Oral aciclovir therapy is rarely associated with neurotoxicity or nephrotoxicity. The safety of oral aciclovir for long-term administration has been established in patients receiving the drug for over 5 years for suppression of recurrent genital herpes.

At standard doses, valaciclovir is a very well-tolerated drug with few significant adverse effects.[12] Headache, nausea and abdominal pain have been reported, but the incidence of these symptoms has not differed between valaciclovir and placebo recipients in clinical studies. A syndrome of thrombotic microangiopathy (TMA) was described in some HIV-infected patients, receiving high-dose valaciclovir (8g/day) for prevention of CMV disease in a clinical trial; a causal relationship between high-dose valaciclovir and TMA has not been proven. The TMA-like syndrome, which is characterized by fever, microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction, has not been observed in immunocompetent patients receiving valaciclovir at approved doses (up to 3g/day). There is no contraindication to using valaciclovir at standard doses in HIV-infected patients.

Significant interactions between aciclovir and other drugs are extremely uncommon. Probenecid decreases the renal clearance of aciclovir and can prolong the plasma excretion half-life. Additive aciclovir-induced nephrotoxicity in patients receiving concomitant cyclosporin-A therapy has been suggested, but does not appear to be clinically important. Lethargy has been reported in a few patients receiving both aciclovir and zidovudine, but a causative role for aciclovir has not been established. Concomitant administration of cimetidine and probenecid reduces the rate of valaciclovir conversion to aciclovir, but the effect is not clinically significant.

Resistance

HSV resistance to aciclovir can develop through mutation of the viral genes encoding thymidine kinase or DNA polymerase. Most aciclovir-resistant clinical HSV isolates are TK deficient and are therefore unable to phosphorylate aciclovir.[13] Aciclovir-resistant HSV isolates are recovered only from immunocompromised patients. The most common clinical presentation of infection caused by aciclovir-resistant HSV is chronic, progressive mucocutaneous lesions. Approximately 5–6% of HSV isolates recovered from HIV-seropositive patients are aciclovir resistant (IC_{50} >2.0µg/ml). Aciclovir-resistant VZV isolates (less frequently encountered than resistant HSV isolates) are recovered almost exclusively from AIDS patients. Most clinical disease caused by aciclovir-resistant VZV has been limited to cutaneous involvement, often characterized by atypical lesions. TK-deficient HSV and VZV isolates will also be resistant to other drugs that require TK for activation, including ganciclovir and penciclovir. The drug of choice for treatment of aciclovir-resistant HSV or VZV disease is foscarnet, a viral DNA polymerase inhibitor that is not dependent on TK for activation.

Penciclovir and famciclovir

Mechanism of action and in vitro activity

Penciclovir, 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine, is an acyclic guanine derivative that is similar to aciclovir in structure, mechanism of action and spectrum of antiviral activity. In HSV-or VZV-infected cells, penciclovir is first monophosphorylated by virally encoded TK and then further phosphorylated to the triphosphate moiety by cellular enzymes. Penciclovir triphosphate blocks viral DNA synthesis through competitive inhibition of viral DNA polymerase. Unlike aciclovir triphosphate, penciclovir triphosphate is not an obligate chain terminator and can be incorporated into the extending DNA chain. Compared with aciclovir triphosphate, intracellular concentrations of penciclovir triphosphate are much higher. For example, the half-life values for penciclovir triphosphate and aciclovir triphosphate in HSV-1 infected cells are 10 hours and 0.7 hour, respectively. However, this potential advantage is offset by a much lower affinity of penciclovir triphosphate for viral DNA polymerase. The *in vitro* activities of penciclovir against HSV-1, HSV-2 and VZV are similar to those of aciclovir, with median IC_{50} values of 0.4, 1.5 and 4.0µg/ml respectively, in MRC-5 cells.[14]

Just as valaciclovir is a prodrug of aciclovir, famciclovir is a prodrug of penciclovir. Because penciclovir is very poorly absorbed, famciclovir (the diacetyl ester of 6-deoxy-penciclovir) was developed as the oral formulation. The first acetyl side chain of famciclovir is cleaved by esterases found in the intestinal wall. On first pass through the liver, the second acetyl group is removed and oxidation catalyzed by aldehyde oxidase occurs at the 6 position, yielding penciclovir, the active antiviral compound.

Pharmacokinetics and distribution

Penciclovir is very poorly absorbed after oral administration. Intravenous infusion of penciclovir at 10mg/kg over 1 hour yields a peak plasma concentration of 12.1µg/ml. Plasma protein binding of penciclovir is <20%. The drug is cleared by renal tubular secretion and passive filtration. The plasma elimination half-life of penciclovir is about 2 hours and approximately 70% of the administered dose is recovered unchanged in the urine.

When administered as the famciclovir prodrug, the bio-availability of penciclovir is about 77%. Following a single oral dose of 250mg or 500mg of famciclovir, peak plasma penciclovir concentrations of 1.9 and 3.5µg/ml are achieved at 1 hour. The pharmacokinetics of penciclovir are linear and dose independent over a famciclovir dosing range of 125–750mg. Food slows famciclovir absorption and lowers the peak plasma penciclovir concentration, but does not alter the AUC value.

Route of administration and dosage

Famciclovir is available as 125mg, 250mg and 500mg tablets. Recommended dosages will vary with indication. The usual dose of famciclovir is 125mg twice daily for episodic therapy of recurrent genital herpes and 500mg three times daily for herpes zoster. The intravenous preparation of penciclovir has not been commercially released. A topical preparation of penciclovir is available as a 1% cream for treatment of HSV labialis.

Indications

Genital HSV infections.

Oral famciclovir reduced the duration of viral shedding and was comparable to oral aciclovir for accelerating healing and symptom resolution in patients with first-episode genital herpes. Famciclovir has not been approved for treatment of initial genital herpes in the United States, but 250mg orally three times daily for 5 days is likely to be effective.

Famciclovir was significantly superior to placebo for treatment of recurrent genital herpes.[15] When therapy was initiated by the patient at the time of symptom onset, famciclovir accelerated the events of healing and time to loss of pain.[15] The recommended dosage of famciclovir for episodic therapy of recurrent genital herpes is 125mg twice daily for 5 days.

Famciclovir is also effective for suppression of recurrent genital herpes.[16] In a multinational study of 455 patients, the time to first genital herpes recurrence was 336

days in the group receiving

TABLE 205-5 -- Oral antiviral therapy for genital herpes.

ORAL ANTIVIRAL THERAPY FOR GENITAL HERPES†			
Drugs	**Initial episode**	**Recurrent episode**	**Suppression**
Aciclovir	200mg 5 times/d (or 400mg tid*) × 10 days	200mg 5 times/d (or 400mg tid*) × 5 days	400mg bid daily
Famciclovir	125mg tid × 10 days*	125mg bid × 5 days	250mg bid daily
Valaciclovir	1000mg bid × 10 days	500mg bid × 3–5 days	500 or 1000mg once daily

† Recommended doses for immunocompetent adults with normal renal function
* This treatment regimen is not approved by the US Food and Drug Administration

famciclovir (250mg twice daily), compared with 47 days in the placebo group.[16] In the same study, 72% of subjects treated with famciclovir 250mg twice daily were recurrence free at 12 months, compared with 22% of placebo recipients.

Three drugs (aciclovir, valaciclovir and famciclovir) with proven efficacy for long-term suppression of recurrent genital herpes are now available. Few data from direct comparative trials have been published to guide the clinician in selecting the most effective treatment (see Table 205.5). All three of the compounds are safe and well tolerated. Considerations in selecting the appropriate drug may include cost and dosing convenience. For any patient, the goal is to establish a suppressive regimen that is effective, economical and convenient. For these drugs, a dose-response relationship exists, meaning that higher total daily doses generally produce more complete suppression. Clinicians may need to titrate the daily dose of the selected drug to identify optimal treatment for an individual patient.

Herpes labialis

Topical 1% penciclovir cream is applied every 2 hours while awake for treatment of herpes labialis. In placebo-controlled trials, topical penciclovir reduced the duration of viral shedding and accelerated lesion healing and pain resolution. Time to lesion healing was 0.7 days faster (4.8 days versus 5.5 days) for penciclovir-treated patients compared to the control group.

HSV infections in AIDS patients

Famciclovir (500mg twice daily) is comparable to aciclovir (400mg five times daily) for treatment of recurrent mucocutaneous (orolabial and anogenital) infections in HIV-infected patients. In a placebo-controlled trial, famciclovir (500mg twice daily) was also shown to be highly effective for suppression of recurrent mucocutaneous HSV infections in HIV-seropositive individuals.[17]

Herpes zoster

Famciclovir has been evaluated for treatment of dermatomal herpes zoster in immunocompetent patients. In a placebo-controlled clinical trial, famciclovir accelerated cutaneous healing and reduced the duration of viral shedding and postherpetic neuralgia.[18] In a subset of subjects over 50 years of age, the duration of postherpetic neuralgia was reduced from a median of 163 days to 63 days in the placebo and famciclovir treatment groups, respectively. [18] In the United States, the recommended dose of famciclovir for uncomplicated herpes zoster is 500mg three times daily. Doses of 250mg three times daily and 750mg once daily are approved in Europe and the United Kingdom.

Three drugs (aciclovir, valaciclovir and famciclovir) are currently available for treatment of uncomplicated herpes zoster in immunocompetent patients (see Table 205.6). The drugs are all well tolerated and appear to be comparable in clinical efficacy. In a large randomized clinical trial, valaciclovir and famciclovir were shown to be therapeutically

TABLE 205-6 -- Oral antiviral therapy for herpes zoster.

ORAL ANTIVIRAL THERAPY FOR HERPES ZOSTER†
Treatment options
• Aciclovir 800mg q4h (5 times daily) × 7–10 days
• Famciclovir 500mg q8h (3 times daily) × 7 days
• Valaciclovir 1000mg q8h (3 times daily) × 7 days

† Recommended doses for immunocompetent adults with normal renal function

equivalent for treatment of herpes zoster.[19] Because of their improved pharmacokinetic profiles and simpler dosing regimens, valaciclovir and famciclovir are preferred over aciclovir for this indication.

Herpes zoster in immunocompromised patients.

In a study of herpes zoster in bone marrow transplant or cancer patients, famciclovir (500mg three times daily) or aciclovir (800mg five times daily) for 10 days were shown to be therapeutically equivalent.[20] Famciclovir was also effective in an open-label study of herpes zoster therapy in HIV-seropositive patients.

Dosage in special circumstances.

Penciclovir is cleared predominantly by renal mechanisms, so adjustments of famciclovir dosing are required in patients with advanced renal insufficiency, as shown in Table 205.7 . In patients with hepatic insufficiency, the rate of conversion of famciclovir to penciclovir is decreased, but the plasma AUC value for penciclovir is not significantly changed; no famciclovir dosage modification in hepatic impairment is necessary.[21] Plasma penciclovir concentrations are slightly higher in elderly patients treated with famciclovir due to age-related reduction in glomerular filtration rates, but dosage modifications on the basis of age are not required. Absorption after topical application is minimal and no dosage modifications are required for use of penciclovir 1% cream. No liquid preparation of famciclovir is currently available and few data regarding use in small children are available. Famciclovir has not been approved for use during pregnancy.

Adverse reactions

Safety data collected from over 3000 patients involved in clinical studies of famciclovir have shown the drug to be very safe and well tolerated.[22] The most frequently reported adverse experiences have included headache, nausea and diarrhea, but the frequency of these events was similar in both famciclovir and placebo recipients.

No clinically significant drug interactions have been noted with famciclovir. Co-administration of famciclovir with cimetidine or theophylline will increase the penciclovir AUC by about 20%. Co-administration of famciclovir and digoxin results in a 19% increase in the peak digoxin concentration, but no change in the AUC.

Topical penciclovir 1% cream is associated with no significant toxicities and has no known drug interactions.

TABLE 205-7 -- Famciclovir dosage modification for renal impairment.

FAMCICLOVIR DOSAGE MODIFICATION FOR RENAL IMPAIRMENT			
Normal dosage regimen	**CrCl (ml/min)**	**Adjusted dosage regimen**	
		Dose (mg)	**Dosing interval (h)**
Famciclovir 500mg q8h	>60	500	8
	40–59	500	12
	20–39	500	24
	<20	250	24
	HD	250	Post-HD
Famciclovir 125mg q12h	=40	125	12
	20–39	125	24
	<20	125	24
	HD	125	Post-HD
Famciclovir 250mg q12h	>40	250	12
	20–39	125	12
	<20	125	24
	HD	125	Post-HD
Famciclovir 500mg q12h	=40	500	12
	20–39	500	24
	<20	250	24
	HD	250	Post-HD
CrCl, creatinine clearance			
FCV, famciclovir			
HD, hemodialysis			
Post-HD, after each hemodialysis			

Resistance

The majority of clinically encountered aciclovir-resistant HSV and VZV isolates are TK deficient and thus will also be resistant to penciclovir, which requires viral TK for activation. However, some HSV strains that are aciclovir resistant by virtue of altered TK or DNA polymerase mutations may retain susceptibility to penciclovir. In general, however, penciclovir or famciclovir should not be considered as appropriate drugs for treatment of infections caused by aciclovir-resistant HSV or VZV.

Other drugs

Vidarabine

Vidarabine (adenine arabinoside) was the first intravenous antiviral drug accepted for widespread clinical use. Intravenous vidarabine was shown to be effective for herpes simplex encephalitis, neonatal HSV infections and for HSV and VZV infections in immunocompromised patients. Vidarabine, however, was not effective for aciclovir-resistant HSV infections in AIDS patients. Vidarabine 3% ophthalmic ointment is used for treatment of HSV keratoconjunctivitis. Vidarabine has now largely been replaced by more effective and less toxic antiviral drugs.

Trifluridine

Trifluridine is a fluorinated pyrimidine nucleoside with good *in vitro* activity against HSV. Trifluridine triphosphate is a competitive inhibitor of HSV DNA polymerase. Trifluridine is too toxic for systemic administration, but has been used successfully as a 1% ophthalmic solution for topical therapy of HSV keratitis. Topical trifluridine has also been used with moderate success for treatment of aciclovir-resistant mucocutaneous infections in AIDS patients.

Idoxuridine

Idoxuridine is an iodinated thymidine derivative with activity against HSV. Use of idoxurdine has been limited to topical application, since systemic administration is associated with significant myelosuppression. Idoxuridine (in topical 1% solution and 0.5% ointment formulations) has been used successfully for treatment of HSV keratitis, but has largely been replaced by topical trifluridine and aciclovir for this indication. Topical application of 15% idoxuridine in dimethyl sulfoxide was shown to shorten the course of herpes labialis in a placebo-controlled trial, and is available in Europe but not in the United States.

Brivudin

Brivudin (bromovinyldeoxyuridine; BVDU) is a highly potent antiviral agent with selective activity against HSV-1 and VZV. The drug has been evaluated for herpes zoster and varicella in both immunocompetent and immunocompromised populations and appears to be therapeutically equivalent to aciclovir. Because of concerns about potential toxicity, commercial development of brivudin has halted in most countries. The drug is available in Germany as a 125mg tablet and as a 0.1% ointment for ophthalmologic use.

n-Docosanol

n-Docosanol is a 22-carbon fatty alcohol with *in vitro* activity against several enveloped viruses, including HSV-1 and HSV-2. The drug acts by interfering with viral entry into target cells. In a multicenter, placebo-controlled study of 743 patients with recurrent herpes labialis (pooled data from two separate studies), 10% docosanol cream or placebo was applied five times a day within 12 hours of onset of prodrome or erythema. Median time to healing was reduced by 17 hours in the docosanol treatment group. n-Docosanol is available over the counter in a 2g tube of 10% cream and is indicated for recurrences of herpes labialis. It is to be applied to the area five times each day with the beginning of prodomal symptoms for a maximum of 10 days.[23]

DRUGS FOR TREATMENT OF CYTOMEGALOVIRUS INFECTIONS

Ganciclovir and valganciclovir

Mechanism of action and in vitro activity

Ganciclovir, 9-[(1,3-dihydroxy-2-propoxy) methyl] guanine, is a nucleoside analogue that is structurally similar to aciclovir, but has a hydroxymethyl group at the 3'

position of the acyclic side chain.[24] This

relatively minor structural modification accounts for enhanced activity of ganciclovir against human CMV and also for the drug's greater toxicity. Ganciclovir triphosphate is a potent inhibitor of herpesvirus DNA replication, acting as both an inhibitor of and a substrate for viral DNA polymerase.[25] In HSV- or VZV-infected cells, monophosphorylation of ganciclovir is induced by viral TK, as also occurs with aciclovir. In CMV-infected cells, ganciclovir monophosphorylation is carried out by a protein kinase encoded by the UL97 gene. The di- and triphosphorylation steps are mediated by cellular kinases. On a molar basis, aciclovir triphosphate is actually a more potent inhibitor of CMV than is ganciclovir triphosphate. However, aciclovir is a poor substrate for phosphorylation by the UL97 gene product; consequently, the concentration of ganciclovir triphosphate in CMV-infected cells is 10-fold higher than that of aciclovir triphosphate. Furthermore, the half-life of ganciclovir triphosphate in CMV-infected cells is 16.5 hours, compared with 2.5 hours for aciclovir triphosphate. Ganciclovir triphosphate does not function as a chain terminator, and can be incorporated into elongating viral DNA (and, to a much lesser extent, human DNA) where it functions to slow DNA chain extension.

Ganciclovir and aciclovir have approximately comparable *in vitro* activity against HSV-1, HSV-2 and VZV. However, ganciclovir is much more active against CMV, with IC_{50} values of 0.1–1.8µg/ml against clinical isolates.

Pharmacokinetics and distribution

Intravenous infusion of ganciclovir at a dose of 5mg/kg yields peak and trough plasma levels of approximately 8 and 1µg/ml. Plasma protein binding is 1–2%. Reported plasma-to-CSF ratios for ganciclovir have ranged from 24% to 70%. Ganciclovir is not metabolized and is cleared by renal mechanisms, with an elimination half-life of about 3 hours. Ganciclovir is poorly absorbed after oral administration, with bio-availability of only 5–9%. Following oral dosing of ganciclovir at 1000mg three times daily, steady-state plasma peak and trough concentrations of 1.2 and 0.2µg/ml are achieved. To overcome the limited oral bio-availability of ganciclovir, a prodrug called valganciclovir has been developed.[26]

Valganciclovir, the L-valyl ester of ganciclovir, is rapidly and almost completely hydrolyzed to ganciclovir in the liver and intestinal wall. Bio-availability of ganciclovir is about 60% from the prodrug formulation and is significantly increased with food administration. Maximum plasma ganciclovir concentrations are 4–5-fold higher than those achieved after oral dosing with the parent drug. Oral valganciclovir doses of 450mg and 875mg once daily for 3 days produced peak plasma ganciclovir concentrations of 3.3 and 6.1µg/ml, respectively. The AUC of ganciclovir after administration of 900mg valganciclovir is about 26µg/ml/h, which is comparable to the AUC following administration of ganciclovir dosed at 5mg/kg intravenously.

Route of administration and dosage

Ganciclovir is available as an oral capsule, an intravenous formulation and as a delayed-release intraocular implant device. Recommended doses will vary with the indication. For treatment of acute CMV disease, the usual dose of intravenous ganciclovir is 5mg/kg every 12 hours. Oral ganciclovir, supplied as 250mg capsules, can be used for maintenance therapy of CMV disease at a dosage of 1000–2000mg three times daily, but has largely been replaced by oral valganciclovir.

Valganciclovir is available as a 450mg tablet. Recommended dose for induction therapy of acute CMV retinitis is 900mg by mouth with food twice daily for a total of 21 days, followed by maintenance therapy at a dose of 900mg by mouth once daily with food.

Indications

CMV retinitis

Ganciclovir was the first drug approved for treatment of CMV retinitis, a sight-threatening disease that occurs in AIDS patients and other immunocompromised hosts. The therapeutic approach for CMV retinitis is to halt disease progression with high-dose induction therapy, then prevent disease relapse by maintenance therapy continued for the duration of the immunosuppression.[27] The usual dose of ganciclovir for induction therapy is 5mg/kg given intravenous every 12 hours for 14–21 days. In uncontrolled studies, ganciclovir therapy halted progression of CMV retinitis in over 80% of patients. Without long-term maintenance therapy, virtually all AIDS patients will experience a relapse of retinitis within 30 days after induction therapy. Continuation of maintenance therapy can extend the interval to first relapse to a median of about 75 days. The usual maintenance regimen with intravenous ganciclovir is 5mg/kg once daily. High-dose oral ganciclovir (4500–6000mg daily) is almost as effective as intravenous ganciclovir for maintenance therapy and may be associated with fewer complications.

For oral therapy of CMV retinitis, ganciclovir has largely been supplanted by valganciclovir. In a study of 160 HIV-positive patients with newly diagnosed CMV retinitis, induction therapy with valganciclovir 900mg orally twice daily was shown to be as effective as ganciclovir given intravenously at 5mg/kg every 12 hours in halting progression of retinitis during the first 4 weeks of therapy. [28] Those receiving induction with valganciclovir experienced more diarrhea than those who received intravenous ganciclovir (19% vs 10%, $p = 0.11$), while those treated with intravenous ganciclovir developed more catheter-related events (9% versus 4%). The incidence of neutropenia was about 14% in both groups.[28] CMV retinitis is currently the only approved indication for valganciclovir.

Intravitreal injections of ganciclovir have been used effectively for treatment of CMV retinitis, although ganciclovir intraocular implants are a better option for patients who cannot tolerate systemic ganciclovir therapy. Median time to relapse of CMV retinitis is longer with ganciclovir intraocular implants then with oral or intravenous ganciclovir and their use should be strongly considered in patients whose retinal lesions are imminently sight threatening.[29] However, there is potential morbidity associated with surgical implantation of the devices, which must be replaced about every 6 months. Unlike systemic ganciclovir therapy, an intraocular implant will not prevent development of CMV retinitis in the contralateral eye or CMV disease in other organs.

Ganciclovir, valganciclovir, foscarnet and cidofovir (see below) are all effective for initial and maintenance therapy of CMV retinitis in AIDS patients (see Table 205.8). All of these drugs can be associated with significant toxicity; drug selection in an individual patient hinges, to some extent, on which adverse effects would be most tolerable. Ganciclovir is primarily myelosuppressive, while foscarnet and cidofovir are nephrotoxic. Ganciclovir would be preferred in a patient who has baseline renal dysfunction or who requires therapy with other nephrotoxic drugs. Conversely, foscarnet might be a better choice in a patient with significant baseline neutropenia. Despite the survival benefits for AIDS patients shown for foscarnet therapy in some studies, most clinicians use ganciclovir or valganciclovir for initial therapy on the basis of its more predictable adverse effects. Combination therapy (e.g. ganciclovir plus foscarnet) may be more effective for CMV disease in selected patients.

CMV gastrointestinal disease

In immunocompromised patients, CMV can cause esophagitis, gastritis, enteritis and especially colitis. Anecdotal data suggest that intravenous ganciclovir is effective for CMV colitis, although data from controlled studies are limited. In a placebo-controlled trial in AIDS

TABLE 205-8 -- Systemic antiviral therapy for CMV retinitis.

SYSTEMIC ANTIVIRAL THERAPY FOR CMV RETINITIS†		
Drugs	**Induction therapy***	**Maintenance therapy***
Ganciclovir	5mg/kg iv q12h × 14–21 days	5mg/kg iv daily or 1000–2000mg po tid daily
Valganciclovir	900mg po bid × 14–21 days	900mg po daily
Foscarnet	90mg/kg iv q12h (or 60mg/kg iv q8h) × 14–21 days	90–120mg/kg iv daily
Cidofovir	5mg/kg iv weekly × 2–3 weeks	5mg/kg iv every other week

† Recommended doses for adults with normal renal function
* Other therapeutic options include intraocular drug implants or intravitreal drug injections

patients with CMV colitis, intravenous ganciclovir (5mg/kg twice daily) reduced virus shedding, improved mucosal appearance by colonoscopic examination and reduced the incidence of extracolonic CMV disease; however, symptom scores between the ganciclovir and placebo groups were similar.[30] A placebo-controlled trial of ganciclovir for CMV gastrointestinal disease in bone marrow transplant recipients also failed to demonstrate symptomatic improvement. Despite these findings, ganciclovir continues to be used for treatment of CMV gastrointestinal disease on the strength of clinical experience. There is no consensus regarding the necessity or duration of maintenance therapy in this setting.

CMV pneumonitis

CMV pulmonary infections following allogeneic bone marrow transplantation are associated with very high mortality rates. In early clinical studies, ganciclovir therapy of CMV pneumonia did not result in improved survival. More recent studies have demonstrated that combination therapy with ganciclovir plus intravenous immune globulin is more effective than either intervention alone, resulting in survival rates over 50%. A commonly used regimen for active CMV pneumonia is intravenous ganciclovir 5mg/kg twice daily plus intravenous immune globulin (0.5g/kg every other day) for 14–21 days, followed by maintenance therapy with ganciclovir 5mg/kg daily for at least 2 more weeks.

Other CMV infections

Case reports and uncontrolled clinical trials have ascribed benefit to ganciclovir therapy for a variety of indications. In solid organ transplant recipients, ganciclovir has been reported to be effective for CMV hepatitis, CMV pneumonia and disseminated CMV syndrome. Improvement in CMV-induced polyradiculopathy and encephalitis has been described in AIDS patients treated with ganciclovir. Controlled studies are required to accurately assess the value of ganciclovir for these indications.

Prophylaxis of CMV disease

Intravenous ganciclovir administered to bone marrow transplant patients either pretransplantation or at the time of engraftment significantly reduced the incidence of CMV disease.[31] [32] However, ganciclovir prophylaxis resulted in significant neutropenia, thus offsetting any survival benefit. An alternative scheme is to withhold ganciclovir until there is early laboratory evidence (e.g. by PCR or antigenemia assay) of CMV activation. [33] This approach permits initiation of therapy before CMV disease becomes symptomatic, while avoiding the risk of neutropenia associated with long-term ganciclovir administration. However, this 'pre-emptive' therapy approach is currently limited by lack of a sensitive, specific and readily available CMV diagnostic assay.

Benefits of intravenous ganciclovir therapy for prophylaxis of CMV infection in solid organ transplant recipients have varied with the transplant type, immunosuppressive regimen and CMV serologic status of the donor and recipient. [34] In general, high-risk patients (donor CMV positive, recipient CMV negative) require long-term (100 days) rather than short-term (28 days) prophylaxis to effectively prevent CMV disease. The 'pre-emptive therapy' approach discussed above has also been successfully employed for management of CMV disease in solid organ transplant recipients.[35] Oral ganciclovir and valganciclovir have shown promise for CMV prophylaxis in solid organ transplant recipients.

Dosage in special circumstances

Because ganciclovir is cleared by renal mechanisms, dosage reduction is necessary in patients with creatinine clearance of <70ml/min (see Table 205.9).[25] About 50% of an administered dose is removed during 4 hours of hemodialysis and dosing after dialysis is recommended. Valganciclovir dosage adjustment is required for patients with creatinine clearance <60ml/min; the drug is not recommended for patients on hemodialysis. No dosage adjustments for hepatic impairment are necessary. Ganciclovir is mutagenic, carcinogenic and causes reproductive toxicity in animal models.[25] Use of ganciclovir or valganciclovir in pregnant or nursing women is not recommended without careful consideration of the risk-benefit ratio. Data on ganciclovir use in children are currently limited, but the drug is being evaluated for therapy of congenital CMV infections.

Adverse reactions

The most important adverse effects of ganciclovir noted in AIDS patients being treated for CMV retinitis were neutropenia and thrombocytopenia. About 40% developed granulocytopenia (absolute neutrophil count <1000/mm^3) and 15% had thrombocytopenia (platelet count <50000/mm^3). Hematologic toxicity is also seen, although less commonly, in organ transplant recipients. Neutropenia and thrombocytopenia are usually reversible when ganciclovir therapy is discontinued. In many patients requiring ganciclovir therapy, neutropenia can be prevented or treated by co-administration of granulocyte colony-stimulating factor. Renal dysfunction has been reported in up to 20% of transplant recipients receiving ganciclovir prophylaxis, although this may be related to co-administration of other nephrotoxic drugs. In animal models, ganciclovir produces significant reproductive toxicity, especially azoospermia.[25] Valganciclovir appears to have similar hematological toxicity to intravenous ganciclovir. Pooled data from two different studies indicate that 50% patients developed granulocytopenia (absolute neutrophil count <1000/mm^3), 35% experienced anemia (hemoglobin <9.5g/dl) and 23% developed thrombocytopenia (platelets <100000/µl). Gastrointestinal complaints were also common: 41% reported diarrhea, 30% nausea, 21% vomiting and 15% abdominal pain.

In vitro, ganciclovir and zidovudine have mutually antagonistic antiviral activity, but this observation has not been shown to be clinically significant. Ganciclovir should be used with caution in

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TABLE 205-9 -- Ganciclovir and valganciclovir dosage modification for renal impairment.

GANCICLOVIR AND VALGANCICLOVIR DOSAGE MODIFICATION FOR RENAL IMPAIRMENT			
Normal dosage regimen	**CrCl (ml/min)**	**Adjusted dosage regimen**	
		Dose	**Dosing interval (h)**
Ganciclovir 5mg/kg iv q12h	=70	5mg/kg	12
	50–69	2.5mg/kg	12
	25–49	2.5mg/kg	24
	10–24	1.25mg/kg	24
	HD	1.25mg/kg	Post-HD (TIW)
Ganciclovir 5mg/kg iv q24h	=70	5mg/kg	24
	50–69	2.5mg/kg	24
	25–49	1.25mg/kg	24
	10–24	0.625mg/kg	24
	HD	0.625mg/kg	Post-HD (TIW)
Ganciclovir 1000mg po q8h	=70	1000mg	8
	50–69	500mg	8
	25–49	500mg	24
	HD	500mg	Post-HD (TIW)

Valganciclovir 900mg po bid	>60	900mg	12
	40–59	450mg	12
	25–39	450mg	24
	10–24	450mg	48
	HD	NR	-
Valganciclovir 900mg po qd	>60	900mg	24
	40–59	450mg	24
	25–39	450mg	48
	10–24	450mg	Twice weekly
	HD	NR	-
CrCl, creatinine clearance			
HD, hemodialysis			
Post-HD, after each dialysis			
TIW, three times weekly			
NR, not recommended			

combination with other myelosuppressive drugs such as zidovudine because of the risk of additive hematologic toxicity. Probenecid can reduce renal clearance of ganciclovir, resulting in clinically significant increases in ganciclovir AUC. Seizures have been reported in patients receiving concomitant therapy with ganciclovir and imipenem.

Resistance

HSV and VZV isolates that are TK deficient and aciclovir resistant will also be cross-resistant with ganciclovir. Ganciclovir resistance *in vitro* is defined as an IC_{50} >6µM (1.5µg/ml). CMV isolates resistant to ganciclovir have been produced in the laboratory and isolated from patients with CMV disease.[36] In a study of 72 AIDS patients treated with ganciclovir, five of 13 culture-positive patients treated for >3 months excreted resistant virus.[37] Ganciclovir-resistant CMV has been identified as a cause of retinitis, encephalitis and poly-radiculopathy in AIDS patients. CMV resistance to ganciclovir is usually secondary to mutations in the UL97 gene, although alterations in the DNA polymerase gene have also been described. UL97 mutants remain susceptible to foscarnet, although polymerase mutants cross-resistant to both ganciclovir and foscarnet have been identified. Foscarnet or cidofovir are therapeutic options for treatment of disease caused by ganciclovir-resistant CMV.

Foscarnet

Mechanism of action and in vitro activity

Foscarnet (trisodium phosphonoformic acid) is an analogue of inorganic pyrophosphate that functions as a noncompetitive inhibitor of herpesvirus DNA polymerase. [38] Foscarnet blocks the pyrophosphate binding site, preventing cleavage of pyrophosphate from deoxynucleotide triphosphates. Viral DNA polymerase is inhibited at foscarnet concentrations 100-fold lower than those required to inhibit cellular DNA polymerase. Unlike the aciclovir-like drugs discussed above, foscarnet is not a nucleoside analogue, does not require intracellular activation by viral kinase and is not incorporated into the viral DNA chain. Therefore, thymidine kinase-deficient HSV and VZV isolates that are resistant to aciclovir will remain susceptible to foscarnet. Foscarnet has *in vitro* activity against HSV, VZV, CMV, EBV and HHV-6. The IC_{50} for most clinical isolates of CMV is in the range of 100–300µM, but varies considerably with the experimental conditions. Foscarnet can also inhibit viral reverse transcriptase and has *in vitro* activity against hepatitis B virus and HIV.[38]

Pharmacokinetics and distribution

Foscarnet has low oral bio-availability (approximately 17%) and is administered only by the intravenous route. Peak plasma concentrations after steady-state dosing at 60mg/kg every 8 hours or 90mg/kg every 12 hours are about 500µM and 700µM, respectively.[38] Plasma protein binding is about 15%. CSF foscarnet levels demonstrate wide interpatient variability, but average about 66% of plasma levels at steady state. Foscarnet is not metabolized and about 80% of an administered dose is excreted unchanged in the urine by glomerular filtration and tubular secretion within 36 hours. About 20% of the foscarnet dose is retained in bone, presumably due to the drug's structural similarity to inorganic phosphate. This results in a complex pattern of drug disposition, in which the initial elimination half-life is about 4.5 hours, followed by a prolonged terminal half-life of about 88 hours as drug is released from bone.[38] Plasma foscarnet levels are reduced about 50% following hemodialysis; dosing after dialysis is recommended.

Route of administration and dosage

Foscarnet is available only as an intravenous formulation. The usual dose for induction therapy of CMV retinitis is 90mg/kg every 12 hours, with a maintenance dose of 90–120mg/kg every 24 hours. When given via a central venous catheter, the drug can be diluted to 24mg/ml; for infusion through peripheral vein catheters, foscarnet must be diluted to 12mg/ml to avoid local phlebitis. The foscarnet dose must be administered over at least 1 hour using an intravenous infusion pump; bolus infusion can result in severe toxicity. A topical 3% foscarnet cream formulation has been evaluated for therapy of herpes labialis, but is not commercially available.

Indications

CMV retinitis

Intravenous foscarnet is approved for treatment of CMV retinitis in immunocompromised patients. In controlled clinical trials, foscarnet therapy was shown to significantly delay progression of CMV retinitis in AIDS patients.[39] As discussed above with ganciclovir, long-term maintenance with foscarnet is required to extend the time to subsequent relapse in patients with CMV retinitis. The recommended foscarnet dosage in patients with normal renal function is 90mg/kg every 12 hours for 14–21 days for induction, followed by 90–120mg/kg daily indefinitely. In a controlled trial comparing foscarnet with ganciclovir for CMV retinitis in AIDS patients, the two drugs were therapeutically equivalent for retinitis, but extended survival (12.6 versus 8.5 months) was shown in the foscarnet group.[40] Foscarnet clearly has antiviral activity against HIV, but it is uncertain whether this accounts for the survival benefit demonstrated in this and other studies. However, foscarnet therapy was significantly more toxic than ganciclovir and patients had to discontinue foscarnet three times more often because of adverse effects.[41] Limited clinical experience has shown intravitreal foscarnet to be effective for CMV retinitis. Foscarnet is an effective alternative therapy for some patients with retinitis caused by ganciclovir-resistant strains of CMV, although dually resistant isolates can rarely occur. Combination therapy with ganciclovir plus foscarnet has been used successfully to treat refractory CMV infections in AIDS patients.[42]

Other CMV infections

Evidence for foscarnet efficacy for CMV infections other than retinitis is derived primarily from uncontrolled studies and clinical experience. Foscarnet

TABLE 205-10 -- Foscarnet dosage modification for renal impairment.

FOSCARNET DOSAGE MODIFICATION FOR RENAL IMPAIRMENT			
Normal dosage regimen	**CrCl (ml/min/kg)**	**Adjusted dosage regimen**	
		Dose (mg/kg)	**Dosing interval (h)**

Foscarnet 90mg/kg q12h	>1.4	90	12
	>1.0–1.4	70	12
	>0.8–1.0	50	12
	>0.6–0.8	80	24
	>0.5–0.6	60	24
	>0.4–0.5	50	24
	<0.4	NR	-
Foscarnet 120mg/kg q24h	>1.4	120	24
	>1.0–1.4	90	24
	>0.8–1.0	65	24
	>0.6–0.8	105	48
	>0.5–0.6	80	48
	>0.4–0.5	65	48
	<0.4	NR	-
CrCl, creatinine clearance			
NR, not recommended			

has been used to treat CMV pneumonia and CMV colitis in AIDS patients. Attempts to use foscarnet for therapy or prophylaxis of CMV infections in bone marrow transplant recipients have met with variable success.

Aciclovir-resistant HSV and VZV infections

Foscarnet is the drug of choice for treatment of infections caused by aciclovir-resistant HSV and VZV.[43] In a controlled trial, foscarnet was clearly superior to vidarabine for treatment of aciclovir-resistant mucocutaneous HSV infections in patients with AIDS.

Dosage in special circumstances

Foscarnet is excreted by renal mechanisms and dosage adjustment is required even for minor degrees of renal insufficiency (see Table 205.10). Serum creatinine should be monitored at least every other day during foscarnet therapy to assess the need for further dose adjustment. Dosage adjustment in hepatic impairment is not required. The safety of foscarnet during pregnancy has not been adequately evaluated and use is not recommended unless no other alternative therapy is available. Little information has been published regarding foscarnet safety and tolerance in neonates and children.

Adverse reactions

The most important adverse effect caused by foscarnet is nephrotoxicity. [41] Dose-limiting renal toxicity occurs in at least 15–20% of patients treated with foscarnet for CMV retinitis. The primary mechanism of renal toxicity appears to be acute tubular necrosis, although interstitial nephritis and crystalline nephropathy have also been described. Loading the patient with intravenous saline prior to foscarnet infusion can help reduce the risk of nephrotoxicity. In most cases, the renal dysfunction is reversible and serum creatinine will return to normal within 2–4 weeks after foscarnet therapy is discontinued. However, irreversible renal failure may occur in patients who are volume depleted or who receive concomitant therapy with other nephrotoxic medications. Foscarnet can induce a variety of electrolyte and metabolic abnormalities, most notably hypocalcemia.[44] Hypercalcemia, hypomagnesemia, hypokalemia and hypo- and hyperphosphatemia have also been reported. The acute decline in ionized serum calcium that can occur with foscarnet infusion may be due to formation of a complex between foscarnet and free calcium.[44] Further depletion of total serum calcium seen with

long-term drug administration may be caused by renal calcium wasting, abnormal bone metabolism, concurrent hypomagnesemia or some combination of these factors. Foscarnet-induced electrolyte disturbances can predispose the patient to cardiac arrhythmias, tetany, altered mental status or seizures. It is mandatory that serum creatinine and electrolyte levels be closely monitored during foscarnet therapy. Foscarnet is much less myelosuppressive than ganciclovir, but anemia was reported in 10–50% of AIDS patients receiving foscarnet. Patients, especially uncircumcised males, may develop genital ulcerations due to local toxicity of high foscarnet concentrations in urine. Nausea and vomiting has been reported by 20–30% of patients receiving foscarnet. Other infrequent adverse effects include headache, diarrhea and abnormal liver function tests. When possible, foscarnet should be administered through a central venous line to avoid peripheral thrombophlebitis.

Specific drug interactions with foscarnet have not been described, although there is significant potential for additive toxicity. Concurrent therapy with foscarnet and intravenous pentamidine can result in severe and potentially fatal hypocalcemia. Concomitant administration of foscarnet with other potentially nephrotoxic drugs such as amphotericin B or aminoglycosides can compound the risk of serious nephrotoxicity. Foscarnet can be safely administered to patients receiving zidovudine, although there may be an increased risk of anemia.

Resistance

Although uncommon, foscarnet-resistant isolates of CMV, VZV and HSV have been encountered in AIDS patients receiving foscarnet therapy. Resistance is due to a mutation in the DNA polymerase gene which means that, in some circumstances, the foscarnet-resistant isolate may remain susceptible to aciclovir or ganciclovir. However, CMV isolates cross-resistant to both ganciclovir and foscarnet (containing both polymerase and UL97 mutations) have been recovered from AIDS patients. Cidofovir may be an effective alternative drug in this setting, but in vitro antiviral susceptibility testing is necessary to guide drug selection.

Cidofovir

Mechanism of action and in vitro activity

Cidofovir is a nucleotide analogue of cytosine monophosphate with potent broad-spectrum antiviral activity. Unlike aciclovir and other nucleoside analogues which require monophosphorylation by viral kinases for activation, cidofovir already carries a phosphonate group and does not require viral enzymes for conversion to cidofovir diphosphate, the active antiviral compound. Cidofovir diphosphate competitively inhibits the DNA polymerases of herpesviruses, thereby blocking DNA synthesis and viral replication. Cidofovir diphosphate inhibits viral DNA polymerases at concentrations much lower than those required to inhibit cellular DNA polymerases, accounting for its selectivity of action.[45] Cidofovir has potent in vitro activity against human CMV, with IC_{50} values in the range of 0.1–0.9µg/ml. Cidofovir retains activity against most CMV clinical isolates that are resistant to ganciclovir. Cidofovir also demonstrates in vitro activity against HSV and VZV (including TK-deficient, aciclovir-resistant isolates), adenovirus, poxvirus (including variola or smallpox virus) and human papillomavirus.

Pharmacokinetics and distribution

Serum cidofovir concentrations are dose proportional over a dosing range of 1.0–10.0mg/kg. Intravenous infusion of cidofovir at a dosage of 5mg/kg produces peak plasma concentrations of about 11µg/ml. The terminal half-life is 2.6 hours. Approximately 90% of the intravenous cidofovir dose is excreted by the kidneys within 24 hours, with clearance involving both glomerular filtration and tubular secretion. At cidofovir doses higher than 3mg/kg, concomitant administration of probenecid can block tubular secretion of cidofovir and reduce its renal clearance. [45] Cidofovir diphosphate and its metabolites have prolonged intracellular half-lives, which permit cidofovir to be effectively administered at extended dosing intervals.

Route of administration and dosage

Cidofovir for intravenous administration is supplied as 375mg of an aqueous solution (75mg/ml). The selected dose is diluted in 100ml of normal saline prior to administration. For induction therapy, the usual dose of cidofovir is 5mg/kg infused over 1 hour once weekly. The dose for maintenance therapy for CMV disease is 5mg/kg administered once every 2 weeks. To minimize nephrotoxicity, patients should receive 1 liter of normal saline intravenously over 1–2 hours immediately prior to cidofovir dose and an additional 1 liter of normal saline immediately following the cidofovir dose. Probenecid is given at a dose of 2g orally 3 hours before the cidofovir dose, then 1 doses at 2 hours and 8 hours after completion of the cidofovir infusion, for a total probenecid dose of 4g. Prodrugs of cidofovir (e.g. cyclic HPMPC) are under development.

Indications

CMV infections

Cidofovir has been approved in the United States for treatment of CMV retinitis. In a study of 48 AIDS patients with CMV retinitis, time to progression of disease was significantly longer in the cidofovir treatment group (120 days) compared with the deferred treatment group (22 days).[46] [47] Cidofovir has also been shown to be effective for CMV retinitis in patients who have relapsed on, or were intolerant to, ganciclovir or foscarnet. Efficacy of intravenous cidofovir was similar to oral ganciclovir plus ganciclovir implant in 61 AIDS patients with CMV retinitis, but adverse effects differed between the two treatment groups.[48] These data indicate that intravenous cidofovir is effective for both initial and salvage therapy of CMV retinitis in AIDS patients.

Cidofovir has a potential role for pre-emptive treatment of CMV disease in bone marrow transplant recipients, but has been evaluated in only a limited number of patients and should currently be considered as second-line therapy.

HSV infections

Because cidofovir is not dependent on thymidine kinase for activation, the drug retains activity against aciclovir-resistant HSV. Published case reports suggest that cidofovir is effective for treatment of aciclovir- and foscarnet-resistant mucocutaneous HSV infections, but data from controlled clinical trials are currently lacking.[49] An investigational topical preparation of cidofovir has also been evaluated for this indication. Cidofovir gel (0.3% or 1%) applied topically to mucocutaneous HSV lesions in AIDS patients resulted in >50% reduction in lesion surface area in half of the cidofovir-treated patients. Six of 20 cidofovir-treated patients (versus 0 of 10 placebo-treated patients) had complete lesion healing.

Human papillomavirus

Cidofovir has potent *in vitro* activity against HPV. Topical preparations and intralesional injections are under evaluation for treatment of laryngeal papillomatosis and genital warts.

Poxvirus infections

Topical 3% cidofovir has been used on an investigational basis to treat severe cases of molluscum contagiosum. Cidofovir has activity against orthopoxvirus infections in animal models and has been suggested as a treatment for smallpox, monkeypox and disseminated vaccinia infections in humans.

Dosage in special circumstances

Because intravenous cidofovir can cause significant nephrotoxicity, initiation of therapy in patients with pre-existing renal dysfunction (serum creatinine >1.5mg/dl, calculated creatinine clearance =55ml/min or proteinuria >100mg/dl [=2+]) is not recommended. Declining renal function during cidofovir therapy mandates dosage adjustment. If the serum creatinine increases by 0.3–0.4mg/dl above baseline, the cidofovir dose should be reduced from 5 to 3mg/kg. If the serum creatinine increases =0.5mg/dl above baseline or if proteinuria =3+ develops, cidofovir therapy should be discontinued. Dosage adjustment in patients with hepatic impairment is not required. Cidofovir is embryotoxic in animals and the drug should not be used during pregnancy unless there are no other therapeutic options. Cidofovir has not been systematically evaluated in children or elderly patients.

Adverse reactions

The most important safety concern with cidofovir therapy is nephrotoxicity. Pretreatment with intravenous hydration and probenecid (which blocks cidofovir tubular secretion) reduces the incidence of nephrotoxicity. In a clinical trial using cidofovir 5mg/kg plus probenecid, proteinuria occurred in five of 41 patients (12%) and elevated serum creatinine levels in two of 41 patients (5%). Neutropenia (ANC <750 WBC/ mm^3) was observed in 15% of cidofovir recipients.[46] Anemia, thrombocytopenia and hepatotoxicity have not been observed with cidofovir therapy. Ocular complications (including iritis, anterior uveitis and hypotony) have been described following intravenous or intravitreal cidofovir administration.

Probenecid, a benzoic acid derivative with a sulfa moiety, can cause allergic symptoms (e.g. fever, chills, rash) in patients allergic to sulfonamides. Patients with a history of severe sulfa hypersensitivity should not be treated with probenecid and, consequently, should not receive cidofovir. Other probenecid-related adverse effects include headache, nausea and vomiting, which can be minimized by administering the drug on a full stomach. In a CMV retinitis trial, probenecid-related adverse effects occurred in 23 of 41 patients (56%) and were dose limiting in three patients (7%).[46]

Cidofovir injections in rats were associated with mammary adenocarcinomas, but surveillance studies in treated patients have not demonstrated any excess frequency of tumors. Cidofovir administration causes embryotoxicity and impaired spermatogenesis in animals; male and female patients are advised to use adequate birth control during and for 3 months after completion of cidofovir therapy.

No specific drug interactions with cidofovir have been described, although concomitant therapy with other nephrotoxic drugs may result in additive toxicity. Probenecid, however, is known to alter the renal excretion of a wide variety of drugs. The dose of zidovudine should be reduced to 50% on days when probenecid administration is planned.

Resistance

Instances of clinical failure of cidofovir therapy due to drug resistance have been reported. CMV resistance to cidofovir results from a mutation in the viral polymerase gene and resistant isolates may exhibit cross-resistance to ganciclovir and/or foscarnet. *In vitro* susceptibility testing is necessary in this circumstance to guide appropriate drug selection.

Fomivirsen

Mechanism of action and in vitro activity

Fomivirsen is a 21-nucleotide phosphorothioate oligonucleotide designed as an antisense molecule with activity against CMV.[50] The oligonucleotide is complementary to mRNA from the immediate-early region 2 of CMV. Antisense inhibition of target gene expression, while necessary for optimal antiviral activity, only partially explains the activity of fomivirsen against CMV. Nonspecific interactions between the oligonucleotide and virus particles may prevent adsorption or lead to inhibition of enzymes required for viral DNA synthesis. Fomivirsen has activity against clinical CMV isolates, including isolates resistant to conventional antiviral drugs, with a median IC_{50} of 0.37µM in tissue culture.

In an open-label, dose-ranging study, increasing doses of fomivirsen were given to 22 AIDS patients (28 eyes) with refractory CMV retinitis. Decreased retinal CMV activity was noted in patients who received 300µg of fomivirsen by intravitreal injection every 1–2 weeks. In another study, 28 AIDS patients with newly diagnosed CMV retinitis (with less than 25% retinal involvement) were randomized to receive prompt treatment with fomivirsen or to defer treatment. In the treatment arm, 18 patients

received 150µg of fomivirsen every week for 3 weeks then every other week. Median time to progression was 71 days in patients who received prompt treatment versus 14 days in the deferred group (p = 0.0056).

Pharmacokinetics and distribution

When cynomolgus monkeys were given 11, 57 or 115µg of fomivirsen intravitreally, maximum vitreal concentrations (ranging from 0.11 to 1.28µM) were achieved 2 days after injection of all doses. For the same dosages, retinal concentrations increased in a logarithmic pattern, with maximal concentration obtained 2 days after injection for all doses, ranging from 0.12 to 0.88µM. For the 115µg dose, the vitreal and retinal half-lives were 22 hours and 78 hours, respectively. Electrophoretic analyses of retina and vitreous specimens indicate the oligonucleotide is metabolized by exonucleolytic cleavage. Both intact drug and its metabolic products diffuse from the vitreous humor to the retina and it is hypothesized that the active metabolism occurs in both compartments.

Route of administration and dosage

Fomivirsen is supplied in single-use vials containing 0.25ml of 6.6mg/ml solution. Dosage for induction therapy of CMV retinitis is 330µg given intravitreally every other week for two doses. Maintenance therapy is 330µg given intravitreally every 4 weeks.

Indications

Fomivirsen is indicated for treatment of CMV retinitis in AIDS patients who have failed or are intolerant of other CMV therapies, including those with CMV that is resistant to ganciclovir and foscarnet. It is not recommended for use in patients who have received intravitreal or intravenous cidofovir. Fomivirsen is not indicated for systemic CMV therapy and will not protect the contralateral eye from involvement.

Dosage in special circumstances

As there is little systemic exposure to fomivirsen after intravitreal injection, no dose adjustments are required. This drug has not been studied in pregnant or lactating women or in pediatric or geriatric populations.

Adverse reactions

The most common adverse reactions reported were increased intraocular pressure and mild to moderate intraocular inflammation of the anterior and posterior chambers. The combined incidence of ocular reactions was 10–12% of patients treated every other week and 20% in patients treated weekly. Other adverse reactions reported in 5–20% patients included abnormal or blurred vision, conjunctival hemorrhage, retinal detachment and retinal edema. Topical steroids have been used with success to ameliorate some of these effects.

Resistance

A fomivirsen-resistant CMV isolate has been developed by exposing a laboratory strain to increasing concentrations of the drug. This resistant virus did not prove to have mutations which would have altered specificity for the fomivirsen target sequence. This provides more evidence that both antisense specific and nonspecific mechanisms are involved in the drug's activity against CMV. There have been no reports of formivirsen-resistant CMV isolates recovered from patients.

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Chapter 206 - Antiviral Agents against Respiratory Viruses

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M2 INHIBITORS

Overview

Amantadine and rimantadine are symmetric tricyclic amines that specifically inhibit the replication of influenza A viruses at low concentrations (<1.0µg/ml) by blocking the action of the M2 protein. M2, an acid-activated ion channel found only in influenza A viruses, is a membrane protein required for efficient nucleocapsid release after viral fusion with the endosomal membrane.

Amantadine and rimantadine share two concentration-dependent mechanisms of antiviral action.[1] [2] Low concentrations of the drugs inhibit the ion channel function of the M2 protein, which inhibits viral uncoating or disassembly of the virion during endocytosis and, in H7 subtypes, alters HA maturation during viral assembly. Amantadine and rimantadine also increase the lysosomal pH, which in turn may inhibit virus-induced membrane fusion events for several enveloped viruses. However, such effects are generally not seen at drug concentrations observed in humans and clinically relevant antiviral activity is confined to influenza A viruses, although studies in chronic hepatitis C are in progress (see Table 206.1).

Resistance

Resistance to the two agents occurs as the result of amino acid substitutions in the transmembrane portion of the M2 protein. Although resistant wild-type virus is uncommonly found (<1%),[3] resistant viruses may rapidly emerge within 2–4 days after the start of therapy in up to 30% of patients.[4] Emergence of resistant virus does not appear to cause a rebound in illness in immunocompetent adults but may be associated with protracted illness and shedding in immunocompromised hosts. [5] Importantly, resistant virus can be spread to others and has caused failures of antiviral prophylaxis under close contact conditions, as in nursing homes and households.[4] The resistant virus appears to retain wild-type pathogenicity and causes an influenza illness indistinguishable from susceptible strains. Cross-resistance occurs to all M2 inhibitors without affecting susceptibility to the neuraminidase inhibitors and ribavirin.

Pharmacokinetics and distribution

Amantadine is rapidly absorbed, with a 53–100% bioavailability, and reachs peak plasma levels of 475µg/l within 4.5 hours after a 100mg dose in healthy adults.[6] The drug is predominantly excreted unchanged in the urine by glomerular filtration and tubular secretion. The plasma elimination half-life is about 11–15 hours in persons with normal renal function. Elimination is markedly prolonged in patients with renal impairment and decreases about 2-fold in the elderly so that dose adjustments are required (Table 206.3). Amantadine is widely distributed with salivary levels equivalent to those of blood and nasal mucus comparable to plasma at 8 hours after dosing.[7] [8] Amantadine crosses the placenta and blood-brain barrier, with cerebrospinal fluid(CSF) levels equal to 56–96% of serum levels, and distributes in breast milk (see Table 206.2).

In experimental human influenza infection, a trough concentration at steady-state of 300µg/l, which corresponds to that observed after 200mg/day dosing, was associated with a lower infection rate than placebo.[8] Adverse events are dose related and daily doses of 200mg in young adults and 100mg in the elderly institutionalized are associated with excess central nervous system (CNS) adverse effects.[6]

Rimantadine has nearly complete oral bioavailability and achieves maximal plasma concentration 3–5 hours after ingestion. Peak concentrations average 416µg/l after a 200mg oral dose. Rimantadine levels in nasal secretions average 1.5 times those of plasma levels. The plasma half-life is long and ranges from 24 to 36 hours. Rimantadine undergoes extensive metabolism, including hydroxylation, conjugation and glucuronidation in the liver, before being excreted in the urine. Only 25% of the parent drug is excreted unchanged in the urine. Dose adjustments are recommended for advanced renal or hepatic failure and older age (see Table 206.3).

Route of administration and dosage

Amantadine and rimantadine come as 100mg tablets and a syrup formulation (50mg/5ml). In adults, the usual dose for treatment or prevention of influenza A infection is 100mg q12h for both drugs (see Table 206.3).

Indications

Amantadine and rimantadine are indicated for the prevention and treatment of influenza A virus illness. Most placebo-controlled studies of these drugs in the management of influenza have been conducted in previously healthy persons. [8]

Prophylaxis

Prophylaxis with amantadine or rimantadine is approximately 70–90% effective in preventing symptomatic influenza A infections.[9] Administration is advised for postexposure prophylaxis in nursing home populations at 100mg/day for 14 days or for at least 7 days after the last culture-confirmed illness in the ward or building; this regimen should be given with concomitant influenza vaccination for those not previously provided.[10] Rimantadine is better tolerated in this population.[11] Seasonal prophylaxis, during the 4–8 weeks of peak influenza virus circulation within the community, can be used for protection of high-risk patients who cannot tolerate immunization, who do not develop an adequate immune response to vaccine or when the strain circulating in the community does not match the vaccine strain. Postexposure prophylaxis in households appears protective when not used in conjunction with treatment of an ill index case.

Treatment

Amantadine or rimantadine therapy reduces duration of fever and symptoms in patients with documented influenza by about 1 day compared with placebo, when the medication is initiated within 48 hours of symptom onset.[12] Treatment is also associated with more rapid functional recovery[12] and resolution of small airways functional abnormalities. Studies comparing the therapeutic activity of

TABLE 206-1 -- In-vitro activity of selected agents used to treat influenza.[26] [49] [50] [51]

IN-VITRO ACTIVITY OF SELECTED AGENTS USED TO TREAT INFLUENZA			
Drug	**Virus type/subtype**	**50% inhibitory concentration (IC_{50})**	
		Cell culture (µM/l)	**Neuraminidase enzyme (nM/l)**

Amantadine[49]	A/H1N1	5.3–7.3	NA
	A/H3N2	1.1–4.4	NA
Rimantadine[49]	A/H1N1	0.6–0.62	NA
	A/H3N2	0.1–3.0	NA
Zanamivir[26]	A/H1N1	0.02–0.07	0.3–0.7
	A/H3N2	0.004–0.241	1.7–4.6
	B	0.03–0.15	1–17.0
Oseltamivir[50]	A/H1N1	0.02–0.94	0.69–2.2
	A/H3N2	0.0006–0.04	0.21–0.56
	B	0.091–0.16	0.8–24.0
Note: Concentrations for inhibition in cell culture are express in µM, whereas those for neuraminidase enzyme inhibition are expressed in nM. Results of representative publications.			

amantadine and rimantadine are few but generally show comparability.[12] Amantadine appears safe and efficacious in reducing length of fever and illness in children older than 2 years of age.[12] Pediatric studies have found variable clinical benefits relative to acetaminophen controls and document the frequent emergence of drug-resistant variants.[12]

Prospective controlled data to support the use of M2 inhibitors in treating severe influenza or in preventing complications are lacking; one retrospective study found no important differences in duration of illness or hospitalization between the amantadine-treated and untreated patients hospitalized with influenza.[13] In hematopoietic stem cell transplant (HSCT) and acute leukemia patients who received therapy with one of the M2 inhibitors, a reduced risk of progression to pneumonia (35% vs 76%) was found compared with no treatment.[14] However, emergence of resistance is common in such patients.[5] One retrospective study of nursing home residents suggested that early treatment might reduce lower respiratory complications.[15] Amantadine is ineffective for treating influenza B or C infections.

TABLE 206-2 -- Pharmacokinetic properties of antivirals with activity against influenza. [7] [31] [23]

PHARMACOKINETIC PROPERTIES OF ANTIVIRALS WITH ACTIVITY AGAINST INFLUENZA								
Drug	**Dose**	**Route**	**C_{max} (µg/l)**	**T_{max} (h)**	**$AUC_{0–12\ hrs}$ (µg/ml·h)**	**$T_{1/2}$ (h)**	**B (%)**	**% Protein binding**
Amantadine[7] [8] *	200mg × 1	Oral (young)	510 (140)	2.1 (1)	10.2 (3.4)	14.4 (6)	62–93	67
		Oral (elderly)	800 (200)	2.2 (2.1)	17.6 (6.5)	19 (9.1)	53–100	
Rimantadine[7] [8] *	200mg × 1	Oral (young)	240 (70)	4.6 (2.1)	9.8 (4.5)	36.5 (17.3)	75–93	40
		Oral (elderly)	250 (50)	4.0 (2.4)	11.5 (3.9)	36.5 (14.5)	NA	
Zanamivir[23] *	16mg × 1	Inhaled	39 (23–69)	0.75 (0.08–2)	0.03 (0.02–0.06)	3.6 (2.2–9.4)	4–17	10
	16mg 6 ×/d × 7 d	Inhaled	54 (34–96)	0.75 (0.25–1)	0.16 (0.09–0.32)	—	4–17	
Oseltamivir[31] *	100mg bid	Oral (18–55 yo)	439 (40.8)	3.5 (1)	3.85 (0.6)	6–10	79	42
		Oral (=65 yo)	575 (83.3)	3.3 (1.4)	4.94 (1.0)	—	—	
C_{max} = maximum serum drug concentration; T_{max} = time to C_{max} ; $T_{1/2}$ = serum elimination half-life; AUC = area under the serum drug concentration versus time for the dose interval; B = bioavailability (% of intravenous C_{max}).								

*Values are mean (SD)
**values are median (range).

Dosage in special circumstances

Dosing of amantadine and rimantadine should be adjusted in the setting of renal failure (see Table 206.3). Neither M2 inhibitor is cleared by hemodialysis. Patients over 65 years of age should have the dose of both medications reduced to 100mg once daily to avoid side effects. Rimantadine needs dose adjustment to 100mg per day for serious hepatic insufficiency. Amantadine and rimantadine are embryotoxic and teratogenic in preclinical tests and amantadine may be associated with birth defects. As a result, neither drug should be used in pregnant women unless the benefits of therapy clearly outweigh the potential risks (pregnancy category C). The recommended pediatric dosage of both amantadine and rimantadine is 5mg/kg/day to a maximum of 150mg/day, divided twice daily in children younger than 10.[10]

Adverse effects and drug interactions

The most common side effects of the M2 inhibitors are minor CNS complaints (anxiety, difficulty concentrating, insomnia, dizziness, headache and jitteriness) and gastrointestinal upset. Patients who receive amantadine may develop antimuscarinic effects, orthostatic hypotension and congestive heart failure at low frequencies. Particularly in the elderly or those with renal failure, serious CNS side-effects due to amantadine, and less often rimantadine, include confusion, disorientation, mood alterations, memory disturbances, delusions, nightmares, ataxia, tremors, seizures, coma, acute psychosis, slurred speech, visual disturbances, delirium, occulogyric episodes and hallucinations.[8] Amantadine causes CNS side-effects in about 15–30% of persons, as well as dose-related abnormalities in psychomotor testing.[8] The incidence and severity of CNS adverse effects are less common with rimantadine.[11] Amantadine and possibly rimantadine may increase the risk of seizures in those with a history of seizures.

Concomitant ingestion of antihistamines or anticholinergic drugs increases the CNS effects of amantadine. Trimethoprim-sulfamethoxazole and triamterene-hydrochlorothiazide decrease the renal clearance of amantadine, which enhances the risk of CNS toxicity. Quinine and quinidine also reduce the clearance of amantadine. Co-administration with monoamine oxidase inhibitors may precipitate life-threatening hypertension. The drug does not appear to interact with the cytochrome P450 system. Cimetidine is associated with 15–20% increases and aspirin or acetaminophen with 10% decreases in plasma rimantadine concentrations, but such changes are unlikely to be of clincial significance.[16] Patients receiving either amantadine or rimantadine along with drugs affecting

TABLE 206-3 -- Agents used to prevent and treat influenza.[10]

AGENTS USED TO PREVENT AND TREAT INFLUENZA				
Drug	**Usual adult dosage+**		**Dose adjustment state**	**Suggested dosage**
	Prophylaxis	**Treatment**		

Amantadine	100mg bid	100mg bid	Age 1–9 years	5mg/kg to max of 150mg in two divided doses
			CrCl 30–50mL/min	100mg qd
			CrCl 15–30mL/min	100mg qod
			CrCl 10–15mL/min	100mg q week
			CrCl <10mL/min	100mg q week
			Age =65 years	100mg qd
Rimantadine	100mg bid	100mg bid	Age 1–9 years*	5mg/kg to max of 150mg in two divided doses
			CrCl <10mL/min	100mg qd
			Severe hepatic dysfunction	100mg qd
			Age =65 years	100mg qd
Zanamivir	2 puffs (10mg) qd*	2 puffs (10mg) bid	No dose adjustment needed	
Oseltamivir	75mg qd	75mg bid	CrCl <30mL/min¶	Treatment: 75mg qd
				Prophylaxis: 75mg qod
			=15kg	30mg bid (2.5ml*)
			15–23kg	45mg bid (3.8ml*)
			23–40kg	60mg bid (5ml*)
			>40kg	75mg bid (6.2ml*)
Recommendations based on those provided by the Advisory Committee on Immunization Practices.[10] § Hemodialysis contributes minimally to clearance.				

+ Duration of treatment is usually 5 days. Duration of prophylaxis depends on clinical setting.
*** Investigational: not approved for treatment in children by the US Food and Drug Administration.
** Investigational: not approved for prophylaxis by the US Food and Drug Administration.
¶ No treatment or prophylaxis dosing recommendations are available for patients undergoing renal dialysis.
* Volume of suspension.

CNS function, such as antihistamines, antidepressants or minor tranquilizers, should be monitored closely.

NEURAMINIDASE INHIBITORS

Overview

Influenza A and B viruses possess a surface glycoprotein with neuraminidase activity whereas influenza C viruses do not. This enzyme cleaves terminal sialic acid residues and destroys the receptors recognized by viral hemagglutinin. This activity is essential for release of virus from infected cells, for prevention of viral aggregates and for viral spread within the respiratory tract.[17] Zanamivir and oseltamivir are sialic acid analogues that potently and specifically inhibit influenza A and B neuraminidases by competitively and reversibly interacting with the active enzyme site.[18] These drugs are active against all nine neuraminidase subtypes in nature including the avian strains of influenza A H5N1 and H9N2 that infected humans (see Table 206.1).

Resistance

Zanamivir and oseltamivir carboxylate resistance in vitro results from mutations in the viral hemagglutinin and/or neuraminidase.[19] [20] In the hemagglutinin variants, mutations in or near the receptor binding site make the virus less dependent on neuraminidase action, whereas neuraminidase mutations directly affect interaction with the inhibitors. The altered neuraminidases typically show reduced activity or stability and the mutated viruses usually have decreased infectivity in animals.[19] The particular neuraminidase mutation determines the degree of resistance and cross-resistance (i.e. R229K causes high-level resistance in oseltamivir but not zanamivir).[19] Several hemagglutin and neuraminidase mutants have been described in immunocompromised patients with prolonged virus shedding.[22] Oseltamivir-resistant variants have been recovered from <1% of treated adults and about 4% of treated children.[18] The possible clinical and epidemiologic significance of such variants requires study and a global Neuraminidase Inhibitor Susceptibility Network has been established to address these concerns.[20]

ZANAMIVIR

Pharmacokinetics and distribution

The oral bioavailability of zanamivir is low (<5%) and most clinical trials have used intranasal or dry powder inhalation delivery. The commercial dry powder formulation is mixed with lactose (5mg zanamivir per 20mg lactose). Following inhalation of the dry powder, approximately 7–21% is deposited in the lower respiratory tract and the remainder in the oropharynx.[23] [24] Median zanamivir concentrations are above 1000ng/ml in induced sputum 6 hours after inhalation and remain detectable up to 24 hours (see Table 206.2). The peak plasma concentration averages 46µg/l after a single 16mg inhalation of zanamivir. The proprietary inhaler device for delivering zanamivir is breath activated and requires a co-operative patient.[25]

In both experimental and natural influenza, once-daily dosing appears protective.[26] Twice-daily administration is therapeutically active but increasing the dose frequency to four times per day does not appear to increase efficacy in treating natural influenza.[26] Intranasal dosing is protective against experimental infection but not natural infection and does not substantially increase the overall therapeutic response to inhaled zanamivir.

Route of administration and dosage

Zanamivir is delivered by inhalation with a proprietary breath-activated device (Diskhaler). The usual adult treatment dose is two inhalations (10mg) twice a day for 5 days.

Indications

Prophylaxis

Although not US Food and Drug Administration approved for prophylaxis, once-daily inhaled zanamivir for 4 weeks was 84% efficacious in preventing laboratory-confirmed illness with fever and 31% effective in preventing influenza infection, irrespective of symptoms.[26] When used for postexposure prophylaxis, inhaled zanamivir for 10 days reduced the risk of secondary influenza illness by 79% in households.[26] In nursing homes experiencing influenza outbreaks, inhaled zanamivir was more effective for prevention of influenza A illness than oral rimantadine, in part because of frequent resistance emergence to the M2 inhibitor.[26]

Treatment

In the USA zanamivir is indicated for the treatment of uncomplicated acute illness due to influenza A and B virus in adults and pediatric patients 7 years and older who have been symptomatic for no more than 2 days. Inhaled zanamivir in adults has consistently shown at least one less day of disabling influenza symptoms and most studies have found a reduction in the number of nights of disturbed sleep, in time to resumption of normal activities and in the use of symptom relief medications. [26] [27] Similar therapeutic benefits have also been shown in children aged 5–12 years.[28] Greatest benefit was noted in patients who were febrile at the time of enrollment, those started on therapy within 30 hours after the onset of symptoms and in adults aged 50 years and older.[26] Zanamivir has also been associated with a 40% reduction in lower respiratory tract complications of influenza leading to antibiotics, particularly bronchitis and pneumonia.[29] It appears generally well tolerated and effective in treating influenza in patients with mild to moderate asthma or, less often, chronic obstructive pulmonary disease (COPD).[27] [29] An uncontrolled study found zanamivir to be safe and possibly effective in allogeneic stem cell transplant recipients, although viral shedding persisted for an average of 2 weeks on therapy.[21] Further studies are needed in immunocompromised populations.

Dosage in special circumstances

Although the plasma elimination half-life increases with creatinine clearance =70ml/min, drug accumulation is negligible after inhalation and dose adjustment is not necessary for renal or hepatic dysfunction. Certain populations, particularly very young, frail or cognitively impaired patients, may have difficulty using the drug delivery system.[25]

Adverse effects and drug interactions

Topically applied zanamivir is generally well tolerated in controlled studies, including those involving patients with asthma and COPD.[27] No difference in adverse events between zanamivir and placebo (lactose) recipients has been found.[27] Less than 5% of zanamivir recipients have reported diarrhea, nausea, sinusitis, nasal signs and symptoms, bronchitis, cough, headache, dizziness and ear, nose and throat infections. Postmarketing reports indicate that bronchospasm may be an uncommon but potentially severe problem, particularly in patients with acute influenza and underlying reactive airways disease.[30] Anecdotal reports of hospitalization and fatality indicate that inhaled zanamivir should be used cautiously in such patients. Current guidelines advise against the use of zanamivir in patients with underlying airway disease, unless the patient is closely monitored and has a fast-acting inhaled bronchodilator available when inhaling zanamivir.[10]

Low bioavailability is associated with low exposure to circulating zanamivir and no clinically significant drug interactions have been recognized. In-vitro studies suggest that zanamivir does not inhibit or induce cytochrome P450 enzymes. The drug does not affect the immunogenicity of concomitant immunization with inactivated virus vaccines. It is uncertain whether inhaled zanamivir might reduce the immunogenicity of intranasal, live-attenuated vaccine if administered concurrently. Although not associated with teratogenic effects in preclinical studies, zanamivir should only be used in pregnancy when the potential benefit justifies the potential risk to the fetus (pregnancy category C).

OSELTAMIVIR

Pharmacokinetics and distribution

Oral oseltamivir ethyl ester is well absorbed and rapidly cleaved by esterases in the gastrointestinal tract, liver or blood. The bioavailability of the active metabolite, oseltamivir carboxylate, is estimated to be approximately 80% in previously healthy persons.[31] Mean peak oseltamivir carboxylate concentrations of 456µg/l are reached at 5 hours after oral administration of 150mg doses in healthy adults and the plasma elimination half-life is 6–10 hours (see Table 206.2). Although drug concentrations over time are 25–35% higher in the elderly at steady-state, no dose adjustment is deemed necessary. Administration with food appears to decrease the risk of gastrointestinal upset without decreasing bioavailability. Both the prodrug and parent are eliminated primarily unchanged through the kidney by glomerular filtration and anionic tubular secretion. The dose should be reduced by half for patients with a creatinine clearance less than 30ml/min.[32] Distribution is not well characterized in humans, but peak bronchoalveolar lavage levels are similar to plasma levels in animals.[31] Drug levels in middle ear fluid and sinus aspirates are similar to those in blood. [31]

There is no clear association between the plasma AUC for the oseltamivir carboxylate drug and viral titer after experimental infection; early therapy of experimental infection is associated with reduced median nasal lavage concentrations of interleukin-6, tumor necrosis factor-a and interferon-? as compared with placebo.[31] In natural influenza, once-daily dosing appears as effective as twice-daily dosing for prevention of influenza illness.[31] Doses of 75mg and 150mg twice daily provide comparable antiviral and clinical effects in treatment of acute influenza A illness[31] and of experimental influenza B in adults.

Route of administration and dosage

Oseltamivir comes as 75mg tablets and as a suspension in bottles containing 25ml of suspension after constitution equivalent to 300mg oseltamivir base. The suspension comes in bottles containing 25ml of suspension after constitution equivalent to 300mg oseltamivir base. The typical adult dose for treatment is 75mg twice daily for 5 days and for prophylaxis is 75mg once daily. Pediatric dosing is based on weight and is outlined in Table 206.3 .

Indications

In the USA oseltamivir is indicated for the treatment of uncomplicated acute illness due to influenza infection in patients 1 year and older who have been symptomatic for no more than 2 days and for the prophylaxis of influenza in adult patients and adolescents 13 years and older.

Prophylaxis

The efficacy of once-daily oseltamivir 75mg for 6 weeks in preventing influenza illness in healthy, nonimmunized adults was 84% and in preventing influenza infection irrespective of symptoms was 50%.[31] In immunized nursing home residents, the efficacy of prophylaxis was

92% against illness compared with placebo.[31] Similar efficacy was seen in a household-contact prophylaxis study,[31] and protection against influenza has been shown in children.[31]

Treatment

Oseltamivir 75mg twice daily for 5 days, when started within the first 2 days of symptoms, was associated with a shorter time to alleviation of illness (29–35 hours shorter) and with reductions in severity of illness, duration of fever, time to return to normal activity, quantity of viral shedding, duration of impaired activity, and complications leading to antibiotic use, particularly bronchitis, compared with placebo in previously healthy adults.[31] Preliminary analyses indicate that early treatment can reduce hospitalizations. In a pediatric study enrolling children between the ages of 1 and 12 years, oseltamivir 2mg/kg twice daily for 5 days significantly reduced illness duration and severity, time to resumption of full activities and the occurrence of complications leading to antibiotic use, particularly acute otitis media.[33] Little published information is available about therapeutic efficacy in elderly or high-risk persons, including those with underlying cardiopulmonary conditions or immunodeficiency.

Dosage in special circumstances

Oseltamivir dose should be reduced to 75mg once a day for treatment and 75mg every other day or 30mg of suspension daily for prophylaxis when a patient has a creatinine clearance of less than 30mg/dl. Doses of oseltamivir should be given after hemodialysis. The safety and pharmacokinetics in patients with hepatic impairment have not been evaluated. Oseltamivir should not be used in pregnant women unless the benefits of therapy clearly outweigh the potential risks (pregnancy category C). The recommended pediatric dosages are listed in Table 206.3 .

Adverse effects and drug interactions

Oral oseltamivir is generally well tolerated and no serious end-organ toxicity has been found in controlled clinical trials. Oseltamivir is associated with nausea, discomfort and, less often, emesis in a minority of treated patients. Nausea and vomiting occur at approximately 10–15% excess in oseltamivir recipients. Gastrointestinal complaints are usually mild to moderate in intensity, usually resolve despite continued dosing and are ameliorated by administration with food.[34] Clinical studies comparing 75mg and 150mg twice daily doses found similar frequencies of adverse events with the two doses. Other infrequent possible adverse events include insomnia, vertigo and fever. Postmarking reports suggest that oseltamivir may be associated rarely with skin rash, hepatic dysfunction or thrombocytopenia.

No clinically significant drug interactions have been recognized. However, probenecid blocks tubular secretion and doubles the half-life of oseltamivir. Studies with amoxicillin, aspirin and acetaminophen have found no clinically important interactions. No interactions with the cytochrome P450 enzymes occur in vitro. Protein binding is below 10%. Oseltamivir should not affect the immunogenicity of concomitant vaccination with inactivated virus but might impair the immunogenicity of concurrent live-attenuated intranasal influenza vaccine.

RIBAVIRIN

Overview

Ribavirin is a guanosine analogue with a wide range of antiviral activity including influenza viruses, respiratory syncytial virus (RSV), parainfluenza viruses and adenoviruses. Ribavirin is rapidly phosphorylated by intracellular enzymes and the triphosphate inhibits influenza virus RNA polymerase activity and competitively inhibits the guanosine triphosphate-dependent 5'-capping of influenza viral messenger RNA. In addition, ribavirin depletes cellular guanine pools.[35] [36] Although it has no antiviral activity when used alone, for the treatment of hepatitis C infection, the combination of ribavirin and interferon-a is more effective than interferon-a alone (see Chapter 207).

Pharmacokinetics and distribution

Oral ribavirin has a bioavailability of 33–45% in adults and children and achieves peak plasma concentrations of 0.6μg/ml 1–2 hours after ingestion of a 400mg dose in adults. Ribavirin has a short initial (0.3–0.7 hours) and long terminal-phase half-life (18–36 hours) and is eliminated by hepatic metabolism and renal clearance.[37] [38] [39] [40] After aerosol administration, plasma levels increase with exposure and range from 0.2 to 1μg/ml. Respiratory secretions have levels of up to 1000μg/ml, which declines with a half-life of 1.4–2.5 hours.

Route of administration and dosage

Ribavirin comes in three formulations: oral, intravenous (investigational in the USA) and aerosol. Ribavirin for aerosolization is available as a 6g/100ml solution, which is diluted to a final concentration of 20mg/ml and delivered by small-particle aerosol for 12–18 hours with a proprietary device (SPAG-2 nebulizer). A higher concentration of aerosol solution (60mg/ml) has been given over 2 hours three times daily in some studies and appears well tolerated. Ribavirin also comes in 200mg tablets and sterile solution for injection.

Indications

Ribavirin aerosol is currently indicated for the treatment of severe RSV infection in children. Trials of aerosolized ribavirin for the treatment of severe RSV infection in infants have shown no consistent effect on duration of hospitalization time or mortality.[41] Earlier studies were confounded by the use of water aerosol as placebo, which may induce bronchospasm. Long-term follow-up of ribavirin recipients has likewise found no consistent benefits on pulmonary function.[41] Current guidelines recommend that aerosolized ribavirin be considered in the treatment of high-risk infants and young children, as defined by congenital heart disease, chronic lung disease, immunodeficiency states, prematurity and age <6 weeks, as well as for those hospitalized with severe illness.[41] [42] Administration of a more concentrated aerosol solution (60mg/ml) over 2 hours thrice daily appears well tolerated and easier to administer.[43] Aerosolized ribavirin has shown minimal efficacy in treating influenza in hospitalized children.[44]

Ribavirin has also been studied for the treatment of RSV and parainfluenza virus infections in immunocompromised patients. Intravenous ribavirin appears to be ineffective in reducing RSV-associated mortality in human stem cell transplant with RSV pneumonia.[45] Aerosolized ribavirin may provide benefit in selected patient groups with less severe RSV disease. Survival is improved when treatment is started before respiratory failure or when infection is limited to the upper respiratory tract.[45] Although no prospective studies of aerosolized ribavirin alone versus combined ribavirin-antibody therapy (IVIG, RespiGam or palivizumab) have been reported, combination therapy may be more effective, particularly when started before severe respiratory distress. [45] In the management of parainfluenza virus (PIV) pneumonia in bone marrow transplant recipients, aerosolized ribavirin failed to improve 30-day mortality or reduce the duration of viral replication relative to no treatment. [45] Intravenous ribavarin may be beneficial in treating PIV or influenza virus infections in some immunocompromized patients.[46] High oral doses of ribavarin (30–60mg/kg/day in divided doses) have been used in early treatment of RSV and PIV infections in HSCT. High oral doses (8.4g over 2 days) provided modest benefit in acute influenza in otherwise healthy adults.[48]

Recently. oral and intravenous ribavirin have been used in treatment of SARS coronavirus illness with uncertain clinical and virologic effects.[48A] [48B]

Dosage in special circumstances

Systemic ribavirin is contraindicated in patients with creatinine clearance of less than 50mL/min and the dose should be reduced by one-third for patients under the age of 10 years. Dose adjustment is needed if there is a substantial decline in hematocrit and the drug should be discontinued if the hematocrit drops below 8.5g/dl. Ribavirin is contraindicated in pregnant women and in male partners of women who are pregnant because of teratogenicity of the drug. Pregnancy should be avoided during therapy and 6 months after completion of therapy in both female patients and in female partners of male patients taking ribavirin (pregnancy category X).

Adverse effects and drug interactions

Systemic ribavirin can cause a dose-related extravascular hemolytic anemia and, at higher doses, suppression of bone marrow release of erythroid elements. Severe anemia may require dose adjustment or cessation or use of erythropoietin. Aerosolized ribavirin can cause bronchospasm, mild conjunctival irritation, rash, psychologic distress if administered in a oxygen tent and, rarely, acute water intoxication. Bolus intravenous administration may cause rigors. Antagonism of both drugs occurs in vitro when ribavirin is combined with zidovudine; the in-vivo significance is unknown.

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Chapter 207 - Drugs to Treat Viral Hepatitis

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INTERFERONS

Interferons (IFNs) are a family of naturally occurring proteins produced by eukaryotic cells that function as cytokines in an early response to viral infection. Interferons do not have direct antiviral activity, but instead induce an antiviral state in exposed cells and activate other immune functions.

Interferons are broadly characterized as type I (IFN-a and -ß), and type II (IFN-?). Interferon-a and -ß have primary antiviral activity while IFN-? has more potent immunoregulatory functions.

Interferons act through cell surface receptors to induce a complex series of intracellular events, with inhibition of viral protein production appearing to be the primary target.[1] A variety of purified and recombinant IFN-a preparations is available for clinical use. The pharmaceuticals are termed 'IFN-alfa' to distinguish them from naturally occurring 'IFN-a'.

Recently, pegylated ('peg') varieties of IFN have become available. Pegylation involves the addition of a polyethylene glycol side-chain to the protein molecule, which extends the half-life significantly. Two formulations are currently available, pegIFN alfa-2a (Pegasys) and pegIFN alfa-2b (Peg Intron). PegIFN alfa-2a has a larger branched peg molecule of about 40kDa in size attached at several sites. [2] PegIFN alfa-2b has a smaller, linear peg molecule of about 12kDa attached at several sites. Their proposed mechanism of action is similar to that of standard IFNs.

Pharmacokinetics and distribution

Standard pharmacokinetic measurements may not be relevant for IFN therapy. Plasma levels of IFN-alfa are often undetectable after subcutaneous or intralesional administration, while the plasma elimination half-life ($t_{½}$) is 2–4 hours after intravenous administration. Interferon-alfa is well absorbed (approximately 80%) following intramuscular administration and peak plasma concentrations occur after approximately 5–8 hours.[3] Interferon-alfa is filtered through the glomeruli and then undergoes rapid proteolytic degradation during tubular resorption. Negligible amounts of IFN are excreted in the urine. A small percentage of the administered dose undergoes hepatic metabolism and biliary excretion. PegIFN alfa-2b reaches maximum concentrations by 20 hours and has a half-life of 40 hours. PegIFN alfa-2a reaches maximum concentrations by 72–96 hours and has a longer $t_{½}$ of 80 hours compared to pegIFN alfa-2b.

Route of administration and dosing

Interferons are administered systemically (subcutaneously or intramuscularly) for the treatment of chronic viral hepatitis and by intralesional injection for the treatment of condylomata acuminata. Dosages vary considerably according to the specific preparation and indication (Table 207.1).

Indications

Chronic hepatitis B virus infection

Interferon is approved for the treatment of chronic hepatitis B virus (HBV) infection. A response to IFN therapy is judged by a loss of HBV DNA and hepatitis B e antigen (HBeAg) from the plasma, along with biochemical and histologic improvements. A meta-analysis of 16 randomized trials examining IFN versus control found that loss of HBeAg occurred in 33% of patients while clearance of HBV DNA occurred in 37% of patients.[4] Control patients experienced these outcomes in only 12 and 17% of cases, respectively. Clinical predictors of response to IFN in patients infected with HBV include low level of HBV DNA, elevated serum transaminases and evidence of active hepatic inflammation.[5] As seen in Figure 207.1 , IFN therapy is associated with significant adverse effects. Recent years have seen a variety of new and effective medications for the treatment of HBV infection with improved side-effect profiles compared to IFN. As a result of these therapies, discussed below, IFN is not considered first-line treatment for HBV infection by most clinicians (see also Chapter 48).

Hepatitis C virus infection

Treatment of acute hepatitis C virus (HCV) infection is still investigational; however, a recent study reported a 98% sustained virologic response rate with IFN monotherapy in this setting.[6] Treatment of chronic HCV infection is recommended in patients experiencing detectable HCV RNA levels higher than 50 IU/ml and with a liver biopsy showing portal or bridging fibrosis and moderate inflammation and necrosis.[7] A sustained virologic response (SVR) of HCV to therapy is defined as a HCV RNA assay <50 IU/ml at 24 weeks after therapy. Evaluation of data from combining results of two large prospective trials shows that IFN monotherapy results in SVR rates of less than 10% after 24 weeks of treatment and SVR rates less than 20% after 48 weeks of therapy. [8] Increasing doses of IFN provide increased responses, with concomitant increases in adverse reactions.

Hepatitis C virus has a half-life of about 3 hours and infection results in the production of about 12 billion virions daily. The dosing and pharmacokinetics of standard IFN described above result in wide variations in serum IFN levels and fluctuating antiviral activity. Pegylation of the IFN molecule maintains rapid absorption and rapid time to peak drug level, while providing a dramatically lengthened half-life. Studies show that the pegylated IFNs provide significantly improved response rates over standard IFNs. Monotherapy with pegylated IFNs yields SVR rates of 25–39% in treatment-naive patients with HCV infection.[9] These improved results over standard IFN apply to both pegIFN alfa-2a as well as pegIFN alfa-2b.[10] [11]

Dosage in special circumstances

Guidelines for adjusting systemic IFN dosage in patients who have renal insufficiency are poorly defined. Only limited data are available regarding administration to patients who have decompensated liver disease, but IFN may be poorly tolerated in this population. In pregnant monkeys, IFN has abortifacient effects and should not be used in pregnant women unless the potential benefits clearly outweigh the potential risks to the fetus.

Adverse reactions

Interferon therapy is associated with an extensive list of toxicities (see Fig. 207.1). Most patients receiving IFN doses of over one

TABLE 207-1 -- Medication dosage regimens.

MEDICATION DOSAGE REGIMENS				
Disease	**Therapy**	**Route**	**Dose**	**Duration**

Chronic hepatitis B	IFN alfa-2b	sc or im	30–35 × 10^6 IU per week administered as either	16 weeks
			5 × 10^6 IU/day or 10 × 10^6 IU three times per week	
	Lamivudine	po	100mg q24h	Optimal duration unknown
	Adefovir dipivoxil	po	10mg q24h	Optimal duration unknown
Chronic hepatitis C	IFN alfa-2a	sc or im	3 × 10^6 IU three times/week	48 weeks*
	IFN alfa-2b	sc or im	3 × 10^6 IU three times/week	48 weeks
	IFN alfacon	sc	9µg three times/week*	48 weeks
	PegIFN alfa-2a	sc	180µg/week	48 weeks
	PegIFN alfa-2b	sc	1.0µg/kg/week†	48 weeks
			or	
			1.5µg/kg/week‡	
	Ribavirin	po	800–1200mg per day††	Co-administer with IFN

** An optimal duration of IFN therapy is not universally agreed upon. Most trials describe 24–48 weeks of IFN or IFN plus ribavirin therapy. Early viral response (EVR) defined as a >2 log drop in HCV viral load after 12 weeks may be predictive of patients who will have a successful response. (http//consensus.NIH.gov/cons/116/091202116cdc_statement.htm (NIH Consensus Statement Sept, 2002)). This duration is not reflected in most package inserts. Readers should examine specific large-scale trials of each regimen for more detailed information.
* Patients who fail therapy with IFN alfacon at standard dose may receive the drug at 15µg sc three times/week for 6 months
† Monotherapy
‡ Dual therapy with ribavirin
†† Optimal dose of ribavirin varies with the co-administration of IFN products as well as patient weight. Optimal dose ranges have not been uniformly determined. Readers should enquire to specific package inserts and recent dose finding trials.

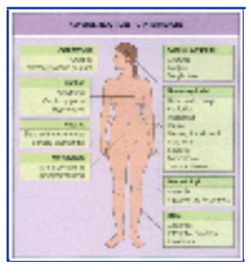

Figure 207-1 Adverse reactions to interferons.

million units experience an 'influenza-like' syndrome characterized by fever, chills, headache, myalgias and arthralgias. These symptoms appear a few hours after the IFN injection, and they usually resolve within 12 hours. The symptoms can often be prevented by premedication with antipyretics. Tolerance develops in many patients with continued therapy. The most frequent dose-limiting adverse effects are leukopenia and thrombocytopenia. Before beginning IFN therapy, a baseline complete blood count, liver function profile, urinalysis, antinuclear antibody screen and thyroid function tests should be obtained.

The adverse events seen with pegylated IFN are similar to those seen with standard IFN. The most common hematologic toxicity associated with pegylated IFNs is neutropenia with as many as 18% of patients requiring dose adjustments as a result.[12] Dose reduction is often helpful in patients experiencing significant generalized or hematologic toxicity. The pegylated IFNs are also associated with psychiatric disturbances including depression, irritability, insomnia and suicidal ideation. Caution is warranted when using IFNs and pegIFNs in patients with a history of a psychiatric disorder.

RIBAVIRIN

Ribavirin is a guanosine analog that has a broad spectrum of antiviral activity. Its mechanism of action is not clearly defined, but it is believed to act against HCV by one or more of the following mechanisms:

¦ enhancing T-cell mediated immune responses to HCV by shifting the balance towards a T-helper-1 response;

¦ inhibiting cellular inosine monophosphate dehydrogenase, thereby decreasing the intracellular guanosine triphosphate pool needed for viral RNA replication;
¦ directly inhibiting HCV polymerase; and
¦ acting as an RNA virus mutagen, thereby reducing viral fitness.[13]

Ribavirin is well absorbed after oral administration, with bioavailability ranging from 33 to 69% of the dose, and about 40% of the drug being eliminated via the kidneys. The major side-effect of ribavirin is hemolytic anemia, which may necessitate discontinuation of therapy. Ribavirin is highly teratogenic and birth control is required if the drug is used by women of child-bearing age or their partners. The benefits of ribavirin monotherapy in HCV infection disappear rapidly when the treatment is discontinued and it is therefore not recommended. As detailed below, ribavirin is used in combination with IFN or pegIFN.

Retrospective analysis of phase III studies of pegIFN plus ribavirin reveal that response is inversely correlated with the patient's body weight. In the study by Manns *et al.*,[12] patients receiving at least 10.6mg/kg of ribavirin had significantly better response rates for the same doses of IFN. This suggests that higher weight-based dosing of ribavirin may lead to improved responses. A randomized clinical trial is now underway to resolve this issue.

COMBINATION THERAPY FOR HEPATITIS C VIRUS INFECTION

Ribavirin given in combination with IFN has been shown to be more effective than the use of IFN alone for the treatment of HCV.[14] An SVR may occur in up to 40% of patients treated with combination therapy compared with approximately 20% of patients treated with IFN alone. Studies combining pegIFN alfa-2b with ribavirin had a 54% SVR compared to a 47% SVR with standard IFN alfa-2b plus ribavirin.[12] The SVR in patients infected with HCV genotype 1, which is associated with a poorer response to therapy, was also improved (42 vs 33%). Trials of pegIFN alfa-2a plus ribavirin also demonstrate excellent responses.[15] Combination therapy for HCV may also be beneficial in patients co-infected with HIV. Preliminary results of a randomized trial in HCV/HIV co-infected patients suggest that pegIFN alfa-2a plus ribavirin is superior to standard IFN alfa-2a plus ribavirin, with 24-week virologic response rates of 44 versus 15%.[16] (See Chapter 125).

THERAPIES IN DEVELOPMENT FOR HEPATITIS C

Hepatitis C virus is a single-stranded (ss) RNA virus of about 9.4kb in length. It is translated into a single polypeptide prescursor, which is cleaved into several functional gene products, including structural proteins, RNA polymerase, helicase and serine protease.[17] Several drugs are in development to target key components of the viral life cycle, including inhibitors of serine protease as well as the helicase enzyme (Fig. 207.2). Ongoing clinical trials are investigating the potential antifibrotic effects of maintenance therapy with pegIFN alfa or colchicine for virologic nonresponders to IFN-based regimens. Interferon-? is also under study as an antifibrotic agent.

NUCLEOSIDE AND NUCLEOTIDE ANALOGS

Nucleosides and nucleotides are the basic components of DNA and RNA in both prokaryotic and eukaryotic organisms. These molecules include a pentose sugar and a base moiety. A nucleotide differs from a nucleoside by the presence of one or more phosphate groups covalently bound to the molecule's sugar group. Intracellular phosphorylation is required before incorporation into the elongating nucleic acid chain. Pharmacologic analogs of these molecules have been developed for use in the treatment of several viral pathogens and

Figure 207-2 Hepatitis C virus life cycle and its inhibition.

function either as nucleic acid chain terminators or as inhibitors of polymerase enzymes. Nucleoside analogs (phosphate absent) include lamivudine, famciclovir and lobucavir, while nucleotide analogs (phosphate present) are represented by adefovir and tenofovir. These drugs inhibit a critical step of the HBV life cycle where the reverse transcriptase activity of its DNA polymerase catalyzes the conversion of RNA to DNA.[18] Resistance to nucleoside analogs can be caused by mutations in the active site of the DNA polymerase enzyme, including the highly conserved amino acid motif YMDD.[19]

Lamivudine

Lamivudine was the first nucleoside analog approved for HBV infection. It is also used for the treatment of HIV infection. Detailed pharmacokinetic and distribution data are provided in Chapter 204 . The dose used for HBV, 100mg q24h, is lower than that used for HIV. Lamivudine therapy is associated with a significant improvement in hepatic histology, normalization of hepatic enzymes and suppression of plasma HBV DNA.[20] In most patients, however, values return to baseline levels when lamivudine is discontinued. The emergence of drug-resistant HBV may limit the value of lamivudine for long-term therapy and may be associated with increases in serum liver transaminases.[21] Regimens combining lamivudine with other active compounds to enhance efficacy and limit the development of resistance are under investigation.[22]

Adefovir

Adefovir is approved for the treatment of HBV infection. Originally developed as an antiretroviral, it is associated with significant renal toxicity at the higher doses needed to inhibit HIV replication and is not approved for that indication.

Pharmacokinetics and distribution

Adefovir is administered as the prodrug adefovir dipivoxil. Unaffected by food, it achieves 60% oral bioavailability. Its half-life is about 12–30 hours and it undergoes renal excretion without significant observed metabolites. It does not substantially affect the cytochrome P450 system.

Routes of administration

Adefovir is approved for oral use only. The drug displays anti-HBV activity at doses above 5mg/day. It is approved for use at 10mg/day for HBV infection.

Indications

Chronic hepatitis B virus infection

Adefovir is highly active against HBV, producing reductions of HBV DNA of >3.5 logs. A phase III study (Adefovir Study 437) randomized patients to adefovir 10mg, 30mg or placebo; HBV viral load reduction, histologic improvement and HBeAg seroconversion rates were superior in the adefovir arms.[23] Furthermore, the drug displays in-vitro efficacy against lamivudine-resistant isolates. [24]

Adverse reactions and interactions

Adefovir appears well tolerated at the 10mg dose. Renal impairment becomes significant, however, at the 30mg dosage.[25] The most common adverse reactions are headache, gastrointestinal upset and elevated transaminases. No significant drug interactions have been documented.

Tenofovir disoprixil fumarate

Tenofovir is an orally bioavailable, nucleotide analog approved for HIV therapy. Detailed pharmacokinetic and distribution data are given in Chapter 204 . Limited data suggest that the drug has activity against lamivudine-resistant HBV isolates.[26] Tenofovir may have potential for the treatment of HBV infection in patients co-infected with HIV.[26A]

Other nucleoside analogs in development

Emtricitabine

Emtricitabine has efficacy against HIV as well as HBV. Its activity against HBV may be greater than that seen with lamivudine. In a dose-ranging study of emtricitabine at 25, 100 and 200mg daily for 48 weeks, 38, 42 and 61% of patients had reductions in HBV DNA levels to below the level of quantification by Digene Hybrid Capture II assay (<4700 copies/ml), respectively, while 32, 38 and 50% in each arm experienced loss of HbeAg.[27]

Entecavir

Entecavir is an orally bioavailable nucleoside analog with a half-life of 110 hours. A dose-ranging study of entecavir at 0.01, 0.1 and 0.5mg versus lamivudine demonstrated reductions in HBV DNA of 2.4, 4.3 and 4.7 logs at each entecavir dose, respectively, compared with a 3.4 log reduction with lamivudine.[28] Preliminary results show efficacy against the lamivudine-resistant YMDD mutant.

Famciclovir (see also Chapter 205)

Famciclovir, an orally administered prodrug of penciclovir, is approved for the treatment of herpes simplex virus and varicella-zoster virus infections. It is effective in animal models of HBV infection and has significant antiviral effects in patients with chronic HBV infection.[29] Famciclovir may not suppress virus as effectively as lamivudine, as evidenced by a lower rate of HBeAg seroconversion.[30] Furthermore, the drug does not appear to suppress lamivudine-resistant HBV mutants.

Lobucavir

Lobucavir is a cyclobutyl analog of guanine with potent in-vitro activity against HBV. In a study of 81 patients with HBV infection, treatment with lobucavir resulted in a 3.5 log decrease in HBV DNA. Levels of HBV DNA were below the level of quantitation in 68% of patients. Unfortunately, the drug appears to be associated with carcinogenic potential in long-term animal studies, and it is not clear whether further drug development will continue.

COMBINATION THERAPY FOR HEPATITIS B VIRUS INFECTION

Studies of combination therapy involving nucleoside analogs plus IFN have yielded conflicting results thus far.[31] These combinations, as well as combinations of nucleoside analogs, await future definitive evaluations before they can be recommended.

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Chapter 208 - Antifungal Agents

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Invasive fungal infections have evolved into important causes of morbidity and mortality in patients with severe underlying diseases. For more than three decades, treatment has been limited to amphotericin B (AmB) deoxycholate with or without flucytosine. Therapeutic options only emerged with the clinical development of fluconazole and itraconazole in the late 1980s. The past 10 years, however, have witnessed a major expansion in our antifungal armamentarium through the introduction of less toxic formulations of amphotericin B, the development of improved antifungal triazoles and the advent of the echinocandin lipopeptides, a new class of antifungal agents that target the fungal cell wall. This chapter reviews the clinical pharmacology of approved and investigational antifungal agents for treatment of invasive fungal infections.

POLYENE ANTIBIOTICS

Amphotericin B deoxycholate

Amphotericin B is a natural polyene macrolide antibiotic and consists of seven conjugated double bounds, an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group (Fig. 208.1). It is amphoteric, virtually insoluble in water, and not orally or intramuscularly absorbed. For parenteral use, AmB has been solubilized with deoxycholate as micellar suspension, and this formulation has been available for now more than 40 years.

Mechanism of action

Amphotericin B primarily acts by binding to ergosterol, the principal sterol in the cell membrane of most fungi, leading to the formation of ion channels and concentration-dependent, cell death (Fig. 208.2). These pores, composed of AmB multimers, form most readily when ergosterol is present in the cell membrane.[1] [2] With less avidity, the compound also binds to cholesterol, the main sterol of mammalian cell membranes, which is believed to account for most of its adverse effects. A second mechanism of action of AmB may involve oxidative damage of the cell through a cascade of oxidative reactions linked to its own oxidation and interactions with lipoproteins with formation of free radicals or an increase in membrane permeability. [3] [4] [5] [6]

Antifungal activity

Amphotericin B has broad-spectrum antifungal activity that includes most fungi pathogenic in humans. Primary resistance has been associated with qualitative or quantitative variations in membrane sterols,[7] but it may also be related to increased catalase activity with decreased susceptibility to oxidative damage.[8] [9] [10] Resistance remains uncommon in *Cryptococcus neoformans* and *Candida* spp. although AmB appears somewhat less active against *Candida guilliermondii, Candida parapsilosis* and *Candida tropicalis. Candida lustitaniae* isolates are often resistant to AmB, and this resistance can develop during treatment.[11] [12] *Aspergillus* spp. and other opportunistic molds, but not the dimorphic molds, tend to have more variable susceptibility to AmB. *Aspergillus terreus, Fusarium* spp., *Pseudallescheria boydii, Scedosporium prolificans*, certain other dematiaceous fungi and *Trichosporon beigelii* may be resistant to AmB at concentrations safely achievable in patients.[11] [13] [14] Acquisition of secondary resistance has been anecdotally reported but its role as a clinical problem has not been fully defined.

Pharmacodynamics

Amphotericin B displays concentration-dependent fungicidal activity against susceptible *Candida albicans, C. neoformans* and *Aspergillus fumigatus* in time-kill assays and exhibits a postantifungal effect of up to 12 hours' duration against *C. albicans* and *C. neoformans* in vitro.[15] [16] [17] Studies in laboratory animals with experimental disseminated candidiasis suggest that peak serum level to MIC ratio correlates best with antifungal efficacy in vivo.[18] These findings support the notion that large doses will be most effective and that achievement of optimal peak concentrations is important.

Pharmacokinetics

After intravenous administration, AmB dissociates from its vehicle and becomes highly protein-bound before distributing predominantly into liver, spleen, bone marrow, kidney and lung. A unique property of AmB is that protein binding in plasma is enhanced with increasing drug concentration.[19] Clearance from plasma is slow with a terminal half-life of 5 days and longer.[20] [21] Concentrations in body fluids other than plasma are generally low; however, despite mostly undetectable concentrations in the cerebrospinal fluid (CSF), AmB is effective in the treatment of fungal infections of the central nervous system. Amphotericin B is mostly excreted as unchanged drug in the urine and feces, and no metabolites have been identified.[20] Dose adjustment of AmB is not necessary in patients with unrelated renal or hepatic dysfunction. Because of its high protein binding, hemodialysis usually does not affect plasma concentrations of AmB. Infants and children appear to clear the drug from plasma more rapidly than adults, as indicated by a significant negative correlation between age and clearance.[22]

Adverse effects

Infusion-related reactions and nephrotoxicity of AmB are major problems and often limit therapy. Infusion-related reactions (fever, rigors, chills, myalgias, arthralgias, nausea, vomiting and headaches) are thought to be mediated by the release of cytokines from monocytes in response to the drug. They can be noted in up to 73% of patients during the first dose, but often improve during continued therapy.[23] Slowing the infusion rate or pre-medication with acetaminophen (10–15mg/kg), hydrocortisone (0.5–1.0mg/kg) or meperidine (0.2–0.5mg/kg) (pethidine, 25–50mg) may blunt these reactions. Continuous 24-hour infusion of AmB further reduces symptoms, but data on the antifungal efficacy of this dosing strategy is limited.[24] Cardiac arrhythmias and cardiac arrest caused by acute potassium release may occur with rapid infusion of under 60 minutes, especially if there is pre-existing hyperkalemia or renal impairment. True allergic reactions are rare.[25]

Amphotericin B-associated nephrotoxicity occurs as a result of alterations of membrane permeability in renal tubular and vascular

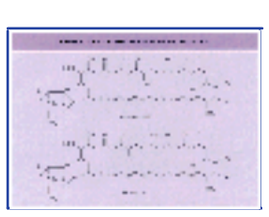

Figure 208-1 Chemical structures of amphotericin B and nystatin A1.

Figure 208-2 Biosynthetic pathway of ergosterol and points of activity by antifungal agents.

smooth muscle cells. Tubular transport defects and decreased glomerular filtration rate caused by vasoconstriction are responsible for potassium and magnesium wasting, tubular acidosis, impaired urinary concentration ability and azotemia. Decreased renal blood flow and recurrent ischemia may lead to permanent structural nephrotoxic effects.[3] Hypokalemia can be quite refractory to replacement until hypomagnesemia is corrected. Tubular acidosis and impaired urinary concentration are rarely of clinical significance. Azotemia is common; in a large prospective clinical trial, the baseline serum creatinine rose by more than 100% in over one-third of 344 unstratified patients receiving AmB for empiric therapy of fever and neutropenia.[23] Risks of developing renal toxicity are related to dose and duration of therapy, concomitant use of nephrotoxic agents such as ciclosporin and amikacin, and pre-existing renal dysfunction.[26] Renal toxicity associated with the use of AmB has the potential to lead to renal failure and dialysis. Often, however, azotemia stabilizes on therapy and is usually reversible after discontinuation of the drug. Avoiding concomitant nephrotoxic agents, appropriate hydration and normal saline loading (0.9% sodium chloride 10–15ml/kg/day) may lessen the likelihood and severity of azotemia.

Other potentially relevant adverse effects of AmB include hypotension, hypertension, flushing, vestibular disturbances and a normocytic, normochromic anemia after chronic administration.[22] Amphotericin B deoxycholate is topically irritating; therefore, a central line should be used for infusion. Local instillation, including intrathecal

administration, can cause focal areas of necrosis and should only be considered in conjunction with expert consultation.[27]

Drug interactions

In rats, AmB decreases the concentration of hepatic microsomal cytochrome P450 and inhibits propafenone metabolism.[28] Drug-drug interactions attributable to shared metabolic pathways have not been described in humans. Hypokalemia may be aggravated by corticosteroids, and hypomagnesemia may become especially profound in cancer patients with platinum-associated nephropathy. Amphotericin B therapy may enhance plasma levels and thereby toxicity of many renally cleared drugs, including aminoglycosides, vancomycin, fluorocytosine and ciclosporin. Cumulative renal toxicity has been seen when amphotericin B is given simultaneously with other nephrotoxic agents e.g., gentamicin, foscarnet or pentamidine. The simultaneous infusion of granulocytes has been associated with acute pulmonary reactions, and should therefore only be used with extreme caution. Treatment with fluconazole followed by AmB has been shown to reduce antifungal susceptibility in vitro and in vivo.[29] The sequential use of an azole followed by AmB should probably be avoided.

Indications and dosing

Despite its toxicity profile, AmB deoxycholate is still considered the drug of choice for the initial treatment of most life-threatening infections caused by susceptible organisms. Depending on both the type of infection and the host, the recommended daily dosage ranges from 0.5 to 1.5mg/kg/day administered over 2–4 hours as tolerated; the standard dosage for empiric therapy in persistently febrile neutropenic patients is 0.5–0.6mg/kg/day.[25] Continuous infusion of AmB deoxycholate may be associated with less toxicity;[24] however, this mode of administration is counterintuitive to the concentration-dependent pharmacodynamics of AmB and is therefore not recommended. Whether the enhanced plasma clearance in infants and young children has implications for dosing remains unknown. Currently, dosage recommendations for all pediatric age groups do not differ from those in adults. Treatment should be started at the full target dosage with careful bedside monitoring during the first infusion to allow for prompt intervention for infusion related reactions.

Therapeutic monitoring

Historically, it has been proposed that a plasma concentration at 1 hour after infusion of twice the MIC of the fungal isolate should be the target for treatment of yeast infections.[30] However, monitoring of AmB concentrations in plasma or CSF appears of little value, since relationships between plasma and tissue concentrations and clinical efficacy or toxicity have not been adequately characterized.[16]

Amphotericin B lipid formulations

During the past few years, three novel formulations of AmB have been approved in the USA and most of Europe:

- AmB colloidal dispersion (ABCD),
- AmB lipid complex (ABLC), and
- a small unilamellar vesicle liposomal formulation (L-AmB).

Because of their reduced nephrotoxicity in comparison to AmB deoxycholate, these compounds allow for the delivery of higher dosages of AmB. However, data from animal models also suggest that higher dosages are required for equivalent antifungal efficacy.

TABLE 208-1 -- Amphotericin B formulations and liposomal nystatin: physicochemical properties and pharmacokinetic parameters.

AMPHOTERICIN B FORMULATIONS AND LIPOSOMAL NYSTATIN: PHYSICOCHEMICAL PROPERTIES AND PHARMACOKINETIC PARAMETERS					
	DAMB	**ABCD**	**ABLC**	**LAMB**	**LNYS**
Lipids (molar ratio)	Deoxycholate	Cholesterylsulfate	DMPC/DMPG (7:3)	HPC/CHOL/DSPG (2:1:0.8)	DMPC/DMPG (7:3)
Mol% drug	34	50	50	10	10
Lipid configuration	micelles	micelles	membrane-like	SUVs	MLVs
Particle diameter (µm)	0.05	0.12–0.14	1.6–11	0.08	0.32
Dosage (mg/kg)	1	5	5	5	4
C_{max} (µg/ml)	2.9	3.1	1.7	58	24
AUC_{0-8} (µg/ml.h)	36	43	14	713	80
VD_{ss} (l/kg)	1.1	4.3	131	0.22	0.24
Cl (l/h/kg)	0.028	0.117	0.476	0.017	0.051
Half-life (h)	39	28	6–18	7–10	2–6
DAMB, amphotericin B deoxycholate; ABCD, amphotericin B colloidal dispersion; ABLC, amphotericin B lipid complex; LAMB, liposomal amphotericin B; LNYS, liposomal nystatin; DMPC, dimiristoyl phosphatidylcholine; DMPG, dimiristoyl phosphatidylglycerol; HPC, hydrogenated phosphatidylcholine; CHOL, cholesterol; DSPG, disteaoryl phosphatidylglycerol; SUVs, small unilamellar vesicles (liposomes); MLVs, multilamellar vesicles (liposomes); C_{max} , peak plasma concentration; AUC_{0-8} area under the concentration vs time curve from time zero to infinity; VDss, apparent volume of distribution at steady state; Cl, plasma clearance; Half-life, apparent plasma half-life during the dosing interval of 24 hours					
Data represent mean values, stem from adult patients and were obtained after different rates of infusion. Concentrations of nystatin were measured in whole blood and are approximately half of those measured in serum					
Modified from Groll et al.[62] and Cossum et al.[45]					

Physicochemical properties and pharmacokinetics

Because of differences in the physicochemical properties of their carriers, each of the lipid formulations confers its own distinct plasma pharmacokinetics properties to AmB. All three formulations, however, preferentially distribute to organs of the mononuclear phagocytic system (MPS), sequester lipid-associated drug within deep tissues and functionally spare the kidney. L-AmB, the only truly liposomal AmB, is composed of small spherical unilamellar lipid vesicles averaging 60–70nm in size. Following infusion the liposomal preparation has a prolonged circulation time in plasma, achieves strikingly high peak plasma concentrations and AUC values, and is only slowly taken up by the MPS. However, most of the AmB in plasma remains liposome-associated, and free unbound drug concentrations are low.[19] [20]

Amphotericin B lipid complex, composed of ribbon-like lipid aggregates, is efficiently opsonized by plasma proteins and rapidly taken up by the MPS to achieve high concentrations of drug in the cellular components of blood, liver, lung and spleen. It exists as a depot in these tissues and free AmB is slowly released. Fungal and inflammatory cell lipases at the site of infection act to release the complexed AmB from the lipid carrier.[31]

Amphotericin B colloidal dispersion, a colloidal dispersion of microscopic disc-shaped particles, also preferentially collects in tissues of the MPS and free drug is slowly released over time. Drug levels in the plasma and kidney are low and terminal elimination half-life is long (Table 208.1).

Whether these distinct pharmacokinetic features translate into different pharmacodynamic properties is largely unknown. More recent experimental comparisons of all four formulations of AmB against defined invasive mycoses suggest potentially important differences in antifungal efficacy depending on agent, dose, type and site of infection.[13]

Safety and antifungal efficacy

Safety and antifungal efficacy of lipid formulations have been demonstrated in phase I and phase II studies in immunocompromised patients with a wide spectrum of

underlying disorders.[32] [33] [34]

The overall response rates in these trials ranged from 53 to 84% in patients with invasive candidiasis and from 34 to 5%, respectively, in patients with probable or documented invasive aspergillosis. Clinical and microbiologic responses were observed in patients with documented invasive fungal infections even when lipid formulations were used as salvage therapy after failure of conventional AmB deoxycholate. Patients with neutropenia responded to therapy with lipid formulations, but, as with other antifungal agents, recovery of neutrophil counts, remission from underlying malignancy and continued antifungal therapy were necessary for resolution of their fungal infections. Several randomized controlled trials have now been completed in which one of the new formulations has been compared with AmB deoxycholate. These studies have consistently shown at least equivalent therapeutic efficacy and reduced nephrotoxicity in comparison with AmB deoxycholate. A meta-analysis of 1149 neutropenic patients from three trials found that prophylactic or empiric use of L-AmB tended to be more effective than AmB deoxycholate in preventing breakthrough fungal infections and that it was associated with less nephrotoxicity, although it did not lead to improved survival.[35] Infusion-related side-effects of fever, chills and rigor appear to be less frequent with L-AmB and may even be increased with ABCD.[23] [36] [37] Several individual cases of substernal chest discomfort, respiratory distress and sharp flank pain have been noted during infusion of L-AmB,[38] and in a comparative study hypoxic episodes associated with fever and chills were more frequent in ABCD recipients than in AmB deoxycholate recipients.[39] Mild increases in serum bilirubin and alkaline phosphatase have been observed with all three formulations, Generally mild increases in serum transaminases and pancreatic enzymes with L-AmB have been observed. However, no cases of fatal liver or pancreatic diseases have been reported.

Indications and dosing

The lipid formulations are indicated for the treatment of patients with AmB-susceptible invasive mycoses that are refractory to AmB deoxycholate and for the treatment of patients who are intolerant to AmB deoxycholate; L-AmB is indicated for empiric therapy of persistently neutropenic patients. Preliminary pediatric pharmacokinetic and safety data indicate no fundamental differences in comparison with adults. The optimal dosages of each formulation for the various type and sites of invasive fungal infections remain to be defined. Based on animal data and the few randomized studies that have used AmB deoxycholate as comparator,[40] [41] we and most other experts in the field consider a dose of 5mg/kg/day of ABCD, ABLC or L-AmB to be equivalent to a dosage of 1mg/kg/day of AmB deoxycholate. Accordingly, an initial dosage of 5mg/kg/day of ABCD, ABLC or L-AmB is currently recommended for treatment of suspected or documented life-threatening infections, and a dosage of 3mg/kg/day is recommended when L-AmB is selected for empiric antifungal therapy in persistently febrile, neutropenic patients. Recent data have also suggested that lipid formulation of AmB at doses of 3mg/kg can be effective in patients with histoplasmosis and in selected patients with candidiasis. [42] [43] Because the favorable therapeutic index of the drug some clinicians have successfully used L-AmB at doses of 10mg/kg and 15mg/kg in patients with recalcitrant invasive fungal infections. The maximum tolerable dose appears to be at least 15mg/kg/day.

Use of lipid formulations of AmB has been limited by their high costs. Current in-vivo and clinical data indicate that L-AmB is the least toxic of the of these agents. However, as L-AmB also carries the highest drug acquisition cost, appreciation of its superior safety profile is often eclipsed by pharmacoeconomic concerns. Comprehensive economic analyses that include hospital and societal costs resulting from excess nephrotoxicity related to the use of conventional AmB deoxycholate appear to indicate that the high cost of lipid formulations of AmB may be supplanted by the even greater cost of AmB deoxycholate-induced nephrotoxicity.

Liposomal nystatin

Nystatin, the first antifungal polyene, is a tetraene-diene macrolide with a structure that is very similar to that of AmB. Like AmB, it binds to ergosterol in the cell membrane of fungi, creating a pore and leading to cell death. Although it is used widely as a topical agent, nystatin is ineffective when given orally and highly toxic when given parenterally (see Fig. 208.1). Although generally less potent on a molar basis, the spectrum of activity of this agent and its in-vitro pharmacodynamics are similar to those of AmB, and it might have activity against some amphotericin B-resistant isolates. [25] [44] An intravenous multilamellar liposomal formulation of nystatin has been developed that has reached clinical trials. The plasma pharmacokinetics of multilamellar liposomal nystatin are unique among the current polyenes and are characterized by comparatively high peak plasma concentrations and rapid elimination from plasma, with a half-life of 5–7 hours.[45] Animal studies have indicated that this formulation is preferentially distributed to lung, liver and spleen, and that the kidney appears to play a major role in excretion.[46] In a phase I study, multilamellar liposomal nystatin was tolerated at multiple dosages of up to 8mg/kg.[47] The compound has demonstrated clinical efficacy in the treatment of candidemia and invasive aspergillosis and as empiric antifungal therapy for neutropenic cancer patients at dosages ranging from 2mg/kg to 4mg/kg.[48] [49] [50] Submission for approval by the US Food and Drug Administration (FDA) is expected in the near future.

FLUCYTOSINE

Flucytosine (5-fluorocytosine, 5-FC) is a low-molecular-weight, water-soluble, synthetic fluorinated analog of cytosine (Fig. 208.3). It has no antifungal activity of its own. It is taken up by the fungus-specific enzyme cytosine permease and converted in the cytoplasm by cytosine deaminase to 5-fluorouracil, which causes RNA miscoding and inhibits DNA synthesis. [51] Although it was originally synthesized as a potential antitumor agent, 5-FC is relatively nontoxic to mammalian cells because of the absence or very low level of activity of cytosine deaminase. Recently, this compound has re-emerged as an antineoplastic agent for use in tumors sensitized by the addition of fungal and bacterial cytosine deaminase genes. In the USA, 5-FC is available only as oral formulation; an intravenous formulation is available in some countries.

Antifungal activity

The antifungal spectrum of 5-FC in vitro encompasses *Candida* spp., *C. neoformans, Saccharomyces cerevisiae* and selected dematiaceous moulds. Flucytosine has little to no activity against *Aspergillus*

Figure 208-3 Comparison of the chemical structures of cytosine, flucytosine and fluorouracil.

spp. and other hyaline molds.[52] [53] Synergistic or additive effects in combination with AmB have been observed against *Candida* spp. and in combination with AmB, fluconazole or the investigational agent posaconazole against *C. neoformans*. [32] [54] With the exception of *Candida krusei*, primary resistance to 5-FC occurs very rarely in *Candida* spp.[55] Resistance to 5-FC, defined as a MIC of 32µg/ml or greater, has been observed in 1.6–2.2% of *C. neoformans* isolates.[56] In contrast to primary resistance, secondary resistance, which occurs predominantly by selection of resistant clones, can evolve rapidly and appears to result from a single point mutation. In *C. neoformans* resistance to 5-FC has been observed in 30–40% of isolates from patients with meningitis who relapsed following monotherapy.[57] Resistance in susceptible fungi may involve either mutations in the enzymes necessary for cellular uptake, transport or metabolism, or competitive upregulation of pyrimidine synthesis. [51] As a consequence, 5-FC is rarely given alone but in combination with AmB or, more recently, fluconazole.

Pharmacodynamics

Flucytosine has demonstrated predominantly concentration-independent fungistatic (99% reduction in colony-forming units) activity against *Candida* spp. and *C. neoformans* in time-kill assays, and prolonged concentration- and exposure-dependent post-antifungal effects of up to 10 hours. [58] [59] Pharmacodynamic studies in mice with experimental disseminated candidiasis have revealed that both the time above the MIC and AUC:MIC ratio were important in predicting efficacy. The C_{max} :MIC ratio was the least important parameter, and maximum efficacy was observed when levels exceeded the MIC for only 20–25% of the 24-hour dosing interval.[60] Thus, lower dosages or less frequent dosing may yield identical antifungal efficacy while further reducing potential toxicities that are mostly dose-dependent.

Pharmacokinetics

Flucytosine is readily absorbed from the gastrointestinal tract, has negligible protein binding and distributes evenly into tissues and body fluids, including the CSF, peritoneal fluid, inflamed joints and the eye. At usual dosages, the drug undergoes little hepatic metabolism and is eliminated predominantly in its active form by glomerular filtration into the urine, with a half-life in plasma of 3–6 hours. Individual dosage adjustment is necessary in patients who have impaired renal function and those undergoing hemofiltration. In patients undergoing hemodialysis, a dose of 37.5mg/kg is recommended following dialysis; in peritoneal dialysis, the compound can

be administered systemically or intraperitoneally.[51] Although the data are limited, impaired liver function does not appear to alter 5-FC disposition.[61] [62] The pharmacokinetics of 5-FC in pediatric patients has not been formally characterized, so that uniform dosing recommendations cannot be made.

Adverse effects

Common adverse effects of 5-FC that occur in 5–6% of patients include gastrointestinal intolerance and reversible elevations of hepatic transaminases and alkaline phosphatase. Rarer side-effects are skin rashes, ulcerative colitis and bowel perforation, blood eosinophilia and crystalluria.[62] Hematologic adverse effects have been reported in 6% of patients receiving oral 5-FC overall; these adverse effects may include neutropenia, thrombocytopenia or pancytopenia. Among 202 AIDS patients receiving combination therapy with AmB and 5-FC for cryptococcal meningitis, the drug toxicity withdrawal rate of 3% was similar to the rate in those receiving AmB monotherapy.[63] Flucytosine-associated adverse effects are usually reversible after discontinuation of the drug or dosage reduction; however, fatal outcomes have been reported.[64] Some of the adverse effects of 5-FC may be due to the conversion of the compound to 5-fluorouracil by the gastrointestinal bacterial flora and the toxic effects of endogenous metabolites.[51] In patients treated with intravenous 5-FC, in whom the drug presumably did not undergo metabolism by the human intestinal microflora, 5-fluorouracil levels were not detected, but some toxicity was still observed.[65] Hematologic adverse effects are less frequent if plasma levels of 5-FC do not exceed 100µg/ml. [66]

Drug interactions

Orally administered, nonresorbable antibiotics and aluminum hydroxide- and magnesium hydroxide-based antacids may delay absorption of 5-FC from the gastrointestinal tract.[62] Flucytosine is not known to interfere with CYP450 enzyme system. However, any drug that can cause a reduction in the glomerular filtration rate may increase 5-FC serum levels and thereby has the potential to enhance 5-FC-associated toxicity. This includes AmB as well as number of antimicrobial agents, anticancer drugs and ciclosporin.[61] Cytosine arabinoside competitively inhibits 5-FC and the two drugs should not be given concomitantly.

Indications and dosing

Owing to the propensity for secondary resistance, 5-FC is generally not administered as a single agent. An established indication for its use in combination with AmB deoxycholate is for induction therapy of cryptococcal meningitis.[63] The combination of AmB deoxycholate with 5-FC can also be recommended for the treatment of candidal infections involving deep tissues, particularly in critically ill patients and when *Candida* spp. other than *C. albicans* are involved.[64] This includes candidal meningitis, endophthalmitis, endocarditis, vasculitis and peritonitis, as well as osteoarticular, renal and chronic disseminated candidiasis.[62] Flucytosine has been reported to delay hematopoietic recovery after cytotoxic chemotherapy and can therefore not be recommended as an addition to AmB for empiric antifungal therapy of persistently febrile neutropenic patients.[67] The combination of 5-FC with fluconazole may be used for cryptococcal meningitis, when treatment with AmB is not feasible. In addition, this combination may also be useful as second-line therapy for individual patients with invasive candidal infections involving aqueous body compartments. Currently, we recommend a starting dosage for both adults and children of 100mg/kg/day in three or four divided doses.

Therapeutic monitoring

Monitoring of plasma concentrations is essential to adjust dosage to changing renal function and to avoid toxicity. Following oral administration, near-peak levels 2 hours postdosing overlap with trough levels as patients reach steady state and are thus sufficient for therapeutic monitoring.[64] Peak plasma levels between 40µg/ml and 60µg/ml correlate with antifungal efficacy and are seldom associated with adverse hematologic effects.[64] [66] The need for monitoring plasma concentrations has limited the use of 5-FC because performance of this test is often restricted to referral laboratories.

ANTIFUNGAL TRIAZOLES

The antifungal azoles are a class of synthetic compounds that have one or more azole rings and a more or less complex side chain, which is attached to one of the nitrogen atoms. The imidazoles miconazole and ketoconazole were the first azoles developed for systemic treatment of human mycoses. Severe toxicities associated with the drug carrier (in the case of miconazole) and erratic absorption and significant interference with the human cytochrome P450 system (in the case of ketoconazole) have limited their clinical usefulness.[62] The subsequently developed triazoles fluconazole and itraconazole (Fig. 208.4), however, have become extremely useful

Figure 208-4 Chemical structures of fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole.

components of the antifungal armamentarium. They possess an expanded spectrum of activity and greater target specificity and are well tolerated overall.

Mechanism of action

The antifungal azoles target ergosterol biosynthesis by inhibiting the fungal cytochrome P450-dependent enzyme lanosterol 14-a-demethylase. This inhibition interrupts the conversion of lanosterol to ergosterol, which leads to accumulation of aberrant 14-a-methylsterols and depletion of ergosterol in the fungal cell membrane (see Fig. 208.2). This alters cell membrane properties and function and, depending on the organism and the compound, may lead to cell death or inhibition of cell growth and replication. In addition, the azoles also inhibit cytochrome P450-dependent enzymes of the fungal respiration chain.[62]

Antifungal activity

Fluconazole and itraconazole are principally active against dermatophytes, *Candida* spp., *C. neoformans*, *T. beigelii* and some other uncommon yeast-like organisms, and dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis* and *Sporothrix schenckii*.[62] These azoles have less activity against *Candida glabrata* and almost none against *C. krusei*.[68] Useful activity against *Aspergillus* spp. and dematiaceous molds is restricted to itraconazole. Itraconazole and fluconazole are quite inactive against *Fusarium* spp. and the zygomycetes.[62] The second-generation triazoles, voriconazole and the investigational agents posaconazole and ravuconazole, are active against *C. albicans* and *C. krusei*, *Aspergillus* spp. and some *Fusarium* spp. Posaconazole is also active against the zygomycetes.[69]

Resistance

Selection and nosocomial spread of azole-resistant *Candida* spp. has become a matter of increasing concern. Resistance in *Candida* spp. is encountered most commonly in form of a primarily resistant species or through selection of resistant subclones during exposure to azoles. Several mechanisms of resistance have been identified, including but not limited to alterations at the target binding site, increased target expression and induction of cellular efflux pumps.[7] Before the advent of highly active antiretroviral therapy, azoleresistant oropharyngeal and esophageal candidiasis was a major clinical conundrum in HIV-infected patients.[70] In addition, emergence of *C. glabrata* and *C. krusei* infections in association with fluconazole prophylaxis has been observed in several bone marrow transplant centers, although frequency and attributable mortality of these breakthrough infections appears to be low overall.[71] Although cross-resistance of *Candida* spp. to antifungal azoles is common,[72] it is not obligate; patients with microbiologic and clinical fluconazole-resistant mucosal candidiasis may respond to itraconazole or newer triazoles. Acquired azole resistance has been documented in a few patients with *C. neoformans* meningitis receiving maintenance therapy. Very little is known, however, about frequency and mechanisms of secondary azole resistance in filamentous fungi.[62]

Fluconazole

Pharmacodynamics

Conventional time-kill assays performed over incubation periods of 24–48 hours in susceptible *Candida* spp. and *C. neoformans* show fungistatic activity of fluconazole with variable concentration-related growth effects.[73] [74] However, with extended incubation of up to 14 days and under nonproliferating growth conditions, fungicidal activity has been observed against *C. albicans*.[75] In serum-free growth media, fluconazole displays no measurable post-antifungal effect against *C. albicans* and *C.*

neoformans, but concentration-dependent post-antifungal effects of 1–3.6 hours were observed in the presence of fresh serum.[16] Pharmacodynamic studies in murine models of disseminated *C. albicans* infection collectively suggest that

the AUC:MIC ratio is the most predictive pharmacodynamic parameter of fluconazole. [76] Overall, the dose-independent pharmacokinetics and the available experimental and clinical data are in support of once daily dosing regimens.

Pharmacokinetics

Fluconazole is available for oral and parenteral use, and it exhibits linear plasma pharmacokinetics that are independent of route and formulation.[77] Steady state is generally reached within 4–7 days with once-daily dosing, but it can be rapidly achieved by doubling the dose on the first day. Protein binding is low, and the drug distributes evenly into virtually all tissue sites and body fluids, including the CSF, brain tissue and the eye. More than 90% of a dose is excreted via the kidney, with approximately 80% recovered as unchanged, active drug and 11% recovered as inactive metabolites. In patients with a creatinine clearance of =50ml/minute, a 50% reduction in dosage is required, and with a creatinine clearance of 21ml/minute, a 75% reduction is needed; the initial loading dose need not be adjusted. Fluconazole is dialyzable; in patients undergoing hemodialysis, 100% of the target dose is given after each dialysis session. In continuous hemofiltration, clearance of fluconazole may be faster, requiring therapy at maximum approved dosages, and in patients with peritoneal dialysis the compound can be administered either systemically or intraperitoneally. Hepatic insufficiency per se does not require dose adjustments, but careful monitoring of additional hepatic toxicity is warranted. [62] [77]

The pharmacokinetics of fluconazole in pediatric age groups reflects developmental changes characteristic for a water-soluble drug with minor metabolism and predominantly renal elimination. Except for premature neonates, in whom clearance is initially decreased, pediatric patients tend to have an increased weight-normalized clearance rate from plasma, which leads to a shorter half-life compared with adults.[78] [79] Therefore, dosages at the high end of the recommended dosage range are necessary for the treatment of invasive mycoses in children.

TABLE 208-2 -- Drug-drug interactions of fluconazole and itraconazole.

DRUG-DRUG INTERACTIONS OF FLUCONAZOLE AND ITRACONAZOLE		
Mechanism and drug involved	**Triazole involved**	**Comment**
Decreased plasma concentration of triazole		
Decreased absorption of triazole	Itra**	Take antacids and antifungal agent at least 2 hours apart
• Antacids, H_2 -antagonists		
• Omeprazole, sucralfate		
• Didanosine, grapefruit juice		
Increased metabolism of triazole	Itra**, flu	Potential for therapy failure; increased potential for hepatotoxicity
• Isoniazid, rifampin		
• Rifabutin, phenytoin		
• Phenobarbital, carbamazepine		
Increased concentration of coadministered drug through inhibition of its metabolism by triazole		
Terfenadine, astemizole, cisapride	Flu‡ , Itra‡	Concom. use prohibited
Lovastatin, simvastatin, atorvastatin	Itra‡ , Flu‡	Concom. use prohibited
Phenytoin	Flu* , Itra*	Monitor levels
Benzodiazepines	Flu† , Itra†	Monitor closely
Carbamazepine	Flu†	Monitor closely
Haloperidol	Itra†	Monitor closely
Rifampin, rifabutin	Flu† , Itra†	Monitor closely
Clarithromycin	Itra†	Monitor closely
Indinavir, ritonavir	Itra†	Monitor closely
Vincristine, vinblastine, vindesine	Itra*	Avoid concom. use
Busulfan	Itra†	Avoid concom. use
All-trans retinoic acid	Flu†	Monitor closely
Nifedipine, felodipine	Itra† , Flu†	Monitor closely
Ciclosporin A, tacrolimus	Flu† , Itra†	Monitor serum level
Sulfonylurea drugs; warfarin; prednisolone	Flu† , Itra†	Monitor closely
Digoxin; quinidine	Itra†	Monitor levels (digoxin)
Zidovudine; theophyllin	Flu†	Monitor closely
Flu, fluconazole; Itra, itraconazole		
Modified from Groll et al.[62]		

‡ contraindicated
* Major significance
† moderate significance

Adverse effects

In adults, fluconazole has been safely administered over prolonged periods of time at dosages of up to 1600mg/day.[80] Compiled data from adult patients who received dosages of 100–400mg/day indicate an overall incidence of significant adverse effects or laboratory abnormalities leading to the discontinuation of the drug of 2.8%. Nausea, vomiting and other gastrointestinal symptoms are seen in <5% of adult patients, skin rashes and headaches in <2% and asymptomatic hepatic transaminase elevations (which are usually reversible) in up to 7%.[81] In pediatric patients of all age groups, fluconazole is generally well tolerated at dosages of up to 12mg/kg/day, with no differences from adults in frequency and profile of adverse events.[82] Severe side-effects, including liver failure and exfoliative dermatitis, have been reported anecdotally.[62] Alopecia can be seen with higher dose fluconazole (400mg/day) given for 2 months or longer; it is reversed by discontinuing therapy or substantially reducing the daily dose.[83]

Drug interactions

Fluconazole undergoes minimal CYP-mediated metabolism, but inhibits CYP3A4 and several other CYP isoforms in vitro and interacts with enzymes involved in glucuronidation, leading to a number of significant drug-drug interactions (Table 208.2).[81] [84] On the other

hand, drugs notorious for hepatic enzyme induction may lead to decreased fluconazole levels and therapeutic failure as the ultimate consequence.[62] Altogether, the number of relevant drug-drug interactions appears to be lower than for ketoconazole and itraconazole.

Clinical indications and dosages

Fluconazole is highly effective for the treatment of superficial and invasive candidal infections, including infections in neutropenic patients.[85] However, in unstable patients and in those who have received antifungal azoles for prophylaxis, AmB remains the agent of choice for initial therapy. Further potential indications for fluconazole include consolidation therapy for chronic disseminated candidiasis, cryptococcal meningitis and infections with *T. beigelii*. Fluconazole is the drug of choice for treatment of coccidioidal meningitis and has effectiveness in nonmeningeal coccidioidal infections;[86] [87] against paracoccidioidomycosis, blastomycosis, histoplasmosis and sporotrichosis, the compound appears comparatively less active than itraconazole.[33] [88] [89] [90] In the prophylactic setting, fluconazole has proven efficacy for primary prevention of invasive candidal infections in high-risk patients with acute leukemia and in patients who have undergone bone marrow or liver transplantation, for primary prevention of cryptococcosis and histoplasmosis in HIV-infected patients with very low CD4+ cell counts, and for secondary prevention of cryptococcisis and coccidioidomycosis in HIV-infected patients. [91] [92] [93]

In adults, the recommended dosage range for treatment of invasive infections is 400–800mg; in the preventive setting 100–400mg is recommended. In pediatric patients of all age groups, the recommended dosage range of fluconazole is 6–12mg/kg/day. However, in view of the faster clearance rate, the larger volume of distribution and the safety profile of fluconazole, 12mg/kg/day may be the more appropriate dosage for treatment of serious infections in full-term neonates and in infants and children. Given the extreme variability in extravascular water content and renal function, particularly in very low birth-weight preterm infants, predictably effective and safe treatment with fluconazole may not be possible in this patient population during the first days of life.

Itraconazole

Pharmacodynamics

Itraconazole exerts species- and strain-dependent fungistatic or fungicidal pharmacodynamics in vitro. Time-kill experiments have demonstrated concentration-independent, fungistatic activity of itraconazole against *Candida* spp. and *C. neoformans*.[16] Against *Aspergillus* spp., however, itraconazole displayed time and concentration-dependent fungicidal activity with >87 to >97% killing within 24 hours of drug exposure.[94] Persistent effects have not been reported thus far, and it remains to be determined which pharmacodynamic parameter best predicts antifungal efficacy in vivo.[16]

Pharmacokinetics

Itraconazole is a high-molecular-weight, highly lipophilic bis-triazole. It is available as capsules, as oral solution in hydroxypropyl-ß-cyclodextrin (HP-ß-CD), and as a parenteral solution that also uses HP-ß-CD as solubilizer. Absorption from the capsule form is dependent on a low intragastric pH and is compromised in the fasting state, and is thus unpredictable in granulocytopenic cancer patients and in patients with hypochlorhydria. Absorption is improved when the capsules are taken with food or an acidic cola beverage. The novel oral solution of itraconazole in HP-ß-CD provides better oral bioavailability, which is further enhanced in the fasting state.[95]

Following oral administration, peak plasma concentrations occur within 1–4 hours; systemic absorption of the cyclodextrin carrier is negligible. With once-daily dosing, steady state is achieved after 7–14 days, but it can be reached more rapidly by doubling the dose over the first 2–3 days. Following administration of intravenous HP-ß-CD itraconazole, drug and carrier rapidly dissociate and follow their own disposition. Peak plasma levels of itraconazole and its carrier occur immediately after completion of the 1-hour infusion. The carrier HP-ß-CD is not significantly metabolized, and virtually 100% is eliminated from plasma within 24 hours in unchanged form via glomerular filtration. Itraconazole is highly protein-bound and is extensively distributed throughout the body. Whereas concentrations in nonproteinaceous body fluids are negligible, tissue concentrations in many organs, including the brain, exceed corresponding plasma levels by 2–10 times.[95] [96]

Itraconazole is extensively metabolized in the liver and is excreted in metabolized form into bile and urine. The major metabolite, hydroxy-itraconazole, possesses antifungal activity similar to that of itraconazole. After oral administration, the plasma concentrations of hydroxy-itraconazole at steady state are 1.5–2 times higher than those of the parent compound, whereas they are considerably lower than those of itraconazole after intravenous dosing.[62] [97] It is important to note that plasma concentrations of itraconazole measured by bioassay are different from those determined by high-performance liquid chromatography, since the latter method usually does not account for hydroxy-itraconazole. The elimination of itraconazole from plasma follows a biphasic pattern; in comparison to single dosing, the elimination half-life at steady state is about twice as long, reflecting saturable excretion mechanisms.[62]

The dosage of oral itraconazole does not need to be adjusted in patients with renal insufficiency or dialysis. However, since the elimination of HP-ß-CD parallels the glomerular filtration rate, intravenous itraconazole is contraindicated in patients with a creatinine clearance of <30ml/minute; no data are available for its use in patients undergoing dialysis. In patients with severe hepatic insufficiency, the elimination half-life of itraconazole can be prolonged, and additional hepatic toxicity or possible drug interactions should be carefully monitored.[95]

Despite considerable variability between patients, the pharmacokinetics of oral HP-ß-CD itraconazole in pediatric patients beyond the neonatal period appear not to be fundamentally different from those in adults.[98] [99] No data are currently available in this population for the capsule form and intravenous HP-ß-CD itraconazole.

Adverse effects

Itraconazole usually is well tolerated, with a similar spectrum and frequency of adverse effects to that of fluconazole. Adverse events leading to the discontinuation of itraconazole occur in approximately 4% of patients treated for systemic fungal infections at dosages of up to 400mg/day. Most observed reactions are transient, and include nausea and vomiting (in <10% of patients), hypertriglyceridemia (in 9%), hypokalemia (in 6%), elevated hepatic transaminases (in 5%), rash or pruritus (in 2%), headaches or dizziness (in <2%) and pedal edema (in 1%).[100] Gastrointestinal intolerance appears to be exceedingly frequent with oral HP-ß-CD itraconazole at dosages exceeding 400mg/day. Only a few cases of more severe hepatic injury or hepatitis have been described. Itraconazole can have negative inotropic effects; because of a possible, albeit low, risk of cardiac toxicity, itraconazole should not be administered to patients with ventricular dysfunction.[101] Oral HP-ß-CD itraconazole was safe and well tolerated in pharmacokinetic studies in pediatric patients.[102] Vomiting (12%), abnormal liver function tests (5%) and abdominal pain (3%) were the most common adverse effects in 103 neutropenic pediatric cancer patients who received the drug at a dosage of 5mg/kg/day or 2.5mg/kg twice daily for antifungal prophylaxis; 18% of patients withdrew from the study because of adverse events.[103]

Drug interactions

In comparison to fluconazole, both the propensity for and the extent of drug-drug interactions are greater with itraconazole (see Table 208.2). Itraconazole is a substrate of CYP3A4, but also interacts with the heme moiety of CYP3A, resulting in noncompetitive inhibition of oxidative metabolism of many CYP3A substrates and increased (and potentially toxic) concentrations of co-administered drugs. Increased metabolism of itraconazole resulting in decreased plasma levels can be induced by drugs known for their induction of hepatic metabolizing enzymes. Patients who receive itraconazole along with one of the drugs listed in Table 208.2 should be followed closely and plasma concentrations, ideally of both compounds, should be monitored carefully. Finally, the systemic availability of itraconazole depends in part on the activity of intestinal CYP3A4 and P-glycoprotein, which contributes to its variable bioavailability after oral administration.

Clinical indications and dosing

Itraconazole is a useful agent for dermatophytic infections, pityriasis versicolor and all forms of cutaneous and mucosal candidiasis; however, its clinical efficacy in invasive candidal infections has not been evaluated. The experience with itraconazole for induction therapy of cryptococcal meningitis is scant, but itraconazole has been used with success for consolidation and maintenance treatment of this condition in patients with HIV infection, although with less success than fluconazole (see Chapter 126). [104] Itraconazole is approved as a second-line agent for the treatment of invasive *Aspergillus* infections; few data exist on its use for first-line treatment in neutropenic patients.[105] Itraconazole may be useful in the management of infections by certain dematiaceous molds,[106] but it has no documented activity against zygomycosis and fusariosis. Itraconazole is the current treatment of choice for lymphocutaneous sporotrichosis and non-life-threatening, nonmeningeal paracoccidioidomycosis, blastomycosis and histoplasmosis. In progressive, nonmeningeal coccidioidomycosis, itraconazole appears at least as active as

fluconazole.[86] However, AmB remains the treatment of choice for most immunocompromised patients and for those with life-threatening forms of endemic mycoses.

Itraconazole has been successfully used as antifungal prophylaxis in patients undergoing stem cell transplantation or intensive chemotherapy.[103] Hydroxypropyl-ß-cyclodextrin itraconazole may reduce the incidence of proven or suspected invasive fungal infections in neutropenic patients with hematologic malignancies, but prophylactic efficacy against invasive aspergillosis has not been convincingly demonstrated thus far.[34] Itraconazole was at least as effective as conventional AmB and was superior with respect to its safety profile as empiric antifungal therapy in persistently febrile neutropenic patients,[107] which has led to the approval of this indication by the FDA.

The recommended dosage range of oral itraconazole is 100–400mg/day (capsules) and 2.5mg/kg twice daily (HP-ß-CD solution). For life-threatening infections, however, more aggressive dosing may be necessary. For such conditions, we recommend a loading dose of 600–800mg/day for 3–5 days followed by a maintenance dose of 400–600mg/day, with monitoring of serum levels. The approved dosage of intravenous HP-ß-CD itraconazole is 200mg twice daily for 2 days, followed by 200mg/day for a maximum of 12 days. Itraconazole is not approved for patients under 18 years of age; based on the available pharmacokinetic data, a starting dosage of 2.5mg/kg twice daily of oral HP-ß-CD itraconazole can be advocated. The recommended dosage range for the capsule formulation is 5–8mg/kg/day with a loading dose of 4mg/kg three times daily for the first 3 days. Data on the use of intravenous itraconazole in pediatric patients are currently lacking.

Therapeutic monitoring

While experimental studies have provided evidence of a relationship between plasma concentrations and antifungal efficacy, the main rationale for monitoring plasma levels has been the erratic oral bioavailability of itraconazole, particularly in neutropenic patients. Historically, the target plasma level for itraconazole has been estimated at 0.25µg/ml (by high-performance liquid chromatography) at trough based on the IC_{90} of a large set of clinical isolates. However, more recent clinical data from a large cohort of patients undergoing intensive chemotherapy for acute leukemia and receiving antifungal prophylaxis with itraconazole have demonstrated a significant statistical association of trough concentrations <0.5µg/ml with the occurrence of invasive fungal infections.[108] We therefore recommend rapid achievement and maintenance of trough levels of =0.5µg/ml when itraconazole is given for prevention or treatment of invasive fungal infections.

Second-generation antifungal triazoles

Further improvements in the structure-activity relationship of antifungal triazoles have led to a new group of synthetic compounds that are collectively known as second-generation triazoles. The FDA has approved voriconazole, and the investigational agents posaconazole and ravuconazole are currently in advanced stages of clinical development. While ravuconazole and voriconazole are structurally related to fluconazole, the structure of posaconazole is similar to that of itraconazole.

Pharmacology

The second-generation triazoles possess enhanced target activity and specificity. They are active against a wide spectrum of clinically important fungi, including *Candida* spp., *T. beigelii, C. neoformans, Aspergillus* spp., *Fusarium* spp. and other hyaline molds, and dematiaceous as well as dimorphic moulds; they have demonstrated efficacy in various animal models of invasive fungal infections. As with itraconazole, these novel triazoles exert fungistatic activity against susceptible yeast-like organisms and strain-dependent fungicidal activity against susceptible filamentous fungi. Fundamental differences in potency, spectrum and antifungal efficacy between posaconazole, ravuconazole and voriconazole have not surfaced, but posaconazole appears to have the best activity against the zygomycetes. Against *Candida* spp. and *C. neoformans*, voriconazole exhibits nonconcentration-dependent pharmacodynamics. Near-maximal fungistatic activity is achieved at a drug concentration of approximately three times the MIC.[109] A post-antifungal effect of up to 4 hours has been observed with *C. albicans*. [110]

Voriconazole is available as a tablet and as parenteral solution that uses sulfobutyl ether-ß-cyclodextrin as solubilizer. Taken in tablet form, voriconazole is rapidly absorbed from the gastrointestinal tract. Although bioavailability is near 100%, steady-state plasma concentrations are achieved more rapidly if an intravenous loading dose is given. Multiple enzymes of the cytochrome P450 system, including CYP2C19, which exhibit genetic polymorphism in certain racial groups, metabolize voriconazole. Drug levels in poor metabolizers, which may include up to 20% of Asian populations, can be significantly elevated. Following its metabolism in the liver, the drug is mostly excreted in urine. In addition to undergoing metabolism by the P450 system, voriconazole inhibits several CYP enzymes. Therefore, there is extensive potential for drug-drug interactions. While all three second-generation triazole agents display some nonlinearity in their disposition, undergo hepatic metabolism and have the potential for drug-drug interactions through their affinity to CYP450 isoenzymes, key pharmacokinetic parameters (oral bioavailability, extent of protein binding, plasma clearance and volume of distribution) vary. Whether these

differences are of clinical significance, however, remains to be elucidated.

Mild and reversible visual disturbances occur in approximately 30% of patients. The mechanism is unknown, but is thought to be retinal in origin. Other common adverse reactions include rashes including phototoxicity, gastrointestinal disturbances and hepatic abnormalities.

Clinical studies

In a randomized, unblinded trial of patients with invasive aspergillosis, initial therapy with voriconazole led to better responses, improved survival and less toxicity than the standard approach of initial therapy with AmB.[111] Voriconazole has been used successfully in the treatment of invasive fungal infections, including aspergillosis in children who were refractory to or intolerant of conventional antifungal therapy.[112] Voriconazole has also been shown to be effective in the treatment of oropharyngeal and esophageal candidiasis in immunocompromised patients.[113] A large randomized multicenter trial has been completed that compared voriconazole (3mg/kg intravenously twice daily or 200mg by mouth twice daily) with liposomal AmB (3mg/kg intravenously) for empiric antifungal therapy. This study showed comparable composite success rates but less proven and probable breakthrough infections, infusion-related toxicity and nephrotoxicity in the voriconazole-treated cohort. However, patients receiving voriconazole had significantly more frequent episodes of transient visual disturbances and hallucination. [114]

For treatment of invasive fungal infections in children and adults an intravenous loading dose of 6mg/kg every 12 hours on day 1 followed by 4mg/kg every 12 hours thereafter is used. Whenever feasible the drug should be given by the oral route at 200mg twice daily (in children weighing less than 40kg, the dose is 100mg twice times daily). Because sulfobutyl ether-ß-cyclodextrin clearance is related to glomerular filtration rate, oral voriconazole should be used in patients whose creatinine clearance is <50ml/min. In patients with mild to moderate hepatic cirrhosis, the standard loading dose is recommended, but the maintenance dose should be halved.

To date, preliminary data from phase II and III studies have been presented for posaconazole and voriconazole. Posaconazole (50–400mg by mouth) was well tolerated and as effective as fluconazole (100mg) in two large randomized comparative studies in HIV-infected patients with oropharyngeal candidiasis.[115] Furthermore, in a salvage study in patients with a variety of invasive fungal infections, response rates in subjects with aspergillosis, fusariosis, cryptococcosis, candidiasis and phaeohyphomycoses after 4–8 weeks of

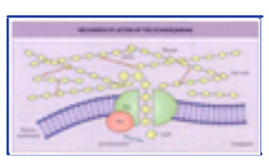

Figure 208-5 Mechanism of action of the echinocandins.

therapy with a dose of 800mg/day by mouth ranged from 44 to 80%.[116] Further clinical trials and development of an intravenous formulation are underway.

ECHINOCANDIN LIPOPEPTIDES

The echinocandins are a novel class of semisynthetic amphiphilic lipopeptides composed of a cyclic hexapeptide core linked to a variably configured lipid side chain. The echinocandins act by noncompetitive inhibition of the synthesis of 1,3-ß-glucan, a polysaccharide in the cell wall of many pathogenic fungi (Fig. 208.5). Together with chitin, the rope-like glucan fibrils are responsible for the strength and shape of the cell wall. They are important in maintaining the osmotic integrity of the fungal cell and play a key role in cell division and cell growth.[117] [118] Caspofungin has recently received approval by the FDA for treatment of refractory invasive aspergillosis and candidal esophagitis. Anidulafungin and micafungin are in advanced stages of clinical development. (Fig. 208.6).

Antifungal activity

The current echinocandins appear to possess very similar pharmacologic properties. All three compounds have potent and broad spectrum, fungicidal in-vitro activity against *Candida* spp. and potent inhibitory activity against *Aspergillus* spp.; their antifungal efficacy against these organisms in vivo has been demonstrated in various animal models. The current echinocandins have variable activity against dematiaceous and endemic mold and are inactive against most hyalohyphomycetes, zygomycetes, *C. neoformans* and *T. beigelii*.[119] Furthermore, all echinocandins have demonstrated preventive and therapeutic activity in animal models of *Pneumocystis carinii* pneumonitis. In-vitro resistance has been observed in fungal isolates overexpressing a gene encoding for a Golgi protein involved in the transport of cell wall components.[120] Primary resistance to echinocandins in otherwise susceptible fungal yeast species is rare, and resistance-induction studies have demonstrated a low potential for secondary resistance in *Candida* spp. The frequency of primary echinocandin resistance among clinical isolates of *Aspergillus* spp. and induction of secondary resistance in vitro have not been studied thus far.

Pharmacodynamics

The echinocandins demonstrate a species-dependent mode of antifungal activity. They have fungicidal activity against most *Candida* spp. but not against *Aspergillus* spp. In the latter, microscopic examination

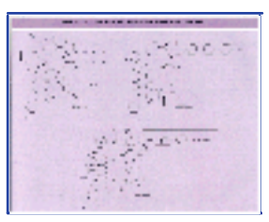

Figure 208-6 Structures of echinocandins currently in clinical practice or in clinical trials.

of exposed hyphae show a dose-dependent formation of microcolonies with progressively truncated, swollen hyphal elements that appear to be cell-wall deficient but that are able to regain their cell walls upon subculture in the absence of the drug. In a process that has been likened to pruning, caspofungin preferentially kills cells at the active centers for new cell wall synthesis within *A. fumigatus* hyphae.[121] These observations indicate differences in functional target sensitivity in both species that are not fully understood. In-vitro pharmacodynamic studies in *Candida* spp. have shown predominantly concentration-dependent fungicidal activity and rate of kill =99.9% reduction in colony-forming units) and concentration-dependent prolonged post-antifungal effects of up to 12 hours.[15] [122] In-vivo pharmacodynamic animal studies suggest similar concentration-dependent activity of the current echinocandins.

Pharmacokinetics

At present, all current echinocandins are available only for intravenous administration. They exhibit dose-proportional plasma pharmacokinetics with a ß-half-life of between 10 and 15 hours, which allows for once-daily dosing. All echinocandins are highly protein bound (>95%) and distribute into all major organ sites, including the brain; however, concentrations in uninfected CSF are low. The echinocandins are metabolized by the liver and slowly excreted into urine and feces; only small fractions are excreted into urine in unchanged form.[123] The pharmacokinetic parameters are generally lower in children than in adults. Drug levels are especially low in smaller, younger children. The ß-half-life in children is reduced by over one-third relative to adults.[124]

Adverse effects and drug interactions

At the currently investigated dosages, all echinocandins are generally well tolerated, and only a small fraction of patients enrolled in the various clinical trials (<5%) discontinued therapy because of drug-related adverse events. The most frequently reported adverse effects include increased liver transaminases, gastrointestinal upset and headaches. As with other basic polypeptides, the echinocandins have the potential to cause histamine release; however, histamine-mediated symptoms have been observed in isolated cases only. The current echinocandins appear to have no significant potential for drug interactions mediated by the CYP450 enzyme system. Caspofungin can reduce the AUC of tacrolimus by approximately 20% but has no effect on ciclosporin levels. However, ciclosporin increased the AUC of caspofungin by approximately 35%; because of transient elevations of hepatic transaminases in single-dose interaction studies, the

concomitant use of both drugs is currently not recommended. Finally, inducers of drug clearance or mixed inducer-inhibitors, namely efavirenz, nelfinavir, nevirapine, phenytoin, rifampin, dexamethasone and carbamazepine, may reduce caspofungin concentrations.

Clinical studies

The clinical efficacy of anidulafungin, caspofungin and micafungin against *Candida* spp. has been investigated in phase II or phase III studies in immunocompromised patients with esophageal candidiasis. All agents were well tolerated without serious adverse events, and all agents achieved therapeutic efficacy that was at least comparable to that of standard agents. A multicenter phase II salvage trial of caspofungin has been completed in 90 patients with definite or probable invasive aspergillosis. The majority of patients had hematologic malignancies or had undergone bone marrow transplantation, and most patients had infections that were refractory to standard therapies. As determined by an independent expert panel, a complete or partial response was observed in 45% of patients receiving at least one dose of caspofungin.[125] In a recent study of adults with invasive candidiasis (predominantly candidemia), caspofungin (70mg loading dose then 50mg/day) was compared with amphotericin B deoxycholate (0.6–1.0mg/kg/day) and found to be equivalent to but better tolerated than amphotericin B.[125A] Caspofungin is also effective and well tolerated in patients with HIV-associated esophageal candidiasis.[126] Given these results, caspofungin should be regarded as a reasonable initial treatment for candida infections. One exception may be infections due to C. parapsilosis, as the echinocandidins are less effective in vitro against this organism.

Caspofungin may be indicated in patients with probable or proven invasive aspergillosis refractory to other approved therapies and in patients who are intolerant of other therapies. The currently recommended dose regimen of caspofungin in adults consists of a single 70mg loading dose on day 1, followed by 50mg/day thereafter, administered by slow intravenous infusion of approximately 1 hour. A daily dosage regimen in the pediatric population has yet to be established. A dose of 50mg/m^2 /day is under current investigation.[124]

TERBINAFINE

Terbinafine is a highly lipophilic and keratophilic allylamine inhibitor of ergosterol biosynthesis. It is a potent noncompetitive inhibitor of fungal squalene epoxidase and prevents squalene epoxidation, an important early step in the synthesis of ergosterol. Treated fungi accumulate squalene and become deficient in ergosterol. Cell death is associated with the development of high intracellular squalene concentrations, which interfere with fungal membrane function and cell wall synthesis.[7] [127] In addition to its antifungal properties terbinafine is also anti-inflammatory and can act as a free radical scavenger.

Terbinafine is active in vitro against a wide range of pathogenic fungi, including dermatophytes, molds, dimorphic fungi, *C. neoformans* and some but not all *Candida* and *Aspergillus* spp.[128] In-vitro studies with *Aspergillus* spp. have shown terbinafine and AmB to have additive, synergistic interactions depending on the isolate. In combination with itraconazole or voriconazole, terbinafine displays potent synergistic interactions against *Aspergillus* spp. Fluconazole also increases the activity of terbinafine in an additive to synergistic fashion.[129] In vitro, combinations of terbinafine with voricoazole can overcome azole resistance in *Candida* spp. Terbinafine in combination with AmB or voriconazole is synergistic against some zygomycetes isolates. Resistance to terbinafine has been observed following a single gene mutation in *Aspergillus*.[130] In *Candida*, cross-resistance following treatment with fluconazole can occur because of upregulation of target enzymes.[131] Multidrug efflux pumps may also reduce susceptibility.[132]

Oral terbinafine is rapidly absorbed, with peak concentration occurring at 60–90 minutes postdose. It is quickly converted to multiple metabolites, which co-exist in plasma with the parent compound. Drug is delivered to peripheral tissues via sebum and by direct diffusion through the dermal layers.[133] It is detected in sebum and hair within the first week of administration and by week 3 in stratum corneum and nail samples. The terminal half-life is approximately 3 weeks and fungicidal concentrations persist in peripheral tissues for weeks to months after administration of the last dose.[134] Increasing age and concomitant hypertension are associated

with higher plasma concentrations, and smokers have lower levels than nonsmokers.[135] When given as 1% cream, terbinafine concentrations in the horny layer of skin far exceed the MICs for common dermatophytes, and effective levels of drug remains in skin well beyond discontinuation of therapy.[136]

Multiple cytochrome P450 enzymes metabolize terbinafine, and agents that affect this system alter drug concentration.[137] Terbinafine competitively inhibits CYP2D6, and elevated levels of desipramine have been observed when the drugs are co-administered. Terbinafine may reduce the level of ciclosporin A. Most of the drug and drug metabolites are eliminated in the urine. The drug is generally well tolerated, but gastrointestinal disturbances, skin rashes and headaches occur occasionally. Rare adverse reactions include hepatobiliary dysfunction, induction and exacerbation of lupus, agranulocytosis and severe skin reactions.

Oral terbinafine at doses of 250mg/day in adults and 62.5–250mg/day in children is effective and generally safe for cutaneous mycoses that warrant systemic therapy.[138] It is highly efficacious as treatment for onychomycosis. Terbinafine at daily doses of 5–15mg/kg for several months was effective in three patients with pulmonary aspergillosis who had failed standard therapy.[139] At this time, however, this agent, cannot be recommended for the treatment of systemic mycoses. Terbinafine 1% cream is effective against a variety of cutaneous mycoses. The length of treatment with either topical or oral formulation depends on the specific infection.

GRISEOFULVIN

Griseofulvin, a metabolic product of *Penicillium*, was the first oral agent available for treatment of dermatomycoses. This compound inhibits fungal cell mitosis and nucleic acid synthesis, and disrupts spindle and cytoplasmic microtubule function.[140] Griseofulvin is active against many dermatophytes, but not all. High-level resistance can develop following drug exposure, and a multiple-layered thick cell wall, which may limit griseofulvin entry, has been observed in resistant isolates.[141] [142] Griseofulvin has extremely low water solubility and moderate lipid solubility. Absorption from the gastrointestinal tract is variable and depends on the amount of dissolved drug that reaches the intestine.[143] Absorption is enhanced by a fatty meal. The bioavailability of the ultamicrosize formulation is higher than that of the microsize formulation. Once absorbed, drug is highly protein-bound. Griseofulvin is detected in the outer layer of the stratum corneum soon after it is ingested and is diffused from the extracellular fluid and sweat. Deposition of drug in growing cells may account for entry into hair and nails. [140] Concentration in plasma peaks at 3–4 hours and in skin blister fluid at 6 hours. The terminal half-life in plasma and in skin blisters is approximately 9–10 hours. During chronic administration, plasma and skin blister levels equilibrate.[144] Griseofulvin is largely metabolized in the liver and degradation metabolites are excreted in the urine. Griseofulvin is also effective in several inflammatory skin conditions, possibly

because of its anti-inflammatory properties mediated by modulation of the expression of cell adhesion molecules on leukocytes and vascular endothelial cells.[145]

Adverse effects include gastrointestinal disturbances, headaches, hepatitis and rashes. Liver and thyroid neoplasia, abnormal germ cell maturation, teratogenicity and embroyotoxicity have been observed in animal studies. The reproductive toxicity as well as the induction of chromosome aberrations in somatic cells may result from disturbance of microtubuli formation. Griseofulvin also induces accumulation of porphyrins and formation of Mallory bodies in hepatocytes. These may represent additional carcinogenic mechanisms.[146] Less common adverse events include exacerbation of lupus, porphyrias and blood dyscrasias. Drug interactions are related to induction of hepatic enzymes and include phenobarbital, oral anticoagulants and oral contraceptives.

For onychomycosis, griseofulvin has been largely supplanted by newer antifungals. Currently, its main indication is for the treatment of tinea capitis and other cutaneous mycoses. Doses of 500–1000mg/day in adults and 15–20mg/kg/day in children have been used successfully. Length of treatment depends on the site and type of infection.

AMOROLFINE

The morpholine derivative amorolfine is currently used only as a topical agent. This compound interferes with ergosterol biosynthesis and leads to the depletion of ergosterol and the subsequent accumulation of sterol precursors within the cell membrane.[147] Amorolfine also weakly inhibits the fungal enzyme squalene epoxidase. In vitro, amorolfine has a broad spectrum of activity that includes dermatophytes, dimorphic, dematiaceous and filamentous fungi, and yeasts.[148] [149] In patients, amorolfine has eradicated infections caused both by *C. albicans* and by a broad range of dermatophytes.[150] In a murine model of dermatophytosis the combinations of amorolfine with griseofulvin, terbinafine, itraconazole and fluconazole were synergistic, but 5-FC appears to be antagonistic in some fungal isolates.[151]

Amorolfine has been formulated as 0.125%, 0.25% and 0.5% creams and as a 5% lacquer. Following application, the amorolfine penetrates the nails rapidly and within 24 hours of contact it exceeds the MIC of most fungi that cause onychomycosis.[152] Following topical application, active concentration of amorolfine is retained in the skin for several days. In experimental models of systemic mycosis, amorolfine shows no significant activity, which may be due to strong protein binding or to rapid metabolism (or both).[147] The bioavailabilty of topical amorolfine is 4–10% and drug is excreted in urine and feces.[153]

Treatment-related adverse events are generally limited to burning, itching, erythema, skin dryness and scaling. Topical amorolfine has been used successfully in a variety of dermatomycoses and for the treatment of onychomycosis. Combination therapy with oral itraconazole or terbinafine may be a promising option for patients with severe disease.[154] [155]

CICLOPIROX

Ciclopirox is a poorly absorbable, synthetic hydroxypyridone antifungal agent. The mechanism of action is related to its affinity for trivalent cations. By chelating essential metal cofactors, ciclopirox inhibits fungal enzymes that are responsible for diverse metabolic processes and ultimately causes membrane and cytoplasmic disruption.[156] Ciclopirox at subinhibitory concentrations impairs candidal adherence to mucosal surfaces.[157] It has potent antifungal activity against a broad range of dermatophytic and nondermatophytic fungi. It is available in a variety of topical formulations, including as 0.77% gel and 8% nail lacquer. It readily penetrates the skin via the epidermis and the hair follicles and achieves the highest concentration in the horny layer.[158] When used as 8% nail lacquer, the drug achieves uniform distribution within the nail-plate after daily use for 1 week, and it reaches a maximum at 3–4 weeks with uniform distribution to all nail layers.[159] Within the nail the drug achieves concentrations in excess of inhibitory and fungicidal concentrations for most pathogens.[156] The bioavailability of topical ciclopirox is 2–5% and absorbed drug is mainly metabolized by glucuronidation. Excretion is predominantly renal with an elimination half-life of approximately 2 hours.[159] [160]

Treatment-related adverse events are uncommon, but local reactions such as pruritus and burning sensation can occur.

Ciclopirox has been used successfully for a variety of cutaneous mycotic infections, mucosal candidiasis and seborrheic dermatitis, and as a treatment for onychomycosis.

FUTURE DIRECTIONS

The past decade has seen a considerable expansion in antifungal drug research and the clinical development of several new compounds targeted against invasive fungal infections. Major progress has been made in defining paradigms for antifungal intervention and in designing and implementing clinical trials. The field of antifungal therapy is currently undergoing accelerated changes. Combination therapy is being explored. Several novel and promising antifungal compounds are currently in the early stages of pre-clinical investigation, and the pursuit of novel biochemical and molecular targets will result in further candidate drugs. In the light of past and present epidemiologic trends, invasive fungal infections are likely to remain a common and important complication in immunocompromised patients. An expanded drug arsenal, elucidation of resistance mechanisms, integration of pharmacokinetic and pharmacodynamic relationships, and combination therapies offer hope for further substantial progress in prevention and treatment.

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Chapter 209 - Antiparasitic Agents

Samuel L Stanley Jr

INTRODUCTION

Antiparasitic agents are used to treat infestations caused by a diverse and complex group of organisms encompassing the unicellular protozoa, which have intricate life cycles often involving more than one host, as well as the helminths, which have highly developed organ systems. Many antiparasitic agents are old drugs that have never been subjected to the rigorous testing of efficacy and safety currently required by agencies in various countries, such as the US Food and Drug Administration. For most of the drugs, information regarding use in pregnancy is totally lacking.

The treatment options by organism or disease entity, along with the recommended adult and pediatric dosages, are listed in Table 209.1 . Agents that are not readily available in the USA are listed in Table 209.2 . Few of the antiparasitic agents have been extensively studied in pregnancy. Table 209.3 divides the drugs into those that are probably safe on the basis of clinical experience, those that are possibly safe on the basis of anecdotal experience or are safe during certain trimesters, and those that are known to be hazardous or for which too little information is known to make a recommendation. In general, however, the decision to use of any of these agents in a pregnant patient must be made on an individual basis, weighing the severity of the illness and the benefit of treatment to the mother against the potential toxicity to the fetus.

ANTIPROTOZOAL AGENTS

AMODIAQUINE

Amodiaquine is a 4-aminoquinoline with antimalarial activity and a mechanism of action similar to that of chloroquine. It is available only as the dihydrochloride salt for oral administration and it undergoes extensive first-pass metabolism in the liver to the active compound desethylamodiaquine, which is widely distributed throughout the body and slowly eliminated. The side-effect profile is similar to that of chloroquine but agranulocytosis and severe hepatitis have been reported with long-term use (as chemoprophylaxis). The activity of amodiaquine against some chloroquine-resistant strains of *Plasmodium falciparum* has led to a modest revival in its use.[6] [7] [8] A recent randomized clinical trial comparing amodiaquine and chloroquine showed superior efficacy for amodiaquine and a similar safety profile in an area with high levels of chloroquine-resistant *P. falciparum*.[9]

AMPHOTERICIN B

Amphotericin B, a polyene antifungal agent, is the drug of choice for primary amebic meningoencephalitis caused by *Naegleria* spp. (see Chapter 244), and it is an alternative drug for leishmaniasis (see Chapter 172).[10] [11] [12] [13] Its pharmacokinetics and side effects are detailed in Chapter 208 . Lipid-associated formulations of amphotericin B have recently been shown to be effective in the treatment of visceral leishmaniasis in India in patients who failed to respond to antimony therapy, single dose therapy with liposomal amphotericin B giving cure rates of more than 92% with minimal toxicity.[12] [13]

ANTIFOLATE AGENTS

Antifolate agents act at various steps in the folic acid cycle. For *Plasmodium* spp., *Toxoplasma* spp. and other sensitive parasites, reduced folic acid derivatives are essential for de-novo pyrimidine synthesis. Unlike mammalian cells, these parasites cannot use preformed pyrimidines. Antifolate agents are most commonly used in combination to block sequential steps in the folic acid metabolic pathway (see Chapter 197).

Pyrimethamine

Pyrimethamine is a diaminopyrimidine that inhibits plasmodial dihydrofolate reductase at a concentration that is 1000 times less than that required to inhibit the mammalian enzyme.[6] [7] [14] [15] [16] It is effective against the erythrocytic stages of all *Plasmodium* spp. that are pathogenic for humans and, in combination with sulfadiazine, clindamycin or atovaquone, it is used for the treatment of *Toxoplasma gondii*. [17] [18] Pyrimethamine also has activity against *Isospora belli*.[19] The drug is available in oral form; it is slowly but completely absorbed, is 85% protein-bound and is extensively metabolized by the liver (< 3% is excreted unchanged). The half-life is 4–6 days.

Although pyrimethamine is available as 25mg tablets, it is almost exclusively used in combination with a sulfonamide (sulfadiazine, sulfadoxine) or a sulfone (dapsone; see below). The dosage for toxoplasmosis is listed in Table 209.1 . Some clinicians give an initial pyrimethamine loading dose of 200mg. In patients who cannot tolerate sulfonamides, clindamycin (1.8–2.4g/day in divided doses) or atovaquone (1.5g q12h) may be substituted. Side effects of pyrimethamine include blood dyscrasias, rash and, very rarely, seizures or shock. At high doses, pyrimethamine causes bone marrow suppression, which can be prevented by concurrent administration of folinic acid.

Trimethoprim

Trimethoprim (TMP) is another diaminopyrimidine that inhibits microbial dihydrofolate reductase. It has activity against

- a variety of bacteria (see Chapter 197);
- *Pneumocystis carinii* (see Chapter 124); and
- the parasites *Isospora belli* and *Cyclospora cayetanensis* (see Chapter 243).[19] [20]

Trimethoprim is readily absorbed, widely distributed and 50% protein-bound. Less than 20% is hepatically metabolized to inactive metabolites and the drug is excreted both in the urine and bile. The half-life is 9–11 hours.

For parasitic infections, TMP is used in fixed combination with sulfamethoxazole (SMX; see below). Side effects include rashes, pruritus, nausea, vomiting, glossitis, elevated liver enzymes, cytopenias, megaloblastic anemia, fever, aseptic meningitis and impaired renal function.

TABLE 209-1 -- Antiparasitic agents and dosages.[†]

ANTIPARASITIC AGENTS AND DOSAGES			
Infection	**Drug**	**Adult dosage**	**Pediatric dosage**
Acanthamoeba (keratitis)			
Drug of choice	Polyhexamethylene biguanide 0.02% (topical) plus 0.1% propamidine isethionate (topical)		
Amebiasis (*Entamoeba histolytica*)			
Asymptomatic			
Drug of choice	Paromomycin	25–35mg/kg/day in three doses for 7 days	25–35mg/kg/day in three doses for 7 days
Alternatives	Diloxanide furoate	500mg q8h for 10 days	20mg/kg/day in three doses for 10 days
	Iodoquinol	650mg q8h for 20 days	30–40mg/kg/day (maximum 2g) in three doses for 20 days
Mild-to-moderate intestinal disease			
Drug of choice	Metronidazole	500–750mg q8h for 5–10 days	35–50mg/kg/day in three doses for 10 days
OR			
	Tinidazole	2g/day for 3 days	50mg/kg (maximum 2g) per day for 3 days
Severe intestinal disease and extraintestinal disease			
Drug of choice	Metronidazole	750mg q8h for 5–10 days	35–50mg/kg/day in three doses for 10 days
OR			
	Tinidazole	600mg q12h or 800mg q8h for 5 days	50mg/kg or 60mg/kg (maximum 2g) per day for 5 days

Amebic meningoencephalitis (primary)			
***Naegleria* spp.**			
Drug of choice	Amphotericin B	1mg/kg/day iv, uncertain duration	1mg/kg/day iv, uncertain duration
***Acanthamoeba* spp.**			
Drug of choice	Pentamidine, ketoconazole, flucytosine		
Balamuthia mandrillaris [*]			
Drugs of choice	Clarithromycin	500mg q8h	
	Fluconazole	400mg q24h	
	Sulfadiazine	1.5g q6h	
	Flucytosine	1.5g q6h	
Sappinia diploides [†]			
Drugs of choice	Azithromycin	250mg q24h	
	Pentamidine	300mg iv q24h	
	Itraconazole	200mg q12h	
	Flucytosine	2.75g q6h	
***Ancylostoma caninum* (eosinophilic enterocolitis)**			
Drug of choice	Mebendazole	100mg q12h for 3 days	100mg q12h for 3 days
OR			
	Pyrantel pamoate	11mg/kg (maximum 1g) for 3 days	11mg/kg (maximum 1g) for 3 days
OR			
	Albendazole	400mg, single dose	400mg, single dose
Angiostrongyliasis			
Angiostrongylus cantonensis			
Drug of choice	Mebendazole	100mg q12h for 5 days	100mg q12h for 5 days
Angiostrongylus costaricensis			
Drug of choice	Mebendazole	200–400mg q8h for 10 days	200–400mg q8h for 10 days
Alternative	Thiabendazole	75mg/kg/day in three doses for 3 days (maximum 3g/day)	75mg/kg/day in three doses for 3 days (maximum 3g/day)
Anisakiasis (*Anisakis* spp.)			
Treatment of choice		Surgical or endoscopic removal	
Ascariasis (*Ascaris lumbricoides*, roundworm)			
Drug of choice	Mebendazole	100mg q12h for 3 days or 500mg, single dose	100mg q12h for 3 days or 500mg, single dose
OR			
	Pyrantel pamoate	11mg/kg, single dose (maximum 1g)	11mg/kg, single dose (maximum 1g)
OR			
	Albendazole	400mg, single dose	400mg, single dose
Babesiosis (*Babesia* spp.)			
Drug of choice	Clindamycin	1.2g q12h iv or 600mg q8h po for 7 days	20–40mg/kg/day po in three doses for 7 days
PLUS	Quinine	650mg q8h po for 7 days	25mg/kg/day in three doses for 7 days
OR			
	Atovaquone	750mg q12h for 7–10 days	20mg/kg q12h for 7–10 days
PLUS	Azithromycin	600mg po daily for 7–10 days	12mg/kg daily for 7–10 days
Balantidiasis (*Balantidium coli*)			
Drug of choice	Tetracycline	500mg q6h for 10 days	40mg/kg/day (maximum 2g) in four doses for 10 days
Alternatives	Iodoquinol	650mg q8h for 20 days	40mg/kg/day in three doses for 20 days
	Metronidazole	750mg q8h for 5 days	35–50mg/kg/day in three doses for 5 days
Baylisascariasis (*Baylisascaris procyonis*)			
Drugs of choice	Albendazole, mebendazole, thiabendazole, levamisole or ivermectin		
Blastocystis hominis			
Drug of choice	Metronidazole	750mg q8h for 10 days	
OR			
	Iodoquinol	650mg q8h for 20 days	
Capillariasis (*Capillaria philippinensis*)			
Drug of choice	Mebendazole	200mg q12h for 20 days	200mg q12h for 20 days
Alternative	Albendazole	400mg/day for 10 days	400mg/day for 10 days
Cryptosporidiosis (*Cryptosporidium parvum*)			

No agent has yet been conclusively proved to be effective in AIDS patients. Nitazoxanide, in the doses listed below, showed efficacy in clearing infection from immunocompetent individuals.			
	Nitazoxanide	500mg q12h for 3 days	age 4–11 years: 200mg q12h for 3 days
			age 1–3 years: 100mg q12h
Cutaneous larva migrans (creeping eruption, dog and cat hookworm)			
Drug of choice	Thiabendazole	Topical administration	Topical administration
OR			
	Ivermectin	150–200µg/kg, single dose	150–200µg/kg, single dose
OR			
	Albendazole	400mg/day for 3 days	400mg/day for 3 days
Cyclosporiasis (*Cyclospora cayetanensis*)			
Drug of choice	TMP-SMX	TMP 160mg, SMX 800mg q12h for 7–10 days	TMP 5mg/kg, SMX 25mg/kg q12h for 7–10 days
Dientamoeba fragilis			
Drug of choice	Iodoquinol	650mg q8h for 20 days	40mg/kg/day (maximum 2g) in three doses for 20 days
OR			
	Paromomycin	25–30mg/kg/day in three doses for 7 days	25–30mg/kg/day in three doses for 7 days
OR			
	Tetracycline	500mg q6h for 10 days	10mg/kg q6h (maximum 2g) for 10 days
Dracunculiasis (*Dracunculus medinensis*, guinea worm)			
Drug of choice	Metronidazole	250mg q8h for 10 days	25mg/kg/day (maximum 750mg) in three doses for 10 days
Entamoeba polecki			
Drug of choice	Metronidazole	750mg q8h for 10 days	35–50mg/kg/day in three doses for 10 days
Enterobiasis (*Enterobius vermicularis*, pinworm)			
Drug of choice	Pyrantel pamoale	11mg/kg, single dose (maximum 1g); repeat in 2 weeks	11mg/kg, single dose (maximum 1g); repeat in 2 weeks
OR			
	Mebendazole	100mg, single dose; repeat in 2 weeks	100mg, single dose; repeat in 2 weeks
OR			
	Albendazole	400mg, single dose; repeat in 2 weeks	400mg, single dose; repeat in 2 weeks
Filariasis			
Wuchereria bancrofti, Brugia malayi			
Drug of choice	Diethylcarbamazine	Day 1: 50mg after food	Day 1: 1mg/kg after food
		Day 2: 50mg q8h	Day 2: 1mg/kg q8h
		Day 3: 100mg q8h	Day 3: 1–2mg/kg q8h
		Days 4–14: 6mg/kg/day in three doses	Days 4–14: 6mg/kg/day in three doses
Loa loa			
Drug of choice	Diethylcarbamazine	Day 1: 50mg after food	Day 1: 1mg/kg after food
		Day 2: 50mg q8h	Day 2: 1mg/kg q8h
		Day 3: 100mg q8h	Day 3: 1–2mg/kg q8h
		Days 4–21: 9mg/kg/day in three doses	Days 4–21: 9mg/kg/day in three doses
Mansonella ozzardi			
Drug of choice	Ivermectin	6mg, single dose	
Mansonella perstans			
Drug of choice	Mebendazole	100mg q12h for 30 days	
OR			
	Albendazole	400mg q12h for 10 days	
Mansonella streptocerca			
Drug of choice	Ivermectin	150µg/kg, single dose	
OR			
	Diethylcarbamazine	6mg/kg/day for 14 days	
Tropical pulmonary eosinophilia			
Drug of choice	Diethylcarbamazine	6mg/kg/day in three doses for 14 days	6mg/kg/day in three doses for 14 days
***Onchocerca volvulus* (river blindness)**			
Drug of choice	Ivermectin	150µg/kg, single dose, repeated every 6–12 months	150µg/kg, single dose, repeated every 6–12 months
Fluke (hermaphroditic) infection			
***Clonorchis sinensis* (Chinese liver fluke)**			
Drug of choice	Praziquantel	75mg/kg/day in three doses for 1 day	75mg/kg/day in three doses for 1 day

OR			
	Albendazole	10mg/kg for 7 days	
***Fasciola hepatica* (sheep liver fluke)**			
Drug of choice	Triclabendazole	10mg/kg, single dose	
Alternative	Bithionol	30–50mg/kg on alternate days for 10–15 doses	30–50mg/kg on alternate days for 10–15 doses
***Fasciotopsis buski, Heterophyes heterophyes, Metagonimus yokogawai* (intestinal flukes)**			
Drug of choice	Praziquantel	75mg/kg/day in three doses for 1 day	75mg/kg/day in three doses for 1 day
***Metorchis conjunctus* (North American liver fluke)**			
Drug of choice	Praziquantel	75mg/kg/day in three doses for 1 day	75mg/kg/day in three doses for 1 day
Nanophyetus salmincola			
Drug of choice	Praziquantel	60mg/kg/day in three doses for 1 day	60mg/kg/day in three doses for 1 day
***Opisthorchis viverrini* (South East Asian liver fluke)**			
Drug of choice	Praziquantel	75mg/kg/day in three doses for 1 day	75mg/kg/day in three doses for 1 day
***Paragonimus westermani* (lung fluke)**			
Drug of choice	Praziquantel	75mg/kg/day in three doses for 2 days	75mg/kg/day in three doses for 2 days
Alternative	Bithionol	30–50mg/kg on alternate for 10–15 doses	30–50mg/kg on alternate for 10–15 doses
Giardiasis (*Giardia lambia*)			
Drug of choice	Metronidazole	250mg q8h for 5 days	15mg/kg/day in three doses for 5 days
Alternative	Tinidazole	2g, single dose	50mg/kg, single dose (maximum 2g)
	Furazolidone	100mg q6h for 7–10 days	6mg/kg/day in four doses for 7–10 days
	Paromomycin	25–35mg/kg/day in three doses for 7 days	
	Quinacrine	100mg q8h for 5 days (max. 300mg/day)	2mg/kg q8h for 5 days (max. 300mg/day)
Gnathostomiasis (*Gnathostoma spinigerum*)			
Treatment of choice		Surgical removal	
OR			
	Ivermectin	200µg/kg/day for 2 days	200µg/kg/day for 2 days
OR			
	Albendazole	400mg q12h for 21 days	400mg q12h for 21 days
Hookworm infection (*Ancylostoma duodenale, Necator americanus*)			
Drug of choice	Mebendazole	100mg q12h for 2 days or 500mg, single dose	100mg q12h for 2 days or 500mg, single dose
OR			
	Pyrantel pamoate	11mg/kg (maximum 1g) for 3 days	11mg/kg (maximum 1g) for 3 days
OR			
	Albendazole	400mg, single dose	400mg, single dose
Isosporiasis (*Isospora belli*)			
Drug of choice	TMP-SMX	160mg TMP, 800mg SMX q6h for 10 days, then q12h for 3 weeks	
Leishmaniasis (*Leishmania mexicana, Leishmania tropica, Leishmania major, Leishmania braziliensis, Leishmania donovani* (kala-azar), *Leishmania infantum*)			
Drug of choice	Sodium stibogluconate	20mg Sb/kg/day iv or im for 20–28 days	20mg Sb/kg/day iv or im for 20–28 days
OR			
	Meglumine antimonate	20mg Sb/kg/day for 20–28 days	20mg Sb/kg/day for 20–28 days
Alternative	Amphotericin B	0.5–1 mg/kg by slow infusion daily or every 2 days for up to 8 weeks	0.5–1 mg/kg by slow infusion daily or every 2 days for up to 8 weeks
OR			
	Lipid-encapsulated amphotericin B	15–20mg/kg (total dose over 5 days or longer)	15–20mg/kg (total dose over 5 days or longer)
	Pentamidine isethionate	2–4mg/kg im daily or every 2 days for up to 15 doses	2–4mg/kg im daily or every 2 days for up to 15 doses
	Paromomycin	Topically q12h for 15 days	
Malaria treatment (*Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae*)			
Chloroquine-resistant *Plasmodium falciparum* (oral regimens)			
Drug of choice	Quinine sulfate	650mg q8h for 3–7 days	25mg/kg/day in three doses for 3–7 days
PLUS			
	Doxycycline	100mg q12h for 7 days	2mg/kg/day for 7 days
OR PLUS			
	Pyrimenthamine-sulfadoxine	3 tablets, single dose on last days of quinine treatment	Aged <1 year, 1/4 tablet; aged 1–3 years, 1/2 tablet; aged 4–8 years, 1 tablet; aged 9–14 years, 2 tablets
OR PLUS			
	Clindamycin	900mg q8h for 5 days	20–40 mg/kg/day in three doses for 5 days
OR			

	Atovaquone/proguanil	Two adults tablets q12h for 3 days	11–20kg: one adult tablet/day for 3 days
			21–30kg: 2 adult tablets/day for 3 days
			31–40kg: 3 adult tablets/day for 3 days
			>40kg: adult dose
Alternatives	Mefloquine	750mg followed by 500mg 12h later	15mg/kg, single dose (if body weight <45kg), followed by 10mg/kg 12h later
	Halofantrine	500mg q6h for three doses; repeat in 1 week	8mg/kg q6h for three doses (if body weight <40kg); repeat in 1 week
	Artesunate	4mg/kg/day for 3 days	
PLUS			
	Mefloquine	1250mg, single dose	
Chloroquine-resistant *Plasmodium vivax*			
Drug of choice	Quinine sulfate	650mg q8h for 3–7 days	25mg/kg/day in three doses for 3–7 days
PLUS			
	Doxycycline	100mg q12h for 7 days	2mg/kg/day for 7 days
OR PLUS			
	Pyrimethamine-sulfadoxine	3 tablets, single dose on last day of quinine treatment	Aged <1 year, 1/4 tablet; aged 1–3 years, 1/2 tablet; aged 4–8 years, 1 tablet; aged 9–14 years, 2 tablets
OR			
	Mefloquine	1250mg, single dose	25mg/kg, single dose (if body weight <45kg)
All *Plasmodium* spp. except chloroquine-resistant *Plasmodium falciparum* and chloroquine-resistant *Plasmodium vivax* (oral regimens)			
Drug of choice	Chloroquine phosphate	1g (600mg base), then 500mg (300mg base) 6 hours later, then 500mg (300mg base) at 24 hours and 48 hours	10mg base/kg (maximum 600mg base), then 5mg base/kg 6 hours later, then 5mg base/kg at 24 hours and 48 hours
All *Plasmodium* spp. (parenteral regimens)			
Drug of choice	Quinidine gluconate	10mg/kg loading dose (maximum 600mg) in normal saline slowly over 1–2 hours, followed by continuous infusion of 0.02mg/kg/minute until oral therapy can be started	10mg/kg loading dose (maximum 600mg) in normal saline slowly over 1–2 hours, followed by continuous infusion of 0.02mg/kg/minute until oral therapy can be started
OR			
	Quinine dihydrochloride	20mg/kg loading dose iv in 5% dextrose over 4 hours, followed by 10mg/kg over 2–4 hours q8h (maximum 1800mg/day) until oral therapy can be started	20mg/kg loading dose iv in 5% dextrose over 4 hours, followed by 10mg/kg over 2–4 hours q8h (maximum 1800mg/day) until oral therapy can be started
Alternative	Artemether	3.2mg/kg im, then 1.6mg/kg q24h	3.2mg/kg im, then 1.6mg/kg q24h
Prevention of relapses (*Plasmodium vivax* and *Plasmodium ovale* only)			
Drug of choice	Primaquine phosphate	26.3mg (15mg base) per day for 14 days or 79mg (45mg base) per week for 8 weeks	0.3mg base/kg/day for 14 days
Malaria prevention			
Chloroquine-sensitive areas			
Drug of choice	Chloroquine phosphate	500mg (300mg base), once per week	5mg/kg base once per week, up to adult dose of 300mg base
Chloroquine-resistant areas			
Drug of choice	Mefloquine	250mg once per week	Weight <5kg, no data; weight 5–9kg, 1/8 tablet; weight 10–19kg, 1/4 tablet; weight 20–30kg, 1/2 tablet; weight 31–45kg, 3/4 tablet; weight >45kg, 1 tablet
OR			
	Doxycycline	100mg/day	2mg/kg/day, up to 100mg/day
OR			
	Atovaquone/proguanil	One adult tablet daily	11–20kg: 62.5mg/25mg
			21–20kg: 125mg/50mg
			31–40kg: 187.5mg/75mg
			>40kg: adult dose
Alternatives	Primaquine	0.5mg/kg base daily	0.5mg/kg base daily
	Chloroquine phosphate	500mg (300mg base) once per week	5mg/kg base once per week, up to adult dose of 300mg base
PLUS			
	Pyrimethamine-sulfadoxine for presumptive treatment	Carry a single dose (3 tablets) for self-treatment of febrile illness when medical care is not immediately available	Aged <1 year, 1/4 tablet; aged 1–3 years, 1/2 tablet; aged 4–8 years, 1 tablet; aged 9–14 years, 2 tablets
OR PLUS			
	Proguanil	200mg/day	Aged <2 years, 50mg/day; aged 2–6 years, 100mg; aged 7–10 years, 150mg; aged >10 years, 200mg
Microsporidiosis			
Ocular microsporidiosis (*Encephalitozoon hellem, Encephalitozoon cuniculi, Vittaforma corneae* (*Nosema corneum*))			
Drug of choice	Albendazole	400mg q12h	
PLUS			
	Fumagillin eyedrops		
Intestinal microsporidiosis (*Enterocylozoon bieneusi, Encephalitozoon (Septata) intestinalis*)			
Drug of choice	Albendazole	400mg q12h	

OR			
	Fumagillin	60mg/day po for 14 days	
Disseminated microsporidrosis (*Enterocytozoon hellem, Enterocytozoon cuniculi, Enterocytozoon intestinalis, Pleistophora* spp.)			
Drug of choice	Albendazole	400mg q12h	
Moniliformis moniliformis			
Drug of choice	Pyrantel pamoate	11mg/kg, single dose, repeat twice 2 weeks apart	11mg/kg, single dose, repeat twice 2 weeks apart
Oesophagostomum bifurcum			
Drug of choice	Albendazole or pyrantel pamoate		
Schislosomiasis (bilharziasis)			
Schistosoma haematobium			
Drug of choice	Praziquantel	40mg/kg/day in two doses for 1 day	40mg/kg/day in two doses for 1 day
Schistosoma japonicum			
Drug of choice	Praziquantel	60mg/kg/day in three doses for 1 day	60mg/kg/day in three doses for 1 day
Schistosoma mansoni			
Drug of choice	Praziquantel	40mg/kg/day in two doses for 1 day	40mg/kg/day in two doses for 1 day
Alternative	Oxamniquine	15mg/kg, single dose	20mg/kg/day in two doses for 1 day
Schistosoma mekongi			
Drug of choice	Praziquantel	60mg/kg/day in three doses for 1 day	60mg/kg/day in three doses for 1 day
Strongyloidiasis (*Strongyloides stercoralis,* threadworm)			
Drug of choice	Ivermectin	200µg/kg/day for 1–2 days	200µg/kg/day for 1–2 days
Alternative	Thiabendazole	50mg/kg/day in two doses (maximum 3g/day) for 2 days	50mg/kg/day in two doses (maximum 3g/day) for 2 days
Tapeworm infection (adult (intestinal stage))			
***Diphyllobothrium latum* (fish), *Taenia saginata* (beef), *Taenia solium* (pork), *Dipylidium caninum* (dog)**			
Drug of choice	Praziquantel	5–10mg/kg, single dose	5–10mg/kg, single dose
Alternative	Niclosamide	2g single dose	50mg/kg, single dose
***Hymenolepis nana* (dwarf tapeworm)**			
Drug of choice	Praziquantel	25mg/kg, single dose	25mg/kg, single dose
Tapeworm infection (larval (tissue stage))			
***Echinococcus granulosus* (hydatid cyst)**			
Drug of choice	Albendazole	400mg q12h for 28 days, repeated as necessary	15mg/kg/day for 28 days, repeated as necessary
Echinococcus multilocularis			
Treatment of choice		Surgical excision	
***Cysticercus cellulosae* (cysticercosis)**			
Drug of choice	Albendazole	400mg q12h for 8–30 days, repeated as necessary	15mg/kg/day (maximum 800mg) in two doses for 8–30 days, repeated as necessary
OR			
	Praziquantel	50mg/kg/day in three doses for 15 days	50mg/kg/day in three doses for 15 days
Alternative	Surgery		
Toxoplasmosis (*Toxoplasma gondii*)			
Drug of choice	Pyrimethamine	25–100mg/day for 3–4 weeks	2mg/kg/day for 3 days, then 1mg/kg/day (maximum 25mg/day) for 4 weeks
PLUS			
	Sulfadiazine	1–1.5g q6h for 3–4 weeks	100–200mg/kg/day for 3–4 weeks
Alternative	Spiramycin	3–4g/day	50–100mg/kg/day for 3–4 weeks
Trichinosis (*Trichinella spiralis*)			
Drug of choice	Corticosteroids for severe symptoms		
PLUS			
	Mebendazole	200–400mg q8h for 3 days, then 400–500mg q8h for 10 days	
Trichomoniasis (*Trichomonas vaginalis*)			
Drug of choice	Metronidazole	2g, single dose or 500mg q12h po for 7 days	15mg/kg/day po in three doses for 7 days
OR			
	Tinidazole	2g, single dose	50mg/kg, single dose (maximum 2g)
Trichostrongyliasis (*Trichostrongylus* spp.)			
Drug of choice	Pyrantel pamoate	11mg/kg, single dose (maximum 1g)	11mg/kg, single dose (maximum 1g)

Alternative	Mebendazole	100mg q12h for 3 days	100mg q12h for 3 days
OR			
	Albendazole	400mg, single dose	400mg, single dose
Trichuriasis (*Trichuris trichiura, whipworm*)			
Drug of choice	Mebendazole	100mg q12h for 3 days or 500mg, single dose	100mg q12h for 3 days or 500mg, single dose
Alternative	Albendazole	400mg, single dose	400mg, single dose
Trypanosomiasis			
***Trypanosoma cruzi* (American trypanosomiasis, Chagas' disease)**			
Drug of choice	Nifurtimox	8–10mg/kg/day in three or four doses for 90–120 days	Aged 1–10 years, 15–20mg/kg/day in four doses for 90 days; aged 11–16 years, 12.5–15mg/kg/day in four doses for 90 days
OR			
	Benznidazole	5–7mg/kg/day for 30–90 days	Aged =12 years, 10mg/kg/day in two doses for 30–90 days
***Trypanosoma brucei gambiense* (West African trypanosomiasis) — hemolymphatic stage**			
Drug of choice	Difluoromethylornithine (eflornithine)	400mg/kg/day iv in four divided doses for 14 days	
OR			
	Pentamidine isethionate	4mg/kg/day im for 10 days	4mg/kg/day im for 10 days
Alternative	Suramin	100–200mg (test dose) iv, then 1g iv on days 1, 3, 7, 14 and 21	20mg/kg iv on days 1, 3, 7, 14 and 21
***Trypanosoma brucei rhodesiense* (East African trypanosomiasis) — hemolymphatic stage**			
Drug of choice	Suramin	100–200mg (test dose) iv, then 1g iv on days 1, 3, 7, 14 and 21	20mg/kg iv on days 1, 3, 7, 14 and 21
Late disease with central nervous system involvement, both *T. brucei gambiense* and *T. brucei rhodesiense*			
Drug of choice	Melarsoprol	2–3.6mg/kg/day iv for 3 days; after 1 week 3.6mg/kg/day iv for 3 days; repeat again after 10–21 days	18–25mg/kg over 1 month; initial dose of 0.36mg/kg iv, increasing gradually to max. 3.6mg/kg at intervals of 1–5 days for total of 9–10 doses
OR			
	Difluoromethylornithine	400mg/kg/day iv in four divided doses for 14 days	
Visceral larva migrans (toxocariasis)			
	Albendazole	400mg q12h for 3–5 days	400mg q12h for 3–5 days
OR			
	Mebendazole	100–200mg q12h for 5 days	100–200mg q12h for 5 days
Organism or disease entity is listed alphabetically. Both adult and pediatric dosages are indicated. Sb, antimony; SMX, sulfamethoxazole; TMP, trimethoprim.			

† *Data modified from Med Lett Drugs Ther 2002:(4):1*[1]

*Based on a single case cited in Abramowicz.[1]
†Based on a single case cited in Gelman *et al.*[2]

TABLE 209-2 -- Availability of antiparasitic agents.

AVAILABILITY OF ANTIPARASITIC AGENTS		
Agent	**Trade name**	**Manufacturer**
Available from the drug service provided by the US Centers for Disease Control and Prevention		
Bithionol	Bitin	Tanabe (Japan)
Dehydroemetine	Dehydroemetine	Roche
	Dametine	Merck
Melarsoprol	Arsobal	Aventis
Nifurtimox	Lampit	Bayer
Sodium stibogluconate	Pentostam	GlaxoSmithKline
Suramin	Germanin	Bayer
Available in the USA from the manufacturer		
Diethylcarbamazine	Hetrazan	Wyeth-Ayerst
Fumagillin	Fumidil B	Mid-Continent Agrimarketing
Nitazoxanide	Cryptaz	Romark Laboratories
Commercially available only outside the USA		
Amodiaquine	Camoquin	Parke-Davis
	Flavoquine	Aventis
Artemether	Artenam	Arenco (Belgium)
Artesunate	(Generic)	Guilin No 1 Factory (China)
Benznidazole	Rochagan	Roche
Diloxanide furoate	Furamide	Boots (UK)
Difluoromethylornithine	Ornidyl	Hoechst Marion Roussel
Flubendazole	Fluvermal, Flumoxane	Janssen
Halofantrine	Halfan	GlaxoSmithKline

Meglumine antimonate	Glucantime	Aventis
Metrifonate	Bilarcil	Bayer
Niclosamide	Yomesan	Bayer
Ornidazole	Tiberal	Roche
Oxamniquine	Vansil	Pfizer
Proguanil	Paludrine	Wyeth-Ayerst, Zeneca
Pyrimethamine-dapsone	Maloprim	GlaxoSmithKline
Pyrimethamine-sulfadoxine-mefloquine	Fansimef	Roche
Quinacrine (discontinued in 1992)	Atabrine	Sanofi
Quinine dihydrochloride	(Generic)	ACF Chemiefarma NV (The Netherlands)
Spiramycin	Rovamycine	Aventis
Tinidazole	Fasigyn	Pfizer
Triclabendazole	Egaten	Novartis
It is often difficult to obtain antiparasitic agents. This is not a comprehensive list; many drugs have multiple trade names and manufacturers, only some of which are included. Agents are divided into those available from the drug service provided by the Centers for Disease Control and Prevention, those available from the manufacturer in the USA and those that are commercially available only outside the USA.		

Sulfonamides

Sulfonamides, which are derivatives of sulfanilamide, interfere with microbial folic acid synthesis by competitively inhibiting the enzyme dihydropteroate synthase.[6] [7] [14] [15] This enzyme is involved in the step in folic acid synthesis that precedes the step blocked by pyrimethamine and TMP. Sulfonamides are separated into four groups:

- short- and intermediate-acting agents;
- long-acting agents;
- agents that are limited to the bowel lumen; and
- topical agents.

TABLE 209-3 -- Safety of antiparasitic agents in pregnancy.*

SAFETY OF ANTIPARASITIC AGENTS IN PREGNANCY
Category 1 drugs: probably safe
Amphotericin B
Azithromycin
Chloroquine
Clindamycin
Dapsone
Paromomycin
Praziquantel
Proguanil
Spiramycin
Category 2 drugs: possibly safe or safe during certain trimesters
Diethylcarbamazine
Ivermectin
Mebendazole
Mefloquine
Metrifonate
Metronidazole
Pyrantel pamoate
Pyrimethamine
Sulfonamides
Trimethoprim
Category 3 drugs: insufficient data or established as unsafe
Albendazole
Artemisinin and derivatives
Atovaquone
Benznidazole
Bithionol
Dehydroemetine
Diloxanide furoate
Doxycycline
Difluoromethylornithine
Emetine
Fumagillin
Furazolidone
Halofantrine
Iodoquinol
Melarsoprol
Niclosamide
Nifurtimox

Oxamniquine
Pentamidine isethionate
Piperazine
Primaquine
Quinacrine
Quinine
Quinidine
Sodium stibogluconate
Suramin
Tetracycline
Thiabendazole
There are no well-controlled studies proving the safety of any of these antiparasitic agents in pregnancy. Category 1 drugs are those for which extensive clinical experience has demonstrated safety in pregnancy. Category 2 drugs are those that have been reported as safe only anecdotally or those that have been used safely in certain trimesters only. Category 3 drugs are those that should not be used because of either inadequate information or documented fetal harm.

** Adapted from Alecrim et al.,[3] Phillips-Howard et al.,[4] and Samuel and Barry.[5]*

Only agents from the first two of these categories are used to treat parasitic diseases; these are generally combined with either pyrimethamine or TMP.

Sulfamethoxazole

Sulfamethoxazole is an intermediate-acting sulfonamide. It is rapidly absorbed, widely distributed, 50–70% protein bound, hepatically metabolized and renally excreted. The half-life is 7–12 hours. It is available in a fixed combination with TMP (see below) for numerous indications.

Sulfadiazine

Sulfadiazine, another intermediate-acting sulfonamide, is also rapidly absorbed, widely distributed (including within the cerebrospinal fluid), 45–55% protein-bound, hepatically metabolized and renally excreted. The half-life is 12 hours. It is used with pyrimethamine in the treatment of toxoplasmosis, as detailed above.

Sulfadoxine

Sulfadoxine, a long-acting sulfonamide, is rapidly absorbed but slowly eliminated and has a half-life of 7–9 days. It is available in a fixed

combination with pyrimethamine (Fansidar) for the prophylaxis and treatment of malaria (see below).

Side effects of sulfonamides

Side effects of sulfonamides are numerous. Nausea, vomiting and anorexia occur in 1–2% of patients. Hypersensitivity reactions include:

- drug eruptions (ranging from morbilliform rash to severe exfoliation);
- fever;
- serum sickness; and
- hepatocellular dysfunction and necrosis.

Acute hemolytic anemia, agranulocytosis and aplastic anemia are rare. Reversible bone marrow suppression is not uncommon in immunocompromised patients, particularly those who have AIDS. Crystalluria can occur with sulfadiazine and can be avoided by increasing fluid intake or alkalinizing the urine.

Fansidar

Fansidar tablets contain pyrimethamine 25mg and sulfadoxine 500mg. Fansidar is used in the treatment of chloroquine-resistant *P. falciparum* infections.[6] [7] [14] [15] [16] Fansidar is no longer recommended for malaria prophylaxis because of the possibility of fatal cutaneous eruptions, including erythema multiforme, toxic epidermal necrolysis and Stevens-Johnson syndrome. These reactions have been attributed to the sulfadoxine component; fatalities have occurred between 1 in 11,000 and 1 in 26,000 users, usually within 5 weeks of starting the agent. Serum sickness, bone marrow suppression, pneumonitis and hepatitis have also been documented in patients taking Fansidar for prophylaxis. There have been no fatalities described with Fansidar used for therapy. *Plasmodium falciparum* resistance to Fansidar has been described in South East Asia, eastern Africa, the Amazon basin, Bangladesh and Oceania.[16]

Fansimef

Fansimef is the combination of pyrimethamine (25mg), sulfadoxine (500mg) and mefloquine (250mg). It has been used in South East Asia and Brazil for both prophylaxis and treatment of chloroquine-resistant *P. falciparum*.

Maloprim

Maloprim tablets contain pyrimethamine 25mg and dapsone 100mg. Dapsone is a sulfone that is used in the treatment of leprosy (see Chapter 154) and *Pneumocystis carinii* pneumonia (see Chapter 124). This combination was used for malaria prophylaxis but is rarely employed today.[21] Hemolytic anemia, bone marrow suppression (including, very rarely, fatal agranulocytosis) and methemoglobinemia are occasionally seen. Dapsone is contraindicated in those who have glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Trimethoprim-sulfamethoxazole

Trimethoprim-sulfamethoxazole (TMP-SMX) is a combination used to treat bacterial infections (see Chapter 197), *P. carinii* (see Chapter 124) and the parasites *I. belli* and *C. cayetanensis*.[19] [20] This combination also has some efficacy against *P. falciparum* but resistance to the TMP component limits its use.[22] [23] It is available as single-strength tablets (80mg TMP and 400mg SMX) and as double-strength tablets (160mg TMP and 800mg SMX). An oral suspension (40mg TMP and 200mg SMX per 5ml) and an intravenous formulation (80mg TMP and 400mg SMX per 5ml vial) are available as well.

The dose for isosporiasis is 1 double-strength tablet orally q6h for 10 days, followed by 1 double-strength tablet q12h for 3 weeks. Immunocompromised patients usually require maintenance therapy of 1 double-strength tablet daily or three times weekly. For cyclosporiasis, 1 double-strength tablet q12h for 7–10 days is generally used but some clinicians extend treatment to 14 days. Immunocompromised patients sometimes require 4 tablets per day and usually need maintenance therapy as well.

Side effects of TMP-SMX include those listed for each of the two component drugs, as detailed above. Dermatologic reactions (3–4%) and gastrointestinal disturbances (3–4%) are the most common side effects in non-immunocompromised patients. For unclear reasons, patients who have AIDS have a much higher rate of

complications, ranging in different series from 45 to 90%.

Trimetrexate

Trimetrexate is a lipid-soluble dihydrofolate reductase inhibitor that was originally developed as a myelosuppressive agent but was found to have antiparasitic activity against *P. carinii* and *T. gondii*.[17] It is available for intravenous injection only. Adverse effects include rash, leukopenia, elevated liver enzymes and a reversible peripheral neuropathy. Folinic acid is administered concurrently to diminish the incidence of bone marrow suppression.

Proguanil

Proguanil is a biguanide that inhibits plasmodial dihydrofolate reductase.[6] [7] [14] [16] Although it is seldom used for therapy because of its slow action, recent studies indicate that it is effective in combination with atovaquone in uncomplicated *P. falciparum* malaria (see below, under Atovaquone).[24] Daily proguanil (200mg) in combination with weekly chloroquine is used for prophylaxis against *P. falciparum* and *Plasmodium vivax*, mainly in sub-Saharan Africa. Prophylaxis failures have been reported in Kenya.[25]

Proguanil is slowly absorbed after oral administration, is 75% protein-bound, is metabolized to the active triazine metabolite cycloguanil, and is excreted in urine (40–60%) and feces (10%). The drug is safe and well tolerated. Pancytopenia has been rarely reported. Nausea, vomiting, abdominal pain, diarrhea and hematuria are associated with the use of high doses.

ARTEMISININ AND ITS DERIVATIVES

Artemisinin, or *qinghaosu*, is a sesquiterpene lactone derived from the leaves of the sweet wormwood *Artemisia annua*.[7] [14] [16] [26] It has been used for centuries in traditional Chinese medicine and is now known to be active against intra-erythrocytic forms of *P. falciparum* and *P. vivax*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Clonorchis sinensis* and *Naegleria fowleri*. It main clinical use has been in the treatment of drug-resistant *P. falciparum* infections.

Artemisinin and two of its derivatives, the water-soluble hemisuccinate artesunate and the oil-soluble methyl ether artemether, are the most rapidly acting of known antimalarials and appear to be quite safe. They are undergoing active study to define their precise role in malaria therapy and are not currently widely available. These compounds can be given by several routes:

- artemisinin is available in oral and suppository forms;
- artesunate is available in oral, intravenous and intramuscular forms; and
- artemether is available in intramuscular form.

They appear to be rapidly absorbed and eliminated, with half-lives ranging from minutes (artesunate) to hours (artemether). The two derivatives are hepatically hydrolyzed to an active metabolite, dehydroartemisinin. These compounds are believed to act by disrupting parasite protein synthesis via the production of oxygen free radicals.

Artemisinin and its derivatives are usually administered in conjunction with a longer acting antimalarial (mefloquine) to decrease the emergence of resistance and enhance efficacy. Artesunate (4mg/kg orally) is usually given q24h for 3 days and is followed by a course of mefloquine. If given as monotherapy, artesunate is

administered for 5–7 days to prevent recrudescence. Parenteral artesunate is administered at a dose of 2mg/kg initially, followed by 1mg/kg at 12–24 hours and then q24h after that. Artemether is given at a dose of 3.2mg/kg intramuscularly initially, followed by 1.6mg/kg q24h. Artemisinin suppositories, which are useful for those unable to take oral medications when there is no access to injectable formulations, can be given at 10mg/kg initially and 4 hours later, followed by 7mg/kg at 24, 36, 48 and 60 hours.

Adverse events include diarrhea, abdominal pain, transient first-degree heart block and reversible mild decreases in reticulocyte and neutrophil counts. Neurotoxicity has been described in animals but not with clinical use in humans. Resistance to artemisinin has occurred in murine malaria, and the resistant parasites also developed cross-resistance to chloroquine, quinine and mefloquine. Newer derivatives, including arteether (an ethyl ether) and the water-soluble artelinic acid, are being developed.

ATOVAQUONE AND ATOVAQUONE/PROGUANIL

Atovaquone, a synthetic hydroxynaphthoquinone derivative, has activity against *P. carinii* (see Chapter 124), *P. falciparum*, *T. gondii* and *Babesia microti*.[16] [24] [27] It interferes with pyrimidine synthesis by uncoupling mitochondrial electron transport. The drug is available only in liquid form. Because of its erratic absorption it is usually administered with a fatty meal. It is hepatically metabolized and excreted in the bile and urine. Atovaquone is an alternative oral agent for the treatment of mild to moderate *P. carinii* pneumonia in those who are intolerant of TMP-SMX, and experimental data indicate that it is synergistic with pyrimethamine or sulfadiazine for *T. gondii* infection. Atovaquone has also been used with azithromycin in the treatment of babesiosis.

The combination of atovaquone (1g q24h) and proguanil (400mg q24h) for 3 days has been used successfully to treat uncomplicated *P. falciparum* malaria in Gabon and Brazil,[24] and a combination pill containing atovaquone 250mg and proguanil 100mg has rapidly become one of the leading drugs for malaria prophylaxis in travellers.[28] A randomized controlled trial between mefloquine and atovaquone/proguanil for malaria prophylaxis in nonimmune travelers found equivalent efficacy for the two agents, with a similar number of adverse events, but fewer adverse effects of moderate or severe intensity were reported in the atovaquone/proguanil group.[29] The adult dosage for prophylaxis is 1 tablet q24h (250mg atovaquone/100mg proguanil), beginning 1–2 days prior to arrival in the malarious area and continuing for 1 week after return. Side effects include rash, nausea, vomiting, diarrhea, headache, fever, anemia, elevated liver function tests, hyponatremia and hyperglycemia.

BENZNIDAZOLE

Benznidazole is a nitroimidazole derivative that is active against *Trypanosoma cruzi* but it has not been as well studied and is relatively toxic.[6] [7] [30] It is the preferred agent for the treatment of Chagas' disease in central Brazil because of strain-susceptibility patterns. It is available in oral form and has a half-life of 12 hours. Side effects include malaise, nausea, photosensitivity rash, peripheral neuropathy, bone marrow suppression and psychiatric disturbances.

CHLOROQUINE

Chloroquine, a 4-aminoquinoline that was first synthesized in 1934 but did not become popular until the end of the Second World War, has been the agent most widely used for treating the erythrocytic stage of uncomplicated malaria caused by *P. vivax*, *Plasmodium ovale*, *Plasmodium malariae* and chloroquine-sensitive *P. falciparum*.[6] [7] [14] [16] Its precise mechanism of action has not been delineated but chloroquine and its metabolites inhibit the ability of the parasite to polymerize the heme moiety of hemoglobin, resulting in toxic levels of free heme.[31] Chloroquine was occasionally used to treat extraintestinal *Entamoeba histolytica* infection but it has been supplanted by metronidazole.

Pharmacokinetics and distribution

Absorption after oral ingestion is excellent (90%), and the volume of distribution is large owing to its extensive tissue sequestration, particularly in the liver, spleen, kidneys and erythrocytes. It is approximately 50% bound to plasma protein and is eliminated slowly. Its half-life of 4–6 days permits weekly dosing for prophylaxis. Chloroquine is metabolized by the liver to the active metabolite desethylchloroquine, but 50% is cleared by the kidneys unchanged. Thus, dosing need not be altered for abnormal renal function but caution must be exercised in patients who have hepatic, gastrointestinal, neurologic or hematologic disorders.

Route of administration and dosage

The drug is formulated as a phosphate, sulfate or hydrochloride salt and is dosed by base content. It can be administered orally or rectally or by intravenous, intramuscular or subcutaneous injection. In the USA, chloroquine is marketed as Aralen phosphate in 500mg salt tablets (equal to 300mg base). The dosage for the treatment and prophylaxis of malaria is given in Table 209.1 . If chloroquine hydrochloride is given intravenously, it must be administered by slow, constant infusion to

avoid the respiratory depression, hypotension, heart block, cardiac arrest and seizures that may occur with transient toxic levels. A dose of 300mg base q8–12h may be given by intramuscular injection.

Adverse reactions

Reversible side effects include headache, gastrointestinal disturbances, blurred vision, dizziness, fatigue and pruritus. Rarer side effects include hair depigmentation, weight loss, myalgias, leukopenia and eczematous eruptions. Very rarely, acute psychosis may occur. Permanent retinal damage has been observed with long-term (longer than 5 years) prophylactic use. The drug is contraindicated in patients who have retinal disease, psoriasis and porphyria. An oral dose of 5g is fatal without immediate mechanical ventilation, epinephrine (adrenaline) and diazepam.

Resistance of *Plasmodium falciparum* to chloroquine

Resistance of *P. falciparum* to chloroquine is ubiquitous in regions where malarial transmission occurs (Fig. 209.1) with the exception of Central America west of the Canal Zone, Mexico, Haiti, the Dominican Republic and much of the Middle East (although there are reports of resistance from Yemen, Oman and Iran). Resistance to chloroquine among *P. vivax* isolates has been reported in Brazil, Colombia, India, Myanmar, Papua New Guinea and Indonesia.[31] [33] [34] A single oral dose of mefloquine (15mg base/kg) has been used successfully in such cases.

CLINDAMYCIN

Clindamycin, a lincosamide antibiotic, is active against bacteria (see Chapter 194), *P. falciparum*, *T. gondii* and *Babesia* spp.[16] [35] [36] It is well absorbed after oral administration and is 90% protein-bound and widely distributed. It is hepatically metabolized and excreted in the urine and bile; its half-life is 2.5–3 hours.

Dosages for malaria and babesiosis are listed in Table 209.1 . For cerebral toxoplasmosis in the case of sulfonamide hypersensitivity, 1.8–2.4g divided into three daily doses is combined with a course of pyrimethamine. Clindamycin has been used in combination with

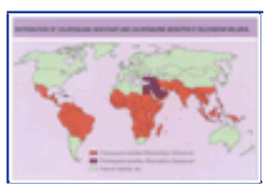

Figure 209-1 Distribution of chloroquine-resistant and chloroquine-sensitive *P. falciparum* malaria. *Adapted with permission from Lobel and Kozarsky.*[32]

quinine for short-course (3-day) treatment of travelers who have *P. falciparum* malaria, with excellent results.[37] Side effects include rash, diarrhea, nausea, vomiting, abdominal pain, pseudomembranous colitis, hepatotoxicity and cytopenias.

DIFLUOROMETHYLORNITHINE

Difluoromethylornithine is an ornithine decarboxylase inhibitor that is effective in the treatment of both early and late sleeping sickness caused by *Trypanosoma brucei gambiense*.[7] [38] [39] It has variable efficacy against *T. brucei rhodesiense* because many strains are resistant. Difluoromethylornithine inhibits ornithine decarboxylase, an enzyme involved in the first step in polyamine synthesis. It is available as the hydrochloride salt for both oral and intravenous administration. It has a half-life of approximately 3 hours, and 80% of the drug is excreted unchanged by the kidneys.

Side effects of difluoromethylornithine include anemia, thrombocytopenia, leukopenia, abdominal pain, nausea, vomiting, weight loss, arthralgias, seizures, hearing loss and alopecia. Overall, difluoromethylornithine is less toxic than other available antitrypanosomal agents but it has not seen widespread use because of its high cost.

DILOXANIDE FUROATE

Diloxanide furoate is a dichloroacetamide derivative that is a luminally active agent used to eradicate cysts of *E. histolytica* in asymptomatic carriers and in those who have mild, noninvasive disease, as well as after treatment with metronidazole in those who have invasive amebiasis.[6] [40] It is not useful in extraintestinal disease. After oral administration, diloxanide furoate is hydrolyzed by intestinal esterases, thus releasing diloxanide, the absorbable component, and the ester furoic acid, which is not well absorbed and thus attains higher intraluminal concentrations in the colon. Both compounds are amebicidal but the mechanism of action is not known. The drug has a half-life of 6 hours, is hepatically conjugated to form a glucuronide and is 60–90% excreted in the urine.

Side effects are mild; they include flatulence and, less commonly, nausea, vomiting, diarrhea, pruritus and urticaria. Because it is relatively inexpensive, diloxanide furoate is an attractive agent for use in developing countries.

EMETINE

Emetine is an alkaloid derived from ipecac, which comes from the root of *Cephaelis ipecacuanha*. This toxic tissue-active amebicide has been used since 1912, primarily for amebic colitis and amebic liver abscess. Dehydroemetine, a synthetic derivative, is less toxic but also less potent. [6] [7] [41] Both drugs have essentially been replaced by the safer nitroimidazoles (metronidazole, tinidazole, ornidazole) and are now used rarely. They are employed as supplemental therapy to metronidazole in individuals who are severely ill with amebic colitis or amebic liver abscess and are not responding well, or as primary therapy in the rare individual who cannot tolerate metronidazole. Because they are not active in the intestinal lumen, therapy with these agents must be followed by treatment with a luminally active drug. Dehydroemetine has also been used for infections caused by S. *mansoni*, *Schistosoma haematobium* and *Fasciola hepatica*. Emetine and dehydroemetine are both available only for intramuscular use. After deep intramuscular injection, they are well absorbed and excreted very slowly in the urine. They act by inhibiting protein synthesis.

The standard dose of dehydroemetine is 1–1.5mg/kg/day, up to a maximum of 90mg/day, for 5–10 days. Common adverse effects of emetine and dehydroemetine include local reactions at the injection site (e.g. pain, stiffness, urticaria and abscesses), nausea, vomiting and diarrhea. Cardiovascular toxicity includes precordial pain, hypotension, tachycardia, arrhythmias, electrocardiographic abnormalities, heart failure and, rarely, sudden death. Headache, myalgias, weakness and polyneuritis may also occur. Hospitalization with electrocardiographic monitoring is required while these drugs are being administered. They are relatively contraindicated in patients who have cardiac or renal disease.

FUMAGILLIN

Fumagillin, a water-insoluble antibiotic derived from *Aspergillus fumigatus*, was discovered in 1949 and originally used in humans as an amebicide. Fumagillin is an inhibitor of parasite RNA synthesis but may also act by inhibiting a key proteinase, type 2 methionine aminopeptidase.[42] A water-soluble preparation (Fumidil B) is used to control nosematosis, a disease of honey bees that

results from infection with microsporidian *Nosema apis*. Topical fumagillin has been used to treat microsporidial keratoconjunctivitis caused by *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Encephalitozoon* (*Septata*) *intestinalis* and, with less success, *Vittaforma corneae* (*Nosema corneum*) in AIDS patients. [43] [44] Studies of oral fumagillin for intestinal microsporidiosis have provided promising results. [45] [46]

FURAZOLIDONE

Furazolidone is a nitrofuran derivative that is commonly used to treat giardiasis in children because of its availability in a liquid form for oral use.[6] [47] Furazolidone also has activity against *I. belli* and *Trichomonas vaginalis* as well as many enteropathogenic bacteria, and is increasingly being used for treatment of *Helicobacter pylori*

infections. The mechanism of action involves damage to DNA. It is well absorbed and is excreted mainly in the urine.

Adverse reactions include diarrhea, fever, nausea and vomiting. Urticaria, serum sickness, hypoglycemia and orthostatic hypotension occur rarely. Furazolidone has disulfiram-like properties and patients should therefore be warned to avoid alcohol. Furazolidone has monoamine oxidase inhibitor activity, but hypertensive crises have not been reported in association with this agent. Furazolidone may cause hemolysis in patients who have G6PD deficiency.

HALOFANTRINE

Halofantrine is an oral synthetic 9-phenanthrene methanol with activity against the intraerythrocytic stages of chloroquine-sensitive and chloroquine-resistant *P. falciparum* and *P. vivax*. [6] [7] [14] [48] It is more active and generally better tolerated than mefloquine but it is poorly absorbed. Ingestion with fatty meals increases absorption. Halofantrine is hepatically metabolized and excreted in feces, with a half-life of 1–2 days for the parent compound and 3–5 days for the active metabolite. Its mechanism of action is poorly understood.

There is some evidence of cross-resistance with mefloquine; therefore halofantrine may not be useful for those patients in areas with mefloquine resistance. Its side effects include prolongation of the PR and QT_c intervals on the electrocardiogram, diarrhea, abdominal pain, pruritus and rash. Because it prolongs the PR and QT_c intervals, halofantrine should not be given to anyone who has conduction defects or to anyone taking other drugs that affect the QT_c interval. Thus, a 28-day interval is recommended between the administration of halofantrine and mefloquine.

IODOQUINOL

Iodoquinol, a halogenated hydroxyquinoline, is a luminal amebicide used to eradicate cysts in patients who have asymptomatic *E. histolytica* infection.[6] [40] It is also given after metronidazole therapy to eradicate cysts in patients who have invasive disease. Iodoquinol is the drug of choice for *Dientamoeba fragilis* infection and is an alternative for *Balantidium coli*.[49] It has been used to treat *Blastocystis hominis*, but the pathogenicity of this protozoan and its need for treatment are controversial.[50] Iodoquinol also has activity against *Giardia lamblia* and *T. vaginalis* but other agents are typically employed. The mechanism of action of iodoquinol is uncertain. It is available in oral form but it is poorly absorbed and should be given with meals. Side effects include nausea, vomiting, diarrhea, abdominal pain, headache, fever, seizures and encephalopathy.

Iodochlorhydroxyquin, a related compound, is better absorbed than iodoquinol but is rarely used because of the high incidence of subacute myelo-optic neuropathy described with its use in Japan in the early 1970s. Because iodoquinol may rarely cause this syndrome when given at high dose or for prolonged periods, treatment recommendations should not be exceeded. For this reason many clinicians, including myself, prefer alternative agents such as paromomycin, metronidazole or diloxanide furoate for these indications.

MACROLIDE ANTIBIOTICS

Spiramycin

Spiramycin is used in Europe to prevent the transmission of *T. gondii* from mother to fetus.[16] The drug is concentrated in the placenta and has been shown to reduce transmission by 60%. It is given at a dose of 1g orally q8h on an empty stomach. If fetal infection has not occurred (as assessed by amniotic fluid polymerase chain reaction testing for *T. gondii*), spiramycin is continued until delivery. Because spiramycin does not cross the placenta well, it cannot be used to treat fetal toxoplasmosis; pyrimethamine and sulfadiazine are recommended in this situation. Oral spiramycin is generally well tolerated; gastrointestinal distress is the main side effect.

Azithromycin

Azithromycin (see Chapter 194), both alone and in combination with pyrimethamine, has recently been shown to be effective in cerebral toxoplasmosis in AIDS patients.[17] It is considered relatively safe in pregnancy but has not been extensively studied in preventing the vertical transmission of *T. gondii*. Azithromycin has antimalarial activity and is effective prophylaxis against *P. vivax*. However, it offers only partial protection (70–80% efficacy) against *P. falciparum* and is therefore not recommended as a first-line agent for prevention of malaria in travelers.[51] Azithromycin has also been used with both quinine and atovaquone for babesiosis. [35] High-dose azithromycin has been used in AIDS patients who have cryptosporidiosis, with variable results.[46] Another macrolide, clarithromycin, when administered for prevention of *Mycobacterium avium* complex disease in AIDS patients, appeared to prevent cryptosporidiosis as well.[52]

MEFLOQUINE

Mefloquine is an fluorinated 4-quinoline methanol derivative of quinine. It is an oral formulation that was developed as part of a search for new antimalarials.[6] [7] [14] [16] [32] [51] It is a blood schizonticide effective against all *Plasmodium* spp. that infect humans, including *P. falciparum* isolates that are resistant to chloroquine and pyrimethamine-sulfadoxine. It is ineffective against exoerythrocytic forms and gametocytes. The mechanism of action is unknown but mefloquine may interfere with the function of *Plasmodium* food vacuoles or inhibit the polymerization of heme. The drug is slowly absorbed, has a bioavailability of 85% and is almost completely protein-bound in plasma. The long elimination half-life of 2–3 weeks allows for weekly prophylaxis. Mefloquine is extensively metabolized and is excreted in bile and feces.

Common side effects at therapeutic doses include nausea, vomiting, dizziness, weakness and dysphoria.[28] [51] [53] Neuropsychiatric reactions, including acute psychosis, sleep disturbances and seizures, have been documented in approximately 0.5% of patients taking therapeutic doses and in less than 0.5% of those taking prophylactic doses. Thus, the drug is not recommended for those who have a history of seizures or psychiatric disorders. Judicious use is suggested for those whose occupations require spatial discrimination and fine motor coordination. Cardiac rhythm and conduction abnormalities and at least one instance of nonfatal cardiac arrest have occurred in patients on ß-adrenergic blockers who took mefloquine; caution should be exercised in any patient who has cardiac disease. Mefloquine should not be co-administered with quinine, quinidine or halofantrine owing to potentially fatal prolongation

of the QT_c interval. Mefloquine may also decrease the response to the live *Salmonella typhi* oral vaccine, and thus the vaccine series should be completed at least 3 days before beginning mefloquine prophylaxis.

Mefloquine resistance in *P. falciparum* isolates has been increasing along the Thailand-Myanmar and Thailand-Cambodia borders, in western Africa and in the Amazon region. In these areas, doxycycline at a dose of 100mg per day or atovaquone/proguanil may be used for prophylaxis. Treatment options include:

- quinine plus tetracycline or doxycycline for 7 days;[54]
- mefloquine plus artesunate;[55]
- mefloquine plus artemether;[56]
- doxycycline plus artesunate;[57]
- mefloquine plus doxycycline;[57] and
- quinine plus clindamycin[37] .

MELARSOPROL

Melarsoprol is a trivalent arsenical compound introduced in 1949 and used for the treatment of late-stage African trypanosomiasis caused either by *T. brucei gambiense* or *T. brucei rhodesiense*.[6] [7] [38] [39] It is also effective in treating the early or hemolymphatic stage of infection but its toxicity prohibits routine use for this stage and it should be used only in patients who have failed to respond to suramin and pentamidine.

Melarsoprol acts by interacting with protein sulfhydryl groups and subsequently inactivating enzymes, a non-specific action that is also responsible for the toxicity of the drug. Melarsoprol, formulated as a 3.6% weight per volume solution in propylene glycol, is given intravenously. A small but adequate amount of the drug penetrates the cerebrospinal fluid, where it is taken up and concentrated by susceptible trypanosomes. Resistant organisms appear to concentrate the drug poorly. Melarsoprol is rapidly excreted in the urine.

Melarsoprol is highly toxic. It is irritating to tissues and care must be taken to prevent extravasation. Fever is commonly seen. Reactive encephalopathy occurs in up to 18% of patients and may be fatal; it usually occurs during the first 3–4 days of therapy.[58] It is manifested by headache, confusion, dizziness, mental slowing and ataxia, with seizures and a progressive decline in mental status, and it is felt to be an immunologic reaction to parasite antigens released during therapy. Corticosteroids have been used to treat the encephalopathy with some success. Very rarely, a hemorrhagic encephalopathy, which is almost always fatal, may occur. Arthralgias, rash, hypertension, proteinuria and hepatic dysfunction have been seen. Abdominal pain and vomiting may be minimized by slow administration of the drug to a patient who is supine and fasting. Erythema nodosum may be precipitated in patients who have leprosy. Hemolysis may be seen in G6PD-deficient patients.

NIFURTIMOX

Nifurtimox, an oral nitrofuran, remains the drug of choice for acute Chagas' disease (American trypanosomiasis), although benznidazole is gaining favor as a first-line agent in some regions with endemic Chagas' disease (see Chapter 173).[6] [7] [30] Nifurtimox has also been used against resistant strains of *T. brucei gambiense*.[38] It acts by inhibiting nucleic acid synthesis by oxygen free radical formation. It is rapidly absorbed, has a half-life of approximately 3 hours, is extensively metabolized by the liver by a first-pass effect that results in low serum and tissue levels, and is excreted by the kidneys. There is considerable geographic variation in responsiveness to nifurtimox; better results are obtained in Argentina and Chile than in Brazil and other countries. Effectiveness in indeterminate-phase and chronic-phase infection is variable and organ damage is not reversible.

Gastrointestinal side effects including nausea, vomiting, anorexia and abdominal pain; weight loss may occur. Neurologic side effects include headache, restlessness, insomnia, disorientation, pares-thesias, polyneuritis, weakness and seizures. Rash, decreased sperm counts and neutropenia have also been described. Adherence to a full 4 months of therapy is often poor, and better agents are needed.

NITAZOXANIDE

Nitazoxanide is a nitrothiazole benzamide derivative with in-vitro activity against a wide variety of bacterial, protozoal and helminthic pathogens. In recent randomized double-blind placebo-controlled clinical trials it showed efficacy comparable to metronidazole in the treatment of giardiasis and amebiasis, and was very successful in eradicating helminths from individuals in Egypt and Mexico.[59] Healthy adults treated with nitazoxanide cleared cryptosporidia from their stool more rapidly than did placebo controls, but the efficacy of nitazoxanide in AIDS patients remains to be established.[59]

NITROIMIDAZOLE DERIVATIVES

Metronidazole

Metronidazole (see Chapter 201) has activity against many anaerobic parasites. It is the drug of choice for the treatment of:

- invasive enterocolitis and liver abscess caused by *E. histolytica* and the rarely reported *Entamoeba polecki*; [6] [7] [36] [37]
- vaginitis caused by *T. vaginalis*;[60] and
- enteritis caused by *G. lamblia*.[40] [47]

It has been used to treat *Blastocystis. hominis* in the stool (although its efficacy remains unproven) and is considered an alternative agent for *Balantidium. coli* infection. Metronidazole is also used in the treatment of infections with the guinea worm, *Dracunculus medinensis*; it decreases inflammation and facilitates worm removal but has no direct toxic effect on the worm itself.

Metronidazole acts as an electron sink under anaerobic or microaerophilic conditions, depriving the parasite of necessary reducing equivalents such as nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). Reduced metronidazole (i.e. drug molecules that have gained electrons) causes a loss of the helical structure of DNA and strand breakage.

Metronidazole is available for oral and intravenous use. It is rapidly and almost completely absorbed orally, has limited protein binding and is widely distributed throughout the body. The half-life is 6–11 hours and metabolism is hepatic. Although excretion is mainly by the kidney, dosage adjustments are seldom needed in renal failure because the metabolites are less active compounds. However, the dosage should be modified in patients who have liver failure.

The most common side effects are headache, metallic taste, dry mouth and nausea. Less frequent are urticaria, pruritus, urethral burning, reversible neutropenia, and vaginal and oral candidiasis. Rarely, patients may experience central nervous system toxicity, including dizziness, vertigo, ataxia, encephalopathy and seizures, as well as peripheral neuropathy. Acute pancreatitis has been reported. Patients should be advised to avoid consuming alcohol because of the disulfiram-like effects of metronidazole, including headache, flushing, abdominal pain and vomiting. Some patients may experience a red-brown discoloration of the urine owing to the presence of metabolites of metronidazole.

Tinidazole and ornidazole

Tinidazole and ornidazole are two other nitroimidazole derivatives (see Chapter 201). Their antimicrobial spectrum is similar to that of metronidazole,[6] [7] [60] and tinidazole has been used successfully for single-dose therapy of amebic liver abscess.[61] Tinidazole has also

been used to treat metronidazole-resistant *Trichomonas* spp. These compounds are well absorbed orally and are widely distributed. Tinidazole has a half-life of 14 hours and ornidazole has a half-life of 12–13 hours; both compounds are probably hepatically metabolized and are excreted primarily in the urine. Generally, these drugs are better tolerated than metronidazole; the main side effects are headache, dizziness and anorexia.

PAROMOMYCIN

Paromomycin (also known as aminosidine) is a non-absorbable aminoglycoside antibiotic (see Chapter 196) that is concentrated in the lumen of the colon. It is active against *E. histolytica, D. fragilis* and *G. lamblia* as well as the cestodes *Taenia saginata*, *Taenia solium*, *Diphyllobothrium latum*, *Dipylidium caninum* and *Hymenolepis nana*.[6] [40] [47] Although often used for the treatment of *Cryptosporidium parvum*, it was no better then placebo in double-blind clinical trials.[62] [63] Paromomycin with methylbenzethonium chloride has been used topically in the treatment of cutaneous leishmaniasis and systemically for visceral leishmaniasis.[10] [64] Paromomycin is available as the sulfate salt for oral administration.

The dose is 25–35mg/kg/day in three divided doses for 7 days. Side effects include cramps, nausea, vomiting, diarrhea, rash, headache and vertigo. Burning may occur with topical preparations.

PENTAMIDINE ISETHIONATE

Pentamidine isethionate, an aromatic diamidine derivative, is effective in the treatment of the early or hemolymphatic stages of sleeping sickness caused by *T. brucei gambiense*, some forms of leishmaniasis and *P. carinii* pneumonia (see Chapter 124 & Chapter 245). [6] [7] [10] [38] It is less effective against *T. brucei rhodesiense*. It has also been used in the treatment of disseminated *Acanthamoeba* spp. infections and babesiosis.[65] [66] The mechanism of action of pentamidine is unclear but it may involve the binding of DNA and the interruption of DNA replication. The drug is available for parenteral and inhalational use; the latter mode is used only in the prophylaxis and treatment of *P. carinii* pneumonia because little of the inhaled drug is absorbed systemically. Parenterally administered pentamidine isethionate penetrates extensively and is excreted slowly from tissues such as liver, spleen, kidneys and adrenal glands. Very little crosses the blood-brain barrier, accounting for the lack of utility of pentamidine in late-stage trypanosomiasis.

Route of administration and dosage

There are different recommendations for dosing pentamidine isethionate. For early *T. brucei gambiense* infection, the US Centers for Disease Control and Prevention (CDC) recommends 4mg/kg per day for 10 days. The World Health Organization (WHO) recommends 3–4mg/kg daily or every other day for 7–10 doses. Because of the rapidity with which *T. brucei rhodesiense* invades the central nervous system, this drug is generally not used for this organism. Pentamidine has also been used for

prophylaxis against infection with *T. brucei gambiense* at a dose of 4mg/kg (to a maximum of 300mg) given every 3–6 months.

For leishmaniasis, the CDC recommends 2–4mg/kg/day or every other day for 12–15 doses; a second course is sometimes given after an interval of 1–2 weeks. Alternatively, the WHO recommends 4mg/kg three times a week for 5–25 weeks or longer. The dosage regimen varies slightly depending on the species of *Leishmania* and the region of the body affected.

Adverse reactions

Pentamidine isethionate may cause toxicity in 50% of patients. Precipitous hypotension with dizziness, dyspnea, tachycardia, headache, vomiting and syncope can occur with rapid intravenous infusion. Intramuscular administration may result in sterile abscesses. Hypoglycemia, which may be life-threatening, pancreatitis, hyperglycemia and diabetes mellitus probably result from a direct toxic effect of pentamidine on pancreatic ß cells. Reversible renal failure occurs in up to 25% of patients. Other side effects include fever, arrhythmias (particularly *torsades de pointes*), hypocalcemia, confusion, hallucinations, leukopenia, thrombocytopenia and elevated transaminases.

PENTAVALENT ANTIMONIAL COMPOUNDS

The pentavalent antimonial compounds are a mainstay of therapy for leishmaniasis and are less toxic than the older trivalent compounds.[6] [7] [10] [67] Sodium stibogluconate has been the most extensively studied and is the only pentavalent antimonial available in the USA. Meglumine antimoniate is used largely in French-speaking countries and parts of Latin America. These compounds appear to inhibit bioenergetic pathways such as glycolysis and fatty acid oxidation in *Leishmania* amastigotes. *Leishmania* strains resistant to pentavalent antimony compounds are becoming more common, especially in India, and this has led to treatment failures and the need for alternative agents.[68]

These compounds are available as aqueous solutions for intravenous or intramuscular use only. Each milliliter of sodium stibogluconate contains the equivalent of 100mg of pentavalent antimony, whereas each milliliter of meglumine antimonate contains 85mg. They are rapidly absorbed and are eliminated in two phases. The first has a half-life of 2 hours but the second is longer, with a half-life of between 33 hours (after intravenous administration) and 76 hours (after intramuscular administration). This slow terminal elimination may result from a conversion to trivalent antimony, which thus may be responsible for the toxicity seen with long-term therapy. Excretion is primarily renal.

Pentavalent antimonials are generally well tolerated. Malaise, nausea, vomiting, abdominal pain, headache, arthralgias, myalgias, fever, rash, elevated transaminases, nephrotoxicity and pancreatitis are seen. Dose-related electrocardiographic changes include T-wave flattening and inversion and QT_c -interval prolongation. Arrhythmias and sudden death have been described with high-dose therapy.

PRIMAQUINE

Primaquine, an 8-aminoquinoline active against hypnozoites of *P. vivax* and *P. ovale* in the liver, is the only agent with the potential for yielding complete resolution of malaria caused by these organisms.[6] [7] [14] [16] Primaquine combined with clindamycin is also effective in the treatment of *P. carinii* pneumonia (see Chapter 124). Recently, primaquine in a dose of 30mg/day showed efficacy as prophylaxis against *P. falciparum* (88% protection) and *P. vivax* (92% protection) malaria.[69] Primaquine acts by interfering with *Plasmodium* mitochondrial function, possibly through its effects on the electron transport chain and pyrimidine biosynthesis. Primaquine phosphate, which is available only in oral form, is rapidly absorbed (bioavailability 96%), widely distributed and hepatically converted to three metabolites, yielding an elimination half-life of 6–7 hours. It is unclear whether the parent compound or the metabolities possess the antimalarial activity.

Primaquine phosphate is formulated in tablets containing 26.3mg of the salt, equivalent to 15mg of the base. Dosages are given in Table 209.1 . Relapse of *P. vivax* after conventional primaquine treatment has been described in up to 30% of cases in Papua New Guinea, the Solomon Islands, Thailand and other parts of South East Asia.[70] Therefore, for cases acquired in South East Asia or Oceania, the dose should be increased to 22.5mg base per day.

The principal toxicity of primaquine is hemolysis in patients who are G6PD-deficient, and thus G6PD levels should be measured before therapy is begun. Headache, nausea, vomiting and abdominal cramps have been reported. At higher doses, mild anemia, cyanosis (due to methemoglobinemia) and leukopenia may occur. Rarely, neurotoxicity, arrhythmias, hypertension and agranulocytosis occur.

QUINACRINE

Quinacrine is an acridine dye derivative that is effective against *G. lamblia*.[6] [40] [47] and it has recently been used as combination therapy with metronidazole for individuals who failed therapy with metronidazole alone.[71] It also has activity against adult cestodes, but for this indication it has been supplanted by less toxic alternatives. In the Second World War, quinacrine was used for malaria prophylaxis and treatment. The mechanism of antiparasitic action is unclear, but the drug has been shown to intercalate with DNA and inhibit nucleic acid synthesis. Quinacrine is available in an oral formulation and is well absorbed and widely distributed. It has extensive tissue binding and has been detected in the urine 2 months after stopping therapy. Its metabolic fate is poorly understood.

The dosage in giardiasis is 100mg q8h for 5–7 days. A second treatment course may be given 2 weeks later. The drug has a bitter taste and may induce nausea and vomiting. Dizziness and headache are also common. Reversible yellow skin discoloration (with spared sclerae) is seen in 4–5% of those treated with quinacrine for giardiasis. Under Wood's light, a bright yellow-green fluorescence distinguishes this side effect from hyperbilirubinemia. Toxic psychosis may occur in 0.1–1.5% of patients. Other rare side effects include blood dyscrasias, ocular toxicity and urticaria. Patients who have psoriasis may experience exfoliative dermatitis. Quinacrine has a disulfiram-like effect, and thus patients should be advised to avoid alcohol consumption while taking it. It also interferes with the metabolism of primaquine, and toxic levels of the primaquine can result from co-administration of quinacrine with primaquine.

QUINIDINE

Quinidine is the dextrostereoisomer of quinine. It is a blood schizonticide and is the parenteral therapy of choice for chloroquine-resistant *P. falciparum* as a result of its wide availability as an antiarrhythmic. [6] [7] [14] [16] [72] It is supplied as the gluconate salt for intravenous use, with a half-life of 6–8 hours. It is 80–90% protein-bound, hepatically metabolized and renally excreted.

During treatment, continuous electrocardiographic and blood-pressure monitoring are recommended. Widening of the QRS complex and prolongation of the QT_c interval may be seen, and hypotension may ensue if the drug is infused rapidly. Other side effects are similar to those of quinine.

QUININE

Quinine is an alkaloid derived from the bark of the South American cinchona tree. It has been used as an antimalarial for over 350 years.[6] [7] [14] [16] It is effective against the asexual blood stages of all four *Plasmodium* spp. that cause malaria in humans, and it is the drug of choice for chloroquine-resistant *P. falciparum* infections. Quinine is also used with clindamycin in the treatment of *Babesia. microti* infection.[31] The basis for the antimalarial activity of quinine is unclear, but three mechanisms have been proposed:

- intercalation with parasite DNA, interrupting replication and transcription;
- interaction with erythrocyte fatty acids, promoting hemolysis and preventing schizont maturation; and
- alkalinization of parasite digestive vacuoles, interfering with hemoglobin degradation.

Pharmacokinetics and distribution

Quinine is available as the sulfate, bisulfate, hydrochloride, dihydrochloride, hydrobromide and ethylcarbonate salts for oral administration and as the dihydrochloride salt for parenteral use. It is rapidly absorbed after oral administration (bioavailability 80%), extensively protein-bound (90%), hepatically metabolized (80%) and renally excreted. The therapeutic range in plasma is 8–15mg/l, which is achieved within 1–3 hours after a single oral dose. The half-life is approximately 11 hours. In cases of severe illness, the volume of distribution decreases, clearance is reduced and the half-life is prolonged. Thus, on a given dosage schedule, plasma quinine concentrations are elevated with acute illness and decrease as the patient improves. Monitoring blood levels is recommended in those who have renal or hepatic

dysfunction; dosage reduction is needed with severe renal failure.

Route of administration and dosage

The oral dose of quinine sulfate (unlike other antimalarial agents, it is dosed by weight of salt) is 650mg salt q8h for 3–7 days. A longer course is preferred for those in areas where *P. falciparum* is less sensitive to quinine, including South East Asia and western Africa.[73]

Intravenous quinine dihydrochloride may be used for severe infections. A 20mg salt/kg loading dose in 5% dextrose is given over 4 hours, followed by 10mg salt/kg over 2–4 hours q8h (maximum 1800mg salt/day) until oral therapy can be given. The loading dose should be omitted in those who have received oral quinine, quinidine or mefloquine during the previous 24 hours. Intravenous quinidine gluconate has become the parenteral therapy of choice worldwide (see above).

Adverse reactions

The term cinchonism refers to a cluster of dose-related and reversible side effects of quinine, including tinnitus, decreased hearing, headache, nausea, vomiting, dysphoria and visual disturbances. Hypoglycemia can occur secondary to quinine stimulation of insulin release in conjunction with parasite consumption of glucose. Skin rashes (urticaria, flushing), pruritus, hepatitis, thrombocytopenia, agranulocytosis and massive hemolysis with hemoglobinuria (with resultant bilirubinuria termed blackwater fever) occur rarely. Quinine can cause respiratory depression in patients who have myasthenia gravis and hemolysis in those who have G6PD deficiency. Myocardial depression, vasodilation and shock may result from rapid intravenous infusion. Overdose can result in delirium, seizures, coma, respiratory depression, cortical blindness, shock and death. An oral quinine dose of 2–8g may be fatal for adults.

SURAMIN

Suramin is a sulfated naphthylamine introduced in 1920 and is used in the treatment of the early or hemolymphatic stage of African trypanosomiasis. [6] [7] [38] It is more effective against *T. brucei rhodesiense* than against *T. brucei gambiense*, for which pentamidine is often used for early disease. Suramin has also been used for prophylaxis in those who have intense exposure. Additionally, suramin is active against the adult forms of *Onchocerca volvulus*, but is rarely used for this infection because of its toxicity.

The mechanism of action of suramin is unclear; it is a poly-anion that inhibits many cellular enzymes. Notably, its antitrypanosomal activity correlates with inhibition of glycerol-3-phosphate oxidase and dehydrogenase, enzymes involved in energy metabolism.

Pharmacokinetics and distribution

Suramin is 99% protein-bound and persists at low levels in plasma for 3 months, which supports its use in prophylaxis. It is not metabolized and is excreted mainly by the kidneys. The large, polar, polyanionic structure affords poor cellular penetration. Very little drug penetrates into the cerebrospinal fluid, accounting for the lack of efficacy of suramin in late-stage disease.

Route of administration and dosage

Suramin is available only for intravenous use. The dosage for trypanosomiasis is given in Table 209.1 For onchocerciasis, a test dose of 100–200mg is followed by weekly infusions of 1g up to a total of 6g. Some investigators administer increasing weekly doses (200mg, then 400mg, and so on to 1g) up to a total dose of only 3–4g, with good efficacy and tolerability.

Adverse reactions

Suramin has a variety of side effects, which are generally more severe in malnourished patients. Immediate reactions include malaise, nausea, vomiting, fatigue, fever, urticaria, shock, loss of consciousness and, rarely, death. Late reactions include fever, rash, stomatitis, exfoliative dermatitis, lacrimation, photophobia, headache and hyperesthesia. Renal dysfunction (hematuria, proteinuria, casts and elevated creatinine), hepatic dysfunction (elevated transaminases and bilirubin), diarrhea, thrombocytopenia and agranulocytosis may occur. Additional side effects during treatment for onchocerciasis include pruritus, dermal edema, papular eruptions, palmoplantar paresthesias and iridocyclitis.

TETRACYCLINES

Tetracycline (see Chapter 200) is used in combination with quinine in the treatment of drug-resistant *P. falciparum* in South East Asia, where resistance to chloroquine, Fansidar and quinine is common.[6] [7] [16] [54] Doxycycline, a longer-acting derivative, is used for malaria prophylaxis in this area, and worldwide in individuals unable to tolerate mefloquine.[32] [51] [74] Tetracycline is also the drug of choice for infection with the ciliate *Balantidium. coli.*

Tetracyclines are well absorbed after oral administration and are probably active against parasite protein synthesis. Side effects include gastrointestinal distress, photosensitivity and vaginal candidiasis.

TRYPARSAMIDE

Tryparsamide, a pentavalent arsenical first described in 1919, is used primarily for the treatment of advanced *T. brucei gambiense* infections resistant to other therapy.[6] [7] It has poor efficacy against *T. brucei rhodesiense*. Side effects of tryparsamide include fever, rash, abdominal pain, vomiting, tinnitus, optic atrophy and blindness, and encephalopathy.

ANTHELMINTIC AGENTS

ALBENDAZOLE

Albendazole is a benzimidazole carbamate that has a broad spectrum of anthelmintic activity, including against *Ascaris lumbricoides*, *Enterobius vermicularis*, *Ancylostoma duodenale*, *Necator americanus*, *Strongyloides stercoralis*, *Echinococcus* spp. and *T. solium* cysticerci.[6] [7] [75] [76] [77] [78] [79] The drug has also been used to treat eosinophilic enterocolitis caused by *Ancylostoma caninum*, *Capillaria philippinensis*, cutaneous and visceral larva migrans, *C. sinensis*, *Gnathostoma spinigerum*, *Oesophagostomum bifurcum* and *Trichostrongylus* spp. It is also used in combination with diethylcarbamazine or ivermectin for mass treatment of lymphatic filariasis (*Brugia malayi* and *Wuchereria bancrofti*).[80] Additionally, it has variable efficacy in the treatment of microsporidiosis caused by *Encephalitozoon hellem*, *E. cuniculi*, *E. intestinalis*, *E. bieneusi*, and *Vittaforma corneae*.[81] Albendazole has some activity against *G. lamblia*.

The mechanism of action is similar to that of mebendazole with blockade of parasite microtubule assembly. Albendazole is poorly soluble in water and should be taken with a fatty meal to enhance absorption. It undergoes extensive first-pass metabolism in the liver, and albendazole sulfoxide is responsible for most of the systemic anthelmintic effects. This metabolite has a half-life of 9–15 hours and is mostly excreted renally.

Single doses of albendazole are generally well tolerated; abdominal discomfort, diarrhea, or migration of *Ascaris* into the mouth and nose occur infrequently. Prolonged, high-dose treatment can be associated with reversible aminotransferase elevations, bone marrow suppression and alopecia.

BITHIONOL

Bithionol is a chlorinated bisphenol that is used for infections with *Fasciola hepatica* but may have been supplanted by triclabendazole. Bithionol is also an alternative agent against *Paragonimus* spp.[6] [7] It has activity against many other flukes but has been replaced by praziquantel. Its mechanism of action is poorly understood. Side effects include anorexia, abdominal pain, nausea, vomiting, headache, dizziness, diarrhea, urticaria and proteinuria, some of which may be allergic responses to liberated fluke antigens.

DIETHYLCARBAMAZINE

Diethylcarbamazine is a piperazine derivative used in the treatment of filariasis. It is microfilaricidal for *W. bancrofti*, *B. malayi* and *Brugia timori*. [6] [7] [76] [82] [83] It appears to be macrofilaricidal for these species as well (i.e. it kills adult worms) and is considered to be the drug of choice for these three infections. Diethylcarbamazine is a key component of mass chemotherapy approaches for the eradication of lymphatic filariasis.[80] It has been used as a sole agent for single-dose therapy, administered long-term as diethylcarbamazine-fortified dietary salt, and as a component of combination single-dose regimens with ivermectin or albendazole. Diethylcarbamazine is also the mainstay of therapy against *Loa loa* and *Mansonella streptocerca*. It has been used to treat tropical pulmonary eosinophilia, supporting the contention that the pulmonary infiltrates in this disorder are due to migrating microfilariae. It has also been used for visceral larva migrans. Diethylcarbamazine is effective in eliminating microfilariae of *O. volvulus* in the skin and eye but the resulting inflammation can cause permanent ocular damage, including uveitis, punctate keratitis and retinal pigment epithelium atrophy. Adult *Onchocerca* worms are not killed, however, and the infection may return once treatment has stopped. Ivermectin has largely replaced diethylcarbamazine for ocular onchocerciasis. Diethylcarbamazine also has activity against *A. lumbricoides*.

The mechanism of action of diethylcarbamazine involves two processes:

- first, filarial muscular activity decreases, probably secondary to hyperpolarization of membranes by the piperazine moiety of diethylcarbamazine; and
- second, diethylcarbamazine alters microfilarial surface membranes by making them more susceptible to host defenses.

Diethylcarbamazine is available as the citrate salt in 50mg tablets. It is rapidly absorbed, widely distributed in the body, hepatically metabolized and renally eliminated. It has a half-life of approximately 10 hours.

Side effects, although common, are usually mild and transient; they include headache, malaise, arthralgias, anorexia, nausea and vomiting. Toxicity can result from the destruction of organisms and release of antigens, which provokes an inflammatory response. This reaction is most severe in patients who are heavily infected with *O. volvulus*. This is termed the Mazzotti reaction and consists of severe pruritus, edema, rash, arthralgias, lymphadenopathy, fever, hypotension, increased eosinophilia, proteinuria and splenomegaly. These symptoms persist for 3–7 days. Nodular swellings along lymphatics and lymphadenitis may occur with *W. bancrofti* and *B. malayi* infections. Patients heavily infected with *L. loa* may experience encephalopathy and other neurologic complications. [84] Pretreatment with corticosteroids may lessen the severity of these inflammatory responses.

FLUBENDAZOLE

Flubendazole is a fluorine analogue of mebendazole and the two drugs have similar spectra of activity.[6] [7] Flubendazole is poorly absorbed after oral administration. It has been used against many of the common intestinal helminths and, with limited success, in the treatment of neurocysticercosis.

IVERMECTIN

Ivermectin is a derivative of avermectin B1, a type of macrocyclic lactone that was discovered in the 1970s as a product of the actinomycete *Streptomyces avermitilis*.[6] [7] [76] [77] [85] Ivermectin is used as a broad-spectrum veterinary agent for infections with helminths and arthropods. Since the 1980s, it has become the drug of choice for onchocerciasis because it kills microfilariae in the skin and the eye while provoking much less inflammation than diethylcarbamazine. The response to ivermectin is rapid and can last for 6–12 months. Adult worms appear to be unaffected by ivermectin, but the drug seems to prevent developing larvae from leaving the uterus. The drug also has activity against *W. bancrofti*, *B. malayi*, *B. timori*, *L. loa*, *Mansonella ozzardi* and *Mansonella streptocerca*, and is being used as a component of mass chemotherapy for lymphatic filiriasis.[80] Ivermectin is effective against *S. stercoralis* and is active against *E. vermicularis*, *A. lumbricoides* and *Trichuris trichiura*. Ivermectin causes tonic paralysis of the helminth musculature but the mechanism of action is poorly understood, although it is known to include ?-aminobutyric acid (GABA) blockade.

Ivermectin is available as 6mg tablets. It is highly protein-bound, has an elimination half-life of 50–60 hours, is concentrated in liver and adipose tissue and is almost entirely excreted in the feces (only 1–2% appears in the urine). Side effects include headache, fever, pruritus, lymphadenopathy, myalgias, arthralgias and, less commonly, orthostatic hypotension.

MEBENDAZOLE

Mebendazole is a benzimidazole carbamate with a broad range of anthelmintic activity.[6] [7] [75] [76] [77] It is active against the larvae and adults of *E. vermicularis*, *A. lumbricoides*, *T. trichiura*, *N. americanus* and *A. duodenale*. It is ovicidal for *Ascaris* and *Trichuris* spp. It is less effective than thiabendazole against *S. stercoralis*. Mebendazole has been used at high doses and for long periods in the treatment of *C. philippinensis*. It can also be used for infections caused by *Angiostrongylus cantonensis*, *Angiostrongylus costaricensis*, *Toxocara canis* and *Trichostrongylus* spp. The drug has activity against adult *Trichinella spiralis*, with some activity against larval forms, and it is currently recommended in the treatment of trichinosis. Mebendazole is also effective in the treatment of certain types of filariasis; it is considered the drug of choice against *Mansonella perstans* (diethylcarbamazine is ineffective), and it has been shown to have efficacy against *L. loa*, *O. volvulus* and *Dracunculus medinensis* infections. The drug has activity against *T. saginata*, *T. solium* and *Hymenolepis nana*, although praziquantel is more effective. Although mebendazole does not eradicate echinococcal infection, the drug prevents progression of existing cysts and the development of new cysts when administered in high dose for a prolonged period. Mebendazole has largely been replaced by albendazole for echinococcosis.

Mebendazole acts by binding parasite tubulin, thus blocking microtubule assembly and interfering with glucose absorption. Susceptible helminths become paralyzed and depleted of energy stores, but death and clearance of the worms from the gastrointestinal tract can take days. Mebendazole, formulated as 100mg tablets, has low

water solubility and is poorly absorbed. It is 95% protein-bound in plasma and undergoes rapid and extensive first-pass metabolism in the liver. Thus, systemic bioavailability is low, accounting not only for its poor tissue levels and relative lack of usefulness in extraintestinal infections, but also for its low rate of side effects.

Abdominal pain and diarrhea may occur after mebendazole administration. The drug also has been reported to prompt the migration of adult *Ascaris* spp. into the mouth and nose. At high doses, reversible bone marrow suppression with neutropenia, alopecia, allergic skin reactions, hepatitis, vertigo and oligospermia occur rarely.

METRIFONATE

Metrifonate is an organophosphate inhibitor of acetylcholinesterase that was originally developed as an insecticide. It has activity against *Schistosoma haematobium*.[6] [7] [86] It is well absorbed orally and metabolized quickly to dichlorvos, an active metabolite. The half-life is 1.5 hours. The dosage is 7.5–10mg/kg orally once every 2 weeks for three cycles. Side effects include nausea, vomiting, vertigo and lethargy. Patients receiving metrifonate should neither be exposed to other insecticides nor receive neuromuscular blocking agents in the 2 days before or after taking metrifonate.

NICLOSAMIDE

Niclosamide is a salicylamide derivative that is active against the cestodes *Diphyllobothrium latum*, *D. caninum*, *H. nana*, *T. saginata* and *T. solium*, as well as the trematodes *Echinostoma* spp., *Fasciolopsis buski* and *Heterophyes heterophyes*.[6] It acts by interfering with oxidative phosphorylation and production of adenosine triphosphate. Treatment failures of *Taenia* spp. with niclosamide have been reported, and praziquantel has been used successfully in these cases.[87] Niclosamide is supplied as 500mg tablets that should be chewed thoroughly because of its very poor absorption. The dosage is 2g as a single dose (or 1g and 1g, given 1 hour apart), except for *H. nana* infection, which requires 2g then 1g q24h for 6 days. Side effects include gastrointestinal distress, dizziness and rash.

OXAMNIQUINE

Oxamniquine is a tetrahydroquinoline that is effective in *S. mansoni* infections.[6] [7] [86] Its mechanism of action is unclear, but it causes adult worms to become paralyzed and dislodged from the veins they inhabit, resulting in subsequent killing by host defenses. The drug is available in 250mg capsules that are rapidly absorbed and extensively metabolized in the liver. The half-life is approximately 2 hours, and 70% of the drug is excreted by the kidneys. Side effects include drowsiness, dizziness, orange-red discoloration of the urine and, rarely, seizures. The drug should be given cautiously to patients who have a history of seizures.

PIPERAZINE

Piperazine has activity against *A. lumbricoides* and *E. vermicularis*.[6] [7] [75] In many parts of the world it has been replaced by less toxic agents such as mebendazole. However, because of its lower cost, piperazine is still frequently used. Piperazine blocks the helminth muscle response to acetylcholine by altering membrane ion permeability and causing hyperpolarization and decreased action potentials. Flaccid paralysis ensues and the worms are eliminated in the stool.

Piperazine is available as the citrate salt in 250mg tablets. There is good oral absorption and a small amount of hepatic metabolism; 60% of the drug is excreted in the urine unmodified. Different dosage schedules exist, but a single dose of 75mg/kg (maximum 4g) per day for 2 days is effective for ascariasis, and a single dose of 65mg/kg daily for 7 days is used for enterobiasis.

Side effects include gastrointestinal disturbances, headache, dizziness and urticaria. Seizures occur rarely, and piperazine is contraindicated in patients who have a history of a seizure disorder. Piperazine and pyrantel pamoate are antagonistic and should not be co-administered.

PRAZIQUANTEL

Praziquantel, a pyrazinoisoquinoline derivative developed in the early 1970s, has broad activity against trematodes and cestodes but not nematodes. [6] [7] [76] [86] All *Schistosoma* spp. that infect humans are susceptible. The drug also has activity against the trematodes *C. sinensis*, *Dicrocoelium dendriticum*, *Echinostoma* spp., *F. buski*, *H. heterophyes*, *Metagonimus yokogawai*, *Metorchis conjunctus*, *Nanophyetus salmincola*, *Opisthorchis viverrini* and *Paragonimus westermani* and other *Paragonimus* spp. *Fasciola hepatica* does not appear to be adequately treated with praziquantel; bithionol is used instead. Praziquantel is effective in treating adult cestodes, including *D. latum* and other *Diphyllobothrium* spp., *D. caninum*, *H. nana*, *Hymenolepis diminuta*, *T. saginata* and *T. solium*. It has been used successfully to treat neurocysticercosis (larval *T. solium*), but it is not useful in echinococcosis. The drug has several actions, including promoting calcium influx and parasite muscle contraction and causing vacuolization and bleb formation in the helminth tegument, thereby activating host defenses.

Praziquantel is available as 600mg tablets that are nearly insoluble in water. There is good oral absorption, 80% protein binding and rapid first-pass metabolism. The half-life is 1.5 hours. About 80% of the drug is excreted in the urine. Side effects are common but transient and include headache, dizziness, nausea, vomiting and abdominal pain. Fever and rashes are occasionally seen.

PYRANTEL PAMOATE

Pyrantel pamoate, a tetrahydropyrimidine that was originally developed as a veterinary anthelmintic, has a broad range of activity in humans.[6] [7] [75] [76] It is considered by many to be the treatment of choice for *E. vermicularis*. It also effective for *A. lumbricoides*, eosinophilic enterocolitis caused by *Ancylostoma caninum*, hookworm, the acanthocephalan *Moniliformis moniliformis* and *Trichostrongylus* spp. It does not have activity against *T. trichiura*. Oxantel pamoate, an *m*-oxyphenol derivative, can be given in a single dose for *Trichuris* sp. Pyrantel pamoate and its analogues act by causing depolarizing neuromuscular blockade and by blocking acetylcholinesterase, which result in spastic paralysis and muscle contracture, respectively, and allow expulsion of the worms.

Pyrantel pamoate is available as an oral suspension of 250mg of pyrantel base per 5ml. It is poorly absorbed. Less than 15% is excreted in the urine and most remains in the feces unmodified. Side effects include headache, dizziness, insomnia, nausea, vomiting, anorexia and abdominal pain. Pyrantel pamoate, which causes depolarization and increased spike frequency in worm muscle cells, should not be given with piperazine, which causes hyperpolarization and a reduction in spike frequency.

THIABENDAZOLE

Thiabendazole is a substituted benzimidazole compound that has better activity against *S. stercoralis* and *Strongyloides fuelleborni* than mebendazole.[6] [7] [75] [76] [77] [86] It is active against *A. costaricensis*, *C. philippinensis*, *D. medinensis*, *Trichostrongylus* spp. and *T. spiralis*. In trichinosis, however, larval stages are often resistant to thiabendazole. The drug is also used in the treatment of both cutaneous and visceral larva migrans. Thiabendazole has some activity against *A. lumbricoides*, hookworm, *E. vermicularis* and *T. trichiura* but mebendazole is less toxic and is thus preferred. The drug acts by inhibiting parasite fumarate reductase, and it may bind tubulin as well.

Thiabendazole is available in tablet and liquid form and, in contrast to other benzimidazole derivatives, is rapidly absorbed. It is extensively metabolized by the liver, has a half-life of 1 hour and is mainly excreted by the kidney.

Side effects are frequent; they include nausea, vomiting, anorexia and dizziness. Pruritus, epigastric pain, headache, drowsiness, giddiness and diarrhea are less common. Rarer still are tinnitus, hallucinations, numbness, seizures, altered olfaction, altered color perception, hypotension, bradycardia, crystalluria, leukopenia, elevated liver enzymes and intrahepatic cholestasis. Allergic manifestations, including fever, angioneurotic edema, erythema multiforme and Stevens-Johnson syndrome, have been described and may result from the release of parasite antigens during treatment. Increased theophylline levels and consequent nausea and vomiting may result from the co-administration of theophylline and thiabendazole.

TRICLABENDAZOLE

Triclabendazole, a benzimidazole derivative used as a veterinary fasciolicide, has been used safely and successfully in cases of human chronic hepatic fascioliasis.[88] [89] It is now considered the drug of choice for human hepatic fascioliasis, but in the USA it is available only by directly contacting Novartis Agribusiness (Basel, Switzerland).

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Chapter 210 - Immunomodulation

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INTRODUCTION

Despite the availability of potent antimicrobial drugs, many infections are still difficult to treat. This is not only the case for infections for which suboptimal or no effective antimicrobial treatment is available, because of intrinsic or acquired resistance of the causative micro-organism, but also those due to failing host defense mechanisms. Based on data from experimental infections and from clinical studies, especially in the neutropenic patient, it is known that antibiotics alone are rarely capable of eradicting pathogenic micro-organisms; components of host defense, especially granulocytes, are required to effectively eliminate the infection, in conjunction with the antibiotics.

Another reason for failure of antibiotic treatment is that the response of the host to infection may be overwhelming. In that situation, proinflammatory cytokines, such as tumor necrosis factor-a (TNF-a), interleukin-1ß (IL-1ß) and IL-8, as well as secretory products of white blood cells, such as elastase, reactive oxygen metabolites and chloramines, can produce serious tissue damage.[1] [2] Not only is it the proinflammatory cytokines which can be hazardous under such circumstances; the anti-inflammatory cytokines may blunt the immune response to such an extent that a state of immunodeficiency ensues. It is, however, impossible to make a simple distinction between the good and bad effects of these cytokines. Under certain circumstances a particular cytokine effect may be beneficial, whereas under other circumstances a similar effect may harm the host.

Thus, there are good reasons to try to enhance or modulate host defenses, particularly in patients with defective host defense mechanisms or when effective drug treatment is not available. If, on the other hand, the host response is overwhelming, attempts may be made to inhibit such a response.

In a general sense, treatments fall into one of the following four categories (or combinations thereof).

- The treatment stimulates the inflammatory response (e.g. by increasing the proinflammatory cytokine status or by augmenting phagocyte or T-cell function).
- The treatment inhibits the counterregulatory, anti-inflammatory response (e.g. by inhibiting anti-inflammatory cytokines such as IL-10, IL-4 or TGF-ß).
- The treatment inhibits the inflammatory response (e.g. by decreasing the proinflammatory cytokine status or by inhibiting phagocyte or T-cell function).
- The treatment promotes the counterregulatory, anti-inflammatory response (e.g. by increasing the status of anti-inflammatory cytokines such as IL-10, IL-4 or TGF-ß).

Stimulation of the proinflammatory cytokine response and inhibition of the anti-inflammatory response are aimed at treatment of specific, difficult-to-treat infections. Inhibition of the proinflammatory response and stimulation of the anti-inflammatory response are especially applicable during overwhelming inflammation, such as the systemic inflammatory response syndrome occurring during sepsis.

These therapeutic approaches have become a great challenge in medicine. However, such interventions may have a large impact on the delicate and complicated cytokine balance and may lead to disturbances that adversely affect the status of the host.

Immunomodulatory treatment has encountered a variety of problems.

- It has turned out to be extremely difficult to determine under which clinical circumstances a certain immunomodulatory effect may be of benefit.
- Many of the immunomodulatory agents have mainly been shown to work *in vitro* or in animal experiments. Many studies have used rather artificial models and often, the infectious challenge has been administered after the immunomodulatory treatment rather than before initiation of therapy.

STIMULATION OF THE INFLAMMATORY RESPONSE

There are, in essence, two ways to augment the inflammatory response. One is to administer an exogenous ('foreign') agent that elicits an inflammatory response, the second is to administer an endogenous substance in recombinant form. So far, the former method has met with very little human application. Among a few exceptions are the addition of an adjuvant (such as alum, monophosphoryl lipid A) to a vaccine, an interferon inducer (such as ampligen[3] and the recently developed cytokine-inducing drug imiquimod, which has been applied in dermatology for topical treatment of genital warts).[4]

An interesting approach that has been tried in humans is the administration of *Mycobacterium vaccae*, a nonvirulent mycobacterial strain, to patients with tuberculosis with the aim of inducing an enhanced type 1 cytokine response (i.e. to induce cytokines like IFN-?). Despite application in a number of controlled trials, it is still controversial whether this approach is effective in tuberculosis.[5]

In experimental animals, a large variety of molecules have been used with the intention of augmenting the inflammatory response, such as bacterial endotoxin, muramylpeptides and glucans.[6] The anti-fungal drug amphotericin B does seem to have such an immunostimulatory effect [7] but it is difficult to demonstrate whether this has added value in clinical terms while treating invasive fungal infections. It is currently unclear whether the administration of nutrients, such as vitamin A and zinc, which have been shown to have a beneficial effect in the treatment of tuberculosis, should be considered as substitution therapy or immunomodulatory treatment.[8]

Colony-stimulating factors

Most clinical research has been performed with the hematopoietic growth factors granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF). [9] In particular, G-CSF and GM-CSF have been studied extensively in patients with congenital or acquired neutropenia. In patients with severe congenital neutropenia, benefit of G-CSF has been shown in terms of prevention of infection and improved quality of life.[10] [11] In cancer patients, these factors have been used both to shorten the duration of chemotherapy-induced granulocytopenia[12] and as adjunctive

TABLE 210-1 -- Immunomodulators for human use.

IMMUNOMODULATORS FOR HUMAN USE
Stimulators of the inflammatory response
• Exogenous agents
? adjuvants (monophosphoryl lipid A; alum)
? interferon inducers (ampligen)
? imiquimod

? amphotericin B
? *Mycobacterium vaccae*
• Endogenous agents
? Colony-stimulating factors (G-CSF, GM-CSF, M-CSF)
? Cytokines (interferon-?, IL-1, IL-2)
? Granulocyte transfusions (G-CSF primed)
Inhibitors of the anti-inflammatory response
Inhibitors of the inflammatory response
? Antiendotoxin strategies (antibodies, polymyxin B, BPI, reconstituted HDL)
? Anticytokine strategies
- Anti-TNF strategies (infliximab, etanercept)
- Anti-IL-1 strategies (IL-1ra)
Stimulators of the anti-inflammatory response
? Anti-inflammatory cytokines (IL-10)
? Glucocorticosteroids
? Macrolides
? Inhibitors of lipid mediators (PAF antagonists, cyclo-oxygenase inhibitors, n-3 fatty acids)
? Activated protein C
? Intravenous immunoglobulin preparations

therapy in patients with neutropenia and fever.[13] However, in patients with acquired neutropenia, the benefits, i.e. prevention and treatment of infection and prolonged survival, have been less impressive than had been anticipated.[9]

Although most of these studies failed to show an effect of CSF therapy on recovery from infection or survival, the potential beneficial role of GM-CSF has been demonstrated in a prospective, randomized, placebo-controlled study of patients with acute myelogenous leukemia.[14] In that study, recombinant GM-CSF was associated with a higher rate of complete response than placebo, longer overall survival and a reduced fungal infection-related mortality rate which was only 2% for those randomized to receive rGM-CSF compared with 19% for those receiving placebo. G-CSF has very few side-effects and this may be due to concomitant anti-inflammatory effects (see below).

Since G-CSF not only augments the number of granulocytes but also activates their microbicidal action and inhibits their apoptotic response, the potential of G-CSF to enhance the host's inflammatory response to infection has been investigated in non-neutropenic conditions. Preclinical studies in animals have yielded favorable effects in bacterial and fungal infections and anecdotal clinical experiences also suggested positive effects.[15] [16] In a small, randomized study in patients with disseminated *Candida* infection, it was suggested that recombinant G-CSF may improve resolution of infection and reduce mortality.[17] Controlled studies in patients with community-acquired pneumonia have failed to demonstrate a survival benefit.[18] It is controversial whether G-CSF may improve clinical outcome in diabetic foot infection, leading to shorter duration of illness and preventing amputation.[19] [20] [21] A multicenter study in chronic recalcitrant sinusitis did not show any clinical benefit.[22] Given the waning interest of the pharmaceutical industry in G-CSF, it is unlikely that further clinical studies with G-CSF in infectious diseases will be performed. The question therefore remains under which conditions recombinant G-CSF should be considered in the treatment of infection.

In the past, several clinical trials have investigated the potential beneficial effect of white blood cell transfusions in patients with refractory neutropenia-related bacterial infections.[23] This modality was abandoned because of the low yield of these transfusions and the toxicity in recipients including fever, chills, hypotension, pulmonary infiltrates, respiratory distress and allo-immunization. Recently, the possibility was raised that administration of rG-CSF to WBC donors would increase their neutrophil to levels that would lead to a higher yield of better quality cells. Donors achieved a 4–10-fold increase of their neutrophil count and the 24-hour post transfusion counts in recipients were favorable.[24] In a recent open study, rG-CSF-elicited WBC transfusions given to cancer patients with neutropenia and documented and refractory fungal infections have been successful. [25] This small pilot study as well as several clinical observations suggest that rG-CSF-enhanced WBC transfusions may be life saving for patients with refractory neutropenia-related fungal infections and are safe to deliver. Likewise, this approach seems to be successful in patients with chronic granulomatous disease (a phagocyte disorder characterized by defective intracellular killing; see below) and refractory pyogenic infection.[26] [27]

The application of recombinant GM-CSF and M-CSF in infectious diseases has been studied less extensively and despite the expectations of many investigators, there is hardly any clinical indication for these drugs, with the possible exception of visceral leishmaniasis.[28] [29]

Interferon-?

Despite many preclinical studies demonstrating useful effects in a variety of bacterial, fungal and parasitic infections, interferon-? (IFN-?) has not gained much favor in clinical medicine. The only established indication for IFN-? is the prevention of infection in chronic granulomatous disease.

Chronic granulomatous disease (CGD) is characterized by a defect in NADPH oxidase in phagocytic cells with a consecutive impairment in synthesis of reactive oxygen species, leading to recurrent pyogenic infections with catalase-positive micro-organisms.[30] Chronic treatment with IFN-? has been shown to reduce the frequency of infections in these patients by more than 70%, without severe side effects. The mechanism of action in CGD is not clear but most likely, the effect of IFN-? is through stimulation of the nonoxidative microbicidal effects of granulocytes and mononuclear phagocytes. Promising results have also been reported in clinical studies involving mycobacterial infections.

Recombinant IFN-? decreases the bacterial load in patients with lepromatous leprosy.[31] [32] Beneficial effects have also been observed in patients with *Mycobacterium avium* complex infection.[33] [34] Recombinant IFN-? has also been investigated as adjunctive therapy in visceral leishmaniasis. Its effect in combination with pentavalent antimony has been disappointing. [35] In a recent study, recombinant IFN-? was suggested to reduce the incidence of opportunistic infections and resulted in a tendency towards increased survival in patients with advanced HIV disease.[36]

In animal experiments, the effects of IFN-? on granulocytes and mononuclear phagocytes are impressive[37] and currently, several clinical trials of IFN-? as a therapeutic agent in mycobacterial and fungal infection are ongoing. Especially now that our understanding of the mechanism of action of IFN-?, its cellular receptor and the role of related cytokines such as IL-12, IL-18 and IL-23 has expanded, the need for such studies is compelling.

Other recombinant cytokines

So far, the use of other recombinant cytokines for therapy of infection has been limited. IL-1, which is effective in enhancing survival

of mice with bacterial and fungal as well as plasmodial infection,[6] has only been used in humans as an anticancer agent.[38] [39] [40] Interestingly, IL-1 seems to be unique in that it enhances survival of lethal bacterial and fungal infections in the absence of neutrophils.[6]

IL-2 has been studied to some extent for treatment of infection. In patients with lepromatous leprosy, who exhibit impaired *Mycobacterium leprae*-specific T-cell proliferation, the administration of recombinant IL-2 may be expected to be beneficial. Indeed, in several small pilot studies, intradermal injection of IL-2 in patients with lepromatous leprosy has lead to increased infiltration of mononuclear cells and reduction of the numbers of viable acid-fast *M. leprae* in the peripheral sites.[41] [42] Interestingly, recombinant IL-12 and IL-2 strongly synergize in restoring both *M. leprae*-specific T-cell proliferation and IFN-? secretion *in vitro*.[43]

Administration of relatively high doses of recombinant IL-2 has shown an effect in HIV-infected persons by increasing their levels of circulating CD4 T cells up to normal

levels.[44] Further placebo-controlled studies have confirmed a significant rise in CD4 cell count, accompanied by a reduction in plasma HIV in recipients of IL-2 relative to control patients, associated with a nonsignificant trend toward improved clinical outcome.[45] The mechanism through which IL-2 increases $CD4^+$ T cells in HIV-infected individuals is unclear. Most $CD4^+$ T cells that expand after IL-2 administration are memory cells, although increase in naïve T cells has been also reported.[46] Of interest, IL-2 administration in combination with highly active antiretroviral therapy (HAART), but not HAART alone, seems capable of reducing the pool of resting memory CD4T cells harboring latent replication-competent HIV.[47]

Although there has been concern that the proinflammatory effects of IL-2 may be harmful in terms of transient increases in plasma viremia after each cycle of cytokine administration, the net effect of IL-2 appears to be beneficial and in several trials, no evidence of increased levels of HIV replication was found after IL-2 administration in the presence of HAART.[48] The toxicity associated with administration of relatively high doses of this cytokine to HIV-infected persons remains a major hurdle.[44] The most common toxic effect is a flu-like syndrome that manifests with fever, chills, fatigue and headache. Recent studies have suggested that low doses of intermittent sc IL-2 induced a stable increase of peripheral CD4 cells that was indistinguishable from those associated with higher, less well-tolerated doses of IL-2.[48] (see also Chapter 140).

INHIBITION OF THE ANTI-INFLAMMATORY RESPONSE

The major anti-inflammatory mediators, such as IL-4, IL-10, TGF-ß, IL-1 receptor antagonist (IL-1ra) and the soluble TNF receptors, are potential targets in immunomodulatory treatment. The net effect of inhibiting these mediators is likely to be an increased proinflammatory response, enhancing the innate host defense to infection. Although such interventions have been studied in experimental infection in animals, e.g. through targeted gene disruption, such approaches have not been undertaken in humans.

IL-10 is considered a prototypic anti-inflammatory cytokine, which inhibits the production of proinflammatory cytokines *in vitro* and *in vivo*. Elevated plasma concentrations of IL-10 have been found in patients with sepsis[49] and its inhibition leads to an increased production of TNF and an enhanced mortality. [50] In contrast, the host defense against localized infection may be enhanced by treatment with anti-IL-10 antibodies. In a murine pneumonia model, antibodies against IL-10 inhibited bacterial outgrowth in lungs and improved survival.[51] There appears to be a fine balance between beneficial and deleterious effects of IL-10, since elimination of IL-10 in a model of septic peritonitis induced by cecal ligation and puncture (CLP) was associated with an increased mortality.[52] [53] These findings are explained by the dual effects of the cytokine network, i.e. whereas in an infected organ a predominantly proinflammatory response contributes to the effective clearance of bacteria, at the systemic level such a response may be harmful to the host. Therefore, during septic peritonitis, endogenous IL-10 impairs bacterial clearance from the peritoneal cavity and facilitates dissemination of bacteria to distant organs, yet attenuates the systemic inflammatory reactions and multiple organ failure associated with this abdominal sepsis syndrome by a mechanism that in part involves inhibition of TNF production. There have been no human trials applying these strategies as yet.

INHIBITION OF THE PROINFLAMMATORY RESPONSE

In recent years, most of the preclinical and clinical studies on immunotherapy of infection have dealt with the inhibition of the proinflammatory response and enforcement of the anti-inflammatory response. These approaches include inhibiting the proinflammatory cytokine TNF-a as well as administration of anti-inflammatory cytokines, such as IL-10. This treatment is based on the notion that an exaggerated and harmful host response may occur during severe infection (Chapter 56), and inhibition of such a response may be beneficial. The therapeutic strategies may interfere at various steps in the inflammatory cascade (Fig. 210.1). First, interference with the infective agent that triggers the host response is a sensible approach. In this respect, prompt antibiotic treatment is a keystone in treatment. Although it has been demonstrated *in vitro* and in animal experiments that antibiotics, especially ß-lactam antibiotics, may liberate endotoxin (lipopolysaccharide, LPS) and hence may be harmful, [54] it has not been convincingly shown that this phenomenon contributes to morbidity in humans.[55] In fact, in fulminant meningococcal septicemia, earlier administration of antibiotics leads to an attenuated mediator response and improved outcome.[56]

Antiendotoxin strategies

Several approaches have been undertaken to block the action of endotoxin. Despite initial optimism, polyclonal as well as monoclonal antibodies against the endotoxin molecule have not met with clinical success.[57] Likewise, an engineered form of the endotoxin-binding substance BPI has not led to a breakthrough. In a randomized study in meningococcal sepsis, this molecule led to a nonsignificant trend towards lower mortality and fewer amputations of affected limbs.[58] A major problem with this study, however, was that the time needed for enrolment of patients led to important delay and to a selection of less severe patients in the trial.[59] Further studies are needed to establish whether early administration of BPI may be beneficial. This points to a key problem with all available interventions, i.e. the timing of the intervention, given the rapidity of evolvement of sepsis.

There are three additional major problems with the antiendotoxin approach. One is that it is impossible in most cases to tell, at the bedside, whether endotoxin plays a role in the clinical illness, i.e. whether the patient has a Gram-negative infection. Fulminant meningococcal sepsis, with its characteristic clinical picture, may be an exception. The second problem is that endotoxin is not the only pathogenetic factor in Gram-negative sepsis; other components of Gram-negative bacilli are also involved. The third problem is that not all endotoxins are equal; for instance, meningococcal LPS is poorly neutralized by substances that do neutralize other endotoxin species, such as polymyxin B and reconstituted high-density lipoprotein.[60]

A subsequent target for treatment may be the site where microbial components interact with host cells. Recently, a new class of recognition molecules has been identified on cells of the host, the toll-like receptors (TLR). These TLR, of which so far at least 10 have been

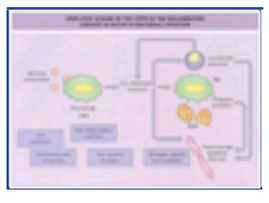

Figure 210-1 Simplified scheme of the steps in the inflammatory cascade as occur in (bacterial) infection. The block arrows indicate sites of intervention.

identified, recognize an array of components of different micro-organisms.[61] One could envisage clinical interference at this level, but tools to do so are not yet available.

Strategies to block proinflammatory cytokines

Based on pioneering work with anti-TNF antibodies in experimental animals challenged with either high-dose bacterial endotoxin or live bacteria, in which impressive reductions of mortality were demonstrated, a series of clinical trials have been performed in sepsis. The anti-TNF strategies in these studies consisted of either murine or humanized monoclonal antibodies against TNF (which only interfere with TNF-a and not with TNF-ß) or a engineered soluble TNF receptor construct (interfering with both types of TNF). None of these studies has met with consistent clinical benefit.[62] Similarly, interference with the actions of IL-1a and ß in sepsis by administration of IL-1ra has been disappointing in a large placebo-controlled trial.[63] There are various explanations for the discrepancy between the results in experimental animals and those in patients. A major explanation is that many of the animal models are not realistic in terms of challenges, timing of microbial challenge and intervention, and endpoints. A second explanation is that the magnitude and the duration of the biological effects of the cytokine intervention had not been studied adequately before the large trials were started.

A third explanation is that the entry criteria in the human studies were too imprecise; based on the idea that sepsis syndrome had a homogeneous mediator response, heterogeneous patients fullfilling the broad entry criteria for sepsis syndrome were enrolled. Another issue is the required endpoint of 28-day mortality; given the complicated course of many very sick and frail patients in the intensive care unit, it is naïve to assume that a short initial intervention will still pay off 4 weeks later. Furthermore, the concept of blocking one cytokine may be too simple, in view of the redundancy within the cytokine network. It is, however, controversial whether blocking more than one cytokine at a time is beneficial.

Finally, the idea that proinflammatory cytokines are only deleterious in sepsis is also an oversimplification. In fact, in the clinical settings in which these anticytokine strategies are effective (rheumatoid arthritis and Crohn's disease), it gradually has become apparent that these treatments enhance the risk for infection. The infections that are seen are not only those caused by facultative intracellular micro-organisms (such as mycobacteria and salmonella), but also by other pathogens. [64] [65] Given the capacity of the various proinflammatory cytokines to activate neutrophils for killing micro-organisms, it is likely that the host defense against common ('extracellular') bacteria (staphylococci, Gram-negative bacilli) is also hampered.

In terms of inhibiting the proinflammatory cytokine response, G-CSF is an interesting molecule. It does not only stimulate proliferation of neutrophils, activate them and inhibit their apoptotic response (as discussed above), but also downregulates the proinflammatory cytokine response. For instance, in human volunteers challenged with LPS, G-CSF pretreatment prevents neutrophil accumulation in the lung. [66] This dual action of G-CSF is not unique and other cytokines, such as IL-6, IL-10 and IFN-?, exhibit pro- and anti-inflammatory effects and even IL-1 has been shown to inhibit TNF production and the expression of cytokine receptors *in vivo*.[67] One way to inhibit cytokine action is to limit their production. This can be done by administration of anti-inflammatory cytokines (such as IL-10, IL-4 and TGF-ß) or by giving glucocorticosteroids. IL-10 has been evaluated as a new adjuvant therapy for several inflammatory diseases.[68] Treatment of Crohn's disease with IL-10 has been found to be associated with a bell-shaped dose-response curve.[69] However, although IL-10 is considered a potent anti-inflammatory cytokine, recent studies have suggested that it also possesses immunostimulatory effects.[70] At a low dose, IL-10 has induced clinical remissions but at higher dose, the beneficial effects are lost. It is hypothesized that these effects may be due to a proinflammatory effect of high-dose IL-10, and this potential IFN-? enhancing effect may especially warrant caution for the use of recombinant IL-10 therapy for Th1-mediated illnesses such as Crohn's disease and rheumatoid arthritis.

Glucocorticosteroids

Glucocorticosteroids have several immunomodulatory effects: not only do they inhibit the production of proinflammatory cytokines, they also inhibit the function of phagocyte and T cells. Two landmark studies demonstrated the ineffectiveness of high dosages of glucocorticosteroids in sepsis.[71] [72] There is, however, a renewed interest in the use of these drugs in sepsis, albeit in lower dosages, based on the concept that septic shock may be complicated by an

TABLE 210-2 -- Uses of systemic glucocorticosteroids in the treatment of infection.*

USES OF SYSTEMIC GLUCOCORTICOSTEROIDS IN THE TREATMENT OF INFECTION		
Infection	**Strength of recommendation#**	**Evidence‡**
Gram-negative sepsis with shock	B	I
Typhoid fever (critical illness)	A	I
Tetanus	B	I
Tuberculous pericarditis	A	I
Tuberculous meningitis	B	II
EBV infection with impending airway obstruction	B	II
Bacterial meningitis (children, *Haemophilus influenzae*)	B	I
Pneumocystis carinii pneumonia with hypoxia	A	I
Acute severe laryngotracheobronchitis (requiring hospitalization)	B	I
Allergic bronchopulmonary aspergillosis	B	II
Chronic effusion after otitis media	B	I

** (Adapted from McGowan et al. [74])*
#A, good evidence to support the recommendation for use; B, moderate evidence to support a recommendation for use. The categories C (poor evidence for or against use), D (moderate evidence against use) and E (good evidence against use) have been excluded from the table.
‡I, evidence from at least one properly randomized controlled trial; II, means evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytic studies, preferably from more than one center, from multiple time-series studies or from dramatic results in uncontrolled experiments.

occult adrenal insufficiency. In a randomized controlled trial in France, the efficacy of this approach has been demonstrated.[73] Glucocorticosteroids have a wider application as immunomodulators in infectious diseases. In Table 210.2 these applications are listed with the strength of the evidence for their use as put forward by the Infectious Diseases Society of America.[74]

The use of glucocorticosteroids has met with side effects that relate to their glucocorticoid, mineralocorticoid and anti-inflammatory effects. The latter are important within the context of this chapter. Their inhibition of influx and function of phagocytic cells (granulocytes and mononuclear phagocytes) enhances the susceptibility to bacterial and fungal infection, while the interference, T-cell distribution and function increase the risk for infection caused by facultative intracellular micro-organisms (such as mycobacteria, *Salmonella* spp. and cryptococci) and viruses (such as cytomegalovirus). Their effect on cytokine production probably impairs both phagocyte and T-cell defense. The degree to which these side-effects occur relates to dose and duration, as has become clear from a meta-analysis of controlled trials of glucocorticosteroid treatment[75] (Fig. 210.2).

Other non-specific inhibitors of inflammation

Macrolides are also able to inhibit the proinflammatory cytokine responses and probably have other anti-inflammatory actions. Although not studied in a randomized controlled fashion, their effect on survival in diffuse panbronchiolitis, a pulmonary disease that occurs in East Asia, points to a potent anti-inflammatory effect.[76] These findings are sustained by animal experiments with macrolides in which bronchial inflammation is modulated.[76]

Since many of the deleterious effects of proinflammatory cytokines are mediated by lipid mediators, such as platelet-activating factor (PAF) and prostaglandins, inhibitors for these secondary mediators have also been studied in sepsis. Controlled trials with PAF antagonists did not change overall mortality of septic shock.[77] [78] [79] [80] Likewise, the prostaglandin inhibitor ibuprofen did not prevent the development of shock or the acute respiratory distress syndrome and did not improve survival. [81] An explanation may be that prostaglandin inhibitors tend to increase the production of the proinflammatory cytokines.[82]

Supplementation with fish oil preparations, a source of n-3 polyunsaturated fatty acids, modulates proinflammatory cytokine production and prostaglandin synthesis. [83] Although impressive effects have been found in lethally infected experimental animals, the effects in

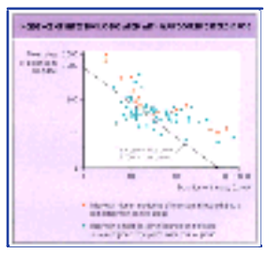

Figure 210-2 Incidence of infection associated with glucocorticosteroid use. In this meta-analysis of 71 placebo-controlled trials of prednisone, all trials in which there was a higher incidence of infection in the treated group (compared with the controls) were located above the isodose line of 700mg. This indicates that, independent of the regimen used in the trial, patients who had a cumulative dose of less than 700mg did not have an increased risk of infectious complications. *(With permission from Stuck et al.[75])*

humans with infections have not been well established.[84] The effects of these supplements seem to differ in different species (humans vs mice).

Intravenous immunoglobulins have been shown to possess anti-inflammatory properties. Although they induce proinflammatory cytokines shortly after infusion their major effect is a downregulation of the inflammatory response, e.g. through induction of IL-1ra and soluble cytokine receptors.[85] So far these complex effects of immunoglobulins have not been used therapeutically in the treatment of infection.

Activated recombinant protein C

Recently, activated protein C as a recombinant product has been investigated as a therapeutic agent in sepsis. Treatment with this product, which has antithrombotic, anti-inflammatory and profibrinolytic properties, was associated with an absolute reduction in the risk of death of 6% in patients with severe sepsis. [81] Further studies are needed to determine the exact indications for this expensive mode of treatment. In addition, the safety in septic patients with severe disseminated intravascular coagulation (such as occurs in fulminant meningococcal sepsis) has to be determined.

CONCLUSION

Despite elegant theoretical concepts and promising preclinical studies, the immunomodulatory adjunctive therapies in the field of infectious diseases have not come to fruition. Although a range of promising molecules is available, we have not yet been able to either discern the niche for them or apply them properly in terms of time and dosage. Another obstacle is that most of the immunomodulatory agents are expensive and hence not available for investigation in developing countries, where infections that are good candidates for immunomodulation (e.g. tuberculosis, leprosy, trypanosomiasis, leishmaniasis, typhoid fever) are prevalent.

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Section 8 - CLINICAL MICROBIOLOGY

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Chapter 211 - Acute Gastroenteritis Viruses

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Acute gastroenteritis constitutes a major cause of morbidity and mortality worldwide (Table 211.1). Infants and young children sustain on average 3–10 episodes per year compared with less than one episode per year for those older than 5 years. In the developing world this represents more than 1000 million cases and 3–4 million deaths every year.[3] These deaths are caused mainly by dehydration, but malnutrition also plays an important role. It has been estimated that 25% of the growth difference between children in developing countries and those in the USA is related to diarrhea. In developed countries, morbidity and mortality caused by diarrheal diseases have declined as economics and sanitation have improved, but 38 million cases still occur annually in the USA, with 2–3.7 million physician visits, 220,000 hospitalizations, 325–425 deaths and at a cost of US$23 billion. [4]

APPROACH TO MANAGEMENT

The goals of management of acute gastroenteritis are prevention and treatment of the two major complications — dehydration and malnutrition (Table 211.2). Children with no or only minimal dehydration can be managed with increased fluid intake and continuation of feeding. Those who have mild or moderate dehydration should receive an oral rehydration solution (ORS). In a few cases (<5%) oral rehydration might not be appropriate and intravenous rehydration should be used instead, for example in patients with severe dehydration, intractable vomiting, high stool output or monosaccharide malabsorption. Traditional ORSs are effective in preventing and treating dehydration, but do not reduce the stool output or duration of the diarrhea. Although cereal-based ORSs do reduce the stool output and duration of the diarrhea, these advantages become clinically insignificant when glucose-based ORSs are combined with appropriate nutritional management. Supplementing ORS with probiotics (*Lactobacillus* GG), oligoelements (zinc) or others (acetorphan) might reduce duration of diarrhea by 24–48 hours; their clinical and public health impact is uncertain at this point, though. There

TABLE 211-1 -- Morbidity and mortality rates of diarrheal diseases in children.*

MORBIDITY AND MORTALITY OF DIARRHEAL DISEASES IN CHILDREN				
	Developing countries			USA-Canada
	Snyder *et al.* (1982)[1]	Claeson & Merson (1990)[2]	Bern *et al.* (1992)[3]	Glass *et al.* (1991)[4]
No. of studies evaluated	24	276	22	4
Episodes/child/year (median)	2.2–3.0	3.3	2.6	1.3–2.5
Diarrheal illnesses/year (millions)	1000	1500	1000	21–37
No. of diarrheal deaths/year	4.6 million	4.0 million	3.3 million	325–425

** Estimates from longitudinal, prospective, community-based studies in developing and developed countries.*

is no reason not to feed children who have gastroenteritis. Full-strength feedings initiated immediately after rehydration improve the weight gain of the children and do not contribute to the severity or duration of the diarrhea compared with diluted or delayed feeding. Despite lactose malabsorption developing frequently during diarrhea, most children can still be fed their usual lactose-containing formulas in small frequent feeds. Human milk has a high lactose content; however, it reduces stool output and hence should be maintained. Milk-cereal and cereal-legume combinations are also safe and efficacious for the nutritional management of diarrhea.

APPROACH TO PREVENTION

The agents of acute gastroenteritis are transmitted by the fecal-oral route, through either direct person-to-person contact or contaminated food or water. The former is more important for the endemic pattern of disease and the latter for outbreaks. Handwashing and hygienic measures prevent person-to-person spread. However, because these measures are difficult to enforce in small children, endemic transmission of the disease continues, even in developed countries. For outbreaks, environmental control (food, water and sanitation) is important and effective. Good handwashing (especially among food handlers), furloughing of ill personnel, thorough cooking of food and disinfection of water and surfaces should prevent or control most outbreaks. For hospitalized patients, enteric precautions (i.e. contact precautions for diapered or incontinent patients, and standard precautions otherwise) must be instituted and continued for at least 48 hours after the resolution of symptoms. Breast-feeding, through a variety of mechanisms, reduces the overall incidence of diarrhea, especially in developing countries. Unfortunately, this protective effect can be overridden by exposure to a heavy inoculum. Continuation of breast-feeding through the diarrhea episode should be encouraged. Probiotics (*Lactobacillus* GG) might confer a modest protective effect.

TABLE 211-2 -- Management guidelines for gastroenteritis.

GASTROENTERITIS: MANAGEMENT GUIDELINES				
		Degree of dehydration		
		None	Mild-moderate	Severe
Assessment	Weight loss (%)	<5	5–10	>10
	Fluid deficit (ml/kg)	<50	50–100	>100
Rehydration (usually accomplished during the first 4h of therapy)	Initial fluid	ORS	ORS	iv Ringer's or 0.9% NaCl
	Amount (ml/kg)	50	50–100	20ml/kg/h
Replacement	ORS 10ml/kg or 2–4oz for each diarrheal stool			
Maintenance	Feedings should start immediately after rehydration. Human milk, lactose-containing and lactose-free formulas; milk staple and solid foods in small frequent feeds are well tolerated by most patients, foster recovery and improve nutritional outcome			
The essential goals of therapy are prevention and treatment of the two major complications, dehydration and malnutrition. ORS, oral rehydration solution.				

TABLE 211-3 -- Acute infectious diarrhea: clinical syndromes.

ACUTE INFECTIOUS DIARRHEA: CLINICAL SYNDROMES		
	Watery	**Dysenteric**
Stools		
Appearance	Watery	Bloody
Volume	Increased: ++/+++	Increased: +/++
Number per day	<10	>10
Reducing substances	0 to +++	0
pH	5.0–7.5	6.0–7.5
Occult blood	Negative	Positive
Fecal polymorphonuclear cells	Absent or few	Many
Mechanisms		
Toxins	Yes	No
Reduced absorption	Yes	No
Mucosal invasion	No	Yes

Complications		
Dehydration	Could be severe	Mild
Others	Acidosis, shock, electrolyte imbalance	Tenesmus, rectal prolapse, seizures
Etiology (Examples)		
	Rotaviruses	*Shigella spp*
	Enterotoxigenic	*Campylobacter spp*
	Escherichia coli	*Entamoeba histolytica*
	Vibrio cholerae	
+: mild; ++: moderate; +++: marked		
Separating the cases of diarrhea into dysenteric or watery is helpful when deciding on the need for laboratory evaluation.		

APPROACH TO DIAGNOSIS

An etiologic diagnosis is not necessary for the management of most cases of gastroenteritis. It might, however, be desirable for clinical, epidemiologic or research purposes. To identify patients that might benefit from a stool culture and antibiotic treatment, it is useful to classify the diarrhea as dysenteric or watery (Table 211.3). The distinction between these two syndromes is usually evident from the patient's history and inspection of the stool. If not, simple and inexpensive tests, such as the presence of occult blood in the stool and fecal leukocytes, should assist in the differentiation. Most diarrheal diseases that need antibiotics (e.g. shigellosis) belong to the dysenteric group, whereas viral gastroenteritis always manifests with watery diarrhea. Some bacterial pathogens can also produce watery diarrhea (e.g. enterotoxigenic *Escherichia coli* and *Salmonella* spp.), but they usually resolve spontaneously.

VIRAL AGENTS OF GASTROENTERITIS

The viruses associated with acute gastroenteritis and their morphologic and clinicoepidemiologic characteristics are shown in Table 211.4 and Table 211.5 . Rotaviruses (RVs) and enteric adenoviruses are large (70–80nm) and have distinct appearances. Astroviruses and caliciviruses (Noroviruses and Sapoviruses) are smaller (27–40nm) and possess well-defined surface structures. They used to be collectively referred to as small round structured viruses (SRSV). Somewhat smaller (20–26nm) viruses with smooth edge and no discernible surface structure were designated 'featureless viruses' or

TABLE 211-4 -- Morphologic characteristics of gastroenteritis viruses.

MORPHOLOGIC CHARACTERISTICS OF GASTROENTERITIS VIRUSES					
Characteristic	**Rotavirus**	**Enteric adenovirus**	**Astrovirus**	**Norovirus**	**Sapovirus**
Family	*Reoviridae*	*Adenoviridae*	*Astroviridae*	*Caliciviridae*	*Caliciviridae*
Virion size (nm)	70–75	70–80	27–32	27–35	27–40
Genome type	dsRNA	dsDNA	ssRNA	ssRNA	ssRNA
Morphology	Triple-shelled, wheel-like capsid, segmented RNA	Icosahedral shape, similar to other adenoviruses	Round, structured, unbroken surface, with pointed star	Round, structured, ragged surface	Round, structured, surface with cup-shaped indentations
ds, double-stranded; ss, single-stranded.					

small round viruses (SRV). These agents resemble enterovirus or parvovirus, and some may be related to them. Rotaviruses and caliciviruses are the main agents of human viral gastroenteritis.

Dehydration or vomiting, especially if it precedes the diarrhea, suggests a viral etiology. However, there is considerable overlap in the clinical manifestations of different viruses and making an etiologic diagnosis requires complex and expensive techniques, unavailable in most clinical centers. Electron microscopy (EM) was originally used to identify all gastroenteritis viruses, and still remains the preferred screening technique, especially when investigating outbreaks. If viral particles are seen by EM, depending on their morphology, more specific tests might follow. Electron microscopy requires the presence of at least one million viral particles per ml of stool; hence, the specimen must be obtained early in the illness (first 2 days). Immunoelectron microscopy (IEM) enhances the sensitivity of EM by using virus-specific antiserum that clumps the particles and makes them easier to visualize.

Immunoassays (IAs) represent simple and rapid methods to detect viruses. Unfortunately, IAs are commercially available only for the

TABLE 211-5 -- Epidemiologic and clinical characteristics of gastroenteritis viruses.

EPIDEMIOLOGIC AND CLINICAL CHARACTERISTICS OF GASTROENTERITIS VIRUSES					
Characteristic	**Rotavirus**	**Enteric adenovirus**	**Astrovirus**	**Norovirus**	**Sapovirus**
Age group	6–24 months	<2 years	<7 years	Adults and children	Children
Mode of transmission	Person-to-person, food, water	Person-to-person	Person-to-person, water, raw shellfish	Person-to-person, water, cold foods, raw shellfish	Person-to-person, water, cold foods, raw shellfish
Disease pattern	Endemic	Endemic	Endemic, outbreaks	Outbreaks, endemic	Endemic, outbreaks
Seasonality	Winter	No	Winter	No	No
Clinical characteristics	Dehydrating diarrhea; vomiting and fever very common	Prolonged diarrhea; vomiting and fever	Watery diarrhea, usually short	Acute vomiting, diarrhea, fever, myalgia, headache, usually short	Rotavirus-like illness in children
Prodrome (days)	2	3–10	1–2	1–2	1–3
Duration of illness (days)	3–8	>7	1–4	0.5–2.5	4
Outpatient prevalence (%)	5–10	4–8	7–8	10–25 endemic, 90 outbreaks	1–10
Inpatient prevalence (%)	35–40	5–20	3–5	Rare	3–5

diagnosis of RVs and enteric adenoviruses. Recent cloning and expression of the viral genomes of most gastroenteritis viruses have made reagents more available for IAs to detect the other agents.

Viral culture is tedious but still an essential technique because it allows characterization of the viruses, study of their pathogenicity and the production of diagnostic reagents. Rotaviruses, adenoviruses and astroviruses can be cultured whereas caliciviruses cannot.

Reverse transcription polymerase chain reaction (RT-PCR) detects and amplifies genetic sequences specific to each pathogen, including unculturable ones. This technique has now been successfully applied to the detection of most gastroenteritis viruses. Other diagnostic tools are polyacrylamide gel electrophoresis (PAGE) and

restriction endonuclease digestion, which can be used to identify RVs or enteric adenoviruses, respectively, on the basis of their characteristic electrophoretic pattern. Finally, dot blot hybridization with complementary DNA (cDNA) has been used to identify adenoviruses and astroviruses. For diagnostic purposes, stool specimens can be stored at 39.2°F (4°C) for 1 week; for longer storage they should be frozen at -70°C. For serologic diagnosis, acute and convalescent specimens are required. An overview of the procedures can be found elsewhere.[5]

ROTAVIRUSES

Identified by Bishop *et al.* in 1973,[6] human RVs have emerged as the main agent of acute gastroenteritis in infants and young children worldwide.

NATURE

Rotaviruses belong to the family Reoviridae. Intact virions measure 70–75nm with a triple-shelled capsid composed of an outer layer (outer capsid), an intermediate layer (inner capsid) and an inner layer (core). Sixty spike-like structures (capsomers) radiating from the inner to the outer capsid, give the virus its characteristic EM appearance (Fig. 211.1) and its name ('rota' is Latin for 'wheel'). Single-shelled particles (55nm) and cores (37nm) can also be seen. Of the three forms, only intact virions are infectious. The core encloses the viral genome, which consists of 11 segments of double-stranded RNA (dsRNA). Each segment encodes for at least one protein: six structural proteins (VP1, VP2, VP3, VP4, VP6 and VP7)

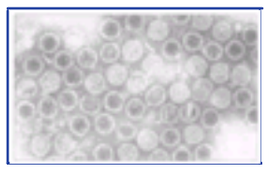

Figure 211-1 Rotavirus. Electron micrograph. *Courtesy of S Spangenberger.*

which are present in the virion; and six nonstructural proteins (NSP1 through NSP6) which are identified only in the cytoplasm of infected cells (Table 211.6). The inner core is made of VP1, VP2 and VP3, with VP2 being the major constituent. VP6 is the only component of the inner capsid. The outer capsid shell is composed of two proteins: VP7 makes about 90% of the outer capsid and is perforated by 132 channels penetrating the virion and reaching the central core; VP4 forms the capsomers, which protrude from the virus surface, pass through VP7 and interact with VP6 (Fig. 211.2).

Based on antigenic specificities, RVs are classified in groups, subgroups and serotypes. Groups and subgroups are determined by VP6. To date seven groups (A–G) and two subgroups (I and II) have been described. Groups A, B and C can infect humans and animals, whereas groups D–G have been found only in animals. Most human infections are caused by group A RVs. Subgroup II is more frequent than subgroup I; however, significant geographic variations occur. The outer capsid proteins VP4 and VP7 determine the serotypes. Those defined by VP7, a glycoprotein, are called G serotypes and those defined by VP4, a protease sensitive protein, are called P serotypes. At least 14 G serotypes (G1–G14) have been described; G1–G4 produce most human infections; however, G8, G9 and G12 infections have also been recognized.[7] [8] Thirteen VP4 serotypes (P1–P13)

TABLE 211-6 -- Rotavirus genome segments and their corresponding viral proteins.*

ROTAVIRUSES' VIRAL PROTEINS			
Genome segment	**Protein product**	**Molecular weight**	**Function/property**
1	VP1	125,000	RNA polymerase
2	VP2	94,000	RNA binding
3	VP3	88,000	Guanylyltransferase, methyltransferase
4	VP4	88,000	Hemagglutinin, neutralization antigen, infectivity
	VP5* and VP8*	529 and 247	Cleavage products of VP4
5	NSP1	53,000	RNA binding
6	VP6	41,000	Subgroup antigen, protection?
7	NSP3	34,000	Binds viral mRNA, inhibits host translation
8	NSP2	35,000	RNA binding
9	VP7	38,000	Neutralization antigen, Ca^{2+} binding protection
10	NSP4	28,000	Enterotoxin, protection?
11	NSP5	26,000	RNA binding, protein kinase, interacts with NSP2 and NSP6
	NSP6	12,000	Interacts with NSP5

VP, viral structural protein; NS, viral nonstructural protein.

** Modified from Estes 2001.*[15]

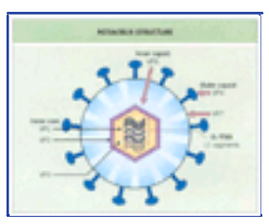

Figure 211-2 Rotavirus structure. Inner core is made of VP1, VP2 and VP3; inner capsid is made of VP6; outer capsid is made of VP7 and VP4. *Modified from Kapikian 2001.*[9]

have been identified; P1, P2, and P5 include two distinct subtypes named: P1A, P1B; P2A, P2B; P5A, P5B.

Nucleic acid hybridization and sequence analysis of the VP4 and VP7 genes permit classification of RVs according to genotypes. There is full concordance between RVs VP7 serotypes and genotypes; thus, RVs classified by either method are simply designated as G1, G2, etc. On the other hand there is poor concordance between VP4 genotypes and VP4 serotypes. For example, 21 VP4 genotypes have been described, and five of them do not have a corresponding serotype; also, the assigned numbers for serotype and genotype classification are not the same. To integrate both classifications, proper designation uses the letter P followed by an open Arabic number to indicate the serotype, and a second Arabic number in brackets to indicate the genotype.[9] For example the human RV strain Wa is designated P1A[8].

EPIDEMIOLOGY

Rotaviruses are the most commonly identified viral enteropathogens among children. In the USA RVs cause 5–10% of all diarrheal episodes and 30–50% of severe diarrhea in children under 5 years of age, resulting in 3.5 million diarrhea cases, 55,000 hospitalizations, 20–40 deaths and a cost in excess of one billion dollars.[10] Worldwide estimates are 130 million episodes, 18 million moderate or severe cases and almost one million deaths annually. The proportion of diarrhea cases caused by RVs decreases from hospital to clinic to community populations, in developed and developing countries (38–89, 10–34 and 6–12%, and 20–46, 10–30 and 10–20%,

respectively), reflecting the tendency of the virus to produce dehydration. Also, RVs account for a larger proportion of cases in developed countries than in developing ones (38–89 versus 20–46%, respectively) and in high-income groups than low-income ones (60 versus 4–30%, respectively).[7]

Rotavirus gastroenteritis is primarily a disease of infants and young children, with peak incidence rates at 6–24 months; it occurs at earlier ages in developing countries. Neonates and adults are affected infrequently unless they are exposed to infected children, in which case 11–70% can become infected. Outbreaks have been described in nursing homes for the elderly, hospital wards and military bases. Travelers' diarrhea is most commonly caused by enterotoxigenic *E. coli*, but RVs have been detected in as many as 20% of these cases. Asymptomatic shedding of RVs occurs in 10–15% of individuals.[7]

In temperate climates RVs appear in characteristic and predictable winter epidemics. In North America, the epidemic starts in Mexico and the southwestern USA in late fall, spreads in a northeast direction and ends in the northeastern USA and the Maritime provinces of Canada in the spring. The reasons for this spread pattern are not clear, but climate, virus characteristics or other factors may play a role.[11] In tropical climates RVs are endemic throughout the year, with some clustering in the cooler, drier months. Group A, serotype G1 strains are most commonly implicated in human disease (54%); other serotypes — G2 (18%), G3 (12%) and G4 (11%) are also seen. Circulating serotypes vary with geographic location and with time. Multiple serotypes can co-circulate during a specific year.[7] Group B RVs have been associated with epidemics of diarrhea among adults in China. Group C RVs have been identified in Central and South America, Europe, Australia and Asia.[7] [12]

Pathogenicity

Person-to-person transmission through the fecal-oral route is the most likely mode of spread of RVs. Fecal excretion starts immediately

before the onset of symptoms and lasts for 5–7 days. The large number of viral particles excreted in feces (approximately one trillion per ml) and the low infective dose (as few as ten RV particles in a child) favors patient-to-patient spread. Contamination of food and water has been implicated in some outbreaks, and fomites may play a role in settings such as day care centers and nurseries. Rotaviruses can survive for 60 days on environmental surfaces at different temperatures (39.2–68°F; 4–20°C) and humidities (50–90%).[13] Respiratory transmission has been suspected in two outbreaks and is supported by the way the annual RV epidemic spreads in North America. However, attempts to isolate RV from respiratory secretions have been mostly unsuccessful.

The virus preferentially infects the mature enterocytes in the villus epithelium of the small intestine. The infected cells change from columnar to cuboidal, with enlarged cisternae of the endoplasmic reticulum and fewer and shorter microvilli. The cells are eventually killed and sloughed off and, with denudation of the tip cells, the villi become shortened. Mononuclear leukocyte infiltration is minimal. These changes occur within 24 hours of infection, start proximally and progress caudally. The major mechanism of diarrhea during RV infections appears to be decreased absorption of salt and water secondary to enterocyte damage and replacement of absorptive intestinal cells by secreting cells from the crypts. Loss of disaccharidases at the damaged brush border results in carbohydrate malabsorption and osmotic diarrhea. The observation of RV diarrhea in the absence of epithelial lesions led to the discovery of a calcium-dependent signal by NSP4 that increases plasma membrane chloride permeability, leading to chloride secretion and secretory diarrhea early in the course of the illness. NSP4 is the first described viral enterotoxin. It is also postulated that RV induces intestinal fluid and electrolyte secretion by activation of the enteric nervous system. [14]

The factors that determine RV pathogenicity are not fully understood. Rotaviruses have tissue and cell-type specific tropism, suggesting the presence of receptors that mediate virus attachment or penetration. The identity of these receptors remain elusive, though. *N*-acetylneuraminic acid (sialic acid: SA) on the surface of the cells was thought to be required for virus binding; however, most RV strains are SA independent. GM1 gangliosides and cellular integrins have also been postulated as RV receptors. The VP4 spike protein mediates viral attachment to the target cells, and its cleavage into VP5* and VP8* by proteases like trypsin is essential for cell penetration. Cell penetration requires also VP7.

Two mechanisms of penetration have been proposed: direct membrane penetration and receptor-mediated endocytosis. Direct membrane penetration appears to be mediated by VP5*. Receptor-mediated endocytosis is supported by EM studies that show uptake of virus particles into coated pits, vesicles and lysosomes. Uncoating of the virus in the enterocytes is mediated by low Ca^{2+} concentrations in the cytoplasm. The next steps of RV replication have been extensively reviewed elsewhere.[15] Host factors, such as age, also influence the pathogenesis of RVs. For example symptoms are more prominent among young hosts. Although animal studies have shown that the quantity of RV-binding receptors on villus epithelial cells decreases with age, a more likely explanation for the age difference in the manifestations of the disease is the acquisition of immunity. Nutritional deficiencies, certain immunodeficiencies or co-infection with bacterial pathogens can increase the severity and duration of RV diarrhea; these situations frequently coexist in the developing world.

The mechanisms underlying immunity against RV infections and illness are not completely understood. Clinical protection may involve local (mucosal) and systemic (serum) antibodies as well as cellular immunity. Serum-neutralizing antibodies against the infecting serotype (homotypic) develop frequently and within 2 weeks of infection. Heterotypic responses (antibodies against different serotypes) also occur, but mostly among adults and vary with the infecting strain; for example G2 produces mainly homotypic antibodies, whereas G1, G3 and G4 produce homotypic and heterotypic responses. Studies have shown a correlation between the presence of homotypic neutralizing antibodies and protection. The duration of homotypic protection is probably longer than that of heterotypic; however, it is both incomplete and short-lived, as shown by the occurrence of reinfections with the same serotype. The role of serum IgM antibodies is unclear. By 3 years of age over 80% of the population has antibodies against RVs, and by 4 years of age this is practically 100%. Antibody levels are high at birth, decline by 3–6 months, rise to a peak at 2–3 years and remain elevated throughout life (probably because of repeat, mostly asymptomatic infections).[7] [12] These serum antibodies do not always prevent the infection, though. Mucosal immunity appears to be more important; it develops 4 weeks after the illness and persists for several months, eventually decreasing with advancing age.[16] Passively acquired mucosal immunity by breast-feeding or orally administered immune globulins has conferred protection to high-risk individuals. Cell-mediated immunity also seems to be important. In mice, RV-specific cytotoxic T cells appear in the intestinal mucosa soon after infection, and mice with severe combined immunodeficiency are able to clear RV infection when reconstituted with CD8T cells, despite their lack of antibodies against the virus.[17]

PREVENTION

Breast-feeding reduces the overall incidence of diarrhea, especially in developing countries. However, its role in the prevention of RV diarrhea has been questioned. Children who have RV diarrhea are as likely to have been breast-fed as those who have non-RV diarrhea. However, the severity and duration of illness might be decreased in breast-fed children.[18]

Strict adherence to enteric precautions and careful handwashing is important to reduce transmission of the virus. Fecal contamination of surfaces and objects occurs frequently in nurseries, pediatric and geriatric wards and day care centers, and, as RVs can survive in the environment for weeks, they should be thoroughly disinfected. Effective disinfectants are 6% hydrogen peroxide, 2500ppm chlorine, 80% ethanol, ethanophenolic disinfectants, ultraviolet radiation and heat; drying and phenolic disinfectants are not effective;[13] hypochlorites are inactivated by fecal organic matter. Nondisposable containers are better cleaned by washing at 176°F (80°C) for at least 1 minute.

The search for an efficacious and safe RV vaccine started 20 years ago and still continues. Multiple vaccine candidates from animal (e.g. bovine, simian) and human (e.g. nursery strains, cold adapted) strains, as well as animal-human reassortants (rhesus-human, bovine-human) have been evaluated in both developed and developing countries with variable results (Table 211.7). The first — and so far only — licensed RV vaccine was RRV-TV. This was a rhesus-human reassortant vaccine containing a mixture of strains with specificities for the four common human RV G serotypes (i.e. G1–G4). Three doses of 4×10^5 PFU of RRV-TV given orally at 2, 4 and 6 months of age showed a protective efficacy of 49% for all RV diarrhea and 80% for severe RV diarrhea, decreasing the need for physician intervention by 73% and basically eliminating all cases of RV dehydration.[19] With these results RRV-TV was licensed for use in the USA in 1998 and was incorporated in the routine immunization schedule. Unfortunately, less than 1 year later the US Centers for Disease Control and Prevention (CDC) suspended the use of this vaccine because of its association with intussusception. Intussusception is a form of intestinal obstruction that normally occurs in 1/2000 children; the risk attributable to the vaccine was estimated as one additional

TABLE 211-7 -- Candidate rotavirus vaccines tested in children.

CANDIDATE ROTAVIRUS VACCINES		
Source	**Strain**	**Specificity**
Animal		

Bovine	RIT4237	G6 P6
	WC3	G6 P5
Rhesus	RRV	G3 P3
Human	M37	G1
	RV3	G3
	89–12	G1 P8
Animal-human reassortant		
Rhesus-human	RRV-TV	G1–G4 P3
Bovine-human	WC3-QV	G1–G4 P1

case/10,000 infants vaccinated, and was most pronounced during the week following administration of the first dose of vaccine.[20] The mechanism by which RRV-TV caused intussusception is unknown at this point. New candidate reassortant vaccines using other parent strains (e.g. bovine) are currently undergoing clinical testing. In addition, nonreplicating RV vaccines have been developed using whole virions, empty capsids, vector-expressed recombinant proteins and cell culture-derived or synthetic peptides. However, the immunogenically important antigens to be included in these products have not yet been defined. Passive immunization and inhibitors of viral replication are also under study.

DIAGNOSTIC MICROBIOLOGY

Antigen detection kits based on enzyme immunoassays (EIAs) and the latex agglutination test are commercially available. They are relatively inexpensive and permit a rapid diagnosis with high sensitivity and specificity (70–100%). Newborns and breast-feeding children might have higher false-positive rates. Samples should be obtained during the symptomatic period to optimize the performance of the test. If samples are not to be processed immediately, they can be stored at 39.2°F (4°C) or frozen. Rotavirus can also be identified by its characteristic appearance on EM; this is especially useful for strains other than group A. The technique is very specific, but it is available only in centers with substantial resources.

Characteristic migration patterns of the RV genome can be detected by PAGE, with good sensitivity (>90%) and specificity (100%). Other diagnostic methods include hybridization of radiolabeled nucleic acid probes to the viral RNA and amplification by PCR. Both are more sensitive than the antigen detection techniques and at least equally specific. Rotavirus can also be cultured in some research centers.

Clinical manifestations

The clinical spectrum of RV infections ranges from asymptomatic to severe disease with dehydration and death. The incubation period is usually less than 48 hours (range 1–7 days). The clinical picture of children attending health care facilities includes:

- vomiting (60–70%), which occurs early in the illness, often as the initial symptom;
- watery diarrhea (96%) without blood or mucus, usually 3–8 stools per day, mean duration 3–4 (up to 10) days;
- fever (60–65%) usually moderate (101.3–103.1°F; 38.5–39.5°C) but can be higher if associated with significant dehydration; and
- abdominal pain.

The disease is self-limiting; the usual total duration is 6–7 days. In severe cases death may occur through dehydration and electrolyte imbalance.[4] [6] [12] Repeated or sequential infections by the same or different serotypes have been documented. Chronic infection has not been described in the normal host.[7] Neonatal RV infections are infrequent, symptomatic in less than 10–20% of cases, and usually mild; severe infections may occur among premature infants and in special care units.

MANAGEMENT

In the management of RV infections, attention should be focused on the prevention or treatment of dehydration, for which ORS should suffice in most cases. A small percentage of patients will present with severe dehydration in shock or coma, and will need intravenous rehydration. Oral immune globulin and colostrum or human milk containing RV antibodies have been used in the treatment of RV diarrhea with good results. The anti-RV titer is low in human preparations, but higher in colostrum from cows immunized against RV. This form of therapy might prove useful for immunocompromised patients and for those with chronic or severe disease. Also, formulas supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduce the incidence of diarrhea and RV shedding in infected children.[21] Interference with the overgrowth of bacteria and promotion of the intestinal immune response to RV have been suggested as the possible mechanisms.

CALICIVIRUSES (NOROVIRUS AND SAPOVIRUS)

When visualized in 1972 by Kapikian *et al.* [22] in specimens from an outbreak of diarrhea that occurred in 1968 in a public elementary school in Norwalk, Ohio, USA, caliciviruses became the first viral agents implicated in human gastroenteritis. After a slow start, recent improvements in detection systems have shown that caliciviruses are the most common agents of outbreaks of gastroenteritis.

NATURE

Caliciviruses are round, approximately 27–40nm diameter viruses, with a positive-sense ssRNA. Initially designated by the location where they were first identified (e.g. Norwalk, Sapporo, Snow Mountain, Hawaii), recent cloning of the Norwalk virus (NV) and other representative strains has allowed development of sensitive molecular diagnostics and improved our understanding of this family. Caliciviruses are members of the family Caliciviridae and, based on genomic organization, the human enteric caliciviruses are now placed into two genera:

- the Norovirus with its type species NV; and
- the Sapovirus with its type species Sapporo virus (SV).[23]

Viruses previously designated SRSVs are mostly assigned to the Noroviruses, and 'classic' human calicivirus falls into both the Noroviruses and the Sapoviruses. The NLVs are furtherly divided into two genogroups (I and II).

It is now apparent that there is great genetic diversity among caliciviruses. The Noroviruses (Fig. 211.3) are 27nm in diameter with a somewhat indistinct, rough outer edge. The Sapoviruses (Fig. 211.4) are about 35nm in diameter, and their virion surface is characterized by 32 cup-shaped (chalice-like) indentations or hollows that may give the appearance of a six-pointed star ('Star of David'). Caliciviruses are unusual among human viruses in that the virions are composed of a single major capsid protein, with a molecular weight of approximately 58–62kDa. Preliminary work suggests that the capsid protein has six distinct regions, which are the determinants of structural and antigenic domains.[5]

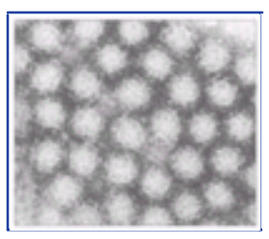

Figure 211-3 Norovirus. Electron micrograph. *Courtesy of C Humphrey, (CDC).*

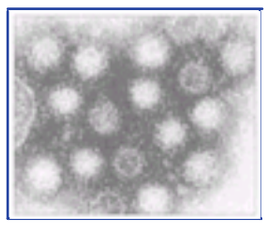

Figure 211-4 Sapovirus. Electron micrograph. *Courtesy of C Humphrey, (CDC).*

EPIDEMIOLOGY

During the 1970s and 1980s limited knowledge of the epidemiology of calicivirus infection emerged when EM, IEM and first generation EIAs were applied to the study of outbreaks of gastroenteritis and experimental infection of volunteers. Nowadays this knowledge is quickly expanding with the introduction of EIAs based on recombinant virus-like particles (VLPs) and the use of RT-PCR.[24] While studies in the 1980s could identify caliciviruses in only 19% of outbreaks in the USA, more recent evaluation of cases of nonbacterial gastroenteritis occurring in the 1990s have identified Noroviruses in 86 (95%) of 90 outbreaks.[25] Caliciviruses are now considered the most common cause of nonbacterial gastroenteritis outbreaks.

The epidemiology and clinical picture of Noroviruses and Sapoviruses are different. Even though there are year-to-year and geographic variations, in general Noroviruses are more frequent than Sapoviruses. Sapporo virus was identified in Sapporo, Japan, as the cause of an outbreak of gastroenteritis in a home for infants. [26] Since, Sapoviruses have been described mainly in young children (e.g. in day care centers and pediatric hospital wards) and less often they have been found in outbreaks affecting adults. The Noroviruses on the other hand infect patients of all ages. The original Norwalk epidemic affected an elementary school with 50% of teachers and students developing gastroenteritis and a secondary attack rate of 32% among household members. Application of newer molecular diagnostics have indicated that besides adults, as previously recognized, children are also frequently affected by Noroviruses. For example, Pang *et al.*[27] found that Noroviruses were implicated in 20% of cases (second only to RV) of non-outbreak, community-acquired gastroenteritis in children in Finland (Sapoviruses were found in 9% of the specimens). Noroviruses outbreaks occur all year round, but recent data suggest a winter peak.

Early outbreaks of Norovirus gastroenteritis were traced to fecally contaminated food or water. Settings where outbreaks have occurred include nursing homes (43%), restaurants (26%), schools (11%) and vacation settings (11%). A mode of transmission was sought in 51 outbreaks, and of these, food was implicated in 37%, person-to-person contact in 20%, consumption of oysters in 10% and water in 6%.[25] Classic caliciviruses (mainly Sapoviruses) were found responsible for 3% of diarrhea episodes in a day care center. Children or the elderly are usually implicated in these outbreaks, rarely adult carers. Some outbreaks have lasted for several weeks. The attack rates during the outbreaks have ranged from 50 to 70%, with frequent asymptomatic infection. Nosocomial outbreaks have been documented in an infant-mother unit and a pediatric hospital. Travelers are at risk for acquiring caliciviruses, although this topic has been rarely evaluated. During the Gulf War, gastroenteritis was the most common illness of soldiers, and 70% of these cases were attributable to Noroviruses.[28] Both Noroviruses and Sapoviruses have worldwide distribution.

PATHOGENICITY

Caliciviruses are ubiquitous and stable in the environment, providing a persistent source of infection. The NV is stable in water chlorinated to 10mg/l (most municipal water systems contain <5mg/l chlorine) and survive freezing and heating to 140°F (60°C), permitting spread in recreational and drinking water and contaminated oysters that have been steamed. The virus is ether-stable, acid-resistant and relatively heat-stable.[22] Asymptomatic shedding of Noroviruses can persist for over 1 week. Food handlers may be an important source of infection. Air-borne transmission by droplets of vomit or through the movement of contaminated laundry has also been suspected. Caliciviruses recently identified in swine and cattle were found to be genetically related to the human caliciviruses, which raises the possibility of animal-to-human transmission.[24]

Very few viruses (<100) are needed for infection; therefore transmission by droplets, person-to-person contact or environmental contamination can occur. Secondary spread to close contacts is frequent. Individuals with O blood group might be more easily infected with NV, while those with B blood group might have decreased risk. Infection is spread by the fecal-oral route either directly or indirectly. Caliciviruses demonstrate great antigenic and genetic diversity providing little cross-protection, so people can be serially infected with various strains. Volunteer studies have established that infection with caliciviruses confers excellent short-term homologous protection. However, the existence of long-term protection is still to be elucidated. Studies of the immunity to caliciviruses is evolving. Serosurveys indicate that levels of antibodies specific for the NV are low during childhood, but rise rapidly during adolescence, reaching 50% by middle age. In developing countries, antibodies are acquired at an earlier age and peak incidence of illness may also occur among younger age groups than in developed nations.[29] Norovirus antibodies peak by the third

week after infection and remain elevated until approximately the sixth week, after which they decline.

Seroprevalence studies of strains now classified as Sapoviruses indicate that antibodies are acquired during early childhood. The peak acquisition occurs between 3 months and 6 years. In London, UK a serosurvey showed that over 70% of children had evidence of infection by the age of 2 years.[30] Mothers of infected infants were rarely affected, suggesting that young adults retain immunity to classic caliciviruses, although the outbreaks among the elderly suggest that immunity may wane with age.

During gastroenteritis caused by the NV the proximal small intestine is affected preferentially, with increased epithelial cell mitoses, villus shortening, crypt hypertrophy, mucosal inflammation (mononuclear cells) and malabsorption of D-xylose, lactose and fat. Adenylate cyclase levels in jejunal mucosa are not elevated (as

occurs in cholera) and fecal leukocytes are not excreted. The gastric secretion of hydrochloric acid, pepsin and intrinsic factor are unaltered, but gastric emptying is markedly delayed, which probably explains the frequent occurrence of nausea and vomiting. These findings persist for at least 4 days after clinical symptoms clear and revert to normal within 2 weeks.

PREVENTION

Handwashing, cleaning of environmental surfaces, thorough cooking of food items, boiling or disinfection of water and furlough for 2 days after resolution of symptoms of ill food handlers should prevent most calicivirus-associated outbreaks. During outbreaks, food, water (including ice) or symptomatic food handlers should be suspected as possible sources. Vigorous handwashing with soap, decontamination of surfaces, use of barriers and disposal of linen contaminated with diarrhea or vomiting should be emphasized. When a water supply is thought to be contaminated, shock chlorine concentrations (>10mg/l for 30 minutes or longer) may be helpful.[12] Vaccines against the NV using recombinant VLPs are under development.[31]

DIAGNOSTIC MICROBIOLOGY

Electron microscopy, especially IEM, were the initial means to identify caliciviruses, and they are still useful in the investigation of outbreaks. The cloning and characterization of the genome of the NV and expression of the capsid antigen in baculovirus (a virus infecting insect cells that is easier to replicate) has provided adequate and abundant reagents suitable for EIAs.[30] Since caliciviruses demonstrate great antigenic and genetic diversity, diagnostic tests need to be able to detect the range of many different virus types. EIAs are suitable for the detection of antibody in serum and antigen in stool both with good sensitivity and specificity.[32] The antibody detecting EIAs appear to be more broadly reactive; IgM and IgA detection systems are under development. As one-half of adults in the USA have pre-existing antibodies to the virus, a single specimen is insufficient to document recent infection. But, if at least one-half of affected persons in an outbreak have a fourfold rise in their antibody titers, a calicivirus can be implicated as the causative agent.[12] More recently, nucleotide primers have been developed suitable for RT-PCR to detect caliciviruses in clinical and environmental samples. Caliciviruses still can not be grown in cell culture. Despite recent advances, further work is sorely needed because current diagnostic methods are not well suited for routine clinical use.

CLINICAL MANIFESTATIONS

Norovirus gastroenteritis usually has an abrupt onset, with explosive watery diarrhea, vomiting and abdominal cramps. In the original Norwalk outbreak diarrhea occurred in 44% of cases and vomiting in 84%. These symptoms are experienced by all age groups, but diarrhea is more prevalent among adults, whereas children experience vomiting more frequently. The incubation period is 24–48 hours, and the mean duration of illness is 12–60 hours. Between 25 and 50% of affected persons also report headache, fever, chills and myalgias. Dehydration and constitutional symptoms can be seen, particularly in the elderly.[12] Of the 93 infants affected in the Sapporo outbreak, 83% had gastrointestinal symptoms, including diarrhea (95%), vomiting (44%) and fever (18%).[26] Infection with the SV is common beginning in infancy and increasing during early childhood.[33] It mimics mild rotaviral illness. The incubation period is 1–3 days, with illness lasting an average of 4 (range 1–11) days. Diarrhea (88%) and vomiting (65%) occur frequently; fever (34%) and upper respiratory symptoms (22%) are less common. In general, Sapoviruses are less common than Noroviruses, seen at younger age and cause milder symptomatology.

MANAGEMENT

No specific therapy is available for calicivirus gastroenteritis. Attention must be paid to fluid and electrolyte balance. The main focus is on the management of dehydration, which can usually be accomplished with oral rehydration.

ENTERIC ADENOVIRUSES

Described in 1975,[34] the role of enteric adenoviruses was difficult to elucidate because of the frequent and prolonged asymptomatic fecal shedding of traditional adenoviruses after respiratory infections. Current techniques distinguish the enteric adenoviruses and have identified them as important etiologic agents of viral diarrhea in both the developed and developing world in hospitalized infants and young children.

NATURE

Human adenoviruses are nonenveloped, dsDNA viruses of the family Adenoviridae (Fig. 211.5). Their size is 70–75nm, with ten structural proteins (5–120kDa). The capsid proteins are arranged as an icosahedron with 20 triangular faces and 12 vertices. Each virion contains 240 hexons and 12 pentons; each penton consists of a base and a fiber. The genus-specific antigen is located in the hexon, but because it resides in the internal part of the capsid it does not elicit protective antibodies. Type-specific antigens are located on the hexon and the fiber; being exposed on the surface of the virion, they give rise to serum-neutralizing antibodies. The fiber is a strong hemagglutinin and elicits inhibition antibodies.[35] Hemagglutination properties allow classification of adenoviruses into six subgroups: A–F. The enteric adenoviruses belong to group F and comprise mainly serotypes 40, 41 and 31. A few other serotypes (e.g. 1, 2, 3, 5, 7 and 12) have also been implicated in gastroenteritis but because they are less frequent their role is less well defined.

EPIDEMIOLOGY

Adenoviruses are identified in 4% of outpatient acute diarrheal episodes and 2–22% of those in hospitalized children.[36] Also, 1–2% of asymptomatic controls shed the virus. Outbreaks have been documented in hospitals and day care centers. In Houston, USA, adenoviruses caused ten of 131 (8%) day care center outbreaks; during the outbreaks an average of 38% of children were infected, one-half of them asymptomatically; the outbreaks lasted 7–44 (mean 24) days,[37] and secondary spread was frequent. Enteric adenovirus infections occur more frequently among children under 2 years of age (median age 12 and 19 months for type 40 and 41, respectively) but

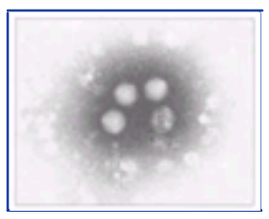

Figure 211-5 Enteric adenovirus. Electron micrograph. *Courtesy of S Spangenberger.*

older children and adults may occasionally be infected. Enteric adenoviruses types 40 and 41 circulate simultaneously and all year long with no obvious seasonality; a slight increase, especially in type 41 cases, might be seen in warmer months.

PATHOGENICITY

The mode of transmission is probably fecal-oral with spread from person to person. Food and water have not been reported as vehicles. The excretion of enteric adenoviruses in stools lasts 10–14 days, on average from 2 days before to 5 days after diarrhea stops. Asymptomatic shedding occurs frequently, but infectivity parallels symptomatic disease. Infection elicits serum-neutralizing and hemagglutination-inhibiting antibodies. Long-term immunity is thought to be acquired during childhood infection.

PREVENTION

Environmental control is important for outbreak control but has little effect on endemic transmission. Enteric adenoviruses survive temporarily in porous and nonporous environmental surfaces, but less efficiently than RVs or hepatitis A virus.[13] Infectious viruses are rapidly inactivated at 132.8°F (56°C) and by exposure to ultraviolet radiation or formalin. No vaccine is under development.

DIAGNOSTIC MICROBIOLOGY

Antigen-detection techniques using adenovirus 40 and 41 type-specific or adenovirus group monoclonal antibodies are commercially available and constitute the most convenient mode of diagnosis. They are relatively inexpensive, technically simple and quick, with a sensitivity and specificity of 98% compared with EM. Some virus variants, however, might not be detected by the assay. Its use should be restricted to diarrheal illnesses of likely viral etiology in the preschool age group or younger, because it has not been validated for other groups. A latex agglutination assay is also available with improved simplicity and speed. Electron microscopy does not discriminate between enteric and respiratory serotypes. Adenoviruses can be cultured, but the enteric serotypes are fastidious and require special cell lines, such as Graham 293 transformed lung fibroblasts or Chang conjunctival cells. The characteristic cytopathic effect generally occurs within 3–7 days but might require up to 28 days. Viral culture is not well suited for routine clinical diagnosis. PCR techniques have been developed for the identification of enteric adenoviruses in stool specimens. [38]

CLINICAL MANIFESTATIONS

Gastroenteritis caused by enteric adenoviruses is similar to that caused by RVs, but less severe and of a more prolonged course. The incubation period is 3–10 days (longer than the 1–3 days seen with other viruses) and illness typically lasts 5–12 days. Diarrhea, usually watery, is the main manifestation, occurring in 97% of patients and with a mean duration of 9 (type 40) to 12 (type 41) days. Prolonged diarrhea (>14 days) occurs in one-third of children and vomiting in 79% (lasting 1–2 days); low-grade fever and respiratory symptoms are occasionally seen. Dehydration is less frequent than with RV infection. An association with intussusception has been proposed but not proved.[39]

MANAGEMENT

No specific therapy is indicated for gastroenteritis caused by adenoviruses. Dehydration is rare and usually mild, so most episodes can be managed with oral rehydration.

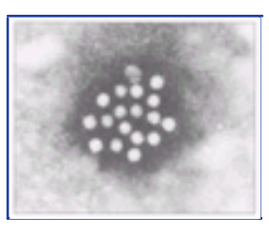

Figure 211-6 Astrovirus. Electron micrograph. *Courtesy of S Spangenberger.*

ASTROVIRUSES

Although astroviruses were well known to cause diarrhea in animals, these agents were not implicated in human gastroenteritis until 1975 when small round viruses were observed by EM in stool samples of hospitalized infants.[40] Astroviruses are now recognized as an important cause of mild gastroenteritis in young children.

NATURE

Astroviruses belong to the family Astroviridae, and are 27–32nm, nonenveloped, single-stranded (ss) RNA viruses (Fig. 211.6). They have smooth edges and characteristically a five- or six-pointed star in the center. Their surface is unbroken and rounded, unlike that of the caliciviruses, which is broken by hollows. Their three structural proteins (31–34kDa, 29–31kDa and 20–24kDa) and their distinctive morphologic and immunologic features distinguish them from caliciviruses.[5] [36] The complete sequence of a strain of human astrovirus serotype 1 has been described.

EPIDEMIOLOGY

Astrovirus infections occur mainly among children aged under 7 years, although adults can be infected and suffer mild disease. Community-based studies show a prevalence of 7–8%, and a 2% excretion rate in asymptomatic individuals. Among children hospitalized who have diarrhea, astroviruses account for 3–5% of cases. Outbreaks have been described in day care centers, hospitals, nursing homes and military facilities. Astroviruses were responsible for 7% of diarrhea outbreaks at a day care center, with about one-half of the infections being asymptomatic. The attack rate during outbreaks is about 50%, and secondary transmission to families occurs in one-third of the cases. Nosocomial outbreaks usually involve children aged under 2 years, with attack rates between 7 and 62%.[41] [42] The largest outbreak of astrovirus infection yet recorded occurred in Japan in 1991, affecting more than 4700 students and teachers in ten primary and four junior high schools; it lasted 5 days and was linked to contaminated food.[43] Sporadic and outbreak astrovirus infections are more prevalent during late fall, winter and early spring in temperate climates; in tropical climates it might be more common during the rainy season.

PATHOGENICITY

Eight human astrovirus serotypes have been identified; serotype 1 strains are the most frequent. The characteristics of immunity to astroviruses are unknown. As reported outbreaks have involved only children and the elderly, young adults may have resistance to infection. By the age of 10 years, 75% of UK children have specific antibodies, and pooled gammaglobulin preparations in the USA, prepared from adult donors, frequently contain antibodies to

astroviruses.[44] In Virginia, USA, 94 and 42% of 6–9-year-old children showed serum antibodies against astrovirus serotype 1 and 3, respectively.[45]

PREVENTION

To prevent or control outbreaks, handwashing is important, especially among food handlers and in day care centers because astroviruses may be shed asymptomatically. Adequate cooking of shellfish should prevent food-borne outbreaks; the virus is inactivated by heating to over 140°F (60°C) for at least 10 minutes. Methanol 70–90% can be used to disinfect surfaces; however, astroviruses are resistant to chloroform or alcohol.[41] No vaccine is available.

DIAGNOSTIC MICROBIOLOGY

Because astrovirus infections are usually mild and self-limiting, the diagnostic methods are not available for routine clinical diagnosis but primarily for epidemiologic studies. Electron microscopy was the only diagnostic tool until EIAs were developed using group-reactive monoclonal antibodies that detect all astroviral serotypes in stools; the sensitivity and specificity (compared with IEM) have been 91 and 96%, respectively.[42] Astroviruses can be grown in human embryonic kidney (HEK), LLC-monkey (Rhesus) kidney cells 2 (LLC-MK2) in the presence of trypsin, and other cells.[5] These systems are not well suited for routine clinical diagnosis, though. Recently, shell vial assays and latex agglutination tests have been developed, which should facilitate diagnosis. RT-PCR has been described to detect all astrovirus serotypes and seems more sensitive than EIA.[44]

CLINICAL MANIFESTATIONS

The clinical manifestations of astrovirus infection are similar to those of RV illness, but less severe. The incubation period is 3–4 days and illness lasts 1–5 days. Mild watery diarrhea is the most common symptom. Less frequent symptoms are fever (20%), vomiting (10%), dehydration (6%) and abdominal pain. Dehydration can be more prominent in children co-infected with bacterial or viral pathogens, and in immunosuppressed (including those who have HIV infection) or malnourished patients. Astroviruses have been associated with hepatitis in animals but not in humans.

MANAGEMENT

No specific therapy is indicated for gastroenteritis caused by astroviruses. Dehydration is rare and usually mild, so it can be treated with oral rehydration. In one case of protracted astrovirus diarrhea in an immunocompromised patient, the use of intravenous immune globulin was beneficial.[46]

OTHER VIRUSES

Torovirus and coronavirus are enveloped viruses, 100–150nm in diameter, with helical symmetry that belong to the family Coronaviridae. Since they do not grow reliably in cell culture, their identification is mostly limited to EM.[5] PCR techniques might hold promise.[47] Toroviruses have been associated with nosocomial out-breaks and have been implicated as the cause of acute and persistent diarrhea in children.[48] [49] Pestiviruses are ssRNA viruses classified as members of the Togaviridae family. They are well-known enteric pathogens of cattle and pigs, pleomorphic and difficult to identify by EM. In a study pestivirus antigen was detected by EIA in the stools of 23% of Arizona Indian children under 2 years of age with gastroenteritis of unknown etiology, compared with 3% of controls. Illness was relatively mild, duration was 3 days and respiratory symptoms were common.[50] Other viruses collectively referred to as small round viruses (such as the Aichi strain and others),[51] parvovirus and picobirnavirus have been identified in children who have gastroenteritis but also from controls so their role as agents of gastroenteritis is not well defined.

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Chapter 212 - Measles, Mumps and Rubella Viruses

Peter Morgan-Capner

INTRODUCTION

The skin and mucous membranes are affected in the course of many infections, bacterial and viral. The viral exanthemata occur largely in childhood, but are not uncommon in adults. The rashes are maculopapular or vesicular, the latter being clinically characteristic of hand, foot and mouth disease and varicella; smallpox has been eradicated, although sporadic outbreaks of monkeypox still occur.[1]

The erythematous rashes present major problems of differential diagnosis.[2] [3] Although there are usually associated clinical features that may be characteristic, there may be considerable overlap, such as with arthralgia in rubella and parvovirus B19, and conjunctivitis in measles and rubella. As some infections such as rubella and measles become increasingly uncommon as a result of successful immunization programs, clinicians become less familiar with their presentations and may not include them in their differential diagnosis.

Other virus infections, such as Epstein-Barr virus infection, which causes infectious mononucleosis, can give generalized rashes, as can bacterial infections such as scarlet fever associated with *Streptococcus pyogenes*, disseminated meningococcal infection and toxic shock syndrome caused by toxin-producing *Staphylococcus aureus*. In some geographic areas, a number of other viruses may present problems of differential diagnosis, such as dengue[4] and other arboviruses.

A further virus infection characteristic of childhood is mumps, although infection in young adults is not uncommon. In adolescents and adults infection may be complicated by involvement of a range of other organs such as testes, ovaries and pancreas; meningitis and encephalitis can be a complication at any age. A wide range of differential diagnoses exists, depending on the symptomatology; some of these may not be infective in origin. As for rubella and measles, infection has been well controlled in some countries by widespread use of live attenuated vaccine.

MEASLES

NATURE

Measles (rubeola) has been identified for about 2000 years, although its infectious nature was not recognized until the mid 19th century when epidemics in island communities were described. It is one of the infectious diseases targeted by the World Health Organization (WHO) for eradication because an effective and safe vaccine is available, natural infection is limited to humans, there is considerable morbidity and mortality, subclinical infection is uncommon and persistent infection is rare.

Measles virus is a member of the *Morbillivirus* genus of the Paramyxoviridae and was first isolated in cell culture in 1954; the first live attenuated vaccine became available in 1963. It is pleomorphic, with a diameter of 150nm or more. Single-stranded RNA is enclosed in a capsid of helical symmetry of 18nm. This is enclosed within an envelope, the surface of which carries the hemagglutinin and fusion proteins. Replication is mainly cytoplasmic, but there is some nuclear involvement. Although there is only one major antigenic type, there has been some genetic drift in the hemagglutinin,[5] and genome analysis can distinguish geographically distinct isolates.[6] These variations are insufficient to escape protection from immunization with current vaccines.

EPIDEMIOLOGY

Measles is endemic worldwide except in those countries in which complete control has been achieved by immunization (Fig. 212.1). Infection in childhood before the impact of immunization was almost 100%, confirming measles as the most infectious of microbial agents. Epidemics occur every 2–3 years, but are less defined in the tropics.

The epidemiology of measles has been profoundly modified by immunization strategies, which have targeted developing nations as well as the industrialized world, with worldwide eradication by 2010 being the aim of the WHO Expanded Programme on Immunization.[7] It is estimated that 1 million or so children die every year from measles, particularly in the developing world. Success has been achieved in parts of the world, however, including South America, the USA and the UK.[8]

High, but not complete, rates of measles vaccination in infancy, without achieving eradication, can lead to a change in epidemiology. Those who are not immunized or are still susceptible after immunization are not exposed to the virus during early childhood, but their continued susceptibility can lead to outbreaks in older children or young adults, as has occurred in both the USA and the UK.[8] [9] These outbreaks, which occur at an age when complications are more frequent, have necessitated mass immunization campaigns and/or the introduction of a second dose of measles vaccine at an older age.

PATHOGENICITY

Infection is spread by droplet from person to person. The incubation period to onset of rash is about 14 days, with prodromal symptoms starting 1–3 days earlier. The maximum infectivity is during the prodrome, although patients are considered to be infective from 4 days before to 4 days after onset of the rash.

After infection, initial replication occurs in the respiratory epithelium with local spread by lymphatics and a primary viremia 2–3 days after infection. A secondary viremia occurs 3–4 days later and lasts for up to 7 days. The peak viremia coincides with the prodromal symptoms. The rash results from the immunologic reaction between the virus antigens and host antibody, with involvement of capillary walls. Intrauterine infection has not been convincingly reported.

PREVENTION

Live attenuated measles vaccine became available in 1963 based on the Edmonston strain, but those in current use such as Schwarz and Moraten strains are further attenuated. Measles vaccine is available in monovalent form, but also combined with mumps and rubella

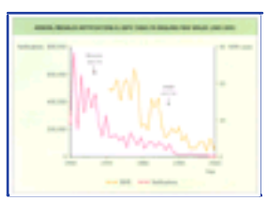

Figure 212-1 Annual measles notifications and subacute sclerosing panencephalitis (SSPE) cases in England and Wales between 1960 and 2000. The number of cases declined from the late 1960s, and there was a further decline after the introduction of MMR vaccine in 1988. *Data from the Office for National Statistics and CDSC.*

vaccine (MMR). The highest efficacy rate, over 90% protection, is achieved by giving vaccine at 12–15 months of age. Earlier administration can result in lower protection because of interference by residual maternal antibody. This is especially problematic for developing countries, where many cases of measles occur in infants under 12 months of age. Attempts to improve efficacy rates at lower ages by using high titer measles vaccines have led to an unexplained and nonspecific mortality at an older age, mainly in girls, and has led to their use being discontinued.[10] Because of a small cohort who miss first immunization, and to boost protection in those with low concentrations of antibody, many countries have introduced a second dose of vaccine at age 4–5 years.

In the patient with HIV infection who is immunocompetent, serious complications from measles vaccine are rare, although seroconversion in individuals who have HIV infection is less likely.[11] Localized pain and tenderness, mild fever (about 10%) and transient rashes (about 5%) may occur, but soon resolve. Encephalitis may occur in one in a million doses, but its causal relationship with the vaccine is uncertain. Measles vaccine is contraindicated in pregnancy because of the theoretic risk from a live vaccine, but is definitely contraindicated in those with impaired cell-mediated immunity because measles-specific complications can occur. Subacute sclerosing panencephalitis (SSPE) occurs in one dose per three million or fewer. Recently, anxieties have been expressed as to whether MMR immunization may lead to inflammatory bowel disease and autism, but the evidence is inconsistent and a direct relationship seems unlikely.[12] [13]

DIAGNOSTIC MICROBIOLOGY

Virus isolation may be attempted from throat swabs, conjunctival swabs, nasopharyngeal aspirates (NPAs) and sedimented cells from urine, but is technically demanding and unreliable. Cell lines susceptible to measles for primary isolation include primary human embryo and monkey kidney, but the cytopathic effect after inoculation is not immediately apparent because multinucleated giant cells can take up to 15 days to develop. Measles virus can also be detected by hemadsorption (monkey, human or guinea-pig red blood cells) and isolation can be confirmed by specific neutralization of the hemadsorption or by immunofluoresence.

Immunofluorescent antigen detection in NPA cells may be performed, and is particularly useful for diagnosing atypical cases or infection in the immunocompromised host. Measles genome may also be detected in cells obtained by throat swab or NPA, and this approach may also be used for determining the geographic origin of the infecting strain.[6]

Serologic diagnosis is dependent on demonstrating seroconversion, rising antibody titer, or detecting specific IgM. Hemagglutination inhibition and complement fixation testing (CFT) have been the established methods; the former detects both specific IgG and specific IgM, but these methods are now largely being replaced by immunoglobulin-specific enzyme immunoassays (EIAs). Measles hemagglutinating antigen and monkey red cells for hemagglutination inhibition have become difficult to obtain, and CFT can give elevated titers that do not indicate recent measles.

Serum should be obtained as soon as possible after onset of rash and, depending on results with this serum, further serum should be collected 10 days later. Specific IgM starts becoming undetectable about 4 weeks after onset. Diagnosis of recent measles can also be achieved by detecting specific IgG and IgM in saliva by antibody

capture techniques.[14]

Subacute sclerosing panencephalitis may be diagnosed by detection of genome or isolation of virus by co-cultivation from brain tissue. A more usual approach, however, is the detection of elevated concentrations of measles antibody in serum and concentrations indicating intrathecal synthesis in cerebrospinal fluid (CSF) by CFT; other serologic approaches may not be reliable or have not been validated for diagnosing SSPE.

For determining past measles infection, the method of choice is plaque reduction neutralization (PRN) assay, but hemagglutination inhibition and specific IgG EIA can also be used. Using PRN, a preexposure titer of >120 is associated with protection against clinically apparent measles.[15]

CLINICAL MANIFESTATIONS

Toward the end of the incubation period the prodromal symptoms of fever and malaise appear and persist for up to 4 days before the rash. Pyrexia may rise to 103–104°F (39.4–40°C) and is often

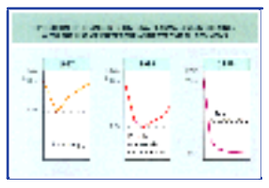

Figure 212-2 Measles. A disseminated erythematous rash can be seen over the trunk and arms.

TABLE 212-1 -- Complications of measles (% of cases).

COMPLICATIONS OF MEASLES				
Age	**USA**			**UK**
	<5 years	**5–19 years**	**>20 years**	**All ages**
Otitis media	14	2	2	5
Pneumonia	9	2	7	4
Encephalitis	0.2	0.1	0.3	0.1
Death	0.3	0.2	0.3	0.25
Hospitalization	25	8	24	1
USA data are broken down into three age groups, whereas UK data are not; however, UK data primarily reflect infection in early childhood. [16] [17]				

accompanied by conjunctivitis, cough and coryza. Koplik's spots are a pathognomonic feature of measles, and can be seen for up to 2 days before rash onset as punctate white spots on an erythematous background on the buccal mucosa.

The rash usually begins on the face and neck, and then evolves to the body and limbs (Fig. 212.2). Although lesions are usually discrete, they can coalesce, and desquamation may occur. The rash usually persists for 5–6 days. Generalized lymphadenopathy and diarrhea are common.

Complications are many, and are most common in younger and older patients (Table 212.1), and those with underlying malnutrition, as in developing countries. In industrialized countries complications are primarily respiratory or neurologic. Pneumonia, either viral or, more usually, secondary bacterial, occurs in up to 4% of patients. Neurologic complications include convulsions, in up to 1% of cases, and encephalitis, which has been reported in 1/1000 cases. Encephalitis usually starts about 1 week after onset of rash, with fever, drowsiness, convulsions, meningeal irritation, vomiting and coma: death occurs in about 15% of patients, and residual neurologic deficit occurs in a further 25%. Overall, estimates of the mortality rate for measles are 1 per 1000 patients or fewer.

Subacute sclerosing panencephalitis manifests from months to years after the initial natural measles or measles immunization; the mean interval is 7 years after natural measles and 3.3 years after immunization. The risk is less after measles immunization (1:1 million) than after natural infection (1:100,000), and is three times more common in boys than in girls. Specific risk factors for the development of SSPE have not been identified. Subacute sclerosing panencephalitis manifests as a progressive intellectual impairment, which may not be noticed for many months, although it is usually apparent in retrospect, and moves to convulsions, motor abnormalities and coma; death is inevitable after a progressive downhill course over months or years.

The immunocompromised patient with T-cell deficiencies, such as those who have leukemia or HIV, is at particular risk. A typical rash is often missing, and infection persists, manifesting as a giant cell pneumonitis or rapidly progressive encephalitis; the fatality rate is high.[18]

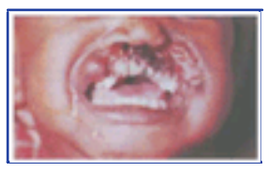

Figure 212-3 Cancrum oris. Necrosis of the upper lip.

Measles in pregnancy seems to carry a higher risk of complications but has not been associated with congenital abnormalities, although there is an increased risk of intrauterine death or premature delivery.[19]

Measles presents special problems in developing countries, where case fatality rates of up to 25% have been described. The high mortality rate is associated with malnutrition and, in particular, vitamin A deficiency.[20] Death usually results from bacterial superinfection, such as pneumonia, or diarrheal illness, although cancrum oris, a progressive oral necrosis, is also seen (Fig. 212.3).

Measles may be attenuated by recent normal immunoglobulin, for instance given after exposure, in younger infants with residual maternal antibody, and in those who have previously received live attenuated vaccine but in whom immunity is incomplete.

Now only of historic interest is 'atypical measles', seen in those immunized in the mid 1960s with a killed measles vaccine and subsequently exposed to natural infection. Various rash appearances were seen, including allergic-type, petechial or vesicular, and these could be accompanied by pneumonia, myalgia, edema and fever.

MANAGEMENT

Postexposure prophylaxis with human normal immunoglobulin is indicated for those who are susceptible and who would be at risk from complications, in particular immunocompromised children and pregnant women. Indeed, one of the greatest benefits to be had from minimizing or eradicating measles in industrialized countries is the impact on immunocompromised children, in whom measles is a substantial risk.

In outbreak situations and in immunocompetent persons, measles vaccine prophylaxis can be considered because there is a rapid development of protection. Administration should be within 3 days of contact.

Uncomplicated measles is managed symptomatically, with vitamin A supplementation for malnourished children. Antimicrobial therapy active against *Streptococcus pneumoniae* and *Haemophilus influenzae* is indicated for presumed bacterial superinfection. In those with major complications, such as pneumonitis in the

immunocompromised, specific antiviral treatment with ribavirin may be of benefit;[21] such treatment is of no value in SSPE, however.

RUBELLA

NATURE

Although rubella had been recognized since the 18th century as a distinct clinical illness, it was not until 1941 that Sir Norman

Gregg, an Australian ophthalmologist, made the association between rubella in early pregnancy and congenital abnormalities.[22] In 1962 the causative virus was isolated, leading to techniques for specific diagnosis and the development of attenuated live vaccines.

Rubella is a single-stranded RNA virus, with an icosahedral nucleocapsid surrounded by an envelope. Replication occurs in the cytoplasm of infected cells. It is the sole member of the genus *Rubivirus* within the family Togaviridae. There are three major virus polypeptides: C, E_1 and E_2 . Polypeptide E_1 is present in the envelope and has hemagglutinating properties. Only one antigenic strain is recognized, and natural infection of species other than humans has not been shown.

EPIDEMIOLOGY

Rubella occurs worldwide, although its contribution to congenital disease in the developing world has not been well quantified.[23]

In countries without an effective infant vaccination policy or a policy that targets females only, rubella remains endemic, with outbreaks in spring and early summer. Epidemics occur every 7–10 years. Although not as infectious as measles, before or without immunization approximately 80–85% of young adults would have had rubella.

PATHOGENICITY

Infection is transmitted by the air-borne route, with patients being potentially infective for up to 1 week before and after onset of the rash. After replication in the upper respiratory tract and local lymph nodes a viremia infects target organs such as skin, joints and placenta. The clinical features in postnatal rubella, such as the rash and arthralgia, primarily result from the immune response to the virus, with virus being found not only in the rash lesions but in surrounding normal skin.

If the patient is pregnant, placental infection can occur; transmission to the fetus is possible but not inevitable.[24] If fetal infection occurs in the first 16 weeks of gestation, persistence of the virus is likely. Virus may still be detectable in congenitally infected infants up to a few years of age, and high concentrations may be excreted in urine or from the throat during the first year of life. The fetal damage may be a consequence of numerous mechanisms, including slowing of cell division, disordered cell differentiation, intimal damage

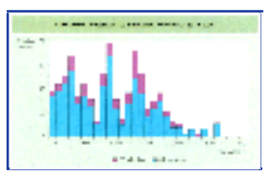

Figure 212-4 Cases of congenital rubella (CR) syndrome and infection in the UK. The number of confirmed cases was substantially reduced by the introduction of MMR vaccine in 1988. *Data from National Congenital Rubella Surveillance Programme and the PHLS Communicable Disease Surveillance Centre.*

of small blood vessels, and, at an older age, immunopathologic mechanisms.

PREVENTION

Passive prophylaxis with human normal immunoglobulin has not been proved to reduce the risk of rubella after contact or fetal infection, although it may attenuate the illness. Control of rubella has resided in using the live attenuated vaccines available since the early 1970s. The vaccine strain that has gained pre-eminence is RA27/3. It induces seroconversion in over 95% of susceptible vaccinees, and protection persists for at least 15–20 years. Vaccine virus can be isolated from the throat of vaccinees, but transmission to susceptible contacts has not been demonstrated. A mild, transient rubelliform rash illness can occur 2–3 weeks after immunization, including arthralgia, which may persist for some weeks and, rarely, some months. [25] If vaccine virus is administered to susceptible women in early pregnancy, fetal infection occurs in perhaps 1% of cases, but fetal damage has not been proved.[26] Although rubella vaccine is contraindicated in pregnancy, and conception should be avoided for 1 month after vaccine administration, if inadvertent administration in pregnancy should occur, the risk is insufficient to justify termination of pregnancy. Rubella immunization presents no significant risk to the individual who has HIV infection.

With rubella being a mild illness, control has focused on preventing infection in pregnant women. Two approaches have been used. First, as exemplified in the UK until 1988, rubella vaccine was targeted at adolescent girls and susceptible women, with rubella remaining endemic in children and men. Although cases of congenital rubella were markedly reduced, there were still 2–3% of women susceptible and at risk of rubella in pregnancy. The alternative approach, as used in the USA and in the UK from 1988, was to combine rubella vaccine with measles and mumps vaccines (MMR), and offer it to all children in the second year of life to eradicate rubella from the community. This policy was supported by identifying and immunizing susceptible women. If uptake rates of more than 90% can be obtained, control of endemic rubella is achievable (Fig. 212.4).

DIAGNOSTIC MICROBIOLOGY

With the clinical diagnosis of rubella being unreliable, laboratory confirmation is required, and is essential if a rash illness occurs in

pregnancy. In addition, in pregnancy it is imperative that contact with rash illness is investigated because subclinical rubella may occur, and this has been proved to be a risk to the fetus.[27] It is debatable whether such investigation of contacts should be pursued if there is a documented history of past detection of rubella antibody or vaccine, but vaccine is not 100% effective in inducing protection and, if there has been only one previous detection of rubella antibody, there is a remote possibility that a laboratory or labeling error could have occurred.

Rubella virus isolation has no role in diagnosing postnatal rubella because it is time-consuming, unreliable and expensive; diagnosis is serologic. The serologic response is detailed in Figure 212.5 ; tests for total rubella antibody (such as hemagglutination inhibition) have been progressively replaced with tests specific for IgG and IgM, particularly EIAs. Serum should be obtained as soon as possible after onset of illness or after contact. Detection of rubella-specific IgG, but failure to demonstrate specific IgM, indicates past primary rubella, or that the illness being investigated is not rubella, or the existence of immunity to primary rubella if it was a contact that was being investigated. If neither rubella-specific IgG nor specific IgM are detected, a later serum is needed. The incubation period after contact may be up to 21 days, and it may take up to 10 days after onset of illness for rubella antibody to be detectable. Hence in the susceptible person it will not be until about 4 weeks after contact that a subclinical illness can be excluded.

If rubella-specific IgM is detected, this is likely to indicate recent primary rubella, but care is needed in interpretation. Rubella-specific IgM reactivity persists for about 1–3 months, but may occur in a range of other infections, including those that would be differential diagnoses of rubella such as parvovirus B19 infection and Epstein-Barr virus infection (infectious mononucleosis). Rubella-specific IgM reactivity may also be nonspecific and may occur in rubella re-infection (see below). Other serologic approaches, such as determining specific IgG avidity, are of value in confirming primary rubella,[28] with specific IgG early after infection being less tightly

bound to antigen (low avidity) than the antibody found in the mature antibody response (high avidity; see Fig. 212.5).

Isolation of rubella virus in cell culture is of value in diagnosing congenital rubella because the infected infant is likely to be excreting high titers of virus in the throat and urine during the first year of life; congenitally infected infants can be highly infectious to susceptible

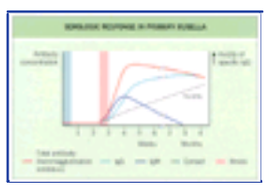

Figure 212-5 Serologic response in primary rubella. The development of specific IgG and IgM and the increasing avidity of specific IgG are illustrated.

contacts until 6 months or so of age. Rubella isolation can be performed in many cell culture lines, primary and continuous, but a cytopathic effect is only produced in a few lines, for example RK 13. Immunofluorescence is probably the best method for detecting virus growth. An alternative but less specific technique is interference, in which the cell culture is challenged with another virus such as echovirus, which will replicate with cytopathic effect in uninfected cultures but not in those in which rubella virus is growing.

The more usual approach to diagnosing congenital rubella is the detection of specific IgM; all congenitally infected infants are positive for the first 3 months of life, and most for the first 6 months.

Occasionally there may be problems with diagnosis or management of rubella in pregnancy and intrauterine diagnosis may be considered. Approaches used, but of limited availability, have included virus isolation and genome detection from amniotic fluid or trophoblast, and detection of specific IgM in fetal blood; this latter method is unreliable before 24 weeks' gestation, however, because the infected fetus may not be capable of an IgM response until that age.

In recent years assays have been developed that detect specific IgM in saliva. These assays are of limited availability, however, and although not sufficiently validated for use in pregnancy, they have been used successfully for clarifying the etiology of rashes in childhood because they are noninvasive and more acceptable for infant sampling.[14]

Irrespective of the success of population immunization and control of rubella in the community, the majority of developed countries have programs in place to identify susceptible women of childbearing age and offer immunization. Consequently, many women are screened for rubella IgG in various health care settings such as occupational health and during antenatal care. Tests for specific IgG are many and include EIAs and latex agglutination. An area of contention is the concentration of rubella-specific IgG taken to indicate protection (or, more correctly, as not necessitating immunization), with opinions varying from any confirmed antibody [possibly as low as 3–5 international units (IU)] up to 15IU; 10IU has been recommended in the USA.[29]

CLINICAL MANIFESTATIONS

In childhood, primary rubella is subclinical in perhaps 50% or more of patients, but in adolescence or older it is usually (90% or more) clinically apparent, although it may be more difficult to recognize in those with dark skin. After an incubation period of up to 21 days, usually 15–17 days, a pinkish-red maculopapular rash develops, usually starting on the face and neck but rapidly spreading over body and limbs (Fig. 212.6). Individual spots may coalesce but the rash usually clears in 3–4 days. Nonspecific symptoms, such as fever, malaise and upper respiratory tract symptoms, may precede and accompany the rash. In childhood the illness is benign and may have no systemic impact. Lymphadenopathy commonly occurs, the suboccipital nodes being frequently involved.

In adolescence and adulthood, rubella is often far more severe, not only because of complications but also because of a more severe systemic illness. Arthralgia is a frequent complication in adults, with women (30% or more) suffering more often than men. Joints frequently involved are those of the hands and wrists; resolution usually occurs in 2–4 weeks but can persist for some months or even years. Thrombocytopenia and postinfectious encephalitis are rare complications, occurring in fewer than 1/5–10,000 patients, with fatal outcome virtually unknown. Even in patients who have HIV infection and other immunocompromised patients, rubella rarely carries any additional risk, although the serologic response may be abnormal. [30]

Figure 212-6 Rubella. A pink macular rash can be seen on the forearm.

TABLE 212-2 -- Features of congenital rubella.

FEATURES OF CONGENITAL RUBELLA
Cardiovascular defects
Persistent ductus arteriosus
Pulmonary artery stenosis
Myocarditis
Ocular defects
Cataracts (unilateral or bilateral)
Pigmentary retinopathy
Micro-ophthalmus
Glaucoma
Iris hypoplasia
Auditory defects
Sensorineural deafness (unilateral or bilateral)
Central nervous system
Microcephaly
Psychomotor retardation
Meningoencephalitis
Behavioral disorders
Speech disorders
Intrauterine growth retardation
Hepatitis/hepatosplenomegaly
Thrombocytopenia, with purpura
Bone 'lesions'

Pneumonitis
Diabetes mellitus
Thyroid disorders
Progressive rubella panencephalitis
The final four features listed become apparent in infancy or later.

Primary rubella in the first 16 weeks of pregnancy presents major risks to the fetus. Consequences for the fetus and the pregnancy include abortion, miscarriage or stillbirth. If the fetus is infected and survives to term, a wide range of abnormalities may be seen (Table 212.2), some of which may be apparent at birth, but others develop later in life as a result of persistent virus infection (e.g. progressive rubella panencephalitis, late-onset deafness) or the immune response (e.g. pneumonitis). The congenital rubella triad comprises cardiac, ophthalmic and auditory lesions, but purpura, intrauterine growth and neurologic problems are also frequent.

The gestation at which the mother suffers her rubella is critical for the outcome. Onset of rubella before conception carries little, if any, risk, [31] whereas from conception to the 12th week the risk is about 90%,[32] although figures vary in different studies. Between 12 and 16 weeks' gestation the risk falls to about 20%, with sensorineural deafness being the only consequence. Beyond 16 weeks' any risk is minimal, with only rare cases of sensorineural deafness.

It has been established for many years that re-infections can occur in those with a past history of natural rubella or successful immunization. Such re-infections are rarely clinically apparent and are usually identified by a serologic response after exposure, which is usually to a close contact such as the patient's own child. Fetal infection and damage can occur after maternal re-infection, but this is rare, with the fetal risk probably being less than 5%.[33] Because of the difference in risk to the fetus, serologically distinguishing subclinical primary rubella from re-infection is critical in early pregnancy, but may be difficult in the absence of past rubella-specific IgG test results because a specific IgM response can occur in both; IgG avidity testing will usually resolve the problem because a re-infection would be characterized by high avidity.

MANAGEMENT

Rubella is a self-limiting illness that usually runs a benign course. Supportive and symptom-relieving therapy is indicated for complications such as arthralgia, and the patient can be reassured as to the unlikelihood of symptoms persisting beyond a few months.

Management of rubella in pregnancy is first dependent on achieving a correct diagnosis, given that despite some apparently characteristic symptoms and findings, such as suboccipital lymphadenopathy and arthralgia, clinical diagnosis is notoriously unreliable. A serum sample must be taken as soon as possible after contact or onset of illness, and the testing laboratory given full details of past testing and/or vaccine, and the circumstances surrounding the investigated illness/contact. On the basis of results and the clinical details, the laboratory will advise on diagnosis and any further testing required. If primary rubella or re-infection in the first 16 weeks of pregnancy is diagnosed, further management will have to take into account social, legal and religious perspectives because in many countries the risk to the fetus is sufficient to justify consideration of termination of pregnancy.

MUMPS

NATURE

Mumps was first identified as a distinct clinical illness by Hippocrates in the 5th century BC, but has only attracted attention in the past two to three centuries, particularly because of the impact of outbreaks in the armed forces. Natural infection is limited to humans and is endemic worldwide.

Mumps virus is a member of the *Paramyxovirus* genus (which also contains the respiratory paramyxoviruses) of the Paramyxoviridae family. It was first isolated in the chick embryo in 1945, with the first live attenuated vaccine being licensed in 1967. On electron microscopy it is pleomorphic (Fig. 212.7) and varies in size from 80 to 350nm, although it is usually approximately 200nm. The envelope is studded with projections containing hemagglutinin/neuraminidase or fusion proteins. Within the envelope the nucleoprotein has helical symmetry with a diameter of 15–19nm. The RNA is single stranded and of negative sense; the nucleocapsid contains an RNA-dependent RNA polymerase. This enzyme enables replication of the RNA in the cytoplasm once the cell has been penetrated. Mature virions are released from the cell by budding. There is only one serologic type although minor strain variations can be identified with monoclonal antibodies.

EPIDEMIOLOGY

Mumps is endemic worldwide, with epidemics every 2–3 years in populations not influenced by widespread use of mumps vaccine. In

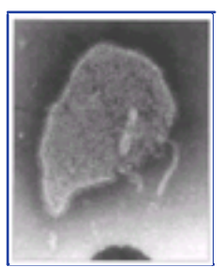

Figure 212-7 Electron micrograph of mumps virus. *Courtesy of Dr A Curry.*

the UK there was a characteristic periodicity of two epidemic years followed by one low-prevalence year (Fig. 212.8). [34] Mumps primarily occurred in 5–9 year olds. When widespread population immunization is introduced, as it was in the USA in 1977 and the UK in 1988, without eradication of the disease, susceptibility in older age groups can increase,[34] leading to a resurgence. This occurred in the USA between 1986 and 1989 in 10–19 year olds, [35] although the incidence later declined.[36] This pattern of changing epidemiology necessitated the introduction of a second dose of vaccine in the preschool or early school years.

PATHOGENICITY

Infection is acquired by droplet spread or direct contact, with initial infection most likely within the upper respiratory tract. Replication in the upper respiratory epithelium is followed by spread to local lymph nodes where further replication is followed by a primary viremia, with seeding of target organs such as parotid glands,

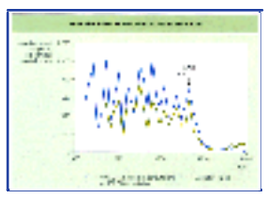

Figure 212-8 Mumps incidence reported to primary care physicians and number of laboratory diagnosed cases in England and Wales between 1962 and 2000. The number of cases declined in response to the introduction of MMR vaccine in 1988. *Data from the PHLS Communicable Disease Surveillance Centre.*

meninges and testes. A secondary viremia occurs at the onset of symptoms.

The incubation period is usually 16–18 days, with infectivity commencing 2 days or so before symptoms and persisting for 5–7 days after onset. Intrauterine infection has been reported only rarely.

PREVENTION

Two live attenuated vaccine strains have been mainly used since 1967: Jeryl Lynn and Urabe. Both are produced in chick embryo cell culture and are available in monovalent form or combined with mumps and rubella vaccine (MMR). The vaccine is usually administered as MMR at 12–15 months of age, with second doses being given later in childhood. The protective efficacy is 75–85%, with clinically apparent mumps in immunized individuals being well documented. Minor complications of mumps vaccine include mild parotitis, fever, and, very rarely, orchitis. Meningitis has been identified as a significant complication with the Urabe strain, however, and this led to its withdrawal in the UK in 1992.[34] [37]

Mumps vaccine is contraindicated in those with previous anaphylaxis to egg and in pregnancy, although adverse implications for the fetus have not been reported. It should also not be given to immunocompromised patients, with the exception of children who have HIV infection, in whom no adverse consequences have been seen.

DIAGNOSTIC MICROBIOLOGY

Virus isolation may be achieved from throat swabs, saliva, CSF and possibly urine. Mumps virus is readily isolated in monkey kidney and Hep2 cells. Although cytopathic effect may sometimes be observed (multinucleated giant cells), isolation is usually confirmed by hemadsorption with chick cells. Identification is by hemadsorption inhibition with specific neutralizing serum. Direct detection of genome can be applied to CSF, and can also be used to distinguish wild-type from vaccine virus.[37] Although feasible, rapid diagnosis by antigen detection in exfoliated upper respiratory cells by immunofluorescence is little used.

Serologic diagnosis is usually achieved by CFT or EIA; neutralization and hemagglutination inhibition testing are now little used. Complement fixation testing uses both a soluble and virion antigen.

Classically, antibody to soluble antigen appears before that to virion antigen, and virion antibody persists longer. This pattern may suggest that single serum diagnosis is feasible (elevated soluble antibody; low or elevated virion antibody), but this approach is highly unreliable because all patterns of antibody development can occur and persist.[38]

Enzyme immunoassay for specific IgG and IgM can be performed, and is relatively reliable, although care must be taken in the interpretation of IgM reactivity because of possible non-specificity. In addition, there is continuing uncertainty as to the possible significance of cross-reacting parainfluenza virus antibody. Saliva specimens can be used for specific IgM detection and are valuable for surveillance of mumps-like illness in the community.[34] For determining past, remote infection CFT has

insufficient sensitivity and EIA for specific IgG is usually used.

CLINICAL MANIFESTATIONS

Toward the end of the incubation period of 16–18 days prodromal symptoms such as pyrexia and malaise develop, to be followed in 24–48 hours by the characteristic enlarged and tender parotid glands. There is often accompanying headache and earache. Recovery is usually complete within 4–5 days. Asymptomatic mumps is common and occurs in about one-third of infections. Parotitis is present in 95% of symptomatic infections and is unilateral in about one-quarter; other salivary glands are involved in about 10% of patients. Parotitis similar to that seen with mumps may also be found in other virus infections, such as coxsackievirus B infection, and blockage of the parotid duct may on occasion give problems with diagnosis.

Complications are common;[39] the risk is the same at all ages except for orchitis and oophoritis, which are virtually limited to post puberty. Complications may manifest 1 week before, at the same time as or 2 weeks after parotid involvement; alternatively, they may manifest with no parotid involvement. The risk of orchitis in the adolescent or adult male is about 35%, with bilateral involvement in one-third of these. There may be some persisting testicular atrophy, but sterility is remarkably uncommon, despite public perceptions. Oophoritis, in contrast, is only observed in about 5% of mumps in adult women and causes lower abdominal pain; it is uncertain whether there may be long-term consequences.

Meningeal involvement, as shown by CSF changes, occurs in about 50% of patients, with signs of meningitis or meningeal irritation in about 1–10%. In about 50% of cases of mumps meningitis there is no parotitis. Characteristic findings in the CSF are a lymphocytosis, raised protein concentrations and, occasionally, a reduced glucose concentration that may lead to a misdiagnosis of early bacterial or mycobacterial meningitis. A surprising feature of the epidemiology of mumps meningitis is that it is three times more common in males than females. In countries in which mumps vaccine has not been used for community control, mumps remains a major cause of viral meningitis; enteroviruses are the other main cause and predominate where mumps has been controlled.

Mumps virus may also cause an encephalitis, presenting as impaired consciousness, fits, aphasia, etc. Two patterns of encephalitis are seen:

- direct involvement of neural tissue; and
- a postinfection encephalitis.

The incidence is about 1/6000 patients, with a mortality rate of 1.4%. Some transient impairment of hearing as a result of a labyrinthitis is not uncommon, with persisting deafness in about 1/20,000 patients. Other complications include pancreatitis, which is usually mild, arthritis, mastitis, thyroiditis and myocarditis. Investigation of renal function will often demonstrate kidney involvement, but there are no clinical consequences.

Mumps in immunocompromised patients seems to carry no undue risk, and persistent infection has not been described. Natural infection with wild-type virus usually gives lifelong protection, although re-infection may occur in 1–2% of those re-exposed.[40]

Infection in the first trimester often leads to abortion, but infection later in pregnancy carries no undue risk, and congenital infection and damage have not been convincingly shown.

MANAGEMENT

Postexposure prophylaxis with normal immunoglobulin is of no benefit, and specific mumps immunoglobulin is no longer available, although it may have reduced the risk of orchitis in adult men. Mumps vaccine administered postexposure is of no value, nor is it of any use in the acute control of outbreaks, although it may be of some use in a continuing epidemic.

Management of infection is symptomatic, with the patient being reassured that recovery occurs in a few days, even when complications are present, although the prognosis should be guarded if encephalitis arises.

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Chapter 213 - Enteroviruses: Polioviruses, Coxsackie viruses, Echoviruses and Enteroviruses 68–71

Heinz Zeichhardt
Hans-Peter Grunert

NATURE

Classification and history

Human enteroviruses comprise one genus in the family Picornaviridae, which also contains the genera rhinovirus, cardiovirus, aphthovirus, hepatovirus and parechovirus.[1] The members of the enterovirus genus that infect humans include the polioviruses, the Coxsackie virus groups A and B, the echoviruses and the enteroviruses 68–71 (Table 213.1). The formerly named enterovirus type 72 has been reclassified as hepatitis A virus in its own genus hepatovirus. Echovirus types 22 and 23 have been reclassified in the new genus parechovirus. Updated information can also be found on the website of the International Committee on Taxonomy of Viruses (www.ncbi.nlm.nih.gov/ICTV/). Human enteroviruses inhabit the alimentary tract and most of them can infect the central nervous system (CNS). In addition, enteroviruses are able to induce a broad spectrum of clinical syndromes (see Table 213.3). For additional information see: Pallansch and Roos,[2] Racaniello[3] and Zeichhardt and Grunert.[4]

The crippling paralytic disease that had been recorded in ancient times was characterized as poliomyelitis with flaccid paralysis by the German orthopedist Heine and the Swedish pediatrician Medin in the 19th century, and was therefore first described as Heine-Medin disease. Evidence for a viral origin of poliomyelitis was shown in 1908 when paralytic poliomyelitis was transmitted to monkeys.[5] The animals were infected with filtered stool from a patient with paralytic disease. It took another 40 years to replace animal inoculation studies by cell culture techniques in order to propagate viruses in primate cell lines. The establishment of in-vitro cell cultures[6] was the major breakthrough for systematic diagnosis and subsequent control of poliomyelitis by vaccination.

Animal inoculation was still the basis for isolation and characterization of another enterovirus in Coxsackie, New York, in 1948. This virus was isolated in suckling mice that had been inoculated with a cell-free filtered stool from children suffering from paralysis.[7] The virus became the first member of the group A Coxsackie viruses because it could not be neutralized by antisera against any of the three poliovirus types. The first group B Coxsackie virus was isolated in 1949.[8]

Further members of the enterovirus group were isolated and characterized by using cell cultures. The first echovirus (enteric, cytopathic, human, orphan) was discovered in 1951.[9] Echoviruses were often isolated from stools of healthy children and could not, therefore, be associated with disease. This is why these enteric viruses were called 'orphan viruses'.

Structure

Enteroviruses are small, spherical and naked RNA viruses (Fig. 213.1). Of all the enteroviruses, poliovirus is the best characterized in its structural and functional features.[3] Poliovirus has a molecular weight of 8.4×10^6 Da (156S, 1.34g/ml buoyant density in cesium chloride). An icosahedral capsid of approximately 30nm in diameter surrounds one molecule of single-stranded RNA. The capsid consists of 60 protomers, each of which contains four nonglycosylated virus proteins. The capsid proteins are virus protein (VP)1 (molecular weight 33.5kDa), VP2 (30.0kDa), VP3 (26.4kDa) and VP4 (7.4kDa).[10] Virus protein 4 is myristoylated at its *N*-terminus. Structure analysis by X-ray crystallography and biochemical accessibility studies has showed that the capsid proteins VP1, VP2 and VP3 are at the capsid surface whereas VP4 is covered inside the capsid shell and has contact to the viral RNA. Virus protein 1, VP2 and VP3 together make up a pseudoequivalent packing arrangement in the capsid, which is also typical for spherical plant viruses. Human enteroviruses therefore seem to be old viruses in evolutionary terms.

Virus protein 1 and VP3 form a depression or canyon (approximately 2.5nm deep and up to 3nm wide) that is oriented around the 5-fold symmetry axis of the capsid.[11] It is proposed that the canyon is the recognition site for the virus-specific receptor; this canyon hypothesis has also been described for the related picornavirus, human rhinovirus 14.[12] The single-stranded genomic RNA has positive-stranded sense and it codes in a single open reading frame for the four capsid proteins and additionally for functional proteins with, for example, RNA polymerase and protease activities (Fig. 213.2).

Antigenicity and neutralization

Poliovirus preparations contain two distinct antigens, the D-antigen and the C-antigen. The D-antigen is characterized by infective or 'native' virus whereas C-antigen comprises noninfective virus with properties of heated antigen. The C-antigens of the three poliovirus types are immunologically cross-reactive whereas the corresponding D-antigens react in a serotype-specific manner.

Four immunodominant antigenic sites at the surface of the poliovirus capsid determine the serotype specificity.[13] Exposed regions of the capsid proteins VP1, VP2 and VP3 compose neutralization antigenic sites at which neutralizing antibodies bind. The major antigenic sites for neutralization of Coxsackie virus type B3 are composed of surface structures of the capsid protein VP2.[14]

Some enteroviruses comprise antigenic relationships as determined by neutralization tests. Partial immunologic cross-reactivity has been observed for:

- poliovirus types 1 and 2;
- Coxsackie virus types A3 and A8, A11 and A15, and A13 and A18; and
- echovirus types 1 and 8, 6 and 30, and 12 and 29.

An increased cross-reactivity can be observed for several enteroviruses in the complement fixation test. This is due to the use of soluble antigen made up of common antigenic sites of the virus proteins that are located in the interior of the virus capsids. Immunoblot techniques confirm such immunologic cross-reactivity between different enteroviruses.[15]

Differences in the composition of the capsid surface define the serotypes of enteroviruses. There are three serotypes of poliovirus (see Table 213.1). Coxsackie virus group A comprises 23 serotypes

TABLE 213-1 -- Serotypes of human enteroviruses.

SEROTYPES OF HUMAN ENTEROVIRUSES	
Viruses	**Serotypes**
Polioviruses	1, 2, 3
Coxsackie viruses	
Group A	A1–22, A24

Group B	B1–B6
Echoviruses	1–7, 9, 11–21, 24–27, 29–33
Other enteroviruses	68–71
Echovirus 8 has been deleted because it is identical to echovirus 1. The following viruses have been reclassified: Coxsackie virus A23 as echovirus 9; echovirus 10 as reovirus type 1; echoviruses 22 and 23 as parechoviruses 1 and 2; echovirus 28 as human rhinovirus 1A; echovirus 34 as Coxsackie virus A24; enterovirus 72 as hepatitis A virus in the new genus hepatovirus. Additional information can be found at the website of the International Committee on Taxonomy of Viruses (www.ncbi.nlm.nih.gov/ICTV/). Echo, enteric cytopathic human orphan.	

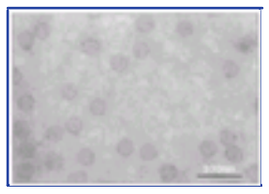

Figure 213-1 Poliovirus type 1. The particles in this electron micrograph are negatively stained with 0.5% uranyl acetate.

(A1–A22 and A24), Coxsackie virus group B has serotypes B1–B6, and the echoviruses consist of 28 serotypes (types 1–7, 9, 11–21, 24–27 and 29–33). The other human enteroviruses are the distinct serotypes 68, 69, 70 and 71.

The antigenic relationships of the enteroviruses are reflected by limited homologies among the RNA and proteins of the different viruses. Sequence identities for different enteroviruses are more than 50% over the genome as a whole. Virus strains within a species have more than 75% sequence identity over the entire genome.[1] Sequence homologies are relatively high for the functional proteins with RNA polymerase and protease activities, whereas sequence heterogeneity is observed for the sequence region coding for capsid proteins. Capsid protein homology is approximately 70% for the three poliovirus Sabin strains and approximately 50% for polioviruses and Coxsackie viruses.[3]

Reactivity to physical and chemical agents and virus stability

A prerequisite for the passage of enteroviruses through the stomach and duodenum is viral resistance to pH levels that are less than 3 as well as to several proteolytic enzymes. Because enteroviruses have no membranous envelope, they are resistant to lipid solvents (e.g. ether, chloroform and detergents). They are also resistant to several disinfectants, including 70% alcohol, 5% lysol and 1% quaternary ammonium components. Chemical inactivation can be achieved by 0.3% formic aldehyde, hydrochloric acid (0.1mol/l) and free residual chlorine (0.3–0.5 parts per million (ppm)) and other halogens (free residual bromine or iodine at a concentration of approximately 0.5ppm for 10 minutes). Because the presence of organic matter with the

Figure 213-2 Organization of the poliovirus genome. The single-stranded genomic RNA of poliovirus (2.4×10^6 Da, approximately 7500 nucleotides) has positive-stranded sense and codes in a single open reading frame for capsid and functional proteins (modified according to Racaniello[3]). The boxes represent the coding region; the lines represent the nontranslated regions (NTR) at the 5'- and 3'-termini (5'- and 3'-NTRs). A small hydrophobic protein is covalently linked to the terminal uracil of the 5'-NTR and called 'virus protein genome linked' (VPg). The 5'-NTR has a significant secondary structure and contains the initiation site for translation at nucleotide position 741 (the internal ribosome entry site). The 3'-NTR (72 nucleotides) is polyadenylated (62 nucleotides on average). The coding region of the genome is translated into a large precursor polyprotein. Region P1 codes for the capsid proteins VP0 (precursor of VP4 and VP2), VP1 and VP3. Regions P2 and P3 code for functional proteins (e.g. 2A codes for a protease, 3B codes for VPg, 3C and 3CD code for proteases, and 3D codes for the RNA polymerase). Three proteases mediate processing of the precursor proteins: protease 2A releases the P1 capsid precursor from the nascent polyprotein, and the proteases 3C and 3CD mediate most of the other cleavages before and during virus assembly. Virus assembly is completed when a single RNA molecule is surrounded by its capsid with 60 protomers, in which the precursor VP0 is then cleaved into mature VP4 and VP2. It is postulated that the viral RNA is involved in this final cleavage.

virus may result in protection against inactivation, the contact time may be prolonged. Enteroviruses can be inactivated by heat at 122°F (50°C) for 1 hour in the absence of magnesium chloride and calcium chloride and by light (in the presence of neutral red, acridine orange, proflavine and other vital dyes). Virus preparations may be stable at 39.2°F (4°C) for several days up to several weeks. At -4°F to -112°F (-20°C to -80°C), enteroviruses can be stored up to several years.

EPIDEMIOLOGY

Transmission

Humans are the only reservoir for human enteroviruses.[2] [4] Because most enteroviruses replicate in the lower or upper alimentary tract, or both, virus spread occurs by both the fecal-oral and respiratory routes. The extensive virus reproduction that occurs in the cells of the gut epithelia leads to massive virus concentrations in the feces (10^6 –10^9 infective virus particles/g feces). Viral spread in the feces can last for weeks or months. Viral spread is usually by way of fecal contamination of fingers, normal household objects (e.g. towels and toys) and food. Nosocomial transmission of enteroviruses typically takes place in newborn nurseries. Fecal-oral transmission is predominant in areas with poor sanitary conditions. Enteroviruses may be found in sewage and transmission by a fecal-water-oral route is possible. Depending on the clinical manifestations, enteroviruses can also be spread by contact infection. For example, hemorrhagic conjunctivitis caused by enterovirus 70 and a variant of Coxsackie virus A24 is effectively transmitted with conjunctival fluid. There is no evidence that enteroviruses are transmitted by sexual routes, blood transfusion or insect bites.

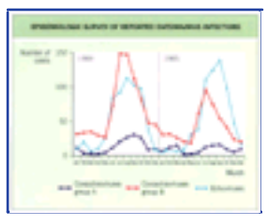

Figure 213-3 Epidemiologic survey of reported enterovirus infections. This epidemiologic survey by the German Association for Prevention of Virus Diseases (DVV) for the years 1984 and 1985 demonstrates that most infections with coxsackieviruses groups A and B and echoviruses in Germany occur during summer and autumn. *Data from Habermehl and Knocke.*

Geographic, seasonal, socio-economic, sex and age factors

Enteroviruses are distributed worldwide.[2] [4] In temperate zones, enterovirus infections typically occur during summer and autumn (Fig. 213.3);[16] in tropical and subtropical areas infections occur throughout the year. Enterovirus infections are most commonly infections of childhood and young children are the main transmitters. More than 90% of enterovirus infections cause no clinical symptoms or signs.

Poor hygiene and low socio-economic conditions increase the risk of enterovirus infections. Diseases caused by enteroviruses occur more frequently in males than females (male:female ratio of 1.5–2.5:1). The clinical signs of most enterovirus diseases are more severe in adults than in children, although newborn and young children may have severe clinical signs when infected with Coxsackie viruses and some echoviruses. For this reason, all efforts must be made to prevent nosocomial infections by these viruses in nurseries.

Epidemics of poliomyelitis frequently occurred before vaccination strategies were introduced with the inactivated Salk vaccine in 1954 and with the oral attenuated Sabin vaccine in 1962. In the prevaccine era, about 10 cases of paralysis per 100,000 population per year were recorded for the USA. The World Health Organization (WHO) still reported as many as 35,251 cases of poliomyelitis worldwide in 1988. (Statistics on global polio cases are available at www.polioeradication.org/.) There were 6349 cases reported for 1998 and only 494 cases for 2001. The WHO-led campaign aims to eradicate poliomyelitis worldwide by the first decade of the 21st century. As a result of vaccination programs, poliomyelitis has been eliminated in the Americas and in Europe. Isolated cases have been reported for The Netherlands, Canada and the USA before 1993 in enclaves of religious groups that are opposed to vaccination. Ten clinically apparent poliomyelitis cases (nine paralytic) occurred in Finland in 1984–85 owing to the occurrence of a genetically altered wild type of poliovirus type 3 against which the Finnish inactivated vaccine induced only partial

immunity. In 1996 an outbreak of paralytic poliomyelitis in Albania, Yugoslavia and Greece resulted in 167 cases of poliomyelitis with 17 deaths.

Some Coxsackie viruses and echoviruses may lead to epidemics. One of the first epidemics observed to be caused by group B Coxsackie viruses was the Bornholm disease epidemic that occurred between 1930 and 1932. The infection caused epidemic myalgia (epidemic pleurodynia) on the island of Bornholm in the Baltic Sea. In the beginning of the 1950s, echovirus 16 was responsible for the so-called Boston exanthema disease, a febrile illness with a rash predominantly occurring in Boston, Massachusetts. Echovirus 9 was responsible for a pandemic of aseptic meningitis in 1955–60. In recent years echovirus 13 outbreaks have taken place in Germany, UK and USA. Large outbreaks of acute hemorrhagic conjunctivitis have been caused by two enteroviruses: enterovirus 70 and a variant of Coxsackie virus A24. From 1969 to 1971, enterovirus 70 spread very quickly in Africa and Asia; acute hemorrhagic conjunctivitis was first recognized in Ghana and after that in other African countries, and it finally spread to Asia. In 1981, enterovirus 70 occurred in the industrialized countries of the West. In 1970–71, acute hemorrhagic conjunctivitis was caused by a variant of Coxsackie virus A24 that originated in South East Asia and was introduced to the Western hemisphere via American Samoa in 1986.

PATHOGENICITY

Virus-specific receptors

The pathogenicity mechanism of enteroviruses is largely determined by their cell tropism. This tropism depends on the specific recognition of the virus by receptors at the surface of susceptible cells (Table 213.2). Several enterovirus receptors can be grouped into receptor families. [28] [29] The receptors for the three types of poliovirus, the six types of group B Coxsackie viruses and some of the group A Coxsackie viruses are members of the immunoglobulin superfamily. The poliovirus receptor[17] [18] is a glycosylated three domain membrane protein that appears in different isoforms (molecular weight 67–80kDa). The receptor for the group B Coxsackie viruses is the coxsackievirus and adenovirus receptor (CAR), a 46kDa glycoprotein that is also used as a receptor by human adenoviruses 2 and 5.[24] Coxsackie viruses A13, A18 and A21 use intercellular adhesion molecule-1 (ICAM-1, CD54) as their receptor.[23] Intercellular adhesion molecule-1 also functions as a receptor for the major group of human rhinoviruses and recognizes as its physiologic ligand an integrin, lymphocyte function-associated molecule-1 (LFA-1), at the surface of leukocytes. The superfamily of integrins contains several enterovirus receptors. Vitronectin (a_v $ß_3$) is the receptor for Coxsackie virus A9.[22] Very late-activating antigen-2 (VLA-2) serves as receptor for echoviruses 1 and 8.[25] An additional membrane protein, decay-accelerating factor (DAF, CD55), is the receptor for echoviruses 6, 7, 12 and 21[26] as well as enterovirus 70.[27]

Further membrane proteins function as specific binding proteins for enteroviruses. They are not limiting factors for virus cell tropism, however, and they might support attachment of viruses at their host cells. Examples of these poliovirus-binding proteins are the lymphocyte-homing protein CD44[19] [20] and other glycosylated membrane proteins with low affinity for poliovirus.[21]

Viral reproduction cycle

The reproduction cycle of polioviruses is the best characterized of that of any of the enteroviruses. The early phase of the poliovirus reproduction cycle can be divided into adsorption, penetration and uncoating; the later phase can be divided into synthesis of virus-specific protein and RNA, virus assembly and virus release from the infected host cell.

Adsorption of poliovirus at the cell surface is regulated by the binding of the poliovirus receptor into the canyon at the capsid surface. The canyon hypothesis proposes that the *N*-terminal domain

TABLE 213-2 -- Enterovirus-specific receptors.

ENTEROVIRUS-SPECIFIC RECEPTORS			
Viruses	**Serotypes**	**Receptor**	**Protein family**
Polioviruses	1, 2, 3	Poliovirus receptor[17] [18]	Immunoglobulin superfamily
		Accessory factors:	
		CD44[19] [20]	
		50kDa and 23–25kDa HeLa cell membrane glycoproteins [21]	
Coxsackie viruses			
Group A	A9	Vitronectin (a_v $ß_3$)[22]	Integrin
	A13, A18, A21	Intercellular adhesion molecule-1[23]	Immunoglobulin superfamily
Group B	B1–6	Coxsackie virus and adenovirus receptor (CAR)[24]	Immunoglobulin superfamily
Echoviruses	1, 8	Very late antigen-2 (a-chain)[25]	Integrin
	6, 7, 12, 21	Decay accelerating factor (CD55)[26]	
Other enteroviruses	70	Decay accelerating factor (CD55)[27]	

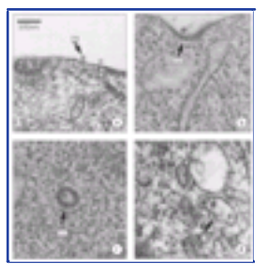

Figure 213-4 Receptor-mediated entry of poliovirus into host cells. The entry of poliovirus into HEp-2 cells is followed by transmission electron microscopy of ultrathin sections of synchronously infected cells.[31] [32] (a) Poliovirus (v) adsorbs at the cell surface immediately after infection (0 minutes after infection). (b) Beginning 1 minute after infection, poliovirus is located at areas of the cell surface that have clathrin-coated pits (cp), at which the surface membrane starts to invaginate. (c) Five minutes after infection, poliovirus is taken up by clathrin-coated vesicles (cv) into the cytoplasm. (d) Between 15 and 20 minutes after infection, poliovirus is within intracellular clathrin-free vesicles or endosomes (e), which are suggested as the sites of viral uncoating.

of the receptor interacts with the canyon, whereas other parts of the receptor are in contact with the capsid surface outside the canyon.[11] [12] [30]

Virus entry into the host cell (penetration) occurs by way of receptor-mediated endocytosis followed by a pH-dependent release of viral RNA from the virus capsid (uncoating; Fig. 213.4).[31] [32] Poliovirus is taken up via clathrin-coated pits after adsorption at the cell surface within 1–5 minutes of infection. After 15–20 minutes, poliovirus reaches acidic compartments in the cytoplasm (endosomes) via clathrin-coated vesicles. The virus capsid undergoes conformational changes during virus entry.[3] The internal capsid protein VP4 is released from the virus and the *N*-terminus of the capsid protein VP1 becomes accessible; this alteration is characterized by antigenic changes. These altered particles have a decreased sedimentation coefficient (135S) and are known as A-particles. An alternative entry mechanism has recently been proposed for poliovirus by which low pH is not required for the release of the viral genome from the capsid.[33]

After the virus has been uncoated, synthesis of viral protein and RNA is initiated by using the viral parental positive-stranded RNA as a template (Fig. 213.5). Viral protein synthesis takes place at the rough endoplasmic reticulum after release of the 'virus protein genome-linked' (VPg) from the viral genome.[13] In contrast to cellular mRNA, enteroviral mRNA has no m^7 G cap group. Initiation of viral protein synthesis takes place in the 5'-nontranslated region, which homes the internal ribosome entry site with the initiation codon AUG beginning at nucleotide position 741 (see Fig. 213.2). The polyprotein is autocatalytically processed by protease 2A via several precursor proteins into the four virus capsid proteins VP1–VP4, the virus-specific proteases 2A and 3C, and the RNA polymerases 3CD and 3D. The replication of viral RNA takes place at the smooth endoplasmic reticulum via negative-stranded RNA copies of the viral genome. During RNA synthesis, multistranded replicative

intermediates occur, from which new positive-stranded RNA is released for further translation and virus assembly.

Viral transcription is regulated by interaction of the mature RNA polymerase 3D with viral and cellular factors. Secondary structural motifs in the nontranslated regions of positive- and negative-stranded RNAs are involved in the initiation of RNA transcription. The virus morphogenesis is characterized by intermediate assembly steps of the virus capsid via precursors and a procapsid. After encapsidation of one molecule of positive-stranded RNA in the provirus, a final proteolytic cleavage of the precursor protein VP0 into VP2 and VP4 finalizes the virus maturation. It is proposed that a morphopoietic factor and smooth membranes are involved in virus maturation.

One reproduction cycle of poliovirus lasts 6–8 hours, at which time up to 10,000 virus particles have been synthesized in a single cell.[2] Light microscopy shows that infected cells are rounded and contain cytoplasmic protrusions (filopodia; Fig. 213.6a,b). [34] The nucleus is pyknotic and the chromatin is condensed. Analysis by scanning electron microscopy reveals that first changes in the cell

Figure 213-5 The reproduction cycle of poliovirus. Receptor-mediated entry of poliovirus is completed by the release of the viral RNA from the virus capsid (uncoating). The syntheses of viral protein and RNA are the next reproduction steps. The viral precursor polyprotein is autocatalytically cleaved by viral proteases, resulting in the viral RNA polymerase and, via several precursor proteins, in the virus capsid proteins (see Fig. 213.2). The viral RNA polymerase mediates viral transcription (i.e. de-novo synthesis of positive-stranded RNA via negative-stranded RNA templates). Maturation of the virus is completed by encapsidation of one molecule of positive-stranded RNA into a capsid with the complete set of proteins VP1, VP2, VP3 and VP4. RI, replicative intermediate.

surface can be observed as early as 3 hours after infection. Eight hours after infection the changes (Fig. 213.6c,d) are characterized by:[35]

- condensation of collapsed microvilli;
- formation of elongated filopodia; and
- a 'rounding up' of the cell.

The nuclear and cytoplasmic alterations are characterized by lobed nuclei with irregular distribution of condensed chromatin and vesicles arranged in clusters in the cytoplasm, as shown by transmission electron microscopy (Fig. 213.6e,f).[35] [36]

Characteristic mitotic changes and chromosomal alterations are induced by poliovirus. The mitosis is enhanced during the early stage of replication. In later stages of infection, the mitosis is arrested in the metaphase (a colchicine-like effect). Chromosomal damage is characterized by single chromatin breaks and pulverization. [37] [38] Poliovirus induces 'shut-off' of the syntheses of cellular protein, RNA and DNA within the first 2 hours of infection. The inhibition of cellular protein synthesis is caused by a proteolytic cleavage of the cellular protein p220.[39] This protein is the initiation factor eIF-4G which, as part of the cap-binding complex eIF-4F, is involved in the initiation of cellular protein synthesis mediated by capped mRNA.

PREVENTION

Vaccination

The introduction of vaccination against the three poliovirus serotypes was a prerequisite for effective prevention of poliomyelitis.[2] [4] The establishment of modern cell culture techniques [6] allowed the development of two poliovirus vaccines. Jonas Salk developed a formaldehyde-inactivated polio vaccine (IPV), which was introduced in 1954 and is administered intramuscularly. In 1962, Albert Sabin introduced an oral polio vaccine (OPV), which consists of live-attenuated viruses. Both IPV and OPV contain the three poliovirus serotypes and induce humoral immunity with circulating antibodies. In addition, OPV induces secretory IgA in the gut owing to the subclinical infection with virus multiplication that it causes in the gastrointestinal tract.

Both vaccines are administered three times several weeks apart in order to give rise to effective titers of neutralizing antibodies. Booster vaccinations are recommended every 10 years for both vaccines. In the temperate zones, it is better not to administer OPV during the summer months when there is a high frequency of other enterovirus infections. Co-infection with other enteroviruses may induce viral interference; this may also occur in the trivalent vaccine when the multiplication of one serotype of poliovirus is reduced by the other types. Such interference may be reduced by the 3-fold administration.

In very rare cases, OPV may gain neurovirulence owing to changes (often point mutations) in the genome of attenuated virus. These mutations occur not only in the genome regions that code for viral capsid proteins and the RNA polymerase but also in the 5'-nontranslated region, which is of importance for the initiation of viral protein synthesis. The Sabin type 3 polio vaccine has been observed to increase in neurovirulence because of a single base change from uracil to cytosine at position 472 in the internal ribosome entry site in the 5'-nontranslated region. [40]

The risk of developing clinical poliomyelitis, including paralysis, after live Sabin vaccination is very small; an incidence of one case of vaccine-associated paralytic poliomyelitis (VAPP) per 1.2 million doses of live vaccine administered has been reported for the USA.[2] Neurovirulent revertants occur mainly with the attenuated vaccine strains of poliovirus types 1 and 3.

All infants and young children should be vaccinated with one of the two vaccines. It is advisable that adults who have not been vaccinated as infants should be vaccinated with IPV followed by OPV. The primary IPV administration helps to prevent VAPP, which has a higher incidence in children and adults than it does in infants. Clinical personnel should be given booster vaccinations at regular intervals. In health care workers, IPV is recommended in order to prevent shedding of attenuated vaccine strains of the virus in clinical

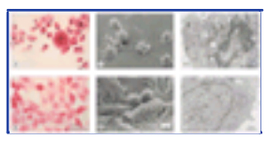

Figure 213-6 The poliovirus-induced cytopathic effect. The cytopathic effect of poliovirus type 1 in monolayers of HEp-2 cells (a, c, e) is demonstrated in comparison to noninfected control cells (b, d, f) by light microscopy, scanning electron microscopy and transmission electron microscopy. [35] (a) Light microscopy of infected cells stained with hemalum-eosin shows rounded cells with pyknotic nuclei and condensed chromatin (8 hours after infection). (b) Light microscopy of control cells. (c) Scanning electron microscopy demonstrates severe rounding of the infected cells (12 hours after infection). The infected cells are characterized by elongated filopodia and microvilli at the cell surface; these are collapsed or even lost. (d) Scanning electron microscopy of control cells. (e) Transmission electron microscopy of ultrathin sections reveals infected pyknotic cell condensation of chromatin arranged in patches in a lobed nucleus and clusters of vesicles in the cytoplasm (8 hours after infection). (f) Transmission electron microscopy of control cells.

surroundings. National health authorities in some countries where poliomyelitis has been eliminated have recently decided to administer IPV instead of OPV because, in these countries, the risk of VAPP is now greater than the risk of infection with wild-type poliovirus.

The WHO-led campaign for eradication of poliomyelitis with the expanded programs of immunization led to a 90% decrease in poliomyelitis worldwide in the years 1988–97.[41] In 2001 only 494 confirmed cases of poliomyelitis were reported. In the Americas and in Europe poliomyelitis is eliminated. Before 1993, very rare local cases of poliomyelitis in Canada and The Netherlands were introduced in ethnic groups refusing vaccination. A prerequisite for global eradication of poliomyelitis is the maintenance of the present high levels of vaccination in developed countries and the induction of immunity in the population of developing countries. For this purpose, national immunization days are performed worldwide. In 1996 on worldwide co-ordinated national immunization days, 420 million children (approximately two-thirds of the world's children under the age of 5 years) were vaccinated against poliomyelitis. The aim is the eradication of poliomyelitis in the first decade of the 21st century.[41]

Passive immunization and hygienic prevention

In contrast to infection caused by polioviruses, infections caused by Coxsackie viruses, echoviruses and enteroviruses 68–71 presently cannot be prevented by vaccination. However, interruption of the routes of virus transmission and prophylactic passive immunization are effective in the prevention of enterovirus infections. Serum that contains antibodies against polioviruses, Coxsackie viruses and echoviruses obtained from convalescing patients can be effective in preventing infections in seronegative patients when administered within 72 hours of exposure. Immunoglobulins can prevent poliovirus infections as well as Coxsackie virus and echovirus infections, which can induce 'fulminant viral sepsis', myocarditis, encephalitis and death in newborn babies when transmitted in neonatal wards.

Nosocomial transmission of enteroviruses is often caused by lack of normal hygienic conditions. Clinical personnel can prevent nosocomial transmission of enteroviruses by obeying hygiene rules and especially by changing coat and gloves between patients and hygienic hand rub and hand washing. Proper removal of feces, including diapers, is of major importance.

Patients infected with poliovirus are not considered to be highly contagious; nevertheless, they should be isolated because of the drastic clinical consequences of infection.

Antiviral chemotherapy

Effective antiviral chemotherapy against enterovirus infections does not yet exist, with the exception of the newly developed antiviral compound pleconaril. Pleconaril shows antiviral activity against different picornaviruses and seems to be a promising new drug candidate for the therapy of enteroviral meningitis. All other antiviral compounds against enteroviruses have currently been proven to show antiviral activity only in cell culture systems. Guanidine and some benzimidazoles specifically inhibit the viral RNA polymerization. Hydrophobic compounds intercalating in the viral capsid protein VP1 stabilize the virus, resulting in inhibition of viral uncoating.[3] [4] [42]

DIAGNOSTIC VIROLOGY

The laboratory diagnosis of enterovirus infections is based on virus detection, including virus isolation and identification, and serologic diagnosis. [2] [43]

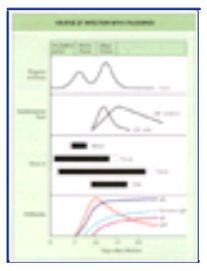

Figure 213-7 The course of infection with poliovirus.

Collection and preparation of specimens

Specimens for virus isolation and identification

Successful virus isolation from clinical specimens depends on proper collection and is related to the time since infection (Fig. 213.7) In general, virus specimens should be collected as soon as possible after onset of clinical symptoms. The specimens should preferably be transported to the laboratory under cooled conditions. If virus isolation cannot be performed immediately after receipt the specimens should be stored at -4°F (-20°C).

Owing to the pathogenesis of enteroviruses with their enteric multiplication and clinical manifestation involving the CNS, the most useful specimens for virus isolation are stools and rectal swabs, throat swabs and washings, and cerebrospinal fluid (CSF). Most enteroviruses can be isolated most effectively from the throat up to 15 days after infection or longer, and from stools and rectal swabs up to 1 month after infection or longer. When there are symptoms involving the CNS, virus isolation is usually successful from the CSF 2–3 weeks after infection. Stools are preferred for virus isolation because virus concentrations in feces are very high (up to 10^6 –10^9 virus particles/g of feces) compared with virus concentrations in other specimens.

In addition, all specimens from target organs allow virus isolation if a biopsy or autopsy specimen is taken during the clinical manifestation of disease. Viruses inducing vesicular rashes (such as Coxsackie viruses types A4, A6, A9, A10 and A16, and enterovirus type 71) can be isolated from the lesions. A Coxsackie virus A24 variant and enterovirus 70 may be responsible for hemorrhagic conjunctivitis and echovirus types 7 and 11 induce conjunctivitis; these viruses can be isolated from ocular samples, either conjunctival swabs or scrapings.

For selected diagnostic purposes, biopsy or autopsy material from the heart, muscle or brain may be successful for isolation of the virus. Autopsy specimens should be collected as soon as possible after death under sterile conditions in order to prevent virus degradation. Depending on the target organ, the order of collection should be CNS or lymph node specimens, specimens from the thorax, specimens from muscles, liver or glands, and finally specimens from peritoneum and gastrointestinal tract. For diagnosing clinical manifestation in the CNS, samples from the pons and medulla oblongata, as well as spinal cord and CSF specimens, are especially likely to yield successful isolation. A planned order of tissue collection is emphasized so that contamination from gastrointestinal contents is avoided.

Virus isolation from blood is successful only during viremia (days 6–9 after infection; see Fig. 213.7) and is therefore not generally attempted because of the short period for collection.

In rare cases, enteroviruses other than poliovirus can be isolated from urine.

Specimens for serologic diagnosis

Detection of virus-specific antibodies should be performed with at least two serum samples. The first, acute-phase serum, should be obtained as soon as possible after the onset of illness. The second, convalescent-phase serum, is usually collected 2–3 weeks later in order to monitor any increase in antibody titer. If there are CNS manifestations, antibody detection in CSF can be useful. Serum and CSF that are free of additives should be used for serologic diagnosis.

Virus isolation and identification

Virus propagation in cell cultures and animals

Virus growth in cell cultures is the preferred method for virus isolation. The human cells that are usually used for propagation of enteroviruses are primary embryonic fibroblasts of the skin and lung, permanent fibroblasts (e.g. MRC-5 cells), permanent amnion cells (e.g. FL cells), and transformed cells such as HeLa, HEp-2 and KB cells. The monkey cells that are most frequently used are primary rhesus or African green monkey kidney cells or permanent monkey kidney cells (e.g. BGM and Vero cells). These standardized cell lines for virus propagation can be obtained from national or international reference institutions (e.g. American Type Culture Collection).

Several types of group A Coxsackie viruses have exceptional growth behavior. Some of these viruses replicate only in a human rhabdomyosarcoma cell line or in newborn mice. Newborn mice are required to isolate Coxsackie virus types A1, A19 and A22.[2] Virus isolation in mice, however, is nowadays restricted to a few reference laboratories worldwide. It should be mentioned that, before the introduction of cell cultures for virus isolation, pathologic lesions in monkeys induced by polioviruses and in mice induced by Coxsackie viruses of groups A and B were the only tools for virus isolation.

Wild-type polioviruses have a tropism that is restricted to primates. Most other enteroviruses, however, also infect laboratory animals such as mice. In order to distinguish between group A and group B Coxsackie viruses before the introduction of cell culture techniques, pathologic lesions in mice were used. In newborn mice, Coxsackie group A viruses cause generalized myositis accompanied by flaccid paralysis. Group A viruses rarely induce CNS alterations. Typically, group B viruses

cause focal myositis and lesions in the brain and interscapular fat pad; myocarditis, endocarditis, hepatitis and pancreatitis may also occur.

Virus neutralization with internationally standardized antiserum pools

Proof of the presence of virus in a specimen is provided by the appearance of the typical cytopathic effect in cell culture. Virus neutralization tests using virus-specific antisera are most commonly used for identification of the virus isolates. Typing of the isolates is performed with pools of internationally standardized hyperimmune equine antisera. These sera were introduced by Lim and Benyesh-Melnick[44] and can be obtained from the WHO Collaborating Center for Virus Reference and Research in Copenhagen. The Lim-Benyesh-Melnick (LBM) sera consist of eight antiserum pools (pools A–H), which can be used for the identification of 42 enterovirus serotypes.

Isolation and identification of selected enteroviruses that cannot be propagated in cell culture are restricted to a few reference laboratories that propagate viruses in animals. For virus typing in animals, additional LBM antiserum pools with neutralizing antibodies (pools J–P) are available. Virus neutralization is characterized by absence of clinical signs in inoculated animals.

Differentiation between wild-type and Sabin vaccine-like poliovirus strains

The differentiation of wild-type strains and Sabin vaccine-like strains of polioviruses is generally performed in specialized laboratories, which use the following techniques:[45]

- determination of growth markers of polioviruses represented by the viral reproductive capacity at elevated temperatures and plaquing capacities;
- intratypic serodifferentiation of virus strains using polyclonal strain-specific antibodies;[46] [47]
- use of monoclonal antibodies against wild-type and vaccine-like viruses for virus neutralization;[47] [48] [49] and
- amplification of viral genome sequences by reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequence analysis.[50] [51]

Virus detection and identification by microscopy

Specialized laboratories perform electron microscopic techniques for direct virus visualization (see Fig. 213.1). The technique of negative staining is restricted to stool specimens, because only this material contains a sufficiently high virus concentration (see above). Direct

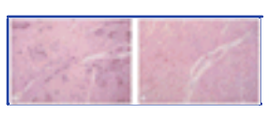

Figure 213-8 Fulminant enterovirus-induced myocarditis. In-situ hybridization of a ^{35} S-labeled enterovirus group-specific cDNA probe to the paraffinembedded autopsy heart tissue of an infant who died of acute enterovirus infection.[53] (a) Autoradiographic silver grains can be clearly localized to distinct infected myocytes, thereby providing the possibility of an unequivocal diagnosis of myocardial enterovirus infection. (b) Hybridization to myocardial cells was not observed when myocardial tissues were hybridized with the ^{35} S-labeled plasmid vector control probe, demonstrating the specificity of in-situ hybridization. Stained with hematoxylin and eosin. *Courtesy of R Kandolf, Tübingen.*

virus identification can be performed by immune electron microscopy. Virus-containing specimens are incubated with virus-specific antibodies and the resulting virus-antibody complexes can be characterized by electron microscopy.

Virus typing in specimens obtained at biopsy and autopsy can be performed by immunofluorescence techniques. Sections of target tissues are incubated with virus-specific antibodies and the resulting immune complexes are detected in a sandwich test with antibodies that are labeled with fluorescent dye and directed against the virus-specific antibody. This laborious sandwich technique is restricted to specialized laboratories.

Detection of virus genome

Techniques for the detection of enterovirus genomes have been introduced for selected diagnostic purposes. However, the traditional immunologic techniques for virus typing by neutralization cannot yet be replaced by molecular biologic techniques. The main reason for the limited use of these techniques in routine diagnostics is the relatively high sequence homology between enteroviruses, which may result in reduced specificity. Molecular biologic techniques nevertheless are very powerful tools for specialized diagnostics:[51] [52]

- the combination of PCR with restriction fragment length polymorphism analysis or sequencing allows virus typing and differentiation between wild-type and Sabin vaccine-like strains of polioviruses;
- oligonucleotide analysis ('fingerprinting') and nucleic acid sequencing is useful for detecting genome variation between different enterovirus isolates, and virus strain-specific oligonucleotide patterns in defined fingerprints detected by two-dimensional gel electrophoresis are useful for epidemiologic surveillance; and
- in-situ hybridization using labeled virus-specific gene probes is a powerful tool for enterovirus genome detection in biopsy or autopsy specimens (Fig. 213.8); the use of Coxsackie virus type B3-specific cDNA has enabled this virus to be identified as a cause of chronic dilated myocarditis,[53] which could not be proven by virus isolation in cell cultures.

The introduction of quantitative methods for nucleic acid amplification (i.e. quantitative PCR) in combination with the introduction

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of standardized international reference material will give new possibilities for the diagnosis of enterovirus infections.

Serologic diagnosis

In order to confirm an infection with enteroviruses, a combination of serologic diagnosis with virus identification is advisable. Recent enterovirus infections can be proven by serologic means by using two kinds of documentation of a virus-specific antibody response.

Analysis of two or more sera from a patient

Ideally, the first serum of the patient should be tested at the beginning of the illness. A second serum should be collected 7–10 days later and tested in parallel with the first serum in order to detect a rise in antibody titer (see Fig. 213.7). A 4-fold or greater rise in antibody titer (i.e. at least two titer steps) proves a recent enterovirus infection.

Detection of virus-specific IgM

The detection of a single high titer of enterovirus-specific IgM proves a recent enterovirus infection. Virus-specific IgM first appears after enterovirus infection (7–10 days after infection) and persists for approximately 4 weeks in 90% of infections (see Fig. 213.7 ; also see below). Common procedures for isolating the IgM fraction of a patient serum from remaining serum proteins are either density gradient ultracentrifugation or column chromatography (i.e. conventional molecular sieve gel chromatography or high pressure liquid chromatography). The isolated IgM fraction is analyzed for virus specificity in neutralization tests.

This laborious and time-consuming isolation of IgM has the advantage that a single serum from a patient is sufficient for detecting 'fresh' virus-specific IgM. This depends on the precondition that this single serum was collected neither within the first 7 days of infection nor too late after infection, when the amount of virus-specific IgM has decreased below the level of detection.

Neutralization test

The virus neutralization test is still the first-choice method for detecting virus-specific antibodies. This test has a very high sensitivity and specificity and it allows the

detection of serotype-specific antibodies, which is in contrast to most other serologic detection methods. The neutralization test is usually performed only in specialized laboratories with the following preconditions:

- an enterovirus infection is suspected because of a defined clinical or epidemiologic situation;
- an enterovirus has already been isolated from a defined patient's material; and
- group-specific antibodies have already been detected with another serologic technique (e.g. with the complement fixation test).

Cytopathic end-point tests that use enterovirus permissive cell cultures in a microtiter system are performed for the detection of the neutralization reaction. The patient's serum or its isolated IgM fraction is geometrically diluted (e.g. 1:10, 1:20 and so on up to 1:320) and each dilution is incubated with a constant infectious dose of a given enterovirus (100 tissue culture ID_{50} is the dose of virus that will induce a cytopathic effect in 50% of the cells). The absence of a cytopathic effect caused by virus neutralization is microscopically detected in comparison with non-neutralized control virus. The serum titer is calculated for the 50% cytopathic end point per inoculation volume against the challenge dose of non-neutralized virus. [43]

Complement fixation test

The complement fixation test is usually performed as a serologic screening method for the detection of enterovirus-specific antibodies. It only allows the detection of enterovirus group-specific antibodies. The use of denatured enterovirus antigen gives rise to immunologic cross-reaction between different enterovirus groups (see above).

Enzyme-linked immunosorbent assay (ELISA) and immunoblot

These serologic methods have not been implemented as routine tests as they have the same limited value as the complement fixation test due to the detection of enterovirus group-specific instead of serotype-specific antibodies.

CLINICAL MANIFESTATIONS

Asymptomatic infections

Most enterovirus infections are silent, mild or subclinical. Between 90% and 95% of poliovirus infections remain asymptomatic whereas only 0.1–1% of infections cause clinical signs involving the CNS, such as paralytic poliomyelitis. Enterovirus passage through the gut may be the reason for the high incidence of inapparent infections because the cells of the gut epithelia normally have a high rate of turnover. Therefore, the virus-induced lysis of the gut cells might be without clinical signs, even though more than 1000 infective virus particles can be reproduced in one infected cell.

Incubation times

The incubation times of all enteroviruses range from 2 to 35 days, with an average of 7–14 days. An exception has been reported for local infections of the eye by enterovirus type 70, which has a short incubation period of 12–30 hours.

Immune response

It is suggested that immunity to enterovirus infections is mediated mainly by humoral and secretory antibodies (see Fig. 213.7). The role of cellular immunity in enterovirus infections has not yet been well analyzed. Humoral neutralizing IgG, IgM and IgA with virus-type specificity prevent hematogenous spread of virus to the target organs. Virus-specific IgM appears first after infection (7–10 days after infection) and persists for at least 4 weeks in 90% of infections. Acquired humoral immunity is mediated by virus-specific IgG, which may persist for several years. After poliovirus infection, production of virus-specific antibodies in the CNS has been observed. Breakdown in the integrity of the meninges may enable serum antibodies to cross the blood-brain barrier and this may be the reason for their appearance in the CNS. Secretory IgA against poliovirus is located mainly in nasopharyngeal and gut tissues; it is induced 2–4 weeks after infection. Secretory IgA induced against each of the three poliovirus types after oral polio vaccination is the main barrier against subsequent wild-type infections because secretory IgA prevents or limits the spread of polioviruses in the alimentary tract.

Clinical syndromes

The lytic infection of host cells with severe cytopathic effects is the basis for the mechanism of pathogenicity of enterovirus infections (see above). Most enteroviruses induce cyclic infections in their host with a viremia and subsequent virus transport to the target organs (especially the spinal cord and brain, meninges, myocardium, skin and liver).[2] [4]

Polioviruses

The mode of infection of polioviruses is the best understood of all the enteroviruses. The mouth is the portal of entry. Beginning shortly after infection, poliovirus is spread via oral and fecal routes as the virus multiplies in the mucosal tissues of the pharynx, the lymphoid tissues (tonsils and Peyer's patches) and the gut. The virus infection is clinically inapparent in 90–95% cases.

TABLE 213-3 -- Clinical syndromes of enterovirus infections.[†]

CLINICAL SYNDROMES OF ENTEROVIRUS INFECTIONS		
Viruses	**Types**	**Clinical syndromes**
Polioviruses	1–3	Abortive poliomyelitis ('minor illness', undifferentiated febrile illness)
		Nonparalytic poliomyelitis (aseptic meningitis)
		Paralytic poliomyelitis ('major illness'), encephalitis (infrequently)
		Postpolio syndrome
Coxsackie viruses		

Group A	2, 3, 4, 5, 6, 8, 10	Herpangina (vesicular pharyngitis)
	10	Acute lymphatic or nodular pharyngitis
	2, 4, 7, 9, 10	Aseptic meningitis
	7, 9	Paralysis (infrequently)
	4, 14, 16	Myocarditis, pericarditis
	4, 5, 6, 9, 16	Exanthema
	4, 5, 9, 10, 16	Hand, foot and mouth disease
	9, 16	Pneumonitis in children
	21, 24	Common cold
	4, 9	Hepatitis
	18, 20, 21, 22, 24	Infantile diarrhea
	24	Acute hemorrhagic conjunctivitis
	Various types	Undifferentiated febrile illness
Group B	1, 2, 3, 4, 5	Pleurodynia
	1, 2, 3, 4, 5	Bornholm disease (epidemic pleurodynia or acute epidemic myalgia)
	1, 2, 3, 4, 5, 6	Aseptic meningitis
	2, 3, 4, 5	Paralysis (infrequently)
	1, 2, 3, 4, 5	Severe systemic infection in infants, meningoencephalitis, myocarditis
	1, 2, 3, 4, 5	Myocarditis, pericarditis, chronic cardiovascular disease
	4, 5	Upper respiratory illness and pneumonia
	5	Exanthema
	2, 5	Hand, foot and mouth disease
	5	Hepatitis
	1, 2, 4	Pancreatitis
	4	Diabetes mellitus
	1, 2, 3, 4, 5, 6	Undifferentiated febrile illness
Echoviruses[*]	1–7, 9, 11, 13–23, 25, 27, 30, 31	Aseptic meningitis
	4, 6, 9, 11, 30; possibly 1, 7, 13, 14, 16, 18, 31	Paralysis (infrequently)
	2, 6, 9, 19; possibly 3, 4, 7, 11, 14, 18, 22	Encephalitis, ataxia, Guillain-Barré syndrome
	2, 4, 6, 9, 11, 16, 18; possibly 1, 3, 5, 7, 12, 14, 19, 20	Exanthema, Boston exanthema disease (echovirus 16)
	4, 9, 11, 20, 25; possibly 1–3, 6–8, 16, 19, 22	Respiratory illness
	7, 11	Conjunctivitis
	1, 6, 9	Epidemic myalgia (infrequently)
	1, 6, 9, 19	Myocarditis, pericarditis (infrequently)
	4, 9	Hepatitis
	Various types	Diarrhea
		Undifferentiated febrile illness
Other enteroviruses	68	Pneumonia, bronchiolitis
	70	Acute hemorrhagic conjunctivitis
	71	Aseptic meningitis
	70, 71	Paralysis
	70, 71	Meningoencephalitis
	71	Hand, foot and mouth disease

† *Adapted from Pallansch and Roos.*[2]
* Echoviruses 22 and 23 have been reclassified as parechoviruses 1 and 2.

Abortive poliomyelitis

Poliovirus can be spread to the draining lymph nodes, most probably with the help of M cells, which are specialized microfold cells overlying Peyer's patches. This leads to a viremia at 6–9 days after infection (see Fig. 213.7). The viremia is characterized by non-specific clinical symptoms such as fever, malaise and sore throat and sometimes headache and vomiting (Table 213.3). This abortive poliomyelitis ('minor illness') occurs in about 4–8% of poliovirus infections.

Nonparalytic poliomyelitis (aseptic meningitis)

Poliovirus can cross the blood-brain barrier by as yet unknown mechanisms to infect its target cells in the CNS. The same prodromal signs as those that occur in abortive poliomyelitis are characteristic of patients who have nonparalytic poliomyelitis, which occurs in 1–2% of poliovirus infections. Between 3 and 7 days after the 'minor illness' these patients develop an illness similar to aseptic meningitis, which is accompanied by high fever, back pain and

muscle spasm. The patients generally show rapid and complete recovery from the disease, which lasts 2–10 days.

Paralytic poliomyelitis

Between 0.1% and 1% of all people infected with poliovirus develop paralytic poliomyelitis, also called 'major illness'. In addition to the clinical signs of 'minor illness' and aseptic meningitis, the 'major illness' comprises flaccid paralysis (involvement of the whole muscle) or paresis (involvement of only some muscle groups) and, in rare cases, encephalitis. Spinal or bulbar damage, or both, is the consequence. The spinal form of poliomyelitis owing to an ascendent infection is more common than the bulbar form. Bulbar poliomyelitis is characterized by damage to the cerebral nerves and vegetative centers and it therefore has a poor prognosis. In spinal poliomyelitis, some recovery of motor function may occur after several months; any remaining paralysis, however, will be permanent.

The very specific extraintestinal tropism of poliovirus to target cells of the CNS is the basis for these severe clinical signs. Polioviruses especially infect the anterior horn cells of the spinal cord but also the dorsal root ganglia, certain brainstem centers, cerebellum, spinal sensory columns and, occasionally, the cerebral motor cortex. Histologic analysis reveals that vascular engorgement is first observed, accompanied by perivascular infiltration with lymphocytes and also polymorphonuclear neutrophils, plasma cells and microglia. Experimental infection of rhesus monkeys has shown that the anterior horn cells are severely damaged by a poliovirus-specific cytopathic effect. The cells are characterized by a diffuse decrease in the size of the Nissl bodies in the cytoplasm (chromatolysis) and nuclear alterations with breakage and condensation of chromatin.[54]

The severity of disease may be increased by certain factors, including male sex, very young and very old age, physical exertion, hypoxia, cold, chronic undernutrition, corticosteroid treatment, irradiation, tonsillectomy, pregnancy, adrenal-related endocrine changes and possibly hypercholesterolemia.[55]

Postpolio syndrome

A small number of infected people develop recrudescence of paralysis and muscle atrophy several decades after their experience with paralytic poliomyelitis. This postpolio syndrome owing to a progressive postpoliomyelitis muscle atrophy is not clearly understood. Postpoliomyelitis muscle atrophy cannot be assigned to persistent poliovirus infection,[2] although some reports have described poliovirus genome sequences in the CNS of these patients as detected by PCR. It is suggested, rather, that the muscle atrophy is a result of the additive effects of physiologic aging in these patients to the long-lasting loss of neuromuscular function resulting from the earlier infection.

Coxsackie viruses, echoviruses and enteroviruses 68–71

The nonpolio enteroviruses are characterized by less specific extraintestinal target organ tropism than the polioviruses and they can therefore induce a wider range of diseases (see Table 213.3). The primary multiplication of the nonpolio enteroviruses takes place in the pharynx and small intestine and viruses are shed in the feces for up to 1 month and in the respiratory secretions for several days. Owing to the broad target organ range, nonpolio enteroviruses can infect the meninges, CNS, myocardium and pericardium, striated muscles, respiratory tract, eye and skin. Enterovirus 69 is the only enterovirus that has not yet been clearly assigned to a disease.

Meningitis and central nervous system disease

Meningitis and mild paresis can be induced by most group A and group B Coxsackie viruses and by echoviruses. As with poliomyelitis 'minor illness', early symptoms are fever, malaise, headache, nausea and abdominal pain; these are followed by meningeal irritation with neck or back stiffness and vomiting before the onset of meningitis and mild paresis. Aseptic meningitis is very often accompanied by a rash. In contrast to the situation in poliovirus infection, the manifestation of the CNS disease is usually milder and patients nearly always recover from paresis. Severe CNS disease, which may be confused clinically with paralytic poliomyelitis, has been described for infections with Coxsackie viruses A7, A9 and B2–B5. Meningoencephalitis is seen (especially in children) in infections with several group B Coxsackie viruses. In 1955–60 a pandemic outbreak of aseptic meningitis was caused by echovirus 9. Encephalitis, ataxia and Guillain-Barré syndrome have been attributed to echovirus infections. In 1969–73 in California, enterovirus 71 was responsible for an epidemic outbreak of aseptic meningitis, meningoencephalitis and paralysis in parallel to hand, foot and mouth disease. In 1998 in Taiwan there was a further large outbreak of enterovirus 71 that affected young children and resulted in 78 deaths, mostly related to encephalitis.[56] A disease similar to poliomyelitis has been observed in some cases of acute hemorrhagic conjunctivitis caused by enterovirus 70.

Pleurodynia

Coxsackie viruses B1–B5 can cause pleurodynia (also known as epidemic myalgia or Bornholm disease), with abrupt onset of fever sometimes preceded by malaise, headache and anorexia. Characteristic clinical signs are severe chest pain, also called 'devil's grip', and abdominal pain, which may be intensified by movement or breathing. Children and adolescents are most often attacked by this disease, which can last from 2 days to 2 weeks and may be accompanied by generalized muscle hypotonia. An epidemic of this disease was first observed on the island of Bornholm (Denmark) in 1930–32. Epidemics predominantly occur during late summer and early autumn. Muscle pain in the lower extremities may be caused by echovirus infections. Patients may suffer from pleurodynia if this myalgia affects the intercostal muscles. Sporadic cases of pleurodynia may be caused by Coxsackie viruses A4, A6, A9 and A10 as well as echoviruses 1 and 6.

Herpangina

Herpangina is mainly caused by group A Coxsackie viruses such as A2–A6, A8 and A10. Infants are most often attacked by herpangina, with abrupt onset of fever, sore throat, vomiting and abdominal pain. Herpangina is characterized by discrete vesicles of different size, sometimes tiny, which most frequently occur on the tongue, anterior pillars of the fauces, the posterior pharynx, the palate, the uvula or the tonsils. Coxsackie virus A10 may also be responsible for lymphatic pharyngitis.

Hand, foot and mouth disease

Coxsackie virus A16 is associated with hand, foot and mouth disease, but Coxsackie viruses A4, A5, A9, A10, B2 and B5 may also cause it. The disease is characterized by vesicles on the hands and feet. The manifestation on the mucosa of the mouth leads to herpangina with generalized vesicular intraoral lesions, which may be ulcerative. Hand, foot and mouth disease caused by enterovirus 71 can be mixed with aseptic meningitis and encephalitis.[56]

Exanthemas

Rubelliform rash may be caused by several group A and B Coxsackie viruses, which most frequently attack young children. Several types of echoviruses are responsible for exanthemas, with a high incidence in infants. These exanthemas can be maculopapular and are sometimes morbilliform or rubelliform; they are accompanied by febrile illnesses or pharyngitis. Echovirus 19 was responsible for an

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epidemic outbreak of a maculopapular rash in Boston, Massachusetts, in the beginning of the 1950s; since then this has been known as 'Boston exanthema disease'.

Respiratory illnesses and acute febrile illnesses

Several types of echoviruses and group A and B Coxsackie viruses are responsible for infections of the upper and lower respiratory tract that cause acute febrile illnesses of short duration without distinctive features. These illnesses predominantly occur during the summer or autumn and often resemble the common cold. In some cases, infections with Coxsackie viruses may cause pneumonia in children and adults and pneumonitis of infants. Children may suffer from pneumonia and bronchiolitis when attacked by enterovirus 68. Enterovirus 71 can lead to an influenza-like illness in children.

Conjunctivitis

Enteroviral conjunctivitis is mainly caused by Coxsackie virus A24 and enterovirus 70. A variant of Coxsackie virus A24 was responsible for an epidemic outbreak of acute hemorrhagic conjunctivitis in Singapore and Hong Kong in 1970–71. This acute hemorrhagic conjunctivitis spread throughout South East Asia and first occurred outside Asia in American Samoa in 1986 (where the incidence was 47%). Acute hemorrhagic conjunctivitis caused by enterovirus 70 first occurred in Africa, South East Asia (including Singapore), Japan and India in 1969–71, with several million cases. After sporadic outbreaks in French Polynesia in 1982, enterovirus 70 was introduced to the USA. Coxsackie virus A24 and enterovirus 70 typically cause local infections of the eye; enterovirus 70, however, can also be responsible for rare manifestations in the CNS with a poliomyelitis-like paralysis. In contrast to all other enterovirus infections, the incubation time of infections with enterovirus 70 is very short (24 hours; range 12–72 hours). Conjunctivitis without hemorrhage can be caused by echoviruses 7 and 11. An epidemic outbreak of conjunctivitis caused by echovirus 7 occurred in Sweden in 1977. It must be emphasized that enterovirus-infected conjunctival fluid can be highly infectious.

Myocarditis and pericarditis

Group B Coxsackie viruses are the main cause of myocarditis, pericarditis and dilated cardiomyopathy among the enteroviruses. In addition, cases of myocarditis and

pericarditis are also described for Coxsackie viruses A4, A14 and A16, and for echoviruses 1, 6, 9 and 19.

Coxsackie viruses and echoviruses may affect the myocardium, endocardium and pericardium. The infected myocardium is characterized by edema, diffuse focal necrosis and signs of acute inflammatory responses. The cardiac disease may be accompanied by meningism and convulsions. Myocarditis in newborns may be fatal (see Fig. 213.8); death occurs in 50% of cases. Young children and adolescents are more often attacked by pericarditis, which in general is less severe than neonatal myocarditis.

Chronic cardiovascular disease

Chronic cardiovascular disease with recurrent pericarditis can be caused by Coxsackie viruses (predominantly B2–B5). Virus-specific IgM may persist for years. Myocytes are persistently infected with Coxsackie viruses, which can be demonstrated by in-situ hybridization.[53] Viral RNA instead of mature virus persists in cardiac myocytes.[53] [57] [58] Long-lasting necrosis in the myocardium may be the consequence of this virus persistence. There are results showing that an autoimmune response is involved in Cocksackie virus-induced chronic cardiovascular disease.[59]

Gastrointestinal disease

Non-specific clinical symptoms caused by several Coxsackie viruses and echoviruses may be accompanied by diarrhea, which may be fatal in newborn babies. Generalized infections with Coxsackie viruses and echoviruses may lead to hepatitis. Pancreatitis has been associated with group B Coxsackie viruses.

Diabetes mellitus

Infections with group B Coxsackie viruses are implicated as a cause of juvenile-onset insulin-dependent diabetes mellitus. Several reports of experimental Coxsackie virus infections of animals discuss an involvement of autoimmunity in diabetes.

Neonatal disease

Nosocomial transmission of Coxsackie viruses and some echoviruses may be responsible for fatal disease in newborn babies, frequently with rapid death. Nursery outbreaks and sporadic infections, mainly with group B Coxsackie viruses and echovirus 11, cause disease within a few days of birth and may lead to an overwhelming systemic infection (viral sepsis) with acute myocarditis or pericarditis, encephalitis and hepatitis. The condition is often hemorrhagic with fatal kidney disorders. Severe diarrhea in young children may cause severe disorders of water and ion balance.

There are case reports suggesting that neonatal diseases may be acquired by intrauterine infection as a result of transplacental transmission and neonatal infection at birth by a contaminated cervix. Maternal infection in the first trimester of pregnancy with Coxsackie viruses A9, B2, B3 and B4 may be associated with fetal abnormalities of the urogenital tract, the gastrointestinal tract, the cardiovascular system and the CNS, but the risk of teratogenesis from infections with Coxsackie viruses or echoviruses cannot yet be estimated.

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Chapter 214 - Hepatitis Viruses

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INTRODUCTION

Viral hepatitis is a major public health problem throughout the world that affects several hundreds of millions of people. Viral hepatitis is a cause of considerable morbidity and mortality, both from acute infection and from chronic sequelae. In the case of infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), these chronic sequelae include chronic active hepatitis, cirrhosis and primary liver cancer.

There is evidence that in addition to the five recognized pathogens, A–E, the so-called non-A–E hepatitis viruses exist. The evidence was based on the observation of short and long incubation periods in post-transfusion hepatitis and in experimental transmission studies; multiple bouts of hepatitis in the same patient; chronic hepatitis not due to HBV, HCV or hepatitis D virus (HDV); chloroform-resistant non-ABC virus; and cross-challenge experiments in susceptible primates.

The hepatitis viruses include a range of unrelated and often unusual human pathogens (Table 214.1).

HEPATITIS A VIRUS

Outbreaks of jaundice have been described frequently for many centuries and the term infectious hepatitis was coined in 1912 to describe the epidemic form of the disease. Hepatitis A virus (HAV) is spread by the fecal-oral route. It continues to be endemic throughout the world and hyperendemic in areas with poor standards of sanitation and hygiene.

NATURE

Classification

Electron microscopy examination of concentrates of filtered fecal extracts from patients in the early stages of infection reveals 27nm particles typical of the Picornaviridae (Fig. 214.1). Hepatitis A virus is a small, unenveloped, symmetric RNA virus and was classified in the genus enterovirus of the family Picornaviridae on the basis of its biophysical and biochemical characteristics. However, after the determination of the entire nucleotide sequence of the viral genome, comparison with other picornavirus sequences revealed limited homology to the enteroviruses and also to the rhinoviruses, although the structure and genome organization is typical of the Picornaviridae. Hepatitis A virus has now been placed as hepatovirus within the heparnavirus genus.

Organization of the Hepatitis A virus genome

The HAV genome comprises about 7500 nucleotides of positive-sense RNA. The RNA is polyadenylated at the 3' end and has a viral polypeptide (VPg) attached to the 5' end. A single, large open reading frame (ORF) occupies most of the genome and encodes a polyprotein with a theoretic molecular mass of M_r 252,000. An untranslated region of around 735 nucleotides precedes the ORF. Secondary structure within this region of the genome may be important for efficient translation of the RNA. There is also a short untranslated region at the 3' end of the HAV genome.

The viral polyprotein is processed to yield the structural polypeptides (located at the amino-terminal end) and the nonstructural viral polypeptides. Many of the features of replication of the picornaviruses have been deduced from studies of prototype enteroviruses and rhinoviruses, in particular poliovirus type 1.

The three-dimensional structures of a number of picornaviruses have been resolved by high-resolution crystallography; polypeptides VP1 (1D), VP2 (1B) and VP3 (1C) are exposed on the surface of the virion, whereas VP4 (1A) is located internally. After release of the structural domain from the polyprotein, the 3C protease cleaves the 1B–1C and 1C–1D junctions to yield VP0 (VP4 plus VP2), VP3 and VP1. The three polypeptides remain associated as a protomer and five protomers assemble to form a pentamer, so that the five copies of the VP1 form the apex. Finally, 12 pentamers assemble around a molecule of viral RNA to form the icosahedral capsid. As the structure locks into place, most copies of VP0 cleave (presumably autocatalytically) to yield VP2 and VP4. However, in the case of HAV it has not been possible to demonstrate VP4, which is predicted to consist of only 23 amino acids.

The functions of some of the other products of the cleavage of the polyprotein, such as 2B, 2C and 3A, are less well understood. Product 3B corresponds to the genome-linked polypeptide VPg, which in other picornaviruses is the primer for the synthesis of both genome-sense RNA and the negative-sense RNA found in replicative intermediates. Polypeptide 3AB may be the precursor of VPg. Finally, the three-dimensional product seems to be the viral replicase and contains the Gly-Asp-Asp motif common to viral RNA-dependent RNA polymerases.

EPIDEMIOLOGY

Viral hepatitis type A (infectious or epidemic hepatitis) occurs endemically in all parts of the world, with frequent reports of minor and major outbreaks. The exact incidence is difficult to estimate because of the high proportion of subclinical infections and infections without jaundice, because of differences in surveillance and because of differing patterns of disease. The degree of under-reporting is very high.

The development of specific serologic tests for hepatitis A has made possible the study of the incidence and distribution of hepatitis A in various countries. These studies have shown that infections with HAV are widespread and endemic in all parts of the world, that chronic excretion of HAV does not occur and that the infection is rarely transmitted by blood transfusion, although transmission by blood coagulation products has been reported. There is no evidence of progression to chronic liver disease.

The seroprevalence of antibodies to HAV has declined since the Second World War in many countries, and infection results most commonly from person-to-person contact, but large epidemics do occur. For example, an outbreak of hepatitis A in Shanghai in 1988 associated with the consumption of clams resulted in almost 300,000 cases.

TABLE 214-1 -- The hepatitis viruses.

THE HEPATITIS VIRUSES			
Virus	**Description**	**Viral group**	**Mode of transmission**
Hepatitis A virus	Small unenveloped symmetric RNA virus	Hepatovirus, in heparnavirus genus	Fecal-oral
Hepatitis B virus	Enveloped double-stranded DNA virus	Hepadnavirus	Blood-blood
			Sexual
Hepatitis C virus	Enveloped single-stranded RNA virus	Related to flavivirus	Blood-blood
Hepatitis D virus	Circular single-stranded RNA virus	Related to plant viral satellites and viroids	Blood-blood
Hepatitis E virus	Unenveloped single-stranded RNA	Related to calciviruses; closely resembles rubella virus and a plant virus (beet necrotic yellow vein virus)	Fecal contamination of water
			Food-borne

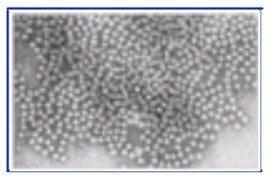

Figure 214-1 Hepatitis A virus. Note the vast number of virus particles present in a fecal extract.

PATHOGENICITY

The incubation period of hepatitis A is 3–5 weeks (Fig. 214.2), with a mean of 28 days. Subclinical and anicteric cases are common and, although the disease

generally has a low mortality rate, patients may be incapacitated for many weeks. There is no evidence of progression to chronic liver damage.

Hepatitis A virus is spread by the fecal-oral route, most commonly by person-to-person contact; infection occurs readily under conditions of poor sanitation and overcrowding. Common source outbreaks are initiated most frequently by fecal contamination of water and food, but water-borne transmission is not a major factor in maintaining this infection in industrialized communities. On the other hand, many outbreaks related to food have been reported. This can be attributed to the shedding of large quantities of virus in the feces by infected food handlers during the incubation period of the illness — the source of the outbreak can often be traced to uncooked food or food that has been handled after cooking. Although hepatitis A remains endemic and common in the developed countries, the infection occurs mainly in small clusters, often with only a few identified cases.

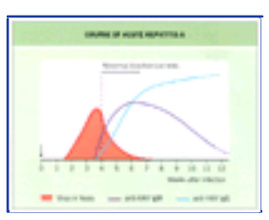

Figure 214-2 Course of acute hepatitis A.

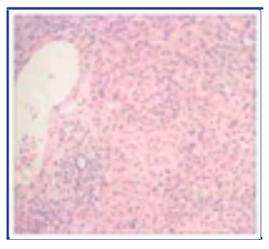

Figure 214-3 Histologic changes in the liver of a patient with acute hepatitis A.

The clinical expression of infection with HAV varies considerably, ranging from subclinical, anicteric, mild illnesses in young children to the full range of symptoms with jaundice in adolescents and adults. The ratio of anicteric to icteric illnesses varies widely, both in individual cases and during outbreaks.

Hepatitis A virus enters the body by ingestion. The virus then spreads, probably by the bloodstream, to the liver, the target organ. Large numbers of virus particles are detectable in feces during the incubation period, beginning as early as 10–14 days after exposure and continuing, in general, until peak elevation of serum amino-transferases. Virus is also detected in feces early in the acute phase of illness but relatively infrequently after the onset of clinical jaundice. Immunoglobulin G antibody to HAV persists and is also detectable late in the incubation period, coinciding approximately with the onset of biochemical evidence of liver damage.

Hepatitis A viral antigen has been localized by immunofluorescence in the cytoplasm of hepatocytes after experimental transmission to chimpanzees. The antigen has not been found in any tissue other than the liver following experimental intravenous inoculation in susceptible nonhuman primates.

Pathologic changes induced by HAV appear only in the liver (Fig. 214.3). These include marked focal activation of sinusoidal lining cells; accumulation of lymphocytes and histiocytes in the parenchyma — these cells often replace hepatocytes that have been lost by cytolytic necrosis, especially in the periportal areas; occasional

coagulative necrosis resulting in the formation of acidophilic bodies; and focal degeneration.

PREVENTION

In areas of high prevalence, most children are infected early in life, and such infections are generally asymptomatic. The later in life that infection occurs, the greater the clinical severity — fewer than 10% of cases of acute hepatitis A in children up to the age of 6 years are icteric, but this increases to 40–50% in the 6- to 14-year-age group, and to 70–80% in adults. Of 115,551 cases of hepatitis A in the USA between 1983 and 1987, only 9% of the cases, but more than 70% of the fatalities, were in those aged over 49 years. It is important, therefore, to protect those at risk because of personal contact with infected people or because of travel to a highly endemic area. Other groups at risk of hepatitis A infection include staff and residents of institutions for the mentally handicapped; day care centers for children; sexually active male homosexuals; intravenous drug abusers; sewage workers; certain groups of health care workers, such as medical students on elective studies in countries where hepatitis A is common; military personnel; and certain low socio-economic groups in defined community settings. Patients with hemophilia should be immunized.

Patients with chronic liver disease, especially if visiting an endemic area, should be immunized against HAV. In some developing countries, the incidence of clinical hepatitis A is increasing as improvements in socio-economic conditions result in infection later in life, and strategies for immunization are yet to be developed and agreed.

Passive immunization

Control of HAV infection is difficult. Because fecal shedding of the virus is at its highest during the late incubation period and the prodromal phase of the illness, strict isolation of cases is not a useful control measure. Spread of HAV is reduced by simple hygienic measures and the sanitary disposal of excreta.

Prevention or attenuation of a clinical illness can be achieved by the intramuscular administration of normal human immunoglobulin that contains at least 100IU/ml of hepatitis A antibody. The dosage should be at least 2IU of hepatitis A antibody per kilo of body weight but in pregnancy or in patients with liver disease this dosage may be doubled (Table 214.2). Immunoglobulin does not always prevent excretion of HAV. The efficacy of passive immunization is based on the presence of hepatitis A antibody in the normal human immunoglobulin, and the minimum titer of antibody required for protection is believed to be about 10IU/l.

Titers of HAV antibody vary among batches of pooled normal human immunoglobulin and the titers are decreasing in batches obtained from pooled plasma of donors in industrialized countries, resulting in clinical cases despite prophylaxis with immunoglobulin.[1] Immunoglobulin

TABLE 214-2 -- Passive immunization with normal human immunoglobulin for travelers to highly endemic areas.

PASSIVE IMMUNIZATION WITH NORMAL HUMAN IMMUNOGLOBULIN FOR TRAVELERS TO HIGHLY ENDEMIC AREAS		
Body weight	**Period of stay less than 3 months**	**Period of stay longer than 3 months**
<55lb (<25kg)	50IU anti-HAV (0.5ml)	100IU anti-HAV (1.0ml)
55–66lb (25–30kg)	100IU anti-HAV (1.0ml)	250IU anti-HAV (2.5ml)
>110lb (>50kg)	200IU anti-HAV (2.0ml)	500IU anti-HAV (5.0ml)

is used most commonly for close personal contacts of patients with hepatitis A and for those exposed to contaminated food. Immunoglobulin has also been used effectively for controlling outbreaks in institutions such as homes for the mentally handicapped and nursery schools. Prophylaxis with immunoglobulin is recommended for people without HAV antibody who are visiting highly endemic areas. After a period of 6 months the administration of immunoglobulin for travelers needs to be repeated, unless it has been demonstrated that the recipient has developed HAV antibodies. Active immunization is recommended, particularly for travelers and for controlling outbreaks, particularly if vaccine is given to all contacts immediately.

Active immunization against hepatitis A

Killed hepatitis A vaccines

The foundations for a hepatitis A vaccine were laid in 1975 by the demonstration that formalin-inactivated virus extracted from the liver of infected marmosets induced protective antibodies in susceptible marmosets on challenge with live virus.[2] Subsequently, HAV was cultivated, after serial passage in marmosets, in a cloned line of fetal rhesus monkey kidney cells, thereby opening the way to the production of hepatitis A vaccines. Later, it was demonstrated that prior adaptation in marmosets was

not a prerequisite to growth of the virus in cell cultures. Several formalin-inactivated hepatitis A vaccines are available, including combined vaccines with hepatitis B vaccine and with typhoid vaccine.

Live attenuated hepatitis A vaccines

The major advantages of live attenuated vaccines (such as the Sabin type of oral poliomyelitis vaccine) include ease of oral administration; relatively low cost, because the virus vaccine strain replicates in the gut; production of both local immunity in the gut and humoral immunity, thereby mimicking natural infection; and the longer term protection afforded.

Disadvantages include the potential of reversion toward virulence, interference with the vaccine strain by other viruses in the gut, relative instability of the vaccine, and shedding of the virus strain in the feces for prolonged periods.

The most extensively studied live attenuated hepatitis A vaccines are based on the CR 326 and HM 175 strains of the virus attenuated by prolonged passage in cell culture.

Two variants of the CR 326 strain have been investigated after passage in marmoset liver in fetal rhesus monkey kidney cells, namely MRC5 and WI-38 cells. Inoculation of susceptible marmosets demonstrated seroconversion, and protection on challenge. Biochemical evidence of liver damage did not occur in susceptible chimpanzees, although a number had histologic evidence of mild hepatitis with the F variant and the vaccine virus was shed in the feces for about 12 weeks before seroconversion. There was no evidence of reversion toward virulence. Studies in human volunteers indicated incomplete attenuation of the F variant, but better results were obtained with the F^1 variant without elevation of liver enzymes.

Studies with the HM 175 strain, which was isolated and passaged in African green monkey kidney cells, showed that this strain was not fully attenuated for marmosets, although it did not induce liver damage on challenge. Further passages and adaptation of HM 175 revealed some evidence of virus replication in the liver of chimpanzees and minimal shedding of the virus into feces. Other studies are in progress in nonhuman primates.

As with vaccine strains of polioviruses, attenuation may be associated with mutations in the 5' noncoding region of the genome, and this affects the secondary structure of the protein compounds. There is also evidence that mutations in the region of the genome encoding the nonstructural polypeptides may be important for adaptation

to cell culture and attenuation. However, markers of attenuation of HAV have not been identified, and reversion to virulence may be a problem. On the other hand, there is also concern that 'over-attenuated' viruses may not be sufficiently immunogenic.

Current candidate live attenuated hepatitis A vaccines require administration by injection. Preparations that may be suitable for oral administration are not available so far.

DIAGNOSTIC MICROBIOLOGY

Various serologic tests are available for HAV, including immune electron microscopy, complement fixation, immune adherence hemagglutination, radioimmunoassay and enzyme immunoassay. Immune adherence hemagglutination, which has been widely used, is moderately specific and sensitive. Several methods of radioimmunoassay have been described, but these have largely been replaced by sensitive enzyme immunoassay techniques.

Only one serotype of HAV has been identified in volunteers infected experimentally with the MS-1 strain of hepatitis A, in patients from different outbreaks of hepatitis in different geographic regions and in random cases of hepatitis A. Several genotypes of the virus are recognized.

Isolation of virus in tissue culture requires prolonged adaptation and it is therefore not suitable for diagnosis.

CLINICAL MANIFESTATIONS

The following description of the acute disease applies to all types of viral hepatitis. Prodromal non-specific symptoms such as fever, chills, headache, fatigue, malaise and aches and pains are followed a few days later by anorexia, nausea, vomiting and right upper quadrant abdominal pain followed by the passage of dark urine and clay-colored stools. Jaundice of the sclera and skin develops. With the appearance of jaundice, there is usually a rapid subjective improvement of symptoms. The jaundice usually deepens for a few days and persists for 1–2 weeks. The feces then darken and the jaundice diminishes over a period of about 2 weeks. Convalescence may be prolonged (see Chapter 48).

HEPATITIS E VIRUS

Retrospective testing of serum samples from patients involved in epidemics of hepatitis associated with fecal contamination of water supplies have indicated that an agent other than HAV or HBV was involved. Epidemics of enterically transmitted non-A, non-B hepatitis in the Indian subcontinent were first reported in 1980, but outbreaks involving tens of thousands of cases have also been documented in the former Soviet Union, South East Asia, northern Africa and Mexico. A huge outbreak occurred in New Delhi in 1956–7, but tests for HAV or HBV were not available then. Infection has been reported in returning travelers.

NATURE

Hepatitis E virus (HEV) is a nonenveloped single-stranded RNA virus that shares many biophysical and biochemical features with caliciviruses.

Morphologically the virus is spherical and unenveloped, measuring 32–34nm in diameter with spikes and indentations visible on the surface of the particle. Confirmation that the virus has been propagated in cell culture is awaited. The virus appears similar to the caliciviruses. However, detailed morphologic studies and the lack of similarities in genome sequence between HEV and recognized caliciviruses suggest that HEV is a single member of a novel virus genus. However, HEV resembles most closely the sequences of rubella virus and a plant virus, beet necrotic yellow vein virus. It has therefore been proposed that these three viruses should be placed in separate but related families.

Genomic organization

Hepatitis E virus was cloned in 1991 and the entire 7.5kb sequence is known. The genome is a single-stranded, positive-sense, polyadenylated RNA molecule, with three overlapping ORFs.

On the basis of the available partial sequence data, it has been suggested that HEV isolates segregate into four major groups based on full-length comparisons. However, more recent studies indicate that HEV may be distributed into at least nine different groups.

EPIDEMIOLOGY

The epidemiologic features of the infection resemble those of hepatitis A. The highest attack rates are found in young adults and high mortality rates of 20–39% have been reported in women infected during the third trimester of pregnancy.[3]

All epidemics of hepatitis E reported to date have been associated with fecal contamination of water, with the exception of a number of food-borne outbreaks in China. Sporadic hepatitis E has been associated with the consumption of uncooked shellfish and has been seen in travelers returning from endemic areas. Hepatitis E virus is an important cause of large epidemics of acute hepatitis, and these, together with a high prevalence of antibody determined by serologic tests, have occurred in the subcontinent of India, South East and central Asia, the Middle East, and northern and western Africa. There have also been outbreaks in eastern Africa and Mexico.

Unexpectedly, the highest prevalence of antibody to HEV is found in young adults and not in infants and children. In some epidemics the antibody has been found more commonly in males, although in most outbreaks the distribution between young adult males and females is equal.

Hepatitis E virus has also been isolated from patients with sporadic acute hepatitis in countries not considered to be endemic for HEV such as the USA, Italy and other European countries and in individuals who had not traveled abroad. There is now evidence that HEV may have an animal reservoir and there are HEV isolates from swine with high sequence identity to human HEV strains isolated from pigs in areas without HEV epidemics. There is recent evidence of a higher prevalence of HEV antibodies among swine farmers, particularly in those with an occupational history of cleaning barns or assisting sows at birth, and also a history of drinking raw milk.

PATHOGENICITY

Virus-like particles have been detected in the feces of infected people by immune electron microscopy using convalescent serum. However, such studies have often proved inconclusive and a large proportion of the excreted virus may be degraded during passage through the gut. Bile was shown to be a rich source. Cross reaction studies between sera and virus in feces associated with a variety of epidemics in several different countries suggest that a single serotype of virus is involved, although two distinct isolates have been recognized and designated as the Burma (B) strain and the Mexico (M) strain. Two other isolates were recently sequenced.

The average incubation period is slightly longer than for HAV, with a mean of 6 weeks.

Studies on HEV have progressed following transmission to susceptible nonhuman primates. Hepatitis E virus was first transmitted to *Cynomolgus* macaques, and a number of other species of monkeys including chimpanzees have also been infected.

PREVENTION

The provision of safe public water supplies, public sanitation and hygiene, safe disposal of feces and raw sewage, and personal hygiene are essential measures.

Passive immunization with immune globulin derived from endemic areas has not been successful. Vaccines are under development.

DIAGNOSTIC MICROBIOLOGY

Serologic tests are necessary to establish the diagnosis. The tests commercially available at present detect anti-HEV IgM in up to 90% of acute infections if serum is obtained 1–4 weeks after the onset of illness, and IgM remains detectable for about 12 weeks. Anti-HEV IgG appears early and reaches a maximum titer 4 weeks after the onset of illness, falling rapidly thereafter.

Tests for HEV RNA by the polymerase chain reaction (PCR) are available in specialized laboratories.

CLINICAL MANIFESTATIONS

Individual cases cannot be differentiated on the basis of clinical features from other cases of hepatitis. In epidemics, most clinical cases will have anorexia, jaundice and hepatomegaly. Serologic tests indicate, however, that clinically inapparent cases occur. The severity of the infection and high mortality during pregnancy have been noted above. Hepatitis E does not progress to chronicity (see Chapter 48).

HEPATITIS B VIRUS

Hepatitis B virus was originally recognized as the agent responsible for 'serum hepatitis', an important and frequent cause of acute and chronic infection of the liver.

NATURE

Hepatitis B virus is a member of the hepadnavirus group, which contains double-stranded DNA viruses that replicate by reverse transcription. Hepatitis B virus is endemic in the human population and hyperendemic in many parts of the world, The virus is transmitted essentially by blood-blood contact and by the sexual route. Mutations of the surface coat protein of the virus and of the core and other proteins have been identified in recent years.[4] [5] [6] [7] [8] [9] [10] Natural hepadnavirus infections also occur in other animals, including woodchucks, beechy ground squirrels and ducks.

Structure and organization of the virus

The hepatitis B virion is a 42nm particle comprising an electron-dense core (nucleocapsid), which is 27nm in diameter, and an outer envelope of the surface protein (hepatitis B surface antigen, HBsAg) embedded in membranous lipid derived from the host cell (Fig. 214.4). The surface antigen, originally referred to as Australia antigen, is produced in excess by the infected hepatocytes and is secreted in the form of 22nm particles and tubular structures of the same diameter.

The 22nm particles are composed of the major surface protein in both nonglycosylated (p4) and glycosylated (gp27) form in approximately equimolar amounts, together with a minority component of the so-called middle proteins (gp33 and gp36), which contain the pre-S2 domain, a glycosylated 55 amino acid amino-terminal extension. The surface of the virion has a similar composition but also contains the large surface proteins (gp39 and gp42), which include both the pre-S1 and pre-S2 regions. These large surface proteins are not found in the 22nm spherical particles (but may be present in the tubular forms in highly viremic people) and their detection in serum

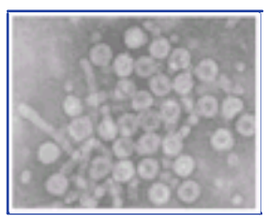

Figure 214-4 Serum from a patient with hepatitis B. The double-shelled particle is the complete virion. Tubular structures and 22nm HBsAg particles are present in small numbers.

correlates with viremia. The domain that binds to the specific HBV receptor on the hepatocyte is believed to reside within the pre-S1 region.

The nucleocapsid of the virion consists of the viral genome surrounded by the core antigen (HBcAg). The genome, which is approximately 3.2kb in length, has an unusual structure and is composed of two linear strands of DNA held in a circular configuration by base-pairing at the 5' ends.

One of the strands is incomplete and the 3' end is associated with a DNA polymerase molecule that is able to complete that strand in the presence of deoxynucleoside triphosphates.

The genomes of more than a dozen isolates of HBV have been cloned and the complete nucleotide sequences determined. Analysis of the coding potential of the genome reveals four ORFs, which are conserved between all of these isolates (Fig. 214.5).

The first ORF encodes the various forms of the surface protein and contains three in-frame methionine codons, which are used for initiation of translation. A second promoter is located upstream of the pre-S1 initiation codon. This directs the synthesis of a 2.1kb mRNA, which is coterminous with the other surface messages and is translated to yield the large (pre-S1) surface proteins.

The core ORF also has two in-phase initiation codons. The 'precore' region is highly conserved, has the properties of a signal sequence and is responsible for the secretion of hepatitis Be antigen (HBeAg).

The third ORF, which is the largest and overlaps the other three, encodes the viral polymerase. This protein appears to be another translation product of the 3.5kb RNA and is synthesized apparently after internal initiation of the ribosome. The amino-terminal domain is believed to be the protein primer for minus strand synthesis. There is then a spacer region followed by the (RNA- and DNA-dependent) DNA polymerase.

The fourth ORF was designated 'x' because the function of its small gene product was not known. However, 'x' has now been demonstrated to be a transcriptional transactivator.

Structure and organization of the virus

There are nine subtypes of HBV, with a common main antigenic determinant, *a.* The nine subtypes described, *ayw*1-*ayw*4, *ayr*, *adw*2, *adw*4, *adrq*$^-$ and *adrq*$^+$, differ in their geographic distribution. Subtyping is employed for epidemiologic studies and to trace nosocomial infection. Traditional subtyping is complemented by classification of different HBV strains into genotypes A–G.

EPIDEMIOLOGY

More than a third of the world's population has been infected with HBV, and the World Health Organization estimates that HBV results in 1,000,000–2,000,000 deaths every year.

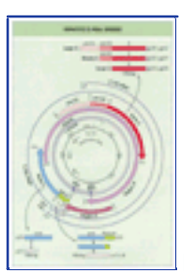

Figure 214-5 Hepatitis B viral genome.

The virus persists in approximately 5–10% of immunocompetent adults and in as many as 90% of infants infected perinatally. Persistent carriage of HBV, defined by the presence of HBsAg in the serum for more than 6 months, has been estimated to affect about 350,000,000 people worldwide.

Although various body fluids (blood, saliva, menstrual and vaginal discharges, serous exudates, seminal fluid and breast milk) have been implicated in the spread of infection, infectivity appears to be especially related to blood and to body fluids contaminated with blood. The epidemiologic propensities of this infection are therefore wide; they include infection by inadequately sterilized syringes and instruments, and transmission by unscreened blood transfusion and blood products, by close

contact and by sexual contact. Transmission of HBV from mother to child may take place — antenatal transmission is rare but perinatal transmission occurs frequently; in some parts of the world (South East Asia and Japan), perinatal transmission is very common.

PATHOGENICITY

The incubation period of hepatitis B is variable, with a range of 1–6 months.

As mentioned above, about 350 million people are carriers of HBV. The pathology is mediated by the cellular immune response of the host to the infected hepatocytes. Long-term continuing virus replication may lead to progression to chronic liver disease, cirrhosis and hepatocellular carcinoma (Fig. 214.6).

In the first phase of chronicity, virus replication continues in the liver and replicative intermediates of the viral genome may be detected in DNA extracted from liver biopsies. Markers of virus replication in serum include HBV DNA, the surface proteins (HBsAg) and a soluble antigen, HBeAg, which is secreted by infected hepatocytes. In those infected at a very young age this phase may persist for life but, more usually, virus levels decline over time. Eventually, in most infected people, there is immune clearance of infected hepatocytes associated with seroconversion from HBeAg to anti-HBe.

During the period of replication, the viral genome may integrate into the chromosomal DNA of some hepatocytes and these cells may persist and expand clonally. Rarely, seroconversion to anti-HBs follows clearance of virus replication but, more frequently, HBsAg persists during a second phase of chronicity as a result of the expression of integrated viral DNA.

Immune responses

Antibody and cell-mediated immune responses to various types of antigens are induced during the infection; however, not all of these are protective and in some instances they may cause autoimmune phenomena that contribute to disease pathogenesis. The immune response to infection with HBV is directed toward at least three antigens: HBsAg, the core antigen and the e antigen. The view that HBV exerts its damaging effect on hepatocytes by direct cytopathic changes is inconsistent with the persistence of large quantities of surface antigen in liver cells of many apparently healthy people who are carriers. Additional evidence suggests that the pathogenesis of liver damage in the course of HBV infection is related to the immune response by the host.

The surface antigen appears in the serum of most patients during the incubation period, 2–8 weeks before biochemical evidence of liver damage or onset of jaundice. The antigen persists during the acute illness and usually clears from the circulation during convalescence. Next to appear in the circulation is the virus-associated DNA polymerase activity, which correlates in time with damage to liver cells as indicated by elevated serum transaminases. The polymerase activity persists for days or weeks in acute cases and for months or years in some persistent carriers. Antibody of the IgM class to the core antigen is found in the serum 2–10 weeks after the surface antigen appears and persists during replication of the virus. Core antibody of the IgG class is detectable for many years after recovery. Finally, antibody to the surface antigen component, anti-HBs, appears.

During the incubation period and during the acute phase of the illness, surface antigen-antibody complexes may be found in the serum of some patients. Immune complexes have been found by electron microscopy in the serum of all patients with fulminant hepatitis, but are seen only infrequently in nonfulminant infection. Immune complexes are also important in the pathogenesis of other disease syndromes characterized by severe damage of blood vessels (e.g. polyarteritis nodosa, some forms of chronic glomerulonephritis and infantile papular acrodermatitis).

Immune complexes have been identified in variable proportions of patients with virtually all the recognized chronic sequelae of acute hepatitis B. Deposits of such immune complexes have also been demonstrated in the cytoplasm and plasma membrane of hepatocytes and on or in the nuclei; the reason why only a small proportion of patients with circulating complexes develop vasculitis or polyarteritis is, however, not clear. Perhaps complexes are critical pathogenic factors only if they are of a particular size and of a certain antigen-antibody ratio.

Figure 214-6 Possible consequences of hepatitis B virus infection in an adult.

Cellular immune responses are known to be particularly important in determining the clinical features and course of viral infections. The occurrence of cell-mediated immunity to HBV antigens has been demonstrated in most patients during the acute phase of hepatitis B and in a significant proportion of patients with surface-antigen-positive chronic active hepatitis, but not in asymptomatic persistent HBV carriers. These observations suggest that cell-mediated immunity may be important in terminating the infection and, in certain circumstances, in promoting liver damage and in the genesis of autoimmunity. Evidence also suggests that progressive liver damage may result from an autoimmune reaction directed against hepatocyte membrane antigens, initiated in many cases by infection with HBV.

Hepatitis B virus and hepatocellular carcinoma

When tests for HBsAg became widely available, regions of the world where the chronic carrier state is common were found to be coincident with those where there is a high prevalence of primary liver cancer (Fig. 214.7). Furthermore, in these areas, patients with this tumor are almost invariably seropositive for HBsAg. A prospective study in Taiwan revealed that 184 cases of hepatocellular carcinoma occurred in 3454 carriers of HBsAg at the start of the study, but only 10 such tumors arose in the 19,253 control males who were HBsAg-negative.[11]

Other case-control and cohort studies and laboratory investigations indicate that there is a consistent and specific causal association between HBV and hepatocellular carcinoma and that up to 80% of such cancers are attributable to this virus. Hepatitis B is thus second

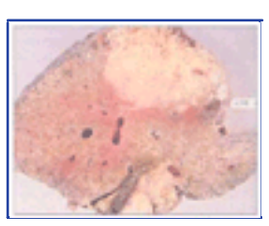

Figure 214-7 Hepatocellular carcinoma.

only to tobacco among the known human carcinogens.[12] Primary liver cancer is the seventh most common cancer in males and the ninth most common in females. Hepatocellular carcinoma is one of the three most common causes of cancer deaths in males in east and South East Asia, the Pacific Basin and sub-Saharan Africa.

Southern hybridization of tumor DNA yields evidence of chromosomal integration of viral sequences in at least 80% of hepatocellular carcinomas from HBsAg carriers. There is no similarity in the pattern of integration between different tumors, and variation is seen both in the integration sites and in the number of copies or partial copies of the viral genome. Sequence analysis of the integrants reveals that the direct repeats in the viral genome often lie close to the virus-cell junctions, suggesting that sequences around the ends of the viral genome may be involved in recombination with host DNA. Integration seems to involve microdeletion of host sequences, and rearrangements and deletions of part of the viral genome also may occur. When an intact surface gene is present, the tumor cells may produce and secrete HBsAg in the form of 22nm particles. Production of HBcAg by tumors is rare, however, and the core ORF is often incomplete, and modifications such as methylation may also modulate its expression. Cytotoxic T lymphocytes targeted against core gene products on the hepatocyte surface seem to be the major mechanism of clearance of infected cells from the liver. Thus, there may be immune selection of cells with integrated viral DNA that are incapable of expressing HBcAg.

The mechanisms of oncogenesis by HBV remain uncertain. Hepatitis B virus may act non-specifically by stimulating active regeneration and cirrhosis, which may be associated with chronicity. However, HBV-associated tumors arise occasionally in the absence of cirrhosis, and such hypotheses do not explain the frequent finding of integrated viral DNA in tumors. In rare instances, the viral genome has been found to be integrated into cellular genes such as cyclin A and a retinoic acid receptor. Translocations and other chromosomal rearrangements have also been observed. Although insertional mutagenesis of HBV remains an attractive hypothesis to explain

its oncogenicity, there is insufficient supportive evidence.

Like many other cancers, the development of hepatocellular carcinoma is likely to be a multifactorial process. The clonal expansion of cells with integrated viral DNA seems to be an early stage in this process, and such clones may accumulate in the liver throughout the period of active virus replication. In areas where the prevalence of primary liver cancer is high, virus infection usually occurs at an early

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age and virus replication may be prolonged, although the peak incidence of tumor is many years after the initial infection.

PREVENTION

The discovery of variation in the epitopes presented on the surface of the virions and subviral particles identified several subtypes of HBV, which differ in their geographic distribution. All isolates of the virus share a common epitope — *a.* This epitope is a domain of the major surface protein, which is believed to protrude as a double loop from the surface of the particle. Two other pairs of mutually exclusive antigenic determinants — *d* or *y* and *w* or *r* — are also present on the major surface protein. These variations have been correlated with single nucleotide changes in the surface ORF, which lead to variation in single amino acids in the protein. Four principal subtypes of HBV are recognized: *adw*, *adr*, *ayw* and *ayr.* Subtype *adw* predominates in northern Europe, the Americas and Australasia, and it is also found in Africa and Asia. Subtype *ayw* is found in the Mediterranean region, eastern Europe, northern and western Africa, the Middle East and the Indian subcontinent. In the Far East, subtype *adr* predominates, but the rarer subtype *ayr* is occasionally found in Japan and Papua New Guinea.

Passive immunization

Hepatitis B immunoglobulin (HBIG) is prepared specifically from pooled plasma with high titer of hepatitis B surface antibody and may confer temporary passive immunity under certain defined conditions. The major indication for the administration of HBIG is a single acute exposure to HBV, such as occurs when blood containing surface antigen is inoculated, ingested or splashed on mucous membranes and the conjunctiva. The optimal dose has not been established but doses in the range of 250–500IU have been used effectively. It should be administered as early as possible after exposure and preferably within 48 hours. The dose is usually 3ml (200IU/ml) in adults. The first dose should not be administered after 7 days following exposure. It is generally recommended that two doses of HBIG should be given 30 days apart.

Results with the use of HBIG for prophylaxis in neonates at risk of infection with HBV are encouraging if the immunoglobulin is given as soon as possible after birth or within 12 hours of birth, and the chance of the baby developing the persistent carrier state is reduced by about 70%. More recent studies using combined passive and active immunization indicate an efficacy approaching 90%. The dose of HBIG recommended in the newborn is 1–2ml (200IU/ml).

Active immunization

The major response of recipients of hepatitis B vaccine is to the common *a* epitope with consequent protection against all subtypes of the virus. First-generation vaccines were prepared from 22nm HBsAg particles purified from plasma donations from chronic carriers. These preparations are safe and immunogenic but have been superseded in some countries by recombinant vaccines produced by the expression of HBsAg in yeast cells. The expression plasmid contains only the 3' portion of the HBV surface ORF, and only the major surface protein, without pre-S epitopes, is produced. Vaccines containing pre-S2 and pre-S1, as well as the major surface proteins expressed by recombinant DNA technology, are undergoing clinical trials.

In many areas of the world with a high prevalence of HBsAg carriage, such as China and South East Asia, the predominant route of transmission is perinatal. Although HBV does not usually cross the placenta, the infants of viremic mothers have a very high risk of infection at the time of birth.

Administration of a course of vaccine with the first dose immediately after birth is effective in preventing transmission from an HBeAg-positive mother in approximately 70% of cases and this protective efficacy rate may be increased to greater than 90% if the vaccine is accompanied by the simultaneous administration of HBIG.

Immunization against HBV is now recognized as a high priority in preventive medicine in all countries and strategies for immunization are being revised. Universal vaccination of infants and adolescents is under examination as a possible strategy to control the transmission of this infection. More than 150 countries now offer universal hepatitis B vaccine, including the USA, Canada and most western European countries.[13]

In a number of countries with a low prevalence of hepatitis B, immunization against HBV is recommended only to groups that are at an increased risk of acquiring this infection. These groups include people requiring repeated transfusions of blood or blood products, people undergoing prolonged inpatient treatment, patients who require frequent tissue penetration or need repeated circulatory access, patients with natural or acquired immune deficiency, and patients with malignant diseases. Viral hepatitis is an occupational hazard among health care personnel and the staff of institutions for the mentally retarded and some semiclosed institutions. High rates of infection with HBV occur in narcotic drug addicts and intravenous drug abusers, sexually active male homosexuals and prostitutes. People working in highly endemic areas are at an increased risk of infection and should be immunized. Young infants, children and susceptible people (including travelers) living in certain tropical and subtropical areas where socio-economic conditions are poor and the prevalence of hepatitis B is high should also be immunized. It should be noted that, in about 30% of patients with hepatitis B, the mode of infection is not known — this is a powerful argument in favor of universal immunization.

Site of injection for vaccination and antibody response

Hepatitis B vaccination should be given in the upper arm or the anterolateral aspect of the thigh and not in the buttock. There are over 100 reports of unexpectedly low antibody seroconversion rates after hepatitis B vaccination using injection into the buttock. In one center in the USA a low antibody response was noted in 54% of healthy adult health care personnel. Many studies have since shown that the antibody response rate is significantly higher in centers using deltoid injection than centers using the buttock. On the basis of antibody tests after vaccination, the Advisory Committee on Immunization Practices of the Centers of Disease Control and Prevention in the USA recommended that the arm be used as the site for hepatitis B vaccination in adults, as has the Department of Health in the UK.

These observations have important public health implications, well illustrated by the estimate that about 20% of the 60,000 people immunized against HBV in the buttock in the USA by March 1985 had failed to attain a minimum level of antibody of 10IU/l and were therefore not protected.

Hepatitis B surface antibody titers should be measured in all people who have been immunized against HBV by injection in the buttock, and when this is not possible a complete course of three injections of vaccine should be administered into the deltoid muscle or the anterolateral aspect of the thigh, the only acceptable sites for HBV immunization.[14]

Apart from the site of injection there are several other factors that are associated with a poor antibody response or no antibody response to currently licensed vaccines. Indeed, all studies of antibody response to plasma-derived HBV vaccines and HBV vaccines prepared by recombinant DNA technology have shown that 5–10% or more of healthy immunocompetent subjects do not mount an antibody response (anti-HBs) to the surface antigen component (HBsAg) present in these preparations (i.e. they are nonresponders) or that

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they respond poorly (i.e. they are hyporesponders). The exact proportions of each group depends partly on the definition of nonresponsiveness and hyporesponsiveness; the usual definitions are a level of less than 10IU/l for nonresponders and 100IU/l for hyporesponders, measured against an international antibody standard.

Hepatitis B surface antibody escape mutants

Production of antibodies to the group antigenic determinant *a* mediates cross-protection against all subtypes, as has been demonstrated by challenge with a second subtype of the virus following recovery from an initial experimental infection. The epitope *a* is located in the region of amino acids 124–148 of the major surface protein and appears to have a double-loop conformation. A monoclonal antibody that recognizes a region within this *a* epitope is capable of neutralizing the infectivity of HBV for chimpanzees, and competitive inhibition assays using the same monoclonal antibody demonstrate that equivalent antibodies are present in the sera of subjects

immunized with either plasma-derived or recombinant HBV vaccine.

During a study of the immunogenicity and efficacy of HBV vaccines in Italy, a number of people who had apparently mounted a successful immune response and became anti-HBs-positive, later became infected with HBV. These cases were characterized by the co-existence of noncomplexed anti-HBs and HBsAg, and in 32 of 44 vaccinated subjects there were other markers of HBV infection.

Furthermore, analysis of the antigen using monoclonal antibodies suggested that the *a* epitope was either absent or masked by antibody. Subsequent sequence analysis of the virus from one of these cases revealed a mutation in the nucleotide sequence encoding the *a* epitope, the consequence of which was a substitution of arginine for glycine at amino acid position 145.[6]

There is now considerable evidence for a wide geographic distribution of the point mutation in HBV from guanosine to adenosine at position 587, resulting in an amino acid substitution at position 145 from glycine to arginine in the highly antigenic group determinant *a* of the surface antigen. This is a stable mutation that has been found in viral isolates from children and adults. It has been described in Italy, Singapore, Japan, Brunei, Taiwan, Hong Kong, India, Germany and the USA;[7] from liver transplant recipients with hepatitis B in the USA, Germany and the UK who had been treated with specific hepatitis B immunoglobulin or humanized hepatitis B monoclonal antibody; and in patients with chronic hepatitis in Japan[8] and elsewhere.

The region in which this mutation occurs is an important virus epitope to which vaccine-induced neutralizing antibody binds, as discussed above, and the mutant virus is not neutralized by antibody to this specificity. It can replicate as a competent virus, implying that the amino acid substitution does not alter the attachment of the virus to the liver cell. Variants of HBV with altered antigenicity of the envelope protein show that HBV is not as antigenically singular as previously believed and that humoral escape mutation can occur in vivo. This finding gives rise to two causes for concern: failure to detect HBsAg may lead to transmission through donated blood or organs, and HBV may infect people who are anti-HBs-positive after immunization. Variation in the second loop of the *a* determinant seems especially important.

Hepatitis B virus precore mutants

The nucleotide sequence of the genome of a strain of HBV cloned from the serum of a naturally infected chimpanzee has been reported.[9] A surprising feature was a point mutation in the penultimate codon of the precore region, which changed the tryptophan codon (TGG) to an amber termination codon (TAG). The nucleotide sequence of the HBV precore region from a number of anti-HBe-positive Greek patients was investigated by direct sequencing PCR-amplified HBV DNA from serum.[10] An identical mutation of the penultimate codon of the precore region to a termination codon was found in 7 of 8 anti-HBe-positive patients who were positive for HBV DNA in serum by hybridization. In most cases there was an additional mutation in the proceeding codon.

Similar variants were found by amplification of HBV DNA from serum from anti-HBe-positive patients in Italy and Greece. These variants are not confined to the Mediterranean region; the same nonsense mutation (without a second mutation in the adjacent codon) has been observed in patients from Japan and elsewhere, along with rarer examples of defective precore regions caused by frameshifts or loss of the initiation codon for the precore region.

Some precore variants may be more pathogenic than the wild-type virus because in many patients with severe chronic liver disease precore variants are found.

DIAGNOSTIC MICROBIOLOGY

Direct demonstration of virus in serum samples is feasible by visualizing the virus particles by electron microscopy, by detecting virus-associated DNA polymerase, by assay of viral DNA and by amplification of viral DNA by various techniques. All these direct techniques are often impractical in the general diagnostic laboratory, and specific diagnosis must therefore rely on serologic tests (Table 214.3).

Hepatitis B surface antigen first appears during the late stages of the incubation period and is easily detectable by radioimmunoassay or enzyme immunoassay. Enzyme immunoassay is specific and highly sensitive and is used widely in preference to radioisotope methods. The antigen persists during the acute phase of the disease and sharply decreases when antibody to the surface antigen becomes detectable. Antibody of the IgM class to the core antigen is found in the serum after the onset of the clinical symptoms and slowly declines after recovery. Its persistence at high titer suggests continuation of the infection. Core antibody of the IgG class persists for many years and provides evidence of past infection.

TABLE 214-3 -- Interpretation of results of serologic tests for hepatitis B virus.

INTERPRETATION OF RESULTS OF SEROLOGIC TESTS FOR HEPATITIS B						
			Anti-HBc			
HBsAg	**HBeAg**	**Anti-HBe**	**IgM**	**IgG**	**Anti-HBs**	**Interpretation**
+	+	-	-	-	-	Incubation period
+	+	-	+	+	-	Acute hepatitis B or persistent carrier state
+	+	-	-	+	-	Persistent carrier state
+	-	+	±	+	-	Persistent carrier state
-	-	+	±	+	+	Convalescence
-	-	-	-	+	+	Recovery
-	-	-	+	-	-	Infection with HBV without detectable HBsAg
-	-	-	-	+	-	Recovery with loss of detectable anti-HBs
-	-	-	-	-	+	Immunization without or recovery from infection with loss of detectable anti-HBc

CLINICAL MANIFESTATIONS

The clinical features of acute infection resemble those of the other viral hepatitides. Acute hepatitis B is frequently anicteric and asymptomatic, although a severe illness with jaundice can occur and occasionally acute liver failure may develop.

Hepatitis A and E viruses do not persist in the liver and there is no evidence of progression to chronic liver damage. Hepatitis B, with or without its satellite hepatitis D, and hepatitis C may be associated with persistent infection, a prolonged carrier state and progression to chronic liver disease, which may be severe. There is an etiologic association between hepatitis B and C viruses and hepatocellular carcinoma. GB virus C (GBV-C) tends to cause persistent infection and further studies are required (see Chapter 48).

HEPATITIS D VIRUS

This virus requires hepadnavirus helper functions for propagation in hepatocytes and is an important cause of acute and severe chronic liver damage in some regions of the world.

NATURE

Hepatitis D virus (HDV) is an unusual single-stranded circular RNA virus with a number of similarities to certain plant viral satellites and viroids.

Delta hepatitis[15] was first recognized following detection of a novel protein, d-antigen (hepatitis D antigen, HDAg), by immunofluorescent staining in the nuclei of hepatocytes from patients with chronic active hepatitis B. Hepatitis D virus is now known to require a helper function of HBV for its transmission. The virus is coated with HBsAg, which is needed for release of the HDV from the host hepatocyte and for entry in the next round of infection.

Two forms of HDV infection are known. In the first form, a susceptible person is co-infected with HBV and HDV, often leading to a more severe form of acute hepatitis caused by HBV. In the second form, a person who is chronically infected with HBV becomes superinfected with HDV. This may cause a second episode of clinical hepatitis and accelerate the course of the chronic liver disease, or cause overt disease in asymptomatic HBsAg carriers. Hepatitis D virus itself seems to be cytopathic and HDAg may be directly cytotoxic.

The HDV particle is approximately 36nm in diameter. It is composed of an RNA genome associated with HDAg, surrounded by an envelope of HBsAg. The HDV genome is a closed circular RNA molecule of 1679 nucleotides and resembles those of the satellite viroids and virusoids of plants, and similarly seems to be replicated by the host RNA polymerase II with autocatalytic cleavage and circularization of the progeny genomes by way of *trans*-esterification reactions (ribozyme activity). Consensus sequences of viroids that are believed to be involved in these processes also are conserved in HDV.[16]

EPIDEMIOLOGY

Hepatitis D is common in some areas of the world with a high prevalence of HBV infection, particularly Italy and other countries bordering the Mediterranean; eastern Europe, particularly Romania; the Middle East; the former Soviet Union; South America, particularly the Amazon basin, Venezuela, Columbia (*hepatitis de Sierra Nevada de Santa Marta*), Brazil (labrea black fever) and Peru; and parts of Africa, particularly western Africa. Antibody to HDV has been found in most countries, commonly among intravenous drug abusers, patients with hemophilia and those requiring treatment by blood and blood products. It has been estimated that 5% of HBsAg carriers worldwide (approximately 18,000,000 people) are infected with HDV. In areas of low prevalence of HBV, those at risk of hepatitis B, particularly intravenous drug abusers, are also at risk of hepatitis D.

The ratio of clinical to subclinical cases of HDV and superinfection is not known. However, the general severity of both forms of infection suggests that most cases are clinically significant. A low persistence of infection occurs in 1–3% of acute infections and in 80% or more of cases of superinfection in chronic HBV carriers. The mortality rate is high, particularly in the case of superinfection, and ranges from 2% to 20%.

PATHOGENICITY

Hepatitis B virus provides a helper function to HDV, which is a defective virus. The histopathologic pattern in the liver is suggestive of a direct cytopathic effect.

Pathologic changes are limited to the liver and histologic changes are those of acute and chronic hepatitis with no particular distinguishing features apart from severity and, in tropical areas in particular, microvesicular steatosis.

It should be noted, however, that the virus was discovered by specific nuclear fluorescence in hepatocytes of patients with chronic hepatitis B.

The modes of transmission are similar to the parenteral transmission of HBV.

PREVENTION

Prevention and control for HDV are similar to those for HBV. Immunization against HBV protects against HDV. The difficulty is protection against superinfection of the many millions of established carriers of HBV. Studies are in progress to determine whether specific immunization against HDV based on HDAg is feasible.

DIAGNOSTIC MICROBIOLOGY

Laboratory diagnosis in acute infection is based on specific serologic tests for anti-HDV IgM or HDV-RNA or HDAg in serum. Acute infection is usually self-limiting and markers of HDV infection often disappear within a few weeks.

Superinfection with HDV in chronic hepatitis B may lead to suppression of HBV markers during the acute phase. Chronic infection with HDV (and HBV) is the usual outcome in nonfulminant disease.

CLINICAL MANIFESTATIONS

The clinical features of hepatitis D are identical to those of hepatitis A (see above). For further manifestations see Chapter 48 .

HEPATITIS C VIRUS

NATURE

Hepatitis C virus (HCV) is an enveloped single-stranded RNA virus that appears to be distantly related (possibly in its evolution) to flaviviruses, although HCV is not transmitted by arthropod vectors.

Hepatitis C virus is unusual because it was identified using molecular methods rather than a conventional virologic approach. Transmission studies in chimpanzees established that the main agent of parenterally acquired non-A, non-B hepatitis was likely to be an enveloped virus some 30–60nm in diameter. Using infected chimpanzee plasma as a starting point, complementary DNA was used to create a library which was screened using serum from a patient with chronic non-A, non-B hepatitis. This approach led to the detection of

a clone that was found to bind to antibodies present in the serum of several patients infected with non-A, non-B hepatitis. Eventually, clones covering the entire viral genome were assembled and the complete nucleotide sequence determined.[17]

Properties of hepatitis C virus

The genome of HCV resembles those of the pestiviruses and flaviviruses in that it comprises around 10,000 nucleotides of positivesense RNA, lacks a 3' poly-A tract and has a similar gene organization. It has been proposed that HCV should be the prototype of a third genus in the family Flaviviridae. All these genomes contain a single large ORF, which is translated to yield a polyprotein (of around 3000 amino acids in the case of HCV) from which the viral proteins are derived by post-translational cleavage and other modifications.

The amino acid sequence of the nucleocapsid protein seems to be highly conserved among different isolates of HCV. The next domain in the polyprotein also has a signal sequence at its carboxyl-terminus and may be processed in a similar fashion. The product is a glycoprotein that is probably found in the viral envelope and is variably termed E1/S or gp35. The third domain may be cleaved by a protease within the viral polyprotein to yield what is probably a second surface glycoprotein, E2/NS1 or gp70. These glycoproteins have not been found in vivo and the molecular sizes have been estimated from sequence data and expression studies in vitro.

Other post-translational modifications, including further proteolytic cleavages, are possible. These proteins are the focus of considerable interest because of their potential use in tests for the direct detection of viral proteins and for HCV vaccines. Nucleotide sequencing studies reveal that both domains contain hypervariable regions. It is possible that this divergence has been driven by antibody selection pressure and that these regions specify important immunogenic epitopes.

The nonstructural region of the HCV genome is divided into regions NS2 to NS5 (Fig. 214.8). In the flaviviruses, NS3 has two functional domains: a protease, which is involved in cleavage of the non-structural region of the polyprotein, and a helicase, which is presumably involved in RNA replication. Motifs within this region of the HCV genome have homology to the appropriate consensus sequences, suggesting similar functions. NS5 seems to be the replicase and contains the Gly-Asp-Asp motif common to viral RNA-dependent RNA polymerases.

Hepatitis C virus consists of a family of highly related but nevertheless distinct genotypes — presently numbering six — and various subtypes with differing geographic distribution and a complex nomenclature. The C, NS3 and NS4 domains are the most highly conserved regions of the genome and therefore these proteins are the most suitable for use as capture antigens for broadly reactive tests for antibodies to HCV. The sequence differences observed between HCV groups suggest that virus-host interactions may be

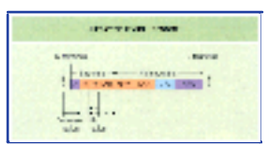

Figure 214-8 Hepatitis C viral genome.

different, which could result in differences in pathogenicity and in response to antiviral therapy.

It is important, therefore, to develop group-specific and virus-specific tests. The degree of divergence that is apparent within the viral envelope proteins implies the absence of a broad cross-neutralizing antibody response to infection by viruses of different groups.

In addition to the sequence diversity observed between HCV groups, there is considerable sequence heterogeneity among almost all HCV isolates in the amino-terminal region of E2-NS1, implying that this region may be under strong immune selection. Indeed, sequence changes within this region may occur during the evolution of disease in individual patients and may play an important role in progression to chronicity.

EPIDEMIOLOGY

Infection with HCV occurs throughout the world. Much of the seroprevalence data are based on blood donors, who represent a carefully selected population. The prevalence of antibodies to HCV in blood donors varies from 0.02% to 1.25% in different countries. Higher rates have been found in southern Italy, Spain, central Europe, Japan and parts of the Middle East, with as many as 19% in Egyptian blood donors. Until screening of blood donors was introduced, hepatitis C accounted for the vast majority of non-A, non-B post-transfusion hepatitis. However, it is clear that, although blood transfusion and the transfusion of blood products are efficient routes of transmission of HCV, these represent a small proportion of cases of acute clinical hepatitis in the USA and a number of other countries (with the exception of patients with hemophilia). Current data indicate that in some 50% of patients in industrialized countries, the source of infection cannot be identified, although 35% of patients have a history of intravenous drug use, and occupational exposure in the health care setting accounts for about 2% of cases. Household contact and sexual exposure do not appear to be major factors in the epidemiology of this common infection. Transmission of HCV from mother to infant occurs in about 10% of viremic mothers and the risk appears to be related to the level of viremia. The possibility of transmission in utero is also being investigated.

PREVENTION

Difficulties in vaccine development include the sequence diversity between viral genotypes and the substantial sequence heterogeneity among isolates in the amino-terminal region of E2-NS1. Neutralizing antibodies have not been clearly defined. The virus has not been cultivated in vitro to permit the development of inactivated or attenuated vaccines (compared with yellow fever vaccines). Much work is in progress employing recombinant DNA techniques.

DIAGNOSTIC MICROBIOLOGY

Successful cloning of portions of the viral genome have permitted the development of new diagnostic tests for infection by the virus. Because the antigen was originally detected by antibodies in the serum of an infected patient it was an obvious candidate as the basis of an enzyme-linked immunosorbent assay (ELISA) to detect anti-HCV antibodies. A larger clone, C100, was assembled from a number of overlapping clones and expressed in yeast as a fusion protein using human superoxide dismutase sequences to facilitate expression. This fusion protein formed the basis of first generation tests for HCV infection.

It is now known that antibodies to C100 are detected relatively late after acute infection. Furthermore, the first generation ELISAs were associated with a high rate of false-positive reactions when applied to low-incidence populations and there were further problems with

some retrospective studies on stored sera. Second-generation tests include antigens from the nucleocapsid and nonstructural regions of the genome. The former antigen (C22) is particularly useful and antibodies to the HCV core protein appear relatively early in the course of infection.

Positive reactions by ELISA require confirmation by supplementary testing using, for example, a recombinant immunoblot assay. Nevertheless, indeterminant results obtained by ELISA represent a significant problem that need resolution. It should also be noted that the time for seroconversion is variable, and that when it can be measured more precisely (e.g. after transfusion) it is generally 7–31 weeks.

The presence of antibodies to specific antigen components is variable and may or may not reflect viremia and, in the case of interferon treatment, a correlation between response and loss of specific antibodies to the E2 component.

Detection and monitoring of viremia are important for management and treatment. Sensitive techniques are available for the measurement of HCV RNA based on reverse-transcription (RT)-PCR amplification, nested PCR, signal amplification using branched DNA analytes and others.

The identification of specific types and subtypes is becoming increasingly important, with observations suggesting that there is an association between response to interferon and particular genotypes and that different types may differ in their pathogenicity.

CLINICAL MANIFESTATIONS

Most acute infections are asymptomatic, and about 20% of acute infections cause jaundice. Fulminant hepatitis has been described. Extrahepatic manifestations include mixed cryoglobulinemia, membranous proliferative glomerulonephritis and porphyria cutanea tarda (see Chapter 48).

Current data suggest that about 80% of infections with HCV progress to chronicity. Histologic examination of liver biopsies from asymptomatic HCV carriers (among blood donors) has revealed none with normal histology; indeed up to 70% have been found to have chronic active hepatitis, cirrhosis, or both (Fig 214.9 and Fig 214.10). Whether the virus is cytopathic or whether there is an

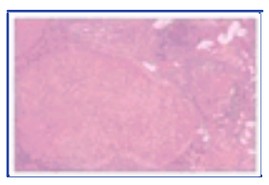

Figure 214-9 Hepatitis C virus active cirrhosis.

Figure 214-10 Acute hepatitis C.

immunopathologic element remains unclear. Infection with HCV is also associated with progression to primary liver cancer. For example, in Japan, where the incidence of hepatocellular carcinoma has been increasing despite a decrease in the prevalence of HBsAg, HCV is now believed to be a major risk factor.

OTHER HEPATITIS VIRUSES

Virus-like particles (referred to by some as candidate hepatitis F virus) have been identified by electron microscopy in the livers and grafts in a subset of British patients with sporadic fulminant hepatitis in whom liver failure recurred about 7 days after grafting.[18] However, subsequent intensive search by advanced molecular techniques have failed to identify a candidate viral agent.

About 30 years ago, a series of transmission studies of human viral hepatitis were initiated in small South American tamarins or marmosets, which were chosen because their very limited contact with humans implied that they were unlikely to have been infected with human viruses.[19]

Serum obtained on the third day of jaundice from a young surgeon (GB) induced hepatitis in each of four inoculated marmosets and was passaged serially in these animals. These important observations remained controversial until the recent application of modern molecular virologic techniques.[20] Preliminary results indicate the identification of two independent viruses, GB virus A (GBV-A) and GB virus B (GBV-B), in the infectious plasma of tamarins inoculated with GB.[21]

GB virus A does not replicate in the liver of tamarins, whereas GBV-B causes hepatitis. Cross-challenge experiments showed that infection with the original infectious tamarin inoculum conferred protection from reinfection with GBV-B but not GBV-A. A third virus, GBV-C, was subsequently isolated from a human specimen that was immunoreactive with a GBV-B protein. GB virus C RNA was found in several patients with clinical hepatitis and was shown to have substantial sequence identity to GBV-A.

A series of studies, including phylogenetic analysis of genomic sequences, showed that GBV-A, GBV-B and GBV-C are not genotypes of HCV and that GBV-A and GBV-C are closely related. GBV-A-GBV-C, GBV-B and HCV are members of distinct viral groups. The organization of the genes of the GBV-A, GBV-B and GBV-C genomes shows that they are related to other positive-strand RNA viruses with local regions of sequence identity with various flaviviruses. The three GB viruses and HCV share only limited overall amino acid sequence identity.[22]

Serologic reagents were prepared with recombinant antigens and limited testing for antibodies and by RT-PCR for specific RNA was carried out in groups of patients, blood donors and other selected people — patients with non-A,B,C,D,E hepatitis, multitransfused patients, intravenous drug abusers and other populations with a high incidence of viral hepatitis. Preliminary studies indicated the presence of antibody to each of the GB viruses in 3–14% of these people. The development and availability of specific diagnostic reagents will establish the epidemiology of these newly identified viruses, their pathogenic significance in humans, and their clinical and public health importance. It should be noted that the virus identified more recently as hepatitis G (HGV) as a new transfusion transmitted agent[23] is now believed to be identical to GBV-C. [24]

The blood-borne nature of GBV-C/HGV has been clearly demonstrated and there is evidence that the virus persists. The association of the virus with liver damage is illustrated by raised levels of alanine transaminase and detectable HGV RNA in a number of patients. However, 40–90% of viremic subjects have normal alanine transaminase levels. In a significant proportion of patients there is co-infection with HBV, HCV, or both. The primary manifestations of

GBV-C/HGV infection may be extrahepatic or hepatic and these may be the result of co-infection with the hepatitis viruses or an (as yet unidentified) hepatotropic agent. The development of sensitive and specific serologic tests for GBV-C/HGV and the application of modern virologic techniques and histologic studies where appropriate will determine the clinical significance of this newly identified virus, which at present appears to be a virus in search of a disease.[25]

A novel human virus was isolated in Japan in 1997 from the serum of a patient with post-transfusion hepatitis and was designated TT virus (TTV) after the initials of the patient (TTV is not an acronym for transfusion-transmitted virus as has been assumed and perpetuated in numerous publications). The virus has a circular single strand DNA, non-enveloped, of negative polarity and is 3965 nucleotides in length. There is 30% diversity in the coding region accounting for numerous genotypes believed to result mainly from recombination. The virus replicates in many tissues, particularly the liver, and is shed into the blood and feces. TT virus DNA is found in saliva (78%), breast milk, semen (60%), cervical swabs and other body fluids of infected persons. The virus is ubiquitous and is found in 20% to more than 90% of the general population and healthy blood donors. There is no evidence of involvement of TTV in acute or chronic liver disease. TT virus is found in farm animals, including chickens (19%), cows (25%), pigs (20%) and sheep (30%), and in other mammals including nonhuman primates.

TTV-like mini virus (TLMV) and several related viruses such as SANBAN, YONBAN and others have been described, but without any disease association in humans. These different viruses have been divided into at least 29 genotypes with sequence divergence of more than 30% from each other and placed into four phylogenetic groups. Since 1999, much attention has been devoted to the SEN virus (again the initials of a patient infected with a virus possibly related to TTV). The SEN virus (SENV) was isolated from an immunocompromised HIV patient with post-transfusion hepatitis of unknown etiology. Eight genotypes of this virus have been described (SENV-A to SENV-H), each differing by at least 25% in nucleotide sequence. SENV-C, SENV-D and SENV-H are supposedly associated with transfusion hepatitis and, although the prevalence of these viruses is common in patients with non-A-E liver disease, a causal association has not been demonstrated and these should not be considered at present as candidate hepatitis viruses.[26]

MANAGEMENT

Although a substantial number of antiviral compounds have been evaluated for the treatment of chronic hepatitis B, interferon-a has been licensed in treatment. Under optimal conditions of careful selection of patients, 30–50% respond at least transiently and about 20% clear the virus, accompanied by improvement in liver function. Combination therapy with nucleoside analogues and other drugs have shown a variable response. Lamivudine and famciclovir are valuable for treatment of hepatitis B chronic liver disease. A comparison of prednisone withdrawal followed by treatment with interferon only did not provide evidence of added benefit for the combined regimen.

Interferon-a is also the only drug approved for the treatment of chronic hepatitis C. Again, the efficacy of interferon is limited in suitable patients, and 15–20% of patients will have a sustained virologic response. Pegylated interferon appears to be more effective, and combination therapy with ribavirin is now regarded as standard of care.

For a more detailed discussion of the management of hepatitis, see Chapter 48 and Chapter 207 .

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Chapter 215 - Herpesviruses

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NATURE

Taxonomy

The herpesviruses comprise a large family of DNA viruses[1] with over 150 individual members. They have been found in almost all species (both vertebrate and invertebrate) in which they have been actively sought. Their widespread occurrence suggests that they first colonized animal species at an early stage of evolution[2] and this ancient colonization has led to adaptation to their natural host so that they are generally highly host specific. This adaption is further exemplified by their high rates of infection (the prevalence of positive serology in adult populations worldwide is 80–90%), the generally mild symptoms associated with primary infection and their strategy for maintaining themselves in a population with a high level of immunity to infection by establishing a latent infection.

On the basis of their biologic properties the viruses comprising the family Herpesviridae are subdivided into three subfamilies — the Alphaherpesvirinae, the Betaherpesvirinae and the Gammaherpesvirinae (Table 215.1).[1] Each of the subfamilies is further subdivided into genera:

- the Alphaherpesvirinae has two genera — Simplexvirus and Varicellovirus;
- the Betaherpesvirinae has three genera — Cytomegalovirus, Muromegalovirus and Roseolovirus; and
- the Gammaherpesvirinae has two genera — Lymphocryptovirus and Rhadinovirus.

The current classification is somewhat subjective because it is based upon biologic properties. In the future, it is likely that a more precise system based on genetic information such as the organization and sequence of reiterated sequences within the genome of the virus (Fig. 215.1), the conservation of selected genes and gene clusters, and possibly comparisons of protein sequences will provide a more objective classification system.[3]

Under the present classification scheme[1] viruses are named according to their natural host species and numbered in accordance with the order in which they were first identified. As many viruses have acquired commonly used names — for example, human herpesvirus 1 is commonly known as herpes simplex virus (HSV) type 1 — the classification is not yet rigorously applied. However, newly discovered viruses are now named in accordance with this classification scheme, for example human herpesviruses 6, 7 and 8. At present eight herpesviruses are known to have the human species as their natural host (Table 215.2). Herpes B virus, a virus belonging to the Simplex genus of the Alphaherpesvirus subfamily and also known as cercopithecine herpesvirus 1 or herpesvirus simiae, is enzootic among old world macaques and usually causes minimal or no morbidity in its natural host. However, B virus infections in humans, mostly because of accidental exposure, may cause severe and often fatal disease.

Structure

The herpesvirus virion often has a pleomorphic appearance when seen by electron microscopy. It measures 150–300nm in diameter

TABLE 215-1 -- Biologic properties of the Herpesviridae.

BIOLOGIC PROPERTIES OF HERPESVIRIDAE	
Common properties	Spheric enveloped virions, 150–200nm in diameter
	Large, linear, dsDNA genome of 125–230kbp
	Synthesis of DNA and assembly of capsid within the nucleus, acquire envelope by budding through nuclear membrane
	Specify a large array of enzymes involved in nucleic acid metabolism and synthesis
	Production of progeny virus results in destruction of the host cell
	Establish latency in their natural host
Alphaherpesvirinae	Variable host range
	Short reproductive cycle
	Rapid spread in cell culture
	Efficient destruction of infected cells
	Establish latency primarily but not exclusively in sensory ganglia
Betaherpesvirinae	Restricted host range (a nonexclusive property of this subfamily)
	Long reproductive cycle
	Infection progresses slowly in culture, frequently forming enlarged (cytomegalic) cells
	Latency in secretory glands, lymphoreticular cells, kidneys and other tissues
Gammaherpesvirinae	Experimental host range limited to family or order of natural host
	In-vitro replication in lymphoblastoid cells
	In-vivo replication and latency in either T or B cells

and is composed of an internal protein nucleocapsid enclosing the double-stranded (ds) DNA genome and an external lipid envelope. The electron microscopic appearance of a typical herpesvirus is shown in Figure 215.2 .

DNA

The genetic information of the virus is encoded by a linear molecule of dsDNA and the size of this molecule varies for different herpesviruses, from approximately 80,000 to 150,000kDa (125–245kbp). The G+C composition of individual herpesvirus DNAs varies from 31 to 75%. The size of individual herpesvirus genomes varies by approximately 10kbp and this is usually attributable to the number of terminal and/or internal reiterated sequences (see Fig. 215.1). Within the virion the DNA is a linear molecule that is tightly wound in the form of a torus. The ends of this molecule appear to attach to

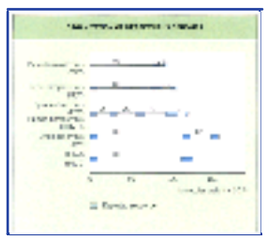

Figure 215-1 Organization of herpesvirus genomes. Inverted repeat sequences in VZV, HSV and CMV allow the genome to recombine in 2,4, and 4 isomers, respectively. Both HSV and CMV have a UL (long unique base sequence) and a US (short unique base sequence) each terminated by two sets of inverted repeated sequences. The repeated sequences allow the UL and US to invert relative to one another, so yielding four isometric forms of DNA. As there is only one set of inverted repeats in VZV, only two isomers of DNA can be produced. Both EBV and HHV-8 have only one isomeric form with several unique regions surrounded by direct repeated sequences.

the inner surface of the nucleocapsid, which prevents the DNA from circularizing until it is released during infection.

The phylogenetic relationship, based on a neighbor-joining analysis of the glycoprotein B gene, between the human herpesviruses and herpes B virus[4] is shown in Figure 215.3 .

Virion polypeptides

Herpes simplex virus type 1 virions contain about 33 virus-specific proteins, but more than double this number are found within an infected cell. The virion polypeptides (VPs) are designated by serial number. The transcription of mRNAs from the genome proceeds from both strands of the genome, in either direction, with evidence of overlapping transcription and of splicing of genes and gene products. [5] There are two sets of *cis*-acting genes embedded in the domains of viral genes. The first set enables binding of cellular transcription

TABLE 215-2 -- Human herpesviruses.

HUMAN HERPESVIRUSES			
ICTV name	**Common name**	**Subfamily**	**Genus**
Human herpesvirus-1	Herpes simplex virus (HSV)-1	Alphaherpesvirinae	Simplexvirus
Human herpesvirus-2	Herpes simplex virus-2	Alphaherpesvirinae	Simplexvirus
Human herpesvirus-3	Varicella-zoster virus (VZV)	Alphaherpesvirinae	Varicellovirus
Human herpesvirus-4	Epstein-Barr virus (EBV)	Gammaherpesvirinae	Lymphocryptovirus
Human herpesvirus-5	Human cytomegalovirus (CMV)	Betaherpesvirinae	Cytomegalovirus
Human herpesvirus-6	Human herpesvirus (HHV)-6	Betaherpesvirinae	Roseolovirus
Human herpesvirus-7	Human herpesvirus (HHV)-7	Betaherpesvirinae	-
Human herpesvirus-8	Kaposi's sarcoma-associated herpesvirus (KSHV)	Gammaherpesvirinae	Rhadinovirus
Two variants, 'a' and 'b', of human herpesvirus 4 and human herpesvirus 6 are known. ICTV, International Committee on Taxonomy of Viruses.[1]			

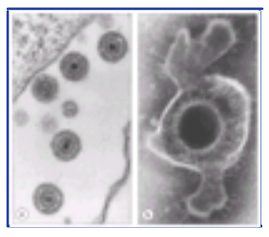

Figure 215-2 Enveloped virus particle. (a) Thin section. (b) Negative staining. These electron microscopic views (×140,000) show HSV. The DNA is surrounded by a nucleocapsid comprised of 162 individual protein subunits (150 hexavalent capsomers and 12 pentavalent capsomers) arranged in the form of an icosahedron. The nucleocapsid is in turn enclosed by the tegument and virus envelope bearing glycoprotein spikes. *Courtesy of Hans Gelderblom*.

factors and *trans*-acting factors to initiate and enhance viral gene expression. The second set enables interaction of genes with regulatory proteins (up- or downregulation). Three rounds of transcription and translation are observed, the so-called:

- ¦ a phase (resulting in the production of 'immediate-early proteins');
- ¦ ß phase (proteins responsible mainly for DNA metabolism); and
- ¦ ? phase (principally structural proteins).

Nucleocapsid

The icosahedral nucleocapsid is 100–110nm in diameter comprising 162 individual capsomers (i.e. 12 pentavalent and 150 hexavalent capsomers). Using electron cryomicroscopy and computer-aided image reconstruction the structure of herpesvirus particles has been determined at a resolution of 26 Å. In HSV individual capsomers are believed to be constructed from the four major capsid proteins VP5, VP26, VP23 and VP19.[6] [7] The interior and exterior of the capsid appear to be linked by transcapsomeric channels.

Figure 215-3 Phylogenetic relationship between the human herpesviruses and herpes B virus. The tree was created by neighbor-joining analysis of the glycoprotein B gene sequences. HVS, herpesvirus simiae (herpes B virus). *Adapted from Schultz, 2000.*[5]

Tegument

The amorphous electron-dense tegument bounded by the nucleocapsid and outer envelope of the virus contains at least eight proteins. These proteins serve important functions after the virus has penetrated the host cell (see below).

Envelope

The viral envelope is an extensively modified form of the original host cell nuclear membrane and bears a series of virus-specified glycoprotein spikes 9–15nm in length (see Fig. 215.2). The glycoprotein spikes define several of the major biologic attributes of the virus. There are major antigenic differences in glycoprotein spikes between the virus species. In HSV there are at least ten glycoproteins on the viral envelope (Table 215.3).

Physical properties

Herpesviruses are thermolabile. In cell culture media the half-life at 86°F (30°C) is between 1.5 and 14 hours. Drying in air leads to desiccation of the viral envelope and loss of infectivity. Ether in water (20%) and other lipid solvents such as 70% alcohol or chloroform rapidly inactivate herpesviruses completely.

EPIDEMIOLOGY

Transmission pathways

Herpesviruses are relatively fragile in that they require a lipid envelope to achieve attachment to and penetration of the host cell. As a consequence these viruses transmit most easily on contact with warm and moist mucosal layers. Two broad groupings of virus can be distinguished:

- those where virus is transmitted most effectively by oral secretions and nongenital contact; and
- those where the virus is transmitted most effectively by genital secretions.

These modes of transmission have a profound influence upon the epidemiology of the individual viruses.

TABLE 215-3 -- Herpes simplex virus glycoproteins.

HERPES SIMPLEX VIRUS GLYCOPROTEINS		
Glycoprotein	**Required for replication in cell culture**	**Function**
gB	+	Forms a dimer, essential for viral entry; induces neutralizing antibody
gC	-	Involved in cell attachment
gD	+	Required after attachment of virus to cell to allow virus entry into the cell
gE	-	In complex with gI; binds Fc portion of antibodies
gG	-	Involved in entry, egress and spread from cell to cell
gH	+	Forms complex with gL (see below); role in entry, egress and cell-to-cell spread
gI	-	gI and gE form a complex for transport to plasma membranes and gI and gE form an Fc receptor
gJ	-	Predicted from DNA sequence only
gK	+	Required for efficient egress (viral exocytosis)
gL	+	Forms complex with gH that is required for transport of gH and gL to the plasma membrane and for viral entry mediated by gH
gM	-	Not known
Information relates to HSV-1; not all glycoprotein species have been identified in other herpesviruses and the size, degree of glycosylation and other post-translational modifications vary for different viruses.		

Prevalence

Herpesviruses transmitted predominantly by oral secretions or nongenital contact are HSV-1, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus (HHV)-6 and HHV-7. Those transmitted predominantly by the sexual route are HSV-2 and HHV-8. The peak incidence for infection transmitted nongenitally is in early childhood (Table 215.4). The peak incidence for infections acquired by genital transmission is in adolescence and early adulthood. The rates of infection by viruses transmitted by the genital route are not usually as high as those for viruses transmitted by a nongenital route. However, for both groups there is a further distinct relation between rates of acquisition, sexual preference and the socio-economic status of the study population.

Geographic aspects

Herpesviruses are distributed worldwide and no animal reservoirs of infection are known for any of the human herpesviruses. Burkitt's lymphoma, which is associated with EBV infection, is endemic only in tropical Africa.[8] Nasopharyngeal carcinoma is also associated with EBV infection and is endemic in Japan and Southern China.[9] A sporadic, non-HIV-associated Kaposi's sarcoma is found predominantly in countries bordering the Mediterranean and in Central Africa.[10]

There is evidence that in tropical areas varicella is less prevalent in childhood than in temperate climatic areas and is more common in adults.[11] Possible explanations for this include the relative isolation of clusters of population in rural areas,[12] epidemiologic 'interference' through infections caused by other viruses, especially HSV,[13] and perhaps decreased efficiency of transmission as a result of the lability of VZV in areas where there is a high ambient temperature.[11]

TABLE 215-4 -- Features of herpesvirus infections.

FEATURES OF HERPESVIRUS INFECTIONS					
Virus	**Peak incidence of primary infection**		**Adult seroprevalence (%)**	**Principal route(s) of transmission**	**Notes**
	Childhood	**Adolescence**			
HSV-1	+++	+	75–95+	Oral secretions, close contact	Overall seroprevalence predominantly determined by socio-economic status
HSV-2	-	+++	4–95	Genital secretions, close contact	Lifetime number of sex partners is predominant influence on rates of seropositivity
VZV	+++	+	90–95	Aerosol, close contact	Epidemic spread in childhood, in tropics relatively more common in adults than children
CMV	++	++	40–95+	Oral secretions, genital secretions	Infection common in infancy, but a significant proportion of women of childbearing age are susceptible; overall seroprevalence predominantly determined by socio-economic status
EBV	++	++	70–95	Oral secretions	Second peak of incidence in early adolescence (glandular fever)
HHV-6	+++	-	>85	Oral secretions	Infection common in infancy — peak age of acquisition 2 years
HHV-7	+++	-	>85	Oral secretions	Infection common in infancy — peak age of acquisition 3 years
HHV-8	-	+++	10–25	Oral secretions, genital secretions	Homosexual men who have AIDS have highest seroprevalence
CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.					

Periodicity

The human herpesviruses are endemic in human populations as a result of their strategy of establishing latent infections. In addition, because of their modes of transmission there is no seasonal variation in the efficiency of transmission. For infections for which reactivation is infrequent (e.g. VZV infection) there may be 'outbreaks' of infection (mini-epidemics) when infection spreads rapidly through a nonimmune population. Outbreaks can also occur if large numbers of susceptible individuals are brought together (e.g. infectious mononucleosis among military recruits or college students).[14]

Determinants of infection

Early acquisition of infection is common for all herpesviruses transmitted by the nongenital route. In a classic study of HSV infection Burnet and Williams[15] showed that there was a clear relationship between the age of acquisition of the virus and socio-economic status. Populations associated with a low socio-economic environment collectively showed earlier acquisition of HSV infection than more affluent populations, although in both groups, infection rates of 90–95% were observed by early adulthood. Primary HSV-1 infection was rare in those over 30 years of age. In recent years several sero-epidemiologic studies have shown a general decrease in the overall prevalence of HSV-1 antibody in developed countries. However, even within individual countries there are variations in seroprevalence, for example the seroprevalence rates are generally higher among inner city residents than among those from rural areas. Here, the major factor determining the seroprevalence is the frequency of direct person-to-person contact rather than the socio-economic status of the individual populations.

If infection does not occur in infancy, transmission routes other than direct oral contact can constitute important risk factors. An example is found in the acquisition of CMV. Although most CMV infections occur in infancy a significant proportion (40–50% in developed countries) of women of child-bearing age are still susceptible to infection and may acquire the infection during pregnancy. Sexual transmission of infection is an important mode of acquisition of infection for these women. In addition, contact with young children of less than 24 months of age is a further important risk factor.

Viruses such as HSV-2 for which the principal mode of transmission is sexual are generally not acquired until the onset of sexual activity in adolescence and early adulthood. The major influence on acquisition of infection appears to be the number of sexual partners and sexual preference of an individual (as might be expected for a sexually transmitted virus). Seroprevalence rates of up to 95% have been reported in some female commercial sex workers. Women are generally infected at an earlier age (15–20%) than men, and rates of infection in women are higher for all age groups including those of 45 or more years of age.[16] Studies among patients attending a genitourinary medicine clinic suggest that only in homosexual men are the rates of infection with HSV-2 comparable to those found in women.

Whereas the seroprevalence of HHV-8 infection is low (<2%) in general populations, except in some parts of Italy, Greece and most of Africa, it is high in groups at high risk of infection such as homosexual men with large numbers of sex partners.[17]

PATHOGENICITY

Replication

The replicative cycle of the virus is illustrated schematically in Figure 215.4 . The initial attachment of a herpesvirus to a host cell is mediated by a glycoprotein or a complex of glycoproteins projecting from the virus envelope interacting with its specific receptor on the host cell.[4]

Initial contact of the herpesviruses with the cell is usually made by low-affinity binding to glycosaminoglycans, preferentially heparan sulphate, on the cell surface. In this way viruses are concentrated at the cell surface, facilitating subsequent binding to a specific receptor, for instance the complement receptor Cr2 (CD 21) in the case of EBV. The virus envelope and cell plasma membranes then fuse,

Figure 215-4 Herpesvirus replication. The tegument proteins effect the shut-down of host cell metabolism. On entry to the nucleus the DNA circularizes and binds a tegument protein and cellular factors to initiate transcription. Transcription and translation occur in three phases: immediate-early, early and late. Capsid proteins migrate into the nucleus and the viral DNA is encapsidated. The viral glycoproteins are extensively modified post-translationally by transit through the Golgi apparatus. The glycoproteins diffuse to the nuclear envelope. The nucleocapsids bud through the modified nuclear membrane and exit the cell via the endoplasmic reticulum or are released on cell lysis.

resulting in the introduction of tegument proteins and viral nucleocapsid into the cell cytoplasm.[18] This process probably involves several, if not all, of the virion surface glycoproteins and is accomplished rapidly.

The tegument proteins serve to both 'disable' the host cell and initiate viral replication. Soon after virus entry, host cell DNA synthesis is shut off, host cell protein synthesis declines rapidly and glycosylation of host cell proteins ceases. In this way the virus ensures that the metabolic machinery of the cell is fully available for virus replication. At the same time the virion nucleocapsid is transported via the cell cytoskeleton to a nuclear pore. At the nuclear pore the viral nucleocapsid breaks down releasing its DNA into the cell nucleus where the linear DNA molecule immediately circularizes.

Transcription of viral DNA takes place within the host cell nucleus involving three classes of mRNA, a, ß and ?, and including both host cell as well as viral proteins. In HSV, to initiate transcription, the circularized DNA must bind a host cell protein (OCT-1) to a *cis*-acting site and the tegument protein a *trans*-inducing factor (a-TIF) binds an additional factor designated C1 (and possibly others). This a-TIF-C1 complex then binds to the OCT-1-DNA complex.[6] a-TIF acts in *trans* to induce a (or 'immediate-early') genes — the first set of viral genes to be transcribed. Immediate-early or a proteins are control proteins that stimulate and regulate all the subsequent steps in the replicative cycle. All but one of the a proteins are regulatory proteins. Their production is essential to stimulate the production of ß polypeptides, which are the enzymes and other proteins involved in viral nucleic acid reproduction (e.g. DNA-dependent DNA polymerase, which reproduces the viral genome). Products of a and ß genes transactivate the translation of the ? genes, which produce the late or ? proteins, the structural proteins for the virion including the viral capsid and glycoproteins.

Productive infection is fatal for the host cell because in the process of viral DNA replication host cell chromosomes are degraded and appear as a chromatin ring around the borders of the nuclear membrane. In addition host cell metabolism is irreversible damaged.

Molecular and cellular basis of pathogenicity

The combination of virion surface glycoproteins and the distribution of cellular receptors provide a partial explanation of the cell and tissue specific tropism of members of the human herpesviruses. A common characteristic of the herpesviruses is their ability to persist in infected cells while expressing only a minimal set of genes needed for latency. After certain stimuli, or when the immune system is suppressed, the virus will be reactivated and undergo replication to produce new viral progeny. In individuals who have deficits in cell-mediated immunity (CMI), infection is more poorly controlled than in those who have intact CMI. Primary infection is in consequence more severe, and reactivation of latent infection is more likely to result in symptomatic disease.

Herpes simplex virus

In vitro, HSV can infect most types of human cells and even cells of other species, but *in vivo* it is host specific, causes lytic infection of fibroblasts and epithelial cells, and establishes latent infection in neurons. In addition, the two biotypes of HSV, HSV-1 and HSV-2, show some predilection for infecting defined anatomic sites — oropharyngeal and genital, respectively. Both viruses are capable of infecting and producing latent infection at either site, but HSV-2 reactivation 'above the waist' and HSV-1 reactivation 'below the waist' are infrequent compared with HSV-1 reactivation 'above the waist' and HSV-2 reactivation 'below the waist',[19] respectively. It is suggested that at the molecular level the site-specific reactivation phenotypes of HSV-1 and HSV-2 depend on the latency-associated transcript (LAT) regions of the viral genome.[20]

Figure 215-5 Pathogenesis of varicella-zoster virus infection.

Varicella-zoster virus

Primary VZV infection starts in the mucosa of the respiratory tract and progresses via the blood and lymphatic system to cells of the reticulo-endothelial system. A secondary viremia after 11–13 days disseminates virus to the skin where the characteristic vesicular lesions of 'chickenpox' are produced (Fig. 215.5). The virus probably establishes latent infection in the dorsal root or cranial nerves during this viremic phase. The molecular basis of latency and reactivation is poorly understood. It has been suggested that differences in the frequency and clinical expression of HSV and VZV recrudescence may be related to the type of cells that are latently infected within sensory ganglia. In one model, [21] HSV latency is restricted to neurons and virus reactivation is induced by non-specific stimuli acting upon the neuron and results in re-infection of a small area of the skin innervated by the neuron. In contrast, VZV latency is mainly established in satellite cells and reactivation requires the spread of virus to neighboring neurons within the ganglia. These neurons act as sources of virus for axonal transport and infection of a wider area of the skin. Further differences in the latency and reactivation of HSV and VZV could relate to the differing structures of the genomes of the two viruses; LATs and neurovirulence genes are encoded in the HSV genome and have no counterpart in the VZV genome.[21]

Epstein-Barr virus

During primary infection EBV establishes a productive infection in the epithelial cells of the oropharynx. Virus is shed in the saliva and gains access to B cells in lymphatic tissue and the blood.[22] A lytic infection leads to the production of EBV proteins, including the early antigens, viral capsid antigen and the glycoproteins of the membrane antigen (Fig. 215.6). Epstein-Barr virus is a B-cell mitogen, stimulating the growth and immortalization of B cells by preventing apoptosis. Epstein-Barr virus infection of B cells also

Figure 215-6 Pathogenesis of Epstein-Barr virus infections. Infection may result in lytic infection of the cell or cell immortalization, which can be distinguished by the production of virus and the expression of different viral proteins and antigens. T cells limit the outgrowth of EBV-infected cells. LMP, latent membrane protein; LP, Epstein-Barr nuclear antigen leader protein. *Adapted from Strauss et al., 1993.*[22]

alters the interaction of the virus with the immune system by enhancing the expression of cell surface proteins such as human leukocyte antigens, adhesion proteins and the CD23 blast antigen.

Cytomegalovirus

Cytomegalovirus is transmitted via infected lymphocytes and mononuclear cells throughout the body. It establishes latent infection in mononuclear leukocytes and organs such as the kidneys and heart. Virus can therefore be transmitted in cells via blood transfusions and organ transplants. Activation and replication of the virus in the ductal epithelial cells of kidney and in secretory glands result in its excretion in body fluids and secretions including semen, saliva, tears, stool, vaginal and cervical secretions, and breast milk. Reactivation of virus in the cervix can result in congenital CMV infection, although more commonly viremia during primary maternal infection is the source of fetal infection. Reactivation usually results in virus shedding without symptoms. However, as for all the human herpesvirus infections in people who have individual deficits in CMI, reactivation is often symptomatic.

Since the original sero-epidemiologic observations,[23] several studies have shown a relationship between CMV infection and atherosclerosis. [24] [25] Such a correlation has also been found for other infectious agents such as *Chlamydia pneumoniae* and *Helicobacter pylori*. The evidence includes increased CMV antibody titers as well as a higher frequency of detection of CMV antigens and sequences in the wall of atherosclerotic vessels. However, results from various studies were not unambiguous, and a causal relationship between CMV and atherosclerosis has not yet been established.

Human herpesviruses 6 and 7

Human herpesvirus-6 was originally isolated from T-cell cultures derived from the blood of patients who had AIDS,[26] and HHV-7 was isolated from the $CD4^+$ T cells of a healthy individual. Both viruses infect and kill $CD4^+$ T cells, just like HIV, yet the outcome of infection is markedly different. In contrast to HIV infection, infection by HHV-6 or -7 is rapidly controlled by the host immune response, and the virus establishes a state of latency. HHV-6 and HHV-7 are closely related to the other member of the human Betaherpesvirinae, CMV (see Fig. 215.3). HHV-6 isolates are classified into two distinct variants, HHV-6A and HHV-6B. Although closely related, consistent differences have been observed in their biologic, immunologic, epidemiologic and molecular properties. HHV-6B is the primary etiologic agent of exanthem subitum,[27] whereas no single disease has been definitively associated with HHV-6A. In the same way, HHV-7 also remains an 'orphan' virus with no firm disease association. Infection with HHV-6 and HHV-7 occurs early in life. The viruses are present in the saliva of most adults and are readily spread by oral secretions. The cellular host range of HHV-6 appears to be extensive. Virus replication in $CD4^+$ T cells is common and there is also limited replication in $CD8^+$ T cells, natural killer cells, monocytes, epithelial cells and brain cells.[28]

Human herpesvirus-7, like HHV-6, grows well in $CD4^+$ T cells, but unlike HHV-6 it uses the CD4 molecule as its receptor. Human herpesvirus-7 also downmodulates expression of CD4. The host range of HHV-7 appears to be more limited than that of HHV-6. It is less cytopathic and grows less rapidly in culture than HHV-6.[28]

Human herpesvirus-8

Using representational difference analysis[29] HHV-8 was initially identified in 90% of AIDS-related Kaposi's sarcoma (KS) lesions and in 15% of non-KS tissues from people who had HIV infection. Following these observations HHV-8 sequences were identified using polymerase chain reaction (PCR) in all forms of KS, including KS from different geographic locations in individuals who had and did not have HIV infection.[10] Human herpesvirus-8 RNA transcripts have been detected in endothelial cells lining vascular spaces, perivascular spindle cells in KS lesions and in extracts of KS tissue. Also, linear HHV-8 DNA has been noted in peripheral blood mononuclear cells from KS patients. [28] These findings suggest that certain cells in KS tumors and also mononuclear cells are able to support the replication of HHV-8.

Large numbers of HHV-8 particles can be found in phorbol ester-stimulated B lymphoma cells by electron microscopy. Nevertheless, the infectivity of the virus has proved difficult to demonstrate in the laboratory. Studies using filtered cell culture fluids from HHV-8-positive $CD19^+$ lymphocytes, KS biopsy material co-cultured with normal $CD19^+$ cells, and fluids from B-cell lymphoma lines activated with phorbol esters have shown the passage of the HHV-8 DNA sequences to fresh uninfected target cells, particularly $CD19^+$ lymphocytes.[28]

Latency

The establishment of latency is central to the success of herpesviruses in maintaining themselves in human populations. During the course of a primary infection a

latent infection is established; the exact site or site(s) varies for each subfamily of herpesvirus:

- Alphaherpesvirinae (HSV-1, HSV-2, VZV) favor neuronal sites of latency;
- Betaherpesvirinae (CMV, HHV-6, HHV-7) are selective for secretory glands, lymphoreticular cells, kidneys and other tissues; and
- Gammaherpesvirinae (EBV, HHV-8) remain latent in either T or B cells (Table 215.5).

Latency permits persistence of the virus in the presence of a fully developed immune response and allows lifelong infection of the

TABLE 215-5 -- Principal sites of latency for herpesviruses.

PRINCIPAL SITES OF LATENCY FOR HERPESVIRUSES		
Virus	**Established (most probable) site of latency**	**Other possible sites of latency**
HSV-1	Neurons (trigeminal ganglia)	Other sensory nerve ganglia, brain, eye
HSV-2	Neurons (sacral ganglia)	Other sensory ganglia
VZV	Neurons (dorsal root ganglia, thoracic nerves, trigeminal ganglia)	Brain
EBV	B cells (epithelial cells of nasopharynx and submandibular salivary glands)	-
CMV	Monocytes, lymphocytes, epithelial cells	Salivary glands, renal tubule cells
HHV-6, HHV-7	T cells	-
HHV-8	?T and B cells, mononuclear cells	-

host. Through periodic reactivation of latent virus and the production of recurrent infection, virus shedding occurs at intervals throughout life, allowing the virus to be spread to new susceptible hosts.

In order to avoid elimination by the host immune system, herpesviruses use various evasion mechanisms. Key to most mechanisms is to reduce or prevent expression of virus-specific peptide-MHC class I complexes at the cellular membrane,[30] thus preventing recognition by cytotoxic T cells that would kill the infected cell. Prevention of peptide-MHC I complexes to reach the cell surface may, for instance, be achieved through interference with the transport of these compexes to the cell surface (HSV) or by induction of degradation of MHC I molecules (CMV).

The molecular events that lead to the establishment of latency are incompletely understood. The viral genome is maintained as an episomal closed circle of DNA (analogous to a bacterial cell plasmid), but how the virus precisely switches from its replicative mode to a latency mode is unclear.

Cell transformation

Some of the human herpesviruses (HSV-1, HSV-2 and CMV) can transform cells in culture, albeit at a very low frequency, and hamster cells transformed by HSV can produce tumors when injected into hamsters. Greater oncogenic potential can be demonstrated for other herpesviruses. For example, EBV-infected human lymphocytes can be transformed into lymphoblast cell lines. All cells that carry the EBV genome express virus-specific nuclear antigens (EBNAs), regardless of whether mature virus is released. There are also clear epidemiologic links to several types of tumor. Infectious mononucleosis can progress to a fatal B-cell lymphoma in boys who are born with an X-linked immunodeficiency; EBV has also been linked to Burkitt's lymphoma and nasopharyngeal carcinoma and to the development of post-transplant lymphoproliferative disease in immunosuppressed solid-organ transplant recipients. The geographic distribution of Burkitt's lymphoma and nasopharyngeal carcinoma mentioned above suggests that factors in addition to virus infection (e.g. a genetic predisposition or an environmental cofactor) may be involved in the production of these cancers.

EBV is also known to be the etiologic agent involved in post-transplant lymphoproliferative disease (PTLD), which comprises a heterogenous group of disorders with variable clinical presentation. PTLD affects 1–5 % of transplant patients and has a very high mortality rate. EBV is also associated with a subset of cases of Hodgkin's disease as demonstrated by the presence of the viral

genome and viral-coded antigens in Reed-Sternberg cells. The exact nature of the association or its relevance to the etiology of Hodgkin's disease need further elucidation.

Human herpesvirus-8 is related to EBV, but is classified in the rhadinovirus subfamily of the Gammaherpesvirinae. The prototype of this subfamily is herpesvirus saimiri, which is not pathogenic in its natural host (the owl monkey), but can induce T-cell lymphomas in other primates. Human herpesvirus-8 contains sequences resembling cyclin D, a cell cycle inducer, cytokines and other human regulatory and DNA metabolism genes. The virus also possesses a Bcl-2-like sequence that could, by preventing apoptosis, produce transformation in a similar manner to that attributed to EBV.[31]

The ubiquitous nature of herpesvirus infections and their establishment of lifelong infections make it difficult to interpret their association with tumorigenic cells. Because of the relatively low efficiencies of transformation *in vitro* it seems likely that herpesvirus infection represents only one part of a complex sequence of events that ultimately leads to neoplasia.

PREVENTION

In a well-controlled environment, such as that of a hospital, prevention of host-to-host transmission for most human herpesviruses is achieved by simple hygiene. In the home or other social situations prevention of transmission by avoiding contact with a person who has evidence of recurrent infection is only partially effective. This is because infectious virus is often excreted before the appearance of overt symptoms of recurrent infection and 'silent' recurrent infections also occur.

Herpesviruses are readily inactivated by a variety of physical and chemical agents (see Physical properties, above) and standard methods of sterilization, including autoclaving, dry heat, ultraviolet or gamma irradiation, and ethylene oxide sterilization are adequate for decontaminating medical equipment. Most common disinfectants (5% phenol, formaldehyde, glutaraldehyde, 1:10,000 quaternary ammonium compounds and 0.3ppm hypochlorites) rapidly inactivate virus. Other virucidal compounds include detergents, chlorhexidine, Merthiolate, sodium azide, ß-propriolactone, ethylene oxide and some proteolytic enzymes. Ultraviolet and X-ray or gamma irradiation also inactivate herpesviruses.

TABLE 215-6 -- **Examples of vaccine under development for immunization to prevent herpes simplex virus-2 infection.**[*]

VACCINES UNDER DEVELOPMENT TO PREVENT HSV-2 INFECTION				
Vaccine type (name)	**Company**	**HSV subunits or mechanisms**	**Adjuvant**	**Stage of research**
Recombinant protein	Glaxo-SmithKline	gD2	MPL	Phase III
Recombinant protein	Chiron	gD2 and gB2	MF59	Phase III halted
Attenuated (DISC)	Cantab	Disabled virus (gH gene deleted)	None	Phase I
DNA (Genevax HSV)	Apollon	Encodes gD2	Bupivacaine (facilitator)	Phase I
Naked DNA	Pharmadigm	Encodes gD2, uses novel myoD promoter	1,25-D3, possibly DHEA	Preclinical
Naked DNA	Vical	Encodes gD2	None	Preclinical
Naked DNA	Merck	Encodes gD2	None	Preclinical
Recombinant protein (Heteroconjugate)	Cel-Sci	T-cell ligands linked with HSV-associated peptides	None	Preclinical

** Adapted from Hanissian, 1998.*[34]

Active immunization

Except for VZV infection (for which a live attenuated vaccine is available) there are currently no licensed vaccines available to prevent herpesvirus infections. Varicella-zoster virus infection causes particular problems in immunosuppressed children. Primary infection in immunosuppressed patients can result in a fulminant, generalized infection or severe respiratory disease. A live attenuated VZV vaccine has been licenced for use in Japan and the USA and is administered using the same schedule as the measles, mumps and rubella vaccine. Breakthrough infections can occur, but usually result in mild illness.[32] In the USA the vaccine is recommended for use for all children over 12 months of age and susceptible healthy adolescents and adults. In most other countries vaccination is only recommended for patients who are immunosuppressed or immunodeficient and who have never had chickenpox. Since its implementation in routine use in the USA, the vaccine has been found to be 97% effective in the prevention against moderately severe or severe disease.[33]

A number of vaccines to prevent HSV-2, CMV and EBV infections are under development or are undergoing clinical trials. The diversity of approaches used is illustrated by the vaccines under development for HSV-2 (Table 215.6).[34] For CMV a candidate vaccine was developed by Plotkin in the 1970s.[35] The vaccine was made from an isolate of a congenitally infected child (the Town strain) after frequent serial passaging in human embryonic fibroblasts. In early studies serconversion was seen in nearly 100% of volunteers, as well as a protective effect against a low-dose challenge with CMV. In addition, the vaccine appeared to provide partial protection as seen after natural infection. However, the vaccine failed to prevent infection of mothers in contact with CMV-excreting children, and therefore failed its primary objective: the prevention of primary infection in pregnancy. Later efforts have focused on the development of recombinant subunits and DNA vaccines; so far, however, with little success.

Attempts to develop an EBV vaccine include the use of purified gp340/220 virus envelope antigen, to which neutralizing antibodies are mainly directed.[36] Although vaccination of tamarins with this antigen preparation protected them against a virus challenge, further attempts to develop this into a vaccine for use in humans have not been successful.

TABLE 215-7 -- Currently used antiherpesvirus drugs.

CURRENTLY USED ANTIHERPESVIRUS DRUGS			
Antiviral drug	**Chemical class**	**Mechanisms of action**	**Target virus**
Aciclovir	Guanosine analog	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Cidofovir	Cytidylic acid analog	DNA polymerase inhibitor	CMV, HSV-1, HSV-2
Famciclovir	Guanosine analog	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
Foscarnet	Pyrophosphate analog	DNA polymerase inhibitor	CMV, HSV-1, HSV-2
Ganciclovir	Guanosine analog	Virus-activated DNA polymerase inhibitor	CMV (HSV-1, HSV-2)
Valaciclovir	Guanosine analog	Virus-activated DNA polymerase inhibitor	HSV-1, HSV-2, VZV
CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.			

Passive Immunization

Varicella-zoster virus hyperimmune globulin (VZVIG) is an effective prophylaxis for babies born to mothers who have chickenpox 5 days before to 4 days after birth. The use of VZVIG in pregnancy, particularly during the first 20 weeks of pregnancy, is also suggested for nonimmune mothers exposed to chickenpox because pregnant women have a greater risk of developing severe pulmonary complications if they contract VZV infection. Prophylactic use of VZVIG is also appropriate to control infection in pediatric units caring for immunocompromised children (see Chapter 8).

In the future the development of human monoclonal antibody preparations may provide more reliable and consistent supplies of immunoglobulins to prevent these and other human herpesvirus infections.

Antiviral prophylaxis

A number of specific antiviral compounds are now available for the treatment of herpesvirus infection and prophylactic use of antiviral chemotherapy to control infection is now well established (Table 215.7).

TABLE 215-8 -- Laboratory diagnosis of herpesvirus infections.

LABORATORY DIAGNOSIS OF HERPESVIRUS INFECTIONS					
Virus	**Disease manifestation**	**Virus culture**	**Serology**	**Antigen detection**	**DNA amplification**
HSV-1	Skin lesions	+++	+	+	+++
	CNS infection	-	++	-	+++
HSV-2	Genital lesions	+++	+	+	+
	CNS infection	-	+	-	+++
VZV	Skin lesions	++	++	++	+++
	CNS infection	-	+	-	+++
CMV	Mononucleosis-like illness	-	+++	-	-
	Neonatal disease	+++	++	-	+++
	Systemic infection in immunocompromised	+	+	++	+++
	CNS disease	-	+	-	+++
EBV	Mononucleosis-like illness	-	+++	-	-
	Systemic infection in immunocompromised	-	+	+	+++
	CNS disease	-	+	-	+++
HHV-6	Exanthema subitum	+	+++	-	-
	CNS disease	-	++	-	+++
HHV-8	Kaposi's sarcoma	-	+	-	+++
CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.					

DIAGNOSTIC VIROLOGY

A key feature of the herpesviruses is their close adaption to their host. In general, primary infection is often asymptomatic or is accompanied by nonspecific mild signs and symptoms. Consequently most primary infections and many recurrent infections are not recognized as herpesvirus infections. Where symptoms are observed, speed in using diagnostic procedures is important because the peak of virus replication and shedding is likely to precede the appearance of symptoms. The diagnostic method chosen (Table 215.8) varies for different herpesviruses and also depends upon the type of infection (whether primary or recurrent), duration of symptoms and clinical manifestations.

Test specimens

If there are visible lesions (HSV-1, HSV-2, VZV) the base of the lesion may be sampled with a dry cotton-tipped swab, which should be placed in virus transport medium and transported to the virus laboratory as quickly as possible. If there are vesicles, vesicle fluid can be aspirated using a fine (intradermal) needle. The fluid should

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then be transported directly to the laboratory for virus detection by electron microscopy, direct immunofluorescent antibody staining, PCR testing or culture.

Viruria and viremia are common during both primary infection and recurrent infection with CMV, EBV and HHV-6, -7 and -8. Urine collected in urine transport medium is therefore a useful specimen (only CMV). Blood collected in anticoagulant can be used to prepare buffy coat preparations that can be used for culture or direct detection techniques. In neurologic disease, cerebrospinal fluid (CSF) and a clotted peripheral blood specimen (for CSF and blood serology) are essential. Clotted blood specimens should be collected during the acute stages of illness and again after 10–14 days.

Virus culture

The fragility of the viral envelope presents a problem if virus is to be cultured. Collection of specimens into appropriate viral transport medium and rapid transportation of specimens to the diagnostic laboratory are essential for successful isolation in cell culture systems. Virus culture is only routinely attempted for HSV-1, HSV-2, VZV and CMV. For those viruses that have fastidious cell culture requirements (e.g. infection of epithelial cells and lymphocytes as for EBV and HHV-6, -7 and -8) virus propagation can be accomplished only with difficulty, and culture of these viruses is not usually attempted in routine diagnostic laboratories. Detection of virus DNA by nucleic acid amplification procedures (see below) is a more practical method for detecting these viruses.

The ability of herpesviruses to replicate in monolayer cell cultures is variable. HSV-1 and HSV-2 can be cultivated in a wide variety of cells of both human and primate origin, including primary, continuous diploid and semicontinuous heteroploid cell lines. In contrast, CMV is fastidious and in cell culture only replicates in primary or semicontinuous (fibroblast) cell cultures of human origin. VZV is intermediate in its fastidiousness. It replicates best in semicontinuous cells of human origin, but may be cultivated with less efficiency in other cells. However, with the advent of molecular diagnostic methods that are more easy to implement in daily, routine diagnostic services, many laboratories are now abandoning their herpesvirus cultures.

Shell vial culture

The efficiency of primary virus isolation and speed of diagnosis can be improved using the so-called 'shell vial' culture. In this technique specimens are inoculated onto monolayers of cells grown on cover-slips. The specimen and shell vial containing the specimen are lightly centrifuged to improve the efficiency of attachment of virus to cells. After 24 or 48 hours of culture, monolayers are fixed (e.g. using cold acetone) and stained with either:

- a virus-specific antibody tagged with fluorescein isothiocyanate and examined by ultraviolet microscopy; or
- a peroxidase-labeled antibody with subsequent dye deposition within cells to identify the agent using light microscopy.

Identification of virus in cell culture

Virus growth in culture can be identified by the appearance of a cytopathogenic effect (CPE) and virus may be further characterized using specific antiserum (i.e. virus neutralization). This labor-intensive and time-consuming technique is now rapidly being replaced by more rapid methods of identification such as shell vial culture, direct immunofluorescent staining of cultures showing CPE and molecular diagnostic methods.

Electron microscopy

The limiting factor in the sensitivity of detecting virus by electron microscopy is the requirement that the sample contains at least 10^6 particles/ml. Electron microscopy is therefore useful in the examination of vesicle fluid from children who have suspected chickenpox and occasionally in the examination of atypical lesions of HSV infection as might be found in eczema herpeticum. The vesicle fluid is mixed with 3% buffered phosphotungstic acid, placed on a formvar and carbon-coated grid, blotted dry and examined in the electron microscope for the characteristic viruses (see Fig. 215.2). Although the morphology of herpesviruses is characteristic, differentiation of HSV-1, HSV-2 and VZV requires immune electron microscopy using virus-specific monoclonal antibodies.

Antigen detection

Antigen detection has a limited but important role to play in the diagnosis of most herpesvirus infections. In general, specimens may contain too little virus to allow reliable detection of antigen. Nevertheless, in a case of vesicular eruption, vesicle fluid (air dried and fixed in cold acetone) can be stained with monoclonal antibodies tagged with fluorescein isothiocyanate to provide a rapid specific diagnosis of infection.

Antigen detection also has an important role in monitoring organ transplant recipients (e.g. bone marrow transplant recipients) for the development of CMV disease. Buffy coat blood samples are examined for CMV pp65 antigen using a monoclonal antibody tagged with fluorescein. This procedure makes it possible to detect and monitor viremia and has been extremely useful to monitor and control CMV infections in various kind of transplant patients. Examination of CSF lymphocytes can provide a diagnosis of CMV infection of the central nervous system (CNS).

A variety of rapid immunoassay tests has been developed for 'within the office' testing for HSV. However, the sensitivity and specificity of these tests are too low to allow their use in diagnosis for hospitalized patients. As with virus cultures, these approaches are now being replaced by rapid molecular diagnostic techniques.

Nucleic acid amplification

The use of molecular diagnostic methods has changed the face of diagnostic virology. With increasing automation and further technical developments the very high sensitivity and the potential for early, rapid diagnosis are now outweighing the well-known disadvantages of technical complexity and lack of robustness, risk of contamination and cost. In addition to many in-house protocols used, a number of nucleic acid amplification systems[37] [38] are now available commercially. They are all based upon either:

- target amplification, for example PCR, ligase chain reaction, nucleic acid sequence based amplification (NASBA) and transcription-mediated amplification (TMA); or
- signal amplification, for example branched DNA amplification (bDNA).

Of these, PCR is currently the most widely used amplification method. Many assay procedures based upon the PCR procedure have been described and the use of PCR for diagnosing neurologic disease caused by the human herpesviruses has become the gold standard.[39] Single (sPCR) and nested (nPCR) PCR procedures have been described, including procedures that detect RNA and those that detect DNA. Both qualitative and quantitative assay procedures are available. Detection of several herpesviruses within the same clinical sample is possible using multiplex PCR where primers are included in the reaction mixture for several different herpesvirus DNA targets. For all these procedures a thorough evaluation of test protocols and rigorous quality control are essential to produce reliable test results.

Many protocols have also been described for extracting DNA from clinical samples. The purpose of DNA extraction is to render the target DNA free from potential inhibitors of the DNA polymerase used in the amplification step, that could result in false-negative PCR test results. As yet, there is no 'universal' extraction procedure

suitable

for all procedures. To determine whether sample inhibition has occurred, PCR test reactions often include an internal control molecule. Usually this is a bacterial plasmid DNA containing regions of DNA equivalent to those in the target DNA where the primers will bind. However, between the primer-binding sequences a 'scrambled' DNA sequence is inserted so that internal control and target DNAs can be distinguished upon amplification.

Detection of amplified DNA is usually accomplished by gel electrophoresis and staining with ethidium bromide. The product can also be detected by hybridization techniques where the dsDNA product is denatured and the ssDNA is hybridized with an oligonucleotide 'probe' sequence.

Progressively this cumbersome post-amplification handling with its inherent contamination risk is being replaced by 'real-time' amplification techniques, where the generation of amplified product is measured during the amplification process itself. Furthermore, the 'real-time' methodology also makes it relatively easy to quantitate the amount of virus present in the patient's specimen. This has been a great step forward in patient management because it allows much better monitoring of the course of disease and treatment of patients by clinicians and virologists.

Nucleic acid amplification techniques are now indispensible for the diagnosis of herpesvirus infections, particularly HSV, of the CNS.[40] [41] Determination of the viral load may be helpful for prognosis.[42] In transplant patients, quantitative 'real-time' assays have become of crucial importance in monitoring the development of CMV and EBV infections and a patient's response to treatment. Further rapid implementation of 'real-time' or other nucleic acid amplification techniques for the detection and quantitation of human herpesviruses is to be expected.

Genotypic analyses (restriction fragment length polymorphism)

Restriction fragment length polymorphism (RFLP) analysis is a specialist procedure of possible value in the investigation of outbreaks of infection and epidemiologic studies. Virus isolates in cell culture or PCR products are labeled with phosphorus-32 and DNA is extracted and digested using a range of restriction enzymes. On gel electrophoresis a series of fragments are segregated. Comparison of the migration of these fragments (RFLP patterns) gives information on the genetic relatedness (or unrelatedness) between strains of virus. Restriction fragment length polymorphism analysis also provides a reference procedure that allows identification and typing of isolates in one procedure.

Virus serology

Complement fixation and indirect immunofluorescence tests are still used in many viral diagnostic laboratories. However, their use is declining in favor of more reproducible and sensitive enzyme-linked immunosorbent assay (ELISA) methodologies.[39] Neutralizing antibody tests for herpesvirus antibodies are not routinely performed in diagnostic laboratories and none of these viruses has hemagglutinating capability.

Many immunoassays are available for the detection of antibodies to HHV-1 to HHV-5 and assays for antibody to HHV-6 to HHV-8 are now becoming commercially available. The antibody-capture ELISA principle is used to detect IgM antibody, but results of IgM tests must be interpreted with care:

- as IgM antibody is the first antibody to be produced in response to infection it is by nature a broadly reactive antibody, so cross-reactivity and false positivity may occur; and
- IgM antibody is produced during both acute (primary) infection and recurrent disease, although the amount produced is generally higher during primary infection.

Quantitation of virus-specific IgG antibody in serial samples (10 or more days apart) often provides a more reliable diagnostic procedure, but it takes considerably longer (up to 2 weeks) to obtain results.

Detection of IgA antibody is not generally carried out, but may have a role in the serologic diagnosis of recurrent herpesvirus infections.[43]

CLINICAL MANIFESTATIONS

With the exception of VZV, primary herpesvirus infections in the immunocompetent host are usually asymptomatic or are associated with a minor illness only, with no specific symptoms such as fever and malaise (Table 215.9). As a consequence, primary herpesvirus infections may not be recognized. When symptoms do occur, herpesvirus infections in the immunocompetent host are normally self-limiting and require only symptomatic treatment. Antiviral therapy is available for a number of herpesviruses, but is usually only indicated for those patients who have more severe disease manifestations or when it is appropriate to minimize the likelihood of complications.

Herpesvirus infections in the immunocompromised host are frequently severe and sometimes life-threatening. Antiviral drug therapy is required for these patients to control the infection (see Table 215.7).

Herpes simplex viruses

Although HSV infections can result in a wide spectrum of disease (Fig. 215.7), mucocutaneous lesions are by far the most common manifestation of primary HSV infections.[44]

Mucocutaneous manifestations

Oropharyngeal infection

Primary oropharyngeal HSV-1 infection usually occurs in childhood and is often asymptomatic. In symptomatic infections acute gingivostomatitis is seen in 10–30% of cases.[45] Lesions on the buccal and gingival mucosa evolve from vesicles to shallow ulcerations on an erythematous base. Frequently the child is unable to eat or swallow liquids because of the significant edema, ulceration of the oropharyngeal membranes and associated pain. Primary HSV gingivostomatitis is usually accompanied by fever and submandibular lymphadenopathy. The incubation period varies from 2 to 12 days (mean 4 days). The duration of clinical illness may extend from 2 to 3 weeks, with virus excretion from the oropharynx for an average of 7–10 days. In many countries the epidemiology of this infection is changing, this change being characterized by an increasing incidence of primary HSV-1 infection later in life. Primary HSV infection in an adolescent or adult is often associated with pharyngitis and a mononucleosis-like syndrome.

The severity and duration of clinical illness in recurrent infection is considerably less than in primary HSV infection. Recurrent orolabial lesions ('cold sores') are heralded by a prodrome of pain, burning, itching or tingling for several hours before the development of the characteristic vesicles. The vesicular stage persists for less than 48 hours and progresses to the ulcerative and crusting stage within 3–4 days. Pain resolves quickly during the same period, and healing is generally complete within 8–10 days. Systemic illness is usually absent in recurrent infection. Recurrence rates are highly variable and the precipitating factors involved are not well defined, but include the type of HSV (HSV-1 is more likely to recur than HSV-2), fever, stress, exposure to ultraviolet light and impaired CMI in the host.

Genital infections

Primary genital herpes usually occurs in adolescents and young adults.[16] Approximately 20–40% of first-episode genital herpes is now due to HSV-1, but the recurrence rate of genital HSV-2 infections is ten times higher than that of genital HSV-1 infections.[46] [47]

TABLE 215-9 -- Clinical manifestations of herpesvirus infections.

CLINICAL MANIFESTATIONS OF HERPESVIRUS INFECTIONS

Agent	Primary infection	Recurrent infection	Complications
HSV-1, -2	Usually asymptomatic; orofacial/genital, mucocutaneous lesions, gingivostomatitis, paronychia	Usually asymptomatic, cold sores, genital herpes, recurrent skin lesions	Meningitis, encephalitis, hepatitis, keratitis, generalized disease (neonates), eczema herpeticum
VZV	Varicella	Herpes zoster, zoster, ophthalmicus, oticus	Pneumonitis, hepatitis, encephalitis, keratitis, postherpetic neuralgia, cerebellar ataxia
CMV	Usually asymptomatic; mononucleosis-like syndrome, fever, malaise, myalgia, lymphadenopathy	Usually asymptomatic	Pneumonia, retinitis, esophagitis, colitis, hepatitis, enteritis
	Neonates: growth and mental retardation, hepatosplenomegaly, microcephaly		
EBV	Usually asymptomatic, infectious mononucleosis	Usually asymptomatic	X-linked proliferative syndrome, persistent mononucleosis-like syndrome, Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoma, encephalitis
HHV-6	Usually asymptomatic; febrile illness exanthema subitum, febrile convulsions	Usually asymptomatic	Meningoencephalitis, pneumonitis, hepatitis
HHV-7	Unknown, occasionally exanthema subitum	Unknown	Unknown
HHV-8	Unknown	Unknown	Kaposi's sarcoma, body cavity lymphoma

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

The incubation period is 3–7 days. The majority of cases (50–70%) are asymptomatic or so mild that the infection is not recognized. The severity and duration of clinical symptoms depend upon both viral and host factors and include the type of virus, past exposure to HSV-1, previous episodes of genital herpes and gender. [46] (See Chapter 76 .)

Primary genital herpes is characterized by the appearance of vesicular lesions — in males usually on the glans penis, on the penile shaft or in the perianal region, and in women involving the vulva, vagina, cervix and perineum. Extragenital lesions (on the buttocks and thighs) occur in 10–20% of patients. The lesions can be very painful and associated with inguinal adenopathy and dysuria. Urinary retention is observed in up to 20% of female patients. Systemic symptoms occur in 40–70% of patients who have primary genital herpes and include fever, malaise, headache and myalgia.

Patients usually present with bilateral pustular or ulcerative lesions on the external genitals that coalesce into larger areas of ulceration. The ulcerative lesions persist for 4–15 days and usually resolve without residual scarring after a phase of crusting and re-epithelialization.

The average duration of primary symptomatic genital herpes is 3 weeks. During this time the pain and irritation caused by the lesions gradually increase during the first week, reach their peak of intensity early in the second week and gradually resolve during the second and third week. Tender lymphadenopathy is usually seen in the second and third week of illness.

Complications of primary genital herpes include aseptic meningitis, which has been reported in up to 36% of women and 12% of men, and sacral radiculopathy, which may lead to urinary retention.[16] [46] In homosexual men primary anal HSV-2 infection is frequently associated with proctitis.

The signs and symptoms of recurrent genital herpes are usually restricted to the genital region and are relatively mild and of shorter duration than for primary disease. Prodromal symptoms are a feature of approximately 50% of episodes. They vary from mild tingling to stabs of pain in the buttocks, legs and hips. As for primary disease, recurrent lesions in women are more often painful and of longer duration than in men (60–90 vs 30–70%, and mean 5.9 vs 3.9 days, respectively).

Other mucocutaneous manifestations

Other mucocutaneous manifestations of HSV infections include classic herpetic lesions — a close crop of vesicles on other areas of the skin, notably of the head, neck and shoulders, or the eyes, where there may be vesiculation of the eyelid and associated conjunctivitis.

Herpetic whitlow is an often painful infection of the finger that can result from auto-inoculation or direct contact with an individual who has an HSV infection. It is therefore an occupational hazard of dentists, doctors and nurses.

In patients who have atopic dermatitis, superinfection with HSV may appear as eczema herpeticum. Most cases are mild with localized lesions, but severe disseminated cases (Kaposi's varicelliform eruption, resembling severe varicella) occur, resulting in a widespread vesicular eruption and are associated with a small but significant risk of fatal disseminated infection.

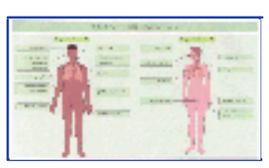

Figure 215-7 Diseases caused by herpes simplex virus-1 and herpes simplex virus-2.

Other manifestations

Herpes simplex virus encephalitis

Herpes simplex virus encephalitis is one of the most severe manifestations of HSV infection.[48] Although rare — incidence 1/250,000 to 1/500,000 population per year — it is the most commonly diagnosed cause of sporadic fatal encephalitis. Either HSV-1 or HSV-2 can cause HSV encephalitis, but approximately 95% of cases are due to HSV-1.[49] It can occur at any time of life and there is no apparent seasonal or geographic clustering of cases. Symptoms include fever, headache, behavioral abnormalities, altered consciousness and focal or generalized seizures suggestive of focal temporal lobe disease. The amount of HSV DNA in the CSF appears to be correlated with prognosis and outcome.[42] Early antiviral treatment is imperative. If untreated, the disease is frequently fatal or results in severe persisting neurologic sequelae (see also Chapter 23).

Neonatal herpes

Neonatal infection is a rare but severe manifestation of HSV infection acquired during delivery or in the immediate postpartum period.[50] It usually occurs in neonates who do not have protective maternal HSV antibody, and HSV-2 infection is usually more severe than HSV-1 infection.

Constitutional signs and symptoms include fever, irritability, seizures, failure to feed and a characteristic vesicular exanthem. Localized neonatal infections mostly involve the skin and are characterized by clusters of discrete vesicles at multiple sites. Localized infection may also occur in the mouth and oropharynx or the eye. In disseminated disease the principal tissues involved are the liver and adrenals, but other organs such as brain, lungs, esophagus, lower gastrointestinal tract, spleen and kidneys can be affected.

If untreated the mortality rate exceeds 50% and children who have disseminated infection have the worst prognosis. Even with prompt administration of specific antiviral chemotherapy survivors often experience neurologic complications. In all cases of neonatal herpes prompt initiation of antiviral chemotherapy is mandatory to prevent the development of long-term neurologic impairment.

Herpes simplex keratoconjunctivitis

Ophthalmic infections with HSV are among the most common causes of corneal blindness.[51] Dendritic ulceration of the cornea is the most characteristic manifestation. Most frequently it is unilateral. Visual acuity is decreased in the presence of ulcers and progression of the disease can result in visual loss. Despite appropriate antiviral treatment attacks can last several weeks to months.

Immunocompromised host

Patients compromised by immunosuppressive therapy (e.g. organ transplant recipients) or patients who have immunodeficiency are at risk of developing a severe primary infection and more severe and frequent recurrent disease. Disease severity is directly related to the degree of immunosuppression or immunodeficiency. Mucocutaneous infections are usually more extensive and severe and take longer to heal, even when appropriate antiviral therapy is initiated. Progression of the infection may involve the respiratory tract, esophagus or gastrointestinal tract.

Varicella-zoster virus

Varicella (chickenpox) and herpes zoster (shingles) are due to infection with the same virus, VZV. Varicella is the usual manifestation of primary infection, herpes zoster of recurrent infection.[52]

Varicella (see Chapter 8)

After an incubation period of 14–15 days (range 10–20 days) a vesicular rash accompanied by low-grade fever and pruritus emerges in successive crops over a 3- to 6-day period. The rash is characterized by maculopapular lesions that vesiculate in about 3–4 days before crusting and scab formation. Scabs may remain in situ for up to 3 weeks.[53]

Varicella is a common childhood exanthematous disease, usually affecting children in their early school years. The exanthem is centripetal rather than centrifugal with most lesions being present on the trunk and proximal extremities. Rashes beginning on the face and hands with subsequent spread to the trunk have been described. Secondary bacterial infection of the lesions as a result of scratching is the most frequent complication. Occasionally cerebellar ataxia, transverse myelitis or Reye's syndrome may complicate the infection. Primary VZV infection occurring in adolescence and adult life is often associated with more severe disease. The rash is often widespread and dense and patients are more likely to develop visceral complications such as pneumonitis, encephalitis and hepatitis.[54]

Primary VZV infection may have a more aggressive course in pregnant women.[55] This frequently includes visceral complications, notably pneumonitis. The fetus is at risk of infection and there is a low risk of severe fetal abnormalities ranging from longlasting rash, limb or dermatomal scarring, to severe neurologic damage.[56] Severe neonatal varicella may occur when maternal varicella presents within 5 days of delivery.

As for other herpesvirus infections, primary VZV infection of immunocompromised patients such as children undergoing cancer chemotherapy or corticosteroid treatment usually has a more severe protracted course with an increased rate of complications, particularly pneumonitis.

Herpes zoster (see Chapter 29)

Herpes zoster is typically a disease of the elderly. Usually there is a prodrome of pain followed within a few days by the development of a unilateral vesicular rash within the dermatome served by the sensory nerve from the affected ganglion.[57] New lesions form over 2–5 days and the rash then pustulates and scabs over 2–3 weeks.

Persisting pain, known as postherpetic neuralgia, is the most common complication of herpes zoster. It can be severe and last for several months. The rate, severity and duration of postherpetic neuralgia are directly related to age. It is uncommon in patients under 50 years of age, but occurs in over 40% of those over 60 years of age.[58]

The sites most commonly affected by herpes zoster are the dermatomes T3 through L3, often the same as those that were most affected during chickenpox. Complications including motor weakness and visceral manifestations may occur, but are unusual. However, in up to 50% of patients where herpes zoster involves the trigeminal nerve ocular manifestations such as conjunctivitis, ulcerative keratitis, uveitis and iridocyclitis occur. Ocular complications are particularly common when the zosteriform lesions extend to the tip of the nose. Herpes zoster oticus, also called the Ramsay Hunt syndrome, consists of lesions on the ear or within the auditory canal, which are sometimes barely visible, and can result in hearing loss and facial paralysis.

Herpes zoster is frequently observed in patients who are immunocompromised, including those undergoing radiotherapy, chemotherapy and other types of immunosuppressive therapy, and those who have immunodeficiency syndromes. In such patients herpes zoster presents with larger, more extensive and hemorrhagic vesicles, which are often multidermatomal. Chronic cutaneous lesions and disseminated infections have been described, particularly in patients who have HIV infection. Meningoencephalitis, pneumonitis or hepatitis can also occur in immunocompromised patients, but are rare.

Cytomegalovirus

CMV is an uncommon cause of disease in people who are immunocompetent. Primary infection may occasionally cause a glandular fever-type illness resembling EBV mononucleosis.[59] In such patients persistent fever, myalgia and asthenia accompanied by atypical lymphocytosis and elevated liver transaminases are common signs and symptoms. Occasionally hepatitis is the presenting symptom. The presence of fever and atypical lymphocytes distinguishes CMV mononucleosis with liver involvement from infections by the usual hepatitis viruses.

In contrast, in patients who have an immature (e.g. fetus or newborn) or compromised immune system both primary and recurrent infection with CMV can result in severe and sometimes fatal disease.

Congenital and perinatal infections

CMV infections are the most common cause of congenital viral infections. [60] Both primary and recurrent infection in a pregnant woman can cause congenital and perinatal disease, but the frequency of severe disease is at least 10-fold higher after primary maternal infection. Overt clinical symptoms occur in 5–10% of cases of intrauterine CMV infection and include growth retardation, hepatosplenomegaly and thrombocytopenic purpura, and less frequently jaundice, microcephaly and chorioretinitis. The prognosis is poor for these infants. Another 10–15% of cases are neonates who are asymptomatic at birth and develop late sequelae, particularly mental retardation and sensorineural hearing loss.

Perinatal infections are nearly always asymptomatic, but neonates occasionally develop CMV pneumonitis, particularly when born prematurely. Severe disseminated disease may follow transfusion of CMV-infected blood products to premature neonates who have CMV-seronegative mothers.

Immunocompromised patients

The pathogenesis and clinical spectrum of CMV disease in patients who are immunocompromised depend upon the cause and degree of immunosuppression. [61] [62] Treatment regimens must take into account the fact that the disease manifestations may have an immunopathologic or viral etiology.

Persistent intermittent fever is often the presenting symptom of CMV infection in patients who have had a transplant. The infection may progress to cause pneumonitis, gastrointestinal disease, hepatitis and retinitis. In recipients of a solid organ transplant, CMV disease most frequently occurs when the donor is CMV-seropositive and the recipient is CMV-seronegative. In bone marrow transplant patients, however, CMV disease most commonly results from reactivation of latent virus in the recipient, usually 20–90 days after the transplant. The most frequent manifestation is CMV pneumonitis. Unless treated very early, preferably before the appearance of

respiratory symptoms, pneumonitis in these patients is often fatal.

Reactivation of latent CMV is usually the cause of CMV disease in patients who have HIV infection, frequently occurring in the later stages of the HIV infection when $CD4^+$ T cell counts are less than $50/mm^3$. The most frequent manifestation is CMV retinitis presenting with blurred vision and decreased acuity (see Chapter 125). [62]

Gastrointestinal disease, hepatitis, encephalitis and pneumonia also occur in patients who have HIV infection. Gastrointestinal disease includes esophagitis, gastritis, enteritis and colitis and is usually associated with fever and weight loss. Although CMV is often detected in respiratory specimens from patients with AIDS who have pneumonitis, the pulmonary process is usually caused by other pathogens, particularly *Pneumocystis carinii*.

Epstein-Barr virus

In most individuals EBV infection does not cause overt disease. [11] Primary infections in adolescents can, however, result in infectious mononucleosis. As for CMV infection, immunocompromised hosts may develop severe manifestations during both primary and recurrent EBV infection. Such severity is exemplified by the role of EBV infection in the causation of lymphoproliferative disease.[63] [64]

Immunocompetent host

Infectious mononucleosis (glandular fever) usually presents as fever, fatigue and malaise accompanied by a sore throat, cervica1 lymphadenopathy, hepatomegaly and splenomegaly. A rash may develop, particularly in those treated with ampicillin. Disease manifestations, especially fatigue and malaise, can persist for several weeks. Relapses and a chronic course have been described but the relationship to EBV infection is uncertain.

The X-linked lymphoproliferative (or Duncan's) syndrome is a rare manifestation of primary EBV infection and occurs in (young) males who have an X-linked genetic defect in their immune response to EBV.[63] Most die from fulminant mononucleosis, fulminant hepatitis or aplastic anemia.

Patients who have Burkitt's lymphoma have large swollen lymph nodes, usually involving the jaw and orbital cavities.[8] The disease occurs mainly in young children. In contrast, nasopharyngeal carcinoma is usually seen in older male patients in South East Asia.[9]

EBV has been associated with Hodgkin's disease. EBV-specific sequences and EBV-induced antigens have been found in Reed-Sternberg cells.[64] The precise role of EBV in the etiology of Hodgkin's disease, however, remains to be established. Other EBV-associated lymphoepithelial tumors have been described in the parotid gland, thymus, stomach, lung and larynx.[64]

Immunocompromised host

Immunocompromised patients are at risk of developing lymphoproliferative disorders following EBV infection. The polyclonal B lymphocyte proliferation is often associated with fever, lymphadenopathy and hepatosplenomegaly,[64] and is frequently life-threatening in severely immunocompromised patients, such as bone marrow transplant patients.

Patients who have AIDS may develop oral hairy leukoplakia, which is characterized by white plaques on the lateral margin of the tongue.[65] EBV is associated with lymphocytic interstitial pneumonia (which is common in pediatric patients who have AIDS) and a rapidly progressing, diffuse encephalitis.

Human herpesviruses-6, -7 and -8

Human herpesviruses-6, -7 and -8 have only been recognized during the past 15 years.[28] Although their etiologic role in the development of a variety of disease manifestations is well documented, their full disease spectrum remains to be determined.

Human herpesvirus-6

HHV-6, variant B, is the cause of exanthema subitum (roseola infantum) in young children.[27] Symptomatic infection is characterized by fever, sometimes associated with a mild respiratory illness and lymphadenopathy, which is followed by the appearance of a fine maculopapular rash spreading from the trunk to the extremities. More recent studies have shown that the primary characteristic of HHV-6 infection in infants is a high fever for 3–4 days, often associated with inflammation of tympanic membranes. Only a small proportion of these (<10%) develops exanthema subitum.[66] Up to 20–25% of hospital admissions of children under 3 years of age with acute febrile illness is due to primary HHV-6 infection.[66] [67] Primary HHV-6 infection has also been demonstrated as a frequent cause of febrile seizures in infants, and accounts for up to one-third of all febrile seizures under the age of 3 years.[67] [68] Recovery is usually rapid and uneventful, although a more protracted and severe course characterized by severe meningoencephalitis, fulminant hepatitis or fatal pancytopenia has been described. In adolescents and adults primary HHV-6 infection can cause a mononucleosis-like illness.

Some recent studies have suggested a role for HHV-6 in the etiology of multiple sclerosis. This is based on the finding by PCR of HHV-6 DNA in the brain. [69] However, literature findings still remain ambiguous, and further studies are needed to define the possible role of HHV-6 in multiple sclerosis.

In severely immunocompromised patients, such as those who have AIDS or received a bone marrow transplant, reactivation of HHV-6 infection mainly has an immunosuppressive effect, subsequent to which other pathogens may cause severe disease. In bone marrow transplant patients HHV-6 reactivation is associated with bone marrow suppression, probably as the result of viral replication in particular progenitor cells.[70] HHV-6 has also been associated with interstitial pneumonia and encephalitis in these patients. Finally, mainly on the basis of *in-vitro* studies, HHV-6 has been reported as a possible cause of or cofactor for certain lymphoproliferative disorders.

Human herpesvirus-7

Although some cases of exanthema subitum appear to be due to HHV-7, a causal association between human disease and HHV-7 infection has not yet been established. HHV-7 has been associated with infant febrile illness as well as subsequent CNS complications. In renal transplant patients evidence has been obtained that HHV-7 may be a cofactor in the development of CMV disease.

Human herpesvirus-8

No association has been recognized between primary HHV-8 infection and specific clinical disease, but it is now generally accepted that HHV-8 has a causal role in KS and a rare form of abdominal B-cell lymphoma, body-cavity associated lymphoma and primary effusion lymphoma.[27] [28] The virus is also found in some cases of multicentric Castleman's disease. The possible involvement of HHV-8 in the development of other endothelial cell-derived tumors requires further investigation.

Herpes B virus

Herpes virus simiae (herpes B virus) causes a benign latent infection in macaques that is analogous to HSV infection in humans.[71] The infection is infrequently transmitted to humans. After an incubation period varying from 3 days to 3 weeks there may be localized pain, redness and vesicular skin lesions near the site of the viral inoculation, followed by localized neurologic symptoms. Encephalopathy is common and fatal in up to 70% of patients. The virus is susceptible to aciclovir and early treatment can be life-saving.[72]

MANAGEMENT

As most herpesvirus infections in the immunocompetent host are self-limiting, patients usually only require supportive care. The type of supportive treatment required varies for different disease manifestations and may consist of rest, hydration, the appropriate use of antipyretics, analgesics, and treatment to soothe skin lesions and to prevent secondary bacterial infection. Effective antiviral therapy is available for more severe cases of infection caused by HSV, VZV or CMV. It is stressed, however,

that although antiviral drugs can help to control a herpesvirus infection they cannot eliminate the infection.

Antiviral drugs

Most antiherpetic drugs are nucleoside analogs[73] that inhibit virus-specified DNA polymerases and terminate DNA chain elongation (see Table 215.7 ; see Chapter 205). The first generation of these drugs — idoxuridine, vidarabine and trifluridine — have only a limited selectivity for the viral DNA polymerase. Due to the interaction of the phosphorylated form of these drugs with cellular DNA polymerases, there are severe adverse effects.

Aciclovir is the prototype of the present generation of antiviral drugs (see Chapter 205). [74] Its high specificity and therefore safety is

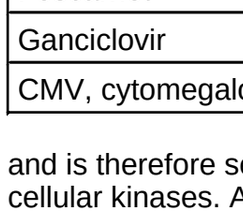

Figure 215-8 Mode of action of aciclovir. Phosphorylation is effected by virus-specified thymidine kinase. The aciclovir monophosphate is then converted to the triphosphate form by cellular kinases. Aciclovir triphosphate binds with high affinity to the virus-specified (but not host derived) DNA polymerase, leading to inactivation of the enzyme's activity. In addition, incorporation of aciclovir in the DNA blocks further chain elongation.

based on its specific interaction with viral and not cellular enzymes (Fig. 215.8). It is an acyclic analog of guanosine that is activated by viral and not the cellular thymidine kinase to serve as substrate for the viral DNA polymerase. Aciclovir is virtually inert as administered. Approximately 90–92% is excreted unchanged by renal excretion with the majority of the remainder appearing as the metabolite 9-carboxymethoxymethylguanine. The serum half-life of the drug is about 2–3 hours. Virus-infected cells appear to be slightly more permeable to aciclovir than noninfected cells, but aciclovir is only entrapped and selectively concentrated within virus-infected cells. This is achieved by phosphorylation effected by virus-specified thymidine kinase. Host cell kinases do not appear capable of effecting this primary phosphorylation to any significant degree. Aciclovir monophosphate cannot traverse cellular membranes

TABLE 215-10 -- Comparative susceptibility of herpesviruses to antiviral drugs.

COMPARATIVE SUSCEPTIBILITY OF HERPESVIRUSES TO ANTIVIRAL DRUGS					
Antiviral drug	**Viral susceptibility**				
	HSV-1	**HSV-2**	**VZV**	**CMV**	**Main target agents**
Aciclovir, valaciclovir	+++	+++	++	-	HSV-1, HSV-2, VZV
Cidofovir	+	+	++	+++	CMV, resistant HSV
Famciclovir	+++	+++	++	-	HSV-1, HSV-2, VZV
Foscarnet	++	++	+	++	CMV, resistant HSV, VZV, CMV
Ganciclovir	+	+	+	+++	CMV
CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.					

and is therefore selectively concentrated in virus-infected cells. The monophosphate is converted to the active antiviral aciclovir triphosphate through the action of cellular kinases. Aciclovir triphosphate is a potent and highly selective inhibitor of viral-specified DNA polymerase. A further but less significant antiviral action is the chain termination effected by the incorporation of aciclovir into the growing viral DNA chain (absence of 2' and 3' carbons of guanosine). Aciclovir is a safe, relatively nontoxic drug and is effective for therapeutic use as well as long-term suppressive treatment.

The recently approved drugs famciclovir and valaciclovir are similar to aciclovir in their mechanism of action, but have different pharmacologic properties. These drugs have improved oral bioavailability, thereby allowing less frequent administration than is required for aciclovir. As with aciclovir, famciclovir and valaciclovir have excellent safety records.

The alternate antiherpetic drugs, ganciclovir, foscarnet and cidofovir have similar mechanisms of action, (i.e. inhibition of the viral DNA polymerase), but generally have a higher toxicity profile, including neutropenia and thrombocytopenia for ganciclovir, and nephrotoxicity for foscarnet and cidofovir. The susceptibility of different herpesviruses to the various antiviral drugs is shown in Table 215.10 .

Antiviral resistance has been described for all of the antiherpes compounds mentioned, particularly when antivirals are administered to immunocompromised patients over extended periods of time. Studies in these patients have shown resistance to aciclovir in 5% and to ganciclovir in 7% of patients.[75] [76] Antiviral resistance is due to mutations in the viral thymidine kinase or in the DNA polymerase.[77] Resistant viruses, however, appear to be less virulent.[78] Significant circulation in the general population has not yet been found.[76] Because of their similar mechanisms of action cross-resistance frequently occurs (e.g. between aciclovir, famciclovir and valaciclovir).

Herpes simplex virus

In several placebo-controlled studies, intravenous, oral or topical administration of aciclovir, famciclovir and valaciclovir have been shown to be effective against mucocutaneous HSV infections (Table 215.11). All three drugs significantly reduce:

- duration of viral shedding;
- time to healing of lesions;
- duration of pain and itching; and
- rate of complications.

The effect is more pronounced for primary or first-episode HSV infections than for recurrent disease in the immunocompetent host. Oral or intravenous administration is generally more effective than topical treatment. Intravenous administration of aciclovir is the treatment of choice for patients who have suspected systemic or neurologic disease due to HSV (e.g. patients who have herpes encephalitis). Antiviral therapy should be initiated as soon as possible

for these patients, even before laboratory confirmation of the clinical diagnosis.

For patients who have frequent severe episodes of genital herpes, a choice can be made between patient-initiated episodic treatment or longer-term suppressive therapy. Episodic treatment must be initiated at the first sign of prodromal symptoms to be of benefit. Long-term suppressive therapy, however, is very effective and can dramatically reduce the frequency of recurrent disease and asymptomatic shedding. [79] [80] Although recurrent disease and intermittent shedding of HSV are not fully prevented,[81] suppressive therapy has been safe, effective and well tolerated in patients treated for more than 6 years. In addition, there is no evidence that it greatly enhances the emergence of antiviral resistance.[81]

Varicella-zoster virus

VZV is significantly less susceptible to aciclovir, famciclovir and valaciclovir than HSV. Therefore, higher dosage regimens are required. Intravenous therapy with aciclovir or oral administration of valaciclovir or famciclovir is indicated for children who have severe manifestations of varicella, particularly those who are immunocompromised (see Table 215.11). Therapy should also be considered for adolescent or adult patients who have varicella because of the increased risk of

complications in these patients. In immunocompetent children the antiviral effect is beneficial only when therapy is started very early after the onset of disease symptoms (<48 hours).

Intravenous aciclovir therapy has become the therapy of choice for patients who have a high risk of developing severe or progressive recurrent VZV disease (herpes zoster). These include patients treated for lymphoproliferative disorders, transplant recipients, other patients who have immunodeficiency conditions and patients who have herpes zoster ophthalmicus and herpes zoster oticus.

The poor bio-availability of aciclovir after oral administration has limited its use for immunocompetent patients who have herpes zoster. Valaciclovir and famciclovir have substantially improved bioavailability, accelerate healing and reduce pain in patients who have

TABLE 215-11 -- **Indications for antiherpesvirus drug treatment.**

INDICATIONS FOR ANTIHERPESVIRUS DRUG TREATMENT			
Antiviral drug	**Target virus**	**Infections**	**Possible side-effects**
Aciclovir, valaciclovir, famciclovir	HSV-1,2	Severe and/or frequent mucocutaneous HSV infection, including genital herpes; herpes encephalitis, herpes keratitis	Headache, nausea, diarrhea
	VZV	Severe cases of varicella, varicella in patients at risk for complications (immunocompromised, adolescents, adults)	Headache, nausea, diarrhea
		Severe cases of herpes zoster, including those at risk of developing postherpetic neuralgia, herpes zoster ophthalmicus, oticus	
Cidofovir	CMV	CMV retinitis	Severe nephrotoxicity
Foscarnet	CMV, HSV-1, HSV-2, VZV; severe disease due to aciclovir-, valaciclovir- and famciclovir-resistant HSV or VZV strains	Severe CMV disease refractory to ganciclovir treatment	Nephrotoxicity, crystalluria
Ganciclovir	CMV	Severe CMV disease in immunocompromised host (e.g. retinitis, esophagitis, colitis, pneumonia, encephalitis)	Neutropenia, thrombocytopenia
CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.			

herpes zoster,[82] [83] and are now licensed for these reasons in many countries. The effect is significant only in patients who are at risk of developing severe disease (i.e. those over 50 years of age who have considerable pain at the onset of symptoms). Furthermore, treatment has to start early (within 72 hours) to be beneficial. Treatment is always indicated for patients who have herpes zoster ophthalmicus or oticus.

Cytomegalovirus

There is no generally accepted indication for using antiviral therapy for immunocompetent patients who have CMV infection. In the immunocompromised host the first line of treatment of CMV disease is, if possible, appropriate reversal of immunosuppressive therapy. Additional, antiviral therapy is often required for these patients (see Table 215.11). For this purpose three drugs are now available — ganciclovir, foscarnet and cidofovir. Because CMV disease frequently recurs after cessation of antiviral therapy in patients who are immunodeficient, lifelong maintenance treatment is often required to prevent or delay disease progression.

Of the available drugs, intravenous ganciclovir is the treatment of choice for immunocompromised patients who have an episode of CMV disease, such as pneumonia, gastrointestinal tract disease or retinitis.[84] For maintenance therapy, particularly for patients who have AIDS and retinitis, there is an approved oral preparation of ganciclovir. Oral valganciclovir (the prodrug of ganciclovir) is recommended (see Chapter 125 and Chapter 205). [85] [86]

In general, foscarnet is less well tolerated than ganciclovir. Its nephrotoxicity is the most common dose-limiting adverse effect and foscarnet is mostly used for patients who have severe adverse effects to ganciclovir or who have ganciclovir-resistant CMV disease.[84]

Experience with cidofovir is limited. Its major benefit is its long half-life, permitting relatively infrequent dosing. The main application of cidofovir therapy has been for patients who have retinitis, using intravitreal administration every 6 weeks, or intravenous infusion once weekly for induction and every other week for maintenance therapy.

Although not very effective in the treatment of CMV infection, prophylactic use of valaciclovir in renal transplant patients has been found to have a preventive effect on the development of CMV disease.[87]

Other herpesviruses

EBV and HHV-6 have shown *in-vitro* susceptibility to various antiherpesvirus drugs such as aciclovir or ganciclovir. However, apart from aciclovir treatment of oral hairy leukoplakia in patients who have AIDS, no significant clinical benefit has so far been demonstrated. As for CMV disease, antiviral drug therapy is probably only effective when the disease is due to a viral rather than an immunopathologic process.

Recent *in-vitro* studies [69b] have shown that for HHV-6, -7 and -8 the most potent, presently available drugs with the highest antiviral selectivity index were:

- for HHV-6 — foscarnet and cidofovir;
- for HHV-7 — cidofovir and foscarnet; and
- for HHV-8 — cidofovir and ganciclovir.

However, so far no data from well-designed clinical studies have been reported to show the effectiveness of these drugs in the treatment of HHV-6, -7 or -8 infections. In addition, a number of experimental compounds, particularly S2242, an acyclic guanosine analog, were also found to have a marked antiviral effect in combination with an acceptable toxicity profile.[88]

Immunotherapy

Options for immunotherapy of herpesvirus-induced disease are limited and require further development, particularly for immunopathologically mediated herpesvirus disease (e.g. as occurs in CMV and EBV infections).

A few studies have shown a beneficial effect for a combination of ganciclovir and intravenous CMV immune globulin over the use of ganciclovir alone in bone marrow transplant recipients who have CMV pneumonia.[89] More recently, promising results have been obtained in the treatment of bone marrow transplant patients with PTLD by infusing donor T cells[90] and by the use of anti-CD20 monoclonal antibodies.[91]

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Chapter 216 - Papillomaviruses and Polyomaviruses

Keerti V Shah

Until recently, these two virus families were grouped together as subfamilies in the papovavirus family because they shared the following characteristics: small size, nonenveloped virion, icosahedral capsid made up of 72 capsomers, double-stranded, circular, supercoiled DNA genome and nuclear site of multiplication. They were separated as different families because they are unrelated genetically and immunologically and are also biologically dissimilar. Papillomaviruses grow on surface epithelia, do not have a systemic phase of infection and are etiologically linked to cancer of the uterine cervix, other lower genital tract cancers and some oropharyngeal cancers. Polyomaviruses, on the other hand, have a viremic phase during which they infect internal organs such as the kidney and the brain and human polyomavirus JC is the etiologic agent for the degenerative central nervous system (CNS) disease progressive multifocal leukoencephalopathy (PML). Polyomaviruses are not linked to naturally occurring cancers.

PAPILLOMAVIRUSES

NATURE

Papillomaviruses are widely distributed in nature; among mammals they infect humans, cattle, dogs, rabbits, monkeys and other species. Some animal papillomaviruses affect both epithelial and fibroblastic tissues and produce fibropapillomas, but human papillomaviruses (HPVs) are strictly epitheliotropic and infect the skin or the mucous membranes.

Papillomaviruses cannot be propagated in cell culture. Therefore, rapid advances in the knowledge about papillomaviruses date from the 1970s, when molecular cloning of the viral genomes allowed comparisons between viruses from different species and from different sites of the same species. The cloned genomes provided reagents for examination of affected tissues for viral sequences.

The papillomavirus particle (Fig. 216.1) is about 55nm in diameter and has a double-stranded, covalently closed, circular genome of about 8000bp. All of the genomic information is located on one strand. The genome is divided into an early region that has eight open reading frames (ORFs E1–E8), a late region that has two ORFs (L1 and L2), and a noncoding long control region (LCR), which contains regulatory elements for viral DNA replication and transcription.

The L1 ORF codes for the major L1 capsid protein, which accounts for most of the virion mass and mediates viral attachment and immune response to infection. The prophylactic HPV vaccine strategies which are being tested are based largely on the use of the L1 protein expressed by recombinant DNA technology and self-assembled as virus-like particles (VLPS). The function of the minor L2 capsid protein is unclear. The early region genes E6 and E7 of high-risk HPVs code for the transforming proteins of the virus that mediate the oncogenic properties of the virus. The E1 gene is required for viral DNA replication and the E2 gene modulates viral transcription. The E5 gene is a membrane protein that interacts with growth factor receptors; it is the main transforming gene for bovine papillomaviruses but not for HPVs. The E4 gene, although it is located in the early region, is expressed late in the virus cycle; it disrupts cytokeratins and probably facilitates virus exit from infected cells. The functions of E3 and E8 ORFs are not known.

EPIDEMIOLOGY

More than 100 individual HPV types have been described to date. [1] They naturally fall into two groups, mucosal HPVs and cutaneous HPVs.

Mucosal human papillomaviruses

The genital tract is the reservoir for mucosal HPVs, except for two HPV types (HPV-13 and HPV-32), which infect the oral cavity. About 40 HPV types infect the genital tract. Genital HPV infections are the most prevalent sexually transmitted pathogens. As many as 30–40% of sexually active young women may have a prevalent HPV infection of the genital tract, as determined by a sensitive, amplification-based DNA detection technique. A history of multiple sexual partners, and having a male sexual partner who has many sexual partners, are the main risk factors for a woman for the acquisition of HPVs. Circumcision may decrease the risk of HPV acquisition in the male.[2] The HPV prevalence reaches its peak in young adults and declines at older ages. HPV infections are largely asymptomatic and of 1–2 years' duration. Infection probably confers partial immunity to re-infection with the same type. The course of HPV infection is altered profoundly by HIV-induced immunosuppression (see Chapter 77).

HPV-6 and HPV-11 are the etiologic agents of genital warts (condylomas), which occur in sexually active individuals, and also of recurrent respiratory papillomatosis (RRP), which may have onset in childhood or in adult life. HPV-16, -18, -31, -45 and some other types account for more than 90% of cervical cancers, as described in later sections of this chapter.

HPV-13 and HPV-32 cause focal epithelial hyperplasia in the oral cavity. This condition is found frequently in indigenous populations, but rarely in other groups. How they are transmitted from person to person is unclear.

Cutaneous human papillomaviruses

Skin warts are transmitted by direct contact with an infected tissue or indirectly by contact with virus-contaminated objects. There is some specificity between HPV type, and site and morphology of the warts; plantar warts most often yield HPV-1, common warts HPV-2 and flat warts HPV-3 and HPV-10.

Epidermodysplasia verruciformis is a rare condition in which an extensive lifelong wart virus infection of the skin is never resolved.[3] The disease may be familial. The warts may be flat or they may be in the form of reddish-brown macular plaques. In about one-third of these patients, malignant transformation occurs in the reddish-brown plaques in areas exposed to sunlight. The epidermodysplasia verruciformis lesions contain a large number of HPV types. Types most commonly associated with skin cancers in epidermodysplasia verruciformis patients are HPV-5 and HPV-8.

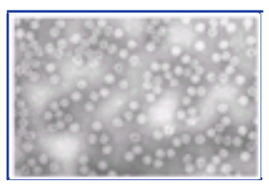


Figure 216-1 Human papillomavirus particles. The particles are nonenveloped, have icosahedral capsids and are 55nm in diameter. *Courtesy of Dr M Reissig.*

Low levels of HPV DNA sequences of a number of HPV types, including several novel types, have been recovered from nonmelanoma skin cancers. HPV sequences are also found in plucked hair roots of healthy skin. It is not clear if HPVs play a role in the development of non-melanoma skin cancers.[4]

PATHOGENICITY

Infectious agent

Papillomaviruses have a high degree of species and tissue specificity. Genital HPVs are rarely detected on skin other than that of the genital tract, but they can infect other mucosal sites in the body such as the aerodigestive tract. Cutaneous HPVs are almost never encountered in the genital tract.

Papillomaviruses cannot be grown in monolayer cell cultures to yield virus particles. Full epithelial differentiation is required for the production of infectious particles.[5] Limited numbers of papillomavirus particles are produced by infection in organotypic cultures of the epithelium or by transplantation of infected human cells to certain sites in immunodeficient mice.

Pathogenesis of cervical cancer

Nearly all cervical cancers originate in the 'transformation zone', located at the lower end of the cervix, where the stratified squamous epithelium of the vagina forms a junction with the columnar cells of the endocervix. The cells in the transformation zone of the cervix must be highly susceptible to the oncogenic effects of HPVs, because cancers arise much less frequently at other sites in the genital tract (vulva, vagina, penis) that are infected with HPVs as frequently as the cervix but do not have an area similar to the transformation zone.

Invasive cervical cancer is preceded by a progressive spectrum of cytologic abnormalities, classified as atypical squamous cells of undetermined significance

(ASCUS), low-grade squamous intraepithelial lesions (LSILs) and high-grade squamous intraepithelial lesions (HSILs). The progression from initial HPV infection to invasive cancer may take 15–20 years.

Although almost all squamous cell abnormalities of the cervix including cervical cancer are the result of HPV infections, the probability of any one HPV infection progressing to cervical cancer is quite small. Most HPV infections produce only transient cytologic abnormalities and are resolved completely without a trace. Cytologic abnormalities are seen in only about 10% of women who are positive

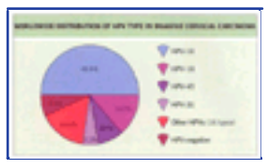


Figure 216-2 Worldwide distribution of HPV type in invasive cervical carcinoma. *The data are based on tests of over 900 cancers from different countries.*[7]

for HPV DNA in the genital tract. A large majority of LSIL regresses completely. In the USA, annually, there are tens of millions of HPV infections but only about 15,000 cases of invasive cancer. The relatively small number of cases is partly the result of treatment of LSIL and HSIL identified from Pap smear screening programs, but it is also, in a large measure, because most HPV infections do not result in significant cervical disease. In countries that have no effective Pap smear screening programs, the number of cases of cervical cancer is still a small fraction of the number of HPV infections in women.

HPVs are found in nearly all lesions spanning the entire spectrum of cytologic abnormalities from LSIL to invasive cancer. The distribution of HPV types changes markedly with increasing severity of disease.[6] Almost all genital HPV types are represented in HPV DNA-positive cytologically normal specimens and in ASCUS and LSIL cases, but only about a dozen HPV types are found in invasive cervical cancer. Of these, four HPV types, HPV-16, -18, -45 and -31, account for nearly 80% of invasive cancers (Fig. 216.2). The genital HPV types

TABLE 216-1 -- Major clinical associations of HPV infections.

MAJOR CLINICAL ASSOCIATIONS OF HPV INFECTIONS		
Disease	**HPV type**	**Transmission**
Cervical cancer		Sexual
High risk	HPV-16, -18, -45, -31	
Intermediate risk	HPV-33, -35, -39, -51, -52 -56, -59, -68, -73	
Low risk	HPV-6, -11, -26, -42, -43, -44, -53, -54, -55, -62, -66	
Cancer of vulva, vagina, anal canal, penis	HPV-16 and others	Sexual
Anogenital warts	HPV-6, -11	Sexual
Juvenile-onset RRP	HPV-6, -11	Mother-child, at birth
Adult-onset RRP	HPV-6, -11	Unclear
Cutaneous warts	HPV-1, -2, -3, -4, -10 and others	Nonsexual contact
Epidermodysplasia verruciformis	HPV-5, -8 and others	Nonsexual contact
Skin cancers	EV HPVs and novel HPVs	Unclear
Focal epithelial hyperplasia of the oral cavity	HPV-13, -32	Nonsexual contact

have therefore been categorized as high-risk, intermediate-risk or low-risk types (Table 216.1) on the basis of their prevalence in invasive cervical cancer.[7]

The oncogenic potential of HPVs is mediated by the E6 and E7 proteins of high-risk HPVs. In laboratory studies, E6 and E7 of high-risk HPVs can each transform mouse 3T3 cells and, together, can immortalize human keratinocytes. Viral constructs containing E6 and E7 genes of high-risk HPVs produce HSIL-like lesions in organotypic cervical epithelium. The E6 and E7 genes are invariably expressed in cells of HPV-associated cervical cancers. The viral genome of HPVs remains episomal in infections that are not associated with cervical cytopathology, as well as in cases of LSIL and in most instances of HSIL. However, in most invasive cancers the viral genome is integrated into the cellular DNA. Integration requires linearization of the circular viral genome, which almost always occurs by a break in the E2 region of the genome. The E6 and E7 genes not only remain intact after integration, but they are released from the inhibitory effect of the E2 protein, and are expressed at high levels in invasive

Figure 216-3 Effect of high-risk HPV E6 on the cell cycle. In normal cells (left), DNA damage results in increased p53 production, which leads to arrest of cell cycle in G1 phase, allowing the cell time to repair DNA damage. In cells infected with high-risk HPVs (right), the HPV E6 mediates degradation of p53, so there is no accumulation of p53, no cell-cycle arrest, and continued cell multiplication. This leads to genetic instability and accumulation of cellular mutations. *Courtesy of Dr TD Kessis.*

cancers. High levels of antibodies to E6 and E7 proteins are markers for invasive cancer.

The molecular mechanisms of cellular transformation by E6 and E7 genes of high-risk HPVs are well understood and are described in detail in excellent reviews.[8] [9] Briefly, the E6 protein complexes with tumor suppressor protein p53 and targets it for destruction via the ubiquitin pathway. The E7 protein complexes with tumor suppressor protein Rb, thereby releasing the transcription factor E2F from its complex with Rb. The E2F activates expression of *myc* and other genes that activate the cell cycle. In the normal cell cycle, tumor suppressor proteins p53 and Rb inhibit cellular proliferation. The functional inactivation of both of these cellular tumor suppressor proteins by the HPV oncoproteins result in continued cell proliferation without time for repair of DNA damage (Fig. 216.3). This leads to genetic instability and accumulation of additional cellular mutations and chromosomal changes. Thus, infections with high-risk HPVs prepare the ground for the cellular genetic alterations that underlie cervical cancer. For example, gain of 3q chromosome was found to be consistently associated with the early stages of invasive cervical carcinoma (Fig. 216.4).[10]

PREVENTION

HPV-related diseases for which preventive strategies are being investigated are cervical and other lower genital tract cancers, genital warts and recurrent respiratory papillomatosis.

Cervical cancer

There are high hopes that HPV-based strategies will help reduce cervical cancer burden in the world. Primary cervical cancer screening by examination of cervical scrapes for DNAs of high-risk HPVs has been shown to have a sensitivity greater than that of Pap smears for the detection of HSIL and cancer.[11] Furthermore, adequate genital

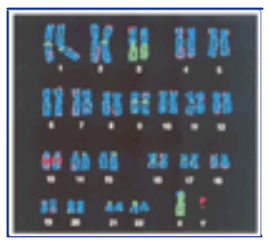


Figure 216-4 Gain of chromosome 3q in early cervical carcinoma. The figure displays a ratio image after comparative genome hybridization, in which normal reference metaphase chromosomes are hybridized with a mixture of differentially labeled tumor DNA (green label) and normal DNA (red label). The chromosomes are ordered in a karyogram-like fashion. Chromosome 3q is gained (over-representation of green label) and chromosomal band 13q21 is lost (over-representation of red label) in this carcinoma. *With permission from Heselmeyer et al.,*[10] *copyright (1996) National Academy of Sciences, USA.*

tract specimens can be self-collected by the women themselves, thus making it possible to avoid a pelvic examination. Assays for genital tract HPV DNAs are useful in the clinical management of mild cervical cytological abnormalities.[12]

Virus-like particles that self-assemble from the L1 capsid protein when it is expressed by baculovirus or yeast recombinant DNA vectors have provided the immunogen for candidate prophylactic vaccines. The VLPs are free of viral DNA and possess conformational epitopes of authentic virions. Immunization with VLPs leads to very high titers of virus neutralizing antibodies and to protection against subsequent HPV infection.[13] The vaccines are expected to provide immunity only to the VLP types included in the vaccine preparation; no cross protection against other types is anticipated. Initial vaccine constructs are for HPV-16 and -18. Several strategies are also being tried for therapeutic immunization, aimed at destroying established lesions of HSIL and invasive cancer. For this purpose, the objective is to generate cytotoxic T cells directed against cells expressing the E6 and E7 proteins of high-risk HPVs.[14] Chimeric vaccines which may have both prophylactic and therapeutic properties are also under investigation.

Genital warts

Although they are benign, genital warts (condylomas) are a significant problem in sexually active populations and especially in immunocompromised individuals. Vaccine strategies, similar to those described above for cervical cancer, but using HPV-6 and HPV-11 VLPs and E6 and E7 proteins, are envisaged for the prevention and treatment of genital warts.

Recurrent respiratory papillomatosis (RRP)

Juvenile-onset RRP results from transmission of HPV-6 and HPV-11 at birth, during fetal passage through an infected birth canal. Potential ways to reduce the incidence of this disease are treatment of condylomas during pregnancy and cesarean delivery when there is an active infection of the maternal genital tract. Vaccines based on HPV-6 and HPV-11 may have a role in the prevention and treatment of juvenile-onset RRP.

DIAGNOSTIC MICROBIOLOGY

Hybridization assays

The presence of HPV in a tissue is ascertained by nucleic acid hybridization assays. The most widely used methods, especially in epidemiologic investigations, are those based on polymerase chain reaction (PCR) amplification. [15] [16] Typically, consensus primers are employed to target conserved regions of the viral genome so that a large number of HPVs are amplified in a single amplification reaction. The PCR products are then screened for different HPVs by hybridization with type-specific oligonucleotide probes, and by a 'generic' probe (a mixture of probes of several HPVs) that would detect many HPVs. The methods have high analytic sensitivity and a high specificity. Recently, this assay has been modified so that the type specific hybridizations are performed in a single step as line blots against probes immobilized on a strip.[17] Hybrid Capture is a signal-amplification based hybridization assay approved by the FDA in the USA. In this assay, genital tract specimens are screened for HPVs in an enzyme-linked immunosorbent assay (ELISA)-type format with two pools of HPV probes, one pool consisting of high-risk HPVs and the other of low-risk HPVs.[18] This test has been found to be useful in 'triaging' women with abnormal Pap smears for colposcopy.[12]

Immunologic assays

Immunologic assays are rarely used for type-specific HPV diagnosis. In most HPV infections, viral particles and capsid proteins are present in very small amounts and so are difficult to detect with antiviral serum. Also, as cervical SILs progress toward higher grade disease and cervical cancer, synthesis of capsid proteins and infectious particles is completely shut down. Type-specific antiserum is not available for any HPV type.

Antibody response to HPVs may be measured using ELISA with virus-like particles.[19] Antibody response to HPV infections is low-titered and detectable in only about 40–50% of infected individuals. The proportion of infected individuals who are antibody positive increases with increased duration of infection and with greater viral load in the genital tract specimens.[20]

Antibodies to E6 and E7 proteins are markers of HPV-associated invasive cancer. These antibodies may be detected by peptide-based ELISA or by radioimmunoprecipitation assays with *in-vitro* synthesized full length E6 and E7 proteins. Elevated levels of E6 or E7 antibodies are found in about 40% of patients who have invasive cancer but in less than 1% of controls.

CLINICAL MANIFESTATIONS

The major clinical associations of HPVs are listed in Table 216.1 . Aspects not covered in the earlier sections of this chapter or in Chapter 77 are discussed below.

Cervical cancer

Cervical cancer is the most common female malignancy in the developing world. Approximately 500,000 cases of cervical cancer occur worldwide per year. The incidence of cervical cancer varies widely in different countries. Cervical cancer has long been known to have all the epidemiologic characteristics of a sexually transmitted disease. Recent evidence from many fields clearly points to the causative role of HPVs in cervical cancer (Table 216.2), making it the first major human cancer with a single infectious etiology.[21] The anticipated use of HPV-based vaccines holds promise for the prevention and treatment of cervical cancer. The sexual behavior of the female, the sexual behavior of her male partners and the availability of an effective Pap smear screening program explain the wide differences in cervical cancer incidence in different countries.[22] In many high-incidence areas, male sexual behavior is the key factor for the high cervical cancer rates among relatively monogamous women.

Other cancers

HPV infections are responsible for the basaloid or warty vulvar cancers that occur in younger women but are not related to the more common typical keratinizing squamous cell carcinomas that occur in older women. They are strongly associated with cancers of the anal canal, vagina and penis. In addition, HPV infections are linked to a subset of oropharyngeal cancers, especially tonsillar cancers.

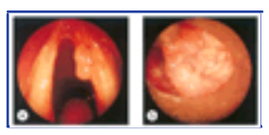
Recurrent respiratory papillomatosis (laryngeal papilloma)

This results from the transmission of HPV-6 and HPV-11 infections from the genital tract to the respiratory tract. For juvenile-onset disease, a large majority of the transmissions occur at birth, during passage of the fetus through an infected birth canal. Some reports suggest occasional intrauterine transmission. About 25% of the cases occur in the first year of life and most of these in the second 6 months. There are progressively fewer cases in each year thereafter.

The most common site of the papilloma is in the larynx on the vocal cords (Fig. 216.5). The tumors are benign but may threaten life if they grow and obstruct respiration. The tumors tend to recur after surgical removal, and in the worst cases operations may be required every few weeks. Operative procedures, especially tracheotomy, may spread the tumor by inadvertently transplanting tumor cells to other sites. Rarely, the tumor may undergo malignant transformation.

TABLE 216-2 -- **Evidence linking HPVs with invasive cervical cancer.**

EVIDENCE LINKING HPVS WITH INVASIVE CERVICAL CANCER	
Epidemiology	• HPVs present in over 90% of cervical cancers both in low-and in high-incidence areas
	• HPV infections precede invasive cancer
	• HPV epidemiology and cervical cancer screening practices together account for differences in worldwide incidence of cervical cancer
Pathogenesis	• HPV genome present in every tumor cell and transcriptionally active
	• HPVs associated with the entire spectrum of cervical neoplasia, from low-grade cytologic abnormalities to invasive disease
	• HPV types most frequent in cancers are the most oncogenic in laboratory assays
	• HPV oncogenes E6 and E7 invariably expressed in cancers
	• Viral genome which is episomal in preinvasive disease, integrates into cellular DNA in invasive cancers
Molecular mechanisms	• Oncoproteins E6 and E7 of high-risk HPVs distort cell cycle and promote genetic instability by degradation of tumor suppressor protein p53 and inactivation of tumor suppressor protein Rb
	• Integration of viral genome in cellular DNA enhances E6 and E7 expression
Observations from the field, the clinic and the laboratory are mutually corroborative in building a compelling case for the HPV etiology of cervical cancer.	

 **Figure 216-5 RRP of juvenile onset.** (a) A respiratory papilloma on the vocal cord of a child. (b) Papilloma obstructing the respiratory tract. *Courtesy of Dr H Kashima.*

MANAGEMENT

The clinical conditions associated with HPV infections are very diverse. Regular Pap smear screening and follow-up of women who have Pap smear abnormalities would greatly decrease the risk of cervical cancer. The preinvasive cervical disease is readily treated with greater than 90% cure rates. Genital and skin warts may regress spontaneously or may be treated with caustic agents (podophyllin), cryotherapy, application of an immunomodulating agent (imiquimod), or by surgical removal. Intralesional or parenteral administration of interferon has been successful in the treatment of refractory genital warts (see Chapter 77).

POLYOMAVIRUSES

NATURE

Polyomaviruses are small, nonenveloped viruses with a diameter of about 42nm. The virions have icosahedral symmetry and 72 capsomers. The viral genome is a circular, covalently closed, supercoiled, double-stranded DNA of about 5000bp. Each DNA strand carries about one-half of the genetic information. The genome is divided into an early region (2.4kb), which codes for large and small T proteins, a late region (2.3kb), which codes for viral proteins VP1, VP2 and VP3, and a non-coding regulatory region (0.4kb), which contains regulatory elements for viral DNA replication and transcription.

EPIDEMIOLOGY

BK virus (BKV) and JC virus (JCV) are two polyomaviruses that infect humans.[23] Infections with both viruses occur in childhood, but infections with BKV occur at an earlier age. In the USA, serologic studies suggest that about 50% of children are infected with BKV by the age of 3 or 4 years, and 100% by 10–11 years. JCV antibody prevalence peaks at about 75–80% by adulthood. The exact mode of transmission of these infections is not known.

Between 1955 and 1961, a large number of people in the USA and elsewhere were exposed to simian virus 40 (SV40), an infection of macaque species, because the virus was an inadvertent contaminant of inactivated poliovirus vaccines that were prepared from virus pools grown in simian kidney cultures. The contaminating SV40 virus was not fully inactivated. Although there have been occasional reports of recovery of SV40 from a number of human cancers, the significance of these observations is unclear.[24]

PATHOGENICITY

The viruses probably multiply at the site of entry (which is not known) and then reach the kidney, the main target organ, by viremia. They undergo further multiplication in the kidney and produce transient viruria. After primary infection, the viruses remain latent in the kidney for an indefinite period of time. In times of immunologic impairment, the viruses may be reactivated in the kidney and virus-infected cells are excreted in the urine. The infected urinary cells have enlarged nuclei and basophilic inclusions. The reactivated viruses may reach other organs, such as the brain. Almost all the diseases associated with BKV and JCV are a result of reactivated infections. The viruses may also persist in B cells and in the brain, after primary infection.

The pathogenesis of progressive multifocal encephalopathy (PML) is discussed below.

PREVENTION

No attempts have been made to prevent primary BKV or JCV infections, which are essentially harmless in immunocompetent individuals.

DIAGNOSTIC MICROBIOLOGY

Tests for BKV and JCV are indicated when urinary cytology reveals abnormal epithelial cells. Differential diagnosis includes urinary tract infections with other viruses, for example cytomegalovirus, and tumor cells from bladder cancer. Tests for BKV and JCV are also indicated in the investigation of cystitis, hemorrhagic cystitis and hematuria.

BKV and JCV can be readily identified by PCR assays or by Southern hybridization of DNA from infected tissue. BKV can be

cultivated in several cell lines of human origin. JCV grows best in human fetal glial cell cultures, which are rich in spongioblasts. Isolation of BKV and JCV in cell cultures is rarely attempted because it is inefficient and cumbersome, and the needed cell types are not readily available.

Cells and tissues infected with BKV or JCV can be identified by immunoperoxidase and immunofluorescence tests with antiviral antiserum. Immunization of rabbits with disrupted viral capsids produces a broadly cross-reactive antiserum. The viruses in urine supernatants can be identified by ELISA. Serologic diagnosis of BKV and JCV infections can be made by hemagglutination-inhibition assays or by ELISA.

CLINICAL MANIFESTATIONS

Progressive multifocal leukoencephalopathy

PML is a fatal, subacute, demyelinating disease of the CNS that occurs as a complication of conditions that impair T-cell immunity, for example HIV infection, lymphoproliferative disorders, chronic diseases and inherited immunodeficiency diseases. In addition, prolonged immunosuppressive therapy, as required for organ transplant recipients, is a risk factor for PML. Until the advent of AIDS, PML was a rare disease with onset typically in the fifth or sixth decades of life. PML cases in patients who have AIDS now outnumber PML cases in all other illnesses combined, and the age distribution of cases has shifted to lower age groups. PML was recognized as an AIDS-defining illness in 1987 and occurs in about 0.7% of patients who have AIDS.[25] Children who have inherited immunodeficiency diseases and children who have AIDS also develop PML.

PML has an insidious onset. Early signs are impairment of speech and vision and mental deterioration. Paralysis of limbs, cortical blindness and sensory impairments are common. Typically, death occurs within 3–6 months of onset. During the course of the disease, the patient is afebrile, with normal cerebrospinal fluid (CSF) and no signs of increased intracranial pressure.

The key event in the pathogenesis of PML is the infection of oligodendrocytes with JCV, which leads to the death of the infected cells. The enlarged nuclei of the oligodendrocytes are the pathognomonic lesion in PML; they contain abundant numbers of JCV viral particles. Oligodendrocytes secrete and maintain myelin; the destruction of the infected oligodendrocytes leads to the characteristic foci of demyelination of PML (Fig. 216.6).[26] The enlarged oligodendrocytes are seen at the periphery of the demyelinating lesion (Fig. 216.7). Giant bizarre astrocytes are seen within the areas of demyelination.

The multifocal nature of the PML lesions suggests that JCV reaches the CNS by the hematogenous route, probably transported by infected B cells. Alternatively, JCV seeded in the normal brain after primary infection may be reactivated by immunosuppression to cause PML. Active PML lesions contain abundant amounts of infectious JCV, JCV proteins and JCV DNA. It is likely that a rearrangement of the regulatory region of the JCV genome facilitates the growth of the virus in the CNS.[27]

Until a few years ago, diagnosis of PML required a brain biopsy but it is now possible to diagnose the disease by noninvasive techniques

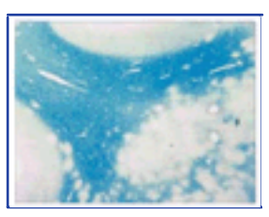


Figure 216-6 Foci of demyelination in PML. The foci in the superior frontal gyrus are the result of PML (Luxol fast blue stain). *With permission from Harrison and McArthur.*[26]

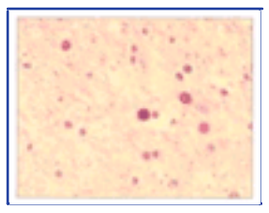


Figure 216-7 Enlarged oligodendrocytes in PML (stained with antiviral serum). *With permission from Harrison and McArthur.*[26]

such as computed tomography or magnetic resonance imaging. The use of the PCR to detect JCV in CSF is still investigational.

BKV-related diseases

BKV infection seems to be responsible for occasional cases of cystitis in healthy children and also for hemorrhagic cystitis, which is a complication of bone marrow transplantation. Atypical primary BKV infection in an immunocompromised child may cause tubulointerstitial nephritis and irreversible renal damage.

A new syndrome, BKV nephropathy, has been recently identified in renal transplant recipients.[28] [29] It may be a complication of newly introduced immunosuppressive drugs. The disease is characterized by extensive BKV multiplication in the tubular epithelium, urinary shedding of a large number of infected epithelial cells, the presence of BKV in the serum, and a loss of allograft function. An unusual tropism of BKV for endothelial cells has been described recently in a fatal case of disseminated BKV infection. [30]

MANAGEMENT

PML is almost always fatal. Nucleic acid based analogs have not been effective in the treatment of PML. For a fuller discussion on treatment options see Chapter 125 . The course of disease may be slowed if it is possible to reduce iatrogenic immunosuppression (e.g. in organ transplant patients). Patients who have AIDS and PML have shown improvement with control of the HIV infection with multidrug therapy including protease inhibitors.

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Chapter 217 - Parvoviruses

Stanley J Naides

NATURE

The family Parvoviridae consists of small, nonenveloped, single-stranded DNA viruses of animals. It is divided into the subfamilies Parvovirinae of vertebrates and Densovirinae of insects. Parvovirinae is further divided into three genuses: *Parvovirus*, *Erythrovirus* and *Dependovirus*. The genus *Parvovirus* consists of a number of nonhuman mammalian viruses including mice minute virus, Aleutian disease virus of mink, feline panleukopenia virus, Kilham rat virus and the bovine, porcine, canine, goose and feline parvoviruses, among others. The Erythroviruses consist of primate parvoviruses that require erythroid precursors for productive infection and notably in the case of B19, the human parvovirus, cause an erythematous rash. Dependoviruses consist of mammalian parvoviruses that require a helper virus for replication. The typical helper virus is adenovirus, hence the name adeno-associated virus (AAV). Human AAVs are candidate gene therapy vectors because they are nonpathogenic and have broad tissue tropism.

Densovirinae are viruses of insects and crustaceans; several have economic importance such as those affecting silk production.

Parvovirus B19 is the only known human pathogen. Cossart and colleagues discovered B19 serendipitously in 1974 during the development of diagnostic tests for hepatitis B virus. B19 was named after the viremic serum (number 19 in panel B) in which a new antigen was detected in Ouchterlony immunodiffusion plates. [1] In short order, the antigen was determined to be a previously unrecognized human parvovirus measuring approximately 23nm in diameter. It appeared icosahedral or spherical on electron microscopy and was non enveloped (Fig. 217.1). Molecular studies have since demonstrated that B19 has palindromic terminal repeats at both ends that are nearly identical. B19 uses a modified rolling hairpin model of replication, as do the other parvoviruses. Progeny virus is assembled in the nucleus and may be observed as intranuclear inclusions. Equal numbers of B19 positive and negative sense DNA strands are encapsidated. B19 utilizes a single strong upstream promoter from which are transcribed message for a 72 kilodalton (kDa) nonstructural protein (NS1), and 84 and 58 kDa capsid proteins (VP1 and VP2, respectively). Transcripts of the capsid proteins utilize alternate start sites, making VP1 identical to VP2 except for the addition of 227 N-terminal amino acids in VP1, often designated the unique VP1 region, uVP1. VP2 is the major capsid protein. VP1 is thought to occupy the five fold axis of symmetry of the icosahedron. Epitopes in uVP1 and adjacent regions are thought to contain the target structures for neutralizing antibodies. NS1 is a helicase and serves to reduce covalently linked monomeric (two gene copies) and dimeric (four genome copies) replicative forms to packageable progeny genome. NS1 is thought to cause transactivation of cellular genes including interleukin 6 and possibly tumor necrosis factor-a (TNF-a), virus-cell interactions that may be important in pathogenesis. Several smaller viral proteins detected during replication have unknown function. Although the B19 genome is generally highly conserved, a variant of unknown significance, designated V9, has been described.[2]

EPIDEMIOLOGY

B19 is found worldwide. It occurs as both sporadic cases and in recurrent epidemics. Transmission is via nasopharyngeal secretions, explaining the frequency of outbreaks in the late winter and spring. Occurrence in susceptible cohorts of school-aged children may also contribute to outbreaks during the school year. However, outbreaks have also been described in summer and fall. Populations of children acquire B19 antibodies in an age-dependent fashion, beginning with entry into schools. Epidemics tend to occur in 3–5-year cycles, the time required for the generation of a susceptible school cohort. Approximately 50% of adults are anti-B19 IgG seropositive, indicating past infection. Illness in susceptible individuals occurs after an incubation period of 7–18 days.[2] IgG antibody is considered protective. B19 is not removed from blood by current solvent detergent-based methods of blood decontamination and therefore pooled blood products may contain infectious B19.[3]

PATHOGENICITY

B19 is typically transmitted through nasopharyngeal secretions. Following an incubation period of 7 days in experimental infections, a brisk viremia of 10^{11} or more viral particles/ml blood occurs. Viral replication causes maturation arrest at the giant pronormoblast stage of erythroid development, likely through viral induction of apoptosis. Nonerythroid lineages may be affected as well, although they are less efficient in supporting B19 replication. In individuals with shortened erythrocyte survival, as in those with inherited or acquired hemolytic anemias, the period of areticulocytosis is associated with severe anemia. On approximately day 11 post infection, specific IgM antibody appears, followed shortly after by anti-B19 IgG.[4] Initially, IgG is directed against VP2 but after 1 week anti-VP1 IgG antibody appears. Failure to produce anti-VP1 IgG results in persistent or recurrent viremia and chronic or recurrent bone marrow suppression. [5]

Two clinical phases of illness are described in experimental infection, but these may overlap in natural infections. In the first phase, fever, malaise and myalgia may occur during the viremia. Areticulocytosis, leukopenia and thrombocytopenia may occur. In the second phase, the appearance of anti-B19 IgM is associated with rash (Fig. 217.2), arthralgia and frank arthritis.[4] In the pregnant susceptible woman, B19 may cross the placenta to cause erythroid maturation arrest, anemia and a high-output congestive heart failure characterized as hydrops fetalis. B19 viral myocarditis as a cause of fetal hydrops has been described.[2]

PREVENTION

Infection control measures include secretion precautions during periods of viremia; some institutions require respiratory precautions. Patients should be considered infectious from 7 days post exposure until either 18 days post exposure or 24 hours after onset of rash or

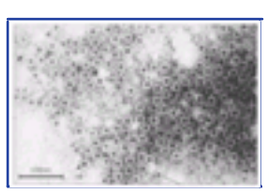

Figure 217-1 Electron micrograph of parvovirus B19 particles showing both full particles (short arrow) and empty capsids (long arrow).

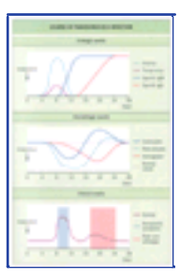

Figure 217-2 Course of parvovirus B19 infection. Volunteers were inoculated nasally with viremic serum and virologic, hematologic and clinical events observed.

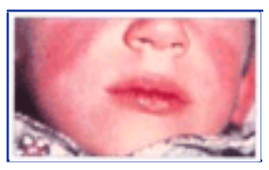

Figure 217-3 Classic 'slapped cheeks' of a child with erythema infectiosum, or fifth disease, caused by parvovirus B19. A lacy macular erythematous eruption is also present on the trunk but not shown. *Courtesy of Dr K Motton.*

arthritis.[6] Immunocompromised individuals with chronic B19 infection tend to have low-titer viremia and are of low infection risk to others. The utility of excusing pregnant workers with frequent exposure to children from work responsibilities during an outbreak is controversial. While a susceptible teacher, for example in a class with five or more children with B19 rash, has a 50% risk of infection,[7] absence from the workplace does not prevent exposure at home or in the community. Secretion precautions and handwashing in the childcare setting remain prudent.

DIAGNOSTIC MICROBIOLOGY

Detection of viremia by immune electron microscopy, DNA hybridization or polymerase chain reaction-based methodologies is diagnostic of B19 infection. Detection of anti-B19 IgM in the setting of acute onset of consistent symptoms is likewise diagnostic (Fig. 217.3). As B19 is not readily grown in tissue culture, viremic serum or recombinant capsid proteins have been used as antigen. The presence of anti-B19 IgG is diagnostically useful only when acute and convalescent samples demonstrate seroconversion. Anti-B19 IgM is usually undetectable by 2 months after infection. Immunocompromised individuals such as those with HIV may have chronic or recurrent viremia followed by poor IgM or IgG responses.[2] Finding B19 DNA in tissues of immunocompetent patients is of questionable diagnostic significance because B19 DNA has been found in about 50% of healthy military recruits in Finland undergoing arthroscopy for joint injury.[8]

CLINICAL MANIFESTATIONS

B19 may cause asymptomatic infections. As many as 70% of childhood infections may be asymptomatic. Individuals with chronic hemolytic anemia, such as sickle cell disease or hereditary spherocytosis, develop transient aplastic crisis during the areticulocytosis phase of the infection. The most common presentation in children is the childhood exanthema erythema infectiosus, or fifth disease, characterized by bright red 'slapped cheek' macular or maculopapular rash. The eruption spreads from the trunk to the extremities in a centrifugal progression, often appearing blotchy or fishnet-like. Activity, heat or sun exposure exacerbates the rash. Headache, fever, cough, sore throat, anorexia, vomiting or diarrhea may occur but is usually mild. Rarely, children have joint symptoms.[2] Adults tend to have either no rash or a subtle one, but a sudden onset, symmetric rheumatoid-like polyarthritis may occur and may be accompanied by a more severe flu-like illness than seen in children. Joint symptoms usually improve within 2 weeks, but in some adults may persist for months to years and are associated with persistent or episodic morning stiffness and arthralgias.[9] In pregnant women the unborn child may develop hydrops fetalis. Immunocompromised individuals may develop chronic or recurrent episodes of anemia, leukopenia or thrombocytopenia. Rarely, isolated anemia, leukopenia or thrombocytopenia may occur in an immunocompetent patient. Hepatitis,

acute fulminant liver failure, myocarditis, vasculitis, Henoch-Schönlein purpura, vesicular skin eruptions, 'socks and gloves' acral rash, purpura with or without thrombocytopenia, benign acute lymphadenopathy, hemophagocytic syndrome, peripheral neuropathy, sensorineural hearing loss, aseptic meningitis and rarely encephalopathy have all been described in association with B19 infection. B19 is the leading cause of pure red cell aplasia in AIDS patients.[2]

MANAGEMENT

Therapy for B19 infection is supportive in most clinical presentations. In immunocompromised patients with persistent B19 infection and chronic or recurrent bone marrow suppression because of failure to make neutralizing anti-VP1 antibody, intravenous immunoglobulin may allow clearing of apparent infection and resolution of bone marrow suppression.[5] Patients with transient aplastic crisis may require transfusion support. Likewise, fetal hydrops may require intrauterine transfusion for anemia. B19 is not considered a cause of congenital anomalies.

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Chapter 218 - Poxviruses

R Mark L Buller

NATURE

Virion morphology, structure and biochemistry

Poxviruses are the largest of all animal viruses and can be visualized under the light microscope, although the details of the virion structure remain obscure. Using negative-staining electron microscopy and/or cryoelectron microscopy, parapoxvirus virions appear ovoid with long and short axes of approximately 260 and 160nm, respectively, whereas all other poxviruses are 'brick'-shaped, with dimensions of 350 by 250nm on average. In clinical specimens two forms of virion have been identified: the intact 'M' (mulberry; Fig. 218.1) form is found mainly in the vesicular fluid, and the 'C' (capsule) form, which appears to be a degenerative 'M' form, is associated with dried scabs.

Electron micrographs of thin sections of poxvirus-infected tissue culture cells have revealed at least three infectious forms of the virion; intracellular mature virions, extracellular enveloped virions and occluded virus in A-type inclusion body.[1]

It has been proposed that the intracellular mature virions form from the membrane structures of the intermediate compartment, and can acquire an additional two membranes from the Golgi, one of which is lost on egress at the plasma membrane. These extracellular enveloped virions are thought to be most important in cell-to-cell spread and systemic disease. In certain poxvirus species the intracellular mature virion can also occlude in a dense protein matrix within the cytoplasm to form an acidophilic inclusion body (A-type inclusion or Downie body), which is thought to protect the virion from the environment.[2] All vertebrate poxviruses have cytoplasmic basophilic (B-type) inclusion bodies, which represent areas of the infected cells that contain large amounts of virus DNA and protein.

Depending on the poxvirus, the genome can range from 130kbp for the parapoxviruses to 300kbp for the avipoxviruses, which is sufficient to encode more than 140 proteins. A schematic intracellular replication cycle based on the poxvirus vaccinia is presented

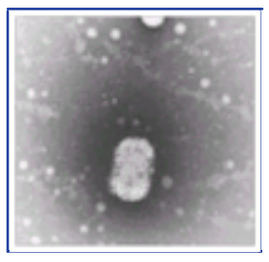

Figure 218-1 A negative-stained M form of molluscum contagiosum virus. Molluscum contagiosum virus from lesion material.

in Figure 218.2 and described in more detail by Moss.[1] The poxvirus virion is notoriously stable in the environment, and this stability is increased by its association with the A-type inclusion bodies or scab material. Thus for poxviruses, fomites must be considered in disease transmission.

Poxviruses pathogenic for humans

Ten poxviruses infect humans (Table 218.1).[2] Except for the 'extinct' variola virus and the increasingly important molluscum contagiosum virus, the poxvirus diseases are zoonoses. With rare exception these zoonotic poxviruses fail to establish a human chain of transmission. Most human poxvirus infections occur through minor abrasions in the skin. Orf, molluscum contagiosum, and monkeypox viruses cause the most frequent poxvirus infections worldwide; the incidence of molluscum contagiosum and monkeypox virus infections is on the rise, the former as an opportunistic infection in late-stage AIDS and the latter as a zoonotic infection in cental Africa. Individuals with atopic dermatitis may be predisposed to poxvirus infections such as molluscum contagiosum, orf or cowpox.

PREVENTION

Because most poxvirus infections are rare zoonoses or, as in molluscum contagiosum virus, cause only superficial, 'nuisance' skin lesions in immunocompetent individuals, there are no recommended prevention strategies other than education of patients and various types of workers as to how the diseases are spread.

MOLLUSCUM CONTAGIOSUM VIRUS

EPIDEMIOLOGY

Geographic range

Molluscum contagiosum virus has a worldwide distribution. Restriction endonuclease analysis of genomic DNA from molluscum contagiosum virus isolates has revealed the existence of at least four virus subtypes or strains, and one recent study suggests the distribution of subtypes can vary geographically.[3]

Prevalence and incidence

For nonsexually transmitted molluscum contagiosum, the disease is more prevalent in the tropics than in Europe. For example, molluscum contagiosum was diagnosed in 1.2% of outpatients in Aberdeen, UK between 1956 and 1963, the mean age of infection was between 10 and 12 years and spread within households and schools was infrequent. On the other hand, in Fiji in 1966, 4.5% of an entire village had the disease, mean age of infection was between 2 and 3 years and 25% of households harbored more than one case.[4] [5] Prevalence of infection in New Guinea was greater than 50% in many villages. In England between 1971 and 1985 there was a 400% increase in cases of genital molluscum contagiosum; the majority of patients were aged 15–24 years, with affected women

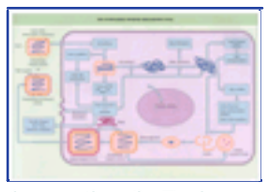

Figure 218-2 The cytoplasmic poxvirus replication cycle. Poxvirus virions containing early RNA transcription machinery attach to, and fuse with, the plasma membrane (uncoating I). Early genes are expressed that code for a variety of functions that modify the host cell for optimal virus replication, attentuate the host response to infection and mediate virus synthetic processes. After further uncoating (II), the virus genome is replicated via concatamers, late transcription factors are expressed from intermediate genes and late gene RNA is synthesized. Late genes encode the early transcription system, enzymes and structural proteins necessary for virion assembly, which commences with the formation of membrane structures in the intermediate compartment and the packaging of resolved unit length genomic DNA. The intracellular mature virion has two membranes derived from the intermediate compartment. It may remain in the cytoplasm or (in certain virus species) become occluded in an A-type inclusion body or become wrapped by a further two membranes in the Golgi and exported from the cell with the loss of one membrane (extracellular enveloped virions). The extracellular enveloped virions are thought to be most important in cell-to-cell spread and systemic disease. This replication scheme is based on the study of the prototypic poxvirus vaccinia.[1] Other poxvirus species probably vary from this model mainly in the types of growth factors and host response modifiers encoded by the virus and the amounts of extracellular enveloped virions produced. *Adapted from Moss.*[1]

being younger than affected men.[5] In the USA between 1966 and 1983 there was a ten-fold increase in cases in patients aged 25–29 years.[5] Molluscum contagiosum is a common and sometimes severely disfiguring opportunistic infection of between 5 and 18% of patients with HIV infection, especially those with severely depressed $CD4^+$ T cell numbers.[6]

Pathogenicity

Transmission

Molluscum contagiosum is observed in children and adults, with spread within this latter group governed in part by sexual practices. Nonsexual transmission is a consequence of infection by direct contact or through fomites. Case histories have suggested transmission from surgeons' fingers, swimming pools, bath towels in gymnasia, contact between wrestlers and as a result of tattooing. [4] Transmission between persons in the absence of fomites requires fairly close contact. Lesions can be commonly observed on opposing surfaces and the virus can be further spread on the person by autoinoculation.

Lesion histopathology

Molluscum contagiosum virus has one of the narrowest cell tropisms of any virus, replicating only in the human keratinocyte of the epidermis.[7] As the virus-infected cell approaches the surface of the skin, the accumulation of progeny virions in a granular matrix in the cytoplasm forces the cell organelles, including the nucleus, to the periphery of the cell. Under light microscopy these cells stain as hyaline acidophilic masses, are referred to as molluscum or Henderson-Patterson bodies and are pathognomonic for disease. The higher magnification of a molluscum body reveals it to be entirely filled with virions, partial virions and debris (Fig. 218.3).

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

Molluscum contagiosum manifests as small clusters of lesions in immunocompetent individuals. The lesions are generally painless; they appear on the trunk and limbs (rarely palms and soles) in the

TABLE 218-1 -- Poxviruses pathogenic for humans.[‡]

POXVIRUSES PATHOGENIC FOR HUMANS			
Genus/Species	**Hosts**	**Geographic distribution**	**Disease**
Molluscipoxvirus	Humans[*]	Worldwide	Molluscum contagiosum, single or multiple skin nodules
Molluscum contagiosum virus			
Parapoxvirus	Sheep[*] , goats, dogs, bighorn sheep, thinhorn sheep, Rocky Mountain goat, chamois, reindeer, musk-ox, Himalayan tahr, Steenbok, alpaca	Worldwide	Orf, localized skin lesions
Orf virus (contagious pustular dermatitis virus or contagious ecthyma virus)			
Pseudocowpox virus (milkers' nodule or paravaccinia)	European cattle[*]	Worldwide	Milkers' nodules, localized skin lesions
Bovine papular stomatitis virus	European cattle[*]	Worldwide	Localized skin lesions
Orthopoxvirus	Rodents[*] , cats, cows, zoo animals	Europe, western Asia	Cowpox, localized pustular lesions
Cowpox virus			
Monkeypox virus	Squirrels[*] , monkeys	Western and central Africa	Monkeypox, rash, generalized disease

Vaccinia virus	Natural host unknown (buffalo† and cattle)	Asia, India, Brazil and laboratory	Buffalopox, localized pustular lesions
Variola virus	Eradicated from humans in 1977	Was worldwide	Smallpox, rash, generalized disease
Yatapoxvirus	Monkeys? Rodents?	Eastern and central Africa	Tanapox, localized nodular skin lesion
Tanapoxvirus			
Yaba monkey tumor poxvirus	Monkeys?	Western Africa	Localized nodular skin lesions
Presented by genus in order of importance for human disease. Alternative hosts are given in parentheses.			

‡ *Adapted from Fenner.*[2]
* Natural reservoir.
† During the smallpox eradication program, water buffalo in India and cattle in Brazil were infected with the local vaccine strain of vaccinia virus, which apparently persists in these animals and occasionally infects humans.
? Putative host.

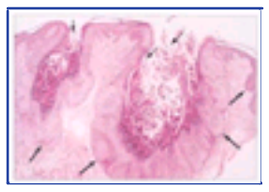

Figure 218-3 Molluscum contagiosum lesion. In this lesion a major and minor umbilicus has formed as a result of the hypertrophy of infected cells and hyperplasia of the basal cells, which caused a severe invagination of the epidermis but no loss of integrity of the basement membrane. The molluscum bodies stain as pink to purple acidophilic hyaline masses up to 37 × 27µm in size. Small arrows: molluscum bodies. Large arrows: epidermis-dermis boundary. (H & E.)

nonsexually transmitted disease. In children, the virus can also be fairly common in the skin of the eyelids, with solitary or multiple lesions, and can be complicated by chronic follicular conjunctivitis and later by a superficial punctate keratitis. [8] There may be an associated erythema 1–11 months after the appearance of the lesion, with no correlation to a history of allergy or eczema.[9] Lesions can persist for as little as 2 weeks or as long as 2 years. Re-infections can be common. As a sexually transmitted disease in teenagers and adults, the lesions are mostly on the lower abdominal wall, pubis, inner thighs and genitalia. As yet there is no solid correlation of virion DNA type with specific pathology or location of lesions (i.e. genital versus nongenital). [10]

In immunocompromised individuals (especially those with HIV infection), the infection is not self-limiting, with more frequent and larger lesions present especially on the face, neck, scalp and upper body. Multiple adjacent lesions sometimes become confluent. Molluscum contagiosum can be considered a cutaneous marker of severe immunodeficiency.

Gross lesion pathology

In immunocompetent patients, molluscum contagiosum virus lesions are epidermal, flesh-colored, raised nodules of 2–5mm in diameter. Occasionally the gross lesions may have a hypopigmented or erythematous halo. Rarely they will present as a large lesion called giant molluscum (>5mm in diameter). Both of these types of lesions usually have an umbilicated center (Fig. 218.4). Although giant molluscum lesions have been reported frequently in severely immunodeficient individuals with HIV infection, they were observed in New Guinea before the introduction of HIV, and therefore may not be a consequence of immunodeficiency.[6] [11]

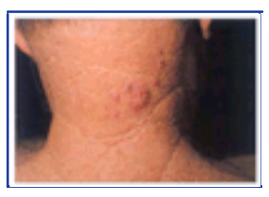

Figure 218-4 Molluscum contagiosum lesions. These are the more typical, but still large, lesions of molluscum contagiosum. *Courtesy of J Burnett.*

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis is usually made clinically based on the gross appearance of the lesions and their chronic nature. Confirmation is easily obtained through the detection of molluscum bodies by hematoxylin and eosin staining of a biopsy or a squash preparation of expressed material from the lesion.

Molluscum contagiosum (especially giant molluscum) can be confused with a number of other disorders such as keratoacanthoma, warty dyskeratoma, syringomas, hidrocystomas, basal cell epithelioma, trichoepithelioma, ectopic hyperplastic sebaceous glands or giant condylomata acuminata, chalazion, sebaceous cysts, verrucas, milia, lid abscess or granuloma on eyelids.[12] [13] [14] In immunodeficient patients, disseminated cutaneous cryptococcosis and cutaneous histoplasmosis can resemble typical molluscum contagiosum. [11] [12] An inflamed molluscum lesion without the association of typical lesions can be mistaken for a bacterial infection.

PARAPOXVIRUSES

The parapoxviruses orf, bovine stomatitis and pseudocowpox generally cause occupational infections of humans, with orf infections being the most common. Because of the clinical similarity of the diseases caused by these agents, they have been referred to collectively as 'farmyard-pox' diseases. No human-to-human transmission of parapoxvirus infections has been reported.

EPIDEMIOLOGY

Geographic range

Orf in sheep and goat populations has been reported in Canada, the USA, Europe, Japan, New Zealand and Africa. Pseudocowpox virus and bovine papular stomatitis virus are both maintained in European-derived dairy herds in all parts of the world.

Prevalence and incidence

In a 1-year New Zealand study 500 meat workers out of a population of 20,000 at risk were infected with orf; those involved in the initial stunning, killing and hanging of the sheep had the highest risk (4%) of infection.[15] Serologic surveys of orf-infected sheep and goat herds yielded orf antibody prevalences of up to 90%. The high seroprevalence of orf antibody in herds is likely attributable to the highly stable nature of the orf virion, which contaminates the pasture.

Pseudocowpox virus and bovine papular stomatitis are probably endemic in all European-derived dairy herds.

PATHOGENICITY

Transmission

Direct transmission of orf virus has been observed as a consequence of bottle-feeding lambs, from animal bites to the hand and contact with sheep and goat products during slaughter. Fomites such as splinters, barbed wire or farmyard surfaces, including soil, feeding troughs or barn beams, have been implicated as sources of virus.

Pseudocowpox virus from lesions on teats of cows is a major source of virus for milkers' nodule of the hand.

Bovine papular stomatitis virus infection of humans occurs generally from lesions confined to the mouth, tongue, lips or nares and occasionally from the teats of infected cattle.

Lesion histopathology

Epidermis

The most striking change in the epidermis is hyperplasia in which strands of epidermal keratinocytes penetrate the dermis.[16] Generally, a mild to moderate degree of acanthosis is detected, and parakeratosis is a common feature. Cytoplasmic vacuolation, nuclear vacuolation and deeply eosinophilic, homogeneous cytoplasmic inclusion bodies often surrounded by a pale halo are also characteristic of the infection. An intense infiltrate of lymphocytes, polymorphs or eosinophils frequently involves the epidermis.

Dermis

A dense, predominantly lymphohistiocytic inflammatory cell infiltrate is present in all cases with marked edema. The most striking feature of the dermis is the massive capillary proliferation and dilatation.

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

The clinical presentation of orf is usually 3–4 weeks after infection. The disease involves the appearance of single or multiple nodules (diameters of 6–27mm),[16] which are sometimes painful, usually on the hands and, less frequently, on the head or neck. Orf infection can also be associated with a low-grade fever, swelling of the lymph nodes and/or erythema multiforme bullosum. Resolution of the disease occurs over 4–6 weeks, usually without complication; however, autoinoculations of the eye can lead to serious sequelae, and enlarged lesions can arise in humans suffering from immunosuppressive conditions, burns or atopic dermatitis.[17] Lesion healing can be complicated by bullous pemphigoid.[18] Re-infections have been documented.[15]

Pseudocowpox virus lesions usually appear on the hands and are relatively painless, but may itch. The draining lymph node may be enlarged. The nodules are gradually absorbed and disappear in 4–6 weeks.[19]

In bovine papular stomatitis, lesions occur on the hands, diminish after 14 days and are no longer evident 3–4 weeks after onset.[20]

Gross lesion pathology

The orf lesion characteristically goes through a maculopapular target stage in which a red center is surrounded by a white ring of cells which is surrounded by a red halo of inflammation (approximately 1–2 weeks after infection; Fig. 218.5); however, patients usually

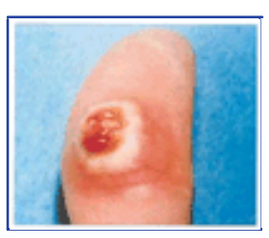

Figure 218-5 A typical orf lesion at the target stage of development. *Courtesy of Andrew Mercer.*

present later when the lesion is at the granulomatous or papillomatous stages.[17]

In pseudocowpox virus infection, milkers' nodules are first observed as round cherry-red papules; these develop into purple, smooth nodules of up to 2cm in diameter and may be umbilicated. The lesions rarely ulcerate.[19]

The lesions of bovine papular stomatitis appear as circumscribed wart-like nodules that gradually enlarge until they are 3–8mm in diameter. [20]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Parapoxvirus disease diagnosis is by clinical (lesion morphology) and epidemiologic evidence (recent contact with cattle or sheep) and electron microscopy of negative-stained lesion material (presence of ovoid particles).[16]

Without knowing the animal source of the infection, orf cannot easily be differentiated from milkers' nodule on the basis of clinical finding, histology or electron microscopy (i.e. disease acquired from sheep is orf and from cattle is milkers' nodule or possibly bovine papular stomatitis).[16] Atypical giant orf lesions in patients who are immunocompromised or suffering from burns or atopic dermatitis may be confused with pyogenic granuloma.[17] [21] Orf can be misdiagnosed as pyogenic granuloma or inflammatory vascular neoplasms.

ORTHOPOXVIRUSES

Although four orthopoxviruses have been shown to cause disease in humans, only two still cause significant human infections. With the global eradication of the disease smallpox in 1977, the causative agent variola virus no longer circulates in human populations, but there is concern that it may be used as a biologic weapon by terrorist groups or rogue nations (see Chapter 6).[22]

In nature, vaccinia virus infections are limited to exposure to a vaccinia-like virus infecting milking buffalos and dairy cattle in Asia and Africa (called buffalopox) and cattle in Brazil (Cantagalo virus). Vaccinia virus (smallpox vaccine) is used also in the immunization of personnel in the laboratory, but is not recommended at this time for the general population due to the occurrence of frequent and sometimes severe complications.[22] Currently only monkeypox and cowpox viruses cause significant human infections, and the incidence of human monkeypox is increasing.

EPIDEMIOLOGY

Geographic range

Monkeypox virus is found in the tropical rain forests of countries in western and central Africa, most notably Zaire. The reservoir of monkeypox virus in nature is most likely the African arboreal squirrels (*Funisciurus* and *Heliosciurus* spp.) and perhaps monkeys.[23] Cowpox virus is endemic in Europe and some western states of the former Soviet Union. Rodents (voles, wood mice and rats) have been implicated as reservoirs of cowpox virus; cows, zoo animals and cats are incidental hosts.[24]

Prevalence and incidence

Intensive surveillance between 1981 and 1986 in Zaire by the World Health Organization confirmed 338 cases of human monkeypox, with the greatest risk of infection to inhabitants of small villages within 100m of tropical rain forests. [25] In recent years there has been an increase in the frequency of human monkeypox in central Africa perhaps due in part to the cessation of vaccination for smallpox.[26]

Although humans have been infected by cows and rodents, the domestic cat is responsible for the majority of human cowpox infections. Between 1969 and 1993 there were approximately 45 human cowpox cases in the UK, three published case histories from Germany and two each from Belgium, Sweden and France.[24]

PATHOGENICITY

Transmission

The exact mode of transmission of monkeypox virus from an animal source to humans is not known but may be via the oropharynx or nasopharynx or through abrasions of the skin or oral cavity. Person-to-person transmission (like eradicated smallpox) is believed to be by the upper respiratory tract, with virus released in oropharyngeal secretions of patients who have a rash.[25] Unlike smallpox, monkeypox person-to-person transmission is very inefficient and rarely surpasses three generations.[25]

Cowpox virus is acquired usually by direct introduction of the virus from an animal source into minor abrasions in the skin; however, 30% of infections show no known risk factor.[24]

Lesion histopathology

Orthopoxvirus lesions are characterized by epidermal hyperplasia, with infected cells becoming swollen, vacuolated and undergoing 'ballooning degeneration'. [24]

CLINICAL MANIFESTATIONS

Signs, symptoms and severity

Approximately 12 days after infection with monkeypox virus, fever and headache occur. This is followed 1–3 days later by a rash and generalized lymphadenopathy. The rash (the number of lesions is variable) appears first on the face and generally has a centrifugal distribution. The illness lasts 2–4 weeks, depending on its severity. The case fatality rate is approximately 12%.[25]

With cowpox virus infection, a lesion, usually solitary, appears on the hands or face; this can be extremely painful, and the patient can present with systemic symptoms, including pyrexia, malaise, lethargy, sore throat and local lymphadenopathy. Complete recovery

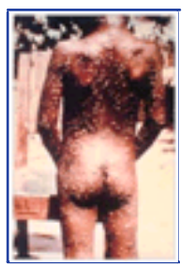

Figure 218-6 Monkeypox rash. A 7-year-old Zairian girl, 2 days after the onset of the rash. *Courtesy of M Szczeniowski.*

takes between 3 and 8 weeks. Person-to-person transmission has not been reported. Complications can include ocular or generalized infections; the latter occurs in patients who have atopic dermatitis, allergic asthma or atopic eczema and in one case was associated with death.[24]

Gross lesion pathology

The monkeypox virus skin lesions begin as macules but rapidly progress to pustules. About 8 or 9 days after the onset of rash the pustules become umbilicated and dry up; by 14–16 days after the onset of the rash a crust has formed. Most skin lesions are about 0.5cm in diameter (Fig. 218.6).[25]

The cowpox lesion appears as an inflamed macule, progresses through an increasingly hemorrhagic vesicle stage to a pustule that ulcerates and crusts over by the end of the second week to become a deep-seated, hard black eschar 1–3cm in diameter (Fig. 218.7). [24]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of monkeypox infection requires clinical (rash), epidemiologic (equatorial Africa) and laboratory (brick-shaped virion in

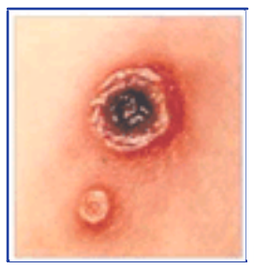

Figure 218-7 Primary and secondary lesions of cowpox. The primary lesion is at the early eschar stage (probably 2–3 weeks after infection), whereas the secondary lesion (below) is at the early vesicular stage. *With permission from Baxby et al.* [24]

scab material) findings. Although the rash with associated lymphadenopathy is usually pathognomonic, the sporadic nature of the disease makes it difficult to arrive at an accurate diagnosis solely on clinical grounds.[25]

Diagnosis of cowpox virus infection is rarely made on clinical findings (lesion morphology and systemic illness) and usually requires laboratory results (brick-shaped virion in scab material). Cowpox should be considered in patients who have had contact with cats and who present in July to October with a painful hemorrhagic vesicle or black eschar, with or without erythema, accompanied by lymphadenopathy and a systemic illness.[24]

Monkeypox can be confused with a number of other conditions that result in a rash:

- chickenpox, although its varicella-zoster lesions are more superficial, appear in crops and have a centripetal distribution;
- tanapox, except the tanapox lesions evolve slowly, are nodular and large, without pustulation; and
- syphilis, although the secondary rash of syphilis does not evolve past the papular stage.[25]

Generalized cowpox can be misdiagnosed as eczema herpeticum, whereas localized cowpox is most frequently misdiagnosed as:

- orf or milkers' nodules, although the parapoxvirus lesion is clinically distinct, and there are often no systemic disease symptoms;
- herpes virus reactivation, even though herpes lesions are not usually hemorrhagic or erythematous and the scab is not so deep-seated and of lighter color; and
- anthrax, although the anthrax lesion is painless and rapidly progresses to the eschar stage (5–6 days).[24]

DIAGNOSTIC MICROBIOLOGY

The appropriate biosafety level precautions must be taken for the handling, transport and processing of infected lesion material.[26]

Molluscum contagiosum virus

For a squash preparation the keratotic dome-shaped molluscum lesion is placed on a regular slide and under a coverslip or second slide. The lesion is flattened and can be examined using light microscopy either directly or after staining with Wright's or methylene blue stains. Round to ovoid molluscum bodies up to 37 × 27 µm are diagnostic of molluscum contagiosum.[4]

For histopathologic analysis, a biopsy specimen is fixed in 10% formal saline and submitted for wax embedding, sectioning at 5µm and staining with hematoxylin and eosin. Microscopic examination should provide a field of view similar to that shown in Figure 218.3 .

Parapoxvirus, orthopoxvirus and yatapoxvirus

Orthopoxvirus (brick-shaped), parapoxvirus (ovoid) and tanapox virus (enveloped brick-shaped) virions can be differentiated from one another by an experienced electron microscopist. Scab material or fluid from the vesicle should be processed by standard methods for negative-staining electron microscopy.[26]

MANAGEMENT

The management of parapoxvirus, orthopoxvirus and yatapoxvirus infections is supportive. Although at this time there are no approved systemic or topical chemotherapeutic agents commercially available for the treatment of poxvirus infections, it is anticipated that cidofovir or related compounds will be approved to treat orf virus, molluscum contagiosum virus, and orthopoxvirus infections.[27] [28] Also for complications arising from the smallpox vaccine, vaccinia immune globulin may be effective. Prevention of secondary bacterial infections through the use of antibiotic ointments is an option. In

the case of cowpox, corticosteroids are contraindicated and may exacerbate the illness. [24] Because of the chronic nature of molluscum contagiosum lesions, one can consider curettage and cryotherapy of lesions. Removal of giant molluscum lesions, but not regular lesions, usually results in scar formation. Molluscum lesions may remit when HIV infection is controlled with anti-HIV therapy.

Curettage with an analgesic

With children, pretreatment of molluscum lesions with lidocaine (lignocaine)-prilocaine for between 1 and 30 hours under plastic occlusion resulted in a decrease in the reported pain on lesion removal with a comedo extractor or curette.

Cryotherapy

Molluscum lesions are treated for 6–30 seconds with a cotton-tipped applicator dipped in liquid nitrogen; this is repeated at 3-week intervals as needed. Cryotherapy is relatively painless, cost-effective and yields good cosmetic results. For patients with HIV infection, this treatment has the added advantage of mitigating the risk of disease transmission to medical personnel.

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Chapter 219 - Rabies

Mary J Warrell
David A Warrell

INTRODUCTION

Rabies is a zoonosis of dogs and other mammals that is occasionally transmitted to humans, causing fatal encephalomyelitis. Rabies virus is a member of the genus *Lyssavirus*, part of the large family of Rhabdoviruses that infect animals and plants. The bullet-shaped virions contain a nonsegmented negative strand of rabies RNA encoding five proteins. A nucleoprotein is coiled with the RNA, a phosphoprotein and an RNA-dependent RNA polymerase to form a helical ribonucleoprotein complex or nucleocapsid. This is covered by a matrix protein. An outer coat is acquired by budding through a host cell membrane, which is studded with the virus-coded glycoprotein bearing club-shaped projections. Rabies virus strains from different vector species and geographic areas can now be distinguished by nucleotide sequencing and monoclonal antibody typing.

Lyssaviruses comprise seven genotypes, of which rabies is genotype 1, and six are rabies related-viruses which may also cause fatal human encephalitis (see Rabies-related viruses, below).[1] [2]

EPIDEMIOLOGY

Animal rabies

Globally, the domestic dog is the most important vector of human rabies, responsible for about 97% of human deaths from this disease. In Asia, most of Africa, and a few urban areas of Latin America, the dog is the dominant vector. Separate reservoirs of enzootic infection also occur in several wild mammalian species (Table 219.1), with occasional overspill into other species. This sylvatic pattern of infection predominates in North America,[2] Europe, southern Africa[3] and parts of the Caribbean. Rabies is enzootic in most parts of the world

Figure 219-1 World distribution of rabies.

(Fig. 219.1). The epizootiology changes constantly, and so local advice should be sought for detailed information.

Human rabies

Estimates of human rabies mortality rates are notoriously unreliable in tropical areas, where about 99% of the deaths occur. In Asia and Africa there are an estimated 40,000–70,000 cases annually. Areas of high incidence include India, Bangladesh, Pakistan and Nepal.

In the USA, 32 cases were reported in the past 11 years, of which 19% were infected abroad, and 75% were associated with indigenous insectivorous bat rabies virus, although only two of 24 had recognized a bat bite.

PATHOGENICITY

Transmission

Human infection is usually the result of a bite by a rabid dog or other mammal. Virus in saliva can penetrate broken skin or intact mucous membranes, and so scratches or licks by a rabid animal may cause infection.

On a few occasions, human rabies has possibly resulted from inhalation of virus in caves that were densely populated by insectivorous bats in the USA, or after laboratory accidents with aerosols of virus. The saliva, respiratory secretions, and tears of rabies patients contain virus,[4] which could infect another person, but the only documented instances of human-to-human transmission have been in eight recipients of corneal grafts from donors in whom rabies had not been suspected. Several women with rabies encephalitis have delivered healthy infants. Only one case of neonatal rabies has been reported, although vertical transmission is well documented in animals.

TABLE 219-1 -- Important rabies vector species.

IMPORTANT RABIES VECTOR SPECIES		
Africa	Domestic dog	Widespread dominant vector
	Black-backed jackals (*Canis mesomelas*)	Southern Africa
	Yellow mongoose (*Cynictis penicillata*)	
	Bat-eared fox (*Otocyon megalotis*)	
Americas	Domestic dog	Mexico, Central America, South America
	Wild canids: Arctic fox (*Alopex lagopus*), red fox (*Vulpes fulva*), gray fox (*Urocyon cinereoargenteus*), Coyote (*Canis latrans*)	Alaska, Canada, New England, Arizona, Texas
	Striped skunk (*Mephitis mephitis*)	Central Canada, central USA, California
	Racoon (*Procyon lotor*)	Mid-Atlantic, northeast, and southeast USA
	Insectivorous bats	USA, South America
	Vampire bat (Desmodontidae)	Southern Texas, Mexico, Central America, South America, Trinidad and Tobago
	Mongoose (*Herpestes* spp.)	Puerto Rico, Grenada, Cuba, Dominican Republic

Asia	Domestic dog	Widespread dominant vector
	Wolf (*Lupus lupus*)	Iran, Iraq, Afghanistan
Australia	Fruit bats (Lyssavirus genotype 7)	-
Europe	Fox (*Vulpes vulpes*)	Eastern Europe, diminishing westward
	Arctic fox (*Alopex lagopus*)	Northern Russian Federation
	Racoon dog (*Nyctereutes procyonoides*)	Poland, Baltic states, Russian Federation
	Wolf	Russian Federation
	Domestic dog	Turkey, Russian Federation
	Insectivorous bats (European bat lyssaviruses genotypes 5 and 6)	Germany, Denmark, The Netherlands, Russian Federation, Poland, Spain, France, Hungary, Switzerland, UK
All vectors carry *Lyssavirus* genotype 1 except where stated.		

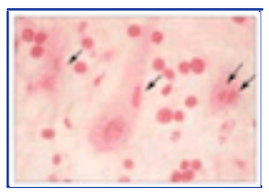

Figure 219-2 Negri bodies in cerebellar Purkinje cells in a human victim of rabies encephalitis. The intracytoplasmic dark-staining Negri bodies are marked with arrows. *Courtesy of the Armed Forces Institute, Bethesda, USA.*

Pathogenesis

Rabies virus will attach to a variety of cellular receptors, but in a bite wound competitive binding to acetylcholine receptors at neuromuscular junctions is probably an important route of entry into the nervous system.[5] During the incubation period, the virus travels to the central nervous system (CNS) by retrograde axonal transport at a rate of 50–100mm/day (in vitro). Infection can be halted experimentally by sectioning the nerve or by applying metabolic inhibitors (e.g. colchicine).

When the virus reaches the brain, massive replication occurs in neurons by budding from intracellular membranes, and accumulation of viral proteins results in the formation of inclusions (Negri bodies; Fig. 219.2). The virus spreads trans-synaptically and remains within neurons where it is hidden from immune surveillance. There is evidence of neuronal dysfunction — neurotransmitter activity is altered and there are electroencephalograph changes. Pathologic features of diffuse encephalomyelitis may be minimal or even absent. The virus then progresses centrifugally via peripheral nerves to many tissues, including the salivary and lacrimal glands, where replication with production of extracellular rabies virus begins (Fig. 219.3).

Immunology

Following infection, rabies virus evades the immune system[6] and no immunologic response can be detected in unvaccinated patients before signs of encephalitis develop. Neutralizing antibody usually appears 1–2 weeks after the onset of symptoms, and a little later in the cerebrospinal fluid (CSF). Rabies-specific IgM is not detectable any earlier than IgG and it is not a sensitive means of diagnosis. The presence of antibody is diagnostic in unvaccinated patients. Specific transformation of peripheral blood lymphocytes has been found in a few patients with furious rabies, but not in paralytic cases. The reverse is found experimentally in rabid mice, and the virus is immunosuppressive to unrelated virus antigens. This immunosuppressive effect is compatible with the minimal histopathologic changes in the brain. Interferon production in humans is very low, and high levels of interferon-a and interferon-ß in animal brains do not protect animals against death. Paradoxically the incubation period was shorter in patients who had had some rabies vaccine; these patients had been inadequately vaccinated. This early death phenomenon can be reproduced experimentally by injecting a little rabies antibody or immune B cells during the incubation period. Clearance of a lethal virus from the brain has been achieved in rodents by treatment with a single glycoprotein-specific neutralizing monoclonal antibody, but the mechanism of recovery is unknown.[7]

CONTROL

The design of an appropriate rabies control strategy[8] depends on surveillance to determine the principal wild or domestic reservoir

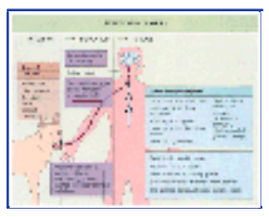

Figure 219-3 Pathogenesis of rabies.

species, which are confirmed by laboratory diagnosis. In some places where the domestic dog was the main vector, including urban areas of Latin America, mass dog vaccination campaigns have radically reduced the incidence of rabies. In contrast, attempts at control by vaccination, poisoning or shooting have been unsuccessful in parts of Africa and Asia where stray or owned, unrestricted dogs are the greatest problem. The immunization of such dogs by distributing oral vaccine in suitable baits is now possible, but is not widely used.

Control of sylvatic rabies must be tailored to particular reservoir species. In Europe the red fox *Vulpes vulpes* is the dominant vector infecting other wild and domestic species. Repeated campaigns to distribute oral live attenuated or vaccinia-recombinant vaccine expressing rabies glycoprotein in baits has almost eradicated terrestrial rabies from western Europe. In North America racoons and coyotes have been similarly immunized, and also jackals in southern Africa. Control of vampire bat rabies, which causes many deaths in cattle in Latin America, is attempted by treating cattle with small doses of anticoagulant to poison the bats. No active measures are taken to control rabies in insectivorous bats in the Americas and Europe, but instructions are issued to encourage people to avoid direct contact with these animals.

CLINICAL MANIFESTATIONS

The incubation period (bite to first symptom) ranges from 4 days to at least 19 years, but in about 75% of cases it is between 20 and 90 days. The only prodromal symptoms that are reasonably suggestive of rabies are itching, pain, or paresthesia, either at the site of the causative bite or radiating proximally from it. After 1–7 days of generally non-specific symptoms, features of either furious or paralytic (dumb) rabies develop.

The pathognomonic symptom of furious rabies is hydrophobia[9] or aerophobia. Jerky and violent contractions of the diaphragm and accessory muscles of inspiration, are associated with an inexplicable terror. This reflex can be provoked by attempts to drink liquid or by a draught of air on the skin. The hydrophobic spasm may end with the patient in opisthotonos, having generalized convulsions and in cardiac or respiratory arrest. Other features of furious rabies are periodic excitement, sometimes with hallucinations or aggression, interspersed with lucid intervals, tachycardia and other arrhythmias, hypersalivation, lacrimation, sweating, and fluctuating temperature and blood pressure. All these features are suggestive of stimulation of the autonomic nervous system as seen in severe tetanus. Conventional neurologic examination may prove surprisingly normal but meningism, cranial nerve lesions, upper motor neuron lesions, fasciculation, and involuntary movements are sometimes detected. Without intensive care, patients usually die, often during a hydrophobic spasm, in the first few days of their encephalitic symptoms.

Paralytic or dumb rabies is probably underdiagnosed but was particularly associated with an epidemic of rabies transmitted by vampire bats in Trinidad in the 1920s and 1930s. After the prodromal symptoms described above, together with fever and headache, local paresthesia and flaccid paralysis develop, usually in the bitten limb, and ascend symmetrically or asymmetrically. There is accompanying pain and fasciculation in the affected muscles and mild objective sensory disturbances. Death follows paralysis of the muscles of deglutition and respiration; there is usually no evidence of hydrophobia or aerophobia. Some patients have survived for as

long as 1 month, even without intensive care.

Patients with paralytic or dumb, or furious rabies whose lives are prolonged by intensive care may develop a variety of respiratory, cardiovascular, neurologic and gastrointestinal complications including fatal cardiac arrhythmias, pneumothorax, cerebral edema, diabetes insipidus, diffuse axonal neuropathy and hematemesis.

Differential diagnosis

Severe and unusual neurologic symptoms, whether encephalitic or paralytic, should suggest the possibility of rabies in any unimmunized person who has had contact with mammals in a rabies endemic area. Rabies patients infected by bats in the USA however, usually deny any contact with a bat.

Furious rabies can be mimicked by the pharyngeal form of cephalic tetanus ('hydrophobic tetanus'), but severe tetanus usually has a shorter incubation period than rabies. In tetanus, there is sustained muscle rigidity, often associated with trismus. Delirium tremens and the excitatory effects of some drugs and plant toxins have been confused with rabies. Paralytic rabies is indistinguishable from many other causes of an ascending paralysis, including postvaccinal encephalomyelitis complicating the use of obsolete nervous tissue rabies vaccines still used in Asia, Africa and Latin America.

Prognosis

Only five documented cases of recovery from rabies, or survival for more than eighteen months, have been reported during the virologic era.[10] No rabies virus or antigen was detected in any of them and all were diagnosed serologically. Two patients were treated after exposure with nervous tissue vaccines and recovered. [11] The other three patients, given either only pre-exposure nervous tissue vaccine or postexposure tissue culture vaccine, were left with profound neurologic deficits.

DIAGNOSIS

Rabies infection can be confirmed by isolating the virus, by identifying antigen, or, in unvaccinated patients, by detecting antibody.[12]

Viral isolation from saliva, respiratory secretions, CSF, tears, or brain biopsy[4] is the ideal method of diagnosis. Inoculation of suckling mice takes 1–3 weeks, whereas tissue culture isolation can be completed in less than 4 days. Virus identification using a panel of monoclonal antibodies or by genetic typing, will indicate the likely vector species and geographic origin of the strain. Rapid intravitam diagnosis can be made by immunofluorescent detection of rabies antigen in frozen sections of full-thickness skin biopsies from hairy areas of the nape of the neck. With controls to ensure specificity and examination of many sections, including bases of hair follicles, the sensitivity is 60–100%, the rate increasing later in the illness.[13] False-positive results have not been reported. Repeated tests should be made until the diagnosis of rabies is excluded. The corneal impression smear test is too insensitive to be useful and false-positive results have occurred. Antigen detection using polymerase chain reaction techniques has proved useful for samples of saliva and CSF.

The usual method of detecting antigen in brain samples is by the immunofluorescent antibody staining of acetone-fixed impression smears, but enzyme immunoassay can be used. Post-mortem needle necropsy of the brain can be taken without a full autopsy by inserting a Vim-Silverman needle via the medial canthus of the eye through the superior orbital fissure, or via the foramen magnum, or via the nose and ethmoid sinus into the brain.

In unvaccinated patients, diagnostic seroconversion usually occurs in the second week of illness.[14] In vaccinated patients, very high levels of antibody both in the serum and in the CSF have been used to make a diagnosis.[11]

MANAGEMENT

The life of patients with rabies encephalitis can be prolonged by intensive care. No effective antiviral or ancillary treatments have been discovered. Patients should be admitted to hospital so that their agonizing symptoms can be palliated with adequate doses of analgesic and sedative drugs. Friends, family and medical staff who have been in close contact with the patient should be given postexposure vaccination to reassure them, even though person-to-person transmission must be extremely rare.

PREVENTION

Post-exposure prophylaxis

In a rabies endemic area, the risk of infection, and hence the decision to give postexposure prophylaxis,[15] [16] depends on the species of animal, its behavior and the circumstances of contact. An unprovoked attack by a known local vector suggests a high risk of exposure to rabies, especially if the animal is unvaccinated, is unusually excitable or is partially paralyzed, or if a wild animal is unusually tame. Vaccinated animals have, however, transmitted rabies. The virus gains access through any bite, scratch, or contamination of broken skin or mucous membrane, but intact skin is an adequate barrier against infection. The risk of infection is greatest from bites on the head, neck, and hands, and multiple bites carry a higher risk than single bites. Before vaccines were available, the mortality rate from all proven rabid dog bites in India was 35–57%.[17]

Strenuous efforts should be made to have the biting animal tested for rabies. The routine immunofluorescent test for antigen may give a false-negative result in 1–2% of rabies cases, and the test is unreliable for rabies-related viruses, so the result of viral culture is important in highly suspicious cases. Specific treatment should not await laboratory results and it should always be started immediately, irrespective of the time since the bite.

Postexposure prophylaxis aims at inactivating rabies virus in a wound and stimulating the immune response to kill the virus before it enters the CNS, where it is protected from immune attack. Post-exposure therapy includes urgent would treatment, and active prophylaxis with vaccine and passive immunization with rabies immune globulin (RIG). Failures of complete optimal treatment started on the day of exposure have only been reported in two patients following bites on the head.

Treatment of mammal bites, scratches or licks

The treatment can be summarized as follows:

1. Scrub vigorously with soap or detergent and water, reaching the depth of the wound, and remove foreign material.
2. Swab with a virucidal agent: povidone iodine, tincture of iodine, or 40–70% ethanol. (The virucidal effect of quaternary ammonium compounds is neutralized by soap, and so they are not recommended.) Local or even general anesthetic may be necessary to allow effective wound cleaning and debridement, especially for children.
3. Suturing of wounds should be avoided or delayed.
4. Tetanus prophylaxis (tetanus immune globulin or toxoid booster plus metronidazole) must not be forgotten. Antibiotic prophylaxis against other potential wound pathogens is recommended.
5. If there is a risk of rabies, give specific therapy immediately.

Rabies vaccines

Vaccines of nervous tissue origin

All human rabies vaccines contain killed virus. Those cultured in sheep or goat brains (Semple vaccines) and suckling mouse brain (SMB) are still used in Asia and Africa. SMB vaccine is also used in parts of South America. These are weak antigens and neurologic reactions still occur (1 in 200 Semple vaccinees). [10] Nevertheless in the absence of a modern vaccine, a nervous tissue product should be used, at least until other treatment can be procured.

Purified tissue culture vaccines

Tissue culture-grown vaccines are used exclusively in North America, most of Europe, China, Thailand, Sri Lanka and the Philippines and increasingly in other

countries. The use of the original expensive human diploid cell vaccine (HDCV) is giving way to cheaper vaccines of equivalent efficacy and safety, including a

German purified chick embryo cell vaccine (PCECV) and a French purified vero cell vaccine (PVRV), which are now widely distributed in endemic areas.

Immunologic response to vaccination

The presence of serum neutralizing antibody is the best available indicator of protection against rabies, and it appears 7–14 days after starting a primary course of a modern rabies vaccine. A level of 0.5I U/ml is considered satisfactory, although the protective level cannot be ascertained in humans. Only the viral surface glycoprotein molecules induce neutralizing antibody. They also stimulate helper T-lymphocyte responses. The rabies nucleoprotein can also induce protection through the induction of non-neutralizing antibody, T-helper lymphocytes and interferon-?. This immunity cross-reacts broadly with other strains of rabies virus and rabies-related viruses, unlike that induced by glycoprotein.

The speed and degree of the antibody response to vaccine varies, probably under genetic influence. A relatively delayed lower level of antibody is found in about 10% of people receiving vaccine; increasing age also impairs the response.

The induction of neutralizing antibody can be accelerated by increasing the amount of antigen (within limits) or by dividing the dose between multiple sites and injecting it intradermally.

Postexposure vaccine regimens

Intramuscular vaccine regimen

The standard regimen is one dose of tissue culture vaccine (1ml or 0.5ml, depending on the product) intramuscularly into the deltoid on days 0, 3, 7, 14 and 28.

Intradermal vaccine regimens

The five-dose intramuscular regimen is unaffordable by the vast majority of patients in developing countries, but economic, safe and effective regimens are now available. The World Health Organization recommends two multiple-site intradermal regimens using specified European vaccines — PVRV, PCECV and HDCV.[15] The manufacturers' instructions must be used for all other vaccines.

An eight-site regimen[18] consists of eight doses of 0.1ml intradermally on day 0 (using a whole vial) on the arms, thighs, and suprascapular and lower abdominal areas; four doses on day 7 on the arms and thighs; and one dose on days 28 and 90 also over the deltoid. Human diploid cell vaccine or PCECV can be used, when the intramuscular dose is 1ml. This has a wide margin of safety because the minimum stipulated antibody response has been achieved by only four of the initial eight intradermal doses. Only four visits are needed. Patients who are anxious about the risk of rabies do not object to the method.

A two-site regimen[15] consists of two intradermal doses (0.1 or 0.2ml depending on whether the intramuscular vaccine dose is 0.5 or 1.0ml) on days 0, 3 and 7; and one intradermal dose on days 28 and 90. The injection is over the deltoid. This has proved effective in thousands of patients who also received RIG treatment in Thailand.

The eight-site regimen induces higher levels of antibody than the two-site regimen from the earliest appearance of antibody onwards.[19] Both require a similar amount of vaccine, less than two intramuscular doses, a saving of 60%. When one ampoule is shared between several patients, a new syringe and needle must be used for each.

Postexposure treatment of previously vaccinated patients

Treatment is always urgent, but, provided that the patient has previously had a complete pre-exposure or postexposure course of tissue culture vaccine or if a neutralizing antibody level has been over 0.5I U/ml at some time, only two doses of intramuscular vaccine are needed on days 0 and 3. Rabies immune globulin treatment is unnecessary.

Side-effects of tissue culture vaccines

The incidence of minor symptoms is very variable.[10] Local pain or erythema occurs in about 15% of people vaccinated, and irritation is more common following intradermal injections. Generalized nonspecific symptoms are reported by about 7% of patients, and transient maculopapular and urticarial rashes are occasionally seen. In the USA, 6% of late pre-exposure booster injections of HDCV have been accompanied by mild immune complex-like disease between three to 13 days later. All of these patients responded to symptomatic treatment. Neurologic symptoms, either Guillain-Barré-like or local limb weakness, are extremely rare, and the incidence following treatment is no greater than those following other routine vaccines. No complications have been observed in pregnancy.

Passive immunization with rabies immune globulin

Every primary postexposure vaccine course should be accompanied by RIG to cover the first 7–10 days before vaccine-induced immunity appears, [15] and it may enhance the T-cell immune response. Rabies immune globulin treatment is especially important after bites on the head, neck or hands, or multiple bites. A single dose of 20 IU/kg of human RIG or 40 IU/kg of equine RIG is given at the same time as the first dose of vaccine. As much as possible is infiltrated into and around the wound, but care is needed when injecting into fingers or other tight tissue compartments. Any remaining vaccine is injected intramuscularly away from the vaccine site. If the volume of RIG is insufficient for infiltration, it can be diluted in saline two- or threefold in order to infiltrate all wounds. Skin testing with RIG does not predict most hypersensitivity reactions, and epinephrine (adrenaline) should always be ready in case of anaphylaxis. Serum sickness occurs in 1–6% of equine RIG recipients, but has not been seen after human RIG therapy.

Pre-exposure vaccination

Pre-exposure vaccination is recommended for anyone at risk of exposure to rabies virus, including veterinary surgeons, animal handlers, zoologists, laboratory staff working with rabies, wildlife officers and people living in or traveling to rabies-endemic areas where dogs are the dominant vector species. A total of three doses of cell culture vaccine are needed on days 0 and 7 and on day 28 (or day 21). The dose can be intramuscular or intradermal. Malarial prophylaxis with choloroquine inhibits the antibody response to vaccine given intradermally, so anyone on antimalarial therapy must have intramuscular injections. A booster dose after about 1 year prolongs the antibody response, which usually lasts 5–10 years. As a prompt secondary immune response to booster doses has proved reliable, repeated booster doses are not recommended in the USA except for those at very high risk, such as laboratory staff.[16] Serologic testing is necessary only if immunosuppression is suspected or to determine the need for booster injections in those at high risk.

RABIES-RELATED VIRUSES

The *Lyssavirus* genus comprises seven genotypes: genotype 1 is classical rabies virus; genotypes 2–7 are rabies related-viruses.[1] [2] Genotype 2, Lagos bat virus, unlike the others, has not been found in humans.

Africa

Genotype 3, Mokola virus, has been identified occasionally in shrews, cats, dogs and rodents in sub-Saharan Africa.[1] [3] [17] Isolation has been reported in two Nigerian children — from the brain of one with fatal encephalitis, without hydrophobia; and from the CSF of another with transient meningitis, but neutralizing antibody was not found in convalescent serum. Genotype 4, Duvenhage virus, is

found rarely in bats in southern Africa, and has caused a rabies-like encephalitis in one patient. The true prevalence of these African infections is uncertain because the diagnosis of rabies encephalitis is normally made on clinical evidence, and the usual diagnostic rabies immunofluorescent test may give a faint result or a negative one with rabies-related viruses.

Europe

Genotype 5, European bat *Lyssavirus* type 1, is found in a variety of insectivorous bats across Europe (see Table 219.1).[1] [2] Genotype 6, European bat *Lyssavirus* type 2, has rarely been isolated from *Myotis* spp. of bats in The Netherlands, Switzerland and the UK. There are four reports of fatal rabies-like encephalitis following bat bites in Europe, and European bat lyssaviruses were confirmed in three of these cases. The most recent was a European bat *Lyssavirus* type 2 infection in Scotland in 2002.

Australia

Genotype 7, Australian bat *Lyssavirus*, was identified in 1996 in flying foxes (*Pteropus*) and other bats, and has caused two fatal human infections indistinguishable from rabies encephalitis.[20] A serologic survey also indicates endemic infection in the Philippines.

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Chapter 220 - Respiratory Viruses

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INTRODUCTION

Respiratory viral infections are among the most common diseases of humans. In the course of a lifetime, every individual will experience several episodes of respiratory viral infections. These illnesses are caused by a heterogeneous group of viruses commonly known as human respiratory viruses (HRVs) which includes adenovirus, coronavirus, reovirus, rhinovirus, respiratory syncytial virus, parainfluenza virus and influenza virus. Other viruses, such as some of the enteroviruses and some of the herpesviruses, can also cause respiratory disease but they are discussed elsewhere in this book.

Respiratory viral infections are largely benign and self-limited. Their impact is largely measured in terms of diminished sense of well-being, exacerbations of underlying chronic cardiopulmonary diseases, loss of productivity, time lost from work or school, increased visits to health care providers and unnecessary prescription of antibiotics with the entailing drug toxicities, emergence of antibiotic resistance and increase in health care cost. Among some subsets of the population such as those at the extremes of age, those with chronic cardiopulmonary diseases or those with primary or secondary immunodeficiencies, these illnesses are not infrequently associated with serious, potentially fatal complications. In recent years, significant progress has been made in our understanding of pathogenesis, natural history, epidemiology, molecular diagnosis, prevention and treatment of these organisms.

The more characteristic illnesses associated with the different respiratory viruses and the populations at higher risk are presented in Table 220.1 . The most common methods for the laboratory diagnosis of viral respiratory infections are summarized in Table 220.2 and Figure 220.1 Figure 220.2 Figure 220.3 Figure 220.4 Figure 220.5

ADENOVIRUS

Nature

Adenoviruses were first isolated in the 1950s from human adenoids and later from respiratory secretions of military recruits with acute febrile respiratory illnesses. Human adenoviruses belong to the family Adenoviridae. More than 50 serotypes have been described and classified into six subgroups (A–F). Approximately one-half of these serotypes are known human pathogens. Virions are nonenveloped icosahedral particles measuring 80–100nm in diameter and containing a linear double-stranded DNA genome. The capsid consists of 252 capsomers, of which 240 are hexons and 12 are pentons (vertices). A fiber with a knob at the end projects from every penton. Virions attach to human cells via interaction of the fibers with a receptor on the cell surface and enter the cell via endocytosis. Virus uncoating occurs in the cytoplasm and the virus core is imported into the nucleus. The transcription and translation of viral DNA depend in part on host cell machinery.

Epidemiology

Although epidemiologic characteristics of the adenoviruses vary by type, the route of transmission is either via respiratory tract, fecal-oral or water-borne. Infections are most common during early childhood, but continue to occur throughout life. Nearly 100% of adults have serum antibodies against multiple serotypes. Adenoviruses have been found to account for 3–5% of acute respiratory infections in children and up to 2% in adults. Close contact in crowded institutions increases the risk for adenovirus infections and outbreaks have been described in day care centers, hospitals, shipyards and military quarters. Adenovirus infections can occur throughout the year but outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring and early summer.

Pathogenesis

Adenoviruses causing respiratory disease can be spread via contact transmission (direct or indirect) and via droplet transmission. Direct contact transmission involves a direct body surface to body surface contact and physical transfer of micro-organisms between a susceptible host and an infected person. Indirect contact transmission involves contact of a susceptible host with a contaminated intermediate object. Adenoviruses can also be transmitted when droplets containing micro-organisms generated from the infected person are propelled a short distance (less than 3 feet) through the air and deposited on the host's conjunctivae, nasal mucosa or mouth. Droplets are particles of respiratory secretions larger than 5µm in diameter that are produced during coughing, sneezing or talking and during the performance of certain procedures such as suctioning and bronchoscopy.

The replicative cycle in the host cells may be lytic or may result in the establishment of a latent infection, primarily in lymphoid tissue. The symptoms of adenovirus disease are due to the lysis of infected epithelial cells and the resulting inflammatory response. The initial immune response is probably the recognition of infected cells by natural killer cells and monocytes, which evokes a cytokine response, followed by induction of cytotoxic T cells, as well as B cells which in turn results in the production of type-specific antibodies. Prolonged shedding of adenoviruses in stools has been recognized even in immunocompetent individuals. Some adenovirus serotypes (especially 12, 18 and 31) have been shown to induce oncogenic transformation in cultured rodent cells. In recent years, intensive research has focused on the use of adenoviruses as gene vectors.[1]

Prevention

An effective oral enteric-coated vaccine has been used to prevent adenovirus serotypes 4 and 7 infections in military recruits. This vaccine is not indicated for the general population. Contact and droplet precautions are indicated for controlling nosocomial transmission of adenovirus infection. Contact precautions include the placing of patients in private rooms, cohorting, use of gowns and globes, hand washing and the use of dedicated noncritical patient care equipment when possible. Droplet precautions include the placing of patients in private rooms, cohorting, the use of masks by the personnel caring for the patient within a distance of 3 feet and

TABLE 220-1 -- Human respiratory viruses: characteristic illnesses and populations at higher risk.

HUMAN RESPIRATORY VIRUSES: CHARACTERISTIC ILLNESSES AND POPULATIONS AT HIGHER RISK		
Virus	**Associated illnesses (most common virus subtype)**	**Patient population at higher risk**

Adenovirus	URI [1–3, 5–7]	Infants, young children
	Pharyngoconjunctival fever [3, 7, 14]	School-aged children
	URI, pneumonia [3, 4, 7, 14, 21]	Military recruits
	Pertussis-like syndrome [5]	Infants, young children
	Pneumonia [1, 2, 3, 4, 7]	Infants, young children, immunocompromised
	Hemorrhagic cystitis [11, 21]	Children, immunocompromised
	Keratoconjunctivitis [8, 11, 19, 35, 37]	All ages
	Gastroenteritis [40, 41]	Children, immunocompromised
	Hepatitis [1, 2, 5, 7, 31]	Immunocompromised
	Meningoencephalitis [7, 12, 32]	Children, immunocompromised
	Disseminated disease [1–5, 7, 11, 21, 31, 32, 34, 35, 39]	Immunocompromised
	Reye's syndrome [3, 7]	Children
Coronavirus	Common cold	All ages
	Otitis	Children
	Pneumonia	Military recruits, elderly, immunocompromised
	Exacerbation of asthma-COPD	Persons with asthma-COPD
Reovirus	Common cold (?)	All ages
	Enteritis (?)	Children
Rhinovirus	Common cold	All ages
	URI	All ages
	Exacerbation of asthma-COPD	Persons with asthma-COPD
	SARS (severe acute respiratory syndrome)	
RSV	URI	All ages
	Pneumonia	Infants, young children, elderly, immunocompromised
	Bronchiolitis	Infants, young children
	Otitis	Children
	Sudden infant death syndrome (?)	Young infants
Parainfluenza	URI	All ages
	Croup	Children
	Otitis media	Children
	Pneumonia	Children, immunocompromised
Influenza	'Flu'	All ages
	URI	All ages
	Pneumonia	Young children, elderly, persons with chronic cardiopulmonary illness, pregnant women, immunocompromised
	Croup	Children
	Otitis media	Children
	Myocarditis	Children
	Toxic shock syndrome	Children
	Reye's syndrome	Children
URI: upper respiratory tract infection		

the use of a mask on the patient while being transported out of the room. Maintaining adequate levels of chlorination is necessary for preventing swimming pool-associated outbreaks of adenovirus conjunctivitis.

Diagnostic microbiology

Virus isolation, antigen detection, polymerase chain reaction assay and serology can be used to identify adenovirus infections. Adenovirus serotyping is usually accomplished by hemagglutination inhibition and/or neutralization with type-specific antisera. The isolation of adenoviruses from body secretions is not sufficient to establish a diagnosis of adenovirus disease as prolonged asymptomatic shedding of adenoviruses can occur. Examination of biopsy or autopsy materials should be pursued whenever feasible in order to identify characteristic cytopathic changes and establish a diagnosis of adenovirus disease, particularly in the setting of immunosuppression where infection with multiple organisms is frequent.

Clinical manifestations

Although adenoviruses mainly cause respiratory illness, they may also cause various other illnesses such as gastroenteritis, keratoconjunctivitis, cystitis, meningoencephalitis and hepatitis, depending on the serotype. Respiratory illnesses associated with adenovirus infections include undifferentiated upper respiratory illness, pharyngoconjunctival fever, pertussis-like syndrome and pneumonia. Adenovirus serotypes 1, 2, 3 and 5 are the most frequent among children. Adenovirus types 4 and 7 and, less often, 3, 14 and 21 are associated with outbreaks of acute respiratory disease in military recruits. Enteric adenoviruses 40 and 41 cause gastroenteritis, usually in children. Epidemic keratoconjunctivitis is associated with

TABLE 220-2 -- Human respiratory viruses.

HUMAN RESPIRATORY VIRUSES				
Virus	**Culture cell lines**	**Characteristic findings**	**Direct assay**	**Serology**

Adenovirus	Primary human embryonic kidney (HEK) and the human lung carcinoma (A549) cell lines are sensitive for a broad range of adenoviruses. Some adenoviruses may require up to 28 days to grow. The continuous epithelial lines, such as Hep-2, HeLa and KB, are sensitive but more difficult to maintain for a long period of time as required by some serotypes	Enlarged, refractile, rounded cells forming grape-like clusters (Fig. 220.1), with some isolates forming a lattice-type arrangement of rounded cells	IF, EIA, and LA	Genus-specific EIA and CF Type-specific HI and NT
Coronavirus	Culture not performed in most laboratories. Primary isolation of human respiratory coronaviruses often requires human fetal tracheal or intestinal organ cultures	Cytocidal coronavirus infections may form multinucleated syncytia, lysis or both	EIA used in research settings	CF, HI, EIA used in research settings
Reovirus	Rarely isolated in the routine diagnostic setting. PRMK, LLC-MK2, HNK, HeLa, MDBK and H292 cell lines may support the growth of this virus	CPE is slow to develop. CPE may be non-specific with increased granularity, and progressive degeneration. HI or IFA can be used for typing	IF used in research settings	CF, NI, NT, EIA used in research settings
Rhinovirus	Human embryonic kidney and human fibroblast cell lines have been used most extensively: WI-38, HFF and MRC-5. HeLa is also sensitive	CPE is usually detected in the first week. CPE consists of rounded, highly refractile cells in loose clusters (Fig. 220.2). CPE permits a presumptive diagnosis of picornavirus infection. Demonstration of lability at pH 3 is used for differentiation from enteroviruses	EIA used in research settings	NT, CF, EIA used in research settings
RSV	Hep-2 is the preferred cell line. Alternatives include HeLa, A549, MRC-5, RhMK and Vero. Calcium and glutamine in the culture medium are important for optimal replication and cytopathic effect	CPE requires 3–7 days or more. Syncytia develops along with non-specific granular degeneration (Fig. 220.3). Confirmation by means of IF (Fig. 220.3) or EIA	IF (Fig. 220.3), EIA, IP	CF, IF, EIA, NT
Parainfluenza	Primary rhesus monkey kidney (PRMK) or MDCK are the preferred cell lines. LLC-MK2, a rhesus kidney heteroploid cell line, and NCI-H292, a human lung carcinoma line, are also sensitive	CPE is variable and non-specific. HPIV-2 and HPIV-3 may induce syncytia formation. Growth may be detected by means of Had (Fig. 220.4), with confirmation and typing by IF (Fig. 220.5), EIA, HAdI or HI	IF, EIA	HI, CF, EIA, NT
Influenza	PRMK and Madin-Darby canine kidney (MDCK) cells are preferred	CPE may be absent or minimal, with non-specific degeneration and granularity. Growth may be detected by means of HAd assay (Fig. 220.4), with confirmation and typing by IF (Fig. 220.5) or EIA	IF, EIA	Type-specific CF, EIA Subtype-specific HI, EIA
IF, immunofluorescence; EIA, enzyme immunoassay; LA, latex agglutination; HAdI, hemadsorption assay; HAd, hemadsorption inhibition; HI, hemagglutination inhibition; NT, neutralization test; IP, immunoperoxidase staining; CF, complement fixation; CPE, cytopathic effect				
Most common methods for laboratory diagnosis: culture, direct assay and serology.				

adenovirus types 8, 19 and 37. Hemorrhagic cystitis is more frequent with types 11 and 21. In children, the occurrence of cerebral edema, fatty liver and liver failure characteristic of Reye's syndrome has been reported in association with adenovirus types 3 and 7. With the exception of pharyngoconjunctival fever, the respiratory symptoms are not specific. The incubation period for the endemic serotypes ranges from 5 to 10 days and the symptoms last approximately 1 week. Chronic lung damage in the form of bronchiolitis obliterans has been reported after infections with serotypes 1, 3, 4, 7 and 21.

Severe and potentially fatal disease has been observed in neonates and individuals with primary or secondary immunodeficiencies.[2] However, cases of severe adenovirus disease have also been reported in apparently normal hosts. Immunocompromised patients, particularly hematopoietic stem cell transplant (HSCT) and solid organ transplant recipients, are susceptible to severe and potentially fatal adenovirus disease,[3] [4] [5] such as protracted hemorrhagic cystitis, encephalitis, nephritis leading to renal failure, hepatitis leading to liver failure, enteritis causing gastrointestinal bleeding, pneumonia leading to respiratory failure or a combination of those illnesses (disseminated disease).

Management

Most infections are mild and require no therapy or only symptomatic treatment. Ribavirin has *in vitro* activity against several adenovirus serotypes but the clinical experience in immunocompromised individuals has produced conflicting results.[5] [6] [7] [8] [9] [10] Aerosolized ribavirin is cumbersome to administer, while intravenous administration may be complicated by the development of hemolytic anemia. Cidofovir is a nucleotide analog with a broad antiviral effect *in vitro*, including against adenoviruses. The drug is administered intravenously every 2 weeks, after two initial weekly doses. Data on the efficacy of cidofovir against adenoviruses are limited to case reports and uncontrolled series.[10] [11] [12] The most significant side-effects of cidofovir include proximal renal tubular acidosis with azotemia, as well as uveitis with hypotony. Concomitant administration of probenecid and normal saline can reduce the risk of renal toxicity.

Figure 220-1 Cytopathic effect caused by adenovirus on Hep-2 cell line culture. (a) Uninoculated cell line. (b) Enlarged, refractile, rounded cells forming grape-like clusters.

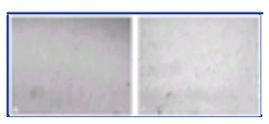

Figure 220-2 Cytopathic effect caused by rhinovirus on human foreskin fibroblasts (HFF) cell line culture. (a) Uninoculated cell line. (b) Formation of small teardrop- to oval-shaped highly refractile cells.

CORONAVIRUS

Nature

Human coronavirus (HCoV) was first isolated in 1965 from a patient with symptoms of the common cold. Virions are pleomorphic and measure 80–200nm in diameter. The outer envelope bears distinctive 'club-shaped' peplomers that correspond to the S (long spike) glycoprotein. The resulting 'crown-like' appearance under electron microscopy gives the family its name. The S glycoprotein is involved in receptor binding and cell fusion and is a major target for neutralizing antibodies. Other structural proteins include the M (matrix) protein, the E (envelope) protein and the N (nucleocapsid) phosphoprotein. In some types, there is also a glycoprotein HE (hemagglutinin-esterase, short spike). Entry occurs via endocytosis and membrane fusion. Replication of the linear single-stranded positive-sense RNA genome takes place in the cytoplasm. HCoV-229E and HCoV-OC43 serve as the prototype human coronaviruses.

Epidemiology

HCoVs are ubiquitous. Serologic studies have suggested that these viruses are a major cause of respiratory disease and account for 10–30% of all common colds. Serologic studies have also suggested that one-half of the infections with coronaviruses are asymptomatic. The peak activity occurs between late fall and early winter. Outbreaks of HCoV-229E in a community occur at 2–4-year intervals and outbreaks of HCoV-OC43 occur in alternate years when the incidence of HCoV-229E infection is low.[13]

Pathogenesis

Intranasal inoculation of volunteers with these viruses results in common colds. Disruption of the ciliated epithelium and ciliary dyskinesia was observed in histological

examinations.[14] Aminopeptidase N (APN), the receptor for HCoV-229E, is expressed on the apical surfaces of respiratory and intestinal epithelium, myelocytic cells, kidney tubular epithelium and synaptic junctions.

Figure 220-3 Cytopathic effect of RSV on Hep-2 cell line culture and identification of RSV antigen by means of IFA. (a) Uninoculated cell line. (b) Syncytia formation in cell line culture. (c) Positive cells coloring green under IF microscope.

Figure 220-4 Identification of hemadsorbing viruses. (a) Uninoculated primary rhesus monkey kidney (PRMK) cell line. (b) Non-specific rounding or clumping of PRMK cells. (c) Positive hemadsorption of guinea-pig red blood cells.

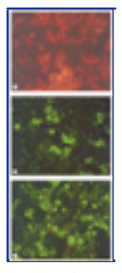

Figure 220-5 Differentiation of hemadsorbing viruses by means of IFA. (a) Negative control. (b) Influenza B positive. (c) Parainfluenza-3 positive, showing a comparatively more finely granular staining.

HCoV-OC43 binds to cells by means of the HE glycoprotein or the viral S glycoprotein, which can recognize 9-*O*-acetylated sialic acid on the cell surface.

Prevention

No specific antiviral drugs or vaccines are currently available. Infection control measures aimed at reducing exposure to respiratory secretions of infected patients should reduce the transmission of these viruses.

Diagnostic microbiology

Although animal coronaviruses can be readily isolated from infected tissues and serially propagated in continuous cell lines, isolation of human coronaviruses from infected individuals is not possible with generally available culture systems. Primary isolation of HCoVs generally requires culture in human fetal tracheal or intestinal organs. The genomes of HCoV-229E and HCoV-OC43 have been sequenced and RT-PCR can be used in the research setting.[15] Most of the population studies of HCoVs have used serologic methods, including complement fixation, hemagglutination inhibition (OC43 only) and recently EIA.

Clinical manifestations

HCoVs are second only to rhinoviruses as a cause of the common cold and the symptoms are indistinguishable. In a study of adult volunteers the mean incubation period was 3.3 days, with a range of 2–4 days. The symptoms lasted for a mean of 7 days, with a range of 3–18 days.[16] HCoVs have also been associated with otitis media, exacerbation of asthma and chronic pulmonary disease and have occasionally been associated with pneumonia, particularly in immunosuppressed patients.[17] [18] Early in 2003 an outbreak of severe pneumonia that originated in China, SARS (severe acute respiratory syndrome) was associated with atypical strains of coronavirus. The case fatality rate was approximately 4%.[110] [111] [112] Coronavirus particles have also been seen (via electron microscopy) in the stools of children and adults with diarrhea and gastroenteritis. The possible role of HCoVs in the genesis of multiple sclerosis (MS) is supported by the observation of coronavirus-like particles in brain tissue of patients with MS and by RT-PCR studies that have identified gene sequences of HCoV-229E and HCoV-OC43 in human brain tissue and cerebrospinal fluid with a higher frequency in MS patients than controls. However, the importance of human coronaviruses as a cause of diseases outside the respiratory tract remains to be determined.

Management

Treatment remains supportive. Potential targets for antiviral therapy are being identified, but no candidate drugs have been tested in clinical trials.

REOVIRUS

Nature

Isolation of the first reovirus was made in 1951, but it was in 1959 that Sabin coined the term reovirus to emphasize the fact that these viruses were isolated from the respiratory and enteric tracts and that they were not associated with any known human disease (Respiratory Enteric Orphan viruses). Reoviruses are nonenveloped viruses of about 65–75nm in diameter. They are composed of two concentric icosahedrically symmetric protein capsids, containing 10 discrete segments of genomic dsRNA. After attachment to cellular receptors, endocytosis of viral particles takes place. Replication occurs in the cytoplasm and results in cell death and lysis. Three distinct serotypes are identified by means of neutralization and hemagglutination inhibition tests (T1L, T2L and T3L).

Epidemiology

A study in which ELISA was used as the diagnostic method showed that the prevalence of antibodies against reovirus increased steadily throughout life from about 35% among 6-month to 1-year-old children to 60% among 11–19-year-olds and to more than 85% among those older than 60 years of age.[19] Infection can be achieved by intranasal inoculation as demonstrated in experiments with volunteers. Reoviruses have been recovered from water supplies and sewage effluents, presumably reflecting contamination of water with human and animal excreta. This could conceivably represent another source of virus for human infection.

Pathogenesis

It has been difficult to provide convincing evidence linking reovirus to specific human diseases. Cellular infection occurs by means of receptor-mediated endocytosis. Replication occurs in the cytoplasm. *In vitro* infection results in cell lysis. Interestingly, it has been observed that some reoviruses selectively replicate in and destroy cancer cells with an activated Ras signaling pathway, raising a possible role in the treatment of human neoplasms such as ovarian and colon cancer.[20]

Prevention

No specific strategies for prevention of transmission of reoviruses have been studied in clinical trials. Observance of appropriate handling of respiratory secretions from infected patients and avoidance of potentially contaminated water supplies could conceivably reduce the transmission of these viruses.

Diagnostic microbiology

The routine search for reovirus in clinical specimens in not recommended. In the research setting, a variety of techniques are available. In addition to identification of characteristic cytopathic effect (CPE) in cell line cultures, the presence of viral antigen can be confirmed using either immunofluorescence or immunocytochemistry. Immunocytochemical techniques can also be employed for the detection of reovirus antigens in tissue specimens. ELISA techniques have been widely used for detection of reovirus antibodies in serum. A variety of molecular techniques, including PCR, can be used for the detection of reovirus in tissues or body fluid samples.

Clinical manifestations

In a study of adult volunteers, intranasal inoculation of reovirus was followed by an increase in serotype-specific antibodies, recovery of the virus from anal swabs and development of respiratory illness in a third of the individuals infected with T1L.[21] The illness began 24–48 hours after the challenge and symptoms lasted for 4–7 days. The predominant symptoms included headache, pharyngitis, sneezing, rhinorrhea, cough and malaise. Because those findings have not been reproduced consistently, there is currently no definite etiological association of reovirus infection with human disease.

Management

Although ribavirin and other compounds exhibit *in vitro* activity against reoviruses, clinical trials seem unlikely to be performed for an organism that remains an 'orphan'.

RHINOVIRUS

Nature

Rhinovirus (RV) was first isolated in the 1950s from nasopharyngeal secretions of patients with the common cold. RV belongs to the Picornaviridae family, which includes the genera Enterovirus (poliovirus, coxsackieviruses groups A and B, echovirus and numbered enteroviruses) and Hepatovirus (hepatitis A virus). Rhinoviruses are small (30nm), nonenveloped particles that contain a linear single-stranded positive-sense RNA genome. The icosahedral capsid is composed of 60 subunits arranged as 12 pentameres. Each subunit is composed of one of each four structural proteins (VP1 to VP4). VP1–3 have exterior projections that are targets for neutralizing antibodies. More than 100 serotypes have been identified. The leukocyte binding protein ICAM-1 is the receptor for the majority of rhinovirus serotypes. The receptor-binding site resides in a depression of the capsid surface that is not accessible to antibodies. After endocytosis, an acid-dependent conformational change of the capsid allows the release of the RNA into the host cell cytoplasm, where viral replication and assembly take place. A polyprotein precursor is processed mainly by two viral proteases designated 2A and 3C. The 2A protease makes the first cleavage between the structural and nonstructural proteins, while the 3C protease catalyzes most of the remaining internal cleavages.

Epidemiology

Rhinoviruses are ubiquitous and infections occur in all age groups. They cause about half of all common colds and are also linked to a variety of complications such as otitis media, sinusitis, bronchitis and exacerbations of asthma and chronic obstructive pulmonary disease. RV infections result in frequent health care contacts, hospitalizations and excess of antibiotic use. These infections occur throughout the year with peaks in the fall and spring and with multiple serotypes circulating simultaneously in a given geographic location. Preschool children commonly introduce the virus to the rest of the family, with a secondary attack rate of about 50%, after an interval of 2–5 days. Extensive studies in susceptible individuals have shown that 95% of challenged subjects become infected and about 75% of them become ill. The major factor determining the risk of illness is the prechallenge level of neutralizing antibodies.

Pathogenesis

In spite of the extensive literature on the transmission of rhinovirus, controversy still exists about the predominant mode of transmission. Most data support the notion that contact transmission, primarily as hand shaking followed by inoculation of the nasal mucosa and lacrimal canals, is the most efficient mode of transmission. RV could survive from a few hours to as long as 4 days on nonporous surfaces and for at least 2 hours on human skin. Although droplet or airborne transmission of rhinovirus infection is possible, prolonged and close exposure is apparently required. There is no good clinical evidence to support the common notion that exposure to cold weather, getting wet or becoming chilled play a role in the genesis of RV illness.

Rhinoviruses attach to respiratory epithelium and spread locally. Most serotypes use ICAM-1 as a receptor for attachment and for subsequent viral uncoating during cell invasion. Some serotypes can upregulate the ICAM-1 expression on human epithelial cells, resulting in an increased susceptibility to infection. Infection is followed by activation of several inflammatory mechanisms including the release of cytokines (particularly IL-8), bradykinins, prostaglandins and histamine, as well as stimulation of parasympathetic reflexes.

The resulting process includes vasodilation of nasal blood vessels, transudation of plasma, glandular secretion and stimulation of nerve fibers, triggering sneeze and cough reflexes. Nasal mucociliary transport is reduced markedly during the illness and may be impaired for weeks. Immunity to individual rhinoviruses is type specific and not long lasting. Protection correlates more with the level of locally synthesized IgA antibodies, which decline within months of infection, rather than with IgG in serum, which may persist for a few years.

Prevention

Hand washing after shaking hands with someone who has a cold or after touching environmental objects potentially contaminated with relatively fresh secretions is strongly recommended. Alcohol-based, waterless antiseptics are also effective for this purpose. Symptomatic patients should be advised to hand wash frequently and to use disposable tissues. The CDC recommends contact precautions for the prevention of nosocomial RV infections. Some institutions also observe droplet isolation precautions.

The diversity of serotypes and the specificity of the immune response have precluded the development of an effective vaccine. A number of drugs have been studied for the prophylaxis of RV infection, including interferon a, pirodavir and recombinant soluble ICAM-1 molecule. However, the practical utility of these prophylaxis modalities remains to be demonstrated.

Diagnostic microbiology

Traditionally, RV infections have been detected by virus isolation in cell culture followed by acid lability testing. Virus isolation requires typically 2–14 days. Presumptive identification of a cell culture isolate as rhinovirus is based on a combination of enterovirus-like cytopathic effect and clinical information.

Further differentiation from enteroviruses is based on the diminished replication of rhinoviruses at 98.6°F compared to that at 91.4°F and at pH 3.0 compared with pH 7.0. ELISA has been developed for the detection of rhinovirus antigens. The use of serological assays is limited by the diversity of serotypes and the lack of a group-specific antigen. Recently developed RT-PCR assays are more rapid and sensitive than isolation techniques for detection of RV RNA in clinical specimens.

Clinical manifestations

Symptoms of the common cold include profuse watery rhinorrhea, nasal congestion, sneezing and quite often headache, sore throat and or cough. The symptoms begin within 8–10 hours of infection and peak around 1–3 days. There is little or no fever. By days 3–5 of the illness, nasal discharge may become mucopurulent from polymorphonuclear leukocytes that have migrated to the infection site in response to cytokines such as IL-8. Resolution of symptoms generally occurs within a week. Complications of RV infection include sinusitis, otitis media and exacerbation of asthma-COPD. Secondary bacterial infections may occur. Recent studies using CT of the sinuses have shown that RV infections can cause the accumulation of secretions in the sinuses, possibly from the passage of nasal secretions into the sinuses during nose blowing.[22] [23] Mounting evidence suggests an association between RV infections and lower respiratory tract illness, especially in immunocompromised individuals.[24] [25] [26] [27] [28] [29]

Management

Symptomatic treatment with decongestants, antihistamines and antitussives is currently the mainstay of therapy. The efficacy of zinc lozenges is controversial and their practical utility is limited because of their metallic taste. A variety of candidate drugs have been studied.[30]

Interferon-a has been found to be effective for the prevention of rhinovirus-associated colds but ineffective for the treatment of established colds. When administered through nasal spray, 80% of secondary RV cold were prevented but some patients developed blood-tinged nasal discharge and concerns about cost-effectiveness have been raised. Pirodavir (R77975) is a capsid-binding antipicornaviral agent with *in vitro* activity against most RV serotypes. Frequent intranasal sprays of pirodavir have been found to be effective in preventing experimentally induced RV illness[31] but ineffective in providing significant clinical benefit in naturally occurring rhinovirus colds. [32] Major recent therapeutic advances include the development of protease 3C inhibitors, recombinant soluble intercellular adhesion molecule (sICAM-1) and capsid-function inhibitors (pleconaril), all of which exhibit potent antirhinoviral activity *in vitro* and varying activity in clinical trials. AG7088, an irreversible inhibitor of rhinovirus 3C protease with *in vitro* activity against a broad range of RV serotypes,[33] is undergoing clinical trials. Recombinant soluble ICAM-1 molecule (Tremacamra) has been found to be effective in reducing the symptoms of experimental common colds in randomized controlled trials. This reduction in symptoms has been apparent regardless of whether the drug was given before or after the challenge with the virus.[34]

Pleconaril binds to a hydrophobic pocket in the viral capsid, inducing conformational changes that lead to altered receptor binding and viral uncoating. Pleconaril is orally bio-available and achieves serum concentrations in excess of those required to inhibit 90% of clinical rhino- and enteroviral isolates *in vitro*. In a placebo-controlled study of rhinovirus infections, pleconaril-treated patients had a 1.5-day reduction in time to resolution of symptoms, significant reduction in symptom severity within 12–24 hours after initiation of therapy and a significant reduction in median viral titers in nasal mucus.[35] The US FDA Advisory Committee has not yet recommended the approval of pleconaril for treatment of the common cold. Additional studies on safety, development of resistance and drug interactions are ongoing.

RESPIRATORY SYNCYTIAL VIRUS

Nature

Respiratory syncytial virus (RSV) belongs to the Paramyxoviridae family of viruses, which also includes parainfluenza viruses, mumps virus and measles virus. RSV was first isolated in the mid-1950s from a symptomatic laboratory chimpanzee during an outbreak of illness resembling the common cold. This virus derives its name from the characteristic formation of multinucleated giant cells in tissue culture.

RSV has a linear single-stranded, negative-sense genomic RNA. Virions are pleomorphic, with both spherical and filamentous forms seen under the electron microscope, and with an average diameter between 120 and 300nm. The envelope consists of a lipid bilayer derived from the host plasma membrane and contains three virally encoded transmembrane surface glycoproteins: the attachment protein G, the fusion protein F and the small hydrophobic SH protein. The F protein can mediate fusion with neighboring cells to form syncytia. The G glycoprotein plays a major role in the process of attachment. Two major types of RSV — A and B — are distinguished serologically based on the antigenic characteristics of the surface glycoprotein G. Other viral proteins include the matrix proteins (M and M2), the major nucleocapsid (N) protein, a phosphoprotein (P) and the major polymerase (L).

Epidemiology

RSV is the most common cause of bronchiolitis and pneumonia among infants and young children worldwide. Virtually all children are infected by the time they reach 3 years of age. Natural immunity is transient and reinfection is common. Crowding in households and day care centers increases the attack rate of RSV. In the USA, it has been estimated that 100,000 hospitalizations and 4500 deaths are related to RSV infection annually. The associated health care expenses are in excess of 300 million dollars per year. In older children and immunocompetent adults, RSV infections are seldom severe and are usually manifested as upper respiratory infection (URI) or tracheobronchitis. Individuals at high risk for severe RSV infection include premature infants (particularly those born at less than 34 weeks of gestation), young infants (particularly those with chronic lung disease, formerly designated bronchopulmonary dysplasia), elderly individuals and immunocompromised subjects.

RSV activity follows a seasonal pattern in temperate zones of the world. In urban centers, annual outbreaks occur during the fall, winter and early spring. In the northern hemisphere most outbreaks peak in February or March. In tropical or subtropical areas, epidemics peak during the rainy season.

Pathogenesis

Humans are the major reservoir. RSV is present in large numbers in the respiratory secretions of symptomatic infected persons and viral titers remain high for about 1 week after the onset of symptoms, followed by a gradual decline that may last for up to 3–4 weeks. The portal of entry is usually the conjunctiva or the nasal mucosa. Although transmission can occur via large droplets, contact transmission predominates.[36] Virus can be cultured for >5 hours on impervious surfaces such as bed rails. Thus, caregivers can contaminate their hands during routine care and transmit the virus by contact to other patients. Neutralizing antibodies to the surface glycoproteins F and G correlate with resistance to reinfection, but protection is far from complete and is of short duration.[37]

Pathologic examination of patients with bronchiolitis has revealed necrosis of ciliated bronchiolar epithelial cells, mononuclear infiltrate and edema of the peribronchial space. Bronchiolar obstruction results from the increased mucous production by goblet cells and the above-mentioned pathologic changes. Partial obstruction results in air trapping, and total obstruction results in atelectasis.

Pathologic examination of patients with pneumonia has revealed marked interstitial inflammation and edema of the lung parenchyma, along with the findings of bronchiolitis. Chronic alterations of pulmonary function tests have been described after RSV infection, but a cause-effect relationship remains to be confirmed.[38]

Prevention

In the household setting, the transmission of RSV may be decreased by means of frequent hand washing and refraining from sharing items such as cups, glasses and utensils with persons who have RSV illness. The CDC recommends contact precautions for the prevention of nosocomial RSV infections. Some institutions also observe droplet isolation precautions. Studies have shown that the implementation of multifaceted infection control strategies results in a reduction of the incidence of nosocomial RSV infection.[39]

Passive immunoprophylaxis is indicated for high-risk children. The available evidence supports the use of RSV-IVIG or palivizumab for the prophylaxis of RSV infections among infants with chronic lung disease (formerly designated bronchopulmonary dysplasia) and premature infants. Passive immunoprophylaxis is not recommended for children with cyanotic congenital heart disease.[40]

RSV intravenous immunoglobulin (RSV-IVIG, RespiGam®) is a human polyclonal immunoglobulin with high RSV microneutralization titers. In placebo-controlled studies, monthly intravenous doses of 750mg/kg during the RSV season significantly reduced the incidence of RSV hospitalizations and the severity of illness among premature infants and infants with bronchopulmonary dysplasia.[41] [42] Palivizumab is a humanized monoclonal antibody that specifically inhibits an epitope at the A antigenic site of the F protein of RSV subtypes A and B. It is dosed monthly at 15mg/kg intramuscularly during RSV season. In a large multicenter placebo-controlled trial involving high-risk infants, palivizumab prophylaxis resulted in a 55% reduction in hospitalization rates and number of hospital days ascribed to RSV infection.[43] Both agents have been well tolerated, with a few adverse effects; however, their high cost necessitates strict guidelines for their use. Cost-saving benefit has not been observed uniformly,[44] [45] [46] [47] [48] perhaps as a result of major differences in the prevalence of RSV lower respiratory tract infections in different countries and regions, suggesting the need for locally adapted recommendations. Palivizumab is easier to administer and does not interfere with the MMR vaccine or varicella vaccine. RSV-IVIG, however, provides additional protection against other respiratory viral illnesses and may be preferred for children receiving replacement IVIG because of

underlying immune deficiency.[43]

The role of passive immunoprophylaxis for the prevention of RSV infections in high-risk adults remains to be defined. Development of an RSV vaccine is ongoing but none is yet available.

Diagnostic microbiology

The gold standard diagnostic method is isolation of the virus from respiratory secretions. Appropriate samples for diagnosis include nasopharyngeal aspirate, wash or swab, tracheal aspirates, bronchioalveolar lavage and biopsy material. Given the lability of the virus, the samples should be kept cold and should be inoculated as quickly as possible. Cell line cultures show characteristics of RSV cytopathic effect after 3–7 days of incubation.

RSV was the first respiratory virus to be readily identified by means of rapid diagnostic tests based on antigen detection. Current methods for rapid detection of RSV antigen include enzyme immunoassay, immunoperoxidase staining, direct and indirect immunofluorescence tests and PCR. Serologic diagnosis requires a convalescent-phase RSV lgG antibody titer at least four times higher than the acute-phase titer.

Clinical manifestations

The incubation period ranges from 2 to 8 days. Illness begins most frequently with rhinorrhea, sneezing and cough. When fever is present it is generally low grade. Most children experience recovery from illness after 8–15 days. During their first RSV infection, 25–40% of infected infants and young children have signs or symptoms of bronchiolitis or pneumonia and 0.5–2% require hospitalization. In contrast, hospitalization will be required in up to 25% of high-risk children such as those with history of prematurity or chronic lung disease. RSV bronchiolitis or pneumonia should be suspected when an infant or young child presents with progressive cough, wheezing and dyspnea during a RSV season. Radiographic findings in patients with bronchiolitis include hyperlucency, diaphragmatic flattening and outward bowing on the intercostal spaces. Findings compatible with pneumonia include interstitial infiltrates and, less frequently, consolidation, particularly in the right upper and middle lobes.

In neonates, the clinical presentation may be atypical with lethargy, irritability or poor oral intake. RSV has been detected in lung tissue of infants with sudden death syndrome, but a cause-effect relationship has not been confirmed. Prolonged pulmonary function deficit and airway hyperreactivity have been reported following RSV infection but it remains unclear whether these abnormalities were present before the infection. Otitis media has also been associated with RSV infection.

Among immunocompetent adults, RSV infection usually manifests with rhinorrhea, pharyngitis, cough, bronchitis, headache, fatigue and fever. In older persons, particularly the institutionalized elderly and those with chronic cardiopulmonary illnesses, severe pneumonia may occur, leading to respiratory failure. Among immunocompromised individuals, RSV has been the most frequently isolated viral respiratory pathogen in surveillance studies performed at large cancer centers.[49] In this setting RSV infection typically begins as a URI and may evolve into lower respiratory tract disease in up to 50% of cases. A particularly high rate of progression to pneumonia (70–80%) has been reported among adult HSCT recipients developing RSV infection within the period prior to engraftment. In such setting the RSV pneumonia resulted in 60–80% mortality, regardless of the inclusion of antiviral drugs in the treatment strategy.

Management

For patients with mild disease, no specific treatment is necessary other than the treatment of symptoms. Children with severe disease may require hospitalization for supplemental oxygen therapy, hydration, nutrition and, sometimes, mechanical ventilation. Improvements in supportive care have made a significant impact on the mortality from RSV bronchiolitis and pneumonia. The use of bronchodilators is likely beneficial for patients with significant bronchospasm. Corticosteroids are commonly used as anti-inflammatory therapy, although there is lack of data demonstrating the benefits of such intervention. Aerosolized ribavirin (Virasole®) was approved in 1986 for the treatment of infants and children with severe RSV disease. The treatment is cumbersome to deliver and its impact on mortality remains controversial.[50] [51]

A combination of passive immunoprophylaxis and ribavirin has been reported to be well tolerated and to improve the outcome of severe RSV infection among bone marrow transplant recipients.[52] [53] In one open trial the mortality was only 22% among nine adults with pneumonia in whom therapy was initiated prior to the onset of profound respiratory failure.[53] Pre-emptive treatment strategies are being investigated primarily in immunocompromised individuals. Delay of immunosuppressive chemotherapy should be considered when severely immunosuppressed individuals, such as HSCT recipients, develop acute RSV infection.

PARAINFLUENZA VIRUS

Nature

Human parainfluenza viruses (HPIVs) were first described in the 1950s, originally as croup-associated viruses. The term parainfluenza was coined because of the associated influenza-like symptoms and the hemagglutinin and neuraminidase activities exhibited by HPIVs. However, HPIVs belong to the Paramyxoviridae family.

The virions are pleomorphic and range in average diameter from 150 to 200nm. They are enveloped particles with a linear single-stranded negative-sense RNA genome. The HPIVs encode two surface glycoproteins: an attachment protein called HN (hemagglutinin-neuraminidase), that binds to sialic acid-containing receptors on host cell surfaces, and a fusion protein F that is involved in the fusion of the viral membrane with the cellular plasma membrane. The HN protein is a multifunctional molecule with binding activity (binding to sialic acid containing surface glycoproteins and glycolipids), neuraminidase activity and fusion promotion activity. The attachment activity can be measured by hemagglutination assay. The neuraminidase activity prevents aggregation of virus particles by removing sialic acid from the virion glycoproteins and facilitates infection in the respiratory tract by digesting the mucin coating of epithelial cells.

These viruses also encode three nucleocapsid associated proteins, NP, P and L, and the nonglycosilated internal protein M. The four HPIVs are segregated in two genera, the Respirovirus (HPIV-1 and HPIV-3) and the Rubulavirus (HPIV-2 and HPIV-4), based on antigenic and structural characteristics, including additional accessory proteins (V,D,W,I,X,C and SH).

Epidemiology

HPIVs are distributed worldwide. Each of the four HPIVs can cause a full spectrum of acute respiratory tract illnesses. HPIV-1 and HPIV-2 are the most common pathogens associated with croup or laryngotracheobronchitis.[54] HPIVs 1, 2 and 3 are also important causes of bronchiolitis and pneumonia among infants and young children.[54] [55] Less is known about HPIV-4. Most children have serologic evidence of infection by 5 years of age. Reinfection is common and occurs throughout life. Most HPIV infections are detected during seasonal epidemics. In a study on an outpatient pediatric population,[56] HPIV-1 occurred in the fall of odd-numbered years, HPIV-2 was less predictable and HPIV-3 appeared yearly with peak activity in spring or summer. In a study of data from the US National Hospital Discharge Survey obtained from 1979 through 1997,[55] 3-month periods of widespread activity for each of the HPIVs were identified: HPIV-1 from September through November of odd-numbered years; HPIV-2 from October through December each year, and HPIV-3 from April through June during 1995 and 1997 and from May through July during 1994 and 1996. The estimated number of hospitalizations associated with HPIV-1–3 infections was 7600 to 48,000 among children aged <1 year and 8100 to 42,600 among children aged 1–4 years. The estimated rates of HPIV-associated hospitalizations ranged from 1.9 to 12 per 1000 children <1 year old and from 0.5 to 2.0 per 1000 children aged 1–4 years.

Pathogenesis

Although droplet transmission can occur, contact is though to be the primary mode of transmission of HPIV infection. This can be direct person to person or indirect through intermediate objects. Inoculation is into the mouth, nasopharynx or oral mucosa. The characteristic tendency to cause laryngotracheobronchitis and croup is not completely understood. Some of the HPIVs can induce syncytia formation but the role of this phenomenon is not well understood. Immune response is complex and includes the production of serum-neutralizing antibodies directed towards epitopes of the HN and F proteins. An important role in the protection against HPIVs infections is played by the mucosal immune response, primarily through the production of IgA antibodies. In animal model studies, it has been demonstrated that the role of $CD8^+$ T cells is critical for virus clearance.

Prevention

General principles for the prevention of respiratory viral infections apply to the prevention of HPIV infections. The CDC recommends contact precautions for the

prevention of nosocomial HPIV infections. Some institutions also observe droplet isolation precautions. No specific vaccine is yet available and no drugs have been tested for prophylactic use.

Diagnostic microbiology

Definite diagnosis relies on the isolation of the virus from respiratory secretions. Other diagnostic methods include detection of viral antigens or nucleic acid, and serologic analysis. Primary isolation and identification, by means of hemadsorption assay, can be followed by typing of the virus by means of immunofluorescence or hemadsorption inhibition. PCR assays can be more sensitive than isolation and antigen detection methods.[57]

Clinical manifestations

Infection in children is associated with an acute febrile illness in up to 80% of cases. Initial symptoms include coryza, sore throat, hoarseness and dry cough. In cases of croup, a brassy or barking cough may progress to stridor and some may progress to airway obstruction. The anteroposterior radiograph of the neck shows glottic and subglottic narrowing ('steeple sign') which differentiate this disease from epiglottitis. In cases of bronchiolitis and pneumonia, progressive cough is accompanied by wheezing, tachypnea and hypoxemia. Chest X-ray examination may reveal air trapping and interstitial infiltrates. Otitis media with presence of HPIVs in the middle ear fluids is common.

In older children and adults, HPIV infections tend to be milder and present as URI. However, HPIV-1 and 3 have been diagnosed by means of serologic assay in a significant number of adults admitted with acute respiratory illness. [58] A few cases of HPIV meningitis have been described. [59] Immunocompromised pediatric and adult individuals such as hematopoietic stem cell,[60] [61] [62] [63] [64] [65] lung[66] and renal[67] transplant

recipients may develop severe HPIV infections with a high rate of progression to pneumonia. The use of corticosteroids was linked with a higher risk of HPIV-3 pneumonia among recipients of hematopoietic stem cell transplantation.[62]

Management

Symptomatic treatment of croup usually includes humidification of air by ultrasonic nebulizer and inhalations of racemic epinephrine. Epinephrine provides rapid but transient relief of the airway obstruction. The anti-inflammatory effect of systemic corticosteroids is advocated to prevent or shorten the period of intubation in severe croup. Patients with bronchiolitis are also treated with bronchodilators. Ribavirin has exhibited *in vitro* activity against HPIVs[68] but data from controlled clinical trials are lacking.

INFLUENZA VIRUS

Nature

The existence of acute, usually self-limited, febrile respiratory illness that occurs in outbreaks of variable severity almost every winter has been known for centuries.[69] [70] [71] The term 'influenza' was derived from Italian in the mid-1300s to indicate that the disease resulted from astrological influences. The etiology of the disease and the explanation for its peculiar behavior remained elusive. It was not until the late 1920s that the etiology of influenza was better understood. Shope[72] showed that swine influenza could be transmitted with filtered mucus, suggesting that the causative agent was a virus. In the 1930s, Smith *et al* isolated the influenza virus from humans with respiratory illness.[73]

Influenza virions are enveloped particles of spherical or elongated shape, measuring 80–120nm in diameter and containing a linear segmented, single-stranded, negative-sense RNA. They belong to the Orthomyxoviridae family of RNA viruses. There are three major antigenic types — A, B and C — that also differ in genetic organization, structure, clinico-epidemiologic characteristics and host range (Table 220.3).

Influenza A virus serves as the prototype strain. The virion has two surface glycoproteins (hemagglutinin and neuraminidase) and a major nucleocapsid glycoprotein (NP) that associates with three other proteins (PA, PB1 and PB2) to form the transcription complex, two matrix proteins (M1 and M2) and two nonstructural proteins (NS1 and NS2). Influenza A virus adsorption to host cells involves the binding of hemagglutinin to sialic acid-containing cell surface glycoproteins or glycolipids. This is followed by virion entry into the

TABLE 220-3 -- Comparison of influenza virus types.

COMPARISON OF INFLUENZA VIRUS TYPES			
Antigenic type	**A**	**B**	**C**
RNA segments	8	8	7
Surface glycoproteins	Hemagglutinin (1 to 15)	Hemagglutinin	HEF: hemagglutinin esterase fusion protein
	Neuraminidase (1 to 9)	Neuraminidase	
Genetic variability	Drifts and shifts	Drifts	Drifts
Human disease	Epidemic, pandemic, sporadic	Epidemic, sporadic	Sporadic
Known hosts	Humans, swine, horses, poultry, sea mammals several avian species	Humans	Humans, swine
Target for current antivirals	M2 protein (amantadine, rimantadine)	Neuraminidase (zanamivir, oseltamivir)	Not available
	Neuraminidase (zanamivir, oseltamivir)		

cell via receptor-mediated endocytosis. The M2 protein ion channel activity leads to the acidification of the vesicle and fusion of the virus membrane to the vesicle membrane, resulting in the release of the viral nucleocapsid into the cytoplasm. Influenza RNA replication and translation depend on viral proteins and cell machinery functions. Budding from the plasma membrane completes assembly. The emerging virions are coated with sialic acid that is then cleaved by the neuraminidase, allowing the virus to be free and able to infect other cells.

Influenza A viruses are divided into subtypes. The current nomenclature system[74] includes the host of origin, geographic location of first isolation, strain number and year of isolation. The antigenic description of the hemagglutinin (HA) and neuraminidase (NA) is given within parentheses (e.g. A/Swine/Iowa/15/30 (H1N1)). By convention, the host of origin of human strains is omitted (e.g. A/Puerto Rico/8/34 (H1N1)). There are 15 HA and nine NA subtypes; the subtypes differ by 30% or more in amino acid sequence homology.

Epidemiology

There is no evidence of persistent or latent infection with human influenza viruses. The virus is maintained in humans by person-to-person spread during acute infection. Influenza virus is also known to infect a variety of birds and mammals (including swine, horses, seals, whales, mink and primates). The current information supports the concept that mammalian influenza viruses are derived from avian influenza reservoirs.[75] [76] [77] Some avian species, such as ducks, are asymptomatic carriers perhaps as a result of virus adaptation over many centuries.

The influenza viruses are unique among the respiratory tract viruses in that they undergo significant antigenic variation. Antigenic drift involves minor antigenic changes in the HA and NA that occur by accumulation of point mutations, resulting in amino acid substitutions in antigenic sites. These changes render the new strain different enough to avoid the immunity induced by previous strains. Influenza epidemics have been recognized as a major cause of morbidity and increased mortality, especially in the very young, the very old, people with chronic cardiopulmonary conditions, pregnant women and immunocompromised individuals. In the USA, epidemics of influenza typically occur during the winter months and are responsible for an average of approximately 16,000 to 220,000 hospitalizations and 20,000 to 40,000 deaths per epidemic.[78] In the tropics, influenza can occur throughout the year and in the temperate regions of the southern hemisphere the majority of influenza activity occurs

during the months of April to September.

During influenza A pandemics, the impact of influenza becomes global and results in significant morbidity and mortality for a broader range of hosts, including young healthy people.[70] The greatest influenza pandemic, so-called Spanish influenza, spread around the globe in 1918–19 after the First World War and resulted in the deaths of approximately 20–40 million persons, more than the war itself. The emergence of pandemic influenza A can be explained by antigenic shifts resulting from genetic reassortment within cells dually infected by human and animal influenza viruses.[79] The exchange of RNA segments between the two viruses occurs in a manner analogous to the exchange of parental chromosomes during meiosis. Interestingly, all major antigenic shifts have originated in China and have been confined to the H1, H2 and H3 subtypes. Avian influenza can be transmitted to humans with swine acting as an intermediary host in which reassortment takes place. However, a direct transmission of a pathogenic avian influenza to humans was observed in Hong Kong in 1997, when 18 people were infected with an avian H5N1 influenza virus and six died of its complications. [80] In addition, it has been observed that the Russian influenza (H1N1), that appeared in northern China in 1977, was identical to the virus that caused a human influenza epidemic in 1950, suggesting that certain influenza strains may remain confined in a natural reservoir for many years and reemerge when the herd immunity has waned.

Pathogenesis

Droplet transmission is considered the primary mode of transmission of influenza. However, scarce data also support the notion that airborne transmission can occur, spread from person to person by direct inhalation of droplet nuclei or small-particle aerosols measuring less than 5µm in diameter. After evaporation, micro-organisms remain suspended in the air for long periods of time and can be dispersed by air currents. Susceptible hosts may inhale the particles within the same room or over a longer distance from the source patient. The extent to which transmission might also occur by contact with virus-contaminated hands or fomites is less well understood.

Acute diffuse mucosal inflammation and edema of the upper airway and bronchi are observed in uncomplicated influenza. The columnar ciliated cells become vacuolated and edematous. This is followed by desquamation of the ciliated and mucus-producing epithelial cells. In cases of influenza viral pneumonia, there is an interstitial pneumonitis with a predominantly mononuclear leukocyte infiltration. The alveolar walls become denuded of epithelium; hyaline membranes form, and the intra-alveolar space becomes filled with exudate and hemorrhage.[81] At a cellular level, influenza virus disrupts protein synthesis and also induces apoptosis.

The immune response against influenza is complex and involves multiple arms of the immune system. Neutralizing antibodies in respiratory secretions are primarily of the IgA isotype[82] and constitute the first line of defense. However, the major role is played by the humoral response via neutralizing antibodies. The level of serum antibody to the HA and NA correlates with resistance to illness and with restriction of the influenza virus replication in the respiratory tract of humans.[83] [84] Interleukin-6 (IL-6), interferon-a and other proinflammatory cytokines are produced in response to influenza infection, with the peak of their production coinciding with the peak of systemic symptoms.[85] The cellular immunity also plays an important role in the immune response to influenza virus. Cytotoxic T cells participate by interacting with B cells and also by destroying cells infected with influenza.[86]

Prevention

Influenza vaccination is the primary method for preventing influenza. To be effective, the vaccine components need to match the circulating strains of the target season. Intensive surveillance of new influenza strains is maintained around the globe in order to make an informed selection of strains for the next yearly influenza vaccine and to detect early on strains that could potentially cause a new influenza pandemic. The primary target groups for annual vaccination are:

- people aged =65 years;
- residents of nursing homes and other chronic care facilities;
- people who have chronic disorders of the pulmonary or cardiovascular systems;
- those who have required regular medical follow-up or hospitalization during the preceding year because of chronic metabolic diseases (including diabetes mellitus), renal dysfunction, hemoglobinopathies or immunosuppression (including immunosuppression caused by medications or by human immunodeficiency virus);
- children and teenagers (aged 6 months to 18 years) who are receiving long-term aspirin therapy and therefore might be at risk for developing Reye's syndrome after influenza infection;
- and women who will be in the second or third trimester of pregnancy during the influenza season.

Vaccination of health care workers and others in close contact with persons at high risk, including household members, is recommended. When feasible, vaccination is also recommended for people aged 50–64 years, because this group has an increased prevalence of high-risk conditions, and for otherwise healthy children aged 6–23 months, because they are at increased risk for influenza-related hospitalization.[78] Depending on vaccine availability, vaccination should also be provided to any person who wishes to reduce the likelihood of becoming ill with influenza.

Vaccination results in reductions of influenza-related respiratory illness and number of physician visits among all groups of age, hospitalization and death among persons at high risk, otitis media among children and work absenteeism among adults. In the United States, the inactivated (i.e. killed virus) vaccine is recommended.[87] The vaccine contains three strains (i.e. two type A and one type B), representing the influenza viruses more likely to circulate in the upcoming winter. The vaccine is made from highly purified, inactivated virions. Subvirion and purified surface antigen preparations are also available. The effectiveness of influenza vaccine depends primarily on the age and immunocompetence of the vaccine recipient and the degree of similarity between the viruses in the vaccine and those in circulation. Local reactions to the vaccine are generally mild. Fever, malaise, myalgia and other systemic symptoms can also occur. The 1976 swine influenza vaccine was associated with an increased frequency of Guillain-Barré syndrome (<10 cases/1,000,000 persons vaccinated).

Live attenuated influenza vaccines can be administered directly to the respiratory tract, avoiding the need for injections and providing a means of inducing the production of IgA by the respiratory mucosa. Strains prepared by reassortment from cold-adapted mutants of both influenza A and B viruses have been widely shown to be well tolerated in both adults and children and to be highly efficacious. [88] [89]

Antiviral chemoprophylaxis is approximately 70–90% effective in preventing illness in healthy adults. Amantadine, rimantadine and oseltamivir are approved for chemoprophylaxis of influenza A virus infections; only oseltamivir is approved for chemoprophylaxis of influenza B virus infections (Table 220.4). Chemoprophylaxis can be considered for persons at high risk who are expected to have an inadequate antibody response to influenza vaccine, and for those with a history of anaphylactic reactions to egg protein. The efficacy of chemoprophylaxis for the prevention of influenza has not been established in immunocompromised patients.

Strategies for prevention of nosocomial transmission of influenza include vaccination programs, the use of chemoprophylaxis and compliance with droplet isolation precautions. Although influenza

TABLE 220-4 -- Comparison of antiviral drugs for prophylaxis and treatment of influenza.

COMPARISON OF ANTIVIRAL DRUGS FOR PROPHYLAXIS AND TREATMENT OF INFLUENZA					
Drug	**Trade name**	**Influenza type**	**Dosing for prophylaxis**	**Dosing for treatment**	**Main side-effects**
Amantadine	Symmetrel®	A	Age 1–9 years: 5mg/k/d po div bid	Age 1–9 years: 5mg/k/d po div bid	Central nervous system
			Age 9 and up: 100mg po bid	Age 9 and up: 100mg po bid	
Rimantadine	Flumadine®	A	Age 1–10 years: 5mg/k/d po qd	Adults: 100mg po bid	Central nervous system
			Age 10 and up: 100mg po bid		
Zanamivir	Relenza®	A and B	N/a	Age > 7 years: 10mg inhaled bid	Bronchial
Oseltamivir	Tamiflu®	A and B	Age =13 years: 75mg po qd	Age 1–12 years: dose per weight*	Gastrointestinal
				Age 13 and up: 75mg po bid	

* For children who weigh "15kg the dose is 30mg bid; for those who weigh 15–23kg, 45mg bid; for those who weigh 23–40kg, 60mg bid; and for those who weigh >40kg, 75mg bid.

can be transmitted via the air-borne route, the use of negative air pressure rooms has not been assessed.

Diagnostic microbiology

Diagnostic tests available for influenza include viral culture, serology, rapid antigen testing and immunofluorescence. Viral culture remains essential for determining the influenza subtypes and strains causing illness and for surveillance of new strains that may need to be included in the influenza vaccine. Because influenza may not produce cytopathic effects in cell culture, the hemadsorption test is used to screen for evidence of viral replication. The early diagnosis of influenza can reduce the inappropriate use of antibiotics and allow the early administration of antiviral therapy. Commercially available rapid influenza diagnostic tests differ in their ability to detect and distinguish between influenza A and B virus infections, methodologies, processing time and cost. The sensitivities of the rapid tests are lower than viral culture of respiratory specimens and a negative result might not exclude influenza virus infection. Rapid influenza tests provide results within 24 hours, while viral culture takes 3–10 days. Appropriate samples for influenza testing include nasopharyngeal or throat swabs, nasal wash or nasal aspirates, depending on which type of test is used. Samples should be collected within the first 4 days of illness. Acute and convalescent serum samples can also be tested for influenza antibodies to diagnose recent infection.

Clinical manifestations

The incubation period is 1–4 days, with an average of 2 days. The typical uncomplicated influenza A syndrome has an abrupt onset with headache, high-grade fever, chills, dry cough, myalgias, malaise and anorexia. In more severe cases, prostration is observed. Respiratory symptoms such as rhinorrhea, nasal congestion and sore throat are present but are overshadowed by the systemic symptoms during the first 3 days of illness. The cough frequently changes from a dry, hacking nature to one that is productive of small amounts of sputum that are usually mucoid but can be purulent. After the fever and upper respiratory tract symptoms resolve, cough and weakness can persist for 1–2 additional weeks. Influenza B virus can cause the same spectrum of disease as that seen after influenza A virus infection, and severe illness can occur. Influenza C virus causes sporadic, usually afebrile upper respiratory tract illness and is rarely associated with severe lower respiratory tract disease.

Influenza attack rates are higher in children compared to adults. High-grade fever, cervical lymphadenopathy, nausea and vomiting are frequent manifestations of influenza in children. Croup is a less frequent manifestation of influenza, limited to children.

Pneumonia complicates influenza predominantly in the elderly, patients with chronic cardiopulmonary disease, pregnant women and immunocompromised individuals. The etiology may be viral, bacterial or mixed viral-bacterial.[90] In primary viral pneumonia, typical influenza is followed by a rapid progression of fever, cough, dyspnea and cyanosis. Physical examination and chest X-ray disclose diffuse bilateral infiltrates consistent with adult respiratory distress syndrome. A combined viral-bacterial pneumonia is more common than primary viral pneumonia. The bacteria most commonly involved are *Streptococcus pneumoniae* and *Staphylococcus aureus*. A third type of complication is a bacterial pneumonia developing after a clinical improvement from influenza. In this case, physical and chest X-ray examinations are more likely to show localized signs of consolidation. Influenza can also exacerbate chronic underlying medical conditions such as asthma, chronic obstructive pulmonary disease and congestive heart failure.

A variety of nonrespiratory complications have been described. Myositis has been reported more frequently in children with influenza B but adults may also be affected and may develop rhabdomyolysis with acute renal failure.[91] Myocarditis and rarely pericarditis[92] [93] have been described in patients with influenza A and B. Reye's syndrome has been reported most frequently after influenza B[94] and has also been described after infections with other viruses such as varicellazoster. The syndrome is characterized by a rapidly progressive non-inflammatory encephalopathy and fatty infiltration of the viscera, especially the liver, resulting in severe hepatic dysfunction. The administration of therapeutic doses of salicylates appears to increase the risk for the development of Reye's syndrome. A wide spectrum of CNS involvement has been observed during influenza A and B virus infection, ranging from irritability and confusion to severe encephalopathy.[95] Toxic shock syndrome has been observed in association with influenza virus infection and is believed to be the consequence of the bacterial exotoxin (TSST-1) secreted by colonizing *Staph. aureus* strains.[96] Febrile convulsions leading to hospitalization occur in children with and without underlying CNS abnormalities.

Influenza viruses can cause severe disease in immunocompromised individuals.[97] [98] [99] [100] [101] [102] [103] Severe lymphopenia, elderly age, receipt of an allogeneic hematopoietic stem cell transplant and concurrent opportunistic infections were identified as independent risk factors for a complicated course in a study of adults with leukemia and adult recipients of hematopoietic stem cell transplant. In that study, significant morbidity and mortality were observed with both influenza A and B viruses. Prolonged shedding of influenza virus and rapid emergence of resistance to amantadine and rimantadine has been observed in immunocompromised persons.[104]

Management

Four antiviral medications — amantadine, rimantadine, oseltamivir and zanamivir — are approved for treatment of influenza A virus infections. Oseltamivir and zanamivir are also approved for treatment

of influenza B virus infections. When administered within 48 hours of symptom onset, antiviral treatment of influenza can reduce the duration of illness by approximately 1 day in healthy adults.

Amantadine and rimantadine inhibit the M2 protein of influenza A virus. Their side-effects include CNS symptoms such as nervousness, anxiety, difficulty concentrating and lightheadedness. Central nervous system side-effects are more frequent with amantadine than with rimantadine.[105] Gastrointestinal side-effects include nausea and loss of appetite. Amantadine and rimantadine are the least expensive antivirals against influenza and have been in use for several years.

Zanamivir and oseltamivir are analogs of sialic acid that were designed to inhibit the neuraminidase of both influenza A and B viruses.[106] Zanamivir is inhaled and may induce bronchospasm in those with underlying lung disease such as asthma and chronic obstructive pulmonary disease. Other less frequent side-effects include diarrhea, nausea, headache and dizziness. Oseltamivir may induce gastrointestinal side-effects, including nausea and vomiting, especially if the drug is not taken with food (see Table 220.4).

Data are limited concerning the effectiveness of amantadine, rimantadine, zanamivir and oseltamivir for the treatment of influenza among persons at high risk for serious complications of influenza. A retrospective analysis of a surveillance study in immunocompromised individuals showed a decreased rate of progression to pneumonia in patients with influenza A treated with amantadine or rimantadine.[107]

HUMAN METAPNEUMOVIRUS

In June 2001, it was reported that a new respiratory virus had been isolated from nasopharyngeal aspirates of 28 young children in The Netherlands. Based on virological data, sequence homology and gene constellation, the virus was classified as a member of the Metapneumovirus genus of the Paramyxoviridae family and was named human metapneumovirus (HMPV). The clinical syndrome was similar to that caused by RSV. Serological studies showed that by the age of 5 years, virtually all children in The Netherlands have been exposed to HMPV and that the virus had been circulating in humans for at least 50 years.[108] Investigators from other countries, including Australia, Canada, the UK and Finland, have reported further evidence that HMPV is an important human respiratory pathogen. The associated clinical presentation includes flu-like illness, bronchospasm and pneumonia. The severity varies from self-limiting mild respiratory illness to respiratory failure requiring mechanical ventilation. Severe lower respiratory tract infections have been observed in very young children, the elderly and immunocompromised patients. [109] The virus shows cytopathic effect in LLC-MK2 (tertiary monkey kidney) cells and molecular methods for rapid detection and identification are being developed.

CONCLUSION

A better understanding of the profound impact that HRVs have on human morbidity and mortality has resulted in more resources being devoted to their study. More efficient diagnostic tools, new antiviral agents, the use of passive immunoprophylaxis, improvements in vaccination programs and in strategies to control the nosocomial transmission of these infections are among the most significant accomplishments.

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Chapter 221 - Retroviruses and Retroviral Infections

Charles AB Boucher

INTRODUCTION

The Retroviridae constitute a large family of viruses that infect both humans and animals. Retroviruses predominantly infect vertebrates, although infections of insects and molluscs have also been described. Retroviral infections may cause a wide spectrum of diseases ranging from malignancies to immune deficiencies and neurologic disorders. However, most retroviral infections occur without any detectable deleterious effect to the host. In spite of the diversity of associated pathology and the broad range of hosts, all retroviruses are united by a common mode of replication and a similar virion structure and genome organization.

This chapter discusses some general features of the retroviruses, such as virion structure, genome organization and taxonomy of retroviruses. Although there have been concerns about the potential clinical significance of porcine retroviruses in the context of xenotransplantation[95] , the main focus of attention is on human retroviruses. During the past decades, the AIDS pandemic has resulted in an upsurge of scientific interest in retroviral infections and related pathology in humans. Several human retroviruses have been identified, of which only HIV and the human T-lymphocyte leukemia viruses (HTLVs) are known to cause clinical manifestations. These two human retroviruses have been well characterized and are discussed in more detail.

NATURE

The electron microscopic view of retroviral particles shows spheric particles approximately 100nm in diameter with an electron-dense core (Fig. 221.1). Retroviruses are enveloped viruses. The envelope consists of a phospholipid double layer derived from the plasma membrane of the host cell. Viral encoded transmembrane glycoproteins are inserted into the envelope, enabling the virus to attach to the host cell (Fig. 221.2). The envelope surrounds the nucleocapsid core, which contains the viral genome: two identical RNA molecules, having the same polarity as mRNA, with which an RNA-dependent DNA polymerase enzyme, reverse transcriptase (RT), is closely associated. Also present in the core is the integrase enzyme.

The genome of retroviruses is approximately 10,000 base pairs (bp) in length. Each end of the genome consists of repeating nucleotide sequences of 4–6bp, called the long terminal repeats (LTRs). The LTRs flank the three genomic regions that encode for sets of structural genes:

- the group antigen (*gag*) region, which codes for the core antigens;
- the polymerase (*pol*) region, which codes for the enzymes protease, RT and integrase; and
- the envelope (*env*) region, which codes for glycoproteins of the envelope.

The structural genes always appear in the same order: *gag-pol-env* (Fig. 221.3).[1] There are several retroviruses that contain additional genes that encode for viral proteins involved in the regulation of several processes in the replication cycle of the virus, such as the rate of transcription, the splicing of mRNA, the transport of mRNA from the nucleus to the cytoplasm and the release of progeny virus. Retroviruses that contain additional genes are called complex retroviruses; examples of these are the HIVs and the HTLVs. The genomes of so-called simple retroviruses only contain a *gag*, *pol* and *env* gene.

The first step in the replication cycle of retroviruses is attachment of the virus to a host cell through interaction of the envelope glycoprotein with a receptor molecule on the host cell surface (Fig. 221.4). This interaction between virus and host cell largely determines the host range of the virus (i.e. which species or cell type the virus will infect). After attachment, fusion of the envelope membrane with the host cell membrane takes place and the nucleocapsid core is released into the cell. However, some retroviruses enter the cell by receptor-mediated endocytosis followed by fusion of the viral envelope with the endosomal membrane.[2]

Following the entry of the nucleocapsid core into the cytoplasm of the host cell, the viral RNA is transcribed into double stranded DNA (dsDNA). This process is catalyzed by the viral enzyme RT. The viral dsDNA, in association with RT and the viral enzyme integrase, is transferred to the nucleus. Subsequently the dsDNA is integrated into the chromosomal DNA of the cell by the action of the enzyme integrase. The integrated viral DNA copy is called provirus.

Using the proviral DNA as template, cellular DNA-dependent RNA polymerases synthesize viral mRNA. Some viral mRNA molecules are spliced and transported to the cytoplasm, where translation yields full-length viral precursor polyproteins. These polyproteins, combined with two copies of unspliced genomic viral RNA, are assembled into immature virus particles. By budding through the plasma membrane the immature particles acquire a bilipid layer envelope. Maturation into infectious and replication-competent virions is accomplished after release of the immature particles from the host cell; this process requires processing of the polyproteins by the viral-encoded protease. Then, a new cycle of replication may start.

As described above, during the replication cycle of retroviruses the viral DNA is integrated in the chromosomal DNA of the host cell. The result of this integration is that the retroviral DNA is now inherited into the progeny of the infected cell. When retroviruses infect germline cells (sperm or egg cells) and the viral DNA is integrated into the chromosomal DNA of these cells, the retrovirus is passed from parent to offspring of the infected host. In this case, the virus is named an endogenous retrovirus. We all carry the remnants of retroviral DNA in our chromosomes as a result of common ancestors who were infected with endogenous retroviruses.

Retroviruses that are not integrated in germline cells of their host are called exogenous retroviruses. The transmission route of exogenous retroviruses is both horizontally and vertically via blood and saliva and by sexual intercourse.

HUMAN ENDOGENOUS RETROVIRUSES

Approximately 1% of the human genome consists of sequences of human endogenous retroviruses (HERVs).[3] Full-length HERVs contain sequences that are homologous to the *gag*, *pol* and *env* genes

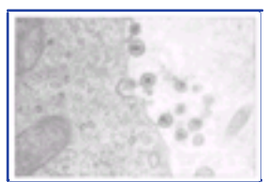

Figure 221-1 Typical electron microscopic view of retroviruses (HIV) showing an electron-dense core. *Courtesy of Dr Piet Joling.*

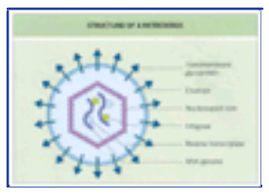

Figure 221-2 Structure of a retrovirus.

Figure 221-3 Genomic organization of three complex retroviruses. The diagram shows HIV-1, HIV-2 and HTLV. The components of the long terminal repeats are shaded. The open reading frames that encode viral proteins are depicted by unshaded boxes. *Adapted from Galasso et al.*[1]

of infectious exogenous retroviruses. The biologic properties and functions of endogenous retroviruses are still under investigation.

In general, HERVs are highly defective proviruses.[3] [4] Expression of HERV mRNA and HERV proteins has been demonstrated in several normal and pathologic tissues, and HERV retroviral particles have been detected by electron microscopy in these tissues.

Nine different HERV families have been described, some of which have as many as 50 copies in the human genome.[3] A description of the specific characteristics of the different HERV families does not fit into the scope of this chapter.

Because it was demonstrated that HERV mRNA and proteins can be expressed, it has been postulated that HERVs might be etiologically involved in human disease. In the literature, numerous pathologic conditions have been related to HERVs, such as inflammatory and degenerative diseases of the nervous system and several autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome and the mixed connective tissue disease). Evidence has been found for HERV antigen expression in some of these conditions. However, no definitive proof for the putative involvement of HERVs in any of these pathologic conditions has been published. The possible role of HERVs in human disease has been reviewed,[4] but more research is required to elucidate the general role of HERVs and their specific role in human disease.

Figure 221-4 Replication cycle of retroviruses.

HUMAN FOAMY VIRUS

In 1971, a human foamy virus (HFV) was the first human exogenous retrovirus to be successfully isolated from human tissue.[5] Human foamy virus is a member of the family of Spuma retroviruses. A clear relationship between HFV and human disease has never been established, although in several publications HFV has been associated with possible involvement in the pathogenesis of disease of the thyroid gland.

Human foamy virus can be isolated from patients who have subacute thyroiditis, also known as granulomatous thyroiditis or de Quervain's thyroiditis. [6] Furthermore, evidence for an association between Graves' disease and HFV infections has been found. The presence of HFV provirus in the peripheral blood mononuclear cells (PBMCs) of 19 out of 29 patients (66%) with Graves' disease has been demonstrated using polymerase chain reaction (PCR)-based techniques.[7] In the same study the PBMCs of 23 control patients were negative for HFV provirus. In another study, it was demonstrated that antibodies directed against the *gag* antigen of HFV reacted with the thyroid glands of all seven patients who had Graves' disease. [8]

In contrast, in two other studies, no HFV provirus was found in the PBMCs of 81 patients who had Graves' disease. In conclusion, more research is needed to establish the putative relation between diseases of the thyroid gland and human Spuma virus infections.

Human foamy virus infections have also been associated with numerous other pathologic conditions, such as the chronic fatigue syndrome, hemodialysis encephalopathy, [9] multiple sclerosis, Guillain-Barré syndrome, amyotrophic lateral sclerosis[10] and Kawasaki's disease. However, again no definitive evidence has been found to support involvement of HFV in any of these diseases.

Several epidemiologic studies using an enzyme-linked immunosorbent assay (ELISA) technique to investigate the distribution of antibodies to the human Spuma virus suggest a high prevalence of the virus in patients from the African continent and low prevalence in Europe. However, in one study HFV provirus could not be detected in the PBMCs of the patients who were serum positive in the ELISA.[11] Therefore, these epidemiologic data need confirmation in additional studies.

In conclusion, more research is needed to resolve the controversy on the epidemiology and virulence of human Spuma viruses.

HUMAN T-LYMPHOCYTE LEUKEMIA VIRUSES

NATURE

Human T-lymphocyte leukemia virus-1 and HTLV-2 are complex retroviruses. In addition to a *gag*, *pol* and *env* gene, they contain four open reading frames in the pX region; these open reading frames have regulatory and accessory functions. [12] Two of these proteins, *rex* and *tax* (see Fig. 221.3), play an important role in regulation of viral replication. The *rex* protein is involved in the stabilization of viral mRNA and the transport of viral mRNA from nucleus to cytoplasm. The *rex* protein also regulates the splicing and processing of viral mRNA. The *tax* protein is a transactivator protein and is also essential for viral replication. By acting on the LTR located 5' to the viral *gag* gene, *tax* induces the transcription of viral mRNA. Apart from regulating viral replication, the *tax* protein is also believed to play a role in oncogenesis through transactivation of cellular proto-oncogenes (see below).

EPIDEMIOLOGY

Seroepidemiologic studies have shown that the highest incidence of anti-HTLV-1 antibodies is found in south-western Japan, ranging from 5 to 35% in endemic areas. Other areas with high incidence are the Caribbean islands, some regions of South and Central America, the south-west Pacific and Papua New Guinea. [2] [11] The general incidence in the USA and Europe is low (0.05%), although an increasing incidence has been reported among homosexuals and intravenous drug users.

The epidemiology of HTLV-2 has been less well studied. It is difficult to distinguish serologically between HTLV-1 and HTLV-2 because of antigenic cross-reactivity. Therefore, HTLV-2 incidence has been studied with the help of a technique based on a PCR using primers specific for the HTLV-2 proviral genome. A high incidence of HTLV-2 has been found among native Americans in Panama and New Mexico and among intravenous drug users in the USA and Italy.[13] [14] [15] [16]

PATHOGENESIS

It is believed that HTLV-1 is transmitted by infected lymphocytes and not as free virus in cell-free body fluids. Three transmission routes for HTLV-1 have been described.

- first, transmission of HTLV-infected lymphocytes from mother to child is a major route; it can occur via the placenta, during birth, or after birth through the mother's milk;
- second, evidence has also been found for transmission from male to female during sexual intercourse — no evidence has been found for sexual transmission of HTLV-1 in the other direction, from female to male; and
- finally, blood transfusions are an efficient transmission route of HTLV-1. [2] [11]

Human T-lymphocyte leukemia virus-1 is a lymphotropic virus that preferentially infects $CD4^+$ T cells. Usually, the leukemic cells in adult T-lymphocyte leukemia (ATL) have the following phenotype: $CD2^+$, $CD3^+$, $CD5^+$, $CD7^-$, $CD4^+$ and $CD8^-$; they also express the activation surface markers CD38 (the interleukin (IL)-2 receptor), CD30 and major histocompatibility complex class II.[17] [18] The cellular receptor mediating the HTLV-1 infection of lymphocytes has not been identified.

The molecular mechanisms by which HTLV-1 is able to induce cell transformation have not been unraveled. Human T-lymphocyte leukemia virus does not contain a typical oncogene. Several other hypothetic mechanisms of HTLV-induced oncogenesis have been formulated.

It has been hypothesized that in HTLV-1-infected T cells the *tax* protein may transactivate cellular growth factor genes such as the IL-2 gene or the IL-2 receptor gene, thus promoting unbridled division of T cells. Human T-lymphocyte leukemia virus-1-infected, immortalized cells have been demonstrated to express high levels of the IL-2 receptor. Normally, in an uninfected person, most of the T cells are in a quiescent state, with low levels of IL-2 production and IL-2 receptor expression, and they only become activated in cases of clone-specific stimulation caused by the presence of an antigen.

Another mechanism of cell transformation that has been postulated is transactivation of proto-oncogenes by the *tax* protein of HTLV-1, such as c-*fos* and the platelet-derived growth factor gene.

Furthermore, in patients who have ATL, several chromosomal aberrations have been described. It is not clear whether these chromosome aberrations are induced by insertion of the HTLV proviral DNA.[2] [11] [19]

However, these hypotheses do not explain why it takes an average incubation time of 20–30 years to develop ATL or why only a small number of HTLV-1-infected people develop clinical manifestations of ATL. More research is required to answer these questions.

DIAGNOSTIC MICROBIOLOGY

The definite diagnosis of ATL is made by demonstrating the presence of HTLV-1 proviral DNA in the DNA of tumor cells using a PCR-based technique.

CLINICAL FEATURES

Human T-lymphocyte leukemia virus-1

Adult T-lymphocyte leukemia

Human T-lymphocyte leukemia virus-1 is considered to be the etiologic agent of ATL, a disease first recognized as a nosologic entity in 1977.[20] Several pieces of evidence established the causal relationship between HTLV-1 and ATL. First, ATL has an identical geographic distribution to that of HTLV-1, having a high incidence in south-western Japan, as was shown in seroepidemiologic studies. [21] Second, all ATL tumor cells contain one or more copies of the HTLV-1 provirus in their genomic DNA. Third, in-vitro infection of human T cells with HTLV-1 results in T-cell immortalization. Finally, HTLV-1 has been demonstrated to be oncogenic in animals.[19]

The development of ATL has been divided into four stages:

- the asymptomatic carrier state,
- the preleukemic state,
- smoldering (or chronic ATL), and
- acute ATL.

Asymptomatic carrier state

Patients in the asymptomatic carrier state are infected with HTLV-1 without any clinical manifestation or abnormal laboratory findings. The majority of HTLV-1-infected people are asymptomatic carriers. The lifetime chance of an infected person developing ATL is about 1%.[22] The first clinical manifestations of ATL generally occur 20–30 years after infection with HTLV-1. The median age of ATL onset is 52.7 years.[23]

Preleukemic state

The preleukemic state is defined by one or more of:

- leukocytosis,
- morphologic abnormalities of the lymphocytes (Fig. 221.5), or
- detection of HTLV-1 provirus by PCR.

Smoldering adult T-lymphocyte leukemia

Smoldering or chronic ATL is characterized by skin lesions and low levels (<5%) of leukemic cells. Patients in this stage may progress towards the acute stage of ATL. The median survival time in chronic ATL is 24 months.

Acute adult T-lymphocyte leukemia

Acute ATL is characterized by high levels (>5%) of leukemic cells, skin lesions, general lymphadenopathy, hepatosplenomegaly and hypercalcemia and immunodeficiencies. Hypercalcemia correlates with poor prognosis and is a result of osteoclast proliferation mediated by IL-1a and a parathyroid-mimicking hormone, both of which are produced by the HTLV-1-infected cells. The median survival of patients with acute ATL is approximately 6 months.

Tropical spastic paresis

Infections with HTLV-1 have also been associated with a neurologic syndrome called tropical spastic paresis, also known as HTLV-1-associated myelopathy (HAM). This disorder is characterized by a

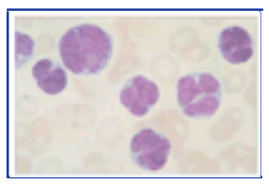

Figure 221-5 Typical 'clover leaf' appearance of nuclei of HTLV-1 induced adult T cell leukemic cells. *Courtesy of Steven M Opal.*

slowly progressive symmetric myelopathy combined with high titers of antibodies to HTLV-1 in plasma and cerebrospinal fluid (CSF). The myelopathy primarily affects the pyramidal tract. The mechanisms by which HTLV-1 infection causes HAM is unclear, but patients show signs of an activated immune system with high levels of immunoglobulins and increased numbers of activated T cells in the blood and CSF, which suggests an immunologic (possibly an autoimmunologic) pathogenesis. There have been reports that patients who have HAM respond to corticosteroids but this is always short-lived and unfortunately no form of therapy has been shown to have lasting benefit.[2] [11]

Ophthalmologic complications

Human T-lymphocyte leukemia virus-1 infections have also been associated with ophthalmologic complications, which are frequently observed in patients suffering from ATL or HAM. These include a wide range of neoplastic, infectious and noninfectious vascular or inflammatory lesions.[24] However, ocular lesions have also been found in patients who are positive for HTLV-1 antibodies but who have no other clinical manifestation of HTLV-1 infection. In a recent study of 93 patients who had HTLV-1 infection (23 asymptomatic and 70 patients who had HAM tropical spastic paresis), 48.4% of the patients were diagnosed with keratoconjunctivitis and 16.1% with uveitis.[25]

Arthropathy

Finally, HTLV-1 infections have been associated with chronic inflammatory arthropathy, both in the presence and in the absence of clinical ATL. Polyarthritis may be the presenting manifestation of HTLV-I-associated ATL. It has been demonstrated that the synovial fluid of patients who have HTLV-1-associated arthritis contains high titers of anti-HTLV-1 antibodies and atypical lymphocytes.[2] [11]

Human T-lymphocyte leukemia virus-2

Human T-lymphocyte leukemia virus-2 infections have been associated with T-cell malignancies, predominantly hairy T-lymphocytic leukemia and chronic lymphatic leukemia. Neurologic complications similar to those seen in HAM or tropical spastic paresis have also been described.

MANAGEMENT

The therapy for ATL usually consists of combined antineoplastic chemotherapy. However, antineoplastic therapies have proved to have only a limited ability to improve the prognosis of ATL. In most patients, resistance of the leukemic cells to the drugs is believed to be the prominent reason for failure to induce a complete remission. However, two promising novel strategies to improve the prognosis of ATL are under investigation. In one study using a combination of zidovudine and interferon-a, a prolonged survival in previously untreated patients was reported. [26] The other a therapeutic approach is directed against the IL-2 receptor on the leukemic cells. Lymphocytes in ATL express high levels of the IL-2 receptor, in contrast to normal resting, non-HTLV-1-infected lymphocytes. A favorable response has been reported in patients treated with antibodies against the IL-2 receptor that are armed with toxins or emitting radionuclides.[27]

HUMAN IMMUNODEFICIENCY VIRUSES

NATURE

The human immunodeficiency viruses HIV-1 and HIV-2 are members of the family of Lentiviruses. Both HIV-1 and HIV-2 are the causative agents of AIDS. For detailed information regarding the clinical manifestations of HIV infection and AIDS (see Chapter 122 , Chapter 124 , Chapter 125 , Chapter 126 , Chapter 127 , Chapter 128 , Chapter 129 , Chapter 130 , Chapter 131 , Chapter 132 , Chapter 133), antiretroviral therapy (see Chapter 139 and Chapter 204) and

Figure 221-6 HIV-1 virion.

therapy evaluation and vaccine strategies (see Chapter 118). This chapter describes viral structure, pathogenesis and epidemiology of HIV.

Virion structure

The electron microscopic view of human immunodeficiency retroviruses shows an electron-dense cone-shaped core. The viral p24 capsid protein functions as a shell for the condensed core, which contains the two identical genomic RNA strands in close association with RT (Fig. 221.6). The viral genome is approximately 9200 bases long and contains nine genes (see Fig. 221.3). Previously it was believed that the p6 protein was also located in the viral core, but this protein does not seem to co-purify with the core structure, suggesting another localization.[28] The viral protein Vpr (in HIV-1) and Vpx (in HIV-2) is also found in the virion and is probably located outside the core.[29] The Vif protein may also be present in the viral particle in close association with the viral core.[30] More research is required to increase our knowledge on the structure of the viral particle and to determine the exact localization of p6, Vpr/Vpx and Vif. The inner part of the viral membrane is covered with a myristylated p17 core protein, which provides the matrix for the viral structure. Glycoprotein spikes project from the virion surface and are anchored into an envelope that consists of a phospholipid bilayer derived from the host cell membrane. The spikes contain two viral glycoproteins (gp), gp120 and gp41. The gp41 acts as a transmembrane protein and its central portion binds to the external surface gp120. In addition to gp41 and gp 120, the viral envelope can contain various proteins derived from the host cell.

Replication cycle

Infection starts with the binding of gp120 glycoprotein to the cellular surface CD4 molecule, which acts as a high-affinity receptor. Recently, two secondary or co-receptors have been identified: CXCR4 (also known as fusin) and CCR5.[31] These co-receptors are members of a cellular transmembrane chemokine receptor family. All HIV strains tested to date use CXCR4 or CCR5, or both, as a co-receptor. However, several other co-receptors for HIV have also been identified, one of which is encoded by the cytomegalovirus. Co-receptor usage determines the cell tropism of the HIV strain. The CCR5 co-receptor is used by HIV strains that in vitro do not cause cell fusion (syncytia) in T-cell lines (non-syncitium-inducing (NSI) strains). Syncytium-inducing (SI) HIV variants use both co-receptors CXCR4 and CCR5. In general, NSI variants are found throughout the complete course of HIV infection, whereas SI variants emerge

only in a later stage of HIV infection. The presence of SI variants in a patient is associated with a more rapid CD4 decline and faster disease progression (see below).

A route of infection independent of the CD4 molecule may also be possible. A variety of host cells lacking the CD4 receptor have been successfully infected in vitro, although infection of CD4 cells seems to be a very inefficient process. The in-vivo significance of the CD4-independent route of entry is yet to be determined.

After binding, HIV enters the cell through fusion of the viral and host cell membranes. Subsequently, the viral core, containing the genome, is released in the cytoplasm of the cell; this involves a process of internalization and uncoating.

During the replication cycle, the first synthesized transcription products are the viral regulatory proteins *nef*, *tat* and *rev* (for a review see Greene *et al.*[32]). The *tat* protein will bind to the transactive response element region on the proviral DNA and, as a consequence, high-level expression of all viral genes occurs. The *nef* protein is required for high-level viral replication in vivo (see Pathogenesis, below), and it decreases CD4 on the cell membrane. The *nef* protein may enhance the efficiency of reverse transcription, but its precise functions have not been completely elucidated.

Through the action of *rev* a switch may occur toward the synthesis of late regulatory and viral structural proteins. The *rev* protein facilitates the nuclear transport of unspliced and incompletely spliced RNA transcripts that contain the *rev*-responsive element, located in the *env* gene. In addition it promotes translation of messages containing the *rev*-responsive element. It remains unclear to what extent the relative amounts of each of these regulatory proteins and their interaction with cellular factors can determine whether the infection of a cell results in a productive cycle or merely a viral latent state.

The *gag* and the *pol* open reading frames (ORFs) overlap. The *pol* ORF is positioned in the -1 frame relative to the *gag* ORF. In the unspliced, full-length mRNA, both reading frames are present. Translation starts at the beginning of the *gag* ORF and continues in most cases until the end of the ORF, and a 55kDa *gag* precursor protein (p55), containing p17, p24, p7, p2, p1 and p6, is made. During translation of full-length mRNA, approximately 5–10% of the ribosomes slip back one nucleotide (frame shift) and gain access to the *pol* ORF, and translation continues. A partial *gag* precursor protein is fused to the *pol* polyprotein, resulting in a *gag-pol* polyprotein. The *pol* gene encodes for three proteins: protease, RT and integrase. Cleavage of the *gag-pol* protein generates protease, RT and integrase. The 99-amino acid protease is coded for at the 5' end of the *pol* gene. HIV protease is active only as homodimer, which is generated by an autolytic process. It is possible that aggregation of two *gag-pol* proteins is required to generate the active form, thereby limiting activation to the budding site. Both *gag* and *gag-pol* polyproteins are post-translationally modified by a covalent attachment of a lipophilic myristyl group onto their aminoterminal glycine by a host cell-derived myristyl transferase. Myristylation may be required for intracellular transport to the cell membrane because, in the absence of myristylation, no infectious particles are produced.

The *gag* precursor protein is cleaved by viral protease into four structural proteins (p17, p24, p7 and p6) and two smaller proteins (p2 and p1). The protein p2 may be important for proper assembly of the virions. The *gag* precursor protein may also play a role in the packaging of viral RNA into the virus particle, specifically through the action of the nucleic acid binding protein p7. This protein binds to a sequence at the 5' end of the viral genome, possibly through the interaction of so-called cystine-histidine motifs. The p6 protein has been shown to be rich in proline, and through binding to p6, Vpr is incorporated in the viral particle. Viruses with a mutation in p6 are not released from the cell surface.

The accessory genes *vif*, *vpr* and *vpu/vpx* influence the viral assembly and budding process, and alterations in these genes leads to a decrease in infectivity. These proteins may be more important for replication in macrophages and primary lymphocytes than for T-cell lines. The protein Vpr can cause cell cycle arrest in the G2 phase, and it is as yet unclear whether this phenomena plays a role in viral replication. The protein Vpu interacts with CD4 and may cause CD4 degradation.[2] [11] The Vif protein is considered to enhance infectivity and the underlying mechanism is still under investigation.[32]

EPIDEMIOLOGY OF HIV AND VIRAL SUBTYPES

Twenty-six nonhuman primates in Africa have been reported to be infected with the retrovirus simian immunodeficiency virus (SIV). Two of these viruses are the cause of AIDS in humans. It has been estimated that they have been transmitted to humans on at least seven different occasions.[33]

The global HIV-1 epidemic is seen as a composite of infections from at least nine genetically distinct subtypes or clades of virus, designated A to H.[34] Together they form the M group.[35] These subtypes can be distinguished on the basis of sequence information of the *env* gene (30% difference) and *gag* gene (15% difference). Two

isolates that are highly divergent from group M have been fully sequenced. The results show that viruses from the new group O are almost as close to HIV-2 as to HIV-1. The distribution of certain HIV-1 subtypes is still geographically limited, but geographic dispersion will contribute to the growing pandemic (see Chapter 115). The distribution of subtypes within countries as well as throughout the world shows that there is no evidence for host specificity for certain subtypes.[35] Dual infection with two subtypes and hybrids between two subtypes have been described in infected individuals.

Five distinct subgroups have been identified for HIV-2, and in analogy with HIV-1 these were designated A to E. The observed viral variation has clear implications for vaccine development and has also implications for development of drugs or treatment of patients. The non-nucleoside RT inhibitors (NNRTIs) inhibit RT activity through binding various amino acids of RT, the most important amino acid being the tyrosine residue at codon 181 for HIV-1 RT. Selection of drug-resistant HIV-1 isolates both in vitro and in vivo results in a substitution at codon 181 of a cysteine. The cysteine substitution gives a considerable increase in the 50% inhibitory concentration for all NNRTIs. Sequence analysis of several HIV-2 RT genes has shown that at codon 181 the cysteine is normally present. The presence of the cysteine variant makes HIV-2 viruses naturally resistant to the NNRTIs.

The *env*-based classification of widely divergent HIV-1 strains into sequence subtypes is also supported by nucleotide sequence analysis of the *gag* genes. The distinction between SI and NSI viruses, conferred by gp120 described in clade B, is also present among other HIV-1 subtypes. Detailed information is available on the prevalence of the different subtypes for certain areas in the world. Information on the prevalence of different clades in a region may give interesting information on the spread of the infection and the possible transmission routes. For instance, in Thailand it has been shown that type A is mainly spreading through sexual intercourse, whereas type B is transmitted among intravenous drug users. Monitoring the prevalence of different clades around the world and searching for possible new clades is important for the use and development of diagnostic procedures. For instance, some screening assays for HIV antibodies have been shown to be relatively insensitive for clade O viruses. The use of diagnostic PCR procedures, especially in children born to mothers who have HIV infection, may be complicated by sequence variation in the region where the primers have to anneal to their substrate. The quantitative molecular assays may also vary in their

sensitivity for different clades. From the perspective of vaccine development, it will also be extremely important to continue to generate information on the worldwide prevalence and geographic distribution of the various clades (see also Chapter 115).[36]

PATHOGENICITY

Transmission

The three major routes of transmission of HIV are by sexual contact or blood or blood products or vertically through maternal-fetal transmission. The first step in infection is binding of the viral particles to submucosal dendritic cells (H). These cells which normally process antigens and present them to immune cells, express a protein termed DC-SIGN. The viral gp120 binds to DC-SIGN with high affinity; subsequently the viral particles are internalized and presented again at the cell surface after the dendritic cells have migrated to the regional lymph tissues and present viral particles to the T cells (see Chapter 120).

It is very likely that the amount of HIV in the inoculating material is the most important factor determining the risk of transmission. The amount of virus is, at least in the blood of the donor, determined by disease stage and antiretroviral therapy. In contrast, data on the relation between the amount of HIV in semen and disease stage is contradictory.[37] Modeling of the epidemic supports the notion that viral load may be the major factor for determining the rate of infection. It has been demonstrated that people who have a primary infection are an important source of transmission.[38] The amount of virus in the blood of people who have a primary infection is generally very high (see below). A prospective study showed that the transmission rate within heterosexual couples tended to increase when the index case was in a more advanced stage of disease, again pointing at an inoculum effect.[39]

Two recent studies showed that the risk of heterosexual transmission is dependent on the HIV RNA concentration in the peripheral blood of the positive partner.[40] [41] In one cohort no cases of transmission were noted if the seropositive partners had HIV RNA concentrations under 1500 copies/ml. In this cohort of 415 couples there was a clear dose-response relation of increased transmission with increasing viral load.[40]

The rate of transmission may also be determined by biologic properties of the virus. Transmission of HIV-2 seems to be considerably lower than of HIV-1 in western Africa. In addition, infection with HIV-2 seems to protect against infection with HIV-1. In a prospective study among commercial female sex workers in Senegal, the HIV-1 infection rate was lower in those workers who were already infected with HIV-2.[42] [43]

Other protective host factors may also play a role, such as the integrity of mucocutaneous membranes of the urogenital tract. Among heterosexual couples the risk of transmission increases when the negative partner had a genital infection.[39] [40]

It has been demonstrated that protection against transmission is also offered by a 32bp deletion in the *CCR5* gene, encoding a cellular co-receptor for HIV (see Replication cycle, above). The CCR5 allele with the 32bp deletion (CCR5 32) is common in the Caucasian population with a frequency of 8080 per 100,000 individuals (13.3% heterozygous, 1.4% homozygous). The CCR5 32 is not found among Asians and Africans. In 1252 homosexual males of the Multicenter AIDS Cohort Study from Chicago none of the HIV-infected participants was homozygous for the CCR5 32 allele, whereas 3.6% of the at-risk but uninfected Caucasian participants were found to be homozygous.[44] This suggests that homozygosity for the CCR5 32 allele is an important protection factor. Heterozygosity has not been found to protect against infection, although it is associated with a delayed disease progression (see below).[44]

Finally, a study among a group of 25 seronegative subjects who had a history of multiple exposures to HIV-1 showed that the $CD4^+$ lymphocytes in a subgroup of patients had a relative resistance to infection with clinical isolates in vitro.[45] These $CD4^+$ lymphocytes produced more chemokines — RANTES, macrophage inflammatory protein (MIP)-1a, MIP-1ß — than sensitive $CD4^+$ lymphocytes. Addition of recombinant chemokines to sensitive $CD4^+$ lymphocytes increased their resistance to infection. These chemokines are the natural ligands of chemokine receptors CCR5 and CXCR4, which have been identified as HIV co-receptors. It has been hypothesized that chemokines compete with HIV for the receptor binding sites, thus inhibiting the efficiency of HIV infection.

Studies addressing transmission from mother to baby have also shown that the risk of vertical transmission increases with a higher viral load in the mother at the time of delivery (as measured as by HIV p24 antigen, HIV-1 RNA, proviral DNA or PBMC viral titer) and with lower $CD4^+$ lymphocyte counts. [46] The current opinion is that the majority of infections of the child occur late during pregnancy and most commonly during delivery, because elective cesarean section lowers the transmission rate by approximately 50%. [47] This notion is supported by the outcome of a multicenter, randomized, placebo-controlled trial of the efficacy and safety of zidovudine in reducing the risk of vertical transmission.[48] A protective effect of treatment was seen despite the fact that the treated women received the study drug only for a median of 11 weeks before giving birth. Moreover, the risk of transmission increases when the fetal membranes rupture more than 4 hours before delivery.[49] [50] This also suggests that most transmissions occur late in pregnancy or during delivery (see Chapter 134).

Incubation period and viral replication

Initially, it was believed that during the asymptomatic or clinically latent phase the amount of viral replication would be very low and the amount of virus present in the body minimal. It has been shown over the past few years that this concept was seriously flawed. Prospective studies show that in most people during the asymptomatic phase of the infection a gradual decline of the $CD4^+$ lymphocytes is paralleled by a gradual rise in viral HIV-1 RNA in the peripheral blood. Moreover, it has been shown that in the asymptomatic phase, virus replicates in the lymphoid tissue in those people who lack cell-free virus in their peripheral blood. Analysis of the viral burden in the peripheral blood compared with the burden in lymphoid tissues shows that the viral load in lymphoid tissues is 1 log higher than in blood. In-situ hybridization studies and electron microscopic analyses have shown that the lymph nodes from asymptomatic carriers can contain high quantities of HIV.[51] Viral particles covered with antibody and complement are trapped by the follicular dendritic cells in the germinal center of the lymph nodes. Thus, the incubation period is characterized by ongoing viral replication.

Viral dynamics and $CD4^+$ lymphocyte depletion

The effects of therapy with two protease inhibitors (ritonavir (10 patients) and indinavir (8 patients)) and a NNRTI (nevirapine (4 patients)) were investigated.[52] In addition to measuring the response of HIV RNA and $CD4^+$ lymphocytes, this study also measured the appearance of drug-resistant mutations in both plasma and PBMCs. The viral clearance rate was calculated (using three methods) and found to range between 1.8 (±0.9) days and 3.0 (±1.7) days. There were no differences observed in viral clearance rates with the three different drugs. In both studies the clearance rate constants were independent of the initial viral loads and the $CD4^+$ lymphocyte counts, suggesting that the viral clearance rate constant is independent of the stage of HIV infection. Monitoring the appearance of drug-resistant mutations over time led to an estimated

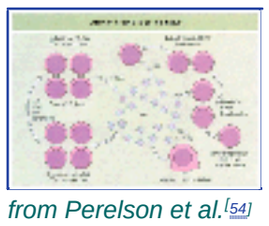

Figure 221-7 Dynamics of HIV-1 infection in vivo. Shown in the center is the cell-free virion population that is sampled when the viral load in plasma is measured. *Adapted from Perelson et al.*[54]

doubling time of approximately 2 days, in accordance with the estimated half-life for HIV in plasma.

In agreement with previous results, an estimate of 2×10^9 $CD4^+$ lymphocytes (about 5% of the total $CD4^+$ lymphocyte population) has been calculated to be produced and destroyed daily. In other studies data have been generated with zidovudine and lamivudine that support the observations described above.[53]

All the data together show that HIV-1 infection is a highly dynamic process in which billions of viruses are being produced (Fig. 221.7). The vast majority of the high number of circulating viruses is derived from continuous rounds of new infections that take place on a daily basis. [54] The contribution of long-living, chronically virus-producing cells is very small. In a review, these insights were placed in the context of genetic variation, pathogenesis and therapy.[55] Our current understanding of the kinetics of HIV infection has changed our views on antiretroviral therapy.

Relationship of viral load and $CD4^+$ lymphocyte levels to disease progression

Several approaches have been developed to obtain information on the amount of virus present in a person with HIV infection and to relate the amount of virus to the duration of the incubation period, rate of disease progression and survival. Both the amount of provirus and the amount of virus in the peripheral blood have been evaluated using quantitative culture techniques and quantitative molecular approaches.

The first quantification of virus was carried out by determination of viral capsid antigen in serum using the HIV p24 or antigen assays. Later, quantitative virus culture assays were developed to measure the amount of culturable virus in serum or plasma.[56] [57] In the first studies, the mean titers ranged from 30 tissue culture infective doses (TCID) for asymptomatic infection to 3200 TCID in patients who have AIDS, but a later study reported higher titers in a small number of patients.[58] Several cross-sectional studies showed a relationship between a more progressed disease stage, lower $CD4^+$ lymphocyte counts and higher plasma titers. Antiretroviral therapy lowers the plasma titers and cellular viral titers.[59] [60] The costs and the need for fast transport of plasma to the laboratory have prevented widespread use of quantitative cultures for estimating the risk of fast disease progression as well as for therapy evaluation. [61]

The amount of provirus has been measured by two methods:

- quantification of the viral titer in patient PBMCs; and
- quantification of the amount of proviral DNA using various PCR-based techniques.

A correlation has been found between a high frequency of positive cells or an increase in the number of positive cells and disease progression in all studies that have addressed the relationship between proviral DNA levels and disease progression, either by comparing patients in different disease stages cross-sectionally[62] [63] [64] [65] [66] [67] [68] or by following patients over time who progressed clinically.[64] [69] [70] [71] The increase observed over time was relatively small (range of 1–2 log). In one longitudinal study of seroconverters, a relationship was found between a high proviral load from the moment of seroconversion and a rapid progression to disease. [70] During the incubation time, the increase in proviral load was relatively small, indicating that the set point obtained around seroconversion determined the subsequent outcome. Similar observations have been made with HIV RNA levels in seroconverters.[72] [73] Two studies were carried out to analyze the amount of proviral DNA in patients infected with HIV-2.[74] [75] The numbers obtained were not significantly different from those in patients infected with HIV-1.

In a recent study the amount of HIV viral DNA was compared between HIV-specific memory cells and other memory cells.[76] It was shown that the HIV-specific memory $CD4^+$ cells contained significantly more HIV viral DNA at all stages of disease, indicating indeed that HIV-specific $CD4^+$ cells are preferentially infected by HIV in vivo.

Several studies have determined the amount and pattern of viral mRNA expression and have correlated these with disease progression and decline in $CD4^+$ -lymphocyte counts.[77] The outcomes of all these studies are remarkably similar. In all studies, independent of the disease stage, viral transcription can be detected. Furthermore, these studies indicate that in patients who show immunologic or clinical deterioration, a shift takes place from a predominantly spliced mRNA pattern to a predominantly unspliced pattern. It has been suggested that this may be caused by post-translational replication block, a model suggesting true in-vivo latency. However, the observed differences in the relative abundance of the different mRNA species may also be caused by de-novo viral replication.[77] Until this type of study is performed on single cells, it will be difficult to find out to what

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extent latency in cells on the basis of post-transcriptional block is a frequent event. The authors of some of these studies advocate the use of quantitative mRNA measurements and pattern analysis as tools for clinical management. It is very unlikely, however, that this type of assay will show an advantage over the well-established methods for quantifying the amount of genomic RNA copies in serum or plasma.

The development of quantitative assays for the determination of viral RNA in serum has revolutionized the field of antiretroviral therapy and our insights into the pathogenesis of disease. The first cross-sectional studies showed that HIV-1 RNA could be detected in all stages of disease and that levels as high as 10^7 copies/ml could be measured.[78] [79] [80] In general, there is a significant correlation between HIV RNA levels and the other quantitative parameters for cell-free virus (extracellular virions), such as HIV p24 antigen, although these parameters are often undetectable.[81] [82] Furthermore, a strong correlation has been found between cell-free HIV RNA levels and cellular RNA levels in PBMCs.[72] The sensitivity and dynamic range of the HIV RNA measurements is much greater, however, and therefore more information has been obtained on the significance of viral load for pathogenesis and therapy evaluation by using the HIV RNA measurements.

Currently, several longitudinal studies on the relationship between HIV RNA load patterns over time and disease progression or $CD4^+$ -lymphocyte decline on four different cohorts of seroconvertors have been performed.[72] [73] [83] [84] [85] These studies showed that a plateau phase or set point is obtained a couple of months after acute HIV infection. This plateau phase is stable during the course of years. A clear variation (2–3 log) was observed, however, in the height of the set point, and this value, obtained some months after seroconversion, was directly related to the ultimate clinical outcome.

With the introduction of the highly active antiretroviral therapy it has become possible to inhibit viral replication completely in some treated individuals. This, however, does not lead to eradication of the infection because pretherapy infected cells not undergoing a full viral replicative cycle can persist. Currently it is not clear how long these latent reservoirs may persist, but most studies estimate that these reservoirs could persist for life.[86]

Indeed, even patients who have supressed their viral replication for as long as 5 years experience a rebound in viral replication if they interrupt their therapy.

This indicates that for eradication of HIV infection alternative strategies need to be developed. The challenge will be to find a way to eliminate the provirus from the T-memory cells, which are currently assumed to represent reservoir cells with the longest survival.

Factors that determine the duration of the incubation period

Presently, it is not clear why the incubation period can greatly differ between individuals and why the decline in $CD4^+$ lymphocyte counts is typically a gradual process (40–80 cells/mm^3 /year).[87] It is likely, however, that multiple factors are involved in determining the time to disease progression. Some factors have been identified. These factors can be divided into viral and immunologic (host) factors (see also Chapter 120).

To get more insight into the factors influencing the incubation period and pathogenesis of HIV infections, studies have been performed on patients with HIV infection who have not shown clinical progression and who have moderate immunologic deterioration, or none at all during long-term follow up (generally up to 10–15 years after

infection).[88] [89] These so-called long-term survivors or long-term nonprogressors seem to have some characteristics in common. The viral burden and proviral burden in the peripheral blood was found to be several orders of magnitude lower than the levels normally found in subjects who have progressive disease.[88] [89] The lymph nodes showed significantly less activation (germinal center formation) than the lymph nodes of control subjects with progressive disease, and furthermore the germinal centers did not show signs of involution and lymphocyte depletion.[89] Viruses could nevertheless be cultured from the mononuclear cells in the lymph nodes, indicating that HIV in these individuals was replication competent.[89]

Three viral factors have been found that seem to play a role in the duration of the incubation time. First, deletions in the *nef* regulatory gene causing a decrease in virulence have been shown to be present in a group of six long-term survivors, all of whom were intravenously infected by blood products from the same donor. However, in another study it was demonstrated that 91.1% of the *nef* genes of 10 long-term nonprogressors had no such deletions. The events described in all cases in which a *nef* deletion was observed are remarkably similar to the events described in rhesus monkeys inoculated with a derivative of the pathogenic SIV containing a 183bp deletion in *nef*. These animals became persistently infected but had low viral burden and normal $CD4^+$ -lymphocyte counts and did not show any sign of disease progression.[90]

Recently, a detailed analysis of a long-term survivor was published. [91] This patient, also infected via a blood transfusion, harbored viruses with only a low frequency of defective *nef* genes, but in contrast the viral population contained inactivating mutations in several other accessory genes (*vif, vpr, vpu, tat* and *rev*).

Thus, some studies show that, in at least in some long-term nonprogressors, specific virologic changes in their HIV cause a decrease in virulence, which may account for their sustained incubation period.

The second viral property that contributes to the rate of disease progression comes from studies that compared the rate of disease development between patients infected with HIV-1 and those infected with HIV-2.[45] [92] These studies suggest that people infected with HIV-2 have a reduced rate of developing a $CD4^+$ lymphocyte count below 400 cells/mm^3 and reduced disease progression compared with people infected with HIV-1. The limitation of this type of data is that the differences may be caused by differences in duration of infection between the HIV-1 and HIV-2 groups. The mechanism responsible for the reduced virulence of HIV-2 needs further research.

A third virologic factor that contributes to the duration of the incubation period is the capacity of HIV to induce syncytia in a MT-2 cell line ex vivo. Prospective studies in the Amsterdam cohort have shown that untreated subjects who switch in viral phenotype from NSI to SI isolates develop a more rapid decline in $CD4^+$ -lymphocyte counts and show a faster progression to AIDS than those who have persisting NSI phenotypic isolates.[93] The SI isolates appeared 2 years before the progression to AIDS.

Several host factors seem to contribute to the duration of the incubation time. The immune response to HIV plays a crucial role in controlling HIV replication in long-term nonprogressors. The cytotoxic T-lymphocyte activity to HIV antigens is believed to protect against disease progression. Recently, several studies have shown that heterozygosity for a deletion of 32bp in the gene encoding the CCR5 HIV co-receptor predicts delayed disease progression. In one study it was shown that 30 months after seroconversion CCR5 32 heterozygotes had a 1.5-fold slower decline in $CD4^+$ T-cell count and a 2.6-fold lower plasma viral load than CCR5 wild-type homozygotes. The predictive value of the CCR5 genotype for disease progression was independent of high HIV RNA plasma load, low $CD4^+$ T-cell counts, low T-cell functionality and the presence of SI variants of HIV.[94]

CLINICAL FEATURES

The hallmark of HIV infection is a selective reduction in the number of $CD4^+$ T cells, resulting in a progressive loss of cellular immunity

and development of AIDS, which is characterized by opportunistic infections and the development of opportunistic malignancies. In addition, several organs may be directly affected by HIV, including the gastrointestinal tract, bone marrow and the central nervous system, resulting in wasting, AIDS dementia, neuropathy and thrombocytopenia. The incubation period of primary HIV infection is approximately 4 weeks. Primary HIV infection is symptomatic in up to 70% cases and is associated with fever, arthralgia, adenopathy, myalgia, truncal (and facial) maculopapular rash and neurologic symptoms, including neuritis, myelopathy and aseptic meningoencephalitis (see Chapter 122). Primary HIV infection is followed by an asymptomatic carrier state of variable duration (median 10 years), after which most patients develop AIDS. The asymptomatic phase of HIV infection is frequently characterized by a persistent generalized lymphadenopathy. On histologic examination, lymph nodes of asymptomatic HIV carriers who have persistent generalized lymphadenopathy show follicular hyperplasia with endothelial cell proliferation, and HIV is found in large amounts on the follicular dendritic cells. Clinical progression toward AIDS is reflected by a rapid decrease in $CD4^+$ lymphocytes and deterioration of the lymph node architecture, from follicular hyperplasia to follicular involution and follicular depletion. The many symptoms and complications of AIDS are beyond the scope of this chapter. For a detailed discussion of the clinical manifestations during late-stage HIV disease, see Chapter 124 Chapter 125 , Chapter 126 , Chapter 127 , Chapter 128 , Chapter 129 , Chapter 130 , Chapter 131 , Chapter 132 , Chapter 133 .

MANAGEMENT

In a majority of patients with HIV infection, highly active antiretroviral therapy consisting of two RT inhibitors and one protease inhibitor results in profound and sustained suppression of HIV replication, increasing $CD4^+$ lymphocyte counts and preventing AIDS-defining illnesses and death. Reduction of plasma HIV RNA concentrations below the level of detection can be achieved for up to 2 years. For a review of the general principles and details of antiretroviral therapy, see Chapter 138 , Chapter 139 , and Chapter 204 .

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Chapter 222 - Zoonotic Viruses

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INTRODUCTION

Zoonotic infections are infections of animals that can naturally infect humans. More than 400 viruses cause clinically important zoonoses and infection is acquired by several different routes. Humans can be infected directly from the host animal species, for example following the bite of a rabies-infected animal, or through inhalation of rodent urine (hantavirus) or by direct contact with rodent urine (Lassa fever). However, the majority of zoonotic infections are spread by arthropod vectors and are classified as arboviruses.

The geographic distribution of the infections described in this chapter depends on many factors and changes over time. Recent examples include West Nile fever, which has recently spread to and become established across the USA. Nipah and hendra viruses, newly recognized causes of encephalitis in humans, have been identified in Australia, Malaysia, Singapore and India. Congo-Crimean hemorrhagic fever has re-emerged in Kosovo, following social dislocation resulting from conflict. Other important zoonotic infections include DNA viruses, such as poxviruses and herpes B virus, and RNA viruses, such as rabies. A number of rare occupational viral zoonoses have been described, including foot and mouth disease, vesicular stomatitis virus and swine vesicular disease.[1] [2]

ARBOVIRUSES

Over 400 viruses that cause human disease are classified as arboviruses. These are distributed worldwide because of their close association with lower vertebrates and insects and their wide host range. Most arbovirus infections are caused by RNA viruses and include members of the togaviruses, bunyaviruses and reoviruses.

Many arboviruses that infect humans cause non-specific symptoms such as acute fever. Three broad clinical patterns are recognized with arbovirus infection:

- fever, rash and arthritis or retinitis;
- encephalitis; and
- viral hemorrhagic fevers (VHFs).

Several comprehensive reviews are available.[3] [4]

The key features of the clinically important arboviruses transmitted to humans by hematophagous arthropod vectors, such as mosquitoes, ticks and *Phlebotomus* flies (sandfly), are given in Figure 222.1 .

Most arboviruses are maintained in complex life cycles that involve nonhuman vertebrate hosts with or without a primary vector. Mosquitoes are the most important arbovirus vector, followed by ticks, sandflies (*Phlebotomus* spp.) and midges (*Culicoides* spp.). These arbovirus life cycles only become evident when humans are bitten directly by the natural enzootic focus, or when they are exposed to a virus that has escaped the primary cycle via a secondary vector or vertebrate host. Many arboviruses have several vertebrate hosts and many can be transmitted by a number of vectors.

Viruses such as urban dengue, urban yellow fever and occasionally urban St Louis encephalitis cause significant viremia and represent the simplest form of epidemic transmission cycle, in which the virus is transmitted directly between mosquitoes and humans (see Fig. 222.1). In some cases the introduction of a secondary vector may allow the transmission of the virus to other nonhuman vertebrate reservoirs (sylvatic cycle), many of which can be regarded as blind-end hosts (Fig. 222.2). Prime examples of sylvatic cycles with multiple hosts are the encephalitides such as Western equine encephalitis, eastern encephalitis and St Louis encephalitis. Many arboviruses are maintained in arthropods, through transovarial transmission. Amplification of the cycle occurs by spread to and from small mammals (Fig. 222.3). The flavivirus infections are discussed in Chapter 23 . The key features of the clinically important arboviruses transmitted to humans by hematophagous arthropod vectors, such as mosquitoes, ticks and *Phlebotomus* flies (sandfly), are given in Table 222.1 . Yellow fever is an example of a mosquito-borne zoonotic virus infection.

YELLOW FEVER

Nature

Yellow fever has historically been one of the great killers of humans. Regrettably, this mosquito-borne virus continues to exact a considerable toll on human populations living in endemic areas even today. Yellow fever is principally responsible for the failure of the French to successfully complete the Panama Canal in the 1880s as the work force was decimated by epidemics of yellow fever. Yellow fever was the first virus shown to be transmissible by a mosquito vector. Pioneering work by Carlos Finlay and Walter Reed are credited with this important discovery, which led to mosquito control efforts and dramatic reductions in the incidence of yellow fever. Yellow fever remains a considerable public health risk to travelers and inhabitants in endemic regions of South America and Africa.

Epidemiology

The World Health Organization (WHO) reported that between 1987 and 1991 there were 18,735 cases of yellow fever worldwide, with 4522 deaths.[5] This represented the greatest number of reported cases over a 5-year period since active reporting began in 1948. Yellow fever primarily occurs in jungle regions of the world and it is estimated that the incidence of yellow fever is grossly under-reported. The WHO estimates that perhaps as many as 200,000 cases of yellow fever occur annually. [6] Countries reporting active cases of yellow fever as of January of 1999 are indicated in Figure 222.4 .[7] Yellow fever has caused significant outbreaks in recent years; in Guinea in 2000, Peru in 2001 and Senegal in 2002. The value of mass vaccination for controlling an outbreak was demonstrated in Guinea. [8]

There are two transmission cycles of yellow fever. The first and most common today is the jungle (or sylvatic) cycle of yellow fever. The jungle transmission cycle consists of a natural reservoir of disease in nonhuman primates with transmission by forest-dwelling mosquitoes. In South America the mosquito primarily responsible for yellow

Figure 222-1 Maintenance of arbovirus cycle involving humans as the primary vertebrate host (e.g. urban dengue and urban yellow fever).

Figure 222-2 Sylvatic cycle of Western equine encephalitis (WEE) and St Louis encephalitis (SLE).

fever transmission to humans is the *Haemagogus* sp. mosquito. In sub-Saharan Africa yellow fever is transmitted in rural areas primarily by *Aedes africanus*. The urban (or epidemic) cycle of yellow fever is related to urban outbreaks transmitted by the human-adapted mosquito *Aedes aegypti*.

All susceptible populations appear to be equally at risk for disease yet most cases occur in children or young men (particularly those involved in the logging industry or construction in heavily forested areas). The increased incidence of yellow fever recently may reflect population expansion and economic development of rainforest areas and inadequate distribution of yellow fever vaccine in regions of the

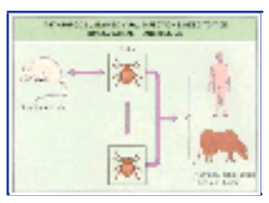

Figure 222-3 Arthropod sustained viral infection linked to tick transovarial transmission.

world where yellow fever remains endemic. Expanding commercial trade and international travel increases the risk of yellow fever outbreaks in nonendemic regions of the world in which the mosquito vector is found. Competent vectors for yellow fever are found in southern USA where the risk for yellow fever outbreaks remains a public health concern.[9]

Pathogenesis

Yellow fever is a prototypic mosquito-borne viral hemorrhagic fever. The virus is a 38nm ssRNA virus with a genome of approximately 4×10^6 Da. The virus is injected into humans during the course of a mosquito bite as the salivary contents of the mosquito contaminate the bite site. Local replication of the virus takes place in human skin and regional lymph nodes followed by viremia and dissemination to multiple organs of the body.

Major pathologic findings are found in the liver, kidney, heart and gastrointestinal tract. The virus infects the liver and causes massive hepatic necrosis. The characteristic pathologic finding is that of mid-zone necrosis with sparing of hepatocytes around the central vein and portal triads (Fig. 222.5). Another characteristic finding is that of Councilman bodies within degenerating hepatocytes (Fig. 222.6). Councilman bodies are acidophilic cytoplasmic deposits and not viral inclusion bodies. Diffuse areas of petechial hemorrhage are found throughout the body with the most marked changes found in the brain, kidney and gastrointestinal tract.

Prevention

The most important preventive measure is to avoid the mosquito vector within endemic regions where yellow fever exists. This can be accomplished by environmental control measures that remove mosquito habitats from areas of human habitation. There is a highly effective preventive vaccine against yellow fever. This is a live attenuated yellow fever vaccine known as vaccine strain 17-D.[10] The vaccine is highly efficacious and results in a prolonged neutralizing antibody response that will persist in the circulation for more than 10 years after immunization. Travelers to yellow fever endemic areas and residents within these areas should be vaccinated to prevent yellow fever.

Diagnostic microbiology

The virus can be isolated from a number of cell lines including Vero cells and AP-61 cells. Virus isolation represents an extreme biohazard and should only be attempted in specialized laboratory facilities.

TABLE 222-1 -- Important arbovirus infections that cause human disease.

IMPORTANT ARBOVIRUS INFECTIONS THAT CAUSE HUMAN DISEASE										
Genus	**Virus**	**Geographic distribution**							**Main hosts**	**Clinical diseases**
		Afr.	**Asia**	**Aust.**	**Eur.**	**N. Am.**	**C. Am.**	**S. Am.**	**Pac.**	
Mosquito vector										
Alphavirus	Western equine encephalitis	-	-	-	-	+	-	+	-	Rodents, birds, marsupials, equines	Encephalitis
	Eastern equine encephalitis	-	-	-	-	+	-	-	-	Wild birds, bats, rodents, equines, reptiles	Encephalitis
	Venezuelan equine encephalitis	-	-	-	-	-	+	+	-	Rodents, birds, marsupials, equines	Encephalitis
	Chikungunya	+	+	-	-	-	-	-	-	Primates, birds, bats, squirrels	Fever, rash, myalgia, polyarthritis
	Ross River	-	-	+	-	-	-	-	+	Large mammals, marsupials	Fever, rash, myalgia, arthralgia, hemorrhagic fever
Flavivirus	Dengue	+	+	+	-	-	+	+	+	Nonhuman primates	Encephalitis
	Japanese B encephalitis	-	+	+	-	-	-	-	-	Birds, pigs	Encephalitis
	St Louis encephalitis	-	-	-	-	+	-	+	-	Rodents mammals	Encephalitis
	Murray Valley encephalitis	-	+	+	-	-	-	-	-	Birds	Encephalitis
	West Nile fever	+	+	-	+	+	-	-	-	Birds	Fever, rash, myalgia, polyarthritis, encephalitis
	Yellow fever	+	-	-	-	-	+	+	-	Primates, marsupials	Hemorrhagic fever
Phlebovirus	Rift Valley fever	+	-	-	-	-	-	-	-	Wild mammals, domestic livestock	Hemorrhagic fever, retinitis, encephalitis
Tick vector										

Flavivirus	Central European encephalitis	-	-	-	+	-	-	-	-	Rodents	Encephalitis
	Russian spring-summer encephalitis	-	-	-	+	-	-	-	-	Rodents	Encephalitis
	Omsk hemorrhagic fever	-	+	+	-	-	-	-	-	Water voles, muskrats	Encephalitis
	Powasson	-	-	-	-	+	-	-	-	Rodents	Encephalitis
Nairovirus	Crimean-Congo hemorrhagic fever	+	+	-	+	-	-	-	-	Wild and domestic mammals	Hemorrhagic fever
Coltivirus	Colarado tick fever virus	-	-	-	-	+	-	-	-		Febrile illness
Sandfly vector											
Phlebovirus	Sandfly fever Naples	+	+	-	+	-	-	-	-	Rodent	Febrile illness, myalgia, conjunctivitis
	Sandfly fever Sicilian	+	+	-	+	-	-	-	-	Rodent	Febrile illness, myalgia, conjunctivitis
	Toscana	-	-	-	+	-	-	-	-	Rodent	Febrile illness, myalgia, conjunctivitis

Serodiagnosis is possible within 1 week of onset of infection with IgM antibodies detectable using enzyme-linked immunoabsorbent assay (ELISA), hemagglutination inhibition or plaque reduction neutralization tests. Virus detection can also be accomplished using reverse transcriptase semi-nested polymerase chain reactions (PCRs) from blood as well as tissue samples.[11]

Clinical manifestations

Human infection with yellow fever follows three discrete phases. The first phase of infection last approximately 72 hours and is initiated by headache, malaise, weakness, nausea and vomiting. The virus is replicating rapidly and high titers of infectious virus are found in the body fluids of patients at this point. This initial period of infection is followed by a brief period of remission where fever and symptoms remit. After 24–48 hours, symptoms recur and are dominated by symptoms of acute hepatic failure and renal failure. Patients develop jaundice and very high fever (hence the name yellow fever) and begin developing clinically overt signs of a hemorrhagic diathesis with petechiae, mucosal hemorrhages and gastrointestinal bleeding. A characteristic physical finding is marked bradycardia in the face of high fever (Fagetis' sign). Patients develop delirium, seizures, coma, acute renal failure and death within 7–10 days of illness. The mortality rate may be as high as 50% in some outbreaks and as low as 5% if optimal medical care is available. [12]

Management

The management of yellow fever remains primarily supportive. Intravenous fluids, blood product support and management of hepatic and renal insufficiency are the mainstays of therapy. Ribavirin does not appear to be effective and no other antiviral

Figure 222-4 Distribution of reported cases of yellow fever, 1 January 1999. Dark purple areas show the worldwide distribution of reported cases.

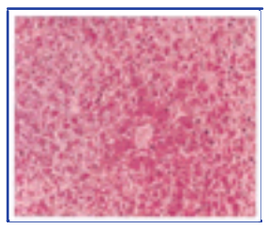

Figure 222-5 Histopathology of yellow fever in the liver. Yellow fever virus causes mid-zone necrosis of the liver tissue with sparing of hepatocytes around the central vain and the portal triads. *From Binford CH, Connor DH. Pathology of tropical and extraordinary diseases. Volume 1. Washington DC: Armed Forces Institute of Pathology; 1976.*

agent has been shown to be useful to treat yellow fever thus far. Patients should be isolated as their blood and body secretions may contain viral particles. Transmission to health care workers, particularly after needlestick injuries, has been described.

WEST NILE VIRUS

Recently human and horse West Nile virus (WNV) infection have been identified in new temperate regions of Europe and North America. Clinical presentation is variable, but severe infection is characterized by encephalitis and a significant mortality rate in humans and horses as well as certain domestic and wild birds. The virus was first isolated from the blood of a febrile women in the West Nile district of Uganda in 1937[13] and was subsequently isolated from patients, birds and mosquitoes, in Egypt in the early 1950s.[14] The

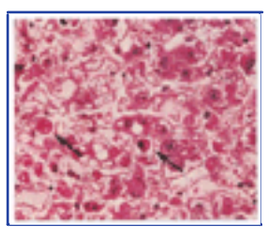

Figure 222-6 Histology of liver tissue in a fatal case of yellow fever. Note the Councilman bodies (arrowed), which are visible within degenerating hepatocytes. *From Binford CH, Connor DH. Pathology of tropical and extraordinary diseases. Volume 1. Washington DC: Armed Forces Institute of Pathology; 1976.*

WNV was first recognized as a cause of human meningoencephalitis in Israel in 1957 and as a cause of equine disease in Egypt and France in the early 1960s.

Virology

The WNV is an RNA virus belonging to the family Flaviviridae (genus flavivirus). It is a member of the Japanese encephalitis complex, which also includes Kunjin, Murray Valley encephalitis and St Louis encephalitis. The WNV virus strains can be divided genetically into two lineages. Only members of lineage 1 have been associated with clinical human encephalitis. Lineage 1 has been isolated from Africa, India, Europe, Asia and North America. Among lineage 1 WNV causing the recent human and equine outbreaks throughout Europe and Asia are closely related to the WNV (strain ROM96) first isolated in Romania and subsequently in Kenya in 1998. The virus

responsible for the outbreak in the USA (NY99) is genetically distinguishable from ROM96, but it is closely related to the virus circulating in Israel form 1997 to 2000 (Isi98). Only the USA and Israel have reported illness and death in humans and animals caused by the WNV strains Isi98 and NY99. The close relations between these strains suggest that the virus causing the outbreak in the USA was imported from the Middle East.

Epidemiology

Since the original isolation of WNV, outbreaks have occurred infrequently in humans, those in Israel (1951–4 and 1957) and South Africa (1974) being the most noticeable. Since the mid-1990s there have been three distinct epidemiologic trends.

First, there has been an increase in disease frequency in humans, notably in Romania,[15] Czech Republic and Russia. Epizootics of disease in horses have occurred in Morocco where 42 of 94 affected horses died, Italy (14 cases in 1998, six died), USA (1999–2002) and the Camargue region of France.

Second, there has been a noticeable increase in severity of human disease, with large outbreaks with significant mortality rates, especially in temperate urban areas. They include Bucharest, Romania (1996, over 400 cases, 17 deaths); Volgograd, Russia (1999, over 800 human cases, 40 deaths); New York City (1999–2000, 83 human cases, nine deaths); and Israel (2000, over 300 human cases, 29 deaths). [16] Since the introduction of WNV into the USA in 1999[17] it has been responsible for the identification of an increased number of cases (1999–2001, 149 human cases, 18 deaths; during the first a months of 2002, 2369 human cases, 139 deaths). The WNV has now been responsible for the spread of human disease westward, found in 34 states of the USA in less than 3 years.

Finally, high avian death rates accompanying human outbreaks have been a significant feature of WNV outbreaks in the USA and Israel.

The principal vectors are mosquitoes, predominantly of the genus *Culex* (*C. pipiens* and *C. restuans*), and the principal amplifying hosts are wild birds. Humans and horses are considered 'end-hosts' or 'incidental hosts'. A striking feature of the initial human epidemic in New York City 1999 was the high number of avian deaths among American crows (*Corvi brachrhynchos*) and other corvids.

Clinical manifestations

The incubation time in humans is reported to be 3–14 days. Symptoms generally last 3–6 days. Reports from earlier outbreaks describe a mild form of WNV infection presenting as a febrile influenza-like illness with sudden onset. This is often accompanied by malaise, anorexia, nausea, vomiting, rash, myalgia, lymphadenopathy and retro-orbital pain. A small proportion of cases (<1%) develop severe illness, such as acute encephalitis, aseptic meningitis or Guillain-Barré syndrome. In the recent outbreak in the USA those patients hospitalized with severe disease reported fever, weakness, gastrointestinal symptoms and flaccid paralysis. A minority of patients with severe disease develop a maculopapular rash or morbilliform rash involving the neck, trunk, arms or legs. Neurologic presentations include ataxia and extrapyramidal signs, cranial nerve abnormalities, myelitis, optical neuritis and seizures, and are more common in those over 50 years of age and in young children.

Diagnostic microbiology

Diagnosis of patients with WNF encephalitis or meningitis is made by the detection of IgM antibody to WNV in serum or cerebrospinal fluid (CSF) collected within 8 days of illness using the IgM antibody capture ELISA (MAC-ELISA). As IgM does not cross the blood-brain barrier, IgM antibody in the CSF strongly suggests central nervous system (CNS) infection. Therefore, the laboratory diagnostic confirmation strategy involves the serologic detection of IgM or IgG ELISA in CSF and serum with subsequent plaque reduction neutralization test (PRNT) confirmation. In addition, highly sensitive reverse transcriptase (RT)-PCRs have been described. Virus isolation on acute CSF samples provides an alternative approach. Antigenic cross-reactivity with other closely related flaviviruses is a major problem in serologic testing, and this underlines the need for confirmation with PRNT. It should be noted that samples from patients recently vaccinated against or recently infected with related flaviviruses (e.g. yellow fever, Japanese encephalitis) may give positive WNV MAC-ELISA results.

Management

There is no specific therapy or vaccine available and management is supportive.

ARENAVIRIDAE

Nature

Members of the Arenaviridae have been isolated from diverse species of rodents, which are their natural hosts, in a wide range of geographic locations. Two arenavirus complexes are recognized — the complex of lymphocytic choriomeningitis virus and Lassa virus (LCMV-LASV), or old world arenaviruses, and the Tacaribe complex, or new world arenaviruses.[18] This broad antigenic classification is supported by more detailed phylogenetic comparisons. The important arenavirus infections of humans are listed in Table 222.2 .

Arenavirus virions range from spheric to pleomorphic and have a mean diameter of 110–130nm. Particles are characterized by a variable number of electron-dense ribosomes (diameter 20–25nm) within the virus particles. They have a lipid membrane with glycoprotein spikes that project 8–10nm from the surface (Fig. 222.7).

TABLE 222-2 -- Arenaviruses known to cause human disease.

ARENAVIRUSES KNOWN TO CAUSE HUMAN DISEASE			
Virus	**Host in nature**	**Geographic distribution**	**Main features of human disease**
Lymphocytic chorio-meningitis virus (LCMV)	*Mus domesticus, Mus musculus*	Europe, Americas, perhaps elsewhere	Isolated 1933; causes lymphocytic choriomeningitis, which usually presents as an aseptic meningitis with a mortality rate <1%
Lassa fever	*Mastomys* spp.	West Africa	Isolated 1969; causes Lassa fever, a severe systemic illness; severe cases suffer shock and hemorrhages; mortality rate 16%
Junin virus	*Calomys musculinus*	Argentina	Isolated 1958; causes Argentine hemorrhagic fever (AHF), which causes a similar illness to Lassa but hemorrhage and CNS disease more frequent; mortality rate up to 30%
Machupo virus	*Calomys callosus*	Beni region of Bolivia	Isolated 1963; causes Bolivian hemorrhagic fever; similar clinical picture to AHF; mortality rate 25%
Guanarito virus	*Zygodontomys brevicauda: Sigmodon aistoni*	Venezuela	Isolated 1990, similar to AHF; mortality rate 25%
Sabia virus	Unknown	Brazil	Isolated 1990; only three human cases described, one fatal; clinical picture probably similar to AHF

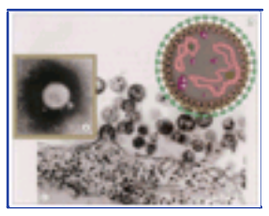

Figure 222-7 Arenaviruses. (a) Negative contrast electron micrograph of Lassa virus. (b) Arenavirus particle showing coiled nucleocapsid ribosomes and glycoprotein spikes. (c) Thin sections of infected Vero cells, showing extracellular virus, budding particles and intracellular inclusions. *Courtesy of G Lloyd, B Dowsett and ASR Featherstone.*

The genome consists of two ssRNA molecules, L (large) and S (small). The L and S RNAs of arenaviruses have an ambience coding arrangement; nucleocapsid (N) is encoded in the viral-complementary sequence corresponding to the 5' half of segment S, whereas the viral glycoprotein precursor (GPC) is encoded in the viral-sense

sequence corresponding to the 3' half of S.[18]

The N protein, which is the most abundant polypeptide, is non-glycosylated and forms a ribonucleoprotein (RNP) complex with the genomic RNA. Two glycosylated proteins, GP-1 and GP-2, are derived by post-transitional cleavage from the GPC. The L segment codes for the L protein, which is an RNA polymerase, and the Z protein, a putative zinc binding protein. a-Dystoglycan has recently been identified as a receptor for LCMV and Lassa fever virus.[19] This belongs to a highly conserved family of proteins found in epithelial, muscle and neurologic cells in humans.

Pathogenesis

In their natural rodent hosts, arenavirus infections occur in two distinct patterns: during the neonatal period, a chronic viremic infection is established; in adult rodents the infection is transient and self-limiting. This lifelong infection does not have major clinical sequelae for the infected rodent. [18]

In humans several arenavirus infections cause severe illness, including meningoencephalitis and hemorrhagic fever, with significant mortality rates. The pathogenesis is not fully understood, and microscopic changes found at autopsy are modest and do not account for the severity of illness. Petechial hemorrhages of the skin are found, and multiple hemorrhages and focal necrosis may be present in internal organs. Serous effusions and interstitial pneumonia have been described. Hemorrhages are more common with South American arenavirus infections. In contrast to these modest pathologic lesions, the physiologic changes are extensive. The state of shock characteristic of severe disease reflects an increase in vascular permeability. The mechanism involved is not fully understood, but it may be an indirect effect of immune mediators

Figure 222-8 Distribution of pathogenic human arenavirus infection in South America and Africa, and year of first isolation of each virus.

on endothelial cells produced by infected macrophages and monocytes, rather than direct viral damage.

Epidemiology

The epidemiology of individual arenaviral disease in humans is dependent on the geographic distribution of infected rodents and the nature of their contacts with humans (Fig. 222.8).

Several distinct patterns are seen. In Lassa fever, which is a common human infection, the reservoir host is *Mastomys* spp., which is a peridomestic rodent found in sub-Saharan Africa. The rodents are persistently infected and contaminate the environment with the virus, which generates infectious aerosols on drying. Other

routes of transmission include direct contact with rodent excretions during the capture and killing of these animals for consumption. The host of junin virus, the cause of Argentine hemorrhagic fever, is *Colomys musculinus*. The natural habitat of this rodent is the hedgerow and transmission occurs at harvest time, presumably because of aerosol transmission during mechanical harvesting.

Infection by LCMV is found worldwide, wherever its natural hosts *Mus domesticus* or *Mus musculus* are infected. The burden of disease is not well established, but LCMV infection is linked with up to 10% of CNS disease of suspected viral origin. Antibody studies show a prevalence of 5% in urban populations in the USA,[20] 9% in urban populations in Germany, and 2.2% in Argentina. An increase in the number of cases is reported in the autumn, which may result from the reservoir host moving into homes for the winter.

Lassa fever is a serious public health problem in West Africa, in two distinct hyperendemic areas — Sierra Leone, Guinea and Liberia and Nigeria. Identification of a recent case in Côte D'Ivoire indicates that infection may be distributed more widely in West Africa.[21] Infections are found throughout the year, but a peak incidence is seen in the dry season. Lassa fever is a more common infection than previously thought, with estimates of up to 400,000 human cases annually and an overall mortality rate of 2%.[22] More severe hospitalized cases have a mortality rate of 16%.[23]

Several hundred cases of Argentine hemorrhagic fever are identified each year, and are associated with the harvest. Most cases occur in adult males.

Bolivian hemorrhagic fever was first identified in 1963–4, when it caused an extensive outbreak in San Joaquin that resulted in 113 deaths. [24] This followed the rodent host entering the small town. However, the disease has since been uncommon, with occasional cases reported in rural communities in those more likely to come into contact with the infected rodent host.

Prevention

Two strategies have been proposed to prevent arenaviral disease in humans — rodent control and vaccination. Rodent control was important in controlling the Bolivian hemorrhagic fever outbreak, by the eradication of *Colomys callosus*. The house mouse, *Mus domesticus*, can be effectively eliminated from homes, thus reducing exposure to LCMV. However, its value in controlling other arenavirus infections has not been established. *Mastomys natalensis* is widespread and it has not proved practical to control rodent populations with living conditions found in West Africa. Both *C. callosus* and *C. musculinus* inhabit rural areas and control is not feasible.

A live, attenuated junin vaccine (Candid-1) has been developed and its efficacy demonstrated in a trial of 6500 volunteers.[25] It is now used for high-risk workers.

Lassa fever is the most important human arenavirus infection and vaccination seems to be the best hope for control. Killed and live vaccines have been developed and evaluated in animal models, and the most promising candidate vaccine is a vaccinia recombinant containing the Lassa virus glycoprotein gene. In challenge experiments this vaccine prevented death from Lassa challenge in guinea pigs and nonhuman primates. No human studies have been performed.[18]

Diagnostic microbiology

All arenavirus infections present similar diagnostic problems; the virus can be isolated early in the disease and this is followed by the later development of IgM and IgG antibody responses. There has been relatively little development of diagnostic assays for these agents because they are relatively rare and geographically localized.

Infection by LCMV is generally diagnosed by the detection of specific antibody. Usually immunofluorescence is used, but an ELISA test has been described. For virus isolation, blood and CSF samples are the most useful. Mice and guinea pigs are the most sensitive culture system, but isolation in Vero cells is more practical.

Lassa fever and several of the South American hemorrhagic fevers cause severe infections and can be transmitted by aerosol. Consequently, they have been classified as biohazard level 4 viruses and work on these agents is confined to the few high-containment laboratories worldwide.

The early clinical diagnosis of Lassa fever is difficult, and because ribavirin is an effective treatment if started early in the disease course, laboratory confirmation of infection is important. For rapid diagnosis either virus culture in Vero E6 cells or genome detection by RT-PCR are used on acute blood samples. Now RT-PCR is the first-line diagnostic test because it enables same-day diagnosis, and the use of guanadinium to extract RNA also inactivates viral infectivity, which makes the sample safe to work on outside the containment laboratory. In a recent study all Lassa fever patients were diagnosed by RT-PCR within 3 days of admission (Fig. 222.9).[26] Detection of Lassa-specific antibody by immunofluorescence is widely used, but antibody rises late in clinical disease and is often not detectable until the second week of illness, which is often too late to effect patient management. Baculovirus-expressed nucleoprotein (NP) has been used for serologic diagnosis and avoids the need to culture virus. Quantitative real-time PCR has now been applied to the diagnosis of Lassa fever, and the results obtained on sequential samples from a recent case are illustrated in (Fig 222.10). The ability to quantify and monitor viral load may have prognostic value and help to guide treatment, but substantive studies are required.

The South American arenaviruses can be cultured from acute blood samples in newborn mice and hamsters or Vero cells. The detection of virus-specific IgG and IgM

by immunofluorescence is widely used, and more recently ELISA using baculovirus-expressed viral proteins to detect antibody and RT-PCR tests have been described.

Clinical features

Arenavirus infections are often mild or subclinical. Even in severe infections, early illness is characterized by non-specific

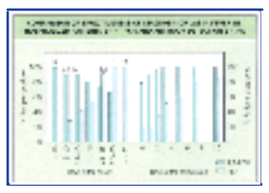

Figure 222-9 Comparison of effectiveness of diagnosis of Lassa fever in hospitalized patients by reverse transcriptase polymerase chain reaction (RT-PCR) and antibody detection by immunofluorescent antibody (IFA). (a) Samples from all patients were stratified with respect to the time since the onset of disease. The number of serum specimens in each group is indicated. (b) Samples were analyzed on a patient-by-patient basis with respect to time since admission to the hospital. *Adapted from Demby et al.* [26]

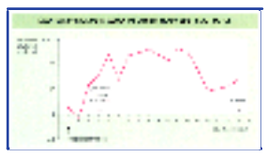

Figure 222-10 Lassa fever viral load in sequential samples from a case measured by real-time polymerase chain reaction.

symptoms, such as fever and myalgia, before the onset of severe systemic disease.

In LCMV infection headache, nausea or vomiting accompany the febrile prodrome. In the second week of illness CNS disease develops in a minority of cases and usually presents as an aseptic meningitis. In severe cases encephalitis can develop and this accounts for a significant minority of hospitalized cases. Full recovery is the most common outcome, but occasional deaths have been reported.

The incubation period of Lassa fever is about 10 days. It generally begins with a gradual onset of fever and malaise. As the disease progresses, abdominal pain, nausea and vomiting become more pronounced and an exudative pharyngitis and conjunctivitis are common. More pathognomonic signs and symptoms develop during the second week of illness, including facial edema, pleural edema, encephalopathy and shock. In severe cases illness is progressive, with shock followed by death. In survivors, defervescence is followed by recovery after 2–3 weeks. Hemorrhagic complications are not uncommon in severe cases and generally present with epistaxis, bruising and conjunctival hemorrhage. In the convalescent period pericarditis has been described. Deafness is now recognized as a late complication.[27] It can be unilateral or bilateral and is not related to the severity of disease; it may be immune mediated.

Argentine and Bolivian hemorrhagic fever present with a similar clinical picture. The progression of illness is more rapid than in Lassa fever, with severe prostration developing 3–4 days after onset. In more severe cases mucous membrane hemorrhage and bruising at intravenous injection sites are seen, which indicate a poor progress. In a few cases substantial neurologic involvement leads to convulsions. Recovery usually starts at the end of the second week of illness.

Management

Lassa fever has caused nosocomial infections in Africa and is the biohazard level 4 agent most commonly imported into nonendemic countries. Consequently, many countries have developed containment guidelines for the management of suspected Lassa fever cases to prevent secondary transmission.[28] [29] Transmission is through direct contact with infected body fluids and little evidence suggests that airborne transmission is important. The guidelines follow a similar approach based on an initial patient-risk assessment, which leads to different levels of investigation, containment and monitoring of close contacts, depending on risk (see also Chapter 183 and Chapter 186a).

Ribavirin, a guanosine analog, is effective in vitro against arenaviruses, but its mode of action is unknown. Ribavirin has been evaluated for the treatment of severe Lassa fever cases and dramatically reduces the mortality rate.[30] Its use for prophylaxis of contacts with Lassa fever cases and for the treatment of other arenavirus infections has been proposed, but no trial reports are yet published.

Treatment of Argentine hemorrhagic fever infections with convalescent human plasma is effective in reducing the mortality rate from 15–30% to <2% if given during the first 8 days of illness. Convalescent plasma could potentially play a role in Lassa fever treatment, but this has not been established in practice. Patient management of arenaviral infections requires general supportive care, with particular attention to fluid balance.

HANTAVIRIDAE

Nature

Hantavirus is the most recently described genus in the Bunyaviridae family and comprises a growing number of isolated viruses, including those that cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). [31] [32] Some recently recognized probable species that have been identified by molecular genetic analysis have not yet been isolated (Table 222.3). Hantaviruses contain a tripartite, single-stranded, negative-sense RNA genome. The three segments are designated as small (S), medium (M) and large (L), and encode for the nucleocapsid (N) protein of 50–53kDa, the two envelope proteins (G1 and G2) of 65 and 74kDa, respectively, and an associated virus of 200kDa.[33]

Epidemiology

The epidemiology of hantavirus disease in humans is dependent on the geographic distribution of persistently infected rodent hosts.[34] The hantaviruses linked with HFRS are associated with specific rodent hosts, each named for the region in which they were first isolated:

- *Apodemus agrarius* (the striped field mouse) for the hantavirus;
- *Rattus norvegicus* (the Norway rat) and *Rattus rattus* (the black rat) for the Seoul virus; and
- *Apodemus flavicollus* (the yellow neck field mouse) for the Dobrava virus.

The hantavirus from Korea, Russia and China and the Dobrava virus from the Balkan countries of Europe (Bosnia, Croatia, Yugoslavia, Albania, Greece and Bulgaria) are associated with the severe form of HFRS, with an estimated mortality rate of 5–15% (see Table 222.3). [35] A moderate form of HFRS is caused by the Seoul virus, which (along with its host) is found virtually worldwide. Serologic evidence for infection with Seoul-like hantaviruses has been found in rodents in major cities within the USA.

TABLE 222-3 -- Characteristics of some known hantaviruses found in Europe, Asia, Russia and India.

CHARACTERISTICS OF SOME KNOWN HANTAVIRUSES FOUND IN EUROPE, ASIA, RUSSIA AND INDIA					
Species	**Human illness**	**Pathology**	**Mortality rate (%)**	**Reservoir**	**Geographic virus distribution**
Hantaan (HTN)	Severe: HFRS, EHF, KHF	Renal	5–15	*Apodemus agrarius* (striped field mouse)	China, Russia, Korea
Dobrava/Belgrade (DOB)	Severe: HFRS	Renal	5–15	*Apodemus flavicollis* (yellow neck mouse)	Balkans
Seoul (SEO)	Moderate: HFRS	Renal	1	*Rattus norvegicus* (Norway rat)	Worldwide

Puumala (PUU)	Mild; NE	Renal	1	*Clethrionomys glareolus* (bank vole)	Europe, Russia, Scandinavia
Khabarovsk (KHB)	?	?	?	*Microtus fortis* (reed mole)	Russia
Tula (TUL)	?	?	?	*Microtus arvalis* (European common vole)	Europe
Thailand (THAI)	?	?	?	*Bandicot indica* (bandicoot rat)	Thailand
Thottapalayan (TMP)	?	?	?	*Suncus murinus* (musk shrew)	India
Topografov (TOP)*	?	?	?	*Lemmus sibircus* (Siberian lemming)	Siberia
? Not yet documented					
EHF, epidemic hemorrhagic fever; HFRS, hemorrhagic fever with renal syndrome; KHF, Korean hemorrhagic fever; NE, nephropathia epidemica.					

* Virus not yet isolated/molecular analysis suggests they are probable hantavirus species

A mild form of HFRS, caused by the Puumala virus, is responsible for nephropathia epidemica (NE) in Scandinavia, the European regions of the former USSR and south of this through much of Europe to France, Spain and Portugal.

The hantavirus associated with HPS, Sin Nombre virus (SNV), has been recognized throughout the USA since its discovery in 1993.[36] Molecular biologic studies have identified similar viruses — Black Creek Canal (BCC), New York, Bayou (BAY) and Andes — that are also associated with HPS illness, that extend their geographic distribution from North America to Mexico and South America (Table 222.4). The distribution of specific host species throughout the Americas suggests a wider distribution of hantaviruses, which potentially could cause further clinical cases.

All rodent hosts are persistently infected with specific hantavirus strains that apparently cause no illness. In wild rodent populations, most infections occur through age-dependent horizontal routes, predominately in mature males. Horizontal transmission among cage mates was experimentally demonstrated via the aerosol route at median infectious doses of 1.0 plague forming units (pfu) for each virus tested. However, although rodents are susceptible to hantavirus through the aerosol route, their susceptibility is increased by inoculation (median infectious doses 0.003–0.016pfu). Vertical transmission from dam to pup has proved negligible or absent, both in wild and experimental settings.

Hantavirus disease, irrespective of strain, is a seasonal disease. This seasonality is associated with population dynamics of the rodent hosts that maintain each virus and their interaction with local human populations. Rodent population cycles vary according to climatic changes and interspecies competition. Rural-associated HFRS in Asia and Europe is linked to human rodent contact during the planting and harvesting of crops (late autumn and early winter). Similarly, in Scandinavia Puumala virus causes most cases of NE during the same period. Both Puumala outbreaks in Scandinavia and the original HPS outbreak in America were associated with increases in the natural rodent population followed by rodent migration from the fields to buildings in response to adverse weather conditions. Alternatively, in Greece, HFRS is observed predominately in the warmer months as a result of increased human outdoor activities, including camping. These cases point to higher risk being experienced by people such as forestry workers and shepherds. In China HFRS induced by Seoul virus appears to be most common in the spring.

The aerosol route of infection is regarded as the most common means of transmission among rodents and to humans. Rodent bites cannot be excluded as a way of maintaining the disease among rodents or occasionally resulting in human infection. Epidemiologic investigations link virus exposure to farming activities, sleeping on the ground and military exercises. Indoor exposure has been linked to rodent infestation of homes during cold weather or to nesting rodents in or near dwellings. Many hantavirus infections are identified among people who live in poor housing conditions or in those who pursue recreational activities such as camping and various water sports. Human-to-human transmission is rare and consequently hantaviruses do not pose a risk to hospital workers. However, recent outbreaks of HPS in South America suggested that minimal exposure to body fluids of infected patients resulted in apparent person-to-person spread.

The annual incidence of HFRS involving hospitalization throughout the world is estimated to be between 150,000 and 200,000 cases. More than half these cases predominate in the endemic center of epidemic hemorrhagic fever in the Chinese provinces of Hubei, Heilongjiang, Jiangxi, Jilin and Shanxi. Annual incidence rates here range from 0.05 to 3.0/1000. Russia and Korea also report hundreds to thousands of cases of HFRS each year. Most of the remaining cases (a few hundred) are reported from Japan, Finland, Sweden, Belgium, The Netherlands, Greece, Hungary, France and the Balkan countries formally constituting Yugoslavia. Mortality rates range from less than 0.1% for HFRS caused by Puumala to approximately 5–10% for HFRS caused by hantavirus.

Several hundred cases of HPS have been reported throughout North and South America (see Table 222.4). [37] Most of the identified cases in America and Canada were caused by SNV. Cases of HPS in south-western USA and South America are caused by molecularly identified strains that include BAY, BCC and Andes viruses.

A number of hantavirus infections have caused several laboratory-associated outbreaks of HFRS, all of which were traced to persistently infected rodents obtained from breeders, to wild-caught naturally infected rodents or to experimentally infected rodents. No illness has been associated with laboratory workers using cell-culture adapted viruses, although asymptomatic seroconversions have been documented.

Pathogenesis

The process by which hantaviruses cause multisystem organ dysfunction syndrome is unclear. Thrombocytopenia, defects in platelet function, transient disseminated intravascular coagulation (DIC) and increased vascular fragility are all thought to play a key role in the

TABLE 222-4 -- Some known hantaviruses found in North and South America.

SOME KNOWN HANTAVIRUSES FOUND IN NORTH AND SOUTH AMERICA					
Species	**Human illness**	**Pathology**	**Mortality rate (%)**	**Reservoir**	**Geographic virus distribution**
Sin Nombre virus (SNV)	Severe: HPS	Pulmonary	50	*Peromyscus maniculatus* (deer mouse)	USA, Canada Mexico
New York (NY)	HPS	Pulmonary	Not recorded	*Peromyscus leucopus* (white-footed mouse)	USA
Black Creek Canal (BCC)	HPS	Pulmonary	Not recorded	*Sigmodon hispidus* (cotton rat)	USA
El Monro Canyon (ELMC)	?	?	?	*Rethrodontomys megalotis* (Western harvest mouse)	USA, Mexico
Bayou (BAY)*	HPS	Pulmonary/renal	?	*Oryzomys palustris* (rice rat)	USA
Andes (AND)*	HPS	Pulmonary	50	*Oligoryzomys longicaudatus* (long tailed pygmy rice rat)	Argentina
Unnamed*	HPS	Pulmonary	?	*Colomys laucha* (vesper mouse)	Paraguay
Rio Segundo (RIOS)*	?	?	?	*Reithrononтomys mexicanus* (Mexican harvest mouse)	Costa Rica
Rio Manore (RIOM)*	?	?	?	*Oligoryzomys mictrotis* (small-eared pygmy rice rat)	Bolivia
Iala Vista (ISLA)*	?	?	?	*Microtis californicus* (Celifornia vole)	USA
Bloodland Lake (BLL)*	?	?	?	*Microtis ochrogaster* (prairie vole)	USA
? Not yet documented					

HPS, hantavirus pulmonary syndrome.

* Virus not yet isolated/molecular analysis suggests they are unique hantavirus strains

early stages of the disease. Hantavirus disease is mainly microvascular in nature and endothelium is the predominant cell type involved. The presence of hantavirus antigen has been demonstrated by immunochemistry in vascular endothelial cells of fatal HFRS infections, experimentally infected rodents and endothelium of lung capillaries in the highly lethal form of HPS.[38] Endothelial damage or dysfunction leads to capillary engorgement, leakage of erythrocytes and increased permeability.

Recent studies implicated the disease process as being immunologically based, with lymphocytes playing a key role, especially T cells. The CD4:CD8 ratio is greatly decreased, although abundant $CD4^+$ and $CD8^+$ lymphocytes have been reported in the lung interstitium of HPS patients.[38] Lymphocyte induction of migrating macrophages and other inflammatory cells results in the production of cytokines, such as tumor necrosis factor (TNF)-a, interleukin (IL)-1 and IL-2, and interferon (IFN)-?, which in turn increases vascular permeability.

Prevention

Prevention of hantavirus infection is dependent upon reducing contact between humans and infected rodents. However, many rodent control programs in known highly endemic areas, such as China, have been ineffective. Guidelines in the USA to control HPS infections have been aimed at reducing rodent access to homes, clean up recommendations of buildings with heavy rodent infestations, and precautions for workers exposed to rodents and for campers and hikers in infected areas.

Although a number of candidate (inactivated, recombinant) vaccines against hantaviruses are under development, none are currently available.

Both wild-caught rodents introduced into laboratories and laboratory-bred colonies have caused a number of laboratory-based infections. Current recommendations directed at the manipulation of hantaviruses call for handling at biosafety containment levels 2 and 3, dependent on the strain. Work with infected laboratory rodent colonies that may lead to the generation of infectious aerosols in urine or feces should be undertaken in facilities that have suitable room ventilation and high-efficiency particulate air (HEPA) filtration, primary containment of animals, and personal protective equipment at a minimum of biosafety containment level 3. Precautions should also ensure suitable decontamination of animal bedding, cages and animal waste.

It is prudent to introduce hantavirus screening of laboratory-bred and wild-caught rodents and cell lines that originate from rodent tissue before use. Such animals and cells should be segregated from other animals, with care being taken to prevent possible aerosolized spread of infectious virus.

Diagnostic microbiology

Diagnosis of hantavirus infection relies upon the recognition of characteristic clinical features, a history consistent with the epidemiology of the disease and serologic confirmation.[37]

Direct isolation of hantavirus from acutely ill patients is prolonged and often unsuccessful and consequently of little diagnostic value. Similarly, the success of antigen-capture ELISA or the application of RT-PCR on acute blood samples is of no practical value for the diagnosis of human infection.

Although the immunofluorescence assay is regarded as a useful diagnostic tool, the method of choice to diagnose acute hantavirus infection is an IgM antibody assay of high sensitivity and specificity.[39] Currently two alternatives are available:

- M antibody capture IgM enzyme immunoassay (EIA) based on the use of cell-culture grown hantavirus antigen; and
- M antibody capture IgM EIA based on baculovirus or *Escherichia coli* expressed full-length nucleocapsid protein.[40]

Both in humans and rodents IgG antibodies persist for life, and therefore the presence of IgG seropositivity may not reflect a recent infection. In addition, a rise in IgG has not always been observed in recent clinical cases. The IgG ELISA or immunofluorescent antibody (IFA) test is well suited for epidemiologic studies, whereas determination of the specific infecting virus is best achieved by focus reduction neutralization tests.

Other methods are also under development to investigate hantavirus:

- hantavirus recombinant immunoblot assay (RIBA), which uses recombinant and synthetic peptides; and
- application of RT-PCR and immunohistochemical staining of antigen contained in formalin-fixed autopsy tissues.[41]

TABLE 222-5 -- Main characteristics of the two severe forms of hantavirus disease.

MAIN CHARACTERISTICS OF THE TWO SEVERE FORMS OF HANTAVIRUS DISEASE		
Characteristics	**Hemorrhagic fever with renal syndrome (HFRS)**	**Hantavirus pulmonary syndrome (HPS)**
Primary target organ	Kidney	Lung
Acute phase	Febrile	Febrile 'prodrome'
Later phases	Shock, hemorrhage	Shock, pulmonary edema
Disease progression	Hypotensive, oliguric, diuresis, convalescence	Diuresis, convalescence
Other clinical and laboratory features	Thrombocytopenia, leukocytosis, proteinuria, hematuria, creatinine >100mmol/l, hemoconcentration, raised transaminases	Thrombocytopenia, leukocytosis, hemoconcentration, shortness of breath, abnormal respiratory rate, lung infiltrates
Mortality rate (%)	1–15	=50

Clinical manifestations

Although HFRS has been clinically recognized and reported in Asia, Russia and Scandinavia for the past century, the causative agent, hantavirus, was not isolated until 1978.[42] The HFRS complex comprises three distant clinical diseases, each infection being caused by a specific hantavirus. [34] [36] The spectrum of disease ranges from non-apparent infection to fulminant hemorrhagic fever with renal failure (sometimes having a fatal outcome). Depending on which hantavirus is responsible for the illness, HFRS can appear as a mild, moderate or severe disease. Fatality rates range from less than 1% for HRFS caused by Puumula virus to approximately 5–10% for HFRS caused by hantavirus (Table 222.5).

The clinical course of pathogenic hantavirus infection comprises an acute febrile illness characterized by variable degrees of hemorrhagic and renal dysfunction. More severe disease involves five overlapping stages — febrile, hypotensive, oliguric, diuretic and convalescent.

The onset of the disease is abrupt and characterized by high fever of 102.2–104°F (39–40°C), chills, intensive headache, malaise, myalgia, dizziness and anorexia. A petechial rash may also appear on the face, neck and trunk. The main laboratory findings include normal-to-elevated white blood count (WBC), decreasing platelets, rising hematocrit and rising proteinuria.

As the febrile phase ends, hypotension can abruptly develop and last for a few hours or days. Prominent features are tachycardia, falling arterial pressure and narrowing pulse pressure, and in severe cases classic shock. Laboratory findings include leukocytosis with a left shift, thrombocytopenia and prolonged bleeding times. Urinalysis shows heavy proteinuria, mild hematuria and hyposthenuria. About one-third of fatalities occur during this phase.

The oliguric phase lasts 3–7 days, during which blood pressure returns to normal or is slightly raised because of hypervolemia. Laboratory findings include normal WBC and platelet counts, initially normal and then depressed hematocrit, continual marked hematuria and elevated blood urea nitrogen and creatinine. Hyponatremia, hyperkalemia and hypocalcemia result from renal failure. Pulmonary edema may occur and fluid management should be handled with care. Almost half of the deaths occur during this phase, often from pulmonary edema or infection, electrolyte imbalance, late shock, or hemorrhage into the brain.

Clinical recovery is signaled by the diuretic phase, with improved renal function and normal clotting. Diuresis over a few hours or days is evident, with an output of 3–6 liters. Fluid management must be maintained to prevent negative fluid balance that may lead to shock. The final (convalescent) phase can last weeks to months before recovery is complete.

Seoul virus infections (mild HFRS) are less severe than those caused by hantavirus. Typically the phases are shorter and difficult to recognize or even absent. The disease is characterized by fever, anorexia, chills and nausea, and vomiting. Palatal injection is common, although other hemorrhagic signs (epistaxis, melena, hematemesis) are observed in fewer than 30% of patients. Laboratory findings include lymphocytosis, thrombocytopenia, microscopic hematuria and proteinuria, and elevation of serum transaminase. Renal involvement is less severe and fatalities are uncommon.

Puumula virus infection (NE) is characterized by a sudden onset of fever accompanied by headache. Characteristically, by the fourth day of illness nausea, vomiting, petechiae in the throat and soft palate, and facial flush and petechial rash have occurred. A mild thrombocytopenia can be observed. Hypotension may be found, but evidence of shock is rare. Onset of oliguria or recognition of renal failure around the sixth day of disease is the main cause of hospital admission. Serum creatinine levels rise and dialysis may be required in 10% of cases. As with other hantavirus infections, the onset of polyuria indicates the recovery process.

First recognized as a severe, often fatal respiratory illness in adults, HPS was characterized as a severe, systemic illness with a non-specific onset, fever, myalgia, cough or dyspnea, gastrointestinal symptoms and headache that typically lasted 3–5 days.[43] Rapid and abrupt onset of noncardiogenic edema, hypotension (systolic blood pressure "80mmHg) then follow, resulting in shock and death in over half of the recognized cases (see Table 222.5). Laboratory findings indicate leukocytosis, an increase in polymorphonuclear leukocytes, left shift, increased hematocrit, thrombocytopenia, prolonged prothrombin and partial thromboplastin time, elevated serum lactate dehydrogenase, decreased serum protein concentrations and proteinuria. Increases in hematocrit and thromboplastin time are considered to be predictors of death. Radiographic examinations show bilateral pulmonary infiltrates and evidence of pleural effusions in the majority of hospitalized patients. Histopathologic features seen in lung tissue reveal mild-to-moderate interstitial pneumonitis with variable degrees of congestion, edema and mononuclear infiltrates. The virus has been identified extensively within endothelial cells of the pulmonary microvasculature, spleen and lymph nodes.

Current understanding of the disease indicates little evidence of renal damage as a feature of this hantavirus infection. The extensive pulmonary endothelial involvement and severe pulmonary edema make clinical management difficult.

Management

Effective management of HFRS and HPS requires early diagnosis, knowledge of disease course and prompt hospitalization. Aggressive clinical management is essential to improve survival. Care should be phase specific, with special attention given to fluid management. Ribavirin can be efficacious in the treatment of HFRS patients, but its treatment of HPS is not proved.

FILOVIRIDAE

Nature

The family Filoviridae consists of two distinctive species, Marburg and Ebola, which cause severe and often fatal hemorrhagic disease in humans and monkeys (see Chapter 183). The viruses have a distinctive filamentous morphology under the electron microscope with a genome that consists of a nonsegmented, negative stranded RNA

Figure 222-11 Filoviruses. (a) Filovirus particle in cross-section (not to scale) showing glycoprotein surface projections, nucleocapsid core inside the envelope. (b) Negative contrast micrograph of Ebola (Reston). (c) Intracellular filamentous particles showing nucleocapsid core. (d) Sections of Ebola (Republic of Congo, formerly Zaire) infected Vero cells, showing extracellular virus, budding particles. *Courtesy of G Lloyd, B Dowsett and ASR Featherstone.*

approximately 19kb in length (Fig. 222.11). Several features of their molecular organization and structure have linked these viruses to members of the Paramyxoviridae and Rhabdoviridae under the taxonomic order Mononegavirales.[41] The virions are composed of a central core formed by an RNP complex, which is surrounded by a lipid envelope derived from the host cell plasma membrane. The RNP is composed of a genomic RNA molecule bound by the NP, virion structural protein 30 (VP30), VP35 and the L protein (RNA transcriptase polymerase). The three remaining structural proteins are membrane-associated — GP, VP24 and VP40 are located on the inner side of the membrane. Among the newly isolated Ebola viruses are differences in molecular structure, pathogenicity and virulence in humans.[44]

Marburg and Ebola were first detected in 1967[45] and 1976,[46] [47] respectively, although human infections since then have been rare. Fresh outbreaks of Ebola infections were identified in Côte d'Ivoire (1994, 1995), Republic of Congo, (formerly Zaire; 1995),[48] and Gabon (1996, 1997, 2001) and Uganda (2000). In addition, importation of Ebola-infected monkeys into the USA (1989–1990, 1996) and Italy (1992) attracted extensive worldwide media coverage and raised public concerns about the potential public health threat of these pathogens through international travel and commerce.[49] [50]

Pathogenesis

The precise mechanisms by which filoviruses cause the most severe forms of VHF are unclear, but there is marked hepatic involvement, DIC and shock, producing extremely high fatality rates (30–90%). In the early stages of infection laboratory findings, such as high aspartate transaminase/alanine transaminase (AST/ALT) ratios, and marked lymphopenia followed by a marked neutropenia with left shift, suggest other extrahepatic targets affected by the virus. As with other VHF infections (HFRS, Lassa, dengue) fluid imbalance and platelet abnormalities indicate endothelial cell and platelet damage

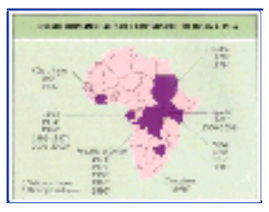

Figure 222-12 Distribution and dates of filovirus outbreaks in Africa.

or dysfunction. Human monocytes and/or macrophages, fibroblasts and endothelial cells support virus replication. Infected monocyte and/or macrophages have been shown in in-vitro models to secret the cytokine TNF-a, which has the ability to increase vascular endothelial cell damage.[51] The data currently available support mediator-induced vascular damage that leads to increased permeability and shock observed in severe cases. Hemorrhage is likely to be caused by reticuloendothelial system damage that cannot be repaired because of platelet and coagulation malfunctions.

Epidemiology

The natural history and reservoirs of filoviruses remain a mystery, as does a detailed understanding of their pathogenesis. In 1967, a fulminating hemorrhagic fever,

Marburg disease, occurred in Marburg and Frankfurt, Germany, and Bosnia, Croatia and Yugoslavia among laboratory workers handling blood and tissue from a shipment of African Green Monkeys (*Cercopithecus aethiops*) soon after being imported from Uganda via London. Among the 31 human cases, 25 of which were primary infections, there were seven (23%) deaths.[45] None of the secondary cases died. Since then, sporadic cases have been reported from South Africa and Kenya (Fig. 222.12).

In 1976, the first known cases of Ebola hemorrhagic fever were identified in southern Sudan (Table 222.6) and in a simultaneous outbreak in northern Republic of Congo (formerly Zaire), with fatality rates of about 60 and 90%, respectively.[46] [47] A further smaller outbreak occurred in the same region of Sudan in 1979, when 22 (65%) of 34 infections proved fatal. Lack of community co-operation hampered control efforts and investigations during an outbreak of Ebola in the Republic of Congo during 2002. Recent instances of Ebola infection were the severe illness of a Swiss zoologist working with infected chimpanzees (*Pan troglodytes verus*) in the Taï National Park in Côte d'Ivoire, West Africa, in late 1994 and a major epidemic in Kikwit, Bandundu Province, Republic of Congo (formerly Zaire), in 1995. Of 315 recorded cases of Ebola hemorrhagic fever a total of 244 died (77% mortality rate).[51] In the later part of 1995 further cases were identified in the Côte d'Ivoire among refugees who originated in Liberia. Finally, three reportedly independent human outbreaks of Ebola have occurred in the forested areas found in northeastern Gabon (1994, February 1996, July 1996, December 2001). Subsequent investigations of the 2001–2 outbreak suggested that cases in villages north of Mekambo resulted from contact with a gorilla whose remains were found to be positive for Ebola virus. A severe outbreak occurred between August

TABLE 222-6 -- **Characteristics of some know filovirus infections in Africa.**

CHARACTERISTICS OF SOME KNOWN FILOVIRUS INFECTIONS IN AFRICA			
Date	**Filovirus Infections**	**Documented fatalities/total cases**	**Main characteristics**
		Marburg	
1967	Marburg, Germany	5/23	Infected through contact with African green monkeys imported from Uganda
1967	Frankfurt, Germany	2/6	Infected through contact with African green monkeys imported from Uganda
1967	Belgrade, Yugoslavia	0/2	Infected through contact with African green monkeys imported from Uganda
1975	Zimbabwe	1/3	Contracted by travelers entering region — source unknown
1980	Mount Elgon, Kenya	1/2	Contracted by travelers entering region — source unknown
1987	Mount Elgon, Kenya	1/1	Contracted by travelers entering region — source unknown
1999	D.R. Congo	52/76 (estimates)	Gold miners near Durba
Total mortality rate		**62/113 (54.5%)**	
		Ebola (Africa)	
1976	Yambuku, D.R. Congo	280/318	Virus when introduced into hospital amplified disease
1976	Maridi, Sudan	151/284	Virus when introduced into hospital amplified disease
1976	England	0/1	Laboratory acquired
1977–8	Tandala, D.R. Congo	1/1	Child infected
1979	Nzara & Yambio, Sudan	22/34	Amplified by nosocomal transmission in hospital
1994	Taï Forest, Côte d'Ivoire	0/1	Worker infected after autopsy of chimpanzee-repatriated to Switzerland
1994	Gabon	32/44	Origin gold mining camps at Mekokou and Andcock. Primarily reported as yellow fever
1995	Kikwit, D.R. Congo	244/315	Included laboratory confirmed cases. Rapid transmission among unprotected health care workers
1995	Taï Forest, Côte d'Ivoire	0/1	Case originated in Liberia ? four additional cases in Liberia
1996 (January–March)	Mayibout, Gabon	21/37	Contact with dead chimpanzees
1996 July & January 1997	Booué & Libreville, Gabon	45/61	Index case hunter living in forest area — infected chimpanzees identified
1996	South Africa	1/1	Epidemiologically linked with outbreak in Gabon. Fatal case — nosocomial infection of nurse by patient transferred from Gabon
2000–1	Uganda	225/425	Occurred in Gulu, Misindi & Mbarara districts of Uganda. Spread associated with attending funerals, community nursing and medical care without personal protective measures
2001–2	Gabon	53/65	Community outbreak evidence suggest contact with dead nonhuman primates (gorilla)
2001–2	Republic of Congo		Outbreak occurred over the border of Gabon and the Republic of the Congo
	Mboma district	20/32	
	Kelle district	23/25	
Total mortality rate		**1118/1645 (68%)**	

TABLE 222-7 -- Recorded filovirus (Ebola (Reston)) infections.

RECORDED FILOVIRUS (EBOLA (RESTON)) INFECTIONS			
Date	**Filovirus infections**	**Documented fatalities/total cases**	**Main characteristics (all isolates originated from same compound)**
1989	Richmond, Virginia, USA	0/4	Reston strain isolated from monkeys imported from Philippines. Serologic evidence of asymptomatic infection among animal workers
1990	Manila, Philippines	0/12	Serologic evidence of asymptomatic infection among animal workers
1992	Siena, Italy	No cases	Reston strain isolated from monkeys imported from Philippines
1996 (April)	Alice Texas, USA	No cases	Reston strain in monkeys imported from Philippines

Total mortality rate	**0/16 (0%)**	

2000 and January 2001 in Uganda (Gulu and Masindi districts) that recorded 423 cases with 225 fatalities.[52] A case from the 1996 infection was treated in South Africa and the source of a fatal nosocomial transmission to a health care worker. All three outbreaks were associated with the deaths of chimpanzees, also thought to be from Ebola virus infection.

Hence, since 1994 West Africa has been considered to be part of the African filovirus map (see Fig. 222.12). Although filovirus outbreaks are rare events, the public health importance lies in their high mortality rate rather than the total number of infections in the 30 years since they were first identified (Table 222.6 and Table 222.7).

Ebola virus has also appeared outside Africa, among shipments of imported cynomolgus monkeys in Reston, Philadelphia and Texas, USA, in 1989, 1990 and 1996, respectively, and Sienna, Italy, in 1992.[49] The monkeys involved in each epizootic event were imported from the Philippines and traced to the same handling facility, where the presence of the virus was also documented. The epizootics indicate that monkeys acutely infected with filoviruses present a major veterinary emergency and possibly could pose a serious hazard to other animals or humans if not recognized in the future. Although an Asian origin of these virus strains cannot be discounted, molecular biologic and serologic studies carried out to date suggest a close similarity with the original Ebola viruses isolated from the 1976 African outbreaks.

Prevention

The imposition of ecologic controls to prevent outbreaks is impossible until the natural history of the viruses has been discovered. It is important that health care workers in areas where hemorrhagic fever exists are aware of the high risk of nosocomial spread if patients are not recognized early and placed in complete isolation.[53] In non-endemic areas an awareness of the changing epidemiology associated with viral re-emergence or emergence should always be considered, bearing in mind the threat and consequences of importation. [54] High-risk patients are those who during the 3 weeks before illness had:

- traveled into areas where VHF occurred recently;
- been in contact with body fluids from a person with VHF; or
- worked in a laboratory environment handling the virus.

(See also Chapter 183 and Chapter 186a). Importantly, filovirus illness is a rare event if patients do not meet any of these criteria.

As nonhuman primates are responsible for the introduction of Marburg into Europe, and of Ebola into the USA and Italy, the management of transportation, quarantine facilities and animal husbandry must ensure that personnel understand the hazards of handling nonhuman primates, and thus reduce the risks of future human outbreaks.[55]

Clinical manifestations

The incubation period for Ebola ranges from 4 to 10 days and for Marburg from 3 to 9 days.[45] The shorter times have been associated with exposure to contaminated needles.[56]

Filoviruses enter the host through close contact with contaminated body fluids. Epidemiologic studies in humans indicate that infection is not readily transmitted by the aerosol route. Although studies in nonhuman primates have established that the aerosol transmission can cause infection,[57] extra care must be taken with contaminated body fluid, tissue, hospital material and waste.

Both Marburg and Ebola virus infections follow a similar pattern of illness. Following exposure, initial symptoms include an abrupt onset with fever, severe frontal headache, malaise and myalgia. Such symptoms are similar to those of many viral illnesses that originate locally and in tropical areas. The initial diagnosis of a filovirus infection is very difficult. Disease progression is characterized by pharyngitis, nausea, severe vomiting of blood and production of bloody stools. Uncontrolled bleeding follows, which can be seen from under the skin, from venepuncture sites and from other orifices of the body. A characteristic maculopapular rash occurs 7–10 days (range 1–21 days) after onset of the clinical disease. If fatal, death occurs 6–9 days after onset of the clinical disease and results from severe blood loss and shock. Convalescence is slow, taking many weeks, and is marked by weight loss, prostration and amnesia of the acute phase of illness. [58]

Clinical laboratory findings in the early acute phase include lymphopenia followed by neutrophilia, marked thrombocytopenia and abnormal platelet aggregation. Serum AST and ALT are elevated and characterized by an AST/ALT ratio of between 10 and 3:1.

Diagnostic microbiology

The clinical diagnosis of Ebola or Marburg should be considered in patients who show acute, febrile illness and have traveled in known epidemic or suspected endemic areas of rural sub-Saharan Africa and Asia, particularly when hemorrhagic signs are present. As the differential diagnosis in the early acute phase of illness is difficult, other causes (malaria, typhoid) should not be excluded and treatment delayed. Laboratory diagnosis carried out on patient specimens is undertaken by more than one procedure to guard against false positives.

Care should be taken when drawing or handling blood specimens at the acute stage of illness because they contain large amounts of infectious virus — the virus is stable for long periods at room temperature at this stage.

Although their morphologic appearance is similar by electron microscopy, Marburg and Ebola are immunologically distinct. The basic diagnostic tool for filovirus infection and the only one to date that has widespread acceptance for the diagnosis of human filovirus disease is IFA, although some epidemiologic serology based on this assay has been regarded as non-specific and unreliable. A rising antibody in paired serum or a high IgG titer (>64) and the presence of IgM antibody together with clinical symptoms compatible with hemorrhagic fever are consistent with a diagnosis of filovirus infection.

Filoviruses can be readily isolated from fresh or stored (-94°F) specimens of blood or serum collected during the acute phase of illness. Vero cells (clone E6) and MA104 have proved the most sensitive for the propagation and assay of Marburg and Ebola viruses. Primary isolation using tissue culture rarely produces a specific cytopathic effect; thus, evidence of infection is confirmed by immunofluorescence staining using antivirus specific monoclonal or polyclonal antibodies. Some strains of Ebola, such as Ebola Sudan, proved difficult to grow in tissue culture and success was improved through interperitoneal inoculation of young guinea pigs. The resultant febrile response coincides with high levels of virus in the blood, which can be easily recovered by tissue culture or examined directly by electron microscopy. The isolation or propagation of filoviruses should not be attempted outside a biosafety containment level 4 laboratory.

During recent epizootic and human African outbreaks, early detection of filovirus infections was considerably improved by the development of an antigen-capture ELISA, amplification of filovirus RNA by RT-PCR, and immunohistochemistry identification of Ebola in skin biopsies. Skin biopsies fixed in formalin can be safely transported to reference facilities for confirmation without the need for low temperature preservation. Recent advances in molecular biology have expressed the NP gene of Ebola virus in a baculovirus expression system. The noninfectious N protein can replace the need for inactivation of infectious virus used in many serologic assays (IFA and ELISA). The diagnostic capabilities of such a system are currently being evaluated.

Management

No specific treatment is available. Treatment is limited to the provision of intensive nursing and effective control of blood volume and electrolyte balance. Shock, renal failure, depletion of blood clotting factors, severe bleeding and oxygen depletion must be managed. Human interferon and human convalescent plasma have been used

to treat patients, but their efficacy is not proved. Ribavirin has no therapeutic value.

Patient management is further complicated by the need to isolate the patient and protect medical and nursing staff.[50] [53] Use of patient isolators is a requirement in many countries, whereas strict barrier nursing techniques are acceptable in others. The latter can be supplemented using high efficiency particulate air filter respirators for protection against aerosols if considered feasible. Particular emphasis should be placed on ensuring that high-risk procedures (such as handling blood, secretions, catheters and introducing intravenous lines) are undertaken under barrier nursing conditions. It is recommended that patients who die from the disease should be buried or cremated promptly.

NIPAH AND HENDRA VIRUSES

Epidemiology

Both Hendra virus (HeV; formerly called equine morbillivirus) and Nipah virus (NiV) are newly recognized hazard group 4 zoonotic viruses.

Two outbreaks of a new zoonotic disease affecting horses and animals in Australia were reported within 1 month of each other in Brisbane (southeast Queensland) and Mackay (central Queensland) in 1994. A third event involving a single fatal equine case occurred near Cairns (North Queensland) in 1999. To date, two humans and 16 horses have died from this disease with acute respiratory failure.[59] [60] [61] Within the subfamily Paramyxovirinae, the extent of nucleotide homology in the N gene between different viruses in the same genus ranges from 56 to 78%, whereas the extent of nucleotide similarity between viruses from different genera is 39–78%. The N genes of HeV and NiV have 78% nucleotide homology, but the two viruses have no more than 49% similarity with any other members of the Paramyxovirinae.[66] Phylogenetic analysis of the N gene sequences show that HeV and NiV form a distinct cluster within the subfamily Paramyxovirinae. The cause of the infection has been identified as HeV, a new member of the family Paramyxoviridae.[62]

Another member of the Paramyxoviridae (NiV) was responsible for an outbreak of severe febrile encephalitis in humans in Malaysia in 1998–9. The outbreak was associated with respiratory illness in pigs and was initially considered to be Japanese encephalitis. Of a total of 256 cases 105 were fatal. In March 1999, a cluster of 11 cases (one fatality) was described in Singapore in abattoir workers who handled pigs imported from the outbreaks regions in Malaysia.[63] Measures to control the concurrent outbreak of respiratory disease in pigs resulted in the culling of over one million pigs (almost half the national pig herd) and had major domestic and international trade implications.

The geographic distribution of both HeV and NiV is currently undefined although Australia and South East Asia are considered to be endemic areas on the basis of the known incidents reported in the literature. Fruit bats of the genus *Pteropus* are considered to be the natural hosts of HeV and NiV[64] and are found in north, east and south-east areas of Australia, Indonesia, Malaysia, Philippines and some of the Pacific Islands. Pigs were the apparent source of infection among most human cases in the Malaysian outbreak of NiV, but other sources, such as infected dogs and cats, cannot be excluded.

Virology

Nipah and Hendra are closely lelated and difficult to distinguish serologically, although they have a diverse geographical distribution.[61] [65] Both NiV and HeV share a nonsegmented, negative-stranded RNA genome and similar genome organization, replication strategy and domain structure in the polymerase proteins to members of the Filoviridae, Paramyxoviridae, Rhabdoviridae and Bornaviridae. While being related to these families they are closely related to the Paramyxoviridae and more specifically the morbilliviruses. The genome organization of NiV and HeV is the same as for the rest of the Paramyxoviridae. There are a total of six transcription units encoding six structural proteins. These are the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) or attachment protein, and large protein (L) or RNA polymerase. They are found on the genome in the order 3'-N-P-M-F-G-L-5'. Evidence suggests that NiV and HeV forms a distinct group of viruses within the subfamily Paramyxovirinae.[65]

Clinical features

The emergence of NiV and HeV has raised clinical concerns in the management of individuals returning from Australia and South East Asia presenting with pyrexia and other non-specific signs and symptoms. The incubation period for NiV and HeV is generally from 4 to 18 days. Onset of disease is usually influenza-like with high fever and myalgia. Sore throat, dizziness, and drowsiness and disorientation have been described. The case fatality rate for clinical cases is about 50% and subclinical infections may be common.

Experimental studies have confirmed the possibility of transmission through close contact with infected body fluids and that aerosol transmission does not seem to be significant. Human-to-human transmission has not been reported and the risk of transmission from horse to human, neither of which are considered natural hosts for the viruses, is thought to be very low. The mode of transmission from animal to animal, and from animal to human is uncertain.

Laboratory diagnosis

Virus isolation has been an important primary diagnostic tool in early outbreaks.

Both NiV and HeV grow well in Vero cells. A cytopathic effect (CPE) usually develops within 3 days but two 5-day passages are recommended before virus isolation is considered to be unsuccessful. However, isolation should only be attempted in a containment level 4 laboratory.

Virus neutralization tests are considered to be the reference standard in the serologic identification of NiV and HeV infection. This procedure needs to be carried out at a containment level 4 laboratory. Sera are added into the media covering virus-inoculated Vero monolayers in a 96-well plate format. Positive results are demonstrated by the inhibition of CPE production by sera.[66]

To reduce the dependence on high containment facilities (CL4), both the indirect IgG and capture IgM ELISA NiV and HeV systems can use irradiated viral antigens purified from cell culture. The level of sample processing involved to make the antigen safe to use outside Advisory Committee on Dangerous Pathogens (ACDP) 4 containment reduces the specificity and sensitivity of the assay.

NiV-specific primers that amplify a 228bp segment of the N gene region of the virus will form the basis of the RT-PCR detection system that has proven of diagnostic value when used in conjunction with specimens (sera, tissue from humans and animals) implicated in HeV and NiV outbreaks.[67] In addition, another published primer pair that amplifies a 200bp region of the matrix (M) protein will form a proven alternative primer set for the RT-PCR detection of HeV originally designed by Halpin *et al.*[64]

Management

As there are neither vaccine nor antivirals for treatment and because the viruses are potentially able to transmit by the aerosol route, both HeV and NiV have been classified as hazard group 4 agents. Working with these agents requires the use of ACDP containment level 4 laboratories. Therefore, with the increasing movement of horses and travelers to endemic areas, both clinicians and veterinarians have increasingly expressed a need to consider these infections as part of the current differential diagnosis.

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Chapter 223 - Prions

Dominique Dormont

Transmissible spongiform encephalopathies (TSEs) are a group of human and animal diseases. In humans, TSEs include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), kuru and fatal familial insomnia (FFI). In animals, scrapie in sheep and goats, feline spongiform encephalopathy, mink transmissible encephalopathy, chronic wasting disease in wild ruminants, and bovine spongiform encephalopathy (BSE), which appeared in the UK in the mid-1980s, [1] belong to the TSE group. These diseases are all transmissible among individuals of the same species and in some cases they may pass from one species to another.

Unconventional micro-organisms known as TSE agents or prions induce TSEs. These agents have biologic and physicochemical characteristics that differ significantly from those of other micro-organisms; for example, they are resistant to inactivation processes that are effective against conventional viruses. Today, the exact nature of TSE agents or prions remains unknown even though it is likely that they consist of protein only. Transmissible spongiform encephalopathies are characterized by the accumulation, within the central nervous system (CNS) of the infected individual, of an abnormal isoform of a particular protein from the host organism, namely the prion protein (PrP).[2]

A dual causality, 'infectious' and 'genetic', of these diseases can be inferred from the various current theories. Indeed, the genetic basis of the familial forms in humans has now been confirmed. Furthermore, transmissibility has been proved in natural situations such as the outbreak of CJD among patients treated with pituitary-derived hormones and the appearance in 1986 of BSE that affected UK cattle. The fact of the transmissibility of TSEs requires strict compliance with safety regulations in sterilization, in the preparation of biologic products for therapeutic use and in the prevention of risks associated with graft processes.

NATURE

The nature of TSE agents is unknown. However, because TSEs are transmissible between different species, there are several animal models. The most common are scrapie in mice and hamsters and most of the experiments have been conducted using these two models, including attempts to isolate and to characterize TSE agents or prions.

The size of TSE agents or prions has been evaluated by utrafiltration to be between 15 and 40nm.[3] These agents aggregate easily because of their hydrophobicity; this could explain the variations in size and density that have been reported in the literature.

The inactivation processes that have been demonstrated to be effective against scrapie agents are those that denature or hydrolyze protein components: treatment with high doses of proteinase K, trypsin[4] or SDS. Diethylpyrocarbonate, guanidium thiocyanate or urea alters infectivity. Conversely, procedures that interact with nucleic acids do not modify infectivity titers: nucleases, ultraviolet treatment, Zn^{2+} hydrolysis[2] and psoralens. Therefore, one may hypothesize that TSE agents either consist of protein only or harbor a small nucleic acid that is protected within a shell that may have a lipidic or lipoproteic nature.

Because numerous experimental data indicate the absence of specific nucleic acid in the infectious fractions, the theory of a self-propagating proteic agent, or 'prion' (for proteinaceous infectious particle), was proposed at the end of the 1970s.[2] Prusiner has purified a 27–30kDa protein specifically associated with infectivity, the PrP^{SC} . This protein is present in the brains of infected individuals in proportion to the level of infection. In 1985, it was demonstrated that PrP^{SC} is an abnormal isoform of a host-encoded normal component of the host,[5] PrP^{C} , that accumulates as a result of a post-translational mechanism in the brains of infected individuals. Differences between PrP^{C} (isolated from normal individuals) and PrP^{SC} (isolated from infected individuals) have been investigated. Because the amino acid sequences of the two proteins are identical, the differences between PrP-c and PrP-res are thought to be at the level of conformation. The sensitivity to proteolytic enzymes varies; PrP^{C} is totally degraded by proteinase K concentrations that only partially degrade PrP^{SC} (Fig. 223.1); and PrP^{SC} is insoluble in detergents, although PrP^{C} is soluble. It is impossible to remove PrP^{SC} from infectious fractions without rendering them noninfectious.[6] The amount of PrP^{C} is 50 times greater in brain than in other organs; this may be a critical parameter of the pathogenesis of TSEs.[5] Other investigators have identified fibrillary structures specific for spongiform subacute encephalopathies in detergent-treated brain extracts from infected individuals, namely scrapie-associated fibrils (SAFs),[7] which are polymers of PrP^{SC} . PrP^{C} is anchored at the cell membrane, in rafts, through a glycosyl phosphatidyl inositol (GPI); its half-life at the cell surface is 5 hours, after which the protein is internalized through a caveolae-dependent mechanism and degraded in the endolysosome compartment. Conversion between PrP^{C} and PrP^{SC} occurs likely during the internalization process; this explains why PrP^{SC} is only detectable in the cytoplasm.

Prion protein amino acid and gene sequences are now well known. In humans, PrP is a 253 amino acid protein. It has two hexapeptides and repeat octapeptides at the *N*-terminus, a disulfide bond and is associated at the *C*-terminus with a GPI, which enables it to anchor to the external part of the cell membrane (Fig. 223.2). The fragment of PrP that is resistant to proteinase K digestion (PrP-res) in infected individuals is between amino acid residues 90 and 233 (molecular weight 27–30kDa). PrP^{C} has two putative sites of glycosylation; thus, three glycoforms of PrP can be described. The relative proportions of these glycoforms and the size of the unglycosylated PrP-res fragment are dependent on the strain of prion.[8] Infrared spectroscopy and circular dichroism have shown that the secondary structure of PrP^{C} is mainly composed of a-helices, whereas PrP^{SC} is mainly ß-sheets;[9] transconformation of a-helices into ß-sheets has been proposed as the structural basis by which PrP acquires pathogenicity in TSEs. Recently, the three-dimensional structures of a normal murine, bovine and hamster PrP have been published:[10] [11] [12] the protein is made of a globular domain (amino acids 121–231), which includes three a-helices and two small antiparrallel ß-sheet

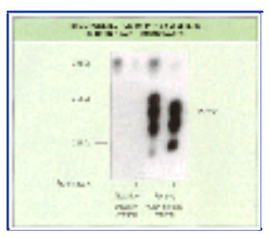

Figure 223-1 Immunodetection of PrP by Western blot in brain homogenates.

structures, and a long flexible tail whose conformation depends on the biophysical parameters of the environment (Fig. 223.3). Crystals of the globular domain of PrP have been obtained; their analysis suggest a possible dimerization of the protein through the three-dimensional swapping of the *C*-terminal helix 3 and rearrangement of the disulfide bond.[13] In-vitro conversion of PrP^{C} into PrP^{SC} is possible in acellular experimental system, and at least one of the biochemical strain characteristics of PrP^{SC} , resistance to PK digestion, can be 'transmitted' to PrP^{C} .[14]

The PrP gene (*PRNP*) is located on the short arm of chromosome 20 in humans (Fig. 223.4). It consists of two exons and one large 10kb intron. The entire coding sequence is within the second exon. The promoter of the PrP gene resembles those of housekeeping genes. Prion protein RNA is 2.1kb in length and is detected at various levels in almost all organs: brain, lung, spleen, heart, etc. (lowest expression level in liver and testicle).[5] Gene expression level is not modulated during TSEs.

Even among the most recent hypotheses, none gives a definite and complete account of the biologic, epidemiologic and clinical observations. Several hypotheses have been advanced:

- the virino hypothesis,[15] which suggests that the agent consists of a nucleic acid, which codes only for its own replication surrounded by host-encoded PrP (thus accounting for the lack of an immune response);
- the unknown conventional virus hypothesis; current concepts of microbiology indicate that this is most unlikely; and
- the protein-only hypotheses that include the seeding model, the chaperone-disease model and the prion theory.

The protein-only hypotheses are supported by the most recent results from transgenic experiments and molecular biology. In these theories, PrP^{SC} is the agent or a major constituent of the infectious fractions.[2] The prion theory postulates that pathogenicity is enciphered into the tertiary structure of PrP^{SC} . Propagation of the abnormal conformation results from the ability of PrP^{SC} to form dimers with PrP^{C} ; this heterodimerization induces a transconfor-mation of PrP^{C} into PrP^{SC} and, therefore, the propagation of this abnormal isoform of PrP (i.e. PrP^{SC}). Recently, it has been suggested that PrP^{C} transconformation into PrP^{SC} requires a host-encoded cellular factor, factor X,[16] which is thought to be a chaperone (Fig. 223.5).

Other theories involving chaperoning molecules[17] or nucleation have also been proposed;[18] in this latter hypothesis, the transformation of PrP^{C} into PrP^{SC} is reversible, PrP^{SC} being stable only when aggregated. Then, binding of PrP^{C} to PrP^{SC} aggregates results in PrP^{C} transconformation. The size of the aggregate then increases until the limit of cohesion of the aggregate, above which a dissociation occurs, giving birth to small seeds efficient for PrP^{C} transconformation. Moreover, the excess of ß-sheet measured in PrP^{SC} could be the consequence of aggregation through the globular domains which could trap a part of the flexible tail inside the aggregate and therefore induce a ß-sheet conformation of a part of the molecule that was not structured before. If demonstrated, this mechanism would not require any transconformation process of PrP.

There are major uncertainties that hinge on the nature of the agent and on the precise level at which the host's genetic background is implicated. The presence of sporadic diseases and familial forms suggests that several diseases probably co-exist under the same designation; some are of infectious origin, whereas others are probably of a metabolic and/or genetic origin. It might therefore be advisable to refer to Creutzfeldt-Jakob syndrome rather than disease in the strict sense of the word.

EPIDEMIOLOGY

Human TSEs are rare diseases, CJD being the most common. Creutzfeldt-Jakob disease can be sporadic (85–90% of cases), familial (10–15% of cases) or iatrogenic (<1%). Its incidence does not differ significantly in countries in which epidemiologic surveillance is performed;[19] incidence ranges between 1 and 1.7 per million inhabitants per year. To date, no link has been described with animal TSE except for the new variant of CJD (vCJD), which occurred mainly in the UK and is thought to

be caused by the BSE agent.[20] Several clusters of CJD have been described in the past, especially in Slovakia and Israel. Progress in molecular genetics has demonstrated that these clusters were in fact inherited forms of CJD. It should be noted that all forms of human TSEs are transmissible, including the genetic diseases.

PATHOGENICITY

Incubation and transmission

Natural and experimental TSEs are characterized by a long incubation phase without clinical symptoms; this silent phase may last as long as 40 years in humans with kuru or infected through extracted growth hormone treatment. Once started, the clinical course of the disease evolves slowly without remission. No inflammatory process is identifiable in blood or cerebrospinal fluid (CSF); none of the usual immunologic stigma or specific signs of chronic viral infections are observed in infected individuals. No virus-like or micro-organism-like structure is identifiable in the brains of infected patients, regardless of which microscopic technique is used. Transmission can be achieved by injecting ultrafiltrates of some organ extracts from infected individuals. The CNS is by far the most infectious; the spleen is also highly infectious in sheep and rodents, but at least 1000 times less so than the brain. In a given genetic background, infectivity depends on the amount injected, the strain of TSE agent and the route of infection; the intracerebral route is the most effective and the oral route the least effective (1 intracerebral route infectious unit = 125,000 oral route infectious units).[21] This has been demonstrated in experimental models, but natural disease-associated agents, especially the BSE agent, may behave differently and be more infectious by a peripheral route (including oral exposure). In-vivo, there are no alterations of the B, T and non-T non-B

Figure 223-2 Human PrP. Representation of the 253 amino acid primary sequence of the human prion protein. Stop tranfert effector (STE) is a sequence that facilitates integration of the protein into the cell membrane.

cells (quantitative or functional) and no antibody against PrP has been detected in natural or experimental diseases; therefore, there is no test available for screening asymptomatic infected individuals. These diseases are always fatal.

Transmissibility is easy in the same species, but is also possible between different species. The strength of the species barrier is variable; for example, BSE is not transmissible to hamsters although it can be transmitted to mice. Several species have never been infected with any TSE agent despite extensive attempts (e.g. rabbit or dog). The major molecular determinant of the species barrier is the homology between the PrP gene of the donor and the PrP gene of the recipient. Other genes play minor roles in TSE agent or prion susceptibility, for example major histocompatibility genes. In humans, the PrP gene exhibits a polymorphism at codon 129; either valine or methionine can be encoded, 50% of the general population being homozygous. Homozygosity at codon 129 has been associated with susceptibility to sporadic and iatrogenic CJD;[22] methionine/methionine homozygosity has also been reported in all the cases of vCJD so far described.

The role of the prion protein

The kinetics of PrP-res accumulation has been studied in a number of experimental animal models. Results have confirmed that

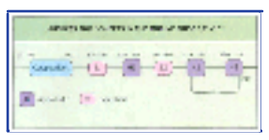

Figure 223-3 a-**Helices and** ß-**sheets in the murine PrP-c 121–231 fragment.** *Adapted from Riek et al.*[11]

accumulation follows the increase in infection. The TSE agent is detectable in the spleen of infected animals soon after a peripheral inoculation; it then appears in the CNS during the second half of the total duration of the experimental disease. In the CNS the infection develops almost exponentially until the appearance of clinical signs and death.

These facts illustrate two main characteristics of the spongiform subacute encephalopathies:

- certain organs of the infected individual, particularly the brain, spinal cord and retina, are heavily infected before clinical signs appear (Table 223.1);[23] and
- these diseases develop without interruption, that is, without any latency of the infectious agent during the asymptomatic phase.

This must be taken into consideration for the safety of biologic products and grafting processes.

The immune system plays a role during the pathogenesis of peripheral infection with TSE agents. It has been demonstrated that immune cells (most probably dendritic cells and macrophages) can be the first site of replication of these agents. In lymphoid structures, infectivity is associated with follicular dendritic cells (FDCs). Neuroinvasion then occurs through the nerve endings that are present in lymphoid organs. It is not known whether PrP^{SC} propagates in the peripheral nervous system (PNS) via a retroaxonal transport or through a propagation of abnormal conformation following a 'domino model' at the surface of the axon; the presence of PrP^{C} is required for agent propagation in the PNS.

The precise function of PrP in healthy individuals remains unknown. Several results obtained in transgenic animals indicate that PrP^{C} might play a role in long-term potentiation, in aging of the Purkinje cells of the cerebellum, in sleep physiology, in oxidative burst compensation (PrP can fix 4 Cu^{++} through its octarepeat domain), in interactions with the extracellular matrix (PrP^{C} can bind to the precursor of the laminin receptor, LRP),[24] in apoptosis and in signal transduction (co-stimulation of PrP^{C} induces a modulation of Fyn kinase phosphorylation).[25]

In TSE-affected individuals, PrP has a determinant role in the incubation time and in the species barrier[26] and its role in pathogenesis is now well established. Transgenic mice lacking *PRNP* expression (i.e. knockout mice that do not express any PrP) are not susceptible to TSE agent or prion infection, demonstrating the key role of PrP in the subacute spongiform encephalopathies.[26] Susceptibility to TSE agents or prions thus depends upon the presence of PrP^{C} on the cell membrane of the host; prions do not propagate in brains that lack PrP^{C} .[27] Moreover, in transgenic animals that express large numbers of *PRNP* copies, it has been demonstrated that incubation time is inversely correlated with PrP-c expression; that is, susceptibility to infection and prion propagation depend on the amount of PrP-c available in the host. Finally, transgenic animals with a PrP gene

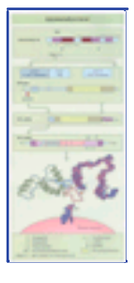

Figure 223-4 Gene structure of the PrP.

mutation equivalent to one described in human familial disease spontaneously exhibit a spongiform encephalopathy that is transmissible under certain conditions.[28]

Neuropathology

Neuropathology of TSEs consists of neuronal death, spongiosis and gliosis with hyperastrocytosis. The precise mechanisms that lead to brain cell damage are not known. Nevertheless, several experimental

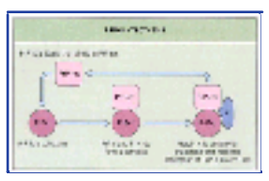

Figure 223-5 Prion hypothesis. *With permission from SB Prusiner, 1996.*

TABLE 223-1 -- **World Health Organization classification of risk associated with scrapie.**

WORLD HEALTH ORGANIZATION CLASSIFICATION OF RISK ASSOCIATED WITH SCRAPIE		
Category	**Infectivity**	**Tissue/body fluid**
I	High	CNS (brain, spinal cord)
II	Medium	Spleen, tonsil, lymph nodes, ileum, proximal colon, placenta
IIIa	Some	Sciatic nerve, pituitary, adrenal, distal colon, nasal mucosa
IIIb	Minimal	CSF, thymus, bone marrow, liver, lung, pancreas, white blood cells
IV	None detectable	Skeletal muscle, heart, mammary gland, colostrum, milk, blood clot, feces, kidney, thyroid, salivary gland, ovary, uterus, testis, seminal vesicle
(Blood might be in category IIb).		

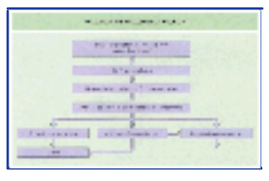

Figure 223-6 Molecular and cellular pathogenesis.

mental results indicate that neuronal death can occur in two ways: by accumulation of PrP^{SC} in vacuoles that fuse and induce cell death through a toxic mechanism; and by apoptosis of noninfected neurons induced by PrP. Indeed, exposure of primary neuronal cell cultures to peptides derived from the 106–126 domain of the PrP molecule induces apoptosis.[29] This apoptosis requires the presence of microglial cells and the presence of PrP^{C} at the cell surface; apoptotic pathway requires NMDA receptor activation. Microglial cells exposed to PrP 106–126 or to PrP^{SC} secrete interleukin(IL)-1ß, IL-6 and other neurotoxic mediators that could participate in neuronal death observed during TSEs. On the other hand, exposure of primary astrocyte cell culture to PrP^{SC} in liposomes induces astrocyte activation and hyperexpression of the glial fibrillary acidic protein (GFAP) gene, which is a biochemical hallmark of natural and experimental TSE (unpublished data). Thus, molecular pathogenesis of brain damage can be summarized as follows (Fig. 223.6).

- PrP^{C} transconformation occurs through direct contact with PrP^{SC} .
- Because of its resistance to proteolysis, PrP^{SC} accumulates in the cytoplasm of infected cells.
- Neuronal death is induced by PrP^{SC} accumulation and PrP^{sc} is released into the extracellular compartment.
- Contact with PrP^{SC} induces either apoptosis of noninfected neurons or infection.
- Contact with PrP^{SC} induces microglial cell activation and release of neurotoxic metabolites.
- Exposure of astrocytes to PrP^{SC} induces activation and hyper-expression of GFAP characteristic of gliosis.

PREVENTION

Several iatrogenic contaminations (Table 223.2) have been reported in the literature.[30] All of them have implicated the CNS as the 'donor tissue' of prions. They have occurred mainly after treatment with extracted pituitary hormones (growth hormone, gonadotropins), dura mater transplantation and the use of surgical instruments contaminated with prions. In several of these cases, transmission has been precisely documented.

In medical practice TSE-associated risk may be, theoretically, at different levels:

TABLE 223-2 -- Iatrogenic contaminations with Creutzfeldt-Jakob disease agent.

IATROGENIC CONTAMINATIONS WITH CJD AGENT			
Mode of infection	**Number of patients**	**Route of entry into brain**	**Mean incubation period**
Instruments			
Neurosurgery	4	Intracerebral	20 months
Stereotactic EEG	2	Intracerebral	18 months
Tissue transfer			
Corneal transplant	2 (+1?)	Optic nerve	18 months
Dura mater implant	115	Cerebral surface	33 months
Tympanic transplant	1	Auditory nerve	48 months
Tissue extract transfer			
Growth hormone	>120	Peripheral	15 years
Gonadotropin	5	Peripheral	13 years

- tissue grafting, including blood transfusion;
- products used in therapy that are extracted from humans, sheep or cattle, including products derived from human blood (drugs and medical devices); and
- hospital sterilization.

The appearance of vCJD in the UK and its possible link with the BSE agent also raises the risk of human contamination by the BSE prion through food or the use of bovine-derived products that are utilized in medicine.

The existence of TSEs has a number of consequences for safety in the use of biologic products in therapy. Such products must be evaluated for the potential risk of transmission of TSE agents; safety will depend on the screening of the donor or the animal source, the purification process (which may include several steps that can inactivate or eliminate TSE agents), the dose and the administration route of the final product (the intracerebral route being the most risky), and the indication for which the product is used. The replacement of biologic products with synthetic/recombinant molecules is desirable whenever possible. For biologic and synthetic molecules a precise evaluation of the therapeutic risk/benefit ratio should be performed.

Patient who have subacute encephalopathy should not be considered as graft or blood donors. Patients belonging to families with cases of familial CJD, GSS or FFI should also be excluded. Finally, it is strongly recommended that individuals with a medical history of treatment with pituitary-derived hormones or dura mater graft be excluded from blood and organ or tissue donation.

Risk associated with vCJD must also be considered, at least in countries in which exposure of the human population to the BSE agent is highly suspected. Although infectivity is restricted to the CNS and retina in classic CJD (sporadic and familial), PrP^{SC} and infectivity can be detected in lymphoid organs of vCJD patients (tonsils, lymph nodes, appendix) in all cases. This implication of the lymphoid system raises several questions, two of which are as follows.

- Are there infectious particles in blood? If so then what is the infectious load?
- Is any surgery involving contact with lymphoid tissue 'at risk' of prion transmission?

These problems are under evaluation at present and the public health consequences of vCJD will depend upon the future number of cases. Nevertheless, precautionary measures have been taken for blood transfusion and the use of plasma-derived products. Some European countries have introduced a systematic leukodepletion of blood donations; in several countries, individuals who spent more than 6 months in the UK between 1980 and 1996 are excluded from blood donation, and British plasma is no longer used for plasma derivative product purification.

Special attention must be given to the sterilization protocols that are currently used in hospitals. Transmissible spongiform encephalopathy agents or prions resist almost all of the physical and biochemical procedures generally used to inactivate conventional viruses. The resistance to disinfectants of TSE agents is exceptional. A level of decontamination compatible with viral safety may be obtained by:

- autoclaving at 273–277°F (134–136°C) for 20 minutes, but susceptibility to autoclaving varies depending on the strain of agent;[20]
- treatment with sodium hydroxide 1 or 2mol/l for 1 hour at 68°F (20°C); and
- treatment with sodium hypochloride (2% available chlorine at least) for 1 hour at 68°F (20°C).

Risks depend on the nature of the patient and/or the type of surgery. High-risk patients include CJD patients, all neurosurgical patients, patients who have a history of treatment with pituitary-extracted hormone and patients from families with documented familial CJD. High-risk procedures include neurosurgery and ophthalmology and otorhinolaryngology surgery. In all these cases (high-risk patients and/or high-risk surgery) decontamination should include at least one of the procedures recommended by World Health Organization. [23] Several countries in Europe stipulate that two of these procedures be applied consecutively. Of course, the definition of 'high-risk patients' may evolve depending upon the spread of vCJD in the human population.

DIAGNOSTIC MICROBIOLOGY

Because the nature of the TSE agent is unknown and because of the lack of a detectable immune response to infection, there is as yet no routine test that permits the diagnosis of infection in asymptomatic individuals or in patients with subacute encephalopathies. Therefore, diagnosis of TSE is most often post mortem. Neuropathology, immunohistochemistry of PrP-res accumulation in the brain and Western blot detection of proteinase K-resistant PrP (see Fig. 223.1) are the most common diagnostic tests that can be used in medical practice. Electron microscopy detection of SAF can also be used for diagnosis. Transmissibility to primates or to rodents can be helpful, but it requires a high level of safety in animal care facilities; moreover, incubation times during primary interspecies passages are often very long.

It is worth noting that neuropathology can be very discrete and that PrP^{SC} accumulation can be undetectable in several parts of the brain; a negative result is inconclusive.

Protein 14-3-3, a protein associated with neuronal death, is detectable in the CSF of human TSE-affected patients.[31] Although not specific of CJD, a positive 14-3-3 test in CSF is a strong argument in favor of a CJD diagnosis when the clinical picture is compatible and when neopterin is normal. It must be kept in mind that 14-3-3 is not elevated in all vCJD and growth hormone-related CJD cases.

CLINICAL MANIFESTATIONS

Creutzfeldt-Jakob disease is a degenerative illness of the CNS (see Chapter 26). It is characterized by a clinically silent latent stage, which may evolve over a period of 20 years or more. Clinical manifestations can vary at the onset of the disease and may include memory loss, abnormalities in movement co-ordination and psychiatric abnormalities. At the full-blown stage of the disease, the clinical picture consists of a severe dementia associated with ataxia, extrapyramidal and pyramidal syndromes, and myoclonia. All of the clinical signs are related to the CNS degeneration. It must be pointed out that few of the clinical symptoms usually associated with infectious diseases (e.g.

fever) are observed in CJD patients. Routine serological testing and nuclear magnetic resonance imaging (except for vCJD) are of little assistance; for example, there are no abnormalities in blood or CSF chemistry or cell populations, except for the frequent detection of neuron-specific enolase and protein 14-3-3 in the CSF. Conversely, the electroencephalogram exhibits pseudoperiodic abnormalities that are constituted by 1–1.5Hz triphasic sharp waves in all derivations in 75% of patients. The evolution of the disease is subacute and always fatal; patients die after a short period of coma. Creutzfeldt-Jakob disease lasts an average of 6 months in adults, but may reach more than 1 year in infants or young adults with iatrogenic disease or with CJD.

MANAGEMENT

No curative or palliative treatment for CJD or other TSEs currently exists. Only a few drugs have proved relatively effective when administered at the time of the experimental infection; they are Congo red, dextran sulfate, polyoxometalates from the tungstoantimoniate family, and amphothericin B and its derivatives.[32] [33] Polyene antibiotics seem the most promising class of drugs that may be suitable for therapy; in experimental scrapie, positive effects on survival are correlated with PrP-res accumulation delay in the brain. This underlines the key role of PrP in the pathogenesis of TSEs.

Unfortunately, none of these drugs are effective once the first clinical signs have appeared; therefore, no drug is suitable for human use.

Recently, treatment with anti-PrP antibodies has been reported as capable of curing infected cells in vitro.[34] Moreover, transgenic animals for anti-PrP antibody have been demonstrated to be less susceptible to peripheral infection than the wild-type controls.[35] This may be the proof of the principle of such a therapeutic approach and may support the possibility of a vaccine against TSEs in the future.

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Chapter 224 - Staphylococci and Other Micrococcaceae

Jan Verhoef
Ad C Fluit
Franz-Josef Schmitz

NATURE

Staphylococci were first observed and cultured by Pasteur and Koch but the first thorough studies on staphylococci were made by Ogston in 1881 [1] and Rosenbach in 1884.[2]

The genus *Staphylococcus* was given its name by Ogston in 1881 when he observed grape-like clusters of bacteria in pus from human abscesses.[1] Three years later, Rosenbach [2] was able to isolate and grow these micro-organisms in pure culture. He gave them the specific epithet *Staphylococcus aureus* Rosenbach because of the yellow-to-orange pigmented appearance of their colonies.[2] Rosenbach showed that *S. aureus* was responsible for wound infections and furunculosis, and that *Staphylococcus epidermidis* was a normal colonizer of the skin. Ever since Rosenbach first described the growth of this 'golden' coccus, surgeons have feared staphylococcal wound infections after surgery.[3] Staphylococci also caused life-threatening disease after trauma and fatal pneumonia during the influenza season, killing young people in the prime of their lives.[4] Therefore, in the pre-antibiotic era, *S. aureus* was known as a major life-threatening pathogen.

Because of penicillin's important contribution to the fight against *S. aureus* disease, the period between 1946 and 1950 was referred to as 'a golden age' for the treatment of staphylococcal disease. However, the rapid spread of resistant staphylococci soon led to a return of this major pathogen.

An excellent up-to-date review on staphylococci is provided by Crossley and Archer.[5]

Taxonomy

The family of Micrococcaceae consists of four genera:

- *Planococcus*;
- *Stomatococcus*;
- *Micrococcus*; and
- *Staphylococcus*.[6]

Planococci are not found in humans, whereas *Stomatococcus* and *Micrococcus* can colonize humans but rarely produce disease.

Staphylococci are Gram-positive nonmobile cocci that characteristically divide in different patterns to form irregular grape-like clusters (Fig. 224.1). They usually produce catalase. They are facultative anaerobes but grow better under aerobic than under anaerobic conditions. Traditionally, they have been divided into coagulase-positive and coagulase-negative staphylococci. The coagulase-negative staphylococci can be subdivided at present into 32 different species (Fig. 224.2). Of the large number of staphylococci, only three are commonly associated with human disease:

- *S. aureus*;
- *S. epidermidis*; and
- *Staphylococcus saprophyticus*.

In addition, *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* must be mentioned since these two species are relatively aggressive and can cause a variety of infections, including foreign body infections, bacteremia, endocarditis, bone infections and abscesses in various organs.

At the genus level staphylococci are a homogenous group of bacteria. On the basis of DNA rRNA hybridization and comparative oligonucleotide analysis of 16S rRNA they appear to be related to the *Bacillus*, *Enterococcus* and *Listeria* cluster (Fig. 224.3).

The micrococci and stomatococci are more related to *Actinomycetes*, although their cytosine-guanine rates vary from 30% to 70%.

Anatomy of the staphylococcus

Capsular polysaccharides

Staphylococci are often surrounded by a loose-fitting polysaccharide capsule. This capsule can be used to identify serotypes. Serotypes 5 and 8 are the most common and many methicillin-resistant *S. aureus* (MRSA) belong to these serotypes.[7]

When isolated from infections, *S. aureus* expresses high levels of polysaccharide but rapidly loses it upon subculture in the laboratory. The function of the capsule is not clear. It may impede phagocytosis (see below) and may therefore contribute to the invasive character of *S. aureus*.

Peptidoglycan

The peptidoglycan layer is the major macromolecule present in the cell wall (Fig. 224.4). The glycan chains are built with approximately 10 alternating subunits of *N*-acetylmuramic acid and *N*-acetylglycosamine. Pentapeptide side chains are attached to the *N*-acetylmuramic acid subunits. The glycan chains are then cross-linked with peptide bridges between the side chains.

Protein A

The surface of all strains of *S. aureus* contains proteins that protrude through the peptidoglycan layer and are anchored covalently. One of these proteins, protein A (Fig. 224.5), has the capacity to bind the Fc fragment of immunoglobulin subclasses IgG1, IgG2 and IgG4.[8] Protein A is not produced by coagulase-negative staphylococci.

Teichoic acid

The teichoic acids are macromolecules of phosphate containing polysaccharides (Fig. 224.6). Teichoic acid is bound both to the peptidoglycan layer and to the cytoplasmic membrane. The polysaccharides are species specific; ribitol teichoic acids are present in *S. aureus* cell walls and glycerol teichoic acids are present in *S. epidermidis*.[9]

EPIDEMIOLOGY

Every human being is colonized with *S. epidermidis*. The normal habitats of these staphylococci are the skin and the mucous membranes. The major habitats of the most pathogenic species, *S. aureus*, are the anterior nares and perineum.

Neonates are readily colonized by *S. epidermidis* and often by *S. aureus*. Newborn infants usually acquire *S. aureus* first on the skin (umbilical area) and later in the nose.[10] [11] Soon after the neonatal period some individuals become permanent carriers, often with the

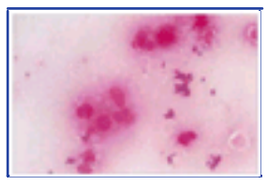

Figure 224-1 *Staphylococcus aureus* in a Gram stain of pus.

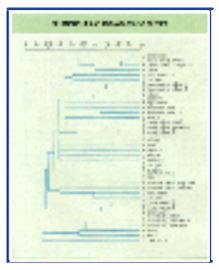

Figure 224-2 Relationship of *Staphylococcus* species. Dendrogram of the DNA relationships of *Staphylococcus* spp. and subspecies based on the relative percentage of DNA-DNA hybridization (reassociation) at optimal conditions. *Adapted with permission from Crossley and Archer.* [5]

same strain. Although some spread of *S. aureus* may occur within a family, generally the established flora of the nose prevents the acquisition of new strains.[12] However, colonization with other strains may occur when antibiotic treatment is given that leads to elimination of the susceptible carrier strain. Because this situation occurs in hospital, patients may become colonized with resistant staphylococci. Carriage rates of *S. aureus* in the nares of people outside hospital varies from 10% to 40%. Hospital patients and personnel have higher carriage rates. The carriage rates of patients may increase

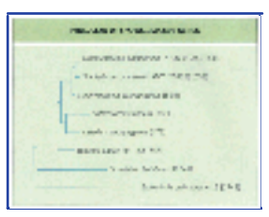

Figure 224-3 Phylogeny of *Staphylococcus* genus based on 16S rRNA sequences. *Adapted with permission from Crossley and Archer.*[5]

during their stay in hospital through colonization and nosocomial transmission. The rates are especially high in patients undergoing hemodialysis and in diabetics, drug addicts and patients who have a variety of dermatologic conditions.[13] [14]

The carrier state is clinically relevant because carriers undergoing surgery have more infections than noncarriers. This has led to the application of mupirocin (a local antibiotic) to the anterior nares in some centers just before open heart surgery to lower the incidence of postoperative wound infection.[15] Dispersers among the carriers are important because dispersers transmit staphylococci not only by direct contact but also by airborne transmission. Heavy perineal carriers almost always disperse large amounts of staphylococci. Staphylococci may accumulate rapidly on the clothes and bedding of dispersers and may disseminate when these fomites are disturbed. Dust particles containing staphylococci may be carried for considerable distances.

Since 1944, an increasing proportion of hospital-acquired *S. aureus* strains have developed resistance to penicillin because of their ability to produce ß-lactamase. By 1950, approximately 80% of hospital-acquired infections were caused by these penicillinase producers.[16] In the mid-1950s, penicillin-resistant *S. aureus* of a new phage type (the so-called phage type 80, see below) was first detected in Australia and then spread rapidly, causing a major pandemic of hospital-acquired infections.[17] Because of the 'resistance advantages' of the new *S. aureus* strain, referred to as 'hospital staph', there were again outbreaks on hospital wards. In 1959, the British Ministry of Health published guidelines for the prevention of further spread of the multiresistant — penicillin-streptomycin-tetracycline-erythromycin (PSTE)-resistant — *S. aureus* strains.[18]

A temporary respite in the war against resistant *S. aureus* occurred in the early 1960s, when Rolinson and Stevens discovered a ß-lactamase-stable penicillin (methicillin).[19] In the following years the introduction of the semisynthetic penicillins (methicillin, oxacillin, flucloxacillin) and the application of infection control measures probably contributed to the spontaneous decrease in the epidemic spread of *S. aureus* strains. However, coinciding with the disappearance of PSTE strains was the appearance of MRSA.

Early MRSA epidemics were reported in the UK in the early 1960s. [20] Later, two surveillance studies conducted by the Cross Infection Reference Laboratory in London showed that the frequency of methicillin resistance in clinical isolates from London hospitals and

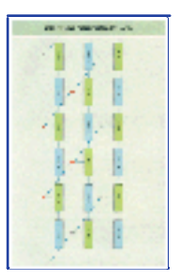

Figure 224-4 Structure of the peptidoglycan layer. The peptidoglycan layer consists of three integral parts. The glycan chains are built with 10–12 alternating *N*-acetylglucosamine (Glu) and *N*-acetylmuramic acid (Mur) subunits jointed with ß-1,4 glycosidic bonds. Vertical pentapeptide side chains are linked to the muramic acids subunits, and the side chains are in turn cross-linked with diagonal intrapeptide bridges. For example, the glycan chains in *Staphylococcus aureus* are cross-linked with pentaglycine bridges attached to L-glycine in one pentapeptide chain and D-alanine in an adjacent chain.

from hospitals in south east England increased until 1963, when it was followed by a stationary phase (below or around 1%) until 1968.[21] The rate of methicillin resistance rose sharply in the period 1968–69 (up to 5%). After another decline in major MRSA epidemics, the first serious hospital outbreaks were reported in 1970. The early MRSA epidemics could be controlled by aminoglycosides. In the late 1970s, however, resistance to gentamicin emerged in *S. aureus* and led to a new wave of hospital outbreaks. [22] Since then the MRSA strains have spread all over the world,[23] not only in hospitals but also in the community.[24] In the USA, the percentage of MRSA in all hospitals in the National Nosocomial Infection Survey system increased from 2.4% in 1975 to 29% in 1991. The incidence varied according to hospital bed capacity, with hospitals of less than 200 beds reporting 14.9% of *S. aureus* isolates as MRSA, and hospitals of 500 beds reporting a 38.3% frequency. [25] Meanwhile, the incidence has increased to nearly 40% MRSA in the USA and 6% in Canada. Within European countries a systematic survey of 27 laboratories documented a wide variation in incidence ranging from less than 1% in northern European countries to over 30–50% in Spain, France and Italy. [26]

Patients at highest risk for MRSA infection are:

- those in large tertiary-care hospitals, particularly the elderly and immunocompromised;
- those in intensive care units;
- burns patients;
- those who have surgical wounds; and

¦ patients who have intravenous lines.

Duration of hospitalization, previous antibiotic treatment and proximity to a patient colonized or infected with the organism also predispose to MRSA infection. In acute care and nursing facilities, colonized and infected patients are the major MRSA reservoir. Person-to-person transmission of MRSA usually occurs via the hands of health care workers.[14]

Typing of staphylococcal isolates

Nosocomial infections caused by *S. aureus*, especially by MRSA, are an increasing problem in hospitals. Quick and reliable typing methods are required to obtain information about the relatedness of individual *S. aureus* isolates in order to implement hygienic measures faster and more effectively. The epidemiologic goal of bacterial typing is to accurately identify the source, extent and mechanisms of transmission of outbreaks of infection. Investigations are typically triggered by an increase in the prevalence of *S. aureus* infection or by noting the appearance of isolates with a distinctive antibiotic susceptibility pattern. A convenient basis for classifying typing methods is to divide them into:

¦ phenotypic techniques — those that detect characteristics expressed by the micro-organisms, such as biotyping, antimicrobial susceptibilities, serotyping, bacteriophage typing, immunoblotting and multilocus enzyme electrophoresis; and
¦ genotypic techniques — those that involve direct DNA-based analyses of chromosomal or extrachromosomal genetic elements, such as plasmid restriction digests, ribotyping, Southern blot analysis, pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR)-based methods.[27]

Bacteriophage typing (Fig. 224.7) has played an important role in the study of many epidemics of *S. aureus* infection.

The temperate bacteriophages of *S. aureus* have a narrow host range, lysing only some strains of the same species, and are therefore useful as typing phages. When phage typing became widespread, it became clear that some phage types were more endemic than others.[28]

Also, molecular typing methods are now being introduced into the study of *S. epidermidis* and *S. aureus*, particularly MRSA. Plasmid analysis has been used extensively with success but it has the disadvantage that plasmids can easily be lost and acquired and are therefore inherently unreliable. Methods designed to recognize restriction fragment length polymorphisms (RFLP) using a variety of gene probes, including rRNA genes (ribotyping), have had limited success in the epidemiology of MRSA. In this technique the choice of both the restriction enzyme used to cleave the genomic DNA and the probes is crucial.

Although there is no definitive typing technique for staphylococci, the preponderance of data indicate that PFGE is currently the single most useful and reliable reference method. This technique, in which genomic DNA is cut with a restriction enzyme that generates large fragments of 50–700kb,[29] is a variation of agarose gel electrophoresis because the orientation of the electric field across the gel is changed periodically. This change of orientation in the electric field enables DNA fragments as large as several megabases to be separated effectively by size. The restriction profiles often demonstrate remarkable

Figure 224-5 Structure and sorting of protein A. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus* and other Gram-positive bacteria. (a) Cell wall sorting of surface proteins consists of four distinct steps, which lead to the proteolytic cleavage of the polypeptide chain between threonine (T) and glycine (G). The carboxyl of threonine is subsequently amide-linked to the free amino group in the pentaglycine cross-bridge of the staphylococcal cell wall. The cell wall linkage of surface proteins in *S. aureus* (b) is compared with that proposed for other Gram-positive bacteria such as *Streptococcus pyogenes* (c) and *Listeria monocytogenes* (d). NacGlu, *N*-acetylglucosamine; NacMur, *N*-acetylmuramic acid. *Adapted with permission from Crossley and Archer.*[5]

Figure 224-6 Structure of teichoic acid and linkage unit attaching teichoic acid to peptidoglycan in *Staphylococcus aureus*. The C2 and C4 positions of the ribitol residues are substituted by D-alanyl and *N*-acetylglucosamine residues. *Adapted with permission from Crossley and Archer.*[5]

variation among different strains, greatly facilitating the analysis and comparison of multiple isolates.

The most obvious 'challengers' to PFGE as the current standard for typing staphylococci are systems based on the PCR. The essential feature of PCR is the ability to replicate a particular DNA sequence rapidly and exponentially. Several different approaches have been proposed (i.e. restriction digestion of PCR products, PCR based on repetitive chromosomal sequences, arbitrarily primed PCR).

The application of molecular techniques to staphylococcal typing has provided a powerful set of new tools that can facilitate both epidemiologic investigations and patient management.[27]

Coagulase-negative staphylococci

The high prevalence of coagulase-negative staphylococci on the skin and the frequent implantation of foreign devices during hospitalization provide these bacteria with an ideal opportunity to cause infection.

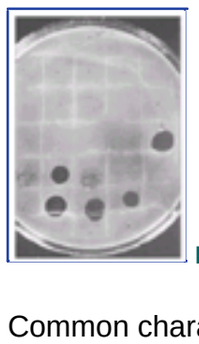

Figure 224-7 Phage type III of *Staphylococcus aureus.*

Common characteristics of coagulase-negative staphylococci infections are as follows.

¦ They are usually nosocomial.
¦ *S. epidermidis* is the most common species involved.
¦ They are associated with implanted foreign devices such as intravascular catheters, prosthetic heart valves or cerebrospinal fluid shunts.

Evaluation of the hospital reservoir of coagulase-negative staphylococci remains problematic because these bacteria form part of the normal skin flora. Although several typing systems have helped to elucidate their epidemiology,[30] evaluate their transmission and define outbreaks of nosocomial infection, little is known about the hospital reservoir of coagulase-negative staphylococci, including that of methicillin-resistant strains (MR coagulase-negative staphylococci). Such strains also serve as a reservoir for antibiotic resistance genes that may be transferred to other Gram-positive organisms, including strains of *S. aureus*. The National Nosocomial Infection Survey (NNIS) of the USA found that, between 1980 and 1989, the incidence of coagulase-negative staphylococci as a cause of nosocomial bacteremias increased from 9% to 27% to become the single most common cause of these infections. Furthermore, NNIS data revealed that during this period the proportion of MR coagulase-negative staphylococci increased from 20% to 60%. Data from the worldwide SENTRY study indicated that, independent of geographic origin, 70–75% of coagulase-negative staphylococci are nowadays resistant to methicillin. Most of these strains were also resistant to multiple additional antimicrobial agents. [26] There is therefore an association between the dramatic increase of coagulase-negative staphylococci as a cause of nosocomial infections and the resistance of these

pathogens to antimicrobial agents.[31]

STAPHYLOCOCCUS AUREUS

PATHOGENICITY

The pathogenesis of *S. aureus* infections is determined by the interaction of *S. aureus* surface-associated and extracellular proteins with host cell determinants. The pathogenesis of staphylococci is multifactorial[32] and therefore it is difficult to determine precisely the role

TABLE 224-1 -- Staphylococcal virulence factors.*

STAPHYLOCOCCAL VIRULENCE FACTORS		
Enzymes	**Toxins**	**Other**
Coagulase	Cytotoxins (a, ß, d, ? leukocidin)	Slime production
Catalase	Exfoliative (epidermolytic) toxin	Capsule
Hyaluronidase	Toxic shock syndrome	Cell wall
Fibrinolysin	Enterotoxin (A–F)	
Lipase		
Nuclease		
Penicillinase		
Overview of enzymatic, toxic and other staphylococcal virulence factors that may participate in the etiology of staphylococcal disease. Several enzymes and toxins are known to play a role in the pathogenesis of staphylococcal infection.		

* *Adapted from Kuroda et al.*[33]

of each individual staphylococcal component in the etiology of staphylococcal disease (Table 224.1).

Adherence

In order to initiate infection, the pathogen must gain access to the host and attach to host cells or tissues (adherence). The *S. aureus* cells express surface proteins that promote attachment to host proteins such as laminin, fibronectin, elastin, vitronectin, fibrinogen and many other molecules that form part of the extracellular matrix. These surface proteins of *S. aureus* are called extracellular matrix binding proteins.[34]

The extracellular matrix molecules are present on epithelial and endothelial surfaces as well as being a component of blood clots. The receptor that promotes attachment to collagen is particularly associated with strains that cause osteomyelitis and septic arthritis.

Antibodies against a fusion protein encompassing a-galactosidase and the domains of fibronectin binding protein may be protective by blocking the binding of *S. aureus* to fibronectin, suggesting a possible therapeutic approach.[35]

Role of adherence in infections associated with medical devices

Staphylococcus aureus and *S. epidermidis* can cause infections associated with indwelling medical devices ranging from simple intravenous catheters to prosthetic joints and replacement heart valves. Soon after biomaterial is implanted in the human body it becomes coated with a complex mixture of host proteins and platelets (Fig. 224.8). Extracellular matrix binding proteins also play a role in the binding of *S. aureus* and *S. epidermidis* to biomaterials. Interbacterial adherence leads to biofilm formation. Recently, a polymer from *S. epidermidis* has been characterized that is involved in the intracellular adhesion between cells, leading to the formation of large cell clusters following the attachment of cells to a plastic surface during biofilm formation. The polymer, known as polysaccharide intracellular adhesin, is a linear homopolymer of ß-1,6-linked glucosamine residues, 80–85% of which are *N*-acetylated.[36]

In addition, *S. aureus* can adhere to the surface of endothelial cells and become internalized by a phagocytosis-like process. It is not clear whether attachment involves a novel receptor or a known surface protein of *S. aureus*. Some researchers think that *S. aureus* can initiate endocarditis by attaching to the undamaged endothelium. Others believe that trauma, even if very minor, is required to promote attachment of bacteria.

Invasion

As soon as microbes invade the tissues, circulating polymorphonuclear leukocytes (PMNLs) are activated. They adhere to endothelial

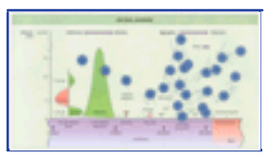

Figure 224-8 Bacterial adherence. The figure illustrates the events associated with bacterial (B) adherence to a biomaterial in relation to time and the molecular sequence in bacterial attachment, adhesion, aggregation and dispersion at substratum surface. A number of possible interactions may occur depending on the specificities of the bacteria or substratum system, the distance from the biomaterial and the stage of adherence. The attachment stage is mediated by non-specific forces. Adhesion is driven by specific adhesin-receptor interactions. The final aggregative step results in a bacterial macrocolony on the biomaterial surface in which the bacteria are firmly adherent to the biomaterial and each other. Bacterial exopolysaccharide blankets the macrocolony and may serve to improve the nutritional microenvironment and protect the bacteria from host defenses. In the dispersion phase, bacteria disaggregate, break loose from the macrocolony and drift free into the bloodstream. *Adapted with permission from Gristina AG. Biomaterial centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588. © 1987 American Association for the Advancement of Science.*

cells and move through the endothelial barrier (chemotaxis). While leukocyte migration occurs, the microbes are opsonized and the microbial surface is coated with antibodies and complement factors for recognition by PMNLs.[37]

Encapsulated *S. aureus* is much more difficult to opsonize than unencapsulated strains. Four mechanisms of opsonization of unencapsulated *S. aureus* strains have been described (Fig. 224.9):

- interaction of PMNLs with *S. aureus* through antibodies against peptidoglycan;
- interaction through antibodies and C3b;
- interaction through C3b generated by direct interaction of peptidoglycan with complement (classic pathway); and
- interaction through direct activation via the alternative pathway.

Opsonization of encapsulated *S. aureus* is largely mediated by antibodies against the *O*-acetyl group of the capsular polysaccharide.[38]

Antibodies bound to specific antigens on the cell wall of bacteria (mostly peptidoglycans) serve as ligands for the attachment of bacteria to PMNLs. Antibodies against

peptidoglycan are opsonic.[39] Peptidoglycan is able to activate the complement system, leading to deposition of C3b on the surface of the bacteria. During *S. aureus* infection, antibodies are also produced against teichoic acid. Their role in opsonization is questionable and is probably indirect via activation of the complement cascade. Antipeptidoglycan antibodies promote phagocytosis in vitro but their opsonic capacity in vivo is unclear as most strains grown under in-vivo conditions contain a capsule (Fig. 224.9 Fig. 224.10 Fig. 224.11).

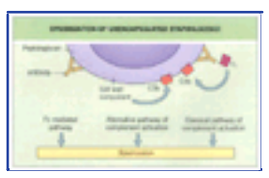

Figure 224-9 Opsonization of unencapsulated staphylococci. Opsonization through complement activation is primarily a function of C3b and iC3b. When antibody (ab) molecules bind to antigen, the antigen-antibody complex activates the first complement component, C1. C1 is then converted into an esterase, initiating the classical pathway. Additionally, some cell-wall components can activate the alternative pathway.

Although the acidic polysaccharides are not activators of the complement pathway by themselves, in the presence of anticapsular antibody they may become activators of the classic and alternative complement pathways, and binding sites for C3b may be created

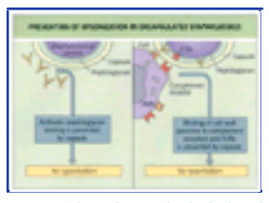

Figure 224-10 Prevention of opsonization in encapsulated staphylococci. (Left) The capsule of *Staphylococcus aureus* prevents binding of antibodies to peptidoglycan: no opsonization. (Right) The capsule prevents binding of opsonins on the cell wall of *Staphylococcus aureus* to complement and Fc receptors on PMNLs.

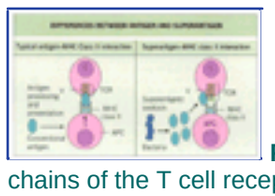

Figure 224-11 Differences between antigen and superantigen. Staphylococcal enterotoxin and TSST-1 act as superantigens, binding directly to MHC class II and the Vß chains of the T cell receptor (TCR) without the need for normal antigen processing.

within the capsular matrix so that these antibodies and C3b finally act as opsonins (see Fig. 224.9).[40]

Opsonization

Opsonization in the absence of antibody and complement

Some bacteria are able to adhere to PMNLs in the absence of antibodies and/or complement through the help of extracellular matrix molecules. These molecules may act as a bridge between PMNLs and bacteria, thus facilitating phagocytosis in the absence of specific opsonins. How important fibronectin is as an opsonin, for example, is not yet known. It is likely that optimal phagocytosis only occurs in the complement process.[41]

Evasion of opsonization

Many staphylococci have developed a defense against opsonophago-cytosis and are therefore able to escape phagocytosis by PMNLs. The most important antiphagocytic defense of staphylococci is an enveloping capsule (see Fig. 224.10). These capsules protect the microbes against PMNLs by interfering with opsonization. Two other cell wall components that help microbes evade the phagocytic defense of the host are peptidoglycans and protein A. Indeed, two mechanisms have been described to explain why encapsulated strains resist phagocytosis in the absence of type-specific anticapsular antibodies (see Fig. 224.10). The capsule shields the peptidoglycan macromolecule from reacting with antibodies and C3b, thereby preventing opsonization. No antibody reaction with peptidoglycan occurs and no complement is activated. It is also possible that, although peptidoglycan reacts with antibodies and iC3b is generated on the peptidoglycan moiety, the capsule prevents the interaction of C3b and IgG on peptidoglycan with the receptors in the membrane of PMNLs.[37]

Protein A probably plays a triple antiphagocytic role in the bacteria-cell recognition process by its binding to the Fc portion of IgG.

- ¦ Extracellular soluble protein A can react with the Fc terminal of IgG molecules of human serum, thereby producing immune aggregates that consume complement, which leads to depletion of complement needed for phagocytosis.
- ¦ Extracellular protein A can bind to the Fc portion of specific anti-staphylococcal antibodies coating the micro-organism with their Fab fragment, thereby preventing further interaction of the complex with the Fc receptor of phagocyte.
- ¦ Cell-bound protein A binds to the Fc fragment of any IgG molecule in its neighborhood, thus eliminating non-specific and specific antibodies.[42]

It has been shown that strains with high levels of protein A are less able to activate complement than strains with low levels of protein A.[43] Indeed, mutants of *S. aureus* lacking protein A are more efficiently phagocytosed in vitro, and studies with mutants in infection models suggest that protein A enhances virulence.

After opsonization, staphylococci are phagocytosed and killed. Some bacteria are usually able to escape killing and are able to survive inside PMNLs. Some strains of *S. aureus* with a special coagulase genotype may be better able to survive inside the PMNL.[33]

Toxins

Staphylococcus aureus can express several different types of protein toxin, which are probably responsible for some symptoms during infections.[44]

a-Toxin

The most potent membrane-damaging toxin of *S. aureus* is a-toxin. It is produced by a large percentage of strains and is expressed as a monomer that forms cylindric oligomeric heptamers and binds to the membrane of susceptible cells. Susceptible cells have a specific receptor for a-toxin that allows low concentrations of toxin to bind resulting in small pores through which monovalent cations can pass. At higher concentrations the toxin reacts non-specifically with membrane lipids, resulting in larger pores through which divalent cations and small molecules can pass. a-Toxin is toxic to a wide range of mammalian cells and animals and is dermonecrotic and neurotoxic.

ß-Toxin

This toxin is a sphingomyelinase that damages membranes containing sphingomyelin. Lysis of sheep erythrocytes by *S. aureus* is caused by ß-toxin. The majority of human isolates of *S. aureus* express ß-toxin but its role in disease is poorly understood. It is produced in high concentration, especially in animal strains, suggesting that it must provide some form of selective advantage to the microbe. This is supported by the fact that ß-toxin-deficient mutants are less virulent. It may play a role in the pathogenesis of bovine mastitis.

?-Toxin and Panton-Valentine leukocidin

Staphylococcus aureus strains produce bicomponent toxins composed of two independently secreted nonassociated proteins. These toxins, ?-toxin and Panton-Valentine leukocidin (PVL), are synergistically

toxic to PMNLs and monocytes and macrophages, and ?-toxin is additionally lytic for red cells from a variety of mammalian species. Recently it was shown that PVL-producing *S. aureus* strains cause rapidly progressive, hemorrhagic, necrotizing pneumonia, mainly in otherwise healthy children and young adults. This pneumonia is often preceded by influenza-like symptoms and has a high mortality rate. [45]

d-Toxin

A variety of features have been attributed to d-toxin.

- It can damage a variety of cell types as a result of its action on cell membranes.
- It causes dose-dependent damage to the bowls of guinea pigs, leading to speculation that it is a mediator of staphylococcal membranous enterocolitis.
- It increases vascular permeability in guinea pig skin, inhibits water absorption and activates adenylate cyclase.
- It has many other effects on various cell systems, such as activation of membrane phospholipase A_2 , stimulation of prostaglandin synthesis, release of lysozyme and ß-glucuronidase from PMNL granules and activation of the acetyltransferase.

Enterotoxins and toxic shock syndrome toxin-1

The enterotoxins, of which there are several serotypes (A, B, C[1] [2] [3] , D, E, G, J, K) cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. Systemically, enterotoxins can cause toxic shock syndrome (TSS). First described in 1978 in association with severe infections with *S. aureus* among children, the major symptoms of TSS are high fever, generalized exanthema, hypotension, multiorgan failure and desquamation of the skin. Menstruation-related TSS is much more common than non-menstruation-related TSS. Most *S. aureus* isolates from patients who have TSS produce toxic shock syndrome toxin (TSST)-1. Enterotoxins B and C cause 50% of nonmenstrual TSS. The TSST-1 is responsible for 75% of TSS, including all menstrual cases.

The TSS can occur as a sequela to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically and the host lacks appropriate neutralizing antibodies. Tampon-associated TSS is not a true infection; rather it is caused by growth of *S. aureus* in a tampon and absorption of the toxin into the bloodstream. The incidence of TSS rose sharply with the introduction of superabsorbent tampons and, although the number of such cases has decreased dramatically, they still occur despite the withdrawal of certain types of tampon from the market.

The enterotoxins and TSST-1 are superantigens. Superantigens (see Chapter 1) stimulate T cells non-specifically without normal antigenic recognition (see Fig. 224.11). Up to one in five T cells may be activated, whereas only 1 in 10,000 are stimulated during normal antigen presentation. It has been postulated that this massive activation of lymphocytes results in cytokine release, which may contribute to some of the features of TSS.

Epidermolytic (exfoliative) toxin

The epidermolytic toxin (ET) causes scalded skin syndrome in neonates, with widespread blistering and loss of the epidermis. The two antigenically distinct forms of the toxin are ETA and ETB. These toxins have protease activity. They have a sequence similarity with the *S. aureus* serine protease, and the three most important amino acids in the active site of the protease are conserved. Changing the active site of serine to a glycine completely eliminates toxin activity. It is therefore possible that the target of the toxins is a very specific protein involved in maintaining the integrity of the epidermis. Recent data indicated that the gene coding for ET is located on a phage and that isolates displaying ET activity are often clonally related.

Other extracellular proteins

Coagulase

Coagulase is an extracellular protein that binds to prothrombin to form a complex called staphylothrombin. The protease activity of thrombin is then activated, resulting in the conversion of fibrinogen to fibrin. This reaction is the basis of the tube coagulase test, in which a clot is formed in plasma after incubation with the *S. aureus*. Coagulase is the classic marker for distinguishing *S. aureus* from *S. epidermidis* in the clinical laboratory. Although there is no direct evidence that coagulase is a virulence factor, it is reasonable to speculate that the bacteria protect themselves from host defenses by causing localized clotting.

Staphylokinase

Many strains of *S. aureus* express a plasminogen activator called staphylokinase. A complex formed between staphylokinase and plasminogen activates plasmin-like proteolytic activity, which causes dissolution of fibrin clots. The mechanism is identical to that of streptokinase, which is used to treat patients who have coronary thrombosis.

Another enzyme produced by staphylococci is catalase, which converts hydrogen peroxidase into nontoxic H_2 O and O_2 . Catalase may contribute to virulence because its production allows staphylococci to evade the killing mechanism of PMNLs.

Regulation of virulence factors

Whole genome sequencing of *S. aureus* strains provided valuable new insights into potential virulence factors and their control. Many staphylococcal products can be involved in the pathogenesis of disease. [33] The expression of these genes is controlled by a number of different global regulators. Best known among these are the *agr* (accessory gene regulator) and *sar* (staphylococcal accessory regulator) loci. The regulation of the expression of various virulence factors is poorly understood but highly complex.

A global regulator may upregulate the expression of one group of virulence factors, downregulate the expression of a second group and have no influence on a third. However, the expression of these genes may also be influenced by other global regulators. Indeed, the expression of one regulator may be influenced by the expression of another. In addition, this regulation network responds to stimuli from the environment (e.g. adhesion to epithelial cells, adhesion to catheter material, presence of other staphylococcal cells). Unraveling this highly complex regulation network may help to explain the different clinical manifestations seen in *S. aureus* infections.

CLINICAL MANIFESTATIONS

Staphylococcal infections are characterized by intense suppurative inflammation of local tissues with a tendency for the infected area to become encapsulated, leading to abscess formation.[46]

Furuncle

The most common staphylococcal infection is the furuncle or boil, which is a localized painful superficial skin infection that develops in a hair follicle or gland. Similar infections at the base of the eyelashes are the common styes. The most common sites for boils are the neck and the buttocks, often as a result of wearing tight clothes. The infected patient is often a carrier of the staphylococcus that causes the infection, usually in the anterior nares. A total of 65% of furuncles, especially those on the head and neck, are caused by a strain indistinguishable from that in the patient's nose, and there are indications that perineal carriage is associated with furuncles on the legs. No specific pathogenicity factors are responsible for the pathology of furuncles. Although about 50% of strains involved in carbuncles and

furuncles have been found to produce PVL, only 2% of all *S. aureus* are producers. The course of infection is usually benign. No specific treatment is needed and most often the infection resolves after spontaneous drainage of the pus or surgical incision.

Chronic furunculosis

Approximately 2–3% of the population have chronic furunculosis, in which repeated attacks of boils are often caused by the same phage type of *S. aureus*. The most appropriate management of patients who have recurrent furunculosis includes a course of an oral antibiotic combined with chlorhexidine washes and the use of an antistaphylococcal intranasal ointment.

Carbuncle

Staphylococcal infection may spread from a furuncle to the deeper subcutaneous tissues, resulting in the development of one or more abscesses known as carbuncles. These abscesses occur mostly on the back of the neck, but may involve other skin sites. They may result in bloodstream invasion.

Bullous impetigo

Certain strains of *S. aureus* can cause bullous impetigo. This is characterized by small bullae, which form and burst. When this occurs in infants it may be described as pemphigus neonatorum.

Bullous impetigo is a highly contagious superficial skin infection characterized by large blisters containing many staphylococci in the superficial layers of the skin. Bullous impetigo is seen most often in infants and children under conditions where direct spread can occur (e.g. sharing of contaminated towels). Impetigo mainly occurs on the face and limbs. Extensive atypical bullous impetigo is associated with HIV infection.

The strains often produce exfoliative toxin and impetigo can therefore be considered as a localized form of scalded skin syndrome. The bullae are the result of epidermolytic toxins, which are serine proteases.

Botryomycosis

Botryomycosis is a rare chronic skin infection that resembles mycetoma or actinomycosis. The lesions may be nodular or granulomatous and may have draining sinuses.

Secondary cutaneous infection

The most common secondary infection occurs in people who have eczema, especially atopic dermatitis. Colonization of lesions in adults approaches 100%; children are colonized only slightly less often. Skin ulcers, especially those of the legs in the elderly, may also become heavily colonized by *S. aureus*. Similarly, hidradenitis suppurativa, a chronic suppurative disease of the skin that occurs in areas bearing apocrine glands, most often the axilla and groin, also yields *S. aureus*.

Paronychia

Another common *S. aureus* infection is paronychia, which involves the soft tissue around the nails.

Deep lesions

Staphylococcus aureus can cause a wide variety of deep tissue, often metastatic, infections. These infections include osteomyelitis, arthritis, endocarditis and cerebral, pulmonary and renal abscesses, and breast abscesses in nursing mothers.

Staphylococcus aureus can also cause bacterial pneumonia, which is almost always secondary (e.g. after influenza or other viral infections).

In many of these situations, diabetes mellitus, leukocyte defects or a general reduction of host defenses by alcoholism, malignancy, old age or corticosteroid or cytotoxic therapy are predisposing factors. Severe *S. aureus* infections, including endocarditis, are particularly common in intravenous drug abusers.

Wound infections

Staphylococcus aureus is a notorious cause of wound infection. Infections can be major complications following surgery. The source may be the patient's own carrier state, other carriers (e.g. doctors or nurses) or other infected patients. Whether a staphylococcal wound infection occurs following surgery depends upon a complex interaction between:

- ¦ host factors, including the status of the immune system and the presence of diseases such as diabetes mellitus;
- ¦ surgical factors, such as the disruption of tissue perfusion that accompanies the surgical procedure and whether foreign bodies are used;
- ¦ staphylococcal factors, including substances that mediate tissue adherence and invasion or that enable staphylococci to survive the host defenses and antibiotics in the tissues; and
- ¦ the use of antimicrobial prophylaxis.

Staphylococcal infections at the site of intravenous lines can result in bacteremia with metastatic infection.

Staphylococcal scalded skin syndrome

This term refers to a group of primarily cutaneous diseases (e.g. generalized scalded skin syndrome, staphylococcal scarlet fever and bullous impetigo) caused by the staphylococcal exfoliatin toxins exfoliatoxin A and exfoliatoxin B.

Toxic epidermal necrolysis (Lyell's disease) is also known as generalized scalded skin syndrome. It results from the production of exfoliatin toxin in a staphylococcal lesion, which can be quite minor. The toxin is absorbed into the bloodstream, and intraepidermal desquamation may occur at remote sites from which *S. aureus* cannot be isolated. The disease is most common in neonates and in children under 8 years of age, and epidemics may occur in nurseries. The face, axilla and groin are most often affected first but the erythema, bullous formation and subsequent desquamation of epithelial sheets can spread to all parts of the body. Generalized scalded skin syndrome is the most severe manifestation of exfoliatin-producing staphylococcal infections.

Milder versions of the same disease are staphylococcal scarlet fever, in which erythema occurs without desquamation, and bullous impetigo, in which there is only local desquamation.

Toxic shock syndrome

Toxic shock syndrome is a serious disease associated with *S. aureus*. As mentioned above it has been most commonly seen in young women during or immediately after menstruation and is associated with the use of highly absorbent intravaginal tampons. In such cases, *S. aureus* grows in large numbers in and around the tampon and liberates TSST-1. The disease is characterized by the development of high fever, vomiting, diarrhea, sore throat and muscle pain. Within 48 hours it may progress to severe shock with evidence of renal and hepatic damage. A skin rash may develop, followed by exfoliation at a deeper level than in scalded skin syndrome. Blood cultures are usually negative.

Staphylococcal food poisoning

Staphylococcal food poisoning results from the production of staphylococcal enterotoxin in food before ingestion. It is an intoxication, not an infection. Because of the heat resistance of the toxin, toxicity persists even if the food is heated to boiling. Ingestion of the food results in acute vomiting and diarrhea within 1–5 hours. There is prostration but usually no fever. Recovery is rapid, except sometimes

in the elderly and in those with other diseases. The diagnosis is suspected on the basis of exposure history and of the typical symptoms (nausea, vomiting, abdominal cramping and diarrhea). Worldwide, 30% of cases of food-borne illness are caused by staphylococci.

DIAGNOSIS

Most staphylococcal lesions contain numerous PMNLs and large numbers of *S. aureus*. These findings are readily demonstrated by a direct Gram smear of pus. This method is also advised for sputum samples from cases of staphylococcal pneumonia and stool specimens from patients who have staphylococcal enterocolitis.

Staphylococci grow aerobically on blood agar and typical colonies of 2mm or more in diameter develop overnight. Rapid and accurate identification of *S. aureus* is important. Rapid methods commonly used by laboratories to differentiate *S. aureus* from other staphylococcal species are:

- the slide coagulase factor reaction; and
- latex agglutination assays.

In the latter, latex particles coated with fibrinogen and IgG are used to detect protein A and clumping factor ('bound coagulase') on the cell surface of *S. aureus*. However, decreased sensitivity has been reported for several tests, especially in identifying MRSA.[47] An additional penicillin binding protein is produced by MRSA that is absent from susceptible strains. It is termed PBP2a or PBP2' and has reduced affinity to ß-lactam antibiotics. Therefore, latex agglutination assays have been developed in order to detect PBP2a. In addition, methods of DNA amplification such as the PCR are becoming increasingly useful for the specific and sensitive detection of *S. aureus* in clinical specimens. In most laboratories the presence of coagulase, coded for by the *coa* gene, is used to identify *S. aureus*. Polymerase chain reactions have been described in which staphylococcus-specific sequences have been amplified for species identification. In these studies DNA was extracted from preincubated colonies or single colonies were picked from agar plates and transferred directly to a PCR assay.[48]

Blood cultures from untreated bacteremia patients are usually positive after overnight incubation.

Deep staphylococcal infection such as osteomyelitis or endocarditis poses special diagnostic problems when the lesion cannot be aspirated. Antibodies to staphylococcal products such as hemolysin, nuclease and cell wall ribitol-teichoic acid can be detected by a variety of immunologic techniques. However, the sera of many individuals who do not have deep staphylococcal infections may also contain such antibodies and there is considerable overlap in the antibody titers of those who are and those who are not infected. Consequently, these assays have a low predictive value and are not cost-effective. These assays are ancillary at best and their results must be interpreted with caution.

MANAGEMENT

Superficial staphylococcal lesions are generally adequately treated by simple drainage of the lesion. Drainage is also important in the treatment of chronic staphylococcal infections.

Acute serious staphylococcal infections (e.g. pneumonia or bacteremia) require immediate antibiotic therapy. Antibiotic susceptibility tests should always be done because of unpredictive staphylococcal susceptibility patterns. A penicillinase-resistant penicillin or cephalosporin is normally used pending the results of a susceptibility test. Infections proved to be caused by strains susceptible to benzylpenicillin are best treated with that antibiotic. There is synergy between cell-wall-active antibiotics and the aminoglycosides when the staphylococcus is susceptible to both. Combinations of a ß-lactamase-stable semisynthetic penicillin and an aminoglycoside are often used for severe systemic infections, particularly in the immunocompromised host. Other antibiotics with good antistaphylococcal activity include clindamycin, rifampin (rifampicin) and fusidic acid.

Some chronic or recurrent infections of the compromised host can be controlled by administration of an oral preparation of one of the penicillinase-resistant penicillins over months or years.

As mentioned, an additional penicillin binding protein, PBP2a, is produced by MRSA. It has reduced affinity to ß-lactam antibiotics. The 2kb gene encoding PBP2a, *mec*A, is carried on a 40–60kb segment of DNA inserted into the staphylococcal chromosome. The added DNA often contains genes encoding resistance to other antimicrobials, insertion-sequence-like elements and genes regulating the expression of *mec*A. Methicillin-resistance of *S. aureus* mediates clinically inadequate susceptibility to all currently available ß-lactam antibiotics, and MRSA is typically resistant to several other antimicrobial agents, including aminoglycosides, chloramphenicol, clindamycin, fluoroquinolones and macrolides. Multiple resistance varies greatly geographically, so in planning treatment it is imperative to carry out susceptibility testing.[49] So far most clinical isolates have been susceptible only to vancomycin, which consequently has become the agent of choice for MRSA infections, although recently several investigators have described clinical isolates of *S. aureus* with diminished sensitivity to vancomycin. In the laboratory, vancomycin resistance has been transferred from *Enterococcus faecalis* to *S. aureus* and is stably expressed.[50] In addition, the first vancomycin-resistant *S. aureus* isolate has been detected in the USA. Probably the *van*A gene, coding for vancomycin resistance, was transferred from enterococci to *S. aureus*.

In addition, *S. aureus* isolates with reduced vancomycin susceptibility were described worldwide (GISA isolates, isolates with reduced susceptibility to glycopeptides). As far as has been studied, all these isolates contained thickened cell walls. Furthermore, all of them, with the exception of the Illinois isolate, showed reduced cross-linking when compared to isogenic revertants. Interestingly, only some of them showed a reduction in D-glutamic acid amidation. The data described so far on GISA strains seem to indicate that, depending on the strain studied, several independent mutations have been accumulated in these strains, which in various combinations lead to the observed resistance phenotype.

Probably, the following mechanisms are associated with the appearance of GISA strain:

- accelerated cell wall synthesis, which leads to a thickened cell wall that is capable of affinity trapping large amounts of vancomycin and shielding the membrane associated lipid II target molecules; and
- most probably caused by the accelerated cell wall synthesis, modifications of the nascent wall precursors, as amidation of the D-glutamic acid cannot be ensured as completely as under nonaccelerated conditions. The same may apply to the cross-linking reaction, although cross-linking will also be reduced because nonamidated precursors are poor substrates for the staphylococcal transpeptidation reaction.

Since lower nonamidation and lower cross-linking both lead to even higher consumption of vancomycin per unit of cell wall weight, they also contribute positively to the GISA resistance phenotype.

Should vancomycin resistance and reduced susceptibility to glycopeptides become widespread, alternative MRSA therapies will be urgently needed. Careless prescription of drugs active against MRSA will inevitably lead to the emergence of resistance and the permanent loss of a valuable agent. Responsible antibiotic prescription therefore mandates close and frequent consultation between the prescribing physician, the medical microbiologist and the infectious disease specialist.

Recently, new antimicrobial substances have been introduced that are valuable antistaphylococcal agents. Synercid® is a new, injectable, water-soluble semisynthetic derivative of pristinamycin. It is composed of a streptogramin B, quinupristin and a streptogramin A, dalfopristin, combined in a 30:70 ratio. Both compounds bind to the 23S RNA of the 50S ribosomal subunit. They act synergistically to inhibit protein synthesis and show good activity against MSRA. The new oxazolidinone linezolid clearly demonstrates an in-vitro activity against MRSA equal to that of vancomycin. In addition to this invitro potency, linezolid also possesses other promising features, most noticeably its lack of cross-resistance with any other mechanisms. Linezolid has been shown to possess a mechanism of action unique among Gram-positive organisms by inhibiting protein synthesis by binding to the 50S ribosomal subunits.

PREVENTION

For patients who have recurrent infection such as chronic furunculosis, preventive measures are aimed at controlling re-infection and, if possible, eliminating the carrier state. For adults, the use of chlorhexidine or hexachlorophene soaps in showering and washing increases the bactericidal activity of the skin. For those individuals or medical personnel found to be a source of infection to patients, anterior nasal carriage can be reduced and often eliminated. Although many different agents and drug

combinations have been used for eradicating nasal carriage of *S. aureus*, there is a consensus that topical mupirocin is presently the regimen of choice.[51] The high-level mupirocin resistance rate is less than 5%. Attempts to remove nasal carriage of MRSA with local antiseptics (e.g. chlorhexidine or povidone iodine) or antibiotics (e.g. bacitracin) have frequently failed. Chemotherapeutic agents for elimination include topical antimicrobials and antiseptics applied directly to the nares or other sites of carriage, systemic agents that achieve adequate concentrations in these sites, and antiseptic soaps for bathing.

Chemoprophylaxis is usually considered mandatory for surgical procedures such as hip and cardiac valve replacements, in which infection with coagulase-positive or coagulase-negative staphylococci can have devastating consequences for the prosthesis and for the patient. Brief high-dose chemoprophylaxis is given around the time of surgery with the intention of preventing superinfections, which often complicate longer periods of antibiotic administration.

More than 30 years have passed since methicillin resistance was first detected in *S. aureus*. Now hospitals worldwide experience MRSA as a major nosocomial pathogen. Three possible strategies in the control of epidemics of staphylococci, especially those of MRSA, have been proposed. [52] They include:

- the 'search and destroy' strategy;
- the *S. aureus* limitation technique (SALT) strategy; and
- the Scutari strategy.

The 'search and destroy' strategy has become the major tactic for MRSA control in countries where the MRSA threat was recognized in the 1980s. In the UK, the Hospital Infection Society and the British Society for Antimicrobial Chemotherapy published MRSA control guidelines in 1986 that were revised in 1990.[53] Included in these guidelines is the installation in hospitals during an outbreak of a dedicated isolation unit. Patients who have MRSA should be nursed in separate rooms or wards by designated personnel using special barrier techniques: the use of gloves and handwashing with an antiseptic detergent, disposable gowns, masks and strict environmental hygiene. This last point is especially important as MRSA is extremely resistant to drying and can survive anywhere (e.g. in tourniquets, mattresses, air-conditioning systems).[54] These guidelines were followed to control the three MRSA epidemics that occurred in the Utrecht University Hospital in The Netherlands.[55]

The SALT strategy stresses the isolation of MRSA-infected patients, whereas MRSA-colonized patients are nursed with precautions for infections only. This would be a rational strategy if one could assume that MRSA carriers do not spread MRSA and that MRSA from infection sites are more virulent. However, there is no indication that MRSA strains differ in virulence. Nevertheless, the SALT strategy may be the only manner in which hospitals with high rates of MRSA can achieve some degree of control.

The Scutari strategy was named after the hospital where Florence Nightingale worked during the Crimean War (1854–1856) and is based on simple hygiene such as handwashing and barrier nursing. This strategy, however, is insufficient to combat MRSA in hospitals. Nevertheless, these general guidelines of simple hygiene can be applied in other settings, such as nursing homes.

Is it worth spending so much time and money to try to prevent the spread of MRSA, a bacterium that often colonizes patients but only causes infections in a minority of cases, most of which can be treated? In those countries where the incidence is still low the answer should be 'yes'. Several authors have proposed that the carriage of MRSA poses an increased risk of infection over the carriage of methicillin-susceptible *S. aureus* (MSSA). Among patients on continuous ambulatory peritoneal dialysis, MRSA carriers have a higher risk for MRSA infections than noncarriers. Perioperative colonization with MRSA significantly increases the risk for postoperative MRSA infection in patients on the intensive care unit. Studying the rate of bacteremia in patients admitted to the intensive care unit, patients colonized with MSSA in the nares had a significantly increased risk for the development of *S. aureus* bacteremia but patients colonized with MRSA had a much higher risk. It seems clear that MRSA carriage constitutes a greater risk for the development of *S. aureus* infection than MSSA carriage.[56] Several studies have failed to show a difference between MRSA and MSSA in terms of virulence. It is therefore most likely that the increased infection rate observed in carriers of MRSA is primarily due to the selection of a more vulnerable group of patients who become carriers of MRSA.

Furthermore, time is running out for the treatment of MRSA infections. Already, the first vancomycin-resistant MRSA has been reported. Increased use of mupirocin as a topical therapy for MRSA carriage may also result in increased mupirocin resistance.[57]

Vaccines and new approaches to combating nosocomial infections

No vaccine is currently available to combat staphylococcal infection. Hyperimmune serum from human volunteer donors or humanized monoclonal antibodies directed toward surface components (e.g. capsular polysaccharide or surface protein adhesions) could both prevent bacterial adherence and promote phagocytosis of bacterial cells. A prototype vaccine based on capsular polysaccharide from *S. aureus* has been administered to volunteers to raise hyperimmune serum, which could be given to patients in hospital before surgery. A vaccine based on fibronectin-binding protein induces protective immunity against mastitis in cattle and might also be used as a vaccine in humans.[58]

STAPHYLOCOCCUS EPIDERMIDIS

PATHOGENICITY

Coagulase-negative staphylococci are frequently isolated from various clinical specimens in the clinical microbiology laboratory. In the past, these isolates were often discarded as contaminants. This situation has radically changed during the past two decades, with coagulase-negative staphylococci now being recognized as an important cause of nosocomial infections.[59]

Intravascular catheters, cerebrospinal fluid shunts, peritoneal dialysates, prosthetic cardiac valves and blood cultures frequently yield coagulase-negative staphylococci. Every isolate from such sources must be carefully evaluated for its clinical significance, although interpretation of the culture remains a challenge to all physicians, even in combination with the clinical presentation of the patient.

In general, coagulase-negative staphylococci become potentially pathogenic as soon as the natural balance between micro-organisms and the immune system is disturbed. This is exemplified in immunocompromised patients such as oncology patients and premature neonates in whom coagulase-negative staphylococcal infections frequently occur in association with the use of intravascular devices. The increase in incidence of infections with coagulase-negative staphylococci is probably related to the increased use of intravascular devices. Furthermore, the presence of large numbers of coagulase-negative staphylococci on the skin, their ability to adhere to biomaterial surfaces and the selection of resistant strains by the use of broad-spectrum antibiotics in hospital have all been cited as possible reasons for the increased rate of infection with coagulase-negative staphylococci.

Of all coagulase-negative staphylococci, *S. epidermidis* is the most frequently isolated species associated with catheter-related infections. The main adhesins responsible for binding *S. epidermidis* to catheters are:

- a capsular polysaccharide; and
- a protease-sensitive surface constituent from the slime-producing strains of *S. epidermidis*.

In addition to promoting adherence to foreign bodies, these adhesins may also protect coagulase-negative staphylococci against phagocytosis and prevent reaction of antibodies to cell-wall components. Specific antibodies to these adhesins may neutralize this shield and are often needed for efficient opsonization of the bacteria. Monoclonal and polyclonal antibodies against *S. epidermidis* adhesins facilitate phagocytosis of homologous *S. epidermidis* strains and protect animals against endocarditis.[60] Most important in the pathogenesis of foreign-body-associated infections is the ability of these bacteria to colonize the polymer surface through the formation of a thick, multilayered biofilm. Biofilm formation takes place in two phases. The first phase involves the attachment of the bacteria to polymer surfaces, which may be either unmodified or coated with host extracellular matrix proteins. In the second phase, the bacteria proliferate and accumulate into multilayered cell clusters embedded in an extracellular material.

The major PMNL receptor for *S. epidermidis* opsonins is the FcRIII receptor.[61] For strains that have a hydrophobic surface structure, however, antibodies alone are not sufficient for opsonization. Either C3b or iC3b is also needed, but deposition or transformation of C3 into the opsonic-active C3b on the surface of *S. epidermidis* is largely facilitated by antibodies. In hypogammaglobulinemic preterm babies, C3b generation by *S. epidermidis* may therefore be impeded and the antibody deficiency state may contribute to the persistence of *S. epidermidis* bacteremia in very-low-birth-weight infants.[62]

CLINICAL MANIFESTATIONS

Catheter-related infections

Staphylococcus epidermidis is the most frequently isolated coagulase-negative staphylococcal species in catheter-related infections, especially in children.[63] These infections have been reported in association with central hyperalimentation catheters, peripheral intravenous catheters, subclavian catheters, Hickmann and Broviac central lines and Swan-Ganz catheters. As can be seen in Figure 224.12 , there are

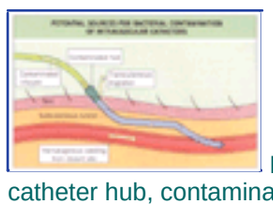

Figure 224-12 Potential sources for bacterial contamination of intravascular catheters. Bacteria gain access to the catheter by the following routes: contamination of the catheter hub, contamination of the infusate, transcutaneous migration and hematogenous seeding.

different potential sources for bacterial contamination of intravascular catheters.

The diagnosis of a catheter-related infection is often a physician's nightmare. Catheters may be colonized without local signs of infection (i.e. without gross evidence of purulence or erythema at the insertion site of the catheter). Furthermore, blood cultures may yield *S. epidermidis* without the patient showing major clinical symptoms.

A diagnosis of catheter-related infection is based on a proper collection of at least three blood cultures from different sites of venepuncture within 24 hours. In addition, culture of the intravascular catheter segment should be performed according to current standards. In most clinical microbiology laboratories, the intravascular segment of the catheter is cultured according to the method of Maki and colleagues.[64] Catheter segments are cultured after being 'rolled' over a blood agar plate and, after incubation for 18 hours at 98.6°F (37°C), the number of colony-forming units (cfu) are counted. Cultures yielding more than 15cfu/blood agar plate are associated with catheter-related bacteremia (see also Chapter 57).

Cerebrospinal fluid shunt infections

Both *S. epidermidis* and *S. aureus* strains are the most frequently encountered isolates in cerebrospinal fluid shunt infections. Infections usually occur within 2 weeks of implantation or manipulation of the shunt. Clinical manifestations are usually non-specific (see Chapter 28).

Peritonitis

About 40% of patients who have continuous ambulatory peritoneal dialysis develop peritonitis during the first year (overall incidence ranging from 0.6 to 6.3 episodes/patient-year). Criteria for diagnosis include a combination of:

- abdominal pain;
- cloudy fluid containing more than 100 white blood cells/mm^3 (the majority of which are PMNLs); and
- a positive dialysate culture.

Staphylococcus epidermidis is by far the most frequently isolated organism in patients who have peritonitis associated with continuous ambulatory peritoneal dialysis.

Endocarditis

Infections of native cardiac valves with coagulase-negative staphylococci are uncommon, accounting for only about 5% of all cases of infective endocarditis. However,

for the majority of cases of nosocomial infective endocarditis. These bacteria are the single most common cause of infections of prosthetic cardiac valves, and *S. epidermidis* is the cause of approximately 40% of cases of prosthetic valve endocarditis (see Chapter 57).

In more than 80% of patients, *S. epidermidis* valve endocarditis is associated with valve dysfunction or persistent fever during therapy. The infection is most frequently located in the valve sewing ring, resulting in complications such as dehiscence, dysrhythmia and obstruction of the valve orifice. Fever usually persists during therapy because the valve ring abscess is relatively protected from antibiotics. *Staphylococcus epidermidis* prosthetic valve endocarditis shows an indolent clinical picture without the classic endocarditis findings such as peripheral emboli and multiple positive blood cultures.

Valve dysfunction and fever are the most frequent symptoms. Most patients are infected at the time of cardiac surgery but the incubation period can vary, with a latency of 2–13 months before symptoms arise.

Orthopedic implant infections

Although the incidence of infection of indwelling prostheses is only 1–5%, such infections are devastating. Usually, infected prostheses must be removed, which may result in organic defects. Fewer than 10% of prosthetic hip and knee infections have been treated successfully without removing the prosthesis. Prosthetic joints may become infected either by local introduction of organisms or by hematogenous seeding. Staphylococci are the most frequently isolated pathogens, causing more than 70% of prosthetic joint infections (see also Chapter 53).

Infections in immunocompromised patients

The coagulase-negative staphylococci have emerged as pathogens in immunocompromised hosts, notably patients on neonatal intensive care units and oncology patients. Most coagulase-negative staphylococci infections in these patients are related to the use of foreign devices, especially central intravascular catheters (see Chapter 100).

Urinary tract infection

Among the coagulase-negative staphylococci, *S. saprophyticus* can cause spontaneous acute urinary tract infection in previously healthy people. With a few exceptions these are women aged 16–25 years. The disease appears soon after the woman becomes sexually active and the symptomatology closely resembles that of urinary tract infection caused by *Escherichia coli* (see Chapter 67).

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Chapter 225 - Streptococci and Related Genera

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Stig E Holm

INTRODUCTION

The genus *Streptococcus* consists of round or slightly oval Gram-positive cocci with a diameter of <2µm. The cocci are often in pairs or chains. The detection of cytochrome enzymes with the catalase test distinguishes members of the catalase-positive family of Micrococcaceae from the members of the family of Streptococcaceae, which are catalase negative. Most of these micro-organisms are facultative anaerobes, but some need carbon dioxide for growth and others may be strictly anaerobic. The major representatives of the genus *Streptococcus* include *Streptococcus* and *Enterococcus*.[1] The further division of streptococci has been the topic of an extensive taxonomic re-evaluation and we still lack a complete picture of the genetic relationship between different subgenera and species. Some of the older classification systems are therefore still in use despite access to more accurate genetic taxonomic methods.

The traditional division of streptococci into a-streptococci, ß-streptococci and ?-streptococci on the basis of the capacity of the bacterial colony to hemolyze erythrocytes in the blood agar medium is still considered the first step in the classification of streptococci. The clear zone produced by lysis of the erythrocytes around the colony is typical of the ß-hemolytic streptococci, but the zone may vary considerably in size (Fig. 225.1). Colonies of a-hemolytic streptococci are surrounded by a zone that is usually green (Fig. 225.2), whereas no color changes (and no hemolysis) are seen around the colonies of ?-streptococci on blood agar plates.

In addition, the colony size of the ß-hemolytic streptococci is sometimes used as a taxonomic tool. Thus, ß-hemolytic streptococci belonging to Lancefield groups A, C and G can be separated into large-colony (>0.5mm diameter) and small-colony (<0.5mm diameter) streptococci. The large-colony forms are usually designated 'pyogenic streptococci'; the small ones are representatives of the *Streptococcus milleri* group.

The further classification of streptococci involves biochemical analysis, including carbohydrate fermentation and detection of enzymatic activities. During the past 10 years genetic methods have been applied increasingly to taxonomic studies of streptococci and related organisms, for instance DNA-DNA and DNA-rRNA hybridization analysis, and small subunit (16S) rRNA sequencing. The latter technique has become the most powerful approach for delineating the phylogenetic interrelation of micro-organisms. Whereas the pyogenic streptococci (groups A, C and G) phylogenetically cluster together, the a-hemolytic streptococci fall into several different phylogenetic groups.

DIAGNOSTIC MICROBIOLOGY

Streptococci can be identified (Table 225.1) on the basis of:

- their hemolytic qualities;
- rapid antigen detection tests;
- serologic assays for detection of cell wall or capsular antigens;
- physiologic and biochemical tests; and
- molecular biologic tests.[2]

Streptococci are catalase negative and fermentation of carbohydrates is accompanied by the production of lactic acid. Streptococci are oxidase negative, a property that, together with the Gram stain, differentiates streptococci from *Neisseria* spp. Nutritional requirements are high; medium containing blood or serum is necessary for their growth (Fig. 225.3). Streptococcal colonies on blood agar can appear as mucoid mat or glistering. This appearance relates to the amount of hyaluronic acid capsule produced by the streptococci. Some of the oral streptococci may form bactericidal substances, so-called bacteriocins, which might inhibit the growth of other (ß-hemolytic) streptococcal species in the surrounding area (Fig. 225.4). [3]

The surface structure of the pyogenic streptococci (groups A, C and G), together with the highly biologically active toxins and enzymes released by the cells, forms the basis of the pathogenesis of these streptococci. The cell wall is composed of a framework of peptidoglycan similar to that found in other Gram-positive bacteria. The group-specific polysaccharide is fixed to peptidoglycan through phosphate bridges, composed of one or several units of glycerol and glycerol rhamnoside.

A rapid, preliminary diagnosis of infections caused by streptococci can be achieved by Gram stain of properly obtained specimens (e.g. sputum, cerebrospinal fluid, pus). The presence of Gram-positive cocci in chains or pairs associated with leukocytes is indicative of the diagnosis of streptococcal infections (Fig 225.5 and Fig 225.6). The skin is not normally colonized by streptococci, but streptococci are part of the commensal oropharyngeal flora; their presence in respiratory specimens collected from patients with pharyngitis has poor predictive value.

Further identification of streptococci may be achieved using extended biochemical and physiologic tests. For this purpose, diagnostic microbiologic laboratories can use standardized, commercially available laboratory kits such as API-20 Strep and Rapid Ig 32 Strep (Fig. 225.7). [4] Generally, these tests have high stability and good reproducibility between different laboratories and there is a database for interpretation of the reaction patterns.

For special epidemiologic purposes, molecular biologic typing techniques may be employed but these are not yet used in routine diagnosis.

ß-Hemolytic streptococci

The ß-hemolytic streptococci have long been considered the main pathogenic streptococci. Table 225.2 illustrates the characteristics of ß-hemolytic streptococci in groups A, C and G, as well as those of streptococci in groups B and F, which may also produce ß-hemolytic colonies on blood agar plates.[2] [5]

Serologic analysis is based on differences in group-specific antigens, which are either carbohydrates or teichoic acids on the cell wall. This allows separation of streptococci into 20 different serogroups (the Lancefield grouping system).[6] Grouping involves a two-stage procedure with rapid acid or enzymatic antigen extraction, followed by immunospecific precipitation or agglutination reactions.

Figure 225-1 ß-**Hemolytic streptococci group A on a blood agar plate.** Note the clear ß-hemolytic zone.

Figure 225-2 a-**Hemolytic streptococci on a blood agar plate.** Note the small greenish zone surrounding the colonies, which is a characteristic of viridans streptococci.

TABLE 225-1 -- Commonly used methods for typing streptococci.

COMMONLY USED METHODS FOR TYPING STREPTOCOCCI		
Serologic methods	M typing and T typing	
	R typing	
	OF typing (inhibition of the opacity reaction) group A streptococci	
	Polysaccharide antigen typing combined with protein antigen typing in group B streptococci	
	Latex agglutination	
Molecular methods	DNA fingerprinting	• M genotyping
		• pulsed-field gel electrophoresis
		• multilocus sequence typing
		• amplified fragment length polymorphism
	Ribotyping	
Other methods	Multilocus enzyme electrophoresis	
	Whole cell protein analysis	
Rarely used methods	Bacteriophage typing	
	Bacteriocin typing	

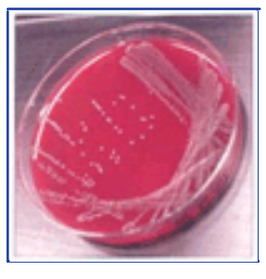

Figure 225-3 ?-**Streptococci on a blood agar plate.** Note the absence of hemolysis.

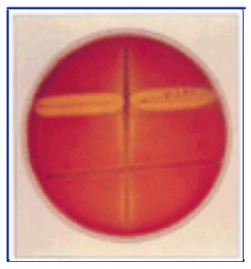

Figure 225-4 Bacterial interference. Inhibition of growth of ß-hemolytic streptococci by an a-hemolytic *Streptococcus* sp. No such inhibition is seen with other streptococci.

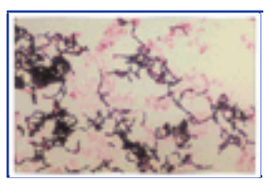

Figure 225-5 Gram-stain of ß-hemolytic streptococci group A.

Commercial kit systems in which group-specific antisera (usually containing A, B, C, D, F or G antibodies) are coupled to latex beads are now widely used for identification of ß-hemolytic streptococci.[7] In a positive test the latex particles clump together; in a negative test they stay separate, giving the suspension a milky appearance (Fig. 225.8).

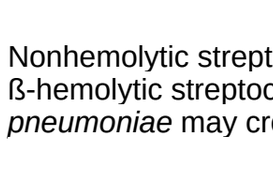

Figure 225-6 Gram stain of a sputum sample infected with *Streptococcus pneumoniae.*

Nonhemolytic streptococci do not possess Lancefield cell wall grouping antigens, but some strains may possess similar antigens that show cross-reaction with ß-hemolytic streptococcal group-specific antisera. Thus, proper assessment of hemolysis is essential for the reliability of the test because, for instance, *Streptococcus pneumoniae* may cross-react with group C latex reagent.

Group A streptococci

Cell wall antigens

From the blood agar plates, suspected hemolytic streptococcal colonies are subjected to Lancefield grouping as described above. Typing of group A streptococcal isolates is reserved mainly for laboratories specializing in streptococcal diagnostics and research. The cell wall proteins of group A streptococci that are used in strain typing include T protein and M protein. T protein is used for classification purposes, although it does not contribute to streptococcal pathogenesis. All group A streptococci carry one or more T antigens, which have a correlation with the M protein. The presence or absence of opacity factor divides the streptococci into two groups, which further facilitates a final typing by the T agglutination test. M antigen, a potent virulence factor, is the basis for the further serologic and genetic identification of more than 100 types.[8] Using specific antisera against these M proteins and sequence typing, more than 90% of fresh isolates of group A streptococcal isolates can be typed. Thus, subclones within group A streptococci carrying the same M protein have been described. However, the discriminatory power of these sera depends on the quality of the antisera and the size of the serum panel. This means that many isolates will be registered as 'nontypable'. This is also true for isolates that have lost the M protein or express only very low amounts of M protein on their surface.

Streptococcal typing by M-genotyping has been useful not only as a tool for general epidemiologic studies but also in analyses of correlation between streptococcal types and a clinical condition (see Clinical manifestations, below). Further discrimination between isolates belonging to the same M-type may be achieved by pulsed-field gel electrophoresis, multilocus sequence typing or amplified fragment length polymorphism.

Rapid antigen detection tests

Nonculture rapid antigen kits for direct detection of group A streptococci in patient samples are widely used.[9] The grouping antigen is extracted from the throat swab using nitrous acid or enzymatic treatment, followed by a procedure to detect the antigen. Most assays use latex agglutination or other immunoassays for antigen detection. It has been shown that these tests have high specificity (>95% in most kits) but variable sensitivity (50–90%) compared with culture techniques. False-negative results are common in the nonhospital setting; specimens from patients with clinical signs of pharyngitis and a negative antigen detection test should undergo routine culturing for streptococcal identification.

The use of rapid diagnostic tests eliminates the need for further epidemiologic analysis and streptococcal surveillance, but the costs of the kits and the extra clinical time spent on carrying out the tests limit their usefulness.

Serology

Determination of antistreptococcal antibodies may be helpful for diagnosing recent streptococcal infections. Streptolysin O is a strong immunogen and antibodies against this factor can be determined for demonstration of a recent infection with group A streptococci.[10] Streptolysin O binds easily to cholesterol and this binding eliminates its hemolytic and immune-stimulating activities. Therefore, in contrast to other streptococcal infections such as pharyngitis, infection in the skin often gives poor immune response to streptolysin O. Deoxyribonuclease (DNase) is produced by group A streptococci in four different antigenic variants, designated A, B, C and D. Most strains produce the B type and antibodies against this can be determined routinely to complement the antistreptolysin O determination in streptococcal infections of the skin; anti-DNase B antibodies appear after exposure to both dermal and respiratory infections. Ideally, two serum samples (acute-phase serum and convalescent serum) are tested for antistreptolysin O and anti-DNase B; a 4-fold increase in the titer is suggested as being significant. However, antibody responses may be depressed in patients receiving early antibiotic treatment for the infection.

The group A streptococci also produce other enzymes such as nicotinamide adenine dinucleotidase (NADase) and hyaluronidase. Antibodies are developed against both these enzymes and anti-hyaluronidase determination can be used in parallel with antistreptolysin O determination to detect a previous streptococcal infection.

Biochemical and enzymatic assays

Streptolysin S, which is heat stable, is responsible for the ß-hemolysis that occurs around the streptococcal colonies in blood agar. The so-called pyr test demonstrates the presence of pyrolidonylamidase, which is almost exclusively produced by ß-hemolytic streptococci of group A and is often used for biochemical characterization of streptococci. It should be mentioned, however, that ß-hemolytic colonies of enterococci can also produce this enzyme. The differentiation between these micro-organisms is often easily done by their differences in colony size and morphology.

The Vogues-Proskauer (VP) test for demonstration of ascetoin production helps to differentiate the small colony forming ß-hemolytic streptococci belonging to the *S. milleri* group, which carry the polysaccharide antigens of Lancefield groups A, C, F and G, from the large colony forming pyogenic streptococci of these serologic groups.

Group B streptococci

Cell wall antigens

The group B streptococci can also be divided into several types based on the differences in the polysaccharide capsule of the organism. These types are designated 1a, 1b and 2–10. These capsular polysaccharides are high molecular weight polymers with straight backbone structures containing short side chains. The composition of the polysaccharide varies but contains galactose, glucose, *N*-acetylgucosamin and *N*-acetylneuraminic acid (sialic acid).[11] The

Figure 225-7 API-20 Strep tests for the identification of streptococci. (a) *Streptococcus equisimilis* ATCC 35666. (b) *Enterococcus faecium* ATCC 35667. (c) *Streptococcus mutans* ATCC 35668. (d) Interpretation scheme.

group B streptococci also have several protein antigens on the surface (designated C, R and X), which can be used to characterize the different serotypes. Their functional significance has not been defined.

Rapid antigen detection tests

Group B streptococci can be diagnosed in cerebrospinal fluid, serum and urine by agglutination assays, in which antibodies against group-specific antigens attached to latex particles recognize the group B streptococcus. These assays cannot be used to screen pregnant women for carriage of group B streptococci. Rapid tests to screen pregnant women include enzyme immunoassays, but because they are not yet sensitive and specific enough, their clinical use is limited.[12]

Biochemical and enzymatic assays

The CAMP assay is used for identification of group B streptococci. It is based on the production of an extracellular protein, the so-called CAMP (Christie, Atkins and Munck-Pedersen) factor. This factor has the capacity to lyse erythrocytes in the presence of staphylococcal ß-lysin. A ß-lysin-producing strain of *Staphylococcus aureus* is streaked at right angles to a streak of the suspected group B streptococci on a sheep blood agar plate. After incubation at 95°F (35°F)

TABLE 225-2 -- Serologic and biochemical differentiation of ß-hemolytic streptococci.[*]

SEROLOGIC AND BIOCHEMICAL DIFFERENTIATION OF ß-HEMOLYTIC STREPTOCOCCI					
Species or group	**Serologic group**	**Test**			
		Voges-Proskauer	**Pyrrolidonyl arylamidase**	**Trehalose**	**Sorbitol**
Streptococcus pyogenes	A	-	+	NA	NA
Streptococcus agalactiae	B	-	-	NA	NA
Streptococcus equi	C	-	-	-	-
Streptococcus equisimilis	C	-	-	+	-

S. zooepidemicus	C	-	-	-	+
Lancefield group G	G	-	-	NA	NA
Streptococcus anginosus	A, C, F, G or none	+	-	NA	NA
+, positive reaction; -, negative reaction; NA, not applicable. *Streptococcus anginosus* is considered a representative of *Streptococcus milleri*.					

** Data from Facklam and Washington.*[5]

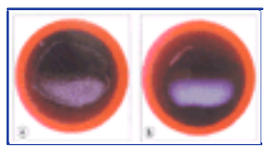

Figure 225-8 Latex agglutination for the identification of ß-hemolytic streptococci group A. (a) Positive agglutination. (b) Negative agglutination.

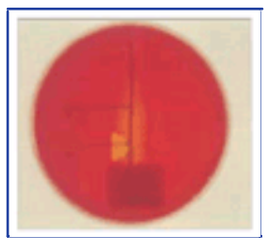

Figure 225-9 The CAMP test. Induced hemolysis of a group B streptococcus culture in the vicinity of a streak of *Staphylococcus* spp.

overnight, an arrowhead-shaped zone of ß-hemolysis will be seen in the area where staphylococcal ß-lysin and CAMP factor meet (Fig. 225.9). Otherwise, group B streptococci can be distinguished by hydrolysis of hippurate or by growth on selective media (e.g. new Granada medium).[13]

Group C and G streptococci

Group C isolates can be differentiated into species on the basis of their capacity to ferment carbohydrates (see Fig. 225.4). However, genetic analysis of group C and G streptococci has shown that human isolates of the group C species *Streptococcus equisimilis* are closely related to group G large colony human isolates and may therefore be classified in the same species. Furthermore, molecular studies have indicated that *Streptococcus zooepidemicus* probably is a subspecies of *Streptococcus equi*.[14] The large colony forming ß-hemolytic streptococci belonging to the Lancefield groups C and G have a disease spectrum and an epidemiology similar to those of the group A streptococci.[15] [16] [17] It should be added that these streptococci are not identified with the quick tests usually employed today to aid streptococcal diagnosis in primary health care. This means that if culture is not performed the diagnosis of throat infections caused by these micro-organisms will be missed.

Group D streptococci (enterococcal and nonenterococcal species)

It is now generally accepted that enterococci are sufficiently different from the other members of the *Streptococcus* genus to represent a separate genus, *Enterococcus*. This is based on the genetic information on *Streptococcus faecalis* and *Streptococcus faecium*, but further analysis by DNA-DNA and DNA-RNA hybridization as well as sequencing by 16S RNA indicate that at least 11 more species should be incorporated into this genus.

Biochemical and enzymatic assays

Most enterococci produce an a-hemolytic (greenish) zone on the blood agar plates but also nonhemolytic colonies as well as complete lysis of the blood around the colonies may be produced by some species (Fig. 225.10). Other characteristics of the enterococcus group, such as growth in broth containing 6.5% sodium chloride and hydrolysis of esculin in the presence of 40% bile salts (bile-esculin medium), help to distinguish between streptococci and enterococci. Furthermore, all strains produce leucinaminopeptidase. In most cases it is possible to extract a cell wall-associated glycerol-teichoic acid, which is identical to the streptococcal group D antigen. The physiologic characteristics of the enterococcus group mentioned above are not restricted to the genus *Enterococcus* and it is now accepted that a number of other micro-organisms may have several or all of these characteristics, including most species of the genus *Pediococcus* and several species of the genus *Leuconostoc* (see Fig. 225.8). *Leuconostoc* spp. can, for example, grow in a bile-esculin milieu and in high salt concentrations, and the group D antiserum test is also positive. However, they show intrinsic resistance to vancomycin, which is not usually the case with *Enterococcus* spp. Additionally, *Streptococcus*

Figure 225-10 *Enterococcus faecalis* on a blood agar plate.

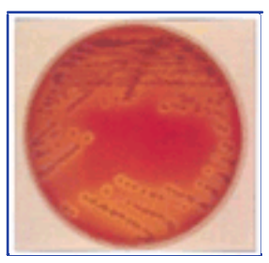

Figure 225-11 Mucoid *Streptococcus pneumoniae* on a blood agar plate.

bovis and *Streptococcus equinus*, of which the primary hosts are animals, belong to the nonenterococcal group D streptococci. *Streptococcus bovis*, unlike the enterococci, does not grow in 6.5% sodium chloride.

a-Hemolytic streptococci

Streptococcus pneumoniae (or pneumococci) are facultative anaerobic bacteria that are usually found as lancet-shaped cells in diploid form or in short chains. In order to be reliable, sputum specimens tested for the presence of pneumococci should be devoid of saliva containing epithelial cells and viridans streptococci, the most prevalent organisms among the oral flora.

Cell wall antigens

Differences in the capsular polysaccharide structure among the isolates allow discrimination between 84 different pneumococcal serotypes. The appearance of pneumococcal colonies depends on the degree of encapsulation; heavily encapsulated strains form larger colonies and appear very mucoid (Fig. 225.11). In Gram stains the capsule is visible as a pink halo or a nonstaining zone around the bacteria. On prolonged incubation, colonies on blood agar plates typically appear as checker pieces surrounded by an intensely greenish color (the a-hemolytic zone).

Rapid antigen detection tests

Soluble pneumococcal capsular polysaccharides can be rapidly detected in infected body fluids by very sensitive immunologic assays. This is especially beneficial in patients who have pneumococcal meningitis. In about half of meningitis patients, cultures may be negative after the administration of even a single dose of antibiotics, yet the antigen detection tests will remain positive.

Biochemical and enzymatic assays

Pneumococci are fastidious and some strains also need carbon dioxide for good growth. They produce an autolytic enzyme (L-alanine-uramylamidase), which has a capacity to hydrolyze the cell wall. This enzyme is activated by detergents such as bile, which results in the lysis of the cell. Optochin (ethylhydrocupreinhydrochloride) inhibits the growth of most pneumococci, in contrast to other a-hemolytic streptococci, and is thus an easy method for distinguishing between these species.

Serology

In routine diagnosis, serologic tests for pneumococcal infections are of limited clinical value because acute and convalescent sera are usually not available and only a limited number of antigens can be used. However, using the C polysaccharide and pneumolysin as antigens, antibody tests have been developed. Unfortunately, antibodies against C polysaccharide develop poorly in children, but in adults an enzyme-linked immunosorbent assay (ELISA) technique based on the C polysaccharide has been valuable for the diagnosis of pneumococcal pneumonia. Determination of antibodies against pneumolysin by ELISA has been applied successfully to establish the pneumococcal etiology in adult patients with pneumonia.

Molecular biology

Development of rapid DNA diagnostic tests such as polymerase chain reaction (PCR) for detection of pneumococci in middle-ear fluid are in progress. [18]

Viridans group streptococci

Traditionally, the name viridans streptococci was given to a-streptococci isolated from the oral region but lacking the Lancefield grouping antigen. The term viridans is not quite correct as this group of streptococci are also nonhemolytic (not producing a green zone of hemolysis in the agar medium around the colonies).

Because viridans group streptococci are capable of inducing significant infections, especially in some high-risk patients, efforts should be made to obtain specimens free of contamination by the normal flora. Determination of the clinical significance of each isolate must be made with caution because such determinations influence the need to direct therapy against these organisms.

Because the viridans streptococci give poor and variable reactions in serologic and conventional physiologic tests and, in many cases, are of relatively low virulence, they have been studied less extensively than the ß-hemolytic streptococci. In selected cases of bacteremia or endocarditis it may be clinically helpful to identify viridans streptococci. The taxonomic classification of these streptococci has caused confusion for a long time but was substantially simplified by a scheme worked out by Facklam and Washington (Table 225.3).[5] The identification of these streptococci has long since been recognized as unsatisfactory, with the result that a significant proportion of these isolates remain misidentified or unidentified. [4] [19] [20]

Biochemical and enzymatic assays

Although they are named viridans, these organisms do not all produce a-hemolysis; the property of green coloration on blood agar is affected by the growth medium used.[4] Commercially developed kits may be of great help in the species identification of viridans streptococci, but misidentification or lack of identification of members of the *S. milleri* group, *Streptococcus oralis*, *Streptococcus mitis*, *Leuconostoc* spp. and other *Streptococcus*-like organisms is a significant

TABLE 225-3 -- Biochemical identification of viridans group streptococci.*

BIOCHEMICAL IDENTIFICATION OF VIRIDANS GROUP STREPTOCOCCI						
Species or group	**Test**					
	Mannitol	**Sorbitol**	**Voges-Proskauer**	**Arginine**	**Esculin**	**Urease**
S. mutans (includes *S. cricetus*, *S. downei*, *S. ferus*, *S. macace*, *S. rattus* (arginine positive), *S. sobrinus*)	+	+	+	-	+	-
S. salivarius (includes *S. intestinalis*, *S. vestibularis*)	-	-	+	-	+	±
S. sanguis (includes *S. gordonii*, groups H and W streptococci)	-	- (+)	-	+	+	-
S. mitis (includes *S. mitior*, *S. oralis*, *S. sanguis* biotype II)	-	-	-	-	-	-
S. anginosus (includes *S. intermedius*, *S. constellatus*, *S. milleri*, DNA groups I and III)	- (+)	- (+)	+ (-)	+	+	-
+, positive reaction; -, negative reaction; ±, some strains positive, other strains negative; + (-), usually positive but occasional exceptions; - (+), usually negative but occasional exceptions.						

** Data from Facklam and Washington.[5]*

TABLE 225-4 -- The viridans group of streptococci.

THE VIRIDANS GROUP OF STREPTOCOCCI	
S. mutans group	*S. mutans, S. sobrinus, S. rattus, S. cricatus*
S. salivarius group	*S. salivarius, S. vestibularis, S. thermophilus*
S. milleri group	*S. anginosus, S. constellatus, S. intermedius*
S. sanguis group	*S. sanguis I, S. gordinii, S. parasanguis, S. crista*
S. mitis	*S. mitior*, *S. oralis* and the previous *S. sanguis II*, *S. mitis*
Current and previous nomenclature.[1]	

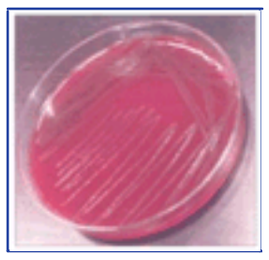

Figure 225-12 *Leuconostoc* on a blood agar plate.

problem. Five species or groups of species can be identified on the basis of various biochemical tests or fermentation capacities (Table 225.4 ; Fig 225.12). [1] [5] [21] *Streptococcus milleri* produces small, pinpoint colonies on blood agar, among which a-hemolytic and a-hemolytic strains and nonhemolytic strains with or without identifiable Lancefield antigens (A, C, F and G) are represented. At least seven distinct species in the *Streptococcus mutans* group are recognized, including *Streptococcus sobrinus, Streptococcus rattus*, and *Streptococcus cricetus*, besides the cariogenic *S. mutans.* The sanguis group, which was formerly designated *Streptococcus sanguis* I, consists of *Streptococcus sanguis, Streptococcus gordoni, Streptococcus parasanguis* and *Streptococcus crista*.

Molecular biology

Molecular biology-based analyses, such as DNA-DNA or RNA-DNA hybridization and restriction fragment length polymorphism analysis of rRNA genes (ribotyping), have helped to clarify the taxonomy of viridans streptococci.[22] The streptococci belonging to the *S. mitis* group were earlier recognized as *Streptococcus mitior, S. sanguis II* and *Streptococcus oralis*. In the *S. milleri* group, the species *Streptococcus anginosus, Streptococcus intermedius* and *Streptococcus constellatus* are now listed, based on similarities found in genetic studies.

Nutritionally variant streptococci (Abiotrophia)

A subgroup of the viridans streptococci has been isolated from patients who have endocarditis and otitis media. These are nutritionally deficient, being dependent on pyridoxal or vitamin B6. They are also designated thiol-dependent streptococci and symbiotic streptococci.[23] These bacteria will not grow on ordinary media, which usually lack pyridoxal, but they will grow in the vicinity of other micro-organisms that produce this substance (e.g. *Staphylococcus aureus, Escherichia coli* and members of several enterobacter groups). Like other viridans streptococci they are normal inhabitants of the oral flora. Nutritionally variant streptococci should be suspected when Gram stains of specimens reveal the presence of cocci but cultures are negative. [24]

Streptococcus-like organisms

Streptococcus-like organisms can be found in the genera *Aerococcus, Facklamia, Pediococcus* and *Leuconostoc*. They have a particular resemblance to the viridans streptococci and share many characteristics with the viridans group of streptococci (Table 225.5). These micro-organisms have a low pathogenic potential but can be found in immunocompromised patients and especially in patients with endocarditis. They can be recovered from wounds, urine and saliva and should be recognized because they may be resistant to vancomycin; this is true for *Pediococcus* and *Leuconostoc* spp. The identification of *Facklamia* spp. is problematic because none of the biochemical testing systems currently include them in their databases.

EPIDEMIOLOGY

Streptococci are widely distributed in nature and can be found in animals and humans as part of the normal flora in the respiratory tract as well as the gastrointestinal and urogenital tracts. They are

TABLE 225-5 -- Characterization of viridans streptococcus-like bacteria.

CHARACTERIZATION OF VIRIDANS STREPTOCOCCUS-LIKE BACTERIA						
	Growth in 6.5% sodium chloride	**Growth in bile esculin**	**Pyrrolidonyl arylamidase**	**Leucinamino-peptidase**	**Hippurate**	**Vancomycin**
Aerococcus spp.	+	V	+	-	+	S
Pediococcus spp.	V	+	-	+	+	R
Leuconostoc spp.	V	V	-	-	+	R
Facklamia spp.	+	-	+	+	V	S
V, variable; S, susceptible; R, resistant.						

also the causative agents of various types of infections with or without late sequelae. [25] [26] [27]

ß-Hemolytic streptococci of groups A, C and G

ß-Hemolytic streptococci of groups A, C and G are often found in the upper respiratory tract, especially in children aged between 5 and 15 years, although people of all ages may be infected. The frequency of streptococcal-induced upper respiratory tract infections and especially pharyngitis and tonsillitis is higher during the early autumn months and the spring.[25] [26] [27] This seasonal variation is fairly constant over the years but fluctuations between years have been noted, especially before the antibiotic era when it was easy to follow the fluctuation in scarlet fever epidemics, which varied widely. Infections in the upper respiratory tract are transferred to the surroundings via droplets and also directly through physical contact; a higher number of streptococcal upper respiratory tract infections is found in highly populated areas in narrow localities. Spread among family members and in classrooms is therefore a common finding. In residential schools and detention centers pharyngeal carriage rates approach 50%. Except in epidemics, skin carriage is not common. However, in certain geographic areas where streptococcal pyoderma is common, skin colonization rates may be as high as 40%. Colonization rates are higher in patients who have skin diseases, such as eczema and psoriasis, and wounds. Children who have streptococcal pharyngitis may excrete this organism in their feces or carry it in the perianal region, which may be the cause of wide-spread streptococcal infections in closed facilities.

Large numbers of pyogenic streptococci are shed in the immediate environment, where the bacteria may be cultivated from clothing as well as sheets and mattresses belonging to the infected person. This is important, especially in the treatment of patients who have skin infections such as impetigo. Recurrent streptococcal throat or skin infections is a common finding within families or institutions. In the search for sources, pets as well as family members are usually incriminated. Generally, pets are not a common source of reinfection because group A streptococci are highly host specific. Otherwise, group C streptococcal infections can be acquired by close contacts with infected horses and group G streptococci can be obtained from dogs, in which group G streptococci are the dominant cause of streptococcal pharyngitis (see Chapter 91).

In a given population only a limited number of M types are prevalent and the population gradually seems to acquire immunity to these. New types seem to enter the population when the immunity against these diminishes below a certain level.

Scarlet fever used to be a frightening disease, but during the past few decades it seems to have had a mild course in the Western world. However, since the mid-1980s a worldwide increase in the incidence of severe group A streptococcal disease has been reported, including rheumatic fever, cellulitis, necrotizing fasciitis and other invasive forms. These serious infections were reported as being associated with the reappearance of M1 and M3 strains and the production of pyrogenic exotoxins A and B although subsequently other exotoxins have also been implicated.[26] [28] [29] Food-borne epidemics (especially carried by milk) were common before pasterurization and were usually caused by group A streptococci, but groups C and G streptococci were also implicated on occasion. Food-borne infection can cause epidemic outbreaks of streptococcal infections such as scarlet fever and pharyngitis.

Recently, *Streptococcus iniae*, a fish pathogen that produces ß-hemolytic colonies on blood agar, has been reported as an additional cause of cellulitis and invasive soft tissue infections.[30]

Group B streptococci

Group B streptococci are less commonly found in the upper respiratory tract, but their presence in the vagina of women aged between 15 and 45 years is rather common. Their numbers fluctuate and are higher before the monthly menstrual period and in pregnancy.[3] [31] [32] Isolation frequencies as high as 30–35% have been recorded in some surveys. During delivery, vaginal colonization causes transmission to the infant in 50–75% of cases. A small number of these infants will develop symptoms of disease (see Clinical manifestations, below).

Enterococci

Enterococci are the most prevalent aerobic cocci in the bowel. Resistance against glycopeptides is emerging, especially in *E. faecium* isolated in intensive care units and nursing homes. This increase in resistance is thought to be associated with the administration of high doses of glycopeptides. In many hospitals, the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) was accompanied by a similar increase in the prescription of vancomycin. In addition, glycopeptides have been used as growth promoters in the livestock industry, thus inducing resistant enterococci in poultry products for human consumption.

Streptococcus pneumoniae

Streptococcus pneumoniae is an obligate parasite in humans, who may be colonized at a very early stage of life. The pneumococcus is a regular inhabitant of the nasopharynx in humans, especially among children, although adults are also frequently colonized. In certain populations carrier rates of 70% are found. In general, the rate of disease in a population depends on the frequency with which invasive serotypes are carried in the nasopharynx. [33] Nasopharyngeal carriage is often accompanied by the development of protection against infection by the same serotype. Carriage rates are inversely related to age and the levels of anticapsular antibody, and thus it is clear that the immune status of the host is an important determinant of the prevalence and longevity of carriage.[33] Like the ß-hemolytic streptococci, pneumococci are spread from person to person by sneezing and coughing. Of the 84 capsular types in the pneumococci, some seem to be more invasive than others (e.g. type 3 is among the most invasive strain types). [34] The capsular types 6a, 6b, 14, 19a, 19f and 23f are the dominant types found in pneumococcal

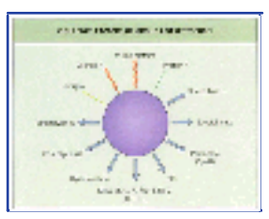

Figure 225-13 Virulence factors of group A streptococci. Spe, streptococcal pyrogenic exotoxins; SIC, streptococcal inhibitor of complement-mediated lysis; Ssa, streptococcal superantigens.

infection during the first 2 years of life, whereas in adults these types and the types 1, 3, 4, 7f, 8, 9, 10a, 11a, 12f, 14, 15b, 17f, 18c, 20 and 22f seem to cause the greatest number of bacteremic infections recorded in the USA.

A viral infection may predispose to a pneumococcal infection; this seems to be the case in children, who very often get pneumococcal otitis media 1–2 weeks after a virus infection. Support for this theory might be the finding in vitro that certain strains of adenovirus enhance the adherence of pneumococci to human respiratory tract epithelial cells.[31] Consequently, pneumococcal infections vary with the season in relation to the frequency and type of virus infections.

Certain penicillin-resistant pneumococcal clones may have a strong epidemic potential owing to their relative resistance to penicillin and other commonly used antibiotics, showing a facilitated spread in subpopulations regularly exposed to both the pneumococcus and to numerous antibiotics.[35]

Viridans group streptococci

Viridans group streptococci are fairly constant in the upper respiratory tract and dominate the aerobic normal flora in this region in the same way that enterococci dominate the aerobic normal flora in the gut. Viridans group streptococci are part of the normal flora of human mucous membranes in the mouth and upper respiratory tract as well as in stool samples.

PATHOGENESIS

Group A streptococci

The virulence factors of group A streptococci comprise both surface structures and proteins released from the cells during growth (Fig. 225.13).

Cell wall antigens

According to some scientists the M protein is a prerequisite for the binding of group A streptococci to epithelial cells in the skin, and F protein, another surface protein, is responsible for the binding to the epithelial cells in the airways, probably by binding to fibronectin.[32] Biochemically, M protein is an a-helicoidal structure similar to some muscle proteins such as myosin and tropomysin (Fig. 225.14 and Fig. 225.15). [36] The M protein has also been shown to cross-react

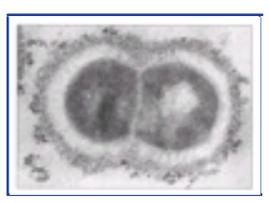

Figure 225-14 Electron microscopy of group A streptococcus. The fuzzy M protein layer can be seen protruding from the cell wall.

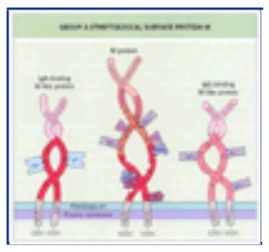

Figure 225-15 Group A streptococcal surface protein M. A, B and C represent various regions of the M protein characterized by different degrees of variability of the protein structure. H, S, A and Fib denote human serum albumin and fibrinogen binding structures, respectively.

immunologically with these contractile proteins.[36] [37] [38] It has been speculated whether this is of significance for the pathogenesis of rheumatic fever. A type-specific immunity is induced during the streptococcal infection and it has been shown in vaccination experiments[38] that purified M protein can be used to induce immunity. Because these antibodies cross-react with human heart muscle proteins, there has been a reluctance to proceed with these experiments in humans. Lately it has been shown that M proteins may also function as superantigens and this has further emphasized that the proteins in the M-protein family may be important virulence factors.[39]

The amount of M protein on the surface of streptococci varies within wide ranges and in some streptococci this antigen cannot be demonstrated. M-negative streptococci are phagocytosed quickly in serum milieu after being opsonized via the alternative complement pathway in the absence of antibodies against the M antigen, whereas M-positive streptococci resist phagocytosis in the absence of type-specific antibodies. The inability of M-positive streptococci to bind to phagocytosing cells efficiently indicates that C3b is not deposited on the surface of the streptococci or it is not accessible to the C3b receptor on the streptococci. The deposition of

C3b is probably regulated by factor H, for which the M antigen has a high affinity. [40]

Several other M-like factors have been identified on the surface of group A streptococci. These have been shown to bind immunoglobulins such as IgG and IgA as well as several other serum proteins, including fibrinogen, plasmin, $ß_2$ microglobulin and factor H. It has also recently been shown that some group A streptococcal strains have the ability to inhibit the activation of complement via the classic pathway by binding C4b to M-protein like structures. The way in which all these factors influence the virulence of the streptococci is not clear at present, but these factors can be seen as an expression of the complex interplay between the host and the micro-organism in group A streptococcal infections.[39] [40]

Another example of this is C5a peptidase, which is a surface protein that by its specific ability to cleave C5a hinders the activation of polymorphonuclear leukocytes and thereby contributes to the protection of the bacteria against phagocytosis. [41]

Extracellular products, toxins and enzymes

Group A streptococci produce a great number of extracellular products (see Fig. 225.13). Streptolysin O is an oxygen-labile hemolysin that is biologically closely related to pneumolysin, tetanolysin and hemolysins from some other micro-organisms. Intravenous injections of this toxin in animals have demonstrated a cardiotoxic effect. It also inhibits chemotaxis, the mobility of neutrophils and the phagocytosis by the macrophages.

Streptokinase (fibrinolysin) is produced by groups A, C and G streptococci. Streptokinase binds to plasminogen in the blood, forming a complex that transforms plasminogen to plasmin, which has a fibrinolytic activity. Purified streptokinase from group C streptococci is commonly used clinically to treat thrombosis in the coronary vessels and the deep veins of the leg. The plasmin formed by the plasminogen-streptokinase complex also activates complement via the alternative pathway. It has been proposed that streptokinase from certain so-called nephrogenic streptococcal strains is related to the nephrogenic capacity of some of these isolates (Fig. 225.16). This explanation of the pathogenesis of nephrotoxicity in these organisms has recently been challenged in an experimental animal model.[42]

The pyrogenic exotoxins (scarlatina toxins) have been proposed as being responsible for the scarlatina erythema. Numerous different toxins are produced; they are designated streptococcal pyrogenic exotoxin (Spe)A, SpeC, SpeF, SpeG, SpeH, SpeJ, SmeZ, mitogenic factor (MF) and streptococcal superantigen (Ssa). (SpeB is now known to be a constitutive cysteine protease and SpeE and MF have been shown to be identical.) Most group A strains produce more than one of these toxins. Several biologic effects have been attributed to the toxins, including pyrogenicity, a decrease in the blood-brain barrier permeability, cardiotoxic effect, T-cell activation and potentiation of the effects of endotoxin. The toxins may be responsible for the development of the shock syndrome that sometimes appears after streptococcal infections.[43] [44] It has been suggested that this reaction, like many of the biologic effects, is induced by the release of cytokines because these toxins function as very active superantigens and thereby stimulate a high level of proliferation of T cells (Fig. 225.17). Patients who develop the toxic shock syndrome have been shown to have very low or undetectable levels of neutralizing antibodies against one or more of these toxins.[44] Development of the streptococcal toxic shock syndrome might be hindered if toxin-neutralizing antibodies could be administered at a very early stage (see Chapter 56).

Groups C and G streptococci

The species *S. equisimilis* (a group C streptococcal species) produces streptolysin O as well as streptokinase, and *S. zooepidemicus* and *S. equi* (also group C streptococci) form hemolysins that differ from streptolysin S and O. Group C streptococci often have a very strong

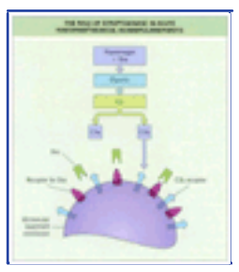

Figure 225-16 The role of streptokinase in acute poststreptococcal glomerulonephritis. Theoretic model of the pathogenesis of acute post-streptococcal glomerulonephritis as induced by streptokinase. The affinity of the streptokinase isotypes (Ska) is probably dependent on differences in the amino acid composition. This model explains the early deposition of C3b in the glomeruli that is typical of the early phase of acute post-streptococcal glomerulonephritis. Ska, streptokinase from Group A streptococci.

antiphagocytic hyaluronic capsule similar to that of group B streptococci. Group C streptococci, like group G streptococci, have not been isolated from patients who have rheumatic fever, but both can cause bacteremia and sepsis. Like the group A streptococci, they have an antiphagocytic M protein on their surface and form several enzymes. They should be regarded as pathogens.

Group B streptococci

Vaginal colonization with group B streptococci is common among adult women and transmission to the infant during childbirth occurs in 50–75% of cases. A small proportion of these infants will be infected (see Chapter 65). This indicates that particular strains or a defect in the immunity of the host play a role in the establishment of infection. It has long been known that antibodies against the capsular polysaccharide are a crucial factor for protection against group B streptococcal infections.[45] [46] In-vitro studies indicate that phago-cytosis and killing by polymorphonuclear leukocytes require the presence of both antibodies and complement. The capsular polysaccharide types 1a, 1b, 2 and 3 are clinically the most important, as they are responsible for infections during the early weeks after birth. Nonencapsulated mutants of group B streptococci belonging to type 3 have been demonstrated to lack virulence in an experimental neonatal rat model. The significance of the capsular polysaccharide for the virulence of group B streptococci was further strengthened by the finding that transplacentally acquired maternal antibodies to the group B streptococcal polysaccharides conferred protection against infection of the baby. Consequently, human experiments have been performed with

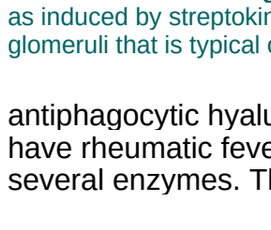

Figure 225-17 Pathogenesis of streptococcal toxic shock syndrome. In this model, patients who have opsonizing antibodies against the M antigen are protected against infection. In the absence of these antibodies, they may develop a serious infection if they are also lacking neutralizing antibodies against the streptococcal pyrogenic exotoxins which, as superantigens, may induce a cytokine cascade. This process will be hindered by the presence of toxin-neutralizing antibodies. The affinity of the toxins to the class II major histocompatibility complex of antigen presenting cells (APC) and T-cell receptors of specific Vß (variable region of ß chain of the T cell receptor) bearing T cells determines the outcome in each individual patient.

polysaccharide-protein conjugate vaccines, which were capable of inducing opsonizing antibodies and killing group B streptococci.[47] The significance of sialic acid as part of the capsular polysaccharide in the inhibition of complement activation adds to the importance of the capsule in the pathogenesis of group B streptococcal disease.

Group B streptococci also produce other potential virulence factors, such as the CAMP factor, hemolysins and neuraminidase. Although these substances may contribute to the pathogenesis, their exact role awaits further studies.

Enterococci

Although members of the genus *Enterococcus* cause many different infections in humans, very little is known about the pathogenic mechanisms involved and the virulence factors carried by these micro-organisms are less well understood than those of many other streptococci. It is clear, however, that infections caused by enterococci are often found in patients who have various types of immunodeficiencies, in patients using mechanically compromising devices, such as intravascular lines and urinary catheters, and in seriously ill patients. The increasing importance of enterococci as nosocomial pathogens may in part be explained by their natural

ability to acquire and exchange extrachromosomal elements encoding virulence traits or antibiotic resistance. Adherence to host tissue may be mediated by enterococcal aggregation substance, fibronectin-binding protein and surface carbohydrates. Extracellular toxins such as cytolysin may also contribute to the pathogenesis.[48]

Streptococcus pneumoniae

Pneumococci are a perfect example of the significance of the role that the mucous lining plays in the inhibition of potentially pathogenic micro-organisms. Thus, pneumococcal infections often occur after a respiratory tract viral infection that has damaged the epithelium, thereby helping the pneumococci to establish themselves in the mucous membranes. Factors that disrupt the normal clearing process in the airways predispose patients to pneumococcal infections in the lungs. Defects in systemic host defenses (i.e. HIV infection, asplenia, immunoglobulin deficiency) may also contribute to pneumococcal infections in the respiratory tract, sometimes causing invasive infections such as bacteremia, meningitis and endocarditis.

The virulence of *S. pneumoniae* is associated with an antiphagocytic capsular polysaccharide.[49] Thus, laboratory strains that have lost the ability to produce a capsule are nonpathogenic. The virulence capacity of pneumococci is neutralized by complement-dependent opsonizing antibodies. Protection is afforded by type-specific antibodies with opsonizing capacity. [34] [49] Not all types of pneumococci are equally invasive. Thus, during the first 2 years of life about 50% of the pneumococcal infections are caused by six serotypes (6A, 6B, 19A, 19F and 23F) and in adults these six types together with 15 others are responsible for 80% of pneumococcal bacteremia in the USA.

Besides polysaccharide, the pneumococci produce a hemolysin similar to the streptolysin O of group A streptococci, as well as hyaluronidase and a neuraminidase. None of these seems to play a direct role in the disease process.[33]

Viridans group streptococci

Viridans group streptococci can be divided into those that have a tendency to induce endocarditis and those that do not. The endocarditis-related species usually produce dextrans and this is true for the species mutants, *S. sanguis* and *S. mititor*, as well as *S. bovis*. A prerequisite for the endocarditis-inducing capacity seems to be a capacity to adhere to endothelial cells in the endocardium and this is in turn related to dextran production. In contrast, *S. salivarius* produces levan as its exopolysaccharide and is usually not related to endocarditis. Dextran production is probably not the only virulence factor because these micro-organisms can cause several other types of infections (see Chapter 59).

Patients undergoing intensive cytotoxic treatment that results in neutropenia are predisposed to severe infections with viridans streptococci, the so-called viridans shock syndrome. The mechanism is not well understood (see Chapter 100).

CLINICAL MANIFESTATIONS

Groups A, C and G streptococci

Group A streptococcus, or *Streptococcus pyogenes*, is considered the most pathogenic species of ß-hemolytic streptococci. Group A streptococci are involved in a wide variety of clinical syndromes, which can be roughly divided into:

- uncomplicated superficial infections,
- severe invasive infections, and
- postinfectious autoimmune sequelae.

Depending on climate and portal of entry, the infections are preferentially related to the upper airways or to the skin.

Uncomplicated superficial infections

Uncomplicated respiratory infections

Group A streptococci are the most prevalent cause of bacterial pharyngitis and tonsillitis, especially in school children aged between 5 and 15 years; however, people of all ages can be affected. Typically in streptococcal pharyngitis, after 1–5 days of incubation, the first clinical symptom is general malaise with excessive tiredness, headache and fever. The tonsils are swollen and red, showing exudates and deposits together with local petechiae. The patient may have

TABLE 225-6 -- Clinical manifestations of sore throat.

CLINICAL MANIFESTATIONS OF SORE THROAT	
Typical streptococcal pharyngitis	**Atypical streptococcal pharyngitis**
Temperature >100°F (38°C)	Cough
Acute throat pain	Hoarseness
Tender lymph nodes in the neck	Watery nose secretion
Redness in pharynx	Conjunctivitis
Tonsillar exudate	
Petechiae	
Sore nose	
Scarlatina	

difficulty in swallowing. Tenderness and enlargement of the anterior cervical lymph nodes are common findings. Based on the symptoms and signs, streptococcal pharyngitis is not easily differentiated from viral infections (Table 225.6). Cough is not a usual finding in streptococcal pharyngitis but is not uncommon in viral infections (see Chapter 31).

When the infecting strain produces one of the so-called pyrogenic (or erythrogenic) exotoxins, scarlet fever may be a complication of streptococcal pharyngitis. Although there are no scarlatinogenic streptococcal types the disease has a higher prevalence in streptococcal infections caused by M types 1, 3, 4, 6, 12, 18, 22 and 66. Group A streptococci are the main causative agents in scarlet fever, but streptococci of groups C and G are isolated from a small number of patients. As well as the signs observed in pharyngitis, scarlet fever is characterized by a diffuse erythematous rash over the trunk, the neck and face and the limbs. The area around the lips is generally free of erythema (circumoral palor). The rash is a diffuse light erythema that blanches on pressure. A white coating on the tongue develops and then resolves, leaving red swollen papillae, the so-called 'strawberry tongue'. Patients usually recover within about 5–7 days. The rash disappears within 10 days, followed by a characteristic discoloration of the skin during the following week. This desquamation is most pronounced on the palms and soles. In certain cases, progression of infection is observed locally causing sinusitis, otitis media, and retropharyngeal or peritonsillar abscesses.

The symptoms of group A streptococcal respiratory infections in young children may have a different clinical appearance. Young children under 3 years usually present with (mucopurulent) nasopharyngitis, which tends to a prolonged course and may be complicated by sinusitis and otitis media.

Uncomplicated skin infections

Streptococci can cause skin infections of various forms and the clinical features are determined by which skin layers are infected:

- infections immediately below the stratum corneum result in impetigo;
- infections in the epidermis can give rise to ecthyma; and
- infections in the dermis give rise to erysipelas and cellulitis.

Pyoderma (impetigo) is most common in young children and is associated with warm, moist climates and poor hygiene (Fig. 225.18). Colonization of the skin occurs first and this is followed by an infection in sites where the integrity of the skin is lost (e.g. in scratches, pierced ear lobes, paronychia or insect bites). Streptococcal infections in more superficial lesions usually result in an impetigenous type of infection, starting with small red papules and developing into vesicles with clear yellow contents. The vesicles change into pustules which, when broken, are covered with a honey-colored crust. Underneath the crust there is a slightly red, blistering surface. The lesions contain numerous bacteria and tend to spread over the body

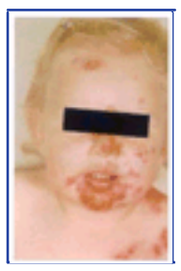

Figure 225-18 Impetigo in a child.

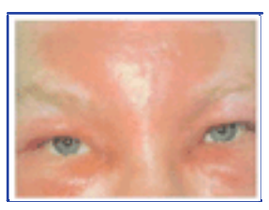

Figure 225-19 Erysipelas. Note the sharp demarcation of the affected skin.

surface, resulting in a multitude of lesions. The name 'impetigo contagiosa' indicates the contagious tendency of this infection, which is especially apparent in closed institutions such as day care centers and in overcrowded conditions. The lesions may heal spontaneously after some weeks and they usually heal without scarring. Strict hygiene precautions are necessary to prevent further spread of the disease.

Erysipelas is an acute superficial cellulitis of the skin that most often involves the face or the limbs (Fig. 225.19). Erysipelas is sharply demarcated and the affected skin is painful and appears red and swollen, with lymphatic involvement. Erysipelas occurs mostly in young children and older adults and is often accompanied by generalized symptoms such as fever and rigors and sometimes by nausea and vomiting. This is usually a relatively mild disease with few complications, but some patients develop pronounced septic symptoms and local complications such as skin necrosis or necrotizing fasciitis (see below; also see Chapter 9 and Chapter 10).

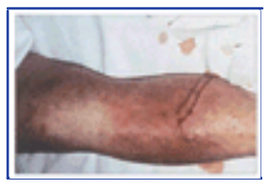

Figure 225-20 Necrotizing fasciitis caused by group A streptococci. There is only moderate erythema but at surgery there was extensive soft tissue damage.

Invasive infections

Invasive group A streptococcal infections occasionally occur. Examples are sepsis (including puerperal sepsis), pneumonia, meningitis and lymphangitis. Puerperal sepsis usually occurs as an ascending genital infection in the postnatal period after obstetric manipulation in combination with poor hygiene. Additionally, group A streptococcus is the second most common cause of nongonococcal septic arthritis after *Staphylococcus aureus*.[29] [50]

Remarkably, the most severe infections, such as necrotizing fasciitis (Fig. 225.20) with or without the toxic shock-like syndrome (analogous to the toxic shock syndrome caused by *Staphylococcus aureus*), have been increasingly seen since the mid-1980s.[26] [28] [29] [43] [44] The skin serves as the main portal of entry, followed by the mucous membranes of the throat, but in many cases the portal of entry is unknown. Early clinical features of group A streptococcal infections, before the development of toxic shock, may be flu-like in nature, with fever, sore throat, vomiting and diarrhea. Sometimes there is a history of blunt trauma. Necrotizing fasciitis is often difficult to diagnose; although redness and bullae may be visible, edema is sometimes the only superficial sign. Discrepancy between the unimpressive clinical observations and the extremely severe pain indicated by the patient is then the only clue to the correct diagnosis. Subsequent surgery may reveal massive tissue necrosis along the fascial planes. In the course of hours or days, toxic shock with hypotension and multiple organ failure may develop. A faint erythematous rash may be seen mainly on the chest. Clinically this disease resembles the staphylococcal toxic shock syndrome (Table 225.7) and it is interesting to note that streptococcal pyrogenic exotoxin A and the enterotoxin B of *Staphylococcus aureus* have a similar molecular structure. Despite adequate antibiotic treatment and intensive care, these infections are characterized by a high mortality rate of between 30% and 80% with up to 50% of the survivors having to have limbs amputated and requiring major debridement of tissue (see Chapter 10). [26] [28] [29]

Autoimmune sequelae

In some cases, streptococcal disease is followed by autoimmune sequelae, either acute rheumatic fever or acute glomerulonephritis.

Rheumatic fever (see also Chapter 60)

Rheumatic fever is seen predominantly during fall and winter in children aged between 5 and 15 years, who also show the highest incidence of streptococcal pharyngitis. Clinical manifestations may

TABLE 225-7 -- Definition for streptococcal toxic shock-like syndrome.

DEFINITION FOR STREPTOCOCCAL TOXIC SHOCK-LIKE SYNDROME
I. Isolation of GAS from:
A. a normally sterile site (e.g. blood, cerebrospinal, pleural or peritoneal fluid, tissue biopsy, surgical wound, etc.)
B. a nonsterile site (e.g. throat, sputum, vagina, superficial skin lesion, etc.)
II. Clinical signs of severity
A. Hypotension: systolic blood pressure=90mmHg in adults or <5th percentile for age in children
and
B. =2 of the following signs
1. Renal impairment: creatinine =177µM for adults or =2 × the upper limit of normal for age. In patients with pre-existing renal disease, a =2-fold elevation over the baseline level.
2. Coagulopathy: platelets =100 × 10^9 /l or disseminated intravascular coagulation defined by prolonged clotting times, low fibrinogen level and the presence of fibrin degradation products.

3. Liver involvement: alanine aminotransferase, aspartate aminotransferase or total bilirubin levels =2 × the upper limit of normal for age. In patients with pre-existing liver disease, a =2-fold elevation over the baseline level.
4. Adult respiratory distress syndrome defined by acute onset of diffuse pulmonary infiltrates and hypoxemia in the absence of cardiac failure or evidence of diffuse capillary leak manifested by acute onset of generalized edema or pleural or peritoneal effusions with hypoalbuminemia.
5. A generalized erythematous macular rash that may desquamate.
6. Soft tissue necrosis, including necrotizing fasciitis or myositis or gangrene.
An illness fulfilling criteria IA and II (A and B) is defined as a *definite* case. An illness fulfilling criteria IB and II (A and B) is defined as a *probable* case if no other etiology for the disease is identified.

vary from mild to highly aggressive. The disease is largely self-limiting. Most patients give a history of preceding pharyngitis. Acute rheumatic fever is a clinical syndrome; there is no specific diagnostic test. Guidelines for the diagnosis of initial attacks of acute rheumatic fever are described in the Jones criteria (Table 225.8). The presence either of two major criteria or of one major criterion and two minor criteria is suggestive of rheumatic fever if a case history of preceding group A streptococcal infection can be documented. Typically, patients present 10–25 days after the preceding streptococcal infection with fever, tachycardia and nonsuppurative inflammatory changes of the joints, heart, skin and subcutaneous tissue. Migratory arthritis is the most common manifestation and can be diagnosed in 70% of patients; carditis is found in about 50% of patients. There is an inverse relationship between the severity of joint involvement and the risk of developing carditis. The carditis is a pancarditis, which means that the pericardium, the myocardium and the endocardium can all be affected. Although carditis may be asymptomatic, clinical symptoms can include:

- heart murmurs,
- arrhythmias,
- cardiac enlargement,
- congestive heart failure,
- pericardial friction rubs, and
- pericardial effusion.

Repetitive group A streptococcal infections tend to cause relapses in these patients, resulting in chronic progressive damage to the cardiac valves. Carditis is the only manifestation of rheumatic fever that has the potential to cause long-term disability or death. In order to prevent valvular destruction, these patients should receive prophylactic penicillin (or alternative antibiotic) therapy.

TABLE 225-8 -- Revised Jones criteria for the diagnosis of poststreptococcal rheumatic fever.

REVISED JONES CRITERIA FOR THE DIAGNOSIS OF POSTSTREPTOCOCCAL RHEUMATIC FEVER		
Major manifestations	**Minor manifestations**	
Carditis	Clinical	Previous rheumatic fever or rheumatic heart disease
Polyarthritis		Arthralgia
Chorea		Fever
Erythema marginatum	Laboratory	Acute phase reactants
Subcutaneous nodules		Leukocytosis
Positive throat culture		Elevated erythrocyte sedimentation rate
Increased titer(s)		Elevated C-reactive protein
Recent scarlet fever		Prolonged P-R interval on electrocardiogram
	Supporting evidence of streptococcal infection	
The presence of two major criteria or of one major and two minor criteria is highly suggestive of rheumatic fever, if supported by evidence of preceding group A streptococcal infection		

Firm, painless, subcutaneous nodules may appear in association with severe carditis, usually over bony surfaces and tendons. Erythema marginatum, a nonpruritic erythematous eruption, may appear on the trunk or the proximal extremities.

In addition, the central nervous system (CNS) may be affected with emotional lability, muscular weakness and rapid, uncoordinated movements (Sydenham's chorea or St Vitus' dance). About 10% of patients develop chorea after a latent period of 3–12 months. The specificity of this disease in relation to rheumatic fever is a matter of controversy. The general distribution of the chorea is interesting. The frequency is equal between the sexes before puberty but after puberty chorea disappears in males and is not seen in military epidemics. In contrast, pregnancy aggravates and exacerbates the disease.

Strains of certain M types are strongly associated with rheumatic fever, especially strains that appear mucoid on blood agar plates. Other equally prevalent serotypes fail to initiate the disease or even to reactivate it in susceptible hosts. Rheumatic fever is associated with pharyngeal strains carrying M proteins of types 1, 3, 4, 5, 6, 12, 14, 18, 19 and 24.

In the developed world, the incidence of rheumatic fever has declined throughout the 20th century, probably owing to improved living conditions, effective antibiotic treatment of upper respiratory tract infections and good health care. However, rheumatic fever is still a problem in developing countries, where it is still the most common cause of acquired heart disease (Table 225.9).

Acute glomerulonephritis

Acute glomerulonephritis is diagnosed on the basis of the clinical presentation in combination with evidence of a recent infection (in the previous 1–3 weeks) with *S. pyogenes*. This syndrome of acute inflammation of the glomeruli comprises oliguria, hematuria, proteinuria, hypertension and edema, but the presentation may be mild. The laboratory findings include proteinuria, hematuria and reduced renal function as evidenced by renal function tests. Decreased serum complement is an important parameter to support the diagnosis.

The toxic effect of streptokinase is thought to be involved in the pathogenesis of acute glomerulonephritis. Autoimmune reactions caused by shared components between glomeruli and streptococcal membranes may also play a role. While the significance of these presumed mechanisms awaits further proof, autoantibodies to glomerular connective tissue components have been identified in patients who have streptococcal glomerulonephritis.

The prognosis in young adults is usually favorable, but sporadically acute disease is followed by chronic glomerulonephritis and subsequent renal failure. The incidence of glomerulonephritis is lower in Western Europe than in the USA, as is the incidence of pyoderma. The

TABLE 225-9 -- Rheumatic heart disease in school-age children in various regions of the world.

RHEUMATIC HEART DISEASE IN SCHOOL-AGE CHILDREN IN VARIOUS REGIONS OF THE WORLD		
Location	**Prevalence per 1000**	**Year(s) of report(s)**
USA	0.6	1965
Latin America	1.0–17.0	1968–1980
Asia	0.4–21.0	1970–1979

Africa	0.3–15.0	1970–1985
Pacific region (except Japan)	1.0–18.6	1978–1985
Japan	0.7	1979

attack rate varies within wide ranges but it seems to decrease substantially in the developed world and many cases can only be detected by slight proteinuria and confirmed by renal biopsies.

Glomerulonephritis is observed more in connection with skin isolates of M types M2, M49, M55, M57, M59, M60 and M61, and throat isolates of M1, M4, M12 and M25. Although the incidence of rheumatic fever has declined dramatically in the developed world, it is still a major concern in developing countries.

Groups C and G streptococci

Groups C and G streptococci can induce similar infections to those caused by group A streptococci, such as pharyngitis. They have been recognized as part of the normal flora of the pharynx, skin, intestinal tract and vagina.[51] [52] Group C streptococci are a common cause of infection in animals, including mastitis in cows. Human infections regularly develop after contact with animals or animal products.[16] [52] Groups C and G streptococci have occasionally been reported as the cause of nosocomial and opportunistic infections, including endocarditis, arthritis, puerperal sepsis and septic abortion, neonatal sepsis, pleuropulmonary infection, peritonitis, meningitis and cellulitis.[51] [52] [53] Malignancy, diabetes, cardiovascular disease and alcoholism are important host determinants of invasive streptococcal infections.[26] [28] [29] [51] [52] [53] [54]

Group B streptococci

Neonatal disease

Group B streptococci (*Streptococcus agalactiae*) are a leading cause of bacteremia and meningitis in neonates (see Chapter 65).[55] Most group B streptococci infections are thought to be transmitted to the infant from the maternal genital tract during delivery.[45]

Early-onset group B streptococcal infection (up to 5 days of age) is associated with a high mortality rate and occurs predominantly in immature neonates whose deliveries have been characterized by complications predisposing to infection (premature onset of labor, rupture of membranes for more than 24 hours before delivery, maternal fever or anogenital colonization of the mother).[55]

With rapid diagnosis, including screening of pregnant women, and better supportive care, mortality has decreased and is now between 15% and 40%. A substantial proportion of children with group B streptococcal sepsis develop meningitis and lumbar punctures should therefore be performed in all infants with suspected sepsis. Pulmonary involvement occurs in 40% of neonates with group B streptococcal sepsis, which cannot be distinguished from hyaline membrane disease on the basis of clinical or radiologic findings.[55]

Late-onset disease (occurring at 7 days of age or older) is infrequently associated with obstetric complications, but is acquired from exogenous sources (e.g. the mother or another infant). Typically, septic patients present with fever, poor feeding and irritability; 80% have meningitis. Death rates are much lower than in early-onset disease, but survivors of meningitis may have permanent neurologic sequelae, including central diabetes insipidus, thermal dysregulation, cortical blindness, deafness, mental retardation and generalized spasticity.[55]

As with group A streptococci, a correlation has been found between certain clinical conditions and the streptococcal type causing the infection. In early-onset neonatal infections, all four of the first serotypes are isolated at about the same frequency, whereas in the late-onset type 3 streptococci are the most prevalent.

Adult disease

In recent years, group B streptococci have been recognized with increasing frequency as a substantial cause of morbidity and mortality among adults. [56] [57] Two groups of adults are at increased risk of group B streptococcal infection: pregnant women and patients with serious underlying disease (e.g. diabetes mellitus, malignancy). [55] [57] The spectrum of group B streptococcal disease in adults includes:[55] [57]

- bacteremia with or without sepsis,
- cellulitis and other soft tissue infections,
- pneumonia,
- arthritis,
- meningitis,
- osteomyelitis,
- pyometritis,
- endocarditis, and
- urinary tract infection.

Mortality is 30–35% and is associated with advanced age (over 60 years).[56] [57]

Postpartum sepsis is generally seen as endometritis or wound infection. Because most young mothers are in good health, the prognosis is excellent when appropriate therapy is started.

Enterococci

Enterococci are the most common aerobic, Gram-positive cocci found in the bowel flora and lower female genital tract of humans and other animals. Initially thought of as merely harmless commensals because of their low intrinsic virulence compared with other organisms such as group A streptococci, enterococci have become the second most common nosocomial pathogen overall. This appears to have been caused by selection of these organisms by the widespread use of broad-spectrum antibiotics, such as the cephalosporins, which lack enterococcal activity, and acquisition of new mechanisms of antibiotic resistance.[58] [59]

Enterococci are commonly associated with urinary tract infections, particularly in patients who have indwelling catheters and are recovered from abdominal and pelvic wound infections and abscesses. However, most clinical studies suggest that patients who have normal host defenses are resistant to enterococcal infections. [58] Furthermore, there is a low frequency of enterococcal bacteremia originating from an intra-abdominal source when antimicrobial agents that do not cover enterococci are used.[58] Enterococcal bacteremia in surgical patients is almost always associated with the ongoing or previous use of antimicrobial agents that are not specific for enterococci.

The most frequently seen infections are infections of the cardiovascular system, with bacteremia related to an intravascular catheter being the most common, [58] [59] although endocarditis also occurs. Other types of infection caused by enterococci include: [59] [60] [61]

- intra-abdominal infections;
- soft tissue and skin infections;
- bone and joint infections, especially in prosthetic joints;
- infections of the CNS associated with shunts;
- deep surgical wound infections;
- urinary tract infections; and
- endometritis.

Classically, enterococcal endocarditis is a disease of older people, with a male predominance as a result of obstructive urinary tract disease; it can also affect those who have prosthetic valves.[59] Enterococcal meningitis is rarely seen; it is associated with underlying disease, immunosuppressive therapy and CNS trauma or

surgery.[61]

It has been reported that 60% of enterococcal infections are nosocomial, with half of them occurring in intensive care units. *Enterococcus faecalis* is responsible for 80–90% of enterococcal infections and *Enterococcus faecium* for 10–20%.[58] [59] [60]

Streptococcus bovis infections are most often manifest as bacteremia or endocarditis. In addition, there is a strong association with underlying colonic malignancy (up to 50% in some series), so all patients with *S. bovis* bacteremia should undergo colonoscopy.

Streptococcus pneumoniae

Streptococcus pneumoniae is an important agent of human disease at the extremities of age and in those who have underlying disease.[62] [63] [64] Pneumococcal disease is most commonly associated with an antecedent viral respiratory infection such as influenza or with chronic conditions such as chronic obstructive pulmonary disease, diabetes mellitus, congestive heart failure, renal failure, smoking and alcoholism. Immunodeficiency such as that caused by splenic dysfunction or splenectomy is an additional risk factor for the development of severe pneumococcal disease because of decreased bacterial clearance and defective production of antibodies.

Streptococcus pneumoniae is the causative agent of the majority of cases of community-acquired pneumonia and otitis media, one of the three most common pathogens in bacterial meningitis and an important cause of sepsis and sinusitis.[33] [62] Bacteremia occurs in 25–30% of patients who have pneumococcal pneumonia and in more than 80% of patients who have pneumococcal meningitis. Children with sickle cell disease and individuals with hypogamma-globulinemia or who have had a splenectomy are at greatly increased risk of fulminating pneumococcal sepsis, which has a high mortality.

Pneumococcal pneumonia

Pneumococcal pneumonia is characterized by an abrupt onset with chills and high fever. Most patients complain of a productive cough with blood-tinged sputum, and chest pain (caused by pleurisy) is common. Physical examination may reveal decreased expiratory excursion as a result of pain, and dullness to percussion. Crackles

are present in nearly all cases. Infiltrations on chest radiograph vary between occupying less than a full segment to lobar consolidation. Empyema, endocarditis, pericarditis, peritonitis and brain abscess are rare complications.[62] [65] Because the disease is related to aspiration, the infection is generally localized in the lower lobes of the lungs.

Recovery is usually rapid after initiation of appropriate antibiotics, with complete resolution in 2–3 weeks. Despite antibiotic therapy, the case fatality rate of pneumococcal pneumonia is about 5–7% in the developed world [33] and can be higher in disease caused by serotype 3, in elderly patients and in cases of bacteremia (see Chapter 34).

Pneumococcal meningitis

Pneumococcal meningitis may follow otitis, sinusitis or bacteremia. It is an infrequent cause of neonatal meningitis, but it is responsible for 15% of cases of meningitis in children and for 30–50% of cases in adults. Mortality and severe neurologic sequelae are more frequent with disease caused by *Strep. pneumoniae* than with the other common causes of bacterial meningitis (see Chapter 22).

Viridans group streptococci

Viridans group streptococci are usually found as normal inhabitants of the oral cavity, the upper respiratory tract and the bowel.[66] When these organisms gain access to the bloodstream, people who have damaged heart valves are at increased risk of endocarditis.[20] Infection is usually endogenous and is preceded by disease, dental extraction or trauma to a mucosal surface. It is often associated with an immunocompromised condition. [67] [68] However, blood cultures that are positive for a-hemolytic streptococci or nonhemolytic streptococci have no clinical relevance in many cases.[69] If the same strain is isolated from more than one blood culture bottle or is isolated on repeated occasions, the assumption of clinical relevance is strengthened (see Chapter 59).

Viridans group streptococci are the most frequent cause of endocarditis and are generally associated with a better clinical outcome than endocarditis caused by staphylococci, enterococci or fungi.[20] A special population prone to develop infections with viridans streptococci is those with hematologic malignancies, who are treated with cytotoxic drugs with high cytotoxicity for mucosal surfaces. The course of infection in these patients may vary from mild to highly severe, with shock, adult respiratory distress syndrome and death.[67]

Streptococcus milleri is a cause of abscesses in association with previous surgery, trauma, diabetes and immunodeficiency. [24] The digestive tract has been recognized as the most likely portal of entry.[70] A notable feature is the preponderance of reported cases of infection in men.[66] [71] [72] *Streptococcus milleri* has been isolated in association with:[24] [66] [70] [71]

- periodontitis,
- odontogenic abscesses,
- sinusitis,
- intracranial abscesses,
- pleuropulmonary abscesses and empyema,
- abdominal infections, including hepatic abscesses, and
- subcutaneous sepsis following human bite wounds.

These bacteria are an infrequent cause of endocarditis and pharyngitis.

Streptococcus bovis can be isolated from patients who have endocarditis and bacteremia. There is a strong association between bacteremia caused by this micro-organism and adenocarcinoma of the colon, as well as neonatal sepsis and meningitis.[73]

The nutritionally variant streptococci (pyridoxal-dependent streptococci) can also cause endocarditis. These bacteria, which are part of the normal oral flora, account for up to 5% of endocarditis cases.[23] [74] These bacteria are the cause of much higher rates of treatment failure, relapse and mortality in endocarditis than other streptococci. [74]

Infections caused by *Leuconostoc* spp. are mostly nosocomial, occurring in immunocompromised patients or neonates. Previous antibiotic therapy or interrupted integrity of the skin or mucosal barriers are usually additional risk factors in patients infected with these micro-organisms. [75] [76]

MANAGEMENT

Groups A, B, C and G streptococci

Penicillin is the traditional first-choice therapy for infections caused by groups A, B, C or G streptococci or pneumococci. *Streptococcus pyogenes* is still extremely sensitive to penicillin and erythromycin is a good alternative in case of penicillin allergy. However, an increase in the prevalence of erythromycin-resistant group A streptococcal strains has been reported in several areas. Resistance occurs in relation to an increased macrolide consumption and may reach 40%.[77]

Acute streptococcal pharyngitis should be treated; amoxicillin is an appropriate choice in the outpatient setting. Generally, there is no need to treat carriers. Persistent oropharyngeal carriage after a complete course of penicillin therapy can occur in up to 30% of patients. ß-Lactamase production by pharyngeal flora as well as penicillin tolerance of the group A streptococci have been suggested as causes of failure of penicillin in the eradication of group A streptococci.

Septic scarlet fever should be treated in order to prevent suppurative and late complications. Again, penicillin is the first-choice therapy but a synergistic effect can be

achieved by addition of an aminoglycoside. In cases of allergy, erythromycin, clindamycin and vancomycin appear to be adequate alternatives.[51]

The object of therapy in patients who have acute rheumatic fever is to reduce inflammation, to decrease fever and toxicity and to control cardiac failure. Depending on the clinical condition of the patient, treatment varies from analgesia to anti-inflammatory drugs (aspirin-like agents or, in severe cases, corticosteroids). Antibiotic treatment of streptococcal infections effectively prevents development of acute rheumatic fever and minimizes the possibility of transmission of rheumatogenic streptococcal strains. On the other hand, a favorable effect of penicillin on prevention of acute glomerulonephritis has never been demonstrated. However, in order to eradicate the nephritogenic streptococcal strain, penicillin should also be administered to these patients. Thus, it is essential to distinguish acute post-streptococcal nephritis from other forms of glomerulonephritis. In fact, no form of treatment is known that influences the generally favorable outcome of acute post-streptococcal glomerulonephritis. Therefore, therapy is symptomatic and usually confined to salt and fluid restriction to prevent circulatory overload. Sometimes, additional treatment including diuretics or antihypertensive therapy is indicated.

In life-threatening infections, such as necrotizing fasciitis and streptococcal toxic shock, a rapid diagnosis immediately followed by adequate treatment is essential. Surgery (debridement of necrotic tissue or even amputation) is the key event in the treatment of necrotizing fasciitis.[26] [28] [29] [43] The combination of penicillin and clindamycin is the therapy of choice. Preliminary data suggest that the addition of intravenous immunoglobulin preparations may be efficacious, although convincing clinical trial data are lacking. Supportive therapy in the intensive care unit, including fluid replacement, inotropics, hemodialysis and resuscitation, is also required (see Chapter 10).

In immunocompromised patients who have serious group C and G streptococcal infections, such as endocarditis, meningitis or septic arthritis, penicillin-aminoglycoside combinations are used.[51] [52] [53]

Failure of antibiotic therapy should prompt investigation to discover underlying disease or undrained foci of infection.

Group B streptococci

Group B streptococci remain susceptible to penicillin G. However, the MIC of penicillin G for group B streptococci is considerably higher (average 0.04µg/ml) than that observed for group A streptococci.[55] Susceptibility to ampicillin and cephalosporins is also uniform, whereas resistance to erythromycin and clindamycin occurs in 1–18% of isolates and tetracycline resistance is seen in 85–92% of recent isolates.[55] [56] Resistance to aminoglycosides is uniform, but the combination of penicillin and gentamicin has synergistic activity and accelerates the killing of group B streptococci *in vitro*.

Because of the inoculum-like effect and the direct relation between delayed sterilization of the CNS and the occurrence of neurologic sequelae, initial therapy of suspected group B streptococcal meningitis should include increased dosages of penicillin to ensure efficacy.

Although the duration of therapy is relatively arbitrary, most investigators recommend parenteral penicillin G for 10 days for bacteremia, 2–3 weeks for meningitis and 3–4 weeks for osteomyelitis or endocarditis.[55]

Enterococci

Antimicrobial therapy for enterococcal infections is complicated because most antibiotics are not bactericidal at clinically relevant concentrations. Combination therapy with a cell wall agent plus an aminoglycoside improves the outcome of enterococcal endocarditis and probably of enterococcal meningitis, but it may not improve the outcome in bacteremia.[59] [61] [78] High-level aminoglycoside resistance, ß-lactamase production, vancomycin resistance and high-level ampicillin resistance are increasing in prevalence.[58] [59] [78]

Enterococcus faecium is typically high-level penicillin and ampicillin resistant and this type of resistance has been reported as a

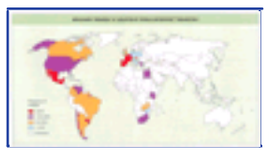

Figure 225-21 Worldwide frequency of isolation of penicillin-resistant pneumococci. Strains with MIC 0.1–2.0µg/ml are said to be intermediate resistant and high-level penicillin-resistant strains are defined as MIC > 2.0µg/ml.

significant predictor of lack of cure.[58] [59] [78] Therapy of enterococcal infections caused by vancomycin-resistant *E. faecium* is very difficult because these organisms are usually resistant to alternative antibiotics currently available.[58] [59] Several types of glycopeptide resistance phenotypes are now known.[58] The VanA phenotype is associated with acquired inducible resistance to both vancomycin and teicoplanin. VanA, which is carried on a transposon (a mobile genetic element), is transferrable to other susceptible enterococci by conjugation. The VanB phenotype, which is chromosomally mediated, inducible and transferrable by conjugation, mediates inducible resistance to vancomycin but not to teicoplanin (see Chapter 189 and Chapter 199). [58]

There has been some interest in the use of quinolones against vancomycin-resistant enterococci. Ciprofloxacin displays bacteriostatic activity against enterococci and synergistic bactericidal activity has been demonstrated in vitro when combined with ampicillin or gentamicin. However, in-vitro activity does not guarantee in-vivo success.[78] The newly developed streptogramins (quinupristin and dalfopristin) and oxazolidinones (linezolid) appeared promising against vancomycin-resistant enterococci.[79] Nitrofurantoin is active against many strains of vancomycin-resistant enterococci and should be useful for urinary tract infections.[78]

Streptococcus bovis should be differentiated from the enterococci because it is usually susceptible to penicillin alone, and both endocarditis and meningitis due to *S. bovis* have a better prognosis.[73]

Streptococcus pneumoniae

Penicillin used to be the first-choice therapy for pneumococcal disease, and in penicillin-sensitive strains it still is. For patients who have penicillin allergy, cephalosporins and erythromycin are valuable alternatives.

Resistance to penicillin is increasing in many parts of the world (Fig. 225.21) and is associated with a decreased affinity of the antibiotic for penicillin-binding proteins present in the bacterial cell wall. Penicillin resistance is thought to be due to horizontal transfer of

genes of altered penicillin-binding proteins with lowered affinity to penicillin and other ß-lactams.

At least 30% of the pneumococcal strains in the USA show intermediate resistance to penicillin (MIC 0.1–2.0µg/ml). Except for meningitis patients, these are readily treatable with increased doses of penicillin.

Of more concern is the appearance of pneumococcal isolates that are regarded as highly resistant to penicillin (MIC =2.0µg/ml). It is suggested that the extended consumption of oral cephalosporins contributes to pneumococcal resistance to penicillin. If these strains are circulating, it might be more reliable to treat severe pneumococcal infections with vancomycin.[65] However, the rate of resistance to other commonly used antibiotics such as erythromycin, tetracycline and trimethoprim-sulfamethoxazole is much greater in penicillin-resistant strains than in penicillin-sensitive strains (see Chapter 189).[35] [62] [80]

Viridans group streptococci

Viridans group streptococci have long been considered uniformly susceptible to ß-lactams, macrolides, lincosamines, rifampin (rifampicin) and vancomycin.[67] Occasionally, however, mostly under the pressure of long-term penicillin therapy, serious infections due to penicillin-resistant or penicillin-tolerant strains have been

reported.[68] [81] In endocarditis, high-dose penicillin in combination with an aminoglycoside is recommended. If MIC values are below 0.12µg/ml, the duration of this combination therapy may be limited to 14 days, but alternatively penicillin monotherapy can be given for 4 weeks. In cases of penicillin allergy, vancomycin is a good alternative.[23] [67] [74] [81] (See also Chapter 59).

Surgical drainage remains central to the management of abscesses (caused by *S. milleri*) and is often augmented by antibiotics.[70] The antibiotic of choice for infections caused by *S. milleri* is penicillin, to which all but a few strains are very sensitive.[66] [70] [71] [72] Suitable alternatives include erythromycin, clindamycin and cephalosporins.[66] [70] [71]

Leuconostoc and *Pediococcus* spp. are vancomycin resistant but are generally susceptible to clindamycin, carbapenems and gentamicin, and most are susceptible to penicillin as well.[75] [82]

PREVENTION

Several of the infections caused by streptococci are followed by complications of such a serious nature that preventive measures should be instituted. Preventive measures can include several modes of action:

- improved hygiene,
- antibiotic prophylaxis, and
- vaccination.

The choice of method should be based on the pathogenicity of the micro-organisms and the reactivity of the host. Special attention should be paid to those patients who have previously known disorders in organs that could be the focus of infection.

Group A streptococci

Resurgence of severe group A streptococcal disease in the USA during the late 1980s has renewed interest in preventive measures for patients who have rheumatic fever. As a new group A streptococcal throat infection can cause further deterioration in an already diseased heart, preventive measures must be undertaken to hinder the further progression of the disease. Penicillin or erythromycin prophylaxis has usually been recommended. Carefully controlled trials in patients who have rheumatic fever have demonstrated convincingly that a monthly intramuscular dose of benzathine penicillin produces a statistically significant reduction in the frequency of recurrent streptococcal infections and subsequent attacks of rheumatic fever. Where benzathine penicillin is no longer available, procaine penicillin may be a suitable alternative. The intramuscular route of penicillin injection is preferred to the oral route because of the higher compliance rates associated with it. The recommended dose is benzathine penicillin intramuscularly 1.2 million units every 4 weeks or penicillin V orally 250mg q12h. It is not clear how long prophylaxis should be continued, but presumably it should be for life.

In contrast, a favorable effect of penicillin prophylaxis in cases of glomerulonephritis has never been demonstrated, and relapses of glomerulonephritis occur at such a low frequency that prophylaxis is not warranted. In rare cases, patients who have streptococcal cellulitis or erysipelas with frequent recurrences should be considered for penicillin prophylaxis.

No vaccine is at present available for protection against group A streptococcal infections, but intensive research is ongoing.

Group B streptococci

Group B streptococci colonize the vagina, vulva and rectum of about 25% of fertile women. This micro-organism therefore represents a threat of bacterial sepsis to the newborn baby, especially in those cases where obstetric complications or prematurity are likely. Although the incidence of serious infections, such as septicemia and meningitis, is only around 1%, antibiotic prophylaxis has been recommended for patients who have obstetric complications, especially if earlier pregnancies have been complicated by group B streptococcal infections. Studies have clearly demonstrated that intrapartum administration of penicillin or ampicillin reduces the transmission of group B streptococci from the mother to the infant, and at least 80% of early-onset group B streptococcal disease can be prevented in this way.[83] Local prophylactic regimens have also been suggested, including vaginal washing with various antiseptics during the last month of pregnancy and at delivery, although the effect of this is still controversial (see Chapter 65).

Another approach is based upon the observation that antibodies toward the capsular polysaccharide of group B streptococci are protective in animal models and appear to correlate also with human immunity. Thus, efforts have been made to prevent group B streptococcal infections by passive or active immunization. Administration of antibodies to the mother before delivery has not given unequivocal results, but the use of vaccine produced by single or multiple group B streptococcal type polysaccharide antigens in many cases has induced protective antibodies. However, the results are often erratic and, although they are promising overall, vaccination against early-onset group B streptococcal neonatal infection is not routinely used. The use of conjugated group B streptococcal capsular polysaccharide-based vaccines may be a step forward.[47]

Streptococcus pneumoniae

In order to control the spread of (penicillin-resistant) pneumococci, restrictive antibiotic policies, isolation facilities and vaccination are indispensable. The currently available pneumococcal vaccines are composed of a mixture of 23 pneumococcal capsular polysaccharides; antibodies against these polysaccharides are protective.[84] However, the 23 vaccine serotypes do not cover more than 63% of the streptococcal types responsible for pneumococcal infections in other parts of the world. The response rate in the healthy adult population is variable.[33] [85] The majority of vaccinated adults respond by producing antibodies against about 75% of the 23 antigens. The poor immunogenicity of the polysaccharide vaccine in people at risk for pneumococcal disease is a major problem; an insufficient response is observed in asplenic or elderly people and in young children. Antibody levels subside with time and may no longer be detectable after 5 years in older adults. Therefore, patients who are

prone to develop pneumococcal disease should be vaccinated every 3–5 years. [62] [63] [85] [86]

The pneumococcal types most commonly found in children are those that seem to be less immunogenic than other types. Furthermore, polysaccharide vaccines do not evoke an antibody response in children aged under 2 years. This problem can at least be partly overcome by preparation of conjugate vaccines, in which the polysaccharides are bound to a protein carrier such as bacterial toxoids, thereby inducing a T cell-dependent immunoreactivity. Further research along these lines is presently being performed. Pneumococcal protein antigens, such as pneumolysin and surface proteins, are currently being examined as candidates for a pneumococcal vaccine, together with the polysaccharide vaccine.

Prevention of endocarditis

There is an obvious risk of endocarditis in patients undergoing dental, surgical or interventional procedures that might induce transient bacteremia. In certain high-risk groups the bacteria can adhere to the heart valves and give rise to endocarditis. This is often the case in patients who have congenital heart failure, rheumatic heart disease, valve prostheses or earlier incidences of endocarditis. In many countries, amoxicillin as a single dose 1 hour before the intervention and ampicillin combined with an aminoglycoside in extended operative procedures are recommended. The prophylaxis is mainly directed against viridans group streptococci when the intervention is localized to the oral cavity and against enterococci if the intervention is directed to the gut or the urinary tract (see Chapter 59).

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Chapter 226 - Aerobic Gram-positive Bacilli

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Timothy Mailman

INTRODUCTION

The aerobic Gram-positive bacilli are a heterogeneous group of bacteria whose members cause a wide variety of infections in both healthy and immuno-compromised individuals, and several organisms have been implicated in outbreak infections. The major pathogens are *Listeria monocytogenes*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Erysipelothrix rhusiopathiae* and *Nocardia* spp. Other organisms discussed are *Arcanobacterium*, *Oerskovia*, *Rhodococcus*, *Rothia* and other corynebacteria.

LISTERIA MONOCYTOGENES

NATURE

Listeria monocytogenes is a short, motile, Gram-positive, non-spore-forming bacillus that is the causative agent in a spectrum of human disease ranging from gastroenteritis to invasive infections — notably in neonates, the elderly and immunocompromised patients. In addition to its importance as a human pathogen, it has a significant association with abortion and encephalitis in sheep and cattle.

The genus *Listeria* is described in *Bergey's Manual of Determinative Bacteriology* as a non-spore-forming, non-acid-fast and nonencapsulated Gram-positive regular short-rod occurring singly or in short chains.[1] *Listeria* is divided into six species based on biochemical properties and DNA relatedness. *Listeria monocytogenes* is distinguished by its ability to produce acid from the sugars L-rhamnose and a-methyl-D-mannoside, lack of acid production from D-xylose and by narrow zones of ß-hemolysis on blood agar. The organism exhibits a positive Christie, Atkins and Munch-Petersen (CAMP) test using *Staphylococcus aureus* or *Rhodococcus equi* as the second indicator hemolytic strain. A positive CAMP test for *L. monocytogenes* differs from that seen for *Streptococcus agalactiae* by producing a rectangular, rather than arrow shaped, zone of accentuated hemolysis (Fig. 226.1a). Other key tests for the identification of *L. monocytogenes* include a positive catalase reaction, tumbling motility when viewed microscopically at room temperature, and an 'umbrella' pattern of motility in soft agar incubated at room temperature (Fig. 226.1b).

Growth of the organism occurs between 39°F (4°C) and 98.6°F (37°C), with fastest growth between 86°F (30°C) and 98.6°F. Cold enrichment techniques or selective agar may be used to isolate *L. monocytogenes* from contaminated specimens but are generally of limited value in the clinical laboratory.

Only *L. monocytogenes* and *Listeria ivanovii* have been associated with significant infection in humans. Six serovars of *L. monocytogenes* have been described and are distinguished on the basis of somatic O and flagellar H antigens. Serovars 1/2a, 4a and 4b are the most common isolates from animals and humans with clinical disease. In the USA, serovars 4b and 1/2a account for 95% of strains, serotype 4b being the most common overall. In Canada serovar 1/2a occurred in 42% of clinical isolates in 1988. Other serovars originate from soil or other environmental sources but are rarely seen as clinical pathogens.

EPIDEMIOLOGY

Reservoir

Listeria monocytogenes is commonly present in soil, decayed matter, wood and other material and is widespread in the natural environment.[2] [3] Spoiled silage appears to be a source of infection to animals. Fecal carriage of *L. monocytogenes* in animals allows recontamination of soil, with subsequent ingestion of contaminated silage to complete the cycle in animals.

Recent evidence suggests that almost all human cases of *L. monocytogenes* are acquired through ingestion of contaminated food. In the early and mid 1980s large outbreaks of *L. monocytogenes* occurred in both pregnant women and immunocompromised hosts. The first outbreak that demonstrated indirect transmission from an animal reservoir was reported from the Atlantic maritime provinces of Canada.[4] In this outbreak, *Listeria*-contaminated sheep manure was used to fertilize cabbages that were placed in cold storage over the winter; clinical disease developed in pregnant women and immunocompromised patients who subsequently consumed the cabbages. Outbreaks of infection attributed to consumption of many foods, including inadequately pasteurized dairy products and contaminated processed meats, have been reported since.[5]

The improved ability to culture *L. monocytogenes* with selective media provides an opportunity to examine food eaten by patients who have sporadic listeriosis. Evidence that most sporadic listeriosis cases result from ingestion of contaminated food was provided by the Listeriosis Study Group at the Centers for Disease Control and Prevention (CDC).[6] [7] Their studies implicated undercooked chicken and soft cheeses as significant sources of disease and are supported by subsequent sampling surveys performed by food regulatory agencies.

Fecal carriage of listeriosis is uncommon but ranges from 1% in hospitalized patients to 26% in household contacts of patients who have listeriosis. In the Canadian outbreak fecal surveys demonstrated carriage in approximately 5% of family contacts.[4] Several studies have shown that fever and gastroenteritis are clinical features of *L. monocytogenes* infection[5] after ingestion of contaminated food. *Listeria monocytogenes* may also cause a self-limited symptomatic gastrointestinal infection.

Occurrence

Active surveillance in the USA suggests an annual incidence of 0.7 cases per 100,000 population,[8] resulting in 1700 cases of listeriosis per year in the USA, with a mortality rate of 40%. Slightly lower figures have recently been reported from Europe. Risk of listeriosis varies among segments of the population. Immunocompromised patients and those at the extremes of age — neonates and the elderly — appear to be at highest risk. Immunocompromised patients comprise two-thirds of adult listeriosis cases. This parallels

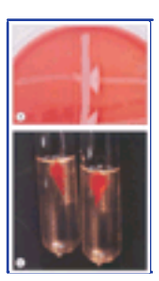

Figure 226-1 Tests for the identification of *Listeria monocytogenes.* (a) CAMP test. Enhanced hemolysis patterns for *L. monocytogenes* (left) and *Streptococcus agalactiae* (right) colonies are shown; these are growing adjacent to a streak of *Staphylococcus aureus* colonies in the center. (b) Demonstration of motility of *L. monocytogenes* grown in semisolid agar at room temperature. Note that the migration of the organism from the central stab is more pronounced at the surface of the soft agar, forming the typical umbrella-shaped pattern in both tubes.

animal models, in which risk of listeriosis is increased by administration of corticosteroids, ciclosporin A and prostaglandins. Renal transplantation appears to be a particularly significant risk factor and a nosocomial outbreak in this population has been reported. Patients who have AIDS have an estimated 1000-fold risk of acquiring invasive listeriosis. Alcoholism, diabetes, cirrhosis, hemochromatosis and decreased gastric acidity are additional risk factors. Community-acquired listeriosis, however, often occurs in patients who have no apparent predisposing conditions.

Incidence of neonatal listeriosis in the USA is approximately 13 per 100,000 live births — approximately 30% of the total number of cases of reported listeriosis. A similar incidence has been reported in studies from Europe. Epidemics of food-borne listeriosis have disproportionately involved perinatal cases. No differences in carriage rates between pregnant women and nonpregnant individuals have been found in fecal and vaginal specimens. Fecal carriage may lead to vaginal colonization and the development of 'late-onset' infection in infants born to healthy mothers.

While most large outbreaks of listeriosis have occurred in the community, case clusters of nosocomial listeriosis in both newborn infants and adults have been described. In infants, the index case typically presents with 'early-onset' infection within 1 week of birth, and subsequent cases develop 'late-onset' listeriosis after the 7th day of life. A dramatic nosocomial outbreak occurred in Costa Rica when an infant who had 'early-onset' listeriosis was bathed in mineral oil that became contaminated with the epidemic strain. A cluster of 'late-onset' disease then erupted after other infants were bathed with the same oil.[9]

PATHOGENICITY

Intracellular growth

Since the 1960s, it has been known that *L. monocytogenes* can survive within macrophages in the liver and spleen. In the late 1980s

Figure 226-2 *Listeria monocytogenes* can invade both 'professional' phagocytic cells — polymorphonuclear leukocytes and monocytes — and nonphagocytic cells. Attachment of the bacteria to the surface of nonphagocytic cells may be determined by heparin sulfate recognition proteins on the surface of the organism. Once internalized, the bacterial product listeriolysin O will lyse the phagosome, liberating the bacteria into the cytoplasm, where they proliferate. After a few hours, actin filaments polarize at one end of the bacterium, propelling it to the internal membrane surface. The organism will break through the membrane to enter the exterior of the cell or an adjacent cell.

it was also appreciated that *Listeria* can infect nonphagocytic cells — epithelial, hepatocellular and fibroblast cell lines — thus providing the organism with an intracellular environment temporarily sheltered from more hostile host defense forces.[10]

Microbial attachment to host cells is the initial step in cell invasion (Fig. 226.2). Recent work has shown that interaction with a heparin sulfate peptidoglycan receptor on the surface of cells is involved in *L. monocytogenes* adhesion.[11] The stage is then set for internalization of the organism. A family of surface proteins known as internalins are secreted in large amounts by all strains of *Listeria* capable of invading host cells.[12] Although these proteins may be an important factor associated with entrance into epithelial cells and hepatocytes, they do not explain intracellular spread and multiplication. The intracellular survival of *Listeria* requires synthesis of a specific hemolysin, termed listeriolysin O. Listeriolysin enables the organism to escape from the phagosome by lysing the phagosomal membrane. Release of *L. monocytogenes* from intracellular vacuoles precipitates both intracellular growth and polymerization of actin of the host cell around *Listeria*. Actin polymerization is important in the cell-to-cell transfer of *L. monocytogenes* and is encoded by the *actA* gene. The liberated organism thrives in the cytoplasm, which provides more abundant growth conditions. Through these mechanisms *Listeria* can enter, grow and spread inside a wide variety of cells including renal epithelial cells, fibroblasts, vascular endothelium, hepatocytes and enterocytes. By producing ActA, *Listeria* causes host-cell actin to assemble into filaments at one end of the bacterium. This 'rocket tail' provides the propulsive force for the organism to move through the cytoplasm. When the bacteria reach the cell membrane, they form filopods that become ingested by adjacent cells.

Acquired immunity

Some 3–4 days after infection begins there is normally a decrease in viable bacteria in the monocyte/macrophage phagocytic system. This heralds the onset of the immune T-cell-dependent stage of antilisterial defense, termed 'acquired resistance'.

The process leading to acquired immunity to *Listeria*, cell-to-cell interaction, cytokines and cytolytic activity is now partially elucidated (Fig. 226.3 and Fig. 226.4). In adult immunocompetent animals, *Listeria* are phagocytosed by 'professional' phagocytes —

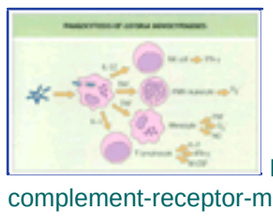

Figure 226-3 Phagocytosis of *Listeria monocytogenes*. Opsonized *L. monocytogenes* is phagocytosed by professional phagocytic cells through a complement-receptor-mediated process. Once ingested into a phagosome of a monocyte or macrophage, most bacteria are quickly killed by oxidative radicals (O_2^-), lysozyme, nitric oxide (NO) and other products of the cell. Tissue macrophages produce a variety of protein products essential for the activation of natural killer (NK) cells (interleukin(IL)-12), T cells (IL-1) and polymorphonuclear (PMN) leukocytes and other monocyte/macrophages (tumor necrosis factor (TNF)-a). These cells in turn produce cytokines, interferons (IFN), interleukins that cause cell proliferation (IL-2; macrophage colony stimulating factor (M-CSF)) or further activation of cell populations involved in elimination of the organism and of infected nonprofessional cells.

macrophages and monocytes; and by 'nonprofessional' phagocytic cells — fibroblasts, hepatocytes, etc. Macrophages take up and process listerial proteins and produce interleukin (IL)-12 and IL-1. Peptides resulting from digestion of *Listeria* are actively processed by the macrophage in the endoplasmic reticulum, where the peptides bind to major histocompatibility complex (MHC) class I molecules.[12] The *Listeria*-peptide-MHC complex is transported to the cell surface, where it is recognized by cytotoxic T cells ($CD8^+$ phenotype) and helper T-cells (Th). In the presence of IL-12, naive Th cells differentiate into Th1 cells, which proliferate, producing IL-2, interferon (IFN)-? and lymphotoxin. [13] Interleukin-12 also stimulates activation and proliferation of natural killer (NK) cells and ?/d T cells. Both cell clones produce IFN-?. Cytotoxic T cells interact with cells bearing *L. monocytogenes* antigen, causing them to lyse. Natural killer cells are further stimulated by IFN-?, inducing an enhanced ability of these cells to lyse infected fibroblasts and hepatocytes (Fig. 226.4).

Cytokines such as macrophage colony-stimulating factor (M-CSF) and tumor necrosis factor (TNF)-a also appear during the first 5 days and have been implicated as mediators of listerial clearance. Peak immunity to *Listeria* is expressed at about the sixth day of infection, which coincides with maximal T-cell synthesis of IFN-?. The role for endogenous IFN-? for resolution for *L. monocytogenes* infection has been clearly shown. [14] Tumor necrosis factor-a enhances a variety of antibacterial or antiparasitic resistance mechanisms. Several cells produce TNF-a, including NK cells; however, monocytes/macrophages are probably the most abundant source. Endotoxin (lipopolysaccharide) and other agents including mitogens, viruses, protozoa and cytokines such as M-CSF, IL-1, IL-2 and IFN-? have been identified as inducers of TNF-a. When administered before infection, TNF-a induces enhanced resistance of the host to bacterial infection. Endogenously produced TNF-a during sublethal *Listeria* infection in adult animals appears to function as an inducer of resistance,[15] suggesting that TNF-a-dependent mechanisms limits intracellular infection early in the course of infection. Down-modulation of the immune

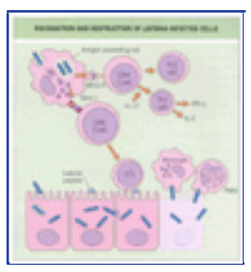

Figure 226-4 Recognition and destruction of *Listeria*-infected cells. Cytotoxic T lymphocytes and NK lymphocytes migrate to the site of infection and recognize and destroy *Listeria*-infected cells by the presence of listerial peptides on the surface of such cells. Intracellular bacteria are then exposed to the extracellular environment. Organisms in the extracellular space are efficiently phagocytosed and killed by polymorphonuclear leukocytes (PMN) and monocytes that have been primed or activated by cytokines (TNF-a, M-CSF, IL-8) and IFN-?.

response is mediated by ?/d T cells, an important role for such cells in controlling unregulated proinflammatory cytokines.[14]

Perinatal listeriosis

The pathogenesis of fetal listeriosis is not clear. Since the heaviest foci for neonatal infection are lung and gut, the fetus is probably infected by swallowing contaminated liquid as well as through the transplacental hematogenous route.[16] An ascending pathway from the lower genital tract may occur; however, infection via the transplacental route is favored by most authors. *Listeria monocytogenes* chorioamnionitis diagnosed by transabdominal amniocentesis (free from vaginal bacterial contamination) has been reported, supporting a maternal-to-newborn blood-borne route of infection.

Host response in the neonate

In newborn animals, susceptibility to *Listeria* is increased and is associated with delayed activation of macrophages. Studies on the afferent and efferent arms of the immune system in newborn mice have shown that macrophage-T-cell interaction is impaired and macrophage activation does not occur. [17]

Natural killer cells also contribute to the early response to *L. monocytogenes* infection. The proportion of mononuclear cells expressing NK cell phenotype and NK cell

activity is decreased at birth, particularly for premature infants. Both NK cell phenotype and function increase rapidly in the weeks after birth.

In adult animals, IFN and agents that induce or augment IFN production confer protection against lethal listeriosis. Synthesis of IFN, IL-2, IL-4 and IL-12, all of which modulate the immune response

and macrophage activation, is deficient in newborns. Although production of these factors may be defective, newborn animals do respond to exogenous IFN; pretreatment with IFN or its inducers protects against subsequent infection.

The ontogeny of cytokine-related host defense mechanisms has not been studied in depth. Tumor necrosis factor is decreased among newborn rats challenged with *L. monocytogenes*.[18] In this study of *L. monocytogenes* infected newborn rats, TNF-a was detected only among animals over 8 days of age. The age at which TNF-a becomes measurable corresponds to the approximate age at which increased resistance to *L. monocytogenes* occurs.

PREVENTION

Food-borne outbreaks of listeriosis are unpredictable and may occur in a wide geographic area. Therefore, reporting of sporadic cases of listeriosis to public health authorities may be the only method of distinguishing sporadic from epidemic disease. The epidemic threshold is unknown and may only be determined in retrospect. Recent studies suggesting that sporadic listeriosis is also food-borne have important public health implications.

Sampling of foodstuffs associated with sporadic cases of listeriosis is not warranted. Case-control studies to determine potential vehicles of transmission in outbreaks may help to define the source, and environmental sampling may be an important part of such outbreak investigations. Strains of *Listeria* from clinical and environmental isolates should be forwarded to a reference laboratory for appropriate epidemiologic typing. At a minimum, serotyping, phage-typing and multifocus enzyme electrophoresis typing should be performed to characterize the epidemic strain.

During an outbreak of listeriosis, high-risk groups such as pregnant women or those who have AIDS who develop a flu-like illness should be empirically treated with ampicillin and an aminoglycoside after appropriate cultures of blood, cerebrospinal fluid (CSF), sputum, rectum and vagina have been obtained. In pregnant women, amniocentesis for diagnosis of chorioamnionitis may be appropriate. If membranes have ruptured and contamination is suspected, use of selective media may enhance the isolation of *Listeria* from these patients.

Identification of early- or late-onset listeriosis in a newborn nursery should prompt appropriate epidemiologic and clinical history taking from the mother as well as postpartum cultures of rectum and vagina. Infection control precautions with gowning, gloves and careful hand-washing will prevent nosocomial transmission between infected infants and is consistent with current procedures for all forms of neonatal sepsis.

The recognition that sporadic cases of listeriosis are primarily food-borne has also prompted publication of preventive guidelines by the CDC (Table 226.1).

DIAGNOSTIC MICROBIOLOGY

Serologic evaluation

The agglutination reaction (Widal test) demonstrates antibodies against somatic O and flagellar H antigens of the various *Listeria* serovars. Unfortunately, because of the antigen complexity of *L. monocytogenes*, no agreement has been reached as to the interpretation of agglutination reactions for diagnostic purposes.

Attempts to demonstrate complement-fixing *Listeria* antibodies date back to the 1930s. In one study, serum samples collected from 32 mothers who had perinatal *Listeria* infection were compared with 128 samples from matched controls.[20] The sensitivity and specificity of the complement fixation test were shown to be 78% and 91%, respectively; however, the positive predictive value was only 75%. Thus,

TABLE 226-1 -- Dietary recommendations for preventing food-borne listeriosis.*

DIETARY RECOMMENDATIONS FOR PREVENTING FOOD-BORNE LISTERIOSIS
• Thoroughly cook raw food from animal sources (e.g. beef, pork and poultry)
• Thoroughly wash raw vegetables before eating
• Keep uncooked meals separate from vegetables, cooked foods and ready-to-eat foods
• Avoid consumption of raw (unpasteurized) milk or foods made from raw milk
• Wash hands, knives and cutting boards after handling uncooked foods
Additional recommendations for people at high risk
• Avoid soft cheeses (e.g. Mexican-style, feta, Brie, Camembert, blue-veined cheeses). There is no need to avoid hard cheeses, cream cheese, cottage cheese or yogurt
• Reheat leftover foods or ready-to-eat foods (e.g. hot dogs) until steaming hot before eating
• Although the risk for listeriosis associated with foods from delicatessen counters is relatively low, pregnant women and immunosuppressed persons should avoid these foods or thoroughly reheat cold cuts before eating
People at high risk are those who are immunocompromised as a result of illness or medications, pregnant women and the elderly.

** Adapted from Centers for Disease Control.*[19]

the complement fixation test can be regarded as reliable only in some patients who have acute *Listeria* infection.

Detection of antibodies to listeriolysin O has been used to diagnose human listeriosis. Sensitivity and specificity of the test were over 90%. Although these results were impressive, the technique is not available commercially and results have not yet been confirmed in other centers.

Isolation of the organism

Cultivation of *L. monocytogenes* is the only reliable means of proving that the cause of an infection is due to *Listeria*. Culture of venous blood, ascitic and other fluids, cervical material, urine, amniotic fluid, lochia and meconium and tissues at biopsy or autopsy offer the best chances for identifying *Listeria* in persons who have an infection. Culture of the stool is not helpful. Feces are positive for *Listeria* in 1–5% of healthy adults.

Microscopic diagnosis may be attempted by use of Gram's stain only in specimens that normally do not contain bacteria — CSF, meconium and tissue smears. The finding of short (sometimes coccoid, and in pairs) Gram-positive rods strongly supports a suspicion of listeriosis and is indicative of this infection (Fig. 226.5). *Listeria monocytogenes* may not Gram stain as well as other Gram-positive organisms. Particularly with longstanding disease or when the patient has received antibiotics, the organisms may appear Gram-negative and be confused with *Haemophilus influenzae* when observed in the CSF. In other instances, *Listeria* has been mistaken for pneumococci or corynebacteria.

The submission of clinical specimens for culture may be facilitated by the use of transport-enrichment fluid. It is, however, advisable to submit specimens such as blood, sputum, CSF and pus without any additives. For patients who have received antibiotics, the use of a commercial antibiotic-removing device in sterile body fluid cultures is useful.

CLINICAL MANIFESTATIONS

The clinical features of listeriosis have considerable variability and may mimic other infections or other disease states. Based on the most common clinical manifestations, four or more clinical groups may be distinguished.

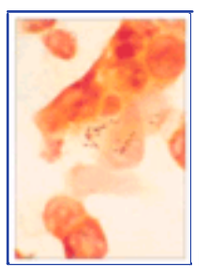

Figure 226-5 Gram stain of clinical specimen showing intra- and extracellular Gram-positive bacilli.

Listeriosis during pregnancy

Susceptibility to *L. monocytogenes* is markedly increased in pregnant animals. The predilection of *Listeria* for the fetoplacental unit and intrauterine infection is well documented.[21] [22] Maternal listeriosis can be transmitted to the fetus by an ascending or transplacental route. Early gestational listeriosis is associated with septic abortion; however, most cases of perinatal listeriosis are found after the fifth month of pregnancy, with premature delivery of a septic or stillborn infant the result.

Maternal influenza-like illness with fever and chills, fatigue, headache and muscle pains often precedes delivery by 2–14 days. Although symptoms in the mother may subside before delivery, infection and fever precipitating delivery is common. Blood cultured from such women can yield *Listeria* at the time of initial symptoms or later. Premature labour in mothers who have listeriosis is common; approximately 70% deliver newborns at less than 35 weeks of gestation. The mortality rate, including stillbirth and abortion, is 40–50%. Early treatment of *Listeria* sepsis in pregnancy, however, can prevent infection and its sequelae.

Listeriosis in the newborn infant

Neonatal listeriosis has become recognized as the most common clinical form of human listeriosis. Infection in the neonatal period is usually divided into two clinical groups defined by age: early- and late-onset infection.

Some clinical and laboratory manifestations of early-onset neonatal listeriosis are outlined in Table 226.2 , which is compiled from clinical cases in which early and late forms of the disease could be differentiated. Classically (as illustrated in Table 226.2), early-onset neonatal listeriosis develops within 1–2 days of life. However, in one outbreak involving 10 infants who had nosocomially acquired listeriosis, an atypical clinical picture was described.[9] Nine of these infants were bathed shortly after birth with mineral oil contaminated with *L. monocytogenes*. Clinical features of infection developed 4–8 days later and were similar to those seen in late-onset infection, insidious onset of illness with fever and meningitis being common.

Evidence of preceding maternal illness is often described in infants who have early-onset disease. Although some symptoms in mothers are vague and non-specific — malaise, myalgia — others are sufficiently

TABLE 226-2 -- Clinical and laboratory features of early-onset listeriosis.

CLINICAL AND LABORATORY FEATURES OF EARLY-ONSET LISTERIOSIS		
Feature		**Proportion of cases (%)**
Male sex		64
Preterm infants (gestation age <35 weeks)		63
Mortality (of live-born infants)		15
Features in infants	Meconium stain	69
	Pneumonia	62
	Anemia	62
	Thromobocylopenia (<150 × 10^9 /l)	35
	Meningitis	21
Source of isolate	Blood	73
Maternal features	Flu-like illness	45
	Blood isolate	35
Age at onset for early-onset listeriosis is under 7 days, but most cases occur at less than 2 days of age. Anemia is defined as hemoglobin less than 14g/dl and/or hematocrit less than 45. Meningitis is based on compatible clinical or autopsy findings or observation of the organism on Gram stain of the CSF. Flu-like illness is defined as the presence of respiratory symptoms, myalgia and fever occurring from 3 days to 1 month before delivery. Blood isolates are defined as of *Listeria monocytogenes* from mother's blood at any time before delivery of the infant.		

distinctive — fever, chills — to alert physicians to the risk of prenatally acquired listeriosis. Blood cultures from such mothers are often positive for *Listeria*.

Although early-onset disease may occur up to 7 days of age, most cases are clinically apparent at delivery, with meconium staining, cyanosis, apnea, respiratory distress and pneumonia. Meconium-stained liquor is a common feature in such infants and may occur at any gestational age, even less than 32 weeks. Pneumonia is also common, but radiographic features are not specific — peribronchial to widespread infiltration. In more longstanding infection, a coarse, mottled or nodular pattern has been described. Assisted ventilation is frequently necessary in such infants. Persistent hypoxia in spite of ventilatory assistance is seen in severely affected infants. Laboratory features are non-specific; a leukocytosis with presence of immature cells may be seen or, if infection is severe, neutropenia. Similarly, thrombocytopenia may also occur. Many infants are anemic, which is perhaps attributable to hemolysin produced by the organism. These laboratory and clinical features will not help to distinguish listeriosis from early-onset group B streptococcal or other bacterial infection.

In severe infection a granulomatous skin rash has been described (Fig. 226.6). Slightly elevated, pale patches measuring 1–2mm in diameter with a bright, erythematous base are seen. If such areas are biopsied, a leukocytic infiltrate with multiple bacteria is found.

Neonatal listerial infection that occurs after 7 days of life is termed late-onset infection. Although there is some overlap between early- and late-onset forms of listeriosis, the clinical pattern of the two is usually distinct. The common clinical and laboratory features of late-onset neonatal listeriosis are shown in Table 226.3 . By far the most common form of *Listeria* infection over this period is meningitis, which is present in 94% of late-onset cases. In many centers, *Listeria* ranks second only to group B streptococci as a cause of bacterial meningitis in this age group, causing approximately 20% of such infections.

Clinical features do not distinguish listerial meningitis in this age group from other causes. A striking predominance of males has been noted in most series. Fever and irritability are predominant clinical

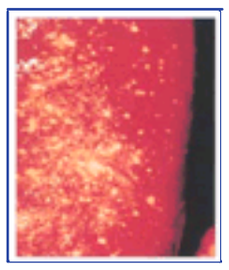

Figure 226-6 Skin rash on premature infant who has sepsis due to *Listeria monocytogenes.* This form of infection, known as granulomatosis infantisepticum, is characterized by disseminated microabscesses on the skin, spleen and liver. The elevated pale patches (1–2mm in diameter) on the skin are clearly seen in contrast to the bright erythema of surrounding skin.

TABLE 226-3 -- Clinical and laboratory features of late-onset listeriosis.

CLINICAL AND LABORATORY FEATURES OF LATE-ONSET LISTERIOSIS		
Feature		**Proportion of cases (%)**
Male sex		67
Signs at presentation	Fever	94
	Irritability	75
	Diarrhea	Occasional
Isolates	CSF	94
	Blood	17
Mean age is 12–20 days.		

features. Often, infants do not appear excessively ill and may therefore elude diagnosis for several days.

Laboratory features of late-onset infection are not specific. Cell count in the CSF is usually high, with a predominance of polymorphonuclear and band forms. Occasionally, in longstanding disease, a relatively high number of monocytes may be seen. Gram stain of CSF may not always suggest a diagnosis, both because the organism may be rare and because the morphology is atypical. Variable decolorization of Gram's crystal violet-iodine stain may result in organisms appearing as Gram-negative rods. The appearance of organisms as illustrated in Figure 226.5 is characteristic of listeriosis in the early phase of severe infection.

Mortality in late-onset newborn infection is generally low, unless diagnosis is delayed by more than 3–4 days after onset of infection. Long-term sequelae and morbidity are uncommon.

Central nervous system infection

Bacteria invade the central nervous system through a variety of mechanisms. For *L. monocytogenes*, invasion of the central nervous system may be mediated by bacterial invasion of endothelial cells at the blood-brain interface followed by cell-to-cell spread of the organism in microvascular endothelial cells. Some investigators have also speculated on a 'Trojan horse' mechanism, whereby monocytes or neutrophils infected with *L. monocytogenes* transport organisms to the central nervous system, where they are released when these cells are lysed.[23] Among adults who have central nervous system infection, a high incidence of prolonged ataxia, hydrocephalus and cerebellar atrophy visualized via computerized tomography (CT) have been reported. Rhombencephalitis and brain abscess have also been seen and appear to have a good prognosis.[22]

Other clinical forms of infection

Papular cutaneous lesions are often observed in newborns when listeriosis is disseminated. These are to be distinguished from the primary skin lesions caused by *Listeria* observed in adults, which are the result of direct contact, such as the handling of a cow's placenta after abortion by a veterinarian or farmer.

In the course of the septicemic form, accompanying conjunctivitis is sometimes observed. Other unusual forms of listeriosis such as endocarditis and septic arthritis have been described in adults but appear to be rare in infants.

THERAPY

Listeria remains susceptible to antibiotics commonly used in its treatment. However, the high mortality rate and risk of relapse have prompted a search for newer therapeutic regimens, including fluoroquinolones, trimethoprim-sulfamethoxazole (co-trimoxazole) and rifampin (rifampicin). Transferable plasmid-mediated antibiotic resistance has been reported, conferring resistance to chloramphenicol, tetracycline and erythromycin.

In-vitro studies

Conflicting reports of in-vitro activity of antibiotics against clinical isolates of *L. monocytogenes* probably reflect a variable pattern of susceptibility for strains as well as differences in laboratory technique. Two large in-vitro studies of antibiotic susceptibility of human clinical isolates of *L. monocytogenes* have been reported, using broth dilution susceptibility methods.[24] [25] Both studies found that the strains represented a homogeneous population susceptible to ampicillin, penicillin, erythromycin and tetracycline. In-vitro results, however, are greatly influenced by methodology — inoculum size, media and definition of end points. As reported in these two studies, the minimal bactericidal concentration (MBC) of antibiotics is often much higher than levels attainable clinically. Thus, most antibiotics tested are bacteriostatic but not bactericidal. Although bacteriostatic antibiotics have been used in the past, bactericidal antibiotics have a potential advantage for patients who have impaired host defense mechanisms, including neonates. Results of cephalosporin antibiotic in-vitro and in-vivo studies have been consistently disappointing. The organism is uniformly highly resistant to all the cephalosporin antibiotics tested.

In-vivo studies

Several combinations of antibiotics have been compared for their bactericidal activity against *L. monocytogenes* in vivo. Animal models appear to provide the only practical method of assessing therapeutic regimens, since large clinical studies in humans are not available. Murine models employing normal adults or immunodeficient animals have been reported.[26] [27] A rabbit model using animals injected intracisternally with *Listeria* has also been described. The model most analogous to neonatal disease has also been described.[28]

In the in-vivo model of neonatal listeriosis, the combination of ampicillin with gentamicin gave significantly better eradication of organisms in spleen than ampicillin alone.[28] Similarly, the combination of trimethoprim and sulfamethoxazole was superior to either drug alone. Reports of efficacy of other antibiotics in vivo are conflicting. Rifampin has been found by some authors to be highly effective in eradicating organisms, while others have found it to be ineffective. Sensitivity of individual strains to rifampin may account for the widely discrepant results. Also, rifampin resistance may develop in vivo when it is used as a single drug. The use of ciprofloxacin

in animal models has not suggested any therapeutic advantage over ampicillin.

Clinical reports

There have been no prospective clinical trials reported for *L. monocytogenes* human infection. Anecdotal reports of single cases or reviews of outbreaks support the conclusions drawn from in-vivo models. In one review of clinical management of 119 cases of listeriosis from three centers in the USA, excellent therapeutic results

were seen for patients treated empirically with penicillin or ampicillin; all had a reduction of fever and clinical improvement. However, patients treated initially with cephalosporins had persistent fever and infection.[2] In the largest assessment of treatment regimens during a single outbreak,[29] lower mortality was reported for children given ampicillin (16% of 57 children) compared to those treated with chloramphenicol, tetracycline or streptomycin (33% of 82 children). Nevertheless, in the absence of controlled clinical trials, a definitive recommendation for treatment cannot be made.

SUGGESTED MANAGEMENT

Listeriosis during pregnancy

If amnionitis is present, initial treatment should be given by intravenous route to assure adequate tissue levels — ampicillin 4–6g per day (divided into four equal doses) plus an aminoglycoside. If amnionitis is not present or if acute symptoms of amnionitis have subsided, then oral antibiotics are probably adequate — amoxicillin 2–3g per day (divided into four equal doses). In both situations, treatment should continue for 14 days. If the patient has a significant allergy to ampicillin, therapeutic options are limited. Erythromycin may be given. The estolate form of this drug should be avoided since there is increased liver toxicity during pregnancy. Trimethoprim-sulfamethoxazole should not be used, since premature delivery of the infant may occur as a consequence of infection, in which case the drug may cause displacement of bilirubin from protein and increase the potential for toxicity in the infant.

Neonatal listeriosis

Ampicillin in combination with an aminoglycoside is the preferred management for early-onset infection. For infants with a body weight under 2000g, administer 100mg/kg per day (divided into two equal doses) for the first week of life. For infants with a body weight over 2000g, administer 150mg/kg per day (divided into three equal doses) for the first week of life. For the second week of life the appropriate daily dosages are 150mg/kg and 200mg/kg for infants under and over 2000g body weight, respectively. Aminoglycoside doses vary with the agent chosen.

For gentamicin the suggested dosages are 5mg/kg per day (divided into two equal doses) for the first week of life and 7.5mg/kg per day (divided into three equal doses) for the second week of life. A treatment duration of 14 days is recommended for early-onset neonatal sepsis due to *L. monocytogenes*; however, a 3-week course of treatment should be given in the uncommon event of early-onset neonatal listeriosis with meningitis.

Listeriosis in infants and adults

Meningitis is commonly present in the late-onset type of neonatal listeriosis and in listeriosis in adults. Delayed eradication of the organism may be seen in such cases. Ampicillin 200–400mg/kg per day (divided into 4–6 equal doses for 3 weeks), in combination with an aminoglycoside, is recommended. [22] Lumbar punctures should be repeated daily until the organism has been cleared. In the event of delayed clearance (more than 2 days), further investigations are indicated and should include CT scan or cranial ultrasound (in infants) to assess for the presence of cerebritis, abscess, rhombencephalitis or intracranial hemorrhage. Treatment should be prolonged to 6 weeks if cerebral pathology is identified.

If the organism persists in the CSF the addition of rifampin or use of trimethoprim-sulfamethoxazole may be considered if the organism is sensitive in vitro. Experience with rifampin and trimethoprim-sulfamethoxazole in the neonatal period and with this organism is limited. Cephalosporin antibiotics have no role in treatment since the organism is uniformly resistant. Vancomycin has been used successfully in patients who are allergic to penicillin.[22]

BACILLUS ANTHRACIS

NATURE

The genus *Bacillus* comprises a variety of Gram-positive, spore-forming bacilli that are differentiated from *Clostridium* spp. by a positive catalase test and the ability to produce endospores in the presence of oxygen. *Bacillus* spp. are found worldwide as inhabitants of soil, water and airborne dust. Some species are part of the normal intestinal flora of humans and animals. Endospores resistant to extremes of heat, pH and salinity are able to survive harsh environmental conditions.

Bacillus anthracis was the first bacterium proved to cause a specific disease when, in 1877, Robert Koch induced anthrax in experimental animals by injecting them with pure cultures of the organism. Today, three well-defined cycles are recognized for the organism: spore multiplication in soil; infection of herbivorous animals; and human infection. Spore multiplication is favored in moist, alkaline soils rich in organic matter. As spore density increases, infection of grazing animals can occur. Human disease is often associated with animal outbreaks. Spores can survive in the soil for decades and contribute to persistence in a geographic area.

Bacillus anthracis can be differentiated from other *Bacillus* spp. by its lack of motility, absence of ß-hemolysis on sheep blood agar and penicillin susceptibility.

EPIDEMIOLOGY

Anthrax is a zoonotic disease of herbivores endemic to many rural areas of the world. Although anthrax spores have been found in soil samples worldwide, the disease has been controlled in most developed countries through animal immunization programs. Human infection occurs incidentally by contact with infected animals or animal products such as hides, hair or wool. Most human cases are related to occupational exposure by agricultural workers, veterinarians or those in the textile industry where animal hides and hair are frequently handled. Transmission to humans usually occurs by direct inoculation of spore-containing material to skin abrasions (cutaneous anthrax) but also via spore aerosolization (inhalational anthrax or 'wool sorter's disease') and ingestion (gastrointestinal anthrax). Accidental infections have occurred in laboratory workers. Person-to-person transmission has not been documented. The incubation period is generally 1–7 days following exposure. Suggestions that delayed onset of disease may occur following exposure to aerosolized spores has led to the current recommendation for a 2-month course of antibiotic prophylaxis following inhalational exposure.[30]

Anthrax remains enzootic in parts of Africa and Asia, despite its decreasing prevalence in the developed world. Multiple epidemics of human disease have been associated with epizootics in agricultural herds. More than 10,000 human cases occurred in Zimbabwe between 1979 and 1985 following an outbreak in cattle.[31] An outbreak in Chad during 1988 resulted in 716 human cases and 88 deaths.[32] Other outbreaks have occurred in the Middle East, South East Asia and the

Indian subcontinent. While most cases have been of cutaneous disease, clusters of inhalational and gastrointestinal anthrax have been reported.[33] Anthrax is probably under-reported in developing nations because of the difficulty in diagnosing gastrointestinal disease and limited public health statistics.

In developed countries, outbreaks have mostly been associated with the handling of imported animal products. An epidemic in a textile factory in Switzerland involved 24 cutaneous cases and one case of inhalational anthrax. The infections were traced to goat hair imported from Pakistan.[34] In North America, intermittent outbreaks occur among wildlife herds but domestic human disease is rare.

The development of anthrax as a biologic warfare and bioterrorism agent has drastically heightened global awareness of the disease. Japan, the USA, the UK, Iraq and the Soviet Union are among countries known to have worked on military applications of anthrax. Its subsequent use in terrorist acts has dissolved the perception of anthrax as merely an uncommon and occupational disease. Health care workers and public health agencies have been re-educated to recognize patterns of illness suggestive of anthrax and to develop contingency plans for epidemics. The lethal inhalational dose for anthrax spores is a millionth of a gram, yielding the potential for a small amount of aerosolized spores to devastate urban populations. These concerns were foreshadowed in April 1979 when anthrax spores were accidentally released from a military microbiology facility in the central Russian city of Sverdlovsk.[35] An anthrax epidemic occurred among people who lived or worked downwind of the site. The 96 cases and 64 deaths that ensued constituted the largest documented outbreak of inhalational anthrax in history. Livestock up to 50km from the site perished from anthrax.

PATHOGENICITY

The virulence of *B. anthracis* is largely mediated by the production of anthrax toxin and encapsulation. Anthrax toxin is an exotoxin composed of three thermolabile proteins, protective antigen (PA), lethal factor (LF) and edema factor (EF), which mediate most of the pathologic findings of anthrax. A capsule containing poly-D-glutamic acid assists the organism in evading phagocytosis. Production of toxin and capsule is mediated by the plasmids pX01 and pX02, respectively.[36]

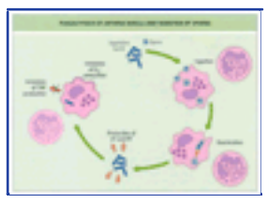

Figure 226-7 Phagocytosis of anthrax bacilli and ingestion of spores. Vegetative bacilli are relatively resistant to phagocytosis by polymorphonuclear leukocytes and monocytes, whereas spores are readily ingested. Once ingested by polymorphonuclear leukocytes or macrophage cells, however, the spores germinate into vegetative forms, which are able to reduce the normal production of bactericidal oxidative radicals by the cell. *Bacillus anthracis* produces lethal toxin (LT) and edema toxin (ET), which are cidal to cells and inhibit TNF-a production and oxidative radical production by phagocytic cells.

Anthrax usually begins by the introduction of spores from the organism through the skin, gastrointestinal tract or respiratory epithelium. Spores may be phagocytosed by tissue macrophages and remain at the site of inoculation or be transported to regional lymph nodes. In either case, spores germinate to vegetative bacilli, which are capable of toxin production. Local damage to tissue results from toxin-mediated edema and tissue necrosis. Toxin, organisms or both may spread via the vascular system to produce systemic disease.

When organisms release anthrax toxin, the PA component binds specific cell-surface receptors, forming a membrane channel that enables EF and LF to enter the cell.

Edema factor is an adenylate cyclase, similar to the adenylate cyclase toxin of *Bordetella pertussis*. Recent studies have demonstrated that activation of EF depends on binding to calmodulin.[37] It combines with PA to form *edema toxin*. In experimental models, cells treated with edema toxin exhibit increased intracellular cyclic adenosine monophosphate (cAMP). In monocytes and macrophages, cAMP regulates TNF-a production and as cAMP rises TNF production decreases. The ensuing cytokine dysregulation produces edema and destruction of local tissues.

Lethal factor combines with PA to form *lethal toxin*, the dominant virulence factor and major cause of death in infected animals. Following entry of lethal toxin into host cells, calcium influx occurs and cellular synthetic pathways are disrupted. Apoptosis and necrosis occur, mediated by protein phosphatases. Cell lysis follows. These tissue effects are seen clinically as the black necrotic lesions of cutaneous anthrax, the hemorrhagic mediastinitis of inhalational anthrax and the necrotic ulcers of pharyngeal or gastrointestinal disease.

Together, lethal toxin and edema toxin contribute to the inhibition of phagocytosis. If ingestion does occur, lethal toxin and edema toxin inhibit the oxidative burst of neutrophils and prevent intracellular killing of ingested bacteria (Fig. 226.7).

PREVENTION

Public health measures for preventing the spread of anthrax have focused on the identification and destruction of diseased animals and quarantine of potentially

infected livestock. Repeated outbreaks in Africa have illustrated that reservoirs of disease in wildlife herds can

be a source of re-introduction to domestic animals. Animal vaccines are available but require annual re-vaccination to maintain efficacy.

Control of anthrax in humans can be achieved by surveillance and control of industrial and agricultural sources of *B. anthracis* by public health authorities. International controls on the export of contaminated animal products has contributed to a decrease in industrial cases of anthrax. Both a live attenuated and a killed vaccine have been developed. The former vaccine has undergone large trials in the former Soviet Union, which demonstrated good protective efficacy. In North America, a cell-free vaccine, BioThrax®, is mandated for all US military personnel and is available for persons who have an occupational risk of acquiring anthrax. The vaccine contains purified protective antigen and can significantly reduce the occurrence of cutaneous anthrax in adults. Protection against aerosol challenge has not been evaluated.[38] Vaccine safety and efficacy have not been evaluated in a postexposure setting.

Postexposure prophylaxis against gastrointestinal and inhalation anthrax has been effective with several antibiotics. In one animal study, antibiotics such as penicillin, ciprofloxacin or doxycycline significantly improved survival when administered after aerosol exposure but before onset of symptoms.[39] Prophylactic antibiotics have also been effective after ingestion of contaminated food. The potential for genetically manipulated strains of military or terrorist origins to exhibit penicillin resistance has made ciprofloxacin or doxycycline the preferred prophylactic options in patients who have such inhalational exposures. The duration of prophylaxis for inhalational anthrax is 60 days.[40]

DIAGNOSTIC MICROBIOLOGY

Diagnostic specimens from patients suspected to have anthrax must be handled in biosafety cabinets to minimize the risk of transmission to laboratory workers. Laboratories should refer presumptive isolates to a reference laboratory capable of the appropriate biosafety containment level and notify public health authorities immediately.

Bacillus anthracis can be isolated from blood, sputum, CSF, vesicular fluid or eschar, gastric aspirates and stool (gastrointestinal anthrax). Blood cultures should be part of the evaluation for all forms of anthrax. Gram staining of clinical specimens may reveal Gram-positive 'boxcar-shaped' *B. anthracis* organisms. M'Fadyean's or India ink stains may be used to visualize the capsule. Direct fluorescent antibody (DFA) testing and polymerase chain reaction (PCR) have been used for rapid diagnosis in clinical specimens but are unavailable in most clinical laboratories.

Bacillus spp. can be cultured on standard media without special nutrient supplementation and typically produce flat, spreading colonies. The filamentous outgrowths from *B. anthracis* colonies are described as forming a 'Medusa-head' appearance. Colonies are gray-white and tenacious. Encapsulation is lost on standard media but may be demonstrated on nutrient agar with 0.8% sodium bicarbonate after overnight incubation in 5% CO_2 . Isolation of *B. anthracis* from non-sterile sites may be achieved with selective media such as polymyxin B-lysozyme EDTA-thallous acetate (PLET) agar or spore selection techniques using heat or alcohol treatment of specimens to kill vegetative cells.

Bacillus anthracis is included with the *Bacillus cereus* group (*B. cereus*, *B. anthracis*, *Bacillus mycoides* and *Bacillus thuringiensis*), whose members are distinguished by production of egg yolk lecithinase. One potential algorithm for *B. anthracis* identification is outlined below.

Presumptive identification criteria

1. From clinical samples such as blood, CSF or skin lesion (vesicular fluid or eschar): large encapsulated Gram-positive rods lacking ß-hemolysis on sheep blood agar;
2. From growth on sheep blood agar: large spore-forming Gram positive rods;
3. Non-motile; and
4. Non-hemolytic on sheep blood agar.

Confirmatory criteria

1. Visualization of capsule; and
2. Lysis by gamma-phage; or
3. DFA assay.

While wild strains of *B. anthracis* are penicillin-susceptible, the potential for genetically altered strains to exhibit penicillin resistance makes this a less reliable trait. Commercial test strips are also available to assist in differentiating *B. anthracis* from other *Bacillus* spp.

Serology has a limited role in acute diagnosis but is useful for epidemiologic studies and as a confirmatory test when the organism has not been isolated. Both an enzyme-linked immunosorbent assay (ELISA) and an electrophoretic immunotransblot (EITB) method have been found to be reliable for detection of antibodies to the capsule and PA respectively. Demonstration of a 4-fold rise in titer between the acute and convalescent phases or a single titer greater than 1:32 is required for confirmation of infection.[38]

Since bacteriologic confirmation is achieved in less than half of anthrax cases, a skin test of cell-mediated immunity to anthrax was developed as a complementary diagnostic tool. An evaluation in the former Soviet Union found the test to have a sensitivity of 81.8% in the first 3 days of illness, 97–99% during the second and third weeks of illness and a sensitivity over 72% up to three decades following recovery — providing a useful method of retrospective diagnosis. [41]

CLINICAL MANIFESTATIONS

Three primary forms of human anthrax are recognized: cutaneous, gastrointestinal and inhalational. Cutaneous anthrax accounts for over 95% of naturally occurring anthrax cases; gastrointestinal anthrax ranks second. Inhalation anthrax is rare. Prior to the outbreak in the Soviet Union in 1979, only 30 inhalational cases had been described.[42]

Clinically, cutaneous anthrax usually begins as a small, pruritic, painless papule 1–10 days after direct contact with infected material. The papule generally develops into one or more vesicles surrounded by a ring of erythema and edema (Fig. 226.8). The vesicles may rupture, exposing an ulcer covered with a black eschar. The word 'anthrax' is derived from the Greek word for coal, in reference to the eschar's dark appearance. Regional lymphadenopathy is also evident and may be severe. Untreated cutaneous anthrax has a fatality rate of 10–20%. However, antibiotic treatment drastically reduces the mortality rate and sequelae.

Gastrointestinal anthrax is associated with ingestion of contaminated meat products that are raw or incompletely cooked. Early symptoms include nausea, vomiting and fever. Severe abdominal pain and bloody diarrhea develop within 2–3 days. Toxemia, shock

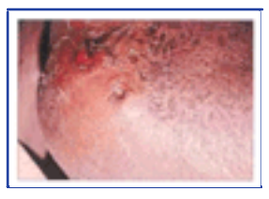

Figure 226-8 Cutaneous anthrax, demonstrating marked erythema, edema and vesicle rupture.

and death can develop shortly after the start of severe symptoms. Lesions can be found anywhere from the oropharynx (pharyngeal anthrax) to the large bowel, with the cecum being frequently involved. Pathologically, a hemorrhagic enteritis is noted, with necrotic ulcerations of the mucosa.

Respiratory anthrax was well characterized during the 1979 outbreak at Sverdlovsk and in more recent cases in the USA.[42] Initial symptoms include sore throat, fever, myalgias and fatigue. Rapid progression to dyspnea, cough, headache, vomiting, chills, weakness, abdominal pain and chest pain is typical. Respiratory failure and shock develop in most patients. The presence of infiltrates is variable on chest radiograph but a widened mediastinum with pleural effusions is a key diagnostic finding and reflects the underlying pathology of hemorrhagic mediastinitis.

In the Sverdlovsk cases, onset of symptoms was typically 3–14 days following exposure. Hospitalized patients were treated with penicillin or other antibiotics, antianthrax globulin, steroid and artificial ventilation. The average hospital stay was 1–2 days for fatal cases and approximately 3 weeks for survivors. Historical data suggest that the mortality rate for untreated inhalational anthrax is approximately 97%. With modern antibiotic treatment and intensive care support, the fatality rate remains at least 75%.

Meningitis may develop following dissemination of cutaneous, gastrointestinal or respiratory anthrax but occurs in fewer than 5% of cases.

MANAGEMENT

Given the potential severity of disease, patients who have suspected anthrax should receive empiric therapy pending diagnostic tests. Historically, penicillin G has been the treatment of choice. Doses of two million units given intravenously every 2 hours have been used in adults. The addition of an aminoglycoside such as gentamicin may confer some improvement in response to treatment. All naturally occurring strains of *B. anthracis* have been sensitive to erythromycin, chloramphenicol, gentamicin and ciprofloxacin. As noted previously, in potential bioterrorism or biowarfare settings, penicillin susceptibility cannot be assured and doxycycline or ciprofloxacin should be used. One recent review has recommended treatment with intravenous ciprofloxacin (400mg q8-12h).[43] Supportive therapy for shock, fluid volume deficit and ventilatory assistance may also be needed (see also Chapter 185). [43]

CORYNEBACTERIUM DIPTHERIAE

NATURE

Corynebacterium diphtheriae is a member of a group of irregular nonspore-forming aerobic Gram-positive bacilli characterized by an ability to grow in the atmosphere of air, lack of endospores and an irregular shape on Gram-stained smears. This group includes human, plant and animal pathogenic species, the human pathogens including *Oerskovia*, *Rothia*, *Actinomyces*, *Arcanobacterium*, *Propionibacterium*, *Arachnia* and *Gardnerella* spp. in addition to *Corynebacterium* spp.

The genus *Corynebacterium* is characterized by pleomorphic Gram-positive bacilli that tend to stick together in parallel groupings and bend when dividing to create 'Chinese letter' arrangements (Fig. 226.9). They are all catalase- and cytochrome-oxidase-positive and can grow in facultative anaerobic conditions.[44]

EPIDEMIOLOGY

Diphtheria, the disease caused by *C. diphtheriae*, was first described in manuscripts of the 6th century AD but was not recognized as a specific

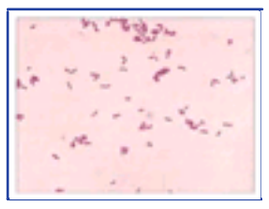

Figure 226-9 Chinese letter and picket-fence appearance of *Corynebacterium* spp. on Gram-stained smears.

disease until Bretonneau defined it in 1826. In the 1880s the bacillus was discovered by Klebs and soon after Koch's postulates were fulfilled. The first successful diphtheria immunization was performed in 1913 by von Behring, at a time when epidemics in Europe and the UK made diphtheria the leading cause of death in children 4–10 years of age. Mass immunization was introduced in the early 1940s, by which time the numbers of cases were already declining. The introduction of diphtheria toxoid immunization was associated with a substantial decrease in reported cases in both Europe and North America for 25 years. A study of an immunization program in Toronto, Canada from 1926–9 showed a 75% reduction in cases of diphtheria in immunized children compared with controls who were unimmunized.[45]

Outbreaks of diphtheria in recent years have been rare. In 1990 an epidemic that began in Moscow rapidly spread to the newly independent states of the former Soviet Union, first in the cities and later into rural areas. Poor immunization practices may have created a large population of susceptible hosts. Toxigenic strains that had not circulated in this part of Europe for about 25 years were finally able to colonize a pool of susceptible hosts large enough to start an epidemic.

Prior to childhood immunization programs, epidemics among European school-aged children occurred about every 25 years through the 17th, 18th and 19th centuries. Speculation that the resurgence of this infectious disease might be due to cyclic intrinsic factors of the micro-organism itself evolved from evidence that non-toxigenic oropharyngeal colonizing strains of *C. diphtheriae* can be made toxigenic by introduction of toxin genes by bacteriophages. Perhaps immunization with diphtheria toxoid does not prevent colonization with non-toxigenic strains of this organism, allowing natural cycling of more virulent strains into a population with waning immunity. The epidemic in the former USSR largely involved the adult population. Poor socio-economic conditions and mass population movements during the dissolution of the Soviet Union may have also contributed to the development of this epidemic. A total of 47,808 cases of diphtheria were reported in the former Soviet Union from 1990–5.[46] Spread of cases by travelers has occurred in western Europe. This is confirmed by the fact that isolates from Finland showed the same prevalent *C. diphtheriae* var. *gravis* strain belonging to the same G1 ribotype as in 91% of a series isolated in northern Russia during this epidemic.[47]

Transmission is generally through contact with infected respiratory droplets or skin wounds. Raw milk and milk products and clothing have been associated with diphtheria transmission. It is transmissible for 2–4 weeks in untreated individuals but with antibiotic treatment the risk of transmission is greatly reduced after the first 48 hours.

PATHOGENICITY

Corynebacterium diphtheriae is not an invasive organism but causes disease by colonizing the skin and upper respiratory epithelium, inducing a mild inflammatory reaction and releasing a potent exotoxin

Figure 226-10 Molecular action of *Corynebacterium diphtheriae.* The B subunit of diphtheria toxin (DT) binds to host cell receptor. Proteolytic cleavage of the B subunit from the A subunit (the active DT) in cell endosome. The DT-A subunit escapes from the endosome into the cytoplasm, where it inhibits the transfer of triple code from mRNA to growing polypeptide at the ribosomes on the endoplasmic reticulum. It does this by modifying the enzyme elongation factor (EF)-2 that catalyzes this mRNA transfer.

that, when taken up by animal cells, inhibits protein synthesis. Locally, the toxin causes tissue necrosis. If circulating toxin antibodies are not present, it will be absorbed into the circulation and transported to distant sites, causing extensive end-organ damage, especially to the kidneys, brain and heart.

On a molecular level, the diphtheria toxin (DT) is synthesized as a 62kDa polypeptide comprising a B subunit that binds to cell receptors and an A subunit that is the active moiety. The DT binds to the host animal cell receptor via its B subunit and is taken up by cellular endosomes at clathrin-coated pit sites (Fig. 226.10). Proteolytic cleavage of DT occurs releasing the A subunit (DT-A; see Fig. 226.10). The acidic condition of the cell's endosome causes conformational change in the B subunits, resulting in opening of cation channels in the endosome membrane and release of the active DT-A moieties into the cytoplasm. There, DT-A inhibits cellular protein synthesis by adenosine diphosphate (ADP)-ribosylation of diphthamide in elongation factor 2-GTP, thus inactivating it. This elongation factor 2 (EF-2) is important for catalyzing the transfer of triple code from messenger RNA to the developing amino-acid polypeptide sequence via transfer RNA at the ribosome. In this way, DT-A acts as a polypeptide chain terminator of host cell protein synthesis, causing an inability of the cell to repair its own membranes and cellular cytotoxicity (see Fig. 226.10). Toxin production by *C. diphtheriae* is induced by a lysogenic bacteriophage ß that co-infects certain strains of this colonizing organism. It is also known that this toxin has DNA fragmentation capabilities and can cause cell cytotoxicity by a mechanism other than inhibition of protein synthesis.[48]

The localized tissue destruction initially forms a gray exudate of dead host cells and white blood cells, which may be removed. Later, with the addition of fibrin and red blood cells, a tough gray-brown pseudomembrane is produced that is difficult to remove and bleeds after such attempts. If the membrane is extensive, it may lead to respiratory tract obstruction. The underlying submucosa is often very edematous. Secondary invasion of bacteria, especially *S. aureus* and *Streptococcus pyogenes*, can occur. End-organ toxin-mediated damage can initiate a myocarditis with edema, mononuclear cell infiltration and fatty changes of heart myofibrils and its conducting system, neuritis and nerve demyelinization and acute tubular necrosis of the kidney.[49]

PREVENTION

The best form of prevention is an active immunization program of children and adults that covers over 90% of the population. Immunization coverage of less than this target leaves enough susceptible people to cause spread in an enclosed population. Infants should receive a primary series of diphtheria toxoid immunization with

other childhood immunizations at 2, 4 and 6 months of age and booster shots at 18 months, 4 years and every 10 years thereafter through adulthood.

Because of an excess of local and systemic reactions, the adult vaccine dosage is usually decreased to 2 Lf units in those over 7 years of age. In an outbreak situation in older children and adults, if no immunization was previously administered, the primary series can be given in two doses 4 weeks apart and the third 6 months later.

Carriers or close contacts of a case of diphtheria should receive not only vaccination with diphtheria toxoid but erythromycin or penicillin prophylaxis for 7 days to prevent subsequent spread of the organism. Eradication of asymptomatic pharyngeal colonization is slow and difficult, with many people excreting organisms for more than 1 month despite antibiotic therapy.[50]

DIAGNOSTIC MICROBIOLOGY

Corynebacterium diphtheriae colonizes the respiratory tract of humans alone. Isolation of the organism requires specialized media containing potassium tellurite (Tinsdale agar) to inhibit other normal flora, including non-*diphtheriae Corynebacterium* spp. After 24–48 hours of incubation at 35°C, gray-black colonies are seen with a surrounding brown halo due to the precipitation of a tellurium sulfide secondary to cysteine breakdown utilizing the bacteria's cysteine desulfatase. Methylene-blue-stained smears will show the pleomorphic club-shaped nonsporulating rods. The organism's name is derived from the Greek word *korynee*, meaning 'club', and *diptheria,* meaning 'leather pouches', referring to the thick, leather-like pharyngeal membranes this organism produces. Other normal flora, *Neisseria* and *Staphylococcus* spp. can also occasionally appear black on this specialized media but have a different morphology on stained smear. In addition, specimens should be streaked onto a blood agar plate to detect *S. aureus* and ß-hemolytic streptococci, which can co-infect tissues with this organism. If transportation to the laboratory is delayed for more than 24 hours, the specimens should be streaked onto a specialized Loeffler transport media agar slant, leaving the swab on top, for later delivery to the laboratory.[51]

Corynebacterium diphtheriae can be distinguished from other *Corynebacterium* spp. biochemically as it is catalase, cysteinase and nitrate reaction-positive; urease, esculin, pyrazinamidase and gelatin hydrolysis reaction-negative; and uses glucose and maltose but not mannitol or xylose sugars for growth requirements.[44] A modification of the normal concentrations of biochemical substrate concentrations and the use of an enriched basal media can allow for rapid 1 hour

identification from a pure subculture of a 18- to 24-hour growth of the organism. [52] A commercial API-Coryne® strip of 20 biochemical substrates may identify this and other *Corynebacterium* spp. within a 24-hour period.

Subdivision of this organism into three biotypes — *gravis*, *mitis* and *intermedius* — can be done using colony size, blood agar hemolysis, morphology on stained smear and biochemical tests. It is also important to establish bacterial toxin production because biotype *mitis* is usually non-toxigenic. Treatment is generally reserved for people carrying toxigenic strains.[44] Recently, in Germany, toxin-producing *C. diphtheriae* var. *mitis* strains have been described that cause invasive disease.[53] Toxin can be demonstrated using the Elek plate, on which the organism and controls are streaked perpendicular to an antitoxin-impregnated filter paper strip embedded in the agar, causing immunoprecipitin lines to form if the organism is a toxin-producer (Fig. 226.11). The PCR technique may be more sensitive by directly detecting toxin genes.[54]

Antimicrobial susceptibility testing of isolates has shown that they are generally susceptible to penicillin, vancomycin and erythromycin but they are methicillin-resistant. Recent isolates from France and Vietnam show that multiresistant strains are becoming increasingly common. Erythromycin resistance was seen in 15% and tetracycline resistance in 20% of 88 strains from Vietnam between 1992 and 1996. [55] Isolates from France between 1987 and 1993 showed a 19% resistance to rifampin.[56]

Multilocus enzyme electrophoresis and ribotyping techniques can be used in molecular characterization of strains of these clinical bacterial isolates to determine whether an outbreak is occurring from a particular clone introduced into a population.[57] Other laboratory diagnostic tests have more limited value and include the Schick test and serology testing to the diphtheria toxin to detect previous exposure to the organism either in the form of a vaccine or infection. The Schick test is an intracutaneous inoculation of a small amount of purified diphtheria toxin. If the subject has circulating antitoxin antibodies, there will be an absence of the redness, tenderness and induration of greater than 10mm that occurs 5 days later. This test is not useful for acute diagnosis of diphtheria, and a negative result does not necessarily indicate that the antitoxin antibody levels present are protective against acquisition of this disease.[49] Indeed, serology testing for antitoxin antibody levels is problematic because protective levels have not been well defined. It is believed that antibody levels of 0.01–0.09IU/ml or more provide some protection.[58]

Figure 226-11 Detection of diphtheria toxin production. The Elek test detects DT production in a bacterial clinical isolate. A paper strip soaked in diphtheria antitoxin is placed perpendicular to a horizontal streak of bacterial growth. If it produces toxin, an arrow-shaped precipitin line develops in the agarose plate.

CLINICAL MANIFESTATIONS (see also Chapter 178)

Respiratory tract disease

After a 2- to 4-day incubation period, colonizing toxigenic strains start producing toxin locally and signs and symptoms of disease start to occur. In nasal disease, typically seen in infants, the illness appears similar to the common cold but then progresses to a serosanguinous and mucopurulent rhinitis. Excoriation of the nares and upper lip and a white septal pseudomembrane may been seen. Spread of the disease to the pharynx occurs next, causing a sore throat, tonsillitis, low-grade temperature and a white to gray pseudomembrane extending from the tonsils to the posterior pharyngeal pillars and nasopharynx. As toxin production continues, there is profound malaise, weakness, cervical lymphadenitis, soft tissue swelling of the neck, causing a 'bull neck', and occasionally palatine paralysis and upper respiratory obstruction with stridor. Further spread of the disease downward to the larynx, causing hoarseness, dyspnea and stridor and later a tracheobronchitis with edema and membrane formation in the lower respiratory tract, will often precede respiratory failure.[49] Indeed, about 60% of 42, mostly middle-aged, adults who died in a review of 1860 cases of diphtheria in St Petersburg, Russia in 1994 had simultaneous involvement of throat, nose and bronchi clinically. Unlike the mortality rate of about 2% for this Russian outbreak, which was mostly in adults, [59] the mortality rate for respiratory tract diphtheria in the USA and Sweden of late, in which more cases of children were involved, has been 10% and 18%, respectively. Clinical manifestations of toxin dissemination after respiratory tract diphtheria occurs as the localized disease is waning. Cardiac toxicity is the next most prevalent cause of death after respiratory failure and neurotoxicity is generally a much later phenomenon that adds to the morbidity from this disease.

Cutaneous disease

Diphtheria involving the skin usually is more common in tropical climates and is associated with infection of pre-existing skin lesions, lower mortality, an indolent and mild course, and superinfection with *Staphylococcus aureus* and *Streptococcus pyogenes*. This disease is characterized by chronic, nonhealing, sharply bordered ulcers that have an adherent gray pseudomembrane over the top of them. Despite their mild clinical course, they produce good protective antitoxin antibodies, do not respond to antitoxin therapy and act as reservoirs for respiratory tract illness, can contaminate objects in the environment and are shed for longer periods of time than pharyngeal colonization. Cutaneous diphtheria is seen in outbreaks among the poor, homeless and alcoholics with close-confined living arrangements. This form of disease may be an important means of person-to-person transmission of diphtheria and is thought to be the means of starting outbreaks of respiratory tract diphtheria.

Myocardial toxicity

When the local disease is improving, 1–2 weeks from onset, up to two-thirds of patients have electrocardiographic evidence of myocardial toxicity, with ST wave alterations and first-degree heart block, but most of them are asymptomatic. Heart failure and arrhythmias occur in up to one-quarter of patients who have severe disease, increasing mortality by three to four times compared with those who do not have these manifestations. Such patients present with dyspnea, weakness, diminished heart sounds, a gallop rhythm, a dilated heart, low cardiac output and conductive disturbance arrhythmias.

A rather uncommon but often fatal cardiac complication of diphtheria is endocarditis. Most reported cases were in patients who had *C. diphtheriae* bacteremia. A total of 33 cases were reported between 1893 and 1993, and at least 20 of those reported since 1950 are associated with non-toxigenic strains. Non-toxigenic strains have been

thought not to be associated frequently with invasive disease but this increased incidence may be due to an increasing prevalence of patients in whom intravenous drug usage is a pre-existing risk factor. Almost half of these cases were children aged 16 years and younger, and a high incidence of septic arthritis and mycotic aneurysms of the lower extremities occurred. Although most patients had a known predisposing factor for endocarditis, such as intravenous drug abuse, valvular heart disease or congenital heart disease, one-third occurred in patients who had no known predisposition. The mitral valve is the most commonly affected heart valve. The mortality rate was about 40%. Very few of these patients received surgical therapy in conjunction with antibiotic therapy and it is unclear whether early surgical intervention would have improved the prognosis of this disease.[60]

Neuronal toxicity

Up to two-thirds of severely involved cases of diphtheria show evidence of neuronal toxicity, presenting early as palatine and pharyngeal paralysis and then cranial palsies involving muscles of the eye, face, pharynx and larynx, causing dysphagia and predisposing these patients to aspiration. The ocular manifestations of diphtheria include blurred vision and failure of accommodation. Later, several weeks after the sore throat, a peripheral motor demyelinating neuritis occurs, starting in the proximal extremities and progressing distally. Generally, there is slow and total resolution of the limb weakness.

MANAGEMENT

The management of a clinical case of diphtheria is described in Figure 226.12 (see also Chapter 178).

Hyperimmune diphtheria antitoxin

This product is made from immunized horses and, because up to 10% of the population shows hypersensitivity to horse serum, this preparation must be diluted 1:1000 with saline and a test dose of 0.1ml administered intracutaneously. An area of redness at the administration site greater than 10mm requires epinephrine (adrenaline) to be given, followed by a desensitization regimen.[49] If no hypersensitivity to horse serum is found, 20,000–40,000 units for pharyngeal or laryngeal disease of less than 48 hours duration, 40,000–60,000 units for nasopharyngeal pseudomembrane formation and 80,000–100,000 units for extensive disease of 3 or more days duration or with neck swelling should be administrated intravenously (or some suggest a spit between intramuscular and intravenous administration) over 60 minutes, as recommended by the American Academy of Pediatrics Red Book Committee. Repeat administrations are of no benefit and may increase the risk of anaphylaxis to horse serum protein.[50]

Antibiotic therapy

Antibiotics do not seem to alter the course of clinical disease but are administered in an effort to terminate toxin production and extension of local disease and prevent spread of the organism to other people. Erythromycin 40mg/kg per day (maximum 2g/day) intravenously or orally in four divided doses has been shown in some studies to be more efficacious than penicillin G (procaine penicillin G 25–50,000U/kg per day intramuscularly in two divided doses for 14 days). Elimination of the organism should be documented by three negative daily cultures after antibiotic therapy before strict isolation is discontinued. Communication of the organism to others can occur up to 4 days after commencement of antibiotic therapy.[50]

Supportive therapy

Strict bed rest is usually advocated during the maximum period of myocardial toxicity, the first 2–3 weeks from onset of illness. Serial

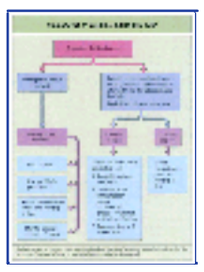

Figure 226-12 Management of a clinical diphtheria case. *Adapted from Farizo.*[61]

electrocardiograms may be useful for earlier detection of this complication. Tracheostomy equipment should be placed by the bedside to be prepared for acute upper airway obstruction. Nasogastric tube feeding may be required to prevent aspiration in the patient who has palatine or pharyngeal paralysis. Long-term immunity is unusual in cases of diphtheria and adequate immunization is required to prevent recurrence of this disease. This is given in the convalescent phase.[50]

OTHER *CORYNEBACTERIUM* SPECIES

Corynebacterium jeikeium

This species is the most commonly isolated *Corynebacterium* sp. from human blood and sterile body fluids. It is a pleomorphic, nonsporulating, aerobic Gram-positive bacillus that forms nonhemolytic, small, whitish colonies on blood agar after 24 hours of incubation. It requires lipid to grow and is unable to reduce nitrate or hydrolyze urea. In addition, it is resistant to most antibiotics, with the exception of being sensitive to vancomycin.[62]

This organism has emerged as a cause of serious nosocomial infections in immunocompromised patients. Predisposing factors for this infection include neutropenia, prior multiple antibiotic usage, prolonged hospitalization and disruption of the skin by surgery or an indwelling catheter. *Corynebacterium jeikeium* heavily colonizes the skin (axillary, inguinal and rectal) of about one-third to one-half of oncology patients for weeks to months but only lightly colonizes the

skin of healthy individuals. The spectrum of infection includes central-line-related septicemias, wound and soft tissue infections, prosthetic joint infection, prosthetic valve endocarditis, continuous ambulatory peritoneal dialysis (CAPD)-related catheter peritonitis, pneumonia, CSF shunt infections and liver abscesses.[63] Recently, there have been reports of infections in immunocompetent patients with this organism.[64]

Treatment involves intravenous vancomycin or ciprofloxacin and the removal of the prosthetic material or catheter.

Corynebacterium urealyticum

Corynebacterium urealyticum (CDC Group D2) is an aerobic Gram-positive bacillus that is similar to *C. jeikeium* in growth characteristics, microbiology and multiple antibiotic resistance profile (often only sensitive to vancomycin) but is rapidly urease-reaction-positive and does not ferment carbohydrates. It is a common cause of alkaline-encrusted cystitis, [65] in which ammonia produced from these urea-splitting bacteria combines with magnesium phosphate. At high pH, ammonium magnesium phosphate crystals precipitate out in the walls of an already damaged bladder and cause ulceration. *Corynebacterium urealyticum* has also been associated with kidney infections and struvite kidney stone production in renal transplant recipients.

Microbiologists should be aware of this organism and not discard urine plates after 24 hours in patients predisposed to such infections (immunosuppressed, urologic or renal transplant patients, especially if they have had repeated urinary tract infections); the plates should be re-examined at 48 hours, when these slow-growing organisms may first become noticed.

Vancomycin is considered the drug of choice to treat this infection. [63]

Corynebacterium pseudodiphtheriticum

This organism is part of the normal oropharyngeal flora, looks like *C. diphtheriae* but does not produce a toxin and, unlike *C. diphtheriae*, possesses both urease and pyrazinamidase enzymes and does not ferment carbohydrates. It has been found to cause endocarditis, necrotizing bronchitis and pneumonia in patients who have chronic disease. Rare case reports of urinary tract infection following renal transplantation, suppurative arthritis and osteomyelitis have been reported.[63]

Corynebacterium minutissimum

Corynebacterium minutissimum has been associated with erythrasma a red-brown pruritic macular skin rash usually found in the intertriginous regions of toes, fingers and axillae. Both the superficial skin lesions and the organism's minute colony growth are noted for their coral-red-orange fluorescence under ultraviolet light produced by a Wood's lamp.[51]

Corynebacterium ulcerans

This organism is morphologically similar to *C. diphtheriae* and is found in the pharynx of persons who drink unpasteurized cows milk or who have had contact with milking cows. In cattle and horses it is part of the normal flora and causes mastitis. This organism can be cultured from the throats of asymptomatic people and occasionally from patients who have an exudative pharyngitis[63] and diphtheria-like illness, in whom the bacterium has been found to produce diphtheria and dermatonecrotic toxins. [66] Both colony morphology and Gram stain of isolates growing on Tinsdale selective medium are indistinguishable from *C. diphtheriae* but the colonies, unlike *C. diphtheriae*, show a narrow zone of hemolysis on blood agar and have a positive urease reaction. The antibiotic susceptibility profile of this organism is similar to that of *C. diphtheriae* in that it is sensitive to most ß-lactam antibiotics and erythromycin.[63]

ERYSIPELOTHRIX RHUSIOPATHIAE

NATURE

Erysipelothrix spp. are short, slender, non-spore-forming Gram-positive rods. While two species of the genus are recognized, *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum*, only *E. rhusiopathiae* has been described as a pathogen in humans. The organism inhabits soil and water worldwide and has been found in mammals, birds, fish and shellfish, presumably following ingestion of contaminated matter. In 1886, *E. rhusiopathiae* was established as the cause of 'swine erysipelas', and the organism continues to have economic importance as a cause of septicemia in swine, calves, lambs, turkeys and other farm animals.[67] Animals may exhibit asymptomatic carriage or disease. Domestic pigs appear to be the major reservoir.

EPIDEMIOLOGY

Infection with *E. rhusiopathiae* occurs worldwide and is considered to be a zoonosis. Transmission to humans usually occurs by direct cutaneous contact with infected fish or animals and manifests as erysipeloid, a localized cellulitis that appears 2–7 days after exposure. Most human infections follow occupational exposure. While persons who handle fish or shellfish appear to be at highest risk, erysipeloid is also more common in abattoir workers, butchers, farmers and veterinarians. The frequent occurrence of erysipeloid in whalers earned it the title 'whale finger'. Systemic *E. rhusiopathiae* infection has been reported following ingestion of undercooked pork. Human-to-human transmission has not been reported.

PATHOGENICITY

Infection of laboratory animals with *E. rhusiopathiae* leads to production of specific antibodies that are protective against re-infection. The virulence of *E. rhusiopathiae* appears to be related to resistance to phagocytosis and to multiplication and survival within cells after uptake. One group described the presence of a capsule on virulent strains when the organism was viewed by electron microscopy.[68] The presence of a capsule appeared to be associated with virulence. Mutant strains lacking the capsule were less virulent than parent strains.[68] Strains with a capsule were relatively resistant to phagocytosis by polymorphonuclear leukocytes unless the organism had been opsonized with organism-specific antiserum.

Virulent *E. rhusiopathiae* can also survive, and subsequently replicate, within macrophages when ingested in the presence of nonimmune serum. Survival within the macrophage appears to be associated with the organism's ability to reduce the production of reactive oxidative products. [69]

DIAGNOSTIC MICROBIOLOGY

In patients who have erysipeloid, organisms are found only in deeper parts of the skin, requiring aspiration or biopsy specimens that include the entire dermal thickness for optimal results. Blood cultures may isolate the organism in patients who have systemic disease. *Erysipelothrix rhusiopathiae* is a short, slender, non-spore-forming Gram-positive rod that grows best on routine blood agar plates in an atmosphere of 5–10% CO_2 at 92°F (35°C). Microscopically, they appear straight or slightly curved and occasionally form long filaments over 60µm long.[70] Unlike *Listeria*, *Bacillus* and *Corynebacterium*, *Erysipelothrix* spp. are catalase-negative. Colonies are small and translucent with a or no hemolysis. Two major discriminatory tests are the production of H_2 S on TSI slants (highly suggestive of *E. rhusiopathiae*) and a 'pipe cleaner' pattern of growth in gelatin stab cultures inoculated at 71.6°F

(22°C). The organism is typically vancomycin-resistant — an uncommon trait among Gram-positive rods. The ability of *E. tonsillarum* to ferment saccharose distinguishes it from *E. rhusiopathiae*. [70]

CLINICAL MANIFESTATIONS

Human infections with *E. rhusiopathiae* have three patterns of presentation: erysipeloid (a localized skin lesion), multiple skin lesions with or without systemic symptoms, or bacteremia/endocarditis.

Erysipeloid constitutes the majority of *E. rhusiopathiae* infections and presents as a subacute cellulitis. Most lesions occur on hands or other areas of the body exposed directly to infected fish or animals. The diagnosis is suggested by a nonsuppurative, insidious cellulitis with severe disproportionate pain — usually in a person who has an occupational exposure. The incubation period is typically 2–7 days. Lesions are usually well demarcated, slightly raised, erythematous and accompanied by swelling of the surrounding tissue. Regional painful lymphadenopathy or lymphangitis may occur. Low-grade fever and arthralgias are present in a minority of cases. Erysipeloid usually resolves within 3–4 weeks of onset without treatment and earlier with appropriate antibiotic treatment.

Untreated, erysipeloid occasionally spreads proximally from the initial lesion to producing multiple noncontiguous lesions. Lesions developing at sites remote from the initial inoculum have also been described, presumably via hematogenous dissemination.

Bacteremia due to *E. rhusiopathiae* is unusual; however, the organism has been identified as a cause of persistent bacteremia and endocarditis in adults.[71] The vast majority of serious infections due to *E. rhusiopathiae* have occurred in men who had a history of occupational exposure such as farming or animal handling.[72] Patients with a history of alcohol abuse or immunosuppression have occasionally been seen with only mild fever in the presence of bacteremia due to *Erysipelothrix*. The possibility of a carrier state of infection in such patients has been proposed.[71] Bacterial endocarditis, particularly involving the aortic valve, has been described. In one review, 40% of patients who have endocarditis had an antecedent erysipeloid cutaneous lesion. Overall mortality due to *E. rhusiopathiae* endocarditis is approximately 40%. The one case of neonatal septicemia that has been described was attributed to cutaneous contamination shortly after birth.[73]

MANAGEMENT AND PREVENTION

Erysipelothrix rhusiopathiae is susceptible to penicillin but resistant to vancomycin.[74] Vancomycin resistance is notable since this drug is commonly used as empiric treatment for bacteremia due to Gram-positive organisms. Therefore, a high index of suspicion for this organism should be maintained for patients who have unusual cutaneous sites of infection or animal exposure. Penicillin is the drug of choice for serious infection caused by *E. rhusiopathiae*; however, the organism is also highly susceptible in vitro to cephalosporins, clindamycin, imipenem and ciprofloxacin.[75]

Prevention is achieved mainly through the use of protective gloves in high-risk occupations.

ARCANOBACTERIUM HAEMOLYTICUM

NATURE AND DIAGNOSTIC MICROBIOLOGY

Arcanobacterium haemolyticum is often overlooked in clinical specimens, as organisms appear similar in morphology to *Corynebacterium* spp. that are resident normal flora of the skin and oropharynx. In fact, the organism was originally named *Corynebacterium haemolyticum*. The name of the genus is derived from the Greek word *arcanus*, meaning 'secretive' or 'mysterious'. Colonies are small, gray-white and take 24–48 hours to appear on sheep blood agar, displaying a narrow zone of ß hemolysis that may not appreciated until a colony is picked off of the plate. A colony Gram stain shows pleomorphic short V- and club-shaped Gram-positive bacilli that, with older growth, appear beaded and streptococcus-like in appearance. The ß-hemolysis and lack of catalase activity distinguish them from *Corynebacterium* spp. In addition, they ferment glucose, lactose, maltose and sucrose but do not reduce nitrate or hydrolyze urea, esculin or gelatin. They are nonmotile and produce a reverse CAMP test with inhibition of the ß-hemolysin activity of *S. aureus*.[76] [77]

EPIDEMIOLOGY

Humans appear to be the primary host for this organism, which causes pharyngitis in teenagers and young adults,[78] as well as cellulitis[79] and chronic skin ulcers, mostly in tropical countries.[80] Although it can be associated with symptomatic pharyngitis, this is uncommon. Pharyngitis associated with this organism is predominantly found in females aged 15–22 years. Rarely, this organism has been found as a cause of septicemia, meningitis, endocarditis, osteomyelitis, sinusitis and cavitary pneumonia in adults who have underlying chronic diseases.[81] One study showed an increase in isolation of this bacterium from throat swabs in spring and autumn.[82] Person-to-person spread by fomites is thought to be the predominant mechanism of transmission, although this has not been adequately studied.

PATHOGENICITY

Evidence that *A. haemolyticum* is a cause of pharyngitis in adolescents and young adults is derived from studies showing its presence in the throats of symptomatic but not asymptomatic subjects. However, some investigators dispute this conclusion. *Arcanobacterium haemolyticum* has been isolated singularly and in association with *S. aureus* and ß-hemolytic streptococci; however, insufficient data exist to rule out concomitant infection with viruses or *Mycoplasma* spp. In a study of seven volunteers who had a pure culture of this organism applied to their pharynx, none became symptomatic in 6 weeks of follow-up despite having heavy pharyngeal colonization.[77] Indeed, several studies have shown an association of *A. haemolyticum* pharyngitis with Epstein-Barr virus infection. Preceding viral colonization may be required in the pharynx before symptomatic infection can occur.[83]

This organism produces phospholipase A and D, lipase, hemolysin and a dermotoxin when injected into the skin of a guinea pig.[77] Occasionally a serologic response has been found in patients who have either skin or pharyngeal infection. The organism can persist intracellularly.[84] Skin biopsies of two patients who had concomitant scarlatiniform-like rash, often seen with the pharyngeal infection, showed no organism or immunoglobulin deposits but a mild lymphocytic perivascular infiltration.[85] This indicates that the rash may be due to a toxin-mediated event similar to the scarlet fever rash of group A streptococci.

CLINICAL MANIFESTATIONS

The signs and symptoms of pharyngitis due to *A. haemolyticum* are listed in Table 226.4 [82] [83] [86] and are similar to those of group A streptococcal pharyngitis with the exception of the rash and presence of more upper respiratory symptoms of dry cough and hoarseness. The rash appears 1–4 days after the onset of pharyngitis and is usually scarlatiniform, appearing first on extensor surfaces of the extremities and then spreading to trunk and chest, sparing palms and soles. It often persists for more than 2 days.[86] Unlike streptococcal scarlet

TABLE 226-4 -- Signs and symptoms of pharyngeal *Arcanobacterium haemolyticum* infection.

SIGNS AND SYMPTOMS OF PHARYNGEAL *A. HAEMOLYTICUM* INFECTION	
Sign or symptom	**Frequency (%)**
Sore throat	97–100
Tonsillar exudate	50–70
Fever	60–80
Dry cough/hoarseness	40–60
Cervical lymphadenopathy	40–67
Rash	50

fever, circumoral pallor does not occur. Less frequently, the rash is maculopapular or urticarial. Skin infections with *A. haemolyticum* were first described in US military personnel stationed in South Pacific islands and have been described as ecthyma, resembling chronic ulcers. Cellulitis and wound infections in these tropical environments often have mixed bacterial infections with this organism.

MANAGEMENT

Arcanobacterium haemolyticum isolates are highly susceptible to erythromycin with MICs of less than 0.01µg/ml. As a result, erythromycin is considered the drug of choice for pharyngitis. As a bacteriostatic compound it is generally inappropriate for therapy of bacteremia. Penicillin is bactericidal but the MICs are more variable and tolerance and antibiotic failure have been documented.[81] Clinical evidence of antibiotic efficacy is anecdotal and largely involves the use of intravenous penicillin to treat *A. haemolyticum* septicemia.

RHODOCOCCUS SPECIES

NATURE

Rhodococcus are aerobic Gram positive bacilli similar to *Nocardia* in that they produce a substrate mycelium in soil environments that fragments into bacillary and coccoid forms. Also like *Nocardia*, *Rhodococcus* is partially acid-fast using the modified Kinyoun stain, because of its mycolic-acid-containing cell wall. Unlike *Nocardia*, it does not produce aerial mycelia and its cell wall composition is different. There are 15 species in this genus and *Rhodococcus equi*, formerly *Corynebacterium equi*, is the predominant pathogen in humans.[87] [88]

EPIDEMIOLOGY

Rhodococcus equi causes bronchopneumonia in foals, cattle, pigs and felines.[89] [90] It is found in soil and herbivore manures[90] and is not part of the normal bacterial flora in healthy humans. Transmission occurs via the respiratory tract and appears to be through contact with soil and animals. Human disease usually occurs in patients who have disturbances of cell-mediated immunity such as AIDS, cancer, transplantation or chronic disease.[91] Human-to-human transmission has not been documented. Many investigators suggest that infection with this organism should warrant testing for concomitant HIV infection.

PATHOGENICITY

Both animals and humans acquire this infection via respiratory tract. There, organisms are taken up by alveolar macrophages, in which they grow intracellularly and cause a granulomatous inflammatory response. This progresses to caseous necrosis of lung tissue. Later there may be hematogenous dissemination from a focal lung infection to brain, liver, kidney, skin, muscle or bone.[92]

DIAGNOSTIC MICROBIOLOGY

Rhodococcus equi produces large, mucoid, salmon-pink colonies on blood agar. Initial Gram staining may reveal Gram-positive coccobacilli. As colonies age, longer Gram-positive bacilli are formed that demonstrate branching and fragmentation into both coccoid and bacillary forms. *Rhodococcus equi* are partially acid-fast, nonmotile and have positive catalase and urease reactions.[87] [88]

CLINICAL MANIFESTATIONS

The overwhelming majority of *R. equi* infections involve the lung. As noted, most patients have an underlying condition such as AIDS or post-transplant immunosuppression that impairs their cell-mediated immunity. Clinical presentation may be insidious, with onset of fatigue, fever and nonproductive cough over several weeks. Chest radiographs often show nodular or cavitary lesions in the upper lung lobes that may mimic lesions due to *Mycobacterium tuberculosis* or *Nocardia* spp.[93] The underlying pathology is a necrotizing pneumonia and *R. equi* lesions have a propensity to show air-fluid levels, which are rarely seen in tuberculosis. Bacteremic pneumonia occurs more frequently in patients who have AIDS and their mortality rate is higher than that of patients who are not infected with HIV.

Fewer than 10% of patients present with extrapulmonary disease, usually abscesses in skin or soft tissues, osteomyelitis, meningitis or endophthalmitis. Recently, several cases of *Rhodococcus* infections have been found associated with medical devices such as central lines, ventriculoperitoneal shunts and chronic ambulatory peritoneal dialysis catheters.[92] [93] [94]

MANAGEMENT

As large numbers of organisms exist both intracellularly in macrophages and in abscesses in patients who have impaired cell mediated immunity, treatment of such infections may be problematic. *Rhodococcus* spp. are commonly resistant to penicillins and cephalosporins or will rapidly develop resistance during therapy. They usually are sensitive to erythromycin, clindamycin, rifampin, vancomycin, imipenem, aminoglycosides, chloramphenicol and fluoroquinolones, [84] but antibiotic therapy alone may not be successful without surgical excision and drainage. Reduction in immunosuppression where possible (i.e. reduction of corticosteroid or ciclosporin dosage).

Antibiotics with enhanced intracellular penetration and concentration abilities such as the macrolide antibiotics and rifampin appear to be the most potent and act synergistically to kill these intracellular bacteria.[95] Duration of therapy is not established but most patients require long courses of at least two antibiotics, even after surgical excision and drainage.

NOCARDIA SPECIES

NATURE

The genus *Nocardia* is a member of the order Actinomycetales, found in soil, plants and decomposing organic material. These organisms are also present in the gastrointestinal tract, oropharynx and skin of animals.

The taxonomy of Actinomycetales has been controversial. In recent editions of *Bergey's Manual of Determinative Bacteriology*, several new species have been recognized, and others reclassified.[96]

Nocardia was first described by Nocard in 1889 and is now divided into several species, only a few of which cause disease in humans — *Nocardia asteroides*, *Nocardia brasiliensis*, *Nocardia otitidiscaviarum*, *Nocardia transvalensis*, *Nocardia farcinica* and *Nocardia nova*. *Nocardia asteroides* accounts for the vast majority of clinical nocardial infection in humans.[97] Ribotyping has been proposed as a tool for taxonomic classification of this organism.[98]

EPIDEMIOLOGY

The incidence of systemic *Nocardia* infection in humans is probably underestimated since there is no comprehensive reporting system. However, based on a survey of US infectious disease physicians, the incidence in the mid-1970s was estimated at between 500 and 1,000 cases annually (2–4/10^6 people).[97] Since immunocompromised patients are profoundly susceptible to the development of nocardiasis, it is likely that the incidence of the disease in the 2000s is far greater than this estimate would suggest. Over the past 20 years there has been a marked increase in the number of immunocompromised patients due to aggressive chemotherapeutic management of cancer and to the epidemic of AIDS.[99]

Although immunosuppression is a major predisposing factor for infection, *Nocardia* spp. may occasionally cause disease in the absence of any predisposing factor. Cases may go undiagnosed because appropriate investigations are not undertaken before antimicrobial therapy is prescribed.

PATHOGENICITY

Nocardia elicits an inflammatory response in the host characterized by infiltration of polymorphonuclear leukocytes, activated lymphocytes and monocytes at the site of infection.

Although the pathogenic mechanisms contributing to elimination of *Nocardia* spp. from the body are poorly understood, a model of nocardiasis in mice shows many similarities to intracellular parasites such as *Listeria monocytogenes* and *Toxoplasma gondii*.[100] In the murine model of *Nocardia* infection, the organism persists for 3–4 weeks in high concentrations in the spleen and liver of intravenously or intraperitoneally infected animals. A large number of macrophages are seen in infected granulomas. Electron microscopic studies demonstrate that these macrophages contain organisms within the cytoplasm in various stages of degeneration. When compared with control cells, macrophages isolated from *Nocardia*-infected animals produce high amounts of TNF-a after ex-vivo stimulation with lipopolysaccharide. Since TNF-a and other cytokines upregulate the phagocytic system, this finding suggests that macrophage activation occurs during *Nocardia* infection. In support of this is the observation that animals treated with TNF-a antibodies fail to eliminate the organism from their liver when this would normally occur. Eradication of the organism from the body therefore appears to depend on TNF-a or its effects on the immune system.

DIAGNOSTIC MICROBIOLOGY

Diagnosis of invasive nocardial infection usually depends on demonstrating the organism in tissue. Specimens of sputum, bronchial wash, exudate, tissue biopsy or CSF should be collected for culture and staining in suspected cases. Gram's stain and a modified Kinyoun stain are recommended. Because the clinical picture is often unclear, clinical material should be examined for other potential pathogens including acid-fast bacilli and fungi.

Although *Nocardia* resemble fungi morphologically, producing a mycelial-like hyphae growth, they exhibit cellular characteristics of bacteria including the presence of procaryotic cellular organization. *Nocardia* spp. grow slowly on laboratory-enriched media. Specimens should be maintained for at least 2–4 weeks before being called negative. *Nocardia* grows well on enriched media such as blood or brain heart infusion agar. However, the microscopic morphology of *Nocardia* is best demonstrated by examining colonies grown on minimal medium such as tap water agar or cornmeal agar without dextrose. A period of 2–3 weeks of cultivation is needed before growth is evident on such media (Fig. 226.13). Colonies may have a smooth, chalky-white appearance; however, gross morphology is variable and may depend on the medium of cultivation and length of inoculation time. The color of *N. asteroides* colonies varies from salmon-pink to orange, while other *Nocardia* spp. vary from orange to tan colored.

The organisms are Gram-positive with short, extensively branched vegetative hyphae that break up into short rods and are less than 1µm in diameter (Fig. 226.14). Aerial hyphae may only be evident when viewed microscopically. Short chains of conidia may be found on the aerial hyphae. Most isolates of *Nocardia* in cultured material are weakly acid-fast by the modified Kinyoun stain.

Although some studies have proposed the use of a complement fixation serologic test to aid in the diagnosis, this is typically available only in research settings. The clinical value of this and other serologic or skin testing remain uncertain.

CLINICAL MANIFESTATIONS

The clinical manifestations of nocardial infection vary from localized to disseminated.

Nocardia spp. are recognized as pathogens among severely immunosuppressed patients such as those who have severe systemic lupus erythematosus,[101] transplant recipients [102] or patients who have advanced HIV infection. [99] Although nocardiasis has been described in patients who have AIDS, it remains relatively uncommon. The clinical manifestations of nocardiasis in HIV-infected patients are nonspecific, with fever, productive cough and weight loss most common. About two-thirds of patients who have systemic nocardial infection with AIDS have the lung as the primary site of infection.

Radiographic features of pulmonary nocardiosis at the time of presentation include unilateral involvement (18 of 22 AIDS patients) with an alveolar pattern (14 out of 22).[100] The upper lobe was most commonly affected. CT scans of patients who have pulmonary

Figure 226-13 Colonies of *Nocardia asteroides,* showing a smooth, chalky-white appearance.

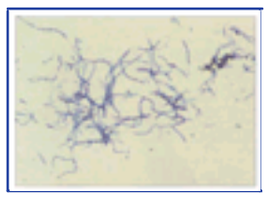

Figure 226-14 *Nocardia asteroides* on Gram stain, showing extensively branched vegetative hyphae that break into short rods.

infection show enlarged nodules or other masses (83%), cavitation (33%), consolidation (33%) and pleural thickening (29%).[104] Among patients who have severe immunosuppression such as AIDS, evidence of cavitation is seen more commonly.

Primary cutaneous infection may be caused by *N. asteroides*, *N. brasiliensis* or *N. otitidiscaviarum*.[104] [105] In contrast to pulmonary or disseminated nocardiasis, cutaneous nocardial infection commonly occurs in immunocompetent individuals. Four clinical types of cutaneous nocardiasis have been described: mycetoma, lymphocutaneous involvement, superficial cellulitis and cutaneous involvement with disseminated disease. Primary cutaneous nocardial infection usually results from traumatic inoculation with the organism. Cutaneous lesions are characteristically painless, slowly progressive and localized (Fig. 226.15).

Rarely, localized infections may become invasive. Such infections have been described both in immunocompetent and immunocompromised subjects and may occur secondary to trauma or direct spread from adjacent anatomic sites.

A total of 131 patients who had *N. asteroides* brain abscess have been described.[106] Only about a third of these patients had a history of an underlying disease or treatment that might have affected the immunocompetence of the subjects. Co-existent *Nocardia* infection outside the central nervous system is common (71%). CT scans using contrast enhancement characteristically show single or multiple ring-enhancing brain abscesses. Most abscesses are located in the supratentorial space. Solitary brain abscess was present in 71 of 121 cases. Patients who had single abscesses had a lower mortality (32%) than those who had multiple abscesses (66%).

MANAGEMENT

In most clinical settings *Nocardia* can be managed effectively with antibiotic therapy. Recommendations for antibiotic therapy of localized or systemic nocardial infection are problematic since no large clinical trials have been undertaken. Moreover, the clinical relevance of in-vitro susceptibility testing has not yet been established. The most commonly used testing method has been the gradient strip diffusion test (E-test, AB Biodisk, Solna, Sweden). Susceptibility testing guidelines have been published by the National Committee for Clinical Laboratory Standards.[107] The most active parenteral

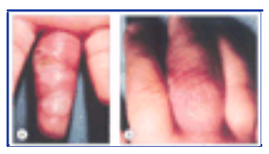

Figure 226-15 Primary cutaneous nocardial infection is characteristically painless, localized and slowly progressive. (a) There is marked swelling and erythema in this child's finger. (b) However, because the finger was painless the child was not brought to medical attention until the infection had progressed to involve the entire finger.

agents for *N. asteroides* are amikacin (95% susceptible), imipenem (88%), ceftriaxone (82%) and cefotaxime (82%). The most active oral agents are sulfonamides (100%), minocycline (100%) and amoxicillin (40%). In many studies the combination of trimethoprim-sulfamethoxazole has been found to be synergistic in vitro. Since both drugs penetrate tissue, including CSF, and are usually well tolerated, the combination of trimethoprim and sulfamethoxazole is often recommended as the first line of therapy.[97] Other drug combinations, such as amikacin-imipenem and amikacin-ceftriaxone, have been proposed for severe infection requiring parenteral therapy.

Nocardial brain abscesses should be managed with a combination of medical and surgical techniques. The largest review on this subject recommended conservative medical treatment if the brain abscess is less than 2cm in diameter. [106] For patients who had larger abscesses or deteriorating conditions, stereotactic aspiration to confirm the diagnosis and decompress the lesion was advocated.

MISCELLANEOUS AEROBIC GRAM-POSITIVE BACILLI

ROTHIA DENTOCARIOSA

This pleomorphic, aerobic Gram-positive bacillus is catalase-reaction-positive, hydrolyzes esculin and forms coccoid spherules in broth culture and branching slender rods on solid agar. It is found as part of the normal flora in the oropharynx, where it has been associated with dental caries and chronic periodontal disease in the immunocompetent host and septicemia, pneumonia and infective endocarditis in the immunocompromised host. This organism remains susceptible to most ß-lactam antibiotics, erythromycin and vancomycin.[108]

OERSKOVIA SPECIES

These aerobic, non-sporulating Gram-positive bacteria are found in soil. They form yellow colonies without aerial mycelia and produce a substrate mycelium that fragments into non-acid-fast, motile rods. They resemble *Nocardia* spp. in their ability to hydrolyze casein, xanthine and hypoxanthine to a varying degree. They have been associated with bacteremias, endocarditis, catheter-associated infections and infections after direct trauma involving soil contact, especially, but not exclusively, in immunocompromised hosts.[109]

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Chapter 227 - Neisseria

Jacob Dankert

INTRODUCTION

The genus *Neisseria* was named after Albert Neisser who observed diplococci in leukocytes in urethral exudates from patients who had gonorrhea in 1879.[1] Three years later Leistikow and Loeffler were able to cultivate the bacterium, termed gonococcus, in pure culture.

Although various species within the Neisseriaceae family can cause disease in humans and various animals, the two well-known pathogenic species for humans are *Neisseria gonorrhoeae*, or gonococcus, and *Neisseria meningitidis* or meningococcus.

Neisseria gonorrhoeae causes gonorrhea, a sexually transmitted disease. Gonorrhea was named by Galen in AD130 after the Greek words *gonor* (seed) and *rhoia* (flow), suggesting that the disease was related to the flow of semen. In the 13th century Maimonides recognized that the urethral discharge of male gonorrhea patients was not semen, but a sexually transmitted disease. Gonorrhea was not clearly distinguished from syphilis until the 19th century. In 1885 Bumm proved the causal relationship between the bacterium and the onset of gonorrhea by inoculating gonococci into volunteers. Although it was known that untreated infection healed spontaneously, treatment was warranted because reinfections were common. Effective therapy became available in the 1930s when sulfonamides were developed and in 1943 when penicillin was produced. Sulphonamide resistance occurred early and rapidly in gonococci. Penicillin-resistant gonococci due to penicillinase production were documented in various parts of the world in 1975 and 1976. The prevalence of strains resistant to penicillin and other antibiotics has risen steadily worldwide. Therefore, the pathogenesis of gonorrhea, the host response to infection and the molecular biology of the gonococcus have been studied intensively over the past two decades.

Neisseria meningitidis is a cause of endemic and epidemic disease in developed and developing countries. Epidemic meningococcal meningitis was first described by Vieusseaux in 1805 in Geneva.[2] Throughout the 19th century periodic epidemics occurred, involving mainly young children and military men in recruit camps. In 1887 Weichselbaum isolated the meningococcus from six of eight cases of primary sporadic community-acquired meningitis. Kiefer described the nasopharyngeal meningococcal carrier state among healthy people in 1896. Recognition of an association between increasing rates of meningococcal carriage and the onset of epidemic disease stimulated studies of the epidemiology and prevention of meningococcal infection. Serum therapy, used by Flexner and Jobling in 1908, resulted in survival from a previously fatal disease. The outcome of meningococcal disease improved drastically by treatment with sulfonamides in 1937. In addition, sulfonamides to eradicate the carrier state were used as prophylaxis to prevent epidemics. Nevertheless, an epidemic in two military bases in California in 1963 could not be aborted by sulfonamide prophylaxis because of the occurrence of sulfonamide-resistant meningococci. This led to the development of vaccines derived from selected meningococcal capsular polysaccharides. Such vaccines have been successfully used in military recruits and have also been given to civilian populations.

A successful vaccine that prevents infection caused by *N. meningitidis* serogroup B, which causes most cases of disease in temperate countries, has not, however, been developed. The absence of a group B vaccine limits the effective control of meningococcal disease. Other problems of meningococcal infection are:

- enhanced susceptibility of certain age groups;
- mechanisms involved in the upper respiratory carrier state leading to invasion of the meningococcus into the bloodstream; and
- reasons for the fulminant nature of the infection in certain patients.

NATURE

Taxonomy

In the 1984 edition of *Bergey's manual of systemic bacteriology*,[3] the family of the Neisseriaceae included the following four genera:

- *Neisseria*,
- *Moraxella*,
- *Kingella*, and
- *Acinetobacter*.

The taxonomy of the family has been extensively revised over the past decade. This has led to the renaming of certain genera and species, as well as the addition of new ones.

The genus *Neisseria* contained 11 species considered as 'true *Neisseria*' recovered from human sources and three animal species called the 'false *Neisseria*'. The genus *Moraxella* had two subgenera — the subgenus *Moraxella* with six species and the subgenus *Branhamella* with four species. The genus *Kingella* contained three species and the genus *Acinetobacter* was made up of various phenotypes.

Studies of the family Neisseriaceae making use of DNA-rRNA hybridization, DNA-DNA hybridization and 16S rRNA sequence analysis have revealed substantial differences between the genera and certain species within each of the four genera.[4] [5] The family Neisseriaceae, as defined in *Bergey's manual of systematic bacteriology*, is genetically heterogeneous. Its members cluster in two separate rRNA superfamilies that exhibit no significant levels of relatedness. The true Neisseriaceae and two *Kingella* spp. belong to the ß-subclass of the Proteobacteria. Because the false *Neisseria, Moraxella, Branhamella* and *Acinetobacter* genera belong to the ?-subclass of the Proteobacteria, recommendations have been made to remove them from the family Neisseriaceae. Molecular genetic techniques have demonstrated that the genera *Eikenella, Simonsiella* and *Alysiella* and four CDC groups (EF-4a and 4b, M-5 and M-6) are close genetic relatives of the true *Neisseria* and two *Kingella* spp.[5] The newly amended family Neisseriaceae, containing true *Neisseria* spp. from humans as well as false *Neisseria* spp., is shown in Table 227.1 . The taxonomic status of the false *Neisseria* is debated, but they are considered as *Neisseria* spp. from animal origin.

Neisseria weaveri and *N. elongata* subspp. *nitroreducens* are the former CDC groups M-5 and M-6, respectively. The true *Neisseria* and both *Kingella* spp. form a cluster with 91% or more DNA homology,

TABLE 227-1 -- Amended classification of micro-organisms within the family Neisseriaceae.

AMENDED CLASSIFICATION OF MICRO-ORGANISMS WITHIN THE FAMILY NEISSERIACEAE						
Neisseria			***Kingella***	***Eikenella***	***Simonsiella***	***Alysiella***
Habitat, humans		Habitat, various animals	Habitat, humans	Habitat, humans	Habitat, humans and animals	Habitat, humans and animals

Urogenital tract	*N. gonorrhoeae*	Oropharynx	Oropharynx and urogenital tract	Oropharynx	Oral cavity	Oral cavity
Oropharynx	*N. meningitidis, N. lactamica, N. sicca, N. subflava, N. flavescens, N. mucosa, N. cinerea, N. polysaccharea, N. elongata*	Monkeys: *N. macacae*; cats: *N. canis*; dogs; *N. weaveri*; guinea-pigs: *N. denitrificans*; iguanas: *N. igaunae*; cats/dogs: CDC, EF-4a, CDC, EF-4b; guinea-pigs: *N. caviae*; sheep/cattle: *N. ovis*; rabbits: *N. cuniculi*	*K. kingae*	*E. corrodens*		
			K. denitrificans			
The taxonomic status of *N. caviae, N. ovis* and *N. cuniculi* is debated.						

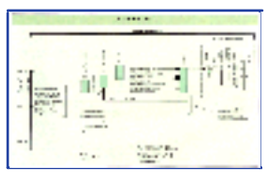

Figure 227-1 Neisseriaceae family. $T_{m(e)}$ is the melting temperature at which 50% of a hybrid is denatured. *(Adapted from Rossau et al.[5])*

but are rather distantly related (Fig. 227.1). The relationship between *Neisseria* spp. is as follows:

- *N. gonorrhoeae* and *N. meningitidis* are very closely related;
- *N. lactamica, N. polysaccharea* and *N. cinerea* are related to *N. gonorrhoeae* and to each other more distantly;
- saccharolytic Neisseriaceae (*N. subflava, N. sicca* and *N. mucosa*) are more distantly related to the other Neisseriaceae; and
- species of animal origin are still more distantly related.

The closest neighbors of the family of Neisseriaceae are the genera *Chromobacterium* and *Aquaspirillium*.

General characteristics of the amended family of Neisseriaceae are that the micro-organisms are coccal, coccoid or rod shaped or exhibit a multicellular micromorphology (*Simonsiella* spp. and *Alysiella* spp.).[4] [5] They are Gram-negative. Several species possess capsules and are fimbriated (piliated). Endospores are not found, and flagella are absent. Members of the genus *Simonsiella* and *Alysiella* show gliding motility. Some *Neisseria* spp., including *N. meningitidis*, may show surface-bound twitching motility. All species are aerobic, but *Kingella* and *Eikenella* spp. also grow under anaerobic conditions. The optimum growth temperature is 89.6–98.6°F. The guanine-plus-cytosine

content of DNA ranges from 41 to 58mol %. Most species are not pathogenic and their main habitat is the mucosal surface of the nasopharynx and oropharynx of humans and various mammals.

Neisseria gonorrhoeae and *N. meningitidis* are primarily pathogens of humans. Both *Kingella* spp. and *Eikenella corrodens* are occasionally isolated from human infections, and are discussed in Chapter 229 .

General characteristics

The genus *Neisseria* is composed of 10 species that may be isolated from humans and 10 species that may be cultured from various animals.[6]

The *Neisseria* spp. are the only true coccal members of the family Neisseriaceae, with the exception of *N. elongata* and *N. weaveri*, which are medium-to-large plump rods that sometimes occur in pairs or short chains. *Neisseria* spp. grow best aerobically in an atmosphere containing 5–10% carbon dioxide at a temperature of 89.6–98.6°F and pH of 7–7.5. The micro-organisms are Gram-negative cocci that typically appear in pairs (diplococci) and occasionally in tetrads or clusters. Diplococci have flattened opposing sides, imparting the characteristic kidney or coffee bean appearance seen in stained smears. Cell size ranges from 0.6 to 1.5µm depending upon the species source of the isolate and the age of the culture.

Neisseria spp. can be grown on several kinds of media, though growth may be inhibited by fatty acids in the medium. Inhibition can be eliminated by adding starch or blood. Blood agar medium or chocolate medium (blood heated at 176–194°F) are suitable media for growing *Neisseria* spp.

Colonies of *N. gonorrhoeae* vary in diameter from 0.5 to 1µm. Colonies of *N. meningitidis* are usually larger (1–µm in diameter) and flatter than those of *N. gonorrhoeae*. Colonies of *N. flavescens* are yellow and opaque and colonies of *N. elongata* have a dry claylike appearance. Colonies of the other nonpathogenic *Neisseria* spp. are similar in size, appearance and consistency to those of the two pathogenic *Neisseria* spp., except those of the saccharolytic *Neisseria* spp. (*N. subflava, N. sicca* and *N. mucosa*). Colonies of these species are larger (1–3µm in diameter), more convex and smooth (*N. mucosa*). Colonies of *N. subflava* and *N. sicca* are opaque and have varying consistency. *Neisseria sicca* adhere to the agar surface and become wrinkled with prolonged incubation. Some nonpathogenic *Neisseria* spp. form a yellow pigment (*N. flavescens*) or a greenish-yellow pigment (*N. mucosa, N. subflava*).

Neisseria spp. are oxidase positive and catalase positive, except *N. elongata*, which is catalase negative.

All species produce acid from a few carbohydrates by oxidation. The ability to produce polysaccharide from sucrose, to produce catalase and deoxyribonuclease, to reduce nitrate and nitrite, and to oxidize the tributyrin fatty acid are also used to identify *Neisseria* spp.

Habitat and prevalence

Neisseria spp. are normal inhabitants of the nasopharyngeal and oropharyngeal mucous membrane of humans and various mammals. [2] [6] *Neisseria meningitidis* is usually carried in the nasopharynx without causing disease. [7] Colonization may persist for many months. One-third of the carriers have chronic carriage for more than 16 months. Carriage may also occur on anogenital mucosal membranes. In children under 4 years of age the carriage rate is 1% or less. Carriage rates rise progressively through the first two decades of life to peak at about 20–25% in late teenage and early adult life, and then slowly decline. During carriage the expression of capsule polysaccharide is absent or low. Therefore, nongroupable (capsular polysaccharide absent) meningococci are predominantly cultured from the nasopharynx of carriers. Meningococcal carriage may not be confined to the mucosal surface.[8] Carriage rate among patients undergoing tonsillectomy as assessed by nasopharyngeal swabbing

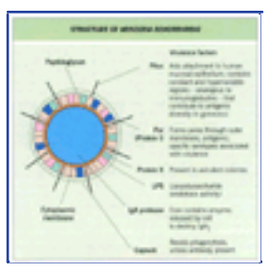

Figure 227-2 Structure of *N. gonorrhoeae.* *(Adapted from Mims et al. [9])*

was 10%, whereas meningococci detected by immunohistochemical methods were present within the epithelial layer and tonsillar tissues from 45% of such patients. These findings show that the conventional swabbing method to determine carriage rate is an insensitive procedure, assuming that the majority of the subepithelial meningococci are viable cells. Carriage induces bactericidal antibodies. Since the development of such antibodies increases progressively at a young age when carriage of meningococci is low, other bacteria with cross-reaction most likely induce bactericidal antibodies. *Neisseria lactamica* carriage elicits bactericidal antibodies against various meningococcal serogroups and serotypes. Carriage of *N. lactamica* is higher than that of *N. meningitidis* in infancy and in young children and is approximately 4% by 3 months of age and peaks at 21% by 18–24 months of age.[7]

Both children and adults can be colonized either by strains of the sucrose-positive *Neisseria* spp. (*N. mucosa, N. perflava, N. sicca*) or by several other *Neisseria* spp. Both patterns of colonization are stable and generally persistent for many months.

Neisseria gonorrhoeae strains are always considered to be pathogenic, in contrast to *N. meningitidis*, of which only some strains can cause meningococcal disease. Gonococci infect those surfaces which are lined with columnar epithelial cells: endocervix, urethra, anogenital and oropharyngeal mucous membranes and conjunctiva.

Both *Kingella* spp. are part of the normal upper respiratory and genitourinary tract flora. *Eikenella corrodens* is a commensal of the human oropharynx and upper respiratory tract.

Structure

Neisseria spp., like other Gram-negative bacteria, possess an inner cytoplasmic membrane, a thin peptidoglycan layer and an outer membrane containing proteins, phospholipids and a lipo-oligosaccharide (LOS), as shown in Figure 227.2 for *N. gonorrhoeae*.[9]

Capsules

Neisseria gonorrhoeae is covered with a loosely adherent capsular-like structure containing high-molecular weight polyphosphate. Its function is unknown.[6]

Neisseria meningitidis also produces polyphosphate loosely adherent to the surface. In contrast to gonococci most meningococci have a polysaccharide capsule.[6] On the basis of structural differences in the polysaccharide, meningococci can be divided into at least 12 serogroups (A, B, C, 29E, H, I, K, L, X, Y, Z and W135). Meningococcal disease-associated serogroups B, C, Y and W135 all have *N*-acetylneuraminic or sialic acid (a common constituent of eukaryotic cell membranes) in their capsular polysaccharide. Sialic acid confers resistance to host complement-mediated attack mechanisms. The disease-associated serogroup A capsule contains *N*-acetyl-mannosamine-1-phosphate. This serogroup causes most of the major epidemics, particularly in less developed countries. Serogroups B and C generally cause endemic disease and occasionally epidemic outbreaks worldwide.

Meningococcal capsular polysaccharides induce specific bactericidal antibodies. Therefore, capsular polysaccharide antigens are used as vaccine materials. The B-polysaccharide is poorly immunogenic, probably because of immunotolerance due to cross-reactivity between this polysaccharide and polysialic acid expressed on host neural cell adhesion molecules.

In the absence of opsonizing antibodies the various capsules render meningococci resistant to phagocytosis. The genetic material responsible for capsule polysaccharide synthesis can be exchanged between meningococci.[10] Serogroup B can thus switch to C or vice versa.[11]

Pili

Pili are hair-like filamentous proteins consisting of repeating peptide subunits (pilin). Pathogenic *Neisseria* spp. have long pili (4300nm in length) protruding through the capsular structures (see Fig. 227.2). Nonpathogenic *Neisseria* spp. have short pili (175–210nm in length) and long pili.

Neisseria gonorrhoeae has pilin protein subunits that have an apparent molecular weight of 16.5–21.5kDa. Gonococci produce one type of pilus; the main subunit is Pil E. CD 46 has been identified as the pili receptor.[12] Pili undergo both phase variation and antigenic variation. Expression of pili is a function of the *pil* gene complex.

Gonococci contain one copy of *pil E* and multiple copies of *pil S*, which are transcriptionally silent. *pil C* encodes a protein involved in pilus assembly. Phase variation involves several mechanisms. Deletions and frame shift mutations produce truncated pilin and a stable P^- -phenotype. Reversible phase variation can be due to changes in the *pil C* region.

Colonial morphology is associated with piliation: cells from small domed colonies are piliated (P^+ and P^{++} colonies) in contrast to cells from large, flat colonies (P^- colonies).

Freshly isolated gonococci from patients carry pili. Initially grown on culture media, gonococci form colony types P^+ and P^{++} . P^- colonies lacking pili predominate after 20 hours of growth. This shift between P^+ or P^{++} to P^- colony types is due to pili phase variation. *In vitro* the frequency of phase variation is high, being 10^{-2} to 10^{-4} , and usually occurs in only a fraction of the bacterial population.

Antigenic variation of pili is due to the variability of pilin sequences residing primarily in the C-terminal half of the protein. The genome of the *pil* gene complex contains many incomplete sequences, which can be recombined with expressed loci on the same chromosome. Lysing gonococci release transforming DNA, which can recombine effectively with partly homologous regions. The frequency of antigenic pili variation may be *in vitro* as high as 10^{-3} per cell in a fraction of the bacterial population.

Neisseria meningitidis expresses two different classes of pili (either class I or class II), which are antigenically and structurally distinct. During infection pili undergo rapid phase shifts and antigenic variation.[13] Unlike gonococci, piliated meningococci form colonies that are indistinguishable from their nonpiliated isogenic forms.

Pili are essential for adhesion to epithelial and endothelial cells and impart tissue tropism.[14] Piliated *Neisseria* spp. adhere to susceptible cells and can initiate infection. Pili can overcome the electrostatic repulsion between the negatively charged mucosal surfaces and also play a role in transformation (the acquisition of homologous and heterologous DNA from the environment).[15] Neisserial competence for transformation is much greater in piliated cells than in nonpiliated cells. Pil E and Pil C are required for DNA uptake.[15]

Outer membrane proteins

The outer membrane proteins of gonococci and meningococci are similar. For both species various proteins are present within or on the surface of the outer membrane.[16] The trimeric proteins provide channels or porins, allowing low-molecular nutrients to diffuse through the outer membrane.

Neisseria gonorrhoeae porin protein is termed Por (formerly protein I). There are two different antigenic forms of Por (Por A and Por B) with molecular weights of 32kDa to 36kDa. Por is LOS associated and shows stable interstrain variation. The antigenic heterogeneity is the basis for serologic typing schemes of gonococci. Strains expressing Por IA are associated with resistance to normal human serum and disseminated gonococcal infection.

Neisseria gonorrhoeae can also express a family of other membrane proteins called protein II (20–28kDa). Colonies of bacteria expressing protein II have a more opaque surface than those not expressing PII. Therefore, PII is designated as an opacity (Opa) protein.[17] Each of the 10–12 Opa proteins is produced from its own *opa* gene. Expression of Opa varies, in contrast to Por, due to high-frequency variation in the *opa* gene because of translational frame shifting. The expression of each gene can be independently switched on and off. These phase transitions occur frequently, yielding expression of up to three different Opa types at one colony (Opa^- , Opa^+ , Opa^{++}). Antigenic variation among the Opa proteins of a strain also occurs.

Opa proteins have an important role in bacterial adherence. Heparin-related compounds[18] and CD 66[19] have been identified as Opa receptors. Cell tropism is determined by the variable expression of the Opa proteins.

Neisseria meningitidis is able to express two porins simultaneously: a class 1 protein, or Por A (44–47kDa), and either a class 2 (40–42kDa) or class 3 (37–39kDa) protein, Por B. [16] Por A is cation selective, whereas class 2 and 3 porins are anion selective. Class 2 and class 3 porins are the equivalent of the gonococci Por protein. The antigenic differences between class 2 and class 3 proteins of different strains are used for serotyping meningococci.

Neisseria meningitidis possesses an outer membrane protein, class 5 protein (26–30kDa), known as opacity protein. So far five variants of protein 5 are known, and from none to all five may be expressed by a single strain. There is no relationship between colonial opacity and the expressions of these proteins in contrast to *N. gonorrhoeae*. As in gonococci, the Opa proteins can undergo antigenic variation during natural infection. Two proteins, Opa and Opc, play a role in attachment of the

bacteria to tissue. CD66e and heparin sulfate proteoglycan are their receptors, respectively. [20] [21] The variable expression of these proteins represents an important cell tropism determinant. The outer membrane of both *Neisseria* spp. also contains a protein which increases in molecular weight upon treatment with a reducing agent. This reduction modifiable protein (Rmp) in *N. gonorrhoeae* is designated as protein III and in *N. meningitidis* as class 4 protein. The protein is immunologically conserved. Rmp antibodies interfere with the bactericidal activity of antibodies directed to other surface antigens. Therefore Rmp antibodies are so-called blocking antibodies. Both *Neisseria* spp. also have a number of environmentally regulated outer membrane proteins. These proteins may

be expressed depending upon the bacterial growth conditions. The iron-regulated proteins are essential for survival of gonococci and meningococci *in vivo*. The transfer-binding proteins (Tbp-1 and Tbp-2) and the lactoferrin-binding proteins (Lbp) mediate internalization of iron into the bacterium.

Lipo-oligosaccharide

Approximately 50% of the outer membrane is LOS. Gonococcal and meningococcal LOS lack the multiple repeating sugars of enterobacterial lipopolysaccharide (LPS). Therefore the neisserial LPS has been termed LOS. Lipo-oligosaccharide is composed of lipid A and a short sugar chain of 8–12 saccharide units, the core oligosaccharide. The lipid A portion anchors LOS in the outer membrane and is the active moiety of endotoxin. The core oligosaccharide is divided into an inner and outer core region. The inner core has a common structure. Lipo-oligosaccharide outer core sugars undergo high-frequency phase and antigenic variation[22] [23] during the course of infection and are used for immunotyping of meningococci.

The terminal structure of LOS is the target for sialylation by bacterial sialyltransferase. Gonococci utilize cytidine-5-monophospho-*N*-acetylneuraminic accid (CMP-NANA) present in the host tissues and add NANA to the LOS. Meningococci synthesize the CMP-NANA. Sialylation protects against the bactericidal activity of normal serum and generates a structure that mimics a structure present on host tissue cells. The neisserial outer membrane continuously sheds vesicles, which occur as blebs. These blebs contain DNA, protein and high levels of LOS, including lipid A. The latter component is implicated in the onset of shock if bacteria gain entrance into the circulation.[24]

Peptidoglycan

The peptidoglycan layer of *N. gonorrhoeae* and *N. meningitidis* may contribute to the inflammatory response, especially in gonococcal disease. In addition, peptidoglycan causes complement activation.

Typing of *N. gonorrhoeae* and *N. meningitidis*

Studies of the pathogenesis and epidemiology of gonorrhea and meningococcal meningitis have been enhanced by the availability of appropriate typing methods.

Characterization of *N. gonorrhoeae* is based on auxotyping and serotyping.[6] Gonococcal strains that have specific requirements for certain nutritional growth factors are known as auxotypes and include prototrophic (none requiring), proline-requiring and arginine-, hypoxanthine- and uracil-requiring (AHU) strains. *Neisseria gonorrhoeae* can be subdivided in 35 auxotypes. Arginine, hypoxanthine and uracil auxotypes are more likely to cause asymptomatic infection and more subtle inflammatory signs than other auxotypes.

Serotyping for *N. gonorrhoeae* is performed using a panel of monoclonal antibodies directed against epitopes on Por (protein I). Strains are divided into two major serogroups, Por IA and IB strains. Subdivision into serovars is based on patterns of reaction with specific protein IA and protein IB antibodies. In total there are at least 55 serovars. Typing systems have been used in epidemiological studies of gonorrhea in communities and in analyzing patterns of antibiotic resistance. Strain typing is also done by analysis of DNA sequence variations, by Opa typing or DNA sequencing of the *por* gene.

The phenotypic classification of *N. meningitidis* is based upon serologic methods to detect antigenic differences on four surface antigens:

- capsular polysaccharide,
- epitopes on class 2 and 3 outer membrane proteins,
- epitopes on the class 1 outer membrane, and
- LOS epitopes.

The 12 serogroups are recognized on the basis of the immune reaction of the polysaccharide capsule. Serotyping is based on the difference in the class 2 and 3 proteins (Por B). Strains are further subdivided into serosubtypes based on differences in the class 1 protein, Por A.

Lipo-oligosaccharides can also be used as typing antigens, giving rise to immunotypes L1, L2, L3, etc. Six different gonococcal LOS immunotypes and 12 different meningococcal LOS immunotypes have been recognized. Among meningococcal serogroups B and C immunotypes L1–9 are found, and in serogroup A immunotypes L10–12. Double epitopes may be recognized depending upon phase variation or the presence of incomplete epitopes on the same bacterium. Using the current typing scheme, a meningococcus is characterized as follows:

- B: serogroup, the capsular polysaccharide composition;
- 15: serotype, class 2/3 (Por B) protein antigen;
- P1.7, 16: serosubtype, class 1 (Por A) protein antigen;
- L 3,7: immunotype, LOS antigen.

Characterization of gonococcal and meningococcal immunotype is relevant for pathogenetic studies. Meningococcal serogroup, serotype and serosubtype characterization are of great value in epidemiologic surveillance.

In addition to the serologic classification of meningococci, other typing methods are used.[25] Multilocus enzyme electrophoresis gives the electrophoretic type (ET). Multilocus sequence typing has been used to show that specific complexes of related hypervirulent strains have caused epidemics.[26] DNA restriction analysis, randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and ribotyping are also used.

EPIDEMIOLOGY

Gonorrhea

Gonorrhea caused by *N. gonorrhoeae* is a common sexually transmitted disease and a worldwide public health problem. Its incidence reflects the lifestyle of the human community. The incidence in most Western European countries is lower than in the USA, with approximately 200 cases/100,000 population in 1992.[27] In the 1995 survey the incidence in the USA was 124 cases/100,000 population.[28] The highest incidence is in countries of the developing world, ranging from 4000 to 10,000/100,000 population. Commonly the highest attack rates occur in 15–25-year-old men and women.

Gonococci are obligate human pathogens. They cannot spontaneously infect animals or reside free in the environment. Gonorrhea is commonly transmitted by sexual intercourse with an infected partner. The risk of acquiring the urethral infection for men is approximately 20% after a single exposure to vaginal intercourse with an infected woman, rising to 60–80% after four or more exposures.

The transmission rate from male to female is approximately 50% per contact, rising to 90% after three exposures. Gonococci may also be transmitted by orogenital contact or rectal intercourse. Perinatal transmission may also occur.

Although gonococci can survive for brief periods outside the human reservoir, transmission is extremely rare. Transmission of purulent vulvovaginitis in prepubescent girls through a contaminated washing glove has been reported, but currently children who have gonorrhea are considered to be victims of sexual abuse by an infected adult.

The major reservoir for continued spread of gonorrhea is the asymptomatic patient. Among infected women 30–50% are asymptomatic or show no symptoms associated with a sexually transmitted disease. Among infected men only 5–10% are asymptomatic. Asymptomatic women and men remain infectious for months. In

addition, people who have symptoms that they ignore may continue

to spread the disease. The rate of transmission of gonococcal infection varies with the type of sexual practice.

The incubation period for gonorrhea is on average 2–7 days, but may vary between 1 and 14 days.

Meningococcal disease

Meningococcal disease is a major worldwide health problem.[29] [30] Among populations of meningococci, different patterns of epidemiological behavior are recognized. These disease patterns are termed endemic, hyperendemic, epidemic and pandemic. The five serogroups A, B, C, Y and W135 account for almost all the disease-causing isolates.

In western Europe and the Americas, endemic meningococcal disease is caused mainly by serogroup B or C organisms occurring at a low level affecting 1–3/100,000 people.[30] [31] Sporadic increases of disease incidence, termed hyperendemic are often localized to a small area or restricted to a particular population or community. For example, hyperendemic outbreaks due to group B have been reported in Brazil, The Netherlands and the UK.[25] [32] Recently, increasing proportions of cases due to serogroup C have been reported from various European countries.[31] [33] Serogroups A and C predominate in China, the Middle East and parts of Africa. In the African 'meningitis belt', disease occurs as major periodic epidemics occurring every 5–12 years and is caused by serogroup A with attack rates of 500/100,000 population or higher.[25] [30] Occasionally, particularly virulent strains arise that cause pandemic outbreaks across continents.[25] [29]

In the USA, Israel and Sweden, disease due to serogroup Y has increased in recent years.[30]

Serogroup B and C generally cause endemic cases and small outbreaks; in addition, distinct groups of genetically related serogroup B and C organisms have been recognized, which spread worldwide. Young adults who socialize frequently at discos and parties may particularly be at higher risk if they smoke.[34] These clusters may contain both serogroup B and C organisms, presumably because of horizontal gene transfer of capsule synthesis genes. As only a small fraction of the clones colonizing the throat of healthy individuals can cause disease, there is a difference in virulence potential of different meningococcal strains.[26]

In the mid-1970s, ET-5 complex serogroup B strains resulted in an increased incidence of meningococcal disease in north west Europe and Central and South America.[25] In other countries in Western Europe, ET-5 (serogroup B) strains were also isolated, although the increase in incidence was much lower. Strains of this cluster caused a severe epidemic in Cuba starting in the 1980s, followed by outbreaks in Florida in 1981 and 1982. The ET-5 strains were isolated only sporadically in North America in the following years, until in 1994 this complex was associated with an increased incidence in the states of Washington and Oregon. In Brazil and Chile the incidence of meningococcal disease rose due to strains of this complex in the mid-1980s and ET-5 strains were isolated in countries in Africa and Asia.[25]

Another cluster that has been identified worldwide is the lineage III. The first strain was isolated in The Netherlands.[32] Since 1980, the number of lineage III isolates has risen in The Netherlands, simultaneously with an increased incidence of disease. Most strains of this genotype are characterized by the serosubtype P1.4 (mostly B:4:P1.4), although lineage III strains with different serosubtypes and nonlineage III strains with P1.4 have been found. Since then B:4:P1.4 strains have been identified in many other European countries, resulting in an increased incidence of disease in Belgium and England and an ongoing epidemic in New Zealand. [25] Meningococci of the ET-37 complex cause much of group C cases worldwide as well as the international outbreaks of W135 disease among pilgrims returning from the Hajj and their close contacts.[35]

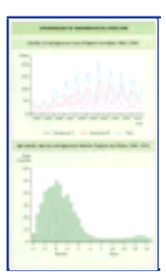

Figure 227-3 Epidemiology of meningococcal infection. *(Adapted from Jones.[36])*

Meningococcal disease irrespective of the serogroup involved is often seasonal in nature. Serogroup A and C disease increases during the dry season in Africa. The number of serogroup B and C cases peaks during the winter months in the developed countries (Fig. 227.3). [36] The reasons for the different epidemiological characteristics of the different serogroups and the cyclic nature of outbreaks are poorly understood.

The incidence of meningococcal disease appears to predominate in children under school age in the Western world (see Fig. 227.3).[36] Infants and children aged between 1 and 4 years account for approximately 50% of the cases. In the meningitis belt young school-aged children represent the peak age group.

Transmission of meningococci is by respiratory droplets, requiring close contact. The attack rate among family members of a clinical case is 1000-fold higher than in the general population. *Neisseria meningitidis* has a particular preference for the nasopharyngeal mucosal membrane.[7] Humans are the only natural host, but the incidence of disease cannot be attributed simply to acquisition of any strain. Invasive disease can be produced by:

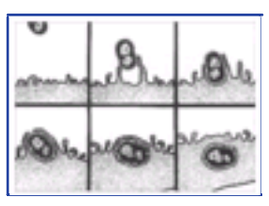

Figure 227-4 Steps in the pathogenesis of *N. gonorrhoeae* infection. *(Reproduced with permission from Weel.[40])*

- a pathogenic organism;
- a susceptible host;
- the influence of a coincidental viral or mycoplasma upper respiratory tract infection; or
- most frequently, a combination of all four.

Although there can be sudden and large changes in the rates of meningococcal acquisition and carriage, there is in general much less variation in rates of carriage than in rates of disease.

PATHOGENESIS

Gonorrhea

Gonococci primarily infect columnar or cuboidal epithelium. In males the anterior urethra is the primary site of infection and in females the endocervix. Colonization may occur, often followed by infection once the mucosal membrane of the genitourinary tract, rectum, oropharynx or eyes has been reached by gonococci. In most cases infection remains restricted to the initial site of colonization, although certain gonococcal strains have the ability to spread to distant sites. The infective dose for the male urethra is over 10^3 gonococci and for the female cervix ranges from 10^2 to more than 10^7 gonococci. [27]

Histopathologic examination of infected mucosa shows gonococci attached to the epithelial cell surface and within the epithelial cells, and occasionally in the submucosal tissue. Fulminant infection is characterized by a vigorous neutrophil response, sloughing of the epithelium, development of (sub)mucosal microabscesses and exudation of pus with gonococci inside polymorphonuclear cells. Several weeks after the start of the infection gonococci can no longer be observed histologically

or recovered by culture.

The first step in the pathogenesis of gonorrhea is the attachment of gonococci to mucosal surfaces and the ability of the gonococci to remain attached despite the continuous flow of body fluids (Fig. 227.4). Gonococci attach to microvilli of nonciliated columnar epithelial cells. Nearly all attached gonococci are piliated. Shortly after initial attachment gonococci become more tightly anchored to the epithelial cell surface. This second stage of binding is in part mediated by the gonococcal PII or Opa proteins to their cell receptors.[18] [20] Opa also mediates the binding of gonococci to each other to form microcolonies, probably aiding the initial colonization.

After attachment there is a critical interaction between gonococci and host defenses.[37] Gonococci have multiple mechanisms by which the bacteria resist innate defense as well as the defense by antibodies elicited by a previous infection. Sialylation of LOS may limit or alter complement binding.[38] The binding of cross-reactive blocking antibodies to Rmp (protein III) hinders the binding of bacterial antibodies. [39] *Neisseria gonorrhoeae* varies its surface proteins, especially pili antigens, by phase variation and antigenic variation.[22] In the case of pili, gonococci have an antigenic repertoire that may be as large as one million different antigenic variants. The antigenic repertoire for protein II (Opa) is smaller. The LOS composition is also changed due to antigenic variation.

Gonococci secrete an IgA protease that specifically cleaves IgA_1 in the hinge region. In response to environmental conditions gonococci synthesize iron-repressed gonococcal outer membrane proteins, which bind lactoferrin and transferrin, providing iron internalization. Cell damage is caused by LOS shed from viable bacteria and peptidoglycan fragments liberated from lysed bacteria.

In vitro it has been shown that after attachment gonococci invade epithelial cells as a result of endocytosis[40] (see Fig. 227.4). Whether this process represents a normal feature of uncomplicated mucosal infection is unclear. Cellular invasion is an important factor in the pathogenesis of a complicated infection such as salpingitis or disseminated infection. Invasion of gonococci is characterized by the formation of intimate contacts between the bacteria and the cell membrane, the zipper mechanism. Por and certain Opas facilitate invasion. Por is inserted into the plasma membrane of eukaryotic cells, probably making pores. Inside the cells the porin protein binds to calmodulin. As a result of this, microfilament-dependent processes are modified.

Por IB variant strains inserting into the host cell membrane remain localized, whereas Por IA variants translocate and are associated with disseminated gonorrhea. In addition, gonococci produce Yop-like substances, that interfere with host cell signal transduction systems and affect the host cell cytoskeleton. Bacteria-mediated rearrangement of actin is associated with forced endocytosis.

Inside the cells gonococci are transported within vacuoles, which coalesce to form larger vacuoles. Por prevents phagolysome fusion. Sheltered from innate host defenses, the gonococci multiply. Vacuoles fuse with the basement membrane. Although the majority of phagocytosed gonococci die, the viable gonococci are discharged into the subepithelial connective tissue.

As gonococci do not produce exotoxins, tissue damage results from the bacterial cell wall components such as LOS and peptidoglycan. Both constituents induce the production of tumor necrosis factor (TNF)-a. The inflammatory reaction is most likely responsible for the local symptoms.

A prominent feature of gonococcal infection is the intimate association with granulocytes, intracellularly as well as extracellularly. Opa+ gonococci adhere to granulocytes, bypassing phagocytosis and killing.[37] Also pili have antiphagocytic properties and sialylation of LOS reduces the opsonization of bacteria. The gonococci are able to resist oxidative killing inside phagocytes and may survive inside the granulocytes by binding of Por to calmodulin and upregulation of catalase production.

Certain strains have the ability to spread. Strains isolated from the fallopian tube lack Opa (protein II). Gonococci from other distant sites have the Por IA type protein. Host factors also play a role in the dissemination of gonococci. People who have terminal complement deficiencies have a high risk of developing bacteremia.[41]

Meningococcal disease

Infection due to *N. meningitidis* commonly results from asymptomatic nasopharyngeal carriage.[7] At any time approximately 10–20% of the population may be carriers of meningococci. Protective antibodies are evoked within 1 week by the meningococcal carrier state. Susceptibility to infection has been shown to correlate with the absence of specific antibodies. Another important host factor playing

a role in the pathogenesis of meningococcal disease is the complement system.[42]

The pathogenic effects of meningococci lie in the ability of the organism to traverse the epithelial lining of the respiratory tract, evade the host immune system and invade the bloodstream (Fig. 227.5).[43] [44] Meningococci either disseminate further into the subarachnoid space or remain circulating in the bloodstream. The mechanisms by which this occurs are only partially understood. *In vitro* studies have shown that meningococci adhere to the host cell receptor CD46, a complement regulatory protein[45] of the microvilli on nonciliated epithelial cells via type IV pili and class 5 proteins. [14] [20] [21] Nonencapsulated strains adhere better than capsulated parent strains. Adherent meningococci cause ciliostasis at a distance, probably due to the induction of TNF-a or interleukin (IL)-1 by bacterial cell wall constituents. Meningococci produce $sIgA_1$ protease, which is though to help bacterial survival on the mucosal membranes. After mucosal adherence meningococci may initiate a bacterial-directed endocytosis.[46] Nonencapsulated meningococci traverse the cytoplasm, whereas encapsulated meningococci are transported in phagocytic vacuoles. The IgA1 protease and PorB may promote the survival of meningococci in the epithelial cell.[30]

In the migration from the mucosa to the deeper tissues the pili are downregulated. However, *in vitro* studies have shown that piliated meningococci associate with endothelial cells more readily than their nonpiliated counterparts. The presence of pili is also associated with a greater degree of cytopathic effects, including disruption of intercellular junctions and rounding up of cells.[43] It is not known why in an unknown percentage of colonized persons pathogenic meningococci are not adequately contained by the local host defenses and traverse the endothelial lining of the vascular bed, probably through widened intercellular junctions.[44] In the bloodstream meningococci may:

- be inactivated by the combined actions of antibody, complement and granulocytes; or
- reach the subarachnoid space; or
- multiply within the circulation.

Little is known of this early bacteremic phase and dissemination to the subarachnoid space. It is thought that there are at least three potential mechanisms by which bacteria may gain access to the subarachnoid space:[44]

- paracellular transport after disruption of tight junctions of the blood-brain barrier in the chord plexus;
- transport within circulating phagocytic cells, such as monocytes; and
- transcellular transport within endothelial cell vacuoles.

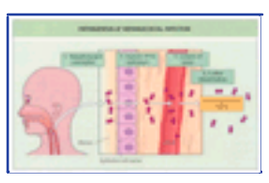

Figure 227-5 Pathogenesis of meningococcal infection. *(Adapted from Virji.[43])*

Systemic meningococcal infection is a bacteremic disease. The number of bacteria/ml of blood has been correlated with the severity of clinical presentation, probably due to the quantity of LOS.[24] Indeed, there is a correlation between clinical outcome and strains of meningococci that shed large amounts of LOS from surface blebs. The subsequent tissue injury is related to the host inflammatory response to LOS; circulating levels of proinflammatory mediators TNF-a, IL-1 and interferon-? at admission are strongly correlated with outcome.

Pathophysiologic sequelae of meningitis are also due to host responses to the presence of bacteria within the subarachnoid space. Host responses are elicited by intact bacteria as well as bacterial constituents such as LOS and peptidoglycan. They include the influx of leukocytes into the subarachnoid space (Fig. 227.6) and the release of soluble inflammatory mediators such as various cytokines, prostaglandins and platelet-activating factor.

Individuals who have inherited deficiencies in the late complement components (C6–C9) have a greatly increased risk of developing meningococcal infection.

Intriguingly, however, they acquire the infection at a much later age, have frequent recurrences and the fatality rate is much lower than for normocomplementemic individuals.[42]

Other genetic defects such as polymorphisms in the mannose-binding lectin[47] [48] and TNF-a may also alter susceptibility to the disease.[49]

PREVENTION

Gonococcal infection

Condoms provide a high degree of protection from acquisition of gonorrhea, as well as other STDs. Other important approaches for prevention of gonorrhea are early diagnosis and treatment, partner notification and screening and case finding. A new approach is the use of topical microbicides for intravaginal or intrarectal use.

Attempts to develop a gonococcal vaccine have been hampered by the high degree of antigenic variability in pili, Opa and LOS of gonococci during the natural course of infection. Porin (protein I), antigenically stable, inducing specific antibodies, is a preferred protein to produce a vaccine.[50]

Figure 227-6 Bacterial meningitis. Exudate of acute inflammatory cells in the subarachnoid space. *Courtesy of P Garen. (Reproduced with permission from Mims et al.* [5] *)*

Meningococcal disease

Prevention of meningococcal disease is based on two different approaches: chemoprophylaxis and the use of vaccines.[30] [51]

Chemoprophylaxis

The aim of chemoprophylaxis is to reduce secondary cases of meningococcal disease and to arrest outbreaks. The risk of a secondary case among close contacts of the index case in the household setting is 150–1000 times higher than that in the general population. Children are at greatest risk, but secondary disease can occur at all ages. Risk is maximal in the week following recognition of the index case but extends for several weeks.

Many of the antibiotics that are useful in treating infection do not effectively eradicate or prevent carriage of meningococci because of their inability to achieve adequate levels in oropharyngeal secretions. A number of agents, however, have been shown to be effective.[30] [52] Minocycline, a tetracycline, can eradicate meningococcal carriage, but its use is associated with an unacceptably high incidence of side-effects, particularly vestibular problems causing vertigo. Rifampin (rifampicin) is widely used as a chemoprophylactic agent now. Carriage is eradicated in 70–95% of the contacts after a 2-day course. Failures may be due to the emergence of meningococci resistant to rifampin. Side-effects include discoloration of soft contact lenses, interference with the oral contraceptive pill and red urine and it is contraindicated during pregnancy. Two other antibiotics have also been shown to effectively eradicate meningococcal carrier status. Ceftriaxone, in a single intramuscular dose, was 97% effective in household contacts after 1–2 weeks after infection. The advantage of ceftriaxone is that it can be used in pregnancy and to small children.

Ciprofloxacin and ofloxacin as a single oral dose have an efficacy of 93–97% in eliminating carriage.[52]

Chemoprophylaxis is recommended for close household contacts of cases and other intimate contacts. In addition, patients who have meningococcal disease should receive chemoprophylaxis before discharge from the hospital, because parenteral antibiotic treatment of meningococcal disease (except ceftriaxone) is unreliable in eliminating meningococci from the nasopharynx.

Vaccines

In the past two decades, increasing effort has been put into the development of new meningococcal vaccines, in particular against serogroup B disease. Two main approaches have been taken, based on polysaccharide or outer membrane protein (OMP) components.

Polysaccharide vaccines

These vaccines have been used successfully to reduce the incidence of infection among military recruits, to reduce the progress of epidemics of group A disease and to protect individuals known to be susceptible because of various complement deficiencies.[53] The vast majority of antibodies appear to bind to epitopes that are affected when the polysaccharide is de-O-acetylated, indicating that O-acetyl groups are critical in a polysaccharide vaccine.[54] However, immunity provided by polysaccharide vaccines is short-lived in adults (as no T-helper response occurs) and no protection is achieved in young children. Polysaccharide vaccines are used by travelers visiting countries with a high incidence of meningococcal disease and in these cases the short duration of the immune response is immaterial. A drawback of polysaccharide vaccines is that there is no vaccine in use against serogroup B meningococci, which causes most cases of disease in temperate countries. Early studies with serogroup B polysaccharide vaccines failed to show evidence of antibody response in human volunteers. There was a reluctance to include the group B polysaccharide in further vaccination approaches because of the possibility of inducing an autoimmune response.[51]

Conjugate vaccines

Following the success of the conjugate vaccines to prevent *Haemophilus influenzae* type b meningitis, serogroup A, C, Y and W135 meningococcal polysaccharides have been conjugated to carrier proteins.[51] Such polysaccharide protein vaccines are immunogenic in young infants. They induce a T cell-dependent response, prime immunologic memory and result in long-term protection. Initial field trials of these vaccines yielded encouraging data. In the United Kingdom meningococcal group C conjugate vaccine was introduced in health care programs in late 1999.[33] Rates of disease caused by serogroup C meningococci fell rapidly in the vaccinated age group.[55]

Age-specific vaccine efficacy had been estimated to be 88% in young children and 95% in young adolescents. Carriage of serogroup C meningococci studied in the vaccinated young adolescents group showed a reduction by 66%.[56] Meningococci have the capacity to exchange genes that induce the enzymes involved in capsular synthesis.[10] [11] It has been documented that the bacteria can switch from serogroup C to serogroup B or vice versa. Capsule switching, replacement by strains having capsular groups not contained in the vaccine providing serogroup specific protection, and replacement by nongroupable strains having the genetic material encoding the capsule but downregulating the capsule expression may occur with the widespread use of monovalent serogroup conjugate vaccines, but have not been established yet.

Bivalent A plus C polysaccharide conjugate vaccines have been assessed in clinical trials. Multivalent polysaccharide A,C,Y and W135 protein conjugate vaccines are being developed.[51]

Outer member protein vaccines

The observation that outer membrane components stimulated cross-reactive antibodies and promoted complement-mediated, bactericidal killing of capsulated strains led to alternative approaches to vaccination against serogroup B disease. The first experimental outer membrane vaccines used membrane preparations as close to the native membrane structure as possible. Detergent extraction was used to remove LOS from outer membrane vesicles obtained from a common disease-causing strain. Such vaccines were used in clinical trials and showed efficacies in the range of 50–80%. However, young children were not protected. There are a variety of novel OMP-based meningococcal vaccines currently under investigation,[51] such as construction of multivalent vaccine strains based on common variants of Por A;

OMPs complexes with group C polysaccharide; immunization with synthetic peptides; use of other organisms as vehicles for the delivery of a meningococcal OMPs; and investigation of vaccine potential of iron-regulated proteins (Tbp-1 and Tbp-2). The rational design of OMP-based vaccines must take into account the antigenic variability of circulating meningococcal strains to determine the most appropriate epitopes for inclusion in a vaccine. Therefore, new vaccine candidates on the basis of conserved antigens are being identified.

Novel vaccine candidates

The completion of the genomic sequencing of a serogroup B meningococcus made it possible to find open reading frames encoding novel surface-exposed proteins. [57] These proteins and those derived from a serogroup A meningococcus and a gonococcus (*N. gonorrhoeae*) were compared to identify similar conserved surface-exposed proteins. Such proteins were cloned, expressed in *Escherichia coli* and antisera were prepared to the recombinant proteins. Genome-derived antigens inducing antibodies which bind to the serogroup B meningococcal surface and eliciting bactericidal activity against serogroup B meningococcus may be vaccine candidates for providing broad protection. [57]

CLINICAL FEATURES

Gonococcal infection

Neisseria gonorrhoeae usually causes an infection of the urethra (urethritis) and cervix (cervicitis).[27] The bacterium may spread to the upper urogenital tract or may disseminate.

In addition, gonococci may cause localized infections at sites outside the urogenital tract.

Urogenital gonococcal infection of men

Acute anterior urethritis

This is the most common manifestation of gonorrhea (Fig. 227.7). It is characterized by a purulent urethral discharge and dysuria.

Acute epididymitis

This is the most common local complication of gonococcal infection and usually presents as gradually increasing unilateral scrotal pain.

Urogenital gonococcal infection of women

Cervicitis

The endocervix is the primary site of urogenital gonococcal infection. The infection is characterized by (muco)purulent discharge and intermenstrual bleeding, but is often asymptomatic.

Acute anterior urethritis

Urethral infection is present in 70–90% of women who have gonococcal cervicitis.

Abscess formation in glands adjacent to the vagina

About 35% of the women who have genital tract gonococcal infection have an infection of Bartholin's glands or Skene's ducts, by which abscess formation may occur.

Ascending gonococcal infection

Endometritis or acute salpingitis develops in 10–20% of women who have lower urogenital gonococcal infection. The high frequency of the asymptomatic infection of the endocervix is an important factor in the development of these complications because the initial infection is neither recognized nor treated. Symptoms of this clinical syndrome of pelvic inflammatory disease (PID) include lower abdominal pain abnormal cervical discharge and bleeding, fever and peripheral leukocytosis. The onset of salpingitis due to gonococci occurs early rather than late in infection and either during or shortly after menstruation. Obstruction

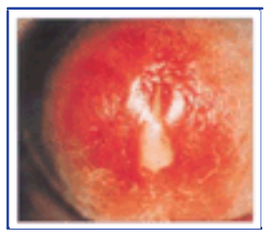

Figure 227-7 Gonococcal urethritis. *Courtesy of J Clay. (Reproduced with permission from Mims et al.[9])*

of the fallopian tubes leading to infertility occurs in 10–20% of patients who have PID after a single episode of gonorrhea.

Gonorrhea in pregnancy

In pregnant women gonorrhea is associated with an increased risk of spontaneous abortion, premature labor, early rupture of fetal membranes and perinatal infant mortality. Pelvic inflammatory disease is uncommon after the first trimester.

Gonorrhea in children

Historically gonococcal infection in children included only ophthalmia neonatorum (acute gonococcal conjunctivitis). Recently, studies have shown that children can acquire gonococcal infection by sexual contact with an infected person. Such infection therefore indicates sexual abuse. In girls the infection often produces a vulvovaginitis. Complications affecting the internal genital organs are rare.

Localized gonococcal infection outside the urogenital tract

Proctitis

Anorectal gonorrhea is present in up to 5% of women who have gonorrhea, although gonococci can be cultured from the anorectal region of 40% of women with gonorrhea. A similar proportion of homosexual men have positive rectal cultures for gonococci. Proctitis is manifested by anal pruritus, tenesmus, purulent discharge or rectal bleeding.

Pharyngeal gonorrhea

Pharyngeal infection occurs in 10–20% of women who have gonorrhea and in 10–25% of homosexual men who have the infection and in 3–7% of heterosexual men.

The infection is due to orogenital sexual exposure. Most cases are asymptomatic and resolve spontaneously.

Acute conjunctivitis

Ocular gonococcal infection is seen in neonates. Ophthalmia neonatorum is acquired during passage through an infected birth canal. In adults ocular infection usually results from autoinoculation of the conjunctiva in a person who has the infection. Gonococcal conjunctivitis is usually severe, with an overt purulent exudate and corneal ulceration.

Disseminated gonococcal infection

In 0.5–3% of infected individuals gonococci invade the bloodstream, resulting in disseminated gonococcal infection.

Dermatitis-arthritis-tenosynovitis syndrome

This infection is characterized by fever, hemorrhagic painful skin lesions, primarily on the hands or feet, tenosynovitis, polyarthralgias and frank arthritis (Fig. 227.8).

Monoarticular septic arthritis

In 30–40% of patients who have disseminated gonococcal infection, gonococci are localized in one joint and cause a purulent arthritis.

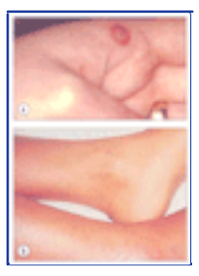

Figure 227-8 Disseminated gonococcal infection. (a) Skin lesions. *Courtesy of JS Bingham.* (b) Arthritis. *Courtesy of TF Sellers Jr. (Reproduced with permission from Mims et al.[9])*

The elbows, wrists, fingers and knee or ankle joints are commonly involved.

Perihepatitis

Acute perihepatitis (Fitz-Hugh-Curtis syndrome) occurs by direct extension of gonococci from the fallopian tube, lymphangitic spread or bacteremic dissemination to the liver capsule.

Endocarditis and meningitis

These complications are reported in 1–2% of patients who have disseminated gonococcal infection.

Meningococcal infection

On presentation 60% of cases have had symptoms for less than 24 hours and 12–20% for less than 2 days. Meningococcal disease may be manifested in various forms.[24] [30] [58]

Meningitis

The most frequent form of meningococcal infection is acute pyogenic meningitis due to inflammation of the meninges. Meningitis may occur with or without meningococcemia. Of patients who have meningococcal disease 75% have meningitis; 40% of them also have bacteremia.

Meningococcemia

Meningococcemia may occur with or without meningitis and may be transient, occult or result in severe sepsis. Bacteremia without meningitis occurs in 7–10% of patients. A distinguishing factor of meningococcemia is the appearance of skin lesions (Fig. 227.9). Initially these lesions appear on the mucous membranes and conjunctivae. Three patterns may be seen:

- a maculopapular rash.
- petechiae, and
- ecchymotic hemorrhagic or necrotic lesions or purpura fulminans.

However, skin lesions may be atypical, evanescent or even entirely absent in patients who have blood culture-positive meningococcal sepsis. Meningococcal sepsis may progress to disseminated intravascular coagulation characterized by increasing petechiae or purpura fulminans, resulting in extensive areas of tissue destruction secondary to coagulopathy, rapid onset of hypotension and adrenal hemorrhage (Waterhouse-Friderichsen syndrome).

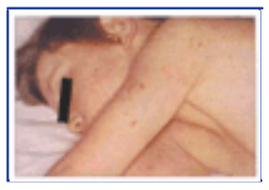

Figure 227-9 Typical rash of meningococcal sepsis. Fine erythematous macules and petechiae are present in some areas.

Chronic meningococcemia

Persistent meningococcal bacteremia is associated with low-grade fever, rash and arthritis (arthritis-dermatitis syndrome).

Respiratory infection

Meningococci are implicated as the etiologic agent in approximately 5–14% of patients who have community-acquired pneumonia. Pharyngitis is associated with recent contact with individuals who are colonized by meningococci and is often a prior symptom and sign of serious meningococcal disease.

Other infections

Neisseria meningitidis may cause other infections as a result of hematogenous spread. Infections due to blood-borne dissemination include endocarditis, pericarditis, arthritis, endophthalmitis, osteomyelitis, conjunctivitis and peritonitis. Arthritis is particularly common with an incidence of 11% in adults and 5% in children.

LABORATORY DIAGNOSIS

Gonococcal infection

Because *N. gonorrhoeae* can cause infection at a variety of body sites, collection of appropriate specimens for diagnosis depends upon the clinical manifestations and the sites exposed. In men suspected of having gonorrhea, urethral exudate is the best specimen. In females cervix swabs are preferred over urethral or vaginal specimens. Swabs coated with charcoal to absorb toxic substance are transported to the laboratory in specific transport media (Stuart's or Amies's). Specimens from the male urethra or the female endocervix should be collected from all cases of suspected gonococcal infection. Both specimens are taken for direct examination and culture. A finding of neutrophils containing Gram-negative cocci in the Gram-stained smear is presumptive evidence of gonococcal infection (Fig. 227.10). Gram stain is highly sensitive and specific for diagnosing genital gonorrhea in men. Gram-stained smears made from endocervix specimens from symptomatic women have a sensitivity of only 40–60% relative to culture, but a high predictive value. In asymptomatic women Gram stain has a low predictive value and is not useful.

Direct nonculture tests are also used. An enzyme-linked immunosorbent assay test detects gonococcal antigen. A nucleic acid probe test is also available. The polymerase chain reaction has also been applied for direct detection of gonococci in clinical specimens. The ligase chain reaction using highly sensitive nucleic acid amplification is used on urine and urogenital specimens. It has proved to be as

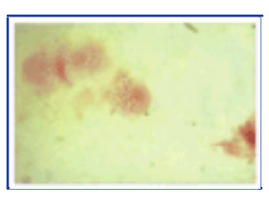

Figure 227-10 Gram stain of a urethral discharge from a male who has gonorrhea. Note the intracellular Gram-negative diplococci with neutrophils.

sensitive and specific as culture. Ligase chain reaction has the advantage of requiring only a freshly voided urine sample, avoiding invasive sampling of the endocervix and urethra.

Culture is used for making a diagnosis of gonococcal infection of the urethra, the endocervix or an extragenital site. Depending upon sexual practices, samples are also collected from the rectum and/or pharynx. In cases of disseminated gonococcal infection blood cultures are obtained and biopsies from skin lesions and joint fluid aspirates are cultured. Maximal recovery of gonococci from infected sites is obtained when specimens for culture are plated directly onto growth medium. If this is not possible or practical, various commercial nonnutritive transport media are available.

Diagnosis of gonococcal infection is made at two levels: presumptive and confirmed. Antimicrobial treatment may be started based on the results of the presumptive tests, but additional tests have to be performed to yield a confirmatory diagnosis.

A variety of enriched selective media are available to grow gonococci. Commonly used media (Thayer-Martin medium and Martin-Lewis medium) contain lysed or heated blood (chocolate agar) supplemented with growth factors and a variety of antimicrobials to suppress the growth of other bacteria and yeast. Specimens taken from sites that are normally sterile are cultured on antibiotic-free media because occasional strains of gonococci are susceptible to the antibiotics added to the growth media. Growth media are placed in a carbon dioxide incubator or candle jar at 95–98.6°F. After incubation for 24–48 hours colonies of gonococci have a characteristic appearance. Isolates on primary culture media are predominantly of the P^{+} and P^{++} (piliated) colony types, being small, glistening and raised. After subculture P^{-} (nonpiliated) colonies appear. They are larger and flatter than P^{+} colonies and there is no glistening. Smears are prepared from suspicious colonies and examined with Gram stain and the oxidase and catalase tests. Gramnegative diplococci that give a positive oxidase and catalase test may be *N. gonorrhoeae*.

For confirmatory identification of gonococci other types of *Neisseria* spp. as well *Branhamella* and *Kingella* spp. have to be considered. Certain characteristics differentiate between species of the genera.

Confirmatory identification tests include carbohydrate utilization test, monoclonal antibody fluorescence test, coagulation test, chromogenic detection of specific enzyme activities or the DNA probe culture confirmation test. Gonococci oxidize glucose (but not maltose, sucrose and lactose). Serologic tests are not used, because none has sufficient sensitivity and specificity for clinical use.

Meningococcal infection

Cerebrospinal fluid (CSF), blood, skin biopsies and aspirates are relevant specimens for the diagnosis of meningococcal disease. Additionally, synovial fluid, sputum and conjunctival and nasopharyngeal swabs may be cultured if clinically indicated. Because meningococci, like gonococci, are susceptible to desiccation and temperature extremes, specimens should be cultured as soon as possible after collection.

For presumptive diagnosis appropriate specimens are examined by Gram stain. Gram-stained smears are made from uncentrifuged CSF if the CSF is cloudy and from centrifuged CSF if the CSF is clear. The probability of detecting Gram-negative diplococci, typically present inside as well as outside polymorphonuclear cells, depends on the number of bacteria present. The majority of the smears will be positive when the CSF bacterial count is 10^5 bacteria per ml or greater. Smears from CSF containing 10^3 bacteria per ml or less will be positive in only 25%; on average 60–90% of cases of CSF specimen will have a positive Gram stain, since the bacterial number per ml CSF ranges from 1.5×10^2 to 6×10^5 (mean 1.3×10^5). Gram-stained smears from petechial skin lesions due to meningococcemia may detect meningococci in more than 70% of cases.

Direct examination of CSF to detect meningococcal capsular polysaccharides can be done by performing direct antigen tests. Currently direct antigen tests contain a polyclonal antibody for serogroups A, C, Y and W135 and a reagent for serogroup B. Using latex agglutination and coagglutination tests, 0.02–0.05mg antigen per ml CSF from infected patients can be detected. The sensitivity of the tests is only about 50%.

The mean CSF leukocyte count is higher in patients who have meningitis than in patients who have meningococcemia. The proportion of polymorphonuclear cells in CSF from patients who have meningitis ranges from 49% to 98% with a mean of 86%. Other CSF abnormalities include low glucose concentration and an elevated protein concentration. In patients partially treated with antibiotics for meningococcal disease the CSF leukocyte count, glucose and protein concentration and the antigen tests remain abnormal for several days, whereas bacteria are not evident on smear and CSF culture is often negative. Blood cultures are positive in only 50% of the patients with meningococcal disease. In those who have received antibiotics prior to the collection of blood for culture, blood cultures are sterile. A nasopharyngeal swab from young children can provide worthwhile information in cases of suspected meningococcal disease. Meningococci are rarely recovered from healthy young children. Therefore, a well-capsulated serogroup B or C meningococcus grown from a throat swab is strong evidence that a meningococcus is the cause of disease.[58]

The aforementioned tests should be accompanied by a culture of specimens on nonselective growth media in order to isolate *N. meningitidis*. It is noteworthy that CSF cultures may be positive even when other tests are negative. Such a finding is encountered particularly in early meningococcal meningitis and in patients who have meningococcemia without clinical evidence of meningitis. Appropriate nonselective media are 5% sheep blood agar (in contrast to gonococci, meningococci grow well on this medium) and chocolate agar. Selective media used to culture nasopharyngeal specimens are the same as those mentioned for gonococci. Most blood culture media support the growth of meningococci, although liquid sodium polyanethole sulfonate (SPS)-containing media may be toxic for some meningococcal strains.

After inoculation, meningococci will grow quite rapidly on agar media in a 3–7% carbon dioxide-enriched atmosphere with rather high humidity at 95–98.6°F. After 18–24 hours of incubation flat, gray-brown, translucent, smooth, 1–3mm in diameter colonies of *N. meningitidis* are present. Mucoid colonies are often serogroup A

or C strains. If sufficient growth is present a presumptive diagnosis is made with a Gram stain, an oxidase test and a catalase test. The finding of Gram-negative diplococci that are oxidase and catalase positive grown from appropriate specimens may be sufficient to initiate antibiotic treatment for meningococcal disease. For confirmatory identification other species of *Neisseria, Kingella* and *Branhamella* have to be taken into account. Characteristics that differentiate *N. meningitidis* from

these species are the production of acid from glucose and maltose. Gonococci acidify only glucose, and *N. lactamica* produces acid from glucose, maltose and lactose, though a number of commensal *Neisseria* spp. may be misidentified as *N. meningitidis* on the basis of carbohydrate oxidation. In order to specify *N. meningitidis* enzyme substrate tests are also used. Meningococci produce ?-glutamylaminopeptidase. In addition, the polysaccharide from sucrose test differentiates between *N. polysaccharea* (polysaccharide positive) and *N. meningitidis* (polysaccharide negative).

A polymerase chain reaction test has been used for the detection of meningococci in CSF or blood. This test may be useful to confirm the diagnosis in patients who have had antibiotic treatment prior to collection of CSF and whose CSF Gram stain, antigen test and culture are negative.

MANAGEMENT

Gonorrhea

Data from the pre-antibiotic era indicate that in males the urethral infection subsides after 2–3 months. Repeated infection, if untreated, leads to stricture of the urethra. Such sequelae are now rare, because the signs of urethritis bring most men to diagnosis and treatment.

In 10–20% of women gonorrhea results in ascending genital infection and PID, manifested by endometritis, salpingitis, tubo-ovarian abscess and pelvic peritonitis. Infertility due to fallopian tube obstructions is the most serious sequela of PID and occurs in 15–20% of women after a single episode and in 50–80% of women after three or more episodes.[27] In addition, disseminated gonococcal infections may occur. In the pre-antibiotic era *N. gonorrhoeae* was a frequent cause of endocarditis and meningitis.

In 1937 sulfonamides were introduced for treatment of gonorrhea. Sulfonamide-resistant strains emerged rapidly and penicillin was traditionally used to treat gonorrhea. Before 1976 *N. gonorrhoeae* was not generally tested for its antimicrobial susceptibility, although gonococcal isolates with a decreased sensitivity for penicillin (MIC >0.1mg/1) had been recovered. This chromosomally encoded penicillin insensitivity is the result of a number of mutations at the *pen A, pen B env* and *mtr* genes. Mutations in the *pen* genes alter penicillin-binding proteins, reducing their affinity for penicillin. The mtr locus is an efflux pump. Mutations of *mtr* and *env* alter the permeability of the gonococcal membrane. Gonococci with this form of penicillin resistance were designated chromosomally resistant *N. gonorrhoeae* (CMRNG). These strains represent 1–6% of all gonococci in the Western world. In 1976 strains of gonococci that were totally resistant to penicillin due to ß-lactamase production were recovered.[27] These isolates contain a plasmid (Pc^r) that carries the genes for a TEM-1 type ß-lactamase. Although the prevalence of such penicillinase-producing *N. gonorrhoeae* (PPNG) remains stable at about 5–10% in Western Europe and the USA, it is no longer common practice to treat patients who have gonococcal infection with penicillin. One of the reasons for this is that failure to cure a case of gonorrhea has implications for the infected patient as well as for public health, because of the possibility for transmission of the disease and the resistant gonococcus. Therefore, antibiotic regimens for gonorrhea require high patient compliance and an efficacy of 100%. In addition, the antibiotic regimens for gonorrhea should have potential efficacy for a concurrent sexually transmitted disease (STD) infection, such as syphilis and STD due to *Chlamydia trachomatis*. Many studies have shown that *C. trachomatis* can be recovered from 15–30% of men who have gonococcal urethritis and from 35–50% of women who have endocervical gonorrhea.[59] In 1985 *N. gonorrhoeae* strains were isolated that showed high-level tetracycline resistance, designated TRNG, so tetracycline is also no longer recommended for the primary treatment of uncomplicated gonococcal infections. This type of tetracycline resistance is due to a conjugative plasmid into which the *tetM* transposon has been inserted. The *tetM* determinant is readily transferable to other gonococci and various other micro-organisms. The prevalence of TRNG among gonococci in Western Europe and the USA is about 5–8%.

Present recommendations for treatment of uncomplicated gonorrhea are based on regimens that provide high efficacy, have potential, efficacy against concurrent sexually transmitted bacterial infections, particularly *Chlamydia trachomatis*, and that can be given as a single dose in order to obtain maximal patient compliance. Typical regimens are ceftriaxone (one intramuscular dose) or a single oral dose of cefixime, ciprofloxacin or ofloxacin.[60] Each of the therapies also includes a single oral dose of azithromycin or doxycycline for 1 week for the treatment of co-infecting *C. trachomatis*. One oral dose of azithromycin is active against both gonococci and *C. trachomatis*. Ciprofloxacin, ofloxacin and doxycycline are contraindicated during pregnancy. Pregnant women who have gonococcal infection are treated with ceftriaxone followed by 7–10 days of erythromycin. In the past decade gonococcal isolates with decreased sensitivity to ciprofloxin and ofloxacin have been isolated, [61] particularly in South East Asia and Africa. The high-level resistance (MIC >1–16mg/l) is chromosomally mediated, mainly due to mutations in *gyr A* (DNA gyrase) and *par C* (topoisomerase IV). The rapid emergence of such resistant strains most likely requires reassessment of ciprofloxacin and ofloxacin for the treatment of gonorrhea in the near future.

Treatment of PID has been performed with a large number of antibiotic combinations. The important role of both gonococci and *C. trachomatis* in producing PID has been elucidated and, although the role of various anaerobes in PID is not ascertained, many studies have shown that anaerobic cover is essential for treatment of PID patients. The recommended regimens now include intravenous cefotetan or cefoxitin plus doxycycline.[60] An alternative regimen is clindamycin or metronidazole plus gentamicin or tobramycin, followed with oral doxycycline or clindamycin to complete 14 days of treatment. The recommended regimen for treatment of outpatients is a 2-week combination of oral ofloxacin and clindamycin or metronidazole. Amoxicillin-clavulanic acid together with doxycycline will also cover the major pathogens responsible for PID.[59] Azithromycin in combination with amoxicilin-clavulanic acid may also give excellent activity against *N. gonorrhoeae*, anaerobes and *C. trachomatis*.

Complicated gonococcal infection in males (acute epididymitis) is treated with a single dose of ceftriaxone plus doxycycline for 10 days or ofloxacin. [60] Treatment of disseminated gonorrhea is always started with ceftriaxone.[60] This treatment is not changed into doxycycline or ciprofloxacin if arthritis or meningitis is present. Children who have gonococcal infection are also treated with ceftriaxone.

Meningococcal disease

The overall case fatality rate of meningococcal disease is 9–12% and has remained fairly stable over the last decades.[30] Approximately 5% of patients who have meningitis, and 25–35% of patients who have meningococcal sepsis, will die. Of the survivors, 11–19% have serious sequelae, such as loss of hearing, scars after skin necrosis and cranial nerve palsies.

After the introduction of the sulfonamides, meningococcal disease could be treated by chemotherapy. Now sulfonamides have no role in the treatment of meningococcal infection, because resistant strains appeared in 1963. The mainstay of treatment for patients who have meningococcal disease is benzylpenicillin.[52] [58] For most cases of uncomplicated meningococcal meningitis a 7-day course is adequate.[58] Since the etiology is not known at admission, ceftriaxone or cefotaxime is used for the first 24–48 hours to cover the possibility of other bacterial pathogens.[58] Routine susceptibility testing is not indicated,[30] although ß-lactamase producing strains have occasionally been recovered. These strains harbor the same plasmid as many PPNG. In addition, there are *N. meningitidis* strains that are not ß-lactamase positive but have decreased sensitivity to penicillin. This diminished sensitivity is due to reduced affinity of penicillin to penicillin-binding proteins 2 and 3, resulting from an altered *pen A*. Although the frequency of relatively penicillin-resistant meningococci is low, continued surveillance is necessary. Cefotaxime or ceftriaxone is used when relatively penicillin-resistant strains are isolated.

A novel approach to treat children with severe meningococcal sepsis is the use of bactericidal/permeability-increasing protein (BPI). BPI is a cationic protein contained within granules of polymorphonuclear leukocytes. Since BPI binds endotoxin, a recombinant fragment of BPI ($rBPI_{21}$) was used to treat children with severe meningococcemia. The results of the first clinical trial showed a beneficial effect of $rBPI_{21}$ in decreasing complications of meningococcal disease. [62]

INFECTIONS CAUSED BY OTHER *NEISSERIACEAE*

The so-called nonpathogenic *Neisseria* spp. colonize the human nasopharynx and oropharynx. They comprise eight species (*N. lactamica, N. cinerea, N. polysaccharea, N. sicca, N. subflava, N. flavescens, N. musosa* and *N. elongata*), which do not grow on the enriched media containing antibiotics used for the isolation of gonococci and meningococci.

Strains of *N. lactamica, N. subflava, N. cinerea* and *N. polysaccharea* can be isolated from the selective media and must be differentiated from meningococci and gonococci. *Neisseria* spp. may be differentiated by their patterns of acid production. Rapid methods have been developed to detect acid production. Without additional tests including chromogenic enzyme substrate tests and serologic tests, however, the eight species may be misidentified as meningococci or gonococci.

Neisseria lactamica, which produces acid from lactose and can thus be differentiated from meningococci, has been isolated from cases of meningitis or sepsis in both adults and children.[63]

Four true *Neisseria* spp., *N. sicca, N. subflava, N. flavescens* and *N. mucosa*, have been reported most frequently with native and prosthetic endocarditis.[63] Most

patients who had endocarditis had underlying heart abnormalities. In many cases the use of intravenous drugs was a risk factor: oral secretions are used as a solvent for the drug or to clean or lubricate the needle before injection, or result in infection of the valve.

Neisseria sicca is associated with cases of native and prosthetic valve endocarditis. It has also been isolated from meningitis cases. It is a rare cause of pneumonia and osteomyelitis.

Neisseria subflava has been isolated from a small number of patients who have endocarditis, meningitis and sepsis.

Neisseria flavescens, producing yellow-pigmented colonies, has been isolated once in association with an outbreak of meningitis. Occasionally this species is the cause of sepsis resembling chronic meningococcemia.

Neisseria mucosa forms large mucoid colonies adherent on the agar and is isolated as an unusual cause of endocarditis, meningitis, ocular infections, cellulitis, pneumonia and empyema.

Neisseria cinerea colonies may resemble gonococcal colonies on selective media. Occasionally this species is recovered from genital sites and may be associated with syndromes similar to those caused by gonococci, such as conjunctivitis in newborns (ophthalmia neonatorum), proctitis and lymphadenitis. Meningitis occurred in a patient who had facial trauma. Pneumonia in immunodeficient patients has also been reported.

Neisseria polysaccharea grows on the selective media and resembles *N. meningitidis* because acid is produced from glucose and maltose. Infections due to this species have not been reported.

Neisseria elongata, a rod-shaped *Neisseria* spp., has been cultured from blood specimens of patients who have endocarditis or sepsis. It has also been associated with wound infections, and osteomyelitis after oral surgery.[63]

The six false *Neisseria* spp. are common inhabitants of the oropharynx of various animals. *Neisseria canis*, first recovered from a healthy dog, is a normal commensal in the upper respiratory tract of cats and has been cultured from wounds due to cat bites.

Neisseria weaveri, a rod-shaped *Neisseria* spp., was formerly called CDC group M-5 and is part of the oropharyngeal flora of dogs. It has been isolated from human wounds due to dog bites.

N. denitrificans and *N. iguanae* are present in the oropharynx of guinea pigs and iguanas, respectively, and have not been associated with human disease.

Centers for Disease Control and Prevention groups EF-4a and 4b are normal inhabitants of the oropharynx of cats and dogs. Most human infections follow dog and cat bites.[63]

In general the commensal *Neisseria* spp. are susceptible to penicillin, ampicillin and tetracyclines. Only *N. mucosa* is penicillin resistant and sensitive to chloramphenicol. Some strains of *N. lactamica* have an altered penicillin-binding protein 2 similar to the penicillin-binding protein 2 in relatively penicillin-resistant *N. meningitidis*. Rare strains of *N. sicca, N. flavescens* and *N. subflava* are penicillin resistant because of production of ß-lactamase. Such strains are a potential source of ß-lactamase genes that are transferable to meningococci and gonococci.

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Chapter 228 - Enterobacteriaceae

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Maarten R Visser

INTRODUCTION

Enterobacteriaceae are ubiquitous organisms that are found worldwide in soil, water and vegetation, and they are part of the normal flora of the gastrointestinal tract of most animals, including humans. Some members of the family Enterobacteriaceae are more frequently associated with disease in humans (e.g. *Shigella*, *Salmonella*, *Yersinia* spp.) than others (e.g. *Escherichia coli, Klebsiella* spp., *Proteus* spp.). These others are members of the normal commensal flora, which in some situations can cause human disease ranging from mild symptoms to severe infections with fatal septicemia. Infections can be of exogenous (e.g. *Salmonella* and *Shigella* spp.) or endogenous origin (e.g. *E. coli*).

NATURE

Taxonomy and nomenclature

The nomenclature and classification of Enterobacteriaceae has always been confusing, with many changes over time. Micro-organisms previously grouped on the basis of biochemical and antigenic properties in certain families may in fact not be closely related.[1]

In recent years, however, techniques of nucleic acid hybridization and nucleic acid sequencing have provided powerful tools that can enable better definition of the evolutionary relationships of all micro-organisms[2] and the relationships of the organisms in one family. Indeed, it is the application of this technology that has led to the current nomenclature.

In addition, molecular information on the complete genome of *E. coli*[3] will provide further valuable insight into the taxonomy of bacteria and will be used to refine current classification schemes.

In 1986, Ewing proposed dividing the family of Enterobacteriaceae into eight tribes that are associated with human diseases (Escherichiae, Edwardsiellae, Citrobactereae, Salmonellae, Klebsiellae, Proteae, Yersiniae, Erwiniae and a group of miscellaneous genera).[4] This tribal concept has been used in some recent descriptions of the Enterobacteriaceae[5] [6] but others have not used it because of lack of diagnostic and taxonomic significance.[2] [5] [7] [8] The classification that is used in this chapter is based on the current bacterial names of the Enterobacteriaceae (see Table 228.4 , Table 228.5 , Table 228.6 , Table 228.7 , Table 228.8 , Table 228.9 , Table 228.10 , Table 228.11 , Table 228.12 , Table 228.13).

Members of the family Enterobacteriaceae show many common properties. They are all Gram-negative, non-spore-forming bacilli, and they are all relatively small (2–3µm × 0.4–0.6µm). They are either motile by peritrichous flagella or nonmotile (e.g. *Shigella* and *Klebsiella* spp.). Some of them are encapsulated.

Enterobacteriaceae grow rapidly on ordinary laboratory media under aerobic and anaerobic conditions. All species utilize glucose fermentatively, often with the formation of either acid or acid and gas. They are oxidase-negative and, with only a few exceptions (certain biotypes of *Pantoea agglomerans* and some *Serratia* spp.), catalase-positive. They reduce nitrates to nitrites.

Anatomy of Enterobacteriaceae

As shown in Figure 228.1 and Figure 228.2 , the major structural compounds of cell wall of Enterobacteriaceae are:

- an inner cytoplasmic membrane;
- the peptidoglycan layer; and
- an outer membrane consisting of two layers — a phospholipid protein layer and an outer lipopolysaccharide (LPS) layer.

Many organisms, such as *Klebsiella* spp. and a number of *E. coli* strains, possess an additional capsular layer (the capsule).

Some Enterobacteriaceae possess flagella, proteinaceous structures that give mobility to the bacteria. In addition, the presence of pili and fimbriae, also proteinaceous in nature, is an important factor in bacterial attachment or adherence to mucosal surfaces.

Cell wall

The cytoplasmic membrane is an important part of the cell envelope, is the boundary between the cytoplasm and environment, and is primarily responsible for regulating the flow of nutrient and metabolic products into and out of the cell. The cytoplasmic membrane is involved in almost every aspect of bacterial growth and metabolism. The chemical units of peptidoglycan, LPS and phospholipids are synthesized by enzymes within this membrane.[9]

Peptidoglycan forms a thinner layer in Gram-negative bacteria than in Gram-positive bacteria. Peptidoglycan consists of a network in which linear amino-sugar chains containing alternating *N*-acetyl-glucosamine and *N*-acetyl muramic acid residues are linked to tetrapeptides. The peptidoglycan layer maintains the shape of bacteria.[10]

The periplasm, or periplasmatic space, lies between the inner and the outer membranes. A number of processes that are vital for the growth and the viability of the cell occur within this compartment.

Changes in the outer membrane or in the LPS allow the release of periplasmatic enzymes and binding proteins, which play a role in protection and interaction of the bacteria and the host defenses.[11]

The outer membrane of Enterobacteriaceae forms an asymmetric bilayer of phospholipids[12] and LPS.[12] [13] The hydrophobic parts of the LPS molecules face the environment and the hydrophilic part interacts with the hydrophilic part of the phospholipid. The LPS molecules generally have three regions: the O-specific polysaccharide chain, the core region and lipid-A moiety.

The O-specific polysaccharide chain is chemically unique for each type of organism and LPS, and it confers serologic specificity on an organism. The O-specific chain normally consists of 20–40 repeating units of oligosaccharides, each containing between two and eight different monosaccharides interlinked with glycosidic bonds. At least 160 different chemical arrangements of O-antigens have been identified in *E. coli* alone.[13] [14] Based on the presence or absence of the O-specific chain, bacterial endotoxin can be smooth (S) or rough (R), named after the appearance of the bacterial colonies on agar plates.

There is uncertainty about the part played by the O antigen in the virulence of the Enterobacteriaceae; however, most of the isolates

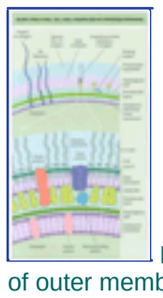

Figure 228-1 Major structural cell wall compounds of Enterobacteriaceae. The figure also illustrates the molecular organization of the outer membrane, and the most likely positions of outer membrane constituents are indicated. Lipopolysaccharide and phospholipid molecules are the major constituents of the asymmetric bilayer. Divalent cations (not indicated) are believed to play important roles in interactions of LPS: Only three types of protein are shown: the pore proteins (LamB protein not shown), OmpA protein and lipoprotein (their interactions with peptidoglycan and lipoprotein are not shown — that such interactions occur cannot be excluded). Several O-antigen chains are much longer than shown here. Enterobacterial core antigen has been omitted for simplicity. The P pili and fimbriae are discussed and illustrated in more detail in Chapter 1 .

from infected sites have smooth LPS. Epidemiologic surveys have demonstrated association between certain O types and clinical infections.[15] It is now generally felt that the O serogroup may act primarily as a marker for a specific cluster of virulence properties needed for a certain infectious process. Certain serogroups possess adhesive factors that are important specifically in urinary tract infection, whereas other serogroups possess colonization factors and toxins that are necessary for the organism to cause gastroenteritis.

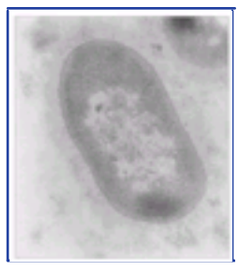

Figure 228-2 Longitudinal section of *Escherichia coli.* The bacterial cell is surrounded by a visible cell wall (outer membrane, cytoplasmic membrane and — in between — periplasm).

The core polysaccharide region of outer membrane, which shows much less variability than the O-specific chain and is often identical in large groups of Gram-negative bacteria, contains deoxy-sugar, 2-keto-deoxyotonic acid and heptose. The core region connects the polysaccharide chain and lipid-A components.

Lipid-A is the most conserved part of LPS and the basic structure is similar in various Gram-negative bacteria. Lipid-A consist of a glucosaminyl-ß-(1–6) glucosamine backbone, which is substituted with six or seven fatty acid residues, all of them saturated. Lipid-A is responsible for the biologic properties of endotoxin. However, lipid-A-associated protein and polysaccharide side chains exert modifying effects and separately may exhibit biologic activities.[16]

Capsule

Two types of capsular polysaccharides are found in Enterobacteriaceae. The M antigen (mucous antigen) or colanic acid is produced by most enteric bacteria, presumably by means of protection against desiccation. Mucous antigen is non-specific and serologically cross-reactive among different micro-organisms. In contrast, K antigen polysaccharides have structures specific to each serotype within a species and presumably help these bacteria to evade phagocytosis.[17] [18]

The presence of K antigen is determined by means of the bacterial agglutination test. It can block the agglutination by specific O antisera; however, these strains become agglutinable when the cells are heated. Two important examples of K antigens are the Vi antigen of *Salmonella typhi* and the K1 antigen of *E. coli,* which is associated with neonatal meningitis and urinary tract infection.[19] [20] Classification of K antigen is now based on electrophoretic mobility, reflecting differences in charge and molecular size.[17]

Flagella

Many members of the Enterobacteriaceae possess proteinaceous structures called flagella, which provide bacteria with mobility.[21]

There are some similarities between several flagellar proteins and proteins involved in either the expression of virulence genes or the export of virulent proteins, or both (e.g. in *Salmonella* spp.).[21] Flagella carry the heat-labile H antigen. The H antigen is dominant to the O antigen; therefore, O-antigen reactivity requires prior denaturation of the H antigen by heating or treatment with acid or alcohol.[17]

Fimbriae

The presence of fimbriae (pili) in bacteria is an important factor for attachment or adherence to the mucosal surfaces of the alimentary, respiratory or genitourinary tract and to red blood cells (causing hemagglutination). These adhesive properties of piliated bacteria play an important role in bacterial colonization of epithelial surfaces and are therefore referred to as colonization factors.[22]

PATHOGENICITY

Normal host defenses limit the majority of bacterial interactions. Therefore, infections caused by members of the Enterobacteriaceae are determined by several virulence factors in the pathogenic strains and the state of the host defenses. Most of the infections are preceded by: colonization of the mucosal site and adherence; evasion of host defenses; multiplication; and host damage.

Virulence factors

Adherence

In order to initiate infection, bacteria must enter the host and attach to host cells. Among Enterobacteriaceae, adhesion is mediated by both fimbrial and nonfimbrial adhesins that are encoded on plasmids and on the bacterial genome (Table 228.1).[23] [24] [25] [26] Most Enterobacteriaceae express the common fimbriae, which can attach to a number of cells; however, the role of this molecule in disease is still unknown.

Fimbriae have been characterized in two different ways, one antigenic and the other functional. Type 1 fimbriae, together with colonization factor antigens CFA/I and CFA/II, are present in enterotoxigenic *E. coli* (ETEC) and are responsible for adherence to the small bowel mucosa[29] ; bundle forming pili (Bfp) are responsible for adherence of enteropathogenic *E. coli* (EPEC) to the small intestine; GvvPG fimbriae mediate adherence of enteroaggregative *E. coli* (EAEC) to the small intestine. Intemin is an adhesin that causes the intimate association found among EPEC and enterohemorrhagic *E. coli* (EHEC). This is associated with the 'attachment and effacement' (E/A) phenomenon, which leads to destruction of the intestinal surface cells[27] [28] of the small as well as the large intestine. The role of nonfimbrial adhesions, other than in uropathogenic *E. coli* (UPEC), are less defined and their role in adherence is not yet clear. The P-fimbriae adhesion factors, known for their ability to bind to P blood group antigens on urinary tract cells, are associated with *E. coli* responsible for urinary tract infections, in particular pyelonephritis and urosepsis.[23] [27] The S-fimbriae, which recognize O-linked sialo-oligosaccharides of glycophorin A, are associated with sepsis and meningitis in neonates.[28]

Phase variation of all fimbrial antigens protects the bacteria from immune-mediated clearance.

The role of adhesins in the pathogenesis of infections caused by Enterobacteriaceae other than *E. coli* is not well characterized. Types 1, 3 and 6 fimbriae have been found in *Klebsiella* spp. but their function as a virulence factor remains largely unknown.[30]

Capsule

The surface of Gram-negative bacteria can play an important role in the protection of the bacterium in a hostile environment. A number of surface antigens appear to be acid polysaccharides; these include the Vi antigen, some of the K antigens of *E. coli* (Table 228.2) and the capsular polysaccharides of *Klebsiella* spp. The capsule provides bacteria with a mechanism for avoiding non-specific host defenses.[31] The K antigen polysaccharides enhance the virulence of invasive bacteria because they counteract the bactericidal action of complement by inhibiting the alternative pathway.

Because the alternative pathway accounts for much of the bactericidal activity of serum and for opsonization in the absence of specific antibodies, it has been suggested that strains with K antigen avoid host defenses more easily, particularly in the early course of infection before the antibody response is mounted. In addition, the capsule may act as a physical barrier to phagocytosis by preventing contact between the bacterium and the phagocytic cell, a result of the anionic and hydrophobic nature of its constituent polysaccharide.[31] [32] Moreover, some of the K antigens are poor immunogens and activators of complement. However, the presence of K antigen alone does not appear to account fully for the virulence of an organism.

Bacterial toxins

Exotoxins

A number of enterotoxins have been identified in Enterobacteriaceae (Table 228.3). Heat-labile enterotoxin (which resembles the cholera toxin) acts by way of activation of cyclic adenosine monophosphate (cAMP), leading to increased electrolyte and fluid secretion and inhibition of resorption. This results in increased fluid in the small intestine. This toxin is produced by enterotoxigenic *E. coli* and occasionally by *Klebsiella* and *Salmonella* spp.[33] [34]

Heat-stable enterotoxin acts via the activation of cGMP in the intestinal epithelium, leading to changes in ion transport, which also result in increased fluid and electrolytes in the intestinal lumen. This toxin is produced by enterotoxigenic *E. coli* and occasionally by *Yersinia enterocolitica* and *Citrobacter freundii*. [33] [35]

Shiga toxin produced by *Shigella flexneri* may elicit a diarrheal prodrome that often precedes bacillary dysentery; however, its role in human disease remains to be defined.[36] Shiga toxin from *Shigella dysenteriae* type 1, a potent cytotoxin, causes capillary dysfunction and focal hemorrhage. This toxin is associated with the hemolytic-uremic syndrome (HUS).[36] Closely related to the Shiga toxin from *S. dysenteriae* type 1 are toxins expressed by enterohemorrhagic *E. coli*. [36]

Hemolysins are present in many species and can cause cell destruction that is not limited to red cells but extends to white cells and other cell types. By killing host cells, hemolysins make iron more available by releasing hemoglobin-bound iron from lysed cells. To release this iron from the host-binding proteins (transferrin and lactoferrin) *E. coli* produces siderophores. a-Hemolysin is produced by pathogenic *E. coli* belonging to a restricted set of O serogroups that are associated with extraintestinal infection.[37]

Endotoxins

As discussed above, Gram-negative bacteria express various macromolecules at their surface. Of these, LPS are of particular microbiologic, immunologic and medical significance.[12] [13] Lipopolysaccharides have been implicated as major factors in the pathogenesis of serious Gram-negative infections (see Chapter 56).[38]

Lipopolysaccharides are released from the bacterial outer membrane during rapid bacterial growth and are released in large quantities on bacterial lysis and death. In animal studies and human volunteer studies,[39] intravenous administration of purified LPS can induce pathophysiologic changes similar to those associated with Gram-negative sepsis and septic shock, including hypotension, metabolic acidosis, coagulation disorders and the progressive failure of multiple organs. These pathophysiologic changes ultimately lead to death.

TABLE 228-1 -- Bacterial adhesins promoting colonization and infections by Enterobacteriaceae.

BACTERIAL ADHESINS PROMOTING COLONIZATION AND INFECTIONS BY ENTEROBACTERIACEAE		
Virulence factor	**Action**	**Organism, site affected and disease**
Bacterial adhesins	Adherence to mucosal surfaces	
P fimbriae		*Escherichia coli* (UPEC): urinary tract infections, pyelonephritis
Type 1 fimbriae		*E. coli*: respiratory tract infections, sepsis
		E. coli (ETEC): gastroenteritis
		Klebsiella pneumoniae: cystitis
Type 3 fimbriae		*Klebsiella* spp.: cystitis, urinary tract infections
Type 6 fimbriae		*Klebsiella* spp.: diseases uncertain
S fimbriae		*E. coli*: sepsis and meningitis in neonates
Bundle-forming pili (Bfp)		*E. coli* (EPEC): gastroenteritis, watery diarrhea
Colonization factor antigens (CFA/I, CFA/II)		*E. coli* (ETEC): gastroenteritis, watery diarrhea in infants and travelers
AAF/I and II fimbriae		*E. coli* (EAEC/some strains): persistent diarrhea in young children, traveler's diarrhea, no inflammation, no fever
Intimin		*E. coli* (EPEC): gastroenteritis, watery diarrhea
		(EHEC): bloody diarrhea
AFA-I; AFA-III; Dr adhesin		*E. coli* (UPEC)
Outer membrane protein		*E. coli* (EIEC)?
		E. coli (EPEC)?
		Proteus mirabilis: urinary tract infection?
		E. coli (EAEC)?
Afimbrial adhesions	Tight binding to host cells	*Klebsiella pneumoniae*: urinary tract infection, infection of cerebrospinal fluid
EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; UPEC, uropathogenic *E. coli*.		

TABLE 228-2 -- Bacterial capsules and infection by Enterobacteriaceae

BACTERIAL CAPSULES AND INFECTION BY ENTEROBACTERIACEAE		
Virulence factor	**Action**	**Organism and disease**
Bacterial capsules	Inhibition of opsonization, prevention of phagocytic uptake	

K antigen		*Escherichia coli* K1: meningitis and sepsis in neonates *Escherichia coli*: invasive extraintestinal infections in adults

The effects of LPS are ascribed to several inflammatory mediators that result from the interaction of LPS with components of both cellular and humoral limbs of the host defense system. Monocytes and macrophages respond to LPS by secreting cytokines, such as tumor necrosis factor and interleukins, that heighten host defense but at the same time activate inflammatory processes that lead to organ failure. Neutrophils play a role in the defense against bacteria but may contribute to the damaging effect of exposure to LPS by enhanced production of oxygen radicals, secretion of proteases and increased cellular adhesiveness.[40] It has been established that the lipid-A part of LPS is responsible for the toxicity.

PREVENTION

Enterobacteriaceae are primarily spread in the hospital from person to person via the hands of hospital personnel.

Escherichia coli diarrheal disease is best controlled by preventing fecal hand-mouth transmission, by stressing the importance of improved hygiene and by introducing appropriate infection-control procedures.

Prevention of *E. coli* infections is difficult because most of the infections (with the exception of intestinal infections) are caused by endogenous bacteria (e.g. community-acquired urinary tract infection) or bacteria acquired during the hospital stay (nosocomial infections). Use of antibacterial chemoprophylaxis in neutropenic patients has reduced the morbidity and mortality attributable to Enterobacteriaceae, especially *E. coli*. [41] For the prevention of nosocomial infections certain risk factors (e.g. the unrestricted use of antibiotics, the unnecessary use of urinary catheters) should be avoided. Unfortunately, many of these factors tend to be found in patients at greatest risk for infections (e.g. immunocompromised patients and patients in intensive care units).

DIAGNOSTIC MICROBIOLOGY

Members of most genera form 'coliform-type' colonies on simple media (i.e. the colonies are circular with a diameter of 1–3mm and have a low convex, smooth-surfaced, colorless to gray and translucent appearance).

For recovery of the Enterobacteriaceae from the specimens containing mixed flora (e.g. fecal samples) several media are used: nonselective media (e.g. blood agar; Fig. 228.3), selective or differential media (e.g. MacConkey agar, *Salmonella-Shigella* agar; Fig 228.4 and Fig 228.5) and enrichment broth to enhance the growth of certain bacteria while inhibiting the growth of other, unwanted bacteria.

On MacConkey (lactose-containing) media (see Fig. 228.4) the colonies may be pink, indicating that the organisms ferment lactose

TABLE 228-3 -- Bacterial toxins and infections by Enterobacteriaceae

BACTERIAL TOXINS AND INFECTIONS BY ENTEROBACTERIACEAE		
Virulence factor	**Action**	**Organism and disease**
Exotoxins		
	Cell destruction	
Hemolysins a, ß	Cell lysis, often leading to cytokine release and inflammatory response	*Escherichia coli*: extraintestinal infection, urinary tract infections, pyelonephritis
Enterotoxins		
Heat-stable toxins (Sta; STb)	Hypersecretion of fluid and electrolytes	*E. coli* (ETEC): gastroenteritis (noninvasive, no inflammation, no fever)
Heat-stable toxins (LT-I; LT-II)		Watery diarrhea in infants and travelers
Verotoxin (VT1 and 2)	Intestinal mucosal destruction	*E. coli* (EHEC): hemorrhagic colitis, diarrhea; hemolytic—ureic syndrome
Enteroaggregative heat-stable toxin (EAST)?		*E. coli* (EAEC), diarrhea
Endotoxin		
Lipoplysaccharide	Complement activation, liberation of cytokines, leukocyte mobilization and degranulation, platelet and coagulation pathway activation	Enterobacteriaceae: fever, sepsis, shock with multiorgan failure

rapidly, or pale, indicating that the organisms either do not ferment lactose or that they cause 'late' fermentation after several days of incubation.[8] This procedure makes possible an immediate presumptive distinction between colonies (e.g. between true intestinal pathogens such as *Salmonella* or *Shigella* spp. and common intestinal commensals). These findings need to be confirmed by other tests.

Differences in biochemical activities provide the main means of differentiating the genus and species.[42] A number of identification methods are readily available from commercial sources (e.g. API-20E system — bioMérieux, 'sHertogenbosch, The Netherlands; Fig. 228.6). These methods give a range of simple biochemical tests, and the biochemical profile for a high percentage of bacterial species can be obtained within 24 hours of incubation at 98.6°F (37°C).[43] The biochemical profile is translated into a numeric code, which can be read from a profile index. Computer-based identification services for unusual organisms are available. The rapid API-20E system allows the identification of Enterobacteriaceae by detection of preformed enzymes in suspension of the test organisms. The rapid system gives a result in 4 hours. Fully automated identification systems, such as Vitek2 (bioMérieux) and Phoenix (BD Diagnostic Systems Europe, France) are also available.

With *E. coli* infections the problem is generally to characterize the pathogenic types from the commensal types. For the isolation of *E. coli* from sterile body sites where its presence indicates infection, such as blood or urine, the standard technique can be used. With the emergence of EHEC as an important pathogen, special media were developed to identify those from feces and food. Most commonly used is sorbitol MacConkey agar, on which some but not all EHEC will appear as nonfermenting colonies, while most other *E. coli* ferment sorbitol.

The diagnosis of diarrheagenic *E. coli* strains depend on identification of virulence characteristics of those strains. This may include in-vitro phenotypic assays that correlate with the presence of specific virulence traits, such as adherence, toxins or the detection of genes encoding those traits. Molecular diagnostic methods are developed to diagnose diarrheagenic *E. coli* strains. These methods allow the differentiation of diarrheagenic strains from nonpathogenic strains of the stool flora and also to make it possible to distinguish one category from another. Nucleic-acid-based probes as well as polymerase chain reaction (PCR) techniques can be applied to isolated bacteria but also directly to fecal samples.[25] [44]

Molecular methods can be used for clinical purposes to detect and identify pathogenic Enterobacteriaceae in order to be able to treat patients and also to perform epidemiologic studies.

CLINICAL MANIFESTATIONS

Escherichia coli is a normal inhabitant of the human gastrointestinal tract that can cause infections under certain conditions. The development and the severity of the infection depends strongly on both the virulence of the bacteria and the state of the host's defense mechanisms. Different strains are associated with different diseases. The versatility of *E. coli* is due to the fact that different strains have acquired different sets of virulence genes. Most of the infections caused by *E. coli*, with the

exception of intestinal infections, are endogenous.

Urinary tract infection (see Chapter 67)

Escherichia coli is a leading cause of urinary tract infection (UTI) in humans.[45] Infecting strains originate from the gastrointestinal tract and several virulence factors have been found to be involved in the pathogenesis of UTI. The most important of these virulence factors are the O-K serotypes, hemolysins and the presence of adhesins for uroepithelial cells.[46] [47]

Urinary tract infections generally start with the colonization of the urethra by *E. coli* strains from the colon or vagina. The bacteria have the ability to adhere to uroepithelial cells. A number of adhesins of uropathogenic *E. coli* strains have been identified.

Type 1 fimbriae may mediate adherence to the bladder cells, and they contribute to virulence in the urinary tract when expressed against the background of a fully virulent uropathogen.[48] These strains do not appear to be specific for uropathogenic strains. The most important type of adhesin, particularly in strains that cause pyelonephritis, are P-fimbriae.[49] They bind specifically to the P blood group antigen, which is present not only on red cells but also on the uroepithelial cells of approximately 99% of people. Uropathogenic strains also have adhesins that are not pili. Some examples are the afimbrial adhesins (AFA I, AFA III) and hemagglutinin that bind to Dr blood group antigen.

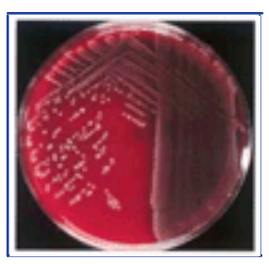

Figure 228-3 Mixed culture of two morphotypes of Enterobacteriaceae (*Escherichia coli* and *Salmonella* spp.) on blood agar plate.

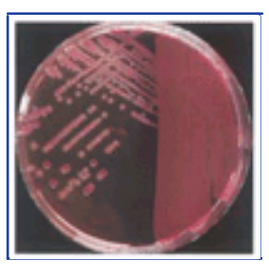

Figure 228-4 Mixed culture of lactosefermenting colonies (red) and non-lactose-fermenting colonies (pale) on MacConkey agar plate.

Figure 228-5 *Salmonella-Shigella* agar plate showing growth of *Salmonella typhimurium* (pale colonies with black pigmentation indicating hydrogen sulfide production).

Figure 228-6 API-20E strip after 24 hours incubation at 95°F (35°C).

Some uropathogenic *E. coli* strains also produce an extracellular toxin, hemolysin.[37] Hemolysin acts generally as a membrane-damaging toxin, leading to the lysis of the cell with subsequent release of cytoplasmic components.

Another important virulence factor is the ability of *E. coli* to acquire iron for growth, and uropathogenic *E. coli* strains have multiple iron-sequestration systems, including a siderophore-based system. Many uropathogenic *E. coli* are encapsulated. [20]

Neonatal meningitis

Some strains of *E. coli* with a capsular antigen (K1) are uniquely associated with neonatal meningitis.[50] The severity of the disease is directly related to the presence, amount and persistence of K1 antigen.[51] The presence of K1 antigen interferes with phagocytosis and, in combination with certain O antigens, shows increased resistance to the bactericidal effect of serum complement.

Escherichia coli K1 antigen is composed of sialic acid residues and shows cross-reactivity with the group B meningococcal polysaccharide capsule. Although colonization of infants with *E. coli* at the time of delivery is common, disease is relatively infrequent.

Intestinal infections (see Chapter 43)

Certain *E. coli* strains are associated with intestinal infections in infants and adults. Most of these strains are the cause of 'traveler's diarrhea'. A characteristic feature of diarrheagenic *E. coli* is their ability to colonize the host's intestinal surface despite host defenses such as gastric acid, peristalsis and competition from other microbial gut flora. This is partly attributed to surface fimbriae for mucosal adherence. Diarrheagenic *E. coli* possess specific fimbrial antigens that increase their ability to colonize the intestine and adhere to the small bowel mucosa — a site that is not normally colonized[25] [52] — once colonization is established. The virulence factors are distinct for each of the groups of diarrheagenic *E. coli.* The versatility of the *E. coli* genome is conferred mainly by two genetic configurations: virulence-related plasmids and chromosomal pathogenicity islands.

There are six main categories of diarrheagenic *E. coli.*[33] Although these categories are quite distinct, they have certain underlying similarities with respect to pathogenesis:

- their virulence properties are encoded on the plasmids;
- they have a characteristic interaction with the intestinal mucosa; and
- they produce enterotoxins or cytotoxins.

Within each category the strains tend to fall within certain O:H serotypes.

Before the availability of DNA techniques, knowledge of the epidemiology of enterodiarrheagenic *E. coli* was based on immunoassays and bioassays for identification of virulence factors such as toxins. These assays were cumbersome and time-consuming. Recent advances in DNA techniques have provided a new approach to

screening a range of virulent genes of *E. coli.*[40] Six major categories of *E. coli* that cause diarrhea have been identified:[33]

- enterotoxigenic *E. coli* (ETEC);
- enteroinvasive *E. coli* (EIEC);
- enterohemorrhagic *E. coli* (EHEC);
- enteropathogenic *E. coli* (EPEC);
- enteroaggregative *E. coli* (EAEC, also known as EaggEC); and
- diffusely adherent *E. coli* (DAEC).

Enterotoxigenic Escherichia coli

Enterotoxigenic *E. coli* causes 'travelers' diarrhea' (in developing countries) by colonizing the small intestine and producing one or more enterotoxin, giving rise to a net secretory state.[29] [53] The heat-labile enterotoxin (LT) is structurally similar to *Vibrio cholera* toxin and, by activation of adenylate cyclase, leads to hypersecretion of fluids and electrolytes into the small intestines. The heat-stable enterotoxin (ST) activates guanylate cyclase and stimulates fluid secretion.

Besides the enterotoxins, three other factors have been used to identify and characterize ETEC: O serogroup, H serogroup and CFAs.[29] The mechanism by which ETEC adheres to small bowel enterocytes is mediated by surface fimbriae (pili). The ETEC fimbriae confer the species specificity of the pathogen; human ETEC possess an array of colonization fimbriae, the CFAs (CFA/I rod-shaped fimbriae; CFA/III; a Bfp CFA/II and CFA/IV). The CFA genes are usually encoded on plasmids, which typically also encode the enterotoxins ST and/or LT.[54]

Infection by ETEC, after an incubation period of 1–2 days, is characterized by diarrhea, nausea and vomiting but usually no fever; the symptoms are usually mild and self-limiting, with a duration of 3–4 days.

Enteroinvasive Escherichia coli

Enteroinvasive *E. coli* causes a dysenteric form of diarrheal illness.[55] Strains of EIEC closely resemble *Shigella* spp. Like *Shigella* spp., they are capable of invading and proliferating within epithelial cells of the large intestine, eventually causing death of the cell. The invasive capacity is dependent on the presence of plasmid coding for the production of several outer membrane proteins involved in invasiveness.[56] Among the genes responsible for invasion are the *mxi* and *spa* loci, which encode a so-called type III secretion apparatus.[56] [57] In addition, both EIEC and *Shigella* spp. elaborate one or more secretory enterotoxins, which may play a role in diarrheal pathogenesis. They do not form enterotoxins, like ETEC. Clinically, the illness is marked by watery diarrhea, often with mucus and leukocytes, a scant bloody stool, severe abdominal cramps and fever. The symptoms are usually self-limiting.

Enterohemorrhagic Escherichia coli

Enterohemorrhagic *E. coli*, also called verocytotoxin-producing *E. coli*, is associated with two syndromes: hemorrhagic colitis, with abdominal pain and bloody diarrhea; and hemolytic-uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia and acute renal failure.[58] [59] *Escherichia coli* O157:H7 has been associated most frequently with hemorrhagic colitis and HUS; however, several other serotypes have also been associated with disease.

Both plasmid-encoded adherence factors for distinct fimbriae and cytotoxin production are important factors in the pathogenesis of disease.[60] Enterohemorrhagic *E. coli* as well as EPEC have the ability to induce a characteristic 'attacking and effacing' (A/E) histopathology on gut enterocytes characterized by localized destruction of brush border microvilli, intimate bacterial adhesion and gross cytoskeletal reorganization.[26] [61] A/E lesion formation is essential for full EPEC and EHEC pathogenicity.

The genes encoding for the A/E histopathology are contained on a 35.6kb pathogenicity island called the locus of enterocute effacement (LEE). The EHEC LEE contains genes encoding intimin type III secretion pathway and some other protein O. Enterohemorrhagic *E. coli* and EPEC strains use the LEE type III secretion system — secretion of virulence factors, some of which are injected (translocated) directly from the pathogen's cytoplasm into the host cell cytosol — to secrete several LEE-encoded proteins. All contacts stimulate the expression of LEE-encoded proteins and the assembling of a protein translocation apparatus (translocon) that provides a continuous channel from the bacterial cytoplasm to the host cell cytosol. The translocon is used to translocate receptor into the host cell, where it becomes inserted into the host membrane; excreted effector proteins are now probably able to make cytoskeletal changes and as a result form A/E lesions.

There are 'typical' EHEC strains, such as O157:H7, that produce stripe-like (Stx) toxins and A/E lesions and possess the 60MDa plasmid; and 'atypical' EHEC strains that do not produce A/E lesions and/or do not possess the 60MDa EHEC plasmid.[25] The development of HUS involves direct cytotoxic action of Stx on renal endothelial cells, but cytokines are probably also indirectly involved in this process.[62]

The symptoms are usually self-limited. They vary in severity from mild symptoms of nonbloody diarrhea to severe hemorrhagic colitis, sometimes ending in death (especially in children and the elderly).

The use of antibiotics for the treatment of severe infection is controversial and a recent study[63] showed that patients treated with antibiotics are at greater risk of developing HUS. The harmful potential is explained by lysis of bacteria and an increase in release of toxins. Recent outbreaks of EHEC disease have raised public concern about the safety of the food supply.[64]

Enteropathogenic Escherichia coli

Enteropathogenic *E. coli* plays a role in infant diarrhea and can also be associated with chronic diarrhea in young children.[65] Plasmid-encoded adhesiveness is the most important factor in the pathogenesis of the disease.[66] Strains of EPEC are responsible for different clinical signs of the infection, depending on the diffuse and localized adherence to cells. The clinical signs range from mild nonbloody diarrhea to more severe diarrhea.

The hallmark of infections due to EPEC is the A/E histopathology similar to that of EHEC (see above), characterized by effacement of microvilli and intimate adherence between the bacteria and the epithelium cell membrane.[26] [61]

In the first step of infection, localized adherence to epithelial cells mediated by Bfp occurs. Bfp is encoded on plasmids called EAF plasmids. In the second step adherence induces a variety of signal induction pathways. Genes responsible for this signal transduction are encoded on a 35kb pathogenicity island (LEE). Intimate adherence of EPEC to epithelial cells is mediated by a 94–97kDa outer membrane protein called intimin. At least four proteins are secreted extracellularly by EPEC and three of them are essential for A/E histopathology.

As with other diarrheal pathogens, the treatment of EPEC diarrhea is to prevent dehydration by correcting fluid and electrolyte imbalances. Antibiotic treatment had been used in many patients, with good results, but multiple antibiotic resistance is common for EPEC.[67]

Enteroaggregative Escherichia coli

Enteroaggregative *E. coli* are an increasingly important cause of diarrhea.[68] They cause nonbloody, watery diarrhea that is often persistent and can be inflammatory. Enteroaggregative *E. coli* have been implicated in sporadic diarrhea in children and adults in both

developing and developed countries, and recently have been reported to cause 'traveler's diarrhea'.

Enteroaggregative *E. coli* are characterized by their ability to adhere to epithelial cells in a characteristic 'stacked-brick' pattern but are otherwise highly heterogeneous. The adherence consist of two phenotypes — diffuse and aggregative. The exact mechanism of pathogenesis is not completely known but adhesins, toxins and several other factors contribute to disease.

Adhesins (plasmid-encoded) are aggregative adherence fimbria I (AAF/I), which is shown to mediate aggregative adherence to epithelial cells and Hep-2 hemagglutination, and to be involved in formation of biofilm. The other type of fimbria, AAF/II, is more associated with diarrhea in children. Other virulence factors include invasion and plasmid-encoded toxin production, but these factors were shown to be present only in some strains. The general problem with *E. coli* infections is

to distinguish the pathogenic from the commensal types.

Diffusely adherent Escherichia coli

Diffusely adherent *E. coli* are now recognized by most authors as an independent category of potentially diarrheagenic *E. coli*. In a number of studies DAEC was associated with endemic pediatric diarrhea lasting longer than 2 weeks. The diffuse adherence to Hep-2 cells is mediated by a unique fimbrial structure known as the F1845 fimbria, which can be found on either bacterial chromosome or a plasmid.

BACTEREMIA AND NOSOCOMIAL INFECTIONS

As a group, Enterobacteriaceae are the most frequent bacterial isolates recovered from both inpatient and outpatient clinical specimens. In 1990–2 Enterobacteriaceae accounted for 30% of pathogens isolated from all infection sites in the Centers for Disease Control and Prevention (CDC) and National Nosocomial Infection Surveillance system.[69]

In the past few years, with the increased use of invasive devices and broad-spectrum antibiotics, Enterobacteriaceae, specifically *E. coli*, have become somewhat less prevalent than Gram-positive cocci as a cause of nosocomial infection. Nevertheless, *E. coli* is still an important cause of hospital-acquired infections despite adequate antibiotic therapy and supportive care.

The increasing antibiotic resistance among *E. coli* resulting in nosocomial infections is a major cause of concern in hospitals. There are several factors that predispose hospitalized patients to such infections: the use of antibiotics that eradicate normal flora, impaired skin and mucous membranes, intubation, intravenous and bladder catheters and surgical procedures. These infections, including UTIs, wound infections, pneumonia, intra-abdominal abscesses and peritonitis, are often followed by bacteremia. However, bacteremia without primary focus is sometimes detected in immunocompromised patients.

Bacteremia

Escherichia coli is the most common cause of nosocomial bacteremia. In 1979 it had an incidence of 2.7 per 10,000 hospital charges.[70] The most common portal of entry of infection for both community-acquired and nosocomial *E. coli* bacteremia is the urinary tract.[71] The overall mortality rate from *E. coli* bacteremia is approximately 20%. Bacteremia that does not originate in the urinary tract tends to have a worse outcome.

Bacteremia is frequently due to pulmonary infections in intubated patients receiving ventilation therapy or to UTIs caused by indwelling urinary catheters. Patients with granulocytopenia due to leukemia, cancer or chemotherapy are at high risk of bacteremia.

Septic shock

Septic shock occurs in approximately 40% of patients with Gram-negative bacteremia. The high frequency of septic shock in bacteriemia caused by Enterobacteriaceae is attributed to the toxic effect on the circulatory system of endotoxin (LPS).[38] [40] Endotoxin within the circulatory system has multiple and complex effects on neutrophils, platelets, complement, clotting factors and the inflammatory mediators in the blood. These complex effects may result in multiple organ failure and eventually in death. Mortality rates from Gram-negative shock range from 40% to 70%.

Urinary tract infections

Urinary tract infections are the most common hospital-acquired infections and *E. coli* is a leading pathogen, implicated in about 25% of all nosocomial UTIs.[69] Recognized major risk factors for hospital-acquired UTIs include urinary catheterization and obstruction to urine flow.

Other sites of hospital-acquired infection

The lungs are a common site of hospital-acquired infection, although it is often difficult to discriminate between *E. coli* colonizing the upper airways and *E. coli* causing pulmonary infection.

Approximately a quarter of surgical infections are caused by Enterobacteriaceae, among which *E. coli* accounts for about 8%.

Nosocomial central nervous system infections occur mainly in neurosurgical patients, neonates and patients undergoing procedures that penetrate the central nervous system. Of postsurgical meningitis, 69% is caused by Gram-negative bacilli, with a majority (70%) of these due to *E. coli.*

MANAGEMENT

Antibiotic therapy for infections with *E. coli* and other Enterobacteriaceae must be guided by an in-vitro susceptibility test and by clinical experience. Although most strains of *E. coli* are still susceptible to the commonly used antibiotics, resistance, especially in hospital-acquired infections, is increasing.

The treatment of infective diarrhea is oral fluid and electrolyte replacement. Antibiotics are generally not recommended and only in chronic or life-threatening infections should antibiotic treatment be considered.[72] The duration and breadth of antimicrobial treatment in other infections caused by *E. coli* or other Enterobacteriaceae depend on the site and severity of the infection. For example, uncomplicated cystitis in otherwise healthy women can be often managed with a single dose of antibiotic, whereas sepsis requires about 10 days' therapy, and prostatitis or deep-seated renal infection requires as much as 6 weeks' treatment.

Conventional approaches to control nosocomial infections caused by Enterobacteriaceae are not always successful. Therefore, antibiotic prophylaxis has been studied to prevent colonization and nosocomial pneumonia in the critically ill, and selective decontamination of the gastrointestinal tract has been investigated as a method of preventing infections by Enterobacteriaceae in ventilated patients in intensive care units.[73] A recent meta-analysis of 22 randomized trials demonstrated that selective decontamination reduced the incidence of respiratory tract infection by 63%.[74] However, in most studies, even when pneumonia rates were reduced, selective decontamination had no effect on the mortality rate or hospital stay. The development of antibiotic resistance is the most feared complication of selective decontamination.[75]

MEDICALLY IMPORTANT GENERA OF ENTEROBACTERIACEAE

Escherichia

Escherichia coli makes up the largest proportion of aerobic Gram-negative bacteria of the intestinal tract. It is a non-spore-forming rod

and is often motile by means of peritrichous flagella. It can grow aerobically as well as anaerobically.

Most strains ferment lactose rapidly, and the colonies on MacConkey medium are smooth, glossy and translucent in appearance and rose pink in color. On blood agar the colonies of some strains are surrounded by zones of hemolysis. Most strains of *E. coli* attack carbohydrates fermentatively, with the production of acid and gas; a few strains will produce acid but not gas. Although most strains of *E. coli* ferment lactose rapidly, there are some strains with a slow fermentation pattern or that are non-lactose-fermenting. Furthermore, *E. coli* is characterized by the ability to reduce nitrates to nitrites. Most of the strains produce indole, give a positive methyl-red reaction and the Voges-Proskauer test is negative. *Escherichia coli* strains do not hydrolyze urea or produce hydrogen sulfide, and phenylalanine deaminase activity is absent. *Escherichia coli* cannot use citrate as the sole source of carbon and will not grow in the presence of potassium cyanide.[2] [42]

Although conventional approaches to identification (e.g. sugar fermentation) are still valid and useful, there are several other methods available now. For example, there are commercially available tests that use rapid chromogenic enzyme substrates, such as the API system or automated methods.[43] Together with growth on selective or differential media, colonial characteristics and cell morphology on a Gram stain, identification can be completed.

Escherichia coli can be subdivided into serogroups, serotypes and biotypes.[1] Two *E. coli* surface compounds form the basis for the serologic classification system: heat-stable somatic O antigen of LPS, and heat-labile flagella H antigen (H stands for *hauch*, the German word for 'breath', because of the mist-like effect around the bacterium caused by the presence of flagella; O denotes *ohne*, 'without', i.e. lacking flagella). The O antigen identifies the serogroup of a strain and the H antigen identifies its serotype. The biotype is determined by the biochemical profile.

To date, 173 different serogroups have been identified, and there are at least 56 identified serotypes.[17] There is some correlation between serogroup and virulence. For example, O86 is commonly found on members of the residual colonic microflora, and members of this serogroup rarely cause disease; on the other hand, group O55 is rarely found in the residual microflora and is almost always associated with disease. If the bacteria is enveloped by a capsule, a capsular (K) antigen is also used for classification. Only a few of the 80 distinct K antigens isolated from natural populations are associated with invasive *E. coli* disease.[18] Strains classified as K1, for example, are a common cause of systemic disease in infants.[17]

Escherichia fergusonii, *Escherichia hermannii* and *Escherichia vulneris* (Table 228.4) are occasionally isolated from patients, but their clinical significance is not well defined. *Escherichia blattae* has been recovered from cockroaches but its role in human infections has not been established. [76]

Shigella

The genus *Shigella* is closely related to the genus *Escherichia*, as shown by genetic analysis. The genetic relationship is so close that it has been advocated that both genera form a single genetic species. However, the distinction between the two genera remains useful from a clinical point of view. The *Shigella* organisms differ from a typical *Escherichia coli* strain in that they are nonmotile, do not ferment lactose and do not produce gas from glucose. One member of the genus *Escherichia*, *E. coli* inactive (formally called 'Alcalescens-Dispar'), has many characteristics similar to those of *Shigella* organisms and they be considered to be a species in between Escherichiae and Shigellae.

Four species of medical importance are traditionally described within the genus *Shigella*: *S. dysenteriae*, *S. flexneri*, *Shigella boydii* and *Shigella sonnei* (see Table 228.4). Members of the species are hard to differentiate from each other by biochemical testing. Using serologic methods, *S. dysenteriae* makes up group A, *S. flexneri* group B and *S. boydii* group C. Each group can be further subdivided into different serotypes, and this is useful for epidemiologic purposes. Serologic cross-reactions with *E. coli* strains and other members of the family of Enterobacteriaceae do occur. *Shigella sonnei* (group D) can be separated from the other *Shigella* spp. by a positive ornithine reaction; this species may show lactose fermentation after incubation for more than 48 hours.

Dysentery caused by *Shigella* spp. occurs worldwide. The annual number of *Shigella* episodes is estimated to be 163.2 million in developing countries and 1.5 million in industrialized countries.[77] In industrialized countries, person-to-person transmission, perhaps via hands, is the most common form of transmission, and *S. sonnei* is now the most frequently isolated species of the Shigellae in these countries.[77] In developing countries, the predominant species is *S. flexneri* and the mode of transmission is often by contaminated water, food or perhaps flies. The low inoculum (less than 200 viable bacteria) needed for disease is often assumed to be one of the factors responsible for the high secondary attack rate during outbreaks. Long-term excretion is uncommon but does occur. Long-term excreters are probably important as a reservoir; in these people, carriage of the *Shigella* strains is often in the colon.

Salmonella

The classification of *Salmonella* organisms has been controversial over the years.[42] Biochemical and genetic studies show so much similarity between *Salmonella* and Arizona group that all strains may belong to a single species. For epidemiologic purposes, serotyping of O antigens (type A, B, C and so on) and of H (flagellate) antigens has resulted in more than 2000 distinct serotypes, which are often named as if they were species (e.g. '*Salmonella typhimurium*' rather than '*Salmonella* serotype *typhimurium*' or even '*Salmonella choleraesuis* subsp. *choleraesuis* serotype *typhimurium*').

The genus *Salmonella* can be subdivided into different groups.[2] More than 99% of clinical isolates belong to subgroup 1 (*S. choleraesuis* subsp. *choleraesuis*; Table 228.5). Other subgroups include *S. choleraesuis* subsp. *salamae* (subgroup 2), *S. choleraesuis* subsp. *arizonae* (subgroup 3a), *S. choleraesuis* subsp. *diarizonae* (subgroup 3a), *S. choleraesuis* subsp. *houtenae* (subgroup 4) and *S. bongori* (subgroup 5). Subgroup 6 has been proposed to contain the subspecies *indica*. The name *Salmonella enterica* has been used by bacteriologists and has caused confusion in the nomenclature of *Salmonella*. [78]

A presumptive identification of '*S. typhi*' can often be made biochemically because these strains are mostly citrate-negative, produce hydrogen sulfide slowly, are ornithine-decarboxylase-negative, do not produce gas from glucose and do not ferment arabinose and rhamnose, in contrast to most other strains of *Salmonella*.

Salmonella gastroenteritis and enteric fever occur worldwide. The reservoir for the serotypes *typhi* and *paratyphi* A, B and C are human, but the other Salmonellae are widely distributed among animals. Some strains are associated with particular animals (e.g. subspecies *arizonae* with reptiles), but exchange of strains between animals is frequent. Transmission is mostly by contaminated water and food, and perhaps by insects such as flies, although direct person-to-person transmission has been described.

Citrobacter

The name *Citrobacter* is derived from the ability of these strains to use citrate as a source of carbon. *Citrobacter* organisms are widely found in environmental samples. Eleven species in this genus have now been listed (Table 228.6). [7] *Citrobacter freundii* is frequently

TABLE 228-4 -- The genera *Escherichia* and *Shigella*. [7]

THE GENERA *ESCHERICHIA* AND *SHIGELLA*	
Name	**Synonym**
Escherichia blattae	
Escherichia coli	
Escherichia fergussonii	CDC enteric group 10
Escherichia hermannii	CDC enteric group 11
Escherichia vulneris	CDC enteric group 1
Shigella boydii	*Shigella* biogroup C
Shigella dysenteriae	*Shigella* biogroup A
Shigella flexneri	*Shigella* biogroup B
Shigella sonnei	*Shigella* biogroup D

TABLE 228-5 -- The genus *Salmonella*.[7]

THE GENUS *SALMONELLA*	
Name	**Synonym**
Salmonella bongori	*Salmonella* subgroup 5
Salmonella choleraesuis subsp. *arizonae*	*Salmonella* subgroup 3a
Salmonella choleraesius subsp. *choleraesius*	*Salmonella* subgroup 1 (includes most serotypes: *S. typhi, S. cholerasuis, S. paratyphi, S. gallinarum, S. pullorum)*
Salmonella choleraesius subsp. *diarizonae*	*Salmonella* subgroup 3b

Salmonella choleraesius subsp. *houtenae*	*Salmonella* subgroup 4
Salmonella choleraesius subsp. *salamae*	*Salmonella* subgroup 2

isolated from nosocomial infections, including hospital-acquired pneumonia, bacteremia, UTIs and wound infections. Nosocomial transmission may be related to antimicrobial resistance; many strains of *C. freundii* produce an inducible broad-spectrum ß-lactamase.[79] In addition, *C. freundii* has been associated with gastroenteritis.[2] [5] New species, formally within the *C. freundii* complex, include *Citrobacter braakii*, *Citrobacter rodentium*,[80] *Citrobacter sedlakii*, *Citrobacter werkmanii*, *Citrobacter youngae*, *Citrobacter gillenii* (genomospecies 10) and *Citrobacter murliniae* (genomospecies 11). [81] Identification of newly described *Citrobacter* spp. by commercial systems has been studied.[82]

Citrobacter koseri (formerly designated *C. diversus*) may be a causative agent of sepsis, meningitis and brain abscesses, especially in young children.[83] Transmission from mother to newborn has been described.[84] Many strains of *C. koseri* produce an inducible class A ß-lactamase, as does *Proteus vulgaris*,[79] although other strains may produce a class C ß-lactamase like *C. freundii*. *Citrobacter amalonaticus* is rarely involved in human disease. It has recently been proposed that melibiose-positive variants of *C. amalonaticus* (biotype 1) should be named *Citrobacter farmeri*.[2]

Klebsiella

The current classification of the genus *Klebsiella* recognizes five species with several subspecies (Table 228.7). All the species are nonmotile. *Klebsiella* strains can be found in the feces of healthy adults.[85] *Klebsiella pneumoniae* is well known as the cause of Friedländer's pneumonia and nosocomial infections. Strains of this species often grow as mucoid lactose-positive colonies on MacConkey agar. The subspecies *rhinoscleromatis* is Voges-Proskauer-negative and does not ferment lactose or several other sugars. It is the causative agent of rhinoscleroma, a granulomatous

TABLE 228-6 -- The genus *Citrobacter*.[7]

THE GENUS *CITROBACTER*	
Name	**Synonym**
Citrobacter amalonaticus	*Levinea amalonatica*
Citrobacter braakii	*Citrobacter freundii*
Citrobacter farmeri	*Citrobacter amalonaticus* biogroup 1
Citrobacter freundii	*Citrobacter freundii*
Citrobacter genomospecies 10	*Citrobacter freundii*
Citrobacter genomospecies 11	*Citrobacter freundii*
Citrobacter koseri	*Citrobacter diversus*
Citrobacter rodentium	*Citrobacter* genomospecies 9,
Citrobacter sedlakii	*Citrobacter freundii*
Citrobacter werkmanii	*Citrobacter freundii*
Citrobacter youngae	

diseases of the nose and associated tissues.[86] *Klebsiella ozaenae* is another biochemically less active subspecies, which is associated with ozena, an atrophic condition of the nasal mucosa. *Klebsiella planticola*, *Klebsiella terrigena* and *Klebsiella ornithinolytica* are uncommon isolates in the clinical setting,[87] and the genus name *Raoultella* has been proposed for these species.[88] Indole-positive strains of *Klebsiella* are classified as *Klebsiella oxytoca*; these strains may cause infections similar to those caused by *K. pneumoniae*. Most strains of *K. pneumoniae* and *K. oxytoca* produce a chromosomal class A ß-lactamase that renders them resistant to ampicillin but susceptible to ß-lactamase inhibition by clavulanic acid and analogues. Since 1982, extended-spectrum ß-lactamases have been described that are mutations of the TEM or SHV type of ß-lactamase; these have increased hydrolytic activity against third-generation cephalosporins or are less inhibited by clavulanate.[79] Genetic information for these enzymes is carried on plasmids, often in combination with other resistance factors such as aminoglycoside-modifying enzymes, and this type of resistance may spread relatively rapidly in settings of increased antibiotic use.

Enterobacter

The species in the genus *Enterobacter* are listed in Table 228.8 . *Enterobacter cloacae* is a common clinical isolate. It is a frequent cause of opportunistic infections and is often acquired in hospital. Antibiotic resistance is frequent, especially against third-generation cephalosporins, by means of an inducible chromosomal ß-lactamase.[89]

Another frequently isolated species in this setting is *Enterobacter aerogenes*. Because this motile strain resembles the genus *Klebsiella* more than other *Enterobacter* organisms, it has been proposed that this species be renamed *Klebsiella mobilis*. [42] A yellow pigment is produced by *Enterobacter sakazakii*, a species that has been described as causing neonatal infections. [90] Another yellow-pigmented species, *Enterobacter agglomerans*, has recently been renamed *Pantoea agglomerans* (see below). *Enterobacter hormaechei*, *Enterobacter cancerogenus* ('*Enterobacter taylorae*'), *Enterobacter intermedium*, *Enterobacter asburiae* and *Enterobacter gergoviae* are among the less common *Enterobacter* spp. that have been isolated from blood cultures and various other specimens from patients with infections.[91] [92] [93] *Enterobacter amnigenus* has also been isolated from human tissue but it is unclear whether it causes disease. [2]

Enterobacter kobei is a recently proposed species[94] that closely resembles *E. cloacae*. Many *Enterobacter* spp. are resistant to second-generation and third-generation cephalosporins by virtue of

TABLE 228-7 -- The genus *Klebsiella*.[7]

THE GENUS *KLEBSIELLA*	
Name	**Synonym**
Klebsiella ornithinolytica	*Klebsiella oxytoca* ornithine positive
Klebsiella oxytoca	
Klebsiella planticola	*Klebsiella travisanii*
Klebsiella pneumoniae subsp. *ozaenae*	*Klebsiella ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*	*Klebsiella pneumoniae*
Klebsiella pneumoniae subsp. *rhinoscleromatis*	*Klebsiella rhinoscleromatis*
Klebsiella terrigena	

an inducible broad-spectrum cephalosporinase encoded by genetic information on the chromosome. These strains may seem susceptible at first isolation, but subsequent isolates may show resistance to these agents.[79] [89]

Serratia

The genus *Serratia* consists of nine species (Table 228.9). [7] *Serratia marcescens* is the one most commonly associated with nosocomial infections, in part because it is often resistant to commonly used ß-lactam antibiotics and in part because it is not fastidious in its growth conditions. This species and other *Serratia* spp. have been

implicated in ocular infections associated with soft contact lenses. [95] Red pigment is formed under suitable conditions by some strains of *S. marcescens*, as well as by *Serratia plymuthica* and *Serratia rubidaea*, two other opportunistic pathogens.[96] [97] Two other species, *Serratia liquefaciens* and *Serratia odorifera*, are less frequently associated with clinical infections.[98]

Serratia grimesii, *Serratia proteamaculans* subsp. *proteamaculans* and *Serratia proteamaculans* subsp. *quinovora* are closely related to *S. liquefaciens*.[5] *Serratia ficaria* and *Serratia fonticola* are rarely involved in human disease.[99]

Proteus

The genus *Proteus* once contained many species but currently only three are listed: *Proteus mirabilis*, *Proteus penneri* and *Proteus vulgaris* (Table 228.10). Characteristics of *Proteus* spp. include their tendency to display swarming motility during growth on nonselective media and their strong urease reaction.[100] This urease activity has been associated with the formation of kidney stones, as UTIs are commonly caused by *Proteus* spp. *Proteus mirabilis* is indole-negative and often susceptible to most common antibiotics, with the exception of nitrofurantoin, whereas indole-positive *P. vulgaris* is more resistant to ß-lactam antibiotics. *Proteus penneri* is closely related to *P. vulgaris* but is indole-negative and can be distinguished from *P. mirabilis* by its negative ornithine reaction. The name *Proteus hauseri* has been proposed for *P. vulgaris* biogroup 3.[101]

Morganella

Morganella morganii is currently the only species in the genus (see Table 228.10). Two subspecies (*morganii* and *sibonii*) can be distinguished by the ability to ferment trehalose.[102] Urinary tract infections and other opportunistic infections are associated with this species. *Morganella morganii* was originally part of the genus *Proteus*.

Providencia

Members of the genus *Providencia* are a diverse group of organisms (see Table 228.10). The two species *Providencia rettgeri* and *Providencia stuartii* may be urease positive (*P. rettgeri* more often

TABLE 228-8 -- The genus *Enterobacter*.[7]

THE GENUS *ENTEROBACTER*	
Name	**Synonym**
Enterobacter aerogenes	*Aerobacter aerogenes*
Enterobacter amnigenus	
Enterobacter asburiae	CDC enteric group 17
Enterobacter cancerogenus	*Enterobacter taylorae*
Enterobacter cloacae	
Enterobacter gergoviae	
Enterobacter hormaechei	CDC enteric group 45
Enterobacter kobei	
Enterobacter sakazakii	

than *P. stuartii*), and both are a frequent cause of UTIs and other opportunistic infections.[103] *Providencia alcalifaciens* has been implicated in gastrointestinal infections in children.[104] *Providencia rustigianii* has rarely been isolated from humans.[2]

Yersinia

The genus *Yersinia* includes three species that can cause a variety of infections in the host (Table 228.11).[105] *Yersinia pestis* is the causative agent of plague.[106] [107] The plague bacillus grows slowly on conventional media. Like infections with other *Yersinia* spp., plague is a zoonosis. The natural reservoir are small animals, and transmission occurs through bites of fleas from these animals, by handling contaminated parts of the animals and by human-to-human transmission from patients with pneumonic plague. Plague has been implicated as a potential biologic weapon, and recommedations for measures to be taken by medical and public health professionals have been developed. [108] *Yersinia pseudotuberculosis* closely resembles *Y. pestis*, and it has even been suggested that the two are a single species. *Yersinia pseudotuberculosis* is associated with mesenterial lymphadenitis, which simulates appendicitis, mainly in children and adolescents. Sepsis with *Y. pseudotuberculosis* has mainly been described in patients with liver disease and other chronic illnesses. *Yersinia enterocolitica* can cause mesenterial lymphadenitis, gastroenteritis, infections in many other tissues and sepsis. Notorious sources of infections include blood transfusion products,[109] in which *Y. enterocolitica* can proliferate at storage temperatures of 39°F (4°C). Serotypes 3 and 9 are most common in Europe, whereas serotype 8 is more frequently encountered in the USA.[76] Complications of *Y. enterocolitica* infection include polyarthritis, erythema nodosum and Reiter's syndrome.

Yersinia frederiksenii, *Yersinia intermedia* and *Yersinia kristensenii* have recently been separated from *Y. enterocolitica*. As with *Yersinia bercovieri*, *Yersinia mollaretii* and *Yersinia rohdei*, their association with human disease is less clear.[110] For a fuller description of the microbiology of *Yersinia* spp. see Chapter 231 .

Edwardsiella

In nature, *Edwardsiella* spp. are found in many cold-blooded animals. *Edwardsiella tarda* has been the cause of liver abscesses, bacteremia and infections in various other tissues.[111] In addition, it has been associated with gastrointestinal infections. It produces large amounts of hydrogen sulfide in culture. The association of *Edwardsiella ictaluri* and *Edwardsiella hoshinae* with human disease is less clear.

Hafnia

Hafnia alvei is presently the only species within the genus *Hafnia*. Fewer carbohydrates are fermented than in *Enterobacter* spp.

TABLE 228-9 -- The genus *Serratia*.[7]

THE GENUS *SERRATIA*	
Name	**Synonym**
Serratia ficaria	
Serratia fonticola	
Serratia grimesii	*Serratia liquefaciens*
Serratia liquefaciens	*Enterobacter liquefaciens*
Serratia marcescens	
Serratia odorifera	
Serratia plymuthica	
Serratia proteamaculans subsp. *proteamaculans*	*Serratia liquefaciens*

Serratia proteamaculans subsp. *quinovora*	*Serratia liquefaciens*
Serratia rubidaea	

TABLE 228-10 -- The genera *Morganella*, *Proteus* and *Providentia*[7]

THE GENERA *MORGANELLA, PROTEUS* AND *PROVIDENTIA*	
Name	**Synonym**
Morganella morganii subsp. *morganii*	*Proteus morganii*
Morganella morganii subsp. *sibonii*	*Proteus morganii*
Proteus mirabilis	
Proteus penneri	*Proteus vulgaris* indole negative
Proteus vulgaris	
Providencia alcalifaciens	*Proteus inconstans*
Providencia rettgeri	*Proteus rettgeri*
Providencia rustigianii	*Providencia alcalifaciens* biogroup 3
Providencia stuartii	*Proteus inconstans*

TABLE 228-11 -- The genus *Yersinia*.[7]

THE GENUS *YERSINIA*	
Name	**Synonym**
Yersinia aldovae	
Yersinia bercovieri	*Yersinia enterocolitica* biogroup 3b
Yersinia enterocolitica	*Pasteurella enterocolitica*
Yersinia frederiksenii	
Yersinia intermedia	
Yersinia kristensenii	
Yersinia mollaretii	*Yersinia enterocolitica* biogroup 3a
Yersinia pestis	*Pasteurella pestis*
Yersinia pseudotuberculosis	*Pasteurella pseudotuberculosis*
Yersinia rohdei	

The organism has been isolated from environmental samples, infected wounds and other tissues, and has been implicated in gastroenteritis.[112] [113] [114] [115]

Pantoea

Pantoea agglomerans was until recently a member of the genus *Enterobacter*. Strains produce a yellow pigment. This species became notorious because of an outbreak of nosocomial bacteremia associated with contaminated intravenous fluids.[116]

TABLE 228-12 -- Miscellaneous enterobacterial genera.[71]

MISCELLANEOUS ENTEROBACTERIAL GENERA	
Name	**Synonym**
Budvicia aquatica	
Buttiauxella agrestis	
Buttiauxella noackiae	CDC enteric group 59
Cedecea davisae	CDC enteric group 15
Cedecea lapagei	
Cedecea neteri	*Cedecea* sp. 4
Cedecea sp. 3	
Cedecea sp. 5	
Edwardsiella hoshinae	
Edwardsiella ictaluri	
Edwardsiella tarda	
Ewingella americana	CDC enteric group 40
Hafnia alvei	*Enterobacter hafniae*
Kluyvera ascorbata	CDC enteric group 8
Kluyvera cryocrescens	
Kluyvera georgiana	CDC enteric group 36/37
	Kluyvera sp. group 3
Leclercia adecarboxylata	*Escherichia adecarboxylata*
	CDC enteric group 41
Leminorella grimontii	CDC enteric group 57
Leminorella richardii	
Moellerlla wisconsensis	CDC enteric group 46
Pantoea agglomerans	*Enterobacter agglomerans*
Photorhabdus luminescens	*Xenorhabdus luminescens*
Rahnella aquatilis	
Tatumella ptyseos	CDC group EF-9
Yokenella regensburgei	*Koserella trabulsii*

	CDC enteric group 45

TABLE 228-13 -- The newer Enterobacteriaceae genera and their distinguishing reactions.[*]

THE NEWER ENTEROBACTERIACEAE GENERA AND THEIR DISTINGUISHING REACTIONS		
Newer genus	**Related genus**	**Selected differentiating reactions**
Buttiauxella	*Escherichia*	Citrate
Cedecea	*Serratia*	DNAase, gelatin, lipase
Ewingella	*Pantoea*	Xylose, arabinose
Kluyvera	*Escherichia*	Citrate
Leclercia	*Escherichia*	Adonitol, lysine, malonate
Leminorella	*Proteus*	Urease, phenylalanine, arabinose
Moellerella	*Providentia*	Lactose, phenylalanine
Rahnella	*Pantoea*	Phenylalanine
Tatumella	*Pantoea*	Mannitol, phenylalanine
Trabumella	*Salmonella*	Ortho-nitrophenyl-ß-galactosidase, dulcitol
Yokenella	*Hafnia*	Voges-Proskauer

** Adapted from Koneman et al.* [5]

Miscellaneous other Enterobacteriaceae

Newer members of the family Enterobacteriaceae have been described in recent years and many have been associated with human diseases.[116] [117] [118] [119] [120] [121] [122] Table 228.12 and Table 228.13 list the newer genera, together with other genera that are closely related and selected reactions to distinguish these strains. In addition to the genera mentioned in these figures, other new genera include *Budvicia*, which are mainly isolated from water sources, *Erwinia*, which are mostly plant pathogens,[123] and *Photorhabdis*. [124]

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Chapter 229 - Pseudomonads and Miscellaneous Gram-negative Bacilli

Eugénie Bergogne-Bérézin

INTRODUCTION

Strictly aerobic Gram-negative bacilli have become increasingly important as human pathogens over the past 20 years.[1] [2] Dominated by *Pseudomonas aeruginosa*, which is well known for its significant pathogenicity for the human host, this large group of saprophytic organisms has undergone confusing taxonomic changes for many years. New definitions of species and genera using modern genotyping analysis, together with reliable identification methods, have resulted in a better knowledge of these bacteria and a significantly increased awareness of their pathogenic role in hospitals and in rare cases of community-acquired infection. In descending order of importance the following are commonly isolated in nosocomial infections:

- *P. aeruginosa*;[1]
- *Acinetobacter baumannii*;[3]
- *Stenotrophomonas maltophilia*;[4] and
- *Burkholderia cepacia*.[5]

Species of *Flavobacterium*, *Comamonas* and *Alcaligenes* groups have only recently (late 1980s) been recognized as potential pathogens. A few other groups less frequently colonize or infect patients (as compared with *P. aeruginosa* and the other groups cited above) in intensive care units (ICUs).[6]

EPIDEMIOLOGY

Because of their ability to grow in environments providing limited nutrients, pseudomonads and other aerobic Gram-negative bacilli are saprophytic organisms and are found in water, soil and various other origins, plants, vegetables, insects and sewage. They may survive in hostile conditions, dry, cold or warm environments, and in a variety of foods such as dairy products, poultry and frozen foods. As nonfastidious organisms, most aerobic Gram-negative bacilli can use a wide variety of substrates as sole carbon and energy sources.[1] [2] [3] Their presence as saprophytes in the human environment, industrial environment (agricultural, food, cosmetic industries) and medical environment (pharmaceutical preparations, antiseptics, warm ICU humidifiers and ventilators) is unavoidable and they have been particularly successful at adapting themselves to new environments created by human activities.

PATHOGENIC ROLE

Aerobic Gram-negative bacilli can be carried as transient commensals in the human body. Although considered as low-virulence organisms, *Pseudomonas* spp. behave as opportunistic pathogens, being responsible for severe infections in hospitals, and are recognized as important human pathogens worldwide.[7]

ANTIBIOTIC THERAPY

Aerobic Gram-negative bacilli are frequently multiresistant to major antibiotics and this contributes to their prominent role in the morbidity and mortality of patients hospitalized in ICUs, oncology units, burn centers and surgery wards.[7] [8] [9] Various mechanisms of resistance have been recognized. Combination antibiotic therapy is often recommended for treatment, on the basis of careful antibiotic susceptibility testing. This chapter deals with *P. aeruginosa* and other potentially pathogenic aerobic Gram-negative bacilli. (Species that are animal or plant pathogens are not included in this review.)

PSEUDOMONAS AERUGINOSA

NATURE

Taxonomic classifications of *Pseudomonas* spp. have evolved considerably over the years. As the traditional phenotypic classifications based on morphologic, biochemical and antigenic characteristics have been replaced by the genotypic classification systems, these groups of bacteria are now much easier to understand. However, divisions and subdivisions based on genetic homologies, DNA hybridizations and rRNA sequence comparisons have undergone designation changes; new genera have been identified such as *Burkholderia* spp. (previously included in *Pseudomonas* spp.).[5] One of the most significant examples of taxonomic changes is the recent conversion of the species initially designated *Pseudomonas maltophilia* in 1961[8] and then *Xanthomonas maltophilia* in 1983[10] to *Stenotrophomonas maltophilia* (Table 229.1).

EPIDEMIOLOGY

The large majority of *Pseudomonas* spp.[7] [11] are ubiquitous organisms and are widely distributed in nature. Their increasing involvement in infections in humans results from multiple factors but mainly the development of antibiotic usage and the resulting selective pressure in favor of inherently or potentially resistant Gram-negative species from the environment.[12] [13] Hospitals, ICUs, immunodepressed patients, invasive procedures and antibiotic usage have provided opportunities for emergence, persistence and transfer of *P. aeruginosa* between patients or from patients to staff and to inanimate reservoirs.

Pseudomonas aeruginosa is also a saprophyte in the normal individual and is by far the predominant aerobic Gram-negative bacillus causing illness in patients who have risk factors and immunosuppressed defense mechanisms. Recent studies using molecular typing methods have shown a variety of environmental sources in hospitals and nursing homes: *P. aeruginosa* isolates from sinks, wash basins and toilets were similar (*exotoxin A* typing) to those isolated from the hands of staff and the urinary tracts of paraplegic patients, and transmission over a 6-month period in an ICU for newborns has been related to a source implicating air valves in the ventilator tubes. It is well recognized that *P. aeruginosa* can be present on surgical and medical material, in antiseptic (quaternary ammonium compounds) or contact lens cleaning solutions, and in ventilatory equipment in ICUs.[7] [11] In humans, few intestinal carriers are found in the general population (about 4%) but they are much more

TABLE 229-1 -- Current nomenclature of non-Enterobacteriaceae, nonfermentative Gram-negative bacilli.

CURRENT NOMENCLATURE OF NON-ENTEROBACTERIACEAE, NONFERMENTATIVE GRAM-NEGATIVE BACILLI		
Main groups (genera)	**Current name**	**Previous name**
Acinetobacter	*Acinetobacter baumannii*	*Acinetobacter anitratus*
	Acinetobacter calcoaceticus	*Acinetobacter anitratus, Acinetobacter calcoaceticus subsp. calcoaceticus*
	Acinetobacter haemolyticus	*Acinetobacter anitratus*
	Acinetobacter johnsonii	
	Acinetobacter junii	
	Acinetobacter lwoffi	*Acinetobacter anitratus, Acinetobacter calcoaceticus subsp. lwoffi*
Alcaligenes	*Agrobacterium tumefaciens*	*Agrobacterium radiobacter* CDC group Vd-3
	Alcaligenes faecalis	*Alcaligenes odorans, Pseudomonas odorans*
	Alcaligenes piechaudii	
	Alcaligenes xylosoxidans subsp. *denitrificans*	*Alcaligenes denitrificans* CDC group Vc
	Alcaligenes xylosoxidans subsp. *xylosoxidans*	*Alcaligenes denitrificans* subsp. *xylosoxidans, Achromobacter xylosoxidans* CDC groups IIIa, IIIb
Burkholderia	*Burkholderia cepacia*	*Pseudomonas cepacia, Pseudomonas multivorans, Pseudomonas kingae* CDC group EO-1
	Burkholderia gladioli	*Pseudomonas gladioli, Pseudomonas marginata*
	Burkholderia mallei	*Pseudomonas mallei, Actinobacillus mallei*
	Burkholderia pickettii	*Pseudomonas pickettii* CDC groups Va-1, Va-2, *Pseudomonas thomasii*
	Burkholderia pseudomallei	*Pseudomonas pseudomallei*
Comamonas	*Comamonas acidovorans*	*Pseudomonas acidovorans*
	Comamonas testosteroni	*Pseudomonas lestosteroni* CDC group EF-19
	Comamonas terrigena	
Chryseobacterium	*Chryseomonas luteola*	*Pseudomonas luteola* CDC group Ve-1
	Chryseobacterium gleum	*Flavobacterium gleum* CDC group IIb
	Chryseobacterium indolongenes	*Flavobacterium indologenes* CDC group IIb
	Chryseobacterium meningosepticum	*Flavobacterium meningosepticum* CDC group IIa
	Chryseobacterium odoratum (Myroides odoratus)	*Flavobacterium odoratum* CDC group M-4f
Flavobacterium	*Empedobacter brevis*	*Flavobacterium breve*
	Flavimonas oryzihabitans	*Pseudomonas oryzihabitans* CDC group Ve-2
	Flavobacterium sp. group IIe	CDC group IIe
	Flavobacterium sp. group IIh	CDC group IIh
	Flavobacterium sp. group IIi	CDC group IIi

Pseudomonas	*Pseudomonas aeruginosa*	
	Pseudomonas alcaligenes	
	Pseudomonas chlororaphis	*Pseudomonas aureofaciens*
	Pseudomonas delafieldii	
	Pseudomonas fluorescens	
	Pseudomonas mendocina	CDC group Vb-2
	Pseudomonas pertucinogena	*Bordetella pertussis* rough phase IV
	Pseudomonas pseudoalcaligenes	*Pseudomonas alcaligenes* biotype B
	Pseudomonas putida	
	Pseudomonas stutzeri	CDC group Vb-1
	Pseudomonas stutzeri-like	CDC group Vb-3
	Pseudomonas sp. group 1	*Pseudomonas denitrificans*
	Pseudomonas-like group 2	CDC group IV-d
Sphingobacterium	*Sphingobacterium mizutaii*	*Flavobacterium mizutaii*
	Sphingobacterium multivorum	*Flavobacterium multivorum* CDC group IIk-2
	Sphingobacterium spiritivorum	*Flavobacterium spiritivorum, Sphingobacterium versatilis* CDC group IIk-3
	Sphingobacterium thalpophilum	*Flavobacterium thalpophilum*
	Sphingobacterium yabuuchiae	*Favobacterium yabuuchiae*
	Sphingomonas paucimobilis	*Pseudomonas paucimobilis* CDC group IIk11
	Weeksella virosa	*Flavobacterium genitale* CDC group II-f
Stenotrophomonas	*Stenotrophomonas maltophilia*	*Xanthomonas maltophilia*
		Pseudomonas maltophilia
The list is limited to those potentially involved in infections.[1] [6] [7]		

common among hospital patients (i.e. up to 18% on average for all hospital populations). Intestinal carriage of *P. aeruginosa* is significantly increased after gastrointestinal surgery, and can reach 73% of patients.[14] Many other carriage sites have been described in hospital patients, including the respiratory tract, genitourinary tract and skin, which can also be the source of dissemination of *Pseudomonas* strains as well as the origin of endogenous contamination (Table 229.2).

TABLE 229-2 -- Carriage in human flora.

CARRIAGE IN HUMAN FLORA				
Organisms	**Respiratory tract**	**Gastrointestinal tract**	**Genitourinary tract**	**Skin, ear, eye, miscellaneous**
Pseudomonas aeruginosa	2+	1+	0	0
Other *Pseudomonas* spp.	0	1+	0	0
Acinetobacter spp.	2+	1+	1+	2+
Burkholderia cepacia	1+	0	0	1+
Alcaligenes faecalis	0	1+	0	1+
Weeksela virosa	0	0	1+	0
Incidence of carriage of aerobic Gram-negative bacilli in human flora (quantitative presence).[2] [15] 2+, frequently isolated; 1+, rarely isolated; 0, not typically isolated.				

PATHOGENICITY

Pseudomonas aeruginosa is responsible for a variety of infections in patients who have many risk factors such as:

- surgery;
- immunosuppression; and
- prolonged hospital stay, mainly in an ICU.

TABLE 229-3 -- Predominant sites and incidences of nosocomial infections.

PREDOMINANT SITES AND INCIDENCES OF NOSOCOMIAL INFECTIONS			
		Hospital wide (%)	**Intensive care unit (%)**
Pseudomonas aeruginosa	Sepsis	18.2*	-
	Nosocomial pneumonia	16.9†	20.8–36.4
	Urinary tract infection	12.0–18.8	11.3
	Surgical wound	8.2	9.5
	CF respiratory infection	70–90% of CF cases	
	Burns — SSTI	15.4	
Stenotrophomonas maltophilia [16] (total 3.7% of 2569 *S. maltophilia* strains)	Sepsis, endocarditis	1.4	
	Surgical wound	15.8	
	Secondary meningitis	-	
	Nosocomial pneumonia	66.4	
	Urinary infection	8.7	
	CF	About 7% of CF lung infections	

Burkholderia cepacia [17] (of 786 strains)	Blood culture	1.8	
	Respiratory infection	78.8	6–20% of CF cases
	Miscellaneous fluids and pus	7.8	
	Urine	3.7	
	Environment	7.6	
Acinetobacter baumannii [18] (of total *Acinetobacter* spp.)	Nosocomial pneumonia		25–29
	Urinary infections		21–30
	Bacteremia, endocarditis		6–9
	Burn infections, wounds		2–12
	CF respiratory infections		Rare
Chryseobacterium meningosepticum [19] (rarely pathogenic)	Neonatal meningitis, sepsis	Predominant sites in neonates	
	Surgical wound, nosocomial pneumonia	Occasional	

Predominant sites of nosocomial infections due to aerobic Gram-negative bacilli and incidences (% of total organisms).[2] [3] [6] [7] CF, cystic fibrosis; SSTI, skin and soft tissue infection.

* % of all Gram-negative bacteremia.[7]

† % of all bacterial species including Gram-positives.[19]

Pseudomonas aeruginosa possesses many virulence factors and various resistance mechanisms, which confer upon it a predominant role as a nosocomial pathogen. Recent surveys have shown that *P. aeruginosa* is responsible for between 10% and more than 20% of all nosocomial infections[7] and that the predominant site of infection is the respiratory tract, although there are many other infection sites (Table 229.3).[13] [14]

PATHOGENESIS

Pseudomonas aeruginosa is pathogenic as a result of toxigenic and invasive properties. It produces many virulence factors that have been characterized and some of them have already been cloned (Table 229.4).[20] The pathogenic factors may act at or away from the site of infection.

At the site of infection

The adhesion factors (pili in nonmucoid strains and/or alginate in mucoid strains) and exoenzyme S play a significant role in the pathogenesis of most respiratory infections; they are particularly involved in pulmonary invasion in cystic fibrosis (CF). Nearly all strains produce pili under favorable conditions; they are uni- or bipolar in distribution and are composed of pili protein (molecular weight 18kDa).[20]

Local enzymatic activities at the site of infection are those of proteases, neuraminidase and phospholipases. They disrupt epithelial cell membranes, phospholipids and protecting cell surface proteins, resulting in tissue damage. Proteases appear to contribute to necrotic skin lesions (ecthyma gangrenosum). Elastolytic activities disrupt the elastin of blood vessels, resulting in hemorrhages. All enzymes contribute to the invasiveness of *P. aeruginosa*. [11] [20] [21]

TABLE 229-4 -- *Pseudomonas aeruginosa* virulence-associated factors.[20] [21] [22]

***PSEUDOMONAS AERUGINOSA* VIRULENCE-ASSOCIATED FACTORS**		
Nature		**Human effects**
Constitutive	Lipopolysaccharide	Cascade of inflammatory events
	Endotoxin	Septic shock
	Mucopolysaccharide capsule in mucoid	Bacterial adhesion to epithelial cells
	Pseudomonas aeruginosa (alginate: polymer of ß-1, 4-D-mannuronic and L-glucuronic acid)	Barrier to antibiotics
		Increased viscosity of bronchial secretions (cystic fibrosis)
	Pili (fimbriae)	Act as adhesins
	Cytoplasmic lectins	PAI specific for D-galactose
	PAI, PAII	PAII specific for D-mannose
Exoproducts	Proteases (alkaline and neutral metalloproteinase)	Damage tissues (active on elastin, collagen, fibrin)
		Digestion of protecting proteins serving as host defenses
	Neuraminidase	Enhances pilin-mediated adherence
	Phenazine pigment (pyocyanin)	Ciliary disruption
	Elastolytic activity (two enzymes Las A, Las B)	Breakdown elastin of blood vessels, hemorrhages
	2 Hemolysins: phospholipase C glycolipid-rhamnolipid (heat-stable)	Disruption of phospholipids of cell membranes, hydrolysis of lung surfactant and ciliostatic action
	Siderophores (pyochelin and pyoverdin)	Help growth in iron-limited condition
		Generation of toxic oxygen-free radicals
	Exotoxin A	Causes tissue damage
		Similar to diphtheria toxin
		Inhibits phagocytes
		Inhibits protein synthesis
	Exoenzyme S (functions as adhesin)	Binding specificity for glycolipids (glycososphingolipid)
	Cytotoxin: leucocidin	Cytopathic effects on leukocytes and alteration of phospholipids of cell membrane
	Antibiotic-inactivating enzymes	ß-Lactamases
		Aminoglycoside-inactivating enzymes

Away from the site of infection

The invasive properties are those of:

- lipopolysaccharide (LPS), an endotoxin responsible for septic shock;
- cytotoxins, which have cytopathic effects on leukocytes; and
- exotoxin A, produced by approximately 90% of strains, which exhibits cytotoxicity by inhibiting protein synthesis, causing intense tissue damage and inhibiting phagocytic activities.

Exotoxin A acts by a similar mechanism to that of diphtheria toxin, but has a different receptor (a 300kDa glycoprotein) on host cells. It is encoded by a single copy of a structural gene, *tox*A, regulated by two genes, *reg*A and *reg*B. It is remarkable that, whereas most Gram-negative bacilli are pathogenic via an endotoxin, *P. aeruginosa* also produces an exotoxin, which enhances the virulence of this pathogen.

Risk factors in patients

A wide variety of *P. aeruginosa* infections of varying severity have been observed. Predominant sites of infections and patient risk factors are directly related to:

- immune status;
- underlying pathologies; and
- hospitalization in an ICU.

Specific factors that determine the site and the severity of *Pseudomonas* infection include:

- extended burns;[23]
- CF in children;[24]
- prosthetic heart valves; and
- intubation or tracheostomy.[13] [25]

PREVENTION

Prevention plays a major role in controlling *Pseudomonas* infections. Preventive measures are based on the identification of sources and modes of transmission of the pathogens (Table 229.5). Numerous guidelines have been established in the USA and in European countries. Isolation policies, administrative and regulatory measures and hospital epidemiology surveillance are increasingly applied to control outbreaks involving *P. aeruginosa*. Among the main guidelines, the aims of three approaches can be summarized as follows:

- elimination of endogenous nosocomial *P. aeruginosa* and reduction of oropharyngeal, intestinal and skin colonization in ICU patients;
- prevention of cross-contamination and control of various sources of *P. aeruginosa* that can be transmitted from patient to patient or from personnel to patient (i.e. proper disinfection and care of catheters, respiratory equipment, humidifiers, endotracheal tubes, dialysis systems, etc.); and
- prevention of contamination in burns patients, surgical wounds and the oropharyngeal area in ventilated patients (i.e. antibiotic prophylaxis in postoperative high-risk patients; for burns patients either systemic antibiotics or local antibiotics or disinfection could be recommended; and aerosolized polymyxin B and/or endotracheal aminoglycosides to prevent *Pseudomonas* pneumonia, which has the highest fatality rate).[13] [24] [25]

In addition, selective digestive decontamination[34] has been advocated in ICU patients; this should prevent colonization of the oropharynx and the gut by potentially pathogenic bacteria, as the digestive tract can be an important reservoir for multiresistant *P. aeruginosa*, and so prevent nosocomial infection. Topical chemoprophylaxis includes nonabsorbable antibiotics, generally polymyxin E, tobramycin (or norfloxacin) and amphotericin B (the latter to control fungal colonization). Most investigations have included coadministration of systemic ceftazidime but a clear consensus about the effectiveness of selective digestive decontamination has not been established, possibly because of the heterogeneous groups of patients and varying oral regimens involved and the inconsistent addition of systemic cefotaxime or ceftazidime (see Chapter 84).

DIAGNOSTIC MICROBIOLOGY

Bacteriology of *Pseudomonas aeruginosa*

Microscopy

Pseudomonas aeruginosa is a thin, motile Gram-negative bacillus that moves relatively fast considering that it has only a single polar flagellum.[1] [11] [15]

It grows easily on simple agar medium and produces characteristic pigments. Its phenotypic characteristics, such as motility, pigment production and positive oxidase reactions, mean that it can be

TABLE 229-5 -- Sources, methods of contamination and risk factors for nosocomial infections due to aerobic Gram-negative bacilli.

SOURCES, METHODS OF CONTAMINATION AND RISK FACTORS FOR NOSOCOMIAL INFECTIONS DUE TO AEROBIC GRAM-NEGATIVE BACILLI			
Organisms and reference	**Settings**	**Mechanism (source)**	**Risk factor/comments**
Pseudomonas aeruginosa[15] [23]	SICU/MICU, HU, BU	Contaminated equipment, solutions, antiseptics; endogenous	Cross-contamination; exposure to broad-spectrum antibiotics; severely ill patients; burns outbreaks
Pseudomonas putida, *Pseudomonas fluorescens*[1] [15] [23]	SICU	Contaminated blood and blood byproducts, antiseptics	Few in wound infections; rare cases of opportunistic infections
Stenotrophomonas maltophilia [2] [15] [16] [27]	Surgery, SICU	Contaminated devices, disinfectants, catheters	Dialysis fluids; exploratory procedures; neutropenia; respiratory devices (CF); tracheostomized patients; backflow from nonsterile tubes
Burkholderia cepacia[2] [15] [17]	ICU	Airborne transmission; contaminated skin preparations, ventilator, thermometer, antiseptic solutions	CF patients; hand carriage; calibration bath (contaminated with 10^5 cfu/ml); immunodepressed patients
Chryseobacterium meningosepticum (*Flavobacterium* spp.)[19] [28] [29]	NICU	Contaminated water, ice, disinfectants, humidifiers	Bacteremia; neonatal meningitis; infected wounds
Alcaligenes xylosoxidans[30] [31] [32]	ICU, HDU	Contaminated chlorhexidine solution, dialysis fluid, aerosols, respirators	Aqueous source; hemodialysis; peritonitis; bacteremia; meningitis; severe underlying disease
Acinetobacter spp.[3] [33]	MICU/SICU	Contaminated ventilators, moist devices, burns; endogenous	Severely ill patients; cross-contamination; outbreaks
BU, burns unit; HDU, hemodialysis unit; HU, hematology unit; MICU, medical intensive care unit; NICU, neonatal intensive care unit; SICU, surgical intensive care unit.			

Figure 229-1 Key to identification of nonfermentative aerobic Gram-negative bacilli. Note that *Burkholderia mallei* has no flagella and is nonmotile; *Pseudomonas aeruginosa* is monotrichous; oxidase-negative organisms use carbohydrates (activity of a-glucosidase, ß-glucosidase, ß-galactosidase, ß-xylosidase); *Alcaligenes* spp. have degenerated peritrichous flagella, which are functional; *Chryseobacterium* spp. produce variably pigmented colonies due to yellowish-orange pigment; and *Stenotrophomonas* spp. (except nonpigmented mutants) produce yellow pigment.

rapidly identified. Details of diagnostic microbiology techniques are shown in Figure 229.1 , Table 229.6 and Table 229.7 .

Electron microscopy

Figure 229.2 clearly shows the very long, thin polar flagellum and the irregular surface of the bacillus.

Culture

Colonies of *Pseudomonas aeruginosa* on agar culture at 98.6°F (37°C) are smooth and flat (Fig. 229.3). The pigments (pyocyanin and fluorescein) give the characteristic blue-green pigmentation due to production of pyocyanin, which is enhanced by culture on King's A medium.

Epidemiologic markers

Reliable epidemiologic tools are needed to trace the geographic spread of strains (Table 229.8).

Conventional phenotypic methods

These are based on biochemical profiles, antibiotic susceptibility patterns, bacteriophage and bacteriocin susceptibilities; more recently developed are outer membrane protein profiles and multilocus

TABLE 229-6 -- Phenotypic identification of aerobic Gram-negative bacilli based on microscopy, pigments and oxidase.

PHENOTYPIC IDENTIFICATION OF AEROBIC GRAM-NEGATIVE BACILLI BASED ON MICROSCOPY, PIGMENTS AND OXIDASE		
Tests	**Characteristics**	
Microscopy	**Gram-negative bacilli**	
Morphology	Thin bacilli: *Pseudomonas aeruginosa*	Coccobacilli (diplobacilli): *Acinetobacter* spp.
Motility	Motile: *P. aeruginosa* (fast motion): *Pseudomonas* spp.	Nonmotile: *Acinetobacter* spp., *Flavobacterium* spp. (*Chryseobacterium*)
Flagella*	Monotrichous, polar unique: *P. aeruginosa; Pseudomonas stutzeri; Pseudomonas pseudoalcaligenes*	None (gliding motility)
	Lophotrichous polar: *Stenotrophomonas maltophilia; Burkholderia cepacia; Pseudomonas fluorescens: Burkholderia pseudomallei*	
	Peritrichous, nonpolar, degenerated: *Alcaligenes faecalis*	
Pigments (on nutrient agar)	*P. aeruginosa/Pseudomonas* spp.	*Flavobacterium* spp. (*Chryseobacterium*) (inconstantly produced)
	Pyocyanin: bluish-green	
	Phenazinic pigment: yellowish-orange (*Pseudomonas aureofaciens*)	Light-yellow, yellowish-orange or bright yellowish-orange; nondiffusible pigment
	Pyoverdin: greenish-yellow (*P. aeruginosa*, *P. fluorescens*, *Pseudomonas putida*)	
	Carotenoïd pigment (*P. stutzeri*)	
	Light-straw yellow pigment (*S. maltophilia*)	
	Yellow-purple (violacein) or brownish in 10% *B. cepacia*	
Oxidase†	Positive:	Negative:
	Pseudomonas spp.	*S. maltophilia* (or weakly and tardily positive)
	Alcaligenes spp.	*Acinetobacter* spp.
	Flavobacterium spp.	
	B. cepacia (rare strains negative)	

* Staining of flagella: Leifson's staining (tannic acid, basic fuchsin) or Rhode's silver-plating staining method.
† Oxidase reaction: on Mueller-Hinton agar, 1% solution of N-dimethyl paraphenylene-diamine (ready-to-use discs or solution prepared in the laboratory).

TABLE 229-7 -- Diagnostic microbiology characteristics differentiating important *Pseudomonas* spp. [15]

DIAGNOSTIC MICROBIOLOGY CHARACTERISTICS DIFFERENTIATING IMPORTANT *PSEUDOMONAS* SPP.						
Characteristics	***Pseudomonas aeruginosa***	***Pseudomonas putida***	***Pseudomonas stutzeri***	***Pseudomonas alcaligenes***	***Pseudomonas pseudoalcaligenes***	***Pseudomonas fluorescens***
Flagella number	1	>1	1*	1	1	>1
Pyocyanin	+ (or v)	-	-	-	-	-
Fluorescent pigments	v	+	-	-	-	+
Growth at 105.8°F	+	-	v	+	+	-
Growth at 39.2°F	-	v	-	-	-	+
Arginine dihydrolase	+	+	-	+	v	+
Oxidase	+	+	+	+	+	+
Denitrification	+	-	+	+	v	- or +
Gelatin hydrolysis	+	-	-	v	v	+
Use of glucose	+	+	+	-	-	+
Use of 2-ketagluconate	+	+	-	-	-	+

Use of L-valine	v	+	+	-	-	NS
Use of ß-alanine	+	+	-	v	v	+
Use of DL-arginine	+	+	-	+	+	+
Guanine + cytosine % content in DNA	67.2	62.5	60.6–66.3	64–68	62–64	59.4–61.3
v, variable; NS, not stated.						

* Lateral flagella produced under certain conditions.

enzyme electrophoresis. Most 'traditional' typing methods are based on unstable properties of the organisms and phenotypic characters may change during the course of an outbreak or during a prolonged period of observation of endemic cases with apparently identical pathogens. However, biotyping, resistance phenotyping and serotyping remain popular methods because they are rapid and easy to

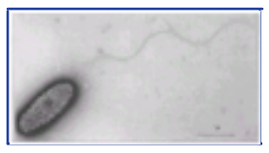


Figure 229-2 *Pseudomonas aeruginosa* monotrichous polar flagellum seen on electron microscopy. *Courtesy of Professor A Marty.*

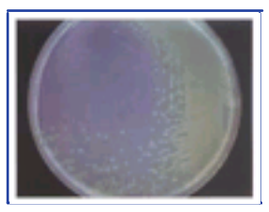


Figure 229-3 *Pseudomonas aeruginosa* colonies on agar medium. *Courtesy of Professor E Bingen.*

TABLE 229-8 -- Epidemiologic markers for *Pseudomonas aeruginosa* typing.

EPIDEMIOLOGIC MARKERS FOR *PSEUDOMONAS AERUGINOSA* TYPING				
Epidemiologic markers		**Principles and characteristics**	**Advantages**	**Drawbacks**
Phenotypic	Biotyping	Utilization of substrates, production of enzymes, biotyping schemes for identification	Rapid, easy to perform, API 20NE panel or automated (Vitek GNI), inexpensive	Unstable, variability of metabolic characters, poorly discriminating
	Resistance phenotype	Antimicrobial susceptibility pattern always carried out, multiple resistance markers	Rapid, easy to perform, standardized (National/International guidelines), early and useful during outbreak	Unstable profiles, plasmid acquisition or loss during an outbreak, derepression of inducible enzymes, mutations, poorly discriminating, unreliable
	Serotyping	Based on somatic O specific antigen (LPS), polyclonal/monoclonal antibodies, 20 serotypes, 17 antisera (IATS)	Rapid, early results, easy to perform, available reagents, inexpensive, most commonly used	50–70% of CF strains nontypable, polyagglutination of some CF strains, reproducibility of anti-LPS monoclonal antibodies is 75%
	Phage typing	Colindale set of 21 phages, cell surface receptors (OM, LPS, slime)	Limited requirements, inexpensive, available reagents	Lack of reproducibility, low discrimination, insensitivity of CF and LPS-defective strains
	Pyocin typing	R, F, S pyocins, specific lytic activity, 105 types, 26 subtypes	Limited requirements, inexpensive	Poor discrimination, complexity of the system, time-consuming technique
Genotypic	Plasmidotyping	Relatively rare plasmid in *Pseudomonas aeruginosa*, plasmids of 1.2–60MDa in 15% of strains	No advantage for *P. aeruginosa* typing	Low frequency, acquisition or loss during epidemics
	Genomic DNA, total DNA	Polymorphism of DNA, REA endonucleases (*Eco*R1, *Hin*dII, *Sma*I), conventional agarose electrophoresis	Good discriminatory power	Large number of fragments making resolution of bands difficult to interpret
	DNA RFLP	Detection of genes coding for exotoxin A (*exo*A). elastase (*las*B). alginate (*alg*D). two probes necessary	Good discriminatory power, good correlation with ribotyping	Laborious techniques, small numbers of isolates can be compared
	Ribotyping (ribosomal DNA)	Three genes coding for rRNA, probes for 16S and 23S RNA, restriction enzymes (*Eco*R1, *Cla*I, *Sal*I)	Universal, excellent reproducibility, stable ribotype patterns within outbreaks	Laborious techniques, sensitivity and specificity not established for *P. aeruginosa*
	Pulsotyping (PFGE)	DNA fingerprinting, restriction enzymes *Dra*I, *Spe*I (fragments >50kb requiring PFGE)	The most specific discriminant technique	Interpretation somewhat delicate, heavy workload
O serotypes internationally recognized; types O18 to O20 not validated yet.[22] [35] [36] IATS, International Antigenic Typing System; OM, outer membrane; PFGE, pulse-field gel electrophoresis; REA, restriction endonuclease analysis; RFLP, restriction fragment length polymorphism.				

perform, are cheap and little equipment is needed; they are useful in emergency situations and are a great help in laboratories with limited facilities.[15] [22]

Serotyping

Pseudomonas aeruginosa is serologically heterogeneous because it possesses many somatic and flagellar antigens. Based upon the

specificity of somatic antigen O (polysaccharide side chains of the LPS), an O antigenic scheme has been internationally recognized with agreement for 17 O serotypes in the International Antigenic Typing Scheme.

Limitations to serotyping include poor discriminating power, polyagglutination for some strains, failure of serotyping in LPS-defective strains and autoagglutination in CF strains. However, serotyping of *P. aeruginosa* remains useful and epidemiologically significant. For example, it has been shown that the most frequent serotypes involved in nosocomial infections are O6, O11, O1 and O3 and that serotype O12 is characterized by its exceptional multi-resistance pattern.[22]

Bacteriophage typing

A large number of bacteriophages active against *P. aeruginosa* attach to specific cell surface receptors: outer membrane proteins, LPS and slime polysaccharide. The

Colindale set of 21 phages is the most popular and constitutes the reference system, with more than 80% of isolates sensitive to these phages. Many *P. aeruginosa* strains are lysogenic and about 10 lysogenic phages may interfere with typing procedures.[22]

Phage typing lacks reproducibility and mucoid and LPS-defective strains are insensitive to phage typing.

Bacteriocin typing

Pyocin particles, identified as R, F and S pyocins (retractile, flexuous and soluble respectively), have been used for typing, on the basis of specific lytic activity, and permit identification of 105 types plus 26 subtype patterns. [37]

Although reproducible (90% of clinical strains typable), pyocinotyping is not a widely used method because of the complexity of the system and inadequate discrimination and reproducibility requiring strict standardization of inoculum and media (reagents are not commercially available).

Multilocus enzyme electrophoresis

This is based upon the electrophoretic motilities of a large number of cellular enzymes. One strain is characterized by a combination of alloenzymes (motility variants) designated 'zymotype'. When applied to *P. aeruginosa* this method has proved complex and time-consuming and is carried out only in specialized laboratories.[35]

Genotypic markers

The many drawbacks and limitations of phenotypic markers have led investigators to develop more reliable typing procedures based upon genomic DNA, and many new epidemiologic tools have become available in clinical laboratories.

Plasmid profile

This is a rapid and simple method for species carrying many plasmids but is relatively useless for *P. aeruginosa* because only 15–20% of the strains carry plasmids; the majority of clinical isolates lack demonstrable plasmids.[36]

DNA fingerprints

DNA fingerprints, which are restriction endonuclease digestions of genomic DNA, can use total chromosomal DNA, resulting in thousands of fragments ranging in size from 2kb to 25kb. The banding patterns for a series of isolates, visualized by ethidium bromide staining in gel, are compared either visually or by scanning densitometry and digitization. Because of the large number of fragments generated, it can be difficult to resolve the bands. DNA restriction fragment length polymorphism may be used to detect specific genes; good discriminatory power, using DNA probes specific for genes coding for exotoxin A (*exo*A), alginate (*alg*D), elastase (*las*B) or pilin (*PAK*), has been observed for differentiating isolates from different patients, provided that at least two DNA probes are used (5% of *P. aeruginosa* strains are deficient in the *exo*A gene).[35] [36]

Ribotyping

This is based on interspecies differences in genomic RNA sequences. Restriction fragment length polymorphisms are detected by differences in the banding pattern (three to six bands for *P. aeruginosa*). Although recognized as a stable and reproducible typing system for *Escherichia coli* and *Haemophilus influenzae,* it seems likely that its sensitivity and specificity are not well established for *P. aeruginosa*.[35] Ribotyping requires combination with another typing method, which involves a great deal of work.

Pulsotyping

Pulsed-field gel electrophoresis uses restriction endonucleases, which cut the chromosome infrequently, producing large fragments (5–800kb) resolved into 10–50 bands by field inversion gel electrophoresis or a contour-clamped homogeneous electric field. Field inversion gel electrophoresis and contour-clamped homogeneous electric field analyses are considered to be the most discriminant tools for establishing the relatedness of strains; isolates that differ by more than three bands are considered to be different, but pulsotyping requires careful interpretation and the techniques for typing require equipment, time and expense.[36]

Other typing procedures

Molecular biology techniques are evolving very rapidly and many new applications are being developed for typing. Polymerase chain reaction has been applied to comparative typing of *P. aeruginosa* — random primed or enterobacterial repetitive intergenic consensus sequences have been used. Both approaches were reproducible and discriminatory.[38] In addition, they require less work and expense than the methods discussed above, and so are becoming methods of choice for fingerprinting strains in the laboratory.

CLINICAL MANIFESTATIONS

Pseudomonas respiratory infections

Pseudomonas *pneumonia*

This generally occurs after endogenous aspiration from a colonized oropharynx, often in patients who have an underlying malignancy; these people carry *P. aeruginosa* in the oral flora more frequently (18–25%) than subjects who do not have malignancies (=5%).

Pseudomonas pneumonia usually occurs in hospitalized patients and the clinical features do not distinguish it from other Gram-negative nosocomial pneumonias. The clinical presentation and radiographic findings (nodular infiltrates, sometimes with cavitation and lesions, predominantly in the lower lobes) are not specific; various degrees of hemorrhagic edematous lung with scattered micro-abscesses and vascular involvement (thrombosis) are often seen on anatomic pathology. The microscopic appearances are of necrosis of alveolar septa (Fig. 229.4) and arterial walls, with areas of focal hemorrhage and, in intact areas, infiltration with macrophages, mononuclear cells and polymorphonuclear leukocytes.[13] [39]

Bacteremic Pseudomonas *pneumonia*

A different lung pathology has been described in bacteremic *Pseudomonas* pneumonia, which occurs in even more severely ill patients who have the risk factors listed in Table 229.9 . The pneumonia is diffuse and bilateral and there is a pleural effusion. On cut section, the lesions are either nodular, hemorrhagic with necrotic foci, or umbilicated nodules surrounded by dark hemorrhage. Intra-alveolar

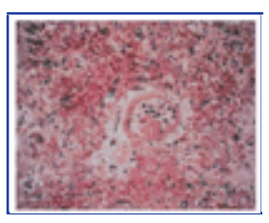


Figure 229-4 Anatomic pathology of *Pseudomonas aeruginosa* pneumonia showing acute inflammatory exudate, necrosis of alveolar membranes and fibrinous thrombosis in a venula. Hematoxylin-eosin stain. *Courtesy of Professor Groussard.*

hemorrhages with patchy alveolar septal necrosis are seen on microscopy. The lesions contain many bacteria but lack a leukocytic reaction. The pneumonia is

accompanied by bloodstream invasion that often spreads to metastatic sites of infection. The pneumonia rapidly progresses, resulting in pulmonary edema and necrotizing bronchopneumonia. The fatality rate is extremely high (up to 80–100% of cases).[20] [21] [39] [40]

Strategies for the treatment of *Pseudomonas* bacteremic pneumonia have emphasized the importance of early initiation of empiric appropriate therapy because the majority of fatal cases occur within the first 48–72 hours. Combination therapy is generally recommended (antipseudomonal ß-lactam and an aminoglycoside), but there is a poor correlation between clinical response and the in-vitro synergistic effects of antibiotics.[26] [41] [42]

Respiratory infections in cystic fibrosis patients

Pathogenesis

Cystic fibrosis is a congenital disease affecting exocrine gland secretions.[43] [44] A protein, the CF transmembrane conductance regulator, which is responsible for transporting chloride across membranes, is abnormal in CF patients and this decreases the electrochemical gradient for sodium ion movement into the CF duct cell. This results in abnormal sodium absorption in airway epithelium and mucous airway secretions contain decreased water and electrolyte concentrations, with twice the normal ratio of macromolecules (mucins) to electrolytes. These glycoprotein mucins form thick tenacious secretions, which obstruct the airways and contribute to the development of progressive suppurative pneumonia (see Chapter 33).

The leading cause of morbidity and mortality in patients who have CF is progressive pulmonary deterioration with chronic necrotizing bronchopneumonia. These pulmonary infections mainly involve *P. aeruginosa*. Cystic fibrosis also causes a wide range of other major organ deficiencies (diarrhea, malabsorption, pancreatic insufficiency) (see Chapter 40g).

Mucoid *Pseudomonas aeruginosa*

In the early 1960s the importance of mucoid variants of *P. aeruginosa* was recognized. Mucoid *P. aeruginosa* are morphologic and

TABLE 229-9 -- Infections due to *Pseudomonas aeruginosa* listed in descending order of incidence.[13] [20] [21] [26]

INFECTIONS DUE TO *PSEUDOMONAS AERUGINOSA* LISTED IN DESCENDING ORDER OF INCIDENCE		
Infection		**Associated factors**
Respiratory infections	*Pseudomonas* pneumonia (30–60% mortality rate)	Mechanical ventilation
		Endotracheal or tracheostomy tube
		Neurologic disease
		Nasogastric tube
		Prolonged stay in intensive care unit
		Broad-spectrum antibiotics
	Bacteremic *Pseudomonas* pneumonia (80–100% mortality rate)	Neutropenia
		Underlying malignant neoplasm
		Chronic bronchiectasis (terminal state)
		Diabetes mellitus
		Severe immunodepression
		Cytotoxic chemotherapy
		Coronary artery disease
		Severe burns
	Respiratory tract infections in people with cystic fibrosis (ultimately fatal unless a pulmonary transplant is carried out)	Presence of the lethal genetic disease cystic fibrosis
		Chronic colonization with *Pseudomonas aeruginosa*
		Progressive lung deterioration
		Altered immune response to *Pseudomonas* spp.
Bacteremia	Primary	Same factors as above
	Secondary	Leukemia, lymphoma
		Intravenous devices
		Intravenous drug abuse
		Trauma
		Prematurity
		Ulceration of the gastrointestinal tract
		Solid organ or bone marrow transplant
		Various endoscopic instrumentation procedures
Skin and soft tissue infections (50–78% mortality rate in burn wound sepsis)		Burn wound sepsis (78% *Pseudomonas* spp.)
		Wound infection
		Ecthyma gangrenosum
		Dermatitis, pyoderma
Urinary tract infections		Acute
		Chronic (obstruction)
Endocarditis		Intravenous drug abusers
		Prosthetic heart valves
Miscellaneous		Meningitis (secondary)
		Brain abscesses
		Bone and joint infections (chronic *Pseudomonas* osteomyelitis)
		Ear infections (otitis externa, malignant external otitis)
		Eye infections (*Pseudomonas* keratitis, endophthalmitis, contact lens keratitis)

functional variants of *P. aeruginosa* characterized by the ability to produce copious amounts of an exopolysaccharide, which is an acetylated polymer of D-mannuronic and L-glucuronic acids (alginate; see Table 229.4). Molecular weights of the polysaccharide vary from 100kDa to 480kDa. It should be distinguished from the 'slime' polysaccharide, which has different biologic properties and is found in nonmucoid *P. aeruginosa* as well as in mucoid *P. aeruginosa*. The controlling genes (*alg*) for the alginate biosynthetic pathway have

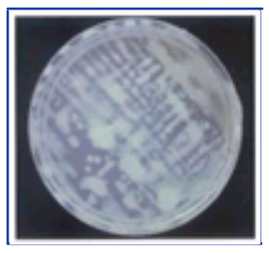


Figure 229-5 Mucoid colonies of a strain of *Pseudomonas aeruginosa* isolated from a patient who has cystic fibrosis. *Courtesy of Professor E Bingen.*

been partially cloned; multiple gene control of alginate production involves several enzymatically controlled steps.[43] It seems likely that most *P. aeruginosa* possess the genetic information for mucoid exopolysaccharide production. They are capable of producing mucoid exopolysaccharide when grown under appropriate environment conditions, such as those present in the CF lung. Figure 229.5 shows colonies of mucoid *P. aeruginosa*.

In vivo, in areas with impaired local defenses, such as in the airways of CF patients, the organism grows in microcolonies surrounded by the thick polysaccharide matrix. In autopsied lungs of people who had CF there are microcolonies adherent to the walls of larger airways and in the alveoli. Mucoid *P. aeruginosa* are present in abundance in foci of active inflammation in small bronchioles but not in destroyed parenchymal areas. This observation is consistent with the simultaneous role of bacterial growth in the active inflammatory process and of toxins produced by *P. aeruginosa* diffusing away from microcolonies. For instance:

- exotoxin A is one of the major factors responsible for tissue injury in the lungs of patients who have CF and they have high titers of antibodies to toxin A; and
- 86% of mucoid *P. aeruginosa* isolates produce proteases, elastase, collagenase and fibrinolysin.

In contrast it seems likely that mucoid *P. aeruginosa* LPS contributes little to the pathogenesis of lung injury but stimulates local inflammation.

Clinical features

The clinical signs, pulmonary functions and radiographic features seen in patients who have CF vary considerably according to the extent of the disease and the frequency of acute exacerbations. A chronic productive cough, wheezing, tachypnea and low-grade fever develop during acute exacerbations. As the pulmonary disease progresses, fever, chest pain and a cough producing abundant purulent viscous sputum are the predominant symptoms of infection. Lung damage results in pulmonary vascular obstruction and a deteriorating pulmonary function. Arterial hypoxemia is progressive and correlates with clinical pulmonary status. Chest radiographs show overaeration, peribronchial thickening, patchy atelectasia and pneumonia. These features are reversible during the early stages of the disease in the first year of infection.

Appropriate management of the disease and acute exacerbations with aggressive antibiotic therapy, chest physiotherapy and nutritional therapy may result in a symptomatic improvement and slow the inevitable progression of pulmonary deterioration.

Pseudomonas bacteremia

Of all nosocomial bacteremias, *P. aeruginosa* accounts for 20–35% of isolates.[13] [40] *Pseudomonas aeruginosa* is particularly common in

TABLE 229-10 -- Anatomic sites of primary infection in *Pseudomonas* bacteremia.*

ANATOMIC SITES OF PRIMARY INFECTION IN *PSEUDOMONAS* BACTEREMIA		
Infection	**Hospital acquired Gram-negative bacilli (%)**	**Community acquired (%)**
Urinary tract	18.8	5.2
Gastrointestinal tract	20.0	4.3
Skin/soft tissue infection	15.4	6.3
Respiratory tract	36.4	27.8
Other	4.8	6.7

** Adapted from MacCue.*[40]

series of patients who have hematologic malignancy or high risk factors (see Table 229.9).

Primary bacteremia

Pseudomonas bacteremia may occur following instrumentation procedures using contaminated equipment or solutions. Equipment used for endoscopic retrograde cholangiopancreatography, intra-aortic balloon pump placement and many other invasive exploratory investigations have been reported as sources of *Pseudomonas* bacteremia. Many cases of bacteremia have also occurred in narcotic addicts.[13] [26]

Secondary bacteremia

Pseudomonas bacteremia is most often related to focal infection (Table 229.10) and the most frequent source is the respiratory tract;[40] the skin and soft tissues, especially in burns patients, and urinary tract are also common sources. Less frequently, colonization of the gastrointestinal tract may precede infection, which develops in the presence of a variety of risk factors such as hospitalization in an ICU, the presence of neutropenia or treatment with cytotoxic chemotherapy; 50% of these patients develop intestinal carriage, which occurs in only 5–15% of the general population, and translocation of *P. aeruginosa* is the potential mechanism for blood invasion and metastatic infection, predominantly in the respiratory tract.

Endocarditis

Endocarditis due to *P. aeruginosa* occurs predominantly in intravenous drug abusers and in those who have prosthetic heart valves. The tricuspid valve is most frequently involved but the aortic or mitral valve and mural endocardium can also be affected.[26] Adherence mechanisms in *P. aeruginosa* (pili, exoenzyme S, alginate) are likely to play a role in the colonization of prosthetic valves, resulting in microcolonies embedded in polysaccharide material and therefore protected from host defenses and antibacterial agents. Similar mechanisms may occur in bacterial colonization of intravenous and intra-arterial catheters.[20] [44]

Skin and soft tissue infections

Burn wound sepsis

Pseudomonas aeruginosa is the most common cause of burn wound sepsis, which is the predominant form of skin and soft tissue infection complicating severe thermal injury. The mortality rate is high (50–78%) despite improvements in management and antibiotic therapy.[26] Colonization of the burned skin surface by *P. aeruginosa* may result from the patient's own flora or from environmental sources. The bacteria penetrate into the subcutaneous tissues via hair follicles and breaks in the burned skin, and may enter with the help of proteolytic enzymes they produce. Other virulence factors (see Table 229.4) make a significant contribution to the severity of the

Figure 229-6 Burned leg that has been superinfected with *Pseudomonas aeruginosa.* *Courtesy of Professor H Carsin.*

Figure 229-7 Burned abdominal wall that has been superinfected with *Pseudomonas aeruginosa.* *Courtesy of Professor H Carsin.*

burn infection, which can be the source of bloodstream invasion. Sepsis, which is clinically characterized by fever or hypothermia, hypotension, oliguria and abdominal distension in addition to the extensive burn eschar, requires specific management in burns centers. Figure 229.6 and Figure 229.7 show extensive burn lesions.

Ecthyma gangrenosum

This is a focal skin lesion that is often associated with *Pseudomonas* bacteremia. The lesion is characterized by an erythema surrounded by hemorrhage, necrosis of skin tissues and bacterial invasion. Other pseudomonal skin lesions may complicate *Pseudomonas* bacteremia, such as subcutaneous nodules, deep abscesses and cellulitis. These extensive and destructive lesions of the skin are particularly seen in neutropenic patients.

Wound superinfections with Pseudomonas aeruginosa

These occur occasionally and their characteristics were described early in the medical literature, particularly because of the blue-green exudate and the colored bandages due to the production of pyocyanin pigments.

Urinary tract infections

Most *Pseudomonas* urinary tract infections are hospital-acquired and associated with either catheterization or surgery or with any cause of obstruction or persistent site of infection (e.g. chronic prostatitis).

Pseudomonas urinary tract infections have no specific clinical presentation but tend to evolve with frequent recurrences and chronic evolution. A characteristic picture of ulcerative or necrotic lesions and multiple renal abscesses is seen in patients who have metastatic bacteremia with urinary tract invasion.[26]

Eye infections

Of all Gram-negative organisms, *P. aeruginosa* is the most common ocular pathogen, despite not forming part of the normal ocular flora. Ocular metastatic infection is rare and the most common source is exogenous. Ocular infections vary from mild (conjunctivitis) to extremely severe (orbital cellulitis). [45]

Keratitis

The most frequent manifestation is keratitis. The predominant predisposing factors of *Pseudomonas* keratitis are prosthetic devices (contact lenses), congenital abnormalities, burns or trauma, altered host defenses (people who have HIV infection) and prematurity. Viral keratitis (herpes simplex) may also be associated with a secondary bacterial infection. The corneal damage results from the exocellular products of *P. aeruginosa* and from strong adhesion to the exposed basement membrane of the epithelium; exotoxins, proteases and phospholipases degrade the corneal stroma, resulting in extensive loss of collagen fibers from the stroma.

Contact-lens associated Pseudomonas *keratitis*

This is common and is mainly observed in association with extended-wear contact lenses, inappropriate disinfecting regimens and poor hygiene. The bacteria may adhere to the lens, resulting in the development of a thick coat of mucopolysaccharide forming the same biofilm as on other prosthetic devices.

Endophthalmitis

This most often results from an endogenous origin, occurring by hematogenous spread from other infected sites. It may also occur after intraocular inoculation of *P. aeruginosa* by either trauma, burns or ocular surgery. Endophthalmitis is an acute fulminant disease, with pain and decreased acuity, which are potentially followed by panophthalmitis. The prognosis is poor without appropriate local and systemic management.[26] [45]

Miscellaneous

Central nervous system infections

These include meningitis and brain abscesses, which can result from either direct inoculation (head trauma, surgery), a contiguous infection (sinus, mastoid) or bloodstream infection. Other occasional sources are spinal anesthesia, lumbar puncture and cancer (the latter being one of occasional causes of central nervous system infections).

Bone and joint infections

These may result from contiguous spread, hematogenous origin or trauma. They occur predominantly in patients who have predisposing factors such as diabetes mellitus, intravenous drug abuse and chronic debilitation. Examples are:

- *Pseudomonas* osteomyelitis, which tends to have a chronic evolution;
- pyarthrosis, which frequently involves the sternoclavicular or sternochondral joints, in drug abusers;
- vertebral osteomyelitis in elderly patients in association with genitourinary instrumentation or surgery (*P. aeruginosa* has a particular affinity for cartilaginous joints of the axial skeleton); and
- *Pseudomonas* osteochondritis of the foot, which occurs in children following puncture wounds (this infection, like other fibrocartilaginous infection sites, involves cartilage, synovium, joint space and contiguous bone).

Pain, swelling, fever and other systemic signs are variable, depending upon the underlying diseases and the immune status of the patient.[20] [21] [26]

The management of bone and joint *Pseudomonas* infections is dependent upon the site of the infection and the immune status of the patient (see Table 229.9).

Ear infections

Pseudomonas aeruginosa is frequently isolated from the external auditory canal, particularly in infants. The significance of the presence of this potential pathogen is not always clear. In some cases, *P. aeruginosa* is involved in a superficial self-limited external otitis, which resolves spontaneously. Occasionally, the pathogen invades the epithelium between cartilage and bone in the lateral portion of the auditory canal, penetrating soft tissue, cartilage and bone.

Malignant external otitis

Malignant external otitis is a severe invasive necrotizing ear infection, clinically characterized by otalgia, otorrhea, early facial nerve paralysis and a swollen erythematous external auditory canal. Adjacent soft tissue is often involved. There is visible extension with cellulitis and bone erosions, the tympanic membrane is generally perforated and there is a purulent discharge.[26] [46]

Pseudomonas aeruginosa is isolated from the external auditory canal and from surgical specimens in all cases of malignant external otitis. Most cases occur in elderly people who have diabetes mellitus, but can occur in infants who have severe underlying diseases.

Management of this severe and extensive ear infection requires prolonged antibiotic therapy, surgical debridement and drainage.[46] The fatality rate is high (about 15–20%). Relapses are frequent and malignant external otitis requires prolonged follow-up.

MANAGEMENT

Antibiotic resistance in *Pseudomonas aeruginosa*

Paradoxically, although *P. aeruginosa* is the aerobic Gram-negative bacillus most frequently involved in nosocomial infection, it is not

TABLE 229-11 -- Resistance mechanisms in *Pseudomonas aeruginosa*.*

RESISTANCE MECHANISMS IN *PSEUDOMONAS AERUGINOSA*					
Antibiotics		**Percentage resistant**[47]	**Percentage resistant**[49]	**Mechanisms**	**Genetic bases**
Penicillins	Ticarcillin	30–40	12	Altered PBPs targets	Chromosomal
	Piperacillin	15–20	22	Altered PBPs targets	Chromosomal
Cephalosporins	Cefsulodin	12–15	-	Reduced permeability	Chromosomal
	Cefoperazone	15–20	-	Reduced permeability	Chromosomal
	Ceftazidime	2–8	6	ß-Lactamase inactivation (83%)	Chromosomal or plasmid mediated
	Cefpirome	5–8	-	ß-Lactamase inactivation (83%)	Chromosomal or plasmid mediated
Monobactams	Aztreonam	5–10	13	ß-Lactamase inactivation	
Carbapenems	Imipenem	10–15	2	Altered protein porin D2, imipenemase	Chromosomal
	Meropenem	10–15	-	Altered protein porin D2, imipenemase	Chromosomal
Aminoglycosides	Gentamicin	30–50	14	Reduced permeability, enzymatic inactivation	Chromosomal or plasmid mediated
	Tobramycin	25–35	4	Reduced permeability, enzymatic inactivation	Chromosomal or plasmid mediated
	Netilmicin	25–40	12	Reduced permeability, enzymatic inactivation	Chromosomal or plasmid mediated
	Amikacin	11–30	2	Reduced permeability, enzymatic inactivation	Chromosomal or plasmid mediated
Fluoroquinolones	Ciprofloxacin	10–50	28	Altered DNA gyrase target, efflux	Chromosomal
	Ofloxacin	10–50			
Fosfomycin		-	50–80	Altered transport system	Chromosomal mutation
Rifampin (rifampicin)		-	-	Altered DNA polymerase target	Chromosomal
Distribution of ß-lactamases in *P. aeruginosa*: plasmid mediated — PSE-1 to PSE-4 36%, OXA (1–10) 25%, TEM-1 and TEM-2 11.5%; inducible chromosomal cephalosporinase. Main modifying enzymes in *P. aeruginosa*: AAC (3)-I, AAC (3)-III; AAC (3)-IV, V; AAC (6')-I, AAC (6')-II; ANT (2''), AAC (6')-I + ANT (2''). PBP, penicillin-binding protein.					

** Data from Chen et al.,[6] Wiedemann et al.[47] and Li et al.[48]*

the most resistant organism among 'pseudomonads' and other aerobic Gram-negative bacilli. A number of ß-lactams considered to be 'antipseudomonal' drugs are still active against this species (Table 229.11). These drugs include:

- semisynthetic penicillins — carboxypenicillins (ticarcillin), ureidopenicillins (piperacillin);
- third-generation cephalosporins (ceftazidime, cefpirome, cefepime). For both penicillins and cephalosporins the percentages of susceptible strains were between 55% and 75% according to recent European[49] [50] and international surveys.[47]
- for carbapenems (imipenem), rates of susceptibility were about 80%; and
- for monobactams (aztreonam) — 70–80% susceptible strains.

Variations occur between countries in relation to different antibiotic usages. Besides natural resistance to many ß-lactam antibiotics, high resistance rates are seen in *P. aeruginosa* with aminoglycosides and fluoroquinolones. The incidence of plasmids in *P. aeruginosa* is relatively low and the major resistance mechanisms are chromosomally mediated. These mechanisms are mainly:

- altered outer membrane permeability (altered protein porins or lack of protein porin OprD);[51]
- production of chromosomally mediated ß-lactamases, inducible cephalosporinases or plasmid-mediated enzymes, including extended spectrum ß-lactamases;[50] [51] [52]
- aminoglycosides inactivating enzymes;[53] and
- active efflux mechanism pumping out different antibiotic classes from the cell.[54]

Resistance during therapy may develop and multiple resistance in *P. aeruginosa* is frequent, resulting in diminished permeability to ß-lactams, increased production of ß-lactamases, aminoglycoside-inactivating enzymes and efflux mechanisms pumping out fluoroquinolones, ticarcillin, aztreonam, cefsulodin, chloramphenicol and tetracyclines.[48] This results in strains with multiresistance profiles that are extremely difficult to eradicate and the choice of a potentially

TABLE 229-12 -- Antibiotic therapy for *Pseudomonas* infections.*

ANTIBIOTIC THERAPY FOR *PSEUDOMONAS* INFECTIONS		
Choices	**Drugs**	**Indications**

Monotherapy	Antipseudomonas penicillins: ticarcillin, piperacillin, azlocillin; Cephalosporins: ceftazidime, cefoperazone, cefpirome; Carbapenems: imipenem; Fluoroquinolones: ciprofloxacin	Limited to nongranulocytopenic patients, non-life-threatening infections, short courses, bacteriologic monitoring
Conventional combinations	Aztreonam, ticarcillin or ceftazidime plus clavulanate and/or sulbactam plus tobramycin; imipenem plus amikacin; ciprofloxacin plus ceftazidime; ciprofloxacin plus fosfomycin	Severe *Pseudomonas* infections — pneumonia, bacteremia, burns (plus topical), malignant external otitis media (plus surgery), central nervous system infection (plus local), cystic fibrosis (plus topical)
Alternatives	Antipseudomonas penicillin plus fluoroquinolone; aztreonam plus aminoglycoside; aminoglycoside plus fluoroquinolone	
Adjuvants	Burns — mafenide acetate (local), local debridement; Cystic fibrosis — mucolytics, passive immunization (anti-TNF MAb, IL-1); vaccines O-polysaccharide-based	
Anti-TNF MAb, monoclonal antibody to tumor necrosis factor; IL-1, interleukin-1.		

** Data from Pollack,[26] Bustamante et al.[41] and Figueredo and Neu.[55]*

efficient antibiotic therapy becomes limited. Serotype O12 has been recognized as one of the most resistant *P. aeruginosa* types; during an outbreak in a burns unit,[23] a serotype O12 strain was resistant to ticarcillin, ceftazidime, aztreonam, imipenem and ciprofloxacin, being only susceptible to amikacin. Paradoxically, 96% of these multiresistant strains were susceptible to fosfomycin.

Management of *Pseudomonas aeruginosa* infection

A summary of appropriate antipseudomonal treatments in various indications is given in Table 229.12 .

Conventional antibiotic therapy of* Pseudomonas aeruginosa *infections

Systemic severe infections, bacteremic pneumonia, life-threatening infections

The antibiotic therapy, either empiric or documented, is based upon an antibiotic combination, such as an aminoglycoside and a ß-lactam with antipseudomonal activity. Before laboratory data and antibiogram are available, a knowledge of local epidemiology of resistance in a particular ward or ICU and antibiotic susceptibilities as established in recent publications should be a great help in choosing a suitable combination.[41] [42] [56] Data shown in Table 229.11 and Table 229.13 indicate the current situation in terms of susceptibilities and minimum inhibitory concentrations (MICs) and should permit an empirical choice to be confirmed by the local clinical laboratory results.

Monotherapy

This has been documented using the following as monotherapy in moderately severe infections:[57]

- a third-generation cephalosporin, preferentially ceftazidime or cefpirome;
- imipenem/cilastatin; or
- a fluoroquinolone.

Agents such as ceftazidime or cefoperazone have been reported to achieve high serum bactericidal levels against bacteria isolated from patients who have *Pseudomonas* pneumonia.

Results from controlled clinical trials have shown that monotherapy is as good as combination therapy in nongranulocytopenic patients.

Antibacterial agents proposed as single-agent treatments of nosocomial pneumonia include imipenem and fluoroquinolones. Monotherapy with quinolones has narrow indications in severe *Pseudomonas* infection; short-term therapy is recommended when quinolones are used as monotherapy, and close bacteriologic monitoring is advised. Therapeutic results with quinolones against *Pseudomonas* infection were initially encouraging. Mild-to-moderate infections in CF patients have been controlled by oral ciprofloxacin when *P. aeruginosa* strains were resistant to ß-lactam agents and aminoglycosides. In some cases patients probably responded to therapy as a result of high tissue concentrations and a favorable ratio of quinolone concentration in vivo to the MICs for the pathogens.[56] However, the susceptibilities of hospital pathogens have evolved, and *Pseudomonas* systemic infections should be monitored carefully for antibiotic resistance.

The major risk of using single-agent treatment for *Pseudomonas* pneumonia is the possible emergence of antibiotic-resistant bacteria during therapy with quinolones or imipenem.

Indications for antibiotic combinations

In microbiologically documented cases of *Pseudomonas* infections, ß-lactam and aminoglycoside combinations offer a broad spectrum of antibacterial activity with synergistic bactericidal effect. Many reports have described in-vitro synergism, and in-vivo studies in animal models of *P. aeruginosa* pneumonia have confirmed the efficacy of such regimens.[58] However, in patients who have *Pseudomonas* nosocomial infections, there were frequent therapeutic problems due to the presence of multiresistant bacteria.[49] In a survey in our hospital, of 762 *Pseudomonas* strains:

- 39% were resistant to all ß-lactams;
- 21% were imipenem resistant;
- 21% were resistant to all aminoglycosides; and
- 37.5% were resistant to all fluoroquinolones.

Among imipenem-resistant strains, however, 41% were susceptible to ticarcillin and ceftazidime. The emergence of resistance has been prevented by using ciprofloxacin with ceftazidime or imipenem in *P. aeruginosa* pneumonia, but the in-vitro effect of combinations is additive or indifferent rather than synergistic. [41] An absence of correlation between in-vitro synergism and clinical outcome with a combination of ciprofloxacin and azlocillin has been observed.[42] When quinolones are combined with ß-lactam drugs (ureidopenicillins, ceftazidime or imipenem), the combination prevents or at least reduces the risk of emergence of resistance. Combinations of fluoroquinolones with fosfomycin have been found to be useful in preventing the emergence of resistant mutants[55] and to be generally synergistic.

Management of specific* Pseudomonas *infections

Central nervous system infections due to *Pseudomonas aeruginosa*

These require bactericidal antibiotics that reach high concentrations in cerebrospinal fluid (CSF) — in *Pseudomonas* meningitis a

TABLE 229-13 -- Antibiotic susceptibility of *Pseudomonas aeruginosa*. [47]

ANTIBIOTIC SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA*				
Antibiotics	**Number of strains tested**	**Range (mg/l)**	**MIC_{50} (mg/l)**	**MIC_{90} (mg/l)**

Beta-lactams	Apalcillin	72	0.25–256	2	256
	Aztocillin	100	0.25–512	8	512
	Aztreonam	100	0.125–128	4	16
	Carbenicillin	76	=2 to =512	64	512
	Cefepime	60	0.5–16	4	16
	Cefixime	322	0.25 to =16	=16	=16
	Cefmenoxime	110	2 to =128	32	=128
	Cefoperazone	100	0.5–128	4	128
	Cefoperazone and sulbactam	72	8 to =128	64	128
	Cefotaxime	100	0.25–512	16	64
	Cefotetan	100	32–64	64	64
	Cefpirome	60	2–32	2	16
	Cefsulodin	110	1 to =128	4	64
	Ceftazidime	110	1–128	4	32
	Ceftazidime	100	0.5–64	2	4
	Ceftizoxime	100	0.5–64	32	64
	Ceftriaxone	99	=0.06 to =16	8	=16
	Imipenem	322	0.25–8	1	2
	Latamoxef	100	2–128	32	128
	Meropenem	100	0.03–32	0.5	2
	Mezlocillin	76	=2 to =512	25	128
	Piperacillin	100	0.5–512	8	512
	Piperacillin and tazobactam	100	0.06–256	4	128
	Temocillin	72	256	256	256
	Ticarcillin	100	0.5–512	32	512
	Ticarcillin and clavutanic acid	100	0.06–512	16	512
Aminoglycosides	Amikacin	100	0.125–2	0.5	2
	Gentamicin	100	0.06–128	0.5	8
	Isepamicin	100	0.125–2	0.5	1
	Tobramycin	100	0.03–32	0.125	4
Quinolones	Ciprofloxacin	100	0.015–2	0.06	0.25
	Enoxacin	100	0.5–4	1	4
	Fleroxacin	100	0.06–32	1	4
	Lomefloxacin	100	0.125–8	0.5	2
	Nalidixic acid	100	=128	=128	=128
	Norfloxacin	100	0.25–2	1	2
	Ofloxacin	100	0.5–8	2	4
	Pefloxacin	100	2–16	8	16
Miscellaneous	Trimethoprim-sulfamethoxazole	25	4 to =128	128	=128

combination of ceftazidime, which is highly concentrated in CSF when the meninges are inflamed, and an aminoglycoside (amikacin, which is the drug with the lowest resistance rates; see Table 229.11). If there is obstruction of the subarachnoid space the aminoglycoside may be instilled directly into the ventricular system. *Pseudomonas* brain abscess is treated surgically together with a similar prolonged antibiotic therapy.[26]

Malignant external otitis

Malignant external otitis, which is an extremely severe infection, must be treated with a combination of local surgical debridement and drainage together with a potent antibiotic therapy combining an aminoglycoside and antipseudomonal ß-lactam.[46]

Pseudomonas burn wound sepsis

This requires a combination of antibiotics, as mentioned above, although there is frequent emergence of resistance due to high bacterial counts and limited access of antibiotic to burn sites. Local measures with topical agents and surgical debridement of necrotic tissue are always applied in addition to systemic antibiotics.[26] [52]

Pseudomonas pneumonia in cystic fibrosis patients

Respiratory infections in CF are among the most serious types of infection, because they occur in children, who suffer for prolonged periods. Eradication of *Pseudomonas* spp. (or other pathogens) from the airways occurs provisionally only, whatever strategy is used. Proper management of acute exacerbations of *Pseudomonas* lung infection requires antibiotic combinations, usually aminoglycosides and ß-lactams (ceftazidime or piperacillin) at larger than usual doses. Although not recommended in children, fluoroquinolones have been used successfully and a combination of ciprofloxacin with fosfomycin has demonstrated in-vitro synergy.[55] Courses of aggressive antibiotic therapy every 3 months in combination with other measures such as mucolytics, antiproteases and topical antibiotic therapy and physiotherapy increase the 5- to 20-year survival of patients who have CF.

Topical antibiotic therapy for nosocomial *Pseudomonas* pneumonia in cystic fibrosis patients

Depending upon their physicochemical characteristics, many injected antibiotics do not achieve significant concentrations in the

lung. Administration of antibiotics directly into the tracheobronchial tree should therefore increase local antibiotic concentrations so that the free drug concentration exceeds the MIC of the pathogen.[59] [60] A good clinical tolerance has been reported for gentamicin, tobramycin and amikacin, although some reduction of the maximum expired volume per second has occasionally been observed. Carbenicillin and ceftazidime were also administered topically but poorly tolerated. Successful inhaled tobramycin therapy has been demonstrated recently in 69 CF centers in the USA.[59]

Among the drawbacks of this route of administration are:

- a low or heterogeneous deposition of antibiotic in lung areas where there is consolidation or atelectasis; and
- resorption of aminoglycoside from the respiratory tract to the blood resulting in a low antibiotic concentration that may promote the emergence of resistant bacteria (this has been observed in *P. aeruginosa* pneumonia when continuous local polymyxin B has been used for prophylaxis).[60]

Nosocomial *Pseudomonas* pneumonia

It has been suggested that parenteral conventional therapy should be combined with direct instillation of aminoglycoside into the respiratory tract via an endotracheal or tracheostomy tube. In a double-blind randomized trial, using endotracheal tobramycin versus placebo, pathogens were eradicated in 68% versus 31% respectively.[61]

OTHER *PSEUDOMONAS* SPECIES

EPIDEMIOLOGY

Among *Pseudomonas* spp. (see Table 229.1), a few may be involved in rare cases of opportunistic infection. Their common habitat is in the natural environment: water, soil and plants. Some of these species, in particular *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas stutzeri*, are widespread in the animal and human environment and are frequently found in the hospital environment; they occasionally contaminate antiseptic solutions, dialysis fluids, transfusion blood and blood byproducts.

DIAGNOSTIC MICROBIOLOGY

Characteristics differentiating *Pseudomonas* spp. are shown in Table 229.7 . This simplified scheme for identification of these bacteria is mainly based on morphologic features (flagella), pigment production and the main metabolic characteristics. The taxonomic status of non-*aeruginosa Pseudomonas* spp. has evolved significantly (see Table 229.1). Some species, such as *P. fluorescens* and *P. putida*, are heterogeneous and several biovars are recognized: five biovars in *P. fluorescens* (I–V) and two biovars in *P. putida* (A and B), which differ in a few metabolic properties.[1] [7] [11]

PATHOGENICITY AND CLINICAL MANIFESTATIONS

Some of these *Pseudomonas* spp. have been isolated from human clinical specimens (blood, urine, stools) and occasional cases of opportunistic infection occur as a result of transfusion, antiseptic use, dialysis and other mechanisms of transmission. *Pseudomonas fluorescens*, a psychrophilic organism, may grow at 39°F (4°C), which favors its presence in blood products.[15] Outbreaks of bacteremia, respiratory infections in CF patients, wound infections and rare cases of community-acquired pneumonia have been reported.[7] These *Pseudomonas* spp. have been also implicated in rare cases of endophthalmitis and keratitis, particularly *P. fluorescens*, *P. stutzeri* and *Pseudomonas paucimobilis* (the last species being rarely cited). Most

TABLE 229-14 -- Epidemiology and pathogenicity of the non-*aeruginosa Pseudomonas* species

EPIDEMIOLOGY AND PATHOGENICITY OF THE NON-*AERUGINOSA PSEUDOMONAS* SPP.		
Habitat and epidemiology	**Pathogenicity**	**Species**
Environment, water, plants; hospital environment; rare opportunistic pathogens	Occasional bacteremia (contaminated blood, solutions); rare cases	*Pseudomonas alcaligenes, Pseudomonas pseudoalcaligenes*
Soil, water, plants; hospital sinks, floor; food spoilage (eggs, meat, fish, milk); opportunistic pathogens	Rarely isolated from clinical specimens; rare cases of isolation in patients with cystic fibrosis, bacteremia, urinary tract infection, wounds	*Pseudomonas putida, Pseudomonas fluorescens*
Ubiquitous, soil, water, sewage water; hospital environment, antiseptics, injectable solutions; relatively more frequent than other non-*aeruginosa*	Outbreaks of pseudobacteremia; frequent isolation from pus, urine, blood, cerebrospinal fluid; contamination of bone marrow transplant	*Pseudomonas stutzeri*
Pseudomonas spp.; opportunist; more susceptible to antibiotics than other species		

cases occur in severely debilitated patients, and these *Pseudomonas* spp. behave as opportunistic pathogens in immunocompromised patients (Table 229.14).[15] [39] [26]

MANAGEMENT

Based on their in-vitro susceptibilities,[47] these organisms can be eradicated by using carbapenems (MICs 0.5–8mg/l) in combination with fluoroquinolones (ciprofloxacin MICs 0.5–16mg/l). Aminoglycosides have poor activity and management of infections due to *P. putida* or *P. fluorescens* is difficult. *Pseudomonas stutzeri* is more susceptible to antibiotics than other species.

ACINETOBACTER SPECIES

NATURE

Acinetobacter spp. are Gram-negative bacteria that are commonly present in soil and water as free-living saprophytes and are also isolated as commensals from skin, throat and various secretions of healthy people. They have emerged as important nosocomial pathogens in outbreaks of hospital infections and rank second after *P. aeruginosa* among aerobic Gram-negative bacilli nosocomial pathogens. The increasing number of these infections and the natural resistance of the strains has led to studies of epidemiology and resistance mechanisms in *Acinetobacter* spp.[3]

EPIDEMIOLOGY

In the natural environment

Acinetobacter spp. are widely distributed in nature and can be found in virtually 100% of soil and freshwater samples when appropriate culture techniques are used.[6] [62] They use a wide variety of substrates as

a sole carbon source. In freshwater samples, less than 1–7.9 × 10^4 cfu/100ml have been recorded, whereas in raw sewage effluents up to 10^6 cfu/100ml have been found. *Acinetobacter* spp. can also be isolated from food and form part of the normal flora of fresh meats; they may contribute to the spoilage process of refrigerated meats.

In the human environment

In hospitals

Acinetobacter spp. have been found in:

- moist situations such as room cold-air humidifiers, tap water, hand washbasins, waterbaths, moist respirometers and all types of ventilatory equipment, which are capable of aerosolizing the organism;
- angiography catheters;
- blood collection tubes; and
- plastic urinals.

The presence of *Acinetobacter* spp. in mattresses in burns units has been reported,[3] [62] and Acinobacter have caused considerable problems on some intensive care units.

Human carriage of Acinetobacter *species*

This has been demonstrated in normal individuals, indicating that the source of nosocomial infections might be endogenous as well as from the external environment. *Acinetobacter* spp. form part of the bacterial flora of the skin and are found in the axilla, groin and toe webs; they can colonize the oral cavity, the respiratory tract and the normal intestine (see Table 229.2).[62] However, the value of this approach has not been independently confirmed and is not widely used.

PATHOGENICITY

Pathogenesis of infections: virulence of *Acinetobacter* species

Several factors may be responsible for the virulence of *Acinetobacter* spp.:

- the presence of a polysaccharide capsule formed of L-rhamnose, L-glucose, D-glucuronic acid and D-mannose that makes the surface of strains more hydrophilic;
- the adhesiveness of *Acinetobacter* spp. to human epithelial cells in the presence of fimbriae and/or mediated by the capsular polysaccharide;
- enzymes (butyrate esterase, caprylate esterase and leucine arylamidase) potentially involved in damaging tissue lipids; and
- the LPS component of the cell wall and the presence of lipid A, which are likely to participate in the pathogenicity of *Acinetobacter* spp.[33]

Host predisposing factors

Susceptible patients include those who have severe underlying conditions such as malignancy, burns and immunosuppression or who have undergone major surgery. Age plays a role in the occurrence of nosocomial infections with *Acinetobacter* spp. in elderly patients and neonates. The settings for infection are medical or surgical ICUs, and renal and burns units. Tubes, catheters and all kinds of artificial devices act as portals of entry at the site of infection (see Table 229.5). In French hospitals, *Acinetobacter* spp. were found in 9.7% of nosocomial infections.[3]

DIAGNOSTIC MICROBIOLOGY

Acinetobacter strains are nonfermentative, nonfastidious, aerobic Gram-negative coccobacilli, usually found in diploid formation or in chains of variable length (Fig. 229.8). They are not motile (*akinetos* means 'unable to move' in Greek), but the cells display a 'twitching

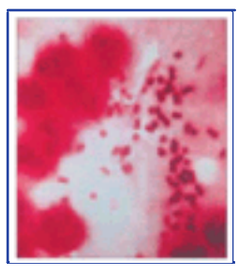


Figure 229-8 Morphology of *Acinetobacter baumannii* on Gram stain. Preparation from a lung infection in mice. *Courtesy of Dr ML Joly-Guillou.*

motility'. Strictly aerobic, they grow well on all common media at temperatures of 68–86°F (20–30°C), but for most strains the optimum is 91.4–95°F (33–35°C). A few species can grow at 105.8°F (41°C) and 111.2°F (44°C) and this is a discriminating character between species. Identification of *Acinetobacter* spp. is based upon:

- oxidase-negative, catalase-positive, indole-negative, nitrate-negative tests; and
- production or not of acid from D-glucose, D-ribose, D-xylose and L-arabinose (used oxidatively as carbon sources; Table 229.15).

Acinetobacter is the only genus with an oxidase-negative test among non motile aerobic Gram-negative bacilli.[3] [7]

Nomenclature of *Acinetobacter* species

This has undergone considerable taxonomic changes. By using modern methods of taxonomy (genetic transformations, DNA hybridizations and rRNA sequence comparisons), the classification proposed by Bouvet and Grimont[63] identified more than 15 genomic species including *Acinetobacter baumannii* (formerly *A. calcoaceticus* var. *anitratus* and *A. glucidolytica non liquefaciens*), *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter johnsonii* and *Acinetobacter radioresistens*.

Species of clinical importance

Most studies have supported the initial observation that *A. baumannii* is the main species associated with outbreaks of nosocomial infection. Other species, *A. haemolyticus*, *A. junii* and *Acinetobacter lwoffi*, have been associated with clinical infections; they can be natural inhabitants of human skin and repeated isolation of non-*A. baumannii* species suggests that they are potential pathogens.[3]

CLINICAL MANIFESTATIONS

The main sites of infections are the respiratory and urinary tracts. Endocarditis, burn infections, and skin and wound sepsis may also occur (see Table 229.3).

Acinetobacter pneumonia

This is common in hospitals, especially in ICUs. Large outbreaks of *Acinetobacter* pneumonia have been described. All patients had severe underlying disease, assisted ventilation and tracheostomy or were intubated. *Acinetobacter* spp. represent 15.6% of the total Gram-negative bacilli involved in nosocomial pneumonia in France. [18]

Community-acquired cases of *Acinetobacter* pneumonia have been reported in middle-aged and elderly people who have a chronic underlying disease or are alcoholic.

TABLE 229-15 -- Identification of the most frequently isolated *Acinetobacter* species. [3] [63]

Genospecies	Growth at	Glucose (acidified)	Hemolysis	Gelatin hydrolysis	Utilization test*			
					β-alanine	DL-lactate assimilation	Citrate	Malonate
A. anitratus (glucose acidified)								
A. calcoaceticus 1	–/35.8°C	+	–	–	+	100% positive strains	+	+
A. baumannii 2	+/41.2°C	+		–	+	+	–	+
Unnamed species 3	–/41.2°C	+/44.4°C	–	–	+		+	+
A. haemolyticus 4	+/44.6°C	+(52%)	+(100%)	+(99%)	–	0% positive strains	+(91%)	–
A. lwoffi (glucose negative)								
A. junii 5	+/44.6°C	–	–	–	–	+	+(82%)	–
Unnamed species 6	+/44.6°C	+(56%)	+(100%)	+(100%)	–	–	+	–
A. johnsonii 7		–	–	–	–	+	+	–
A. lwoffi 8/9	+/44.6°C	–	–	–	–	+	–	–
A. radioresistens 12†	+/44.6°C	–	–	–	–	–	+	+

* Eleven other assimilation tests of carbon and energy sources can be used[3]; data not shown for unnamed genospecies 10 and 11.
† Five other unnamed genospecies have been identified – 13, 14, 15, 16, 17 – and are being studied.

Red indicates a positive result, and white a negative result. The numbered genospecies are based on DNA-DNA hybridizations. *A. alcaligenes* is hemolytic.

Urinary tract infection

Several reviews[9] [18] [62] have described *Acinetobacter* spp. in 2–61% of nosocomially acquired urinary tract infections. A recent investigation in France has indicated an overall incidence of 30.5% of acquired urinary tract infection.[18]

Meningitis

Nosocomial meningitis is an infrequent manifestation of *Acinetobacter* infection. The first case report was in 1908 by Von Lingelsheim and the organism was designated *Diplococcus mucosus*, later renamed *Mima* by Debord (1939) because of its resemblance to *Neisseria meningitidis*. Cases of *Acinetobacter* meningitis have been reported after neurosurgical procedures, but rare cases of primary meningitis, especially in children, have also occurred.[3]

Miscellaneous

Skin and wound infections, abscesses, sepsis, endocarditis, peritonitis and burn wound infections have been reported.[3]

MANAGEMENT

Antibiotic resistance

Since 1980 successive surveys have shown increasing resistance in clinical isolates of *Acinetobacter* spp.[3] [9] [18] [64] High proportions of strains are resistant to most commonly used antibacterial drugs including aminopenicillins, ureidopenicillins, cephalosporins of the first generation (cephalothin) and second generation (cefamandole), cephamycins such as cefoxitin, most aminoglycosides, aminocyclitols, chloramphenicol and tetracyclines. Differences in susceptibilities of *Acinetobacter* isolates as a function of countries and areas could be attributable to environmental factors and different patterns of antibiotic usage.[3] [64] [65]

Management of *Acinetobacter* infections

Only a few antibiotics are active in the treatment of *Acinetobacter* spp. infections. A few ß-lactams might be used after careful in-vitro susceptibility testing:

- ticarcillin combined with sulbactam (the latter being a ß-lactamase inhibitor often active by itself against *Acinetobacter* spp.);[3] [66]
- ceftazidime; and
- most often imipenem, which is by far the most active drug for treating *Acinetobacter* infection.

Combination therapy is always recommended, combining a ß-lactam with an aminoglycoside, a fluoroquinolone or rifampin (rifampicin). The addition of ß-lactamase inhibitors, clavulanic acid to ticarcillin, or sulbactam to a third-generation cephalosporin, may significantly enhance the ß-lactam activity. In a recent study,[64] pneumonia and sepsis due to *A. baumannii* were treated using synergistic combinations of imipenem plus either amikacin or tobramycin (14 cases) or ampicillin-sulbactam with tobramycin and ticarcillin-clavulanate with tobramycin (four cases), underlining the importance of ß-lactamase inhibitors in combination therapy in *Acinetobacter* infections.

BURKHOLDERIA SPECIES

NATURE AND EPIDEMIOLOGY

Burkholderia spp. were transferred from the genus *Pseudomonas* (see Table 229.1). Three species are recognized as opportunistic agents involved in nosocomial infections:

- *Burkholderia cepacia* (type species),
- *Burkholderia gladioli*, and
- *Burkholderia pickettii*.

Two species are responsible for specific infections in horses (glanders) — *Burkholderia mallei* and *Burkholderia pseudomallei* — and are occasionally transmitted to humans, resulting in a disease named melioidosis (the organism was identified by Whitmore in 1913). [5]

All *Burkholderia* spp. are genetically related on the basis of DNA-DNA homologies, 16S rRNA sequences and phenotypic characters. They are ubiquitous organisms, being widespread in water, soil and plants, and are present in the human environment. *Burkholderia cepacia* is an environmental organism that has no specific nutritional requirements and survives for months in water, sinks, antiseptic solutions (chlorhexidine, quaternary ammoniums, povidone-iodine), pharmaceutical products, dialysis fluid and various injectable solutions. It may even survive on environmental surfaces[11] and may be found in nebulizers, ventilatory equipment and many other medical and dental devices (see Table 229.5).

PATHOGENICITY

Although weakly virulent with a limited invasive capacity, *B. cepacia* has become an important nosocomial pathogen. Clinical manifestations are dependent upon the source of contamination. Most cases and outbreaks of *B. cepacia* infection have been bacteremias with septic shock, nosocomial urinary tract infections, peritonitis (in dialysis units), endophthalmitis and keratitis, and nosocomial pneumonias.[26] [39] [44] [67] All infections due to *B. cepacia* have been described in immunocompromised patients, who have usually been hospitalized in ICUs. The predominant site of infection is the respiratory tract (see Table 229.3), mainly in patients who have CF.[44] [68] [69] [70]

Burkholderia cepacia in severe respiratory infections in cystic fibrosis patients

The pathogenesis of *B. cepacia* in severe respiratory infections in people who have CF has not been extensively studied. Virulence factors suspected in *B. cepacia* could be mainly exoproducts (proteases, lipases, exopolysaccharides), which act in addition to the LPS forming part of O antigen, responsible for severe pneumonia and sepsis in CF patients.[44] [17] Cases of fulminant and extensive necrotizing pneumonia with a rapidly fatal outcome have been observed.[71]

Other *Burkholderia* species

Burkholderia pickettii *and* Burkholderia gladioli

These are ubiquitous organisms that can be found in water and soil and may play a role as nosocomial pathogens. Rare outbreaks of infection have been described and emergence of multiresistance is a potential problem.[11] [70]

Burkholderia mallei *and* Burkholderia pseudomallei

These are environmental organisms predominantly found in Asia, Africa and South America. *Burkholderia mallei* is not motile but is genetically related to *B. pseudomallei*. The latter, an animal pathogen, can be transmitted to humans; sepsis with severe pneumonia and subacute pulmonary cavitating disease are the main human forms of melioidosis (see Chapter 175). These infections are severe and have high fatality rates.[26]

DIAGNOSTIC MICROBIOLOGY

Identification tests for *Burkholderia* spp. are summarized in Table 229.16 .

MANAGEMENT

The susceptibility of strains to aztreonam, ceftazidime, ceftriaxone and fluoroquinolones suggests the use of combinations of drugs belonging to these classes for treating these severe infections, particularly in people who have CF. Imipenem is not the most active ß-lactam (MICs 1 to =32mg/l) and aminoglycosides have variable efficacy (MICs 2 to >32mg/l). However, aminoglycoside plus imipenem may be a synergistic combination.[11] Highly multiresistant strains can be isolated from CF during exacerbations of pulmonary infection; consecutive sequences of antibiotic treatments[70] [71] may have exerted a selective pressure leading to a therapeutic 'dead end'. In those cases, inhaled antibiotic therapy[59] [60] associated with an intravenously administered antibiotic combination can control pulmonary exacerbation by *B. cepacia*.

STENOTROPHOMONAS MALTOPHILIA

NATURE AND EPIDEMIOLOGY

Stenotrophomonas maltophilia is an emerging opportunistic pathogen that belonged to the *Pseudomonas* genus, was transferred

TABLE 229-16 -- Diagnostic microbiology of *Burkholderia* species.

DIAGNOSTIC MICROBIOLOGY OF *BURKHOLDERIA* SPECIES			
Characteristics	*Burkholderia cepacia*	*Burkholderia mallei*	*Burkholderia pseudomallei*
Cell length (µm)	1.6–3.2	1.4–4.0	1.5
Flagella number	>1	0	>1
Pigment (diffusible)			
Oxidase			
Poly-β-hydroxybutyrate accumulation			
Gelatin	v		
Lipase (Tween 80 hydrolysis)		v	
Denitrification			
Arginine dihydrolase			
Growth at 105.8°F	v		
Guanine + cytosine content in DNA (%)	67.4	69.0	69.5

Main characteristics differentiating species.[1] [11] Red indicates a positive result, and white a negative result. v, variable.

to the *Xanthomonas* group[10] and then received a new designation, *S. maltophilia*, [4] belonging in the newly defined genus *Stenotrophomonas*. Isolated from soil, plants, water and raw milk, this ubiquitous bacterium is common in the hospital environment.[27] It has often been isolated from ventilatory equipment (thermal humidifying units) and from moist respirometers, as well as from dialysis fluids and antiseptic solutions (see Table 229.5).

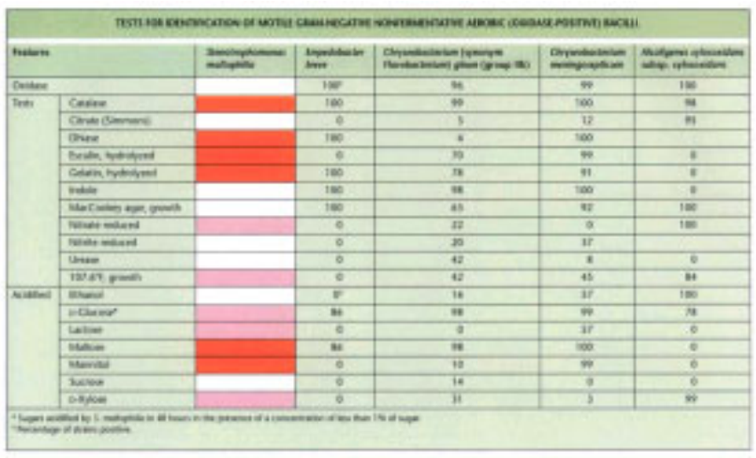
DIAGNOSTIC MICROBIOLOGY

These bacteria are characterized by the presence of a single or a small number of polar flagella (motile bacteria), frequently pigmented colonies (yellow or yellowish orange) and oxidase-negative reaction.[4] [11] [15] (Table 229.17 ; also see Fig. 229.1 and Table 229.6). *Stenotrophomonas maltophilia* acidifies sugars (except for rhamnose or mannitol) and is generally proteolytic. Its identification tests are shown in Table 229.17 .

CLINICAL MANIFESTATIONS

Stenotrophomonas maltophilia can be isolated from patients who have respiratory tract infections, endocarditis, bacteremia, meningitis and urinary tract infections, and can also be implicated in severe cutaneous infections (ecthyma gangrenosum similar to that due to *P. aeruginosa*), cellulitis and abscesses. It produces proteolytic enzymes and other pathogenic extracellular enzymes such as DNAse, RNAse, elastase, lipase, hyaluronidase, mucinase and hemolysin, which contribute to the severity of *S. maltophilia* infection in immunodepressed patients in ICUs.[2] [4] [27] There is a high incidence of infection with *S. maltophilia* in patients who have cancer, leukemia or lymphoma, and *S. maltophilia* is increasingly implicated in pulmonary superinfections in patients who have CF (see Table 229.3). It can be underlined that *S. maltophilia* is a rapidly developing agent of nosocomial infection, with an increasing incidence in respiratory infections in CF patients. Another species, identified as *Stenotrophomonas africana*, is an emergent pathogen, a strictly aerobic, nonproducer of oxidase close to *S. maltophilia*. It has been isolated in east Africa from the CSF of an HIV-positive patient with meningoencephalitis. This organism is one of the most resistant bacteria, susceptible only to ciprofloxacin and colistin.[72]

TABLE 229-17 -- Tests for identification of motile Gram-negative nonfermentative aerobic (oxidase-positive) bacilli.



Clinically important species of the genera *Stenotrophomonas*, *Chryseobacterium-Flavobacterium* group and *Alcaligenes* (*Stenotrophomonas maltophilia* is not in fact oxidase-positive). Genera and species of *Empedobacter breve, Chryseobacterium gleum* and *Chryseobacterium meningosepticum* are derived from the genus *Flavobacterium*. Red indicates a positive result, white a negative result and pink a variable result.[1] [2] [4]

Stenotrophomonas maltophilia is frequently associated with other bacterial species at sites of infection but is increasingly isolated as the sole pathogen.

MANAGEMENT

Resistance

Stenotrophomonas maltophilia is naturally resistant to most antibiotics as a result of chromosomally mediated mechanisms, poor permeability of the outer membrane and naturally produced inactivating enzymes. [6] [67]

- Many ß-lactamases have been described in *Stenotrophomonas maltophilia* and it is susceptible only to latamoxef and combinations of ticarcillin plus clavulanic acid or piperacillin plus tazobactam. It is naturally resistant to imipenem and meropenem as a result of carbapenemase production.
- Only a few strains are susceptible to gentamicin, neomycin and kanamycin. Resistance to aminoglycosides is probably plasmid-mediated. *Stenotrophomonas maltophilia* is poorly susceptible to quinolones.

- Resistance to quinolones is generally associated with resistance to chloramphenicol and to doxycycline in more than 50% of cases and is associated with alteration of outer membrane proteins.[16]
- An active efflux mechanism has been also demonstrated.[67]

Stenotrophomonas maltophilia is variably susceptible to minocycline, rifampin and trimethoprim-sulfamethoxazole.

Management of infections due to *Stenotrophomonas maltophilia*

Infections due to *S. maltophilia* require combination therapy including rifampin plus a fluoroquinolone or plus ß-lactams (mezlocillin, ceftazidime or cefotaxime; MICs 8, 128 and 16mg/l respectively). Combinations decrease the MICs of ß-lactams.[67] [73]

MISCELLANEOUS AEROBIC GRAM-NEGATIVE BACILLI

Many other aerobic Gram-negative bacilli either derived from the *Pseudomonas* group or originating from dispersed groups of environmental organisms have been identified. Some are increasingly involved in human infection.[5] [11] [72] [73] These genera and species have undergone many taxonomic changes; some have been identified recently, and the wide use of analysis of ribosomal 16S RNA gene sequences has allowed a clearer taxonomic position to be established for most aerobic Gram-negative bacilli. The following section includes a short description of the pathogenic role of those involved in human infections and of the management of these infections. For easy reading, the generic groups are described in alphabetical order (Table 229.18). Species cited in the list are those isolated from human infections (see also Table 229.1).

ACHROMOBACTER spp.

See *Ochrobacterium* spp.[74]

TABLE 229-18 -- Current and previous nomenclature of miscellaneous aerobic Gram-negative bacilli.

CURRENT AND PREVIOUS NOMENCLATURE OF MISCELLANEOUS AEROBIC GRAM-NEGATIVE BACILLI		
Main groups (genera)	**Current name**	**Previous name**
Achromobacter	*Ochrobactrum anthropi*	*Achromobacter* Vd
Aeromonas	*Aeromonas enteropilogenes*	*Aeromonas trota*
	Aeromonas ichtiosemia	*Aeromonas veronii*
	Aeromonas salmonicida	*Haemophilus piscium*
	Aeromonas caviae	-
Agrobacterium	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*
	Agrobacterium tumefaciens	
	Pantoea agglomerans	*Agrobacterium gypsophilae*
Alcaligenes (see Table 229.1)	*Alcaligenes faecalis type 1*	
Alcaligenes xylosoxidans	*Alcaligenes odorans*	
Achromobacter xylosoxidans		
Bergeyella	*Bergeyella zoohelcum*	*Weeksella zoohelcum*
Burkholderia (see Table 229.1)	*Burkholderia cepacia*	
Burkholderia mallei		
Burkholderia pseudomallei	*Pseudomonas kingii*	
Pseudomonas mallei		
Pseudomonas pseudomallei		
Calymmatobacterium	*Calymmatobacterium granulomatis*	*Klebsiella granulomatis*
Capnocytophaga	*Capnocytophaga ochracea*	*Bacteroides ochraceus*
	Capnocytophaga gingivalis	-
	Capnocytophaga sputigena	-
Chromobacterium	*Chromohalobacter marismortui*	*Chromobacterium marismortui*
Chryseobacterium (see Table 229.1)	*Chryseobacterium meningosepticum*	*Flavobacterium meningosepticum*
Chryseomonas (formerly *Flavobacterium*)	Chryseomonas luteola	Pseudomonas luteola
Comamonas	*Comamonas terrigena*	*Pseudomonas terrigena*
	Comamonas testosteroni	-
	Delftia acidovorans	*Comamonas acidovorans*
Ochrobactrum	*Ochrobactrum anthropi*	*Achromobacter* Vd
	Ochrobactrum intermedium	*Ochrobactrum* nov. sp.
Oligella	*Oligella urethralis*	*Moraxella urethralis*
Plesiomonas	*Proteus shigelloides*	*Plesiomonas shigelloides*
Ralstonia	*Ralstonia picketii*	*Burkholderia picketii*
	Ralstonia eutropha	*Pseudomonas/Alcaligenes eutrophus*
Shewanella	*Shewanella hanedai*	*Alteromonas hanedai*
	Shewanella putrefaciens	*Pseudomonas/Alteromonas putrefaciens*
Sphingobacterium (see Table 229.1)	*Sphingobacterium mizutae, S. multivorum*	*Flavobacterium* spp.
Stenotrophomonas	*Stenotrophomonas maltophilia*	*Xanthomonas maltophilia*
	Stenotrophomonas africana	-
Weeksella	*Bergeyella zoohelcum*	*Weeksella zoohelcum*
Only species seen as pathogenic in humans are listed. This table completes and/or modifies the taxonomic data in Table 229.1 .		

AEROMONAS spp.

Originally included in the Vibrionaceae group, *Aeromonas* spp.[70] [76] form their own family (the Aeromonadaceae). Their natural habitat is water, fish and other aquatic animals, and the gut of pigs, dogs and cats. At least seven species have been recognized, four of which are the most pathogenic for humans (see Table 229.18).

Microbiology

These organisms are motile, Gram-negative rods (one single polar flagellum), oxidase- and catalase-positive and facultatively anaerobic. They grow easily at low temperatures (between 41°F/5°C and 72–82°F/22.2–27.8°C) on nutrient agar. Selective growth media that contain ampicillin has been formulated, as ampicillin inhibits the growth of *Aeromonas* spp. at more than 32mg/l.

Pathogenic role

Aeromonas spp. isolated from contaminated water produce enteric infections with diarrhea, abdominal pain and fever. *Aeromonas* spp. present in the hospital environment may also be responsible for systemic infections, bacteremia, meningitis, peritonitis, cholecystitis and liver infections, all of which occur generally in patients who have severe underlying disease or immunodepression.

Aeromonas hydrophila strains have been involved in nosocomial infections. They may also be transmitted by sanguivorous leeches used in plastic or graft surgery, being responsible for severe cellulitis, osteitis and pyomyositis.

Management

Aeromonas spp. are resistant to most ß-lactams; they produce several ß-lactamases, including a carbapenemase, which confers resistance to imipenem. Infections are treated using third-generation cephalosporins, aminoglycosides, tetracyclines, fluoroquinolones and trimethoprim-sulfamethoxazole. [72]

AGROBACTERIUM spp.

Initially included in the group of *Alcaligenes* spp. (see Table 229.1), these bacteria are considered as emerging pathogens but only one species, namely *Agrobacterium radiobacter*, has been involved in human infections.[72] [73]

Microbiology

These phytopathogenic organisms, present in water, soil and environmental plants, are strictly aerobic coccobacilli, motile with peritrichous flagella (one to six). They grow easily on conventional media and produce oxydase and catalase; these bacteria are identified by using commercial identification kits.

Pathogenic role

Oncogenic in plants, *Agrobacterium* spp. have not been found to be oncogenic in humans, but they have been implicated in bacteremia, endocarditis, catheter infection, peritonitis and urinary tract infections. A limited number of cases have been cited in the literature.

Management

Agrobacterium spp. are susceptible to cephalosporins (second- and third-generation), ticarcillin, imipenem, tetracyclines, colistin, trimethoprim-sulfamethoxazole and fluoroquinolones, but outcomes of treatment depend on underlying pathologies in infected patients.

ALCALIGENES spp.

See Table 229.1 , Table 229.5 , Table 229.17 and Table 229.18 and Figure 229.1 .

Epidemiology

The natural habitat of *Alcaligenes* spp. is the same as that of *Pseudomonas* spp. In the hospital environment, *Alcaligenes faecalis* and *Alcaligenes xylosoxidans* can be isolated from various environmental sources, such as respirators, hemodialysis systems, intravenous solutions and disinfectants.[1] [2] [3] [30] [77]

Microbiology

These are short, Gram-negative rods (0.5–2.6µm), strictly aerobic and motile with one to eight peritrichous (nonpolar) flagella, usually described as degenerated (see Fig. 229.1 and Table 229.6). They are oxidase-positive and catalase-positive. *Alcaligenes faecalis, Alcaligenes piechaudii* and *A. xylosoxidans* subsp. *denitrificans* are not saccharolytic. The only saccharolytic species is *A. xylosoxidans* subsp. *xylosoxidans*. Not all *Alcaligenes* spp. possess specific physiologic or biochemical characteristics (see Table 229.17) and those most commonly involved in nosocomial infections are *A. faecalis* and *A. xylosoxidans*.

Pathogenic role

Alcaligenes spp. have been isolated from blood, feces, sputum, urine, cerebrospinal fluid, wounds, burns and swabs taken from throat, eyes and ear discharges. *Alcaligenes* spp. strains do not seem to possess any specific virulence determinants. They are infrequent causes of hospital-acquired infection in patients who have severe underlying disease. Rare cases of peritonitis, pneumonia, bacteremia, meningitis and urinary tract infections are found in the literature. In many instances the organism is considered to be a colonizer. Nosocomial outbreaks of infection are usually associated with an aqueous source of contamination.[30] Recent findings have underlined the fact that *Alcaligenes* spp. are predominantly isolated from respiratory tract specimens and that recovery of these organisms from the sputum of CF patients is associated with an exacerbation of pulmonary symptoms.[31]

Management

Alcaligenes spp. are resistant to aminoglycosides, chloramphenicol and tetracyclines; they are variably susceptible to trimethoprim-sulfamethaxazole and newer ß-lactams. *Alcaligenes xylosoxidans* has been shown to be susceptible to ureidopenicillins, latamoxef, imipenem and some fluoroquinolones (ciprofloxacin, ofloxacin). There have been several reports of multiple ß-lactam resistance to broad-spectrum penicillins in *A. xylosoxidans* due to constitutive ß-lactamase production; three different types of cephalosporinase and the presence of other ß-lactamases have been demonstrated. [32] [77] Treatment of *Alcaligenes* infection requires combination therapy including expanded-spectrum ß-lactams (piperacillin, imipenem) and recent fluoroquinolones (ciprofloxacin, sparfloxacin) or trimethoprim-sulfamethaxazole.

BERGEYELLA spp.

See *Weeksella* spp.

CALYMMATOBACTERIUM sp.

One known species, *Calymmatobacterium granulomatis*, an aerobic Gram-negative bacillus, remains of uncertain taxonomy. The only reservoir of the organism is humans and its main epidemiologic characteristic is its presence intracellularly in macrophages of the lesions in donovanosis (granuloma inguinale). This sexually transmitted disease is endemic in Asian countries, India, South America, Australia and Africa. *Calymmatobacterium* has not been cultured yet (only 15 successful isolations) since it does not grow on conventional media; knowledge on the microbiology of this organism is limited. The diagnosis is based on bacterial inclusions found in biopsies (Giemsa or Wright staining). Extensive granuloma inguinale is seen in pregnant women and in HIV patients. Antibiotic treatment requires lipid-soluble drugs that can penetrate intracellularly and the first-line therapy is a combination of trimethoprim-sulfamethoxazole, tetracycline or thiamphenicol. Additional options include lincomycin, chloramphenicol or gentamicin. [72] [78]

CAPNOCYTOPHAGA spp.

These Gram-negative, strictly aerobic organisms are oxydase- and catalase-negative (group DF-1) or positive (group DF-2). Group DF-1 includes three species — *Capnocytophaga ochracea, Capnocytophaga gingivalis* and *Capnocytophaga sputigena* (see Table 229.18) — found as commensals of the human oral cavity. They can be responsible for cervicofacial infections and, in patients who have valvular lesions or prosthetic valves, endocarditis and bacteremia. *Capnocytophaga canimorsus*, a commensal of the oral cavity of dogs, has been cited as responsible for human infection after dog bites (septicemia, endocarditis).

These bacteria grow easily on blood agar and identification is based on conventional tests. Treatment of infection uses penicillins, cephalosporins, clindamycin and fluoroquinolones but *Capnocytophaga* spp. are resistant to aminoglycosides.[72]

Figure 229-9 Colonies of *Flavobacterium-Chryseobacterium* group grown on Mueller-Hinton agar. *Courtesy of Professor H Monteil.*

CHROMOBACTERIUM spp.

These bacteria of uncertain taxonomic position and designation have a limited pathogenic role. They are motile by one to four subpolar flagella. Grown on agar plates, colonies are violet and the bacterium has some fermentative activities on sugars and is proteolytic. Found in soil and water in tropical countries, *Chromobacterium violaceum* is occasionally pathogenic in humans, causing occasional pyogenic or septicemic infection.[2] [15]

CHRYSEOBACTERIUM spp.

Chryseobacterium spp. (formerly *Flavobacterium* spp.; see Table 229.1) are ubiquitous organisms that can be found in the hospital environment. Epidemiologic studies have traced the bacterial source to contaminated water, ice machines and humidifiers. Phenotypic markers used for the delineation of outbreaks of *Chryseobacterium meningosepticum* infections were serology based on the O antigenic type; nine O serovars have been identified (A–H and K).

Microbiology

These organisms grow at between 41°F (5°C) and 86°F (30°C) and strains isolated from human specimens can grow at 98.6°F (37°C). On nutrient agar they produce colonies of 1–2mm in diameter, which are frequently pigmented light yellow or yellowish orange (nondiffusible pigment; Fig. 229.9). The metabolism is strictly aerobic and sugars are metabolized by the oxidative pathway — except for *Chryseobacterium odoratum (Myroides odoratus)* and *Sphingobacterium multivorum*, which do not acidify glucose. Indole-positive species (i.e. *Empedobacter breve, C. meningosepticum, Chryseobacterium gleum*) are usually strongly proteolytic; esculin, citrate and urease tests are variably positive (see Table 229.17). [15] [28] [29] [72] [73]

Clinical manifestations

Infections due to Chryseobacterium meningosepticum *and* Chryseobacterium indologenes

These have been isolated in sepsis, meningitis and endocarditis. Meningitis due to *C. meningosepticum* (still designated under its previous name, *Flavobacterium*) has been often observed in neonates but infrequently in immunocompromised patients.[28] [29] [19] *Chryseobacterium meningosepticum* has been isolated from adults suffering from pneumonia, postoperative bacteremia and meningitis, usually in patients who had severe underlying pathologies.[29] [19] Rare cases of community-acquired *C. meningosepticum* pneumonia have been cited in the literature.[79]

Infections with *Flavimonas oryzihabitans* (an emerging Gram-negative aerobic bacillus previously designated *Pseudomonas oryzihabitans* and closely related to *Chryseobacterium* spp.; see Table 229.1) have been reported.[80] *Chryseobacterium indologenes* has been cited in association with infections due to indwelling devices.[29] *Chryseomonas luteola* (previously designated *Pseudomonas luteola*), *Comamonas testosteroni* and *Comamonas acidovorans* have been implicated in bacteremia, catheter infections, prosthetic valve endocarditis and peritonitis in patients who are being treated with continuous ambulatory peritoneal dialysis.[72]

Management

These bacteria are generally resistant to aminoglycosides (MIC >16mg/l), third-generation cephalosporins, antipseudomonal penicillins (mezlocillin, piperacillin, ticarcillin), aztreonam, imipenem, erythromycin and tetracycline. The most active antibiotics are rifampin and clindamycin (MICs 1–4mg/l). Ciprofloxacin has proven effective for treating pneumonia in pediatric patients. Cases of neonatal sepsis have been treated with clindamycin combined with piperacillin.[73] [81] Susceptibility to ß-lactams can be recovered by combining ß-lactamase inhibitors with ß-lactam antibiotics.

COMAMONAS spp.

These aerobic Gram-negative oxydase-positive bacilli are seldom implicated in human infections. Rare cases of catheter-induced bacteremia (*C. testosteroni, Delftia acidovorans*), conjunctivitis (*C. testosteroni*) and otitis media (*D. acidovorans*) have been cited in the literature. These straight or slightly curved rods are motile by means of tuft of polar flagella. They grow easily on standard media and are susceptible to piperacillin, cefotaxime, imipenem and ciprofloxacin.[72]

OCHROBACTRUM spp.

Derived from the genus *Achromobacter*, two species have been recognized as having a medical role, *Ochrobactrum anthropi* and *Ochrobactrum intermedium*. These environmental organisms are considered as opportunistic pathogens. A few recent publications have pointed out their role in nosocomial infections, induced by contaminated catheters.[72] [82] Endocarditis, postoperative cases of endophthalmitis and necrotizing fasciitis have been cited as well. These nonfastidious bacteria are easily grown on conventional media and identified by using classical biochemical tests. The major problem regarding these organisms is their resistance to most ß-lactams, since they produce ß-lactamases of AmpC class 1. They are susceptible to imipenem, aminoglycosides and fluoroquinolones but strains of *O. intermedium* have been cited as resistant to tobramycin and colistin.

OLIGELLA sp.

This genus was created in 1987 and *Oligella urethralis* was derived from *Moraxella urethralis*. These small rods, often occurring in pairs, develop slowly on blood agar and exhibit a limited metabolic activity. They are oxidase- and catalase-positive. Their potential pathogenic role is limited to the genitourinary tract.[72]

PLESIOMONAS sp.

Only one species, *Plesiomonas shigelloides*, until recently designated *Proteus shigelloides*, is present in the environment (water, soil) in tropical and subtropical areas. Its pathogenic role in humans is controversial but rare cases of gastroenteritis, septicemia and neonatal meningitis have been cited in Japan and the USA. Raw oysters and contact with contaminated water or with aquatic animals have been implicated. *Plesiomonas* sp. is not a strictly aerobic organism and may grow on selective media used for isolation of Enterobacteriaceae. Its production of oxidase and other biochemical characteristics

permit its identification. Antibiotic testing of *P. shigelloides* shows a general resistance to penicillins and aminoglycosides but this organism is susceptible to cephalosporins, imipenem and fluoroquinolones, the latter antibiotics being very active in treating gastrointestinal infections.[83]

RALSTONIA spp.

Ralstonia picketii was derived from *Burkholderia picketii* or *Pseudomonas picketii,* and *Ralstonia eutropha* from *Alcaligenes eutrophus* (see Table 229.18). Both are emerging pathogens isolated from infections in immunodepressed patients or from respiratory tract infections in CF patients.[72] [73]

SHEWANELLA spp.

Derived from *Alteromonas* spp. or *Pseudomonas* spp., *Shewanella putrefaciens* belongs to the Vibrionaceae class but grows in media used for Enterobacteriaceae and produces SH_2 , which may result in confusions with *Salmonella* spp. or *Proteus* spp. This bacterium is present in the environment and has been isolated from otitis media, intra-abdominal infections and bacteremia, most cases occurring in immunodepressed patients. Treatment of these infections is based on third-generation cephalosporins, imipenem, ciprofloxacin, aminoglycosides, trimethoprim-sulfamethoxazole and tetracyclines. [72]

SPHINGOBACTERIUM spp.

Two species (see Table 229.18) of *Sphingobacterium* are derived from *Flavobacterium* spp. (see Table 229.1). They are characterized by colonies that develop a yellow pigment after a few days at room temperature. Their presence in the hospital environment and in most aquatic sources is frequent but their clinical significance is limited to rare cases of opportunist infection.

WEEKSELLA spp.

Designated currently *Bergeyella* spp. and deriving from *Flavobacterium* spp., these organisms include *Weeksella virosa* (see Table 229.1) and *Weeksella zoohelcum*. Both grow as pigmented colonies (brown or yellow). Their pathogenic role is doubtful and is limited to local infection after animal bites or genitourinary tract infection.[73]

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Chapter 230 - Curved and Spiral Bacilli

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INTRODUCTION

The curved and spiral bacilli are a heterogeneous group of bacteria that share little morphology.

Helicobacter pylori was previously called *Campylobacter pylori*[1] and in the first part of the 20th century *Campylobacter* was called *Vibrio. Campylobacter, Helicobacter* and *Vibrio* spp. all produce infections of the gastrointestinal tract. Moreover, they are all Gram-negative organisms and have a similar, curved shape. Using nucleic acid sequence determination of 16S rRNA, the genera *Campylobacter* and *Helicobacter* have been classified (together with *Arcobacter, Sulfurospirillum* and *Wolinella*) as members of the superfamily VI of Gram-negative bacilli (Table 230.1).[2] Only those bacteria belonging to this superfamily that are involved in human infections are discussed in this chapter.

Treponema, Borrelia and *Leptospira* are all members of Spirochaetales. These spirochetes are thin, helical, Gram-negative bacteria.

CAMPYLOBACTER SPP., *HELICOBACTER PYLORI* AND *VIBRIO CHOLERAE*

CAMPYLOBACTER SPP.

Nature

Campylobacter spp. are micro-aerophilic, Gram-negative, curved rods which obtain their energy by using fatty acids and amino acids rather than carbohydrates, and are adapted to life in mucus of the digestive tract. With the genera *Arcobacter* and *Sulfurospirillum*, they form the family *Campylobacteraceae*. At least 15 species and six subspecies have been differentiated. Not all, however, cause disease in humans.[3] *Campylobacter jejuni* and *Campylobacter coli* are responsible for enteric infections and are the most common Campylobacters found in humans. *Campylobacter fetus* is the third most frequently isolated, but is mostly involved in systemic diseases. The other species (*Campylobacter lari, Campylobacter upsaliensis*) occur only anecdotally. They also lead to enteric infections.

Epidemiology

Campylobacter spp. infections can be considered as zoonoses, because the primary reservoir for *Campylobacter* spp. is animals. These bacteria are essentially present in the digestive tract of animals, especially birds, where they do not cause disease. They can cause septic abortion in cattle. Humans can become infected by ingesting contaminated food or water or through contact with infected animals, including pets. The majority of *Campylobacter* spp. infections are sporadic, although outbreaks do occur. *Campylobacter* enteritis is generally more common than *Salmonella* and *Shigella* enteritis and is a major cause of travelers' diarrhea. Infections can be caused by the ingestion of undercooked, contaminated poultry or contaminated milk as well as by cross-contamination of foods which will be consumed raw. It has been estimated that there are 2.4 million cases annually in the USA.[4] Transmission of the disease from human to human has also been described but seldom occurs. In temperate countries there is a peak incidence in summer and early autumn, although infections occur throughout the year.[5] The highest incidence is found in infants and young children, with a second peak in young adulthood. The incidence in developing countries, with less hygienic living conditions, is even higher than in developed countries and direct transmission from poultry to humans seems to occur.

Pathogenesis

Campylobacter bacilli are acid sensitive. Because of the relative barrier imposed by the gastric acid environment, infection is more likely to occur when large numbers of bacteria are ingested. Histologic examination of gut biopsies obtained from patients who have *Campylobacter* enteritis reveals inflammation and edema of the mucosa, with infiltration of neutrophils in the lamina propria. Lesions are mainly restricted to the ileum and colon.

In vitro co-culture of epithelial cells with *C. jejuni* has shown that these bacteria can adhere and penetrate into the cells. Pili, flagellin (major antigen), outer membrane proteins and lipopolysaccharides (LPS) could play the role of adhesins. *C. jejuni* is also able to synthesize proteins which may play a role in internalization and cytoskeletal rearrangement. *C. jejuni* can survive in vacuoles and induce IL-8 synthesis. Its translocation may occur by transcellular as well as paracellular means.[6] In addition, *C. jejuni* produces a cytolethal distending toxin (cdt) acting on the cell cycle and leading to apoptosis. Nevertheless, the numerous pathogenicity studies performed have not determined a specific mechanism. Recent knowledge of the whole genomic sequence of *C. jejuni* [7] should bring insight to this field. The molecular mimicry of human ganglioside with the LPS molecules present in strains of *C. jejuni* expressing the O:19 antigen has been implicated in the association of *C. jejuni* infection with the Guillain-Barré syndrome.[8]

Immune persons in endemic areas, where infections are frequent, can become asymptomatic carriers. Infection can have a protracted course in the case of reduced resistance, such as in patients suffering from hypogammaglobulinemia. In HIV-infected patients, opportunistic infections with atypical *Campylobacter* spp. also suggest a role for cellular immunity.

An interesting mechanism to avoid elimination by the immune system has been discovered for *C. fetus*. Almost all *C. fetus* strains express a surface protein that abrogates complement C3b binding. This prevents opsonization, thereby conferring resistance to killing by phagocytes and adding to the pathogenicity of the species.[9]

Prevention

Preventive measures for *Campylobacter* spp. infections include adequate disinfection of drinking water supplies, adequate heating of contaminated food and reinforcement of hygiene in the kitchen in order to avoid cross-contamination. Eradication of the animal reservoir is impossible but adequate measures taken in poultry farms and abattoirs can decrease the level of contamination. Development of vaccines is an alternative. Research in this direction has been boosted

TABLE 230-1 -- Clusters of the rRNA VI superfamily of Gram-negative bacilli.*

CLUSTERS OF THE rRNA VI SUPERFAMILY OF GRAM-NEGATIVE BACILLI		
rRNA group I	**rRNA group II**	**rRNA group III**
Campylobacter jejuni	*Arcobacter butzleri*	*Helicobacter pylori*
Campylobacter coli	*Arcobacter cryaerophilus*	*Helicobacter acinonyx*
Campylobacter hyointestinalis	*Arcobacter nitrofigilis*	*Helicobacter bilis*
Campylobacter fetus	*Arcobacter skirrowii*	*Helicobacter bizzozeronii*
Campylobacter upsaliensis		*Helicobacter canis*
Campylobacter concisus		*Helicobacter cinaedi*
Campylobacter curvus		*Helicobacter felis*
Campylobacter gracilis		*Helicobacter fennelliae*
Campylobacter helveticus		*Helicobacter hepaticus*
Campylobacter hyoilei		*Helicobacter muridarum*
Campylobacter lari		*Helicobacter mustelae*
Campylobacter mucosalis		*Helicobacter nemestrinae*
Campylobacter rectus		*Helicobacter pametensis*
Campylobacter showae		*Helicobacter pullorum*
Campylobacter sputorum		CLO-3
Bacteroides ureolyticus		*Gastrospillum hominis*
		Wolinella succinogenes
B. ureolyticus is placed in this group based on 16S rRNA. CLO, *Campylobacter*-like organism.		

** Adapted from Koneman et al.*[105]

by the discovery of an association between *Campylobacter* enteritis and the Guillain-Barré syndrome, because the latter can cause permanent disability in a significant number of cases. Vaccines using killed whole-cell preparations have been successful in animal models and experiments with humans are under way. [10]

Diagnostic microbiology

The curved motile rods can be demonstrated in a fecal sample using Gram staining or dark-field microscopy. Culturing *Campylobacter* spp. necessitates special conditions, sometimes difficult to implement.[11] Cultures are commonly performed with an atmosphere comprising 5% oxygen, 10% carbon dioxide and 85% nitrogen. Some species, such as *Campylobacter rectus* and *Campylobacter hyointestinalis*, also require hydrogen in the atmosphere for growth. Selective culture media contain antibiotics such as cefoperazone to suppress the growth of normal intestinal bacteria and blood or charcoal to neutralize inhibiting factors such as oxygen radicals. Commonly used media are Skirrow's, Butzler's, Campy (cefoperazone-vancomycin-amphotericin) agar and cefoperazone charcoal deoxycholate agar. An important disadvantage of selective media is that bacteria such as *C. hyointestinalis, C. fetus* and *C. upsaliensis*, which are sensitive to antibiotics used in these selective media, can be missed. To circumvent this problem, membrane filtration of feces can be performed to eliminate contaminants followed by culturing on nonselective media. However, this filtration technique is less sensitive than direct plating.

Although the most important species (i.e. *C. jejuni* and *C. coli*) grow at 107.6°F, some species (i.e. *C. fetus*) grow best at 98.6°F and will be missed when cultures are only incubated at 107.6°F. Typically, colonies with a gray color and growing flat and confluently are visible after 2–3 days of culture. Definite identification of suspect colonies is performed using standard biochemical tests showing positive oxidase and catalase tests for *C. jejuni* and *C. coli*.

Campylobacter jejuni is the only *Campylobacter* spp. that is capable of hydrolyzing hippurate, which is essential for its differentiation from other Campylobacters, especially *C. coli*. Growth at 77°F is essential for diagnosing *C. fetus*. Given the high level of resistance of Campylobacters to quinolones, the nalidixic acid susceptibility test is no longer a key test in *Campylobacter* identification. Molecular identification (PCR, sequencing) is now being performed more frequently in this group of bacteria.

The presence of *Campylobacter* spp. can also be determined using PCR directly on feces. Typing of isolates is important for epidemiologic studies. More than 60 serotypes of *C. jejuni* and *C. coli* have been identified with the Penner O typing system but molecular typing methods are now commonly used, including PCR-RFLP of *fla* genes, macrorestriction of the genome and AFLP.[12] Serology can also be helpful in diagnosing a *Campylobacter* spp. infection because serum IgG and IgM levels start to rise in response to infection 5 days after infection and reach a peak 2–4 weeks later. This is the essential method to diagnose the Guillain-Barré syndrome due to *C. jejuni*. Immunoglobulin A is also produced and excreted in the gut lumen.

Clinical features

Most *Campylobacter* spp. infections manifest as acute enteritis.[13] The ensuing diarrhea can vary from modest to voluminous stools that may be watery or bloody. The infection can also run a subclinical course, especially in hyperexposed populations. Disease will develop 1–3 days after ingestion of the bacilli and symptoms usually disappear after 1 week. Stool samples typically remain positive for *Campylobacter* spp. for several weeks. In most cases, *Campylobacter* enteritis is a self-limiting disease and it tends to be more severe in patients at the extreme ends of age. Fever, malaise and abdominal pain may precede diarrhea or may be the most predominant signs. Infection with *Campylobacter* spp. gives rise to inflammation of the gut mucosa. The accompanying pain and fever may also lead to disease resembling Crohn's colitis or ulcerative colitis. When pain is the major feature of the infection, differentiation from appendicitis may be difficult. When fever is the major feature, differentiation from *Salmonella* enteritis may be difficult. *Campylobacter jejuni* can grow in bile and can occasionally cause acute cholecystitis and pancreatitis. [14]

Only a few patients who have a *C. jejuni* infection develop systemic disease. Bacteremia can occur, but this is considered rare and generally occurs in patients who have an underlying disease.[15]

As with other pathogenic bacteria, a postinfectious syndrome may occur after *C. jejuni* infection. One is an acute reactive arthritis which is very similar to the complication seen after enteritis caused by *Salmonella* spp., *Shigella* spp. or *Yersinia* spp. and is associated with the presence of the HLA-B27 antigen.

Another important complication of *Campylobacter* enteritis is the Guillain-Barré syndrome. This syndrome is an acute demyelinating disease affecting the peripheral neurons and is characterized by an ascending paralysis. [8] *Campylobacter jejuni* enteritis is the infection most frequently observed before Guillain-Barré syndrome and occurs in 20–40% of cases. The risk of developing Guillain-Barré syndrome after *Campylobacter* spp. infection is estimated at 1 per 2000 infections. Major neurological sequelae exist in 20% of the cases.

Campylobacter coli infections are very similar to infections with *C. jejuni*, but they tend to follow a less severe course.

Infections with *C. fetus* tend to disseminate from the intestine, especially in patients who have conditions that cause impaired immunity, such as chronic alcoholism, diabetes mellitus, malignancies and HIV infection, and in the elderly. Systemic *C. fetus* infections can lead to endocarditis, thrombophlebitis, meningitis and septic abortion.

Campylobacter upsaliensis, C. lari and *C. hyointestinalis* can also cause enteritis. *Campylobacter consisus, C. gracilis, C. curvus, C. mucosalis, C. rectus, C. showae, C. sputorum* and *Bacteroides ureolyticus* can be associated with periodontal infections.

Management

Disease management is primarily symptomatic. Depending on the severity of the diarrhea, fluid replacement can be performed with oral rehydration fluids or with saline infusions. Antibiotic intervention is especially effective early in the disease and is of benefit in cases of prolonged illness, recurrent disease and secondary sepsis, for example in patients who have reduced resistance. The first-choice antibiotic is erythromycin (500mg q6h orally for 2 weeks) or another macrolide, resistance being low for this class of antibiotics. Ciprofloxacin (500mg q12h orally for 2 weeks) can also be used, but a high incidence of resistance to quinolones exists (see Chapter 43). Other alternatives include tetracycline and amoxicillin-clavulanic acid. Gentamicin in association with amoxicillin-clavulanic acid is the antibiotic of choice for systemic diseases.

HELICOBACTER PYLORI

Helicobacter pylori was cultured for the first time in 1982, from the stomach which was previously thought to be sterile. [16] Currently this bacterium is considered to be the most important bacterium responsible for chronic infections. It is also the first bacterium known to be involved in a cancer in humans.

Nature

Helicobacter spp. are spiral-shaped, Gram-negative bacilli with between five and seven terminal flagella. They are micro-aerophilic and use amino acids and fatty acids rather than carbohydrates to obtain their energy. At present, about 17 species of *Helicobacter* have been identified, only eight of which cause disease in humans (Fig. 230.1). *Helicobacter pylori* is the third bacterium for which the entire genome has been sequenced[17] and the first for which the genome of two strains has been sequenced.[18]

Epidemiology

The prevalence of an *H. pylori* infection is strongly linked to the socio-economic level of the community.[19] The infection rate decreases as the socio-economic level increases. Infection usually occurs in childhood and the bacilli persist in the stomach for decades and possibly for life. The socio-economic level of the family into which a child is born and raised is a more important risk factor than his or her socio-economic status in adult life. The corresponding risk factors are poor sanitation, poor education and sharing a bed.[20] Given the substantial improvement in socio-economic conditions in

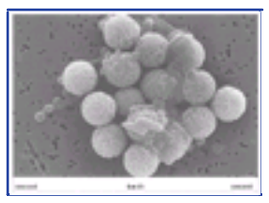

Figure 230-1 Cultured *H. pylori* in coccoid and bacilli forms, bound to immunomagnetic beads. *Adapted from Murray et al.* [106]

developed countries during recent decades, there has been a gradual decrease in the acquisition of the infection. Because the infection is lifelong, a cohort effect is present: the oldest people in a population are infected more often than the youngest. [21]

The current prevalence of *H. pylori* infections in the 20–29 year age group in Europe shows a gradient decreasing from east to west and from south to north. The prevalence in young adults in Western countries does not exceed 20%. The incidence of *H. pylori* infection is low in adulthood (less than 0.5%), but higher in developing countries than in the West. Although the source of the infection is known (i.e. the stomach of humans), the mode of transmission remains uncertain: it may be fecal-oral or oral-oral. Vomiting may play an important role.

Pathogenesis

Helicobacter pylori is adapted to the acid milieu of the stomach: it produces urease, which breaks down the urea diffusing from the mucosa and buffers the pH around the bacterium. *Helicobacter pylori* moves into the mucus and produces different kinds of adhesins that allow it to adhere very specifically to mucus cells. *Helicobacter pylori* appears in the duodenum when metaplasia of these mucus cells is present and disappears from the stomach when intestinal metaplasia is present.

Helicobacter pylori can persist by escaping host-defense mechanisms. For example, it synthesizes catalase and superoxide dismutase enzymes, which destroy bactericidal products from inflammatory cells. Moreover, urease increases the pH of the phagolysosomal compartment, thereby disturbing phagocyte function. It has also been proposed that the large amount of released antigens could saturate local antibodies. In addition, *H. pylori* triggers a response from T helper (TH)l lymphocytes with IgG production and inflammation, whereas a TH2 lymphocyte response would be more appropriate.[22]

Helicobacter pylori strains do not all share the same virulence factors; some produce the vacuolating cytotoxin A (VacA) and some possess a pathogenicity island. VacA may be responsible for the epithelial cell damage observed in *H. pylori* infection. The main action of VacA is on the mitochondria, vacuole formation being the consequence of the late endocytosis compartment when weak bases are present, in this case, NH_4 Cl. [23] The sequence of the *vacA* gene is not homogeneous. There are two main regions of variation: the signal sequence (s) and the midregion (m). Typing has been proposed: s1-ml, s1-m2 and s2-ml, corresponding to high, low and no production of toxin, respectively. The pathogenicity island is named *cag* and has the *cagA* gene as a marker. It is a 40kB fragment containing 27–31 open reading frames. Six of them have sequence similarities to genes coding for a type IV secretion system, i.e. a complex protein structure that allows the bacterium to inject compounds into eukaryotic cells. The CagA protein is one of them which is phosphorylated and leads to a reorganization of the actin cytoskeleton. The other effect associated with the *cag* pathogenicity island is an increased production of IL-8 via an NF?B pathway.[24] Nevertheless, *cag*-negative *H. pylori* also induce inflammation, which stresses the fact that other factors present in all *H. pylori* strains may also be involved. Lipopolysaccharide moieties of the outer membrane of *H. pylori* may be identical to Lewis x and y antigens. Such antigen mimicry could confer autoimmunity and play a role in the pathogenesis of, for example, gastric atrophy.[25]

Prevention

Because the route of transmission is unclear, it is difficult to take preventive measures. The first attempts at developing a vaccine using recombinant urease as the only antigen have not been successful in humans. Other vaccine candidates antigens are under study. Mucosal administration seems to be the most popular route.

Diagnostic microbiology

Invasive methods for diagnosing *H. pylori* infection depend on endoscopy to obtain biopsies. These biopsies can then be processed for histological examination and stained with hematoxylin-eosin, Giemsa stain or silver stain. *Helicobacter pylori* is usually abundant and its typical morphology and the presence of polymorphs make diagnosis easy. The use of immunoperoxidase staining can be considered when atypical bacilli are detected. The examination of a smear is quick and can be performed in the endoscopic ward using either dark-field examination of a wet smear or Gram staining.

Because *H. pylori* is a fragile organism, transport conditions are extremely important for culture. Biopsies can be transported in saline for 3–4 hours, but (commercially) available transport medium must be used if preservation up to 24 hours is necessary. The media must be maintained at a low temperature (<50°F) before plating. Biopsies must be ground with a homogenizer and plated on media enriched with 5–10% blood and antibiotics to inhibit growth of nonrelevant bacteria. The plates must then be incubated for up to 10 days in a micro-aerobic atmosphere. Growth usually occurs within 3–4 days. *Helicobacter pylori* colonies are easily identified by their morphology and their urease, catalase and oxidase activities.

The urease test is specific for *H. pylori*. A color change is observed if *H. pylori* urease is present when the biopsy is introduced into a medium containing urea and a pH indicator. Tests using semisolid agars show an optimal sensitivity after 24 hours. In contrast, strip tests show a high sensitivity after just 2 hours.

Polymerase chain reaction for diagnosing *H. pylori* infection does not require specific transport conditions and can be performed with a urease test kit sent by mail. Several genes can be targeted: urease, 16S rRNA, 29kDa antigen, *cagA* gene or *vacA* gene. Recently a real-time PCR has been developed which allows detection of both *H. pylori* and its resistance to macrolides. Primers target the 23S rRNA gene and the dissociation curve of the hybrids indicates the mutation present.

Less invasive methods for obtaining material are aspiration of gastric juice or a capsulated string. Diagnosis can also be made using noninvasive tests. The urea breath test measures urease production by *H. pylori*. Samples of breath air are collected by having the patient blow into a tube before and 30 minutes after ingestion of ^{13}C-labeled urea. The tubes can be maintained for months and sent by mail to a laboratory that has a mass spectrometer in order to measure the ^{13}C:^{12}C ratio.

Antibodies are mainly detected by enzyme-linked immunosorbent assay (ELISA). There are numerous kits commercially available. Immunoblot methods can also be used.

Detection of specific antibodies has also been proposed but shows a lower sensitivity than in blood. It is also possible to detect *H. pylori* antigens in stools using ELISA. Polyclonal antibodies were first used as a reagent. A second generation of tests employs monoclonal antibodies and gives excellent results.

Since none of the tests are perfect in terms of sensitivity, a combination of tests is recommended.[26] All these tests have a comparable sensitivity except for the smear examination, the agar-based urease and rapid serology tests which are inferior. The urea breath test is ideal for follow-up after eradication therapy. Serology cannot be used for this purpose because the antibody titer may be high for months after the disappearance of *H. pylori*.

Clinical features

The presence of *H. pylori* in the stomach is always accompanied by inflammation of the mucosa. However, this infection is not always symptomatic. Duodenal ulcer, gastric ulcer, gastric carcinoma, gastric lymphoma and some nonulcer dyspepsia syndromes can develop. Symptoms of these diseases (e.g. epigastric pain and dyspepsia) are not specific.

Duodenal ulcer

Duodenal ulcer occurs in subjects who are infected by *H. pylori* and also have gastric metaplasia in the duodenal bulb, which will be colonized with *H. pylori*.[27] Hyperproduction of acid following a decrease in somatostatin, and an increase in gastrin production are observed. Antral gastritis is the usual pattern of histological lesions. Smoking and infection with *cag*-positive *H. pylori* strains are important risk factors.

Gastric ulcer

In contrast, gastric ulcer occurs in patients who have multifocal gastritis or pangastritis leading to a decreased acid production by the corpus. The lesions have been

explained by a retrodiffusion of hydrogen ions in the gastric wall. A vascular factor is also probably important. Gastric ulcer in Western countries is about five times less frequent than duodenal ulcer and occurs in older people. Smoking, dietary factors (e.g. high salt intake) and infection with *cag*-positive *H. pylori* strains are important risk factors. In some instances, gastric ulcer may be a precursor of gastric carcinoma, motivating endoscopic follow-up.

Gastric carcinoma

The incidence of gastric carcinoma is currently decreasing in developed countries. This decrease has been attributed to a decrease in the rate of *H. pylori* infection. Indeed, gastric carcinoma is virtually absent when gastric mucosa is normal.[28] Most gastric carcinomas are of the intestinal type and are thought to result from chronic gastritis followed by atrophy, intestinal metaplasia and dysplasia ultimately leading to carcinoma. These events occur over several decades. They can be reproduced in an animal model, the Mongolian gerbil infected with *H. pylori*. [29] A very early acquisition of the bacterium, infection with *cag*-positive strains and dietary factors (e.g. high salt consumption and low vitamin intake) are risk factors for this evolution. The diffuse type of gastric cancer does not follow the pattern described above, but it is associated with *H. pylori* infection.

Gastric lymphoma

Gastric lymphoma involves mucosa-associated lymphoid tissue (MALT). The stomach is normally free of lymphoid follicles; they only occur when *H. pylori* is present.[30] T cells stimulated by *H. pylori* antigens trigger a monoclonal B-cell proliferation, giving rise to lymphoid follicles. *H. pylori* infection is responsible for 80% of gastric MALT lymphoma.

Nonulcer dyspepsia

Nonulcer dyspepsia occurs in dyspeptic patients in whom no organic lesion is found at endoscopy. *Helicobacter pylori* is found in about 50% of the cases in Western countries. In most of these cases, however, the presence of *H. pylori* is probably incidental, with only about 10% of cases being the consequence of *H. pylori* infection.[31]

Other diseases

Helicobacter pylori infection has been implicated in children's diseases including iron deficiency anemia, growth retardation and recurrent abdominal pain. It may also be a risk factor for myocardial infarction, possibly because of the long-lasting chronic inflammation induced.[32]

Management

Eradication of *H. pylori* in duodenal ulcer and gastric ulcer avoids relapses, increases the healing process and normalizes the mucosa and gastric physiology. In low-grade gastric MALT lymphoma, eradication

---

leads to a disappearance of the lesions. Nevertheless, follow-up is needed for several years. Eradication of *H. pylori* at an early stage can have a positive effect in gastric carcinoma. Nevertheless, surgery and other cancer treatments remain essential.

The optimal regimen to eradicate *H. pylori* consists of two orally administered antibiotics for 7–10 days with an antisecretory drug. The combinations favored are:

- clarithromycin (500mg q12h) and amoxicillin (1g q12h), or
- clarithromycin (250mg q12h) and metronidazole (500mg q12h).

Most studies have been performed with omeprazole. However, lansoprazole and pantoprazole and rabeprazole are also effective. A double dose of proton pump inhibitors is also usually given.[33] Currently, resistance to amoxicillin is seldom found, whereas resistance to macrolides occurs in up to 20% of the strains and resistance to metronidazole in 10–80%. Follow-up is performed 4–6 weeks after treatment has been stopped.

In case of failure, the recommended second-line treatment is a quadruple therapy with metronidazole, tetracycline, bismuth salts and a proton pump inhibitor for a week, or the use of the new compound ranitidine-bismuth citrate, plus two antibiotics.[33] The use of dual therapy (e.g. a proton pump inhibitor with amoxicillin or clarithromycin) is no longer recommended. The main risk factors for treatment failure are the resistance of *H. pylori* and lack of patient compliance (see Chapter 42 for further discussion on gastritis).

VIBRIO CHOLERAE

Nature

Vibrio cholerae is a Gram-negative, comma-shaped rod belonging to the family *Vibrionaceae*. Its natural habitat consists of fresh-water and salt-water environments. Based on differences in the composition of the major cell wall antigen (O), 139 serotypes have been differentiated. *Vibrio cholerae*, which belongs to either serogroup O1 or serogroup O139, has been associated with epidemic cholera. *Vibrio cholerae* serogroup O1 can be subdivided into El Tor and classic biotypes as well as the Ogawa, Inaba and Hikojima serotypes. Other serogroups of *V. cholerae*, in addition to nontoxigenic *V. cholerae* O1 or O139, do not cause epidemic cholera; they may, however, cause individual cases of diarrhea (see Chapter 43).

Epidemiology

Cholera has raged in seven pandemics since 1817. It is possible that an eighth is superimposed on the seventh. The fifth and sixth pandemics have been explored and were caused by the classic biotype and originated in the Indian subcontinent. The seventh and current one is caused by the El Tor strain and began in 1961 in Indonesia. It has gradually affected most of Asia and Africa and is found incidentally in parts of Europe. In 1991, this pandemic reached South America and has since spread throughout Latin America (Fig. 230.2).[34] Persons who have blood group O are at higher risk of El Tor cholera than those with blood group A, B or AB.[35] This is particularly important for Latin America, where 73% of the population carries the blood type O. An episode of classic cholera protects nearly entirely against recurrent cholera of either biotype, but an episode of El Tor cholera does not protect against future attacks. In 1992, a novel *V. cholerae* variant O139 (synonym Bengal), which has the same origin as the El Tor strain, emerged in southern Asia. This 1992 epidemic was the first one caused by a serogroup other than O1, and it occurred in populations assumed to be largely immune to *V. cholerae* O1. The impact of a *V. cholerae* O139 infection on the risk of a subsequent *V. cholerae* infection, either O1 or non-O1, has not yet been determined. The Bengal strain has the potential to spread pandemically. It has now affected areas throughout the Indian subcontinent, neighboring states and other

Figure 230-2 Spread of the *Vibrio cholerae* O1 El Tor pandemic in Central and South America, 1991–94. *(Adapted from Tauxe et al. [35])*

parts of Asia. Cases occurring as far away as the USA and Western Europe have also been described.

V. cholerae, including serogroups O1 and O139, exist as natural inhabitants of aquatic ecosystems and are therefore facultative human pathogens. Within the marine environment they attach to zooplankton and are able to form biofilm, facilitating environmental persistence. It was suggested but largely debated that *V. cholerae* can switch into a viable but nonculturable state. It is likely that the aquatic environment harbors different virulence-associated genes scattered among environmental vibrios, which possess a lower virulence potential than the epidemic strains. The clustering of such genes in a proper combination could lead to the emergence of new *V.*

cholerae strains with epidemic potential.[36]

Pathogenesis

Infection starts with ingestion of water or food contaminated with *V. cholerae*. The infectious dose is high due to the acid sensitivity of the bacteria. Persons who have impaired gastric acidity or who take acid-suppressing medication have an increased risk of infection. In addition, *Helicobacter pylori* gastritis is also associated with an increased risk of cholera.[37] The surviving bacteria adhere to and colonize the small intestine epithelial cells, producing the cholera toxin and causing acute watery diarrhea. In the intestine *V. cholerae* is faced with growth inhibitory substances like bile salts and defense factors like complement, defensins, against which it has developed survival strategies. The powerful enterotoxin then released is a 68kDa protein consisting of an active (A) subunit and five binding (B) subunits. These B subunits attach to the GM1 ganglioside receptor at the lining of the mucosal cells (Fig. 230.3). The A subunit triggers a cascade of reactions involving cyclic adenosine monophosphate, prostaglandins, 5-hydroxytryptamine and calmodulin.[38] This results in an increased level of intracellular cAMP leading to an increase in intestinal chloride secretion and a decrease in sodium chloride absorption. The outcome is a passive watery excretion that leads to diarrhea. The volume typically exceeds 1 liter per hour in adults and 10ml/kg/h in children. Since cholera results from a locally acting enterotoxin, it is not accompanied by systemic manifestations caused by a cytokine-induced acute-phase reaction.

Molecular knowledge of pathogenesis has recently improved. The most important colonization factor was identified as a type IV pilus named toxin-coregulated pilus (TCP). The genes required for TCP synthesis are located on a pathogenicity island (PI) named Vibrio PI.[39]

Figure 230-3 Mechanism of action of *Vibrio cholerae* toxin. The toxin binds to the GM1 ganglioside receptor on the intestinal mucosal cell membrane via the binding (B) subunits (a). The active portion of the A subunit enters the cell and activates adenyl cyclase (b), which results in the accumulation of cyclic adenosine monophosphate (cAMP), derived from adenosine triphosphate (ATP), along the cell membrane (c). The cAMP causes active secretion of sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-) and water (H_2O) out of the cell into the lumen of the intestine (d). *Adapted from Murray et al.*[106]

The genes encoding the cholera toxin are enclosed by a phage named CTX integrated into the large chromosome of the two *V. cholerae* chromosomes.[40] The surface antigens (capsule, LPS, outer membrane proteins) are important virulence factors.

A unique regulatory system co-ordinates the expression of TCP and cholera toxin. Interestingly, it was recently shown that quorum sensing systems control virulence genes in *V. cholerae*.[41]

The complete genome sequence of the two chromosomes of *V. cholerae* O1 El Tor, now available,[42] will help further dissection of cholera pathogenesis.

Prevention

Cholera is difficult to eradicate from water and is likely to remain a serious threat to public health for some time. Measures to prevent cholera include separating sewage and drinking water systems, disinfection of drinking water and food, hygiene measures (e.g. handwashing with soap), active case finding and effective case management with the use of oral rehydration. Breast-feeding provides important protection to infants, not because of transmission of maternal antibodies but because of the lower exposure to contaminated food and water. During epidemics, the bodies of persons who died of cholera should be disinfected and buried rapidly and the consumption of food at gatherings, including funerals, should be discouraged. The currently licensed, parenteral, killed cholera vaccine is no longer recommended by the World Health Organization because of its limited protective efficacy. To induce mucosal immunity, oral vaccines, both inactivated (whole cell/B-subunit) and live (CVD103-HgR), have been developed; these show no side effects and a longer lasting protection than the parenteral vaccine.[43] Vaccines that include the B-subunit of the toxin provide cross-protection against heat-labile enterotoxigenic *Escherichia coli*, a result of the close relationship between the two toxins. CVD103-HgR produces higher resistance to the homologous classic strain than to the El Tor strain. Attenuation of El Tor has led to several new candidate vaccines that have proven safe and highly effective against El Tor cholera.[44] Because immunity against the O1 type is not protective against O139, *V. cholerae* O139 type vaccines are urgently needed. To date, one live, attenuated candidate vaccine, Bengal-15, has shown a protective efficacy as high as 83%.

Diagnostic microbiology

The diagnosis of cholera is confirmed when *V. cholerae* is identified in a stool culture on thiosulfate citrate bile salts sucrose agar directly or after enrichment, taking advantage of *V. cholerae*'s tolerance to alkaline conditions. For rapid diagnosis, dark-field examination of a fresh, unstained stool specimen is highly sensitive and specific: a characteristic finding is the 'shooting star' phenomenon caused by the motility of the single polar flagellum of the organism. Once identified at the species level, additional tests include serotyping for O antigens and the 3 antigen variants recognized, as well as biotyping to differentiate classic and El Tor biotypes. A rapid, colorimetric immunodiagnostic method is available for the detection of *V. cholerae* O1. The immunochromatographic strip test may be the simplest, most rapid and most sensitive and specific test for the detection of *V. cholerae*. Two rapid immunodiagnostic test kits based on the use of monoclonal antibodies have been developed for the direct detection of *V. cholerae* O139: a coagglutination test and Bengal DFA (a direct fluorescent-antibody test). In epidemic settings in developing countries, a bacteriological diagnosis is not indicated in all suspected cases because the management of dehydrating diarrhea is guided by the extent of fluid loss rather than by the nature of the infecting organism. In contrast, the diagnosis should be confirmed in individual cases of suspected cholera in the developed world.

Clinical features

In Latin America, well-established case management has kept fatality rates low (at about 1%). In contrast, cholera in Africa has been sporadic and as a result less well recognized. The fatality rates there also tend to be higher (about 10%).[45] Children are more likely to have only subclinical infection or mild diarrhea, whereas adults tend to develop more severe disease and require hospitalization. Cholera is in principle a self-limiting disease if dehydration is sufficiently remedied. Cholera gravis is a voluminous painless watery diarrhea that can lead to dehydration and even death within a few hours. The case fatality rate for untreated cholera gravis is 50%. Symptoms include nausea, vomiting (especially early in the illness) and muscle cramps, followed by signs of hypovolemic shock.

Cholera stool is not malodorous and is often described as 'rice water' in character because it contains small flecks of mucus but little fecal matter; bloody stool is not suggestive of cholera. Because *V. cholerae* does not invade the epithelial lining of the intestine, there is little inflammatory response and hence the stool contains few if any leukocytes and patients are afebrile. The degree of dehydration is based on physical signs and measuring stool, vomit and urine output.

Complications from cholera (e.g. acute tubular necrosis) are mostly due to inadequate rehydration. Metabolic abnormalities may also occur, including hypokalemia as the result of potassium loss, acidosis from both bicarbonate loss and increased lactate production resulting from anaerobic glycolysis, and hypoglycemia due to deficient gluconeogenesis resulting in seizures and other neurologic abnormalities. Hyperventilation (Kussmaul breathing) may occur as a result of the metabolic acidosis. Shock from cholera can precipitate abortion in pregnant women, although this is less likely to occur if rehydration is prompt.

Management

The management of cholera patients consists of two components:

- rehydration, which is critical; and
- antimicrobial therapy, which is optional and intended to shorten the duration of the illness and prevent spread of the disease.

Effective fluid replacement can be expected to reduce mortality to less than 1% of the severely affected individuals. Mild and moderately dehydrated patients can be treated using oral rehydration solution (ORS), which has three basic components: sugars, salts and water. Physiologically, the sugar acts as the vehicle whereby salts can be absorbed by the mucosal cell; water follows passively.[46] Patients on ORS should remain on ORS until the diarrhea stops. Severely dehydrated patients should be treated intravenously. When intravenous solutions and equipment are not available, severely dehydrated patients may be treated through a nasogastric tube.

Antibiotic treatment serves to shorten the illness and save rehydration fluids. Incomplete courses have contributed to antibiotic resistance. Options are guided by local sensitivity patterns. These are tetracycline 250mg q12h for 7–10 days or amoxicillin 250mg q6h for 5 days. Tetracyclines and quinolones are not recommended during pregnancy. During epidemics, prophylactic antibiotics should be used for the immediate family only and should be limited to a single-dose therapy.

In conclusion, it can be said that during the last decade of cholera research two important findings have emerged: the characterization of a novel serogroup (O139) which circumvented established immunity against O1 and the discovery that cholera toxin genes were encoded on the genome of a bacteriophage. These two characteristics illustrate how bacterial pathogens can rapidly evolve through the acquisition of horizontally transferred genetic elements.[47]

Other pathogenic *Vibrio* spp.

Vibrio parahaemolyticus is a halophilic, or salt-requiring, *Vibrio* sp. that has been related to food poisoning and ingestion of raw or inadequately cooked seafood. The epidemiology results from the ubiquitous presence of the organism in coastal waters. The median incubation time is estimated at 23 hours with a range of 5–92 hours; secondary cases are rare. Preventive measures include the use of boiled water for cooking food. It is not known whether clinical disease confers immunity. There is no effective vaccine available. Stool culture on selective thiosulfate citrate bile salts sucrose agar demonstrates distinct opaque green colonies; final identification is made using standard biochemical tests. The clinical picture ranges from mild, watery diarrhea with low-grade fever to frank dysentery. Specific treatment is not required in most cases because the illness is self-limiting and no benefit has been established from antimicrobial agents.

Other less common halophilic *Vibrio* spp. include *Vibrio vulnificus, V. alginolyticus, V. fluvialis, V. hollisae* and *V. damsela*. Molecular methods, especially PCR, can be used for identification or detection of these species, including in the environment. In contrast to the *Vibrio* spp. discussed so far, *V. vulnificus* and *V. alginolyticus* are more associated with soft tissue wound infection and sepsis than with diarrhea.[48] *Vibrio vulnificus* may be considered an emerging pathogen and its virulence is the strongest among the noncholera *Vibrio* spp. Pathogenicity is generally reserved for the immunocompromised host and is related to disease states that exhibit high serum iron levels, including liver disease. Prevention involves avoidance of contaminated salt water. Immunocompromised patients should be warned against the ingestion of raw oysters and shellfish.[33] At higher latitudes, severe *V. vulnificus* infections have been reported in association with very hot weather conditions and seawater temperatures above 68°F. A vibrio polysaccharide conjugate vaccine may be useful in the management of *V. vulnificus* infections. Antibodies that react with the capsular polysaccharide of the organism are detectable in infected patients and in persons without known exposure to the organism, suggesting that cross-reacting antibodies are present in the general population.

Vibrio vulnificus infections present as two main clinical syndromes:

- primary sepsis secondary to ingestion of raw oysters; and
- localized infection from wound exposure to salt water in which the organism lives.

Both syndromes demonstrate characteristic skin lesions of the trunk and extremities. These present as hemorrhagic bullae and progress to necrotic ulcerations. Necrotizing fasciitis of the foot associated with *V. vulnificus* can cause death within 48 hours and has an overall mortality rate of 50%, even with appropriate antibiotic and surgical treatment.[49] Besides these two syndromes, *V. vulnificus* may also cause acute diarrhea in those on antacid therapy. Early suspicion is critical, because *V. vulnificus* is not always susceptible to aminoglycosides, and tetracycline is the first-choice treatment.

Vibrio alginolyticus may cause bacteremia and death in immunocompromised hosts. Among immunocompetent hosts, it may cause cellulitis and otitis media in swimmers and fishermen.

Vibrio mimicus, a nonhalophilic vibrio, produces a clinical spectrum that is indistinguishable from that of *V. parahaemolyticus*. The epidemiology of *V. mimicus* reflects a global distribution; outbreaks have been associated with heavy contamination of water sources. Prevention involves adequate purification of water sources and proper cooking practices. The diagnosis is confirmed by identifying the agent on thiosulfate citrate bile salts sucrose agar and subsequent specific antiserum testing. Treatment is limited to fluid and electrolyte replacement.

TREPONEMA SPP., *BORRELIA* SPP. AND *LEPTOSPIRA* SPP.

The order of the Spirochaetales is divided into the families Spirochaetaceae and Leptospiraceae. These families include, among others, the genera *Treponema, Borrelia* and *Leptospira* (Table 230.2).

TREPONEMA SPP.

Nature

The genus *Treponema* includes *Treponema carateum*, the causative agent of pinta, and *Treponema pallidum*. The latter species is subdivided into;

- *T. pallidum* subsp. *pallidum*, the causative agent of human venereal and congenital syphilis;
- *T. pallidum* subsp. *pertenue*, the cause of yaws; and
- *T. pallidum* subsp. *endemicum*, the cause of bejel.

Treponemata are motile, thin, spiralliform bacteria (Fig. 230.4).

Treponema pallidum

Epidemiology

In the majority of cases, *T. pallidum* subsp. *pallidum* is transmitted by sexual intercourse, with a moderate to high probability of transmission

TABLE 230-2 -- Order Spirochaetales.

ORDER SPIROCHAETALES			
Spirochaetaceae		**Etiologic agent**	**Human disease**
Family	Genus *Cristispira*		None
Spirochaetaceae	Genus *Serpulina*		None
	Genus *Spirochaeta*		None
	Genus *Treponema*	*Treponema pallidum* subsp. *pallidum*	Syphilis
		Treponema pallidum subsp. *endemicum*	Bejel
		Treponema pallidum subsp. *pertenue*	Yaws
		Treponema carateum	Pinta
	Genus *Borrelia*	*Borrelia recurrentis*	Epidemic relapsing fever
		Many *Borrelia* spp.	Endemic relapsing fever
		Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii	Lyme borreliosis
Family	Genus *Leptonema*		None
Leptospiraceae	Genus *Leptospira*	*Leptospira interrogans*	Leptospirosis
The genera *Treponema, Borrelia* and *Leptospira* contain pathogens associated with human infections. The genera *Cristispira, Serpulina, Spirochaeta* and *Leptonema* are not known to cause disease in humans.			

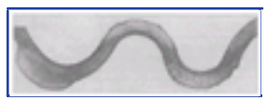

Figure 230-4 Helical structure of *T. pallidum* with the periplasmic flagella. *From Binford and Connor.*[107]

during contact between susceptible and infectious sexual partners (see Chapter 75). It can also be transmitted by kissing, transfusion of fresh blood, placental passage or during birth. Patients are most contagious early during the illness when fresh lesions exist. As a sexually transmitted disease, syphilis has the highest incidence among young adults.

A high incidence was observed in homosexual men until the mid-1980s when, in response to the threat of HIV infection, changes in sexual behavior (e.g. increased condom use) led to a lower incidence. During the 1990s, a resurgence of syphilis among male homosexuals occurred because the tenets of safe sex education gave way to the reacceptance of risky behavior. This, combined with the uncertainty of immunity to reinfections, helps ensure the long-term persistence of syphilis. [50] All patients who have syphilis should be screened for other sexually transmitted pathogens because many patients carry more than one.

Pathogenesis

Treponema pallidum passes through the abraded skin or mucous membrane and spreads throughout the body. The incubation time is proportional to the size of the inoculum. The immunoevasiveness of *T. pallidum* is largely the result of its unusual molecular architecture; its outer membrane contains a paucity of poorly immunogenic proteins, the *T. pallidum* rare outer membrane proteins (TROMPS), whereas its highly immunogenic lipoproteins are anchored predominantly in the periplasmic leaflet of the cytoplasmic membrane.[51]

The presence of granulocytes and macrophages can be demonstrated in a primary chancre, and CD8+ cytotoxic lymphocytes are found in both primary and secondary lesions.[52] The pathologic hallmark of syphilis is endarteritis obliterans, which consists of concentric endothelial and fibroblastic proliferation. In late syphilis, endarteritis affects the vasa vasorum and leads to the formation of gummas with their typical necrotic, coagulated center, most notably in the ascending aorta and the meningeal arteries.

Prevention

Along with individually oriented strategies, such as case finding, partner notification and presumptive treatment, the use of epidemiologic information is recommended when designing community-oriented, population-based strategies, which should include (but not be limited to) selective mass treatment in high-prevalence populations.[53] Widespread treatment of syphilis with penicillin in the USA, beginning in the mid-1940s, led to a decline of more than 85% in its incidence. The increased incidence seen after 1955 was attributed to the relaxation of control measures and increased sexual promiscuity. Surveillance data from Switzerland suggest that the incidence of infection with *Neisseria gonorrhoeae*, *T. pallidum* and *Chlamydia trachomatis* has declined notably since the 1980s. This is a result of population-based strategies, which include more widespread treatment with specific antibiotics and changes in behavior education, leading to a major increase in safe

sex practices, including condom use, in combination with the control of drugs and alcohol abuse. The cost effectiveness of antenatal screening for syphilis has been shown in various settings.[54] A vaccine for syphilis is not available for public health purposes.

Diagnostic microbiology

The lack of a method for *in vitro* culturing of *T. pallidum* necessitates the use of alternative methods (e.g. animal inoculation, dark-field microscopy or serology). Serologic tests, divided into two classes, do not become positive until several week after the appearance of the lesion. The nontreponemal tests detect antibodies to cardiolopin; examples include the Venereal Disease Research Laboratory (VDRL) test, the rapid plasma reagin (RPR) test, the automated reagin test (ART) and the syphilis screen. Because of their ease of use and low cost, these tests are applied for screening purposes. Reactivity in these tests generally indicates host tissue damage that may not be specific for syphilis. False-positive tests may occur in the elderly, drug addicts and patients who have chronic infections and autoimmune disease. Confirmation is required with treponemal tests such as the fluorescent treponemal antibody absorbed (FTA-ABS) test and the *T. pallidum* hemagglutination (TPHA) test. The TPHA and FTA-ABS are good markers for screening potential blood donors who have high-risk sexual behavior because they are more sensitive than the reagin tests. Syphilis Screen is claimed to be an alternative for the TPHA method.[55] After 3 months and following treatment, the non-specific cardiolipins begin to decline, whereas the FTA-ABS remains positive for life. In suspected neurosyphilis, VDRL should be performed on the cerebrospinal fluid (CSF); in unexpected cases, a positive FTA-ABS should be confirmed by a second sample. A number of direct antigen, ELISA and PCR techniques

are currently being developed.[56] Recently, a specific and highly sensitive reverse transcriptase PCR was developed for detecting very low numbers of organisms. Syphilis serology findings (both RPR and TPHA) may be altered in the presence of HIV infection, although in at least one study the serologic response to therapy was similar in HIV-positive and HIV-negative patients.[57] The high prevalence of neurosyphilis in HIV-infected patients who have syphilis warrants examination of the CSF regardless of the stage of illness.

Congenital syphilis is often a presumptive diagnosis based on serology, because confirmation requires the identification of *T. pallidum* in fetal or neonatal tissue or in the placenta, by either histopathology or PCR.

Clinical features

Local symptoms develop as primary syphilis following an incubation period of 10–90 days. This involves a painless, indurated or firm, sharply demarcated chancre (ulcus), usually distinctly different from the more painful, soft and bleeding ulcers caused by *Haemophilus ducreyi* (chancroid). The most common site is the coronal sulcus or prepuce in males and the labia or cervix in females. Primary lesions may also occur on the lips, breasts, mouth and anus. These local lesions heal in 3 months. These symptoms may be followed by regional lymphadenopathy.

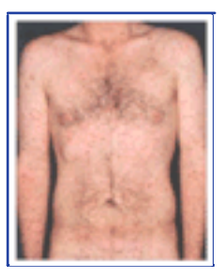

Figure 230-5 Secondary syphilis with typical skin rash. *(Reproduced with permission from Habif.[58])*

TABLE 230-3 -- Treatment options for treponematosis.

TREATMENT OPTIONS FOR TREPONEMATOSIS				
		Antibiotic regimen		
Bacterium	**Clinical features**	**First choice**	**Second choice**	**Third choice**
Treponema pallidum subsp. *pallidum*	Syphilis, all stages except neurosyphilis	Benzathine penicillin G 2.4MU once weekly im for 3 weeks	Doxycycline 200mg q12h po for 2–4 weeks	Erythromycin 500mg q6h po for 2–4 weeks
	Neurosyphilis	Penicillin G 2MU q4h iv for 2 weeks	Ceftriaxone 1000mg q24h iv or im for 2 weeks	Doxycycline 200mg q12h po for 2–4 weeks
Treponema pallidum subsp. *pertenue*	Yaws (frambesia)	Benzathine penicillin G 1.2MU single dose im	Doxycycline 100mg q12h po 10–14 days	
Treponema pallidum subsp. *endemicum*	Bejel	Benzathine penicillin G 1.2MU single dose im		
Treponema carateum	Pinta	Benzathine penicillin G 1.2MU single dose im	Doxycycline 100mg q12h po 10–14 days	

If the primary infection is untreated, secondary syphilis may develop 2–8 weeks later, manifesting as a generalized, dry, nonpruritic rash that persists for 2–6 weeks. Patients are highly contagious during this stage (Fig. 230.5). The rash heals spontaneously, but it relapses in 25% of cases and may be accompanied by condylomata lata in the axillae or anogenital region and by mucous patches in the mouth, pharynx and cervix. Concurrent symptoms of secondary syphilis include generalized lymphadenopathy, low-grade fever, arthralgia, malaise, hepatitis, nephritis and pleuritis. As such, secondary syphilis may mimic other infectious diseases (e.g. brucellosis, rickettsiosis and certain viral diseases).

The rash and the other symptoms may disappear gradually in 1–3 months, marking the beginning of tertiary syphilis or the latent stage. Approximately one-third of the patients go on to develop granulomatous inflammation (gummas) in various organs, cardiovascular lesions (classically an aortic aneurysm), Charcot joints and the involvement of the central nervous system (neurosyphilis).

Management

Penicillin G is the drug of choice. A recent study showed that the addition of amoxicillin and probenecid did not improve the outcome, whether or not patients were co-infected with HIV.[59] Current recommendations for the management of early syphilis remain unchanged and a lumbar puncture is not necessary unless neurologic signs or symptoms are present. Because the effectiveness of the nonpenicillin alternative treatment regimens for syphilis (tetracycline or erythromycin) has not been well studied and because these regimens require the patient to comply with 15–30 days of oral therapy, desensitization of penicillin-allergic patients and treatment with penicillin rather than the use of oral therapy is preferred. Tetracycline-resistant bacteria have been increasingly found since the early 1950s. Treatment options are summarized in Table 230.3 . *Treponema pallidum* infection in the fetus during early pregnancy can be successfully treated by maternal treatment. The same treatment recommendations apply to pregnant women with penicillin as the preferred agent.

Nonsyphilitic treponematoses

Nature and epidemiology

Treponema pallidum subsp. *pertenue, T. carateum* and *T. pallidum* subsp. *endemicum* are morphologically indistinguishable from *T. pallidum* and cause yaws, pinta and bejel, respectively. The distribution of yaws spans the warm and humid tropics; pinta is limited to equatorial America and bejel is prevalent in arid subtropical and temperate climates. The prevalence of yaws decreased from 50–100 million

Figure 230-6 The papillomatous skin lesions of yaws. *(Reproduced with permission from Peters & Giles.[60])*

infected people during the 1950s to fewer than 2 million during the 1970s because of a mass treatment campaign sponsored by the World Health Organization. Yaws has been virtually eliminated in the Americas but it has re-emerged in western Africa and areas of the western Pacific. Pinta is limited to remote areas of Colombia, Central America and Mexico. Bejel is prevalent in parts of Africa and western Asia (see Chapter 156).

Pathogenesis and prevention

The treponemata that cause these three diseases are transmitted by direct contact among children living under unhygienic circumstances. The contacts of patients may be treated with antibiotics. Vaccines for public health use are not available.

Diagnostic microbiology and clinical features

Yaws is characterized by early papillomatous skin lesions and periostitis, late destructive skin lesions (including hyperkeratosis) and bone and skin gummata (Fig. 230.6). As in syphilis, the diagnosis can be confirmed directly by dark-field microscopy or fluorescent antibody staining, or serologically by the VDRL test, the RPR test, the treponemal FTA-ABS test or the specific microhemagglutination antibody tests. The incubation period is 3–5 weeks.

Pinta is suspected in patients from endemic areas who present with papulosquamous or depigmented skin lesions. The diagnosis is confirmed by dark-field microscopy or serologic tests (VDRL, FTA-ABS) that become positive after the lesions appear. The incubation period is about 1–3 weeks.

Bejel is suspected in patients from endemic areas who present with early mucous membrane and skin lesions and late gummata of skin and bone.

Management

Long-acting penicillin G is recommended for patients and their contacts in a single dose of 1.2 million units for those over age 10 years and 600,000 units for children below 10 years of age (see Fig. 230.6).

BORRELIA SPP.

Lyme disease

Borrelia spp. have gained importance during the past 25 years. This genus has become a well-known pathogen to many medical specialists since the first description of Lyme disease and the discovery that *Borrelia* spp. are the causative agents.

Nature

All *Borrelia* spp. are loosely coiled, left-handed bacteria characterized by the presence of a protoplasmic cylinder that is surrounded by a cell membrane, a periplasmic flagellum and an outer membrane (Fig. 230.7). The genome has been sequenced and is relatively small, with 150 megabases comprising a linear chromosome and plasmids. Three

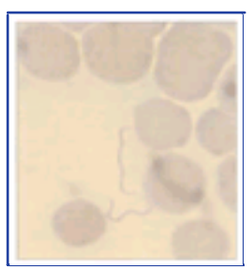

Figure 230-7 Giemsa stain of blood with *B. burgdorferi.* *Adapted from Murray et al.[106]*

species have been associated with disease in humans: *B. burgdorferi sensu stricto, B. afzelii* and *B. garinii*, which together form the *Borrelia burgdorferi sensu lato* complex.[61] In Europe, *Borrelia afzelii* and *Borrelia garinii* are predominant but *B. burgdorferi sensu stricto* can also be encountered. In North America, only infections with *B. burgdorferi sensu stricto* have been observed.

Epidemiology

Lyme disease surveillance data, collected since 1982, show that it is the commonest vector-borne infection in the USA. *Borrelia burgdorferi* is transmitted by ticks of the *Ixodes ricinus* complex. The principal vector in Europe is *I. ricinus*, in north-eastern and mid-west USA *Ixodes scapularis*, in the western coastal states *Ixodes pacificus*, and in Asia *Ixodes persulcatus*. Deer serve as hosts for *I. ricinus*. Small rodents, rats and mice serve as transitional hosts for the tick in its larval and nymph stages. Humans are more or less accidental hosts. The total developmental cycle from egg to adult takes 2 years. The tick is active during the summer and early autumn. In their larval stage, 1–4% of ticks are infected; in their nymph and adult stages, up to 25% are infected. In endemic areas Lyme disease is an important health problem in school-aged children, with a reported incidence of clinical Lyme disease of 10.1 cases per 1000 person-years and a reported incidence of asymptomatic *B. burgdorferi* infection of 3.8 cases per 1000 person-years.

Pathogenesis

Most tick bites do not cause infection and if they do, serious illness is rare. Early during the illness, the distribution of the spirochete is widespread. This is facilitated by the capacity of *B. burgdorferi* to resist elimination by phagocytes, to adhere to epithelial and brain cells, to cross intracellular junctions and penetrate cytoplasm, and to induce tumor necrosis factor (TNF)-a and interleukin (IL)-1b.[62] *Borrelia burgdorferi* actively attaches to, invades and kills human B and T cells. The cellular immune response seen locally in erythema migrans is mainly composed of $CD4^+$ lymphocytes, $CD8^+$ lymphocytes and macrophages. The immune response is suppressed initially, but it is heightened 3–6 weeks later at the peak of the specific IgM response, which is usually directed toward the spirochetal 41kDa flagellar antigen. This IgM response is associated with polyclonal B-lymphocyte activation, including elevated total serum IgM levels, cryoglobulins, circulating immune complexes, rheumatoid factor and antinuclear antibodies or anticardiolipin antibodies. After a further 6–8 weeks, a specific IgG response develops to different spirochetal antigens. At this stage, affected tissues show lymphocyte and plasma cell infiltration and signs of vascular damage.

Prevention

Preventing tick-borne diseases such as Lyme disease can be accomplished by tucking trousers into socks, covering the neck and head (especially of small children) and inspection of the body for ticks after a possible exposure. The use of environmental modifications (e.g. removal of leaf litter, deer fencing or eradication, and the application of insecticides around residential areas) are all effective for various periods of time. Since no scientific evidence is present supporting antibiotic prophylaxis after exposure, adverse affects and resistance development should discourage this approach.[63] The Food and Drug Administration approved in 1998 the LYMErix

(Glaxo Smith Kline) vaccine for persons 15–70 years of age. The vaccine is composed of recombinant OspA (outer surface protein A) and aims at the elimination of the spirochete in the gut of the tick before entry in the host.[64] Efficacy in preventing Lyme disease was 49% and 76% after two and three injections, respectively.[65] A theoretical concern is the induction of inflammatory arthritis by vaccination although no proven cases have been documented. All strains in the USA have been shown to express the same OspA variant; in Europe, however, a polyvalent vaccine will be obligatory.[66] [67]

Diagnostic microbiology

Lyme disease is primarily a clinical diagnosis for which erythema migrans is pathognomonic (Fig. 230.8). [68] It should be suspected in young patients from endemic areas who present with facial palsy or unexplained heart block. Because of the scarcity of organisms, infection can rarely be proven from samples other than the skin on histologic staining. Culturing *B. burgdorferi* from erythema migrans lesions or infected ticks is relatively easy. *Borrelia burgdorferi* grows as fastidious microaerophilic bacteria, at 91–92% F in Barbour-Stoenner-Kelly II medium. Serologic tests serve only as an adjunct to the clinical diagnosis because false positivity may occur. Enzyme-linked immunosorbent assays are preferred to the indirect fluorescent antibody staining method because of their higher sensitivity and reproducibility.[69] False-positive results and considerable variation within and between laboratories have been reported. Despite these shortcomings, the CDC has developed criteria for a two-step approach to determine antibodies against *B. burgdorferi* using ELISA and Western blotting.[65] These criteria apply only for the situation in the USA since antibody production against *Borrelia* spp. other than *B. burgdorferi sensu stricto* is less predictable. None of the laboratory tests can be used to estimate the extent of infection, its prior duration or its prognosis. In general, antibody response is slow and may persist for many years after treatment.

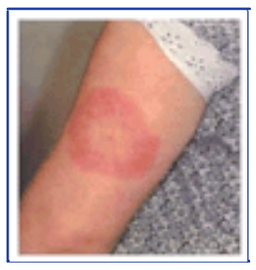

Figure 230-8 Typical erythema migrans rash. *Adapted from Murray et al.*[106]

Borrelia burgdorferi DNA has been demonstrated with PCR in skin biopsies from patients who have erythema migrans and acrodermatitis chronica atrophicans, in CSF from patients who have neuroborreliosis and in synovial fluid and synovial tissue from patients who have Lyme arthritis. [70] Urine is an important source of *Borrelia* DNA in patients who have erythema migrans, acrodermatitis chronica atrophicans, neuroborreliosis and Lyme arthritis.[71] In general, molecular diagnostics for Lyme disease should be reserved for patients with a high clinical probability in the early phase of disease before seroconversion.[72]

Clinical features

Although erythema migrans, the major presenting feature of the illness, is similar in all parts of the world, differences in disease manifestations, disease severity and frequency occur. These differences may reflect regional variation in the prevalence of genospecies of *B. burgdorferi*. In general, a wider spectrum of disease presentations has been noted in Europe than in North America. Borreliosis may evolve in three clinical stages.[73] The clinical heterogeneity results in the tendency to overdiagnose Lyme disease. Stage 1 may remain asymptomatic, whereas stages 2 and 3 may not occur in sequence or may not develop at all. A summary of the clinical manifestations is given in Table 230.4 .

Stage 1 (localized infection)

Three days to a month after the tick bite, a flu-like illness develops together with erythema migrans. Erythema migrans may also be the sole presenting feature. It begins at the site of the tick bite, expands from a macule or papule to a large circular lesion of 3–60cm diameter and fades spontaneously in the course of weeks. Most commonly, the lesions are found on the trunk or lower extremities. Differential diagnoses for erythema migrans include streptococcal and staphylococcal cellulitis, hypersensitivity reactions to arthropod bites, plant dermatitis, tinea and granuloma annulare.

Stage 2 (early dissemination)

Multiple secondary lesions may occur several days after the first lesion of erythema migrans and result from the spread of the organism. Lymphadenopathy and malaise may be evident. After an interval of weeks or months, neurologic abnormalities occur in 10–15% of cases. The cranial nerves can be affected, particularly the facial nerve, resulting in a Bell's palsy. Lymphocytic meningitis develops in 5% of cases. Even less frequent complications include meningoencephalitis, chorea, myelitis, radiculitis and peripheral neuritis. A lymphocytoma, which is sometimes painful, especially when exposed to cold, may also develop. These symptoms may disappear spontaneously or become chronic or relapsing. Transient arthritis or arthralgia may occur as well. At this stage, 10% of the patients develop myocarditis, with atrioventricular blocks of various degrees, which may resolve spontaneously or require a temporary pacemaker.

Stage 3 (persistent infection)

Months to years after the primary exposure, 50% of the patients who have not received adequate treatment develop monoarthritis or oligoarthritis, which becomes chronic in 10–20% of cases. Late complications may also include chronic encephalomyelitis, uveitis and acrodermatitis chronica atrophicans.

Management

Stage 1 and mild manifestations of stage 2 can be treated with oral doxycycline or amoxicillin for 2–3 weeks; early treatment may prevent the development of antibodies.[76] Treatment with doxycycline has the advantage that human granulocytic ehrlichiosis (HGE) is treated as well. Cefuroxime axetil can serve as an alternative for patients allergic to doxycycline or amoxicillin. Macrolides should only

TABLE 230-4 -- Clinical features of Lyme borreliosis.*

CLINICAL FEATURES OF LYME BORRELIOSIS			
System	**Localized (stage 1)**	**Early infection, disseminated (stage 2)**	**Late infection, persistent (stage 3)**
Skin	Erythema migrans	Secondary erythema migrans, lymphadenosis benigna cutis	Acrodermatitis chronica atrophicans
Musculoskeletal		Migratory pain in joints, tendons, muscle, bone; brief attacks of arthritis	Prolonged attacks of arthritis, arthritis, peripheral chronic enthesopathy, joint subluxation below the lesions of acrodermatitis chronica atrophicans
Neurologic		Meningitis, cranial neuritis, Bell's palsy, motor or sensory radiculoneuritis, encephalitis	Chronic encephalomyelitis, spastic parapareses, mental disorders, dementia
Heart		Atrioventricular block, myopericarditis, pancarditis	
Lymphatic	Regional lymphadenopathy	Regional or generalized lymphadenopathy	
Eyes		Conjunctivitis, iritis	
Liver		Hepatitis	
Respiratory		Nonproductive cough	
Renal		Microscopic hematuria or proteinuria	

'Constitutional symptoms'	Minor	Fatigue, malaise	Fatigue

** (Adapted from Steere[74] and Blaauw.[75])*

TABLE 230-5 -- Treatment options for borreliosis.*

TREATMENT OPTIONS FOR BORRELIOSIS				
		Antibiotic		
Bacteria	**Clinical features**	**First choice**	**Second choice**	**Third choice**
Borrelia recurrentis	Recurrent fever	Doxycycline 100mg 1 dose po	Erythromycin 500mg 1 dose po	Tetracycline 500mg 1 dose po
Borrelia spp.		Doxycycline 100mg q12h po 5–10 days	Erythromycin 500mg q6h po 5–10 days	
B. burgdorferi	Stage 1	Doxycycline 100mg q12h 14–21 days	Amoxicillin 500mg q8h 14–21 days	Cefuroxime axetil 500mg q12h 14–21 days
	Stage 2 and 3 mild Cranial nerve palsy, 1st or 2nd degree heart block, arteritis	Doxycycline 100mg q12h 14–28 days	Amoxicillin 500mg q8h 14–28 days	Cefuroxime axetil 500mg q12h 14–28 days
	Stage 2 and 3 serious disease Meningitis, radiculopathy, 3rd degree heart block	Ceftriaxone 2g once daily 14–28 days	Cefotaxime 2g q8h 14–28 days	Penicillin G 18–24 million units iv/d given q4h 14–28 days

** Adapted from Speelman et al.[106]*

be used when patients are intolerant to the aforementioned drugs. The more serious complications of stage 2 and stage 3 such as meningitis, radiculopathy and third-degree atrioventricular heart block will require parenteral antibiotics such as ceftriaxone. Intravenous penicillin G or cefotaxime can serve as an alternative. Oral doxycyline is probably equally effective for the treatment of neuroborreliosis (Table 230.5).[77] Prophylactic treatment is not recommended, especially if the tick has been attached for less than 24 hours.

Borrelia recurrentis

Nature and epidemiology

The louse-borne epidemic relapsing fever, caused by *Borrelia recurrentis*, is transmitted by the human body louse and is associated with unhygienic living circumstances. The tick-borne endemic relapsing fever is caused by different *Borrelia* spp. and is transmitted by the *Ornithodorus* tick.

Pathogenesis and prevention

When lice are crushed, *B. recurrentis* is released and penetrates the skin or mucous membranes. Organisms can be detected in the blood when the patient has a fever. They sequester in internal organs in the afebrile periods. Mutational changes in the antigenic properties of the organism and consequent antibody production account for the relapses, which end when sufficient borrelicidal antibody has been developed.

Preventive measures are similar to those recommended for Lyme disease and involve the elimination or avoidance of the

spirochete vector. A vaccine is not yet available for public health purposes.

Diagnostic microbiology

Diagnosis is based on the clinical presentation and detection of the bacteria in the blood. Spirochetes are found in the majority of wet or stained blood smears. They have also been detected in the CSF of patients who have signs of central nervous system involvement. Up to 10% show positive serologic tests for syphilis.

An early clinical diagnosis of louse-borne relapsing fever is not difficult during epidemics. In contrast, the initial differential diagnosis in an isolated case includes many different diseases such as malaria, typhoid fever, leptospirosis and dengue fever.[78]

Clinical features

Epidemic louse-borne relapsing fever and endemic tick-borne relapsing fever have very similar clinical manifestations; heterogeneity reflects differences in, for example, spirochete strains and the condition of the patient. Louse-borne relapsing fever and tick-borne relapsing fever both have a very acute onset of high fever with chills and muscle and joint aches. Headache and lethargy may be accompanied by neurologic findings, including meningitis and seizures leading to coma. Photophobia and iridocyclitis may develop, causing permanent damage to the sight. Coughing may result from bronchitis or pneumonia. A 2-day truncal rash may occur at the end of the first febrile period. Abdominal tenderness with enlargement of liver and spleen may be associated with jaundice, lymphadenopathy and hemorrhagic diathesis.

The first bout of fever usually lasts 3–6 days, sometimes with fatal complications including myocarditis, cerebral hemorrhage and hepatic failure. The fever may suddenly reappear after a 7–10-day interval. Louse-borne disease typically has a longer incubation period and a more elongated cycle of febrile and afebrile periods, and it is associated with a single relapse. Tick-borne disease manifests with multiple relapses that taper off in duration and intensity.

Management

First-choice antibiotic is doxycycline 100mg q12h for 5–10 days. One 500mg dose of erythromycin is the second-choice option. Antibiotic treatment may be accompanied by a Jarisch-Herxheimer reaction that cannot be prevented by prior corticosteroid therapy.[79] and that reflects clearance of the spirochetemia. It may be advisable to give the first dose of antibiotics at a lower dose (e.g. erythromycin 250mg) to limit the severity of the Jarisch-Herxheimer reaction (see Chapter 182 for further management guidelines).

LEPTOSPIRA INTERROGANS SPP.

Nature

Leptospira interrogans spp. are thin, motile, coiled bacilli. The serotypes of the *Leptospira interrogans* sp. group include *icterohaemorrhagiae, canicola, pomona, autumnalis, grippotyphosa, hebdomadis, ballum* and *australis*. [80] Each of these has a different natural habitat. For example, *L. interrogans* serotype *icterohaemorrhagiae* is most commonly found in rats, *L. interrogans* serotype *canicola* in dogs and *L. interrogans* serotype *pomona* in pigs.

Epidemiology

Leptospirosis is a zoonosis that occurs worldwide (see Chapter 181). In the USA, rats are the most common vector for human infection, followed by dogs, livestock,

rodents, wild mammals and cats. Humans are dead-end hosts because person-person transmission is very rare. The peak incidence is during the summer and early autumn; young adult men often become infected, for example, after recreational exposure to contaminated water. Leptospirosis has long been considered an occupational disease affecting farmers, veterinary surgeons and abattoir workers through indirect contact with infected animals via urine-contaminated water or soil. [81]

Pathogenesis

Leptospira spp. penetrate the intact mucous membranes or abraded skin, enter the bloodstream and spread to all parts of the body, including the CSF and eyes. Liver damage is subcellular and may lead to jaundice. Renal failure may result from a direct toxic effect of the leptospires on the tubules. Meningeal irritation may occur when the leptospires enter the CSF. They are abundant during the early stage of the meningeal disease and disappear when serum antibodies develop. A chronic or recurrent uveitis may result from leptospires persisting in the corpus vitrium of the eye. Myalgia, particularly of the calves, may be an early sign, and pathologic changes such as polymorphonuclear infiltration of the muscle occur late.[82]

Prevention

Effective control of leptospirosis is difficult because leptospires can establish a symbiotic relationship with many hosts, even if they have been vaccinated, and persist in the renal tubules (with excretion in the urine) without causing illness or pathologic changes in the kidneys. In addition, wild animals represent a reservoir from which domestic animals are continually infected. Widespread vaccination of livestock and pets in the USA has reduced the incidence of infection in some species, although adequately vaccinated dogs can still infect humans.[83] Immunization of rice fieldworkers in China with polyvalent vaccines has been practiced. In France a vaccine against serovar *icterohaemorrhagiae* is available for human use.[84] Prohibition of recreational activities in contaminated waters and measures to reduce the number of rats have been successful in reducing the incidence of disease. Prevention can be considered using 200mg doxycycline once a week for heavily exposed individuals such as soldiers who train in the jungle.[85]

Diagnostic microbiology

Isolating the organism from patient material or the detection of seroconversion confirms the clinical suspicion of leptospirosis. Leptospires can be isolated from blood or CSF during the first 10 days of illness. During the second week, they appear in the urine and can be detected in biopsies from various organs. Tween 80-albumin is viewed as the best available medium.[86] Cultures should be maintained in the dark for up to 6 weeks at 82–86°F. Leptospires grow in semisolid media in a ring 0.5–1cm below the surface, appearing 6–14 days after inoculation. They remain viable in heparinized blood for more than 1 week and can be mailed to a reference laboratory for identification.

The laboratory technique most commonly used to diagnose leptospirosis is a two-step serologic procedure, although antibiotic treatment may interfere with antibody development and account for false-negative results.

Macroscopic slide agglutination is used for screening purposes, followed by microscopic agglutination test (MAT) with live antigens for determining antibody titers and identifying serotypes. Both tests use pools of antigens representative of most serogroups. Nevertheless, cross-reactions often occur and serologic tests cannot accurately identify the serotype responsible for infection. Currently, the sensitivity and specificity necessary for reliable clinical diagnosis is best achieved using a battery of 23 antigens. The presumptive diagnosis may be based on a microscopic agglutination titer of 1:100 or greater or on a positive slide agglutination test. Agglutinins appear after 1 week of illness and decline after the third or fourth week. For areas in the world where laboratory resources are limited a dipstick

assay using a broadly reactive leptospiral antigen on a solid support for the detection of IgM antibodies was demonstrated to have satisfactory specificity and sensitivity. [87]

Other techniques to identify leptospires include dark-field examination and silver staining. Polymerase chain reaction has proven valuable as a rapid, sensitive and specific means of diagnosing leptospiral infection, especially during the first 10 days of illness when the clinical expression of the disease may be confusing. [88]

Clinical manifestations

Leptospirosis may evolve subclinically or clinically. It has an incubation period of approximately 7–12 days; clinical illness typically follows a biphasic course. During the initial phase of 4–7 days, patients present with sudden onset of fever, severe general malaise, muscular pain, especially in the calves, conjunctival congestion and leptospires can be isolated from most tissues. Two days without fever follow. During the second phase of up to 30 days, leptospires are still detectable in the urine, kidney and vitreous body. Circulating antibodies emerge and meningeal inflammation, uveitis and rash develop.[89]

Icteric leptospirosis, or Weil's disease, occurs in 10% of the clinically ill patients; 90% remain anicteric. Icteric leptospirosis can be caused by any type of leptospire including *L. interrogans* subtype *icterohaemorrhagiae*. Prominent features are renal and liver malfunction, hemorrhage and impaired consciousness; the mortality rate is 5%.[90] The combination of a direct serum bilirubin level of less than 20mg/100ml (342mmol/l), a marked elevation in serum creatine phosphokinase and an elevation in transaminase (SGOT and SGPT0 of less than 200 units is suggestive of the diagnosis.[91] Hepatocellular necrosis rarely occurs and no residual hepatic dysfunction ensues. Hepatomegaly is found in 25% of cases. The severity of the illness probably reflects the degree of the vasculitis.

Anicteric leptospirosis is a more common and less severe form of leptospirosis. It has a sudden onset and is accompanied by remittent fever and chills, persistent headache, severe myalgia, malaise, prostration and abdominal pain with nausea and vomiting lasting up to 7 days. During the following so-called immune stage, the fever virtually subsides. Delirium and meningism, however, may develop. Several organ systems can be affected, including the lungs, which may show pulmonary infiltrates, and the eyes.[92] A rash may develop as well. Distinct erythematous pretibial lesions can be found in the Fort Bragg fever syndrome, which is caused by *Leptospira interrogans* serotype *autumnalis*.

Management

Severely ill patients should be treated with intravenous penicillin or amoxicillin for 7 days (Table 230.6). [93] Less severely ill patients should receive oral doxycycline for 7 days.[94] Severe disease is not limited to adults but may also affect children, who benefit from antibiotics even late during the illness.[95] Careful monitoring and supportive therapy are important in order to prevent possible complications, including renal failure, hypotension and major hemorrhage.

SPIRILLUM MINUS

Rat-bite fever can be caused by either *Streptobacillus moniliformis* or *Spirillum minus*. The first description of the spirillum dates back to 1888 when Carter reported a spiral organism which he called *Spirillum minor* in the blood of wild rats. Later the organism was named *Spirocheta morsus muris* and in 1924 the organism was renamed *Spirillum minus*.[96]

Nature

The literature on *Spirillum minus* is very puzzling since largely conflicting properties of the organism have been reported. The basic

TABLE 230-6 -- Treatment options for leptospirosis.*

TREATMENT OPTIONS FOR LEPTOSPIROSIS		
	Antibiotic	
Bacteria	**First choice**	**Second choice**
Leptospira icterohaemorrhagiae, copenhageni (Weil's disease)	Penicillin G 1 MU q4-6h iv for 7 days	Doxycycline 100mg q12h po for 7 days
Leptospira hardjo (Milker's disease)	Alternative: Amoxicillin 500mg q6h for 7 days	
Leptospira grippotyphosa		

** Adapted from Speelman et al.*[106]

problem lies in the fact that the description of the organism is purely morphological. [97] No biochemical properties or nucleotide sequence data are available. *Spirillum minus* is a Gram-negative spiral microorganism. It measures 0.2–0.5µm in width and 3–5µm in length. It possesses two to three windings and is actively motile by means of bipolar tufts of flagella.

Epidemiology and pathogenesis

Spirillum minus can be detected in the oropharynx of rats and small rodents. Infections occur throughout the world. Data on incidence are lacking so the widely held belief that infections with *S. minus* are more often seen in the Pacific region cannot be substantiated. Transmission from human to human has not been documented.

Prevention

No studies have been reported on the use of prophylactic antibiotics after a rat bite.

Diagnostic microbiology

The spiral bacteria can be made visible using dark-field microscopy or Giemsa staining of blood smears, smears from eschars or lymph node aspirates. *Bergey's manual of determinative microbiology* states that the organism cannot be cultured on artificial media but can be detected in the blood of mice or guinea-pigs 3 weeks after intraperitoneal injection of clinical samples.[97] A practical problem concerning this approach is the fact that these animals are the natural reservoir of these spirilla. Animal inoculation studies should include control animals and preinoculaton evaluation for spirilla.[98] Most reports in the literature do not describe if such controls were performed. Diagnosis of *S. minus* infection in the literature is based on either clinical presentation, [99] direct visualization of the organism[100] or animal inoculation.[98] These spirilla could not be cultured on artificial media. Other reports indicate an easily cultivable *S. minus* as an etiologic agent for endocarditis[101] [102] and recurrent fever.[103] These spirilla could be cultured on simple media such as blood agar and an antibiogram could be established. Further characterization of the spirilla is warranted to determine if we are dealing with one or multiple species or even genera.[104] No specific serological test can be used; however, the VDRL is positive in 50% of cases.

Clinical features

Spirillum minus rat bite fever is also called sodoku: 'so' means rat and 'doku' means poison in Japanese.[96] The initial lesion of the rat bite usually heals promptly. One to 36 days after the exposure the patient becomes ill and develops fever. At the site of the bite, the wound reactivates and an eschar develops. Regional lymphadenitis is present in

the majority of cases. A violaceous maculopapular rash on palms and soles, very similar to the one seen in syphilis, is a common feature. If untreated, relapsing fever will develop. As compared to the rat-bite fever caused by *Streptobacillus moniliformis*, sodoku has a longer incubation period and arthritis is not a frequent event.

Management

Penicillin is the treatment of choice although no clearcut guidelines can be presented about dose and duration. A Jarisch-Herxheimer reaction can occur after initiation of antibiotic therapy. Tetracycline can be used for patients with an allergy for penicillin.

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Chapter 231 - Gram-negative Coccobacilli

Mary PE Slack

INTRODUCTION

The Gram-negative coccobacilli that are important human pathogens include *Bordetella*, *Brucella*, *Francisella*, *Haemophilus*, *Legionella*, *Pasteurella* and *Yersinia* spp. *Actinobacillus*, *Cardiobacterium*, *Eikenella*, *Kingella* and *Moraxella* (*Branhamella*) spp. occasionally cause human disease. All of these genera except *Yersinia* are fastidious, requiring special nutrients and growth factors for isolation. *Yersinia* spp. are members of the Enterobacteriaceae and are not exacting in their growth requirements.

The genera *Brucella*, *Francisella*, *Pasteurella* and *Yersinia* cause zoonotic infections in humans. *Legionella* infections are acquired through exposure to environmental contamination. *Haemophilus* and most *Bordetella* infections arise through person-to-person transmission. Rarely *Bordetella bronchiseptica*, an animal pathogen, produces opportunistic human infections.

BORDETELLA SPECIES

NATURE

Bordetella spp. are minute Gram-negative coccobacilli. They often demonstrate bipolar staining. They may be nonmotile or motile by means of peritrichous flagella. They are strictly aerobic and the optimum temperature for growth is 95–98.6°F (35–37°C). No acid is produced from carbohydrates. There are seven species in the genus; these differ in their fastidiousness and growth requirements. *Bordetella pertussis* is the most exacting and requires special media for isolation. *Bordetella parapertussis* is slightly less exacting. *Bordetella bronchiseptica* and *Bordetella avium* will grow on ordinary laboratory media. *Bordetella pertussis* and *B. parapertussis* show homogenous clonality. *Bordetella avium* is a distinct species, the G + C content of its DNA being 62mol%, whereas the other species have a G + C content of 66–70mol%. The mol% of guanine and cytosine (G + C) bases in the DNA is the measurement of the genetic unrelatedness of bacterial species. The relatedness of the newly identified species *B. hinzii*, *B. holmesii* and *B. trematum* to *B. pertussis* has not yet been determined.

Bordetella pertussis and *B. parapertussis* are human pathogens of the respiratory tract causing pertussis or whooping cough. *B. bronchiseptica* and *B. avium* are primary respiratory tract pathogens of birds and mammals. *B. bronchiseptica*, *B. avium*, *B. hinzii*, *B. holmesii* and *B. trematum* may infrequently cause infections in humans, particularly in the immunocompromised host.

EPIDEMIOLOGY

Pertussis is highly contagious, being transmitted via aerosolized droplets of respiratory secretions, and in the prevaccine era nearly all children became infected between the ages of 1 and 5 years. Attack rates range from 50% for school contacts to 80–90% for close family contacts. Patients disseminate organisms for weeks or months and are highly infectious in the non-specific catarrhal and early paroxysmal stages of the infection. It is therefore easy for the infection to spread to susceptible individuals before the possibility of whooping cough is considered. There is little evidence of asymptomatic carriers.

Whooping cough is still a major disease worldwide and an important cause of death in malnourished children. It is estimated that there are over 51,000,000 cases each year worldwide and 600,000 deaths.[1] In most populations the disease is endemic, with epidemics occurring every 4 years in late winter and spring. There is no animal reservoir.

With the use of whole-cell pertussis vaccine the incidence of pertussis in children aged 1–5 years declines sharply but there is an increase in the incidence of the disease in children younger than 1 year. Unfortunately, these young children are those at greatest risk of morbidity and mortality from pertussis. Females are more likely to be infected than males.[2] Adults with waning vaccine-induced immunity are increasingly also suffering from pertussis, which often goes undiagnosed because the infection may be atypical. It has been suggested that up to 30% of adults with a prolonged cough may be suffering from pertussis.

There are three serotypes of *B. pertussis* pathogenic for humans, which contain agglutinins 1,2; 1,2,3; and 1,3. Strains may switch serotype both in vitro and in vivo. For this reason, a genotypic method of classification, based on techniques such as restriction fragment length polymorphism (RFLP) or pulsed-field gel electrophoresis (PFGE) is preferable.

PATHOGENICITY

Bordetella spp. possess a common cell wall somatic O antigen. There are 14 capsular polysaccharide types or factors. Factor 7 is found in all species of *Bordetella*. Factors 1–6 are specific to *B. pertussis*. Factor 1 is a lipo-oligosaccharide; it may be involved in attachment. Pertactin is an outer membrane protein that is also involved in adhesion. *Bordetella pertussis* produces a number of other factors that may play a part in pathogenesis [3] [4] (Table 231.1).

Filamentous hemagglutinin is a protein that is involved in the binding of *B. pertussis* to receptors on respiratory cilia. Pertussis toxin is a four-polypeptide toxin with many biologic activities. It acts as a histamine-sensitizing factor and a lymphocytosis-promoting factor, enhances insulin secretion and is also a potent adjuvant. It is composed of two subunits, A and B. Subunit B mediates binding of the toxin and subunit A carries out the biologic activities of the toxin. Pertussis toxin acts by activating adenyl cyclase, which results in increased cyclic adenosine monophosphate (cAMP) in the host cell. The toxin is only expressed by *B. pertussis*. *Bordetella parapertussis* and *B. bronchiseptica* contain the toxin gene but it is not expressed. Adenylate cyclase toxin is an extracytoplasmic protein that can enter leukocytes. Once inside the cells, adenylate cyclase toxin is activated by calmodulin to catalyze the production of cAMP from adenosine triphosphate (ATP). The increased cAMP interferes with leukocyte function. Dermonecrotic toxin is a heat-labile toxin

TABLE 231-1 -- Virulence factors of *Bordetella pertussis.*

VIRULENCE FACTORS OF *BORDETELLA PERTUSSIS*			
Toxin	**Synonyms**	**Composition**	**Action**
Filamentous hemagglutinin	FHA	Protein	Adhesion
Pertactin	PRN	Protein (outer membrane)	Adhesion
Pertussis toxin	PT	Protein	Activation of cAMP, HSF, LPF, IAP
			Impairs leukocyte function
			?Adhesion
Adenylate cyclase toxin	ACT	Protein	Activation of cAMP
			Interference with leukocyte function
			Hemolytic
Dermonecrotic toxin	DNT, heatlabile toxin	Polypeptide	Vascular smooth muscle contraction
			Focal necrosis
Tracheal cytotoxin	TCT	Glycopeptide	Ciliostasis
			Inhibition of DNA synthesis
			Lethal to ciliated epithelial cells
Lipopolysaccharide	LPS	Lipopolysaccharide	Endotoxin
Agglutinogens	Fimbriae, FIM	Protein	Adhesion
With the exception of pertussis toxin, similar toxins are expressed in *B. parapertussis* and *B. bronchiseptica*. cAMP, cyclic adenosine monophosphate; HSF, histamine sensitizing factor; IAP, islet-activating protein; LPF, lymphocytosis promoting factor.			

that can cause vascular smooth muscle contraction resulting in focal ischemic necrosis. Tracheal cytotoxin is a peptide derived from the bacterial peptidoglycan. It causes ciliostasis, inhibits DNA synthesis and kills ciliated epithelial cells, which are then sloughed off. The lipopolysaccharide (LPS) of *B. pertussis* probably also plays a part in pathogenicity.

Following inhalation, *B. pertussis* adheres to the ciliated epithelium of the trachea and bronchi. Adhesion is mediated by filamentous hemagglutinin, pertactin and

possibly pertussis toxin. The bacilli then begin to multiply, producing pertussis toxin, which disrupts cell function; tracheal cytotoxin, which inhibits ciliary motion; and adenylate cyclase toxin, which interferes with phagocytosis. The dermonecrotic toxin causes local necrosis. The organisms remain localized on the respiratory epithelium. They do not invade.[5] [6]

PREVENTION

Prevention of pertussis depends on immunization. The vaccines generally used are killed suspensions of whole bacterial cells adsorbed with aluminum hydroxide, which acts as an adjuvant and also results in fewer adverse reactions. The vaccine should be administered routinely to infants. To be effective the vaccine must contain all three agglutinogens, 1, 2 and 3. Pertussis vaccine was introduced into the UK during the 1950s and resulted in a steady decline in the size of pertussis epidemics until the mid-1970s.

Many concerns have been voiced about the safety of whole-cell vaccines and fears of possible neurologic sequelae. In the UK these fears resulted in a dramatic fall in the rate of vaccine uptake and three large epidemics of pertussis occurred in the late 1970s (Fig. 231.1). Since that time, parental confidence in the vaccine has gradually been restored and the number of cases has again declined. There is no strong evidence that whole-cell pertussis vaccine does produce long-term adverse effects but it may trigger the appearance of pre-existing neurologic problems. A severe adverse reaction to a previous dose of vaccine is a contraindication for further doses. Vaccination should be delayed in children who are unwell.

Acellular pertussis vaccines have been developed. They contain inactivated pertussis toxin and filamentous hemagglutinin. Some also contain fimbrial agglutinogen. Acellular vaccines are currently licensed and being used in the UK, USA, Japan, Germany and Italy. They appear to be safe and effective and may soon replace whole-cell

Figure 231-1 Whooping cough notifications — cases and deaths. Figures are for England and Wales 1940–2000. *Data supplied by the Public Health Laboratory Service Communicable Disease Surveillance Centre.*

vaccines.[7] [8] Because the incidence of pertussis in older children and adults is increasing there may be a case for using acellular pertussis vaccine to boost waning vaccine-induced immunity in older age groups.

DIAGNOSTIC MICROBIOLOGY

With careful sampling and culture techniques *B. pertussis* can be recovered for up to 3 months after the onset of the illness. Ideally, a flexible pernasal swab should be used to collect material. This is far superior to 'cough plates' and postnasal swabs. The material collected should be plated immediately on a suitable medium. If this is not possible it should be placed in a charcoal transport medium.[9] The traditional Bordet-Gengou agar, a potato infusion agar containing 10% glycerol and 20% sheep blood, has the disadvantage of a short shelf life. Better growth is obtained on charcoal blood agar, which can be made selective by the addition of cephalexin.

Figure 231-2 Colonies of *Bordetella pertussis* on charcoal blood agar.

TABLE 231-2 -- Differentiation of species of *Bordetella* found in clinical material.

DIFFERENTIATION OF SPECIES OF *BORDETELLA* FOUND IN CLINICAL MATERIAL				
Species	**Growth on blood agar**	**Motility**	**Oxidase**	**Urease**
Bordetella pertussis	-	-	+	-
Bordetella parapertussis	+	-	-	+
Bordetella bronchiseptica	+	+	+	+

Cultures should be incubated in a moist aerobic atmosphere at 98.6°F (37°F) for at least 5 days. After 3–5 days of incubation, typical 'bisected pearl' colonies appear (Fig. 231.2). Suspect colonies should be checked by Gram-staining and identified using slide agglutination with type-specific antisera. *B. pertussis* and *B. parapertussis* can be differentiated by a series of simple tests (Table 231.2). *B. bronchiseptica* can be identified using a commercial galley test kit such as API 20NE.

Direct immunofluorescence can be applied to respiratory secretions to give a rapid diagnosis.[9] False-negative results may occur because of host antibody adsorbed to the organism. The sample must therefore be pretreated with enzymes. False-positive results may be obtained because of cross-reactions with other organisms such as staphylococci. A polymerase chain reaction (PCR) method may be used to improve the diagnosis of pertussis[10] and has the advantage that it can give a positive result even in the absence of viable organisms.

Immunoglobulin (Ig)M and IgG antibodies in serum or IgA in respiratory secretions can be assayed using an enzyme-linked immunosorbent assay (ELISA) technique. A diagnosis of whooping cough relies on a rising titer of IgG and IgM antibody in two samples collected at least 5 days apart, or a single raised level of IgM or IgG antibodies. However, the antibody response may be slow and weak, especially in young children. Cross-reactions with other organisms and the long-term persistence of pertussis antibodies can produce false-positive results.

CLINICAL MANIFESTATIONS

In a typical case, after an incubation period of 7–14 days, the patient develops red eyes, a runny nose, a mild cough and sneezing ('catarrhal stage'); this is a picture indistinguishable from many viral upper respiratory tract infections. After about 1 week the cough becomes more severe and the patient experiences paroxysmal bouts of a severe, hacking cough. A paroxysm consists of repeated coughing followed by an inspiratory gasp as the patient finally breathes in. This is the characteristic 'whoop'. Paroxysms may be triggered by a variety of stimuli, including cold air and loud noises. The child may become cyanosed during a paroxysm and can suffer fatal hypoxia. Often the child vomits at the end of a paroxysm and is left exhausted. The 'paroxysmal stage' may last for 1–4 weeks. This is followed by a lengthy 'convalescent stage' as the paroxysms decline and the patient slowly recovers. Many cases, but not all, develop a lymphocytosis. Pertussis is most severe in very young infants. In this age group the presentation may be atypical, with no characteristic paroxysmal whooping. Similarly, partially immunized children and adults may have an atypical form of pertussis.[11] [12]

The principal complications of pertussis are secondary infections, notably pneumonia and otitis media, and mechanical damage, including subconjunctival hemorrhage and diaphragmatic herniation during paroxysms. Encephalopathy and fits may also occur.

Bordetella parapertussis is a cause of acute bronchitis or pertussis-like infections in children.[13]

Bordetella bronchiseptica may rarely cause pneumonia, meningitis or whooping cough in highly immunocompromised patients.[14]

MANAGEMENT

The most important aspect of management is supportive care, ensuring that the patient is adequately hydrated, oxygenated and fed. Erythromycin is the antibiotic of choice and appears to reduce the severity and duration of the disease. Trimethoprim-sulfamethoxazole (co-trimoxazole) is an alternative for older patients who are unable to tolerate erythromycin. Corticosteroids may be indicated in infants who have life-threatening disease. Secondary bacterial infections should be treated with appropriate antibiotics.

BRUCELLA SPECIES

NATURE

Brucella spp. are small, nonmotile, nonsporing, noncapsulated Gram-negative coccobacilli. They are aerobic, but some strains require 5–10% carbon dioxide for primary isolation. Growth on culture media is improved by the addition of serum or blood. Growth in vitro is slow and primary isolation may require up to 4 weeks incubation. *Brucella* spp. are catalase-positive but oxidase activity, urease activity and hydrogen sulfide production are variable. They are facultative intracellular parasites that infect a wide variety of domestic and wild animals. The G + C content of DNA is 58–59mol%. DNA-DNA hybridization shows more than 95% homology between all types of *Brucella*. They are to be regarded as a single species, *Brucella melitensis*, with multiple biovars. In practice it is more useful to refer to separate species, which have differing host specificities. Thus, there are six species of *Brucella*, four of which can infect humans (Table 231.3). *B. melitensis* is the most virulent and is responsible for the majority of human infections. Both *Brucella abortus* and *Brucella suis* cause considerable morbidity in countries where brucellosis persists in domestic animals.

EPIDEMIOLOGY

Brucella spp. can infect a wide variety of domestic and wild animals. Brucellosis is a true zoonosis because humans acquire the infection directly or indirectly from infected animals. About 500,000 cases of human brucellosis are reported annually worldwide but this almost certainly is a gross underestimate of the true incidence of the disease.

Brucellosis is prevalent in Mediterranean countries, the Middle East, the Gulf of Arabia, the Indian subcontinent and Central and South

TABLE 231-3 -- Species of *Brucella* pathogenic to humans.

SPECIES OF *BRUCELLA* PATHOGENIC TO MAN			
Species		**Primary host**	**Humans as secondary host**
Brucella abortus (biovars 1–6, 9)		Cattle	+
Brucella melitensis (biovars 1–3)		Goats, sheep, camels	+
Brucella suis	biovars 1–3	Pigs	+
	biovar 4	Reindeer	+
	biovar 5	Small rodents	+
Brucella canis		Dog, fox, coyote	+

America. Some countries have eradicated brucellosis, including the UK, much of northern Europe, Australia, New Zealand and Canada.

In their natural hosts, *Brucella* spp. tend to cause chronic infections that are mild or asymptomatic. The bacteria localize in the reproductive tissues of ruminants, which are rich in mesoerythritol. Erythritol stimulates the multiplication of *Brucella* spp. This accounts for the main symptoms of brucellosis in animals — sterility or abortion. Erythritol is not present in human tissue. *Brucella* spp. are shed in large numbers in the products of conception, in urine and in milk, and when the animals are slaughtered. Humans are infected either by direct contact with infected animals or animal products or indirectly by ingesting infected milk or dairy produce.

In endemic areas the majority of cases occur in dairymen, herdsmen, abattoir workers, butchers and veterinary surgeons. Children may become infected in rural areas of developing countries if they live in close proximity with domestic animals. The general public generally acquires the infection through the ingestion of unpasteurized milk and milk products such as fresh soft cheese. Case-to-case transmission in humans is very rare. Self-inoculation with live *Brucella* vaccine is a recognized risk among veterinary surgeons. Laboratory workers are at risk of acquiring brucellosis through the inhalation of aerosols.[15] This has occurred when handling as yet unidentified Gram-negative coccobacilli without adequate safety precautions.

Brucella melitensis is responsible for the majority of human infections and is primarily food-borne. The primary hosts for *B. melitensis* are sheep and goats. *Brucella abortus* and *B. suis* infections are generally sporadic and occur in people who work with cattle or pigs respectively.[16] *Brucella canis* infections are the least common in humans and are generally laboratory-acquired.

PATHOGENICITY

Brucella spp. are facultative intracellular parasites, surviving and multiplying within cells of the reticuloendothelial system. The organisms enter the body by inhalation, ingestion or after penetration of intack skin, abrasions or the conjunctival mucosa. The major virulence determinant is the LPS of smooth strains (S-LPS). Organisms possessing S-LPS are more resistant to killing by normal serum. Soon after entry into the body, the bacteria are ingested by polymorphonuclear and mononuclear phagocytes. Virulent *Brucella* spp. can survive and multiply within these cells by releasing AMP and guanine monophosphate (GMP), which inhibit the bactericidal myeloperoxidase-peroxide-halide system of the phagocytes. Other factors that contribute toward the intracellular survival of *Brucella* spp. are the production of copper-zinc superoxide dismutase, which prevents oxidative destruction, S-LPS and stress-induced proteins that promote survival within macrophages. [17] [18]

After ingestion by phagocytes, the organisms proliferate in the local lymph nodes. The infection spreads hematogenously to tissues rich in elements of the reticuloendothelial system, including the liver, bone marrow, lymph nodes and spleen. Organisms may also localize in other tissues, including joints, the central nervous system, the heart and the kidneys.

In humans, *Brucella* spp. infection results in the formation of minute granulomata consisting of epithelioid cells, polymorphonuclear leukocytes, lymphocytes and some giant cells. In *B. melitensis* infections the granulomata are very small but there is often marked toxemia. *Brucella suis* is often accompanied by chronic abscess formation in joints and the spleen.

Endotoxin and hypersensitivity to *Brucella* antigens may cause some of the symptoms of brucellosis, including fever and weight loss. Antibodies against the *Brucella* LPS appear within a few days of the onset of the acute phase of the disease and are important in preventing re-infection. However, cell-mediated immunity, particularly the production of activated mononuclear phagocytes, is more important in promoting recovery.

Macrophages process *Brucella* antigens and present them to T cells, which excrete lymphokines, thus inactivating the bactericidal mechanisms of macrophages. The T-cell-derived lymphokines are also responsible for attracting cells to the foci of infection, resulting in the formation of granulomata. At the same time as the development of cell-mediated immunity, delayed-type hypersensitivity to numerous protein antigens can be demonstrated.

PREVENTION

The ideal method of prevention of human brucellosis is to eliminate the disease from domestic livestock. This has been achieved in several countries. An eradication campaign first focuses on mass vaccination of susceptible animals to reduce the incidence of brucellosis in domestic animals. *Brucella abortus* strain 19 vaccine is used for cattle and *B. melitensis* strain Rev-1 vaccine is used for sheep and goats. In the second phase of the campaign, infected animals are detected, using skin tests for sheep and serologic tests on milk or blood samples for cattle. Any infected animals must be compulsorily slaughtered, with financial compensation for their owners. The movement of animals must also be carefully controlled. All these measures require a program of effective education to ensure the co-operation of all those working

with susceptible animals.

Where eradication has not been achieved, people who are occupationally exposed should minimize the risk of infection by wearing protective clothing, gloves, goggles and masks. These measures are particularly important when handling animals that have recently given birth or aborted.

In the past, live, attenuated animal vaccines have been given to workers at high risk of contracting brucellosis. However, these vaccines may produce infection in humans and therefore are far from ideal. Recent work on pure mutants of *B. melitensis* has given promising results in animals but clinical trials in humans have not been undertaken. *Brucella* spp. are killed after heating at 140°F (60°C) for 10 minutes. Boiling or pasteurizing milk will therefore eliminate the risk of transmission via dairy products.

Laboratory-acquired infections can be prevented by handling all cultures and material possibly infected with *Brucella* spp. in a containment level 3 laboratory and in a category I or III exhaust protective cabinet.

DIAGNOSTIC MICROBIOLOGY

A definitive diagnosis depends on the isolation of *Brucella* spp. from cultures of blood, bone marrow or tissue taken early in the disease. Bone marrow cultures are reported to give a slightly higher yield than peripheral blood cultures but, since modern blood culture methods can produce positive results in up to 80% of cases, bone

marrow cultures are not generally required or advocated. Biphasic Castaneda bottles are traditionally recommended to reduce the risk of contamination during subculturing. Cultures should be incubated at 98.6°F (37°F) for at least 6 weeks before they are discarded. The modern semiautomated blood culture techniques (e.g. Bactec®, Bact-Alert®) are suitable for the isolation of *Brucella* spp. and are in use in many diagnostic laboratories. The lysis centrifugation method also gives good results. These commercial systems may give positive results in a matter of days rather than weeks, depending on the initial inoculum of organisms in the sample.

Positive cultures should be subcultured onto serum dextrose agar or a similar medium. Selective culture media are not necessary for human blood cultures but may be required for contaminated material. *Brucella* spp. produce small, smooth, translucent colonies after 48 hours of incubation at 98.6°F (37°C) in air plus 5–10% carbon dioxide.[19] The identity of the organisms can be confirmed by agglutination with monospecific antisera and lysis by specific Tbilisi bacteriophage.

Commercial galley-test identification systems may misidentify *Brucella* spp. as *Moraxella phenylpyruvica*, [15] and isolates should be sent to a reference laboratory for further tests to determine the biovar of the strain. This classification is based on the production of hydrogen sulfide and the tolerance of the organism to the bacteriostatic dyes basic fuchsin and thionin.

A PCR for diagnosing brucellosis has been described but awaits standardization and evaluation.[20] Antigen detection is another potential diagnostic tool.

Many serologic tests have been described for brucellosis. Interpretation of results is often difficult since both IgG and IgM antibodies can persist for a long time. A sample of serum should be collected as early as possible in the course of the disease. The serum agglutination test (SAT) using *B. abortus* strain 119 is widely used. False-negative results can occur if serum samples are not diluted beyond 1:320, owing to a prozone phenomenon. False-positive reactions have also been reported owing to cross-reactivity with strains of *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica* and *Francisella tularensis*. The SAT measures both IgM and IgG. To measure the quantity of specific IgG the serum is treated with 2-mercaptoethanol to inactivate IgM. The SAT will not detect antibodies to *B. canis*. Enzyme-linked immunosorbent assay has the advantage that it can quantify IgM, IgG and IgA antibodies. Immunoglobulin G and IgA antibodies seem to be the best indicators of acute infection. A rise in antibody titer is observed in more than 97% of patients who have acute brucellosis and positive blood cultures. An initial IgM antibody response is followed after 1–2 weeks by a rise in IgG. During recovery the IgG antibody titers slowly decline over a period of months but IgM antibodies may persist at a low level in the serum for several years. Sustained IgG antibody titers or a second rise in IgG antibody levels are seen in chronic infection or relapse. Immunoblotting analysis of *Brucella* cytoplasmic antigens may be a way of differentiating active infection from past infection.[21] [22]

CLINICAL MANIFESTATIONS (see also Chapter 180)

Brucellosis is a systemic infection that can affect any tissue of the body. The protean, vague clinical symptoms are generally not specific for brucellosis.[23] The possibility of brucellosis should be considered in any fever of unknown origin, especially in patients who have occupational exposure or have recently traveled in endemic areas.

In about 50% of cases, the onset is acute following an incubation period of 2–8 weeks. An acute presentation is more commonly seen with *B. melitensis* than with the other species. The patient develops 'flu-like' symptoms of headache, myalgia, anorexia, depression, sweats, weight loss and fever. The fever often shows diurnal variation

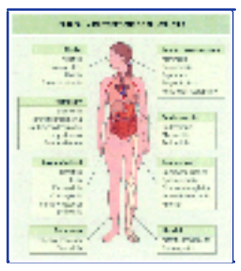

Figure 231-3 Clinical manifestations of brucellosis.

and may come and go over a few weeks or months if the patient is untreated. This 'undulant' fever pattern is not invariably seen. On examination the patient may have hepatosplenomegaly and lymphadenopathy. The white cell count is often raised and liver function tests may be abnormal.

Some patients present with focal or localized infections (Fig. 231.3). Almost any organ of the body can be involved. Given this myriad of clinical features the only unequivocal way of diagnosing brucellosis is to isolate the organism from blood or tissue cultures (see above).

With appropriate treatment the majority of patients will recover within a few weeks or months. Relapses of infection are, however, fairly common. In some patients the symptoms persist for 12 months or more. This is called chronic brucellosis, an ill-defined condition in which a definitive diagnosis is often elusive. It is usually due to the persistence of a deep-seated focus of infection in a bone, joint, kidney, liver or spleen. Chronic brucellosis has similar features to the chronic fatigue syndrome. Typically, the patient suffers from malaise, depression and a fever, which comes and goes in 2- or 3-week cycles.

MANAGEMENT

Before the introduction of antibiotics, brucellosis was a chronic, relapsing illness. A number of antimicrobial agents are active against *Brucella* spp. in vitro but they are not always clinically effective. *Brucella* spp. tend to be sequestered intracellularly, so agents that achieve adequate intracellular concentrations should be used. It is also necessary to give a prolonged course of treatment to reduce the rate of relapsing infection (see also Chapter 180).

Treatment with doxycycline and rifampin (rifampicin) for 6 weeks is recommended for treating acute brucellosis in adults. Doxycycline plus streptomycin (or gentamicin) is an alternative and may result in fewer relapses. The combination of a quinolone, such as ofloxacin,

plus rifampin looks promising but awaits adequate clinical trials.[24] Children, in whom tetracyclines are contraindicated, can be treated with a combination of trimethoprim-sulfamethoxazole and an aminoglycoside. *Brucella* endocarditis and meningitis have been successfully treated with long-term courses of a combination of doxycycline, rifampin and trimethoprim-sulfamethoxazole. The majority of cases of *Brucella* endocarditis will also require surgical intervention. In the case of meningitis, treatment should be continued for 6–9 months.

FRANCISELLA SPECIES

NATURE

Francisella spp. are very small, faintly staining, pleomorphic Gramnegative, nonmotile and non-spore-forming coccobacilli. They are strictly aerobic, oxidase-negative and weakly catalase-positive. The organisms are surrounded by a thin, lipid-rich capsule. They attack carbohydrates slowly, producing acid but no gas. *Francisella tularensis* requires cysteine or cystine for growth and grows slowly at 98.6°F (37°C) on suitably enriched media. The G + C content of the DNA is 33–36mol%.

Francisella tularensis causes tularemia in animals and humans. Within the species *F. tularensis* there are several biovars or subspecies, which differ in their virulence and their epidemiology (Table 231.4). *Francisella philomiragia* was previously classified as *Yersinia philomiragia*. It is of low virulence to humans. *Francisella tularensis* biogroup *novicida* causes infections in laboratory animals but is of low virulence for humans.

EPIDEMIOLOGY

Francisella tularensis is distributed throughout the world but the majority of human infections occur in the northern hemisphere between latitudes 30° and 71°. It is well recognized in North America, Scandinavia and Russia. It has not been described in the UK. The organism is found in many species of wild and domestic animals, birds, fish and blood-sucking arthropods.

In the USA the most important reservoirs of *F. tularensis* are rabbits, hares, muskrats and ticks. In Scandinavia and Russia, rodents (such as voles and mice) and mosquitoes are important reservoirs. The modes of transmission to humans include tick or mosquito bites, contact with infected animal tissues, inhalation of an infectious aerosol or ingestion of contaminated meat or water. There is an increased risk of tularemia in certain occupations that bring people into contact with infected material.

There are approximately 200 cases each year reported in the USA, with over 50% occurring in the southern and southern-central states of Missouri, Oklahoma, Arkansas, Texas and Kansas. Most infections occur in males. There are more cases in summer (when there is a greater risk of exposure to biting insects) and in winter when animals are hunted and trapped.[25] [26]

TABLE 231-4 -- ***Francisella*** **species and biogroups.**

FRANCISELLA **SPECIES AND BIOGROUPS**					
Species	**Biogroup**	**Synonyms**	**Virulence in humans**	**Virulence in rabbits**	**Main animal hosts**
Francisella tularensis	*tularensis*	Type A neoarctica	+++	+++	Rabbits, hares, ground squirrels
F. tularensis	*palearctica*	Type B holoarctica	++	+	Rodents, beavers, muskrats
F. tularensis	*novicida*	(formerly *F. novicida*)	+	+	Rodents, rabbits
F. philomiragia		(formerly *Yersinia philomiragia*)	+	?	Muskrats
All the groups listed are found in North America; *Francisella tularensis* biogroup *palearctica* is also found in Asia and Europe.					

PATHOGENICITY

The infectious dose in humans depends on the route of entry. It can be as low as 10–50 organisms when inoculated through the skin or by inhalation. The infecting dose rises to 10^8 when the organisms are ingested. In most cases infection follows an insect bite or contact with infected animal products. Laboratory workers are at high risk through handling infected laboratory animals or cultures of the organism. *Francisella tularensis* is a hazard group 3 pathogen. *Francisella tularensis* biogroup *tularensis* is more virulent to animals and humans than biogroups *palearctica* and *novicida*.

After entry into the body, the organisms spread to the regional lymph nodes, from where they may disseminate via the lymphatic system or bloodstream to involve multiple organs. There is probably a transient bacteremia at this early stage. In affected animals large numbers of bacilli are present in the cells of the liver and the spleen. Focal necrosis occurs in affected tissue and granulomata may develop. The granulomata may sometimes caseate, giving an appearance similar to tuberculosis.

Francisella tularensis is a facultative intracellular parasite and can survive for prolonged periods within macrophages, hepatocytes and endothelial cells. Intracellular survival is associated with a failure of phagosome-lysosome fusion. Recovery from tularemia depends on cell-mediated immunity, which is directed against the protein antigens of the organism. The bacilli are protected from humoral antibodies, which are directed against carbohydrate antigens. [27]

Pathogenic strains of *F. tularensis* possess an antiphagocytic capsule. Loss of the capsule results in decreased virulence. Like other Gram-negative bacilli, *F. tularensis* has endotoxin activity.

PREVENTION

Prevention of tularemia is best achieved by avoiding exposure to the organism. Gloves, masks and goggles should be worn when skinning or eviscerating animals. Animals that look sick should be left intact. Game meat should be thoroughly cooked and fresh water that is possibly contaminated should not be drunk. Ticks should be promptly removed and chemical insect repellants may be used.

A live attenuated vaccine that induces both humoral and cell-mediated immunity is available for high-risk groups such as laboratory staff working on *F. tularensis*.[28] Live attenuated vaccine must the administered by scarification and does not induce complete protection but will reduce the severity of accidental infection. New tularemia vaccines based on individual components of *F. tularensis* are currently being developed and are attracting considerable interest, since *F. tularensis* is a potential biologic weapon.[29]

DIAGNOSTIC MICROBIOLOGY

Isolation of *F. tularensis* from clinical material is potentially hazardous and it is imperative that the laboratory is notified if tularemia is suspected so that appropriate containment precautions can be

taken. *Francisella tularensis* is a category 3 pathogen and should only be knowingly handled in a containment level 3 facility and in a class I or class III exhaust protective cabinet by trained and experienced staff.

The detection of *F. tularensis* in a Gram-stained smear of aspirates or other samples is rarely successful. Direct immunofluorescence of tissue smears is more sensitive and specific. A PCR method has been described that can be applied to blood samples. However, this does not appear to be any more sensitive than culture.

The organism requires cysteine or cystine for growth. Specialized media such as cysteine-glucose-blood agar have been devised, but *F. tularensis* will grow on some media that are routinely used for other organisms. Chocolate blood agar is often supplemented with cysteine (e.g. Iso VitaleX) and will then support the growth of *F.*

tularensis. Similarly, *F. tularensis* will grow on buffered charcoal yeast extract agar (BCYE), which is normally used to isolate *Legionella* spp. Occasionally, strains of *F. tularensis* will grow on ordinary laboratory media. It is therefore imperative to warn the laboratory if tularemia is suspected to ensure that proper containment precautions are taken when processing the specimens.

Cultures should be incubated at 98.6°F (37°F) with additional carbon dioxide in the atmosphere of incubation. *Francisella tularensis* grows slowly, taking 3–5 days to produce visible colonies, and cultures should be incubated for 3 weeks before being discarded as negative. The addition of penicillin to the medium will suppress the overgrowth of contaminants.

Colonies of *F. tularensis* are blue-gray, round, smooth and somewhat mucoid. They are a-hemolytic on blood-containing media. A slide agglutination method using commercial antiserum or a fluorescent antibody technique can be used to confirm the identity of the organism. Biochemical tests are of little value. Further subspeciation should only be undertaken in a reference laboratory.

However, most cases of tularemia are diagnosed serologically. An agglutination test using killed *F. tularensis* antigen is generally used. A

Figure 231-4 Clinical types of tularemia.

4-fold rise in antibody titer or a single titer of more than 1:160 is considered diagnostic. Immunoglobulin G and IgM antibodies can persist for months or years and it may be very difficult to distinguish past from current infection. Infections with *Brucella* spp., *F. novicida* and *F. philomiragia* can give rise to antibodies that cross-react with *F. tularensis*. An ELISA test has been described.

CLINICAL MANIFESTATIONS

The clinical manifestations of *F. tularensis* infection depend on the portal of entry, the virulence of the infecting organism and the immune status of the individual host. After 3–5 days of incubation the patient develops an abrupt onset of flu-like symptoms — fever, chills, malaise, anorexia and tiredness. The cases are classified clinically into six types, based on the site of the infection and the presence of skin lesions and lymphadenopathy. This division is somewhat artificial because there is considerable overlap between the types (Fig. 231.4) (see Chapter 177).[30]

The most common presentation is ulceroglandular tularemia, which accounts for 70–85% of cases. The patient has a painful red papular skin lesion at the site of entry of the organism, which proceeds to ulcerate with regional lymphadenopathy. There is an accompanying systemic illness with fever, headache, sweating and myalgia. In oculoglandular tularemia (1–2% of cases) the organisms are inoculated via the conjunctival route, resulting in a severe conjunctivitis and regional lymphadenopathy. Oropharyngeal tularemia (2–4%) occurs when the primary invasion is via the oropharynx. There is a severe exudative pharyngitis and regional lymphadenopathy.

In some cases there is regional lymphadenopathy and no local skin lesion. This glandular form of tularemia occurs in 5–10% of cases. Other cases present with a systemic illness but no local skin lesions or lymphadenopathy. This typhoidal form of tularemia accounts for 5–15% of cases. Many of these patients have serious underlying illnesses and the infection may follow an acutely fulminating course. Pneumonic tularemia (5–20% of cases) may follow inhalation of contaminated aerosols or may occur as a complication of bacteremic dissemination of organisms in any of the other groups.

The severity of the infection is highly variable and ranges from a mild afebrile or asymptomatic self-limiting infection to fulminant sepsis. Milder cases are often associated with biogroup *palearctica* infections.

Tularemia may result in chronic debility lasting for several months, with fatigue and suppuration of infected lymph nodes. The overall mortality rate is 1–3% with antimicrobial treatment, but typhoidal tularemia and secondary pneumonic tularemia carry a higher morbidity and mortality.[31]

Francisella tularensis biogroup *novicida* infection results in a milder form of tularemia. *Francisella philomiragia* may cause pneumonia, sepsis and meningitis in patients with underlying conditions, including chronic granulomatous disease, myeloproliferative disorders or near-drowning in salt water.

MANAGEMENT

Streptomycin remains the drug of first choice for treating all forms of tularemia. Gentamicin is an acceptable alternative.[25] Tetracycline and chloramphenicol have been used to treat infections but both are bacteriostatic for *F. tularensis* and there is a high rate of relapse when these agents are used. *Francisella tularensis* produces a ß-lactamase, and ß-lactamase-stable ß-lactams, such as cefotaxime, ceftriaxone and imipenem, may be of use, although they have not been fully evaluated. Other potentially useful but unproven agents include erythromycin and fluoroquinolones such as ciprofloxacin (see Chapter 177).[31]

TABLE 231-5 -- Species of *Haemophilus* associated with infections of humans.

SPECIES OF *HAEMOPHILUS* ASSOCIATED WITH INFECTION OF HUMANS									
Species	**Requirement for**			**Hemolysis**	**Acid from**				
	X factor	**V factor**	**CO_2**		**Glucose**	**Sucrose**	**Lactose**	**Mannose**	**Xylose**
H. influenzae	+	+	-	-	+	-	-	-	+
H. influenzae biogroup *aegyptius*	+	+	-	-	+	-	-	-	+
H. haemolyticus	+	+	-	+	+	-	-	-	±
H. parainfluenzae	-	+	-	-	+	+	-	+	-
H. parahaemolyticus	-	+	-	+	+	+	-	-	-
H. aphrophilus	h	-	+	-	+	+	+	+	-
H. paraphrophilus	-	+	+	-	+	+	+	+	-
H. segnis	-	+	-	-	w	w	-	-	-
H. ducreyi	+	-	-	-	-	-	-	-	-
h, requires hemin for primary isolation; w, weak reaction.									

HAEMOPHILUS SPECIES

NATURE

Haemophilus spp. are small, pleomorphic, nonmotile, nonsporing Gram-negative rods or coccobacilli. They are aerobic and facultatively anaerobic. Growth is often enhanced by the addition of 5–10% carbon dioxide to the incubation atmosphere. The oxidase and catalase reactions vary among the species. *Haemophilus* spp. require one or both of two accessory growth factors (X and V). X factor can be provided by hemin, protoporphyrin IX or other iron-containing porphyrins. X-dependent *Haemophilus* spp. cannot synthesize protoporphyrin from d-aminolevulinic acid, a process involving several enzyme-mediated steps, some or all of which may be defective. V factor is nicotinamide adenine dinucleotide (NAD) or NAD phosphate or certain unidentified precursors of these compounds. It is essential for oxidation-reduction processes.

The differential requirements for X and V factors are important criteria for defining *Haemophilus* spp., which are obligate parasites of humans and animals. There are eight species of *Haemophilus* associated with infections in humans (Table 231.5). *Haemophilus influenzae* is the major human pathogen in the group. Some strains of *H. influenzae* have a polysaccharide capsule, of which there are six distinct antigenic types, designated a–f. The most important is type b, which has a capsule consisting of polyribosyl ribitol phosphate (PRP). *Haemophilus influenzae* type b (Hib) strains are associated with the majority of invasive infections.

Haemophilus influenzae biogroup *aegyptius* (formerly *Haemophilus aegyptius*) is a cause of epidemic conjunctivitis and Brazilian purpuric fever. *Haemophilus ducreyi* is the causative agent of chancroid. *Haemophilus aphrophilus*, *H. paraphrophilus* and *H. parainfluenzae* are occasionally implicated in infective endocarditis and in brain and liver abscesses.

The genus *Haemophilus* is a member of the family Pasteurellaceae, which also encompasses the genera *Pasteurella* and *Actinobacillus*. The G + C content of DNA in *Haemophilus* spp. is 37–44mol%.

EPIDEMIOLOGY

Haemophilus influenzae is an obligate parasite of human mucous membranes; it is not found in any other animal species. It colonizes the throat and nasopharynx, and to a lesser extent the conjunctivae and genital tract. The respiratory tract is colonized by *Haemophilus*

TABLE 231-6 -- Spectrum of infections caused by *Haemophilus influenzae*.

SPECTRUM OF INFECTIONS CAUSED BY *HAEMOPHILUS INFLUENZAE*		
Infections	**Age group affected**	**Strains**
1. Invasive infections:	90% children <4 years old	90% type b
Meningitis	10% older children and adults	10% ncHi
Epiglottitis		1% types e, f
Pneumonia		
Septic arthritis		
Osteomyelitis		
Cellulitis		
Bacteremia		
2. Neonatal and maternal sepsis	Neonates	Over 90% ncHi
	Parturient mothers	
3. Noninvasive respiratory infections, otitis media, sinusitis, conjunctivitis, acute exacerbations of chronic bronchitis	Children and adults	Over 90% ncHi

spp. — mainly *H. parainfluenzae* and noncapsulated strains of *H. influenzae* (ncHi) — in 25–75% of healthy people. Carriage of Hib is found in 3–5% of healthy people. Immunization of infants with conjugate Hib vaccine results in a reduction in the rate of nasopharyngeal colonization by Hib.[32]

Haemophilus influenzae is associated with two types of infection, invasive infections and noninvasive infections (Table 231.6), which have distinctive epidemiologic profiles.[33] The commonest invasive infection caused by *H. influenzae* is meningitis, but epiglottitis, pneumonia, bone and joint infections and cellulitis also occur. These bacteremic infections are usually caused by Hib. Rarely, other capsular serotypes, especially a, e and f, and ncHi, are associated with invasive infections.

Haemophilus influenzae type b meningitis principally affects children aged 2 months to 2 years. Epiglottitis occurs in slightly older children, with the peak incidence being in children aged 2–3 years. Risk factors for invasive disease are mainly socio-economic and include overcrowding, attendance at day care centers, chronic illness and lack of access to good health care facilities.

Invasive Hib infections are distributed worldwide but the incidence varies from country to country with very high incidence rates being found in certain racial groups, for example Apache and Navajo Native

TABLE 231-7 -- **Annual incidence of *Haemophilus influenzae* type b meningitis.**

ANNUAL INCIDENCE OF *HAEMOPHILUS INFLUENZAE* TYPE b MENINGITIS	
Country	**Incidence**
England	25
Finland	26
USA (Texas, North Carolina, Maryland, California)	19–69
Australia (New South Wales, Victoria)	20–25
Hong Kong Chinese	2.7
Gambia	60
Alaskan Inuit	282
Apache Native Americans	254
Australian Aborigines	454
Figures for incidence per 100,000 children under the age of 5 years and are from various countries and ethnic groups prior to the introduction of routine immunization.	

Americans, Alaskan Inuit and Australian Aborigines (Table 231.7).[34] In these racial groups the peak incidence of infection occurs at a younger age. By contrast, a

very low incidence rate has been reported in Hong Kong Chinese. There is seasonal variation, with most cases occurring in the winter months.

The introduction of vaccines effective against Hib in infants has dramatically reduced the incidence of invasive disease in children in countries using this vaccine, and consequently the epidemiology is changing.

The noninvasive infections of the respiratory tract associated with *H. influenzae* include otitis media, sinusitis, acute exacerbations of chronic bronchitis and conjunctivitis. The majority of these infections are caused by ncHi and many occur in adults. There is often an underlying physiologic or anatomic abnormality. These infections have a worldwide distribution and all of them are more common in the winter months.

Haemophilus influenzae biogroup *aegyptius* is associated with an acute form of seasonal conjunctivitis and septicemia (Brazilian purpuric fever) in hot climates; these conditions occur in children.[35]

PATHOGENICITY

Haemophilus influenzae is transmitted by aerosols of respiratory secretions or by direct contact with contaminated material. The primary event is colonization of the nasopharynx. Prior infection with respiratory viruses (e.g. influenza) promotes colonization and subsequent infection by *Haemophilus* spp. The microbial factors that promote colonization include fimbriae, LPS, IgA1 protease and a ciliotoxin.[36] Noninvasive infections of the respiratory tract result

TABLE 231-8 -- Conjugate *Haemophilus influenzae* type b (Hib) vaccines.

CONJUGATE *HAEMOPHILUS INFLUENZAE* TYPE B (Hib) VACCINES			
Scientific name	**Product name**	**Carbohydrate**	**Protein carrier**
PRP-T	Act Hib (Aventis Pasteur)	PRP	Tetanus toxoid
	Infanrix Hib (Glaxo SmithKline)		
HbOC	Hib TITER (Wyeth)	Oligosaccharide	Nontoxic mutant diphtheria toxin (CRM_{197})
PRM-OMPC	Pedvax HIB (Merck)	PRP	*Neisseria meningitidis* group B outer membrane protein complex
PRP-D	ProHIBit (Connaught)	PRP	Diphtheria toxoid

from contiguous spread of organisms colonizing the respiratory tract. Acute sinusitis, otitis media and acute exacerbations of chronic bronchitis usually follow a viral infection. This predisposes to secondary infection with potentially pathogenic components of the local resident bacterial flora by mechanisms including obstruction to the outflow of respiratory secretions, depression of local immunity and suppression of mucociliary clearance. The majority of these infections are caused by ncHi. Possession of the outer membrane protein P2 is an important virulence factor for ncHi. Immunoglobulin A1 protease and ciliotoxin appear to be important in the pathogenesis of pneumonia.

Invasive infections, notably meningitis and epiglottitis, are mainly caused by Hib and result from invasion of the bloodstream. The pathogenesis of these infections has been elegantly elucidated using an infant rat model.[37] The capsule of type b *H. influenzae* is the single most important determinant of virulence for invasion because it protects the organism from phagocytosis and complement-mediated lysis. The rarity of infections in the first 2 months of life correlates with the presence of maternal antibodies to PRP and the occurrence of infection in early infancy with the absence of antibodies having such specificity. As the mean level of PRP antibodies in the population rises, so Hib infections become less common. It is unclear whether natural antibody production is stimulated by exposure to Hib or to some other organism (e.g. *E. coli* K100) that possesses cross-reacting antigens.

Brazilian purpuric fever is caused by a single clone of *H. influenzae* biogroup *aegyptius*.[35] The LPS phenotype is a critical determinant of virulence for *H. influenzae* biogroup *aegyptius*.

PREVENTION

Vaccination

Conjugate vaccines have now been developed in which the PRP is covalently linked to a protein carrier (Table 231.8).[38] These vaccines produce a lasting anamnestic response, which is not age-related. They can be given effectively to infants as young as 2 months of age and are also effective in high-risk groups of patients who give a poor response to polysaccharide vaccines. The Hib vaccine can be given concurrently with diphtheria-tetanus-pertussis immunization. In the UK, Hib vaccine is thus administered at 2, 3 and 4 months of age. Most countries except the UK and the Republic of Ireland give a booster dose in the second year of life. Countries where Hib vaccine is routinely offered have witnessed a dramatic decline in the occurrence of Hib infections (Fig. 231.5).[39] [40]

Administration of Hib conjugate vaccine has the important additional effect of reducing nasopharyngeal colonization with Hib.[34]

Haemophilus influenzae type b vaccine is recommended after splenectomy, or for patients who have functional asplenia, together with pneumococcal vaccine and meningococcal vaccine.

There is as yet no effective vaccine for ncHi infections.

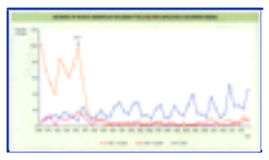

Figure 231-5 Incidence of invasive *Haemophilus influenzae* type b and noncapsulated *H. influenzae* disease. Figures are for the period from October 1990 to June 1997 in England.

Chemoprophylaxis

Oral rifampin for 4 days is recommended for eradicating carriage of *H. influenzae* and has been used to prevent secondary infection in household and nursery contacts. Its efficacy is unproven. Unvaccinated siblings of a case who are more than 4 years old and less than 10 years old should be immunized with Hib conjugate vaccine. Unvaccinated siblings less than 4 years old should be offered both chemoprophylaxis and vaccine. Children less than 4 years of age who have invasive Hib disease should also be offered both vaccine and chemoprophylaxis in order to eliminate nasopharyngeal carriage, because disease sometimes fails to induce immunity in the very young. Widespread use of the vaccine may soon render chemoprophylaxis unnecessary.

DIAGNOSTIC MICROBIOLOGY

Gram-stained smears of cerebrospinal fluid, pus, sputum or aspirates from joints, middle ear or sinuses may provide a rapid diagnosis. *Haemophilus influenzae* tends to stain poorly and dilute carbol fuchsin is a better counterstain than neutral red or safranin.

Rapid tests for Hib capsular antigens, such as latex agglutination or a PCR-based method, can be applied to clinical material. False-positive latex agglutination results have been reported in cere-brospinal fluid samples collected from children who had recently received Hib vaccine.

Specimens should be plated on appropriate culture media as soon as possible. Chocolate agar is the most commonly used medium for culturing *Haemophilus* spp. It can be used without further supplementation for specimens from normally sterile sites. The addition of bacitracin to chocolate agar will assist the recovery of *H. influenzae* from respiratory samples because it will suppress the growth of other respiratory organisms.[41] Good growth can also be obtained on certain transparent

media that contain blood extracts (e.g. Levinthal's agar or Fildes peptic digest agar).

The cultures should be incubated at 98.6°F (37°C) in an aerobic atmosphere enriched with 5–10% carbon dioxide. After 24 hours of incubation, colonies of ncHi are small, circular, smooth and pale gray. Capsulated strains produce somewhat larger, mucoid colonies, which have a characteristic seminal odor. On clear media, such as Levinthal's agar, colonies of capsulated strains exhibit iridescence when viewed obliquely with transmitted light. Type b capsulated strains can also be detected by the presence of precipitin haloes on transparent media containing hyperimmune Hib antiserum.

Confirmation of the identity of colonies depends on demonstrating a requirement for X factor, V factor, or both. This is usually performed as a disk test. The culture is plated onto a nutrient agar that is deficient in both X and V factor, and paper disks containing X factor alone, V factor alone, and both X and V factors are placed on the surface of the agar. After overnight incubation, growth is observed around the disks supplying the required growth factors (Fig. 231.6).

Haemophilus influenzae strains should be typed to determine their capsular serotype. This is normally carried out by slide agglutination using type-specific antisera. This method is prone to misinterpretation, with problems of autoagglutination, cross-reacting antisera and observer error. The definitive method of typing *H. influenzae* is capsular genotyping using a PCR-based method.

CLINICAL MANIFESTATIONS

Invasive infections

Meningitis is the commonest and most serious manifestation of systemic infection with *H. influenzae* (see Table 231.6). It is a disease of infants, principally between the ages of 2 months and 2 years.

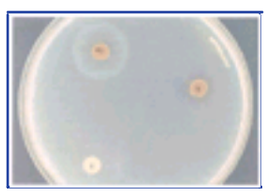

Figure 231-6 Growth factor requirement of *Haemophilus influenzae.* Strain of *H. influenzae* sown on Columbia agar plate. Filter paper disks containing X factor, V factor, and both X and V factors have been placed on the surface of inoculated plate, but colonies of *H. influenzae* grow only around the disk with both X and V factors.

There is often a history of a preceding viral upper respiratory tract infection or otitis media. There are no clinical features that distinguish *Haemophilus* meningitis from other forms of bacterial meningitis. Up to 20% of survivors of *Haemophilus* meningitis have long-term neurologic sequelae — notably sensorineural deafness. *Haemophilus* meningitis can also occur in neonates, [42] older children and adults. In neonates the causative organism is almost invariably ncHi and the presentation can resemble group B streptococcal sepsis. In adults the infection may be secondary to head injury, neurosurgery, chronic sinusitis or mastoiditis. Both ncHi and Hib may be responsible. [43]

Epiglottitis is a life-threatening infection that is almost always caused by Hib. It occurs in slightly older children, aged 2–5 years, with a peak incidence in those aged 2–3 years. Typically there is a rapid onset of sore throat, fever and dyspnea, which may progress to airway obstruction requiring emergency tracheostomy and ventilation. Examination of the larynx is potentially hazardous because it may provoke respiratory obstruction. The diagnosis should be based on the clinical findings confirmed by blood culture results (see also Chapter 31).

Other less common manifestations of invasive *H. influenzae* disease include pneumonia, periorbital cellulitis (which is generally unilateral), septic arthritis and osteomyelitis, or a bacteremia without any obvious focus of infection. *Haemophilus* pneumonia is a major cause of mortality among young children in the developing world.

Noninvasive infections

A variety of local infections, principally in the respiratory tract, may be seen. These include otitis media, sinusitis, conjunctivitis and acute exacerbations of chronic bronchitis (see Chapter 33). Most of these infections are caused by noncapsulated strains of *H. influenzae*. *Haemophilus influenzae* is an important cause of purulent conjunctivitis. This may be sporadic but can occur in outbreaks. Severe conjunctivitis associated with severe sepsis and a high mortality was first described in 1984 in Brazilian children aged 1–4 years. The causative organism is a clone of *H. influenzae* biogroup *aegyptius*, which is indistinguishable in routine laboratory tests from ncHi.

Rarely *H. influenzae* may be associated with other infections, including urinary tract infections, cholecystitis, salpingitis and epididymo-orchitis. Many of these unusual infections are associated with pre-existing damage, including the presence of urinary stones or gallstones.

Haemophilus parainfluenzae, *H. aphrophilus* and *H. paraphrophilus* may be associated with infective endocarditis, meningitis and cerebral abscesses.[44] These species form part of the HACEK group (see below).

Haemophilus segnis is occasionally associated with acute appendicitis.

MANAGEMENT

Cefotaxime, ceftriaxone and related third-generation cephalosporins are the antibiotics of choice for treating *Haemophilus* meningitis. They are bactericidal for *H. influenzae*, achieve high concentrations in the meninges and cerebral tissues, and have proved highly effective in clinical practice.

Chloramphenicol was formerly the treatment of choice. Resistance may be encountered but in most parts of the world this remains uncommon. Ampicillin is also effective but resistance due to a ß-lactamase is now encountered in an unacceptably high percentage of strains.[45] For example, 25% of Hib strains in the UK are ß-lactamase-producing. In view of this, ampicillin should no longer be used as a single agent in *Haemophilus* meningitis in countries where ampicillin resistance is prevalent.

Antibiotic therapy is only one component of the clinical management of meningitis and full supportive care is required to achieve the most favorable outcome. Skilled medical and nursing care is essential in cases of epiglottitis to maintain the patient's airway.

The risk of infection is increased 500-fold for close contacts of a case of invasive Hib disease. Close contacts should be given rifampin chemoprophylaxis and, where appropriate, offered Hib vaccine (see above).

For the treatment of less serious respiratory infections, oral antibiotics including amoxicillin, amoxicillin-clavulanate, tetracycline, erythromycin or newer macrolides such as clarithromycin or azithromycin are all effective. ß-Lactamase-mediated amoxicillin resistance may again be a problem. In the UK, 20% of ncHi isolates are ß-lactamase producers. Occasional strains are resistant to ampicillin by an intrinsic mechanism.

HAEMOPHILUS DUCREYI

EPIDEMIOLOGY

Haemophilus ducreyi is the causative agent of the sexually transmitted disease chancroid, or soft sore.[46] Chancroid is endemic in tropical areas, particularly central and southern Africa, South East Asia, India and the Caribbean. It occurs sporadically in other parts of the world and recently there have been major outbreaks in Canada and the USA. It is more common in nonwhite, uncircumcised males and in low socio-economic groups living in poor hygienic conditions. Infection may be asymptomatic or inapparent in females. Genital ulcers, including chancroid, are associated with increased transmission of HIV.

PATHOGENICITY

Haemophilus ducreyi has not been isolated from nonhuman sources and is sexually transmitted. The organisms gain access via a break in the epithelium and establish infection in a focal area of mucosa or skin in the genital tract. The pathogenesis of chancroid is poorly understood. Potential virulence factors include fimbriae, LPS, cytotoxins and outer membrane protein (OMP).[47] [48]

PREVENTION

The use of condoms dramatically reduces the transmission of *H. ducreyi*. All sexual partners should be identified by contact tracing and treated. Education is vitally important.

DIAGNOSTIC MICROBIOLOGY

Specimens should be collected from the base and margins of ulcers using a saline-moistened swab or by aspirating a bubo. On Gram-stained examination pleomorphic coccobacilli or short rods may be seen. They are more commonly extracellular but may be intracellular. Characteristically they resemble 'shoals of fish'. However, ulcer material is generally contaminated with other bacteria and direct Gram-staining identifies only about 10% of culture-positive cases.

Haemophilus ducreyi is a fastidious organism that grows slowly on chocolate agar. In general a suitable medium contains a growth supplement such as IsoVitaleX and is made selective by the addition of vancomycin. [49] [50]

Cultures should be incubated for up to 5 days at 95–97°F (35–36°C) with added moisture and carbon dioxide.

The appearance of colonies of *H. ducreyi* varies with the medium used. Typically, after 24 hours incubation the colonies are pinpoint, yellow-gray and translucent or semiopaque. Cultures often appear mixed, with a variety of colonial forms. The colonies are very cohesive and can be pushed across the agar surface with an inoculating loop.

Haemophilus ducreyi requires X factor but not V factor for growth. It can be identified using a number of rapid test systems, including API-ZYM, Minitek and RapIDNH.

A variety of noncultural techniques have also been developed for *H. ducreyi* including indirect immunofluorescence, ELISA-based antigen detection and PCR-based methods.[51]

CLINICAL MANIFESTATIONS

After exposure there is an incubation period varying from 1 day to several weeks (average 5–7 days). Initially, tender papules develop on an area of genital skin or mucosa, surrounded by a zone of erythema. After 2–3 days the papules become pustular and then break down to form sharply circumscribed, painful ulcers with ragged, undermined edges. The base of the ulcer bleeds easily when it is scraped. Ulcers may be single or multiple. The ipsilateral regional inguinal lymph nodes become painful and enlarged. If left untreated, nodes may become fluctuant, forming an abscess or bubo. In some cases, buboes may spontaneously rupture, leading to chronic sinus formation (see Chapter 78).

TABLE 231-9 -- Differentiation of HACEK species.

DIFFERENTIATION OF HACEK SPECIES											
Species	**Growth on blood agar**	**Requirement for**		**CO_2**	**Hemolysis**	**Oxidase**	**Catalase**	**Indole**	**Urease**	**Nitrate reductase**	**Acid from glucose**
		X factor	**V factor**								
Haemophilus aphrophilus	-	+	-	+	-	-	-	-	-	+	+
Haemophilus paraphrophilus	-	-	+	+	-	+	-	-	-	+	+
Haemophilus parainfluenzae	-	-	+	-	-	+	±	v	v	+	+
Actinobacillus actinomycetemcomitans	+	-	-	+	-	+	+	-	-	+	+
Cardiobacterium hominis	+	+*	-	+*	-	+	-	+	-	-	+
Eikenella corrodens	+†	+*	-	+	-	+	-	-	-	+	-
Kingella kingae	+‡	-	-	-	(+)	+	-	-	-	-	+
V, reactions vary with the different biotypes.											

* Requirements on primary isolation; usually lost on subculture.
† Colonies pit the agar.
‡ Pitted colonies.

MANAGEMENT

Most isolates of *H. ducreyi* are susceptible to erythromycin and the newer macrolides azithromycin, clarithromycin and roxithromycin. A 1-week course of oral erythromycin is well established as an effective treatment for chancroid. A single dose of azithromycin[52] or ceftriaxone appears to be effective but there have been reports of treatment failures with ceftriaxone in HIV-positive men in Kenya. Indeed, HIV-positive patients may respond poorly to any treatment for chancroid.

The susceptibility of *H. ducreyi* to antimicrobial agents varies from one geographic area to another.[53] Many strains are ß-lactamase producers and resistance to

tetracycline is common. Trimethoprim-sulfamethoxazole resistance is being increasingly seen, especially in the Far East.

Other agents that show excellent in-vitro activity against *H. ducreyi* include quinolones such as ciprofloxacin, rifampin, spectinomycin and amoxicillin-clavulanate.[54]

Buboes should be drained to prevent sinus formation.

HACEK GROUP

NATURE

The HACEK group of organisms includes *H. parainfluenzae*, *H. aphrophilus*, *H. paraphophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*. They are all small, fastidious, pleomorphic Gramnegative bacilli that form part of the normal commensal flora of the oropharynx and are infrequent causes of human infections. They are slow-growing, their growth being enhanced by the use of enriched culture media and an increased carbon dioxide tension. Some biochemical properties that can be used to differentiate the individual species are listed in Table 231.9 .

Cardiobacterium hominis and *E. corrodens* usually require hemin (X factor) and CO_2 on primary isolation and may be misidentified as *Haemophilus* spp.; these requirements are lost on subculture. The colonies of *E. corrodens* have a characteristic 'bleach-like' odor and often 'pit' the surface of the agar. Colonies of *K. kingae* produce slight ß-hemolysis on blood agar.

CLINICAL MANIFESTATIONS

Collectively, the HACEK group of organisms causes 3% of all cases of infective endocarditis[55] and is responsible for a variety of other infections (see Chapter 59).

Actinobacillus actinomycetemcomitans was originally thought to be a secondary pathogen in actinomycotic infections caused by *Actinomyces israelii*. It is now recognized as a cause of periodontal infections, brain and other soft tissue abscesses, osteomyelitis and infective endocarditis.[56]

Cardiobacterium hominis has been implicated in cases of natural and prosthetic valve endocarditis[57] but rarely causes other types of infection.

Eikenella corrodens tends to cause indolent infections, often in association with other organisms. Infections associated with *E. corrodens* include periodontitis, human bite wounds, sinusitis, meningitis, brain abscess, pulmonary infections, pelvic infections, endocarditis, osteomyelitis and septic arthritis.[58] [59] [60] Clenched fist injuries are the most serious human bite wounds and are sustained when the knuckles of the assailant's hand strike the teeth of the adversary.

Kingella spp. (*K. kingae* and *K. denitrificans*) are associated with endocarditis and osteoarticular infections. Since the introduction of routine Hib immunization in the USA, and consequent decline in Hib septic arthritis, *K. kingae* has emerged as a major cause of septic arthritis in children under 2 years of age.[61]

Haemophilus parainfluenzae, *H. aphrophilus* and *H. paraphophilus* may all cause endocarditis. *Haemophilus parainfluenzae* has been reported as a rare cause of the spectrum of infections associated with *H. influenzae*: meningitis, epiglottitis, septic arthritis, osteomyelitis, pneumonia and bacteremia. *Haemophilus aphrophilus* may cause brain abscesses, septic arthritis, osteomyelitis or meningitis. *Haemophilus paraphrophilus* has been implicated in cases of epiglottitis, brain abscess and osteomyelitis.

PREVENTION

Approximately 60% of cases of endocarditis caused by HACEK organisms are associated with poor oral hygiene, bad dentition or recent dental work.[55] Prevention of endocarditis should therefore be based on good dental and oral hygiene in patients at risk of endocarditis.

MANAGEMENT

The treatment of endocarditis caused by HACEK organisms should be based on antimicrobial susceptibility tests, including tests for ß-lactamase activity. Since many of these organisms are slow-growing, such tests can be problematic. Currently, third-generation cephalosporins, including cefotaxime or ceftriaxone, are the drugs of choice for treating endocarditis caused by the HACEK organisms.

Eikenella corrodens is generally susceptible to penicillin, but consideration should be given to the possibility of ß-lactamase-producing aerobic and anaerobic organisms when treating mixed infections, such as human bites.

LEGIONELLA SPECIES

NATURE

Legionella spp. are slender, aerobic, noncapsulated, non-spore-forming Gram-negative rods. In clinical material they often appear as coccobacilli but they tend to form elongated filamentous forms on some culture media. They are motile with polar flagella. *Legionella* spp. stain poorly with Gram stain. Basic fuchsin is a better counterstain for these organisms. They do not attack carbohydrates, and nitrate is not reduced. *Legionella* spp. are nutritionally fastidious and will not grow on ordinary culture media. A suitable medium for their isolation is buffered charcoal yeast extract (BCYE) supplemented with L-cysteine, a-ketoglutarate and ferric ions. The cell wall contains branched-chain fatty acids with high concentrations of ubiquinones, which give characteristic profiles by gas-liquid chromatographic analysis.

TABLE 231-10 -- *Legionella* species associated with disease in humans.

***LEGIONELLA* SPECIES ASSOCIATED WITH DISEASE IN HUMANS**	
Species	**Number of serogroups**
Legionella pneumophila	15
Legionella micdadei	1
Legionella bozemanii	2
Legionella dumoffii	1
Legionella longbeachae	2
Legionella anisa	1
Legionella birminghamensis	1
Legionella cincinnatensis	1
Legionella feeleii	2
Legionella gormanii	1
Legionella hackeliae	2
Legionella jordanis	1
Legionella lansingensis	1
Legionella maceachernii	1
Legionella oakridgensis	
Legionella parisiensis	1
Legionella sainthelensi	2
Legionella tucsonensis	
Legionella wadsworthii	1

There are more than 40 species and 64 subgroups in the genus *Legionella*. More than 15% of human infections are caused by *Legionella pneumophila* serogroup 1, but other serogroups and other species, particularly *Legionella micdadei*, have also been implicated (Table 231.10).

EPIDEMIOLOGY

Legionella spp. are distributed worldwide. They are found in natural bodies of fresh water — streams, rivers, ponds, lakes and thermal pools — in moist soil and mud. They have even been found in the canopy of the rain forest. The organisms are able to survive in moist environments for long periods of time and can withstand temperatures of 32–154°F (0–68°C) and a pH range of 5.0–8.5 They can survive chlorination and can thus enter water supply systems and proliferate in thermal habitats, including air-conditioning cooling towers, hot water systems, shower heads, taps, whirlpool spas and respiratory ventilators. The organisms are found in biofilms on the surfaces of these systems, where they are far less susceptible to the effects of biocides and chlorine. The growth of *Legionella* spp. is aided by co-existing micro-organisms, which provide nutrients, and free-living amebae, in which the *Legionella* spp. can reside and multiply. [62]

The exact incidence of *Legionella* infections is difficult to determine. There is no doubt that exposure to *Legionella* spp. is a fairly frequent event and serologic surveys suggest that asymptomatic infection and seroconversion are common. Large-scale surveys of pneumonia suggest that *Legionella* spp. cause 2–5% of community-acquired pneumonia and up to 30% of nosocomial pneumonia.

Cases may be sporadic or occur as part of an outbreak. The original description of Legionnaires' disease was of an outbreak of a febrile respiratory illness in delegates at an American Legion convention in Philadelphia in 1976. A total of 221 people developed pneumonia and 34 died.[63] Retrospective studies have revealed that the first proven case occurred in 1947 and the first known epidemic was in 1965 in Washington, DC.

Sporadic cases are reported throughout the year, but most cases of epidemic infection occur in the summer and autumn, presumably because warmer weather encourages proliferation of the bacteria in water. The disease tends to occur in the middle-aged and elderly, especially in people who have impaired respiratory and cardiac function, who are heavy smokers or who are immunocompromised. Cases have been documented in children. Person-to-person spread has not been demonstrated. Surgery is a major predisposition in nosocomial infection, with transplant patients at the greatest risk.

Legionella is a good example of an organism that has been present in the environment for a long time but has been brought into close contact with humans as a result of technical developments. Organisms are disseminated via contaminated water droplets from nebulizers and humidifiers and in aerosols from cooling towers, whirlpools and evaporative condensers. Infection arises from inhalation of contaminated aerosols, direct instillation during surgery or possibly by ingestion of contaminated water. Hospital equipment that has been rinsed in tap water prior to use may be a source of infection.

PATHOGENICITY

Legionella spp. residing in water are transmitted to humans via aerosols. Droplet nuclei of less than 5µm will reach the alveoli, where the organisms are ingested by alveolar macrophages. *Legionella* is a facultative intracellular parasite and multiplies freely within the macrophages. The bacteria bind to the alveolar macrophages via complement receptors and are engulfed in phagosomes. Phagolysosome fusion is inhibited by an unknown mechanism that prevents intracellular killing. The bacteria multiply within the phagosomes. Eventually the cell ruptures, releasing bacteria, which are then taken up by other macrophages. This process produces chemotactic substances that attract polymorphonuclear leukocytes and monocytes. The inflammatory response results in a destructive pneumonia.

The virulence factors of *L. pneumophila* have not been fully determined. A 24kDa protein, macrophage infectivity potentiator, appears to be required for virulence. The major outer membrane protein may be important in the uptake of *Legionella* spp. by macrophages. A variety of extracellular enzymes are produced by *Legionella*,

including proteases, esterases and hemolysins, but these have not been shown to be important virulence factors.[64] The virulence of *L. pneumophila* may be enhanced by multiplication in amebae.

PREVENTION

Prevention of legionellosis depends upon identification of the environmental source and reduction of *Legionella* colonization. The most commonly used method of decontamination is periodic superheating and flushing of water supplies. This method is particularly useful for urgent disinfection during an outbreak. Hyperchlorination is no longer recommended because chlorine decomposes in hot water and *Legionella* spp. are relatively chlorine-tolerant. Biocides are relatively ineffective. The most effective method appears to be copper-silver ionization units, which generate metallic ions that disrupt and kill bacterial cells.[65] Despite these costly measures it is often impossible to eliminate *Legionella* spp. from water supplies.[66]

New water systems should be designed to minimize the risk of heavy colonization with *Legionella* spp., avoiding 'dead spaces', stagnation, materials that support the growth of *Legionella* spp. and the build-up of sediment. Regular monitoring for *Legionella* spp. is also advisable, particularly in high-risk areas.

Several putative vaccines have been developed that induce cell-mediated immunity in guinea pigs. These may be an option in the future for highly susceptible patients.

DIAGNOSTIC MICROBIOLOGY

Definitive diagnosis of legionellosis depends on culturing *Legionella* organisms, detecting *Legionella* antigens in body fluids or demonstrating a serologic response to the infection.[67] A PCR method is also available but does not appear to be any more sensitive than culture. A PCR test can be successfully used to identify *Legionella* in water samples.

Legionella spp. can be cultured from sputum, endotracheal aspirates, bronchoalveolar lavages, lung biopsies and pleural fluid. Sputum samples should not be diluted in saline, because this can prove inhibitory to some *Legionella* spp., but in distilled water. Gram-stained smears of the sample will reveal poorly staining Gram-negative rods, which are suggestive of legionellosis. Direct immunofluorescence of the smear using a monoclonal antibody to a major outer membrane protein common to all the serogroups of *L. pneumophila* can give a rapid provisional diagnosis but this test is not as sensitive as culture and will only detect *L. pneumophila*.

Cultures must therefore also be carried out. The sample should be cultured on a specific medium such as BCYE. *Legionella* spp. do not grow on blood agar. It is possible to render BCYE semiselective by the inclusion of antibiotics, but this may prove inhibitory to some strains of *Legionella* spp. It is therefore prudent to culture samples on both BCYE and BCYE supplemented with antibiotics. Contaminated material, such as sputum, can be heated at 122°F (50°C) for 30 minutes to suppress the growth of other less heat-stable organisms prior to inoculating the culture plates. Cultures should be incubated at 98.6°F (37°C) in 2–5% carbon dioxide for up to 14 days. *Legionella* spp. are slow growing, and on primary isolation it may take 3–5 days for colonies to appear. Colonies of *Legionella* spp. have a ground-glass appearance, which may be white, gray, pale blue, or purple-tinged (Fig. 231.7). Often the colonies will fluoresce under ultraviolet light.

Colonies that grow on BCYE but not blood agar, that are catalase-positive and have a characteristic Gram-stain morphology should be regarded as presumptive *Legionella* spp. *Legionella pneumophila* hydrolyzes hippurate, starch and gelatin but in general biochemical tests are not of great help in identifying *Legionella* spp. Serologic typing using direct or indirect fluorescent antibody tests with polyclonal or monoclonal antibodies will confirm the identity of clinical isolates.

Urine samples can be examined for *Legionella* soluble antigen using an ELISA test. This test is specific for *L. pneumophila* serogroup

Figure 231-7 Colonies of *Legionella pneumophila* on BCYE agar, showing the typical ground-glass appearance. *With permission from Harrison TG, Taylor AG (eds). A laboratory manual for Legionella. Chichester: Wiley; 1998.*

1 (which causes 70–80% of human infections) and can give a rapid diagnosis. However, it will not detect infections caused by other *Legionella* spp. The antigen persists in the urine for 1–3 weeks but some patients continue to excrete antigen for months.

Serologic tests can be used to make a diagnosis of legionellosis. The most commonly used technique is an indirect fluorescent antibody test, but other techniques, such as ELISA or a rapid micro-agglutination test, are also available. A 4-fold or greater rise in antibody titer or a single titer of 256 or more indicates *Legionella* infection. It may take weeks or even months for the antibody titer to rise, and antibodies can persist for years, so serology is less useful than culture or antigen detection in the diagnosis of acute cases. Serology is much more useful in epidemiologic studies. The only validated serologic test is for *L. pneumophila* serogroup 1. Cross-reacting antibodies may be found in patients who have *Campylobacter* spp. infection.

CLINICAL MANIFESTATIONS

Asymptomatic *Legionella* infections are relatively common, as demonstrated by seroepidemiologic surveys.

Symptomatic *Legionella* infection can present in two distinct ways: a severe pneumonia — Legionnaires' disease — and an acute influenza-like illness known as Pontiac fever.[68]

Legionnaires' disease

The most common presentation is an acute pneumonia,[69] which accounts for 5% of community-acquired pneumonia and up to 30% of nosocomial pneumonia (see also Chapter 34). After an incubation period of 2–10 days there is an acute onset of fever, malaise, myalgia and headache, followed by a nonproductive cough and dyspnea. Confusion can be a prominent feature, and abdominal pain and diarrhea may also be present. The severity of the illness ranges from a mild infection not requiring hospitalization to life-threatening multilobar *Legionella* pneumonia and respiratory failure. The infection may progress to involve many organs, and renal and hepatic dysfunction are commonly seen. Many of the manifestations are not specific for Legionnaires' disease and may occur in other infective pneumonias. Characteristic features suggestive of *Legionella* spp. are hyponatremia (serum sodium less than 130mmol/ml) and a Gram-stained smear of respiratory secretions showing many polymorphs but few if any visible organisms. Legionnaires' disease should be considered in any pneumonia that has failed to respond to ß-lactam antibiotics.

In previously healthy people, the mortality is approximately 10%, but the mortality rate may be 30–50% in immunocompromised patients or those in whom there is a delay in making the diagnosis.

The chest radiograph characteristically shows patchy interstitial inflammation. Initially this is unilateral and involves the lower lobe. The initial infiltrates progress to areas of consolidation over a few days. Pleural effusions are commonly seen.

Bacteremic dissemination may occur, particularly in immunocompromised patients. Cellulitis, pancreatitis, endocarditis and sinusitis have all been reported.

Cases of wound infection and prosthetic valve endocarditis due to *Legionella* infection have been described. These are generally nosocomial infections.

Pontiac fever

Pontiac fever[68] is an acute, self-limited, flu-like, febrile illness that occurs 1–2 days after exposure. It is thought to represent a hypersensitivity reaction following acute exposure. The illness lasts for 2–5 days and there is no evidence of pneumonia. The chest radiograph is clear. The condition resolves spontaneously. Pontiac fever has a high attack rate, in contrast to Legionnaires' disease, in which only a small proportion of people exposed to the organism develop the disease.

MANAGEMENT

Azithromycin, ciprofloxacin or high-dose erythromycin is the treatment of choice. In the patient who is acutely ill with Legionnaires' disease, azithromycin or a quinolone should be administered together with rifampin (rifampicin), although it should be noted that good comparative clinical trials in this area are lacking.

Cases of Pontiac fever resolve spontaneously without the need for antibiotic therapy.

MORAXELLA SPECIES

NATURE

The genus *Moraxella* is composed of two subgenera, *Moraxella* and *Branhamella*. [70] *Moraxella* (*Moraxella*) spp. are short, oxidase-positive, catalase-positive, DNA-ase-negative Gram-negative coccobacilli that often appear in pairs. They are strictly aerobic and asaccharolytic. The G + C content of DNA is 40–40mol%.

Moraxella (*Moraxella*) *lacunata* is associated with conjunctivitis. *Moraxella* (*Moraxella*) *non-liquefaciens* is a commensal in the upper respiratory tract and may be a secondary invader in respiratory infections. *Moraxella* (*Moraxella*) *osloensis* is a common commensal in the genital tract and may be misidentified as *Neisseria gonorrhoeae*. Unlike *N. gonorrhoeae*, *M. osloensis* grows well on blood agar. *M. osloensis* has been reported in cases of septic arthritis, osteomyelitis and bacteremia. These three organisms will not be further discussed.

Moraxella (*Branhamella*) spp. are Gram-negative diplococci that morphologically and phenotypically resemble *Neisseria* spp. They are aerobic, oxidase-positive, catalase-positive, DNA-ase-positive and asaccharolytic. The G + C content of DNA is 40–45mol%. *Moraxella* (*Branhamella*) *catarrhalis* is now recognized as an important cause of upper and lower respiratory tract infections in children and adults.

EPIDEMIOLOGY

Moraxella (*Branhamella*) *catarrhalis* is exclusively found in humans. The nasopharyngeal carriage rate of *M. catarrhalis* is 1–3% in healthy adults, but up to 75% of infants and young children are colonized with this organism. This remarkable disparity remains unexplained. Nasopharyngeal carriage rates are significantly higher in the autumn and winter months. It also appears that the carriage rate is higher in adults who have chronic lung disease than in healthy adults.

PATHOGENICITY

The majority of *M. catarrhalis* infections result from contiguous spread of organisms colonizing the upper respiratory tract. Little is known of the events that alter the host-pathogen relationship and cause a shift from asymptomatic colonization to infection in the upper or lower respiratory tract.

The virulence factors of *M. catarrhalis* have not been fully elucidated. The majority of strains possess pili or fimbriae, which play a role in adherence to host mucosal cells. The outer membrane lipooligosaccharide (LOS) is probably a virulence factor. Three serotypes of LOS have been identified. Some strains are serum-resistant, by virtue of interference with the formation of the membrane attack complex of complement. There is some evidence that *M. catarrhalis* possesses a polysaccharide capsule. *M. catarrhalis* synthesizes a significant amount of histamine, which results in decreased mucociliary clearing and increased colonization of the respiratory tract. [71]

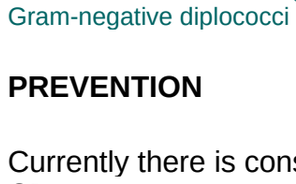

Figure 231-8 Gram-stained smear of sputum containing *Moraxella catarrhalis*. Sample from a patient suffering an acute exacerbation of chronic bronchitis showing Gram-negative diplococci and leukocytes (*Moraxella catarrhalis*).

PREVENTION

Currently there is considerable interest in the development of a vaccine to prevent *M. catarrhalis* infections based on OMPs including UspA, CopB, OMP B1 and OMP CD.

DIAGNOSTIC MICROBIOLOGY

Moraxella catarrhalis grows well on blood agar and chocolate agar, producing small, nonhemolytic, grayish-white colonies that slide across the agar surface, like a hockey puck, when pushed with a bacteriologic loop. It may be difficult to distinguish *M. catarrhalis* from commensal *Neisseria* spp. in respiratory tract specimens, and the use of selective media may be necessary. Gram-stained films reveal Gram-negative diplococci but *M. catarrhalis* often resists destaining and thus may appear to be Gram-positive (Fig. 231.8). The bacteria are oxidase-positive. *M. catarrhalis* is DNA-ase-positive, nitrate-reductase-positive and hydrolyzes tributyrin, tests that differentiate it from the majority of *Neisseria* spp.

A PCR test has been devised that can be applied to clinical material without the need for bacterial culture. This test has been successfully used to detect *M. catarrhalis* in middle ear effusions and appears to be more sensitive than culture.

CLINICAL MANIFESTATIONS

Moraxella catarrhalis is an important cause of otitis media and sinusitis in children, being the third most common cause of these conditions after *Streptococcus pneumoniae* and *H. influenzae*.

In adults *M. catarrhalis* is associated with acute exacerbations of chronic bronchitis, being the third most common isolate after *H. influenzae* and *S. pneumoniae*. *M. catarrhalis* may cause pneumonia, especially in the elderly and is associated with a poor prognosis. Lower respiratory tract infection with *M. catarrhalis* is more common in the winter months.

There have been several reports of nosocomial outbreaks of lower respiratory tract infections due to *M. catarrhalis* in respiratory units.[69]

Moraxella catarrhalis may occasionally cause invasive infections, including bacteremia, meningitis and endocarditis.

MANAGEMENT

Before 1977 no ß-lactamase-positive isolates of *M. catarrhalis* had been reported. By 1980 75% of *M. catarrhalis* isolates in the USA were ß-lactamase-positive and now virtually all strains of *M. catarrhalis* are ß-lactamase-positive. There are two *M. catarrhalis* ß-lactamases, BRO-1 and BRO-2, which are unique to this genus. There is some evidence that, in mixed infections, *M. catarrhalis* ß-lactamases can protect other respiratory pathogens such as *S. pneumoniae* and *H. influenzae* from the action of ß-lactam antibiotics. The ß-lactamases of *M. catarrhalis* are chromosomal and inducible; therefore ampicillin therapy should be avoided.

Many infections due to *M. catarrhalis* can be treated with oral antibiotics, including amoxicillin-clavulanate, trimethoprim, sulfamethoxazole, tetracyclines, (contraindicated in children aged under 7 years), cefaclor, clarithromycin, azithromycin and ciprofloxacin.

PASTEURELLA SPECIES

NATURE

Pasteurella spp. are very small, nonmotile, non-spore-forming Gram-negative bacteria that are coccoid, oval or rod-shaped. They often exhibit bipolar staining. They are aerobic and facultatively anaerobic. Most species are catalase-positive and slowly oxidase-positive. They attack carbohydrates readily, forming acid but no gas. Nitrates are reduced to nitrite. *Pasteurella* spp. will grow on ordinary laboratory media at 98.6°F (37°C). The G + C content of the DNA is 40–45mol%.

The family Pasteurellaceae encompasses the genera *Pasteurella*, *Actinobacillus* and *Haemophilus*. There are 11 species within the genus *Pasteurella*. The species *Pasteurella multocida* is further subdivided into subspecies on the basis of acid production from dulcitol and sorbitol. Most human infections are caused by *P. multocida*.

EPIDEMIOLOGY

Pasteurella spp. are distributed worldwide. They are commensals or parasitic organisms in the upper respiratory tract and gastrointestinal tracts of many domestic and wild animals and birds.

Pasteurella multocida is found in the oropharynx of 50–90% of domestic cats, 50–70% of dogs and 50% of pigs. It is the cause of hemorrhagic sepsis in cattle and water buffalo, pneumonia in pigs and sheep, snuffles in rabbits and fowl, and cholera in chickens, ducks and turkeys. It is generally carried asymptomatically by cats and dogs but may infect bites inflicted on these animals by feline or canine assailants. Humans become infected through contact with infected animals.

Pasteurella multocida can remain viable in soil and water for up to 4 weeks and may survive in animal carcasses for up to 3 months. Most human infections are caused by *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica*. *Pasteurella canis*, *Pasteurella dagmatis* and *Pasteurella stomatis* have also been associated with human disease (Table 231.11).

PATHOGENICITY

Pasteurella multocida is transmitted to humans by contact with infected animals. The most common source of infection is bites, scratches, or licks from cats or dogs (see Chapter 91).[72] Respiratory tract infections in those handling animals may result from airborne transmission. In a few patients it is not possible to document an animal source of the infection.

TABLE 231-11 -- Species of *Pasteurella* associated with infections in humans.

SPECIES OF *PASTEURELLA* ASSOCIATED WITH INFECTIONS IN HUMANS
Pasteurella multocida subsp. *multocida*
Pasteurella multocida subsp. *septica*
Pasteurella canis
Pasteurella dagmatis
Pasteurella stomatis

Virulence in *P. multocida* is associated with the degree of encapsulation. Strains with large capsules are more resistant to phagocytosis in vivo. There are four capsular types — A, B, D and E — and 11 somatic antigens.

PREVENTION

The only way of effectively preventing *Pasteurella* infections is to avoid contact with domestic or wild animals. Animal bites should be cleaned promptly and devitalized tissue should be debrided. Prophylactic antibiotics may be indicated after cat and dog bites, particularly if the patient is immunocompromised or diabetic or the wound affects the hands or face.

DIAGNOSTIC MICROBIOLOGY

A clinical history of animal bites will, in most instances, alert the laboratory to the possibility of *Pasteurella* spp. Samples should be cultured on blood agar plates at 98.6°F (37°C) for 24 hours. *Pasteurella multocida* grows as small, nonhemolytic colonies. Gramstained films reveal very small Gram-negative coccobacilli. The organisms often show bipolar staining in methylene blue preparations. The identity of the organism can be confirmed by a series of biochemical and serologic tests and serology (Table 231.12). [73]

CLINICAL MANIFESTATIONS

Pasteurella infections in humans usually present as soft tissue infections. The onset is acute, several hours after being bitten or scratched by a domestic or wild animal. Open wounds may be infected by animal licks. The area is erythematous, painful and swollen. In nearly 50% of cases the infection spreads to deeper tissues, leading to tenosynovitis, abscess formation, septic arthritis or osteomyelitis. There may be an accompanying regional lymphadenopathy or lymphangitis. The severity of the infection is often greater than one would suspect on first inspection. Some *Pasteurella* infections present as respiratory infections, which may be chronic. In other cases the patient presents with a bacteremic infection, which may or may not result in a localized focus of infection (Fig. 231.9).[73] [74]

TABLE 231-12 -- Differentiation of *Yersinia* and *Pasteurella* species.

DIFFERENTIATION OF *YERSINIA* AND *PASTEURELLA* SPECIES										
Species	**Growth on MacConkey agar**	**Oxidase**	**Catalase**	**Motility at 22°C**	**ONPG**	**Acid (no gas) production from**		**Indole**	**Urease**	**Ornithine decarboxylase**
						Sucrose	**Maltose**			
Yersinia pestis	+	-	+	-	+	-	+	-	-	-
Yersinia enterocolitica	+	-	+	+	+	+	-	-	+	+
Yersinia pseudotuberculosis	+	-	+	+	+	-	+	-	+	-
Pasteurella multocida	-	±	+	-	-	+	-	+	-	+

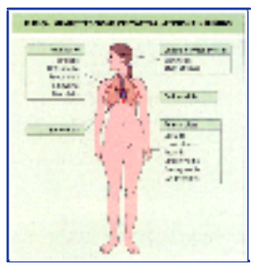

Figure 231-9 Clinical manifestations of *Pasteurella* infection in humans.

Approximately one-third of patients who have bacteremia have underlying cirrhosis.

MANAGEMENT

Animal bites should be carefully cleaned, irrigated and debrided. Penicillin is the treatment of choice for *Pasteurella* infections. Other agents with good activity include ampicillin, amoxicillin-clavulanate, cefuroxime and ciprofloxacin. Many infected animal bites have a polymicrobial etiology and it is prudent to use a regimen that provides good cover against anaerobes and streptococci as well, such as amoxicillin-clavulanate. A strain of *P. multocida* that produced ROB-1 ß-lactamase has been reported.

Prophylactic antibiotics for cat and dog bites are sometimes advocated but their value is not clearly established (see Chapter 91).

YERSINIA SPECIES

NATURE

Yersinia spp. are members of the Enterobacteriaceae. They are short, pleomorphic Gram-negative rods or coccobacilli, which often exhibit

bipolar staining. *Yersinia pestis* is nonmotile. Other species are nonmotile at 98.6°F (37°C) but motile at temperatures less than 86°F (30°C) by means of peritrichous flagella. They are aerobic and facultatively anaerobic, oxidase-negative and catalase-positive. They are nonlactose fermenters. *Yersinia pestis* is urease-negative, and *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are both urease-positive. *Yersinia* spp. grow on simple laboratory media and are tolerant of bile salts. The optimal temperature for growth is 82–86°F (27.8–;30°C). They do not form spores or capsules, but *Y. pestis* produces a capsule-like envelope. They share antigens with other members of the Enterobacteriaceae.

The genus *Yersinia* contains three important human pathogens associated with zoonotic infections — *Y. pestis* causes plague, and *Y. pseudotuberculosis* and *Y. enterocolitica* give rise to infections known as yersiniosis.

Because these two diseases are very different they are described separately.

PLAGUE

Epidemiology

Yersinia pestis causes plague, an infection that has afflicted humans since biblical times. It was known and feared as 'the Black Death' in the Middle Ages. Over the centuries there have been about 150 recorded epidemics and pandemics of plague. The pandemic of plague that swept across Europe in the 14th century killed about one-third of the total population of continental Europe and England. The 'Great Plague' of 1665 killed more than 100,000 people in London. In the 20th century plague is still found in many parts of the world, including China, Mongolia, South East Asia, India, central and southern Africa, the Middle East, South America and the southwestern states of the USA. Major epidemics or pandemics no longer occur, possibly because of improved standards of hygiene, more effective rodent control and the use of insecticides. Worldwide, approximately 3000 cases of plague were reported in 1994.[75] Males and females are equally affected.

Pathogenicity

Plague is a zoonotic infection. It is transmitted between its natural hosts, wild rodents, by rodent flea bites or by the consumption of infected animal carcasses. The rodents may succumb to plague or become carriers of the organism. This carrier state ensures the endemicity of plague in some parts of the world. *Yersinia pestis* persists in many sylvatic rodent species, including ground squirrels, tree rats, voles and prairie dogs. Urban rats — *Rattus rattus* and *Rattus norvegicus* — are the most important reservoirs of plague for human infections.

Humans are accidentally infected when they are bitten by a plague-carrying rodent flea. Occasionally, humans are infected by handling infected animal tissues. Only rarely, during an epidemic, does person-to-person spread occur. The most efficient vector is the oriental rat flea, *Xenopsylla cheopsis*, but there are over 30 species of rodent fleas capable of transmitting plague. Occasionally, infection may pass from patient to patient via the human flea, *Pulex irritans*. This may have been an important vector in some of the medieval epidemics. During an epizootic there is a build-up of infections in the rodent population and many may die. It is at this time that human infections are more likely to occur, since the rodent fleas turn to humans in search of food (Fig. 231.10).

The flea vector becomes infected by feeding on the blood of a diseased rodent. It ingests *Y. pestis* with the blood meal. The organisms multiply within the stomach of the flea. At temperatures of less than 80°F (26.7°C), *Y. pestis* produces coagulase and a blood clot is formed. This fibrinous clot, which contains viable *Y. pestis*, blocks the proventriculus of the flea. When the hungry flea attempts to feed

Figure 231-10 Transmission of plague.

from another rodent or human, the fresh blood meal, together with thousands of *Y. pestis* bacilli, is regurgitated back into the bite wound. Thus the infection is transmitted to a new host. When the ambient temperature is over 80°F (26.7°C), *Y. pestis* does not produce coagulase and may form fibrinolysin, so a clot does not form and blockage and regurgitation do not occur. Transmission of *Y. pestis* is therefore uncommon during hot, dry seasons.

On entering the new host, the bacilli migrate via the lymphatics to the regional lymph nodes. In the flea, *Y. pestis* produces only a small amount of envelope antigen (F-1 antigen). The ingested organisms are readily phagocytosed by polymorphonuclear phagocytes and mononuclear phagocytes. *Yersinia pestis* is a facultative intracellular parasite and resists killing within the mononuclear phagocytes. *Y. pestis* can multiply within these cells, where it elaborates F-1 envelope antigen. If the macrophages are lysed, the re-enveloped organisms are able to resist phagocytosis and can multiply freely in the extracellular environment. The regional lymph nodes become hot, tender and inflamed and undergo hemorrhagic necrosis. This produces the black buboes characteristic of plague. The infection then spills into the bloodstream and may rapidly spread to the liver, lungs and spleen. If the lungs are affected, a severe pneumonia develops and large numbers of plague bacilli are expectorated into the air.[76]

There are a number of putative virulence factors — F-1 antigen consists of a glycoprotein (F-1A) and a carbohydrate-free protein (F-1B). It is antiphagocytic and stimulates protective immunity in mice and humans.

Other important virulence factors are V–W antigens and *Yersinia* outer membrane proteins (YOPs).[77] These protein antigens are produced at 98.6°F (37°C) in the presence of low concentrations of calcium. They appear to be important both for intracellular survival and multiplication and for extracellular survival by virtue of their antiphagocytic activity. The LPS of *Y. pestis* is in part responsible for hemorrhagic necrosis and vascular collapse, but the mechanism is again poorly understood (Table 231.13). Coagulase and fibrinolysin are produced by *Y. pestis*. They aid survival and dissemination within the invertebrate host.

TABLE 231-13 -- Virulence factors of *Yersinia pestis.*

VIRULENCE FACTORS OF *YERSINIA PESTIS*		
Factor	**Composition**	**Pathogenic role**
F-1 antigen 'envelope'	Glycoprotein F^1 A + protein F^1 B	Antiphagocytic

V antigen	Protein	Antiphagocytic
+		
W antigen	Lipoprotein	(Aids intracellular survival)
YOPs	Proteins	Antiphagocytic
LPS	Lipopolysaccharide	Hemorrhagic
		Focal necrosis

Prevention

Prevention depends on effective control of the rodent and flea populations that harbor *Y. pestis*. Fleas can be controlled by the use of insecticides in domestic settings. A variety of measures have been used to control rats in urban environments (e.g. ratproofing houses) and to prevent rats migrating on ships or airplanes. It is, however, virtually impossible to eliminate sylvatic rodents, which remain a reservoir for plague.

A formalin-killed vaccine is effective against bubonic plague but not pneumonic plague. It is recommended for travelers to endemic areas, workers who handle rodents and laboratory staff working with *Y. pestis*. Protection is only short-lived and booster doses are required for those who continue to be exposed to the risk of infection. A subunit vaccine containing recombinant F1 and V antigens is under development and appears to be protective in an animal model.[78]

Diagnostic microbiology

Plague should be suspected in febrile patients exposed to flea bites or rodents in endemic parts of the world. It is important to notify the laboratory if plague is suspected so that appropriate precautions can be taken. There is a considerable risk of laboratory-acquired infection. Material suspected of containing *Y. pestis* must be handled in a containment level 3 laboratory in a category I or III exhaust protective cabinet by experienced, trained personnel. Smears taken from bubo aspirates, blood, sputum or cerebrospinal fluid should be examined using Gram's stain and methylene blue. Gram's stain will reveal Gram-negative pleomorphic coccobacilli with rounded ends. Methylene blue stain will demonstrate bipolar staining. *Yersinia pestis* appear light blue with dark blue polar bodies. Smears can also be examined by direct immunofluorescence.

Material can be cultured on blood agar or MacConkey agar or in broth cultures. Cultures should be incubated at 80°F (26.7°C). Tiny, translucent, nonhemolytic colonies appear on blood agar after 24 hours. After further incubation the colonies enlarge and become opaque. *Yersinia pestis* grows rather poorly in MacConkey agar and the colonies tend to autolyze after 2–3 days. In fluid culture *Y. pestis* tends to form chains.

The identity of presumptive colonies of *Y. pestis* can be confirmed using cultural and biochemical tests. Alternatively, definitive identification can be made by direct immunofluorescence of the organisms. A serologic diagnosis can be made using a passive hemagglutination test using tanned sheep red cells to which F-1 antigen has been adsorbed, or a complement fixation test. A 4-fold or greater rise in titer or a single titer over 1:16 in convalescent serum indicates plague infection. For large-scale seroepidemiologic surveys an ELISA test for antibodies to F-1 antigen can be used.

Strains of *Y. pestis* can be typed using bacteriophage typing.

TABLE 231-14 -- Plague syndromes.

PLAGUE SYNDROMES	
Syndrome	**Clinical features**
Bubonic plague	Fever
	Painful lymph node
	Bubo
Pneumonic plague	Primary following inhalation of *Yersinia pestis* or secondary to bubonic plague
	Fever
	Cough
	Dyspnea
	Hemoptysis
	± bubo
Septicemic plague	Fever
	Shock
	Purpura
	Distal gangrene
	No bubo
Plague meningitis	Secondary to bubonic plague

Clinical manifestations

There are a number of distinct clinical presentations of plague (Table 231.14) (see also Chapter 176).

Bubonic plague

The most common presentation is bubonic plague. Following a flea bite, which is often on the lower extremities, the bacteria proliferate in the regional lymph nodes for 2–8 days. The patient then develops a fever of sudden onset and an intensely painful swelling in a group of lymph nodes. This is the bubo. The most common site for the primary bubo is the groin. From this site the bacteria disseminate throughout the body in a transient bacteremia. Some patients will develop a secondary pneumonia or sepsis. Most patients do not develop skin lesions. Untreated patients may die within 2–4 days of the onset of symptoms.

Pneumonic plague

In some patients who have bubonic plague the infection spreads to the lung via the bloodstream. The patient develops a high fever and cough, chest pain and hemoptysis. Less commonly, pulmonary plague may be the primary event following the inhalation of infected droplet nuclei from patients who have pneumonic disease. Patients who have primary pulmonary plague do not have buboes. Pneumonic plague is highly contagious because large numbers of plague bacilli are expectorated during coughing. This form of plague carries a higher mortality than bubonic plague.

Septicemic plague

In some cases a flea bite is followed by proliferation of *Y. pestis* in the circulation without any localization in lymph nodes and bubo formation. This form is more common in children and is often rapidly fatal.

Other manifestations of plague include a meningitis secondary to inadequately treated bubonic plague. Overall, the mortality of untreated plague is more than 50%.

Management

The treatment of choice remains streptomycin. It is essential to institute therapy as soon as possible because untreated plague runs a rapidly fulminating course. With prompt effective antimicrobial therapy the mortality rate is reduced from over 50% to 6%. Streptomycin resistance has been reported in some parts of the world. Oral tetracycline is an alternative but it is contraindicated in

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children and during pregnancy. Chloramphenicol may also be used and is the treatment of choice for plague meningitis. All cases of plague should be reported to national health authorities and to the World Health Organization (see Chapter 176).

YERSINIOSIS

Yersiniosis encompasses a wide spectrum of infections caused by *Y. enterocolitica* and *Y. pseudotuberculosis*. The clinical features range from gastroenteritis, enterocolitis, or measenteric adenitis to sepsis.

Epidemiology

Yersinia enterocolitica is harbored in the gastrointestinal tract of a wide range of mammals, including rodents, cattle, sheep, pigs, cats and dogs. Infected animals tend to become chronic carriers and excrete large numbers of bacilli, which can contaminate water and dairy products. Humans are infected by eating inadequately cooked meat (especially pork) or other food contaminated with *Yersinia* spp. Infection may also arise following contact with an infected domestic animal. In the USA, outbreaks associated with dairy products, chocolate and milk have been reported. Rarely, water is the source of the infection. The ability of *Y. enterocolitica* to grow at 39°F (4°C) means that refrigerated meat and meat products can become a potent source of infection. Infections are more common in children than adults. Most cases occur in the autumn and winter. *Yersinia enterocolitica* sepsis has been reported following the transfusion of blood that has been stored for more than 3 weeks at 39°F.

Yersinia enterocolitica is a well-recognized cause of abdominal pain and diarrhea in Scandinavia and northern Europe. It is less commonly seen in the USA. Cases have been reported from other parts of the world. In Europe, infections are generally sporadic and the predominant serotypes are 03 and 09. In the USA, sporadic infections are less common but food-borne outbreaks caused by serotypes 03 and 09 have been described.[79]

Yersinia pseudotuberculosis infection is less commonly seen. The organism is harbored in the gastrointestinal tract of rodents, farm animals and birds. Human infections have been reported from all parts of the world but, as with *Y. enterocolitica*, it is more common in northern Europe than elsewhere. Infection is generally seen in children and affects males more frequently than females. The majority of infections occur in the winter.[80]

Pathogenicity

Yersinia enterocolitica generally gains entry to the body via the gastrointestinal tract. The infecting does is 10^8 –10^9 organisms/ml. Within the ileum the organisms adhere to the mucosa and incite an inflammatory response. They also invade mucosal cells, Peyer's patches and macrophages, where they can survive intracellularly and multiply. This results in a severe diarrhea and abdominal pain mimicking acute appendicitis. There may be necrosis of Peyer's patches, ulcerative ileitis or mesenteric adenitis.

The organisms may disseminate through the body, producing sepsis and splenic and hepatic abscesses. A reactive polyarthritis can occur, particularly in HLA-B27-positive patients.[81]

Yersinia pseudotuberculosis generally gives rise to mesenteric adenitis. Both species produce V–W and YOPs antigens, which are essential for virulence, and an outer membrane protein, invasin, which is involved in adherence to and invasion of cells.

Prevention

Prevention of yersiniosis depends on good animal husbandry and careful slaughtering techniques to minimize contamination of meat with orofecal material. Meat should not be consumed raw and should not be stored at 39°F (4°C) for prolonged periods before consumption. There is no effective vaccine.

Diagnostic microbiology

Material for culture will include stool specimens, blood, lymph nodes and food samples. Specimens should be cultured on blood agar and MacConkey agar at 80°F (26.7C) for 24 hours. Heavily contaminated samples such as feces can be subjected to a cold enrichment technique whereby the sample is inoculated into phosphate-buffered saline and incubated at 39°F (4°C) for 3 weeks or more. The broth is subcultured at weekly intervals on to MacConkey agar or a special selective medium, CIN (cefsulodin, irgasan, novobiocin) agar. *Yersinia pseudotuberculosis* is rarely isolated from feces.

Presumptive colonies of *Yersinia* spp. are identified using a range of physical and biochemical tests (see Table 231.12). The serotype can be determined using slide agglutination tests.

A serologic diagnosis depends on demonstrating a significant rise of serotype-specific antibodies.

Clinical manifestations

A variety of clinical presentations have been documented in yersiniosis (Table 231.15). The commonest form is acute gastroenteritis due to *Y. enterocolitica*. [81] [82] This is often indistinguishable from *Salmonella* or *Campylobacter* gastroenteritis, and the patient complains of abdominal pain, diarrhea and vomiting. The incubation period is probably 3–7 days. The commonest presentation of *Y. pseudotuberculosis* infection is mesenteric adenitis, which can mimic acute appendicitis.

Some patients develop a reactive polyarthritis a few days after the onset of enteritis. This is associated with the presence of HLA-B27. Erythema nodosum occurs in about one-third of patients.

Sepsis is uncommon and is generally associated with a variety of underlying conditions. These include diabetes mellitus, cirrhosis, old age, malignancy, hemachromatosis, multiple blood transfusions and iron overload, particularly when treated with deferoxamine. Patients who have sepsis may develop hepatic or splenic abscesses, endocarditis or meningitis.

The mortality of *Yersinia* sepsis is 50%.

Management

Yersinia enteritis and mesenteric adenitis do not generally require antimicrobial chemotherapy. Sepsis, extraintestinal foci of infection and enteritis in immunocompromised patients should be treated with antimicrobials. *Yersinia enterocolitica* is generally resistant to penicillin, ampicillin and first-generation cephalosporins. Aminoglycosides, quinolones, tetracyclines and third-generation cephalosporins have all been used successfully to treat *Y. enterocolitica* infections. *Yersinia pseudotuberculosis* is usually sensitive to benzylpenicillin and ampicillin.

TABLE 231-15 -- Clinical manifestations of yersiniosis.

CLINICAL MANIFESTATIONS OF YERSINIOSIS

Yersinia enterocolitica	***Yersinia pseudotuberculosis***
Gastroenteritis	Mesenteric lymphadenitis
Mesenteric lymphadenitis	Ulcerative ileitis
Ulcerative ileitis	Septicemia
Septicemia	Erythema nodosum
Hepatic or splenic abscesses	
Reactive polyarthritis (HLA-B27)	
Reiter's syndrome	
Erythema nodosum	
Meningitis	
Exudative pharyngitis	
Note that septicemia in *Yersinia enterocolitica* infection is associated with underlying conditions, including diabetes mellitus, cirrhosis, malignancy, hemochromatosis, old age, anemia, iron overload and deferoxamine treatment.	

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Chapter 232 - Anaerobic Bacteria

Itzhak Brook

INTRODUCTION

Infections caused by anaerobic bacteria are common, and may be serious and life-threatening. Anaerobes are the predominant components of the bacterial flora of normal human skin and mucous membranes,[1] and are therefore a common cause of bacterial infections of endogenous origin. Because of their fastidious nature, these organisms are difficult to isolate from infectious sites and are often overlooked. Delay in appropriate therapy against these organisms often leads to clinical failures. Their isolation requires appropriate methods of collection, transportation and cultivation of specimens.[2] [3] [4] Treatment of anaerobic bacterial infection is complicated by the slow growth of these organisms, which makes diagnosis in the laboratory only possible after several days, by the often polymicrobial nature of the infection and by the growing resistance of anaerobic bacteria to antimicrobial agents.

NATURE

Anaerobic bacteria do not grow on solid media in the presence of room air (10% carbon dioxide and 18% oxygen), whereas facultative anaerobic bacteria grow both in the presence and in the absence of air. Microaerophilic bacteria grow poorly or not at all aerobically, but grow better under 10% carbon dioxide or anaerobically. Anaerobes can be divided into strict anaerobes that are unable to grow in the presence of more than 0.5% oxygen and moderate anaerobes that are capable of growing at between 2 and 8% oxygen.[4] Anaerobes generally do not possess catalase, but some clinical isolates produce superoxide dismutase that can protect them from oxygen.

The clinically important anaerobic bacteria are:

- six genera of Gram-negative rods (*Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila* and *Sutterella* spp.);
- Gram-positive cocci (primarily *Peptostreptococcus* spp.);
- Gram-positive spore-forming (*Clostridium* spp.) and nonspore-forming bacilli (*Actinomyces, Propionibacterium, Eubacterium, Lactobacillus* and *Bifidobacterium* spp.); and
- Gram-negative cocci (mainly *Veillonella* spp.) (Table 232.1).[4] [5] [6]

The frequency of recovery of anaerobic bacterial strains differs in various infectious sites (Table 232.2). Mixed infections caused by numerous aerobic and anaerobic organisms are observed commonly in clinical situations (Fig. 232.1). [2] [3]

The taxonomy of anaerobic bacteria has changed in recent years because of improved characterization methods using genetic studies.[4] [6] The ability to differentiate between similar strains enables better characterization of type of infection and predicted antimicrobial susceptibility.

The species of anaerobes most frequently isolated from clinical infections are, in decreasing frequency:

- Gram-negative bacilli,
- Gram-positive cocci,
- *Clostridium* spp.,
- *Fusobacterium* spp.,
- Gram-positive bacilli (*Eubacterium, Lactobacillus, Propionibacterium, Actinomyces* and *Bifidobacterium* spp.), and
- Gram-negative cocci (*Veillonella* and *Acidaminococcus* spp.).[2] [3] [4]

Approximately 95% of the anaerobes isolated from clinical infections are members of these genera. The remaining isolates belong to species not yet described, but these usually can be assigned to the appropriate genus on the basis of morphologic characteristics and fermentation products. The frequency of recovery of anaerobic strains differs in various infectious sites.

EPIDEMIOLOGY

Most infections are caused by anaerobes that belong to the normal flora. Only when the situation is out of balance and the local defenses are decreased in capacity, anaerobic infection can then occur. Some anaerobic infections are caused by contamination of

TABLE 232-1 -- Predominant anaerobic bacteria.

PREDOMINANT ANAEROBIC BACTERIA	
Gram-positive cocci	*Peptostreptococcus* spp.: *P. magnus*, *P. asaccharolyticus*, *P. prevotii*, *P. intermedius*, *P. anaerobius*, *P. micros*
	Microaerophilic streptococci (not true anaerobes)
Gram-positive nonspore-forming bacilli	*Propionibacterium* spp.: *P. acnes*, *P. propionicum*, *P. granulosum*
	Eubacterium tentum
	Bifidobacterium spp.: *B. eriksonii*, *B. dentium*
	Actinomyces spp.: *A. israelii*, *A. naestundii*, *A. viscosus*, *A. odontolyticus*, *A. meyerii*
Gram-positive spore-forming bacilli	*Clostridium* spp.: *C. perfringens*, *C. ramosum*, *C. septicum*, *C. novyi*, *C. histolytica*, *C. sporogenes*, *C. difficile*, *C. bifermentans*, *C. butyricum*, *C. innocuum*, *C. sordellii*, *C. botulinum*, *C. tetani*
Gram-negative bacilli	*Bacteroides fragilis* group: *B. fragilis*, *B. thetaiotaomicron*, *B. distasonis*, *B. vulgatus*, *B. ovatus*, *B. uniformis*
	Other *Bacteroides* spp.: *B. gracilis*, *B. ureolyticus*
	Pigmented *Prevotella* spp.: *P. melaninogenica*, *P. intermedia*, *P. denticola*, *P. loescheii*, *P. corporis*, *P. nigrescens*
	Other *Prevotella* spp.: *P. oris*, *P. buccae*, *P. oralis* group (*P. oralis*, *P. buccalis*, *P. veroralis*), *P. bivia*, *P. disiens*
	Porphyromonas spp.: *P. asaccharolytica*, *P. gingivalis*, *P. endodontalis*
	Fusobacterium spp.: *F. nucleatum*, *F. necrophorum*, *F. gonidiaformans*, *F. naviforme*, *F. mortiferum*, *F. varium*

TABLE 232-2 -- Anaerobic bacteria most frequently encountered in specific infection sites.

ANAEROBIC BACTERIA MOST FREQUENTLY ENCOUNTERED IN SPECIFIC INFECTION SITES		
Organism		**Infection site**
Gram-positive cocci	*Peptostreptococcus* spp.	Respiratory tract, intra-abdominal and soft-tissue infections
	Microaerophilic streptococci (not obligate anaerobes)	Sinusitis, brain abscesses
Gram-positive bacilli	Nonspore-forming:	
	Actinomyces spp.	Intracranial abscesses, chronic mastoiditis, aspiration pneumonia, head and neck infections
	Propionibacterium acnes	Shunt infections (cardiac, intracranial), infections associated with foreign body
	Bifidobacterium spp.	Chronic otitis media, cervical lymphadenitis, abdominal infections
	Spore-forming:	
	Clostridium perfringens	Soft-tissue infection, sepsis, food poisoning
	Clostridium septicum	Sepsis, neutropenic enterocolitis
	Clostridium difficile	Colitis, antibiotic-associated diarrheal disease
	Clostridium botulinum	Botulism
	Clostridium tetani	Tetanus
	Clostridium ramosum	Soft-tissue infections
Gram-negative bacilli	*Bacteroides fragilis* group	Intra-abdominal and female genital tract infections, sepsis, neonatal infections
	Pigmented *Prevotella* and *Porphyromonas* spp.	Orofacial infections, aspiration pneumonia, periodontitis
	Prevotella oralis	Orofacial infections
	Prevotella oris-buccae	Orofacial infections, intra-abdominal infections
	Prevotella bivia, Prevotella disiens	Female genital tract infections
	Fusobacterium nucleatum	Orofacial and respiratory tract infections, brain abscesses, bacteremia
	Fusobacterium necrophorum	Aspiration pneumonia, bacteremia

wounds by soil containing anaerobes. Such is the case with some *Clostridium* infections. *Clostridium difficile* enterocolitis can lead to epidemics in the hospital. In geriatric wards and nurseries in particular, these bacteria are transmitted by hands from one patient to another. *Clostridium botulinum* causes food poisoning due to the presence of the toxin produced by *C. botulinum* in food.

Gram-positive spore-forming bacilli

Anaerobic spore-forming bacilli belong to the genus *Clostridium*. Morphologically, the clostridia are highly pleomorphic, ranging from short, thick bacilli to long filamentous forms, and are either ramrod

Figure 232-1 Gram stain of a perirectal abscess caused by polymicrobial aerobic and anaerobic flora.

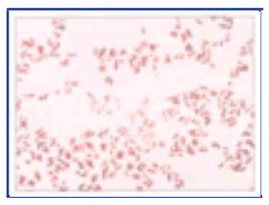

Figure 232-2 Gram stain of *Clostridium perfringens.*

straight or slightly curved. The clostridia found most frequently in clinical infections are *Clostridium perfringens* (Fig. 232.2), *Clostridium septicum, Clostridium ramosum, Clostridium novyi, Clostridium sordellii, Clostridium histolyticum, Clostridium fallax, Clostridium bifermentans* and *Clostridium innocuum*.

Clostridium perfringens, the most commonly recovered *Clostridium* isolate, is an inhabitant of soil and of intestines of humans and animals, and is the most frequently encountered histotoxic *Clostridium* species. This micro-organism, which elaborates a number of necrotizing extracellular toxins,[7] [8] is easily isolated and identified in the clinical laboratory. It can be characterized in direct smears of a purulent exudate by the presence of stout Gram-variable rods of varying length, frequently surrounded by a capsule. *Clostridium perfringens* can cause a devastating illness with a high mortality rate. Bacteremia caused by *Clostridium* spp. is associated with extensive tissue necrosis, hemolytic anemia and renal failure.

Clostridium septicum has often been found to be associated with malignancy. The intestinal tract is thought to be the source of the organism, and most of the isolates are recovered from blood and subcutaneous tissue.

Although *C. botulinum* is usually associated with food poisoning, wound infections caused by this organism are being recognized with increasing frequency. Proteolytic strains of types A and B have been reported from food poisoning and wound infections. Infant botulism occurs with types A, B and F.[7] Disease caused by *C. botulinum* is usually an intoxication produced by ingestion of contaminated food (uncooked meat, poorly processed fish, improperly canned vegetables) containing a highly potent neurotoxin.[8] The polypeptide neurotoxin is relatively heat labile, and food containing this toxin may be rendered innocuous by exposure to 212°F (100°C) for 10 minutes. Infection of a wound with *C. botulinum* occurs rarely and can produce botulism.

Clostridium difficile has been incriminated as the causative agent of antibiotic-associated and spontaneous diarrhea and colitis.[9] *Clostridium tetani* is found in soil and is rarely isolated from human feces. Infections caused by this bacillus are a result of contamination of wounds with soil containing *C. tetani* spores.[8] The spores will

germinate in devitalized tissue and produce the neurotoxin that is responsible for the clinical findings.

Gram-positive, nonspore-forming bacilli

Anaerobic, Gram-positive, nonspore-forming rods comprise part of the microflora of the gingival crevices, the gastrointestinal tract, the vagina and the skin. Several distinct genera are recognized: *Actinomyces, Arcanobacterium, Atopobium, Bifidobacterium, Eubacterium, Lactobacillus, Mobiluncus, Propionibacterium* and *Pseudoramibacter*. The most frequently recovered species are *Propionibacterium, Eubacterium, Bifidobacterium* and *Lactobacillus.*

The *Actinomyces, Arcanobacterium* and *Bifidobacterium* spp. are Gram-positive, pleomorphic, anaerobic to microaerophilic bacilli.

Actinomyces israelii, Actinomyces naeslundii and *Propionibacterium propionicum* are normal inhabitants of the human mouth and throat and are the most frequent

cause of actinomycosis. The organisms have been recovered from intracranial abscesses, chronic mastoiditis, aspiration pneumonia and peritonitis.[3] [5] The lesions of actinomycosis occur most commonly in the tissues of the face, neck, lungs, pleura and ileocecal regions. Bone, pericardial and anorectal lesions are less common, but virtually any tissue may be invaded; even disseminated infection with bacteremia has been described.

Most organisms of the genus *Eubacterium* and anaerobic lactobacilli seem to be nonpathogenic. They are almost invariably isolated as part of mixed flora in the oral, vaginal and gastrointestinal areas. These organisms have been recovered most commonly in infections associated with predisposing or underlying conditions that include malignancy, previous surgery, immunodeficiency, diabetes mellitus, the presence of a foreign body, dental extraction and broad-spectrum antibiotic therapy.[10]

Propionibacterium spp. ordinarily are not pathogens, but can be found in association with implanted cardiac prostheses or central nervous system (CNS) shunts or as a cause of endocarditis on previously damaged valves. They have been recovered from parotid and dental infections, brain abscesses, conjunctivitis associated with contact lens, peritonitis and pulmonary infections. The most common species, *Propionibacterium acnes*, may be isolated from blood cultures but is associated only rarely with bacteremia or endocarditis. Because these organisms are part of the normal skin flora, they are common laboratory contaminants or may grow in blood cultures from skin contamination if the skin surface has been improperly decontaminated before the blood sample is drawn. *Propionibacterium acnes* can cause bacteremia, especially in association with shunt infections,[11] and is believed to play a role in the pathogenesis of acne vulgaris.

Gram-negative bacilli

Bacteroides fragilis group are the most frequently recovered species of Bacteroidaceae in clinical specimens (Fig. 232.3). *Bacteroides fragilis* group are resistant to penicillins, mostly through the production

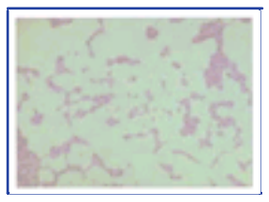

Figure 232-3 Gram stain of *Bacteroides fragilis.* *Courtesy of Mike Cox.*

of ß-lactamase. They include ten members — the most commonly isolated are *B. fragilis* (the most commonly recovered member), *Bacteroides thetaiotaomicron, Bacteroides distasonis, Bacteroides ovatus* and *Bacteroides vulgatus*. These organisms are part of the normal gastrointestinal flora[1] and predominate in intra-abdominal infections and other infections that originate from the gut flora (i.e. perirectal abscesses, decubitus ulcers).[3] [5]

Pigmented *Prevotella* spp. (*Prevotella melaninogenica* and *Prevotella intermedia*), *Porphyromonas* spp. (*Porphyromonas asaccharolytica*) and nonpigmented *Prevotella* spp. (*Prevotella oralis, Prevotella oris*) are part of the normal oral and vaginal flora and are the predominant Gram-negative anaerobic species isolated from respiratory infections and their complications, aspiration pneumonia, lung abscess, chronic otitis media, chronic sinusitis, abscesses around the oral cavity, human bites, paronychia, brain abscesses and osteomyelitis.[12] *Prevotella bivia* and *Prevotella disiens* are important isolates from obstetric and gynecologic infections.

Fusobacterium species

Fusobacterium spp. are moderately long and thin organisms with tapered ends, and have typical fusiform morphology (Fig. 232.4). The species of *Fusobacterium* seen most often in clinical infections are *Fusobacterium nucleatum, Fusobacterium necrophorum, Fusobacterium mortiferum* and *Fusobacterium varium. Fusobacterium nucleatum* is the predominant *Fusobacterium* sp. from clinical specimens, and is often associated with oral, pulmonary and intracranial infections.[13] *Fusobacterium* spp. are frequently isolated from abscesses, obstetric and gynecologic infections, blood and wounds.

A growing resistance of Gram-negative anaerobic bacilli to penicillins has been noticed in the past decade.[14] These include the pigmented *Prevotella* and *Porphyromonas, Prev. oralis, Prev. disiens, Prev. bivia* and *Fusobacterium* spp. The main mechanism of resistance is through the production of the enzyme ß-lactamase. Testing for antimicrobial susceptibility and the ability to produce ß-lactamase of all Gram-negative anaerobic bacilli can assist in the selection of proper antimicrobials.

The recovery rates of the different anaerobic Gram-negative bacilli in infected sites are similar to their distributions in the normal flora.[3] [5] *B. fragilis* group were more often isolates in sites proximal to the gastrointestinal tract (abdomen, bile), pigmented *Prevotella* spp. were more prevalent in infections proximal to the oral cavity (bones, sinuses, chest), and *Prev. bivia* and *Prev. disiens* were more often isolates in obstetric and gynecologic infections (see Table 232.2). Knowledge of this common mode of distribution allows for logical choice of antimicrobials that are adequate for the therapy of infections in these sites.

Gram-positive cocci

The species most commonly isolated are *Peptostreptococcus magnus, Peptostreptococcus asaccharolyticus, Peptostreptococcus anaerobius, Peptostreptococcus prevotii* and *Peptostreptococcus micros.*

Figure 232-4 Gram stain of *Fusobacterium nucleatum.* *Courtesy of Mike Cox.*

Additional anaerobic cocci include *Coprococcus, Peptococcus, Ruminococcus sarcina* and *Staphylococcus saccharolyticus*. These organisms are part of the normal flora of the mouth, upper respiratory tract, intestinal tract, vagina and skin.

These organisms can be isolated in all types of anaerobic infections. They also predominant in all types of respiratory infections (including chronic sinusitis, mastoiditis, acute and chronic otitis media, aspiration pneumonia and lung abscess), and necrotizing, subcutaneous and soft tissue infections.[15] They are generally recovered mixed with other aerobic or anaerobic organisms, but in many cases they are the only pathogens recovered. This may be of particular significance in cases of bacteremia. Microaerophilic Gram-positive cocci include *Streptococcus anginosus, Streptococcus constellatus, Streptococcus intermedius* and *Gemella morbillorum.* Microaerophilic streptococci are of particular importance in chronic sinusitis and brain abscesses. They are also recovered from obstetric and gynecologic infections and abscesses.[16]

Gram-negative cocci

There are three genera described as anaerobic Gram-negative cocci: *Veillonella, Acidaminococcus* and *Megasphaera* spp. There are two described species of *Veillonella* and only one each of the other two genera. *Veillonella* is the most frequently involved of the three genera and *Veillonella* spp. are part of the normal flora of the mouth, vagina and the small intestine of some persons. Although they are rarely isolated from clinical infections, these organisms have been recovered occasionally from almost every type of anaerobic infection. [17]

PATHOGENICITY

Anaerobes as part of the normal flora

The human body mucosal and epithelial surfaces are colonized with aerobic and anaerobic micro-organisms.[1] Differences in the environment, such as oxygen tension, pH and variations in the ability of bacteria to adhere to these surfaces, account for changing patterns of colonization. Microflora also vary in different sites within the body system, as in the oral cavity; for example, the micro-organisms present in the buccal folds vary in their concentration and types of

TABLE 232-3 -- Normal flora.

NORMAL FLORA			
Site	**No. of organisms/g fecal material**		**Predominant anaerobic bacteria**
	Aerobes	**Anaerobes**	
Skin	-	-	*Propionibacterium acnes*
			Peptostreptococcus spp.
Mouth/upper respiratory tract	10^8 –10^9	10^9 –10^{11}	Pigmented *Prevotella* and *Porphyromonas* spp.
			Fusobacterium spp.
			Peptostreptococcus spp.
			Actinomyces spp.
Gastrointestinal tract:			
Upper	10^2 –10^5	10^3 –10^7	*Bacteroides fragilis* group
			Clostridium spp.
Lower	10^5 –10^9	10^{10} –10^{12}	*Peptostreptococcus* spp.
			Bifidobacterium spp.
			Eubacterium spp.
Female genital tract	10^8	10^9	*Peptostreptococcus* spp.
			Prevotella bivia
			Prevotella disiens
For predominant anaerobic bacteria in the gastrointestinal tract, all organisms can be found both in the upper and lower gastrointestinal tracts.			

strains from those isolated from the tongue or gingival sulci. However, the organisms that prevail in one body system tend to belong to certain major bacterial species, and their presence in that system is predictable. The relative and total counts of organisms can be affected by various factors, such as age, diet, anatomic variations, illness, hospitalization and antimicrobial therapy. However, these sets of bacterial flora, with predictable pattern, remain stable through life, despite their subjection to perturbing factors. Anaerobes outnumber aerobic bacteria on all mucosal surfaces, and certain organisms predominate in the different sites (see Table 232.3).

Knowledge of the composition of the flora at certain sites is useful for predicting which organisms may be involved in an infection adjacent to that site and can assist in the selection of a logical antimicrobial therapy, even before the exact microbial etiology of the infection is known. Recognition of the normal flora can also help the clinical microbiology laboratory to choose proper culture media that will be selective in inhibiting organisms that may interfere with the growth of certain anaerobic bacteria.

The anaerobic microflora of the skin is largely made up of the genus *Propionibacterium*[11] (mostly *Propionibacterium acnes*) and, to a lesser extent, *Peptostreptococcus* spp. The perineum and lower extremity may harbor members of the colonic and vaginal flora.

The microflora of the upper airways, including oral cavity, nasopharynx and oropharynx, is complex and contains many types of obligate anaerobes. The distribution of bacteria within the mouth seems to be a function of their ability to adhere to the oral surfaces. The differences in numbers of the anaerobic microflora probably occur because of considerable variations in the oxygen concentration in parts of the oral cavity. The ratio of anaerobic bacteria to aerobic bacteria in saliva is approximately 10:1. The total count of anaerobic bacteria in the saliva and elsewhere in the oral cavity reach 10^7 –10^8 bacteria/ml. The predominant anaerobic bacteria in the upper airways include *Fusobacterium* spp. (especially *F. nucleatum*), pigmented *Prevotella* and *Porphyromonas* spp., *Prev. oralis* and *Peptostreptococcus* spp. These organisms also predominate in oropharyngeal, otolaryngologic and pulmonary infections.

The gastrointestinal flora varies in bacterial concentration at different levels. The stomach acidity reduces the number of organisms swallowed from the oropharynx. The stomach, duodenum, jejunum and

proximal ileum normally contain relatively few bacteria. However, the flora becomes more complex, and the number of different bacterial species increase in the distal portion of the gastrointestinal tract. However, interruption in intestinal motility may result in an increase in the number of anaerobic and aerobic bacteria. The bacterial counts in the small intestine are relatively low, with total counts of 10^2 –10^5 organisms/ml. The organisms that predominate up to the ileocecal valve are Gram-positive facultative, whereas below that structure *Bacteroides, Peptostreptococcus* and *Clostridium* spp., and coliform bacteria are the major isolates.[2] The mean number of bacteria in the colon exceeds 10^{11} bacteria/g fecal material. Approximately 99.9% of these bacteria are anaerobic (ratio of aerobes to anaerobes is 1:1000–10,000). In the colon 300–400 different species or types of bacteria can be found, many of whom are not yet identified.

The female genital flora is composed of mixed aerobic and anaerobic flora. However, the concentration and type of bacteria is less stable than that of the gastrointestinal flora and can be influenced by antimicrobial therapy, pregnancy and gynecologic surgery. A concentration of 10^8 organisms/ml is found during the reproductive years. Changes occur in the number of organisms at the various stages of the menstrual cycle.[18] The predominant aerobic organisms are aerobic lactobacilli, and the predominant anaerobic bacteria are anaerobic *Lactobacillus, Peptostreptococcus, Prevotella* and *Bacteroides* and *Clostridium* spp. Other anaerobic isolates include *Porphyromonas, Fusobacterium, Bilophilia, Bifidobacterium, Actinomyces, Eubacterium* and *Propionibacterium* spp. Enterobacteriaceae can be found in postmenopausal flora. Bacterial vaginosis is associated with an increase in the number of anaerobic flora and a decrease in the concentration of lactobacilli.[19]

Most infections caused by anaerobic bacteria originate from the endogenous mucosal membrane and skin flora. An exception is *C. difficile*, the major cause of antibiotic-associated colitis. Anaerobes belonging to the indigenous flora of the oral cavity can be recovered from various infections adjacent to that area, such as cervical lymphadenitis, subcutaneous abscesses and burns in proximity to the oral cavity, human and animal bites, paronychia, tonsillar and retropharyngeal abscesses, chronic sinusitis, chronic otitis media, periodontal abscess, thyroiditis, aspiration pneumonia and bacteremia associated with one of the above infections.[3] [5] The predominant anaerobes recovered in these infections are *Prevotella, Porphyromonas, Fusobacterium* and *Peptostreptococcus* spp., which are all part of the normal flora of the mucous surfaces of the oropharynx (Fig. 232.5).

A similar correlation exists in infections associated with the gastrointestinal tract. Such infections include peritonitis after rupture of a viscus, liver and spleen abscess, abscess and wounds near the anus, intra-abdominal abscess, and bacteremia associated with any of

Figure 232-5 Recovery of anaerobic bacteria in different infectious sites.

these infections.[3] [5] The anaerobes that predominate in these infections are *B. fragilis* group, *Clostridium* spp. (including *C. perfringens*) and *Peptostreptococcus* spp.

Another site where a correlation exists between the normal flora and the anaerobic bacteria recovered from infected sites is the genitourinary tract. The infections involved are amnionitis, septic abortion and other pelvic inflammations. [3] [5] The anaerobes usually recovered from these sites are species of anaerobic Gram-negative bacteria and *Peptostreptococcus* spp. Organisms belonging to the vaginal-cervical flora are also important pathogens of neonatal infections. They can be acquired by the newborn before delivery in the presence of amnionitis or during passage through the birth canal.

Conditions predisposing to anaerobic infection

The clinical situations that predispose to anaerobic infections include exposure of the sterile body sites to a high inoculum of indigenous mucous membrane flora. Poor

blood supply and tissue necrosis lower the oxidation and reduction potential and favor the growth of anaerobic bacteria. Any condition that lowers the blood supply to an affected area of the body can predispose to anaerobic infection. Therefore, trauma, foreign body, malignancy, surgery, edema, shock, colitis and vascular disease may predispose to anaerobic infection. Other predisposing conditions include diabetes mellitus, splenectomy, immunosuppression, hypogammaglobinemia, neutropenia, leukemia, collagen vascular disease and cytotoxic drugs. Previous infection with aerobic or facultative organisms may make the local tissue conditions more favorable for the growth of anaerobic bacteria. The human defense mechanisms may also be impaired by anaerobic conditions and anaerobic bacteria. The noted effects include impairments in phagocytosis and intracellular killing (often caused by encapsulated anaerobes [20] and by succinic acid produced by *Bacteroides* spp.), inhibition of chemotaxis (by *Fusobacterium, Prevotella* and *Porphyromonas* spp.), degradation of serum proteins by proteases (by *Bacteroides* spp.) and production of leukotoxins (by *Fusobacterium* spp.).[21]

Suppuration, abscess formation, thrombophlebitis and gangrenous destruction of tissue associated with gas formation are the hallmarks of anaerobic infection (Table 232.4). Anaerobes are especially common in chronic infections, and they are commonly seen after failure of therapy with antimicrobials that are effective against them, such as aminoglycosides, trimethoprim-sulfamethoxazole (cotrimoxazole) and earlier quinolones.

Certain infections are very likely to involve anaerobes as important pathogens, and their presence should always be assumed. Such infections include brain abscess, oral or dental infections, human or animal bites, aspiration pneumonia and lung abscesses, peritonitis after perforation of viscus, amnionitis, endometritis, septic abortions,

TABLE 232-4 -- Clinical signs of anaerobic infection.

CLINICAL SIGNS OF ANAEROBIC INFECTION
• Infection adjacent to a mucosal surface
• Foul-smelling discharge
• Necrotic gangrenous tissue and abscess formation
• Free gas in tissue
• Bacteremia or endocarditis with no growth on aerobic blood cultures
• Infection related to the use of antibiotics effective against aerobes only
• Infection related to tumors or other destructive processes
• Infected thrombophlebitis
• Infection after bites
• Black discoloration of exudates containing *Bacteroides melaninogenicus*, which may fluoresce under ultraviolet light
• 'Sulfur granules' in discharges caused by actinomycosis
• Clinical presentation of gas gangrene
• Clinical condition predisposing to anaerobic infection (after maternal amnionitis, perforation of bowel, etc.)

tubo-ovarian abscess, abscesses in and around the oral and rectal areas, pus-forming necrotizing infections of soft tissue or muscle and postsurgical infections following procedures on the oral or gastrointestinal tract or female pelvic area. Certain solid malignant tumors, such as colon, uterine and bronchogenic carcinomas, and necrotic tumors of the head and neck, have the tendency to become infected with anaerobic bacteria.[22] The anoxic conditions in the tumor and exposure to the endogenous adjacent mucous flora may predispose to these infections.

Virulence factors

Anaerobes contribute to the severity of infection through their synergy with their aerobic counterparts and with each other.[23] Anaerobic bacteria generally take longer than aerobic bacteria to become virulent. This is because some of the major virulence factors of certain anaerobic bacteria (i.e. the production of a capsule) are expressed only after the infection has become chronic.[20]

Anaerobic bacteria possess several virulence factors that assist them to adhere and invade epithelial surfaces. These factors include the presence of surface structures (such as capsule polysaccharide or lipopolysaccharide), production of superoxide dismutase and catalase, immunoglobulin proteases, coagulation promoting, spreading factors (such as hyaluronidase, collagenase and fibrinolysin), adherence factors, and the production of toxins.[21] Other factors that enhance the virulence of anaerobes include mucosal damage, oxidation-reduction potential drop and the presence of hemoglobin or blood in an infected site.

An indirect pathogenic role of some anaerobes is their ability to produce the enzyme ßlactamase. Several anaerobic bacteria can

TABLE 232-5 -- Principal ß-lactamase-producing anaerobes.

PRINCIPAL ß-LACTAMASE-PRODUCING ANAEROBES
• *Fusobacterium* spp.: *F. nucleatum*, *F mortiferum*, *F. varium*
• Pigmented *Prevotella* and *Porphyromonas* spp., *Prevotella oralis* group
• Other *Prevotella* spp.: *P. oris*, *P. buccae*, *P. bivia*, *P. disiens*
• *Bacteroides fragilis* group, *Bacteroides splanchnicus*
• *Bilophila wadsworthia*
• *Clostridium* spp.: *C. ramosum*, *C. clostridioforme* and *C. butyricum*

produce ß-lactamase (Table 232.5). ß-lactamase-producing bacteria (BLPB) can protect not only themselves but also other penicillin-susceptible organisms from the activity of penicillins. This can occur when the enzyme ß-lactamase is secreted into the infected tissue or abscess fluid in sufficient quantities to degrade the ß-lactam ring of penicillin before it can kill the susceptible bacteria.[24]

In-vitro and *in-vivo* studies have demonstrated protection of penicillin-susceptible bacteria from penicillin by aerobic and anaerobic BLPB (i.e. protection of group A streptococci by *Staphylococcus aureus* or *Bacteroides* spp.).[24] The predominant anaerobic BLPB are pigmented *Prevotella* and *Porphyromonas, Bacteroides* and *Fusobacterium* spp.

PREVENTION

Proper therapy of acute infections can prevent the occurrence of chronic infections where anaerobes predominate. In settings where anaerobic infections are expected to occur, such as intra-abdominal and wound infections after surgery, proper antimicrobial prophylaxis reduces the chance of such infections.

Prevention and early therapy of conditions that may lead to anaerobic infection can reduce their rate. Preventing oral flora aspiration by improving the neurologic status of the patient, repeated suctioning of oral secretion, improving oral hygiene and maintaining lower stomach pH can reduce the risk of aspiration pneumonia and its complications. Skin and soft-tissue infections can be prevented by irrigation and debridement of wounds and necrotic tissue, drainage of pus and improvement of blood supply.

Prophylactic therapy before surgery is generally administered when the operative field is expected to be contaminated by the normal flora of the mucous membrane at the operated site. Cefazolin is effective in surgical prophylaxis in sites distant from the oral or rectal areas, when anaerobic cover is not required. Cefoxitin or cefotetan

are used in procedures that involve the oral, rectal or vulvovaginal surfaces because their spectrum includes the anaerobic flora likely to be encountered.

DIAGNOSTIC MICROBIOLOGY

Collection of specimens for anaerobic bacteria

The proper management of anaerobic infection depends on appropriate documentation of the bacteria causing the infection. Without such an approach, the patient may be exposed to inappropriate, costly and undesirable antimicrobial agents with adverse side-effects. Some laboratories may fail to recover certain or all of the anaerobes present in a specimen. This situation can occur particularly when the specimen is not promptly placed under anaerobic conditions for transport to the laboratory. If care is not taken to avoid specimen contamination with normal flora, anaerobes may not be recovered.

Appropriate cultures for anaerobes are especially important in polymicrobial infections.[4] Techniques or media that are inadequate for isolation of anaerobes because of a lack of an anaerobic environment or because of an overgrowth of aerobic organisms, can be misleading. This may cause the clinician to direct therapy toward only the isolated aerobic organisms.

The most acceptable documentation of an anaerobe is through recovery of anaerobic organisms from the infected site. Three essential elements require the physician's cooperation with the microbiology laboratory for appropriate documentation of anaerobic infection: collection of appropriate specimens, their expeditious transportation and careful laboratory processing.

Specimens must be obtained free of contamination so that saprophytic organisms or normal flora are excluded and culture results can be interpreted correctly. Because indigenous anaerobes are often

TABLE 232-6 -- Methods for collection of specimens for anaerobic bacteria.

METHODS FOR COLLECTION OF SPECIMENS FOR ANAEROBIC BACTERIA	
Infection site	**Methods**
Abscess or body cavity	Aspiration by syringe and needle
	Incised abscesses: syringe or swab (less desirable); specimen obtained during surgery after cleansing the skin
Tissue or bone	Surgical specimen using tissue biopsy or curette
Sinuses or mucus surface abscesses	Aspiration after decontamination or surgical specimen
Ear	Aspiration after decontamination of ear canal and membrane; in perforation, cleanse ear canal and aspirate through perforation
Pulmonary	Transtracheal aspiration, lung puncture, bronchoscopic aspirate (using double lumen catheter and quantitative culture)
Pleural	Thoracentesis
Urinary tract	Suprapubic bladder aspiration
Female genital tract	Culdocentesis after decontamination, surgical specimen
	Transabdominal needle aspirate of uterus
	Intrauterine brush (using double lumen catheter and quantitative culture)

present on the surfaces of skin and mucous membranes in large numbers, even minimal contamination of a specimen with normal flora can give misleading results. On this basis, specimens can be designated according to their acceptability for anaerobic culture to the acceptable or the unacceptable category. Materials that are appropriate for anaerobic cultures should be obtained using a technique that bypasses the normal flora (Table 232.6). Unacceptable or inappropriate specimens can yield normal flora also and therefore have no diagnostic value.

Acceptable specimens (Table 232.7) include blood specimens, aspirates of body fluids (pleural, pericardial, cerebrospinal, peritoneal and joint fluids), urine collected by percutaneous suprapubic bladder aspiration, abscess contents, deep aspirates of wounds and specimens collected by special techniques, such as transtracheal aspirates or direct lung puncture. Specimens of the lower respiratory tract are difficult to obtain without contamination with indigenous flora. Double-lumen catheter bronchial brushing and bronchoalveolar lavage, cultured quantitatively, can be useful. Direct needle aspiration is probably the best method of obtaining a culture, and the use of swabs is much less desirable. Specimens obtained from sites that are normally sterile may be collected after thorough skin decontamination, as is the case for the collection of blood, or spinal, joint or peritoneal fluids.

Transportation of specimens

Prompt delivery of specimens to the laboratory to allow for microbiologic processing is essential. Various transport devices are available that generate an oxygen-free environment. These systems generally contain an oxygen-free environment provided by mixture of carbon dioxide, hydrogen and nitrogen, plus an indicator that illustrates aerobic conditions. The specimens should be placed into an anaerobic transporter as soon as possible after collection. Aspirates of liquid specimen or tissue are always preferred to swabs, although systems for the collection of all culture forms are commercially available.

Liquid specimens may be inoculated into a commercially available anaerobic transport vial. A plastic or glass syringe and needle may

TABLE 232-7 -- Appropriate and inappropriate specimens for anaerobic culture.

APPROPRIATE AND INAPPROPRIATE SPECIMENS FOR ANAEROBIC CULTURE	
Inappropriate	**Appropriate**
Feces or rectal swabs	All normally sterile body fluids other than urine (e.g. blood, pleural and joint fluids)
Throat or nasopharyngeal swabs	
Sputum or bronchoscopic specimens	Urine obtained by suprapublic bladder aspiration
Routine or catheterized urine	Percutaneous transtracheal aspiration or direct lung puncture
Vaginal or cervical swabs	Culdocentesis fluid obtained after decontamination of the vagina
Material from superficial wound or abscesses not collected properly to exclude surface contaminations	Material obtained from closed abscesses
Material from abdominal wounds obviously contaminated with feces (e.g. an open fistula)	Material obtained from sinus tracts or draining wounds

TABLE 232-8 -- Bacteriologic findings suggestive of anaerobic infection.

BACTERIOLOGIC FINDINGS SUGGESTIVE OF ANAEROBIC INFECTION
• Organisms seen on Gram stain that cannot be grown in aerobic cultures
• Typical morphology for anaerobes on Gram stain
• Anaerobic growth on proper media containing antibiotic-suppressing aerobes
• Growth in anaerobic zone of fluid or agar media

• Gas, foul-smelling odor in specimen or bacterial culture
• Characteristic colonies on anaerobic plates
• Colonies of pigmented *Prevotella* or *Porphyromonus* spp. may fluoresce red under ultraviolet light, and older colonies produce a typical dark pigment

also be used for transport. After the specimen is collected and all air bubbles are expelled from the syringe and needle, the needle tip should be inserted into a sterile rubber stopper. Because air gradually diffuses into the plastic syringe, no more than 30 minutes should elapse before the specimen is processed. This inexpensive transport device for liquid specimens is especially useful in the hospital situation where it can be rapidly transported to the microbiology laboratory.

Swabs may be placed in the sterilized tubes containing carbon dioxide or prereduced, anaerobically sterile Carey and Blair semisolid media. Tissue specimens or swabs can be transported in an anaerobic jar or in a Petri dish inside a sealed plastic bag that can be rendered anaerobic by use of a catalyzer.

Laboratory diagnosis

Certain findings are suggestive of anaerobic infection (Table 232.8). However, laboratory diagnosis of anaerobic infections begins with the examination of a Gram-stained smear of the specimen (see Fig. 232.1). The appearance of the Gram-stained organisms will give important preliminary information regarding types of organisms present, suggest appropriate initial therapy and serve as a quality control on the final culture analysis. The laboratory should be able to recover all of the morphologic types in the approximate ratio in which they are seen.

The techniques for cultivation of anaerobes should provide optimal anaerobic conditions throughout processing. Detailed procedures of these methods can be found in microbiology manuals.[4]

Figure 232-6 Anaerobic glove-box used in the microbiology laboratory for processing of specimens and identifying anaerobic bacteria. *Courtesy of Mike Cox.*

Briefly, these methods could be the prereduced tube method, the anaerobic glove-box technique (Fig. 232.6), which provides an anaerobic environment throughout processing, or the anaerobic jar or bag systems, which are more simplified.

As a minimum requirement for the recovery of anaerobes, specimens should be inoculated onto enriched blood agar medium (containing vitamin K1 and hemin) and a selective medium (for *Bacteroides* spp.), such as laked sheep blood agar with kanamycin and vancomycin, should be used. The use of selective media along with a nonselective one increases the recovery rate and can shorten the time to identification of organisms.

Although prereduced vitamin K1 enriched thioglycolate broth is generally used as a back-up culture, this media alone should never be used as a substitute for a solid media. The major limitation of liquid media is the possibility of overgrowth of slow-growing strict anaerobes by rapid-growing aerobic and facultative organisms. Selective media can supplement nonselective media in increasing the bacterial yield and facilitating recovery.

Cultures should be placed immediately under anaerobic conditions and incubated for 48 hours or longer. Plates should then be examined for approximate number and types of colonies present. Each colony type should be isolated, tested for aerotolerance and identified.

An additional period of 36–48 hours is generally required to identify completely the anaerobic bacteria to a species or genus level using biochemical tests. Kits containing these biochemical tests are commercially available. These are good with fast growing anaerobes (i.e. *B. fragilis* and C. *perfringens*). Rapid enzymatic tests have recently been introduced. This panel test enables an identification of the anaerobes after only 4 hours of aerobic incubation and seems to be as good as the biochemical tests. Other rapid tests that have potential use and can also be used directly on clinical isolates are the direct fluorescent microscopy and direct gas liquid chromatography. Direct fluorescent microscopy can identify *Actinomyces* spp and *P. propionica*. This method is inaccurate in identification of *B. fragilis* group, pigmented *Prevotella* and *Porphyromonas* spp. Gas-liquid chromatography of metabolites can be employed to assist in the identification of anaerobes. Direct cellular fatty acid analysis using gas liquid chromatography with capillary column can also be useful. Nucleic acid probes and polymerase chain reaction methods are also being developed for rapid identification of anaerobic bacteria.

Most clinical microbiology laboratories are able to identify the major anaerobic bacteria. Peptostreptococci are generally not speciated because they are generally susceptible to commonly used antimicrobials. *Clostridium* spp. can be identified by the presence of spores and their ability to survive 30 minutes exposure to ethanol or heating to 176°F (80°C) for 10 minutes. Speciation requires biochemical testing. Nonspore-forming Gram-positive bacilli can be speciated by gas liquid chromatography and biochemical tests. *Propionibacterium* spp., which are often a contaminant, can be separated from other nonspore-forming Gram-positive bacilli by using a catalyze test and indole reaction. *Bacteroides fragilis* group organisms grow on 20% bile and are generally catalase positive. Pigmented *Prevotella* and *Porphyromonas* spp. produce black or brown pigment within a week when growing on rabbit blood agar medium. *Fusobacterium* spp. have a distinct morphology on Gram stain and, in contrast to *Bacteroides* spp., are susceptible to kanamycin.

Identification of an anaerobe to a species level is often cumbersome, expensive and time-consuming, taking up to 72 hours. Identification is most helpful in selecting an antibiotic against a species that has predictable antibiotic susceptibility. The level of speciation adequate for identifying an anaerobe is often controversial.

Occasionally, species identification of an organism will provide the diagnosis, as with *C. difficile* in patients who have colitis or *C. botulinum* in infants with botulism. However, because most anaerobes are endogenous, there are rarely epidemiologic reasons to perform complete identification. Identifying *B. fragilis* group, which often cause bacteremia and septic complications, has significant prognostic value.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of anaerobes has become less predictable. Resistance to several antimicrobials, especially by anaerobic Gram-negative bacilli and *Fusobacterium* spp., has increased over the past decade.[13] It is important, therefore, to perform susceptibility testing for anaerobes recovered from sterile body sites, those with particular epidemiologic or prognostic significance (e.g. *C. difficile*), or those that are clinically important and have variable susceptibilities.

Screening of anaerobic Gram-negative bacilli isolates (particularly *Prevotella, Bacteroides* and *Fusobacterium* spp.) for ß-lactamase activity may be helpful. This can provide information regarding their penicillin susceptibility. However, occasional bacterial strains may resist ß-lactam antibiotics through other mechanisms.

Routine susceptibility testing of all anaerobic isolates is extremely time-consuming and in many cases unnecessary. Susceptibility testing should be limited to anaerobes isolated from blood cultures, bone, CNS and serious infections, as well as to those isolated in pure culture from properly collected specimens. Antibiotics tested should include penicillin, a broad-spectrum penicillin, a penicillin plus a ß-lactamase inhibitor, clindamycin, chloramphenicol, a second-generation cephalosporin (e.g. cefoxitin), metronidazole and a carbapenem (e.g. imipenem).

The method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) includes agar dilution testing, microbroth and macrobroth dilution.[25] Newer methods include the E-test and the spiral gradient end-point system.

CLINICAL MANIFESTATIONS

Anaerobic bacteria have been recovered in infections at all anatomic locations. However, their frequency of isolation and the type of bacterial isolates depend on the microbial flora at their source, or at the adjacent mucocutaneous sites.

Central nervous system infections

Anaerobic bacteria can cause a variety of intracranial infections. These organisms induce brain abscess, subdural empyema and infrequently cause epidural abscess

and meningitis. The main source of brain abscess is an adjacent, generally chronic infection in the ears, mastoids, sinuses, oropharynx, teeth or lungs.[26] Ear or mastoid infection tend to spread to the temporal lobe or cerebellum, whereas facial sinusitis often causes abscess of the frontal lobe.

Hematogenous spread often occurs after dental, oropharyngeal or pulmonary infection. Rarely bacteremia of another origin or endocarditis can lead to such infection.

Meningitis caused by anaerobes is rare and can follow respiratory infection or develop as a complication of a cerebrospinal fluid shunt. Shunt infections are generally caused by skin flora such as *Prop. acnes*, [11] or in instances of ventriculoperitoneal shunts that perforate the gut, by anaerobes of enteric origin (i.e. *B. fragilis*).[27] *Clostridium perfringens* has been reported as a cause of brain abscesses and meningitis after head injuries or after intracranial surgery.[2]

The anaerobic bacteria generally recovered from brain abscesses that complicate respiratory and dental infections include *Prevotella, Porphyromonas, Bacteroides, Fusobacterium* and *Peptostreptococcus* spp. Microaerophilic and other streptococci are also often isolated. Actinomyces are less frequently encountered.

TABLE 232-9 -- Aerobic and anaerobic bacteria isolated in head and neck and upper respiratory tract infections.

AEROBIC AND ANAEROBIC BACTERIA ISOLATED IN HEAD AND NECK AND UPPER RESPIRATORY TRACT INFECTIONS			
Type of infection		**Aerobic and facultative organisms**	**Anaerobic organism**
Otitis media and mastoiditis:	acute	*Streptococcus pneumoniae*	*Peptostreptococcus* spp.
		*Haemophilus influenzae**	
		*Moraxella catarrhalis**	
	chronic	*Staphylococcus aureus**	Pigmented *Prevotella* and *Porphyromonas* spp.*
		*Escherichia coli**	*Bacteroides* spp.*
		*Klebsiella pneumoniae**	*Fusobacterium* spp.*
		*Pseudomonas aeruginosa**	*Peptostreptococcus* spp.*
Peritonsillar and retropharyngeal abscess		*Streptococcus pyogenes*	*Fusobacterium* spp.*
		*Staphylococcus aureus**	Pigmented *Prevotella* and *Porphyromonas* spp.*
Recurrent tonsillitis		*Streptococcus pyogenes*	*Fusobacterium* spp.*
		*Haemophilus influenzae**	
		*Staphylococcus aureus**	
Suppurative thyroiditis		*Streptococcus pyogenes*	Pigmented *Prevotella* and *Porphyromonas* spp.*
		*Staphylococcus aureus**	*Peptostreptococcus* spp.
Sinusitis:	acute	*Haemophilus influenzae**	*Peptostreptococcus* spp.
		Streptococcus pneumoniae	
		*Moraxella catarrhalis**	
	chronic	*Staphylococcus aureus**	Pigmented *Prevotella* and *Porphyromonas* spp.*
		Streptococcus pneumoniae	*Fusobacterium* spp.*
		*Haemophilus influenzae**	*Bacteroides fragilis* group*
Cervical lymphadenitis		*Staphylococcus aureus**	Pigmented *Prevotella* and *Porphyromonas* spp.*
		Mycobacterium spp.	*Peptostreptococcus* spp.
Postoperative infection disrupting oral mucosa		*Staphylococcus* spp.*	*Fusobacterium* spp.*
		Enterobacteriaceae*	*Bacteroides* spp.*
		Streptococcus pyogenes	Pigmented *Prevotella* and *Porphyromonas* spp.*
			Peptostreptococcus spp.
Deep neck abscesses and parotitis		*Streptococcus* spp.	*Bacteroides* spp.*
		Staphylococcus spp.*	*Fusobacterium* spp.*
			Peptostreptococcus spp.*
Odontogenic complications		*Streptococcus* spp.	Pigmented *Prevotella* and *Porphyromonas* spp.*
		Staphylococcus spp.*	*Peptostreptococcus* spp.
Oropharyngeal: Vincent's angina and necrotizing		*Streptococcus* spp.	*Fusobacterium necrophorum**
		Staphylococcus spp.*	

* Organisms that have the potential of producing ß-lactamase

At the stage of encephalitis, antimicrobial therapy accompanied by measures to control the increase in the intracranial pressure can prevent the abscess formation. Once an abscess has formed, surgical excision or drainage may be needed, combined with a long course of antibiotics (4–8 weeks). Some neurosurgeons advocate complete evacuation of the abscess, whereas others advocate repeated aspirations. [28] In patients who have multiple abscesses or in those who have abscesses in essential brain areas, repeated aspirations are preferable. High-dose antibiotics for an extended period may represent an alternative approach in this group of patients and have often replaced surgical drainage.[28]

Because of the difficulty involved in the penetration of various antimicrobial agents through the blood-brain barrier, the choice of antibiotics is restricted. The antimicrobials advocated for these infections are metronidazole, penicillins, carbapenems and

chloramphenicol. However, the choice may vary according to the specific isolates and their susceptibilities. A significant improvement in the mortality rate has been associated with the introduction of computed tomography (CT) and use of metronidazole therapy.

Head and neck and upper respiratory tract infections

Anaerobic bacteria can be recovered from a variety of head and neck and upper respiratory tract infections and predominate more in their chronic forms (Table 232.9). These include chronic otitis media, sinusitis and mastoiditis, tonsillar, peritonsillar and retropharyngeal abscesses, all deep neck space infections, parotitis, thyroiditis, odontogenic infections, and postsurgical and nonsurgical head and neck wounds and abscesses. The predominant organisms are of oropharyngeal flora origin and

include *Prevotella, Porphyromonas, Bacteroides, Fusobacterium* and *Peptostreptococcus* spp.

Most dental infections involve anaerobes. These include endodontal pulpitis and periodontal (gingivitis and periodontitis) infections, periapical and dental abscesses, and perimandibular space infection.[29] [30] Pulpitis may progress to an abscess and eventually involve the mandible and other neck spaces. In addition to the organisms mentioned above, microaerophilic streptococci and *Streptococcus salivarius* can also be involved.

Vincent's angina (or trench mouth) is a distinct form of ulcerative gingivitis; the causative organisms include *Fusobacterium* spp. and anaerobic spirochetes.[2]

Deep neck infections after oral, dental and pharyngeal infections are usually polymicrobial. These include mediastinitis after perforation of the esophagus, extension of retropharyngeal abscess or cellulitis, and dental abscess. [31]

Otitis media

Anaerobes were isolated in 5–15% of patients who had acute otitis media[32] and 42% of culture-positive aspirates of patients who had serous otitis media.[33] The predominant isolates in acute otitis media were *Peptostreptococcus* spp. and *Prop. acnes*. These organisms, as well as Gram-negative anaerobic bacilli, were recovered in serous otitis media.

Several studies reported the recovery of anaerobes in about 50% of the patients who have chronic suppurative otitis media.[3] [5] [34] [35] The infection is often polymicrobial and the predominant anaerobes were Gram-negative bacilli and peptostreptococci, and the common aerobes were *Pseudomonas aeruginosa* and *S. aureus*. Many of these organisms can produce ß-lactamase and might have contributed to the high failure rate of ß-lactam antibiotics in the therapy of this infection. Anaerobes were isolated from 23 out of 24 (96%) specimens of chronic mastoiditis[36] and from most patients who have intracranial abscesses that complicate chronic suppurative otitis media.[3] [5] [26]

Anaerobes were recovered from infected cholesteatomas.[37] [38] The production of organic acids by anaerobic bacteria may promote the process of bone destruction in cholesteatoma.[38] Because infected cholesteatoma contains bacteria similar to those recovered from chronically infected ears, it may serve as a nidus for chronic infection.

Sinusitis

Sinus disease may develop when allergy, viral infection, or anatomic obstruction occurs, preventing normal drainage. In the first stages of infection, the most common pathogens are similar to those recovered in acute otitis media: *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis*. Anaerobic organisms become involved as the infection becomes chronic and the levels of tissue oxygen decline. [39] The transition in the bacterial flora from aerobic to anaerobic flora has been demonstrated.[40] An elevated serum antibody level to *Prevotella* and *Fusbacterium* spp. was demonstrated in patients with chronic sinusitis.[41] Although anaerobes are generally isolated from only about 7% of patients who have acute sinusitis (generally in maxillary sinusitis secondary to periodontal infection), they can be isolated from up to 67% of patients who have chronic infection.[3] [5] [42] An average of three anaerobes per aspirate were recovered in patients who had chronic sinusitis.[43]

Sinus infection may spread via anastomosing veins or contiguously to the CNS. Complications include orbital cellulitis[44] meningitis, cavernous sinus thrombosis, and epidural, subdural and brain abscesses. [3] [5]

Parotitis

Viral parotitis can be caused by paramyxovirus (mumps), Epstein-Barr virus, coxsackievirus, HIV, and influenza A and parainfluenza virus. Acute suppurative parotitis is generally caused by *S. aureus*, streptococci and, rarely, aerobic Gram-negative bacteria. Anaerobes, mostly *Peptostreptococcus, Bacteroides*, and pigmented *Prevotella* and *Porphyromonas* spp., have also been recognized as an important cause of this infection.[45] Empiric antibiotic therapy should be directed against both aerobic and anaerobic bacteria. Surgical drainage may be indicated when pus has formed.

Cervical lymphadenitis

The anterior cervical, the submandibular or the posterior cervical nodes are the most prevalent sites of infection. The most common causes in children are viruses. The organisms causing acute unilateral infection associated with facial trauma or impetigo are *S. aureus* and *Streptococcus pyogenes. Bartonella henselae* and mycobacterial infections are important in chronic infections. Anaerobes (mostly *Fusobacterium* and *Peptostreptococcus* spp.) were isolated in about 25% of the infections, often in pure culture.[46] Their recovery was associated with a primary dental, periodontal, or tonsillar infection.

Thyroiditis

Anaerobic Gram-negative bacilli and peptostreptococci were identified as causative agents in thyroiditis.[3] [5] [47] *Eikenella corrodens* and *Actinomyces* spp. were also reported.

Infected cysts

Thyroglossal duct, branchial cleft and dermoid cysts, cystic hygromas and laryngoceles can become infected. The organisms that can cause these infections can originate from either the skin or the oropharynx. *Staphylococcus aureus* and *S. pyogenes* are the predominant aerobic isolates, whereas pigmented *Prevotella* and *Porphyromonas* spp. are the predominate anaerobes. [48]

Infection after head and neck surgery

Infections after head and neck surgery are caused by the exposure of the surgical site to the oropharyngeal flora, by the decreased blood supply and by the presence of necrotic tissues. They are common after surgery for malignant tumors. The wounds are generally infected by polymicrobial aerobic and anaerobic flora; the average number of isolates varies from one to nine (average six).[49] The most common isolates are peptostreptococci, *S. aureus,* anaerobic Gram-negative bacilli (i.e. *Bacteroides* spp.), *Fusobacterium* spp. and Enterobacteriaceae. The presence of this flora warrants the use of antimicrobial agents that are effective against these organisms in the prophylaxis and therapy of this infection.[50]

Tonsillitis

Indirect evidence is mounting that anaerobes are involved in both acute and chronic tonsillitis. The evidence is mainly derived from

studies of complications of anaerobic tonsillitis (i.e. bacteremia, abscesses) where anaerobes play a major role. The organisms associated with the infection are *Fusobacterium* spp., peptostreptococci and Gram-negative anaerobic bacilli. Polymicrobial flora predominate in peritonsillar and retropharyngeal abscesses, where the number of isolates is between one and 12 (average five anaerobes and two aerobes).[3] [5] [51] Anaerobes have also been isolated from the cores of tonsils of children with recurrent group A ß-hemolytic streptococci (GABHS) infection[24] [42] [52] and non-GABHS tonsillitis.[52] These organisms can be isolated from 25% of suppurative cervical lymph nodes and are mostly associated with the presence of dental or tonsillar infections.[46] Anaerobic organisms were associated with thrombophlebitis of the internal jugular veins, which often causes postanginal sepsis.[3] [5]

The pathogenic role of anaerobes in the acute inflammatory process in the tonsils is also supported by several clinical observations:

- their recovery in tonsillar or retropharyngeal abscesses often without any aerobic bacteria;[51]
- their isolation from tonsils in Vincent's angina;[5]
- the recovery of encapsulated pigmented *Prevotella* and *Porphyromonas* spp. in acutely inflamed tonsils;
- the isolation of anaerobes from the core of recurrently inflamed non-GABHS tonsils;[52] and

¦ the response to antibiotics in patients who have non-GABHS tonsillitis. [53] [54]

Furthermore, immune response against *Prev. intermedia* can be detected in patients who have non-GABHS tonsillitis;[55] and against *Prev. intermedia* and *F. nucleatum* in patients who recovered from peritonsillar cellulitis or abscesses [56] and infectious mononucleosis.[57]

Metronidazole therapy alleviated the symptoms of tonsillar hypertrophy and shortened the duration of fever in patients who had infectious mononucleosis. [53] Because metronidazole has no antiviral or aerobic antibacterial efficacy, suppression of the oral anaerobic flora may contribute to diminishing the inflammation induced by the Epstein-Barr virus. This is supported by the increased recovery of *Prev. intermedia* and *F. nucleatum* during the acute phases of mononucleosis. [58]

Recurrent pharyngotonsillitis and failure to eradicate the GABHS with penicillin can be a serious clinical problem. One explanation for penicillin failure is that repeated administrations result in selection of BLPB.[24] ß-lactamase-producing strains of pigmented *Prevotella* and *Porphyromonas* spp., *B. fragilis*, *Fusobacterium* spp., *H. influenzae* and *Staph. aureus* were isolated from the tonsils of more than 75% of children with recurrent GABHS tonsillitis[24] [42] [59] [60] and from 40% of children with non-GABHS tonsillitis.[52] Similar organisms were recovered from patients who had adenoiditis and adenoid hypertrophy. [61]

The recovery of these bacteria in more than three-quarters of the patients who had recurrent GABHS tonsillitis,[24] [59] [60] the ability to measure ß-lactamase activity in the core of these tonsils[62] and the response of patients to antimicrobials effective against BLPB (i.e. clindamycin or amoxicillin plus clavulanic acid)[24] [63] [64] support the role of these aerobic as well as anaerobic organisms in the inability of penicillin to eradicate GABHS tonsillitis.

Pleuropulmonary infections

Aspiration of oropharyngeal secretions or gastric contents, and severe periodontal or gingival disease are the most prevalent risk factors for developing anaerobic pleuropulmonary secretion. The infection can progress from pneumonitis into necrotizing pneumonia and pulmonary abscess, with or without empyema.[65] The lesions tend to develop in the dependent pulmonary segments, in either of the superior segments of the lower lobes and in the posterior segments of the upper lobes. The infection is generally polymicrobial where the causative organisms of community-acquired infection (in 60–80% of cases) are aerobic and anaerobic members of oropharyngeal flora. The anaerobes isolated are *Prevotella*, *Porphyromonas*, *Fusobacterium* and *Peptostreptococcus* spp., and the aerobes are a-hemolytic streptococci and microaerophilic streptococci (Table 232.10).[66] Anaerobes can also be recovered in about one-third of patients who have nosocomial-acquired aspiration pneumonia and pneumonia associated with tracheostomy with and without mechanical ventilation,[67] where they are generally recovered mixed with Enterobacteriaceae, *Pseudomonas* spp. and *Staph. aureus*. Specimens for culture should be obtained in a manner that will avoid their contamination by the oral flora. They can be obtained using bronchoalveolar lavage, bronchoscopy via bronchial brush protected in a double-lumen plugged catheter (using quantitative cultures in the last two methods), percutaneous transtracheal aspiration, lung biopsy and thoracentesis (of empyema fluid). Management of these infections includes drainage of the pleural space, in the presence of empyema, and antimicrobials effective against the anaerobic and aerobic bacteria (see Chapter 36).

Intra-abdominal infections

Secondary peritonitis and intra-abdominal abscesses generally occur because of the entry of enteric micro-organisms into the peritoneal cavity through a defect in the wall of the intestine or other viscus as a result of obstruction, infarction or direct trauma. Perforated appendicitis, inflammatory bowel disease with perforation and gastrointestinal surgery are often associated with polymicrobial infections caused by aerobic and anaerobic bacteria, where the number of isolates can average 12 (two-thirds are generally anaerobes). Diverticulitis and its complications is also associated with such an infection.[68]

The initial infection that follows perforation is peritonitis. This is a synergistic infection in which more than one organism is involved. Characteristically, the more types of bacteria that can be isolated, the graver the morbidity. The specific micro-organisms involved in peritonitis are generally those of the gastrointestinal tract flora where anaerobes outnumber aerobes in the ratio 1:1000–10,000.[1] Of the more of the 400 bacterial species that constitute the gut flora, only the virulent ones survive in the peritoneum to cause the infection. The more distal the perforation in the gastrointestinal tract, the more numerous are the types and number of organisms that spill into the peritoneal cavity. An average of 11.6 organisms per specimen (8.5 anaerobes and 3.1 nonanaerobes) was recovered in a study of 71 patients with gangrenous and perforated appendicitis.[69] The predominant aerobic and facultatives are *Escherichia coli* and *Streptococcus* spp. (including *Enterococcus* spp.), and the most frequently encountered anaerobes are the *B. fragilis* group, and *Peptostreptococcus*, *Clostridium*, *Fusobacterium* and *Eubacterium* spp. (see Table 232.10).[3] [5]

Intra-abdominal infections are characteristically biphasic: in the initial stages a generalized peritonitis occurs, which is primarily associated with *E. coli* sepsis, and in the later stages, in which the infection is contained and intra-abdominal abscesses emerge, *B. fragilis* can be recovered.

The clinical manifestations of secondary peritonitis are a reflection of the underlying disease process. Fever, diffuse abdominal pain, nausea and vomiting are characteristic. Physical examination reveals signs of peritoneal inflammation, including rebound tenderness, abdominal wall rigidity and decrease in bowel sounds. These early findings may be followed by signs and symptoms of shock.

The manifestations of shock from a ruptured viscus merge with those of peritonitis and may be followed by toxemia, restlessness and irritability, a higher temperature, an increase in pulse rate, chills and convulsions. Biliary tract infection is caused by *E. coli*,

TABLE 232-10 -- Aerobic and anaerobic bacteria isolated in various types of infections.

AEROBIC AND ANAEROBIC BACTERIA ISOLATED IN VARIOUS INFECTIONS		
Type of infection	**Aerobic and facultative organisms**	**Anaerobic organism**
Pleuropulmonary	*Staphylococcus aureus*[*] viridans streptococci	Pigmented *Prevotella* spp. (*P. denticola*, *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, *P. loeschii*)
	Pseudomonas aeruginosa[*]	
	Enterobacteriaceae[*]	Nonpigmented *Prevotella* spp. (*P. oris*, *P. buccae*, *P. oralis*)
		Fusobacterium nucleatum
		Peptostreptococcus spp. (*P. micros*, *P. anaerobius*, *P. magnus*)
		Bacteroides fragilis group
		Nonspore-forming Gram-positive rods (*Actinomyces*, *Eubacterium*, *Lactobacillus* spp.)
Intra-abdominal	*Escherichia coli*	*Bacteroides fragilis* group
	Enterococcus spp.	*Bilophila wadsworthia*
	Pseudomonas aeruginosa[*]	*Peptostreptococcus* spp. (especially *P. micros*)
		Clostridium spp.
Female genital tract	*Streptococcus* (groups A, B, others)	*Peptostreptococcus* spp.
	Escherichia coli	*Prevolella* spp. (especially *P. bivia*, *P. disiens*)
	Klebsiella pneumoniae	*Bacteroides fragilis* group
	Neisseria gonorrhoeae (in sexually active patients)	*Clostridium* spp. (especially *C. perfringens*)
	Chlamydia spp. (in sexually active patients)	*Actinomyces*, *Eubacterium* spp. (in intrauterine contraceptive device-associated infections)
	Mycoplasma hominis (in postpartum patients)	

Skin and soft tissue	*Staphylococcus aureus*	*Peptostreptococcus* spp. (*P. magnus*, *P. micros*, *P. asaccharolyticus*)
	Streptococcus (*Strep. milleri* group, groups A and B, viridans group)	Pigmented *Prevotella* spp.†
	Enterococcus spp.‡	*Actinomyces* spp.
	Enterobacteriaceae‡	*Fusobacterium nucleatum*†
	*Pseudomonas aeruginosa**	*Bacteroides fragilis* group‡
		Clostridium spp.‡

* Recovered in hospital-acquired infection
† After exposure to oral flora
‡ After exposure to colonic flora

Klebsiella and *Enterococcus* spp. Anaerobes (mostly *B. fragilis* group, and rarely *C. perfringens*) can be recovered in complicated infections associated with carcinoma, recurrent infection, obstruction, bile tract surgery or manipulation.[70]

Laboratory studies reveal an elevated blood leukocyte count in excess of 12,000/mm^3 with a predominance of polymorphonuclear forms. Radiographs of the abdomen may reveal free air in the peritoneal cavity, evidence of ileus or obstruction and obliteration of the psoas shadow. Diagnostic ultrasound, gallium and CT scanning[71] may be useful in detecting appendiceal or other intra-abdominal abscesses. Postoperative wound infections can occur after appendectomy.

Appropriate management of mixed aerobic and anaerobic intra-abdominal infections requires the administration of antimicrobials that are effective against both aerobic and anaerobic components of the infection[2] [5] as well as surgical correction and drainage of pus.[72] Single and easily accessible abscesses can be drained percutaneously, thus avoiding a surgical procedure. The outcome of the infection depends on a variety of factors that include the general condition of the patient (as measured by the Apache score[73]), the site of perforation, the bacteriology of the infection and the antimicrobial chosen for therapy. The principle of using antimicrobial coverage effective against both the aerobic and anaerobic offenders has become the cornerstone of practice and its efficacy has been confirmed by numerous studies.[72]

The choice of therapy should cover Enterobacteriaceae and anaerobes (mainly *B. fragilis* group), and can be achieved by combination or single-agent therapy. Single-agent therapy provides the advantage of avoiding the ototoxicity and nephrotoxicity of aminoglycosides and may be less expensive. However, a single agent may not be effective against hospital-acquired resistant bacterial strains, and the use of a single agent is devoid of antibacterial synergy, which may be important in immunocompromised hosts. However, for otherwise healthy persons, when therapy is initiated without a long delay, single agents provide adequate therapy. An anti-Enterobacteriaceae agent, such as an aminoglycoside, a quinolone or a third-generation cephalosporin, plus an anti-anaerobic agent, such as clindamycin, metronidazole or cefoxitin, is used as combination therapy. Single agent therapy includes a carbapenem (i.e. imipenem, meropenem), or a penicillin plus a ß-lactamase inhibitor (i.e. ticarcillin-clavulanate). The need to add therapy directed at *Enterococcus* spp. is controversial and some authorities advocate

adding such a drug (i.e. amoxicillin or vancomycin). However, these organisms are isolated in only 10–20% of cases, and rarely in pure culture (see also Chapter 47).

Antimicrobial prophylaxis before colonic surgery includes either an oral preparation such as erythromycin plus neomycin, or a parenteral antimicrobial such as cefoxitin. Use of prophylaxis has reduced the rate of postsurgical wound infection (see also Chapter 190). [74]

Female genital tract infection

Female genital tract infections involving anaerobes are polymicrobial and include the following: bacterial vaginosis; soft-tissue perineal, vulvar and Bartholin gland abscesses; endometritis; pyometra; salpingitis; tubo-ovarian abscesses; adnexal abscess; pelvic inflammatory disease, which may include pelvic cellulitis and abscess; amnionitis; septic pelvic thrombophlebitis; intrauterine contraceptive device-associated infection; septic abortion; and postsurgical obstetric and gynecologic infections.[3] [5] Obtaining proper cultures is often difficult, and avoiding their contamination by the normal genital flora can be achieved by use of culdocentesis, laparoscopy or quantitative endometrial cultures of transcervical samples obtained with a telescoping catheter.

The predominant anaerobes include *Prev. bivia, Prev. disiens*, and *Peptostreptococcus, Porphyromonas* and *Clostridium* spp. *Bacteroides fragilis* group is less often isolated in these infections than in intra-abdominal infection. *Actinomyces* spp. and *Eubacterium nodatum* are commonly isolated in infections associated with intrauterine devices. *Mobiluncus* spp. may be involved with bacterial vaginosis.[3] [5] [75] The aerobic organisms also isolated mixed with these anaerobes include Enterobacteriaceae, *Streptococcus* spp. (including groups A and B), *Neisseria gonnorhoeae* and *Chlamydia* spp. (in sexually active females) and *Mycoplasma hominis* (see Table 232.10).

Clinical findings associated with the presence of anaerobes include gas in the tissues, abscess formation and foul-smelling discharge. Management of polymicrobial pelvic infection includes the use of antimicrobials effective against all potential aerobic and anaerobic pathogens. Additionally, coverage against sexually transmissible pathogens should be provided. The therapeutic regimens include

Figure 232-7 Infected diabetic ulcer.

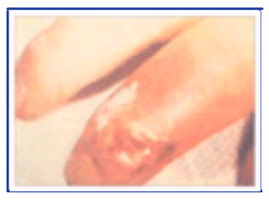

Figure 232-8 Human bite wound.

doxycycline or a macrolide in combination with cefoxitin, cefotetan, clindamycin or metronidazole.

Skin and soft-tissue infections

Skin and soft-tissue infections that generally involve anaerobes include superficial infections, such as infected cutaneous ulcers, cellulitis, pyoderma, paronychia, hidradenitis suppurativa and a variety of secondary infected sites. These include secondary infected diaper rash, gastrostomy or tracheostomy site wounds, infected subcutaneous sebaceous or inclusion cysts, eczema, scabies or kerion infections, and postsurgical wound infection.[3] [5] [76] [77] [78]

Subcutaneous tissue infections that may also include skin involvement include cutaneous and subcutaneous abscesses, breast abscess, decubitus ulcers, infected diabetic (vascular or trophic) ulcers (Fig. 232.7), bite wound (Fig. 232.8), anaerobic cellulitis (Fig. 232.9) and gas gangrene, bacterial synergistic gangrene, infected pilonidal cyst or sinus, Meleney's ulcer and burn wound infection.[77] [78] Anaerobic soft-tissue infections that occur deeper are necrotizing fasciitis (Fig. 232.10), necrotizing synergistic cellulitis, gas gangrene and crepitus cellulitis.[79] These infections can involve the fascia and the muscle surrounded by the fascia, and can induce myositis (Fig. 232.11) and myonecrosis.

The organisms recovered from soft-tissue infections vary according to the type of infections. However, the location and the circumstances leading to the infection can

influence the type of the organisms involved in the infection. Cultures from lesions involving

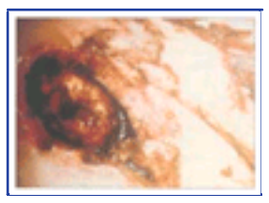

Figure 232-9 Polymicrobial necrotizing cellulitis. The initial lesion is a reddish-brown bled and is accompanied by local tenderness.

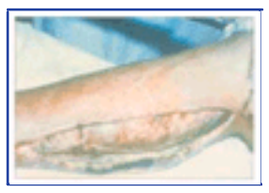

Figure 232-10 Necrotizing fasciitis caused by multiple aerobic and anaerobic bacteria. The fascia inspected through a surgical incision is swollen and dull in appearance, with areas of necrosis. A thin, brownish discharge exudes from the wound, with no pus.

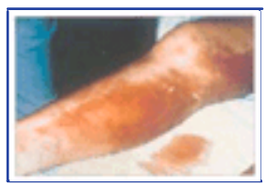

Figure 232-11 Anaerobic streptococcal myositis involving muscle and fascial planes.

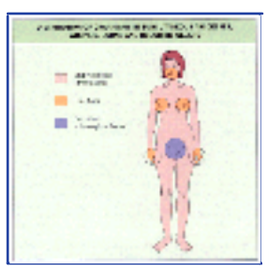

Figure 232-12 Distribution of organisms in subcutaneous abscesses, wounds, burns and decubitus ulcers.

anaerobes frequently contain bacterial species that are members of the 'normal flora' of the region of the infection (Fig. 232.12).

Aspirates from wounds and subcutaneous tissue infections and abscesses of the rectal area (decubitus ulcer, perirectal abscess) or those that originate from the gut flora (i.e. diabetic foot infection) tend to yield organisms found in the colonic flora.[3] [5] [77] [78] [79] These include *B. fragilis* group, *Clostridium* spp., Enterobacteriaceae and *Enterococcus* spp. In contrast, infections in and around the oropharynx, or those that originate from that site, generally contain members of the oral flora (i.e. paronychia, bites, breast abscess). These include pigmented *Prevotella* and *Porphyromonas, Fusobacterium* and *Peptostreptococcus* spp. Skin flora organisms such as *Staph. aureus* and *Streptococcus* spp., or nosocomially acquired organisms can be isolated at all body sites (see Table 232.10). In addition to oral flora, human bite infections often contain *Eikenella* spp. and animal bites harbor *Pasteurella multocida*.[80]

Infections involving anaerobes are generally polymicrobial, and in some (i.e. decubitus ulcers, diabetic foot ulcer) they are often complicated by osteomyelitis or bacteremia.[81] Deep tissue infections such as necrotizing cellulitis, fasciitis and myositis often involve *Clostridium* spp., *S. pyogenes* or a polymicrobic combination of aerobic and anaerobic bacteria. They often contain gas in the tissues and putrid-like pus with a gray thin quality, and are associated with a high rate of bacteremia and mortality.[79] [82]

Management of deep-seated soft-tissue infection includes surgical debridement, drainage and vigorous surgical management. Improvement of oxygenation of the involved tissues through enhancement of blood supply and administration of hyperbaric oxygen, especially in clostridial infection, may be helpful, although there is no formal evidence demonstrating the benefit of hyperbaric oxygen.

Osteomyelitis and septic arthritis

Osteomyelitis caused by anaerobic bacteria is often indistinguishable from infection caused by aerobic bacteria. Anaerobes are especially notable in oesteomyelitis of the long bones after trauma and fracture, osteomyelitis related to peripheral vascular disease, and decubitus ulcers and osteomyelitis of cranial and facial bones. [81] [83] Most of these infections are polymicrobial.

Anaerobic osteomyelitis of cranial and facial bones is generally caused by spread from a contiguous soft-tissue source or from sinus, ear or dental infection. The high number of anaerobes within the oral flora accounts for their improtance in cranial osteomyelitis. The predominance of intestinal anaerobes in pelvic osteomyelitis has been related to their spread from decubitus ulcers.[5] The anaerobes in osteomyelitis associated with peripheral vascular disease access the involved bone from adjacent soft-tissue ulcers. Osteomyelitis of long bones is generally caused by hematogenic spread, trauma or the presence of a prosthetic device.

Anaerobic streptococci and *Bacteroides* spp. are the most common organisms at all sites, including osteomyelitis associated with bites and cranial infection. Pigmented *Prevotella* and *Porphyromonas* spp. are especially prevalent in skull and bite infections, whereas members of the *B. fragilis* group are associated with vascular disease or neuropathy. Fusobacteria, which are members of the oral flora, are most frequently isolated from bites and from cranial and facial infections. Clostridia are most often found in long bones, especially in association with environmental wound contamination after trauma. Because clostridia colonize the lower gastrointestinal tract, they may contaminate compound fractures of the lower extremities.

Septic arthritis caused by anaerobic bacteria is uncommon, and is often associated with hematogenous and contiguous spread of infection, trauma and prosthetic joints.[81] [84] Most cases of septic arthritis caused by anaerobes are monomicrobial. The predominant isolated anaerobes are peptostreptococci and *Prop. acnes* (often in prosthetic joint infection), *B. fragilis* and fusobacteria (often in infections of hematogenic origin), and clostridia (associated with trauma).

Bacteremia

The incidence of anaerobes in bacteremia is 5–15%.[82] [85] The most prevalent blood culture isolates are *B. fragilis* group (over 75% of anaerobic isolates). Other common isolates include *Clostridium* spp. (10–20%), *Peptostreptococcus* spp. (10–15%), *Fusobacterium* spp. (10–15%) and *Prop. acnes* (2–5%).

The specific organisms involved in bacteremia largely depend on the portal of entry and underlying disease. Recovery of *B. fragilis* group and clostridia is mostly associated with a gastrointestinal source, pigmented *Prevotella* and *Porphyromonas* spp. and fusobacteria with oropharynx and pulmonary sources, fusobacteria with the female genital tract, *Prop. acnes* with foreign body, and peptostreptococci with all sources, but especially with oropharyngeal, pulmonary and female genital tract sources. The predominance of these isolates in conjunction with the specific sources is related to the origin of the primary infection and the endogenous flora at the infection site.

The predisposing factors to anaerobic bacterial bacteremia include malignant neoplasma; hematologic disorders; organ transplant; recent gastrointestinal, obstetric, or gynecologic surgery; intestinal obstruction; decubitus ulcers; dental extraction; the newborn; sickle cell disease; diabetes mellitus; postsplenectomy; and the use of cytotoxic agents or corticosteroids.[3] [5]

The clinical presentations of anaerobic bacteremia are similar to those seen with aerobic infection, except for the signs of infection at the portal of entry of infection. It commonly includes fever, chills, hypotension, leukocytosis, shock, disseminated intravascular coagulation and anemia. Features typical of anaerobic infection include metastatic lesions, hyperbilirubinemia, and suppurative thrombophlebitis. Mortality rate varies between 15 and 30% and is improved with early and appropriate

antimicrobial therapy and resolution, when present, of the primary infection.

MANAGEMENT

The recovery from an anaerobic infection depends on prompt and proper management. The principles of managing anaerobic infections include neutralizing toxins produced by anaerobes, preventing their local proliferation by changing the environment and hampering their spread into healthy tissues.

Toxin neutralization by specific antitoxins may be employed, especially in infections caused by *Clostridium* spp. (tetanus and botulism). Controlling the environment is achieved by debriding of necrotic tissue, draining the pus, improving circulation, alleviating the obstruction and increasing the tissue oxygenation. Certain types of adjunct therapy such as hyperbaric oxygen (HBO) may also be useful. The primary role of antimicrobials is in limiting the local and systemic spread of the organism.

Hyperbaric oxygen

There is controversy regarding whether HBO should be used in infection of spore-forming Gram-positive anaerobic rods. There are several uncontrolled reports that demonstrated efficacy in individual cases.[3] [5] [86] However, because no well-controlled studies are available, the efficacy of HBO is unproved. Using HBO in conjunction with other therapeutic measures is not contraindicated, except when it may delay the execution of other essential procedures. Topical application of oxygen-releasing compounds may be useful as an adjunct to other procedures.

Surgical therapy

In many cases surgical therapy is the most important and sometimes the only form of treatment required, whereas in others surgical therapy is an important adjunct to a pharmacologic approach. Surgery is important in draining abscesses, debriding necrotic tissues, decompressing closed space infections and relieving obstructions. When surgical drainage is not used, the infection may persist and serious complications can develop.

Antimicrobial therapy

Appropriate management of mixed aerobic and anaerobic infections requires the administration of antimicrobial drugs effective against both the aerobic and the anaerobic components. A number of factors should be considered when choosing appropriate antimicrobial agents. They should have efficacy against all target organisms, induce little or no resistance, achieve sufficient levels in the infected site, and have minimal toxicity and maximum stability.

Antimicrobials often fail to cure the infection. Among the reasons for this are the development of bacterial resistance, achievement of

Figure 232-13 Susceptibility of anaerobic bacteria to antimicrobial agents.

insufficient tissue levels, incompatible drug interaction and the development of an abscess. The environment of an abscess is detrimental to many antibiotics. The abscess capsule interferes with the penetration of drugs, and the low pH and the presence of binding proteins or inactivating enzymes (i.e. ß-lactamase) may impair their activity. The low pH and the anaerobic environment within the abscess are especially unfavorable for the aminoglycosides and quinolones. However, an acidic pH, high osmolarity and an anaerobic environment can also develop in the absence of an abscess.

When choosing antimicrobials for the therapy of mixed infections, their aerobic and anaerobic antibacterial spectrum (Fig. 232.13) and their availability in oral or parenteral form should be considered (Table 232.11). Some antimicrobials have a limited range of activity. For example, metronidazole is active only against anaerobes and therefore cannot be administered as a single agent for the therapy of mixed infections. Others (i.e. carbaepenems) have wide spectra of activity against Enterobacteriaceae and anaerobes.

The selection of antimicrobial agents is simplified when reliable culture results are available. However, this may be difficult to achieve in anaerobic infections because of the problems in obtaining appropriate specimens. For this reason, many patients are treated empirically on the basis of suspected, rather than established, pathogens. Fortunately, the types of organisms involved in many anaerobic infections and their antimicrobial susceptibility patterns tend to be predictable. However, the pattern of resistance to antimicrobials may vary in a particular hospital and resistance to antimicrobial agents may emerge while a patient is receiving therapy.

The susceptibility of the *B. fragilis* group to the frequently used antimicrobial drugs has been studied systemically over the past two decades. These surveys have failed to reveal strains resistant to chloramphenicol or metronidazole and minimal resistance (<1%) to imipenem or the combinations of a penicillin and ß-lactamase inhibitors, but resistance to other agents varies. The rate differs among various medical centers and generally increases with extensive use of some antimicrobial agents (penicillins, cephalosporins and clindamycin).[87]

Aside from susceptibility patterns, other factors influencing the choice of antimicrobial therapy include the pharmacologic characteristics of the various drugs, their toxicity, their effect on the normal flora and bactericidal activity. Although identification of the infecting organisms and their antimicrobial susceptibility may be needed for selection of optimal therapy, the clinical setting and Gram-stain preparation of the specimen may indicate the types of anaerobes present in the infection as well as the nature of the infectious process.

TABLE 232-11 -- Antimicrobial drugs recommended for the therapy of site-specific anaerobic infections.

ANTIMICROBIAL DRUGS RECOMMENDED FOR THE THERAPY OF SITE-SPECIFIC ANAEROBIC INFECTIONS			
	Surgical prophylaxis	**Parenteral**	**Oral**
Intracranial	Penicillin (vancomycin)	Metronidazole* (chloramphenicol)	Metronidazole* (chloramphenicol)
Dental	Penicillin (erythromycin)	Clindamycin (metronidazole* , chloramphenicol)	Clindamycin, amoxicillin + CA (metronidazole* chloramphenicol)
Upper respiratory tract	Cefoxitin (clindamycin)	Clindamycin (chloramphenicol, metronidazole*)	Clindamycin, amoxicillin + CA (chloramphenicol, metronidazole‡
Pulmonary	NA	Clindamycin* (chloramphenicol, a penicillin + BLI§ , a carbapenem)	Clindamycin* (chloramphenicol, metronidazole,‡ amoxicillin + CA)
Abdominal	Cefoxitin (clindamycin*)	Clindamycin* , cefoxitin* , metronidazole* (a carbapenem, a penicillin + BLI)	Clindamycin* , metronidazole* (chloramphenicol, amoxicillin + CA)
Pelvic	Cefoxitin (doxycycline)	Cefoxitin§ , clindamycin* (a penicillin + BLI§ metronidazole§)	Clindamycin§ (amoxicillin + CA§ , metronidazole§)
Skin	Cefazolin# (vancomycin)	Clindamycin, cefoxitin (metronidazole* + methicillin)	Clindamycin, amoxicillin + CA (metronidazole‡)
Bone and joint	Cefazolin# (vancomycin)	Clindamycin, a carbapenem (chloramphenicol, metronidazole* , a penicillin + BLI)	Clindamycin (chloramphenicol, metronidazole*)

Bacteremia with BLPB	NA	A carbapenem, metronidazole (cefoxitin, a penicillin + BLI)	Clindamycin, metronidazole (chloramphenicol, amoxicillin + CA)
Bacteremia with BLPB	NA	Penicillin (clindamycin, metronidazole, cefoxitin)	Penicillin (metronidazole, chloramphenicol, clindamycin)
Therapies are given as drug(s) of choice (alternative drugs). BLI, ß-lactamase inhibitor; BLPB, ß-lactamase-producing bacteria; CA, clavulanic acid; NA, not applicable.			

* Plus a penicillin
‡ Plus a macrolide (i.e. erythromycin)
* Plus an anti-aerobic bacilli agent (aminoglycoside)
§ Plus doxycycline
# In location proximal to the rectal and oral areas use cefoxitin

Antimicrobial agents

Some classes of agents have poor activity against anaerobic bacteria. These include the aminoglycosides, the monobactams and the currently available quinolones. Antimicrobial agents suitable for use in controlling aerobic infections are discussed in more detail below.[88] [89]

Penicillins

Penicillin G (benzypenicillin) is still the drug of choice against most non-BLPB. These include anaerobic streptococci, *Clostridium* spp. and nonsporolating anaerobic bacilli, and most non-ß-lactamase-producing Gram-negative anaerobic rods (i.e. *Bacteroides, Fusobacterium, Prevotella* and *Porphyromonas* spp.).[88] However, in addition to the *B. fragilis* group, which is known to resist the drug, many other anaerobic Gram-negative rods are showing increased resistance. These include *Fusobacterium* spp., pigmented *Prevotella* and *Porphyromonas* spp. (prevalent in orofacial infections), *Prev. bivia* and *Prev. disiens* (common in obstetric and gynecologic infections), and *Bilophila wadsworthia* and *Bacteroides splanchinus*. Resistance to penicillin of some *Clostridium* spp. through production of ß-lactamase has also been noted. These included *C. ramosum, C. clostridioforme* and *C. butyricum*.

The increase in the number of penicillin-resistant bacterial strains has important implications for antimicrobial therapy. Many penicillin-resistant bacteria can produce enzymes that degrade penicillins or cephalosporins by releasing the enzyme in the area of the infection. Therefore, these organisms may protect not only themselves but also penicillin-sensitive pathogens. Penicillin therapy directed against a susceptible pathogen might therefore be rendered ineffective by the presence of BLPB.[24]

The use of combinations of ß-lactamase inhibitors (such as clavulanic acid, sulbactam, or tazobactam) plus a ß-lactam antibiotic (ampicillin, amoxicillin, ticarcillin or piperacillin) can overcome this phenomenon in organisms that produce a ß-lactamase that can be bound by the inhibitor. However, if other mechanisms of resistance emerge, blockage of the enzyme ß-lactamase will not prevent resistance. Other mechanisms of resistance include alteration in the porin canal through which the antimicrobial penetrates into the bacteria and changes in the penicillin-binding protein that inhibit binding of the drug into the bacterial cell wall.

The semisynthetic penicillins, carbenicillin, ticarcillin, piperacillin and mezlocillin are generally administered in large quantities to achieve high serum concentrations. These drugs have good activity against Enterobacteriaceae and most anaerobes in these concentrations. However, they are not absolutely resistant to ß-lactamase produced by Gram-negative anaerobic bacilli.

Cephalosporins

The efficacy of cephalosporins varies against *Bacteroides* spp.[88] [89] The activity of the first-generation cephalosporins against anaerobes is similar to that of penicillin G, although on a weight basis they are less active. Most strains of the *B. fragilis* group and many *Prevotella* and *Porphyromonas* spp. are resistant by virtue of cephalosporinase production. The second-generation cephalosporin cefoxitin is relatively resistant to this enzyme and is therefore the most effective cephalosporin against the *B. fragilis* group. However, 5–15% of *B. fragilis* group may be resistant, reflecting hospital use pattern. Because of its wide antibacterial coverage, it is often used for the therapy and prophylaxis of mixed infections. Cefoxitin is relatively inactive against most species of *Clostridium* (including *C. difficile*); *C. perfringens* is an exception. Other second-generation cephalosporins, such as cefotetan and cefmetazole, have a longer half-life than cefoxitin. These two agents are as effective as cefoxitin against *B. fragilis*, but have poor efficacy against other members of the *B. fragilis* group (i.e. *B. thetaiotaomicron*). Third-generation cephalosporins have activity against *Bacteroides* spp. that is inferior to that of second-generation cephalosporins.

Carbapenems (imipenem, meropenem, ertapenem)

The ß-lactam carbapenems imipenem and meropenem have excellent activity against a broad spectrum of aerobic bacteria and anaerobic bacteria, including ß-lactamase-producing *Bacteroides* spp. and Enterobacteriaceae and *Pseudomonas* spp.[89] Resistance of *B. fragilis* group is very race (<1%). Ertapenem has similar efficacy, but is not active against *Pseudomonas* spp. and *Acinetobacter* spp.[90]

Chloramphenicol

Chloramphenicol shows excellent *in-vitro* activity against most anaerobic bacteria, and resistance is rare. The drug is also effective against many Enterobacteriaceae and Gram-positive cocci. However, the experience of using this drug in intra-abdominal sepsis was disappointing. The toxicity of chloramphenicol, the rare but fatal aplastic anemia and the dose-dependent leukopenia, limit its use, especially in neutropenic patients.

Clindamycin and lincomycin

Clindamycin and lincomycin are effective against anaerobes and have good activity against aerobic Gram-positive cocci.[88] Clindamycin has the broader coverage against anaerobes, including ß-lactamase-producing *Bacteroides* spp. Resistance of *B. fragilis* group is 5–10%, and some *Clostridium* spp. other than *C. perfringens* are resistant. Antibiotic-associated colitis caused by *C. difficile* was first described after clindamycin therapy. However, colitis has been associated with many other antimicrobial agents, including penicillins and cephalosporins.

Metronidazole

Metronidazole has excellent activity against anaerobes. However, its antibacterial efficacy is limited to anaerobic bacteria. Microaerophilic streptococci, *Prop. acnes* and *Actinomyces* spp. are often resistant. Concern was raised about the carcinogenic and mutagenic effects of this drug; however, these effects were shown only in one species of mice and were never substantiated in other animals or humans.[3] [5]

Macrolides (erythromycin, azithromycin, clarithromycin)

The macrolides erythromycin, azithromycin and clarithromycin have moderate-to-good *in-vitro* activity against anaerobic bacteria other than *B. fragilis* group and fusobacteria. They are active against *Prevotella* and *Porphyromonas* spp., microaerophilic and anaerobic streptococci, Gram-positive nonspore-forming anaerobic bacilli and certain clostridia. They show relatively good activity against *C. perfringens* and poor or inconsistent activity against Gramnegative anaerobic bacilli.

Glycopeptides (vancomycin, teicoplanin)

The glycopeptides are effective against all Gram-positive anaerobes (including *C. difficile*), but are inactive against Gram-negative bacilli.

Tetracyclines

Tetracycline is of limited use because of the development of resistance by all types of anaerobes, including *B. fragilis* group. The newer tetracycline analogs doxycycline and minocycline are more active than the parent compound. Because there is significant resistance to these drugs, they can be used if the organisms are susceptible or in less severe infections in which a therapeutic trial is feasible.

Quinolones

The first generation of fluoroquinolones such as ciprofloxacin and ofloxacin are inactive against most anaerobic bacteria. However, some broad-spectrum quinolones, which have recently become clinically available or are under active development, have significant anti-anaerobic activity. Quinolones with low activity against anaerobes include ciprofloxacin, ofloxacin, levofloxacin, fleroxacin, pefloxacin, enoxacin and lomefloxacin. Compounds with intermediate anti-anaerobic activity include sparfloxacin and grepafloxacin. Trovafloxacin, gatifloxacin and moxifloxacin yield low minimum inhibitory concentrations (MICs) against most groups of anaerobes. Quinolones with the greatest *in-vitro* activity against anaerobes include clinafloxacin and sitafloxacin.[91] The use of the quinolones is restricted in growing children and pregnancy because of their possible adverse effects on the cartilage.

Other agents

Bacitracin is active against pigmented *Prevotella* and *Porphyromonas* spp. but is inactive against *B. fragilis* and *Fusobacterium nucleatum*. [2] Quinupristin-dalfopristin shows antibacterial activity against anaerobic organisms tested, including *C. perfringens* and *Lactobacillus* and *Peptostreptococcus* spp.[92] Linezolid is active against *F. nucleatum*, other fusobacteria and *Porphyromonas, Prevotella* and *Peptostreptococcus* spp. [93] Little clinical experience has been, however, gained in the treatment of anaerobic bacteria using these agents.

Choice of antimicrobial agents

The parenteral antimicrobials that can be used in most infectious sites (Table 232.11 , Table 232.12) are clindamycin, metronidazole, chloramphenicol, cefoxitin, a penicillin (e.g. ticarcillin, ampicillin, piperacillin) and a ß-lactamase inhibitor (e.g. clavulanic acid, sulbactam, tazobactam), and a carbapenem (e.g. imipenem, meropenem, ertapenem).

An agent effective against Gram-negative enteric bacilli (e.g. aminoglycoside) or an antipseudomonal cephalosporin (e.g. cefepime) are generally added to clindamycin, metronidazole and, occasionally, cefoxitin, when treating intra-abdominal infections to provide coverage for these bacteria. Failure of therapy in intra-abdominal infections has been noticed more often with chloramphenicol and, therefore, this drug is not recommended.

Penicillin can be added to metronidazole in the therapy of intracranial, pulmonary and dental infections to cover for microaerophilic streptococci, and *Actinomyces* spp. A macrolide (i.e. erythromycin) is added to metronidazole in upper respiratory infections to treat *Staph. aureus* and aerobic streptococci. Penicillin is added to clindamycin to supplement its coverage against *Peptostreptococcus* spp. and other Gram-positive anaerobic organisms.

TABLE 232-12 -- Antimicrobial drugs of choice for anaerobic bacteria.

ANTIMICROBIAL DRUGS OF CHOICE FOR ANAEROBIC BACTERIA		
	First choice	**Alternative**
Peptostreptococcus spp.	Penicillin	Clindamycin, chloramphenicol, cephalosporins
Clostridium spp.	Penicillin	Metronidazole, chloramphenicol, cefoxitin, clindamycin
Clostridium difficile	Vancomycin	Metronidazole, bacitracin
Gram-negative bacilli (BL-)	Penicillin	Metronidazole, clindamycin, chloramphenicol
Gram-negative bacilli (BL+)	Metronidazole, a carbapenem, a penicillin and BL inhibitor, clindamycin	Cefoxitin, chloramphenicol, piperacillin
Gramnegative bacilli include *Bacteroides fragilis* group and *Prevotella*, *Porphyromonas* and *Fusobacterium* spp. BL, ß-lactamase.		

Doxycycline is added to most regimens in the treatment of pelvic infections to provide therapy for chlamydia and mycoplasma. Penicillin is still the drug of choice for bacteremia caused by non-BLPB. However, other agents should be used for the therapy of bacteremia caused by BLPB.

Because the duration of therapy for anaerobic infections, which are often chronic, is generally longer than for infections caused by aerobic and facultative anaerobes, oral therapy is often substituted for parenteral therapy. The agents available for oral therapy are limited and include clindamycin, amoxicillin plus clavulanic acid, chloramphenicol and metronidazole.

Clinical judgment, personal experience, safety and patient compliance should direct the physician in the choice of the appropriate antimicrobial agents. The duration of therapy generally ranges between 2 and 4 weeks, but should be individualized depending on the response. In some cases, such as lung abscesses, treatment may be required for as long as 6–8 weeks, but can often be shortened with proper surgical drainage.

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Chapter 233 - Mycobacteria

Clark B Inderlied

INTRODUCTION

In his review article entitled 'The White Plague', MF Perutz recounted that Cardinal Richelieu (1585–1642), Heinrich Heine (1797–1856), Frédéric Chopin (1810–49), Anton Chekhov (1860–1904), Franz Kafka (1883–1924), George Orwell (1903–50) and Eleanor Roosevelt (1884–1962) all shared a common fate.[1] Each of these famous people died of tuberculosis. In some cases their disease was not understood or only poorly understood, while in other cases management of the disease was inadequate, often because of a lack of effective therapeutic agents. Indeed, while the virulence and transmissibility of *Mycobacterium tuberculosis* are critical factors, there are additional factors that influence the occurrence and spread of this disease.

Clearly, the presence of a concurrent disease (especially AIDS) as well as the overall state of person's health and host defense mechanisms are critical determinants of the morbidity and mortality rates of tuberculosis. However, even more complex factors strongly influence the incidence of tuberculosis including poverty, ignorance and indifference about the disease, and the lack of access to health care. Indeed, in the World Health Organization (WHO) 2002 report on global tuberculosis control, 22 countries were identified as having a 'high burden' of tuberculosis. For example, while 62% of the world's human population (estimated at 6.1 billion persons in 2000) live in these 22 countries, nearly 80% of cases of tuberculosis worldwide occur in these countries. Nevertheless, even within the 'high burden' countries the incidence of tuberculosis varies from a low of 68 cases to more than 584 cases per 100,000 population. The nearly 9-fold difference in the incidence of tuberculosis reflects the plethora and complexity of factors that influence the spread and incidence of tuberculosis.

The goals of the WHO global tuberculosis control program are to detect 70% of tuberculosis cases in the high burden countries and to successfully treat 85% of the identified cases. Although the WHO sponsored program is focused on tuberculosis, many of the principles of diagnosis, treatment and control of tuberculosis pertain to other nontuberculous infections. The laboratory diagnosis of tuberculosis and other mycobacterial diseases is an important element of the effective treatment and control of all of these diseases and is the focus of this chapter.

NATURE

Lehmann and Neumann first introduced the genus *Mycobacterium* into the scientific literature in 1896. The subsequent history of the genus in terms of our knowledge and understanding of these micro-organisms has been profoundly influenced by the fact that only one of the more than 40 currently recognized species has been a devastating cause of human disease and suffering for time immemorial: *M. tuberculosis*. Indeed, Wayne[2] noted in his discussion of mycobacterial speciation that taxonomic studies of the genus have been undoubtedly skewed because of the focus on the need for the detection and accurate identification of *M. tuberculosis*. Thus, studies of microbial physiology, structure, genetics and diagnostic tools have focused on *M. tuberculosis* and secondarily on *Mycobacterium leprae*. Nevertheless, during the past several years other species of mycobacteria, notably the rapidly growing mycobacteria and the *M. avium* complex, have been the subject of extensive investigations largely because of the increased incidence of human disease and increased awareness of disease caused by these mycobacteria.

All members of the genus *Mycobacterium* are aerobic, nonsporeforming, nonmotile single cell bacteria. In general, the size of mycobacteria are significantly smaller than other types of bacteria and cell shape varies from coccobacilli to elongated rods, but pleomorphic morphology is common. The most prominent feature of mycobacteria that is uniformly present and distinctive to the genus is the complex, lipid-rich cell envelope. Indeed, it is the complex cell envelope of mycobacteria that confers upon these bacteria the property of 'acid-fastness' (i.e. resistance to decolorization when stained with carbolfuchsin and decolorized with dilute hydrochloric acid). The mycobacterial cell envelope has been the subject of extensive investigations because of the chemical uniqueness of the structure, the importance of the envelope to the biology of the micro-organism, and the role of envelope antigens in immunobiology and diagnostics.

Mycobacteria are classified with the Gram-positive bacteria, but most mycobacteria do not stain well with Gram's stain. Mycobacteria possess a cell wall polysaccharide that resembles that of Gram-positive bacteria; however, the mycobacterial peptidoglycan contains lipids in place of proteins and polysaccharides.[3] Furthermore, the mycobacterial envelope contains a plasma membrane that is quite similar in structure and function to the plasma membrane of other bacteria except for the presence of lipoarabinomannan (LAM), lipomannan and phosphatidylinositol mannosides.[3] The cell wall component of the envelope confers size, shape, protection against osmotic pressure, and probably protects the plasma membrane from deleterious molecules in the environment of the cell. The peptidoglycan confers cell shape while the next layer of the envelope, arabinogalactan esterified to the mycolic acids (fatty acids), is largely believed to restrict permeability.

At the exterior surface of the cell envelope are the mycosides, a mixture of glycolipids. The mycosides confer the agglutination serotype of a strain and influence colony morphology and may influence virulence. The mycosides are 'in charge of public relations for mycobacteria' and in this capacity are analogous to the 'O' antigens of Gram-negative bacteria.[4] In 1947, Middlebrook first described growth of tubercule bacilli in the shape of serpentine cords ('cording'), and for many years cording was correlated with virulence and was considered to be a distinctive feature of *M. tuberculosis* (Fig. 233.1). However, it is now known that several species of mycobacteria display cording and the correlation with virulence, if any, is unclear. Cord factor appears to be a mixture of mycolate-containing molecules including the original cord factor associated with *M. tuberculosis*, trehalose 6,6'-dimycolate. Thus, the structure of the mycobacterial cell envelope (Fig. 233.2) includes a plasma membrane, a peptidoglycan layer, an arabinogalactan layer esterified to an uneven mycolate layer, and a glycolipid layer. Lipoarabinomannan and a

Figure 233-1 Microscopic clusters of three different species of mycobacteria. (a) Serpentine cording of *Mycobacterium tuberculosis*. (b) Cross-banding of *Mycobacterium kansasii*. (c) Loose clusters of *Mycobacterium avium* complex. *Photomicrographs taken from Attorri et al.* [5]

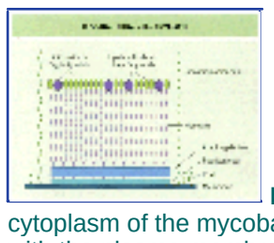

Figure 233-2 Mycobacterial cell envelope. This model displays the asymmetric array of the structural elements extending from the plasma membrane that surrounds the cytoplasm of the mycobacterial cell. The arabinogalactan is covalently linked to the peptidoglycan, which along with the lipoarabinomannan and phosphatidylinositol mannosides (PIM) are associated with the plasma membrane. The cell wall lipids are shown in a possible arrangement with the mycolates linked to the arabinogalactan. Two classes of polar lipids with medium and short chain fatty acids complement the varying hydrocarbon chains of the mycolates to create an even cell envelope. There is evidence for a small number of porins within the hydrophobic bilayer. *Adapted from Brennan and Draper.*[3]

small number of porins traverse the width of the mycobacterial envelope. As in other bacteria the mycobacterial porins provide a mechanism for the passage of low molecular weight molecules from the environment into the intracellular matrix.

Many species of mycobacteria are capable of producing carotenoids and this feature can be an important aid in the presumptive identification of an unknown mycobacterial isolate. The principal carotenoids produced by mycobacteria are carotenes and xanthophylls and it appears that all mycobacteria are at least capable of producing some carotenoids. However, the production of carotenoids is strongly influenced by the composition of the media and growth conditions. Light has a significant effect on pigment production for some species. Scotochromogens (e.g. *M. scrofulaceum*) produce pigment in the absence of light whereas

photochromogens (e.g. *M. kansasii*) produce pigment when stimulated by light (Fig. 233.3). Nonphotochromogens (e.g. *M. avium* complex and *M. tuberculosis*) may or may not produce carotenoids, but most strains usually do not. The

Figure 233-3 *Mycobacterium kansasii,* a photochromogenic mycobacterium, grown on Löwenstein-Jensen medium in light. *Courtesy of S Froman and A Gaytan.*

function of mycobacterial carotenoids is largely unknown, but some evidence suggests that carotenoids provide protection from phototoxic effects.

The genome of mycobacteria is large (3–5.5×10^9 Da) and like other prokaryotes is arranged as a closed circle. The nucleotide base composition of mycobacterial DNA is extraordinarily rich in guanine (G) and cytosine (C; i.e. 66–71 mol% GC). Most of the environmental species of mycobacteria, in particular *M. avium* complex, *M. scrofulaceum* and the rapid growers, contain plasmids. The size and number of plasmids vary; the function(s) of the plasmids are largely unknown, but may include resistance to heavy metals. There has been some evidence and considerable speculation that plasmids in mycobacteria include DNA sequences that encode a variety of virulence factors. However, plasmids would appear to have no role in antibiotic resistance or virulence in *M. tuberculosis*, since the majority of *M. tuberculosis* strains do not contain plasmids.

A phylogenetic tree, based on 16S rRNA sequence information of selected species of slowly growing mycobacteria is shown in Figure 233.4 .[6] The lack of separation of 'species' within the *M. tuberculosis*

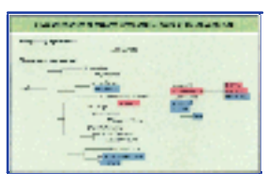

Figure 233-4 Phylogenetic tree of selected mycobacteria, based on 165 rRNA sequence information, including species whose genome sequence is completed (red) or is in progress (green). *Figure adapted from Brosch et al.*[6]

complex reflects that these mycobacteria should be viewed more as pathovars or subspecies. Overall, however, this analysis of genetic relatedness confirms the classical classification scheme based on pigmentation and growth rate. However, genome sequence information has already revealed interesting information about differences within the *M. tuberculosis* complex. For example, comparisons of *M. tuberculosis* H37Rv (a human isolate that is also virulent in mice) with bacille Calmette-Guérin (BCG) Pasteur revealed that 18 variable sequence regions are missing in BCG Pasteur. Perhaps even more intriguing is the presence of two large tandem duplications in BCG Pasteur, whereas other BCG strains lack one of these duplications and neither of the duplications are seen in H37Rv. Furthermore, the genome size of *M. bovis* is significantly smaller than that of *M. tuberculosis*, which seems at odds with the fact that *M. bovis* has a significantly wider host range than *M. tuberculosis*.[6] Indeed, sequence information has placed in doubt the longheld belief that *M. tuberculosis* evolved from an animal pathogen (i.e. *M. bovis*) and indicates that *M. tuberculosis* was always a human pathogen.[7]

EPIDEMIOLOGY

Tuberculosis

Tuberculosis is the leading cause of death worldwide that can be attributed to a single infectious disease agent.[8] [9] [10] The vast majority of tuberculosis cases are found in the developing world and disease occurs predominantly during the prime period of life from 15 to 59 years of age. The estimated incidence of tuberculosis worldwide in 2000 was 8.7 million cases, of which nearly 80% (6.9 million) were found in 22 high-burden countries (Fig. 233.5).[11] Conversely, less than 3% of the worldwide incidence of tuberculosis is found in the industrialized countries. [12] The mortality rate due to tuberculosis closely tracks the worldwide incidence with the highest mortality rate again in South East Asia and Africa. Overall, tuberculosis is estimated to cause over 25% of avoidable adult deaths in the developing world. Indeed, before the development of effective chemotherapy, the mortality rate for tuberculosis was 50–60%. However, following the discovery of isoniazid and then rifampin (rifampicin), the majority of pulmonary and extrapulmonary tuberculosis became treatable. Nevertheless, the treatment of tuberculosis remains problematic because of the lack of or intermittent access to therapy, poor adherence to therapy and the emergence of resistance.

Leprosy

Epidemiology of leprosy (Hansen's disease) is difficult to assess accurately because of the lack of a diagnostic skin test, inability to cultivate the leprosy bacillus in vitro and the nature of the geographic distribution of endemic disease. Leprosy appears to be primarily spread from human to human through nasal secretions. Skin contact is most likely an uncommon route of transmission.[13] Although there has been speculation that an insect vector may be responsible for the spread of some leprosy, this has not been demonstrated. It does appear that transmission by any route requires prolonged exposure to an infected individual. However, the transmission of leprosy is similar to tuberculosis in that, while prolonged exposure is the most common risk factor, a single exposure involving coincident but otherwise singly rare circumstances can result in the transmission of disease. Treatment appears to decrease infectiousness significantly. The global incidence of leprosy is estimated to be between 5.5 and 6.5 million patients.[4] The vast majority of patients are in the developing world, predominantly South East Asia and mostly in India. The care and treatment of leprosy have two equally difficult components. The first is treatment of active disease, especially in patients with physical deformities or at risk of developing deformities. The second is care of effectively treated patients, but with disabling or debilitating deformities due to the pathology associated with the various manifestations of this disease.

Mycobacterium avium complex

Mycobacterium avium complex (MAC) disease occurs as a disseminated disease usually, but not exclusively, in people with HIV infection. *Mycobacterium avium* complex also causes pulmonary disease in immunocompetent patients and cervical lymphadenitis in normal hosts, mostly children. It is ubiquitous in the environment, including and especially soil and water, but also in certain foods (e.g. hard cheese). Portals of infection are probably both gastrointestinal and pulmonary by ingestion of contaminated food or water and inhalation of aerosols. Disseminated MAC disease was rarely reported before the first reports of opportunistic infections and AIDS, published in the early 1980s. Based on complete follow-up information (even at the peak of the AIDS epidemic in the USA disseminated MAC was infrequently the AIDS-defining opportunistic infection) disseminated MAC disease occurs in 20–35% per year of people with HIV infection. [14] However, even this is an historic figure and MAC prophylaxis (rifabutin, azithromycin or clarithromycin) and the

Figure 233-5 Estimated tuberculosis incidence rates, 2000. *Data and map adapted from World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO Report 2002, Switzerland, WHO/CDS/TB/2002.295.*[11]

use of highly active antiretroviral therapy (HAART) has significantly decreased the incidence of MAC disease in people with HIV infection. The incidence of disseminated MAC disease in children with HIV infection is nearly the same as in adults and the manifestations of disseminated MAC disease in these patients are virtually identical to those in adults. *Mycobacterium avium* complex pulmonary disease is seen worldwide with an overall incidence of about 1/100,000 persons. The incidence of pulmonary disease appears to be increasing, but this may be due in part to increased surveillance and awareness by the medical community. Although

MAC pulmonary disease has been associated with a history of chronic lung infection including cystic fibrosis, many patients have no identifiable risk factors except age. *Mycobacterium avium* complex cervical lymphadenitis is mostly a pediatric disease and primarily seen in children under 5 years of age. There are several reports of disseminated MAC disease in children who do not have HIV infection, but it seems likely that these children have a very specific immunodeficiency that causes the patient to be at risk for MAC infection.

Rapid growers

Although there are over 30 species of rapidly growing mycobacteria, the vast majority (>90%) of disease that occurs in humans is caused by three species: *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium abscessus*. These species are important causes of cutaneous, pulmonary and postsurgical wound infections especially following catheter placement, augmentation mammaplasty and cardiac bypass surgery. Disseminated disease is rare and almost invariably occurs in immunocompromised patients, but disease is not common in people with HIV infection.

The distribution of rapidly growing mycobacteria appears to be worldwide. The three species associated with human disease have been isolated from drinking water as well as natural waters. In addition, these mycobacteria are commonly found in animal drinking water, waste water and soil. The ubiquitous nature of these micro-organisms, their resistance to disinfectants including relative resistance to chlorine, and their ability to grow on unusual substrates such as polyhalogenated phenols account for the fact that rapidly growing mycobacteria are found as contaminants of medical equipment, prosthetic valves and disinfectants.

The incidence of pulmonary disease caused by rapidly growing mycobacteria is unclear (80% by *M. abscessus*), but an increased awareness of these species as human pathogens is leading to greater insight into the true incidence of disease. Nevertheless, the isolation of rapidly growing mycobacteria from respiratory tract specimens must be interpreted with great caution because contamination and colonization are common. True infection is usually associated with chronic pulmonary disease such as cystic fibrosis, previous mycobacterial lung disease and bronchiectasis due to previous respiratory infection. Rapidly growing mycobacteria are intrinsically resistant to antituberculous agents including isoniazid, rifampin and pyrazinamide.

PATHOGENICITY

The pathogenicity of mycobacterial infections in the immunocompetent host has as much to do with the immune response of the host as with destructive virulence properties of the mycobacterial pathogen. Thus, the principal virulence factor of mycobacteria is the ability to invade and persist or replicate within macrophages. Tubercle bacilli are believed to attach to macrophages by binding to the mannose receptor on the macrophage via the mycobacterial cell envelope LAM. Alternatively tubercle bacilli can indirectly bind via CR1/CR3 complement receptors or Fc receptors.

The bacilli enter the macrophage by phagocytosis and once internalized the bacilli are surrounded by a membrane-bound vacuole to

form a nascent phagosome. With the maturation of the phagolysosome, bacilli are exposed to a variety of antimicrobial factors including reactive oxygen intermediates, hydrolytic activities and a highly acidic pH. Cytokine-activated macrophages may also produce reactive nitrogen intermediates, although this has been conclusively shown only in murine macrophages. Virulent mycobacteria obstruct the maturation of phagosomes, inhibiting acidification, and can escape from the phagolysosome into the cytoplasm. Other bacilli appear able to adapt to life within the phagolysosome.

Recent evidence indicates that the maturation of the phagolysosome varies depending on the bacterial pathogen and varies even between intracellular pathogens.[15] The composition of the phagosome membrane is determined in part by the infecting mycobacteria; for example, phagosomes containing *M. avium* do not contain the vacuolar H^+ -ATPase that is needed for acidification of the phagosome. Phagosomes containing either *M. tuberculosis* or *M. avium* bacilli are frequently incompetent for fusion with endocytic organelles, suggesting a mechanism for the block in phagolysosome fusion. Inhibition of acidification can occur as a consequence of the exclusion of a proton pump (as with *M. avium*) or fusion with a lysosome that does not contain a pump or perhaps by the release of an ammonia metabolizing enzyme such as glutamine synthetase. Once mycobacteria have modified or adapted to the intracellular environment the bacilli may proliferate, but the mechanism of intracellular replication of mycobacteria is poorly understood.

The immunopathogenesis of mycobacterial infections primarily involves a cell-mediated immune response. This response includes the activation of macrophages to identify and inhibit or kill mycobacteria and the detection and lysis of phagocytes in which mycobacteria are in a state of growth and replication (Fig. 233.6).

Mycobacterial antigens are presented by antigen-presenting cells (monocyte/macrophage lineage) resulting in secretion of interleukin (IL)-2 and clonal proliferation of $CD4^+$ and $CD8^+$ lymphocytes (a/ß T cells). Mycobacterial antigens arising from the phagosome are presented by major histocompatibility complex (MHC) class II molecules while antigens arising from the cytoplasm are presented by MHC class I molecules.[15] This difference determines, in part, the fate of the antigen-presenting cell, stimulation or destruction, because the presentation of antigen arising from the cytoplasm indicates that the cell has failed to control the mycobacteria. The release of interferon (IFN)-? by the clonally expanded CD4 cells, but also to a lesser extent by CD8 cells, natural killer (NK) cells and ?d cells, activates macrophages. This initiates a cascade of events including the hydrolysis of vitamin D that leads to further activation of macrophages and the release of tumor necrosis factor (TNF)-a by the activated macrophages. The sequence of cellular responses at the site of mycobacterial infection appears to be polymorphonuclear granulocytes (PNGs), NK cells, ?d cells and then a/ß T cells; however, with time the a/ß T cells become predominant in the cellular response. The sequence of recruitment is probably determined by the proximity of the cells to the site of infection; for example, ?d cells are likely to be one of the first cells recruited to the site of *M. tuberculosis* infection in the lung. The PNGs produce highly proteolytic enzymes that cause tissue liquefaction while each of the T-cell types possesses cytotoxic activity. Activated macrophages have an increased capacity for seeking out, engulfing and destroying mycobacteria, but the production of TNF-a by macrophages contributes to tissue necrosis and, therefore, to the immunopathology of the disease.

The activity of macrophages to resist mycobacterium multiplication, which determines in part host susceptibility or host resistance to tuberculosis, is determined by the presence of the gene *Bcg/Ity/Lsh* in mice with its human homolog *NRAMP1* (natural resistance-associated macrophage protein 1).[17] [18]

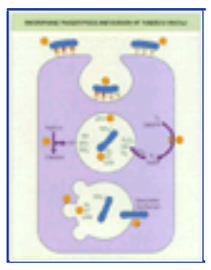

Figure 233-6 Macrophage phagocytosis and evasion of tubercle bacilli. The tubercle bacilli bind via lipoarabinomannan (LAM) (1) or complement receptors (2); phagocytosis occurs (3) with the activation of an oxidative burst (4); glycolipids (GL), sulfatides (ST) and LAM can downregulate the oxidative burst (5); reactive nitrogen intermediates may play a role in antimycobacterial activity (6), as does the acidic pH of the phagolysosome (7). Finally, the production of ammonia by tubercle bacilli may diminish the effect of reactive nitrogen intermediates (8) and contribute to the failure to form a phagolysosome fusion (9). Tubercule bacilli may evade the antimycobacterial activities of the phagolysosome by producing a hemolysin that releases the bacilli into the cytoplasm (10). NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; SOD, superoxide dismutase. *Adapted from Chan and Kaufmann.* [16]

On the other hand, mycobacteria are equipped to resist oxidative killing by phagocytic cells. This resistance is controlled by the *katG* promoter in the micobacterial genome. The *katG* gene encodes an enzyme with catalase, peroxidase and peroxynitritase activities.[19]

Granulomas develop as a mycobacterial infection becomes more chronic. Granulomas are aggregates of activated macrophages that take on epithelial cell-like morphology (epithelioid cells), and typically lymphocytes are found at the periphery of the granuloma. Although the formation of a granuloma effectively contains the mycobacteria where they can be killed, bacilli can persist within giant cells (fused epithelioid cells) and the granuloma may never sterilize. With time a fibrotic wall (collagen) will encapsulate the granuloma and the center of the granuloma may become necrotic with a cheese-like appearance, which is the source of the descriptive word caseation. Hippocrates termed granulomas 'tubercles' because they were similar in morphology to the tubers of plants, hence the name tuberculosis.[4]

Finally, in immunocompetent hosts and to lesser degree immunocompromised hosts (e.g. people with HIV infection may be totally anergic), there is a delayed-type hypersensitivity (DTH) reaction to *M. tuberculosis* infection. In addition, a DTH reaction probably occurs in most mycobacterial infections, but certainly with *M.*

avium-intracellulare, *M. leprae* and *M. ulcerans*. The DTH that occurs in response to *M. tuberculosis* infection is the basis for the tuberculin skin test commonly referred to as the purified protein derivative (PPD) test.

DIAGNOSTIC MICROBIOLOGY

The detection and identification of mycobacteria in clinical specimens is largely still considered to be a subspecialty in clinical microbiology that requires considerable expertise, especially for the accurate identification and susceptibility testing of uncommon mycobacterial species. Nevertheless, the technology available to assist in the laboratory diagnosis of mycobacterial infections has improved remarkably. Semi-automated culture systems are available for the primary culture of clinical specimens. Nucleic acid amplification assays are commercially available for the direct detection of *M. tuberculosis* in clinical specimens and nucleic acid probes have been commercially available for several years for the rapid identification of *M. tuberculosis*, *M. avium* complex, *M. avium*, *M. intracellulare*, *M. kansasii* and *M. gordonae* from culture.

Susceptibility testing of *M. tuberculosis* is more standardized and several molecular assays have been described for the rapid detection of antimicrobial resistance, especially rifampin resistance in *M. tuberculosis*, but molecular methods for detecting resistance have yet to become routinely used in clinical mycobacteriology laboratories.[20] [21] [22]

In discussing diagnostic mycobacteriology it is important to emphasize two general principles of laboratory medicine. First, the quality of any clinical test is highly dependent on the quality of the specimen, including the time of collection in the course of the disease, the appropriateness and sufficiency of the specimen, and the prompt and proper transport of the specimen to the laboratory. Second, the intended purpose of the test, as it was designed and verified, must be understood by the physician who orders the test. Was the test designed to screen for disease, to provide a definitive diagnosis based on a clinical index of suspicion, to confirm another test, to monitor therapy (test of cure), or to provide epidemiologic information? Although any one test may have multiple purposes, most tests have been designed and evaluated for a limited purpose. Use of a test for a purpose for which it was not designed and evaluated could be misleading. For example, nucleic acid amplification assays for the direct detection of *M. tuberculosis* in clinical specimens were evaluated as diagnostic assays, not as tests of cure or for monitoring response to therapy.

Laboratory diagnosis

The laboratory diagnosis of mycobacterial infections often consists of five steps:

- first, mycobacteria can be directly detected in clinical specimens by microscopic examination and molecular assays;
- second, mycobacteria can be detected in clinical specimens by culture using a variety of liquid and solid media;
- third, the isolated mycobacteria can be identified using morphologic, biochemical and molecular tests;
- fourth, the activity of antimicrobial agents can be measured using in-vitro tests; and
- fifth, strains of mycobacteria can be characterized by molecular or other methods for epidemiologic and public health purposes.

The isolation of *M. tuberculosis* strains resistant to multiple antibiotics and the increase in the incidence of tuberculosis in the USA for a short time led to concerns about the length of time (commonly believed to be at least 4–8 weeks) necessary for a conventional laboratory diagnosis. Therefore, in providing laboratory services for the diagnosis of tuberculosis and other mycobacterial diseases, one must recognize the need for prompt as well as accurate information. Salfinger[23] led in designing and implementing 'fast-track' mycobacteriology services, initially in response to an acute need during an outbreak of multidrug resistant *M. tuberculosis* in New York City. However, even before the 'fast-track' era, it was possible to provide mycobacteriology services in a reliable and prompt manner. All that was required was the appropriate diligence and awareness of the laboratory, physicians and public health officials.

Specimen collection

The most critical step in the laboratory diagnosis of any infectious disease occurs before the specimen arrives at the laboratory. The quality, quantity, timing, transport and appropriateness of the specimen have a greater impact on the outcome of a laboratory test than almost any other factor (Table 233.1). Mycobacterial infections can be localized or disseminated, or disseminated with foci of infection that are more likely than other body sites to yield mycobacteria. Once a specimen has been collected it is important to transport the specimen in a container that is sterile, leak-proof and wax-free. The specimen must be accompanied by a request form or transmittal slip that includes the patient's name, medical record number, date and time collected, name of the laboratory, diagnosis, therapy, type of specimen, and test(s) requested. It is important that physicians notify the laboratory if they suspect an uncommon or fastidious mycobacterial infection such as *M. ulcerans*, *M. marinum* or *M. haemophilum*. These and other species of mycobacteria have growth requirements that are significantly different from the majority of other species of mycobacteria isolated from clinical specimens.

Sputum

Sputum is the best specimen for the diagnosis of pulmonary tuberculosis. Expectorated sputum specimen should be collected early in the morning on three occasions. Sputum should be collected directly into a wide-mouthed, sterile, plastic container (wax free) with a tight fitting cap. The specimen should be immediately transported to the laboratory or held at 32–39.2°F (0–4°C) until processed. Prompt processing of sputum is required because of the potential for overgrowth of normal respiratory tract microflora. Alternative respiratory tract specimens are induced sputum, endotracheal aspiration, bronchial washings or aspirates taken during bronchoscopy, laryngeal swabs (not a simple throat swab) and gastric lavage. Bronchoscopes must be carefully cleaned and decontaminated after collecting specimens from a patient with suspected tuberculosis.

Gastric lavage

Gastric lavage (for swallowed sputum) is useful for collecting specimens from patients who for a variety of reasons are unable to produce sputum by other means. Gastric lavage is frequently used to collect specimens from young children. In performing a gastric lavage it is common to suggest to patients that they sip water or swallow a small amount of ice to help pass the gastric tube into the stomach. However, *M. gordonae* is frequently found in many hospital water supplies and especially in ice machines, and use of such contaminated water during the collection of a gastric aspirate may result in a false-positive smear or culture. A gastric lavage must be promptly sent to the laboratory because it must be processed as soon as possible or neutralized with 10% sodium carbonate to avoid loss of mycobacteria due to gastric acidity. As with expectorated sputum specimens, gastric lavage specimens should be collected early in the morning, before eating, and on three separate occasions.

TABLE 233-1 -- Specimen types and requirements for the diagnosis of mycobacterial infections.

SPECIMEN TYPES AND REQUIREMENTS FOR DIAGNOSIS OF MYCOBACTERIAL INFECTIONS			
Specimen type	**Requirements**	**Collection device**	**Note**
Abscess fluid	10ml*	Syringe with cap	Dry swab unacceptable
Blood	10ml	SPS tube, Isolator, Bactec 13A	EDTA tube unacceptable, treat coagulated blood as tissue
Fluids (pleural, pericardial, peritoneal)	10–15ml*	Sterile container, syringe	
Bone	Bone chip	Sterile container, no fixative	Formalin fixed unacceptable
Bone marrow	100mg* 1–3ml aspirate	SPS tube, 1.5ml Isolator	Aspirate volumes >5ml may be primarily peripheral blood
Bronchoalveolar lavage or bronchial washing	=5ml	Sterile container	Avoid contamination of bronchoscope with tap water — possible false positive
CSF	10ml*	Sterile tube	Smear is STAT
Gastric lavage	5–10ml	Sterile container, collect in morning before arising and before eating	Neutralize with 10% carbonate, if delayed processing

Lymph node	Whole node or part, caseous part	Sterile container	Formalin fixed unacceptable
Skin biopsy	Sterile container, aspirate in syringe	Biopsy at periphery or aspirate from under margin of lesion	Note if suspect *M. ulcerans*, *M. haemophilum*, or *M. marinum* — need special culture conditions or extended incubation
Sputum	5–10ml	Sterile, wax-free container	Use sterile hypertonic saline for induced sputum, avoid exposure to tap water
Stool	=1g	Sterile, wax-free container	Do *not* freeze
Tissue bropsy	1g	Sterile container	Caseous portion, formalin fixed unacceptable
Transtracheal aspirate	10ml*	Syringe with cap	
Urine	50ml*	Sterile container, syringe with cap	24-hour urine, catheter urine are unacceptable
SPS, sodium polyanetholsulfonate.			

* As much as possible up to or in excess of the weight shown

Urine

Three early morning midstream urine specimens should be collected into a sterile plastic container (wax-free) with a leak-proof cap. Large volumes of urine can be concentrated by filtration, but 24-hour urine should not be used because of contamination and dilution of any mycobacteria present. True positive urine cultures for mycobacteria almost invariably indicate infection elsewhere in the patient.

Blood and bone marrow

Blood, bone marrow aspirates or cores are ideal specimens for the diagnosis of disseminated MAC, disseminated *M. tuberculosis*, and other disseminated mycobacterial infections. Blood should be processed using an agent that causes blood cell cytolysis and the cell-free mycobacteria collected by centrifugation using either an Isolator tube (Wampole, Cranbury, NJ) or a sodium polyanethol sulfonate (SPS) tube.[24] Blood collected in an SPS tube must be treated with a lytic agent such as desoxycholate and then concentrated by centrifugation before inoculating media. Alternatively, blood can be collected into an SPS tube and transferred directly to a Bactec460 TB 13A medium or other semi-automated systems (Table 233.2) for the detection of *M. avium* complex. Bone marrow aspirate can be collected in a pediatric Isolator tube, but bone marrow core should be processed as tissue. Bone marrow specimens should also be inoculated into a liquid medium such as Bactec460 TB 12B or Middlebrook 7H9. Blood and bone marrow for culture should not be collected in an EDTA tube; however, an albumin-citrate-dextrose (ACD) tube can be used if an SPS tube is not available.

Other fluids

Cerebrospinal fluid (CSF), pleural, pericardial, synovial and ascitic fluids as well as pus and bone marrow aspirates may be submitted for smear and culture. *Mycobacterium tuberculosis* meningitis is a medical emergency and CSF specimens should be collected, transported and processed in a manner that reflects the urgent nature of such a diagnosis. Aseptically collected sterile body site fluids should not be decontaminated, but these specimens can be concentrated. Fluids should be promptly processed and inoculated into a liquid growth medium as well as solid media.

Tissues

Tissue should be submitted for both histology and mycobacteriology. Tissue is preferred over necrotic material or pus for culture, therefore, it is important to have fresh tissue and not swabs submitted to the microbiology laboratory for smear and culture. Formalin-preserved tissue should be submitted for histologic studies. Although not suitable for culture, formalin-preserved and paraffin-embedded tissue can be submitted for polymerase chain reaction (PCR) analysis, especially if acid-fast bacilli are observed on microscopic examination of the tissue sections. [25] [26]

Feces

Feces are not a particularly useful specimen for the diagnosis of tuberculosis or other mycobacterial infections, with the exception of suspected gastrointestinal tract infection with MAC in HIV-positive patients. However, the recovery of MAC from feces is poor (low sensitivity) even in HIV-positive patients. If *M. avium* is isolated from the feces the positive predictive value of the culture is high, meaning that the patient is likely to develop disseminated disease.[27] In order to avoid overgrowth of normal gastrointestinal tract microflora, feces must be rather harshly decontaminated, but this is likely to also decrease the yield of mycobacteria.[28] The use of a selective medium such as Mitchison's selective medium may assist in the recovery of mycobacteria from feces.

TABLE 233-2 -- Semi-automated systems for the detection of *Mycobacterium tuberculosis* and other mycobacteria in clinical specimens.

SEMI-AUTOMATED SYSTEMS FOR THE DETECTION OF *MYCOBACTERIUM TUBERCULOSIS* AND OTHER MYCOBACTERIA IN CLINICAL SPECIMENS			
Instrument/medium	**Manufacturer**	**Detection system**	**SIRE/PZA susceptibility testing**
Bactec 9000MB MYCO/F medium + supplements (modified 7H9 broth)	Becton Dickinson Microbiology Systems, Sparks, MD, USA	Detects changes in O_2 concentration in the growth medium using an O_2 sensor based on fluorescence quenching	No/No
Bactec MGIT 960 (modified 7H9 broth)	Becton Dickinson Microbiology Systems, Sparks, MD, USA	O_2 sensor based on fluorescence quenching	Yes/Yes
ESP Culture System II/ESP Myco (modified 7H9 broth)	TREK Diagnostic Systems, Westlake, OH, USA	Detects production and consumption of gas by measuring changes in partial pressure of gas phase of culture medium	Yes/No
MB/BacT	BioMerieux, Durham, NC, USA	Detects changes in CO_2 concentration in gas phase of the growth medium using a colorimetric sensor and reflected light	Yes/No
Bactec 460/Bactec 12B and Bactec 13A (modified 7H9 broth)	Becton Dickinson Microbiology Systems, Sparks, MD, USA	Detection of radioactively labeled CO_2 released during the catabolism of ^{14}C-labeled palmitic acid in the growth medium	Yes/Yes
PZA, pyrazinamide; SIRE, streptomycin, isoniazid, rifampin and ethambutol.			

Processing contaminated specimens

Most respiratory tract specimens are expected to be contaminated with normal respiratory tract microflora. In addition, the mucin matrix of these specimens makes the specimens difficult to process and mucin both protects and traps micro-organisms. Such contaminated specimens must be treated to liquify the specimen and release any micro-organisms trapped in the mucin or cells. Then, the specimen is decontaminated to reduce or eliminate other micro-organisms that would be likely to overgrow the mycobacteria that might be present in the specimen. The most common mucolytic agents used to digest sputum are *N*-acetyl-L-cysteine (NALC) and dithiothreitol (DTT or sputolysin). Both NALC and DTT are unstable in air and must be prepared fresh; sodium citrate is usually added to NALC/DTT to chelate heavy metals that may inactivate these compounds. Both NALC and DTT have sulfhydryl groups that reduce the disulfide bridges of the glycoprotein that confers upon sputum its mucilaginous property. A combination of NALC and sodium hydroxide is most commonly used for digesting and decontaminating sputum. Other agents used

for digestion and decontamination are zephiran (benzalkonium chloride)-trisodium phosphate, oxalic acid, cetylpyridinium chloride (CPC)-sodium chloride, and sulfuric acid.

There is no ideal method for digesting and decontaminating sputum and other respiratory tract specimens and invariably mycobacteria are lost during decontamination. Indeed, it has been estimated that up to one-third of the mycobacteria present in a sputum specimen may be lost during decontamination. If *M. gordonae* is naturally found in the environment of the laboratory, one should expect about 5% of clinical specimens processed in the laboratory will be contaminated with this mycobacterium. If less than 5% *M. gordonae* contamination is observed, the decontamination procedure may be too harsh. Detailed descriptions of the various procedures for digesting and decontaminating sputum and other contaminated specimens have been well described elsewhere.[29] [30]

Processing uncontaminated specimens

Aseptically collected tissues and fluids from normally sterile body sites usually do not require processing before inoculation of cultures. Certain fluids such as pericardial or pleural fluid may require the addition of an anticoagulant to the collection container. The primary concern about specimens from sterile body sites is the quantity of specimen submitted for smear and culture. Swabs usually contain insufficient material for culture and 1g of tissue or 10ml of fluid are ideal. To improve recovery of mycobacteria, centrifugation or filtration should be used to concentrate large volumes of fluids.

Centrifugation

Although the chemical components of the digestion and decontamination procedure are critical to specimen processing and the successful recovery of mycobacteria, the concentration of mycobacteria by centrifugation is a frequently overlooked component of the procedure that may contribute to poor recovery if not performed properly. Two aspects of the centrifugation step are important:

- the sedimenting efficiency or relative centrifugal force (RCF); and
- the heat generated during centrifugation.

The sedimenting efficiency should be 95% and the RCF should be at least 3000G for 15–20 minutes. Higher RCF values can be used; however, the type of centrifuge tube used will also determine the maximum permissible RCF. Aerosols generated during centrifugation are a high-risk factor for transmission of tuberculosis to laboratory workers. Therefore, specimens must be placed in aerosol-free canisters or in an aerosol-free centrifuge. Manipulation of the supernatant must follow appropriate biohazard protection guidelines.

Culture

A variety of solid and liquid media are available for the culture of mycobacteria from clinical specimens. It is prudent to use several media to ensure recovery and provide good growth for subsequent identification and susceptibility testing. The macroscopic and microscopic growth of mycobacteria may vary with the type of media and these differences in morphology, rate of growth and pigmentation are valuable in making a presumptive identification (see Fig. 233.1).

Several semi-automated systems are designed to detect mycobacteria in clinical specimens (Table 233.2) in addition to the radio-respirometric system developed in the late 1970s by Middlebrook and commercially developed as the Bactec460 TB 460 system in the early 1980s by Becton Dickinson Co. In the various studies that were performed to evaluate these newer systems, comparisons were usually made with conventional culture (e.g. Löwenstein-Jensen medium) and the Bactec460 TB system. Indeed, the Bactec460 TB 460 system became a 'gold standard' for the rapid detection of mycobacteria in semi-automated systems. In the Bactec460 TB 460 the growth of mycobacteria is detected by measuring the release of $^{14}CO_2$ from ^{14}C-labeled palmitate, which is the primary carbon source in the Bactec460 TB 12B culture medium. The limitations of

the Bactec460 TB 460 system are that the medium includes a radioactive substrate, the media are expensive and the system is surprisingly labor intensive.

Information about the performance of the new semi-automated systems, the studies published to date indicate that these systems (Becton Dickinson's Bactec460 TB 9000MB and Bactec MGIT 960, Trek Diagnostic System's ESP II and bioMerieux's MB/BacT) perform well in comparison with conventional culture and the Bactec 460 system.[31] [32] [33] [34] [35] [36] [37] [38] [39] [40] [41] [42] [43] [44] The new systems are convenient, do not require the use of radioisotope, and are truly labor-saving. In comparison with the Bactec460 TB 460 system the newer systems are 5–10% less sensitive and the time to detection is the same or 1–5 days longer. It is important to emphasize that a liquid medium, of one of the types described here, as well as a solid medium must be included in the laboratory diagnosis of mycobacterial infections to ensure maximum recovery (sensitivity).

Additional manual culture systems used for the detection of mycobacteria are the Septi-Chek acid-fast bacillus (AFB) biphasic system, the MB Redox tube system and the Mycobacteria Growth Indicator Tube (MGIT). These are manual culture systems, although the MGIT system has been recently adapted to a semi-automated detection system. The Septi-Chek AFB system is a biphasic medium consisting of 30ml of Middlebrook 7H9 broth and a triangular-shaped plastic paddle with Middlebrook 7H11 agar on one side and Middlebrook 7H11 with NAP and chocolate agar on the remaining surfaces. Comparison studies with conventional culture and the Bactec460 TB 460 system indicated that the Septi-Chek AFB system is reliable and practical.[45] [46] MB Redox tubes contain 4ml of modified Kirchner medium (Kirchner base medium plus glucose, horse serum, a vitamin combination and OADC (oleic acid, albumin, dextrose and catalase)). In addition, the medium contains a mixture of antimicrobial agents (polymyxin B, amphotericin B, carbenicillin and trimethoprim) and a redox indicator (tetrazolium salt). Mycobacteria reduce the tetrazolium salt to a pink-, red- and violet-colored formazan. The accumulation of reduced dye on the surface of the bacilli results in the appearance of visible microcolonies. The MGIT culture is a highly enriched Middlebrook 7H9 broth that contains a disk of silicon rubber impregnated with ruthenium pentahydrate that acts as a fluorescence-quenching oxygen sensor. Growth is detected by exposing

TABLE 233-3 -- The *Mycobacterium tuberculosis* complex.†

THE *MYCOBACTERIUM TUBERCULOSIS COMPLEX*			
Species	**Geographic distribution**	**Taxonomic features**	**Identifying features**
M. tuberculosis	Widespread, humans are the definitive host		TCH-resistant, PZA-susceptible, nitrate reductase activity positive, aerobic
M. bovis	Widespread, broad host range including cattle, nonhuman primates, goats, cats, dogs, buffalo badgers, possums, deer and bison. Humans are viewed as a 'spillover' host	While probably more properly viewed as a subspecies of *M. tuberculosis*, epidemiologic and economic factors presently compel separation of species	TCH-susceptible, PZA-resistant, nitrate reductase activity negative, microaerophilic
M. bovis BCG	Nonvirulent vaccine strain	First reported in 1908 as a nonvirulent strain of *M. tuberculosis*; appears to be a regulatory mutant of *M. bovis*	TCH-susceptible, PZA-resistant, nitrate reductase activity negative, aerobic
M. africanum		Probably more properly viewed as a subspecies or biovar of *M. bovis*	TCH-susceptible, PZA-resistant, nitrate reductase activity negative, microaerophilic
M. microti	Usually considered strictly an animal pathogen (e.g. voles), severe human cases were reported in 1998*	Probably more properly viewed as a subspecies or pathovar of *M. tuberculosis*	
M. canetti	East Africa, but probably worldwide. Appears to have a very limited incidence	Taxonomic status is unclear, but PRA results suggest a distinct taxonomic cluster	Smooth colony variant of *M. tuberculosis*, PZA-resistant
PRA, PCR restriction analysis; PZA, pyrazinamide; TCH, thiophen-2-carboxylic acid hydrazide.			

† *Adapted from Grange[4] and Goh et al. [54]*
* From van Soolingen *et al.*[55]

an inoculated MGIT tube to ultraviolet light and examining for fluorescence, which is an indication of growth and oxygen consumption. The performance of these aforementioned manual systems varies in comparison with the Bactec 460 standard, but most investigators have concluded that each of these systems, in combination with conventional solid media, are a satisfactory substitute for the Bactec.[40] [47] [48] [49] [50] [51] Indeed, deciding factors are more likely to reflect differences in cost, convenience and safety, availability and workload.

Identification

In 1958, Ernest Runyon proposed that by dividing mycobacteria into four groups based on the rate of growth and pigmentation one could make a preliminary identification. Rapid growers were separated from slowly growing mycobacteria according to the time required to produce clearly visible colonies (=7 days for rapid growers). Slowly growing mycobacteria were separated based on pigment production: photochromogenic, scotochromogenic (Fr. Gk *skotos*, darkness) and nonphotochromogenic. Forty years later Dr Runyon's simple scheme, now the Runyon groups, remains a valuable guide for the initial identification of mycobacteria isolated by culture, and the logic of this classification has been corroborated by 16S rRNA sequence analysis. [52] Indeed, the correlation between an initial classification into a Runyon group and the final identification ranges from 87% (nonphotochromogens) to 97% (rapid growers).[53] The first challenge for the clinical mycobacteriology laboratory is to distinguish between an isolate that is in the *M. tuberculosis* complex (Table 233.3) and an isolate that is a nontuberculous mycobacterium, a taxonomically imprecise but clinically important distinction. In many ways clinical mycobacteriology remains an arcane field and experience is a critical factor in assuring the accurate identification of mycobacteria, especially the nontuberculous mycobacteria (Table 233.4).

Nature and appearance of cultures

The growth of *M. tuberculosis* on Middlebrook 7H10 or 7H11 agar at 95°F (35°C) is usually detected in 2–3 weeks. The colonies are beige-colored, rough, dry, corded, flat and with irregular borders (Fig. 233.7). On egg media such as Löwenstein-Jensen or Ogawa media the colonies are frequently warty and granular and with time heap into a cauliflower shape (Fig. 233.8). Although the growth of

TABLE 233-4 -- Nontuberculous mycobacteria.*

NONTUBERCULOUS MYCOBACTERIA							
Clinical disease/specimen/site	**Occurrence**	**Species**	**Growth rate**	**Recovered from environment**	**Geographic distribution**	**Risk factors**	**Microbiologic features/diagnostic tools**
Cutaneous, wound	Common	*M. chelonae*	Rapid	Yes	Widespread		
Cutaneous, wound	Common	*M. fortuitum*	Rapid	Yes	Widespread	Central catheter postsurgical wound	Rapid growth (<7 days, especially on subculture), not pigmented, growth in salt and MacConkey agar is temp dependent
Cutaneous, wound	Common	*M. haemophilum*	Slow	Yes?	Australia, Europe, North America	AIDS, organ transplant, lymphoma, children	Growth requires hemin or ferric ammonium citrate, growth at 86–89.6°F (30–32°C), growth stimulated by CO_2 , colony variants occur, may be missed
Cutaneous	Common	*M. marinum*	Rapid/slow	Yes	Widespread	Skin abrasion or trauma	Photochromogen, slow-to-rapid growth, initial culture =89.6°F (32°C), colony variants occur
Cutaneous, ulcers	Common	*M. ulcerans*	Slow	Yes	Ghana, Uganda, South East Asia, Tropics, Central and South America, Australia	Normal host, third most prevalent mycobacterium isolated from human specimens and continuing to emerge	Culture — slow growth at 77 and 98.6°F (25 and 37°C), HPLC, microaerophilic, mycolic acids similar to *M. marinum*
Cutaneous, wound	Uncommon	*M. abscessus*	Rapid	Yes			
Cutaneous, wound	Uncommon	*M. avium complex*	Slow	Yes			
Cutaneous	Uncommon	*M. kansasii*	Rapid	Yes			
Cutaneous	Uncommon	*M. smegmatis*	Rapid	Yes			
Cutaneous, wound	Unknown	*M. conspicuum*	Slow	No			Culture, HPLC, 16S rRNA
Cutaneous Urine Fluids (BAL, joint) Blood	Unknown	*M. immunogenicum*	Rapid	Yes	Unknown	Respiratory pseudo-outbreaks (contaminated bronchoscopy equipment). Newly described species	Culture 77–86°F (25–30°C), 16S rRNA or DNA hybridization, *M. abscessus-chelonae*-like
Cutaneous	Unknown	*M. novocastrense*	Rapid	Yes		Potential human pathogen	Culture, pigmented (yellow), HPLC, 16S rRNA, *M. marinum*-like
Disseminated		*M. xenopi*	Slow	Yes	Worldwide	AIDS	
Disseminated	Common	*M. avium complex*	Slow	Yes	Widespread	AIDS, hairy cell leukemia, congenital immunodeficiencies	Slow growth (>7 days), nonpigmented but pigmented colonies occur, colony variants isolated from blood, bone marrow, other biopsies
Disseminated	Common	*M. chelonae*	Rapid	Yes	Widespread		
Disseminated Blood or bone marrow	Common	*M. haemophilum*	Slow	Yes?	Australia, Europe, North America	Common and continuing to emerge	Culture (requires hemin), HPLC, GLC, TLC, 16S rRNA
Disseminated	Common	*M. kansasii*	Rapid	Yes	Europe, USA		
Disseminated	Uncommon	*M. abscessus*	Rapid	Yes			
Disseminated Blood, bone marrow, liver, spleen	Uncommon	*M. genavense*	Slow	Yes	Widespread, isolated from pet birds	Disease in humans established, especially in AIDS, malnourished	
Disseminated	Uncommon	*M. malmoense*	Very slow	Yes		AIDS	

Disseminated	Unknown	*M. celatum*	Slow	No	Europe, USA	Disease in humans established	Culture, nonphotochromagen, HPLC, TLC, cross-reacts with GenProbe Mtb probe, 16S rRNA
Disseminated, blood	Unknown	*M. conspicuum*	Slow	No			Culture, HPLC, 16S rRNA
Disseminated Spinal osteomyelitis Lymphadenitis	Unknown	*M. lentiflavin*	Slow	No		Disease in humans established, especially HIV and AIDS	Culture, 16S rRNA
Disseminated Lymph nodes, spinal fluid, pericardial and peritoneal fluids	Unknown	*M. triplex*	Slow	No	Unknown	Disease in humans established	Culture, HPLC, 16S rRNA, *M. simiae-avium*-like
Intestinal		*M. paratuberculosis*	Slow	Yes			Cause of Johne's disease in cattle
Lymphadenitis	Common	*M. avium* complex	Slow	Yes	Widespread	Children	
Lymphadenitis	Common	*M. malmoense*	Very slow	Yes	UK, Northern Europe, Scandinavia	Childhood	Slow growth (6 weeks), subculture growth is similarly slow, easily missed
Lymphadenitis	Common	*M. scrofulaceum*	Slow	Yes	Widespread	Children	
Lymphadenitis	Uncommon	*M. abscessus*	Rapid	Yes			
Lymphadenitis	Uncommon	*M. chelonae*	Rapid	Yes			
Lymphadenitis	Uncommon	*M. fortuitum*	Rapid	Yes			
Lymphadenitis	Uncommon	*M. haemophilum*	Slow	Yes?			
Lymphadenitis Soft tissue	Unknown	*M. bohemicum*	Slow	Yes	Unknown	Potential pathogen	Culture, GLC, 16S rRNA
Lymphadenitis	Unknown	*M. heidelbergense*	Slow	No	Germany, unknown?	Potential pathogen	Growth, HPLC, PRA
Lymphadenitis Pulmonary	Unknown	*M. tusciae*	Slow	Yes	Tuscany, unknown?	Potential pathogen	Culture, HPLC, 16S rRNA
Lymphadenitis Disseminated	Unknown	*M. interjectum*	Slow/rapid	No		Disease in humans established	HPLC, TLC, 16S rRNA
Pulmonary	Common	*M. abscessus*	Rapid	Yes	Widespread, predominantly USA		Rapid growth (<7 days), not pigmented
Pulmonary	Common	*M. avium complex*	Slow	Yes	Widespread	Chronic lung disease, cystic fibrosis	Slow growth (>7 days), usually not pigmented, colony variants
Pulmonary	Common	*M. kansasii*	Rapid	Yes	Localized USA, UK, Europe	Alcoholism, AIDS, chronic lung disease	Photochromogen, intensely pigmented, large, beaded, acid-fast, elongated bacilli
Pulmonary	Common	*M. malmoense*	Very slow	Yes	Localized UK, Northern Europe	Pneumoconiosis, silicosis, industrial dust disease. Common and continuing emerge	Slow growth (6 weeks), subculture growth is similarly slow, easily missed
Pulmonary	Common	*M. xenopi*	Slow	Yes	Localized UK, Europe, Canada	Underlying lung disease. Common and continuing to emerge	Culture, slow growth, pigmented, growth at 113°F (45°C), 'birds nest' colony
Pulmonary	Uncommon	*M. asiaticum*	Slow	Yes	Localized Australia, USA		
Pulmonary	Uncommon	*M. fortuitum*	Rapid	Yes			
Pulmonary	Uncommon	*M. gordonae*	Slow	Yes	Widespread	Chronic lung disease, but frequent contaminant and rare cause of disease	'Tap-water' scotochromogen
Pulmonary	Uncommon	*M. haemophilum*	Slow	Yes?	Unknown, seems localized		
Pulmonary	Uncommon	*M. shimoidae*	Slow		Australia, Japan		
Pulmonary	Uncommon	*M. simiae*	Slow	Yes	Israel, Thailand, France, USA, Australia	Underlying lung disease, pneumonia	Photochromogen, slow but good growth at 98.6°F (37°C), produces niacin
Pulmonary	Uncommon	*M. szulgai*	Slow	Yes?	Europe, Japan, USA	Underlying disease, alcoholism	Scotochromogen, slow growth at 77–98.6°F (25–37°C), no growth at 107.6°F (42°C)
Pulmonary, sputum	Unknown	*M. alvei*	Rapid	Yes	Unknown, first isolated and described in Spain	Potential human pathogen	GLC, DNA hybridization
Pulmonary	Unknown	*M. branderi*	Slow	No	Unknown	Potential pathogen	Culture, chromatography, 16S rRNA
Pulmonary	Unknown	*M. brumae*	Rapid	Yes	Unknown	Potential human pathogen	Culture, arylsulfatase-negative
Pulmonary	Unknown	*M. celatum*	Slow	No	Europe, USA		Culture, nonphotochromagen, HPLC, TLC, cross-reacts with GenProbe Mtb probe, 16S rRNA
Pulmonary, sputum	Unknown	*M. confluentis*	Rapid	No	Unknown	Potential human pathogen	Culture, brown pigment, chromatography, 16S rRNA
Pulmonary	Unknown	*M. conspicuum*	Slow	No	Europe	Disease in humans established	

Pulmonary	Unknown	*M. heckeshornense*	Slow	No	Unknown	Potential pathogen	Culture, GLC, TLC, HPLC, *M. xenopi*-like organism
Pulmonary	Unknown	*M. interjectum*	Slow/rapid	No		Disease in humans established	
Pulmonary	Unknown	*M. intermedium*	Slow/rapid	No	Unknown	Disease in humans established	Culture, photochromogen, GLC, TLC
Pulmonary	Unknown	*M. kubicae*	Slow		Unknown	Potential human pathogen	Culture, HPLC, 16S rRNA
Pulmonary, sputum?	Unknown	*M. mageritense*	Rapid	No	Spain, unknown	Potential human pathogen	DNA hybridization, 16S rRNA
Pulmonary, wounds	Unknown	*M. mucogenicum*	Rapid	Yes	Unknown	Newly described, occurrence unknown	Culture, HPLC, GLC, 16S rRNA
Pulmonary	Unknown	*M. triplex*	Slow	No	Unknown	Disease in humans established	Culture, HPLC, 16S rRNA, *M. avium* complex-like
Urine	Unknown	*M. hassiacum*	Rapid	Yes	Unknown	Nonpathogen?	Culture, scotochromagen, HPLC, PRA
Wound	Unknown	*M. branderi*	Slow	No	Unknown	Disease in humans established	Culture, HPLC, GLC, TLC, 16S rRNA
Wound, soft tissue, blood	Unknown	*M. fortuitum* 3rd biovariant	Rapid	Yes		Newly described, occurrence unknown	Culture, 16S rRNA, includes *M. septicum*
Wound, pulmonary	Unknown	*M. goodii*	Rapid	No	Unknown	Newly described, occurrence unknown, nosocomial?	Culture, pigmented/HPLC, 16S rRNA, *M. smegmatis*-like
Wound, osteomyelitis	Unknown	*M. wolinskyi*	Rapid	No	Unknown	Post-traumatic and postsurgical.	Culture, HPLC, 16S rRNA, *M. smegmatis*-like
						Newly described, occurrence unknown	
BAL, bronchoalveolar lavage; GLC, gas liquid chromatography; PRA, PCR restriction analysis; TLC, thin layer chromatography.							

** Information compiled and adapted from a variety of sources including Falkinham,[56] Grange,[4] Kiehn and White,[57] American Thoracic Society[58] and Brown-Elliot et al.[59]*

Figure 233-7 Primary isolate of *M. tuberculosis* grown from sputum on Löwenstein-Jensen medium displaying characteristic beige, rough and dry-appearing growth. *Courtesy of S Froman and A Gaytan.*

Figure 233-8 Primary isolate of *Mycobacterium tuberculosis* grown from sputum on Löwenstein-Jensen medium displaying 'cauliflower' or verrucose colonies. These are also characteristic of other mycobacteria including MAC and rapid growers, especially as a culture ages. *Courtesy of S Froman and A Gaytan.*

M. tuberculosis is quite distinct and it is not unreasonable for an experienced mycobacteriologist to make a presumptive report of *M. tuberculosis* isolated based on the rate and appearance of growth, the identification must always be confirmed by biochemical, chromatographic or nucleic acid probe tests.

Three species of mycobacteria have optimal growth temperatures less than 95–98.6°F (35–37°C): *M. ulcerans*, *M. marinum* and *M. haemophilum*. If these species are suspected the cultures should be incubated at 86°F (30°C). In addition, *M. haemophilum* requires hemin for growth and, therefore, specimens suspected to harbor this species should be inoculated onto chocolate agar, Middlebrook 7H10 agar with hemolyzed blood or 7H10 agar with a hemin-containing paper strip.

The most common clinically significant mycobacterium isolated in many clinical microbiology laboratories is MAC. *Mycobacterium avium* complex isolated from blood or sputum appears as non-pigmented colonies within 10–14 days after inoculation. When grown on Middlebrook 7H10 or 7H11 agar two colony variants are commonly observed. One variant is smooth, opaque and domed while the second variant is flat and transparent. The distinction between variant types diminishes with the age of the culture and eventually the colonies become verrucose and many develop pigment. The initial distinction between colony types is important because the transparent variants tend to be more resistant to antimicrobial agents and these colonies should be picked for susceptibility testing. The colony variant types are not observed when MAC is grown on egg-based media.

Growth rate

Mycobacteria are commonly separated into rapid growers and slowly growing based on whether growth appears before or after 7 days of incubation. It is important to note that the most accurate measure of growth rate is made with subcultures. Harsh digestion-decontamination of a specimen may significantly increase the time it takes for a rapid grower to appear on primary culture.

Acid-fast stain

An acid-fast stain remains the most rapid, convenient and least expensive method for directly detecting mycobacteria in clinical specimens. Although an acid-fast stain is not specific, the presence of acid-fast bacilli in a specimen from a patient with signs and symptoms of tuberculosis or another mycobacterial infection is an important guide to effective treatment and the initiation of public health measures. Furthermore, an acid-fast stain can be used to monitor the response to therapy and quickly assess patient infectiousness, and is used to confirm that a culture is acid-fast. The sensitivity of the acid-fast stain has been estimated to be 5000–10,000 bacilli/ml of sputum while culture is estimated to be 100- to 1000-fold more sensitive[53] (i.e. the sensitivity of an AFB stain may be only 22–78% compared with culture).[51] The threshold for the detection of MAC in bone marrow by either immunoperoxidase (Fig. 233.9) or Kinyoun staining is of a similar magnitude.[60] The importance of the acid-fast stain and the need for a prompt turnaround time cannot be over-emphasized. In laboratories that do not perform cultures, an acid-fast stain can be performed using a specimen treated for a short time with 5% sodium hypochlorite. If specimens are sent to a reference laboratory, insist that acid-fast stain results are reported within 24 hours of specimen collection.[61]

Acid-fastness is a distinctive feature of mycobacteria that is shared with only a few other bacteria such as *Nocardia* spp. and *Rhodococcus* spp., and parasites such as

Cryptosporidium spp. and *Isopora* spp. The three stains that are commonly used to detect acid-fast bacteria are Ziehl-Neelsen, Kinyoun and auramine-rhodamine fluorochrome stains. With each of these procedures, acid-fastness is defined as resistance to decolorizing with acid-alcohol (e.g. 3% hydrochloric acid in 95% ethanol). When mycobacteria are stained with Gram's crystal violet and safranin they often appear as beaded Gram-positive bacilli or fail to stain at all. It is important to note, however, that culture media, incubation conditions and the age of the culture influence the acid-fastness of mycobacteria. The critical role of the mycobacterial cell wall in acid-fastness is underscored by the observation that isoniazid causes a loss of 'acid-fastness' in susceptible strains of *M. tuberculosis*.

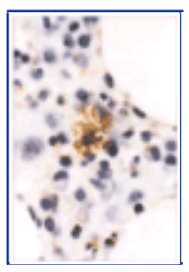

Figure 233-9 Immunoperoxidase stain of *Mycobacterium avium* complex in the bone marrow of a patient who has HIV infection and disseminated disease. The presence of mycobacteria in the bone marrow and blood of a patient is the microbiologic hallmark of disseminated MAC disease. The presence of mycobacteria in bone marrow sections using either an immunoperoxidase stain or a conventional acid-fast stain requires the presence of approximately 1.5×10^4 bacilli/g of bone marrow.

The preparation of the smear is the critical first step in performing an acid-fast stain. Using a glass slide cleaned with ethanol, make a smear of approximately 1×2cm of a single specimen or isolate. For CSF specimens previously concentrated by centrifugation, three drops of the concentrate are placed on a clean glass slide, one drop at a time. The drop is allowed to air dry before the next drop is added. The slide is heated at 149–167°F (65–75°C) for 2–24 hours or alternatively 176°F (80°C) for 15 minutes — it is important to note that heat fixing may not kill all the mycobacteria on the slide. Mycobacteria appear brightly fluorescent against a dark background in the auramine-rhodamine stain and the increased sensitivity of the fluorescence stain compared with the other stains is used to rapidly screen slides at a lower (250–450×) magnification. However, fluorescent objects must be examined at 800–1000× to confirm morphology and positive fluorescent smears should be confirmed with the Ziehl-Neelsen or Kinyoun stains. The Ziehl-Neelsen stain requires that the carbolfuchsin stain be heated for the stain to penetrate the mycobacterial cell. In contrast, heating is unnecessary with the Kinyoun stain because the concentration of basic fuchsin and phenol have been increased to ensure that the basic fuchsin penetrates the

TABLE 233-5 -- Biochemical features of the most common clinical isolates of *Mycobacterium* spp.

BIOCHEMICAL FEATURES OF THE MOST COMMON CLINICAL ISOLATES OF *MYCOBACTERIUM* SPP.									
Species	**Pigment**	**Niacin**	**Nitrate reductase**	**154.4°F (68°C) catalase**	**Semiquantitative catalase (>45mm)**	**Tween 80 hydrolysis**	**Iron uptake**	**Arylsulfatase**	**Urease**
M. tuberculosis	N	+	+	-	-	-	-	-	+
M. kansasii	P	-	+	+	+	+	-	-	+
M. avium complex	N/S	-	-	+	-	-	-	-	-
M. fortuitum	N	-	+	+			+	+	
M. chelonae	N	V	-	V			-	+	
M. abscessus	N		-				-	+	
N, none; P, photochromogenic; S, scotochromogenic; V, variable.									

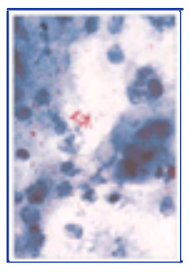

Figure 233-10 Ziehl-Neelsen acid-fast stain of sputum containing 4+ tubercle bacilli. *Courtesy of S Froman and A Gaytan.*

mycobacterial cell wall. Using a 100× oil immersion lens, 100–300 fields of a properly prepared smear and stain should be examined. It should be remembered that examining even 100–300 fields, only 1–4% of a 1×2cm smear would be examined. Mycobacteria usually appear as slender rod-shaped bacilli; however, pleomorphic shapes are common and range from coccoid to long rods with curves or bends (Fig. 233.10 ; see Fig. 233.1).

Conventional biochemical tests

The salient biochemical features used in the conventional identification of mycobacteria are shown in Table 233.5 . Although many of these tests have been supplanted by other more convenient or rapid tests, the conventional biochemical tests represent a 'gold standard'. Details about the many biochemical tests used in the conventional identification of mycobacteria are described in a variety of sources and Wayne and Sramek[62] described a strategy for the use of these tests.

Although biochemical testing is a reliable and accurate approach to the identification of mycobacteria, alternative methods have largely supplanted biochemical tests for the identification of most

mycobacteria commonly isolated from clinical specimens. These alternative methods include high performance liquid chromatography (HPLC), the Bactec460 TB NAP test, nucleic acid probes and nucleic acid sequencing.

Developed in the late 1960s, HPLC is used in many sections of the clinical laboratory for a variety of analytes including the detection and quantitation of antimycobacterial agents.[63] In addition, HPLC has proven to be a useful and reliable technology for the rapid identification of mycobacteria.[64] Other applications of HPLC have been developed for the direct detection of mycobacteria in smear-positive clinical specimens or positive liquid cultures.[65] For the identification of mycobacteria, HPLC is used to analyze mycolic acids extracted from an unknown organism. The mycolic acids are extracted by saponification and then derivatives are prepared with either a phenyl ester or a fluorescent compound for detection by ultraviolet absorption or fluorescence spectroscopy. The HPLC pattern of an unknown is then compared with a library of known patterns usually facilitated by a decision analysis system.[66] The equipment cost and the expertise required to perform HPLC analyses has restricted use of this technology mostly to large hospital, and reference and public health laboratories. Alternative chromatographic methods that can be used in the identification of mycobacteria include thin-layer chromatography of lipids and capillary gas chromatography of mycobacterial fatty acids. Instrumentation and software for the identification of mycobacteria as well as other micro-organisms using whole cell fatty acid analysis is commercially available (e.g. Microbial Identification Systems, Newark, DL).

Nucleic acid probes

Nucleic acid probes are commercially available (AccuProbe, GenProbe, San Diego, CA) for the identification of five of the most common species of mycobacteria isolated from clinical specimens: *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, MAC, *M. gordonae* and *M. kansasii*. The AccuProbe culture confirmation test is based on the use of DNA probes that are complementary to species-specific rRNA. The rRNA is released from the mycobacteria using a combination of a lysing reagent, sonication and heat. The rRNA-containing lysate is reacted with a species-specific DNA probe that is labeled with an acridinium ester. If a stable RNA-DNA

duplex forms, the acridinium label is protected from a selection reagent while free acridinium label is inactivated. Stable duplexes are detected by the addition of an alkaline hydrogen peroxide solution, which in combination with the bound acridinium ester generates chemiluminesence. The amount of light emitted is proportional to the amount of probe hybridized to the target rRNA. The amount of light produced is measured in a luminometer as relative light units (RLU). The AccuProbe test cannot be used for the direct detection of mycobacteria in clinical specimens; the test is for culture identification only. Ribosomal RNA from a rare number of MAC isolates (identified based on biochemical characteristics or 16S rDNA sequence analysis) will not hybridize to the MAC probe. These strains of *M. avium* may represent a third genospecies within the MAC.[67] *The M. tuberculosis* complex AccuProbe test does not differentiate between the species of the *M. tuberculosis* complex. The MAC AccuProbe test does not differentiate between *M. avium* and *M. intracellulare*. In general, the AccuProbe tests are considered to be highly reliable and simple to perform. In addition, the AccuProbe tests can be successfully combined with the semi-automated culture systems (Table 233.2).[68] [69] [70] [71]

Molecular methods

Molecular methods have been described and in many instances are commercially available for:

- detecting and identifying mycobacteria directly in clinical specimens;
- identifying culture isolates; and
- identifying strains of a mycobacterium for epidemiologic purposes.

Molecular methods have also been developed to detect antimicrobial resistance in mycobacteria based on a knowledge of the genetic basis of resistance.

Two molecular tests are commercially available in the USA for the direct detection of *M. tuberculosis* in clinical specimens:

- the Amplicor *M. tuberculosis* Test (Roche Diagnostic Systems, Inc.); and
- the Amplified *M. tuberculosis* Direct Test (MTD; GenProbe, Inc.).

Both these tests are based on a knowledge of prokaryotic 16S rDNA sequence information. 'Signature' sequences (unique rDNA or rRNA sequences that serve to distinguish one organism or group of organisms from another) have been determined for a majority of clinically relevant, slowly growing mycobacteria.[72] The Amplified *Mycobacterium tuberculosis* Direct (MTD) test detects viable and nonviable tubercle bacilli in a clinical specimen. The test uses transcription-mediated amplification and a hybridization protection assay to qualitatively detect all members of the *M. tuberculosis* complex. Mycobacterial rRNA is released by sonication from bacilli present in a clinical specimen, the rRNA is denatured with heat and transcribed into cDNA with a reverse transcriptase. One part of the oligonucleotide used in the reverse transcription step includes a promoter region for a high efficiency RNA polymerase (e.g. phage T7 polymerase); the other part of the oligonucleotide includes a 16S rRNA target-specific sequence. As a result of cycles of denaturation, reverse transcription and RNA synthesis there is an amplification of the mycobacterial rRNA. The repetitive cycles of denaturation and synthesis can be performed under isothermal conditions — 107.6°F (42°C) — because the RNase H activity associated with the reverse transcriptase used in the assay destabilizes RNA-DNA duplexes. *Mycobacterium tuberculosis* complex-specific amplified sequences are detected using the GenProbe hybridization protection assay. The probe in this assay is an acridinium ester-labeled single-stranded DNA molecule that is complementary to the amplified *M. tuberculosis* complex-specific rRNA sequences. Stable RNA-DNA duplexes protect the acridinium label (using a proprietary selection agent) and hybridized label is measured as chemiluminesence in a luminometer.

When initially approved by the US Food and Drug Administration, the assay was limited to use with AFB stain-positive specimens from untreated patients suspected of having tuberculosis. Furthermore, the patients must have received less than 7 days of therapy or, if previously treated, the patient must not have had therapy in the previous 12 months. However, in September 1999, an enhanced version of the GenProbe MTD test was approved for use with respiratory specimens, regardless of the AFB stain results. In several studies the negative predictive value for the enhanced MTD test was 99% or higher. Nevertheless, the MTD test should be performed in conjunction with a routine mycobacterial culture in order to:

- identify subspecies (e.g. *M. tuberculosis* vs *M. bovis*);
- detect mycobacteria other than *M. tuberculosis*; and
- provide growth for susceptibility testing.

The presence of large numbers of nontuberculous mycobacteria in a specimen may generate a weak false-positive reaction (low relative light units) with the MTD test. The MTD test should be performed only with sediments prepared using a standard NALC/NaOH procedure for decontamination and concentration. An acid-fast stain should always be performed on the same specimen at the time the MTD test is performed.

The Amplicor *M. tuberculosis* test is a PCR assay designed for the qualitative detection of *M. tuberculosis* complex DNA in concentrated sputum specimens, bronchial specimens, bronchial alveolar

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aspirates or washes and endotracheal aspirates. The PCR assay targets a 584 base pair (bp) sequence within the 16S rDNA that is amplified with oligonucleotide primers conjugated to biotin. Amplified DNA is detected by capturing single-stranded forms of the PCR-amplified DNA with a species-specific probe bound to bovine serum albumin that is, in turn, bound to the wells of a microtiter plate. The DNA duplex of probe and amplified target DNA are detected with an avidin-horse radish peroxidase conjugate in a colorimetric enzyme assay. The result is measured as a 450nm optical density read in a microtiter plate spectrophotometer. The amplification reaction includes deoxyuridine in place of deoxythymidine along with the three other nucleotide triphosphates. In this manner the carryover of previously amplified target DNA can be eliminated because the PCR reaction mix includes uracil-N-glycosylase (UNG), an enzyme that degrades DNA that contains deoxyuridine. The UNG itself is denatured in the first cycle of amplification when the temperature reaches 131°F (55°C). Both the GenProbe MTD and Roche Amplicor assays perform extremely well (=95% sensitivity and specificity) with smear-positive specimens; however, only the GenProbe test has been approved for use with AFB stain-negative specimens. An algorithm for the use of these nucleic acid amplification tests is shown in Table 233.6 , which is based on the recommendations of the Centers for Disease Control and Prevention, USA. In considering the use of these tests it is important to realize that AFB-staining is itself only 40–60% sensitive and, of course, is not specific for *M. tuberculosis*. Furthermore, while culture is commonly accepted as the 'gold standard', in reality it too is an imperfect standard and is only 70–80% sensitive when compared with all other laboratory data, patient history and clinical observations.

Another important application of molecular methods has been for epidemiologic studies of *M. tuberculosis*, *M. avium* and other species of mycobacteria. Two methods were initially used:

- restriction endonuclease analysis (REA) using field inversion or pulse field electrophoresis to separate large DNA fragments generated by restriction enzymes with infrequent restriction sites; and
- fragment length polymorphism analysis of the number and pattern of certain insertion sequences (IS) or repetitive DNA elements present in a collection of potentially related strains.

TABLE 233-6 -- Algorithm for the use and interpretation of nucleic acid amplification tests for tuberculosis.*

ALGORITHM FOR THE USE AND INTERPRETATION OF NUCLEIC ACID AMPLIFICATION TESTS FOR TUBERCULOSIS				
	Specimen	**Smear**	**NAA**	**Interpretation/response**
?	1st	-	-	Repeat smear and culture, do not repeat NAA
	2nd	-	X	Repeat smear and culture, do not repeat NAA
	3rd	-	X	Presume patient to not have tuberculosis
?	1st, 2nd or 3rd	+	+	Presume patient to have tuberculosis. Unless NTM are a consideration, the NAA test adds little diagnostic value
?	1st	+	-	If inhibitors of NAA test not present, test 2nd specimen
	2nd	+	-	If inhibitors not present, patient presumed to have NTM
	1st	+	-	If inhibitors present, NAA test is of no diagnostic value NAA up to two additional specimens

?	1st	-	MTD+	Test 2nd specimen. Note Roche Amplicor test is *not* approved for testing smear-negative specimens.
	2nd	-	MTD+	Presume patient has tuberculosis
	2nd	-	MTD-	Test 3rd specimen
	3rd	-	MTD+	Presume patient has tuberculosis
	3rd	-	MTD-	Rely on clinical judgment
Respiratory specimens (e.g. sputum) should be collected on three different days for AFB smear and culture. The nucleic acid amplification (NAA) test (Roche Amplicor *Mycobacterium tuberculosis* Test or GenProbe Amplified *Mycobacterium Tuberculosis* Direct Test) should be performed on the first specimen, first smear-positive specimen, or as indicated in the table. MTD, *Mycobacterium tuberculosis* direct test; X, test not performed; NTM, nontuberculous mycobacteria.				

** Adapted from Centers for Disease Control and Prevention.*[73]

REA is cumbersome because the patterns generated are complex and sometimes difficult to reproduce. IS6110 analysis for the identification of strains of *M. tuberculosis* is based on differences in the copy number of this genetic element. IS6110 is a 1355bp insertion sequence that was first identified in 1990, and was found to be widely distributed in strains of *M. tuberculosis*. There are 0–20 copies of IS6110 in most strains of *M. tuberculosis*. In strains of *M. tuberculosis* with a small number of IS6110 sequences, the elements are uniformly distributed throughout the genome. This factor can limit the epidemiologic value of IS6110 analysis involving such strains because the resulting patterns often do not discriminate between strains. However, a majority of strains of *M. tuberculosis* carry several copies of IS6110 and the distribution of these IS elements within the genome appears to be stable over several months to years. Slight variations in the distribution of IS6110 elements can usually be tolerated in focused epidemiology studies.[74] Insertion sequence analysis is conceptually straightforward, but technically subject to operator error or variations in protocol. Therefore, standard methods of analysis and interpretation have been developed to ensure comparability of results.[75] Basically, the method involves extraction of genomic DNA, restriction of the DNA with an appropriate enzyme (e.g. *Pvu*II) and electrophoretic separation of the restriction fragments (RFLP analysis). The IS6110 pattern is revealed by Southern hybridization using a labeled fragment of the IS6110 sequence. A computer-based method of analysis of the IS6110 patterns was described by Heersma *et al.*[76] A drawback to this method is the relatively large amount (about 2µg) of genomic DNA that is required for analysis. Therefore, PCR-based typing methods were developed that have comparable discrimination and reproducibility to RFLP analysis. Spoligotyping uses primers to PCR amplify the 36bp direct repeats (DR) in the genomic DR region of *M. tuberculosis* DNA.[77] The resulting PCR products are hybridized to 43 different oligonucleotides fixed to a membrane. The 43 oligonucleotides were derived from the sequences of the spacer DNA between the DRs. However, in general spoligotyping is used as a screen for IS6110 analysis. Other methods of analysis include polymorphic GC-rich sequence (PGRS) RFLP typing, [78] genome sequence-based fluorescent-amplified fragment length polymorphism analysis (FAFLP)[79] [80] and PCR-based typing.[81]

ANTIMICROBIAL RESISTANCE AND SUSCEPTIBILITY TESTING

Resistance

Antimicrobial resistance in mycobacteria is fundamentally a reflection of the large populations of mycobacteria present in infected tissues and fluids and the frequencies of individual gene mutations that result in a resistant phenotype. In pulmonary tuberculosis there are 10^7 –10^9 bacilli in lung cavities, but only 10^2 –10^4 bacilli in hard caseous lesions. In disseminated *M. avium* disease the level of bacteremia ranges from 1 to 10^6 colony forming units (cfu) per milliliter of blood, but may be orders of magnitude higher in bone marrow and other tissues. Therefore, drug resistance is a more common occurrence in cavitary tuberculosis than noncavitary disease and resistance in disseminated *M. avium* complex infections develops rapidly when patients are given macrolide monotherapy. Antimicrobial resistance in *M. tuberculosis* is classically defined as a significant difference in the activity of an antimycobacterial between a wild-type strain and another strain. A wild-type strain is defined as a strain isolated from a patient before treatment and less than 1% of a population of that strain is resistant to any antimycobacterial agent. Resistance emerges as a consequence of individual mutations in mycobacterial genes that lead to a structural or functional change such that an antimycobacterial agent is no longer active against that strain. [16] For example, there is compelling evidence that resistance to isoniazid results from a mutation or a combination of

TABLE 233-7 -- Mycobacterial genes with mutations associated with antimicrobial resistance.*

MYCOBACTERIAL GENES WITH MUTATIONS ASSOCIATED WITH ANTIMICROBIAL RESISTANCE					
Antimicrobial Agent	**Species**	**Gene**	**Proportion of resistance (%)**	**Product**	**References**
Rifampin	*M. tuberculosis*	*rpoB*	>96	ß subunit of RNA polymerase	[84]
	M. africanum				[85]
	M. leprae				[86]
	M. avium				
Isoniazid	*M. tuberculosis*	*katG*	}	Catalase peroxidase	[83]
					[22]
					[86]
Isoniazid/ethionamide	*M. tuberculosis*	*inhA* locus		*envM* analog	[87]
				3-ketoacyl-acyl carrier protein reductase analog	
Isoniazid	*M. tuberculosis*	*ahpC*		Subunit of alkyli hydroperoxide reductase	[88]
	M. leprae				[89]
					[90]
Isoniazid	*M. tuberculosis*	*acpM (kasA)*		ß-ketoacyl ACP synthase	[91]
Ethambutol	*M. tuberculosis*	*embB*	47–65	Arabinosyltransferase	[92]
Streptomycin	*M. tuberculosis*,	*rpsL*	70	Ribosomal protein S12	[93]
	M. smegmatis				[94]
					[55]
Streptomycin	*M. tuberculosis*	*rrs*	70	16S rRNA	[94]
					[96]
Pyrazinamide	*M. tuberculosis*	*pncA*	72–97	Pyrazinamidase	[97] [98]
					[99]
Fluoroquinolone	*M. tuberculosis*,	*gyrA*	75–94	DNA gyrase A subunit	[100]
	M. smeginatis				[101]
Azithromycin/clarithromycin	*M. avium*,	V domain 23S rRNA	95	235 rRNA	[102] [103] [104]
	M. intracellulare,				
	M. chelonae				
	M. abscessus				
Proportion of resistance represents the estimated percentage of resistance that can be accounted for by mutations in the respective genes; mutations in *katG*, *aphC*, *inhA* and/or *kasA* collectively probably account for 90% of isoniazid resistance.					

** Adapted from Musser. [22] Percentage figures taken in part from Alcaide and Telenti.[21]*

mutations in the *katG*, *ahpC*, *inhA* or the *kasA* genes of *M. tuberculosis* (Table 233.7).[82] [83]

Resistance to rifampin is a result of a mutation within an 81bp (27 amino acid) sequence of the core region of the *rpoB* gene (RNA polymerase ß subunit) and streptomycin resistance has been attributed to mutations in either the *rrs* gene (16S rRNA gene) or the *rpsL* gene (ribosomal protein S12). Quinolone resistance has been ascribed to mutations in the *gyrA* genes[100] and pyrazinamide resistance to mutations in the *pncA* gene that encodes pyrazinamidase/nicotinamidase activity.[97] [99] The targets for the major classes or types of antimycobacterial agents are shown in Figure 233.11 . Antibiotic resistance does not appear to transfer between strains of mycobacteria by either plasmid exchange or resistance transfer factors. The *M. tuberculosis* multidrug resistance phenotype (minimally resistant to isoniazid and rifampin) appears to be entirely the result of accumulation of individual mutations. Intrinsic resistance to antimicrobial agents is also common in both slowly and rapidly growing mycobacteria. In most instances this form of resistance appears to be the result of the impermeability of the mycobacterial cell envelope. For example, most MAC isolates are resistant to rifampin despite the fact that the isolate has a wild-type *rpoB* gene. However, *M. avium* resistance to isoniazid may reflect the lack of an effective antimicrobial activity rather than or in addition to a lack of permeability.[107] [108] Both *M. chelonae* and *M. abscessus* are intrinsically resistant to quinolones, while *M. fortuitum* is susceptible.

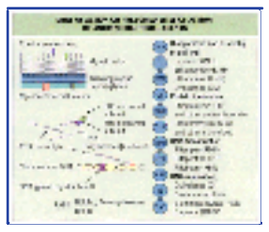

Figure 233-11 Sites of action or presumed sites of action of antimycobacterial agents. DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; PABA, *p*-aminobenzoic acid; PAS, *p*-aminosalicylic acid. *Figure adapted from Parsons et al.*[105] *and Young.*[106]

TABLE 233-8 -- Antimycobacterial agents ranked by clinical use.

ANTIMYCOBACTERIAL AGENTS RANKED BY CLINICAL USE			
Species	**Primary or first choice**	**Secondary or second choice**	**Notes**
M. tuberculosis	INH, RMP, PZA, SM, EMB	Amikacin, ciprofloxacin, levofloxacin or sparfloxacin	Treatment plan varies depending on incidence of INH resistance in community, if MDR (INH plus RMP resistance), patient adherence (viz. DOT), other factors
M. bovis	INH, RMP, EMB		Uniformly PZA resistant, 9–12 months therapy recommended by some. Confirm identity of isolate, sometimes nontuberculous mycobacterium
M. leprae	Dapsone, RMP, clofazimine, clarithromycin	Ethionamide, prothionamide, minocycline, pefloxacin, sparfloxacin	Treatment plan varies depending on paucibacillary or multibacillary disease
M. avium	Azithromycin or clarithromycin, ethambutol, rifabutin	Amikacin, streptomycin, moxifloxacin	Isoniazid resistance is usual. Drugs and does vary depending on immunocompetency, disseminated or pulmonary or other, and treatment vs prophylaxis
M. intracellulare			
M. chelonae	Amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline or minocycline, sulfonamides	Imipenem, levofloxacin, tobramycin (*M. chelonae* only)	All resistant to INH, PZA, RMP, SM, EMB and clofazimine. EMB may be useful for treating *M. smegmatis.* Contamination with these species is common, confirm clinical significance
M. fortuitum			
M. abscessus			
M. kansasii	RMB, INH, EMB	Pyridoxine, clarithromycin, rifabutin, sulfamethoxazole	PZA resistance is uniform. Only test RMP; testing of INH and EMB can be misleading. Clarithromycin resistance occurs. Treatment for 12 or more months
M. scrofulaceum	Surgical excision without chemotherapy	Azithromycin, clarithromycin	INH and PZA resistant
M. ulcerans	Surgical excision, RMP, amikacin	EMB, trimethoprim-sulfamethoxazole, SM, ciprofloxacin, sparfloxacin	Chemotherapy rarely effective
M. marinum	Clarithromycin, minocycline, doxycycline, RMB, EMB, trimethoprim-sulfamethoxazole		INH and PZA resistant
M. haemophilum	Clarithromycin, rifabutin, ciprofloxacin. Surgical debridement		Limited clinical experience
M. simiae	Treat like *M. avium* complex		INH, RMP, EMB and PZA resistant
M. xenopi	Azithromycin, clarithromycin, RMP	Rifabutin, SM	
M. celatum	Clarithromycin		RMP resistant
M. genavense	EMB, RMP, rifabutin, clarithromycin	Amikacin, clofazimine	
M. gordonae	INH, RMP, EMB	Amikacin, ciprofloxacin	Common contaminate, confirm clinical significance
First-choice agents are expected to be active against wild-type isolates (i.e. from untreated patients); second-choice agents are less preferred, usually due to toxicity, expense or unclear efficacy. DOT, directly observed therapy; EMB, ethambutol; INH, isoniazid; MDR, multiple drug resistance; PZA, pyrazinamide; RMP, rifampin; SM streptomycin. (See also Chapter 202 .)			

Antimicrobial agents

There are a variety of antimicrobial agents available for the treatment of mycobacterial infections; however, until recently almost all clinical studies of these agents were focused on *M. tuberculosis* and to a lesser extent *M. leprae*. The emergence of MAC as an important opportunistic infection associated with HIV infection and increased recognition that rapidly growing mycobacteria are a significant cause of human disease led to an expansion in our knowledge about the activity of certain agents against these and other mycobacteria. Infections caused by rapidly growing mycobacteria must be treated with antimicrobial agents other than the primary antituberculous agents. *Mycobacterium avium* complex is resistant to isoniazid and only variably susceptible to rifampin, but MAC infections can be successfully treated with a macrolide and at least one other agent such as ethambutol. For most other species there is often only limited information about the effectiveness and efficacy of antimycobacterial agents, perhaps with the exception of *M. kansasii* and *M. marinum*. The antimicrobial agents recommended for the treatment of various mycobacterial infections are shown in Table 233.8 ; however, it is not always appropriate to perform susceptibility tests on all of the agents because of a lack of standardized procedures and well-established interpretive criteria (see also Chapter 202).

Susceptibility testing

The susceptibility testing of mycobacteria and the definition of antimicrobial resistance have been influenced by decades of focus on *M. tuberculosis*. Thus, there is more information about antimicrobial agents and the susceptibility testing of *M. tuberculosis* than for all other species of mycobacteria. Indeed, the susceptibility testing of many nontuberculous mycobacteria has been largely extrapolated from experience with *M. tuberculosis*, including adoption of the same interpretive criteria. In some instances this extrapolation has proven to provide useful and reliable information, but in other situations this practice can be misleading. Therefore, with the exception of the routine testing of *M. tuberculosis* isolates against the first-line antimycobacterial agents (isoniazid, rifampin, ethambutol, streptomycin and pyrazinamide), susceptibility testing of mycobacteria should be performed in laboratories with extensive experience in this aspect of clinical mycobacteriology. Although the laboratory can provide valuable guidance in the interpretation of results, the application of those results to the treatment of a patient with an uncommon mycobacterial infection is likely to require the involvement of a physician with experience in the management of such infections.

Critical concentrations

The most common method of susceptibility testing *M. tuberculosis* is based on the concept of 'critical concentrations' of antituberculosis agents and the percentage of resistant tubercle bacilli within a test population. Critical concentrations for antituberculosis agents were established on an empiric clinical basis. Therapeutic success was unlikely if the proportion of drug-resistant mutants within a population of *M. tuberculosis* isolated from a patient exceeded a threshold of 1% at a concentration of the antituberculosis agent that was known to be therapeutically effective against a 'wild-type' or fully susceptible strain. The susceptibility test method used to measure the percentage of resistance is, therefore, referred to as the 'proportion method'. The critical concentration may not be the same as the peak serum concentration of a drug and only recently has there been an interest in applying the use of minimum inhibitory concentration (MIC) testing to mycobacteria.[109] However, there are no standardized methods for MIC testing of mycobacteria and the testing of *M. tuberculosis* continues to follow the conventions of critical concentrations and the 1% growth inhibition threshold.

Test methods

Four methods are used for susceptibility testing of *M. tuberculosis*:

- agar proportion method,
- radiometric (Bactec460 TB) proportion method,
- absolute concentration method, and
- resistance ratio method.

The agar proportion and radiometric proportion methods are widely performed in the USA, UK and Western Europe and the following discussion will focus on these methods.

Agar proportion method

The proportion method can be applied as either a direct or an indirect test. In the direct test, a specimen that is positive for acid-fast bacilli by stain is used as the source of inoculum for the susceptibility test. The specimen is inoculated directly onto the test agar media with and without drugs. In the indirect test, a pure culture of acid-fast bacteria is used as the inoculum source for the susceptibility test. On average, the results of the direct test are available 3–4 weeks before the results of an indirect test. The results of a direct test should be confirmed with the indirect test. Inoculating several dilutions of a standardized suspension of mycobacteria onto Middlebrook 7H10 agar plates is the basis of the agar proportion method. The number of cfu that grow on the drug-containing plates or quadrants are compared with the number of cfu on a drug-free plate or quandrant. If the number of cfu that grow on drug-containing medium exceeds 1% of the total number of cfu on the drug-free medium, then the isolate is considered 'resistant' to that drug at that concentration. Some advocate the use of Middlebrook 7H11 agar because some drug-resistant strains of *M. tuberculosis* do not grow on the 7H10 medium.

Radiometric proportion method

The radiometric proportion method is simply an adaptation of the agar proportion method to the Bactec460 TB 12B medium and radiometric measurement of growth inhibition. The test isolate is inoculated into Bactec460 TB 12B medium with or without the addition of test drug. The concentration of mycobacteria inoculated into medium without drug is 100-fold less than the concentration inoculated into Bactec460 TB media with drug. If a drug inhibits the growth of the test strain such that the growth of the control (no drug with 100-fold fewer organisms) reaches a growth threshold before growth in the drug-containing medium reaches that same threshold, then the test isolate is considered susceptible to that drug at the concentration tested. Although partial inhibition of growth occurs, more commonly an isolate of *M. tuberculosis* is either fully susceptible (no growth) or fully resistant (growth exceeds the control). Nevertheless, the growth of resistant isolates may be particularly slow and a report that an isolate is susceptible to a drug should be made only after a full period of incubation. Resistance can be reported as soon as growth in the presence of a drug is detected and exceeds the growth of the control, but the report should be preliminary until the performance of the controls can be assured.

It is prudent to consider the limitations of the Bactec460 TB method. In some clinical situations the Bactec method might be best considered as a screening test because the method does not allow an estimate of the percent of resistant bacilli and is vulnerable to major errors (false susceptibility or resistance) due to mixed populations of mycobacterial species. Indeed, when a multiple drug resistance isolate of *M. tuberculosis* is detected for the first time using the Bactec460 TB method, the identity of the isolate should be confirmed and the presence of a contaminant or mixed culture should be ruled out before proceeding with the testing of secondary agents. Although the importance of promptly reporting a multiple drug resistant isolate of *M. tuberculosis* cannot be overstated, the consequences of a false report of multiple drug resistance must be recognized as well.

Pyrazinamide testing

Pyrazinamide is only active at an acidic pH, and therefore susceptibility tests for this drug must be performed in media with a pH of 5.5–6.0. Methods have been developed for testing pyrazinamide using either Middlebrook 7H10 agar or Bactec460 TB 12B media adjusted to pH 5.5 or 6.0, respectively. The Bactec460 TB method is more convenient and the higher pH is less toxic to *M. tuberculosis* isolates.[76] It should be noted that pyrazinamide monoresistance is rare in *M. tuberculosis* and virtually all *M. bovis* isolates are intrinsically resistant to pyrazinamide. Therefore, pyrazinamide testing of *M. tuberculosis* could be restricted to isolates that are known to be resistant to isoniazid or rifampin and the occurrence of pyrazinamide monoresistance is suggestive that the isolate is *M. bovis*.

Other methods

Three semi-automated mycobacteria culture systems have procedures for testing the primary antituberculosis agents (see Table 233.2), but only the MGIT system can be used to test PZA.[77] The absolute concentration method consists of inoculating media with and without antimycobacterial agents with a carefully controlled inoculum containing 2×10^3 -1×10^4 cfu of mycobacteria. Resistance is defined as growth that is greater than a certain number of cfu (usually 20) at a particular drug concentration and the drug

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concentrations must be precisely confirmed for each batch of media. The resistance ratio method is similar to the absolute concentration method except that a second identical series of tubes are inoculated with the standard *M. tuberculosis* H37Rv strain. The susceptibility test results are expressed in terms of the ratio of the MIC of drug necessary to inhibit the growth of the test isolate of *M. tuberculosis* to that of the standard H37Rv strain. The advantage of this method is that small batch-to-batch variations in the test media can be disregarded because the results are normalized using the H37Rv strain.

Mycobacterium avium complex, *Mycobacterium kansasii* and other slowly growing mycobacteria

In-vitro susceptibility testing of MAC and most of the other nontuberculous mycobacteria, using the methods and interpretive criteria described for *M. tuberculosis*, has little value as a guide to antimicrobial treatment. One important exception is *M. kansasii*, for which in-vitro results based on the interpretive criteria used with *M. tuberculosis* correlate with clinical efficacy. Interpretive criteria have been proposed for clarithromycin and azithromycin, macrolides with proven efficacy in the

treatment of *M. avium* and other nontuberculous mycobacteria. For many uncommon nontuberculous mycobacteria (e.g. *M. simiae* and *M. szulgai*) there are few clinical cases, often initially confused with tuberculosis, to form a basis for interpretive criteria. Indeed, it is often difficult to distinguish between contamination, colonization, infection and disease with many of the nontuberculous mycobacteria, especially the rapidly growing mycobacteria.

In general, the in-vitro susceptibility testing of MAC has limited value primarily because of the lack of a correlation with clinical response and, therefore, the lack of interpretive criteria. The important exceptions are for azithromycin, clarithromycin and roxithromycin because these macrolides have proven clinical and microbiologic efficacy in the prophylaxis and treatment of MAC disease with interpretive criteria based, at least in part, on monotherapy trials in humans. Although wild-type MAC is uniformly susceptible to macrolides, macrolide resistance develops quickly with monotherapy. An analysis of these resistant isolates showed that over 95% of clinically significant macrolide resistance in MAC is a consequence of mutations in the V-domain of the 23S rRNA gene.[107] [108] Therefore, clinically significant macrolide resistance can be defined as a MIC for clarithromycin and azithromycin at pH 6.8 of =64µg/ml and =512µg/ml, respectively.[110]

Mycobacterium avium complex isolates from patients with breakthrough azithromycin, roxithromycin or clarithromycin prophylaxis can be tested against one macrolide. Testing one drug is sufficient, since all evidence indicates that resistance crosses between these macrolides. If a patient has not received macrolide prophylaxis, it is unnecessary to perform a susceptibility test on initial MAC isolates from blood or tissue to guide treatment. However, establishing baseline MIC values for a MAC isolate may prove valuable in interpreting susceptibility test results for a subsequent isolate from the same patient weeks or months later. Susceptibility testing is also warranted if a patient relapses, if the infection is intractable or if the clinical situation is desperate. Testing may assist in deciding to add drugs; however, macrolide treatment should probably be continued even in the face of resistance.

The interpretation of in-vitro test results for ethambutol should not be attempted at this time. Ethambutol is commonly used as a 'second' agent in the treatment of MAC to prevent macrolide resistance,[111] [112] but ethambutol has little or no therapeutic activity alone against MAC.[113] The drug does, however, increase the activity of other agents including macrolides and this may influence the mutation frequency.[114] The National Committee for Clinical Laboratory Standards (NCCLS) now recommends that MAC be tested using either a Bactec 460 or microtiter method.[115]

Mycobacterium marinum is predictably susceptible to rifampin and ethambutol; alternative agents are amikacin and kanamycin as well as tetracycline, doxycycline, minocycline, ciprofloxacin, clarithromycin and trimethoprim-sulfamethoxazole (co-trimoxazole). Routine susceptibility testing of *M. marinum* isolates using methods and interpretive criteria described for *M. tuberculosis* appears to be inappropriate and the methods and interpretive criteria for testing rapidly growing mycobacteria are more likely to provide clinically useful results. Wild-type isolates of *M. haemophilum* are susceptible to quinolones, rifamycins, clarithromycin and azithromycin, and resistant to pyrazinamide, ethambutol and are likely to be resistant to isoniazid and streptomycin.[116] *Mycobacterium simiae* is highly resistant to antimycobacterial agents; however, there are exceedingly few cases of disease to base any firm conclusions about susceptibility and clinical usefulness. Based on an animal test system, clarithromycin in combination with ethambutol and perhaps a quinolone such as ofloxacin is effective. [117] Testing *M. gordonae* isolates is usually inappropriate because actual disease is quite rare and contamination quite common. Before testing such isolates the following questions should be asked.

- Is the isolate a true *M. gordonae*?
- Is there convincing evidence that the isolate is playing a role in the disease?

Rapid growers

Although four methods have been described for measuring the invitro susceptibility of rapidly growing mycobacteria, the NCCLS now recommends only the broth microdilution method.[115] Broth microdilution provides a quantitative result and better supports the growth of *M. chelonae*. The broth microdilution method is essentially a modification of a standard method for nonmycobacteria that grow aerobically as described, for example, by Brown *et al.*[118] Commercially prepared broth microdilution panels can be used if the appropriate drugs are available at the necessary concentrations. Alternatively, broth microdilution panels can be prepared in house. The agents that should be tested are listed in Table 233.9 . The inoculum can be either a subculture in broth or prepared directly by picking colonies from a plate. Typically the plates are incubated for 3–5 days at 86°F (30°C) and longer periods of incubation should be avoided because of potential drug instability. The MIC is defined as the lowest concentration of antimicrobial agent that completely inhibits visible growth. The testing of rapidly growing mycobacteria should be restricted to laboratories with more extensive experience testing these mycobacteria.

MANAGEMENT AND PREVENTION

In general the treatment of mycobacterial infections requires the use of multiple antimicrobial agents administered over several months (e.g. 6–9 months). Patients with an underlying immunodeficiency or other complicating medical condition may require even longer periods of treatment, or in the case of people with HIV infection, some advocate lifelong treatment. Multiple agents are used to prevent the emergence of resistance,[119] but increasingly in the hope of achieving a synergistic effect and improved outcome. Disseminated and localized MAC infections can be successfully treated with a macrolide (clarithromycin) or an azalide (azithromycin), usually in combination with at least one additional agent such as ethambutol, but also rifabutin, amikacin or a quinolone. Indeed, clarithromycin and azithromycin have emerged as valuable chemotherapeutic agents for the treatment of several nontuberculous mycobacteria, if not as first-line agents, then as second-line agents.[58] For example, clarithromycin may be useful in the treatment of infections caused by

TABLE 233-9 -- Antimicrobial agents to test against selected mycobacteria as adapted from National Committee for Clinical Laboratory Standards (NCCLS). [115]

ANTIMICROBIAL AGENTS TO TEST AGAINST SELECTED MYCOBACTERIA		
Antimicrobial agent	**Susceptible (µg/ml)**	**Resistant (µg/ml)**
M. tuberculosis		
Primary agents		
INH	0.05–0.2	
RMP	0.5	
PZA	20	
EMB	1–5	
Secondary agents		
Streptomycin	8	
Capreomycin	1–50	
Kanamycin	5	
Cycloserine	5–20	
Ethionamide	0.6–2.5	
PAS	1	
Alternative agents		
Rifabutin	0.06–8	
Rifapentine		
Amikacin	1	
Ciprofloxacin	0.25–3	
Ofloxacin	0.5–2.5	
***M. avium* complex**		

Clarithromycin	=16	=64
Azithromycin	=128	=512
***M. kansasii* and selected other slowly growing mycobacteria**		
RMP	1–2	
Rifabutin	0.5–2	
Ethambutol	5	
Isoniazid	5	
Streptomycin	10	
Clarithromycin	32	
Amikacin	10	
Ciprofloxacin	2	
Trimethoprim-sulfamethoxazole	2/38	
Rapidly growing mycobacteria		
Amikacin	=16	=64
Cefoxitin	=16	=128
Ciprofloxacin	=1	=4
Clarithromycin	=2	=8
Doxycycline	=1	=16
Imipenem	=4	=16
Sulfamethoxazole	=32	=64
Tobramycin	=4	=16
Susceptible (µg/ml) for *M. tuberculosis* refers to the typical MIC for a susceptible isolate. Susceptible and resistant breakpoints for other mycobacteria refer to the NCCLS recommended breakpoints.		

M. chelonae, *M. abscessus*, *M. leprae*, *M. kansasii*, *M. marinum* and *M. malmoense*. Rapidly growing mycobacteria are resistant to the conventional antimycobacterial agents, but can be effectively treated with amikacin, cefoxitin, imipenem, ciprofloxacin, augmentin, sulfonamides and doxycycline as well as clarithromycin (see Chapter 38).

Prophylaxis with isoniazid for 6–12 months is recommended for people with significant exposure to drug-susceptible tuberculosis or following conversion of their skin test (see Chapter 37 and Chapter 202). In the latter situation, isoniazid is used more as preventive therapy than prophylaxis. Prophylaxis or preventive therapy is especially important in children, who are at greater risk than adults for extrapulmonary tuberculosis including meningitis. Clarithromycin, azithromycin and rifabutin are effective prophylactic agents for disseminated MAC infection in people with HIV infection with $CD4^+$ lymphocytes counts below 100 cells/mm^3 . The evidence now seems compelling that a restoration of $CD4^+$ cells to levels above this threshold, in response to treatment of HIV infection with highly active antiretroviral agents, is protective against disseminated MAC infection (see Chapter 129). [120]

CLINICAL MANIFESTATIONS

Tuberculosis in the most common form is a chronic pulmonary disease classified as either primary or post-primary disease. Post-primary disease can be a consequence of either reactivation (endogenous infection) or re-infection (exogenous infection). By far, the most common (95%) route of infection is inhalation of infectious droplet nuclei, but exposure to *M. tuberculosis* bacilli neither always lead to infection nor are all patients with disease infectious. Risk of infection is directly related to the number and distribution of tubercle bacilli in the inhaled and respired air, emphasizing the importance of infectious droplet nuclei to airborne transmission. Unless a patient receives prophylaxis, symptomatic disease eventually occurs in 5–10% of infected patients. The appearance and extent of disease varies with only one-half of infected patients developing disease within the first 2 years. Hematogenous spread of tubercle bacilli from the lung probably invariably occurs, but the bacteremia is usually occult and usually does not produce symptoms or disease. Nevertheless, hematogenous dissemination accounts for the occurrence of extrapulmonary involvement of lymph nodes, kidneys, reproductive organs, bones and gastrointestinal tract (see Chapter 37).

Leprosy is a chronic disease of the skin, nerves and mucous membranes. The immunologic response (e.g. hypersensitivity) becomes an important component of the pathogenesis of the disease. The clinical manifestations of leprosy have been separated into six categories according to the Ridley-Jopling classification scheme.[13] This classification system is both a clinical classification based on the nature and severity of symptoms and a histopathologic classification. The classification groups are:

- (1) polar tuberculoid,
- (2) borderline tuberculoid,
- (3) borderline,
- (4) borderline lepromatous,
- (5) lepromatous (subpolar), and
- (6) lepromatous polar.

Lepromatous leprosy is the most severe form of the disease with numerous skin lesions involving the face and nose. At one pole, lepromatous leprosy, acid-fast bacilli are numerous and present in immature macrophages while at the other pole, tuberculoid leprosy, macrophages have matured into epithelioid cells (see also Chapter 154).

Mycobacterium arium complex disease is frequently viewed as disease in two groups of patients, persons with and without underlying HIV infection. Although there are some similarities in the manifestation of MAC disease in both groups, the mortality rate, extent and pathologic manifestations of disease are frequently dissimilar.[121] Disseminated MAC disease in people with HIV infection is a progressive illness characterized by intermittent fever, sweats, weakness, anorexia and weight loss. Patients may have nausea, diarrhea and vomiting along with abdominal pain. The microbiologic hallmark of MAC disease is a positive blood or bone marrow culture; however, duodenal, rectal, spleen or liver biopsies may be diagnostic (Fig. 233.12). The level of the bacteremia ranges from intermittently culture positive to 10^6 cfu/ml. The level of infection of bone marrow may be orders of magnitude higher than in blood.[122]

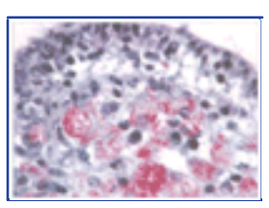

Figure 233-12 Acid-fast stain of a section of small intestine from a patient who has HIV infection and disseminated *Mycobacterium avium* disease. The photomicrograph shows many acid-fast bacilli within a villus tip of the intestinal tract biopsy. The cuboidal cells at the periphery of the tip are in disarray and appear abnormal with cell nuclei not evenly distributed at the base of the cells. There is no evidence of granuloma, but the overwhelming number of mycobacteria may be partially obscuring the host's cellular response. The histopathology is consistent with the symptoms of patients with MAC gastrointestinal tract infections, including abdominal pain, diarrhea and wasting. *Courtesy of LS Young.*

Disseminated MAC infection in people with HIV infection clearly decreases survival and treatment with a macrolide and another agent improves survival and the quality of life. MAC disease in people with HIV infection may be focal including pulmonary infection, peripheral lymphadenitis and cutaneous infection (see Chapter 129). The majority (=90%) of disseminated MAC disease is caused by *M. avium*; however, other species should be considered for symptomatic patients with negative cultures,

such as *M. triplex*, *M. genavense* and *M. conspicuum* or, for patients with cutaneous infections, *M. haemophilum* (see Table 233.4). In patients without underlying HIV infection, MAC can cause pulmonary disease, usually in patients with a history of chronic pulmonary disease, including patients with chronic obstructive pulmonary disease and cystic fibrosis. Symptoms are varied and non-specific, including chronic productive cough, dyspnea, sweats, malaise and fatigue (see Chapter 38).

Distinction between infection with MAC and transient colonization may be difficult. *Mycobacterium intracellulare* is isolated about as frequently as *M. avium*, but the isolation of *M. avium* has been associated with a poorer prognosis.

Mycobacterium arium complex lymphadenitis in immunocompetent children usually presents as an insidious, painless, unilateral process involving one or more lymph nodes. Mycobacteria isolated from infected lymph nodes are mostly (60–80%) MAC with the remainder being *M. scrofulaceum* and *M. tuberculosis* (see Chapter 39). *Mycobacterium arium* complex lymph node infection of children over 12 years of age is rarely simple lymphadenitis and may indicate disseminated disease and immunodeficiency. Disseminated MAC disease in HIV-negative patients is usually associated with congenital immunodeficiency, immunosuppression, malignancy or a specific immunodeficiency such as a deficiency in IFN-? production or IFN-? receptors. Disseminated MAC disease with visceral involvement has been associated with a high (82%) mortality rate in children without HIV infection.

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Chapter 234 - Mycoplasma and Ureaplasma

Per-Anders Mårdh

INTRODUCTION

The first mycoplasma strain was isolated in 1936 from a female genital tract specimen and was most likely a strain of *Mycoplasma hominis*.[1] Since then a number of other *Mycoplasma* spp. have been recovered from the human genital tract and from other organ systems. They have been found in otherwise sterile samples such as blood, cerebrospinal fluid (CSF) and synovial fluid, as well as in pus from abscesses in a variety of organs.[2] [3]

The mycoplasmas and ureaplasmas taxonomically belong to the order Mycoplasmatales in class Mollicutes together with organisms of the families Acholeplasmataceae and Spiroplasmataceae. Apart from being human pathogens, some organisms of the order Mollicutes are economically important plant and animal pathogens.

The initial division of mycoplasmas into different species was based on inhibition of growth of the organisms on agar media by homologous antibodies. In later studies, sequencing of the genome of mycoplasmas has confirmed the relevance of the initial classification method.

The role of *Mycoplasma pneumoniae* in primary atypical pneumonia became established in the 1940s; earlier it was believed that the condition was caused by a virus, known as the 'Eaton agent'. Chanock and Hayflick succeeded in 1961 in isolating the organism on an agar medium.[4]

First recovered by Tully and Taylor-Robinson in 1981,[5] *Mycoplasma genitalium* is the most recently recognized human mycoplasma pathogen. It has been regarded as a urogenital pathogen, for example causing nongonococcal urethritis (NGU).[6] [7]

Ureaplasma urealyticum was first discovered in 1954 by Shepard.[8] It was isolated from the urethra of men who had signs of urethritis.[2] [3] Its role in NGU has, however, remained controversial and some studies indicate that *M. genitalium* may instead be the responsible agent in such cases.[6] *Ureaplasma urealyticum* is an otherwise common inhabitant of the genital tract of healthy people. Ureaplasmas have been associated with low-birth-weight infants and can cause neonatal meningoencephalitis.[9]

Apart from the species mentioned above, several other species of *Mycoplasma* occur in the human indigenous flora of the mouth and the genital tract (e.g. *Mycoplasma buccale, M. faucium, M. lipophilum, M. orale* and *M. salivarium*).

NATURE

Mycoplasma and ureaplasma measuring 400–500nm are the smallest organisms so far identified as being capable of reproducing in broth and on agar media; on the latter they form colonies barely visible to the naked eye.[10] They also have the smallest known genome of any 'free-living' organisms (i.e. 4.5 × 10^8 Da). The genome has been completely sequenced for some species.[11] The guanine and cytosine (GC) content is low (i.e. approximately 25% as compared with the approximately 80% found in eubacteria).

The organisms are regarded as so unique that they form their own taxonomic class (i.e. Mollicutes), which refers to their 'soft skin' (they lack the rigid cell wall found in eubacteria) and to their pleomorphic shape (Fig 234.1 and Fig 234.2). Thus, they differ from eubacteria by lacking peptidoglycan, which gives the latter a rigid shape and a strong osmotic stability. The mycoplasmas are surrounded by a 8–10nm thick protoplasma membrane. The absence of peptidoglycan and ß-lactam receptors (i.e. for penicillins and cephalosporins) explains their resistance to these antimicrobial agents interfering with cell wall integrity.

Interestingly, two of the human pathogens, namely *M. genitalium* and *M. pneumoniae*, have an adherence organ in contrast to the rest of the species of the family Mycoplasmataceae. [5]

Ureaplasma urealyticum derives its name from the fact that it processes the enzyme urease. Previously, the organism was called T-strain mycoplasma as it produced tiny (T) colonies in comparison to those of mycoplasmas. *Ureaplasma urealyticum* has remained the single species in the genus *Ureaplasma*, although there have been several proposals for a differentiation based, for example, on biochemical and serologic characteristics. Fourteen different serotypes of *U. urealyticum* have been described. One biovar of human ureaplasmas (biovar no. 1) has been called *Ureaplasma parvum*.[12]

EPIDEMIOLOGY

Mycoplasma hominis

This organism has been isolated from the fallopian tubes of women with laparoscopic signs of acute salpingitis (i.e. pelvic inflammatory disease (PID)), and in whom an antibody response to the organism could be demonstrated.[13] [14] The organism seems to be able to spread through the cervical canal via the uterine cavity as well as by the lymphatic vessels of the parametrium to the tubes (Fig. 234.3). The relative role of *M. hominis* and *M. genitalium* in endometritis and salpingitis is currently under investigation.

Mycoplasma hominis is a common finding in women who lack a lactobacilli-dominated vaginal flora and who present with a mixture of bacterial species in the vagina. Many of these species are strictly anaerobic, most of which normally occur in the intestinal flora. Up to 75% of all women with a flora change, such as that seen in bacterial vaginosis (BV), are carriers of *M. hominis*.[15] A role for *M. hominis* in both BV and non-specific vaginitis has been considered, but has been difficult to prove. One of the reasons is that *M. hominis* can also occur in the vagina of women with vaginal flora changes who do not fulfill the criteria for BV.[16] The recovery rates of *M. hominis* (and of *Mobiluncus* spp.) in women with BV, trichomoniasis, vulvovaginal candidosis and in a group of healthy women are shown in Table 234.1 .

Salpingitis can occur as secondary infections of the fallopian tubes in women in whom the tubes have been primarily damaged by sexually transmitted disease agents, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.[17] *Mycoplasma hominis* may increase the risk for tubal occlusion and infertility, if infection has already damaged the tubes.

Mycoplasma hominis has been recovered from blood of women who have had a septic (febrile) abortion.[18] A significant change in

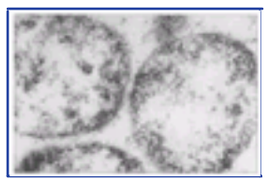

Figure 234-1 Electron micrograph of a mycoplasma organism. It has a diameter of approximately 300nm and lacks a rigid cell wall containing peptidoglycan.

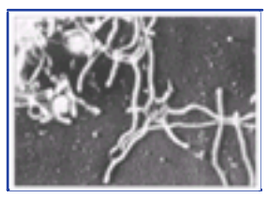

Figure 234-2 Electron micrograph of a mycoplasma organism producing filamentous structures in a broth culture. This gives the organism the appearance of a fungal mycelium. ('Myco' in mycoplasma refers to this feature.)

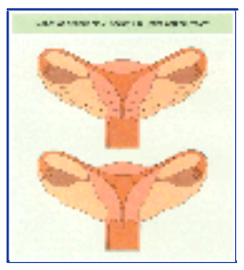

Figure 234-3 Modes of spread of *Mycoplasma hominis* to the upper genital tract. The canalicular spread to the tubes is indicated in the lower panel of the figure and the lymphatic spread to the parametria in the upper one.

the antibody titer to the organism has been found in such patients. *Mycoplasma hominis* has also been recovered from blood cultures of women with postpartum fever. These findings have been associated with a specific antibody response and a positive culture from the amniotic surface of placentas of such cases. A pure culture of

TABLE 234-1 -- Recovery of *Mycoplasma hominis, Mobiluncus mulieris* and *Mobiluncus curtisii.*

RECOVERY OF *MYCOPLASMA HOMINIS, MOBILUNCUS MULIERIS* AND *MOBILUNCUS CURTISII*				
Study group	**No. tested**	**No. of persons positive for**		
		M. hominis	***M. mulieris***	***M. curtisii***
Bacterial vaginosis	62	31	51	47
Trichomoniasis	13	3	0	0
Candidiasis	37	1	0	6
Healthy women	25	5	2	6
Partners to BV cases	47	8	1	5
Samples taken from women with bacterial vaginosis (BV), vulvovaginal candidiasis and trichomoniasis, and from healthy females and male partners of women with BV.				

M. hominis has been obtained from synovial fluid of a woman postpartum who had septic arthritis.

Mycoplasma hominis has also been recovered from the blood and CSF of newborns with signs of sepsis and meningoencephalitis.[15] [19] [20] There is a series of case reports in the literature of infants infected by *M. hominis* with malformation of the central nervous system, such as spina bifida, but also in cases without any malformation.[15] A significant antibody response to *M. hominis* has been detected in infants who have signs of pneumonia and from whom *M. hominis* has been isolated from the upper respiratory tract. In stillbirth, the organism has been recovered from lung and liver tissue.

There is no evidence that either *M. hominis* or *U. urealyticum* is the cause of bartholinitis as earlier believed.

Mycoplasma genitalium

Mycoplasma genitalium has been claimed to play an etiologic role in acute and possibly also in chronic cases of NGU.[6] Further studies have supported an independent role of *M. genitalium* and *Chlamydia trachomatis* in NGU in men.[7] Likewise, *M. genitalium* has been recovered from the urethra of HIV-infected men.[21] It can be found in urethral cultures from men with and without urethral discharge. There is no evidence of a role for the organism in chronic prostatitis.

In a recent study of women in the second and third trimesters of pregnancy, *M. genitalium* was recovered from vaginal lavage samples in 1.6% of the 500 women who had reached the second trimester. This percentage had increased in those who returned for repeated examination in the third trimester. The corresponding percentages for *M. hominis* were 10.8 and 22.3, respectively.[22]

Mycoplasma genitalium is likely a cause of cervicitis and endometritis[23] which may induce pre-term birth. Carriage of *M. genitalium* was associated with mucopurulent discharge, smoking, frequent vaginal douching and a history of miscarriage.[24] On the other hand, there seems to be no evidence of an association with bacterial vaginosis.

Mycoplasma genitalium antibodies were detected in 40% of women with PID, in whom microimmunofluorescence tests indicated a 4-fold or greater titer change within 1 month of clinical onset of upper genital tract infection.[25] Such antibodies were detected by immunoblotting in approximately half of the women with proven tubal factor infertility, and there was a correlation between the occurrence of specific anti-mycoplasma antibodies and tubal scarring.

Mycoplasma genitalium has also been associated with pneumonia and arthritic conditions.[26]

Mycoplasma pneumoniae

Mycoplasma pneumoniae infection is one of the most frequent causes of atypical pneumonia.[26] [27] [28] [29] Endemic infections occur on

a regular basis in between epidemic outbreaks which, at least for some decades, occurred in many countries every 3–5 years. However, this sequence pattern has become less distinct in many countries during recent decades.

Young adults are particularly affected by *M. pneumoniae*. The incubation period, as indicated by case-to-case transmission studies, is estimated at a mean of 20 days (range 11–25 days). The reported attack rates in closed communities, such as in families, have been 17–66%. [26] [30]

Ureaplasma urealyticum

The prevalence of *U. urealyticum* in the genital tract is associated with sexual experience.[2] Disappearance of the organism from the genital tract after long-term sexual abstinence has been documented.

There have been a large number of studies on the possible role of *U. urealyticum* in NGU, since it was first recovered from the urethra of US military men by Shepard.[8] One major problem in evaluating its possible role in nongonococcal, nonchlamydial urethritis has been to find suitable comparison groups as the organism is a very common member of the indigenous microbial flora of the male urethra. A quantitative approach in culture studies of *U. urealyticum* in NGU cases has been undertaken. If the organism establishes itself as a pathogen, it is likely to increase in number. However, such studies have not been consistent and are therefore difficult to interpret.

Furthermore, an extensive etiologic investigation is required to prove a role of *U. urealyticum* in NGU, including highly sensitive diagnostic tests for *Chlamydia trachomatis* and *M. genitalium*.[7] However, these organisms were not known at the time when many of the NGU studies were performed. Phenotyping and serotyping of ureaplasma isolates and antibody tests have not confirmed a pathogenic role for *U. urealyticum* in NGU. The difference in susceptibility of *U. urealyticum* and other possible causative agents of urethritis has been utilized in attempts to establish the role, if any, of *U. urealyticum* in NGU.[2] [3] However, none of the studies have yielded conclusive results.

Ureaplasma urealyticum often recolonizes the genital tract of individuals after attempts to eradicate it by antibiotic therapy. This may be seen as a criterion for the organism belonging to the indigenous human microbial flora, meaning that it does not represent an exogenous pathogen.

Like most organisms found in the urogenital tract of men, *U. urealyticum* has also been proposed to be a causative agent of nonbacterial prostatitis. Cultures of urethral secretion, voided urine as well as expressed prostatic fluid do not prove an etiologic role of ureaplasmas in prostatitis because of the risk of contamination of the samples from nonprostatic sites. Likewise, antibiotic therapeutic trials cannot easily prove such a role of the agent in nonbacterial prostatitis.

Ureaplasma urealyticum is found in the lower genital tract in up to 75% of all women of reproductive age. It may ascend to the fallopian tubes.[13] Treatment of infertile couples with tetracyclines has provided inconclusive support for a role for *U. urealyticum* as a cause of infertility.

The higher recovery rate of *U. urealyticum* from babies with low birth weight than from infants of normal weight has remained a puzzling observation. There is also a statistical correlation of carriage of *U. urealyticum* with pre-term birth. Several studies have shown that *U. urealyticum* is a comparatively common isolate, not only from the urethra [31] but also from amniotic fluid in women with adverse pregnancy outcome, such as premature birth.[24] The organism may be recovered from the respiratory tract of newborns. Respiratory distress syndrome, the need for assisted ventilation, severe respiratory insufficiency and death of newborns have been found to be more common in those colonized than not colonized by *U. urealyticum* in the nasopharynx or elsewhere in the upper respiratory tract. Studies have not proved an etiologic role of *U. urealyticum* in chronic lung disease in persons born pre-term.

It is an important observation that *U. urealyticum*, like *M. hominis*, can cause meningoencephalitis in newborns. Such infections should be considered as a differential diagnosis in any investigation of meningitis in newborns, particularly when the CSF glucose level is normal.[15] [20]

PATHOGENICITY

Pathogenetic mechanisms are not known for the vast majority of *Mycoplasma* spp. One obvious exception is *M. neurolyticum*, which produces a neurotoxin, explaining the course of infections by this animal pathogen.

Mycoplasmas are generally epiparasites, attaching to eukaryotic cell surfaces. However, the fact that *M. genitalium, M. fermentans* and *M. penetrans* can grow intracellularly means that the organisms can be protected from many host defense mechanism. For example, in experimentally infected vero cell cultures *M. genitalium* penetrated the cells. This capability may give the organism the ability to persist in AIDS patients, from whom the organisms have been recovered (see below).[21] [32] [33] [34] [35] [36] [37] [38]

Both *Mycoplasma pneumoniae* and *M. genitalium* have a flask-like appearance under the electron microscope.[5] They both have an adherence organ. These structures probably represent a pathogenic mechanism. The adherence structure has a 170kDa epitope, which is shared by *M. pneumoniae* and *M. genitalium*. A distinct binding site for *M. pneumoniae* on mononuclear cells has also been identified. *Mycoplasma pneumoniae* is motile and produces hydrogen oxide, which may contribute to its pathogenicity.

Both *M. hominis* and *M. genitalium* are able to cause salpingitis experimental infections in subhuman primates.[39] [40] Oviduct inoculation of *M. genitalium* in grivet monkeys and marmosets resulted in a moderate-to-severe endosalpingitis with consequent adhesions between the mucosal folds. The changes were similar to those found in experimental infections by *M. hominis* and *C. trachomatis* as well as being consistent with alterations seen in women naturally infected with these agents who had developed PID.

In tissue cell cultures of human fallopian tubes infected by *M. hominis*, a pathologic swelling or so-called 'ballooning' of the cilia has been seen (Fig. 234.4). Whether a similar phenomenon occurs in vivo in women who have contracted the organism is, however, not known.

In mice, estradiol predisposes animals to infection by *M. hominis*. Progesterone induces susceptibility to *M. genitalium* in the murine genital tract when the organism has been installed intravaginally. However, this does not account for experimental infection with *M. hominis* in this animal model.

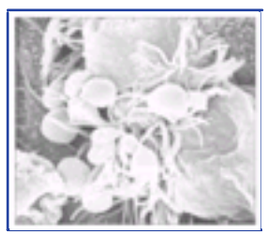

Figure 234-4 Swelling ('ballooning') of cilia in tissue cell cultures experimentally infected by *Mycoplasma hominis.*

Mycoplasma pneumoniae causes a polyclonal stimulation of both B and T lymphocytes and results in cytokine production. A marked increase in the total serum IgM is often seen in people infected by *M. pneumoniae*, often by as much as 100% or more.[30] [41]

Antibodies directed against the blood group antigen (i.e. cold agglutinins) may occur in *M. pneumoniae* infection and may, although very seldom, cause hemolytic anemia and intravascular coagulation, which may be fatal.[26] [27]

The development of antibodies to a number of tissue antigens (e.g. to brain[42] and kidney tissue epitopes) occurs as a transient autoimmune phenomenon in persons infected by *M. pneumoniae*, often without any obvious pathologic effects.

Ureaplasma urealyticum has the ability to split urea, which has been considered to be a mechanism by which the organism could cause stone formation in the urinary tract, including calculi formation in the kidney pelvices. Calculi formation has been seen in female rats experimentally infected by *U. urealyticum* after bladder installation of the organism. It is not clear, however, whether colonization by ureaplasmas occurs more easily in persons who have already developed stones in the urinary tract.

Ureaplasma urealyticum also expresses a specific immunoglobulin A1 protease that cleaves human immunoglobulin into Fab and Fc fragments, the clinical relevance of which is unknown.

Colonization with *U. urealyticum* in experimentally infected female mice is enhanced by estradiol therapy.

There have been several observations of a correlation between low birth weight and culture positivity for genital mycoplasmas and/or ureaplasmas from the nose and throat in newborns.[2] [3] The influence on pregnancy outcome has remained difficult to explain. Therapeutic trials in which the organisms have been eradicated from the lower genital tract of pregnant women resulting in an increased birth weight of their offspring is an even more puzzling observation. Confounding, unrecognized factors might have played a role.

Mycoplasmas and AIDS

It has been claimed that *M. fermentans* might play a role in AIDS.[33] [34] [36] When first recovered from AIDS patients, the isolates of *M. fermentans* were named *M. incognitus*. The organism has been detected in urine samples and antigen of the organism has been detected in blood samples of HIV-infected persons.

Mycoplasma genitalium has been recovered from genital samples of AIDS patients.[21] In contrast to many of the infections observed in untreated AIDS patients, no

relation between *M. genitalium* positivity on one hand and $CD4^+$ lymphocyte count, HIV p24 antigenemia and any opportunistic infections on the other has been demonstrated, and it is no longer believed that mycoplasma have any causative role.

PREVENTION

Prevention of mycoplasmal genital infections should follow the principles of any sexually transmitted infection, including partner notification and treatment of infected partners at the same time as the index case to prevent 'ping-pong' infections. However, there are no data proving the positive effect of such interventions.

As there is a markedly increased risk of tubal occlusion with each new episode of salpingitis and thereby a risk of infertility, eradication of *M. hominis* may be indicated in females who are vaginal carriers of the organism and who have a history of PID.

Treatment of pregnant women who are carriers of *M. hominis* and *U. urealyticum* to prevent obstetric and perinatal infections has been recommended, but there is no hard evidence for such a recommendation. It is unclear whether *M. genitalium* should be screened for and whether therapy should be given both to carriers and their sexual partners.

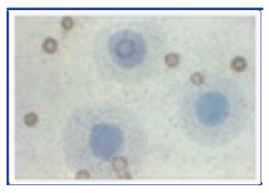

Figure 234-5 Colonies of *Mycoplasma hominis* on PPLO agar with 'fried egg' appearance.

Prevention of mycoplasmal (i.e. *M. pneumoniae*) respiratory tract infections is generally not feasible.

DIAGNOSTIC MICROBIOLOGY

Mycoplasmas can be recovered on special pleuropneumonia-like organism (PPLO) agar media, where they produce colonies with a characteristic 'fried egg' morphology (Fig. 234.5). Growth inhibition on agar media by species-specific antibodies (lysis of organisms) has been used for speciation of mycoplasmas. Growth inhibition in broth cultures by specific antisera (e.g. in tests for interference with arginine metabolism by growing mycoplasmas) has also been used for this purpose. The latter test, however, requires reading the result at an unpredictable time after initiating the test, making it impractical to use.[10]

The suitability of each batch of mycoplasma medium should be checked before use (e.g. serum components may be toxic to the mycoplasma organisms). A number of commercial media are available. Solid media should be incubated at 36°C or 96.8°F in an atmosphere of 5% carbon dioxide (for ureaplasmas the concentration may be as high as 15%) and 95% nitrogen.[10]

Polymerase chain reaction (PCR) has become more frequently used for diagnosing mycoplasma infections.[31] [43] Specific primers that assess not only the occurrence of the organism but also its species can be obtained. Anti-*M. pneumoniae* IgM antibodies are often not detected in pneumonia caused by the agent.[44] Also, secretory IgA antibodies to *M. pneumoniae* occur in cases with other types of respiratory tract infections and in healthy controls. Culture facilities are generally not available and, even if they are, cultures are often negative in cases where serologic tests indicate a current infection by the agent.

Mycoplasma pneumoniae

Attempts to diagnose *M. pneumoniae* infections by culture usually fail. If successful, it takes weeks before colonies (Fig. 234.6) can be detected when inspected under a microscope. *Mycoplasma pneumoniae* can be distinguished from other human *Mycoplasma* spp. by its

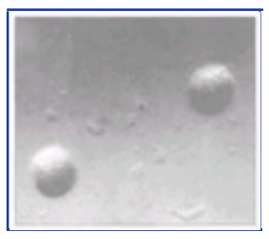

Figure 234-6 Colonies of *Mycoplasma pneumoniae* on PPLO agar with 'golf ball' appearance.

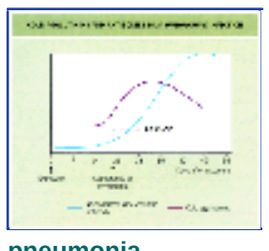

Figure 234-7 Kinetics of appearance of cold agglutinins and specific antibodies to *Mycoplasma pneumoniae* related to time after being taken ill with *M. pneumoniae* pneumonia.

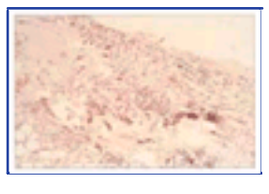

Figure 234-8 Section of parametrium from which *Mycoplasma hominis* was recovered. The patient had parametritis and developed an antibody response to the organism.

ability to adsorb red blood cells, resulting in a mulberry-like appearance of the colonies.

Serology and detection of cold agglutinins have played a more important role in diagnosis of *M. pneumoniae* than culture studies (Fig. 234.7). [26] Detection of IgM antibodies to *M. pneumoniae* has a limited diagnostic value for detecting current infection. Cold agglutinins occur before specific antibodies to *M. pneumoniae* appear (see Fig. 234.7). However, detection of cold agglutinins alone has low sensitivity and specificity in patients with community-acquired pneumonia. The possibility of diagnosing *M. pneumoniae* pneumonia by one seropositive sample plus a positive cold agglutinin test has been stressed.[27] At admission, an IgM antibody response to *M. pneumoniae* has been shown in 86% of patients with *M. pneumoniae* pneumonia. However, some have questioned the diagnostic value of such antibody tests in routine practice. Approximately 20% of patients with such pneumonia have not developed a specific antibody response to *M. pneumoniae* at their first visit. Antibodies to a number of tissue antigens develop during the course of a *M. pneumoniae* infection,[42] the clinical significance of which has remained unestablished.

Progress in the diagnosis of infections by *M. pneumoniae* has been achieved by the use of nucleic acid-based techniques [43] [45] (e.g. ^{125}I-labeled DNA probe), directed against sequences specific for *M. pneumoniae* rRNA (GenProbe) or probes. The test has the disadvantage of requiring metabolically active organisms but it has been claimed to be more sensitive than enzyme immunoassays. PCR tests can detect only 10^2 –10^3 organisms/ml, but adequate internal controls are necessary to avoid false-negative results. The usefulness of PCR tests is limited in the post-therapy situation, as dead *M. pneumoniae* organisms may be detected. Such organisms are shed for up to 1 month after finishing antibiotic therapy. A comparison of the outcome of cultures, PCR tests and serology in suspected *M. pneumoniae* cases showed that, in 21 cases in which cultures were performed, 19 were found to harbor the organism and 14 of the same 21 cases were seropositive for the organism. Only two of another 62 culture- and serology-negative cases were positive for *M. pneumoniae* in a study using a PCR assay. Both sputum and pharyngeal swabs may be tested by PCR to prove a mycoplasma etiology in pneumonia cases.

Mycoplasma pneumoniae DNA has also been detected in intestinal biopsy samples of persons with inflammatory bowel disease (e.g. in cases of Crohn's disease and

in those with ulcerative colitis as well as in healthy controls).[46]

Thus, there are obvious difficulties in diagnosing *M. pneumoniae* infections, even when laboratory support makes use of PCR assays. The use of more than one diagnostic test may be helpful. The clinical picture may also be misleading. For example, there may be difficulties in distinguishing between mycoplasmal and chlamydial infections.[47] The diagnostic difficulties have been stressed by the US Practice Guidelines for the Management of Community-acquired Pneumonia, which state that it is not possible to diagnose *M. pneumoniae* infection with any degree of accuracy in a routine health care situation.[28]

Mycoplasma hominis

Mycoplasma hominis is easy to isolate on agar media.[10] Isolates can be typed by homologous antisera, causing growth inhibition. The capacity of *M. hominis* to metabolize arginine can be tested by the production of ammonia as an end-product, the presence of which results in a pH increase that can be visualized by an indicator strip. Colonies of *M. hominis* produce pinpoint nonhemolytic colonies on blood agar plates. Polymerase chain reaction tests may also be used.[32]

Mycoplasma genitalium

Mycoplasma genitalium is difficult to grow on artificial media, which is why the introduction of PCR-based methods for its detection has been important. The organism is susceptible to thallium acetate. This may explain why it has been detected only recently, as thallium acetate has often been added to mycoplasma media to reduce any overgrowth of eubacteria and fungi. [7]

Ureaplasma urealyticum

Growth in a broth medium containing urea and an indicator causing a color change has been used to diagnose ureaplasmas.[10] This test is useful for detection of ureaplasmas in genital samples. Tests for the urea-splitting capability of ureaplasmas can also be carried out on agar media, where the color of colonies of *U. urealyticum* turns dark brown after adding the test substrate. Ureaplasmas do not grow in the presence of thallium acetate, which is often used in mycoplasma media to avoid overgrowth of other microbes. Using 16S rRNA gene-based PCR tests, *U. urealyticum* is more often detected in the lower female genital tract than by culture.[32]

CLINICAL MANIFESTATIONS

Women with *M. hominis* salpingitis may be afebrile and present with a malodorous vaginal discharge that is often characterized as 'having a smell of rotten fish'. They may have palpatory pain over the adnexa and pain at movement of the cervix during vaginal examination. However, as in PID of any etiology, mycoplasmal PID may be more or less asymptomatic.[13] *M. hominis* may cause parametritis (Fig. 234.8).

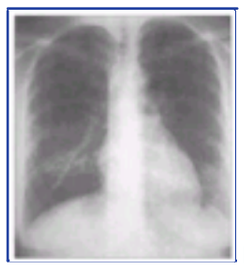

Figure 234-9 *Mycoplasma pneumoniae* pneumonia. There were few signs on auscultation.

Mycoplasma hominis sepsis may be suspected in women who have aborted and who develop fever, particularly in cases in which bacterial blood cultures are negative for bacterial pathogens.

Symptoms of *M. pneumoniae* infections are initially malaise, muscle pain, headache, fever, throat pain, chills and nonproductive cough. The latter occurs in up to 90% of cases.[26] [28] [29] [41] [48] The symptoms and signs of mycoplasma pneumonia are more like those of a viral than a bacterial pneumonia. However, cough and headache do occur more often, and rhinitis less often, in mycoplasmal than viral pneumonia cases. Later, often after 1–2 weeks, cough may become productive and, although less commonly, hemorrhagic. The expectorate is often sparse. Cough may continue during the convalescent period. Tracheitis, bronchitis and tracheobronchitis may occur, but less often than pneumonia. Severe deterioration of the general condition may occur with high body temperature, due to the development of generalized aveolitis.

Findings on auscultation are often sparse or even normal, even when there are obvious radiographic changes. The alterations are usually unilateral and found in a lower lobe (Fig. 234.9). Chest radiography usually shows multiple interstitial or alveolar consolidation in one of the lower lobes, often radiating from the hilum. A solid lobular consolidation may be seen and there may be pleuritis. The chest radiographic findings may be mistaken for a viral pneumonia. Symptoms of fatigue may last for many months, and in children this may result in poor performance at school.[49] (see Chapter 34)

In children, the clinical course of a *M. pneumoniae* infection usually runs a milder course than in adults.[28] The infection in children may even pass as a clinically silent or relatively asymptomatic upper respiratory infection that ones pay no attention to. In elderly persons, the infection may manifest itself as pharyngitis, laryngitis or bronchitis rather than pneumonia.

Truly asymptomatic *M. pneumoniae* infections are uncommon. Only a small percentage of such infections run a clinically silent course as evidenced from serologic findings. Transient carriage of *M. pneumoniae* may occur after repeated exposure in those who have developed a cellular immunity to *M. pneumoniae*.

Mycoplasma pneumoniae infection has been associated with a large number of other conditions (Table 234.2) although in many cases evidence to support an etiologic role for *Mycoplasma* is uncertain.[30] [41] [48] [50] [51] [52] [53]

Death in infections with *M. pneumoniae* is very unusual. In such cases, either intravascular coagulation or complications from the central nervous system have been diagnosed. At autopsies of

TABLE 234-2 -- Clinical manifestations of *Mycoplasma pneumoniae* infection.

CLINICAL MANIFESTATIONS OF *MYCOPLASMA PNEUMONIAE* INFECTION	
Respiratory tract	Pharyngitis, laryngitis, acute bronchitis, bronchopneumonia
Skin and mucosa	Maculopapular and vesicular exanthema, urticaria, purpura, erythema nodosum, erythema multiforme, Stevens-Johnson syndrome
Central nervous system	Meningitis, meningoencephalitis, acute psychosis, cerebellitis, Guillian-Barré syndrome?
Parenchymatous organs	Pancreatitis, diabetes mellitus, non-specific reactive hepatitis, subacute thyroiditis?
Miscellaneous	Hemorrhagic bullous myringitis, hemolytic anemia, pericarditis, thromboembolism?
Some associations remain uncertain.	

persons who died of *M. pneumoniae* infection, diffuse interstial pneumonia has been demonstrated. Other autopsy findings have included bronchial edema. Histology has revealed macrophage, lymphocyte and plasma cell infiltrates in bronchial tissue.

The broad spectrum of complications described in *M. pneumoniae* infections must be considered in the light of recent studies that have found that nucleic acid tests for *M. pneumoniae* may often be positive even in healthy individuals. That is, without serologic evidence of an ongoing acute infection, it is difficult to confirm any etiologic relationship to various conditions.

MANAGEMENT

Mycoplasmas are resistant to ß-lactam antibiotics as they lack a cell wall and penicillin-binding proteins. They are generally susceptible to tetracyclines. Tetracycline resistance in *M. hominis* is, however, common in certain areas[54] and is often due to decreased intracellular uptake of the drug.[55]

Children should not be given tetracylines. Erythromycin is the drug of choice for children, except for infections by *M. hominis*, which has natural resistance to this antibiotic. The reason for this diversity from all other species of *Mycoplasma* is unknown. Clindamycin is a suitable alternative for *M. hominis* infections in newborns.

Azithromycin and clarithromycin are alternative drugs for the treatment of *M. pneumoniae* infections in adults. Intravenous treatment of *M. pneumoniae* pneumonia has not proved to be superior to oral therapy (see Chapter 34).

Mycoplasma genitalium has an antibiotic susceptibility pattern similar to that of *M. pneumoniae* (i.e. being susceptible to macrolides and tetracyclines).[56] [57] Azithromycin has been recommended for therapy of *M. genitalium* infections.

Aminoglycosides and chloramphenicol are second- and third-line therapy alternatives. Chloramphenicol has, however, been used successfully to treat meningoencephalitis in newborns infected by tetracycline-resistant strains of *M. hominis*.

A number of antibiotics are less active against mycoplasmas than macrolides and tetracyclines (e.g. quinolones, such as cinoxacin, ciprofloxacin and norfloxacin). Sparfloxacin is the most active quinolone against *M. genitalium*.[57] Mycoplasmas, like ureaplasmas, are resistant to sulphonamides and trimethoprim-sulfamethoxazole.

Ureaplasmas are known to have a similar susceptibility pattern to mycoplasmas. However, they are usually susceptible to erythromycin, in contrast to *M. hominis*.

Mycoplasma pneumoniae pneumonia in adults can be treated with a macrolide or tetracycline.

For younger patients, apart from those infected by *M. hominis*, erythromycin can be given for 10–14 days with a dose adjusted to the weight of the infant or child under treatment.[28]

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Chapter 235 - Rickettsia and Rickettsia-like Organisms

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INTRODUCTION

The family Rickettsiaceae was established to accommodate fastidious, obligate intracellular, pleomorphic coccobacilli that could not survive for long outside the host cell and were transmitted to vertebrate hosts by arthropods. Members of the family usually stained weakly with Gram's stain but stained well with Giemsa or Gimenez stains. Cross-reactivity with antisera indicated that they shared common antigens, although this did not always lead to cross-protection against infection. Two organisms, *Rochalimaea* (now *Bartonella*), which was capable of growth in cell-free media, and *Coxiella*, which was able to survive outside a host cell for long periods of time, were regarded as rickettsias because of their morphologic similarity to other members of the family and because of their association with arthropod vectors. The family was further divided into three tribes, Rickettsiae, Ehrlichiae and Wolbachiae on the basis of pathogenicity for humans, pathogenicity for other vertebrate animals or exclusive association with insects, respectively. All human pathogens then known were classified in the tribe Rickettsiae.

Molecular techniques are now used to classify bacteria. Phylogenetic trees, constructed from 16S rRNA gene sequences, show three major groups of rickettsias, each belonging to a different subdivision of the Proteobacteria. The genera *Rickettsia*, *Ehrlichia* and *Anaplasma* belong to the a_1 subdivision, the genus *Bartonella* is found in the a_2 subdivision, and these two groups are themselves only distantly related to *Coxiella*, which belongs to the ? subdivision (Fig. 235.1).

Within the genus *Rickettsia*, three groups were originally recognized: the typhus group, the spotted fever group and the scrub typhus group. Molecular techniques show close affinity between the typhus and spotted fever groups, confirming their status as members of the same genus. On the basis of differences in ultrastructure and DNA composition the causative organism of scrub typhus, *Rickettsia tsutsugamushi*, has been reclassified into a new genus, *Orientia*. Some members of the genus *Ehrlichia* are now known to infect humans and cause diseases that were previously unrecognized. Finally, an increasing number of members of the genus *Bartonella* are being recognized as human pathogens and the cause of an expanding array of diseases. The field of rickettsiology is thus undergoing rapid change and expansion, with recognition of new organisms, of new relationships between rickettsias and other bacteria, and of new diseases.

The rickettsias are found on all continents, occupying many different ecologic niches and are associated with a variety of arthropod and vertebrate hosts. Human rickettsioses may be subclinical, may cause mild, self-limiting disease or may cause severe, life-threatening disease. In general, early treatment greatly decreases the severity of infection, with consequent reduction in the risk of mortality. Unfortunately, early diagnosis is seldom easy. Tick bites are often painless and go unnoticed. Rickettsioses are clinically non-specific, and isolation of organisms requires specialized techniques and carries a risk to laboratory staff. Serodiagnosis is simpler and safer, but the commonly used Weil-Felix agglutination test has low sensitivity and specificity. Even with more reliable serologic tests, false-negative results are common early in infection. A high index of suspicion is therefore critical in detecting rickettsioses. Fortunately, most infections respond rapidly to therapy with widely available antibiotics.

SPOTTED FEVER GROUP RICKETTSIAS

NATURE

The spotted fever group (SFG) rickettsias are intracellular coccobacilli, of 1µm × 0.3µm, that are found within the cytoplasm, and sometimes the nucleus, of the host eukaryotic cell. Ultrastructural studies show that the cell membrane has an inner leaflet that is thicker than the outer leaflet, a characteristic that distinguishes *Rickettsia* from related genera. The cell wall has a high content of lipopolysaccharides (LPS), which are highly immunogenic and are responsible for the serologic cross-reactivity of different SFG rickettsias. The LPS also confers cross-reactivity with *Proteus* and *Legionella*. High-molecular-weight proteins can be recognized in cell-wall preparations using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and specific antigenic characteristics have been demonstrated by Western blot.[1]

The SFG rickettsias do not grow on cell-free media but will grow in animals, including guinea pigs and embryonated chicken eggs, and in tissue cultures. Members of the group are often distinguished using reactivity with homologous and heterologous murine antisera in the micro-immunofluorescence (MIF) test. The reliability of this is

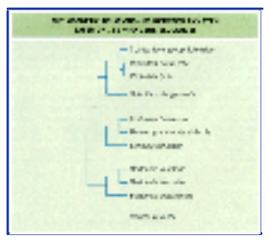

Figure 235-1 Phylogenetic relationships between rickettsias based on 16S rRNA gene sequences.

unclear, however. If molecular criteria were strictly applied, many of the currently recognized 'species' would have to be considered serotypic variants of *Rickettsia rickettsii*, despite the differences in epidemiologic, antigenic and pathogenic characteristics.

Before 1984, only six SFG rickettsioses were recognized: Rocky Mountain spotted fever, Mediterranean spotted fever, Siberian tick typhus, Queensland tick typhus, rickettsialpox and Israeli spotted fever. Another eight new tick-borne rickettsioses have been described in recent years: Japanese spotted fever, Chinese spotted fever, Flinders Island spotted fever, Astrakhan fever, African tick bite fever and three so far unnamed spotted fevers (caused by *Rickettsia mongolotimonae*, *Rickettsia helvetica* and *Rickettsia slovaca*). In addition, the ELB agent, transmitted by cat fleas, is now recognized as *Rickettsia felis*, and is the causative agent of flea typhus in southern USA and parts of Mexico.[2] The list will undoubtedly continue to enlarge as more investigations are completed.

Sequence analysis of genes coding for outer membrane proteins indicate that there are three main complexes within the SFG:[3]

- cluster I: *Rickettsia conorii* complex, including Malish, M1, Moroccan and Indian tick typhus strains as well agents of Astrakhan fever and Israeli tick typhus;
- cluster II: *Rickettsia africae*, *Rickettsia parkeri*, *Rickettsia sibirica* and *R. mongolotimonae*; and
- cluster III: *Rickettsia aeschlimannii*, *Rickettsia rhipicephali*, *Rickettsia japonica*, *Rickettsia massiliae* and *Rickettsia montanensis*.

Other rickettsias, including *Rickettsia rickettsiae*, *R. japonica*, *R. slovaca* and Thai tick typhus rickettsias, show greater divergence in gene sequences and do not fit within these groups.

EPIDEMIOLOGY

With the exception of *Rickettsia akari* (transmitted by mites) and *R. felis* (transmitted by fleas), ticks are the vectors of most SFG rickettsias. The prevalence of infected ticks may be high.[4] Ticks probably become infected when they feed on rickettsemic wild or domestic animals and the rickettsias then multiply rapidly in many organs of this host. Infection of the ovaries and oocytes of a female tick leads to transovarial transmission, with at least some eggs becoming infected. Thereafter infections are transmitted trans-stadially, probably to all stages of the tick, and each time the larvae, nymphs or adults feed they may transmit the infection to the vertebrate host. Because all of the stages may be infected with rickettsias, ticks are regarded as both reservoirs and vectors of SFG rickettsial infection. Tick bites usually occur during occupational or recreational activity, or following the introduction of infected ticks into the home by domestic animals. Transmission of infection, however, has also been reported during the manual removal of ticks from domestic animals.

Most of the SFG rickettsial diseases of humans have limited geographic distribution (which is reflected in the names used to describe them; Table 235.1) and different tick species may be important transmitters in these different regions (Fig. 235.2). Increasing international traffic, and possibly movement of vectors on hosts such as birds, has resulted in a number of apparently imported cases of SFG rickettsial infection.[5] Transmission through the use of contaminated needles is suggested by the finding that intravenous drug users may be at increased risk of *R. akari* infection.[6]

PATHOGENICITY

Ticks and mites inject organisms into the host with their saliva. At the site of the bite the rickettsias localize in contiguous endothelial cells. Entry into the cells appears to be by receptor-mediated attachment and phagocytosis. Organisms move out of the phagolysosome,

Figure 235-2 *Amblyomma hebraeum*, a typical tick vector of spotted fever group rickettsial infection.

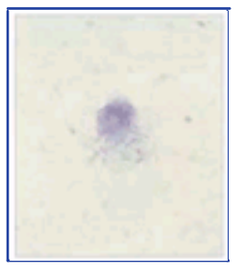

Figure 235-3 Gimenez stain of tissue culture cells infected with spotted fever group rickettsias.

probably by means of a phospholipase, into the cytoplasm and start to divide by binary fission (Fig. 235.3). Dilation of rough endoplasmic reticulum can be observed after about 48 hours. The bacteria can move between adjacent cells via cytoplasmic extrusions, and movement is associated with polymerization of actin molecules. The resulting vascular damage results in moderate to severe lymphohistiocytic vasculitis, increased vascular permeability leading to edema, and cutaneous necrosis. As an eschar forms at the bite site and organisms escape from infected cells and spread via lymphatics to endothelial cells in blood vessels throughout the body. The

resulting widespread cell death, vasculitis and increased vascular permeability result in the typical 'spotted fever' and 'tick typhus' diseases.

PREVENTION

Preventive measures include tick control programs for domestic and wild animals and the use of tick repellents on clothing and skin. Vaccines are effective in laboratory animals but have not been developed for use in humans. Antibiotic prophylaxis for people exposed to tick bites is not recommended as tetracyclines may be bacteriostatic and so only delay the onset of disease rather than prevent it.

TABLE 235-1 -- Global distribution of spotted fever group rickettsias.

GLOBAL DISTRIBUTION OF SPOTTED FEVER GROUP RICKETTSIAS			
Geographic distribution	**Human disease**	**Organism**	**Principal vector(s)**
Africa			
Mediterranean	'Mediterranean spotted fever'	*Rickettsia conorii*	*Rhipicephalus sanguineus*
	'Boutonneuse fever'		
Sub-Saharan Africa	'Kenyan tick typhus', 'South African tick-bite fever'	*Rickettsia conorii*	*Rhipicephalus simus*
			Haemaphysalis leachii
			Rhipicephalus mushamae
	'African tick bite fever'	*Rickettsia africae*	*Amblyomma hebraeum*
			Amblyomma variegatum
Morocco	None known	*Rickettsia aeschlimannii*	*Hyalomma marginatum*
Central African Republic	None known	*Rickettsia rhipicephali*	*Rhipicephalus* spp.
	None known	*Rickettsia massiliae*	*Rhipicephalus lunulatus*
			Rhipicephalus sulcatus
			Rhipicephalus mushamae
America			
USA	'Rocky Mountain spotted fever'	*Rickettsia rickettsii*	*Dermacentor andersoni*
			Dermacentor variabilis
	None known	*Rickettsia belli*	*Dermacentor variabilis*
	None known	*Rickettsia montanensis*	*Dermacentor andersoni*
			Dermacentor variabilis
	None known	*Rickettsia parkeri*	*Amblyomma maculatum*
	None known	*Rickettsia rhipicephali*	*Dermacentor* spp.
	None known	*Rickettsia peacocki*	*Dermacentor andersoni*
	'Rickettsial pox'	*Rickettsia akari*	*Allodermanyssus sanguineus*
	'Rocky Mountain spotted fever'	*Rickettsia amblyommii*	*Amblyomma americanum*
Mexico, southern USA	Flea typhus	*Rickettsia felis*	*Ctenocephalides felis*
Brazil	'Brazilian spotted fever'	*Rickettsia rickettsii* (*R. sibirica*?)	*Amblyomma cajennense*
Guadeloupe	'Tick-borne fever'	*Rickettsia africae*	*Amblyomma variegatum*
Australasia			
Australia	'Queensland tick typhus'	*Rickettsia australis*	*Ixodes holocyclus*
Tasmania	'Flinders Island spotted fever'	*Rickettsia honei*	*Ixodes tasmani*
Asia			
Former Soviet Asia	'Siberian tick typhus'	*Rickettsia sibirica*	*Dermacentor nuttali*
			Dermacentor marginatus
			Dermacentor silvarum
			Dermacentor sinicus
			Haemaphysalis concinna
Japan	'Japanese spotted fever'	*Rickettsia japonica*	*Dermacentor taiwanensis?*
Thailand	'Thai tick typhus'	'TTT rickettsia'	*Rhipicephalus sanguineus*
			Ixodes spp.
China	'Chinese spotted fever'	*Rickettsia heilongjiangjii*	*Dermacentor silvarum*
		Rickettsia hulinii	*Haemaphysalis concinna*
		Rickettsia mongolotimonae	*Hyalomma asiaticum*
India	'Indian tick typhus'	*Rickettsia conorii*	*Rhipicephalus sanguineus*
Astrakhan	'Astrakhan spotted fever'	'Astrakhan SFG rickettsia'	*Rhipicephalus sanguineus*
			Rhipicephalus pumulio
Israel	'Israeli spotted fever'	'ISF rickettsia'	*Rhipicephalus sanguineus*
Europe			
Mediterranean coast	'Mediterranean spotted fever'	*Rickettsia conorii*	*Rhipicephalus sanguineus*
	'Boutonneuse fever'		
European Russia	'Siberian tick typhus'	*Rickettsia sibirica*	*Dermacentor nuttali*
			Dermacentor marginatus
			Dermacentor silvarum
			Haemaphysalis concinna

Germany	Flea typhus	*Rickettsia felis*	Not known
Slovakia, Switzerland, France, Portugal	Febrile illness, meningoencephalitis	*Rickettsia slovaca*	*Dermacentor marginatus*
Switzerland, France, Sweden	Fever, myocarditis	*Rickettsia helvetica*	*Ixodes ricinus*
France	Fever, eschar, lymphangitis	*Rickettsia mongolotimonae*	Not known
	None known	*Rickettsia rhipicephali*	*Rhipicephalus sanguineus*
France, Greece, Portugal	None known	*Rickettsia massiliae*	*Rhipicephalus sanguineus*
	Rhipicephalus turanicus		
Russia	Rickettsialpox	*Rickettsia akari*	*Allodermanyssus sanguineus*

DIAGNOSTIC MICROBIOLOGY

Early diagnosis is necessary for prompt and appropriate therapy, a factor that significantly reduces morbidity and mortality. A history of tick-bite, the presence of an eschar at the bite site, fever, headache and rash are the classic features of SFG rickettsioses, but these may occur together in only 50–75% of patients. Tick bites are usually painless and may go unnoticed, particularly if they occur in the anogenital region or among the hairs on the scalp. The lesion at the bite site may be confused with traumatic, parasitic, bacterial or viral skin lesions. Fever, headache and rash may be due to a variety of causes. There are, therefore, no clinical or epidemiologic findings that are reliable in diagnosis.

Spotted fever group rickettsias can be isolated from blood or from biopsies of the eschar, using rodents or embryonated chick eggs. Organisms may not, however, be detected for a week or more after inoculation, and special laboratory facilities are needed. Close attention to safety is required in order to prevent transmission to laboratory workers. The centrifugation-shell vial technique, where samples are centrifuged on to a cell monolayer, may reduce the time for detection to 3–4 days.

Rickettsial antigens can be detected in endothelial cells in dermal blood vessels by direct immunofluorescence staining of frozen skin sections from the eschar or sites affected by rash. Direct immunofluorescence may also be used to detect antigens in circulating endothelial cells isolated from whole blood. Even greater sensitivity may be achieved with polymerase chain reaction (PCR) assays, using various primers.[7] These technologies are, however, only rarely available in diagnostic laboratories.

The classic serologic test for rickettsial infection is the Weil-Felix test, which relies on the antigenic cross-reactivity between epitopes in the LPS of *Rickettsia* spp. and in particular strains of *Proteus* spp. Although very simple, and widely available, the test has low specificity and sensitivity and is regarded as unacceptable for accurate diagnosis. There are a number of other tests such as latex agglutination, hemagglutination, indirect fluorescent antibody (IFA) and the

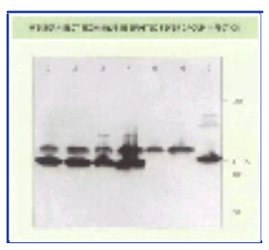

Figure 235-4 Western blot technique in spotted fever group infection. Western blot of pooled mouse antisera to *Rickettsia africae* — human isolate (lane 1), *Rickettsia africae* — tick isolate (lanes 2–4), *Rickettsia conorii* — Kenyan strain (lane 5), *Rickettsia conorii* — Moroccan strain (lane 6) and Israeli SFG rickettsia (lane 7). Molecular masses (in thousands) are shown.

enzyme-linked immunosorbent assay (ELISA).[7] The latter two show high sensitivity and can be adapted to detect different antibody isotypes, which may be useful in acute infections. There is extensive cross-reactivity of sera with the different species of SFG rickettsias, and so IFA or ELISA cannot be reliably used to make a specific diagnosis. Recently, dried blood spots have been shown to be effective for serologic testing.[8]

Demonstration of rising titers in paired sera is more reliable than examination of a single specimen, although this is seldom of benefit since treatment must be initiated as early as possible. Western blotting may detect significant antibody titers one day earlier than IFA or ELISA, but patients who present soon after the onset of symptoms may have antibody titers below the threshold of detection by any test (Fig. 235.4). Kinetic studies show that IgM and IgG antibodies appear 3–10 days after the appearance of symptoms, and peak titers are reached after 3–4 weeks. Titers then decline slowly, and IgM and IgG are still detectable after 1 and 4 years, respectively. Administration of therapy within 2 days of the onset of symptoms may abrogate antibody production.

CLINICAL MANIFESTATIONS

The SFG rickettsioses are usually named after the geographic region in which they are detected (e.g. 'Rocky Mountain spotted fever', 'Mediterranean spotted fever', 'African tick bite fever', 'Indian tick typhus', etc.). After an incubation period of 4–10 days, common clinical features in Rocky Mountain spotted fever include:[9]

- an eschar or '*tache noire*' at the site of tick attachment;
- regional lymphadenopathy;
- fever (100.4–104°F (38–40°C));
- severe headache;
- chills;
- joint and muscle pain;
- malaise; and
- general weakness.

Within 3–4 days of the onset of fever, a maculopapular rash may develop on the trunk and extremities, frequently but not consistently including the soles and the palms. If untreated, the fever persists for 6–12 days and the rash may still be visible for up to 3 weeks. In African tick bite fever, and other SFG infections, pyrexia, headache and lymphadenopathy may be common but a rash is seen infrequently. [10]

Many organs may become involved, with hepatomegaly and splenomegaly and gastrointestinal symptoms such as vomiting, diarrhea and abdominal discomfort. Nephritis and renal failure may occur, and cardiac and circulatory abnormalities such as dysrhythmia, myocarditis, pericarditis, heart failure, deep venous thrombosis and embolism have been described. Pulmonary disorders such as pneumonitis and pleuritis and neurologic signs including impaired consciousness, seizures and vertigo may occur. [11]

Hematologic abnormalities include anemia and thrombocytopenia, with neutropenia in the acute phase of the disease and leukocytosis in the later stages. Biochemical changes include decreased levels of protein, particularly albumin, sodium, potassium and chloride during the first 10 days of the disease. Alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase are usually elevated. Creatinine phosphokinase and lactic dehydrogenase are also often raised in acute infection. Cytokine abnormalities may also be seen, with raised levels of macrophage colony stimulating factor, interleukin (IL)-1ß, IL-10, interferon-? and tumor necrosis factor-a.

Predisposing factors for the development of severe infections include initiation of treatment after the seventh day of illness, old age, diabetes, cardiac insufficiency, heavy smoking, alcoholism, chronic liver disease and glucose-6-phosphate dehydrogenase deficiency.

MANAGEMENT

Based on in-vitro susceptibility testing and in-vivo experience, doxycycline is the currently recommended drug of choice for the treatment of the SFG rickettsioses.[12] [13] The most commonly used regimen is 200mg daily for 2–5 days or until 24 hours after defervescence. Two single 200mg doses of doxycycline are also effective.

Ciprofloxacin has been shown to be at least as effective as doxycycline. Treatment for 5 days with the macrolide josamycin has been successful in children and pregnant women in whom quinolones are not considered safe. Other macrolides, such as clarithromycin, have been shown to have in-vitro activity against a number of rickettsias[14] and may be useful in treatment. Treatment failure has been reported with chloramphenicol. The aminoglycosides, ß lactams, erythromycin and trimethoprim-sulfamethoxazole are ineffective, and the latter is in fact contraindicated as it may stimulate bacterial growth and increase the severity of disease. Studies indicate there may be considerable heterogeneity in susceptibility to rifampin (rifampicin), and this may explain discrepancies in the outcome of treatment with this antibiotic.[12]

TYPHUS GROUP RICKETTSIAS

NATURE

Rickettsia typhi is the etiologic agent of endemic or murine typhus and *Rickettsia prowazekii* is the agent of epidemic typhus. They are both small, Gram-negative coccobacilli and, as with other members of the genus *Rickettsia*, their outer walls have an inner leaflet that is thicker than the outer. A polysaccharide slime layer may cover the outer leaflet, although its function is unknown and it is not associated with virulence.

There is considerable sharing of antigens by these two rickettsias and, while this gives cross-immunity, it creates problems in serologic surveys. The surface antigens include both high-molecular-weight proteins and low-molecular-weight LPS. The latter do not react with monoclonal antibodies to LPS of SFG rickettsia, suggesting structural differences. Combinations of immunofluorescence tests and Western blots can help to distinguish epidemic and endemic typhus in most cases. [15] Species specific epitopes occur on a 120kDa major surface protein, and monoclonal antibodies to these can distinguish *R. typhi* from *R. prowazekii*.

The complete genome sequence of *R. prowazekii* has been published, although less is known about *R. typhi*. [16]

EPIDEMIOLOGY

Endemic typhus, caused by *R. typhi*, is transmitted by the rat flea *Xenopsylla cheopsis* and is distributed throughout the world, but particularly in developing countries where there is close contact between humans and rodents. *Rickettsia prowazekii*, causing epidemic typhus, is transmitted in the feces of infected body lice, *Pediculus humanus* var. *corporis*, and occurs in epidemic foci where conditions are disrupted by poverty, famine, wars or natural disasters (Table 235.2). The historic association between epidemic typhus and war has been noted by many authors but, in recent years, the disease has been described mainly from disaster areas in Africa, such as Burundi,[17] and from outbreaks in Central and South America. Epidemic typhus occurs occasionally in developed countries and, while many such infections are probably imported, some appear to be locally acquired.[18] Older people, who may have had contact with epidemic typhus years before, may serve as a source of epidemics in areas in, for instance, eastern Europe, where conflict and political turmoil have disrupted normal living conditions.

TABLE 235-2 -- Comparison of features of endemic and epidemic typhus.

COMPARISON OF FEATURES OF ENDEMIC AND EPIDEMIC TYPHUS		
	Endemic typhus	**Epidemic typhus**
Causative agent	*Rickettsia typhi*	*Rickettsia prowazekii*
Vector	Rat flea	Human louse
Occurrence	Worldwide	Outbreaks associated with war, famine
Onset	Slow	Sudden
Eschar	Rare	Rare
Lymphadenopathy	Rare	Rare
Rash	Mascular, becoming papular	Macular, becoming petechial or necrotic
CNS symptoms	Rare, mild	Common, sereve
Duration	1–2 weeks	3–4 weeks
Mortality	<5%	Up to 60%
Recrudescence	Does not occur	Brill-Zinsser disease may occur
Diagnosis	Weil-Felix (OX 19), immunofluorescent assay, extensive cross-reactivity	

PATHOGENICITY

After multiplication at the injection site, there is hematogenous spread to endothelial cells of capillaries and small veins throughout the body. The rickettsias invade the cytoplasm of host cells but not the nucleus. Unlike SFG rickettsias, *R. prowazekii* grows to large numbers without causing significant injury until cell lysis. Polymerization of actin filaments is not a characteristic of typhus group infection, suggesting that these rickettsias do not migrate through the cytoplasm or move directly from one cell to another. Fibrin and platelet deposition in infected blood vessels leads to perivascular infiltration, and this may lead to local thrombosis, which affects many organs but particularly the heart, kidney and liver.

PREVENTION

Vector control with insecticides such as permethrin powder (1%) at doses of 30–50g for an adult and 15–25g for a child is the mainstay of protection. Insecticide should also be applied at 125–250mg/m^2 to kill lice in clothes and bedding. For endemic typhus both the normal murine host of the vector and the vector itself are the target of control measures. Insecticides can be used in buildings where rodents abound, and when the flea population has been reduced traps are used to limit the rodent population. Insecticide applied around the traps ensures control of fleas leaving the dead host. A recombinant 120kDa protein has been shown to confer protection against *R. prowazekii* in laboratory animals but there is little information on safety and efficacy in humans. Older vaccines, produced in yolk sacs, are protective but may induce non-specific reactions in recipients.

DIAGNOSTIC MICROBIOLOGY

A history of exposure to the vector is a crucial aid in the diagnosis of typhus. Isolated cases of epidemic typhus occur rarely,[19] [20] and usually there is a focus associated with war, famine or other disaster.[17] Organisms can be recovered from blood, using cell or animal cultures, and shell-vial assays using human embryonic lung fibroblasts may be useful. Organisms are highly infectious and safety facilities are essential.

Antibodies to typhus group rickettsias develop within 1–2 weeks of illness in the majority of patients. In the Weil-Felix test there is a rise in the agglutination titer with OX19 *Proteus vulgaris* antigen, but the low specificity of this test makes it unreliable. More sensitive and specific tests, such as IFA and ELISA, are available. There is extensive cross-reactivity between *R. typhi* and *R. prowazekii*, and infections can be distinguished by serology sometimes only with difficulty.[15] Cross-reactivity with some SFG rickettsial antigens may also occur, adding further to the difficulties of accurate serologic diagnosis. Detection of DNA sequences by PCR has been described[18] and has been used to detect *R. prowazekii* in cerebrospinal fluid.[19]

CLINICAL MANIFESTATIONS

The clinical features of endemic and epidemic typhus are similar, although the former is a milder disease. After an incubation period of 7–14 days, there is a high fever (>102°F (>39°C)), with headache, backache, chills, malaise and generalized pain. In epidemic typhus the onset is sudden but in endemic typhus signs of the infection develop over several days. Eschars, lymphadenopathy and splenomegaly are unusual features. A macular rash develops between day 5 and day 7 of the illness but it

may be absent in 50% of endemic typhus cases, especially in children. Unlike SFG rickettsial infections, the rash is more prominent on the trunk than on the limbs and rarely involves the face, palms or soles. In epidemic typhus, a pink macular rash usually spreads from axillary regions to the trunk and limbs.[20] The rash may become petechial or necrotic.

Invasion into the central nervous system is characteristic and confusion, coma, meningitis, delirium or manic symptoms develop toward the end of the first week, especially, although not exclusively, in epidemic typhus. Other features of infection include pulmonary (pneumonitis, bronchitis, bronchiolitis), cardiovascular (myocarditis, hypotension, tachycardia) and renal (oliguria) dysfunction. Similar but milder symptoms occur in endemic typhus.

If untreated, symptoms persist for 1–2 weeks (endemic typhus) or 3–4 weeks (epidemic typhus). In epidemic typhus the mortality can be over 40% but in endemic typhus mortality is low and is usually restricted to the elderly or immunocompromised. Recovery in epidemic typhus is slow, with convalescence lasting several months, while in endemic typhus defervescence and recovery occurs within 2–3 days of commencing therapy.

Recrudescent typhus (Brill-Zinsser disease) can occur years after patients show clinical recovery from epidemic typhus, usually at a time of immunodepression. Relapses present as a mild febrile illness, often without a rash, and diagnosis requires a history of previous rickettsial disease. The site and mechanism of survival of rickettsias within the body is not known, but they remain virulent and are transmissible to lice. Testing, contact tracing and the implementation of delousing programs may be needed to prevent outbreaks arising from recrudescent epidemic typhus.

MANAGEMENT

In-vitro antibiotic tests show both *R. typhi* and *R. prowazekii* are susceptible to tetracyclines, chloramphenicol and erythromycin. Resistance to erythromycin may emerge rapidly in vivo, and treatment failure has been reported in Brill-Zinsser disease with azithromycin. As with other rickettsial diseases, doxycycline is the recommended therapy in typhus infections, and quinolones may be suitable alternatives. Patients become afebrile within 3 days of starting treatment, but treatment should continue for 2–3 days longer to prevent relapse.

SCRUB TYPHUS

NATURE

Unlike true *Rickettsia* spp., the outer leaflet of the cell membrane of the agent of scrub typhus is thicker than the inner leaflet. This and other structural, immunologic and genotypic characteristics led to the reclassification of this agent as *Orientia tsutsugamushi*. [21]

Orientia tsutsugamushi is a weakly Gram-negative, obligate intracellular coccobacillus that can be cultured in fertilized eggs or in cell monolayers, where it forms plaques. In human cells, the bacteria are 1.2–1.6µm by 0.5–0.6µm, but in insect cells they may grow considerably longer (up to 4µm). Escape from the intracellular environment is via projections on the cell surface and is not necessarily related to cell damage.

Different strains can be recognized on the basis of serologic reactivities but they are not associated with a specific vector, geographic area or host species. Indeed, different serotypes of *O. tsutsugamushi* have been detected in the same host at the same time. An immunodominant 56kDa major outer membrane protein is found in all isolates, although there may be variation in amino acid sequences in this protein.

EPIDEMIOLOGY

Scrub typhus is a disease that occurs most frequently in South East Asia, but it has been reported from the Indian subcontinent, Australia and from Astrakhan in central Asia. Infections may be endemic or seasonal, depending to a large extent on the numbers of the vector, namely mites of the genus *Leptotrombidium*. These mites feed on vertebrates only in their larval stage, the nymphs and adults feeding on organic matter in the soil. The larva or 'chigger' takes only a single meal, and so can infect only one host. In the mite, rickettsias multiply in many organs, and are transmitted transovarially to the offspring. They do not, however, infect spermatogonal cells and so are not transmitted from male to female. Sex ratio distortions occur in some infected mite populations, with the almost complete absence of males. The mechanism for this is not known, but other related bacteria are associated with parthenogenesis and male-killing in insects.

In areas where ideal climatic conditions, vegetation and hosts are present, hyperendemic 'mite islands' may occur. In these areas transmission of scrub typhus is rapid and frequent.[22]

The usual vertebrate hosts for the mites are rats, mice and voles. In naturally infected rodents, rickettsemia may last several months. The significance of rickettsemia is unknown, however, since chiggers feed on tissue juices rather than blood, and the majority of mite infections are maintained by transovarian transmission rather than by infection from vertebrate reservoirs.

The recovery of viable *O. tsutsugamushi* from packed red cells has raised the possibility of transmission via blood transfusion from asymptomatic donors[23] and cell filtration or the use of psoralen photochemistry have been suggested as ways of preventing this.

PATHOGENESIS

Organisms are introduced into the skin by the bite of the mite, and adherence to cell surfaces is mediated by specific polypeptides of about 54–56kDa. Monoclonal antibodies directed against these can differentiate serotypes that may show differing degrees of virulence. *Orientia* induce host cell phagocytosis in order to enter cells and then escape to the cytoplasm, through which they migrate using microtubules.[24] Endothelial cells are the main targets of *O. tsutsugamushi*, and cell culture studies indicate that they induce apoptosis.[25]

Multiplication occurs in the endothelial cells of many organs, and organisms have been recovered from the heart, lung, brain, kidney, pancreas and skin. Spread is via the lymphatics and blood stream and, while initially regional, lymphadenopathy quickly becomes generalized. Proinflammatory cytokines such as IL-6 and IL-8 may be stimulated during infection.

PREVENTION

No vaccines are available for scrub typhus, and prevention is by use of insect repellents such as DEET (diethyl toluamide) or DEPA (diethyl phenyl acetamide) to reduce the risk of being bitten, and both of these compounds can be used on clothing. Rapid removal of mites is recommended since they do not feed until several hours after attachment.

DIAGNOSTIC MICROBIOLOGY

Scrub typhus should be suspected in patients from endemic areas who present with high fever and lymphadenopathy. Hematologic signs — a neutropenia and/or mononucleosis — are not specific, and most infections are diagnosed by serology. The Weil-Felix test detects cross-reacting antibodies that agglutinate the OXK strain of *Proteus*, probably through common epitopes on membrane LPS molecules. The test is rapid and simple but unreliable, with a sensitivity of less than 50%. The IFA is more specific but requires at least four antigenically distinct strains of *O. tsutsugamushi*, because responses may be serotype-specific.[26] Hemagglutination using recombinant 56kDa protein has been found to be sensitive and specific, and rapid testing for IgM and IgG antibodies by an immunochromatography flow assay has been described.[27] A PCR assay uses primers derived from the gene coding for the 56kDa protein, and can be used to detect bacteria in blood with high sensitivity.[23] [28]

CLINICAL MANIFESTATIONS

The presentation of scrub typhus depends on features of both host and pathogen and can vary from a mild, inapparent infection to a fulminant, rapidly fatal disease. After the bite of an infected mite, incubation takes 1–3 weeks. A vesicular lesion forms at the site of the bite in about 75% cases, particularly in visitors to infective areas, and develops into a necrotic eschar. Systemic disease is indicated by a sudden onset high fever (>102°F (>39°C)), with headache, myalgia and regional and generalized lymphadenopathy, the latter particularly in patients who do not develop an eschar. A maculopapular rash appears about 5–6 days after onset in up to 80% cases in some studies.[29] Purpuric or hemorrhagic lesions are rare and transient. In the absence of treatment, defervescence starts by the end of week 3.

Neurologic signs such as lethargy, asthenia, confusion and delirium may occur. Respiratory symptoms are reported frequently, and radiologic abnormalities may be common.[29] Retinal vein occlusion with retinal hemorrhage, gastrointestinal vasculitis, pericarditis, myocarditis, acute renal failure and encephalomyelitis have all been reported as serious complications of scrub typhus.

Recently it was shown that there was a significant reduction in HIV-1 viral load during acute *Orientia* infection. [30] Moreover, sera from HIV-1 negative patients who had scrub typhus had potent HIV-1 suppressive effects in vitro, indicating the release of HIV-1 suppressive agents during scrub typhus infection. These findings are under further investigation.

MANAGEMENT

As with other rickettsial infections, tetracycline antibiotics are the drugs of choice in treatment. With doxycycline defervescence usually occurs within 24 hours. Resistance may occur in some areas, such as northern Thailand. Rifampin or azithromycin may be useful alternatives, and chloramphenicol may be effective.

HUMAN EHRLICHIOSIS

NATURE

The tribe Ehrlichiae was originally established to include veterinary pathogens such as *Anaplasma marginale, Cowdria ruminantium, Ehrlichia canis* and *Ehrlichia phagocytophila.*. The first human infection with an *Ehrlichia* species, thought to be *E. canis*, was described in 1987 and, shortly after this, retrospective examination of sera from patients who had suspected SFG rickettsioses demonstrated many who had antibodies reactive with *E. canis* antigen. Prospective studies showed that human ehrlichiosis occurred frequently in some parts of the USA.[31] The application of tissue culture techniques led to the isolation of an *Ehrlichia* species from the blood of a febrile soldier at Fort Chaffee, Arkansas and its description as a new species, *Ehrlichia chaffeensis*.[32] The two species, *E. canis* and *E. chaffeensis*, share a number of antigens, and it is likely that at least some reports of *E. canis* infection in humans may be serologic cross-reactivity.

Sequence analysis of ehrlichial 16S rRNA and other genes suggests that there are three related genogroups: the *Ehrlichia*, the *Anaplasma* and the *Neorickettsia* (Table 235.3). Changes in nomenclature have been proposed to unify members of each of the genogroups into a single genus.[33] The agent of human granulocytic ehrlichiosis (HGE) is so similar to *E. phagocytophila* and *Ehrlichia equi* that all three are included in a single species, *Anaplasma phagocytophila*. It remains to be seen if this proposed classification becomes widely accepted.

It is now recognized that there are two etiologically and epidemiologically distinct forms of disease: human monocytic ehrlichiosis (HME) and HGE. *Ehrlichia chaffeensis* is the main agent of HME. While the other species that invade monocytes, such as *E. canis*, may be human pathogens, their role in HME is at best a minor one. The agent of HGE is a member of the *E. phagocytophila* group, and both this agent and *Ehrlichia ewingii* invade granulocytes.[34] Serologic evidence from Japan suggests that *Ehrlichia muris* may also occasionally infect humans, but no isolates have been made.

The characteristic structures of ehrlichias, visible by light microscopy, are the morulas that lie within vacuoles in host cells (Fig. 235.5). Electron microscopy has shown the morulas to be aggregates of bacteria with two distinct morphologic forms. Larger (1µm in diameter) reticulate forms have evenly dispersed nucleoid filaments and ribosomes, while smaller forms (0.6µm in diameter) have filaments and ribosomes that are condensed in a central mass. These small forms are often actively dividing by binary fission. The cell wall of members of the genus *Ehrlichia* has outer and inner leaflets of equal thickness.

EPIDEMIOLOGY

Both HME and HGE have been detected in clinical samples from the USA and Europe. While there is serologic evidence of these infections in Africa, South America and Israel, the organisms are closely related and cross-reactions occur within and between groups. Isolation of organisms and/or molecular identification of pathogens will be needed to confirm the distribution of these two infections. It is probable that many infections go unrecognized because of the lack of specific clinical features and the difficulty of diagnosis. A prospective surveillance study of hospitalized febrile patients in Georgia showed that the incidence of human ehrlichiosis was six times that of Rocky Mountain spotted fever.[31]

TABLE 235-3 -- Genogroups of ehrlichias.

GENOGROUPS OF EHRLICHIAS				
	Human infection	**Animal infection**	**Vector**	**Host cell**
Group I: ***Ehrlichia* group**				
Ehrlichia chaffeensis	Yes	Deer	*Amblyomma*	Monocyte/macrophage
Ehrlichia canis	Possibly	Dogs	*Rhipicephalus*	Monocyte/macrophage
Ehrlichia ewingii	Yes	Dogs	*Amblyomma*	Neutrophil
Ehrlichia muris	Possibly	Vole	?	Monocyte/macrophage
Ehrlichia ruminantium	No	Cattle, sheep, goats	*Amblyomma*	Endothelium
Group II: ***Anaplasma* group**				
Anaplasma bovis	No	Cattle	*Boophilus*	Erythrocyte
Anaplasma platys	No	Dogs	*Rhipicephalus*	Neutrophil
Anaplasma phagocytophila (incl. *Ehrlichia phagocytophila, E. equi* and 'HGE' agent)	Yes	Horses, cattle, sheep, deer	*Ixodes*	Neutrophil
Group III: ***Neorickettsia* group**				
Neorickettsia helminthoeca	No	Dogs	Fish	Macrophage
Neorickettsia sennetsu	Yes	No	Possibly fish	Monocyte/macrophage
Neorickettsia risticii	No	Horses	Trematode larvae	Monocyte/enterocyte

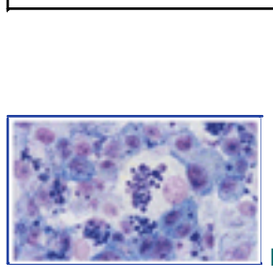

Figure 235-5 Multiple morulas of *Ehrlichia canis* in culture DH82 cells.

Surveys of tick populations using monoclonal antibodies or PCR have shown *Amblyomma americanum*, the 'lone star tick', to be the main vector of *E. chaffeensis* in the USA. The vectors of HGE are *Ixodes* ticks in both the USA and Europe. In some areas, both HGE and *Borrelia burgdorferi* may be found in the same tick at the same time.[35] Reservoirs of infection are found in many vertebrates, including deer, dogs and rodents. Ticks probably become infected in their larval and nymph stages, and the infection can be transmitted transstadially, so that both nymphs and adults can transmit the infection. There is no evidence for transovarian transmission of ehrlichias.

PATHOGENICITY

Both *E. chaffeensis* and the HGE agent are introduced into the skin by the bite of a tick. From there the organisms spread hematogenously to their respective target cells. *Ehrlichia chaffeensis* is taken into the phagosomes of macrophages and monocytes in the liver, spleen and lymph glands. Human granulocytic ehrlichiosis organisms are thought to primarily infect granulocyte precursor cells in the bone marrow rather than to invade mature peripheral neutrophils. The agent of HGE does, however, stimulate IL-8 production, inducing neutrophil migration to sites of infection.

Animal studies show that ehrlichiosis has an impact on immune function, with macrophage necrosis, inhibition of phagocytosis and killing, downregulation of

immunoglobulin production, and decreased lymphocytic responses to mitogens.[36] An immunopathologic basis for HGE has also been suggested in mouse models of infection. [37] Patients who are infected with *E. chaffeensis* show a lymphocytopenia with an abnormal predominance of ?dT cells in the peripheral circulation[38] and neutrophils infected with HGE show inhibited superoxide anion generation.[39] The mechanisms of these immunologic abnormalities and their clinical significance is not well understood, but autopsy studies of patients who had HGE have shown that many patients had opportunistic lung infections, suggesting that impaired host defenses are a primary feature of human ehrlichiosis.

PREVENTION

There are no vaccines for ehrlichiosis and prevention is based on avoiding areas where ticks are common or the use of tick repellents. Tick control programs in wild and domestic animals may be beneficial in reducing the risk of exposure. Daily removal of ticks may not be effective in preventing infection as transmission may occur within 24 hours of tick attachment.[40]

DIAGNOSTIC MICROBIOLOGY

Ehrlichiosis should be included in the differential diagnosis of patients who have acute febrile illness where there has been a history of exposure to ticks. Hematologic abnormalities, particularly a leukopenia and thrombocytopenia, would be consistent with this diagnosis but there are no pathognomonic signs. The presence of arthralgias and/or an elevated C-reactive protein may help differentiate ehrlichiosis from other infections such as tick-borne encephalitis. Careful examination of peripheral blood or buffy coat smears may show morulas in macrophages or neutrophils but in most cases such examinations are fruitless. The majority of cases of human ehrlichiosis have been diagnosed on the basis of serology, using IFA or ELISA, or detection of specific DNA sequences. Using IFA with *E. chaffeensis* antigen, antibody titers rise during the third week of illness, peak and then decline rapidly about a year later. Serology has been negative,

however, in a number of patients confirmed to have ehrlichiosis using PCR, and the sensitivity of IFA may be low. Cell-free recombinant antigens of *E. canis* have been described, and may become useful in serologic tests for HME.[41] Serologic tests for HGE using *E. equi* infected horse neutrophils or cultured human isolates also detect antibodies within 2 weeks after onset of symptoms, and peak titers are reached during the first month. Antibodies may still be detected in about 50% of patients after 1 year.[42] The *E. phagocytophila* group is, however, characterized by antigenic diversity, and this may lead to interlaboratory differences in interpretation of serology assays. Recombinant antigens, derived from *E. phagocytophila* or HGE isolates, have been used to develop rapid serologic assays, and these may give more consistent results. [43] Specific sequences for PCR, using primers from the 16S rRNA gene sequences, have been described for both *E. chaffeensis* and HGE, but diagnostic procedures continue to be revised. [44]

CLINICAL MANIFESTATIONS

Studies of seroconverters suggest that two-thirds of human ehrlichial infections are asymptomatic. In symptomatic cases, there is a moderate to severe illness, with the majority requiring hospitalization. There are no clinically distinct diagnostic features but fever, headache, myalgia, anorexia, vomiting and nausea are common. Skin rash, cough, pharyngitis, diarrhea, abdominal pain and confusion are reported from less than 25% of patients. Involvement of the central nervous system may lead to seizures and coma, and myocardial damage has been recorded. The symptoms are similar in both human monocytic and granulocytic ehrlichiosis, although rash is very rare in the latter. The mortality is probably less than 5% in HME, but may be higher in HGE.[45] In untreated patients, symptoms persist for 3–11 weeks before resolving, leaving a long lasting immunity to further infection.

Both leukopenia and thrombocytopenia are laboratory common findings, with an increase in band neutrophil counts in early infection. A reduced lymphocyte count may be the most significant hematologic finding in HGE culture positive cases. [46] A mildly elevated hepatic transaminase activity may occur.

MANAGEMENT

In-vitro studies have shown that *E. chaffeensis* is susceptible to doxycycline and rifampin but is resistant to quinolones, aminoglycosides, erythromycin and trimethoprim-sulfamethoxazole. Studies with *E. phagocytophila* show a similar picture, except that the organisms are

Figure 235-6 Colonies of *Bartonella henselae* on blood agar. *Courtesy of R. Birtles.*

susceptible to quinolones.[47] Administration of doxycycline leads to defervescence in 2–3 days in over 90% of patients and so this is the antibiotic of choice. With other antibiotics, the fever may persist for up to 7 days. Persistence of viable ehrlichias after treatment is known to occur in animal infections, even after 6 weeks of treatment, and has been reported from at least one human case. The significance of persistent ehrlichiosis in humans is not known. Rifampin has been successfully used to treat HGE in a pregnant woman.

BARTONELLOSIS

NATURE

The *Bartonella* are Gram-negative rods that belong in the a_2 subgroup of the class Proteobacteria. [48] They can be grown in tissue culture and also on cell-free media containing blood. Identification of *Bartonella* is based on genomic analysis, as the organisms are biochemically inert.

There are now 19 species in the genus, each with a mammalian reservoir host and each probably transmitted by an arthropod vector.[49] The principal human pathogens are *Bartonella quintana*, *Bartonella henselae* (Fig. 235.6) and *Bartonella bacilliformis*. Six other species have been implicated in human disease: *Bartonella elizabethae* and *Bartonella vinsonii* subsp. *berkhoffii* have been found to cause endocarditis, *B. vinsonii* subsp. *arupensis* was isolated from a febrile patient who had heart valve disease, *Bartonella grahamii* has been implicated in cases of neuroretinitis, *Bartonella clarridgeiae* has been implicated in cat scratch disease and *B. washoensis* has been identified as a cause of myocarditis (Table 235.4).

EPIDEMIOLOGY

Infections with *B. bacilliformis* have been reported only from valleys in the Andean regions of Peru and Ecuador, where sandfly vectors of the genus *Lutzomyia* occur. Infections with *B. quintana* and *B. henselae* have been reported from North America, Europe, the Middle East, Africa and Asia, and so are presumed to occur worldwide. Both the body louse *P. humanus* var. *corporis* and the head louse *P. humanus* var. *capitis* are experimentally capable of transmitting *B. quintana*, but epidemiologic evidence is that natural transmission is effected only by the body louse. In the louse, the organism is extracellular in the gut lumen and causes no pathology. They are, however, excreted in large numbers in louse feces. Humans are the only known natural animal host of *B. quintana*, and even under experimental conditions primates are the only susceptible animals. This contrasts with *B. henselae*, which has frequently been isolated from domestic cats. Cats can transmit the infection to humans in bites and scratches, and the presence of *B. henselae* in cat fleas indicates that these may also be a source of human infection. *Ixodes* ticks infected with *Bartonella* closely related to human pathogens have been described, and some infections may be transmitted this way.[50]

PATHOGENICITY

Microscopic examination of tissue infected with *Bartonella* frequently shows tumor-like capillary lobules indicating neovascular proliferation, and these lesions regress during antibiotic therapy. Experimental studies show that contact with a number of *Bartonella* species enhances proliferation of endothelial cell lines.[51] These observations suggest that *Bartonella* directly stimulates angiogenesis, possibly through secretion of one or more angiogenic factors, and there is some evidence that production of these is mediated through extrachromosomal elements. [52]

TABLE 235-4 -- *Bartonella* spp. associated with human disease.

***BARTONELLA* SPP. ASSOCIATED WITH HUMAN DISEASE**			
***Bartonella* sp.**	**Disease**	**Vector**	**Reservoir**
B. bacilliformis	Oroya fever	*Lutzomyia*	Human
	Verruga peruana		
B. quintana	Trench fever	*Pediculus humanus*	Human
	Bacteremia		
	Endocarditis		
	Bacillary angiomatosis		
B. henselae	Cat scratch disease	Cat flea	Cats
	Endocarditis		
	Bacteremia		
	Bacillary angiomatosis		
	Juvenile arthritis		
B. vinsonii subsp. *berkhoffii*	Endocarditis	Fleas and ticks	Dogs
B. vinsonii subsp. *arupensis*	Endocarditis	Not known	Rodents
B. elizabethae	Endocarditis	Fleas	Rodents
B. clarridgeiae	Cat scratch disease	Cat flea	Cats
B. grahamii	Neuroretinitis	Not known	Rodents
B. washoensis	Myocarditis	Not known	Rodents
Other species (e.g. *B. vinsonii* subsp. *vinsonii*, *B. tribocorum*, *B. weissii*, *B. alsatica*, *B. taylorii*, *B. doshiae*, *B. schoenbuchii*, *B. capreoli* and *B. koehlerae*) may be found in rodents, rabbits and ruminants but have not so far been associated with human infections.			

PREVENTION

Control of the arthropod vectors and avoidance of close contact with cats and cat fleas are the only preventive measures available.

DIAGNOSTIC MICROBIOLOGY

Recovery of *Bartonella* from blood requires prolonged incubation using highly enriched media and/or cultivation in endothelial cell lines. The timing of blood sampling may be important since the numbers of bacteria in the blood stream varies. Primary isolation may require a month or more of culture in a humid (80%), CO_2 -enriched (5%) atmosphere using media containing horse or rabbit blood. The addition of fetal calf serum and maintenance of hemin concentration within the range 50–250µg/ml improves growth. For *B. bacilliformis* an incubation temperature of 82.4°F (28°C) is preferred.

Primary isolates produce rough colonies deeply embedded in the agar. On subculture, growth is more rapid, with smooth, shiny colonies developing in 5 days or less. Organisms will also grow in liquid media, and acridine orange staining of culture samples is useful because of the slow growth and because little or no CO_2 is produced. Suspect isolates can be presumptively identified on the basis of slow growth, Gram stain appearance and lack of biochemical activity. Specific identification of isolates is usually based on molecular analysis[52] [53] or serologic typing.[54]

Serologic diagnosis can be made using hemagglutination, IFA and ELISA. There is little cross-reactivity with other rickettsias, but cross-reactions with *Coxiella* and *Chlamydia* spp. have been described. Removing LPS from antigen preparations improves the specificity. Serology is particularly useful in endocarditis, where culture may take several weeks.[55] Cross-reacting protein antigens are common to different species of *Bartonella*, and serologic tests cannot reliably distinguish between *B.*

quintana and *B. henselae* infections. Polyvalent murine antisera and monoclonal antibodies can, however, be used to differentiate *Bartonella* species isolated in the laboratory[56] .

Polymerase chain reaction assays, based on primers for the 16S rRNA gene, can be used to detect *Bartonella* in clinical specimens.

CLINICAL MANIFESTATIONS

A number of different clinical conditions have been associated with *Bartonella* infections.

Trench fever

A relapsing fever accompanied by severe pain in the legs became prominent as a disease among troops serving in the trenches during the First World War and the etiologic agent is now named *B. quintana*. Infection occurs by penetration of organisms in excreta of body lice into skin broken from scratching as a result of intense pruritus. The incubation period is usually 2–3 weeks, and infection leads to sudden onset of fever, retro-orbital headache, weakness, pain in the legs, shivering, intestinal disturbance and insomnia. The severity of symptoms increases over the first 2–3 days. There may be neck stiffness and the disease may resemble meningitis. Intense pain in the tibia is characteristic, and is exacerbated by cold and damp conditions. Pyrexia is often periodic, recurring about every 5 days (hence the name *quintana*), with each attack being less severe than the previous one. Laboratory findings are non-specific, with a polymorphonuclear leukocytosis, anemia and disturbance of liver function. The disease is usually not fatal, although it may persist for 4–6 weeks and is very debilitating. More recently, trench fever has been described in refugees in Burundi,[17] and also as so-called 'urban trench fever' among louse-infested homeless persons.[57]

Bacteremia and endocarditis

In a study of homeless, chronic alcoholics, bacteremia with *B. quintana* persisted for more than 10 days in 4/10 patients, and untreated bacteremia persisted for over 8 weeks.[58] Prolonged bacteremia with *B. henselae* is also common and, in HIV-infected patients, this may be asymptomatic or be associated with an insidiously developing, prolonged symptom complex of malaise, fatigue, weight loss and recurring fevers. Both *B. henselae* and, more frequently, *B. quintana* have been associated with endocarditis,[59] with extensive damage to heart valves, fibrosis and calcification suggestive of prolonged infection. Failure to detect more common pathogens from the blood of endocarditis patients should alert the physician to the possibility of *Bartonella*-induced endocarditis.

Cat scratch disease

Cat scrath disease, caused by *B. henselae* and perhaps other *Bartonella* spp., is now considered to be the most common cause of chronic, benign lymphadenopathy in children and young adults. Epidemiologic evidence suggests that transmission is through cat scratches, bites or contact with cat fleas and studies in the USA have shown that up to 40% of domestic cats have an asymptomatic *B. henselae* bacteremia, which may persist for more than 1 year. In humans the initial lesion develops as a nonpruritic vesicle, papule or pustule, usually on the head, neck or arms, 3–10 days after a scratch or bite. The lesion heals without scar formation within a week. Regional lymphadenopathy may persist for 2–4 months before resolving spontaneously. Transient mild systemic symptoms include fever, malaise, fatigue, headache, anorexia and weight loss. Atypical manifestations include conjunctivitis with pre-auricular lymphadenopathy, hemolytic anemia, hepatosplenomegaly, glomerulonephritis, pleural effusion and neurologic abnormalities and severe chest and pulmonary diseases have been reported.[60]

Arteriolar proliferation and widening of the arteriolar walls, reticulum cell hyperplasia, multiple microabscesses, frank abscess formation and tubercle-like granulomas may be seen on histology of affected lymph nodes. Organisms can be seen using Warthin-Starry stain in clumps or as filaments in the walls of vessels and epithelial cells and free in necrotic debris. Dissemination of organisms can result in the formation of granulomas, identical to those described in lymph nodes, in bone, liver, mesenteric lymph nodes and/or spleen.

Bacillary angiomatosis

Bacillary angiomatosis and epithelioid angiomatosis are vascular proliferative diseases that usually, but not exclusively, involve the skin and are associated with immunodeficiency (Fig. 235.7). Both *B. quintana* and *B. henselae* have been detected in patients in Europe and North America. A similar condition, verruga peruana, is caused by *B. bacilliformis*, but has a limited geographic distribution. Cutaneous lesions may be single or multiple red or purple papules that increase in size and become nodular and bleed profusely when punctured. They may be superficial, dermal or subcutaneous and sometimes involve the oral, anal, conjunctival or intestinal mucosa. While they may resemble lesions of Kaposi's sarcoma, there is no evidence that herpesvirus is involved in the vascular proliferation.[61] Other organs that may be affected include bone marrow, spleen, liver and lymph nodes. Severely immunocompromised patients, particularly those who have HIV, may develop peliosis hepatitis, characterized by cystic,

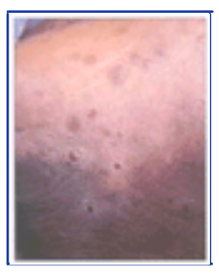

Figure 235-7 Skin lesions of bacillary angiomatosis. *Courtesy of P. Kelly.*

blood-filled spaces in the hepatic parenchyma. Histology shows masses of bacteria in dense clumps of granular material.

Concurrent lymphadenopathy is more frequent in *B. henselae* infected patients, while concurrent central nervous system disorders are more frequent in *B. quintana* infected patients. [62] [63] The main risk factor in *B. henselae* bacillary angiomatosis patients is contact with cats and fleas, while homelessness, poor socio-economic status and contact with lice are the main risk factors in *B. quintana* patients.

Carrion's disease

Carrion's disease, caused by the intra-erythrocytic organism *B. bacilliformis*, was first described in 1907. The disease is restricted geographically to the Andean regions of Peru and Ecuador, and occurs in two forms: a febrile anemia ('Oroya fever') and an angioprolific cutaneous disease ('verruga peruana') that resembles bacillary angiomatosis.

MANAGEMENT

Most *Bartonella* isolates are susceptible in vitro to ß-lactams (except oxacillin and cephalothin), rifampin, chloramphenicol, macrolides and tetracyclines but susceptibility to clindamycin, quinolones and trimethoprim-sulfamethoxazole is more variable. Clinical trial data are scarce, but most report a poor relationship between in-vitro susceptibility and in-vivo efficacy. Recommendations on treatment depend to a larger extent on clinical presentation rather than on etiology. In immunocompetent patients who have cat scrath disease there is no evidence that antibiotic therapy reduces the duration of symptoms. The use of antibiotics in immunosuppressed patients, however, is associated with rapid clinical improvement. *Bartonella quintana* bacteremia has been reported to respond well to doxycycline, ceftriaxone and macrolides.[57] The majority of patients who have bacillary angiomatosis respond initially to amoxicillin, aminoglycosides or trimethoprim-sulfamethoxazole but relapses occur frequently. The efficacy of tetracyclines and erythromycin appears to depend on the duration of therapy, with fewer relapses occurring in patients treated for more than 1 month. There are few data on treatment of *Bartonella* endocarditis but complex regimens using combinations of ß-lactams, aminoglycosides and rifampin have been used.[57] Valve replacement is almost always essential because of the extent of damage to the valves by the time a diagnosis is made.

Q FEVER

NATURE

'Query' or Q fever was first recognized as a disease of humans in abattoir workers in Queensland, Australia in the 1930s and it is now known to occur worldwide. The causative agent, *Coxiella burnetii*, is an obligate intracellular organism that requires the acidic environment within the phagolysosome of eukaryotic cells for its metabolism. While originally included within the Rickettsiaceae, *C. burnetii* has always been regarded as an unusual member of this family because of unique features of its life cycle and epidemiology. It is more closely related to organisms such as *Legionella* and *Francisella* than to *Rickettsia* spp.

Morphologically, *C. burnetii* is a Gram-negative, intracellular, pleomorphic coccobacillus 0.2–1.0µm in diameter. The organism may occur as a small-cell variant (SCV) or large-cell variant (LCV). The SCV is a compact, small rod with a very electron-dense center of condensed nucleoid filaments. The LCV is larger and less electrondense, and has a clear, periplasmic space between the outer and cytoplasmic membranes. A dense, endospore-like body 130–170nm in diameter might be found at one pole of some LCV. The SCV are

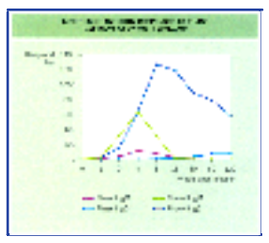

Figure 235-8 Kinetics of antibody responses to phase variants of *Coxiella burnetii*.

metabolically inactive and are the extracellular form of the organism. Once within a phagolysosome, the SCV is activated by the acidic environment and forms an LCV. The LCV is the metabolically active intracellular form of the bacterium, and undergoes sporogenic differentiation to produce resistant, spore-like forms of the bacterium. These develop to SCVs, which are released when the cells lyse.

In addition to this morphologic change, there is also phase variation, which is associated with changes in the LPS of the outer membrane. Phase I organisms can be readily isolated from acutely infected animals, and remain virulent, while phase II organisms, obtained after serial passage in eggs or tissue culture, are of low virulence. Phase II organisms revert to phase I following passage in a vertebrate host. There are no distinct morphologic changes associated with this phase variation, and SDS-PAGE studies show only minor changes in immunogenic protein profiles (Fig. 235.8).

EPIDEMIOLOGY

With the notable exception of New Zealand, *C. burnetii* infections occur worldwide. The ability of *C. burnetii* to survive extreme environmental conditions for many years, and the low infective dose, results in ready transmission of infection, probably in most cases by inhalation of infectious aerosol particles. While cattle, sheep and goats are the most important animal reservoirs, *C. burnetii* has also been detected in fish, birds, rodents, marsupials, snakes, tortoises and many domestic animals, and has been recovered from ticks and other arthropods. Naturally infected cattle, sheep or goats carry large numbers of bacteria in blood and tissues, and they excrete viable organisms in milk. In most cases these animal infections are asymptomatic. While abortion occurs only rarely, the placenta of an infected animal frequently contains large numbers of organisms. It is contamination of the environment from these sources that usually precedes transmission to humans.

Because the minimum infective dose is a single viable organism, [64] exposure need only be minimal. Most human cases of Q fever occur sporadically but outbreaks occur with high-risk occupations (farmers, hunters, workers in meat or milk processing plants, slaughterhouses, veterinary schools, etc.). Outbreaks have also been reported in such disparate groups such as inhabitants of a village through which infected sheep were herded, golf players on a course previously used as a sheep pasture, military personnel coming into contact with infected hay and poker players exposed to a parturient cat. Extracellular *C. burnetii* are extremely resistant to desiccation, low or high pH, disinfectants and ultraviolet light, and may remain infective in aerosols for up to 2 weeks and in the soil for as long as 5 months. Amebae may also be infected with *C. burnetii* and may be another reservoir for contamination of the environment. [65] Ticks are probably important only in maintaining infections in small rodents and lagomorphs. There is little evidence for direct tick transmission to humans, but organisms may be excreted in large numbers in tick feces, leading to environmental contamination.

In addition to inhalation of viable *C. burnetii* in aerosols, infection may also occur through ingestion of infected milk and meat products. Most such infections result in seroconversion without symptoms, however. *Coxiella burnetii* has been isolated from the human placenta and breast milk, and transmission may also be from mother to child. Sexual transmission of Q fever has recently been reported.[66]

PATHOGENICITY

Monocytes and macrophages are target cells for *C. burnetii*, and alveolar macrophages are the usual primary target. Attachment to the host cell is followed by passive entry into the phagolysosome, where production of a potent acid phosphatase protects the organism from enzyme attack.[67] Disturbance of cytokine regulatory processes, allowing survival and replication of *C. burnetii* within host cells, is also important in establishing infection.[68]

In animals, the great majority of infections produce no obvious pathology, apart from inflammation in the uterus and placenta, where there may be massive replication of *C. burnetii* during the final stages of pregnancy.

Monoclonal antibodies react specifically with epitopes on isolates associated with acute (Nine Mile strain) and chronic (Priscilla strain) infections. Plasmids that code for surface proteins may also be associated with virulence. For example the plasmid QpHI is found in isolates from acute infection, while the plasmid QpRS is found in isolates from patients who have endocarditis. Other studies suggest, however, that differences in host response are more important that genomic variation in determining the clinical outcome of infection.

PREVENTION

The extent to which Q fever organisms contaminate the environment, the ability of these organisms to survive harsh conditions and the low infective dose means that prevention through the use of environmental measures is difficult. Some measures, such as the avoidance of unpasteurized dairy products, may prevent some forms of transmission. Q fever can be prevented in animals using vaccines prepared from extracted subunits of phase I cells, and vaccines may also be useful for people at high risk when they can be identified.[69] Vaccines prepared from phase II organisms have been shown to prevent abortion in animals, but vaccinated animals may still transmit infection to humans.

DIAGNOSTIC MICROBIOLOGY

The suspicion of acute Q fever is not dependent on a specific history of exposure to animals, since organisms persist in the environment for many years. Symptoms are not pathognomonic and may readily be confused with influenza or with other rickettsial diseases. Because organisms are highly infectious, all specimens from patients who have suspected Q fever should be handled with extreme care. *Coxiella* can be recovered from blood, urine and other body fluids during acute infection, using mice or vero cell lines, although only laboratories with adequate safety facilities should attempt this. The shell vial assay may

improve recovery of organisms from patients who have endocarditis. A PCR assay, using primers derived from the *htpAB*-associated sequence, has been used to detect organisms in a variety of specimens, including heart valves and milk.[70] *Coxiella burnetii* plasmid DNA has also been identified in human serum specimens by PCR.[71]

Serologic diagnosis is more usual, and is especially useful in areas of high endemicity. While complement fixation and IFA have been used, ELISA is useful for epidemiologic screening and as a diagnostic test. The stage of the infection may be distinguished by using isotype-specific tests, and phase specific antigens. Immunoglobulin M antibodies reactive with phase II *C. burnetii* appear rapidly, reach high titers within 14 days and persist for 10–12 weeks. Immunoglobulin M antibodies reactive with phase I antigens are usually at a much lower titer during acute infection. Immunoglobulibn G antibodies reactive with phase II antigens reach peak titers about 8 weeks after the onset of symptoms, while those reactive with phase I antigens develop only very slowly and remain at lower titers than antibodies to phase II antigens, even after a year. In chronic Q fever, where there is persistence of organisms, the IgG titers to phase I and phase II antigens may both be high, and the presence of IgA antibody to phase I antigen is usually, although not exclusively, associated with chronic infection. Thus elevated levels of IgG (>1/200) and IgM (>1/25) to phase II but not phase I antigens indicates acute infection, while high titers of IgG (1/800) and IgA (>1/50) to phase I antigen is more predictive of chronic infection. An analysis of three isotype-specific assays (IgG, IgM and IgA) is therefore more useful than total antibody assays in laboratory investigations of suspected Q fever.[72] Moreover, patients who have increased levels of IgA2, rather than IgA1, to phase II antigens appear to be at high risk of developing endocarditis.[73]

CLINICAL MANIFESTATIONS

The incubation period depends on the route of exposure, the inoculum dose and the age of the patient, but is usually about 3–4 weeks. A variety of clinical manifestations may be recognized. The most frequent of these are mild fever (>99.5°F (>37.5°C)), headache, chills, sweating, cough, nausea and bradycardia relative to body temperature[69] . A maculopapular rash develops in about 20% of acute infections. The fever usually subsides gradually during week 1, with recovery by week 3 of the illness. There is an inverse relationship between severity of disease and age, and infections in children may go unnoticed. Other frequently occurring presentations include pneumonia and hepatitis, and the prevalence of these may vary geographically. Possible reasons for this geographical variation include strain characteristics, the source, the route and the dose of infection and a variety of host factors.

Clinical recovery usually occurs by the third week but *C. burnetii* organisms may persist in the tissues much longer, and can be recovered from tissues years or even decades after primary infection. A post-Q-fever fatigue syndrome, associated with cytokine dysregulation and presenting with fatigue, myalgia, arthralgia, night sweats, mood change and sleep disturbance, may occur in up to 20% of patients. Chronic Q fever may result in osteomyelitis or encephalitis but there is a high risk of endocarditis, particularly in patients who have pre-existing valvular disease. Q fever endocarditis has a poor prognosis despite therapy. Myocarditis, splenic rupture, meningoencephalitis and pericarditis are all rare manifestation of acute Q fever.[74]

Management

In-vitro studies have shown that rifampin, doxycycline and oxytetracycline and quinolones inhibit the growth of *C. burnetii*, although within the acidic environment of the phagolysosome other agents such as ceftriaxone and fusidic acid may be more effective. Prompt treatment of acute Q fever with doxycycline or tetracycline reduces the duration of fever, but it is not known whether this correlates with elimination of organisms. Combinations of doxycycline with either hydroxychloroquine, or ofloxacin taken daily for 18–36 months has been recommended for chronic infections and may prevent development of endocarditis. [75] The treatment of Q fever endocarditis is problematic. Prolonged regimens that include doxycycline and either rifampicin, trimethoprim-sulfamethoxazole or lincomycin have been reported to be effective but organisms can still be demonstrated in tissues months or even years later.

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Chapter 236 - Chlamydia

Pekka Al Saikku

INTRODUCTION

The disease of trachoma was described for the first time some 3000 years ago. Chlamydial inclusions were first observed in patients with trachoma in 1908 by Halberstaedter and Prowazek[1] but the causative agent was isolated in chicken egg yolk sacs only in 1957.[2] Although the association was found before the First World War, it was not until the 1970s that the prominent role of the trachoma agent in sexually transmitted disease (STD) was realized and soon its importance in pelvic inflammatory disease (PID) was revealed.[3] [4]

The causative agent of psittacosis ('parrot fever'), transmitted by caged birds, was discovered in the 1930s. In several animal diseases, similar small bacteria that multiply in inclusions were found, and later it was realized that they were related to lymphogranuloma venereum and the trachoma agent. In the 1960s, these agents were finally classified on the basis of their multiplication cycle and the common group antigen as belonging to the genus *Chlamydia*, which was further divided, on the basis of glycogen content of inclusions, into two species: *C. trachomatis* (trachoma and human STD strains) and *C. psittaci* (which included all the other strains).[3]

Once serologic diagnosis of psittacosis was available, it became apparent that 'bird-transmitted pneumonia' could be transmitted without any apparent contact with birds. A chlamydial strain that had been isolated in 1965 as a result of trachoma studies in Taiwan was found to be responsible for the majority of chlamydial respiratory infections in humans. It represented a new chlamydial species, *C. pneumoniae*, and is in fact the most common chlamydial species infecting humans.[5]

The diseases seldom occur abruptly and they are not easily recognized; rather, chlamydial infections may run an insidious, chronic course, causing severe damage only after years of infection. Moreover, their obligatory intracellular growth makes normal bacteriologic analysis difficult.[6] The sequencing of whole genomes of several chlamydial species[7] has provided new possibilities for studying these organisms and our concepts of the nature of chlamydiae are changing accordingly.

NATURE

Current classification of chlamydial species is rapidly evolving and there are proposals to name new species and even new genera.[8] The genus *Chlamydia* includes *C. trachomatis* as a human pathogen with relatives found in the mouse (*C. muridarum*) and pig (*C. suis*). The genus *Chlamydophila* includes *C. pneumoniae* as a human pathogen with relatives reported in the horse and koala. *Chlamydophila psittaci* is a pathogen of birds, *C. abortus* and *C. pecorum* are pathogens of ruminants, *C. felis* is a pathogen of cats and *C. caviae* is found in guinea pigs. All except the last species also cause diseases in humans.

Two new families are proposed, Parachlamydiaceae and Simkaniaceae. Both currently contain a single species and *S. negevensis* seems to be common in humans. Its possible association to human diseases is still poorly understood.[9] The closest relatives of *Chlamydia* spp. are small aquatic bacteria that, like *Chlamydia* spp., do not use peptidoglycan as the structural component of the infective particle (Table 236.1).

Chlamydiae are small, Gram-negative bacteria that are obligatory intracellular parasites. They are not cultivable on synthetic media. They exist in nature in two forms: a non-replicating, infectious dense particle called the elementary body (EB); and a loose, larger, intracellular form, the reticulate body (RB), which is able to multiply by binary fission but is noninfectious (Fig. 236.1).[10] The EB is a spherical particle about 300nm in diameter; it can be stored at -94°F (-70°C) in sucrose-containing buffers and cultured in cell cultures.

Chlamydiae have a double layer membrane of Gram-negative bacteria with a periplasmic space. They are usually devoid of peptidoglycan, but seem to use it when dividing.[11] The circular genomes of *C. pneumoniae* and *C. trachomatis* contain 1,230,000 and 1,039,000 base pairs, respectively, with just over 1000 potential genes.[7] Species-specific plasmid is present in 10 copies in *C. trachomatis*,[12] but is not found in the human type of *C. pneumoniae*. The major outer membrane protein (MOMP) forms the outer membrane of the particle with some minor proteins, of which two are rich in cysteine. *Chlamydia trachomatis* has nine and *C. pneumoniae* 21 genes for putative membrane proteins (PMPs). Cysteine-rich proteins apparently have replaced the peptidoglycan with their S-S bridges in maintaining the rigidity of the chlamydial membrane. On electron microscopy, the surface can be seen to consist of a hexagonal pattern with substructures made up of a few rosettes with short spikes (Fig. 236.2).[13]

The MOMP of *C. trachomatis* contains four variable regions, which divide it into about 20 immunotypes denoted alphabetically. [14] The MOMP of *C. pneumoniae* is more homogeneous and the surface structure differs from that of *C. trachomatis*. Chlamydial lipopolysaccharide (LPS) is of rough type, weakly endotoxic and situated in the outer membrane.

The multiplication cycle is best known in the case of *C. trachomatis*. The EBs attach to the surface of susceptible cells and are passively engulfed by activity of the cell. *Chlamydia trachomatis* lymphogranuloma venereum biotype is wrapped by heparan sulfate that is taken from the cell by the chlamydial organism, which can then use heparin receptors on the cell surfaces;[15] *C. pneumoniae* and *C. psittaci* may also use heparin in the attachment. An oligosaccharide containing mannose residues that are covalently bound to MOMP may also participate in the attachment process.[16]

Chlamydiae can prevent the fusion of the endocytic vacuole with lysosomes and travels on microtubules joining to the exocytic pathway. The EB envelope changes, transforming the metabolically inactive inert EB into the metabolically active RB, which is 0.8–1µm in diameter. In the process, the disulfide bridges are reduced and MOMP acts as a porin. DNA, RNA and proteins are synthesized within the RB, which divides successively by binary fission. The endosomal walls intercept nearby trans-Golgi vesicles that incorporate sphingomyelin and glycerophospholipids into the swelling chlamydial inclusion (Fig. 236.3 and Fig. 236.4) Metabolically active RBs acquire

TABLE 236-1 -- General properties of *Chlamydia*.

GENERAL PROPERTIES OF *CHLAMYDIA*
• Obligatory intracellular Gram-negative parasites
• Elementary body-reticulate body cycle
• Lack of peptidoglycan
• Common group antigen (lipopolysaccharide)
• Tendency to persistent infections
• Immune defense participates in the development of lesions

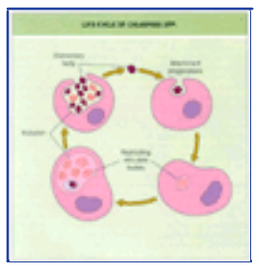

Figure 236-1 Life cycle of *Chlamydia* spp.

Figure 236-2 Structure of *Chlamydia* spp. *Courtesy of Dr A Matsumoto.*

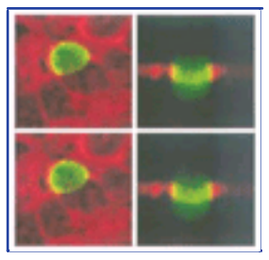

Figure 236-3 Chlamydial inclusion in confocal stereomicroscopy. *Chlamydia pneumoniae* cultured in human line (HL) cells are shown. Fluorescein isothiocyanate (FITC)-labeled anti-LPS monoclonal antibody strain. *Courtesy of Dr A Laurila.*

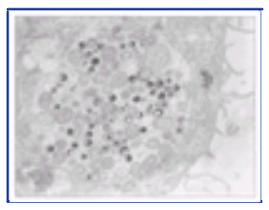

Figure 236-4 Electron microscopy of chlamydial inclusion. Reticulate bodies and transition stages to dense elementary bodies are shown. *Chlamydia pneumoniae* cultured in HL cells. *Courtesy of Dr CH von Bonsdorff.*

nutrients and metabolic building blocks, including nucleotides and ATP ('energy' parasites) from host cells.[17] Chlamydial LPS is present in RBs and seems to attach to host cell membranes.

Chlamydiae use their type III secretion system to gain control over the host cell. Apoptosis is prevented during the growth period but induced in nearby inflammatory cells.[18] Eventually, the inclusion body may contain several thousand chlamydial RBs. At the end of the replication cycle, chlamydiae start to condense into EBs and apoptosis and proteolysis are promoted in order to liberate the new infectious particles. Recently, the presence of a chlamydial cytotoxin has been demonstrated.[19] Sometimes the mature inclusion is expelled intact but usually the inclusion bursts open, releasing infective chlamydial particles and the cell dies. The whole cycle takes 2–3 days. Depletion of tryptophan or iron or the presence of penicillin or interferon-? in subinhibitory concentrations induces an aberrant, slowly metabolizing form of RB, which can persist for long periods. In chronic infections this may be the dominant form of chlamydia.[20] The microscopic appearance of chlamydial inclusion varies with different strains, and the inclusions of *C. trachomatis* contain glycogen.

EPIDEMIOLOGY

Chlamydia trachomatis is transmitted by direct contact and via vectors such as flies and contaminated towels in poor sanitary conditions in trachoma-endemic areas. Newborn infants contract the infection from their mother, sometimes before birth, and can infect their siblings via excretions. In industrialized countries, *C. trachomatis* is by far the most common bacterial STD. The incidence is greatest in young sexually active people and varies widely among different areas, from very low levels in Scandinavian countries to 15–20% in some urban areas of other developed countries (see Chapter 74).

Chlamydia pneumoniae is a common respiratory pathogen worldwide. In tropical countries, infections are common during the first

years of life, especially in urban slums. In industrialized countries, children begin to seroconvert at school age at the rate of 10% each year.[21] The rate seems to depend on population density, but at the age of 15 years 25–50% of populations have demonstrable antibodies against the agent. The antibody prevalence continues to rise; nearly all elderly people have measurable antibodies. Because antibodies are lost by a few years after an acute infection, this steady increase points to repeated infections during life and to possible chronic infections. Spread via droplets has been proposed. Asymptomatic carriers are found but their number is disputed and there is a possibility that only some people are effective transmitters of the agent. In sparsely inhabited areas in high latitudes, *C. pneumoniae* causes epidemics at intervals of 5–7 years. In lower latitudes, the interval between epidemics is shorter and an endemic situation can be found near the equator. In military garrisons with susceptible recruits, the epidemics are prolonged and can last 6 months.[22]

Avian *C. psittaci* is contagious in dried droppings of diseased birds for several days and is typically transmitted by the inhalation of dust. Patients are usually turkey or duck farmers, plant processors or persons who have contact with diseased caged or wild birds. Person-to-person transmission occasionally occurs. Infections caused by *C. psittaci* strains from mammals are less well known. Ovine abortion strains are known to cause septic abortions in pregnant women who are in contact with affected farm cattle. [23] Pet cats can be a source of *C. felis* infections. *C. psittaci* strains are well known for their capacity to cause severe laboratory infections.

C. pneumoniae, like *Legionella*, is able to multiply in amebae[24] but the significance of this property in epidemiology is so far not known.

PATHOGENICITY

Owing to their unique life cycle, in which they parasitize only living, metabolically active cells, chlamydiae are not harmless commensals.[3] [25] It is not known whether the pathogen stays dormant inside cells, protected from antibodies, for prolonged periods, only to be activated later by some signal, or whether there is a special form of chronically infecting *Chlamydia* spp.[26] In chronic persistent infections in vitro, it is possible that chlamydiae respond with morphologically abnormal forms,[27] in which internal proteins such as heat shock proteins (hsps) are produced and antigens of mature EBs are diminished.[20]

Chlamydiae are potent inducers of cytokines, such as interferon-? and interleukin-1, which prevent the multiplication of the agent in vitro.[17] In chronic infections such as trachoma, there are findings that point to diminished cell-mediated immunity and increased humoral immunity.[28] Chlamydial immunity is relatively short-lived and immunotype specific and reinfections are common. In repeated chlamydial infections,[29] there is a hypersensitivity component, apparently caused by common internal cross-reactive proteins. Chlamydial hsp60, a protein produced in chronic infections, has been blamed for the hypersensitivity reactions seen.[30] Moreover, it is related to the host's hsp60 and may initiate autoimmune reactions.[31]

In chronic chlamydial infections, chlamydia can persist in non-cultivable form and damage results partly from persistent inflammation.[25] Blinding trachoma[32] was thus named after *C. trachomatis* and long-term sequelae of lymphogranuloma venereum (rectal strictures, elephantiasis and esthiomena) have been known for some time,[33] but infertility and ectopic pregnancies caused by tubal occlusion have only recently been associated with chronic *C. trachomatis* infection. [4] *Chlamydia pneumoniae* (Fig. 236.5) is able to disseminate inside white blood cells in the circulation[34] [35] and multiply in vascular tissue.[36] Human hsp60 and chlamydial hsp60 co-localize in atherosclerotic plaques.[37] Chlamydial LPS induces cytokine and adhesin responses and formation of foam cells in macrophages.[38]

Figure 236-5 Chronic chlamydial infection in mouse lung. Note the infiltration of mononuclear cells. Hematoxylin and eosin stain. *Courtesy of Dr A Laurila.*

PREVENTION

Vaccines against *Chlamydia* spp. have been under development for 40 years without evident success. *Chlamydia trachomatis* has several immunotypes, making development of a vaccine complicated, and the protective antigens of *C. pneumoniae* are not known. Knowledge of the complete genome and progress in immunology may alter this situation in the future.[39]

Mass medication campaigns have been used against trachoma in Africa and there are local projects to eradicate the agent with generalized antibiotic courses for the whole population.[40] However, improvement in living standards would be a better long-term preventive measure. Washing the face every second day with clear water and wiping it with a towel that is not shared with other people is effective.[41] Dirty water and shared towels are effective disseminators of the infection from eye to eye. Latrine hygiene is effective in controlling flies that spread trachoma. Using an undamaged condom protects against genital chlamydial infection.

Active tracing and treatment have been effective against sexually transmitted *C. trachomatis*. The new nucleic acid-based tests have raised the question of general screening in order to eradicate *C. trachomatis* from populations. In the USA screening of sexually active women under 25 years of age is recommended (see Chapter 73).[42]

DIAGNOSTIC MICROBIOLOGY

The clinical features of chlamydial infections, such as their slow onset, the low numbers of infectious agents present and the cross-reactions seen in serology, make usual diagnostic methods difficult.[43] [44]

Culture

Culture was originally the gold standard in *C. trachomatis* diagnosis but it is an unsatisfactory standard for *C. trachomatis* and even more so for *C. pneumoniae*. Moreover, culture of *C. psittaci* is a well-known cause of laboratory infections.

Samples for culture should contain live cells from the diseased area and this can be problematic if the pathogen has invaded to deeper tissues. The sample is collected in a medium that contains sucrose, aminoglycosides, vancomycin and antifungal agents. McCoy cell lines and green monkey kidney cell lines are commonly used for *C. trachomatis* and Hep2 and HL cells are commonly used for *C. pneumoniae*. Centrifugation onto cells and the addition of cytostat

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(usually cycloheximide) into the growth medium are needed for optimal growth. Multiwell plates are suitable for mass isolation attempts, but vials are better protected from cross-contamination. *Chlamydia trachomatis* can be stained by Lugol's iodine stain. Immunofluorescence staining gives a more clear-cut result and, when using LPS-specific monoclonal antibody, stains all *Chlamydia* spp. The culture result is available in 2–3 days, but blind passage can improve sensitivity.

Chlamydia trachomatis can be present in clinical samples in sufficient amounts to be toxic for cell cultures. The situation is quite different with *C. pneumoniae*, because as a rule only a few inclusions are found in primary isolations, pointing to the paucity of *C. pneumoniae* in throat epithelium collected by swabbing. Nasopharyngeal swabs for *C. pneumoniae* rather than throat swabs have been recommended in some studies.

Antigen detection

All enzyme immunoassay (EIA) kits for the diagnosis of *C. trachomatis* measure the common LPS group antigen that is present in all chlamydiae and produced in large amounts during the growth cycle. Several commercial kits, some fully automated, are available and widely used. Because massive microbial contamination can cause false-positive reactions, positive results should be confirmed. Confirmatory EIA tests are available, but in the case of *C. trachomatis*, EBs can be concentrated from the positive sample and visualized with direct fluorescent antibody (DFA) staining. Lipopolysaccharide-EIA kits can probably also be used for the detection of *C. pneumoniae* LPS from respiratory tract samples, but they have not been systematically tested.

Direct fluorescent antibody staining has been successfully used in *C. trachomatis* diagnosis. This test seems to be marginally more sensitive than EIA; moreover, the quality of the sample can be controlled in the stained smear. However, the interpretation demands expertise and is tiring to the reader, and therefore the test is often used only in the confirmation of EIA tests. In this circumstance, samples that are weakly positive (under the cut-off range) should be tested with DFA staining, which will reveal the true positives among the weakly positive samples. The test is insensitive in the demonstration of *C. pneumoniae* in throat swabs. The pharyngeal microbial flora is quite variable, and the search for very small numbers of EBs is difficult. *Chlamydophila psittaci* can also be seen in smears. The lack of species-specific monoclonal antibodies for *C. psittaci* necessitates the use of anti-LPS antibody, and the results can then be difficult to interpret, especially if the number of EBs is small.

Serology

In individual *C. trachomatis* infections, serologic tests are often of questionable value, although serology has been important in epidemiologic and disease association studies. The infection is often superficial without detectable seroconversion. Proper antibiotic therapy can also prevent antibody formation. Serology has been the method most commonly used for *C. psittaci* and *C. pneumoniae*. The need for paired samples considerably lessens the value of serology in acute situations.

The time-honored complement fixation (CF) test has traditionally been used for the diagnosis of chlamydial respiratory tract infections. The CF test is sensitive in psittacosis, but in *C. pneumoniae* infections it is sensitive only in primary infections of young adults.[22] The use of the CF test in *C. trachomatis* infections is limited to lymphogranuloma venereum.

Enzyme immunoassay tests based on chlamydial EBs or chlamydial LPS (group antigen) are commercially available but have not gained wide popularity. In acute infections, in most cases they demonstrate seroconversions satisfactorily. Synthetic peptides should enable EIA to be made species-specific in the future.

Microimmunofluorescence (MIF) testing, if properly done, can differentiate between species and even immunotypes. It is, however, demanding and can be performed in relatively few laboratories. Chlamydial antibodies are rare in young children, but at school age antibodies against *C. pneumoniae* begin to rise rapidly. *Chlamydia trachomatis* antibodies start to appear at the sexually active age and peak at about 30 years of age. They are more often found in females than in males; the opposite is seen with *C. pneumoniae* antibodies, where males predominate. Microimmunofluorescence testing seems, for the moment, to be the most convenient test for the serologic diagnosis of an acute *C. pneumoniae* infection.[44] In primary infections the diagnosis can be obtained from the first sample that contains IgM antibodies specific for *C. pneumoniae*. The possibility of a false-positive reaction due to IgM rheumatoid factor should always be kept in mind, especially in elderly patients. In elderly patients undergoing reinfections, the rapid response can be missed if the first serum sample is not collected early enough after the onset of disease. Apart from the situation in which strong group reaction interferes with the result, an experienced reader can interpret the reaction to differentiate not only the species but also the immunotype. In MIF testing, strains of *C. pneumoniae* react much more uniformly than do those of *C. trachomatis*. In *C. trachomatis* infections, MIF testing is usable in lymphogranuloma venereum, perihepatitis and infant pneumonitis, and it can give a clue to the triggering agent in reactive arthritis.

Detection of nucleic acids

The use of nuclei acid-based detection systems has altered the former concept of culture as the 'gold standard' of *C. trachomatis* diagnosis[42] and is replacing other diagnostic methods. Three methods have already appeared on the market, based on polymerase chain reactions (PCR), ligase chain reaction or transcription-mediated amplification. A great advantage is the possibility of using mailed morning void urine, tampons and self-collected samples for detection.

In the case of *C. pneumoniae* commercial PCR kits are not currently available and in-house PCR kits vary widely in their sensitivity. [43] In acute infections, they seem to be more sensitive than isolation. Upper respiratory tract samples can be negative and sputum is preferred. [44]

Diagnosis of chronic chlamydial infections

Diagnosing chronic chlamydial infections is problematic. The number of infective organisms can be small and the site of infection can be difficult to reach. Culture often remains negative, especially if the sample is from a peripheral site. Antibody responses can be quite variable or even lacking. Persistently elevated titers, especially of IgA, have been suggested as a marker, but their value in individual diagnosis is doubtful. Enzyme immunoassay tests have been inferior when compared to the MIF test. Antigen detection by immunohistochemistry is more sensitive and diagnostic but, like nucleic acid-based methods (e.g. amplification, in-situ hybridization), they are currently used in research laboratories only.[44] The value of demonstrating circulating immune complexes that contain chlamydial antigens in blood samples is under evaluation, as is the value of detecting nucleic acid in circulating white blood cells. Both markers, however, can also be found in healthy persons and are affected by age and season.[34] [45]

CLINICAL MANIFESTATIONS

The diseases associated with chlamydial infection are listed in Table 236.2 .

TABLE 236-2 -- Diseases associated with *Chlamydia spp.*, or in which a possible association has been proposed.

DISEASES ASSOCIATED WITH *CHLAMYDIA* SPP., OR IN WHICH A POSSIBLE ASSOCIATION HAS BEEN PROPOSED				
Species	**Infection**	**Distribution**	**Disease**	**Incidence**
Chlamydia trachomatis	Acute infections	Females	Cervicitis	About 30%
			Endometritis	Common
			PID	10–70%
			Perihepatitis, splenitis, appendicitis	Isolated case reports
			Bartholinitis	Rare
		Males	Urethritis	10–30%
			Epididymitis	In young males
		Both sexes	Conjunctivitis	Isolated case reports
			Reactive arthritis	Common cause
		Neonates	Conjunctivitis	<10%
			Infant pneumonitis	By definition
	Chronic infections		Trachoma	By definition
			Chronic PID	Common
Chlamydia pneumoniae	Acute infections		Pneumonia Endemic	10%
			Pneumonia Epidemic	up to 50%
			Acute bronchitis	5%
			Sinusitis	<5%
			Otitis media	Isolated case reports
			Upper respiratory tract infection	~10% of school children annually
			Carditis	Isolated case reports
			Vasculitis (erythema nodosum)	Isolated case reports
			Reactive arthritis	Isolated case reports
	Chronic infections		Chronic obstructive lung disease	10–50%
			Asthma	?
			Sarcoidosis	?
			Atherosclerosis	up to 50%
Chlamydia psittaci			Psittacosis-ornithosis	By definition
			Infectious abortion	Rare

Chlamydia trachomatis infections

Genital infections in females (see Chapter 74)

Chlamydia trachomatis is the most important cause of bacterial genital infections in women. Infected women often remain asymptomatic carriers and can thus transmit the disease. The prevalence of carriers varies widely depending on the population (2–20%). Immunotypes E–K and B are usually found in genital infections.

Vaginitis due to *C. trachomatis* is seen only in childhood and after the menopause. Women aged 15–25 years are particularly susceptible to *C. trachomatis* cervicitis, both for anatomic reasons and because of the slow development of immunity. It cannot be differentiated clinically from cervicitis caused by other agents, although mucopurulent discharge and hypertrophic follicular formations in an edematous and friable epithelium ('genital trachoma') are typical of the disease (Fig. 236.6).[46] One of the causes of 'aseptic', culture-negative cystitis in women is *C. trachomatis*. [47] *Chlamydia trachomatis* has been isolated as pure culture in cases of bartholinitis. It is much more common in patients with endometritis.[48] Plasma cell infiltration is the typical microscopic hallmark in the chronic form of this disease.

Pelvic inflammatory disease is the most important complication of female genital infection with *C. trachomatis*, and in areas where the prevalence of gonorrhea has decreased *C. trachomatis* is the most common cause of PID. Clinically, chlamydial PID cannot be differentiated from gonococcal PID, but in chlamydial PID the patients tend to be younger and the onset of the disease is slower with a less marked fever. Markers of acute infection, such as erythrocyte sedimentation

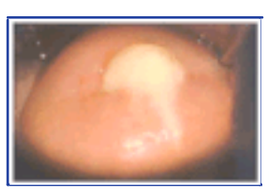

Figure 236-6 Mucopurulent cervicitis caused by *Chlamydia trachomatis*. *Courtesy of Dr J Paavonen.*

rate (ESR) and C-reactive protein, are clearly elevated. Infection can proceed to the abdominal cavity and cause 'sterile' peritonitis with ascites.

Typically, however, the symptoms come from more localized areas of infection. Acute perihepatitis (Fitz-Hugh-Curtis syndrome) is commonly caused by *C. trachomatis*

and this should be kept in mind when young female patients complain of right upper quadrant

abdominal pain. In perihepatitis, the capsule of the liver, not the liver tissue itself, is inflamed, typically leading to 'violin-string' adhesions. Periappendicitis and perisplenitis have also been reported.

Chlamydial PID often goes unrecognized. As a result, the patient is not treated, infection becomes chronic and over years can severely damage the fallopian tubes. Almost 50% of patients with tubal occlusions and elevated chlamydial antibody titers deny any episode of PID. Ectopic pregnancies and infertility problems are the most important complications of genital chlamydial infections in women.[49] Chronic chlamydial infection has been associated with fetal death, pre-term labor and late-onset puerperal fever.[50]

Genital infections in males

One-third of cases of nongonococcal urethritis are caused by *C. trachomatis*. The incubation period is 6–12 days, which is longer than for gonorrhea, and the course is milder than in gonorrhea. Asymptomatic carriers of *C. trachomatis* are also more common (up to 10% in some areas) than asymptomatic *N. gonorrhoea* carriers. Treatment aimed at gonococci does not affect *Chlamydia* spp. and the majority of postgonococcal urethritis cases are due to simultaneously acquired *C. trachomatis* infection.

The role of *Chlamydia* spp. in prostatitis is unclear. Cultures are negative but chlamydial nucleic acids and antigens have been demonstrated in prostatic tissue. It has not been proven that chlamydiae are a cause of azoospermia or infertility in men. However, epididymitis in young males is most commonly caused by *C. trachomatis*, and if the infection is bilateral this can cause infertility.

Infections in newborns

Chlamydial infection can be transmitted in utero and cause serious disease in the pre-term infant. Half the children born to a chronically infected mother are infected during birth. Topical prophylaxis is not effective and mothers should be treated before birth. Inclusion conjunctivitis of the newborn (ophthalmia neonatorum; Fig. 236.7) develops in 50% of the infants, usually after they have been discharged from hospital. The disease is milder than gonococcal ophthalmia neonatorum and there is no danger of perforation; only minute scarring can be seen. The disease is usually unilateral, but edema of both eyelids can be marked. *Chlamydia* spp. can also infect the nasopharynx, middle ears, lungs, intestine and vagina of the newborn.

Infant pneumonitis is a well-characterized complication.[51] The disease starts at 3–12 weeks of age with a stuffy nose, difficulty in breathing and a gradually worsening staccato cough. The infant is usually afebrile, but chest radiographs may show marked hyperexpansion and prominent striations in the lungs. Specific IgM levels

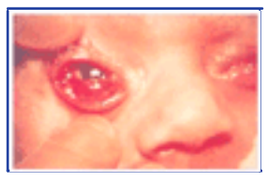

Figure 236-7 Ophthalmia neonatorum. *Courtesy of Dr M Puolakkainen.*

are elevated, as may those of eosinophils. Although the radiograph findings are often marked, the short-term prognosis is good and only rarely does the patient require intensive therapy. In the long term, however, the lungs seem to be damaged and these children are sensitive to repeated respiratory tract infections and often go on to develop asthma.[52] *Chlamydia trachomatis* is a rare cause of respiratory tract infections after 6 months of age. There are reports in the literature of pharyngeal carriage of *C. trachomatis* in adults, but the agent has not been clearly associated with pharyngitis.

Eye infections

Trachoma, which is due to *C. trachomatis*, is still a great scourge in developing countries, where it is the most common preventable cause of blindness.[32] About 500 million people are victims of this disease and 7 million are blind as a result of the chronic follicular conjunctivitis it causes. Although the pathogenesis of trachoma has been intensively studied, it is not known why only some of the children exposed to repeated inoculations by chlamydial agents (immunotypes A–C) develop chronic disease. The clinical features of the chronic disease include a pannus over the cornea and scarring of the lids by entrichiasis (eyelashes turn inwards and scrape the cornea). Trachoma occurs in poorly developed areas with a low standard of sanitation and lack of washing facilities.

Occasionally, genital *C. trachomatis* infection can lead to 'inclusion conjunctivitis', because of self-inoculation or oral sex with an infected partner. Follicular formation is seen, but corneal lesions are rare. Infection is usually unilateral and associated with palpebral edema. The disease is typically diagnosed only after topical treatments have failed. Although the first *C. pneumoniae* strains to be isolated were from eyes, conjunctivitis is rarely seen in *C. pneumoniae* infections.

Lymphogranuloma venereum

Chlamydia trachomatis immunotypes L_1 , L_2 and L_3 differ from other *C. trachomatis* strains in their invasive nature and in belonging to the lymphogranuloma venereum biovar. Instead of multiplying in epithelial cells, they multiply in the reticuloendothelial system. Inguinal lymph nodes are the target organs, but the agent spreads systemically and can affect various organs. Sequelae include strictures in pelvic lymphatic vessels, which may lead to elephantiasis, and rectal strictures. The disease is not common in industrialized countries, but it is important in some tropical areas (see Chapter 78). [33]

Chlamydophila pneumoniae and *Chlamydophila psittaci* infections

Chlamydophila pneumoniae and *C. psittaci* multiply in similar target cells. The patterns of disease they cause are identical except that the ovine abortion agent of *C. psittaci* can cause infectious abortion in pregnant women and acute infections due to *C. psittaci* tend to be more severe than those caused by *C. pneumoniae*.

Respiratory infections

In children the typical disease picture is a mild upper respiratory tract infection, but pneumonia has been reported, even in infants. This pneumonia should not be confused with *C. trachomatis* infant pneumonitis, in which the parents must be treated for their STD. Chlamydial pneumonias typically have an insidious onset, fulminant psittacosis being an exception. Epithelial cells and mononuclear phagocytes are target cells and the result is pneumonitis without purulent sputum. If the sputum turns purulent, there is likely to be an accompanying bacterial infection. Except for true psittacosis and in elderly patients with underlying predisposing disease, the prognosis is good, but convalescence can be prolonged by persisting fatigue. There is a tendency for relapses.[53]

C. pneumoniae can cause sinusitis and otitis, but dry cough with mild pharyngitis is often the most common manifestation. [22]

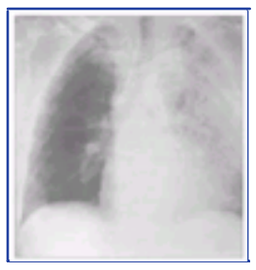

Figure 236-8 *Chlamydia pneumoniae* reinfection pneumonia in a 63-year-old male.

C. pneumoniae is the cause of acute bronchitis in 5% of cases.[5] Infection can be asymptomatic, especially in children, but the number of carriers is unknown.

In young adults, 10% of cases of *C. pneumoniae* infections are pneumonias, which often have an insidious-onset pharyngitis followed by a mild pneumonia. The throat is hoarse and there is a persistent dry cough and usually a low-grade fever. Erythrocyte sedimentation rate and C-reactive protein are elevated, but the white cell count is usually normal or only mildly elevated. Differential diagnosis from other causes of pneumonia is impossible on clinical grounds. Chlamydial pneumonia cannot be reliably differentiated from pneumococcal pneumonia on chest radiograph, because chlamydial pneumonia can have a lobar pattern as well as its more usual pattern of 'atypical' pneumonia (Fig. 236.8) (see Chapter 34).

Generalized* Chlamydophila pneumoniae *and* Chlamydophila psittaci *infections

Chlamydophila pneumoniae and *C. psittaci* are able to multiply in mononuclear phagocytes and this enables dissemination into the circulation. Headache and confusion are common in severe infections, and meningoencephalitis and Guillain-Barré syndrome have been described. In some patients liver enzymes are elevated and proteinuria is present. Endocarditis, myocarditis and pericarditis have been found.[54] All of these symptoms have been described in the absence of any preceding overt respiratory infection.

C. pneumoniae infection has a tendency to remain persistent and low levels of the agent have been demonstrated in the lungs of healthy young adults by immunohistochemistry.[55] *Chlamydophila*-specific IgA levels are elevated in serum and especially in the local secretions of patients with chronic obstructive lung disease.[56] Exacerbations associated with acute *C. pneumoniae* infections have been reported [57] but more often there seems to be chronic carriage without seroconversion; the agent is detected in pulmonary secretions by using PCR and is shown to be abundant in histologic samples by immunohistochemistry and electron microscopy.[55] [58] The extent to which this carriage participates in the inflammatory process is not known.

Recent studies have also suggested an association between *C. pneumoniae* and asthma, in both adults and children.[59] Favorable outcomes in therapeutic trials with antibiotics effective against *C. pneumoniae* have been claimed in adult-onset asthma,[60] where the presence of specific IgA antibodies again seems to be a better marker of chronic infection than IgG. The role of the agent in childhood asthma is uncertain, but carriage demonstrated by both culture and PCR has been reported, as has *C. pneumoniae*-specific IgE and local IgA antibodies. The first large intervention trial against *C. pneumoniae*-associated asthma in adults with antibiotics left the question open.[61]

One of the most unexpected findings in *Chlamydia* research has been the association of *C. pneumoniae* with atherosclerosis.[62] If proven, it will be of enormous significance.

Over 50 seroepidemiologic surveys have repeatedly demonstrated that the presence of elevated antibodies against *C. pneumoniae* is associated with an increased risk of having a cardiac event.[63] [64] The magnitude of the increased risk is dependent partly on the prevalence of antibodies in control population; it is greatest in young populations during periods between epidemics. The risk increases usually about 2-fold but higher risks have been reported. The antibody findings are further supported by the presence of circulating immune complexes containing *C. pneumoniae* antibodies and antigens, pointing to an access of chlamydial components into the circulation.[45] Final demonstration of the common presence of the agent in the atherosclerotic lesion has been obtained by electron microscopy, immunohistochemistry, nucleic acid demonstration by PCR, in-situ hybridization[65] and even isolation of the agent from lesions.[35] [66] [67]

The important question is does this presence have an effect on the atherosclerotic process? There is a possibility that *C. pneumoniae* is simply an innocent bystander in atherosclerotic lesions. However, it is a nonmotile intracellular pathogen, able to multiply in macrophages, endothelial cells and smooth muscle cells.[36] This multiplication is accompanied by induction of cytokines, adhesion molecules, proteases and oxidative substances as the host defense mechanisms against chlamydial infection. Infected macrophages tend to become foam cells, the characteristic pathologic feature of the atherosclerotic plaque.[38] Several well-known risk factors for coronary heart disease are also associated with a chronic *C. pneumoniae* infection, such as smoking, elevated cholesterol levels, lowered levels of high-density cholesterol[64] and elevated blood pressure.[68] Continuous induction of cytokines may be responsible for this association. Recent animal experiments point to the possibility that it could even initiate the process[69] and that this could be prevented with antibiotics. Recent preliminary human intervention trials with macrolides[70] [71] [72] [73] [74] [75] or doxycycline[76] have given partly contradictory results.

If chlamydial infection is a significant factor in the development of atherosclerosis, it would open new perspectives in the battle against this disease and even the prospect of the development of an 'anti-atherosclerosis vaccine' is possible.

Chlamydia-*associated arthritis*

Chlamydiae have long been known to trigger reactive arthritis which is typically seen after an episode of urethritis in males (Reiter's syndrome — a similar association is elusive in females) and in cases of *C. pneumoniae* and *C. psittaci* after a respiratory tract infection. Patients usually have HLA-B27 antigen and a complete Reiter's triad of urethritis, conjunctivitis and arthritis can occasionally be seen. Although chlamydial cultures are usually negative, recent studies have demonstrated chlamydial particles in synovial fluid and metabolically active chlamydia in synovial tissue, which is not the original concept of reactive arthritis. This could also explain why prolonged antibiotic treatment is effective in chlamydial arthritis[77] but not in enterobacterial reactive arthritis. *Chlamydia trachomatis* seems to be the most common cause of chlamydial arthritis. Whether this is a reflection of differing tissue tropism of the different *Chlamydia* spp. is not known.

Chlamydiae in cancer and chronic neurologic processes

By the 1930s, *C. trachomatis* lymphogranuloma venereum biovars had been associated with anorectal cancers and subsequently they were associated with cervical dysplasia and cancer. Recently, these observations have been repeated and there seems to be an

association between *C. trachomatis* infection and invasive squamous cell cervical carcinoma.[78] In smokers, *C. pneumoniae* has been associated with squamous cell carcinoma and small cell carcinoma in lungs.[79] The situation is analogous to the association between *Helicobacter pylori* and gastric cancer and it deserves further study. There are some claims that *C. pneumoniae* is associated with chronic neurologic processes such as multiple sclerosis and Alzheimer's disease, but this has remained unproven.[80]

MANAGEMENT (see Chapter 18 , Chapter 34 and Chapter 74)

Chlamydiae are sensitive to tetracyclines, macrolides, azalides and newer fluoroquinolones. An antimicrobial effect is also seen with rifampin (rifampicin), clindamycin and chloramphenicol. Chlamydiae are resistant to aminoglycosides, vancomycin and cephalosporins.

Chlamydiae contain penicillin-binding proteins and amoxicillin has been recommended for *C. trachomatis* cervicitis or carriage during pregnancy.[81] However, there is a danger of infection becoming chronic. Single-dose 1g azithromycin is replacing the traditional 7–10 days' tetracycline or macrolide treatment in uncomplicated genital chlamydial infections.[82] In complications such as PID, longer treatment periods of 2 weeks are recommended, and a possible polymicrobial origin must be considered. In *C. trachomatis* conjunctivitis, whether neonatal or adult, topical treatment is not sufficient. *C. pneumoniae* can be very resistant to therapy and in pneumonia 3-week macrolide or doxycycline courses have been recommended. In chronic chlamydial infections, prolonged treatment courses of 1–3 months have been used. Persistent forms seem to be very resistant to treatment and in intervention trials even 1-year courses have been tried.

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Chapter 237 - Opportunistic Fungi

Andy IM Hoepelman

INTRODUCTION

Fungal infections have become increasingly important clinically because of the rising incidence of immunocompromised patients as a result of infection, malignancy (especially leukemia), chemotherapy and immunosuppressive therapy, especially in transplantation medicine and in critical care. Moreover, the use of current antimicrobial prophylactic strategies has likely contributed to the changing epidemiology of invasive mycoses. Table 237.1 gives a list of medically important fungi that can cause disseminated infection in humans. Table 237.2 shows the variables that likely account for the current trends in the epidemiology of opportunistic fungal infections.

CANDIDIASIS

NATURE

Candida spp. are yeast-like fungi that can form true hyphae as well as pseudohyphae. They are ubiquitous in soil and food and can be found as normal commensals on skin and mucosal membranes of the human gastrointestinal, genitourethral and respiratory tracts.

Candida spp. that under certain conditions are clinically important are *C. albicans, C. guilliermondii, C. krusei, C. parapsilosis, C. tropicalis, C. kefyr, C. lusitaniae, C. rugosa, C. dubliniensis* and *C. glabrata* (also called *Torulopsis glabrata*).

The fungal origin of these lesions was first proposed in 1839. [1]

EPIDEMIOLOGY

Since the introduction of antibiotics in the 1940s, there has been a sharp increase in the incidence of candidal infections. In a survey of 180 hospitals from 1980 to 1990, *Candida* spp. were the sixth most commonly isolated nosocomial pathogens, most frequently cultured from the urinary tract (46%).[2] *Candida* spp. were the fourth most common cause of bloodstream infection (BSI; 8%), and were associated with a mortality of 29% (compared with 17% for BSIs caused by nonfungal pathogens).[2] [3]

In addition, there is an increasing shift toward non-*albicans* spp. in candidemia. A surveillance program of BSIs in the USA, Canada, Latin America and Europe from 1997 through 1999 detected 1184 episodes of candidemia in 71 medical centers (32 in the USA, 23 in Europe, nine in Latin America and seven in Canada). Overall, 55% of the yeast BSIs were due to *C. albicans*, followed by *C. glabrata* (20%) and *C. parapsilosis* (15%), *C. tropicalis* (9%) and miscellaneous *Candida* spp. (6%).[4]

Changes in the frequency of invasive candidiasis are most notable in the following subgroups of patients: those hospitalized in critical care units, patients with hematologic malignancies, hematopoietic stem cell transplant recipients and organ transplant recipients.[5] In a recent review of 74 published studies,[6] [7] the most important risk factors for nosocomial colonization and infection with *Candida* spp. were as follows: underlying diseases (hematologic cancer (OR 1.7–45), renal failure (OR 1.4–22), hepatic failure (OR 7–42)); invasive procedures or devices (central venous or arterial catheter (OR 5.8–26), urinary catheter (OR 13)); interhospital transfer of a patient (OR 21); and prolonged use of antibiotics (OR 1.7–25), especially vancomycin (OR 275). In mice and humans, vancomycin has indeed been shown to enhance gastrointestinal colonization with *C. albicans*.[2]

Among hematopoietic stem cell transplant recipients, an overall decrease has been documented in the frequency of candidal infections, as well as a shift toward isolation of non-*albicans* species of *Candida*.[8] [9] It has been proposed that use of fluconazole as antifungal prophylaxis largely accounts for these trends.[8] Antifungal prophylaxis with fluconazole during neutropenia and acute graft-versus-host disease (until day 75 after transplantation) was associated with a significant reduction in the incidence of invasive candidiasis and improved survival rates.[9] However, although *C. albicans* was the most common colonizing isolate before transplantation, resistant species such as *C. krusei* and *C. glabrata* were isolated after transplantation and exposure to fluconazole.[9] In another study, fluconazole prophylaxis was the most important determinant for the relative increase in isolation of *C. krusei* (OR 27.07) and *C. glabrata* (OR 5).[8] A meta-analysis of 16 randomized controlled trials, however, showed that fluconazole prophylaxis in neutropenic non-bone marrow transplant patients did not decrease fungus-related mortality or systemic fungal infections.[10]

Among organ transplant recipients, invasive candidal infections are most relevant for liver and pancreas transplant recipients. An overall decline in the incidence of invasive candidiasis has been noted in liver transplant recipients, even in the absence of systemic antifungal prophylaxis; many centers now report incidences of under 10%.

Molecular typing has shown that, in the majority of cases, candidemia arises from an endogenous origin after previous colonization. This holds true for *C. albicans* and most non-*albicans* spp. except for infections with *C. parapsilosis*, which are thought to arise mainly from infected biomaterials, intravenous fluids or the hands of health care workers.[3] Also, human-to-human transmission (patient-to-patient, nurse-to-patient and between sexual partners) has become increasingly important.

Recurrence of oropharyngeal candidiasis in patients who have AIDS has been shown to be mostly due to recurrence of the same strain (relapse), although infection with a new strain also occurs.[3]

PATHOGENICITY

Intact barrier function is an essential feature of host defense against candidiasis. The virulence of the *Candida* spp. has been shown to correlate with their ability to adhere to epithelial cells (especially *C. albicans*) or plastic polymers such as intravascular or urethral catheters (*C. tropicalis*). The fungus is capable of secreting proteinases and lipases that can assist invasion, although the clinical importance of these enzymes is not clear.[11]

After candidal invasion of the dermis or bloodstream, neutrophils constitute the first line of defense, followed by monocytes and eosinophils, which can kill *Candida* spp. via oxidative and nonoxidative

TABLE 237-1 -- Fungi causing disseminated infection in humans.

FUNGI CAUSING DISSEMINATED INFECTION IN HUMANS	
Fungi	**Medically important species**
Penicillium	*Penicillium marneffei*
Candida	*C. albicans, C. guilliermondii, C. krusei, C. lusitaniae, C. parapsilosis, C. pseudotropicalis, C. rugosa, C. stellatoidea, C. tropicalis, Torulopsis (or Candida) glabrata*
Cryptococcus	*C. neoformans var. neoformans, C. neoformans var gattii*
Aspergillus	*A. fumigatus, A. flavus, A. niger, A. terreus, A. nidulans*
Mucorates	*Rhizopus oryzae, R. arrhizus, R. rhizopodiformis, Rhizomucor pusillus, Absidia oryzae, A. corymbifera, A. ramosa, Mucor circinelloides*
Histoplasma	*H. capsulatum*
Coccidioides	*C. immitis*
Blastomyces	*B. dermatitidis*
Sporothrix	*S. schenckii*
Fusarium	*F. solani, F. oxysporum, F. moniliforme, F. dimerum, F. chlamydosporum, F. anthophilum*
Trichosporon	*T. beigelii, T. capitatum*
Geotrichum	*G. candidum*
Rhodotorula	*R. rubra*

Dermatiaceous molds (phaeohyphomycetes)	*Pseudallescheria boydii* (scedosporium apiospermum), *Bipolaris*, *Alternaria*, Scedosporium prolificans

TABLE 237-2 -- Variables that likely account for the current trends in the epidemiology of opportunistic fungal infections.[5]

VARIABLES THAT LIKELY ACCOUNT FOR THE CURRENT TRENDS IN THE EPIDEMIOLOGY OF OPPORTUNISTIC FUNGAL INFECTIONS
Increasing number of susceptible hosts
Greater laboratory expertise in the detection and identification of fungi
Use of new transplantation modalities for hematopoietic stem cell transplantation (e.g. $CD34^+$ selected autografts and peripheral blood stem cell transplantation)
Evolution in organ transplantation practices
Advances in surgical technology
Use of corticosteroid-sparing regimens and an overall conservative approach to immunosuppression
Use of novel immunosuppressive agents
Use of antimicrobial prophylactic practices, e.g. use of fluconazole for antifungal prophylaxis, ganciclovir for cytomegalovirus prophylaxis, quinolones for Gram-negative bacterial prophylaxis

pathways. Patients who have neutropenia are particularly at risk of developing candidiasis, which underscores the importance of neutrophils in host defense against this fungus. The clinical outcome of infection is primarily determined by the host defense status. Clinical and experimental data suggest that:

- an impairment of acquired cellular immunity (e.g. in HIV infection) predisposes mainly to mucocutaneous candidiasis (gastrointestinal and vaginal); and
- impaired innate immunity, especially neutrophil function, with or without impaired T-cell function, is the major risk factor for the development of systemic candidiasis.

Figure 237-1 Colonies of *Candida albicans* on culture plate.

In the rare chronic mucocutaneous candidiasis syndrome, various combinations of defect in T cell response to *Candida* spp. have been described [12] (see Chapter 98).

The role of humoral acquired immunity in the body's defense against candidal infection remains to be determined.[11]

DIAGNOSTIC MICROBIOLOGY

In general, a diagnosis of candidiasis faces the dual problem of:

- differentiating between colonization and infection, and
- under-detection of deep-seated infection.

In culture, *Candida* spp. grow rapidly at 77–98.6°F (25–37°C) on simple media. On special culture media, hyphae or elongating pseudohyphae are formed.

Candida spp. grow in routine ventilated blood culture flasks and on agar plates as smooth creamy white colonies (Fig. 237.1). A differential culture medium (CHROMagar Candida) can distinguish between *C. albicans* and certain non-C. *albicans* spp.

The yield from blood cultures in disseminated disease using conventional methods is low (15–30%), but improved culture techniques such as biphasic cultures and lysis-centrifugation have greatly improved the diagnostic capacity.[13] [14] Recently developed new methods to detect fungi more rapidly in blood cultures are BACTEC and BacT/Alert (Table 237.3). Therefore, appropriate communication about any clinical suspicion of candidemia to the diagnostic laboratory is of paramount importance.

Specimens for microscopic evaluation give a better diagnostic yield after treatment with 10% potassium hydroxide, which lyses epithelial cells. The demonstration of blastoconidia (budding yeast), hyphae and pseudohyphae is highly suggestive but not diagnostic for tissue invasion.[13] Staining with calcofluor white (the preferred method) is a sensitive method for the detection of fungi, but requires a fluorescent microscope. Alternatives to the calcofluor white stain are Gram stain (fungal elements stain Gram positive) and the germ tube test, which enables identification of *C. albicans* (but not most non-*albicans* spp.) by showing the formation of hyphal elements within 90 minutes.[13]

Nonculture methods being developed and evaluated for mycotic infections include polymerase chain reaction (PCR), galactomannan (GM) antigenemia, Western blot (WB) to detect antibodies, and detection of the fungal metabolites D-arabinitol and (1,3)-ß-D-glucan. Sample preparation for PCR from blood specimens depends on fractionation of peripheral blood, its pre-incubation in blood culture broth or a total DNA method, which does not rely on fractionation or pre-incubation. Targets for PCR of fungi in the 18S or ITS2 subunits of the ribosomal RNA genes facilitated the design of *Aspergillus* and *Candida* genus and species probes.

TABLE 237-3 -- Detection of fungal infections.

DETECTION OF FUNGAL INFECTIONS			
Diagnostic technique	**Major features**	**Useful**	**Not useful**
Microscopy/histopathology	Rapid	Histopathologic identification of:	Cannot give a specific species classification for:
	Relies on distinctive appearance of organism	*Cryptococcus*	*Aspergillus*
		Blastomyces	*Candida*
		Histoplasma	
		Coccidioides	
Culture-based methods			
• Traditional culture	Inexpensive	*Cryptococcus* grows rapidly	Slow growth for most endemics
		Aspergillus — tissue sample	Poor sensitivity for *Candida* and *Aspergillus* blood samples
• Automated blood culture methods	Early detection of growth	*Candida* and BSIs	No value for *Aspergillus*
	Capital expense	*Cryptococcus* and *Histoplasma*	
Nonculture methods			

• Antigen	Sensitive and specific	*Cryptococcus* and *Histoplasma*	No reliable tests for other mycoses
		Aspergillus-galactomannan may be useful	
• Antibody	Moderately sensitive and specific	Endemic mycoses	No reliable tests for opportunistic fungi
• PCR	Still experimental	Potential use for *Candida* and *Aspergillus*	

TABLE 237-4 -- General patterns of susceptibility of *Candida* species[*]

GENERAL PATTERNS OF SUSCEPTIBILITY OF *CANDIDA* SPECIES						
***Candida* species**	**Fluconazole**	**Itraconazole**	**Flucytosine**	**Amphotericin B**	**Caspofungin**	**Voriconazole**
C. albicans	S	S	S	S	S	S
C. tropicalis	S	S	S	S	S	S-I
C. parapsilosis	S	S	S	S	S-R	S
C. glabrata	S-DD to R	S-DD to R	S	S-I	S	S
C. krusei	R	S-DD to R	I-R	S-I	S	S
C. lusitaniae	S	S	S	S to R	S	S

** (Adapted from Rex et al.[27])*

The measurement of anticandidal antibodies in the serum of patients does not allow a distinction between colonization and local infection from disseminated disease.[14] The diagnostic sensitivity and specificity reported in the literature of various enzyme immunoassays for cell wall mannan tested on a mixed patient population ranged from 53% to 100% and from 89% to 100%, respectively. [14] [15] [16] The diagnostic yield increases significantly with repeated sampling.

The performance of a rapid test based on the ratio of D/L-arabinitol in urine has been disappointing. Many patients were positive, positivity was shown late in infections and many infections due to *C. krusei* remained undetected due to nonproduction of arabinitol.

Molecular diagnosis by PCR appears very promising since fungal DNA can be detected in the blood of infected patients before conventional methods. Furthermore, a broad range of yeasts and molds can be identified to species level. Automation of sample preparation and use of real-time PCR systems will help standardize the procedure and reduce false-positive results due to contamination.[16] Candidal strain typing is mainly used in hospital infection control and for epidemiologic investigation.

Susceptibility testing and drug dosing

Intensive efforts to develop standardized, reproducible and clinically relevant susceptibility testing methods for the fungi have resulted in the development of the NCCLS M27-A methodology for susceptibility testing of yeasts.[17] Data-driven interpretive breakpoints using this method are available for testing the susceptibility of *Candida* spp. to the most important antifungals (Table 237.4). Several features of these breakpoints are important. First, these interpretive breakpoints should not be used with other methods without extensive testing. Second, these breakpoints place a strong emphasis on interpretation in the context of the delivered dose of the azole antifungal agent. The novel category S-DD (susceptible-dose/delivery dependent) indicates that optimizing dosage and bioavailability is critical to successful therapy. In the case of fluconazole, both human and animal data suggest that S-DD isolates may be treated successfully with 12mg/kg/day. In the case of itraconazole, oral absorption is somewhat unpredictable and achievement of blood levels of 0.5g/ml (as determined by high-performance liquid chromatography) appears important to successful therapy. Finally, these breakpoints have been developed on the basis of data from two groups of infected patients: patients with oropharyngeal and esophageal candidiasis and patients with invasive candidiasis (mostly non-neutropenic patients with candidemia).

Reliable and convincing interpretive breakpoints are not yet available for amphotericin B. The NCCLS M27-A methodology does not reliably identify amphotericin B-resistant isolates. Although these methods are as yet insufficiently standardized to permit routine use, several generalizations are becoming apparent. First, amphotericin B resistance appears uncommon among isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis*. Second, isolates of *C. lusitaniae* most often demonstrate readily detectable and clinically apparent amphotericin B resistance. However, the exact frequency of this event is uncertain and not all isolates are resistant. Third, a growing body of data suggests that a significant proportion of the isolates of *C. glabrata* and *C. krusei* may be resistant to amphotericin B. Importantly, delivery of additional amphotericin B by use of a lipid-based preparation may not always be adequate to overcome this resistance.

CLINICAL MANIFESTATIONS

Clinical manifestations of candidal infections are also discussed in Chapter 111 .

The clinical manifestations of candidal infection can be divided into mucocutaneous infections and deep-seated infections. Mucocutaneous infections include thrush, candidal esophagitis, nonesophageal gastrointestinal candidiasis, candidal vaginitis and cutaneous candidiasis syndromes. Deep-seated infections include chronic disseminated candidiasis (hepatosplenic candidiasis), candidemia and candidiasis of various organ systems.

Mucocutaneous candidiasis

The most common clinical manifestation of candidal infection is oral thrush (acute pseudomembranous candidiasis), which presents as curd-like plaques on examination. It is diagnosed by the demonstration of yeast hyphae and pseudohyphae in a Gram-stained direct smear, 10% potassium hydroxide preparation, calcofluor preparation or culture of scrapings. Oral thrush should alert the physician to the possibility of an underlying disease.

Candidal esophagitis (Fig. 237.2) is frequently associated with AIDS, lymphoma or leukemia, although it can occur in people who are not immunocompromised. In up to 30% of cases of esophagitis there are no oral lesions on examination.

Of women with vulvovaginal candidiasis 75% have no risk factors. Diagnosis is made on the basis of the combination of clinical symptoms, microscopy with 10% potassium hydroxide (include a wet preparation as well to exclude *Trichomonas vaginalis* and clue cells) and/or culture. The vaginal pH in candidiasis should be in the normal range (4.0–4.5); a pH higher than 4.7 indicates bacterial vaginosis, trichomoniasis or a mixed infection.

Chronic mucocutaneous candidiasis is a relatively rare disease characterized by protracted and persistent infections with *Candida* spp. of skin, mucosal membranes, hair and nails, and it is frequently associated with endocrinopathies or autoimmune disorders. Severe disease may prove fatal, usually due to bacterial sepsis. Disseminated candidiasis is a rare complication.[12]

Deep organ infection

Disseminated candidiasis and candidemia constitute major clinical and diagnostic problems. Overall, in more than 50% of cases, fungal blood cultures remain negative,[13] although increased blood volume,

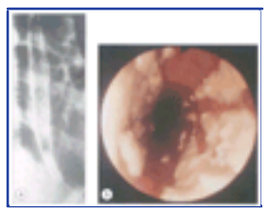

Figure 237-2 Candidal esophagitis. (a) Double-contrast radiography in candidal esophagitis. (b) Endoscopy of same patient.

ventilation of culture flasks and Isolator lysis-centrifugation cultivation have improved the diagnostic yield to 82%.[14] The value of surveillance cultures is controversial, although absence of *C. albicans* in surveillance cultures has a high predictive value for the absence of disseminated candidiasis in patients who have leukemia or lymphoma or in bone marrow transplantation patients.[2] [14] However, isolation of *C. tropicalis* in surveillance cultures is highly suggestive of disseminated infection because more than 50% of these patients will develop candidemia. Skin and eye lesions (Fig. 237.3) are present in only 10% of cases of disseminated candidiasis. In general, only 15–40% of cases are diagnosed early enough to initiate therapy.

For a detailed clinical discussion of disseminated candidiasis, candidemia and chronic disseminated or hepatosplenic candidiasis (Fig. 237.4), see Chapter 111 .

Virtually every organ can be infected by *Candida* spp.

Candidal meningitis and encephalitis usually occur as a complication of disseminated candidiasis. In disseminated candidiasis, 62% of cases developed candidal myocarditis,[13] with electrocardiographic changes mimicking infarction and supraventricular tachycardias.

Candida spp. are the major cause of fungal endocarditis, with up to 41% of cases caused by non-*albicans* spp. Blood cultures are positive in 54% of patients; additional means of diagnosis are culture of the cardiac vegetation (73% sensitivity) and histologic examinations.[18a]

Candidal pneumonia (Fig. 237.5) is usually associated with disseminated candidiasis. Primary candidal pneumonia is rare. Diagnosis is based on transbronchial biopsy.

In abdominal surgery, heavy growth of *Candida* spp. in the first culture (intraoperative or from abdominal drain) or an increasing

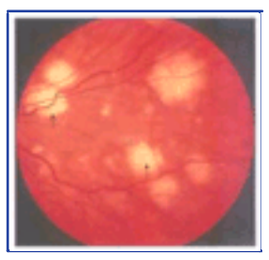

Figure 237-3 Retina of a patient who has lesions (arrows) due to disseminated candidiasis.

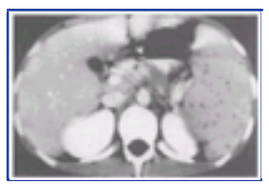

Figure 237-4 Hepatosplenic candidiasis. Multiple abscesses can be seen.

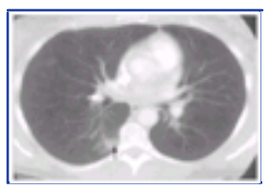

Figure 237-5 CT scan showing enlarged right hilar lymph node (top arrow) and a posterior infiltrate (bottom arrow) due to *Candida albicans.*

fungal load in serial cultures has been shown to be highly predictive of the development of candidiasis.

Infection of the urinary tract with *Candida* spp. is difficult to discriminate from colonization or from disseminated candidiasis. Microscopic urine analysis does not discriminate, unless renal casts containing yeasts are found, and quantitation of candiduria (such as is used for bacterial urinary infections) is not a reliable indicator of deep infection. Any patient who has persistent candiduria without a recent history of urinary tract instrumentation should be evaluated for diabetes mellitus, renal insufficiency or genitourinary tract abnormalities. [18]

'*Candida* hypersensitivity syndrome'

For many years, *C. albicans* has been mentioned as the cause of a chronic syndrome called *Candida* hypersensitivity syndrome, also known as chronic candidiasis, *Candida*-related complex and 'the yeast connection'.[19] Symptoms include general malaise, fatigue and non-specific gastrointestinal, genitourinary and neuropsychiatric complaints. The syndrome is said to be caused by intestinal and vaginal fungal overgrowth, inflammation, production of fungal toxins and invasion of mucous membranes. Therapy usually consists of a rigorous 'yeast elimination' diet and long-term antifungal treatment.

TABLE 237-5 -- Drugs approved for treatment of systemic fungal diseases.

DRUGS APPROVED FOR TREATMENT OF SYSTEMIC FUNGAL DISEASES				
Class	**Generic name**	**Brand name**	**Available formulation(s)**	**Year initially approved**
Polyene	Amphotericin B	Fungizone (Apothecon Products, Princeton, NJ)	Intravenous, oral solution	1958
Polyene	Amphotericin B lipid complex	Abelcet (Liposome, Princeton, NJ)	Intravenous	1995
Polyene	Amphotericin B cholesteryl sulfate	Amphotec (SEQUUS Pharmaceuticals, Menlo Park, CA)	Intravenous	1996
Polyene	Amphotericin B liposomal	AmBisome (Fujisawa Healthcare, Deerfield, IL)	Intravenous	1997
Pyrimidine	Flucytosine	Ancoban (ICN Pharmaceuticals, Costa Mesa, CA)	Oral tablet, intravenous	1972
Azole	Ketoconazole	Nizoral (Janssen Pharmaceutica, Titusville, NJ)	Oral tablet	1981
Azole	Fluconazole	Diflucan (Pfizer, New York, NY)	Intravenous, oral tablet, oral suspension	1990
Azole	Itraconazole	Sporanox (Janssen Pharmaceutica, Titusvilie, NJ)	Intravenous, oral capsule, oral solution	1992
Azole	Voriconazole	Vfend (Pfizer, New York, NY)	Intravenous, oral tablet	2002
Echinocandin	Caspofungin	Cancidas (MSD, Whitehouse Station, NJ)	Intravenous	2001

In an effort to characterize the disease, 100 individuals who had chronic fatigue syndrome were evaluated, including eight who had been given a diagnosis of 'yeast connection'. No differences in laboratory or physical findings, including candidal skin testing, could be detected between patients and control subjects. [20] A prospective randomized clinical trial comparing nystatin treatment with placebo failed to show an improvement in the systemic complaints [21] and analysis of the diet demonstrated nutritional imbalances that could lead to the development of nutritional deficiencies if the diet were followed over a prolonged period of time. The American Academy of Allergy and Immunology reviewed the literature and concluded that the *Candida* hypersensitivity syndrome is 'speculative and unproven'.[22]

MANAGEMENT

Recently the mycoses study group of the Infectious Diseases Society of America (IDSA) published practice guidelines for the treatment of fungal infections. [17] [23] These will be followed throughout this part of the chapter. The current drugs approved for treatment of systemic fungal diseases in the USA are listed in Table 237.5 . Their mechanism of action is shown in Fig. 237.6 .

Mucocutaneous candidiasis

Oropharyngeal candidiasis

Initial episodes can be treated with clotrimazole troches (one 10mg troche 5 times daily) or nystatin (available as a suspension of 100,000 U/ml (46ml 4 times daily) or as flavored 200,000 U pastilles (one or two 4–5 times daily) for 7–14 days). Oral fluconazole (100mg/day for 7–14 days orally) is as effective as and in some studies superior to topical therapy. Itraconazole solution (200mg/day for 7–14 days orally) is as efficacious as fluconazole. [24] Ketoconazole and itraconazole capsules are less effective than fluconazole because of variable absorption. Suppressive therapy is effective for the prevention of recurrent infections, but to reduce the likelihood of development of antifungal resistance it should be used only if the recurrences are frequent or disabling. Fluconazole-refractory oropharyngeal candidiasis will respond to itraconazole (200mg/day orally, preferably as the solution) approximately two-thirds of the time. Amphotericin B oral suspension (1ml 4 times daily of the 100mg/ml suspension) is sometimes effective in patients who do not respond to itraconazole. Intravenous amphotericin B (0.3mg/kg/day) is usually effective and may be used as a last resort in patients with refractory disease. Denture-related disease may require thorough disinfection of the denture for definitive cure.

Figure 237-6 Targets and mechanisms of action of antifungal drugs.

Esophageal candidiasis

Systemic therapy is required for effective treatment of esophageal candidiasis. Although symptoms of esophageal candidiasis may be mimicked by other pathogens, a diagnostic trial of antifungal therapy is often appropriate before endoscopy to search for other causes of esophagitis. A 14- to 21-day course of either fluconazole (100mg/day orally) or itraconazole solution (200mg/day orally) is highly effective. Suppressive therapy should be used occasionally in patients with disabling recurrent infections. Fluconazole-refractory esophageal candidiasis should be treated with itraconazole solution (200mg/day orally). Intravenous amphotericin B (0.3–0.7mg/kg/day as needed to produce a response) may be used in patients with otherwise refractory disease.

In mainly HIV-positive patients with esophageal and oropharyngeal candidiasis, the new antifungal agent caspofungin in a dosage of 35 or 50mg/day showed superior efficacy compared with low-dose amphotericin B (0.5mg/kg/day). This compound, representing the first clinically studied agent from a new class of antifungals inhibiting the synthesis of ß-(1,3)-D-glucan in the fungal cell wall, was extremely well tolerated.[25]

For *Candida*-associated denture stomatitis, the treatment of choice is fluconazole (50mg daily for 2 weeks) combined with chlorhexidine.[26]

Candidal vulvovaginitis

This may be classified into complicated (severe, resistant species, impaired host defenses) and uncomplicated forms. Uncomplicated vaginitis is seen in 90% of patients and responds readily to short-course oral or topical treatment with any of the therapies listed above, including the single-dose regimens. In contrast, the complicated vaginitis seen in 10% of patients requires antimycotic therapy for 7 days. Azole therapy is unreliable for non-*albicans* spp. of *Candida*. *Candida glabrata* and the other non-*albicans* infections frequently respond to topical boric acid 600mg/day for 14 days or topical flucytosine. Azole-resistant *C. albicans* infections are extremely rare.

Recurrent vaginitis is usually due to azole-susceptible *C. albicans*. After control of causal factors (e.g. uncontrolled diabetes), induction therapy with 2 weeks of a topical or oral azole should be followed by a maintenance regimen for 6 months. Suitable maintenance regimens include fluconazole (150mg orally every week), ketoconazole (100mg/day), itraconazole (100mg every other day) or daily therapy with any topical azole.

The persistent immunologic defect of chronic mucocutaneous candidiasis requires a long-term approach.[12] Systemic therapy is needed and all of the azole antifungal agents have been used successfully. The dosages required are similar to those used for other forms of mucocutaneous candidiasis. As with HIV-infected patients, development of resistance to these agents has also been described.

Systemic candidiasis

Candidemia and intravenous catheter-related candidal infections

Candida BSIs are frequently associated with clinical evidence of the sepsis syndrome and high associated attributable mortality. In addition, hematogenous seeding may compromise the function of one or more organs.

In the past, there was controversy regarding whether every candidemia should be treated. Currently, the consensus is that all patients with a positive blood culture should be treated, even if the sample is drawn from a catheter.[17] Additionally, indwelling intravascular catheters should be removed or changed, preferably without using a wire for replacement. The evidence for this recommendation is strongest in the non-neutropenic patient population. In neutropenic patients, the role of the gut as a source for disseminated candidiasis is evident from autopsy studies, but in an individual patient it is difficult to determine the relative contribution of gut versus catheter as the primary source of fungemia. An exception is made for fungemia due to *C. parapsilosis*, which is very frequently associated with catheters.

The standard treatment for candidemia was amphotericin B (0.7–1.5mg/kg/day) with or without 5-FC, but recent clinical trials have shown that for stable non-neutropenic and stable neutropenic patients who have not previously been exposed to azoles, fluconazole (at 400mg/day, not to exceed 6mg/kg intravenously for the first week) can be as effective as amphotericin B and is less toxic.[27] For neutropenic patients who have evidence of deep organ infection or for unstable patients, amphotericin B with or without 5-FC is advised. If fluconazole is used in these groups, a higher dose up to 800mg/day (12mg/kg) is preferable. Three lipid formulations of amphotericin B have been developed and approved for use in humans: amphotericin B lipid complex (ABLC), amphotericin B colloidal dispersion (ABCD) and liposomal amphotericin B (Ambisome). Only ABLC and liposomal amphotericin B have been approved for use in proven candidiasis. These approvals are for second-line therapy of patients who are intolerant of or refractory to therapy with conventional amphotericin B (defined in one study using ABLC as: failure of 500mg amphotericin B, initial renal insufficiency, creatinine clearance <25ml/min), a significant rise in creatinine (to 2.5mg/dl for adults or 1.5mg/dl for children) or severe acute administration-related toxicity. Open-label therapy of candidemia with ABCD at 26mg/kg/day has been successful. In a randomized trial, ABLC at 5mg/kg/day was found to be equivalent to 0.6–1.0mg/kg/day amphotericin B as therapy for nosocomial candidiasis (mostly candidemia). Trials with caspofungin and voriconazole in candidiasis are underway. A recent study showed that caspofungin is at least as effective as amphotericin B for the treatment of invasive candidiasis. [27A]

Antifungal treatment should be continued for at least 2 weeks after the last positive blood culture or after the clinical symptoms have subsided, and patients should be followed for 3–6 months to detect long-term sequelae due to hematogenous seeding.

An increased incidence of the less fluconazole-sensitive *C. krusei* and *C. glabrata* in neutropenic patients has been associated with frequent use of fluconazole as prophylaxis.[8] [9] This warrants caution in treating candidemia in immunocompromised patients with fluconazole before the *Candida* sp. has been identified (germ tube test), especially if there has been previous fluconazole exposure.

Data on the use of itraconazole in candidemia are limited. Unlike fluconazole, itraconazole also acts against *Aspergillus* spp., which is a theoretic advantage over fluconazole in neutropenic patients.

If 5-FC is added to amphotericin B, the dose (on average 100mg/kg/day) should be regularly adjusted according to peripheral blood cell counts, nomograms for renal functions or serum 5-FC levels (under 50–100µg/ml) to prevent bone marrow toxicity. Because of the rapid development of resistance, 5-FC should not be given as monotherapy.

Recommended treatment of candidal meningitis or encephalitis has been high-dose (0.7–1mg/kg) amphotericin B combined with flucytosine. The flucytosine dose should be adjusted to produce serum levels of 40–60µg/ml. The role of fluconazole remains to be determined. Data on the use of intrathecal amphotericin B or fluconazole are sporadic and this treatment should be reserved for severe therapy-refractory cases. Intracranial shunts should be removed. The occurrence of a brain abscess worsens the prognosis considerably. The indication for surgery remains to be determined but surgical drainage, if feasible, is probably advisable.[17]

Candidal endocarditis should be treated with combined antifungal treatment (amphotericin B plus 5-FC) and early valve replacement. Without surgical intervention, the mortality is high (90%); with combined surgical and medical treatment, the mortality has dropped to 45%.[17] Because of the high relapse rate of candidal endocarditis, continuation of amphotericin B plus 5-FC for at least 6–10 weeks (but possibly much longer) after surgery has been recommended. Because recurrences have occurred years later patients should be followed up for at least 2 years. Primary therapy with fluconazole has been successfully used on occasion, but fluconazole is more often employed as part of a long-term suppressive regimen.

In urinary candidiasis, the clinical circumstances dictate the management because candiduria can represent colonization, cystitis, pyelonephritis, disseminated candidemia or a fungus ball. If the candiduria is not catheter related and diabetes mellitus, neutropenia, urinary tract obstruction and urinary tract abnormalities have been ruled out and the patient is otherwise in good physical condition, observation is justified. Postcatheter candiduria in the stable patient usually resolves without treatment as well. In contrast, most patients who are neutropenic or who have had a recent renal transplant or have ureteric stents need systemic treatment with either amphotericin B or fluconazole (provided that *C. krusei* and *C. glabrata* have been ruled out). Treatment is not invariably indicated for catheter-related asymptomatic candiduria (eradication of candiduria in almost 40% of patients). Catheter-related candidal cystitis, where the catheter cannot be removed, can be treated with fluconazole 50–200mg/day for 2–3 days or a single intravenous dose of amphotericin B (0.3mg/kg). A recently completed placebo-controlled trial found that fluconazole at 200mg/day for 14 days hastened the time to a negative urine culture, but that 2 weeks after the end of therapy the frequency of a negative urine culture was the same in both treatment groups (60% for catheterized patients and 73% for non-catheterized patients).

Bladder irrigation with amphotericin B (50g/ml in sterile water or 5% glucose administered continuously over a three-way Foley catheter for 5–7 days) has been shown to clear the infection temporarily in over 80% and can be used as a diagnostic 'wash-out' test for systemic or renal candidiasis but is not recommended as definitive treatment. If there is a fungus ball in the urinary tract, then the treatment is surgical.

There are two major syndromes of peritoneal candidiasis. In disease related to peritoneal dialysis catheters, catheter removal is usually required for successful therapy. Both amphotericin B and fluconazole have been used successfully. Intraperitoneal amphotericin B has been associated with painful chemical peritonitis and should in general be avoided.

Candida peritonitis may also develop in association with surgical or traumatic injury to the gut wall. In this setting, *Candida* is usually part of a polymicrobial infection and case series suggest that therapy directed toward *Candida* is indicated, particularly when it is isolated as part of a complex infection or in an immunocompromised patient (as opposed to isolation in association with promptly repaired acute traumatic injury). The required duration of therapy for all forms of *Candida* peritonitis is not well defined and should be guided by the patient's response. In general, 2–3 weeks of therapy seems to be required. Surgical patients with recurrent gastrointestinal perforation are at increased risk for *Candida* peritonitis and may benefit from prophylactic antifungal therapy.

Chronic disseminated candidiasis (hepatosplenic candidiasis) is difficult to treat; this syndrome is not acutely life-threatening but does require prolonged therapy to produce a cure. Fluconazole at 6mg/kg/day is generally preferred in stable patients. Amphotericin B at 0.6–0.7mg/kg/day may be used in acutely ill patients or patients with refractory disease. Some but not all experts recommend an initial 1–2 week course of amphotericin B for all patients, followed by a prolonged course of fluconazole. Therapy should be continued until calcification or resolution of lesions, particularly in patients receiving continued chemotherapy or immunosuppression. Premature discontinuation of antifungal therapy may lead to recurrent infection.

Prophylaxis

Antifungal prophylaxis is highly controversial because the relationship between candidal colonization and dissemination is often not clear and azole prophylaxis may select for *Candida* spp. that are resistant to azoles.

Randomized, prospective, placebo-controlled trials have shown that systemically active antifungal agents can reduce the rate of development of invasive *Candida* infections in high-risk patients. The best data have compared fluconazole at 400mg/day with placebo in bone marrow transplant recipients.[9]

No consensus exists over the prophylactic use of azoles in other neutropenic patients.[17] Also, prophylaxis for high-risk (=2 risk factors such as retransplantation, creatinine <2mg/dl, choledochojejunostomy, intraoperative use of =40 units of blood products, fungal colonization within the first 3 days after transplantation) liver transplant recipients is advised.[17] The risk for candidiasis following pancreatic transplantation may be comparable to that following liver transplantation. The risk of invasive candidiasis following transplantation of other solid organs appears to be too low to warrant systemic prophylaxis.

Apart from pharmacologic prophylaxis, simple measures such as handwashing by medical personnel are of paramount importance, especially in preventing the spread of *C. parapsilosis*.[3]

ASPERGILLOSIS

NATURE

Aspergillus spp. can cause disease in humans by:

- colonization and subsequently allergic reactions,
- colonization of pre-existing cavities (fungus ball or aspergilloma), or
- tissue invasion.

Aspergilli are found ubiquitously in organic debris such as hay, decaying vegetation (compost piles), soil, potted plants, in pepper and spices and in construction sites. The genus *Aspergillus* contains over 250 species, although only a few are associated with disease.

EPIDEMIOLOGY

Worldwide, aspergillosis is the most common invasive mold infection. [28]

In a 10-year autopsy survey,[29] the overall incidence of invasive aspergillosis was 1.4%. In the subgroup of immunocompromised patients, however, the incidence was found to be 10.7% and was highest in liver transplant recipients and patients who have hematologic malignancies. Mini-outbreaks of aspergillosis in immunocompromised patients have been reported in association with local construction work.[30] The most common sites of infection are the lungs and the brain. In patients who have AIDS, invasive aspergillosis is often diagnosed at autopsy. Surveys of clinically diagnosed disease report an incidence of 0.9–8.6%,[31] all before the highly active antiretroviral therapy (HAART) era.

The reported prevalence of allergic bronchopulmonary aspergillosis (ABPA) in patients who have asthma varies from 6% to 28%. In cystic fibrosis, 6–25% of patients are reported to have ABPA.[32]

PATHOGENICITY

The main route of infection in aspergillosis is airborne, via inhalation of the 2.5–3.0µm conidia (spores), which settle in lungs, nose or paranasal sinuses. However, most studies suffer from a lack of correlation between air spore counts and the rate of infection. Very recently, the hospital water supply has been recognized as a source of infection.[33] Other routes of infection are traumatized skin, especially due to burns, insertion openings of indwelling intravenous catheters and intravenous drug use. No patient-to-patient spread has been described.

The main risk factors for invasive aspergillosis include prolonged profound granulocytopenia ($<0.5 \times 10^9$ /l), especially in bone marrow transplant recipients, high-dose corticosteroid therapy, broad-spectrum antibiotic therapy, chronic granulomatous disease, AIDS with a $CD4^+$ lymphocyte count below 50/l, and treatment with cytotoxic drugs such as ciclosporin. Less commonly encountered risk factors are diabetes mellitus, alcohol excess, influenza, prematurity and exposure to aspergilli in large quantities.[34]

In chronic granulomatous disease, phagocytes are unable to generate the respiratory burst to kill micro-organisms. The fact that these patients and patients who have neutropenia are at risk of developing aspergillosis underscores the importance of phagocytes in host defense. The role of T-cell-mediated immunity in aspergillosis is not clear. In the past, HIV infection was not considered to be an independent risk factor for aspergillosis, but this view has recently been challenged, even though patients who have late-stage HIV infection ($CD4^+$ lymphocyte count <50/l) frequently have other risk factors as well, such as neutropenia or treatment with glucocorticosteroids or broad-spectrum antibiotics.[34]

Alveolar macrophages and neutrophils play a major role in the defense against aspergilli.[35] Alveolar macrophages form the first line of defense by ingesting and killing conidia, even in the absence of opsonins. When conidia escape and germinate to form hyphae, granulocytes will adhere to and kill the fungus. The conidia that have not been cleared may germinate in the alveolar spaces and hyphal forms invade the pulmonary tissue and vasculature, leading to hematogenous dissemination to other organs (brain). However, blood cultures are rarely positive. Conidia that stay in the dormant state can survive for many days in the reticuloendothelial system. Killing of aspergilli by phagocytes is impaired by corticosteroids.

The role of humoral immunity is double-edged. In ABPA, aspergilli elicit an anti-aspergillus IgE antibody response, which evokes a type I immediate hypersensitivity reaction. Anti-aspergillus IgG antibodies have been demonstrated in ABPA, aspergilloma and in the convalescence phase of invasive aspergillosis. These antibodies do not seem to play a significant role in host defense.

The fungus secretes various metabolic products (gliotoxin) and enzymes, such as phospholipases, hemolysin and elastase, which may play a role in virulence.[34] However, the biologic significance of these agents is not clear, since molecular studies in which gene-deletion mutants have been constructed do not show diminished virulence.

DIAGNOSTIC MICROBIOLOGY

Aspergillus is a mold that grows with dichotomously branching septate hyphae (Fig. 237.7). Only pathogenic *Aspergillus* spp. are able to grow in temperatures between 95°F (35°C) and 98.6°F (38°C) and *A. fumigatus* can even grow at 127.4°F (53°C). *Aspergillus fumigatus* (80–90%) and *A. flavus* (5–10%) account for the majority of all *Aspergillus* infections. Other infections are caused by *A. niger* (1–2%), *A. terreus* (2–5%) and *A. nidulans* (<1%).

Aspergilli can be isolated from the sputum of 1–6% of healthy individuals. Also, *Aspergillus* colonization is found in patients who have chronic lung disease, cigarette smokers and those who have HIV infection. However, in immunocompromised patients who do not have AIDS, positive sputum cultures have a high positive predictive value for invasive aspergillosis, even without chest radiograph abnormalities. The positive predictive value may be as high as 80–90% in patients with leukemia or bone marrow transplants.

The negative predictive value of sputum cultures in these patients is low. Growth of aspergilli in cultures from nasal swabs of leukemic patients who have neutropenia has been shown to be predictive of invasive pulmonary aspergillosis.

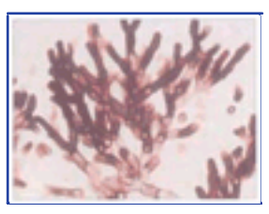

Figure 237-7 *Aspergillus* hyphae.

In invasive aspergillosis, positive blood cultures for aspergilli are rare. In neutropenia, *Aspergillus* spp. are responsible for 8% of all fungemias and there is an overall mortality of 56%. Patients who undergo cardiac surgery have a risk of developing fungemia and *Aspergillus* endocarditis, probably due to contamination of the operating room air.[34]

Even though *Aspergillus* spp. can grow on standard bacteriologic media, mycologic media will give a higher yield and are therefore recommended. A potential diagnostic and management problem is pseudofungemia (i.e. contamination of blood specimens of uninfected patients in the laboratory). For patients at risk, however, culture results have to be taken seriously until proven otherwise.[34]

Because of the ubiquitous nature of the organism, establishing a definitive diagnosis of disease caused by *Aspergillus* is difficult. The use of antibodies against *Aspergillus* to diagnose invasive aspergillosis has produced conflicting results. Patients in high-risk groups are too frequently falsely seronegative. With antibody or antigen testing, serial assays appear more valuable than isolated tests, but specific recommendations about frequency of testing have not been established. Several promising assays have been developed to detect *Aspergillus* galactomannan in urine, sera, cerebrospinal fluid and bronchoalveolar lavage specimens by EIA, ELISA and immunoblot. These appear more sensitive and reproducible than earlier latex agglutination methodology. Studies with an EIA system commercially available in Europe for detection of *Aspergillus* galactomannan reported positive predictive values of 54% and negative predictive values of 95%, largely among bone marrow transplant recipients in France.[36] [37] A problem in some studies with antigen testing was that case detection occurred too late to be useful, although early detection has been noted in some studies with ELISA methodology. In some studies, true positives were more frequent among the high titer results and false positives more common in children.

In addition, assays that use PCR have been reported to detect fragments within the 18S rRNA of *Aspergillus* spp., the 135-bp fragment in the mDNA of *Aspergillus* spp. and a 401-bp fragment in the rDNA complex of *A. fumigatus*, and PCR assays may prove more sensitive than antigen detection.[16] However, few of these PCR assays have been tested with body fluids in prospective trials of invasive aspergillosis, and reproducibility must be verified.

Therefore, a diagnosis of invasive aspergillosis is ideally based on a combination of culture results and histologic proof of tissue invasion.

CLINICAL MANIFESTATIONS

Clinical manifestations of aspergillus infections are also discussed in Chapter 111 .

Aspergillosis can manifest as either fungal colonization, which can cause allergic disease and saprophytic disease (aspergilloma), or invasive disease.

The spectrum of disease caused by *Aspergillus* spp. includes:

¦ noninvasive aspergillosis — sinotracheobronchial colonization, allergic (bronchopulmonary) aspergillosis, aspergilloma (i.e. secondary saprophytic colonization of pre-existing cavities) and obstructing bronchial aspergillosis (mainly in patients who have AIDS); and
¦ invasive aspergillosis with pulmonary involvement such as acute bronchopneumonia, cavitation or slowly progressive bronchopneumonia, or extrapulmonary involvement of paranasal sinuses, central nervous system (CNS), skin, bone and heart.

Allergic bronchopulmonary aspergillosis

Allergic aspergillosis can either manifest as an extrinsic allergic alveolitis, causing a hypersensitivity pneumonitis in nonatopic patients, or it can cause ABPA in atopic individuals.

For a diagnosis of ABPA, Rosenberg and Wang established seven criteria:[32]

¦ episodic bronchial obstruction (asthma),
¦ immediate cutaneous reactivity to *A. fumigatus*,
¦ elevated total serum IgE (<150IU/ml),
¦ precipitating antibodies to *Aspergillus* antigen,
¦ proximal bronchiectases,
¦ history of infiltrates on chest radiography, and
¦ peripheral blood eosinophilia at the same time as infiltrates on chest radiography.

The diagnosis of ABPA was felt likely if the first six diagnostic criteria were present, and the presence of all seven made the diagnosis certain. The presence of central bronchiectases in ABPA signifies more permanent lung damage. [32]

Aspergilloma

Aspergillomas are fungus balls that colonize pre-existing cavities in the lungs, and occasionally in the sinuses and the nose. They can cause massive hemoptysis, which is fatal in 10% of cases.[34] Other symptoms can include fever, malaise and weight loss. A pulmonary computerized tomography (CT) scan may show a cavitary mass surrounded by air. Microscopic and cultural identification of *Aspergillus* spp. in a sputum smear and high antibody titers to *Aspergillus* support the diagnosis. Over 95% of patients have detectable IgG antibodies to *Aspergillus*, and antibody levels usually decrease after successful surgical removal of the aspergilloma.

Invasive aspergillosis

Invasive aspergillosis is a life-threatening aggressive disease that affects immunocompromised patients and is described in detail in Chapter 111 .

If a pulmonary infiltrate (Fig. 237.8) occurs during granulocytopenia, it is more likely to be due to invasive aspergillosis than if an infiltrate develops during recovery from neutropenia, which is more

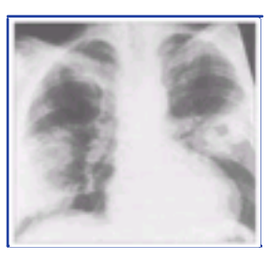

Figure 237-8 *Aspergillus* pneumonia.

likely to represent a resolving bacterial pneumonia.[38] If invasive pulmonary aspergillosis is suspected in an immunocompromised patient, then a chest CT scan or magnetic resonance imaging (MRI) should be performed, even if the chest radiograph shows no abnormalities. Typical abnormalities on CT scan in aspergillosis are a halo sign (low radiographic density around an area of infiltration), air crescent lesions or a cavitary lesion or consolidation combined with pneumothorax, although *Mucorales* can cause similar lesions.

A retrospective study of 300 immunocompromised patients, evaluating the diagnostic value of cytology from bronchoalveolar lavage (BAL) fluid for the diagnosis of invasive pulmonary aspergillosis, reported a sensitivity of 64%, a specificity of 99%, a positive predictive value of 84.2% and a negative predictive value of 97%. Compared with cytology, the positive and negative predictive values for culture from BAL fluid were slightly less favorable.[37] Colonization with aspergilli occurs, but an immunocompromised patient who has *Aspergillus* spp. in sputum or BAL fluid and fever or radiographic infiltrates that are not responding to antibiotic treatment warrants antifungal treatment.[37] [38]

For definite proof of invasive aspergillosis, additional biopsy results are important. Transbronchial biopsies are often negative in invasive aspergillosis due to sampling error, and so percutaneous or open lung biopsies may be required.

Recent studies published in abstract only show that a combination of HRCD scan of the thorax in combination with galactomannan detection in BAL has a high positive

predictive value.

Chronic invasive (necrotizing) aspergillosis

Chronic invasive aspergillosis is a more indolent form of pulmonary aspergillosis. This syndrome may be seen with aspergillomas, at the interface of the fungus ball and normal lung. Presumably, the slowly progressive nature of this infection is a function of the host immune response, which is enough to barely hold the organism in check, but not to completely kill it. CIA is usually seen in middle-aged and elderly patients with documented or suspected underlying lung diseases like COPD, inactive tuberculosis, previous lung resection, radiation therapy, pneumoconiosis, cystic fibrosis, lung infarction, or, rarely, sarcoidosis. It also has been described in patients with mild immunosuppression, including those with diabetes mellitus, those with poor nutrition, those undergoing low-dose corticosteroid therapy, and those with connective tissue diseases such as rheumatoid arthritis and ankylosing spondylitis Cough, hemoptysis, weight loss of 1 to 6 months' duration and low grade fever may be seen. The chest x-ray shows an infiltrative process in the upper lobes or the superior segments of the lower lobes. A fungal ball may be seen in nearly one half of the cases. Adjacent pleural thickening is a characteristic finding and may be an early indication of a locally invasive process. However, interpretation of radiologic studies may be complicated by the presence of concomitant lung disease, since this is the setting in which chronic invasive aspergillosis usually presents.

The diagnosis is confirmed by a histologic demonstration of tissue invasion by the fungus and the growth of Aspergillus species on a culture. However, the yield of transbronchial biopsy specimens or percutaneous aspirates is relatively poor, and a thoracoscopic or open-lung biopsy is rarely performed in these patients. So, a clinical diagnosis of CNA could be made using the following criteria:

- ¦ Clinical and radiologic features consistent with the diagnosis;
- ¦ Isolation of Aspergillus species by culture from sputum or from bronchoscopic or percutaneous samples; and
- ¦ Exclusion of other conditions with similar presentations, such as active tuberculosis, atypical mycobacterial infection, chronic cavitary histoplasmosis, or coccidioidomycosis.

Treatment with antifungal medications is indicated once the diagnosis is made.[38A] The response to antifungal therapy is generally favorable. Surgical resection is generally reserved for healthy young patients with focal disease and good pulmonary reserves, patients not tolerating antifungal therapy, and patients with residual localized but active disease despite adequate antifungal treatment. In the initial series by Binder et al,[38B] 90% of patients who underwent surgical resections had good responses. However, there were significant postoperative complications, and one patient died. The long-term prognosis for patients with CNA is not well-documented. In the original series, 73% of the patients were alive 1 to 2 years following therapy, and the majority of deaths were due to other causes.

Extrapulmonary aspergillosis

Extrapulmonary dissemination of pulmonary aspergillosis, especially to the brain, is common in the immunocompromised host. Meningitis due to *Aspergillus* spp. can occur, but it is rare. A multivariate discriminant analysis of autopsy-proven fungal infections of the CNS demonstrated that a combination of pulmonary infiltrates and focal neurologic disease in an immunocompromised patient is more likely to be caused by *Aspergillus* spp. than by either *Candida* spp. or *Cryptococcus neoformans*. Definite diagnosis is made by biopsy.[38]

Virtually any organ can be infected by *Aspergillus* spp. The fungus may cause local infection in the ear or eye (endophthalmitis, keratomycosis). Direct bony invasion can occur or hematogenous spread causing osteomyelitis. In the gastrointestinal tract, aspergillosis can lead to fatal perforation. Black necrotizing skin lesions can be a sign of disseminated aspergillosis.

As in candidiasis, aspergilli can form fungal balls in the urinary tract, which present as renal colic.

Fungal sinusitis

Fungal sinusitis (Fig. 237.9) should be considered in all patients who have chronic sinusitis. [39] Clinical features and predisposing conditions are listed in Table 237.6 . It is important to distinguish between noninvasive and invasive sinusitis because the latter can progress through invasion into soft tissue, cartilage and bone into the palate and nose or it can invade cerebral blood vessels, resulting in ischemic infarction and direct infection of the brain.

Chronic indolent invasive sinonasal infections occur in immunocompetent hosts in regions with high levels of environmental spores, such as the Sudan, Saudi Arabia, and other tropical or desert areas, and occasionally in patients with diabetes in other locales. *Aspergillus flavus* is the most common causative agent of these infections, in contrast to the frequent isolation of *A. fumigatus* from sites of infection in immunocompromised hosts. These infections have a progressive clinical course over months to years, with invasion of the surrounding tissues: the ethmoid sinuses, orbit and subsequent cranial bone osteomyelitis and intracranial extension.

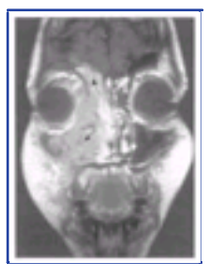

Figure 237-9 *Aspergillus* sinusitis. Involvement of right maxillary sinus with extension to adjacent structures and the brain (arrows). Coronal section.

TABLE 237-6 -- Key clinical points in the diagnosis and treatment of fungal sinusitis.*

KEY CLINICAL POINTS IN THE DIAGNOSIS AND TREATMENT OF FUNGAL SINUSITIS				
Type	**Clinical clues**	**Most common causes**	**Diagnosis**	**Initial management**
Noninvasive fungal sinusitis	Immunocompetent patient: intractable symptoms despite adequate treatment for bacterial sinusitis; allergic rhinitis, asthma, nasal polyps. Calcifications in sinus on CT; proptosis in children	Hyaline molds; *Aspergillus* spp.; *Fusarium* spp.; dematiaceous molds; *Bipolaris* spp.; *Curvularia lunata; Pseudallescheria boydii*	Aspiration of sinus contents should be followed by silver impregnation staining and culture of aspirate; sinus contents often have the consistency of peanut butter or cottage cheese; in patients who have diabetes mellitus or other conditions involving immunocompromise, biopsy of healthy and diseased mucosa and bone should be considered to rule out tissue invasion	Surgery is necessary to establish drainage and to remove impacted mucus, polyps or fungus ball
Invasive fungal sinusitis	Fever, headache, epistaxis and cough in an immunocompromised patient; diabetes mellitus; hemochromatosis; protein-calorie malnutrition; leukemia; neutropenia. Nasal mucosal ulcer or eschar; calcifications in sinus on CT; orbital apex syndrome; proptosis in adults	Hyaline molds, zygomycetes; *Rhizopus oryzae; Cunninghamella bertholetiae; Aspergillus* spp.; *Fusarium* spp.; dematiaceous molds; *P. boydii*	Early endoscopic evaluation should be followed by biopsy of healthy and diseased mucosa and bone; sinus contents should be cultured; aspiration of sinus contents should be followed by silver impregnation staining and culture of aspirate; if the results of endoscopic evaluation are negative, open biopsy should be performed immediately	Emergency surgery is necessary to remove necrotic and devitalized tissue; treatment with amphotericin B should be initiated on demonstration of tissue invasion and before culture results become available; immunosuppression should be reversed, including discontinuation of corticosteroids and treatment of iatrogenic neutropenia

** Adapted from DeShazo et al.[39]*

Radiologic findings associated with fungal sinusitis are calcifications and loss of bony sinus margins, as well as features that are common in bacterial sinusitis, such as air-fluid levels of more than 8mm of mucoperiosteal thickening.

To distinguish between invasive and noninvasive fungal sinusitis, adequate tissue biopsies as described in Table 237.6 are necessary. The presence of hyphae excludes chronic bacterial sinusitis. In superficial fungal disease, hyphae are found only in mucopurulent material within the sinus. In invasive fungal infection, hyphae penetrate into the sinus submucosa, blood vessels or bone.

The main differential diagnosis is zygomycosis.

Aspergillosis and AIDS

Aspergillosis and AIDS is discussed in Chapter 126 .

MANAGEMENT

In ABPA, the treatment of choice is systemic corticosteroids. Generally, prednisone is advised, 0.5mg/kg for 2 weeks followed by gradual tapering, although no randomized studies have been performed. Parameters for monitoring the treatment response are pulmonary function, chest radiograph and serum IgE concentration.

Another approach to the treatment of ABPA is to eradicate *Aspergillus* spp. from the airways. A recently completed double-blind, randomized, placebo-controlled trial[40] for ABPA showed that itraconazole 200mg twice daily for 16 weeks resulted in statistically significant differences in ability to ameliorate disease, as assessed by the reduction in corticosteroid dose and IgE and the improvement in exercise tolerance and in pulmonary function. Itraconazole may be useful as a corticosteroid-sparing agent. Detailed therapeutic guidelines are provided by Stevens *et al.*[41]

For aspergillomas, the natural history is variable, and therapy must be individualized. A conservative approach is prudent. Surgical resection (or where available, selective radiologic embolization of the feeding vessels) is indicated for hemoptysis. Spontaneous resolution of pulmonary aspergillomas has been reported.

The medical treatment options for invasive pulmonary aspergillosis are listed in Table 237.7 . For neutropenic patients, the isolation of *Aspergillus* spp. from any site warrants immediate antifungal treatment. The same is true for any sudden intracranial event in this group, especially in the presence of pulmonary infiltrates, with or without fever.[38]

The largest therapeutic experience is with amphotericin B deoxycholate, which should be given at maximum tolerated doses (e.g. 1–1.5mg/kg/day) and should be continued, despite modest increases in serum creatinine levels. Lipid formulations of amphotericin are indicated for the patient who has impaired renal function or who develops nephrotoxicity while receiving deoxycholate amphotericin.

Itraconazole, the only triazole presently licensed for the treatment of aspergillosis, has recently become available for intravenous administration. In neutropenic patients with hematologic malignancies and refractory fever, intravenous itraconazole has shown at least equivalent efficacy (47% vs 38%) to D-AmB.[42] Not surprisingly, it was better tolerated (withdrawal due to toxicity 19% vs 38%) and showed less nephrotoxicity than D-AmB. Beyond that, about one-third of patients given intravenous itraconazole could be switched to the oral formulation after a median of 9 days of treatment.

Caspofungin has also shown significant efficacy as a salvage antifungal in 63 patients with documented invasive aspergillosis refractory or intolerant to conventional antifungals.[43] Based upon the clearly documented response to caspofungin in 41% of these patients, the drug was approved in early 2001 by the Food and Drug Administration (FDA) for the treatment of patients with aspergillosis refractory or intolerant to other licensed antifungals.

The US Mycoses Study Group and the Invasive Fungal Infections Co-operative Group of the European Organization for Research and Treatment of Cancer recently reported that in patients with proven or probable invasive aspergillosis, voriconazole is significantly superior to AmB with respect to response rates (53% vs 31%) and survival (71% vs 58%).[44] Walsh *et al.*[45] were able to show that voriconazole was as effective as L-AmB for empiric therapy in neutropenic patients with fever refractory to broad-spectrum antibiotics. The rate of breakthrough fungal infections was significantly lower in patients treated with voriconazole compared even with those receiving L-AmB (1.9% vs 5%). However, the response rates (defined as defervescence during neutropenia and absence of breakthrough

TABLE 237-7 -- First- and second-line therapy for invasive aspergillosis.*

FIRST- AND SECOND-LINE THERAPY FOR INVASIVE ASPERGILLOSIS		
Generic (trade) name	**Dosage**	**Comments**
Amphotericin B deoxycholate	0.8–1.25mg/kg/day iv	High doses in neutropenia; significant interaction with ciclosporin
Itraconazole	Intravenous form, recently available 200mg iv qd; 200mg q8h for 4 days, then 200mg q12h po (for cerebral disease 400mg q12h)	If patient can eat and does not take hepatic cytochrome P450-inducing co-medication; significant interaction with ciclosporin; measure itraconazole serum levels; new itraconazole cyclodextrin oral solution has better bioavailability; limited data on iv use >14 days
Voriconazole	Loading 6mg/kg twice daily, thereafter 3mg/kg twice daily or 200mg orally twice daily	At least as effective as liposomal amphotericin B for emperical antifungal therapy in febrile neutropenia[45] More effective than D-AmB for invasive aspergillosis[45]
Caspofungin	70mg iv on day 1	FDA approved for refractory aspergillosis
	50mg iv thereafter	
Lipid-formulations of amphotericin (all iv)		
Liposomal amphotericin (AmBisome)	1–5mg/kg/day	Less toxic than amphotericin B deoxycholate, but higher dosage needed to be equally effective
Amphotericin B colloidal dispersion (ABCD, Amphotec)	4–6mg/kg/day	Less toxic than amphotericin B deoxycholate, but higher dosage needed to be equally effective; expensive
Amphotericin B lipid complex (ABLC, Abelcet)	5mg/kg/day	Less toxic than amphotericin B but higher dosage needed to be equally effective; expensive

* *Adapted from Denning.*[28]

fungal infections and no withdrawal due to adverse events and survival) were disappointing in both groups (26% vs 31%). Two issues with voriconazole must be taken into account: the occurrence of transient visual disturbances in about 30% of patients, and the considerable number of drugs showing important interactions with voriconazole.[45]

The optimal duration of therapy is unknown and dependent on the extent of invasive aspergillosis, the response to therapy and the patient's underlying disease(s) or immune status. A reasonable course would be to continue therapy to treat microfoci, after clinical and radiographic abnormalities are resolving, cultures (if they can be readily obtained) are negative, and reversible underlying predispositions have abated. Duration of therapy should be guided by clinical response rather than any arbitrary total dose. Continuation of antifungal therapy through reinduction cancer chemotherapy, or resumption of antifungal therapy in patients with apparently resolved fungal infection who are about to receive reinduction chemotherapy, is worthy of consideration. The ultimate response of these patients to antifungal therapy is largely related to host factors, such as the resolution of neutropenia and the return of neutrophil function, lessening immunosuppression and the return of graft function

from a bone marrow or organ transplant, as well as the extent of aspergillosis when diagnosed.

PREVENTION

If untreated, the mortality of invasive aspergillosis reaches 100%.[28]

Patients who have cancer and who develop invasive aspergillosis during cytotoxic chemotherapy have been shown to have a 50% risk of recurrence during subsequent cytotoxic chemotherapy cycles. Therefore, empiric administration of amphotericin B or voriconazole at the onset of granulocytopenia with fever or even before the development of neutropenia has been advocated if the patient has a positive history of invasive aspergillosis or residual cavitary disease.

Preventive measures include reduction of environmental exposure of patients from sources of infection and antifungal prophylaxis. Specialized air-handling systems capable of excluding *Aspergillus* spores, such as high-efficiency particulate air (HEPA) filtration with or without laminar air flow ventilation, have proven to be very efficacious. Given recent data, the water systems should be evaluated in facilities with high-risk patients. Targeted antifungal prophylaxis for hematologic patients who are at high risk for developing invasive fungal infections is not currently recommended.

CRYPTOCOCCOSIS

NATURE

Cryptococcosis is a systemic infection caused by the encapsulated yeast-like fungus *Cryptococcus neoformans*. As early as 1894, Otto Busse described 'corpuscular' tumor-like lesions caused by 'coccidia species'. Since then *C. neoformans* has been known by a variety of names, including *Saccharomyces neoformans* and *Torula histolytica*. Of the existing cryptococcal species, only *neoformans* is known to be pathogenic.

According to the chemical structure of the cryptococcal polysaccharide capsule, four serotypes can be distinguished in three varieties:

- serotype A: *C. neoformans* var. *grubii*;
- serotype D: *C. neoformans* var. *neoformans*; and
- serotypes B and C: *C. neoformans* var. *gattii*.

The sexual form of *C. neoformans* is classified as *Filobasidiella neoformans*, and has two mating phenotypes: a- and *a*-mating type. In clinical isolates, the a-mating type is always predominant.[46]

Cryptococcus neoformans var. *neoformans* and *grubii* are ubiquitous. They are found year round, especially in aged pigeon droppings, and affect mainly the immunocompromised host.

Cryptococcus neoformans var. *gattii* has a tropical and subtropical distribution and is found mainly in Australia, South America, parts of Africa, South East Asia, southern Europe and southern California, where it is associated with the river red gum tree (*Eucalyptus camaldulensis*) and the forest red gum tree (*Eucalyptus tereticornis*). Recently, a Vancouver Island outbreak was detected. *Cryptococcus neoformans* var. *gattii* infects mainly healthy individuals. The peak incidence of disease coincides with the flowering season of the eucalyptus tree, namely November through February.

EPIDEMIOLOGY

Cryptococcal meningitis is the most common life-threatening fungal infection in people who have HIV infection.

The incidence of cryptococcosis in patients who have AIDS in the USA, western Europe and Australia has been estimated to be 6–10%, but a recent analysis of survival data of patients who have AIDS in the USA before the introduction of HAART demonstrated a decrease in the cryptococcosis-associated death rate from 7.7% in 1987 to 5% in 1992,[47] probably due to the widespread use of azoles for candidiasis. Since the introduction of HAART, a further decline in incidence of cryptococcosis has been seen.[48]

In sub-Saharan Africa, the estimated incidence of cryptococcosis in AIDS patients is 15–30%. In Zimbabwe, cryptococcosis constitutes the AIDS-defining illness in 88% of HIV-infected patients, where it currently represents the most important cause of meningitis in adults.[49] In people who have AIDS, almost 100% of cases are caused by *C. neoformans* var. *grubii*, even in areas where *C. neoformans* var. *gattii* is endemic.[50] In France, 21% of *C. neoformans* infections are caused by serotype D, which is associated with a higher incidence of skin lesions and a lower incidence of meningitis than serotype A.[51] In the immunocompetent host, 70–80% of cryptococcal infections are caused by *C. neoformans* var. *gattii*. [52]

For unknown reasons, the incidence of cryptococcal meningitis in children is much lower than statistically expected.[50]

Use of novel immunosuppressive agents may also have a role in the changing frequency, spectrum and clinical presentation of opportunistic mycoses. With the declining incidence of *C. neoformans* infection in HIV-infected patients treated with HAART, organ transplant recipients have re-emerged as one of the leading groups of immunocompromised patients at risk for cryptococcal infections.[3]

PATHOGENICITY

Cryptococcus neoformans is a facultative intracellular organism. Its pathogenicity depends upon the immune status of the host, virulence factors of the *C. neoformans* strain and the size of the inoculum.

Risk factors for cryptococcosis include (in decreasing order of frequency):

- HIV infection (especially with a low $CD4^+$ lymphocyte count),
- corticosteroid treatment,
- organ transplantation,
- chronic leukemia,
- lymphoma, and
- sarcoid, even without corticosteroid treatment.

The main fungal virulence factors consist of its:

- polysaccharide capsule,
- production of melanin and mannitol,
- alpha mating type, and
- mannoprotein-4.[53]

The polysaccharide capsule is known to interfere with phagocytosis, antigen presentation and leukocyte migration, and it can activate immunosuppressive T cells.[46] Most clinical cryptococcal strains develop large capsules during infection.[46] The cryptococcal metabolic products melanin and mannitol can function as antioxidants that can protect the yeast against oxidative attacks of phagocytes. It is not known why the alpha mating type of *C. neoformans* is correlated with enhanced virulence.[50] An interesting and still incompletely explained observation is that more than 95% of in vivo and in vitro isolates are of the alpha mating type. Moreover, studies in mice showed that this mating type was also more virulent.[50]

No direct animal-to-human cryptococcal transmission is known. Human-to-human transmission has only been described through infected corneal transplants. Laboratory technicians working with *C. neoformans* and pigeon breeders have a high incidence of positive skin tests, but active cryptococcal disease in these groups is extremely rare, except for a local cryptococcoma after accidental direct inoculation with yeasts. Infection with *C. neoformans* is thought to occur through inhalation of poorly encapsulated or acapsular organisms. Once the yeast is inhaled, it starts to regenerate its capsule. The first line of defense consists of alveolar macrophages, followed by infiltration of neutrophils and later T cells, natural killer cells and monocytes. Neutrophils can kill opsonized cryptococci in vitro. There is evidence that the capsular polysaccharide of *C. neoformans* plays an active role in interfering with neutrophil migration into the subarachnoid space during inflammation,[54] [55] despite the presence of the neutrophil attractant interleukin (IL)-8 in the cerebrospinal fluid (CSF).[56] Recently, we have identified mannoprotein-4 as a novel capsular antigen prematurely activating neutrophils and desensitizing them toward a chemoattractant challenge. [53]

However, the role of neutrophils in the host defense against *C. neoformans* is not clear because patients who have severe neutropenia are not at risk of developing cryptococcosis. Experimental data have shown that functional T cells are essential in restricting cryptococcal spread. With intact T-cell function, confinement of yeasts and granuloma formation has been observed. Occasionally, *C. neoformans* can be found in granulomatous pulmonary lesions and reactivates upon decreasing host

resistance and appears analogous to the primary complex in tuberculosis. [57]

The role of antibodies in the defense against *C. neoformans* is not clear, but passive immunization with anticapsular antibodies has been shown to prolong survival in mice infected with *C. neoformans*. Antibodies do play a role in opsonization and antibody-dependent cellular cytotoxicity, as well as clearance of deleterious levels of capsular polysaccharide.

A case-controlled study of the human antibody response to *C. neoformans*, comparing the serum antibody profiles of HIV-infected persons who did (HIV+/CM+) or did not (HIV-infected controls) develop cryptococcal meningitis (CM) and HIV-uninfected persons with samples obtained from the Multicenter AIDS Cohort Study, was performed. Compared with the HIV+/CM+ group, the HIV-infected control group had significantly lower levels of total IgM, IgA and antibodies expressing a certain VH3 determinant. The HIV-infected control group manifested an increase in immunoglobulin levels with a decrease in CD4 lymphocytes. The findings suggest a possible association between reduced expression of certain immunoglobulin subsets and HIV-associated CM.[58]

DIAGNOSTIC MICROBIOLOGY

Cryptococcus neoformans grows on routine laboratory media such as Sabouraud agar, producing white-to-cream colored mucoid colonies, which develop within 36–72 hours (Fig. 237.10). Unlike

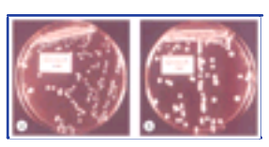

Figure 237-10 ***Cryptococcus neoformans.*** (a) Thinly encapsuiated. (b) With a thick capsule.

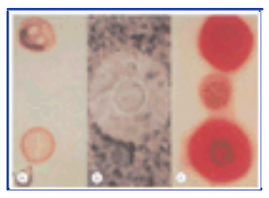

Figure 237-11 ***Cryptococcus neoformans.*** (a) Cytologic preparation of CSF, (b) India ink preparation, (c) Mayer's mucicarmine stain. *With permission from Jaster and Malecha. Copyright 1996 Massachusetts Medical Society. All rights reserved.*

nonpathogenic cryptococci, *C. neoformans* replicates at 98.6°F (37°C). For blood cultures, the lysis-centrifugation (Isolator) method has been recommended. Because *C. neoformans* is susceptible to cycloheximide, media containing cycloheximide should not be used. A rapid test to identify *C. neoformans* is the urease test because cryptococci produce large amounts of urease in the presence of urea (as do all basidiomycetous yeasts). To distinguish *C. gattii* from *C. neoformans* var. *neoformans*, glycine-L-canavanine-bromothymol blue agar can be used.

Microscopically, *C. neoformans* can be distinguished from other yeasts by its capsule, which is visualized by an India ink preparation (Fig. 237.11). The preparation is made by mixing equal volumes of sample fluid and ink, and ideally one should be barely able to read a newspaper through the preparation. The preparation typically shows budding yeast with a double refractive wall, distinctly outlined capsule and refractive inclusions in the cytoplasm. Occasionally, short hyphal yeast forms can be seen.

The laboratory diagnosis of cryptococcosis is rather straightforward compared with that of other fungal infections (see Table 237.3).

Latex agglutination tests (or ELISA) for cryptococcal antigen in body fluids are sensitive and specific and detect all serotypes. Due to differences between various commercial kits, absolute titer values are not interchangeable. In general, however, a positive test at a dilution of greater than 1:4 is highly suggestive of cryptococcal infection. False-positive results can be caused by rheumatoid factor or (rarely) through cross-reaction with other micro-organisms such as *Trichosporon beigelii* and the bacterium *Capnocytophaga canimorsus* or contamination of the specimen with agar or agarose in the laboratory. In CSF, false-negative results can be caused by either low or high antigen titers (prozone effect, especially in patients who have AIDS) or because of immune complex formation. Pronase pretreatment of the sample reduces both prozone reactions and rheumatoid factor interactions. Antigen testing in CSF is more sensitive than India ink preparation or culture. In patients who have pulmonary cryptococcosis without dissemination, serum samples may test negative for cryptococcal antigen. However, in these cases, *C. neoformans* antigen is likely to be positive in BAL fluid. Cryptococci are occasionally isolated from the sputum. In patients who are not immunocompromised it is safe to keep the patient under close observation without starting treatment. In all other cases, a careful search for infection of other sites should be made (including CSF examination), and there should be a low threshold for initiating therapy.

Antibodies to *C. neoformans* have no diagnostic value and are found in healthy people as well as in those with cryptococcosis. Presumably, antibodies have a favorable prognostic value when they become positive during convalescence in patients who do not have AIDS. In pulmonary cryptococcosis, antibodies may interfere with antigen testing due to the formation of complexes, necessitating pronase pretreatment of the samples.

For epidemiologic purposes, individual cryptococcal strains can be biotyped (e.g. AFLP or RAPD) or fingerprinted by restriction analysis or enzymatic methods.

CLINICAL MANIFESTATIONS

Clinical manifestations of cryptococcosis are also discussed in Chapter 111 and Chapter 126 .

Although *C. neoformans* usually enters the body through the lungs, the main site of infection is the CNS. However, any other organ can be involved, mainly skin, bone, prostate and eye.

The clinical picture of cryptococcosis in patients who have AIDS resembles that in severely immunocompromised patients who do not have HIV infection. In patients who have AIDS, the fungal burden is usually higher and they have a higher frequency of positive blood and urine cultures, more extraneural sites of infection and a higher relapse rate, and fewer CSF inflammatory cells.[56] In the rare cases where patients who have AIDS are infected with *C. neoformans* var. *gattii*, the clinical picture resembles that of *C. neoformans* var. *neoformans* or *grubii*.

In the immunocompetent host, disease is usually more focal, with cryptococcoma formation (especially with *C. neoformans* var. *gattii*) and disease is more often confined to the lungs.[52] Although survival is higher for infection with *C. neoformans* var. *gattii*, it causes more neurologic complications, residual disease and relapses than *C. neoformans* var. *neoformans* and *grubii*. [51] [52]

Pulmonary involvement

The clinical picture of pulmonary cryptococcosis depends upon the immune status of the host. In the immunocompetent host, one-third of patients are asymptomatic, and in some cases isolation of *C. neoformans* may represent colonization. The majority of patients present with pulmonary symptoms such as cough (54%), chest pain (46%) and sputum production (32%). If the cryptococcosis is confined to the lungs, cultures and antigen titers in CSF, blood and urine can be negative.

Compared with the immunocompetent host, cryptococcosis in the immunocompromised patient who does not have HIV infection has a more rapid course with early dissemination. In an analysis of 41 cases, 83% of patients presented with constitutional symptoms such as fever and malaise, and 44% presented with chest pain.[59]

Patients who have HIV infection and pulmonary cryptococcosis are almost invariably symptomatic, presenting with fever (84%), cough (63%), dyspnea (50%), weight loss (47%) and headache (41%). They have disseminated disease, as shown by positive cultures and antigen testing in CSF, blood and urine. Most patients have low $CD4^+$ lymphocyte counts, often less than 100,000/ml.

Pulmonary cryptococcosis is diagnosed through antigen detection or culture of expectorated sputum, BAL, transbronchial lung biopsy (Fig. 237.12) or needle

aspiration. In all cases, serum and CSF analysis for antigen and cryptococcal culture should be performed to assess dissemination.

Central nervous system involvement

If the CNS is involved in cryptococcosis, both the brain and the meninges are usually diffusely affected. In the immunocompromised patient, cryptococcomas and focal signs of disease are rare. Approximately 70–90% of patients present with signs of subacute meningitis

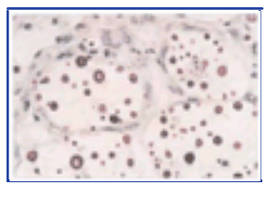
Figure 237-12 Histology of pulmonary cryptococcosis.

or meningoencephalitis: headache, fever, irritability, dizziness, memory loss, personality change, somnolence, confusion or obtundation. Classic signs of meningitis such as nuchal rigidity are often absent. Papilledema is seen in one-third and cranial nerve palsies in one-fifth of cases. Symptoms may wax and wane over weeks to months and are often non-specific.

Cryptococcal meningoencephalitis in immunocompromised patients who do not have HIV infection resembles the clinical picture in patients who do have HIV infection, with three exceptions:

- the duration of symptoms is usually shorter in patients who have AIDS, due to paucity of the inflammatory response and high fungal burden;
- patients who have AIDS present more often earlier in the disease with a second site of infection such as lungs, skin or blood; and
- concomitant illness such as infections (e.g. due to *Toxoplasma gondii*) or neoplasms is more likely.

Because focal neurologic symptoms due to cryptococcosis occur in only 10% of patients who have HIV infection, these symptoms should alert the physician to concomitant pathology. Patients who are successfully treated for cryptococcosis but who present with insidious mental impairment, ataxia or other neurologic signs should be evaluated for hydrocephalus by CT. Shunting is indicated for hydrocephalus.

Analysis of CSF in cryptococcal meningitis will typically yield the following findings:

- high opening pressure (reflecting raised intracranial pressure),
- CSF glucose level that may be either low or normal, and
- elevated or normal protein levels.

Usually, the CSF contains remarkably few cells with mononuclear predominance, especially in patients who have AIDS.[56] Cryptococci can be cultured or identified directly through staining with India ink (see above, under Diagnostic microbiology); cryptococcal antigen levels in CSF and serum are almost always positive.

Cerebral CT findings can be normal in 50% of patients who have cryptococcal meningitis, regardless of whether they have HIV infection. In patients who do not have AIDS, the CT may reveal hydrocephalus, gyral enhancement and/or multiple focal nodules with or without contrast enhancement. In patients who have AIDS, diffuse cortical atrophy is more common. Cerebral MRI appears to be more sensitive than CT in cryptococcal meningoencephalitis. The finding of pseudocysts and choroidal ependymal granulomas (cryptococcomas) is thought to be relatively specific for cryptococcosis. Lesions due to *C. neoformans* var. *gattii* tend to be fewer in number, bigger in size and surrounded by edema as compared with those caused by *C. neoformans* var. *neoformans* and *grubii*.

Other sites of infection

About 10–15% of patients will develop skin manifestations, which can present in many forms. For unknown reasons, serotype D (var. *neoformans*) is relatively more often associated with skin lesions than serotype A.[51] In patients who do not have AIDS, skin lesions can be the sole site of infection; however, in patients who have AIDS, cryptococcal skin lesions are almost always a sign of disseminated disease. A diagnosis of cryptococcal skin disease is confirmed by biopsy.

The eye is a frequent site of infection and any part of the eye may be involved. Visual loss is a distinct threat, especially in cases of endophthalmitis, although some patients have visual loss without signs of endophthalmitis, possibly due to optic neuritis or elevated intracranial pressure. Early diagnosis and rapid treatment are essential to preserve sight.[50]

From 5% to 10% of patients have bone lesions, which are mostly osteolytic and must be distinguished from cold abscesses (e.g. due to tuberculosis) or neoplasms.

Many other body sites can be affected by *C. neoformans*. In men, the prostate gland is thought to serve as an extraneural reservoir and source of relapse.

Interestingly, solid organ transplant recipients receiving tacrolimus were significantly less likely to have CNS involvement than were those receiving a different agent (ciclosporin or azathioprine), on the basis of immunosuppression (78% vs 11%, respectively). On the contrary, skin, soft tissue and/or osteoarticular involvement was significantly more likely in patients receiving tacrolimus (66%) than in patients receiving a different agent.[3]

MANAGEMENT

In isolated pulmonary cryptococcosis and other non-CNS disease in patients who do not have HIV infection, patients can be treated according to their risk and disease severity. In the immunocompetent asymptomatic patient who has minor lesions on the chest radiograph and no extrapulmonary dissemination, careful observation or fluconazole monotherapy (200–400mg/day for 3–6 months) is justified because many undergo spontaneous regression. Every patient who has symptomatic or disseminated disease or a compromised immune system should be treated with antifungal medication. In all cases, lumbar puncture should be performed to exclude meningeal involvement.

There is no consensus about the treatment schedule for pulmonary cryptococcosis and other non-CNS disease. For patients who have extensive lobar consolidation or mass lesions (more frequent with *C. neoformans* var. *gattii*), surgical resection of the lesions can be warranted.

All patients who have cryptococcal meningitis should be treated, regardless of their immune status, because 10–20% will either die or develop serious neurologic sequelae.

For patients who have cryptococcal meningitis and AIDS, the antifungal regimen of choice is high-dose amphotericin B (0.7mg/kg/day) plus 5-FC (100mg/kg/day) for 2 weeks, followed by consolidation therapy with fluconazole 400mg/day for 8–10 weeks, and subsequent lifelong maintenance therapy at 200mg/day. This regimen has been shown to reduce the mortality from about 15% to 6% in the first 2 weeks in a recently published double-blind randomized multicenter trial.[60] Combination therapy of amphotericin B and flucytosine will sterilize CSF within 2 weeks of treatment in 60–90% of patients.

The addition of flucytosine to amphotericin B was independently associated with earlier CSF sterilization, and 5-FC is thought to be important in preventing early relapse. Response rates are lower if CSF cultures at 2 weeks are still positive and these patients may require longer courses of induction therapy. For consolidation therapy, itraconazole (400mg/day) compared only slightly less

TABLE 237-8 -- Preferred treatment options for cryptococcal disease in HIV-negative patients.*

PREFERRED TREATMENT OPTIONS FOR CRYPTOCOCCAL DISEASE IN HIV-NEGATIVE PATIENTS
Pulmonary and other non-CNS disease
Mild-to-moderate symptoms or culture-positive specimen from this site
Fluconazole, 200–400mg/day for 6–12months
Itraconazole, 200–400mg/day for 6–12months
Amphotericin B, 0.5–1mg/kg/day (total 1000–2000mg)
Severe symptoms and immunocompromised hosts
Treat like CNS disease
CNS
Induction/consolidation: amphotericin B 0.7–1mg/kg/day plus flucytosine 100mg/kg/day for 2 wk, then fluconazole 400mg/day for minimum 10 wk
Amphotericin B 0.7–1 mg/kg/day plus flucytosine 100mg/kg/day for 6–10 wk
Amphotericin B 0.7–1mg/kg/day for 6–10 wk
Lipid formulation of amphotericin B 3–6mg/kg/day for 6–10 wk
The clinician must determine whether to follow lung therapeutic regimen or CNS (disseminated) regimen for treatment of infection in other body sites (e.g. skin). When other disseminated sites of infection are noted or the patient is at risk for disseminated infection. It is important to rule out CNS disease. Duration of therapy is based on resolution of disease.

** Adapted from Saag et al.[59]*

favorably to fluconazole, which makes it a reasonable alternative to fluconazole for maintenance therapy (at 10 weeks after diagnosis, fluconazole maintenance therapy resulted in 72% negative CSF cultures vs 60% with itraconazole[59]). Weekly intravenous amphotericin B has been shown to be inferior to fluconazole in preventing relapse of cryptococcal disease in patients who have AIDS.

It has been shown that maintenance therapy can be safely discontinued if the CD4 cell count is >200 for 3 months.[61]

For patients who have cryptococcal meningitis (Table 237.8), but who do not have AIDS, the regimen of choice is less well defined. Treatment with low-dose amphotericin B plus 5-FC has proved to be as effective and less toxic than amphotericin B alone in this group and it is likely that the above-mentioned regimen of 2 weeks of amphotericin with 5-FC intravenously followed by high doses of fluconazole for 8 weeks will also be effective in patients who do not have HIV infection. A total treatment duration of 4–6 weeks has been successful, but the relapse rate may be lower with longer treatment courses or azole consolidation therapy.

It is advisable to follow patients closely over the first 6–12 months because most relapses occur in the first year after treatment. In male patients who do not have AIDS, positive urine culture after prostatic massage can predict a high risk for recurrence and prolonged oral treatment is advisable in these cases.

For those who have pre-existing neutropenia, amphotericin B (0.7–1.0mg/kg/day) monotherapy can be given to avoid additional bone marrow toxicity. Fluconazole as monotherapy is associated with a higher mortality in the first 2 weeks, with a slower CSF clearing rate than with amphotericin B.

For patients with elevated baseline opening pressure, lumbar drainage should remove enough CSF to reduce the opening pressure by 50%. Patients should initially undergo daily lumbar punctures to maintain CSF opening pressure in the normal range. When the CSF pressure is normal for several days, the procedure can be suspended. Occasionally, patients who present with extremely high opening pressures (>400mmH_2 O) may require a lumbar drain, especially when frequent lumbar punctures are required to or fail to control symptoms of elevated intracranial pressure. In cases where repeated lumbar punctures or use of a lumbar drain fail to control elevated pressure symptoms, or when persistent or progressive neurologic deficits are present, a ventriculoperitoneal shunt is indicated. There are no data to support the routine use of direct intraventricular therapy (e.g. via an Ommaya reservoir).

Despite wide use of triazole (and other antifungal) drugs, drug resistance has not yet been a serious concern in cryptococcocal infections.

PROGNOSIS

For patients who do not have AIDS or cancer, the mortality rate due to cryptococcal infections is about 25–30%. After initial curative treatment, 20–25% of patients who do not have AIDS relapse. Among cured patients 40% have significant neurologic deficits such as visual loss, cranial nerve palsy, motor dysfunction, personality change and decreased mental function due to chronic increased intracranial pressure or hydrocephalus. Mortality in patients after solid organ transplantation is, given the current antifungal regimens, still high at 40%.[5]

For patients who have AIDS, the mortality rate during initial therapy has been 10–25%, and 30–60% of patients die within 12 months. The relapse rate without maintenance treatment is 50–60%. Currently, the prognosis is mainly determined by the response to HAART. The prognosis for patients who have a malignancy is worse than for patients who have AIDS, but this probably reflects the course of the underlying disease rather than the cryptococcosis.[50]

The most important prognostic predictor of early mortality in cryptococcal meningitis is the mental status of the patient at presentation.[62] Also, the prognosis is adversely affected by a high fungal burden and a poor inflammatory response.

Adverse prognostic clinical features in patients who do not have HIV infection are listed in Table 237.9 . [63] Other adverse clinical prognostic signs include altered mental status and blood pressure changes.

PREVENTION

Prospective controlled trials indicate that fluconazole and itraconazole can reduce the frequency of cryptococcal disease among

TABLE 237-9 -- Adverse prognostic clinical features in cryptococcal meningitis in patients who do not have HIV infection.

ADVERSE PROGNOSTIC CLINICAL FEATURES IN CRYPTOCOCCAL MENINGITIS
• Initial positive India ink examination of CSF
• High CSF opening pressure
• Low CSF glucose
• Low CSF leukocyte count (<20/µl)
• Cryptococci isolated from extraneural sites
• Initial CSF (or serum) antigen titer >32
• Corticosteroid treatment or lymphoreticular malignancy
Recurrent cryptococcal disease
• Abnormal CSF glucose concentration after =4 weeks of therapy
• Absence of anticryptococcal antibodies
• Post-treatment CSF (or serum) cryptococcal antigen titer of =8
• No decrease in antigen titers during therapy

• Daily corticosteroid treatment =20mg prednisone after completion of antifungal therapy
A retrospective study of patients who had AIDS demonstrated only a prognostic value of antigen levels in the CSF, not in serum. There is substantial variability in titers with the different antigen detection kits used.

patients who have advanced HIV disease. However, the majority of HIV specialists recommend that antifungal prophylaxis not be used routinely to prevent cryptococcosis because of the relative infrequency, the lack of survival benefits associated with prophylaxis, possibility of drug interactions, potential antifungal drug resistance and cost.[59]

MUCORMYCOSIS AND INFECTIONS BY OTHER ZYGOMYCETES

NATURE

Mucormycosis refers to disease caused by fungi belonging to the order Mucorales. Other names for the disease include phycomycosis and zygomycosis, the latter including diseases caused by Entomophthorales. Both Mucorales and Entomophthorales belong to the class Zygomycetes.

The major forms of zygomycosis include rhinocerebral, pulmonary, cutaneous, gastrointestinal and disseminated diseases. *Rhizopus, Mucor, Rhizomucor* and *Absidia* are the most common organisms that cause zygomycosis in humans.

EPIDEMIOLOGY

Although these organisms are ubiquitous and grow in decaying organic material, mucormycosis is a rare disease and occurs almost exclusively in patients who have an underlying disease, with the exception of *Apophysomyces elegans*, a newly described species, that has been reported as a causative agent of zygomycosis, especially in immunocompetent patients.

Risk factors for mucormycosis are listed in Table 237.10 . Diabetes mellitus and neutropenia are the most commonly encountered risk factors.

PATHOGENICITY

Infection with Mucorales usually occurs through inhalation of spores or deposition of spores in the nasal turbinates. In cutaneous mucormycosis, direct inoculation of abraded skin can result in disease with invasion of subcutaneous tissue.[64]

After pulmonary infection, the first line of defense is provided by alveolar macrophages. In animal studies, alveolar macrophages from healthy mice have been shown to inhibit germination of *Rhizopus oryzae* spores. In contrast, alveolar macrophages from corticosteroid-treated mice or diabetic mice fail to inhibit spore germination and the mice are rapidly killed by pulmonary and disseminated disease.

Neutrophils play an important role in the second line of host defense. It is not known how diabetes mellitus or corticosteroids interfere with elimination of the fungi. Normal human serum can

TABLE 237-10 -- Risk factors for mucormycosis.*

RISK FACTORS FOR MUCORMYCOSIS
• Diabetes mellitus, especially with ketoacidosis
• Immunosuppression, especially corticosteroid treatment
• Iron overload with or without deferoxamine treatment (e.g. hemodialysis, hemochromatosis)
• Hematologic disease, especially neutropenia
• Intravenous drug use (CNS mucomycosis)
• Sustained skin trauma (cutaneous mucormycosis)
• Kwashiorkor (gastrointestinal mucormycosis)

** From Sugar[64] and Yohai et al. [66]*

inhibit the growth of *Rhizopus* spp., unlike serum from patients who have diabetic ketoacidosis, which enhances fungal growth. The precise mechanism is unknown, although alteration in the availability of iron due to the decreased pH has been suggested.[66] Iron or iron bound to circulating deferoxamine (desferrioxamine) is used by Mucorales for replication. This is underscored by the clinical observation that patients who receive deferoxamine (e.g. in renal failure with hemodialysis or patients who have iron overload) are at risk of developing mucormycosis.[66]

As in aspergillosis, a hallmark of mucormycosis is angio-invasion, resulting in thrombosis and tissue necrosis. The fungus has a predilection for veins over arteries.[66]

DIAGNOSTIC MICROBIOLOGY

Mucorales grow at temperatures of 77–131°F (35–55°C) with an optimal temperature of 82.4–86°F (30°C). Clinical isolates will grow at 98.6°F in the laboratory 2–5 days after incubation under aerobic conditions.[64] Cycloheximide inhibits their growth, and so culture media containing cycloheximide should not be used.

On light microscopy (Fig. 237.13), Mucorales have irregularly shaped, nonseptate, broad (10–20mm in diameter) hyphae with right-angle branching, visualized with hematoxylin and eosin staining, periodic acid-Schiff reaction or Grocott-Gomori methenamine-silver nitrate stains. In tissue samples, Mucorales are often found near blood vessels and surrounded by a neutrophilic infiltrate.

CLINICAL MANIFESTATIONS

A detailed description is provided in Chapter 111 .

The clinical manifestations of mucormycosis can be divided into seven syndromes:

- rhinocerebral,
- pulmonary,
- cutaneous,
- gastrointestinal,
- CNS,
- disseminated, and
- miscellaneous (e.g. bones, kidney, heart or mediastinum).

The clinical manifestations depend upon the underlying disease. In patients who have diabetic ketoacidosis, rhinocerebral disease is the most common manifestation. Leukemic patients who have neutropenia are susceptible to rhinocerebral, pulmonary and disseminated disease. Children who have kwashiorkor (proteincalorie malnutrition) are especially at risk of developing gastrointestinal mucormycosis. Patients treated with deferoxamine because

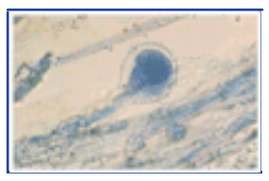

Figure 237-13 Lactophenol cotton blue preparation of *Absidia corymbifera.*

of iron or aluminum overload mainly present with disseminated mucormycosis.

In general, clues to the diagnosis of mucormycosis are signs of vasculitis with tissue necrosis, such as a black discharge or eschar on the skin, palate or nasal mucosa. Also, any radiographic imaging that reveals a lesion that surrounds vessels without a mass effect in an immunocompromised patient is suggestive of mucormycosis.

A biopsy with culture and microscopic evaluation is needed in order to discriminate between mucormycosis, aspergillosis and infection caused by Gram-negative bacteria such as *Pseudomonas* spp. The angio-invasion of the fungus causes an unusually low tendency to bleed during diagnostic surgery, which should alert surgeons to the diagnosis.[66]

If the organism is isolated by culture only, the clinician should differentiate between colonization, contamination and infection.

About 60% of rhinocerebral mucormycosis cases occur in diabetic patients and it is a rapidly fulminant disease, presenting with fever, nasal mucosal ulceration or necrosis, sinusitis (in 26% as an early sign), headache and facial pain or orbital involvement. Typically, when a ketoacidotic diabetic patient who has decreased consciousness does not wake up within 24–48 hours after correction of serum glucose and electrolytes, (rhino)cerebral mucormycosis should be considered and the palate should be inspected for a black necrotic eschar resulting from extension of the disease towards the oropharynx.

In rare cases, rhinocerebral disease may follow a chronic course. [66]

The pulmonary form of mucormycosis is the third most commonly occurring pulmonary opportunistic mycosis, especially in severely neutropenic hematologic patients or those who have diabetes mellitus. It presents with cough, fever, chest pain and dyspnea and progresses rapidly. In diabetics, a less fulminant form of pulmonary mucormycosis may occur.

A diagnosis of pulmonary mucormycosis is often made by bronchoscopy. Chest radiographs will underestimate the actual amount of tissue damage. The appearance of an air crescent sign on chest radiography or CT may herald a potentially fatal hemoptysis and warrants surgical and medical intervention.[67]

Cutaneous mucormycosis usually occurs in people who have sustained trauma (including indwelling postoperative catheters) or who have an underlying illness such as diabetes mellitus. Occasionally, cutaneous mucormycosis is a manifestation of systemic disease.

Gastrointestinal mucormycosis is primarily found in patients who have extreme malnutrition. All segments of the gastrointestinal tract can be involved. The clinical symptomatology mimics an intra-abdominal abscess.

Central nervous system mucormycosis is rare and occurs most frequently as a direct extension from the nose or paranasal sinuses, or is associated with intravenous drug use.

Cerebral lesions appear on CT scanning as low-density masses with variable peripheral enhancement. Gadolinium-contrast MRI can suggest cavernous sinus thrombosis and thrombosis of other vessels as indirect signs of infection, or ocular muscle involvement may be demonstrated.

MANAGEMENT

Mucormycosis is a rapidly progressive disease that warrants immediate aggressive combined surgical and medical treatment. All devitalized tissue should be removed, if necessary repeatedly, followed by reconstructive surgery in a later phase. Optimal treatment of the underlying disease is vital, including rapid correction of diabetic ketoacidosis. Intravenous amphotericin B in high (initial) doses of 1–1.5mg/kg/day is the treatment of choice, with a total dose around 2.5–3g. Several case histories report positive results with lipid formulations of amphotericin B.[68] A case report describes successful treatment of disseminated mucormycosis in a neutropenic patient with a combination of surgery, ABLC and G-CSF.[69]

Other less common treatment modalities reported in the literature with presumed but unproven effect include local irrigation with amphotericin B, addition of rifampin (rifampicin) or tetracycline to amphotericin treatment and hyperbaric oxygen. Despite occasional successful case reports, the currently registered azoles have no significant in-vitro activity against mucormycosis.

PROGNOSIS

The prognosis of mucormycosis depends largely upon the underlying disease, and in general the prognosis is better for those who have diabetes mellitus. The overall mortality has been over 50%, but with early aggressive surgery combined with medical treatment, this mortality has been reduced to less than 20%. Adverse prognostic factors in rhinocerebral disease include hemiparesis or hemiplegia, bilateral infections, nondiabetic co-morbidity and extensive facial necrosis (Fig. 237.14).[66] An overall mortality of 80% has been reported in pulmonary mucormycosis, but varies widely according to the underlying disease and extension of the infection.[70]

Gastrointestinal mucormycosis has a high mortality and is usually diagnosed at autopsy.

PENICILLIOSIS

NATURE

Penicilliosis is caused by the fungus *Penicillium marneffei*, a dimorphic mold with yeast-like growth in tissue.

The fungus is endemic in South East Asia and was originally isolated from the bamboo rat *Rhizomys sinensis*. It causes deep-seated infections in humans and rodents.

EPIDEMIOLOGY

Before the AIDS era, most patients in endemic regions (northern Thailand and rural south eastern China) affected with penicilliosis had no known underlying disease. Now, the infection mainly affects patients who have HIV infection and is recognized as an AIDS-defining opportunistic infection. In Thailand's Chiang Mai province it is the third most common HIV-related opportunistic infection (after tuberculosis and cryptococcosis).[71]

PATHOGENICITY

Penicillium marneffei is a facultative intracellular organism that can survive and replicate in macrophages. The route of infection is not known but it is assumed to be airborne or through ingestion, and

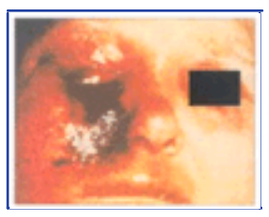

Figure 237-14 Patient with mucormycosis. Ocular invasion by Mucor in a patient with diabetes mellitus and ketoacidosis.

occasionally infection occurs through local inoculation. *Penicillium marneffei* evokes three patterns of tissue response:

- in the immunocompetent host, granuloma formation with central necrosis;
- suppurative abscesses are found in various organs; and
- in the immunocompromised host, an anergic necrotizing reaction in lung, liver and skin is seen, with diffuse infiltration of macrophages in tissues with proliferating yeast.

Antibody-mediated immunity does not seem to play a major role in host defense.[72]

Penicilliosis affects all ages and both sexes, although 90% of the cases reported in the English literature are male.[72] Although the fungus has been repeatedly isolated from the bamboo rat, human contact with or consumption of the rat does not appear to be a risk factor. In contrast, a recent history of exposure to soil is significantly associated with the acquisition of human penicilliosis, so a common reservoir of infection for rats and humans has been suggested.[73]

DIAGNOSTIC MICROBIOLOGY

In culture, *P. marneffei* is the only thermally dimorphic *Penicillium* spp. The fungus grows as a mold at 77°F (25°C) and looks grayish and downy. It produces a distinctive red diffusible pigment, which is visible on agar media. At 98.6°F (37°C) it grows as a yeast on Sabouraud glucose agar with cerebriform colonies that do not produce the red pigment.

On microscopic examination (Fig. 237.15), *Penicillium* spp. appear as short, septate, branching hyphae as well as sausage-shaped cells that divide by fission instead of budding, and may show a central septum, which distinguishes it from *Histoplasma capsulatum*. *Penicillium* spp. can be identified both inside macrophages and extracellularly when tissue preparations are stained with periodic acid-Schiff, Wright's or Giemsa stain. Recently, a monoclonal antibody has been developed that enables immunohistochemical identification of the fungus. [72]

CLINICAL MANIFESTATIONS

Clinical manifestations of penicilliosis are also discussed in Chapter 126 .

In patients who are not immunocompromised, the clinical picture may strongly resemble that of tuberculosis or histoplasmosis (e.g. suppurative lymphadenitis, fever, weight loss, anemia and a nonproductive cough). In patients who have HIV infection, the disease is usually disseminated, affecting skin, reticuloendothelial system, lung and gut. Other tissues that may be involved are liver and spleen, kidney, bone, joints and pericardium. In contrast to histoplasmosis and tuberculosis, adrenal involvement and CNS infections are rare. The molluscum contagiosum-like lesions of skin and mucosa indicate disseminated disease (Fig. 237.16).[72]

Chest radiographs show patchy infiltration and sometimes abscess formation. Abdominal CT scanning often demonstrates hepatomegaly or hepatosplenomegaly, but the diffuse microabscesses that cause the hepatomegaly are usually indistinguishable.

The diagnosis is made by culturing *P. marneffei* from blood, bone marrow, skin scrapings or liver biopsy specimen or by identifying the organism microscopically in a touch smear of a skin biopsy or bone marrow aspirate.

MANAGEMENT

The treatment of penicilliosis is amphotericin B (0.5–1.0mg/kg/day for 2 weeks), followed by itraconazole 200–400mg/day for 6 weeks for people who do not have HIV infection, and indefinitely for patients who have HIV infection. [69] [71] In in-vitro studies, fluconazole

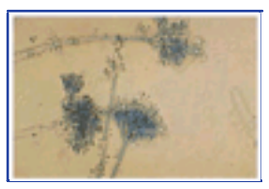

Figure 237-15 Lactophenol cotton blue preparation of *Penicillium brevi compactum.*

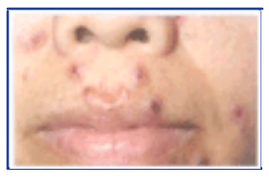

Figure 237-16 HIV-infected patient with *Penicillium marneffei* infection with molluscum contagiosum-like lesions.

appears to be less active. If treated appropriately, the reported response rate ranges between 59%[68] and 75%.[69]

Prevention with itraconazole in advanced HIV disease is successful, but does not provide survival benefit.

FUSARIOSIS

Fusarium spp. are found in soil with a worldwide distribution. Infections with *Fusarium* spp. (*Fusarium verticillioides* is, after *Fusarium solani*, the most common species) are rare and can lead to localized or disseminated disease. Examples of localized disease include keratomycosis, endophthalmitis, peritonitis due to implanted catheters for chronic ambulatory peritoneal dialysis, paronychia, invasive nasal infection and post-traumatic lesions of the bone, joint or skin.[73] Disseminated fusariosis has been described in a study of 97 patients.[74] These infections are supposedly airborne or inoculated through breakdown of the skin barrier. Most patients have prolonged and severe (<100 neutrophils/ml) neutropenia. The disease mimics aspergillosis and presents with an abrupt onset of fever, often combined with sinusitis, painful ecthyma gangrenosum-like skin lesions, pulmonary involvement and myalgia.

Histopathologic tissue examination with PAS or Gomori methenamine-silver staining reveals vascular invasion by hyphae

with infarction and necrosis. Contrary to aspergillosis, 50–70% of cases with disseminated fusariosis have positive blood cultures.[74] *Fusarium* spp. grow rapidly on agar media but growth is inhibited by cycloheximide. The response of *Fusarium* spp. to amphotericin B and itraconazole is limited. Newer treatment modalities such as lipid formulations of amphotericin B, voriconazole and the adjuvant use of growth factors (G-CSF and GM-CSF) may be promising.[74] Localized infection usually responds to treatment, but disseminated infection has a high mortality, especially if the underlying myelosuppression is not reversed. Moreover, successfully treated disseminated fusariosis tends to recur with repeated bone marrow suppression.

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Chapter 238 - Systemic Fungi

Thomas G Mitchell

INTRODUCTION

Systemic mycoses are customarily acquired by inhaling exogenous fungi that may disseminate to other internal organs, as well as the skin. The etiological agents tend to be restricted to rather circumscribed geographical areas. Consequently, the prevalence and geographic distribution of these mycoses are highly delimited. The term systemic mycoses is not exclusive, as many opportunistic (Chapter 237) and subcutaneous (Chapter 239) mycoses may also exhibit systemic clinical manifestations. Although systemic mycoses may be caused by a number of exogenously acquired fungi, this chapter will focus on the four most common primary systemic mycoses: blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis. All are caused by thermally dimorphic fungi. The fungi that cause coccidioidomycosis and histoplasmosis exist in nature, where they are associated with dry soil or soil mixed with guano. The agents of blastomycosis and paracoccidioidomycosis are presumed to exist in nature, but their habitats have not been clearly defined. Inhalation of any one of these fungi can lead to pulmonary infection, which may or may not be symptomatic. Dissemination may occur to other parts of the body. Except for a few extremely rare cases, there is no evidence of transmission among humans or animals. Table 238.1 compares some of the epidemiological features of these systemic mycoses.

Although most symptomatic cases of blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis occur in patients without significant pre-existing and predisposing disease, individuals with defects of cell-mediated immunity have long been recognized to be at risk for these mycoses if they are exposed by residence or travel in the appropriate endemic areas. In recent years, the incidence of each of these mycoses as opportunistic mycoses in AIDS patients has sharply increased. Indeed, the AIDS pandemic has revealed a new endemic and systemic, dimorphic, opportunistic pathogen, *Penicillium marneffei*; both the agent and the disease are confined to Asia (see Chapter 126).

Blastomycosis, coccidioidomycosis, histoplasmosis and paracoccidioidomycosis are caused respectively by the following dimorphic fungi: *Blastomyces dermatitidis, Coccidioides immitis* (or *Coccidioides posadasii*), *Histoplasma capsulatum* and *Paracoccidioides brasiliensis*. Each species grows as a mold in nature or the laboratory at temperatures below 98.6°F (37°C). On routine fungal culture media at 77–86°F (25–30°C), they produce mycelial colonies that vary in texture, pigment and growth rate, but may be indistinguishable from each other or many saprophytic molds. The ecology and geographic distribution of these fungi are summarized in Table 238.2 . Microscopically, the hyphae are uniform in width, hyaline (not pigmented), branched, septate and similar in appearance except for the production of asexual spores or conidia, which are helpful aids to their identification. In the host or under the appropriate growth conditions *in vitro*, they convert to a distinctive form of growth that is found in tissue: *B. dermatitidis, H. capsulatum* and *P. brasiliensis* produce characteristic budding yeast cells and *C. immitis* and *C. posadasii* produce spherules.

BLASTOMYCOSIS

Blastomycosis is a chronic infection characterized by granulomatous and suppurative lesions but the clinical manifestations may be protean. The disease is undoubtedly initiated by inhalation of air-borne conidia of the etiologic agent, *Blastomyces dermatitidis*. From the lung, dissemination may occur to any organ, but preferentially to the skin and bones. The disease has been called North American blastomycosis because many early cases were confined to the United States, Canada and Central America. Although the prevalence continues to be highest on the North American continent, autochthonous cases of blastomycosis have been documented in Africa, South America and Asia. In some parts of the United States it is even endemic for humans and dogs.

Blastomycosis was first described in its chronic cutaneous form in the 1890s by Gilchrist.[1] Many early case descriptions were undoubtedly confused with coccidioidomycosis, cryptococcosis (European blastomycosis) and paracoccidioidomycosis (South American blastomycosis) until Benham established the etiology of blastomycosis in 1934.[2] Eventually, Schwarz & Baum gathered histopathologic evidence to confirm that both cutaneous and systemic manifestations originate in the lung.[3] Primary cutaneous infection has been demonstrated only rarely. Thus, blastomycosis is primarily a pulmonary infection characterized by secondary spread to the skin and other parts of the body. However, the respiratory episode may be completely asymptomatic.

NATURE

Cultural characteristics

Blastomycosis is caused by a single dimorphic species, *B. dermatitidis*. The sexual or teleomorphic form is *Ajellomyces dermatitidis*. At temperatures below 95°F (35°C), *B. dermatitidis* grows as a mold, producing a colony of uniform, hyaline, septate hyphae and conidia. On Sabouraud's glucose agar at 77°F (25°C), different isolates of *B. dermatitidis* vary in their rate of growth, colony appearance and degree and type of conidiation. Colonies usually require at least 2 weeks to reach full development. Many strains produce a white, cottony mycelium that becomes tan to brown with age (Table 238.2). On enriched media at 98.6°F (37°C), *B. dermatitidis* converts to growth in the yeast form and produces colonies that are folded, pasty and moist.

Microscopic appearance

The mycelial form produces abundant conidia from short lateral conidiophores on the aerial hyphae (Fig. 238.1). The conidia are spherical, ovoid or pyriform in shape, smooth walled and up to 10µm in diameter. Thick-walled chlamydospores, 7–18µm in diameter, may also be observed. Because the colony and conidia of *B. dermatitidis* may be confused with many other fungi, identification must be confirmed by conversion to the characteristic yeast form. This conversion can be accomplished by *in vitro* cultivation on rich

TABLE 238-1 -- Epidemiological features of fungal infections.[25]

EPIDEMIOLOGICAL FEATURES OF FUNGAL INFECTIONS				
Feature	**Blastomycosis**	**Coccidioidomycosis**	**Histoplasmosis**	**Paracoccidioidomycosis**
Geographic areas of high endemicity	Yes	Yes	Yes	Yes
Saprophytic form (<95°F): hyaline, septate hyphae	Yes	Yes	Yes	Yes
Predominant tissue forms	Yeasts	Spherules	Yeasts	Yeasts
High infection rate in endemic areas	?	Yes	Yes	Yes
At least 90% of infections are initiated by inhalation	Yes	Yes	Yes	Yes
Approximate percentage of infections that are asymptomatic	?	=60	>95	>95
At least 90% of infections are self-limited	?	Yes	Yes	Yes
Approximate percentage of immunocompetent patients	>90	>90	>90	>90
Approximate percentage of males among patients with disease	50–90	75–90	80–90	95
Relative frequency of disease among AIDS patients in the endemic areas	Rare	Common	Common	Infrequent

TABLE 238-2 -- Summary of systemic mycoses.

SUMMARY OF SYSTEMIC MYCOSES					
Mycosis	**Etiology**	**Ecology**	**Geographic distribution**	**Mycelial form***	**Tissue form**
Blastomycosis	*Blastomyces dermatitidis*	Unknown (riverbanks?)	Endemic along Mississippi, Ohio & St Lawrence River valleys & south-eastern USA	Slow to moderate growth rate. Colonies are white to tan, flat, velvety or cottony. Hyaline septate hyphae and short conidiophores bearing single globose to pyriform conidia, 2–10µm	Thick-walled yeasts with broad-based, usually single, buds, 8–15µm
Coccidioidomycosis	*Coccidioides immitis* and *C. posadasii*	Soil	Semiarid regions of southwestern USA, Mexico, Central & South America	Moderate to rapid growth rate. Colonies are white to brown, flat to wooly. Hyaline s eptate hyphae and arthroconidia, 3 × 6µm.	Spherules, 10–80µm or larger, containing endospores, 2–4µm
Histoplasmosis	*Histoplasma capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*	Bat & avian habitats (guano); alkaline soil	Global; endemic in Ohio, Missouri & Mississippi River valleys; central Africa (var. *duboisii*)	Slow growth rate. Colonies are white to brown, powdery to wooly. Hyaline septate hyphae, tuberculate macroconidia, 8–16µm, and small oval microconidia, 3–5µm	Oval yeasts, 2 × 4µm, intracellular in macrophages
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*	Unknown (soil?)	Central & South America	Slow growth rate. Colonies are flat to velvety, white to brown. Hyaline, septate hyphae and rare globose conidia and chlamydospores	Large, multiple budding yeasts, 15–30µm or larger

* Colony descriptions are for typical isolates grown at 77°F on Sabouraud's glucose agar.

medium (e.g. brain-heart infusion, chocolate agar or Kelly's medium) at 98.6°F (37°C) or by animal inoculation. Under these conditions, *B. dermatitidis* grows as a thick-walled spherical yeast that usually produces single buds (Fig. 238.2). The bud and parent yeast have a characteristically wide base of attachment and the bud often enlarges to a size equal to that of the parent cell before it separates. Yeast cells are multinucleated and normally range in size from 8 to 15µm, although some cells reach a diameter of 30µm.

Sexual reproduction

The teleomorphic state (i.e. the sexual or perfect form) of *B. dermatitidis* was discovered by McDonough and Lewis.[4] Based on its sexual apparatus and resultant ascospores, *B. dermatitidis* was renamed *Ajellomyces dermatitidis*; it has been placed in the same genus as the teleomorph of *H. capsulatum*. Both species, as well as the sexual forms of the dermatophytes, are closely related and classified in the ascomycetous family Gymnoascaceae. *Ajellomyces dermatitidis* is heterothallic and requires two compatible (opposite) mating types for sexual reproduction. Although the two mating types possess different antigens, they are similar in many other respects, including pathogenicity. Mating compatibility has been used to confirm that a single species is responsible for blastomycosis among dogs and humans and probably also for cases in North America and Africa.

EPIDEMIOLOGY

Ecology

The natural habitat of *B. dermatitidis* has not been resolved. Assuming that most cases of blastomycosis are acquired by inhalation of exogenous, infectious particles, *B. dermatitidis* must reproduce in nature and generate air-borne cells. However, there have been too few isolations of *B. dermatitidis* from the environment to establish its ecological niche. Over the years, *B. dermatitidis* has been isolated, but never repeatedly, from soil samples collected in rural environments, including a chicken house, a cattle crossing and

Figure 238-1 *Blastomyces dermatitidis,* mycelial form in culture at 86°F. This shows hyaline, septate, branching hyphae and short conidiophores bearing solitary oval-to-piriform conidia.

Figure 238-2 *Blastomyces dermatitidis,* yeast form in hematoxylin and eosin-stained section of microabscess from a cutaneous lesion. This shows large spherical yeasts characterized by thick, highly refractile cell walls, single budding and a broad attachment between the parent yeast cell and bud.

several river banks. Positive samples were collected in Wisconsin from a beaver dam associated with a large outbreak of blastomycosis and at another outbreak from a fishing site. Several clusters of cases, with or without recovery of *B. dermatitidis* from environmental samples, have been associated with river banks, which suggests an association with fresh water and soil.[5] [6] [7] [8] However, a recent study showed that the genotypes of environmental isolates proximal to outbreaks were not prevalent among the case isolates.[9]

Dogs are commonly infected and numerous reports have documented isolated cases in other animal species, but there is no evidence that an animal reservoir exists to perpetuate *B. dermatitidis*. It probably resides in nature in a protected and dormant condition during most of the year, until it is stimulated by a suitable climatic or other specific but transient event to propagate and produce conidia that become air borne and infectious.

Geographic distribution

Because *B. dermatitidis* is not readily recoverable from nature and an adequate skin test antigen is not available for conducting population surveys of exposure, the geographic distribution of blastomycosis has been estimated from reports of human and canine cases. The endemic area extends broadly eastward from states that border the Mississippi River. Blastomycosis is endemic in southern Canada east of Manitoba and, in the United States, in Illinois, Wisconsin, Minnesota, Ohio, the Atlantic coastal states and the south-eastern states, with the exception of Florida. Blastomycosis is rare in the New England and western states. The highest incidence occurs in Arkansas, Kentucky, Illinois, Louisiana, Mississippi, North Carolina, Tennessee and Wisconsin. Within these areas, local pockets of high endemicity have been identified. Canine cases exhibit the same endemic pattern as those of humans. Indeed, blastomycosis may occur more frequently in dogs than in humans.[5] [10]

Clinical reports have also documented autochthonous cases in both northern and southern Africa, as well as India, Israel, Mexico and Venezuela. Reports of the infection occurring elsewhere are dubious because the diagnosis was questionable or the patients had a history of travel to an endemic area.

At least 11 outbreaks of blastomycosis have been documented.[5] [6] [7] [8] [11] [12] Each outbreak consisted of a cluster of cases that occurred at approximately the same place and time. All but one area was rural. Seven outbreaks occurred during the fall and winter, and four in the summer. These outbreaks have provided helpful insights regarding the ecology of *B. dermatitidis* and natural history of blastomycosis.

Incidence

The data from large, clinical reviews indicate that blastomycosis occurs more frequently during middle age and in males. Although the disease can occur at any age, 60% of patients were between the ages of 30 and 60 years. Among the hundreds of cases compiled in several reviews, less than 4% of the patients were under 20 years of age and blastomycosis was rarely reported in children. However, in more recent studies, especially involving outbreaks, blastomycosis occurred equally in both sexes, and two-thirds of the patients associated with outbreaks have been children (<16 years old).

The male-to-female ratios reported in several surveys involving hundreds of patients vary from 6:1 to 15:1, but lower ratios have been reported in smaller studies and the overall sex ratio of outbreak cases is approximately 1. Perhaps both sexes are equally susceptible to acute blastomycosis, but males are more inclined to develop chronic or disseminated disease. The other systemic mycoses caused by exogenous fungi also occur more often in males (Table 238.1) and male animals are more susceptible (or less resistant) than females to challenge with *B. dermatitidis*. Blastomycosis may be similar to histoplasmosis in that children are equally susceptible to infection and disease, but the sex-related differences are manifested after puberty.

Genetic or racial differences in attack rates have not been confirmed. Socio-economic and occupational data have associated blastomycosis with poverty, malnutrition, manual labor, agriculture, construction work and exposure to dust and wood. The incidence of blastomycosis among immunocompromised patients, including patients with AIDS, is relatively low but apparently rising.[13] [14]

PATHOGENICITY

Strains of *B. dermatitidis* vary in their virulence for experimental animals, but the explanation for these differences has not been resolved.[15] Among cell wall components, evidence has linked experimental virulence of strains of *B. dermatitidis* with the amount of

a-1,3-glucan in the cell walls. This polysaccharide has been shown to vary in strains of *H. capsulatum* and *P. brasiliensis* that differ in virulence.[16]

The best characterized virulence factor is the WI-1 (also called BAD1) cell wall adhesin and immunodominant antigen. This protein contains multiple copies of a

25-amino acid tandem repeat, which binds to CR3 complement receptors and elicits both antibodies and cell-mediated immune responses.[17] [18] [19] Compared with less virulent mutants, the wild type contains less surface-bound WI-1. Although the mechanisms whereby WI-1, as well as a-1,3-glucan, contribute to virulence have not been fully elucidated, Klein and associates have postulated that shedding of WI-1 by virulent strains may neutralize macrophage defenses by occupying receptors, diminishing their ability to bind yeast cells.[17] [18] [20] Many strains of *B. dermatitidis* from Africa lack WI-1 and are less virulent than New World isolates.[19]

Pathogenesis

Almost all cases of blastomycosis originate in the lung. In the alveoli, *B. dermatitidis* induces an inflammatory response characterized by the infiltration of both macrophages and neutrophils and leading to the subsequent formation of granulomata. Both conidia and yeast cells are susceptible to the killing mechanisms of neutrophils and macrophages.[21] The accumulation of neutrophils presents a suppurative component that is uncharacteristic of most mycoses and other chronic diseases. Neutrophils and cell-mediated immunity combine to marshal effective resistance to blastomycosis. Unlike coccidioidomycosis and histoplasmosis, blastomycosis is relatively rare in patients with cellular immunodeficiencies.

If the pathogenesis of blastomycosis resembles that of histoplasmosis and coccidioidomycosis, most infections may be subclinical and resolve spontaneously. However, because specific and sensitive skin test antigens are lacking, the extent of exposure to *B. dermatitidis* in the general population has not been determined. In addition, calcification is uncommon and there is little radiologic or histopathologic evidence of residual blastomycotic lesions. The best evidence for the existence of subclinical blastomycosis derives from the reported outbreaks, where specific serological and skin test reactions documented exposure to *B. dermatitidis* in the absence of symptoms.[22]

Alternatively, based on the apparent scarcity of *B. dermatitidis* in nature and the limited evidence for subclinical infections, blastomycosis may be a rare and usually serious disease.[23] This hypothesis is supported by some of the studies of experimental canine blastomycosis. In humans, the primary pulmonary lesion may be inapparent to severe. If inapparent, dissemination to the skin and bones may follow. If the pulmonary episode is severe, the generalized systemic disease may develop, with the potential involvement of multiple organs.

PREVENTION

There are no clear guidelines for the prevention of blastomycosis. However, the WI-1 antigen may be an excellent vaccine candidate for future study.

DIAGNOSTIC MICROBIOLOGY

Blastomycosis is best diagnosed by direct examination or positive culture of sputa, skin lesions or other specimens. On most routine culture media, *B. dermatitidis* produces a mold with variable macroscopic features and conidia. Its identification is confirmed by growth on a rich medium at 98.6°F (37°C) and conversion to the characteristic yeast form. Alternatively, the identity of a culture may be established by an immunodiffusion test to detect a *B. dermatitidis*-specific

TABLE 238-3 -- Species-specific exoantigens for the identification of systemic dimorphic fungal pathogens.[1] [21]

SPECIES-SPECIFIC EXOANTIGENS FOR THE IDENTIFICATION OF SYSTEMIC DIMORPHIC FUNGAL PATHOGENS	
Fungus	**Exoantigens**
Coccidioides immitis	HS, F, HL
Histoplasma capsulatum	h, m
Blastomyces dermatitidis	A
Paracoccidioides brasiliensis	1, 2, 3
Exoantigens are detected by precipitin lines of identity in immunodiffusion tests of concentrated culture supernatant fluids versus reference antigens and antisera.[25] [120]	

antigen (antigen A) in a concentrated overnight, aqueous extract of the colony on solid medium or in the supernatant fluid of a short-term broth culture of the isolate. The formation of a precipitin line of identity with reference antisera and control antigen identifies the specific exoantigen extracted from the isolate. This method is rapid and applicable to nonsporulating cultures. As indicated in Table 238.3 , the technique has also been expanded to identify other dimorphic pathogens. In addition, a DNA probe is commercially available to identify cultures of *B. dermatitidis*, as well as other agents of systemic mycoses.[24]

In culture at 98.6°F (37°C) or in tissue, *B. dermatitidis* forms large single-budding yeast cells with highly refractory cell walls and a broad attachment between the bud and parent yeast cell. As noted below, in fresh specimens or histopathological sections, the yeasts can be readily observed and identified.

Since the tests for complement fixing antibodies and delayed-type skin reactivity lack specificity and sensitivity, they are not helpful unless the patient is negative to heterologous fungal antigens; even then, positive, monospecific serological tests to *B. dermatitidis* may not indicate active infection. Serological tests for antibodies to the specific antigen A, which can be detected by the immunodiffusion test or an enzyme immunoassay, are more suggestive of infection, but negative tests do not exclude blastomycosis. Table 238.4 summarizes the conventional serological tests for antibodies to *B. dermatitidis* and other systemic fungi.

Microscopic examination

In calcofluor white or KOH preparations of pus, exudate, sputum, bronchial lavage fluid or other specimens, a diagnosis can be established by detecting the characteristic yeast cells of *B. dermatitidis*. The yeasts are large (8–15µm in diameter) and typically thick walled. The cell wall is highly refractile and often resembles a double wall. Budding usually occurs singly. The bud is attached to the parent cell by a broad base and enlarges to the size of the parent yeast before it is detached. These features, as depicted in Figure 238.1 and Figure 238.2 , are pathognomonic for blastomycosis. Unsuspected cases of blastomycosis are occasionally diagnosed from respiratory specimens submitted for routine cytology. In tissue stained with hematoxylin and eosin, the yeast cytoplasm stains darkly and the cell wall appears colorless. The yeasts are multinucleated. They are often abundant in cutaneous lesions and these specimens are usually positive on direct examination. If the yeasts are sparse, fungal cell wall stains, such as periodic acid-Schiff or methenamine silver, are helpful.

Rarely, small forms are seen in tissue. These cells are typical of *B. dermatitidis* in shape and budding but only 2–5µm in diameter. Their multinucleation may help to differentiate them from *Cryptococcus neoformans* and *H. capsulatum* var. *duboisii*.

TABLE 238-4 -- Summary of serologic tests for antibodies to systemic dimorphic fungal pathogens in patients without AIDS.

SUMMARY OF SEROLOGIC TESTS FOR ANTIBODIES TO SYSTEMIC DIMORPHIC FUNGAL PATHOGENS IN PATIENTS WITHOUT AIDS					
Mycosis	**Test**	**Antigen**	**Sensitivity and value**		**Limitation/specificity**
			Diagnosis	**Prognosis**	
Blastomycosis	CF	By	Less than 50% of cases positive; reaction to homologous antigen only is diagnostic	Fourfold change in titer	Highly cross-reactive
	ID	Bcf	Up to 80% of cases positive, i.e. A band	Loss of A band	More specific than CF test
	EIA	A	Up to 90% of cases positive (titer =1:16)	Change in titer	92% specificity

Coccidioidomycosis	TP	C	Early primary infection; 90% cases positive	None	None
	CF	C	Titer =1:32 = secondary disease	Titer reflects severity (except in meningeal disease)	Rarely cross-reactive with H
	ID	C	More than 90% cases positive, i.e. F and/or HL band		More specific than CF test
Histoplasmosis	CF	H	Up to 83% of cases positive (titer =1:8)	Fourfold change in titer	Cross-reactions in patients with blastomycosis, coccidioidomycosis, cryptococcosis, aspergillosis; titer may be boosted by skin test with H
		Y	Up to 94% of cases positive (titer =1:8)	Fourfold change in titer	Less cross-reactivity than with H
	ID	H(10X)	Up to 85% of cases positive, i.e. m or m and h bands	Loss of h	Skin test with H may boost m band; more specific than CF test
Paracoccidioidomycosis	CF	P	80–95% of cases positive (titer =1:8)	Fourfold change in titer	Some cross-reactions at low titer with aspergillosis and candidiasis sera
	ID	P	98% of cases positive (bands 1, 2 and/or 3)	Loss of bands	Band 3 and band m (to H) are identical

A, antigen A of *B. dermatitidis*; Bcf, culture filtrate of *B. dermatitidis* yeast phase; By, yeast phase of *B. dermatitidis*; C, coccidioidin; CF, complement fixation; EIA, enzyme immunoassay; H, histoplasmin; ID, immunodiffusion; P, culture filtrate of *P. brasiliensis* yeast phase; TP, tube precipitin; Y, yeast phase of *H. capsulatum*. [27] [102] [103]

Culture

Clinical specimens are cultured on any of several fungal media. Sabouraud's agar, the standard medium used for morphologic descriptions of pathogenic fungi, is composed of 4% glucose, 1% neopeptone and agar. A modified Sabouraud's agar, containing 2% glucose, is a more effective isolation medium. Inhibitory mold agar is a complex medium designed for optimal recovery of pathogenic fungi. For the culture of nonsterile specimens, such as sputum, skin or urine, antibiotics are included in the media, usually cycloheximide and chloramphenicol or gentamicin, to inhibit saprophytic fungi and bacterial contaminants. Inhibitory mold agar contains all three additives. Cultures should be incubated at 86°F (30°C) or room temperature for at least 4 weeks. Primary colonies are white to brown, variably textured molds, and they produce potentially infectious conidia. The identification of *B. dermatitidis* is confirmed by detecting the A exoantigen (Table 238.3), by subculturing on an enriched medium, such as brain-heart infusion blood agar or Kelly's medium at 98.6°F (37°C), for subsequent conversion to the yeast form (Fig. 238.2) or by hybridization with a specific DNA probe.[25]

Some laboratories employ direct animal inoculation as a method of rapidly isolating and identifying *B. dermatitidis*. Mice are injected intraperitoneally with the clinical specimen and their peritoneums are examined for typical yeast cells 1–2 weeks later.

Skin test

Delayed-type hypersensitivity to *B. dermatitidis* has been detected by skin tests with both whole cell and culture filtrate antigens (blastomycin). However, the commercial blastomycin was abandoned because it lacked specificity and sensitivity. More promising antigen preparations have been developed, but they are not available for routine human skin testing.

Serology

The most useful serologic procedure is an enzyme immunoassay (EIA) for antibodies to antigen A. With titers >1:16, the sensitivity and specificity of this test were shown to be >77% and >92%, respectively,[26] [27] and EIA titers reflected the severity of disease (Table 238.4). More recent evaluation of a commercial EIA reported sensitivity of 83% and false-positive results with sera from patients with histoplasmosis, but only rarely with sera from patients with other mycoses.[28] In this study, the EIA was much more sensitive than the immunodiffusion test. Using reference reagents, precipitins to antigen A can also be detected by an immunodiffusion (ID) test, as indicated in Table 238.4 . Measurement of CF antibodies to various antigen preparations of *B. dermatitidis* has not proved reliable.

CLINICAL MANIFESTATIONS

Two classic forms of blastomycosis are recognized: pulmonary, often with dissemination, and chronic cutaneous blastomycosis. A wide variety of symptoms, pathology and radiographic appearances may be observed in blastomycosis. The most common symptoms include cough, weight loss, chest pain, skin lesions, fever, hemoptysis and localized swelling. After the lung, the most frequently involved organs are the skin, bones and genitourinary tract, followed by the central nervous system, liver or spleen. Less often, the lymph nodes, thyroid, heart, adrenal, omentum, gastrointestinal tract, muscles and pancreas may become infected.[14] [29] [30] [31] [32] [33]

Pulmonary blastomycosis

Primary pulmonary blastomycosis may be asymptomatic or present as acute or subacute pneumonia, ranging from mild to severe. Cases associated with outbreaks have confirmed that spontaneous recovery can follow primary blastomycosis. [32] [34] However, the possibility of subsequent reactivation cannot be excluded. Even if nonapparent, dissemination may spread to the skin, bones or other sites. If the pulmonary episode is severe, the generalized systemic disease may develop and may involve multiple organs. Cases associated with outbreaks have indicated that the incubation period is 3–12 weeks and that spontaneous recovery can follow primary blastomycosis. Overall, most cases occur in adults and in males, but in pediatric blastomycosis, children of both sexes are equally susceptible. Most pediatric cases are recognized as acute pulmonary blastomycosis.[5] [30]

Patients with symptomatic, primary pulmonary infection may present with symptoms of mild respiratory infection, including cough, chest pain and high fever, as well as numerous other complaints. The primary pulmonary infection may persist locally, spread to other organ(s), or both. Alternatively, the pulmonary lesion may heal by fibrosis and absorption, leaving no residual evidence of infection. In patients whose pulmonary lesions have resolved, dissemination, generally to the skin, may already have occurred. If the pulmonary focus becomes more severe, an acute to chronic lung infection may develop. Patients with chronic pulmonary blastomycosis usually present with cough, low-grade fever, loss of weight, night sweats and other problems. The most common forms of pulmonary involvement are infiltration, cavitation, pneumonia or nodules.[22] [35] A variety of pulmonary manifestations may be observed and none of the radiographic presentations are consistent enough to be diagnostic of blastomycosis.

Chronic cutaneous blastomycosis

In chronic cutaneous blastomycosis, the initial skin lesion presents as one or more subcutaneous nodules that eventually ulcerate. Lesions are most common on exposed skin surfaces, such as the face, hands, wrist and lower leg. Spread may occur by extension to the trunk or other areas and may require weeks or months for the ulcerative process to evolve. If untreated, elevated, granulomatous lesions with advancing borders will develop in time. The yeast cells can be found in microabscesses near the dermis. Extensive, often verrucous, epithelial hyperplasia overlying the abscesses may develop and resemble carcinoma. These extensive cutaneous lesions are characteristically discolored and crusty and they tend to heal and scar in the central, older areas. The active microabscesses found at the leading edge of the lesion can be aspirated or biopsied and the typical yeast cells of *B. dermatitidis* can be observed on direct microscopic examination (Fig. 238.3).

Disseminated blastomycosis

Dissemination may be widespread in blastomycosis. The most frequently involved extrapulmonary sites are the skin, bones, genitourinary tract, central nervous system and spleen. Less frequently, the liver, lymph nodes, heart and other viscera are infected. The progressive systemic form of blastomycosis develops in patients with

unresolving pulmonary infection, but the degree of pulmonary involvement is not related to the extent of dissemination. This infection may be chronic, with few organisms present, or multiple pulmonary foci may be demonstrable at the time generalized systemic disease develops.

From the lungs, the yeasts disseminate with a characteristic predilection for the skin and bones. Skin lesions may be more severe than those in chronic cutaneous blastomycosis and are seen in about 75% of the cases. Overall, skeletal involvement is observed in approximately a third of patients. Osteomyelitis and, in some cases,

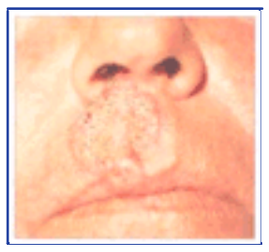

Figure 238-3 Initial cutaneous lesion of blastomycosis.

draining sinuses to the skin develop and should be examined for the presence of characteristic yeast cells. Because of the frequency of bone involvement and because almost any bone can be affected, whenever blastomycosis is diagnosed, a complete radiographic examination is advisable. Arthritis may develop by extension from infected bone or by direct dissemination from the lung without bone infection. In up to 22% of patients, the urogenital tract is involved, especially the prostate, male genitalia, kidney and adrenals. Metastasis to the central nervous system, resulting in meningitis or brain abscess, occurs in up to 10% of patients.

Primary cutaneous blastomycosis

This form of blastomycosis is initiated by traumatic autoinoculation or contamination of an open wound with the infectious material. The pathogenesis differs considerably from the other forms of blastomycosis. The lymphatics and regional lymph nodes are involved, but the infection remains localized and often resolves without treatment. Several cases of accidental cutaneous inoculation have occurred in veterinarians who were examining or performing autopsies on diseased dogs. Traumatic inoculation leading to primary, subcutaneous disease can occur, albeit rarely, with any of the systemic fungi discussed in this chapter. The natural history and pathology are similar to subcutaneous mycoses (see Chapter 239) and systemic dissemination is rare in the immunocompetent host.

Blastomycosis and AIDS

Despite the consistent prevalence of blastomycosis in its endemic area, patients with HIV infection are not commonly infected. Because of the presumed rural reservoir of *B. dermatitidis* or its low census in nature, patients with AIDS may be exposed less often than similar patients in the endemic areas for coccidioidomycosis or histoplasmosis. Alternatively, the host defenses against *B. dermatitidis* may be less dependent upon cell-mediated immunity. The cases of blastomycosis and AIDS that have been reported include the presentation of acute miliary disease in the lungs and a relatively higher frequency of CNS disease.[36] [37] Blastomycosis in compromised patients is being seen more frequently and has a much poorer prognosis.[13] [37]

MANAGEMENT

As demonstrated by several outbreak cases, primary blastomycosis in immunocompetent individuals may not require therapy. Nevertheless, such patients with a confirmed diagnosis of primary pulmonary infection that is mild and resolves spontaneously without treatment must be closely observed for at least 2 years following the primary infection because of the possibility of reactivation blastomycosis. Patients with

protracted, severe or progressive primary infection, chronic pulmonary or disseminated blastomycosis require treatment.[30] [38] Depending upon the manifestations of disease and the integrity of the underlying host defenses, chemotherapeutic success rates with amphotericin B, ketoconazole or itraconazole currently vary between 70% and 95%. Survival in patients with AIDS and other immunocompromising conditions is about half this figure.[37] [39] Recent guidelines recommend itraconazole for mild to moderate blastomycosis and amphotericin B for life-threatening disease and/or patients with AIDS.[39]

Ketoconazole

Ketoconazole was the first azole recommended for immunocompetent patients with mild to moderately severe disease (e.g. blastomycosis that is neither life threatening nor involving the central nervous system). An oral dose of 400–800mg per day for at least 6 months is effective in most adult patients, but patients must be closely followed because relapses have occurred on ketoconazole.[39] Successful treatment of blastomycosis has been achieved in 80–90% of adult patients. The adverse effects of ketoconazole include liver toxicity and reversible hormonal imbalances (e.g. gynecomastia), as well as nausea, pruritus, dizziness or headache. Itraconazole has supplanted ketoconazole because it offers improved absorption. tolerance and efficacy.[39]

Itraconazole

Recent trials have confirmed the efficacy of itraconazole for the treatment of mild to moderate, nonmeningeal, nonlife-threatening blastomycosis. When such adult patients were given oral dosages of 200–400mg/day for at least 6 months, the rate of cure or improvement was 95%. Itraconazole should be taken with meals and patients are treated for approximately 6 months with follow-up for 1 year or longer. Toxicity from itraconazole occurs in less than one-third of patients, is usually mild and rarely necessitates cessation of therapy. The most common side-effects are gastrointestinal symptoms, usually nausea, vomiting or diarrhea. Minor reactions include weakness, dizziness, headache, chills, fever, tinnitus, skin rash, pruritus, paresthesia and, rarely, transient, modest elevation of transaminase. Any relapse may be amenable to a second course of itraconazole.

Compared with ketoconazole, itraconazole is equally or more effective and better tolerated. For example, gastrointestinal symptoms were more than twice as frequent among patients receiving ketoconazole than itraconazole, although these symptoms occur more often in association with higher doses. Several treatment failures with ketoconazole have been successfully managed with itraconazole. The recommended pediatric regimen of itraconazole is 5–7mg/kg/day.[30] [39]

Fluconazole

Fluconazole, at 400 or 800mg/day for at least 6 months, is an effective alternative for the treatment of nonlife-threatening cases of blastomycosis, especially in patients who may not have responded to another drug.[40] Its only advantage over itraconazole may be in patients with involvement of the CNS, which is penetrated well by fluconazole.

Amphotericin B

Blastomyces dermatitidis is quite susceptible to amphotericin B (AMB), which is the recommended treatment for patients with life-threatening or severe disease (e.g. involvement of multiple organs), those with meningitis, or blastomycosis in immunocompromised patients, as well as patients who do not respond to an azole.[39] A total dose of 2–2.5g is required to eradicate all the organisms, as the relapse rate is significant if <1.5g is administered. The protocol for administration of AMB and monitoring renal function are the same as its application for other mycoses (see Chapter 208). An initial test dose of 1 or 5mg in adults is administered intravenously in a solution of 5% glucose, deoxycholate and buffer. The dose is gradually increased by 5–10mg/day to a maximal daily administration of 0.5–1.5mg/kg body weight.

Most patients experience adverse side effects, including renal dysfunction, fever, anorexia, phlebitis, hypokalemia, nausea, chills, headache and anemia. Administration is interrupted when blood levels of urea nitrogen reach 40–50mg/dl or 3.0–3.5mg/dl of creatinine.

Lipid emulsions of AMB have the advantage of delivering higher doses with diminished toxicity. Adult respiratory distress syndrome due to overwhelming pulmonary blastomycosis requires aggressive treatment with AMB. Blastomycosis in patients with AIDS may require suppressive therapy, following the initial course of treatment with AMB, similar to the management of cryptococcosis and histoplasmosis in patients with AIDS. Suppressive treatment in AIDS patients would be 200–400mg/day itraconazole, or possibly 800mg/day fluconazole for CNS disease.[39]

For children, the test dose of 0.1mg/kg (maximum dose 1mg) should be followed by 0.25mg/kg at 4 hours later and 0.5mg/kg after 24 hours. Beyond 48 hours, 1mg/kg/day is administered to reach a total dose of >30mg/kg. Pediatric, as adult patients, must be closely monitored for adverse reactions.

Blastomycosis has been reported in pregnant women, and the infection may or may not be transmitted to the infant. Pregnant patients have been treated with AMB without congenital or toxic effects in the fetus. Systemic treatment with azoles must be avoided in pregnant women because of their teratogenic potential. In the endemic area, blastomycosis should be considered in any neonate with a reticulonodular lesion on chest film or whose mother has evidence of blastomycosis.

Surgery

Corrective surgery may be necessary as an adjunct to antibiotic treatment. Because of the occurrence of relapse or reactivation blastomycosis, patients should be observed for years after treatment and resolution of disease.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis is caused by either of two indistinguishable species, *Coccidioides immitis* or *Coccidioides posadasii*, dimorphic fungi that normally live in well-defined geographic areas. Both the agents and coccidioidomycosis are almost entirely limited to these endemic regions. *Coccidioides immitis* was first described in 1892 by an Argentinian pathologist. Alejandro Posadas, who examined tissue from a fatal case and named the orgnaism *Coccidioides*, meaning '*Coccidia*-like', because of the spherules found in tissue.[41] [42] The species name, 'immitis,' means not mild. Indeed, most early cases were diagnosed at autopsy and until 1930, the disease was erroneously thought to be invariably severe and disseminated.

It was recognized quite early that coccidioidomycosis is confined to the south-western United States, contiguous regions of northern Mexico and specific areas of Central and South America. The natural reservoir was established by isolating *C. immitis* from soil samples collected throughout the endemic areas, and the environmental conditions under which the fungus was propagated were described. From mycelial culture filtrates, the skin test antigen coccidioidin was developed and used to detect exposure to *C. immitis* or *C. posadasii* and to conduct population surveys of skin reactivity. The more common primary form is a mild, respiratory ailment, also called valley fever or San Joaquin Valley fever.

NATURE

Etiology and dimorphism

The new species, *C. posadasii*, was recently recognized by Fisher *et al.*[43] They compared the genotypes of isolates of *C. immitis* from different geographical regions and, based on their phylogenetic analyses of these populations, determined that the majority of isolates outside California belong to a different species, which they named *C. posadasii* to honor Dr Posadas. Since *C. posadasii* has a broader geographical distribution, it is the more likely cause of the majority of cases of coccidioidomycosis. Although these species differ in their geographical distribution and genotype, any differences in their phenotypes or pathogenicity have yet to be delineated. Because the two species cannot be distinguished by simple laboratory tests, the more familiar name, *C. immitis*, will continue to be used.

The life cycle of *C. immitis* (or *C. posadasii*) encompasses at least four distinct morphologic structures that are produced under different conditions. In nature and in the laboratory, either species grows as a mold, producing hyaline, branching, septate hyphae. As the culture ages, characteristic arthroconidia are formed, usually, but not invariably, in alternate hyphal cells (Fig. 238.4). With time, the arthroconidial chains fragment to release the unicellular, barrel-shaped arthroconidia, the ends of which often retain remnants of cell wall material from the disintegrated adjacent cells. Arthroconidia are approximately 3 × 6µm in size, easily air borne and small enough to be inhaled into the alveoli. They are highly resistant to desiccation, temperature extremes and deprivation of nutrients and may remain viable for years. Under appropriate growth conditions, the arthroconidia will germinate to recycle the saprophytic mycelial form (Fig. 238.4).

Following their inhalation, the arthroconidia become spherical. In the infected host, *C. immitis* exists as spherules — spherical thick-walled structures, 15–80µm in diameter that are filled with a few to several hundred endospores (Fig. 238.5). As a spherule enlarges, the nuclei undergo mitosis, the cytoplasm condenses around these nuclei and a cell wall forms around each developing endospore. At maturation, the spherule ruptures to release its endospores. The endospores are 2–5µm in size and may, in turn, enlarge to form mature spherules. Hyphae as well as spherules may form in the tissues and appear in sputum of patients with coccidioidal cavities of the lungs.

Cultural characteristics

On routine mycological media, such as inhibitory mold or Sabouraud's agar, at the usual incubation temperature of 77–86°F (25–30°C), different isolates of *C. immitis* produce a wide variety of colony types. Colonies may be white, gray or brownish in color, with a powdery, wooly or cottony texture. Because numerous infectious arthroconidia are produced in culture and can be readily aerosolized in the dry state, cultures of *C. immitis* must be handled with extreme caution to prevent accidental exposure. Tubes or plates should be opened only under a safety cabinet that protects both the laboratory worker and the environment.

Spherules can be produced in the laboratory on a complex medium at 104°F (40°C) under 20% CO_2 . However, *in vitro* growth of the tissue phase even under optimal conditions is seldom extensive.

EPIDEMIOLOGY

In the USA, the geographic areas endemic for coccidioidomycosis and from which *C. immitis* can be isolated from the soil correspond to the Lower Sonoran life zone. These areas are characterized by a semi-arid climate, alkaline soil and characteristic indigenous desert plants and rodents. The endemic foci in Mexico, Argentina and other scattered areas of Central and South America are associated with

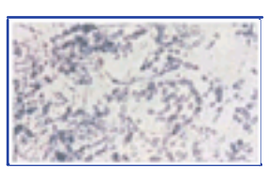

Figure 238-4 *Coccidioides immitis,* mycelial form in culture at 86°F. This shows hyaline, septate, branching hyphae and chains of arthroconidia, often in alternating cells.

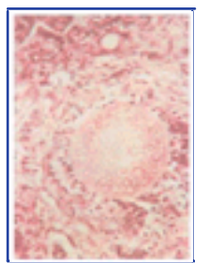

Figure 238-5 *Coccidioides immitis,* spherule in hematoxylin- and eosin-stained section of lung lesion. This shows refractile cell wall and internal endospores.

ecologically similar environments. Although *C. immitis* grows in the laboratory over a wide range of temperature, pH and salt concentration and requires only glucose and ammonium salts to grow, it has never become established in soils outside the endemic area, despite being transmitted to other locations by infected animals and fomites. Although *C. immitis* is inhibited by other micro-organisms, cultivated soil or treatment with various chemicals, none of these factors fully explains its restricted habitation. The mycelia, which can be found several inches beneath the soil surface, are recovered at the surface after the spring rains. As the weather becomes hot and dry, the mycelia convert to infectious arthroconidia and this accounts for the peak infection rate during the summer. In the endemic area, natural infections also occur among indigenous fauna, such as desert rodents, dogs and cattle.

Inhalation of the arthroconidia of *C. immitis* leads to infection and acquisition of a positive delayed-type hypersensitivity response to coccidioidin. More than half of these infections are benign and most of the others are symptomatic but self-limited (see Table 238.1). Approximately 1% of these cases will develop progressive pulmonary disease, dissemination or both. Some individuals

have an increased risk of developing disseminated disease following primary infection (Table 238.5). These include persons in certain ethnic groups: Filipinos, African

Americans, Latin Americans, and Native Americans. This ethnic predilection for severe disease clearly indicates the importance of the genetic background of the host in mounting an effective immune response to infection.[44] In addition to ethnicity, males, women in the third trimester of pregnancy, persons with a cellular immunodeficiency (including AIDS), and individuals at the age extremes are more susceptible to severe disease (see Table 238.5).

The areas of endemicity defined by case reports and by isolation of *C. immitis* from soil have been confirmed by skin test surveys with coccidioidin. Within the endemic areas, which include portions of the south-western USA (California, Arizona, New Mexico, Nevada, Utah and Texas) and north-western Mexico, the percent reactivity varies; some of the highest rates are found in Phoenix and Tucson, Arizona, and Kern County, California. Isolated cases of coccidioidomycosis occurring outside the established areas of endemicity have been attributed to fomite transmission of the arthroconidia or to patient travel through the endemic area.

Numerous outbreaks of primary infection have been reported among individuals simultaneously exposed to a heavy aerosol of arthroconidia. Coccidioidomycosis is therefore considered an occupational hazard for construction workers, archeology students and others who work the soil in the endemic areas. In a similar manner, many cases of acute disease developed subsequent to a severe wind storm in California in 1977, when contaminated soil was blown from the San Joaquin Valley far north and west, exposing large populations of unsensitized individuals. A similar epidemic of coccidioidomycosis produced a marked increase in Kern County in 1991–92. [45] The explanation for this increase has baffled investigators, but it may have been caused by concurrent earthquakes and high winds. Risks for severe pulmonary disease include diabetes, cigarette smoking, low income and old age.[46] Recently an outbreak was described among Navy SEALs who trained for 6 weeks in Coalinga, California. Forty-five percent (10 out of 22) had serologic and radiographic evidence of acute coccidiodomycosis, and all were symptomatic.[121]

PATHOGENICITY

Using reverse genetics, Cole and his colleagues have identified antigens and potential virulence factors that are elaborated by *C. immitis*. The cell wall of the infectious particle, the arthroconidium, has three distinct layers.[47] *In vivo*, potent cell wall antigens are released when the arthroconidia develop into spherules.[48] Although arthroconidia and endospores are readily engulfed by alveolar macrophages, killing is enhanced by activation of

TABLE 238-5 -- Risk factors for disseminated coccidioidomycosis.

RISK FACTORS FOR DISSEMINATED COCCIDIOIDOMYCOSIS
Age Infants and elderly
Sex Male
Genetics Filipino > African-American > Native American > Hispanic > Asian
Serum CF antibody titer >1:32
Pregnancy Late pregnancy and postpartum
Delayed-type hypersensitivity skin test Negative
Depressed cell-mediated immunity Malignancy, chemotherapy, steroid treatment, HIV infection

macrophages with the appropriate T cells or cytokines.[49] When stimulated by spherules, leukocytes from both patients and skin test-positive subjects secrete potentially protective cytokines, such as interferon-? and IL-12.[50] [51]

Investigators have identified several potential virulence factors. For example, *C. immitis* produces a serine proteinase with broad specificity for host substrates, such as elastin, collagen, IgG and IgA. [52] [53] Other proteinases have also been detected.[16] [54] These proteinases are thought to contribute to the development of spherules and release of endospores.

Many of the patients who develop disseminated coccidioidomycosis have depressed cell-mediated immunity. There is a marked inverse relationship between the antibody titer (see below) and specific cell-mediated immunity, as measured by skin test and, *in vitro*, by the numbers of $CD4^+$ and $CD8^+$ T cells, the responsiveness of T cells to mitogens or antigens, and the production of cytokines. In severe coccidioidomycosis, patients have elevated antibody titers and circulating immune complexes and depressed cellular immunity. This condition has been related to increased antigen burden, populations of suppressor cells, immune complexes and impaired lymphocyte circulation.[55] Immune complexes are detected in serum of patients with coccidioidomycosis and correlate with the severity of disease.[56] In the mouse model of experimental coccidioidomycosis, specific anergy is correlated with the amount of coccidioidal antigen present.[57] Recovery often leads to restoration of immune functions. However, the impaired cellular immune responses are likely governed by whether Th1 or Th2 responses predominate early in infection.[55] [58] [59] In strains of mice that differ in susceptibility to *C. immitis*, protective responses are correlated with the secretion of interferon-?, which is a Th1-associated cytokine and potent activator of macrophages.[60] Conversely, much of the immunopathology may be attributable to excess production of TNF-a. Perhaps the ethnic predisposition to disseminated coccidioidomycosis (Table 238.5) is related to genetic control of the T cell response to *C. immitis*.

PREVENTION

Coccidioides immitis cannot be eliminated from the soil, but public health efforts to reduce the dust associated with dispersion of the arthroconidia are helpful in areas of high endemicity.[61] Another approach has focused on the development of a vaccine for persons at risk. In mice and humans, cell-mediated immunity to *C. immitis* confers excellent protection against disease. Spherule-derived vaccines in the past were not successful, but several new approaches to identify specific candidate epitopes are currently under investigation.[58] [62]

DIAGNOSTIC MICROBIOLOGY

Direct examination

A definitive diagnosis of coccidioidomycosis requires the finding of spherules of *C. immitis* in sputum, draining sinuses or tissue specimens (Fig. 238.5). Clinical exudates should be examined directly in 10% or 20% potassium hydroxide, with or without calcofluor white, and tissue obtained from biopsy can be stained with hematoxylin and eosin or special fungal stains, such as Gomori methenamine silver or the periodic acid-Schiff stain, which stain fungal cell walls black or reddish, respectively. Direct microscopic examination of cutaneous or deep tissue specimens, either in calcofluor/KOH preparations or histologic sections, yields positive results in approximately 85% of proven cases. However, sputum specimens are positive by direct examination or culture in less than half of the cases.

Culture

Clinical specimens are cultured on inhibitory mold, Sabouraud's agar or other routine fungal medium, as described above under Blastomycosis. Colonies of *C. immitis* develop within 1 or 2 weeks and are examined microscopically for the production of characteristic arthroconidia. Microscopic preparations of mycelia should always be prepared under a biosafety hood. The identification of *C. immitis* may be confirmed by the production of spherules *in vitro* by incubation in a complex medium at 104°F (40°C) with 20% CO_2 or by animal inoculation (e.g. intraperitoneal injection or mice or intratesticular inoculation of guinea-pigs). An easier method of confirmation involves the exoantigen test described in the discussion of Blastomycosis. Production of exoantigen F confirms the identity as *C. immitis* (see Table 238.3). This rapid method can be used with young, nonsporulating cultures.[25] [63] [64] Alternatively, DNA-based identification is available with a commercial system.[24] [63]

With the exception of tissue scrapings, biopsies and surgical specimens, cultures are more often positive than microscopic examinations of clinical material. However, use of both procedures will optimize the opportunity to establish a diagnosis. Between 25% and 50% of sputa, bronchial washes, spinal fluids and urine specimens yield positive cultures. Positive blood cultures are infrequent but significantly associated with acute, disseminated coccidioidomycosis and high mortality.

Skin tests

As noted above, coccidioidin, which is a crude but standardized toluene extract of a mycelial culture filtrate, is used for skin testing. A delayed-type hypersensitivity reaction is elicited, and a positive test is defined as induration exceeding 5mm in diameter. Another *C. immitis* antigen, prepared from cultured spherules and termed spherulin, is more sensitive but less specific than coccidioidin. Skin testing with either antigen does not induce or boost an immune response. The skin test becomes positive within 2 weeks after the onset of symptoms and before the appearance of antibodies and often remains positive indefinitely. A positive reaction has no

diagnostic significance without a history of conversion, but a negative test can be used to exclude coccidioidomycosis, except in patients with severe disseminated coccidioidomycosis who may have become anergic. Indeed, a negative skin test in confirmed cases is associated with a grave prognosis. Conversely, a positive skin test in healthy subjects implies immunity to symptomatic reinfection.

Serologic tests

As indicated in Table 238.3 , tube precipitins or latex agglutinins measure specific IgM antibodies. They are produced early and assist in the diagnosis of primary infections. They are detected by a sensitive tube test that becomes positive in 90% of patients within 2 weeks after the appearance of symptoms and disappears in most cases by 4 months. Therefore, a positive tube precipitin (TP) test indicates active primary (or reactivation) coccidioidomycosis. Results obtained with the original TP method correlate quite well with those obtained with the more rapid and convenient latex particle agglutination test; the latter procedure is more sensitive but less specific than the TP test. The TP antigen, a component of coccidioidin, is heat stable at 140°F (60°C), whereas the antigen detected in the complement fixation (CF) test is heat labile.

The CF test, which measures IgG antibodies to coccidioidin, is a powerful diagnostic and prognostic tool. Because the CF test becomes positive more slowly and persists longer, the presence of CF antibodies may reflect either active infection or the recovery stage. The CF titer correlates with the severity of disease. Most patients with secondary coccidioidomycosis develop a titer of 1:16 or higher, whereas in nondisseminated cases, the titer is almost

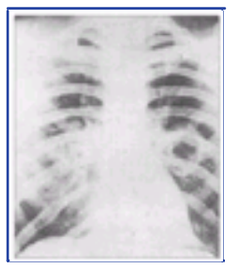

Figure 238-6 Coccidioidomycosis, showing hilar lymphadenopathy and a cavity in the left lung.

invariably lower. Therefore, a critical titer of 1:32 or higher reflects active, disseminated disease. However, a lower titer does not exclude disseminated disease because many patients, such as those with single extrapulmonary lesions, notably coccidioidal meningitis, do not develop high titers.

Multiple serum specimens are most helpful because a change in the CF titer reflects the prognosis: the CF titer declines with recovery and eventually disappears. A rising titer indicates active, uncontrolled infection and a poor prognosis. A stable or fluctuating titer often indicates the presence of a recalcitrant or stabilized lesion. An exceptional situation is coccidioidal meningitis, in which only half of the patients have a titer of 1:32 or higher. However, most of these patients will have a positive CF test in their spinal fluid, which is equally valuable.

The immunodiffusion (ID) method can be used to detect both TP and CF antibodies by using reference antisera and heated (TP only) and unheated antigen. Antibodies to two specific heat-labile antigens, termed F (or CF) and HL, may be detected. The CF antigen has been determined to be a chitinase.[65]

CLINICAL MANIFESTATIONS

Primary coccidioidomycosis

Following inhalation of arthroconidia, the primary infection in most individuals is asymptomatic. Others may develop flu-like symptoms: fever, chest pain, cough or weight loss. Radiographic examination often reveals discrete nodules in the lower lobes. Primary pulmonary coccidioidomycosis has an incubation period of 10–16 days and usually resolves without complication in 3 weeks to 3 months. A small percentage of patients retain cavities (Fig. 238.6), nodules or calcifications, but endogenous reactivation of residual pulmonary lesions is rare in immunocompetent individuals.

Up to 20% of patients with primary coccidioidomycosis manifest allergic reactions, usually erythema nodosum (Fig. 238.7) or erythema multiforme, which appear with the primary symptoms, are very painful and persist for approximately 1 week. These allergic manifestations are associated with strong immunity and a good prognosis.

Secondary coccidioidomycosis

Disseminated or secondary coccidioidomycosis usually develops within a few months as a complication of the primary form. The numerous manifestations of secondary coccidioidomycosis include chronic and

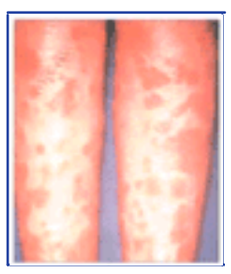

Figure 238-7 Allergic manifestations of infection with *Coccidioides immitis.* Erythema nodosum on the lower legs.

progressive pulmonary disease, single or multiple extrapulmonary dissemination or generalized systemic infection. Chronic pulmonary coccidioidomycosis usually involves a single, thin-walled cavity, but patients may develop enlarging or multiplying nodules or cavities.

Dissemination may be fulminant or chronic, with periods of remission and exacerbation. Extrapulmonary lesions most frequently involve the meninges, skin or bone. Chronic cutaneous coccidioidomycosis develops from initial lesions that usually appear on the face or neck and that, over a period of years, evolve into thick, raised, verrucous lesions with extensive epithelial hyperplasia.[66] Bone involvement may accompany generalized systemic disease. Both osteomyelitis of long bones, vertebrae and other bones and arthritis may develop.[67] Draining sinus tracts may evolve from subcutaneous and osseous lesions.

Coccidioidomycosis and AIDS

Coccidioidomycosis is the AIDS-defining illness for many patients, who commonly present with fever and chills, weight loss and night sweats.[68] [69] After pulmonary disease, coccidioidal meningitis is a frequent complication. Serologies are often negative in AIDS patients and the mortality rate is high. Diffuse pulmonary disease and low $CD4^+$ lymphocyte count (<50/μl) carry a poor prognosis.[68]

MANAGEMENT

Symptomatic treatment is usually adequate for the patient with primary coccidioidomycosis, although ketoconazole may reduce the symptoms. However, if the primary infection is persistent or severe, or if there is evidence of dissemination, ketoconazole or itraconazole should be administered at 400mg/day for 2–6 months.

The chronicity of the disseminated disease usually requires prolonged therapy, which may increase the chances of undesirable side effects. Successful treatment with ketoconazole may require continuous administration for more than a year. Chronic coccidioidal meningitis is often treated with fluconazole, which has excellent penetration across the blood-brain barrier. Some cases may require the intrathecal administration of chemotherapy. Grave disease is treated with amphotericin B in the usual formulation or as a lipid preparation to provide a higher dosage with reduced toxicity. For patients with AIDS, the total dose of amphotericin B (1.0–1.5mg/kg/day) should be at least 1.0g, after which a maintenance regimen of itraconazole or fluconazole may be started. Patients treated with amphotericin B or azoles are more likely to relapse if the delayed skin test becomes negative and the CF antibody titers equal or exceed 1:256.[70]

HISTOPLASMOSIS

Histoplasmosis, the most prevalent cause of pulmonary fungal disease in humans and animals, is caused by the thermally dimorphic fungus, *Histoplasma capsulatum*. The infection occurs worldwide and is initiated by inhalation of *H. capsulatum*. The incidence varies considerably, being negligible in many parts of the world but pronounced in local regions wherein most of the native population have been infected. Ninety-five percent of the infections are inapparent and are detected only by the manifestation of residual lung calcification(s), delayed hypersensitivity to *H. capsulatum*, or both. As one of the more common primary mycoses, throughout history, its existence has been inferred on the basis of descriptions of the pathogenesis and natural history of disease.

Around the turn of the last century, when histoplasmosis was discovered, the appearance of the organism within macrophages in histopathologic sections of infected tissue resembled encapsulated protozoa, hence the name, *Histoplasma capsulatum*.[71] The fungal etiology of histoplasmosis was subsequently confirmed and even though *H. capsulatum* is neither a parasite nor encapsulated, the name, by precedence and taxonomic custom, remains. With the discovery of a sexual reproduction cycle in *H. capsulatum*, it was transferred to the family Gymnoascaceae within the subdivision Ascomycotina. Because of the similarity between the sexual reproductive structures of the teleomorphs of *H. capsulatum* and *Blastomyces dermatitidis*, whose teleomorph, *Ajellomyces dermatitidis*, had been discovered earlier, *H. capsulatum* was transferred to that genus as *Ajellomyces capsulatus*.[72] [73] The name *H. capsulatum* is retained by common usage and remains appropriate as the fungus is clinically isolated in its asexual or anamorphic state. The close relationship between *H. capsulatum* and *B. dermatitidis* has been confirmed by molecular evolutionary studies comparing ribosomal DNA sequences.[74] Of three closely related varieties of *H. capsulatum*, most human cases are caused by *H. capsulatum* var. *capsulatum*, while *H. capsulatum* var. *duboisii* is found in Africa (see below), and *H. capsulatum* var. *farciminosum* causes infection in horses.

The extent of unapparent infection was not appreciated until the 1940s. During extensive skin test surveys for tuberculosis in student nurses from various parts of the country, radiologists observed small calcifications in the lungs of many apparently healthy individuals who were nonreactive to old tuberculin.[75] [76] Subsequent skin testing with histoplasmin, an antigen from *H. capsulatum* analogous to old tuberculin, revealed a high correlation between delayed skin test reactivity and pulmonary calcifications in tuberculin-negative individuals. The availability of skin test antigens for histoplasmosis has provided a very useful epidemiologic tool.

NATURE

Histoplasma capsulatum is a thermally dimorphic fungus. At temperatures below 95°F (35°C), it grows as a mold, often white or brown in color, and at 98.6°F (37°C), it grows as a yeast with small, heaped and pasty colonies. *Histoplasma capsulatum* characteristically grows slowly. Under optimal conditions, the mold colony develops after 1 or 2 weeks and conidia are produced shortly thereafter. However, cultures of clinical specimens sometimes require incubation periods of 8–12 weeks before there is detectable growth. Primary isolates are often brown and become white with prolonged cultivation.[25]

Both microconidia and macroconidia are produced at temperatures below 95°F (35°C). The microconidia are borne singly on short conidiophores, globose and 1–5µm in diameter. Upon dehydration, the conidia are easily dislodged by air currents and aerosolized. Their small size enables the microconidia to transmit the infection. The

macroconidia, or tuberculate chlamydospores, are very distinctive. Mature forms are large, 8–16µm in diameter, spherical thick-walled structures with projections radiating from the cell wall (Fig. 238.8). Young macroconidia are smooth walled and the echinulations develop as the conidia mature.

At 98.6°F (37°C), the mold converts to growth as a small, budding yeast. Conversion is often difficult to effect *in vitro* but is enhanced by a rich, complex medium, such as brain-heart infusion agar. Hyphal cells may form buds directly or develop enlarged, transitional cells, which subsequently produce buds. Microconidia may also convert to budding yeast cells. The yeast cells are small, ellipsoidal, approximately 1–3µm by 3–5µm in size, and virtually identical to the yeasts observed *in vivo* within phagocytes (Fig. 238.9).

Biochemical studies of the yeast and mycelial forms of *H. capsulatum* have detected significant differences in the relative amounts of chitin, a-glucan and ß-glucan of the cell walls. The three RNA polymerases in each growth form are also different. Morphogenesis in *H. capsulatum* involves a protein, histin, isolated from the cytoplasm of mycelial form. Histin inhibits RNA polymerase and may help to regulate the temperature-dependent control of transcription. An inhibitor, such as histin, may be operative in the mycelia, because protein synthesis is considerably reduced during mycelial growth.

In *in vitro* studies of the crucial mycelium-to-yeast conversion at 98.6°F (37°C), Maresca *et al.* confirmed the importance of cysteine.[77] [78] This conversion proceeds in three stages:

1. during the first 40 hours at 98.6°F (37°C), respiration gradually decreases and the intracellular amino acid pools become almost depleted;
2. the cells remain dormant for 4–6 days;
3. then concentrations of intracellular cysteine and other amino acids increase and respiration is restored.

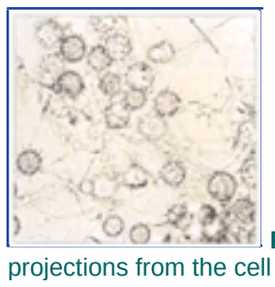

Figure 238-8 *Histoplasma capsulatum,* mycelial form in culture at 86°F. This shows hyaline septate branching hyphae, microconidia and large spherical macroconidia, with projections from the cell walls.

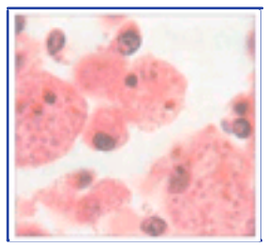

Figure 238-9 *Histoplasma capsulatum.* Small yeast cells packed inside macrophages in a Giemsa-stained smear of bone marrow aspirate.

Cysteine and cystine are essential for the transition to yeast cells. These compounds and other reducing agents appear to stimulate the mitochondrial electron transport chain, probably via a unique cysteine oxidase. This mycelium-to-yeast conversion is inhibited by elevated cyclic AMP, which may also be regulated intracellularly by the action of sulfhydryl agents.[77] Recent research from several laboratories has identified yeast form-specific genes that may be involved in the regulation of dimorphism and consequently virulence.[79] [80] [81] [82] [83]

In studies of virulent strains, the sulfhydryl blocking agent, *p*-chloromercuriphenylsulfonic acid (PCMS), irreversibly inhibited the conversion of mycelium to yeast at 98.6°F (37°C), but mycelial growth continued at 98.6°F (37°C).[84] PCMS-treated mycelia also failed to infect mice. Therefore, mycelium-to-yeast transformation appeared necessary for pathogenicity but not for growth at 98.6°F (37°C). PCMS-treated mycelia may provide a vaccine for histoplasmosis and indicate the essential changes for transformation.[84]

Sexual reproduction

As noted above, discovery of its sexual reproductive cycle led to reclassification of the teleomorph *A. capsulatus*, which closely resembles the teleomorph of *B.*

dermatitidis. [72] Being heterothallic, *A. capsulatus* requires strains of two opposite (compatible) mating types for sexual reproduction. Although the mating types, designated '+' and '-', are equally prevalent among soil isolates, many more infections are caused by the '-' type, which suggests a linkage of this allele with pathogenicity.[85]

Biotypes

Biotyping may facilitate epidemiological studies and several DNA-based methods have been developed to identify and compare strains of *H. capsulatum* from different sources and with different phenotypes. Isolates that differ in virulence for animals have been compared with strains from the environment and with isolates from patients with and without AIDS.[86] [87] [88] In addition, phylogenetic analyses have identified major subpopulations of *H. capsulatum* that correspond with geographic groups.[89]

EPIDEMIOLOGY

Ecology

In nature, *H. capsulatum* lives in soil with a high nitrogen content and is associated with bat and avian habitats. *H. capsulatum* has been isolated many times from bird roosts, chicken houses, bat caves and similar environments. Conidia, when dry, are easily air borne and spread by wind currents, as well as by birds and bats. *H. capsulatum* is most prevalent in the environment where the infection is most endemic, namely in the Ohio-Mississippi Valley — in Missouri, Kentucky, Tennessee, Indiana, Ohio and southern Illinois. This region also has the highest population of starlings, which tend to congregate in large numbers. The excrement from these birds provides an ideal medium for the enrichment of *H. capsulatum*. In South America, the chief reservoir appears to be chicken coops and bat caves.

In cases of histoplasmosis from Africa, both *H. capsulatum* and a stable variant, *H. capsulatum* var. *duboisii*, have been isolated. Infections elsewhere are due to the global variety, *H. capsulatum* var. *capsulatum, H. capsulatum* var. *duboisii* causes African histoplasmosis, which is distinguished from the usual infection by:

- a greater frequency of skin and bone lesions;
- diminished pulmonary involvement;
- pronounced giant cell formation; and
- larger, thick-walled yeast cells in tissue.

Although these clinical features are characteristic and reproducible, *H. capsulatum* var. *duboisii* cannot be reliably differentiated *in vitro* from the type species on the basis of morphology, physiology

and antigenic composition. Indeed, both forms of histoplasmosis are the same species, as *H. capsulatum* var. *duboisii* mates with *H. capsulatum* var. *capsulatum*, and its sexual form is identical to *A. capsulatus*.

Another variety, *H. capsulatum* var. *farciminosum*, is associated with cutaneous, subcutaneous and lymphangitic lesions in horses.[90]

Histoplasmin skin test

The antigen, histoplasmin, is produced by growing the mycelial phase of *H. capsulatum* in a specialized broth medium. The filtrate from the culture is dialyzed, the concentration is standardized and 0.1ml of the appropriate dilution is injected intradermally. A positive reaction is indicated by induration of >5mm diameter after 48 hours.

A positive test, if specific, denotes previous sensitization to *H. capsulatum*. Without a history of prior negativity, the positive test has no diagnostic significance. Histoplasmin is a crude, polyvalent mixture of antigens, only some of which are specific for *H. capsulatum*. Because some antigenic determinants are shared by other pathogenic fungi, cross-reactions can occur. For example, some individuals sensitive to *B. dermatitidis* or *C. immitis* will give a false-positive reaction to histoplasmin. Therefore, along with histoplasmin, it is routine to administer coccidioidin in the USA or coccidioidin and paracoccidioidin in South America. A reaction to a single antigen is generally considered specific. Reactions to two antigens may be caused by sensitization to one or both, although the larger reaction is often considered more specific.

Incidence

Much of the knowledge concerning the prevalence of histoplasmosis has been derived from extensive skin test surveys conducted since the 1950s all over the world. The region with the highest level of reactivity is the central USA, along the river valleys of the Ohio, Mississippi, St Lawrence and Rio Grande rivers, where in some locales 80–90% of the population may be skin test positive by the age of 20 years. Foci of high reactivity exist elsewhere in the world, such as southern Mexico, Indonesia, the Philippines and Turkey. In the USA alone, projections based on skin test surveys have led to estimations that more than 40 million people have been exposed with 500,000 new infections every year. Of these, perhaps 55,000 to 200,000 cases will be symptomatic, 1500–4000 will require hospitalization annually and 25–100 deaths will occur. These projections were made prior to 1980 and do not reflect the increasing incidence of opportunistic histoplasmosis in patients with AIDS.

Outbreaks

Outbreaks or epidemics of acute respiratory histoplasmosis result from the simultaneous exposure of a large number of people. These epidemics are not caused by direct spread among humans or animals. The experience of youths on Earth Day, 1970, in Delaware, Ohio, is more ironic than most, but otherwise typical of these epidemic outbreaks.[122] The young people gathered to reclaim an abandoned park and, in so doing, overturned several truckloads of soil, which was enriched with starling feces and contaminated with an enormous quantity of *H. capsulatum* conidia. Several cases of acute respiratory histoplasmosis followed inhalation of this heavy inocula of aerosolized microconidia. Many similar episodes have been documented: the sudden release leads to multiple exposure of a heavy inoculum that has accumulated in a dormant environment. Silos, air-conditioning units contaminated with bird droppings and accumulations of guano in caves, attics or parks have all been implicated as reservoirs for *H. capsulatum* in outbreaks of this type. Perhaps the largest outbreak occurred in Indianapolis between the fall of 1978 and 1979.[91] It is estimated that more than 100,000 persons were infected during this time, resulting in over 300 hospitalized cases and at least 15 deaths. The incidence of disseminated histoplasmosis and the fatality rate were unusually high. The environmental source of the fungus was not determined. Indeed, *H. capsulatum* was not recovered from any of the soil samples collected at the most likely site, where an abandoned amusement park had been recently dismantled.

Males develop symptomatic histoplasmosis more often than females, and approximately 75% of cases occur in males. Before puberty, the attack rate for males and females is identical and the percentage of positive skin test reactors is the same for both sexes at all ages. These epidemiologic data suggest that either adult males are inherently more susceptible to the disease or females are more resistant. Severity of disease and mortality are greater at the age extremes, in infancy and after the age of 50.

In addition to humans, many wild and domestic animals are susceptible to histoplasmosis. Some animals, including the bat, may act as vectors to disseminate the organism in nature.

PATHOGENICITY

All clinical forms are believed to evolve from the same natural history. Microconidia are inhaled from an exogenous source and penetrate to the alveoli, where they convert to small, budding yeast cells. This temperature-dependent morphogenesis is related to the virulence of strains of *H. capsulatum*. [92] [93] The yeasts are readily phagocytized by alveolar macrophages. At this stage, the yeast-laden macrophages may be cleared through the upper respiratory tract. They may disseminate through the circulation, spreading the yeasts to other reticuloendothelial organs, and/or they may invoke a tissue response *in situ*. The tissue reaction may involve an early influx of neutrophils and lymphocytes, but the pyogenic inflammatory response gives way to epithelioid cell tubercle formation. In the course of these various possible reactions, the intracellular yeasts may or may not be inactivated by the phagocytes (see below).

The conversion of *H. capsulatum* to the yeast form at 98.6°F (37°C) appears to be essential for pathogenicity. As noted above, treatment of the mycelial form with PCMS blocks morphogenesis and reduces virulence, but does not inhibit survival at 98.6°F (37°C).[84] The expression of yeast-specific genes has been correlated with

virulence and thermal tolerance.[80] [83] [92] [94]

Pathogenesis

After being phagocytized, the yeast cells of *H. capsulatum* survive intracellularly by a calcium-dependent process, block acidification within the phagolysosome and multiply within macrophages.[83] [95] However, macrophages from immunized animals, as well as normal macrophages activated by immune lymphocytes or cytokines, restrict the growth of intracellular yeasts.[96] [97] In experimental, self-limited murine histoplasmosis, various parameters of cell-mediated immunity are depressed during the height of antigen (yeast) burden, suppressor T cells and macrophage-like suppressor cells are detected and production of IL-1 and IL-2 is impaired.[98] [99] Concomitant with resolution of the infection, the number of suppressor cells in the spleen diminishes and T helper cells increases. These correlations of competent cell-mediated immune responses with resistance to infection are supported by the clinical data. Similar to coccidioidomycosis, there is an inverse relationship between the magnitude of the cell-mediated immune response, as measured by delayed-type hypersensitivity or *in vitro* lymphoblastogenesis, and high levels of specific antibody, which correlate with the severity of the disease. Additional evidence suggests that TNF-a may be crucial in the induction of protective T-cell responses.[55] [100]

DIAGNOSTIC MICROBIOLOGY

Microscopic examination

Histoplasmosis can be diagnosed on finding the yeast cells in clinical material. Suitable specimens include sputa, tissue from biopsy or surgical specimens, spinal fluid and blood. The buffy coat of a blood specimen may reveal yeast-filled macrophages. Bone marrow obtained when patients are febrile may contain yeast cells. Smears of infected sputum, blood, marrow or tissue that have been fixed with methanol and stained with the Wright or Giemsa stain will reveal the characteristically small, ellipsoidal yeast cells (approximately 2 × 4µm) inside macrophages. With either stain, the larger end of the yeast cell contains an eccentric, red-staining mass (see Fig. 238.9).

Culture

Sputum specimens should be collected early in the morning and purulent or sanguineous portions should be selected for culture. A bronchial wash is even more likely to be positive. Nonsterile specimens (e.g. sputum, skin or urine) should be cultured on a blood-enriched medium and inhibitory mold or Sabouraud's agar with antibiotics (cycloheximide and chloramphenicol or gentamicin) and incubated for at least 4 weeks at 77°F (25°C) or 86°F (30°C). Because *H. capsulatum* may grow very slowly, cultures should be incubated for up to 12 weeks, if possible, before discarding as negative. If a sporulating mold develops, *H. capsulatum* can be identified by the presence of its characteristic macroconidia (see Fig. 238.8) and by conversion to the yeast from by growth on an enriched medium at 98.6°F (37°C). Alternatively, conversion to the yeast may be effected by growth in tissue cultures, such as HeLa cells, or by animal inoculation, such as the intraperitoneal injection into mice. Occasional isolates of *H. capsulatum* will not produce conidia, but it should be possible to identify these variants by conversion to the yeast form, by the detection of *H. capsulatum*-specific exoantigens (see Table 238.3) or by using a specific DNA probe.[24]

In endemic areas or in cases where histoplasmosis is suspected, specimens should be inoculated on multiple media, such as Sabouraud's agar without antibiotics at 77–86°F (25–30°C), Sabouraud's agar with antibiotics (cycloheximide and chloramphenicol, gentamicin or penicillin and streptomycin) at 77–86°F (25–30°C), brain-heart infusion agar with 5% sheep blood and antibiotics at 77–86°F (25–30°C), and brain-heart infusion agar with 5% sheep blood without cycloheximide at 98.6°F (37°C). The pH of these media should be near neutrality, since *H. capsulatum* is inhibited below pH 6.

In disseminated cases, the lysis-centrifugation method is recommended for culturing blood, although transient fungemia may be observed in patients with acute pulmonary histoplasmosis. Blood volumes of 10ml are added to a tube containing a mixture of anticoagulants and reagents to lyse the blood cells; the tubes are then centrifuged and the pellet, which contains any yeast cells in the blood, is inoculated onto plates of inhibitory mold agar and other media. Lysis-centrifugation is the most sensitive and rapid method to recover fungi from blood, especially *H. capsulatum*.[101]

Skin test

The skin test antigen, histoplasmin, is a valuable epidemiologic tool. Within weeks after infection, most persons develop a positive skin test and this reactivity usually persists for many years. The diagnostic value of the skin test is minimal. With most patients, only a history of conversion from negative to positive is diagnostic. A negative reaction can be used to rule out active histoplasmosis in the immunocompetent subject, but patients with anergy may be falsely negative. Without a prior history of a negative skin test, a positive reaction is meaningless except in infants, in whom a positive test can be presumed to result from recent or current infection. Because of its limited diagnostic value and the possibility that the skin test may confound the antibody titration (see below), skin testing with histoplasmin should be avoided in most patients.

Serology

Specific antibodies to *H. capsulatum* antigens can be detected during infection. Two serologic tests are now widely accepted because of their convenience, availability and utility: the measurement antibodies by complement fixation (CF) and the immunodiffusion (ID) test for precipitins. Both tests may be helpful in the diagnosis and prognosis of histoplasmosis, provided the results are properly interpreted (see Table 238.4).

The CF test is routinely performed under standard conditions for measuring fixation of complement by the classic pathway.[102] [103] CF tests are performed to detect antibodies to two antigens of *H. capsulatum*: histoplasmin and a standardized suspension of killed yeast cells. Because of the possibility of cross-reactivity, patient sera are tested concomitantly against other fungal antigens, such as coccidioidin, spherulin, *B. dermatitidis* or *Paracoccidioides brasiliensis*. Serum antibodies specific for *H. capsulatum* antigens can be detected by the CF test 2–4 weeks following exposure. Most laboratories perform the CF test on twofold dilutions of patient serum, beginning with a dilution of 1:8. With resolution of the infection, the antibody titer gradually declines and disappears (i.e. titer <1:8), in most cases by 9 months. The CF test with either *H. capsulatum* yeast or mycelial (histoplasmin) antigen is very sensitive and 90% of patients are positive (i.e. titer >1:8).[91] A titer of 1:32 that persists or rises over the course of several weeks indicates active disease in patients with an established diagnosis of histoplasmosis. Unfortunately, in sensitive patients, the skin test antigen may boost the CF antibody titer to histoplasmin and the elevated titer may remain for as long as 3 months. Therefore, the CF test, which can deliver results as rapidly as the skin test, is preferable for diagnostic purposes. However, a positive CF test, even in high titer, is not by itself diagnostic, as the results can be caused by cross-reacting antibodies. If a patient's serum is reactive to more than one fungous antigen or if it is anticomplementary, the ID test should be conducted.

Precipitins can be detected by immunodiffusion (ID) of serum and antigen in agarose. The antigen is histoplasmin in 10-fold the concentration used for the CF test. The ID test becomes positive in up to 80% of patients with histoplasmosis by the third or fourth week of infection. This test, while less sensitive and requiring a longer time to become positive, is more specific than the CF test. Precipitin lines or bands specific for *H. capsulatum* are detected by the formation of lines of identity with reference serum. There are two specific precipitin bands, m and h. The m line, which is observed more frequently, appears soon after infection and may persist in the serum for up to 3 years following recovery. The h band, which forms closer to the serum wells, is more transient. Because it disappears soon after the disease, the presence of serum antibodies to the h antigen is better correlated with active infection. As with the CF titer, the m band may be boosted by the administration of the histoplasmin skin test, and the boosting effect may last up to 3 months.

An excellent radioimmunoassay (RIA) test for antigenemia and antigenuria has been developed by Wheat and colleagues.[104] [105] Tests for polysaccharide antigen in the serum have been detected in 79% of patients with disseminated histoplasmosis and 97% had a positive test for antigen in the urine. After treatment with AMB, serum titers dropped in all the patients and urine titers dropped in 91% of patients. Testing either serum and urine had prognostic value, detecting relapses in AIDS patients. [104] This RIA is commercially available from the Histoplasmosis Reference Laboratory (Indianapolis, IN 46202). The antigen can also be measured in CSF or bronchoalveolar lavage fluid specimens. However, the test lacks optimal specificity

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because patients with other systemic mycoses have been shown to yield positive tests for the antigen in urine.[106]

CLINICAL MANIFESTATIONS

The manifestations of infection with *H. capsulatum* are numerous. The initial pulmonary episode may be acute or chronic or dissemination may occur by hematogenous

or lymphatic spread from the lungs to other organs. Several clinical classifications have been devised, but none is completely satisfactory. Most normal individuals are able to contain the infection. The granulomata that form may undergo fibrosis, and residual scars may remain in the lungs or spleen. Resolution appears to confer some immunity to reinfection. This process occurs without symptoms in 95% of all persons with acute, primary histoplasmosis, whether disseminated or confined to the lung.

Acute pulmonary histoplasmosis

Patients with acute pulmonary histoplasmosis manifest symptoms ranging from a mild influenza-like illness that clears spontaneously to a moderate or severe disease. In healthy hosts, the degree of involvement and symptomatology seems to correlate with the size of the inoculum inhaled. In the previously sensitized individual, such reinfection exposure results in a shorter and milder infection with minimal histopathology. The incubation period varies from one to several weeks. A moderate disease is characterized by cough, chest pain, dyspnea and hoarseness. In more severe cases, patients have fever, night sweats and weight loss. Occasionally, yeast cells may be observed in the sputum. Radiologic examination may reveal multiple lesions scattered throughout the lungs and in patients with active disease, hilar lymphadenopathy is usually present (Fig. 238.10). Pulmonary lesions due to *H. capsulatum* resolve slowly. Healing may be complete or with fibrosis but, typically, calcification occurs.

The differential diagnosis includes other systemic mycoses, tuberculosis, bacterial bronchiectasis and lymphoblastoma. An experienced radiologist can differentiate between the calcifications of histoplasmosis and tuberculosis. Calcifications produced by *H. capsulatum* are more regular, with halos, and may be found in the liver and spleen as well as in the lungs. Miliary calcifications may also occur. Calcifications are produced more rapidly in children than adults. Single, solitary, uncalcified coin lesions are also produced and are similar to those seen in tuberculosis. These resemble neoplastic lesions and are often removed surgically. Another tuberculosis-like pulmonary manifestation usually found in the adult lung is a histoplasmoma, which may be 2–3cm in diameter and contains a central necrotic area encased in a fibrotic capsule. Calcification begins in the center of the lesion and is followed by the development of concentric rings of fibrosis and calcification.

Chronic pulmonary histoplasmosis

This form is seen most often in adult males. It is considered to be an opportunistic complication of underlying chronic obstructive lung disease with emphysema and abnormal pulmonary spaces. With small emphysematous air spaces, transient pneumonitis develops and infection of large bullous spaces may result in cavitary histoplasmosis. Symptoms of the latter may be indistinguishable from those of chronic cavitary tuberculosis. The chronic form is secondary to the underlying pulmonary disease. It may develop immediately after primary inhalation or after years of apparent quiescence. Pathologic and immunologic evidence suggests that the late onset results from reactivation of an old lesion rather than exogenous reinfection. Chronic pulmonary histoplasmosis is usually apical. Patients experience a low-grade fever, productive cough, progressive weakness and fatigue. Chest films show centrilobular or bullous emphysema. Prognosis depends upon control of the underlying disease as much as on treatment for histoplasmosis.

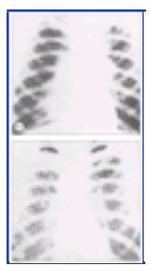

Figure 238-10 Histoplasmosis. (a) Acute pulmonary histoplasmosis showing hilar lymphadenopathy and diffuse infiltrates. (b) Hilar lymphadenopathy and miliary calcifications throughout both lungs.

Disseminated histoplasmosis

The gamut of clinical forms and pathology observed in pulmonary histoplasmosis can also occur in any other part of the body. The yeast cells are probably disseminated throughout the body while inside macrophages. The most common sites of involvement, after the lung, are the reticuloendothelial tissues of the spleen, liver, lymph nodes and bone marrow. However, lesions have been documented in almost every organ. Dissemination may be completely benign and unapparent except for the presence of calcified lesions, usually in organs of the reticuloendothelial system.

Alternatively, disseminated histoplasmosis may be acute and progressive. In such cases, the pulmonary symptoms are insignificant and patients may have splenomegaly and hepatomegaly, weight loss, anemia and leukopenia. Granulomatous lesions and macrophages packed with yeast cells can be observed throughout the liver, spleen, marrow and, quite often, the adrenals. Acute progressive histoplasmosis is often fulminant and rapidly fatal — ultimately every organ can become diseased. This form of histoplasmosis is an opportunistic disease associated with compromised cell-mediated immunity, such as in patients with AIDS or those receiving immunosuppressive drugs and those with underlying lymphomatous neoplasia. In most cases, the compromising condition serves to reactivate a quiescent lesion that was originally acquired years earlier. Within the endemic area, infants with histiocytosis may develop disseminated histoplasmosis that is characteristically fulminant. Chronic disseminated histoplasmosis may evolve from protraction of the acute disease. This form is progressive, with eventual involvement of every organ, especially the mucocutaneous areas around the eye, tongue and anus.

Histoplasmosis and AIDS

In a recent prospective study in the endemic area, the annual incidence of subclinical and asymptomatic histoplasmosis among

patients with AIDS was 4.7%. Three-quarters of the cases were symptomatic and histoplasmosis was the initial AIDS-defining condition in most of them.[107] The survival time for patients with AIDS was significantly shorter for those with disseminated histoplasmosis. Significant risk factors were identified for the development of histoplasmosis in AIDS patients: environmental exposure to likely sources of *H. capsulatum* (chicken coops), positive CF antibodies to histoplasmin, chronic medical condition(s), herpes simplex infection and a $CD4^+$ lymphocyte count of <150,000/ml.[107] [108]

Other forms

Very rarely, chronic disseminated histoplasmosis develops following primary inoculation of the skin or mucocutaneous tissue. The lungs are not involved in these cases, as the organisms are typically introduced following the contamination of a traumatic wound. Such infections may be anatomically localized, as with some ocular cases, or they may chronically progress with involvement along the draining lymphatics.

Another clinical condition, probably unrelated to *H. capsulatum*, is 'presumed ocular histoplasmosis syndrome' or POHS. Patients with POHS exhibit characteristic choroidal lesions, macular subretinal membranes and peripapillary atrophy.[109] Although POHS is usually associated with a positive histoplasmin skin test, its cause is unknown. Patients with histoplasmosis may develop ocular lesions, but POHS is not seen in patients with active histoplasmosis.

MANAGEMENT

Most primary pulmonary infections with *H. capsulatum* go undetected and require no treatment. Symptomatic cases in immunocompetent persons are alleviated with itraconazole or ketoconazole. With progressive pulmonary or disseminated disease, the treatment of choice for mild to moderate or stable disease is itraconazole.[110] Severe cases of any form are treated initially with AMB followed by itraconazole. The regimen of AMB is similar to that applied to other systemic mycoses and a total dose of 1.5g AMB is recommended. Recovery following treatment with AMB is generally faster and fewer relapses occur than are experienced with blastomycosis and coccidioidomycosis. Arrested pulmonary lesions are often removed surgically. Less severe cases in nonimmunocompromised patients may be treated with itraconazole. AIDS patients with histoplasmosis may be placed on maintenance therapy with itraconazole at 200mg twice per day.[110] [111] Cases of meningitis due to *H. capsulatum* are treated with amphotericin B followed by fluconazole.

PARACOCCIDIOIDOMYCOSIS

Paracoccidioidomycosis (South American blastomycosis) is an infection caused by the thermally dimorphic fungus, *Paracoccidioides brasiliensis*. The infection is restricted to endemic areas of Central and South America. It is a chronic, granulomatous disease that begins with a primary, pulmonary, usually unapparent infection that disseminates to produce ulcerative granulomata in the mucosal surfaces of the nose, mouth and gastrointestinal tract. In addition to the skin and lymph nodes, the infection may spread to the internal organs.

NATURE

Colonies of *P. brasiliensis* grow very slowly, requiring 2 or 3 weeks of incubation to reach a diameter of 1–2cm. The macroscopic features of the mold colony are variable and nonspecific. Various asexual reproductive structures are produced by *P. brasiliensis*, including chlamydospores, arthroconidia and singly borne conidia. In the absence of conidia, which may not be produced for 10 weeks

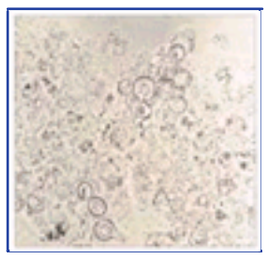

Figure 238-11 ***Paracoccidioides brasiliensis.*** Large multiply budding yeast cells in a potassium hydroxide preparation of a scraping of cutaneous paracoccidioidomycosis.

in culture, the mycelia and colony may be indistinguishable from *B. dermatitidis* or many saprophytes.

The yeast form can be induced by cultivation on a rich medium at 95–98.6°F (35–37°C). The yeasts are readily identified by their unique appearance. As shown in Figure 238.11 , the yeasts produce multiple buds and each is attached to the parent yeast by a narrow base. The yeast cells are large, up to 30µm in diameter, and have thinner walls than the yeasts of *B. dermatitidis*. These forms have been described as 'pilot wheels'.

EPIDEMIOLOGY

The natural habitat of *P. brasiliensis* has not been proven, but is presumed to be soil. The organism has been recovered only sporadically and not repeatedly from soil in Venezuela and Argentina. Like *B. dermatitidis*, its natural existence and life cycle in nature are unknown. There is no evidence of an animal vector or transmission of the infection. Natural disease does not occur in either wild or domestic animals, although experimental infections can be established in mice and bats after inhalation of an infectious inoculum. Human infections are presumed to follow exposure to the organisms from an exogenous source. Most patients are males (>90%), agricultural workers, often malnourished, and usually 30–60 years of age.

Thousands of cases of paracoccidioidomycosis have been reported from Brazil, Venezuela, Colombia and lesser numbers from Argentina, Ecuador and other South and Central American countries, with the exception of Chile and the Caribbean nations.[112] Discrete endemic foci exist within this broad area of geographic distribution. However, all cases are isolated and outbreaks have not been observed. The endemic zones are associated with moderate temperatures 57–86°F (14–30°C) and rainfall, elevation of 500 to 6500 feet, subtropical forests and river valleys, but not all areas fitting this description have paracoccidioidomycosis.

Skin test surveys have been conducted with various antigens derived from *P. brasiliensis*. These paracoccidioidins exhibit cross-reactivity with histoplasmin and it is difficult to interpret double reactions of equal size in the same individual. As with the skin test antigens of the other dimorphic, systemic pathogens, paracoccidioidin elicits a delayed, indurative reaction that indicates previous exposure. The percentage of reactivity in the endemic areas varies up to 75% and occurs equally in both men and women. Significant risk factors for infection (i.e. positive skin test) include agricultural occupations, association with certain aquatic environments and contact with bats.[113]

Many patients with paracoccidioidomycosis are malnourished and exhibit depressed cell-mediated immune responses.

PATHOGENICITY

P. brasiliensis, like the other systemic fungi, causes disease in males more frequently than females (Table 238.1), although skin test surveys have revealed comparable reactivity between the sexes, implying equal exposure. Sex-linked differences may be associated with the generally more potent cellular immunity of females. Limited studies indicate that physiological concentrations of sex hormones do not directly inhibit these fungi. However, a protein from the mycelial cytosol of *P. brasiliensis* has been shown to bind estrogen but not testosterone or other hormones. Binding blocks conversion of the mycelium to yeast at 98.6°F (37°C) and may explain the resistance of females to paracoccidioidomycosis.[114]

Once yeast cells of *P. brasiliensis* have developed in the lung, yeast cell wall polysaccharides, such as a-glucan, are associated with virulence and the ability to stimulate granulomata.

DIAGNOSTIC MICROBIOLOGY

Microscopic examination and culture

Sputum, tissue or scrapings of mucocutaneous lesions may reveal the multiply budding yeast cells that are pathognomonic for *P. brasiliensis* (Fig. 238.11). Specimens should be cultured at 77–86°F (25–30°C) on inhibitory mold agar, Sabouraud's agar with antibiotics, Sabouraud's agar without cycloheximide and on brain-heart infusion blood agar at 95–98.6°F (35–37°C). The yeast form often grows better at 95°F (35°C) or 96.8°F (36°C) than 98.6°F (37°C).

Serologic tests

A number of antigens and serological procedures have been evaluated. [115] The ID test is extremely useful. As indicated in Table 238.4 , nearly 100% of patients have at least one of three specific precipitin lines (designated 1, 2 and 3) detected by identity with reference serum. The ID test also has prognostic value, as the bands disappear with clearing of the infection and the number of bands is somewhat correlated with the severity of the disease.[116] The CF test is quantitative and useful for assessing prognosis, but cross-reactions occur with other fungi.

CLINICAL MANIFESTATIONS

The initial contact with *P. brasiliensis* occurs by inhalation. This episode is unapparent and the organism becomes quiescent for an indefinite period, which may be several decades in some individuals, or the lesion may resolve, perhaps with scarring. This asymptomatic infection results in the acquisition of a positive delayed skin test reaction to paracoccidioidin. The eventual development of symptomatic disease depends upon the host-fungal interaction, namely, the integrity of the cell-mediated immunity of the individual, environmental conditions (e.g. temperature, nutrients), host conditions (e.g. age, sex, state of nutrition), and the virulence of the strain of *P. brasiliensis*.

Acute or subacute disease

Patients under 30 years of age may develop an acute, progressive infection characterized by lymphonodular lesions in the lung.[117] This juvenile form is rare. The yeasts may disseminate to the reticuloendothelial

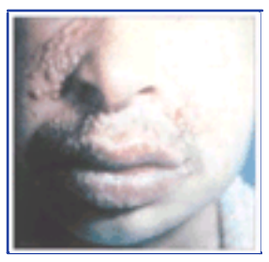

Figure 238-12 Cutaneous and mucocutaneous paracoccidiodomycosis.

tissue, lymph nodes, liver, spleen, skin, bone, joints or other organs. The severity and duration of the illness depend upon the extent of organ involvement, but it may be fatal within a period of several weeks or months.

Chronic disease

More than 90% of cases are of this 'adult' type and develop from the latent form, usually after several years. Lesions may be localized in the lung or metastasis may occur from the lung to other organs, particularly the skin and mucocutaneous tissue, lymph nodes, spleen, liver, adrenals and combinations thereof. Mucocutaneous, often petechial, lesions frequently develop on the corners of the mouth, lips, gingiva or tongue (Fig. 238.12). Pulmonary lesions are granulomatous nodules that may cavitate but rarely calcify.[118]

MANAGEMENT

Patients should be assessed for malnourishment, alcoholism and extensive tobacco use, all of which exacerbate the disease. Since many of the antifungal drugs are effective against *P. brasiliensis*, the initial treatment choice may reflect the expense and local availability of antifungal agents.[119] Sulfonamide derivatives are among the earliest but less effective drugs. Trimethoprim-sulfamethoxazole (TMP-SMX) is the currently recommended formulation of this type. The azoles are much more effective. Some of the initial clinical trials with ketoconazole demonstrated its efficacy against paracoccidioidomycosis. Itraconazole is currently the drug of choice; a clinical cure rate approaching 100% is achievable with a daily dose of 100mg for 6 months. Relapses are rare. Fluconazole has also been shown to be effective, producing cures in about 90% of patients who received 200–400mg/day for 6 months. Although AMB is highly effective against paracoccidioidomycosis, it should be reserved for patients who fail to respond or cannot tolerate one of the azoles. The total dose usually required is 2g or less. After initiating therapy, serologies are checked every few months to monitor the effectiveness of treatment. Some clinicians recommend a maintenance regimen of sulfa-doxin, TMP-SMX or ketoconazole (200mg/day) for up to 1 year after serological tests become negative.[119]

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Chapter 239 - Subcutaneous Mycoses

Malcolm D Richardson

Although subcutaneous fungal infections exhibit extraordinary heterogeneity, they have certain features in common — infection is usually acquired from nature and not from infected humans or animals, and the endemic areas are delineated by an ecosystem that consists of altitude, temperature, rainfall, type of soil and type of vegetation. Most patients belong to low socioeconomic groups or live in rural areas. Subcutaneous mycoses arise from inoculation of soil or vegetation into the skin by minor trauma, and most patients have an occupation connected either with agriculture or an outdoor activity and do not use appropriate footwear.

The group of fungi that cause the majority of subcutaneous infections in humans are termed black molds.[1] Black molds are a heterogeneous group of darkly pigmented (dematiaceous) fungi, widely distributed in the environment, that occasionally cause infection in humans. The taxonomy and terminology of dematiaceous fungal infections is baffling. The term chromoblastomycosis was introduced in 1922 and was later modified in 1935 to a broader term 'chromomycosis'. More recently, the term 'phaeohyphomycosis' was proposed to cover 'all infections of cutaneous, subcutaneous and systemic nature caused by hyphomycetous fungi that develop in the host tissues in the form of dark walled dematiaceous septate mycelial elements'. In 1981, the term was further expanded to include deuteromycota and ascomycota whose tissue forms are filamentous and dematiaceous. This certainly excludes infections by fungi that produce thick-walled 'sclerotic bodies' in the tissues and are classically labeled as chromoblastomycosis. The line of demarcation is, however, only histopathologic and very thin because some of the fungi (e.g. *Exophiala dermatidis*), in addition to mycelial forms, produce rounded structures closely resembling sclerotic bodies. Thus, there has been plenty of overlap in the nomenclature of these cases, especially during the 1970s and 1980s.

The clinical spectrum of infection includes mycetomas, chromoblastomycosis, sinusitis and superficial, cutaneous, subcutaneous and systemic phaeohyphomycosis. During the past few years, there have been reports of infections caused by black molds in previously healthy individuals and in immunocompromised patients. Molecular studies have contributed to our understanding of the epidemiology of these infections. In addition, data on antifungal susceptibility tests have become available. Surgical excision and antifungal therapy (usually itraconazole) remain the standard treatment for these infections.

Many standard texts contain excellent reviews of all the infections described here.[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] Specific references and reviews are cited here where new information, particularly concerning diagnosis and therapy, supercedes that found in the general texts.

NATURE

Chromoblastomycosis

Chromoblastomycosis (or chromomycosis) is a chronic localized infection of the skin and subcutaneous tissue, most often involving the limbs. It is characterized by raised crusted lesions. It may be caused by a number of brown-pigmented (dematiaceous) fungi.

Entomophthoramycosis

Rhinofacial conidiobolomycosis is a chronic mycosis affecting the subcutaneous tissues. It originates in the nasal sinuses and spreads to the adjacent subcutaneous tissue of the face, causing disfigurement. Basidiobolomycosis is a chronic subcutaneous infection of the trunk and limbs. For an exhaustive review of entomophthoramycosis refer to Ribes *et al.*[12]

Lobomycosis

Lobomycosis is characterized by slowly developing variably sized cutaneous nodules after a traumatic event. The dermal nodules manifest as either smooth, verrucose, or ulcerated surfaces that can attain the size of a small cauliflower-like keloid. The onset of the disease is generally insidious. The increase in size or number of lesions is a slow process, progressing over a period of 40–50 years. The lesions are composed of granulomatous inflammatory tissue containing numerous globose or subglobose to lemon-shaped, yeast-like fungal cells singly or in simple and branched chains.

Mycetoma

Mycetoma is a chronic suppurative infection of the skin, subcutaneous tissue and bone. It usually affects the hand or foot and may be caused by various fungi (eumycetoma) or actinomycetes (actinomycetoma). The micro-organisms are inoculated into subcutaneous tissue by minor trauma. A characteristic feature of mycetoma is the production of grains in the infected tissue; these grains are compact masses of fungal or actinomycete elements, and they discharge to the outside through sinus tracts.

Phaeohyphomycosis

Phaeohyphomycosis is a rare infection caused by dematiaceous fungi, involving the skin and subcutis, paranasal sinuses or central nervous system (CNS). Phaeohyphomycosis refers to subcutaneous and deep-seated infections caused by brown-pigmented (dematiaceous) molds that adopt a septate mycelial form in tissue. This term was also created to separate various clinical infections caused by dematiaceous molds from the distinct subcutaneous infection known as chromoblastomycosis. Unlike mycetoma and chromoblastomycosis, phaeohyphomycosis is not limited to the skin or subcutaneous tissues, and elicits a wider variety of inflammatory responses.

Phaeohyphomycosis is characterized by a nodule, cyst, or pyogranuloma. Histopathologically, the lesions show brown-walled hyphae in the dermis, subcutis, or sometimes in the epidermis.

Rhinosporidiosis

Rhinosporidiosis is a chronic granulomatous infection of the mucous membranes, especially the nasal mucosa, caused by *Rhinosporidium seeberi*.

Sporotrichosis

Sporotrichosis is a subacute or chronic infection caused by the dimorphic fungus *Sporothrix schenckii*.[13] [14] [15] [16] After implantation, this organism can cause cutaneous or subcutaneous infection, which

commonly shows lymphatic spread. Occasionally, widespread disseminated infection also occurs.

EPIDEMIOLOGY

Chromoblastomycosis

Chromoblastomycosis is encountered mainly in arid parts of tropical and subtropical regions. Most cases occur in Central and South America, but chromoblastomycosis has also been reported in South Africa, Asia and Australia. Another major focus appears to be Madagascar.[17]

Chromoblastomycosis is caused by various dematiaceous fungi, to which a number of names have been given. There is therefore a great deal of confusion in the nomenclature used by various authors. The most frequently involved etiologic agents, beginning with the most common, are *Phialophora verrucosa*, *Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Cladosporium carrionii* and *Rhinocladiella aquaspersa* (*Ramichloridium cerophilum*). Sporadic cases of chromoblastomycosis can also be caused by other dematiaceous molds. These organisms form characteristic thick-walled, dark brown muriform sclerotic cells in tissue.

The etiologic agents of chromoblastomycosis are widespread in the environment, being found in soil, wood and decomposing plant matter. Human infection usually follows the traumatic inoculation of the fungus into the skin. Minor trauma, such as cuts or wounds due to thorns or wood splinters, is often sufficient. The disease is most prevalent in rural parts of warmer climates where people go barefoot. There is no human-to-human transmission.

Chromoblastomycosis is unusual in children and adolescents. Except in Japan, men contract the disease much more frequently than women, reflecting the importance of occupational exposure. Men have a greater opportunity for soil contact and predisposition to injury while working in the fields. The majority are aged 30–50 years. The rarity of the disease in children exposed to the same environmental conditions as adults suggests a long period of latency.

A number of collections of case reports have been published.[18] [19] [20] An illustrative example is a study of 51 cases of chromoblastomycosis detected in a 17-year period, all of which were clinically and mycologically proven by direct examinations, cultures and biopsies. Most cases were males (36 of 51; 70%), the mean age was 35 years and farmers predominated (74%); the most frequent lesions were in the lower limbs (54%). Major clinical presentations were nodular (41%) and verrucous (26%). The principal etiologic agent isolated was *F. pedrosoi* (90%). Overall results of the various treatments were as follows: 31% were cured, 57% improved and 12% failed. The best results were obtained with cryosurgery for small lesions, with itraconazole for large ones, and in some cases the combination of both treatments.

Another case series reviews the clinical features and response to therapy in patients with chromoblastomycosis in the state of Rio Grande do Sul, Brazil.[19] Case records of 100 patients with skin lesions caused by chromoblastomycosis, who were treated between 1963 and 1998, were reviewed. The cases were confirmed by histopathology and culture. There was a predominance of male patients (4:1) and of white farmers whose ages ranged from 50 to 59 years, with lesions on their lower limbs. Most of them were from the northern regions of the state. The average time between the appearance of the disease and medical diagnosis was 14 years. The verrucous type proved to be the most frequently reported lesion (53%). Thorn wounds were associated with the disease in 16% of the cases. Lesions uncommon to some parts of the body were also reported. In two of the cases, cutaneous lesions caused by paracoccidioidomycosis and chromoblastomycosis were found in the same patient. Epidermoid carcinoma was found in the same parts of the body affected by chromoblastomycosis. Eumycotic mycetoma and chromoblastomycosis were associated. *Fonsecaea pedrosoi* was found in 96% of the cases and *P. verrucosa* in 4% of the cases. Severe cases of chromoblastomycosis with intense skin

involvement (e.g. lesions with carcinoma) were observed. Statistical analysis showed recrudescence of the disease in 43% of cases despite the treatment used.

Entomophthoramycosis

Entomophthoramycosis occurs mainly in the tropical rain forests of East and West Africa, South and Central America, and South East Asia.

Conidiobolus coronatus (*Entomophthora coronata*), the causative organism of rhinofacial conidiobolomycosis, lives as a saprophyte in soil and on decomposing plant matter in moist, warm climates. It can also parasitize certain insects.

The most widely held view is that *Basidiobolus ranarum* is the sole agent causing basidiobolomycosis, and that *B. meristosporus* and *B. haptosporus* are synonyms of the former; not all authors are of this opinion, however. *Basidiobolus ranarum* has been recovered from soil and decaying vegetation; it has also been isolated from the gut of frogs, toads and lizards that had apparently swallowed infected insects. It is still uncertain how the disease is acquired and what is the length of incubation. Inoculation through a thorn prick or an insect bite has been suggested, as has contamination of a wound or other abrasion. The infection is most common in children. More detailed aspects of entomophthoramycosis caused by *B. ranarum* can be found in the review by Gugnani. [21]

For an exhaustive review of the epidemiology of entophthoramycosis refer to Ribes *et al.*[12]

Lobomycosis

In lobomycosis, the onset of the disease is generally insidious and difficult to document. The increase in size and number of lesions is a slow process; it can take 40–50 years. This latency period often makes it important to note the patient's history of travel or stay in areas of endemicity to arrive at a proper diagnosis. The history often reveals the cause being a trauma, for example an arthropod sting, a snake bite, a cut from an instrument, or a wound acquired while cutting vegetation. The causal agent of lobomycosis appears to be saprobic in aquatic environments, which probably plays an extremely significant part in its life cycle.

The human disease is endemic in the tropical zone of the New World and has been reported in central and western Brazil, Bolivia, Colombia, Costa Rica, Ecuador, Guyana, French Guiana, Mexico, Panama, Peru, Surinam and Venezuela. There have been isolated cases reported in Holland and a doubtful case in Bangladesh. Identification of the disease in dolphins widened the geographic distribution of the disease. Seven cases of lobomycosis involving two species of dolphins, namely marine dolphins (*Tursiops truncatus*) and marine freshwater dolphins (*Sotalia fluviatilis*), have been reported in Florida, the Texas coast, the Spanish-French coast, the South Brazilian coast and the Surinam River estuary. Although lobomycosis in dolphins has been reported in the USA, only one human case has been reported from the USA.[22]

All attempts to isolate the fungus from lesions of infected people have failed. In the dermis it appears as spheric or elliptic budding cells. Although it is accepted that the infection is exogenous in origin, the natural habitat of the causal fungus remains unknown.

The organism gains entry through the skin; it develops in situ for an unspecified period (several years) and then reaches the subcutaneous tissue. The disease is most prevalent in men aged 30–40 years; it is much less common in women and children.

Mycetoma

Mycetomas are most common in arid tropical and subtropical regions of Africa and Central America, particularly those areas bordering the great deserts. However, sporadic cases have been reported from many parts of the world. The countries surrounding the Saharan and Arabian deserts form the most important endemic area, not only because of the number of new cases occurring each year, but also because of the diversity of causal organisms. Mycetoma is also endemic in certain regions of India and in Central and South America.

Mycetomas are caused by various actinomycetes and fungi that occur as saprophytes in the soil or on vegetation. Individual species of fungi or actinomycetes are often associated with particular geographic areas. About six species of fungi are common causes of eumycetoma and five aerobic actinomycetes are common etiologic agents of actinomycetoma. The geographic distribution of these environmental organisms is influenced by climate.

In the arid regions of the tropics and subtropics, the most frequent etiologic agents are *Madurella mycetomatis*, *Actinomadura madurae*, *Actinomadura pelletieri* and *Streptomyces somaliensis*. These organisms are encountered in the great deserts of Africa and Asia and on their fringes, as well as in south-eastern Europe. In the relatively humid mountain regions of Latin America, *Nocardia brasiliensis* is the predominant organism while *Madurella grisea* is a less prominent cause of infection.

In the occasional cases of mycetoma that occur in temperate regions, the principal isolates have been *Pseudallescheria boydii*, *A. madurae* and *M. mycetomatis*. Other organisms that have occasionally been implicated as causes of mycetoma include *Leptosphaeria senegalensis*, *Neotestudina rosatii*, *Pyrenochaeta romeroi*, *Exophiala jeanselmei*, *Acremonium* spp., *Aspergillus nidulans*, *Nocardia asteroides* and *Nocardia caviae*.

Mycetomas occur more frequently in men than in women. Adults aged between 20 and 50 years are the most commonly affected, although cases in children have also been reported. Most patients come from rural districts in the tropics and subtropics, but cases often occur in some countries with a temperate climate, such as Romania.

Trauma is a critical factor in acquisition of the infection. The organisms may be implanted at the time of injury, or later as a result of secondary contamination of the wound. Traumas are often due to vegetable matter (grasses, wisps of straw, hay). In the tropics and subtropics thorny trees such as the acacia are abundant and are often used for fuel. Wounds caused by the thorns may facilitate the entry of soil organisms, or the causative agents may grow on the thorns and be implanted directly into the subcutaneous tissue. It is not surprising, therefore, that mycetomas affect mainly the feet of country-dwellers who walk barefoot.

The vast majority of organisms causing mycetomas are saprophytes of the external environment; *Nocardia* spp. exist in the soil; other species are encountered not only in the soil, but also on living and dead plants. However, little is known about their behavior outside the human host. A recent study of 264 cases of mycetoma in West Bengal illustrates the epidemiology of the causative organisms.[23] Between 1981 and 2000, 264 cases of mycetoma were diagnosed clinically and microbiologically at the Calcutta School of Tropical Medicine. Retrospective analysis of the records revealed that the ratio of actinomycetomas and eumycetomas was 197:67; the male to female ratio was 183:81. Ninety-four cases occurred in the 1980s and 170 in 1990s, with significantly more infections of *Actinomadura* spp. and fewer with *N. caviae* during the last decade. Pricking was the most common injury associated with eumycetomas. A total of 196 infections were in exposed body parts and 68 in covered areas. The localization of mycetomas differed significantly according to sex, incidence of actinomycetomas or eumycetomas, and obvious history of trauma. Exposed area cases were more common among agricultural workers, while covered area mycetomas were almost always actinomycetomas with a remarkably lower incidence of *N. caviae*, *A. madurae* and *M. grisea* infections. The peak age of onset was between 16 and 25 years. The delay of diagnosis for the 80th percentile of cases was around 6 years for cases caused by *N. brasiliensis* and *Streptomyces* spp.; 8 years for *N. caviae* and *N. asteroides*; and 10 years for *M. grisea* and *Actinomadura* spp. From the history of trauma in 130 patients, the 80th percentile incubation period was calculated for *N. brasiliensis, N. caviae* and *N. asteroides* as 3 years; for *Actinomadura* spp. 7 years; and for *M. grisea* 9 years. The minimum incubation period for all organisms was around 3 months.

Phaeohyphomycosis

Black molds are widely encountered in soil and wood. In addition, some organisms can produce yeast-like synanamorphs that adapt to aqueous environments. Typically, the infection is acquired by the inoculation of the fungus through a penetrating injury. In addition, other possible portals of entry have been suggested, including the inhalation of spores with lung or sinus invasion, the ingestion of contaminated food or water with subsequent penetration through the gastrointestinal tract, contamination of the skin at the insertion of a vascular catheter, and contamination of the catheter itself. Some cases of systemic infection have no apparent portal of entry.

Phaeohyphomycosis has a worldwide distribution, but subcutaneous infection is most often seen in the rural population of tropical parts of Central and South America. Most cases of cerebral or paranasal sinus infection have been reported from USA. There is little information on the incidence of phaeohyphomycosis. In a population-based surveillance conducted over 2 years in the San Francisco Bay area, the incidence of infection due to black molds was one case per million per year.[24]

The number of organisms implicated as etiologic agents of phaeohyphomycosis is increasing. More than 80 different molds, classified in 40 different genera, have been

incriminated. These fungi have often been given different names at different times, and there is therefore a great deal of confusion in the nomenclature used in different reports.

Among the more important etiologic agents, *Alternaria*, *Bipolaris*, *Curvularia*, *Exophiala*, *Exserohilum* and *Phialophora* spp. and *Xylohypha bantiana* can be included. Many of these organisms are found in soil or decomposing plant debris; others are plant pathogens. The most important predisposing factor for cutaneous and subcutaneous infection is exposure to contaminated material present in the environment (decaying wood, plants).

Human infection follows inhalation or traumatic implantation of the fungus. In addition to these agents of phaeohyphomycosis, others are being reported. For example, *Colletotrichum* spp., which are common plant pathogens, have been reported as a cause of subcutaneous phaeohyphomycosis in patients undergoing chemotherapy for hematologic malignancies and may cause life-threatening phaeohyphomycosis in immunosuppressed patients.[25]

Rhinosporidiosis

Rhinosporidiosis is endemic in India[26] and Sri Lanka, as well as in South America and Africa. Occasional cases have been reported from the USA, South East Asia and other parts of the world. The etiologic agent is an endosporulating organism, *Rhinosporidium seeberi*. So far, all attempts to isolate this fungus from lesions have failed. In tissue, large, thick-walled sporangia (spherules) are formed. Large numbers of spores are produced within the sporangia and, when the spores are mature, they are released through a pore in the wall. Each spore may develop to form a new sporangium. Little is known about the natural habitat of *R. seeberi*, but it

is believed that stagnant pools of water may be the source of human infection.

The disease is most prevalent in rural districts, particularly among people working or bathing in stagnant water (such as rice fields). Men are more commonly affected than women.

Sporotrichosis

Sporotrichosis is worldwide in distribution, but occurs most frequently in temperate humid climatic regions. At present, the largest number of reported cases comes from the North American continent.[27] Other regions where the infection is endemic include South America,[28] South Africa and South East Asia.

The causative agent is a dimorphic fungus, *Sporothrix schenckii*, which is found in the soil and on plants and sphagnum moss. It grows in nature as a mycelium, but in tissue it forms small, budding cells.

Infection usually follows the traumatic introduction of the fungus into the skin. Minor trauma, such as abrasions or wounds from thorns or wood splinters, may be sufficient. In occasional cases, infection follows spore inhalation.

It is not clear whether the infection is more common among men than women. Incidence in the different age groups is also variously assessed, but children are less often affected than adults.

Sporotrichosis is most prevalent among people who handle soil or plant materials, such as gardeners, florists, mineworkers and carpenters. For this reason sporotrichosis has been regarded as an occupational disease. Sporotrichosis is not transferred from human to human, and the multiple cases that sometimes occur in families or closed communities are usually due to common exposure to the same exogenous source of contamination.

PATHOGENICITY

Chromoblastomycosis

The causative fungi require implantation through the skin into subcutaneous tissue. The lesion appears at the site of skin trauma or puncture wound. However, the inoculation may have occurred so long before that no history of injury can be elicited. In general, the disease remains localized to the area surrounding the initial infection. In rare cases, hematogenous spread to the brain, lymph nodes, liver, lungs and other organs is observed.[9]

Entomophthoramycosis

Pathogenicity of the causal organisms is a reflection of inoculum size and frequency of exposure in endemic areas. For an exhaustive review of the pathogenicity of the agents causing entomophthoramycosis refer to Ribes *et al.*[12]

Lobomycosis

Lobomycosis develops following trauma to the skin, but in most clinical histories the event is so minimal that it is not remembered. The disease runs an extremely slow course and years may elapse before the patient seeks medical advice.

Mycetoma and phaeohyphomycosis

The organisms causing these conditions are not regarded as being pathogenic. Typically, the infection is acquired by the inoculation of the fungus through a penetrating injury

Rhinosporidiosis

Studies on the virulence of *R. seeberi* have not been carried out. Nothing is known about the mode of infection. It is most likely that trauma is an essential factor in the initiation of disease. Spores of *R. seeberi* are not able to penetrate intact epithelium. Because the nose and eyes are the most common sites of the disease it is suggested that the organisms are transmitted in dust and water.

Sporotrichosis

Sporothrix schenckii usually enters the body through traumatic implantation, but occasionally the fungus is introduced through inhalation of the conidia. Because the infection can also be hematogenously disseminated, it may be that the yeast cells are able to resist phagocytosis and intracellular killing by host effector cells, although in-vitro data suggest that the yeast cells are readily killed in the presence of human serum. Host defense mechanisms in response to *S. schenckii* have not been extensively studied.

PREVENTION

Avoidance of skin penetration is the best means of preventing chromoblastomycosis, entomophthoramycosis and phaeohyphomycosis. Suitable footwear will help to prevent chromoblastomycosis.

Very little is known about the ecology of *Loboa loboi* so it is difficult to recommend specific methods of contact and prevention for lobomycosis.

The causative agents of mycetoma normally live as saprophytes in the soil. Because the most common site for mycetoma is the foot it is reasonable to assume that the wearing of appropriate footwear would prevent infection. Avoidance of trauma to the hands and other areas is difficult to encourage because most infections seem to be related to outdoor activities.

Rhinosporidiosis can be prevented by avoiding eye and nose contact with contaminated dust and water.

Occupations that predispose persons to sporotrichosis include gardening, farming, masonry, floral work, outdoor labor and other activities involving exposure to contaminated soil or vegetation such as sphagnum moss or roses. Wearing gloves and protective clothing while carrying out these activities may therefore prevent traumatic implantation of the fungus through the skin.

DIAGNOSTIC MICROBIOLOGY

Chromoblastomycosis

Microscopic examination of wet preparations of pus, scrapings, or crusts from lesions can permit the diagnosis of chromoblastomycosis if clusters of the characteristic small, round, thick-walled, brown-pigmented sclerotic cells are seen (Fig. 239.1). These cells are often divided by longitudinal and transverse septa.[6]

The definitive diagnosis of chromoblastomycosis depends on the isolation of the etiologic agent in culture. Identifiable olive-green or brownish-black mycelial colonies can be obtained after incubation at

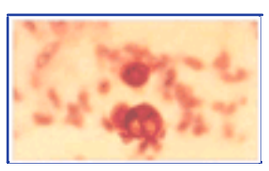

Figure 239-1 Chromoblastomycosis. Thick-walled, septate, dematiaceous muriform cells. *With permission from Richardson MD, et al. Slide atlas of fungal infection: subcutaneous and unusual fungal infections, Oxford: Blackwell, 1995.*

77–86°F (25–30°C) for 1–2 weeks, but cultures should be retained for 4 weeks before being discarded. Identification of the individual etiologic agents is difficult. Serologic techniques have been shown to detect cases of chromoblastomycosis caused by *C. carrionii*.[29]

Entomophthoramycosis

Microscopic examination of smears or tissue from the nasal mucosa will reveal broad, nonseptate, thin-walled mycelial filaments.

The culture of causal organisms of entomophthoramycosis is difficult. To optimize the recovery of fungus from clinical material, specimens must be inoculated on the largest possible number of media; they should be incubated at 77–95°F (25–35°C).

For an exhaustive review of the diagnosis of entomophthoramycosis refer to Ribes *et al.*[12]

Lobomycosis

The etiologic agent of lobomycosis is an obligate pathogen of humans and lower mammals that has yet to be isolated and grown in vitro; therefore, nothing is known of its basic cultural characteristics and growth. Diagnosis is based on demonstrating the presence of globose, thick-walled, yeast-like cells ranging from 5 to 12µm in diameter in lesion exudate or tissue sections. The organism multiplies by budding, and thus mother cells with single buds are often observed. However, characteristic sequential budding leads to the production of chains of cells that are linked to each other by a tubular connection, or isthmus. Budding may occur at more than one point on a cell, giving rise to branched or radiating chains of cells. These thick-walled, hyaline, spherical cells with chains of cells interconnected by tubular connections are the basis on which a diagnosis of lobomycosis rests. The thick-walled, budding hyaline cells with catenate chains of conidia can be readily observed in tissue smears or exudates mounted in 10% potassium hydroxide or in Calcofluor white preparations.

Microscopic examination of specimens of pathologic material will reveal numerous hyaline, round or ovoid cells with an average diameter of 9µm (Fig. 239.2). These cells closely resemble the yeast forms of *Paracoccidioides brasiliensis* or *Histoplasma duboisii*. The cells are enclosed in a double-contoured membrane and are capable of budding. They often form chains and appear to be joined together by bridge-like structures within the chain. If the individual elements show multiple budding, the chains are divided into branches.

The epidermis is irregular in thickness, with parakeratotic zones and sometimes ulcerations and crusts. The dermis underlying it shows hypertrophic and partly hyalinized bundles of connective tissue. Between these bundles are granulomatous infiltrates that contain numerous yeasts, which are either located extracellularly or phagocytosed by mononuclear and polynuclear cells. Intracytoplasmic

Figure 239-2 Lobomycosis. Yeast cells are attached to each other in short chains. Nonbudding and single-budding cells are also present.

asteroid bodies have been observed in some giant cells. Their nature is unknown and they have been confused with the asteroid bodies seen in sporotrichosis. They are, however, different and unrelated structures.[30]

A case report describing the first case of lobomycosis diagnosed in the USA illustrates further the histopathologic features of this disease.[22] The patient had traveled to the Angel Falls in Venezuela 7 years earlier where he had been exposed to the high pressures of the falls. This resulted in a pustule and surrounding erythema on the skin of his right chest wall. The lesion gradually increased in size and had the appearance of a keloid. After an uncomplicated excision the patient recovered completely. Examination of the tissue sections showed a nodular inflammatory infiltrate of foamy histiocytes, multinucleated giant cells and scattered lymphocytes. Throughout the infiltrate were numerous globose or subglobose, lemon-shaped cells that measured 5.0–11.0µm in diameter. Many cells showed thick refractile walls and reproduced by single and multiple budding. The buds were attached to the mother cell by narrow tubular connections, giving a beaded appearance. There were many chains of cells showing narrow tubular connections characteristic of *L. loboi*.

Loboa loboi has never been successfully cultured. This distinguishes it from *P. brasiliensis*, which it closely resembles morphologically. The globose and subglobose budding cells of *L. loboi* resemble budding cells of *P. brasiliensis* in tissue. However, the central mother cells of *P. brasiliensis* become large and thick-walled compared to the daughter cells, which remain smaller. In contrast, yeast cells of *L. loboi* remain consistent in diameter, giving rise to branching chains of blastoconidia. In addition, the cell wall of *L. loboi* contains constitutive melanin,[31] which can be detected by the use of the Fontana-Masson histologic stain. The walls of cells of *P. brasiliensis* are not known to contain melanin. *Loboa loboi* has never been cultured in vitro. On the other hand, *P. brasiliensis* can be grown in artificial culture and is known to be a dimorphic pathogen.

Molecular methods have been used in an attempt to characterize the causative agent of lobomycosis.[32] Fungal-specific primers targeted for highly conserved genomic nucleic acid sequences were used in a polymerase chain reaction (PCR) to amplify DNA from lobomycosis lesions in a bottlenose dolphin. Sequence alignments of this DNA possessed high homology to fungal ribosomal DNA sequences found in the genus *Cladosporium*. When used for in-situ hybridization, the riboprobe transcribed from a cloned PCR-generated fragment bound to *L. loboi* cells. These results support the hypothesis that *L. loboi* in dolphin tissue is a fungus.

A new monotypic genus, *Lacazia*, with *Lacazia loboi* as the type species, was recently proposed by Taborda *et al.*,[31] to accommodate the obligate etiologic agent of lobomycosis in mammals. The continued placement of *L. loboi* in the genus *Paracoccidioides* as *Paracoccidioides loboi* O.M. Fonseca et Lacaz was found to be taxonomically inappropriate. The older name *Loboa loboi* Ciferri *et al.* was considered to be a synonym of *P. brasiliensis*.

Mycetoma

The diagnosis of mycetoma depends on the identification of grains. These should, if possible, be obtained by puncture from a softened, but not ulcerated, nodule with a syringe. Failing this, grains can be obtained with a dissecting needle or by aspiration from the secretion flowing from a sinus.[33] If there is no pus flowing from the

lesion, small fragments of tissue should be removed. If possible, between 20 and 30 grains should be obtained; these should be rinsed in sterile saline before being cultured.

Gross examination of the grains may give a clue to the etiologic diagnosis. Black grains suggest a fungal infection; minute white

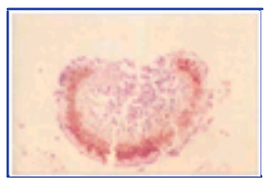
Figure 239-3 Granule of *Madurella mycetomatis.* The granules have a deeply pigmented periphery of compact hyphae. Randomly oriented, poorly pigmented fungal elements in the interior of the granule are less compact.

grains often indicate a *Nocardia* infection; and larger white grains the size of a pinhead may be of either fungal or actinomycotic origin. Small, red grains are specific to *A. pelletieri*, but yellowish-white grains may be actinomycotic or fungal in origin. Their shape, consistency and structure must be carefully determined.

Direct microscopic examination will confirm the diagnosis of mycetoma, and will also reveal whether the causative organism is a fungus or an actinomycete. [33] Actinomycotic grains contain very fine filaments (<1µm diameter), whereas fungal grains contain short hyphae (2–4µm diameter), which are sometimes swollen. This can be seen by direct microscopic examination of crushed grains in potassium hydroxide, but it is much more readily observed in stained histologic sections (Fig. 239.3).

Although the identification of the causal agents of mycetoma can often be deduced from the morphologic characteristics of the grains, it is also important to isolate the organism in culture. Agar plates should be inoculated with several grains (or with secretion or tissue fragments) and incubated at 77–86°F (25–30°C) and at 98.6°F (37°C). The most commonly used agar medium is Sabouraud's agar, without antibiotics but with cycloheximide for isolation of actinomycetes, and with antibiotics but without cycloheximide for fungal agents. Alternative media for isolation of actinomycetes include brain-heart infusion or blood agar.

Cultures should be retained for up to 6 weeks before being discarded. The actinomycetes grow much more slowly than the fungi.

Madurella mycetomatis is the commonest cause of eumycetoma in Sudan and other countries in tropical Africa. Currently, the early diagnosis of mycetoma is difficult. In attempting to improve the identification of *M. mycetomatis* and, consequently, the diagnosis of mycetoma, a specific oligonucleotide primer based on the sequence of the internal transcribed spacer (ITS) regions spacing the genes encoding the fungal ribosomal RNAs has been described.[34] The ITS regions were amplified with universal primers and sequenced, and then two sets of species-specific primers were designed that specifically amplify parts of the ITS and the 5.8S ribosomal DNA gene. The new primers were tested for specificity with DNA isolated from human mycetoma lesions and DNA extracted from cultures of *M. mycetomatis* reference strains and related fungi as well as human DNA. To study the genetic variability of the ITS regions of *M. mycetomatis*, ITS amplicons were obtained from 25 different clinical isolates and subjected to restriction

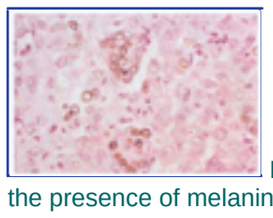
Figure 239-4 Subcutaneous phaeohyphomycosis caused by *Bipolaris spicifera.* The fungal elements are stained with Fontana-Masson, which accentuates and confirms the presence of melanin or melanin-like pigment in the fungal cell walls.

fragment length polymorphism (RFLP) analysis with *Cfo*I, *Hae*III, *Msp*I, *Sau*3AI, *Rsa*I and *Spe*I restriction enzymes. Restriction fragment length polymorphism analysis of the ITS region did not reveal even a single difference, indicating the homogeneity of the isolates analyzed during the study.

Phaeohyphomycosis

One common factor among these fungi is their melanin formation in the cell wall in culture and, in most cases, in human tissue. Microscopic examination of stained histopathologic sections or wet preparations of clinical material, such as pus or skin scrapings, can permit the diagnosis of phaeohyphomycosis if brown-pigmented septate mycelium with occasional branching is seen (Fig. 239.4).

Identification of the etiologic agent is essential for correct management, and this depends on its isolation in culture. Identifiable mycelial colonies can be obtained after incubation at 86°F (30°C) for 1–3 weeks. No serologic tests are available.

Rhinosporidiosis

Microscopy of clinical material reveals round or ovoid organisms that, depending on age, vary in diameter with a prominent wall.[35] [36] [37] These mature forms are known as sporangia. Sporangia measure up to 350µm in diameter and have a cell wall measuring about 5µm. The sporangia may be filled with endospores (Fig. 239.5). The immature forms of the organism are known as trophocytes; they are smaller than sporangia, have a relatively thinner wall and do not contain endospores. Trophocytes mature into sporangia. Endospores develop within sporangia. Endospores are released upon maturity and thereafter develop into trophocytes.

Rhinosporidium seeberi has traditionally been classified as a fungus on the basis of morphologic and histochemical characteristics. Recent molecular studies have generated conflicting results. The organism may be either a cyanobacterium or a protist. Using consensus PCR, a portion of the *R. seeberi* 18S rRNA gene directly from infected tissue has been amplified. [38] Analysis of the aligned sequence and inference of phylogenetic relationships showed that *R. seeberi* is a protist from a novel clade of parasites that infect fish and amphibians. Fluorescence in-situ hybridization and *R. seeberi*-specific PCR showed that this unique 18S rRNA sequence is also present in other tissues infected with *R. seeberi*. These data support the *R. seeberi* phylogeny recently suggested by others. *Rhinosporidium seeberi* is not a classic fungus, but rather the first known

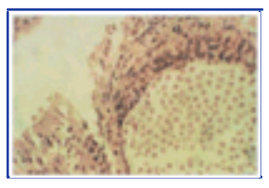
Figure 239-5 Rhinosporidiosis.

human pathogen on the basis of a previously aligned dataset of the DRIPs clade (named after the organisms *Dermocystidium*, the rosette agent, *Ichthyophonus* and *Psorospermium*), a novel clade of aquatic protistan parasites (*Ichthyosporea*).

Immunologic methods have been used to identify the causal agent of rhinosporidiosis in situ where the immunolocalization of *R. seeberi* antigens using sera from individuals infected with *R. seeberi* and tissue from Sri Lankan patients with rhinosporidiosis was investigated.[39] The tissues were fixed and evaluated by transmission electron microscopy for the presence of *R. seeberi* sporangia. The tissue samples were reacted with the patients's sera and then labeled with protein A colloidal gold (PACG) for immunolocalization. It was found that the PACG had fixed to antibodies that specifically recognized an internal electron lucent layer situated immediately under the mature sporangium's wall. The endospores and the juvenile and intermediate sporangia did not undergo PACG labeling. This study found that the expression of this antigen occurs only in the final developmental stages of *R. seeberi* mature sporangia. The data may explain why circulating antibodies to *R. seeberi* were not detected before in studies that used endospores as antigen in immunoassays. This appears to be the first report in which an antigenic material with a potential role in

the immunology of rhinosporidiosis has been described.

Sporotrichosis

Direct examination of clinical material, such as pus or tissue, is often disappointing because the organism is seldom abundant. However, it can be of value if conducted with painstaking care. The detection of typical ovoid or cigar-shaped cells or asteroid bodies of *S. schenckii* will confirm the diagnosis (Fig. 239.6).

The definitive diagnosis of sporotrichosis depends on the isolation of the etiologic agent in culture. Clinical material should be inoculated onto several media, including Sabouraud's agar, and incubated at 72–77°F (22–25°C). Identifiable mycelial colonies will appear in 2–5 days. The color usually changes from cream or light brown to dark brown or black with age. Confirmation of the identification depends on the morphologic characteristics of the mycelial form and its conversion to the yeast form on blood agar at 98.6°F (37°C).

At present, serologic tests do not have a significant role in the diagnosis of sporotrichosis. Tube agglutination and latex particle agglutination tests can be used to detect antibodies to *S. schenckii*, but are more helpful in diagnosing the unusual extracutaneous forms of sporotrichosis than in detecting cutaneous infection.

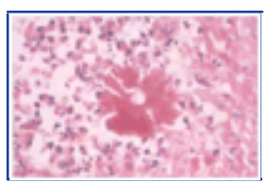

Figure 239-6 Asteroid body in cutaneous sporotrichosis. A yeast-like cell of *Sporothrix schenckii* with faintly basophilic, retracted cytoplasm is intimately surrounded by elongated spicules of Splendore-Hoeppli material.

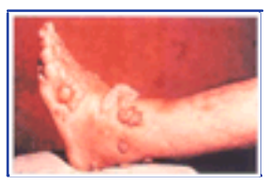

Figure 239-7 Chromoblastomycosis lesions on the foot and ankle. The typical raised, crusted, verrucous lesions seen on the foot and ankle.

CLINICAL MANIFESTATIONS

Chromoblastomycosis

The lesions of chromoblastomycosis are usually unilateral and occur mainly on the exposed parts of the body, particularly the feet and lower legs (Fig. 239.7).[20] Other less common sites include the hands, arms, shoulders and neck (Fig. 239.8). The initial lesion is a painless papule or nodule on an erythematous and occasionally verrucous base. The condition may also present as an abscess surrounded by infiltration or as a psoriasiform lesion with erythema and scaling. As the disease develops, the affected limb becomes enlarged. Small satellite nodules may occur at the edge of the original lesion. Itching often occurs and it may be severe.

The primary lesion develops very slowly, its diameter increasing by only 1–2mm per year. It is firm and elastic in consistency and colored red or violet verging on gray. There is a warty, papillomatous margin surrounding a center that may be flat, smooth, or scaly, with areas of scarring.

Later in the disease, the lesion may become pedunculated or ulcerated (if bacterial superinfection occurs). However, the lesions usually retain a warty, dry character.

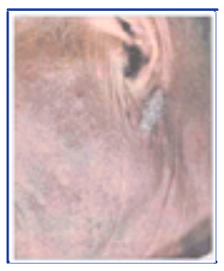

Figure 239-8 Chromoblastomycosis lesions on the ear. A crusty lesion on the lower part of the external ear.

Secondary lesions may appear, especially along the lymphatics draining the site of infection; here again, development is slow and symptoms are few. The lymph nodes are only involved if there is superimposed bacterial infection.

In some cases the skin lesions may spread and involve an entire limb. In other cases there is spontaneous resolution, leaving behind scars that are atrophic and abnormally pigmented or, by contrast, hypertrophic and contracting.

Cutaneous dissemination is rare, but may follow autoinoculation from scratching. Lymphatic or hematogenous spread may also occur. In elderly patients the lesions may spread to involve the skin of the trunk, whereas the scalp is rarely involved. The nails may also be damaged as a result of infection of the matrix and nailbed.

Rare complications of chromoblastomycosis include carcinomatous transformation. Metastatic lesions may occur in the oral mucosa, lymph nodes, bones and other tissues.

Usually lesions on the upper limbs are erythematosquamous, psoriasiform and of low relief, whereas those on the lower limbs are more exuberant. The latter may be associated with sclerosing inflammation of the subcutaneous tissue and result in elephantiasis.

In endemic areas the unilateral development of vegetative, atrophic and scarred lesions on a lower limb is suggestive of chromoblastomycosis. The condition must be distinguished from a number of other fungal infections including blastomycosis, lobomycosis, paracoccidioidomycosis, phaeohyphomycosis, rhinosporidiosis and sporotrichosis. It must also be differentiated from protothecosis, leishmaniasis, verrucous tuberculosis, certain leprous lesions and syphilis. On the upper limbs the erythematosquamous lesions can be confused with psoriasis or subacute or discoid lupus erythematosus. Mycologic and histologic investigations are indispensable for confirmation of the diagnosis.

Entomophthoramycosis

Entomophthoramycosis is a chronic subcutaneous infection of the trunk and limbs. The subcutaneous swelling that characterizes this disease is usually localized to the buttock and thighs, but may also be found on the arm, leg or shoulder.

The initial swelling may be rapid or slow in onset and is hard and painless. The spread is slow but relentless, and a large mass is formed that is attached to the skin but not to the underlying tissue (unlike *Conidiobolus* infection). This is a disfiguring infection, but the skin covering the lesions does not ulcerate. Lymphatic obstruction may occur and can result in massive lymphedema. There is no functional

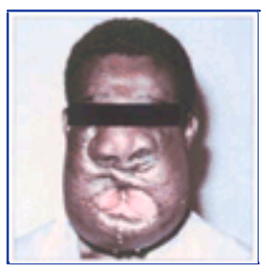

Figure 239-9 Rhinofacial conidiobolomycosis. *With permission from Richardson MD, et al. Slide atlas of fungal infection: subcutaneous and unusual fungal infections, Oxford: Blackwell, 1995.*

impairment as long as the joints are not blocked by the volume of the swellings. The underlying bone and joints are not affected by the disease.

The disease is most common among adult males, particularly those living or working in tropical rain forests. Infection is acquired through inhalation of spores, or their introduction into the nasal cavities by soiled hands.

Very rarely, *B. ranarum* can cause gastrointestinal basidiobolomycosis.[40]

Conidiobolus infection generally begins with unilateral involvement of the nasal mucosa. The most common nasal symptom is obstruction, but frequent nose bleeding can occur and is evidence of the development of a nasal polyp in the anterior region of the inferior turbinate. Subcutaneous nodules then develop in the nasal and perinasal regions and may be associated with epidermal lesions.

The spread of the infection is slow but relentless. It is usually confined to the face, and the development of gross facial swelling involving the forehead, periorbital region and upper lip is very distinctive (Fig. 239.9). As a rule, the lesions are firmly attached to the underlying tissue, although the bone is spared. The skin remains intact. Spread to the lymph nodes has been reported. Even if, in advanced cases, the diagnosis is obvious from the appearance, mycologic and histologic examinations are essential for its confirmation.

The disease can be diagnosed with confidence on the basis of appearance and the results of the mycologic and (in particular) the histologic examination. Specimens must be taken from the subcutaneous tissue where the infection develops.

For an exhaustive review of the clinical manifestations of entomophthoramycosis refer to Ribes *et al.*[12]

Lobomycosis

Lobomycosis is an indolent infection that first manifests as a papule or small nodule of normal pink skin color or with a grayish tinge. The nodule then proliferates and, by partial or total coalescence, may form extensive multilobar lesions. The disease spreads by peripheral extension or autoinoculation from scratching, or it may follow the draining lymphatics, especially in elderly people.

The lesions are located in the dermis and subcutis and may form massive tumors, which are firm and resistant to pressure at the outset, but which later become hard and fibrous and resemble a keloid. If there is ulceration, depressed scars may remain; their surface is smooth and shiny in places, owing to atrophy of the underlying epidermis, and wrinkled and fissured elsewhere.

The disease may be symptomless or cause itching and burning, and trauma to the affected area may be especially painful. The most common sites of infection are the coolest parts of the body — the

limbs, face, ears and buttocks. The lesions may cover a whole limb. If the head is involved, the patient may be so grossly disfigured as to be completely excluded from social life. With a few exceptions there are no adenopathies.

Lesions may be keloid scars or irregular fibrous changes of the skin without secretion. Leprosy, leishmaniasis and chromoblastomycosis can produce similar lesions. Mycologic and histologic examination will confirm the diagnosis.

Mycetoma

Mycetoma is a chronic, suppurative infection of the subcutaneous tissue and contiguous bones. The lesion appears to begin at a site of minor trauma and continues to spread locally over the ensuing months and years.

The clinical features of the disease are fairly uniform, regardless of the type of organism causing it. Eumycetoma follows a slower and generally less destructive course than actinomycetoma. Spread to the internal organs and involvement of the regional lymph nodes is rare, occurring in no more than 2–5% of cases.

The feet are by far the most common site of involvement and account for two-thirds or more of cases (Fig. 239.10). Other sites include the lower legs, hands, head, neck, chest, shoulder, arms and abdomen.

In most cases, the first sign of the disease is a small, hard, usually painless, subcutaneous nodule that is not attached to the underlying tissue. It is covered by taut thinned skin, which is reddish-violet in color. A number of small nodules may coalesce to form a larger and frequently multilobar nodule.

Over the ensuing months the nodule begins to soften on the surface, caves in, ulcerates and partly empties, discharging a viscous, purulent fluid containing grains. If there is little fluid, the grains may not escape. The lesion then broadens out at the surface and also spreads inward to infect muscles and bones. The lesions, which are covered with depigmented and scarred skin, present as swellings, which are often covered with a crust. Later, the lesions develop sinus tracts that discharge pus and blood containing the characteristic grains.

The infection slowly spreads to adjacent tissue, including bone; this often causes considerable deformity. Mycetomas of the feet make the arches convex, thus preventing the toes from touching the ground. However, the general health of the patient is not affected. Pain, burning and pruritus may occur but are usually mild. Depending on the location and size of the lesion, and also on whether there is any bone involvement, limb function may be impaired.

Radiologic examination is useful in determining the extent of bone destruction. Abnormalities include periosteal reactions, sclerosis,

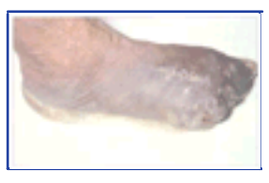

Figure 239-10 Mycetoma of the foot.

endosteal reactions, cortical erosions and joint destruction. Computerized tomography scanning is also helpful in delineating the extent of lesions.

Bacterial superinfection is not uncommon and is largely responsible for adenopathies and impairment of the general health. Visceral and especially cerebral metastases are the most serious complications; they cause cachexia and are often fatal. Fortunately, they are rare.

In most cases, the diagnosis of mycetoma of the feet presents no problems, but it may be difficult if other body sites are involved, particularly in regions where the disease is not endemic and if no grains have been discharged at the time of examination.

The characteristic feature of mycetoma is the presence in a fistulated swelling of grains that are found to contain actinomycotic or fungal filaments. This finding

distinguishes mycetoma from chromoblastomycosis, cutaneous tuberculosis, certain syphilitic or leprous lesions, botryomycosis and other conditions.

Phaeohyphomycosis

Phaeohyphomycosis can be divided into a number of distinct clinical forms, including subcutaneous infection, paranasal sinus infection, cerebral infection and invasive and systemic disease.[1] [41] The disease spectrum of noncutaneous phaeohyphomycosis includes sinusitis, pulmonary disease, CNS infection, ocular disease, arthritis, osteomyelitis, fungemia, endocarditis, peritonitis and gastrointestinal disease.

Subcutaneous phaeohyphomycosis

Subcutaneous phaeohyphomycosis usually follows the traumatic implantation of the fungus into the subcutaneous tissue. Minor trauma, such as cuts or wounds from thorns or wood splinters, is often sufficient. The principal etiologic agents include *E. jeanselmei*, *Exophiala spinifera*, *Exophiala dermatitidis* (*Wangiella dermatitidis*), *Phialophora richardsiae* and *Phialophora parasitica*.

The lesions occur mainly on the arms and legs. Other less common sites include the buttocks, neck and face. The initial lesion is a firm, sometimes tender, subcutaneous nodule that may enlarge slowly to form a painless cystic abscess. Lesions are attached to the skin but not to the underlying tissue or bone. Unless the cyst ruptures, the overlying skin remains unaffected. In immunosuppressed patients with subcutaneous phaeohyphomycosis, the lesions are more likely to drain through sinuses.

Phaeohyphomycosis in transplant recipients

Infection of subcutaneous tissue by black fungi has only been reported in six transplant patients, all of whom were solid organ recipients.[42] These patients presented with indolent, localized infections at least 1 year after transplant, while on maintenance immunosuppressive regimens. They were cured by surgical resection, either alone or in conjunction with antifungal agents. A further case report illustrates the features of subcutaneous phaeohyphomycosis, occurring in a bone marrow transplant recipient receiving high doses of immunosuppressive agents, in whom widespread subcutaneous infection due to *E. jeanselmei* was not eradicated by repeated resections and therapy with amphotericin B and flucytosine. [42] The infection was eventually cured after addition of itraconazole to the therapeutic regimen. Results of in-vitro testing of the isolate for susceptibility to a combination of amphotericin B, flucytosine and itraconazole confirmed the potential role of combination antifungal therapy in the setting of refractory infection.

Local recurrence of subcutaneous phaeohyphomycosis in transplant recipients after medication or surgical treatment is also seen. An illustrative case is a 42-year-old woman who had undergone a bilateral lung transplant who developed pheohyphomycotic cysts with local recurrence and then was successfully treated by local

excision with pre- and postsurgery oral itraconazole treatment.[43] Simple excision or excision with postsurgery oral itraconazole resulted in local recurrence in this patient. Local excision with pre- and postsurgery oral itraconazole was effective in preventing the local recurrence. The authors conclude that local excision with pre- and postsurgery oral itraconazole can be used to treat these patients with recurrent phaeohyphomycosis.

Paranasal sinus infection

This form of phaeohyphomycosis is becoming more common. The principal etiologic agents include *Alternaria* spp., *Bipolaris spicifera*, *Bipolaris hawaiiensis*, *Curvularia lunata* and *Exserohilum rostratum*.

It is a slowly progressive condition that may remain confined to the sinuses or spread to contiguous structures. Affected people usually complain of longstanding symptoms of allergic rhinitis, nasal polyps or intermittent sinus pain. Patients present with nasal obstruction and facial pain, with or without proptosis. The sinuses are filled with a thick, dark, tenacious, inspissated mucus.

Computerized tomography scanning is the best method for evaluating the extent of the infection. The typical finding is a large mass filling one or more of the sinuses.

Alternaria and *Curvularia* spp. occasionally cause necrotic lesions of the nasal septum in patients with leukemia or AIDS.

Cerebral phaeohyphomycosis

This form of phaeohyphomycosis may follow hematogenous dissemination of infection from the lungs, or it may result from direct spread from the nasal sinuses. Involvement of the CNS carries a poor prognosis. The routes of infection have not been clearly established, but the most likely port of entry seems to be the respiratory tract, although direct inoculation into the brain and extension from the sinuses, ear and pulmonary infections have also been reported. Most cases are due to *X. bantiana* (*Cladosporium trichoides*). Other etiologic agents include *Bipolaris* spp. and *E. dermatitidis*. Many cases of cerebral infection with *X. bantiana* have occurred in people with no obvious predisposing factors.

The symptoms of cerebral phaeohyphomycosis are gradual in onset. Persistent headache is the most common presenting symptom. The most frequent clinical findings include focal neurologic signs, hemiparesis and fits. Fever is minimal or absent. Chest radiographs are normal.

Computerized tomography scans of the head will often reveal a unilateral, well-circumscribed lesion, with the frontal lobes of the brain being the most common location. Cerebrospinal fluid (CSF) findings are varied. The opening pressure may be raised, the protein concentration may be increased, the glucose concentration may be reduced, and pleocytosis may be present. It is most unusual to recover the fungus from the CSF. The diagnosis is seldom established until neurologic resection is performed.

Cutaneous infection

Alternaria spp. have been seen in and isolated from crusted, ulcerated or scaling skin lesions. Many of these infections have followed traumatic implantation and a substantial proportion have occurred in leukemic patients or transplant recipients. The arms and legs are the more common sites of infection.

Other forms of phaeohyphomycosis

Dematiaceous molds have caused endocarditis after valve insertion or replacement, and peritonitis in patients on continuous peritoneal dialysis.[40] Post-traumatic osteomyelitis and arthritis have also been reported. Fungemia due to black fungi is unusual.[40] Fever without a clear source of infection is the most frequent presentation. In a series of 23 cases occurring in a tertiary hospital, fever was the most frequent clinical manifestation, and only one patient developed signs of deep-seated infection, with a clinical picture of necrotizing pneumonia similar to that caused by *Aspergillus* spp.[40]

The lesions of subcutaneous phaeohyphomycosis can be confused with the small initial lesions of chromoblastomycosis, sporotrichosis, blastomycosis, coccidioidomycosis and paracoccidioidomycosis, as well as with cutaneous leishmaniasis. Lymphangitic spread of sporotrichosis and the development of verrucous lesions in the other conditions makes the distinction easier.

In people who are not immunosuppressed, the clinical presentation of phaeohyphomycosis of the paranasal sinuses cannot be distinguished from that of *Aspergillus* infection. In immunosuppressed patients, *Aspergillus* sinusitis is a fulminant and often lethal condition, unlike pheohyphomycotic sinusitis. However, both groups of organisms can cause black necrotic lesions of the nasal septum.

Bacterial brain abscess is the most common initial diagnosis in patients with cerebral phaeohyphomycosis. In occasional patients, the diagnosis of cryptococcosis, histoplasmosis, coccidioidomycosis or sporotrichosis must be excluded.

Rhinosporidiosis

Rhinosporidium seeberi causes the production of large polyps or wart-like lesions that occur predominantly on the mucous membranes. The nasal mucosa is affected in more than 70% of cases. The onset of the disease in the nose is insidious and the patient remains unaware of its presence until symptoms of obstruction develop. In some cases, the patient complains of itching and unilateral coryza. Rhinoscopic examination will reveal papular or nodular smooth-surfaced lesions that gradually become pedunculated and acquire a papillomatous or proliferative appearance. They are pink, red, or violet in color. The polyps may obstruct the nasal passages, particularly in the event of even slight trauma. If located low in the nostril, they may protrude and hang onto the upper lips. If they are sited in the posterior part of the fossa, they may partially obstruct the pharynx or larynx and cause dysphagia or dysphonia and dysphonia and dyspnea.

In some cases the eyes are affected, the lesions being located on the conjunctiva. Initially these are small, flat granulations that may grow to form multilobed polyps of a pale pink color. At the same time there is diffuse vascular dilatation, photophobia and lacrimation, which is often due to involvement of the lacrimal sac and duct.

The ears may also become involved; depending on their size and location, these polyps may impair hearing.

Lesions may also develop on the male genital organs (the penis and, in exceptional cases, the urethra) and on the vulva and vagina in women. They may resemble flat or acuminate condylomas; lesions in the anus present as polyps and may sometimes be mistaken for hemorrhoids.

Cutaneous rhinosporidiosis, which is very rare, is generally due to spread from a neighboring mucosal lesion. It presents initially as minute papillomas; these gradually become larger and pedunculated. The surface is irregular, verrucous and polypous.

Cutaneous lesions are usually asymptomatic, but depending on their location (especially if they are on the sole of the foot) or their size they may cause pain. The surface of ulcerated rhinosporidiosis lesions is dotted with white spots, which are more readily discerned when depressed with a glass spatula; on microscopy these are seen to be sporangia.

Dissemination to the internal organs or bones is rare. In most cases the general health of the patient is unimpaired.

The appearance of pedunculated or unpedunculated polyps or nodules covered with white dots on the nasal mucosa or the conjunctiva should suggest the diagnosis of rhinosporidiosis. The condition must be distinguished from cryptococcosis, cutaneous tuberculosis, leprous lesions, leishmaniasis and treponematoses.

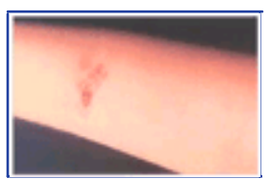

Figure 239-11 Localized lesion in sporotrichosis. The most common clinical presentation is a localized cutaneous or subcutaneous lesion, which develops at the site of implantation of the etiologic agent, *Sporothrix schenckii*.

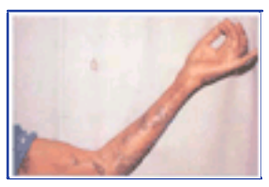

Figure 239-12 Lymphagitic spread of sporotrichosis. The spread is from a primary digital lesion up the dorsal surface of the forearm.

Sporotrichosis

The clinical manifestations of sporotrichosis are rather variable, which helps to explain the large number of different classification schemes that have been proposed.[14] [16] The most common clinical presentation is a localized cutaneous or subcutaneous lesion (Fig. 239.11). Lymphatic spread may then lead to the development of further cutaneous lesions (Fig. 239.12). Much less commonly, the fungus may cause infection of the lungs, joints, bones, eyes and meninges. Widespread disseminated infection has been reported in patients with diabetes, alcoholics, drug abusers and patients with AIDS (Fig. 239.13).

Cutaneous sporotrichosis

Cutaneous sporotrichosis tends to affect exposed sites, mainly the limbs and especially the hands and fingers. The right hand is affected more frequently than the left. The initial lesion develops at the site of implantation of the fungus. It is a painless nodule that is movable at first but that later becomes attached to the neighboring tissue. The skin turns red then violaceous, and the nodule breaks down to form an ulcer, which discharges a serous or purulent fluid. The edge of the ulcer is often irregular and it may become edematous, vegetative and crusted.

After a period of a few days to several weeks, the primary lesion may become surrounded by satellite lesions, or further nodules

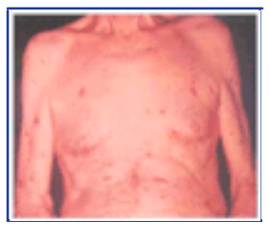

Figure 239-13 Multiple lesions in sporotrichosis. These skin lesions are a result of hematogenous spread.

along the course of the draining lymphatics may develop. These soon become palpable and ulcerate through to the skin. In most cases, however, the lymphangitis heals or remains static for a long time without ulcers forming. In most cases the regional lymph nodes are not involved. However, this is not an invariable rule. Any involvement of these lymph nodes is evidence of a superimposed bacterial infection and they may ulcerate in turn.

Apart from these very typical lesions, sporotrichosis may present a different clinical picture. Extension over large areas of skin, often described as the disseminated cutaneous form, may occur. Flat, infiltrated or papulopustular or nodulopustular lesions may develop. Whether oozing, proliferative, papillomatous or verrucous, the lesions of sporotrichosis are generally painless but often pruritic. Several ulcers may be interconnected by subcutaneous fistular passages. Confluent lesions may form a purulent and warty plaque with a continually expanding margin, whereas the center becomes atrophied, smooth and shiny.

Primary cutaneous lesions may heal spontaneously, leaving behind unsightly and even disfiguring scars, which may be a functional impediment. However, secondary lesions may persist for several years.

Extracutaneous sporotrichosis

Pulmonary sporotrichosis is a rare but well-recognized condition. It may be primary, following the inhalation of spores, and may be accompanied by enlargement of the hilar or tracheobronchial lymph nodes. It may, however, also be of a secondary character, caused by hematogenous dissemination. The symptoms are non-specific and include a productive cough, fever and weight loss. Hemoptysis may occur and can be massive and fatal. The course may be chronic. The typical radiologic finding is a single, nodular upper lobe lesion, which may or may not cavitate. The natural course of the lung lesion is gradual progression to death.

Most patients with osteoarticular sporotrichosis also have preceding cutaneous lesions. This condition presents as stiffness and pain in a large joint. The onset is indolent. In almost all cases of arthritis, the knee, elbow, ankle or wrist is involved. Osteomyelitis seldom occurs without arthritis; the lesions are usually confined to the long bones nearby affected joints.

Endophthalmitis, although very rare, may result in blindness; chorioretinitis has also been reported. Cases of meningitis have also been seen.

The development of a cutaneous lesion on the limbs following trauma is suggestive of sporotrichosis, particularly if the patient is resident in an endemic region. The development of multiple ulcers along lymphatics is also suspicious. At a later stage of development sporotrichosis must be distinguished from mycoses such as blastomycosis, chromoblastomycosis and paracoccidioidomycosis, and from leishmaniasis, verrucous tuberculosis and tertiary syphilis. The diagnosis ultimately depends on mycologic and histologic examination.

MANAGEMENT

There are no trials comparing different strategies for the treatment of infection caused by black fungi. Treatment depends on the clinical form of the disease and is reviewed by Silveira and Nucci.[1] Cutaneous and subcutaneous phaeohyphomycosis are usually treated with complete surgical excision of the lesion, resulting in complete cure in the majority of cases. In addition, various antifungal agents have been used. Itraconazole is considered the drug of choice, and the dose has ranged from 200 to 600mg daily. The duration of treatment is not established. Although unusual, progression or recurrence of disease, even with adequate itraconazole serum levels, has been observed Whenever possible, surgical resection is also recommended for lesions in other organs, in association with an antifungal agent.

A number of reviews expanding many of the guidelines given here have been published.[1] [44] [45]

Chromoblastomycosis

Chromoblastomycosis is a difficult condition to treat.[46] Surgical excision should be reserved for small lesions;[47] it carries a high risk of local dissemination and should only be attempted in conjunction with antifungal treatment.

There is no ideal antifungal treatment for chromoblastomycosis. The most commonly used drug is flucytosine (150–200mg/kg per day given as four divided doses), but resistance is a frequent problem during long-term treatment. Amphotericin B is not effective as monotherapy but appears to be effective in combination with 5-fluorocytosine (5FC). Ketoconazole is effective in combination with 5FC. Fluconazole is reported to be successful.[48] [49] [50]

Much better results have been obtained when 5FC (4g/day given as four divided doses) is combined with oral thiabendazole (1g/day given as two divided doses). Treatment should be continued for at least 1 month after clinical cure is obtained. Itraconazole (400mg/day) has given promising results in a few patients. Itraconazole is particularly effective when combined with liquid nitrogen cryotherapy. [51] [52] Recent trials have shown terbinafine (50mg/day) to be effective, even in chronic cases. [17] The local application of heat to the lesions may be beneficial.

Entomophthoramycosis

Rhinofacial conidiobolomycosis

Treatment has so far been disappointing. Surgical resection of infected tissue is seldom successful and it may hasten the spread of infection. The condition can be treated with saturated potassium iodide solution (up to 10ml q8h as tolerated) or amphotericin B. Long-term results are poor.

Patients with rhinofacial conidiobolomycosis treated with fluconazole (200mg/day for 4 months) have been completely cured or have exhibited considerable improvement. Some patients have responded to combination treatment with amphotericin B and terbinafine,[53] or a combination of itraconazole and fluconazole.[54]

Basidiobolomycosis

The therapy of choice still appears to be saturated potassium iodide solution (30mg/kg per day) which should be given for 6–12 months. Oral ketoconazole (400mg/day) has sometimes been successful, but amphotericin B has seldom been helpful. Surgical resection is not curative.

Ribes *et al.*[12] provides an exhaustive review of the treatment of entomophthoramycosis.

Lobomycosis

Antifungal drugs are ineffective. Amphotericin B, griseofulvin, sulfonamides and ketoconazole have been employed without adequate clinical responses.

Cure can only be achieved by surgical excision, the extent of the lesions permitting. Care must be taken during surgery of lobomycosis to avoid contaminating healthy tissue. Cryosurgery has also been used. Unfortunately, however, recurrence after excision is common. In advanced cases, the extensive excision required to remove the lesion may not be justified if the infection is not life-threatening. In cases involving larger areas of infection, treatment with clofazimine is recommended. At present, the disease does not have a satisfactory medical treatment. The course of the disease is slow and chronic and the prognosis is poor. Lobomycosis never heals spontaneously and is never fatal, but it may be a very serious impediment.

Mycetoma

Early actinomycetomas (and some late and advanced cases) respond well to treatment.[47] The drug of choice is streptomycin sulfate (1000mg/day intramuscularly). This should be combined with trimethoprim-sulfamethoxazole (co-trimoxazole) 960mg q12h in cases caused by *S. somaliensis*, *A. pelletieri* or *N. brasiliensis*. Other regimens include trimethoprim-sulfamethoxazole and amikacin, and streptomycin combined with either dapsone or trimethoprim-sulfamethoxazole. If no response is seen after 3 weeks of treatment, other regimens can be substituted. These include streptomcyin and rifampin (rifampicin), or streptomycin and sulfadoxine plus pyrimethamine. Therapy must be continued for months or even years. In favorable cases, edema and tenderness regress, discharge of secretion and grains diminishes, and sinuses dry up and close.

Even after symptoms and clinical signs have disappeared, the disease has become clinically silent and laboratory tests have become normal, it is recommended that treatment be continued for the same period of time as was required to achieve these results.

The response of eumycetoma to antifungal treatment is disappointing. *Madurella mycetomatis* and *P. boydii* infections have been known to respond to ketoconazole (400mg/day), but it is essential to test liver function before starting and during treatment with this drug. Long-term treatment with itraconazole has resulted in improvement in *M. grisea* mycetoma. The fungal agents causing mycetoma are resistant to amphotericin B and 5FC. Terbinafine has been successful in the treatment of maduromycetoma.

Mycetoma due to *Acremonium* spp. and *P. boydii* appear to respond to fluconazole (200mg daily for 10–12 weeks).

Surgical excision is the method of choice if the eumycotic lesions are small enough for total removal to be possible. Amputation is often required in advanced cases with bone involvement, particularly when there is no response to drug treatment. Prostheses and rehabilitation are indispensable in every case of mutilating surgery.

Phaeohyphomycosis

Subcutaneous phaeohyphomycosis

Incision and drainage of subcutaneous lesions is seldom successful. Surgical resection is required. Treatment with amphotericin B has

cured or improved unresectable lesions, but later relapse has been common. Itraconazole and terbinafine have been successful in the treatment of phaeohyphomycosis.

Paranasal sinus infection

Complete surgical debridement combined with amphotericin B treatment is essential to halt the progression of this form of phaeohyphomycosis. Even so, it is not uncommon for the condition to recur. The need for repeated debridement is most evident in patients with disabling symptoms or erosion of the bone separating the paranasal sinus from the brain.

Oral treatment with itraconazole (100–400mg/day) appears promising, although the optimum dosage and duration of treatment has not been defined.

Necrotic nasal septum lesions due to *Alternaria* spp. or *Curvularia* spp. have been cured after surgical excision.

Cerebral phaeohyphomycosis

In no case has a patient survived without surgical resection of the lesion. Treatment with amphotericin B on its own is ineffective. Lesions that have not been completely removed have usually proved fatal. A single case of cerebral phaeohyphomycosis due to *Cladosporium* spp. responded partially to fluconazole (300mg daily for 5 weeks).

Cutaneous infection

Surgical debridement of cutaneous lesions combined with parenteral amphotericin B is the most effective method of treatment. Topical antifungal treatment is seldom helpful.

Other forms of phaeohyphomycosis

Too few patients have been treated for firm recommendations to be possible. However, the response to amphotericin B has been partial at best. Surgical resection of lesions or oral treatment with itraconazole should be considered.

In in-vitro tests the new azole antifungals, including voriconazole, appear to be as active as itraconazole against a number of agents of phaeohyphomycosis.

Rhinosporidiosis

The treatment of choice is surgical excision. No drug treatment has proved effective. If left untreated, the polyps will continue to enlarge slowly. In very rare cases, widely disseminated or deep-seated visceral lesions may develop. Spontaneous remission is unusual.

Sporotrichosis

Saturated potassium iodide solution remains the treatment of choice for patients in developing countries who contract cutaneous sporotrichosis, owing to its ease of administration and low cost.[55] [56] The starting dose is 1ml q8h, and this is increased to 4–6ml q8h. Treatment should be continued for at least 1 month after clinical cure is obtained, which may take 2–4 months. Intolerance (iodism) is frequent and consequently therapy is often stopped.

If the patient cannot tolerate potassium iodide, oral itraconazole (100–200mg/day) can be used.[57] [58] Treatment should be continued for up to 6 months. The most appropriate therapeutic regimen appears to be 200mg/day during the entire treatment period, or 200mg/day at the beginning of treatment, reduced to 100mg/day after improvement. Many cases treated in this way are cured relatively quickly. Cutaneous sporotrichosis has also been successfully treated with terbinafine (125mg/day).[59] Oral ketoconazole has given poor results.

Local heat, on its own or in combination with drug treatment, has been shown to improve cutaneous lesions. Besides thermotherapy, the simple warming of diseased limbs in winter months has proved helpful.

Amphotericin B has cured some patients with extracutaneous forms of sporotrichosis, but failures are common. In cases of arthritis or osteomyelitis, better results have been obtained when the drug has been combined with surgical debridement.

Itraconazole (400mg/day) has given good results in patients with extracutaneous infection, especially in those who have not responded to fluconazole. [60] The Mycoses Study Group of the Infectious Diseases Society of America has recently reviewed the treatment options for sporotrichosis and published detailed practice guidelines.[61] Their recommendations were derived primarily from multicenter, nonrandomized treatment trials, small retrospective series and case reports. The treatment of choice for fixed cutaneous or lymphocutaneous sporotrichosis is itraconazole 100–200mg/day for 3–6 months.

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Chapter 240 - Superficial Fungal Pathogens

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The majority of superficial fungal infections are caused by three groups of fungi: dermatophytes, *Candida albicans* and *Malassezia* spp.[1] Dermatophytes, the agents of dermatophytosis (ringworm, athlete's foot) are members of the phylum Ascomycota. Within that phylum, they are related to the order Onygenales, along with systemic pathogens such as *Histoplasma* and *Coccidioides*. In conventional biologic classification they are related to the genus *Arthroderma*; in the unique dual nomenclature of fungal anamorphs (asexual states), they are members of the genera *Trichophyton, Microsporum* and *Epidermophyton* (Fig. 240.1).

NATURE

Candida albicans, the principal agent of candidiasis, is an anamorphic (asexual) yeast also related to the Ascomycota. It and some related *Candida* spp. are mammalian commensals (normally harmless colonizers) related to the class Endomycetes, which also contains the bread yeast *Saccharomyces*.

Malassezia spp. are yeast anamorphs related to the phylum Basidiomycota, but not closely related to other human pathogens in this phylum such as *Cryptococcus* and *Trichosporon* spp. They are lipophilic (lipid-requiring) body surface commensals and agents of the skin disease tinea versicolor (pityriasis versicolor). In addition, they may be causal in the allergically mediated condition seborrheic dermatitis.

Other fungi cause less common types of superficial disease. Yeasts in the genus *Trichosporon* may cause white piedra, superficial colonizations of the scalp, and axillary and public hair shafts. The melanized yeast-like organism *Hortaea werneckii* (also known by several older names all ending in '*werneckii*') causes the dark skin lesions of tinea nigra. The rare hair shaft colonization black piedra is caused by the ascomycete *Piedraia hortae*. Finally, approximately 35 nondermatophytic filamentous fungi and a few yeasts have been authenticated as causing onychomycosis (i.e. fungal nail disease). Most prominent among these are members of the genera *Scytalidium, Aspergillus, Scopulariopsis, Fusarium* and *Onychocola*.

EPIDEMIOLOGY

Dermatophytosis is among the most common of all communicable diseases. Because many cases are never brought to medical attention, fully reliable incidence figures do not exist. Onychomycosis, in which the great majority of cases are dermatophytic, [2] may account for 10–30% of all superficial mycoses.[3] [4] In the USA Medicare insurance for seniors (=65 years old) in 1 year paid for consultations involving 662,000 patients, or 2.7% of the senior population, at a cost of over US$43,000,000. Less than 10% of those aged under 30 years and more than 20% of those aged over 75 years had tinea unguium (dermatophytic onychomycosis; Table 240.1) in population studies.[5] Tinea capitis in children is common in some areas. In an urban center in the USA up to 4% of children presenting with non-dermatologic complaints at a clinic were infected by *Trichophyton tonsurans*, whereas up to 30% of asymptomatic adults accompanying infected children were carriers.[6] A study of 224 children in a Philadelphia school reported 3% symptomatic and 14% asymptomatic, infected children. [7] Tinea pedis has been found to affect 1.5% of pediatric patients, 5.9% of 11–15 year olds[6] and up to 45% of adult marathon runners.[8] There is a significant age-dependent association between sporting activities and pedal dermatophytosis.[9] Many of the most prevalent dermatophytes are cosmopolitan, but certain species, especially agents of tinea capitis, have defined endemic regions (see Table 240.1).

Cutaneous candidiasis is particularly common in infants, at least 10% of whom have candidal skin colonizations. Fifty per cent of this colonized group go on to become symptomatic, usually with candidal diaper dermatitis.[6] Many adults harbor an indigenous strain of *C. albicans*; up to 30% of healthy women, for example, are culture positive for this species in vaginal swab samples, at least when pregnant or taking oral contraceptives.[1] The lower gut may serve as a reservoir when other body sites are free of *C. albicans*. Normal skin only rarely yields *C. albicans* but the yeast may rapidly colonize chronically moist or damaged skin, including moist dermatophyte lesions.

Malassezia spp. have been found as skin commensals in over 90% of humans surveyed.[1] In the temperate zone they only occasionally proliferate to the point of causing the finely scaly maculae of tinea versicolor, but in the tropics the prevalence may reach 50% of the population.[1] *Malassezia* spp. in patients who have HIV and other immunocompromised patients may cause pustular hair follicle inflammations referred to as *Malassezia* folliculitis, and may be associated with an increased prevalence of seborrheic dermatitis. [10] In atopic individuals, *Malassezia* spp. growing commensally on skin may serve as a triggering allergen in atopic dermatitis. [11] A possible association between *Malassezia* and neonatal acne or cephalic pustulosis is under investigation.

White piedra is found worldwide, but is now uncommon because of modern hygiene. Black piedra is native to South East Asia, the Pacific and South America, and affects other primates in addition to humans. It is uncommon when discouraged but is cultivated in some cultures.[1] Tinea nigra is of tropical or subtropical origin; about 150 indigenous US cases were reported between 1950 and 1988, in addition to many more cases acquired by travelers, mostly in the Caribbean.[1] *Scytalidium* infections of nails, soles or palms are usually acquired in the tropics. *Scytalidium dimidiatum*, the most common infectious species, has rapidly growing pantropical forms as well as a distinctive slow-growing Indian subcontinent form that may be transmitted among family members in the Indian Diaspora. Most other agents of nondermatophytic onychomycosis are cosmopolitan saprobic molds and, despite their opportunistic potential, are more frequently seen as insignificant contaminants of body surfaces than as etiologic agents.[12]

PATHOGENICITY

Dermatophytes normally infect only the keratinized stratum corneum of the epithelial skin layers. They degrade keratin and other skin

Figure 240-1 *Trichophyton rubrum* (heavily conidial Afro-Asiatic variant). Typical micromorphology of a dermatophyte in pure culture, showing micro- and macroaleurioconidia.

proteins.[13] They are restricted to the stratum corneum by cellular immune components, and by the iron limitation and other effects mediated by transferrin.[14] A 2-fold higher prevalence in males than females may reflect inhibition by progesterone. Development of one type of dermatophytosis, tinea imbricata, requires homozygosity for

TABLE 240-1 -- Definition and distribution of dermatophytoses.

DEFINITION AND DISTRIBUTION OF DERMATOPHYTOSES			
Area of infection	**Disease name**	**Prevalent agents**	**Geographic area**
Scalp	Tinea capitis	*Trichophyton tonsurans*	Cosmopolitan, especially urban Americas, Latin America
		Microsporum canis	Cosmopolitan; predominant dermatophyte in parts of south and east Europe
		Trichophyton violaceum	Endemic to Middle East, North Africa
		Trichophyton soudanense	Endemic to sub-Saharan Africa
Beard area	Tinea barbae	*Trichophyton rubrum, Trichophyton verrucosum*	Cosmopolitan
Glabrous skin (trunk, limbs, face)	Tinea corporis	*Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes* complex, *Microsporum canis, Epidermophyton floccosum*	Cosmopolitan
		Trichophyton megninii	Endemic to Portugal, Sardinia
Scalp, glabrous skin	Favus	*Trichophyton schoenleinii*	Relict in rural Africa, central Asia; formerly widespread
Palms	Tinea manuum	*Trichophyton rubrum, Trichophyton mentagrophytes* complex	Cosmopolitan

Groin	Tinea cruris	*Trichophyton rubrum, Trichophyton mentagrophytes* complex, *Epidermophyton floccosum*	Cosmopolitan
Soles, toe webs	Tinea pedis	*Trichophyton rubrum, Trichophyton mentagrophytes* complex, *Epidermophyton floccosum*	Cosmopolitan
Nails	Tinea unguium (dermatophytic onychomycosis)	*Trichophyton rubrum, Trichophyton mentagrophytes* complex	Cosmopolitan

an otherwise uncharacterized recessive allele;[1] involvement of an autosomal dominant susceptibility allele has been proposed in the common chronic form of *Trichophyton rubrum* infection. [15] An indication of the relative importance of lymphocytes in host defense is seen in HIV infection, in which helper T-cell counts below 100 cells/ml correlate with a marked increase in onychomycosis,[16] including the unusual 'proximal white' form. Chronic dermatophytosis in normal patients may be mediated by locally immunosuppressive fungal carbohydrates as well as incapacitation of local cellular immunity by an immediate hypersensitivity response[17] and activation of a Th2 pattern of immune response.[18]

Dermatophytes differ in their host interactions. Anthropophilic dermatophytes (Table 240.2), specific to human disease, are distinguished from zoophilic dermatophytes, which have specific animal associations but may be transmitted to humans, and from geophilic dermatophytes, which are occasionally pathogenic to humans or animals but primarily grow on decaying keratinous material. The *Trichophyton mentagrophytes* complex (Fig. 240.2), containing four sibling-species distinguishable by molecular study, has both anthropophilic and zoophilic variants. Infection of humans by zoophilic dermatophytes usually elicits a pronounced inflammatory response.[14] Such inflamed lesions may resolve spontaneously, unlike the often chronic lesions of anthropophilic dermatophytoses.

The common anthropophilic dermatophytes include lower body dermatophytes associated with sites other than the scalp, and dermatophytes

TABLE 240-2 -- Common and formerly common dermatophytes and their infectivity.

COMMON AND FORMERLY COMMON DERMATOPHYTES AND THEIR INFECTIVITY		
Anthropophiles (type of scalp infection if characteristic)	**Zoophiles (type of human scalp infection) [principal hosts]**	**Geophiles (type of human scalp infection)**
Epidermophyton floccosum	*Microsporum canis* (ectothrix) [cat, dog]	*Microsporum gypseum* (ectothrix)
Microsporum audouinii (ectothrix)	*Microsporum gallinae* [fowl]	*Microsporum praecox*
Microsporum ferrugineum (ectothrix)	*Microsporum nanum* [swine]	*Microsporum racemosum*
Trichophyton concentricum	*Microsporum persicolor* [vole]	*Microsporum vanbreuseghemii*
Trichophyton megninii (ectothrix)	*Trichophyton equinum* [horse]	The listing of geophiles does not include species that have not been well substantiated to cause human disease. These species are not properly considered dermatophytes according to Ajello[19]
Trichophyton mentagrophytes complex (phylogenetic species *Trichophyton interdigitale*)	*Trichophyton mentagrophytes* complex (phylogenetic species *Trichophyton interdigitale, Trichophyton mentagrophytes, Trichophyton erinacei*) [rodents, rabbits, hedgehogs]	
Trichophyton rubrum	*Trichophyton simii* [monkey]	
Trichophyton schoenleinii (favus)	*Trichophyton verrucosum* [cattle]	
Trichophyton soudanense (endothrix)		
Trichophyton tonsurans (endothrix)		
Trichophyton violaceum (endothrix)		

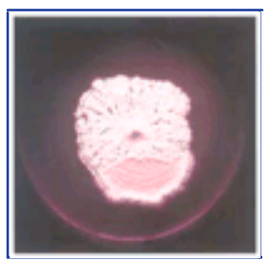

Figure 240-2 *Trichophyton mentagrophytes* complex: a zoophilic (animal-associated) isolate showing the 'granular' colonial morphology typical of such isolates. The strain depicted has been shown through mating to belong to the phylogenetic species *Trichophyton interdigitale* (sexual state called *Arthroderma vanbreuseghemii*), one of several genetic sibling species now known to make up the *T. mentagrophytes* complex.

matophytes strongly adapted for tinea capitis, less commonly causing other tineas. *Trichophyton rubrum, T. mentagrophytes* complex and *Epidermophyton floccosum* are the common lower body species. Tinea capitis dermatophytes consist of two major groups distinguished by their colonization of hair (see Table 240.2). In the 'ectothrix' group, hair shafts are externally ensheathed with masses of arthroconidia (spores). In the 'endothrix' group, the hair shafts are heavily invested internally by arthroconidia. An anomalous scalp infection, favus, caused by the now rare *Trichophyton schoenleinii*, is distinguished by the presence of follicles transformed into scutula, that is, pits lined with fungal mycelium.

Tinea capitis agents primarily cause new infections in children, and may cause dramatic outbreaks. *Microsporum audouinii* infections spontaneously resolve at 15–19 years of age, but most endothrix agents cause lifelong asymptomatic infections in some adult carriers[7] and occasionally cause new tinea corporis or other infections in adults. New anthropophilic tinea capitis infections are usually acquired via shared headgear, bedding or grooming and haircutting instruments. Adults who acquire new infections caused by endothrix species usually have intimate contact with infected children. Anthropophilic lower body dermatophytoses are often acquired via the feet, either from family members or in communal aquatic or exercise facilities. After infecting the feet, these fungi may go on to infect other body sites.

Zoophilic dermatophytes (see Table 240.2) usually cause tinea corporis or tinea capitis in humans. They may be transmitted directly from infected animals or from fomites, such as fence posts in cattle yards. *Microsporum canis* may cause limited outbreaks among humans before virulence is attenuated.[20]

Candida albicans is often acquired in the birth canal or in infancy from care givers. Generally, an individual harbors only one or two strains. Cutaneous candidiasis is predisposed to by warm, moist conditions with abrasion, especially in the diaper rash of infancy but also in adult occupations that involve wet hands. In the latter cases, paronychia or interdigital erosion frequently results. Intertriginous candidiasis occurs in moist body folds and is exacerbated by diabetes mellitus or obesity. Chronic mucocutaneous candidiasis (CMCC), in which skin and mucosa are extensively colonized by *C. albicans*, results from inherited defects in cellular immunity.[21] Particular associated conditions include congenital thymus dysplasias, poly-endocrine deficiencies and leukocyte dysfunctions such as chronic granulomatous disease.[1] In this latter disease, the impaired production of toxic oxygen species prevents killing of *Candida* spp. within phagocytes.

Malassezia spp. are also generally acquired as commensal surface flora in early infancy. They primarily use fatty acids secreted by the skin. Corticosteroid use, Cushing's disease, malnutrition and immunosuppression may contribute to an increased frequency of tinea versicolor; HIV infection and miscellaneous

immunosuppressions predispose to folliculitis.

Nondermatophytic onychomycosis caused by genera other than *Scytalidium* is strongly predisposed to by advanced age.[22]

PREVENTION

Prevention of infections caused by anthropophilic dermatophytes adapted for tinea capitis can be achieved by a combination of sanitation and detection of asymptomatic carriers. In *Microsporum* spp. outbreaks, a Wood's light can be used to screen potentially infected individuals rapidly; lesions usually fluoresce yellow-green. The same technique can also be used to detect animal carriers when the zoophilic *M. canis* is involved. Subclinical cases are more difficult to detect in nonfluorescent *Trichophyton* outbreaks; conventional microscopy and culture analysis of slightly scaly areas in individuals likely to be infected are required. In all cases in which tinea capitis fungi are involved, infected individuals must be treated promptly and their contacts must be educated not to share materials that contact the scalp. Fomites may endure on surfaces in contaminated rooms for months and belatedly cause new infections unless adequate disinfection is performed. Strategies for dealing with institutional outbreaks have been summarized.[14]

Lower body anthropophilic dermatophytosis, for example *T. mentagrophytes* complex tinea pedis, may be prevented to some extent by minimizing barefoot walking in public showers, swimming pool and hot tub peripheries, gymnasia, barracks and similar localities. However, familial genetic susceptibility and organism carriage may outweigh exposure in public places as a risk factor in the most common lower body dermatophytoses, *T. rubrum* tinea pedis and onychomycosis.[15]

Occupational animal handlers may reduce the risk of zoophilic dermatophytosis by wearing protective gloves.

Prevention of the more severe forms of epidermal candidiasis is linked to prevention of immunodepression and will not be dealt with here. Persons whose occupations involve frequent wetting of hands should wear protective gloves. Good hygiene and prompt change of diapers may aid in preventing candidal diaper rash.

Good hygiene is instrumental in the prevention of white piedra and may also help to prevent tinea versicolor.

DIAGNOSTIC MICROBIOLOGY

Superficial mycotic infections are best tested by the combination of direct microscopy for fungal elements in hydroxide (KOH, NaOH) slides, and fungal culture. Direct microscopy may be facilitated by the use of fluorescent fungal cell wall dyes such as calcofluor or Congo red. Histopathology performed with fungal stains has been suggested as an alternative to 'KOH and culture' for onychomycosis, but the ability of this technique to distinguish dermatophyte from nondermatophyte infections has received only preliminary investigation.

Dermatophytes in direct microscopy show cylindrical fungal filaments that may include chains of rounded arthroconidia. At times filaments may be converted entirely into arthroconidia, such as within or around hair shafts in tinea capitis. In superficial white onychomycosis caused by *T. mentagrophytes* complex members, microscopy may may show 'frondose branching', an appressed, palmate spreading of fungal hyphae between keratin lamellae. In nails, soles and palms, all morphologies of dermatophyte filaments overlap with those of other potentially infectious fungi, especially *Scytalidium* spp.

Generally, dermatophyte filaments from the growing fronts of infection, such as the peripheral 'ring' in classic ringworm and adjacent to the nailbed in tinea unguium, will be alive and will stain with vital stains such as neutral red. [23] Those from prior growth fronts, such as centers of ringworm lesions or extruded, distal nail, tend to be senescent and will not yield a culture. For this reason, sampling tinea at its extending margin, and subungual onychomycosis at the juncture of nail and nailbed, is recommended. Tinea corporis is sampled by using a scalpel to remove skin scale from the advancing lesion margin after light disinfection with an ethanol swab. In subungual onychomycosis, heavily contaminated debris is scraped from the undersurface of the nail and discarded; then, after swabbing with ethanol, friable material is scraped from the nail undersurface as close as possible to the nailbed. Clippings may also be taken. In superficial white onychomycosis, white, opaque areas on the upper nail surface are scraped. As with subungual onychomycosis, superficial debris is scraped and discarded before sample collection. In tinea pedis of the sole and tinea manuum, in which growth fronts may not be apparent, the affected area should be scraped broadly so that the whole is represented in the sample.

Even with expert sampling approximately 10–25% of nail samples positive for fungal filaments in direct microscopy fail to yield a culture.[23] That such a finding accurately reflects the active infection can be confirmed by vital staining,[20] and should not be mistaken for a technical 'false-negative' culture result. Also, approximately 45–50% of toenails and 60% of fingernails clinically suggestive of onychomycosis fail to yield any evidence of fungal infection.[24] Dermatophyte colonization in very degraded nails may be partially supplanted by secondary invaders such as *Pseudomonas* bacteria and *Scopulariopsis* mold (the latter may also appear as a sole agent of onychomycosis or as a contaminant), and the original etiologic agent may be difficult to trace.

Hair from tinea capitis or barbae is plucked so that the hair root, the best source of inoculum, is included. In 'black dot' tinea capitis, in which hairs are fragile, a few hair roots may be extracted with the tip of a scalpel and included along with surface scrapings of the lesion. *Microsporum* scalp lesions typically show strong yellow-green fluorescence under a Wood's light and can be presumptively diagnosed in this manner. In zoophilic tinea corporis and other lesions with vesicles or bullae, the apices of these may be clipped and included in the sample. Moist, suppurating lesions may be sampled with a swab. Swab samples may make direct microscopy difficult and are best evaluated by culturing.

For culture, scrapings or fragments from clippings are usually planted on Sabouraud agar supplemented with antibacterials and the selective antifungal cycloheximide. Common commercial equivalents include Mycosel, Dermasel and Mycobiotic. Dermatophytes and *C. albicans* are among the small minority of fungi able to grow well on these media. Potato glucose or potato flake agar with cycloheximide is preferred by some laboratories because the distinctive pigmentation of some species is enhanced. A cycloheximide-free medium is generally used for nails, and also be isolated from at least some of these sites. The most common of such media are plain Sabouraud agar supplemented with antibacterials only, and two restrictive media: Littman oxgall agar and inhibitory mold agar. These media, unlike plain Sabouraud agar, inhibit fast-growing contaminant molds from overgrowing the slower growing etiologic agents.

The characteristics and criteria used in identifying dermatophytes have been summarized.[25] [26] [27] Since most dermatophyte species are susceptible to similar therapies (duration of therapy may vary, e.g. between *Microsporum* spp. and the more rapidly inhibited endothrix

Trichophyton spp. in tinea capitis), species identification is salient primarily to recognize situations in which animal hosts or familial or institutional carriers constitute potential sources of re-infection and continuation of outbreaks. Zoophilic dermatophyte species and the endothrix tinea capitis agents are most notorious in these situations, but unusual outbreaks of anthropophilic lower body dermatophytoses may also be detected and controlled through species identification.

Candidiasis in skin and nails is recognized in direct microscopy by the presence of budding yeast cells and candidal-type filaments; the yeast cells bud through a narrow constriction, unlike *Malassezia* yeasts, and the filaments give rise to budding cells on side branches, unlike dermatophyte filaments. Although filaments indicate an invasive condition in cutaneous candidiasis, and are usually found in any genuine case of infection, masses of budding cells alone may be seen in some nail specimens. In fingernails, this often indicates nearby paronychia that was not sampled directly. In some cases, however, especially with toenails, yeast cells merely indicate harmless growth of *Candida* spp. (other than *C. albicans*) of the normal skin flora in crevices in onychomycotic or traumatized nails. *Candida albicans* should normally be identified when isolated from skin; there are a number of inexpensive, specific, rapid tests for this species such as rice-tween, cornmeal-tween or oxgall medium tests for formation of chlamydospores under a cover slip. *Candida* spp. other than *C. albicans* need not be identified to species level from superficial sites except in rare cases in which they are isolated from material with conclusive evidence of invasive yeast infection (e.g. formation of filaments with lateral budding cells) in direct microscopy. Because *C. albicans* commonly contaminates moist dermatophyte lesions without significantly exacerbating symptoms, and because normal flora yeasts may proliferate harmlessly in nail crevices, the laboratory gold standard for any diagnosis of cutaneous yeast infection is the specific presence of yeast-type filamentous elements within cutaneous tissue in direct microscopy.

Tinea versicolor is recognized in direct microscopy by the rounded yeast cells and short hyphal fragments ('spaghetti and meat balls') of *Malassezia* spp. Culture is normally unnecessary and may be problematic when attempted. Although there is considerable research interest in quantitative cultural analysis of *Malassezia* spp. in seborrheic dermatitis, clinical diagnosis based on symptoms remains the gold standard.

Other purely microscopic diagnoses not requiring culture include black and white piedra. Tinea nigra evinces distinctive dark filaments in direct microscopy (Fig. 240.3), but is also readily cultured to yield the etiologic agent, *H. werneckii*. No other organism shows melanized, two-celled yeasts budding from annellidic apertures heavily fringed with collarette remnants. Mycology laboratories

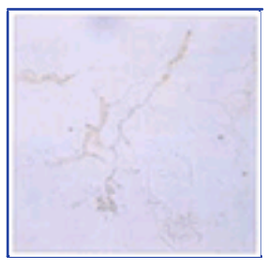

Figure 240-3 Direct microscopy (NaOH slide) of palm skin showing distinctive dark fungal filaments of *Hortaea werneckii*, causal agent of tinea nigra.

must also recognize the microscopically distinctive corynebacterial and actinomycetous elements found in erythrasma, trichomycosis axillaris and pitted keratolysis.[1] All intertriginous skin samples should be stained with methylene blue preparations to detect erythrasma. In specimens from this infection, methylene blue deeply stains delicate branching filaments less than 1µm in diameter, often seen breaking up into smaller bacillary or coccoid forms. The poorly understood '*Dermatophilus*-like' agent of pitted keratolysis[1] also stains in methylene blue, and shows a mixture of coccoid cells and short filaments, at least some of which appear tapered and, in broader regions, subdivide to give rise to a second or third longitudinal series of cells. In trichomycosis axillaris, hair is heavily ensheathed in corynebacterial cells (Gram-positive rod and coccus forms less than 1µm in diameter). These may be readily distinguished in crushed preparation in hydroxide from the over 2µm in diameter arthroconidia and yeast cells seen in the macroscopically similar white piedra.

The correct diagnosis of onychomycosis caused by nondermatophyte molds can be especially challenging. These nondermatophyte filamentous fungi can easily be ascertained as causing onychomycosis if distinctive morphologic elements such as conidiophores are seen in addition to filaments in direct microscopy of nail specimen, or if a fungus from warm latitudes, such as a *Scytalidium*, is isolated in an area in which only such fungi occur in infected patients. Most cases are more ambiguous. A fungal species such as *Aspergillus sydowii* may be isolated either as a contaminant or as an etiologic agent, and filaments seen in direct microscopy may be either nonviable dermatophyte elements or genuine nondermatophyte elements. Therefore, even exclusive and heavy isolation of such a nondermatophyte from a specimen positive for fungal filaments does not guarantee that the nail is infected by the same nondermatophyte.[12] The current gold standard, which may not be easy to attain in practice, is:

- first to demonstrate fungal elements in direct microscopy compatible with the suspected agent; and
- second, in culture, to show, through correlating the results of two nail samples collected at least 1 week apart, that the nondermatophyte in question consistently grows from the diseased nail.

True mixed infection by a dermatophyte and a nondermatophyte may occur, but again can only be demonstrated scientifically by showing a consistent presence of the latter in more than one serial sample.

CLINICAL MANIFESTATIONS

Tinea capitis

The organisms that cause the 'noninflammatory type' of tinea capitis (Fig. 240.4) include *T. tonsurans, M. canis, M. audouinii* and

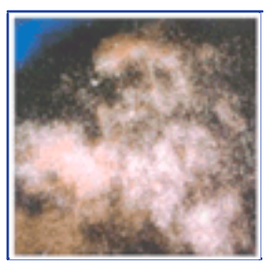

Figure 240-4 Tinea capitis, noninflammatory type. *Courtesy of Dr Natalie Roholt.*

Microsporum ferrugineum. There may be variable erythema with scaling that may be minimal or clinically significant, such that scale is consistently present. One or more well-demarcated patches may be present.

The inflammatory type of tinea capitis is often caused by zoophilic or geophilic species (see Table 240.2). The inflammatory changes range from a pustular folliculitis to multiple follicular abscesses, or even a kerion. A kerion may be fluctuant with a boggy feel to it. Pus may discharge from one or more points. Within the kerion there may be patchy or complete hair loss. In some instances the kerion resolves with scarring alopecia. A patient who has a kerion may have a tender, full neck as a result of cervical lymphadenopathy. Other symptoms and signs may include fever, neck ache and pruritus of the scalp.

Tinea capitis may also manifest as 'black dot' disease, which is usually caused by endothrix organisms, such as *T. tonsurans* and *Trichophyton violaceum*. The hair is brittle and breaks off at the scalp surface with the free end of the in-situ portion of the hair manifesting as a 'black dot'. There may be a variable amount of inflammation and scale present.

The favus type of tinea capitis is caused by *T. schoenleinii*; less commonly *T. violaceum* or *Microsporum gypseum* may cause a similar presentation. Within the scalp there are typically yellow crusts and scale, known as scutula. The condition may progress to involve large portions of the scalp, with yellow, adherent scale and crusting, and cicatricial alopecia.

Tinea barbae

Three types of tinea barbae exist: inflammatory, superficial and circinate. In the inflammatory type the lesions may be pustular, boggy or nodular, with alopecia. The superficial type can manifest as a folliculitis with papules and pustules. In the circinate variety there may be an active border with vesicles and pustules, and central scaling.

Tinea faciei (tinea corporis of the face)

Typically there is an annular lesion with erythema and scaling, with central clearing. The lesion may be pruritic and the patient may give a history of the lesion worsening after exposure to the sun.

Tinea corporis

Generally the lesions are annular with central clearing. The margin is typically erythematous with scaling, and sometimes with papules, pustules or vesicles. Pigmentary change may be seen with hypopigmentation or hyperpigmentation of the lesion. The inflammatory change may be more pronounced when the infecting organism is zoophilic. Sometimes the dermatophytes infect the hair follicle, with the formation of inflammatory perifollicular nodules or pustules, also known as Majocchi's granuloma. Infrequently, nodular granulomatous changes or subcutaneous spread may occur.

Tinea cruris

The eruption can be symmetric or asymmetric, unilateral or bilateral. The patient may complain of pruritus. The lesion may demonstrate an active border with erythema, scaling, papules and vesicles. When the causal organism is *T. rubrum* there may be a reservoir of infection, such as the nails or soles of feet. With *E. floccosum* infection there is no reservoir. When infection is caused by *Candida* spp. satellite pustules are less likely to be present in adults than in children.

Tinea pedis

The three major clinical presentations are the interdigital, the 'moccasin' or chronic hyperkeratotic, and the vesiculobullous (Fig. 240.5). Interdigital tinea pedis is the most common presentation, usually with involvement of the web space between the fourth and fifth toes or the third and fourth toes. The clinical presentation may vary from

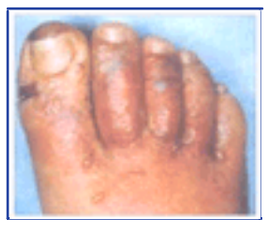

Figure 240-5 Tinea pedis, vesiculobullous variety. *Courtesy of Dr Natalie Roholt.*

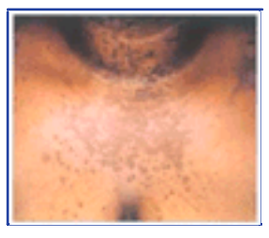

Figure 240-6 Tinea versicolor. *Courtesy of Dr Natalie Roholt.*

relatively asymptomatic mild scaling to maceration, erythema and exudative changes. In the plantar, moccasin-type tinea pedis there may be scaling, hyperkeratosis and a variable degree of erythema. Sometimes the fungal infection manifests as vesicles or bullae that may be seen on the instep of the foot or the mid-anterior plantar surface.

Tinea nigra

This superficial fungal infection caused by *H. werneckii* may appear as a scaly, asymptomatic, brown-black hyperpigmentation of the palms or soles of the feet. The palm and sole or both soles may be involved. The main differential diagnosis is benign and malignant melanocytic lesions.

Piedra

In black piedra, hard black nodules of varying sizes firmly adhere to hair. Generally scalp hair is involved, although hair at any site may be affected. In white piedra, soft white nodules are of varying sizes and are less adherent to the hair shaft. The infection is present within and on the exterior of the shaft, resulting in weakening of the hair. There is no fluorescence under Wood's light. In immunocompromised individuals disseminated infection may occur with skin lesions.

Tinea versicolor (pityriasis versicolor)

The lesions are macular, with fine scale and varying degrees of erythema. There may be hypo- or hyperpigmentation (Fig. 240.6). The usual site of involvement is the upper trunk and upper extremities. The eruption may occur at other sites, including the face, scalp, neck, palms and in areas of occlusion. In inverse tinea versicolor the flexural area may be involved.

Lesions of *Malassezia* folliculitis are generally seen on the trunk and upper extremities. The lesions are typically perifollicular, inflammatory papules and pustules that are often pruritic.

Candidiasis

The cutaneous and mucosal manifestations of candidiasis are varied and include oral candidiasis, balanitis and vulvovaginal candidiasis. Cutaneous candidiasis may colonize occluded areas or folds of the skin producing infection in flexural sites (*Candida* intertrigo), diaper dermatitis, inframammary dermatitis and infection in fingerweb areas (erosio interdigitalis blastomycetica). Clinical manifestations include erythema, scaling, maceration, and satellite vesicles and pustules. Candida miliaria may occur on the trunk of febrile patients after sweating.[28] Pustules or vesicles develop from which the organism may be recovered. A folliculitis may also occur, for example, in the perioral area.

Candida spp., particularly *C. albicans*, may be associated with nail infection. *Candida* paronychia is more common when there is frequent contact with water. Typically there is erythema, swelling and tenderness of the paronychial area. The cuticle may be separated from the surface of the nailplate. Application of gentle pressure on the paronychial surface can result in the expression of pus from underneath the proximal nailfold. Concomitant bacterial infection may be present. For example, with *Pseudomonas aeruginosa* infection a greenish discoloration of the nail can be expected. Uncommonly, distal and lateral subungual onychomycosis may be caused by *Candida* spp., particularly involving the fingernails. Congenital CMCC tends to feature both severe cutaneous candidiasis, including paronychia, and oral candidiasis, with manifestations usually seen in infancy or early childhood. *Candida* granulomas may be present. The nails may be thickened and dystrophic, with the entire nailplate being involved.

Onychomycosis

Onychomycosis is a fungal infection of the nailbed with secondary involvement of the nailplate. The five main types of onychomycosis are:

- distal and lateral subungual onychomycosis (DLSO; Fig. 240.7),
- superficial white onychomycosis (SWO),
- proximal subungual onychomycosis (PSO),
- endonyx onychomycosis (EO), and
- *Candida* onychomycosis (CO).

In DLSO the distal portion of the nail is infected initially with subungual hyperkeratosis and onychomycosis. In lateral onychomycosis the infection initially involves the lateral portion of the nailbed and nailplate. In DLSO the most common causative organism is *T. rubrum*. As DLSO has a similar clinical presentation whether caused by dermatophytes or nondermatophytes it is important to obtain a nail sample for mycologic examination so that the causative organism can be identified. In SWO the infection involves the superficial portion of the nailplate, the most common organisms being members of the *T. mentagrophytes* complex and *Aspergillus, Fusarium* and *Acremonium* spp. In PSO the infection initially involves the proximal

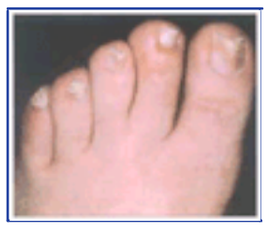

Figure 240-7 Onychomycosis (tinea unguium), distal and lateral subungual type. *Courtesy of Dr Natalie Roholt.*

nailbed and nailplate, with *T. rubrum* being the most common causative organism. Endonix onychomycosis, involving the nailplate but not the nailbed, is mainly caused by *Trichophyton soudanense*. [29] It gives affected nails a milky appearance without hyperkeratosis. Proximal white superficial onychomycosis is characteristically seen in patients who are immunocompromised, for example, those who have AIDS. *Candida* onychomycosis is typically found in patients who have CMCC. It may also occur in individuals who spend time with their hands in water, for example, bar-tenders or home-makers.

MANAGEMENT

In tinea capitis the causative organism should be identified whenever possible. If it is a zoophilic organism then the animal source should be treated. When the organism is anthropophilic, close contacts should be examined. Griseofulvin has been widely used to treat tinea capitis. The dose is 15–20mg/kg/day given for 6–8 weeks. In some countries a liquid formulation is available. Ketoconazole may have an efficacy similar to that of griseofulvin and has been used in patients who have responded poorly to griseofulvin. More recently terbinafine and itraconazole have been reported to be effective and safe. It may be possible to shorten the duration of therapy with the newer antifungal agents. Adjunctive measures may include cutting scalp hair short and using a shampoo such as ketoconazole or 2.5% selenium sulfide. If possible, old items of headgear should be discarded, the practice of sharing items such as caps and helmets discouraged and old combs thrown away or boiled in water, as appropriate.

With tinea barbae, tinea faciei, tinea corporis, tinea cruris and tinea pedis the source of infection should be identified and treated, whenever possible. In many instances the use of a topical antifungal agent such as an imidazole or an allylamine may suffice. Possible choices include ketoconazole, ciclopirox olamine or terbinafine creams. Some of the topical antifungal agents also have antibacterial and anti-inflammatory properties. Other topical antifungal agents, for example, tolnaftate and haloprogin, have a limited spectrum of activity. Generally, the topical antifungal agent should be applied over a small area beyond the active infection.

In instances in which the eruption involves a large area, when there is poor response (and the diagnosis has been confirmed to be a fungal infection), or if the infection involves the hair follicles and deeper cutaneous structures, then an oral agent should be considered. The traditional antifungal agent has been griseofulvin. In adults, ultramicrosize griseofulvin 330mg/day may be given for 2–4 weeks to treat tinea corporis and for 4–8 weeks in tinea pedis. In tinea pedis the dose may have to be increased to 330mg q12h. The newer antifungal agents itraconazole, terbinafine and fluconazole have also been reported to be effective. Itraconazole is a triazole that may be used as pulse therapy, 200mg/day for 1 week, to treat tinea corporis and tinea cruris. For tinea pedis the dosage of itraconazole pulse therapy is 200mg q12h for 1 week. Itraconazole may also be given as continuous therapy, 200mg/day for 2 weeks to treat tinea corporis and tinea cruris and for 4 weeks to treat tinea pedis. Terbinafine is an allylamine that is also effective in the treatment of tinea infections. The dosage regimen is 250mg/day given for 2–4 weeks to treat tinea corporis and tinea cruris and for 2–6 weeks when tinea pedis is present. In some instances a shorter duration of therapy may suffice. Reports of fluconazole used for the treatment of tinea infection suggest that a dose of 150mg once a week for 2–4 weeks may be beneficial.

For the management of onychomycosis, any co-existing tinea pedis should be identified and treated. The patient should be asked to cut the diseased nail as far back as possible, keep feet dry and clean, and wear socks made of absorbent material and, when possible, wear footwear that allows the feet to 'breathe'. The patient should use foot

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powders judiciously and be encouraged to use appropriate footwear when walking on communal areas that may harbor a high density of fungal inoculum, for example swimming pools, gymnasium floors or health spas. Griseofulvin is a narrow-spectrum agent that is ineffective against *Candida* spp. and nondermatophyte molds. When griseofulvin is used to treat dermatophytes, cure rates have been low (3–30%) and relapse rates high (40–60%). Therapy for fingernail and toenail onychomycosis lasts for 6–9 months and 9–18 months, respectively. With the newer antifungal agents terbinafine, itraconazole and fluconazole the response rates are higher and treatment is given for a shorter duration. These agents have a favorable adverse effects profile. The recommended dosage for terbinafine is 250mg/day given for 6 and 12 weeks for fingernail and toenail onychomycoses, respectively. With itraconazole the dosage schedule is 200mg/day given as continuous dosing for 6 and 12 weeks for fingernail and toenail onychomycoses, respectively. When itraconazole is given as pulse dosing the treatment regimen is 200mg q12h for 1 week per month given for two and three pulses for fingernail and toenail onychomycoses, respectively. Fluconazole has been given as 150mg once a week for 7–12 months to treat toenail onychomycosis. Topical agents have recently received renewed attention as alternatives in the treatment of minimal-to-moderate onychomycosis. Traditional topical therapies produced less than desirable results in onychomycosis treatment; this may, in part, have been due to the method of application because creams, gels, powders and water- or oil-based lotions can easily be wiped, rubbed and washed off. Recently, with the development of nail lacquers such as amorolfine and ciclopirox, topical agents have shown increased efficacy rates and patient compliance. Amorolfine is available in many countries for the treatment of onychomycosis, but is not approved in the USA. Ciclopirox nail lacquer 8% is approved in the USA for the treatment of mild-to-moderate onychomycosis without lunula involvement. Surgical interventions such as nail avulsion may be considered as part of a combination therapy with antifungal drugs when a single nail is diseased; however, such procedures are used infrequently, particularly with the advent of the newer, more effective oral antifungal agents.

In tinea nigra, therapy includes topical azoles. Griseofulvin is ineffective. The management of piedra includes shaving the infected hair. Spontaneous remission may occur. Topical antifungal agents have been reported to be beneficial. The management of tinea versicolor includes topical products such as 2.5% selenium sulfide shampoo, ketoconazole shampoo, other topical azoles or terbinafine, and keratolytic agents. Griseofulvin and oral terbinafine are ineffective. Short-term therapies with ketoconazole and itraconazole may be effective. The management of candidiasis depends upon the particular clinical form and is covered in detail elsewhere (see Chapter 62 , and Chapter 111 and Chapter 126). [30]

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Chapter 241 - Pneumocystis

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Dana Milatovic

NATURE

Pneumocystis carinii was first described by Chagas, who misidentified the organism as the sexual state of *Trypanosoma cruzi*.[1] For years it was classified as a protozoon. However, detailed ultrastructural analysis and molecular analysis of 16S-like rRNA[2] and mitochondrial DNA from the organism revealed that it is phylogenetically closely related to the Ascomycetes and Basidiomycetes and thus is now classified as a fungus.

However, *Pneumocystis carinii* has features that are atypical for a fungus. It has the morphologic appearance of a protozoon: thick-walled cysts and amoeboid trophozoites. Furthermore, it contains only two copies of the rRNA gene, whereas most fungi contain hundreds of copies. It is resistant to antifungal agents affecting the outer sterol membranes of fungi (e.g. amphotericin or azole drugs) because it lacks ergosterol and contains cholesterol instead. Nevertheless, its similarity to fungi may ultimately prove therapeutically exploitable. In fact, the echinocandins that inhibit ß-glucan synthase of fungi are very effective in a rat model of *Pneumocystis* pneumonia.

It has become apparent that there is considerable genotypic and antigenic variation among *P. carinii* isolated from different host species and among *P. carinii* found in the same host species.[3] However, controversy remains as to whether these differences establish that multiple species of *P. carinii* exist. At present, the different variants of *P. carinii* are referred to as 'special forms'. Using a trinomial nomenclature system, human *P. carinii* is now known as *P. carinii* special form *hominis* (*P. carinii* sp. f. *hominis*). Until now, molecular epidemiologic studies have identified more than 50 genotypes of human-derived *P. carinii*.

The lack of an effective culture system for *P. carinii* has hampered efforts to study the antigenic composition of the organism. However, two antigen complexes have been identified that appear to be important in pathogenesis. The highly immunogenic major surface glycoprotein (MSG, also known as glycoprotein A or gp120) is encoded by a multicopy gene family. Expression of the MSG genes is regulated by a single expression site: the upstream conserved sequence (UCS). As a result the MSG shows considerable antigenic variation. This may be the mechanism enabling the organism to evade the host immune system. In fact, analysis of pulmonary samples of patients who had *Pneumocystis carinii* pneumonia (PCP) demonstrated that multiple MSG genes were expressed in a given host and that different patterns of MSG expression were seen among different patients.[4] Another glycoprotein that migrates in a 35–45kDa band appears to be important in eliciting humoral response to *P. carinii* in humans.[5]

EPIDEMIOLOGY

Serologic studies have shown that nearly 85% of all children undergo a *P. carinii* infection early in life, often being accompanied by mild respiratory symptoms.[6] [7] Up to now no environmental reservoir has been identified. Therefore it seems that humans are the only reservoir for *P. carinii* sp. f. *hominis*. As a result, for years the most accepted hypothesis for pathogenesis has been that the organisms remain latent within the lung until the host becomes severely immunocompromised and reactivation of *P. carinii* occurs. However, recent epidemiologic studies suggest that re-infection with *P. carinii* may also play a role.

The 'latency theory' is supported by several observations:

- *P. carinii* is carried in apparently healthy immunocompetent animals;
- refractoriness to grow in culture media suggests that it cannot grow outside of the mammalian host;
- host specificity implies co-evolution during long-term carriage; and
- *P. carinii* continuously changes its antigenic composition, which is consistent with the capacity for long-term carriage in an immunocompetent host.[3]

Although several studies using polymerase chain reaction (PCR) have demonstrated asymptomatic carriage of *P. carinii* in non-immunocompromised individuals,[7] [8] this does not necessarily prove long-term colonization.[9] Other data suggest that exposure to *P. carinii* can result either in direct clearance of the micro-organism from the airways, temporary latency or, depending on the immune status, in PCP. In the laboratory, immunosuppressed rodents acquire infection shortly after inhalation of *P. carinii*. Electrophoretic karyotyping identifies *P. carinii* in the lungs of naive animals as the same as those in chronically infected animals, from which infections are acquired.[10] When immunosuppression is withdrawn, these rats appear capable of clearing the organism completely from their lungs. Analysis of internal transcribed spacer (ITS) and mitochondrial large-subunit genes of *P. carinii* isolates in bronchoalveolar lavage (BAL) fluid from HIV patients during several episodes of pneumonia revealed that recurrence could also be attributed to de-novo infections.[11] [12] In approximately 50% of patients who had recurrent episodes of PCP, the sequence types observed at the second episode were different from those of the first, suggesting the occurrence of both reactivation of a previously acquired infection and re-infection from an exogenous source.[13] [14]

A finding complicating epidemiologic studies is that latency and infection are not monoclonal. Analysis using PCR of BAL specimens from 212 patients who had PCP revealed that 23% were infected with a single *P. carinii* genotype, 50% with two and 27% with more types.[15] [16] Moreover, the number of genotypes found in respiratory samples does not represent the number of genotypes found in autopsy lungs. These results suggest that there is heterogeneity and compartmentalization of *P. carinii* genotypes within the lungs.[17] [18]

The organism is probably not acquired from zoonotic sources because of host restriction based on distinct (anti)genetic subgroups.[19] Although *P. carinii* DNA has been found in samples of outdoor air, no environmental reservoir for *P. carinii* has been identified. Geographic studies of HIV-associated PCP cases have shown residential areas with significantly lower risks of PCP versus clustering of cases, suggesting an environmental factor.[20]

Studies using molecular techniques support person-to-person spread, which was suggested by earlier reports on clusters and

outbreaks of PCP among oncology and transplant patients.[21] *Pneumocystis carinii* DNA was detected in respiratory samples of health care workers and those with the closest occupational contact with patients who had PCP were more likely to have detectable *P. carinii* DNA.[22] Furthermore, analysis of air filters in hospitals suggests that transmission is airborne; corresponding genotypes were found in BAL specimens from PCP patients and air filters from their rooms.[23] [24] Currently available information justifies the isolation of patients who have PCP from individuals who are at increased risk of acquiring the infection.

PATHOGENICITY

In-vivo studies indicate that *P. carinii* is acquired by the airborne route. Once inhaled, the organism adheres to the type I alveolar cell of the lung and interacts with alveolar macrophages through the extracellular proteins fibronectin, vitronectin and surfactant protein D, which bind the MSG of *P. carinii* (Fig. 241.1). After several weeks of cortisone treatment, small clusters of *P. carinii* can be detected in alveolar spaces throughout the lungs of previously uninfected rats. Later, the alveoli become completely filled with organisms, suggesting that replication is slow but proliferation in the lung is extensive.

Two distinct morphologic forms of the organism are detected during infection. One is a small pleomorphic trophozoite that constitutes the majority of micro-organisms in

the lung and the other is a

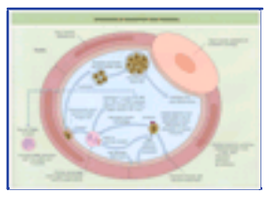
Figure 241-1 Pathogenesis of *Pneumocystis carinii* pneumonia. IFN, interferon; NO, nitric oxide; PMN, polymorph nuclear cell; SpA, surfactant protein A.

more mature, larger, thick-walled cyst that contains up to eight intra-cystic bodies. Examination of infected lung by light microscopy typically shows foamy alveolar exudate consisting of degenerative cell membranes, surfactant and host proteins (Fig. 241.2). A modest number of alveolar macrophages are present. As infection progresses, septal hypertrophy occurs and there is an accumulation of interstitial edema and mononuclear cell infiltration.[25] Pathophysiologic features have also been detected by electron microscopy and include subepithelial bleb formation resulting from increased alveolar-capillary permeability, degenerative changes in type-I alveolar cells and effacement of the basement membrane. Consequently, serum, fibrin and other proteins diffuse into the alveoli.[26]

Surfactant plays a major role in the pathogenesis of PCP; intra-tracheal instillation of surfactant improves oxygenation and pulmonary architecture in immunosuppressed laboratory animals with PCP. Furthermore, during experimental infection, there are alterations in phospholipid content of the surfactant (diminished phosphatidylcholine and increased sphingomyelin). This can be explained by the observation that the organism has uptake and catabolism of lipids[27] and that the surfactant protein A level, which regulates secretion and absorption of surfactant phospholipids, is increased. The exact relation of these changes to lung injury is unknown, although deficiency of surfactant phospholipids likely results in abnormalities in alveolar surface tension, lung distensibility and ventilation-perfusion matching. Surfactant protein A plays another role; it binds to the

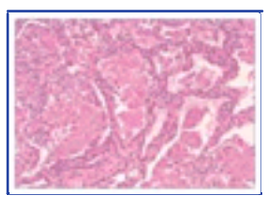
Figure 241-2 Hematoxylin and eosin stained section of lung tissue. Characteristic intra-alveolar foamy exudates can be seen in this patient, who has *P. carinii* pneumonia. *Courtesy of Michael Koss.*

mannose residue the MSG[28] and thereby enhances attachment of the organism to type I pneumocytes, interfering with the phagocytosis of *P. carinii* by alveolar macrophages.[29]

The physiologic consequences of these pathologic abnormalities include the early impairment of gas exchange because of increased alveolar-capillary permeability, which is measured by decreased membrane diffusing capacity of carbon monoxide and ultimately decreased compliance, total lung capacity and vital capacity. Lung function may be further impaired by the host inflammatory response. In severe combined immunodeficiency (SCID) mice with PCP, reconstitution of the immune system by bone marrow transplantation or infusion of $CD4^+$ lymphocytes resulted in clearance of *P. carinii* from the lungs[30] but also in early deaths from a hyperinflammatory local response. That the inflammatory response can be deleterious is also found in humans; a decrease in oxygenation occurs often soon after initiation of specific anti-*P. carinii* therapy that is blunted by adjunctive corticosteroids. Similarly, an increased number of neutrophils in BAL in patients is associated with a poor outcome.

Multiple studies have supported an important role for inflammatory mediators in the pathogenesis of PCP.[31] [32] [33] Production of interleukin (IL)-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-a, leukotriene B_4 and phospholipase A_2 are all increased in AIDS patients who have PCP. In individuals who have PCP, IL-8 is elevated in BAL fluid and correlates with BAL fluid neutrophilia, $P(A\text{-}a)O_2$ and severity of illness; therapy with corticosteroids resulted in a reduction in IL-8 levels.[34] Conversely, although TNF-a expression by alveolar macrophages is also decreased by corticosteroid administration, TNF-a appears to be essential in the host defense against *P. carinii*.[35]

Macrophages play an important role in clearing *P. carinii* from the lungs and preventing the development of PCP. *Pneumocystis carinii* is phagocytosed by the alveolar macrophages either after it is opsonized by specific antibodies (Fc-receptor mediated) or after binding by the mannose receptor (MR) to the MSG of *P. carinii*. Recent studies have demonstrated[36] [37] that alveolar macrophage MR activity is downregulated in HIV patients and that functional MR is shed from the macrophage cell surface. This soluble MR was detected in cell-free alveolar fluid from patients who had PCP, but not in alveolar fluid from healthy controls. When purified *P. carinii* organisms were incubated with soluble MR-containing supernatants, they were phagocytosed less readily by alveolar macrophages than were control organisms.[38] This suggests that *P. carinii* enhances the shedding of MR from the macrophage cell surface and binds to the 'free' MR, resulting in inhibition of phagocytosis.

In nude mice, SCID mice and medically immunosuppressed rodents, the common immunologic deficit predisposing these animals to PCP is severe depletion in T-cell function. Adoptive transfer of spleen T cells from phenotypically normal mice into *P. carinii* infected nude mice reduced lung cysts and resolved PCP.[39] Other adoptive transfer experiments established that helper L3T4 lymphocytes (equivalent to human $CD4^+$ lymphocytes) were necessary to reconstitute delayed-type hypersensitivity for *P. carinii* but suppressor lymphocytes were necessary for complete expression of resistance to the organism. Administration of monoclonal antibody to $CD4^+$ lymphocytes to conventional BALB/c mice and reconstituted SCID mice inoculated with *P. carinii* resulted in PCP and inability to eradicate the infection, respectively. These experiments all provide inferential evidence that depletion of $CD4^+$ lymphocytes is necessary for the development of PCP.

In humans who do not have HIV infection, PCP frequently occurs in association with lymphoreticular malignancies, certain congenital disorders and chemotherapy with ciclosporin or corticosteroids that have in common variable defects in T-cell function. By contrast, adults who have pure agammaglobulinemia rarely develop PCP. Most AIDS patients have $CD4^+$ lymphocyte counts in the range of 50–75/mm^3 at the time of their first episode of PCP, and more than 90% of episodes occur when counts are below 200/mm^3 . If patients who have less than 200 $CD4^+$ lymphocytes/mm^3 are not given prophylaxis, the risk of developing PCP is 8.4% at 6 months, 18.4% at 12 months and 33.3% at 36 months. Thus, diminished T cell function and/or numbers is most likely the critical immune deficit necessary for this opportunistic infection to occur.

Recent studies suggest that antibodies may also be important components of host defense to *P. carinii*.[40] In adoptive transfer of T cells and spleen cells from immunocompetent donors into SCID mice, B cells were necessary, in addition to T cells, for recipient mice to resolve pre-existing PCP. Both in mice and humans, intense reactivity of monoclonal antibodies from tracheobronchial lymph node B-cells[41] and a serum antibody response were found[5] following recovery from PCP. In HIV-infected patients both local antibodies (IgA) and the serum antibody levels were lower and the ability to mount an antibody response to the *P. carinii* infection was impaired, suggesting that the poor antibody response may contribute to the tendency of HIV-infected patients to have PCP.[42] [43] Thus, although T-cell-mediated immunity may be the central component of the host response to *P. carinii*, humoral immunity may also have an important role in certain aspects of protection against infection.

CLINICAL MANIFESTATIONS

The clinical manifestations of PCP in patients who do not have AIDS are similar to those in AIDS patients (covered in detail in Chapter 124). Although there can be great variability, non-AIDS patients typically present more acutely, often within days of their initial symptoms.[44] Up to 20% of patients who have corticosteroid-associated PCP present during stable dosing with corticosteroids, but severe symptoms typically appear abruptly upon rapid tapering of corticosteroid therapy. These events probably represent activation of immune mechanisms that had been inhibited by the high doses of corticosteroids.

The cardinal symptoms are fever, cough and shortness of breath. [44] [45] Early in the course of the disease, dyspnea may occur only on exertion and careful questioning is required to elicit a recent decrease in exercise tolerance. Cough is typically nonproductive, but small amounts of clear sputum are common, whereas purulent sputum and hemoptysis are rare in the absence of concurrent infection. Chest tightness and a dull retrosternal ache that is worse with inspiration may also occur.

Physical examination may be completely normal, particularly in individuals who have mild disease. Tachypnea, tachycardia and respiratory distress tend to signify advanced disease and a poorer prognosis.

Chest radiographs typically demonstrate fine, bilateral interstitial infiltrates extending from the hilum, but this appearance may be seen with many other pneumonias.

Normal or minimally abnormal radiographs may occur early in disease or in lung transplant recipients.[46] Atypical radiographic abnormalities have been frequently reported in

AIDS patients but also occur in non-AIDS patients and include localized or unilateral infiltrates, cavitary, cystic or honeycomb lesions, hilar enlargement with lymphadenopathy, spontaneous pneumothorax, predominant upper lobe disease, solitary nodules, small pleural effusions and linear opacities.[47] Dissemination to brain, kidney, adrenal and thyroid glands, liver, intestines, spleen and eye have been reported and require biopsy for definitive diagnosis. A high index of suspicion and appropriate stains of any involved organ will yield the diagnosis.

No single routine laboratory test either excludes or is pathognomonic for PCP. Elevated serum lactic dehydrogenase (LDH) and eosinophilia[48] are frequently found, particularly during severe episodes, but these tests are non-specific and there are multiple other causes for eosinophilia or raised LDH levels in immunocompromised individuals. Impaired oxygenation is frequently present and, when arterial blood gases are normal, diffusing capacity for carbon monoxide, arterial oxygen saturation during exercise and gallium scintigraphy are potentially helpful tests but are non-specific. The diagnosis ultimately depends on demonstration of the organism in respiratory secretions or lung tissue (see Diagnostic microbiology, below). Patients who have very low $CD4^+$ counts (<100 cells/mm^3) and/or BAL neutrophilia have a poorer prognosis. The overall mortality of PCP in both HIV and non-HIV patients is approximately 40%.[49]

DIAGNOSTIC MICROBIOLOGY

Bronchoscopy with BAL is the diagnostic procedure for obtaining pulmonary secretions with which all other tests are compared. Bronchoalveolar lavage involves wedging the bronchoscope into a peripheral nondependent airway (usually the right middle lobe), instilling 100–250ml non-bacteriostatic saline as 20–30ml aliquots and aspirating fluid after each aliquot is instilled. When approximately 50ml of fluid has been recovered, the specimen is centrifuged and the pellet is stained for *P. carinii*. In 86–97% of patients, *P. carinii* organisms are detected by this procedure.

Several approaches may improve yield. Bilateral BAL resulted in a diagnosis in 31 (94%) out of 33 patients compared with 51 (84%) out of 61 historical control patients undergoing BAL only on one side.[50] Similarly, the combination of multiple-lobe, site-directed BAL plus monoclonal antibody staining increased diagnostic yield from 80% to 98%.[51] In patients who had focal infiltrates, the combination of site-directed BAL and transbronchial biopsy led to a diagnosis in 90% of patients who had a prior nondiagnostic BAL.[52]

In contrast to BAL, induced sputum specimens are easily obtained by ultrasonic nebulization of hypertonic saline, although care must be taken because many patients experience arterial oxygen desaturation during the procedure. When the sediment is appropriately stained and slides are reviewed by experts, the procedure has a sensitivity of 15–94% with corresponding negative predictive values that have also varied widely (39–96%). The procedure is laborious for respiratory therapists, but positive results usually obviate the need for bronchoscopy. Fluorescent staining with monoclonal antibodies for *P. carinii* (Fig. 241.3) may increase the yield to over 90%.[53] With the use of PCR techniques, the sensitivity of induced sputum examination may approach that of BAL (see below).

As for BAL, the yield of induced sputum may be lower for patients receiving aerosol pentamidine because the numbers of organisms may be reduced. In one study of 54 patients, the diagnosis was confirmed by induced sputum in 64% of those receiving aerosol pentamidine compared with 92% of those not receiving the aerosol (p <0.02).[54] By contrast, the respective yields were 63% compared with 64% in another trial. [55]

When transbronchial biopsies are obtained without crush artefact and contain at least 25 alveoli, the diagnostic yield is similar to that

Figure 241-3 Monoclonal immunofluorescent stain. Monoclonal immunofluorescent stain of a BAL specimen (Merifluor stain) demonstrating *P. carinii* cysts that stain with a characteristic apple-green fluorescence. *With permission from Meridian Diagnostics.*

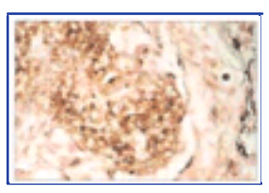

Figure 241-4 Gomori's methenamine silver stain of lung tissue. Gomori-stained section of lung tissue showing characteristic *P. carinii* cysts.

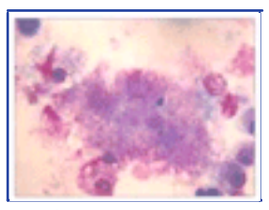

Figure 241-5 Wright-Giemsa stain of sputum. Wright-Giemsa stained specimen of an induced sputum specimen showing clusters of *P. carinii* organisms. *Courtesy of Maria Appleman.*

of BAL. If both BAL and transbronchial biopsies are obtained, the diagnostic sensitivity is additive and approaches 100%. However, pneumothoraces or bleeding may occur in up to 10% of patients undergoing bronchoscopic biopsy. Thus, many pulmonologists prefer to perform only BAL with the initial bronchoscopy. If the first procedure is nondiagnostic, BAL is repeated and transbronchial biopsies are obtained if coagulation indices permit. However, one study using monoclonal antibody staining suggests that biopsy does not increase the yield of BAL and may only increase the chance of complications. [50]

Open lung biopsy can also lead to the diagnosis of PCP but is rarely needed because of the high yield with sputum induction and BAL. It is usually reserved for patients in whom bronchoscopy has been non-diagnostic because of technical problems with the procedure or where transbronchial biopsies are contraindicated because of bleeding disorders or concurrent management with mechanical ventilation.

Traditionally Gomori's methenamine silver stain is used to identify *P. carinii* cysts in patient samples (Fig. 241.4). Its diagnostic sensitivity and specificity both generally exceed 95%. However, up to 10% false negatives may occur. The procedure requires 4–6 hours but modifications have reduced processing time to 1–2 hours. The cyst wall can also be stained by toluidine blue, with the advantage that it is more rapid than standard silver stains.

Wright-Giemsa (Fig. 241.5) and Diff-Quik stains have been used to stain trophozoites, nuclei of cysts and intermediate forms and can be

completed within 30 minutes. However, organisms will be missed in 10–15% of cases. The methodology also allows polymorphonuclear neutrophils to be identified in BAL fluid, a finding associated with more severe impairment in pulmonary physiology and lower survival.

Direct and indirect immunofluorescent staining using monoclonal antibodies (Fig. 241.3) are highly sensitive, with yields in excess of 90% for BAL specimens,[51] [53] and are more sensitive for sputum samples than silver or Wright-Giemsa stains. Although false positives occasionally occur, this has become the diagnostic procedure of choice in many clinical laboratories.

The development of the PCR has increased the sensitivity of detection of *P. carinii* in patient samples substantially. Consequently, the PCR can also yield a positive result in asymptomatic individuals who are colonized by *P. carinii*. In several studies analysis of respiratory samples by PCR (single and nested) revealed higher

numbers of false-positive results and lower positive-predictive values than microscopy for non-HIV immunocompromised and immunocompetent patients. Even with HIV patients, in whom the differences between the diagnostic results of microscopy and single and nested PCRs were quite marginal, microscopy and single PCR performed better than nested PCR with regard to specificity and positive predictive value.[56] [57] [58] [59] Although the PCR technique has no additional value in analysis of BAL fluid, it can improve diagnostic sensitivity of induced sputum and oral washes up to the same level as that of BAL specimens.[60] [61] [62] At present, it appears reasonable to reserve PCR for epidemiologic research and diagnosis in induced sputum and oral wash specimens that prove negative by immunofluorescence.

PREVENTION

The indications for primary and secondary chemoprophylaxis of PCP in HIV-infected patients are well known and are covered in Chapter 123 and Chapter 124 . In HIV-negative immunocompromised patients no general guidelines for PCP prophylaxis exist and the decision to administer prophylaxis depends on the degree and duration of the immunosuppression and on local institutional trends in PCP incidence. Patients at risk for PCP for whom chemoprophylaxis should be considered include:

- those who have primary immunodeficiency;
- those who have severe malnutrition;
- after organ transplantation;
- those who have persistent $CD4^+$ counts of below $200/mm^3$; and
- in cytotoxic or immunosuppressive treatment of cancer, connective tissue disorders and other diseases.[63] [64]

The incidence of PCP is highest during the first year after solid organ transplantation, except for patients who have lung transplants, who continue to have higher risks more than a year after transplantation.[65] The risk of PCP in transplant patients is also increased in cases of invasive cytomegalovirus disease, during intensive immunosuppression for allograft rejection (corticosteroids) and during periods of neutropenia.[66] [67] Prophylaxis should also be considered for more than 1 year after bone marrow transplantation for patients receiving corticosteroids as well as in those who have hematologic relapse or extensive chronic graft-versus-host disease.[68] Corticosteroids are recognized as an independent risk factor if given for longer than 4 weeks at a level equivalent to 20mg of prednisone (prednisolone) daily.[69] In non-HIV patients, determination of $CD4^+$ lymphocyte counts can help to identify patients who are at risk for PCP; 91% of patients have fewer than 300 $CD4^+$ lymphocytes/mm^3 at the time of diagnosis of PCP.[70] [71]

First choice of prophylaxis consists of one double-strength (960mg) tablet of trimethoprim-sulphamethoxazole (TMP-SMX) per day.[72] [73] One single-strength tablet (480mg) daily or one double-strength tablet three times per week is also effective. For pediatric patients who have acute lymphoblastic leukemia, TMP-SMX (150mg/m^2 TMP plus 750mg/m^2 SMX, to a maximum of 160mg TMP and 800mg SMX) given q12h for three consecutive days per week has similar efficacy to daily administration.[74] Regimens of TMP-SMX would also be expected to provide some protection against toxoplasmosis, *Nocardia asteroides, Listeria monocytogenes*, bacterial pneumonia and urinary tract infections. Some patients have to discontinue TMP-SMX prophylaxis because of adverse reactions (mostly gastrointestinal symptoms and skin rashes). For these individuals dapsone (100mg/day) alone, or dapsone (50mg/day) in combination with pyrimethamine (50mg/week) and leucovorin (25mg/week) could be an alternative.[75] [76] Aerosolized pentamidine (300mg once per month) may also be effective, but is more expensive.[72] [72] [77] Atovaquone suspension (1500mg or 750mg daily) has also proven effective after bone marrow and liver transplantation respectively.[78] [79] Other drug regimens have been considered but there is insufficient evidence to recommend their use.

Furthermore, judging from the current state of knowledge about the epidemiology and transmission of *P. carinii*, direct contact of PCP patients with other immunocompromised patients should be avoided.

MANAGEMENT

The management of patients who have AIDS-associated PCP is covered in detail in Chapter 124 . In HIV-negative patients, management should consist of TMP-SMX in a dosage of 15–20mg/kg/day TMP and 75–100mg/kg/day SMX in three or four divided doses (orally or intravenously). Initially, therapy should be given intravenously until clinical improvement ensues and the patient is able to tolerate oral intake well. Therapy should be continued for 21 days; shorter courses of therapy (i.e. 14–17 days) should be reserved for patients who have mild disease or who respond rapidly. Adverse events are considerably less common in non-AIDS patients[44] and include rash, fever, nausea and vomiting, leukopenia and hepatitis.

Sulfamethoxazole inhibits dihydropteroate synthase (DHPS) in the folic acid synthesis pathway. Although mutations in the DHPS genes are found more in patients who are taking sulfa or sulfone prophylaxis,[80] [81] [82] they are not invariably associated with failure of treatment or prophylaxis. Likewise, the majority of patients who have mutations respond well to sulfa or sulfone therapy. [82] [83]

Intravenous pentamidine is the primary alternative agent for non-AIDS patients who are intolerant of TMP-SMX. The standard dosage is 4mg/kg q24h. Because of problems with pain and sterile abscesses, the intramuscular route should not be used. Adverse events are common and occasionally life-threatening. They include hypotension, nausea and vomiting, ventricular dysrhythmias, renal failure, pancreatitis, hypoglycemia, hyperglycemia/diabetes mellitus (which can be permanent and require chronic insulin therapy), leukopenia, thrombocytopenia and hypocalcemia.

Trimethoprim-dapsone, clindamycin-primaquine, atovaquone and trimetrexate are proven alternatives for the treatment of PCP in AIDS patients but have not been studied in non-AIDS patients. Similarly, the role of adjunctive corticosteroids for non-AIDS PCP has not undergone controlled prospective investigation. As the pathogenic mechanisms are likely to be similar for HIV and non-HIV patients, it would be reasonable to consider use of prednisone (40mg q12h for 5 days, 40mg q24h for 5 days, then 20mg q24h for the remainder of the duration of PCP therapy) for any severely ill patient (PO_2 <70mmHg or (P(A-a)DO_2 >35mmHg).[84] For indivi-duals who develop symptomatic PCP upon tapering of ongoing corticosteroid therapy, a resumption of the previous dose of corticosteroids should be considered during their initial antimicrobial therapy, with subsequent tapering after clinical improvement occurs.

Development of future treatments may include artificial surfactant, deferoxamine or echinocandins.

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Chapter 242 - Protozoa: Intestinal and Urogenital Amebae, Flagellates and Ciliates

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This chapter discusses the amebae, flagellates and ciliates that parasitize the intestinal and urogenital systems of humans (Table 242.1). With the exception of *Trichomonas vaginalis*, all of the organisms live in the intestinal tract. Intestinal protozoa vary in pathogenicity and prevalence but, with rare exceptions, all have a worldwide distribution.

NATURE

Amebae, flagellates and ciliates are single-celled eukaryotic organisms belonging to the subkingdom or phylum Protozoa. In general, the organisms related to human infection change their form and function from the active, feeding trophozoites to the resting cyst form. All protozoa contain a nucleus, often with a karyosome near its center. The cytoplasm is composed of the endoplasm, which immediately surrounds the nucleus, and the ectoplasm, which functions as the locomotion apparatus. Reproduction is relatively simple and is accomplished through repeated asexual multiplication by binary fission. Currently, it is not completely clear whether *Blastocystis hominis* should be classified with the protozoa or is actually a fungus-like organism within the stramenopile group; however, it will be discussed in this chapter. [1]

EPIDEMIOLOGY

Intestinal amebae, flagellates and ciliates are transmitted through fecally contaminated food, water or other materials. Prevalence is correlated with socio-economic conditions and higher infection rates occur in people who have poor personal hygiene or who live in areas with poor sanitation. Contaminated water supplies are a particular problem because the usual levels of chlorination may not kill cysts. Filtration is required. Endemic and epidemic disease has been traced to water supplies that use surface water which either is not filtered or has improperly functioning filters.

These organisms generally have a cystic stage that develops when conditions in the environment are unfavorable for continued multiplication. The cyst wall is thicker than the trophozoite membrane and thus provides protection. Once these cysts have been transferred to a new host, usually by fecal-oral contamination from person to person, the organisms excyst. Excystation factors include osmotic changes in the environment, enzymatic action of the enclosed organism on the inner surface of the cyst wall and, among the parasitic protozoa, favorable pH and enzymatic action of the host tissues. Transmission of those protozoa with no identified cyst stage has not been totally explained.

Entamoeba histolytica

Infections with *E. histolytica* are seen worldwide and are more prevalent in the tropics. In 1997, over 500 million people were estimated to be infected with *E. histolytica*, 50 million had extensive symptoms, including colitis or extraintestinal abscesses, and there were 100,000 deaths.[2] For every case of invasive disease diagnosed, there are at least 10–20 asymptomatic individuals excreting infective cysts. Population groups with a higher incidence of amebiasis include people from the developing world or recent immigrants from there to the developed nations.

Social changes seen in the late 1960s — open expression of homosexuality, increased sexual contacts, increased frequency of sexual activities and anonymity of sexual partners — contributed to dramatic increases in sexually transmitted organisms, including *E. histolytica*. A number of clinical syndromes have been recognized and referred to as 'the gay bowel syndrome'. Published reports confirm that *E. histolytica* is one of the major pathogens in the gay bowel syndrome. However, some feel that this term 'gay bowel syndrome' is neither gay specific, confined to the bowel, nor a syndrome.[3] Clinical presentations within the homosexual community often differ from those seen in the heterosexual population and almost 50% of all homosexual men found to be infected with *E. histolytica* are asymptomatic. Studies have confirmed the lack of correlation between symptoms and the presence of *E. histolytica* in this risk group.[4] However, it is an accepted fact that the ameba morphologically identified as *E. histolytica* is actually two separate and distinct species. *E. histolytica* is the pathogenic species and is considered the etiologic agent of amebic colitis and extraintestinal amebiasis, while *E. dispar* is the nonpathogenic species and does not invade tissue or cause intestinal symptoms. This may explain the lack of correlation between symptoms and the presence of the morphological identification of *E. histolytica* in the group of homosexual men studied. Therefore, in epidemiologic studies prior to the development of specific reagents for the identification of true *E. histolytica*, those organisms identified as *E. histolytica* were actually *E. histolytica/E. dispar*.

Epidemiologic evidence of sexual transmission of *E. histolytica* has grown significantly since the early 1970s, particularly in New York City and San Francisco. In San Francisco, the incidence of reported symptomatic intestinal amebiasis increased by over 1000% from 1978 to 1988 among homosexual men between 20 and 39 years of age.[4] It appears that approximately 30% of urban homosexual men may be infected with *E. histolytica* (*E. histolytica/E. dispar*), a sharp increase over the estimated rate of less than 5% seen in the general population within the USA. Direct oral-anal contact (anilingus) leads to fecal exposure and oral contact with a variety of intestinal pathogens. Although anilingus has been listed as a key risk factor in potential exposure, transmission can also occur during oral-genital sex after anal intercourse has occurred. Active heterosexuals can acquire infection with *E. histolytica* through sexual activities that provide an opportunity for fecal-oral contamination. The key factor is not necessarily homosexuality, but the frequency of sexual activity and potential for fecal-oral contact. In a more recent study from Japan, symptomatic amebiasis in the east-south east area of Tokyo is a disease that predominantly afflicts males and the high rates of patients who engaged in male homosexual or bisexual practices suggest that amebiasis is likely to be a sexually transmitted disease in homosexual or bisexual men. These infections were confirmed to be true *E. histolytica*, primarily zymodeme II.[5]

TABLE 242-1 -- Amebae, flagellates and ciliate that parasitize the intestinal and urogenital systems of humans.

AMEBAE, FLAGELLATES AND CILIATE THAT PARASITIZE THE INTESTINAL AND UROGENITAL SYSTEMS OF HUMANS		
Type	**Species**	**Pathogenicity***
Amebae	*Entamoeba histolytica*	+
	Entamoeba dispar	-
	Entamoeba hartmanni	-
	Entamoeba coli	-
	Entamoeba polecki	-
	Entamoeba gingivalis†	-
	Endolimax nana	-
	Iodamoeba bütschlii	-
	Blastocystis hominis	±
Flagellates	*Dientamoeba fragilis*	+
	Giardia lamblia	+
	Trichomonas vaginalis‡	+
	Pentatrichomonas (Trichomonas) hominis	-
	Trichomonas tenax†	-
	Chilomastix mesnili	-
	Enteromonas hominis	-
	Retortamonas intestinalis	-
Ciliate	*Balantidium coli*	+

* Pathogenic = +; nonpathogenic = -; ± = pathogenicity controversial
† Body site: mouth
‡ Body site: urogenital system

With the advent of AIDS and the subsequent modifications in sexual practices within homosexual communities, the incidence of *E. histolytica* infection has decreased. In recent years, the increased incidence of disease caused by coccidian parasites, *Isospora belli* and *Cryptosporidium parvum* and the microsporidia, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* has become much more of a problem in patients who have AIDS.

In certain urban areas (Mexico City, Mexico; Medellin, Colombia; and Durban, South Africa), the incidence of invasive disease is considerably higher than in the rest of the world. Contributing factors may include poor nutrition, tropical climate, decreased immunologic competence of the host, stress, altered bacterial flora in the colon, traumatic injuries to the colonic mucosa, alcoholism and genetic factors.

The human is the reservoir host for *E. histolytica/E. dispar* and can transmit the infection to other humans, primates, dogs, cats and possibly pigs. The cyst stages are very resistant to environmental conditions and remain viable in the soil for days. The asymptomatic cyst passer who is a food handler is thought to play the most important role in transmission.

It has been postulated that a colonization-blocking vaccine could eliminate *E. histolytica* as a cause of human disease, particularly as humans serve as the only significant reservoir host, and a number of potential protective antigens are being investigated.[6] [7] [8]

Blastocystis hominis

Blastocystis hominis is probably transmitted via the fecal-oral route through contaminated food or water; the cysts survive in water for up to 19 days at normal temperatures.[9] [10] Although other modes of transmission are not defined, the incidence and apparent worldwide distribution suggest the traditional route of infection. Prevention would involve improved personal hygiene and sanitary conditions.

Giardia lamblia (intestinalis)

Although some authorities have changed or are considering a species name change to *G. intestinalis*, we have decided to maintain *G. lamblia* for this chapter. Transmission is by ingestion of viable cysts, and contaminated food and drink are the usual sources. This infection is found most frequently in children or in groups that live in close quarters.[11] [12] Also there may be outbreaks caused by poor sanitation facilities or breakdowns; travelers and campers often experience such outbreaks.[13] [14] There is also an increase in the prevalence of giardiasis in the male homosexual population, probably as a result of anal and/or oral sexual practices.[15]

Decreased gastric acid production may predispose people to infection with *G. lamblia*. Normal gastric acidity may act as a barrier to infection; patients who have had a gastrectomy are prone to infection with *G. lamblia*. Reduction in gastric acid also occurs as a result of malnutrition and both factors may, as a group, increase susceptibility to infection with this organism. Impairment of the host's immune system may also play a role.

Lower incidence of giardiasis in infants up to 6 months of age may be associated with breast-feeding and some protection against infection via secretary IgA; however, lower incidence may also be related to a decreased exposure to *G. lamblia* in breast-fed infants.

Giardiasis is one of the common causes of travelers' diarrhea and is worldwide in distribution. Visitors in areas endemic for *Giardia* spp. are more likely to become symptomatic than the inhabitants of that area; this is probably because the latter have developed immunity from previous, and possibly continued, exposure to the organism. A number of outbreaks in the USA have been attributed to resort or municipal water supplies, such as Oregon, Colorado, Utah, Washington, New Hampshire and New York.[16] [17] [18] High infection rates were also reported from hikers and campers who drank stream water. Because some of these areas were remote from human habitation, infected wild animals, especially the beaver, were suspected of being the source.[13] [17] [19] [20] [21] Surveys show human infection rates of 2–15% in various parts of the world.

Because of potential wild animal reservoirs and possible domestic animal reservoir hosts, measures in addition to personal hygiene and improved sanitation have to be considered.[22] [23] Iodine is recommended as an effective disinfectant for drinking water, but it must be used according to directions.[24] [25] Filtration systems have also been recommended, although they have certain drawbacks, such as clogging.

Isoenzyme studies, used for parasite identification and classification, have provided information related to organism pathogenicity, possible implication in water-borne outbreaks and the potential cause of human disease. The examination of isoenzyme patterns of *G. duodenalis* (*G. lamblia*) obtained from humans and animals showed no obvious correlation between clinical symptoms and isoenzyme patterns. These studies also demonstrated significant differences between isolates from within a single region and those from other distant geographic locations.[26] [27]

Dientamoeba fragilis

Dientamoeba fragilis infection is commonly associated with enterobiasis and it has been suggested that *D. fragilis* may infect *Enterobius* spp. eggs and thus bypass gastric acidity. Although clinical infections with *D. fragilis* occur, they are not often reported. This is probably because the infection is self-limiting, stool examination is not requested and laboratory identification is difficult. The incubation period for clinical disease is not clearly defined.

Trichomonas vaginalis

Infection with *T. vaginalis* is acquired primarily through sexual intercourse; asymptomatic men therefore need to be diagnosed and treated. The organism can survive for some time in a moist environment such as damp towels and underclothes; however, this mode of transmission is thought to be very rare.

Balantidium coli

Domestic pigs are probably the most important reservoir host for human infection with *B. coli*. In areas in which pigs are the main domestic animal, the incidence of human infection can be quite high (e.g. New Guinea). Human infection is fairly rare in temperate areas, although once the infection is established, it can develop into an epidemic, particularly when environmental sanitation and personal hygiene are poor. This situation has been seen in mental hospitals in the USA. Preventive measures involve increased attention to personal hygiene and sanitation, as the mode of transmission is ingestion of infective cysts through contaminated food or water.

PREVENTION

Transmission of the majority of the intestinal protozoa occurs through ingestion of infective cysts, which can be acquired from food, water and person to person by the fecal-oral route. These infections tend to be found more frequently in groups that live in close quarters or in certain population groups. There may be outbreaks due to poor sanitation facilities or breakdowns as evidenced by infections in travelers and campers; certainly this has been found for giardiasis. Although amebiasis is usually associated with poor sanitation and underdeveloped areas of the world, sexual transmission has also been documented, mainly among urban homosexual men. Prevention in this group is directly related to limiting sexual practices that provide an opportunity for fecal-oral contamination. The single most effective practice that prevents the spread of infections with intestinal protozoa, particularly in the childcare setting, is thorough handwashing by the children, staff and visitors. In the case of *D. fragilis*, it has been suggested that the trophozoites may infect *Enterobius* spp. eggs, thus allowing protection from gastric acidity; however, under most circumstances, total prevention of enterobiasis and/or infection with *D. fragilis* is neither realistic nor possible. *T. vaginalis* is acquired primarily though sexual intercourse, hence the need to diagnose and treat asymptomatic males. The organism can also survive for some time in a moist environment such as damp towels and underclothes; however, this manner of transmission is considered rare.

Although incomplete epidemiologic investigation and reporting make it difficult to determine the significance of the water-borne transmission of giardiasis accurately, the water-borne route seems to be more important for this protozoan than for other more commonly recognized water-borne pathogens, with the possible exception of *Cryptosporidium parvum*. Iodine has been recommended as an effective disinfectant for drinking water, but it must be used according to directions. Because of the potential for wild animal and possibly domestic animal reservoir hosts related to *Giardia* spp., measures in addition to personal hygiene and improved sanitary measures have to be considered and implemented. If appropriate procedures are followed, conventional water filtration should trap most protozoan parasites, including *Giardia*. One should avoid swallowing water when in lakes, rivers, pools or hot tubs; also do not drink directly from lakes, rivers, streams or springs. Filtration devices

for hikers and campers are also available; however, one should look for 'reverse osmosis', 'absolute 1 micron', 'Standard 53', and the words 'cyst reduction' or 'cyst removal'. Boiling water, at a rolling boil, for 1 minute is sufficient to kill organisms, including *Giardia* spp. and *Cryptosporidium* spp. It is also important to thoroughly wash all fruits and vegetables if eating uncooked, use safe water for washing food, peel fruit and avoid unpasteurized milk or dairy products.

PATHOGENICITY

Some of the intestinal protozoa are nonpathogenic and produce no disease; however, microscopists must be able to distinguish pathogenic from nonpathogenic species. The presence of nonpathogenic species indicates that the person has been exposed to fecal contamination. Several species can cause mild to severe gastrointestinal symptoms, and *E. histolytica* may produce extraintestinal lesions. However, pathogenic or potentially pathogenic protozoa do not always produce symptoms or they may remain after symptoms have resolved. Asymptomatic individuals may serve as reservoirs for the infection. Detection of a potentially pathogenic protozoan does not necessarily prove that the organism is causing the illness. Patients may have diarrhea caused by other organisms such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* or rotavirus. The pathogenicity of some species (e.g. *B. hominis*) has been questioned and *E. histolytica* is now considered to be two separate organisms, one pathogenic (*E. histolytica*) and one nonpathogenic (*E. dispar*). *Trichomonas vaginalis*, a urogenital flagellate, is also considered pathogenic and may cause mild to severe vaginitis and other urogenital problems.

Entamoeba histolytica

Although many people worldwide are infected with *E. histolytica*, only a small percentage develops clinical symptoms. Morbidity and mortality caused by *E. histolytica* vary, depending on geographic area, organism species (*E. histolytica* vs *E. dispar*) and the immune status of the patient.

In 1961, with the development of successful axenic culture methods requiring no bacterial co-culture, sufficient numbers of organisms could be obtained for additional studies. Approximately 15 years later, reports indicated that *E. histolytica* clinical isolates could be classified into groups using starch gel electrophoresis and review of banding patterns related to specific isoenzymes. [28] The four isoenzymes are glucophosphate isomerase (GPI), phosphoglucomutases (PGM), malate dehydrogenase (ME) and hexokinase (HK), indicating that there are pathogenic and nonpathogenic strains (zymodemes) of *E. histolytica*. On the basis of analysis of thousands of clinical isolates, the zymodeme patterns were thought to be genetic rather than phenotypic.

During the 1980s and 1990s several publications reviewed the issues regarding pathogenic *E. histolytica* versus nonpathogenic *E. dispar*. On the basis of current knowledge, pathogenic *E. histolytica* is considered to be the etiologic agent of amebic colitis and extraintestinal disease, whereas nonpathogenic *E. dispar* produces no intestinal symptoms and is not invasive in humans. [29] [30] [31] Diamond & Clark[32] redescribed the two species as *E. histolytica* (Schaudinn 1903), which is the invasive human pathogen, and *E. dispar* (Brumpt 1925), which is noninvasive and does not cause disease.

Blastocystis hominis

In spite of the published literature, the true role of this organism in terms of colonization or disease and the relevance of organism numbers are still somewhat controversial. In one recent study of patients with irritable bowel syndrome, there was a set of patients in whom the presence of *B. hominis* did not appear to be incidental.[33] The first report of a possible relationship between intestinal obstruction and a concomitant *B. hominis* infection has also been published recently. [34] In patients with other underlying conditions, the symptoms may be more pronounced.[35] There is evidence to indicate there are several ribodeme types and there may be a relationship between ribodeme type and pathogenicity, only some of which will be responsible for increased intestinal permeability and symptoms.[36] [37]

Giardia lamblia (intestinalis)

The majority of individuals infected with *G. lamblia* are asymptomatic. Preliminary studies indicate that there may be two different strains of *G. lamblia*, group A and group B, associated with different degrees of virulence. Group A appears to be more pathogenic and is associated with symptomatic infection. Isoenzyme and molecular studies also support the differences between these two groups.[38] [39] [40]

Dientamoeba fragilis

Although its pathogenic status is still not well defined, *D. fragilis* has been associated with a wide range of symptoms. This uncertainty could be due in part to the existence of pathogenic and nonpathogenic variants. Evidence for two genetically distinct forms has been obtained using PCR-restriction fragment length polymorphism analysis of ribosomal genes.[41] [42]

Trichomonas vaginalis

T. vaginalis is site specific and usually cannot survive outside the urogenital system. After introduction, proliferation begins, with resulting inflammation and large numbers of trophozoites in the tissues and the secretions. Nutrient acquisition and cytoadherence, immune system evasion and regulation of virulence genes are virulence factors associated with pathogenesis. [43] It appears that interference with trichomonads, mucin receptors and proteinases may form a strategy to prevent colonization with this pathogenic flagellate.[44]

DIAGNOSTIC MICROBIOLOGY

Because intestinal symptoms are non-specific, diagnosis requires laboratory identification of the organisms present. Immunodiagnostic methods for antibody detection are useful for the diagnosis of extraintestinal amebiasis, but their utility is limited for intestinal disease.

Organism morphology varies and species characteristics often overlap. For reliable identification, microscopists must be able to differentiate all species regardless of their potential for causing disease. Special attention will be given to the clinically significant intestinal pathogens, especially *E. histolytica*, *B. hominis*, *G. lamblia*, *D. fragilis* and *B. coli*.

Identification of amebae in fecal specimens

Trophozoites and cysts are diagnostic stages of the amebae and either or both stages can be detected in feces. Microscopists must be able to distinguish trophozoites and cysts from epithelial cells, polymorphonuclear leukocytes and macrophages, as well as from pus cells, yeasts, pollen, moulds, and vegetable and crystalline artefacts.

Trophozoite motility in physiologic saline mounts of fresh material and cytoplasmic inclusions, such as erythrocytes in trophozoites and chromatoid bodies in cysts, can be observed. Iodine solutions are used for temporary cyst stains of fresh or fixed specimens. Cyst characteristics are less variable than those of trophozoites and species of cysts can often be identified in iodine-stained wet mounts. However, examination of permanent stained smears using oil immersion (×1000) is recommended for definitive identification of trophozoites and cysts. Size is not a reliable feature for species differentiation of either trophozoites or cysts except when separating *E. histolytica/E. dispar* from *E. hartmanni*.

The microscopist must observe the cytoplasmic and the nuclear characteristics of several organisms before making a final identification. Although cysts are more easily identified than trophozoites, several cysts (particularly if they are immature) should be observed to ensure that the identification is reliable. If two species are identified, there should be distinct populations of each.

Sometimes, although amebic organisms are seen, species cannot be identified. In these instances, the laboratory should report 'unidentified ameba trophozoites (or cysts)'. If the genus can be determined but the species cannot, 'unidentified *Entamoeba* trophozoites or cysts' should be reported and another specimen should be requested.

Entamoeba histolytica

Intestinal infection is usually diagnosed by the microscopic identification of organisms in feces or in sigmoidoscopic material from ulcerations (Fig. 241.1). Nonpathogenic amebae can be confused with pathogens (Fig 242.2 , Fig 242.3 , Fig 242.4 , Fig 242.5). Only trophozoites are found in tissue lesions, but both

trophozoites and cysts may be found in the intestinal

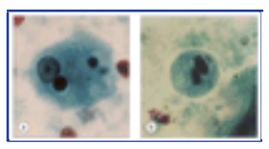
Figure 242-1 *Entamoeba histolytica.* (a) Trophozoite containing ingested red blood cells (the presence of red blood cells confirms the organism is the true pathogen, *E. histolytica*). (b) ***Entamoeba histolytica/E. dispar***, cyst containing four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: from the cyst morphology, it is not possible to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*.

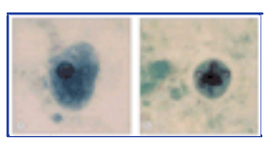
Figure 242-2 *Entamoeba hartmanni.* (a) Trophozoite. (b) Cyst containing up to four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: *E. hartmanni* measures less than *E. histolytica/E. dispar*, the trophozoite is <12µm and the cyst is <10µm.

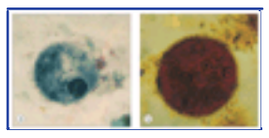
Figure 242-3 *Entamoeba coli.* (a) Trophozoite containing a single nucleus in which the karyosome is eccentric (tends to be centrally located in *E. histolytica/E. dispar*). (b) Cyst contains more than five nuclei (d'Antoni's iodine).

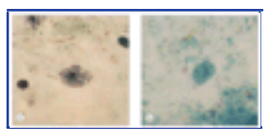
Figure 242-4 *Endolimax nana.* (a) Trophozoite containing a single nucleus with no peripheral chromatin (large karyosome only) and vacuolated cytoplasm. (b) Cyst containing four nuclei (three easily visible) (trichrome stain).

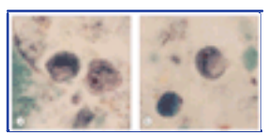
Figure 242-5 *Iodamoeba bütschlii.* (a) Trophozoite containing a single nucleus with a large karyosome and cyst containing a single nucleus and a large glycogen vacuole. (b) Cyst containing a single nucleus and a large glycogen vacuole — note the size of the karyosome (trichome stain). There is also a *Blastocystis hominis* central body form present at the lower left of the image.

lumen. Some patients who have invasive disease may have only trophozoites in fecal specimens and examination of permanent stained smears may be required to establish the diagnosis.

Suspected amebic abscesses are often diagnosed by positive serologic tests. Aspirates of abscesses or intestinal lesions may contain amebic trophozoites, sometimes with ingested erythrocytes seen in direct wet mounts or in permanent stains such as trichrome or iron-hematoxylin stains.[45] [46] Other species of intestinal amebae are not pathogenic but must be differentiated from *E. histolytica*. *Entamoeba polecki* is seen occasionally in refugees from South East Asia and may be confused with *E. histolytica*. [45] *Entamoeba gingivalis* is a common inhabitant of the oral cavity, particularly in patients who have poor oral hygiene. It resembles *E. histolytica* but has no known cyst stage. As a result, trophozoites of *E. gingivalis* may lead to the misdiagnosis of amebic lung abscess by morphologic examination of pulmonary material, especially sputum.

Currently, there are several immunoassays that can be used to identify organisms in the genus *Entamoeba* (*E. histolytica/E. dispar* group) and other reagents that can differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. The antigen detection enzyme-linked immunosorbent assay (ELISA) kits are based on specific amebic adhesin molecules found in the feces of people infected by either *E. histolytica* or *E. dispar*. The second ELISA reagent is able to detect the adhesin produced by *E. histolytica* in

feces. Another immunoassay product is available for the detection of *E. histolytica* and *E. dispar* in fecal specimens; however, this kit does not differentiate between the two organisms, but is specific for the *E. histolytica/E. dispar* group.[47] These procedures are simple, sensitive and specific. It is important to remember that these kits require fresh stool specimens; specimens preserved in any of the routine stool collection fixatives are not acceptable. Polymerase chain reaction methods are also being developed for the differentiation of *E. histolytica* from *E. dispar*.[48]

Serologic testing for intestinal disease is not recommended unless the patient has true dysentery; even in these patients, the titer (e.g. indirect hemagglutination) may be low and difficult to interpret. The definitive diagnosis of intestinal amebiasis should not be made without demonstrating the organisms. When extraintestinal disease is suspected, serologic tests are much more relevant. Indirect hemagglutination and indirect fluorescent antibody tests (FAs) have been reported positive with titers of =1:256 and =1:200, respectively, in almost 100% of cases of amebic liver abscess.[49] Positive serologic results, in addition to clinical findings, make the diagnosis highly probable. In the absence of rapid serologic tests for amebiasis (tests with very rapid turnaround times for results), the decision as to causative agent often must be made on clinical grounds and on results of other diagnostic tests such as scans.

Histologic diagnosis of amebiasis can be made when trophozoites within the tissue are identified and differentiated from host cells, particularly histiocytes and ganglion cells. Periodic acid-Schiff staining is often used; the organisms will appear bright pink with a green-blue background (depending on the counterstain used). Hematoxylin and eosin staining will also allow typical morphology to be seen. As a result of sectioning, some organisms will exhibit the evenly arranged nuclear chromatin with the central karyosome and some will no longer contain the nucleus.

Blastocystis hominis

The characteristic form of *B. hominis* that is usually seen in human fecal specimens varies in size from 6 to 40µm and contains a large central body resembling a vacuole, which may be involved with carbohydrate and lipid storage. The amebic form can occasionally be seen in diarrheal fluid but may be difficult to recognize. Generally, *B. hominis* will be identified on the basis of the typical round form containing the central body.[45] Routine stool examinations are very effective in recovering and identifying *B. hominis* (Fig. 242.6), although the permanent stained smear is the procedure of choice because the examination of wet preparations may not easily reveal the organism. The organisms should be quantitated on the report form, that is, as rare, few, moderate or many. It is also important to remember that other possible pathogens should be adequately ruled out before a patient is treated for *B. hominis*.

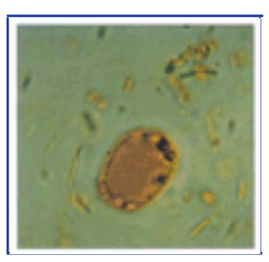
Figure 242-6 *Blastocystis hominis.* (a) Central body form with large 'empty' area (appears like a vacuole) with multiple nuclei around the edges (d'Antoni's iodine). (b) Three central body forms with the large empty area surrounded by nuclei (trichrome stain).

Identification of flagellates in fecal specimens

The flagellates are a more diverse group than the amebae. The type of motility, shape, number of nuclei and other characteristics, such as an undulating membrane, sucking disk, cytostome, spiral groove, and the number and location of flagellae, are important characteristics used to identify flagellate trophozoites. The organism shape and size, number and position of nuclei, and absence or arrangement of fibrils are used to identify flagellate cysts.

In some cases, species can be determined by the examination of either direct or concentrated wet mounts. Species of cysts may be identified in iodine-stained mounts. However, permanent stains are always recommended for every stool specimen submitted; organisms identified in wet preparations may not represent all types of organisms present.

Giardia lamblia (intestinalis)

Diagnosis is usually established by the demonstration of cysts or, occasionally, trophozoites in feces (Fig. 242.7). Nonpathogenic flagellates can be seen in Figure 242.8 . Because of the variable shedding of organisms, several stool specimens should be examined before the infection is ruled out. In some cases, a series of stools can be examined and be negative and yet the patient still has giardiasis. It is important for the laboratory and the clinician to recognize this fact. Permanent stains are recommended for the definitive diagnosis of this infection.

When *G. lamblia* organisms are not found in stool specimens, duodenal aspirates, string test mucus or biopsied mucosal tissue can be examined. The string test[45] is used to collect mucus from the duodenal area and its use may be less traumatic for the patient than other methods. Materials obtained by drainage, aspiration or the string test can be examined by simple, direct wet mounts. Biopsy tissue may be processed and stained by the usual histopathologic methods; however, before preservation, a fresh imprint smear of the mucosal surface on a slide can be made and stained with trichrome or Giemsa stain.

Immunoassay methods (enzyme immunoassay, FA, immunochromatographic assay) are available and may be appropriate in test ordering situations.[49A] [49B] Education of the medical staff will be mandatory to ensure that tests are appropriately ordered and that there is complete understanding of the limits of the information generated (test results limited to absence or presence of *G. lamblia*). Recently, industrial companies and municipalities have shown a great deal of interest in these reagents. This is particularly relevant when the water sources are used for drinking and/or recreational purposes. [45]

Dientamoeba fragilis

Permanent stains are required to diagnose this infection and multiple specimens may be required because shedding varies from day to day.

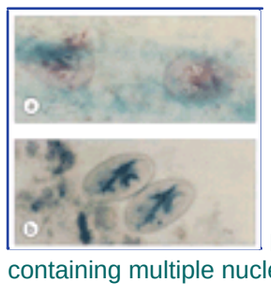

Figure 242-7 *Giardia lamblia.* (a) Trophozoites in mucus — note the sucking disk area, linear axonemes, curved median bodies and two nuclei (trichrome stain). (b) Cysts containing multiple nuclei, linear axonemes and curved median bodies (iron-hematoxylin stain).

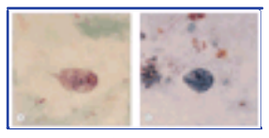

Figure 242-8 *Chilomastix mesnili.* (a) Trophozoite with single nucleus and clear feeding groove/cytostome. (b) Cyst containing single nucleus and curved fibril called the 'shepherd's crook' (trichrome stain).

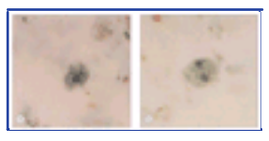

Figure 242-9 *Dientamoeba fragilis.* (a) Trophozoite with single nucleus fragmented into a 'tetrad' configuration. (b) Trophozoite containing two nuclei, each showing fragmented chromatin (trichrome stain). There is no known cyst form for this organism.

The delicately staining trophozoites are usually binucleate, although the nuclei may be in different planes of focus (Fig. 242.9). The nuclei tend to be fragmented and can often be seen in a 'tetrad' formation. They must be differentiated from the trophozoites of *Endolimax nana*, *Iodamoeba bütschlii* and *Entamoeba hartmanni*. Nuclear characteristics, the presence of binucleate forms, tremendous size variation, and the absence of cysts aid in identification of this organism.

A permanent stained smear should be examined for every stool specimen submitted to the laboratory for an ova and parasite examination.[45] [46] If this approach is not used, then many infections with this organism can be missed. If a laboratory periodically finds and identifies *G. lamblia* but never sees *D. fragilis*, then collection and diagnostic methods should be reviewed.

Identification of flagellates in urogenital specimens

Trichomonas vaginalis

Infections with *T. vaginalis* are usually detected by finding the motile trophozoites in wet mounts of vaginal fluid, prostatic fluid or sediments of freshly passed urine. In wet mounts, the trophozoites move with a jerky motion and possess an undulating membrane, which extends only one-half the length of the organism (Fig. 242.10). In old urine specimens, the organisms may be dead or badly distorted and thus cannot be identified or may be confused with host cells.

Specimens include vaginal fluid, scrapings or washings. They may be examined in a saline wet mount or as a stained smear, or the material can be cultured. Although some consider that wet mount examinations are as efficient as cultures in revealing infections, current evidence suggests that cultivation methods are superior.[50] [51] Immunofluorescent and ELISA methods have also been described. Organisms

Figure 242-10 *Trichomonas vaginalis.* Trophozoites showing axostyle, flagella and part of the undulating membrane (smaller organism) (Giemsa stain). There is no known cyst form for this organism.

may be difficult to recognize in permanent stains; however, if a dry smear is submitted to the laboratory, Giemsa or Papanicolaou stain can be used. Chronic *T. vaginalis* infections may cause atypical cellular changes that can be misinterpreted, particularly on the Papanicolaou smear. Organisms are routinely missed on Gram stains. The number of false-positive and false-negative results reported on the basis of stained smears strongly suggests that identification should be confirmed by observation of motile organisms, either from the direct wet mount or from appropriate culture media.

For culture, it is mandatory that the specimen be collected correctly, immediately inoculated into the proper medium and properly

incubated. Excellent methods are available using plastic envelopes containing appropriate media. This envelope approach allows both immediate examination and culture in one self-contained envelope. These systems are commercially available and serve as the specimen transport container, the growth chamber during incubation and the 'slide' during microscopy.[45] [50] [51] [52]

Monoclonal antibodies and DNA probe procedures for the detection of *T. vaginalis* have been reported as being very effective.[53] [54] An enzyme immunoassay has been developed for the detection of the *T. vaginalis* antigen from vaginal swabs.[55] [56] The predictive value of a positive test was 82% and that of a negative test was 99.3%. Commercial products based on these methodologies should be very helpful in diagnosing this infection. Serologic tests have been tried; however, none are commercially available.

Identification of ciliates in fecal specimens

Balantidium coli

In human feces, *B. coli* trophozoites are readily recognized by their large size, their shape and their rapid, rotating motion. Cysts are less easily identified, but they usually cause few diagnostic problems. The morphology of trophozoites and cysts is seen in Figure 242.11 .

Examination of direct saline mounts is the most practical method of detecting these protozoa. Cysts can be recovered by concentration, but in human infections trophozoites are usually more numerous than cysts. Iodine-stained mounts and permanent stains are of little value because the organisms tend to overstain and may resemble helminth eggs and/or debris.

CLINICAL MANIFESTATIONS (see Chapter 46)

In the descriptions of diseases, the clinical manifestations noted refer to findings in patients who have symptomatic disease and do not necessarily refer to findings in every person infected with the parasite species.

Amebae

Eight species of intestinal amebae may live in the cecum and colon of humans: *E. histolytica*, *E. dispar*, *E. hartmanni*, *E. polecki*, *E. coli*, *E. nana*, *I. bütschlii* and *B. hominis*. However, of these, only *E. histolytica* and *B. hominis* are thought to cause symptoms.

Entamoeba histolytica

Infections with the true pathogen, *E. histolytica*, are classified as amebiasis irrespective of whether the person exhibits symptoms. Outbreaks have occurred in the USA, usually from contaminated food or water. *Entamoeba* spp. isolated from patients who have clinical

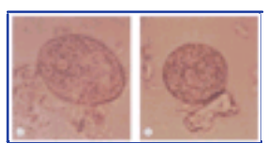

Figure 242-11 *Balantidium coli.* (a) Trophozoite — note the cilia around the edges ('fuzzy football'). (b) Cyst — note the cilia are difficult to see within the cyst wall (d'Antoni's iodine — light preparation, pale color).

disease have been shown by isoenzyme analysis to differ from nonpathogenic organisms and *E. histolytica* will be used to designate pathogens, whereas *E. dispar* will designate those that are nonpathogenic. The incubation is variable, from days to weeks or even months.

Asymptomatic infection

Individuals harboring *E. histolytica/E. dispar* may have either a negative or weak antibody titer and negative stools for occult blood and may be passing cysts that can be detected if a routine ova and parasite examination is performed. Although trophozoites may also be found, they will not contain any phagocytized red blood cells. Isoenzyme analyses of organisms isolated from asymptomatic individuals generally indicate that the isolates belong to nonpathogenic zymodemes (*E. dispar*).[28] Generally, asymptomatic patients never become symptomatic and may excrete cysts for a short period. This same pattern is found for patients who have either nonpathogenic or pathogenic zymodemes.

Intestinal disease

Although the exact mode of mucosal penetration is not known, studies suggest that amebae have enzymes that lyse host tissue, possibly from lysosomes on the surface of the amebae or from ruptured organisms. Cysteine proteinases and activation of host cell protein tyrosine phosphatases (PTPases) and dephosphorylation also play a critical role in the pathogenesis of invasive amebiasis caused by *E. histolytica*.[57] [58] [59] Amebic ulcers usually develop in the cecum, appendix or adjacent portion of the ascending colon and can also be found in the sigmoidorectal area. From these primary sites, other lesions may occur. Ulcers are usually raised, with a small opening on the mucosal surface and a larger area of destruction below the surface, thus described as 'flask shaped'. The mucosal lining may appear normal between ulcers. The incubation period may vary from 1 to 4 months and in an endemic area it is impossible to determine exactly when the exposure took place. Symptoms may range from none to those mimicking ulcerative colitis. Patients who have colicky abdominal pain, frequent bowel movements and tenesmus may present with a gradual onset of disease. With the onset of dysentery, bowel movements characterized by blood-tinged mucus are frequent (up to 10/day). Although dysentery may last for months, it usually varies from severe to mild over that time and may lead to weight loss and prostration. In severe cases, symptoms may begin very suddenly and include profuse diarrhea (over 10 stools/day), fever, dehydration and electrolyte imbalances. Acute illness may mimic appendicitis, cholecystitis, intestinal obstruction or diverticulitis. In some people, an increased frequency of bowel movements with or

without blood and mucus may occur. A chronic form, amebic colitis, produces symptoms similar to those of ulcerative colitis or other forms of inflammatory bowel disease, with diarrhea, sometimes bloody, occurring over months to years, sometimes alternating with periods of constipation or normal bowel function. Some patients who have amebiasis have been misdiagnosed as having ulcerative colitis. Another, less common form of intestinal disease, ameboma, is produced by the growth of granulomatous tissue in response to the infecting amebae, resulting in a large local lesion of the bowel that radiologically resembles a carcinoma.

Other than trophozoites containing ingested red blood cells, we have no way of differentiating pathogenic *E. histolytica* from nonpathogenic *E. dispar* based on morphologic differences, so our approach to reporting and therapy may not change in the near future. Any *E. histolytica* organisms should be reported to the physician and, providing the trophozoites do not contain ingested red blood cells, should be reported as *E. histolytica/E. dispar*. There are molecular-based diagnostic reagents that can confirm the group *E. histolytica/E. dispar* and other reagents that can indicate the species identification (*E. histolytica* or *E. dispar*). Although these reagents are relatively expensive, they may become more widely used in the next few years.[60]

Hepatic disease

Infections with *E. histolytica*, with or without a history of gastrointestinal symptoms, may result in hematogenous spread of the organisms from the submucosa to the liver via the portal system, resulting in amebic abscesses of the liver. This occurs in up to 5% of patients who have symptomatic intestinal amebiasis. Approximately 40% of patients who have amebic liver abscess do not have a history of prior bowel symptoms and in some patients *E. histolytica* may not be present in stool at the time liver disease becomes manifest. Onset of symptoms may be gradual or sudden; upper right abdominal pain with fever from 100° to 102°F (38–39°C) is the most

consistent finding. Weakness, weight loss, cough and sweating are less common. There is hepatomegaly with tenderness; however, liver function tests may be normal or slightly abnormal, with jaundice being very rare. There may be changes at the base of the right lung caused by the elevated diaphragm. The abscess can be visualized radiologically, sonically or by radionuclear scan and the majority of patients have a single abscess in the right lobe of the liver. The most common complication is rupture of the abscess into the pleural space. An abscess can also extend into the peritoneum and through the skin. Hematogenous spread to the brain, as well as to lung, pericardium and other sites, is possible.

Entamoeba histolytica cysts and trophozoites are found in the stools of only a few patients who have liver abscess. Usually 60% of these patients have no intestinal symptoms or any previous history of dysentery.

Blastocystis hominis

When *B. hominis* is present in large numbers in the absence of other parasites, bacteria or viruses, it may be the cause of diarrhea, cramps, nausea, fever, vomiting and abdominal pain and may require therapy. In patients who have other underlying conditions, the symptoms may be more pronounced. Incidence of this organism is found in a higher percentage of stools submitted for parasite examination than expected, especially when compared with percent positive stools for other intestinal protozoa. In symptomatic patients in whom no other etiologic agent has been identified, *B. hominis* should be considered a possible pathogen. When organisms number more than five per high-power field (×400), they should be quantitated and reported.[61] However, in one study, there did not appear to be any specific symptoms associated with *B. hominis* infection and the presence of larger numbers in the stool was not associated with more severe symptoms.[62] Other studies suggest that when a symptomatic *B. hominis* infection responds to therapy, the improvement probably represents elimination of some other undetected pathogenic organism (*E. histolytica*, *G. lamblia*, *D. fragilis*). [63] Data from other geographic areas indicate that although it is commonly seen in stool samples, *B. hominis* is thought to be nonpathogenic.[62] *Blastocystis hominis* inhabits the large intestine and organisms are passed in feces. Three morphologic forms have been described: amebic, granular and vacuolated.[64] The vacuolated ('central body') form is most commonly seen in fecal specimens.

Although *B. hominis* may be found in up to 25% of stool specimens examined, only occasional patients have clinical symptoms and the issue of pathogenicity may be explained by the confirmation of various ribodeme types. Different patient presentations can thus be explained, based on the evidence that there are several ribodeme types, only some of which will be responsible for increased intestinal permeability and symptoms.[36] [37] *Blastocystis hominis* may be suspected as the etiologic agent when the complete battery of parasitologic, bacterial and viral tests on stools has failed to disclose any agent other than *B. hominis*. Although most of these patients have numerous *B. hominis* organisms in the stool, organism numbers are not always elevated in symptomatic patients. The predominant and virtually only symptom has been persistent, mild diarrhea.

Infection is diagnosed by finding the familiar spherical or ovoid form with a large central vacuole with nuclei and various other organelles arranged around the periphery (see Fig. 242.6). *Blastocystis hominis* organisms can be demonstrated by any of the methods used for the diagnosis of intestinal parasite infections; however, the permanent stained smear is recommended.

Although we normally do not quantitate protozoa on the report form, this organism should be reported and semiquantitated (rare, few, moderate or many) from the examination of the permanent stained smear. As the published literature is somewhat controversial on the relationship between organism numbers and patient symptoms, quantitation may be helpful for the clinician. The percentage of people who harbor this parasite can range from about 10% to over 50% in some areas of the world.

Flagellates

Two species, *G. lamblia* and *D. fragilis*, cause clinically significant intestinal disease, and *T. vaginalis* is a frequent cause of vaginitis.

Giardia lamblia (intestinalis)

Because the acute stage usually lasts only a few days, giardiasis may not be recognized as the cause and the condition may mimic acute viral enteritis, bacillary dysentery, bacterial or other food poisonings, acute intestinal amebiasis or travelers' diarrhea (toxigenic *E. coli*). However, the type of diarrhea plus the lack of blood, mucus and cellular exudate is consistent with giardiasis.

Although organism numbers in the crypts of the duodenal mucosa may reach very high densities, they may not cause any pathology. Organisms have been seen in biopsy material inside the intestinal mucosa, whereas others have been seen only attached to the epithelium. In symptomatic cases, there may be irritation of the mucosal lining, increased mucus secretion and dehydration.

In the compromised patient, conditions and genetic factors associated with *G. lamblia* infection may include hypogammaglobulinemia, protein or caloric malnutrition, previous gastrectomy, histocompatibility antigen HLA-B12, gastric achlorhydria, blood group A, differences in mucolytic proteins, immunoglobulin deficiencies and reduced secretory IgA levels in the gut. Trophozoites infect the upper small intestine but do not invade the tissues to produce ulcers. Infection may elicit a variety of symptoms or may be

asymptomatic. The incubation period is variable, ranging from a few days to several weeks, with an average of about 9 days. In acute giardiasis, symptoms include nausea, upper intestinal cramping or pain, and malaise. There is often explosive, watery diarrhea, characterized by foul-smelling stools. These symptoms are accompanied by flatulence and abdominal distention. The acute stage of clinical giardiasis may be followed by a chronic stage or the chronic type of infection may be the first indication of infection. In such infections there is flatulence and abdominal distention. Patients may also exhibit belching, nausea, anorexia, vomiting and symptoms of heartburn. Fever and chills may be present, but to a lesser degree. Chronic disease must be differentiated from amebiasis and other intestinal parasite infections with *D. fragilis*, *C. parvum*, *Cyclospora cayetanensis*, *I. belli*, the microsporidia and *Strongyloides stercoralis* and from inflammatory bowel disease and irritable colon. On the basis of symptoms such as upper intestinal discomfort, heartburn and belching, giardiasis must also be differentiated from duodenal ulcer, hiatal hernia and gallbladder and pancreatic disease.

In some patients, the cysts may be excreted in stools in a variable pattern, although the reasons for this are not clear. This variable shedding of cysts may occur even when there are classic symptoms of disease and numerous trophozoites in the upper small intestine.

Dientamoeba fragilis

Symptoms have been reported more frequently in children than in adults and are predominantly diarrhea and abdominal distention. Outbreaks in daycare centers have been described. Nausea, vomiting and weight loss have been recorded in from one-third to one-fifth of the cases reported in the literature.[65]

Trichomonas vaginalis

The infection in men is generally asymptomatic, but 25–50% of infected women exhibit symptoms, which include dysuria, vaginal itching and burning and, in severe infections, a foamy, yellowish-green discharge with a foul odor. In many women the infection becomes symptomatic and chronic, with periods of relief in response to therapy. Recurrences of infection and disease may be caused by reinfection from an asymptomatic sexual partner or by failure of the drug metronidazole to eliminate the parasite completely. Symptomatic infections in men are rarely reported but include prostatitis, urethritis, epididymitis and urethral stricture. Rarely, *T. vaginalis* infections occur in ectopic sites and parasites may be recovered from areas of the body other than the urogenital system. *Trichomonas* spp. have been reported in pulmonary infections, presumably from oral trichomonads.

Ciliate

Balantidium coli

The symptoms of infection with *B. coli* are similar to those of amebiasis: lower abdominal pain, nausea, vomiting and tenesmus. Chronic infections may manifest with cramps, frequent episodes of watery, mucoid diarrhea and rarely with bloody diarrhea. Chronic infections have been known to last for several months. In tropical areas in which the parasite is endemic, the infection often is severe in patients who may also have other parasitic, bacterial or viral infections or who are undernourished.

Balantidium coli causes colonic ulcers similar to those caused by *E. histolytica*, but it does not spread to other organs.

MANAGEMENT

Entamoeba histolytica

Although carriers usually harbor amebae with nonpathogenic isoenzyme patterns (*E. dispar*), pathogenic patterns also may be found in these individuals. At present, test methodologies that differentiate between *E. histolytica* and *E. dispar* are not used routinely by all diagnostic laboratories. The diagnosis of *E. histolytica/E. dispar* infection is most often based on organism morphology. For this reason, in general, patients in the USA who harbor this organism are treated, regardless of the presence or absence of symptoms.

There are two classes of drugs used in the treatment of amebic infections: luminal amebicides, such as iodoquinol, paromomycin or diloxanide furoate, and tissue amebicides, such as metronidazole, chloroquine or dehydroemetine. Because there are differences in each drug's efficacy, it is important that the laboratory report for the physician indicates whether cysts, trophozoites or both are present in the stool specimen.

Asymptomatic patients

Patients found to have confirmed *E. histolytica* in the intestinal tract, even if they are asymptomatic, should be treated to eliminate the organisms. Both diloxanide furoate and iodoquinol (650 mg 3 times a day for 20 days; pediatric dose, 30–40 mg/kg/day (max 2g) in 3 doses for 20 days) or paromomycin (25–35 mg/kg/day in 3 doses for 7 days; pediatric dose, 25–35 mg/kg/day in 3 doses for 7 days) are available for treatment of patients who have cysts in the lumen of the gut. A study involving 14 years' experience in the USA using diloxanide furoate for treating asymptomatic cyst passers indicated that the drug is safe and effective and may be particularly well tolerated in children.[66] However, diloxanide furoate is generally not available commercially, but can be compounded by several pharmacies (500mg 3 times a day for 10 days; pediatric dose, 20mg/kg/day in 3 doses for 10 days).[67] In general, these treatments are ineffective against extraintestinal disease. If the patient is passing trophozoites and cysts, the recommended treatment is metronidazole (500–750mg 3 times a day for 7–10 days; pediatric dose, 35–50mg/kg/day in 3 doses for 7–10 days) or tinidazole (not marketed in the USA; 2g per day in 3 divided doses per day for 3 days; pediatric dose, 50mg/kg (max 2g) per day for 3 days).[67] [68]

Mild to moderate disease

In mild to moderate disease, metronidazole (Flagyl) should be given as indicated above when tissue invasion occurs, regardless of the tissue involved. Drugs directed against the lumen organisms should also be used in these cases (as indicated for iodoquinol or paromomycin above).

Severe intestinal disease

Metronidazole (750mg 3 times a day for 7–10 days; pediatric dose, 35–50 mg/kg per day in 3 doses for 7–10 days) or tinidazole (not marketed in the USA; 800mg 3 times a day for 5 days; pediatric dose, 60mg/kg per day (max 2g) for 5 days) should be used for therapy.[67] Some also recommend the use of a luminal drug (see iodoquinol or paromomycin above).

Hepatic disease

Metronidazole plus one of the luminal drugs should be used to treat hepatic disease. There are also other combinations that can be used; some physicians use emetine, in which case the patient must be monitored very carefully for possible cardiotoxicity. The importance of using both luminal and tissue amebicides was emphasized in a study that reviewed the enteric phase of 50 patients who had amebic liver abscess.[66] The prevalence of asymptomatic colonization was 72% (36 out of 50); however, isoenzyme analysis indicated that all of these isolates were pathogenic. In patients treated with metronidazole (tissue amebicide), the clinical response of the hepatic lesions was 100%; failure to eliminate the organism from the bowel in 20 out of 36 patients led to second bouts, with invasive disease and intestinal colonization. Also, these carriers constituted a public health hazard.[67]

Blastocystis hominis

Although there is not a great deal of clinical evidence, there have been studies on the *in vitro* susceptibility of *B. hominis* to numerous drugs.[64] At present, metronidazole appears to be the most appropriate drug. Di-iodohydroxyquin (Yodoxin) has also been effective, and dosage schedules for these two drugs are as recommended for other intestinal protozoa. Another option would be trimethoprim-sulfamethoxazole. The development of a new drug sensitivity assay may improve our ability to evaluate the activities of various drugs against this organism scientifically.[68]

Giardia lamblia (intestinalis)

If giardiasis is diagnosed, the patient should be treated. In the majority of patients, metronidazole is the drug of choice. Metronidazole is not recommended for pregnant women; although not absorbed and not highly effective, paromomycin may be used to treat giardiasis in pregnancy. Tinidazole has proved more effective than metronidazole as a single dose.[69] Furazolidone is another option, but has been reported to be mutagenic and carcinogenic.

Dientamoeba fragilis

Clinical improvement has been observed in adults receiving tetracycline; symptomatic relief was reported in children receiving di-iodohydroxyquin, metronidazole or tetracycline. Current recommendations include iodoquinol, paromomycin or tetracycline. As symptomatic relief has been observed to follow appropriate therapy, *D. fragilis* is probably pathogenic in infected individuals who are symptomatic.

Trichomonas vaginalis

Metronidazole is recommended for urogenital trichomoniasis, although resistance to this and to other 5-nitroimidazoles has been reported.[70] Sexual partners should be treated to avoid immediate reinfection.

Balantidium coli

Tetracycline is the drug of choice, although it is considered investigational. Iodoquinol or metronidazole may be used as alternatives.

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Chapter 243 - Protozoa: Intestinal Coccidia and Microsporidia

Rainer Weber

Intestinal coccidia and microsporidia have increasingly gained attention as etiologic agents of HIV-associated diarrhea. These organisms, however, are not only opportunistic pathogens, but are also the cause of common, worldwide intestinal infections in immunocompetent children and adults. Also, newly described microsporidia are recognized as causing disseminated infections in immunocompromised patients.

NATURE

Intestinal coccidia

The intestinal coccidia are obligate intracellular protozoal parasites that belong to the phylum Apicomplexa, suphylum Sporozoa, and infect small intestinal enterocytes. Species of four genera (*Cryptosporidium*, *Cyclospora*, *Isospora*, *Sarcocystis*) are pathogenic in humans.[1] [2] [3]

The most frequent agents of human cryptosporidiosis are the human and the bovine genotype of *Cryptosporidium parvum*, although *C. meleagridis*, *C. felis*, and the canine genotype of *C. parvum* have also been identified in stools of patients with HIV infection and in HIV-seronegative Peruvian children.[1] [4] *Isospora belli* has been identified as the only accepted cause of human isosporiasis.[3] Intestinal organisms that were previously termed blue green algae, cyanobacterium-like bodies, or coccidia-like bodies were recently characterized as belonging to the coccidians, and are now named *Cyclospora cayetanensis*.[2]

Microsporidia

The term 'microsporidia' is a nontaxonomic designation commonly used to describe a group of obligate intracellular protozoa belonging to the phylum Microsporidia. More than 140 microsporidial genera and almost 1200 species have been identified that are parasitic in every major animal group. To date, seven genera (*Enterocytozoon*, *Encephalitozoon*, *Nosema*, *Pleistophora*, *Vittaforma*, *Trachipleistophora* and *Brachiola*) and unclassified microsporidia (referred to collectively as *Microsporidium*) have been implicated in human infections.[5] [6] [7] [8]

EPIDEMIOLOGY

Cryptosporidium species

Cryptosporidial infections have been detected on all continents. Cumulative prevalence rates are between 1 and 3% in industrialized nations and between 5 and 10% in developing countries.[9] [10] Children, particularly those less than 2 years of age, have a higher prevalence of infection than adults. Seroepidemiologic studies indicate that cryptosporidiosis may be more common than is estimated based upon surveys of fecal oocyst shedding. Seroprevalence rates in developed countries range between 25 and 35%, and in developing countries they are up to 65%. In severely immunodeficient patients with HIV infection, cryptosporidiosis is among the most important causes of chronic diarrhea, accounting for 10–20% of diarrheal episodes.[11]

Cryptosporidial oocysts are transmitted by the fecal-oral route and infection may be acquired from contaminated surfaces, ground and recreational water, pets and farm animals (particularly cattle and sheep), contaminated foods and person-to-person contact, including transmission between household members, sexual partners, children in day care centers and nosocomial infections involving both medical care staff and patients. An increasing number of outbreaks of cryptosporidial infections attributed to drinking water have been reported, including an outbreak in Milwaukee, USA in 1993 that affected over 400,000 persons. Ingestion of as few as ten oocysts may cause diarrhea.[12]

Cyclospora species

Cyclospora spp. have been identified worldwide in stool specimens of immunocompetent and immunocompromised patients, but appears to be most common in tropical and subtropical areas. The parasite is transmitted by the fecal-oral route and infection is most probably acquired from contaminated water or food.[13] It is not known whether animals can be infected and serve as sources for human infection. Direct person-to-person transmission is unlikely because excreted oocysts require days to weeks to become infectious. Warm temperatures and high humidity facilitate sporulation.

Isospora species

Isospora belli is endemic in many parts of Africa, Asia and South America, and is particularly common in patients from developing countries who have AIDS and chronic diarrhea; for example, it occurs in 10–20% of such patients in Haiti or Africa. Modes of transmission are not known but it is assumed that they comprise water and/or food that contains oocysts.

Microsporidia

Reported human infections are globally dispersed. Although microsporidiosis appears to occur most frequently in persons infected with HIV, it is emerging as an infection in otherwise immunocompromised hosts and in immunocompetent individuals. [5]

The sources of microsporidia infecting humans and modes of transmission are uncertain. Because microsporidial spores are released into the environment via stool, urine and respiratory secretions, possible sources of infection may be persons or animals infected with microsporidia. Ingestion of microsporidial spores is the most probable mode of transmission. Transmission by the aerosol route has also been considered because spores have been found in respiratory specimens of patients who have *Encephalitozoon* spp. infection.[14] Epidemiologic and experimental studies in mammals suggest that *Encephalitozoon* spp. can be transmitted transplacentally from mother to offspring, but no congenitally acquired human infections have been reported.

PATHOGENICITY

Cryptosporidium species

Cryptosporidium spp. develop intracellularly at the microvillous border of enterocytes (Fig. 243.1). Infected cells lack microvilli at

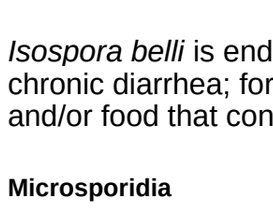

Figure 243-1 Intestinal cryptosporidial infection. Transmission electron micrograph of duodenal tissue of a patient with HIV infection showing two different developmental stages of *Cryptosporidium* spp. on the brush border of the mucosal surface: mature schizont with merozoites (right), undifferentiated zygote (left). *Cryptosporidium* spp. develop intracellularly just under the plasma membrane of the host cell. *Courtesy of MA Spycher.*

the site of parasite attachment and the mucosal surface appears disrupted. In immunocompetent individuals, infection is usually limited to the intestine. In immunocompromised patients, organisms are found throughout the entire gastrointestinal tract and within epithelial cells of the biliary tree, the pancreatic ducts and the airways. In the intestines, cryptosporidial infection induces atrophy, blunting or loss of villi, crypt hyperplasia, and infiltration of lymphocytes, neutrophils, plasma cells, and macrophages into the lamina propria. Cryptosporidial infection has been associated with marked reduction in the brush border enzyme activities, including sucrase, lactase, and maltase deficiency, with impaired absorption of vitamin B12 and D-xylose, and with increased permeability of the intestinal epithelium to organic molecules. Malabsorption and intestinal injury appear to correlate with the number of organisms infecting the intestine.[15] No specific virulence determinants of the parasite have been clearly linked to direct or indirect damage of intestinal host tissues. Putative virulence factors include molecules that are involved in parasite attachment to host cells and host cell membrane disruption. [16]

The immune responses to cryptosporidial infection are poorly understood, but probably include humoral and cellular processes. Persistent cryptosporidiosis has been observed in patients who have hypo- or agammaglobulinemias and those with T-cell deficiencies. Animal studies and epidemiologic data strongly indicate the

importance of the systemic cellular immunity, particularly $CD4^+$ lymphocytes, in modulating cryptosporidial infection.[1]

Isospora species

Isospora belli develops within parasitophorous vacuoles deep in the cytoplasm of the enterocyte. Histologic abnormalities associated with isosporiasis range from minimal changes of the small intestinal architecture to marked villous atrophy, crypt hyperplasia, and inflammatory infiltrates in the lamina propria consisting of eosinophils, neutrophils, lymphocytes and plasma cells. The mechanisms by which these changes occur are unknown. As a result of the intestinal injury, malabsorption and steatorrhea have been documented.[3]

Cyclospora species

Cyclosporiasis is associated with villous atrophy, crypt hyperplasia, and inflammatory infiltrates. The mechanisms that lead to the clinical features are unknown.

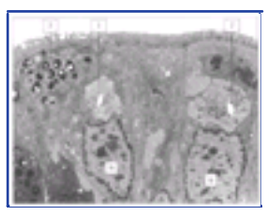

Figure 243-2 Intestinal *Enterocytozoon bieneusi* infection. Transmission electron micrograph showing duodenal epithelium of a patient with HIV infection who has *Enterocytozoon bieneusi* infection. The different developmental stages between the enterocyte nuclei (N) and the microvillous border include: (1) a proliferative plasmodium; (2) late sporogonial plasmodia; and (3) mature spores. *Courtesy of MA Spycher.*

Microsporidia

Microsporidiosis has been associated with abnormalities in structure and function of infected organs but how the different microsporidial species cause disease is not sufficiently understood.[5]

Enterocytozoon bieneusi

Enterocytozoon bieneusi infection generally appears to be limited to intestinal enterocytes (Fig. 243.2) and biliary epithelium. Patients who have severe cellular immunodeficiency appear at highest risk of developing microsporidial disease but little is known about immunity to *E. bieneusi* infection. It is not understood whether microsporidial infection in these patients is primarily a reactivation of latent infection acquired before the state of suppressed immunity or whether microsporidial disease is caused by recently acquired infection.

Encephalitozoon species

Encephalitozoon cuniculi and *E. hellem* infect a variety of cells including epithelial and endothelial cells, fibroblasts, kidney tubule cells, macrophages and possibly other cell types in numerous mammalian hosts, for example rabbits, rodents, carnivores, monkeys and humans.[17] In mammals, they usually cause latent asymptomatic or chronic mildly symptomatic infection, but interstitial nephritis and severe neurologic disease caused by central nervous system vasculitis and granulomatous encephalitis may occur. The parasites are able to persist in their animal hosts despite an active immune response. Microsporidial infection activates antibody production, although antibodies alone do not appear to yield protection. The role of a competent cellular immune response in suppressing microsporidial multiplication has been established experimentally. The pathogenesis of human *Encephalitozoon* infection has yet to be defined. Rare histologic and clinical investigations in immunodeficient patients have indicated that *E. cuniculi* and *E. hellem* usually cause disseminated infection in this patient group.[14] [17]

PREVENTION

Cryptosporidiosis

Cryptosporidial oocysts are remarkably resistant to many common disinfectants, including chlorine-based compounds. Adequate filter

systems are required to guarantee the complete removal of cryptosporidia from water supplies. A water disinfection device delivering germicidal ultraviolet (UV) light for *Cryptosporidium* oocyst inactivation yielded promising results.[18]

Cyclosporiasis, isosporiasis

Avoiding food or water that may be contaminated with feces may prevent cyclosporiasis and isosporiasis, but details of the sources of infection and the modes of transmission are unknown.

Microsporidiosis

Laboratory experiments indicate that the thick-walled spores may survive in the environment for months or years depending on the temperature and humidity. Exposure to recommended working concentrations of most disinfectants, boiling and autoclaving seems to kill *Encephalitozoon* spp. spores but no data are available for *Enterocytozoon* spp.

DIAGNOSTIC MICROBIOLOGY

Stool examination by light microscopy is the most important test to diagnose intestinal coccidia and intestinal microsporidia. In many laboratories, tests for *Cryptosporidium* spp., *Isospora* spp., *Cyclospora* spp. and microsporidia must be specifically requested because the general request of 'stool for O & P' (ova and parasites) often does not mean that the specific methods to detect these organisms are applied. Microsporidial species that cause systemic infection are best detected in urine sediments.

Cryptosporidium species

Examination of stool specimens by light microscopy

To visualize the *Cryptosporidium* spp. oocysts in fecal smears, acid-fast staining (e.g. modified cold Kinyoun technique; Fig. 243.3) and the immunofluorescence (IF) technique are among the most sensitive, specific and widely used methods. The IF detection procedure is more sensitive than the acid-fast staining but the difference in sensitivity may not be of clinical relevance when watery stools of patients with HIV infection are examined. These patients usually excrete an amount of oocysts that can easily be detected using acid-fast staining. The oocysts should be measured in order to distinguish *Cryptosporidium* spp. (4–6µm in diameter) from *Cyclospora* spp. (8–10µm). Enhanced sensitivity of stool examination can be obtained by concentrating oocysts, preferably with the formalin-ethyl acetate (FEA) technique.

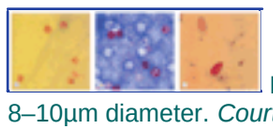

Figure 243-3 Acid-fast stained smears of fecal specimens showing intestinal coccidia. (a) *Cryptosporidium* spp., round, 4–6µm diameter. (b) *Cyclospora* spp., round, 8–10µm diameter. *Courtesy of EG Long.* (c) *Isospora belli*, elliptical, 23–33µm long and 10–19µm wide. Modified Kinyoun stain.

The exact sensitivity of the coprodiagnostic techniques is not known but some data raise questions about the widely held belief that these techniques are sufficient to meet the needs of clinicians and epidemiologists.[19] The minimum number of oocysts in human stool specimens that can be detected by the FEA stool concentration technique and the IF staining procedure was found to be unexpectedly high: 5000–10,000 oocysts per gram of stool in watery stool specimens and 10,000–50,000

oocysts per gram of stool in formed stool. Examination of multiple specimens may be necessary, because clinical studies have shown that examination of single stool specimens may have an insufficient diagnostic yield. Furthermore, in a prospective analyses of jejunal biopsies in patients who have AIDS, *Cryptosporidium* spp. were present in more than 10% of patients whose stool examinations were negative. These results, however, were in contrast to another study that only identified organisms on biopsy in one-third of patients shedding *Cryptosporidium* spp. in their feces.

Stool antigen detection techniques have been developed but the sensitivity of currently available tests is not better than microscopic techniques. Molecular diagnostic methods have improved diagnostic yields but currently are not ready to use in routine laboratories.[20] [21]

Cytologic diagnosis

Aspiration of duodenal fluid or small intestinal brushing can be used for diagnosis when upper endoscopy is performed. Examination of centrifuged duodenal aspirates under the microscope may be the most sensitive diagnostic procedure.

Histologic examinations

Cryptosporidium spp. appear basophilic by examination under the light microscope of small intestinal tissue sections stained with hematoxylin and eosin. The intracellular organisms seem to project into the intestinal lumen because of their apical extracytoplasmic localization. Under electron microscopy the unique ultrastructural features of different developmental stages of the parasite can be seen (see Fig. 243.1), but this is rarely necessary for diagnostic purposes.

Serology

Specific IgM or IgG antibodies to *Cryptosporidium* spp. can be detected within 2 weeks of onset of symptoms in most patients. In the majority of patients IgG titers may persist for long periods. Serologic testing is mainly used as an epidemiologic tool and has no diagnostic application, particularly because antibody persistence limits its use in the diagnosis of acute infection.

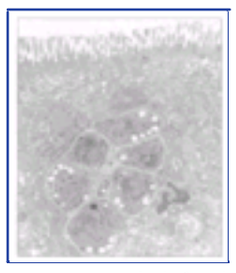

Figure 243-4 Intestinal *Cyclospora* infection. Transmission electron micrograph of duodenal epithelium obtained from a patient with HIV infection who has *Cyclospora cayetanensis* infection. A mature schizont filled with numerous merozoites is shown. *Courtesy of AM Deloul and FP Chatelet.*

Cyclospora species

Diagnosis of *Cyclospora* spp. is dependent on detection by light microscopy of the refractile oocysts, 8–10µm in diameter, in wet mounts of fresh stool specimens or in acid-fast stained smears prepared from stool concentrated by the FEA sedimentation.[22] Acid-fast stained oocysts vary in appearance from faint pink to deep red, and with many organisms remain as unstained spheres (see Fig. 243.3). The sensitivity of the coprodiagnostic techniques is unknown. In many patients' specimens, however, the number of oocysts detected per slide is low, indicating that not all symptomatic patients excrete a large enough number of oocysts for laboratory detection to be assured. Examination of small intestinal tissue of patients who have *Cyclospora* infection often did not reveal any parasites. The presence of intracellular parasites has rarely been documented in aspirated duodenal or jejunal fluid and on duodenal and jejunal biopsy (Fig. 243.4).

Isospora species

Diagnosis of *I. belli* is usually achieved by detection under the light microscope of the parasite oocysts in wet preparations or acid-fast stained smears of concentrated stool specimens (see Fig. 243.3). Repetitive stool examinations may be necessary because the parasite may be excreted intermittently or in low numbers. Histologic examination of small intestinal tissue sections may reveal the parasite within enterocytes.

Microsporidia

Diagnosis of microsporidial infection is dependent on morphologic demonstration of the organisms themselves. This can be difficult because of the organisms' small size and staining properties hamper visualization of the spores and developing stages using routine staining techniques. The spores, the stages by which microsporidia are usually identified, are small, ranging in size from 1 to 3.5µm in diameter.

Evaluation of patients who have suspected microsporidiosis should begin with examination of body fluids by light microscopy using special staining techniques. Definitive species identification of microsporidia is made using electron microscopy, antigenic analysis, and molecular analysis. Collection of fresh material (without fixative) may be useful for cell culture and for future molecular analysis. [5] [23]

Examination of stool specimens

In patients who have suspected enteric microsporidiosis, examination of stool specimens by light microscopy is the first step. It is at

Figure 243-5 Detection of microsporidia in stool samples. Smear of a formalin-fixed, unconcentrated stool specimen of a patient who has AIDS and chronic diarrhea, showing pinkish-red-stained spores of *Enterocytozoon bieneusi*. Chromotrope staining (oil immersion).

least as sensitive as examination of biopsy specimens. Detection of microsporidial spores requires adequate illumination and magnification [i.e. ×630 or ×1000 magnification (oil immersion)], and special staining methods. The two most commonly used stains are the chromotrope stain, which appears to be the most specific (Fig. 243.5),[24] and chemofluorescent agents, which might be more sensitive but may produce false-positive results.[25] An epidemiologic comparison of these techniques resulted in the recommendation to screen specimens with chemofluorescent agents and to confirm the results with the chromotrope stain.

The differences in spore size between *Enterocytozoon* (1–1.5µm in diameter) and *Encephalitozoon* spp. (2–3µm in diameter) often permit a tentative diagnosis of the genus from examination of stool under the light microscope. The microsporidian should be identified to the level of genus by electron microscopy or molecular analysis because *Encephalitozoon* spp. have a propensity for dissemination, and have a different drug sensitivity pattern compared with *Enterocytozoon bieneusi*.

Immunofluorescent procedures for diagnosis of *Encephalitozoon*-like microsporidial spores are promising but not widely available. Diagnostic application of polyclonal antibodies in fecal samples has been hampered by background staining, cross-reactions with yeast and bacteria, and low sensitivity. A few monoclonal antibodies against *Encephalitozoon* spp.[26] and one against spores of *Enterocytozoon bieneusi* have been generated.[27]

Histologic examination

Among patients with HIV infection who suffered from chronic diarrhea, stool examinations proved as sensitive as endoscopic evaluation for all pathogens except cytomegalovirus and *Leishmania*.[11] Examination of duodenal and terminal ileal tissue has resulted in detection of microsporidia but the parasites are rarely found in colonic tissue sections.

Only highly experienced pathologists have reliably and consistently identified microsporidia in tissue sections using routine techniques such as hematoxylin and eosin stain. Ultra-thin plastic sections stained with methylene blue-azure II-basic fuchsin or with toluidine blue may facilitate detection but these techniques are not routinely used. In our experience, tissue Gram stains such as Brown-Brenn or Brown-Hopps have proved to be the most useful for the rapid and reliable identification of HIV-associated microsporidia in routine paraffin-embedded tissue sections (Fig. 243.6). Others prefer a silver stain (Warthin-Starry stain) or the chromotrope-based staining technique.

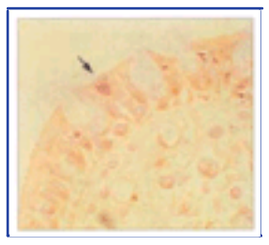

Figure 243-6 Intestinal *Enterocytozoon bieneusi* infection. Terminal ileal tissue obtained by ileocolonoscopy in a patient who has AIDS and chronic diarrhea caused by *Enterocytozoon bieneusi* infection. Gram-positive or Gram-labile microsporidial spores (arrow) are found in supranuclear location within small intestinal enterocytes. Brown-Brenn stain.

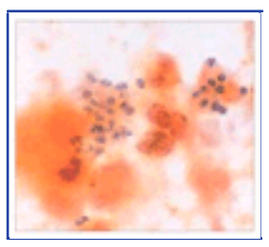

Figure 243-7 Detection of microsporidia in urine sediment. Urine sediment from a patient who has AIDS and disseminated *Encephalitozoon hellem* infection, showing Gram-labile intracellular and extracellular microsporidial spores. Gram stain (oil immersion).

Cytologic diagnosis

Microsporidial spores have been detected in sediments of duodenal aspirate, bile or biliary aspirates, urine (Fig. 243.7), bronchoalveolar lavage fluid, cerebrospinal fluid (CSF) and in smears of conjunctival swabs, sputum and nasal discharge.[5] Microscopic examination of centrifuged duodenal aspirate obtained during endoscopy appears to be the most sensitive technique for diagnosis of intestinal microsporidiosis. Because microsporidial infection often involves multiple organs, detection of microsporidia in virtually any tissue or bodily fluid should prompt a thorough search of other sites. In particular, urine specimens of patients suspected of having disseminated microsporidiosis should be examined.

Electron microscopy

Microsporidial ultrastructure is unique and pathognomonic for the phylum and, with rare exceptions, ultrastructural features can distinguish between most genera of microsporidia (Fig. 243.8 ; see Fig. 243.2).

Serology

Serologic assays (including carbon immunoassay, indirect IF test, enzyme-linked immunosorbent assay and Western blot immunodetection) have been useful in detecting antibodies to *E. cuniculi* in several species of animals, but reliable serologic tests for diagnosis of human microsporidiosis are lacking. This lack of availability is partly because *Enterocytozoon* spp. have not been continuously propagated in cell culture or laboratory animals.

Cell culture

Encephalitozoon spp., *Nosema* spp., *Trachipleistophora hominis*, *Vittaforma corneae* and *Brachiola algerae* have been isolated using cell culture systems, but these tests are fastidious and costly, and the

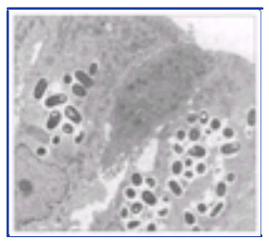

Figure 243-8 *Encephalitozoon intestinalis* (formerly *Septata intestinalis*): developing spores within enterocytes separated by a fibrillar matrix. *Encephalitozoon intestinalis* develop within parasitophorous vacuoles, unlike *E. bieneusi*, which develops in direct contact with enterocyte cytoplasm. *Courtesy of MA Spycher.*

most common human species, *E. bieneusi*, has not been continuously propagated.

Molecular techniques

Universal panmicrosporidian and species-specific primer pairs that amplify the short regions of the small subunit rRNA gene have been developed, [17] [28] but, at present, these techniques are confined to research laboratories. Diagnosis and identification of *Enterocytozoon bieneusi* and the different *Encephalitozoon* spp. have been successfully performed with fresh stool specimens, formalin-fixed stool specimens, intestinal tissue obtained by endoscopic biopsy, urine specimens and other body fluids.[17] [29] *In-situ* hybridization to visualize *E. bieneusi* in tissue sections has been developed.

CLINICAL MANIFESTATIONS

Cryptosporidiosis

For cryptosporidiosis the mean incubation period between infection and onset of symptoms is approximately 7–14 days (range 5–28 days). The severity and duration of illness varies depending on the immune status of the hosts. Children, the elderly and individuals with nutritional deficiencies may suffer from a more severe and prolonged disease, as is observed in immunodeficient patients. Whether cryptosporidiosis in immunocompromised patients is mainly a reactivation of latent infection or whether clinical illness is caused by recently acquired infection is not known.

Immunocompetent patients

In immunocompetent patients, cryptosporidia cause a self-limiting, usually watery, diarrhea lasting 10–14 days (range 2–28 days), but the clinical presentation varies from asymptomatic shedding of oocysts to severe disease that may last up to 3 months.[12] Patients often complain of abdominal pain, flatulence, loss of appetite, nausea and vomiting, and may suffer from low-grade fever, anorexia, malaise, weakness, fatigue, myalgias and headaches. The diarrhea and abdominal pain are often made worse by eating. Cough appears significantly more frequent in children with cryptosporidiosis than in children with diarrhea of another etiology, but the parasite has rarely been documented in the airways of immunocompetent individuals. Single case reports of pancreatitis associated with cryptosporidiosis and reactive arthritis have been described. Poorly understood lasting adverse effects of infection in children in developing children may include deficits of linear growth, even if

cryptosporidial infections is otherwise symptomless.[30]

Immunocompromised patients **(see Chapter 127)**

In immunocompromised patients, particularly individuals with HIV infection, cryptosporidiosis is a more severe, often chronic and

incurable illness that can be life-threatening. The main clinical presentation is watery diarrhea that can lead to severe dehydration, electrolyte depletion, malnutrition and weight loss. In addition, infection of the biliary tract and the gallbladder, resulting in acalculous cholecystitis, sclerosing cholangitis ('AIDS cholangiopathy') and stenosis of the papilla of Vater, frequently occurs. Infection of the epithelial cells of the respiratory tract, including sinuses, is increasingly described, but it is not clear whether this finding is of clinical relevance because most of these patients have a concomitant respiratory infection. Systemic cryptosporidiosis has not been described.

The clinical course of HIV-associated cryptosporidiosis is highly variable. Four clinical patterns of disease have been identified:

- asymptomatic shedding of oocysts;
- transient diarrhea with transient or chronic shedding of oocysts;
- chronic diarrhea; and
- fulminant disease that leads to cachexia and death within months.

Most patients who have severe illness have $CD4^+$ lymphocyte counts below 50 cells/mm^3 . Spontaneous clinical recovery may occur and is mainly correlated with higher $CD4^+$ lymphocyte counts, but a benign course of the diarrhea may also occur in severely immunodeficient patient. Clinical observations suggest that cryptosporidial disease is more severe if co-infections caused by other enteropathogens are present. Concurrent dual or multiple intestinal infection is found in up to 50% of patients who have HIV-associated cryptosporidiosis.

Cyclosporiasis

The spectrum of *Cyclospora*-associated illness is not yet fully defined but it may range from asymptomatic carriage of the organism to severe and prolonged diarrhea in immunocompetent and immunocompromised patients. Patients who have AIDS tend to have a more prolonged and severe illness, and *Cyclospora*-associated cholangiopathy has been described in this group.

The incubation period between infection and onset of symptoms ranges between 2 and 11 days and is usually about 7 days. In symptomatic infections the main clinical manifestation is mild to severe watery diarrhea, accompanied by abdominal cramps, bloating, increased flatus, nausea, anorexia, substantial weight loss and fatigue. Vomiting is less common and about 25% of the patients report fever and myalgias.

If not treated, diarrhea is self-limiting but may last for 1 month or longer (range 2–107 days), and remissions and relapses may occur during gradual resolution of the illness. *Cyclospora* infection does not appear to provide lasting immunity.

Isosporiasis

In immunocompetent persons, *I. belli* infection causes self-limited watery diarrhea accompanied by malaise, anorexia, cramping abdominal pain, weight loss and, less frequently, low-grade fever. In immunocompromised patients, the illness is more severe and prolonged, or chronic if untreated. Also, acalculous cholecystitis and two single cases of disseminated extraintestinal isosporiasis in patients with AIDS have been reported.

Microsporidiosis in immunocompromised patients

The spectrum of clinically manifest microsporidial infection is diverse and includes intestinal, ocular, muscular, cerebral, respiratory and urinary tract disease (Table 243.1). [5] The most prevalent microsporidial disease is HIV-associated chronic diarrhea.[11] Disseminated microsporidial infections are being increasingly recognized in patients who have AIDS.

Diarrhea, cholangitis, acalculous cholecystitis

Two microsporidial species — *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* — cause chronic diarrhea and wasting, cholangiopathy

TABLE 243-1 -- Microsporidial species pathogenic in humans, and clinical manifestations.

MICROSPORIDIAL SPECIES PATHOGENIC IN HUMANS, AND CLINICAL MANIFESTATIONS	
Microsporidial species	**Clinical manifestations**
Enterocytozoon bieneusi	Diarrhea, wasting syndrome
	Cholangiopathy, cholangitis, acalculous cholecystitis
	Sinusitis, bronchitis, pneumonitis
Encephalitozoon intestinalis (formerly *Septata intestinalis*)	Diarrhea
	Cholangiopathy, cholangitis, acalculous cholecystitis
	Sinusitis, bronchitis, pneumonitis
	Urinary tract infection, nephritis
	Bone lesions; nodular cutaneous lesions
Encephalitozoon cuniculi	Disseminated infection
	Keratoconjunctivitis
	Sinusitis, pneumonitis
	Urinary tract infection, nephritis
	Hepatitis
	Peritonitis
	Symptomatic and asymptomatic intestinal infections
	Seizures, encephalitis
Encephalitozoon hellem	Disseminated infection
	Keratoconjunctivitis
	Sinusitis, bronchitis, pneumonia
	Nephritis, ureteritis, cystitis, prostatitis, urethritis
Pleistophora spp.	Myositis
Trachipleistophora hominis	Myositis, keratoconjunctivitis, sinusitis
Trachipleistophora anthropophthera	Disseminated infection including brain, heart, kidneys

Brachiola connori (formerly *Nosema connori*)	Disseminated infection
Brachiola vesicularum	Myositis
Brachiola algerae (formerly *Nosema algerae*)	Keratitis
Vittaforma corneae (formerly: *Nosema corneum*)	Keratitis
	Disseminated infection
Nosema ocularum	Keratitis
Microsporidium ceylonensis	Corneal ulcer
Microsporidium africanum	Corneal ulcer

and acalculous cholecystitis in patients who have HIV infection or who are otherwise immunodeficient, particularly when $CD4^+$ lymphocyte counts drop below 50–100/µl.

Enterocytozoon bieneusi is estimated to be one of the most important HIV-associated intestinal pathogens, present in 5–30% of those with otherwise unexplained diarrhea. The main symptoms are chronic nonbloody diarrhea, anorexia, weight loss and bloating. Some patients experience intermittent diarrhea and a few excrete microsporidial spores without having diarrhea. The stool is watery or soft, and diarrhea seems to be worsened by most foods. Some of the patients report

abdominal pain or nausea and vomiting. Laboratory evidence for intestinal malabsorption is common. *E. bieneusi* itself is not immediately life-threatening, but diarrhea is debilitating, and weight loss may lead to cachexia, which is a significant cause or cofactor in the deaths of many patients. Up to one-third of patients who have intestinal microsporidiosis have dual or multiple co-infection with other intestinal pathogens. The parasite has also been detected in the biliary tree and/or gallbladder of patients who have cholangitis and acalculous cholecystitis. Imaging procedures often reveal dilatation of both intrahepatic and common bile ducts, irregularities of the bile duct wall and gallbladder abnormalities such as wall thickening, distention or the presence of sludge.[5] [11]

Encephalitozoon intestinalis primarily causes diarrhea, and the parasite may also spread into the biliary tract and gallbladder, causing cholangitis and cholecystitis. In contrast to *Enterocytozoon bieneusi*, systemic dissemination to kidneys and other sites may occur. [31]

Systemic microsporidiosis

There are three microsporidial genera, *Encephalitozoon* spp., *Trachipleistophora* spp. and *Vittaforma corneae*, which have been found to disseminate in severely immunodeficient patients who have HIV infection.[6] [17] [32] [33] Disseminated infection caused by *Brachiola connori* (formerly *Nosema connori*) was found at autopsy in a 4-month-old athymic boy reported in 1973.

Encephalitozoon spp. was initially identified in patients who had AIDS and keratoconjunctivitis.[34] Subsequently the spectrum of recognized *Encephalitozoon*-associated disease has expanded to include keratoconjunctivitis, bronchiolitis, sinusitis, pneumonitis, nephritis, ureteritis, cystitis, prostatitis, hepatitis, peritonitis, diarrhea and encephalitis.[14] [17] Clinical manifestations may vary substantially, ranging from an asymptomatic carrier state to organ failure.

Trachipleistophora anthropophthera was identified at autopsy in cerebral, cardiac, renal, pancreatic, thyroid, hepatic, splenic, lymphoid and bone marrow tissue of two patients who had AIDS and initially presented with seizures.[32]

Urinary tract infection

Predominant genitourinary signs and symptoms caused by *Encephalitozoon* infections have been observed. Clinical manifestations included asymptomatic microhematuria, urethritis, prostatitis, acute cystitis and interstitial nephritis associated with dysuria, gross hematuria and progressive renal insufficiency.[14]

Respiratory tract infection

In most patients who have respiratory tract microsporidial infection, including bronchiolitis, pneumonia and progressive respiratory failure, intestinal or systemic microsporidiosis was also present. Single cases of patients have been reported in whom sinusitis causing nasal obstruction and persistent mucopurulent nasal discharge caused by *E. bieneusi* or *Encephalitozoon* spp. was a predominant manifestation of systemic microsporidiosis.

Keratoconjunctivitis

HIV-associated ocular microsporidiosis caused by *Encephalitozoon* spp. is restricted to the superficial epithelium of the cornea and conjunctiva. Most patients exhibit bilateral coarse punctate epithelial keratopathy (Fig. 243.9), conjunctival inflammation resulting in redness and foreign body sensation, decreased visual acuity and photophobia. In patients who initially present with symptomatic keratoconjunctival microsporidiosis, dissemination of the parasite may be common, but clinical manifestations other than keratoconjunctivitis may be mild or absent.[35]

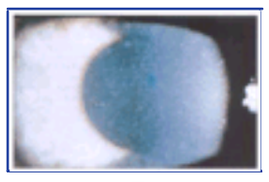

Figure 243-9 Keratopathy caused by *Encephalitozoon hellem.* Slit-lamp demonstration of punctate epithelial keratopathy in a patient who has AIDS and keratoconjunctivitis caused by *Encephalitozoon hellem*. Ocular microsporidiosis can often be diagnosed by examination under the light microscope of a smear obtained by a nontraumatic conjunctival swab. *Courtesy of M Diesenhouse and DA Schwartz.*

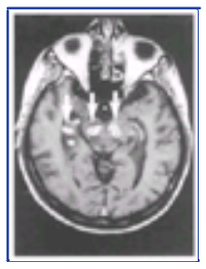

Figure 243-10 Cerebral microsporidiosis caused by *Encephalitozoon cuniculi* in a patient with HIV infection. The MRI shows multiple small contrast-enhancing, mostly ring-like, partly micronodular, lesions in hippocampal, mesencephal and intracortical regions (arrows), partly accompanied by slight edema, and congestion of the right ethmoid sinus. *Encephalitozoon cuniculi* was isolated from CSF. *From Weber et al.,[36] with permission from Massachusetts Medical Society.*

Cerebral microsporidiosis

Microsporidia was first accepted as the etiologic agent of a neurologic disorder in two children, reported in 1959 and 1984. Both presented with seizures and might have had impaired immune responses. *Encephalitozoon cuniculi* and *Trachipleistophora anthropophthera* were detected in CSF or brain tissue of patients with HIV infection who presented with fever and somnolence or seizures and mental decline. Magnetic resonance imaging disclosed multiple small, contrast-enhancing, mostly ring-like lesions localized to the hippocampus, mesencephalon and cerebral cortex (Fig. 243.10).[32] [36]

Myositis

Myositis caused by *Pleistophora* spp. has been described in an HIV-seronegative patient and in a patient with HIV infection, both of whom had severe cellular immunodeficiency. Newly characterized microsporidia, *Trachipleistophora hominis* and *Brachiola vesicularum*, were detected in muscle biopsies of patients who had

AIDS and presented with myositis.[6] [8]

Microsporidiosis in immunocompetent persons

Enterocytozoon bieneusi and *Encephalitozoon* spp. are associated with self-limiting watery diarrhea in immunocompetent adults as well as in children, particularly among persons who reside or have traveled in tropical countries.[37] An unexpectedly high prevalence (17%) of intestinal microsporidiosis due to *Enterocytozoon bieneusi* was found in HIV-seronegative elderly patients in Spain.[38]

Deep stromal infections of the cornea caused by different microsporidial species have been described in otherwise seemingly healthy persons who presented with severe keratitis or a corneal ulcer (see Table 243.1).

MANAGEMENT

Cryptosporidiosis

Among immunocompetent patients, cryptosporidiosis is usually self-limiting. In patients who have AIDS, case series and a randomized, controlled trial indicate that treatment with oral paromomycin (500mg q6h for at least 4 weeks) may result in decreased oocyst shedding and improved intestinal function and morphology,[39] but other investigators have not found any clinical benefit.[40] Case observations in patients who have AIDS suggest that maintenance therapy with paromomycin (1000–2000mg/day) may prevent relapses or improve the clinical course but controlled data are not available.

Randomized, double-blind trials found beneficial effects of nitazoxanide: At doses of 1–2g daily for 2 weeks, parasitologic cure was reported in 65% of Mexican patients who had AIDS.[41] Age-dependent doses of 100–500mg twice daily reduced the duration of diarrhea and oocyst shedding in HIV-seronegative children and in adults in Egypt.[42] *In-vitro* studies have indicated that nitazoxanide activity may be enhanced by the co-administration of azithromycin or rifabutin,[43] but clinical experience of such combinations is lacking. Hyperimmune bovine colostrum has been found in *in-vitro* experiments, in animal studies and in single patients to reduce or eradicate cryptosporidia, but these results could not be reproduced in larger human studies. Symptomatic treatment of diarrhea may include drugs that affect gut motility such as loperamide, diphenoxylate, opiates, somatostatin and octreotide. Immune reconstitution following initiation of potent antiretroviral therapy of patients who have HIV infection results in cessation of oocyst shedding and diarrhea, but cryptosporidial infection is controlled rather than cured because failure of antiretroviral therapy often results in relapse of cryptosporidiosis.[44]

Cyclosporiasis

A placebo-controlled trial showed that trimethoprim-sulfamethoxazole [TMP-SMX (co-trimoxazole); double-strength TMP 160/SMX 800mg q12h for 7 days] was clinically successful and shortened oocyst shedding in immunocompetent patients who have cyclosporiasis.[45] In this study, 3 days of treatment with TMP-SMX was not sufficient to eradicate cyclospora. Trimethoprim 160/SMX 800mg q6h for 10 days cured HIV-associated cyclosporiasis but the relapse rate was high (43%). Maintenance therapy with double-strength TMP-SMX three times per week did prevent relapses in these patients. There are no known alternatives to TMP-SMX.

Isosporiasis

Immunocompetent and immunocompromised patients respond promptly to therapy with TMP-SMX (double-strength TMP 160/SMX 800mg q6h for 10 days).[46] Alternatively, pyrimethamine 75mg/day plus folinic acid 10mg/day may be successful. To prevent the high rate of relapses in patients who have AIDS, maintenance therapy with double-strength TMP-SMX, three times per week, or pyrimethamine 25mg/day plus folinic acid 5mg/day, is recommended.

Microsporidiosis

Albendazole, fumagillin, its analog TNP-470, and nikkomycin Z have been found to inhibit completely or partially the replication or spore germination of *Encephalitozoon* spp. and *Vittaforma corneae* propagated in cell cultures, but did not destroy mature microsporidial spores, so that these may sustain infection.[47] [48] [49] Numerous other antiprotozoal drugs and antibiotics have been tested *in vitro*, with negative findings. *In-vitro* systems to investigate *Enterocytozoon bieneusi* are not available.

Little information on clinical experience in the therapy of human microsporidiosis is available, and only two controlled treatment trials have been conducted,[49] [50] confirming previous case observations which indicated that albendazole can result in clinical cure of HIV-associated encephalitozoonosis in parallel with the cessation of spore excretion. In contrast, albendazole is not effective for the treatment of *Enterocytozoon bieneusi* infection and does not reduce the parasite load although previous observations had suggested that clinical improvement may occur in some patients. Oral purified fumagillin was recently used in a pilot study and subsequently in a small randomized trial to treat HIV-associated diarrhea due to *E. bieneusi*. Fumagillin appeared to eradicate the parasite transiently in many patients but serious adverse events and parasitic relapse were observed.[49] [51] Recent experience with the antitumor necrosis factor-a agent thalidomide or potent antiretroviral treatment strategies in persons with HIV infection indicate that a modulation or improvement of the local or systemic immune function may lead to parasite clearance in this patient group.[44]

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Chapter 244 - Protozoa: Free-living Amebae

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NATURE

The concept that small free-living amebae, particularly *Acanthamoeba* spp. have the potential to cause disease in humans was developed by CG Culbertson of the Indiana University School of Medicine and the Eli Lilly Laboratories in 1958. The basis of this observation was a chance discovery of an ameba growing in a batch of monkey kidney cell cultures that were to be used for growing polio virus for the development of polio vaccine. This ameba, described as *Acanthamoeba* sp. (Lilly A-1), was found to produce, on intracerebral, intraspinal and intranasal inoculations, meningoencephalitis in cortisone-treated monkeys and mice.[1] [2] [3] This isolate is now called *Acanthamoeba culbertsoni*.

The first case of amebic meningoencephalitis in humans, attributed initially to *Acanthamoeba* although later the causative organism was identified as a species of *Naegleria*, was described in Australia in 1962. It is now known that besides *Acanthamoeba* spp., two other free-living amebae, *Naegleria fowleri* and *Balamuthia mandrillaris*, also cause central nervous system (CNS) disease in humans and other animals.[1] [2] [3] [4] Recently, however, *Sappinia diploidea*, a saprophytic ameba that has been previously isolated from the fecal specimens of lizards, elks and bisons, was identified in a brain biopsy specimen of a previously healthy 38-year-old man who developed visual disturbances, headache and a seizure.[5] This suggests that there are cases of human infections caused by free-living amebae other than *Acanthamoeba*, *Balamuthia* and *Naegleria* spp. that may have been either misdiagnosed or unrecognized.

Taxonomy

The free-living amebae are classified under the subphylum Sarcodina and superclass Rhizopodea.[2] The sarcodinian rhizopods are a heterogeneous group of amebae that include the free-living *Acanthamoeba, Naegleria, Balamuthia, Hartmannella* and *Vahlkampfia* spp. and others such as the parasitic amebae (e.g. *Entamoeba histolytica*) that move by producing cytoplasmic bulges, the lobopodia, from the surface of the body. In contrast to *E. histolytica*, which is a mitochondria-lacking ameba that causes gastrointestinal disease, *Naegleria, Acanthamoeba* and *Balamuthia* are mitochondria-bearing amebae that cause diseases of the CNS of humans and animals, which almost always lead to death. The term amphizoic amebae indicates the ability of these amebae to exist as free-living in nature and as parasites within host tissue; this differentiates them from the truly parasitic *E. histolytica*.[6]

Although several species of *Naegleria* have been described, so far only one species, *N. fowleri* (*Naegleria aerobia* and *Naegleria invadens* are non-valid synonyms), is known to infect the human CNS. Several of the more than 20 species of *Acanthamoeba* that have been described so far cause not only a chronic granulomatous CNS disease in humans and other animals, but also infect the cornea (*Acanthamoeba* keratitis), the skin, the nasal sinuses and pulmonary tissues. The disease caused by *Acanthamoeba* spp. has been described as granulomatous amebic encephalitis (GAE). *Balamuthia mandrillaris*, the only known species of *Balamuthia,* causes GAE and skin infections in humans and other animals.[1] [2] [3] [4] [7]

Naegleria fowleri

Naegleria fowleri is also described as an ameboflagellate because it has a transient flagellate stage in its life cycle in addition to a feeding and dividing form, the trophozoite, and a resistant cyst stage (Fig. 244.1). The trophozoite, measuring 10–25µm, normally feeds on bacteria and multiplies by binary fission. However, it is able to differentiate into a pear-shaped biflagellate stage in response to sudden changes in the ionic concentration of its environment. When the conditions become unfavorable the trophozoite differentiates into a resistant cyst stage. The trophozoite is usually uninucleate; the nucleus is spherical and contains a large, centrally placed, dense nucleolus. Additionally, numerous dumbbell-shaped mitochondria, vacuoles, lysosomes and ribosomes are present within the cytoplasm. Cysts are round and contain a single nucleus with a central dense nucleolus; the dense cyst walls are plugged with one or more flat pores. The cysts are 7–14µm in diameter, with a mean of 10µm.[2] [6]

Acanthamoeba spp.

In 1930 Aldo Castellani isolated an ameba from a yeast culture; it was later named as *Acanthamoeba castellanii*.[1] Currently more than 20 species of *Acanthamoeba* have been described.[8] The acanthamebae are also called opportunistic amebae because they produce disease principally in immunodeficient individuals.

The life cycle of *Acanthamoeba* spp. consists of two stages: a feeding and reproducing trophozoite stage and a resistant cyst stage (Fig. 244.2). The trophozoites feed on bacteria and detritus present in the milieu and multiply by binary fission. They are uninucleate and are 15–45µm in size. The nucleus has a centrally placed, large, densely staining nucleolus. The cytoplasm is finely granular and contains numerous mitochondria, ribosomes and lysosomes. Cysts are 10–25µm in size and are double walled. The outer wall or the ectocyst is wrinkled and contains protein and the inner wall, the endocyst, is usually stellate, polygonal, oval or spherical and contains cellulose. Pores covered by opercula are present at the point of contact between the ectocyst and the endocyst. Cysts are uninucleate and possess a centrally placed dense nucleolus.[6] [8]

Balamuthia mandrillaris

Balamuthia mandrillaris, previously called leptomyxid ameba, has two stages in its life cycle (Fig. 244.3). The trophozoite is irregular in shape and measures from 12 to 60µm with a mean size of about 30µm. It is usually uninucleate, but binucleate forms are occasionally seen. The nucleus contains a large centrally placed nucleolus. Occasionally, in infected human tissues, trophic stages containing a large nucleus with two or three nucleolar bodies have been observed. Cysts are also uninucleate, more or less spherical, and range in size from 12 to 30µm with a mean of 15µm. Cysts appear to be double walled with a wavy ectocyst and a spherical endocyst when viewed under the light microscope. However, ultrastructurally

Figure 244-1 ***Naegleria fowleri.*** The trophozoite can be differentiated from the cyst by its characteristic lobopodial locomotion; both are taken from culture. Differential interference contrast.

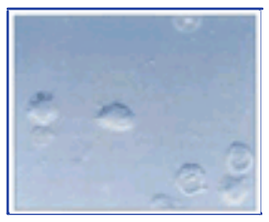

Figure 244-2 ***Acanthamoeba castellanii.*** The trophozoite has spiny acanthopodia whereas the cyst has an outer wrinkled ectocyst and a stellate endocyst; both are taken from culture. Differential interference contrast.

Figure 244-3 ***Balamuthia mandrillaris.*** The trophozoite is irregularly shaped whereas the cyst is spherical; both are taken from culture. Differential interference contrast.

the cysts are tripartite with an outer thin and irregular ectocyst, an inner thick endocyst and a middle amorphous fibrillar mesocyst.[4] [7]

Cultivation

Acanthamoeba spp. and *N. fowleri*, but not *B. mandrillaris*, can be cultivated on non-nutrient agar plates coated with a suitable Gram-negative bacteria such as *Escherichia coli* or *Enterobacter aerogenes*. The amebae will feed on the bacteria, multiply and differentiate into cysts within a few days. They can be easily subcultured by transplanting a small piece of agar containing trophozoites and/or cysts onto a fresh agar plate coated with bacteria as before. *Naegleria fowleri* and *Acanthamoeba* spp. can also be grown successfully on mammalian cell cultures. *Balamuthia mandrillaris* will not grow on bacteria-coated agar plates. However, it can be isolated from infected human or animal tissue by inoculating monkey kidney or human lung fibroblasts with the triturated tissue and from which a continuous culture can be established by periodic transfers.

Naegleria fowleri and *Acanthamoeba* spp. can be grown axenically (bacteria free) in a complex chemical medium. Although several different formulations are available, in our laboratory we use a modified version of Nelson's medium that contains a 0.5% solution of liver digest, 0.5% glucose and a low osmolarity buffered salt solution supplemented with 3–5% fetal bovine serum. *Acanthamoeba* spp. can also be easily grown in a medium composed of 2% proteose peptone, 0.5% yeast extract and 0.1% glucose made up in a low osmotic buffered salt solution with or without serum. Additionally, *N. fowleri* and several species of *Acanthamoeba* have also been grown in a chemically defined medium consisting of several different amino acids, vitamins, hemin and salts.[1] [2] *Balamuthia mandrillaris* has also been grown in an axenic medium, and this has facilitated the screening of various pharmaceutical agents. [9]

EPIDEMIOLOGY

Naegleria fowleri is widely distributed throughout the world and has been isolated from fresh water, thermal discharges of power plants, heated swimming pools, hot springs, aquariums, soil, dust in air, sewage and even from nasal passages of children. *Naegleria fowleri* is thermophilic and grows well, even at temperatures of up to 113°F (45°C). It is therefore not surprising that primary amebic meningoencephalitis (PAM) cases have occurred in the hot summer months when many people engage in aquatic activities in lakes, ponds and swimming pools that may harbor these amebae in large numbers. In colder climates these amebae will probably encyst and remain dormant in the sediments of fresh water lakes, ponds, rivers and swimming pools.[1] [2] [3] [10]

Acanthamoeba spp. have been isolated from soil, sewage, fresh, brackish and sea water, bottled mineral water, cooling towers of electric and nuclear power plants, physiotherapy pools, jacuzzis, heating and ventilating and air conditioning units, eye wash stations, dialysis machines, dust in the air, bacterial, fungal and mammalian cell cultures, contact lens paraphernalia, the nose and throat of patients who have respiratory complaints and healthy individuals, and biopsy or autopsy specimens of cornea, lungs, nasal sinus, skin and CNS tissue of humans and animals. *Acanthamoeba* spp. have been known to be hosts for *Legionella* spp., *Mycobacteria* spp. and echoviruses, which signifies the importance of these amebae to public health. [1] [2] [3]

Cases of GAE may occur at any time of the year and therefore transmission has no relation to climatologic changes.

Balamuthia mandrillaris has not as yet been isolated from the environment; it is, however, believed to have the same habitat as *Acanthamoeba* spp. and *N. fowleri*. *Balamuthia mandrillaris* has been isolated from biopsy and autopsy specimens of humans and other animals.[4] [7]

PATHOGENICITY

Naegleria fowleri

Primary amebic meningoencephalitis occurs in active healthy children and young adults with no known history of immune disorder. Most patients who have PAM have a history of swimming, diving or playing under water; the ameba enters through the nasal passages and the nasal mucosa is the initial site of the primary lesions. The route of invasion into the brain is through the fila olfactoria of the olfactory nerves. The trophozoites cross the cribriform plate and reach the subarachnoid space, which is richly vascularized and bathed in the cerebrospinal fluid (CSF), thus constituting an ideal medium for their proliferation and subsequent dissemination into the brain parenchyma and other areas of the brain.[1] [2] [3] [11]

Naegleria fowleri is a highly pathogenic and virulent ameba. However, the minimum number of amebae required to cause infection

and death in humans is not known. Experiments with infection have shown that mice, when just a few amebae are instilled intranasally, die of PAM in a similar fashion to humans. However, different isolates of *N. fowleri* vary in their degree of virulence: some are highly virulent whereas other isolates are only moderately virulent. Furthermore, any isolate can become avirulent on prolonged cultivation in an axenic medium. It is believed that the probability of infection in nature may depend upon the number and virulence of the amebae, the temperature and the type and source of the nutrient available to the amebae in the environment. [2]

The trophozoite of *N. fowleri* is highly phagocytic and induces necrosis of the CNS tissue. It is believed to ingest human tissue directly by producing a food cup or amebostome and by producing lysosomal hydrolases and phospholipases that degrade myelin. It has also been shown experimentally that the amebae exert a contact-dependent cytolysis mediated possibly by a multicomponent system that consists of a heat-stable hemolytic protein, heat-labile cytolysin and/or phospholipase A enzyme.[1] [2] [12]

The incubation period of PAM varies from 2 to 15 days depending on the size of the inoculum and the virulence of the amebae. In experimental infections with a mildly virulent *N. fowleri*, the incubation period has been as long as 3–4 weeks.[2]

Acanthamoeba spp.

Granulomatous amebic encephalitis, whether caused by *Acanthamoeba* spp. or *B. mandrillaris*, usually occurs in chronically ill, debilitated individuals, in immunosuppressed patients, those who have received broad-spectrum antibiotics or chemotherapeutic medications and those who have AIDS. [1] [11] [13] The pathogenesis of GAE is complex and poorly understood. It is believed that the immunity is predominantly T-cell mediated and therefore depletion of $CD4^+$ and T-helper lymphocytes permits the growth and development of the amebae. The incubation period is unknown and several weeks or months may elapse before the disease becomes apparent. The respiratory tract or the skin may act as the portal of entry. One hypothesis is that ulceration of the skin may enable the amebae to enter.[1] The route of invasion to the brain must be via the bloodstream because there are no lymphatic channels within the brain.[1] [13] Furthermore, trophozoites and cysts are often seen around blood vessels and within necrotic CNS tissue. The acanthamoebae are known to secrete enzymes such as lysosomal hydrolases, aminopeptidases and phospholipases, which may contribute to CNS damage. [1] [2]

Acanthamoeba *keratitis*

Acanthamoeba keratitis (AK) is the inflammation of the cornea caused by ocular trauma or by contact lens wear and the use of non-sterile homemade saline solution that has been contaminated with bacteria and fungi, which support the growth and multiplication of *Acanthamoeba* spp. The latter may secrete proteolytic and collagenolytic enzymes that may damage the corneal epithelium and thus contribute to the pathogenesis of AK. If proper treatment is not provided, AK may lead to a vascularized scar within a thin cornea, causing impaired vision or perforation of the cornea and loss of the eye.[14] [15]

PREVENTION

Primary amebic meningoencephalitis

The trophic and the cyst forms of *N. fowleri* are susceptible to chlorine and are killed at 1mg/l, provided the water temperature is 78.8°F (26°C) or below. If, however, the water temperature is above 78.8°F the chlorine concentration may need to be increased to 2mg/l. It is therefore important for swimming pools to be maintained properly with adequate chlorination at all times. As it is not possible to disinfect natural bodies of waters such as lakes and ponds in which *N. fowleri* may be found, appropriate warnings should be posted, particularly during the hot summer months.[1] [2]

Granulomatous amebic encephalitis

New and creative preventive measures need to be formulated because GAE produced by *Acanthamoeba* spp. and *B. mandrillaris* occurs principally in immunocompromised individuals. As *Acanthamoeba* spp. can grow and colonize hot water tanks, jacuzzis, filters used in heating, ventilating and air conditioning units, and in-line filters used for purifying portable water supplies and eye wash stations, periodic inspection of these systems is recommended.[1] [2] [3]

Acanthamoeba keratitis

It is recommended that eye care professionals educate patients about the proper care and use of contact lenses. Contact lenses and contact lens paraphernalia, particularly the solutions, should be kept meticulously clean. Contact lens wearers should follow the directions and recommendations of the manufacturers and eye care professionals. Additionally, contact lenses should not be used during swimming or other water sports.[14]

DIAGNOSTIC MICROBIOLOGY

Primary amebic meningoencephalitis

In individuals who have PAM, the CSF is characterized by pleocytosis, with a predominance of polymorphonuclear leukocytes but without bacteria. The CSF pressure is elevated (100–600mmHg). Glucose concentration may be slightly reduced or normal, but the protein content is elevated, ranging from 100mg/100ml to 1000mg/100ml. Computed tomography scans show obliteration of the cisterns around the midbrain and the subarachnoid space over the cerebral hemispheres. Differential diagnosis of PAM from acute pyogenic or bacterial meningoencephalitis is dependent upon the detection of the ameba in the CSF *in situ* under a microscope. Smears of CSF should also be stained with Giemsa or trichrome stains for the delineation of the characteristic nuclear morphology. Gram stain is not useful. Care must be taken to differentiate amebic trophozoites from macrophages. Many cases have been diagnosed retrospectively based on examination of hematoxylin and eosin-stained sections or immunofluorescent tests.[1] [2] [3] [11] Serologic tests are of no value in the diagnosis of PAM because most patients die too early (within 5–7 days) in the disease process to mount a detectable immune response.

Granulomatous amebic encephalitis

Examination of the CSF in patients who have GAE reveals lymphocytic pleocytosis with mild elevation of proteins and normal levels of glucose. Visual detection of *Acanthamoeba* spp. trophozoites in the CSF has rarely been reported. However, *Acanthamoeba* spp. have been identified in brain biopsies from several patients. Computed tomography scans and magnetic resonance imaging are nonspecific and of limited value in the diagnosis. Single or multiple heterogeneous, hypodense, non-enhancing, space-occupying lesions that involve the basal ganglia, cerebral cortex, subcortical white matter, cerebellum and pons may be encountered, suggesting a brain abscess, brain tumor or intracerebral hematoma. Brain and skin biopsies are important diagnostic procedures. *Acanthamoeba* spp. can be easily cultured from the brain, skin, lung and corneal tissue by placing a portion of the tissue that has been minced on non-nutrient agar plates coated with a layer of Gram-negative bacteria. Specimens for culture should be processed as quickly as possible. The incubation temperature depends upon the source of the samples. The agar plate should be incubated at 86°F (30°C), if the

specimens originate from cornea or skin, but at 98.6°F (37°C) if the specimens are from the brain, the lung or any other internal organ. Amebae, if present in the samples, will feed on bacteria and multiply by binary fission. If the plates are examined under the light microscope, distinctive track marks with an ameba at the end of each track may be seen. The amebae will differentiate into cysts after a few days of incubation. Amebae can be identified to the level of genus on the basis of the characteristic morphology of trophozoites and cysts. Additionally, *Naegleria* spp. can be identified if flagellates appear within 2–4 hours of a loopful of amebae from the agar plate being suspended in distilled water. Identification to the species level, however, is difficult on the basis of morphology alone; non-morphologic methods such as serology, isoenzyme analysis or DNA profiles therefore need to be used.[2] [6] [16] [17] [18]

Balamuthia mandrillaris will not grow on bacterized agar plates. However, *B. mandrillaris* can be isolated from the CNS by inoculating monkey kidney cell culture with brain extract[4] [7] and subsequently growing the amebae in an axenic medium.[9]

Acanthamoeba keratitis

For the diagnosis of AK, deep corneal scraping and biopsy is recommended. Unfixed specimens should be processed for culture. Smears should also be prepared and fixed with methanol, Schaudinn's fixative or a spray-on fixative, and stained with Giemsa-Wright or Hemacolor, or with Wheatly's or Masson's trichrome stain. Hemacolor staining is quick and stains the distinctive cyst wall pinkish-red. The trichrome stains the nucleolus of the trophozoite reddish-pink and the cytoplasm greenish-purple and is therefore useful in differentiating trophozoites of *Acanthamoeba* spp. from the host cells.[11] Confocal microscopy has also been used recently to diagnose AK.[19]

CLINICAL MANIFESTATIONS

Primary amebic meningoencephalitis

The first case of PAM was reported in 1965.[1] [2] [3] Although at that time the case was thought to be caused by *Acanthamoeba* sp., it is now considered to be caused by *N. fowleri*. It is believed that more than 200 cases of PAM have occurred worldwide as of March 2002. Further, as many as 100 cases have been reported in the USA alone. Primary amebic meningoencephalitis has also been described in a South American tapir and cattle.[1]

Primary amebic meningoencephalitis is an acute, fulminating and usually fatal CNS disease that occurs mainly in healthy young adults and children with a recent history of watersport activities. Primary amebic meningoencephalitis has a rapid onset and a short incubation period that lasts from 3 to 7 days. It is characterized by the sudden onset of bifrontal or bitemporal headaches, fever, nausea, vomiting and stiff neck. Nuchal rigidity usually occurs with positive Kernig's and Brudzinski's signs. Abnormalities in taste or smell and cerebellar ataxia may be seen early but photophobia occurs late in the clinical course. An increase in intracranial pressure has been reported in most patients. Generalized seizures leading to lethargy, confusion, coma and death within 48–72 hours have been reported in a number of patients.[1] [11] Focal myocardial necrosis has also been reported.

Pathologic features

At autopsy the cerebral hemispheres are swollen and edematous. The olfactory bulbs and the orbitofrontal cortices are necrotic and hemorrhagic. Because of increased intracranial pressure, uncal and cerebellar tonsillar herniations are usually seen. The arachnoid membrane is severely congested with scant purulent

exudate. Amebic trophozoites are usually seen within the Virchow-Robin spaces with minimal or no inflammatory reaction (Fig. 244.4). Cysts are not seen

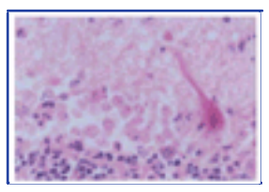

Figure 244-4 CNS section demonstrating numerous trophozoites of *Naegleria fowleri.* Note the absence of cysts. (H & E.)

within the CNS lesions. Necrotizing angiitis may occasionally be seen.[1] [2] [11]

Granulomatous amebic encephalitis produced by *Acanthamoeba* spp. and *Balamuthia mandrillaris*

Granulomatous amebic encephalitis caused by *Acanthamoeba* spp. occurs principally in individuals who are immunosuppressed (either iatrogenically or because of AIDS) and GAE caused by *B. mandrillaris* occurs in either the very young or very old and in individuals who have AIDS. As of 1 April 2002, more than 200 cases (114 caused by *Acanthamoeba* spp. and 93 caused by *B. mandrillaris*) of GAE had occurred worldwide. In the USA as many as 82 cases attributable to *Acanthamoeba* spp. (60 in people with AIDS) and 44 attributable to *B. mandrillaris* had occurred. Additionally, several cases of GAE caused by *Acanthamoeba* spp. and *B. mandrillaris* have been described in gorillas, a baboon, monkeys, dogs, sheep and cows.[1] [3] [20]

The clinical manifestations and pathologic features of GAE are similar regardless of which of these two organisms is the cause. It is an insidious disease and has a long and protracted clinical course. Clinical signs include personality changes, headache, low-grade fever, nausea, vomiting, lethargy, hemiparesis, seizures, depressed levels of consciousness and coma. Third and sixth cranial nerve palsies may be seen in some patients. Clinically, GAE may mimic bacterial leptomeningitis, tuberculous or viral meningitis, or single or multiple space-occupying lesions. Cerebellar ataxia and diplopia have been described in some patients. Pneumonitis with the presence of trophozoites and cysts within pulmonary alveoli has also been described.[1] [11] In most cases of GAE, however, final diagnosis is made at autopsy.

Pathologic features

The cerebral hemispheres are edematous. Encephalomalacia with multifocal areas of cortical softening and hemorrhagic necrosis may be seen. Multifocal necrotic lesions may also be seen in the posterior fossa structures, midbrain, thalamus, brainstem and cerebellum. Trophozoites and cysts of the infecting organisms are seen, most often in the necrotic lesions in basal ganglia, midbrain, brainstem and cerebral hemispheres (Fig. 244.5). Microglial nodules may be seen within the necrotic tissues. Occasionally, angiitis may be seen with perivascular cuffing by inflammatory cells, chiefly lymphocytes, a few plasma cells and macrophages. In patients who have advanced AIDS there is very little inflammation. Trophozoites and cysts can easily be identified by light microscopic examination of the tissue

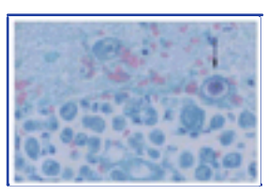

Figure 244-5 Brain section showing numerous trophozoites and a cyst (arrow) with typical features of *Acanthamoeba culbertsoni.* (Masson's trichrome.)

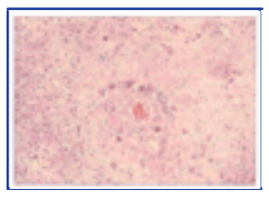

Figure 244-6 Trophozoites and cysts of *Acanthamoeba* sp. surrounding a blood vessel in a skin biopsy of a patient who has AIDS. A diffuse but modest inflammatory reaction is seen.

sections. Also in patients who have AIDS, multiple ulcerations of the skin with acute and chronic inflammation may be seen and the ulcers may contain trophozoites and cysts of the infecting ameba (Fig. 244.6). The kidneys, prostate gland, adrenal glands, lungs and liver may also be involved, suggesting hematogenous dissemination. The ulcerated skin may serve as a portal of entry for the amebae in some patients. Several cases of skin infection without dissemination into the CNS have also been reported.[1] [21] [22]

In general, the trophic and cyst stages of *Acanthamoeba* spp. and *B. mandrillaris* look similar in formalin-fixed and hematoxylin and eosin stained sections under the light microscope. In some patients, however, differential identification of *B. mandrillaris* can be made if the nuclei of the amebae in the sections possess two or three nucleolar elements because these are not seen in *Acanthamoeba* spp. (Fig. 244.7). A definitive identification may be arrived at by carrying out immunofluorescence analysis of the tissue sections using rabbit anti-*Acanthamoeba* spp. or anti-*B. mandrillaris* sera. The cyst wall of *B. mandrillaris* is characteristically tripartite and hence can be identified definitively by electron microscopy analysis of brain sections.[1] [4] [7]

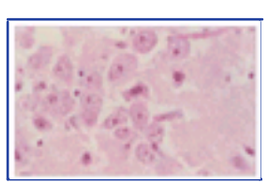

Figure 244-7 CNS section showing many trophozoites of *Balamuthia mandrillaris.* More than one nucleoli can be seen within the nuclei of the trophozoite.

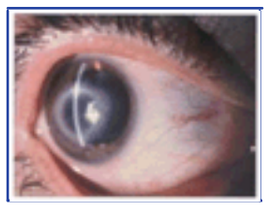

Figure 244-8 *Acanthamoeba* keratitis. Note the typical central or paracentral ring infiltrate. *Courtesy of Dr Theodore.*

Acanthamoeba keratitis

Acanthamoeba keratitis is a sight-threatening chronic inflammation of the cornea. It is associated with contact lens wear and the use of homemade nonsterile saline solution, corneal abrasion or trauma. Although the first case of AK in the USA was reported in a farmer from south Texas with ocular trauma of the right eye,[3] [14] wearing contact lenses and using nonsterile homemade saline was found to be a risk factor for AK in 1985 because most of the 24 patients reported in the previous 2 years were contact lens wearers.[3] [14] By December 1996, more than 700 cases of AK were estimated to have occurred worldwide.

The hallmark of AK is severe ocular pain, photophobia, a central or paracentral 360° stromal ring infiltrate (Fig. 244.8), recurrent breakdown of corneal epithelium with a waxing and waning clinical course and a corneal lesion refractory to the usual antibacterial, antiviral and antimycotic medications.[14] [15]

When examined under the light microscope, corneal scrapings and/or sections of biopsied corneal tissue reveal trophozoites and cysts of *Acanthamoeba* spp. infiltrated between the lamellae of the cornea (Fig. 244.9). Polymorphonuclear leukocytes, eosinophils, lymphocytes, macrophages and plasma cells, have also been seen occasionally. Ulceration, descemetocele formation and perforation are often seen in the later stages of AK.[11] [14] [15]

MANAGEMENT

Primary amebic meningoencephalitis

Primary amebic meningoencephalitis has almost always resulted in death. A few patients, however, have survived, probably because of

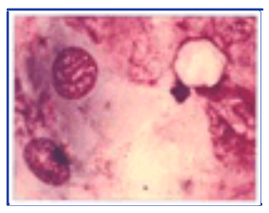

Figure 244-9 Characteristic star-shaped morphology of the cyst of *Acanthamoeba* sp. demonstrated by a corneal scraping stained with Hemacolor. *Courtesy of Dr Theodore.*

early diagnosis and aggressive treatment with amphotericin B, miconazole and rifampin (rifampicin).[23] Amphotericin B and miconazole can be administered intrathecally or intravenously, alone or in combination, and rifampin can be given orally. A 9-year-old girl from California, one of the patients who survived this disease in the USA, was treated as follows: intravenous amphotericin B 1.5mg/kg body weight per day in two divided doses for 3 days and then 1mg/kg for 3 days; intrathecal amphotericin B 1.5mg/day for 2 days followed by 1mg/day for 8 days; intravenous miconazole 350mg/m^2 body surface area per day in three divided doses for 9 days; intrathecal miconazole 10mg/day for 1 day followed by 10mg every other day for 8 days; and oral rifampin 10mg/kg per day in three divided doses for 9 days. The patient was also given sulfisoxazole (sulphafurazole) q6h for 3 days, but this was discontinued once *N. fowleri* was identified as the cause of the disease. Dexamethasone and phenytoin were given to lower the intracranial pressure and seizure activity, respectively. Amebae disappeared from the CSF after 3 days of treatment. Although cell counts and biochemistry of the CSF were abnormal for several months afterwards, the patient recovered completely without any neurologic sequelae.[23] It has also been reported that a boy from Texas, USA, recovered from PAM after intravenous amphotericin B and treatment in a hyperbaric chamber.[24]

Granulomatous amebic encephalitis

There is as yet no effective treatment for GAE and therefore the prognosis is poor.[1] [3] [25] [26] [27] The disease has been identified in only a very few patients before death. Three such patients who were diagnosed, however, have reportedly recovered from this disease. *Acanthamoeba rhysodes* was isolated from the CSF of a Nigerian patient with neurologic disease who showed clinical improvement after treatment with sulfamethazine. He was discharged from the hospital but was lost to follow-up.[2] [3]

Acanthamoeba culbertsoni was isolated from the CSF of an Indian patient with CNS impairment who recovered after treatment with chloramphenicol.[2] [3]

A 7-year-old girl from Barbados with a 'brain tumor' recovered without any neurologic sequelae after surgical excision. *Acanthamoeba healyi* was isolated from the 'tumor' and also demonstrated in the hematoxylin and eosin-stained, formalin-fixed sections. The patient also received oral ketoconazole treatment for 8 months postoperatively.[28] [29] It is known from *in-vitro* experiments that several drugs, including pentamidine, propamidine, dibromopropamidine, miconazole, paromomycin, neomycin, ketoconazole and 5-fluorocytosine, have inhibitory effects on several isolates of *Acanthamoeba*.[3] [30] According to one study a combination of pentamidine and azithromycin may be effective in treating GAE.[9] Prognosis of patients who have disseminated skin infections without CNS involvement is, however, good. In an immunocompromised (without HIV/AIDS) patient with *A. rhysodes* skin infection, skin ulcers healed after the following treatment regimen: cleansing of skin ulcers twice daily with chlorhexidine gluconate solution followed by the application of 2% ketoconazole cream. The patient was also given 4mg/kg intravenous pentamidine isethionate for 1 day. Because of pentamidine toxicity the dosage was cut to 2700mg and given over a 4-week period. Thereafter, he was given itraconazole orally at a dose of 200mg daily for 8 months, resulting in the total healing of the skin lesions.[21]

***Acanthamoeba* keratitis**

Treatment of AK with topical application of polyhexamethylene biguanide or chlorhexidine gluconate in combination with propamidine isethionate appears to be the treatment of choice, as several patients have been treated successfully in this way.[31] [32] Many patients have also been treated with topical application of propamidine isethionate 0.1% and dibromopropamidine, in conjunction with neosporin. Ketoconazole and clotrimazole appears to be effective *in vitro* and *in vivo*. Other reports of successful treatment with 0.1% hexamidine di-isethionate eye drops have been reported. Some patients have also been treated successfully with combinations of topical propamidine and miconazole and systemic ketoconazole, or topical clotrimazole or oral itraconazole with topical miconazole.[33] [34] Debridement of the cornea, penetrating keratoplasty and corneal grafting have also been performed with good results in some patients. Recurrence of AK has been reported after corneal transplantation, probably caused by cysts of *Acanthamoeba* spp. still present in the corneal stroma.

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Chapter 245 - Blood and Tissue Protozoa

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Emilio Bouza

Organisms of the genera *Plasmodium, Babesia, Toxoplasma, Leishmania* and *Trypanosoma* are the most prevalent protozoa able to invade blood and human tissues. They cause diseases of immense socioeconomic impact, most of them having a limited (although extensive) geographic distribution.[1]

MALARIA

Malaria means bad air in Italian, reflecting its association with marshy areas. It is caused by intraerythrocytic parasites first recognized by Laveran in 1880.

NATURE

Human malaria is caused by four species of *Plasmodium* parasites: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*.

The life cycle of *Plasmodium* spp. in humans is shown in Figure 245.1 . The sporozoite form of the parasite is cleared from the circulation in less than 1 hour and infects hepatocytes (intrahepatic cycle). Because the number of infected liver cells is low, there are no symptoms or signs of disease at this stage. The parasite cells multiply in the infected hepatocytes (liver schizonts), and after an average incubation period of 5–20 days the liver schizonts rupture. The young parasite cells liberated into the circulation invade red cells where they grow (trophozoites) and divide (schizonts). Parasites of the species *P. vivax* and *P. ovale* may persist for months or even a few years as intrahepatocytic schizonts in a 'dormant' state (hypnozoites) before a new intraerythrocytic cycle begins. The earliest parasitic stage in the erythrocyte is the trophozoite (*trophos*, nourishment), a growing ring of cytoplasm with a nucleus on one side. As the parasite grows, a brownish-green pigment (hemozoin) accumulates in the red cell. In the schizont stage (*schizo*, to split), the nucleus of the plasmodium cell divides repeatedly giving rise to 8–24 daughter cells called merozoites (*mero*, separated part). The red cell disintegrates, releasing the merozoites into plasma where they invade new erythrocytes, to repeat the intraerythrocytic asexual cycle. After a few intraerythrocytic cycles, some trophozoites, instead of becoming schizonts, undergo sexual differentiation and develop into large uninucleate female gametocytes (macrogametocytes) and male gametocytes (microgametocytes). The gametocytes undergo no further development in humans, and eventually degenerate or are destroyed unless a susceptible mosquito feeds on them. Sexual reproduction takes place in the stomach of the mosquito (sporogonic cycle), and as a result infective sporozoites accumulate in the salivary gland of the mosquito from where they are injected into humans in the next blood meal.

EPIDEMIOLOGY

Malaria incidence is estimated to be 500 million cases/year and malaria causes at least 2 million deaths (4% of all deaths). Children account for more than 1 million of these deaths, and malaria causes 15–25% of all deaths in children under 5 years of age. About 80% of cases occur in sub-Saharan Africa, and up to 30,000 travelers from Europe and North America contract the disease each year.

Malaria transmission occurs when an infected *Anopheles* mosquito bites a susceptible individual. Infections are more likely to be acquired during the wet season in the tropics or spring and autumn in temperate climates. Figure 245.2 shows the geographic distribution of the disease.

In highly endemic areas, the disease is acquired in early childhood and the whole population is re-infected periodically throughout life.

PATHOGENICITY

The production of disease by *Plasmodium* spp. is complex and not fully understood, but essentially consists of hemolytic anemia and an impaired microcirculation (see also Chapter 166).

The anemia in malaria is due to the rupture of parasitized erythrocytes, removal of parasitized and unparasitized erythrocytes by the spleen, capillary sequestration and bone marrow dyserythropoiesis. Bleeding and, in some cases, disseminated intravascular coagulation (DIC) may contribute to the pathogenesis.

The pathogenesis of microvascular flow impairment is controversial. It is characterized by the 'packing' of red blood cells containing mature forms of the parasite in the capillaries and venules. Hemozoin or, more recently, phospholipids have been proposed as the 'toxins' that trigger the interleukin (IL) cascade and subsequent febrile reaction. Antibodies to these phospholipids and tumor necrosis factor (TNF)-a levels are elevated in malaria and correlate with the prognosis in *P. falciparum* malaria.

Several genetic and immunologic factors offer protection against malaria or against the more severe forms of the disease. The absence of Duffy antigen in red blood cells, a trait that predominates in West Africans and derived populations, prevents *P. vivax* malaria.

In patients with hereditary elliptocytosis, in those with glycophorin C deficiency (Leach phenotype) as well as in those who are heterozygous for sickle cell disease, it has been observed that the incidence of malaria is lower than expected for the general population of a given area. Other hemoglobinopathies such as thalassaemias or glucose-6-phosphate dehydrogenase (G6PD) deficiency may offer a less clinically evident level of protection.

Untreated infected patients eventually develop curative immunity against the parasitizing strain. As several strains usually circulate in any given area, re-infection is extremely common. When the patient has successive infections from a number of strains, a certain degree of immunity to the infection develops. This partial immunity prevents severe disease, conferring tolerance to high parasitemias. The immune protection reached after repeated infections eventually disappears if re-infections do not occur for years. Iron deficiency, which is very common in holoendemic areas, seems to protect against severe disease.

PREVENTION

The prevention of disease can be approached individually or as a wider disease control program. Complete eradication of the disease

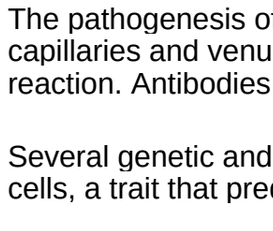

Figure 245-1 *Plasmodium vivax* life cycle. The life cycle starts when an infected mosquito feeds, inoculating the sporozoite form of the parasite, which infects the hepatocytes. The parasite cells then multiply (liver schizonts) and are liberated into the circulation to invade red cells where they grow (trophozoites) and divide (schizonts). Some trophozoites differentiate into gametocytes infective for mosquitoes.

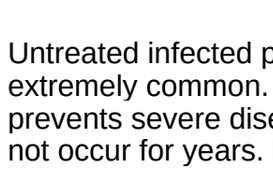

Figure 245-2 World distribution of malaria and areas of drug resistance, 2001.

is now considered to be unfeasible. The development of a vaccine has been attempted for decades with little success.

Personal preventive measures depend upon the estimated risk of infection based on itinerary, duration of stay, type of activities, accessibility to medical facilities, age and health. Counseling may then include protection from mosquito bites and appropriate chemoprophylaxis. The traveler must understand the importance of adherence

to preventive methods, but be aware that no method provides absolute protection and that symptoms may appear weeks or months after leaving the endemic area. Individuals at risk of developing severe disease such as infants, pregnant women or patients who have serious diseases should be discouraged from going to endemic areas. Appropriate protection against biting insects is strongly recommended.

In the few remaining areas of chloroquine-sensitive malaria, chloroquine prophylaxis is recommended. In chloroquine-resistant areas mefloquine, chloroquine-proguanil, atovaquone-proguanil or doxycycline may be recommended. The frequency of central nervous system (CNS) events with mefloquine prophylaxis is a matter of concern. Mefloquine prophylaxis usually should be taken for 1 week before entering a malarious area, while in the malarious area and for 4 weeks after leaving the area. Long-term (several months or years) prophylaxis is considered safe for chloroquine and mefloquine, and probably for doxycycline.[2] In areas of chloroquine resistance, strict personal antimosquito measures and chloroquine with proguanil is usually recommended to pregnant women. Mefloquine prophylaxis, which provides better protection, can be given after week 20 of pregnancy and is well tolerated. [3] Pregnancy must be avoided while on doxycycline prophylaxis. In areas of mefloquine resistance (parts of Thailand, Cambodia and Myanmar) doxycycline can be recommended for prophylaxis (Table 245.1).

Standby treatment is an alternative for selected patients. The standby treatment of choice for most areas would be atovaquone-proguanil. After leaving the endemic area, primaquine (15mg of base/day for 14 days) can be administered to eliminate any potential hypnozoites if exposure to *P. vivax* or to *P. ovale* infection has been intense.

Updated information on malaria risks and prophylaxis can be obtained from the US Centers for Disease Control and Prevention (CDC)[4] on a 24-hour toll-free telephone service (tel: (1) 877 FYI TRIP; fax: (1) 88 232 3299), or UK Travel Clinic, Hospital for Tropical Disease (tel: +44 171 388 9600), or through the internet (http://www.cdc.gov/travel/). The World Health Organization (WHO) also provides useful on-line information (http://www.who.int/ith).

TABLE 245-1 -- Malaria chemoprophylactic drug dosage.

MALARIA CHEMOPROPHYLACTIC DRUG DOSAGE		
Drug	**Adults**	**Pediatric**
Chloroquine phosphate	300mg base weekly (=500mg product)	5mg/kg base weekly
Mefloquine	250mg salt weekly	5mg/kg salt weekly
Doxycycline	100mg/day	2mg/kg/day Not for those under 8 years old
Proguanil	200mg daily	4mg/kg daily
Atovaquone ± proguanil	Atovaquone 250mg and proguanil 100mg daily	Atovaquone 62.5mg and proguanil 25mg. 11–20kg: 1 pediatric tablet daily (one extra tablet for each extra 10kg increase). Not for those under 11kg body weight
The maximum pediatric dosage is the adult recommended dose.		

DIAGNOSTIC MICROBIOLOGY

Laboratory confirmation of malaria is still based on microscopic examination of Giemsa-stained blood smears (see Chapter 166). The detection limit is around 50–500 parasites/µl of blood depending on the skill of the microscopist. Alternatively, visualization of plasmodia can be accomplished by fluorescent microscopy of capillary-tube centrifuged blood stained with acridine orange — quantitative buffy coat (QBC) — a slightly more sensitive method than thick films.[5]

If malaria is suspected and the first smear is negative, examinations should be repeated three times at 12-hour intervals, because in synchronous falciparum malaria the mature forms are sequestered from the circulation.

Parasitologic differential diagnosis should be made between the four *Plasmodium* spp. and *Babesia* spp. If a *Plasmodium* parasite cannot be identified to species level, it should be treated immediately as *P. falciparum*. The slides should then be sent to a reference laboratory and G6PD testing should be requested. The decision on treatment of possible intrahepatic hypnozoites using primaquine can be delayed until those results arrive.

Polymerase chain reaction (PCR) is highly specific and sensitive, detecting up to 0.3 parasites/µl of blood.[6] It has been found clinically useful in the detection of low parasitemia (as in the malarial hyper-reactive syndrome) and multiple-species infections.[7]

Antibody detection may be useful in the diagnosis of hyper-reactive malarial syndrome and for retrospective diagnosis. Recently developed immunochromatographic dipstick assays permit a rapid nonmicroscopic diagnosis of most cases of malaria. Their performance varies depending on the detected antigen, the level of parasitemia and the infecting *Plasmodium* spp. Compared with PCR tests devices that detect *P. falciparum* histidine-rich protein-2 have a sensitivity of 88% and a specificity of 97%. The test may persist positive weeks after successful therapy. The detection of aldolase or parasite lactate dehydrogenase permit the detection of high parasitemias of nonfalciparum malaria. In this device mixed infections would key out as *P. falciparum*. [8]

Serial parasite counts are recommended to monitor therapy;[9] 48 hours after the initiation of treatment, parasite counts are expected to be lower than 25% of initial counts. Quinine-related

TABLE 245-2 -- Features associated with a poor prognosis in *falciparum* malaria.*

FEATURES ASSOCIATED WITH POOR PROGNOSIS IN *FALCIPARUM* MALARIA		
Clinical	**Serum biochemistry**	**Hematology**
Agitation	? Glucose	Leukocytosis
Hyperventilation	? Bicarbonate	Severe anemia (PCV <15%)
Sustained body temperature	? Lactate	Platelets <50 × 10^6 /ml
>102.2°F(39°C)	? Creatinine	Fibrinogen <200mg/dl
Hypothermia	? Total billrubin	Prothrombin time >3 seconds
Bleeding	? AST 3×	Partial thromboplastin time ?
Coma	? ALT 3×	Parasitemia > 10^5 /ml
Convulsions	? 5-nucleotidase	Pigment-containing parasites >20%
Anuria	? CPK	Pigment-containing neutrophils >5%
Jaundice	? Myoglobin	
Shock	? Urate	
In patients who have premunition the parasitemia level has less prognostic value. CPK, creatine phosphokinase; PCV, packed cell volume; sAST, serum aspartate aminotransferase; sALT, serum alanine aminotransferase; 3×, three times normal.		

* *Adapted from White.*[11]

antimalarials do not kill gametocytes and so their presence in blood does not mean treatment failure. Recently, it has been shown that parasite lactate dehydrogenase is useful for monitoring patient progress.[10] Common laboratory findings are progressive anemia, moderate-to-severe thrombocytopenia and a normal white blood cell

count. Metabolic lactic or ketotic acidosis and other biochemical findings can be observed in severe disease (Table 245.2). Reaginic tests for syphilis may become positive.

CLINICAL MANIFESTATIONS

Malaria must be suspected in any febrile patient who has been in an endemic area in the previous 3 years. Occasionally, autochthonous cases are produced in nonendemic areas through transfusion of blood or blood products, sharing needles, maternal-fetal transmission or infection from an aircraft-transported infective *Anopheles* mosquito.

Before the onset of fever, non-specific symptoms such as malaise, headache, myalgia, fatigue, abdominal discomfort, dry cough, nausea, vomiting and diarrhea can occur, misleading the diagnosis.

Fever, the hallmark of malaria, usually develops 2 weeks after the infective bite, and in 95% of cases within the first 6 weeks and occasionally may follow the classic paroxysmal patterns:

- quartan pattern (peaks on days 1, 4, 7, etc.) — characteristic of *P. malariae;* or
- the tertian pattern of fever (peaks on day 1, 3, 5, etc.) — characteristic of all other species.

The absence of a typical fever pattern should not be a reason for disregarding malaria.

Physical examination may reveal tachycardia, splenomegaly, liver enlargement and jaundice. Malaria does not cause lymph node enlargement. In uncomplicated malaria, discrete hemolytic anemia, leukopenia and thrombocytopenia and other minor abnormalities of routine laboratory tests are usual.[11]

COMPLICATIONS

Patients infected with *Plasmodium* spp. other than *P. falciparum* rarely die of the infection in the developed world. The fatalities caused by 'benign' malaria are usually related to severe chronic anemia or rupture of an enlarged spleen. Patients infected with *P. malariae* may develop nephrotic syndrome.

On the other hand, *P. falciparum* infection must always be considered a life-threatening condition. The definition of severe malaria and complicated malaria is, however, controversial. On practical grounds, in developed countries where malaria is not endemic it is our practice to treat every patient who has *P. falciparum* malaria as an inpatient. Intravenous treatment is prescribed if the patient is seriously ill, regardless of strict fulfillment of the WHO definition of severe malaria.[12]

The most important complication of acute falciparum malaria is CNS involvement. Cerebral malaria is defined strictly as unrousable coma. Less severe neurologic manifestations are common, and high fever alone can cause confusion and delirium and, in children, seizures. In cerebral malaria, focal signs are uncommon and physical examination shows symmetric encephalopathy. Cerebrospinal fluid (CSF) is usually unrevealing. Untreated cerebral malaria is uniformly fatal. If adequately treated, the mortality ranges from 15 to 25%. There are persistent neurologic sequelae in 10% of children and 3% of adults.[13] A rare complication called postmalaria neurologic syndrome has been defined in patients recently recovered from *Plasmodium falciparum* malaria who have negative blood films at the time of onset of neurological or neuropsychiatric symptoms. Clinical manifestations emerge a few days or weeks after recovery from malaria and can be confused with mefloquine neurotoxicity.

Non-neurologic complications of malaria include renal failure, heart failure, acute pulmonary edema, adult respiratory distress syndrome, shock, coagulation disorders, severe anemia, hypoglycemia, metabolic acidosis, drug-related toxicity, malaria relapses and malarial hyper-reactive syndrome.

Relapse is defined as the reappearance of malaria originating from dormant liver schizonts, whereas recrudescence is the reappearance of disease after a partially effective treatment. Relapses can be expected in 50% of patients who have *P. vivax* or *P. ovale* infection who do not receive primaquine.

A condition called tropical splenomegaly or hyper-reactive malarial syndrome is occasionally seen in countries where malaria is endemic. It is defined as the presence of an enlarged spleen (often massive), raised IgM levels and high levels of antiplasmodium antibodies in patients who have negative smears for *Plasmodium* spp. and for whom no other cause of splenomegaly can be elicited.[14]

MANAGEMENT

Antimalarial therapy must be started in most cases without delay. The selection of treatment depends upon the parasite species and the expected resistance for that area. Details of drug regimens can be found in Chapter 166 .

Oral chloroquine is the treatment of choice for uncomplicated *P. falciparum* infections from chloroquine-sensitive areas, and for most *P. malariae*, *P. vivax* and *P. ovale* infections. Malaria due to the latter two species should also be treated with primaquine to eliminate hypnozoites. If the patient is pregnant or has G6PD deficiency, primaquine should not be used and any eventual relapses should be treated with chloroquine.

Occasionally, infections due to *P. vivax* are not cured by standard treatment. Chloroquine-resistant *P. vivax* and primaquine failure have been reported in areas of Asia and in Colombia.[15] It should be suspected if adequately treated *P. vivax* infection reappears within 1 month of treatment. In such a case mefloquine should be administered. If parasitemia reappears more than 1 month after the initial treatment, then primaquine failure should be suspected and more primaquine (22.5mg/day to a total cumulative dose of 6mg/kg) should be administered after chloroquine retreatment.

Uncomplicated *P. falciparum* infection from chloroquine-resistant areas can be treated with mefloquine taken with abundant water. The main side-effects are neuropsychiatric and cardiac. Intravenous physostigmine may reverse the severe neuropsychiatric side-effects of mefloquine.[16] Vomiting and diarrhea are associated with treatment failure. An acceptable alternative is 1 week of treatment with oral quinine, but lack of compliance may be a problem. When quinine is given combined with doxycycline, 3 days of treatment are equally effective. Pyrimethamine-sulfadoxine is a simple well-tolerated alternative, but parasite resistance has been reported from most endemic regions. Chloroquine plus clindamycin is also effective. Halofantrine is similar to mefloquine, but its cardiac toxicity is considered unacceptable if other alternatives are available. Atovaquone plus proguanil has proved efficacious for the treatment of uncomplicated, multidrug-resistant falciparum malaria.[17]

In some areas of Thailand, Myanmar and Cambodia, *P. falciparum* strains that have a higher level of resistance are common. Chloroquine or mefloquine does not prevent infections by these strains and their response to quinine, mefloquine and halofantrine is poor, unless these drugs are combined with other drugs. In these areas artemisin derivatives, such as artesunate, artemether or arteether, have been used extensively with remarkable success. Artesunate followed by mefloquine is the recommended treatment for malaria acquired in these areas. An alternative therapy is quinine plus doxycycline (clindamycin may be substituted for doxycycline in children

under 8 years of age and in pregnant women). In severe malaria, intramuscular artemether is as effective as quinine and less toxic.[18] [19] In severe *P. falciparum* infections, antimalarial treatment should be started parenterally because shock, vomiting and impaired swallowing may interfere with absorption. Therapy can be switched to oral administration after initial recovery,

Hyper-reactive malaria syndrome is treated with standard doses of antimalarials expected to be effective for the area of acquisition, followed by chemoprophylaxis while in the endemic area. Splenectomy is indicated if severe hypersplenism persists after months of continuous prophylaxis.

Supportive treatment is an essential part of the management of severe malaria. Seizures must be promptly treated or prevented in children and in adults with severe disease. Corticosteroids are not recommended for cerebral malaria. [20]

BABESIOSIS

Human babesiosis is an uncommon tick-borne parasitic zoonotic disease that was first described by Babes in Rumanian cattle in 1888. It was not reported as a human disease until 1957.

NATURE

The genus *Babesia* belongs to the family Piroplasmida and comprises over 70 species that parasitize mammals and birds. Infection in cattle is widespread and it is an economically important disease. Four species are the main potential human parasites:

- *Babesia bovis* and *Babesia divergens* in Europe;
- *Babesia microti* in the USA; and
- a *Babesia*-like piroplasm, designated WA1, that has been recently described in a patient from Washington State and might be the cause of the cases acquired on the west coast of the USA.[21]

EPIDEMIOLOGY AND PATHOGENICITY

Babesiosis in humans occurs predominantly in the USA, although a few cases have occurred in Europe and in other areas. It is highly endemic in the coastal areas of Massachusetts, Connecticut and New York State, where seroprevalence rates reach 4–6%, indicating that subclinical infections are common. In these areas rodents are the main reservoir and humans are usually infected by the bite of an infected nymph or *Ixodes dammini* tick. Transfusions of blood or blood products have caused a few cases. In Europe recent data indicate that most infections are not detected.[22]

PREVENTION

General recommendations include avoidance of known endemic areas, especially in the warm months of the year. Clothes should be sprayed with tick repellents before going on outdoor activities and on return the skin should be thoroughly examined for the presence of ticks.

DIAGNOSTIC MICROBIOLOGY

Babesia spp. are intraerythrocytic parasites that may resemble plasmodium trophozoites, but the hemozoin pigment is never present in babesiosis. Schizonts and gametocytes are not formed. Multiple erythrocyte parasitization is common and organisms are occasionally arranged in a characteristic Maltese cross shape (Fig. 245.3).

Parasitemia usually persists, demonstrable by light microscopy, for 3–12 weeks.

Serologic diagnosis can be made using indirect immunofluorescent antibody (IFA) assay. A serum titer of at least 1/256 is diagnostic of

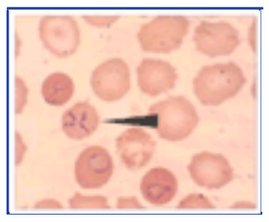

Figure 245-3 *Babesia* spp. Single and multiple intraerythrocytic parasites can be seen. The arrow marks a typical Maltese cross.

recent *B. microti* infection. Within a few weeks, most patients who have active infection have serum titers equal to or higher than 1/1024, falling over a few months to levels under 1/256.[23] Due to the lack of cross-reactivity, individuals exposed to the infection on the West coast of the USA should be tested also for antibodies to the *Babesia WA1* species.

PCR tests are more sensitive than microscopy but are not commonly available.

CLINICAL MANIFESTATIONS

The clinical forms of babesiosis vary widely depending upon the immune status of the host and the species of *Babesia* involved.

In the American Northeast, the symptoms of *B. microti* infection (Nantucket fever) range from a non-specific flu-like self-limited febrile illness to severe hemolytic anemia. It is most severe in those who are older or asplenic. The usual incubation period is 1–3 weeks. The onset is gradual with non-specific symptoms such as malaise, fatigue, anorexia, headache, emotional lability, myalgias and arthralgias. Blood leukocytes are not elevated and platelets are usually decreased. There may be a hemolytic anemia. The mortality rate is less than 10% of symptomatic cases.

The European cases typically occur in asplenic patients who present with a high fever and severe hemolytic anemia, and the infection is usually fatal. [24]

MANAGEMENT

Early treatment is recommended for all diagnosed cases to prevent long-term sequelae and potential transmission through blood donation.

The standard treatment is clindamycin, 600mg q8h plus 650mg of quinine q8h; both drugs are given for 7–10 days. Combination of atovaquone (750mg q12h) and azithromycin (500mg on day 1 and 250mg/day thereafter) for 7 days is also effective and is associated with fewer adverse reactions.[25] Chloroquine is not recommended. Blood transfusion and general supportive treatment may be needed for severe disease. Exchange transfusion has been used in an attempt to decrease parasite load, but its usefulness has not been demonstrated. It is recommended that co-infection by other tick-transmitted micro-organisms be ruled out.

TOXOPLASMOSIS

Toxoplasmosis is a widespread zoonosis, caused by the coccidian parasite *Toxoplasma gondii*, first recognized in 1908. *Toxoplasma* (from the Greek, meaning 'shaped as a bow') is a one-species genus.

Humans are usually infected orally, developing few, if any, symptoms of infection. The infection persists for life without signs of disease. However, during immunosuppression, quiescent parasites multiply, resulting in neurologic disease or, more rarely, other organ manifestations. Congenital transmission is a cause of major concern because it is frequently associated with severe disease in the newborn (see Chapter 65).

NATURE

Toxoplasma gondii is a parasite of many species of mammals and birds. The main forms of the parasite life cycle are oocysts, tachyzoites and bradyzoites.

The oocysts result from the sexual multiplication that takes place exclusively in the intestinal epithelium of cats and other felines. They are present in feline feces, and after maturing in the environment become the source of infection for noncarnivorous animals.

In the secondary host (all hosts but felines) *T. gondii* cells have two forms:

- rapidly multiplying tachyzoites (*tachy*, rapid), and
- 'dormant' bradyzoites (*brady*, slow).

When the host is infected by oocysts, tachyzoites or bradyzoites, a disseminated infection by tachyzoite forms takes place. Tachyzoites multiply inside the host cells, which rupture when 8–32 tachyzoites are produced. The tachyzoites released infect new cells. When the immune response controls the infection, some surviving parasites persist for many years in tissue cysts, which are sacs containing hundreds or thousands of *T. gondii* bradyzoites. They represent the parasite reservoir present in secondary hosts. Tissue cysts can be found in any tissue, but are most common in muscle and brain.

Cats become infected from eating birds, small rodents or other sources of raw meat. Humans and other animals become infected from either oocysts in soil and contaminated food or tissue cysts in raw meat. When acute infection occurs during pregnancy, tachyzoites are able to infect the placenta and, in a second step, the fetus.

EPIDEMIOLOGY

Toxoplasma gondii cysts are present in 5–92% of lamb meat and in a lower percentage of beef and pork meat. The prevalence of oocyst-shedding cats has been estimated at 1%. Infection rates in humans are very variable and depend upon eating habits and environmental factors. The prevalence increases progressively with age. In the 1990s the seroprevalence in women of child-bearing age with no obstetric history ranged between 37 and 58% in central Europe, Northern Africa and Australia, while in America and sub-Saharan Africa the range was 51–77%, and in India, South East Asia and China it was 4–39%.[26]

Human disease caused by *T. gondii* is much more restricted than the infection itself, and is generally a consequence of either intrauterine acquisition or immunocompromise.

PATHOGENICITY AND IMMUNITY

Toxoplasma gondii tachyzoites invade cells from nearly every organ, where they survive inside parasitophorous vacuoles. Resistance to the infection has been found to be enhanced by interferon (IFN)-? and diminished by IL-6. Extracellular tachyzoites are lysed by complement combined to specific antibody, and $CD4^+$ and $CD8^+$ T cells, lymphokine-activated killer and natural killer cells play a major role in infection control.

PREVENTION

Toxoplasmosis can be prevented at three different levels:

- prevention of primary infection;
- prevention of vertical transmission and congenital disease; and
- prevention of disease in infected immunocompromised individuals.

To prevent primary infection, the exposure to parasite can be reduced by health education (Table 245.3). No vaccine is presently available. Maternal immunity due to toxoplasmosis passed before conception protects the fetus from the infection. Immunodeficient patients receiving trimethoprim-sulfamethoxazole (co-trimoxazole)

TABLE 245-3 -- Recommendations to prevent toxoplasmosis.

RECOMMENDATIONS TO PREVENT TOXOPLASMOSIS
Do not eat raw or undercooked meat or eggs
Cured or smoked meat and sausages are considered safe. Freeze at -4°F (-20°C) for 1 or more days as an additional precaution
While handling raw meat, avoid touching the mouth or contaminating other food; normal hygienic washing of hands and utensils will suffice
Consume only pasteurized or ultra-heat-treated sterilized milk and dairy products
Wash or peel fruits and vegetables to be eaten uncooked
Control insect pests and their access to foodstuffs
Use gloves for gardening or handling sand where cats usually defecate
Avoid living with cats; if unfeasible, clean cat litter trays with nearly boiling water daily
Infection in cats can be reduced if they do not use raw meat, birds and rodents as a source of food

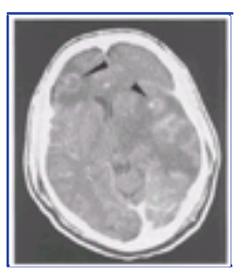

Figure 245-4 Toxoplasmic encephalitis in person who has AIDS. A cranial CT scan shows bilateral contrast-enhanced ring lesions with peripheral edema and mass effect.

as prophylaxis for *Pneumocystis* infection are substantially protected from toxoplasmosis.

Toxoplasma gondii IgG serology should be included in the initial work-up of pre-transplant patients, and transplantation of a solid organ from a seropositive donor to a seronegative recipient should be avoided if possible. If such a transplant is performed, then the recipient should have anti-*T. gondii* treatment for at least 2 months.

Individuals who have HIV infection and are seronegative for *T. gondii* should avoid being exposed to the parasite (Fig. 245.4), and should be periodically screened for seroconversion. For those patients who have HIV infection and are seropositive for *T. gondii*, further serologic tests do not provide useful information.

Maternal screening is controversial.[27] [28] Serologic screening is aimed at detecting acute maternal infection. It is, however, sometimes difficult to establish an early and reliable diagnosis of acute maternal or fetal infection. When acute infection is diagnosed in a pregnant woman, anti-*T. gondii* treatment, further assessment of fetal infection and abortion are offered.

DIAGNOSTIC MICROBIOLOGY

The diagnosis of *T. gondii* infection is usually made by serologic methods alone. The parasite can also be demonstrated by PCR, examination of stained tissue or fluid smears, cultivation in tissue cultures [29] and experimental animal inoculation.

Immunoglobulin G serology is widely used to screen for past infection. Immunoglobulin M appears earlier than IgG, declines faster and is a marker of relatively recent infection. In cases of toxoplasmic lymphadenopathy, 90% were IgM positive when tested within the first 4 months after the onset of lymphadenopathy and 22% remained positive when tested more than 12 months after the onset. In some cases, reactive IgM was undetectable.[30] [31] As the functional affinity of specific IgG antibodies is initially low and increases during subsequent weeks, the measurement of high-avidity antibodies helps to discriminate between recent (less than 3 months) and past infection. To diagnose a suspected case of acute toxoplasmosis IgG and IgM serology should be ordered in paired serum samples 3–4 weeks apart. If no antibodies are detected or if there is no seroconversion, acute toxoplasmosis can reasonably be ruled out. Avidity tests should be ordered if results are equivocal or IgM is positive.

CLINICAL MANIFESTATIONS

There are three different forms of toxoplasmosis: congenital infection, acute extrauterine infection and toxoplasmosis of the immunocompromised host.

Congenital toxoplasmosis

Toxoplasmosis acquired in utero can be asymptomatic or may produce signs of disease that can be present at birth.

The risk of congenital toxoplasmosis depends upon the time of acquisition of acute maternal infection.[32] Vertical transmission of *T. gondii* increases with gestation age (15–25% in the first trimester of pregnancy, 30–54% in the second and 60–65% in the third). Conversely, the severity of congenital disease is increased when infection occurs in early pregnancy. Signs of infection at delivery are present in 21–28% of those infected in the second trimester and up to 11% of those infected in the third trimester. Overall, 10% are born with severe disease.

Clinical manifestations of congenital toxoplasmosis include strabismus, chorioretinitis, encephalitis, microcephaly, hydrocephalus, psychomotor retardation and convulsions, as well as non-specific manifestations such as anemia, jaundice, hypothermia, thrombocytopenia, diarrhea and pneumonitis. The characteristic triad of hydrocephalus, cerebral calcifications and chorioretinitis resulting in

Figure 245-5 Fundoscopic image of toxoplasmic chorioretinitis. Most cases presenting in adults represent a late manifestation of a congenitally acquired toxoplasmosis.

mental retardation, epilepsy and impaired vision is the most severe and extreme form of the disease. Cerebral lesions may calcify, providing retrospective evidence of congenital toxoplasmosis.

Toxoplasma gondii chorioretinitis in immunocompetent patients is frequently secondary to congenitally acquired infection. The proportion of congenitally infected asymptomatic individuals who develop chorioretinitis later in life (usually in the first four decades) is unknown, but may well be over two-thirds (Fig. 245.5). Up to 30% of patients relapse after treatment.

Acquired toxoplasmosis

In 90% of cases, no clinical symptoms are apparent during acute infection. Most symptomatic patients present with enlarged lymph nodes that are located mainly in the head or neck area. Malaise and low-grade fever are present in less than 50% of symptomatic cases. Although *T. gondii* organisms are not usually seen, the histologic picture of acute *T. gondii* lymphadenopathy is usually diagnostic (Piringer-Kuchinka lymphadenitis). Occasionally, the lymph node histopathology can be mistaken for that of cat-scratch disease, other infections and lymphoma.[33]

Rare manifestations of acute infection include chorioretinitis, myositis and heart, lung, liver or CNS symptomatic involvement.

Toxoplasmosis in the immunocompromised host

Toxoplasmosis is a serious disease in patients who have profound immunosuppression such as those who have received a transplant or who have AIDS (see Chapter 127).

In transplant patients, the incidence and severity of the disease depends upon previous exposure to the parasite of donor and recipient, the type of organ transplanted and the degree of immunosuppression induced. The disease can be due to reactivation of a chronic silent infection or an acute primary infection acquired from the transplanted organ. The risk of developing toxoplasmosis for seronegative recipients of an organ from a seropositive donor is 57% for heart, 20% for kidney and minimal for liver transplants, reflecting the organ tropism of tissue cysts. [34] The disease manifests as a systemic disease with diverse degrees of multiorgan involvement including pneumonitis, carditis, hepatitis, myositis and encephalitis. In cardiac transplant recipients signs of myocarditis and pericarditis predominate.[35]

In patients who have AIDS, the clinical manifestations are usually related to CNS dysfunction or ocular lesions. Myocarditis is frequently found at autopsy, but is rarely clinically apparent. Infections of lung and other organs have also been reported.

Patients who become infected with *T. gondii* risk developing toxoplasmosis when their $CD4^+$ T cell count is less than 100 cells/mm^3 . The onset of *T. gondii* encephalitis is usually subacute. Fever and malaise usually precede the first neurologic symptoms. Headache, confusion, neuropsychiatric manifestations, seizures or other focal signs strongly suggest the diagnosis. Diagnosis is based upon clinical

data, neuro-radiology — magnetic resonance imaging (MRI) is the procedure of choice — and response to therapy. PCR testing of cerebrospinal fluid may be diagnostic too. *Toxoplasma gondii* encephalitis in patients who have AIDS usually presents as multiple abscesses in the basal ganglia or the gray-white matter junction with a contrast-enhanced ring (see Fig. 245.4). Diffuse *T. gondii* encephalitis is an uncommon rapidly fatal form.

In people who have HIV infection there is no good serologic marker of active toxoplasmosis. The presence of anti-*T. gondii* antibody means previous exposure and therefore present infection, but not necessarily disease. The absence of specific antibodies makes toxoplasmosis unlikely.

MANAGEMENT

Congenital toxoplasmosis

In gestational toxoplasmosis, the drug therapy may be intended to treat the mother, the fetus or the newborn (Fig. 245.6).[36] Spiramycin (not available in the UK) is a macrolide antibiotic that concentrates in the placenta, reducing placental infection by 60%.[37] It does not pass consistently through the placental barrier and it is used to decrease vertical transmission. Spiramycin 3g/day q8h should be given to pregnant women who have an acute infection from diagnosis to delivery unless fetal infection is proved. In such a case, the maternal regimen must be switched to sulfadiazine 4g plus pyrimethamine 25mg and folinic acid 15mg a day until delivery.[38] The risk of serious disease in early infections outweighs any consideration of potential teratogenic effects of antifolates used in the first trimester of pregnancy. All infected newborns should have anti-*T. gondii* treatment (sulfadiazine 50mg/kg q12h plus pyrimethamine 1mg/kg/day and folinic acid 5mg/kg/day for at least 6 months). No treatment regimen seems to decrease the rate of chorioretinitis

To confirm fetal infection, ultrasound fetal examination and sampling the amniotic fluid for *T. gondii* PCR and culture are recommended.[39] Fetal blood sampling obtained by cordocentesis has been commonly used to detect fetal antibodies and for *T. gondii* culture. Cordocentesis had an inherent risk of fetal loss of 2–3% and has been replaced by PCR testing of amniotic fluid.

Termination of pregnancy is commonly offered to women who seroconvert in the first 8 weeks of pregnancy and to those infected in the first 22 weeks of pregnancy when fetal infection is confirmed. A more conservative approach recommends abortion only if there is ultrasonographic evidence of hydrocephalus, although a small percentage of cases with neurologic disease will then be born.[36] [40]

Toxoplasmosis complicating a previous pregnancy is not an indication for prophylaxis during a subsequent pregnancy. Women may be reassured that they are not at greater risk of toxoplasmosis affecting the new pregnancy.

Acquired toxoplasmosis

Symptomatic *T. gondii* infection in nonpregnant immunocompetent individuals does not need to be treated unless there is visceral involvement or the patient has been infected in a laboratory accident.

Toxoplasmosis in the immunocompromised host

Standard anti-*T. gondii* treatment is sulfadiazine 4g/day q6h plus pyrimethamine as a first 100mg loading dose followed by 50mg/day and folinic acid 15mg/day for 6–8 weeks. If brain edema is of relevance corticosteroids should be given, but the clinical response to anti-*T. gondii* therapy would be masked by the unspecific improvement due to the reduction of edema. If the patient has not improved in the first 2 weeks of therapy a control computerized tomography (CT) or MRI scan should be repeated to review the empiric diagnosis. A repeat scan should be carried out after completing treatment. With

Figure 245-6 Toxoplasmosis risk management in pregnancy.

this regimen 80% of patients survive, although 50% of the survivors will have neurologic sequelae. Serious adverse effects to the medication can be expected in 40% of patients. In cases of intolerance to sulfadiazine, 75–100mg/day of pyrimethamine alone or with clindamycin has been used (see also Chapter 113 and Chapter 127).

Secondary prevention

Approximately 80% of AIDS patients relapse after an initial episode of toxoplasmosis. Daily treatment with pyrimethamine 50mg plus sulfadiazine 1g and folinic acid 10–15mg should be given to reduce the relapse rate to 5–10%. Current US Public Health Service (USPHS)/Infectious Diseases Society of America (IDSA) guidelines (http://www.hivatis.org) recommend that secondary prophylaxis may be discontinued in patients with sustained (>6 month) increase in CD4T cells (>200 cells/mm^3) (see Chapter 123 and Chapter 127).

LEISHMANIASIS

Leishmaniasis is the term used to describe a group of chronic parasitic diseases caused by a number of species of the genus *Leishmania*. The infection can be localized in the skin or mucous membranes or disseminated in the reticuloendothelial system. It is usually a zoonosis transmitted by Phlebotominae sandflies, which are endemic in areas of Southern Europe, Asia, Africa and the Americas.

NATURE

The genus *Leishmania*, along with the genus *Trypanosoma*, belongs to the order Kinetoplastida, which comprises flagellated protozoa that possess a characteristic extranuclear DNA mass called the kinetoplast.

Leishmania parasites are classified into two broad groups:

- New World *Leishmania* spp.; and
- Old World *Leishmania* spp.

Closely related species are grouped into complexes (Table 245.4). Members of *Leishmania braziliensis* complex have been renamed *Leishmania* subgenus *viannia*.

Sandflies are small (3–4mm), hairy mosquito-like insects, and transmit the disease. Old World sandflies belong to the genus *Phlebotomus*; New World species belong to the genera *Lutzomyia* and *Psychodopygus*.[41]

Sandflies become infected when taking a blood meal containing infected macrophages, which are present in blood or skin. Leishmania live in the digestive tract of the insect as flagellated parasites (the promastigote form). The parasites divide in the midgut of the insect except for the *viannia* group, which multiply in the hindgut. The highly motile promastigotes migrate to the buccal cavity of the insect, which regurgitates them on biting, thereby infecting a vertebrate host. Promastigotes rapidly penetrate macrophages and transform into an aflagellated oval body measuring 2–3.5mm across and called an amastigote (literally, without whip). Amastigotes live as obligate intracellular parasites inside the macrophages, where they multiply and are phagocytosed by other mononuclear cells when the host cell ruptures. The infection may disseminate to internal organs, spread locally to mucous membranes or persist as a chronic skin lesion.

TABLE 245-4 -- Summary of the epidemiology of leishmaniasis.

EPIDEMIOLOGY OF LEISHMANIASIS				
Complex	**Main species**	**Reservoir**	**Geographic distribution**	**Clinical disease**
Leishmania donovani	*L. donovani*	Humans	India, Bangladesh, Burma, Sudan, Kenya, Somalia	VL, PKDL, CL
	L. infantum	Dogs	A belt from Portugal to China	VL, CL
	L. chagasi (infantum)	Foxes, dogs, opossums	South of Mexico to northern South America	VL, CL
Not included in a complex	*L. major*	Rodents	Arid areas north of the Sahara, Arabia and Central Asia, sub-Saharan savanna	CL, LR
	L. tropica	Humans	Towns in East Mediterranean countries, Middle East and Central Asia	CL, LR
	L. aethiopica	Hyraxes	Highlands of Ethiopia and Kenya	CL, DCL, MCL
Leishmania mexicana	*L. mexicana*	Forest rodents	Tropical forests of Southern Mexico, Belize and Guatemala	CL (chiclero ulcer)
	L. amazonensis	Forest rodents	Tropical forests of South America	CL, DCL
	L. venezuelensis, L. garnhami, L. pifanoi	Unknown	Venezuela	CL
Leishmania braziliensis	*L. braziliensis*	Forest rodents, domestic animals	Tropical forests of South and Central America	CL, MCL, VL
	L. guyanensis	Sloths, arboreal ant-eaters	Guyana, Surinam, areas of Brazil	CL (forest pian), MCL
	L. panamensis	Sloths	Panama, Costa Rica, Colombia	CL (bejuco ulcer), MCL
	L. peruviana	Dogs	Areas of Peru and Argentina	CL (uta)
CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; LR, leishmaniasis recidivans; MCL, mucocutaneous leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis; VL, visceral leishmaniasis. Common names are in parentheses. Currently, there is no firm concensus on the species classification.				

EPIDEMIOLOGY

Leishmaniasis is endemic in 88 countries (Fig. 245.7). The world prevalence and annual incidence have been estimated to be 12 and 2 million people, respectively. The incidence of all forms of the disease is steadily increasing.

The vast majority of patients are infected by sandflies, but rare cases in which infection is transmitted by blood transfusion have been reported. Transmission by sharing needles among intravenous drug abusers has been proposed. [42]

PATHOGENICITY

The internalization of leishmania in the macrophage results from the interaction of complement CR3 and fucose-mannose receptors with the parasite major surface protein (Gp63) cysteine proteinases, and membrane lipophosphoglycan. Parasite survival inside the parasitophorous vacuole is facilitated by iron superoxide dismutase and acidic phosphatase secreted by the parasite. In vitro the parasite inhibits inducible nitric oxide synthase activity and nitric oxide production by macrophages. In animal models, susceptibility to infection is mediated by two subsets of $CD4^+$ T cells, named T-helper (Th)1 and Th2:

- Th1 lymphocytes produce IFN-?, which activates macrophage destruction of parasites; and
- Th2 lymphocytes produce lymphokines, which inhibit Th1 activity and activate an ineffective humoral response.

The $CD8^+$ T cells also play a major protective role. The host's immune system, parasite species and strain-dependent factors interact to create the full spectrum of clinical disease (see Chapter 172).

Figure 245-7 Geographic distribution of leishmaniasis. Visceral leishmaniasis (VL): 90% of cases occur in India, Bangladesh, Sudan and Brazil. Mucocutaneous leishmaniasis (MCL): 90% of cases occur in Bolivia, Brazil and Peru. Cutaneous leishmaniasis (CL): 90% of cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria.

DIAGNOSTIC MICROBIOLOGY

The laboratory diagnosis can be made by demonstrating *Leishmania* by microscopy or culture, detecting parasite DNA by PCR techniques or demonstrating immune response by serology or leishmanin cutaneous tests.

To study skin lesions, smears can be prepared from the tissue juice of the raised edge of an ulcer. The sample can be aspirated with a needle or scraped with a blade. Parasites can be detected by microscopy or culture in only 30% of cases of leishmaniasis recidivans, 50% of cases of mucocutaneous leishmaniasis (MCL) and 70% of cases of cutaneous leishmaniasis (CL).

In cases of visceral leishmaniasis (VL) sternal bone marrow aspirate is the sample of choice for parasitologic diagnosis (62–100% sensitivity). Splenic fluid aspirate has a higher diagnostic rate (95–100%), but splenic rupture may complicate the procedure. Other samples such as blood, lymph nodes or liver tissue may be of value. To increase positive yield, samples should be routinely cultivated in special media. Species and strain identification can be accomplished by monoclonal antibody staining, PCR and isoenzyme typing.[43]

Serologic testing is useful in the diagnosis of VL and can be of value in cutaneous forms of leishmaniasis. The indirect fluorescent antibody test (IFAT) is sensitive and group specific and its sensitivity is 80–100% in VL patients who do not have HIV infection and 77–89% in MCL. In CL, low titers can occasionally be found. Latex and direct agglutination tests are useful for low-technical testing on field working conditions. Serologic cross-reactivity has been found in the sera of patients who have leprosy, tuberculosis, Chagas' disease, African trypanosomiasis, malaria and schistosomiasis. Refined enzyme-linked immunosorbent assay (ELISA) tests have shown promising results in VL and CL.[44] A proportion of individuals who have HIV infection and VL have persistently negative serology. The IFAT is positive in 22–57% of cases, whereas ELISA combined with IFAT is positive in 55–71% and the Western blot *Leishmania* test is positive in 83% of cases.[45] Polymerase chain reaction testing is superior to bone marrow culture and microscopy.[46] The detection of parasite antigens, that do not depend on serologic response, may eventually prove very useful.

Leishmanin tests (Montenegro reaction) assess cutaneous reactivity against a phenolized promastigote suspension. It is a useful epidemiologic marker of past infection. The test is almost invariably negative in patients who have VL, and in 80% becomes positive 6 months after successful treatment.[47] In localized cutaneous forms the test becomes positive within 3 months of appearance of the lesion and remains positive for life. In diffuse cutaneous leishmaniasis (DCL), this test is universally negative.

CLINICAL MANIFESTATIONS

Most cases of leishmanial infection are probably subclinical. The disease produced can be classified in two groups: VL (also called kala-azar) and CL. Cutaneous disease can be divided into Old World CL, New World CL and MCL. Less frequent forms include DCL, cutaneous recidivans leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL) (see also Chapter 172).

Visceral leishmaniasis

The population at risk includes children under 5 years of age, immunocompromised patients and those who are malnourished. The incubation period is usually 2–8 months. Most patients do not recall having a suggestive skin lesion during the previous months. The onset is usually as an insidious febrile illness, but can be sudden with a high fever and chills. An enlarged spleen and moderately enlarged liver and lymph nodes can be detected on physical examination. As the disease progresses, signs of anemia, ecchymoses and abdominal discomfort become apparent. Anemia is due to splenic sequestration, hemodilution, bone marrow infiltration, hemolysis and bleeding. Thrombocytopenia and leukopenia are also common. In advanced disease, massive spleen enlargement, emaciation, ascites, subcutaneous edema, diarrhea, bleeding and severe anemia develop.

The mortality rate of untreated disease is 85–95%. Intercurrent infections are the cause of death in most cases.

Visceral leishmaniasis in immunocompromised patients

Patients who have HIV infection and immunosuppressed transplant recipients have an increased risk of developing leishmaniasis. In Southern Europe, the prevalence of leishmaniasis among AIDS patients is 2–9%. This represents 10,000 times the rate in the general population.[48] Several years may elapse from exposure to development of the disease. Immunodepressed patients may develop VL when infected with strains that in immunocompetent individuals cause only CL. Atypical manifestations of VL encountered in these patients include localized lesions on bony prominences (Fig. 245.8), tongue, larynx, intestinal tract and lung, and Kaposi's sarcoma. Most of these atypical manifestations have also been recognized in non-AIDS patients who have advanced disease. In immunocompromised patients the disease almost invariably relapses after treatment.[49]

Old World cutaneous leishmaniasis

There are four forms of CL with distinctive clinical and immunologic features.

Oriental sore or localized CL appears on the site of the sandfly bite after approximately 2–4 weeks. The lesion begins as an asymptomatic papule, which enlarges and forms a well-circumscribed ulcer with a raised violaceous border. The lesion spontaneously resolves over a period of months, leaving a depressed scar. Three species produce most cases: *Leishmania tropica*, *Leishmania major* and *Leishmania aethiopica*.

Leishmaniasis recidivans presents as a chronic skin lesion that appears on the skin areas where an oriental sore has been. It has also been called 'chronic relapsing CL' and 'chronic lupoid leishmaniasis' because it closely resembles lupus vulgaris both clinically and histologically.[50]

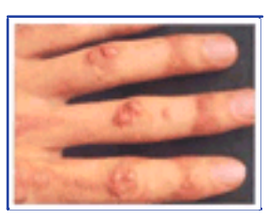

Figure 245-8 Cutaneous manifestation of kala-azar in AIDS.

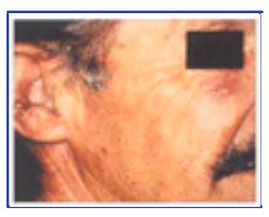

Figure 245-9 New World cutaneous leishmaniasis.

Diffuse cutaneous leishmaniasis is a rare form of diffuse skin infection characterized by heavy parasitization of skin macrophages and absence of an effective cellular response. The lesions do not ulcerate.

Post-kala-azar dermal leishmaniasis consists of symmetric macules or nodules on the face and sometimes on the trunk and extremities of patients who have had VL.

New World cutaneous leishmaniasis

Cutaneous leishmaniasis and MCL in the Americas take several clinical forms. The most notorious are chiclero ulcer, uta, cutaneous diseases caused by *Leishmania (Viannia) braziliensis* and MCL.

Chiclero ulcer caused by *Leishmania mexicana* was originally seen in latex (chicle) collectors who entered the forest. The lesion appears as a destructive ulcer of the pinna of the ear or the face and progresses over months (Fig. 245.9).

In uta, a disease of young children caused by *Leishmania peruviana*, the skin ulcer heals in 3–6 months leaving a disfiguring scar, usually on the face.

The cutaneous diseases caused by *Leishmania (viannia) braziliensis* complex consists usually of multiple shallow ulcers that have a tendency to metastasize along the lymphatic vessels. They evolve to mucocutaneous disease in 5% of cases. Untreated, such diseases persist for years. Relapses after treatment are common.

Mucocutaneous leishmaniasis, a markedly disfiguring, life-threatening disease, is typically caused by *L. braziliensis* and appears during the first 10 years after resolution of cutaneous disease. It consists of a pseudotumoral destructive lesion. It usually starts in the nasal mucosa and spreads to oronasopharyngeal mucosa, larynx and skin of the lips and nose. Death may be due to pneumonia, laryngeal obstruction, secondary sepsis or starvation. Spontaneous cure is rare and response to therapy is poor. Differential diagnoses include carcinoma, syphilis, yaws, rhinoscleroma and rhinosporidiasis.

MANAGEMENT

Treatment schemes can be divided in two groups — those applied to localized CL and those for extensive disease (VL, MCL and DCL).

Most cutaneous sores will slowly resolve spontaneously providing lifelong immunity. Indications for treatment are infection by *L. braziliensis* complex and sores in potentially disabling sites. Surgical excision and cryotherapy are suitable for small lesions. Heating at 131°F (55°C) for 5 minutes using infrared radiation cured 90% of cases of oriental sores.[51] Infiltration of the sore with antimonials is also effective: 0.5–1.5ml of parenteral solution is carefully injected into the edges and base of the ulcer and three doses are given 3 days apart. Systemic treatment is recommended for all forms of *L. v. braziliensis* complex infections (see Chapter 172).

Alternative treatments include topical aminosidine (paromomycin) ointment (once daily for 1 month), ketoconazole (600mg/day for 4 weeks), dapsone (200mg/day for 4 weeks) and oral allopurinol (5mg/kg q6h) plus probenecid (500mg q6h) for 1 month. Some immune enhancing methods have also been found to be effective. [52]

For VL pentavalent antimonials have been the first choice of treatment for years. There are two commercial preparations with similar efficacy and toxicity:

- meglumine antimoniate, and
- stibogluconate sodium.

The recommended dosage of antimony is 20mg/kg/day. The duration of treatment is 1 month for VL and 2–3 months for DCL and PKDL. If the patient is not thrombocytopenic, intramuscular administration is recommended. Intravenous drug should be infused over 2 hours to prevent cardiac arrhythmia. If higher doses are used, cardiac monitoring is needed. Serum amylase concentration should be periodically measured because pancreatitis is frequently seen in patients who have HIV infection. Other common side-effects are arthralgia, myalgia, an exfoliative rash and abdominal cramps. In Bihar state (India) antimonial resistance is now widespread. The membrane of leishmania amastigotes contains ergosterol. For this reason, antifungals that interfere with the ergosterol pathway can be used in leishmaniasis. Amphotericin B deoxycholate, and meglumine have similar rates of initial cure, and relapse-free intervals differing in the type of toxicity.[53]

New lipid-associated amphotericin compounds are theoretically targeted against leishmania because the amphotericin B-lipid complexes are cleared from blood by the monocellular/macrophagic system where the parasite persists. None of these preparations has been tested against conventional amphotericin B, but as the treatment is of shorter duration and toxicity is diminished, it will probably be preferred where costs are not a major factor.[54] [55] Present recommended cumulated dosage of liposomal amphotericin B is at least 18mg/kg (3mg/kg per day for 5 days, followed by 3mg/kg on day 10).[56]

Parenteral administration of aminosidine, a toxic aminoglycoside with parasiticidal activity has been used combined with antimonials for antimonial-resistant strains after amphotericin failure. Miltephosin (an alkyl phospholipid derivative developed as a treatment for breast cancer) is a new promising oral treatment.[57]

Continuous suppressive treatment with one dose of antimonials every 2–4 weeks is indicated for patients who have HIV infection and leishmaniasis after a full course of treatment.[58] Amphotericin every 2–4 weeks has also been used. Pentamidine might prevent relapses when used as prophylaxis against *Pneumocystis carinii* infection. When suppressive therapy was discontinued after successful antiretroviral therapy relapses were observed only in patients with CD4 counts under 350 cells/mm^3 . [59]

TRYPANOSOMIASIS

Trypanosomes produce two clinically and epidemiologically different diseases that are presented separately:

- American trypanosomiasis or Chagas' disease, and
- African trypanosomiasis or sleeping sickness.

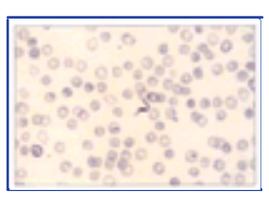

Figure 245-10 ***Trypanosoma brucei.*** Note the central nucleon, an undulating membrane and a clump of mitochondrial DNA called kinetoplast.

TABLE 245-5 -- Features of different types of human trypanosomiasis.

FEATURES OF HUMAN TRYPANOSOMIASIS			
	Trypanosoma brucei gambiense, Trypanosoma brucei rhodesiense	***Trypanosoma cruzi***	***Trypanosoma rangeli***
Geographic distribution	Africa	Central and South America	
Name of disease produced	Sleeping sickness	Chagas' disease	Nonpathogenic
Main affected organ	Brain	Heart, colon, esophagus	None
Vector (common name)	*Glossina* spp. (tsetse fly)	Reduviidae (kissing bugs)	
Mode of transmission	Bite (insect saliva)	Insect feces	Bite (insect saliva)
Multiplying form in humans	Trypomastigote	Amastigote	Trypomastigote

When a human host is infected the organisms multiply in the inoculation area, then pass into the blood, and in a third phase they localize in some internal organs causing chronic disease.

Trypanosomes pass through the morphologic stages of amastigote and trypomastigote (Fig. 245.10). The differences between the species that can be found in humans are summarized in Table 245.5 .

CHAGAS' DISEASE (AMERICAN TRYPANOSOMIASIS)

Chagas' disease is named after Carlos Chagas, who discovered *Trypanosoma cruzi* and described its life cycle and the clinical features of the disease.

The disease starts as an acute febrile illness followed by a long asymptomatic period and a chronic symptomatic stage characterized by distention and abnormal function of the heart and gastrointestinal tract.

Epidemiology

Chagas' disease is endemic in most Latin American countries. Low-income families of rural areas are most frequently affected and up to 20% of the population have the infection in the most affected regions. The WHO estimates that there are 16–18 million people who currently have the infection and that 2–3 million of them have chronic disease. It causes more than 45,000 deaths/year (see Chapter 173).

Pathogenicity

Hemipterous insects of the Reduviidae family (kissing bugs) are responsible for transmission. The parasite has a sylvatic cycle maintained by reduviids that feed on opossums and other wild animals, and which occasionally bite humans. It may also persist in a domestic cycle maintained by reduviids that live in the cracks of mud huts, feeding on humans, house rodents and domestic animals. In some areas, these two cycles overlap. As asymptomatic individuals may harbor a low number of parasites in their blood for many years, transmission by blood product transfusion is a major cause for concern. Oral transmission through breast milk or contaminated food and transplacental transmission are less common routes of infection.

Triatomid bugs infect when taking a blood meal from a host infected by *T. cruzi*. The parasite develops in the gut of the insect and infective trypomastigotes are released with insect feces. *Triatoma* spp. bugs bite humans at night and defecate soon after they are engorged, contaminating the skin with trypomastigotes that penetrate through skin erosions or mucosal surfaces (typically the conjunctiva). The parasite multiplies inside cells as amastigotes. Some amastigotes differentiate into trypomastigotes, which disseminate through the circulation and may eventually be eaten by a reduviid bug.

Although the histopathology of Chagas' disease is characteristic, the pathophysiologic mechanism that leads to these changes is not clearly understood. As a result of an inflammatory process (and perhaps a neurotoxin) the conducting system of the heart and the myenteric plexus are damaged. The mononuclear cell response gradually controls the infection, but does not usually eradicate it. In the late stages of the disease, only small numbers of parasites are found in cardiac muscle and the gastrointestinal tract, and autoimmune phenomena are probably responsible for the continuous progression of heart muscle damage. Neuronal depletion of the myenteric plexus leads to asynchronous peristaltic movement and, in the most severe cases, the esophagus, colon and other internal ducts dilate to enormous size.

Prevention

General improvement in housing standards or at least the use of noncracking plaster and the regular use of residual insecticides is recommended to eliminate domestic vectors. In endemic countries, blood donations should be serologically screened for trypanosome infection. Where this is not feasible, the addition of gentian violet to a bag of blood will 'trypanosome-sterilize' it.[60]

Diagnostic microbiology

Direct parasitologic diagnosis can be obtained by visualizing the parasite in blood or tissue, demonstrating it in culture or by xenodiagnosis. Conventional serology and PCR are especially useful for patients who have undetectable parasitemia.

Parasitemia is usually detectable in the acute phase of disease by staining the buffy coat. Needle aspiration or slit-skin smear of cutaneous inoculation lesions may be positive. The parasite can also be grown in leishmanial culture media.

Xenodiagnosis is the most sensitive method of parasite demonstration. [61] Fasted, laboratory-reared, triatomid bugs are placed in a box with a gauze on one side. The box is briefly applied to the forearm of the subject to let the bugs feed. After 10–30 days, the parasite can be found in the intestinal contents of the bugs. In acute

disease xenodiagnosis is nearly always positive, but in the chronic stage of disease it is positive in only half of the patients tested.

Serology is a reliable and sensitive method of diagnosis. It usually becomes positive 1 month after infection and remains positive for life.[62] The presence of antibodies that bind to the epitopes of living trypomastigotes (called lytic antibodies) is indicative of persisting infection. Cross-reactivity exists with leishmaniasis, malaria, toxoplasmosis, pemphigus foliaceus, infectious mononucleosis and *Trypanosoma rangeli* and mycobacterial infections.

Newly developed PCR techniques may be especially useful in defining parasitologic cure, diagnosing indeterminate-stage patients and assessing suspected cross-reacting serology or combined infections.[63]

Clinical manifestations

The disease evolves through three different clinical stages: acute infection, asymptomatic period and chronic disease (see Chapter 173).

Acute infection is symptomatic in only one-third of those infected. The severity and intensity of signs are greatest in younger patients, but the prevalence of infection increases with age. Symptomatic patients may develop an inflammatory nodule at the site of inoculation and an enlarged satellite lymph node. A characteristic feature is the presence of periocular edema due to conjunctival inoculation of *T. cruzi* (Romaña's sign). Manifestations of acute disease are fever, generalized lymphadenopathy, moderate hepatosplenomegaly, erythematous rash and myocarditis. Most patients survive the acute stage and gradually become asymptomatic within a few months. Congenitally infected newborns present with myocarditis or meningoencephalitis and usually die within days.

The indeterminate form is the asymptomatic period during which the infection can only be detected by serologic methods, although some degree of esophageal dysfunction or cardiac disease may be found when appropriate diagnostic methods are used.

The chronic symptomatic stage is characterized by the chagasic megasyndromes that develop in one-third of those infected, usually 5–15 years after infection (Fig. 245.11 and Fig. 245.12). Chronic cardiomyopathy is eventually present in 27%, digestive manifestations in 6% and neurologic disorders in 3% of infected individuals.

Congestive heart failure, arrhythmias and embolic disease characterize chagasic cardiomyopathy,[64] which is the most common cause of death in Chagas' disease. The presence of any of these conditions with suggestive epidemiologic data strongly suggest *T. cruzi* infection.

Esophageal disorders include achalasia, cardiospasm and enlargement. Megacolon results in constipation and abdominal pain. Associated complications include esophagitis, esophageal hemorrhage, esophageal cancer, volvulus and obstructive intestinal disease.

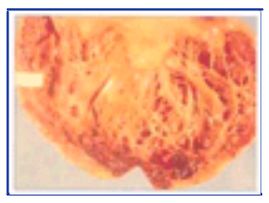

Figure 245-11 A dilated heart from a fatal case of chagasic cardiomyopathy. Chronic heart disease is the commonest cause of death in Chagas' disease. *Courtesy of Dr J Cohen, Cordoba, Argentina.*

Figure 245-12 Chagasic megacolon at autopsy. Grossly enlarged colon as shown here or other duct dilatation is characteristic of chronic Chagas' disease. *Courtesy of Dr J Cohen, Cordoba, Argentina.*

Latent disease reactivates in immunocompromised patients. In one series of AIDS patients who had Chagas' disease, 87% developed encephalitis and 21% acute myocarditis.[65]

Management

Antiparasitic therapy is clearly recommended only for the acute forms and parasitemic immunocompromised patients. Nifurtimox and benznidazole are the only two parasiticidal drugs used against *T. cruzi* (see Chapter 173).

Nifurtimox is seldom available. Benznidazole dosage is 7.5mg/kg up to 5g/day q12-24h given for 2 months. Some preliminary data suggest that the use of benznidazole in the early chronic stages and the use of allopurinol (600–900mg/day for 2 months) in chagasic heat disease may be beneficial.[66] Chagas' disease has traditionally been considered to be a contraindication for heart transplantation, but with the use of a low ciclosporin dosage, 80% survival at 24 months has been achieved.[67] Esophageal disease is treated with cardiac dilatation in early cases. Surgery is reserved for severe esophageal or colonic dilatation.

INFECTION BY *TRYPANOSOMA RANGELI*

Trypanosoma rangeli is an American parasite transmitted by triatomids. In some areas of Central America, human parasitization by *T. rangeli* is six times more frequent than that by *T. cruzi*. Although regarded as nonpathogenic to humans, it is medically important as a source of misdiagnosis of Chagas' disease.

AFRICAN TRYPANOSOMIASIS

Two African trypanosomes of the *T. brucei* complex infect humans:

- *Trypanosoma brucei gambiense*, which usually produces chronic infections; and
- *Trypanosoma brucei rhodesiense*, which tends to produce a more acute disease.

After an initial stage of parasitization restricted to the bloodstream, some individuals develop encephalitis. One of its most striking symptoms is a somnolence/insomnia disorder after which the disease was named 'sleeping sickness' (see Chapter 157).

Epidemiology

The vector of *T. brucei* subspecies is a fly of the genus *Glossina* (tsetse fly), which is geographically confined to tropical Africa. The incidence of the disease is unknown, but may be around 200,000 cases/year.

Several *Trypanosoma* spp. infect wild and domestic animals and the infection is an important economic problem.

Trypanosoma b. rhodesiense is transmitted by *Glossina morsitans*. This fly lives in the dry savannas of East Africa, feeding mainly on the wild ungulates, which represent the main reservoir. *Trypanosoma b. gambiense* is endemic in West Africa. Humans are considered to be the main reservoir. Transmission usually occurs in the shaded riverine areas where *Glossina palpalis* and *Glossina tachinoides* live and breed.

Trypanosoma brucei brucei is an animal pathogen that is unable to infect humans.

Nature

The vector fly is infected when taking a blood meal containing trypanosomes. The ingested parasites transform and multiply in the insect midgut and migrate to the salivary glands from where new hosts are infected by biting. In the human host the parasite multiplies asexually in the interstitial space and spreads through the lymphatic vessels to the lymph nodes and blood.

On human blood smears, two different morphologies can be observed:

- a short, stumpy form that is infective for *Glossina* spp; and
- a slender, actively multiplying, flagellated form.

Amastigote forms are not usually seen.

Pathogenicity

The parasite can produce hundreds of antigenically different surface glycoproteins serially. This process enables it to elude the host immune response, resulting in a characteristic fluctuating parasitemia pattern throughout the course of infection. After a variable period, the parasite reaches the CNS, causing meningitis and choroid plexus breakdown. Large amounts of IgM are produced in the serum and CSF. Characteristic plasmacytic morular cells can be seen in tissues, blood, bone marrow and CSF. Autoimmune phenomena, circulating immune complex deposition and increased vessel permeability play an important role in pathogenesis (see Chapter 157).

Prevention

Massive clearance of *Glossina* spp. habitats, insecticide spraying, destruction of wild game, periodic mass treatment and enforced relocation of entire populations were successfully used in the past. A more recent development for control is the use of *Glossina* spp.-attracting traps. Active surveillance is recommended periodically in areas of *T. b. gambiense* and during epidemics in *T. b. rhodesiense* areas. Travelers visiting endemic areas should be warned of the type of activities that may increase the chances of being bitten by tsetse flies and report any possible exposure. Mosquito repellents may be useful.

Diagnostic microbiology

The diagnosis of the disease essentially depends upon the careful microscopic examination of blood or CSF samples. Given the fluctuating nature of parasitemia, daily samples should be taken for 2 weeks to rule out parasitemia. Lymph node and bone marrow aspiration may be positive in cases with a low parasitemia. In-vitro culture and miniature anion exchange column test are also feasible. Serologic tests to detect antibodies against invariant surface antigens or against the most prevalent variant surface glycoproteins and PCR testing are available in some reference laboratories.

Clinical manifestations

In African trypanosomiasis, the disease develops in two stages:

- a hemolymphatic stage that spares the nervous system; and
- a second stage defined by CNS involvement.

The disease produced by *T. b. gambiense* usually develops chronically whereas in *T. b. rhodesiense* infection the disease tend to be more acute, without a clear distinction between stages.

An infective bite by a *Glossina* spp. fly may produce an inoculation chancre. Within days or weeks, the parasite disseminates to the blood. In the Gambian form of the disease, an enlarged neck lymph node may be present (Winterbottom's sign) and the patient may remain asymptomatic for years. In the hemolymphatic stage, the symptoms may be few and non-specific: undulant fever, headache, malaise, weight loss, anemia, edema, arthralgia, diarrhea and pruritus.

On physical examination there may be erythematous circinate papules on the trunk, disproportionate pain to soft tissue pressure (Kerandel's sign) and discrete enlargement of lymph nodes, liver and spleen. Pancarditis is common in the more acute forms, when it constitutes a major cause of death. Some degree of anemia, thrombocytopenia, leukocytosis, hypogonadism, renal disease and thymus atrophy may be seen.

The CNS manifestation that gave the disease its name is the disappearance of the circadian distribution of sleep and wakefulness, which are therefore fragmented throughout the day and night.[68] Other CNS manifestations include altered reflexes, paresthesiae, pareses, dyskinesia, choreoathetosis, epilepsy, slurred speech, mood changes, lethargy, delirium and psychosis. Without treatment, nearly all patients will develop neural involvement and die (see also Chapter 157).

Management

The choice of antiparasitic drug will depend upon whether the CNS is infected. Pentamidine or suramin can be used when the CNS is spared from infection. Melarsoprol and eflornithine are effective for both hemolymphatic and neural stages. Suramin dosage is 20mg/kg/day (maximum 1500mg), one intravenous injection every 5 days for 25 days. *Onchocerca volvulus* co-infection should be ruled out or treated before treatment of trypanosomiasis. Pentamidine dosage is 4mg/kg/day, one dose every second day for 20 days. *Trypanosoma b. rhodesiense* and some strains of *T. b. gambiense* do not respond to pentamidine.

Melarsoprol, a toxic trivalent arsenical derivative, has been the treatment of choice for patients who have CNS involvement for five decades. A number of empiric, dosing schemes have been used.[69] Relapse after treatment occurs in up to 6% of cases. Arsenic-related encephalopathy occurs in 10% of cases and prednisone treatment may reduce its incidence. The mortality rate of this side-effect largely depends upon the supportive treatment available, but is generally above 50%.[70]

Eflornithine (a-difluoromethylornithine) is an inhibitor of polyamine biosynthesis. Adult dosage is 400mg/kg/day in four divided doses for 1–4 weeks. Although it is less toxic than melarsoprol, drug shortages, high costs and the lack of comparative studies have relegated its use to a second-choice therapy.

General supportive treatment, anticonvulsant preventive therapy, and early recognition and treatment of associated parasitic and bacterial infections are essential.

With adequate treatment, most extraneural disease is cured. In the presence of CNS infection, cure rates are above 80%. All treated patients should be carefully followed up twice yearly for at least 3 years.

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Chapter 246 - Helminths

John H Cross

Helminths are the most common parasites infecting humans. The world population is over six billion and there are probably a similar number of helminthic infections occurring in humans. They are transmitted to humans by food, water, soil and arthropod and molluscan vectors. Once established, they can be found in all organs, particularly intestines but also in liver, lungs, blood and occasionally in brain and other organs. This chapter describes some of these worms, their biology, epidemiology, pathogenicity, clinical aspects, diagnosis, management and prevention.

NATURE

The word helminth comes from the Greek meaning worm and refers to all types of worms, both free-living and parasitic. The major parasitic worms are found primarily in two major phyla:

- the Nematoda or roundworms; and
- the Platyhelminthes or flatworms, which are divided into the Trematoda or flukes and the Cestoda or tapeworms (Table 246.1).

Structurally the helminths have an outer covering called a cuticle or tegument. It may be tough or delicate and essentially protects the organism, especially from digestion, while in the host intestinal tract. This structure may also possess spines, hooks, cutting plates and stylets used for attachment or to aid in penetration. There are also suckers or acetabula used by the flatworms for attachment. Some species have lytic glands near the mouth and the secretions digest host tissue for food or lyse tissues during migration.

The helminths have digestive systems of various types, excretory systems and massive reproductive systems. Most of the trematodes are hermaphroditic, one worm possessing both male and female reproductive organs; the schistosomes, on the other hand, have separate sexes. Each segment of a tapeworm has both male and female sex organs, whereas the nematodes have separate sexes. Some worms produce larvae, but most produce eggs, which pass in the host's excrement. Worms in the circulatory system produce larvae, which are carried in the blood, are ingested by blood-sucking arthropods and are then transmitted to the next host via the bite. The life cycles of the parasites are highly variable, from simple direct egg transmission to complex cycles involving one or more intermediate hosts (Fig 246.1 , Fig 246.2 , Fig 246.3 , Fig 246.4). The classification of the worms is based upon internal and external structures, and reproductive stages of eggs, larvae and adults.

EPIDEMIOLOGY

Helminthic infections are the most common parasites of humans. The highest prevalence occur in tropical countries with poor or inadequate food supplies, and where insect, molluscan and other invertebrate vectors abound and sanitary conditions are poor.

Nematodes

Intestinal nematodes

Over one billion people, primarily children, are infected with *Ascaris lumbricoides*. In some areas of Indonesia infection rates are as high as 90%.[1] Nearly one billion people are infected with hookworm, *Necator americanus, Ancylostoma duodenale* or both. The former has a worldwide distribution, whereas the latter is found in certain parts of Africa, China, India and Japan. Hookworm prevalences are highly variable, being 35% in the Philippines and 90% in Indonesia, with infection rates highest in adult males.[1] Up to 800 million people are infected with *Trichuris trichiura*; both sexes and all age groups are infected and in the Cameroons 3.1–97.3% of 22,000 children were found to be infected.[2]

Strongyloides stercoralis, like other soil-transmitted helminths, is endemic in areas with high humidity, warm temperatures and poor sanitation. The prevalence of this parasite varies from 10% to 20% in areas of Africa, South America and Asia. In surveys conducted in Indonesia and the Philippines using the Harada-Mori filter paper test-tube technique on single-stool specimens the prevalence rates were usually less than 1%.[1] [3] There are reports, however, of rates as high as 25% in areas with concomitant high prevalence of human T-lymphocyte leukemia/lymphoma virus-1 virus infections.[4]

Pinworm or *Enterobius vermicularis* is a common nematode in temperate regions with an estimated 200 million infections worldwide. It is a parasite that most individuals acquire in childhood, but it is not uncommon in adults in a family with infected children. It is a common nematode infection in the USA. A high prevalence of pinworm is also reported in male homosexuals. [5]

Tissue nematodes

Larva migrans is a syndrome caused primarily by larvae of nematode parasites of lower animals. Larvae of dog hookworms such as *Ancylostoma braziliensis* and *A. caninum* penetrate human skin and migrate through this tissue causing a creeping eruption or cutaneous larva migrans. This occurs worldwide, but mostly in areas with warm, moist climates. *Ancylostoma caninum* can also cause an enteritis in humans, especially in Australia.[6] The larvae of dog and cat ascarids or *Toxocara* spp. cause visceral larva migrans in children when embryonated eggs are ingested. The larvae provoke granuloma formation, often in the central nervous system (CNS) and eye. Seroprevalence rates for *Toxocara* antibodies are high in England and France.

Although there are now seven recognized species of *Trichinella*, the only species that is important to humans is *T. spiralis*. This species has a worldwide distribution in the temperate regions,[7] whereas the other species are reported from animals in Africa (*T. nelsoni*), Arctic regions (*T. nativa*) and Palearctic and Nearctic regions (*T. pseudospiralis, T. britovi, T. murrelli*) and the Australian region (*T. papuae*). *Trichinella* spp. are ubiquitous and found in a wide variety of animal life. Transmission occurs among animals by the ingestion of *Trichinella*-infected meat. All types of carnivous mammals and birds are susceptible to infections. Most human infections are obtained by eating infected pigs, boars and other wild animals. Reports of trichinosis are sporadic, usually among groups who have participated in a communal meal. An epidemic in France reported several thousand with some deaths after the consumption of 'steak tartare' prepared from imported horse meat.[8]

TABLE 246-1 -- The major parasitic worms.*

THE MAJOR PARASITIC WORMS

Nematodes (roundworms)	Intestinal		
	Ascaris lumbricoides	*Necator americanus*	*Trichuris trichiura*
	Enterobius vermicularis	*Strongyloides stercoralis*	*Capillaria philippinensis*
	Ancylostoma duodenale	*Trichostrongylus* spp.	
	Tissue		
	Trichinella spiralis	*Angiostrongylus cantonensis*	*Phocanema* spp. (larvae from saltwater fish)
	Visceral larva migrans (*Toxocara canis or Toxocara cati*)	*Angiostrongylus costaricensis*	*Contracaecum* spp. (larvae from saltwater fish)
	Ocular larva migrans (*Toxocara canis or Toxocara cati*)	*Gnathostoma spinigerum*	*Capillaria hepatica*
	Cutaneous larva migrans (*Ancylostoma braziliensis or Ancylostoma caninum)*	*Anisakis* spp. (larvae from saltwater fish)	*Thelazia* spp.
	Dracunculus medinensis		
	Blood and tissues (filarial worms)		
	Wuchereria bancrofti	*Onchocerca volvulus*	*Dirofilaria immitis* (usually lung lesion; in dogs, heartworm)
	Brugia malayi	*Mansonella ozzardi*	
	Brugia timori	*Mansonella streptocerca*	*Dirofilaria* spp. (may be found in subcutaneous nodules)
	Loa loa	*Mansonella perstans*	
Platyhelminthes (flatworms)	Intestinal		
	Diphyllobothrium latum	*Hymenolepis nana*	*Taenia solium*
Cestodes (tapeworms)	*Dipylidium caninum*	*Hymenolepis diminuta*	*Taenia saginata*
	Tissue (larval forms)		
	Taenia solium	*Echinococcus multilocularis*	*Spirometra mansonoides*
	Echinococcus granulosus	*Multiceps multiceps*	*Diphyllobothrium* spp.
Trematodes (flukes)	Intestinal		
	Fasciolopsis buski	*Heterophyes heterophyes*	
	Echinostoma ilocanum	*Metagonimus yokogawai*	
	Liver/lung		
	Clonorchis (opisthorchis) sinensis	*Paragonimus westermani*	*Paragonimus skrjabini*
	Opisthorchis viverrini	*Paragonimus mexicanus*	*Paragonimus* spp.
	Fasciola hepatica	*Paragonimus heterotremus*	
	Blood		
	Schistosoma mansoni	*Schistosoma japonicum*	*Schistosoma mekongi*
	Schistosoma haematobium	*Schistosoma intercalatum*	

** Adapted from Garcia LS. Classification of human parasites. Clin Infect Dis 1997;25:21–3.*

The 'firey serpent of Medina' or *Dracunculus medinensis* (also known as the guinea worm) is reported from 17 African countries, Yemen, Saudi Arabia, India and Pakistan. Ten million people may be infected, and in some areas 50% of a population may have the infection every year. Sex prevalences are variable, but most infection occurs in those aged 15–40 years. Transmission is seasonal and closely related to rainfall, when the population of the major copepod vectors (*Cyclops* spp.) are abundant.

Angiostrongyliasis, or parastrongyliasis, is caused by the molluscan-borne nematodes *Angiostrongylus cantonensis* and *A. costaricensis*. The former is endemic in Asia, the Pacific Islands, Australia, India, Africa, the Caribbean and Louisiana in the USA. *Angiostrongylus costaricensis* is reported in Latin America and Texas, but most human infections are reported from Costa Rica. Human infection with *A. cantonensis* is reported primarily from Taiwan and Thailand with infection acquired from eating the snails *Achatina fulica* in Taiwan and *Pila ampullacae* in Thailand. In Taiwan most infections are in children of both sexes while in Thailand the parasitosis is seen mostly in adult males.[9] One human infection has been reported in a young boy in Louisiana.[10] *Angiostrongylus costaricensis* is usually seen in children who accidently ingest the slug *Vaginulus plebeius* on vegetation. Rats (*Rattus* spp.), cotton rats (*Sigmodon* spp.) and rice rats (*Oryzomys* spp.) are natural hosts for *A. costaricensis*.

Anisakiasis is caused by third-stage larvae of *Anisakis simplex* and *Pseudoterranova decipiens*, which are acquired by eating species of raw marine fish and squid. The adult worms, related to *Ascaris*, are found in marine mammals worldwide, but most infections in humans have occurred in Japan and other countries where people eat uncooked marine fish and squid.

Several *Gnathostoma* spp. larvae can invade human tissue and most infections are reported from Thailand and Japan, and occasionally Mexico. *Gnathostoma spinigerum* is the most common species in Thailand. *Gnathostoma hispidium* has also been found in humans, although there are fewer infections. Dogs and cats are the natural hosts for adult worms. Copepods serve as the first intermediate host, and fish, frogs, snakes and chicken are the second intermediate hosts. Infections are more common in adults of both sexes.

Blood and tissue nematodes

The filarids are a group of nematodes located in the lymphatics, subcutaneous and cutaneous tissues. There are eight major filarids of humans transmitted by arthropods in tropical and subtropical parts of the world.

Wuchereria bancrofti is the most widespread, being endemic in Asia, Africa, Central and South America and the Pacific Islands. Prevalence rates are highly variable depending upon the vector mosquito population, temperature, humidity, a susceptible human population and environmental sanitation. An estimated 115 million people may be infected worldwide, with 50 million in Africa, 62 million in Asia, 2 million in the Pacific Islands and less than 1 million in Latin

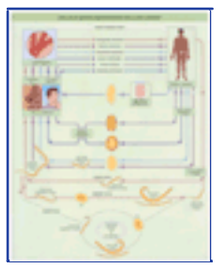

Figure 246-1 Life cycles of important human roundworms: adults living in the intestines.

Figure 246-2 Life cycles of important human roundworms: adults living in tissues.

Figure 246-3 Life cycles of important human flukes: adults living in the liver, lungs, intestines and blood.

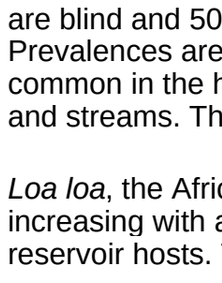

Figure 246-4 Life cycles of important human tapeworms: adults living in the intestines.

America.[11] There are different vectors in different endemic areas; the most extensive is *Culex quinquefasciatus*, whereas *Aedes polynesiensis* is the important vector in the Pacific Islands. There is a nocturnal periodicity associated with the feeding habits of the mosquitoes, except in the Pacific where there is a diurnal periodicity and the mosquito vector feeds in the daytime. Although a limited number of monkeys have been experimentally infected with *W. bancrofti* [12] no naturally infected animal reservoirs have been documented.

Brugia malayi is found only in rural Asia and infects approximately 13 million[11] people. Many of the islands of Indonesia are endemic, with prevalence rates from less than 1% to over 30%.[13] The major mosquito vectors are *Mansonia, Anopheles* and *Aedes* spp. and cats and *Macaca* and *Presbytis* monkeys are known reservoir hosts. Most strains of brugian filariasis are periodic, but some microfilariae exhibit subperiodic periodicity.

Brugia timori is found only in the Lesser Sunda Islands of Indonesia. A prevalence rate of 25% was reported in a population on the island of Flores.[14] *Anopheles barbirostris* is a major mosquito vector and, although no naturally infected reservoirs have been found, cats and Mongolian gerbils have been experimentally infected. [15]

Onchocerca volvulus is endemic in 26 countries in Africa and six in Central and South America. Approximately 18 million people are infected and as a result 270,000 are blind and 500,000 severely disabled.[19] Infections are more common in males than females, reflecting male occupations and their higher exposure to vector flies. Prevalences are also higher in areas close to running water where the blackfly *Simulium damnosum* and related species breed. In the Americas infections are more common in the highlands 1000–4000 feet above sea level, whereas in Africa infection is common below 1000 feet. Infections decrease with distance from the rivers and streams. There are no animal reservoirs and there is no microfilarial periodicity.

Loa loa, the African eye worm, is endemic in West and Central Africa where *Chrysops* spp. are present. Prevalence rates vary from 8% to 40%, with infections increasing with age; 20–30 million people live in endemic areas. Transmission by the day-biting female deerflies is highest during wet seasons. Monkeys serve as reservoir hosts. The microfilariae are diurnal.

Three *Mansonella* spp. are found in humans:

- *M. streptocerca* is found in Africa;
- *M. perstans* in West Africa and parts of South America; and
- *M. ozzardi* in Latin America.

Culicoides spp. serve as vectors for all three species and *Simulium* spp. also transmit *M. ozzardi*. Monkeys in Africa serve as reservoir hosts. Infections are highest in adult males and some areas report prevalence rates as high as 80%. There is no microfilarial periodicity.

Trematodes (see Chapter 168)

Over 40 million people worldwide are infected with food-borne trematodes; the most important occur in the liver, lungs, blood and intestines.

Liver/lung trematodes

The opisthorchid liver flukes are reported from Asia and Europe. The Chinese liver fluke, *Clonorchis sinensis*, is reported from China, Japan, Korea, Taiwan and Vietnam. Infections are highest in older males and females and there is a tendency toward familial aggregations. An estimated 7 million people are infected, with prevalence rates in China of 15–24.6%, in Korea 11.7–30.8% and in Japan 2.9%. Infections are highest in the snail and fish intermediate hosts in the warmer months. Snails in the families Hydrobiida, Melaniidae, Assimineidae and Thiaridae are first intermediate hosts, and fish, primarily of the family Cyprinidae, are second

intermediate hosts. *Opisthorchis viverrini* is endemic in Thailand, Laos and Vietnam.

The infection is found in an estimated 10 million people in all age groups increasing from childhood to adulthood, and in both sexes. Prevalences vary from 7.3% to 53.2% in Thailand. *Bithynia* spp. are important snail hosts and *Cyclocheilichthys* spp. of freshwater fish are important second intermediate hosts. Dogs, cats and other fish-eating mammals are reservoir hosts.[17]

Fasciola hepatica, the sheep liver fluke, is endemic in most sheep-raising areas of the world. Human infections are increasing and are most common in parts of Europe, South America and the Middle East. Approximately 2.4 million people are infected. Sheep, goats and cattle are the natural hosts, with infection rates varying from 25% to 92% in countries such as Bolivia. The prevalence in humans reaches 65% in Bolivia, 24–53% in Ecuador, 10% in Peru and 2–17% in Egypt.[20] Infections are more common in adults who usually acquire infection from eating watercress in salads.

Although over 40 species of *Paragonimus* are described, only a few infect humans and only one, the Oriental lung fluke, *Paragonimus westermani*, is responsible for the most serious disease. It is reported from China, Taiwan, Japan, Korea, the Philippines and Thailand. Other species occur in China, Thailand, Mexico, Ecuador, Peru and parts of Africa. The parasitosis is acquired by eating metacercariae-laden crustaceans: crabs and crayfish. In some areas the juice from crabs is used for seasoning and traditional medicine. The juice may contain metacercariae. There are 20 million suspected infections in the Chinese, and over 600,000 elsewhere.[20] There are many snail hosts, with *Semisulcospira* spp. being major vectors in China and Korea. The crabs *Eriocheir* and *Potamon* spp. and the crayfish *Cambaroides* spp. are important second intermediate hosts. Dogs, cats and other carnivorous mammals are reservoir hosts. Children often obtain the infection by eating crabs on the way home from school.

Intestinal trematodes

There is a very large number (about 70) of intestinal flukes and they are reported mostly in Asians who acquire infections because of eating raw food. *Fasciolopsis buski*, the giant intestinal fluke, is reported from China, Taiwan, Thailand, Indonesia, Bangladesh and India and occasionally from Indonesia. Over 200,000 infections have been reported in China and 10,000 in Thailand.[20] Pigs are the reservoir host, and snails such as *Segmentina, Hippeutis* and *Gyraulus* spp. are important vectors. Cercariae from snails encyst on water plants such as water caltrop, watercress, water bamboo and water chestnut. These are eaten uncooked and infections occur most often in children.

Other important intestinal flukes include *Echinostoma, Heterophyes* and *Metagonimus* spp. There are an estimated 150,000 cases of echinostomiasis, 240,000 cases of heterophyiasis and 650,000 cases of metagonimiasis, most in China, Korea and Japan. Echinostome metacercariae are acquired from snails, fish and other aquatic animals, whereas *Heterophyes* and *Metagonimus* infective stages occur in fish. All ages and both sexes are infected as a result of eating the second intermediate hosts uncooked.

Blood trematodes

Schistosomiasis is endemic to many tropical areas of the world:

- *Schistosoma japonicum* is found in Asia;
- *S. haematobium* and *S. mansoni* in Africa and the Middle East; and
- *S. mansoni* in South America and some islands in the Caribbean (see Chapter 167).

Other species of less importance are *S. mekongi* and *S. intercalatum*, which are focally located in South East Asia and Africa, respectively.

It is estimated that there are over 200 million infections, with the prevalence and intensity of infections being highest in children

aged 5–15 years. Infection rates depend upon water contact in endemic areas. Despite the widespread distribution of snails and frequent opportunities for water contact, high transmission only occurs at a few sites. It is important to identify these sites.[21] Snail vectors are members of the genera *Bulinus* spp. for *S. haematobium*, *Biomphalaria* spp. for *S. mansoni* and *Oncomelania* spp. for *S. japonicum*. *Bulinus* and *Biomphalaria* spp. are strictly aquatic whereas the *Oncomelania* are amphibious snails. There are some animal hosts for *S. mansoni*, but humans are the most important source of infection. Humans are also the most important host for *S. haematobium*, whereas many domestic and wild animals are a reservoir host for *S. japonicum*. Although *S. japonicum* is endemic on Taiwan, the strain of parasite on the island will not infect humans. It is considered to be a zoophilic strain.[22]

Cestodes

Cestode or tapeworm infections are acquired by ingestion of intermediate hosts containing the infective larval stages:

- the beef tapeworm *Taenia saginata* and pork tapeworm *T. solium* are acquired by ingesting the cysticercus stage in beef or pork;
- the fish tapeworm *Diphyllobothrium latum* is acquired by eating raw fish infected with the plerocercoid or sparganum stage; and
- *Hymenolepis nana* and *H. diminuta* are rodent tapeworms that use fleas and beetles as intermediate hosts (see Chapter 168).

Taenia saginata is reported worldwide, with high infection rates in Africa and Asia. Most infections occur in adults. The cysticercus is found only in bovids; however, in parts of Asia, especially in Taiwan, pigs are a recognized source of infection for a strain of the parasite, namely *T. saginata (asiatica)*.[23]

Taenia solium is found sporadically in pigs worldwide, with many human cases reported from Mexico and Central and South America, South West Asia and Africa. Human cysticercosis is also present in these areas. Certain areas of Irian Jaya in Indonesia report many cases of cysticercosis because of the habit of eating uncooked pork.

Hymenolepis nana is the most common tapeworm in North America and is particularly reported in children worldwide. *Hymenolepis diminuta* is not common but reported occasionally in South East Asia.

There is a high prevalence of *D. latum* in humans in Scandinavia, Finland, Alaska, Canada and Northern USA. Other species of *Diphyllobothrium* are reported from Japan and South America.

Sparganosis or infections with the larval stage of *Spirometra* spp. is reported occasionally from many parts of the world. Infection is acquired by ingesting copepods or second intermediate host (frogs, toads or other aquatic animals). Infections in southern Asia are attributed to the use of fresh animal tissues as poultices.

Echinococcus granulosus and *E. multilocularis* are associated with human hydatid disease, whereas *E. vogeli* and *E. oligarthrus* rarely cause disease in humans. *Echinococcus granulosus* is the most important and found primarily in sheep-raising areas. Strains of the parasite are also found in goats, swine, cattle, horses and camels.

The parasitoses are found worldwide, with canines serving as the definitive host and animals such as sheep as intermediate hosts. Humans acquire infection by ingesting eggs from dogs, whereas dogs acquire the infection by eating sheep liver and other organs containing cysts with large numbers of scolices. Infections usually occur in the young and the cysts develop over a period of years.

Echinococcus multilocularis has a limited distribution in dogs, foxes, wolves and cats as the reservoir hosts and larval stages in wild rodents, especially voles. The parasite, however, is reported to be moving southward in the USA. Rare human infections are acquired by the ingestion of eggs passed by the canines. Infection with any of the *Echinococcus* spp., however, is related to poor sanitary conditions in populations with a low level of education and in people closely associated with canine reservoir hosts.

PATHOGENICITY

Helminths seem to live in peaceful coexistence with their hosts and usually cause few problems. However, if there are many worms, there may be severe disease and

symptoms. The parasites, especially in the intestines, can cause obstruction and possibly perforation. The worms also secrete or excrete toxic substances, which affect the tissues. Lytic substances, such as those secreted by hookworms to obtain blood, can induce inflammation and there may be changes due to malabsorption and competition for nutrients. Larval stages may be more pathogenic, secreting antigenic substances that cause hypersensitivity, while the antigens promote antibody production and cellular immune responses. The most serious aspect of helminthic infection is that the worms end up in ectopic locations. Worms in the wrong place can be highly pathogenic.

Helminths have intricate life cycles and disease is usually associated with the larval migratory pathways and the final habitat of adult worms in the host. The amount of pathology and disease varies with the parasitosis.

Nematodes living in the intestine

Enterobius vermicularis

The life cycle of *Enterobius vermicularis* is presented in Figure 246.1 . Pinworm resides in the large bowel, where mating occurs. Gravid females migrate out of the anus and deposit eggs on the perianal skin. The adult males die after copulation and the females usually after depositing eggs. The perianal region becomes itchy and scratching can lead to excoriation and weeping. Eggs under the fingernails in children lead to reinfection when the fingers are placed in the mouth. Eggs can be released onto the bedsheets and become disseminated throughout the household when the sheets are shaken. The eggs will survive for 2 weeks in a humid and cool environment. When eggs are ingested they hatch in the intestine and the larvae migrate to the large intestine to mature in 6 weeks. Adults may migrate into the vagina, cervix, uterus and fallopian tubes and peritoneal cavity. Granulomas caused by the adult worms are reported in the abdominal cavity. Young girls may experience urinary tract infection and vulvovaginitis caused by wandering female worms or the presence of eggs on the vulva.

Ascaris lumbricoides

Infection with *Ascaris lumbricoides* can occasionally be highly pathogenic. The life cycle is presented in Figure 246.1 . The adult male worm may measure 15–31cm by 2–4mm and the female 20–35cm by 3–6mm. The female can live for a year or more and produce 240,000 eggs/day. The eggs pass in the feces and embryonate in the soil in 10–14 days. Upon ingestion the egg hatches in the intestine and the liberated larva penetrates the mucosa and passes to the liver via the portal vessels and then to the lungs. After a few weeks the larva penetrates the alveolar air sac, passes up the pulmonary tree, is coughed up and swallowed. The worms become sexually mature in the small intestines and produce eggs in 60–75 days.

Migrating larvae in a previously infected sensitized person can cause inflammatory lesions in the liver. Large numbers of larvae can cause an intense tissue reaction, including inflammation and the development of granulomatous lesions involving eosinophils, epithelioid cells and macrophages. This is referred to as *Ascaris* pneumonitis.

Adult worms in the intestines cause little disease unless in large numbers or if the worms become erratic and migrate into vital organs or exit from one of the orifices. Heavy infections can also cause intestinal obstruction, intussusception, volvulus, blockage of the bile ducts and cholangitis and intestinal perforation.

Trichuris trichiuria

Trichuris trichiuria has a direct life cycle (see Fig. 246.1). Adult females in the large intestine deposit eggs in the fecal stream and after a few weeks in the soil they become embryonated. When eggs are swallowed they hatch in the intestine and the larva moves down the bowel to the colon. The worms bury their narrow anterior end into the mucosa with the wider posterior end extended freely into the lumen of the colon. The females lay eggs after 3 months and produce 3000–10,000/day. The worm's longer, thinner, anterior end contains rows of cells (stichocytes) surrounding the esophogeal tube. The females measure 35–55mm and the males 30–45mm. The males have a curved tail with copulatory spicules.

Heavy infections can cause local irritation, diarrhea, cramps, edema and blood loss; the worms suck blood and there is seepage at the attachment sites. A prolapsed rectum is not uncommon, especially in children. There may be eosinophilia along with nutritional and weight loss.

Hookworms

A number of hookworm species can enter the human body, but most are natural parasites of animals other than humans (i.e. zoonotic parasites). The species from lower animals usually cause a creeping eruption or cutaneous larva migrans. Only two hookworms are considered to be important human species:

- *Necator americanus*; and
- *Ancylostoma duodenale*.

Both species measure about 10–13mm for females and 6–11mm for males and can be 0.4–0.6mm in width. The anterior mouths have cutting plates or teeth and the males have bell-shaped bursal rays at the posterior end that are used for holding females during copulation. The vulva is mid-central and eggs pass from the females into the fecal stream and out with host feces onto the soil. Female worms pass 5000–10,000 (*N. americanus*) and 10,000–20,000 (*A. duodenale*) eggs/day. In warm, moist climates the eggs embryonate in the soil and rhabditform larvae are released. Within a few days the larvae molt and develop into filariform larvae or the infective stage. The larvae climb to the top of the soil or grass, cling together and wait for a human host to come by. They usually penetrate the skin between the toes or other body surfaces. Some *A. duodenale* larvae may be ingested in water and enter the oral mucosa. The larvae are carried by the blood to the heart and then the lungs where they remain for a period, break out into the alveoli, pass up the pulmonary tree and are swallowed. The parasite matures in the small intestine, copulates and produces eggs in about 5 weeks (see Fig. 246.1).

The infections can remain active for as long as 13 years. Penetration of larvae into the skin causes ground itch and at times a secondary, bacterial infection may develop. Ground itch is caused when hookworm or other larvae enter the skin by contact with the ground, which results in irritation, erythema, edema and papulovascular eruption. Larvae in the lungs also produce minute focal hemorrhage and pneumonitis. The attachment of adult worms can cause small erosive lesions, hemorrhage, tissue cytolysis and neutrophilic infiltration. The worms change attachment sites regularly, leaving old sites oozing blood and plasma. Iron deficiency anemia and blood loss result from long-term infection with a large number of worms.

Pathologic changes occur in the bone marrow due to blood loss. Liver function may change as a result of anemia, as well as reduced capacity for albumen synthesis. Fatty deterioration of heart, liver and kidney may also occur. Hookworm disease with iron deficiency anemia, hypoproteinemia and hepatosplenomegaly may contribute to thousands of deaths each year.

Strongyloides stercoralis

Strongyloides stercoralis is a unique nematode having a parasitic life cycle as well as a free-living cycle, with males and females present in the free-living cycle (see Fig. 246.1). Only females are present in the parasitic life cycle, however. The parasitic females measure 1.5–2.5mm, and have a long cylindric esophagus. The vulva is in the posterior third of the body and the paired uteri contain thin-shelled eggs.

The eggs are deposited in the small intestinal epithelium and hatch soon after release. The first-stage or rhabditiform larva passes in the feces and develops into a second-stage and then a third or infective-stage filariform larva after a few days. The third-stage larvae penetrate the skin and migrate through the body and via the blood to the lungs; after several days they migrate up the respiratory tree and are swallowed. The larvae enter the intestinal mucosa and mature, and the female worms produce eggs parthenogenetically. Some larvae transform into infective forms in the bowel, penetrate the mucosa, migrate and develop into adults. This is internal autoinfection and can lead to hyperinfection in the immunocompromised host. Some rhabditiform larvae transform into rhabditoid free-living male and female adults in the soil and reproduce. The eggs hatch, releasing rhabditiform larvae, which develop into filariform larvae that can enter the skin of a host.

Cellular immunity is responsible for keeping infections under control, but when immunity is affected by disease or immunosuppression, the parasite multiplies unabated and this leads to hyperinfection and dissemination. Larvae entering the skin can cause inflammation with lymphocytic and eosinophilic infiltration. Larvae in the lungs cause eosinophilic infiltration and hypersensitivity reactions.

Pneumonia and hemorrhage result from hyperinfection. The intestinal mucosa is edematous and covered with mucus in cases of chronic strongyloidiasis. A cellular infiltration with eosinophils and monocytes and reactions around the worms may be seen in the lamina propria. Mucosal atrophy and flattening of villi as well as fibrotic changes with ulcerative enteritis may result from long-term infections. In disseminated infections the parasite is found in many organs, with secondary bacterial infection when bacteria are carried by the larvae from the intestines.

Capillaria philippinensis

Intestinal capillariasis is a disease in which the parasite can multiply in the digestive tract. *Capillaria philippinensis* is a tiny worm; females measure 2.5–5.3mm and males 1.3–3.9mm. The anterior body is narrow and consists of an esophagus surrounded by rows of cells or stichocytes forming the stichosome. The posterior is slightly wider and contains reproductive organs and digestive tract. The females deposit eggs, which must reach water where they embryonate. When these are eaten by small freshwater fish the eggs hatch and the larvae develop into infective stages in 3 weeks. When humans eat the fish the larvae mature in 2 weeks. After mating the females first produce larvae that will mature in the gut, whereas the second-generation worms produce thick-shelled eggs, which pass out in the feces. The parasites can multiply rapidly, producing thousands of progeny.[24] If the patient is not treated the disease is usually fatal. Over 200,000 worms were recovered from 1 liter of bowel fluid at one autopsy. The worms enter the crypts and cause atrophy, the villi are flattened and denuded, the mucosal glands are denuded and the lamina propria is infiltrated with inflammatory cells. Other organs are

also affected by malnutrition and hypokalemia. Most of the pathology is in the jejunum. [25]

Nematodes living in tissues

Trichinella spiralis

Trichinella spiralis is the only species in the genus of any importance; the other six species are parasites of lower animals and rarely infect humans. The parasites are related to other trichiurids in having a slender anterior and a wider posterior end. Stichocytes line the stichosome in the anterior end. Female worms measure 2–4mm and males 1–1.5mm. Infections are acquired by eating uncooked muscle containing encysted larvae from infected animals, usually pigs.

The larvae are digested from the cyst, pass to the small intestines and burrow beneath the epithelium where they develop into adults, re-enter the gut lumen and reproduce. The larvae produced enter the gut wall, are picked up by the blood and carried throughout the body to striated muscle where they become encysted. The adult worms cause a transitory enteritis and malabsorption, eosinophilia and excessive secretion of mucin. The larvae entering the muscle cell cause alterations in morphology, which results in the characteristic 'nurse cell'. Morphologic and molecular changes occur in the cell until it eventually becomes calcified. The larvae can remain alive for many years in the cell. Chronic inflammatory cells may surround the parasitized muscle cell. Vasculitis may cause periorbital edema and neurologic manifestations and there may be a trichinal myocarditis.

Filarial nematodes

The filarial nematodes are long and slender and are found in the lymphatics, tissues and body cavities. The life cycles of some of the filarids are presented in Figure 246.2 . Microfilariae are produced by female worms and arthropods are the vectors (see Chapter 170).

Wuchereria bancrofti, Brugia malayi and *Brugia timori*

The lymphatic filarids *W. bancrofti, B. malayi* and *B. timori* produce microfilariae that usually appear in the blood between 2200 and 0200 hours (nocturnal periodicity). *Wuchereria bancrofti*, found in some of the Pacific Islands, produces microfilariae that appear in the blood in the daytime (diurnal periodicity). Mosquitoes obtain blood at night and the larvae develop into the infective stage in 10–14 days. At the next feeding the infective larvae migrate from the thoracic muscles of the mosquito to the proboscis and crawl into the hole made by the bite. The larvae migrate in the host until they reach the definitive habitat and develop into adults. The worms copulate and the females produce microfilariae; the life cycle of *W. bancrofti* is a few months longer than that of *B. malayi*. Infections may persist for several years.

The pathogenesis of lymphatic filariasis has been a matter of debate and a model integrating various aspects of the parasitosis has been proposed. [16] It has also been suggested that *Wobachia*, an endosymbiont of filarids, may be associated with the pathogenesis. On the other hand, the pathology of lymphatic filariasis may be associated with immunologic responsiveness. [17] Some people who have the infection are microfilaremic but without antibodies or disease, whereas other are amicrofilaremic and have antibodies. There is an inflammatory stage with lymph channel irritation. Adult worms can be found in the lymph vessels, primarily the axillary, epitrochlear, inguinal and pelvic nodes as well as those in the testis, epididymis and spermatic cord. Attacks of lymphangitis and lymphadenitis may develop, followed by orchitis, funiculitis and epididymitis, especially in bancroftian infections. Blockage of the lymph flow by the inflammatory reaction and granuloma formation leads to lymphatic varicoses and lymphedema. Hyperplasia of the connective tissue and cellular infiltration and induration of the tissue may take place, with thickening and verrucous changes in the skin leading to elephantiasis.

Tropical pulmonary eosinophilia, 'Weingarten's syndrome', occurs in some endemic areas and is caused by granulomas containing degenerating microfilariae in the lung, spleen and lymph nodes. Patients have a cough, asthma, pulmonary infiltration, elevated eosinophils and antifilarial antibodies, but no microfilariae.

Onchocerca volvulus

Onchocerca volvulus adults are coiled in fibrous tissue nodules in the subcutaneous tissue. The females may reach 50cm in length and 0.5mm in diameter and the microfilariae produced are released into the interstitium of the skin. Males are smaller, being 5cm in length. Microfilariae are picked up by biting flies or black flies of the genus *Simulium* and after developing to the infective stage the larvae are introduced into a host at the next feeding. The larvae migrate through the tissue and after many months settle down, usually in pairs, and become encapsulated (see Fig. 246.2).

The microfilariae leave the nodule and migrate through the dermis, provoking a dermatitis; microfilariae in the eye chamber may lead to blindness. There may be a loss of skin elasticity in the pelvic region leading to hanging groin. Eye lesions begin with punctate keratitis and small opacities in the cornea caused by degenerating microfilariae, which evoke an eosinophilia. Sclerokeratitis follows. The endosymbiotic *Wobachia* bacteria in the worm may be associated with the pathogenesis of river blindness.[18] In Africa and Venezuela the nodules are located on the trunk and limbs, whereas in Mexico and Guatemala the nodules are usually on the head.

Loa loa

The African eye worm *Loa loa* actually moves through subcutaneous tissue and often traverses the conjunctiva. The males are 3–4cm and the females 4–7cm in length and 3–5mm wide. Microfilariae have a diurnal periodicity and are ingested by species of *Chrysops* at the time of a blood meal. The larvae develop to the infective stage in 10–13 days and enter the host through the hole made by the bite at the next feeding (see Fig. 246.2). The adult worms cause little damage as they migrate quickly through the tissue. Transient swellings (Calabar swellings), a subcutaneous edema caused by an allergic reaction to metabolic products of the worms or dead worms, may occur. Eosinophilic hypersensitivity reactions develop to the worms and microfilariae along with lymphadenitis.

Mansonella ozzardi, Mansonella perstans and *Mansonella streptocerca*

These are tissue parasites found in the mesenteries, visceral fat, skin and deep connective tissue. Microfilariae are found in the blood and skin and deep connective tissue. The vectors are species of *Culicoides* and *Simulium* (see Fig. 246.2). Most infections provoke little pathology except for articular pain, inguinal adenopathy, skin rash and dermatitis.

Dracunculus medinensis

Dracunculus medinensis is similar to the filarids. The females measure up to 120cm long and 2mm wide and the males 2cm. The worms mature in the connective tissue; gravid females then migrate to the subcutaneous tissue where they cause ulcers through which larvae are released when the lesion is immersed in water. The larvae are ingested by copepods and develop into infective larvae in 2 weeks. When the copepods are ingested in drinking water the larvae are liberated, penetrate the

digestive tract and develop into adults in the connective tissue in a year (see Fig. 246.2). A painful localized reaction develops around the ulcer and multiple blisters and tracts may also develop. The tracts may become secondarily infected with bacteria. Once the uterus is emptied the worms may withdraw and die in the tissue. On the other hand, degeneration

of the worm may lead to subcutaneous abscess and eventual calcification.

Angiostrongylus cantonensis

Angiostrongylus cantonensis is found naturally in the lungs of rats. The larvae produced by the females move up the pulmonary tree, are swallowed and pass out in the rat feces. Molluscs (snails and slugs) serve as intermediate hosts and, when eaten by rats, the larvae are digested out of the snail tissue, migrate from the gut to the brain, and after 3 weeks migrate to the lungs. Larvae are produced by adult females a few weeks later. Humans who ingest snails or slugs acquire the infective larvae, which migrate to the central nervous system (CNS) and cause eosinophilic meningitis. The females reach 2–3cm and the males 1–2cm in length and 0.2–0.3mm in width. In most human infections the larvae die in the brain and cord and elicit an inflammatory reaction in the meninges. Eosinophils, monocytes and foreign body giant cells infiltrate around the dead worms. Tissue necrosis is also evident. Larvae are reported in the eye, and are often recovered from cerebrospinal fluid (CSF). Adult worms have been found on a few occasions in the pulmonary artery.[9]

Anisakis simplex and Pseudoterronova decipiens

Anisakis simplex larvae measure 2–3cm by 0.3–0.6mm and *Pseudoterranova decipiens* 2–3cm by 0.3–1.2mm. They are found in the muscle and body cavity of marine fish and squid. When eaten by marine mammals (e.g. whales, seals, porpoises) the worms mature in the stomach and the females deposit eggs in the feces. In the ocean, a larva hatches from the egg and is eaten by a small marine crustacean. When the crustacean with a third-stage larva is eaten by fish the freed larva migrates to the abdominal cavity. When infected fish are eaten raw by humans the liberated larvae penetrate the stomach or small intestine and provoke a foreign body reaction around the worm. The submucosa becomes edematous with massive cellular infiltration. An abscess develops, characterized by necrosis and hemorrhagic and eosinophilic infiltration.

Gnathostoma spinigerum

Gnathostoma spinigerum is a robust nematode with a large globose head surrounded by rows of spines. The females measure 1–1.5cm by 1–2.5mm and the males 1–2.5cm by 1–2mm. The adults are coiled in the wall of the digestive tract of dogs and cats. Eggs pass in the feces, embryonate in fresh water and are ingested by copepods. The larvae hatch and develop into the second larval stage. When the copepod is eaten by a second intermediate host, the third-stage larva develops, and when this is eaten by a dog or cat the worm penetrates the gut wall and matures. Humans who eat infected fish, frogs or other aquatic food, raw or fermented, acquire the infection and the larvae migrate through the tissue. In the skin there are patches of edema, which last a few days and reappear elsewhere. The areas may be large and itchy with a rash and pain, producing lesions similar to those of cutaneous larva migrans. There is peripheral eosinophilia. The CNS may be invaded, resulting in an eosinophilic myeloencephalitis with eosinophilic pleocytosis and bloody and xanthochromic CSF. The eye may also be invaded, causing palpebral edema, exophthalmos and subconjunctival hemorrhage.

Larva migrans

Cutaneous larva migrans is caused by dog or cat hookworm larvae that enter the skin and are unable to complete the life cycle. They either migrate through subcutaneous tissue or encyst in the tissue and the larvae may cause serpiginous erythematous tracts. The tracts eventually become dry and encrusted.

Visceral larva migrans is caused by the dog and cat ascarid larvae (*Toxocara* spp.) that escape from eggs that are accidentally swallowed. The larvae migrate through the tissue and encyst as second-stage larvae. The migrating larvae produce tracts with hemorrhagic necrosis and eosinophilic and lymphocytic infiltration. Migration into the liver causes enlargement and it becomes studded with nodules containing eosinophils and plasma and giant cells. There may be interstitial eosinophilia in the lungs. Focal lung granulomas may develop around disintegrating larvae. Larvae may also enter the eye and cause endophthalmitis and retinal detachment and some larvae may enter the CNS and heart (see Chapter 174).

Trematodes living in liver, lungs, intestines and blood

Although many trematodes infect humans, only a few are considered to be important pathogens. These flat worms are found in all organs, especially the intestines, and a few are found in the liver, lungs and blood. They are hermaphoditic except for the schistosomes. The life cycles of the important trematodes are presented in Figure 246.3 .

The opisthorchid liver flukes

Clonorchis sinensis, Opisthorchis viverrini and *Opisthorchis felineus* are found in the bile ducts. The worms are flat or leaf-like and about 1.5cm long and tapered at both ends. There are two branched testes at the posterior end. The eggs produced pass down the bile ducts to the intestine and out with the feces. In water, snails of a number of species (*Parafossarulus, Thiara* and *Bithynia*) serve as the first intermediate host; they ingest the egg and the miracidium is released from the egg. After reproducing by polyembryony, cercariae are produced that leave the snail and encyst as metacecariae in freshwater fish (cyprinids or carp). When the fish is eaten raw or improperly cooked the metacercariae are digested out of the fish and migrate down the bowel and then into the bile passages. There may be mechanical irritation, inflammation of the bile ducts with metaplasia and proliferation of the epithelium and periductal fibrosis. Pancreatic ducts may show epithelial metaplasia and partial obstruction. There is often biliary stone formation and bacterial superinfection. Cholangiocarcinoma is a possible complication and DNA carcinogens may be associated with such tumor formation. There may also be an association with nitrosamines, which are commonly found in Asian foods.[26]

Fasciolopsis buski

Fasciolopsis buski is the largest intestinal trematode of humans, measuring 5–7cm by 8–20mm by 1–3mm. It has an oral and ventral sucker, two large ceca, two branched testes and a central and coiled uterus. Eggs pass in the feces into water and the hatched miracidium enters a specific planorbid snail. Cercariae released from the snail attach to aquatic vegetation and form into metacercariae. When eaten the metacercariae excyst and attach to the mucosa of the small intestine. Large numbers of worms cause bleeding at attachment sites, excess mucus secretion and obstruction. Toxic secretions and excretions may be absorbed, causing generalized edema and cachexia. Death can result from massive infection.

Fasciolia hepatica

Fasciolia hepatica is acquired by eating aquatic vegetation on which the metacercariae are attached. Upon ingestion the metacercariae are released and penetrate the gut wall, traverse the peritoneal cavity, pass through the liver capsule into the liver parenchyma and into the bile duct. The migratory stages are characterized by hepatomegaly and hemorrhagic necrotic worm tracts. Eosinophils and other inflammatory cells are present. In chronic infections there may be mechanical irritation, because of the large size of the fluke and toxic metabolites, and hyperplasia of the biliary epithelium resulting in obstruction in some cases. Worms may also re-enter the liver parenchyma. The worms are large, 4cm in length and 1.5cm wide, with a large cephalic cone at the anterior end. The egg passes

down the bile duct to the intestines and out with the feces. It hatches in the water and the miracidium enters into snails of the genus *Lymnea*. Cercariae are released and encyst on all varieties of aquatic vegetations.

Paragonimus westermani

Paragonimus westermani usually resides in pairs in the lung. They are reddish brown, plump bodied and shaped like coffee beans, and measure about 1.2cm long, 0.6cm wide and 0.4cm thick. They have two large branching testes in the posterior half of the body. Eggs enter the alveoli, are coughed up and swallowed, and pass

out in the feces or in sputum. The egg hatches in water, enters a specific snail and multiplies, and released cercariae enter crabs and crayfish where the metacercariae encyst. When the infected crustacean is eaten the metacercariae are released from the cyst, penetrate the gut wall and enter into the peritoneum, diaphragm and lung.

Young migrating worms produce local hemorrhage and cellular infiltration. These worms may settle in ectopic locations and evoke a pronounced tissue reaction. In the lung and other locations a leukocytic infiltration develops around the worm and fibrous tissue infiltrates to form a cyst wall. Eggs may migrate into pulmonary tissue and other locations and evoke granulomatous reactions. Cerebral paragonimiasis often develops, with hemorrhage, eosinophils, a yellowish exudate and Charcot-Leyden crystals. Some lesions may eventually calcify.

Other species of *Paragonimus* can also infect humans and invade subcutaneous tissue and the abdominal cavity.

Other intestinal fluke infections

Most intestinal fluke infections are innocuous unless there is a large number of worms. Bleeding and ulceration, inflammation and excess mucus secretion may result from *Echinostoma* spp. and heterophyid infections. The eggs of the heterophyids are tiny and may be carried by the lymphatics and venules to ectopic locations such as the heart and evoke granulomas. Eggs of the intestinal flukes pass in the feces and hatch in water and the miracidia enter snails. Cercariae emerging from the snails enter fish and other aquatic animal life and when these animals are eaten the metacercariae excyst and develop into adults in the small intestine.

Human schistosomes

Human schistosomes are found in the venous bloodstream. Eggs are passed into water and the miracidia released search out specific snails in which to continue the life cycle. Cercariae released from the snails penetrate the skin and the larval schistosomulae migrate to the lungs and then to the mesenteric and vesical veins. Eggs carried back through the mesenteric veins to the liver are responsible for granuloma formation. The liver becomes enlarged, especially the left lobe. The spleen may become enlarged and portal hypertension develops and leads to obstructive liver disease, esophageal varices and ascites. Egg granulomas also form, especially in the large intestine.

Gastrointestinal symptoms such as diarrhea with blood, mucoid stools and a protein-losing enteropathy are not uncommon. In urinary schistosomiasis hematobia egg granulomas develop in the bladder, possibly leading to cancer. Inflammation and fibrosis of the urethral wall lead to stenosis, irregular dilatations and hydronephroses. Secondary bacterial infections are common and can lead to renal failure and death. *Schistosoma haematobium* eggs may reach the lung and cause fibrosis and lead to cor pulmonale. *Schistosoma japonicum* is responsible for cerebral schistosomiasis, and spinal cord involvement is reported in *S. mansoni* infections.

The schistosomes are the only trematodes with separate sexes. They have oral and ventral suckers. The females are 1.5–2.5cm in length and 0.2–0.3mm in diameter. The male is 0.5–2.0cm and, although flat, usually curls up to form the gynecophoral canal in which the female lies. the female leaves the male, crawls to the small venules close to the lumen of the gut or urinary bladder and deposits eggs. The miracidium releases enzymes to work with the spines on the *S. mansoni* and *S. haematobium* eggs to digest the tissue and enter the lumen of the intestine and bladder. Some eggs are carried back via the venules and veins to the liver. The life cycles of the human schistosomes are presented in Figure 246.3 .

Cestodes living in the intestines and tissues

Adult tapeworms residing in the intestines do not cause serious disease, but larval stages of two species are highly pathogenic. The adults in the intestines pass eggs, which are taken up by an intermediate host (Fig. 246.4).

Eggs of *T. saginata* and *T. solium* are ingested by bovids and swine and the larval or cysticercus stages develop in muscles. When the meat is eaten insufficiently cooked the cysticerci are released and develop into adults in the small intestine.

The life cycles of the fish tapeworm *D. latum* and related species involves two intermediate hosts: a copepod and a fish. The egg hatches in water and the corricidium is taken in by a copepod and develop into a procercoid larva. When eaten by a fish the larva develops into a pleroceroid larva and when the fish is eaten the larva becomes an adult in the intestine.

Hymenolepid cestodes often require an insect (beetles and fleas) as an intermediate host. *Hymenolepis nana* can also be acquired by ingestion of the egg. Some strains of *H. nana* are also capable of internal autoinfection of humans.

Morphologically, all tapeworms have a scolex and neck, and immature, mature and gravid proglottids. The scolex of *D. latum* has a sucking groove as a hold-fast organ, whereas the other tapeworms have a scolex with four suckers, and *T. solium* and *H. nana* have a rostellum on the scolex with rows of hooklets. Each mature proglottid is hermaphroditic with both male and female sex organs. The lengths and width of each species varies with the longest being *D. latum* (2–15m), followed by the taeniids (1–4m), *H. nana* (1.5–4cm) and *H. diminuta* (1–6cm).

Usually, only one *Taenia* spp. in the intestines causes little disease. There may be some irritation at the site of attachment. Infection with more than one worm may cause intestinal and appendiceal blockage. At times the worms or proglottids block the pancreatic or bile ducts. Toxic substances from the worms may be responsible for systemic manifestations. The fish tapeworm causes decreased vitamin B12 absorption and eventual macrocytic and megaloblastic anemia.

Larval stages of tapeworms also infect humans (Fig. 246.5). Infection with the plerocercoid larva of diphyllobothriid species such as *Spirometra* is the cause of sparganosis. The larvae acquired from eating aquatic animals or drinking copepods in water penetrate the gut wall and migrate through the tissue. Animal poultices used in Asia are another source of infection. The larvae cause painful inflammatory swellings or transient lesions. The parasite may invade the eye causing periorbital edema.

Humans who become infected with the eggs of *T. solium* may develop cysticercosis. The larvae invade the muscles, brain, eye and skin. Although the cyst may remain intact and dormant for years, it may eventually break and the released fluids cause granulomas and calcification. Subcutaneous nodules are palpable and their presence usually indicates CNS involvement. Neurocysticercosis is responsible for arachnoiditis with pleocytosis and increased protein in the CSF. Intense inflammation may develop around dead or dying parasites.

Echinococcosis or hyatid disease develops in humans who accidentally ingest the eggs of the dog tapeworms *E. granulosus* or *E. multilocularis*. The egg hatches in the duodenum and the oncosphere

Figure 246-5 Life cycles of important human tapeworms; adults living in tissues and intestines and blood: humans are accidental hosts.

penetrates the gut and becomes established in the liver and other organs. A cyst develops brood capsules and protoscolices proliferate from the inner germinal epithelium of the brood capsule. An outer laminated acellular limited membrane forms and the cyst develops over many years. Unilocular cysts develop with *E. granulosus* and multilocular alveolar cysts with *E. multilocularis*. Cysts develop in many organs, especially the liver, lungs, brain and bones. A host inflammatory reaction produces fibroblasts, giant cells and mononuclear and eosinophilic cells. A fibrotic capsule eventually develops. The cyst may become calcified and the parasites destroyed. Rupture of the cyst releases fluids that can cause anaphylactic shock and the scolices may spread to set up new foci in other areas. The adult

worms are parasites of canines and the intermediate host can be any animal, but most often are sheep for *E. granulosus* and rodents for *E. multilocularis*.

PREVENTION

Most parasitic infections can be prevented if good sanitary practices are followed. The soil-transmitted nematodes can be controlled and possibly eradicated with proper disposal of feces. This is true for most intestinal parasites, even those that require an intermediate host. The disposal of fecal matter into water bodies containing molluscan intermediate hosts perpetuates trematode infections. The construction and use of sanitary privies will go a long way toward preventing infections of all types, but in most endemic areas these practices are usually not followed and indiscriminate defecation is the trend. Infection with skin-penetrating helminths such as hookworm and *Strongyloides* spp. can be prevented by wearing shoes and protective clothing.

Arthropod-borne helminthiasis, such as filariasis, can be prevented by the use of insecticides in houses. Breeding areas of the vectors can also be treated with insecticides. The use of repellents will also help, as will sleeping under insecticide-impregnated bed nets.

The avoidance of water known to harbor snail vectors will prevent schistosome infections. The destruction of snail breeding areas and the use of molluscicides will help to control infections.

The ingestion of raw foods should be restricted in endemic areas. The eating of raw fish is a practice in many countries, and if this is discouraged certain trematode and cestode infections could be prevented. The cooking of marine fish would prevent anisakiasis. Similarly the cooking of aquatic vegetation would prevent trematode infection such as fascioliasis and fasciolopsiasis. Undercooked beef and pork are responsible for trichinosis and tapeworm infections and through cooking will destroy the parasites. Freezing foods will kill most parasites and in the future irradiation of foods will make food safe.[27] Microwave treatment, however, is not considered adequate for destroying parasites.

The use of anthelmintics can be used to eliminate helminthic infections. Mebendazole, albendazole and praziquantel have been used in mass treatment campaigns for some nematode and trematode infections. Antifilarial drugs have been used in mass treatment programs and have even been included in table salt distributed to population group. Reduction of infections in a population will be of significant benefit and will augument control and eventual eradication programs. These methods along with education programs would be a definite adjunct to prevention and control.

DIAGNOSIS

The diagnosis of helminthic infections depends upon the location of the parasite in the body. The majority of worms are in the digestive tract and the diagnosis is made by stool examination. Blood is examined for parasites in the circulatory system, and urine and sputum are examined for worms in the genitourinary systems and respiratory passages. Tissue parasites are diagnosed by biopsies and by immunologic methods. Many techniques have been developed to aid in the diagnosis and most of these have been presented in detail.[28]

Stool specimens (see Chapter 165)

Intestinal parasites are detected by examination of one or preferably more than one stool specimen.[29] The stools should be collected in a clean dry container and examined as soon as possible after being passed. A direct examination is made by making wet films on a clean microscope slide. A small portion of feces is mixed with a drop of saline on the slide and examined under low power (×100) and high power (×450) magnification, if needed. The preparation should not be too thick; it should be thin enough to read newsprint through it. Iodine stain preparations can also be used, especially for protozoan infections, but do not add a great deal to the observation of helminth eggs and larvae. If a stool specimen cannot be examined within a few hours after passage it should be placed into 10% formalin or another preservative. The examination can then be done when it is more convenient.

Concentration techniques are also available. The simplest is sedimentation of feces in a tube or sedimentation flask and the sediment is then examined microscopically. The formalin-ethyl acetate technique, in which the stools are mixed with saline and passed through gauze or a screen, is widely used. The material collected is centrifuged several times in saline and finally formalin. Ethyl acetate is added and the preparation is centrifuged slowly. The detritus layer and supernatant are poured off and the sediment examined microscopically. The zinc sulfate flotation method is another simple technique in which small samples of feces are mixed thoroughly with zinc sulfate (1.18 sp gr or 1.2 sp gr for formalized stools). This may be centrifuged or permitted to stand for a period and the surface fluids examined for eggs. This method is unsatisfactory for operculated eggs, however. Eggs from various helminths as well as larvae of hookworm and *Strongyloides* are depicted in Figure 246.6 and Figure 246.7 .

The Kato method to examine stools has been modified into the Kato-Katz technique for estimating the number of eggs in the feces. Screened feces are placed into a small hole in a template, which delivers 41.7mg feces onto a microscope slide. A Cellophane square soaked in a glycerine-malachite green is placed onto the feces and the preparation is permitted to stand for 30–60 minutes. The glycerine clears the fecal material and eggs can be seen against a green background. This technique is especially good for thick-shelled eggs such as those of *A. lumbricoides, T. trichiura* and *S. mansoni*. The preparation should be examined within 30 minutes for thin-shelled eggs such as hookworm, which may dissolve if they stand for too long. The number of eggs observed is multiplied by 24 to obtain the number of eggs/g of feces. The Kato method uses only the glycerine-soaked Cellophane and is not quantitative.

Fecal cultures can be used to recover nematode larvae, especially of the hookworms and *Strongyloides* spp. The feces are mixed with charcoal with a small amount of water and placed into a Petri dish with moist filter paper lining the bottom of the dish. This is kept at room temperature for several days and the larvae will migrate to the surface of the charcoal. The culture is placed into a Baermann apparatus consisting of a funnel with rubber tubing attached to the stem and a pinch-clamp closing the tube. A sieve is placed into the top of the funnel and lined with gauze. The funnel is filled with warm water and the culture placed onto the gauze. The apparatus stands at room temperature for 10–12 hours and the fluid is drawn off through the tubing into a glass flask. Larvae can be collected from the flask and examined microscopically to determine the species.

The Harada-Mori technique is also used to culture feces and recover larvae. Filter paper strips are coated on one side with feces

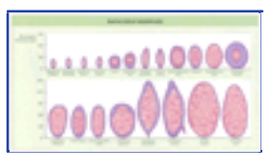

Figure 246-6 Relative size of helminth eggs. HHS Publication No. (CDC 89-8116)

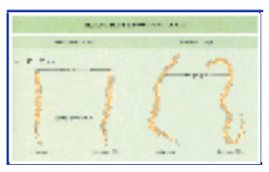

Figure 246-7 Hookworm and *Strongyloides* larvae. HHS Publication No. (CDC 89-8116)

and placed into a tube containing a small amount of water. The tube is kept upright so that the filter paper is kept moist by capillary action. After 4–10 days the filter paper is removed and the water examined for larvae.

An agar plate culture for *Strongyloides* spp. has been developed.[30] A few grams of feces are placed into the center of the agar and the plate is incubated at room temperature for 48 hours. Tracks are made by the parasite in the agar and the larvae can be recovered by a Pasteur pipette and placed onto a microscope slide for identification.

Eggs of some parasites can be picked up on perianal swabs. Swabs such as scotch tape or paddle swabs are placed on the perianal area and then placed under a microscope for examination. Pinworm, tapeworm and *A. lumbricoides* eggs can be recovered.

Eggs of *Schistosoma* spp. can be found in feces using some of the above-mentioned examinations. An egg hatching technique can also be used in which feces are placed into a sidearm flask and water added until it reaches a level just below the sidearm. When the suspension settles more water is added until the level is above

the sidearm. A dark cloth or bag is placed over the flask without covering the sidearm. The sidearm is then placed into the light and hatching miracidia from the schistosome eggs will be attracted to the light. They can be examined microscopically.

Specimens can be collected directly from the duodenum and examined for helminthic infections. Material collected by duodenal aspiration can be examined microscopically for *Strongyloides* and *Ascaris* spp. and flukes.

The Entero-Test or string capsule is also available for examining duodenal specimens. A gelatin capsule containing a string is swallowed and the end of the string taped to the cheek. The string in the capsule contains a small weight that carries the string down the duodenum after the capsule dissolves. After 4 hours the string is pulled up and the mucus adhering to the string is placed onto a microscope slide and examined.

Tapeworm proglottids are at times found in stool specimens. These segments should be washed in water and placed between two microscope slides. The slides are than tied together with string and the specimens placed into a fixative — alcohol, formalin, acetic acid (AFA). After fixation the worms can be stained and examined for specific identification. If the proglottids have rosette-shaped uterine branches the worm can be identified as a diphyllabothriid. The number of uterine branches of *Taenia* spp. can be counted and the specimen identified as *T. solium* (6–11) or *T. saginata* (15–20). India ink can be injected into the uterine pore and the branches are then more easily counted.

Adult trematodes found in feces should be washed and placed between two microscope slides that are then tied together with string. The specimens are than placed into a fixative such as AFA. After fixation the worms can be stained and examined for speciation.

Sputum specimens

Sputum to be examined for eggs or larvae should be mixed with 3% sodium hydroxide, centrifuged and the sediment examined.

Urine specimens

Urine specimens will sometimes help in the diagnosis of *S. haematobium* eggs or microfilariae of *W. bancrofti* and *O. volvulus*. The urine specimen is centrifuged and the sediment examined.

Blood samples

Several of the filarial infections can be diagnosed by the examination of drops of blood from finger or ear lobe pricks. The blood is placed onto a microscope slide and covered with a coverglass and examined under low power for thrashing microfilariae. For specific identification thick blood smears are made with several drops of blood, the smear is permitted to dry for several hours and then stained with Giemsa stain. Identifications are based upon for example microfilarial sizes, cephalic space and tail nuclei. Microfilariae of *W. bancrofti*, *B. malayi*, *B. timori*, *L. loa* and *Mansonella* spp. are present in the blood. *Onchocerca volvulus* microfilariae are found in skin snips.

Blood should be obtained at night for most lymphatic filariae and in the daytime for *L. loa* and *Mansonella* spp. Microfilariae can also be recovered from blood by membrane filtration. In some cases of lymphatic filariasis adult worms may be seen by ultrasound and the worms can be seen 'dancing' in the lymph channels.[31]

Cerebrospinal fluid

Cerebrospinal fluid is examined for evidence of helminthic infections. Eosinophilic pleocytosis is suggestive of angiostrongyliasis, paragonimiasis, cysticercosis, gnathostomiasis and schistosomiasis. Larvae of *A. cantonensis* are reported in CSF, especially in children, and *T. spiralis* larvae can be found in the CSF in very heavy infections. The CSF is centrifuged and the sediment examined for evidence of a helminth infection.

Tissue specimens

Tissue specimens may be taken and examined for helminthic infections. Muscle biopsies can be sectioned, stained and examined microscopically for *T. spiralis*, or the muscle can be pressed between to two glass slides and examined for trichina larvae.

Rectal and bladder biopsy specimens can be examined for schistosome eggs. Skin biopsies may reveal the presence of microfilariae of *O. volvulus* or *Mansonella* spp. Fluid obtained from suspected hydatid cysts, although dangerous to obtain, may reveal scolices of *E. granulosus*. Biopsies can also be made when anticipating a diagnosis of gnathostomiasis, sparganosis or infection with another migrating helminth.

Immunodiagnostic tests

There are myriad immunodiagnostic tests available to aid in the diagnosis of helminthic infections. Immunologic tests, however, only offer a presumptive diagnosis, and the finding of the worms, eggs or larvae provides the definitive diagnosis. Immunologic tests are useful when the parasite is not demonstrated such as in cases of toxocariasis and in some cases of cysticercosis and echinococcosis. Immunodiagnostic tests have been developed for most parasitic disease to detect antibodies as well as antigens. Techniques in molecular biology have also been emerging and may eventually become valuable tools in detecting helminthic infections.[32]

Detailed information on immunologic and molecular methods in the diagnosis of parasitic diseases has been presented elsewhere.[33] Older tests such as complement fixation are still of value in the diagnosis of cysticercosis, paragonimiasis and schistosomiasis, especially for indicating active infections. Gel diffusion has been used in fascioliasis and the circumoval precipitation test (COPT) is used to detect antibodies in cases of schistosomiasis. In the latter, eggs of schistosomes develop precipitates around the shell in the presence of antibody-positive sera.

The enzyme-linked immunosorbent assay (ELISA) is currently one of the most widely used tests for many parasitoses. Indirect fluorescent antibody is also used, especially on tissue and parasite sections, to detect antigenic material. The indirect hemagglutination test along with EIA (enzyme immunoassay) is used for hydatid disease and EIA for cysticercosis. An EIA test is now recommended for strongyloidiasis, toxocariasis and other larval migrans. It is also of value in the diagnosis of paragonimiasis, schistosomiasis, trichinosis, angiostrongyliasis, gnathostomiasis and filariasis. *Taenia*-specific antigens can be detected in human feces by ELISA.[34]

The immunoblot or Western blot is being applied to the diagnosis of most helminthiases, especially fascioliasis, schistosomiasis, paragonimiasis and cysticercosis. Molecular diagnostics have been reported for most species of parasites, and mostly applied to research laboratories; the detection of DNA by the polymerase chain reaction (PCR) is reported for the most important human helminths. For example, a PCR assay has been developed to detect *W. bancrofti* DNA in blood[35] and a PCR to determine the presence of *W. bancrofti* DNA in vector mosquitoes.[36]

CLINICAL MANIFESTATIONS

An intestinal helminthiasis is usually unnoticed unless a large worm is found by the patient in the feces.

Pinworms

Pinworms generally cause little disease, but in children infections lead to perianal itching, eczema, nose-picking, pica and loss of sleep. Worms may migrate into the vagina, uterus and fallopian tubes and peritoneal cavity. Pinworms can be responsible for recurrent urinary tract infections in young girls.

Ascariasis

Ascariasis is innocuous unless the worms become erratic and migrate into vital organs or pass out of one of the orifices — nose, mouth or anus. Erratic ascariasis may be caused by anesthesia, trauma or some medications, such as tetrachloroethylene. A bolus of a large number of worms can cause volvulus or intussusception,

abdominal distress,

pneumonitis and cough, and disease is often associated with malnutrition and decreased growth.

Trichuriasis

Severe trichuriasis can lead to abdominal discomfort, dysentery, colitis, anemia and bloody stools. There may be nutritional loss, weight loss and malnutrition. Rectal prolapse occurs with some infections.

Hookworm

Hookworm larvae entering the skin may provoke a ground itch and secondary bacterial infections. Migrating larvae can cause pneumonitis and eosinophilia, and worms in the intestine can cause abdominal pain, nausea, vomiting and diarrhea. Large numbers of worms sucking blood cause iron deficiency anemia, hypoproteinemia and hepatosplenomegaly, and children will have impaired mental, physical and sexual development. Blood loss can be as high as 0.3ml/day/adult *Ancyclostoma duodenale*. Severe malnutrition develops, especially with concomitant infections with *A. lumbricoides* and *T. trichura*

Toxocariasis (see Chapter 174)

Toxocariasis (visceral larva migrans) causes hypereosinophilia, hepatomegaly and symptoms of chronic pulmonary inflammation with cough. There may also be visual difficulties due to retinochoroiditis or peripheral retinitis. Dog hookworm larvae in the skin cause cutaneous larva migrans and serpingous tunnels may present with intense pruritus. The lesion becomes erythematous and sometimes vesicular. *Ancylostroma caninum* may cause eosinophilic lesions in the intestines leading to abdominal pain.[6]

Strongyloides stercoralis

Early infections with *S. stercoralis* are similar to hookworm infections, causing ground itch and pneumonitis. Worms in the intestines cause inflammation, irritation, diarrhea, intestinal bleeding and melena. Sprue-like symptoms may also develop, along with malabsorption, weight loss and eosinophilia. Dermal lesions may occur at irregular intervals with urticarial eruptions on the buttocks, termed larval currens. Immunocompromised people, especially those with HTLV-1 associated lymphoma, are at risk of developing disseminated strongyloidiasis and present with severe enteritis, a protein-losing enteropathy, bronchitis, pneumonia, pleural effusion, cough and a blood-tinged sputum with rhabditiform larvae. Eosinophilia is usually absent in these cases. Death may result. Disseminated strongyloidiasis may also present as Gram negative pneumonia or meningitis, or as a polymicrobial bacteremia, with bacteria being spread by worms migrating from the bowel.

Capillariasis philippinensis

Capillariasis philippinensis leads to a protein-losing enteropathy, malabsorption, electrolyte imbalance, weight loss, wasting and death. Patients initially present with abdominal pain, borborygmi and diarrhea. Untreated infections usually lead to death.[24]

Trichinosis

Trichinosis is asymptomatic except when a large number of trichina larvae are ingested. After the worms become adults in the small intestines, symptoms of gastroenteritis develop, with nausea, abdominal pain, anorexia, diarrhea, fever and weight loss. Larvae are produced after copulation and these are able to enter the blood and are carried to striated muscles. During the migratory phase, muscle and joint pain develop, followed by periorbital edema and eosinophilia. Larvae passing through the heart can cause cell destruction, acute inflammatory changes and interstitial myocarditis. The infection can also cause neurologic and pulmonary complications. Dead larvae in the muscle eventually become calcified.

Lymphatic filariasis (see Chapter 170)

Symptoms associated with lymphatic filariasis are associated with the host's immune status; little immunity is associated with more severe disease. Early symptoms are fever, lymphangitis, lymphedema and lymphadenitis, which may be transitory and occur periodically. Scrotal involvement leads to orchitis, hydrocele and chyluria. Worms may block the lymph flow by an inflammatory reaction and the development of granulomas around the worms and these lead to lymphatic varicoses and eventual skin fibrosis, thickening and elephantiasis. Renal lesions may also occur, with microfilariae in the urine and chyluria. There may be enlargement of the legs, arms, scrotum, mammary glands and vulva. Tropical pulmonary eosinophilia with fever, splenomegaly, pulmonary infiltrates and hypereosinophilia is associated with *W. bancrofti* and *B. malayi* infections. Microfilariae are absent and are probably destroyed by host immune mechanisms in chronic infections.

Subcutaneous migration of *L. loa* may cause transient swelling known as Calabar swellings, which are a local reaction to the worm and its products. It is characterized by fever, eosinopilia and the urticarial swellings, which are more common in Caucasians. Migrating adult worms may pass over the bridge of the nose or through the conjunctiva across the eyeball. Migration of the worms in abnormal locations can cause symptoms in the scrotum, bowel, kidney and heart, but not usually in natives living in the African endemic areas. Visitors suffer more than indigenous populations.

Oncocercomas due to *O. volvulus* develop over bony prominences. Some nodules develop in deeper tissue and are unnoticeable. Dermatitis and blindness are caused by the microfilariae. Recent investigations suggest that the inflammatory response in the eye is induced by the endosymbiontic *Wobachia* bacteria released by dead microfilariae. [18] Lymph nodes in the groin may show lymphocyte depletion and fibrosis, followed by 'hanging groin,' especially in Africans. In the Americas nodules commonly develop on the upper part of the body, whereas in Africa the nodules develop on lower parts of the body.

Mansonellosis may cause:

- dermatitis (*M. streptocerca*);
- swellings on the hands and face, joint pains and itching (*M. perstans*); and
- allergic manifestations, articular pain, itching, headache, lymphadenopathy and eosinophilia (*M. ozzardi* in the Americas).

Guinea worm

Guinea worm in the subcutaneous tissue elicits allergic manifestations such as itching. When the female worms get close to the skin there is a localized erythema followed by pruritus, nausea, vomiting, diarrhea or asthmatic symptoms. The worm will secrete a toxic substance that causes a blister, which breaks when the area comes in contact with water, and the female releases larvae when the lesion opens up. The area becomes painful. The worm will eventually die and is resorbed or becomes calcified.

Angiostrongylus cantonensis and *Angiostrongylus costaricensis*

Migrating third-stage larvae of *A. cantonensis* may cause vague symptoms of gastroenteritis, vomiting, headache and fever. Once the worms reach the CNS they cause headache, nausea, vomiting, stiff neck, myalgia, pain and paresthesia. Coma may be a feature of heavy infections. Worms have been seen in the eyes of some patients. There may be paralysis of eye muscles. Frequent signs are abnormal tendon reflexes including abnormal Achilles' reflex, a positive Kernig's sign and impaired sensorium and vision. Infections with a large number of worms may be fatal.[9]

Abdominal angiostrongyliasis due to *A. costaricensis* resembles acute appendicitis. A tumor-like mass is palpable in the right lower quadrant. There may be fever, diarrhea or vomiting, along with eosinophilia.[37]

Gnathastome and anisakid larvae

Shortly after ingestion of gnathostome larvae there are symptoms of nausea, vomiting, pruritus and urticaria, and at times upper abdominal pain. Larval invasion into the liver will cause right upper quadrant tenderness and changes in liver function. In the lung there is a pulmonary infiltration and pneumothorax in patients with pleural infusion. Larvae migrating through the subcutaneous tissue and skin cause a rash along with red pruritic painless swellings. Invasion of the CNS often leads to death.

Anisakid larvae in the throat may lead to a 'tickle-throat', causing cough, but when they invade the mucosa of the intestinal tract they provoke eosinophilic granuloma formation, severe abdominal pain, nausea, vomiting and diarrhea. Usually, only a single worm is involved and the symptoms disappear when it is removed.

Trematodes

The trematodes, like the nematodes, cause little disease unless a large number of worms are involved. The liver flukes (*C. sinensis*, *O. viverrini*, *O. felineus*) in the bile ducts may cause symptoms early in the infection such as hepatomegaly, jaundice, diarrhea, anorexia, epigastric pain and fever. Repeated infection over a period of years may lead to ductal fibrosis, obstruction, cholangitis, cholecystitis and cirrhosis, and in some patients cholangiocarcinoma.

The sheep liver fluke *F. hepatica*, because of its large size, may block the bile ducts and cause cholangitis. Toxic secretions cause fever, chills, jaundice, an enlarged tender liver, cough, vomiting, abdominal symptoms and eosinophilia. Young worms may attack the pharyngeal mucosa, causing bleeding and edema. This occurs after eating raw sheep liver containing the parasite. The condition has been called halzoun.

The large intestinal fluke *F. buski* may cause intestinal blockage and toxemia when there is a large number of worms. Eosinophilia is common and ulcers may develop, which often hemorrhage. It causes abdominal distension, hunger pains and increased appetite, diarrhea and a foul-smelling yellowish stool. Allergic manifestations, nausea, vomiting, ascites and cachexia develop as a result of toxins secreted by the worms. Other intestinal flukes cause little disease, but tiny eggs of the heterophyids may enter the mucosa and are carried to ectopic locations such as the brain and heart, provoking granuloma formation. Echinostomes may produce inflammation and ulceration with diarrhea and abdominal pain.

The young lung fluke of *P. westermani* produces little disease during migration, but once established in the lung may cause fever, dyspnea, cough, chest pain and the production of a rusty sputum. At first the disease is often thought to be tuberculosis. The worms may enter the cranial cavity and invade the brain causing fever, headache, nausea, vomiting and visual disturbances, convulsions and meningeal signs.

Schistosome infections **(see Chapter 167)**

There may be petechial hemorrhages at the site of penetration where schistosome cercariae enter the skin. There will be localized edema and pruritus. After a few weeks there are toxic or allergic reactions and symptoms include fever, nausea, abdominal pain, rigor, urticarial rashes and eosinophilia. This acute stage is known as Katayama syndrome. In the chronic stage granulomas have formed around the eggs in tissue and hepatomegaly develops and the spleen becomes enlarged. This is followed by esophageal varices and finally ascites. Intestinal disease, usually with *S. mansoni* and *S. japonicum*, may involve the entire intestine or more often the large bowel. There may be abdominal cramps, tenderness and bloody mucoid stools. A protein-losing enteropathy, weight loss and anemia may also develop.

Features of *S. haematobium* infection are dysuria, urinary frequency and hematuria. Eosinophils in the urine are not uncommon. Heavy infections, with the deposition of many eggs in the bladder tissue, can lead to squamous cell carcinoma.

Pulmonary involvement can be a feature of all schistosome infections, and causes cor pulmonale with dyspnea, cough and hemoptysis.

Cerebral manifestations are common in oriental schistosomiasis, with symptoms of lethargy and confusion followed by speech difficulties and optical field defects. *Schistosoma mansoni* occasionally causes the spinal cord symptoms of a transverse myelitis, usually in the lumbar region. Flaccid paralysis of the lower limbs is also reported.

Tapeworm (see Chapter 168)

Tapeworm, even the larger ones, in the small intestines provoke few symptoms. Patients usually become aware of being infected only when worm segments are passed in the feces. If *D. latum* attaches to the proximal portion of the jejunum vitamin B12 deficiency results, with the development of pernicious anemia. This was seen in Finland but is now very rare. Sparganosis associated with other diphyllobothriid species causes painful inflammatory swellings, which may be transient. Spargana in the eyes cause intense reactions and periorbital edema.

Taenia spp. in the intestine are asymptomatic in most patients, but some have hunger pains, abdominal discomfort and indigestion. Patients usually become symptomatic when proglottids are found in the feces or passing from the anus. Eosinophilia is not uncommon. Cysticercosis or infection with larvae of *T. solium* is a serious disease, especially if there are cysticerci in the CNS. Cysticerci in the muscle are asymptomatic, but can give rise to myositis, fever and eosinophilia. The cysticerci usually die and become calcified. Cysticerci in the eye cause visual symptoms such as a decrease in visual acuity, retinal edema and hemorrhage. Neurocysticercosis results in arachnoiditis with CSF pleocytosis and an increase in CSF pressure. Obstructive hydrocephalus, cerebral infarction, epilepsy, papilledema, vomiting, headache, a toxic gait and intellectual deterioration may also develop. Dead or dying parasites may exacerbate symptoms, and location of the parasite in the CNS is responsible for a variety of CNS symptoms.

Hyperinfections with *H. nana* may cause diarrhea, loss of appetite, abdominal pain, headache, weakness, and at times epileptoid convulsion, dizziness and eosinophilia. *Hymenolepis diminuta* may cause diarrhea.

Hydatid disease **(see Chapter 169)**

There are usually no symptoms with hydatid disease until the cysts arelarge and in vital organs. Many years are usually required for a cyst to reach a significant size. Cysts in the liver may put pressure on the bile ducts and blood vessels. Large cysts in the lung can cause cough, shortness of breath and chest pains. Involvement of the CNS results in different symptoms depending upon the location of the cyst. Leaks of hydatid fluid sensitize the patient and can cause anaphylactic shock. A cyst in the eye causes proptosis.

MANAGEMENT (see Chapter 209)

Management of helminthic infection is variable and depends upon:

- the specific parasite;
- its location in the host; and
- the number of worms involved.

Usually anthelmintics are effective, but in some cases surgical or other intervention may be necessary. Follow-up examination and anthelmintic treatment may be required. The currently used anthelmintics are discussed in the *The Medical Letter*:[38]

- the soil-transmitted helminths *A. lumbricoides, T. trichiuria* and hookworm, found in the intestines, respond to mebendazole 100mg q12h for 3 days, pyrantal pamoate 11mg/kg once or albendazole 400mg once;
- *Strongyloides* spp. infections will respond to thiabendazole 50mg in two doses for 2 days or ivermectin 200mg/kg/day for 1–2 days; albendazole 400mg/day for 3 days may also be effective; the hyperinfection syndrome requires prolonged treatment;
- children with a history of erratic ascariasis should be treated with pyrantal pamoate first because some anthelmintics cause erratic ascariasis;
- pinworm infections are easily treated with mebendazole 100mg once followed by another dosage of 100mg in 2 weeks; pyrantal pamoate 11mg/kg or albendazole 400mg repeated 2 weeks later are also effective;
- mebendazole 200mg q12h for 20 days or albendazole 200mg q12h for 10 days along with fluid and electrolyte replacement are recommended for intestinal

capillariasis; treatment is repeated in the case of relapses by giving mebendazole for 30 days or albendazole for 20 days;[24] and
¦ the use of anthelmintics in trichinosis is debatable; symptomatic treatment with corticosteroids is recommended and mebendazole at dosages of 200–400mg q8h for 3 days then 400–500mg q8h for 10 days, or albendazole 400–800mg may provide some benefit.

Hookworm disease with anemia may require blood transfusion and long-term treatment with ferrous sulfate until hemoglobin levels become normal.

The two anthelmintics used to treat lymphatic filariasis are:

¦ diethylcarbamazine (DEC); and
¦ ivermectin (see Chapter 170).

Diethylcarbamazine can be given in dosages of 6mg/kg for 10–12 days. Filarial lymphangitis and side-effects can be managed with antihistamines and antipyretics. Tropical pulmonary eosinophilia is treated with DEC, 6mg/kg in three doses for 21 days from diagnosis. Single-dose treatment with DEC or ivermectin, or both, repeated at 6-month intervals for a few years shows promise for both the treatment and control of lymphatic filariasis.[39] Treatment of filariasis with an antibiotic may affect the endosymbiontic *Wobachia*, which may have an affect on the fertility of the parasite.[17]

In the past DEC has been used to treat onchocerciasis, but has been replaced with ivermectin because of severe side-effects. The recommended dose of ivermectin is 150mg/kg once, repeated at 3- to 12-month intervals for a few years.

Loiasis can be treated by surgical removal of the worm as it passes over the nose or the eye. Diethylcarbamazine is effective, first in low dosages of:

¦ 50mg on day 1;
¦ 50mg q8h on day 2;
¦ 100mg q8h on day 3; and
¦ 6mg/kg/day in three doses on days 4–21.

Antihistamines and corticosteroids are used in case of side-effects. Ivermectin in a single dose of 200mg/kg caused a decrease in microfilariae. Mebendazole may also be useful. Diethylcarbamazine can be used as prophylaxis.[40]

Mansonella spp. infections do not usually require treatment, but DEC or mebendazole and possibly ivermectin can be used. Ivermectin is reported to be effective against *M. streptocerca*.[41]

Angiostrongyliasis caused by *A. cantonensis* is usually self-limiting in light infections. Some investigators recommend symptomatic treatment and not an anthelmintic because drugs can kill the worms too quickly and cause more pathology. However, albendazole and mebendazole have been reported to be effective for pediatric cases in Taiwan.[42]

Angiostrongylus costaricensis infection is treated surgically. Similarly gnathostome infections are treated surgically, but the use of albendazole shows promise.[43] Anisakiasis is also treated surgically or by removal of the worms from the stomach by gastroscopy. No specific anthelmintic treatment is needed for transient anisakiasis.

Visceral larva migrans due to *T. canis* is usually self-limiting and symptomatic treatment and corticosteroids are beneficial. However, DEC 6mg/kg/day for 7–10 days, albendazole 400mg q12h for 3–5 days or mebendazole 100–200mg q12h for 5 days have been reported to be of some value. Cutaneous larval migrans can be treated with thiabendazole either topically or as 50mg/kg/day in two doses. Ivermectin 150–200mg/kg once or albendazole 200mg q12h for 3 days have also been used (see Chapter 174).

Fluke infections

Nearly all fluke infections are treated with praziquantel, 75mg/kg/day for 1–2 days. The Chinese liver fluke *C. sinensis* responds to praziquantel. *Opisthorchis viverrini* and *O. felineus* are also treated with the drug.

Albendazole 10mg/kg/week is also effective against *C. sinensis* and may also be useful for the other opisthorchiids.

The intestinal flukes *F. buski, H. heterophyes, M. yokogawai* and the echinostomes can be treated with praziquantel 75mg/kg/day in three doses for 1 day. Tetrachlorethylene 0.1ml/kg is also effective.

Praziquantel is ineffective in treating the sheep liver fluke, *F. hepatica*. Bithional 30–50mg/kg on alternate days for 10–15 doses was used in the past, but is no longer being manufactured in Japan. Triclabendazole 10mg/kg once has been shown to be effective[44] and is the drug of choice. Dehydroemetine 1mg/kg/day for 10 days has been used, but is more toxic than other drugs.

Praziquantel is effective against *Paragonimus* spp. in dosages of 75mg/kg/day in three doses for 2 days. Biothional had also been used.

The three major schistosomes, *S. mansoni, S. japonicum and S. haematobium*, respond to praziquantel (see Chapter 167). The dosage for *S. mansoni* and *S. haematobium* is 40mg/kg in two doses for 1 day, and for *S. japonicum* 60mg/kg/day in three doses for 1 day. Oxamniquine 15mg/kg once is effective against *S. mansoni* and metrifonate 10mg/kg once every other week for a total of three doses is effective against *S. haematobium*. The latter regimen may be effective against *S. mansoni* located in the perivesical plexis. Surgery is often required for esophogeal varices; splenectomy and splenorenal shunting will relieve portal pressure. Concomitant bacterial infection associated with schistosomiasis should be treated with antibiotics following antischistosome therapy. Schistosome dermatitis should be treated palliatively with antipruritics and antihistamines.

Cestode infections (see Chapter 168)

Praziquantel is effective for treating cestode infections. Adult *T. saginata, T. solium* and *D. latum* can be treated with praziquantel 10mg/kg once. An alternative drug for *T. saginata* and *D. latum* is niclosomide 2g in four chewable tablets. However, this drug causes side-effects, the proglottids disintegrate and the scolex is not easily found. Treatment may have to be repeated a few months later. Paramomycin 1g q4h for 4 days may be used, but there may be gastrointestinal disturbances. Atebrine, 0.8g divided in half and given in half-hour intervals, will often provide the whole worm intact. It is given on an empty stomach and a saline purge is given 2 hours after treatment.

The recommended treatment for *T. solium* is praziquantel; other drugs may be deleterious to the proglottids, releasing eggs, which may lead to cysticercosis.

The recommended treatment for cysticercosis is albendazole, 15mg/kg in 2–3 doses over 8–28 days, repeated as necessary. Praziquantel is also used in a dosage of 50mg/kg/day in three doses for 15 days. The cyst may also be surgically removed. Surgery (and not chemotherapy) is recommended for intraocular cysticercosis.

Sparganosis is also treated surgically or with praziquantel with a total dose of 120–150mg/kg over 2 days.[45] *Hymenolepis nana* is treated with praziquantel 25mg/kg once and *H. diminuta* with 10mg/kg once or niclosamide 2g in a single dose.

Surgery was the recommended treatment for hydatid cysts until albendazole was found to be effective at a dosage of 400mg q12h for 28 days, repeated if necessary after 14 days for up to 12 cycles. Percutaneous drainage with ultrasound guidance in addition to albendazole has been found to be valuable in the treatment of hepatic hydatid cysts. *Echinococcus multilocularis* is more difficult to treat, but albendazole should be effective (see Chapter 169).

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Chapter 247 - Arthropods

Thomas R Fritsche

NATURE

The phylum Arthropoda is the largest and most varied of the animal phyla, consisting of more than one million described species among a total of 30 million or more. An exceedingly small number of this total directly or indirectly affect human health, but those that do are responsible for significant morbidity and mortality. Species responsible for human disease include representatives of all major arthropod classes (Table 247.1).

PATHOGENICITY

Direct tissue invasion

Arthropods responsible for superficial tissue invasion (referred to as infestation) include chigoe fleas and scabies mites, whereas invasion of deeper body tissues and cavities (referred to as infection) may be produced by dipteran larvae (maggots) and pentastomids. Tissue invasion of any sort by maggots is referred to as myiasis, and may occur either in living or devitalized tissues, depending upon the species.[1] [2] [3]

Envenomation and hypersensitivity reactions

Humans are continually subjected to the stings and bites of many different arthropods. Reaction to these attacks varies greatly and depends upon:

- the species involved;
- the type of saliva or venom being introduced into the wound;
- the location of the wound; and
- an individual's physiologic response, which may vary depending upon previous sensitization.

Arthropod stings and bites cause only local tissue reactions in most people, but some people have serious systemic reactions, including anaphylaxis.[4] [5] [6]

Vesication

Some of the larger tropical species of millipedes are capable of spraying an irritating and vesicating (blister-causing) chemical, which is used for defensive purposes, from glands located on each body segment.[7] Blister beetles are so named because of the presence of the vesicating compound cantharidin in their body fluids. Upon handling these attractive beetles, cantharidin may be released, resulting in the appearance of fluid-filled skin blisters several hours following exposure.[1] [8] [9]

Transmission of infectious agents

During the act of feeding, many species of arthropods are capable of either mechanically or biologically transmitting agents responsible for serious infectious disease (Table 247.2). Filth flies, which include the common house fly *Musca domestica,* and cockroaches have been incriminated in the mechanical transmission of the agents of bacillary dysentery, cholera, typhoid, viral diarrhea, amebic dysentery and giardiasis, pinworms and tapeworms. Arthropod vectors responsible for the biologic transmission of infectious agents may serve either:

- as simple amplification vehicles for the organism; or
- in a more complex role involving changing life cycle stages of certain parasites.[1] [3] [9] [10]

PREVENTION

Principles of prevention are directed at limiting skin and mucous membane exposure to crawling and flying arthropods and their emanations. This may be accomplished by avoiding locations serving as a habitat for those arthropods posing the greatest threat to an individual such as:

- gardens, fields, orchards or unclean picnic areas that attract bees, wasps and yellowjackets;
- crawl spaces, wood sheds or outdoor privies, which may harbor venomous spiders or scorpions;
- domiciles conducive to cockroach, bedbug or kissing bug habitation; and
- outdoor environments known to be a source of ticks and biting diptera.

Remaining indoors during evening hours lessens the risks associated with exposure to mosquitoes. The wearing of light-colored clothing, especially long pants, long-sleeved shirts and closed-toed shoes when working outdoors or pursuing outdoor recreation is less attractive than dark clothing, short pants, short-sleeved shirts and opened-toed shoes to flying insects and allows the wearer to more readily recognize the presence of ticks and other crawling arthropods. Gloves also provide additional protection when gardening, but the use of scented lotions, perfumes or soaps should be discouraged. [8] [11]

In addition to these simple measures, the use of insect repellents is an important preventative when the threat of exposure to noxious or serious pests cannot otherwise be easily mitigated. A variety of chemical repellents are available on the market. Those containing the compound *N,N*-diethyl-*M*-toluamide are among the most widely used and come in a variety of preparations for use on clothing and skin in concentrations varying from about 10 to 100%. The use of high-concentration products on children is not advised because there are reports of associated seizures. [8] [12]

Use of mosquito nets during night-time hours and headnets at other times is mandatory in areas where mosquitoes are plentiful, especially in the tropics and subtropics, where they serve as vectors for malaria, filariae, viruses and other infectious agents. Impregnation of nets with insecticides has proved to be especially effective in decreasing the transmission rates of malaria in highly endemic areas. [13]

Desensitization regimens are highly effective in preventing anaphylaxis in individuals who have become sensitized to bee and wasp venoms. Venom immunotherapy is usually indicated for those who have a history of moderate-to-severe anaphylaxis, positive venom skin tests and are at risk of subsequent stings. It is not indicated for those who experience transient pain and local swelling.[5] Despite the use of desensitization, up to 15% of individuals whose initial anaphylactic reaction is judged to be severe continue to be at risk for anaphylaxis if re-stung. [14] Immunotherapy reagents are also available and may prove useful for individuals with intractable allergies to, for example, cockroaches and dust mites.[8]

TABLE 247-1 -- Arthropods of medical importance and the threats they pose to human health.

ARTHROPODS OF MEDICAL IMPORTANCE AND THE THREATS THEY POSE TO HUMAN HEALTH

Class	Subclass	Order	Pathogenic potential
Insecta (insects)		Anoplura (sucking lice)	Biting pests, local hypersensitivity, biologic vectors
		Siphonaptera (fleas)	Biting pests, local hypersensitivity, biologic vectors, superficial tissue invasion (*Tunga penetrans*)
		Dictyoptera (cockroaches)	Mechanical vectors, allergies
		Hemiptera (bedbugs, kissing bugs)	Biting pests, local and rarely systemic hypersensitivity, biologic vector (kissing bugs)
		Hymenoptera (bees, wasps, ants)	Envenomation, local and systemic hypersensitivity including anaphylaxis, biting pests (ants)
		Coleoptera (beetles)	Mechanical and biologic vectors, urticating hairs (larvae), vesicating fluids (adults)
		Lepidoptera (moths, butterflies, caterpillars)	Urticating hairs and venomous spines (larvae), urticating hairs and scales (adults)
		Diptera (flies, mosquitoes, biting midges)	Biting pests, local hypersensitivity, mechanical and biologic vectors, tissue invasion (myiasis)
Arachnida (arachnids)	Scorpiones (scorpions)		Envenomation with neurotoxins
	Araneae (spiders)		Envenomation with neurotoxins and necrotoxins
	Acari (ticks, mites, chiggers)		Biting pests, local hypersensitivity, allergies (mites), biologic vectors, superficial tissue invasion (*Sarcoptes scabiei*), neurotoxins (ticks)
Diplopoda (millipedes)			Vesicating fluid
Chilopoda (centipedes)			Envenomation, local and rarely systemic hypersensitivity
Crustacea (crustaceans)		Copepoda (copepods, water fleas)	Biologic vectors
		Decapoda (crabs, crayfish)	Biologic vectors
Pentastomida			Tissue and body cavity infection

CLINICAL MANIFESTATIONS, EPIDEMIOLOGY AND DIAGNOSTIC MICROBIOLOGY

Specimens recovered by physicians or submitted by patients may take the form of intact organisms or parts of organisms, skin scrapings, tissues, sputum, urine, stool, articles of clothing, bedding, carpeting and foodstuffs. It is not uncommon for patients to submit arthropods recovered from the toilet bowl following a bowel movement or urination, raising concern that they may have an intestinal or bladder infection. Such an occurrence is usually coincidental and not related to infection. Although the more common forms of medically important arthropods, especially ectoparasites (fleas, lice, mites and ticks) may be readily identifiable, detailed identification of other arthropods, especially larval forms, may require consultation with a specialist in entomology. Repeated examinations fail to reveal the presence of arthropods or parasites in cases of delusional parasitosis; it is imperative that the affected individual's domicile is thoroughly examined to rule out the possibility of unrecognized mite infestations following abandonment of an attached rodent or bird's nest.[10] [15]

Insects

Insects comprise more than 90% of all described arthropod species and include many of the vectors responsible for the transmission of serious infectious diseases.

Sucking lice, order Anoplura

Sucking lice are dorsoventrally flattened, wingless insects that have characteristic claws on the ends of each leg, which allow attachment to body hairs or clothing. All species suck blood and may cause unexplained dermatitis as a result of their repetitive feeding activities and chronic exposure of the skin to louse saliva and feces. Although named for their primary site of attachment, they do not always remain confined to that location.

The head louse, *Pediculus capitis,* and the body louse, *P. humanus*, are indistinguishable to the non-specialist. They are longer than they are wide and grow to about 3mm in length (Fig. 247.1). Biologic differences are apparent; only *P. humanus* transmits the agents of epidemic typhus, trench fever and relapsing fever (see Table 247.2).[3] Infestations with both species occur among people living in crowded conditions who have little opportunity for bathing and laundering. Children of school-age are at particular risk for acquiring head lice through the sharing of caps, clothing and combs.[2] [16] Lice eggs (known as nits) are typically 1mm long and when unhatched have an intact operculum (Fig. 247.2). Head louse nits are deposited primarily on hair shafts close to the scalp, especially along the nape of the neck, whereas nits of body lice are deposited primarily upon clothing. Because such objects as hair casts, dander, hair spray and fungal hair infections may mimic nits, differentiation is important. Transmission of body lice occurs primarily through the sharing of infested clothing and bedding because they tend to deposit their eggs in clusters, especially along seams or waistbands.

The public louse, *Phthirus pubis,* is distinctly different from the others; it is rounder, measuring up to 2mm in diameter, the abdomen is more crab-like, and their first pair of legs is significantly smaller and more slender than the other pairs (Fig. 247.3). Pubic lice and their nits are found primarily on pubic hairs, but may extend to the chest, armpit and facial hair. Transmission occurs primarily

TABLE 247-2 -- Summary of the major arthropod genera involved in the biologic transmission of infectious diseases.*

SUMMARY OF THE MAJOR ARTHROPOD GENERA INVOLVED IN THE BIOLOGIC TRANSMISSION OF INFECTIOUS DISEASES				
Class	**Subclass/order**	**Genus**	**Etiologic agent**	**Disease**

Insecta	Anoplura	*Pediculus*	*Rickettsia prowazekii*	Epidemic typhus
			Bartonella quintana	Trench fever
			Borrelia recurrentis	Epidemic relapsing fever
	Siphonaptera	*Xenopsylla*	*Yersinia pestis*	Plague
			Rickettsia typhi	Murine typhus
		Nosopsyllus	*Rickettsia typhi*	Murine typhus
		Ctenocephalides	*Dipylidium caninum*	Dog tapeworm disease
	Hemiptera	*Panstrongylus, Rhodnius, Triatoma*	*Trypanosoma cruzi*	Chagas' disease
	Coleoptera	*Dermestes, Tribolium, Tenebrio*	*Hymenolepis nana, H. diminuta*	Hymenolepiasis
	Diptera	*Aedes*	Flaviviruses	Dengue, yellow fever
			Other arboviruses	Febrile illness, encephalitis
		Anopheles	*Plasmodium* spp.	Malaria
			Brugia malayi	Filariasis
			Arboviruses	Febrile illness, encephalitis
		Culex	*Wuchereria, Brugia*	Filariasis
			Arboviruses	Febrile illness, encephalitis
		Culicoides	*Mansonella* spp.	Filariasis
		Glossina	*Trypanosoma brucei*	African sleeping sickness
		Chrysops	*Los loa*	Loiasis
			Francisella tularensis	Tularemia
		Simulium	*Onchocerca volvulus*	Onchocerciasis
			Mansonella ozzardi	Filariasis
		Phlebotomus, Lutzomyia	*Leishmania* spp.	Leishmaniasis
			Bartonella bacilliformis	Bartonellosis
			Phlebovirus	Sandfly fever
Arachnida	Acari (ticks)	*Ixodes*	*Borrelia burgdorferi*	Lyme disease
			Ehrlichia spp.	Human granulocytic ehrlichiosis
			Babesia spp.	Babesiosis
			Arboviruses	Encephalitis
		Dermacentor	*Rickettsia rickettsii*	Rocky Mountain spotted fever
			Rickettsia siberica	Siberian tick typhus
			Francisella tularensis	Tularemia
			Coltivirus	Colorado tick fever
			Other arboviruses	Encephalitis
		Amblyomma	*Rickettsia rickettsii*	Rocky Mountain spotted fever
			Rickettsia conorii	Boutonneuse fever
			Ehrlichia chaffeensis	Human monocytic ehrlichiosis
			Francisella tularensis	Tularemia
		Rhipicephalus	*Rickettsia rickettsii*	Rocky Mountain spotted fever
			Rickettsia conorii	Boutonneuse fever
		Haemaphysalis	*Rickettsia siberica*	Siberian tick typhus
			Rickettsia conorii	Boutonneuse fever
			Arboviruses	Encephalitis, Kyasanur Forest disease
		Hyalomma	*Rickettsia siberica*	Siberian tick typhus
			Arbovirus	Crimean-Congo hemorrhagic fever
		Ornithodoros	*Borrelia* spp.	Relapsing fever
	Acari (mites)	*Leptotrombidium*	*Rickettsia tsutsugamushi*	Scrub typhus
		Liponyssoides	*Rickettsia akari*	Rickettsialpox
Crustacea		Decapods	*Paragonimus* spp.	Paragonimiasis
		Copepods	*Diphyllobothrium* spp.	Diphyllobothriasis
			Dracunculus medinensis	Guinea worm disease
			Gnathostoma spinigerum	Gnathostomiasis

** Adapted from Fritsche, 1999.*[10]

Figure 247-1 Adult *Pediculus capitis*, the human head louse. Note the prominent claws for grasping hair.

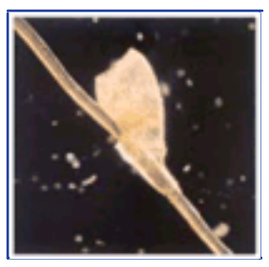

Figure 247-2 Egg or nit of human head louse attached to a hair shaft. Note that the operculum is missing indicating previous emergence of the larval louse.

during sexual intercourse. The life cycle for all human lice averages 3–4 weeks.[2] [9]

Fleas, order Siphonaptera

Fleas are small (1–2mm), laterally compressed, wingless ectoparasites with long muscular legs adapted for jumping great distances (Fig. 247.4). Fleas that attack humans include both blood-sucking pests (many species) and tissue-penetrating jiggers or chigoes. Infestations commonly occur with exposure to domestic animals and pets; the most pestiferous species are the dog flea (*Ctenocephalides canis*), the cat flea (*C. felis*) and the human flea (*Pulex irritans*). Some individuals become highly sensitized to flea bites, whereas others are unaffected. Fleas probe their hosts repeatedly, often leaving clustered bite sites, which may produce intense itching with papule formation in sensitized individuals. Cat and dog fleas are also the usual intermediate hosts for the tapeworm *Dipylidium caninum* and less frequently for *Hymenolepis diminuta* and *H. nana*. Because larvae of these species often develop in an animal's bedding or in carpets and furniture, eradication may require fumigation and cleaning of these articles. The Oriental rat flea, *Xenopsylla cheopis*, is an extremely important species because it transmits the plague bacillus *Yersinia pestis* and the agent of murine typhus, *Rickettsia typhi* (see Table 247.2). Although normally parasitizing several species of rats, this flea readily attacks humans should the rodent host die.[1] [3] [8]

Tunga penetrans, the jigger or chigoe flea, is found in both Central and South America and regions of tropical Africa. The female flea attaches to and embeds itself in the skin, especially between the toes and under the toenails, where it grows to the size of a small pea.

Figure 247-3 Adult *Phthirus pubis*, the human crab louse. The bodies of these lice are rounder and more crab-like than those of head or body lice. *With permission from ASM Press.*

Figure 247-4 Adult *Ctenocephalides canis*, the dog flea. Note the strong muscular hind legs.

After the eggs are discharged, the flea dies, prompting an inflammatory response and possible secondary bacterial infection. Tungiasis is diagnosed by identifying the dark portion of the flea's abdomen (displaying the respiratory spiracles) protruding from the skin surface of an enlarging lesion.[1] [8] [9]

Cockroaches, order Dictyoptera

Cockroaches are nuisance pests that have closely adapted themselves to human habitation, sharing our food, shelter and warmth. In addition to mechanically transmitting pathogenic bacteria, they may also spread hepatitis virus and polioviruses, intestinal protozoa and enteric nematodes. Allergies and asthma may develop in some individuals following exposure to their excreta, cast skins or body parts.[8]

Cosmopolitan species include *Blattela germanica* (the German cockroach), *Blatta orientalis* (the Oriental cockroach) and *Periplaneta americana* (the American cockroach).

Control programs include limiting entry to buildings, denying access to food, and the use of pesticides.[9] [17]

Bedbugs and kissing bugs, order Hemiptera

Bedbugs (family Cimicidae) and kissing bugs (family Reduviidae) are blood-sucking insects that have a long narrow proboscis, which is folded underneath the body when not in use. Bedbugs (*Cimex lectularius* and *C. hemipterus*) are reddish-brown, dorsoventrally flattened, wingless insects that are approximately 5mm in length (Fig. 247.5). They are cosmopolitan in distribution and will attack almost any mammal, feeding primarily at night. During daylight hours they hide under mattresses, loose wallpaper and floorboards. Although not

known to transmit disease, bedbug bites may cause painful wheals or bullae, depending upon an individual's sensitivity to their saliva.[1] [3] [18]

Kissing bugs (genera *Triatoma, Rhodnius, Panstrongylus*) have a cone-shaped head on a narrow neck and an abdomen that is widened in the middle. These insects are black or brown and some have orange and black markings on the abdomen. They average 1–3cm in length and unlike bedbugs have well-developed wings for flight. Like bedbugs, kissing bugs are relatively painless feeders on vertebrates and produce similar skin reactions. In Mexico and Central and South America they transmit the agent of Chagas' disease, *Trypanosoma cruzi*, in the feces, which is secondarily inoculated into the skin by the human host while scratching.[1] [9]

Assassin bugs are closely related to kissing bugs and are also able to transmit *T. cruzi*. Unlike kissing bugs, assassin bugs have less conspicuous body markings and produce a painful bite.

Bees, wasps and ants, order Hymenoptera

Hymenopterans are social insects that readily defend their nests when disturbed. In nonreproductive females the ovipositor is modified as a stinger capable of injecting venom for use in the capture of prey or for defense. The venom of bees (genera *Apis, Bombus*), paper wasps (genus *Polistes*), hornets (genus *Vespa*) and yellowjackets (genera *Vespula, Paravespula* and *Dolichovespula*) causes only transient swelling and discomfort in most individuals, but may be responsible for systemic reactions, including anaphylaxis, in others who have been previously sensitized. The africanized honey bee was introduced into Brazil in 1956 and has since spread throughout parts of South, Central and North America. These bees, which are more easily provoked than other honey bees, exhibit massive stinging behavior, although their venom is no more toxic than that of the European honey bee.[5] [8]

Many species of ants are problematic for humans because of their abilities to bite, and some groups, such as harvestor ants (genus *Pogonomyrmex*) in North America, fire ants (genus *Solenopsis*) in North and South America, bull ants (genus *Myrmecia*) in Australia and *Myrmica* ants elsewhere are capable of giving painful stings, to which some individuals may develop serious allergic reactions.[19] Fire ants in particular are easily disturbed and will attack in large numbers.[8]

Beetles, order Coleoptera

Although beetles are perhaps best known as pests of agricultural crops, some species can give a painful bite, and others, especially the blister beetles, can exude vesicating fluids, including cantharidin, that cause dermatitis or blister formation. Cantharidin is the chief component of the aphrodisiac known as 'Spanish Fly'. The larvae of

Figure 247-5 Adult bedbug *Cimex lectularius.* *With permission from ASM Press.*

certain larder beetles have barbed urticating hairs, which may be responsible for dermatitis or, if ingested, irritation of the gastrointestinal tract. Larval and adult larder and grain beetles can also serve as intermediate hosts for the rodent and human tapeworms *H. diminuta* and *H. nana*.[1] [3] [8]

Moths and butterflies, order Lepidoptera

Certain larvae (caterpillars) of lepidoptera possess urticating hairs or spines capable of injecting venom when handled. Although most effects of these toxins remain localized to the skin and can produce a severe local burning sensation, systemic effects, including shock and paralysis, have been reported.[8] Adult tussock and gypsy moths are known to have urticating scales and hairs that can cause dermatitis, eye irritation and respiratory tract irritation, especially among forestry workers.[20]

Flies, mosquitoes and midges, order Diptera

Diptera are characterized by the presence of a single pair of membranous wings. Among all arthropods, they are responsible for the greatest share of human disease through their blood-sucking activities, direct tissue invasion by larval forms (myiasis) and mechanical or biologic transmission of infectious agents (see Table 247.2). Bites from a variety of flies, mosquitoes and biting midges often cause local irritation from sensitivity to the saliva and, in some individuals, systemic reactions. In addition to blood-sucking activities, the repeated attacks themselves can be physically and psychologically damaging.

Myiasis may occur in an accidental, facultative or obligatory fashion and involve cutaneous, auricular, ocular, nasopharyngeal, gastrointestinal or genitourinary sites. Pseudomyiases occur occasionally and can cause significant psychologic distress, but usually result from the contamination of sputum, urine or stool samples with fly eggs following improper collection or storage.[8] [10]

Accidental and facultative myiasis

The house fly, *Musca domestica*, has no requirement for developing in mammalian tissue, yet is occasionally found in dead tissue or under plaster casts. This type of accidental myiasis is not uncommon, but is rarely clinically significant. Facultative myiasis is most often caused by blowflies and flesh flies, which ordinarily feed on dead tissues, but may move into adjacent viable tissues or infest exposed ulcers or traumatic wounds.

Obligatory myiasis

Obligatory myiasis is caused by certain species that develop only in living tissues. Those species that infect humans are all of zoonotic origin. The human botfly, *Dermatobia hominis,* develops in boil-like subcutaneous lesions with the posterior end of the maggot appearing at the skin surface (Fig. 247.6). This species is most commonly found in individuals who have spent time in Central or South America or, less frequently, Africa. Other botflies that occasionally infest humans are those parasitic on domestic and wild animals, including the horse botfly *Gasterophilus intestinalis*, cattle botfly *Hypoderma bovis*,

Figure 247-6 Larva of *Dermatobia hominis*, the human botfly, following recovery from a furuncular skin lesion. *With permission from J Brad Thomas, David L Bergeron and James J Plorde.*

the sheep botfly *Oestrus ovis* and rodent botflies of the genus *Cuterebra*.[21]

The tumbu fly (*Cordylobia anthropophaga*), found in sub-Saharan Africa, also causes a furuncular-type of myiasis. Eggs of this species are usually laid on the ground or on hanging laundry and larvae rapidly penetrate the skin upon contact.

The most serious obligatory myiasis is caused by the Old World screw-worm, *Chrysomya bezziana,* and the New World screw-worm, *Cochliomyia hominivorax*. These species lay their eggs directly on their cattle hosts, usually on wounds or near the nostrils. The larvae actively feed and move through living tissues. Human infections can be particularly destructive if the larvae invade the eye, nose or mouth. A variety of other species are also known to be responsible for traumatic obligatory myiasis in humans.[3] [8] [9] [22]

Arachnids

Medically important arachnids include the scorpions, spiders, ticks and mites. Scorpions and spiders are best known for their abilities to inject poisonous venoms, whereas ticks and mites are best known as vectors for viral, bacterial and protozoal pathogens.[1] [11] [18]

Scorpions, subclass Scorpiones

Scorpions are predatory in nature and paralyze their intended victim with a neurotoxic venom from the sting, which may also be used for defensive purposes. Toxicity to humans varies depending upon the species. Many elicit no more reaction than that of a bee sting, but some species are deadly, causing over 1000 deaths annually, especially among children. Poisonous species occur in the western hemisphere, Europe, Africa and the Middle East.[1] [3] [4] [8]

Spiders, subclass Araneae

Spiders lack a tail with attached stinger, but instead have fang-like chelicerae among their mouthparts, through which venom can be expressed. Although the majority of spiders are venomous, few have chelicerae capable of penetrating human skin; among those that can most envenomations produce no more than transient local effects with pain, numbness, erythema and swelling (e.g. wolf spiders in the genus *Lycosa* and tarantulas, among many others). More serious disease results from those species capable of producing local or systemic arachnidism with potent dermonecrotic enzymes or neurotoxins, respectively.[8]

Necrotic arachnidism occurs worldwide and is produced most commonly by a number of species in the genus *Loxosceles*, especially the cosmopolitan *L. reclusa* (brown recluse), whereas in South America *L. laeta* is well known for producing severe dermonecrosis. Variously named violin, fiddleback or recluse spiders, they measure 1–2cm long, are tan to dark brown in color and have a darkened violin-shaped marking oriented base forward on the dorsum of the cephalothorax. When present in homes they are reclusive in their habits, preferring undisturbed areas. Their bite is painless and often goes unrecognized until several hours later when the

area becomes red, swollen and painful. The venom contains multiple enzymes including sphingomyelinase D and is both dermonecrotic and hemolytic, producing variable degrees of cutaneous necrosis and sloughing of the involved skin over several days. The resulting lesion may be difficult to heal and subject to secondary infection. Systemic reactions such as hemolysis and acute renal failure occur rarely.[1] [3] [8] Other spider genera implicated in producing necrotic arachnidism include the hobo spider *Tegenaria agrestis* in Europe and the Pacific Northwest of North America, crab spiders (genus *Sicarius*) in Africa and sac spiders (genus *Chiracanthium*), which are found worldwide.[4] [23]

Widow spiders (genus *Latrodectus*) are found worldwide and are responsible for systemic arachnidism through the action of a potent neurotoxin capable of producing weakness, myalgia, paralysis and convulsions, and occasionally death. Latrodectism is one of the

Figure 247-7 Female *Lactrodectus mactans*, the black widow spider. Note the characteristic red hourglass marking on the underside of the abdomen. *With permission from New England Journal of Medicine 1994;331:777.*

leading causes of death from arthropod envenomations worldwide, with published mortality rates varying from < 1 to 6%.[4] *Latrodectus mactans,* the black widow, and *L. geometricus*, the brown widow, are among the most widespread species. Female widow spiders vary in appearance by species, being glossy black to brown with a characteristic red or orange hourglass-shaped marking on the underside of the abdomen, and displaying a leg span of 3–4cm (Fig. 247.7). They live in protected locations such as woodsheds, basements and outdoor privies. [3] [8] Other spiders producing dangerous neurotoxins include the funnel web spiders (genus *Atrax*) in Australia and armed spiders (genus *Phoneutria*) in South America.

Ticks, subclass Acari

Unlike spiders and scorpions, ticks have a fused cephalothorax and abdomen and a characteristic toothed hypostome for feeding. Tick development progresses through four stages — egg, larva, nymph and adult — with a blood meal required for progression. Humans usually acquire ticks in grassy or brushy areas in close proximity to the usual animal hosts. All species are obligate blood-sucking ectoparasites and are important vectors of viral, bacterial and protozoal pathogens to humans and domestic animals (see Table 247.2). Their feeding activities may also produce local tissue damage and blood loss, especially in livestock and wildlife, or tick paralysis, a syndrome caused by a neurotoxin secreted by a tick's salivary glands that produces ascending flaccid paralysis and toxemia. The symptoms may closely mimic those of Guillain-Barré syndrome, poliomyelitis or botulism. Removal of the attached tick, often found in an unnoticed location such as above the hair line on the nape of the neck, usually results in resolution of the symptoms within hours to days.[24] [25]

Species affecting humans include members of the family Ixodidae (hard ticks) and Argasidae (soft ticks):

- hard ticks have anteriorly directed mouthparts and a sclerotized plate or scutum, on the dorsum, with the scutum covering the entire dorsum of the male, but only the anterior portion in the female, allowing the body to swell when engorged (Fig. 247.8); and
- argasid ticks have a soft leathery body lacking a scutum and ventrally directed mouthparts that are not visible when viewed from above (Fig. 247.9).

Unengorged ticks are generally 2–5mm long, but may enlarge to several times this size following engorgement. Engorged hard ticks may mimic soft ticks, so care must be exercised in their identification.[24] Most ticks found crawling on or embedded in human skin are hard ticks. Soft ticks tend to feed only briefly and then often at night.

Figure 247-8 Nonengorged adult female *Dermacentor variabilis*, the common dog tick, of the family Ixodidae (hard ticks). *With permission from Northwest Infectious Disease Consultants.*

Figure 247-9 Nonengorged adult *Ornithodoros hermsi*, of the family Argasidae (soft ticks). *With permission from Northwest Infectious Disease Consultants.*

Disease transmission is produced most commonly by hard ticks in six genera (see Table 247.2). *Ixodes* spp., primarily *I. scapularis* and *I. pacificus* in North America and *I. ricinus* and *I. persulcatus* in Europe and parts of Asia, are responsible for transmitting the agents of Lyme disease, babesiosis and human granulocytic ehrlichiosis (Fig. 247.10). The latter is an emerging rickettsial-like disease that produces an influenza-like illness that varies from being subclinical to fatal. Old World *Ixodes* spp. are also capable of transmitting encephalitis-producing arboviruses. *Dermacentor variabilis* and *D. andersoni* are vectors for the agents of Rocky Mountain spotted fever, tularemia and Colorado tick fever in the New World, whereas *D. marginatus*, *D. silvarum* and *D. nuttalli* are capable of transmitting Siberian tick typhus and encephalitis-producing arboviruses in Europe and Asia.[3] [8] [11] [26]

Ticks in the genera *Amblyomma* and *Rhipicephalus* are found worldwide and are capable of transmitting Rocky Mountain spotted fever in the New World. *Rhipicephalus* ticks also transmit boutonneuse fever in southern Europe and Africa, whereas *Amblyomma* ticks have recently been implicated as vectors of *Ehrlichia chaffeensis*, the agent of human monocytic ehrlichiosis. Infection with this organism produces an illness similar to that of human granulocytic ehrlichiosis and the disease has been documented widely in the USA as well as in Europe and Africa. [8] [26]

Haemaphysalis ticks transmit Siberian tick typhus in parts of Eurasia, boutonneuse fever in Africa, and arboviruses responsible for encephalitis in parts of Eurasia and Kyasanur Forest disease in India.

Hyalomma ticks are widespread in parts of Europe, Africa and Central Asia and transmit both Siberian tick typhus and the viral agent of Crimean-Congo hemorrhagic fever.[3] [8]

Soft ticks of the genus *Ornithodoros* occur in many parts of the New and Old worlds and are important vectors of the relapsing fever spirochetes (*Borrelia recurrentis* and related forms; see Table 247.2). [11]

Figure 247-10 Nonengorged adult female *Ixodes scapularis*, the blacklegged tick or deer tick, of the family Ixodidae (hard ticks). *With permission from Northwest Infectious Disease Consultants.*

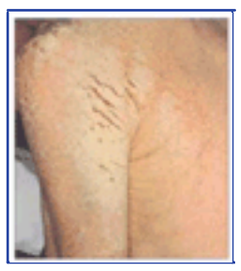

Figure 247-11 Crusted Norwegian scabies in a patient who has AIDS. *With permission from New England Journal of Medicine 1994;331:777.*

Mites, subclass Acari

Mites are microscopic (usually <1mm) arachnids that are widely distributed in the environment. Medically important species may:

- attack humans directly;
- serve as vectors for infectious diseases; and
- cause dust allergies.

Most humans are naturally infested with both *Demodex folliculorum* and *D. brevis*, the follicle mites. Follicle mites are minute (0.1–0.4mm) elongated parasites with stubby legs that can be recovered from hair follicles and sebaceous glands. They are common incidental findings on histologic skin preparations. Although their presence has been associated with various skin conditions, they are commonly found in healthy individuals as well, which makes their significance hard to assess.[27]

Sarcoptes scabiei, the itch or mange mite, is of greater medical importance because of its ability to create serpiginous tunnels through the upper layers of the epidermis. Transmitted through personal contact, these mites are found primarily in the interdigital spaces and the flexor surfaces of the wrists and forearms, and less commonly in other areas, including the breasts, buttocks and external genitalia. Inflammation and intense itching results from the tunneling activity and from the deposition of eggs and excreta. Clinical manifestations vary depending upon the degree of sensitization to the parasites and their products. Lesions often become secondarily infected. A generalized dermatitis occurring in the presence of thousands of mites, typically in elderly or immunocompromised individuals, is known as crusted or Norwegian scabies (Fig. 247.11). Diagnosis is made by placing skin scrapings collected from tunneled areas in 20% potassium hydroxide or mineral oil for clearing and

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Figure 247-12 Adult *Sarcoptes scabiei*, the human itch or mange mite, seen in skin scrapings. *With permission from New England Journal of Medicine 1994;331:777.*

examining under the microscope. Detection of eggs, six-legged larvae and eight-legged nymphs or adults is diagnostic, but may be difficult to demonstrate (Fig. 247.12).

A number of animal mite species can attack humans for a blood meal, either as larval forms or as adults, when the normal mammalian or bird hosts are not available.[15] [28] [29] Larval chigger mites (family Trombiculidae) are problematic in many parts of the world because their saliva can produce large wheal-and-flare reactions with intense itching. Often red in color, these tiny six-legged larvae commonly attach to the skin in areas where clothing is restrictive such as ankles, waistline, armpits and wrists. In parts of Asia and Australia trombiculid mites are vectors for transmitting the agent of scrub typhus (see Table 247.2).[3] [9]

Classes of lesser medical importance

Millipedes and centipedes

Millipedes are worm-like arthropods with numerous apparent body segments, each with two pairs of legs, that are commonly found in and under decaying vegetation.

Many species produce vesicating secretions composed of quinonoids and parabenzoquinones from glands located on each body segment. Larger tropical forms (up to 25cm) can squirt these secretions over several centimeters when handled roughly. Exposure of the skin or mucous membranes to these fluids may produce a burning sensation and blister formation. Exposure of the eye may result in periorbital 'burns', conjunctivitis and keratitis, which may progress to corneal perforation and blindness.[7] [8]

Centipedes are flatter than millipedes, have only one pair of legs per body segment and display long antennae. They are fast moving and can inflict a painful sting from a pair of forward-directed pincers that are modified from the first pair of legs. The larger species (26–45cm) found in subtropical and tropical regions are able to penetrate human skin when handled, giving a painful burning sting with a local tissue reaction. Although systemic reactions can occur in individuals who have been previously sensitized, fatalities are rare.[3] [8]

Crustaceans

Crustaceans, most notably crabs, crayfish and microscopic copepods are of medical importance by serving as hosts and vectors for larval stages of several different helminths (see Table 247.2). [1] [18]

Pentastomes

Pentastomes or tongue worms are arthropods of uncertain affinities that possess few distinctive morphologic characteristics. Larval stages resemble mites and have occasionally been reported producing liver and lung infections in humans in Asia and Africa. Adult stages are wormlike organisms that live in the nasal passages of certain predatory reptiles, birds and mammals; they have been recovered from the nasopharynx of individuals from the Middle East and Africa where they are responsible for an obstructive condition known as halzoun.[1] [18]

MANAGEMENT

Medical management of most exposures to arthropods involves:

- removal of the offending organism if attached or embedded;
- palliative treatment for most bites and stings;
- use of antihistamines for local and systemic allergic phenomena and epinephrine (adrenaline) for serious systemic reactions; and
- administration of appropriate antivenoms for exposure to certain spider bites and scorpion stings.

Most embedded chigoe fleas and maggots can be removed as a minor surgical procedure either with a sterile needle, curettage or excision; use of mineral oil alone to occlude the spiracles (breathing tubes) of maggots at the skin surface may result in their spontaneous emergence. Care must be taken to ensure complete removal or serious secondary infection may result. Similarly, removal of the mouthparts of attached ticks is important as transmission of infectious agents may continue for a period of time should they remain embedded. Tweezers may be used to grasp the tick as close as possible to the skin surface followed by the use of steady pressure in a direction perpendicular to the skin.[8] [11]

Physiologic responses to the large variety of arthropod bites and stings varies significantly between individuals. Exposure to the excreta, molted skins and body parts of cockroaches, lice, fleas, bedbugs, scabies mites and dust mites may produce sensitization. Treatment is usually directed at counteracting the effects of histamine and other vasoactive compounds, released either by mast cells as a consequence of previous sensitization or introduced as a component of hymenopteran venom. Administration of oral, and occasionally parenteral, antihistamines may be indicated, depending upon the severity of symptoms. The development of systemic allergic reactions, most commonly associated with bee and wasp stings, may result in anaphylaxis and irreversible shock. Angioedema of the upper airway may produce occlusion, whereas pulmonary edema and bronchial constriction may result in respiratory failure, requiring the immediate administration of epinephrine and/or bronchodilators. [3] [4] [8]

Treatment for the effects of biting arthropods and exposure to the spines and urticating hairs of lepidoptera and coleoptera includes palliative remedies such as antipruritic lotions, creams and ointments, and, if more severe, a short course of systemic corticosteroids. Respiratory allergies, usually developing following sensitization to dust mites or cockroaches, are treated using inhaled corticosteroids or cromolyn sodium (sodium cromoglicate), although removal or control of the offending allergen should be attempted.

Bites or stings that involve neurotoxic or necrotic venoms are managed by counteracting the effects of the venom and providing supportive treatment. Antivenoms for widow spider bites are commercially available. Antivenoms for bites by South American *Loxosceles* spiders and stings by scorpions are available locally in endemic areas.[3] [4]

Eradication of body lice, head lice, pubic lice and scabies mites often becomes a challenge for both patient and physician and

reports of insecticide-resistant strains have made the task more complex. Mechanisms of resistance are unclear and the possibility of reinfestation or noncompliance with treatment regimens must be taken into account. Generally:

- body lice are treated with a combination of pyrethrins with piperonyl butoxide;
- head lice and public lice are treated using 1% permethrin lotion or lindane (1% gamma benzene hexachloride lotion or shampoo); and
- scabies infestations are usually treated with 5% permethrin cream, 10% crotamiton lotion or cream or 1% lindane lotion or cream.

With any of these compounds there is a possibility of allergic skin reactions; in addition lindane has the potential for central nervous system toxicity, especially in children and the elderly.[3] [8]

Management of delusional parasitoses are invariably difficult and time-consuming, requiring a team approach involving the family practitioner, dermatologist and psychiatrist. Treatment may include the use of psychoactive drugs such as pimozide or risperidone.[30]

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Figure 1-1 Viral genomes.

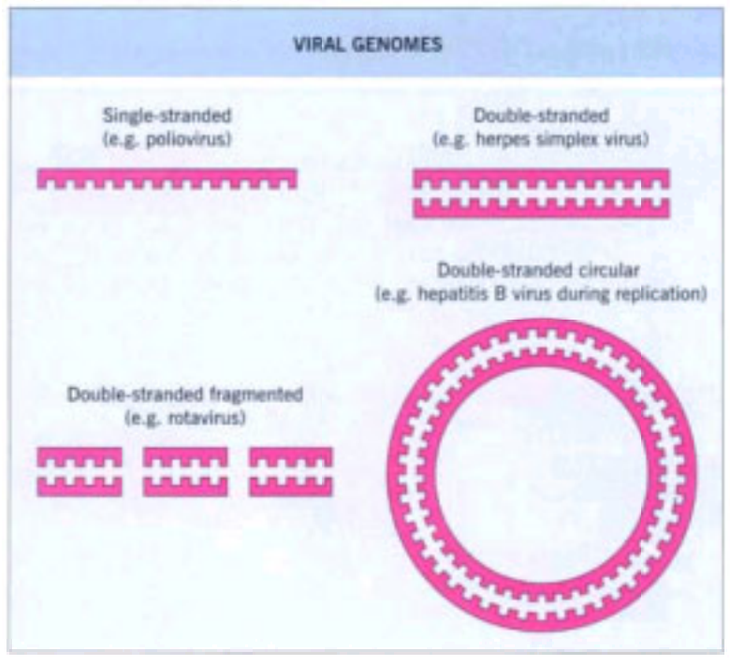


Figure 1-2 Examples of virions. Adenovirus is an icosahedral DNA virus without an envelope; fibers extend from the 12 points of the icosahedral coat; DNA forms a ribbon-like molecule. Approximate size 80nm. HIV-1; glycoprotein (GP) molecules protrude through the lipid membrane; the icosahedral capsid encloses a vase-shaped nucleocapsid, in which the diploid RNA is enclosed. Approximate size 100nm. Influenza virus is an enveloped RNA virus, containing nucleocapsid of helical symmetry; spikes of hemagglutinin and neuraminidase protrude from the lipid bilayer. Approximate size 100–200nm. Rabies virus is a helical RNA nucleocapsid with a bullet-shaped lipoprotein envelope, in which approximately 200 GPs are embedded. Approximate size 150nm. (The diagram is not to relative scale.) *Adapted from Collier and Oxford*[4] *by permission of Oxford University Press.*

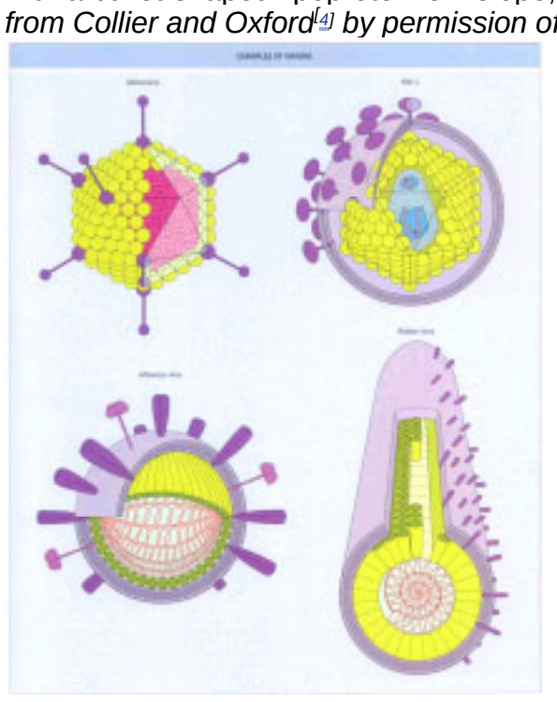


Figure 1-3 Viral 'lifestyles'.

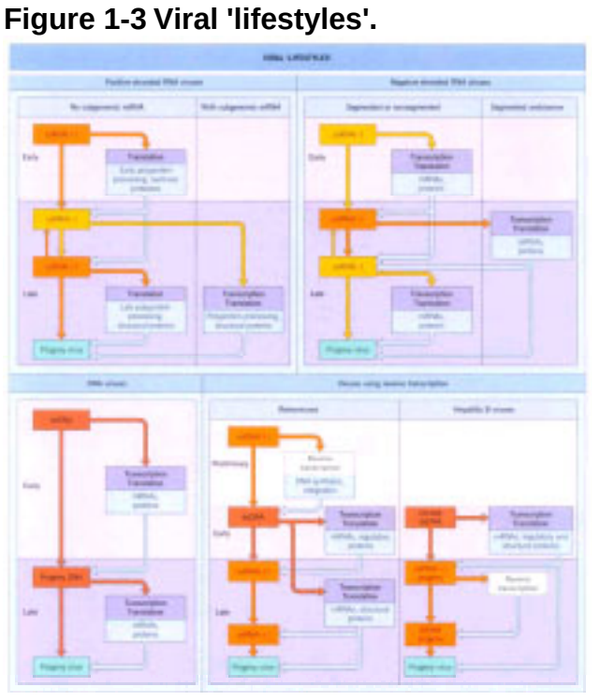


Figure 1-4 Bacterial cell walls. (a) *Mycoplasma pneumoniae* has a single membrane, made up of phospholipids and membrane proteins. (b) In Gram-positive organisms the cytoplasmic membrane is covered with a thick layer of peptidoglycan; chains of lipoteichoic acid protrude outside. (c) The cell wall of a Gram-negative rod is more complex. The layers are: the cytoplasmic membrane; the periplasmic space; a layer of peptidoglycan, which is thinner than that in Gram-positive bacteria; and an asymmetric outer membrane. The inner leaflet of the outer membrane is made of phospholipids. The outer leaflet has lipopolysaccharides as its principal lipids; porins, which are channel-forming proteins often organized as trimers, allow the penetration of hydrophilic molecules through the outer membrane. (d) The peptidoglycan of *Staphylococcus aureus* has polysaccharide chains ('backbone') that are alternating residues of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). Tetrapeptides are attached to MurNAc and are linked together by pentaglycines bridging the L-lysine of each tetrapeptide chain to the D-alanine of the neighboring one.

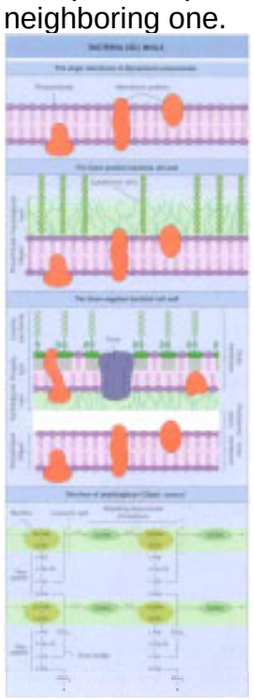


Figure 1-5 Transcription and translation in bacteria (*Escherichia coli*).

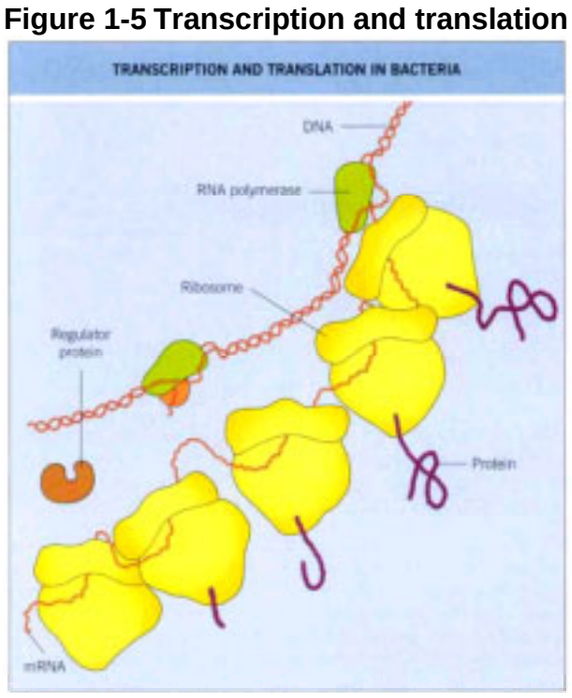


Figure 1-6 Genetic information in bacteria. This example is *Escherichia coli*. Additional genetic information may be supplied by extrachromosomal elements such as plasmids or bacteriophages. Bacteria may carry a variety of these 'mobile genetic elements', which may transfer readily from one cell to another. The electron micrograph shows a 8.65kb *E. coli* plasmid that confers sulfonamide and streptomycin resistance (left) and a single-stranded derivative of the plasmid (right).

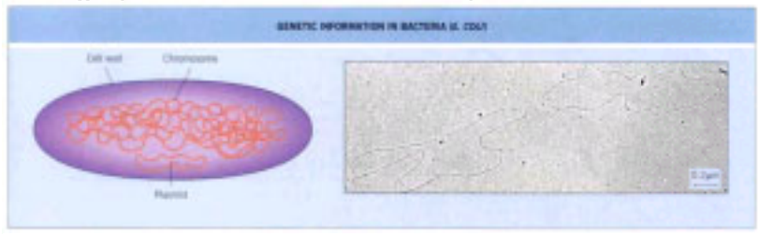


Figure 1-7 Flagella and motility in bacteria.

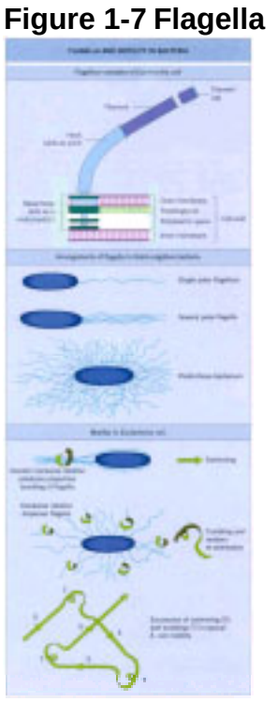


Figure 1-8 Contamination of humans by micro-organisms. Many parts of the body are colonized by normal flora, which can be the source of endogenous infection. Large numbers of micro-organisms are found in moist areas of the skin (e.g. the groin, between the toes), the upper respiratory tract, the digestive tract (e.g. the mouth, the nasopharynx), the ileum and large intestine, the anterior parts of the urethra and the vagina. Other routes are interhuman transmission of infections and exposure to exogenous contamination.

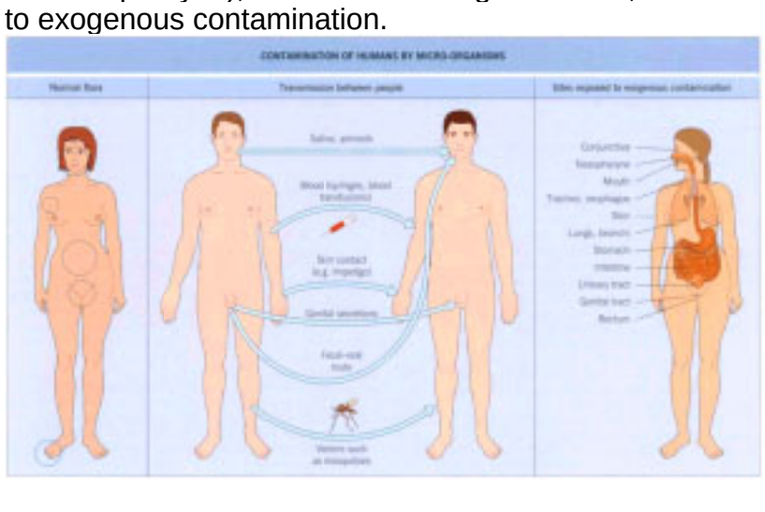


Figure 1-9 Bacterial adherence.

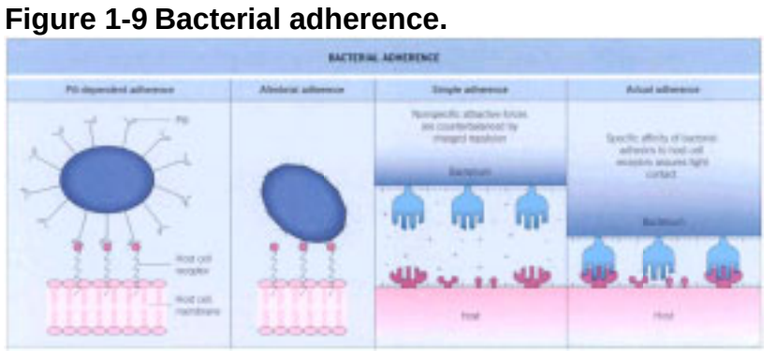


Figure 1-10 Structure of P-pilus in *Escherichia coli.*

Figure 1-11 Cell wall of *Streptococcus pyogenes.* The proposed model of the M protein is based on current sequence and structural data. ARP, immunoglobulin A receptor protein; FcR, receptor for the Fc portions of immunoglobulin. *Adapted from Kehoe.*[14]

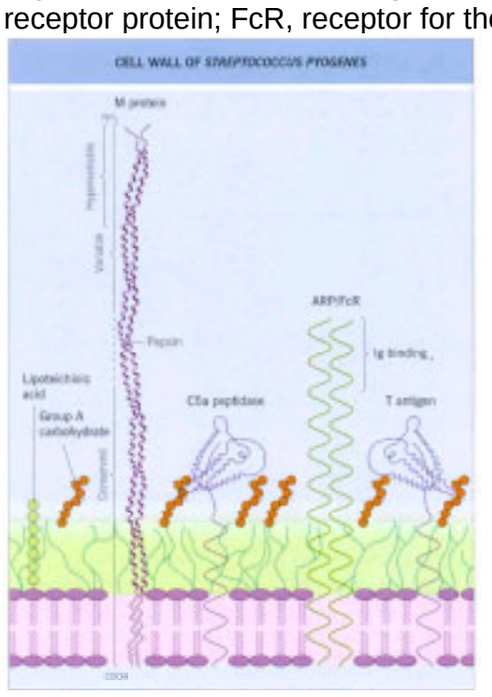


Figure 1-12 Opsonization and phagocytosis of bacteria. Bacteria are covered with IgG, specific for surface antigens. Bound IgG interacts with the phagocyte Fc?-receptor and pseudopods are formed, engulfing the bacterium into the host cell.

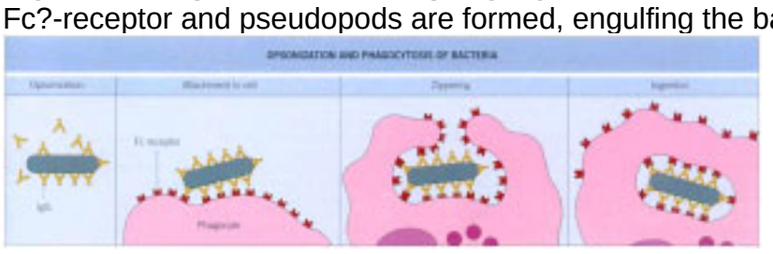


Figure 1-13 Enteropathogen-M cell interactions. (a) An uninfected M cell, enclosed between two adjacent enterocytes. The basolateral side forms a pocket where lymphocytes and macrophages are located. (b) Enteroadherent *Escherichia coli* forms microcolonies at the M cell surface, but is not internalized. (c) *Vibrio cholerae* undergoes transcytosis but is efficiently phagocytosed in the submucosa. (d) *Campylobacter jejuni* and *Yersinia* spp. undergo transcytosis, replicate in the submucosa and disseminate. (e) *Salmonella* spp. are transported across M cells, leading to destruction of the M cell. (f) *Shigella flexneri* is endocytosed by M cells, escapes into the cytoplasm, replicates, is propelled by actin tails and spreads to adjacent enterocytes. *Adapted from Siebers and Finlay.*[17]

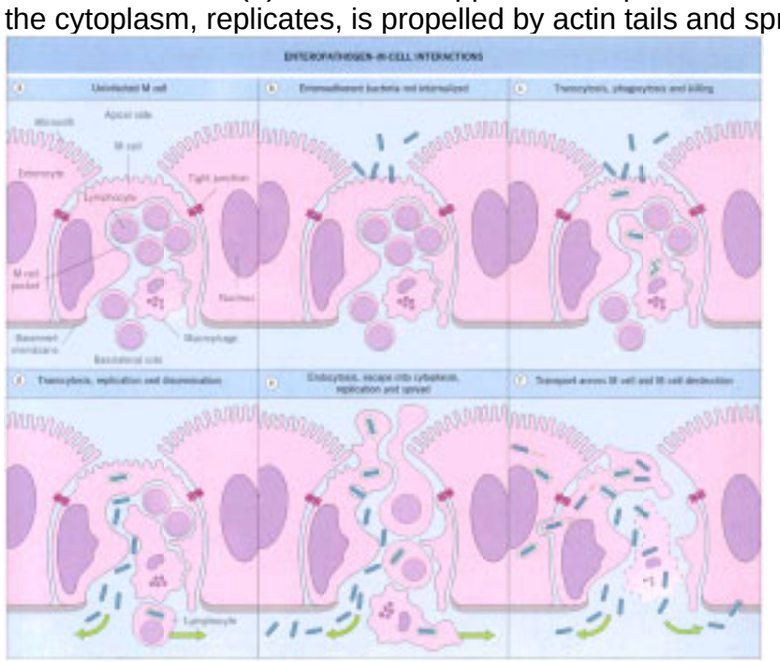


Figure 1-14 Genetic organization of the invasion region in *Salmonella* and *Shigella* spp. Identical patterns indicate topologically conserved blocks of genes. Each genus has genes that are unique. Despite remarkable genetic similarities, the invasion strategies of the two bacteria are quite different (see Fig. 1.13). *Adapted from Galan.*[19]

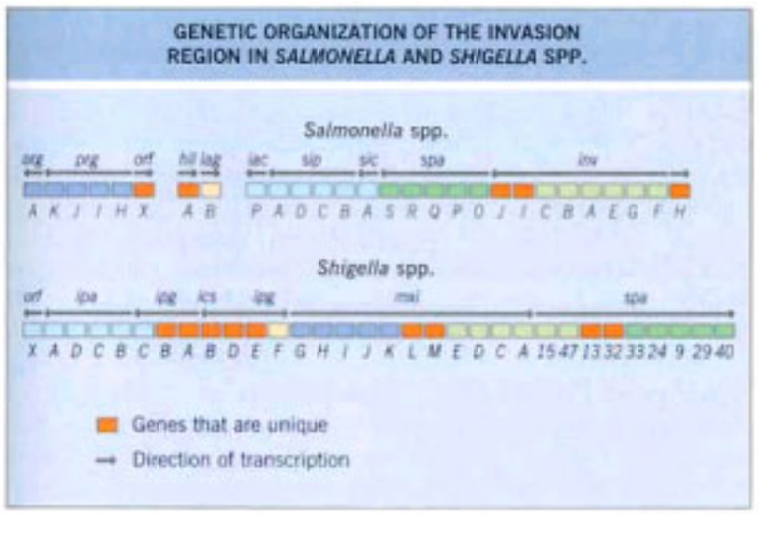


Figure 1-15 Actin-based motility in *Listeria monocytogenes.* The bacterium moves forwards at the rate of actin-filament growth behind the pathogen. *Adapted from Sanders and Theriot.*[27] The EM shows a section of a CaCo-2 cell infected with *Listeria monocytogenes*; the bacterium protrudes into the cytoplasm of an adjacent cell; protrusion is limited by a double membrane (arrowheads).

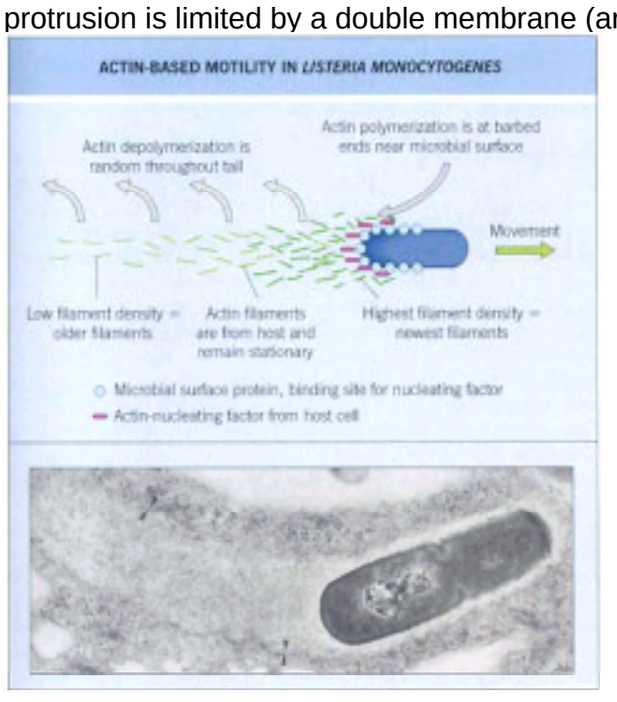


Figure 1-16 Intracellular life cycle of *Listeria monocytogenes*.

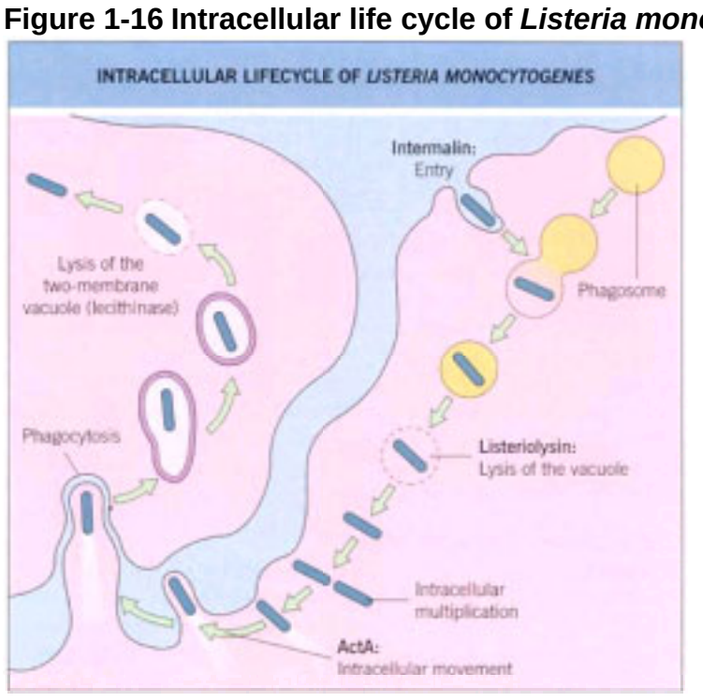


Figure 1-17 Action of bacterial toxins. (a) *Xenopus* oocyte treated with the cytolytic delta toxin (perfringolysin) of *Clostridium perfringens*. (b) Rabbit erythrocyte exposed to a very small quantity of streptolysin-O, produced by *Streptococcus* A,C,G. Hemoglobin escapes from sites of membrane rupture. *Courtesy of Dr J Alouf.*

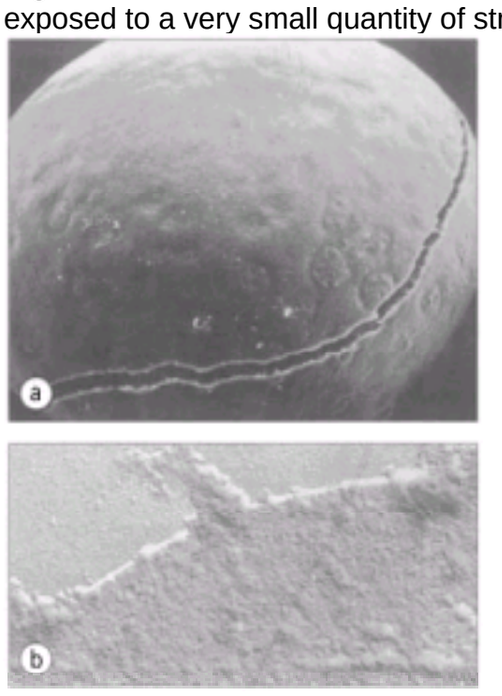


Figure 1-18 Diphtheria toxin synthesis and mode of action. (Top) The 25-residue leader sequence is cleaved off by the bacterial leader peptidase; the A and B subunits are generated from the precursor protein by a 'trypsin-like enzyme'. Once in the cytoplasm of a targeted eukaryotic cell, the A chain, responsible for ADP-ribosyl transfer, is disconnected from the B chain, responsible for receptor binding and membrane insertion. (Bottom) The B chain binds to a specific receptor on the eukaryotic cell. After endocytosis, acidification in the endosome induces insertion of the B chain into the endosomal membrane and translocation of subunit A into the cytosol, where it catalyzes the ADP ribosylation of EF-2. As a result, protein synthesis is inhibited and the targeted cell dies.

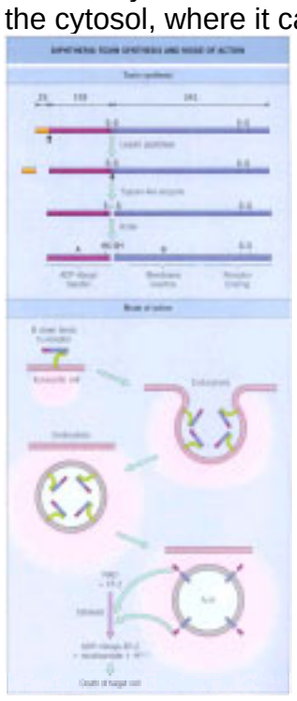


Figure 1-19 Iron regulation of diphtheria toxin synthesis. High iron concentrations in the environment repress the synthesis of diphtheria toxin: when bound to iron, DtxR-Fe acts as a transcriptional repressor of the *tox* gene.

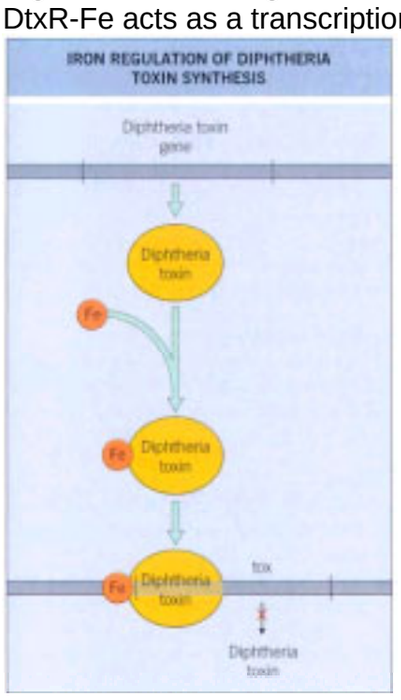


Figure 1-20 Apoptosis induced by Sendai virus. Morphologic changes in the apoptotic Sendai infected cell (right) include the typical condensation of chromosomal DNA. *Courtesy of Dr Dick Compans and Dr Kiyoshi Tanebayashi.*

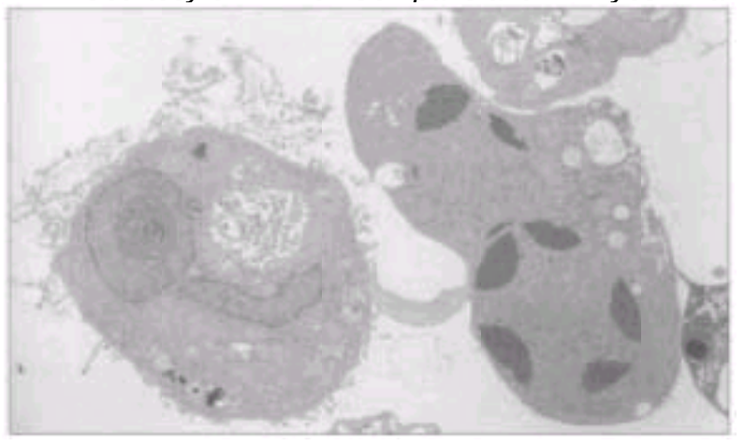


Figure 1-21 Virus-induced cytopathic effects.

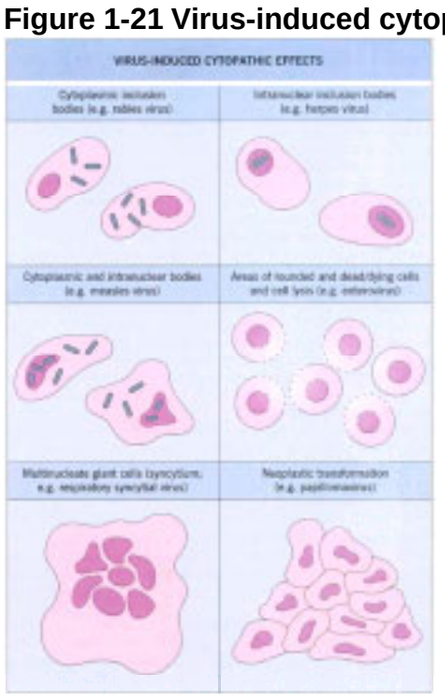


Figure 1-22 Phagocytosis and bacterial resistance to killing.

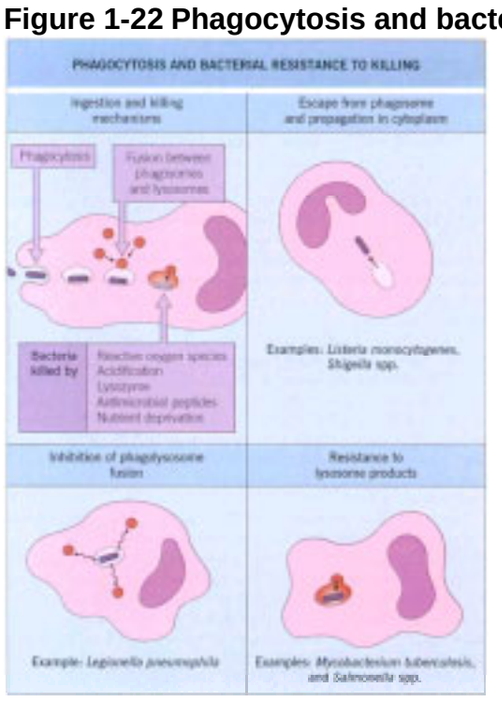


Figure 1-23 Mechanism of resistance to macrophage antimicrobial peptides by *Salmonella* spp. *Salmonella* produces the SapA (A) peptide, which complexes with host cell antimicrobial peptides. Other proteins encoded by the *sap* locus (SapB, SapC and SapD) are required for the transport of the SapA-antimicrobial peptide complex into the cytosol where the antimicrobial peptide is degraded.

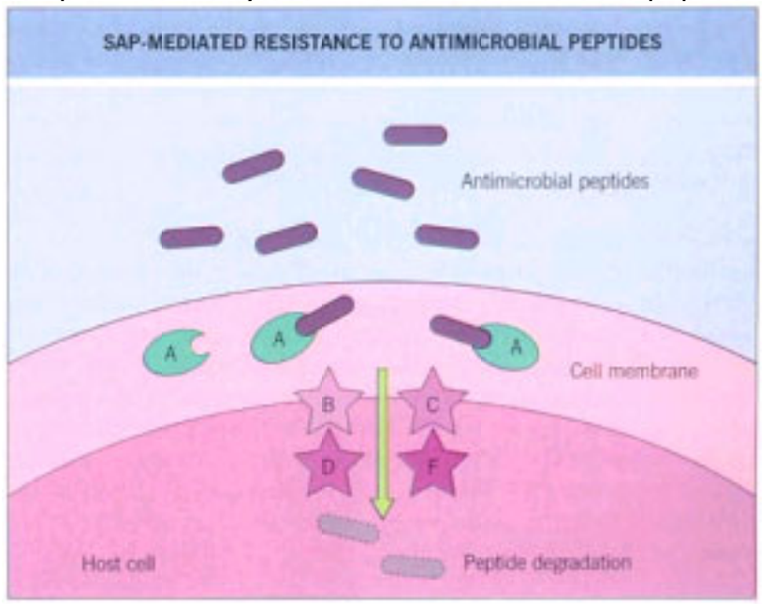


Figure 1-24 Antigenic and phase variations in microbial pathogens. Three mechanisms are shown. (Top) Exchange of DNA between nonexpressed copies of *pil*S and the expressed gene *pil*E in *Neisseria gonorrhoeae* can change the expressed antigen. (Middle) A switch mechanism is responsible for the (mutually exclusive) production of type A and type B flagella in *Salmonella typhimurium*. Phase variation depends on the orientation of a DNA fragment adjacent to the type A flagella gene. When A is expressed (a) from the promoter in the invertable fragment, the repressor for the type B flagella is expressed at the same time. As a consequence the type B flagella gene is repressed. Inversion of the DNA fragment abolishes expression of the A-repressor gene and the B-repressor gene (b). In this situation type B flagella are produced. (Bottom) Antigenic shift by gene reassortment results from infection of a single cell by two different virions.

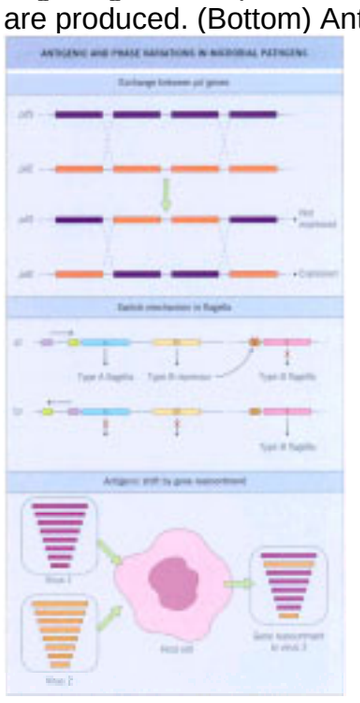


Figure 2-1 Pathways of inflammation induced by microbial components. Components of Gram-negative organisms (LPS) and Gram-positive bacteria (peptidoglycan) can activate similar pathways. C3a, biologically active soluble cleavage product of the activation of complement factor 3; C5a, biologically active soluble cleavage product of the activation of complement factor 5; DIC, disseminated intravascular coagulation; IFN, interferon; IL, interleukin; PMNL, polymorphonuclear leukocyte; TNF, tumor necrosis factor.

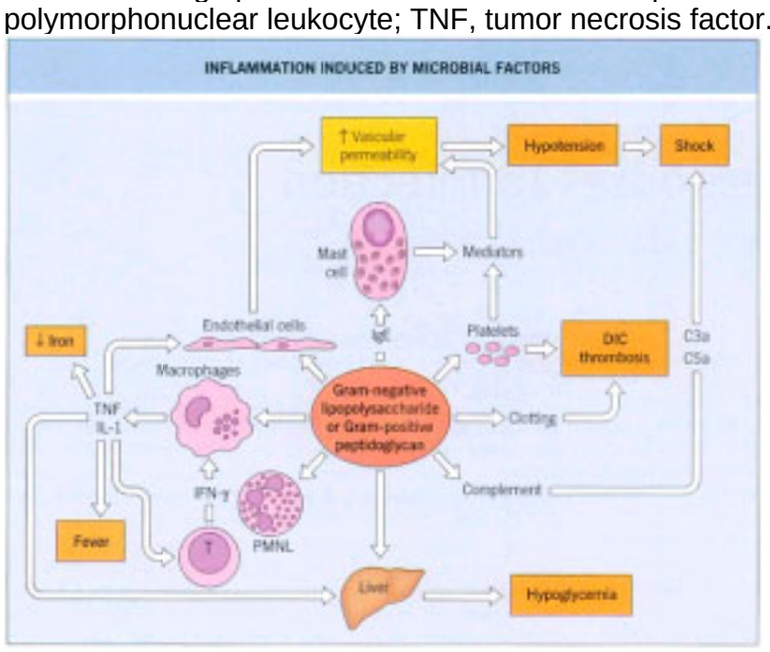


Figure 2-2 Systemic inflammatory response syndrome, septic shock and multiple organ dysfunction syndrome.

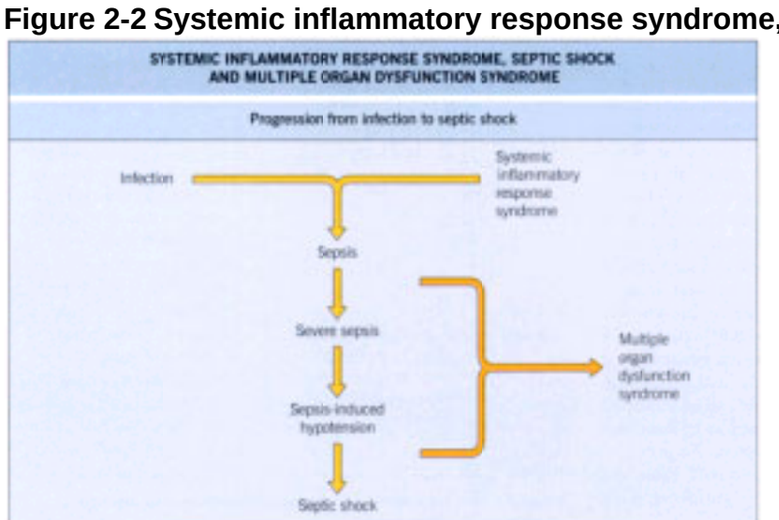


Figure 2-3 Tertian and quartan malarial fever patterns.

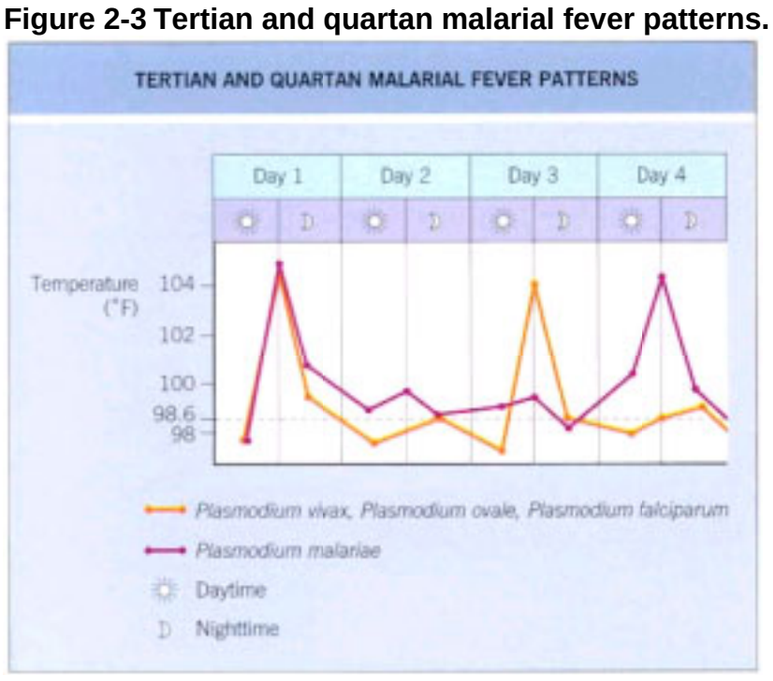


Figure 2-4 Normal diurnal variation in temperature. *These data are from eight healthy volunteers (see Chapter 80).*

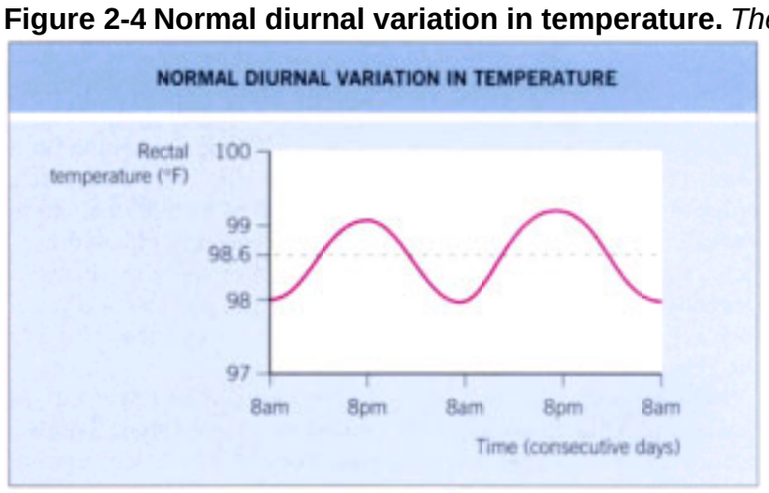


Figure 2-5 Mechanisms of fever. Fever may be induced either by exogenous pyrogens, such as microbes or their toxins, or by endogenous pyrogens.

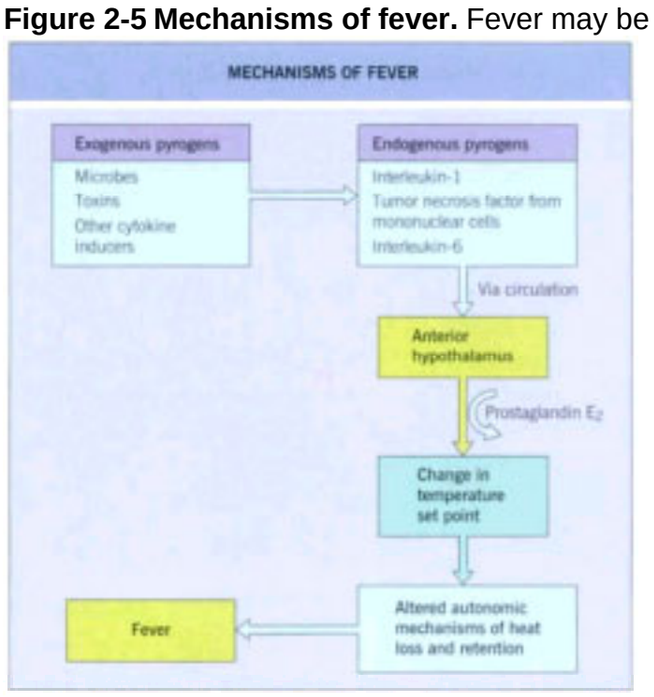


Figure 2-6 High and low temperature set points. The temperature set point is the temperature at which the animal modifies temperature by moving in the temperature gradient chamber. The increased high and low set points in the animals challenged with *Aeromonas hydrophila* result in higher than normal temperature. The data were obtained 3–6 hours after injection with saline or *A. hydrophila*. *Data from Vaughan et al.*[10]

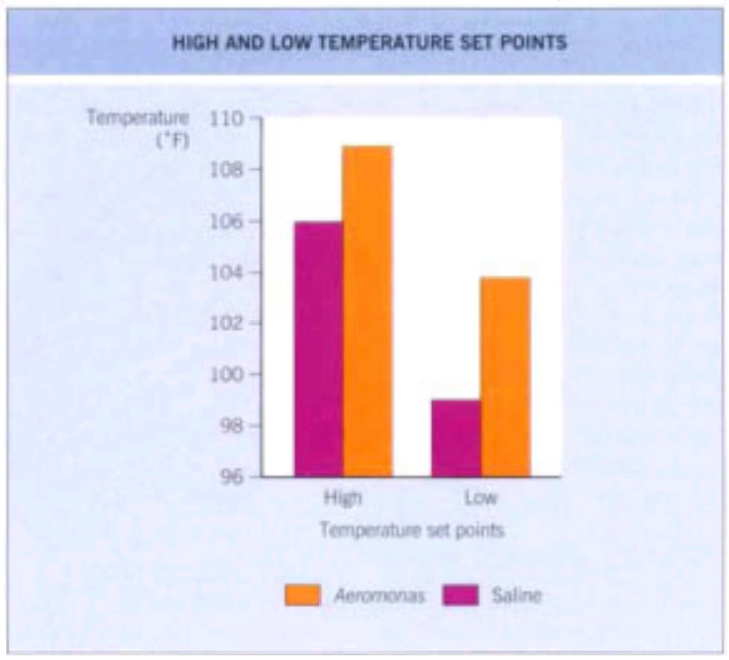


Figure 2-7 Survival of lizards injected with *Aeromonas hydrophila* at varying ambient temperatures. *Data from Kluger et al.*[7]

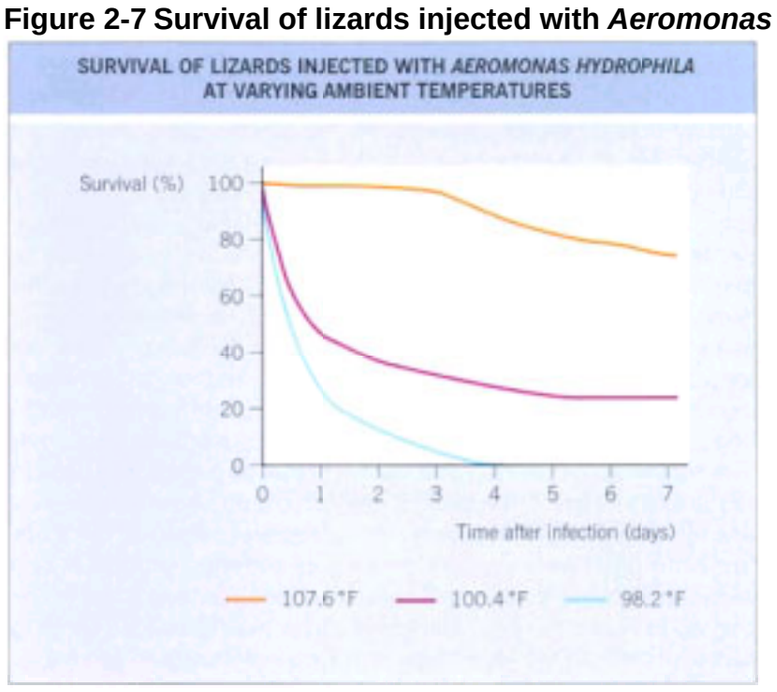


Figure 2-8 Signaling pathways that involve leptin.

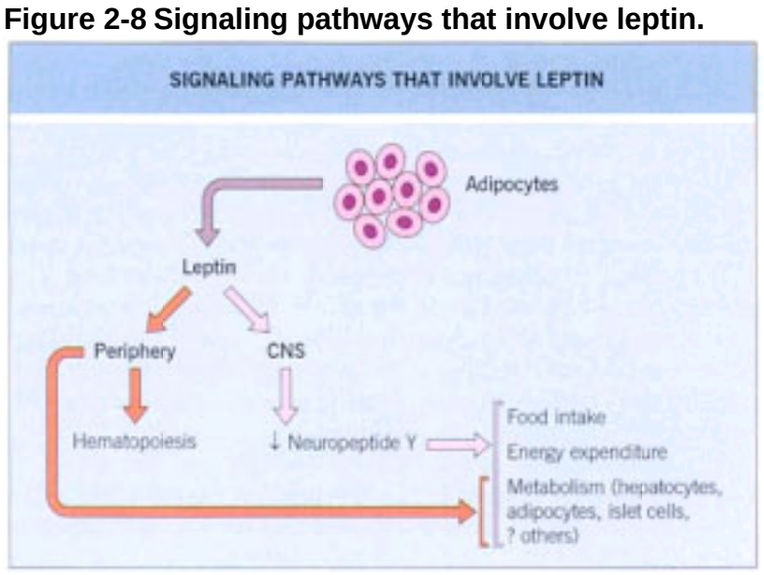


Figure 2-9 Production of acute-phase proteins after infection. Infection causes a rapid increase in the production of these proteins.

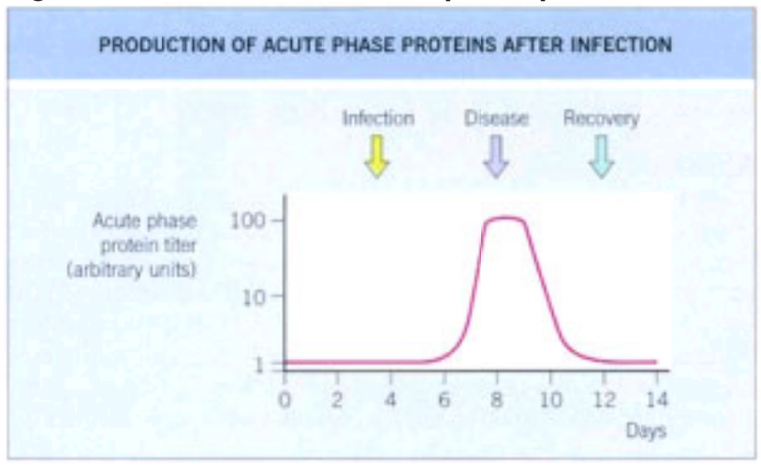


Figure 2-10 Acute cation response in infection and inflammation.

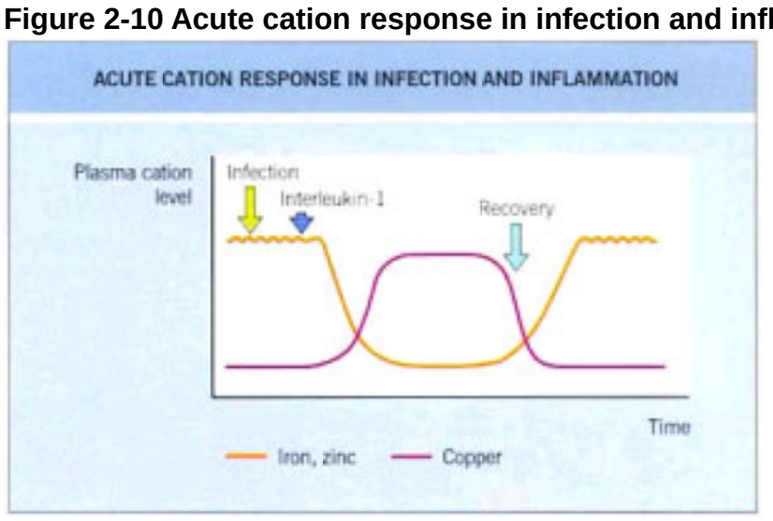


Figure 2-11 Microbial gene regulation via *Fur* gene.

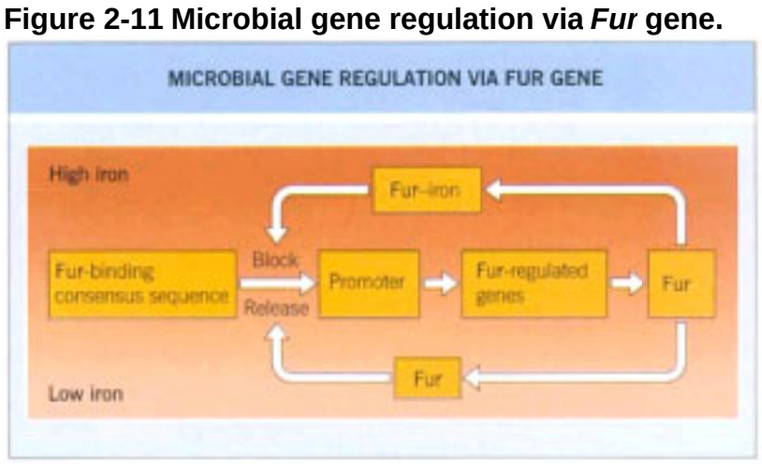


Figure 2-12 Pathologic effects of infection. B, B derived lymphocytes; IFN, interferon; IL, interleukin; PMNL, polymorphonuclear leukocyte; T, T lymphocytes; TNF, tumor necrosis factor.

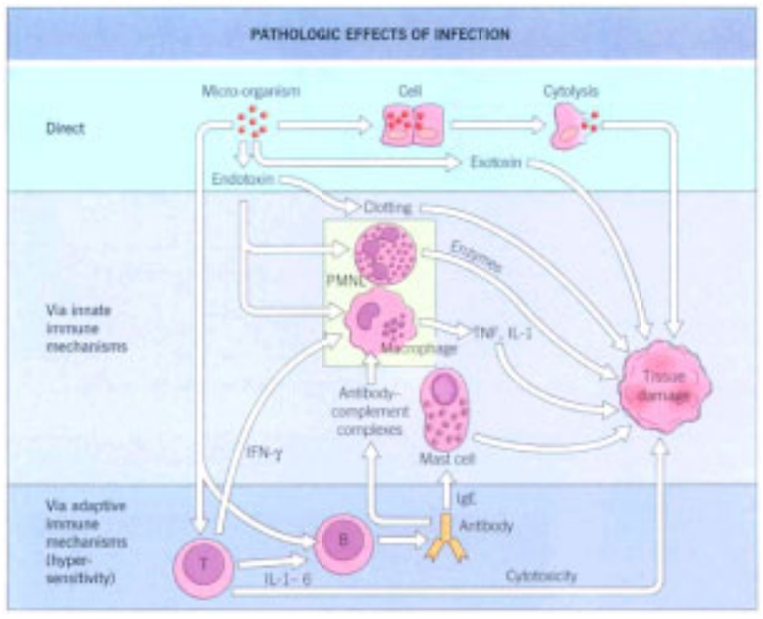


Figure 2-13 Invasion of micro-organisms across the intestinal mucosa.

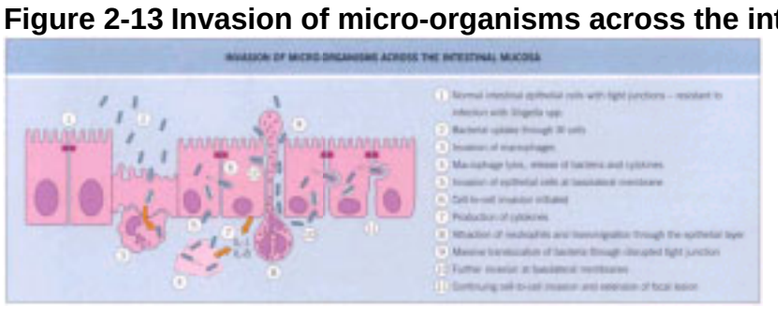


Figure 2-14 Various mechanisms adopted by micro-organisms to avoid phagocytosis.

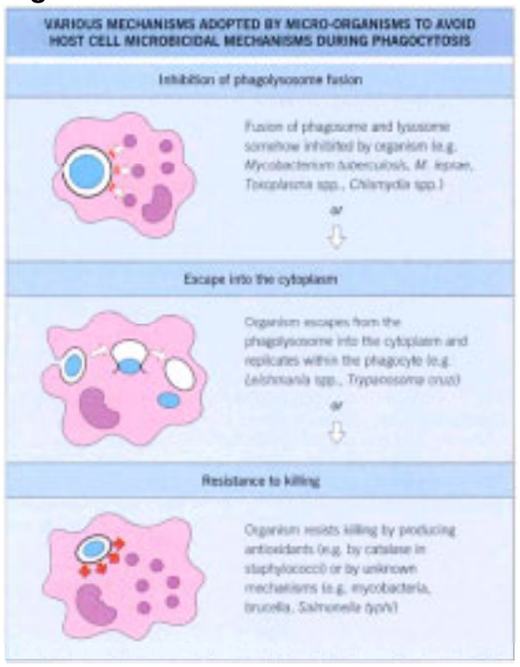


Figure 2-15 Nonimmunologic host defenses.

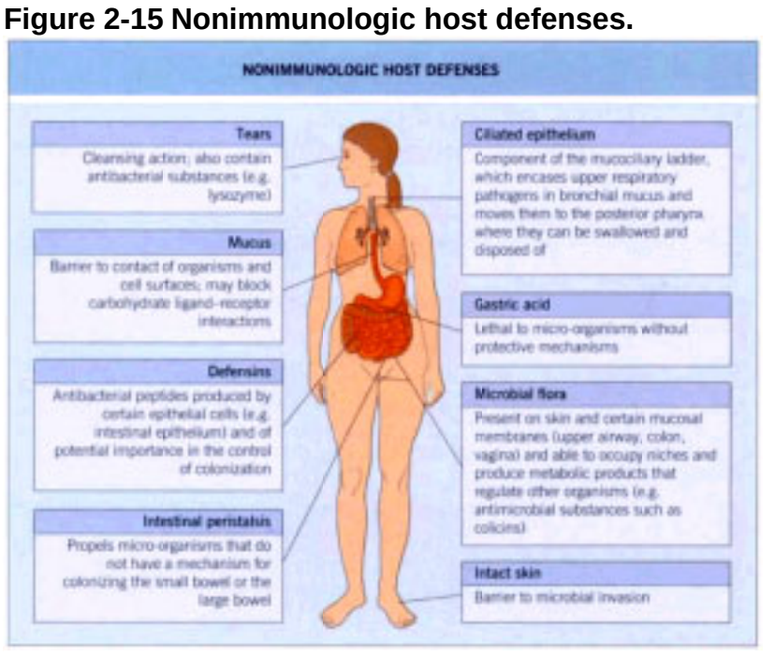


Figure 2-16 Life cycle of *Trypanosoma cruzi.*

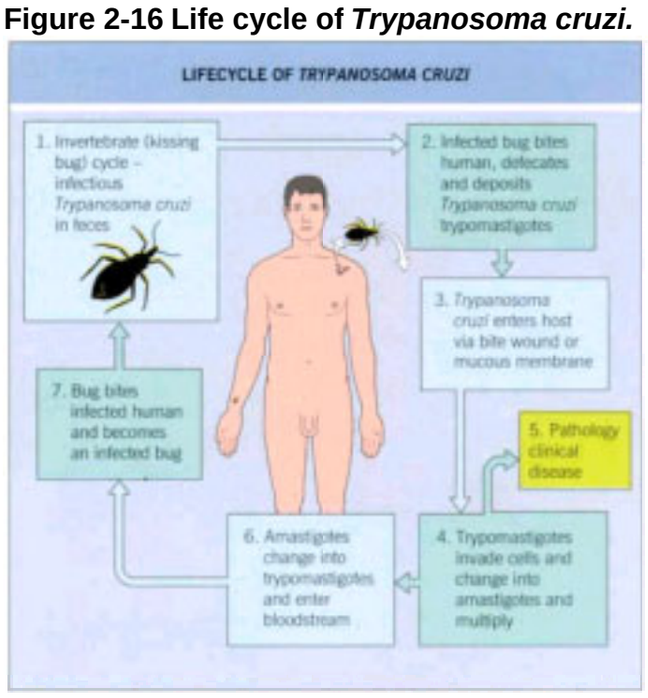


Figure 2-17 Microbial response to acid production by the host.

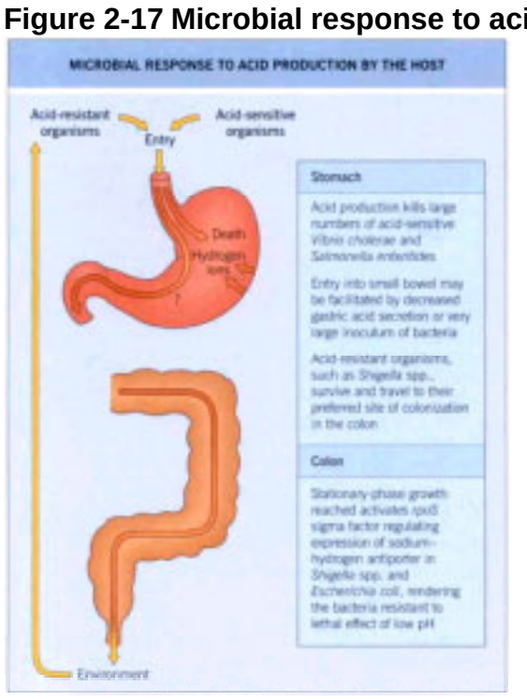


Figure 2-18 Normal flora of the gastrointestinal tract.

Figure 2-19 Lipopolysaccharide activation of macrophages via lipopolysaccharide binding protein (LBP), CD14, Toll-like receptor (TLR) 4, and MD2. IL, interleukin; TNF, tumor necrosis factor.

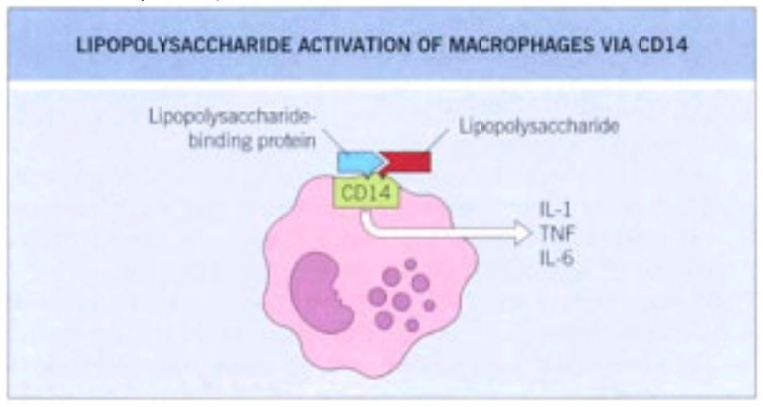


Figure 2-20 Effect of lipopolysaccharide-binding protein (LBP) and bactericidal permeability-increasing protein (BPI) on survival of *Escherichia coli.* (Top) Survival of *E. coli* in the presence of increasing concentrations of LBP or BPI. (Bottom) Synergistic effect on *E. coli* survival of LBP and a recombinant 23kDa *N*-terminal fragment of BPI (rBPI23). *Data from Horwitz et al.* [68]

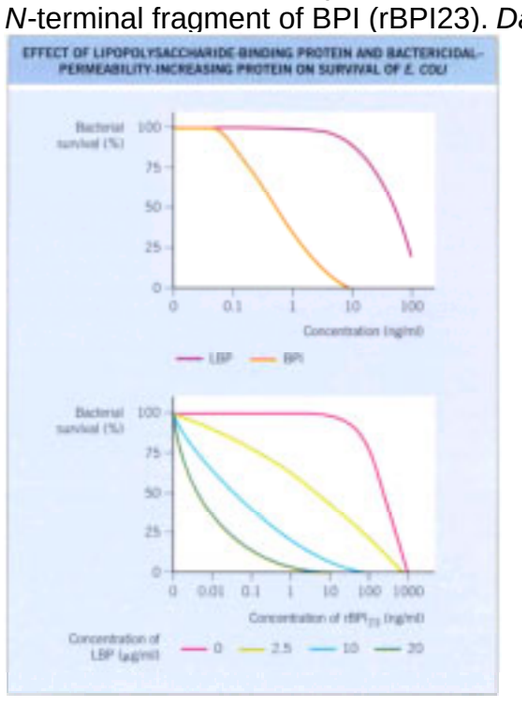


Figure 2-21 Pathogenesis of AIDS. The pathogenesis begins with the binding of HIV to CD4 receptors on the regulatory cells of the immune system.

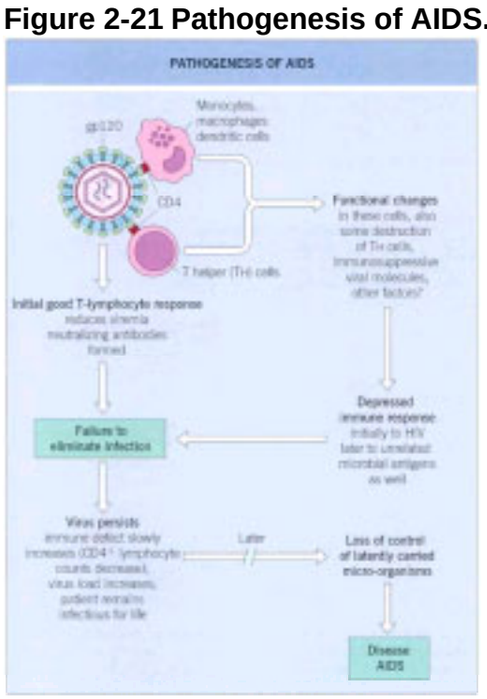


Figure 2-22 The process of phagocytosis.

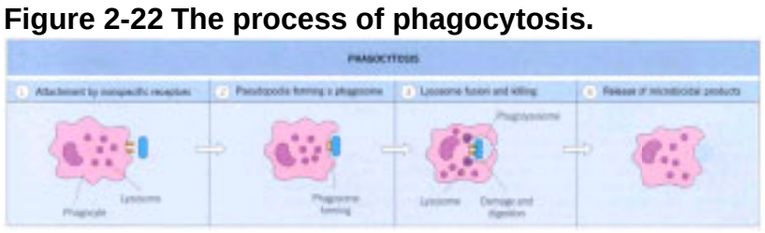


Figure 2-23 Effect of antibody and complement on the rate of clearance of virulent bacteria from the blood. Phagocytosis is greatly potentiated if the microbes are coated with antibody and complement.

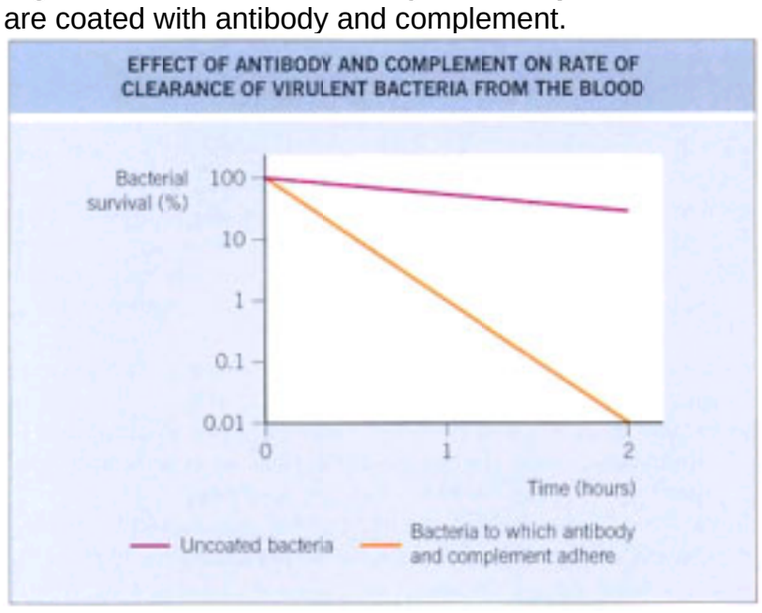


Figure 2-24 The interaction between bacteria and phagocytic cells. This is facilitated by a variety of molecules, the precise nature of which may determine whether uptake occurs and whether killing mechanisms are triggered.

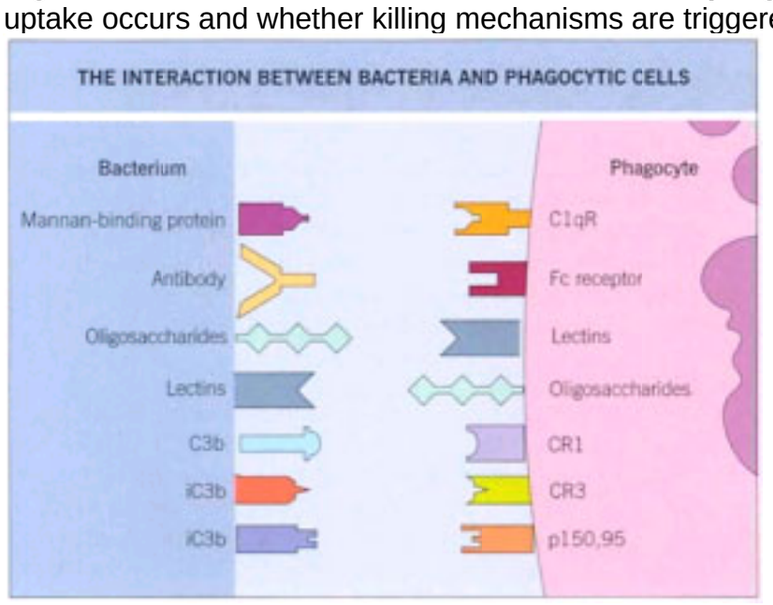


Figure 2-25 Binding of selectins to carbohydrates expressed on various cells. HEV, high endothelial venules.

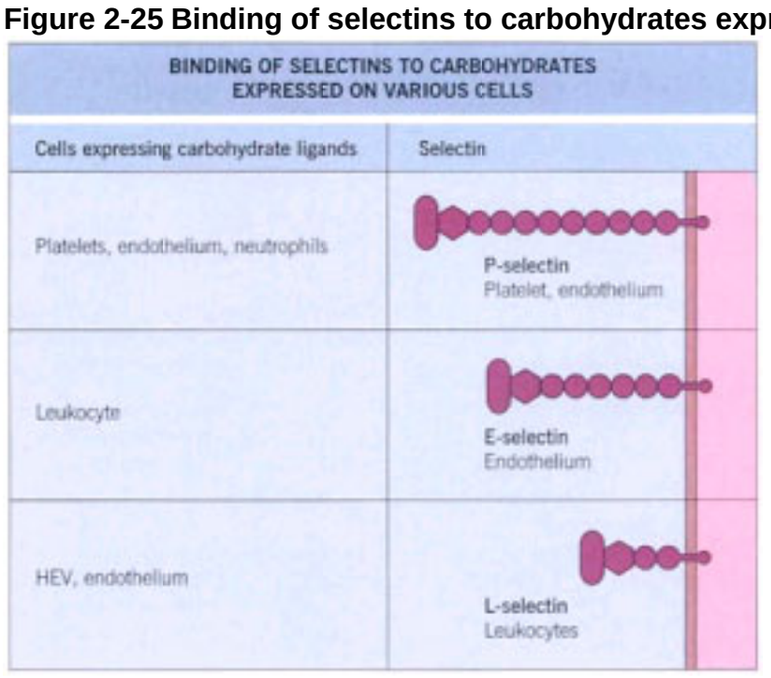


Figure 2-26 Modulation of leukocyte adhesion. There are four ways in which leukocyte binding to endothelium can be enhanced. LFA-1, lymphocyte function associated antigen-1.

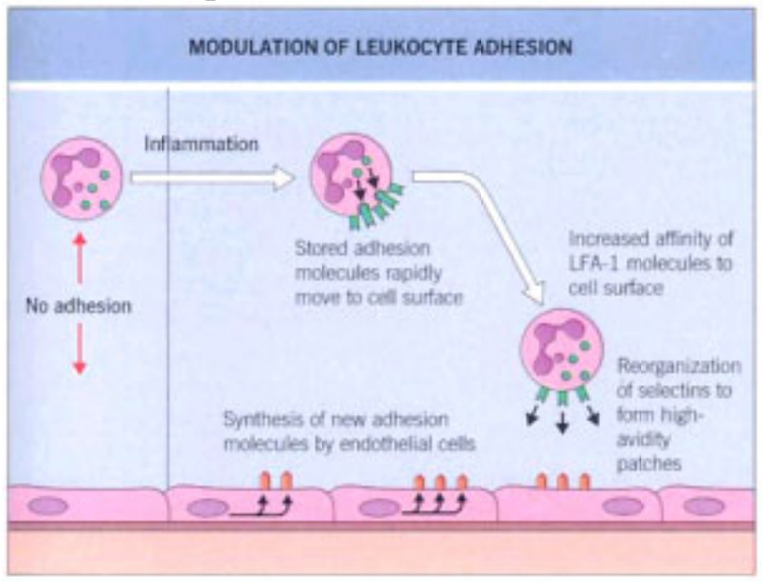


Figure 2-27 Primary and secondary responses to the same antigen during infection.

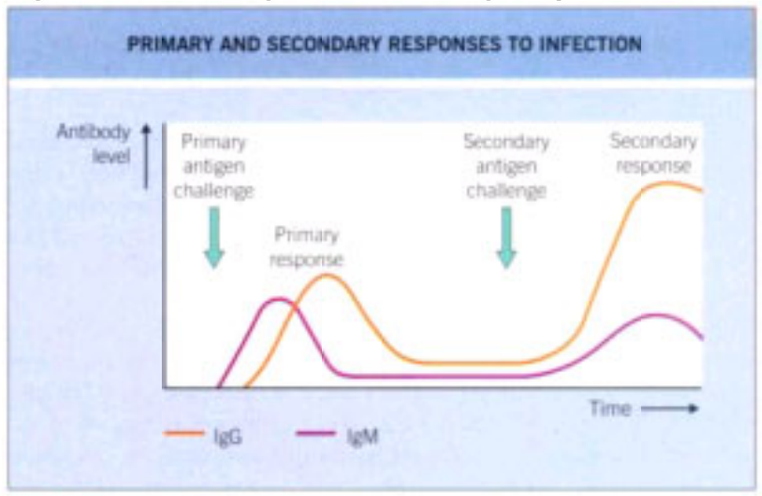


Figure 2-28 Affinity of the antibody responses following primary and secondary antigen challenge.

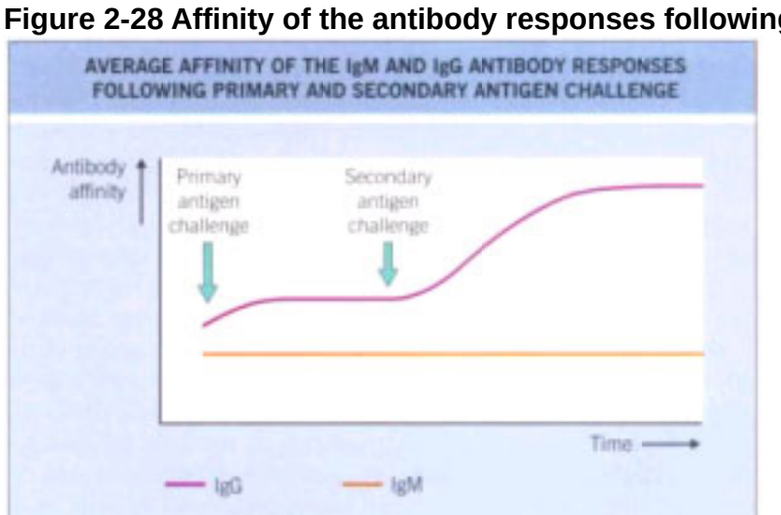


Figure 2-29 Examples of host-pathogen interactions blocked by antibody.

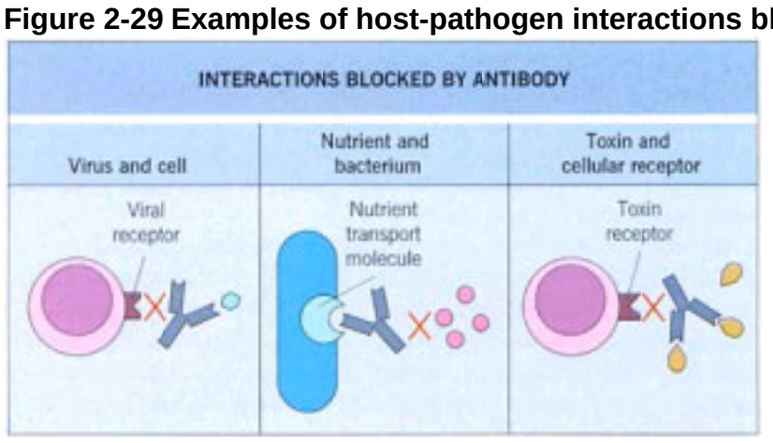


Figure 2-30 Antibody-dependent cell-mediated cytotoxicity. Different effector cells bind to the surface of the target cell via their receptor for antibody.

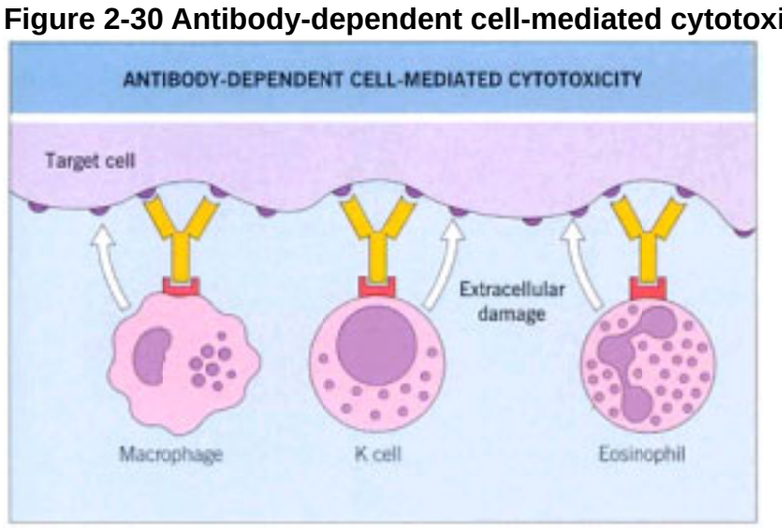


Figure 2-31 Dual role of antibody in the immune reaction to schistosomes. Following contact with the schistosome antigen, mast cells sensitized with anti-schistosome IgE release chemotactic factor, which attracts eosinophils. When the eosinophils arrive they are able to bind to the antibody-coated worm via their Fc receptors and damage the parasite.

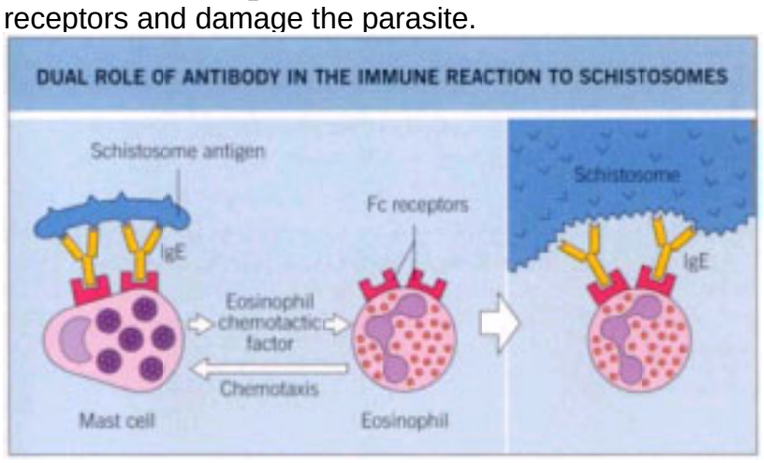


Figure 2-32 Cytotoxic T-lymphocyte response. Cytotoxic T lymphocytes expressing CD8 recognize antigen and major histocompatibility complex (MHC), enabling them to bind target cells.

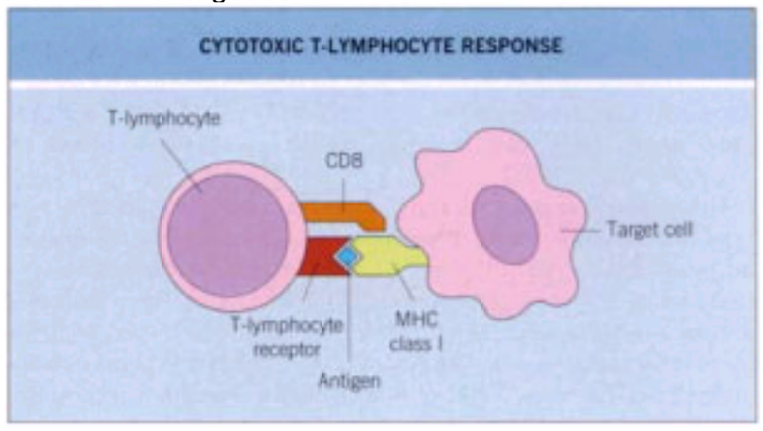


Figure 2-33 The release of interferon (IFN)-? from natural killer (NK) cells. IL, interleukin; TNF, tumor necrosis factor.

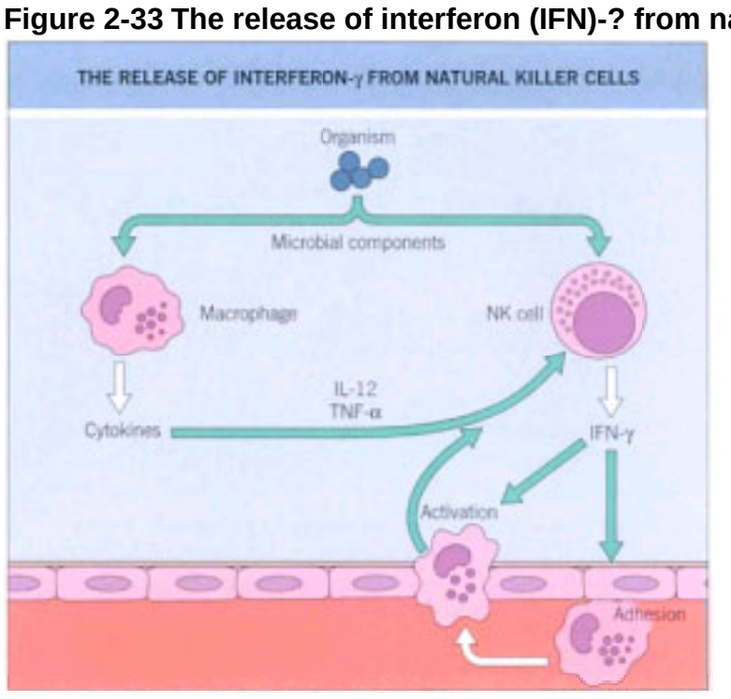


Figure 2-34 T-helper (Th)1 and Th2 lymphocyte imbalance in leishmaniasis. Leishmaniasis is characterized by deficient interferon (IFN)-? production and inhibition of its action. IL, interleukin.

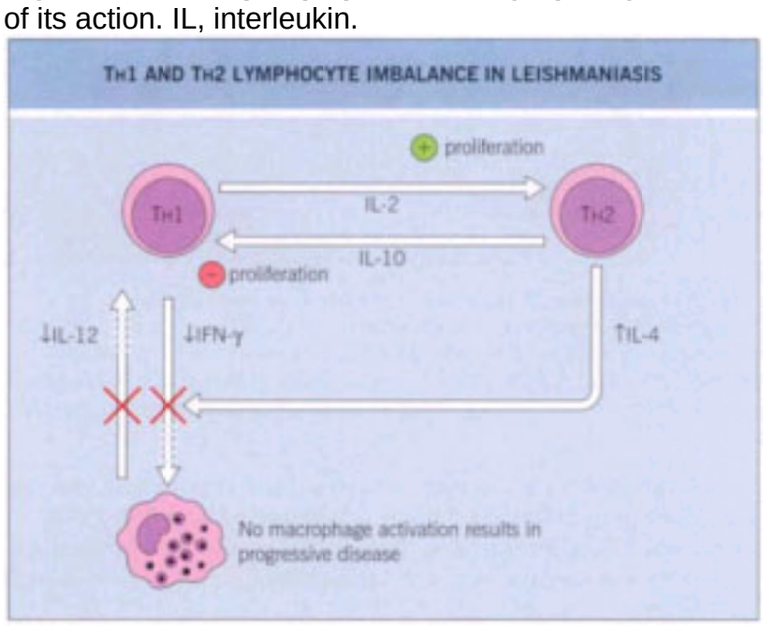


Figure 3-1 Water supply and sanitation. Coverage by region, 1990 and 2000. *Adapted from WHO*[9] .

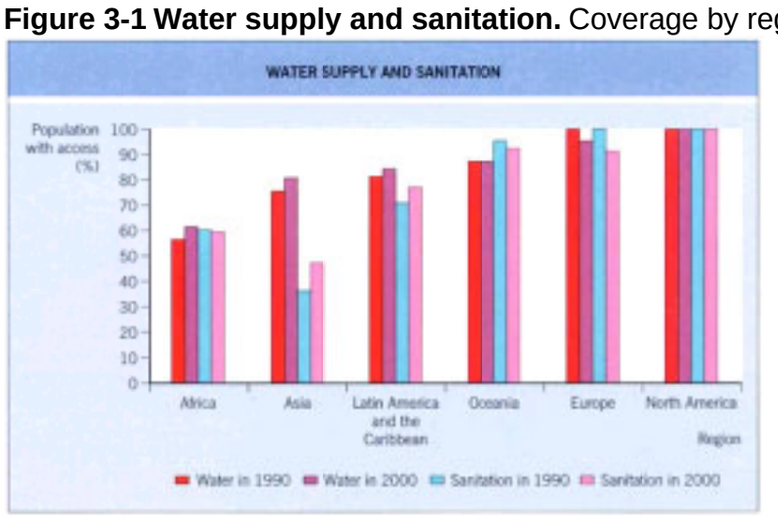


Figure 3-2 Global urban and rural water supply and sanitation, 1990 and 2000. *Adapted from WHO.*[9]

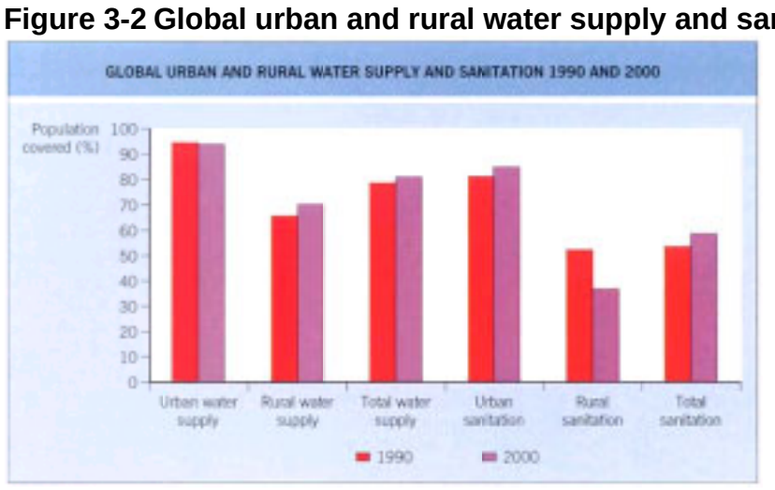


Figure 3-3 The host-agent-environment triad. *Adapted from WHO.*[9]

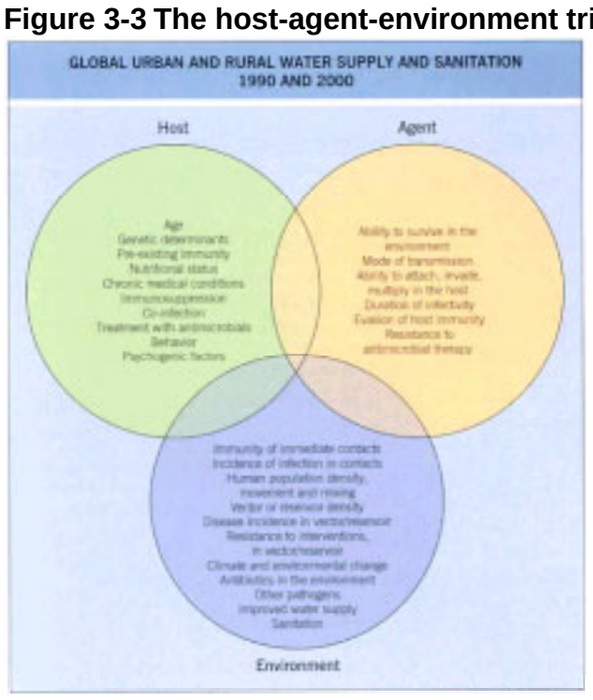


Figure 3-4 The basic reproductive number, R_0 — threshold for invasion. The left side shows $R_0 = 3$ and the right side $R_0 = 1$. *Modified from Begg and Gay.*[32]

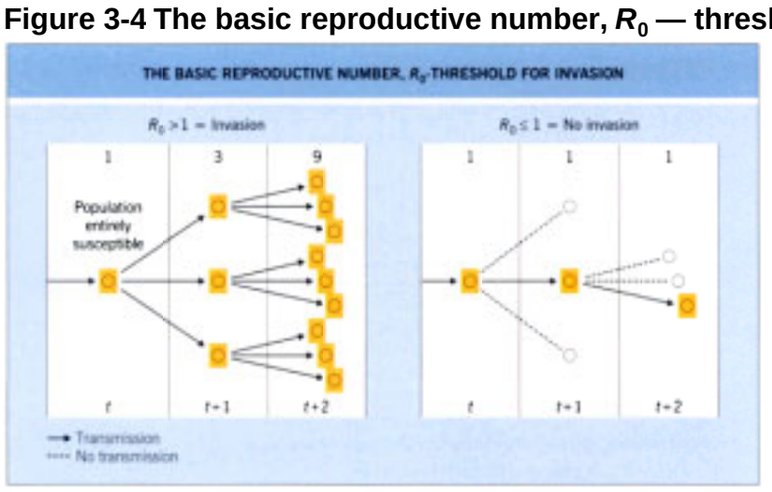


Figure 3-5 Global polio incidence. Global and regional summaries of reported cases of poliomyelitis worldwide from 1990–2001. *Modified from WHO.*[43]

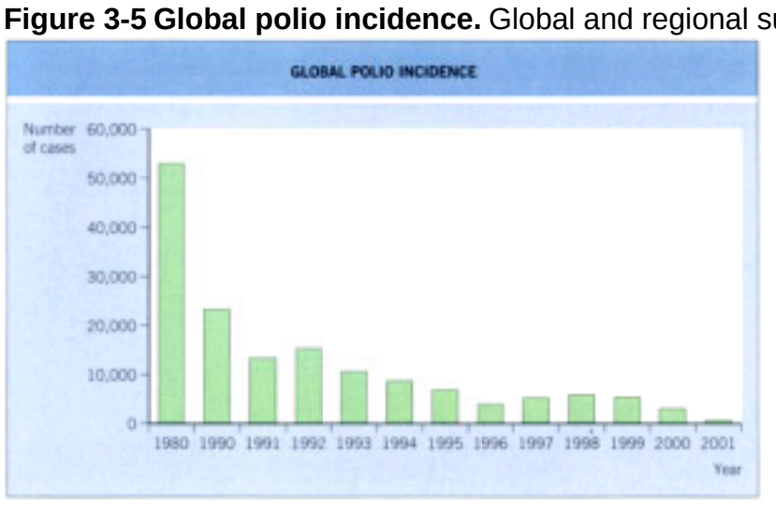


Figure 3-6 Recommended childhood immunization schedule for the USA, 2003. This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of 1 December 2002, for children through age 18 years. DTaP, diphtheria, tetanus, acellular pertussis; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; MMR, measles, mumps, rubella; OPV, oral polio vaccine; PCV, pneumococcal conjugate vaccine; PPV, polysaccharide pneumococcal vaccine; Td, tetanus, low dose diphtheria; Var, varicella. *Redrawn with permission from CDC.*[39]

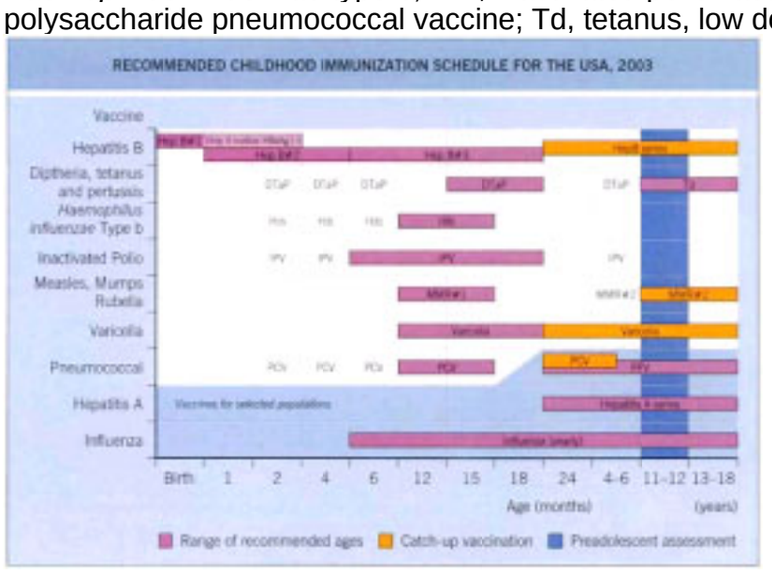


Figure 3-7 Vaccine vial monitor showing stages of exposure. The information delivered by the vaccine vial monitor is simple. If the inner square is a lighter color than the outer reference ring than the vaccine can be used. If the inner square is the same color as the outer ring or darker than it, then the vaccine should not be used. *From WHO.*[53]

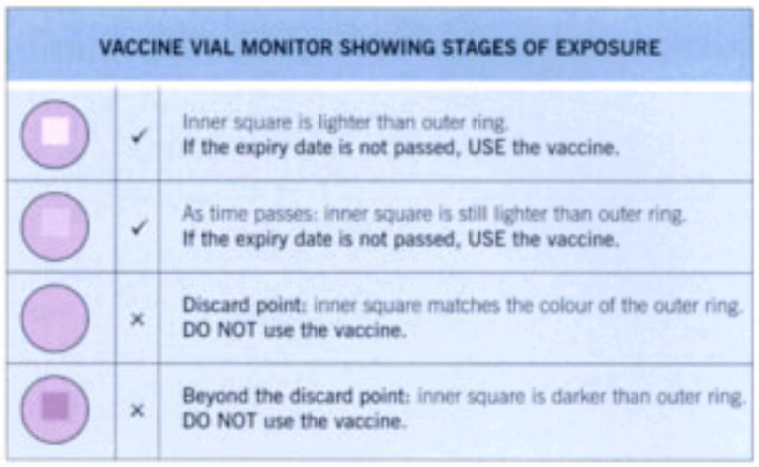


Figure 4-1 Interactions among humans, disease vectors and the environment that contribute to disease emergence. *Source: Institute of Medicine. Emerging infections: microbial threats to health in the United States. Washington DC: National Academy Press; 1992.*[3] *(In March 2003, the Institute of Medicine published a successor to this report, entitled Microbial threats to health: emergence, detection, and response.)*

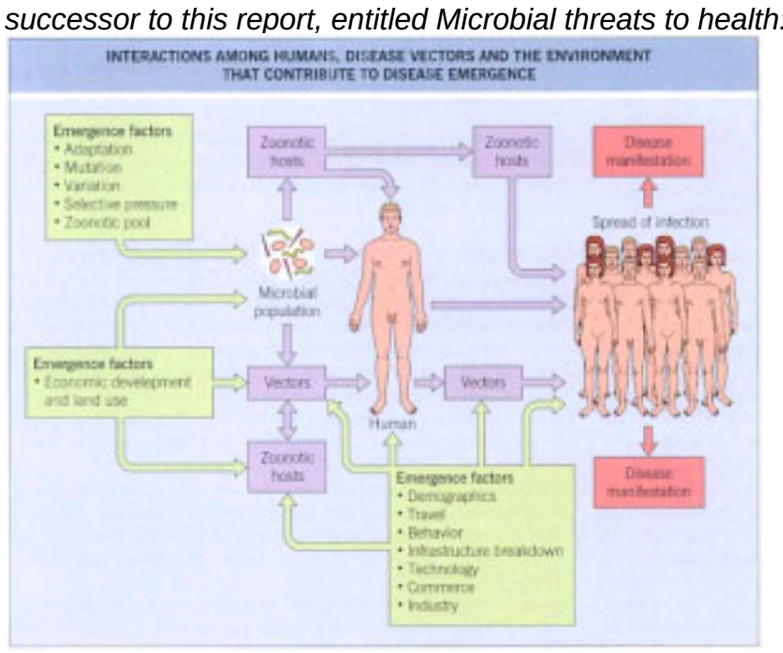


Figure 4-2 Spread of West Nile virus in the United States. In the 4 years since West Nile virus was first reported in the USA (in New York City), it has been detected in 44 states. *Source: National Center for Infectious Diseases, Centers for Disease Control and Prevention.*

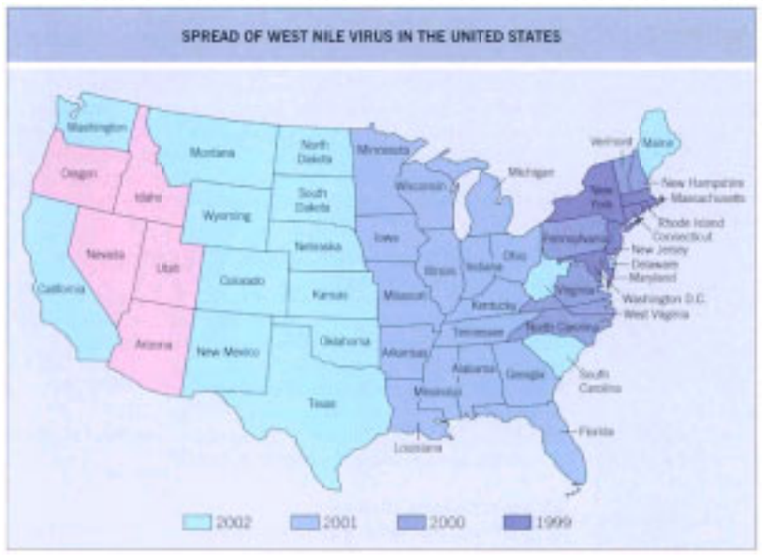


Figure 4-3 International tourist arrivals, 1950–99. International travel has increased by 160 million tourist arrivals since 1990, from 500 million to more than 660 million in 1999. In 1950, 15 countries received nearly 100% of the 25 million international tourist arrivals. In 1999, at least 70 countries and territories each received more than one million international tourist arrivals. The rate of growth in international arrivals in Africa alone was 7.8%, nearly twice the world average. ('Tourism' in these statistics includes business travel.) *Source: World Tourism Organization. WTO tourism highlights 2000, August 2000.*

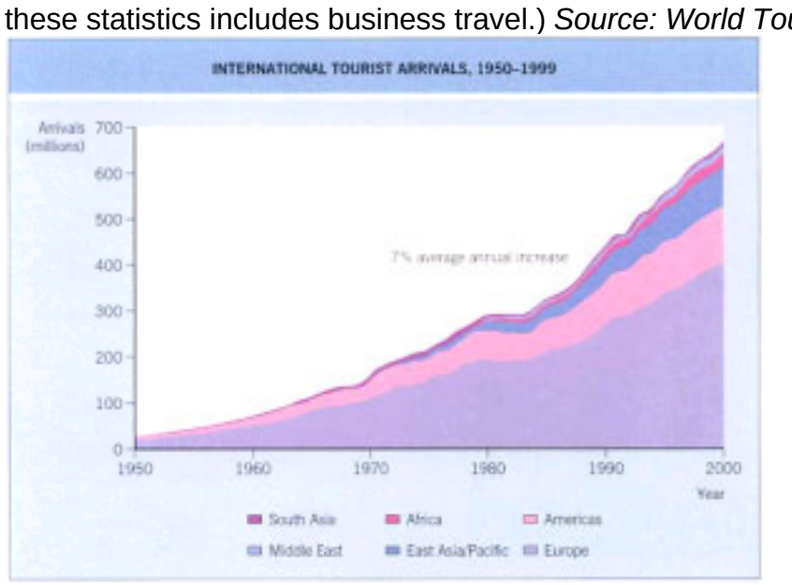


Figure 4-4 Leading infectious killers. *Source: World Health Organization. Overcoming antimicrobial resistance. Geneva: WHO.*

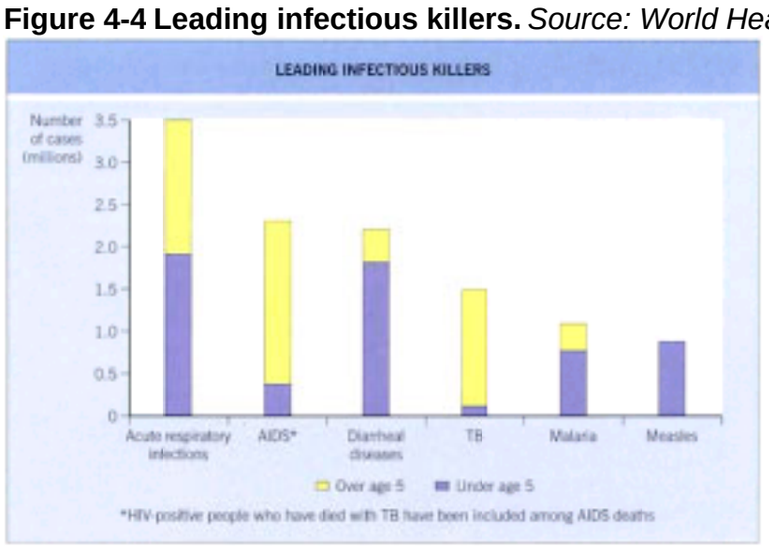


Figure 4-5 Estimated AIDS incidence (*adjusted for reporting delays), deaths and prevalence, by quarter-year of diagnosis/death — United States, 1981–2000. AIDS incidence increased rapidly through the 1980s, peaked in the early 1990s and then declined. The peak of new diagnoses was associated with the expansion of the AIDS surveillance case definition in 1993 (CDC. HIV/AIDS surveillance report, 2000;1 2[1]). As of 1996, sharp declines were reported in AIDS incidence and deaths. From 1998 through June 2000, AIDS incidence and deaths leveled off and AIDS prevalence continued to increase. Throughout the epidemic, approximately 85% of persons diagnosed with AIDS were aged 20–49 years. *Source: Centers for Disease Control and Prevention. HIV and AIDS — United States, 1981–2000. MMWR 2001;50(21);430–4.*

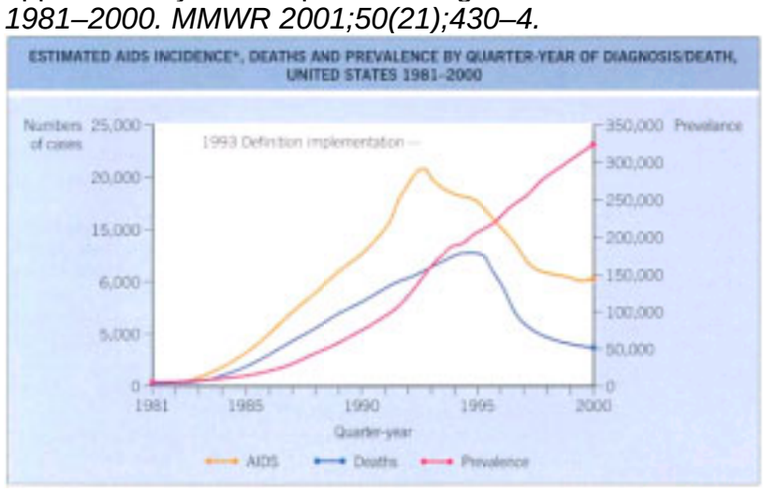


Figure 4-6 Adults and children estimated to be living with HIV/AIDS at the end of 2001. *Source: UNAIDS and World Health Organization, 2001 (http://www.unaids.org/barcelona/presskit/epigraphics/epicore_en4_0602.GIF).*

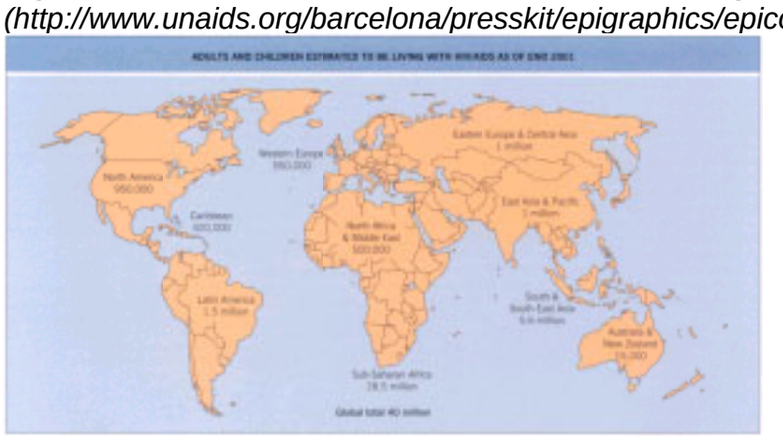


Figure 5-1 Fluorescence micrographs of a formalin fixed intestinal biopsy from a patient with Whipple's disease. (a) Confocal micrograph demonstrating hybridization of a fluorescent *Tropheryma whipplei* rDNA probe (blue) to bacterial rRNA in a small intestine biopsy from a patient with Whipple's disease. Yo-pro dye (green) highlights cell nuclei and an anti-vimentin antibody (red) labels the cytoskeletal protein of human mesenchymal cells. Bacteria localize to the lamina propria and appear abundant in the extracellular spaces. Macrophages and polymorphonuclear leukocytes infiltrate the lamina propria of this enlarged villus. (b) Confocal micrograph of Whipple's disease intestine showing clumps of small bacillary bodies in the lamina propria stained with Yo-pro nucleic acid dye in green (small arrow), and a human epithelial cell nucleus (large arrow). The extremely small size of the Whipple bacillus makes detection difficult using standard methods.

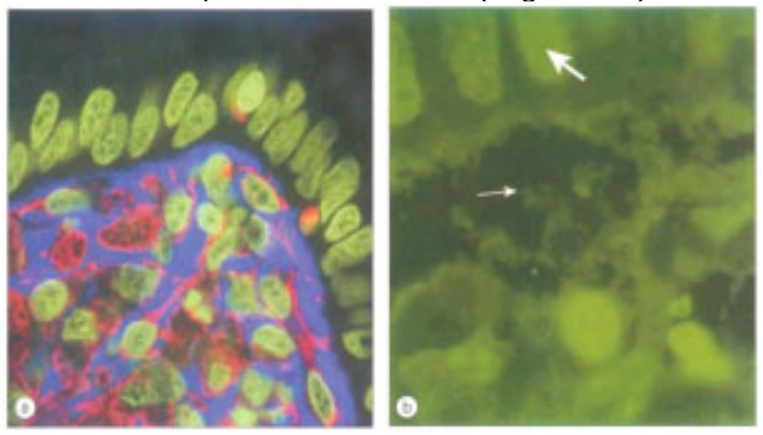


Figure 6-1 Imaging abnormalities associated with anthrax. (a) Chest radiograph demonstrating widened mediastinum due to inhalational anthrax. *Courtesy CDC and Dr PS Brachman.*

Figure 6-2 *Bacillus anthracis.* (a) *Bacillus anthracis* appearing as Gram-positive bacilli. (b) The typical 'jointed bamboo-rod' appearance of the organism from blood cultures. *Courtesy CDC and Dr William A Clark.*

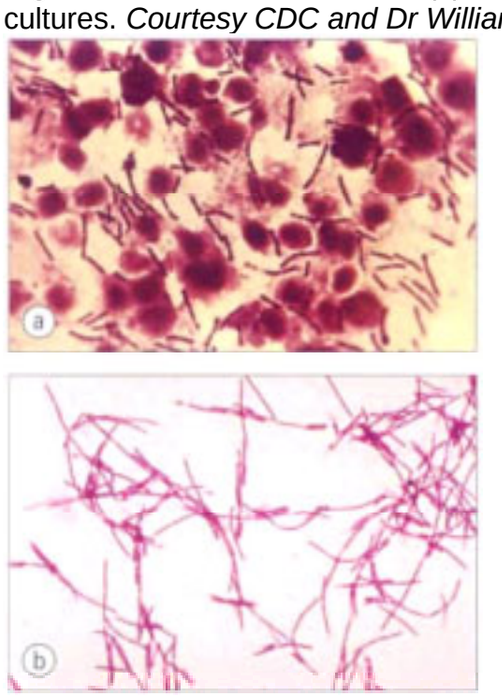


Figure 6-3 Third day of rash in smallpox. Additional lesions continue to appear and some of the papules are becoming obviously vesicular. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

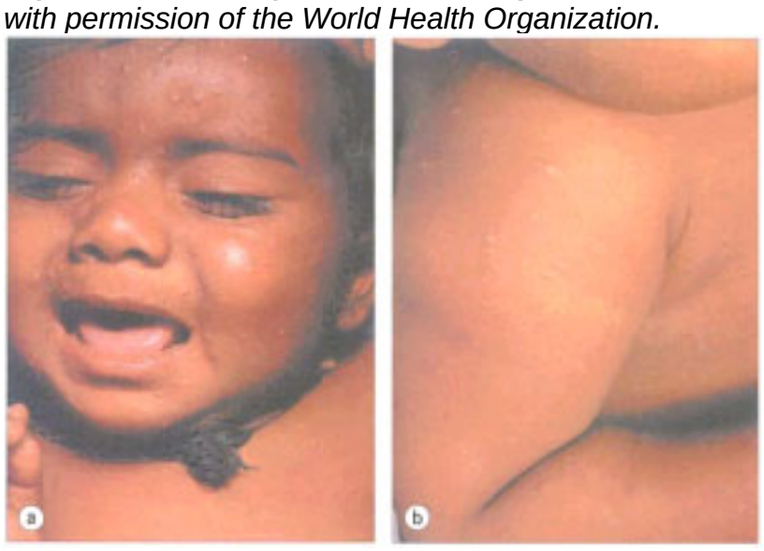


Figure 6-4 Fifth day of rash in smallpox. Almost all the papules have now become vesicular or pustular, the truly 'vesicular' stage usually being very brief. Some of the lesions on the upper arm show early umbilication. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

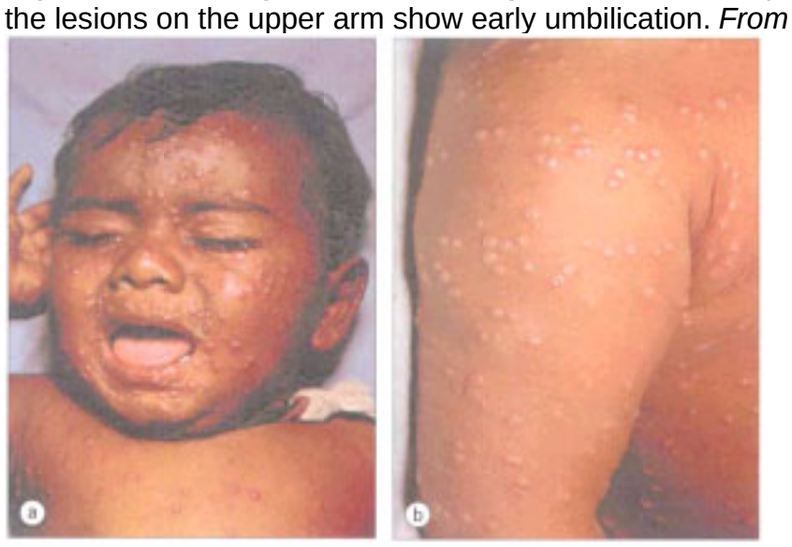


Figure 6-5 Eighth day of rash in smallpox. This case is now clearly classified as discrete ordinary-type smallpox. In the confluent subtype of ordinary-type smallpox the lesions would have been confluent on the face and forearms: in the semiconfluent subtype they would have been confluent on the face but not on the forearms. *From Fenner et al.,*[20] *with permission of the World Health Organization.*

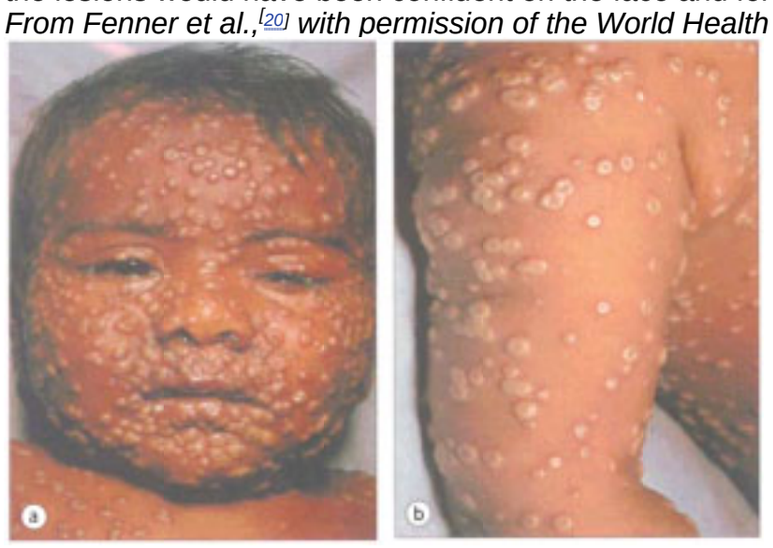


Figure 6-6 Twentieth day of rash in smallpox. The scabs have separated except on the palms of the hands and the soles of the feet, leaving depigmented areas. *From Fenner et al., [20] with permission of the World Health Organization.*

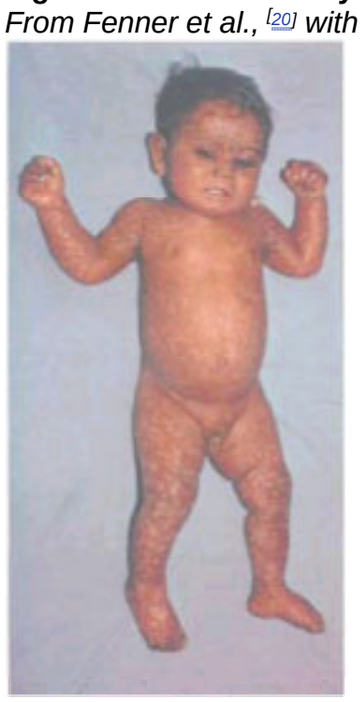


Figure 7-1 The microbial revolution of the 1990s: bacterial genome sequencing. (1) Construction of a random gene library. (2) Random sequencing of thousands of clones. (3) Closure phase (which can be very labour-intensive). (4) Collation and annotation of the final sequence *(reproduced from Moxon[30] with permission from The Lancet)*

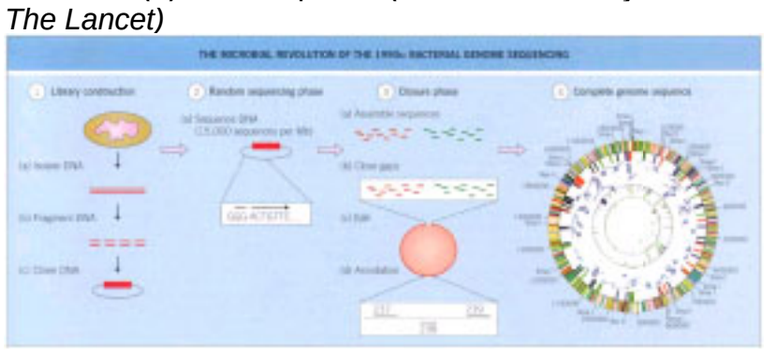


Figure 7-2 How a whole-genome sequence of a pathogenic microbe can be used to develop a candidate vaccine. (1) Representation of lipopolysaccharide (LPS), a major surface antigen of *H. influenzae* and a potential vaccine candidate. The lipid A (endotoxin) is inserted into the cell envelope of the bacterium to which are attached inner and outer core saccharides (uppermost in figure). These oligosaccharides are important in the interactions of the bacterium with host cells. (2) Whole-genome sequence of *H. influenzae* Rd that includes all of the genes involved in the biosynthesis of *H. influenzae* LPS of this strain (reproduced with permission from Fleischmann et al[1]). (3) DNA sequences (or their deduced amino-acid sequences), publicly available in databases (e.g. GenBank, EMBL), are used as probesto search and identify homologues (candidate LPS genes) in the *H. influenzae* complete genome sequence. (4) These candidate *H. influenzae* LPS genes can be further investigated to confirm or reject their role in LPS biosynthesis through appropriate experimental methods. (5) Oligonucleotide primers can be constructed to obtain multiple copies of each of the candidate LPS genes using polymerase chain reaction (PCR). (6) The PCR amplified candidate LPS genes are cloned into a suitable plasmid vector and mutations are constructed. In this example, the candidate LPS gene has been disrupted by an insertion of a cassette of DNA containing a gene for kanamycin resistance; this also acts as a selectable marker. (7) The cloned mutant gene is introduced into *H. influenzae* by transformation (allelic replacement); the phenotype of the parent strain and its mutant can then be compared to determine whether there are differences in LPS phenotype. (8) Immunoblotting is used to compare the reactivity of a monoclonal antibody (specific for LPS) in the parent and mutant strains; in this example, the LPS of the mutant has lost its capacity to bind to the monoclonal antibody, whereas colonies of the parent strain bind this antibody. This provides strong evidence in support of a function for this gene in LPS biosynthesis. (9) Further tests of biological function on parent and mutant can be done to further characterise the role of LPS, for example, its role in virulence. (10) Information on the many genes involved in LPS biosynthesis provides detailed information on the structure and its potential for use in vaccine development. *(Reproduced from Moxon[30] with permission from The Lancet.)*

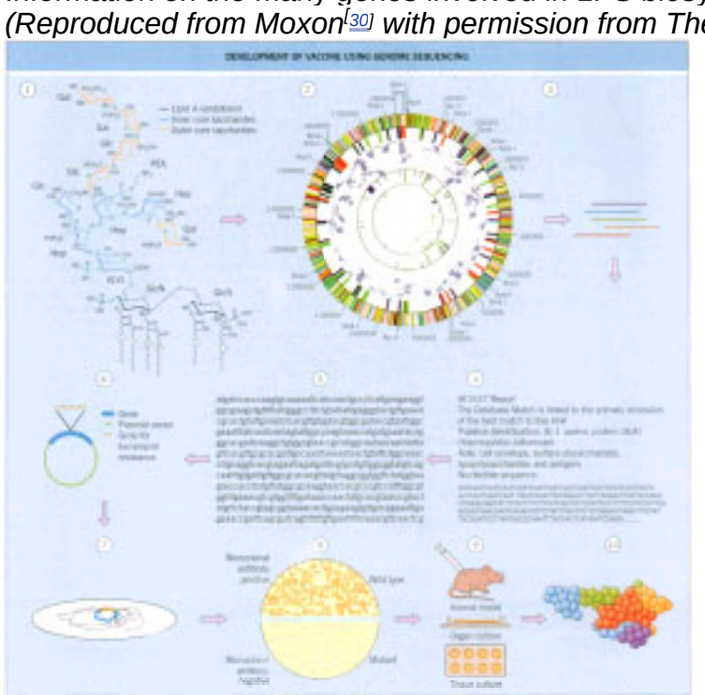


Figure 7-3 Assignment of gene function.

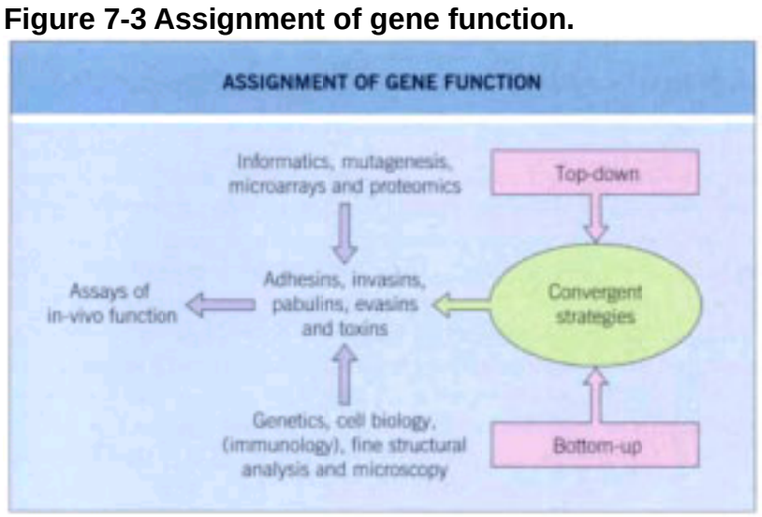


Figure 7-4 Comparing gene expression using a DNA microarray. The color of each spot relates to the level of expression of every gene on the microarray. *Further details can be obtained from http://www.ifr.ac.uk/safety/microarrays*

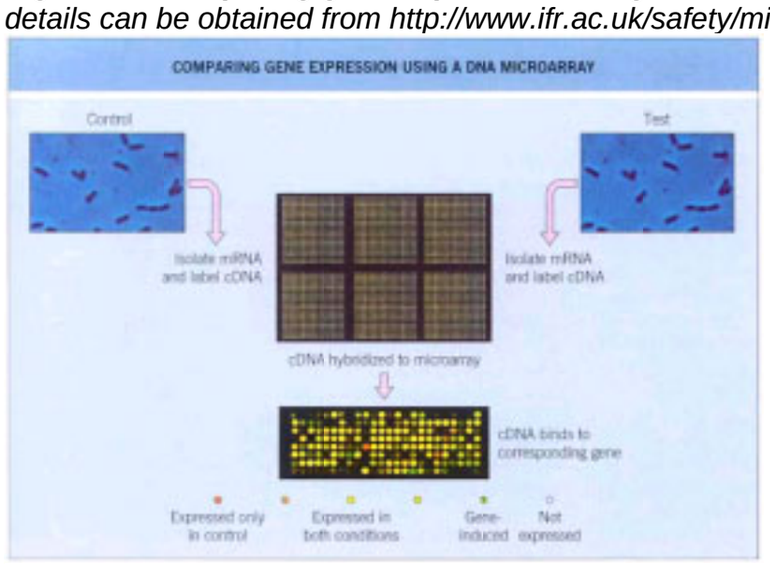


Figure 8-1 Incidence of measles in the USA 1960–90. The effect of measles and measles/mumps/rubella immunization on the incidence of measles and of SSPE in the USA, showing that both are prevented by the live attenuated vaccines.

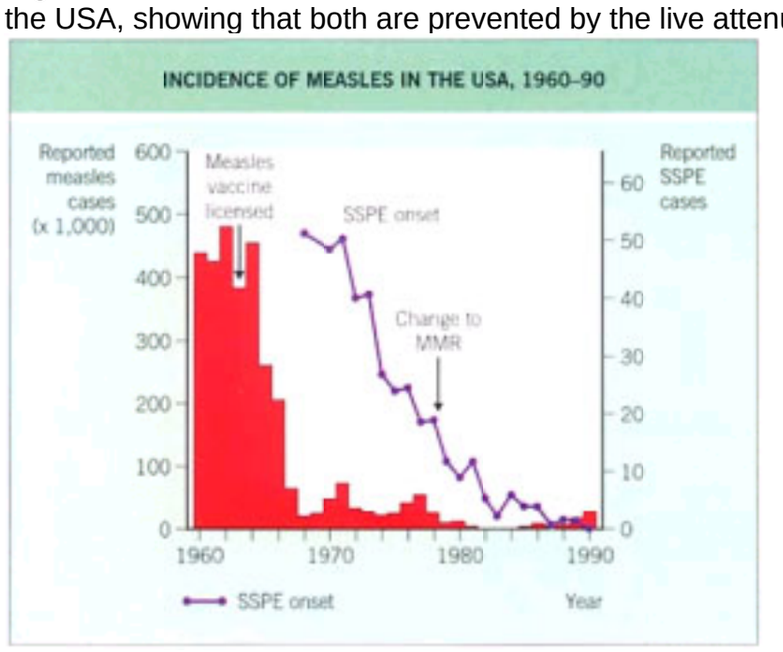


Figure 8-2 The acute rash of measles. Marked conjunctivitis accompanied the maculopapular skin lesions in this unimmunized adult.

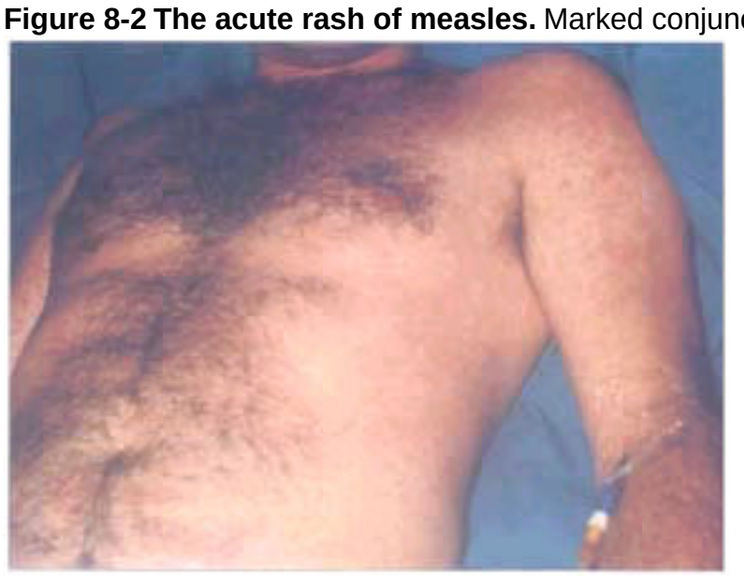


Figure 8-3 Secondary invasion by *Staphylococcus aureus* in measles. Ill-defined basal opacities were seen in a patient who had moderate respiratory failure; *S. aureus* was isolated from sputum (same patient as in Fig. 8.2).

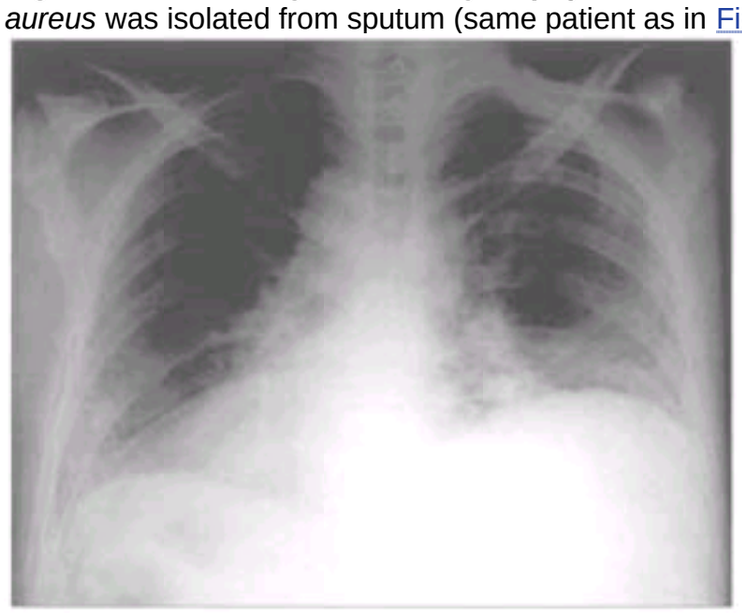


Figure 8-4 Secondary infections complicating measles. Perioral infection and paranasal herpes simplex lesion in a 2-year-old girl.

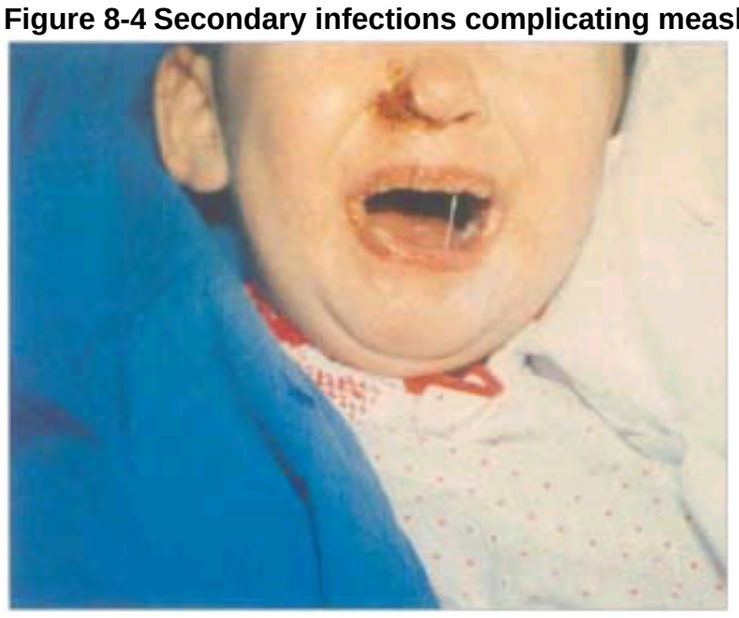


Figure 8-5 Incidence of rubella in England and Wales. The effects of different immunization programs are shown. *Data from Bannister et al. Infectious disease, 2nd ed. Oxford: Blackwell Science; 2000: 234.*

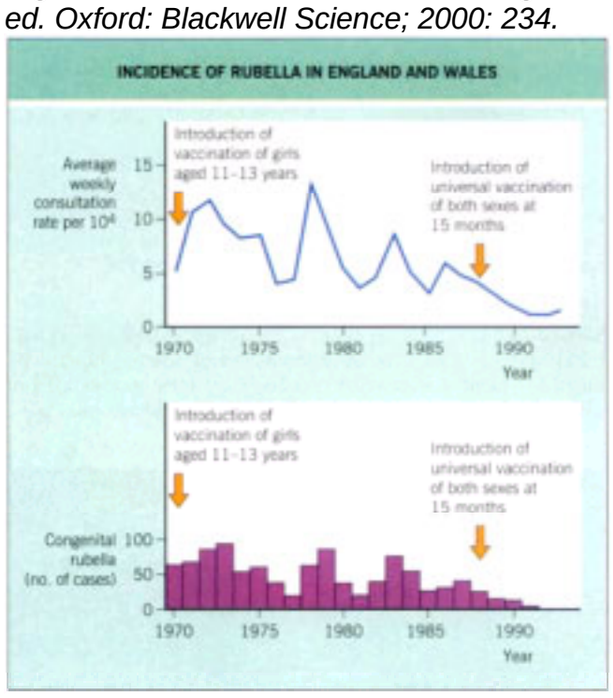


Figure 8-6 Rubella. This patient had a typical early maculopapular rash, irritating conjunctivitis and painful occipital lymphadenopathy.

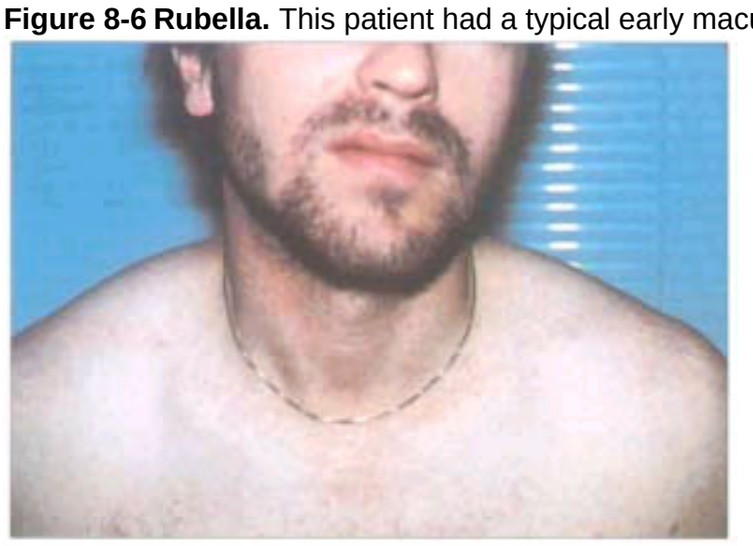


Figure 8-7 The lesions of varicella. Papules, vesicles and pustules, some of which are beginning to crust from the center, are seen.

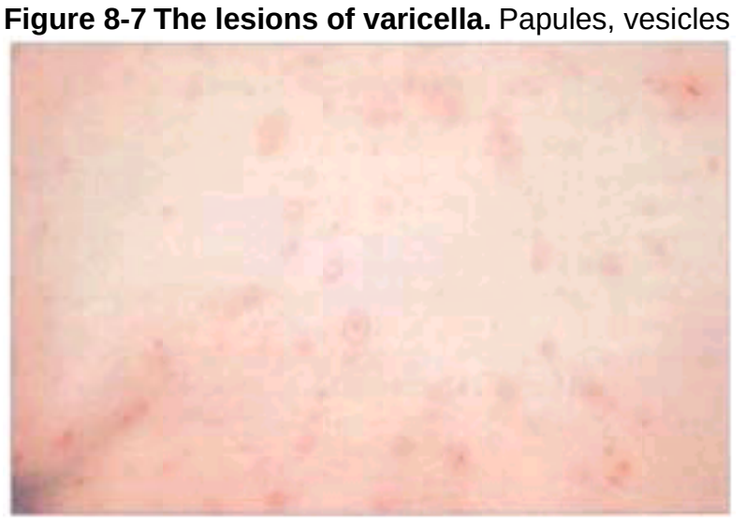


Figure 8-8 Course of varicella in a 54-year-old woman. Low arterial oxygen saturation precedes respiratory and hemodynamic failure.

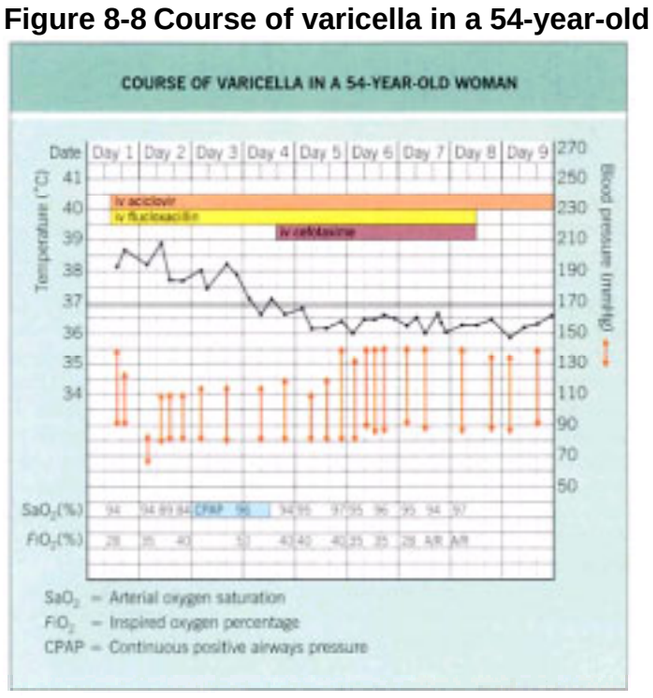


Figure 8-9 The rash of herpes zoster. It can be seen that the lesions occur in groups, with coalescence of lesions in the larger groups.

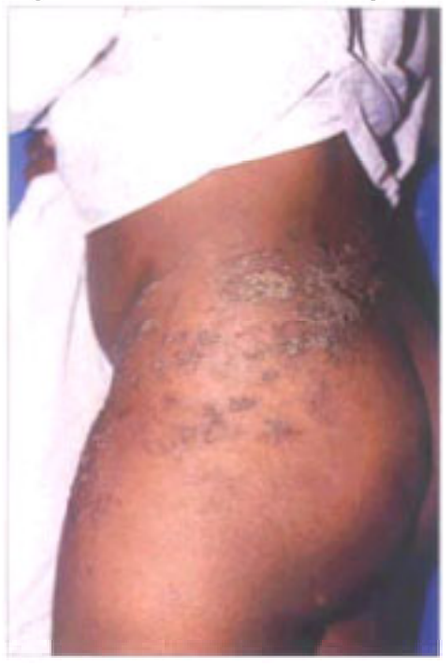


Figure 8-10 Secondary staphylococcal infection of varicella lesions. Staphylococcal pyrogenic exotoxin has caused a 'scalded skin' type of lesion surrounding the infected spots. *Courtesy of Dr MG Brook.*

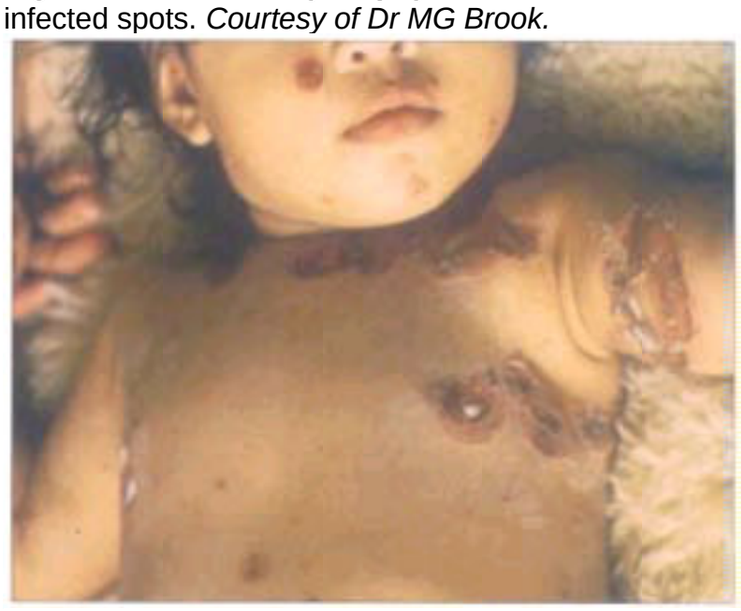


Figure 8-11 Varicella-zoster virus infection. Infection in a patient who had a history of varicella in childhood, had recently had a thymectomy and was taking high-dose prednisone (prednisolone): initially localized lesions on the neck were quickly followed by an extensive, varicella-type rash.

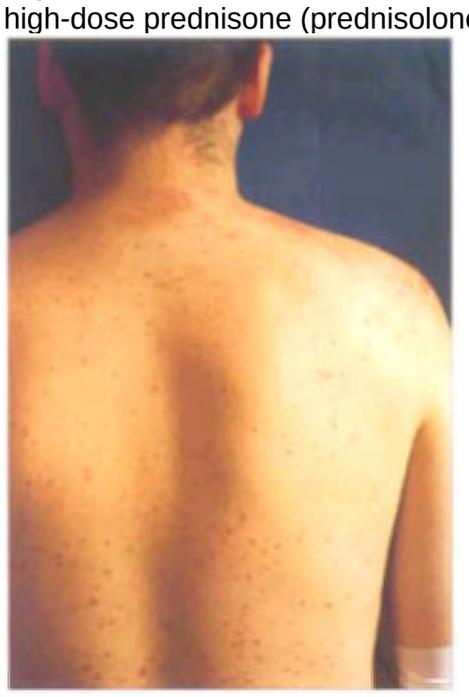


Figure 8-12 Hand, foot and mouth disease. Typical vesicles are seen on the foot of a 3-year-old child.

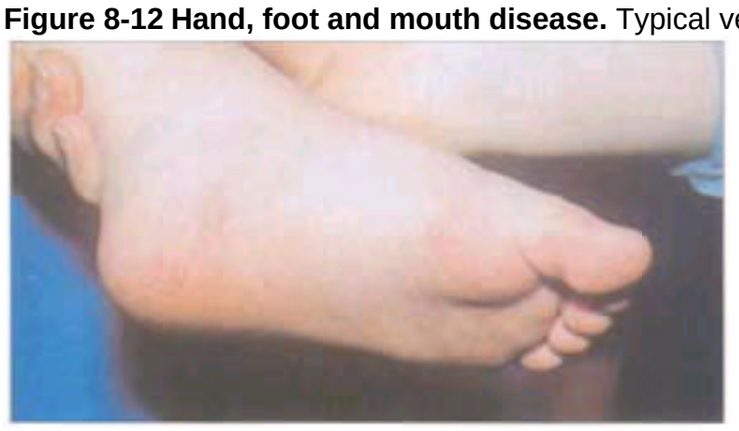


Figure 9-1 Cutaneous infection at the previous insertion site of an intravenous catheter. Organisms from the skin were likely introduced into the dermis and subcutaneous tissue at the time of catheter insertion. Many of these infections remain superficial, but in this patient suppurative thrombophlebitis with bacteremia ensued.

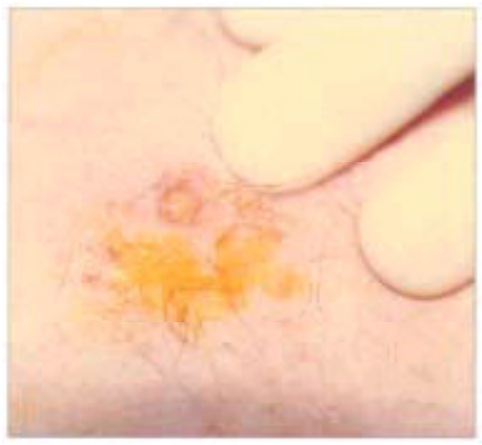


Figure 9-2 Diffuse skin involvement. Petechial lesions in a patient with *Staphylococcus aureus* bacteremia, endocarditis and acute aortic insufficiency.

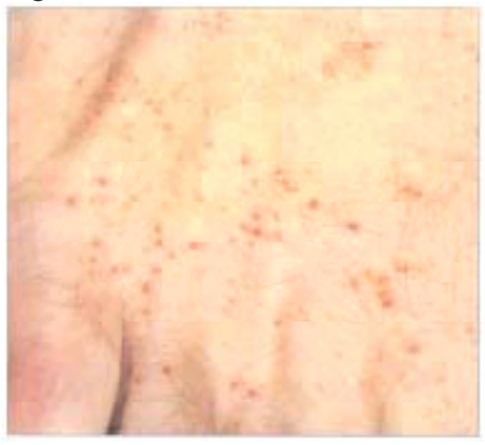


Figure 9-3 Carbuncle of the buttock caused by *Staphylococcus aureus.* This large carbuncle developed over the course of 7–10 days and required surgical drainage plus treatment with antibiotics. The patient had previously experienced numerous episodes of *Staph. aureus* cutaneous abscesses. He carried the staphylococci in his anterior nares.

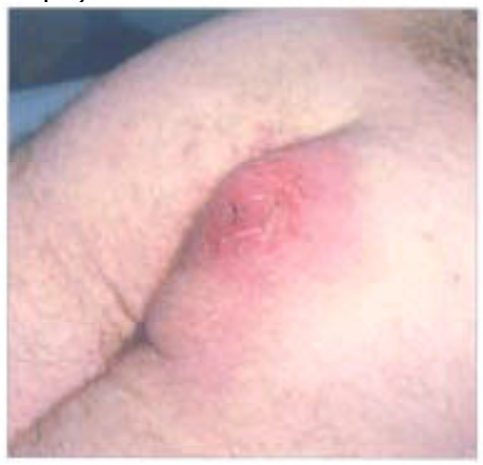


Figure 9-4 Staphylococcal nasal carriage. This patient had a small staphylococcal abscess beneath the mucosa of the nose, illustrating how *Staphylococcus aureus*, which colonizes the nares, can infect skin and submucosa. Intact mucosa is highly resistant to infection; such infections usually occur as a result of defects in the mucosal membranes or via hair follicles inside the nose.

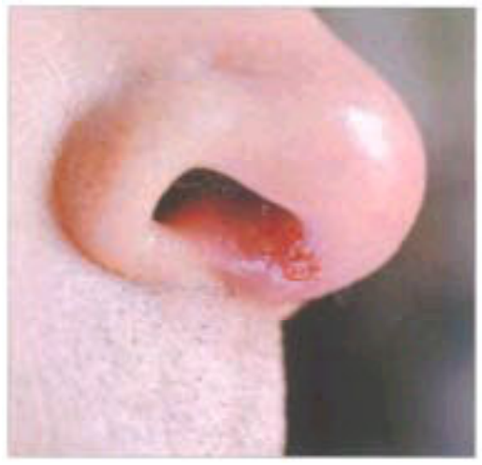


Figure 9-5 Swimmer's itch. Diffuse folliculitis can be caused by *Pseudomonas aeruginosa* (hot tub folliculitis), schistosomes (swimmer's itch) or *Staphylococcus aureus* (folliculitis). This young man had been fishing in an alkaline lake in the western part of the USA. He had been fishing from a 'float tube' and had exposed only his hands and arms to the water. The rash was associated with severe itching. Although his white blood count was not elevated 35% of the white cells were eosinophils.

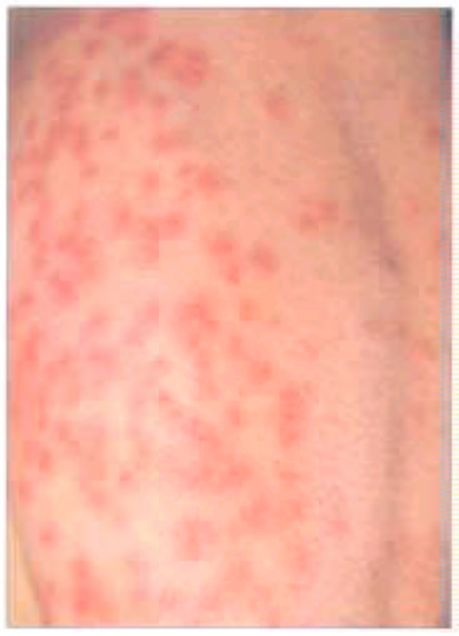


Figure 9-6 Staphylococcal scalded skin syndrome. Flaccid bullae occur as single or multiple lesions. Examination of a frozen tissue section reveals that the cleavage plane is at the stratum corneum. This disease must be distinguished from toxic epidermal necrolysis (see Fig. 9.7).

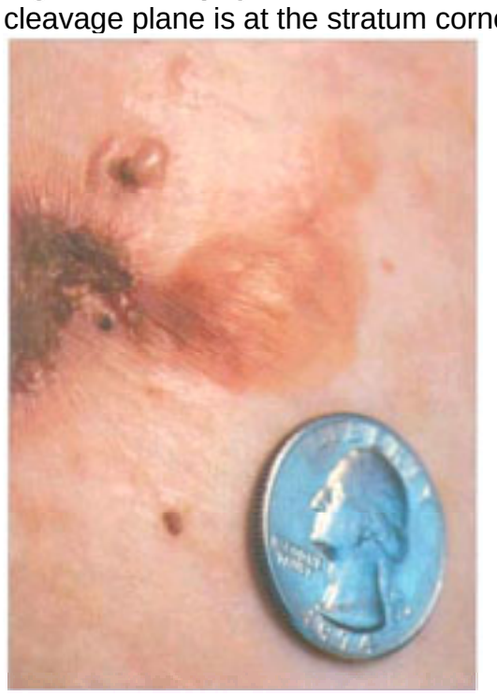


Figure 9-7 Toxic epidermal necrolysis. This picture shows a skin slough (Nikolsky's sign), which resulted when lateral pressure was applied by the thumb in a plane parallel to the skin surface. This disorder is more common in adults, has a high mortality rate and is usually caused by medications.

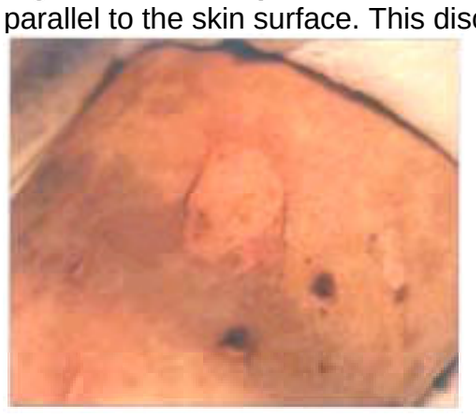


Figure 9-8 Impetigo. (a) Impetigo in a homeless man. Both *Staphylococcus aureus* and group A streptococci were cultured from these lesions. (b) Impetigo, with initial vesicles changing to crusts.

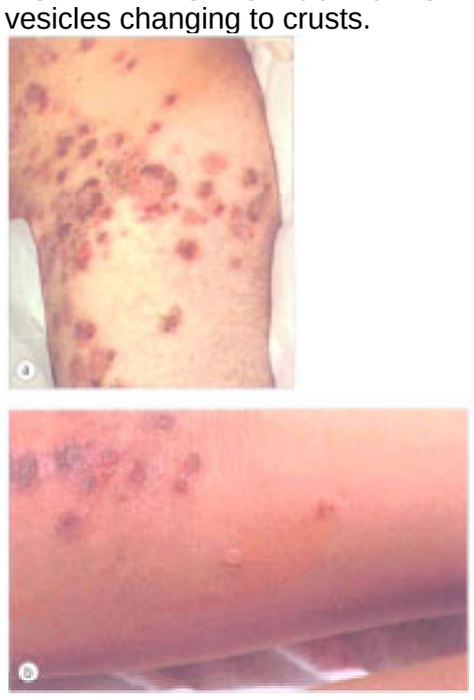


Figure 9-9 Erysipelas. This form of cellulitis is caused by *Streptococcus pyogenes* and is most common in the elderly. Unique characteristics include a fiery red or salmon color, well-demarcated edges, desquamation after 5–7 days and location on the face or lower extemities. This picture was taken 48 hours after treatment with penicillin when the brilliant red salmon color had evolved to a reddish blue color. On the second day of treatment patients usually have less pain and fever subsides, but swelling may be more extensive.

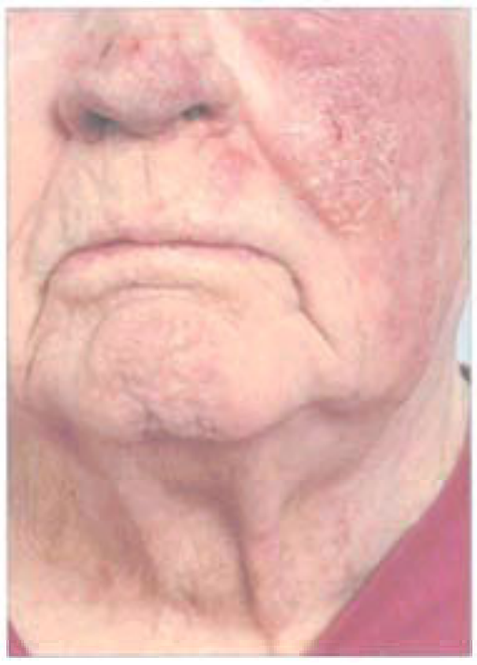


Figure 9-10 Cellulitis. In contrast to erysipelas, cellulitis is a pink color rather than brilliant red and has indistinct margins. *Staphylococcus aureus* and group A, C and G streptococci are the most common etiologies. Many other bacteria may cause cellulitis (see Table 9.1).

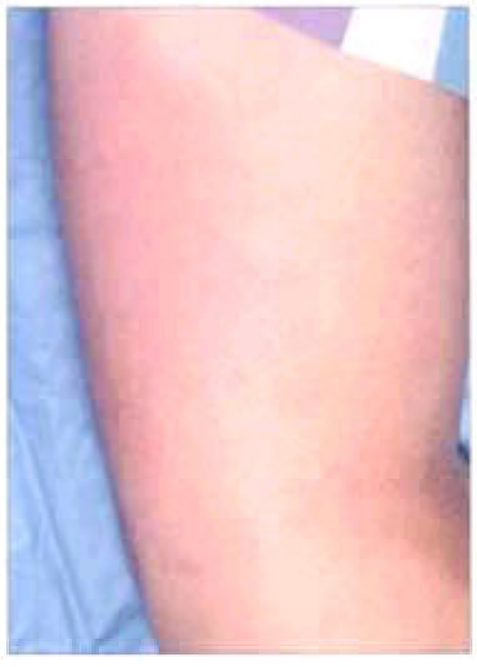


Figure 9-11 Cellulitis. (a) This case was caused by *Staphylococcus aureus* and is spreading centripetally from a central localized focus of infection. The redness and swelling characteristic of cellulitis are apparent over the upper eyelid. (b) The cellulitis has developed from a localized staphylococcal abscess formed in a meibomian gland (chalazion).

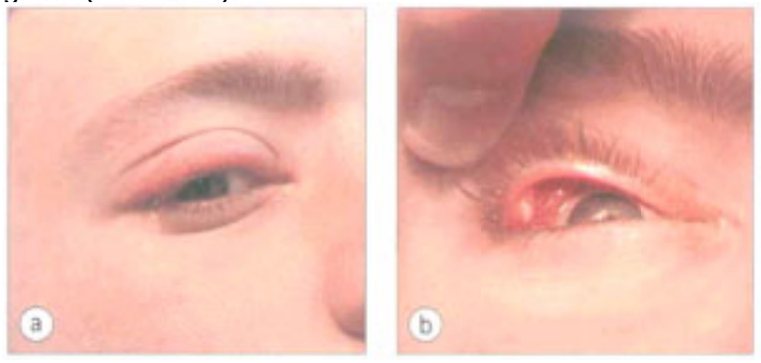


Figure 9-12 *Staphylococcus aureus* cellulitis of the nose. The focal lesion began in a hair follicle inside the nose, with redness, swelling and pain. Rarely, such lesions on the nose are complicated by extension into the cavernous sinus via veins draining the central part of the face.

Figure 9-13 Lymphangitis. Cellulitis caused by group A streptococci began below the knee and rapidly spread; about 4 hours later lymphangitis had spread up the inner aspect of the thigh.

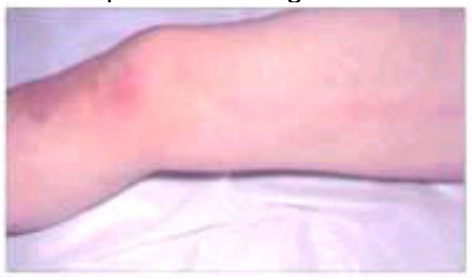


Figure 9-14 Cellulitis of the lower leg associated with chronic venous insufficiency. Streptococci of groups A, B, C and G are the most common isolates. Group B streptococci seldom cause cellulitis in previously healthy hosts, but should be considered in people who have peripheral vascular disease or diabetes mellitus.

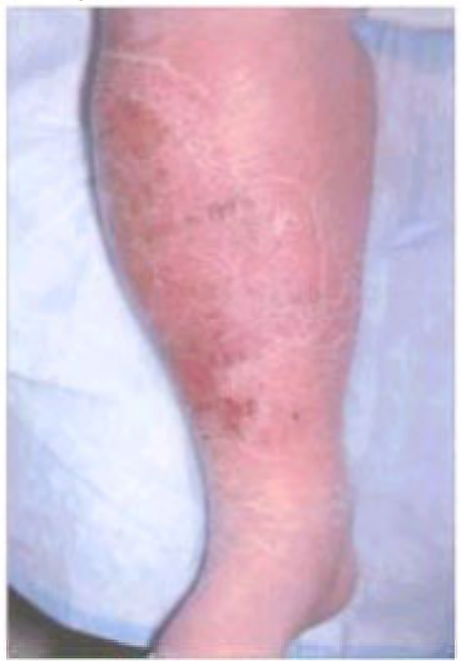


Figure 9-15 Gram stain of purulent material demonstrating *Staphylococcus aureus.* The microbial etiology of cellulitis may be suspected based upon signs, symptoms and history; however, definitive diagnosis requires Gram stain and culture. If there is no portal of entry, aspiration or even punch biopsy of cellulitic skin yields a positive culture in only 20% of cases.

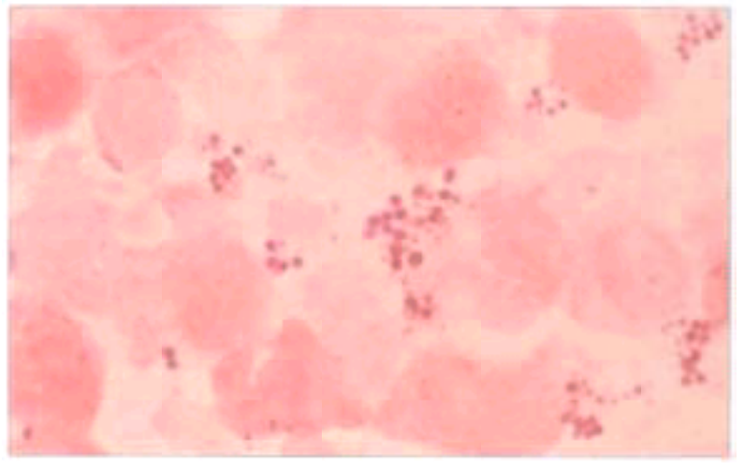


Figure 9-16 Cellulitis at the elbow associated with olecranon bursitis. (a) Pale pink erythema on the inner aspect of the elbow. (b) Careful inspection demonstrates a focal infection over the point of the elbow. Fluid aspirated from the olecranon bursa yielded a pure culture of *Staphylococcus aureus*.

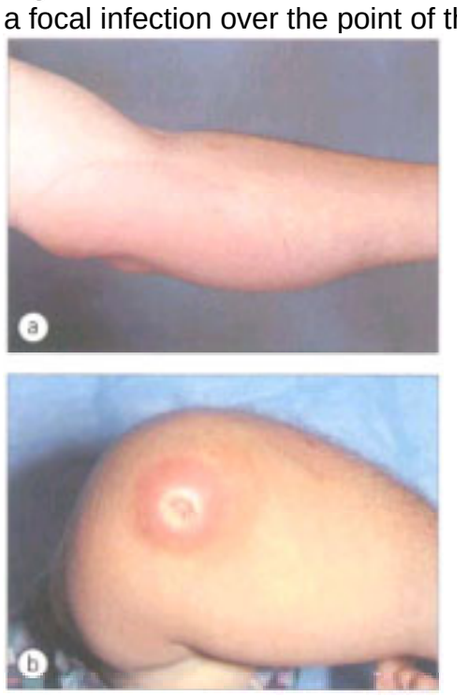
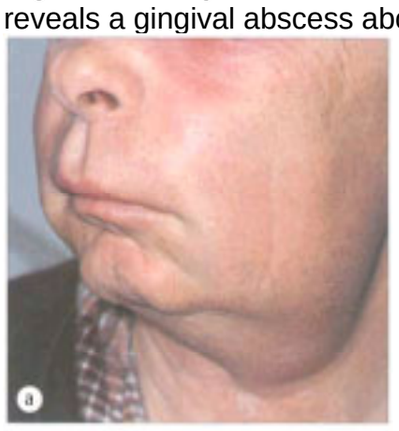


Figure 9-17 Erythema and swelling of the face due to a tooth abscess. (a) Swelling of the face, on inspection resembling periorbital cellulitis. (b) Further inspection reveals a gingival abscess above the patient's left upper canine tooth.



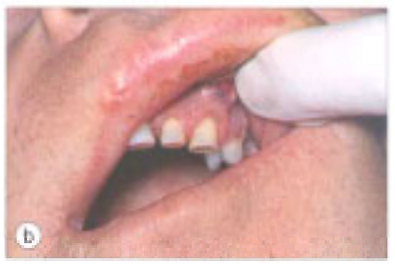


Figure 10-1 Differential diagnosis of infections involving muscle and fascia. Red, severe; orange, moderate; yellow, mild-to-moderate; blue, mild; white, none.

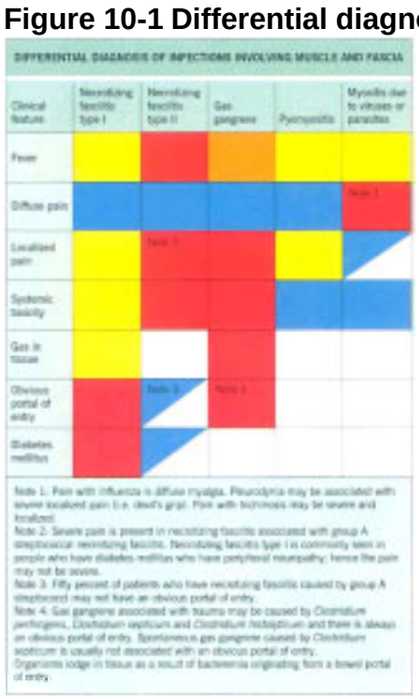


Figure 10-2 Histopathologic examination of tissue from a patient who has necrotizing fasciitis with extension into the underlying musculature. Note the absence of acute inflammatory cells in the area of muscle necrosis. When present, infiltrating granulocytes can be seen at the interface between normal and necrotic tissue and are often massed within small postcapillary venules.

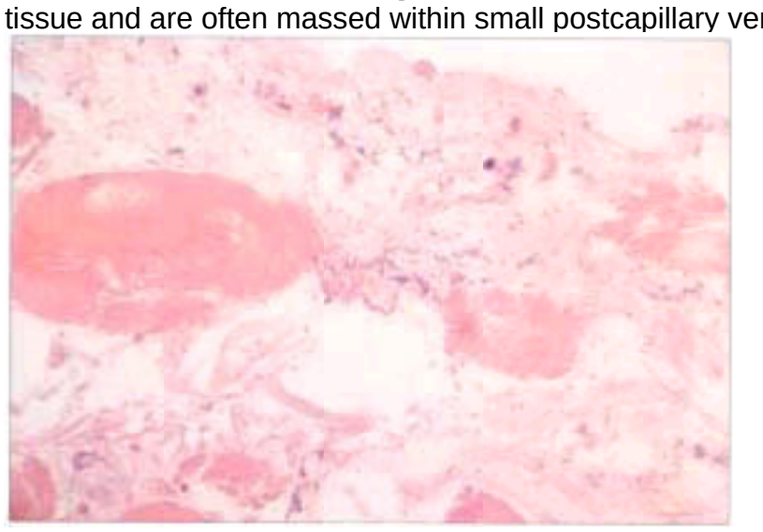


Figure 10-3 Ludwig's angina. Infection begins with a break in the mucosal lining in the oropharynx; oral bacterial flora invade the soft tissues at the base of the tongue and penetrate through the floor of the mouth and into soft tissue of the neck. The floor of the mouth is elevated and patients talk as though they have a 'hot potato' in their mouth. Potential airway obstruction is a major concern. Although patients usually respond to penicillin, surgical consultation should be obtained and CT or MRI scans are useful for determining whether a necrotizing process is present.

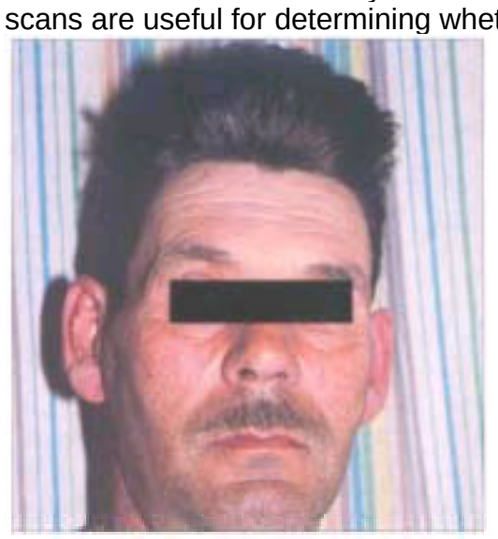


Figure 10-4 Computerized tomography of a soft tissue infection of the neck. This infection is caused by group A streptococci, which invaded as a rare complication of a previous 'strep throat'. Surgical drainage yielded a pure culture of group A streptococci and established a diagnosis. The patient was treated with intravenous penicillin for 10 days and made a good recovery.

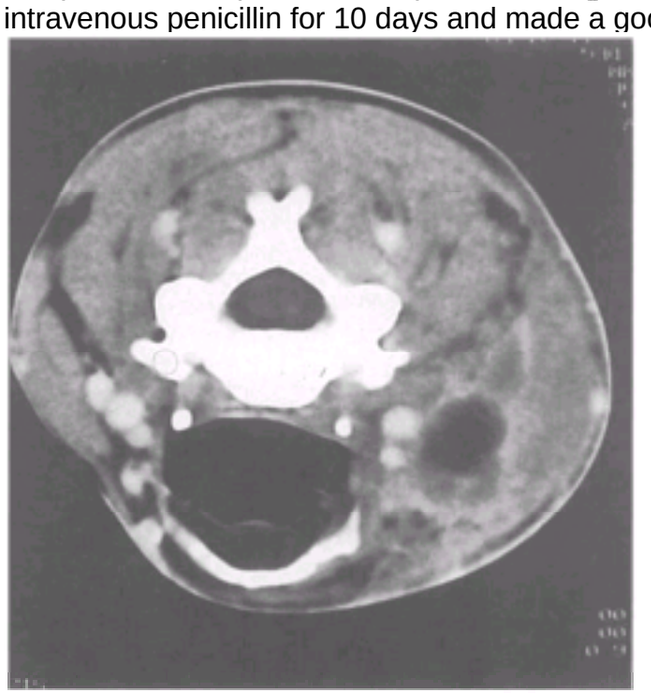


Figure 10-5 Type II necrotizing fasciitis caused by group A streptococci. (a) This patient was a 60-year-old man who had type II diabetes mellitus and who had a 3-day history of malaise, diffuse myalgia and low-grade fever. Over the course of 2–3 hours the pain became excruciating and was localized to the calf. During this time the calf swelled. Note that the skin over the anterior shin looks relatively normal, but that two small purple bullae are present. (b) Extensive necrotizing fasciitis was present on surgical exploration. In addition, myonecrosis was present beneath the fascia. The patient developed profound hypotension, acute respiratory distress syndrome and renal failure. He died despite aggressive surgical and medical management. There was no definable portal of entry, yet group A streptococci were grown from deep cultures and from blood.

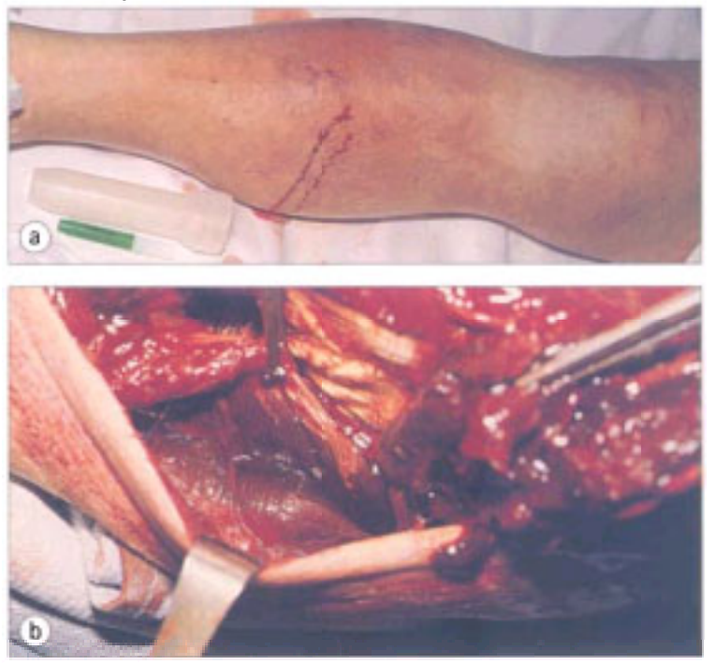


Figure 10-6 Postpartum sepsis due to group A streptococci. The patient was a 24-year-old woman who delivered a normal child. Thirty-six hours after delivery she developed fever, leukocytosis with marked left shift and increasing low abdominal pain. This MRI demonstrates swelling of the uterus, although not out of proportion for a recent delivery. There was no gas in the tissue. An emergency laparotomy revealed necrosis of the mucosa of the uterus, necrotizing fasciitis and myonecrosis of the uterus.

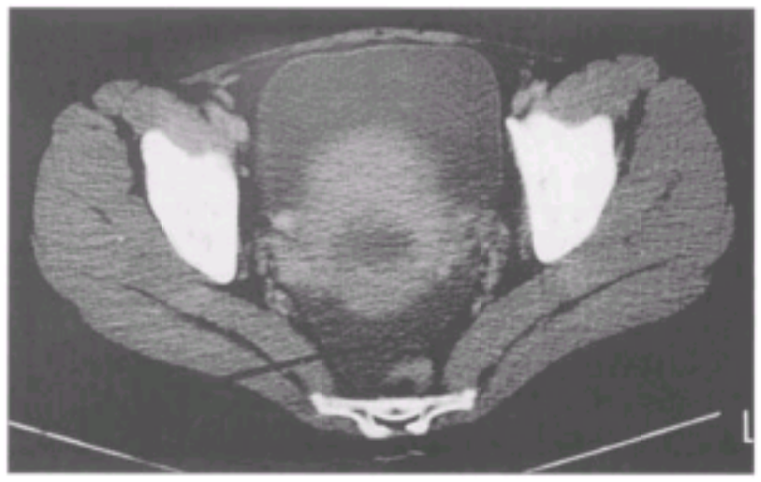
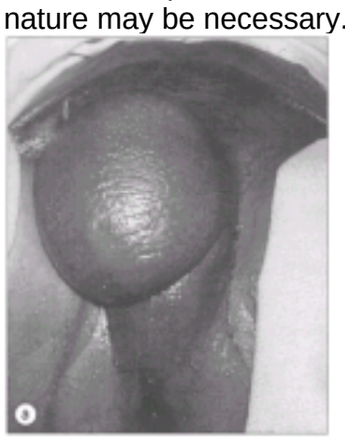


Figure 10-7 Type I necrotizing fasciitis. A 24-year-old man had been in good health but was awakened with severe perineal pain. (a) This photograph was taken 3 hours later. Note the massive swelling of the scrotum. (b) Soft tissue radiograph shows gas in the tissues of the thigh, buttocks, scrotum and anterior abdominal wall. Surgical inspection revealed brownish fluid in the scrotum, with gray, dull-colored, friable fascia but normal underlying musculature. Cultures grew *Enterococcus faecalis*, *Bacteroides fragilis*, *Escherichia coli* and anaerobic streptococci. The patient was treated with ampicillin, clindamycin and gentamicin for 3 weeks and surgical drains were placed in the scrotum, buttocks, thigh and anterior abdominal wall. There was an excellent clinical response. In some cases, surgery of a more radical nature may be necessary.



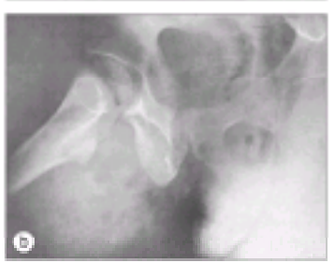


Figure 10-8 Colonies of *Clostridium perfringens* growing on an anaerobic blood agar plate. Theta toxin causes the clear zone of hemolysis closest to the colony. A second area of partial hemolysis is caused by a-toxin, an enzyme with phospholipase C activity.

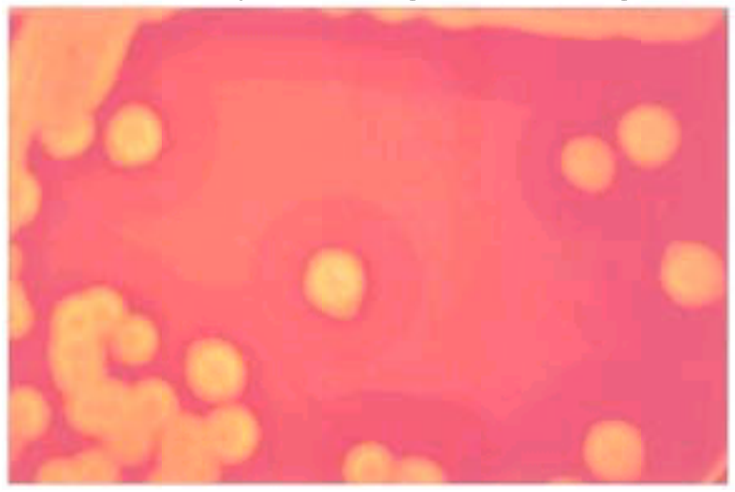


Figure 10-9 Extensive gas gangrene of the arm due to *Clostridium perfringens.* A 35-year-old man sustained a knife wound to the forearm. He did not seek medical care, but 36 hours later experienced severe pain in the upper arm and came to the emergency room. There was extreme tenderness of the arm and crepitus was easily demonstrated. A radiograph also demonstrated gas in the deep soft tissues. Surgical debridement and antibiotics were instituted, but later amputation at the level of the shoulder was necessary. A pure culture of *C. perfringens* was grown from the deep tissues.

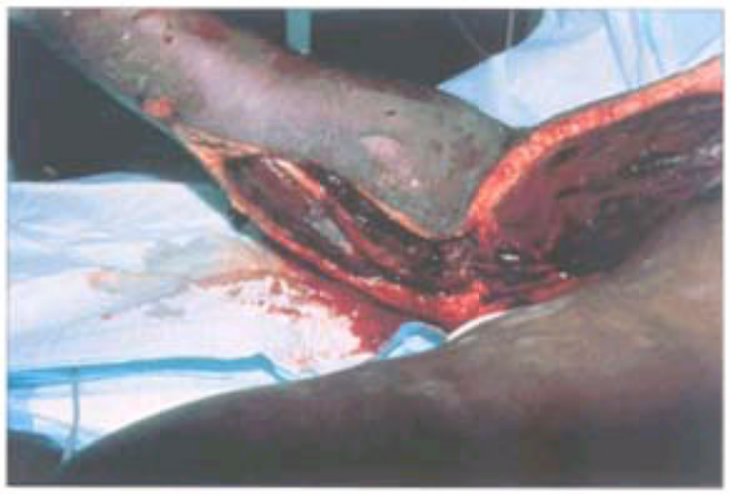


Figure 10-10 *Clostridium perfringens* in a patient who has extensive gas gangrene. (a) Tissue Gram stain of tissue removed from the arm of the patient described in Figure 10.9 . Note that the bacteria are rod shaped but gram variable. Note also that there are few if any acute inflammatory cells at the site of infection. (b) Transmission electron micrograph of *C. perfringens*. Note the endospores.

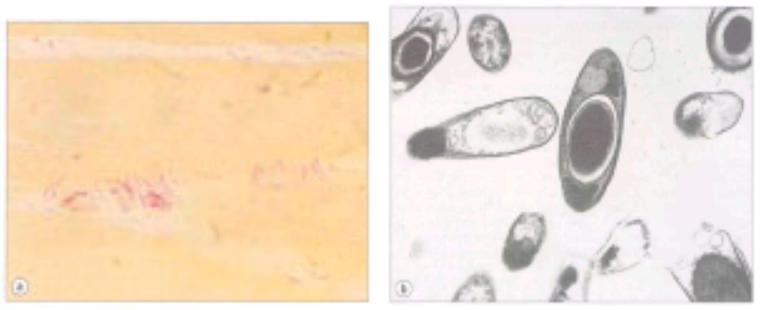


Figure 10-11 Spontaneous necrotizing fasciitis due to *Clostridium septicum.* This patient developed the sudden onset of severe pain in the forearm. Swelling rapidly ensued and he sought medical treatment. Crepitus was present on physical examination and gas in the soft tissue was verified with routine radiographs. Immediate surgical debridement revealed necrotizing fasciitis but sparing of the muscle. Note the purple-violaceous appearance of the skin. See also Figure 10.12 .

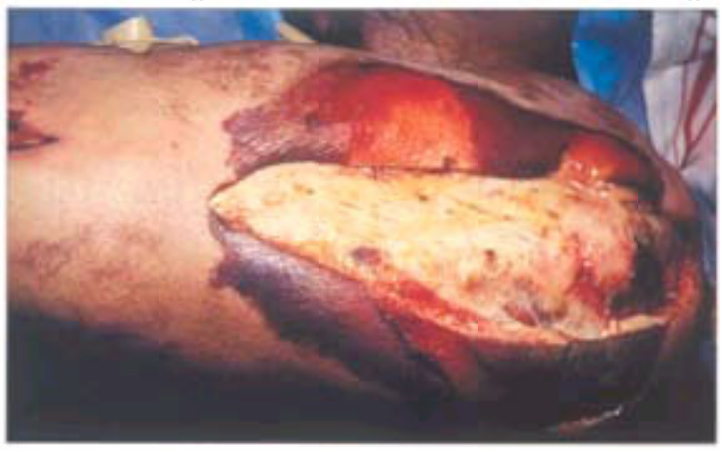


Figure 10-12 Colonic carcinoma in a patient who has spontaneous gas gangrene caused by *Clostridium septicum.* The patient described in Figure 10.11 was found to have a mass in the colon. Surgical resection revealed an adenocarcinoma, which probably served as a portal of entry for the *Clostridium septicum* bacillus. Hematogenous seeding of the forearm resulted in spontaneous gas gangrene.

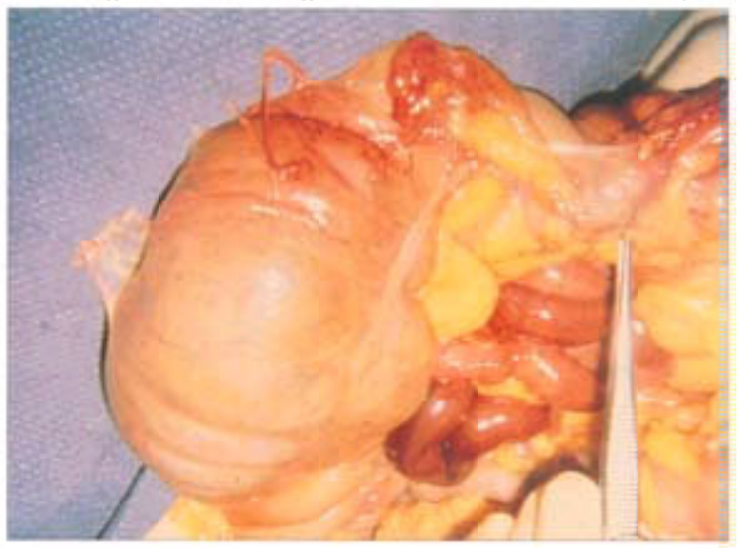


Figure 10-13 MRI scan showing high signal STIR sequence (consistent with marked edema) in the adductor muscles of the patient's left leg in a patient with *Staphylococcus aureus* bacteremia (arrow). At operation, necrotic and infected muscle was decompressed and debrided. This represents the 'woody' stage, prior to muscle liquefaction and the formation of frank abscesses.

Figure 11-1 Typical lesions of papular urticaria, caused in this case by bedbug bites.

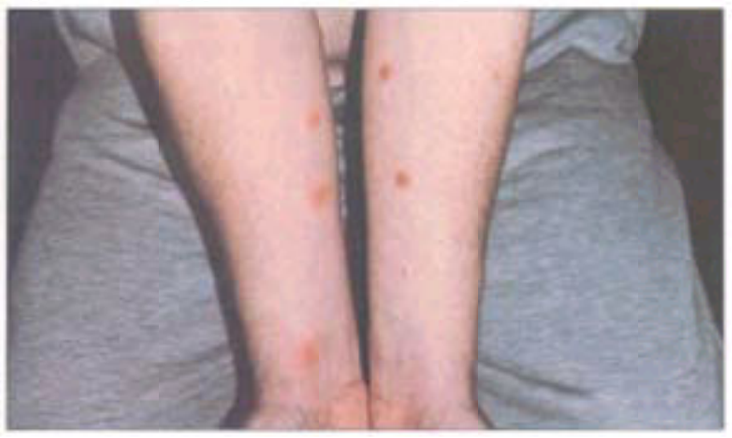


Figure 11-2 Mosquito bites with secondary staphylococcal and streptococcal infection.

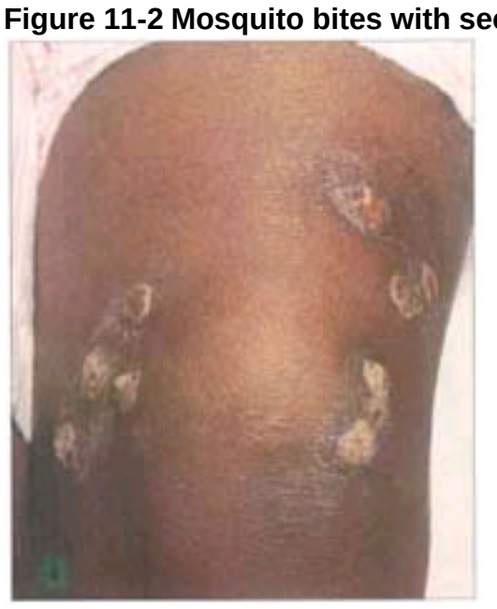


Figure 11-3 Ixodid tick after feeding on the host for several days.

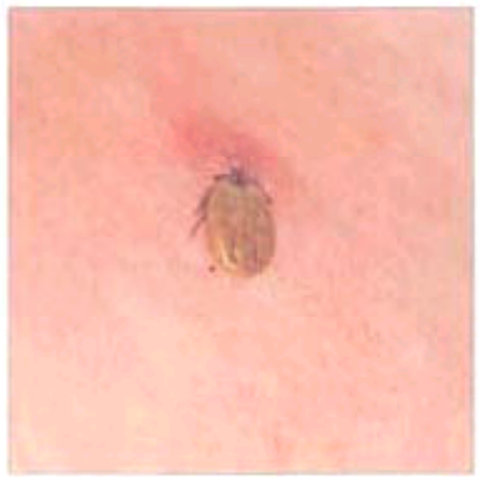


Figure 11-4 Tick-bite eschar associated with African tick typhus.

Figure 12-1 Scrofuloderma in a 60-year-old patient. A biopsy confirmed tuberculoid granulation tissue and the patient responded very well to antituberculous therapy.

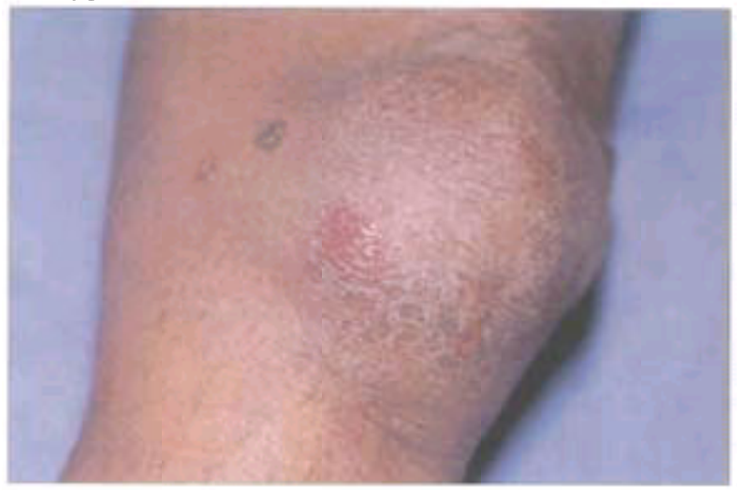


Figure 12-2 Erythema induratum on the back of the leg of a 45-year-old woman.

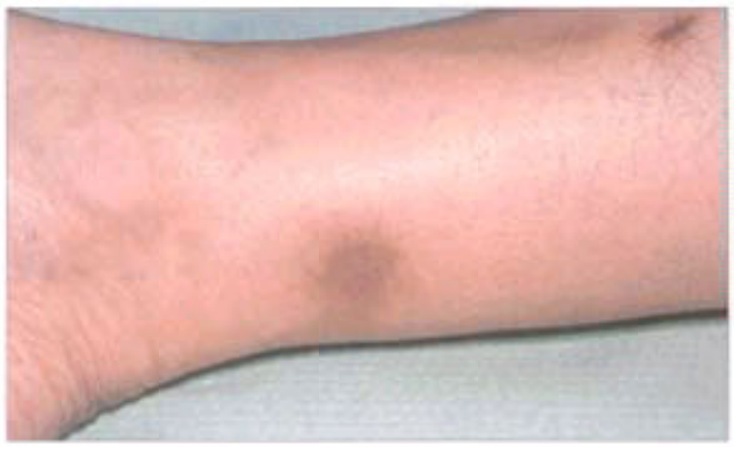


Figure 12-3 Tertiary syphilis on the face of a 56-year-old woman.

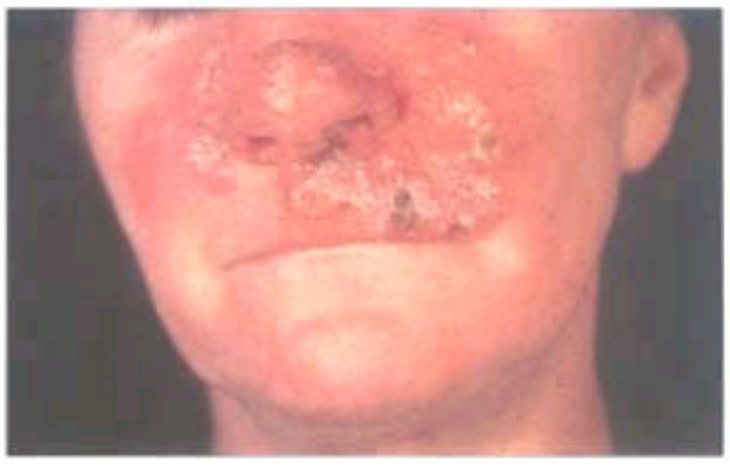


Figure 12-4 Secondary yaws showing papular and rather vegetative lesions on the anterior chest wall.

Figure 12-5 Cutaneous cryptococcosis in a renal transplant patient. These lesions started as nodules that then rapidly ulcerated. Computerized tomography of the patient's brain showed no abnormality but *Cryptococcus neoformans* was grown from the cerebrospinal fluid.

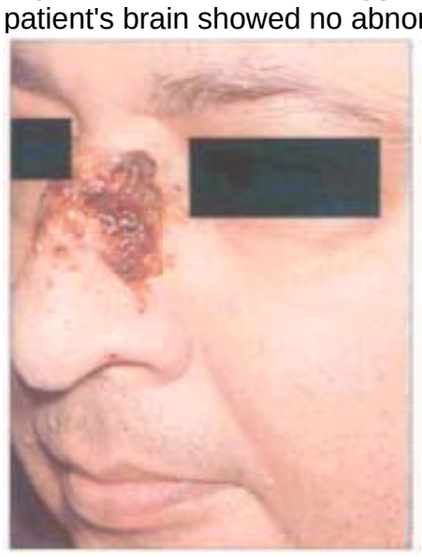


Figure 12-6 Kaposi's sarcoma in a 20-year-old man who had AIDS. One lesion on the patient's back had been treated with radiotherapy, resulting in disfiguring pigmentation at the site of treatment.

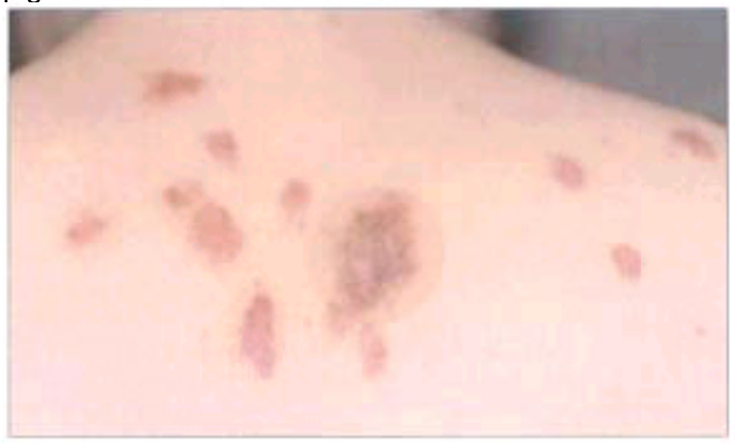


Figure 12-7 Lepromatous leprosy, with multiple symmetric lesions on the face, giving a leonine facies.

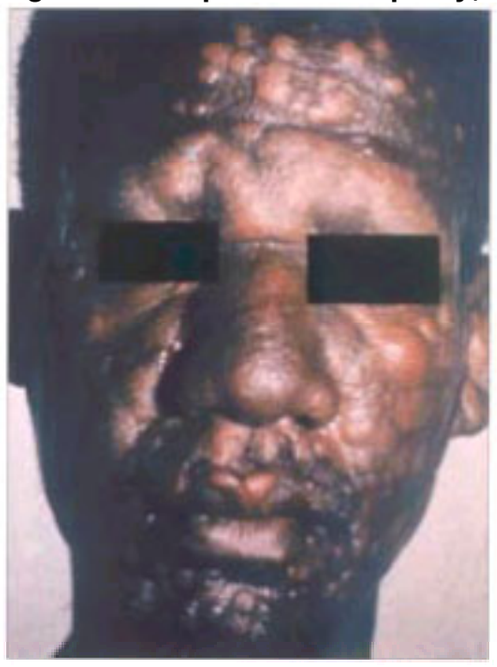


Figure 12-8 Erythema multiforme showing target lesions and bullous lesions on the palms of the hands.

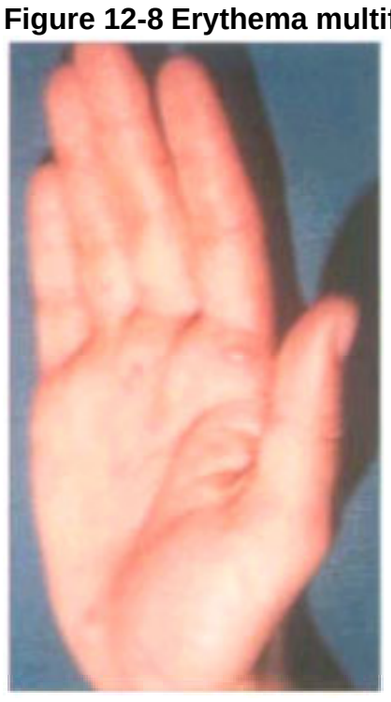


Figure 12-9 Erythema nodosum on the lower legs. On investigation this patient was found to have a negative Mantoux even at 1 in 100 but the erythema nodosum subsided when the patient was started on antituberculous therapy.

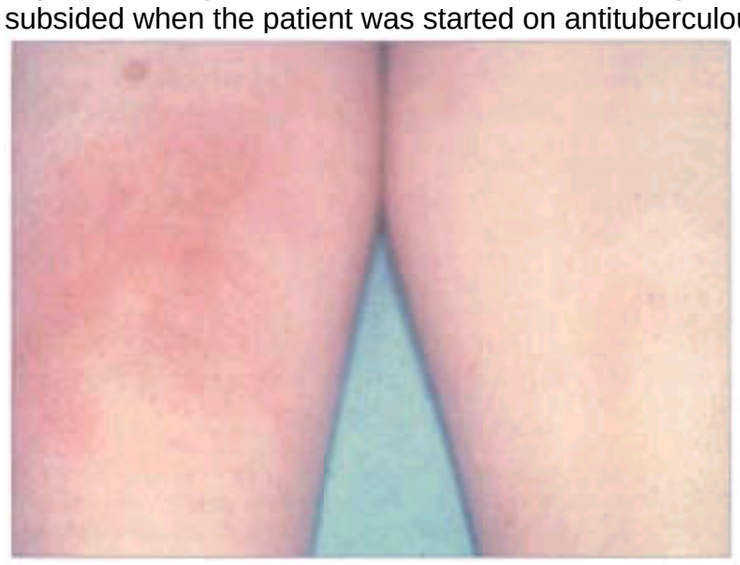


Figure 12-10 Acute leukocytoclastic vasculitis showing bullous lesions on the lower leg. This patient was found to have a high antistreptolysin titer.

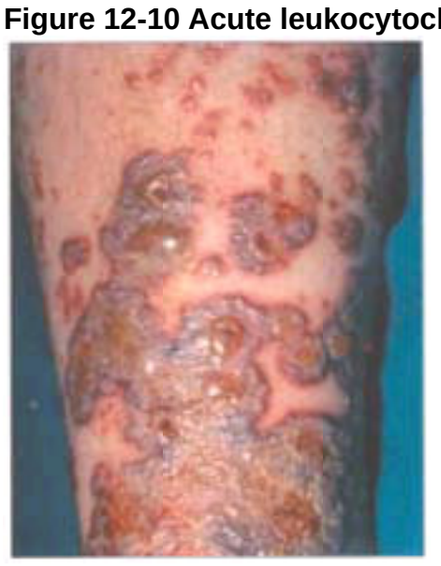


Figure 13-1 Tinea barbae due to *Trichophyton verrucosum.*

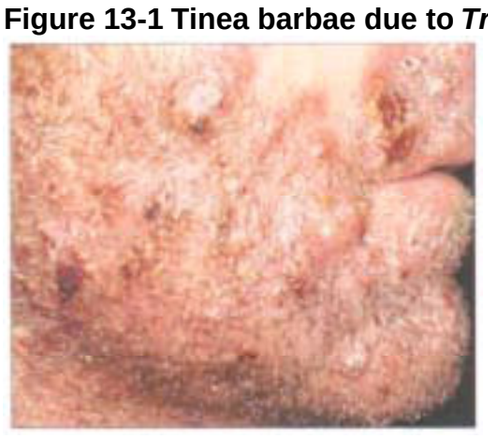


Figure 13-2 Tinea corporis due to *Trichophyton mentagrophytes* var. *mentagrophytes*.

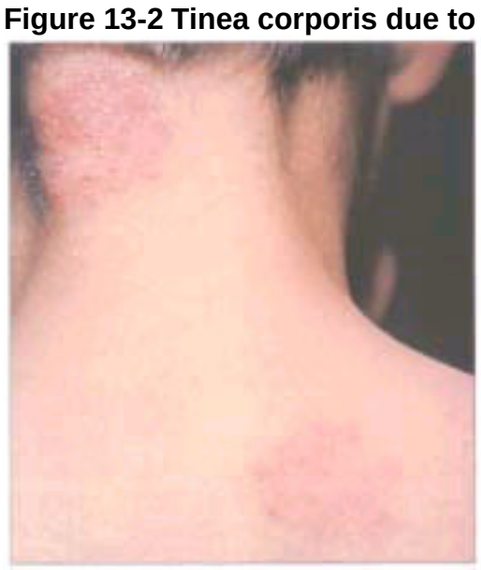


Figure 13-3 Moccasin tinea pedis due to *Trichophyton rubrum.*

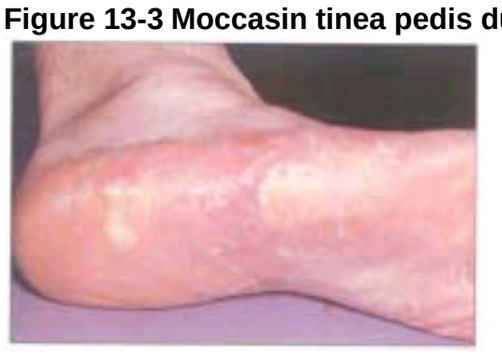


Figure 13-4 Patterns of fungal nail disease.

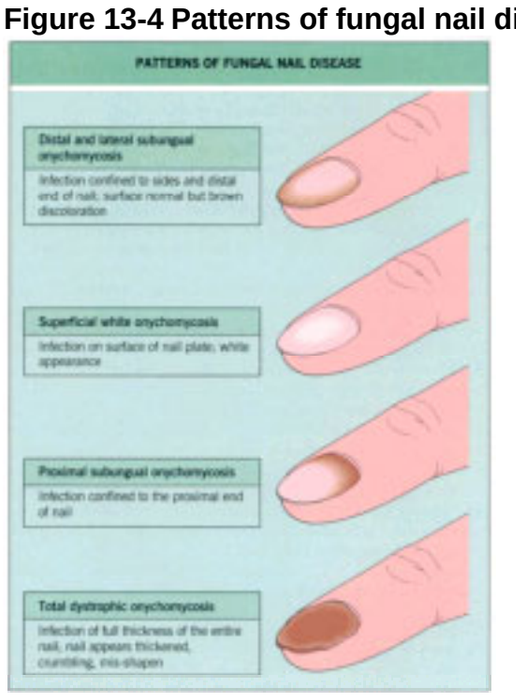


Figure 13-5 Total dystrophic onychomycosis due to *Trichophyton rubrum.*

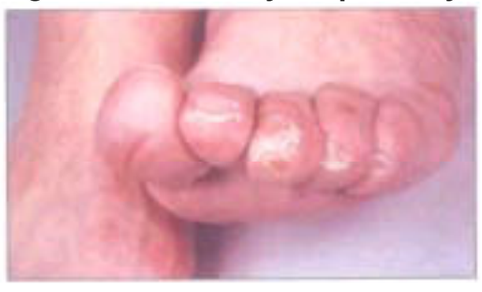


Figure 13-6 Interdigital candidiasis.

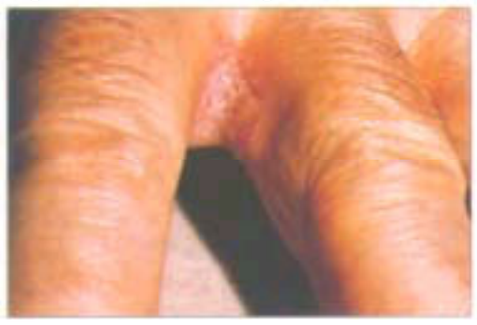


Figure 13-7 Pityriasis versicolor showing depigmented lesions.

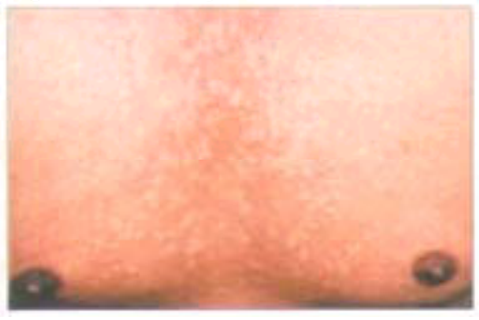


Figure 13-8 Onychomycosis due to *Scytalidium dimidiatum* (*Hendersonula toruloidea*).

Figure 14-1 Adult *Rhipicephalus sanguineus.*

Figure 14-2 Maculopapular rash in Mediterranean spotted fever.

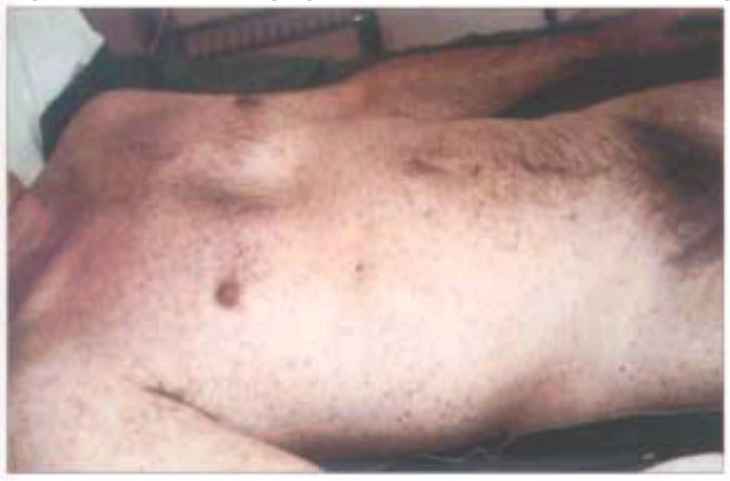


Figure 14-3 Two Inoculation eschars on the legs of a patient who presented with African tick bite fever.

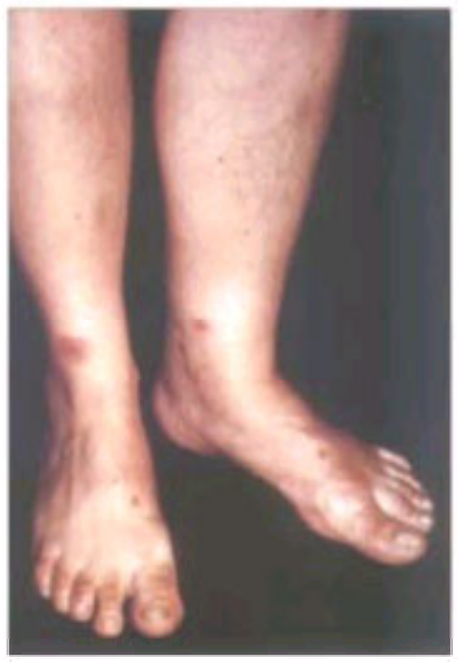


Figure 14-4 Adult *Dermacentor marginatus.*

Figure 14-5 Eschar inoculation on the scalp of a patient who presented with *Rickettsia slovaca* infection.

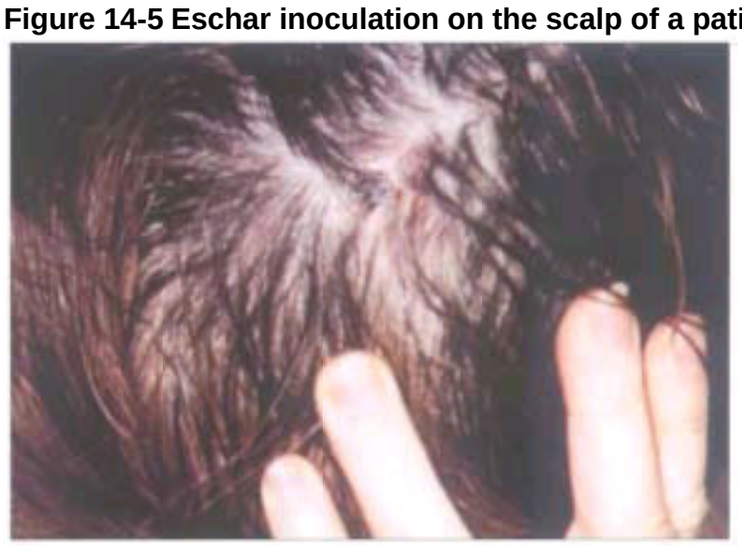


Figure 15.d-1 Severe recurrent cellulitis associated with obesity.

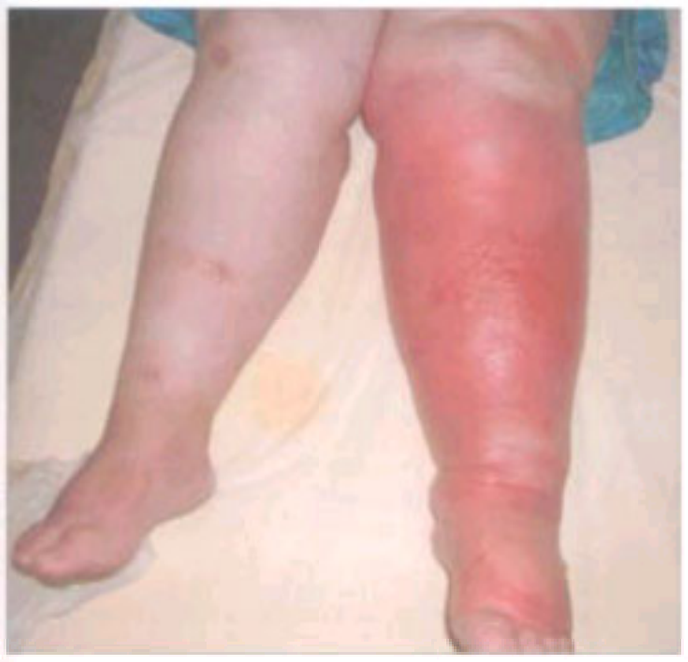


Figure 15.d-2 Severe recurrent cellulitis in a lymphedematous leg following radical surgery for rhabdomyosarcoma.

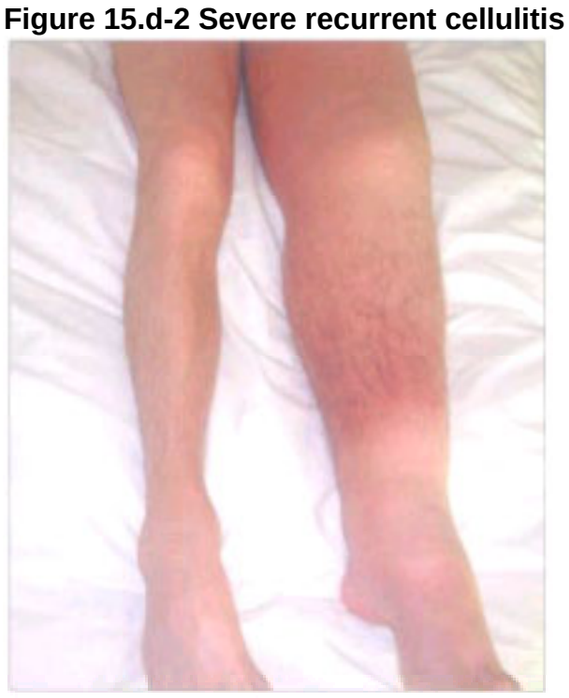


Figure 15.d-3 Injecting drug user with severe recurrent cellulitis of the left arm.

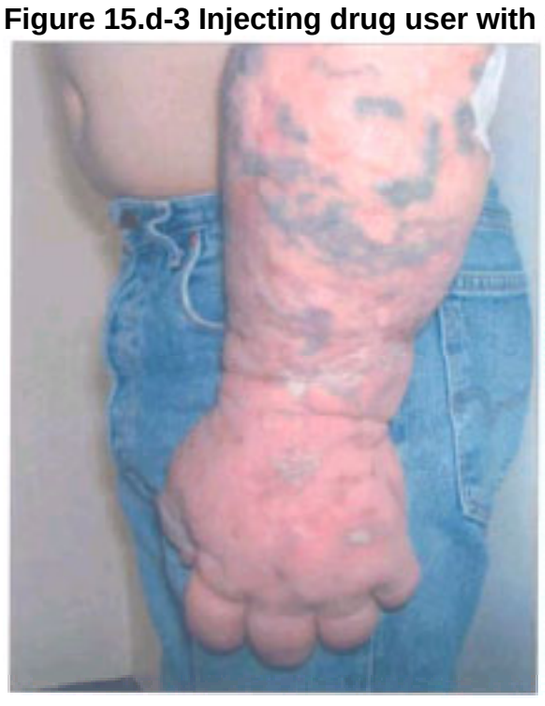


Figure 16-1 The human lymphatic system.

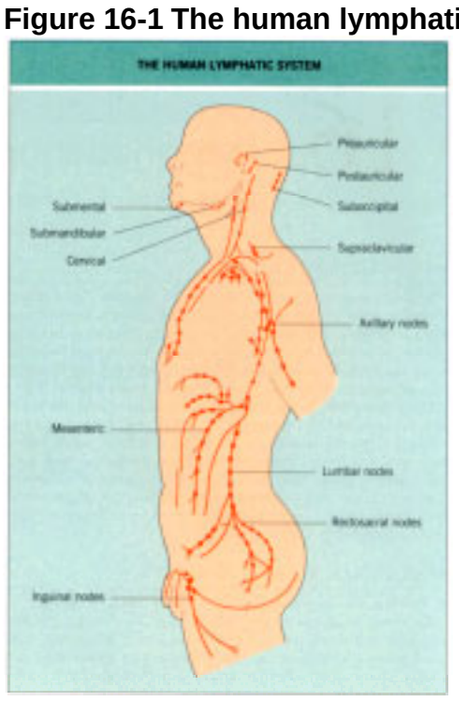


Figure 16-2 The lymph node.

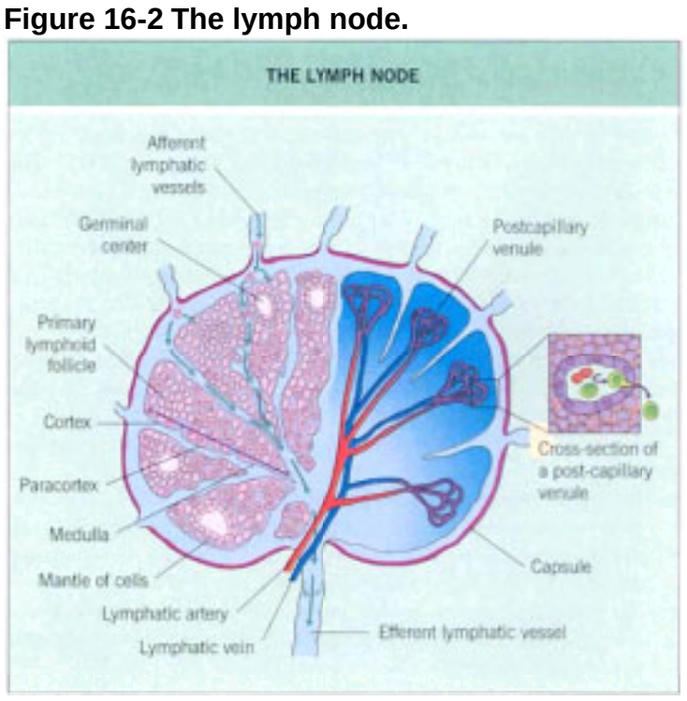


Figure 16-3 Tuberculous lymphadenitis of the axilla.

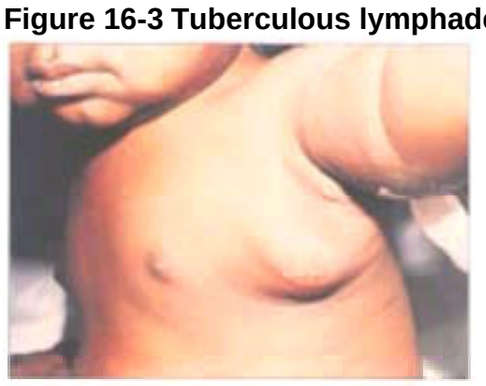


Figure 16-4 Groove sign of lymphogranuloma venereum. There is cleavage of extensive lymphadenopathy by the inguinal ligament.

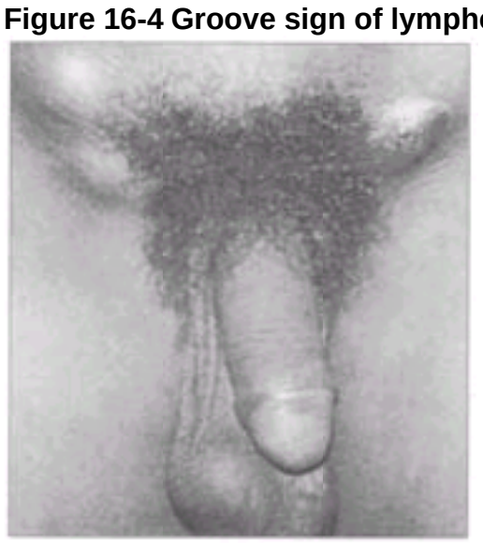


Figure 16-5 Bubonic plague. Femoral lymph nodes matted together to form the classic bubo.

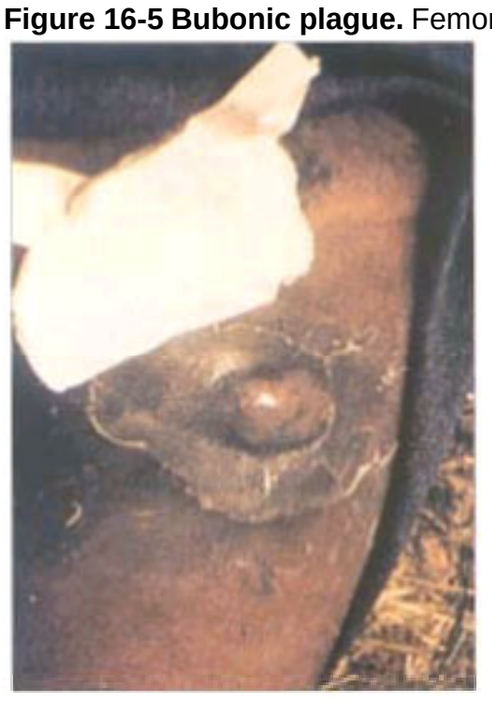


Figure 16-6 Pulmonary tuberculosis.

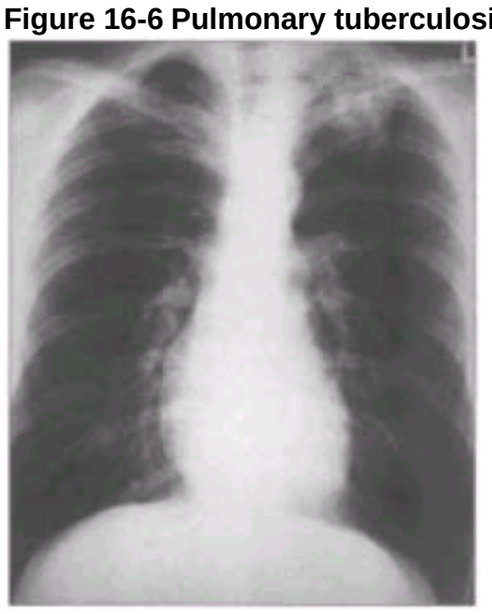


Figure 16-7 Posterior cervical lymphadenopathy in infectious mononucleosis.

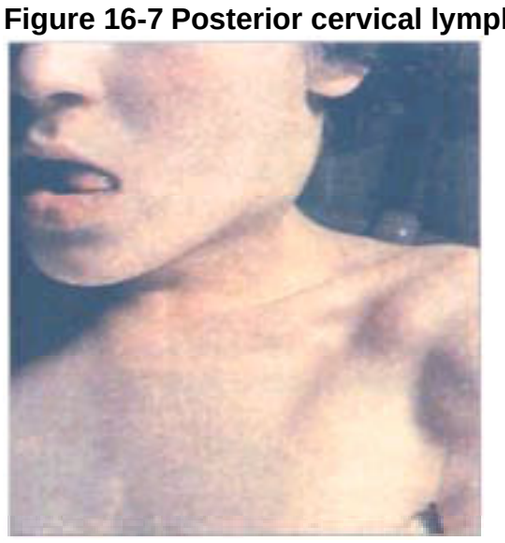


Figure 16-8 Romaña's sign in acute Chagas' disease. There is unilateral edema of the eyelid accompanied by conjunctivitis and auricular lymphadenopathy.

Figure 18-1 Typical microbial keratitis. Note accumulation of inflammatory cells at the dependent part of the anterior chamber of the eye (hypopyon) and mid-corneal defect. *Courtesy of Myron Yanoff.*

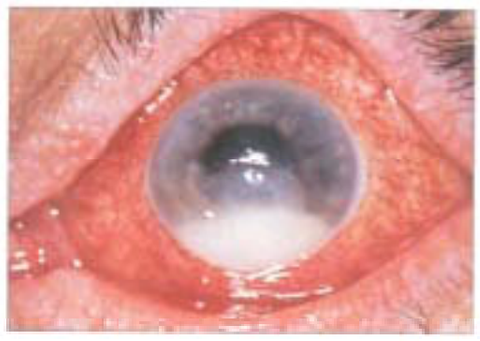


Figure 18-2 Diagnosis of keratitis.

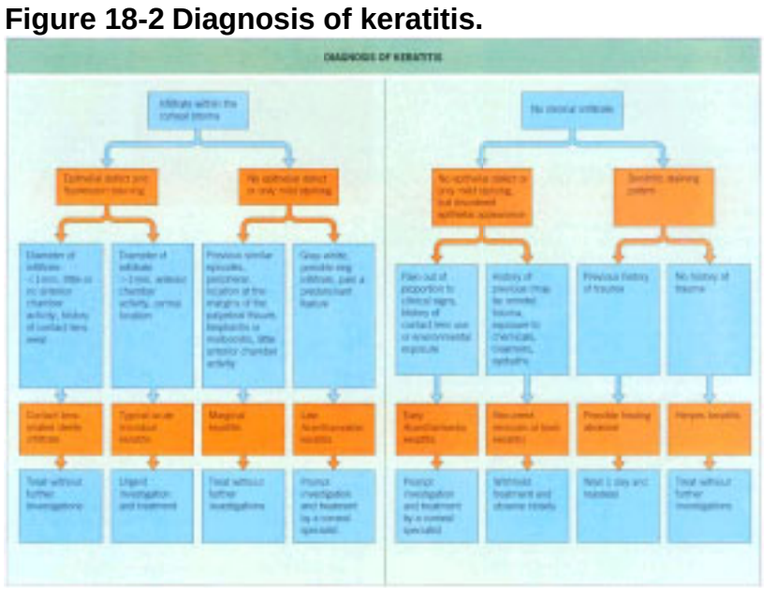


Figure 18-3 Fungal keratitis. The corneal surface looks rough, and there are several satellite lesions best seen here at the periphery on the left side of the cornea. *Courtesy of Myron Yanoff.*

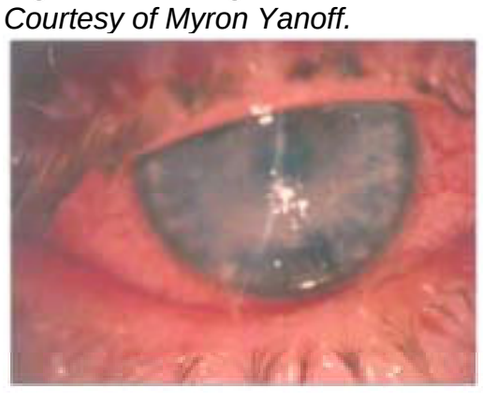


Figure 18-4 Herpes simplex virus dendritic keratitis, showing branching epithelial lesions seen (a) without staining and (b) with rose bengal staining. Rose bengal stains the devitalized cells at the edges of the dendritic lesions. *Courtesy of Myron Yanoff.*

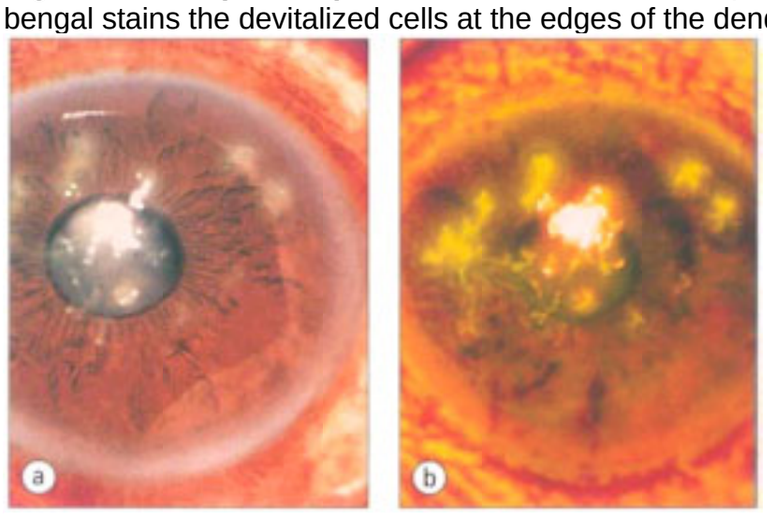


Figure 18-5 Herpes zoster ophthalmicus. Inflamed right periorbital skin, with conjunctivitis and a lesion on the nose. *Courtesy of Myron Yanoff.*

Figure 19-1 Corneal edema and fibrinous anterior chamber exudate in a traumatic foreign body-induced endophthalmitis. Any posterior vitreal or retinal view is obscured by the anterior corneal and aqueous haze.

Figure 19-2 B-scan ultrasound of the eye showing total vitreous opacity of a severe endophthalmitis in patient seen in Figure 19.1 . This horizontal 'cut' through the eye shows the normally 'transparent' vitreous cavity to be filled with inflammatory debris, but there is no obvious retinal detachment.

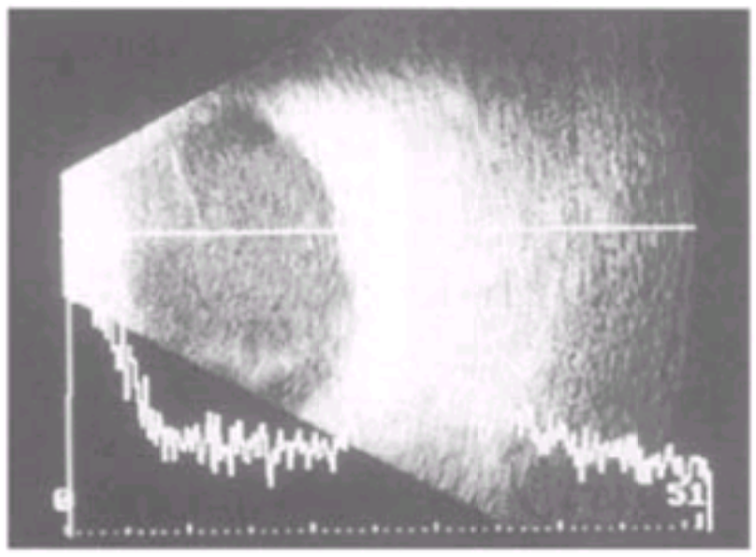


Figure 19-3 A dense vitreous abscess in advanced endophthalmitis. This partially treated postoperative endophthalmitis has vitreous cellular and protein deposits obscuring the retinal view.

Figure 19-4 The typical posterior capsular opacities seen in a late-onset *Staphylococcus epidermidis* endophthalmitis. These deposits are actual coccal colonies, which are frequently removed at subsequent vitrectomy surgery to open up the capsular bag to intraocular antibiotics.

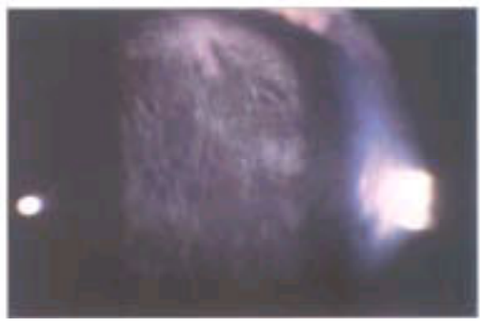


Figure 19-5 A 'quiet' endogenous fungal endophthalmitis with small hypopyon. This eye is relatively quiet with little chemosis, injection and pain, but has a small hypopyon and some small fungal 'balls' on the temporal iris.

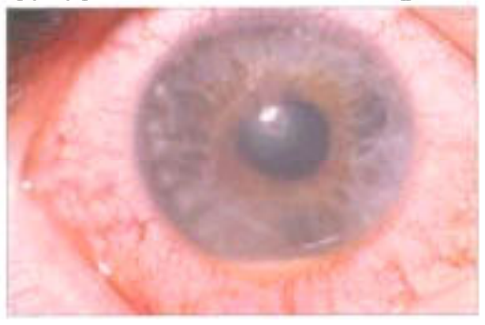


Figure 19-6 The degraded ophthalmoscopic retinal view obtained in patient seen in Figure 19.5 . The corneal edema and anterior chamber activity make vitreous and retinal observation difficult.

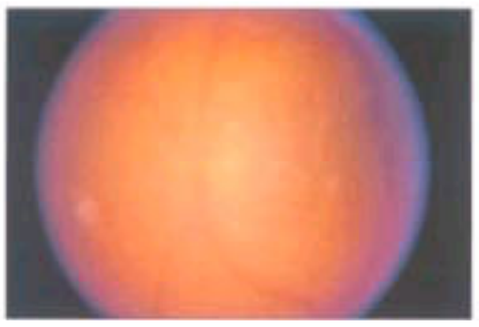


Figure 19-7 Fungal 'fluff balls' on the iris seen in a fungal endophthalmitis. Although these are not pathognomonic, their appearance raises the real possibility that the infection is of fungal origin.

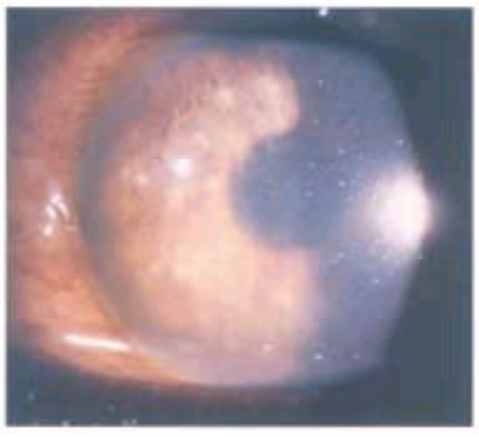


Figure 19-8 Horizontal computerized tomography scan section of eye seen in Figure 19.1 and Figure 19.2 revealing metallic intraocular foreign body in vitreous cavity (arrow). This CT scan demonstrates that the vitreous opacity seen by ultrasound is 'invisible' to this investigation.

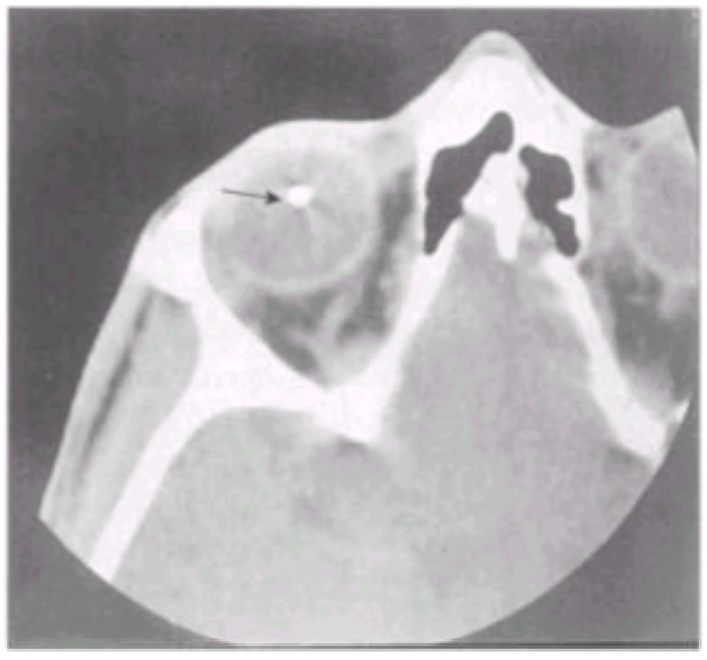


Figure 20-1 Major structures of the eye.

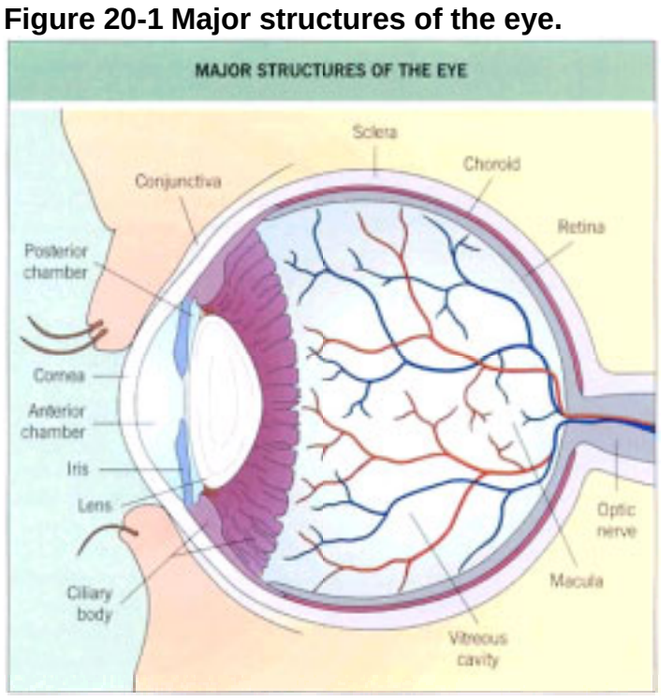


Figure 20-2 Retinal inflammatory vascular sheathing (vasculitis). This case occurred secondary to sarcoidosis.

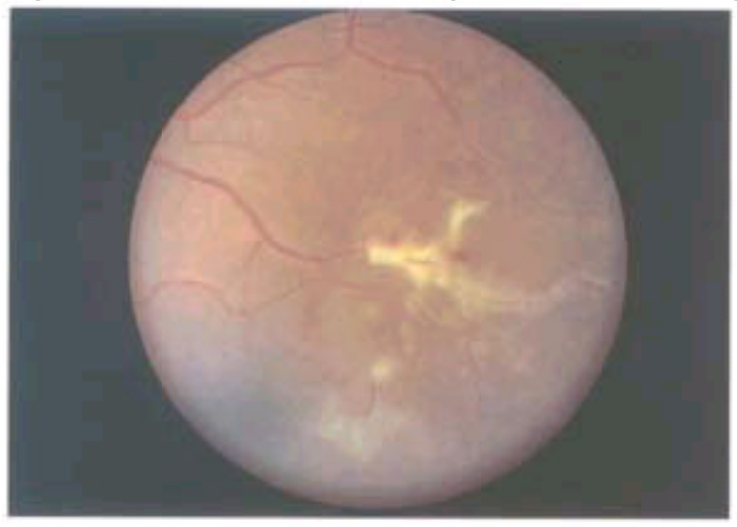


Figure 20-3 Hypopyon. The finding of a hypopyon (layered inflammatory cells in the anterior chamber of the eye) usually denotes a severe anterior uveitis.

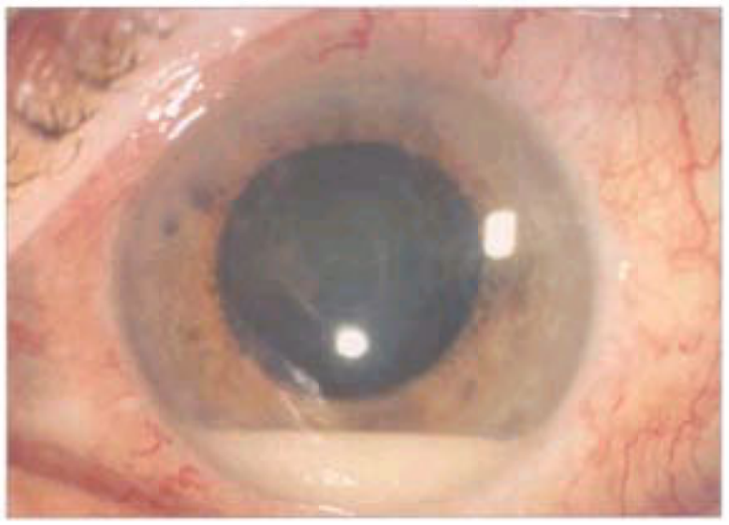


Figure 20-4 Salt-and-pepper fundus. The pigment alterations in the macula give a 'dirty' appearance to the retina. The lesion occurred following congenital rubella infection. The vertical black line across the fovea is a focusing stick.

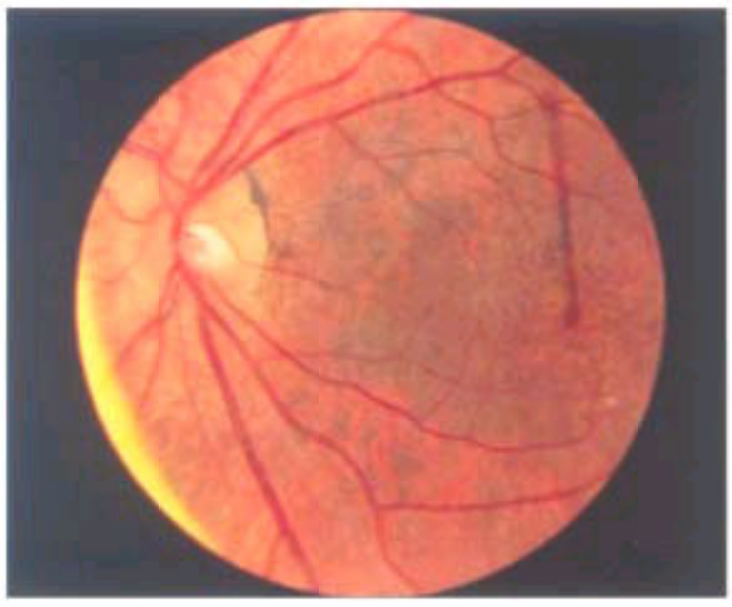


Figure 20-5 Macular retinitis. This patient has subacute sclerosing panencephalitis.

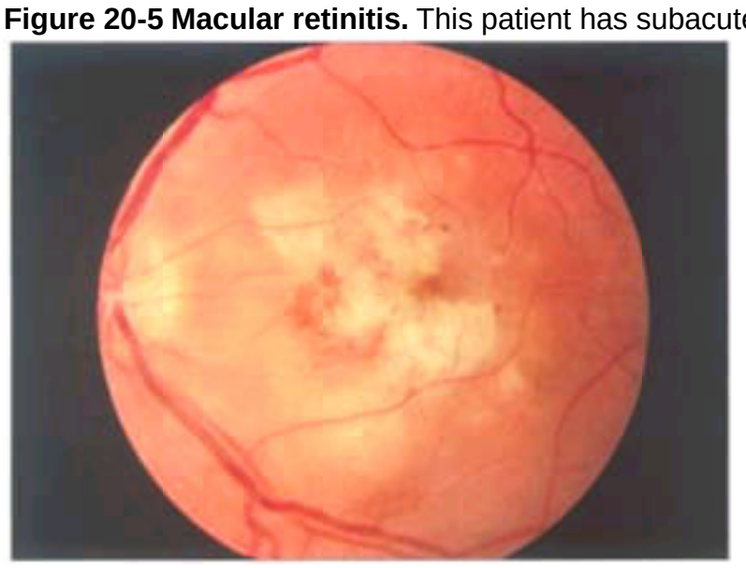


Figure 20-6 Neuroretinitis with a macular star associated with *Bartonella* infection. Note the swelling of the optic disk with hard exudate in the macula in the so-called 'stellate' pattern.

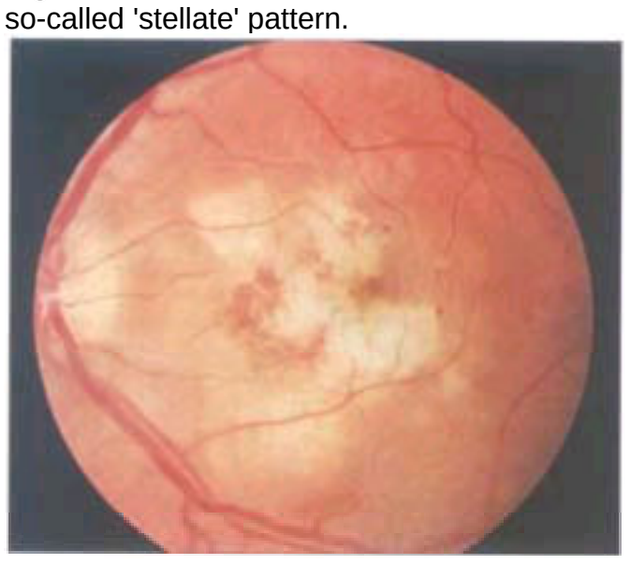


Figure 20-7 A focal area of superficial retinitis and vitritis. This occurred secondary to *Candida* albicans.

Figure 20-8 The classic ocular findings of previous histoplasmosis. Note the peripapillary atrophy and punched-out yellowish chorioretinal scars. An old choroidal neovascular membrane is present in the center of the histoplasmosis scar temporal to the macula.

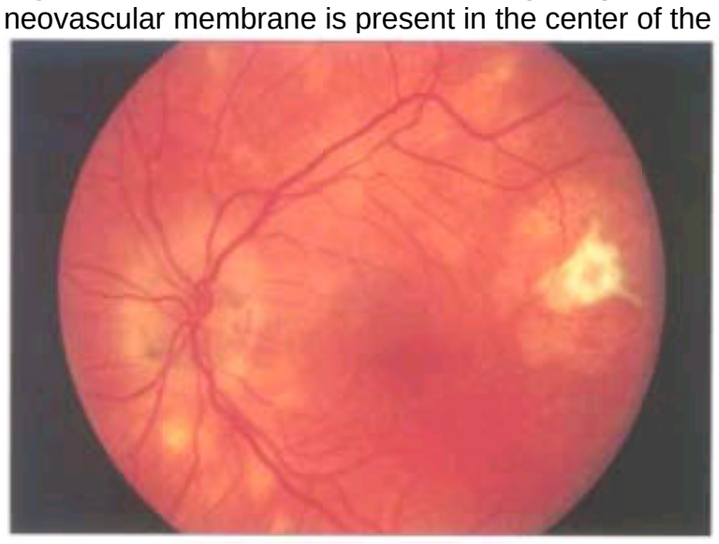


Figure 20-9 A *Nocardia* chorioretinal abscess. This abscess is in the macula of a patient on systemic immunosuppression following heart transplant.

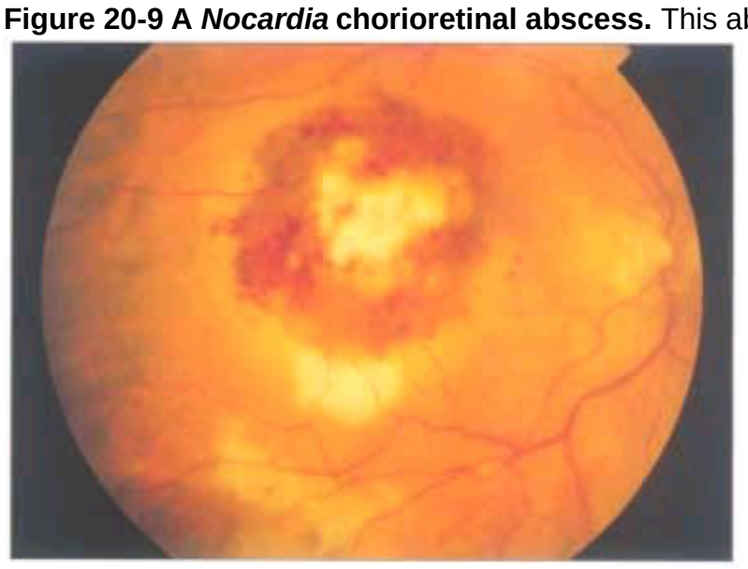


Figure 20-10 Ocular toxocariasis. (a) The posterior pole of a left eye affected by toxocariasis. There is severe macular distortion and dragging of the retina toward a granuloma in the inferotemporal retinal periphery. (b) The periphery of the inferotemporal retina in the same eye showing the granuloma.

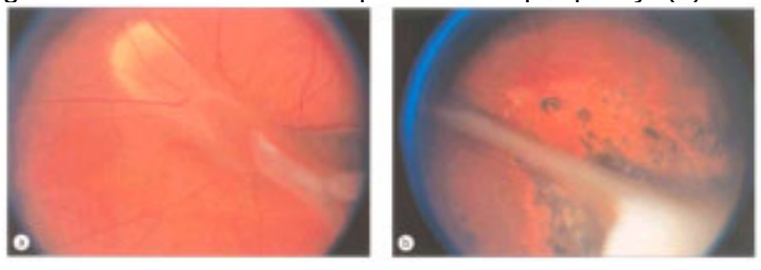


Figure 20-11 An old, inactive congenital macular toxoplasmosis scar. The patient's vision was 20/400.

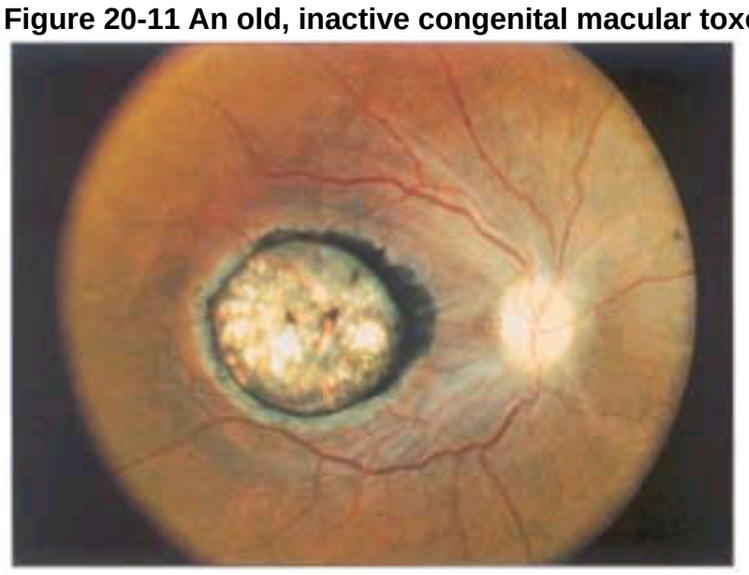


Figure 20-12 A reactivated area of retinal toxoplasmosis. The lesion is the area of whitening and is adjacent to an old scar, just temporal to the macula of the left eye.

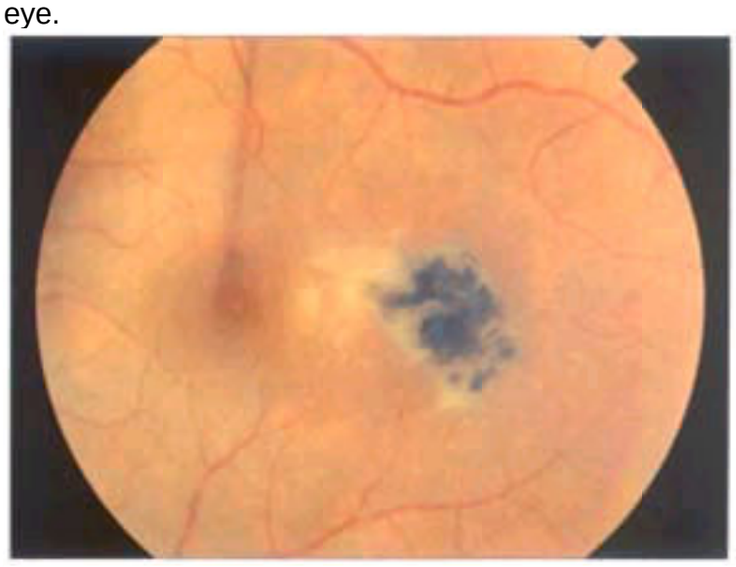


Figure 20-13 Typical appearance of the retina in the acute retinal necrosis syndrome. There is dense peripheral retinal whitening with a geographic border. Satellite lesions are common. The view is hazy owing to vitritis.

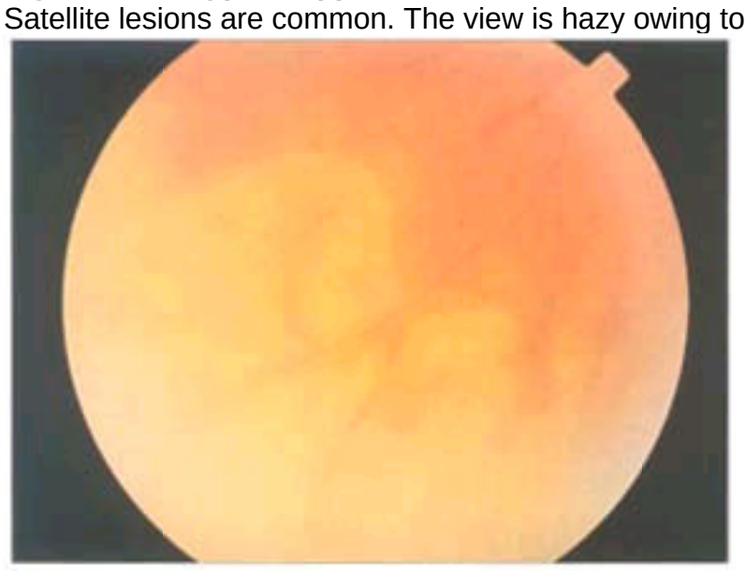


Figure 20-14 Cytomegalovirus infection with granular retinal whitening along the major blood vessels with mild hemorrhage. The view is clear because there is only mild vitritis.

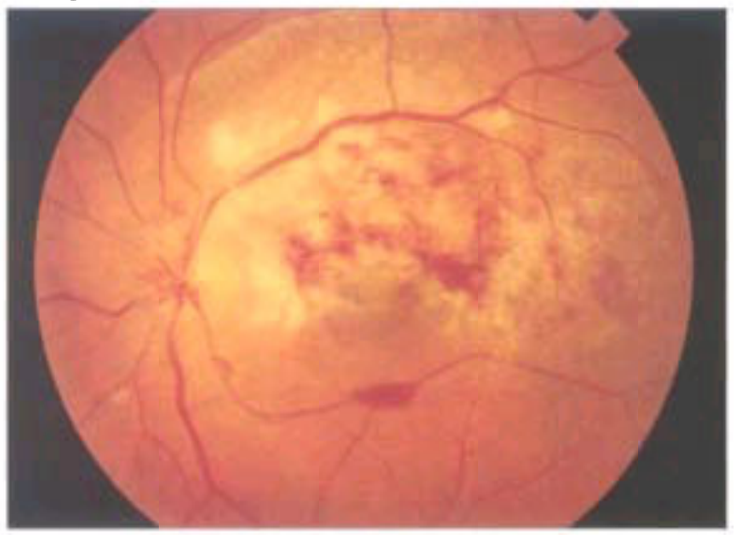


Figure 20-15 HIV retinopathy. There are multiple superficial white patches in the retina (cotton-wool spots). These do not affect vision and typically wax and wane over time.

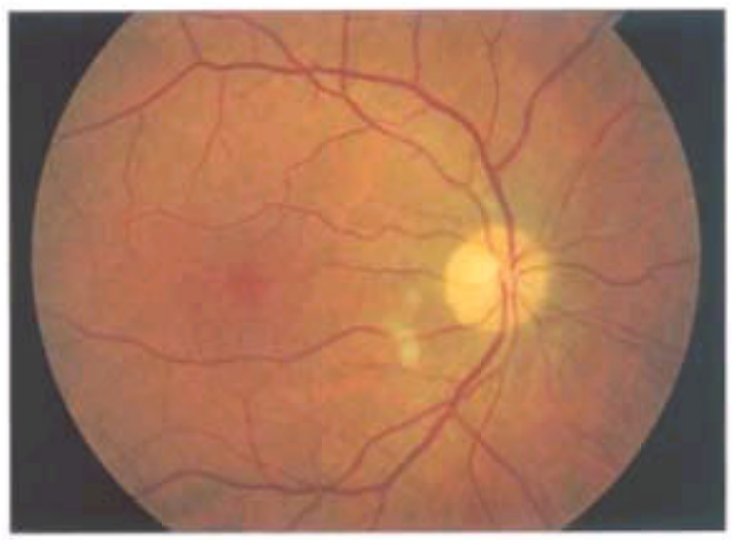


Figure 21-1 Frost suture. A 5.0 silk suture is placed at the upper lid margin through half the tissue thickness (above) and used to tape the lid shut (below).

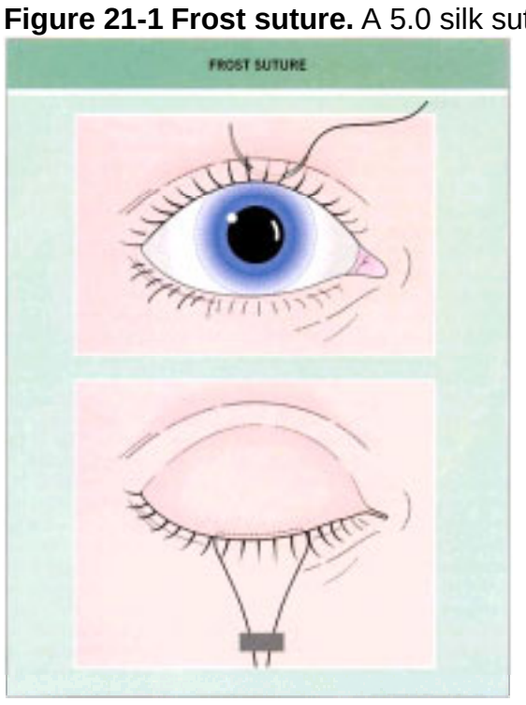


Figure 22-1 Pathogenesis of meningitis.

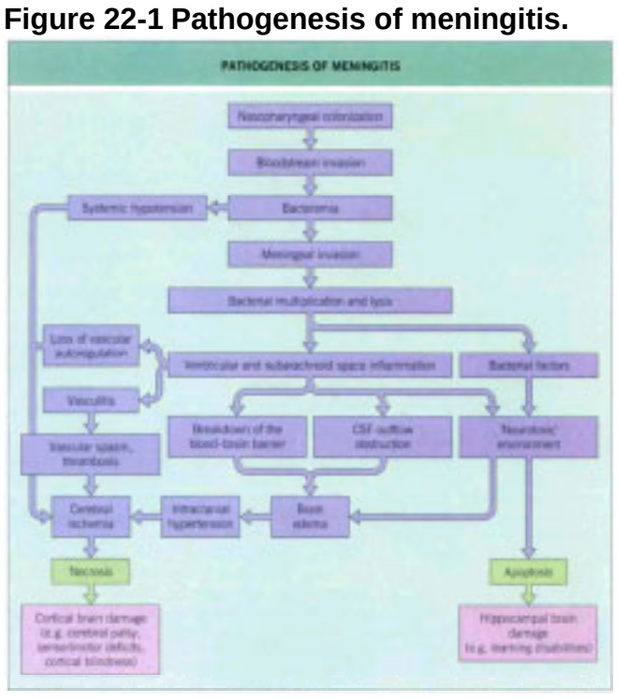


Figure 22-2 Subarachnoid and perivascular inflammation in meningitis. The inflammation in the subarachnoid space extends into the Virchow-Robin spaces along the vasculature.

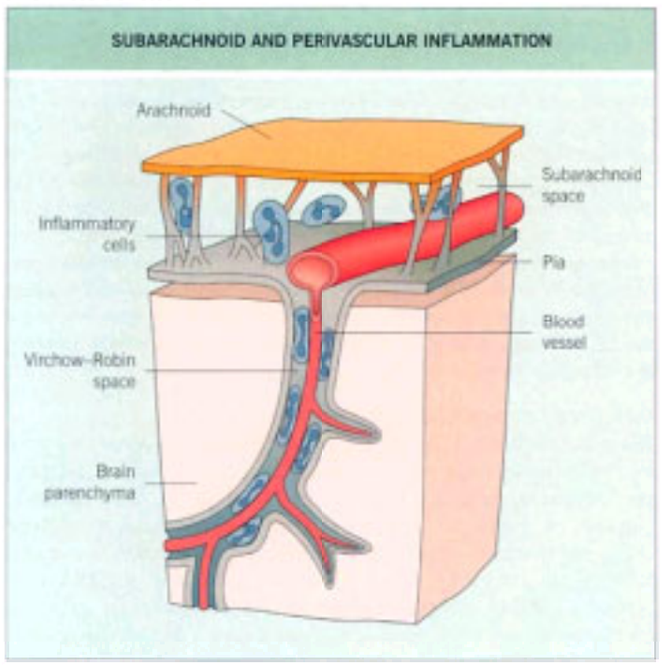


Figure 22-3 Arachnoid membrane in purulent meningitis. Close-up view of the arachnoid membrane covering the purulent subarachnoid space, with penetrating blood vessels exhibiting inflammatory vasculitis and thrombosis. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

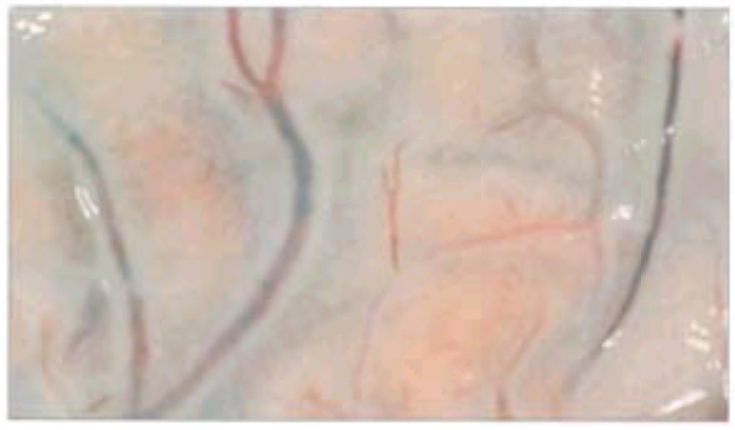


Figure 22-4 Brain with inflammatory exudate covering the cortical hemispheres in purulent meningitis. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

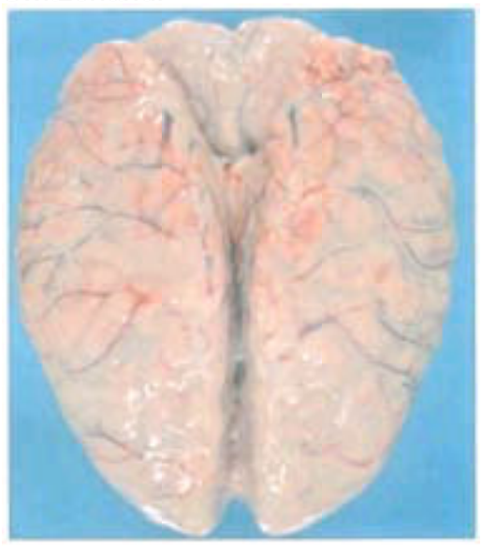


Figure 22-5 Histopathology of the subarachnoid space in meningitis. Note the inflammatory involvement of the blood vessels and the small vessel leading into the brain parenchyma surrounded by inflammation in the Virchow-Robin space. *Courtesy of Dr M Tolnay, University of Basel, Switzerland.*

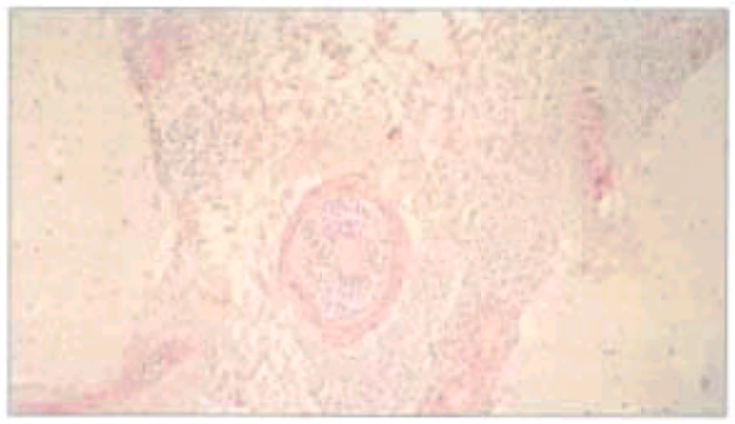


Figure 22-6 Skin lesions in acute meningococcemia. Characteristic purpura with petechiae and ecchymoses in a patient who has fulminant sepsis and meningitis due to *Neisseria meningitidis*. *Courtesy of Professor W Zimmerli, University of Basel, Switzerland.*

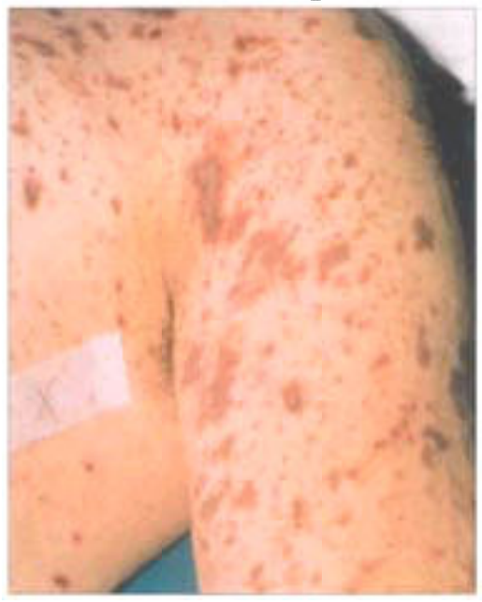


Figure 23-1 Common viral etiologies of central nervous system infection (relative prevalence by month).

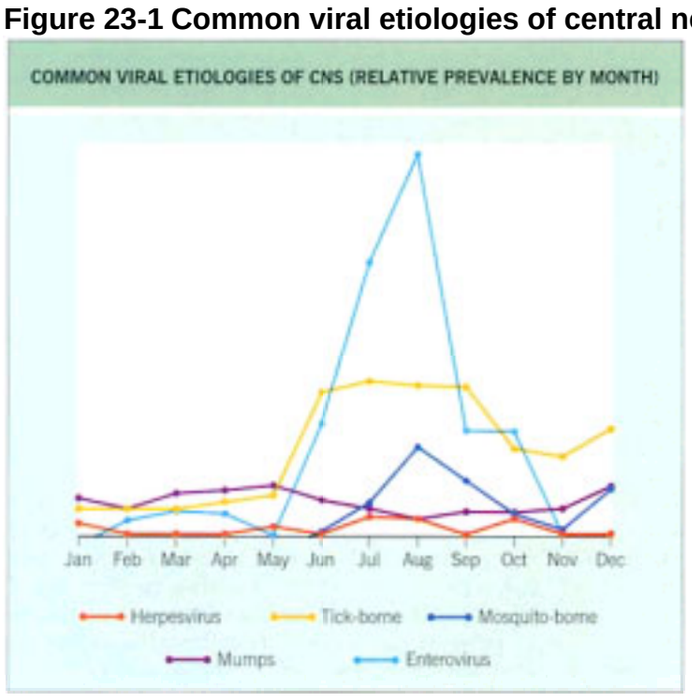


Figure 23-2 Hematogenous spread of viral pathogens to the central nervous system.

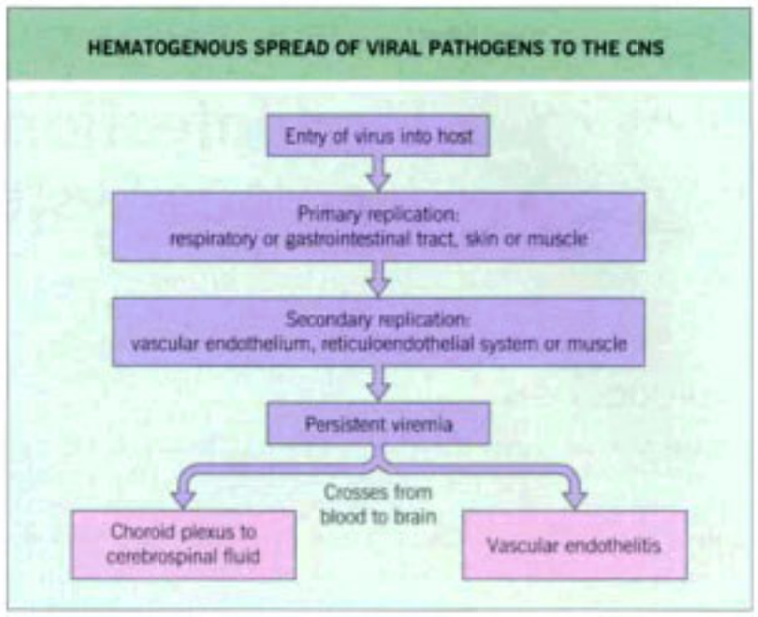


Figure 23-3 Pathogenesis of viral central nervous system infections: neuronal transmission.

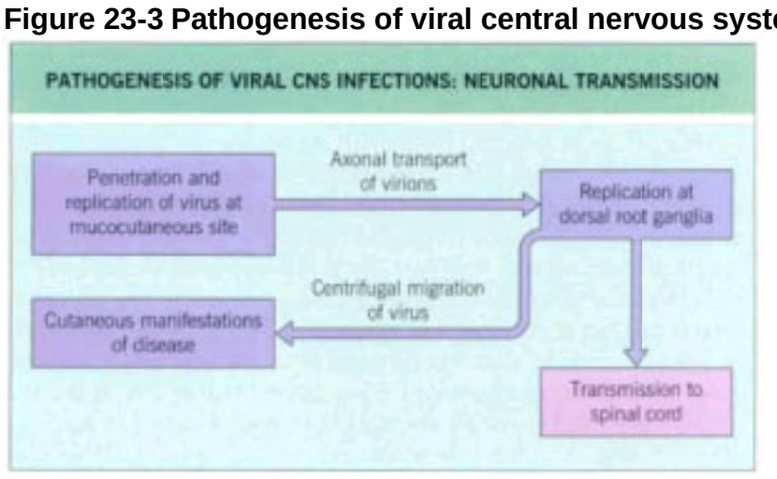


Figure 23-4 Herpes simplex encephalitis. Basilar view of herpes simplex encephalitis showing (a) hemorrhagic necrosis of temporal lobes. (b) Coronal section of brain from patient with herpes simplex encephalitis. [49]

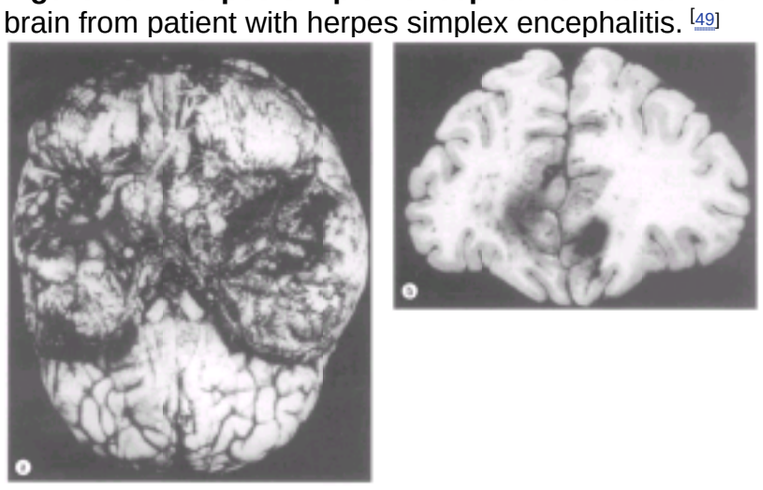


Figure 23-5 Approach to the patient who has presumed viral central nervous system disease.[56]

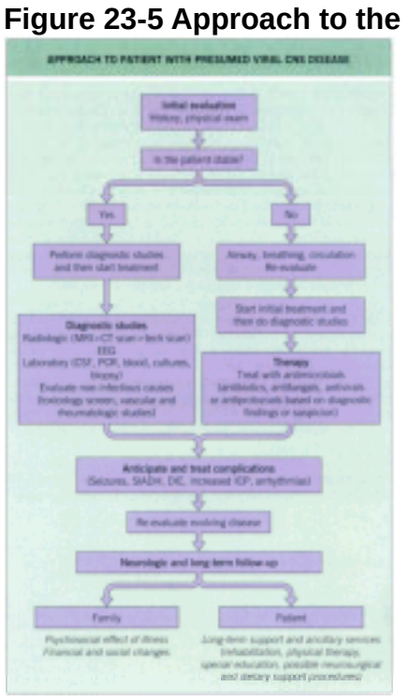


Figure 24-1 Anatomic relationships between potential contiguous sources of infection and sites at which focal pyogenic central nervous system infections may occur.

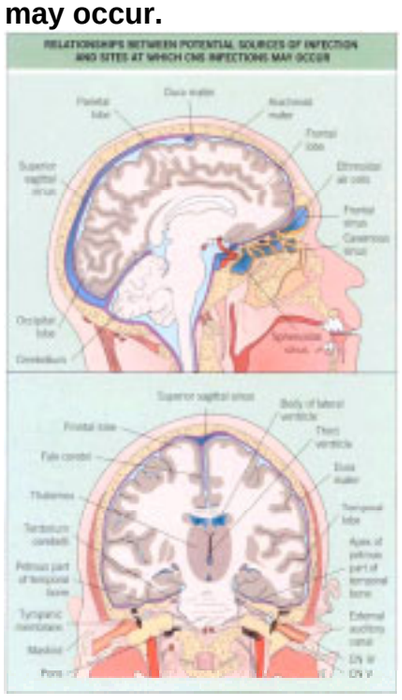


Figure 24-2 Contrast-enhanced CT scan of the head in the coronal projection of a 43-year-old man with an atrial septal defect that persisted after attempts at surgical repair. The patient presented with seizures after undergoing dental work for which he did not receive antimicrobial prophylaxis. Note the ring-enhancing lesion in the right frontoparietal region with edema and mass effect.

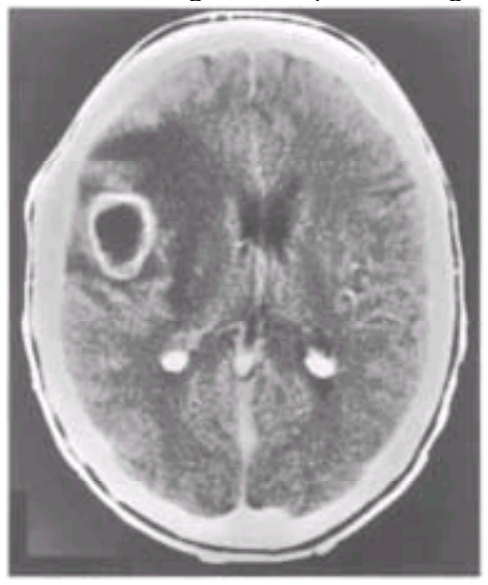


Figure 24-3 Contrast-enhanced CT and MRI scans of the head in the coronal projection of a 43-year-old woman with headaches after a recent fall on her head. (a) CT scan image reveals a cystic ring-enhancing lesion in the left cerebellum. Note the prominent bone artefact. (b) T1-weighted MRI scan image reveals an enhancing cystic lesion in the left cerebellum with significant surrounding edema. Bone artefact is absent. Both CT and MRI scans were felt to be most consistent with a primary or metastatic neoplasm, but culture of material obtained at stereotactically guided aspiration grew *Staphylococcus aureus*.

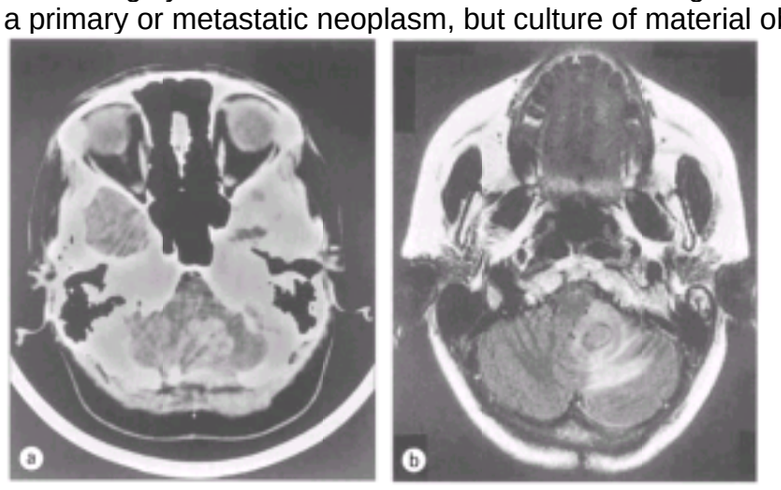


Figure 24-4 Contrast-enhanced CT scans of the head in the coronal projection of a 66-year-old woman with a group B streptococcal brain abscess demonstrating evolution of the abscess during and after surgical and antimicrobial therapy. (a) The original scan demonstrates a hypodense necrotic center surrounded by an enhancing capsule and hypodense edema. (b) Seven weeks later, after stereotactically guided aspiration and a full course of antimicrobial therapy, the central cavity can no longer be seen, although the enhancement and surrounding edema persist to a small degree.

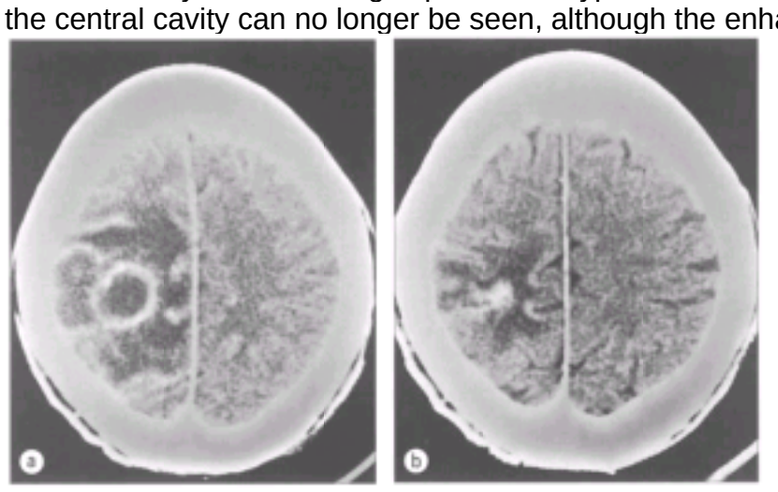


Figure 24-5 Contrast-enhanced CT scan of the head in the coronal projection of a 23-year-old man with fever and headache. There is a small isodense extra-axial fluid collection in the subdural space on the right, with significant mass effect shown by right-to-left midline shift and effacement of the right lateral ventricle. There was also opacification of the frontal and ethmoid sinuses, suggesting sinusitis as the source of this subdural empyema.

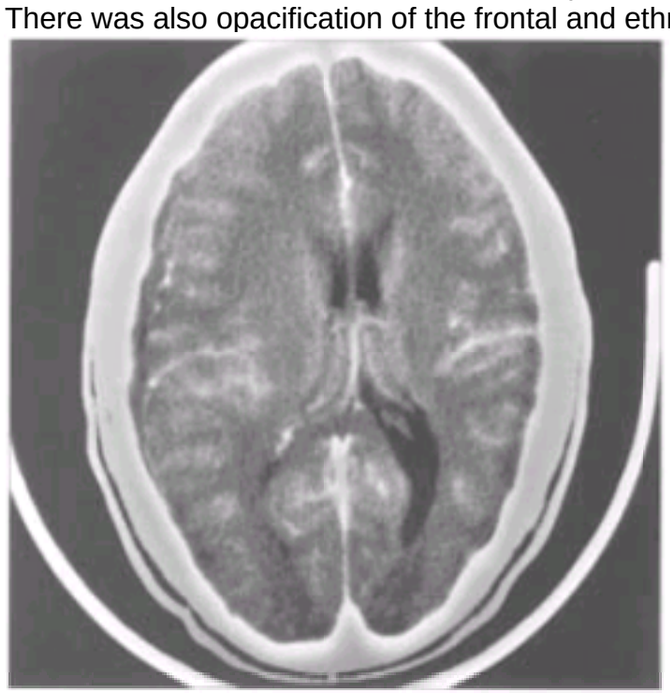


Figure 24-6 Contrast-enhanced CT scan of the head in the coronal projection of a 19-year-old man with otitis media who presented with sinus congestion 1 week earlier. Plain films of the sinuses revealed opacification of the right maxillary and ethmoidal sinuses and an intracranial air-fluid level. Note the intracranial gas in the right frontal region abutting a hypodense region in the epidural space with ring enhancement and surrounding edema, representing an intracranial epidural abscess.

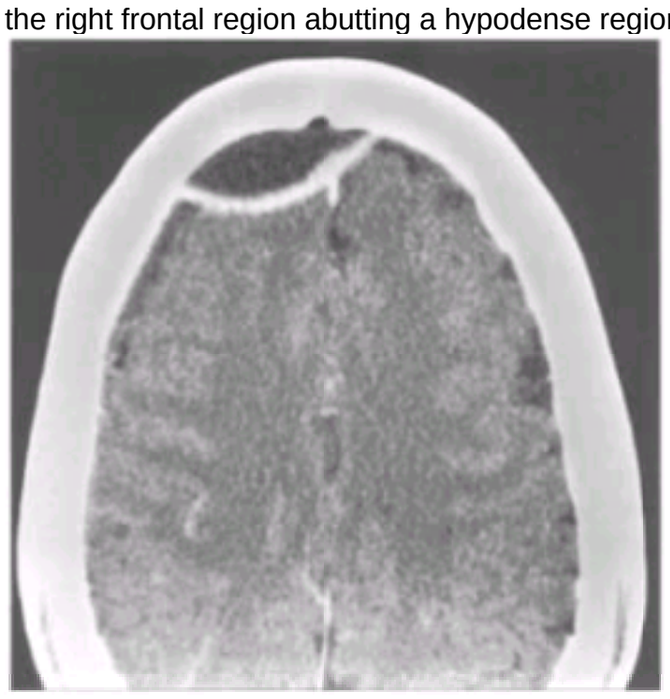


Figure 24-7 Contrast-enhanced MRI scan of the head in the sagittal projection of a 29-year-old man with sinus congestion and headache. There is non-uniform signal intensity of the cavernous venous sinuses, indicating cavernous sinus thrombosis. The sphenoid, ethmoidal and maxillary paranasal sinuses also demonstrated abnormal signal intensity.

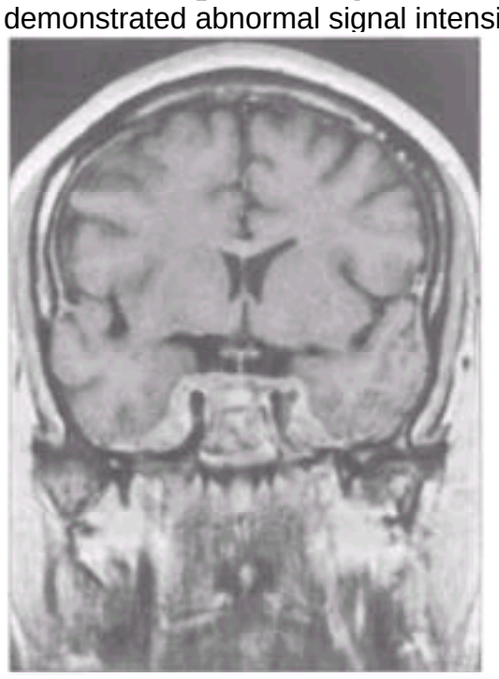


Figure 24-8 Contrast-enhanced MRI scan of the spine in the coronal projection of a 28-year-old man with a 1-week history of headache, fever and sweats. Physical examination demonstrated meningismus but no focal neurologic deficits. Scans in the sagittal section demonstrated a substance nearly isointense with the spinal cord and running nearly the length of the cord. This scan clearly demonstrates impingement and anterior displacement of the cord by the spinal epidural abscess.

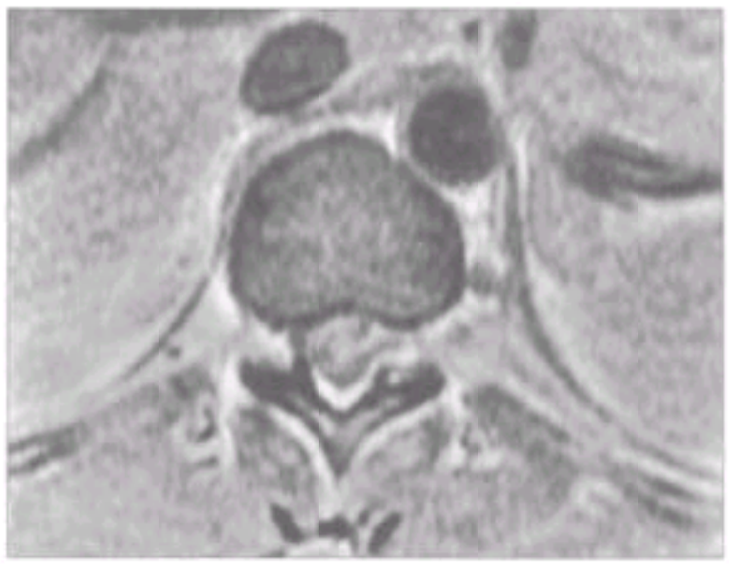


Figure 25-1 Reported number of tetanus cases in the USA (1947–97).

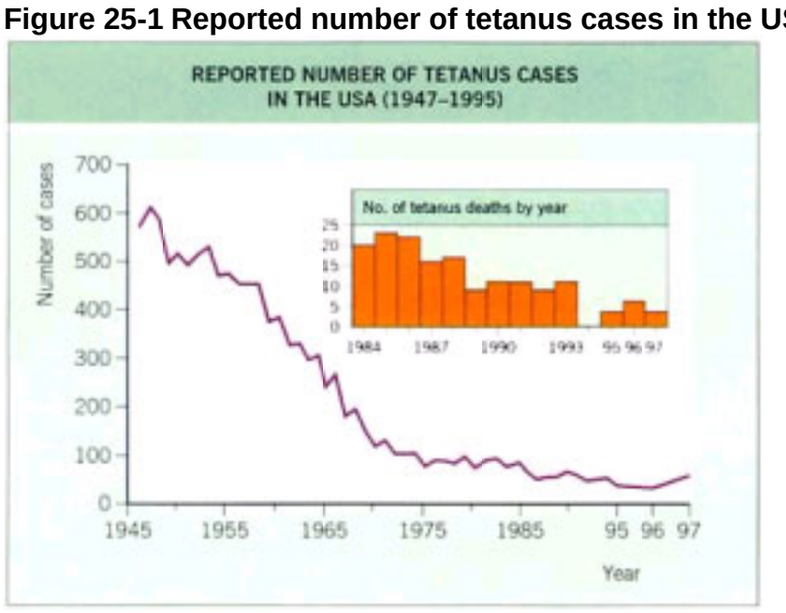


Figure 25-2 Sites of action of tetanospasmin. Tetanospasmin (TS) is produced by *Clostridium tetani* at the site of the wound and binds and internalizes at the neuromuscular junction into the a motor neuron. It then travels by retrograde axonal flow to the cell body and diffuses out into the synapses and extracellular space of the CNS. It enters other neurons and travels further into the CNS. Its major effect is to inhibit transmitter release from the glycinergic presynaptic inhibitory neuron but it can also inhibit release of transmitters at the excitatory synapses and of acetylcholine at the neuromuscular junction.

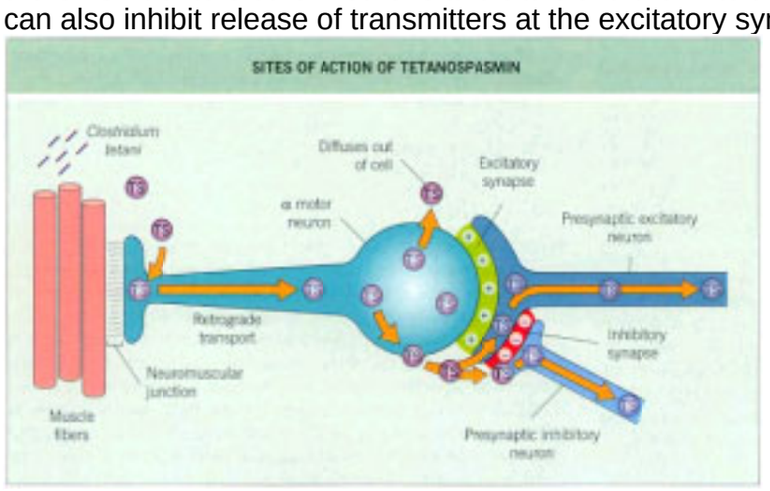


Figure 25-3 Facial spasm and risus sardonicus in a Filipino patient who has tetanus.

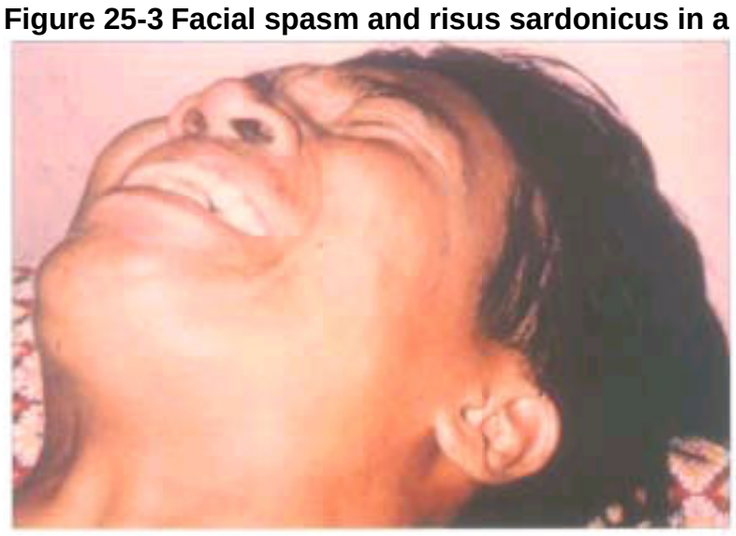


Figure 25-4 Botulinum toxin in its derivative and activated forms.

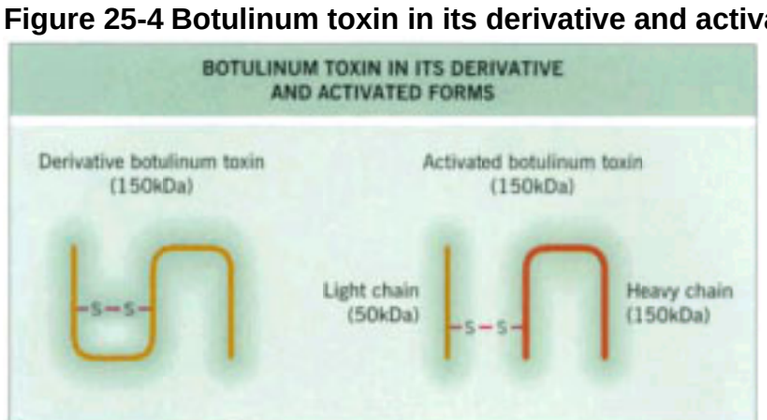


Figure 25-5 Action of diphtheria toxin. The binding subunit attaches to the cell surface and the toxin enters the cell. After endocytosis the toxin is cleaved and the active A subunit is released. This then catalyzes the cleavage of nicotinamide adenine dinucleotide (NAD) and the transfer of adenine diphosphate ribose (ADPR) to EF-2. EF-2 is essential for ribosomal reactions at the acceptor and donor sites, whereby the mRNA code is transferred via tRNA to an amino acid sequence and the building of a polypeptide chain. EF-2-ADPR is incapable of adding amino acids to a polypeptide chain and protein synthesis is stopped.

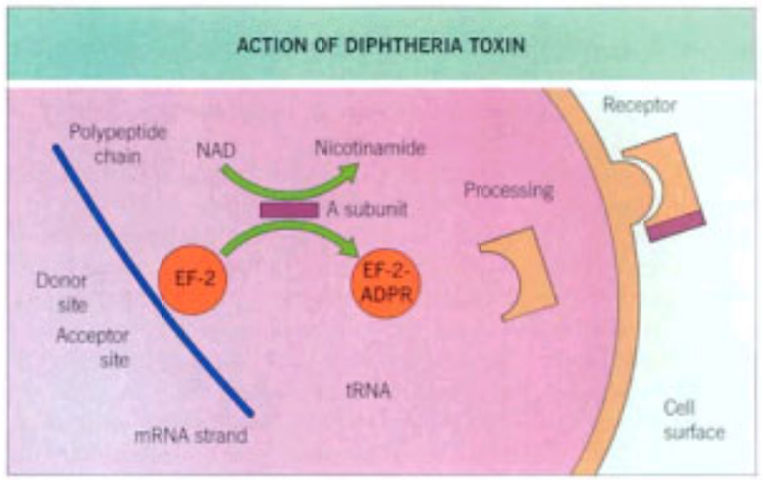


Figure 26-1 Structure of the human prion protein. Model of glycosylated human prion protein indicating positions of *N*-linked glycans (blue), the single disulfide bond linking helixes 2 and 3, and the glycosylphosphatidylinositol (GPI) anchor to the outer surface of the cell membrane. *Courtesy of Dr Richard Sessions and Mr Ray Young.*

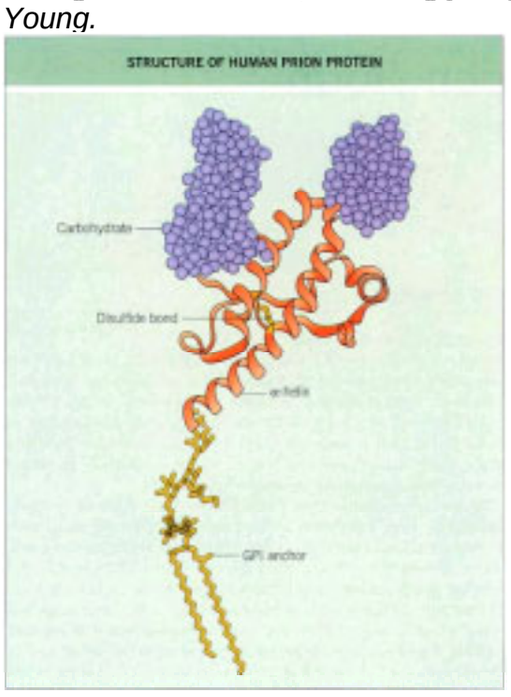


Figure 26-2 Prion propagation. Schematic representation of a possible mechanism for prion propagation. The predominantly a-helical form of the normal prion protein (PrP^{C}) proceeds via an unfolded state (a) to refold into a predominantly ß-sheet form (ß-PrP) (b). Prion replication may require a critical 'seed' size. Further recruitment of ß-PrP (c) or unfolded PrP (d) occurs as an essentially irreversible process. *Courtesy of Prof Tony Clarke and Mr Ray Young.*

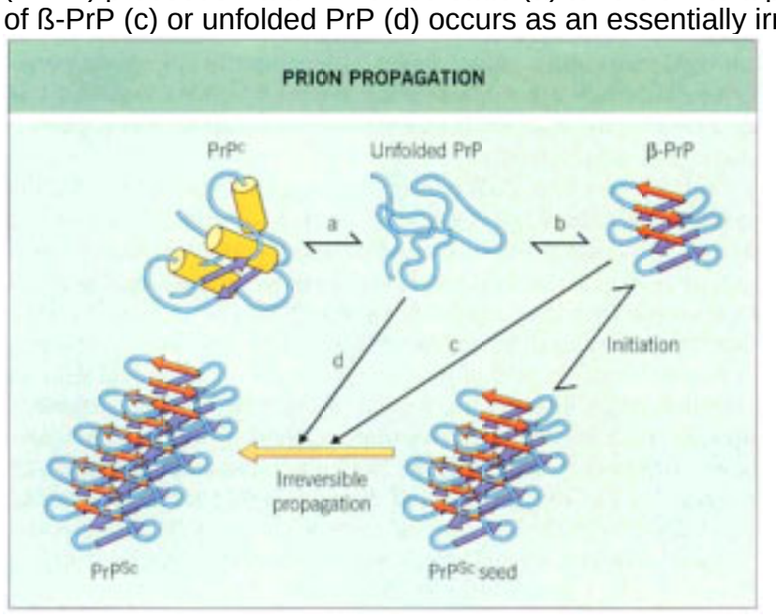


Figure 26-3 Pathogenic mutations and polymorphisms in the human prion protein. The pathogenic mutations associated with human prion disease are shown above the PrP coding sequence. These consist of 1, 2 or 4–9 octapeptide repeat insertions within the octarepeat region between codons 51 and 91, a deletion of 2 octapeptide repeats; and various point mutations causing missense amino acid substitutions. Point mutations are designated by the wild-type amino acid preceding the codon number, followed by the mutant residue, using single-letter amino acid conventions. Polymorphic variants are shown below the PrP coding sequence. Deletion of one octapeptide repeat is not associated with disease.

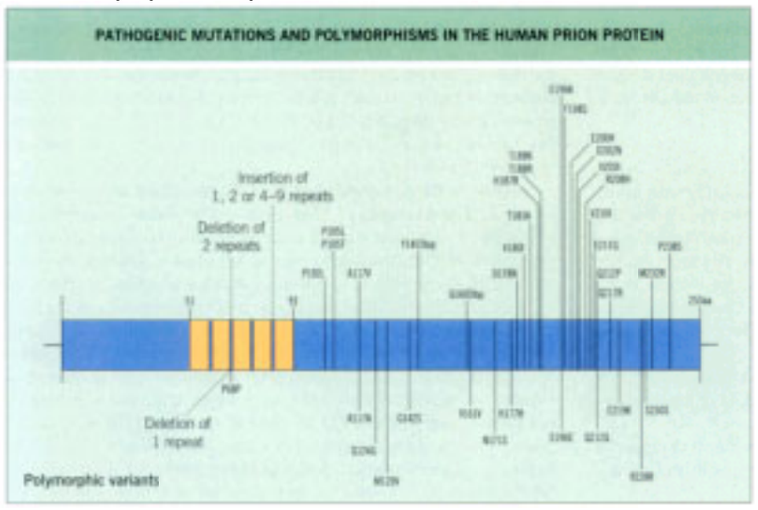


Figure 26-4 Axial T_2 -weighted MRI brain demonstrating high signal bilaterally in the posterior thalamus (arrowed) — the 'pulvinar sign' in a patient with vCJD.

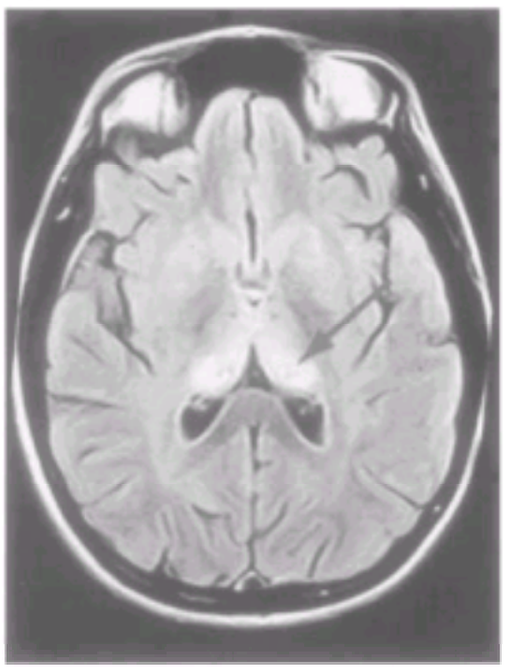


Figure 26-5 Western blot demonstrating the presence of PrP^{Sc} in brain and tonsil from a patient with vCJD. The presence of PrP^{Sc} is revealed after proteinase K (PK) treatment, which digests the normal form of PrP (PrP^{C}) but not the pathological form (PrP^{Sc}). *Courtesy of Ms Susan Joiner and Dr Andy Hill.*

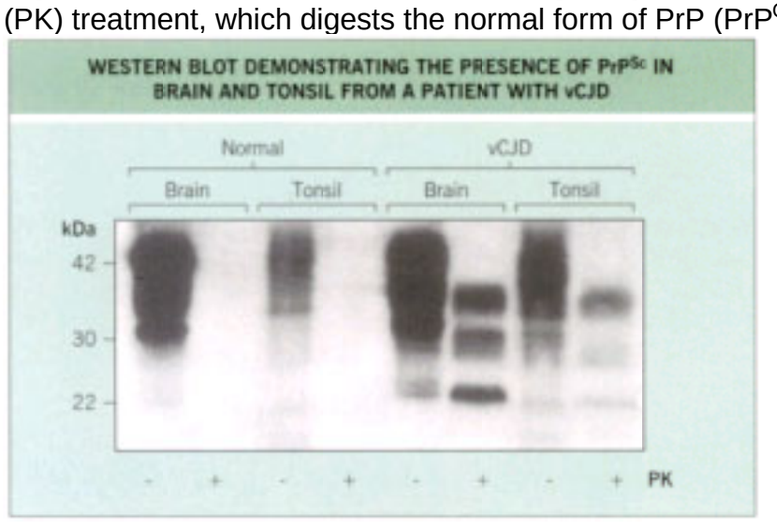


Figure 26-6 Neuropathology of prion disease — histopathological findings in vCJD. (a) Florid plaques, a characteristic feature of vCJD pathology. They consist of a round amyloid core (arrowed) surrounded by a ring of vacuoles (hematoxylin and eosin stain). (b) Spongiform degeneration in prion disease. This area shows severe vacuolization (spongiosis), there is severe neuronal loss and many strongly reactive astrocytes (arrowed) (hematoxylin and eosin stain). (c) Immunostaining of the pathological prion protein. The specimen is pretreated to denature the normal PrP and staining with a prion protein antibody reveals the presence of plaques (P) and synapses (S) staining positively for PrP^{Sc} . (d) Detection of pathological prion protein in the follicular dendritic cells in a tonsil. Accumulation of prion protein in lymphoreticular organs, such as spleen, tonsils or appendix, is a specific finding in vCJD and is not present in other forms of CJD. Therefore tonsillar biopsies can be used to specifically diagnose vCJD when clinical symptoms are only emerging. *Courtesy of Dr Sebastian Brandner.*

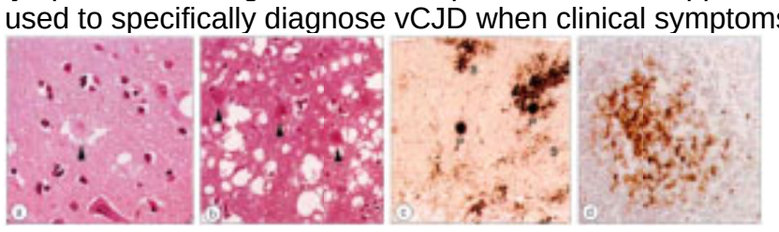


Figure 26-7 The tissue distribution of PrP^{Sc} in vCJD compared to classic CJD. In vCJD PrP^{Sc} is found in lymphoreticular tissue as well as brain and spinal cord. Using highly sensitive immunodetection methods, PrP^{Sc} has also been found in the optic nerve, retina, adrenal gland and rectum. *Courtesy of Dr Jonathon Wadsworth and Mr Ray Young.*

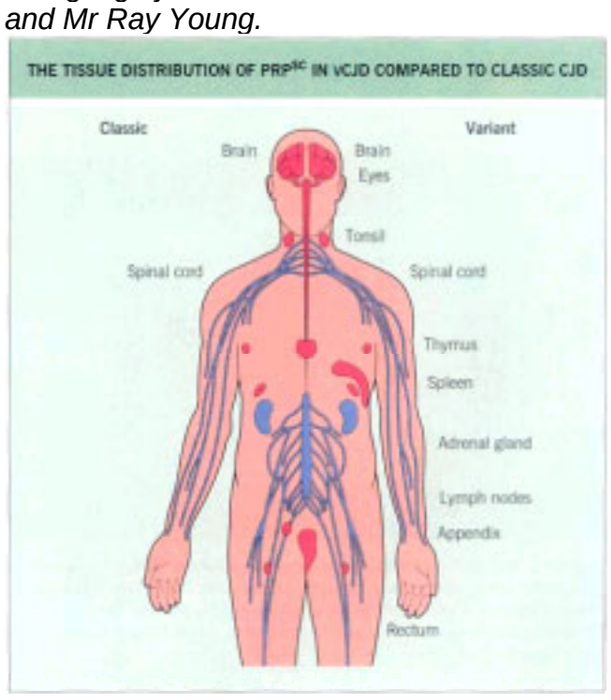


Figure 27-1 Postinfectious encephalomyelitis. (a, b) MRI of brain at presentation showing asymmetric demyelination of cortical white matter. Basal ganglia were also involved bilaterally (not shown). (b) At 7 weeks, the distribution is more symmetric. (c) Residual lesions at 4 months, with full clinical recovery.

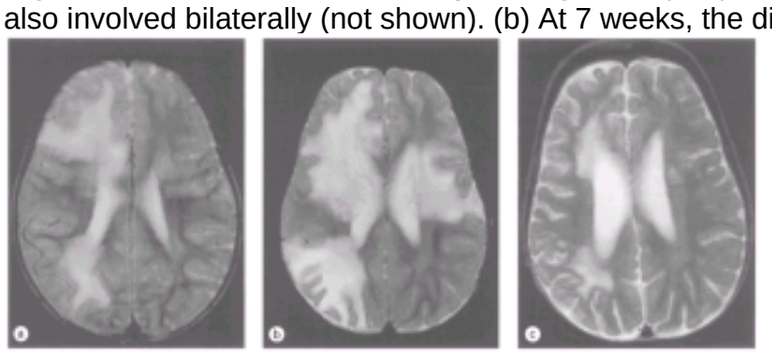


Figure 28-1 Routes of drainage of ventriculoperitoneal and ventriculoatrial shunts. Ventriculoperitoneal shunts drain CSF from the cerebral ventricles to the peritoneal cavity via catheter tubing implanted superficially over the rib cage. The lower end of the peritoneal catheter lies free in the abdomen. Ventriculoatrial shunts drain CSF via a convenient neck vein such as the jugular and the superior vena cava to the right atrium.

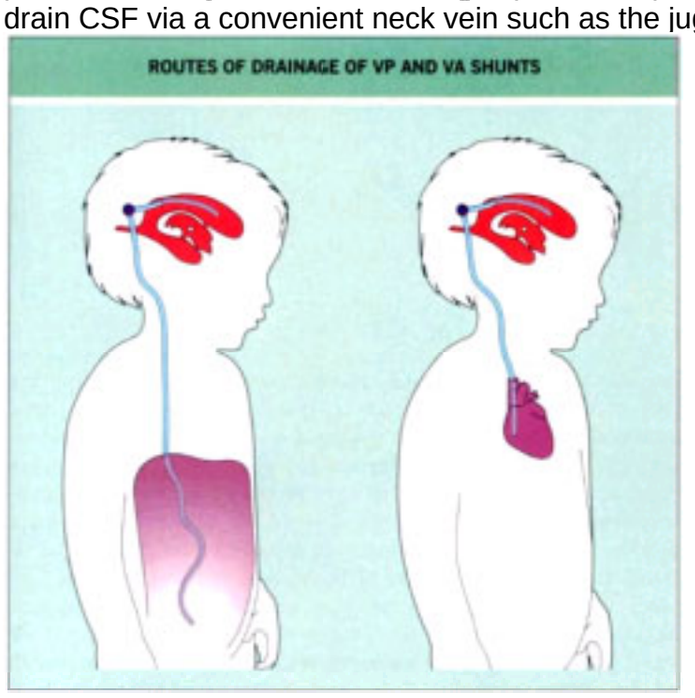


Figure 28-2 External shunt infection in a premature infant with poor nutritional status. The infection can be caused by organisms introduced at surgery or they may gain access through minor skin abrasions and pressure necrosis. Differing from the more common internal shunt infections, they are usually caused by *Staphylococcus aureus* and constitute a wound infection enhanced by a foreign material.

Figure 28-3 Cystic obstruction of a ventriculoperitoneal shunt caused by shunt infection with *Staphylococcus epidermidis.* Bacteria and bacterial products entering the peritoneal cavity via the shunt catheter evoke an inflammatory response involving the greater omentum, which seals off the catheter outlet. The resulting cyst fills with CSF, giving rise to recurrence of the hydrocephalus. Cystic obstruction can occur from noninfective causes but unlike those cases caused by infection, which present within 6–9 months of surgery, they can arise at any time.

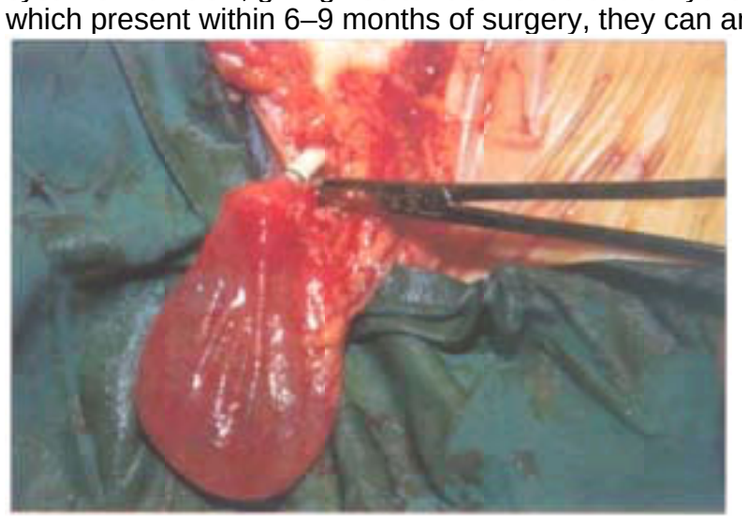


Figure 29-1 Global annual poliomyelitis cases reported to the World Health Organization 1974–99.

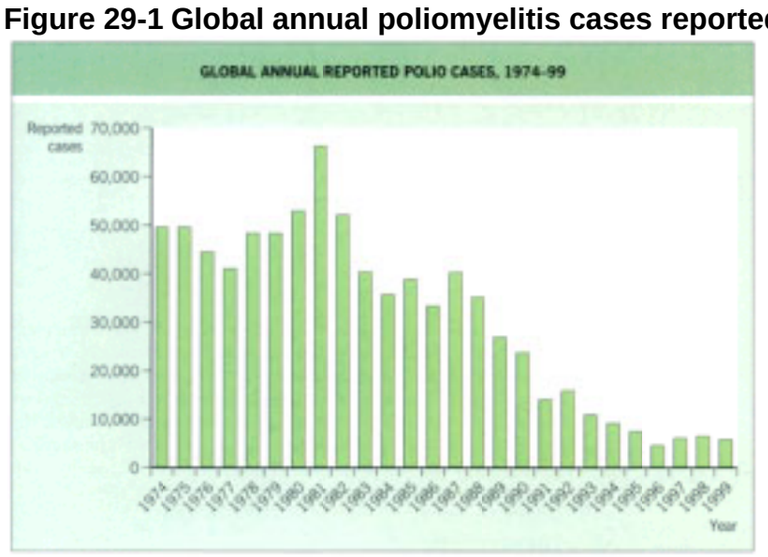


Figure 29-2 Total number of cases of paralytic poliomyelitis and vaccine-associated poliomyelitis in the USA 1960–98.

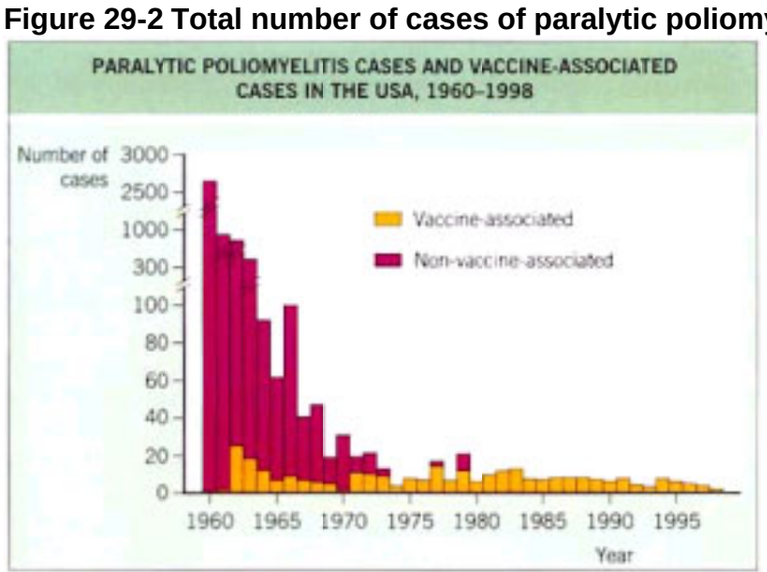


Figure 29-3 Anterior horn in poliomyelitis. Damaged and destroyed anterior horn neuron cell bodies are surrounded by an inflammatory infiltrate (hematoxylin & eosin stain).

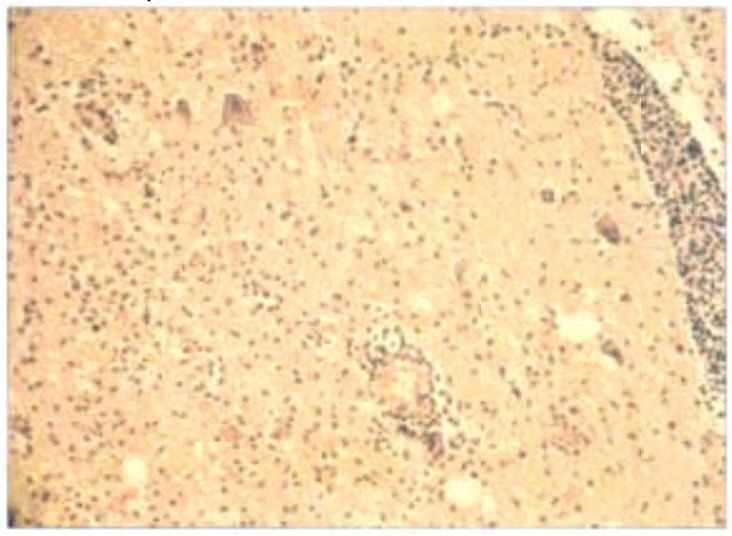


Figure 29-4 Incidence of herpes zoster at different ages.[12]

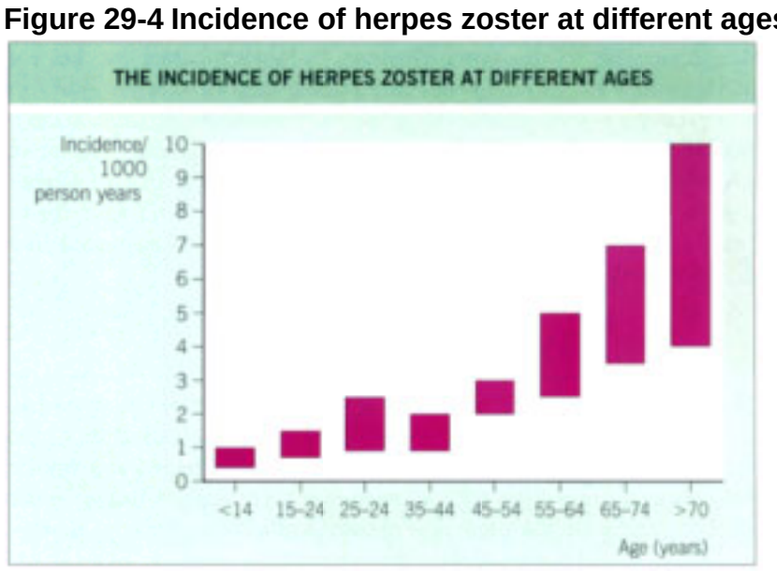


Figure 29-5 Typical dermatomal rash of herpes zoster.

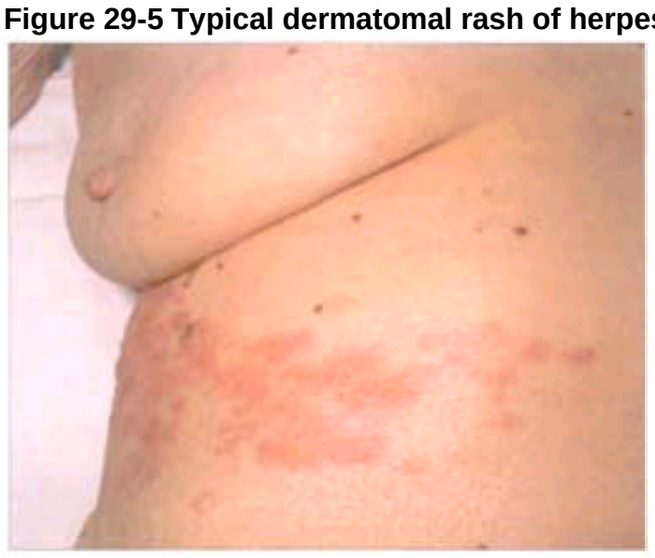


Figure 29-6 Hutchinson's sign. When the rash of herpes zoster involves the skin at the tip and side of the nose it indicates that the nasociliary branch of the trigeminal nerve is involved and there is an increased risk of uveal tract inflammation and ocular damage.

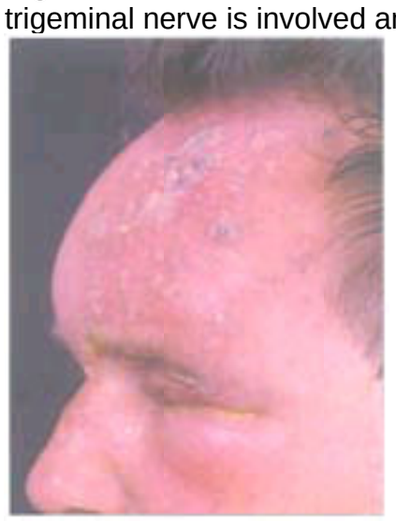


Figure 29-7 Life cycle of HTLV-I. HTLV-1 infection is initiated by cell-free virions or, more commonly, by cell-to-cell virus transmission. The two RNA genome copies are converted into double-stranded DNA provirus by the viral enzyme reverse transcriptase and the proviral DNA is integrated into the host chromosome. Transcription is activated by the viral Tax protein. In the early stages of infection both Tax and Rex proteins are produced. The Rex protein directs the preferential transport of unspliced or singly spliced viral messages to the cytoplasm for translation into structural proteins for virion assembly.

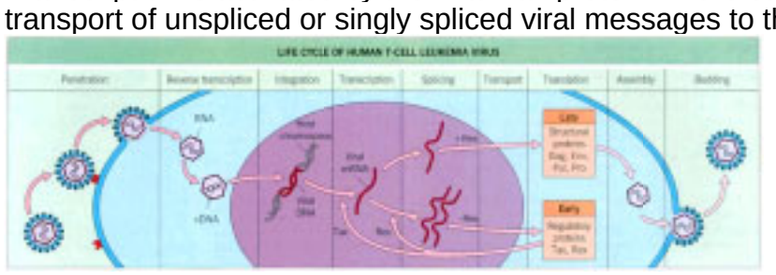


Figure 30.a-1 Cerebral abscess. Contrast-enhanced axial CT demonstrating two cerebral abscesses with surrounding edema and mass effect. Thin, smooth, enhancing capsules surround cavities of nonenhancing necrotic tissue. *Courtesy of Dr I Colquhoun.*

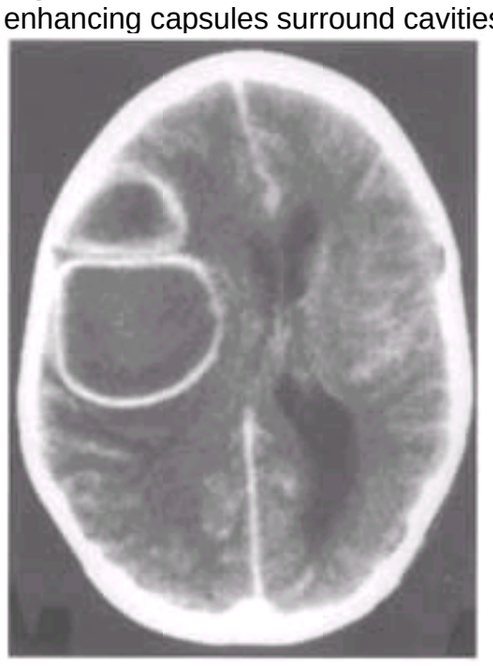


Figure 30.a-2 Extradural empyema. Contrast-enhanced T1-weighted coronal MRI demonstrating a lentiform extradural collection surrounded by enhancing dura mater. *Courtesy of Dr K Chong.*

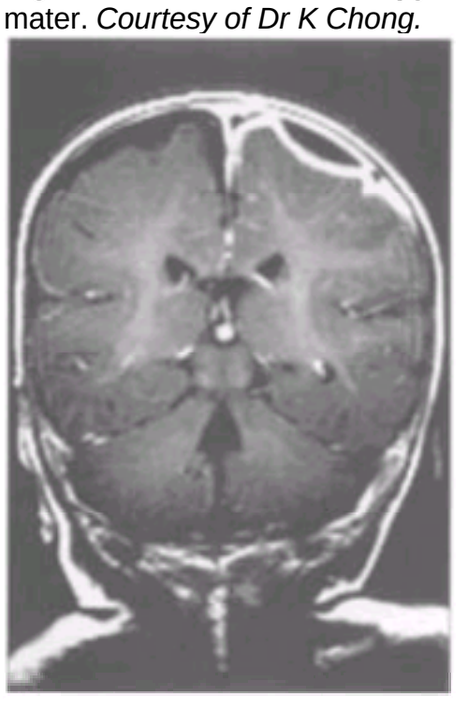


Figure 30.a-3 Herpes simplex encephalitis. T2-weighted axial MRI demonstrating characteristic involvement of medial temporal lobes with high-signal edema. *Courtesy of Dr K Chong.*

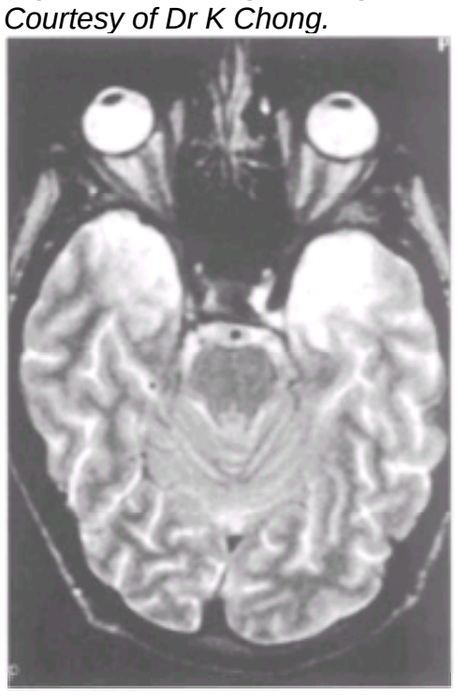


Figure 31-1 Incidence and median age of HIB disease. Disease type and geographic location per 100,000 children less than 5 years of age are shown.

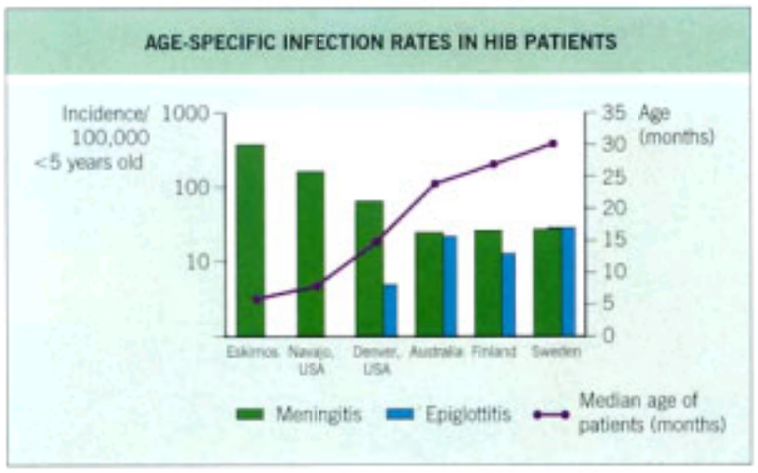


Figure 31-2 Epstein-Barr virus (mononucleosis or glandular fever) pharyngitis.

Figure 31-3 Adenoviral pharyngitis.

Figure 31-4 Adenopathy associated with EBV.

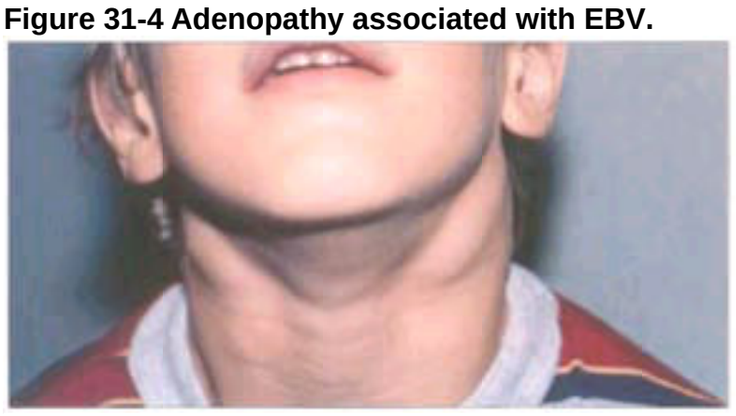


Figure 31-5 Herpes simplex virus stomatitis.

Figure 31-6 Pharyngitis associated with GAS infection. Exudates are not always present.

Figure 31-7 Scarlet fever. Skin rash and pharyngitis associated with GAS infection.

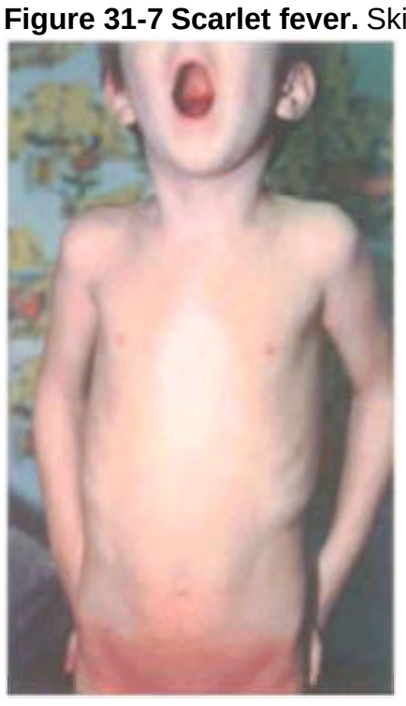


Figure 31-8 Facial rash associated with GAS infection.

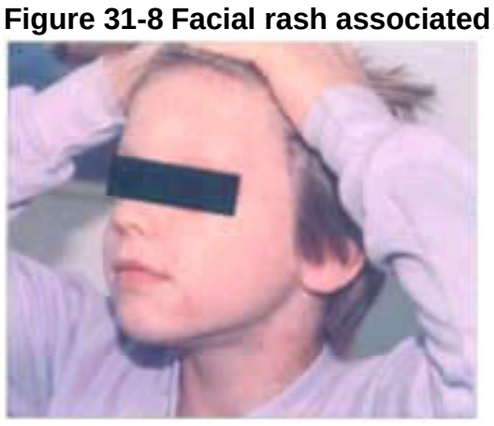


Figure 31-9 Child with epiglottitis. *Courtesy of Intensive Care Unit, Royal Children's Hospital.*

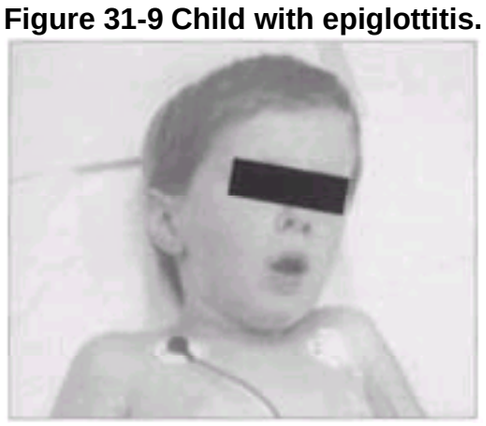


Figure 31-10 Acutely inflamed epiglottis associated with HIB. The epiglottis protrudes upwards and is cherry red from the bottom of the figure. *Courtesy of Intensive Care Unit, Royal Children's Hospital.*

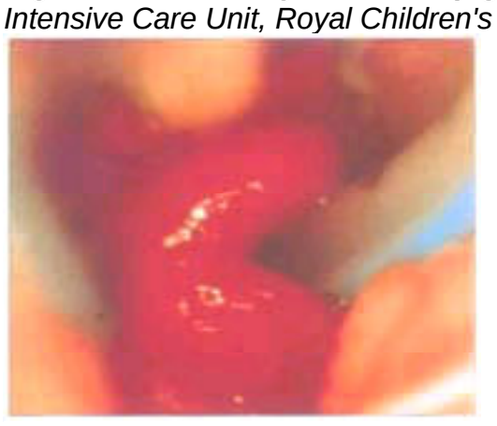


Figure 31-11 Lateral neck radiograph of a child with acute epiglottitis demonstrating an enlarged hypopharynx due to forward neck extension and an enlarged 'thumb-shaped' epiglottis (arrow). *Courtesy of Dr Donald Frush.*

Figure 32-1 Pathogenesis of acute otitis media.

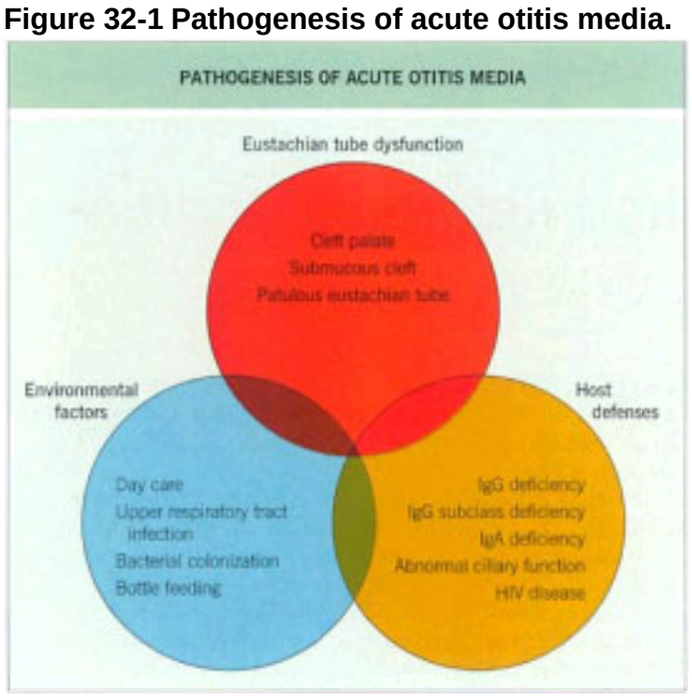


Figure 32-2 Microbiology of acute otitis media in the USA, eastern and central Europe and Israel. Culture results of middle ear aspirates from children. *Adapted from Jacobs et al.*[26]

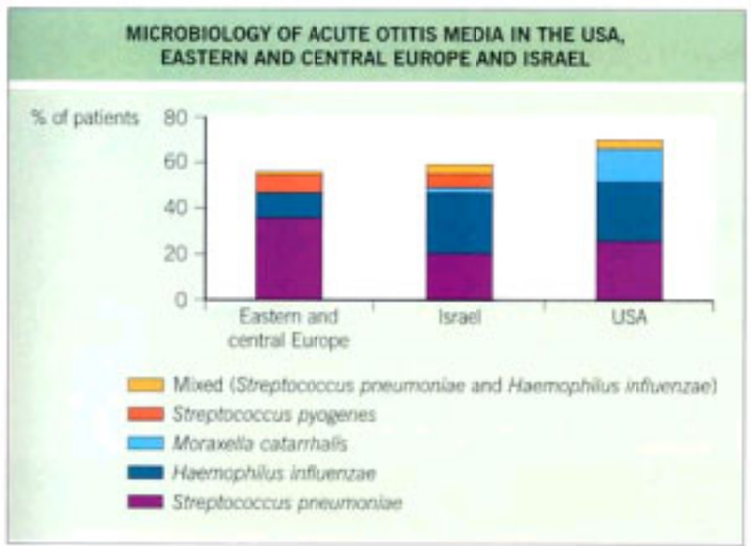


Figure 32-3 Resolution of middle ear effusion after acute otitis media. *Adapted from Klein et al.*[1]

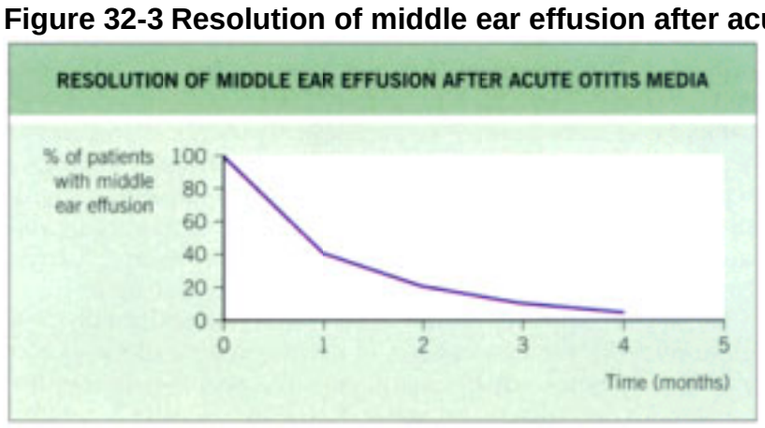


Figure 32-4 Acute sinusitis with facial swelling and periorbital edema.

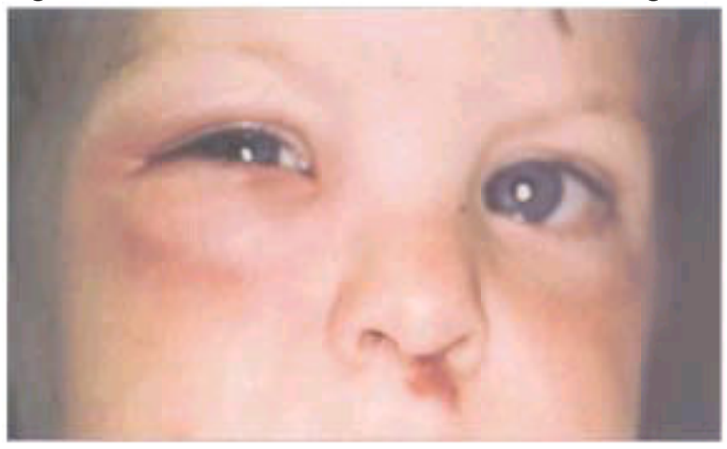


Figure 32-5 Left maxillary sinusitis.

Figure 32-6 Computerized tomography scan of a patient who has right subperiosteal abscess (arrow) adjacent to the lamina papyracea. Note the partial opacification in the right ethmoid sinus.

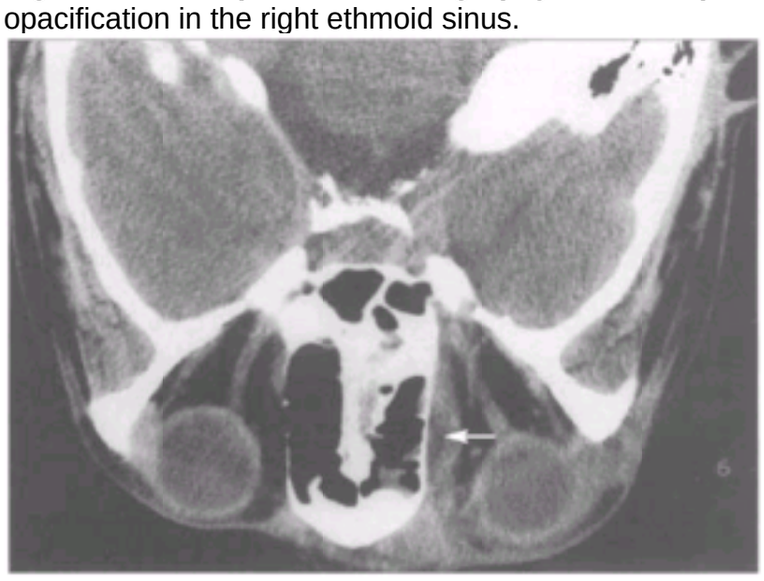


Figure 32-7 Predicted percentages of acute otitis media due to susceptible, intermediate and resistant *Streptococcus pneumoniae* in Boston, Massachusetts, USA.

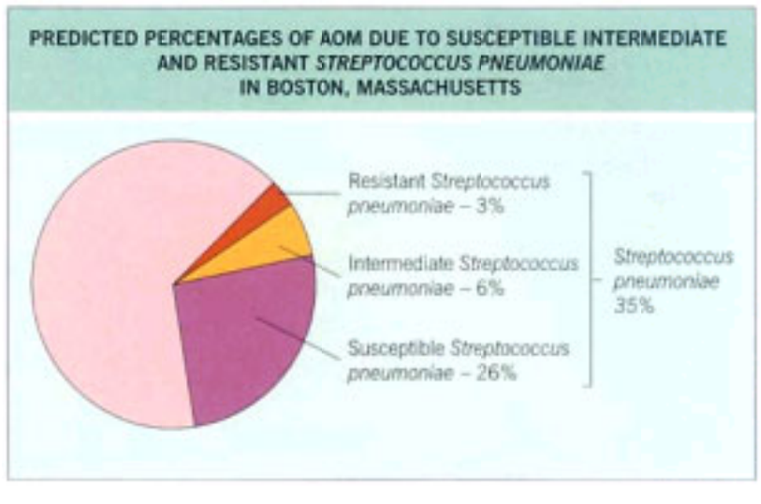


Figure 33-1 (a) Bronchial wall from normal patient. Normal pseudostratified columnar epithelium with few goblet cells overlies smooth muscle and a submucosal gland. Cartilage is at the bottom of the figure. H&E stain, 100× original magnification. (b) Bronchial wall from a patient with chronic bronchitis. Hyperplastic epithelium with mucous cell metaplasia overlies a hypertrophied submucosal gland. H&E stain, 100× original magnification.

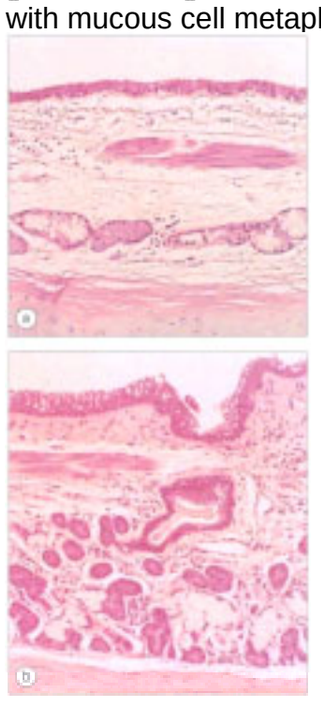


Figure 33-2 Median survival age of patients who have cystic fibrosis. The median survival age has increased dramatically. *Data from Cystic Fibrosis Foundation, Bethesda, MD.*

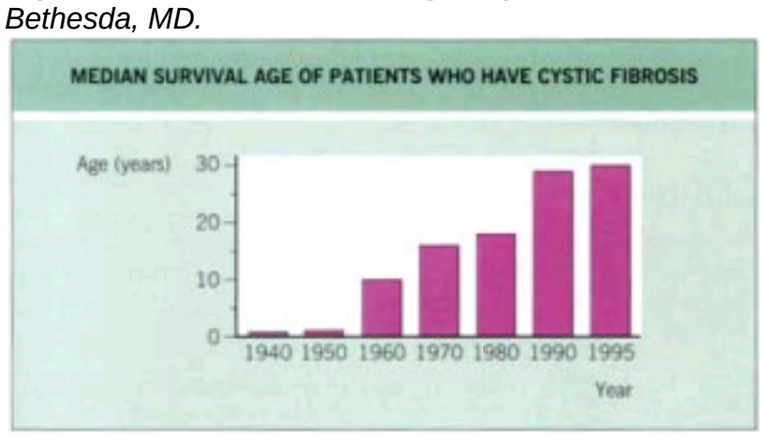


Figure 33-3 Representation of the CFTR model, based on structural, hydropathy and expression studies. The membrane-spanning domains are arranged in groups of six, each associated with a nucleotide-binding fold. These features are similar to those of the multidrug resistance 'P' glycoprotein. The 'R' domain is unique to CFTR.

Figure 33-4 CFTR mutations are divided into classes based on the mechanisms of dysfunction. See text for detailed descriptions. *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.*

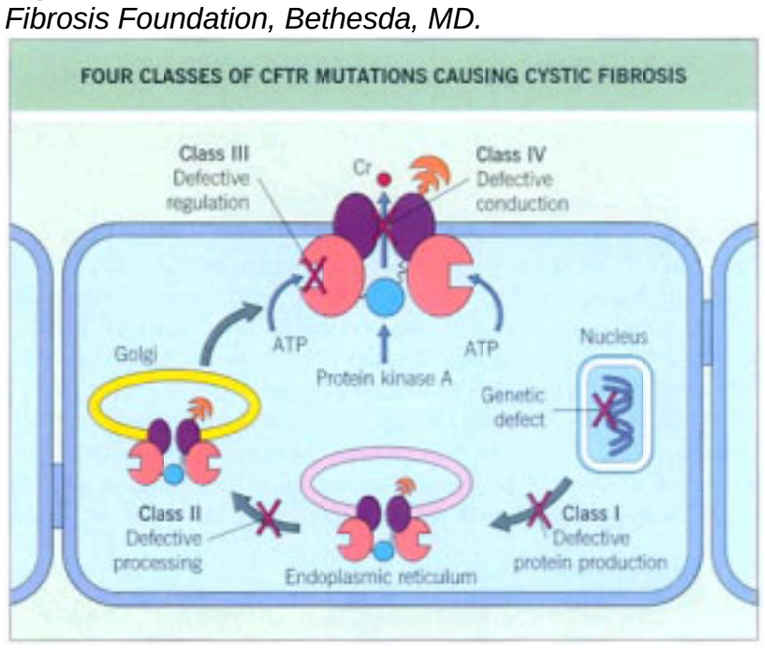


Figure 33-5 Age-specific infection rates in cystic fibrosis patients. Bacteria isolated from CF sputum samples vary with age and demonstrate the trend toward *Pseudomonas aeruginosa* as the dominant pathogen. *Figure provided by Cystic Fibrosis Foundation, Bethesda, MD.*

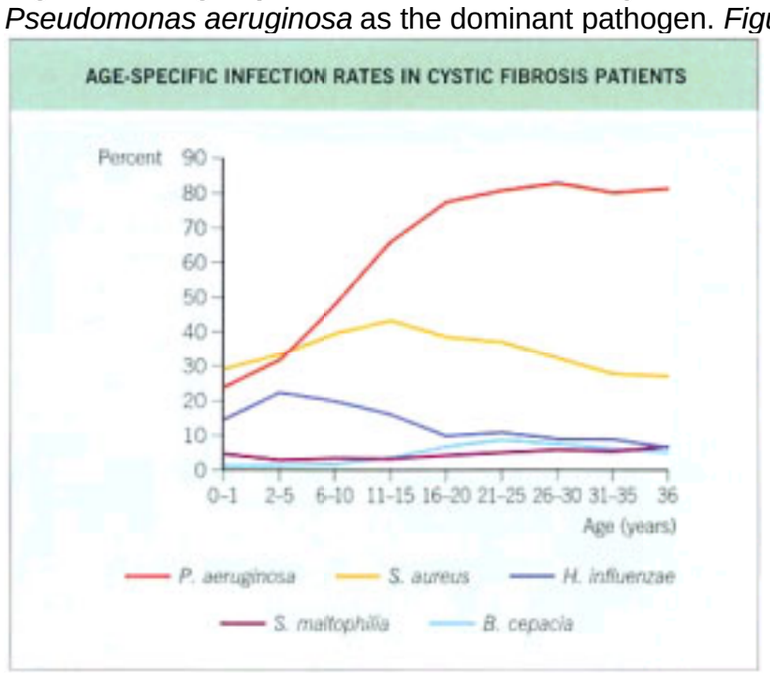


Figure 33-6 A CF bronchiole is completely occluded by mucoid secretions and surrounded by fibrotic tissue. H&E stain, original magnification 400X.

Figure 33-7 A CF submucosal gland demonstrates marked hypertrophy and dilated gland ducts with mucoid secretions. The surface epithelium has marked goblet cell metaplasia. H&E stain, original magnification 100X.

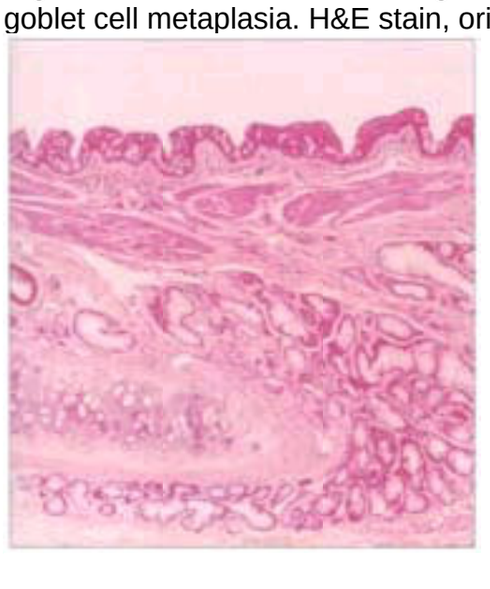


Figure 33-8 A typical postero-anterior chest X-ray of a 24-year-old man with CF. The lungs are hyperinflated due to airway obstruction and bronchiectasis. The right upper lobe atelectasis is chronic.

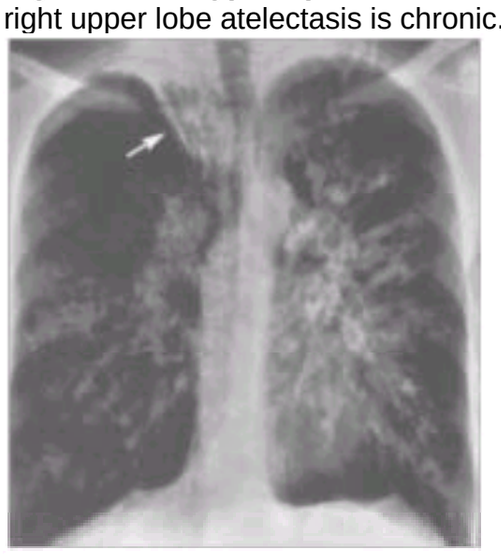


Figure 33-9 Typical clinical course in CF. Serial pulmonary function measurements (FEV_1) demonstrate a typical clinical course. Measurements are connected by solid lines during therapy for acute exacerbations. The lower bars indicate periods of hospital treatment. Pulmonary function decreases and the exacerbations are more frequent and less responsive to treatment in advanced disease.

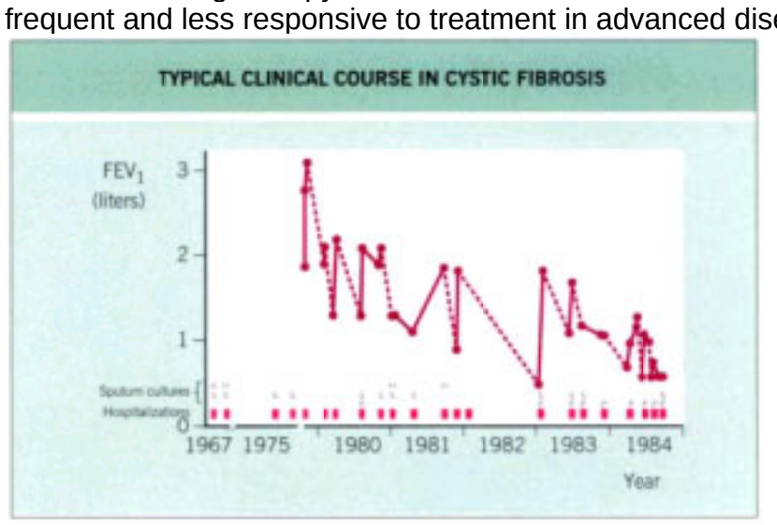


Figure 34-1 Seasonal pattern of respiratory pathogens in the northern hemisphere. Most respiratory pathogens are commoner in the winter months with the notable exceptions of *Legionella* spp. and *Coxiella burnetii* (Q fever). *With permission from Macfarlane J. Community-acquired pneumonia. Br J Dis Chest 1987;81:116–27.*

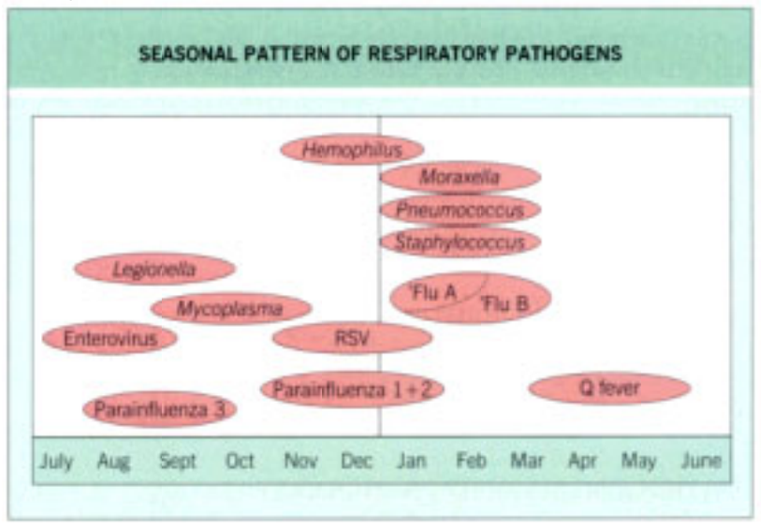


Figure 34-2 Multiple discrete areas of consolidation with abscess formation is a classic feature of *Staphylococcus aureus* pneumonia. *With permission from Macfarlane JT, Finch RG, Colton RE. A colour atlas of respiratory infections. London: Chapman & Hall; 1993.*

Figure 34-3 Rate of resolution of radiographic pulmonary shadows after CAP. Pneumonia can take 4–8 weeks to clear radiographically even in less severe cases such as *Mycoplasma* pneumonia. More severe pneumonias such as *Legionella* and bacteremic pneumococcal pneumonia take longer to resolve, sometimes up to 6 months. *Data from Macfarlane et al.*[30]

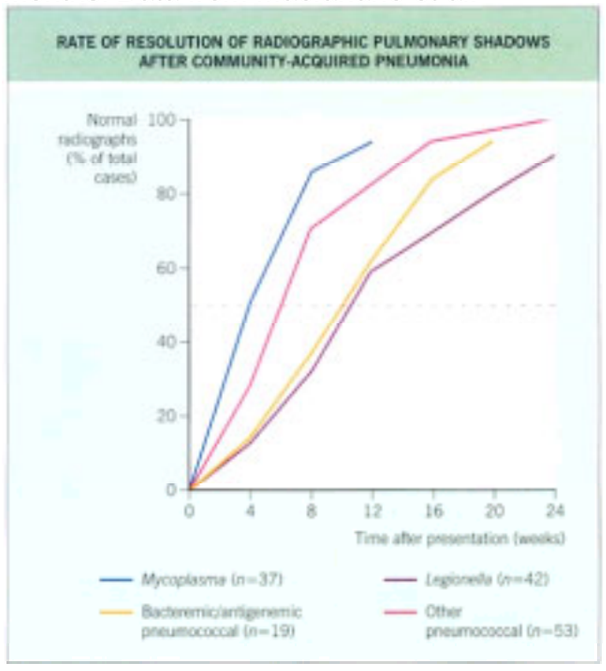


Figure 34-4 Algorithm for the management of CAP. *Adapted from the Nottingham City Hospital CAP Guidelines, 1996.*

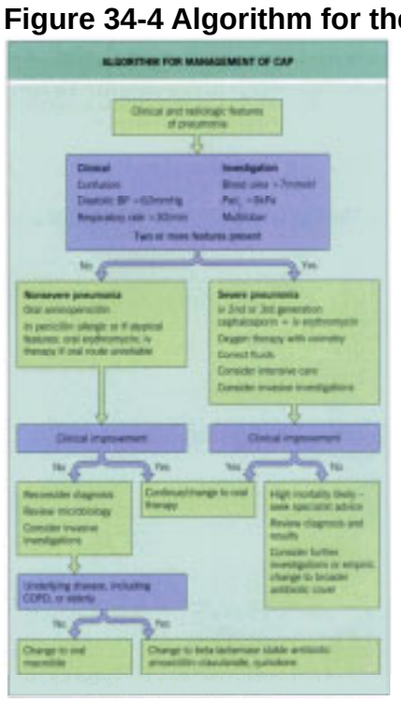


Figure 34-5 Chest radiograph showing a right sided lobar pneumonia with right empyema in a 25-year-old man who was training for the British rowing team. Prompt treatment with intercostal drainage ensured that he recovered without loss of lung function.

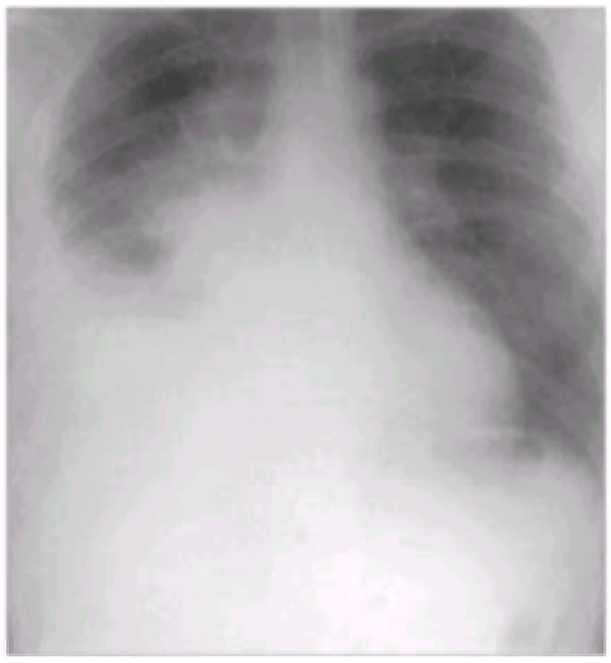


Figure 35-1 Factors involved in the pathogenesis of hospital-acquired pneumonia. *Reproduced from Macfarlane JT. Pneumonia. Medicine International 1986; 3 by kind permission of the Medicine Publishing Company.*

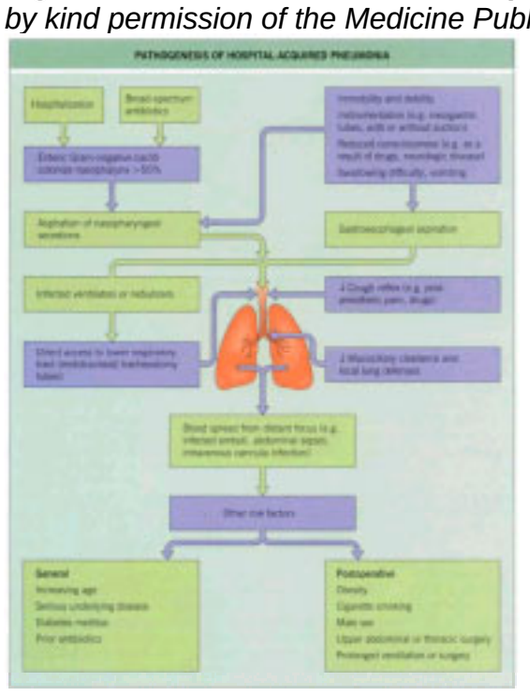


Figure 35-2 Algorithm for classifying patients who have hospital-acquired pneumonia to provide a basis for empiric antibiotic management. *Adapted from Figure 1 in the American Thoracic Society consensus statement.*[1]

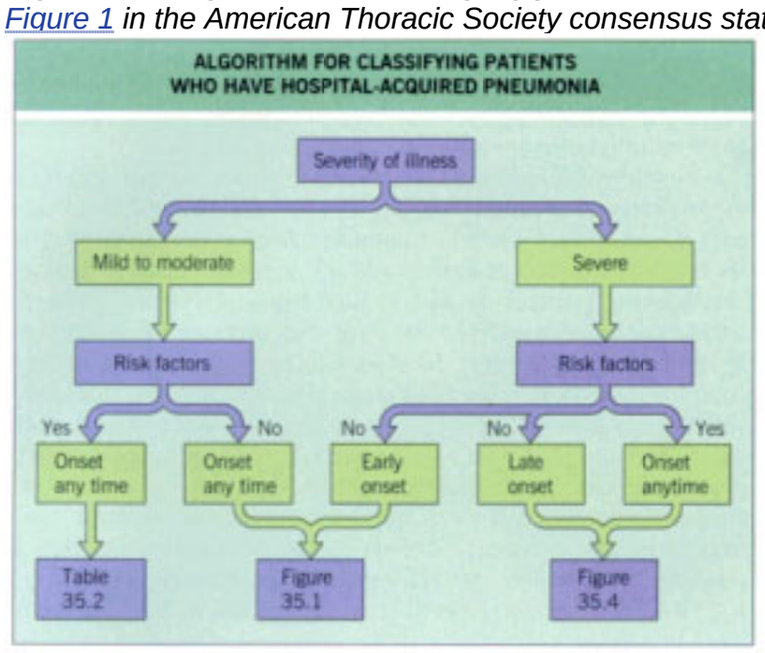


Figure 35-3 Pleural fluid, if present, should always be sampled in a patient who has pneumonia to assess etiology. In this case, purulent fluid was detected suggestive of an empyema. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

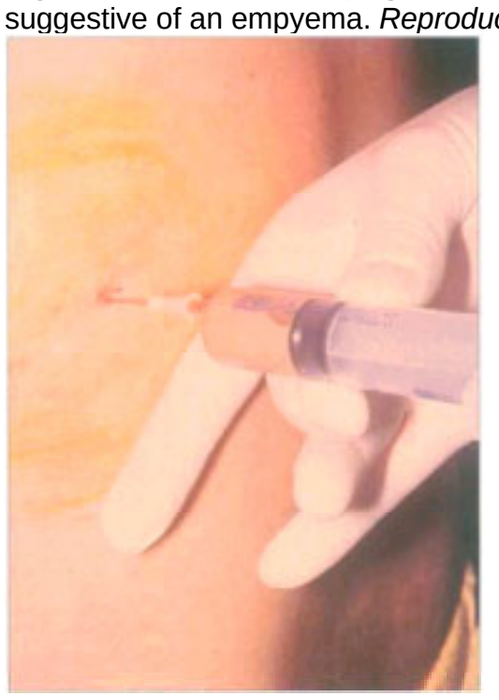


Figure 35-4 An extended protected specimen brush protruding from the end of a fiberoptic bronchoscope. Note the outer plastic sheath (arrow 1) and the inner protective yellow plastic cover (arrow 2) with the microbiologic brush pushed out (arrow 3). The protective gelatin plug occludes the end of the outer cover (arrow 4) until ejected before obtaining the specimen. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

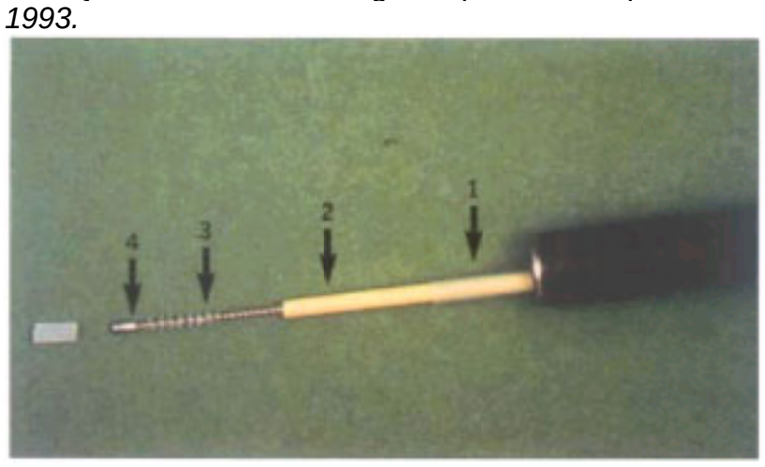


Figure 35-5 Limited bronchoalveolar lavage of right middle lobe using radiopaque solution. Note the alveolar filling pattern. *Reproduced from Macfarlane JT, Finch RG, Cotton RE. A Colour Atlas of Respiratory Infections. London: Chapman & Hall; 1993.*

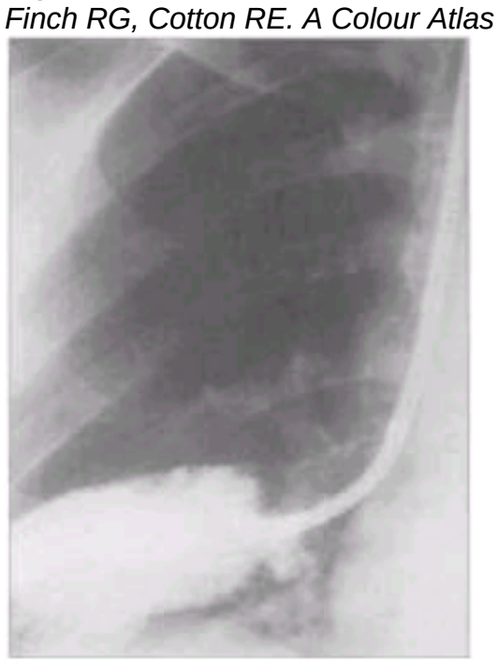


Figure 35-6 A diagnostic algorithm for the management of ventilator-associated pneumonia, depending on whether an empiric treatment approach or an investigation approach, (using quantitative culture of bronchoscopically or nonbronchoscopically obtained specimens), is adopted. *Adapted from Ioanas et al.[27] and Grossman et al.[40]*

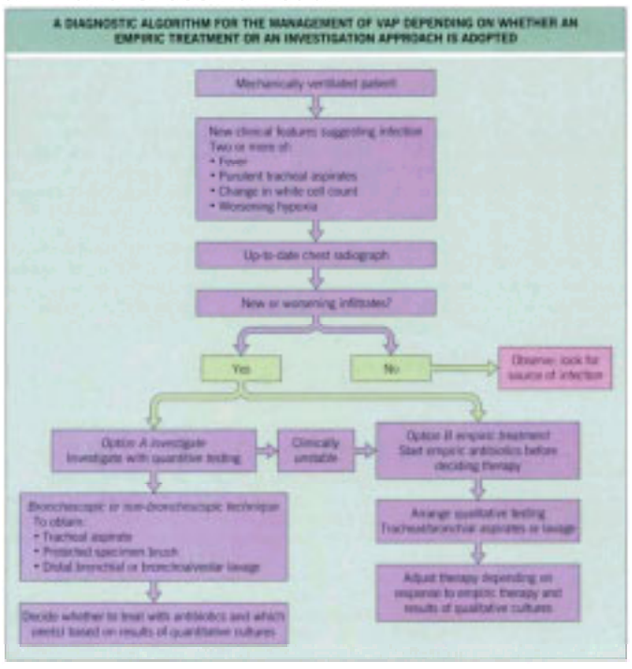


Figure 36-1 Causes of LRTIs in adults. Oropharyngeal streptococci and anaerobes, *Staphylococcus aureus*, Enterobacteriaceae, *Pseudomonas aeruginosa*, the dimorphic fungi (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*) and mycobacteria frequently cause necrosis and subsequent abscess formation.

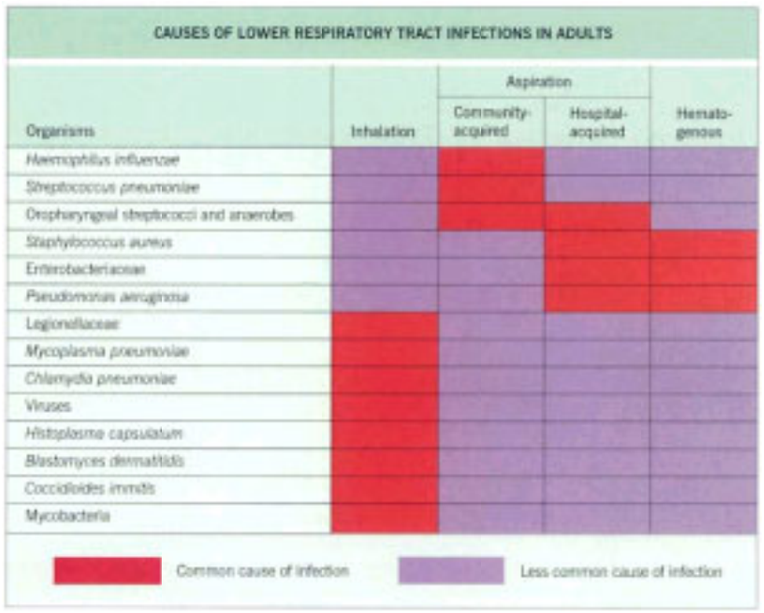


Figure 36-2 Cross-section of a lung abscess.

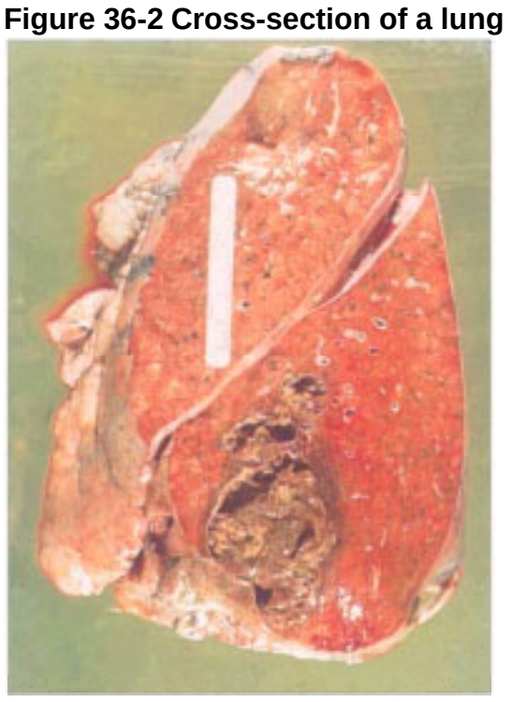


Figure 36-3 A lung abscess showing an air-fluid level.

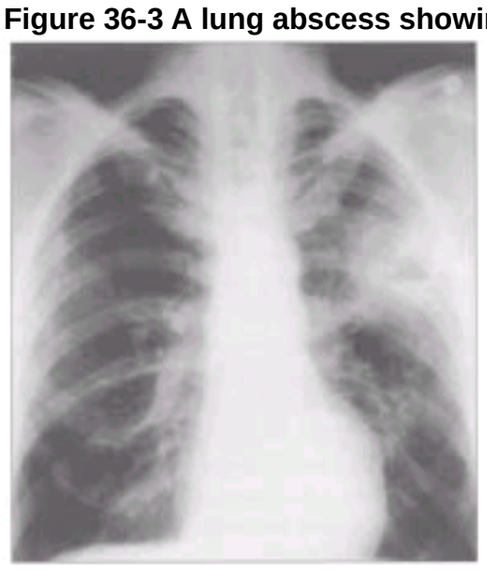


Figure 36-4 A lung abscess associated with a multinodular bronchogenic carcinoma.

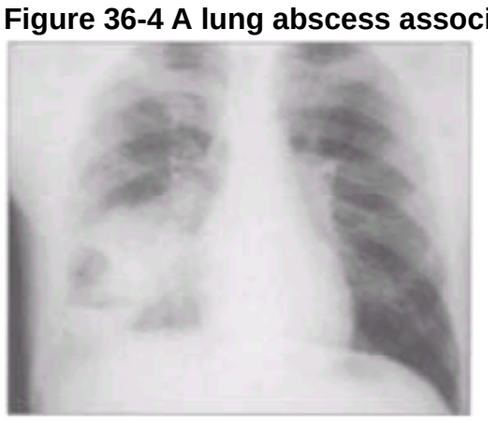


Figure 36-5 Computerized tomography scan of a lung abscess showing an air-fluid level.

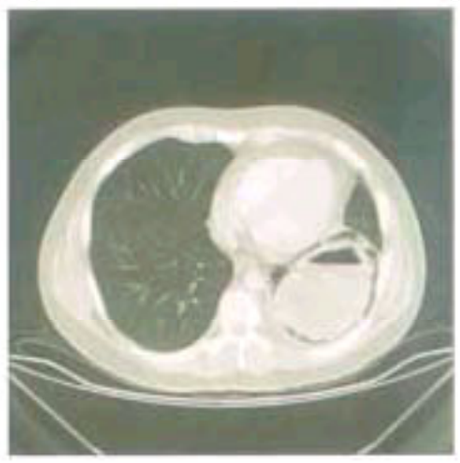


Figure 36-6 Gram stain of lower respiratory tract secretions. The patient had a lung abscess caused by oropharyngeal streptococci and anaerobes.

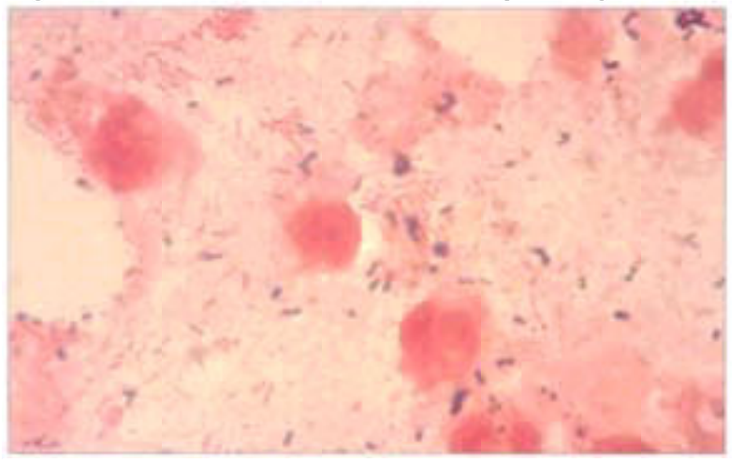


Figure 36-7 Antimicrobials for bacterial lung abscesses and empyemas. Information for *Streptococcus pneumoniae* is for penicillin-susceptible strains — selected cephalosporins or vancomycin should be used for resistant strains. Information for *Staphylococcus aureus* is for methicillin-susceptible strains — vancomycin should be used for resistant strains. Vancomycin is the drug of choice for ß-lactam-resistant Gram-positive organisms.

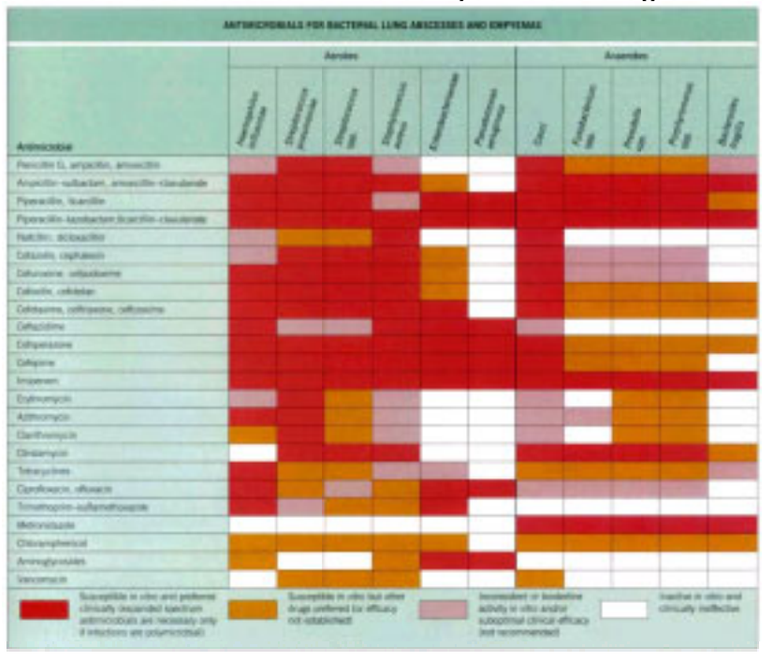


Figure 36-8 Duration of therapy for lung abscess. Response of patients who had lung abscess to penicillin G (17 patients) and clindamycin (16 patients).

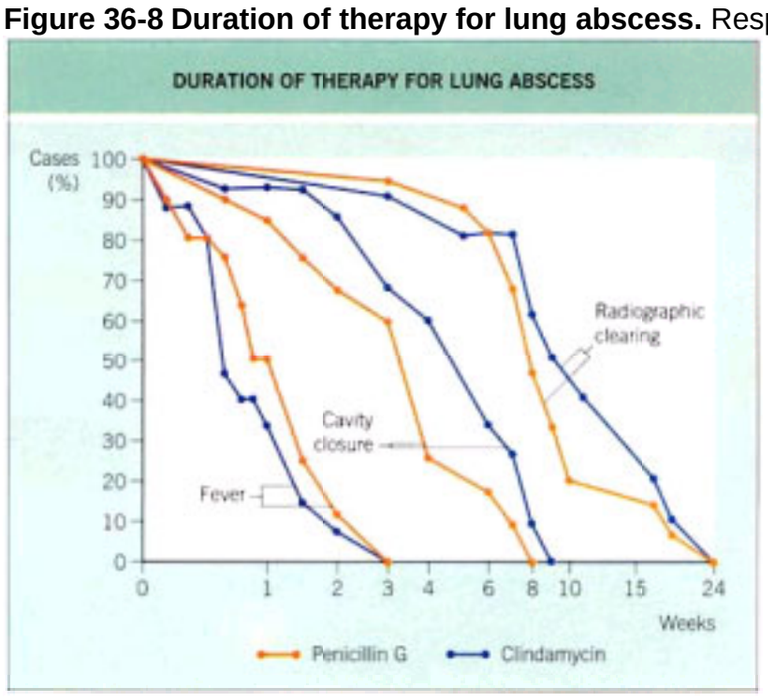


Figure 37-1 Estimated tuberculosis incidence rates worlwide. *Reproduced with permission from the World Health Organization.*

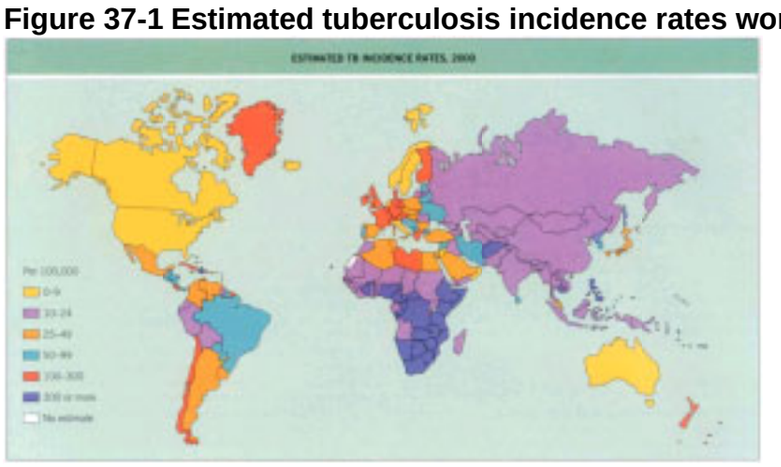


Figure 37-2 Estimated incidence rates of adults co-infected with TB and HIV. *Reproduced with permission from the World Health Organization.*[4]

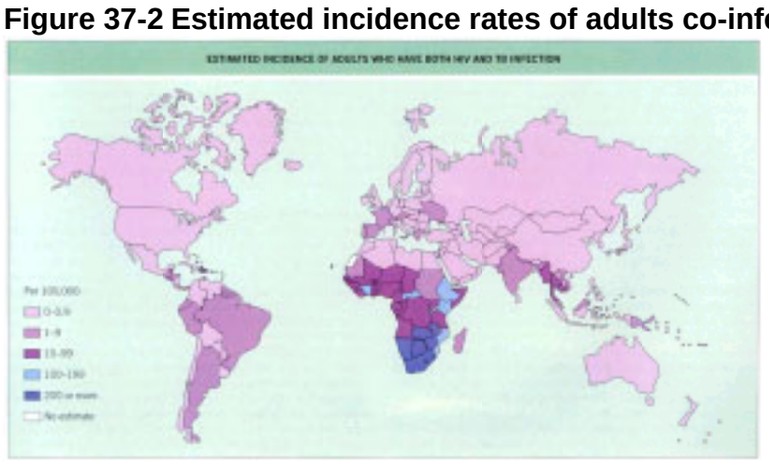


Figure 37-3 Estimate of the incidence of multidrug-resistant TB (MDR-TB) in newly diagnosed cases of infection. *Reproduced with permission from the World Health Organization.*[17]

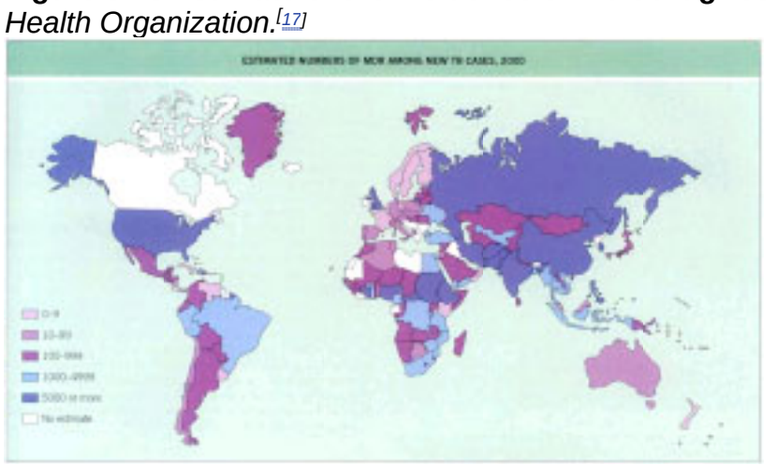


Figure 37-4 Detailed histology of the tuberculous granuloma. Monocytic cells and smaller T cells are shown together with multinucleate giant cells on the edge of an area of caseation.

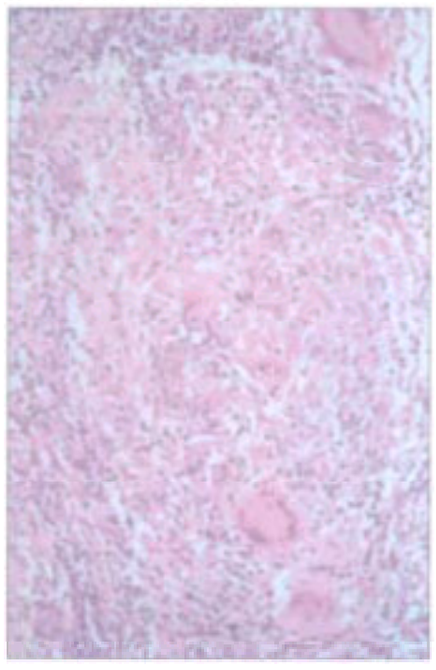


Figure 37-5 Phagocytosis of *Mycobacterium tuberculosis* by macrophages. Phagocytosis initiates many critical pathways involved in host defense to infection.

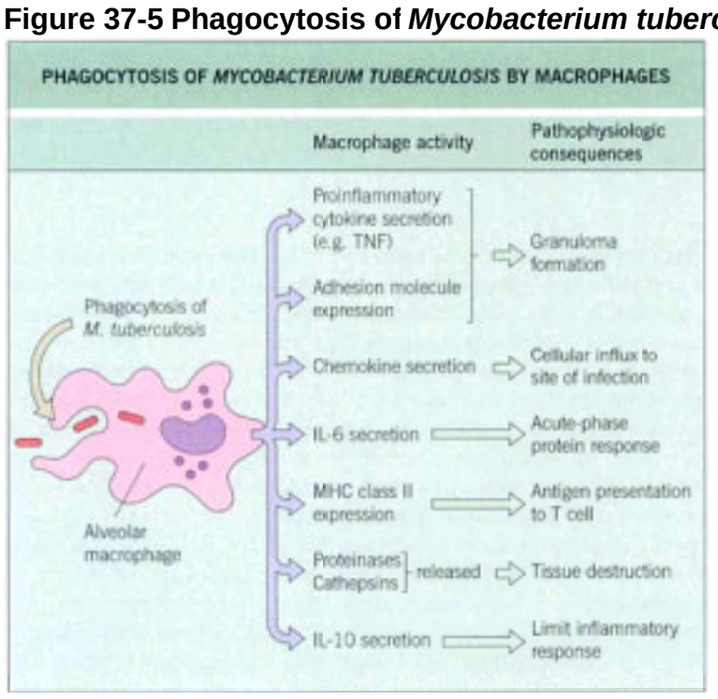


Figure 37-6 A Heaf test grade 2 response. *With permission from James DG, Studdy PR, A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

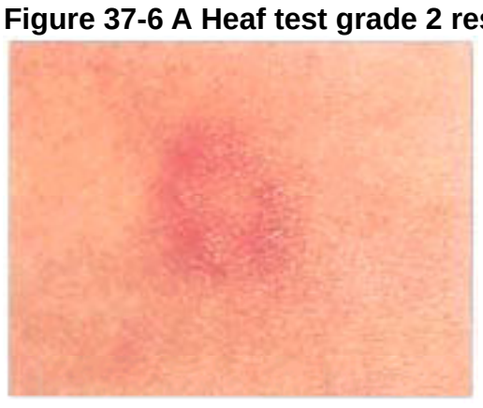


Figure 37-7 BCG response at 6 weeks. (a) Clinical evidence of a cell-mediated immune response is clearly apparent at 6 weeks. (b) The healed BCG scar. *With permission from James DG, Studdy PR, A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

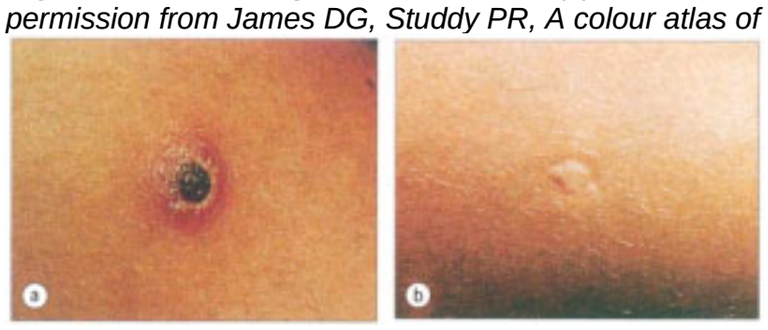


Figure 37-8 Chest radiograph of pulmonary tuberculous pneumonia. (a) Posteroanterior and (b) lateral chest radiographs of a patient with TB presenting as a consolidation. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

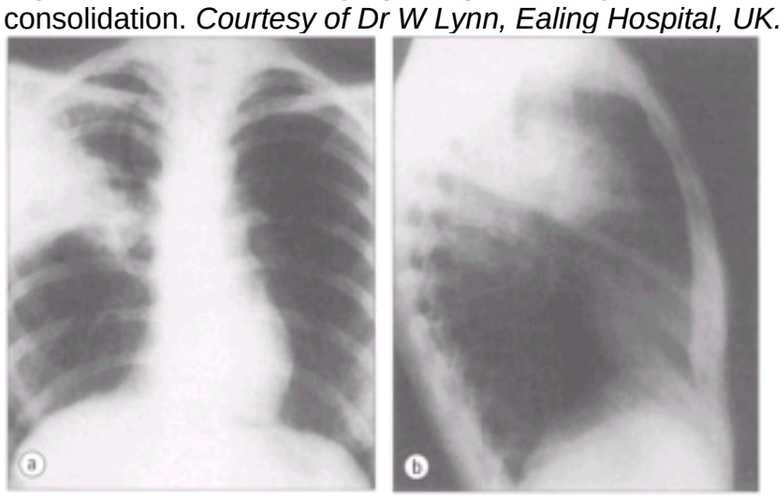


Figure 37-9 Choroidal TB. Choroidal disease is a manifestation of TB which is very highly suggestive of miliary disease. *With permission from James DG, Studdy PR. A colour atlas of respiratory diseases, 2E. London: Mosby; 1992.*

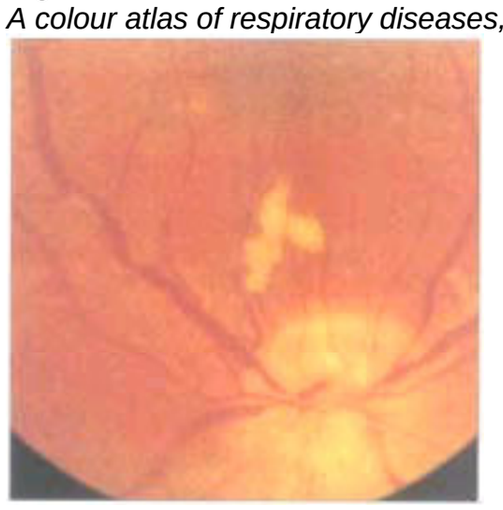


Figure 37-10 Miliary TB. Chest radiograph of miliary TB showing characteristic mottled shadowing throughout both lung fields. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

Figure 37-11 Vertebral TB. Tuberculosis of the spine or Pott's disease. Kyphosis is secondary to anterior destruction of vertebral bodies resulting in wedging of adjacent vertebrae and loss of disk space clearly seen by radiography. *(a) and (b) Courtesy of Professor J Cohen, Brighton, UK; (c) Courtesy of Dr A Wightman, with permission from Edmond RTD, Rowland HAK, Welsby PD, A colour atlas of infectious diseases, 3E. London: Mosby; 1995.*

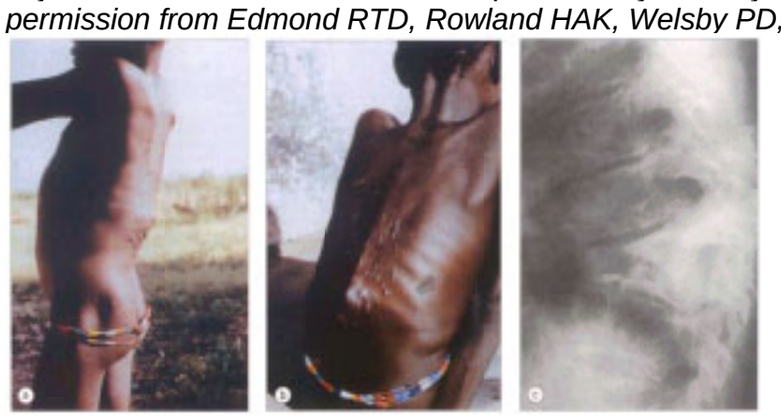


Figure 37-12 Abscess formation in TB. This patient had multiple TB abscesses particularly affecting the psoas and quadriceps muscle groups.

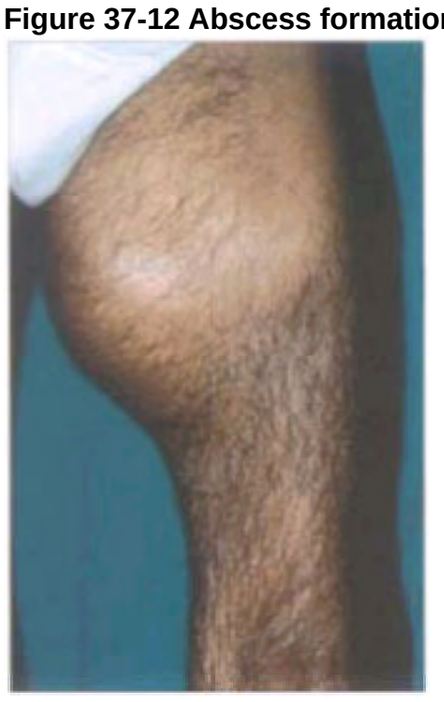


Figure 37-13 2-Fluoro-deoxy glucose positron emission tomography (FDG-PET) scan in bilateral apical pulmonary TB (arrowed). Tracer is excreted via the renal tract and is clearly seen in the bladder.

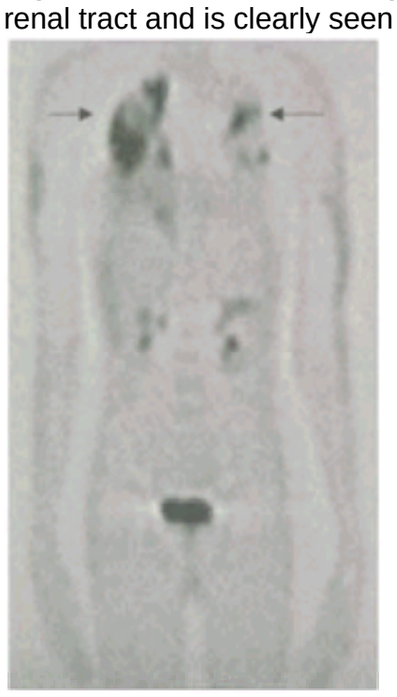


Figure 37-14 Ziehl-Neelsen-stained sputum specimens containing *Mycobacterium tuberculosis.* *Courtesy of Dr F Ahmed, Ealing Hospital, UK.*

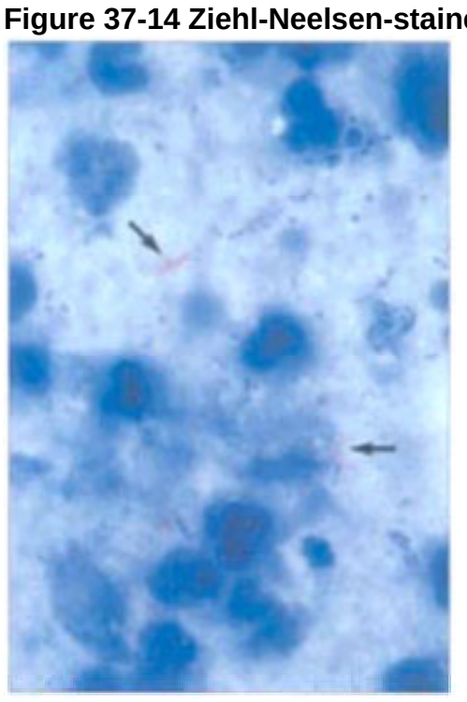


Figure 37-15 *Mycobacterium tuberculosis* in sputum detected by auramine staining. *Courtesy of Mr M Croughan, with permission from Edmond RTD, Rowland HAK, Welsby PD, A colour atlas of infectious diseases, 3E. London: Mosby, 1995.*

Figure 37-16 Rifampin urine testing. Patients should be warned that rifampin turns urine and other body secretions orange. This fact can be helful in monitoring compliance with drug treatment. *Courtesy of Dr W Lynn, Ealing Hospital, UK.*

Figure 38-1 DNA fingerprint patterns of 10 Mycobacterium abscessus isolates from an outbreak of M. abscessus infection in a hemodialysis center. Lanes 1–5 are identical strains of *M. abscessus* recovered in blood cultures from five different patients, indicating a common source of infection. Lanes 6–10 are the same strain of *M. abscessus* recovered by sampling a water treatment system contaminated by *M. abscessus*, the source of the patients' isolates. *Courtesy of Dr Yansheng Zhang and Dr Richard J Wallace Jr.*

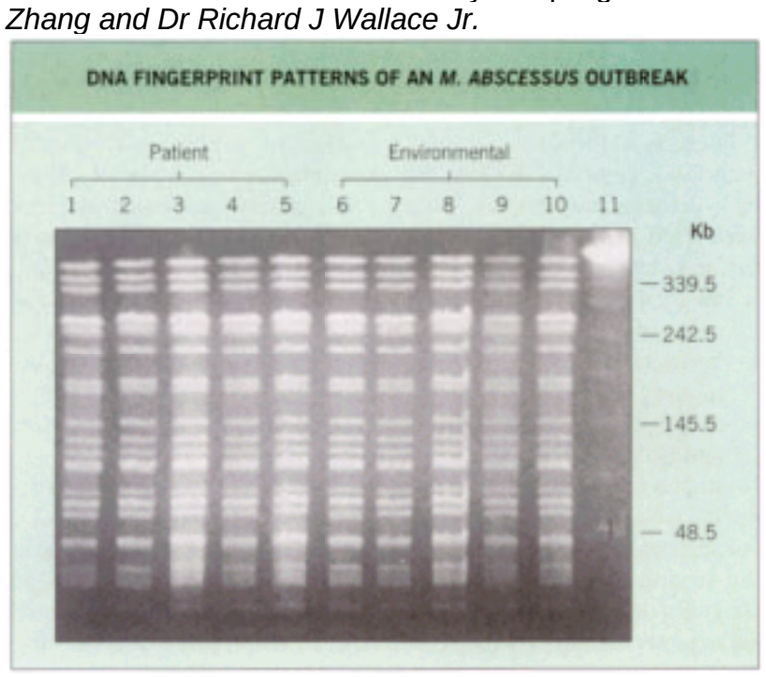


Figure 38-2 DNA fingerprint patterns of nine *Mycobacterium avium* complex (MAC) respiratory isolates (lanes 1–9) collected over an approximately 7-year period from a 67-year-old woman with nodular- bronchiectatic MAC lung disease. Each of the MAC isolates has a distinct DNA fingerprint pattern that suggests repeated reinfection by new MAC strains rather than relapse of infection from a single MAC strain.

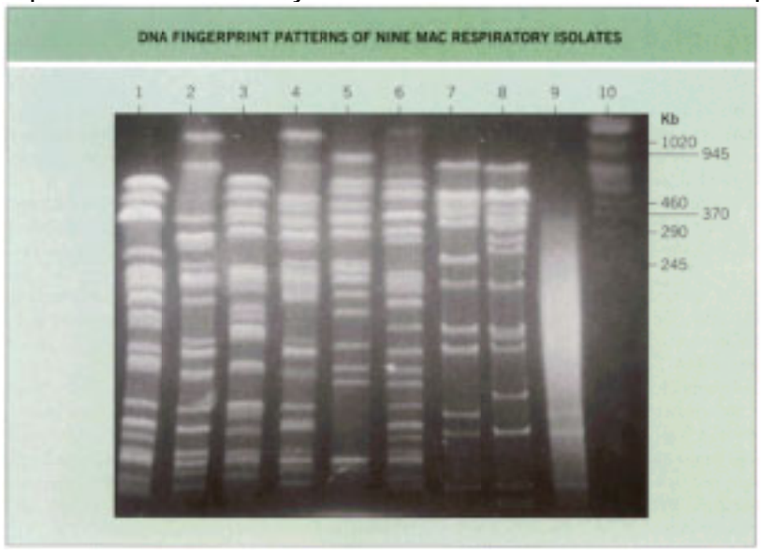


Figure 38-3 Cytokine pathways involved in mycobacterial infection and host response. Mycobacteria infect macrophages and stimulate production of interleukin (IL)-12. Interleukin-12, composed of p35 and p40 subunits, recognizes its receptor on T cells or natural killer (NK) lymhocytes, causing phosphorylation of the cytoplasmic kinases, Tyk2 and Jak2. This results in phosphorylation of signal transducer and activator of transcription (STAT)4, leading to production of interferon (IFN)-?, tumor necrosis factor (TNF)-a and granulocyte-macrophage colony stimulation factor (GM-CSF). Interleukin-12 also stimulates production of IL-2, which feeds back on the T or NK lymphocyte. Interferon-? binds to its receptor on the macrophage, causing aggregation of IFN-?R1 and IFN-?R2 and phosphorylation of the cytoplasmic Janus kinases, Jak1 and Jak2. Consequently, cytosolic STAT1 is phosphorylated, homodimerized and transported to the nucleus, where it upregulates IFN-? responsive genes. Interferon-? and GM-CSF stimulate macrophages to produce TNF-a, which in conjunction with IFN-? drives forward the production of IL-12 and reactive oxygen intermediates, such as superoxide and nitric oxide. *Adapted with permission from Holland SM. Host susceptibility factors in mycobacterial infection: genetics and body morphotype. Infect Dis Clin North Am 2002;16:163–86.*

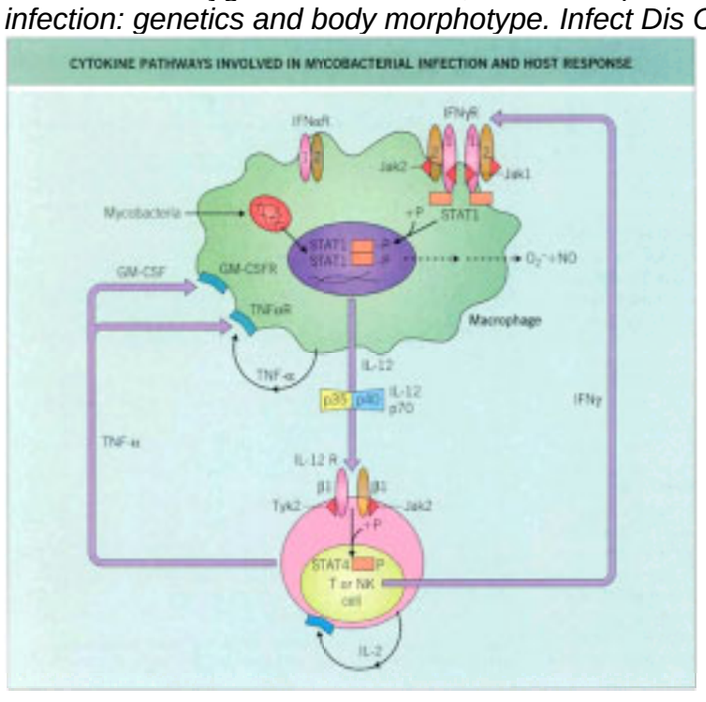


Figure 38-4 Apical cavitary infiltrates similar to those caused by pulmonary tuberculosis in a 60-year-old male cigarette smoker with *Mycobacterium avium* complex lung disease.

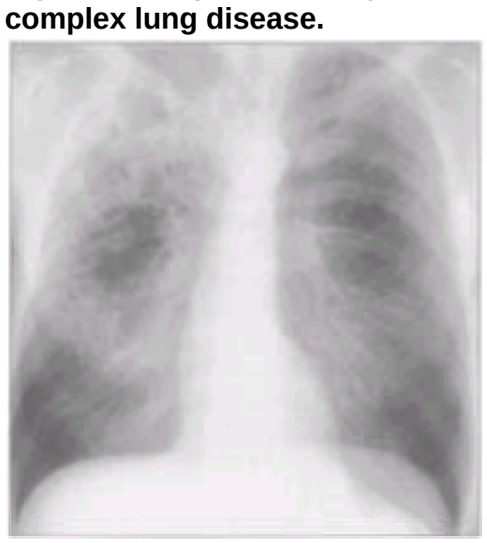


Figure 38-5 *Mycobacterium avium* complex lung disease. (a) Interstitial nodular midlung field infiltrates (right>left) in a 64-year-old female with MAC lung disease. (b) Chest CT scan from a 52-year-old woman with MAC lung disease demonstrating three abnormalities that are common in MAC lung disease: bronchiectasis, a cavity and small (<5mm) nodules.

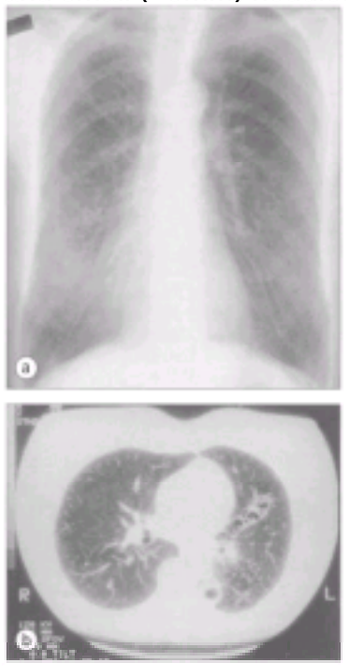


Figure 38-6 Far-advanced *Mycobacterium kansasii* bilateral lung disease in a 42-year-old male cigarette smoker with extensive cavitary destruction of the left upper lobe.

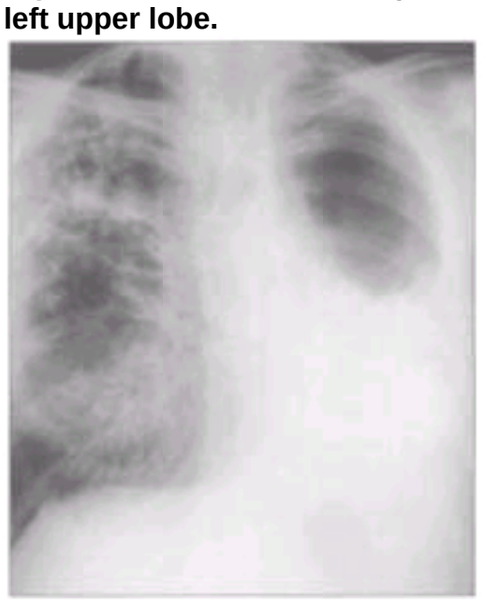


Figure 38-7 Disseminated *Mycobacterium chelonae* disease. This manifests itself here by subcutaneous nodules on the lower extremities in an 81-year-old woman receiving high-dose corticosteroids for rheumatoid arthritis.

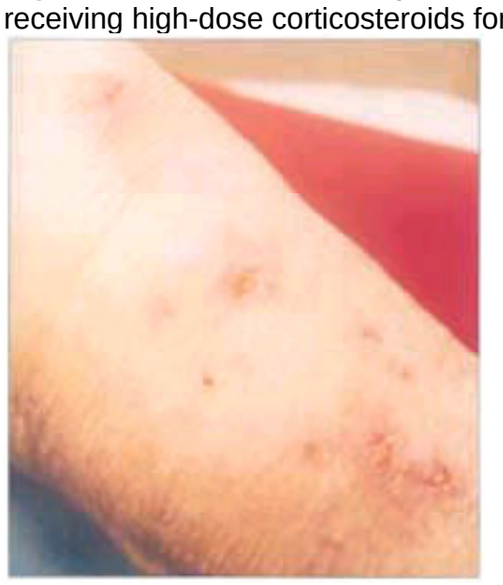


Figure 38-8 Nodular lesions on the hand caused by *Mycobacterium marinum.* This 45-year old man contracted *M. marinum* after penetrating trauma while cleaning his boat in salt water.

Figure 39-1 Endemic distribution of histoplasmosis in the Americas. This is based upon skin testing surveys.

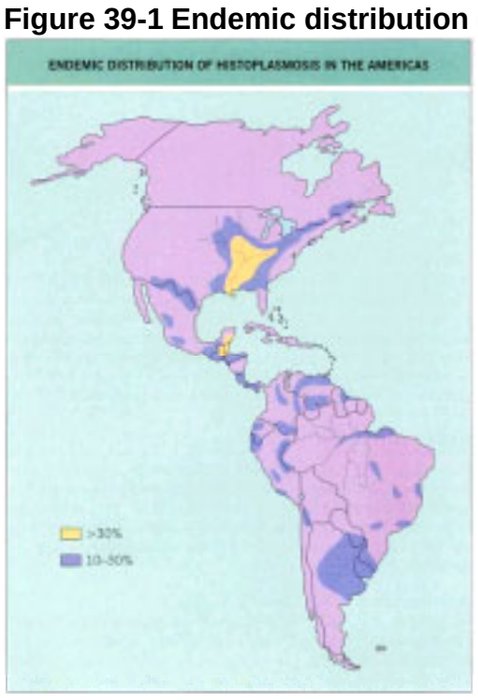


Figure 39-2 Cytologic specimen from bronchoalveolar lavage fluid showing intracellular *Histoplasma capsulatum.*

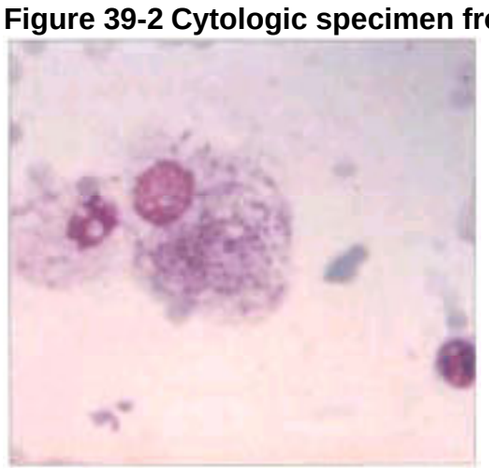


Figure 39-3 Pulmonary coccidioidomycosis. Granuloma showing typical spherules.

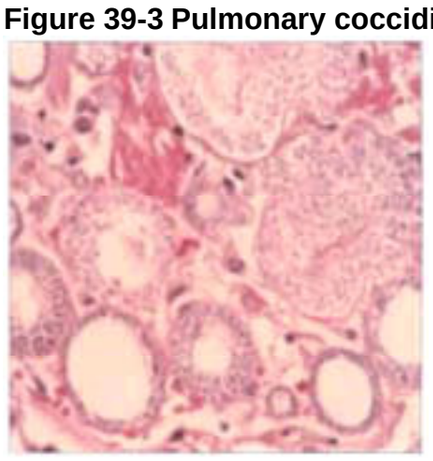


Figure 39-4 Cutaneous blastomycosis. (a,b) Skin lesions caused by *Blastomyces dermatitidis* in a normal host. (c) A large cutaneous ulcer in a patient who has multiple myeloma.

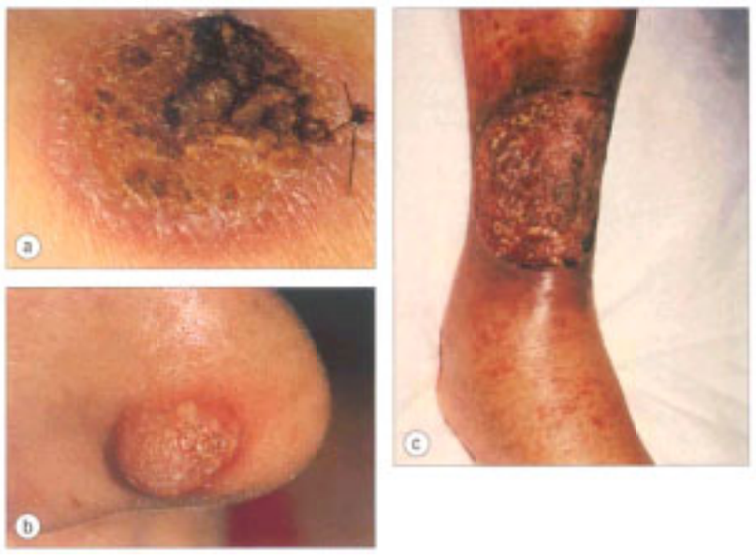


Figure 40.a-1 Abram's needle. The curved arrow shows clockwise rotation of the inner cylinder to close the side hole.

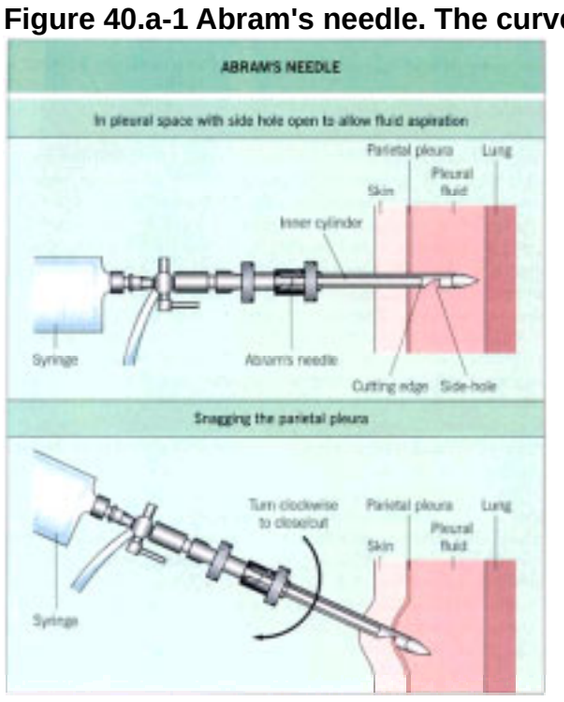


Figure 40.e-1 Management of elevated tests of liver function if pre-treatment liver function is abnormal. *Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.*

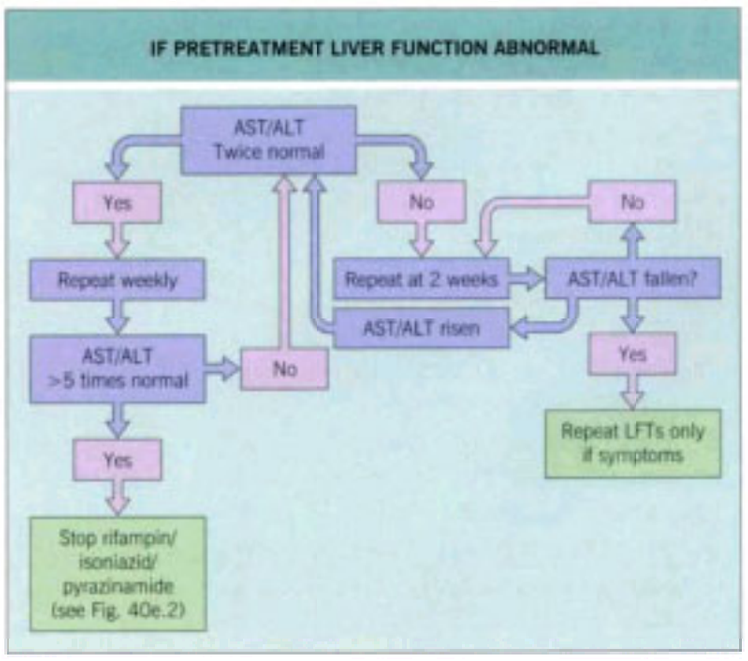


Figure 40.e-2 Management of elevated tests of liver function if antituberculosis drugs (rifampin/isoniazid/pyrazinamide) stopped because AST/ALT over five times normal or bilirubin elevated. *Derived from Ormerod LP, Skinner C, Wales J. Hepatotoxicity of antituberculosis drugs. Thorax 1996;51:111–13, with permission from BMJ Publishing Group.*

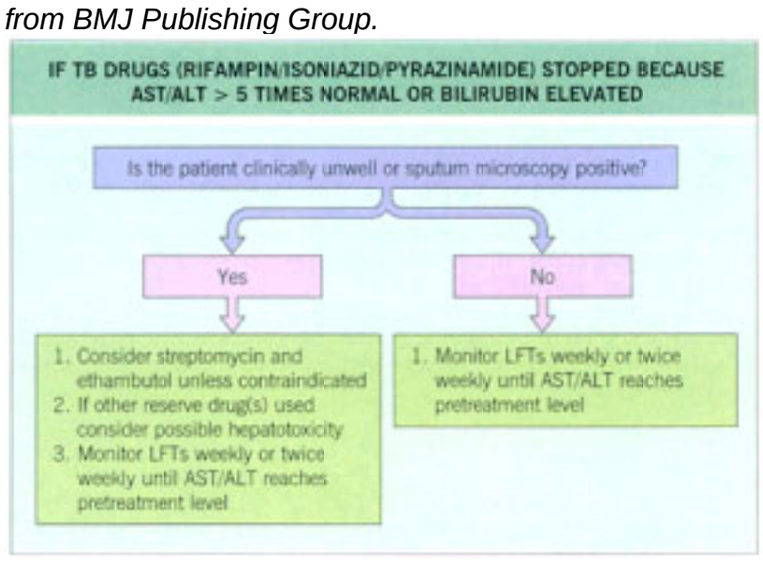


Figure 40.f-1 A schematic representation of the role played by bacteria in COPD. There is limited evidence at the present time for the steps indicated by broken arrows which suggest that inflammation might be caused by lower airway bacterial colonization (LABC) and that airway infections might lead to progression of COPD.

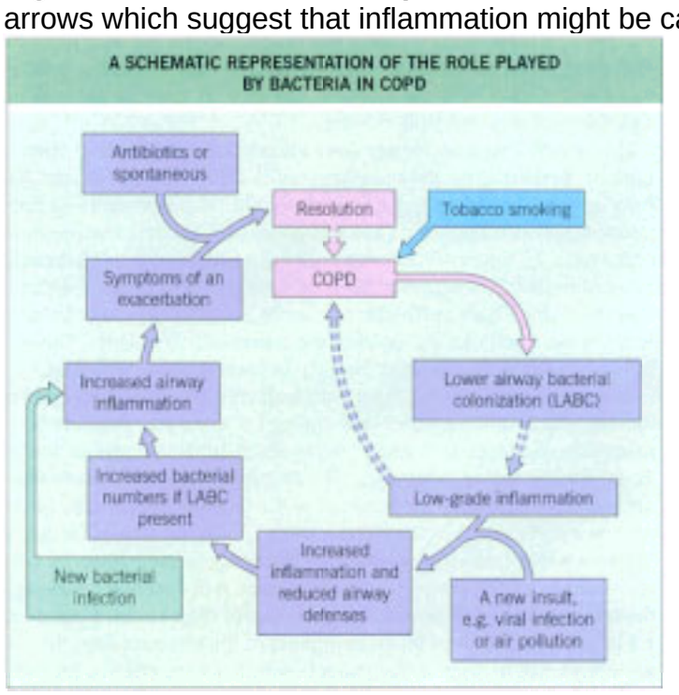


Figure 40.f-2 A decision tree outlining how choices about whether or not to prescribe an antibiotic for patients with airway diseases can be made. There is very little evidence that one antibiotic is superior to another, but concern about antibiotic resistance will influence choice in severe cases. An antibiotic which is resistant to ß-lactamase enzyme and potent against the major pathogens should be chosen. Ciprofloxacin is indicated when there is concern about *Pseudomonas aeruginosa*. Evidence shows that this bacterium is usually only a consideration in patients with FEV1 =50% predicted, although in my own experience it is only patients with even more severe airflow obstruction in whom it occurs or in those patients who also have bronchiectasis.

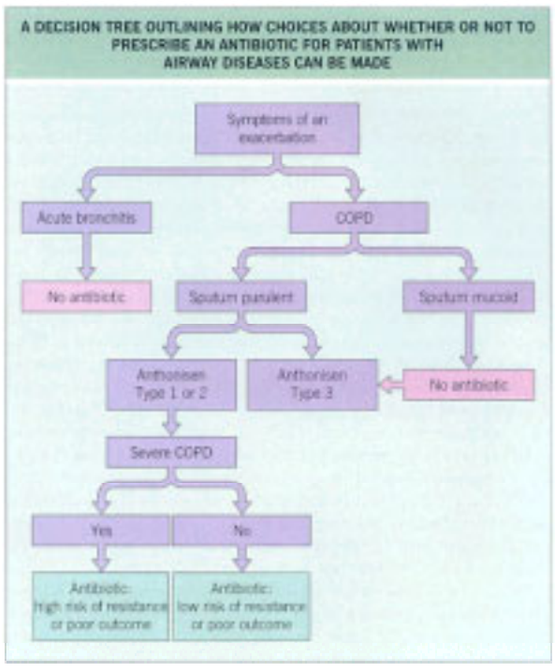


Figure 41-1 Spread of dental infection. A spreading tooth abscess will encroach upon the nearest cortical plate and its subsequent spread depends on the relationship of that site to muscle attachment. *Adapted from Peterson.*[8]

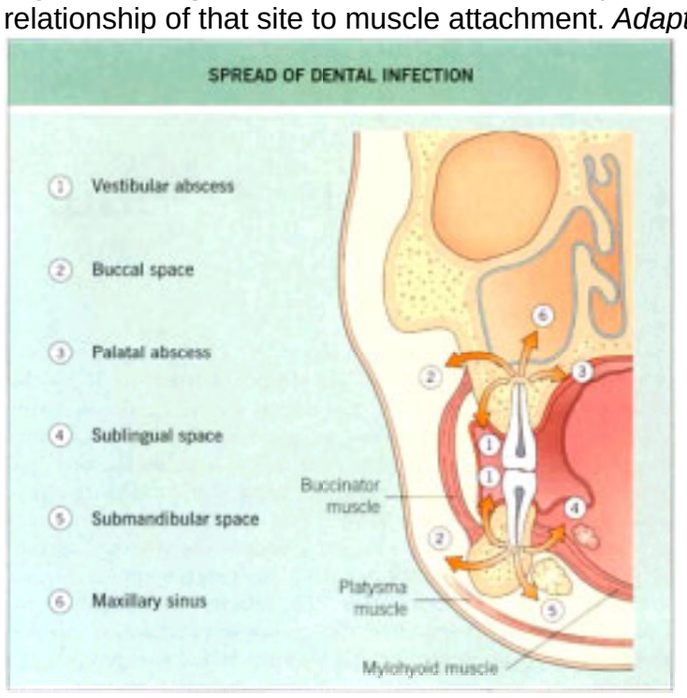


Figure 41-2 Painful vestibular abscess. *Courtesy of Professor I Brook.*

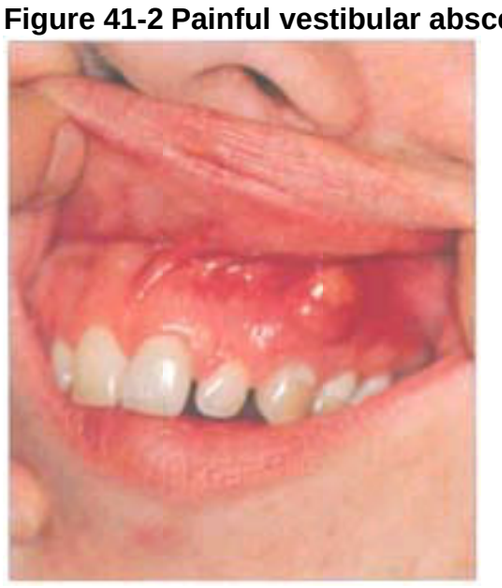


Figure 41-3 Buccal space abscess originating from right lower molar infection. The buccal space lies between the buccinator muscle and the overlying skin and fascia. *Courtesy of Professor I Brook.*

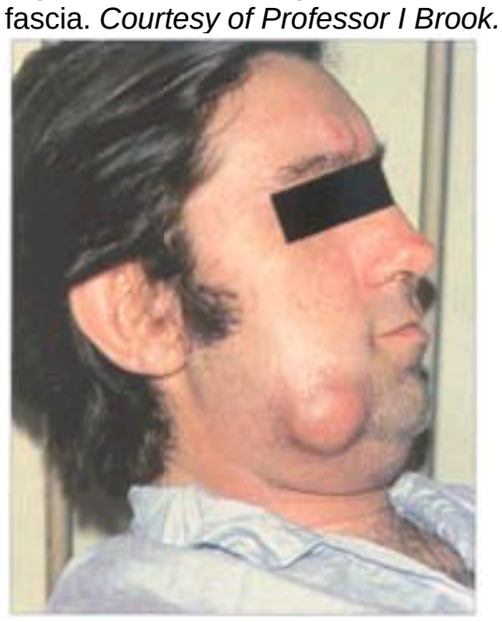


Figure 41-4 Submandibular abscess originating from a 2nd molar tooth infection. *Courtesy of University of Sheffield School of Dentistry, UK.*

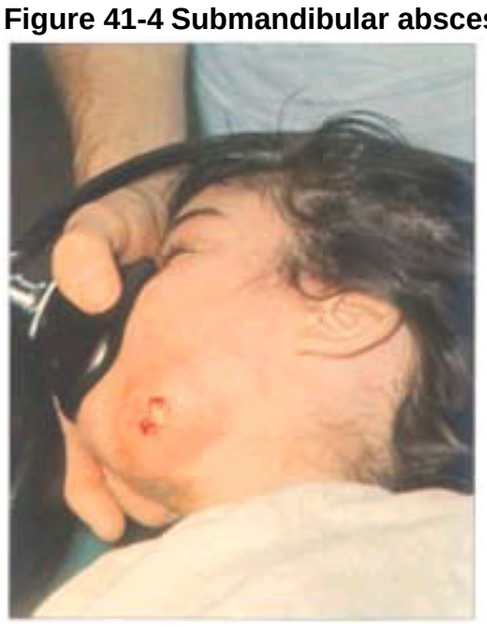


Figure 41-5 Ludwig's angina. (a) This patient had painful cellulitis within the submandibular and sublingual spaces. (b) Brawny edema was present within the floor of the mouth, pushing the tongue upwards. *Courtesy of University of Sheffield School of Dentistry, UK.*

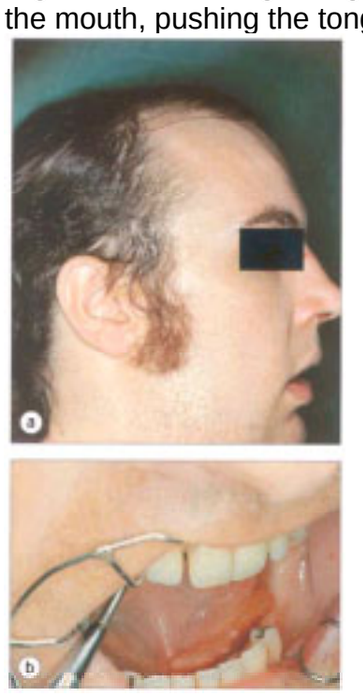


Figure 41-6 Cavernous sinus thrombosis. A patient who displays evidence of severe orbital swelling caused by obstruction of orbital veins is shown. In this patient, the originating focus was infection of soft tissues of the nose. *Courtesy of University of Sheffield School of Dentistry, UK.*

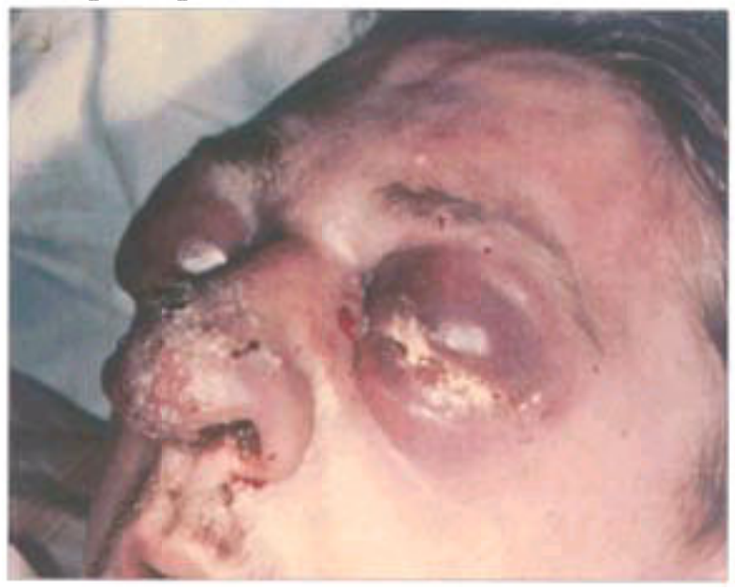


Figure 41-7 Retropharyngeal abscess. Lateral radiograph of the neck in a patient who has a retropharyngeal abscess, showing gross expansion of prevertebral soft tissue. *Courtesy of Mr R Bull.*

Figure 41-8 Actinomycosis. (a) This patient had chronic disease over the mandible which (b) healed with several months of antibiotics, leaving a residual chronic sinus. *Courtesy of Professor I Brook.*

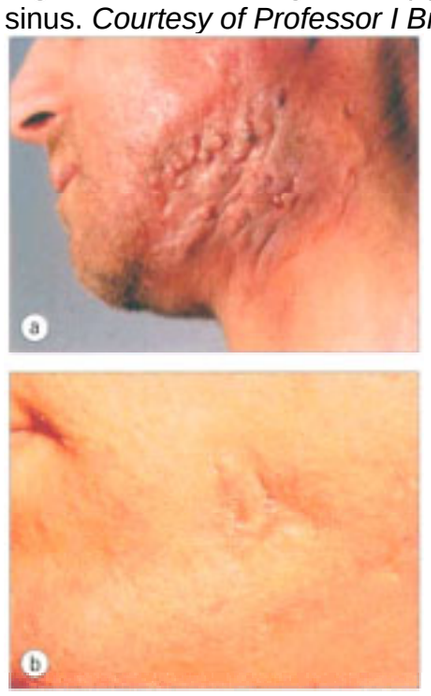
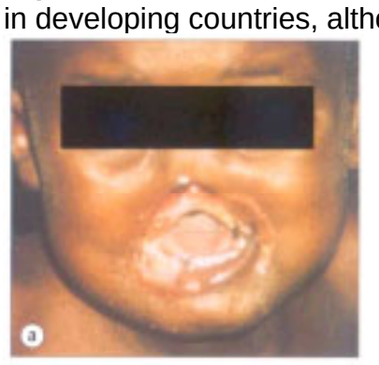


Figure 41-9 Acute necrotizing gingivitis. *Courtesy of Professor I Brook.*

Figure 41-10 Noma. This is a destructive process extending from oral structures, which is a sequel of necrotizing gingivitis and (a) is seen most commonly in patients in developing countries, although (b) occasionally it is seen in the elderly debilitated in developed countries. *Courtesy of Professor I Brook.*



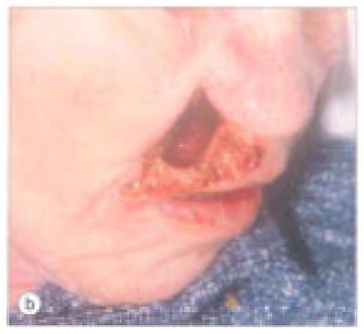


Figure 41-11 Primary HSV-1 stomatitis.

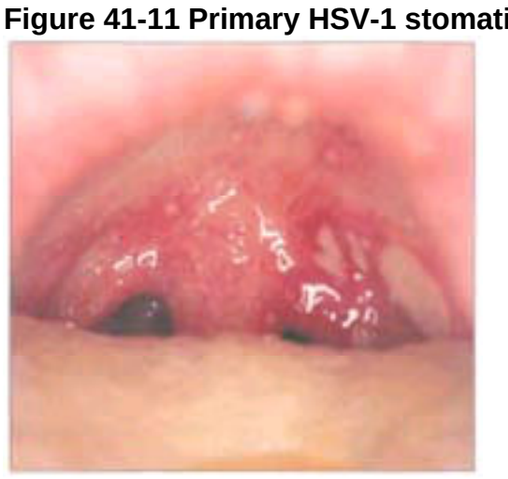


Figure 41-12 Herpangina in a teenager with severe throat pain.

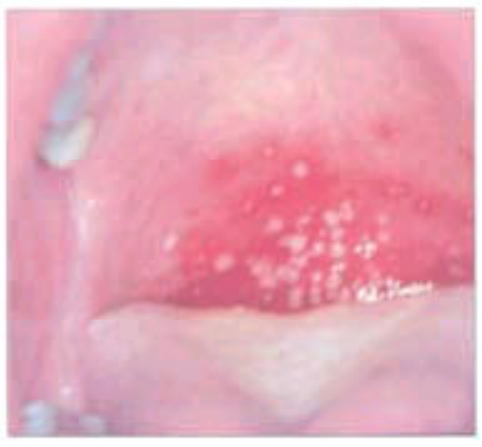


Figure 41-13 Suppurative parotitis (a) in a diabetic patient who had a recent history of dehydration secondary to diabetic ketoacidosis. (b) Pus was manually expressed from Stensen's duct from which *Staphylococcus aureus* was cultured. *Courtesy of Dr E Ridgway.*

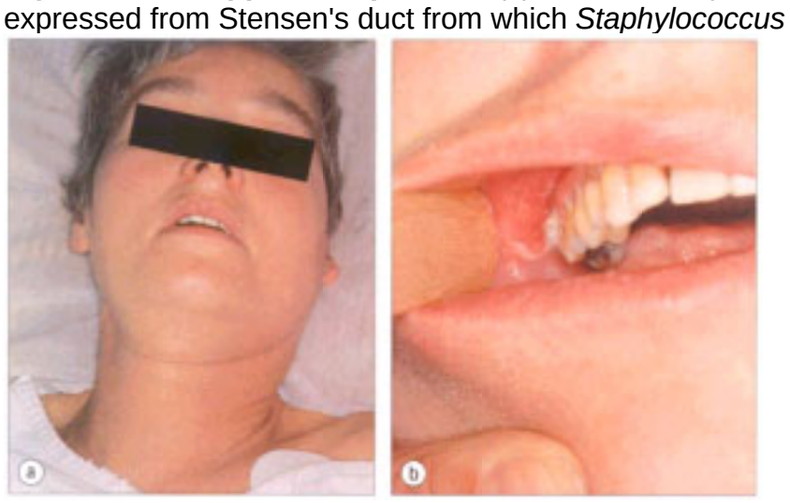


Figure 42-1 Endoscopic pictures of the stomach and duodenum. (a) Erythema of the gastric antrum. This appearance correlates poorly with histologic gastritis and may be a normal finding. (b) Duodenal ulceration. (c) Gastric ulcer. Note the clot in the base indicating recent bleeding and high risk of rebleed and the endoscope entering the stomach through the cardia.

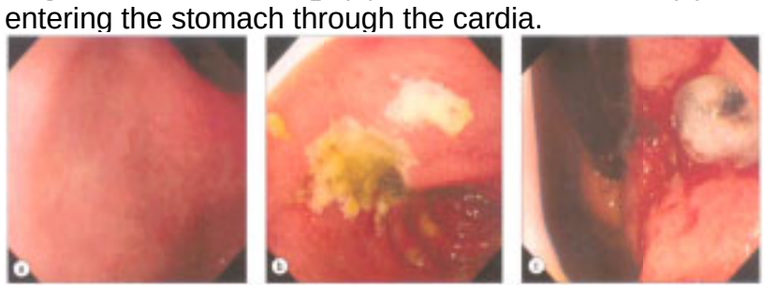


Figure 42-2 Prevalence patterns of *Helicobacter pylori.* Prevalence of *H. pylori* infection in 10 developing countries (Group 1) and 10 developed countries (Group 2). *Adapted with permission from Pounder and Ng.*[5]

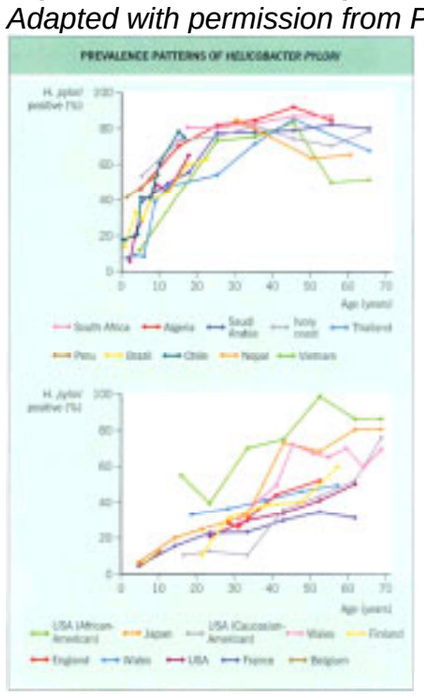


Figure 42-3 Appearances of *Helicobacter pylori* and NSAID antral gastritis. (a) Antral gastritis in *H. pylori* infection with active (neutrophil) and chronic inflammation of the lamina propria and glands. The epithelial surface is typically ballooned. *Helicobacter pylori* organisms are not readily apparent on a hematoxylin and eosin stain. (b) Antral gastritis associated with NSAID use. Foveolar hyperplasia with a mild chronic inflammatory infiltrate and smooth muscle cells are seen in the lamina propria. *Courtesy of Dr MM Walker.*

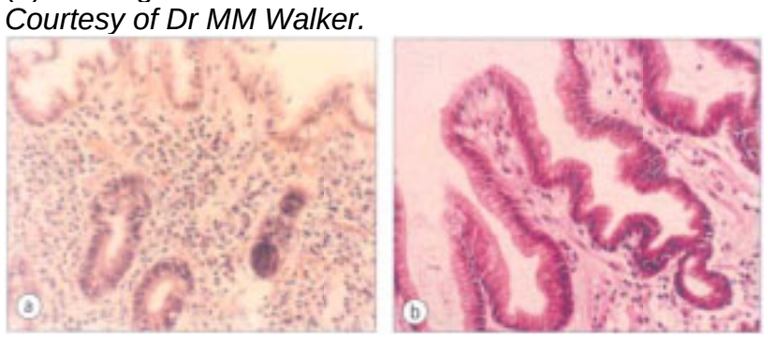


Figure 42-4 *Helicobacter pylori* (Gimenez stain). Other special stains that can be used are the modified Giemsa stain or a silver stain such as Warthin-Starry stain. *Courtesy of Dr MM Walker.*

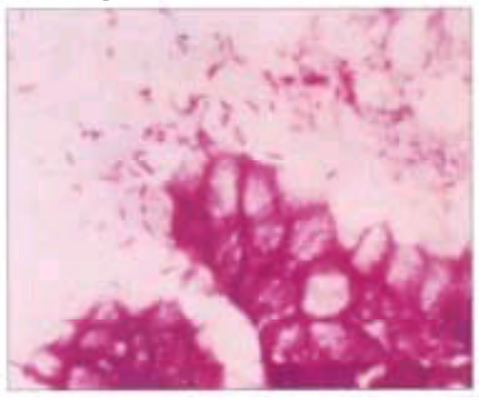


Figure 42-5 Decision algorithm for the management of duodenal ulcer disease diagnosed at upper gastrointestinal endoscopy.

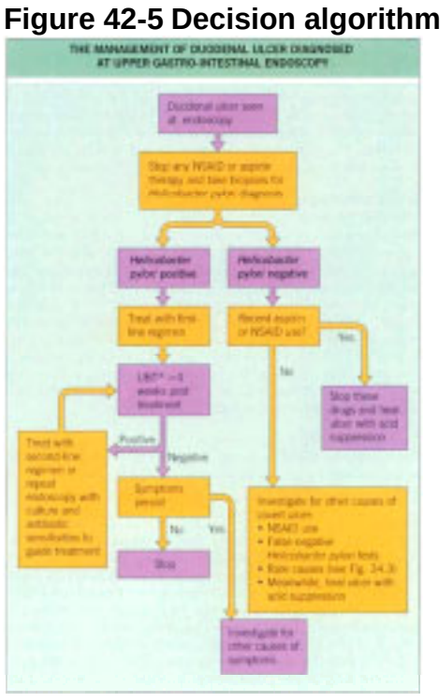
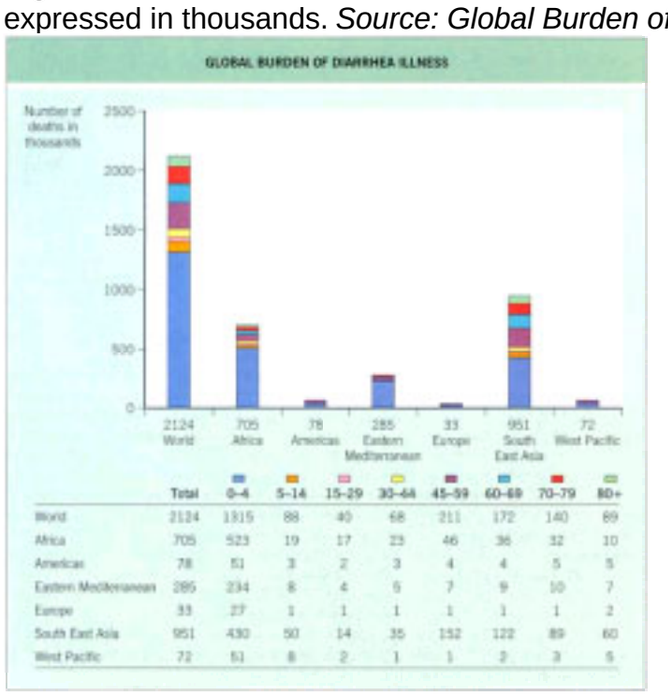


Figure 43-1 Global burden of diarrhea illness. Estimated number of deaths secondary to diarrhea in the year 2000, by age group and world regions. Numbers are expressed in thousands. *Source: Global Burden of Disease 2000, World Health Organization.*



	Total	0–4	5–14	15–29	30–44	45–59	60–69	70–79	80+
World	2124	1315	88	40	68	211	172	140	89
Africa	705	523	19	17	23	46	36	32	10
Americas	78	51	3	2	3	4	4	5	5
Eastern Mediterranean	285	234	6	4	5	7	9	10	7
Europe	33	27	1	1	1	1	1	1	2
South East Asia	951	430	50	14	35	152	122	89	60
West Pacific	72	51	8	2	1	1	2	3	5

Figure 43-2 Pathogenic mechanism of diarrhea caused by EPEC. Figure shows attachment of EPEC to the enterocyte, villous destruction and formation of the A/E lesion.

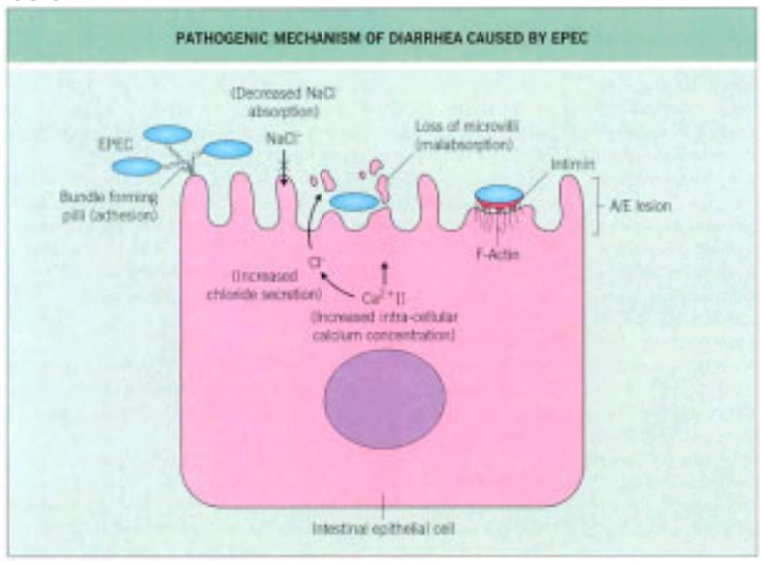


Figure 43-3 Pathogenic mechanism of toxin-mediated diarrhea.

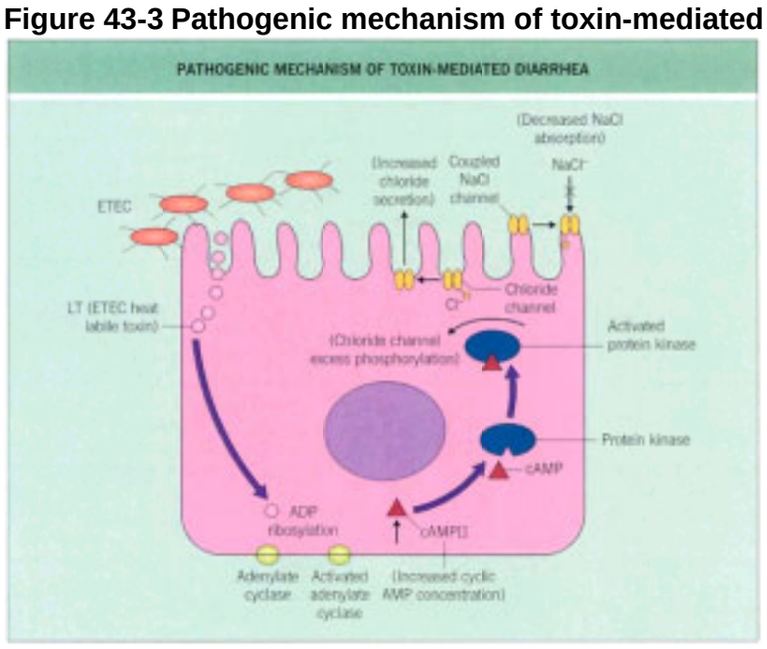


Figure 44-1 The pathogenesis model for hospital-acquired *Clostridium difficile*-associated diarrhea (CDAD). This 'three-hit' model of *C. difficile* pathogenesis shows that exposure to antibiotics establishes susceptibility to infection. Once susceptible, the patient may acquire nontoxigenic (nonpathogenic) or toxigenic strains of *C. difficile* (the second 'hit'). Acquisition of toxigenic *C. difficile* may be followed by asymptomatic colonization or *C. difficile*-associated disease, depending on one or more factors (the third 'hit'). Inadequate anamnestic IgG response to toxin A produced by toxigenic *C. difficile* strains is an important host factor determining disease outcome.[9]

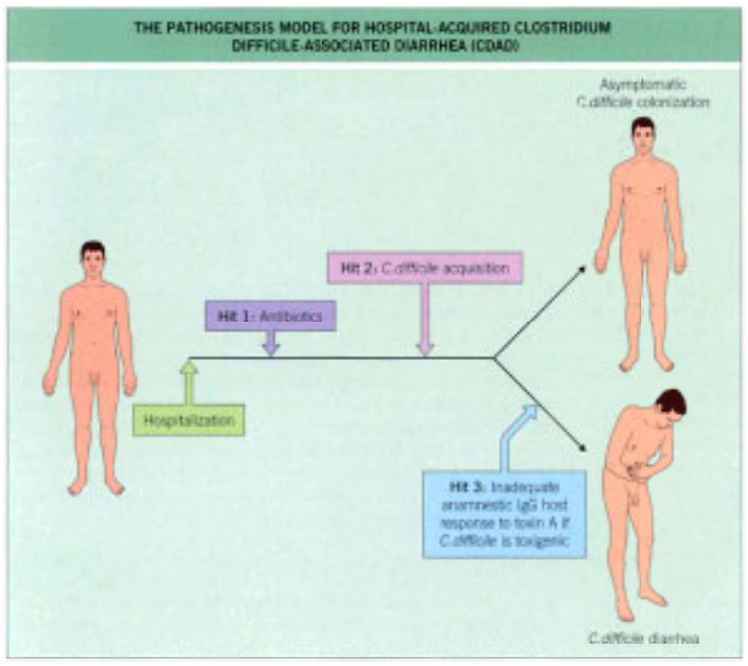


Figure 44-2 Endoscopic view of multiple pseudomembranes covering the colon in a patient with PMC.

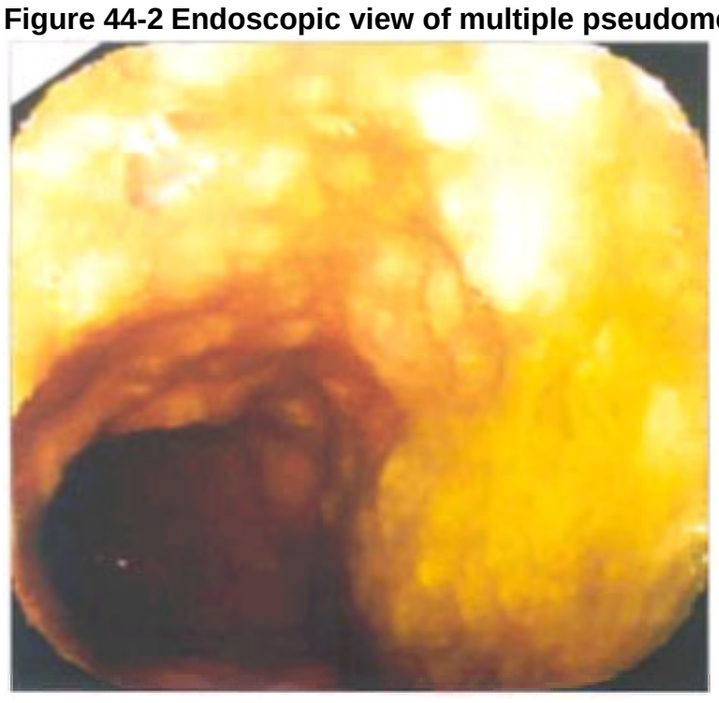


Figure 44-3 CT scan of the abdomen in a patient with fulminant PMC. The colonic and rectal walls are markedly thickened with fluid-filled colon and rectum.

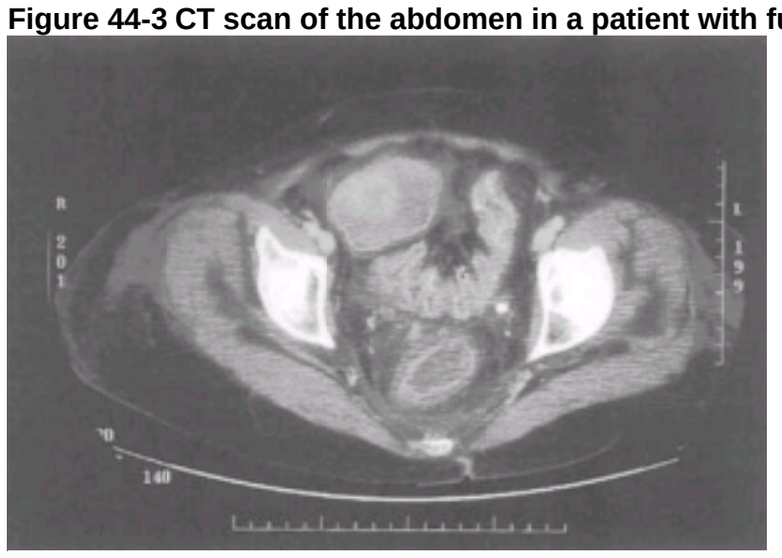


Figure 45-1 Section from a PAS-stained duodenal biopsy. There are numerous (pink-staining) PAS-positive macrophages in the lamina propria.

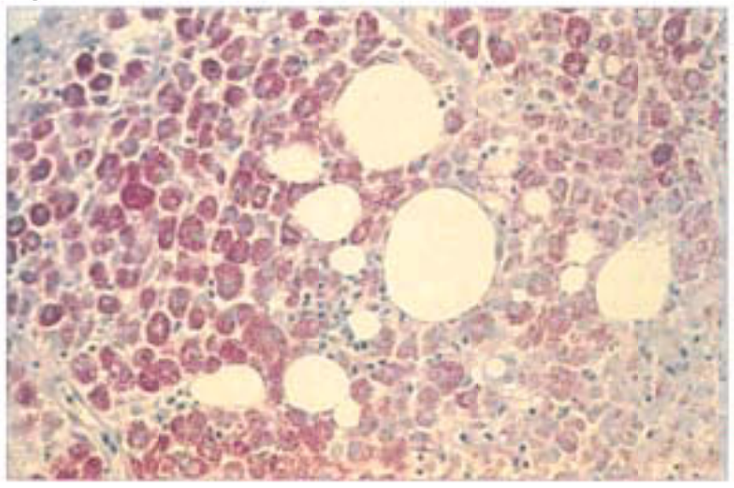


Figure 45-2 Electron micrograph of small intestinal mucosa demonstrating the typical appearance of the Whipple's disease bacillus. *Courtesy of Professor H Hodgson.*

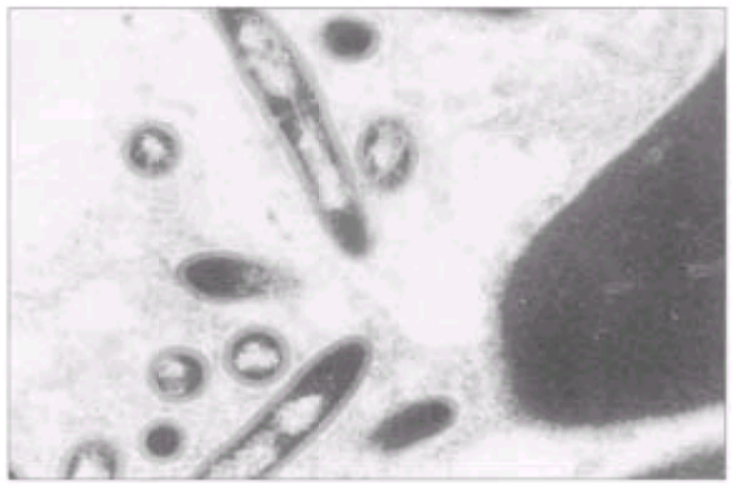


Figure 45-3 Phylogenetic relations of the Whipple's disease bacterium, *Tropheryma whipplei.*

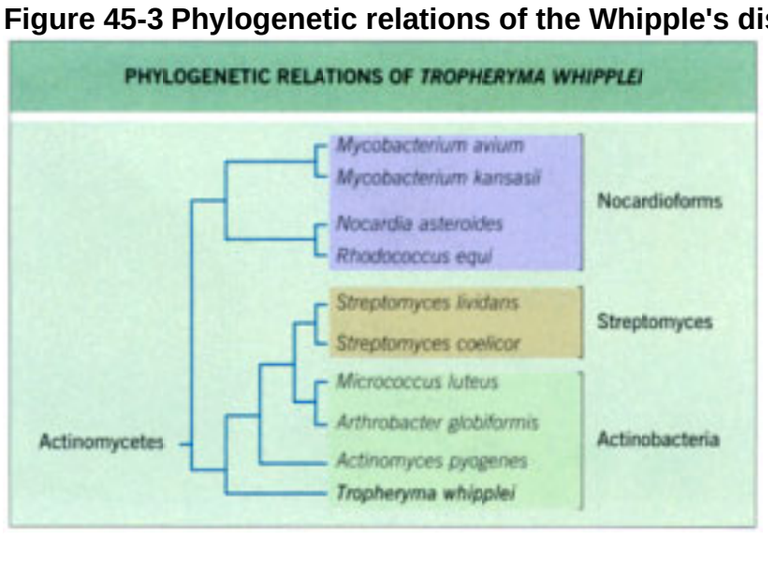


Figure 46-1 Adult beef tapeworm (*Taenia saginata*) passed in a patient's feces.

Figure 46-2 *Ascaris lumbricoides* ovum in feces. The ovum measures 50–70mm × 40–50mm and is elliptical. The rough albuminous coat gives it a mammillated appearance.

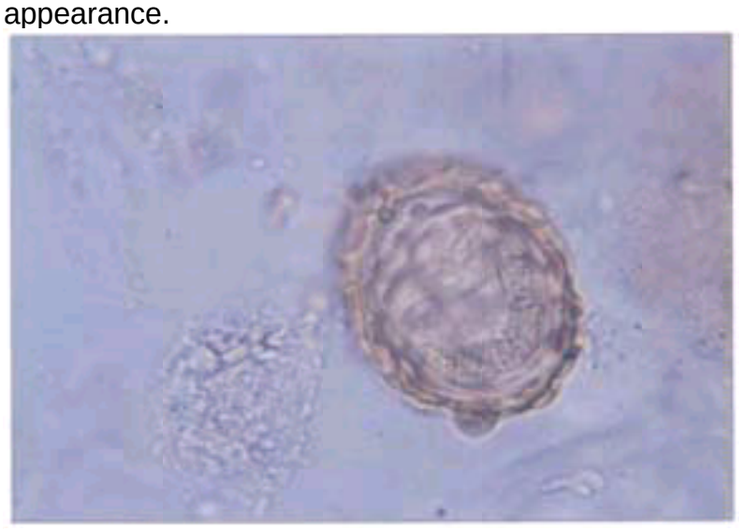


Figure 46-3 Life cycle of *Strongyloides stercoralis*.

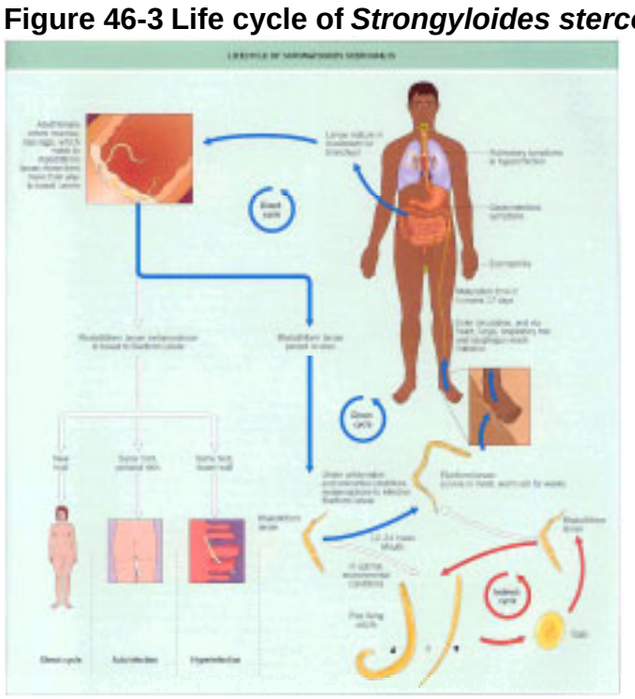


Figure 46-4 *Strongyloides* larvae.

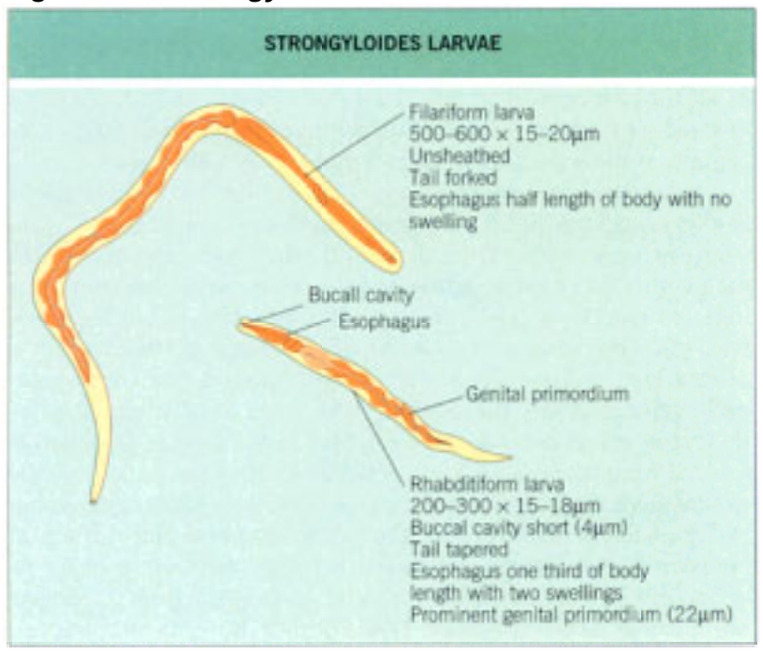


Figure 47-1 (a) Left lateral decubitus radiograph demonstrating free air outlining the liver. (b) Pneumatosis coli. (c) Air in the portal vein.

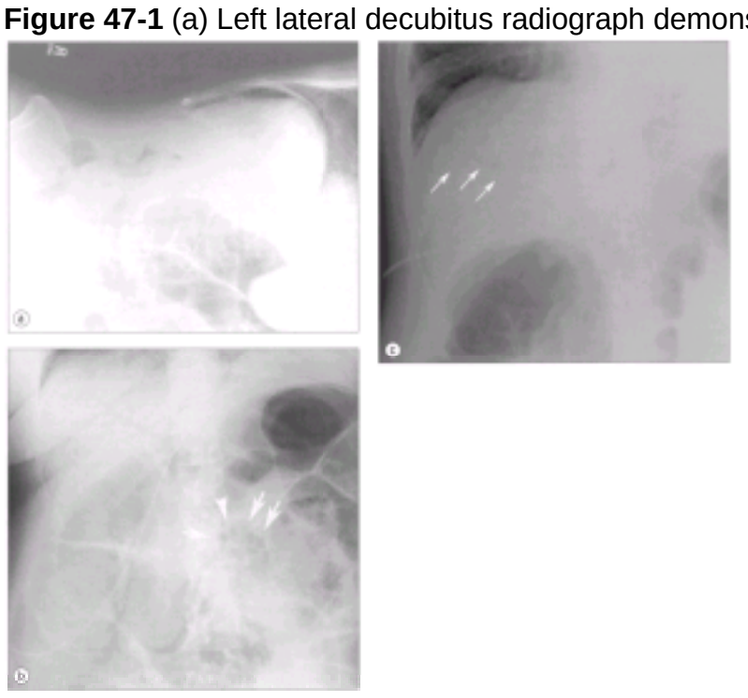


Figure 47-2 Diverticular abscess (a) before and (b) after drainage. A small fistula is seen that required further drainage but did not interfere with planned interval colectomy.

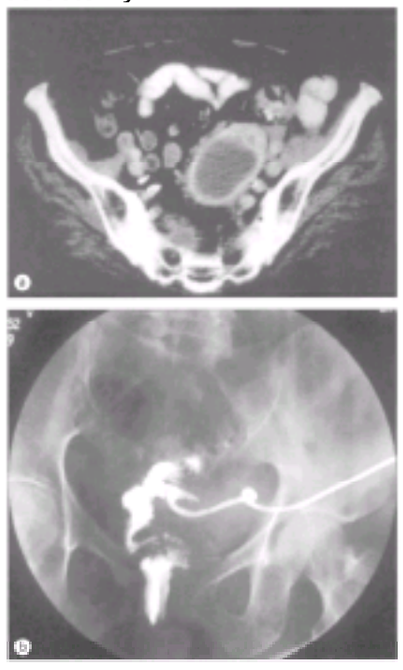


Figure 47-3 Multiple intra-abdominal abscesses following appendectomy of perforated appendicitis.

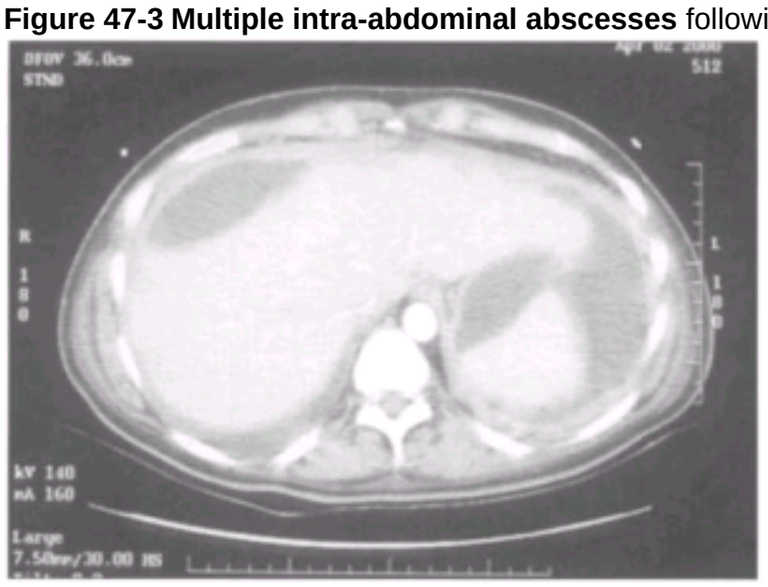


Figure 47-4 Pancreatic drainage for lesser sac infection during the course of acute necrotizing pancreatitis. The difficulty in removing necrotic debris through the narrow catheter is suggested by the abscess's persistence. Follow-up operative intervention was required.

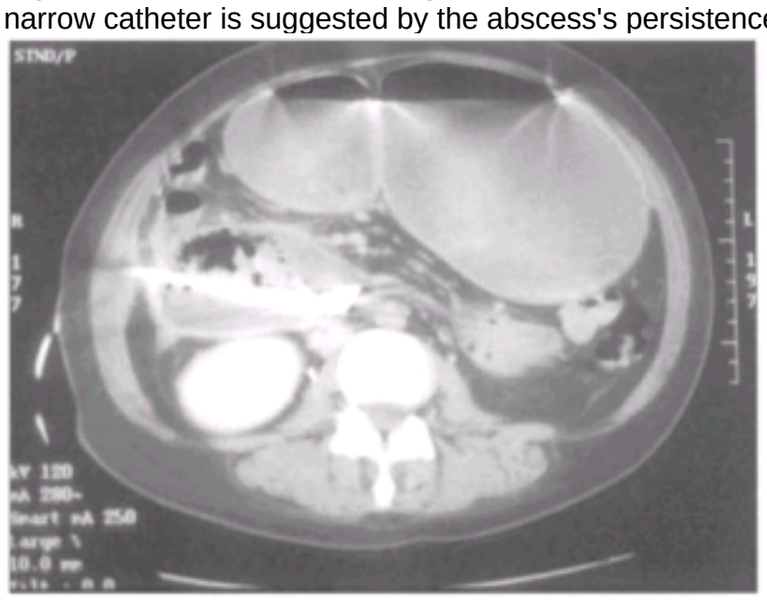


Figure 48-1 Seroprevalence of hepatitis E IgG in selected world populations.

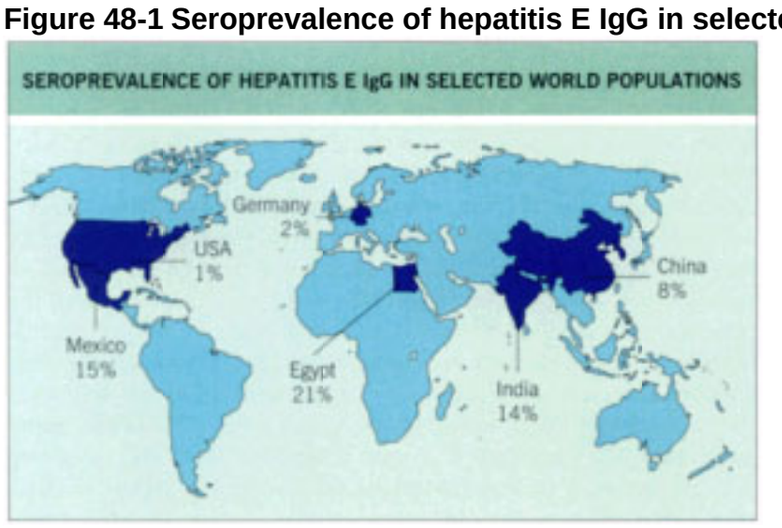


Figure 48-2 Hepatitis B virus replication. Viral DNA present in the nucleus is transcribed to RNA, which then acts as a template for protein synthesis (viral coat-HBsAg and viral proteins essential for infectivity and replication-HBcAg). Viral particles are then assembled and secreted from the cell cytoplasm. For every complete virion a large number of incomplete particles derived from HBsAg alone are exported. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen.

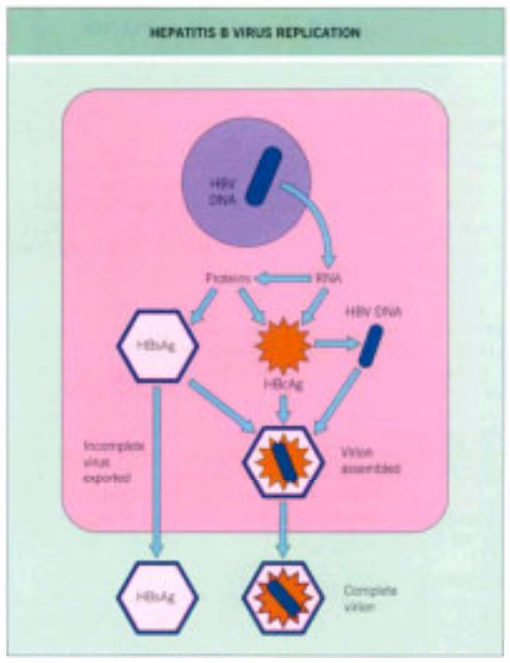


Figure 48-3 Phases of chronic hepatitis B infection. Initial infection is usually in childhood and has a long immune tolerant phase. Immune recognition then develops which can allow inactivation of hepatitis B by either clearance of infected cells (with associated liver cell damage) or suppression of viral antigen expression on infected hepatocytes. This produces viral inactivation. In a significant proportion of patients where this has occurred, a third phase develops, where replication of HBV resumes due to viral escape mutants, leading to HBV DNA again appearing in serum and the risk of chronic liver disease.

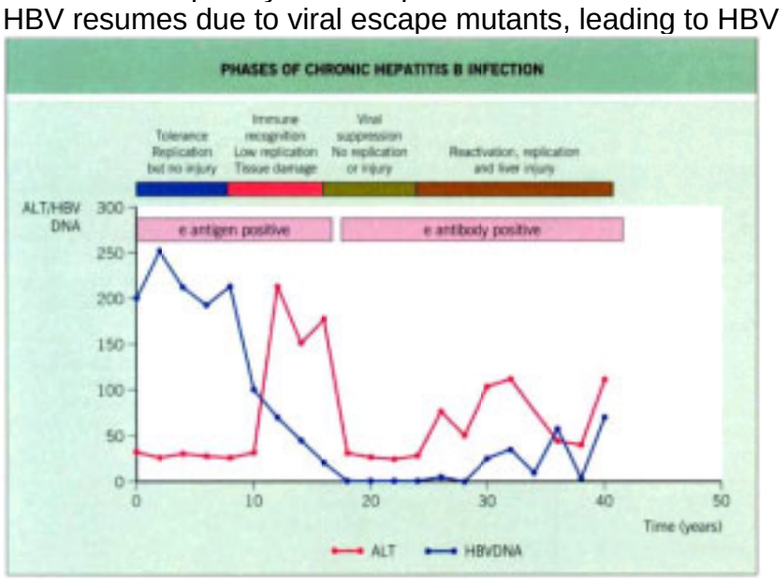


Figure 48-4 Facial stigmata of chronic liver disease. This woman presented with serologically proven acute hepatitis A virus infection. She has multiple stigmata of chronic liver disease including facial spider nevi and was shown to have pre-existing cirrhosis.

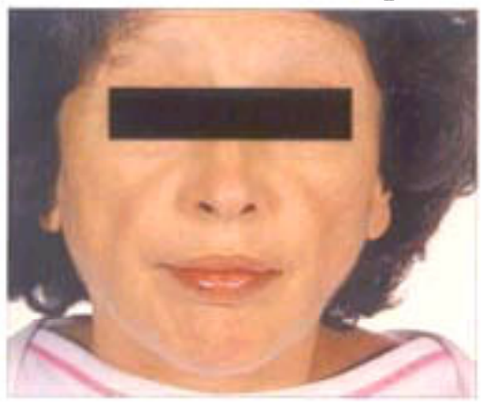


Figure 48-5 Vasculitic rash in a patient who has mixed cryoglobulinemia.

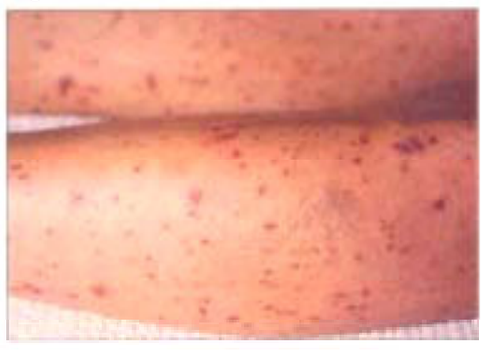


Figure 48-6 Endoscopic view of esophageal varices.

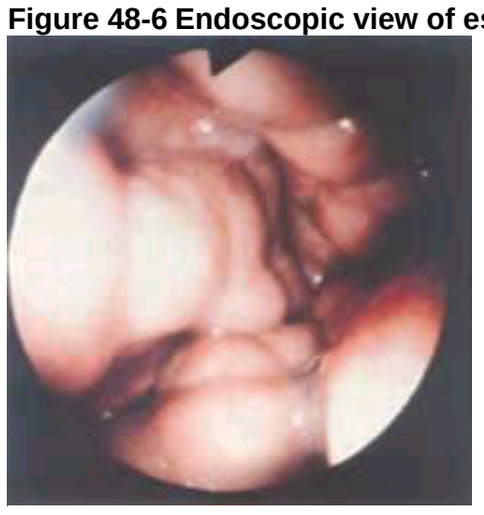


Figure 48-7 Surgical histology of a nodule of hepatocellular carcinoma in a patient undergoing liver transplantation for hepatitis C cirrhosis.

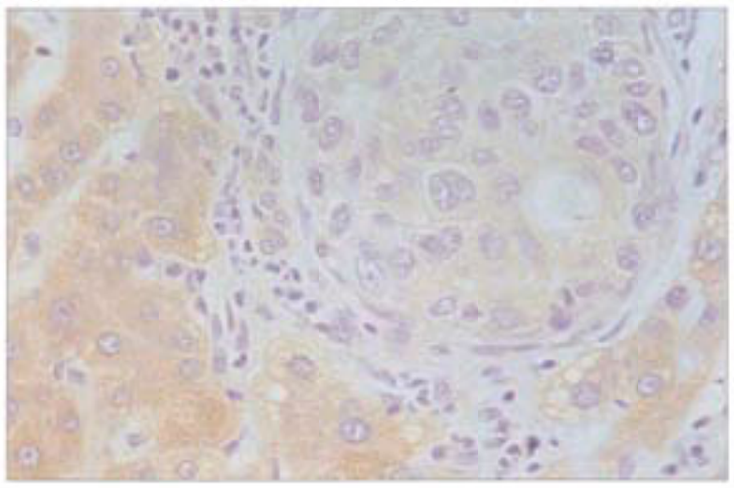


Figure 48-8 Computed tomography scan following lipiodol injection into the hepatic artery showing hepatocellular carcinoma in the cirrhotic liver of a hepatitis B virus-positive male. Lipiodol is selectively retained in the tumor.

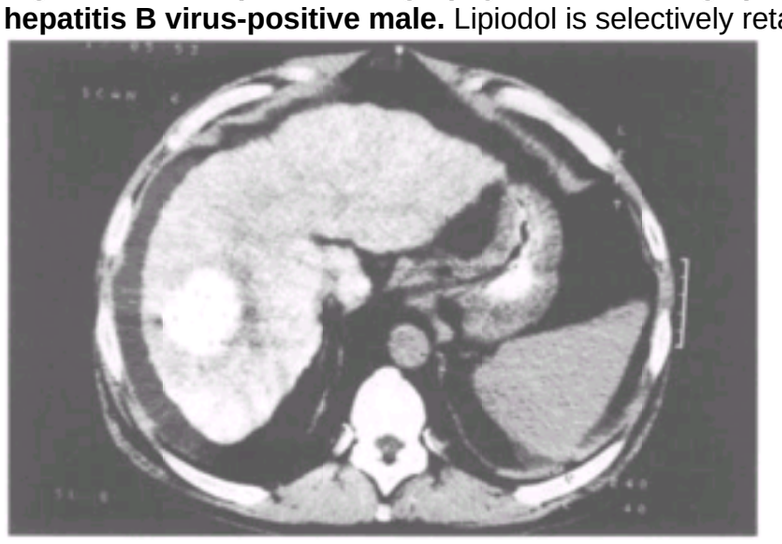


Figure 48-9 Serologic markers in acute self-limiting hepatitis B virus infection. HBV, hepatitis B virus; HBeAg, hepatitis B e antigen. Measurements in arbitrary units.

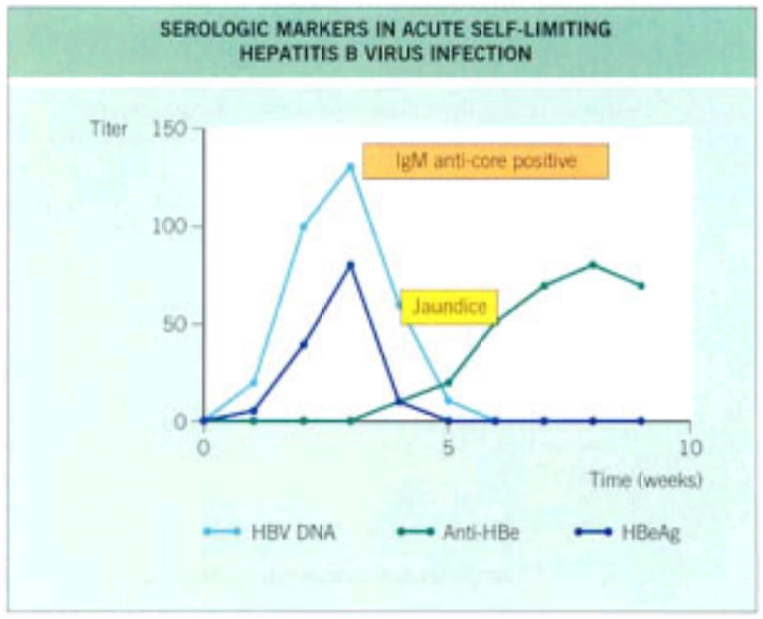


Figure 48-10 The appearance of hepatitis C virus RNA, anti-hepatitis C virus and elevated ALT in acute hepatitis C virus infection. HCV, hepatitis C virus; ALT, alanine transaminase. Measurements in arbitary units.

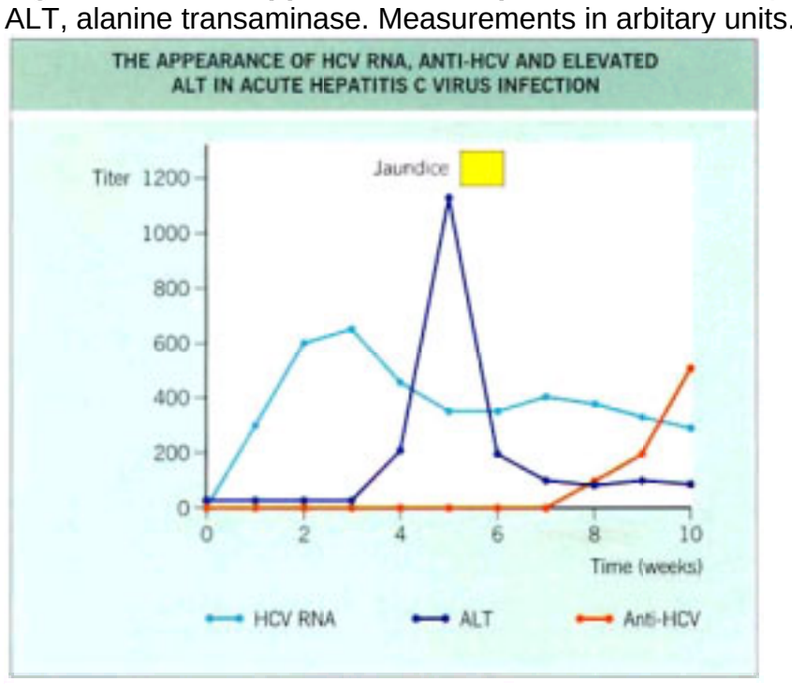


Figure 48-11 Production of hepatitis B core antigen and hepatitis B e antigen and the generation of e antigen-negative mutants. Hepatitis B virus (HBV) has a closed circular genome that contains insufficient bases to produce all its required proteins. It therefore uses different start points for transcription, enabling it to use the same base sequence to produce different proteins. Two important proteins, e antigen and core antigen, are produced by transcription of the same region with overlap; e antigen is produced from the core protein by cleavage at a specific site.

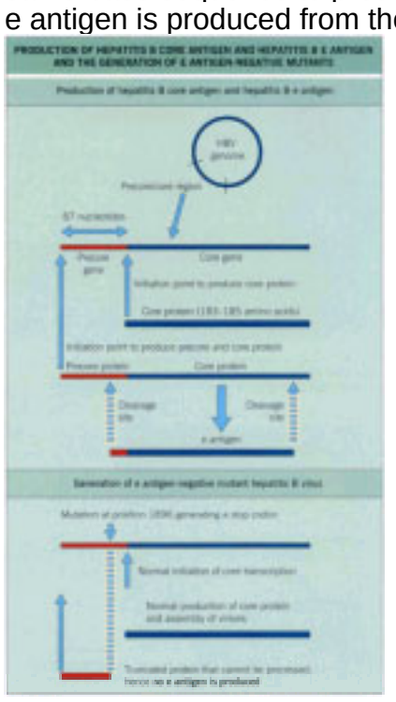


Figure 48-12 Response to interferon therapy in hepatitis B virus infection. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

Figure 48-13 Late seroconversion after interferon therapy for hepatitis B virus infection. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

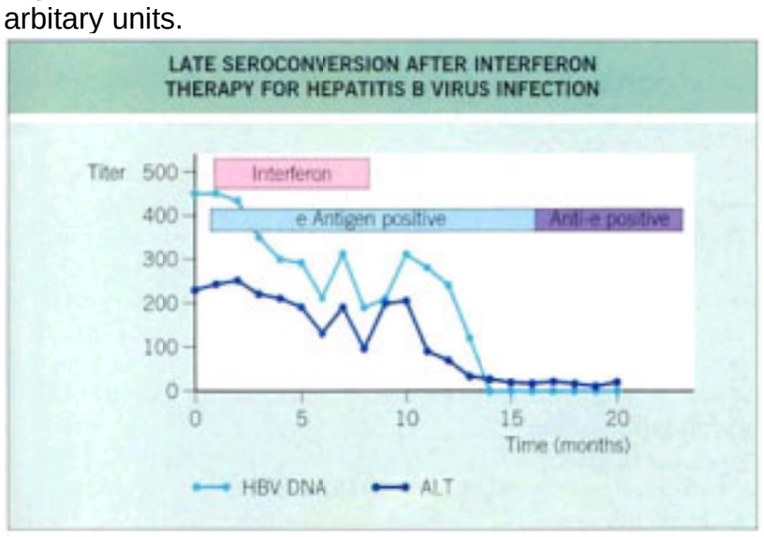


Figure 48-14 Relapse of eAg-negative hepatitis B virus after interferon therapy. HBV, hepatitis B virus; ALT, alanine transaminase. Measurements in arbitary units.

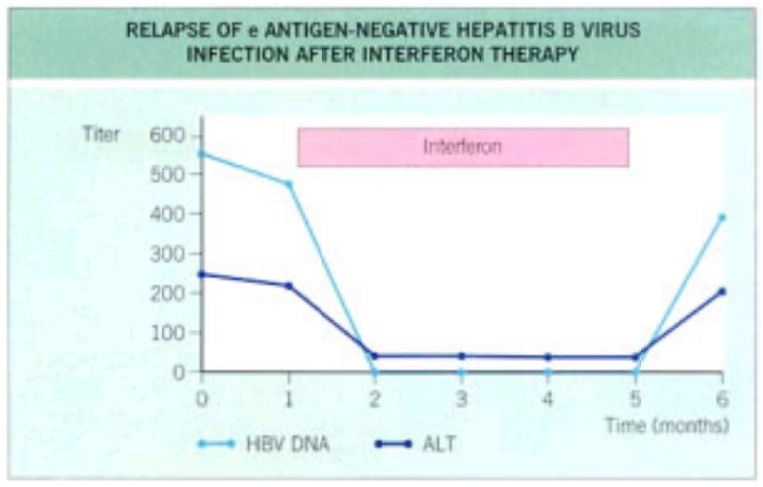


Figure 48-15 Lamivudine therapy in fibrosing cholestatic hepatitis due to recurrent hepatitis B after liver transplantation. HBV, hepatitis B virus. Measurements in arbitary units.

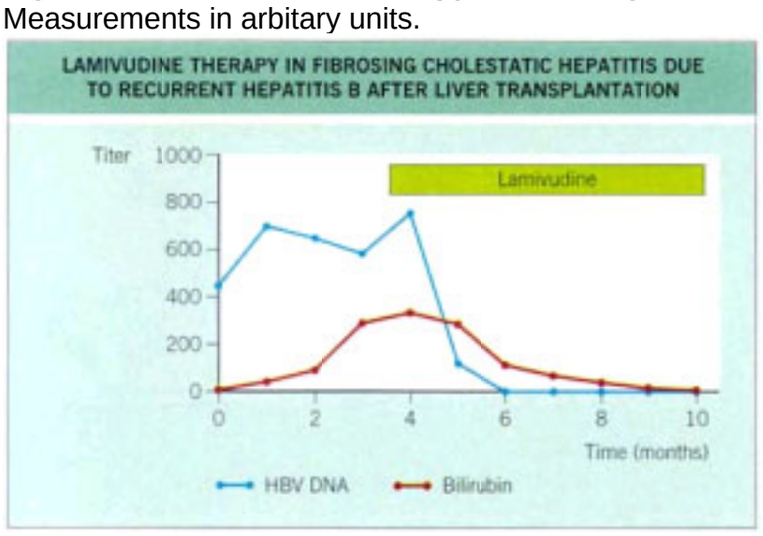


Figure 48-16 Outcome of lamivudine therapy for chronic hepatitis B (e antigen positive patients). e antigen seroconversion and the development of viral resistance both occur.

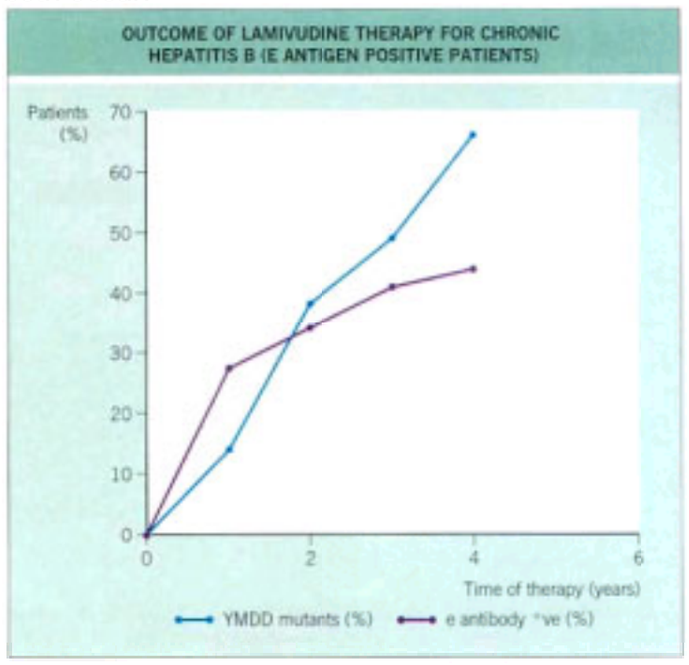


Figure 48-17 Factors predicting response to standard interferon and ribavirin therapy in chronic HCV infection.

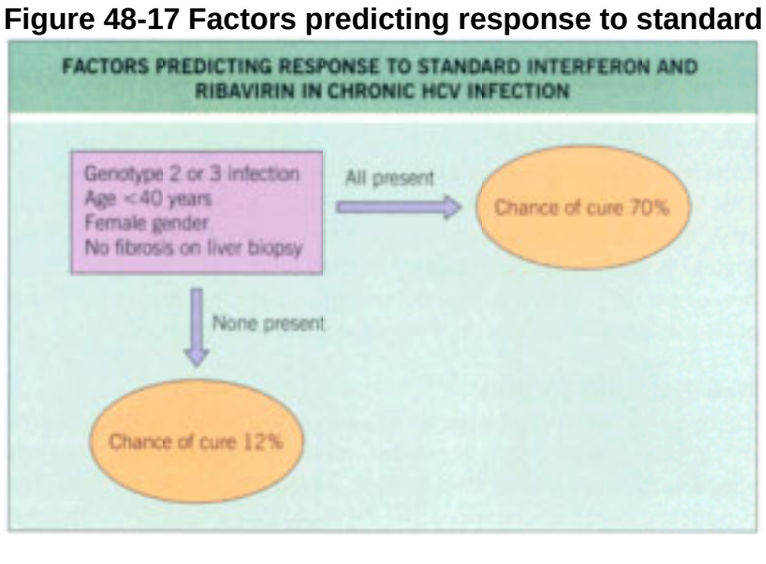


Figure 48-18 Hemolysis with ribavirin — effect of dose reduction.

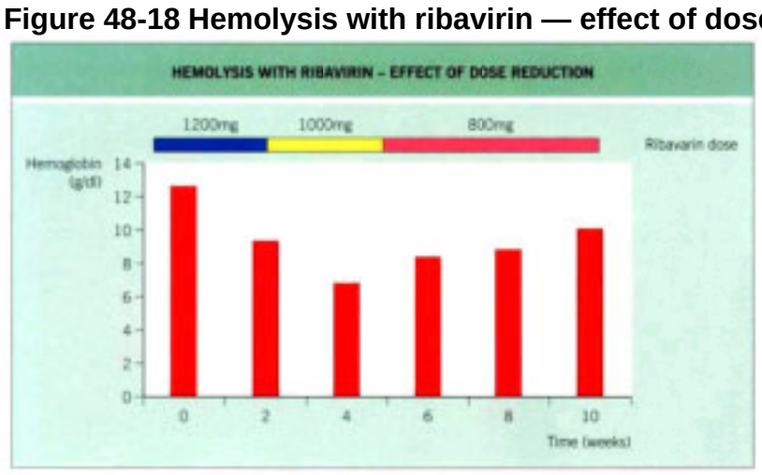


Figure 48-19 Serum interferon levels: pegylated interferon-a versus standard interferon-a.

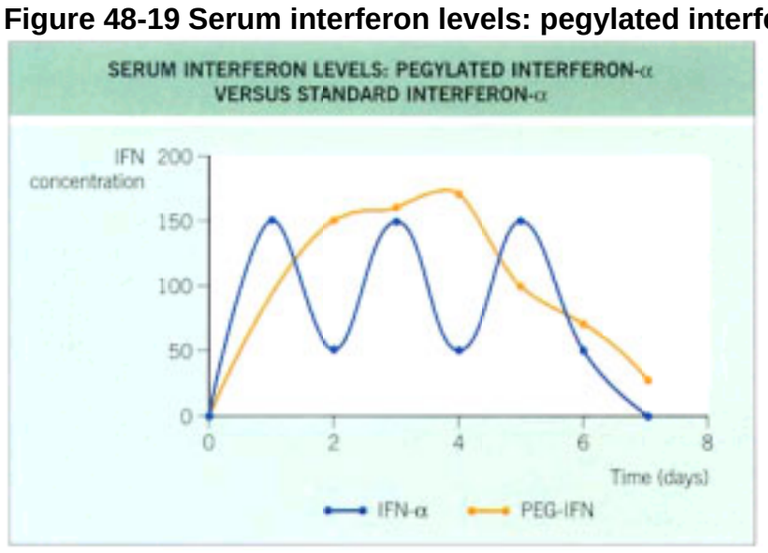


Figure 48-20 PEG-interferons as monotherapy in interferon-naive patients with and without cirrhosis.

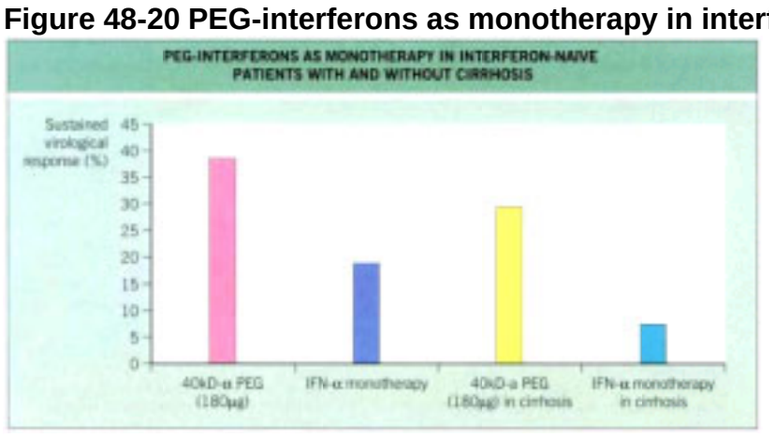


Figure 48-21 Results or therapy with pegylated interferon and ribavirin compared to standard therapy. All based on 12 months' duration of therapy.

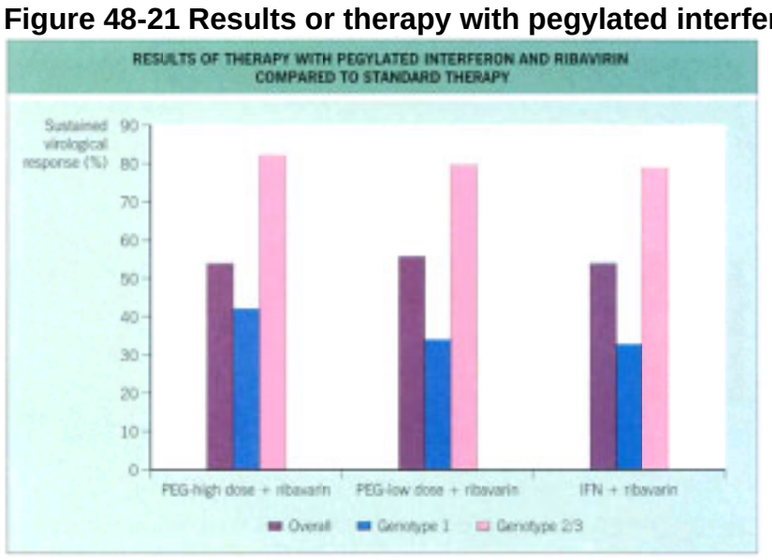


Figure 49-1 Routes of infection.

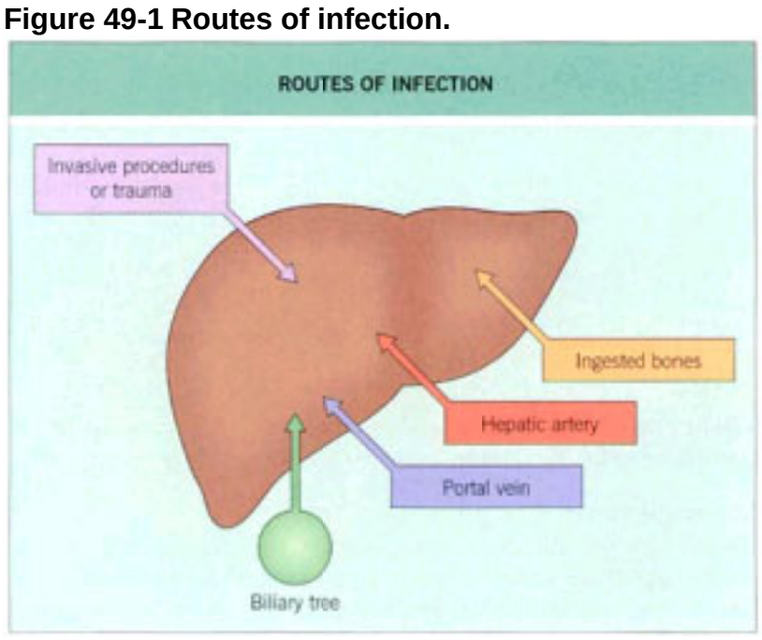


Figure 49-2 Schistosomiasis of the liver. A refractile schistosome ova is located in a portal tract and is associated with an eosinophil-rich granulomatous inflammatory reaction.

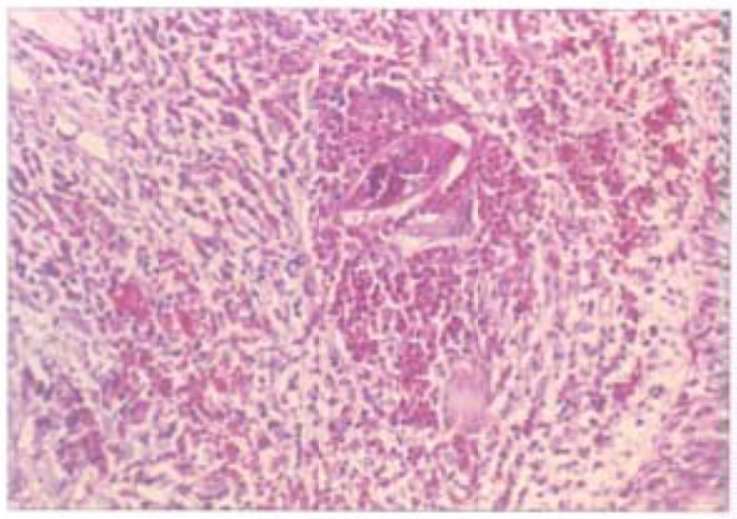


Figure 49-3 Hydatid disease. Hydatid cyst of the liver.

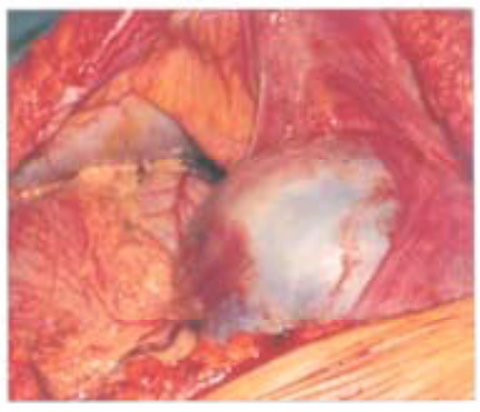


Figure 49-4 Hydatid disease. Hydatid 'daughter cysts'.

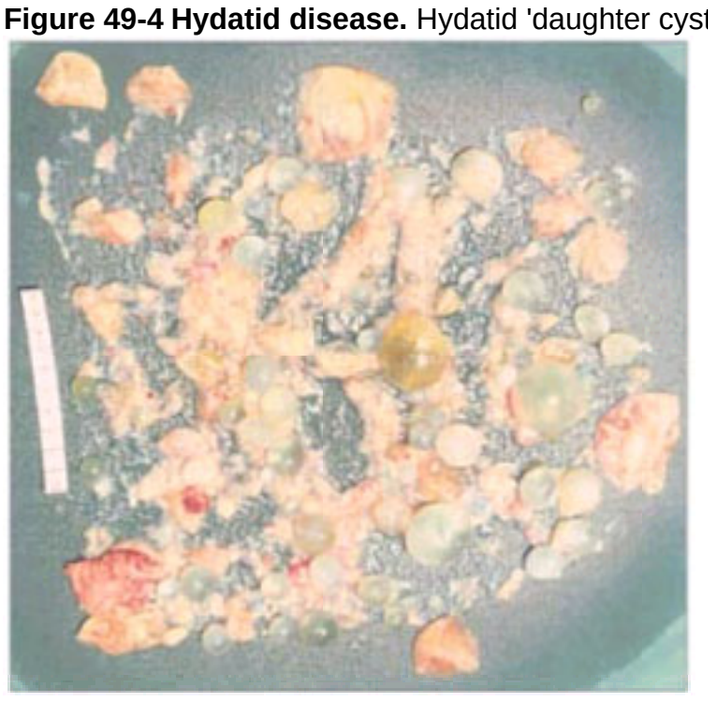


Figure 50.a-1 Point source outbreak.

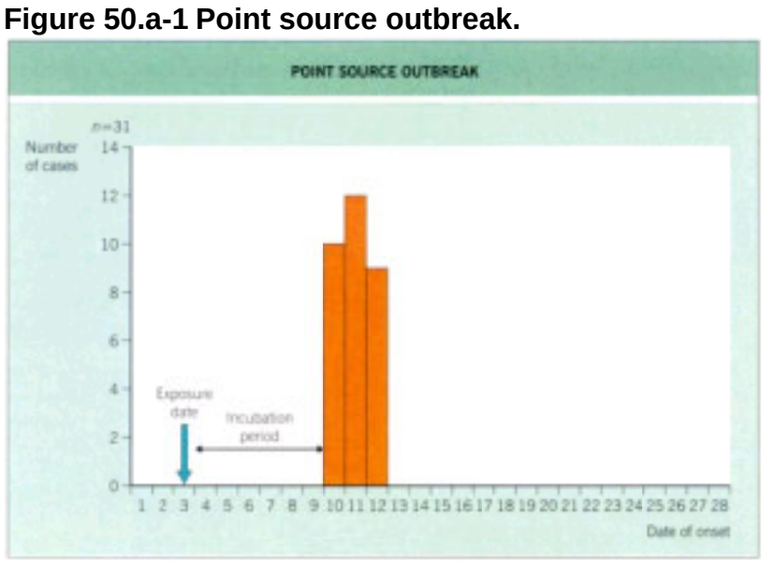


Figure 50.a-2 Common source outbreak.

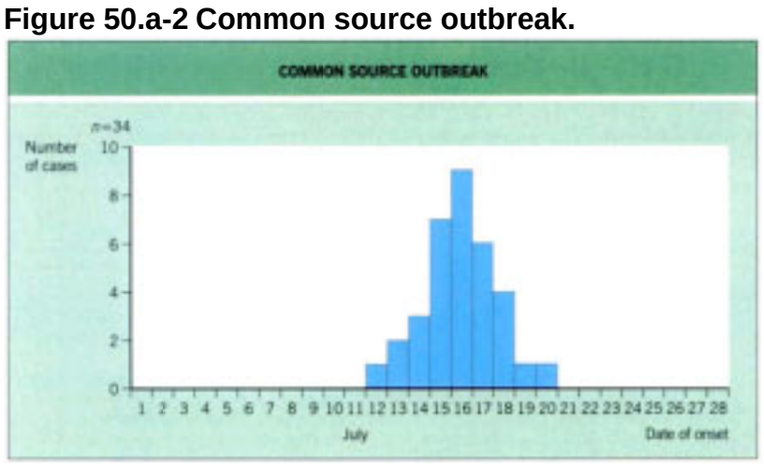


Figure 50.a-3 Propagative outbreak.

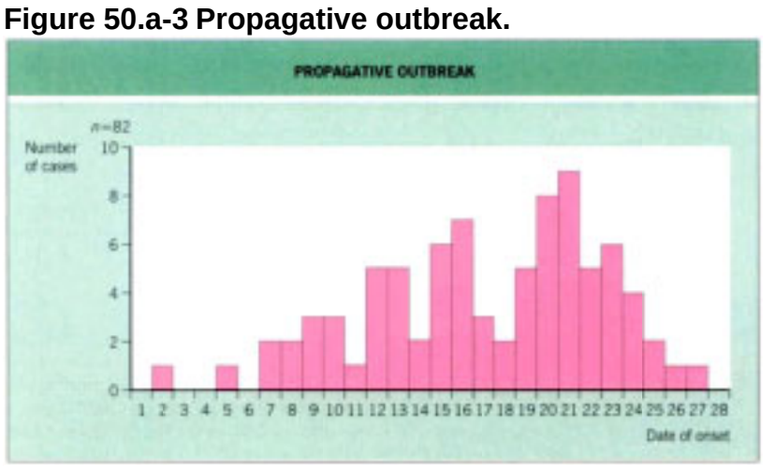


Figure 51-1 Tomogram of right knee of a patient who has *Staphylococcus aureus* septic arthritis and periarticular osteomyelitis. Note the mixed sclerosis and lytic changes suggestive of osteomyelitis.

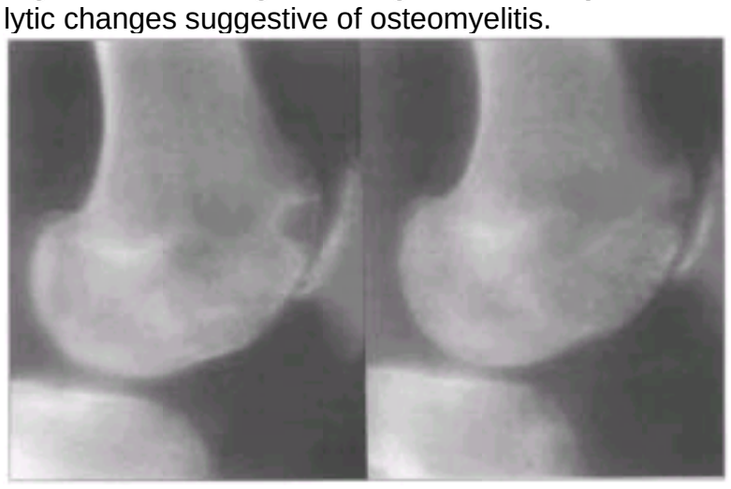


Figure 51-2 MRI scan of right knee of a patient who has *Staphylococcus aureus* septic arthritis. Note the soft tissue inflammation and a joint effusion.

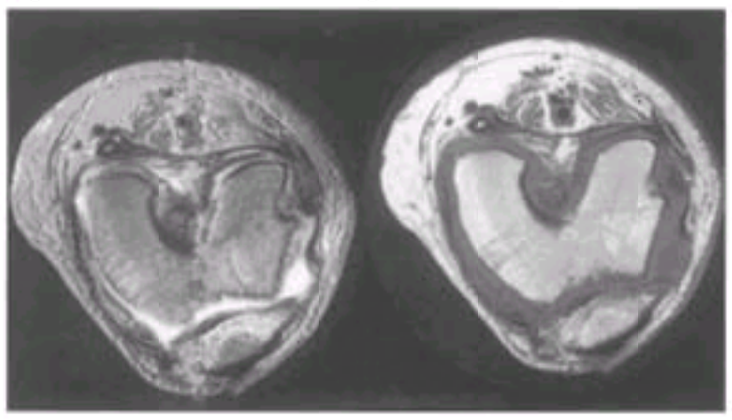


Figure 51-3 Intraoperative photograph of right knee of a patient who has ***Staphylococcus aureus*** **septic arthritis.** Note the damaged joint and dark brown, boggy and hyperemic synovium.

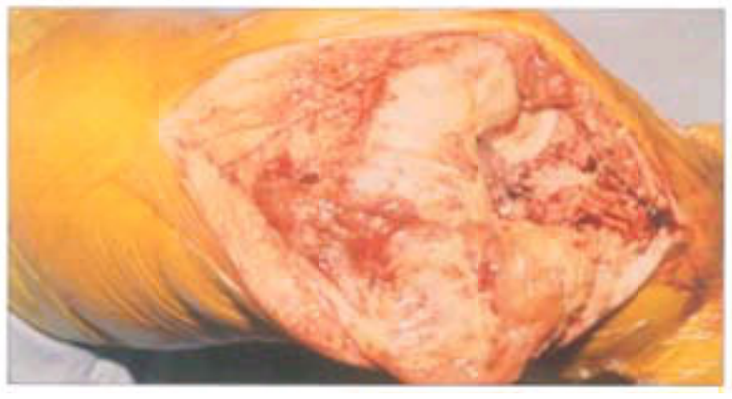


Figure 52-1 Acute hematogenous osteomyelitis in preschool children. *Data from Gillespie.*[1]

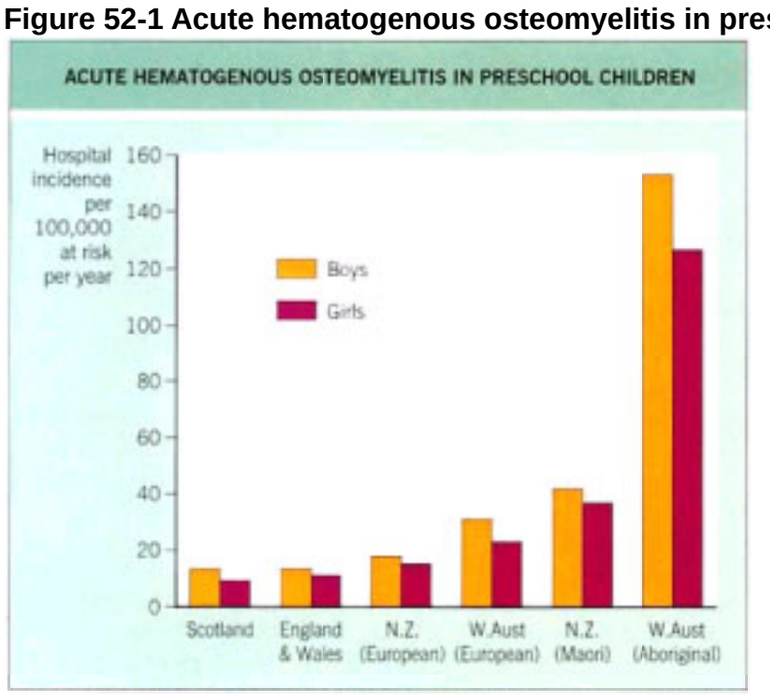


Figure 52-2 Endosteum of bone showing staphylococci near the endosteal haversian canal. In-vitro incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). *From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.*

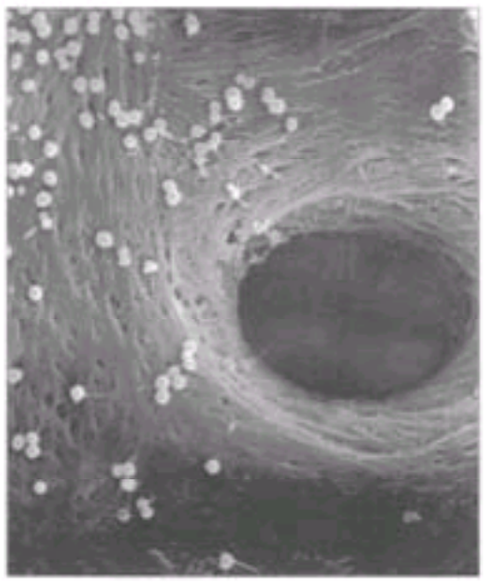


Figure 52-3 Staphylococci enmeshed in glycocalyx near the haversian osteum. In-vitro incubation of bone chips with *Staphylococcus aureus* interrupted at 48 hours (scanning electromicrograph). *From Norden CW, Gillespie WJ, Nade S. Infections in bones and joints. Blackwell Scientific Publications; 1994, with permission.*

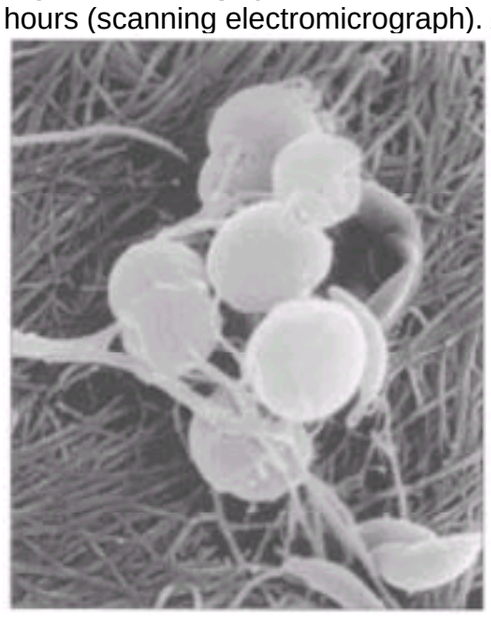


Figure 52-4 Anatomic classification of osteomyelitis in adult long bones. *Adapted with permission from Mader JT, Calhoun J. Osteomyelitis. In: Mandel G, Bennet J, Dolin R, eds. Infectious diseases. New York: Churchill-Livingstone; 1995:1039–52.*

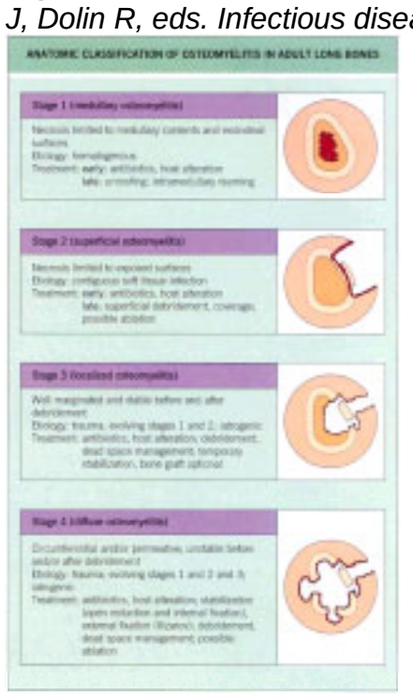


Figure 52-5 Chronic osteomyelitis. The patient is a 30-year-old man who was born in Pakistan and who, as a child, had chronic osteomyelitis caused by *Staphylococcus aureus*. He is asymptomatic now except for occasional pain in the hip and a limp. The radiograph shows destruction of the femoral head and acetabulum, chronic changes in the femoral shaft and fusion of the right hip joint. *Courtesy of Dr Joseph Mammone.*

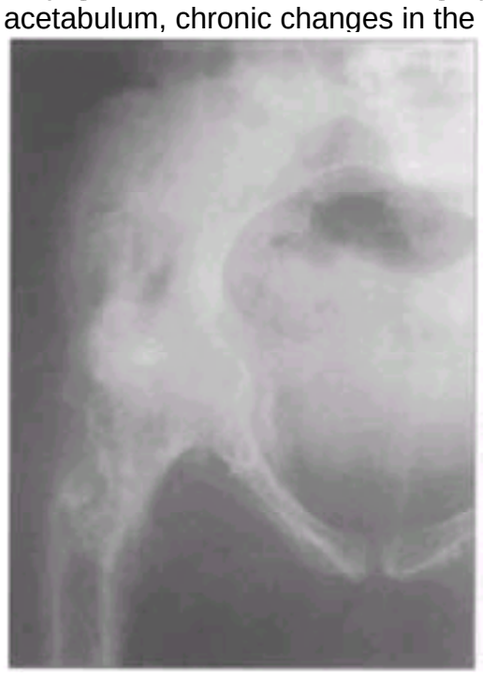


Figure 52-6 Chronic active osteomyelitis in the femur. This case of osteomyelitis was secondary to a fracture and open reduction and internal fixation 30 years before. This axial, contrast-enhanced, fat-suppressed T1-weighted MRI scan shows cortical thickening and a focal intraosseous fluid collection with an enhancing rim, communicating via a sinus tract to the surface of the thigh (arrow).

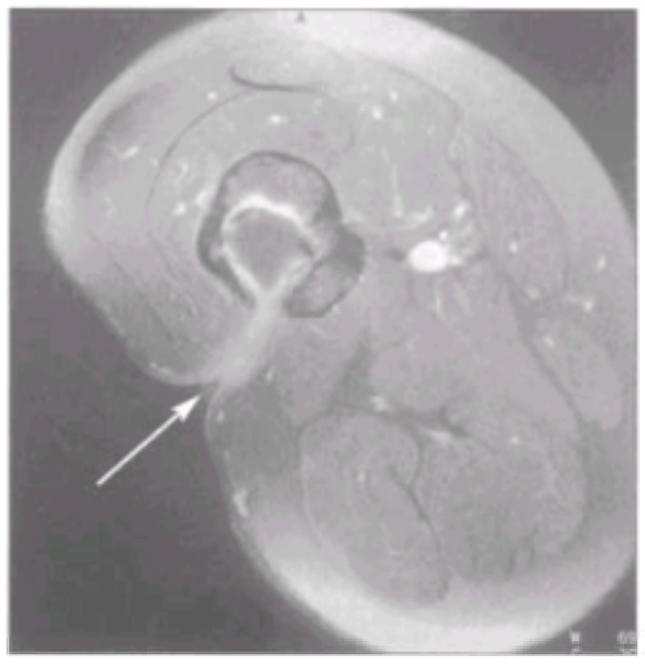


Figure 52-7 Vertebral osteomyelitis. A sagittal, contrast-enhanced convential spin echo MRI scan (T1-weighted) demonstrates a posteriorly located epidural abscess at the L4–L5 vertebral level with an enhancing rim and displacement of the nerve roots anteriorly. *Courtesy of Dr Joseph Mammone.*

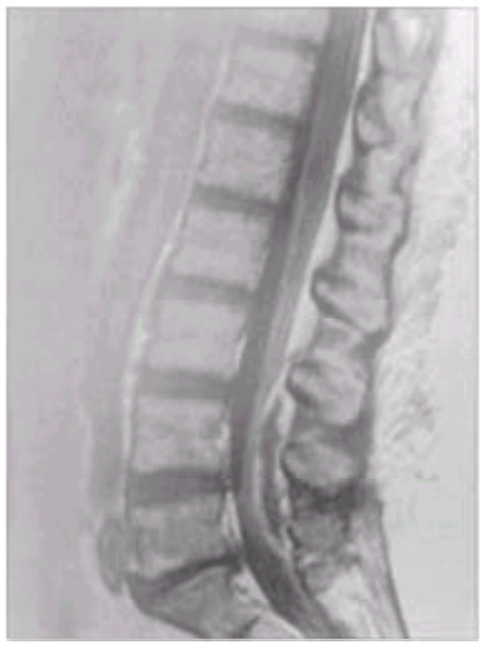


Figure 52-8 Vertebral osteomyelitis. A sagittal, turbo spin echo MRI scan (T2-weighted) from the same patient as the scan in Fig. 52.7 . *Courtesy of Dr Joseph Mammone.*

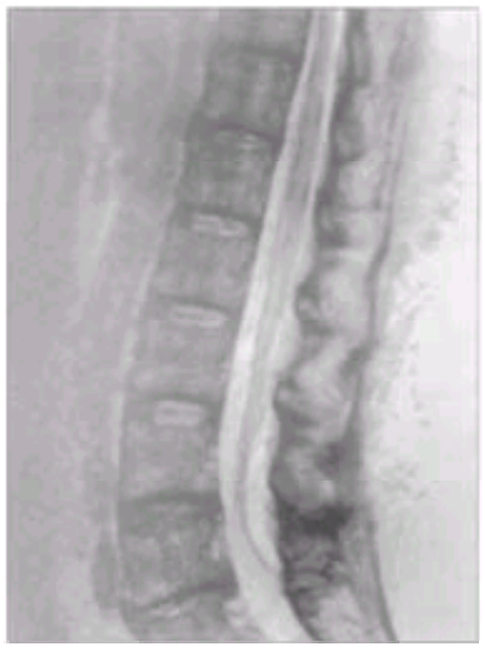


Figure 52-9 Vertebral osteomyelitis. A myelogram showing posterior compression of the spinal cord by an inflammatory mass. Note the involvement of adjacent vertebral endplates and the intervertebral disc. *Courtesy of Dr Joseph Mammone.*

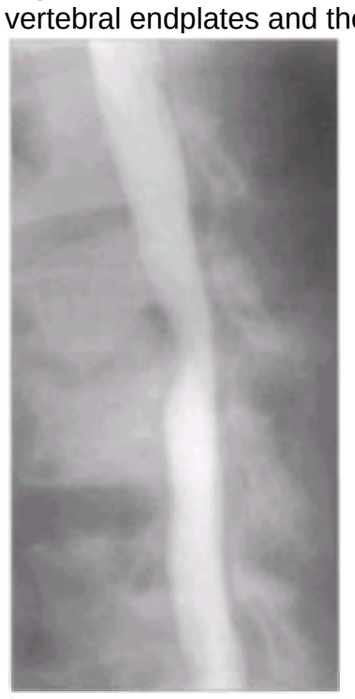


Figure 52-10 Osteomyelitis in a diabetic patient. Diabetic patient with osteomyelitis and destruction of proximal second phalanx and metatarsal as well as second metatarsal-phalangeal joint. *Courtesy of Dr Joseph Mammone.*

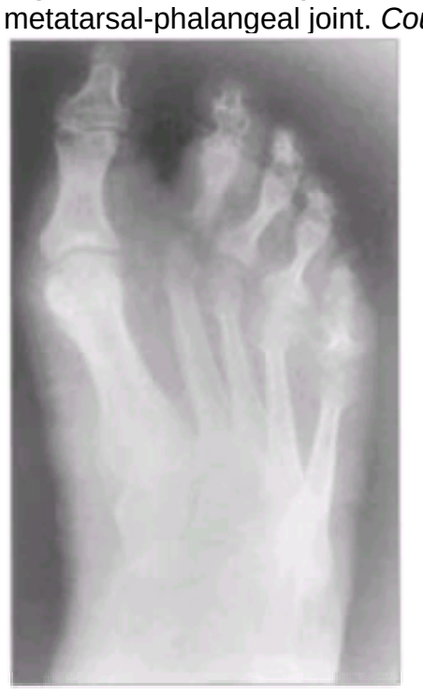


Figure 52-11 Investigation and management of chronic osteomyelitis.

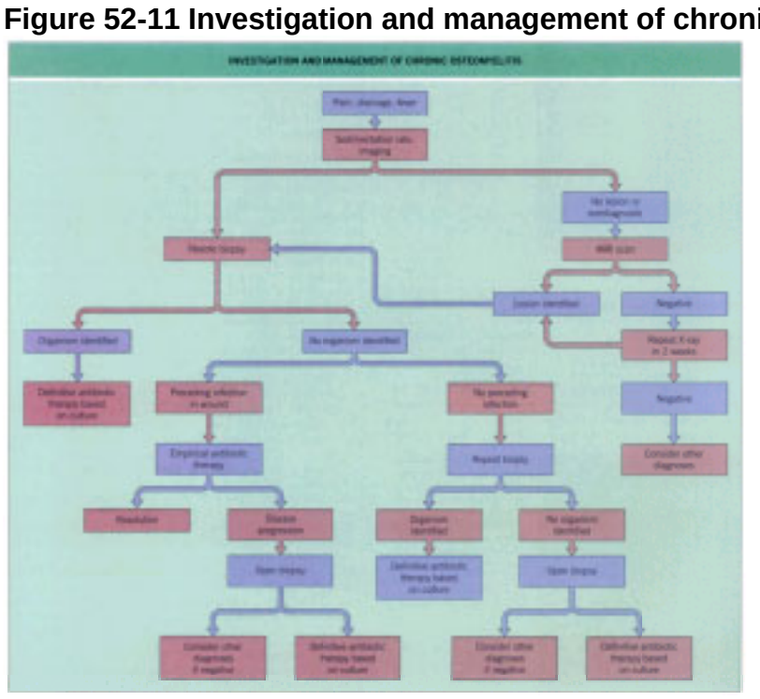


Figure 52-12 Twenty-four-hour bone scintigram of the hands. The patient is a 50-year-old diabetic with a draining ulcer at the top of the right thumb (arrow). A biopsy grew *Staphylococcus aureus*. There is intense uptake in distal first phalanx and in multiple neuropathic joints. *With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.*

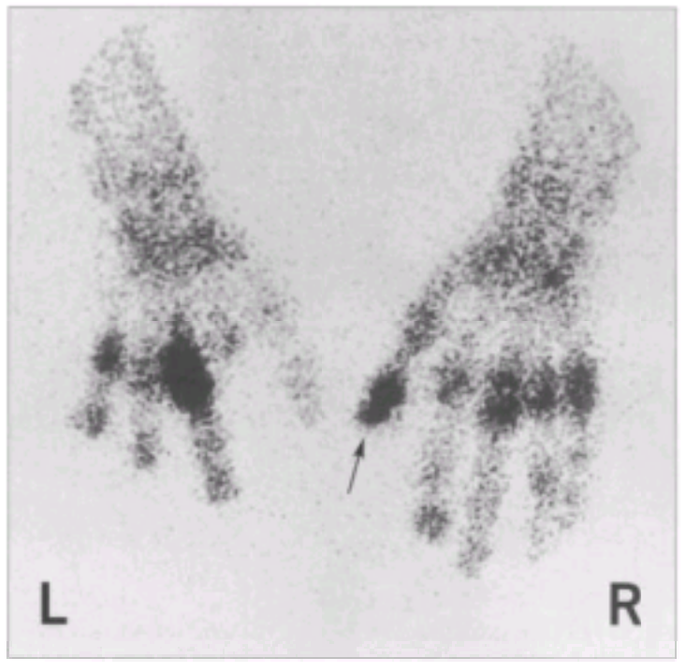


Figure 52-13 Leukocyte scintigram of the hands. This scan is from the same patient as the scan in Fig. 52.12 . Again there is intense uptake in distal first phalanx, but there is no accumulation of leukocytes in the multiple neuropathic joints. *With permission from Jacobson AF, Harley J, Kipsky B, Pecoraro R. Diagnosis of osteomyelitis in the presence of soft tissue infection and radiologic evidence of osseous abnormalities. AJR Am J Roentgenol 1991;157:807–12.*

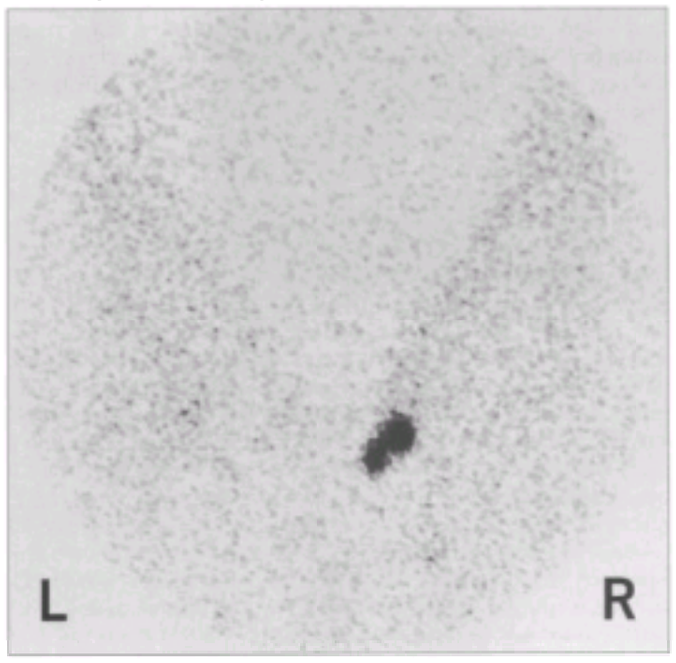


Figure 52-14 T1-weighted image of the foot. The scan reveals forefoot amputation and a normal signal in distal tibia, talus and posterior calcaneus. The interior portion of the calcaneus has edema. The remainder of the tarsal bones have been destroyed and replaced by a pale, heterogeneous inflammatory mass.

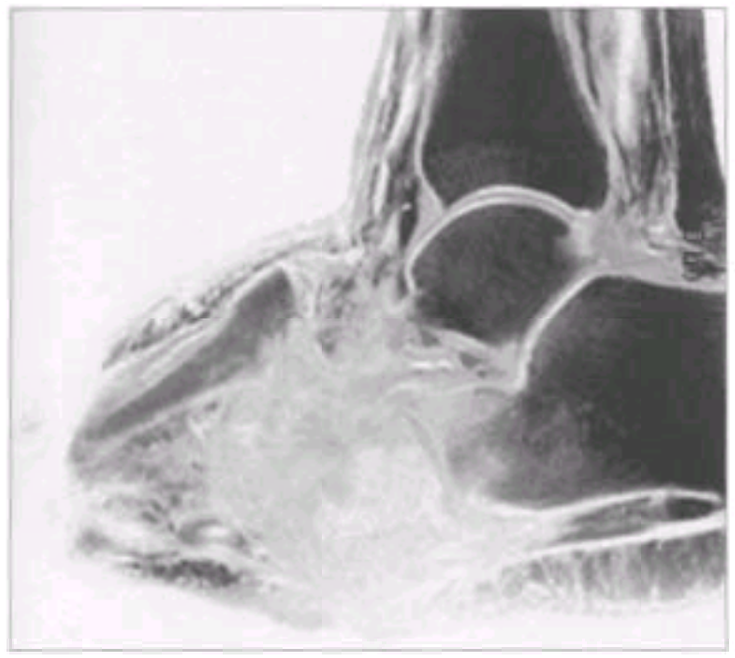


Figure 52-15 Diabetic foot infections. Algorithmic approach to diagnosis and management. *Adapted with permission from Lipsky et al.*[3]

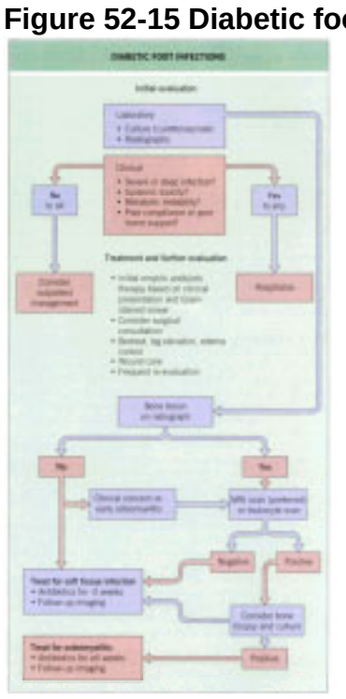


Figure 53-1 Bacterial adherence to the implant, bacterial multiplication on the surface and biofilm formation. An ineffective host response triggers bone resorption, contributing to loosening, which is accelerated by wear particles and the host response to them.

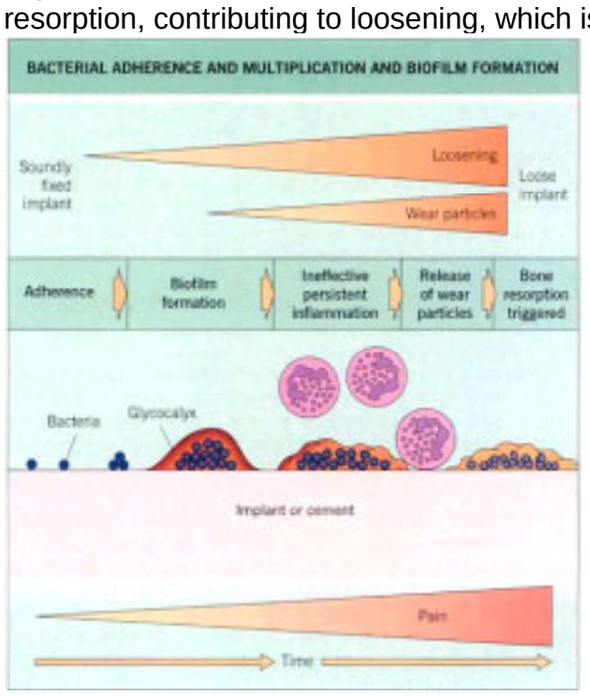


Figure 53-2 Histologic features of infection. Periprosthetic tissue from a clinically infected total hip replacement from which multiple specimens grew an indistinguishable organism. Numerous neutrophils are present in the tissue.

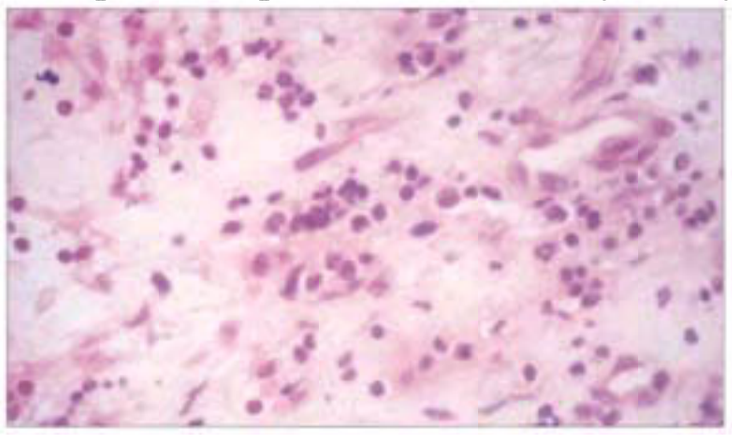


Figure 53-3 An acutely infected knee replacement. The site was washed out but the infection failed to resolve. At reoperation the implant was found to be loose and it needed to be removed. *Staphylococcus aureus* was grown from deep specimens.

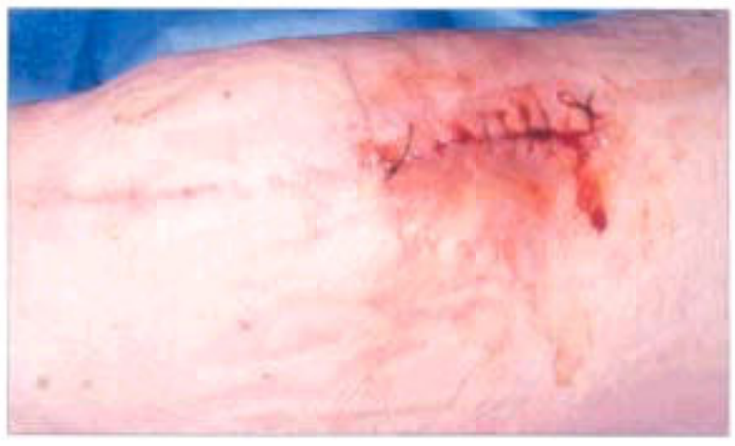


Figure 53-4 A sinus tract discharging from an infected total hip replacement. *Staphylococcus aureus* was grown from deep specimens. Note the Koebner phenomenon; this patient's psoriasis was probably a significant risk factor for infection.

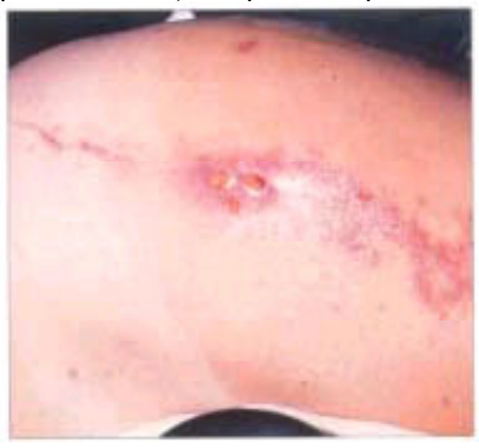


Figure 53-5 Implant loosening in late infection. (a) Radiograph of the infected hip shown in Figure 53.4 . There is an obvious radiolucent line at the bone-cement interface. This implant required revision. (b) Radiograph of a loose knee replacement, showing resorption of bone beneath the tibial component, a cause of instability. Coagulase-negative staphylococci were grown from multiple deep specimens.

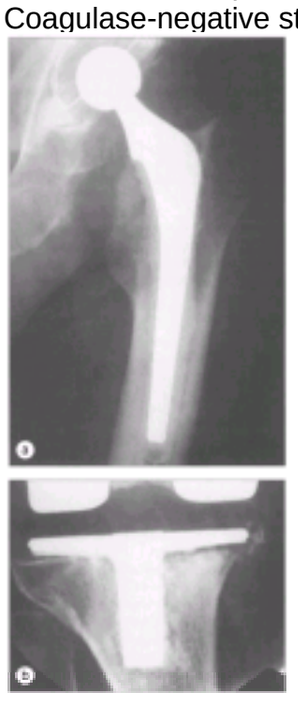


Figure 54-1 Life cycle of *Ixodes scapularis* (also known as *Ixodes dammini*). The life cycle spans 2 years. Eggs hatch in the spring; six-legged larvae develop and feed once in the summer, acquiring *Borrelia burgdorferi* from their preferred host, the white-footed mouse. Next spring, the larvae molt into eight-legged nymphs, which feed once; mice are the preferred host, humans not being necessary for the ticks' life cycle. The nymphs molt into adult male and female ticks; mating often occurs while the female feeds on a deer, and the male may remain on the deer, the female falling off and then laying eggs. Adapted, with permission, from an illustration by Nancy Lou Makris in Rahn and Malawista.[30]

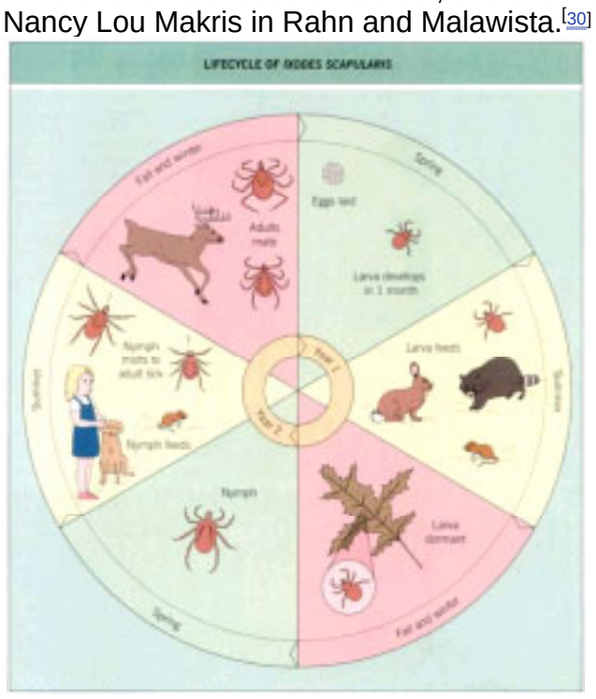


Figure 54-2 ***Ixodes scapularis.*** Larva, nymph, adult male and adult female. *Courtesy of Pfizer Central Research.*

Figure 54-3 Erythema migrans. A typical annular, flat, erythematous lesion with a sharply demarcated border and partial central healing. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

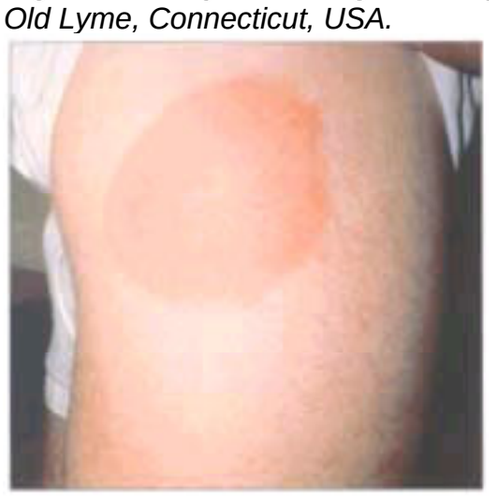


Figure 54-4 Erythema migrans. A lesion with variation in color and a target-like appearance. The bite site is visible in the center. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

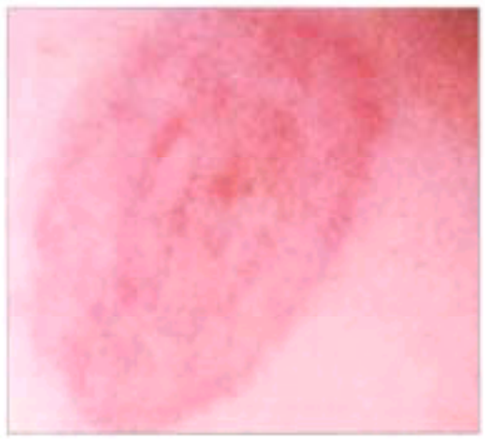


Figure 54-5 Erythema migrans. A lesion with a dusky center, a common variant. *Courtesy of Dr Steven Luger.*

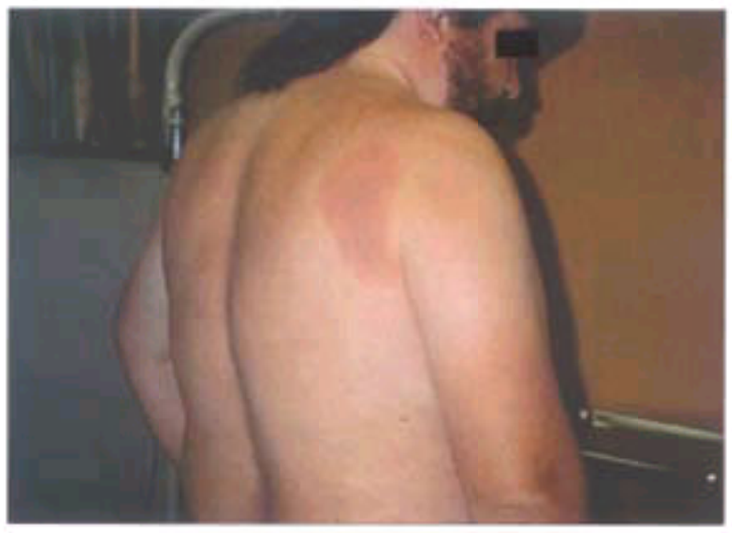


Figure 54-6 Multiple erythema migrans lesions. Lateral (a) and posterior (b) views of the same patient with multiple erythematous macules of EM. Secondary lesions result from hematogenous spread. They may occur anywhere in the body. Secondary lesions are usually of uniform color and lack in duration. *Courtesy of Dr Steven Luger, Old Lyme, Connecticut, USA.*

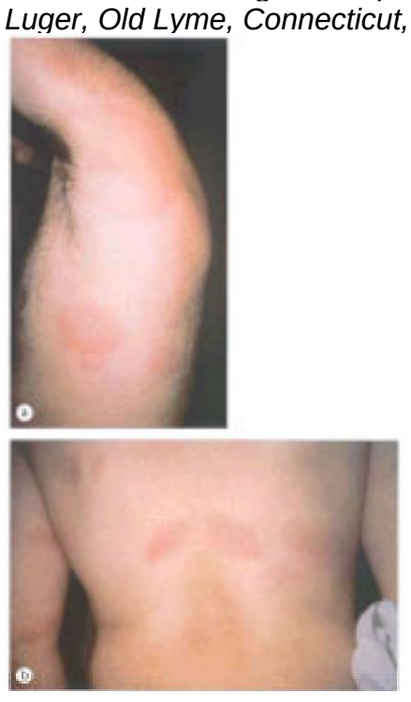


Figure 54-7 Acrodermatitis chronicum atrophicans. Typical inflammatory bluish-red lesions of acrodermatitis chronicum atrophicans. Lesions usually occur on acral portions of extremities. *Courtesy of Dr Eva Asbrink.*

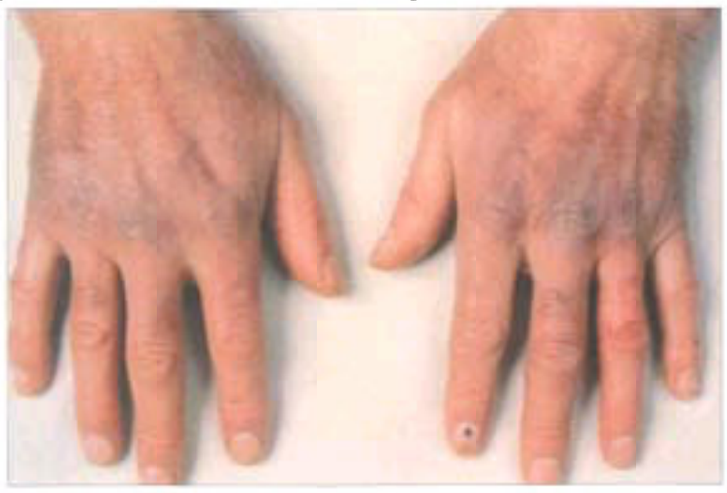


Figure 54-8 The usual serologic response in Lyme disease. Specific IgM becomes detectable 1–2 weeks after symptom onset and the appearance of erythema migrans. The later appearance of IgG is frequently concurrent with systemic manifestations. IgG is nearly always elevated with late disease. Typically, and even in untreated patients, IgM falls over 4–6 months; persistence for longer than this predicts later manifestations.

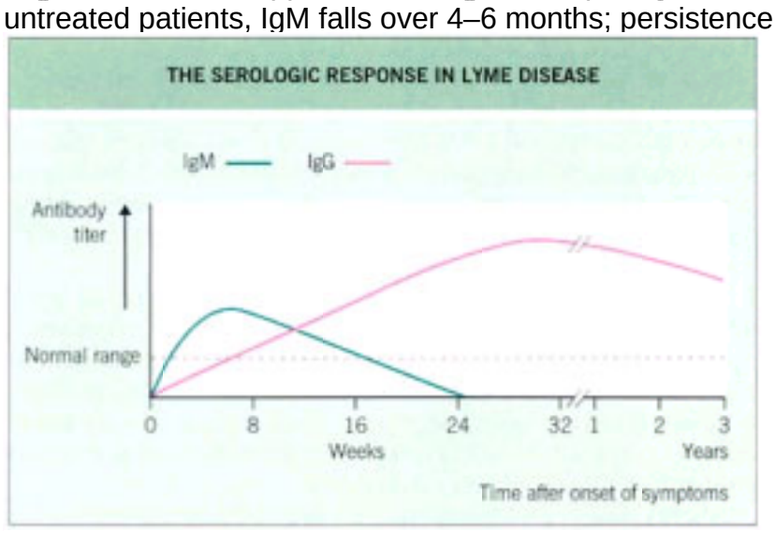


Figure 56-1 Potential risk factors leading to sepsis.

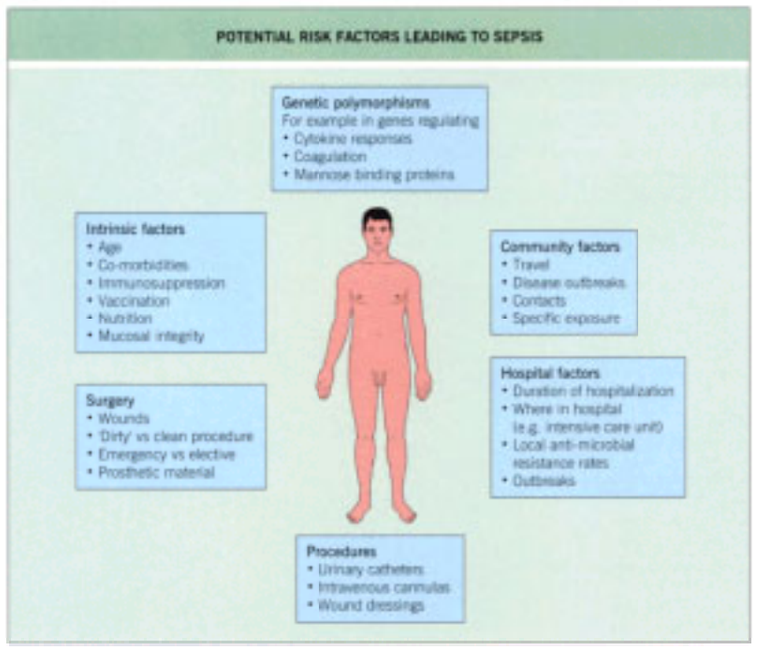


Figure 56-2 Positive blood cultures in severe sepsis. *Data from Bochud et al.* [42]

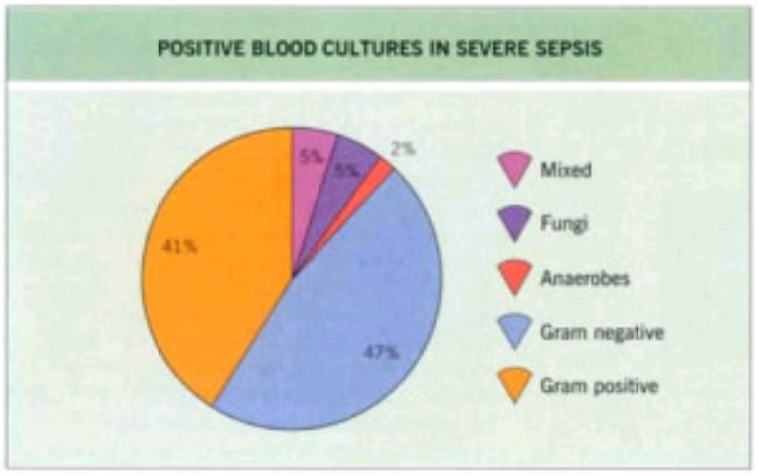


Figure 56-3 The Gram-negative bacterial cell wall. In the cell wall of a Gram-negative bacteria such as *Escherichia coli*, the inner membrane is composed of phospholipids and membrane proteins and is separated from the outer membrane by the periplasmic space and peptidoglycan. Lipopolysaccharide (expanded box) is found only in the outermost leaflet of the outer membrane with the lipid A moiety in the membrane and the polysaccharide (O) side chain directed outwards. Lipid A is highly conserved across Gram-negative bacteria and consists of a phosphorylated diglucosamine backbone decorated with six or seven acyl side chains. Dephosphorylation or deacylation of lipid A abrogates its toxicity. Lipid A is covalently linked to an inner core of sugar residues that is relatively well conserved across species and antibodies directed against the core may protect against challenge with heterologous Gram-negative bacilli. The core is followed by an outer polysaccharide chain, of repeating sugar residues, that varies between different of bacterial strains (O antigen). Antibodies directed against the O antigen will only protect against challenge with that individual bacterial strain. Gal, D-galactose; Glc, D-glucose; GlcNAc, *N*-acetyl-D-glucosamine; KDO, 3-deoxy-D-manno-octuylosonic acid; Hep, L-glycero-D-mannoheptose.

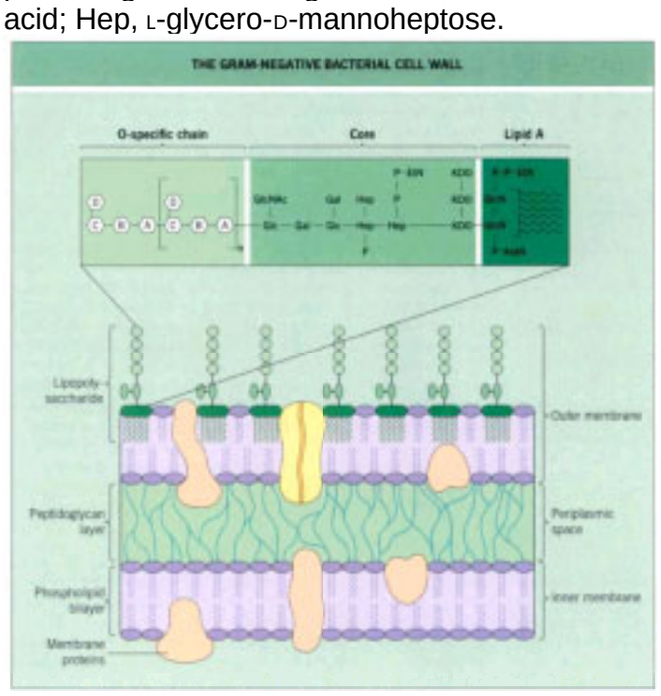


Figure 56-4 CD14 and toll receptor pathway of cellular activation by bacterial lipopolysaccharide. Schematic representation of events at the inflammatory cell surface. Lipopolysaccharide (LPS), either free or as part of lipoprotein complexes, is bound by LPS-binding protein (LBP) in the fluid phase. The LPS-LBP complex binds to the cell surface receptor CD14 on neutrophils and macrophages. CD14 lacks an intracellular domain and acts as a co-receptor presenting LPS to toll-like receptor 4 (TLR4), leading in turn to activation of intracellular signalling and gene activation. MYD88, myeloid differentiation factor 88; NF?B, nuclear factor kappa B; I?B, inhibitor of kappa B; IRAK, interlkeukin 1 receptor-associated kinase; TRAF6, tumour necrosis factor receptor-associated factor 6; MAP3K, mitogen-activated protein 3-kinase

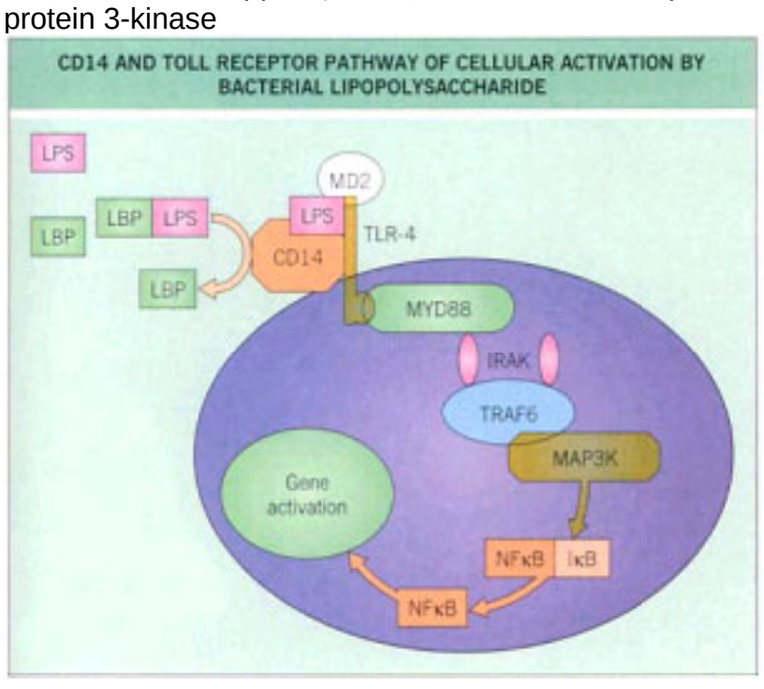


Figure 56-5 T-cell activation by superantigens. In the conventional response to bacterial antigens (bottom) the antigen is processed and presented by the antigen-presenting cell (APC) in association with MHC II. Only T cells with the correct antigen recognition site can then be activated (i.e. this is a highly antigen-specific process). Superantigens (top) are able to bypass this process by bridging between MHC II and the Vß subunit of the T-cell receptor. Thus the entire population of T cells expressing that particular Vß subunit can be activated; this can be up to 20% of the total T-cell population. *Adapted from Sriskandan and Cohen. The pathogenesis of septic shock. J Infect 1995;30:201–6.*

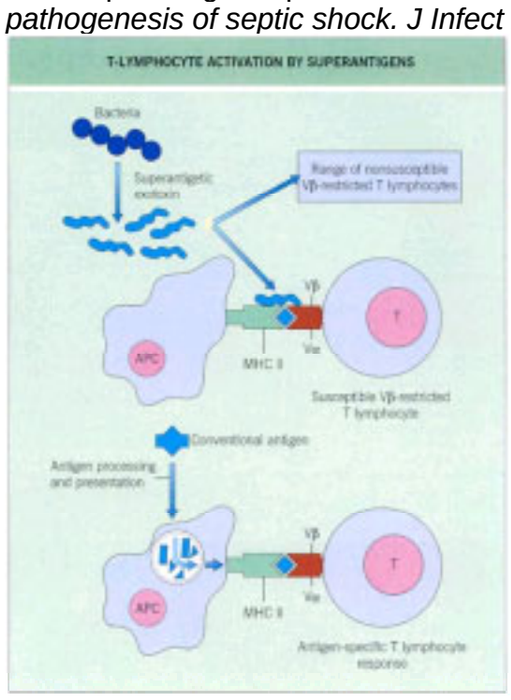


Figure 56-6 Interaction of inflammatory pathways in severe sepsis. ARDS, adult respiratory distress syndrome; IFN-?, interferon-?; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; LBP, LPS-binding protein; LPS, lipopolysaccharide; NO, nitric oxide; TGF-ß, transforming growth factor-ß; sTNFr, soluble TNF receptor.

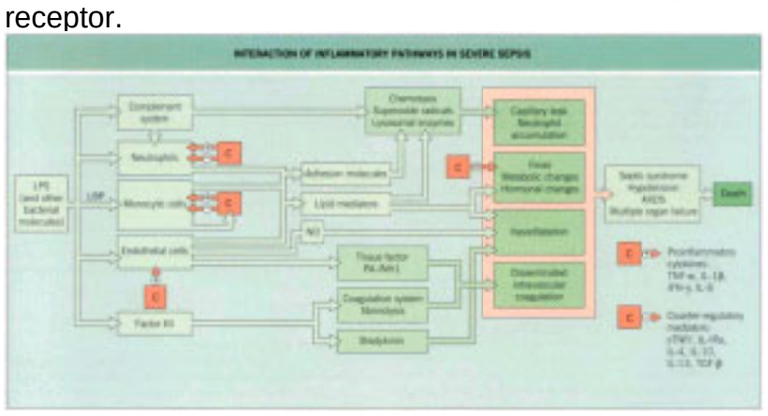


Figure 56-7 Organ failure in severe sepsis. Organ dysfunction and finally organ failure is the result of cellular hypoxia and acidosis. Hypoxia and acidosis result both from a failure of oxygen delivery due to disturbances in circulation and limitation of the cellular ability to use oxygen as a result of mitochondrial dysfunction.

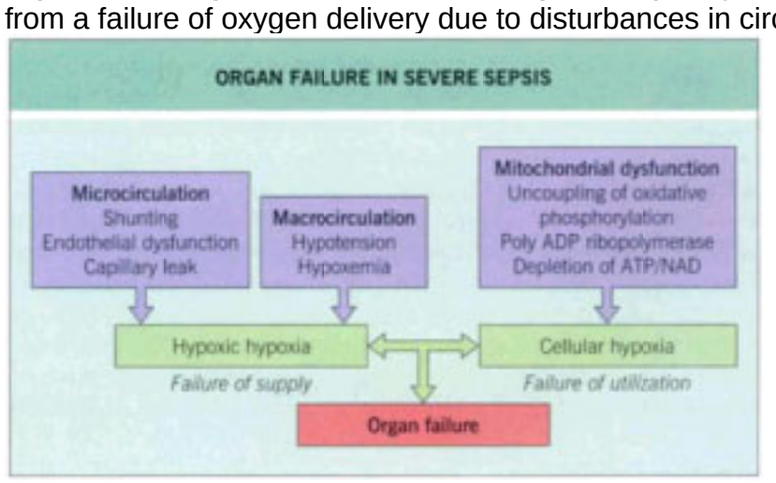


Figure 56-8 Systemic manifestations of Gram-negative bacterial sepsis. Observation chart of a 49-year-old woman admitted to hospital with a suspected drug fever. For the first 24 hours she was observed without antimicrobial chemotherapy and demonstrated a persistent fever and tachycardia (sepsis). At this point she suddenly became confused, hypotensive and oliguric indicating the development of severe sepsis. The underlying cause was an *Escherichia coli* bacteremia from an unsuspected urinary tract infection. She responded to fluid replacement and antibiotics and made a full recovery.

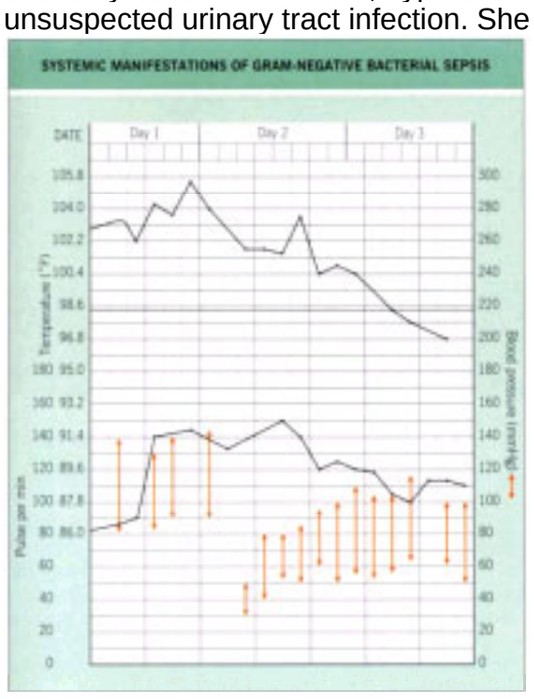


Figure 56-9 Toxic shock syndrome. A 30-year-old woman presented with a short history of fever, breathlessness, diarrhea and abdominal pain. She was pyrexial and hypotensive with a blanching macular rash noted on day 2. Blood cultures grew a Group A ß-hemolytic streptococcus. Laparotomy revealed 800ml of peritoneal pus with no intestinal perforation consistent with spontaneous bacterial peritonitis. She required ventilatory support plus inotropes to maintain blood pressure for 6 days and developed evidence of disseminated intravascular coagulation (DIC) and adult respiratory distress syndrome (ARDS). She made a gradual recovery complicated by an enterococcal urinary tract infection, finally leaving hospital 27 days after presentation. The group A streptococcal isolate from her blood was subsequently shown to release streptococcal mitogenic exotoxin Z (SMEZ) a potent superantigen. *Courtesy of P Gothard and S Sriskandan, Hammersmith Hospital, London.*

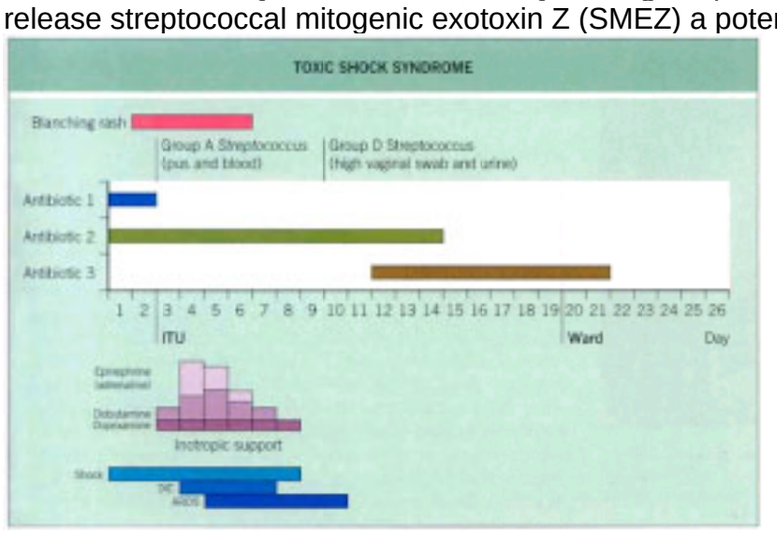


Figure 56-10 Cutaneous changes in meningococcal infection. (a) Petechial hemorrhages are the hallmark of meningococcal infection and may be found on the periphery or as in this case the conjunctivae. (b) In severe disease the purpura may become confluent (purpura fulminans) and lead to severe digital gangrene. *Courtesy of J Cohen, Brighton.*

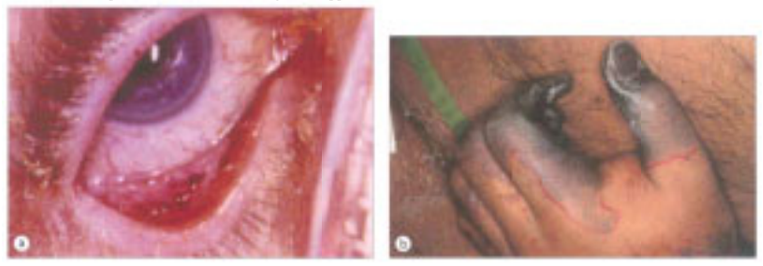
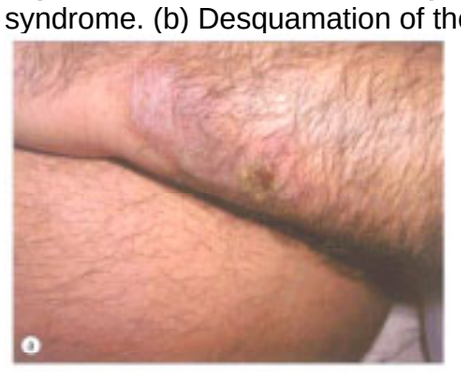


Figure 56-11 Cutaneous changes in toxic shock. (a) Localized infection at the edge of a patch of eczema in a patient presenting with staphylococcal toxic shock syndrome. (b) Desquamation of the palm following an episode of staphylococcal toxic shock. *Courtesy of M Jacobs, London.*



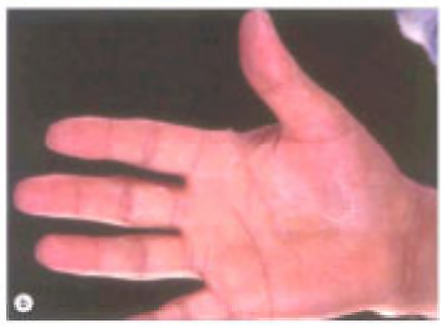


Figure 56-12 Acute tubular necrosis. The tubules are dilated with flattened epilithial cells, and contain debris; the glomerulus is not greatly affected. Hematoxylin and eosin. *With permission from Williams JD et al., Clinical Atlas of the Kidney. London: Mosby.*

Figure 56-13 Acute hemorrhagic necrosis of the adrenal glands (Waterhouse-Friderichsen syndrome). Both adrenal glands of a child with meningococcal septicemia show hemorrhagic necrosis leading to acute adrenal failure. *With permission from Stevens A, Lowe J. Pathology. London: Mosby; 1995.*

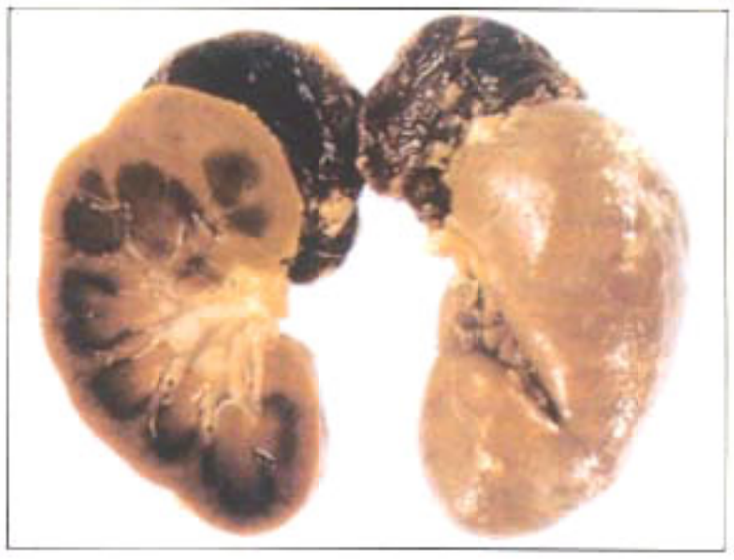


Figure 56-14 Radiologic detection of occult foci of infection in severe sepsis. Plain abdominal radiograph in a diabetic with an *Escherichia coli* urinary tract infection and pyelonephritis who had developed hypotension and oliguria. The radiograph reveals gas around the kidney due to a perinephric abscess requiring urgent drainage.

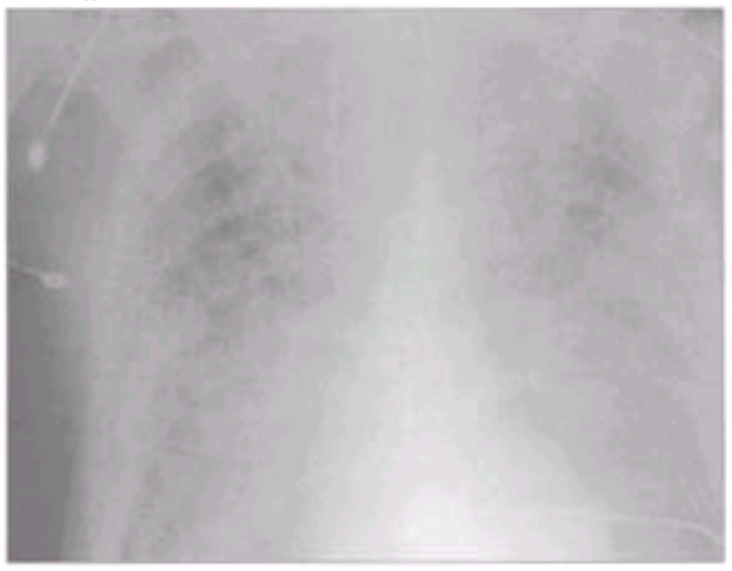


Figure 57-1 Potential sources of infection of a percutaneous intravascular device (IVD). These include contiguous skin flora, contamination of the catheter hub and lumen, contamination of infusate and hematogenous colonization of the IVD from distant, unrelated sites of infection. HCW, health care worker. *From Crnich and Maki.*[23]

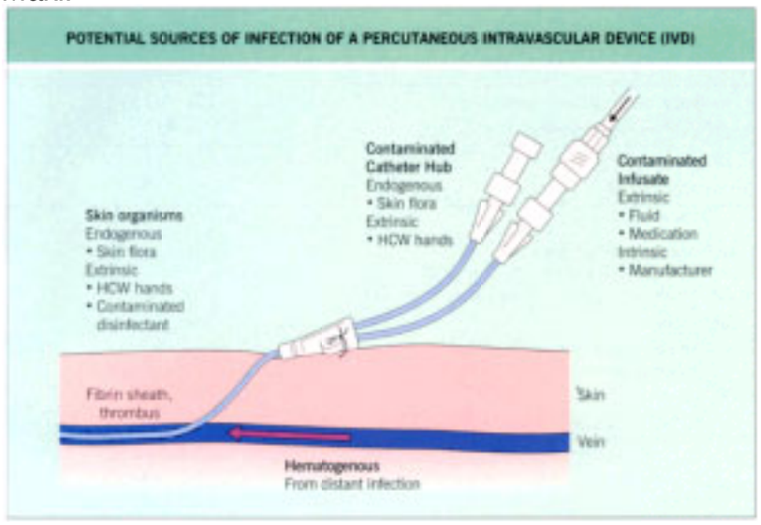


Figure 57-2 Microbial profile of intravascular device-related bloodstream infection. Based on an analysis 159 published prospective studies, *from Maki et al.*[10]

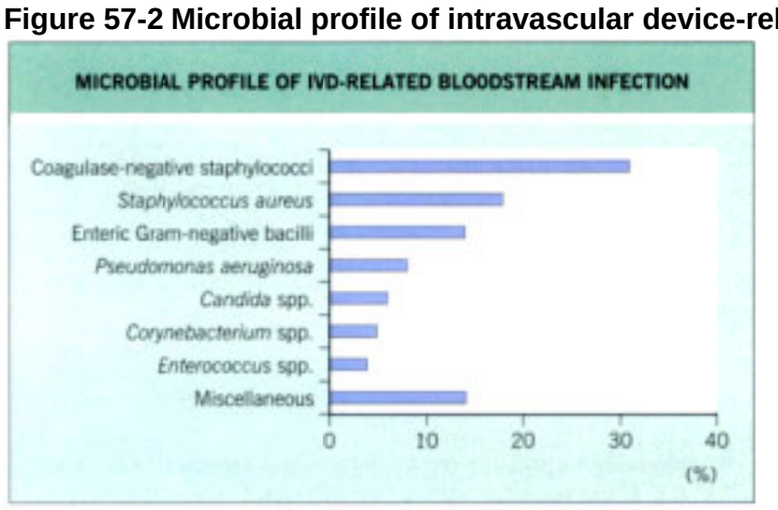


Figure 57-3 Thrombus extracted surgically from distal subclavian vein. *From Andes et al.* [29]

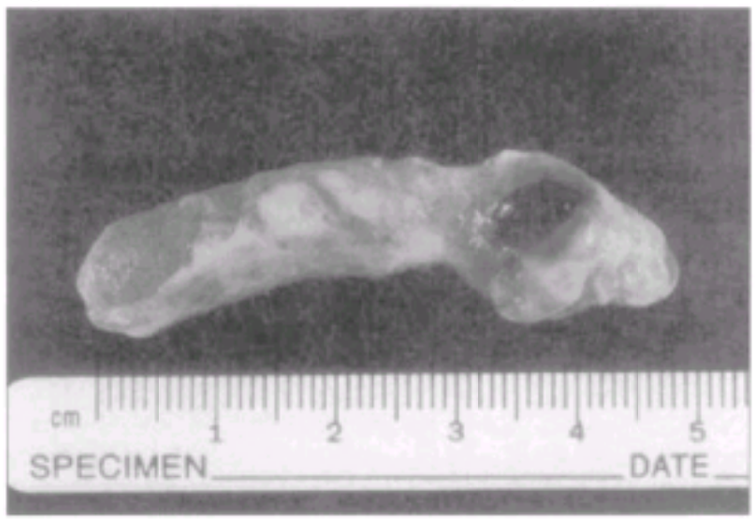


Figure 57-4 Microbial profile of infections of prosthetic arterial grafts by location of the implanted graft. *From Goëau-Brissonniere and Coggia.*[43]

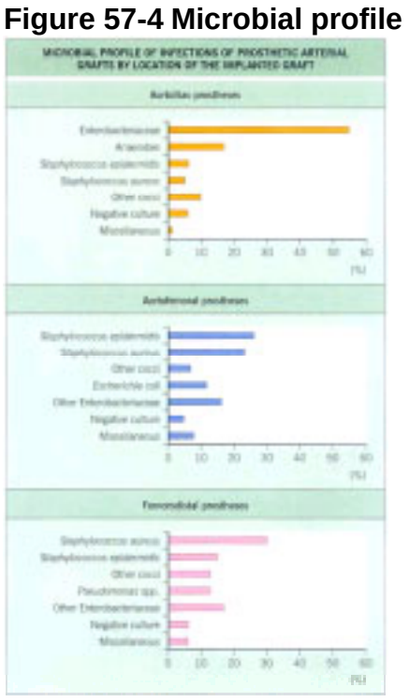


Figure 57-5 Contrast computerized tomography of the pelvis demonstrating perigraft fluid in a patient with an infected left-sided aortoiliac graft (arrow).

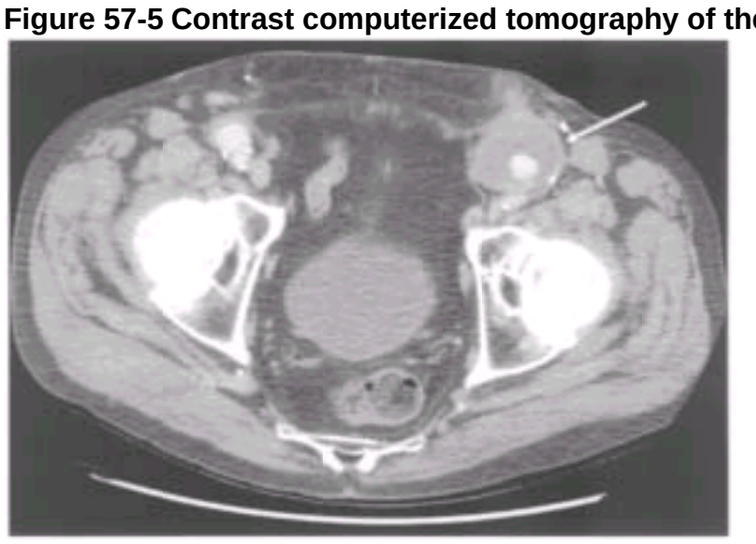


Figure 58-1 Acute viral myocarditis, with a characteristic mononuclear infiltrate.

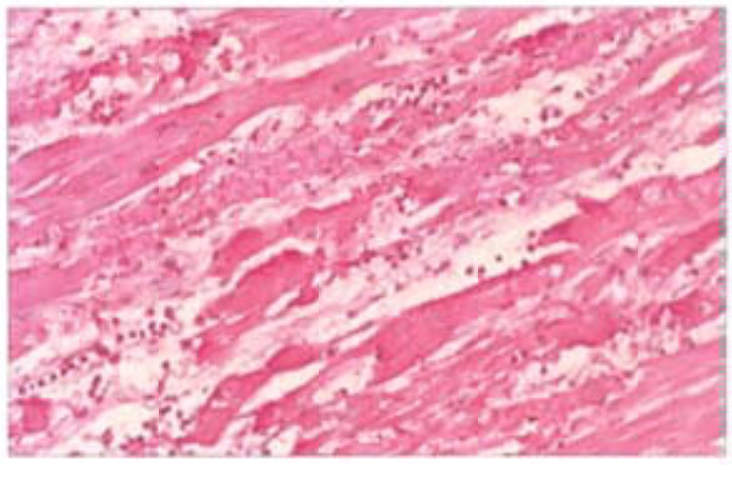


Figure 58-2 Heart at autopsy of a patient who had acute suppurative pericarditis. The parietal pericardium has been stripped from the specimen, revealing a 'bread and butter' appearance.

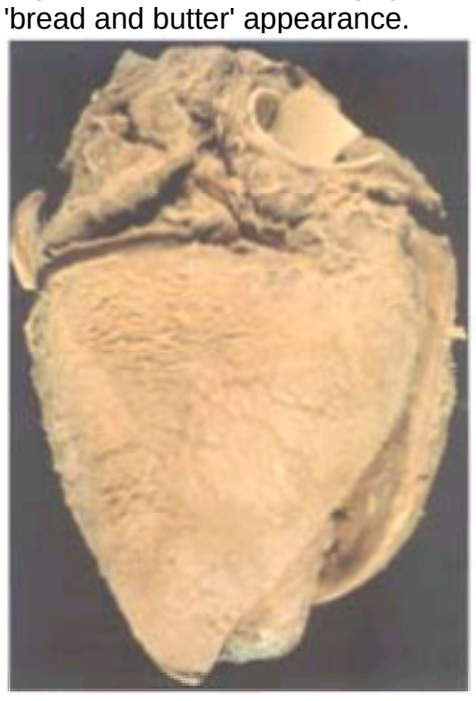


Figure 58-3 Cardiomegaly in a patient who has pericarditis. The presence of a 'water-bottle' heart on this plain film suggests a large pericardial effusion.

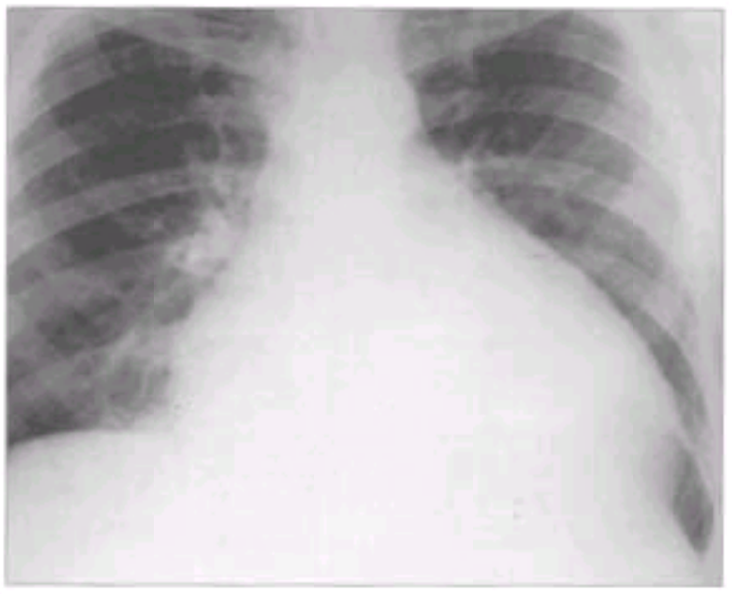


Figure 58-4 Electrocardiogram of a patient who has early acute pericarditis. Note the presence of diffuse ST segment elevation and PR depression in the inferolateral leads (arrows). 25mm/s; 10.0mm/mV; F–W 0.05–100.

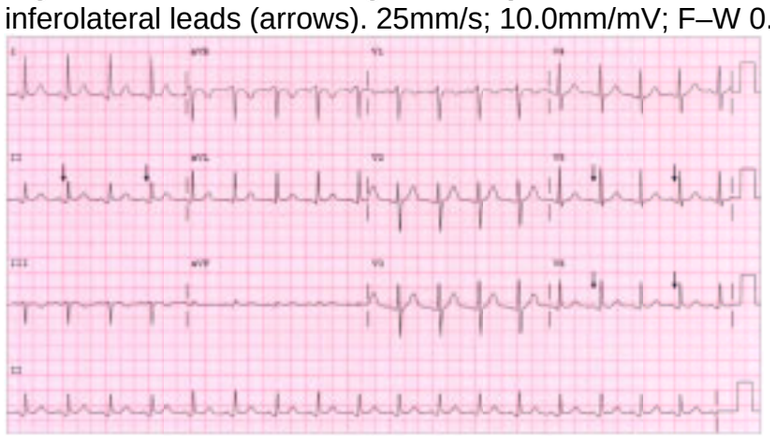


Figure 58-5 Chest CT of a patient who has a large crescent-shaped pericardial effusion.

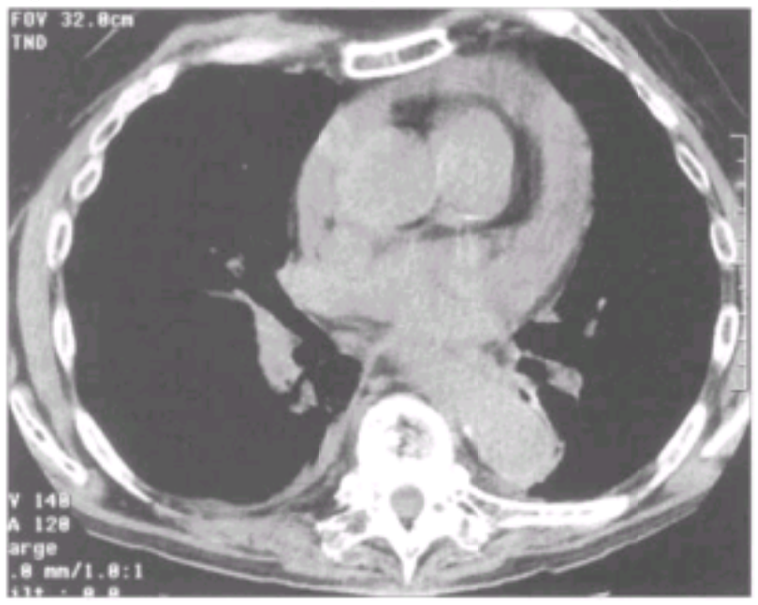


Figure 59-1 Scanning electron microscopy of a rabbit aortic valve leaflet. (a) A normal valve, covered by a monolayer of endothelial cells. (b) The meshwork of fibrin and platelets covering a damaged valve. Mechanical lesions of the valve were created by inserting a catheter through the right aortic carotid and across the aortic valve.

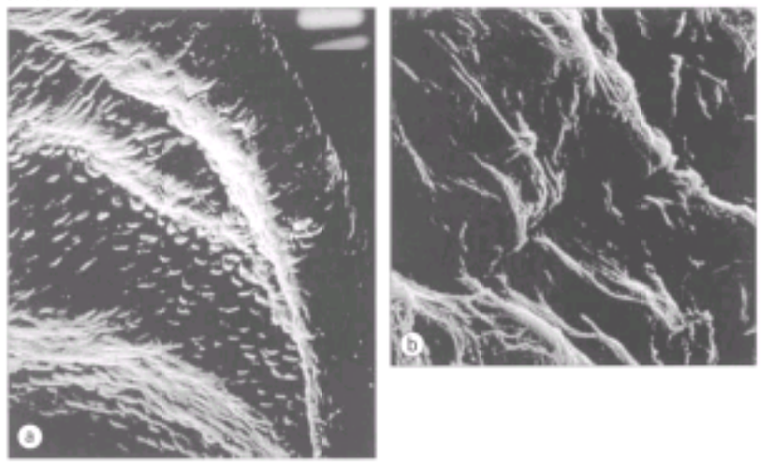


Figure 59-2 Colonization and infection of an endothelial lesion by *Staphylococcus aureus.* Exposure of the subendothelial matrix triggers the deposition of platelet-fibrin clots and other plasma-soluble and matrix proteins, including fibrinogen, fibrin, fibronectin and thrombospondin. Triggering of the coagulation cascade is also mediated by tissue factor, which contributes to platelet activation and the constitution of a nonbacterial thrombotic vegetation. *Staphylococcus aureus* is equipped with a wealth of surface determinants that may promote binding and colonization of nonbacterial thrombotic vegetations. The best known of these are fibrinogen-binding protein (or clumping factor), fibronectin-binding protein and coagulase. These factors are likely to mediate direct and/or indirect attachment to vascular lesions and promote infection.

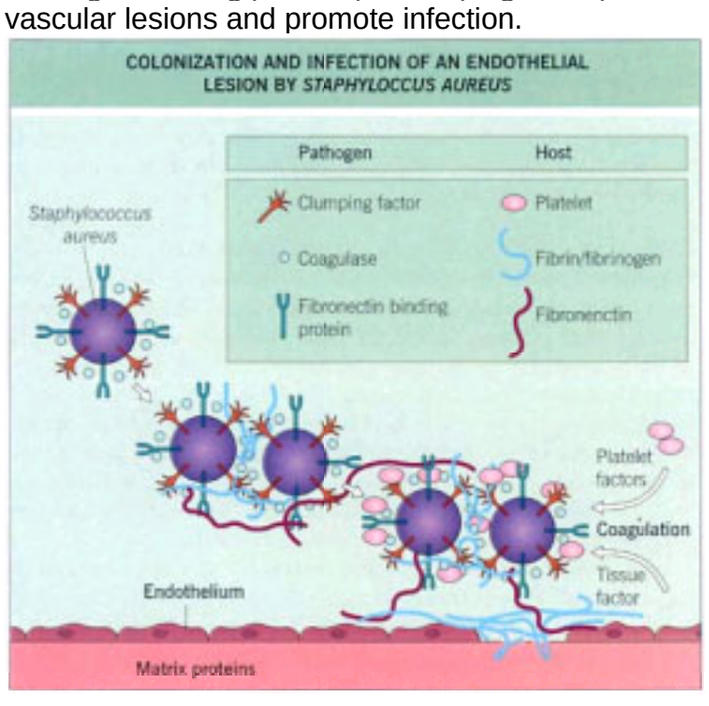


Figure 59-3 Microscopic appearance of a vegetation from a patient suffering mitral valve infective endocarditis due to *Streptococcus sanguis.* The purple area represents clusters of streptococci packed within a fibrin-platelet meshwork. Professional phagocytes are essentially absent from the lesion.

Figure 59-4 Transesophageal echocardiography of a mitral valve infective endocarditis. (a) Mass attached to anterior leaflet. (b) Prolapse of the leaflet due to rupture of chordae tendinae. The echo is from the patient shown in Figure 59.3 . Arrows indicate a large pediculated vegetation, which oscillates from the left ventricle to the left atrium during systole. *Courtesy of Dr X Jeanrenaud.*

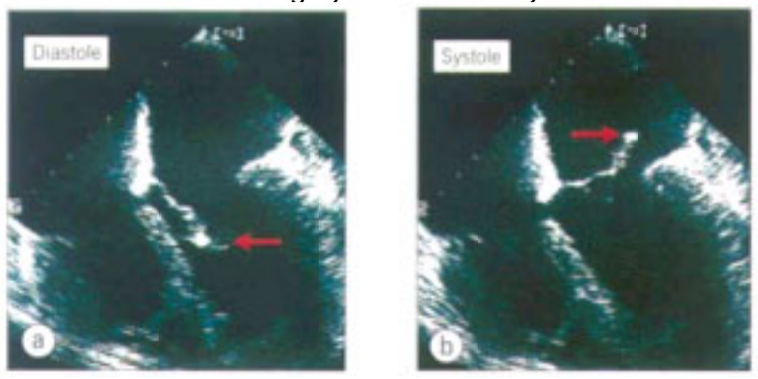


Figure 59-5 Aortic valve of a patient undergoing emergency valve replacement for acute endocarditis caused by *Staphylococcus aureus.* In this patient, emergency valve replacement was mandatory because of multiple embolizations and acute heart failure. Here, the aorta has been opened and the valve is viewed from its upper side. The lower tweezers are holding a valve leaflet covered with vegetations. Next to the upper tweezers, a portion of a cuspid with a normal appearance can be distinguished.

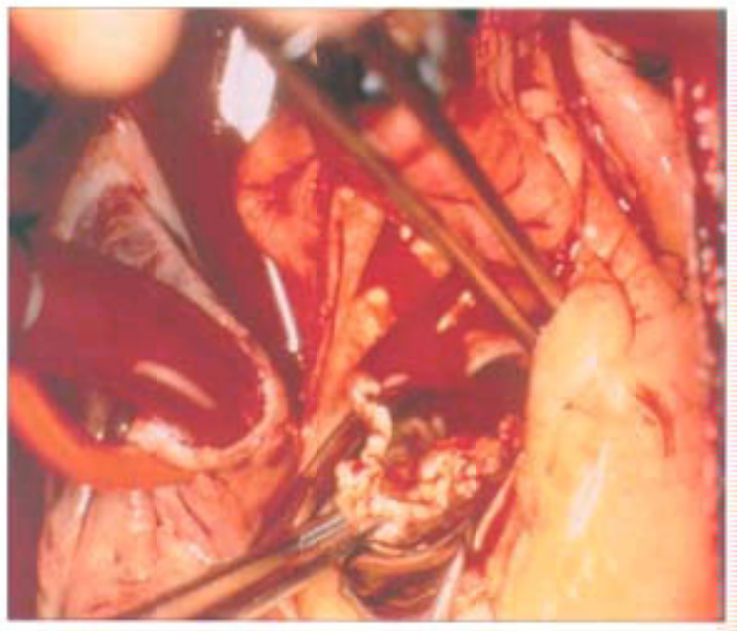


Figure 59-6 Skin lesions (Janeway spots) on the foot (a) and septic emboli of the retina (b), the results of peripheral emboli in acute endocarditis caused by *Staphylococcus aureus.* These occurred in the patient described in Figure 59.5 and were present on admission to hospital.

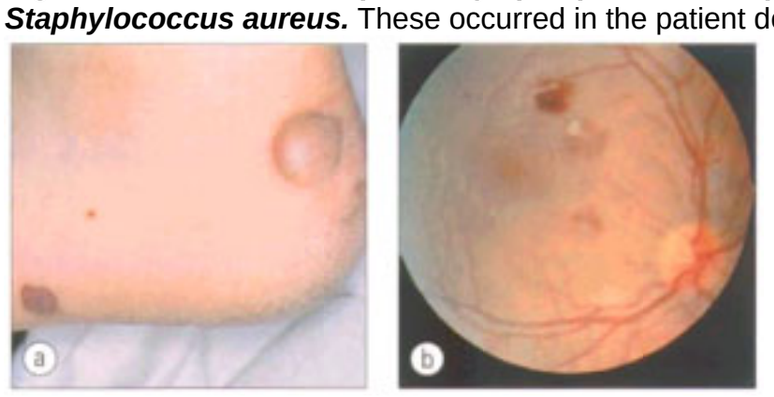


Figure 59-7 A cerebral abscess (a) and multiple abscesses and ischemic necroses of the spleen (b), the result of peripheral emboli in acute endocarditis cause by *Staphylococcus aureus.* Again these occurred in the patient described in Figure 59.5 ; they developed after admission to hospital.

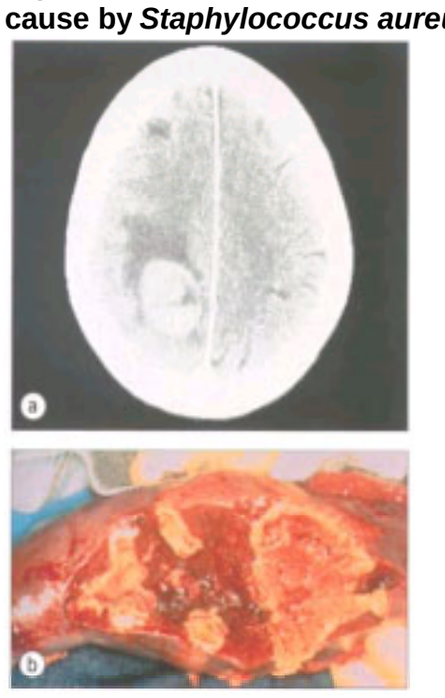


Figure 59-8 Osler node on the thumb during subacute endocarditis. This was a rounded, tender, inflamed mass about 5mm in diameter.

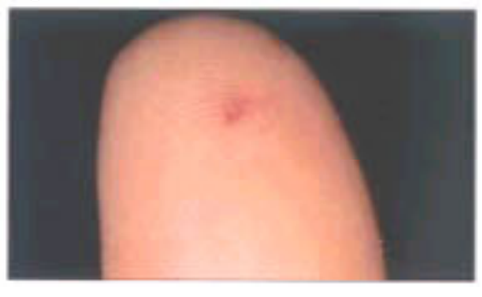


Figure 59-9 Blood culture-negative aortic valve endocarditis caused by *Tropheryma whippelii* in a 48-year-old patient. The patient was admitted to hospital for acute abdominal pain. Abdominal surgery revealed an ischemic necrosis of the transverse colon, presumably due to arterial embolization. An echocardiogram revealed exuberant vegetations on the aortic valve. All blood cultures were negative. Emergency valve replacement was performed for cardiac insufficiency. *Tropheryma whippelii* infection was identified by histology and by using broad-spectrum PCR amplification of the surgical material. The patient was treated with long-term (>1 year) trimethoprim-sulfamethoxazole (see Table 59.5). *With permission from Bugon and Moreillon.*[49]

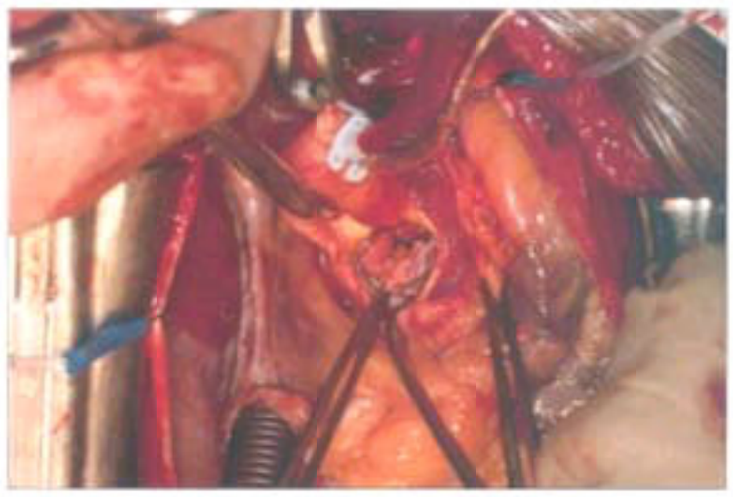


Figure 60-1 (a) Chest radiograph of a 15-year-old boy who had multiple recurrences of acute rheumatic fever, showing gross cardiac enlargement and failure. He had mitral regurgitation and stenosis, and aortic regurgitation and stenosis. He died 2 days after this radiograph was taken, of intractable cardiac failure. (b) Postmortem cardiac examination of the same boy, showing thickened, shortened mitral valve cusps with calcific vegetation and thickened chordae tendinae. *(Photographs kindly provided by Professor Bart Currie, Darwin, NT, Australia.)*

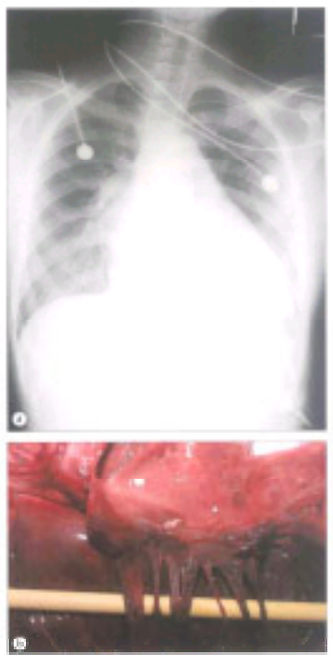


Figure 60-2 Electrocardiographic changes in a young adult with acute rheumatic fever, showing evolution over 18 days from complete heart block to second-degree (Wenckebach) block to first-degree block and then to normal sinus rhythm. *(Reproduced with permission from Bishop W, Currie B, Carapetis J, Kilburn C. A subtle presentation of acute rheumatic fever in remote northern Australia. Aus NZ J Med 1996;26:241–2.)*

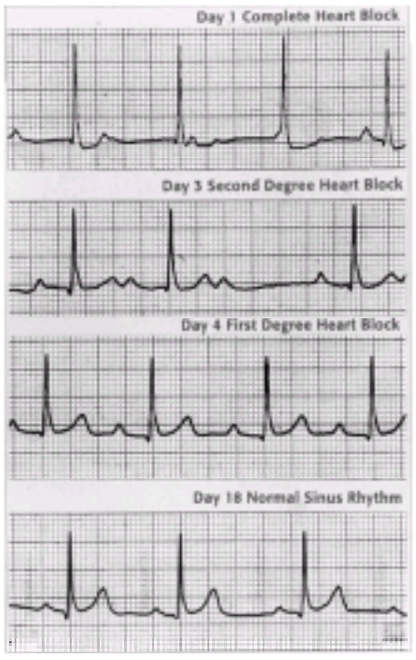


Figure 60-3 Erythema marginatum on the trunk of an 8-year-old Caucasian boy. The pen mark shows the location of the rash approximately 60 minutes previously. *(Photograph kindly provided by Associate Professor Mike South, Royal Children's Hospital, Melbourne, Australia.)*

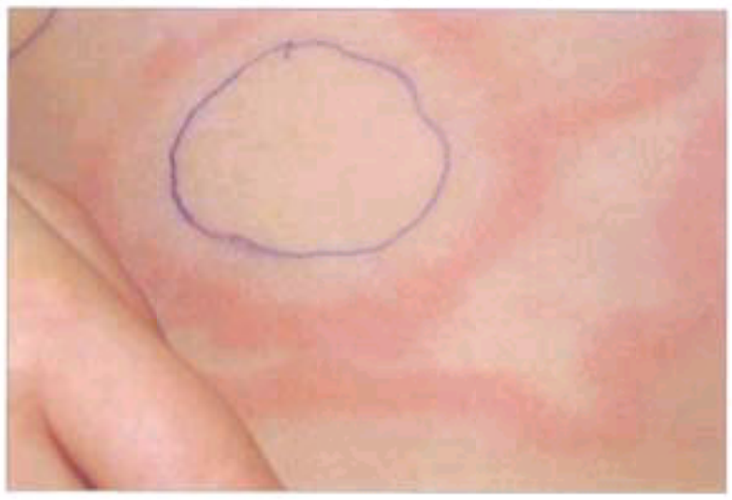


Figure 62-1 Typical *Candida* vulvovaginitis with bilateral symmetric erythema and edema of vestibule and labia.

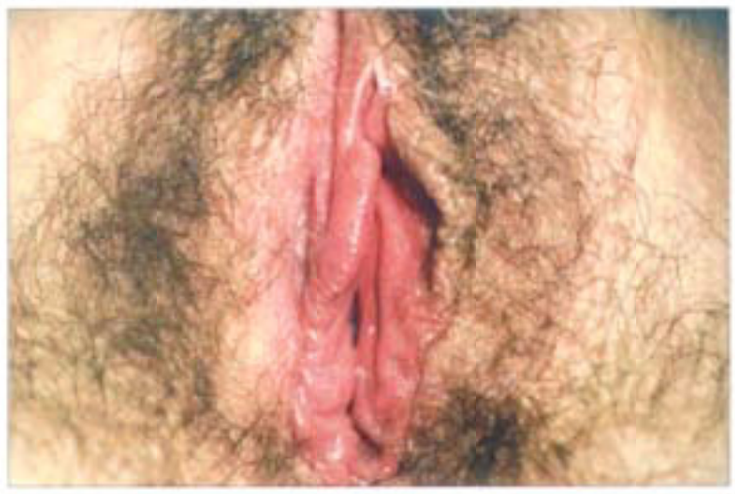


Figure 62-2 Severe *Candida* vulvovaginitis with bilateral painful fissure formation in the vulva.

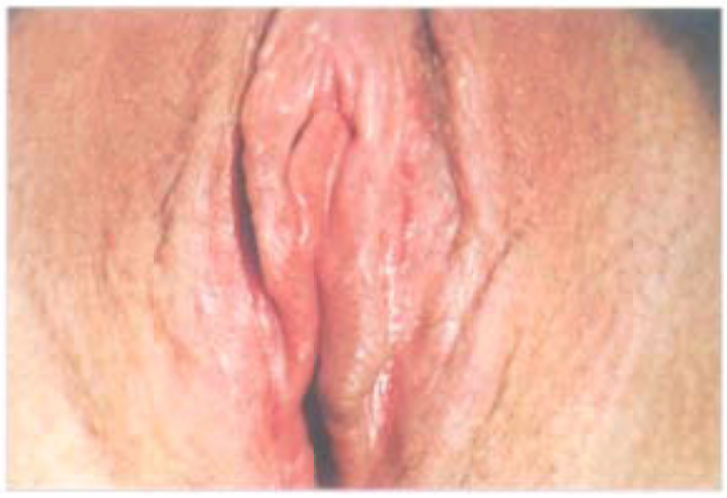


Figure 62-3 Vulvar psoriasis resulting in pruritus vulva and misdiagnosed as *Candida vulvovaginitis*.

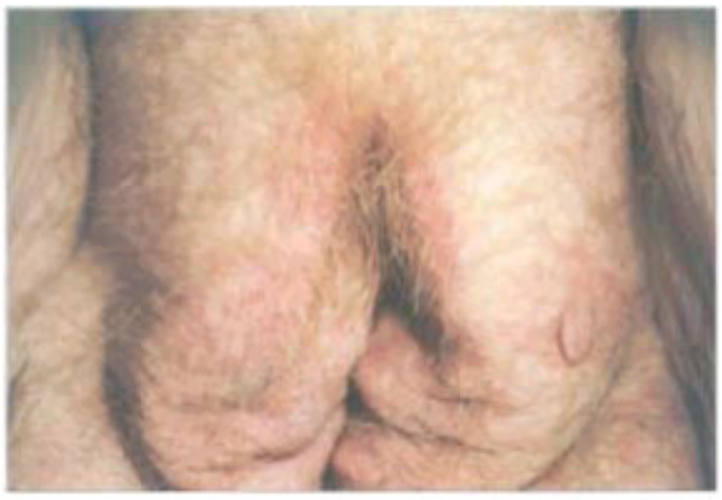


Figure 66.C-1 Management of neonates whose mothers received IAP. The flow chart summarizes the management of neonates born to mothers who have received IAP for early-onset GBS-disease (2002 revision). Full diagnostic evaluation includes full blood count and differential white cell count, blood culture and chest radiograph. A lumbar puncture is performed at the discretion of the clinician. Limited evaluation includes full blood count and differential white cell count plus blood culture. The duration of IAP stated applies only to penicillin, ampicillin and cefazolin prophylaxis.

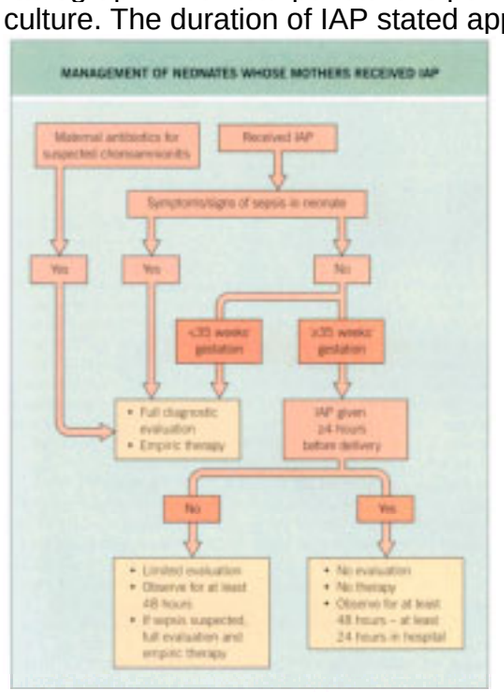


Figure 66.d-1 Managing exposure to varicella during pregnancy.

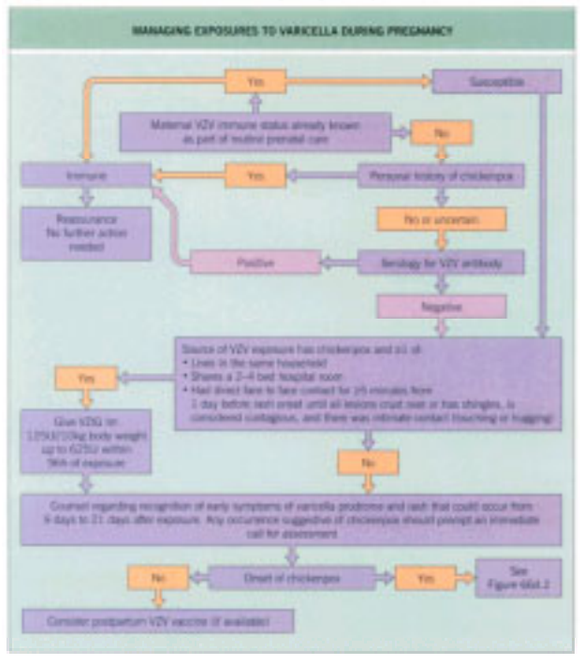


Figure 66.d-2 Managing chickenpox in the immunocompetent pregnant woman.

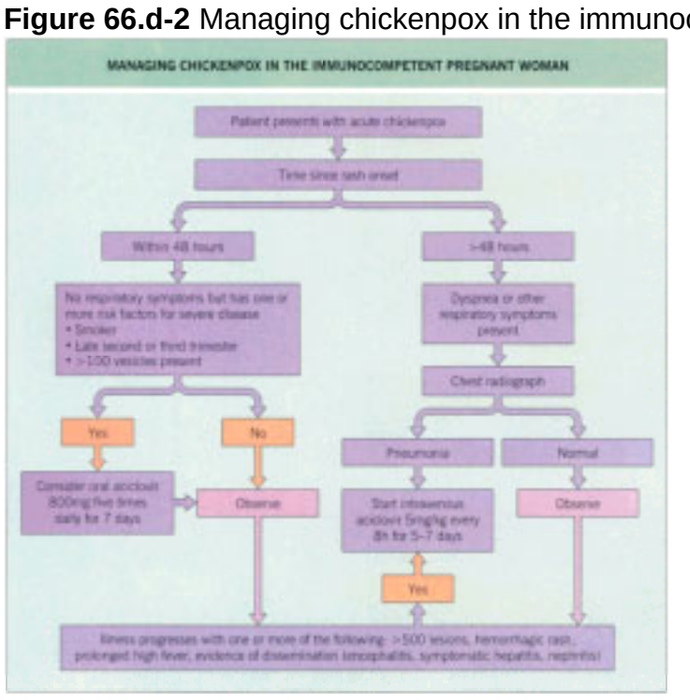


Figure 67-1 Pathogenesis of cystitis. Factors favoring bacterial persistence and infection include bacterial binding to bladder mucosa (fimbriae), and high bacterial growth rates despite high osmolarity and urea concentrations and low pH. Factors favoring bacterial elimination include high urine flow rate, high voiding frequency, bactericidal effects of bladder mucosa, secreted proteins that bind to fimbrial adhesins and the inflammatory response. IL, interleukin; TNF, tumor necrosis factor.

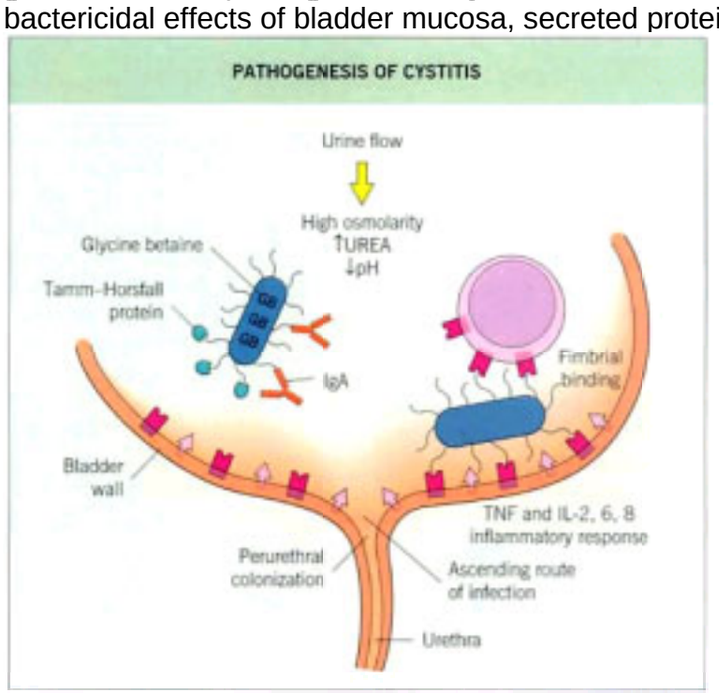


Figure 67-2 Treatment of uncomplicated cystitis in a nonpregnant woman.

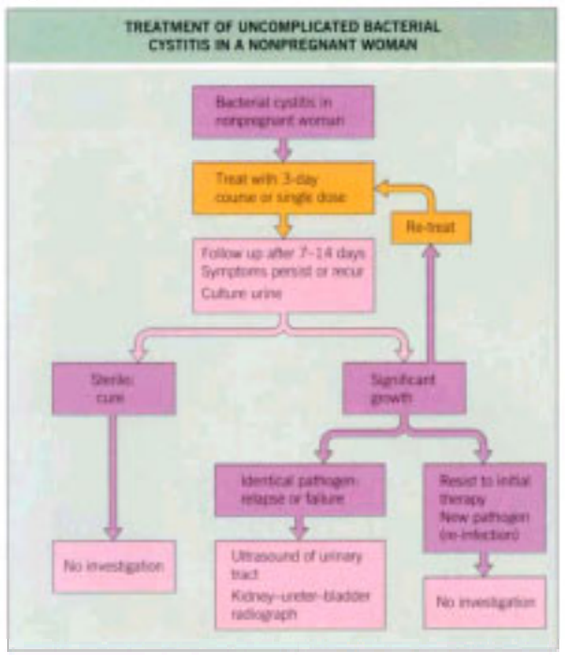


Figure 67-3 Treatment of recurrent bacterial cystitis in a nonpregnant woman.

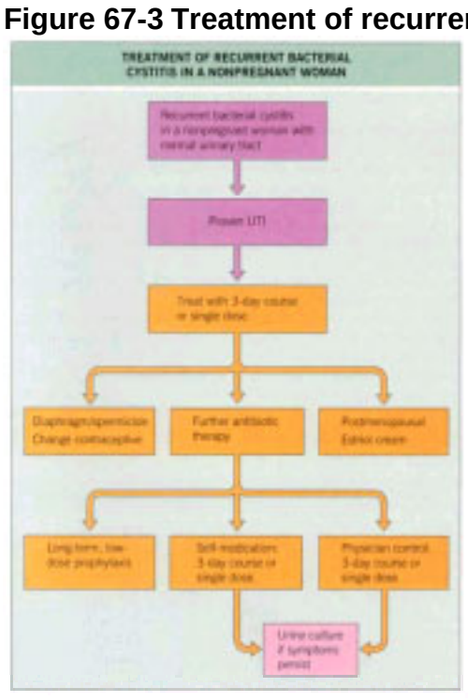


Figure 67-4 Treatment of asymptomatic bacteriuria in pregnancy.

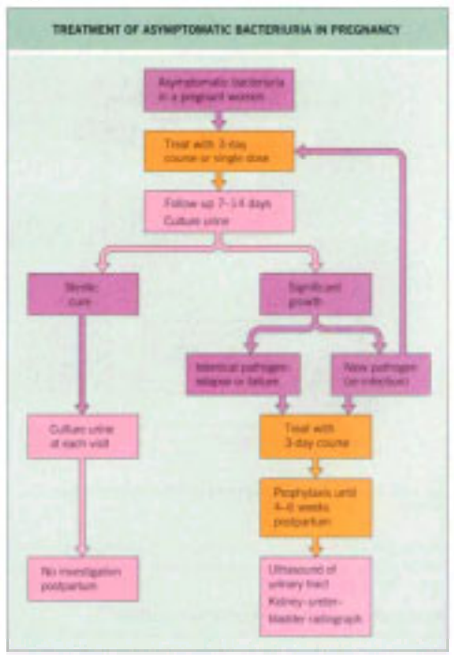


Figure 68-1 Meares and Stamey[17] localization technique to diagnose chronic bacterial prostatistis. Prostate secretion can be more readily obtained if the patient has not ejaculated for approximately 3–5 days before the examination.

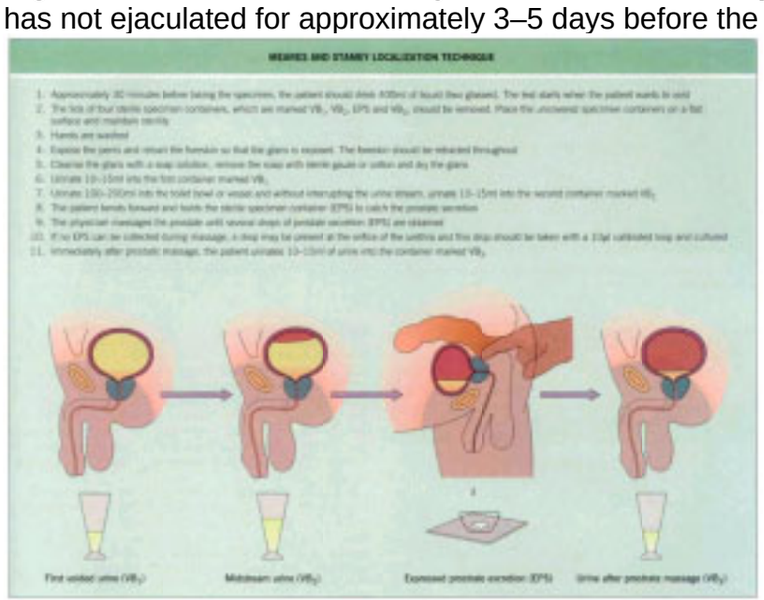


Figure 68-2 Diagnostic criteria of chronic bacterial prostatitis by expressed prostatic secretion analysis. LDH, lactate dehydrogenase; PAF, prostatic antibacterial factor.

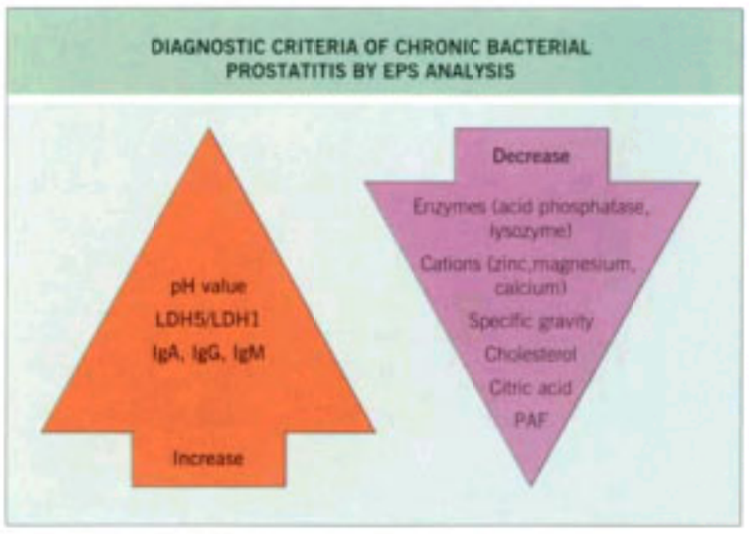


Figure 68-3 Transrectal ultrasonography of the prostate with diffuse calcifications (prostatitis calcarea).

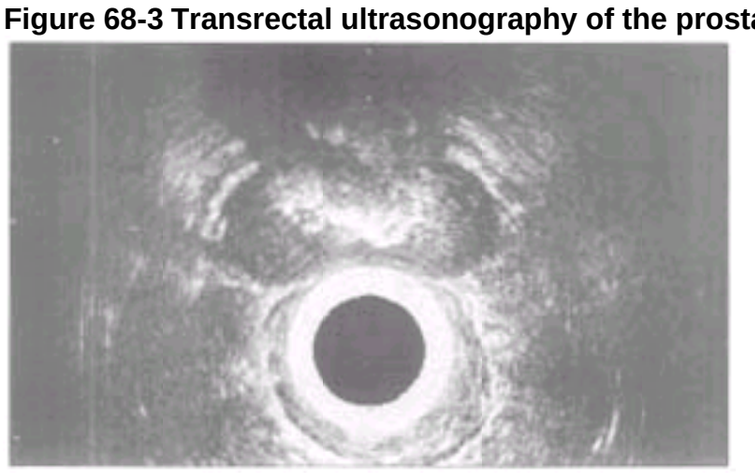


Figure 68-4 Diagnostic management in patients who have prostatitis-like symptoms.

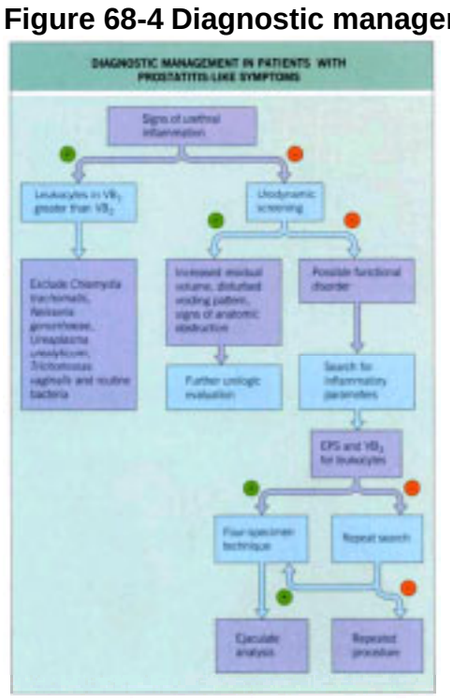


Figure 69-1 Uropathogenic strain of *Escherichia coli.* Note the typical virulence properties, including adhesive fimbriae, cytotoxins, lipopolysaccharide (LPS), capsular polysaccharide, the aerobactin system and outer membrane proteins important in serum resistance. Bacterial interactions with host cells trigger cytokine production, inflammatory cell infiltration and bacterial internalization within epithelial cells. Internalized bacteria can multiply intracellularly and stimulate sloughing, rupture, necrosis or apoptosis of host cells.

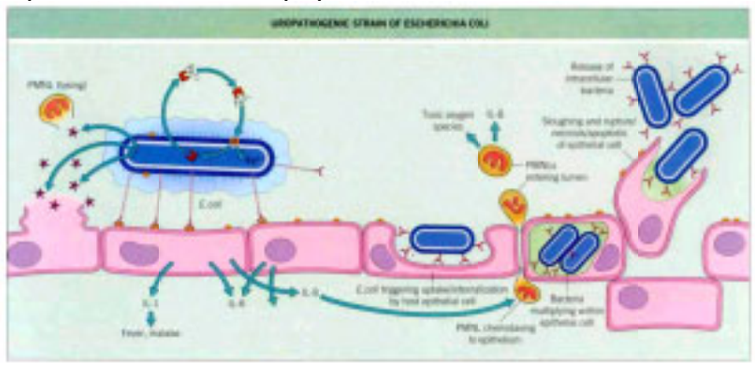


Figure 69-2 Acute pyelonephritis. Note interstitial edema, tubules packed with PMNLs and a diffuse interstitial acute inflammatory infiltrate in this autopsy specimen from a diabetic patient who had refractory *Escherichia coli* urosepsis.

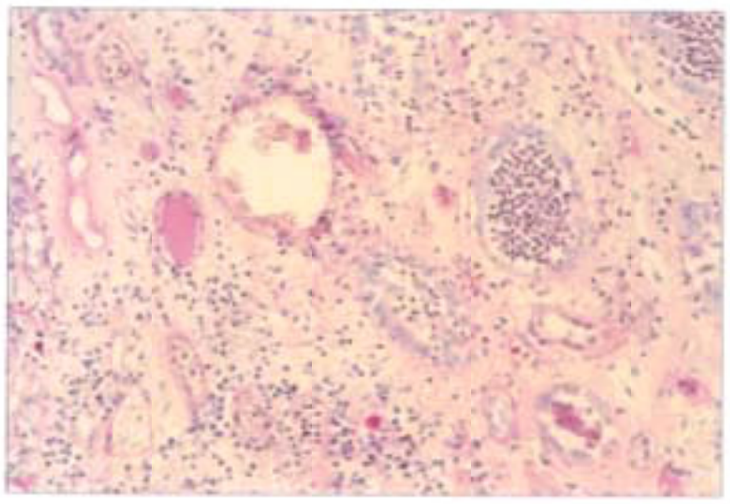


Figure 69-3 Emphysematous pyelonephritis. Cortical necrosis (solid arrow), diffuse cortical hemorrhage (open arrow) and dilatation of the collecting system (arrowheads) in a nephrectomy specimen from a diabetic patient who received combined medical/surgical therapy and survived emphysematous pyelonephritis due to an unusual pathogen, namely *Candida albicans*.

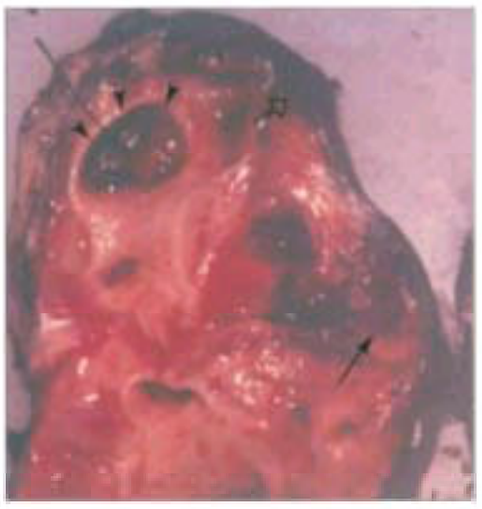


Figure 69-4 Acute papillary necrosis (arrows) in an autopsy specimen from a diabetic patient who died from refractory *Escherichia coli* urosepsis. Necrotic papillae (arrows) failed to take up formalin, so appear pink, in contrast to the grayish-tan formalinized tissue.

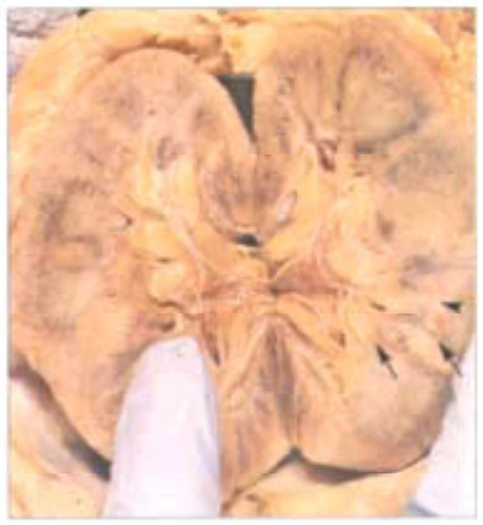


Figure 69-5 Febrile urinary tract infection with white blood cell count of 36,000/ml (girl, 3 years). (a) Precontrast CT scan: left kidney is diffusely swollen; parenchymal attenuation is the same as that of the right kidney. (b) Postcontrast CT scan: wedge-shaped regions of hypoenhancing parenchyma in the left kidney are most pronounced in the posterior portion. Inflamed parenchyma enhances from 32 to 93 Hounsfield units (HU), whereas normal kidney enhances from 33 to 140HU. The right kidney shows normal cortical enhancement and pronounced medullary blush. *With permission from Talner.*[45]

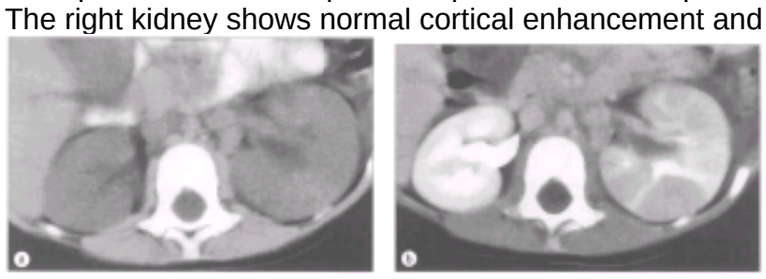


Figure 69-6 Woman with clinical signs of acute pyelonephritis. (a) Precontrast CT scan: focal bulge present in anterolateral aspect of left kidney. Attenuation is the same as that of normal kidney parenchyma. (b) Postcontrast CT scan: rounded and streaky regions of hypoenhancing parenchyma in the left kidney are most pronounced anterolaterally. Attenuation in the region of interest (cursor) was 22HU on precontrast scans and increased to 93HU on postcontrast scans. Normal parenchyma increased from 25–130HU. *With permission from Talner.*[45]

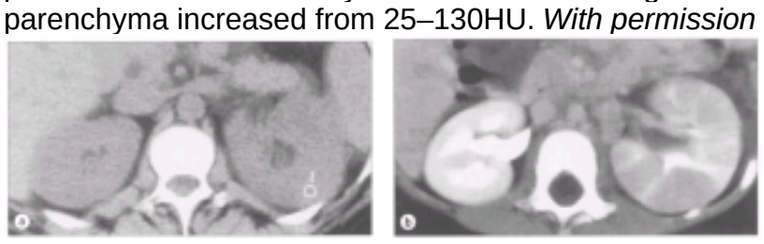


Figure 69-7 Acute pyelonephritis with small intrarenal abscess. (a) Precontrast CT scan shows small region of low attenuation (arrows). (b) On the postcontrast CT scan, the abscess (A) fails to enhance at all. Surrounding inflamed parenchyma bulges and enhances less than adjacent normal parenchyma. (c) Follow-up CT scan obtained after prolonged antibiotic therapy. The abscess has resolved without drainage. The focal swelling is gone but the parenchyma still shows hypoenhancement. *With permission from Talner.*[45]

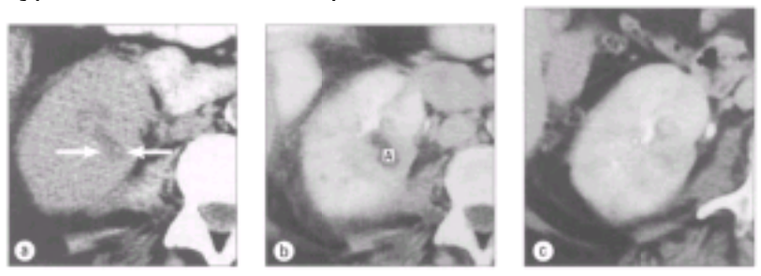


Figure 69-8 Renal abscess perforating into subcapsular and perinephric spaces (woman, 29 years). (a) Postcontrast CT scan. Dumbbell-shaped nonenhancing region laterally in right kidney represents parenchymal abscess breaking through subcapsular and perinephric spaces. Note marked thickening of perinephric fascia posterolaterally. (b) CT section obtained caudal to (a). Note thickening of perinephric inflammation. At this level there is a small pararenal abscess pocket adjacent to the liver. *With permission from Talner.*[45]

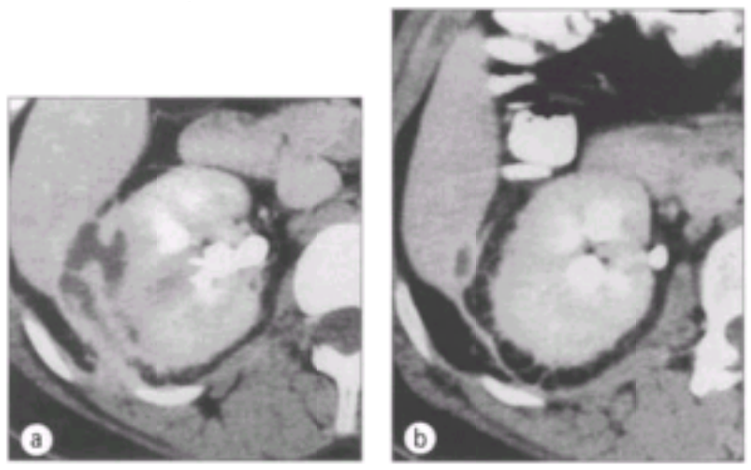


Figure 70-1 Nephrocalcinosis complicated by relapsing *Pseudomonas aeruginosa* infection. Plain film of the abdomen showing multiple bilateral renal stones. This 31-year-old woman has had recurrent stone formation since the age of 18 despite dietary manipulation and repeated lithotripsy. Urinary infection with *P. aeruginosa* was identified at 24 years of age with subsequent recurrent episodes of symptomatic upper tract infection. She has been maintained on suppressive ciprofloxacin therapy for years with control of symptomatic infection.

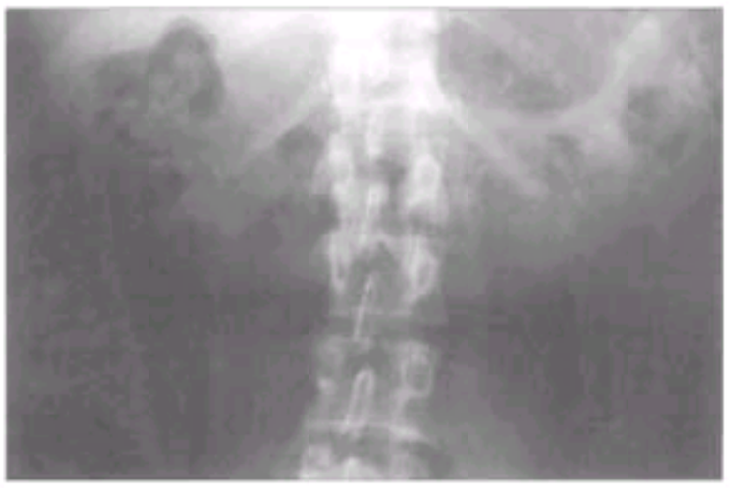


Figure 71-1 Granuloma formation in kidney biopsy. *Courtesy of Robert F Peterson.*

Figure 71-2 Chronic tuberculous nephritis with almost complete destruction of the kidney *Courtesy of Robert F Peterson*.

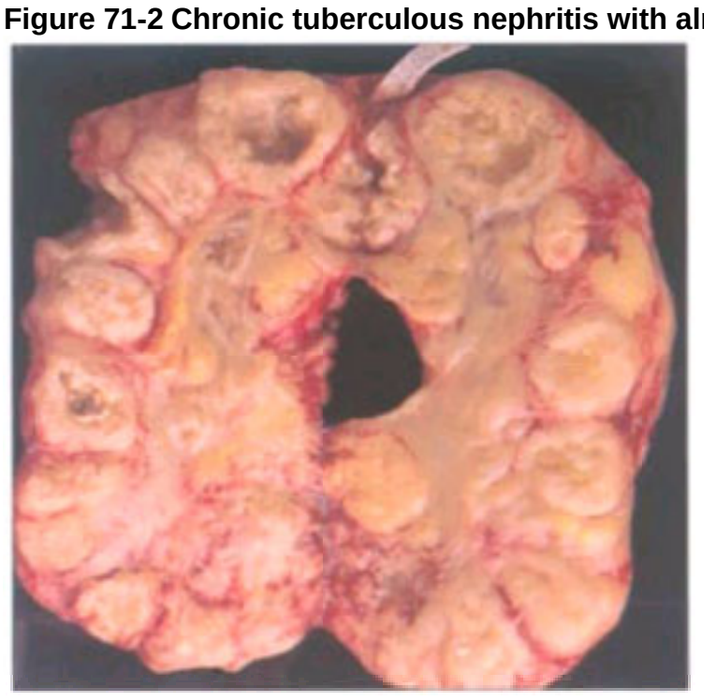


Figure 71-3 Diffuse acute tuberculous nephritis with abscess formation. *Courtesy of Robert F Peterson.*

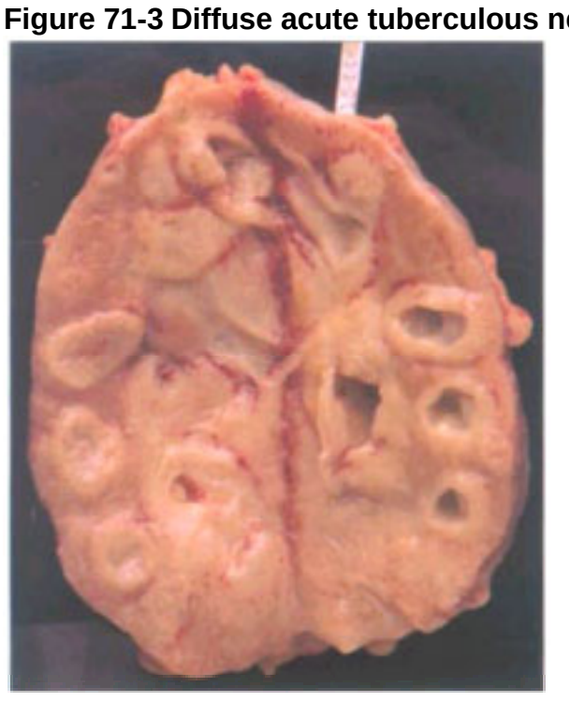


Figure 71-4 Chronic tuberculous nephritis with destruction of all landmarks and pelvic calculus. The arrow shows a fistulous tract delineated by the wooden probe. *Courtesy of Robert F Peterson.*

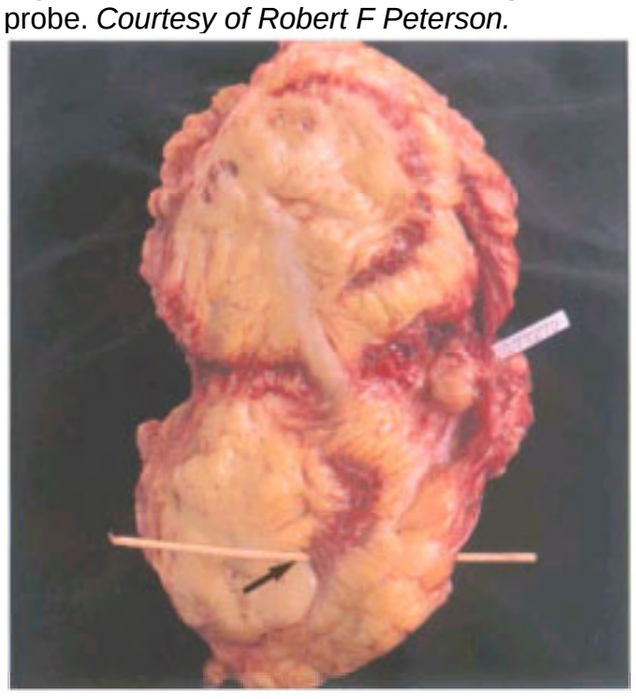


Figure 71-5 Intravenous pyelogram of left kidney with bivalved surgical pathology specimen.

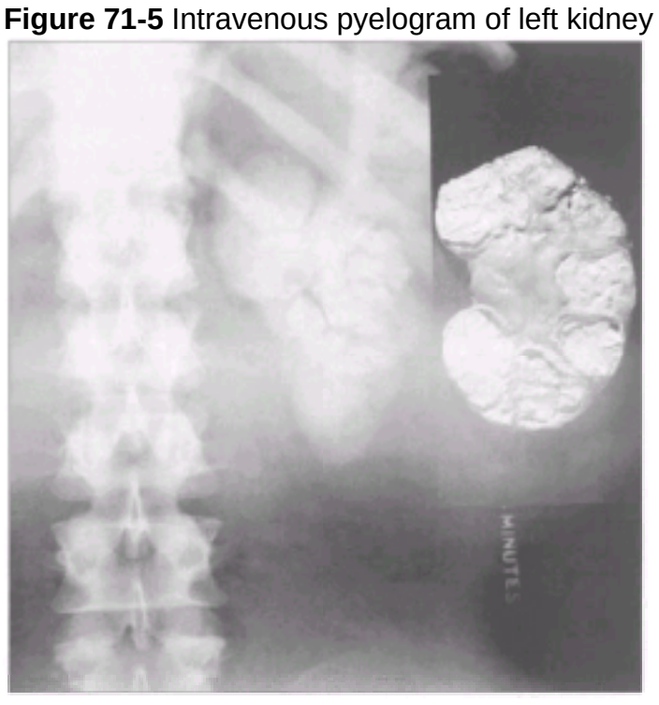


Figure 71-6 Laparoscopic views in genitourinary tuberculosis. (a) Free and loculated ascites and fine fibrous adhesions. (b) Miliary nodular exudate in the anterior wall.

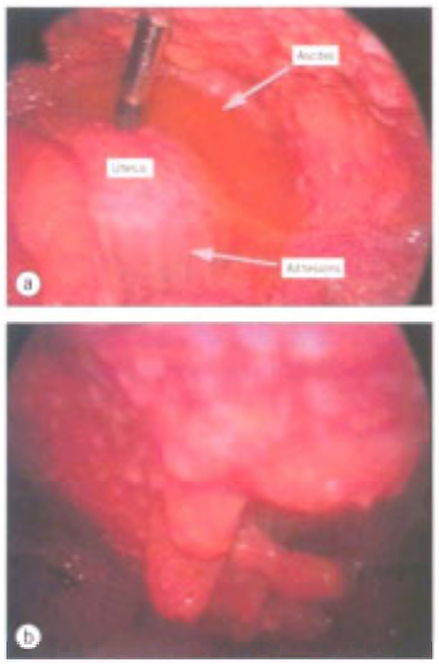


Figure 73-1 Healthy life lost — top ten causes in young adults aged 15–44 years.

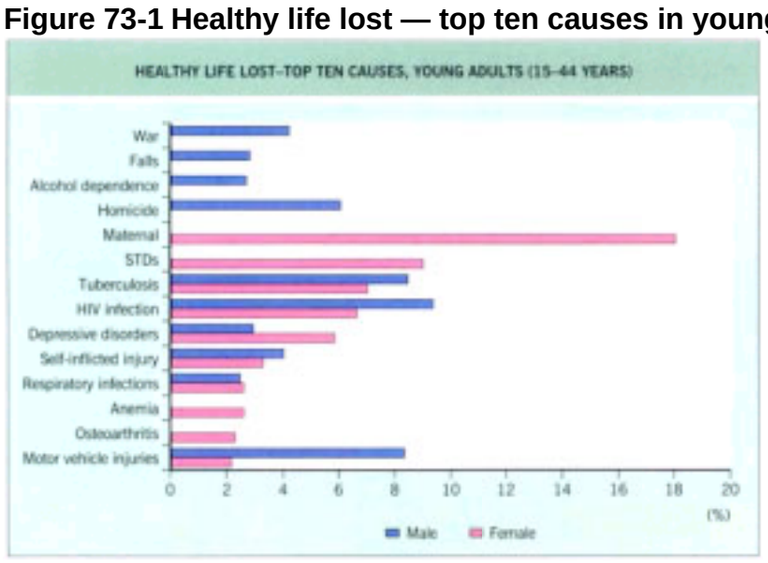


Figure 73-2 Estimated new cases of curable STD among adults, 1999.

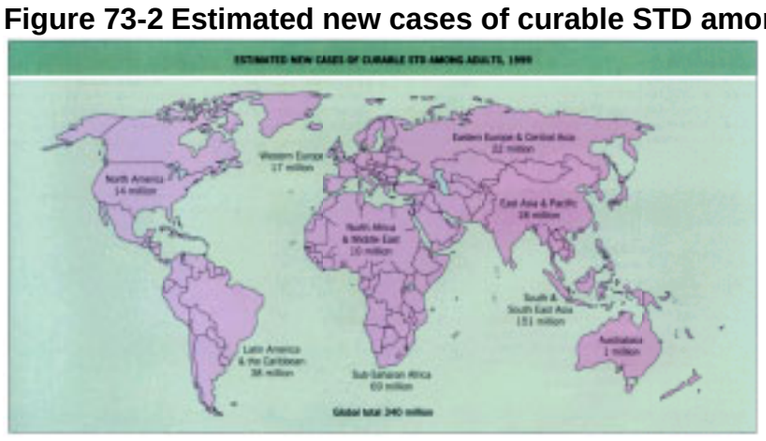


Figure 73-3 Diagnoses of uncomplicated gonorrhea by sex in England and Wales 1990–2000. ** Data for homosexually acquired infection in males available from 1994 onwards only.*

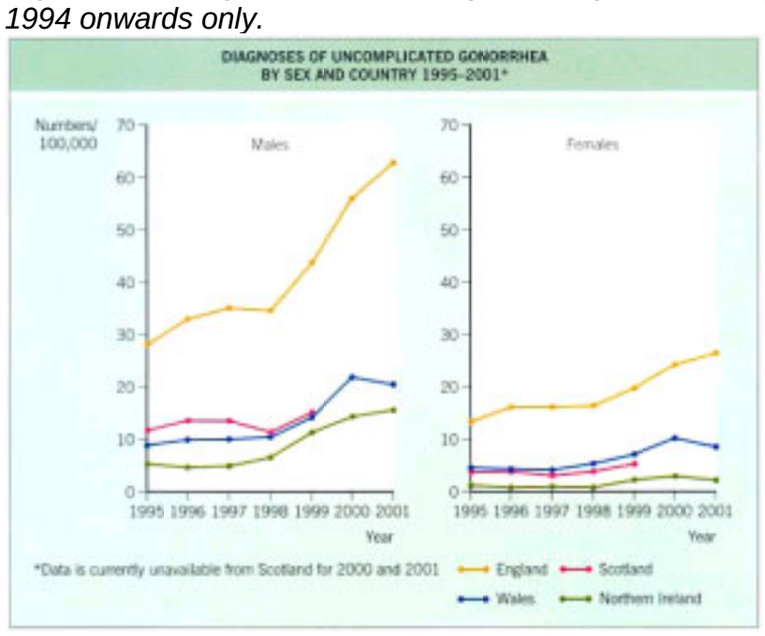


Figure 73-4 New diagnoses of selected STIs in men who have sex with men in England and Wales 1995–2000.

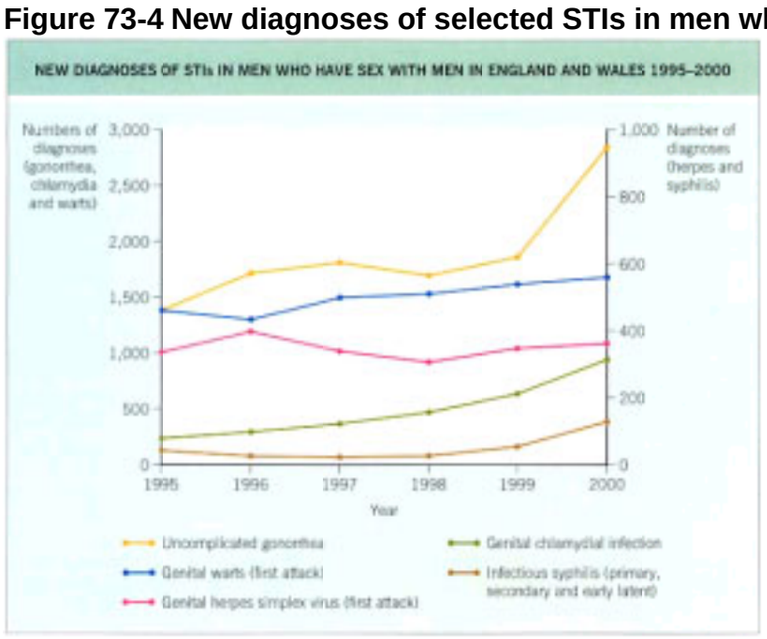


Figure 73-5 Gonorrhea rates by race and ethnicity in the USA 1981–2000 and the Healthy People Year 2010 objective. Nat Am/AK Nat, American Indian/Alaska Natives.

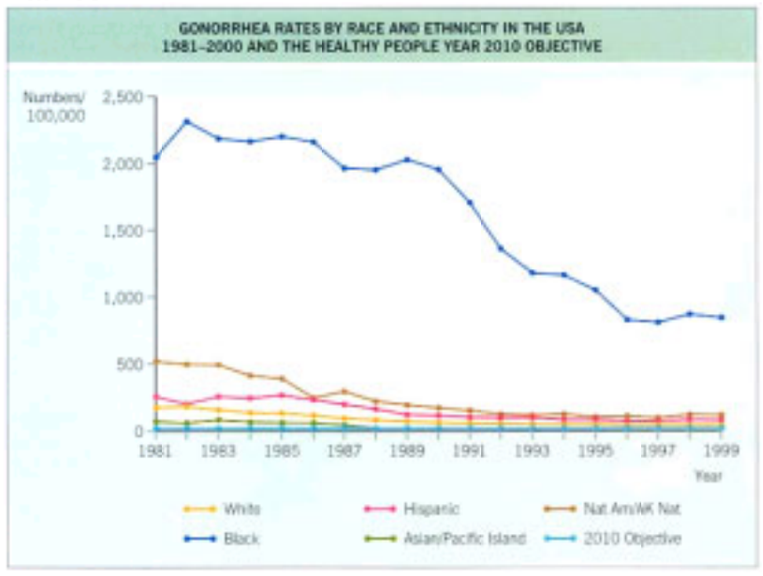


Figure 73-6 Annual incidence of syphilis in Belarus, Estonia, Kazakhstan, the Russian Federation and Ukraine 1991–2000.

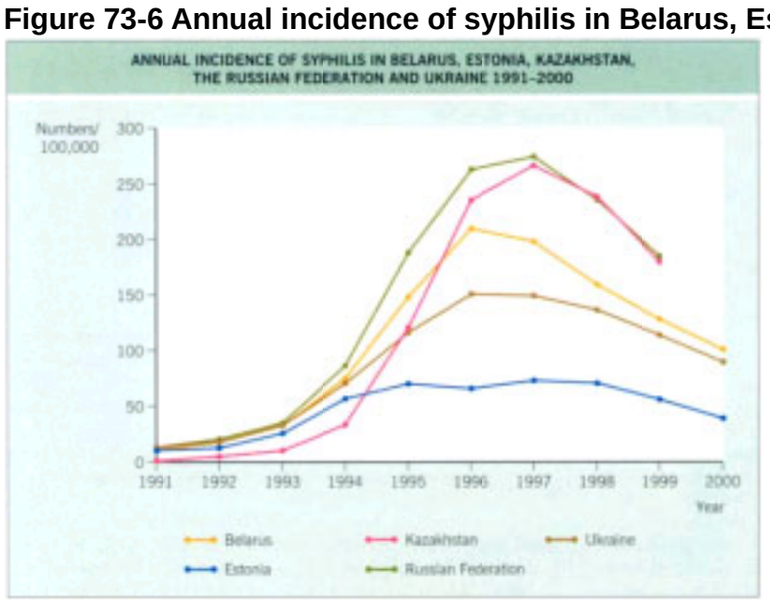


Figure 73-7 Diagnoses of uncomplicated genital chlamydial infection in genitourinary medicine clinics by sex and age group in the UK 1995–2000. *Data are currently unavailable from Scotland for 2000 and from Northern Ireland for 1996 and 1997. *Data from the PHLS and Scottish ISD(D)5 Collaborative Group (ISD, SCIEH and MSSVD).*

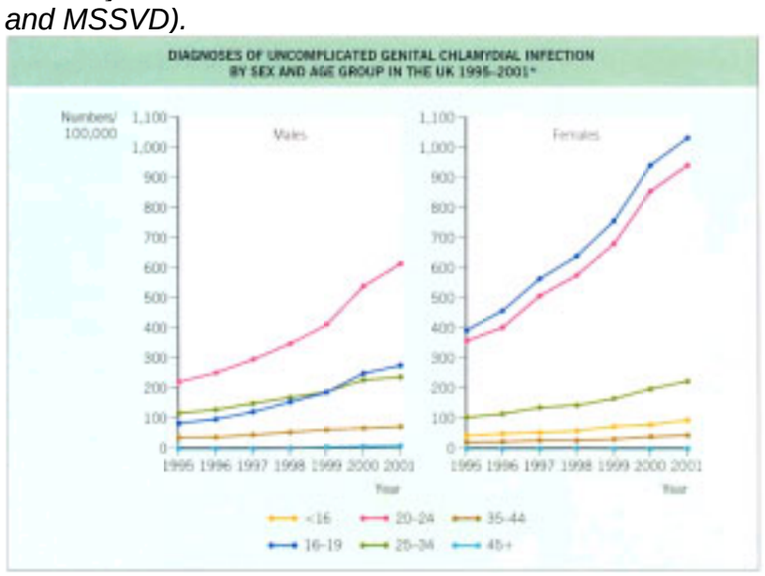


Figure 73-8 Sexually transmitted diseases in women in Africa.

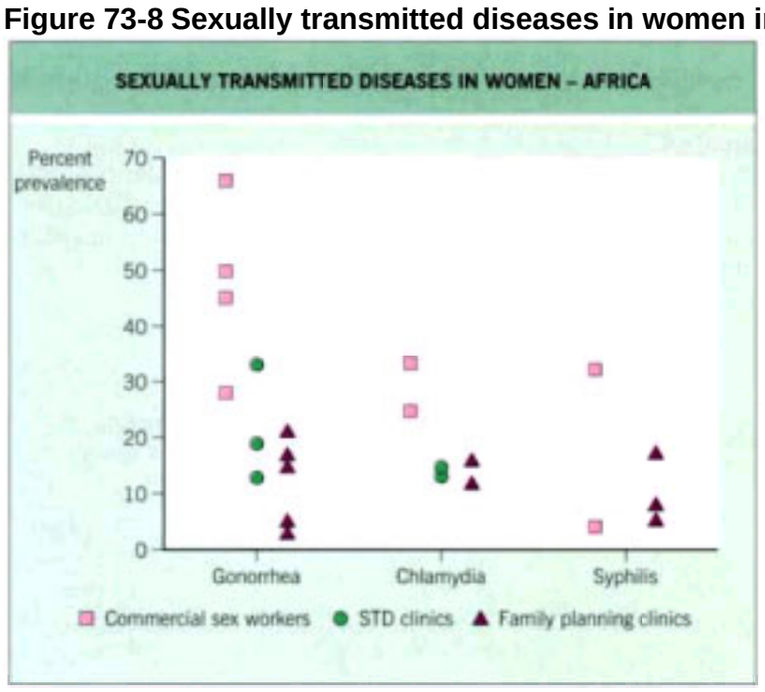


Figure 73-9 Health care-seeking behavior for an STD.

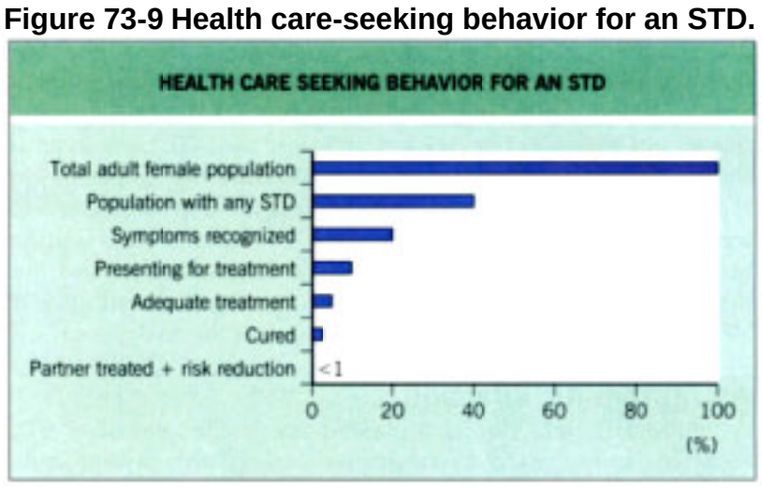


Figure 74-1 Trends in gonorrhea rates among men aged 15–34 years in the USA, 1981–2000. Rates are cases per 100,000 population. *Source: Sexually Transmitted Disease Surveillance, 2000.*[1]

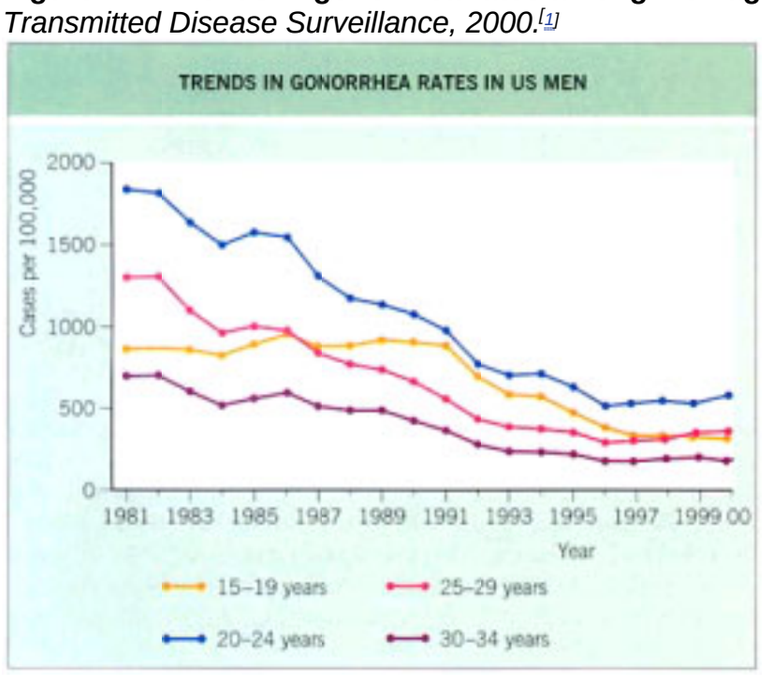


Figure 74-2 Rates of chlamydial infection in the USA, 2000. Rates are cases per 100,000 population. *Source: Sexually Transmitted Disease Surveillance, 2000.*[1]

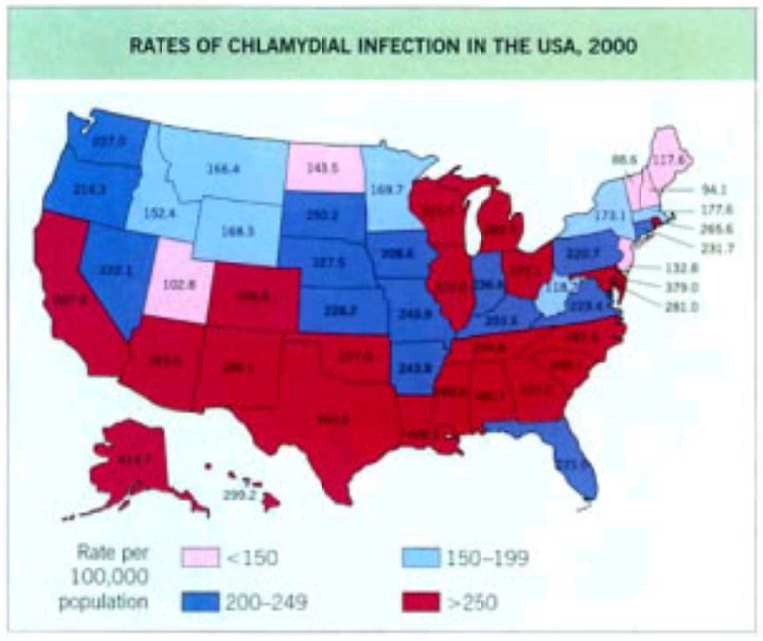


Figure 74-3 Important components of the outer membrane of *Neisseria gonorrhoeae*. Porin is the major outer membrane protein. Reduction modifiable protein (Rmp) is the target of blocking antibodies that prevent bactericidal antibodies from binding to porin. Pilin and opacity protein (Opa) are important in adhesion. Lipo-oligosaccharide (LOS) stimulates PMN response and, when sialylated, blocks antibody-mediated killing.

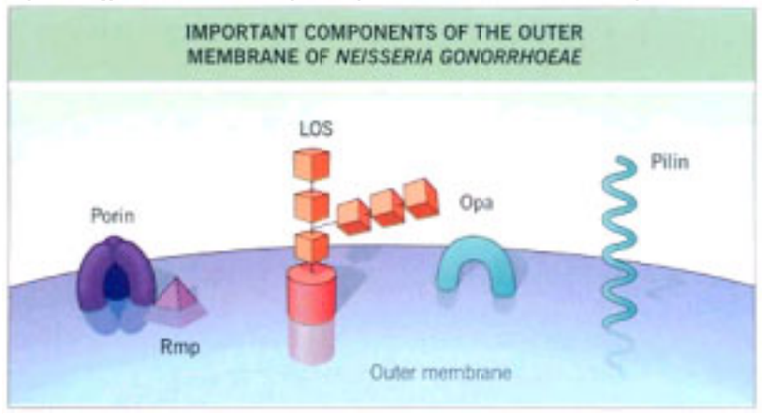


Figure 74-4 The life cycle of *Chlamydia trachomatis*. The elementary body (EB) invades the host cell and then reorganizes into the metabolically active reticulate body (RB) while in a phagosome. The reticulate body multiplies and the resultant reticulate bodies reorganize into elementary bodies, which are released by rupture of the host cell.

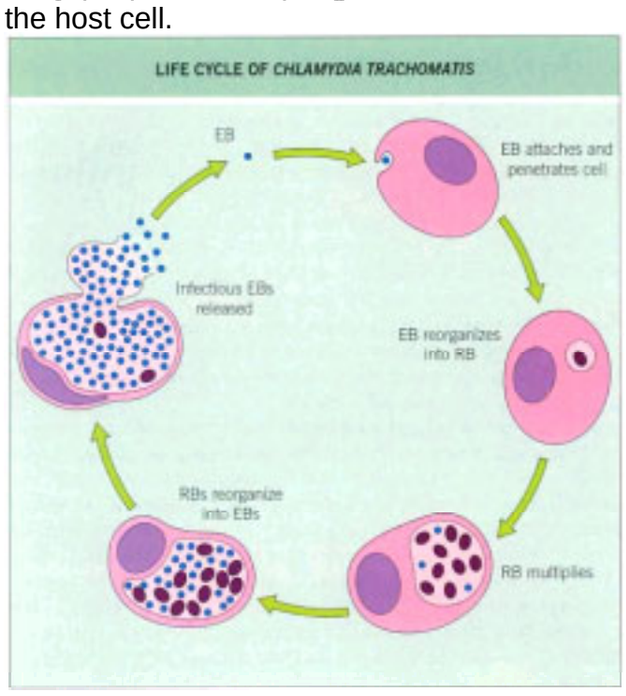


Figure 74-5 Procedure for obtaining a urethral specimen and preparing a smear for Gram stain. (a) If no discharge is present at the meatus, collect a specimen by inserting the urethral swab 2–4cm into the urethra and rotating for 5 seconds. (b) Roll the swab on a glass slide; rolling the swab preserves cell morphology.

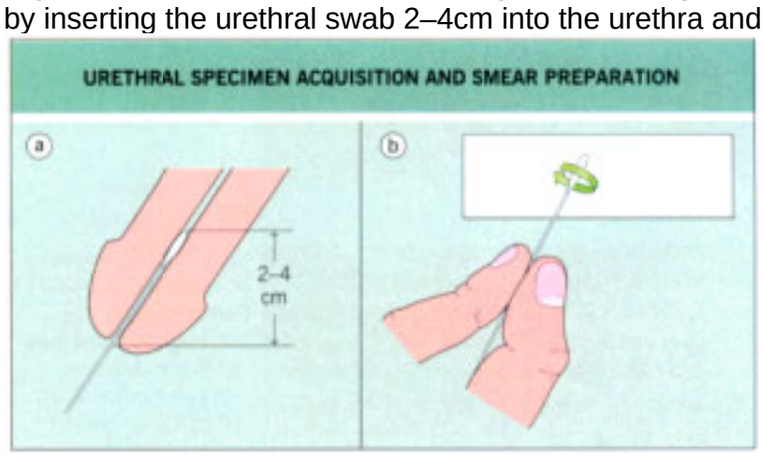


Figure 74-6 Nongonococcal urethritis. Gram-stained smear of urethral discharge containing many PMNs but no visible bacteria.

Figure 74-7 Gonorrhea. Gram-stained smear of urethral discharge containing numerous PMNs and Gram-negative intracellular diplococci consistent with *Neisseria gonorrhoeae*.

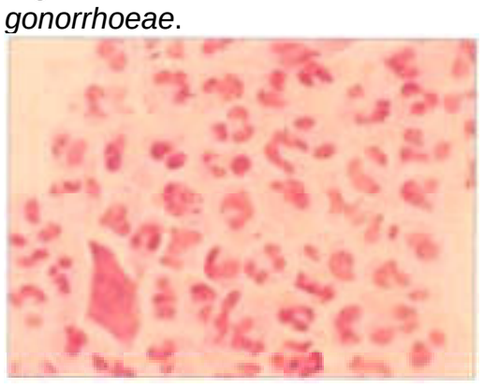


Figure 74-8 Trichomonal infection. Saline mount of *Trichomonas vaginalis* (arrow); characteristic ovoid shape and flagella can be seen.

Figure 74-9 A simplified approach to the diagnosis and management of symptomatic urethritis. hpf, high-power field; STS, serologic test for syphilis.

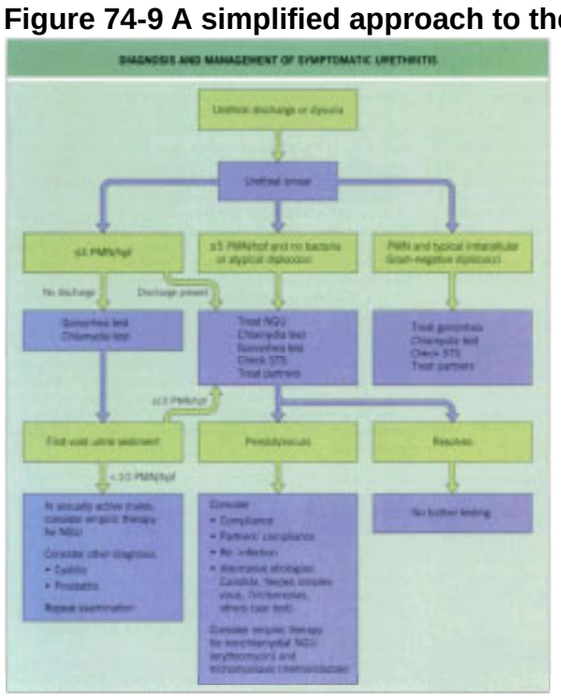


Figure 75-1 Diagnoses of syphilis in England and Wales. Primary, secondary and early latent infection seen in genitourinary medicine clinics. (a) 1931–2000 and (b) 1990–2000.

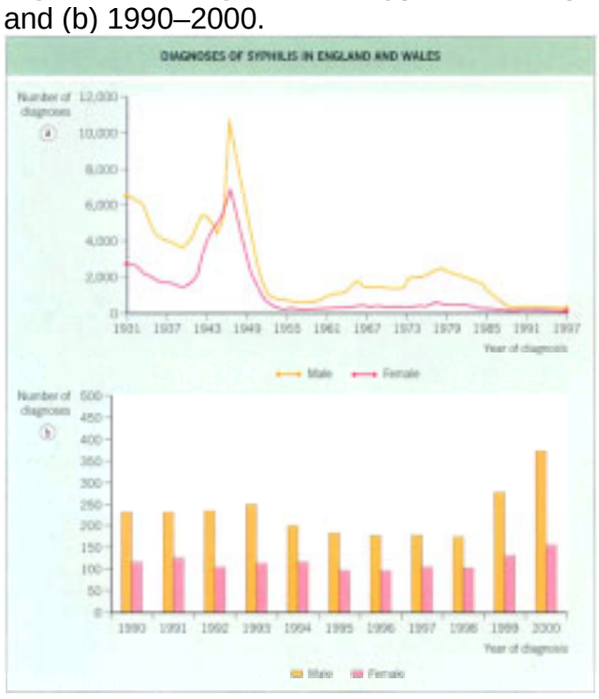


Figure 75-2 Clinical stages and presentation of syphilis.

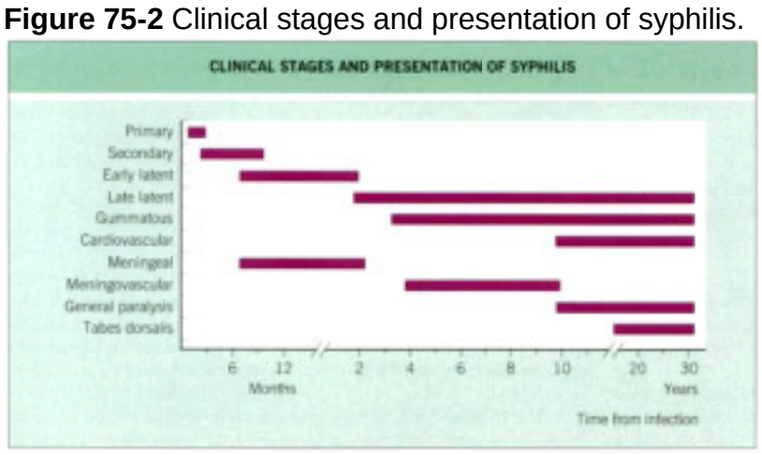


Figure 75-3 Primary chancre in coronal sulcus in primary syphilis. A typical solitary lesion with raised everted edges, central ulceration and undermined base. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

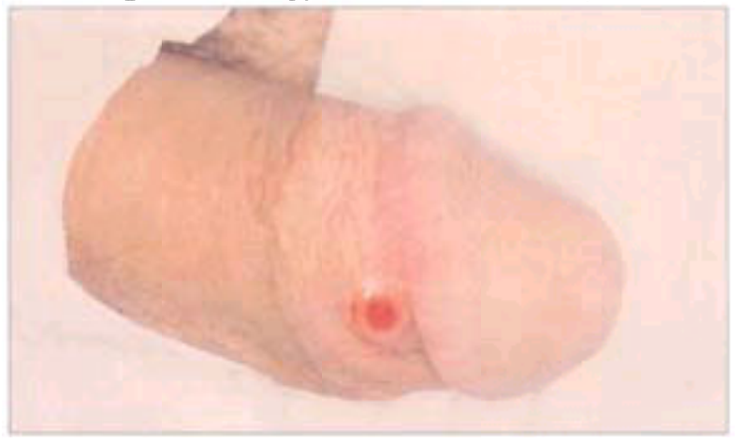


Figure 75-4 Chancre of upper lip in primary syphilis. The chancre shows the characteristic features of a raised, rolled and everted edge; central ulceration; and a granular base. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

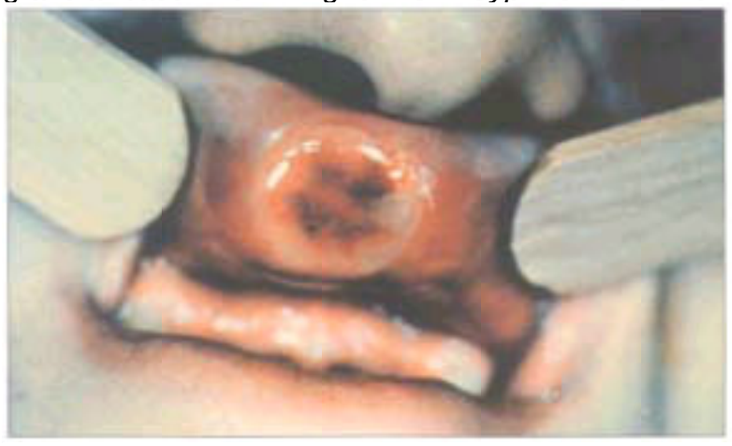


Figure 75-5 Maculopapular rash on trunk in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

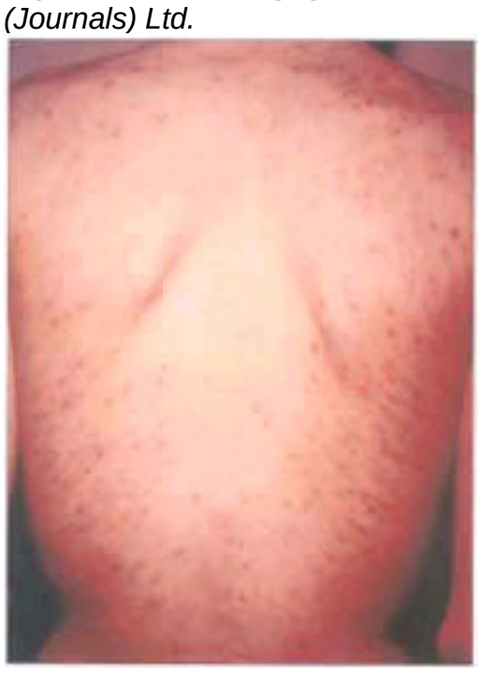


Figure 75-6 Plantar syphilid in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

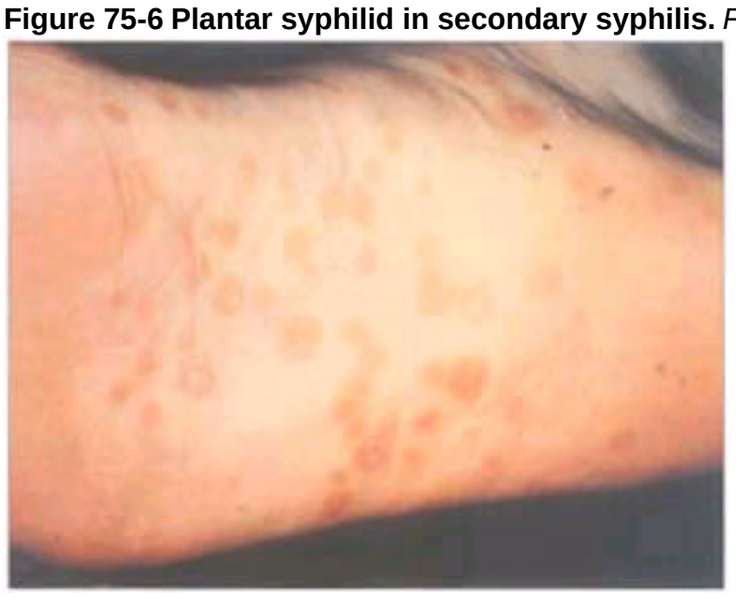


Figure 75-7 Split papules at angle of mouth and mucous patch on lower lip in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

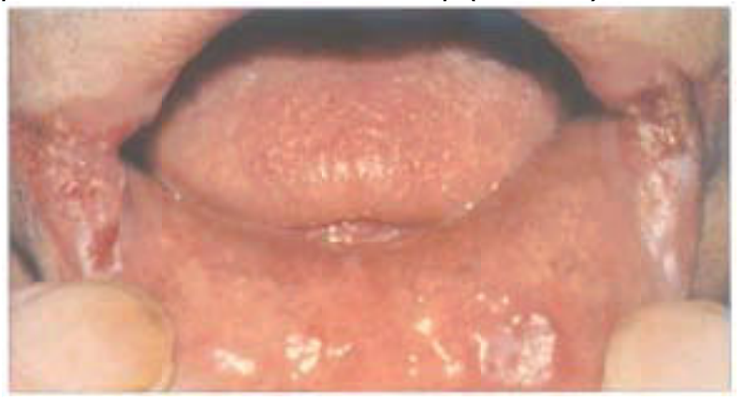


Figure 75-8 Maculopapular rash extending into axilla in secondary syphilis. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

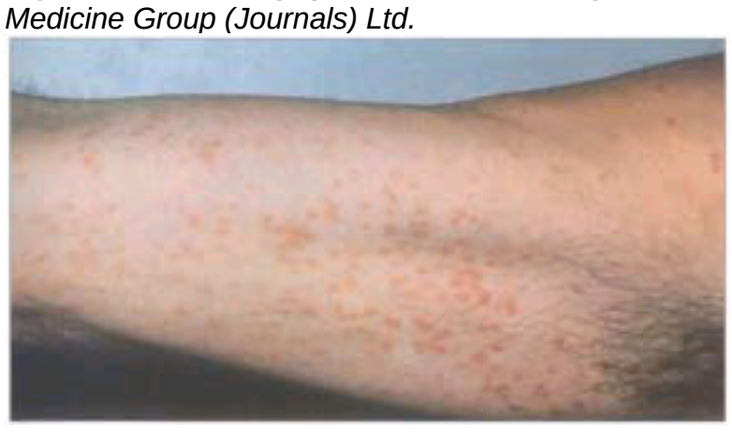


Figure 75-9 Cutaneous nodular gummas of upper arm in tertiary gummatous syphilis. The lesions have a serpiginous outline. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

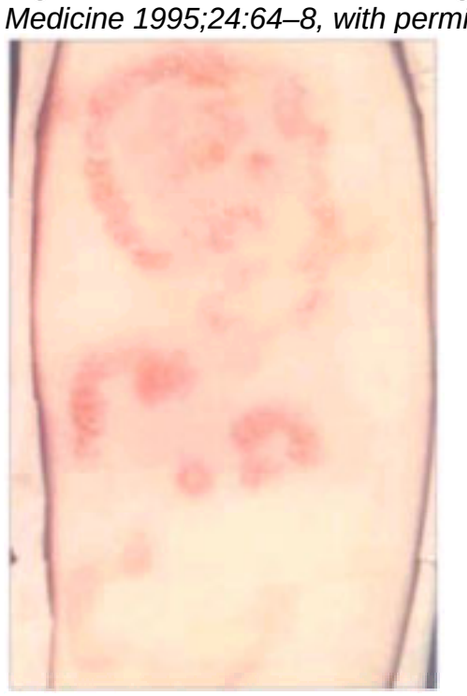


Figure 75-10 Multiple gummatous ulcers of lower leg in tertiary gummatous syphilis. The lesions have a punched-out appearance with 'wash-leather' slough overlying a base of granulation tissue. They show a tendency for peripheral healing with thin tissue-paper scars. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

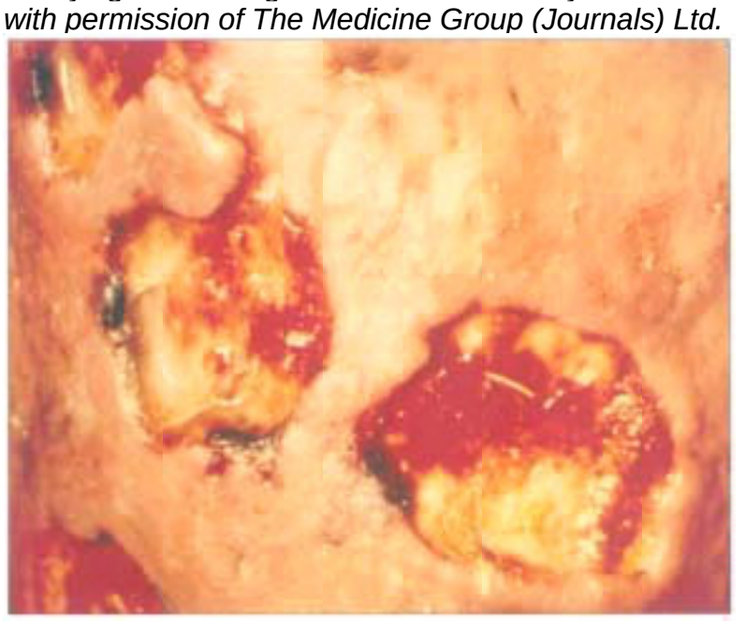


Figure 75-11 Typical facies in late congenital syphilis. There is frontal bossing, an underdeveloped maxilla, a prominent jaw and a depressed nasal bridge, with multiple gummatous ulcers of scalp. *From Kinghorn GR. Syphilis. Medicine 1995;24:64–8, with permission of The Medicine Group (Journals) Ltd.*

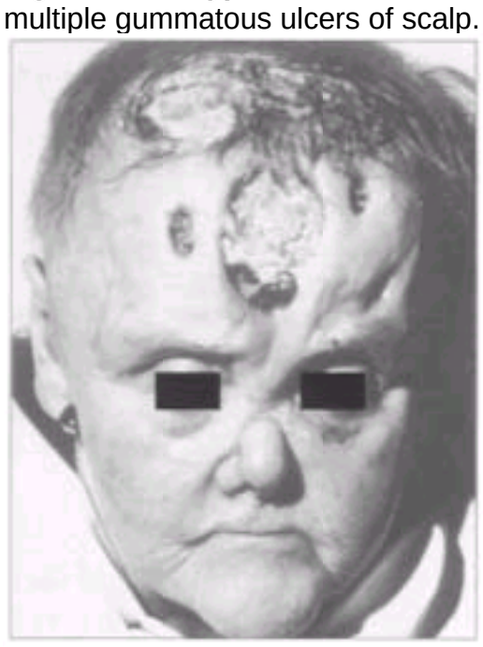


Figure 76-1 The herpes simplex virion. This consists of a dsDNA core surrounded by a capsid, an amorphous tegument layer and a lipid envelope with numerous glycoprotein spikes. The overall diameter is 150–200nm. Virus replication takes place within the nucleus of the infected cell. The envelope is gained as the virion passes through the nuclear membrane. Replication of virus within the host cell results in cell lysis and destruction. Latent virus do not cause neural cell lysis within ganglia.

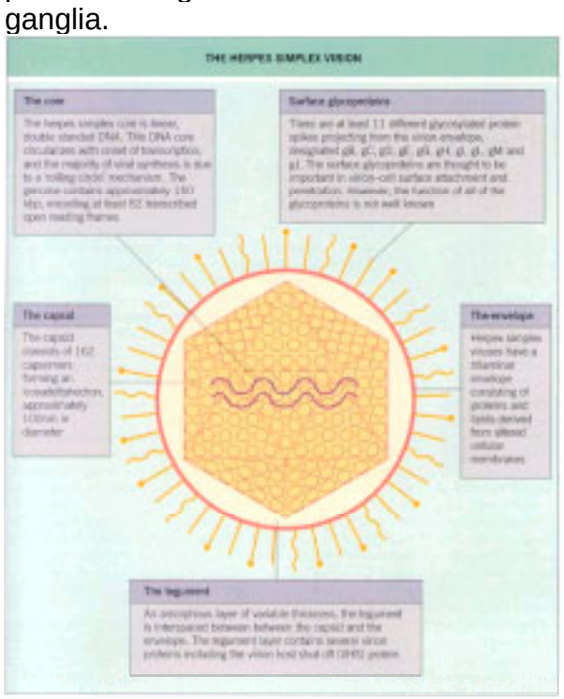


Figure 76-2 Histology. Section of human skin showing typical HSV virus effects: multinucleated giant cells (arrowhead) and intranuclear inclusion (arrow).

Figure 76-3 Primary HSV. Multiple painful, erythematous, ulcerating lesions on shaft and head of penis. Exudative crust is visible over one lesion.

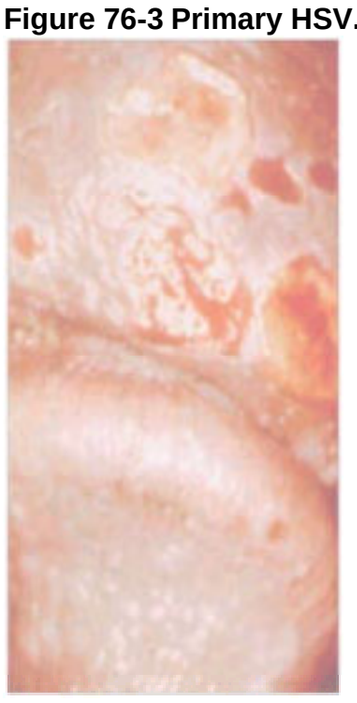


Figure 76-4 Frequency of asymptomatic and unrecognized HSV-2 in four major US cities.[4] [31] [37] [38] [39]

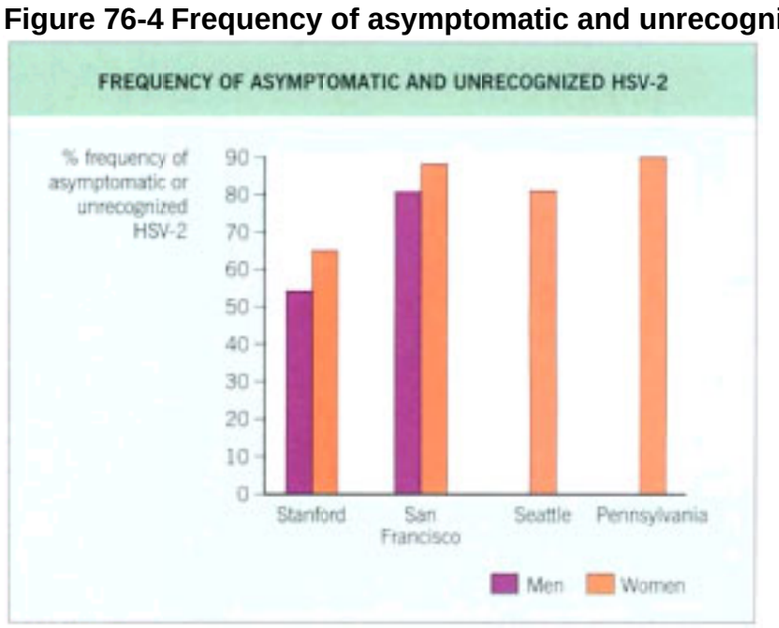


Figure 77-1 Normal squamous cells and inflammatory cells. (Pap stain). *Courtesy of Dr William H Rogers.*

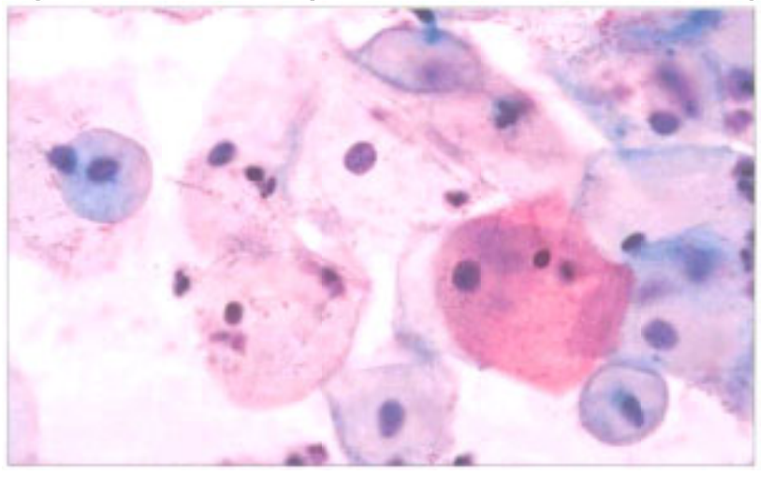


Figure 77-2 Atypical squamous cells of undetermined significance. Here the cells are slightly enlarged and irregular relative to the cells in Figure 77.1 and contain perinuclear clear areas suggestive, but not diagnostic, of HPV infection. *Courtesy of Dr William H Rogers (Pap stain).*

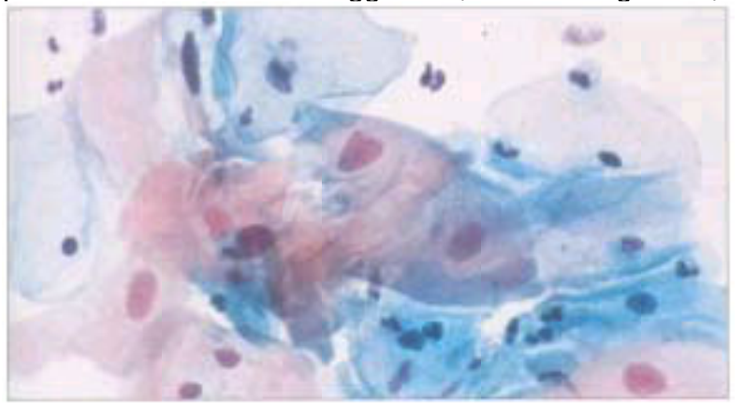


Figure 77-3 Low-grade squamous intraepithelial lesion. In this case, the lesion would classically be called a mild dysplasia; the cell in the center of the photograph has a nucleus that is enlarged more than four times the size of the surrounding normal squamous cells. In addition, the nucleus has irregular nuclear outlines and hyperchromasia. *Courtesy of Dr William H Rogers (Pap stain).*

Figure 77-4 High-grade squamous intraepithelial lesion. This contains small cells with an increased nuclear to cytoplasmic ratio and marked nuclear hyperchromasia; in the classic terminology, this would be considered a severe dysplasia or CIN III. *Courtesy of Dr William H Rogers (Pap stain).*

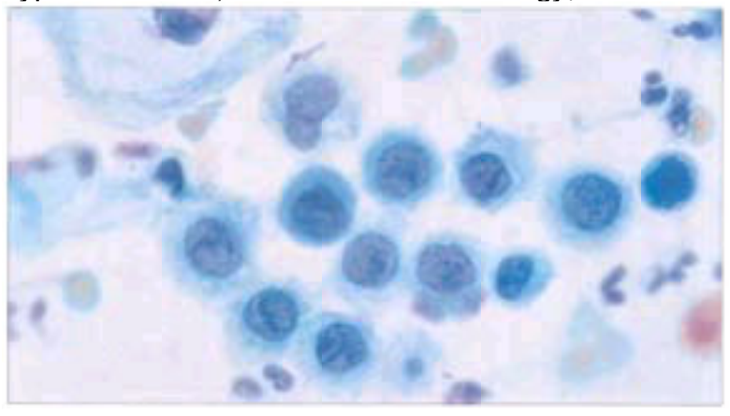


Figure 77-5 Carcinoma in situ. The abnormal hyperchromatic cells have indistinct cell borders and form a pseudosyncytial arrangement. *Courtesy of Dr William H Rogers (Pap stain).*

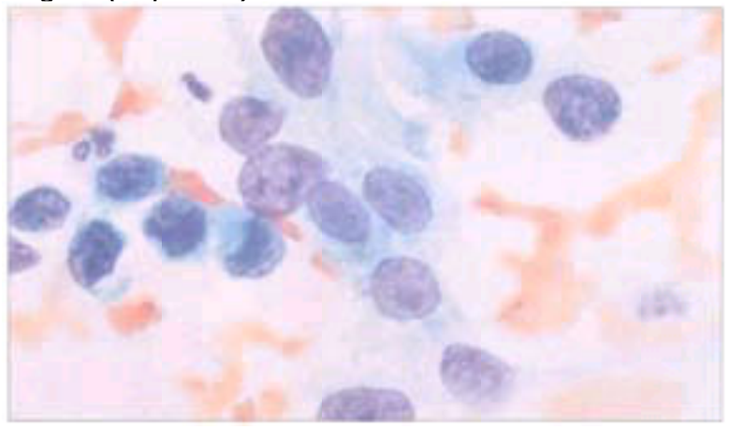


Figure 77-6 Squamous cell carcinoma. This shows highly atypical, enlarged, abnormal keratinized cells. *Courtesy of Dr William H Rogers (Pap stain).*

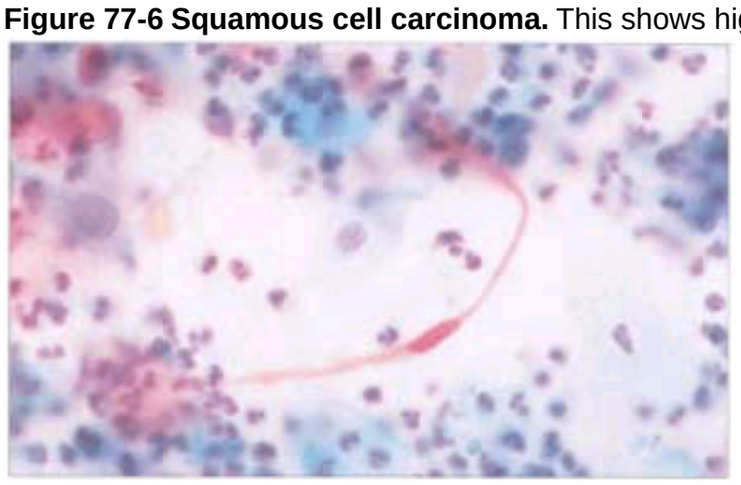


Figure 77-7 Genital HPV infection. (a) Vulvovaginal HPV infection. (b) Penile HPV infection.

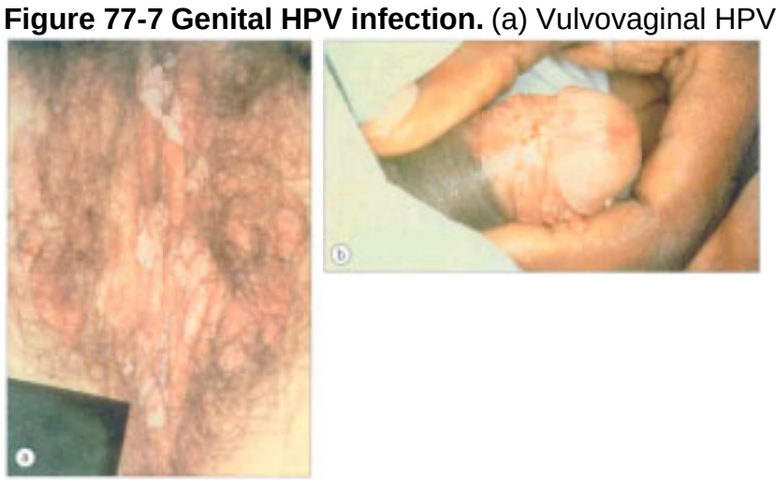


Figure 77-8 Cervigrams. (a) Normal cervix. (b) Cervix with ectopy. (c) Cervix with microglandular hyperplasia.

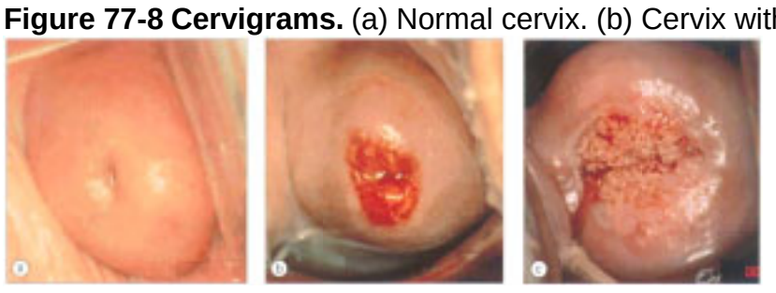


Figure 78-1 Lymphogranuloma venereum causing unilateral vulvar lymphedema and inguinal buboes.

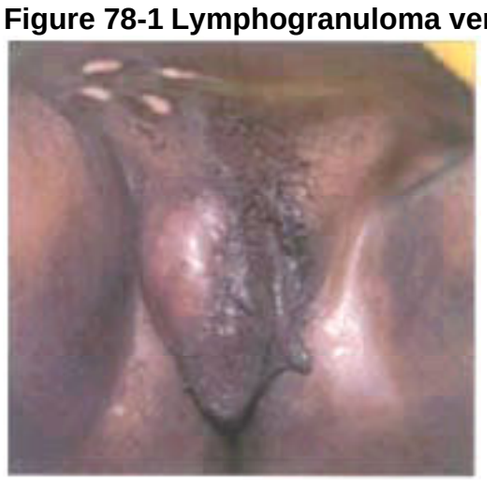


Figure 78-2 Chancroid ulcer. (a) Before and (b) after the performance of a swab, demonstrating the friability of the ulcer base.

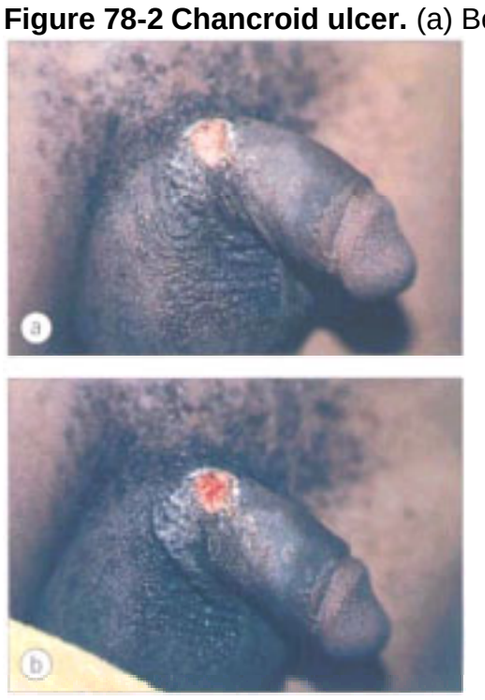


Figure 78-3 Typical chancroid ulcer. Unilateral lymphadenitis and demonstration of the aspiration of a bubo.

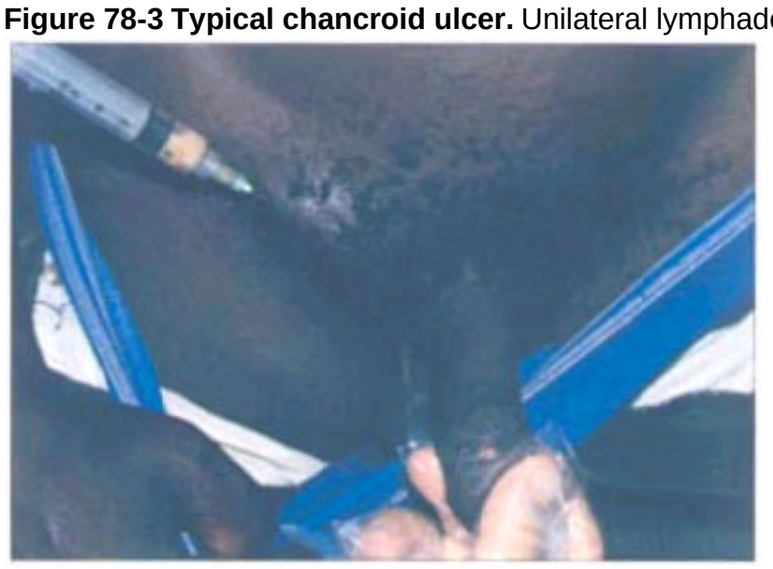


Figure 78-4 Phagedenic chancroid with extensive tissue destruction.

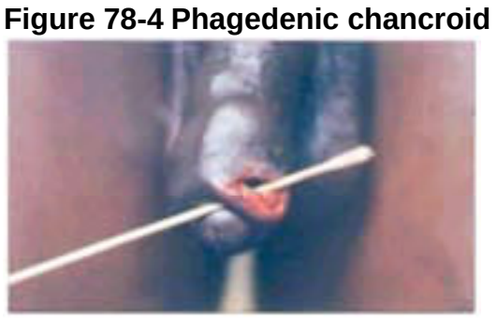


Figure 78-5 Healed inguinal bubo with scar formation from previous chancroid infection.

Figure 78-6 Granuloma inguinale. The chronic, granulomatous, beefy-red ulcer without suppuration is typical. *Photo kindly supplied by J K Maniar.*

Figure 78-7 Granuloma inguinale. Lack of treatment has permitted progressive destruction of the scrotum. *Photo kindly supplied by J K Maniar.*

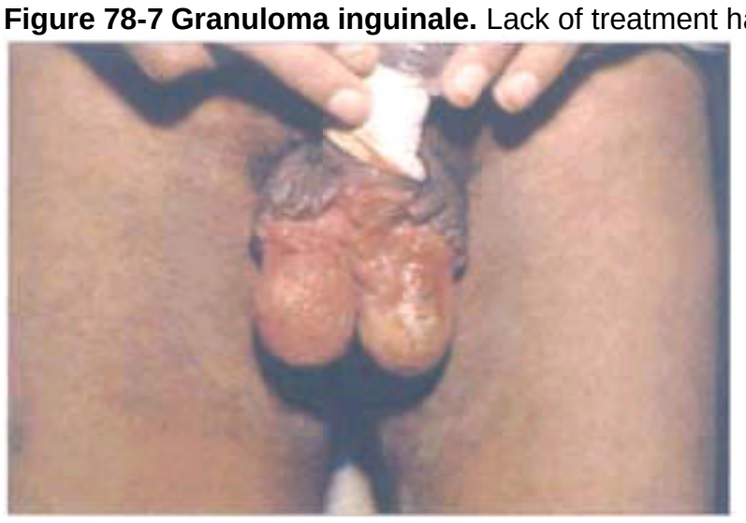


Figure 79.a-1 Clinical and microscopic evaluation of recurrent vaginitis.

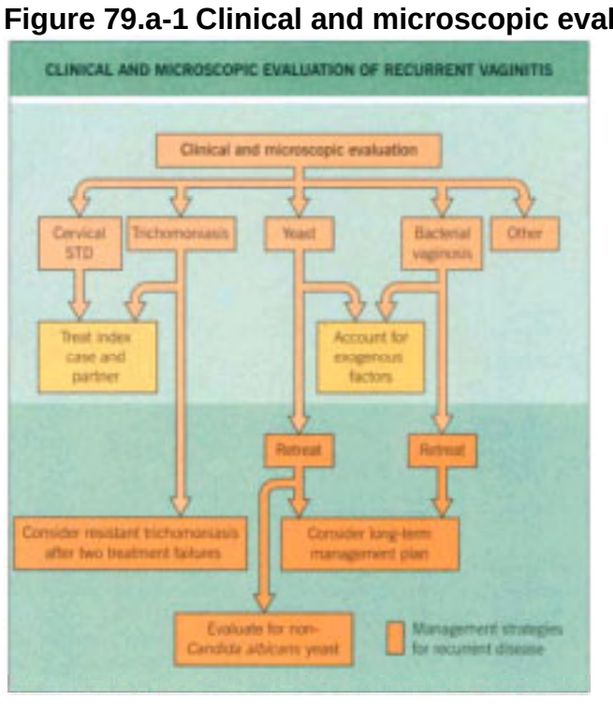


Figure 79.b-1 Diagnostic assignment and cause of infertility.

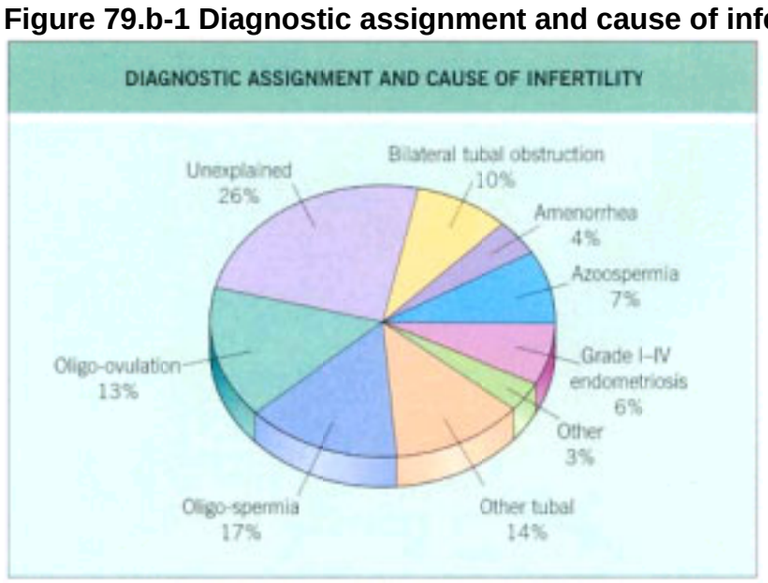


Figure 80-1 Distribution of baseline oral temperatures in healthy men and women. Frequency distribution of 700 baseline oral temperatures obtained during two consecutive days of observation in 148 healthy young volunteers. Arrow indicates location of 98.6°F (37°C). *With permission from Mackowiak et al.*[21] *Copyright 1992, American Medical Association.*

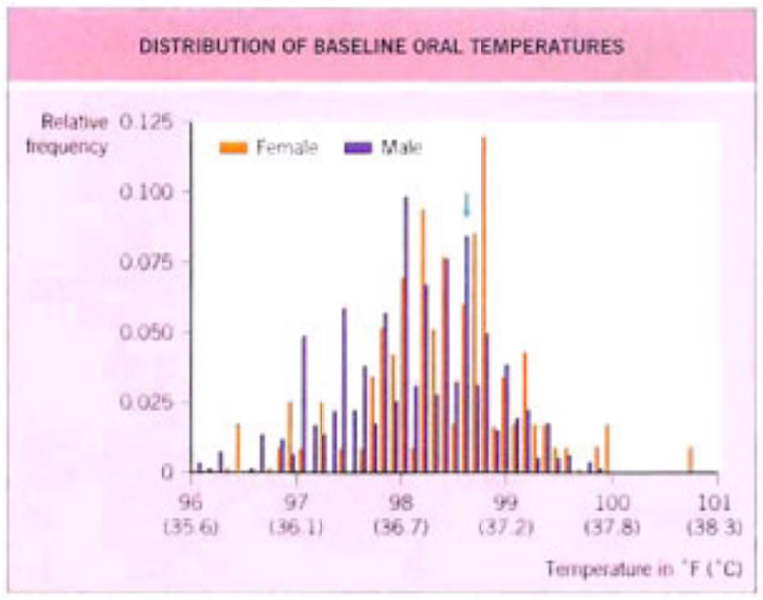


Figure 80-2 Sagittal view of the brain and upper spinal cord. The figure shows the multisynaptic pathway of skin and spinal thermoreceptors through the spinothalamic tract (STT) and reticular formation (RF) to the anterior hypothalamus, the preoptic region and the septum. *Redrawn with permission from Mackowiak.*[1]

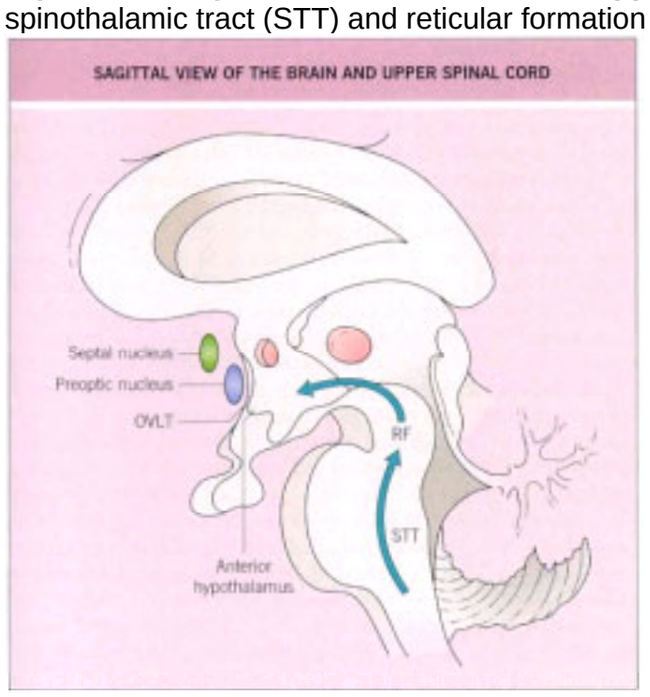


Figure 80-3 Events during fever and defervescence. When pyrogens are present in the preoptic region the whole body and neuronal responses are as shown by the purple lines. Upper: Normally the firing rates of warm-sensitive and temperature-insensitive neurons functionally overlap at 98.6°F (37°C), the set-point of thermoregulatory neurons. During pyrogen inhibition of warm-sensitive neurons this overlap occurs at the raised set-point of 102.2°F (39°C). Center: During fever, heat production is initially great but, as body temperature rises towards 102.2°F, heat production diminishes and should cease at 102.2°F. Lower: During fever initiation, shivering causes an increase in hypothalamic temperature and then ceases as the temperature reaches 102.2°F. During defervescence the hypothalamus activates heat loss responses such as sweating. *Adapted with permission from Boulant.*[23]

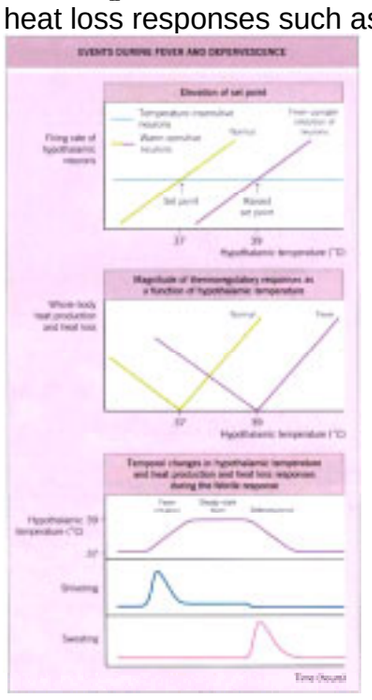


Figure 80-4 Production of fever. Microbial agents, inflammatory agents and some cytokines induce the synthesis and release of pyrogenic cytokines from a variety of cells. These cytokines, in turn, trigger specialized endothelial cells of the hypothalamic vascular organs, which release PGE_2 . Elevated PGE_2 then brings about increases in cyclic adenosine monophosphate (cAMP) monoamines and calcium in the thermoregulatory center of the anterior hypothalamus, resulting in a resetting of the thermostatic temperature from normothermia to febrile levels. These neurotransmitters then activate the vasomotor center, which brings about vasoconstriction (heat conservation) and increased heat production, both resulting in an increase in blood temperature (fever). Regardless of the cause of fever, antipyretics inhibit this pathway by preventing the increase in cytokine-mediated PGE_2 production in the hypothalamus. IFN, interferon.

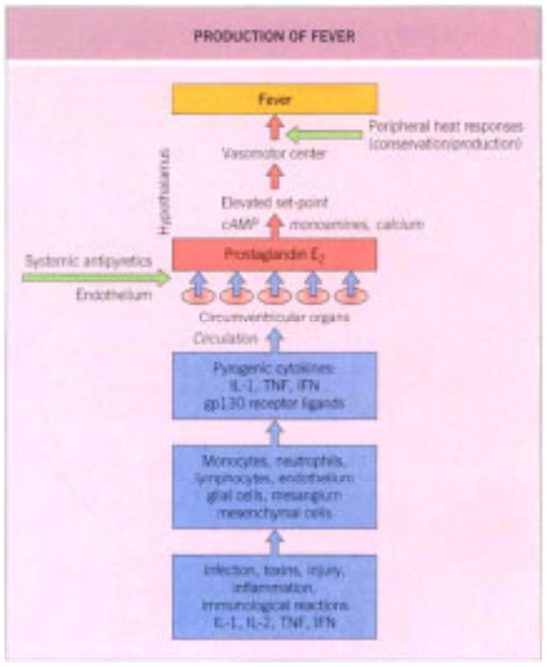


Figure 81-1 The early focal rash of smallpox. This is the fifth day of the fever and the third day since the rash began to appear.

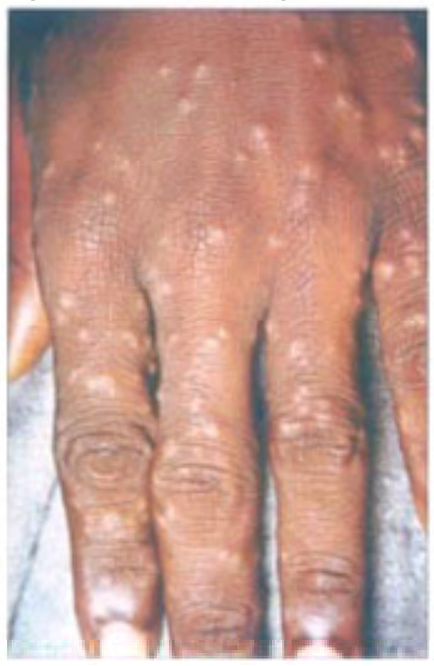


Figure 81-2 Fully developed, almost pathognomonic hemorrhagic rash of meningococcal sepsis.

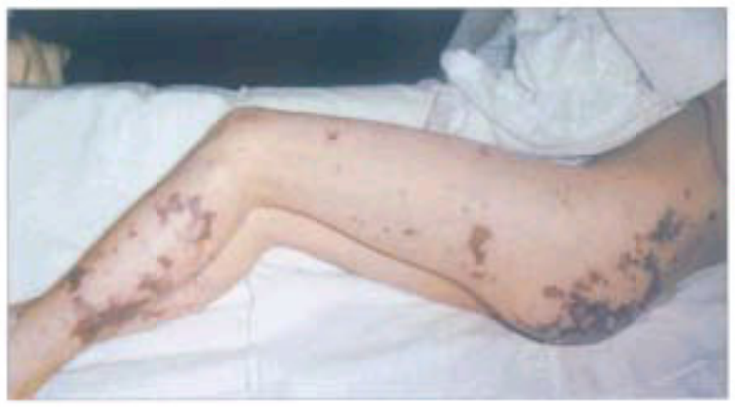


Figure 81-3 Very early rash of meningococcal sepsis. A few petechiae only, but meningococcal sepsis nonetheless. It can progress to the appearance of Figure 81.2 within minutes or hours. This is the window of opportunity for early treatment.

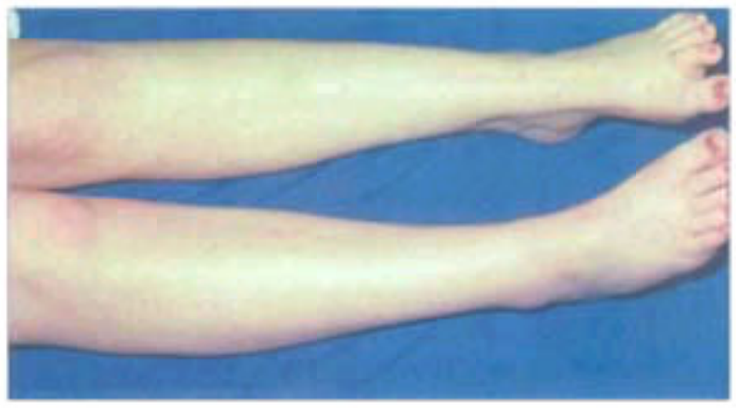


Figure 81-4 Hand, foot and mouth disease. This shows the scanty lax vesicles found at these sites. There is often a maculopapular rash too, especially on the buttocks.

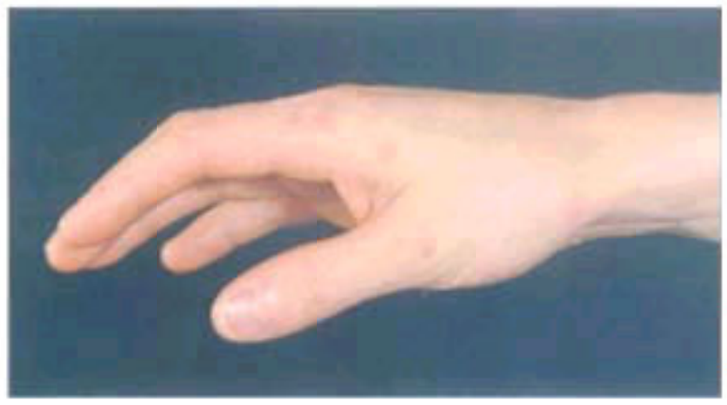


Figure 81-5 Purpuric skin lesions in staphylococcal endocarditis.

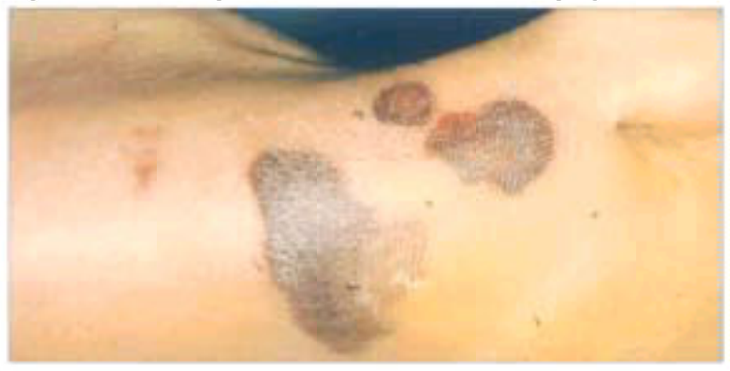


Figure 81-6 Oral signs in hand, foot and mouth disease.

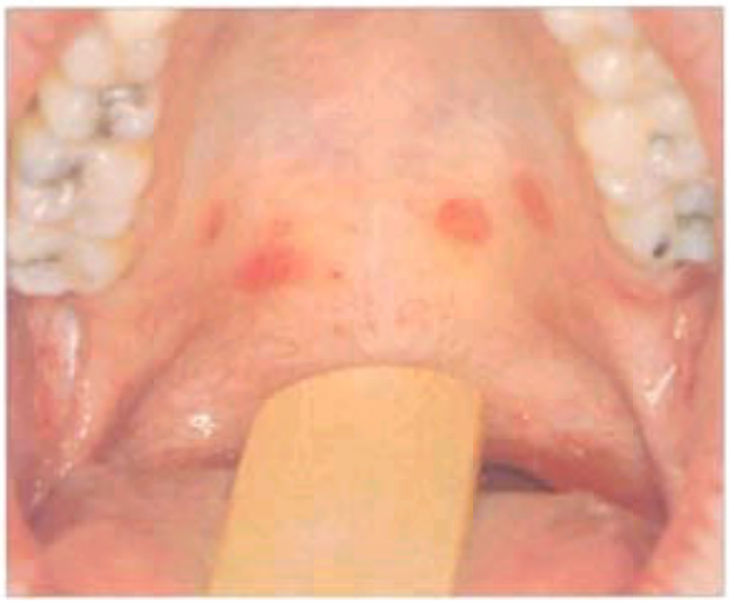


Figure 81-7 Decision pathway for the management of acute febrile illness.

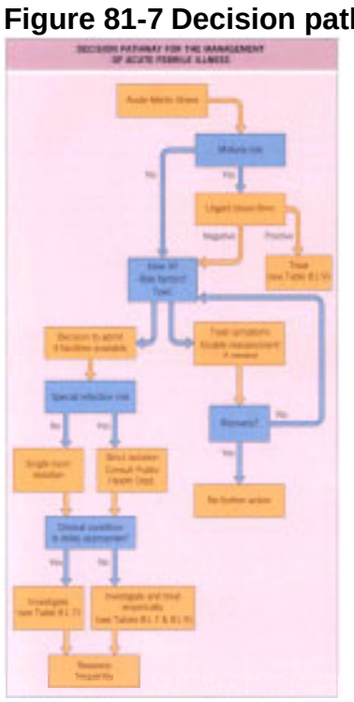


Figure 81-8 Chest radiograph of a patient with *Mycoplasma* pneumonia. Respiratory symptoms are often scanty or absent in the first few days of the illness.

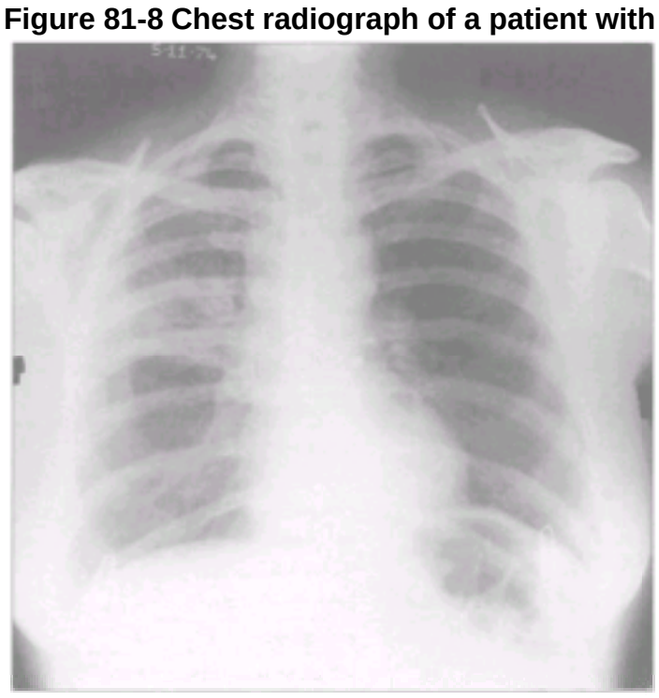


Figure 83-1 Complexity of modern health care systems.

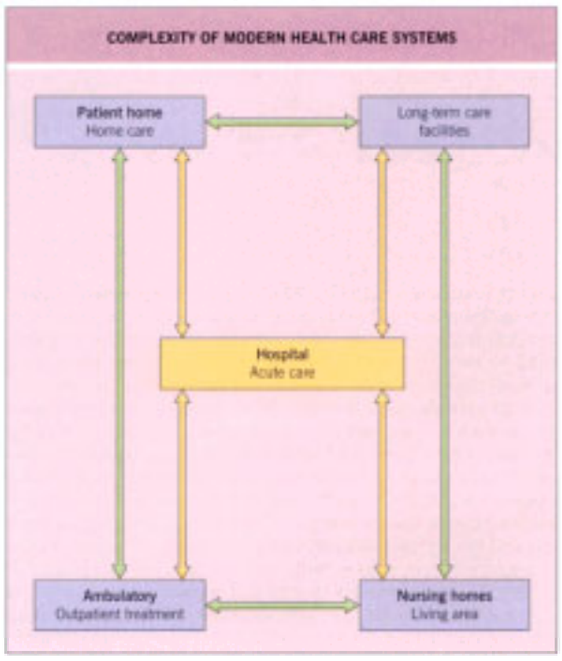


Figure 83-2 Trends in average length of hospital stay, patient case-mix, and nosocomial infection; Yale-New Haven Hospital, 1988–1995. (a) Trends showing the decrease in average length of hospital stay and the increase in the mean diagnosis-related group case-mix index. During the study period, there was a significant decrease in the mean length of stay, from 7.3 to 6.0 days (p = 0.01), and a concomitant increase in the mean diagnosis-related group case-mix index, from 1.03 to 1.24 (p = 0.001). (b) Nosocomial infection rates, assessed by repeated prevalence surveys, remained unchanged (p = 0.43) over the study period, but rates of nosocomial bloodstream infection increased (p = 0.05) corresponding to increased medical device use (p = 0.001, not shown). *From Weinstein et al.*[10] *, with permission.*

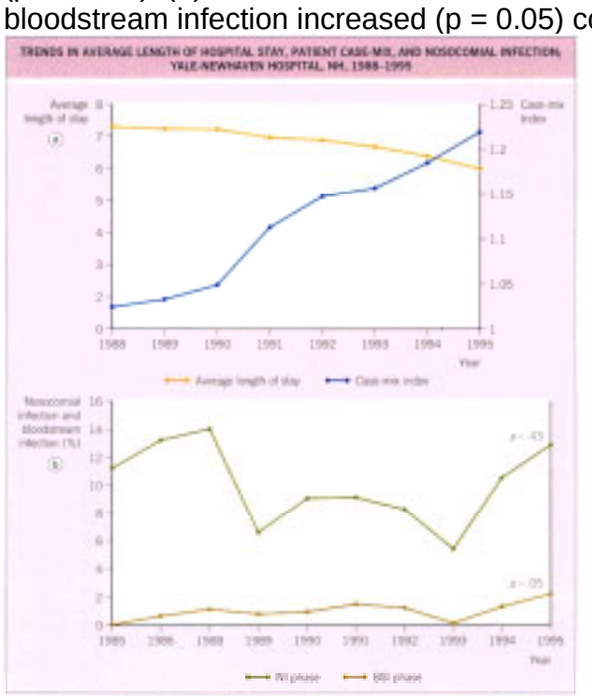


Figure 83-3 Leading sites in acute, subacute and long-term care settings. *From Sax et al.,[3] with permission.*

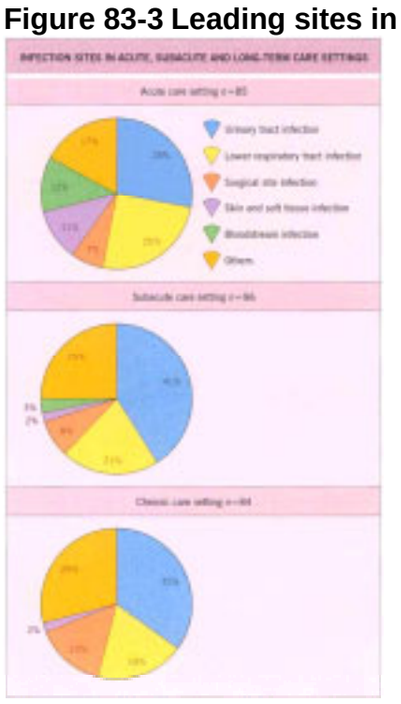


Figure 83-4 Outpatient antibiotic use in different developed countries. *From Harbarth et al., [15] with permission.*

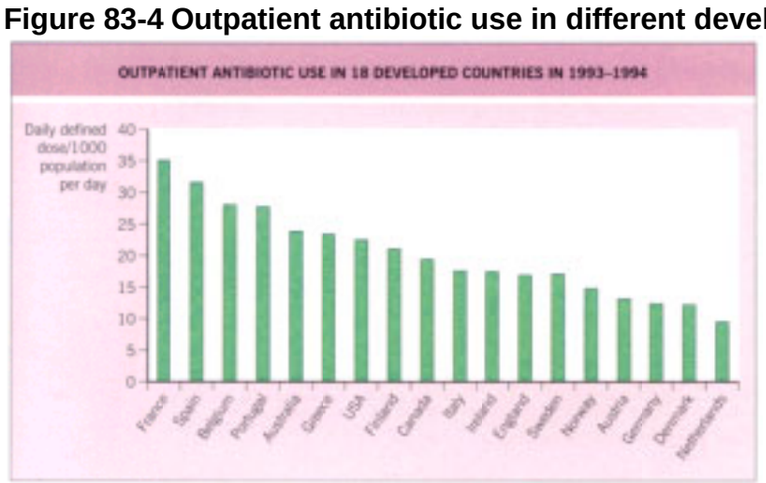


Figure 83-5 The essential causes of infection.

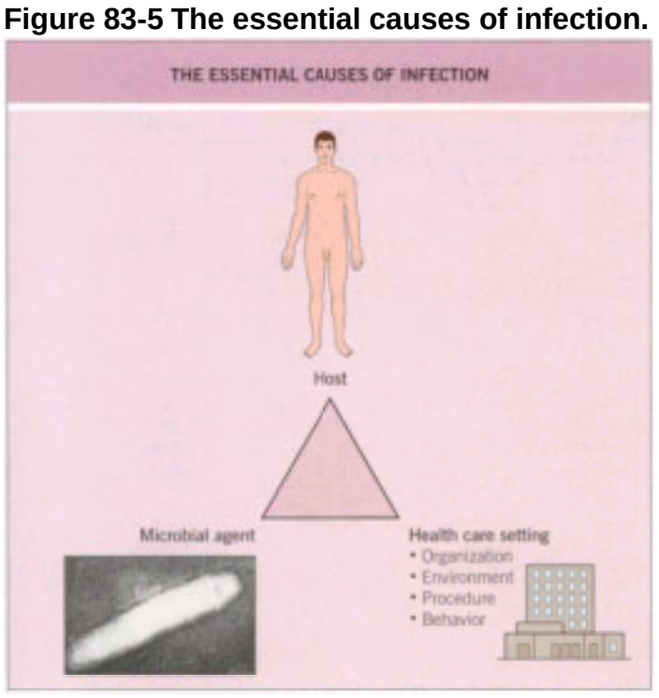


Figure 83-6 Relationship between antibiotic consumption and penicillin-resistant pneumococci. Total use of antibiotics (in daily defined dose (DDD) per 1000 population) is indicated (horizontal axis) and percent of penicillin-resistant *Streptococcus. pneumoniae* isolates. *Data aggregated from several studies and kindly provided by Dr S Harbarth. From Harbarth et al.,*[15] *with permission.*

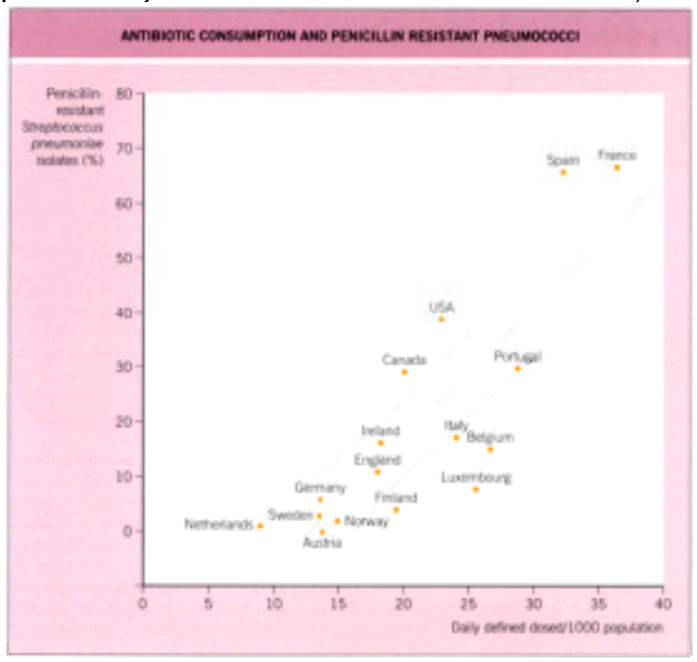


Figure 83-7 Current and future structure of infection prevention programs.

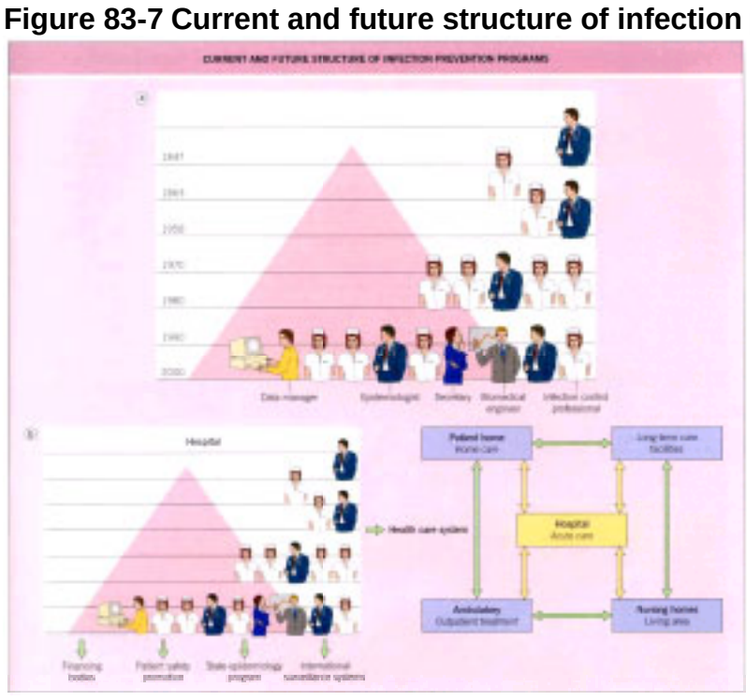


Figure 84-1 Antimicrobial resistance. Summary of antimicrobial resistance among common pathogens identified from ICU patients with nosocomial infections in hospitals participating in the CDC's NNIS. *From: http://www.cdc.gov/ncidod/hip/NNIS/ar_surv99.pdf*

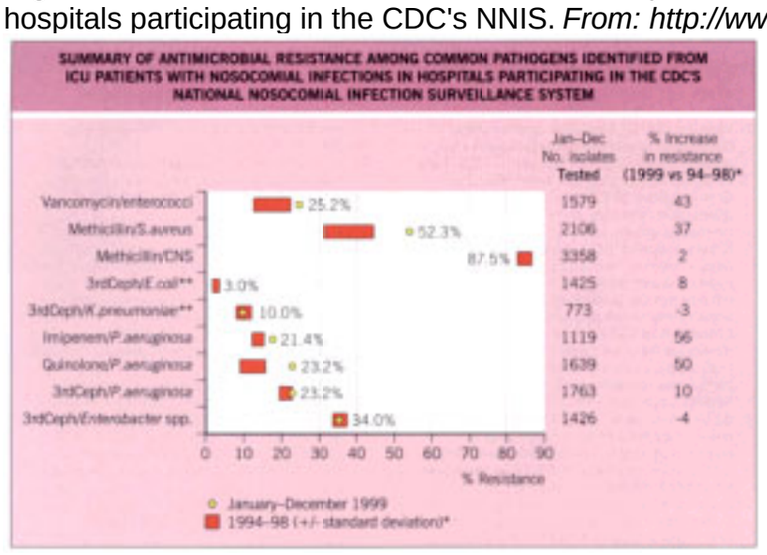


Figure 85-1 Predicted mortality rate based upon total burn size and age.

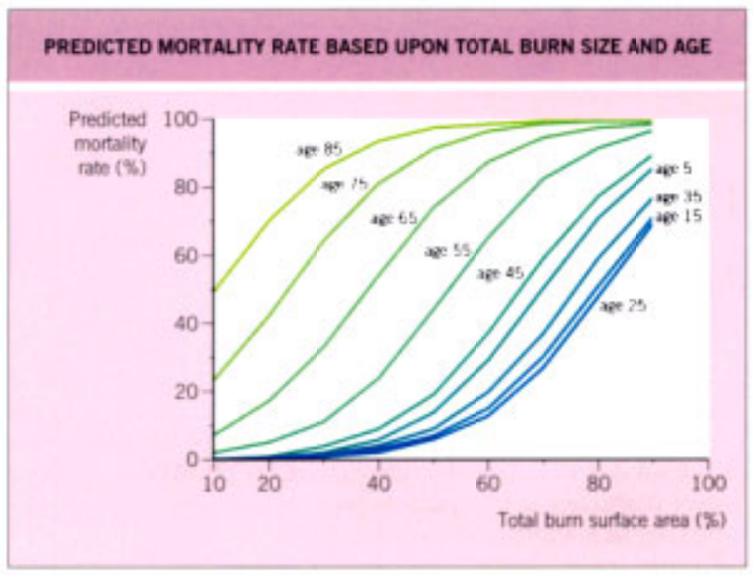


Figure 85-2 Incidence and outcome of *Pseudomonas aeruginosa* bacteremia over a 20-year period.

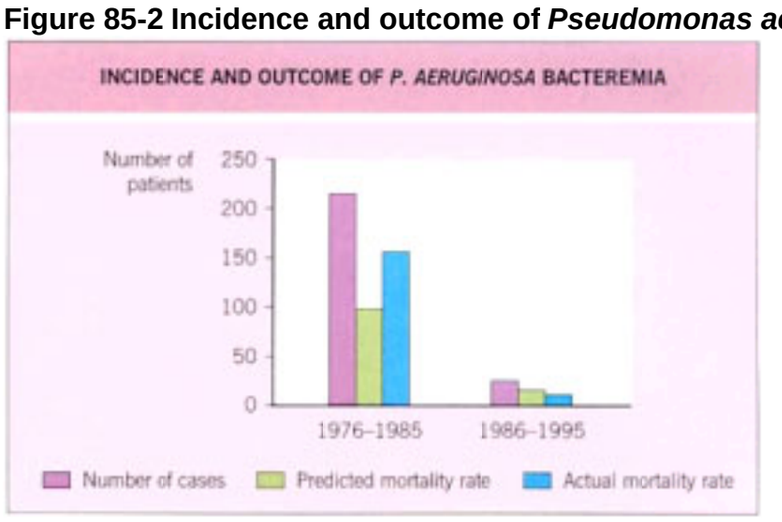


Figure 85-3 Types of infections in burn patients (1986–1995).

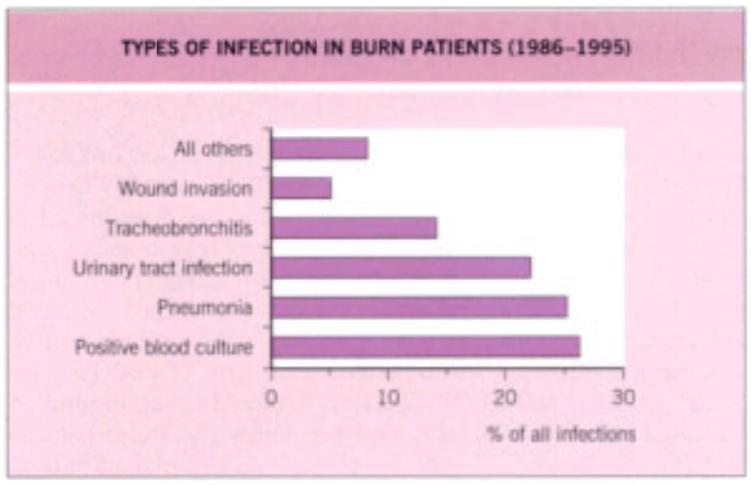


Figure 85-4 Causes of infection.

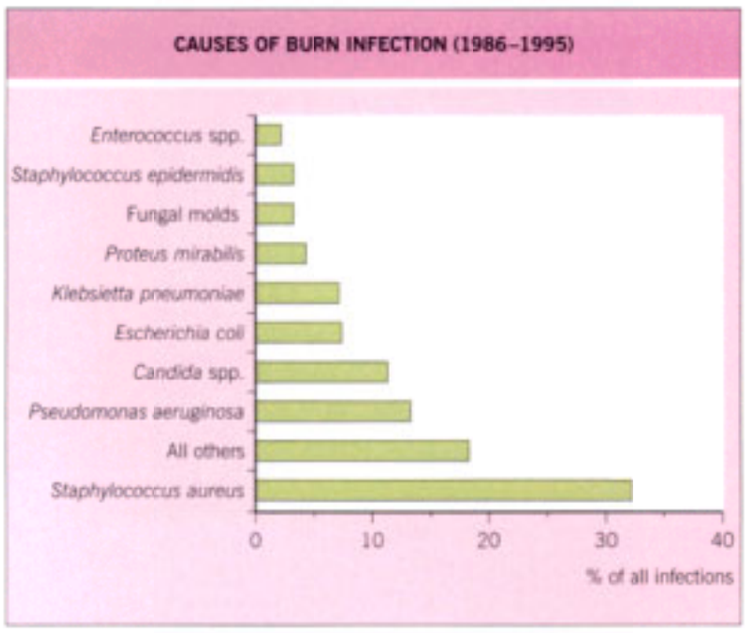


Figure 85-5 Contact plate used for wound surveillance cultures. The culture media is lifted out of the Petri dish by the attached sterile gauze, placed in contact with the burn wound, and then returned to the Petri dish for incubation.

Figure 85-6 Clinical appearance of suppurative chondritis.

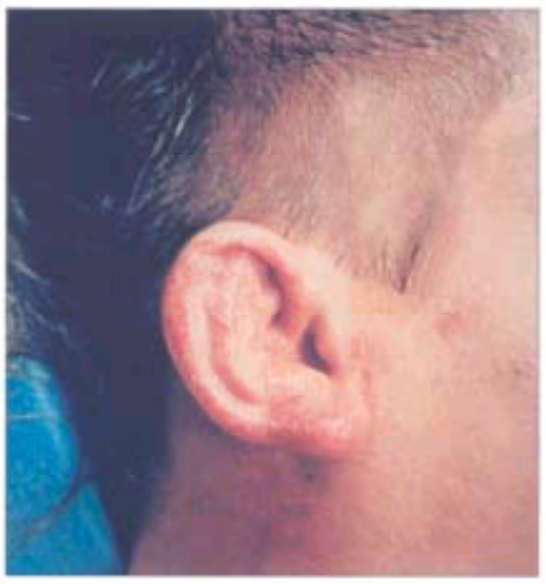


Figure 85-7 Bivalve excision of infected cartilage.

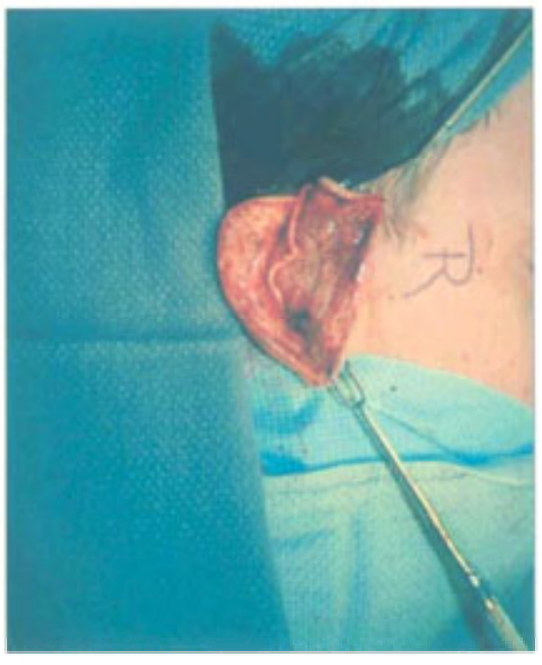


Figure 85-8 Vein resection for suppurative thrombophlebitis.

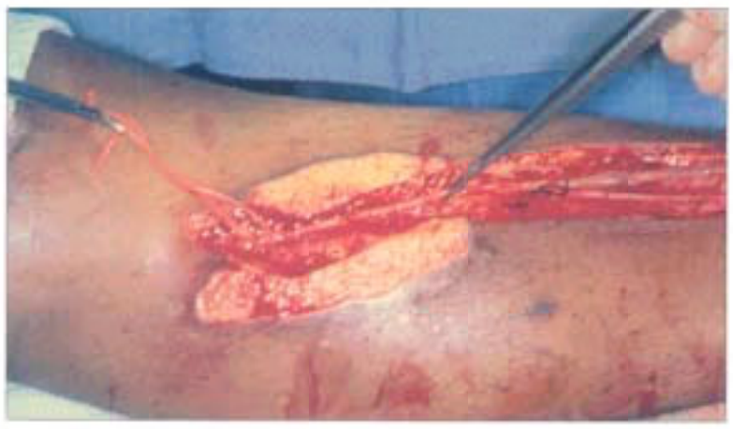


Figure 85-9 Suppurative thrombophlebitis. (a) Histologic section of excised vein demonstrating thrombus. (b) Micro-organisms (Gram-positive cocci) present within the thrombus (arrow).

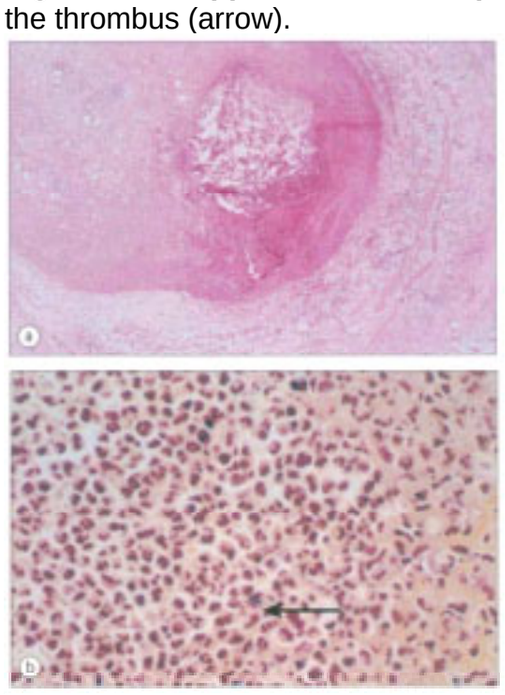


Figure 85-10 Day of onset of infection in burn patients.

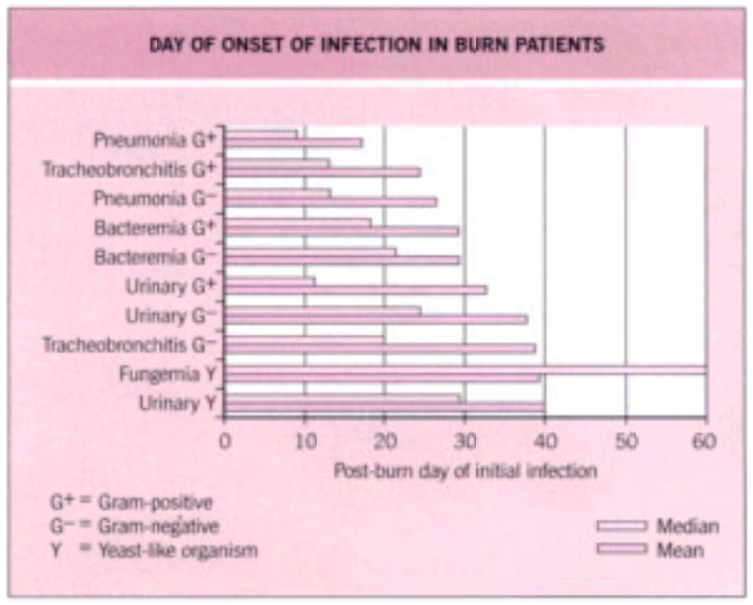


Figure 85-11 Invasive pseudomonal burn wound infection, stage 2C.

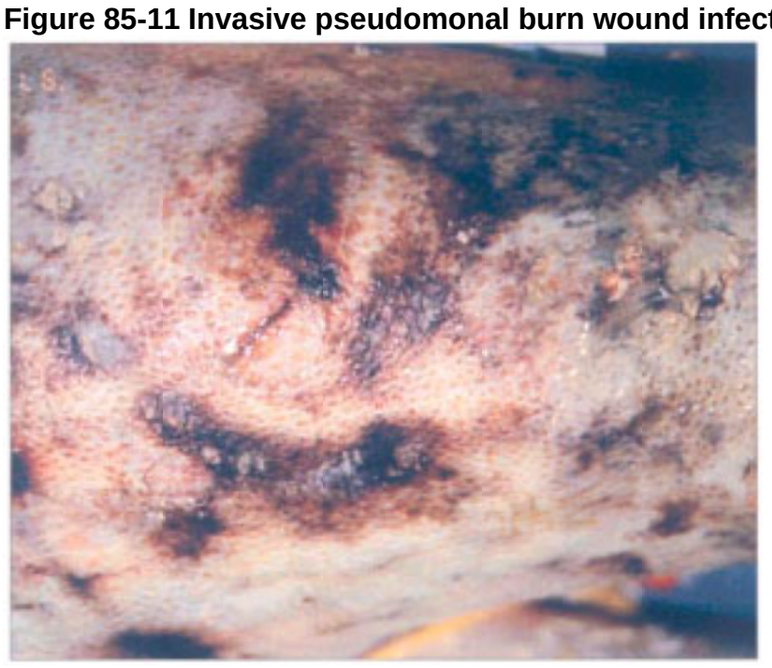


Figure 86-1 Conditions associated with systemic inflammatory response syndrome. The complex, overlapping relationship between infection and inflammation.

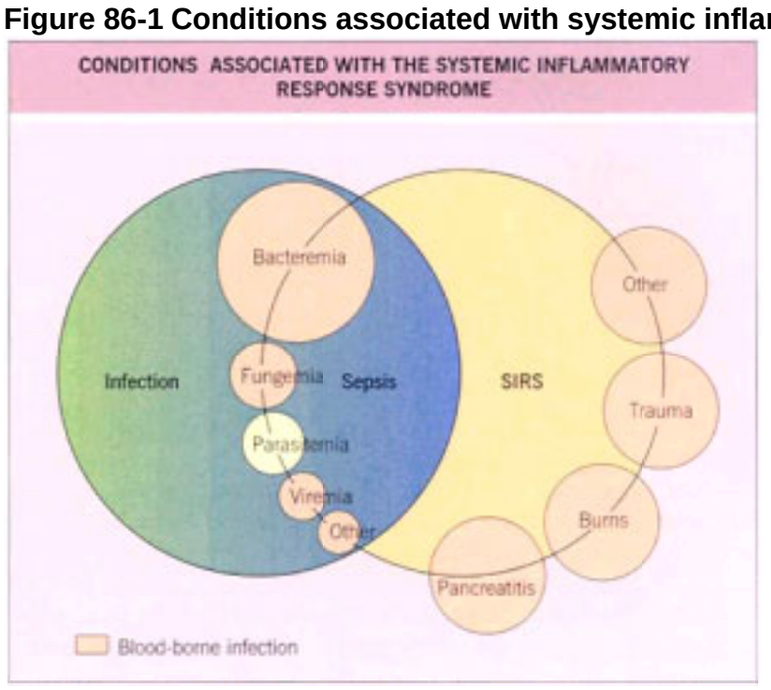


Figure 86-2 Hypothetical interactions of proinflammatory and anti-inflammatory responses. Pathogenesis of immunosuppression, nosocomial infection, organ dysfunction and outcome.

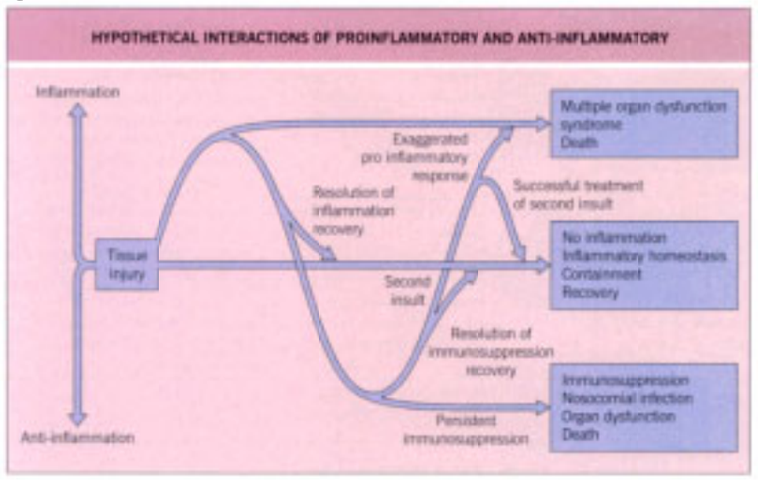


Figure 87-1 Record trend, 1992–2001, in bloodstream infection in Belgian hospitals by pathogen group.

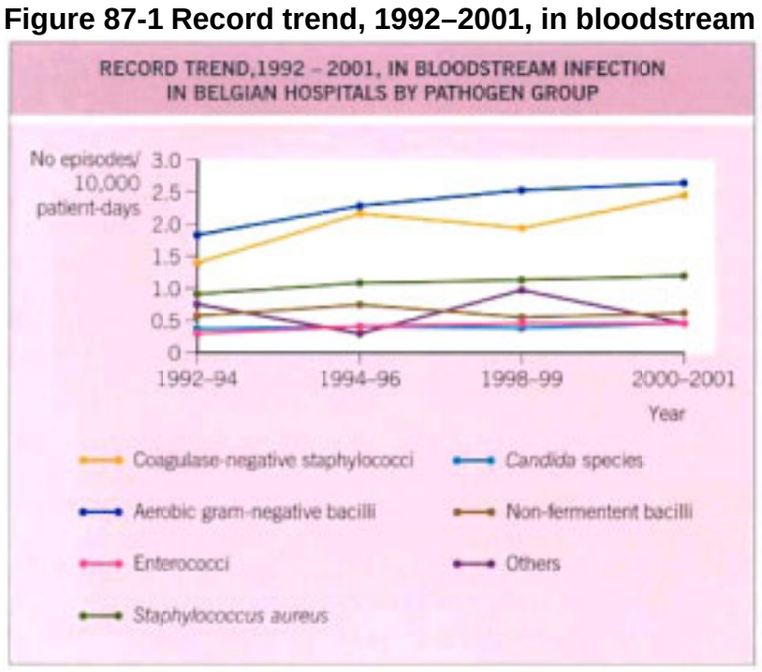


Figure 87-2 Trends in bloodstream infection rates by intensive care unit type and year. National Nosocomial Infection Surveillance System, USA, 1990–99.[13]

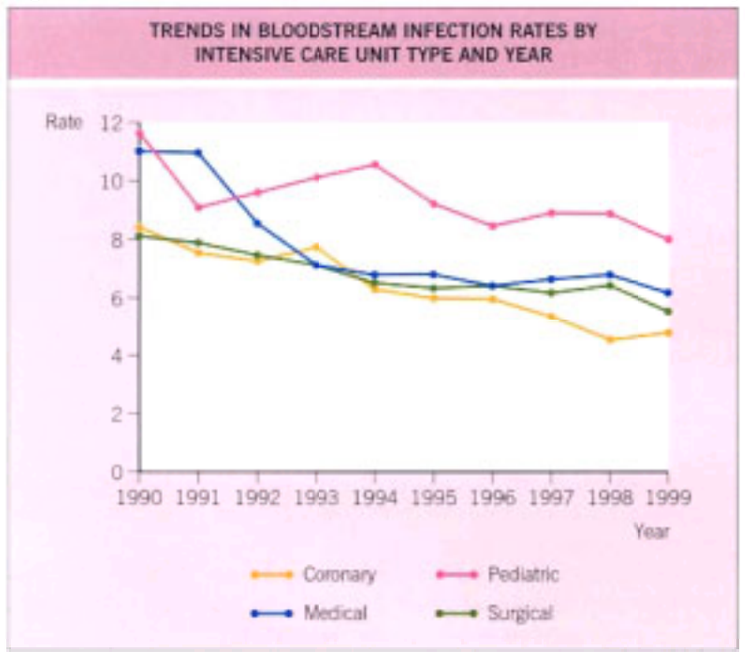


Figure 87-3 Endogenous and exogenous sources of hospital infection. Hospital infection can originate from an endogenous source or from an exogenous route. Endogenous infection may occur by translocation of resident microflora secondary to a breach in host defense (top). Exogenous infection may result from transmission from patient to patient or from health care worker to patient. It can also occur after exposure of a susceptible patient to a contaminated environmental source, such as inadequately disinfected medical devices (bottom).

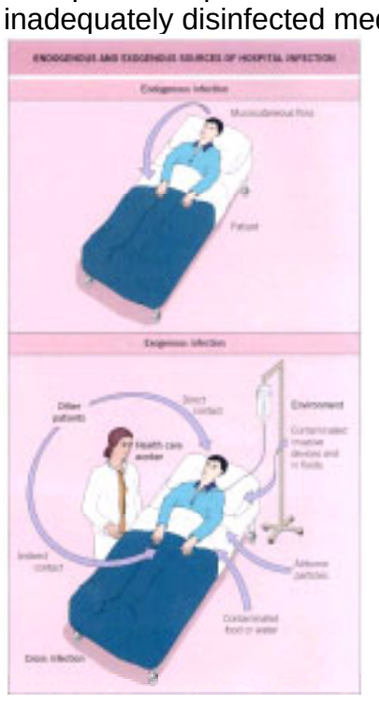


Figure 87-4 Catheter exit site infection in a patient with central venous catheterization through the jugular vein. *Courtesy of Dr F Jacobs.*

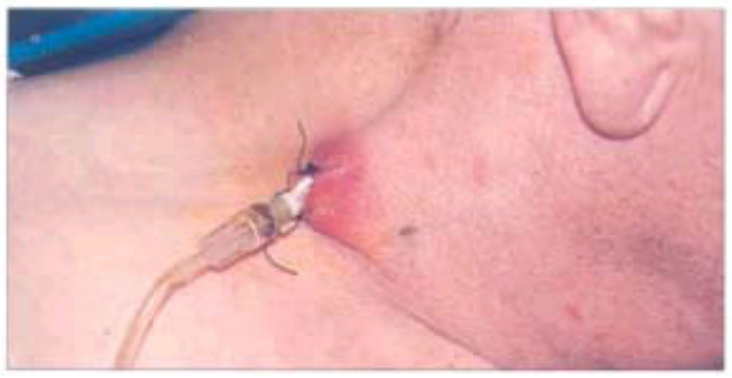


Figure 87-5 Severe clinical conditions are often associated with invasive life-support devices (a) causing multiple disruptions of mucocutaneous barriers. (b) Oropharyngeal and nasogastric tubes; (c) wound drainage; (d) urinary indwelling catheter; (e) central venous catheter.

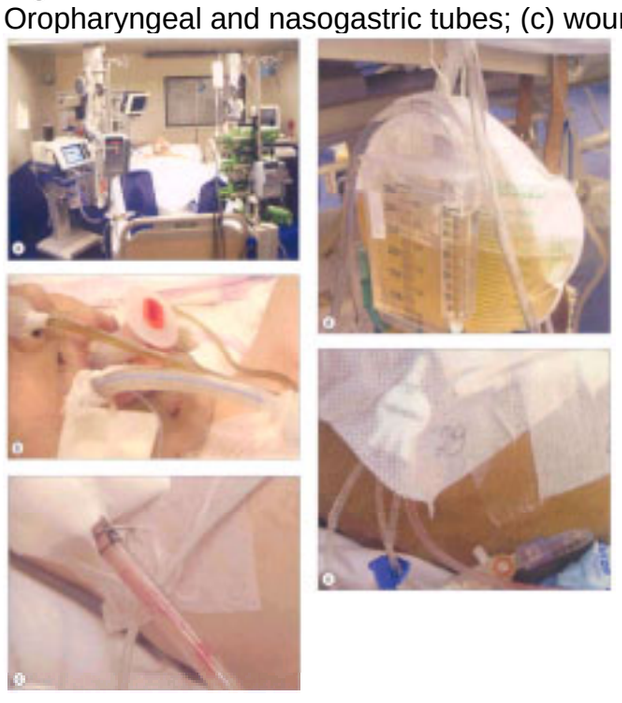


Figure 87-6 Distribution of outbreaks of hospital infection by pathogen. Distribution of outbreaks of hospital infection identified by Medline search from 1997 to June 2002, by category of pathogen. Bacteria caused 65% of outbreaks, of which 61% were Gram negative, 36% Gram positive and 3% mixed bacterial pathogens.

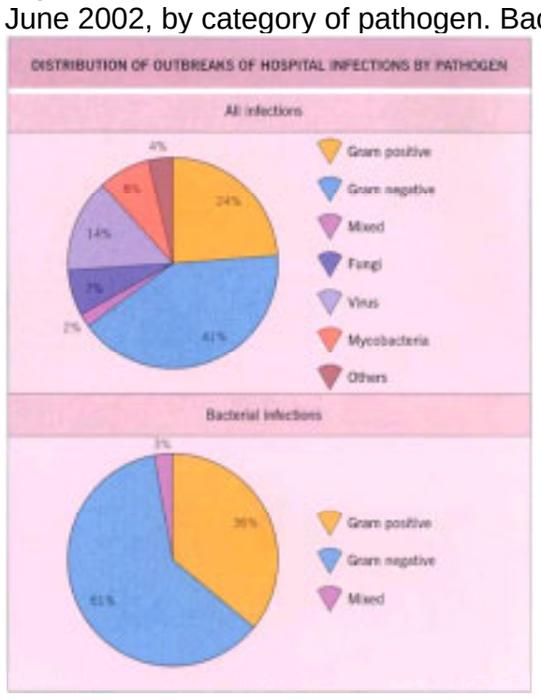


Figure 87-7 Etiologic agents of nosocomial bloodstream and surgical site infections in Belgian hospitals. National Program for the Surveillance of Infection in Hospitals, 1992–95.

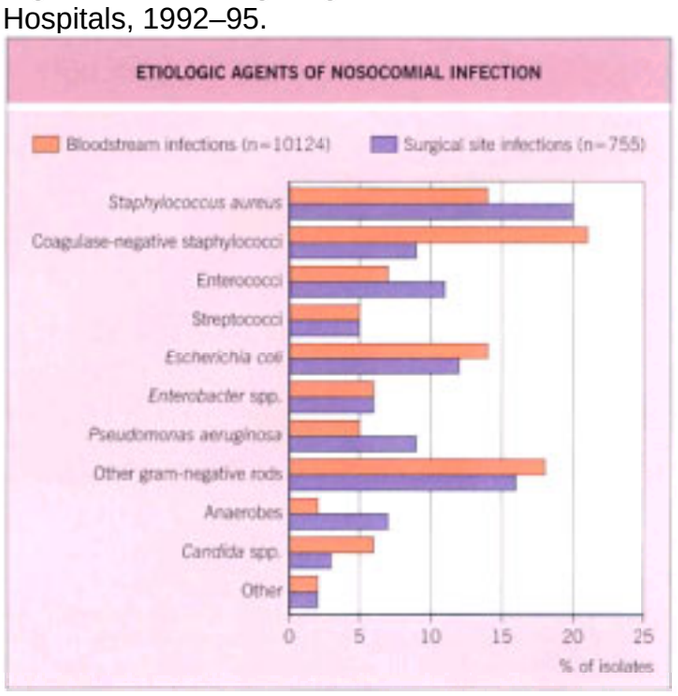


Figure 87-8 Selected antimicrobial resistant pathogens associated with nosocomial infections in intensive care unit patients. Comparison of resistance rates from January-December 2000 with 1995–99, NNIS Surveillance. CNS, coagulase-negative staphylococci; 3rd ceph, resistance to 3rd generation cephalosporins.

Figure 87-9 Demonstration of nosocomial transmission of a clone of methicillin-resistant *Staphylococcus aureus.* PFGE pattern of macrorestriction fragments cleaved with the rarely cutting enzyme *Sma I* of genomic DNA from MRSA. The same genotype (type D4) is found in isolates from 12 patients and 2 colonized health care staff, indicating that some members of staff may be involved in transmission in an intensive care unit department. Unrelated genotypes are also observed (B2–G1). Size marker, kb: kilobase pairs.

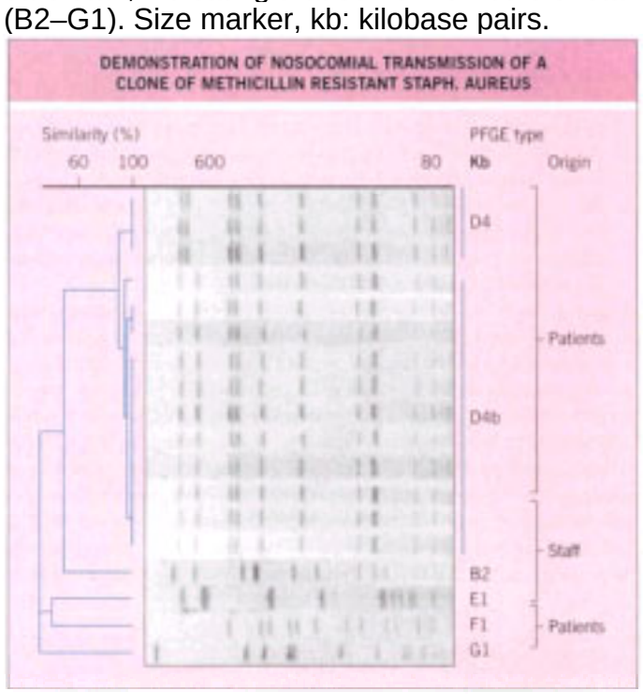


Figure 87-10 Restriction analysis of bacterial genome polymorphism. Chromosomal DNA is released after lysis of the bacterial cells. Restriction endonucleases that recognize commonly occurring sites will cut DNA in many small fragments. After conventional electrophoresis, these fragments are transferred onto a membrane and revealed by hybridization with labeled probes (Southern blot analysis). Alternatively, DNA can be restricted by endonucleases that recognize only rarely occurring sites. The few large, or macrorestriction, fragments are then separated by PFGE analysis.

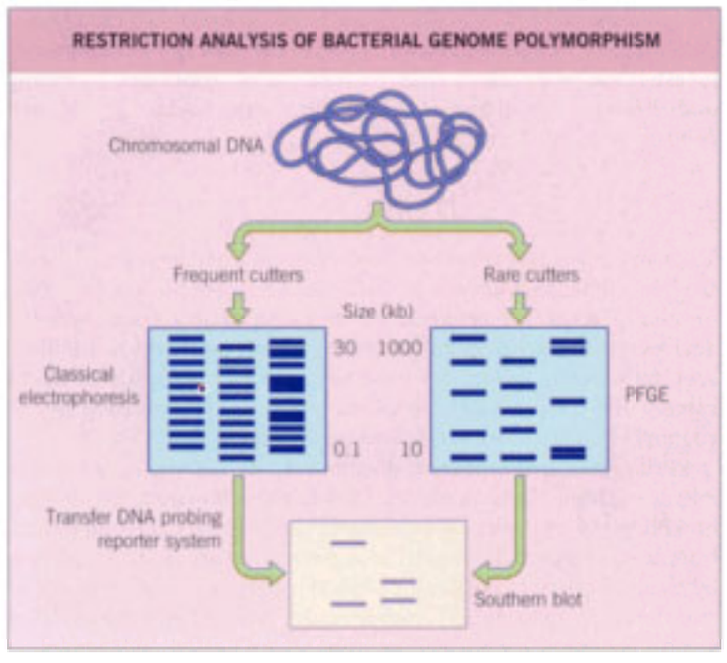


Figure 88-1 Example of organization and interdisciplinary roles in an EHS.

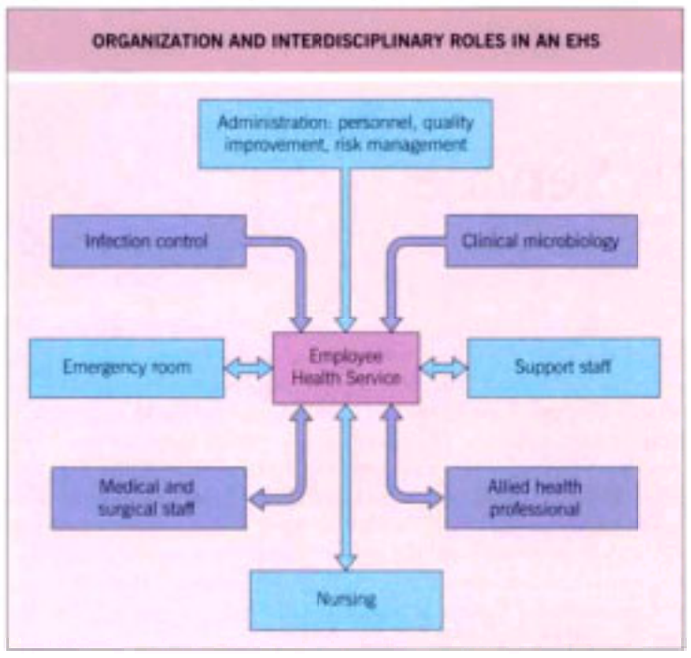


Figure 92-1 Examples of routes by which zoonoses are acquired.

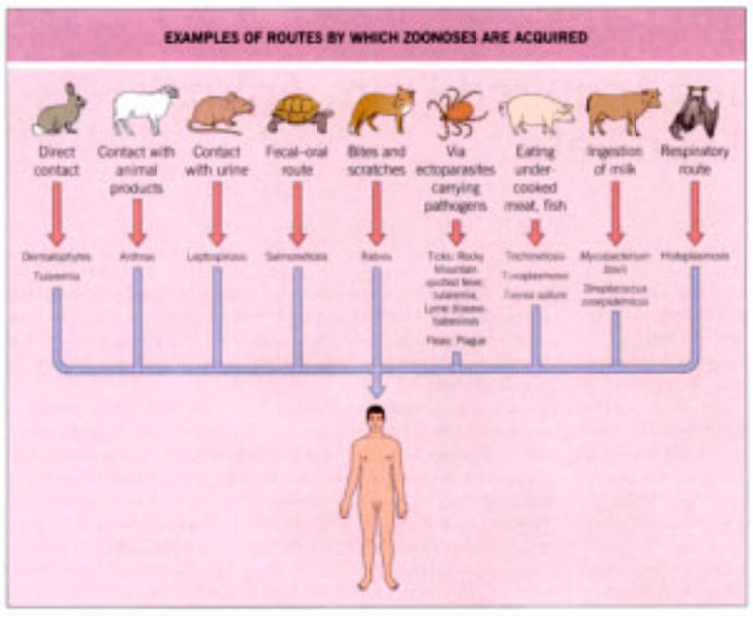


Figure 93-1 Water source in a developing country at a refugee camp.

Figure 93-2 An example of a pit latrine.

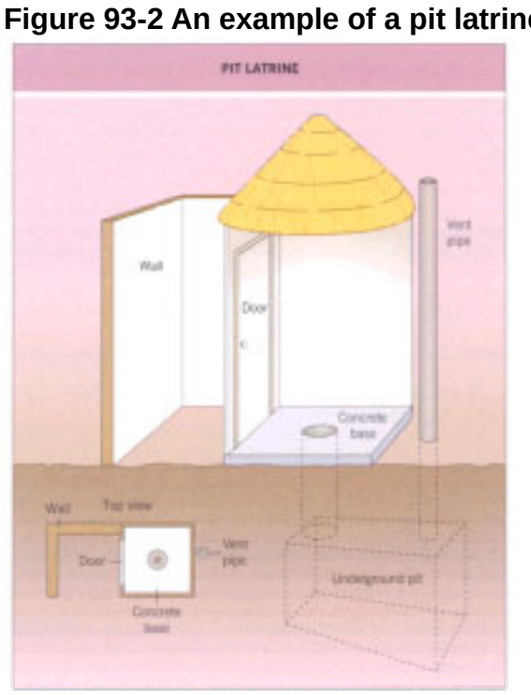


Figure 93-3 A cholera ward in Peru. *Courtesy of Dr J Sanchez.*

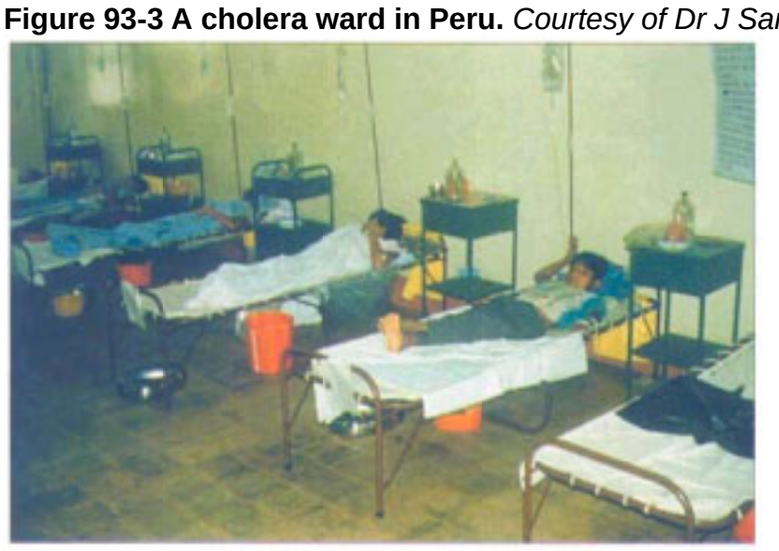


Figure 93-4 Electron micrograph of small round-structured viruses.

Figure 93-5 Amebic dysentery. A postmortem specimen. Note discrete flask-like ulcers with areas of hemorrhage.

Figure 93-6 Histology of infective colitis caused by *Campylobacter jejuni.*

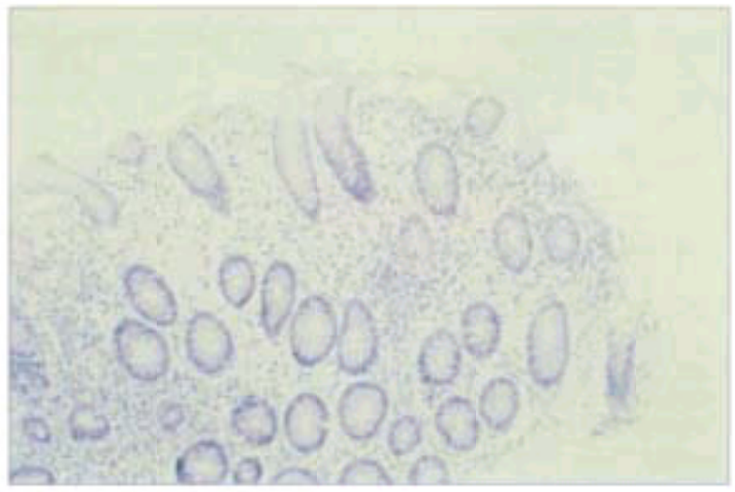


Figure 93-7 Unfertilized egg of *Ascaris lumbricoides*.

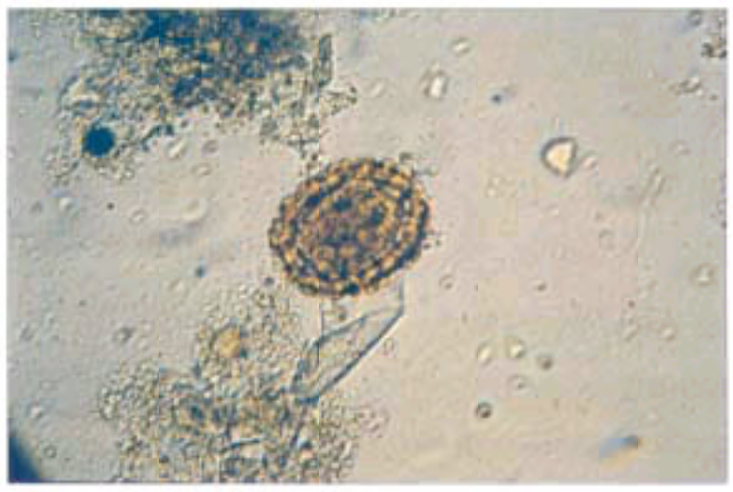


Figure 95-1 Potential health effects of stress. Indirect (behavioral) and direct (neural and neuroendocrine) mechanisms through which psychologic distress can alter the onset and course of infectious disease.

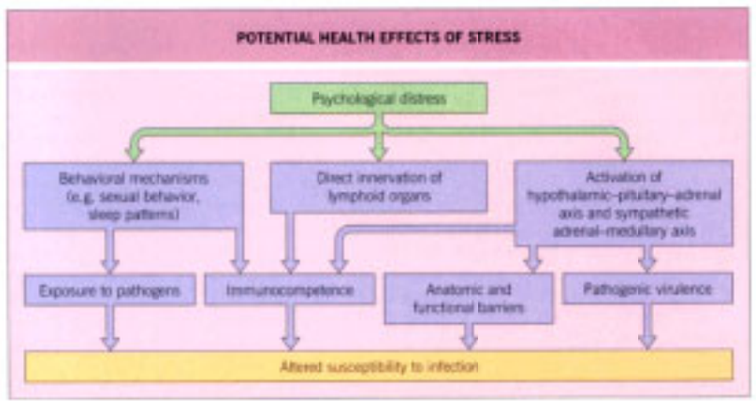


Figure 96.b-1 Characteristic evanescent rash of Still's disease.

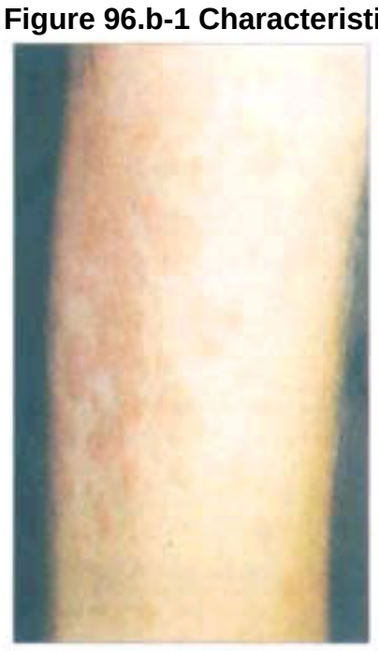


Figure 96.b-2 Clinical features associated with recurrent fever of unknown origin. SLE, systemic lupus erythematosus; FMF, familial Mediterranean fever.

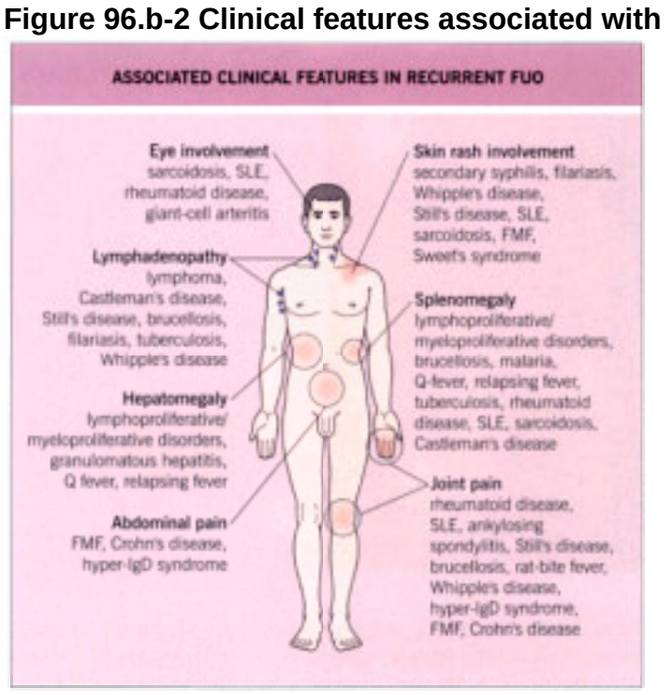


Figure 96.d-1 Typical centrifugal distribution of the rash in smallpox. *Courtesy CDC and Dr Paul B Dean.*

Figure 96.d-2 Patient with smallpox, Kosovo, Yugoslavia epidemic, March and April 1972. The scabs will eventually fall off leaving marks on the skin that will become pitted scars. The patient is contagious until all scabs have fallen off. *Courtesy CDC and Dr William Foege.*

Figure 96.f-1 Contact evaluation: circles of exposure. Circles are constructed based on length of time of exposure and intensity of exposure. The innermost circle represents the highest risk; individuals in this circle are the first priority for screening. Circles of risk are constructed with each having less exposure — and thus less risk — until the PPD conversion rate in a circle is equivalent to the prevalence of LTBI in the local population.

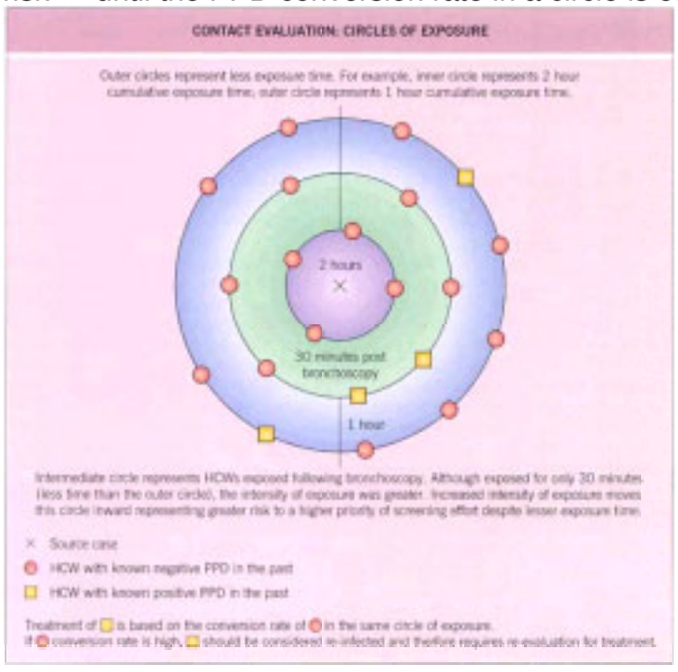


Figure 97-1 Toll-like receptors and identified ligands to date. The identified ligands that bind and signal via TLRs are shown here. Note that TLR2 and TLR6 combine to form a functional heterodimer to recognize peptidoglycan, and probably further combinations of TLRs will be identified, which will increase the diversity of ligands that are recognized. LAM, lipoarabinomannan.

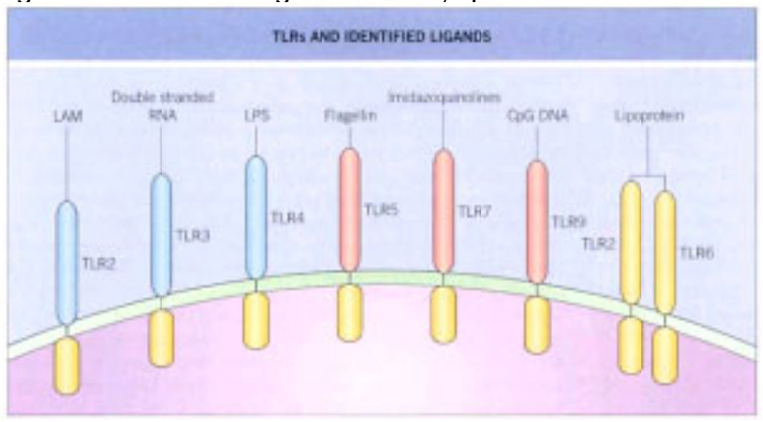


Figure 97-2 Pathways for the generation of the major antimicrobial effector mechanisms of macrophages. This shows the pathways for the generation of ROIs from NADPH oxidase, a process initiated by FcR cross-linking, or exposure of cells to IFN-?. Similar activation can also induce the production of RNIs from L-arginine, catalyzed by inducible nitric oxide synthase (iNOS). Also listed are various examples of bacterial genes or gene products that are known to counteract components of these pathways.

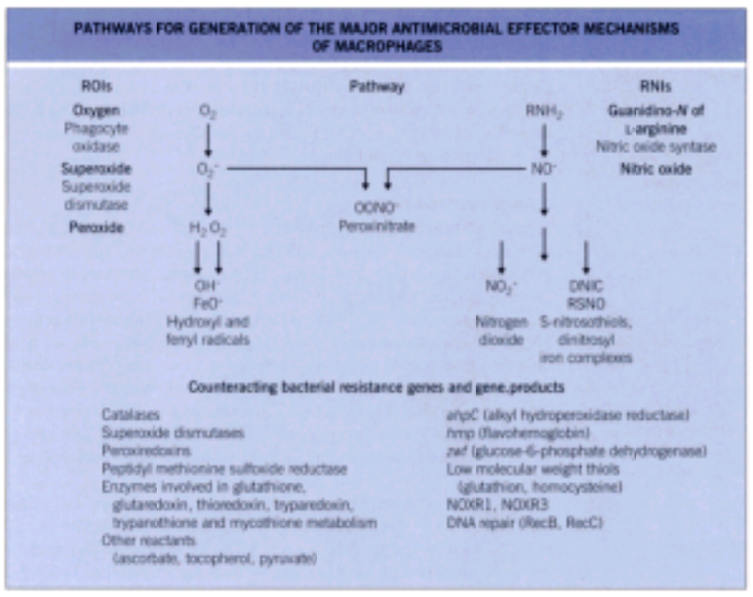


Figure 97-3 Pathways of antigen processing for activation of T-cell subsets. (a) CD4 T cells: within the APCs secreted or somatic antigens are digested by vacuolar proteases, which generate peptides of 15–22 amino acids in length — these peptides are loaded onto major histocompatibility complex (MHC) class II molecules in a specialized compartment before being transported to the cell surface. (b) CD8 T cells: endogenous antigens are cleaved in the cytoplasm by the proteasome to generate peptides of a final length of 8–9 amino acids — these are loaded onto MHC class I molecules in the endoplasmic reticulum (ER) and transported to the cell surface in association with ß$_2$ -microglobulin (ß$_2$ m). (c) CD1-restricted T cells: glycolipid antigens are presented by CD1 molecules on the surface of APC. Recent observations suggest that some processing events are required that involve components of the MHC class II pathway, but that antigen is loaded onto CD1 in the ER, similar to the MHC class I. (d) ?d T cells: to date no processing event or presentation molecule has been identified, but there is some suggestion that the nonpolymorphic Qa-1 molecule can function in antigen presentation. CTL, cytotoxic T lymphocyte; TAP, transporter associated with antigen processing.

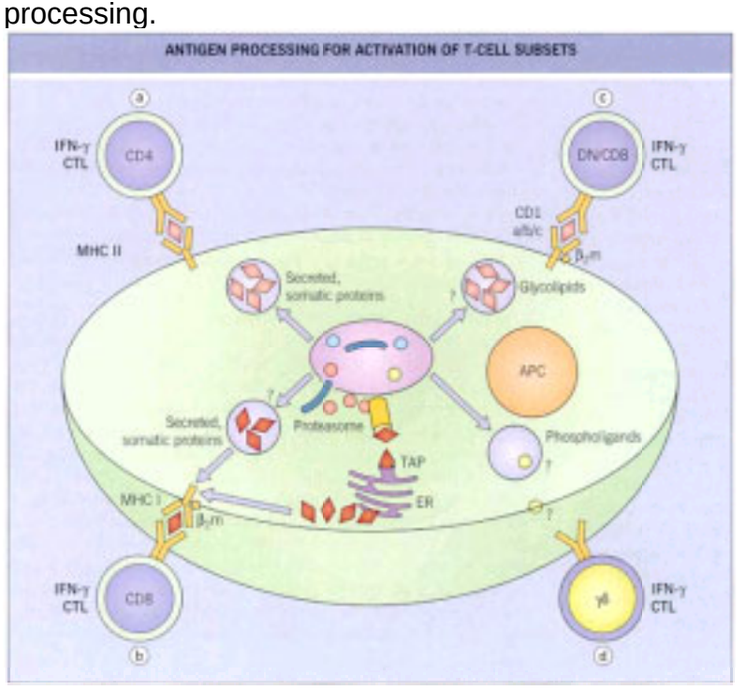


Figure 97-4 Differentiation of CD4 T-helper cell subsets and their effector functions in infection. Under the influence of cytokines produced by dendritic cells (DC) and NK cells, Th0 cells differentiate into Th1 cells (promoted by IL-12 and IL-18) or Th2 cells (promoted by IL-4). These differentiated T cells produce a characteristic pattern of cytokines, which perform various effector functions to eliminate pathogens. The major cytokine produced by Th1 cells is IFN-?, which promotes macrophage activation, critical in the elimination of intracellular pathogens. Th2 cells produce IL-4 and IL-5, which are critical for B-cell maturation and immunoglobulin class switching and hence are important in the control of helminths and extracellular pathogens. Note that via the production of these cytokines, each subset of Th cells can downregulate the differentiation of the other. DC1, myeloid dendritic cells derived from $CD14^+$ monocytes, which produce mainly IL-12; DC2, lymphoid dendritic cells derived from $CD4^+$ precursors, which produce mainly IL-4.

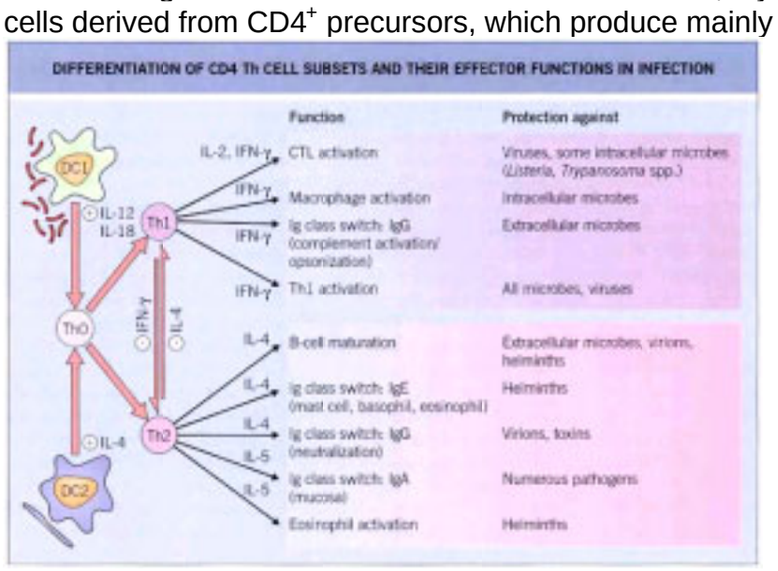


Figure 97-5 Kinetics of the immune response and the cell populations involved. Summary of the kinetics of the immune response to infection, from the recognition of microbial agents that initiates events, through to the generation of an effector T-cell response. The effector cytokines have been colored red for inhibitory and green for stimulatory.

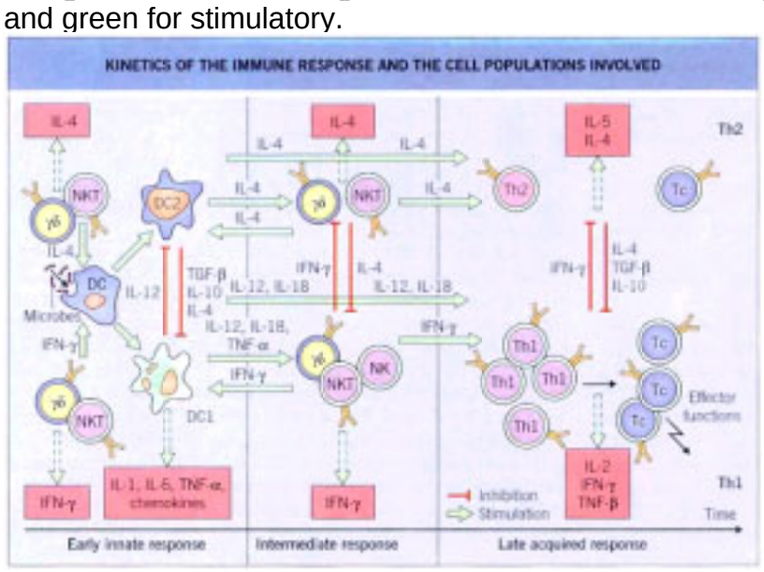


Figure 98-1 Neutrophil granules. Neutrophils contain primary, secondary and tertiary granules, each of which has specific contents that are produced at different points in myeloid ontogeny. The larger, azurophilic primary granules contain a host of proteins, only some of which are listed here. Note that secondary granule deficiency affects both all secondary granule contents as well as the primary granule defensins. Primary granules fuse in Chediak-Higashi syndrome, along with a smaller number of secondary granules, to give the characteristic cell inclusions.

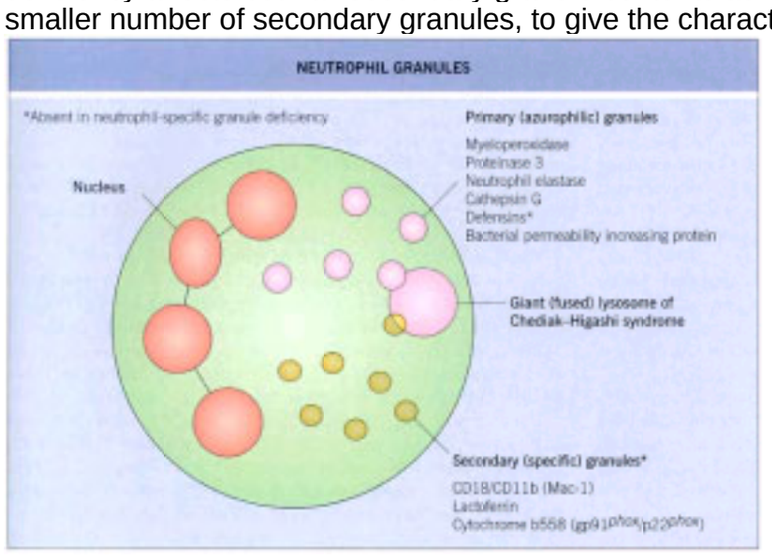


Figure 98-2 The NADPH oxidase. Cellular activation leads to assembly of the nascent NADPH oxidase by joining of secondary granule membrane and cytosolic components. The generation of an intravacuolar charge is rectified by potassium influx, which in turn liberates neutrophil elastase (NE) and cathepsin G (CG) from their associated matrix (**). As classically conceived, an ingested organism is shown degrading its own hydrogen peroxide, thus eliminating a supplement to the defective metabolic pathway in CGD. However, the pathologic relevance of the role of catalase in bacterial or fungal virulence is unclear.

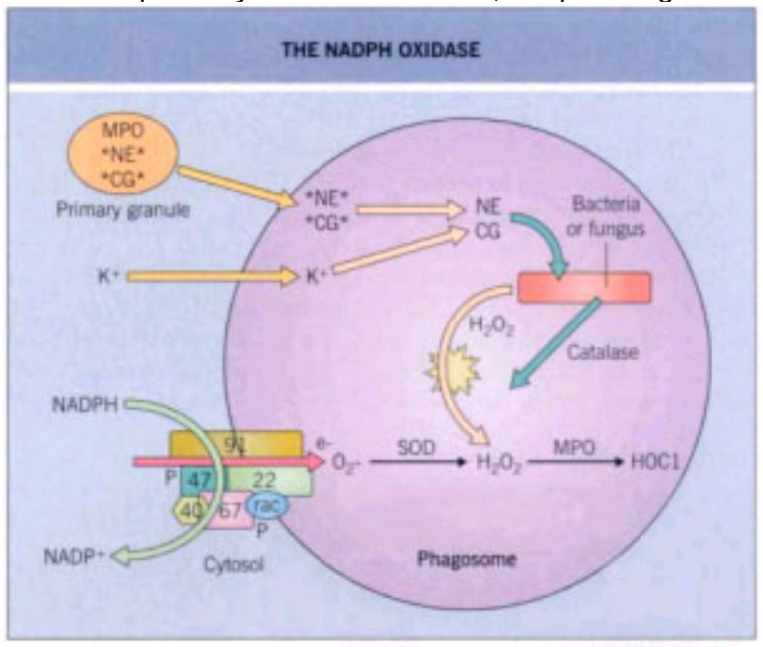


Figure 98-3 Manifestations of chronic granulomatous disease. (a) Pneumonia due to *Aspergillus* spp. can present subtly, both clinically and radiographically. This patient was asymptomatic but had multifocal pneumonia due to *Aspergillus fumigatus*. (b) Wound dehiscence typically presents 5–10 days postoperatively. It is an exuberant granulation tissue on biopsy and is best treated with short courses of corticosteroids. (c and d) Liver abscesses due to staphylococci are quite common in CGD ((c) arrow) and typically require *en bloc* resection because of their dense, granulomatous nature (d).

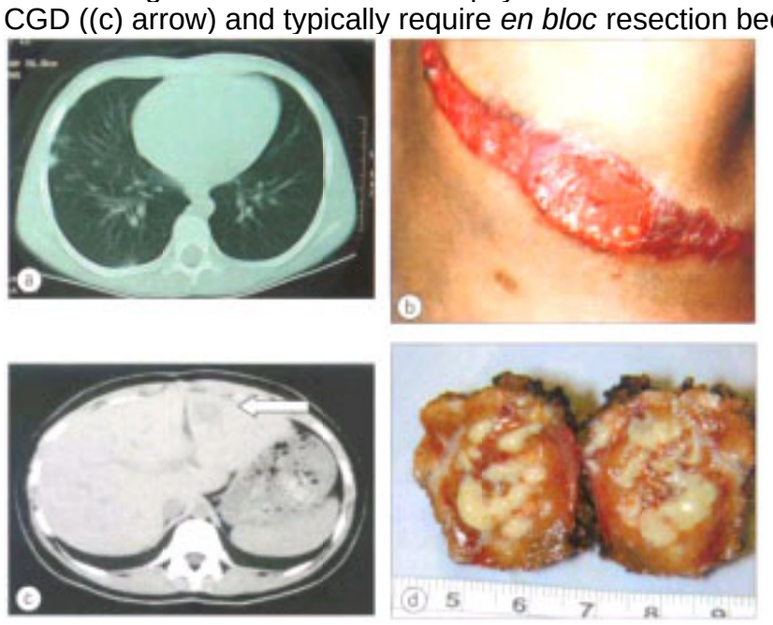


Figure 98-4 Leukocyte adhesion. Neutrophils sample the endothelium in the postcapillary venules through a series of receptors. Depicted here are the selectin and integrin pathways, which are critical for neutrophil adhesion (monocytes and eosinophils have other ligand and receptor options). CD15s binds to selectins on the endothelium with loose adhesion, allowing closer sampling of the endothelium for tight adhesion in the setting of endothelial activation, mediated through the integrins. Neutrophil integrins bind to intercellular adhesion molecules (ICAMs).

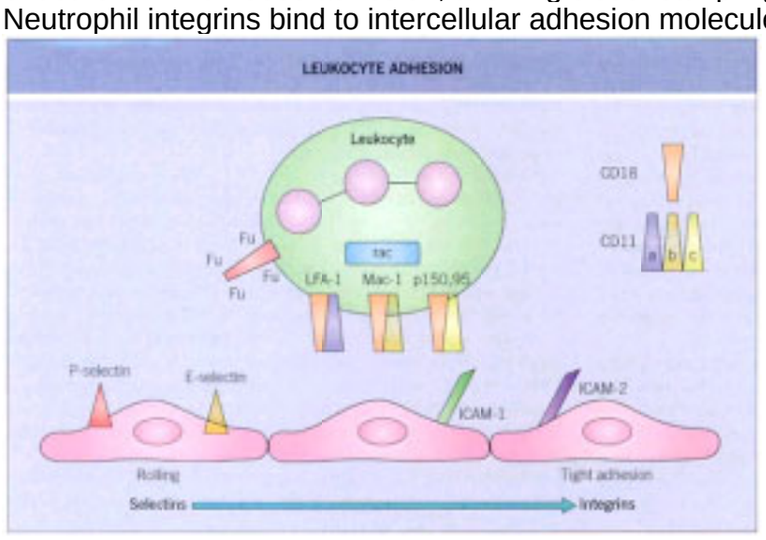


Figure 98-5 Examples of leukocyte adhesion deficiency type 1. (a) A biopsy from the bowel of a patient with extensive inflammation and intestinal ulceration. Note the abundance of neutrophils intravascularly (arrow) but the paucity of neutrophils in the parenchyma. (b) Characteristic dystrophic or 'cigarette paper' scarring following skin ulceration in a boy who has LAD1.

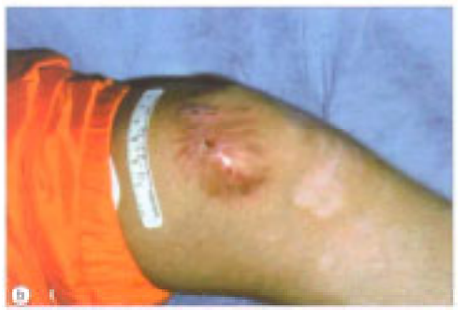


Figure 98-6 Critical cytokine pathways in the control of mycobacteria. Mycobacteria and salmonellae stimulate the elaboration of IL-12 by infected macrophages, leading to the production of IFN-? by T cells and NK cells. IFN-? in turn stimulates macrophages to produce TNF-a and IL-12. The critical signaling molecules STAT1 and NEMO are also indicated. IL-12-independent pathways for IFN-? production are also indicated (IL-15, IL-18), which work in concert with IL-12 for lymphocyte stimulation. Other, as yet undefined pathways are also suggested.

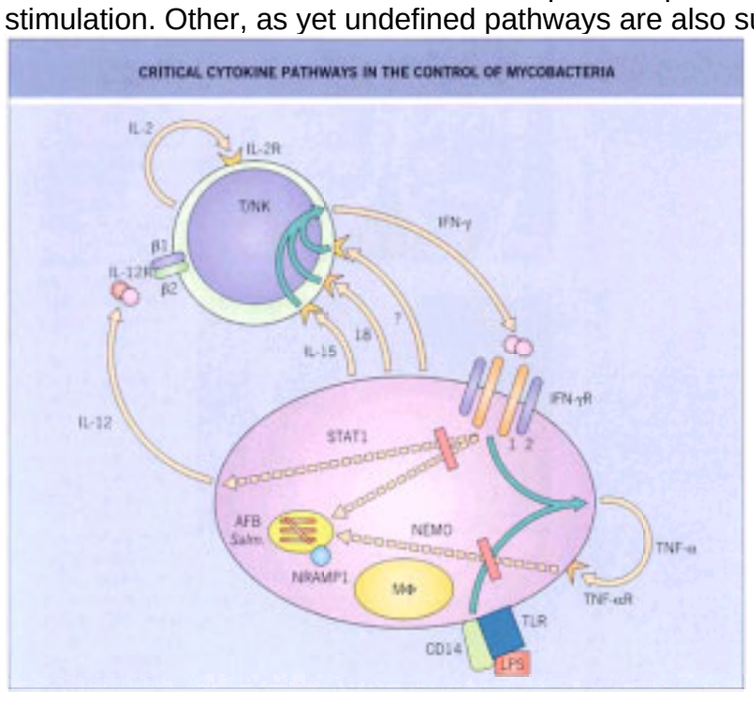


Figure 98-7 In-vitro and clinical aspects of IFN-?R1 deficiency. (a–c) Flow cytometry for the IFN-?R1 on peripheral blood monocytes. Dotted lines indicate the background fluorescence of the sample with an irrelevant antibody. (a) Normal IFN-?R1 fluorescence intensity. (b) Monocytes from a child who has complete IFN-?R1 deficiency. Note the lack of specific staining for IFN-?R1. (c) Increased intensity of IFN-?R1 staining on monocytes from a patient who has MAC osteomyelitis. (d) Chest radiograph from the same patient showing extensive right lung infection with MAC. (e) The corresponding magnetic resonance image of his distal femora (arrow indicates the infected lesion).

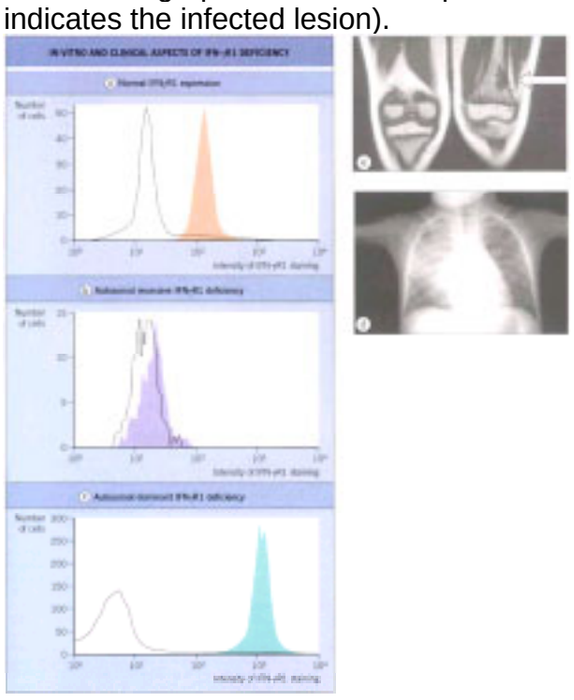
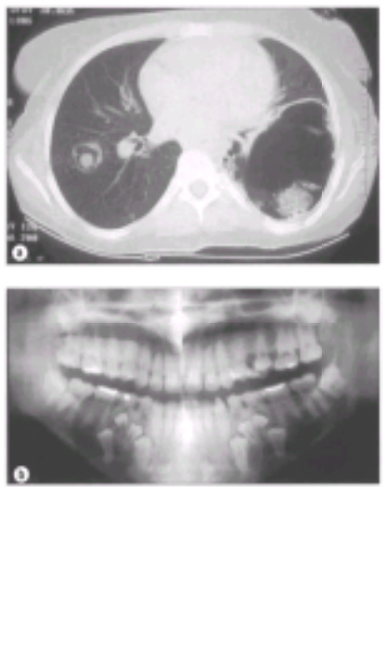


Figure 98-8 Clinical manifestations of hyper-IgE (Job's) syndrome. (a) Computerized tomogram of the chest showing characteristic postinflammatory pneumatocele formation. Note the development of bilateral aspergillomata with inflammation of the cavity walls. (b) A panoramic radiograph of the dentition of a 33-year-old woman who has HIES shows the characteristic retention of primary teeth due to failure of deciduation. (c) Extensive scoliosis is demonstrated in this radionuclide bone scan of a 25-year-old woman who has HIES. A list of some of the characteristic features of the syndrome is included in Table 98.2 .



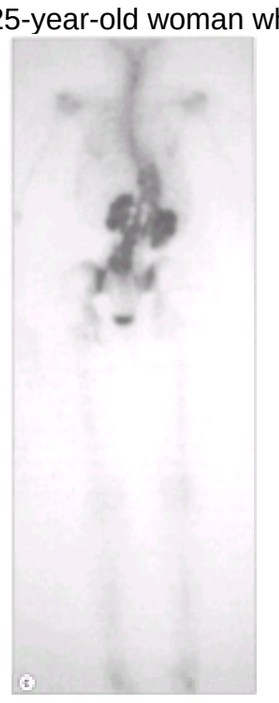


Figure 98-9 Distribution of severe combined immunodeficiency. Distribution of diagnoses in 100 consecutive SCID babies at the Children's University Hospital, Brescia, Italy.

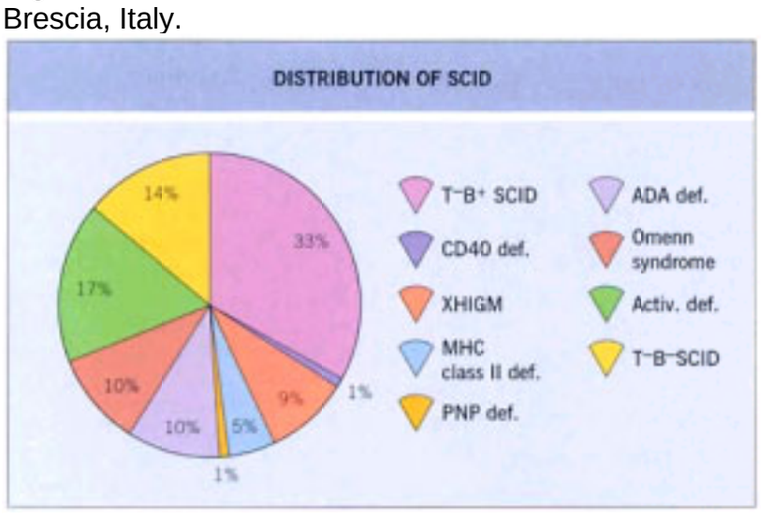


Figure 98-10 Typical severe combined immunodeficiency baby. Note the wasting and malnutrition.

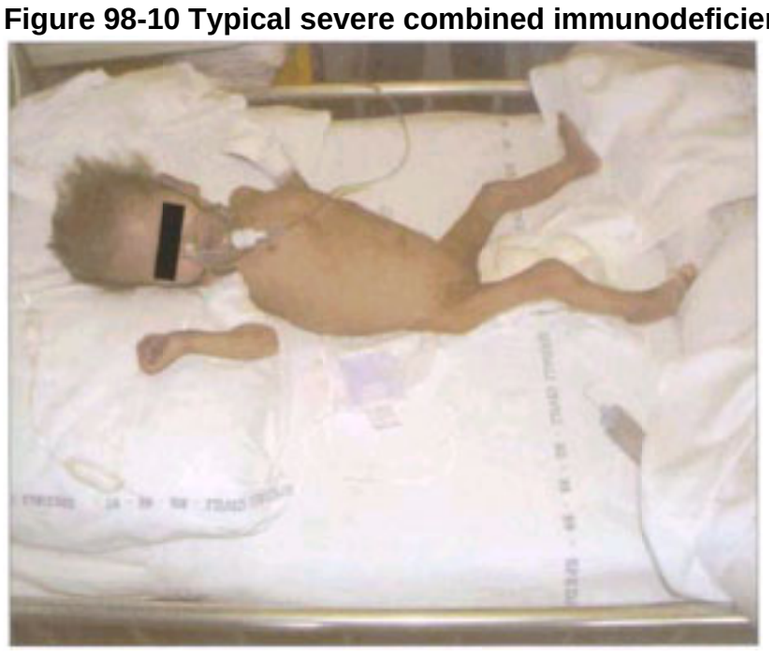


Figure 98-11 Diagnosis of severe combined immunodeficiency. Clinical and laboratory elements useful in the diagnosis of SCID.

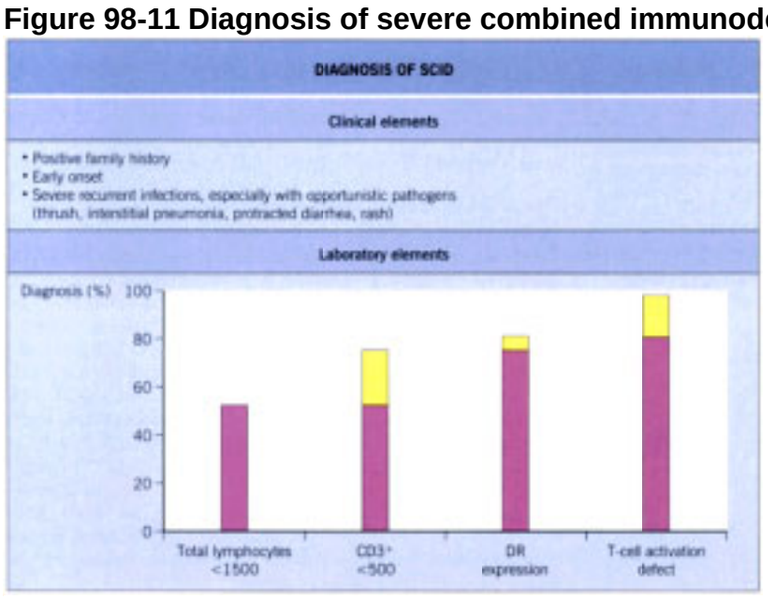


Figure 98-12 Axillary BCGitis in a T-B^{+} severe combined immunodeficiency baby who has generalized BCG infection. Note the extensive cutaneous ulceration.

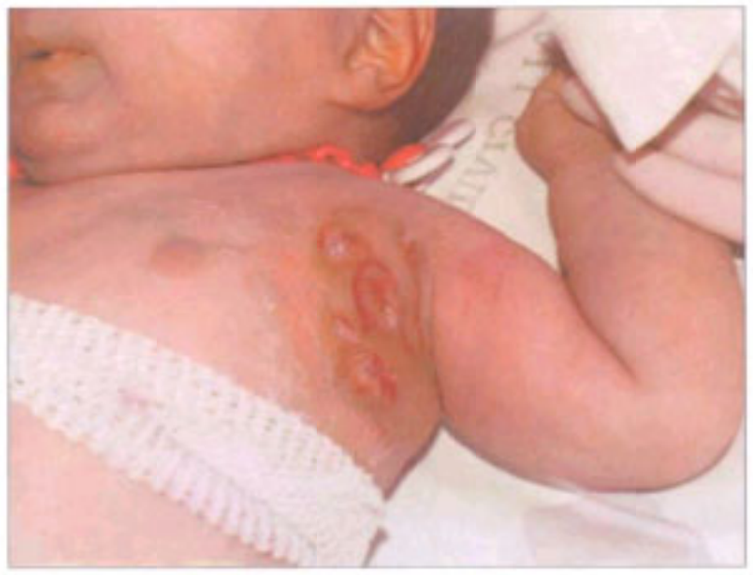


Figure 98-13 Gene defects and lymphocyte development. Model of lymphocyte development with the relative gene defects that may lead to immune deficiencies shown in red (for details see text).

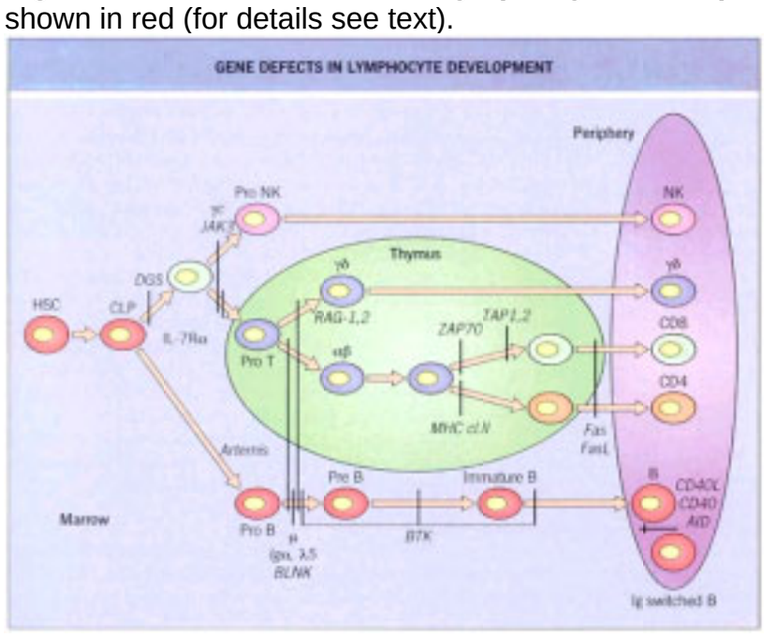


Figure 98-14 Receptors using the ? chain/JAK3 pathway. The six interleukin receptors that use the common ? chain/JAK3 pathway for signaling.

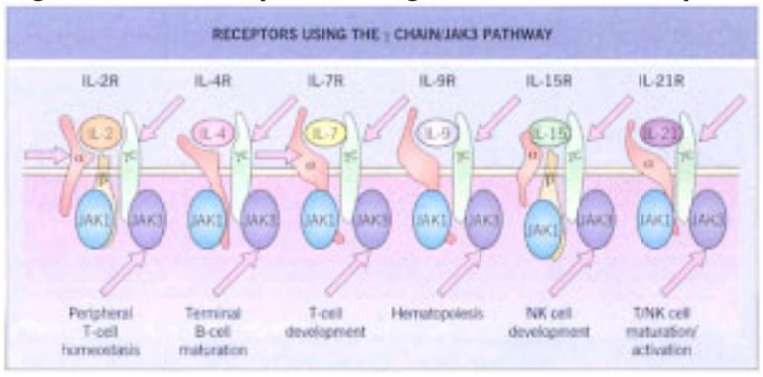


Figure 98-15 ?**c/JAK3/STAT signaling.** ?c/JAK3/STAT signaling is initiated by binding of the cytokine to its receptor. Heterodimerization (1) of the receptor chains (one IL-specific chain and the ?c) allows for reciprocal tyrosine phosphorylation (2a) of JAK3 and other JAK molecules, leading to their activation. Activated JAKs then phosphorylate (2b) tyrosine residues on both receptor subunits, creating docking sites for the STATs. These signaling elements are themselves tyrosine phosphorylated by the JAKs (2c), in order to dimerize and translocate to the nucleus (3), where they bind to consensus sequences within regulatory regions of cytokine-inducible genes and act as transcription factors driving transcription of the target genes.

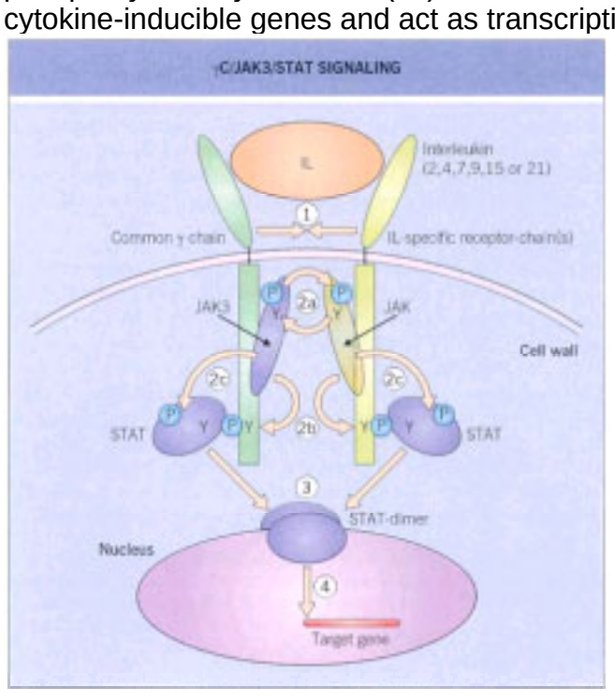


Figure 98-16 V(D)J recombination process. Diagram showing the V(D)J recombination process with the genes involved in T^- B^- SCID and Omenn syndrome.

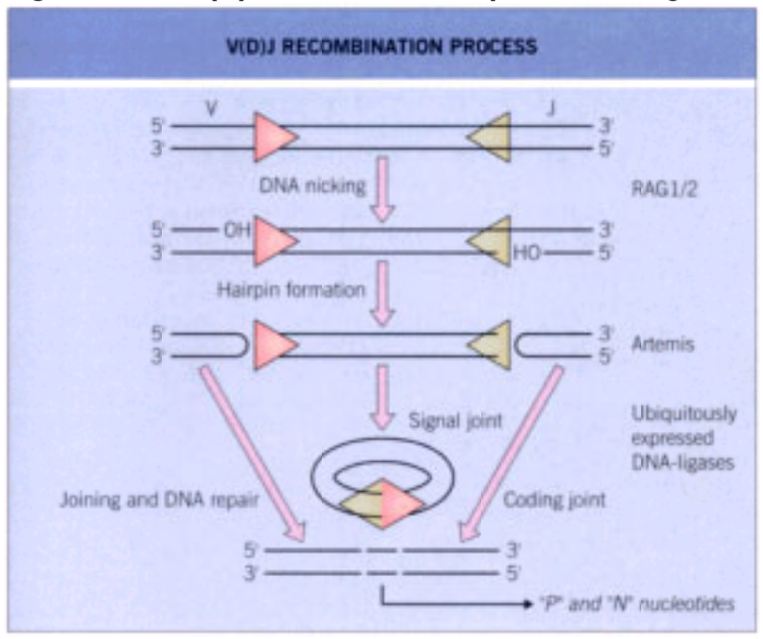


Figure 98-17 A baby who has Omenn syndrome. Note the diffuse erythema.

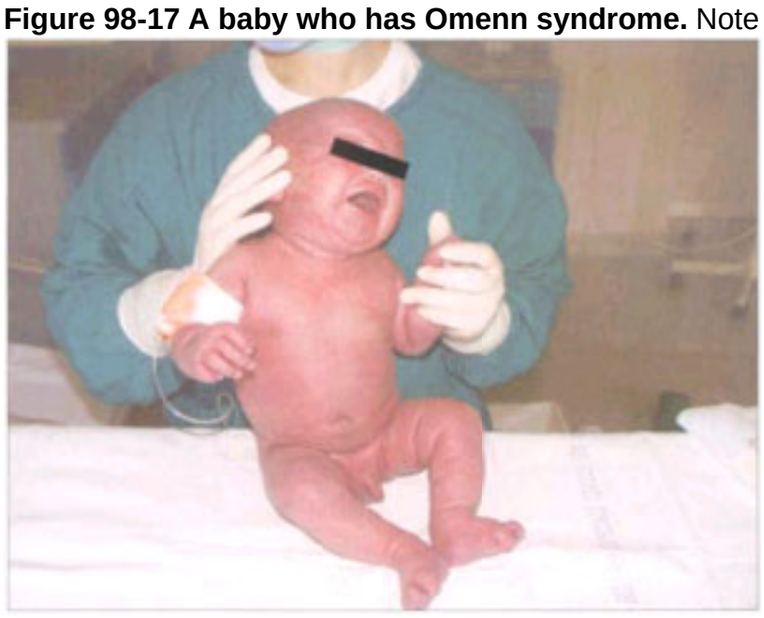


Figure 98-18 T cell receptor signaling. Stimulation of T cells through the CD3/TCR complex results in activation of p56lck, a src-tyrosine kinase, that mediates tyrosine-phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3-?, -d, -epsilon and -? chains. ZAP-70, an intracellular tyrosine kinase, is then recruited into the CD3/TCR complex through binding of its SH2 domains to phosphorylated ITAMs of the ? chain. ZAP-70 itself becomes phosphorylated by Src-family protein tyrosine kinases. Phosphorylation triggers ZAP-70 activation, allowing phosphorylation of downstream signaling molecules such as linker for activation of T cells (LAT) and SLP-76.

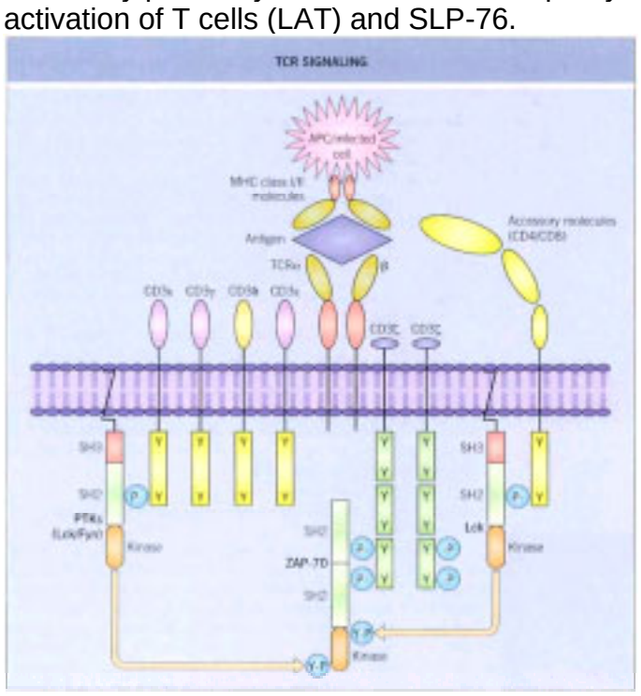


Figure 98-19 The major histocompatibility complex class II promoter complex. Mutations in these transcription factors account for the various complementation groups identified in MHC class II deficiency.

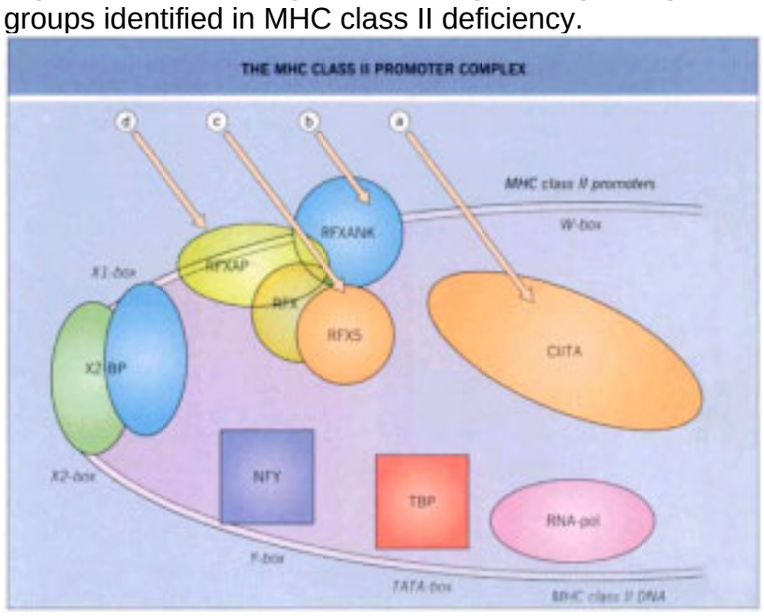


Figure 98-20 Blockage of B cell maturation. Model of B cell differentiation with the various proteins involved in the different stages shown below. The vertical red lines indicate a maturation block caused by the molecules indicated above.

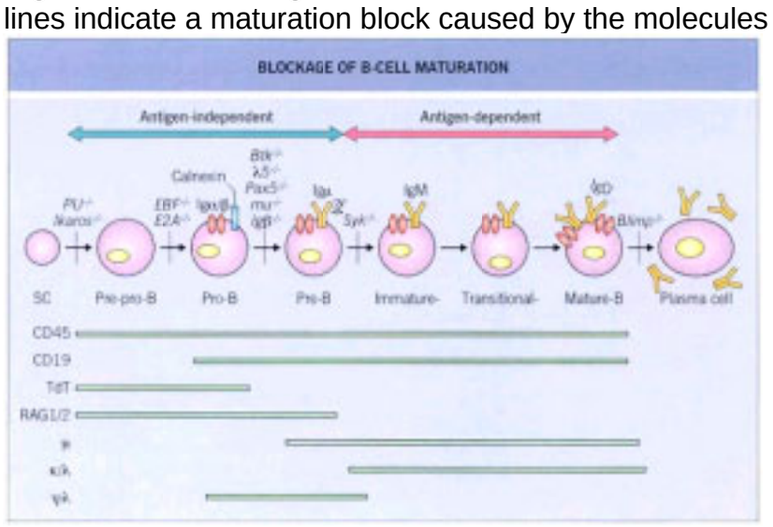


Figure 98-21 Fas/Fas ligand apoptosis system. The Fas/FasL apoptosis system and the various forms of ALPS caused by defects in it.

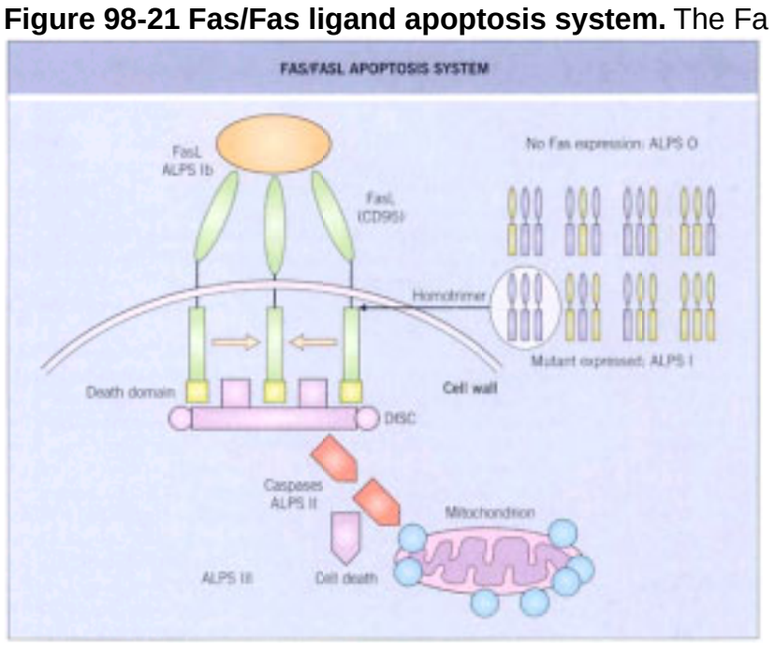


Figure 99-1 Mechanism of action of the different immunosuppressive agents currently in use in clinical practice.

Figure 99-2 Mechanism of action of ciclosporin A. Ciclosporin A inhibits T-cell activation by interfering with calcineurin. It is a lipophilic undecapeptide reversible inhibitor of T-cell activation that acts by interfering with calcineurin. The T-cell receptor induces signaling through an elevation in concentration of Ca^{2+} in the cytoplasm. This activates the transcription factor AP-1. The Ca^{2+} binds to calcineurin, which in turn dephosphorylates the cytoplasmic form of NFAT. Then NFAT migrates into the nucleus and forms a complex with AP-1. This complex can induce the transcription of genes required for T-cell activation including IL-2. In the presence of CsA a complex forms with cyclophilin (Cyp). The CsA-Cyp complex can bind to calcineurin, blocking its ability to activate NFAT, and therefore inhibiting T-cell activation.

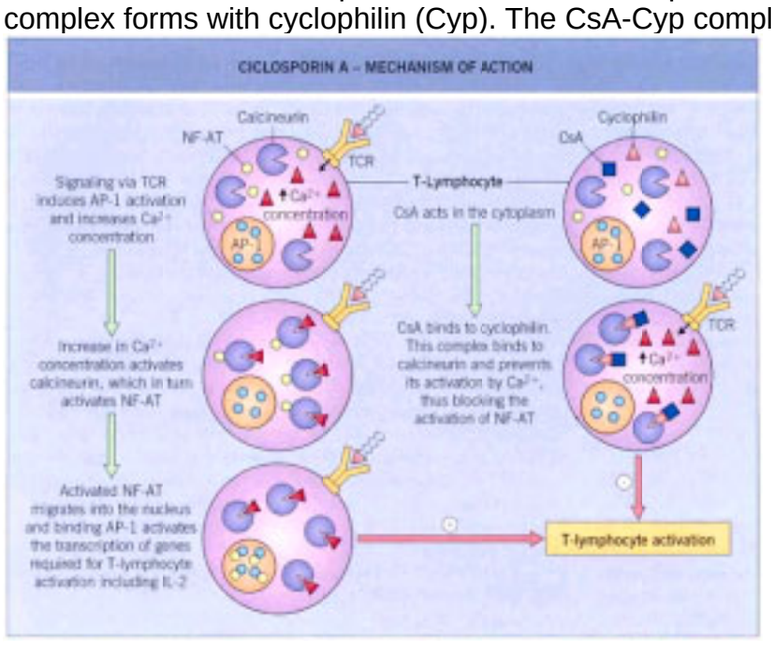


Figure 99-3 Mechanism of inhibition of purine synthesis by mycophenolic acid. MPA inhibits inosine monophosphate dehydrogenase (IMPDH) and leads to the depletion of guanosine nucleotides. PRPP, 5-phosphoribosyl-1(a)-pyrophosphate; ribose-5P, D-ribose-5'-phosphate.

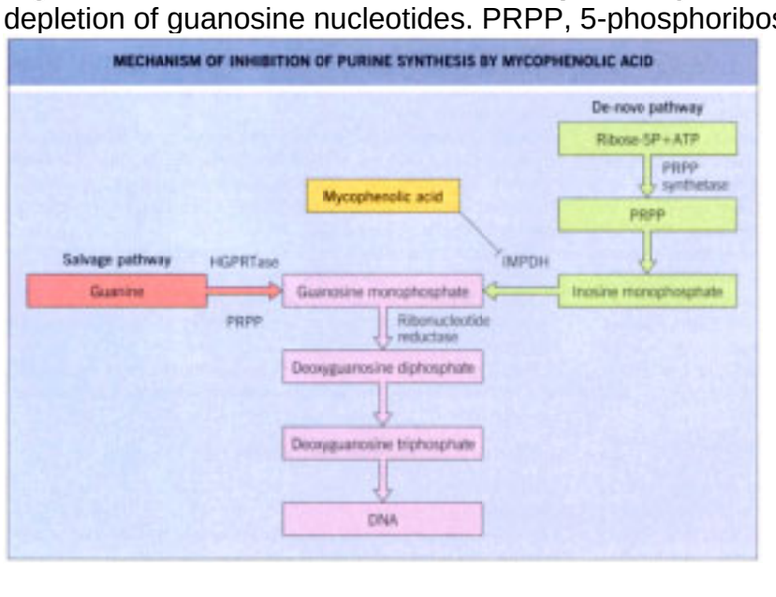


Figure 100-1 Single-organism bloodstream infections due to Gram-negative and Gram-positive bacteria in febrile neutropenic patients. European Organisation for Research and Treatment of Cancer — International Antimicrobial Therapy Group (EORTC-IATG) studies (1973–2000). *Adapted from reference*[8] .

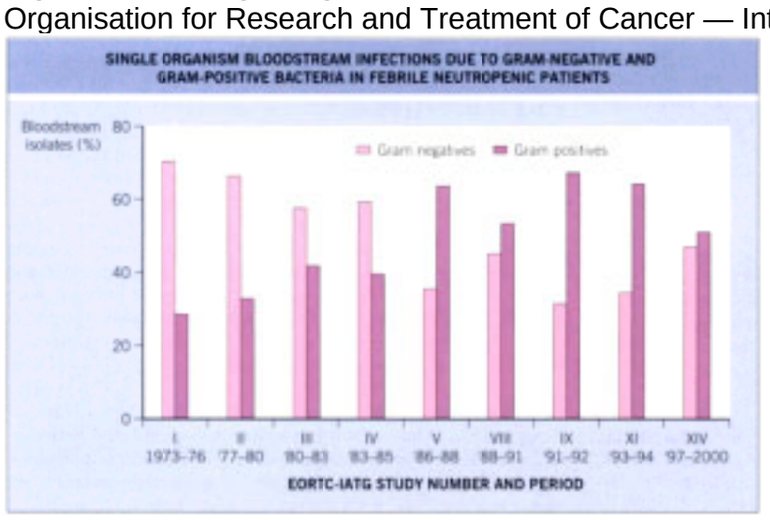


Figure 100-2 Incidence of infection according to duration and severity of neutropenia. *Adapted from reference* [3] .

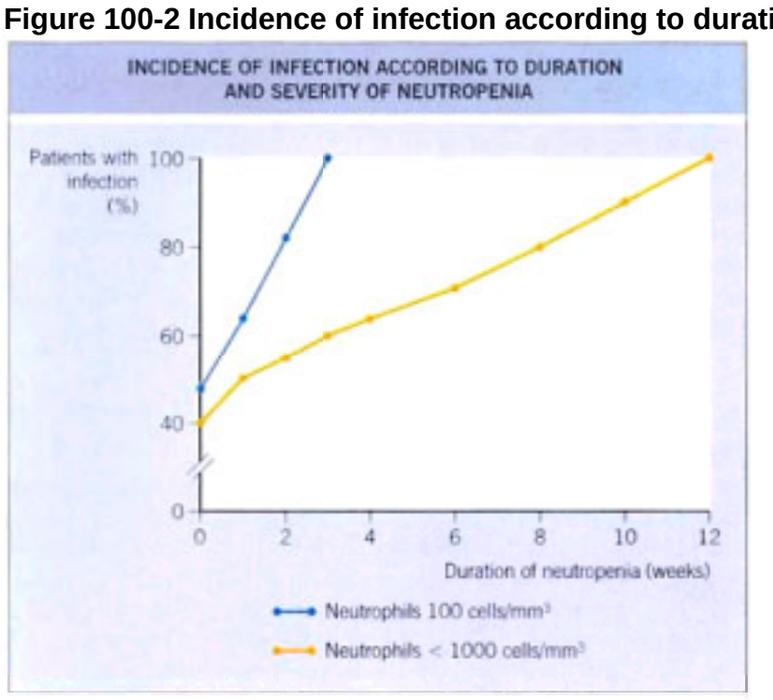


Figure 100-3 Causes of fever in neutropenic patients with hematological malignancies (n=1773) or solid tumors. *Data are derived from four consecutive EORTC-IATG studies conducted between 1991 and 2000 and from a North American study conducted between 1992 and 1997.*[17] [18] [19] [20] [21]

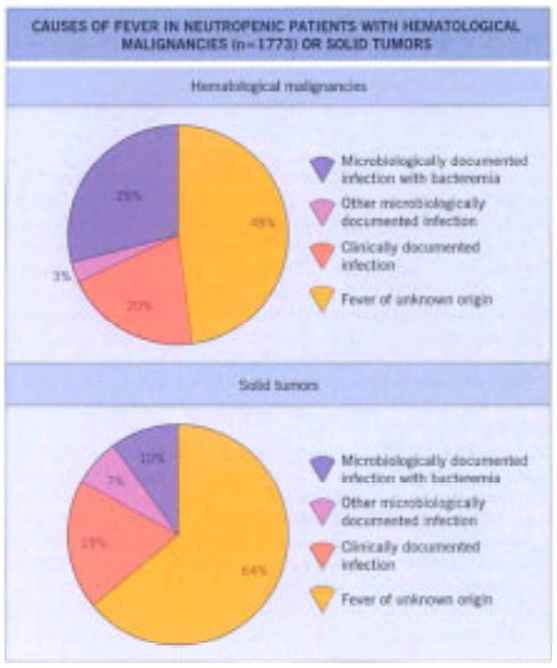


Figure 100-4 Sites of infection in febrile neutropenic patients with hematological malignancies. *Data are derived from three consecutive EORTC-IATG studies conducted between 1991 and 2000.*[17] [18] [19]

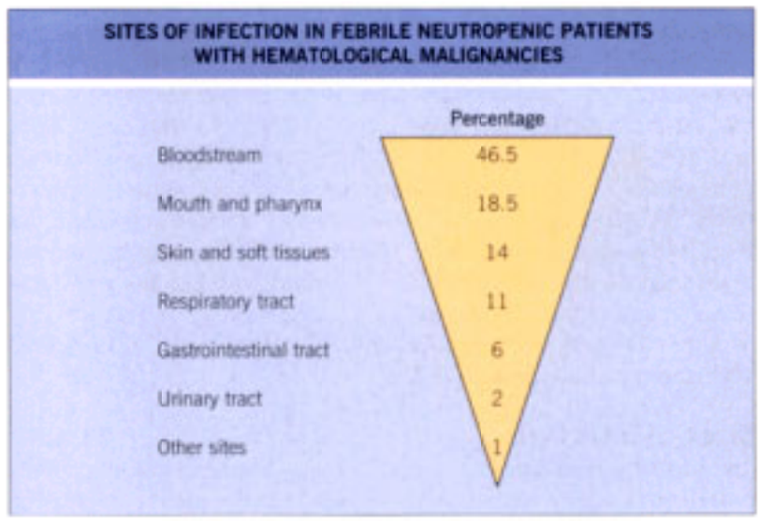
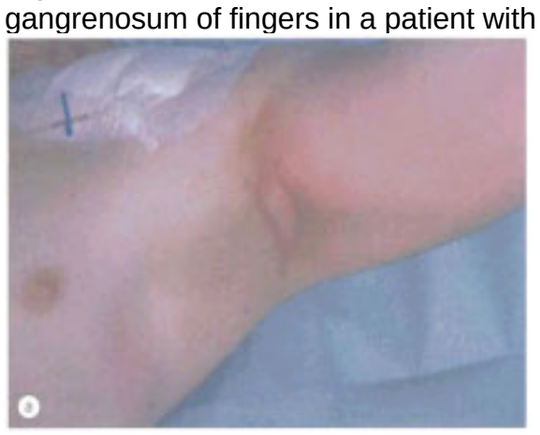


Figure 100-5 Cutaneous bacterial infections in neutropenic patients with acute leukemia. (a) Axillary *Pseudomonas aeruginosa* hydradenitis. (b) Ecthyma gangrenosum of fingers in a patient with *P. aeruginosa* sepsis.



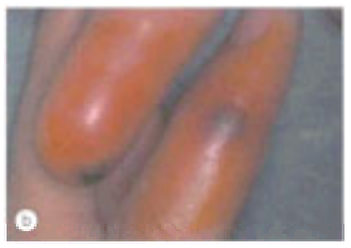


Figure 100-6 Cutaneous manifestations of disseminated fungal infections in leukemic patients. (a) *Aspergillus terreus*, back. (b) *Candida tropicalis*, arm. (c) *Fusarium*, leg. (d) *Pseudallescheria boydii*, leg.

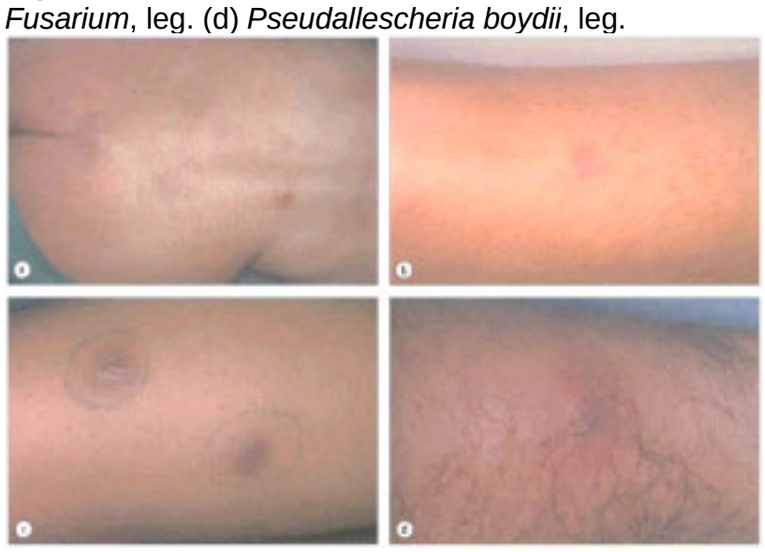


Figure 100-7 Disseminated *Staph. aureus* infection in a leukemic patient. (a) Muscular abscess, MRI. (b) Retinal infectious lesion with macular involvement and secondary bleeding (arrow), fundoscopy.

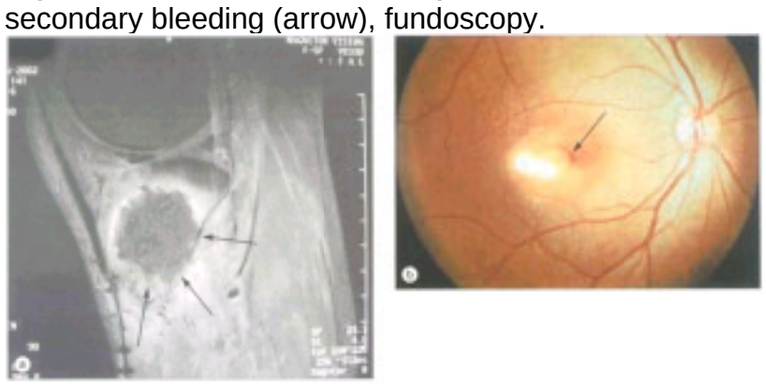


Figure 100-8 Ethmoidal sinusitis due to *Aspergillus fumigatus* in a leukemic patient. The MRI shows a bone destruction with invasion of the orbit, optical nerve (upper arrow) and central nervous system (lower arrow).

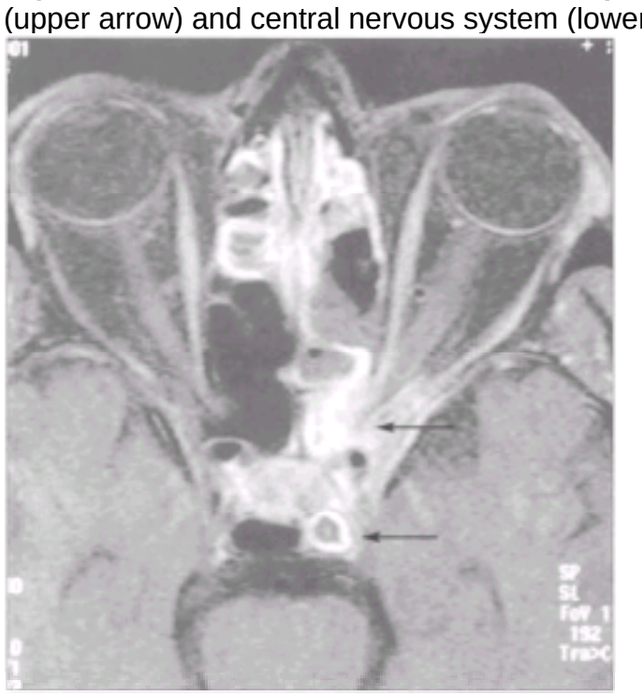


Figure 100-9 Invasive pulmonary aspergillosis in a patient with acute lymphoblastic leukemia. (a) Early stage with halo sign (arrow) during neutropenia, CT scan. (b) Late stage with air crescent sign (arrow) after bone marrow recovery, CT scan.

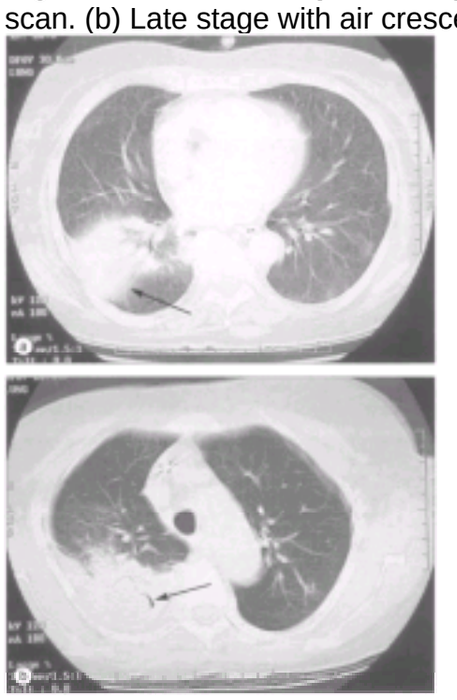


Figure 100-10 Disseminated candidiasis in a patient with acute myelogenous leukemia. (a) Multifocal nodular lung lesions, CT scan. (b) Multiple hepatosplenic abscesses, CT scan.

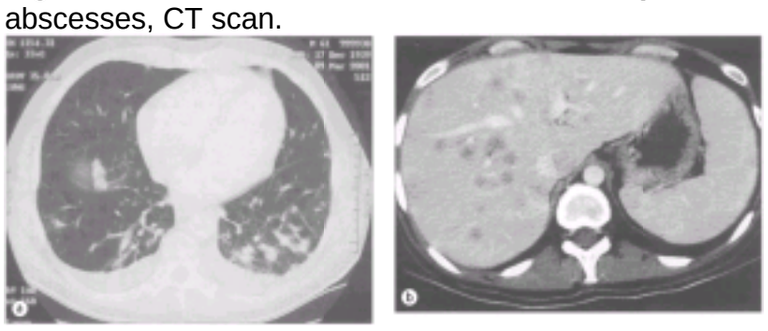


Figure 100-11 Pulmonary infections in neutropenic patients with hematological malignancies. (a) Interstitial pneumonia due to influenza virus. (b) Viridans streptococcal bacteremia with acute respiratory distress syndrome.

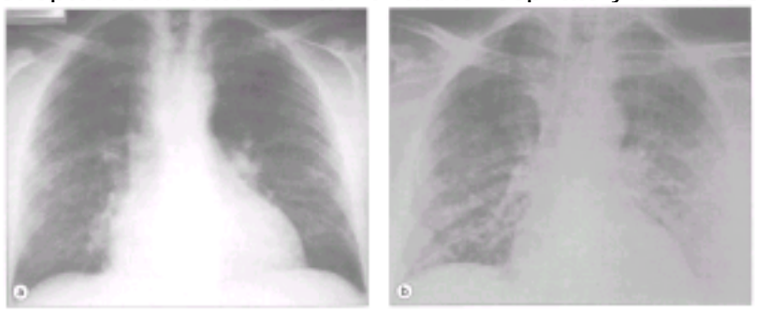


Figure 100-12 Neutropenic enterocolitis in a leukemic patient. Prominent thickening of a segment of ileum, CT scan (arrow).

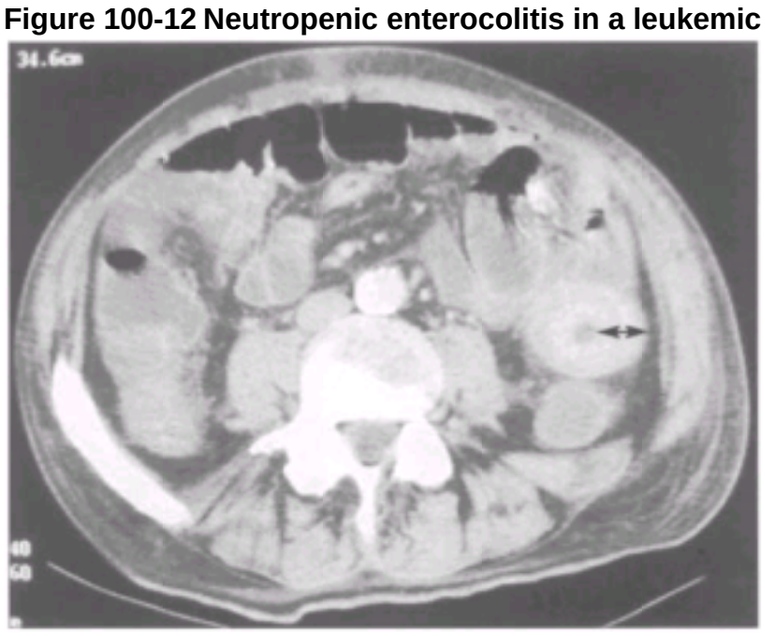


Figure 100-13 Relative risk of infection, fever and death in neutropenic patients with hematological malignancies treated with single-agent fluroquinolone prophylaxis. Yellow circles and black bars show relative risks with 95% confidence intervals in patients receiving fluoroquinolone prophylaxis compared to patients receiving placebo or other regimens. Adapted from reference [26] .

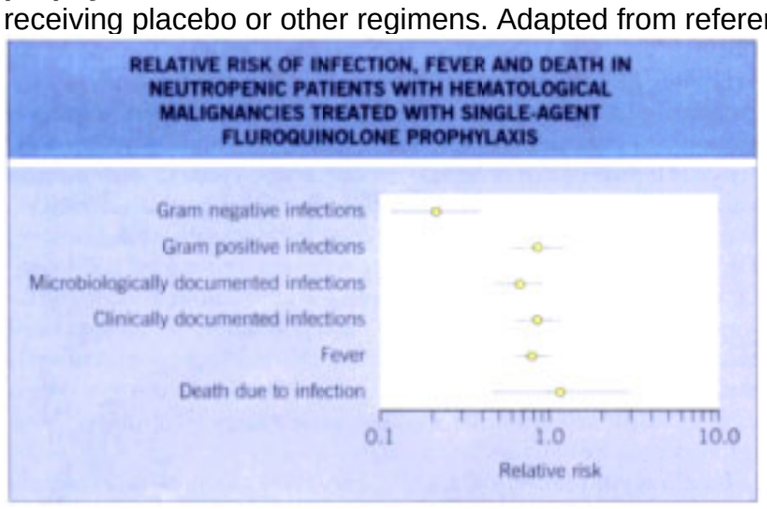


Figure 100-14 Risk assessment and selection of oral or intravenous empirical antibiotic therapy in febrile neutropenic cancer patients. Severe sepsis and septic shock are defined according to reference[37] . *Adapted from reference[6] .*

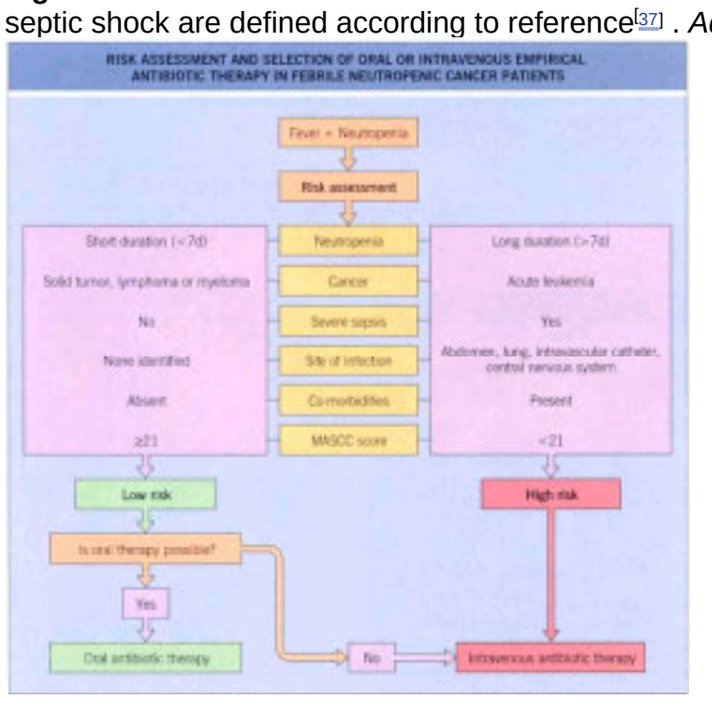


Figure 100-15 Choices of empirical intravenous antibiotics in high-risk febrile neutropenic cancer patients.

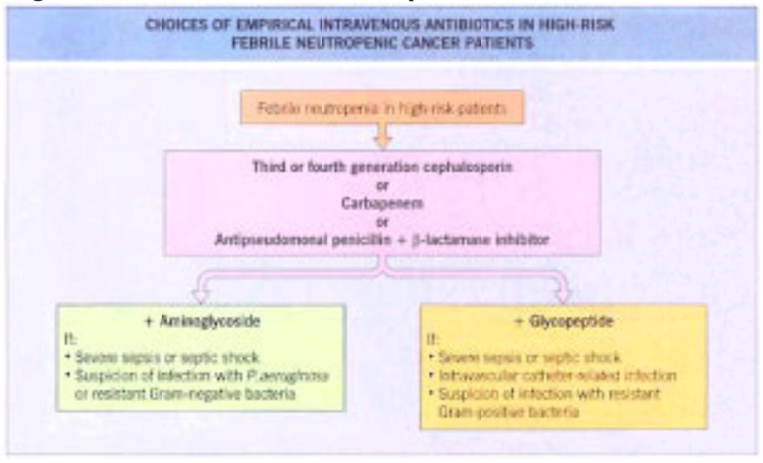


Figure 100-16 Algorithm for adjustment of empirical antimicrobial therapy based on results of microbiological cultures and clinical response.

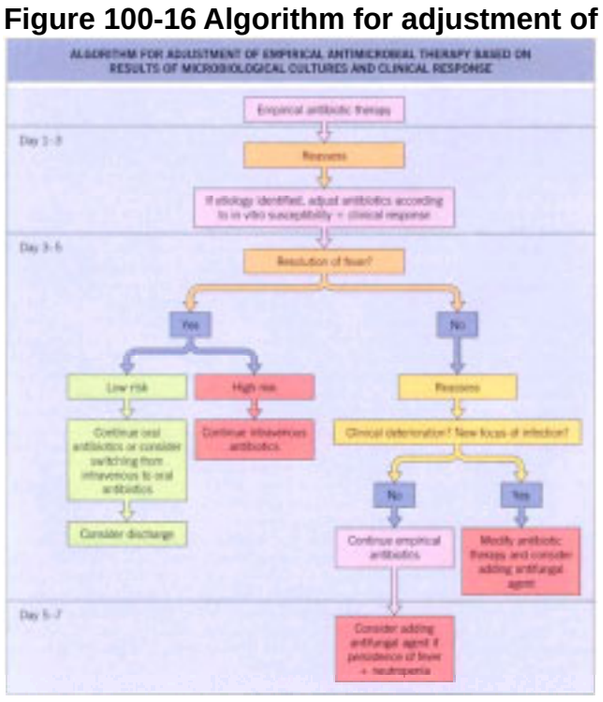


Figure 101-1 Primary risk periods for infections after hematopoietic stem cell transplantation. Typical risk periods for the most common infections after each type of HSCT are shown. Risks are based on typical prophylaxis strategies, which include trimethoprim-sulfamethoxazole (co-trimoxazole) for *Pneumocystis carinii*, screened or filtered blood products and ganciclovir for CMV, aciclovir for herpes simplex virus, and fluconazole for candidemia. *Adapted from Bowden.*[54]

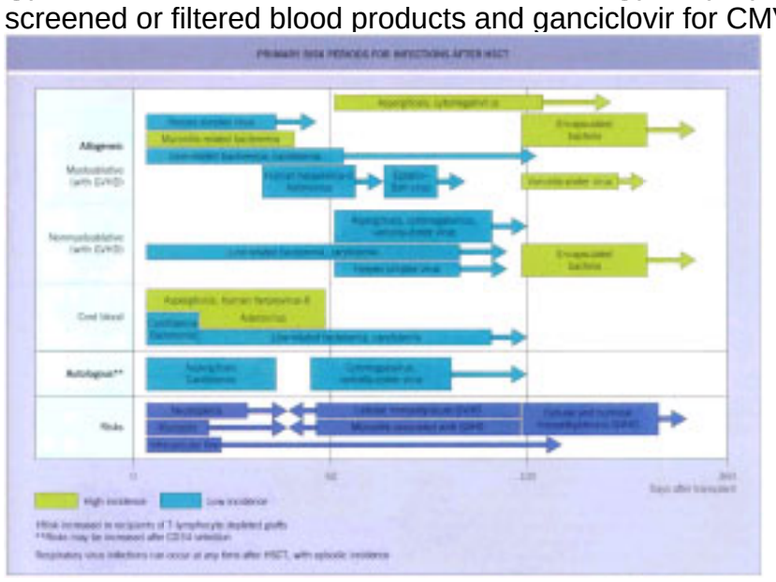


Figure 101-2 Probability of fungal infections in the late 1980s compared to late 1990s at the Fred Hutchinson Cancer Research Center. Probabilities of proven candidemia, and proven or probable aspergillosis are shown. *Data from the 1980s are provided by Dr Raleigh Bowden; data from the 1990s are abstracted from Marr et al.*[40] [69]

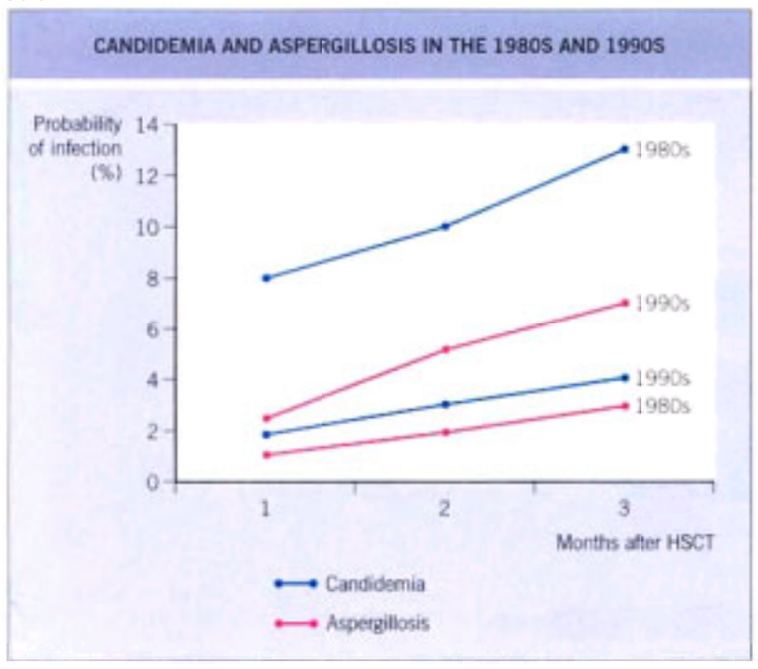


Figure 101-3 Risk factors for aspergillosis after allogeneic hematopoietic stem cell transplantation. Specific risks are demonstrated according to primary risk period (shaded) and day of transplantation. [10] [13] [73] Day 0 represents day of receipt of stem cells. CML-CP, chronic myelogenous leukemia in chronic phase, LAF, laminar airflow.

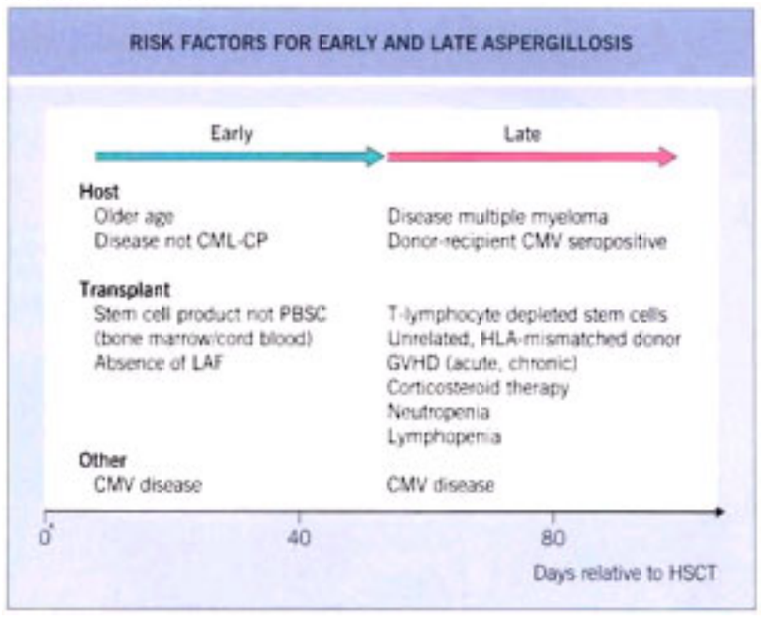


Figure 102-1 Timetable of infection in the organ transplant recipient. The typical infections are plotted by time following transplantation. Note the occurrence of bacterial and candidal and some viral infections in the first month. This is followed by predominantly viral infections between 1 and 6 months, and then fungal and chronic viral infections after 6 months, largely in patients who have chronic rejection and who are receiving more intensive immunosupression. Infections that deviate from this schema suggest a higher level of immunosuppression or a more intense environmental exposure. *Reproduced with permission from Fishman and Rubin.*[3] *CMV, cytomegalovirus; EBV, Epstein-Barr virus; PTLD, post-transplantation lymphoproliferative disease; VZV, varicella-zoster virus.*

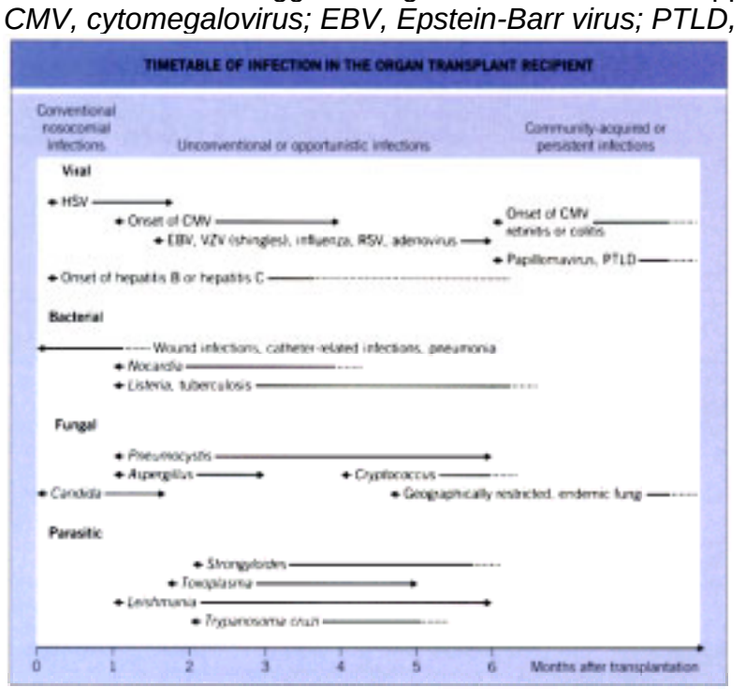


Figure 104-1 Postsurgical mediastinitis due to *aureus* in a HT recipient.

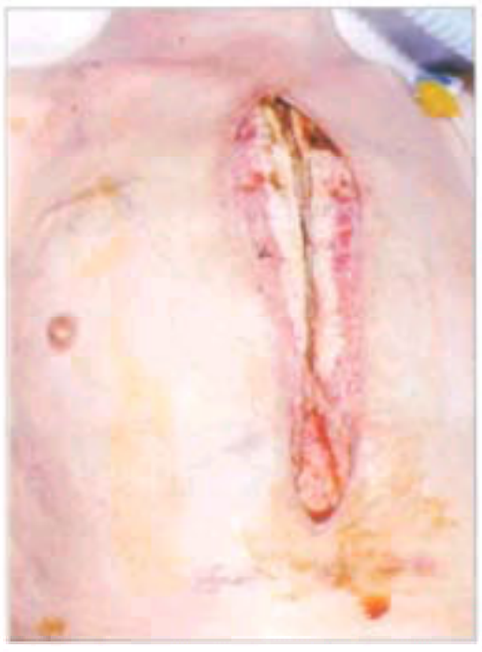


Figure 104-2 Kaposi's sarcoma in a HT recipient. Primary infection by HHV-8 was demonstrated.

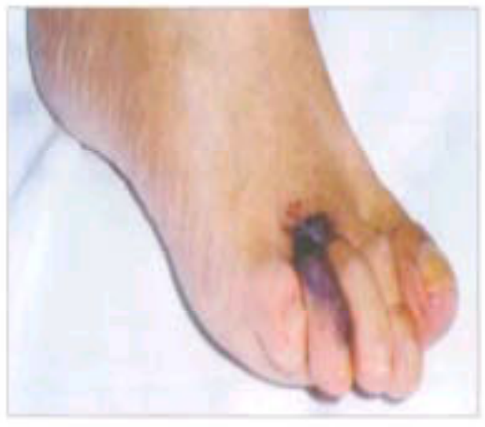


Figure 104-3 Bilateral invasive aspergillosis in a HT recipient.

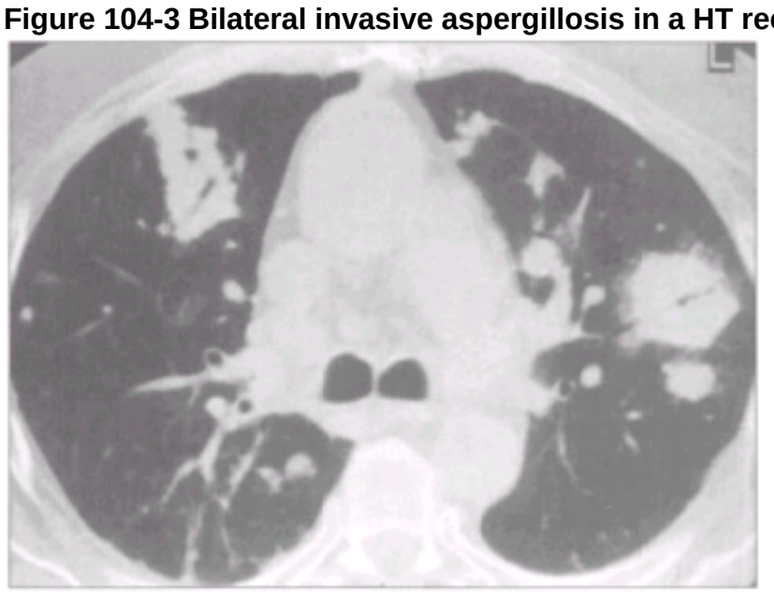


Figure 105-1 Natural history timeline of infections following liver transplantation in the absence of antimicrobial prophylaxis.

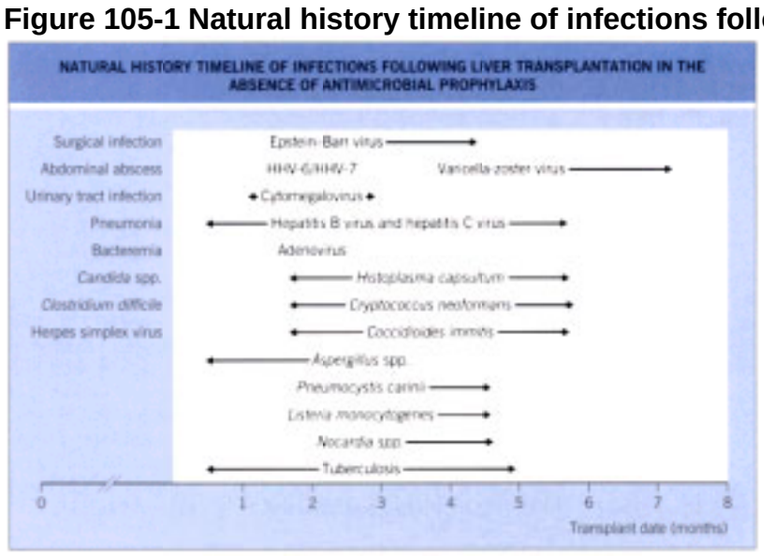


Figure 106-1 ^{99m} Technetium voiding cystourethrograms. (a) Filling and voiding phases of normal ^{99m} Tc DTPA VCUG. The large arrow represents the duodenal segment of the bladder-drained pancreas transplant. The small arrow represents retrograde flow of radioisotope into the pelvis of the transplanted kidney. (b) Abnormal ^{99m} Tc VCUG. Persistence of radioisotope in the peritoneal cavity is consistent with bladder leak (multiple white arrow heads). Although sensitive for diagnosing urine leak, a radioisotope VCUG does not localize the site of leak. A follow-up contrast VCUG may provide localization, if necessary. (c) Abdominal CT scan demonstrating an enteric leak. The pancreas (white arrow) and a peripancreatic fluid collection (black arrow) are seen. Air and contrast material in the fluid collection are diagnostic of an enteric leak. The absence of these findings, however, does not rule out the possibility of a leak.

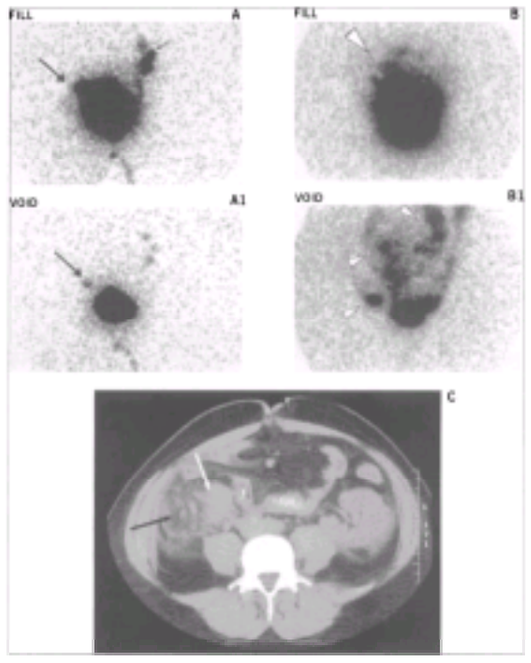


Figure 106-2 Infected false aneurysm of a pancreas transplant. (a), (b) Arteriogram of an infected false aneurysm originating from the ligated superior mesenteric artery stump of the pancreas transplant. (c) Triphasic CT reconstruction (90° rotation) demonstrating the feeding vessels of the false aneurysm in relation to the pancreas transplant.

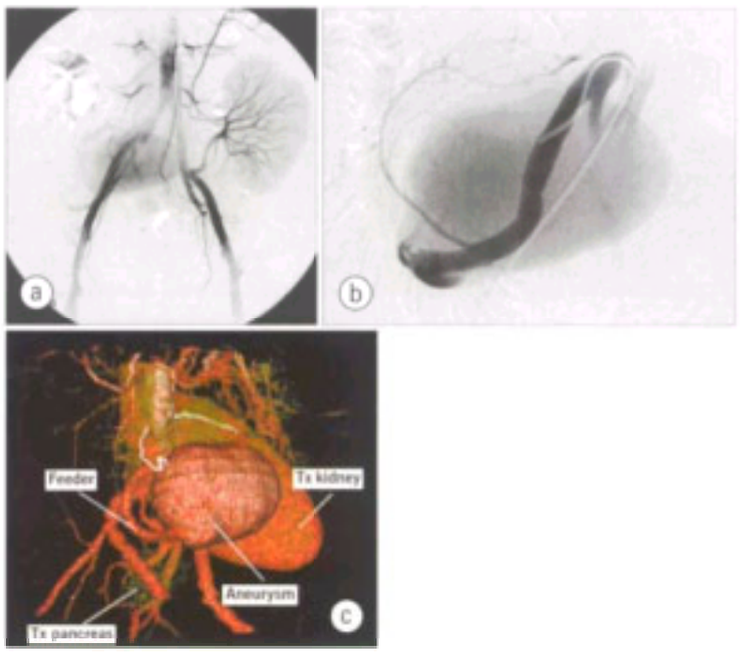


Figure 106-3 Pancreatic pseudocyst. (a) A CT scan demonstrating a pseudocyst of a pancreas transplant in the left pelvis (arrow). (b) Percutaneous drainage of a pancreas transplant pseudocyst. Injection of radiographic contrast material (black arrow) demonstrates the pancreatic duct (arrowhead) in communication with the pancreatic pseudocyst.

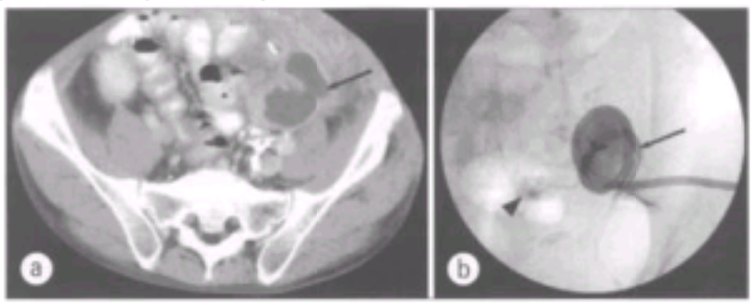


Figure 106-4 Algorithm for evaluating enterically drained pancreas transplant patients with abdominal pain.

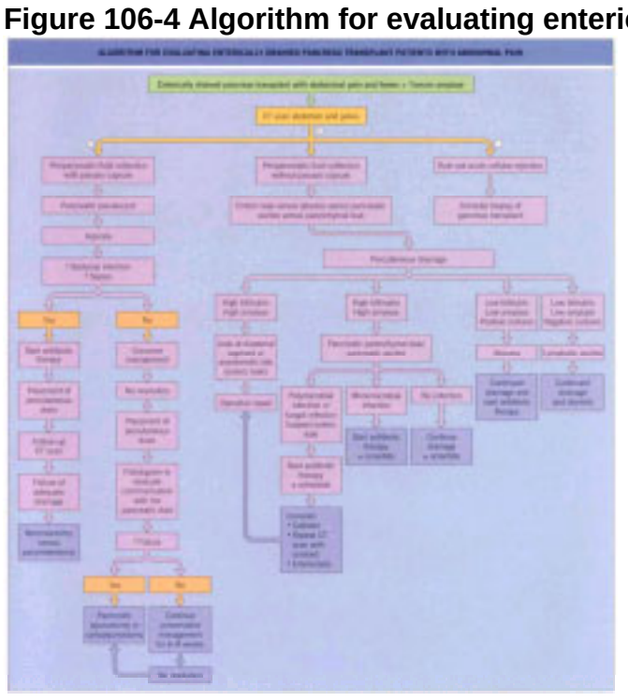


Figure 108-1 A large necrotizing lesion caused by herpes simplex type-1 in a patient who has a teratoma. Herpetic stomatitis is common in immunosuppressed patients and is often atypical; any ulcerating lesion in the perioral region should be considered to be herpetic until proved otherwise.

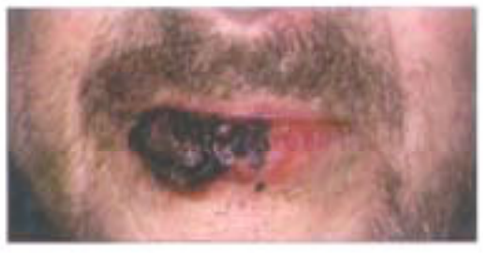


Figure 108-2 Extensive dermatophyte infection in a bone marrow transplant recipient. Many other infections (and graft-versus-host disease) can give a similar appearance but the diagnosis is quickly established by biopsy and microscopy. This condition is limited to the skin but nevertheless requires systemic antifungal therapy.

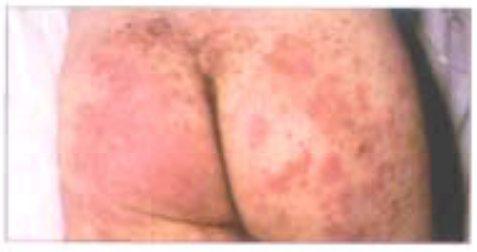


Figure 108-3 Extensive skin lesions caused by *Mycobacterium chelonae* in a patient who had polyarteritis nodosa. The lesions were palpable but not especially painful.

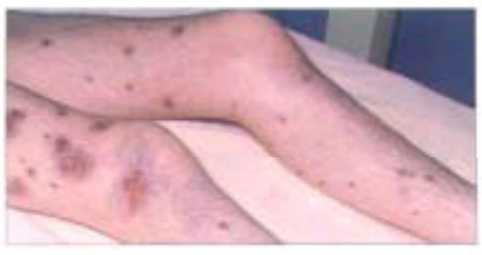


Figure 109-1 Normal splenic architecture in the adult human. PLS, periarterial lymphatic sheath; PA, penicillary arteriole; MZ, marginal zone (B lymphocytes predominate); WP, white pulp (T cells predominate); RP, red pulp (vascular cords and venous sinuses). Hematoxylin and eosin stain.

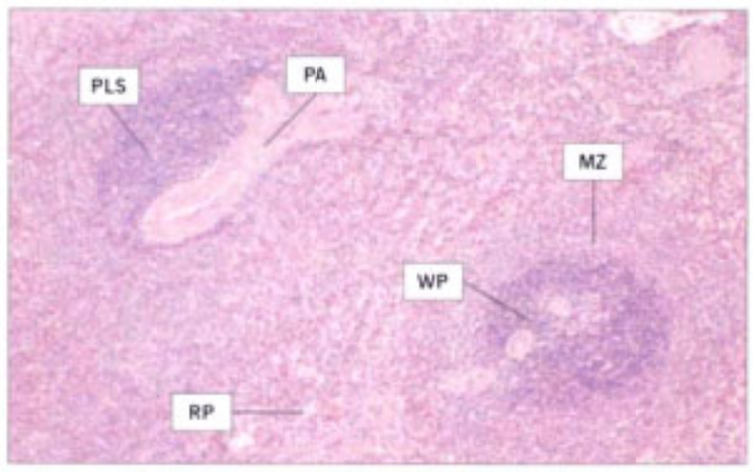


Figure 109-2 Peripheral blood smear of a patient who has pneumococcal sepsis and meningitis. Note polymorphonuclear leukocyte with several bacterial diplococci in the cytoplasm. Wright stain.

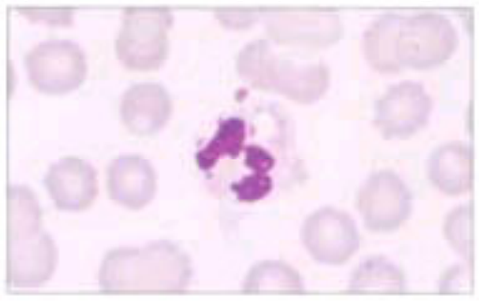


Figure 109-3 Gram stain of cerebrospinal fluid of a patient who has pneumococcal meningitis. Note numerous Gram-positive cocci in pairs and a single lymphocyte.

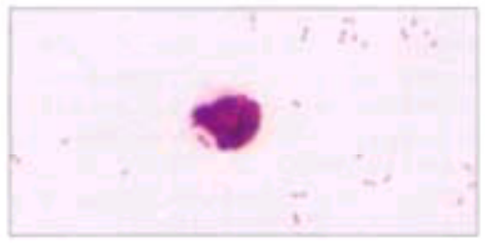


Figure 111-1 A T2-weighted MRI of the liver in a 24-year-old woman being treated for lymphoma who has chronic disseminated (hepatosplenic) candidiasis. The hepatic lesions are central densities surrounded by zones of lower signal intensity which create a 'bull's eye' appearance. Smaller lesions are also visible in the spleen, particularly in the subcapsular region.

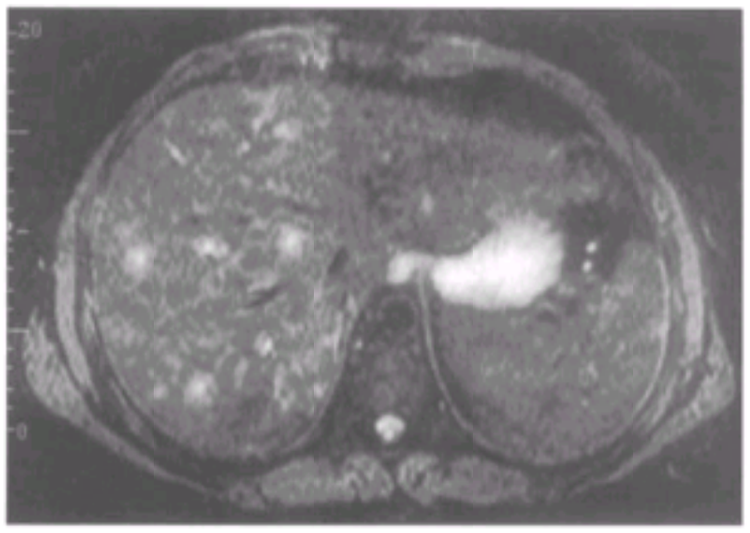


Figure 111-2 Aspergillosis. (a and b) A chest radiograph to evaluate persistent neutropenic fever in a patient with relapsed leukemia showed subtle increased markings in the right lower lung. (c) A chest CT scan obtained the same day showed a significant area of consolidation not apparent on the chest radiograph. Bronchoalveolar lavage grew *Aspergillus fumigatus*. This case illustrates the appropriate use of a chest CT scan in patients with persistent neutropenic fever at high risk for invasive mold infection. (d) Invasive pulmonary aspergillosis in another patient showing necrosis and invasive hyphae (Gomori methenamine silver).

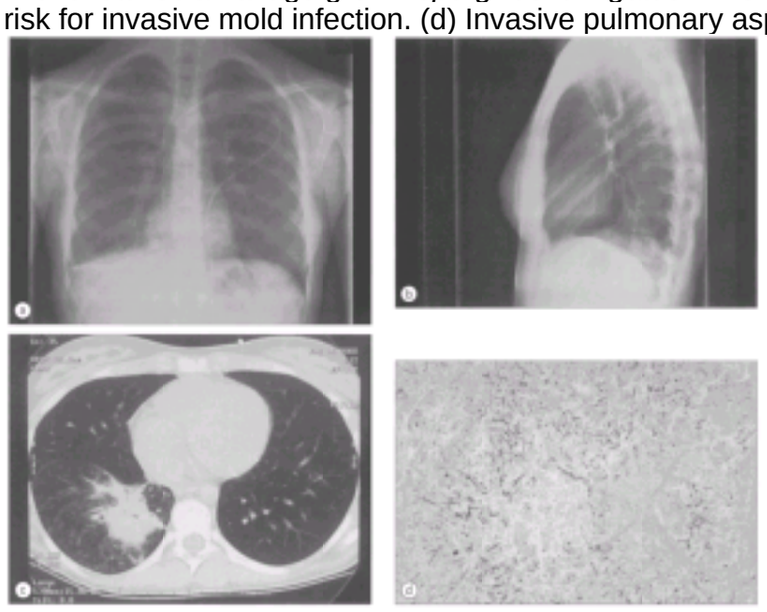


Figure 112-1 Hemorrhagic labial HSV lesions in a patient with leukemia and chemotherapy-induced thrombocytopenia. Diagnosis of HSV disease may be difficult and needs confirmation by virus culture or PCR.

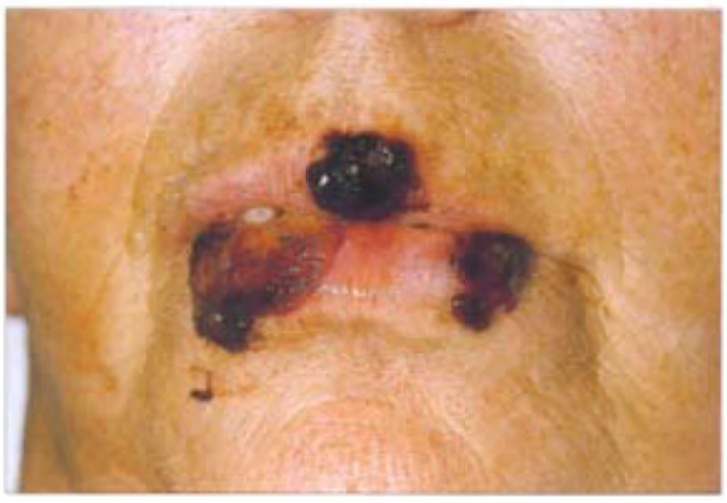


Figure 112-2 Aciclovir-resistant perineal ulcerative HSV lesions after allogeneic SCT.

Figure 112-3 Mechanisms of HSV and VZV resistance to antiviral drugs. Most cases of HSV and VZV resistance are due to mutations in the gene of the virus-encoded thymidine kinase, which result in a reduced or abrogated conversion of aciclovir to aciclovir monophosphate. This leads to low or absent levels of aciclovir triphosphate, the active metabolite that inhibits the viral DNA polymerase and acts as DNA chain terminator. Viral replication may then occur despite aciclovir therapy. Foscarnet and cidofovir do not require viral thymidine kinase-dependent intracellular activation. Resistance to these two drugs may occur if HSV or VZV resistance is caused by mutations in the viral DNA polymerase gene *(adapted from*[8] *)*.

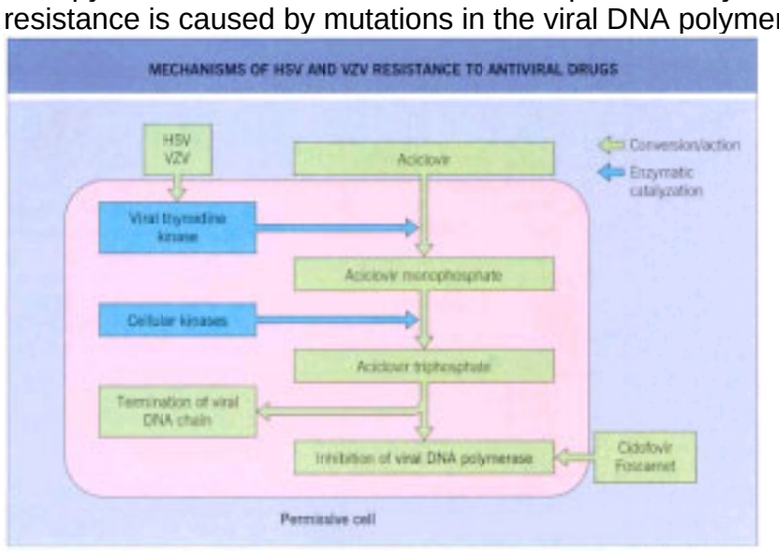


Figure 112-4 (a,b) **Disseminated cutaneous lesions in a patient with chronic lymphocytic leukemia who developed fatal varicella despite rapid initiation of intravenous aciclovir therapy.** Varicella lesions are numerous and hemorrhagic.

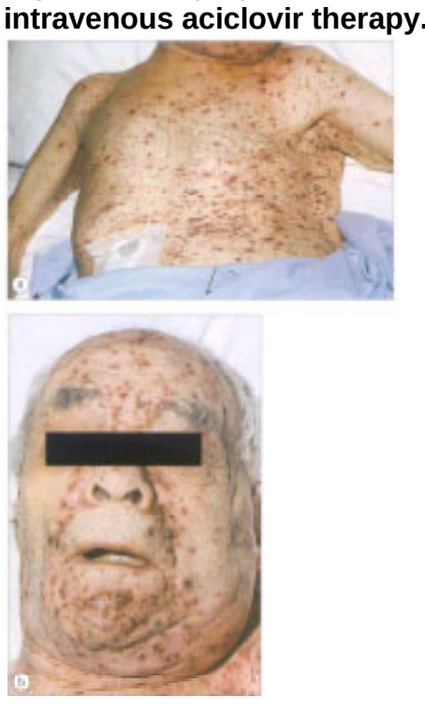


Figure 112-5 Varicella pneumonia in a renal transplant recipient with bilateral lung infiltrates on chest radiography.

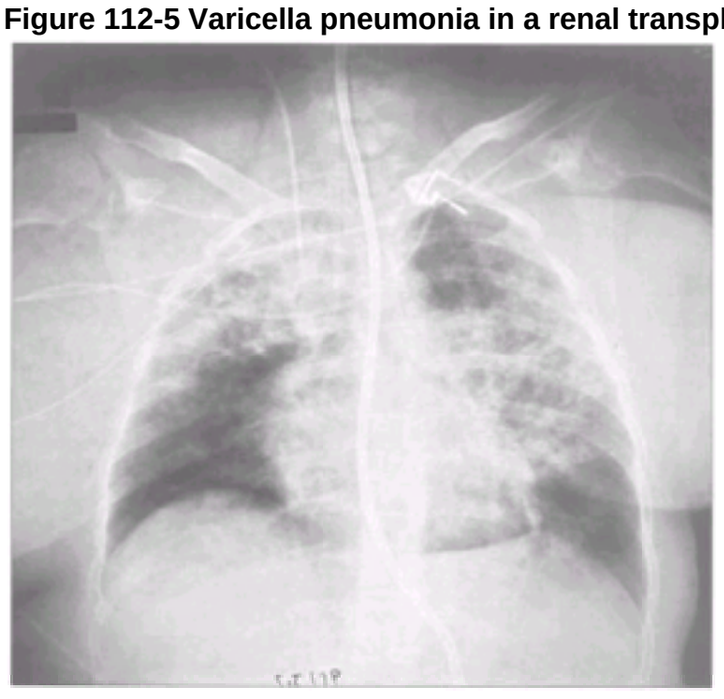


Figure 112-6 Herpes zoster in cervical dermatomes in a patient 5 months after allogeneic SCT for leukemia.

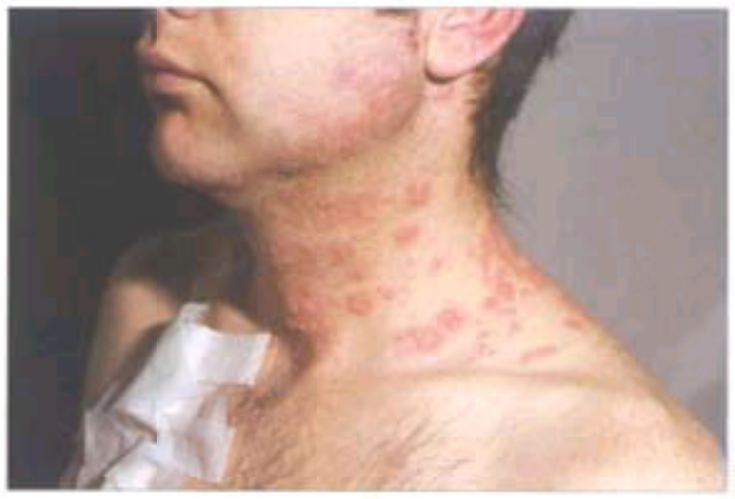


Figure 112-7 Positive CMV antigenemia assay. Three peripheral blood leukocytes containing the pp65 CMV antigen show positive immunoperoxidase staining.

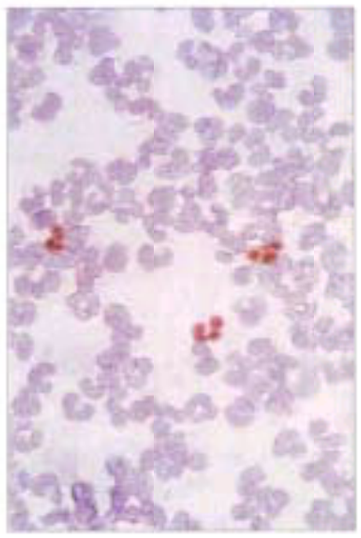


Figure 112-8 CMV pneumonia in an allogeneic SCT recipient with diffuse bilateral interstitial lung infiltrates on chest radiography.

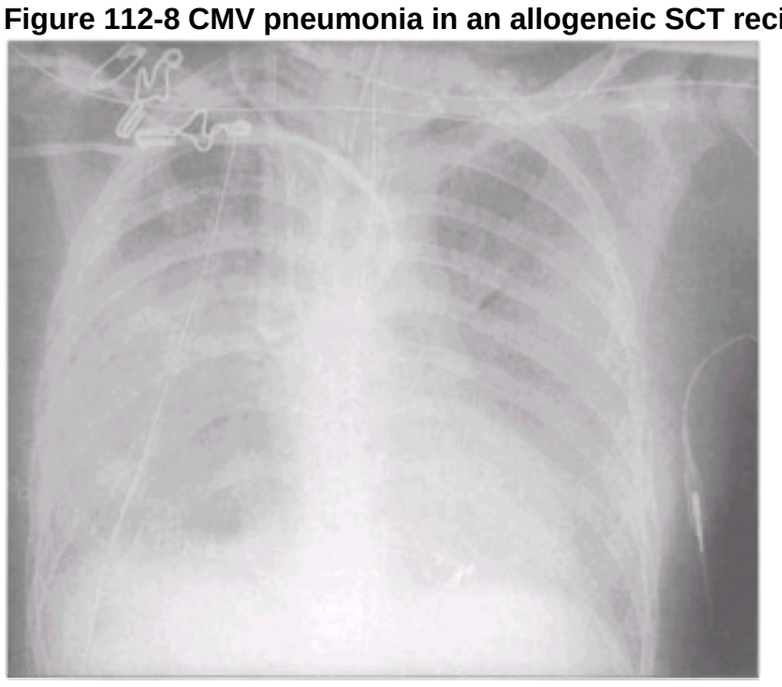


Figure 112-9 Mechanisms of CMV resistance to antiviral drugs. Most cases of CMV resistance are due to mutations in the viral UL97 gene encoding for the phosphotransferase, which result in a reduced or abrogated conversion of ganciclovir to ganciclovir monophosphate. This leads to low or absent levels of ganciclovir triphosphate, the active metabolite that inhibits the viral DNA polymerase. Viral replication may then occur despite ganciclovir therapy. Foscarnet and cidofovir do not require viral phosphotransferase-dependent intracellular activation. Resistance to these two drugs may occur if CMV resistance is caused by mutations in the viral DNA polymerase gene *(adapted from[8]).*

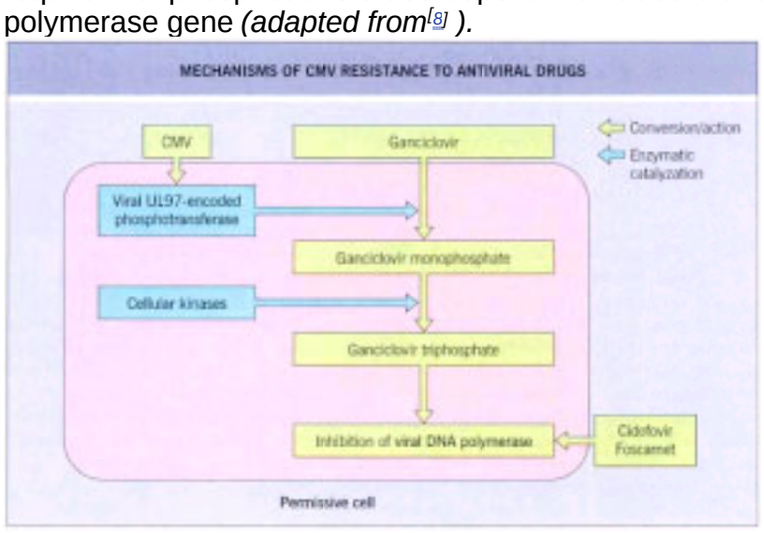


Figure 113-1 Giemsa-stained smear of bronchoalveolar lavage from a bone marrow transplant patient with disseminated toxoplasmosis. Tachyzoite form is demonstrated (arrows).

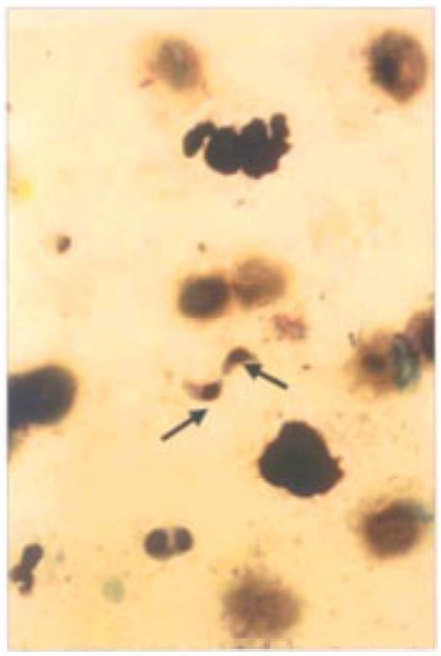


Figure 113-2 Hematoxylin-eosin stain of the cyst form of *T. gondii* in brain (arrows).

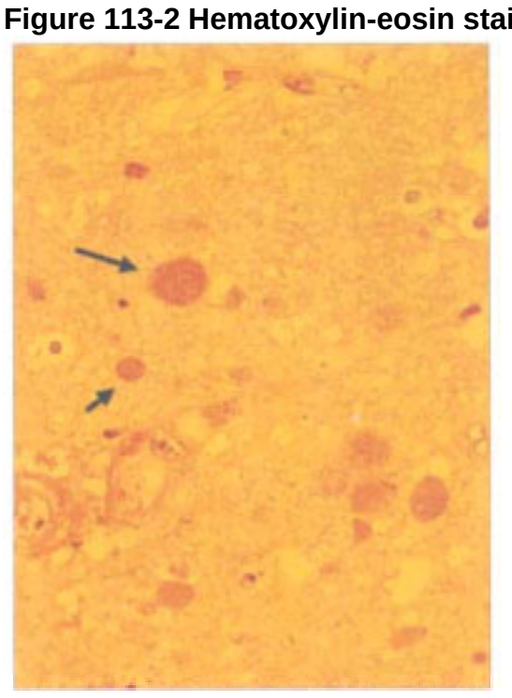


Figure 113-3 Magnetic resonance imaging of the brain in an autologous bone marrow transplant patient with toxoplasmic encephalitis (TE). Enhancing lesions are shown (arrows).

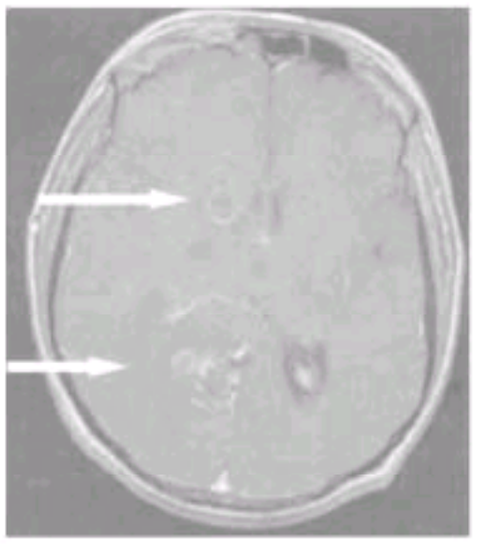


Figure 115-1 Young women and men (aged 15–24 years) estimated to be living with HIV/AIDS, 2001. Total at end of 2001, 40 million.

Figure 115-2 Percentage of newly reported AIDS cases by race/ethnicity, USA.

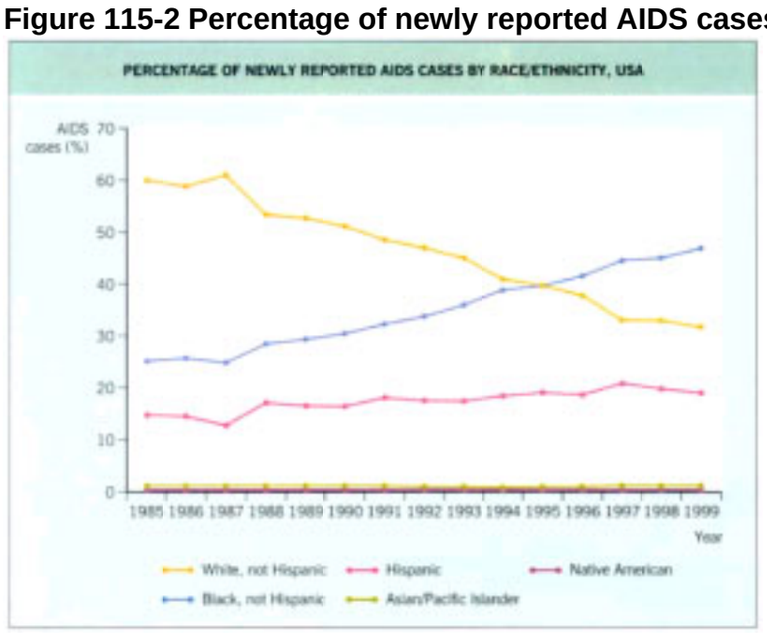


Figure 115-3 Adult/adolescent AIDS cases by transmission group, western Europe.

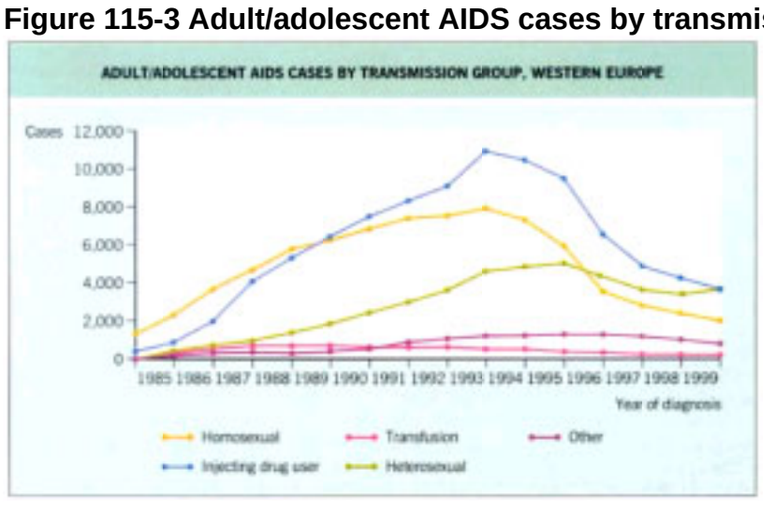


Figure 115-4 HIV prevalence among pregnant women, selected provinces, South Africa.

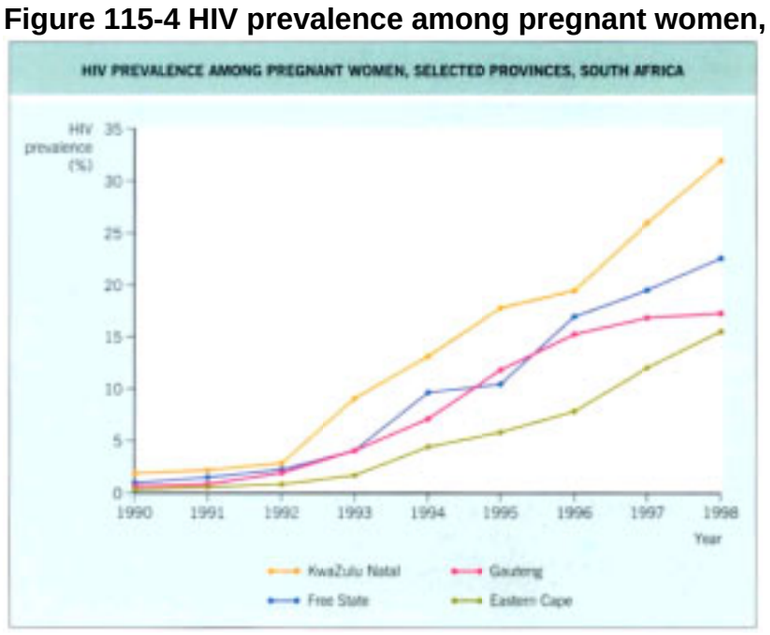


Figure 115-5 Proportion of new HIV infections in injecting drug users.

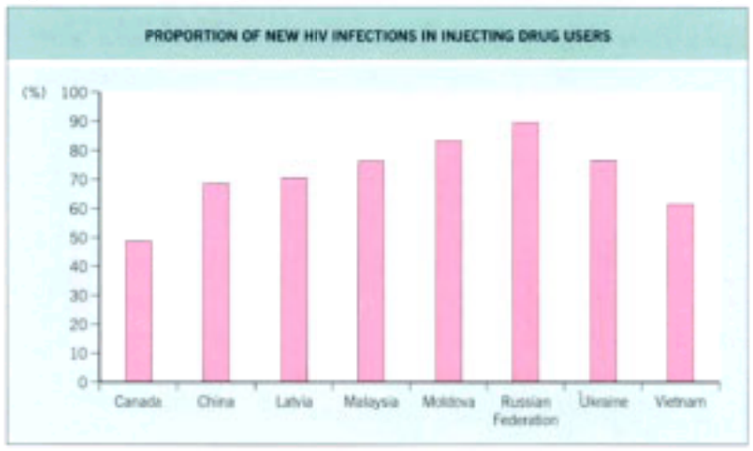


Figure 115-6 Impact of the epidemic on children with HIV-infected mothers. *Model of Global Orphan Project, data from Brazil.*

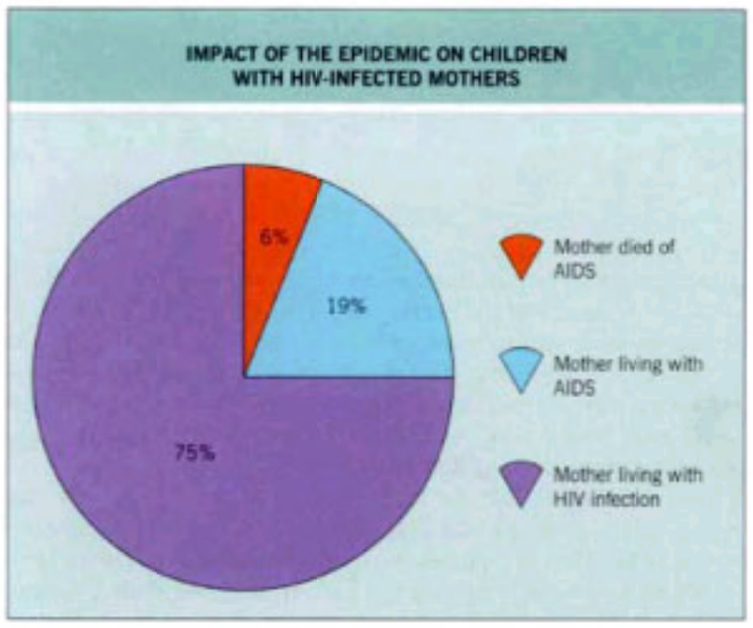


Figure 115-7 HIV prevalence among young women, African studies. Seropositivity among women aged 15–19 years and 20–24 years in selected community surveys in sub-Saharan Africa, 1995–2000.

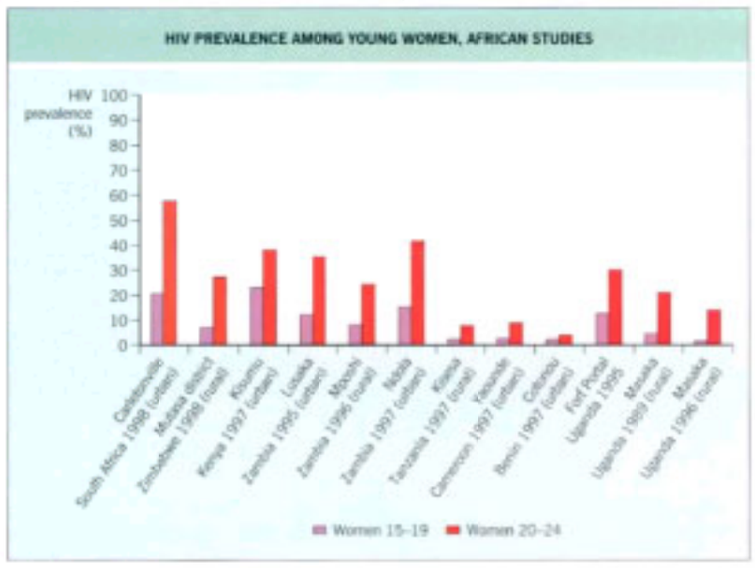


Figure 115-8 Percentage of adolescents who had their first sexual experience before age 15 years.

Figure 115-9 HSV-2 among young people in a South African town. Seroprevalence among male and female adolescents aged 15–24 years by number of lifetime sexual partners.

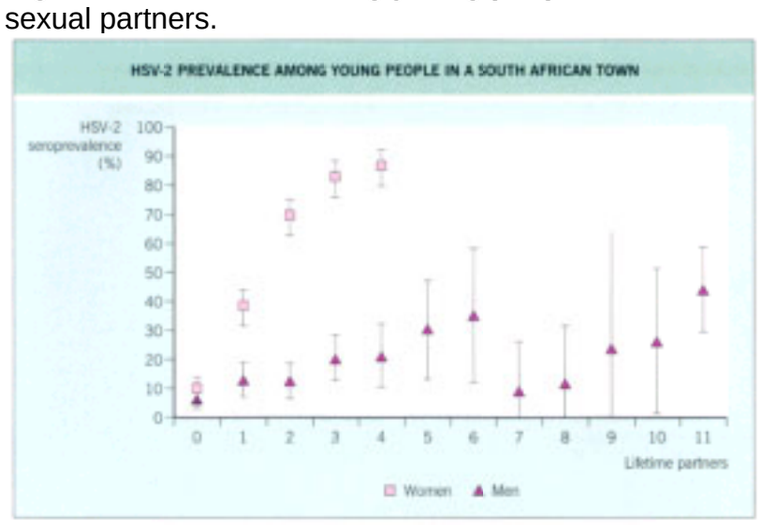


Figure 115-10 Estimated prevalence of HIV-1 *env* subtypes by region, 1998.

Figure 115-11 Trends in HIV prevalence in selected populations. HIV seroprevalence among pregnant women in Dakar and Kampala and among military conscripts in Thailand, 1989–1999.

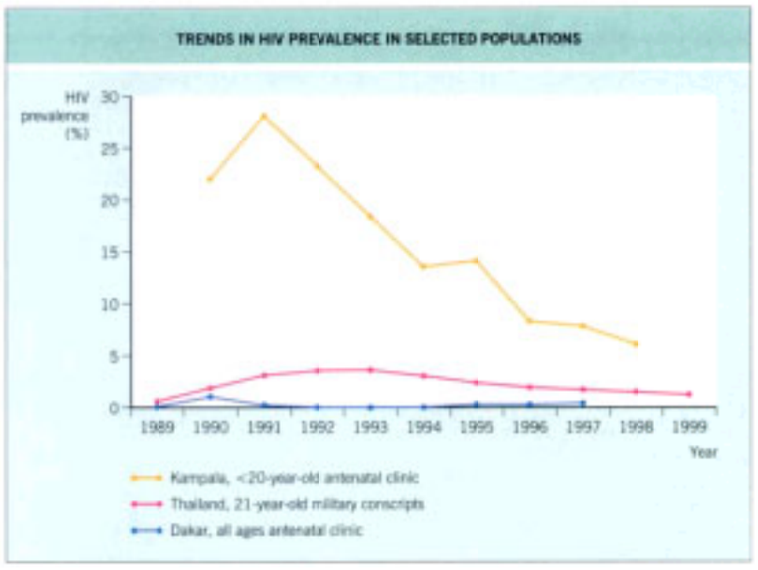


Figure 115-12 Changes in life expectancy in selected sub-Saharan countries. Projected life expectancy at birth, 1950–2000.

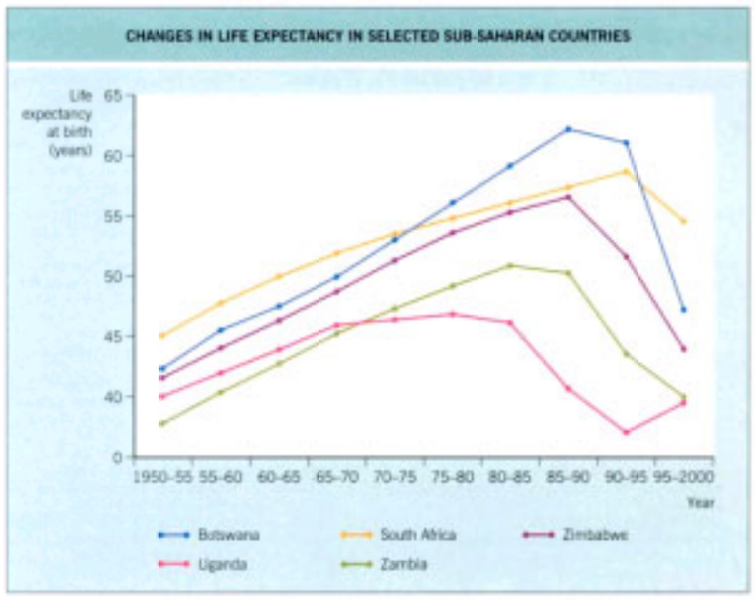


Figure 115-13 Projected population structure with and without AIDS, Botswana, 2020.

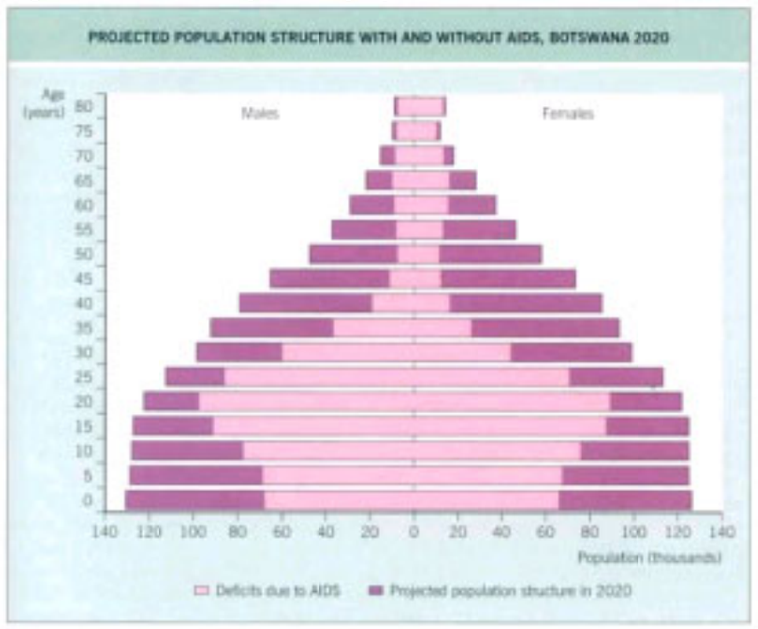


Figure 116-1 Approaches to preventing HIV transmission.

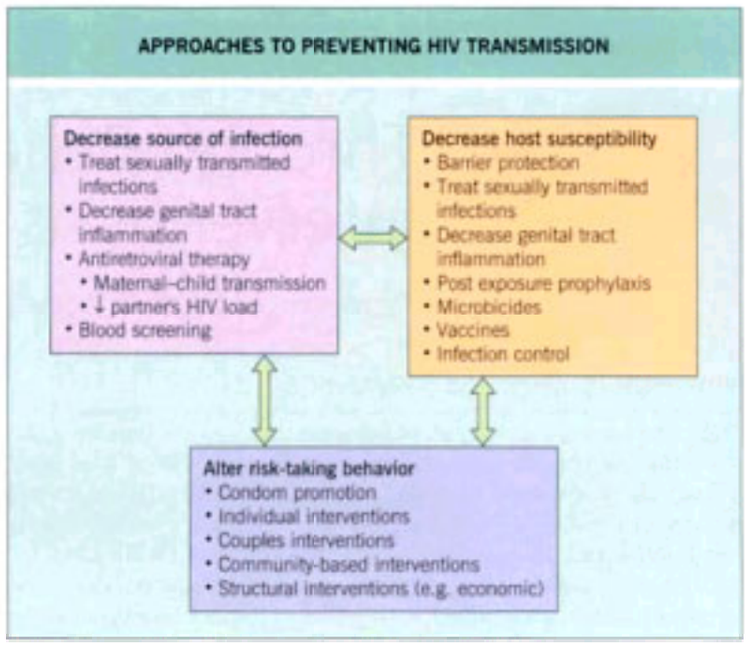
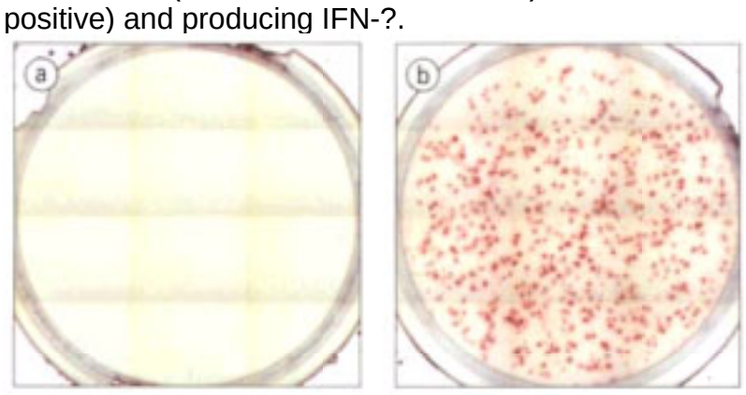


Figure 118-1 Two newer methods for studying responses to HIV vaccines. (a) and (b) are representative negative and positive wells (respectively) from an interferon-? (IFN-?) ELISPOT assay. Mononuclear cells are separated from blood, placed in wells coated with antibody against IFN-?, and stimulated overnight with (b) or without (a) peptides from the antigen of interest. On stimulation, antigen-specific T cells release IFN-?; the captured IFN-? is stained using a second antibody with an enzymatic tag to develop a color change, much like a conventional ELISA test. A colored spot appears for each cell producing IFN-?. The lower panels are representative of the result from a cytokine flow cytometry (CFC) assay. Whole blood from an HIV^{+} patient is cultured without (c) or with (d) peptides from the HIV *gag* gene in the presence of an inhibitor of Golgi secretion. Newly synthesized cytokine within the cells, in addition to cell surface markers, are then stained using fluorescently labeled antibodies and analyzed on a fluorescence activated cell sorter (FACS). The cells in (c) and (d) have been labeled for CD3 (general T-cell marker), CD4 (helper class of T cells) and CD69 (activation marker); they are also permeabilized and stained to reveal intracellular IFN-?. The cells staining for CD3 but not CD4 (i.e. CD8 or effector T cells) were selected using automated software. The upper right quadrant indicates which of the CD8 cells are newly activated (CD69 positive) and producing IFN-?.



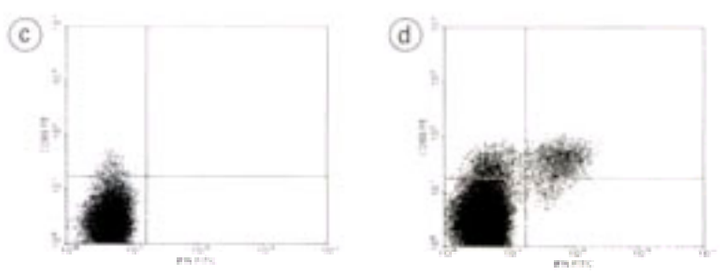


Figure 120-1 Kinetics of viral load and immune response during the phases of HIV-1 infection. After HIV-1 exposure, initial virus replication and spread occur in the lymphoid organs, and systemic dissemination of HIV-1 is reflected by the peak of plasma viremia. A clinical syndrome of varying severity is associated with this phase of primary HIV-1 infection in up to 70% of HIV-1-infected persons. Downregulation of viremia during the transition from the primary to the early chronic phase coincides with the appearance of HIV-1-specific cytotoxic T cells and with the progressive resolution of the clinical syndrome. The long phase of clinical latency is associated with active virus replication, particularly in the lymphoid tissue. During the clinically latent period, $CD4^+$ T cell counts slowly decrease, as does the HIV-1-specific immune response. When $CD4^+$ T cell counts decrease below H 200 cells/µl (i.e. when overt AIDS occurs), the clinical picture is characterized by severe constitutional symptoms and by the possible development of opportunistic infections and/or neoplasms.

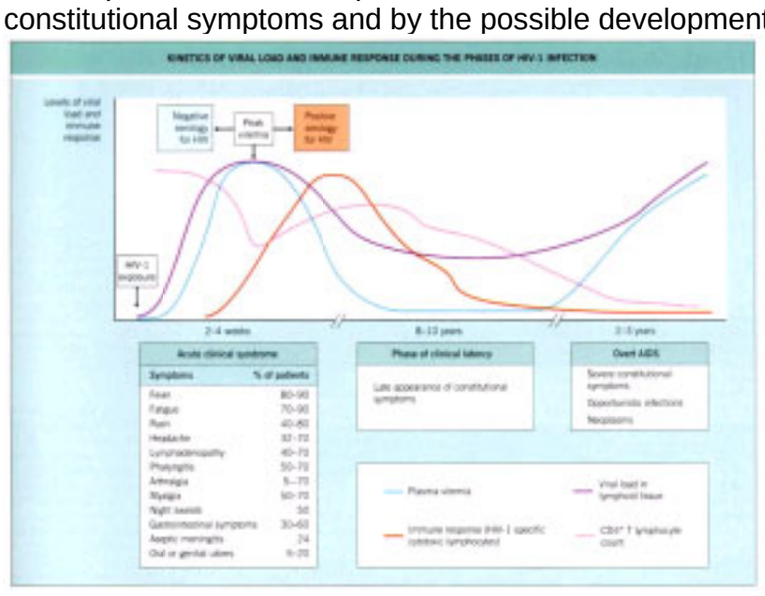


Figure 120-2 Changes in viral load, CD4$^+$ T cells and immune response in the different natural courses of HIV-1 infection. Typical progressors represent 60–70% of the total HIV-1-infected population, rapid progressors represent 10–20%, slow progressors represent 5–15% and long-term nonprogressors represent 1%.

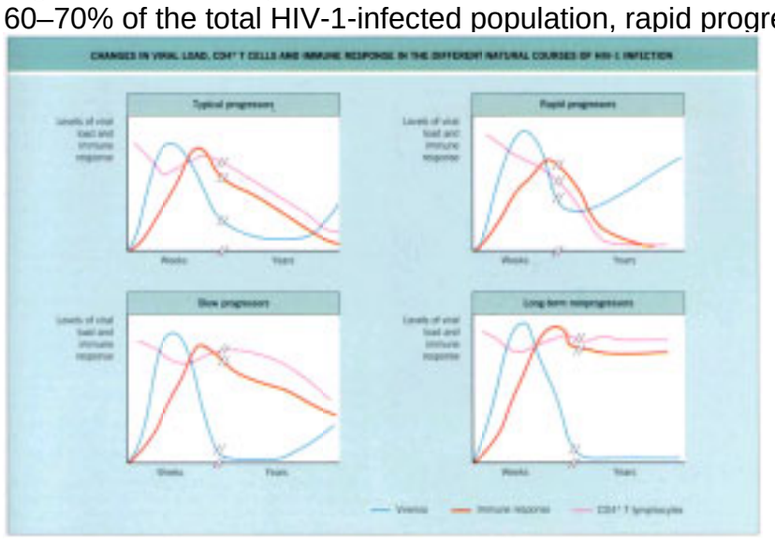


Figure 120-3 Transmission of HIV-1 at the mucosal surface. After entry at the mucosal epithelium, Langerhans cells, also named epidermal dendritic cells, can either be infected by R5 (macrophage (M)-tropic) strains of HIV-1 or pick up HIV-1 virions. Epidermal, DC-SIGN-negative dendritic cells are thought to select for the M-tropic viruses that are the most frequently transmitted variants. The M-tropic HIV-1 carried by epidermal dendritic cells can bind to additional subepithelial DC-SIGN-positive dendritic cells. DC-SIGN can capture HIV-1 on the cell surface of dendritic cells without allowing viral entry. It is thought that DC-SIGN-positive dendritic cells play the major role in the delivery of virus to T cells, thus greatly amplifying HIV-1 infection.

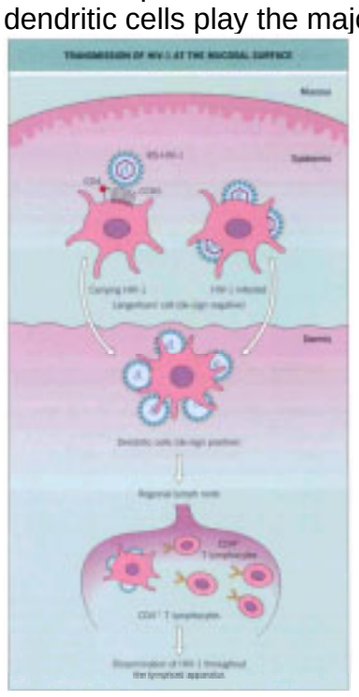


Figure 120-4 Changes in virus distribution within lymph nodes during the transition from the acute to the chronic phase of HIV-1 infection. (a) During the initial weeks of primary HIV-1 infection, the virus is detected in lymphoid tissue as individual virus-expressing cells. This is shown by in-situ hybridization for the detection of HIV-1 RNA (white dots indicate HIV-1 RNA-positive cells). (b) Numerous individual virus-expressing cells seen in the acute phase. (c) Elevated virion levels are found in the circulation in the acute phase. (d) After transition to the chronic phase of the disease, virions trapped in the follicular dendritic cell network become the dominant form of HIV-1, as shown by in-situ hybridization for the detection of HIV-1 RNA (diffuse white areas indicate virus trapped within follicular dendritic cells). (e) Binding of virions on the extracellular surface of follicular dendritic cells in the chronic phase. (f) The number of circulating virions is dramatically reduced in the chronic phase.

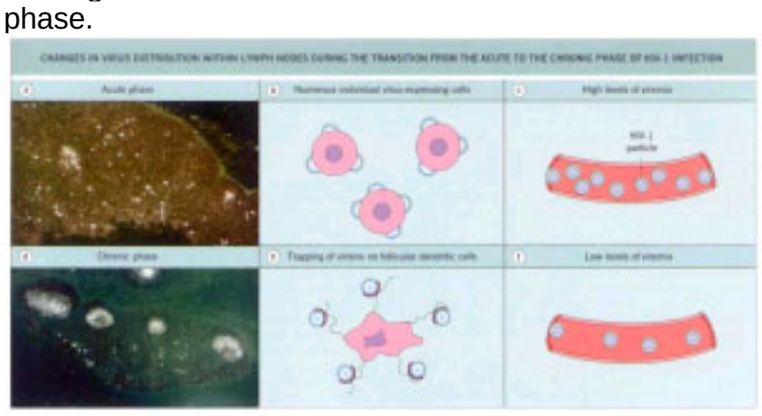


Figure 120-5 Three-phase decay model of virus replication. Viremia below 50 HIV-1 RNA copies/ml plasma does not correspond to complete suppression of virus replication. Residual viremia (5–10 HIV-1 RNA copies/ml plasma) may still be detected 48 weeks after initiation of HAART. However, it is still unclear whether HAART induces complete suppression of virus replication and, if it does, what duration of HAART is needed to achieve this goal.

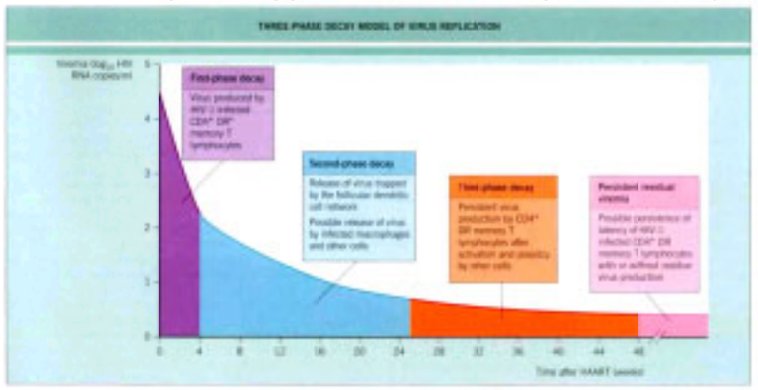


Figure 121-1 HIV-1 virion. The glycoprotein gp120 constitutes the outer envelope of the virus and is noncovalently linked to the transmembrane protein gp41. The matrix protein (p17) bridges the envelope protein with the cone-shaped structure formed by the capsid protein (p24). The viral genomic RNA and processed nucleocapsid (NC; p7) and Pol proteins, reverse transcriptase (RT) and integrase (IN), are located inside the capsid core. PR, protease.

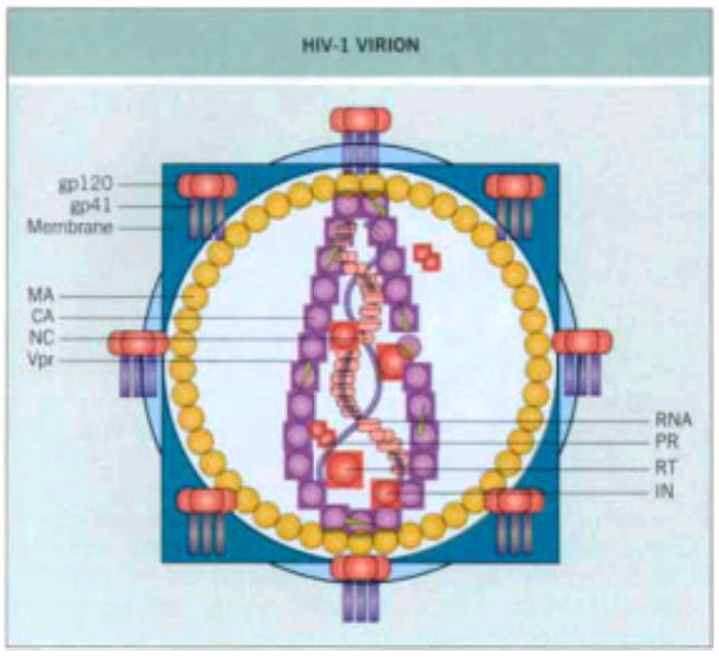


Figure 121-2 Genetic organization of HIV-1 and known functions of gene products. The structural genes are *gag, gag-pol* and *env*. Catalytic proteins are encoded by the *pol* gene. Regulatory proteins are translated from fully spliced mRNA. Within the HIV-1 genome, there are additional open reading frames that flank the *env* gene and encode several regulatory proteins including Vif, Vpr, Tat, Rev, Vpu and Nef. LTR, long terminal repeat; PIC, pre-integration complex consisting of viral cDNA, IN, RT, Vpr, MA and NC.

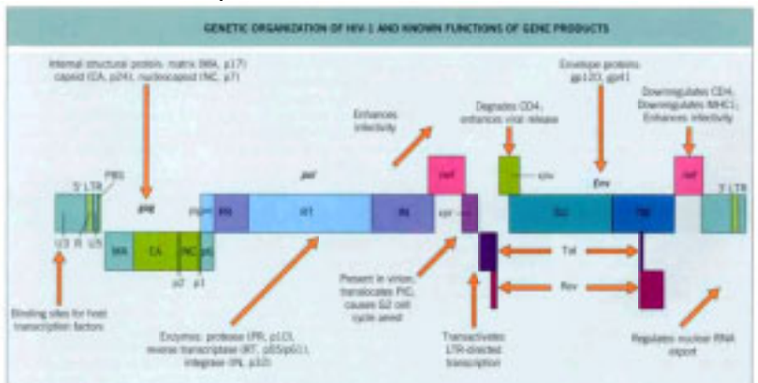


Figure 121-3 Processing of Gag and Gag-Pol proteins. The Gag proteins are initially translated as a 55kDa polyprecursor. Proteolytic processing of Pr55Gag generates several mature products. The catalytic proteins, including reverse transcriptase (RT), integrase (IN) and protease (PR), are first produced as a Gag-Pol precursor (Pr160Gag-Pol) through a translational frameshift mechanism; Pr160Gag-Pol is then cleaved to the smaller enzymatically active subunits.

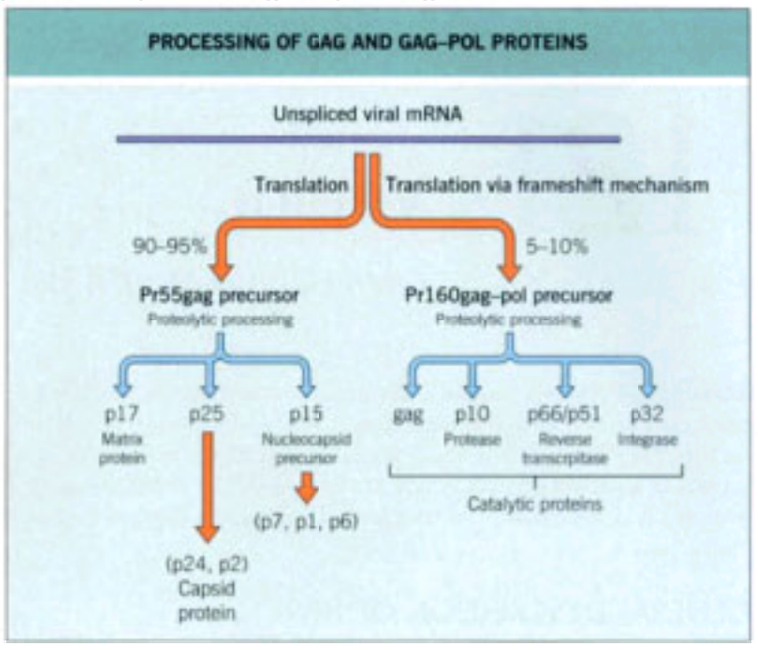


Figure 121-4 The HIV-1 life cycle. The diagram shows the various stages involved, including entry, uncoating, reverse transcription, integration, expression of proviral genome, viral assembly and particle release.

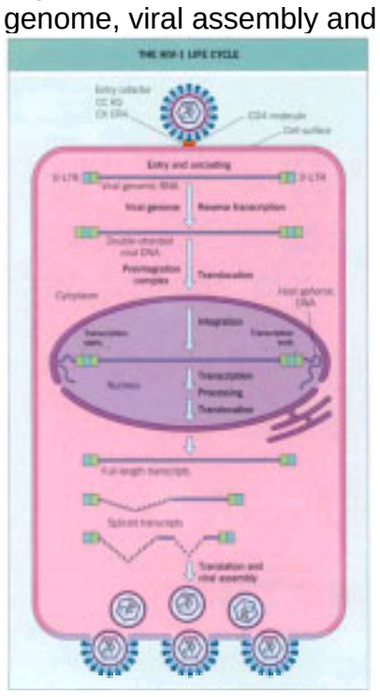


Figure 122-1 Maculopapular rash during primary HIV infection.

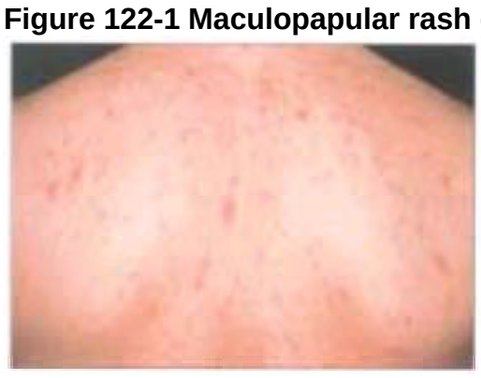


Figure 122-2 Acneiform lesions during primary HIV infection.

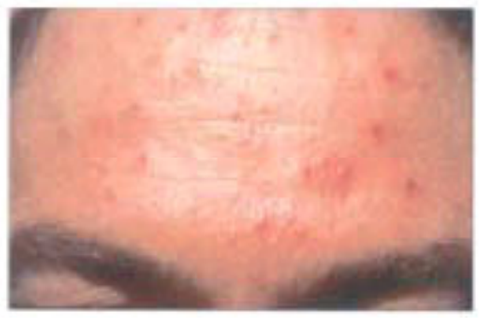


Figure 122-3 Penile ulcer during primary HIV infection.

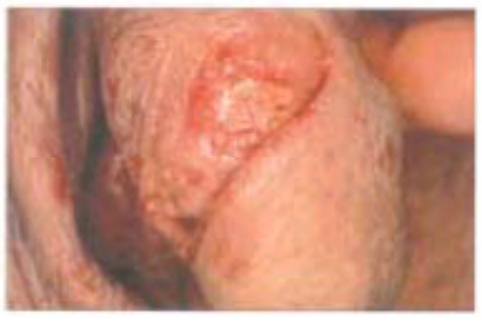


Figure 122-4 Mucosal ulcerations during primary HIV infection.

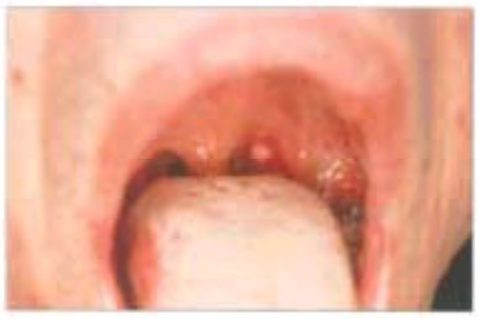


Figure 122-5 Successive Western blots during primary HIV infection. Note that on September 30, 1986, when the patient presented with fever, rash, meningitis and subclinical hepatitis, the screening enzyme-linked immunosorbent assay (ELISA) test for HIV antibodies was negative, while the Western blot showed only a single weak band corresponding to the p24 antigen. CSF, cerebrospinal fluid; ASAT, aspartate transaminase; ALAT, alanine transaminase; H, hepatitis (A or B); ND, not done.

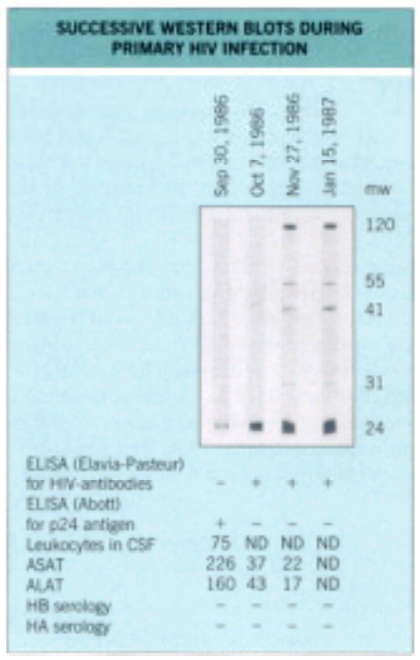


Figure 122-6 Changes in lymphocyte subpopulations and HIV viremia during primary HIV infection.

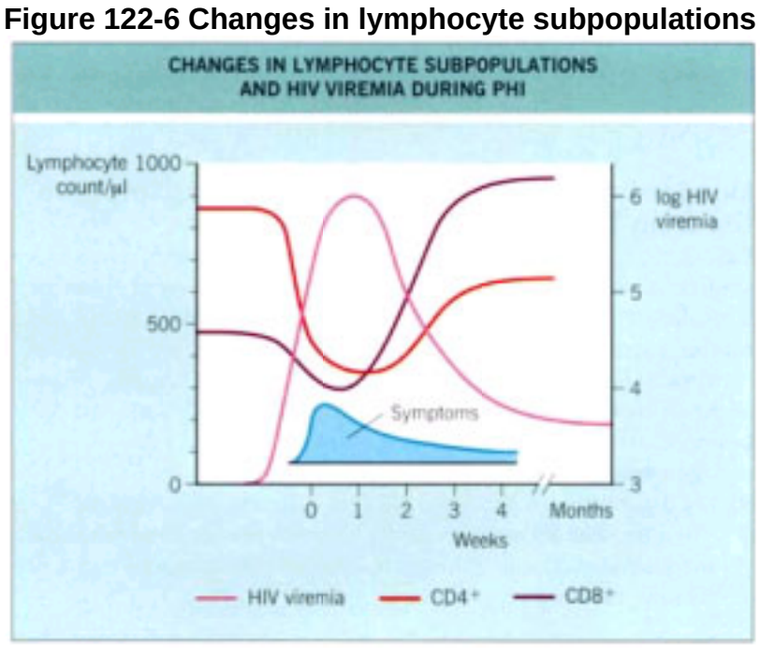


Figure 123-1 Incidence of opportunistic infections among patients who have HIV infection. CHU Saint-Pierre, 1985–2001.

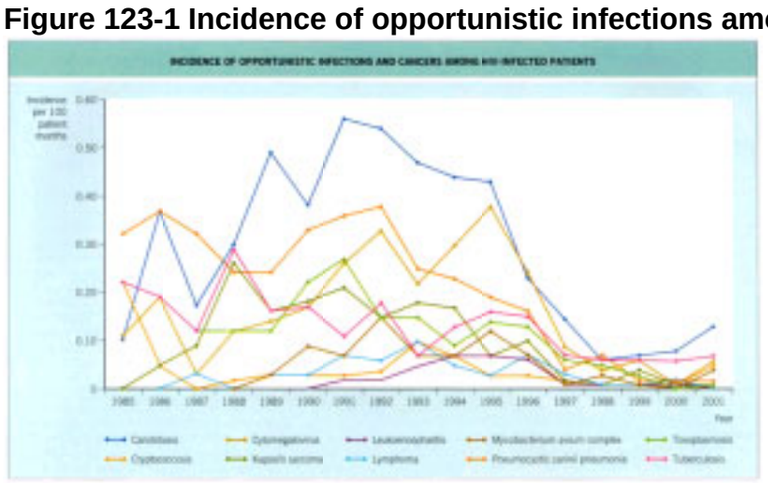


Figure 123-2 Association between opportunistic infections and CD4$^+$ cell count.

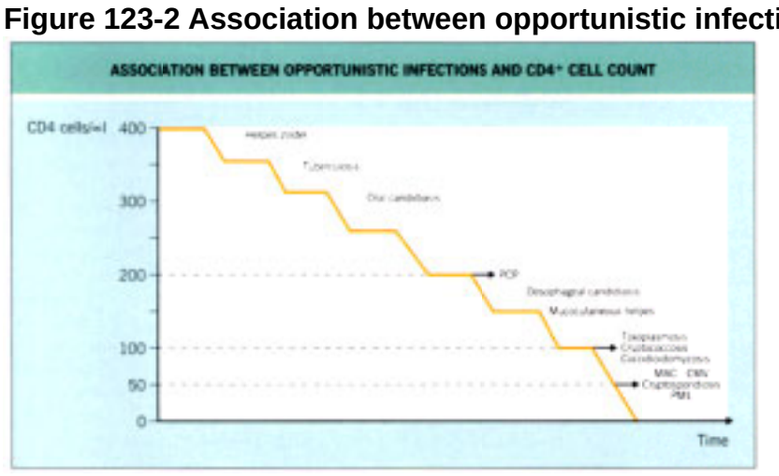


Figure 124-1 Mild *Pneumocystis carinii* pneumonia. There are bilateral micronodular lesions.

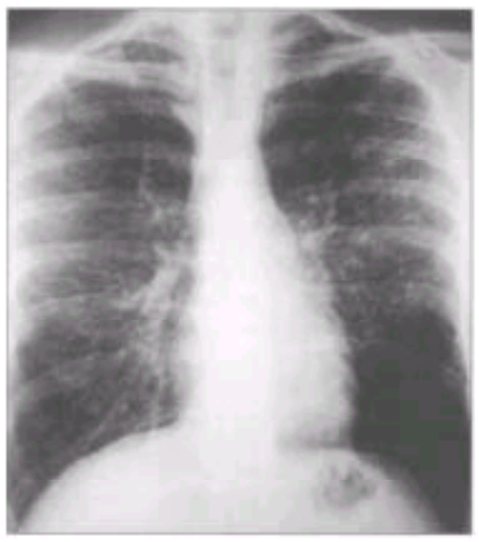


Figure 124-2 Severe *Pneumocystis carinii* pneumonia. This shows an extensive alveolar interstitial infiltrate with consolidation of the left lung and upper right lobe. *Courtesy of A Cabié.*

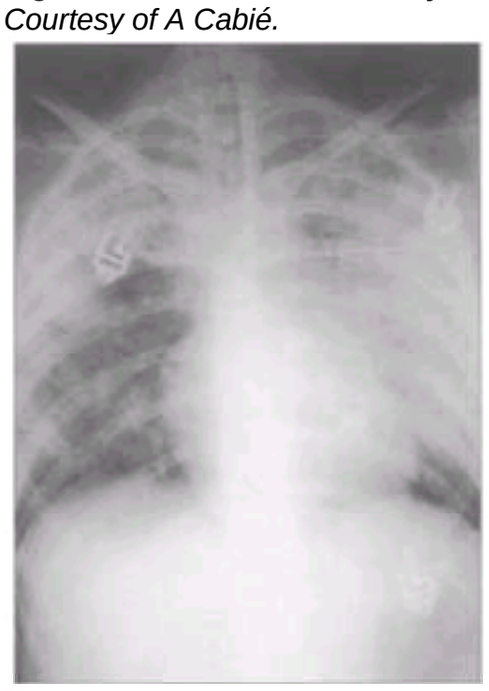


Figure 124-3 Six weeks after treatment of moderate *Pneumocystis carinii* pneumonia. Chest CT shows large, thin-walled bullae of the right lung.

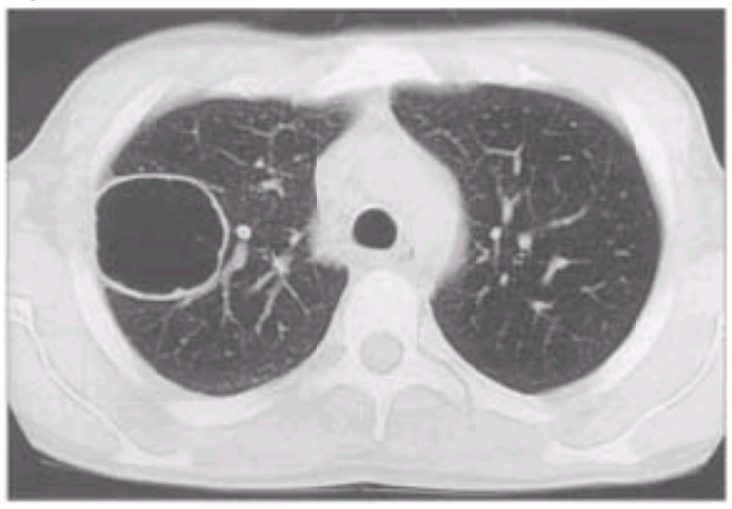


Figure 124-4 Splenic *Pneumocystis carinii* **pneumonia.** Abdominal CT shows multiple ring-enhancing abscess-like round formations. *Courtesy of C Bazin.*

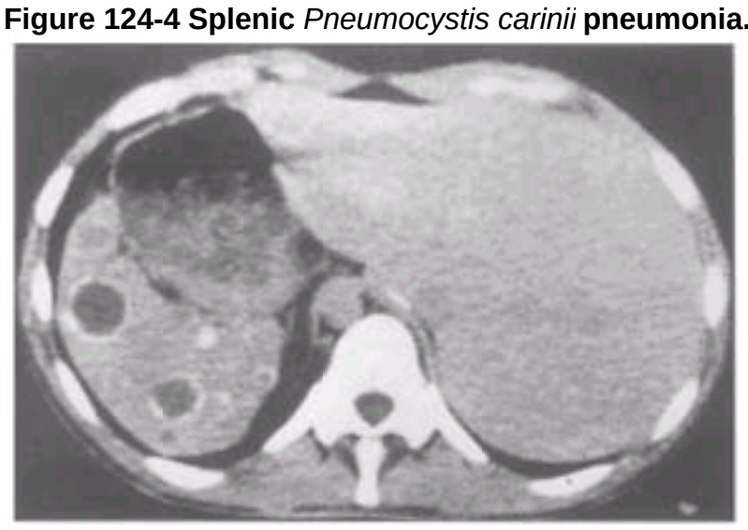


Figure 125-1 Cytomegalovirus retinitis, with characteristic perivascular hemorrhages and exudates.

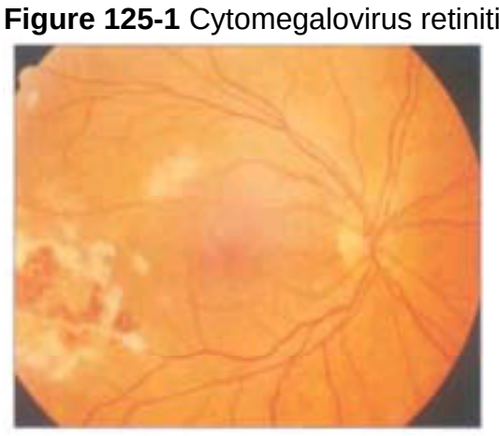


Figure 125-2 Severe perianal aciclovir-resistant herpes simplex virus 2 infection. (a) Untreated appearance. (b) Healing and re-epithelialization after treatment with foscarnet and institution of HAART.

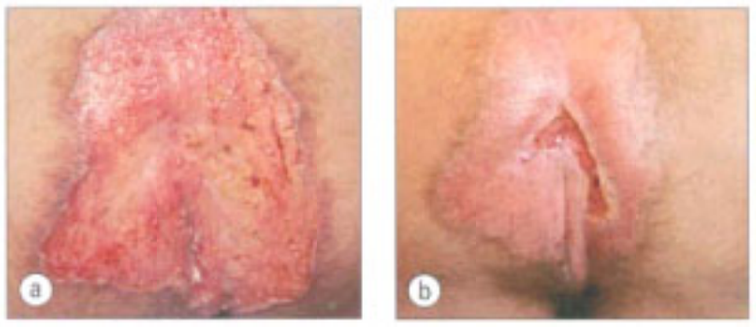


Figure 125-3 Herpes zoster in the T10 dermatome. *Courtesy of Professor Anthony J Pinching.*

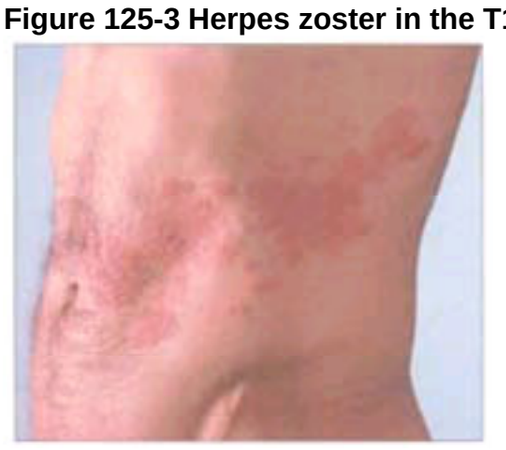


Figure 125-4 Progressive multifocal leukoencephalopathy. MRI scan showing frontal and occipital white matter lesions. *Courtesy of Dr Jane Anderson.*

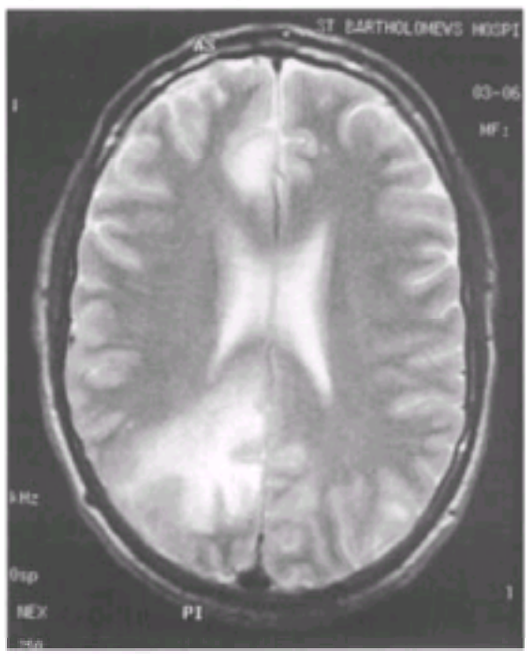


Figure 126-1 Pseudomembranous oral candidiasis ('thrush').

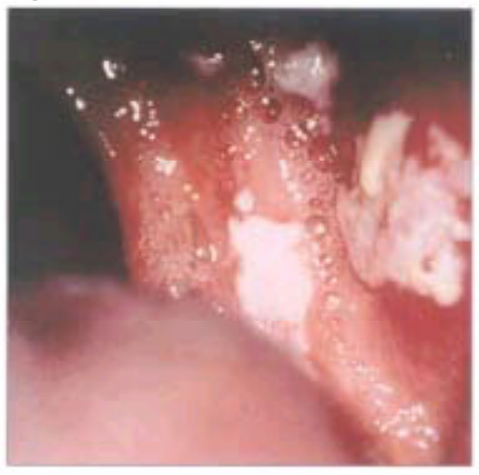


Figure 126-2 Cutaneous cryptococcosis. This lesion is typical of the skin lesions of most endemic mycoses that occur in patients who have AIDS, and such a lesion may therefore also be seen in an AIDS patient who has disseminated histoplasmosis or penicilliosis.

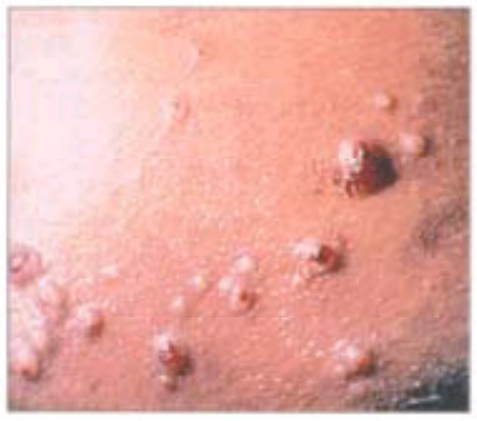


Figure 126-3 Algorithm for the diagnosis and treatment of cryptococcal meningitis.

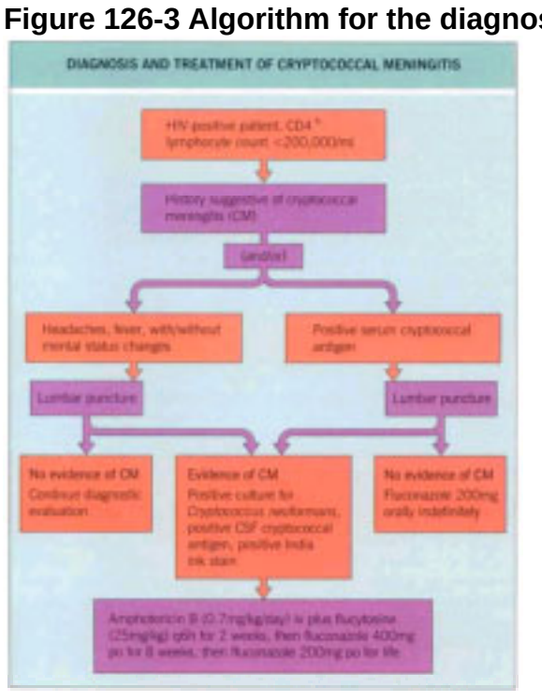


Figure 126-4 North American Endemic areas for histoplasmosis and coccidioidomycosis.

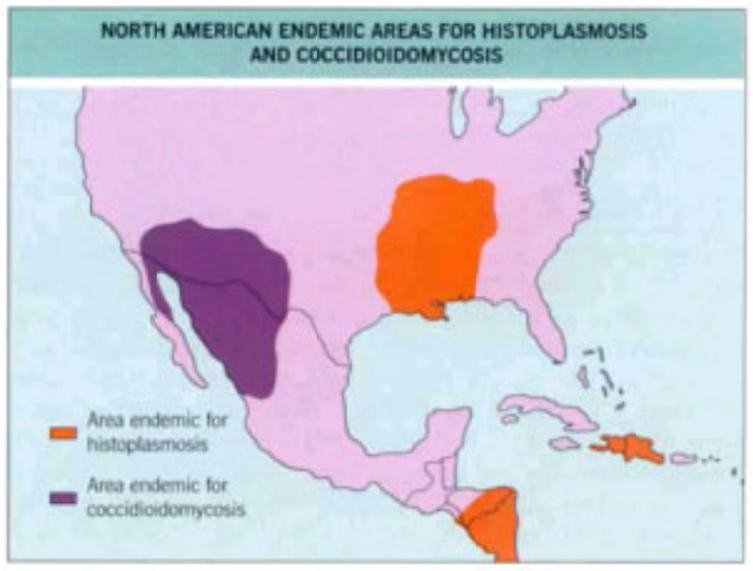


Figure 126-5 Endemic area for penicilliosis.

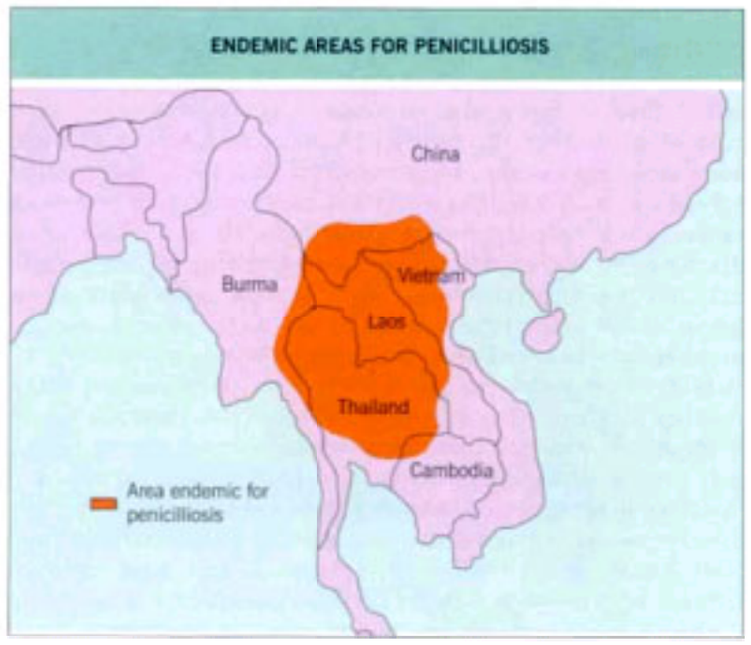


Figure 127-1 Diagnosis and management of cerebral toxoplasmosis in HIV-positive patients.

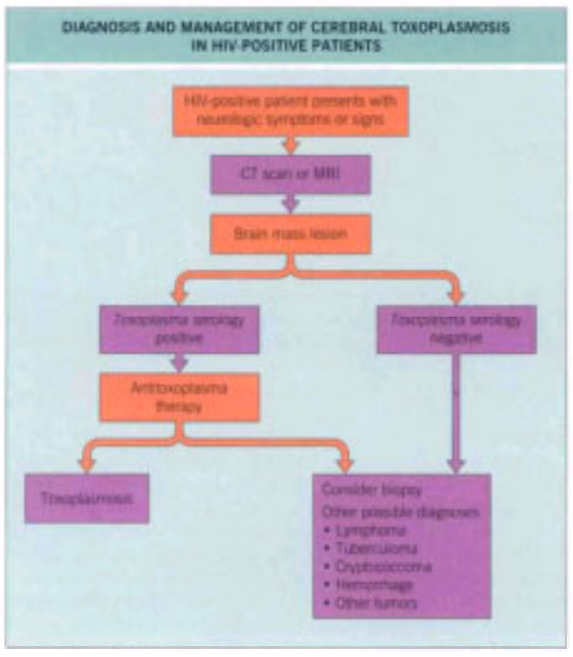


Figure 127-2 Toxoplasmic retinitis. (a) Diagnosis from fundoscopy. (b) Evolution after antitoxoplasma therapy.

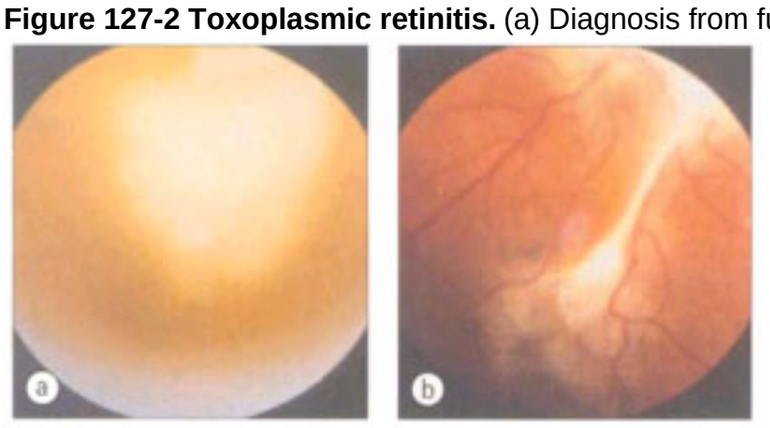


Figure 127-3 Toxoplasmic encephalitis. (a) CT scan at diagnosis. (b) Evolution of the disease after 42 days of treatment with pyrimethamine-sulfadiazine.

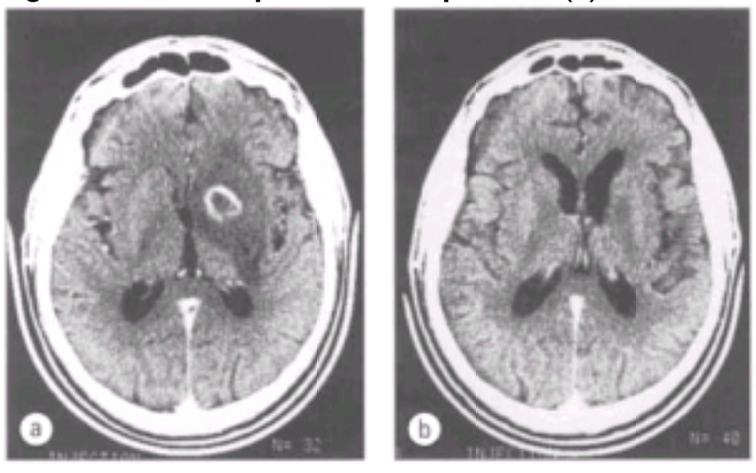


Figure 128-1 Community-acquired pneumonia in former drug users. Kaplan-Meier estimates of the cumulative probability of community-acquired pneumonia in HIV-negative and HIV-positive former drug users. *Adapted from Boschini et al.*[3]

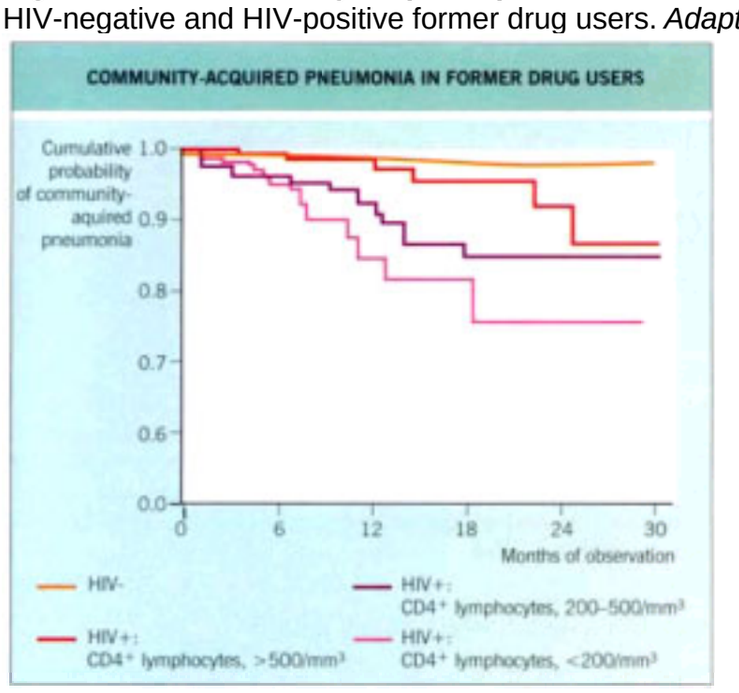


Figure 128-2 Percentage of patients responding to pneumococcal polysaccharide vaccine antigens. *Adapted from Rodriquez-Barradas et al.*[20]

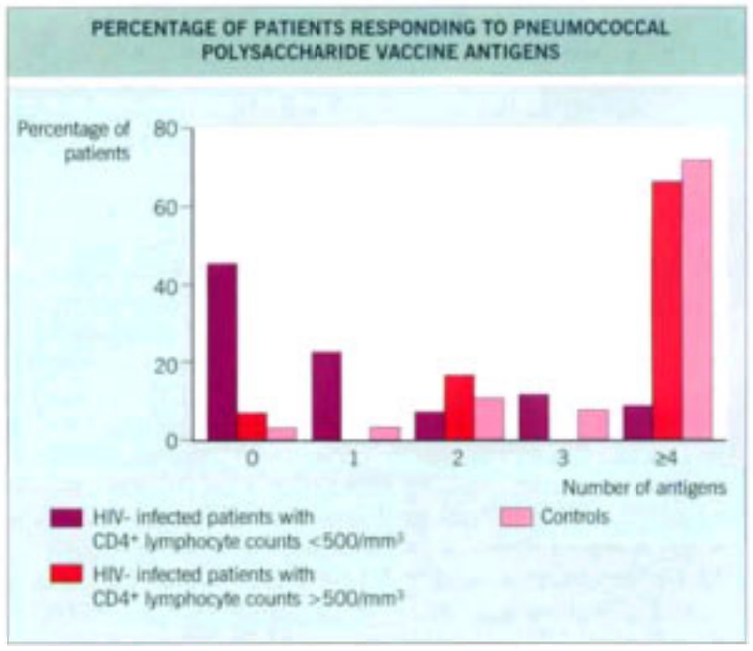


Figure 128-3 Typical appearance of bacillary angiomatosis. *Courtesy of Ciro Martins, MD.*

Figure 128-4 Rates of bacterial pneumonia in in HIV-positive and HIV-negative patients. The rates are shown by baseline $CD4^+$ lymphocyte count. *Data from Hirschtick et al.*[4]

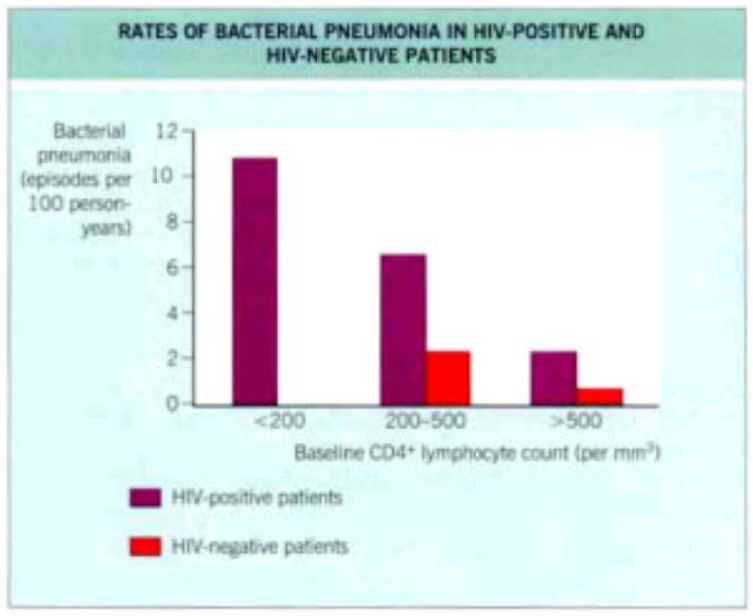


Figure 128-5 Months between diagnosis of pneumococcal bacteremia and diagnosis of AIDS. The data relate to 37 patients as reported by Redd *et al.*[53]

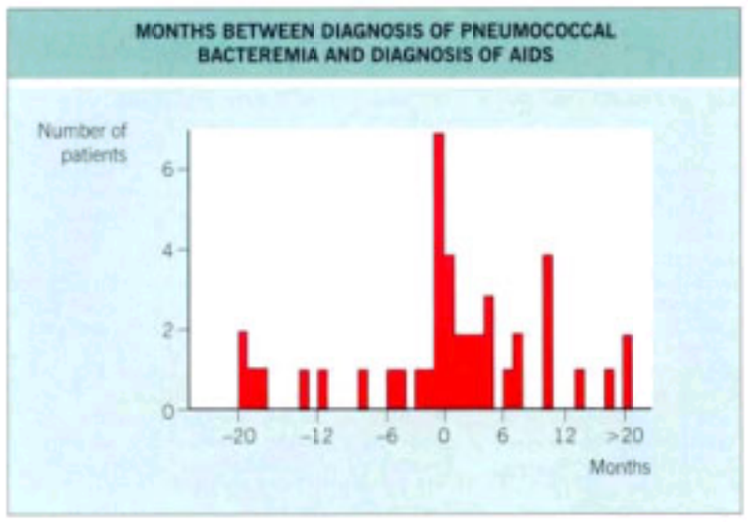


Figure 129-1 Estimated global distribution of adults co-infected with HIV and *Mycobacterium tuberculosis*, to mid-1993. *From the World Health Organization Tuberculosis Program. Redrawn with permission from Snider et al.*[2]

Figure 129-2 Tuberculosis and *Mycobacterium avium* infection after introduction of highly active antiretroviral therapy. Incidence among HIV-positive patients. Between 1995 and 1997 the use of HAART became general practice in Europe. *Redrawn with permission from Kirk et al.*[5]

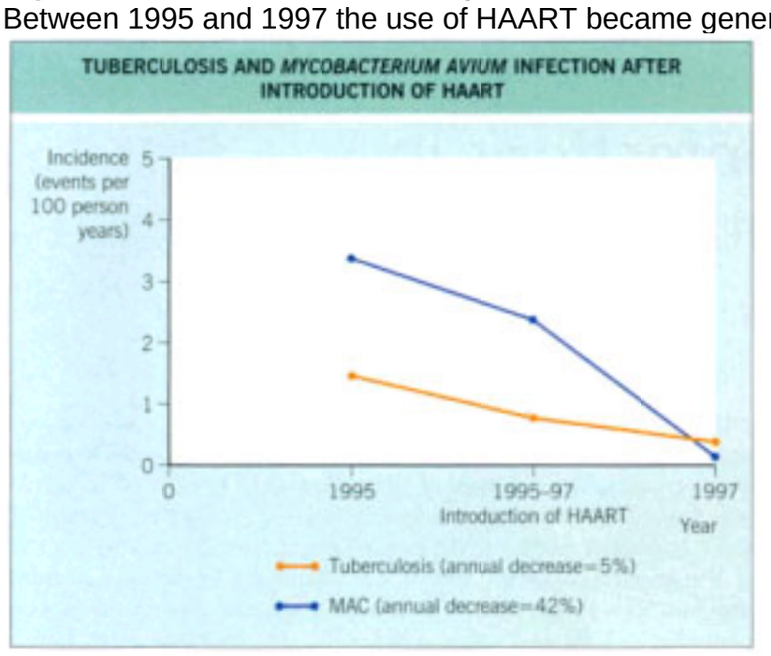


Figure 129-3 Tuberculosis incidence in recipients of highly active antiretroviral therapy, Hospital Clinic of Barcelona. (a) The incidence rate per 100 HIV-infected patients per year, separated into three different groups by $CD4^+$ lymphocyte level. (b) Tuberculosis incidence, 2000, expressed per 100,000 persons per year. It is notable that the incidence of tuberculosis is higher in HIV patients in all $CD4^+$ lymphocyte strata, as compared with the general population.

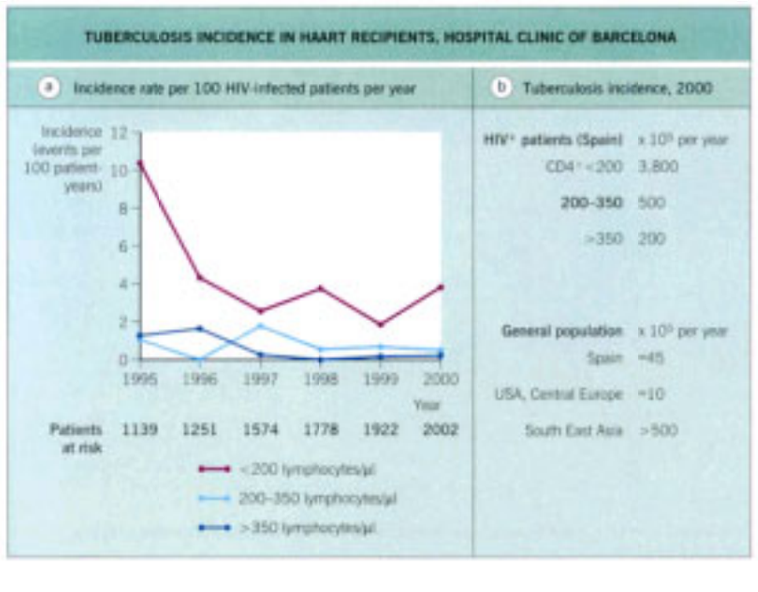


Figure 129-4 CD4$^+$ count and opportunistic infection. Relation between CD4$^+$ lymphocyte count and the main opportunistic infections in HIV-infected patients. *Data from Hospital Clinic AIDS unit, Barcelona. CAN, esophageal candidiasis; CMV, cytomegalovirus infection; LEIS, disseminated leishmaniasis; MAC, Mycobacterium avium complex infection; PCP, Pneumocystis carinii pneumonia; TBE, extrapulmonary tuberculosis; TBP, pulmonary tuberculosis; TOXO, central nervous system toxoplasmosis. Redrawn with permission from Miro et al.*[7]

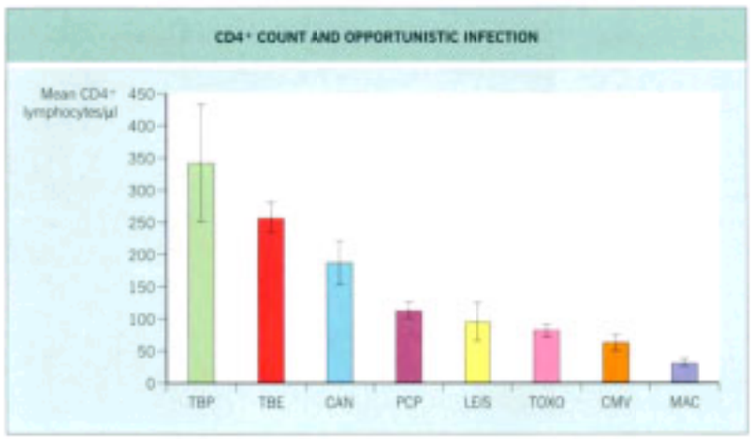


Figure 129-5 Influence of HIV infection on tuberculosis epidemiology and strategies against spread. HIV^+ , HIV-infected patients; ICH, immunocompetent host; PPD, purified protein derivative; SP, smear-positive.

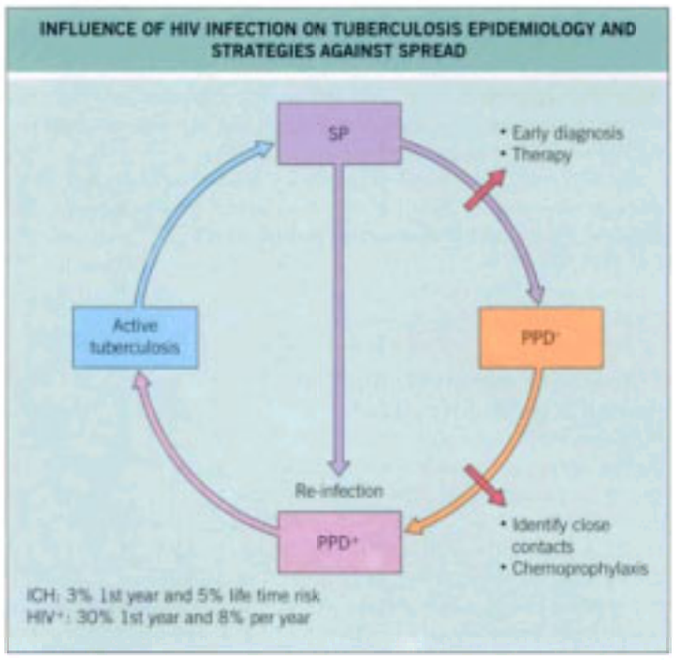


Figure 129-6 Characteristic upper lobe cavity on chest radiograph in an HIV patient who has tuberculosis.

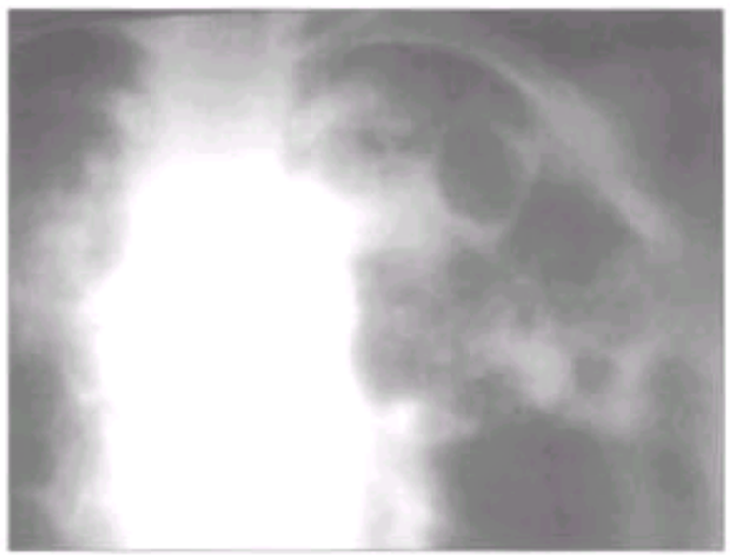


Figure 129-7 Laterocervical adenopathy in a HIV-infected patient. The needle aspiration demonstrated abundant AFB.

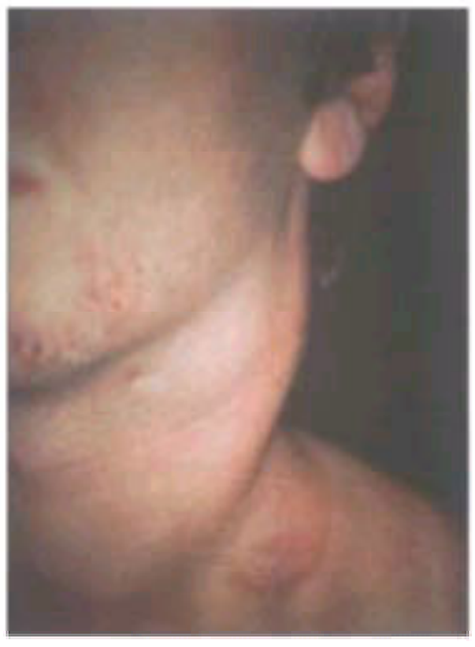


Figure 129-8 Abdominal CT scan of an HIV-infected patient who has tuberculosis. Multiple retroperitoneal lymph nodes (arrow) are typical findings.

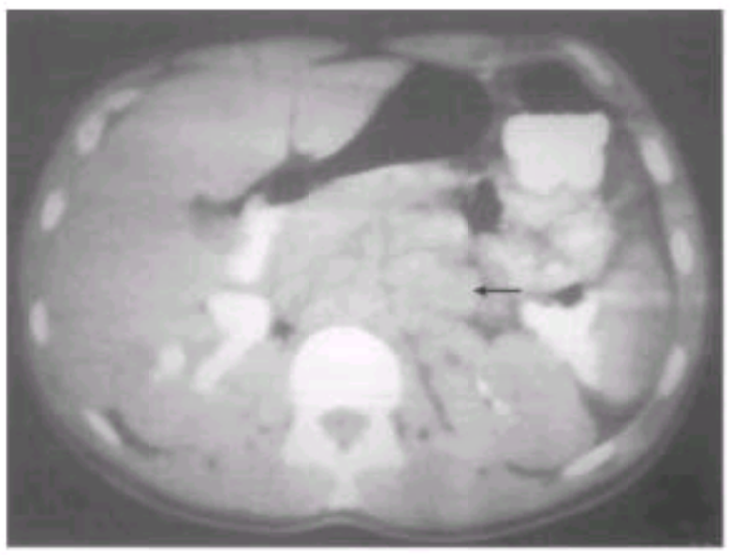


Figure 129-9 Chest radiograph of HIV-infected patient who has miliary tuberculosis.

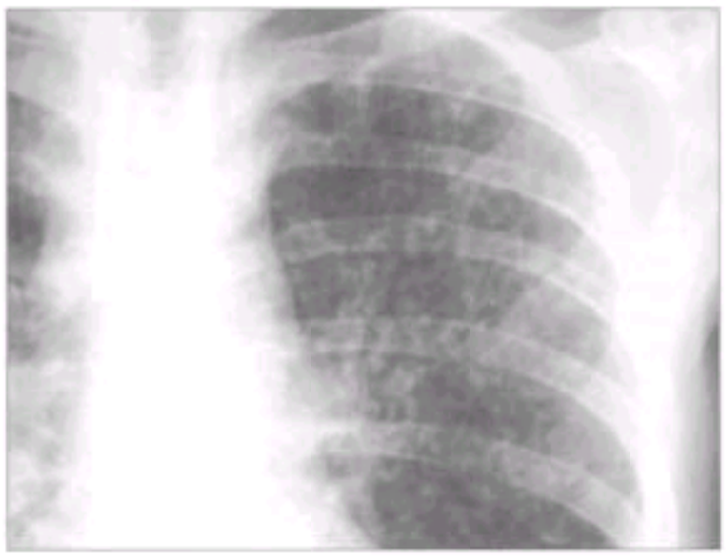


Figure 129-10 Multiple cerebral cortical densities (tuberculomas, arrows) on CT scan of a patient who has tuberculosis of the central nervous system.

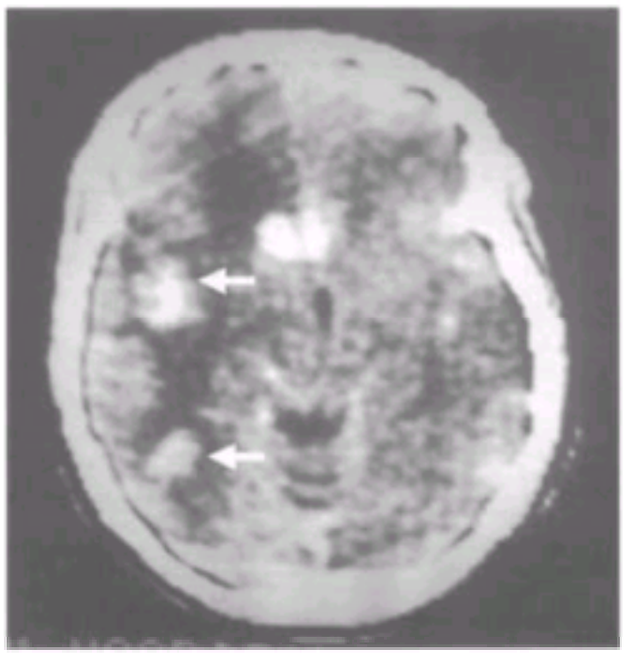


Figure 129-11 Management strategies for patients who have HIV infection and tuberculosis. EFV, efavirenz; NRTI, nucleoside reverse transcriptase inhibitor; RBT, rifabutin; RIF, rifampin.

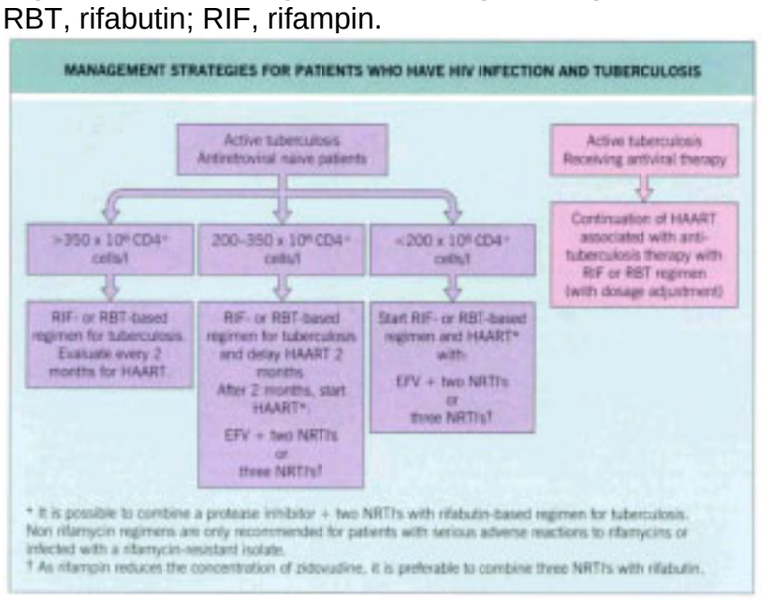


Figure 130-1 Kaposi's sarcoma. There are large confluent hyperpigmented patch-stage lesions with lymphedema.

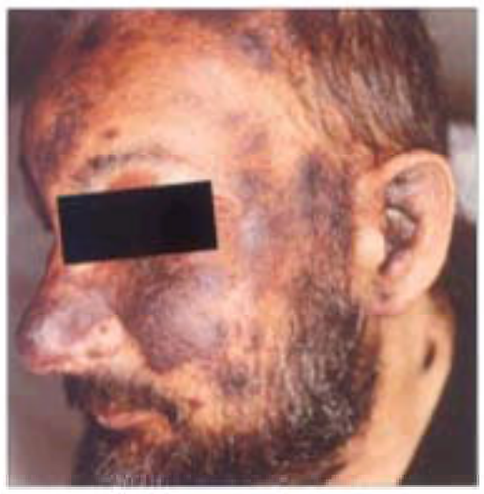


Figure 130-2 Non-Hodgkin's lymphoma. Bulky disease in the gingiva.

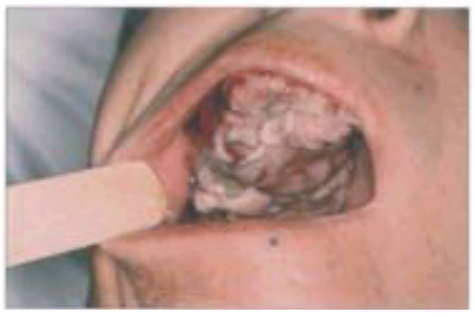


Figure 131-1 Weight loss associated with opportunistic infections. Weight chart of one patient who had episodes of rapid weight loss and partial recovery coinciding with episodes of acute opportunistic infection — *Pneumocystis carinii* pneumonia (PCP) and cytomegalovirus (CMV) — and one patient who had chronic progressive weight loss associated with cryptosporidial diarrhea. Reproduced with permission by the American Journal of Clinical Nutrition © Am L Clin Nutr. American Society for Clinical Nutrition.

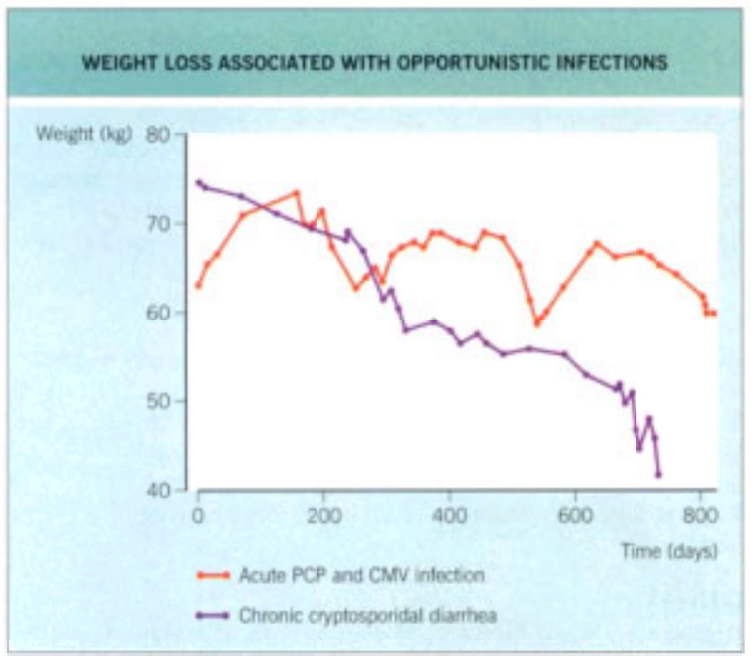


Figure 132-1 Acute exanthem of HIV infection. There is morbilliform eruption involving the trunk and extremities. The eruption is similar to a morbilliform drug eruption and to other viral exanthemata.

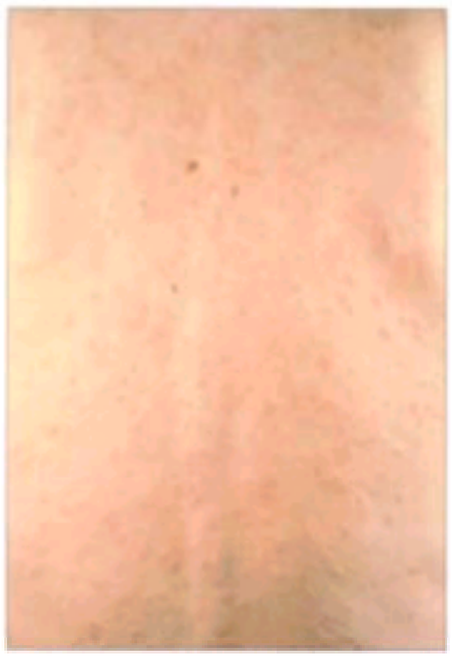


Figure 132-2 Herpes zoster. A painful linear-zosteriform eruption of vesicles on an erythematous base is characteristic of herpes zoster. The eruption may be persistent and verrucous lesions are not uncommon.

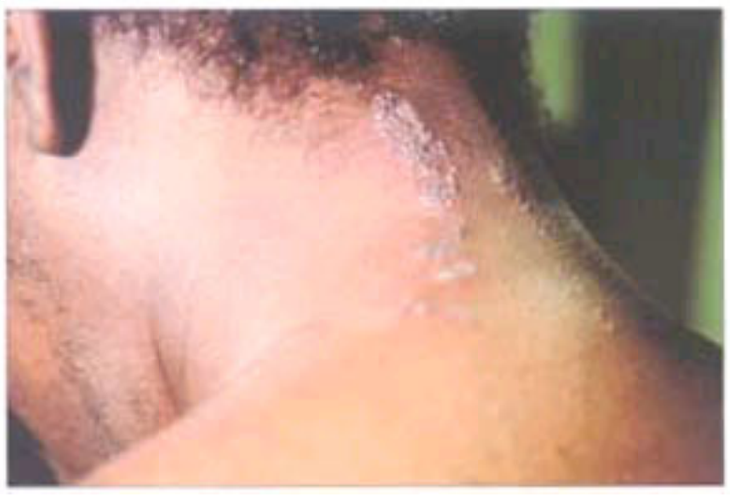


Figure 132-3 Human papillomavirus infection. Human papillomavirus infections are common in HIV-infected patients. They may have unusual features, as demonstrated here, and may be refractory to therapy.

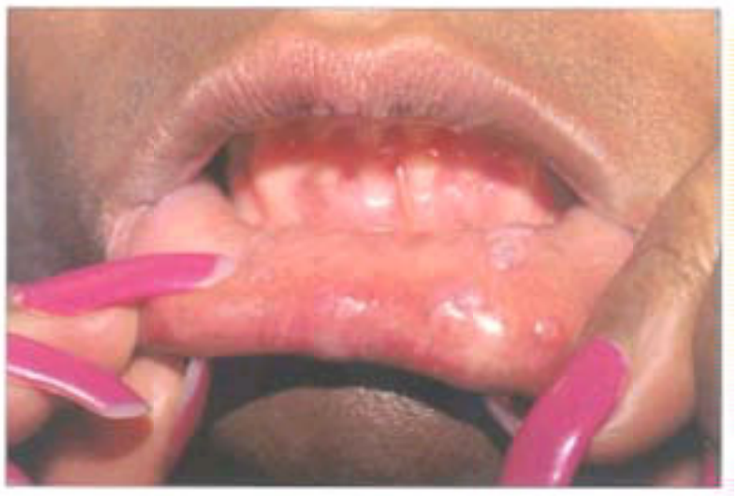


Figure 132-4 Molluscum contagiosum. These lesions are characteristically translucent, waxy papules with central umbilication.

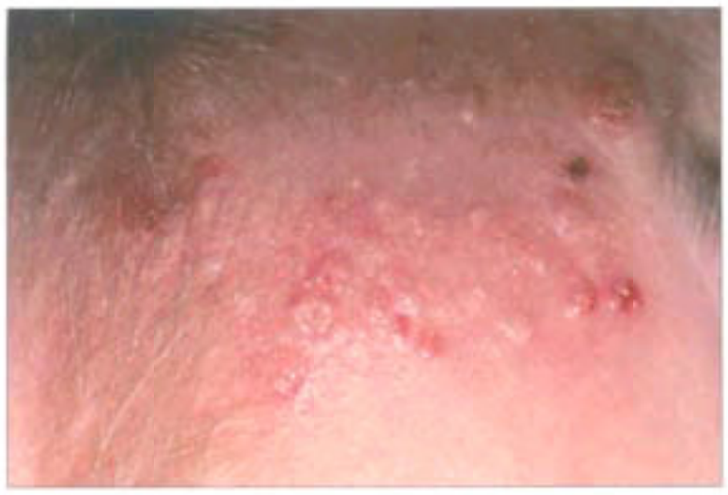


Figure 132-5 Bacillary angiomatosis. There are elevated vascular papules of the glabrous skin. When incised, these lesions bleed profusely.

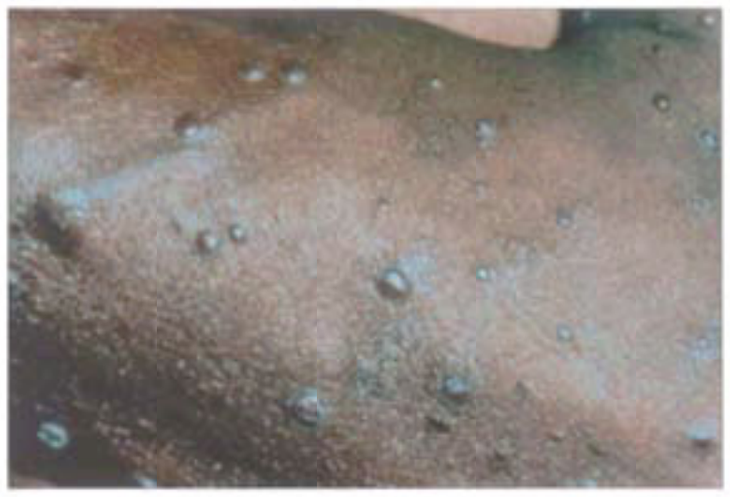


Figure 132-6 Cutaneous histoplasmosis. These lesions are characteristically quite nondescript and may simulate other infectious disorders and verrucous neoplastic conditions.

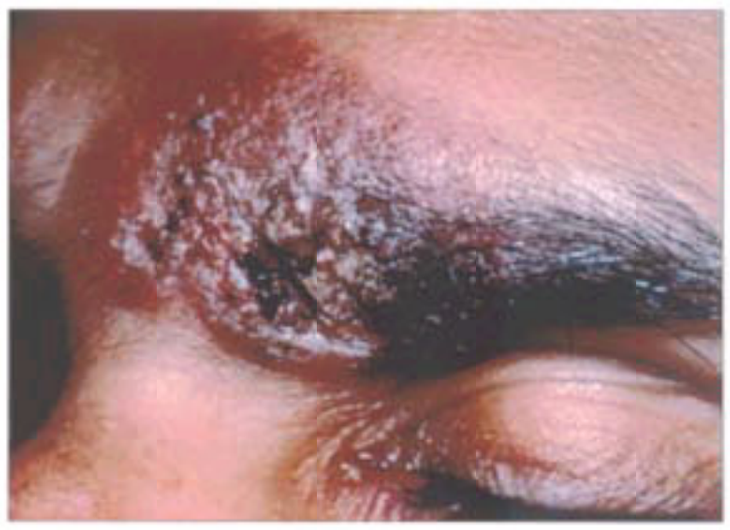


Figure 132-7 Seborrheic dermatitis. Note the characteristic greasy scale and the erythematous plaques involving the face, especially the nasolabial folds and the eyebrows.

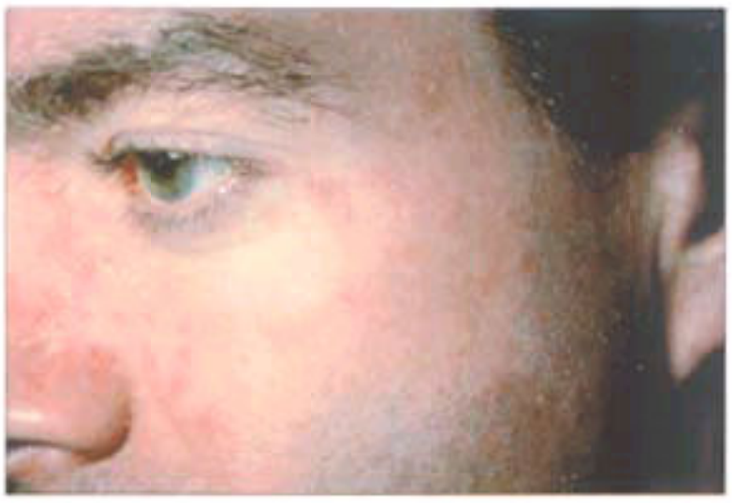


Figure 132-8 Eosinophilic folliculitis. There are follicular papules, many of which have been excoriated, involving the upper trunk and the face. Histologically, numerous eosinophils are present within the follicular ostia.

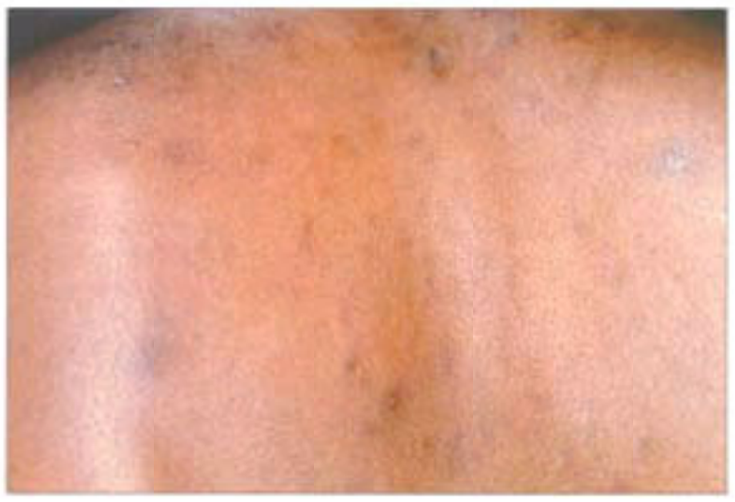


Figure 132-9 Kaposi's sarcoma, plaque stage. There is an erythematous plaque, which is linear in shape, arranged along skin cleavage lines.

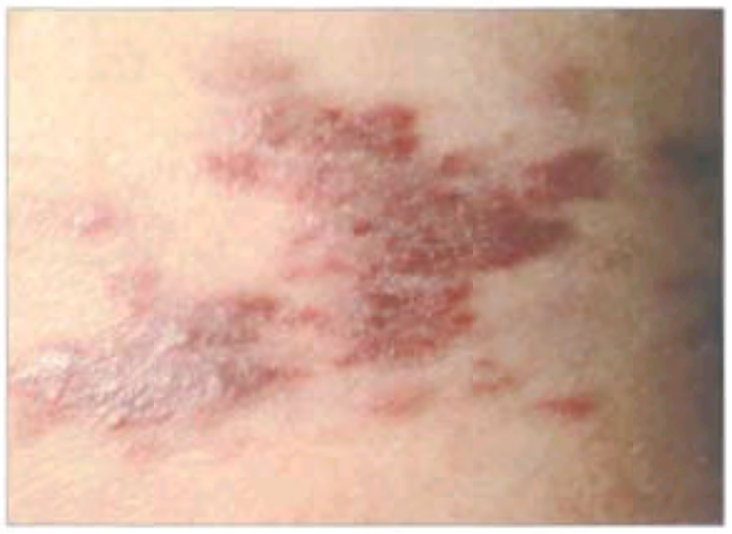


Figure 132-10 Morbilliform drug eruption. There is a diffuse eruption of fine pink macules and papules, which have coalesced, involving the trunk and the extremities. The most common cause of these eruptions is trimethoprim-sulfamethoxazole.

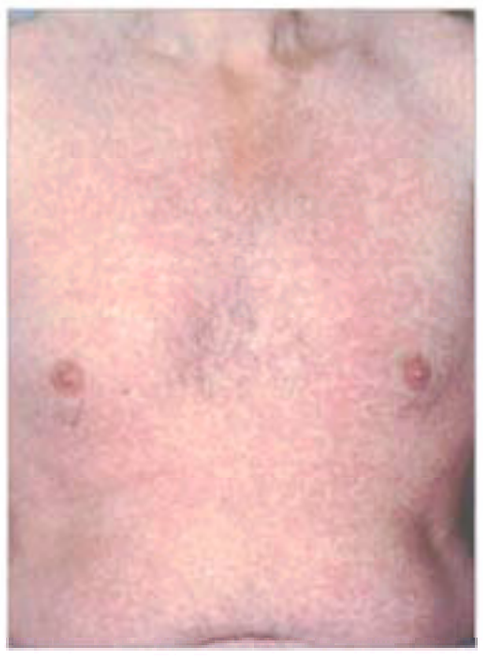


Figure 133-1 Annual AIDS deaths since 1982. Deaths in the USA are contrasted with those in sub-Saharan Africa.

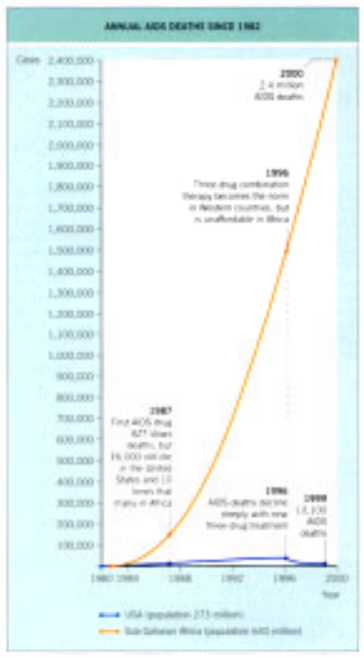


Figure 133-2 Risk of death from AIDS in developing countries. Lifetime risk of AIDS death among 15-year-old boys, assuming unchanged or halved risk of becoming infected with HIV, in selected countries.

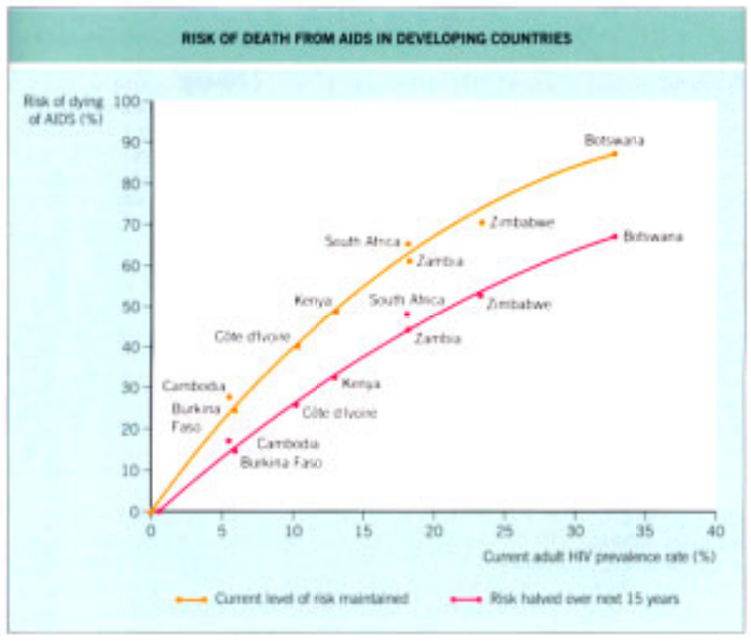


Figure 137-1 Positive HIV-1 Western blot. The binding of the patient's antibodies to viral antigens coated on the strip is revealed by an enzyme-labeled antihuman globulin. gp160, gp120 and gp41 are *env* gene products. p55, p24 and p17 are *gag* gene products. p68, p52 and p34 are *pol* gene products. MW, molecular weight of the viral proteins.

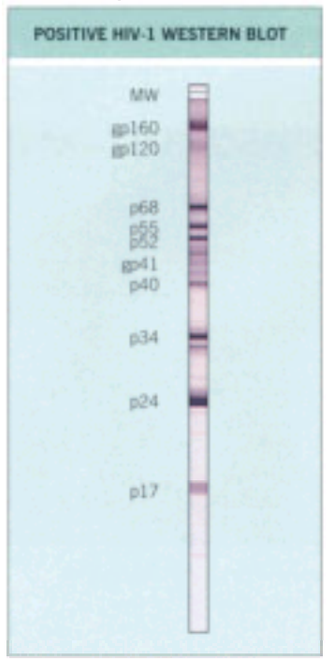


Figure 137-2 Syncytia formed by infection of MT-2 cells with an X4 (syncytium-inducing) isolate of HIV-1.

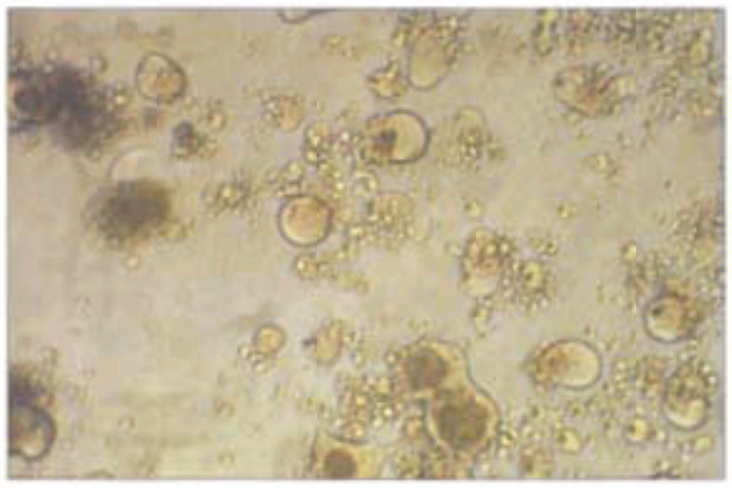


Figure 137-3 Relationship between co-receptor usage and cellular tropism of HIV-1.

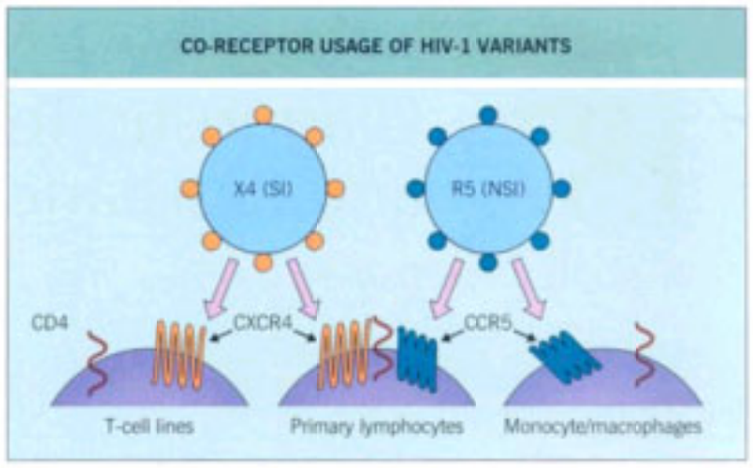


Figure 137-4 HIV-1 drug resistance assay. RT, reverse transcription reaction; cDNA, complementary DNA; PR-RT, protease and reverse transcriptase gene; IC_{50} , 50% inhibitory concentration.

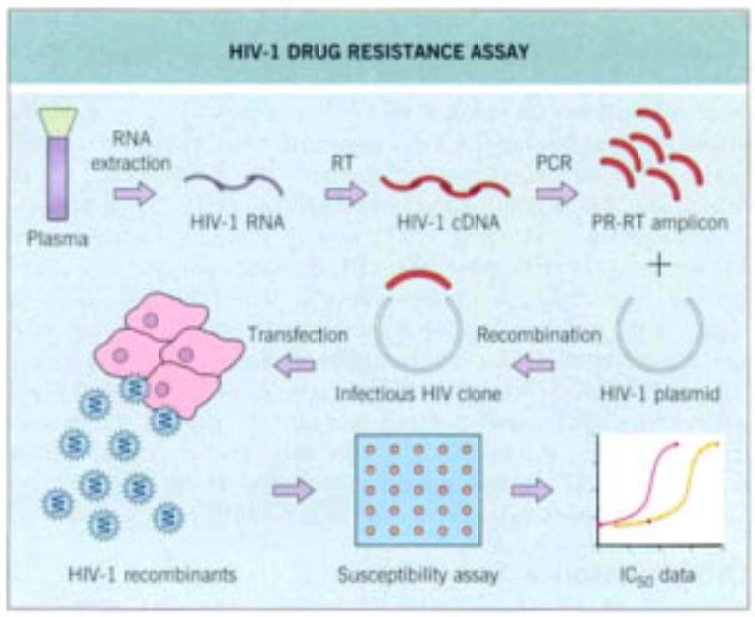


Figure 137-5 Kinetics of viral markers during primary HIV-1 infection. The first positive viral marker is plasma RNA 11–12 days after infection. p24 Antigenemia is detectable on day 14 or 15. The first anti-HIV antibodies are detectable by third-generation ELISAs on days 20–21. (Pink, plasma HIV RNA; purple, p24 antigenemia; blue, anti-HIV antibody).

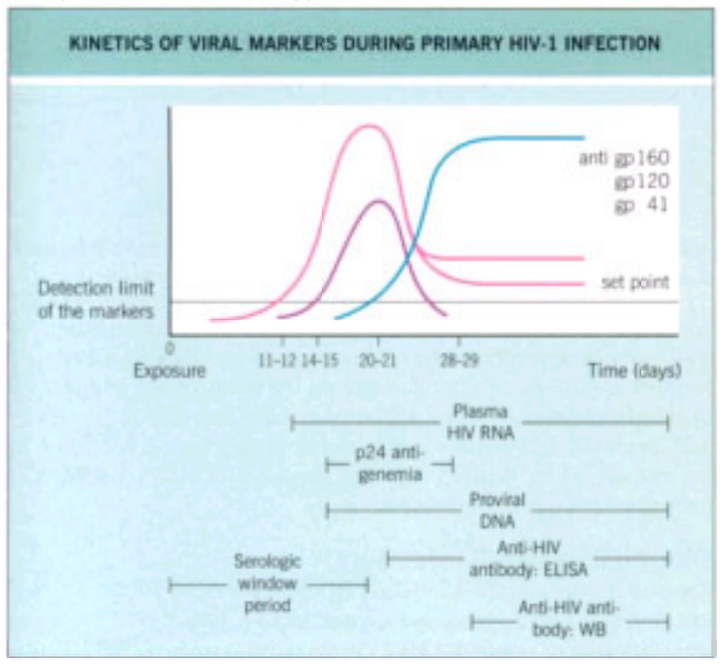


Figure 137-6 Western blot reactivity in one HIV-1 seroconverter. Lanes 1 and 2, negative (NC) and positive controls (PC). Lanes 3–10, serial samples collected on days (D) 0, 2, 3, 5, 7, 12, 22 and 30. Day 0 corresponds to the first collected sample. Anti-p24 was the first antibody detected, rapidly followed by anti-gp160, p55, p40 and gp120. Later, gp41 and p18 are weakly reactive.

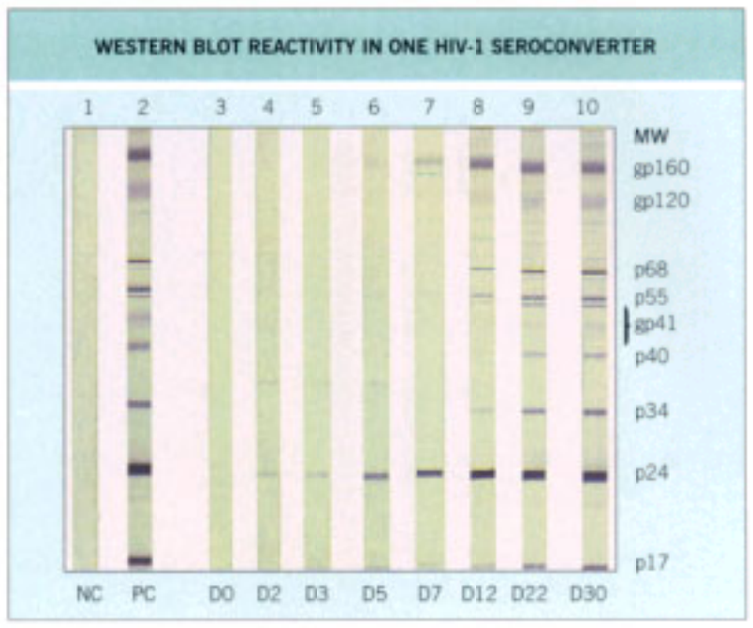


Figure 137-7 Different Western blot patterns of nine samples. Samples 1, 2 and 3 are from recent seroconverters infected by HIV-1 subtype B. Western blot is weakly reactive on gp41 and *pol* products. Patient 4 has a slight decrease in *gag* products by WB. Patients 5 and 6 are fully WB reactive. Patient 7 is a recent seroconverter infected by HIV-1 subtype A. gp120 and gp41 are weakly reactive. Patient 8 is infected with HIV-1 group O. The WB pattern is highly suggestive of infection by a variant, with no *env* reactivity and strong *pol* reactivity. Patient 9 is in the terminal phase of AIDS, with disappearance of *gag* reactivity.

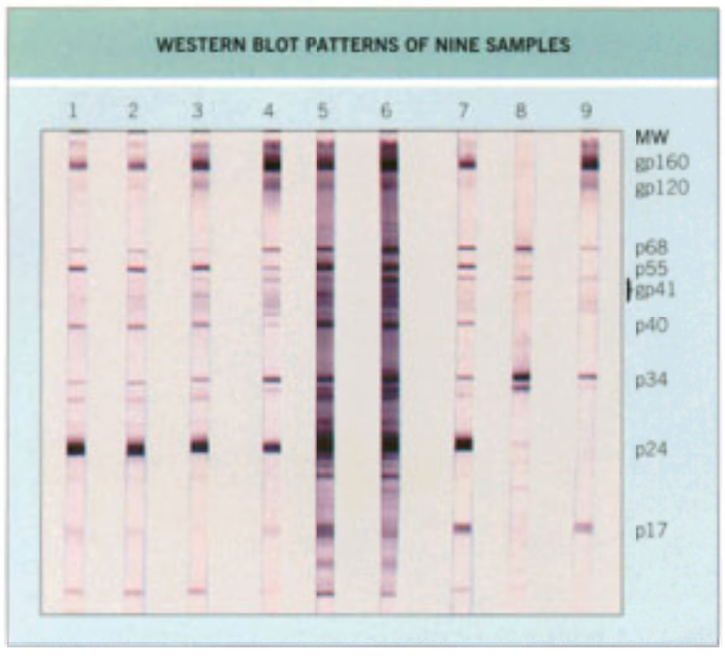


Figure 137-8 Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance. Wild-type amino acids are shown above the bar, and mutant amino acids are shown below the bar. Numbers indicate amino acid position. Vertical bars indicate cross-resistance. Bold-face indicates major protease inhibitor resistance mutations. Complete explanation of figure and footnotes available at www.iasusa.org. *From D'Aquila et al.*[28] *, reprinted with permission.*

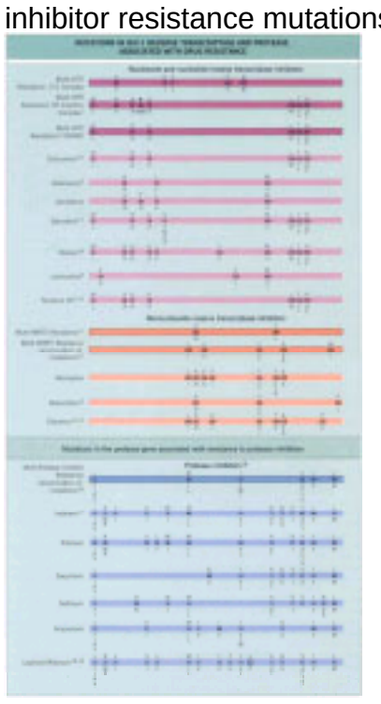


Figure 137-9 Relationship of trough drug concentration (C_{min}) to the 50% inhibitory concentration (IC_{50}) required for viral inhibition. IQ, inhibitory quotient (C_{min} :IC_{50}).

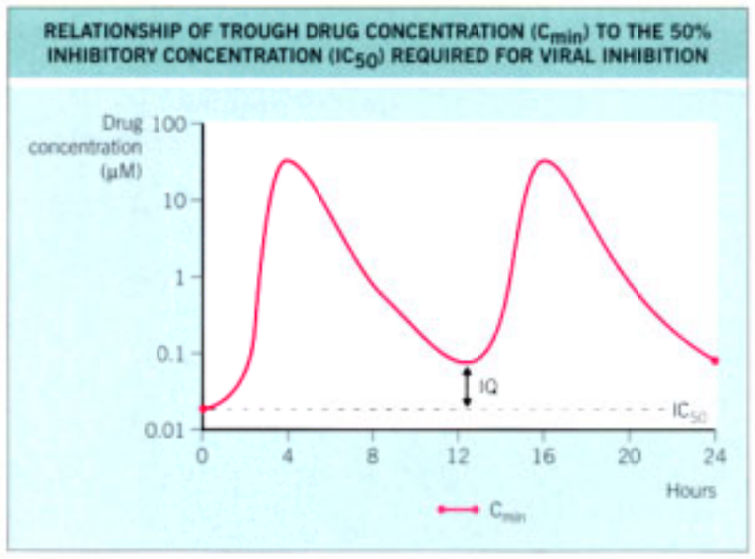


Figure 138-1 Prognostic contribution of viral load determinations at different CD4$^+$ levels. For a given CD4$^+$ lymphocyte count, individuals with high viral load will have a more rapid decline in CD4$^+$ count and therefore a worse clinical prognosis (demonstrated by the downward shift of the survival curve). The viral load is an independent predictor of rapidity of disease progression. A more aggressive virus phenotype — syncytium-inducing versus non-syncytium-inducing — may also adversely affect prognosis, as may viral strains that are resistant to antiretroviral medications.

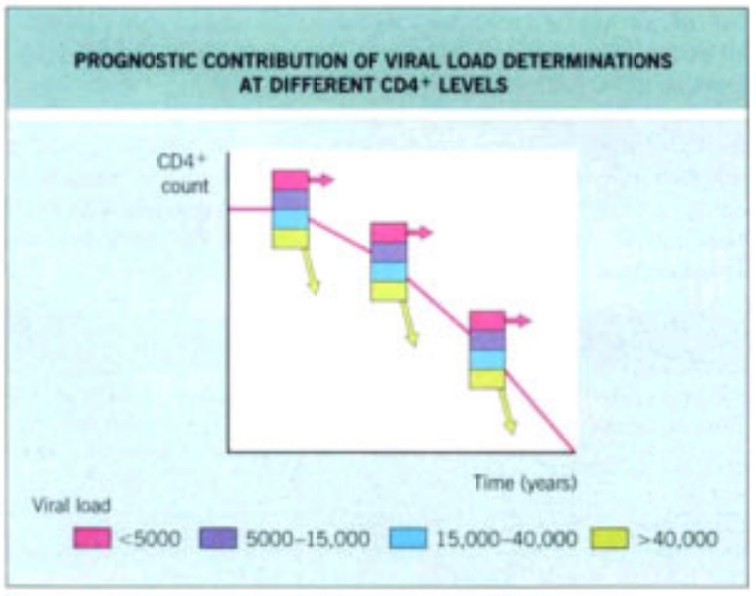


Figure 138-2 Approach to antiretroviral therapy. PI, protease inhibitors.

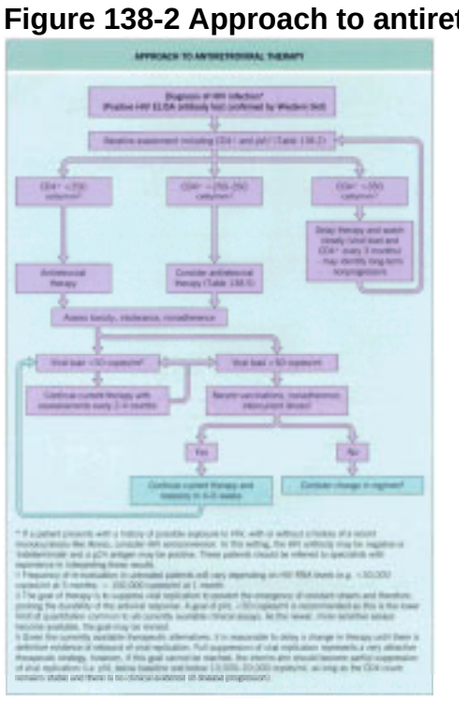


Figure 139-1 Possible sites of intervention in the inhibition of HIV replication.

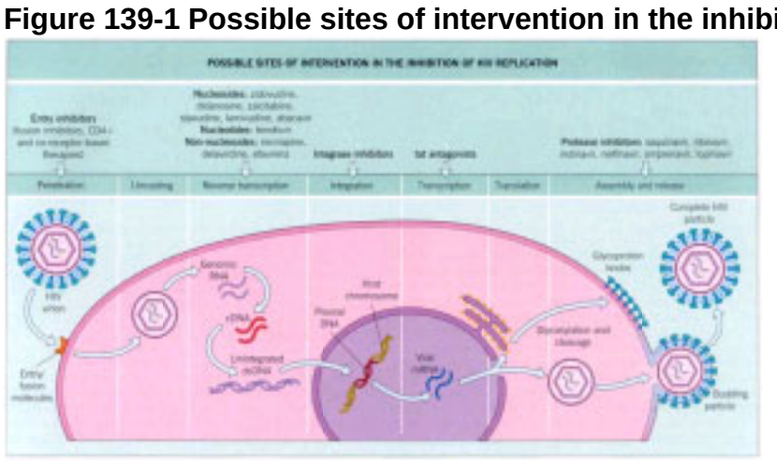


Figure 139-2 Evolution of changes in HIV RNA plasma levels obtained with the use of different antiretroviral regimens.

Figure 141.a-1 A way to explain troughs, mutants and staunch compliance. Relationship between lack of patient adherence to treatment, insufficient drug levels and emergence of resistance. *Adapted with permission from Mascolini M. The drugs we've got ... the drugs we're getting ... beyond blood: a three part look at the Fourth Conference on Retroviruses and Opportunistic Infections. J Int Assoc Physicians AIDS Care 1997;3:30.*

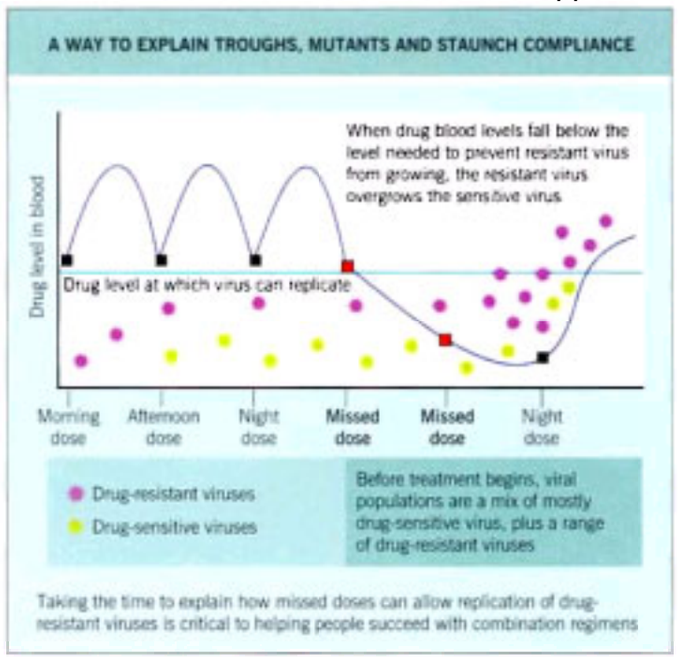


Figure 141.c-1 Involvement of putative liver lesions in HIV-HCV co-infection.

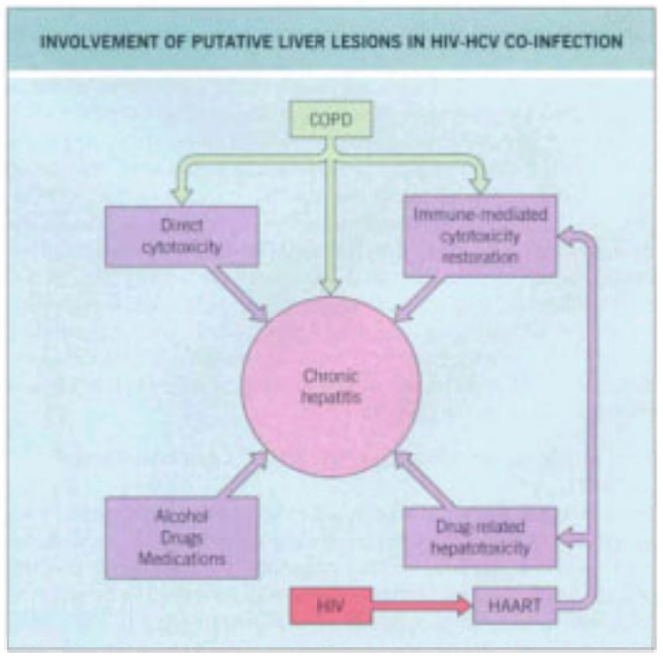


Figure 141.c-2 Therapeutic options in HIV-HCV co-infected patients.

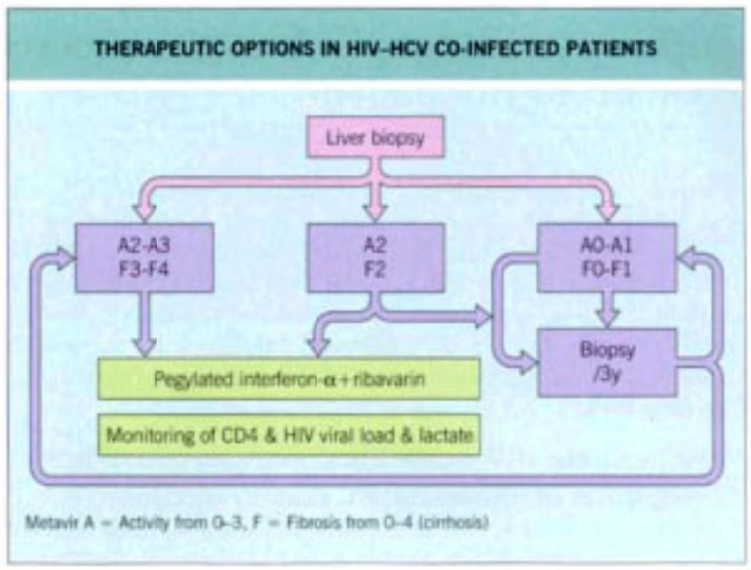


Figure 142-1 Life cycles of some important pathogens.

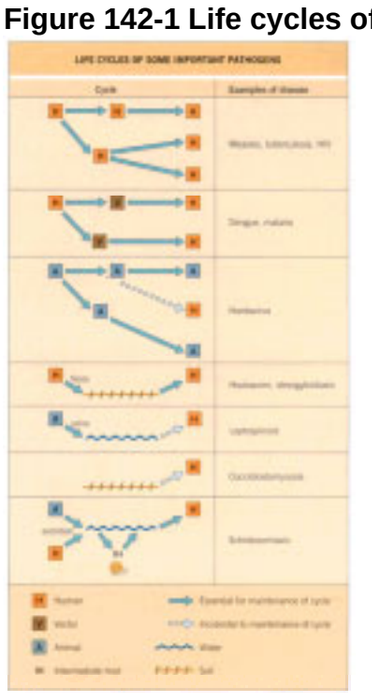


Figure 142-2 Areas of the USA thought to be endemic for malaria during the years 1882–1912.

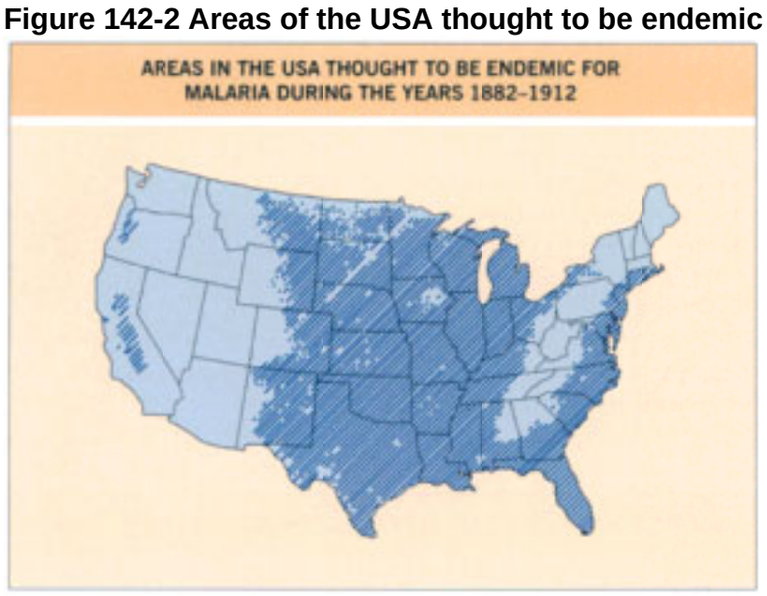


Figure 142-3 Worldwide distribution of schistosomiasis.

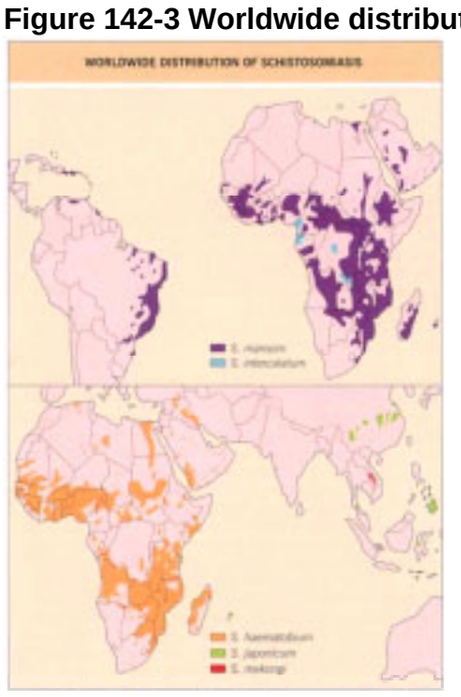


Figure 142-4 Rates of tuberculosis in England and Wales by crowding index (1992). *Adapted from Bhatti et al.*[14]

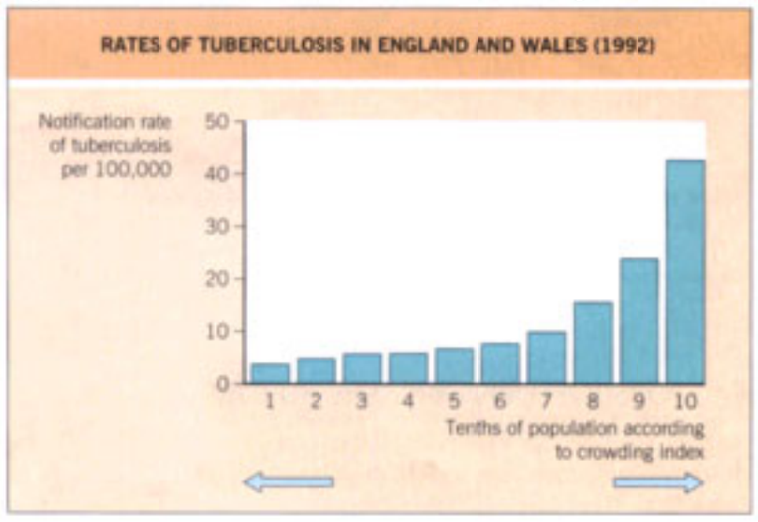


Figure 142-5 Meningitis belt in Africa.

Figure 142-6 Areas reporting dengue fever and areas with a competent vector (2002). Many areas with a competent vector do not report dengue epidemic activity. *Data from the Centers for Disease Control and Prevention.*[16]

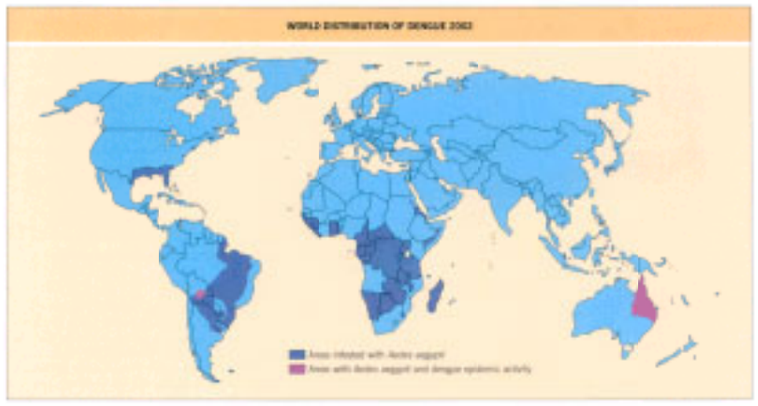


Figure 142-7 Worldwide distribution of malaria (2001). *Data from the Centers for Disease Control and Prevention.* [16]

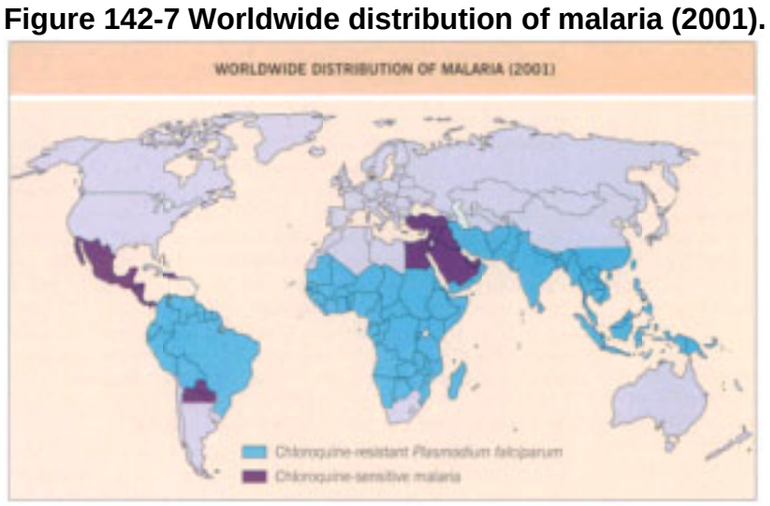


Figure 143-1 Estimated monthly incidence of health problems for travelers to tropical areas. *Adapted from reference* [18] .

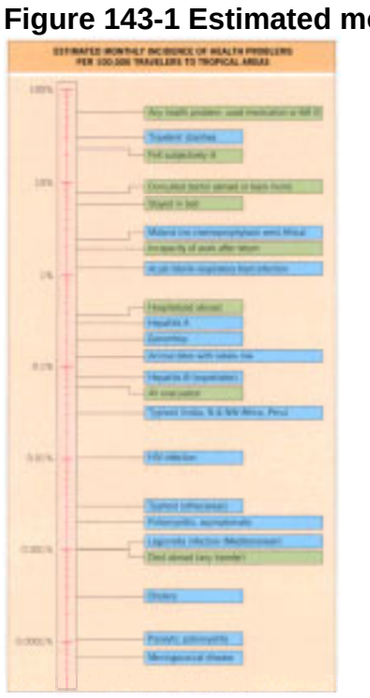


Figure 144-1 Food-borne transmission probably accounts for the majority of diarrheal illness in travelers and expatriates. The high rates of shigellosis and campylobacteriosis compared with cholera among travelers in cholera-endemic areas suggest that they may be better able to avoid exposure to contaminated water than to contaminated food.

Figure 144-2 A suggested approach to the evaluation and management of acute diarrhea in the returned traveler. The most likely pathogens for each scenario are given.

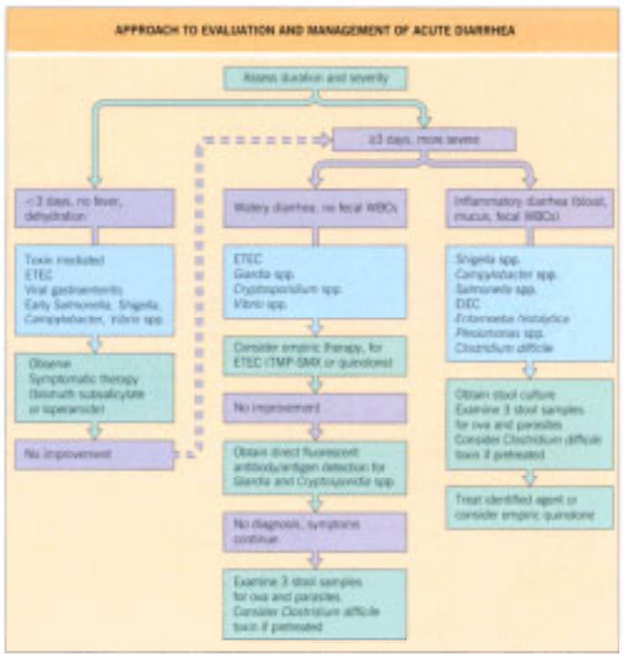


Figure 144-3 A suggested approach to the evaluation and management of chronic diarrhea in the returned traveler. The presence of significant weight loss or evidence of malabsorption should influence the pace and aggressiveness of the evaluation. This approach is designed for use in a Western setting.

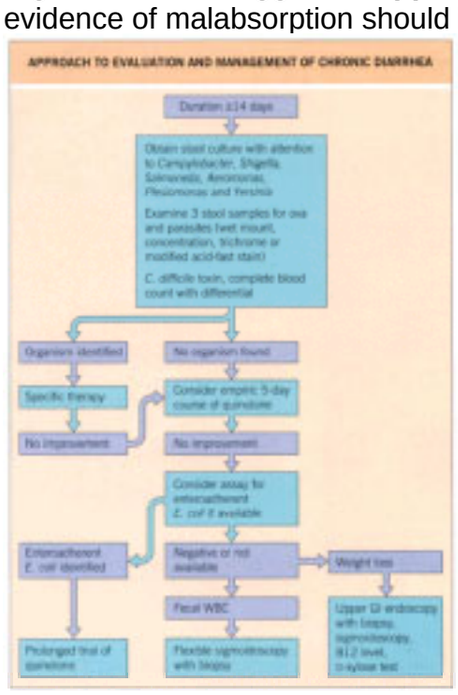


Figure 145-1 Temperature pattern in a patient with dengue fever.

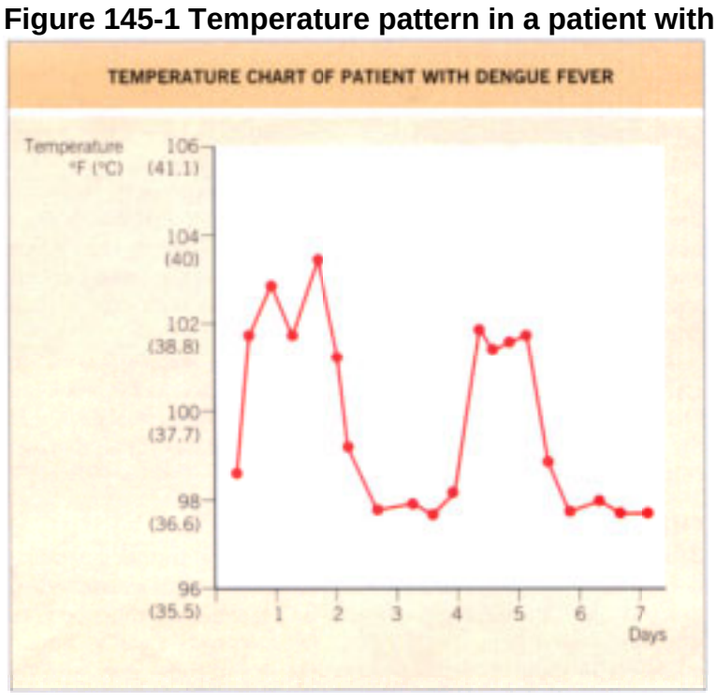


Figure 146-1 Rose spots. In *Salmonella typhi* and *S. paratyphi* infections (enteric fever) classic rose spots of 1–3mm diameter can be found, especially on the abdominal wall, lower thorax and back of the trunk. These are small erythematous macular lesions, which tend to come and go during infection. *Courtesy of Anthony Bryceson.*

Figure 146-2 Trypanosomal rash. In fair-skinned individuals, each peak of fever may be accompanied by a remarkable skin eruption in the form of annular patches of erythema. In other cases, the rash may be more generalized, as seen here on the sixth day of an infection with *Trypanosoma brucei rhodesiense*. *Courtesy of Anthony Bryceson.*

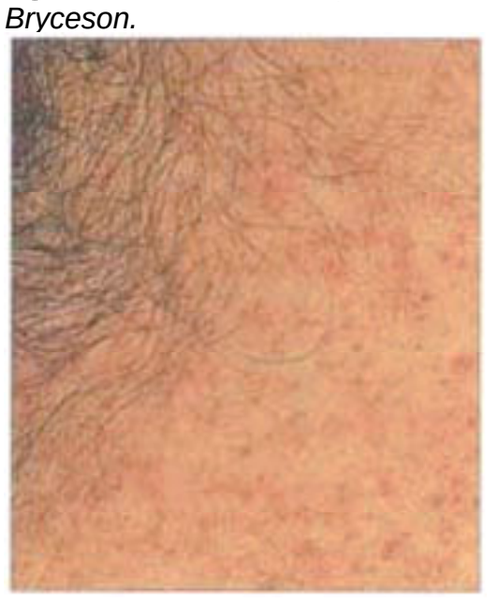


Figure 146-3 Subconjunctival hemorrhages and jaundice in leptospirosis. Asymptomatic or atypical infection probably occurs in 90% of cases and, in some tropical areas, leptospirosis may account for up to 15% of all patients with undiagnosed pyrexia. While this form can be mild, the infection may develop into a generalized septic form with confusion within 1–2 weeks. This is characterized by fever, myalgia and often subconjunctival hemorrhages. The patient illustrated was in the second week following the onset of symptoms. The most dangerous form (Weil's disease) may be very severe and can involve several organs, with jaundice, renal failure, haemorrhagia, vascular collapse and obtundation.

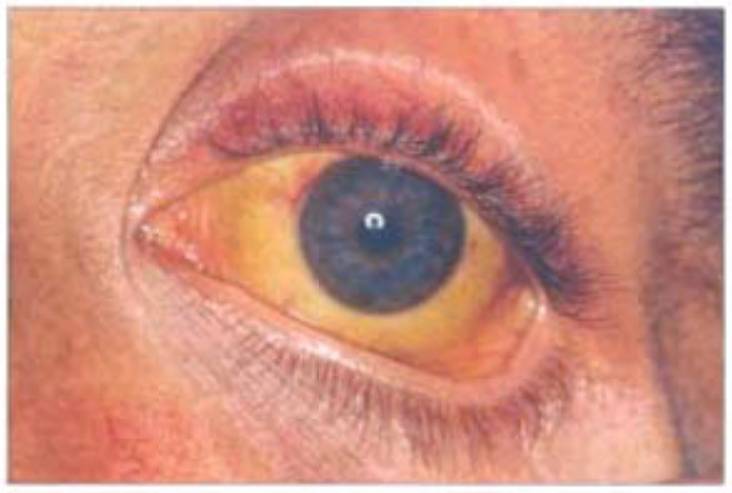


Figure 146-4 Meningococcal septicemia. Meningococcal infections are common in the tropics, most notably in the relatively dry 'meningococcal belt' of sub-Saharan Africa, stretching from Senegal and the Gambia in the west to Ethiopia in the east. The nonblanching skin rash and the petechiae and purpura are often difficult to see in individuals with pigmented skin, as is demonstrated in this patient.

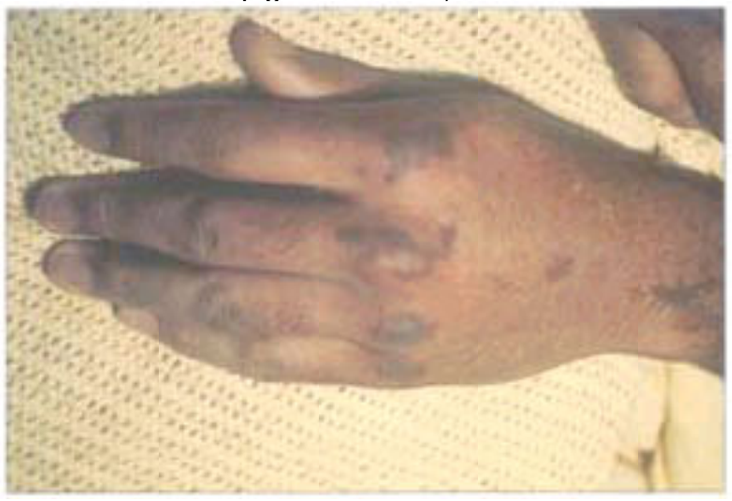


Figure 146-5 Section of *Angiostrongylus cantonensis* larvae in meninges of human brain. Humans may be infested by ingesting third-stage larvae in raw or inadequately cooked intermediate hosts such as snails, prawns, frogs or fish, or in contaminated salads. The larvae migrate to the brain, where they cause eosinophilic meningitis or meningoencephalitis. The diagnosis is aided by the serologic examination of paired specimens, using a specific antigen from adult worms. *Courtesy of Colonel JC Crook, RAMC.*

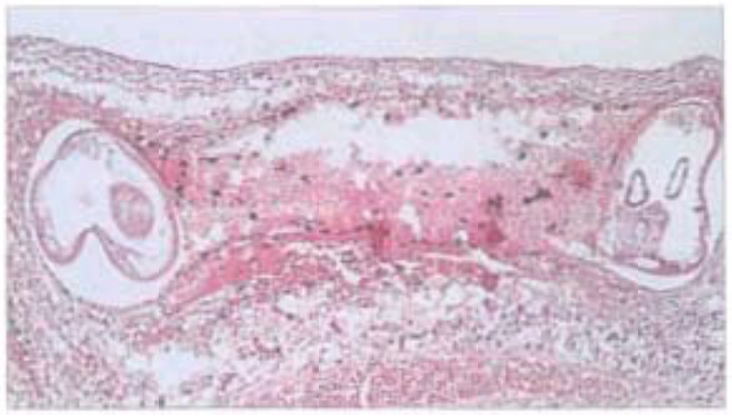


Figure 146-6 Management of a confused or comatose traveler who has suspected viral hemorrhagic fever, malaria or an HIV-related problem.

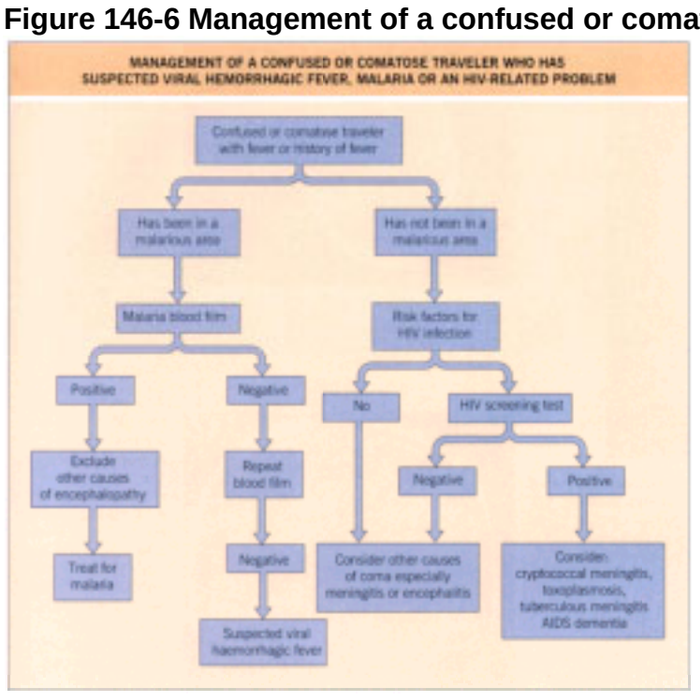


Figure 146-7 Peripheral blood film of a patient with past malaria. The patient had returned 5 days previously from Malawi, having been treated there for malaria. At this stage no parasites were visible on the film. She was confused and delirious on admission and was found to have a profound acidosis (pH 6.98) and acute renal failure, thought to be due to the malaria. She was hypotensive, which suggested a secondary bacterial infection, and was found to have a *Salmonella* sepsis. Secondary bacterial infections are not uncommon in severe malaria associated with immunosuppression. The only evidence on blood film for malaria was the presence of malarial pigment (hemozoin) in many of the neutrophils, one of which is demonstrated here (arrow).

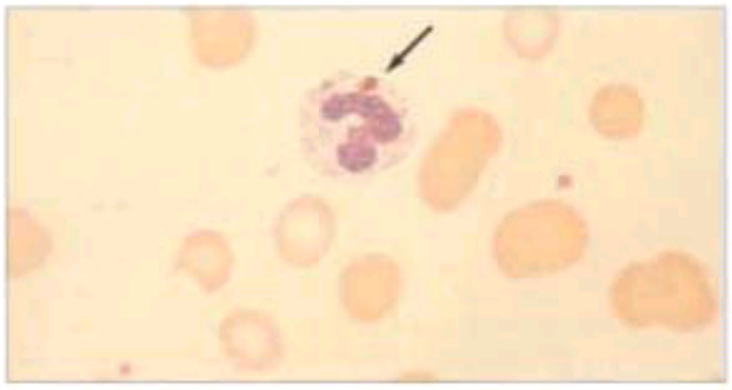


Figure 146-8 Toxoplasmosis. This patient presented to the hospital confused, with a right-sided stroke. An astute doctor requested an HIV test, which was positive. The CT scan carried out with injected contrast shows a typical ring-enhancing lesion in the left internal capsule with considerable surrounding edema. Further lesions were noted on different sections. The *Toxoplasma* IgG was positive and the patient made a full response to anti-*Toxoplasma* therapy.

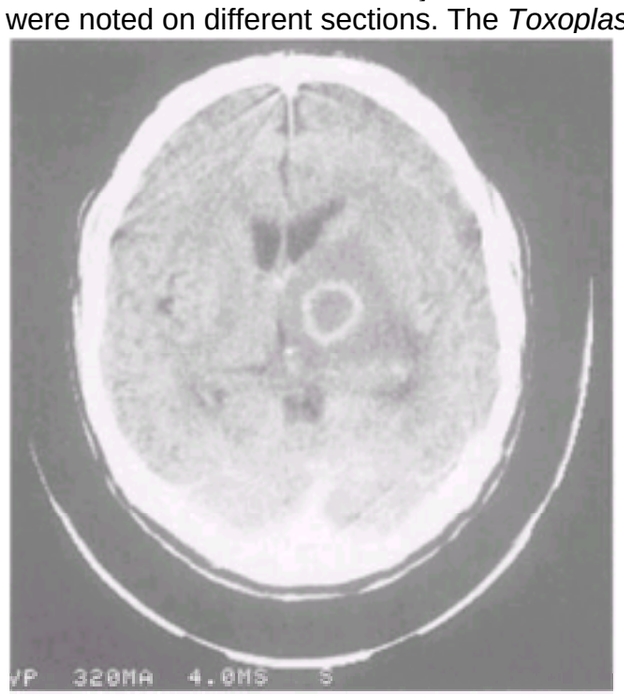


Figure 146-9 Tuberculous meningitis. A CT scan of the brain showing increased uptake of contrast around the vessels at the base of the brain in the circle of Willis in a patient with tuberculous meningitis. Obtundation in a patient presenting with tuberculous meningitis is an indication for the use of steroids.

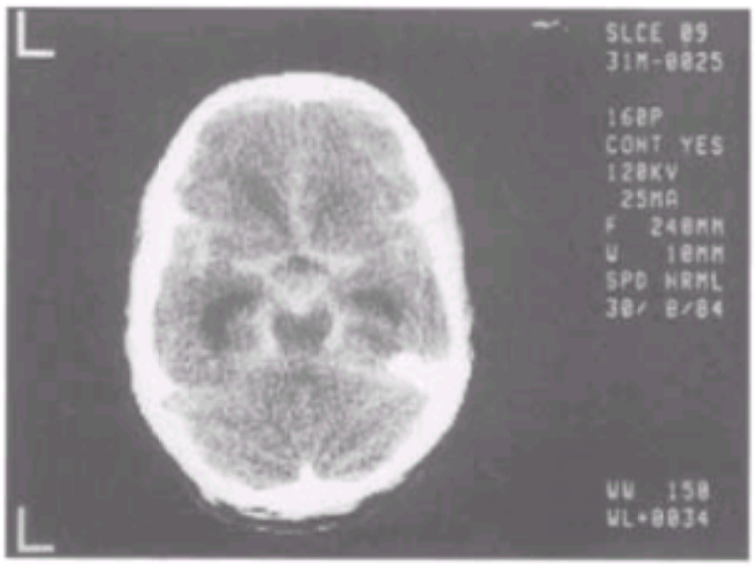


Figure 146-10 Coronal section of brain in cerebral cysticercosis. This magnetic resonance imaging scan shows a cyst with a surrounding white area of edema. While this 22-year-old man presented with focal convulsions of his left arm, edematous lesions placed more centrally, especially within the brain stem, can lead to both confusion and coma.

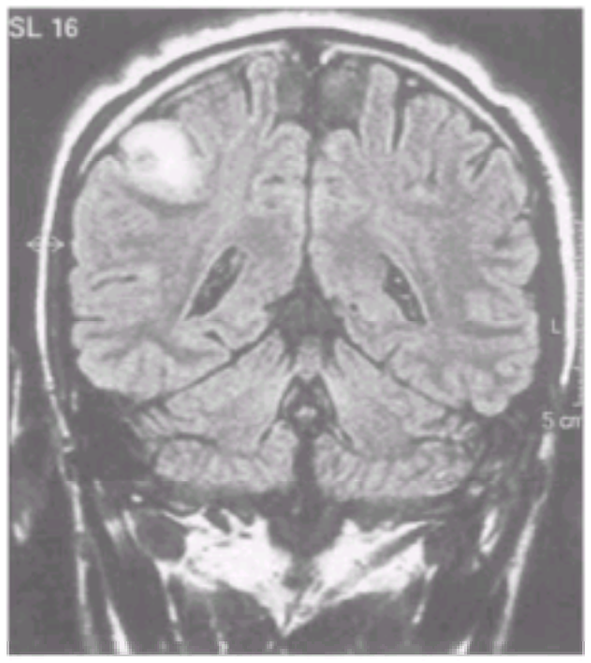


Figure 147-1 Pityriasis versicolor. The rash shows confluent scaly macules.

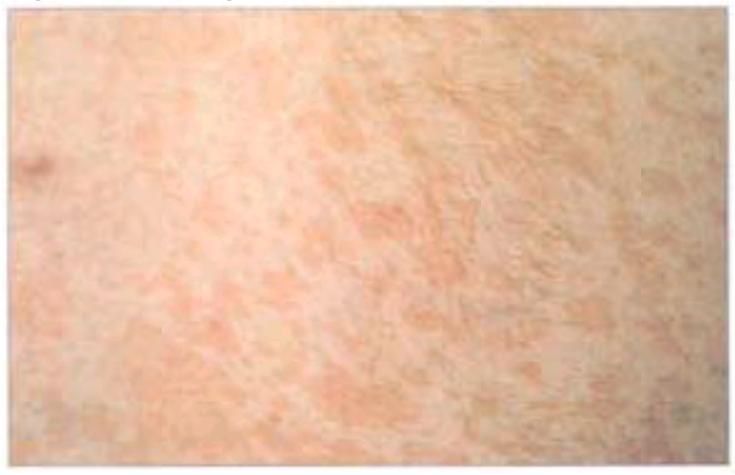


Figure 147-2 Onychomycosis caused by *Scytalidium dimidiatum* in a traveler.

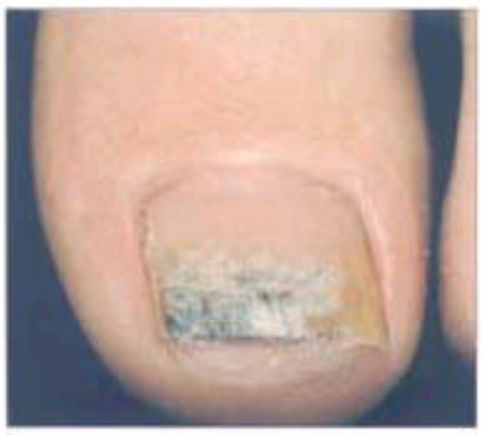


Figure 147-3 Acute papular onchodermatitis. There is a combination of dermal edema and a papular rash.

Figure 147-4 Tropical ulcer. (a) Acute. (b) Chronic.

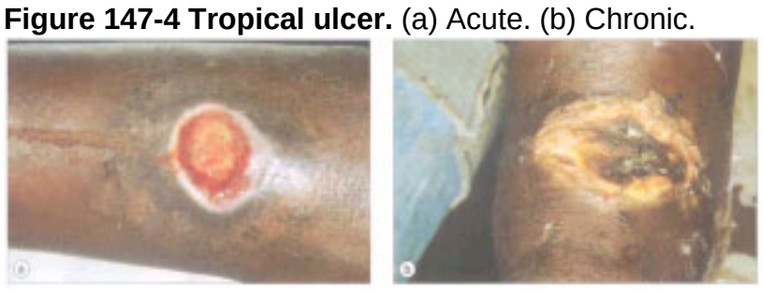


Figure 148-1 Chancroid. Characteristic purulent lesion with ragged borders, friable base and surrounding inflammation.

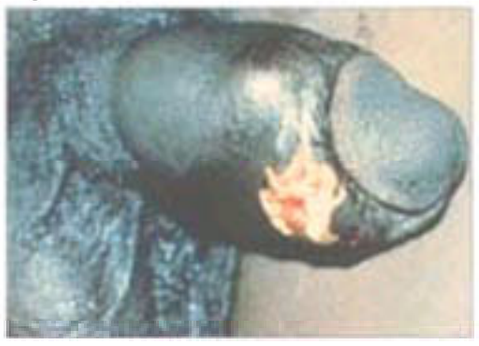


Figure 149-1 Distribution of human malaria.

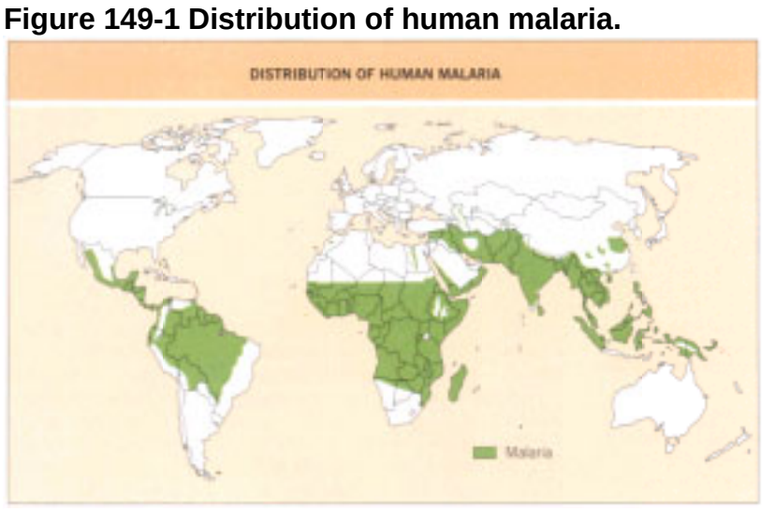


Figure 149-2 Distribution of some viral hemorrhagic fevers that cause jaundice.

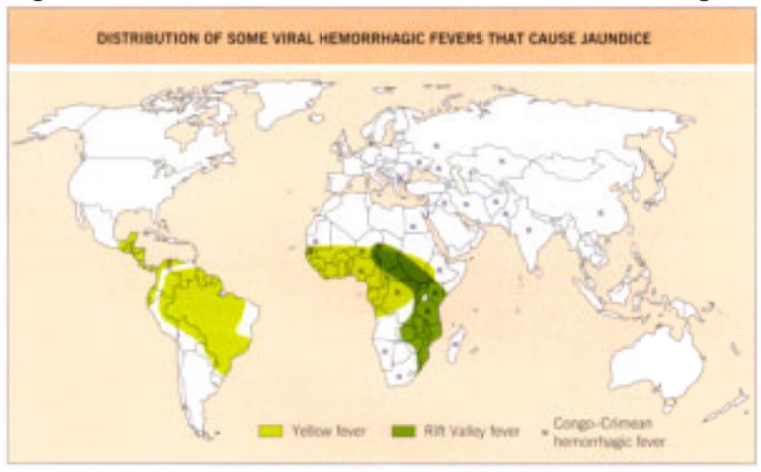


Figure 151-1 Respiratory illness in southern Asia (February 1985 to December 1987). *Data modified from reference [5] .*

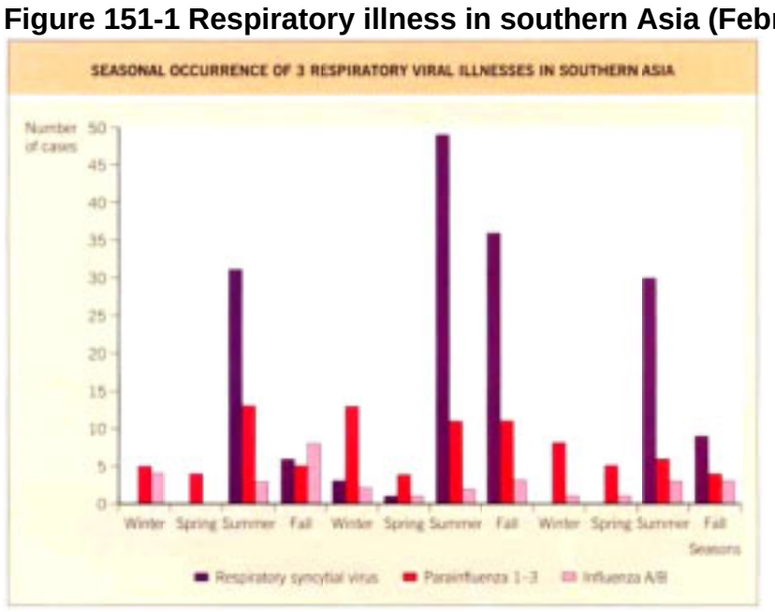


Figure 151-2 Significant tuberculin skin tests in passengers and one flight crew member in the rear section of a Boeing 747 flight from Chicago to Honolulu.

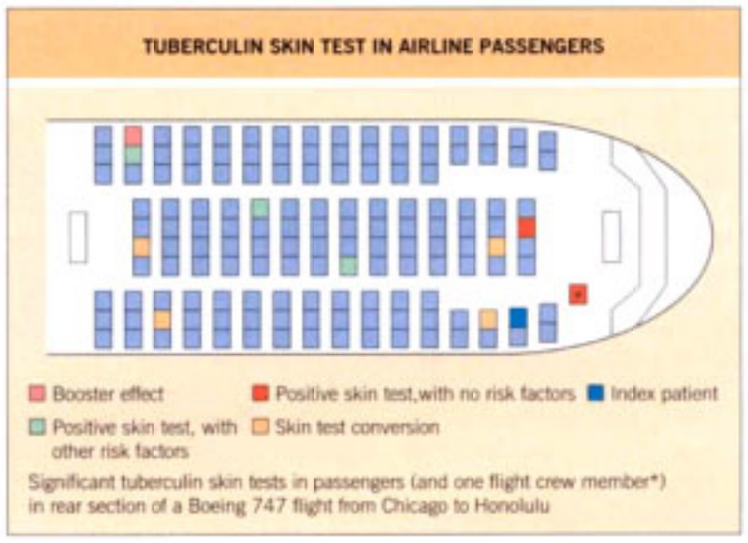


Figure 151-3 Diagnostic/treatment flow chart for cough in travelers.

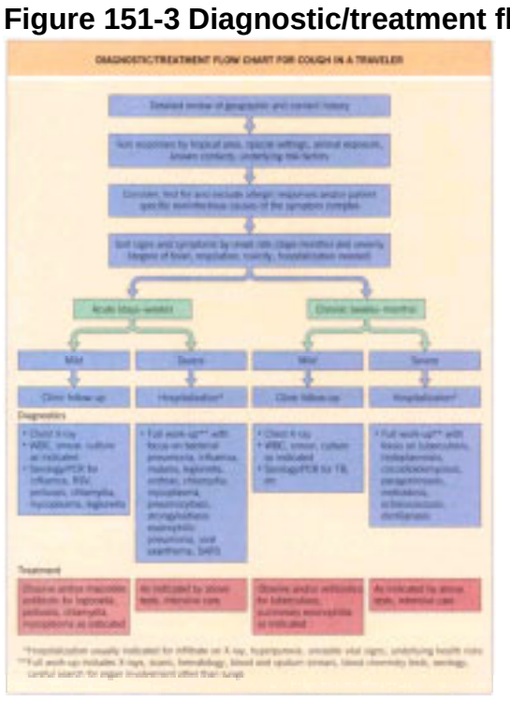


Figure 152-1 Early rash of dengue fever.

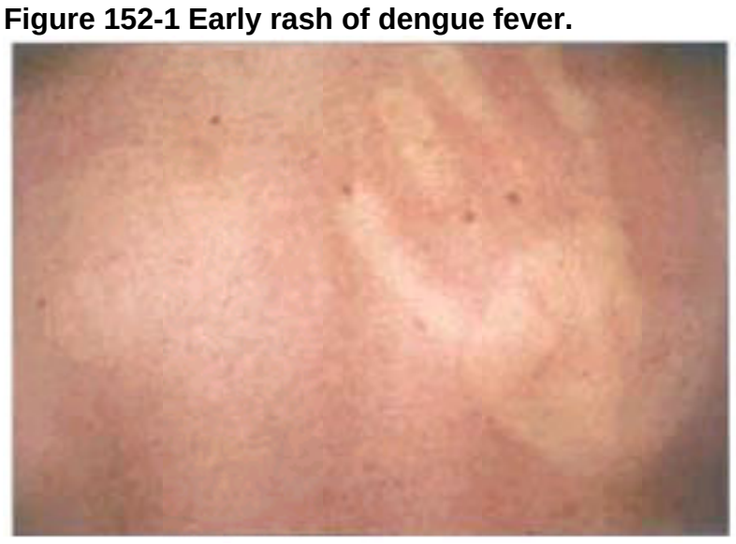


Figure 153-1 Animal rabies cases by geographic region for 1998. A total of 32,342 cases are displayed here. According to WHO sources in the 34th World Survey (Via Rabnet document, 2000, WHO/CDS/CSR/APH/99.6), based upon data from 110 countries reporting out of 193 members, wildlife rabies predominates in some regions, such as the USA and Canada, whereas dogs remain a significant reservoir in many other countries. Values shown are percentages. (Note: Rabies has been diagnosed among bats in Australia but these do not appear in the above report.)

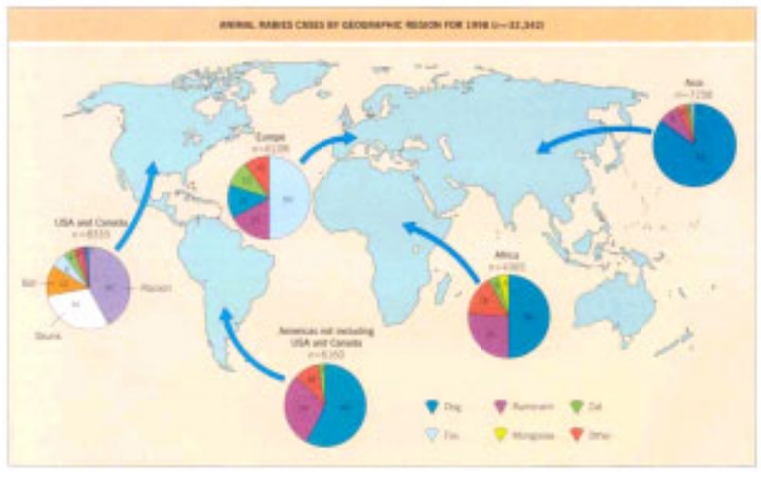


Figure 153-2 A preventable case of rabies in a returned traveler. The patient had been traveling for 6 months. She did not receive pre-exposure rabies vaccine and despite multiple opportunities, did not receive postexposure prophylaxis.[10]

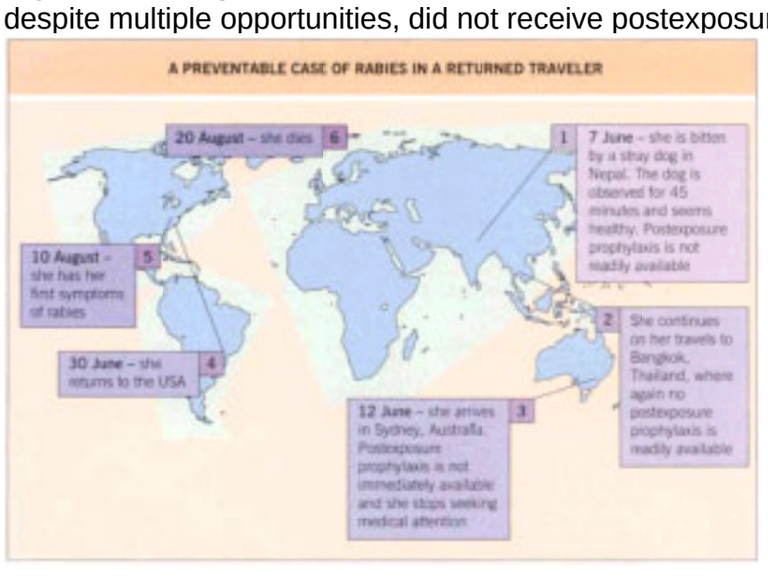


Figure 154-1 The clinical-immunologic spectrum of leprosy. This reflects the underlying host immunity as measured by the T-cell and antibody responses to *M. leprae*. Spontaneous fluctuations in the immune response are responsible for reversal reactions and erythema nodosum leprosum (ENL). TT, tuberculoid leprosy; BT, borderline tuberculoid; BB, mid-borderline leprosy; BL, borderline lepromatous leprosy; LL, lepromatous leprosy; IFN, interferon; IL, interleukin.

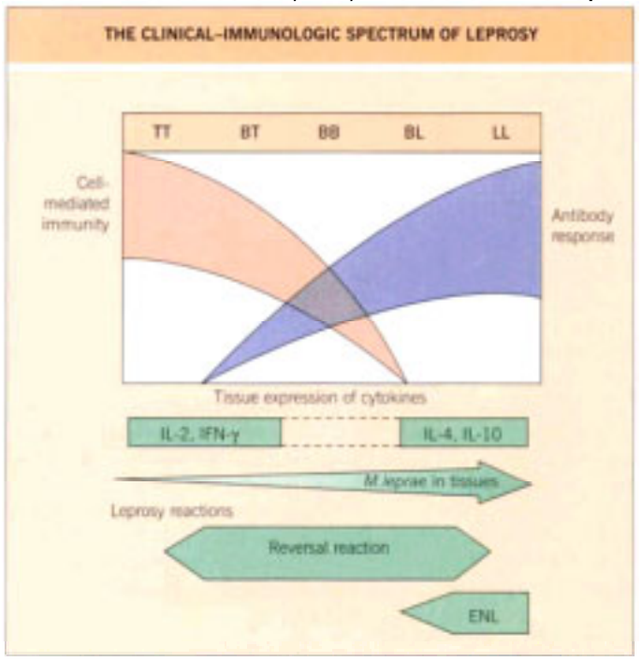


Figure 154-2 Tuberculoid leprosy. Single hypopigmented anesthetic plaque with raised border and dry surface.

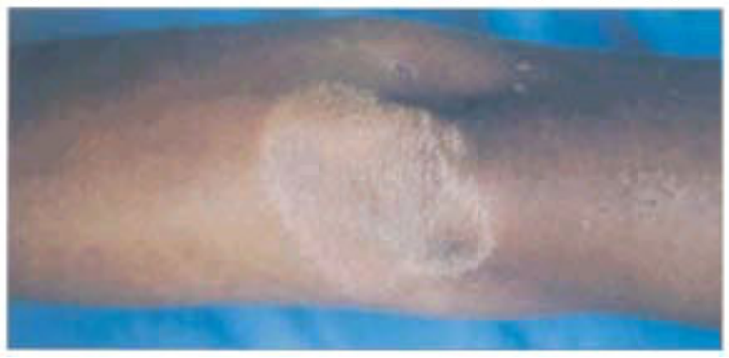


Figure 154-3 Borderline tuberculoid leprosy. Three large well-defined erythematous patches with reduced sensation, spreading borders and satellite lesions.

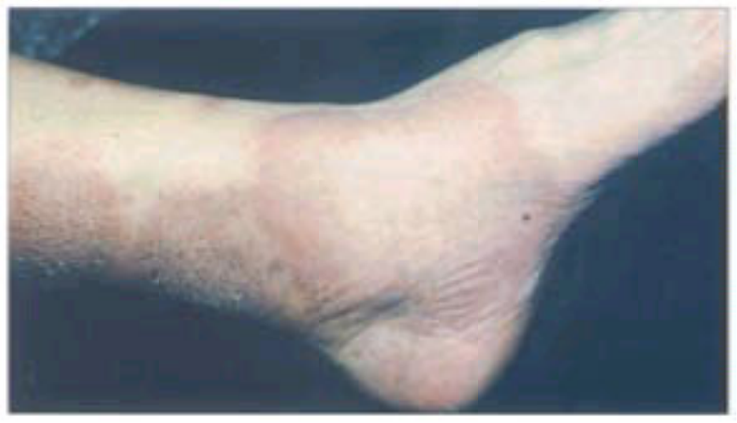


Figure 154-4 Mid-borderline leprosy. Characteristic target lesion with raised erythematous annular border and 'punched-out' central area with impaired sensation.

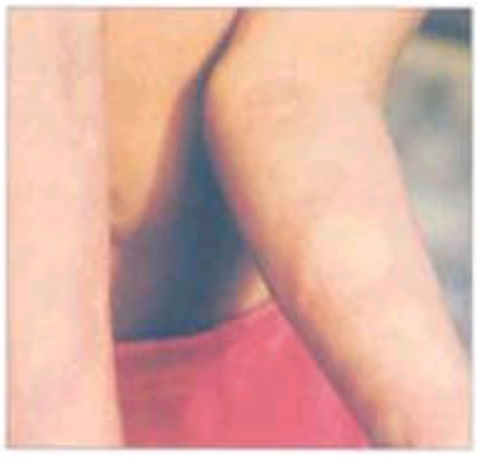


Figure 154-5 Lepromatous leprosy. Multiple, small, slightly erythematous macules with intact sensation and symmetric distribution. The skin smears of both the lesions and intervening skin are positive for acid-fast bacilli.

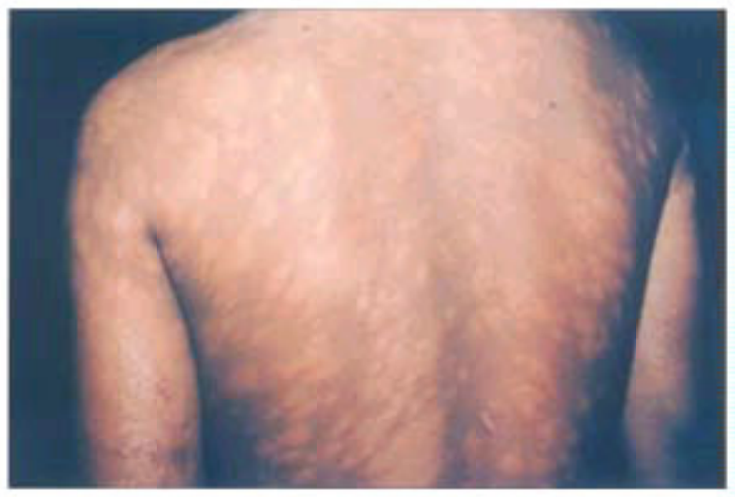


Figure 154-6 Nodular lepromatous leprosy. Diffuse infiltration of the skin by multiple nodules of varying size, each teeming with bacilli.

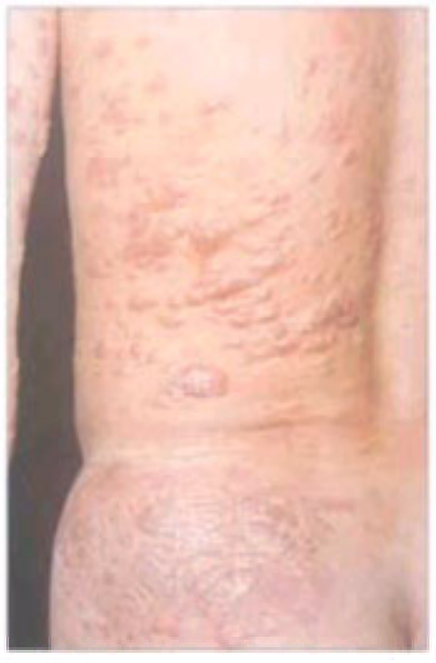


Figure 154-7 Reversal reaction. Erythema and edema in the facial lesions of a patient who has borderline-tuberculoid leprosy undergoing an upgrading reversal reaction.

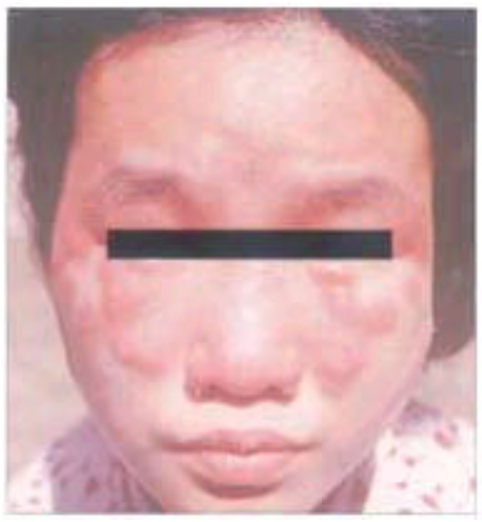


Figure 154-8 Erythema nodosum leprosum. Tender papules associated with fever, arthralgia and acute neuritis in a patient who has lepromatous leprosy.

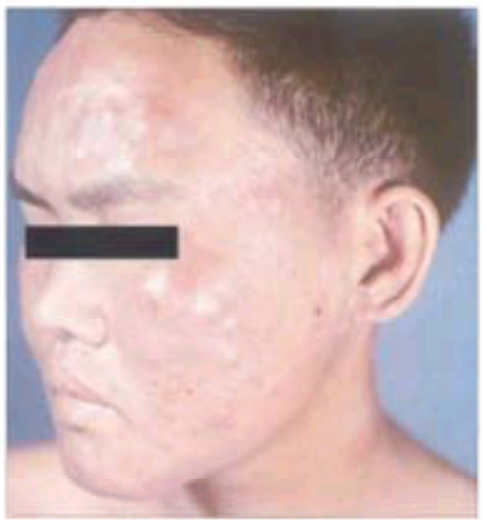


Figure 155-1 Adult *Pediculus humanus corporis* (the body louse) feeding. These insects are not only a source of considerable skin irritation but also the vectors of epidemic typhus and trench fever. *Courtesy of Dr med H Lieske. With permission from Peters and Pasvol.*[7]

Figure 155-2 The 'crab louse', *Phthirus pubis.* The crab louse, which is commonly acquired during sexual intercourse, infests not only the public region but also other sites, including the eyelashes. *Courtesy of Dr med H Lieske. With permission from Peters and Pasvol.*[7]

Figure 155-3 Third instar larva of *Cordylobia anthropophaga*, the Tumbu fly. The powerful mouth hooks, with which the larva feeds, are seen as long, dark bars. *With permission from Peters.*[1]

Figure 155-4 Adult female *Dermatobia hominis.* The fly lays eggs on blood-sucking insects such as mosquitoes or on ticks. After about 1 week larvae hatch from the eggs to infest the skin of a human or other warm-blooded host, which is fed on by the phoretic host. *Courtesy of Dr AJ Shelley. With permission from Peters.*[1]

Figure 155-5 Second instar larva of *Dermatobia hominis* after surgical removal. The characteristic rows of dark spines are seen clearly. *Courtesy of Dr RP Lane. With permission from Peters and Pasvol.*[7]

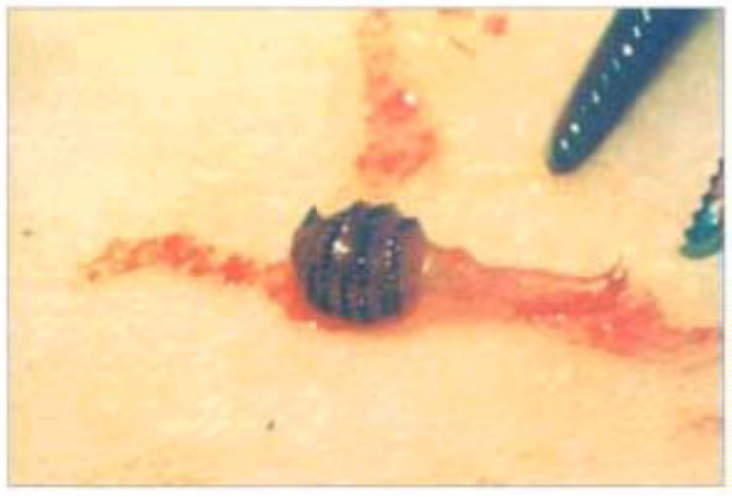


Figure 155-6 Cavity left in the skin of the back of a woman who returned to Europe from an African holiday with multiple furuncular lesions caused by larvae of *Cordylobia anthropophaga.* Note the marked surrounding inflammation. *Courtesy of Professor T Rufli. With permission from Peters.*[1]

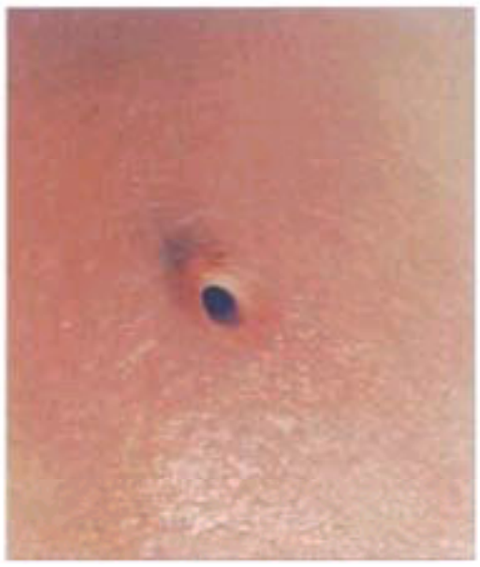


Figure 155-7 Cutaneous larva migrans ('creeping eruption') due to invasion of infective larvae of the dog hookworm *Ancylostoma caninum.* This condition may be mistaken for infestation with the larvae of ectoparasitic arthropods. The lesions respond rapidly to treatment with albendazole or ivermectin. *With permission from Peters and Pasvol.*[7]

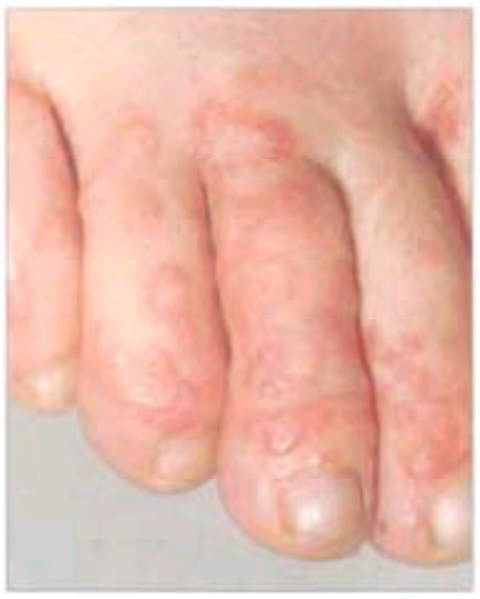


Figure 155-8 Extracting a larva of *Cordylobia anthropophaga* after covering it with paraffin. The pair of black spiracles can just be seen in the center of the posterior tip of the larva. *Courtesy of Professor A Bryceson. With permission from Peters and Pasvol.*[7]

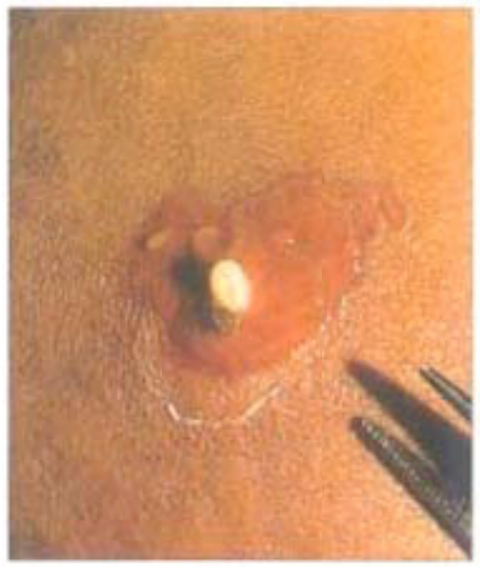


Figure 155-9 Surgical extraction of a gravid female *Tunga penetrans.* Care must be taken not to disrupt the abdomen and release the eggs. *Courtesy of Professor C Curtis. With permission from Peters.*[1]

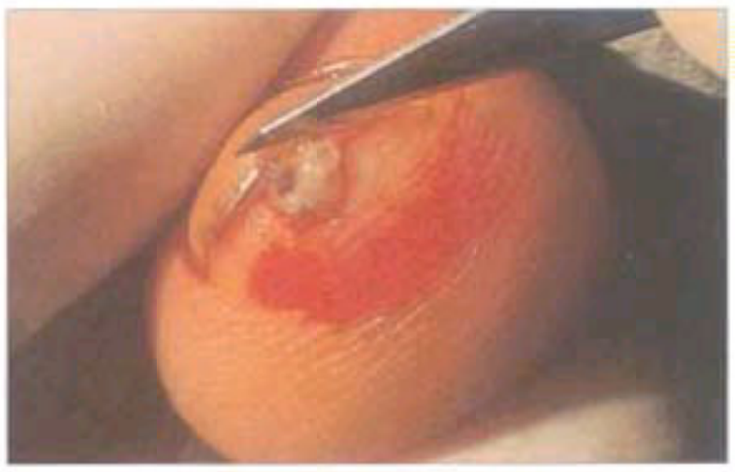


Figure 156-1 Mixed early yaws lesions: papillomata, ulceropapillomatous lesions and squamous macules. *Courtesy of WHO, Geneva.*

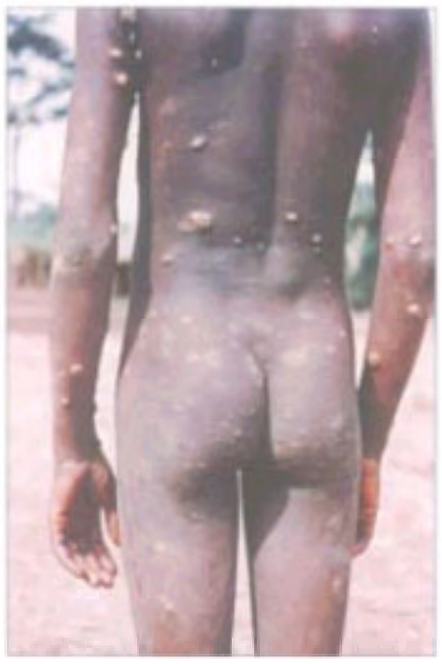


Figure 156-2 Gangosa in late stage of yaws (rhinopharyngitis mutilans; occurs also in endemic syphilis). *Courtesy of WHO, Geneva.*

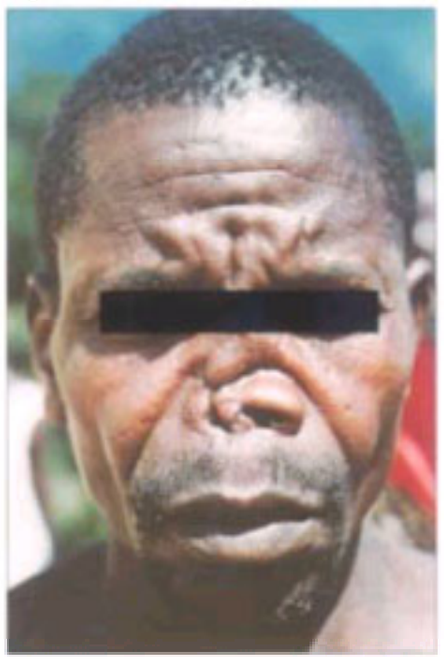


Figure 156-3 Angular stomatitis (also called split papules) of endemic syphilis; these lesions are also found in early yaws. *Courtesy of Dr GM Antal.*

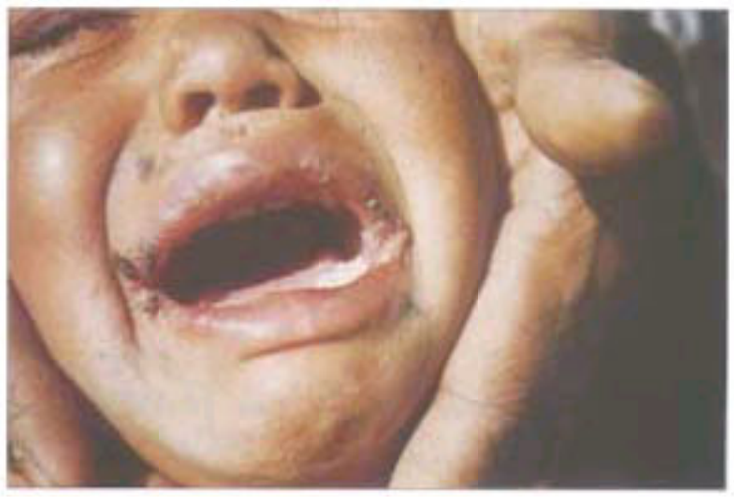


Figure 157-1 The causative organisms of sleeping sickness in humans. Reproduction of Dutton's original drawing of *Trypanosoma brucei gambiense* from the blood of a man. The organisms possess nucleus kinetoplast and flagella, and their relative size can be assessed from the red blood cell diameter of 7µm.

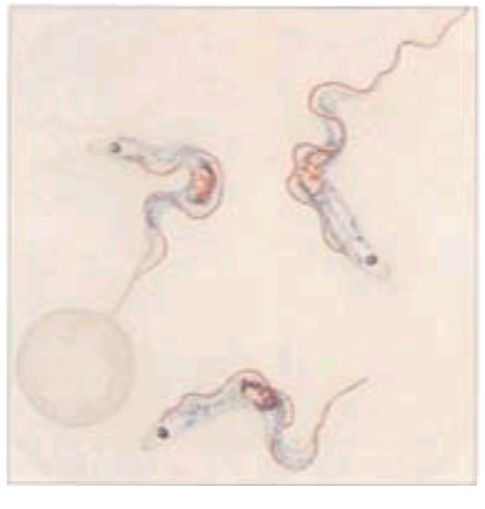


Figure 157-2 Endemic areas for trypanosomiasis.

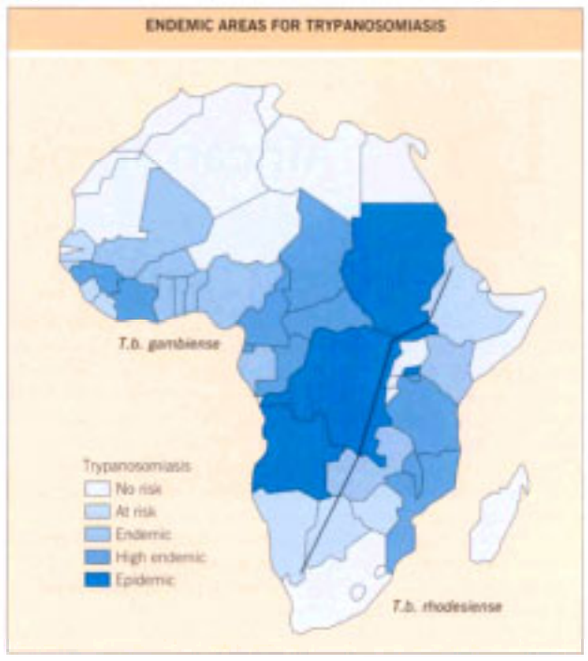


Figure 157-3 Typical chancre of a patient infected with *Trypanosoma brucei rhodesiense*.. The chancre develops at the site of the infecting fly bite.

Figure 157-4 Emergency treatment center Uganda. This center was established to provide facilities for the care and treatment of patients with sleeping sickness during the epidemic in Busoga.

Figure 157-5 Comatose terminal stage patient with sleeping sickness. Note the degree of cachexia.

Figure 157-6 Treatment of a patient with an intravenous injection of melarsoprol. It is vital to adhere to the schedules of treatment and to have a scrupulous technique of injection in order to avoid destruction of local tissue as a result of leakages of the melarsoprol suspended in propylene glycol.

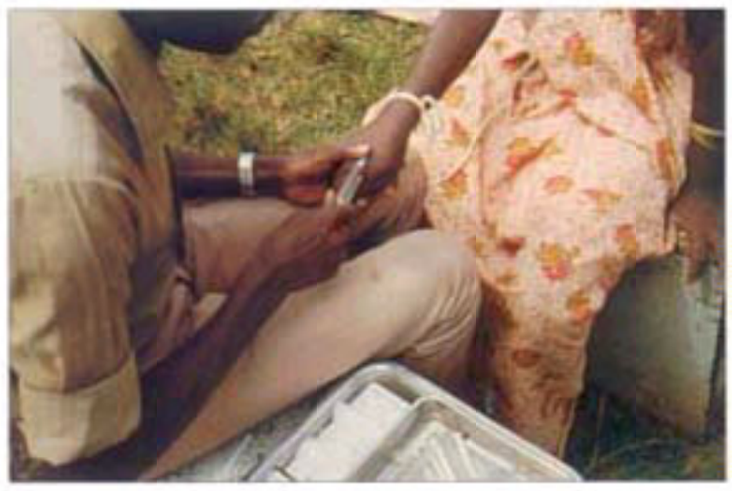


Figure 158-1 Potential routes to the brain and spinal cord for parasitic protozoa and helminths. Collateral circulation (e.g. in hepatosplenic schistosomiasis with portal hypertension) allows ova to embolize via portopulmonary anastomoses to the lung and thence to the systemic circulation. Batson's vertebral venous plexus allows retrograde access to the spinal cord and brain by parasites and/or ova.

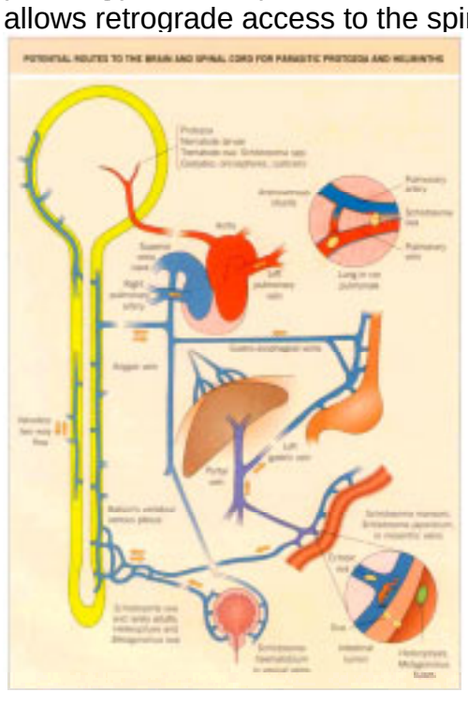


Figure 158-2 Toxoplasma abscess. (a) This CT brain scan shows a toxoplasma abscess in the left internal capsule, compressing the lateral ventricles. Contrast demonstrates a typical ring-enhancing effect. (b) Same patient after 17 days of treatment with pyrimethamine and sulfonamide showing resolving abscess.

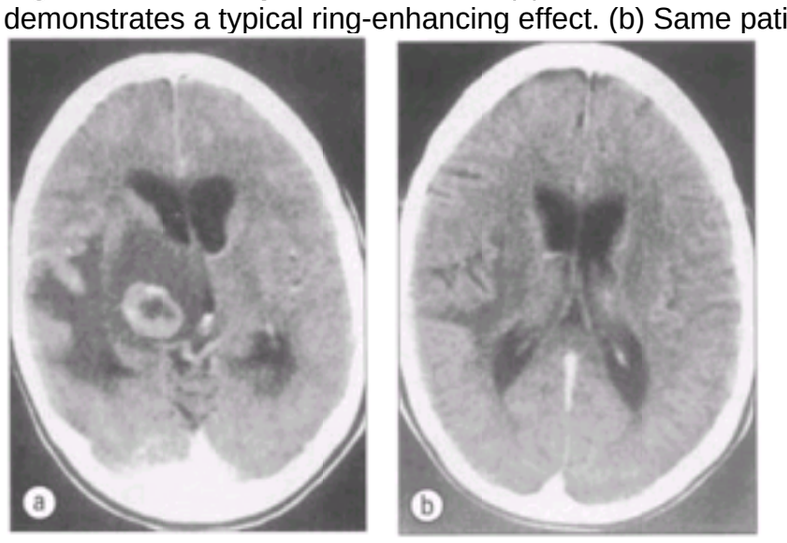


Figure 158-3 Coronal MRI of brain showing living cysticercus, with the scolex appearing as a hyperintense center ('pea in the pod' appearance). There is no visible inflammatory reaction.

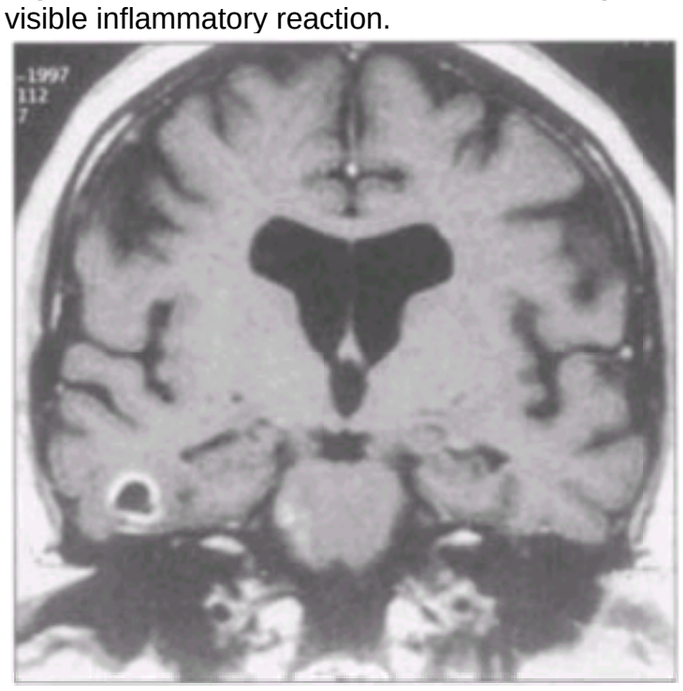


Figure 158-4 Cerebral cysticercus. (a) This MRI of brain shows a dying cysticercus surrounded by intense inflammation. (b) Post-contrast MRI T2-weighted image demonstrating the isointense wall of the cyst and surrounding hyperintense edema.

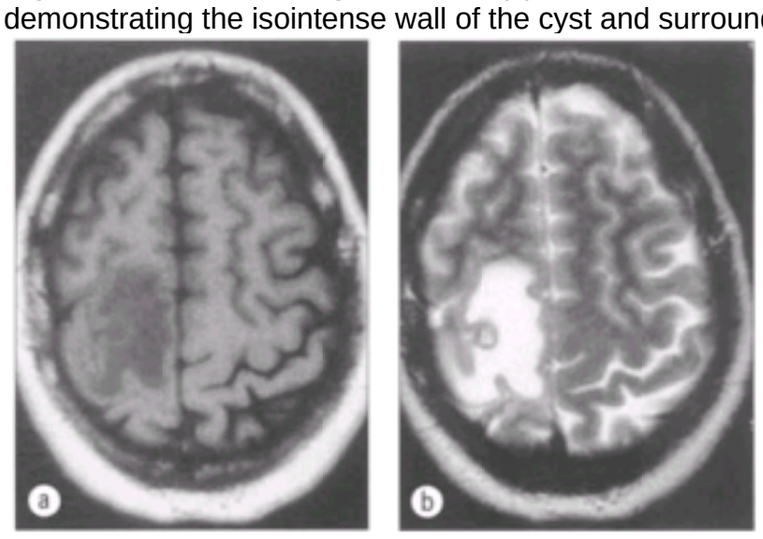


Figure 158-5 Extensive necro-hemorrhagic lesions of the right cerebral hemisphere in an AIDS patient with reactivated acute Chagas' disease. *Courtesy of Professor L Chimelli.*

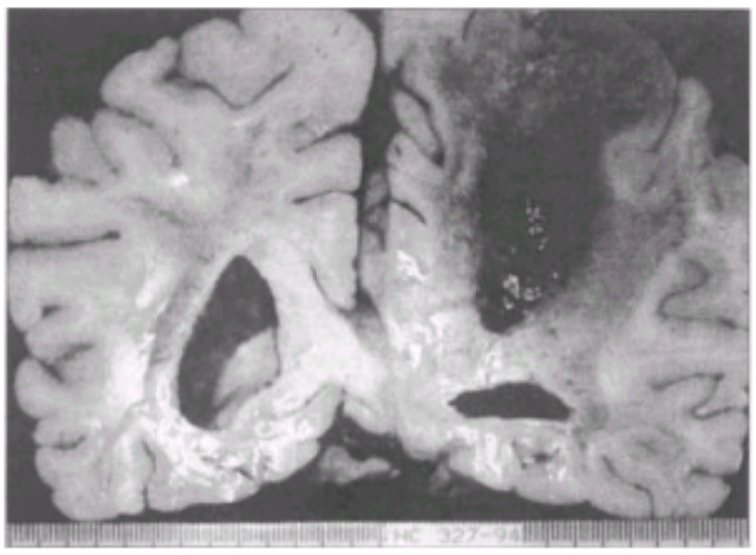


Figure 158-6 MRI of the spinal cord of a 9-year-old Omani boy with acute schistosomiasis mansoni, who presented with transverse myelitis (vertical scale in cms). Extensive cord edema from the first thoracic vertebral level to the conus medullaris is present (arrows). He recovered and was ambulant after 2 weeks treatment with prednisone and praziquantel.

Figure 159-1 The African meningitis belt.

Figure 159-2 Meningococcal septicemia with purpura fulminans. (a) In an infant and (b) in an adult.

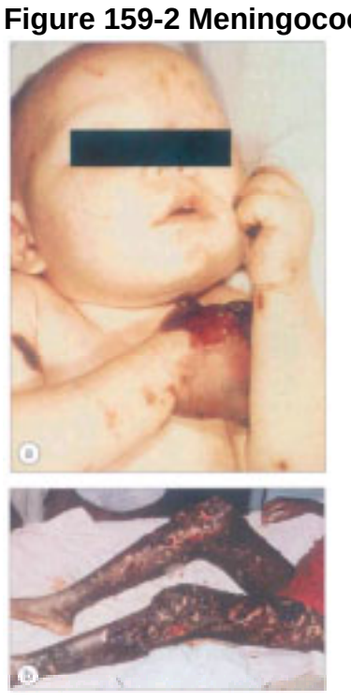


Figure 160-1 Current distribution of active trachoma (WHO).

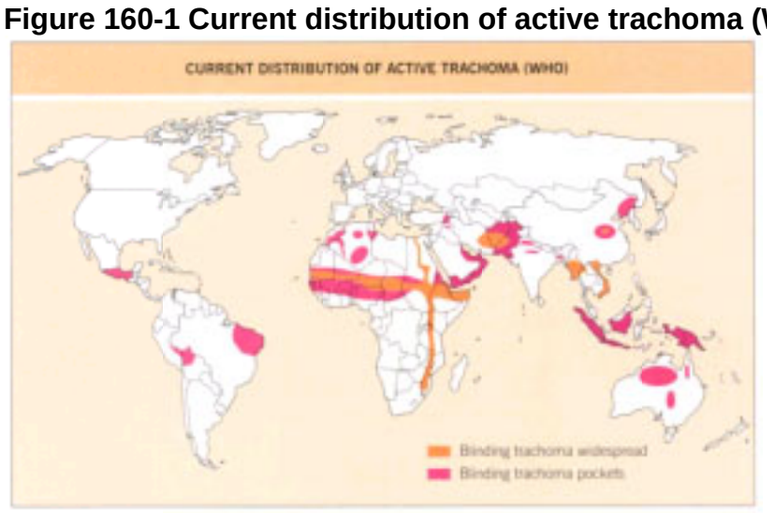


Figure 160-2 Everted eyelid showing follicular trachoma (TF). *Courtesy of the WHO Program for the Prevention of Blindness.*

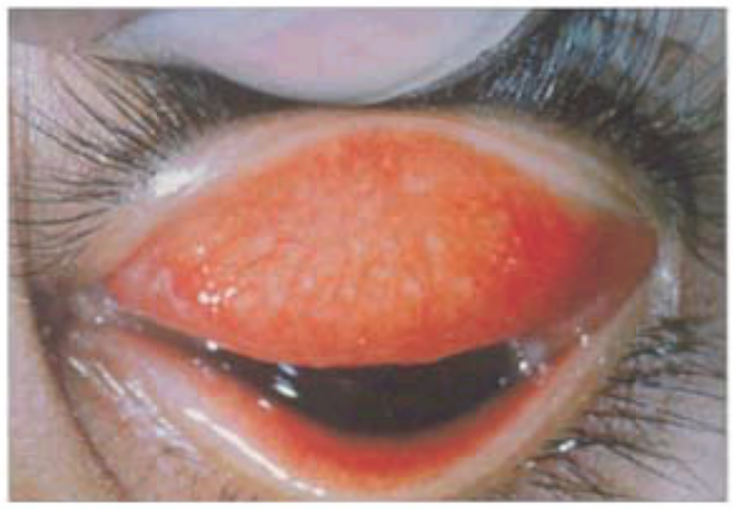


Figure 160-3 Everted eyelid showing intense inflammatory trachoma. Follicles are also present.

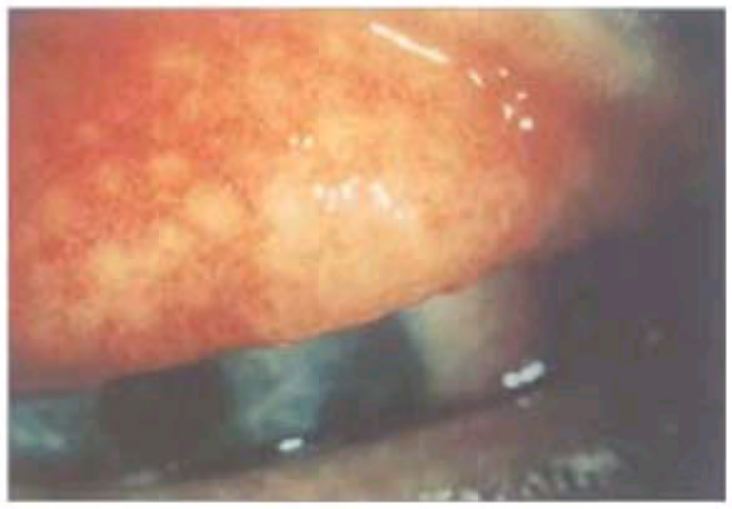


Figure 160-4 Everted eyelid showing trachomatous scarring (TS). There are also Herbert's pits visible at the corneoscleral junction. *Courtesy of the WHO Program for the Prevention of Blindness.*

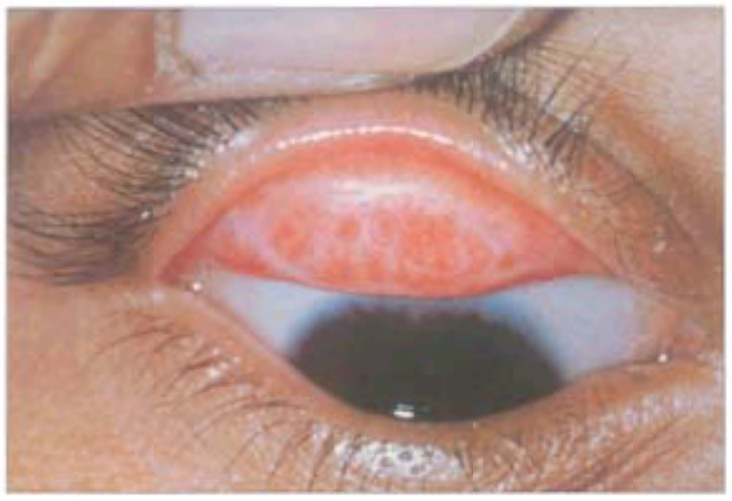


Figure 160-5 Trachomatous trichiasis (TT) and secondary corneal opacity. *Courtesy of the WHO Program for the Prevention of Blindness.*

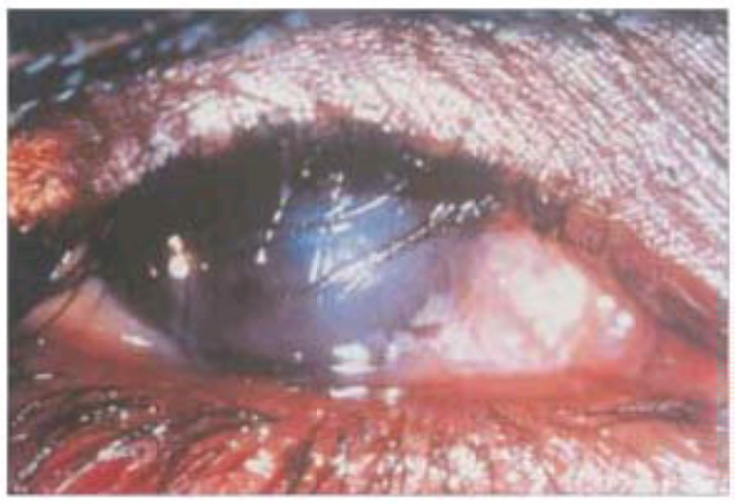


Figure 160-6 Typical dendritic ulcer caused by herpes simplex virus, visualized with fluorescein staining.

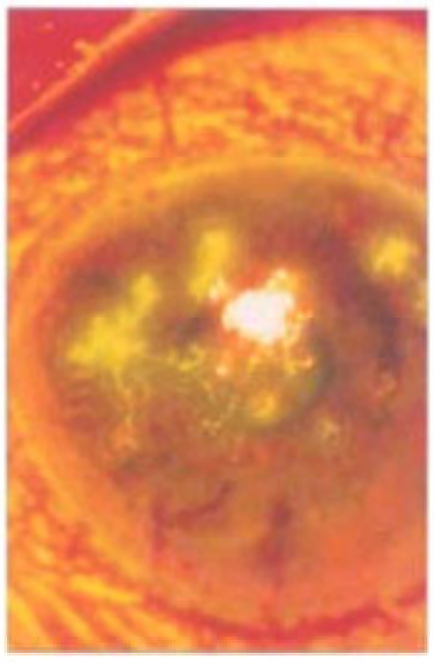


Figure 161-1 Severe dehydration from cholera. Decreased skin turgor in a severely dehydrated cholera patient. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

Figure 161-2 Oral rehydration. The patient is immediately given ORS to correct her dehydration. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

Figure 161-3 Complete recovery from cholera after rehydration. The patient 24 hours later is completely rehydrated on ORS alone. *Courtesy of the International Centre for Diarrhoeal Diseases Research, Bangladesh.*

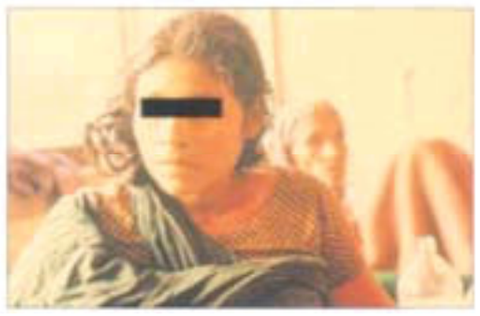


Figure 162-1 Global distribution of tropical malabsorption and sprue. Tropical malabsorption and sprue in central equatorial Africa remains largely unexplored or unreported.

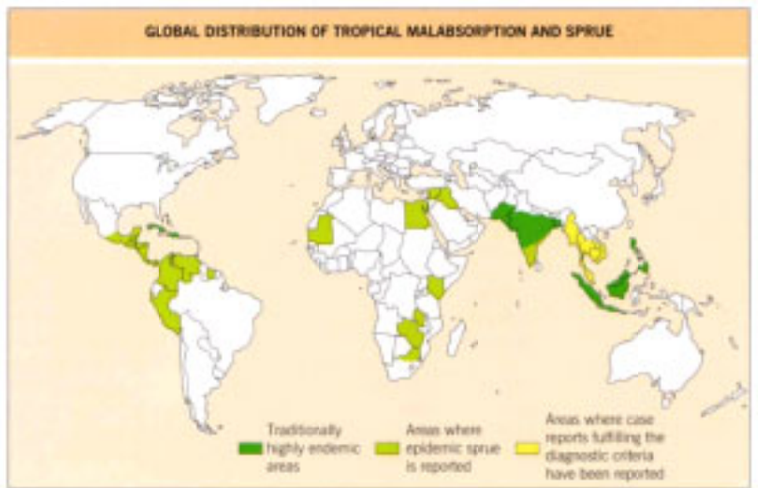


Figure 163-1 Perforating typhoid ulcer of the terminal ileum. This large, necrotic ulcer covered in dark brown slough is on the antimesenteric side of the distal ileum. A gloved fingertip is seen within the large perforating ulcer that caused the patient's death. *Reproduced with permission.*[22]

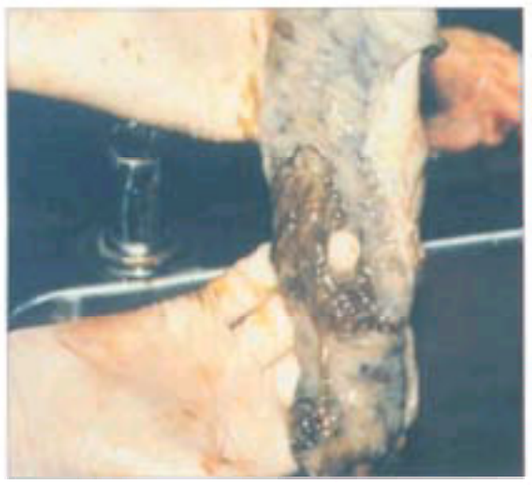


Figure 163-2 Suggested pathogenesis of typhoid. A Schwartzman-type reaction occurs when Peyer's patches are re-exposed to Salmonella typhi following initial uptake, hematogenous dissemination and return to small intestine via gallbladder.[4]

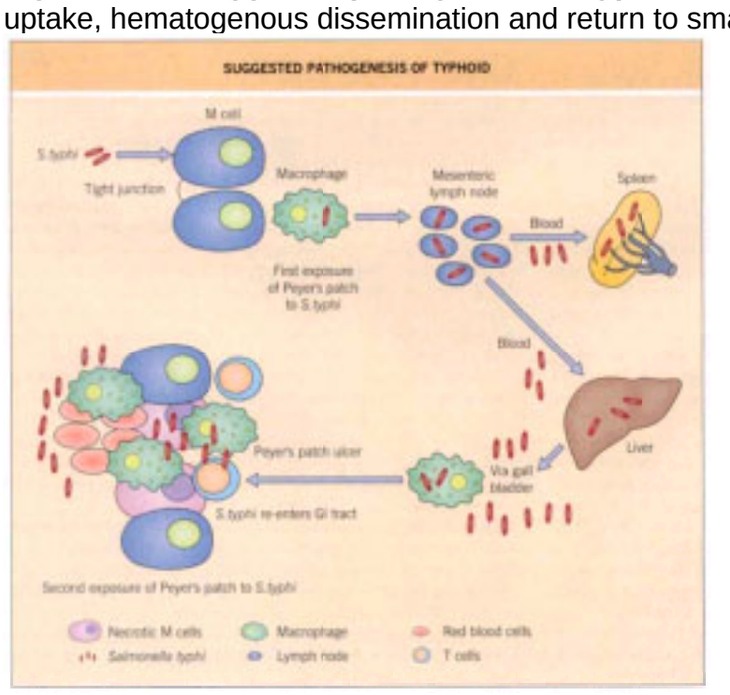


Figure 163-3 Typhoid rose spots. The abdomen is the best place to look for these lesions, which appear at the end of the first week and are usually quite sparse. They usually take the form of pink macules that blanch when the skin is stretched, but they may take on a more purpuric, nonblanching character, as in this patient. *Reproduced with permission.*[22]

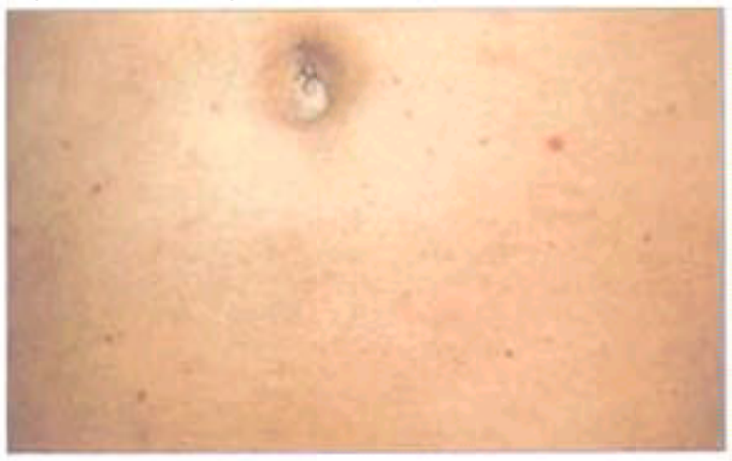


Figure 164-1 Pathology specimen from a fatal case of human amebic colitis. Deep ulcerations into the submucosa have produced abundant hemorrhages. *Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaria de Salud.*

Figure 164-2 Experimental amebic liver abscess. Two characteristic granulomas can be observed with several trophozoites (arrowheads) around its necrotic center (N) and epithelioid cells limiting the lesion, surrounded by an area of fibrosis (F).

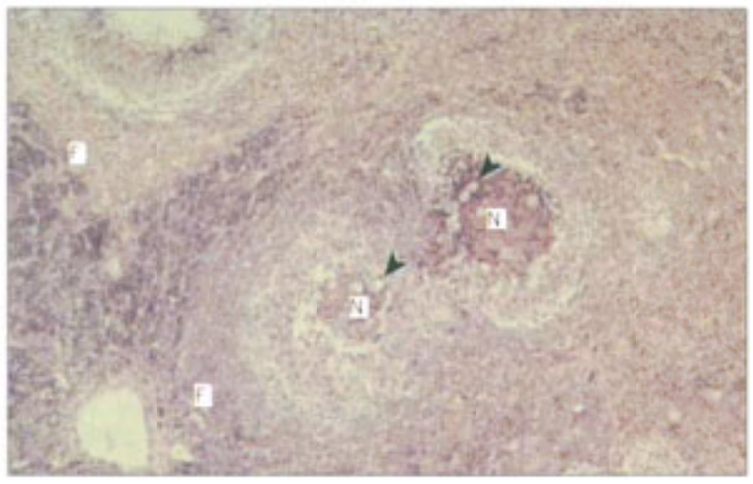


Figure 164-3 Human amebic liver abscess. Multiple abscesses, one cavitated, can be observed occupying virtually all lobes of the liver parenchyma, which is replaced by a semisolid material. *Courtesy of Dr Jesús Aguirre García, Hospital General de México, Secretaría de Salud.*

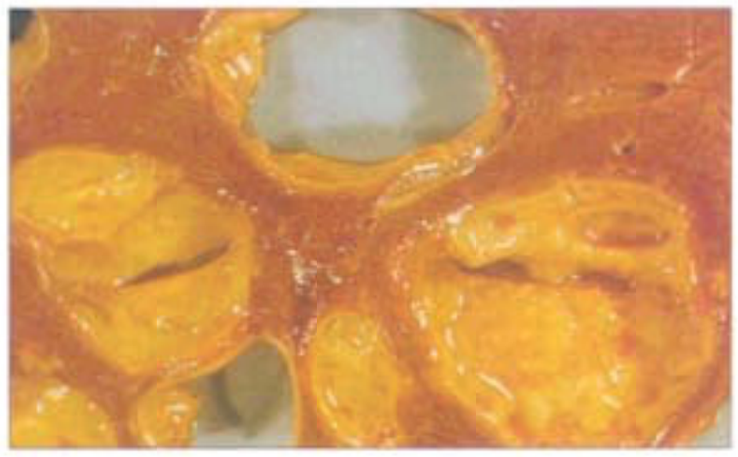


Figure 165-1 Ovum of *Clonorchis.* Large, operculated ova in fecal specimen.

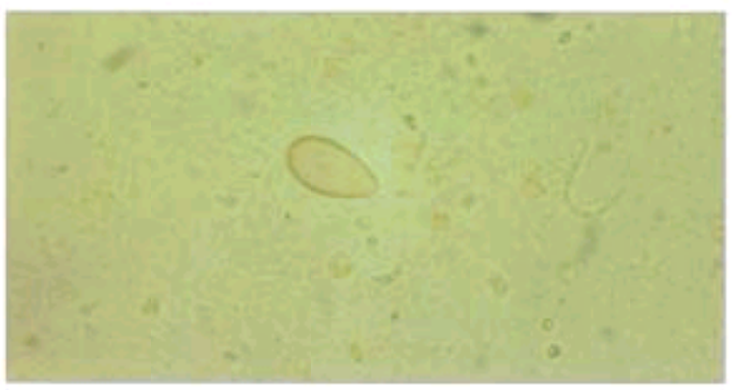


Figure 165-2 Laboratory examination of fecal specimens.

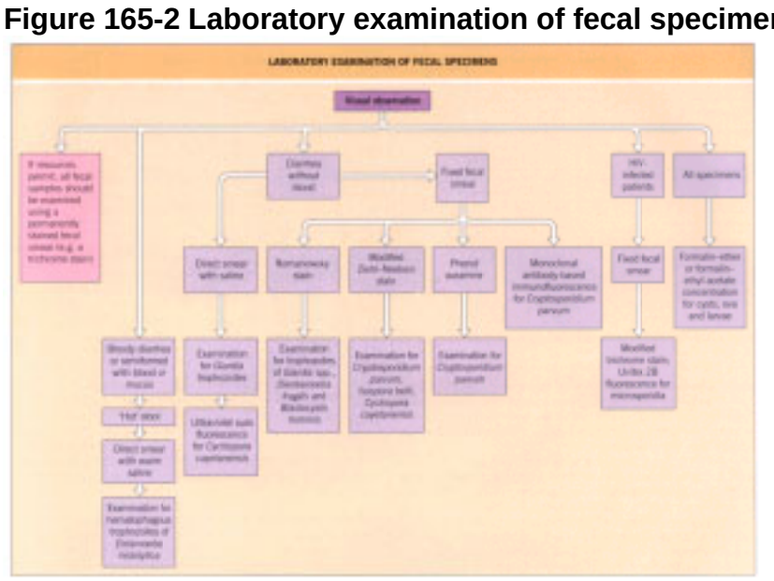


Figure 165-3 Key to the identification of helminth eggs. *Adapted from the World Health Organization.*[1]

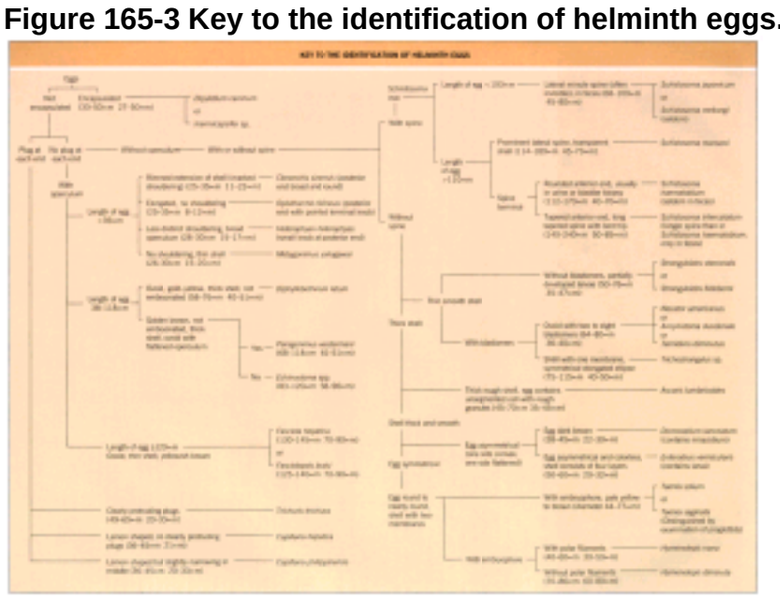


Figure 165-4 Ovum of *Diphyllobothrium latum* in a fecal specimen.

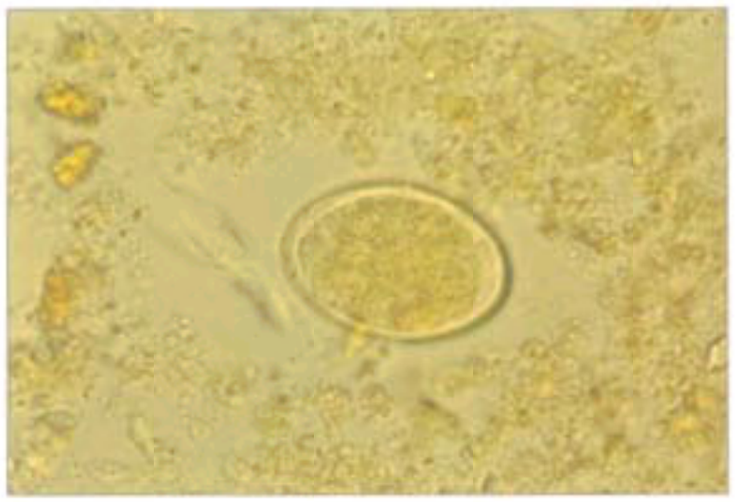


Figure 165-5 Numerous rhabditiform larvae of *Strongyloides* in a duodenal juice specimen.

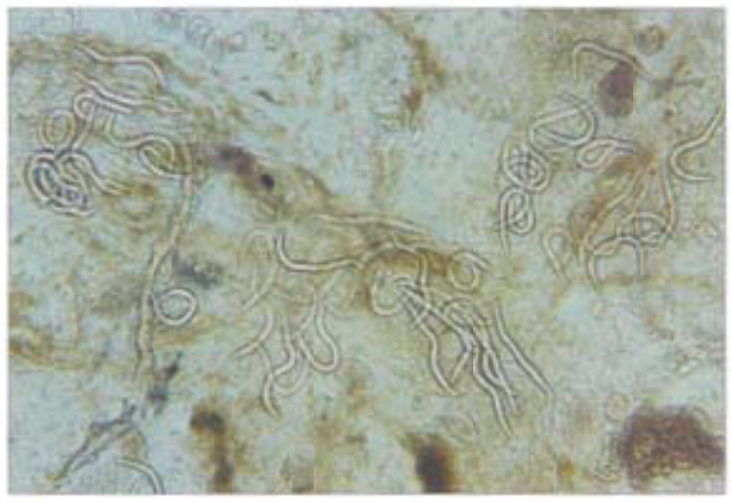


Figure 166-1 Distribution map of malaria. Despite intensive control measures over the past 50 years, malaria is still widely distributed in the tropics and subtropics. The breakdown of large-scale vector control operations and the emergence of multidrug-resistant parasites have even led to an increase in the incidence of malaria in some regions. O, areas where malaria has disappeared, been eradicated or never existed; +, areas with limited risk; + +, areas where malaria transmission occurs. *(Adapted from WHO 1999, Map No. WHO 99419 EF.) Courtesy of Dr C Lavaissiere.*

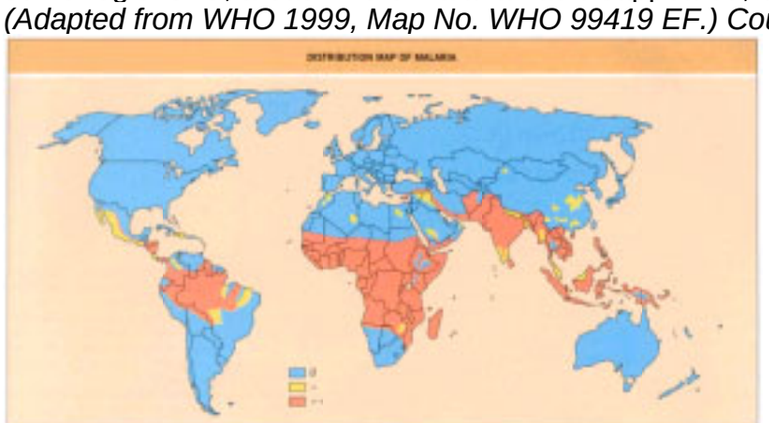


Figure 166-2 Major cell interactions in the pathogenesis of falciparum malaria. The injected sporozoites invade hepatocytes. Merozoites released from rupturing liver schizonts invade red cells. The parasite matures via the ring to the trophozoite to the erythrocytic schizont stage. Such schizonts can bind to uninfected red cells (rosette formation) or to the endothelial cells lining the postcapillary venules (cytoadherence). When the mature schizont ruptures, 'toxin'-like molecules are released which induce the release of proinflammatory cytokines such as TNF-a.

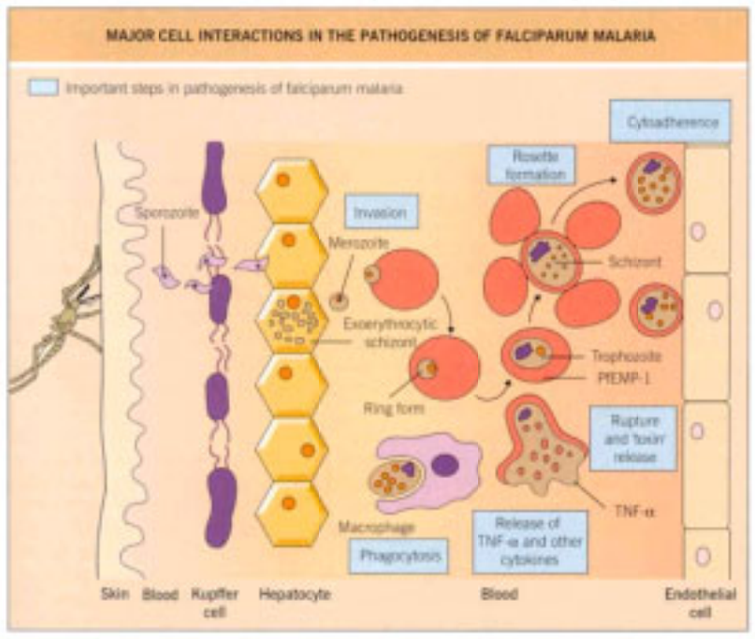


Figure 166-3 Retinal hemorrhage in severe *falciparum* malaria. Examination of the fundus is important in the physical examination of a patient with severe *falciparum* malaria as it can give some indication as to prognosis. In this case the hemorrhage is near the macula. Such hemorrhages have been found in as many as 18–30% of patients with cerebral malaria. In children, additional changes of extramacular whitening and changes in which the vessels turn white in isolated segments, often at branch points, occur.

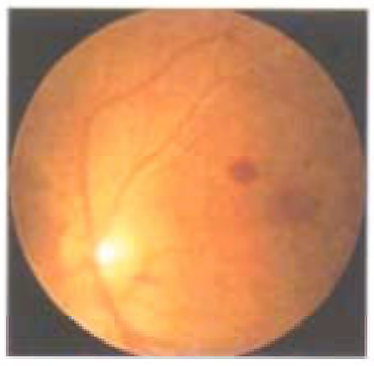


Figure 166-4 Massive hepatosplenomegaly in a patient with severe malarial anemia due to *P. falciparum.* This CT scan of the abdomen was taken in a traveler from West Africa who, after a prolonged history of fevers, presented with a hemoglobin concentration of less than 50g/l. The scan shows a massively enlarged liver, the left lobe of which is encircling an equally enlarged spleen.

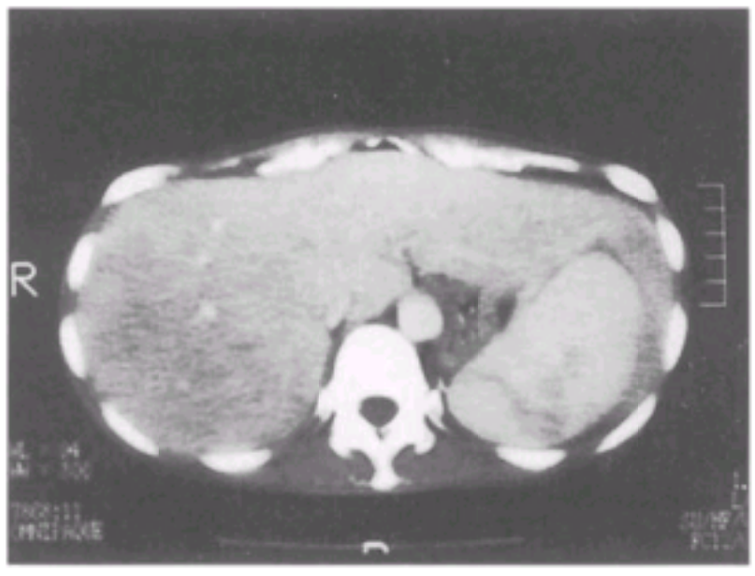


Figure 166-5 Chest radiograph of a patient with acute respiratory distress sydrome (ARDS) due to *falciparum* malaria. This X-ray shows new, bilateral, diffuse, homogeneous pulmonary infiltrates without cardiac failure, fluid overload, chest infection or chronic lung disease in an adult with severe *falciparum* malaria. The prognosis is poor. This condition is rare in children.

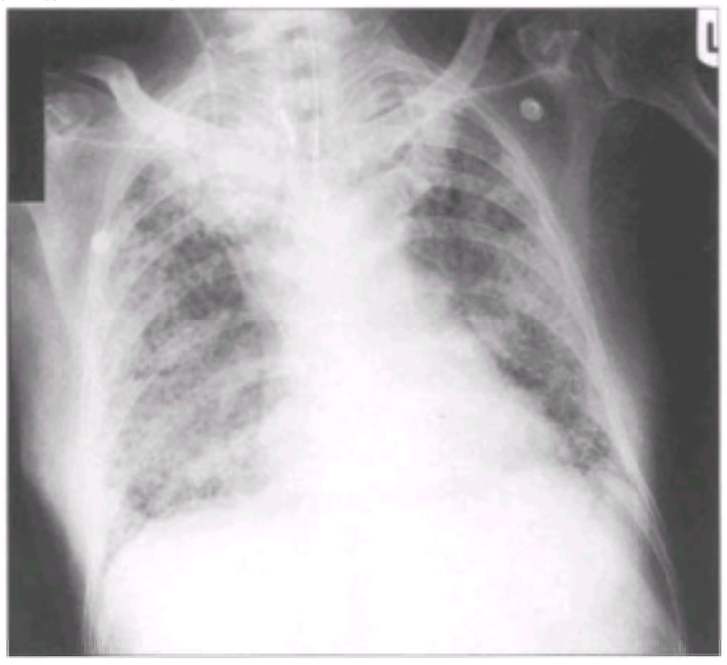


Figure 166-6 Disseminated intravascular coagulation in *falciparum* malaria. Bleeding into the skin seen in a patient with a thrombocytopenia, a prolonged prothrombin time, increased fibrinogen degradation products and hypofibrinogenemia. The patient had no signs of cerebral malaria.

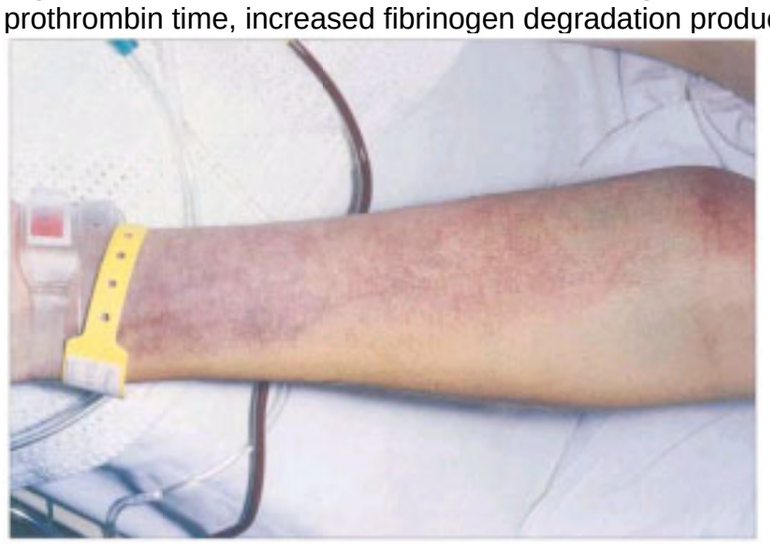


Figure 166-7 Blackwater fever. Urine specimen on admission (left) and days 2, 3 and 4 in a cross-Africa traveler with *falciparum* malaria on quinine treatment, showing the characteristic dark urine of blackwater fever, which showed gradual clearing. The same patient presented with a fever 1 week later and when treated presumptively for malaria with quinine, developed dark urine once again. Renal function was only mildly impaired.

Figure 166-8 Thin blood films from patients with malaria. (a) Delicate small ring forms of *Plasmodium falciparum* showing multiply infected red cells and a characteristic 'appliqué' form in the uppermost parasite in the central red cell where the parasite appears as if it is applied to the surface, rather than within the red cell. (b) Ring forms of *P. falciparum* in a heavy infection and where the pH of the stain is 7.2 rather than 6.7 showing the irregular, basophilic Maurer's clefts in the cytoplasm of infected cells characteristic of *P. falciparum.* (c) Very early trophozoites of *P. falciparum* in the peripheral blood film of a patient with severe disease. The relative size and presence of pigment indicate the greater maturity of the parasite and may indicate a poorer prognosis. (d) Peripheral blood film from a patient with *vivax* malaria showing mixed ring and schizont forms. The ring forms are far more fleshy and ameboid and the cytoplasm of the infected cell shows the characteristic regular and eosinophilic Schüffner's dots, which help in diagnosis. (e) Peripheral blood film from a patient with *ovale* malaria showing a small ring form on the left, which could quite easily be mistaken for *P. falciparum*. The larger central parasite has enlarged the cell into an oval shape and has also formed a fimbriated fringe at the upper pole of the cell. (f) Peripheral blood film from a patient with *malariae* malaria showing the characteristic rosette schizont with daughter merozoites (usually eight) around a central piece of pigment (*hemazoin*). The ring forms of this species characteristically form a band stretching across the width of the red cell.

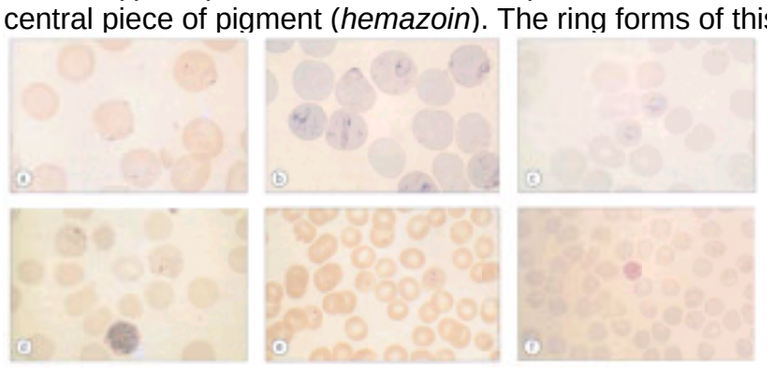


Figure 167-1 The basic pathologic lesions of intestinal schistosomiasis. A liver biopsy is depicted with egg deposition, granuloma formation and fibrosis in the periportal areas. *Courtesy of Professor MA Madwar.*

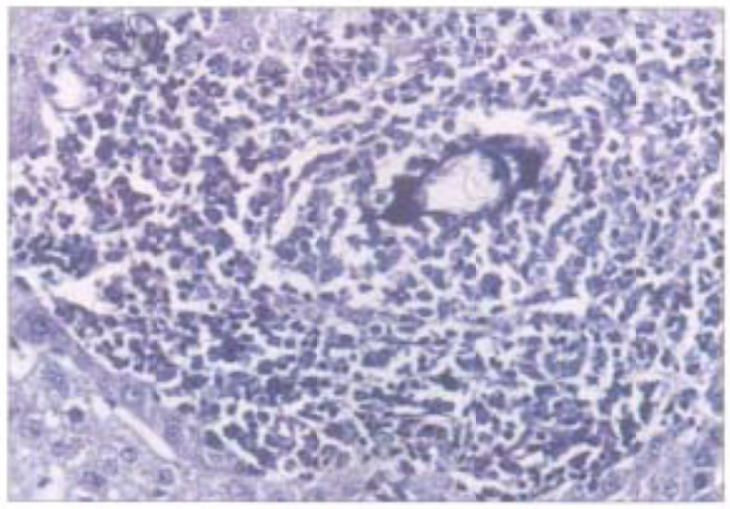


Figure 167-2 Late manifestations of disease caused by *Schistosoma mansoni* infection. Note marked ascites and collateral circulation on anterior abdominal wall. *Courtesy of Professor MA Madwar.*

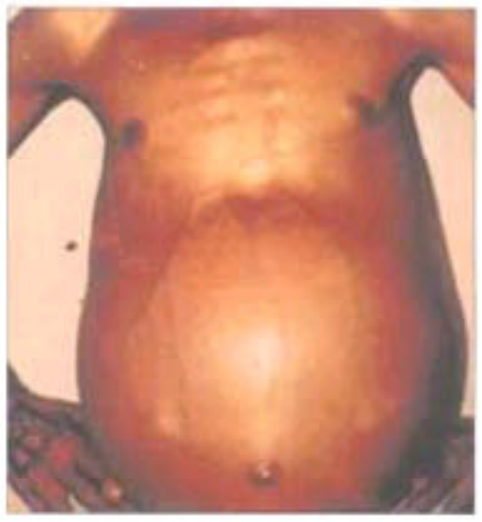


Figure 167-3 Advanced liver fibrosis in portal tracts (clay pipe-stem). *Courtesy of Professor MA Madwar.*

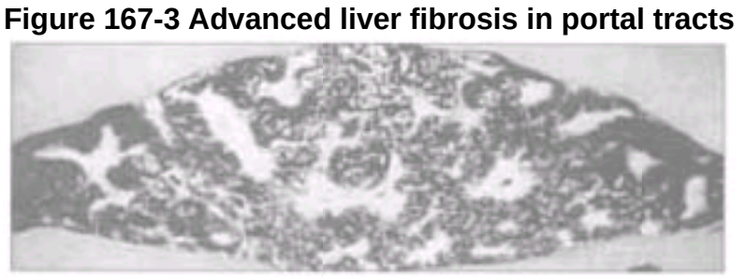


Figure 168-1 Cysticerci in the brain.

Figure 168-2 Computerized tomography appearances in neurocysticercosis. Viable cysts appear as radiolucent defects (arrowhead). The central protoscolex appears as a radiodense spot in about 50% (small arrow). Cysts that show ring enhancement are probably degenerating. Calcified cysts (large arrow) are dead and will not benefit from specific therapy.

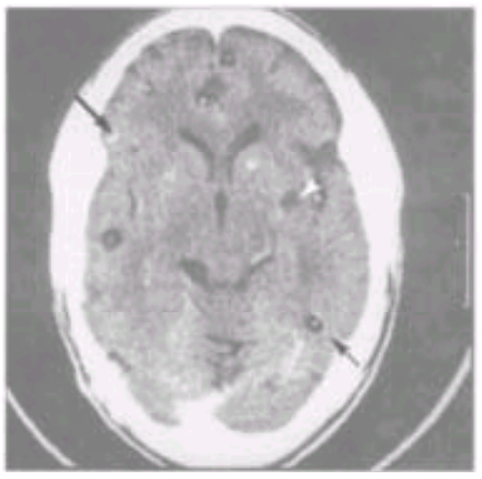


Figure 168-3 A sparganum worm dissected from an inguinal mass.

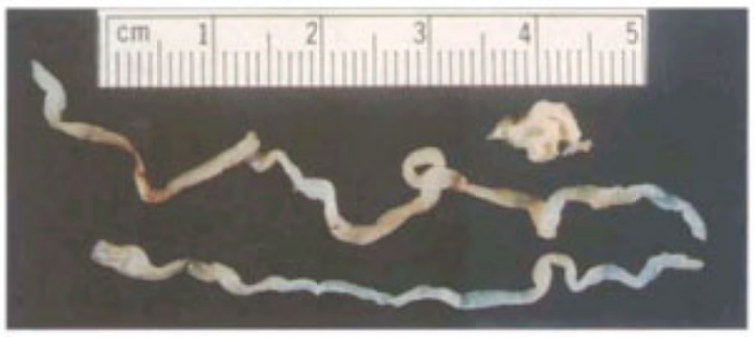


Figure 168-4 *Taenia saginata.* A mature worm may be over 33 feet (10m) long.

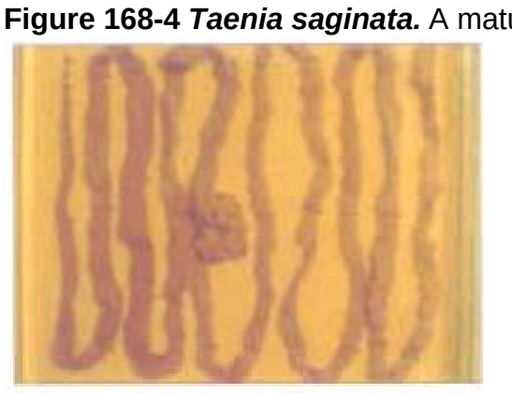


Figure 168-5 An adult liver fluke (*Clonorchis sinensis*). The worm is typically about 0.75 inches (2cm) in length.

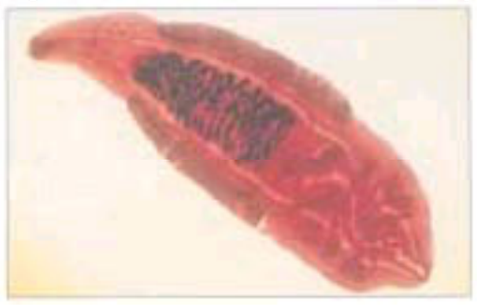


Figure 168-6 Low-power section of a human bile duct containing adult *Clonorchis sinensis* flukes.

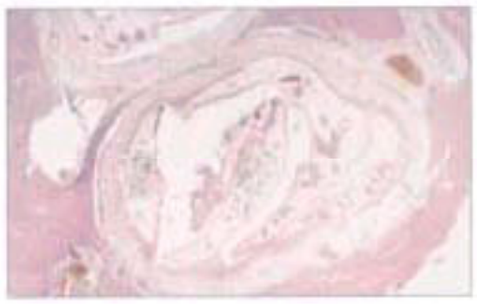


Figure 168-7 Shadowing in the right upper zone with cavitation in a 6-year-old-boy with an African lung fluke infection (*Paragonimus uterobilateralis*).

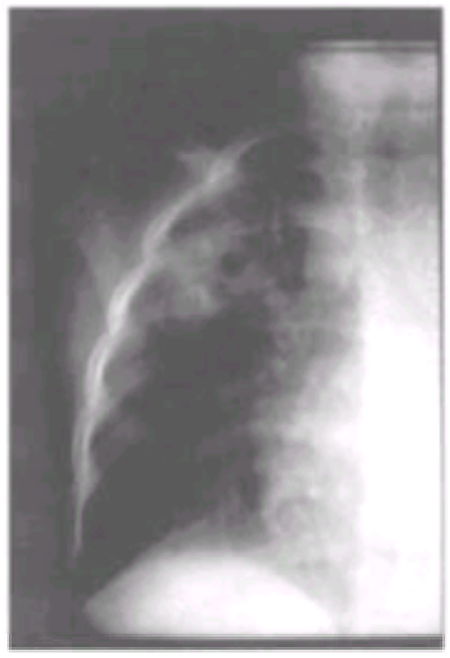


Figure 169-1 Life cycle of *Echinococcus granulosus.* Adult tapeworms parasitize the small intestine of definitive hosts, mainly dogs (1). Parasite proglottids and eggs are shed with the feces (2), such eggs being infectious for intermediate hosts including humans (3). Hydatid cyst formation occurs predominantly in the liver (4), but also in lungs and other organs. Imaging techniques such as CT (5) demonstrate well-delineated, fluid-filled, usually unilocular bladder-like lesions. Internal daughter cysts may be visible in larger cysts as septated segments within the primary cyst. Histologically, the cyst by itself consists of a very thin inner germinal and nucleated layer with a predominantly syncytial structure (6). The germinal layer is externally protected by an acellular laminated layer of variable thickness. The endogenous formation of brood capsules and protoscolices is a prerequisite for completion of the life cycle (6), which occurs when definitive hosts ingest protoscolex-containing hydatid cysts.

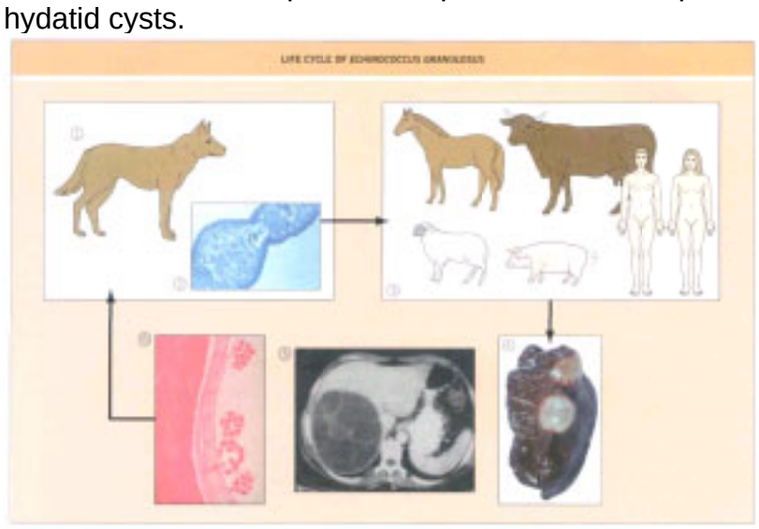


Figure 169-2 Primary sites of metacestode development in humans. Organ distribution of the primary sites of metacestode development for *Echinococcus granulosus* (cystic echinococcosis) and *Echinococcus multilocularis* (alveolar echinococcosis) in human disease.

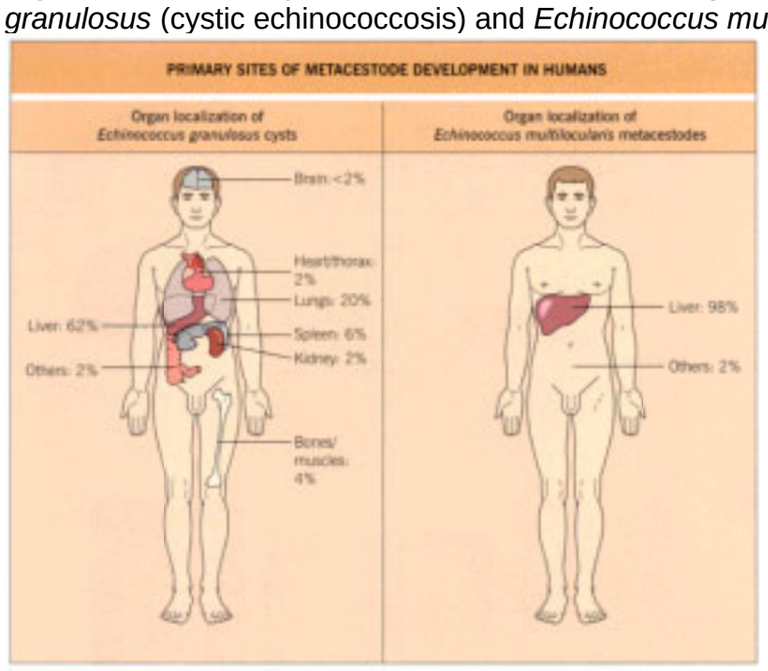


Figure 169-3 Life cycle of *Echinococcus multilocularis.* This involves predominantly foxes as definitive hosts (1) and occasionally other carnivores such as domestic dogs or house cats. Egg production by the tapeworm starts as early as 28 days after infection (2). Eggs must be ingested by a suitable intermediate host (3), including humans and various rodent species (4). As a result, the parasite metacestode primarily becomes established in the liver. Macroscopically, the typical lesion is characterized by a dispersed mass of fibrous tissue with a multitude of interconnected vesicles ranging from a few millimeters to centimeters in size (5). The lesion often contains focal necrotic zones with scattered calcifications, as demonstrated by CT (6). Histologically, the hepatic lesion consists of a conglomerate of small vesicles and cysts demarcated by a thin laminated layer with or without an inner germinal layer and, predominantly in the rodent intermediate host, protoscolex formation (7). Oral ingestion of protoscolex-containing metacestodes by definitive hosts completes the life cycle.

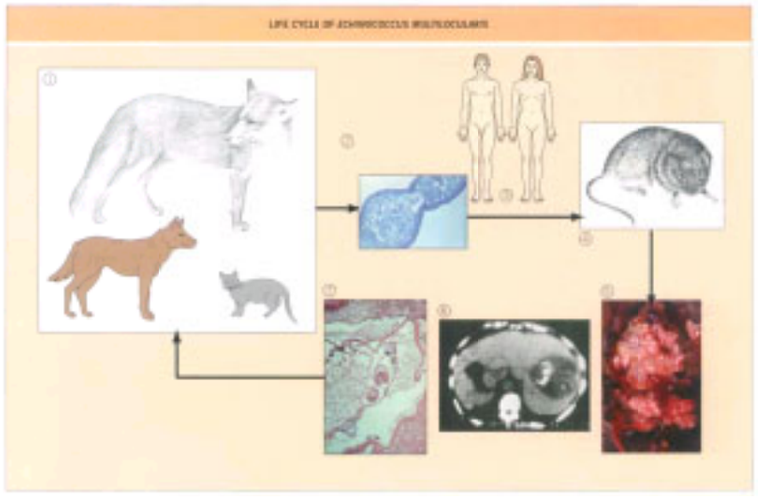


Figure 170-1 Elephantiasis. (a) Already advanced elephantiasis in a 14-year-old Indian girl who has bancroftian filariasis. Although such clinical expression of filarial disease is more commonly seen in adults, infection in endemic areas is usually established in early childhood. (b) Scrotal elephantiasis in an adult man who has bancroftian filariasis.

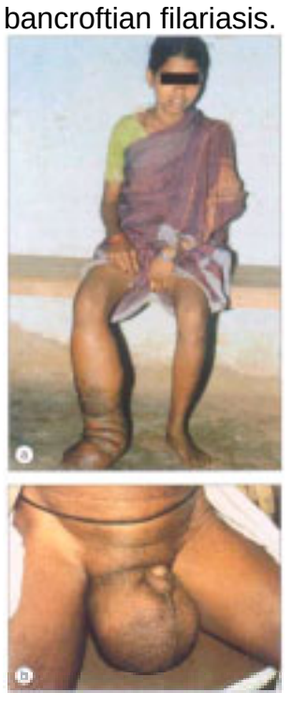


Figure 170-2 Onchocerciasis. Evidence of excoriation caused by the patient's trying to relieve the maddening pruritus caused by onchocerciasis. Note also the marked dermal atrophy associated with chronic infection.

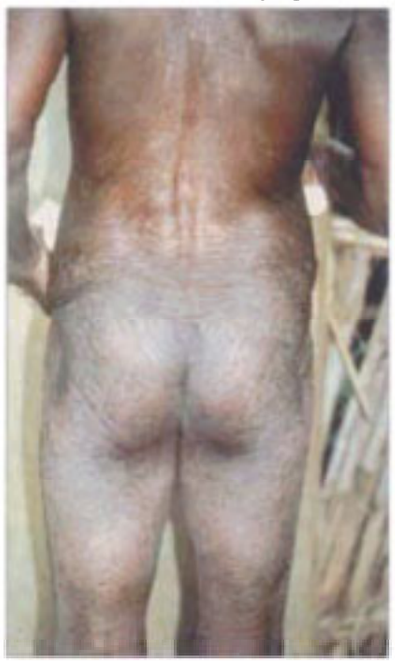


Figure 170-3 *Loa loa* adult worm. The worm has been teased from the subcutaneous tissue after incision was made through a small pruritic papule (0.5cm in diameter) in an expatriate patient who had loiasis. Such papules can occur spontaneously or after treatment with DEC.

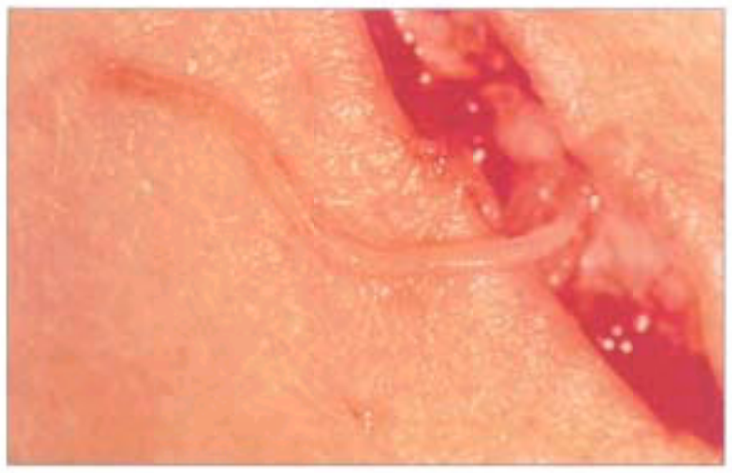


Figure 171-1 Erythrocyte passage into the venous sinuses of the spleen. A red cell passing between endothelial cells (arrow shows direction of movement) from the cordal tissue to the vascular sinus. Sickle cells do not have this deformability, so they accumulate in the spleen. Modified from Weiss.[1]

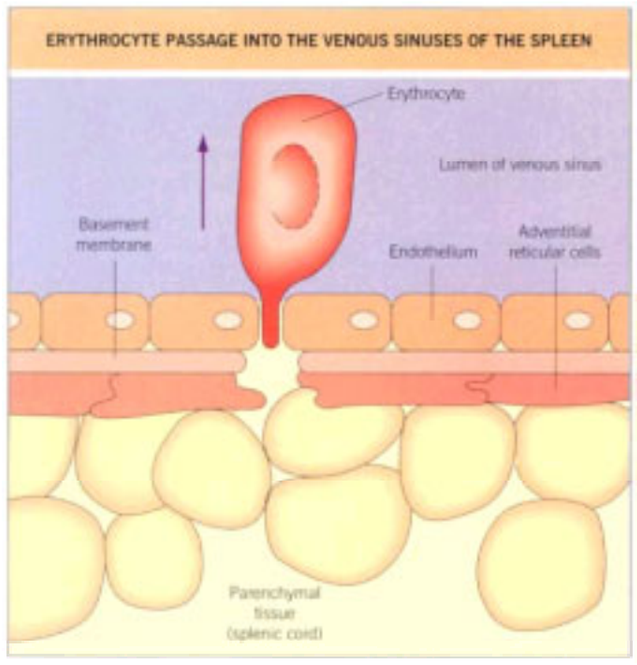


Figure 171-2 ^{99m} Technetium sulfur colloid scans (posterior view) in a 2-year old child with SS disease and splenomegaly. (a) The scan shows hepatic uptake but no splenic uptake. (b) A repeat scan 6 days after a blood transfusion shows restoration of splenic uptake of colloid. From Pearson *et al.*[5]

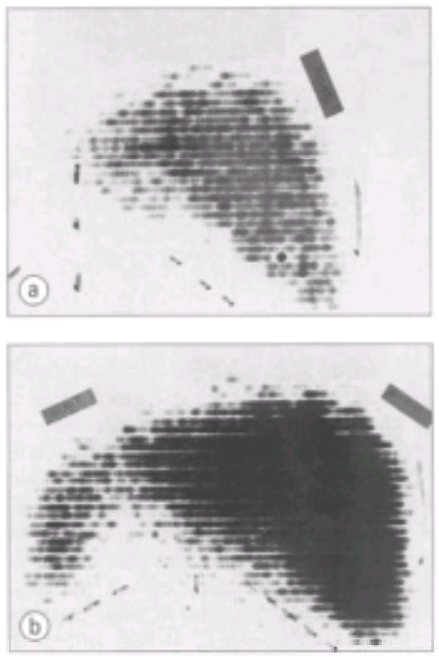


Figure 171-3 Experience with pneumococcal sepsis in Jamaican children from birth to 6 years of age. Pre-trial experience shows that sepsis episodes commenced at 6 months and 9 or 10 episodes occurred before 3 years. During penicillin prophylaxis from 6 months to 3 years, no episodes occurred but seven episcodes occurred after cessation of penicillin. The numbers by the arrows refer to pneumococcal serotypes.

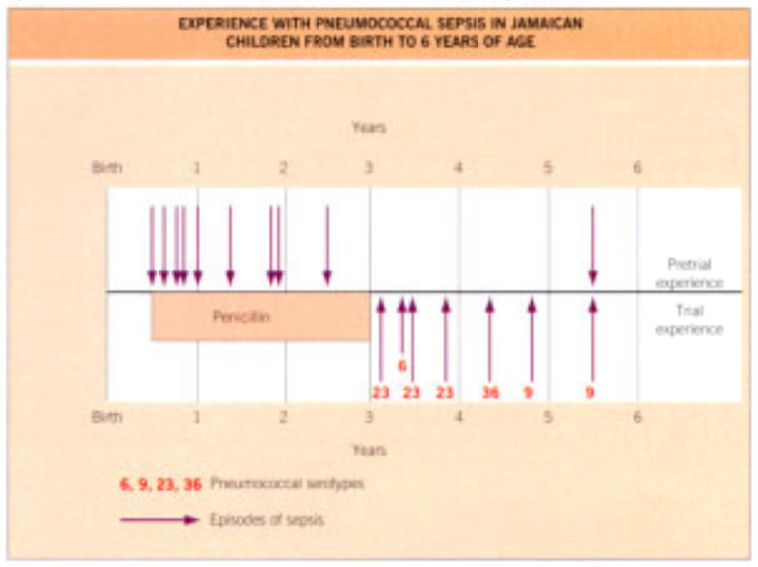


Figure 172-1 Global distribution of visceral leishmaniasis. More than 90% of VL cases occur in India/Nepal/Bangladesh, Sudan/Ethiopia and Brazil.

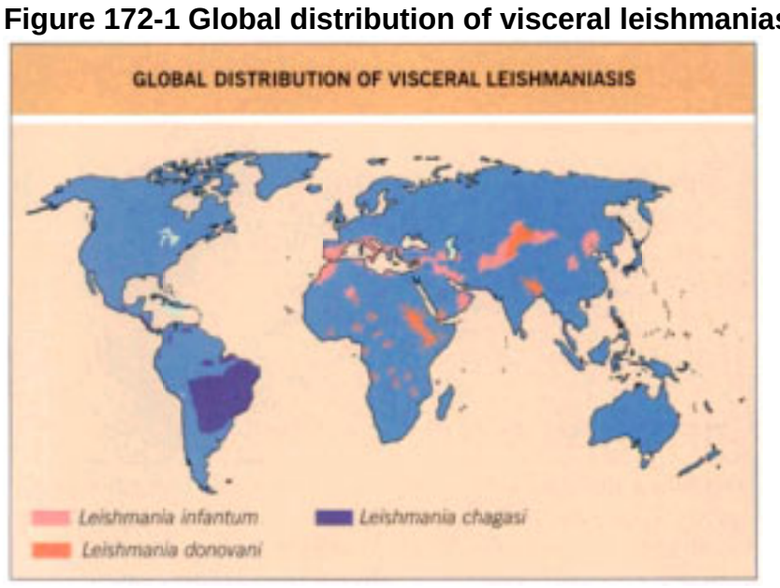


Figure 172-2 Global distribution of cutaneous leishmaniasis. More than 90% of CL cases occur in the regions of Brazil/Peru, Algeria, Saudi Arabia and Syria/Iraq/Iran/Afghanistan.

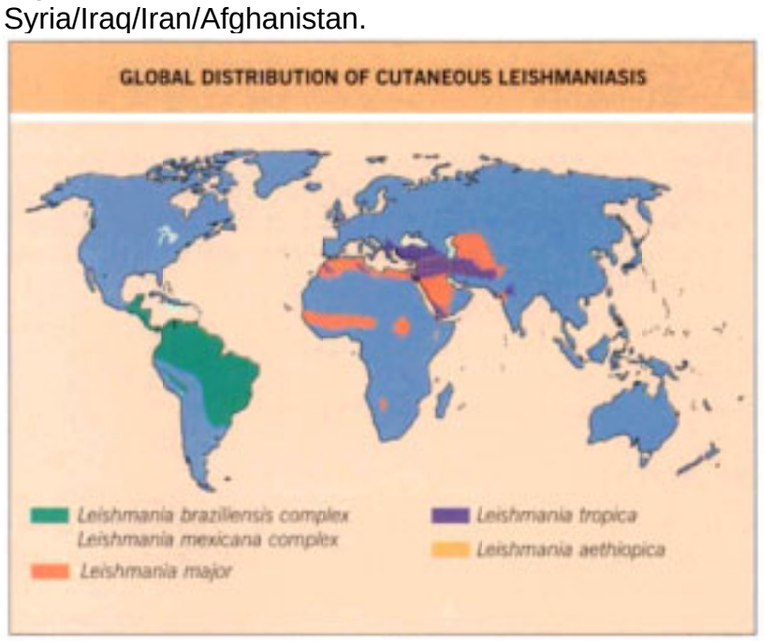


Figure 172-3 Visceral leishmaniasis. (a) Hepatosplenomegaly and pallor in a 29-year old Italian man. (b) Splenomegaly and pallor in a 23-year old Angolan. Both complained of weight loss, fatigue and fever of several weeks' duration.

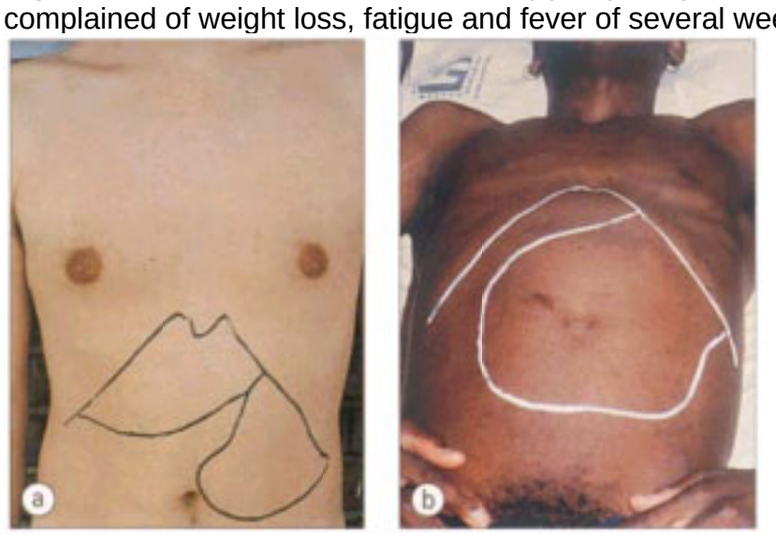


Figure 172-4 Cutaneous leishmaniasis. *Leishmania tropica* recidivans leishmaniasis lesions on the face and forearm of a Syrian girl. These had been present for 4 years with slow healing in the center and multiple recurrences despite courses of intralesional meglumine antimonate.

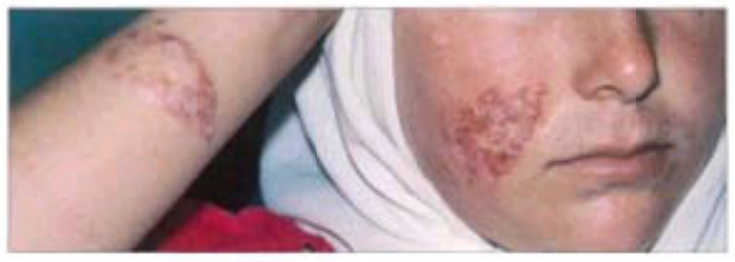


Figure 172-5 Mucocutaneous leishmaniasis. A young man from Peru who had a 2-year history of slow enlargement of the lips and ulceration of the nostrils. *Courtesy of Professor Luis Valda Rodriguez.*

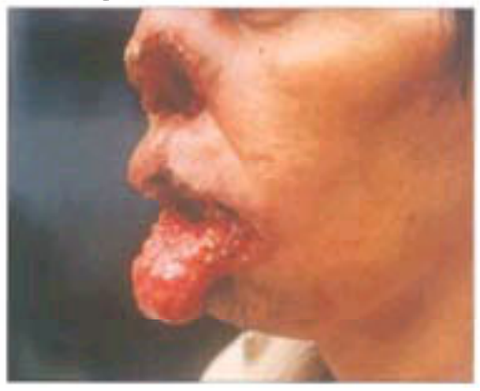


Figure 172-6 Amastigotes (Leishman-Donovan bodies) in bone marrow aspirate from a patient who had *Leishmania infantum* visceral leishmaniasis and AIDS. The nucleus and kinetoplast stain deeply with Giemsa and give the organism its characteristic appearance. *Histoplasma* spp. are the main source of mistaken identification in bone marrow smears, but lack these structures. Amastigotes measure 2–3μm in length and are found within macrophages in tissue sections, but usually lie free in smears because infected macrophages burst as they are smeared.

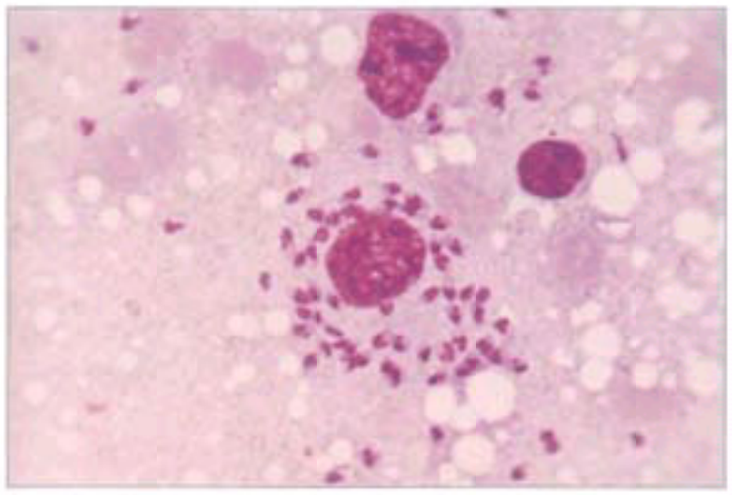


Figure 172-7 Splenic aspiration. The picture shows a splenic aspirate being performed under field conditions on a child suffering from *Leishmania donovani* kala-azar in south Sudan. The procedure is simple, painless and safe if the prothrombin time is normal and the platelet count is above 40×10^9 /l. Palpate the spleen and mark its outline. Using a 1.25 inch (30mm) long 21-gauge needle attached to a 5ml syringe, penetrate the skin over the spleen. Withdraw the plunger 1 ml and plunge the needle into the spleen upwards at an angle of 45° and withdraw immediately, maintaining suction. The tiny amount of material obtained is sufficient for culture and smear. *Courtesy of Drs Robert Wilkinson and Jill Seaman.*

Figure 172-8 Slit skin smear. The picture shows a slit skin smear being taken from the edge of a chronic *Leishmania infantum* ulcer obtained in Malta. Smears are taken from the raised edge of the ulcer or center of the nodule, where amastigotes are most abundant. The skin is cleaned and then firmly pinched throughout the procedure to squeeze away blood. A 5mm-long and 3mm-deep incision is made and then the scalpel is turned through 90° and the blade is used to scrape the edge of the slit. A line of tissue scrapings is gently streaked on to a slide and the process is repeated until two or three lines of scrapings are present on at least two slides. Further scrapings and fluid oozing from the pinched slit are put into culture medium.

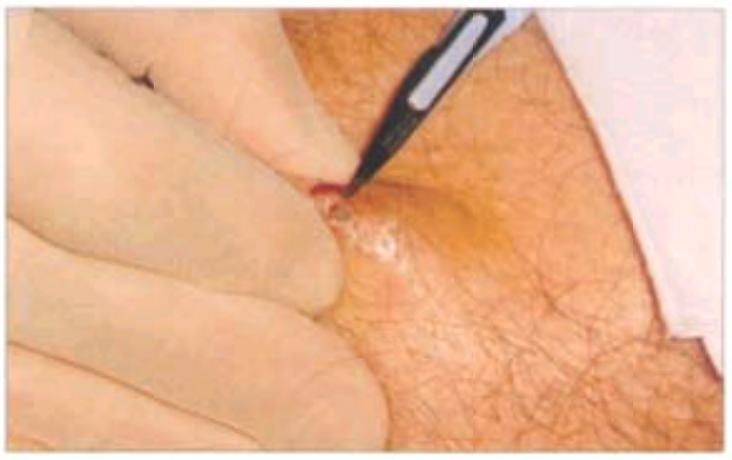


Figure 173-1 *Trypanosoma cruzi* C-shaped trypomastigote in Giemsa-stained thin blood film.

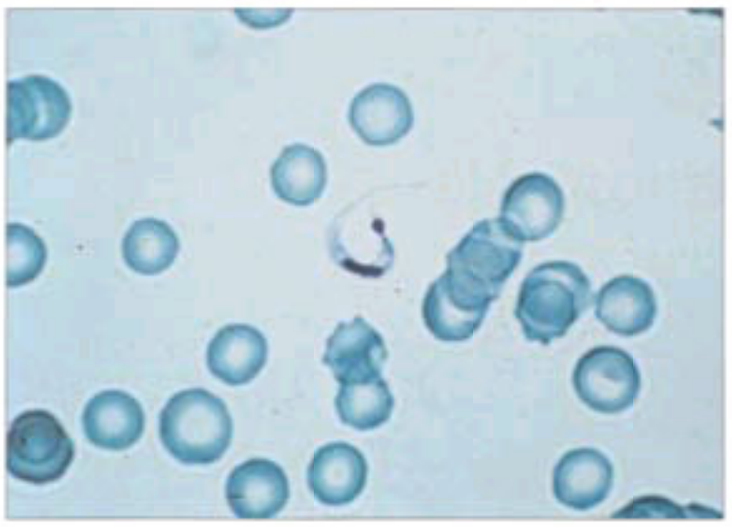


Figure 173-2 Apical aneurysm of the left ventricle in chronic Chagas' disease. *Courtesy of Dr JS Oliveira.*

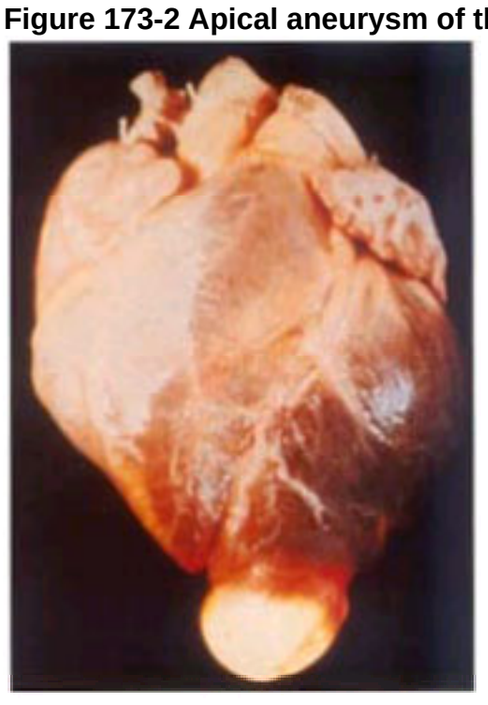


Figure 173-3 Mega-esophagus on radiograph. *Courtesy of Dr JS Oliveira.*

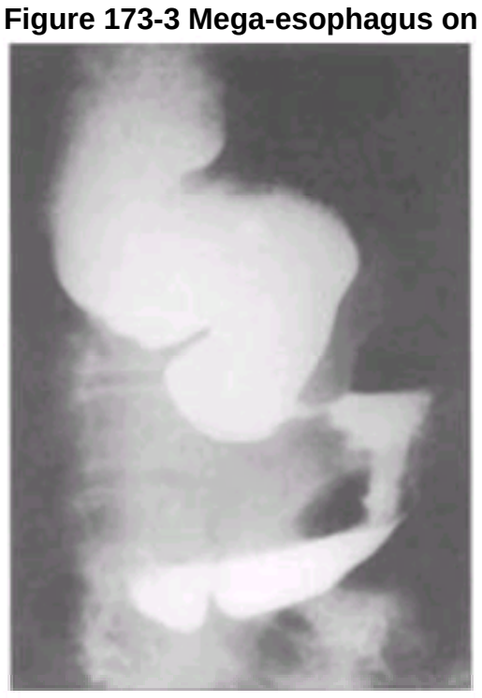


Figure 173-4 Megacolon. *Courtesy of Dr JS Oliveira.*

Figure 173-5 Romaña's sign.

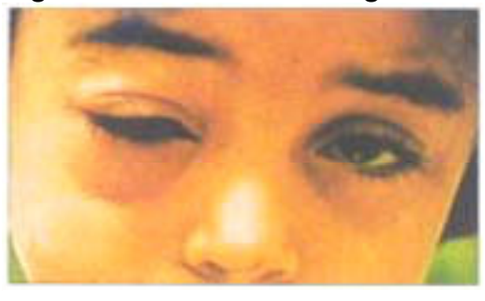


Figure 173-6 Diagnosis of Chagas' disease. ELISA, enzyme-linked immunosorbent assay; IFAT, indirect fluorescent antibody test; IHAT, indirect hemagglutination test.

Figure 173-7 Modified Duhamel-Haddad procedure for surgical correction of megacolon.[9] [19] [27]

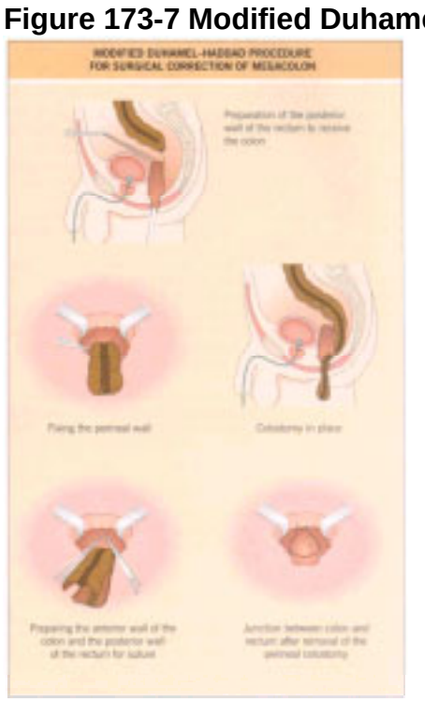


Figure 174-1 Fully embryonated egg of *Toxocara canis* hatching. To the right are two unfertilized eggs.

Figure 174-2 Life cycle of *Toxocara canis*. This demonstrates the importance of transplacental transmission in maintaining canine infection, and the role of young dogs in transmitting infection to humans.

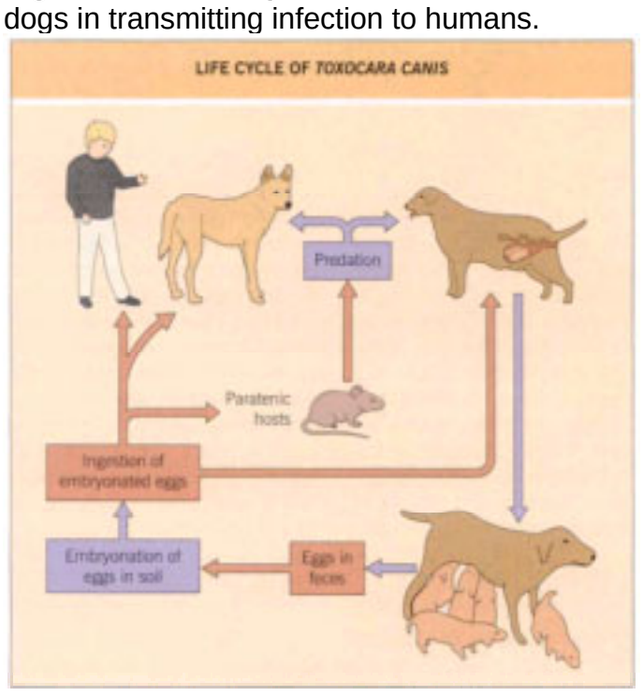


Figure 175-1 World distribution of *Burkholderia pseudomallei* and *Burkholderia pseudomallei*-like organisms. The boundaries are political and are not intended to define the true limits of distribution. *With permission from Dance.*[1]

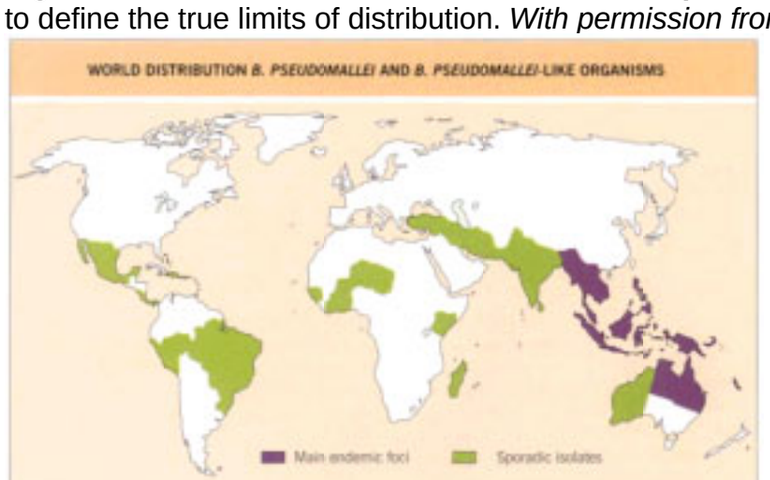


Figure 175-2 Chest radiograph of patient who has septic melioidosis. Note the multiple areas of consolidation scattered throughout both lung fields (blood-borne pneumonia). *With permission from Prof N J White.*

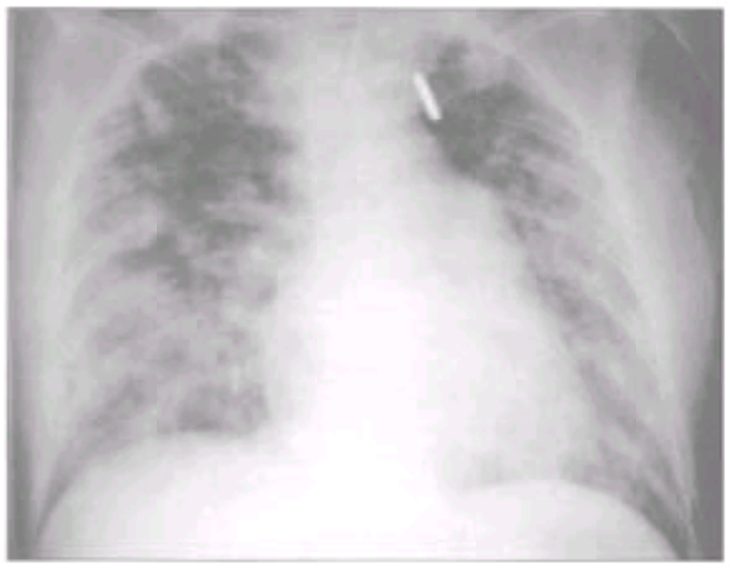


Figure 175-3 Acute suppurative parotitis. This form of melioidosis accounts for around one third of pediatric cases in north east Thailand, but has rarely been reported elsewhere. *With permission from Dance et al.*[8]

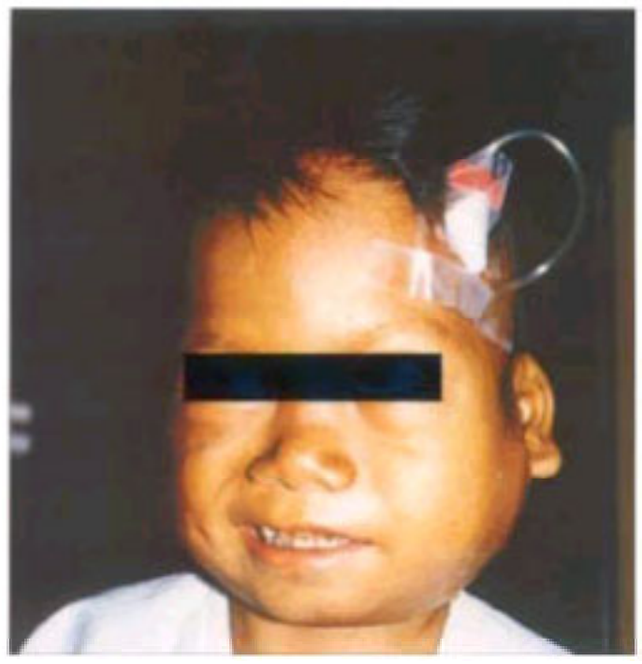


Figure 176-1 Transmission cycles of *Yersinia pestis*.

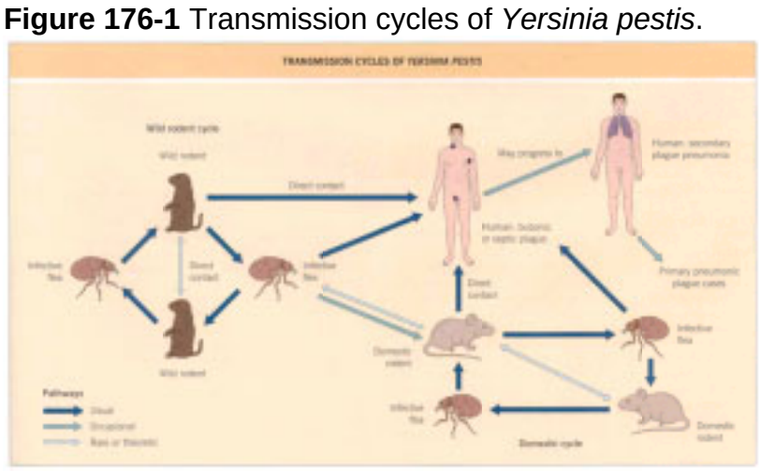


Figure 176-2 Global distribution of plague. Compiled from sources of the WHO, the Centers for Disease Control and Prevention, and the individual countries.

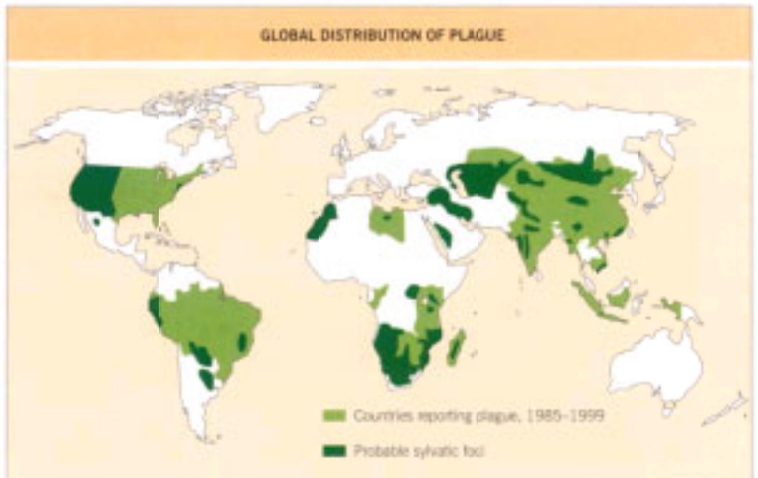


Figure 176-3 Left inguinal and femoral buboes, demonstrating surrounding edema and overlying desquamation.

Figure 176-4 Septic plague patient who demonstrated disseminated intravascular coagulation, bleeding into the skin and acral gangrene as a late manifestation.

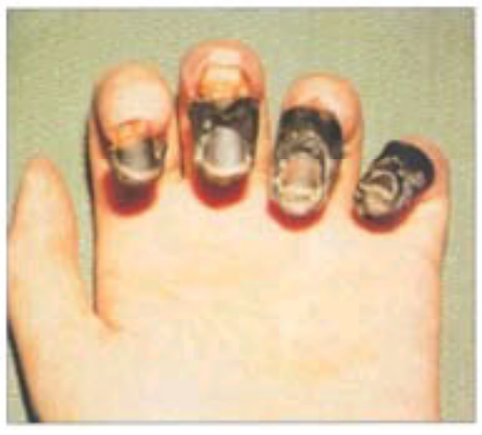


Figure 176-5 Chest radiograph of a patient who has primary plague pneumonia, showing extensive infiltrates in the right middle and lower lung fields.

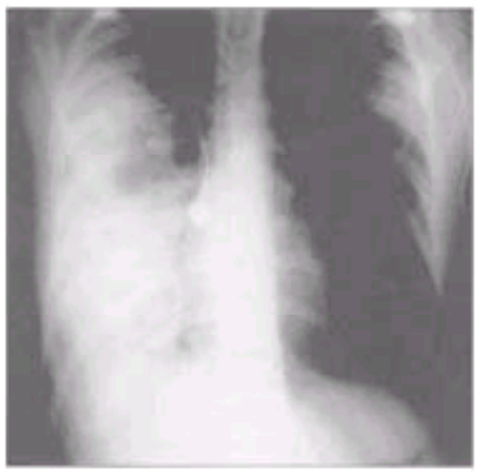


Figure 177-1 Life cycles of *Francisella tularensis.* The two major life cycles in nature are shown. In cycle (a), which is dominant in North America, *F. tularensis* is maintained predominantly among lagomorphs and hard ticks. In cycle (b), which is dominant in Eurasia, *F. tularensis* is principally maintained among cricetine rodents, especially field voles and mice, water voles and other aquatic rodents. Humans are incidental hosts that are infected by tick vectors and by the bites of flies or mosquitoes that have contaminated mouthparts, by direct contact with infected animal carcasses or other contaminated materials, by ingestion of contaminated matter or by inhalation of infectious aerosols or dusts.

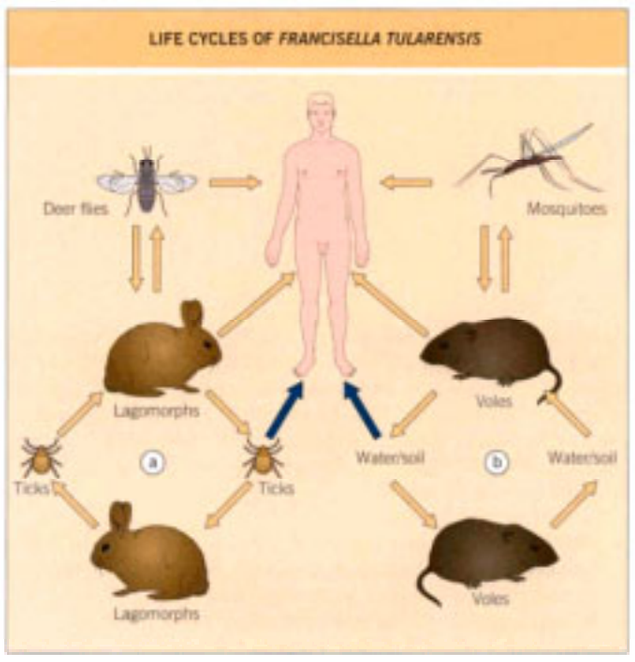


Figure 177-2 Reported cases of tularemia per 100,000 population in the USA, 1990–2000.

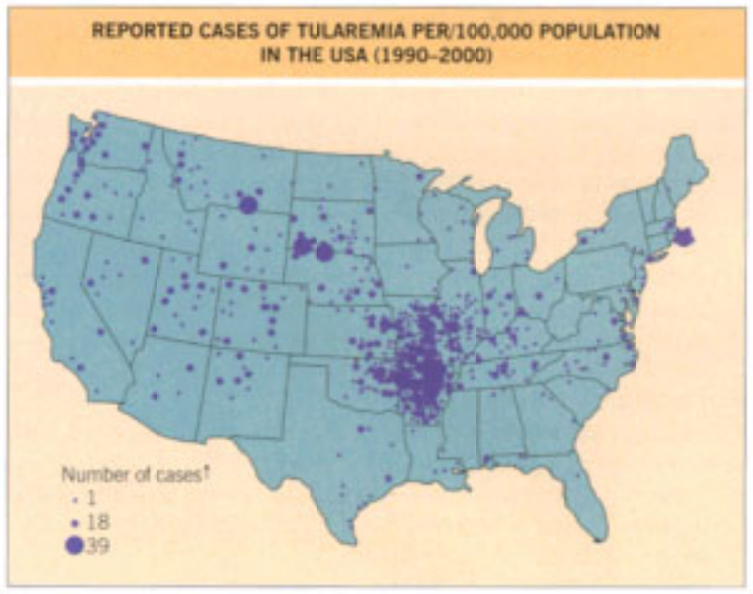


Figure 177-3 Tularemic ulcer with eschar formation after percutaneous inoculation of *Francisella tularensis*.

Figure 178-1 Reported cases of diphtheria 1980–2000. Cases reported to the WHO worldwide and within the WHO European Region.

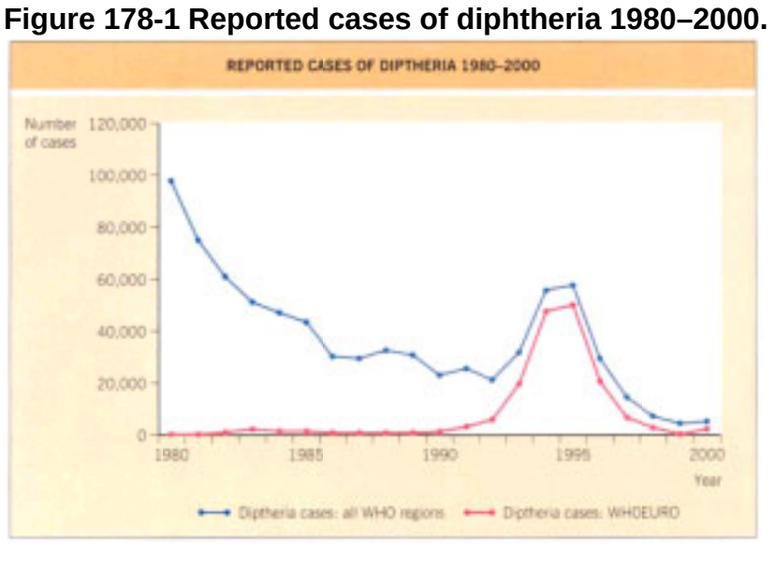


Figure 178-2 Characteristic diphtheria pseudomembrane in a child. *Courtesy of Dr Norman Begg.*

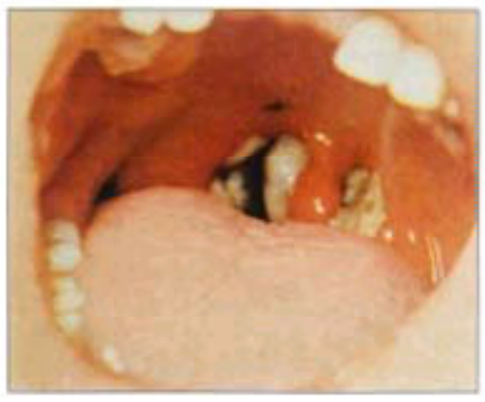


Figure 178-3 Characteristic diphtheria lesion of the lower limb, showing the classic rolled, 'crater-like' edge and eschar.

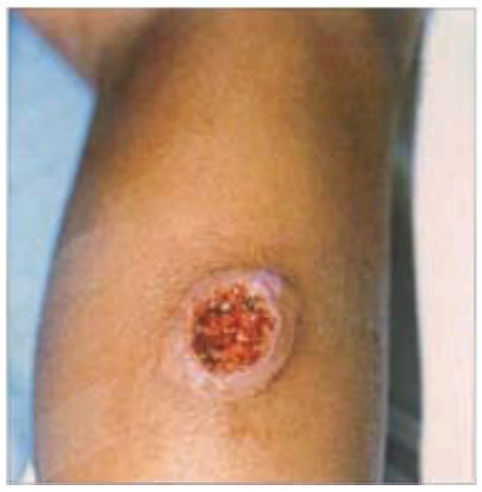


Figure 179-1 Macular rash in a patient with scrub typhus.

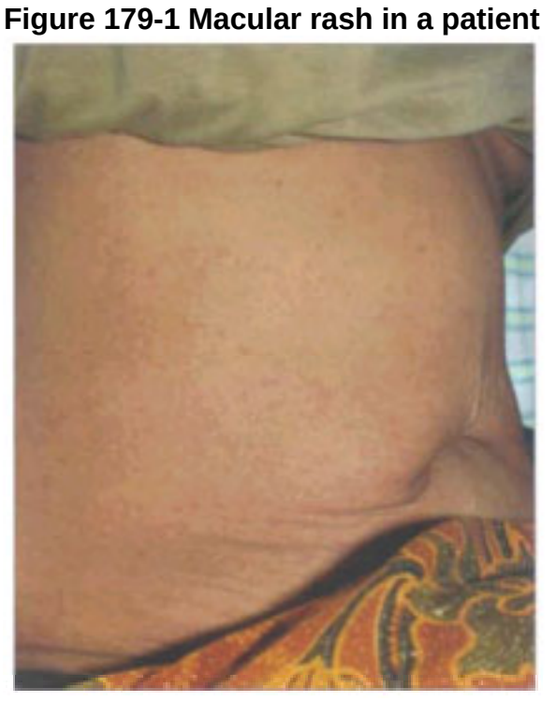


Figure 179-2 Eschar at the bite site, a hallmark of rickettsial diseases.

Figure 180-1 CT scan of fine needle aspiration of paraspinal abscess (arrow) in a patient with brucellosis.

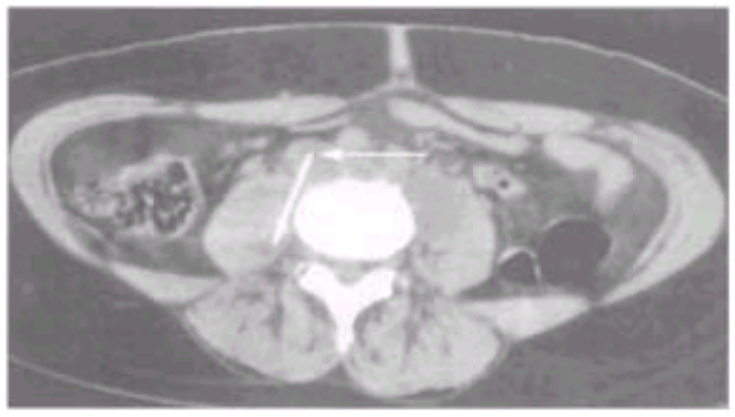


Figure 180-2 Radiograph of the lumbar spine in a patient who has discitis and spondylitis of L_{3-4} caused by brucellosis. Note the reduced disc space and the destruction of the upper articular margins of L_4 (arrows).

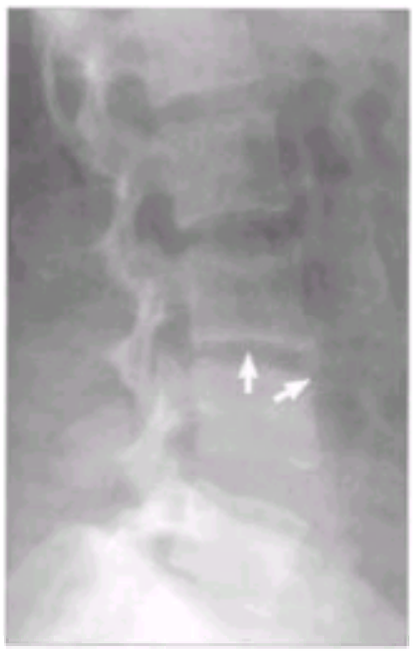


Figure 181-1 Conjunctival suffusion and jaundice.

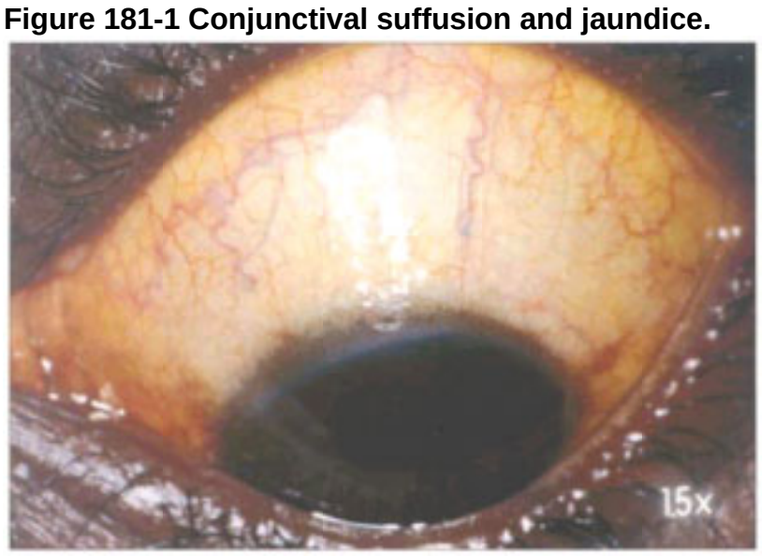


Figure 182-1 Ethiopian patient who has louse-borne relapsing fever 4 days after the start of febrile symptoms, showing subconjunctival hemorrhages and jaundice.

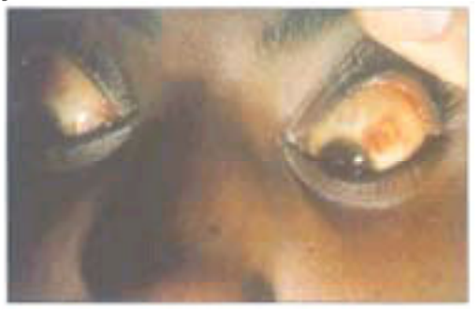


Figure 182-2 Plasma cytokine profiles in louse-borne relapsing fever. These profiles are from an Ethiopian patient treated with penicillin (at time 0 on the horizontal axis). There is a sharp increase in (TNF)-a, IL-6 and IL-8 concentrations at the start of the phase of violent rigors.

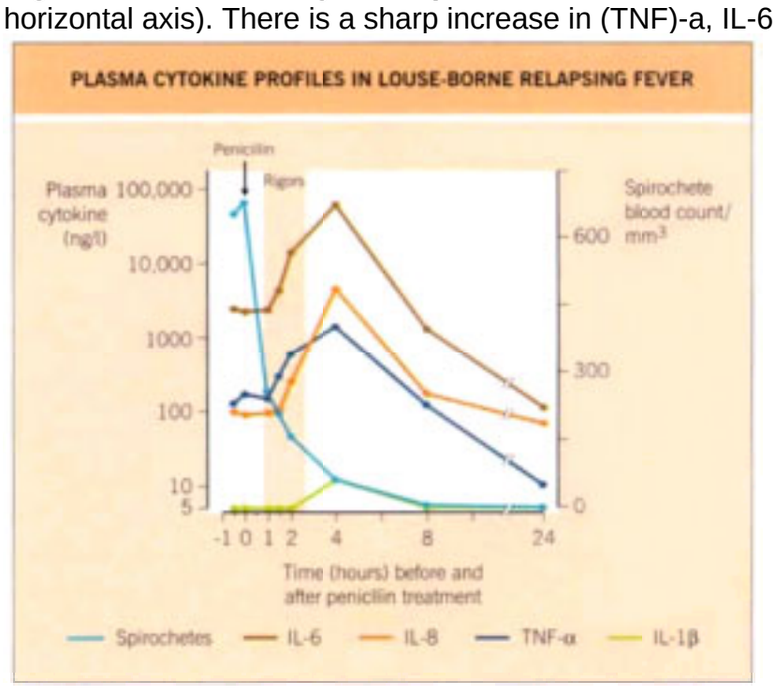


Figure 182-3 Thin blood smear from an Ethiopian patient who has louse-borne relapsing fever (Giemsa stain), showing numerous spirochetes.

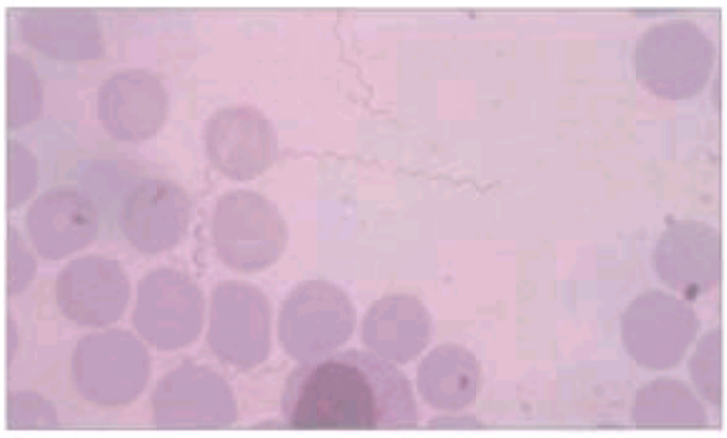


Figure 184-1 Geographic distribution of dengue.

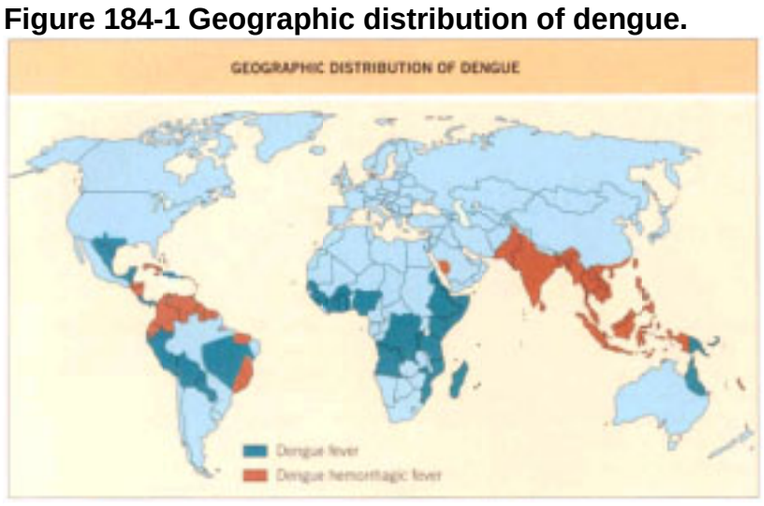


Figure 184-2 Typical clinical course of dengue hemorrhagic fever.

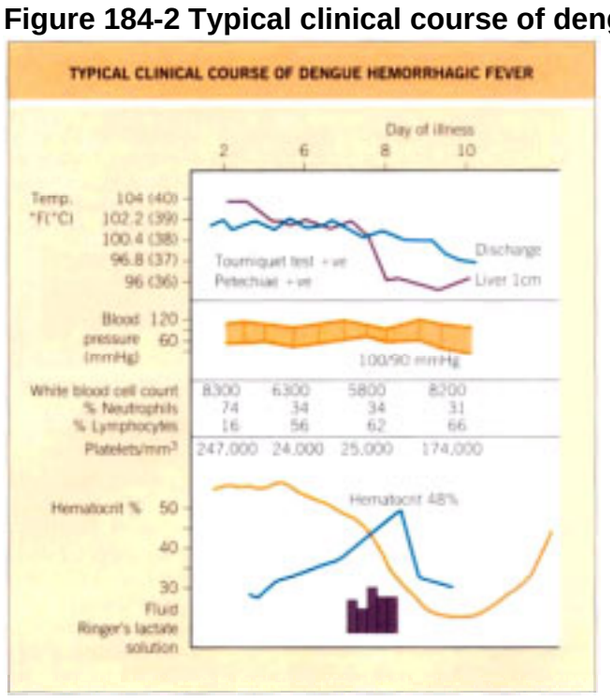


Figure 184-3 Dengue hemorrhagic fever/dengue shock syndrome. Case definition, clinical staging and treatment strategies for DHF/DSS.

DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME			
	Case definition	Grading	Management
DHF DSS	• Fever of 2 or more days duration • Hematocrit increase ≥20% recovery level • Thrombocytopenia ≤100,000/mm³ • Narrow pulse pressure (≤20mmHg) or hypotension for age	I Positive tourniquet test II Bleeding manifestations III Circulatory failure IV Profound shock	• Oral rehydration fluids in outpatient department • If hematocrit increases, hospitalize • Monitor pulse rate, hematocrit, urine output Infuse 5% dextrose in Ringer's lactate 7ml/kg/h • If hematocrit increases, add colloid • With improvement reduce infusion rate to avoid overhydration

Figure 185-1 A cutaneous anthrax lesion with extensive erythema and hemorrhagic bullae on the wrist.

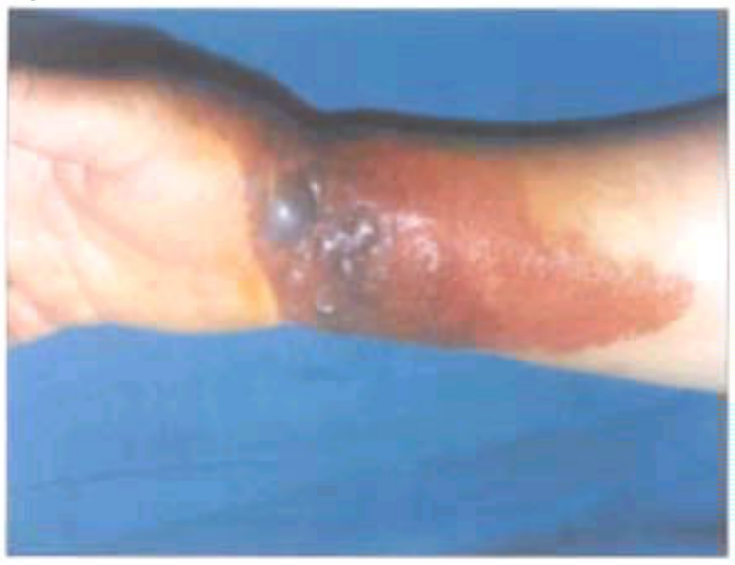


Figure 185-2 Cutaneous anthrax. (a) A well developed lesion on the right forearm (third day of disease). (b) The extension of the skin lesion in the same patient on the sixth day. Extensive edema, induration and bullous changes have occurred over the last 3 days despite antibiotic therapy. Antibiotic therapy does not prevent inflammatory reactions.

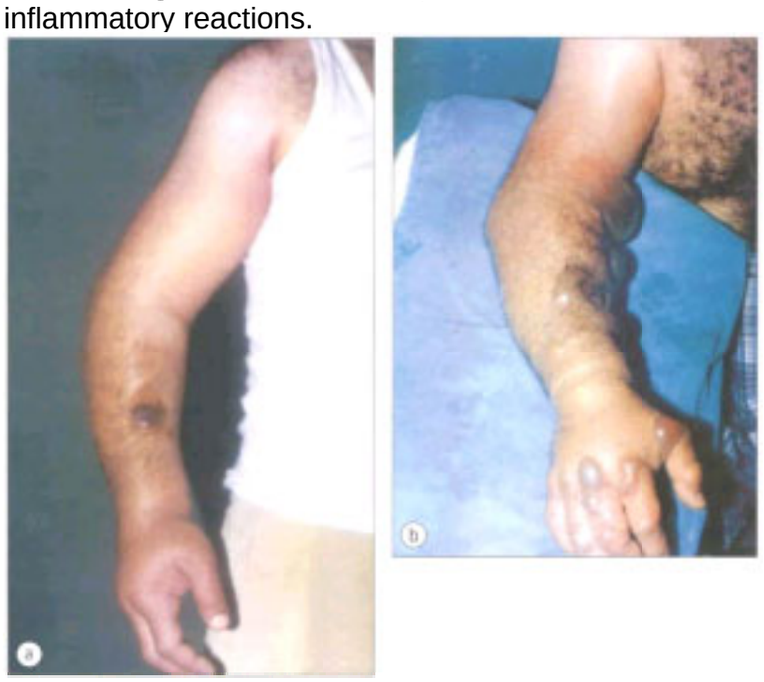


Figure 185-3 A dried black anthrax eschar on the eyelids on the 15th day of therapy (third week of the disease). The lesion healed leaving a deep scar. *Courtesy of Professor O Ural, Konya, Turkey.*

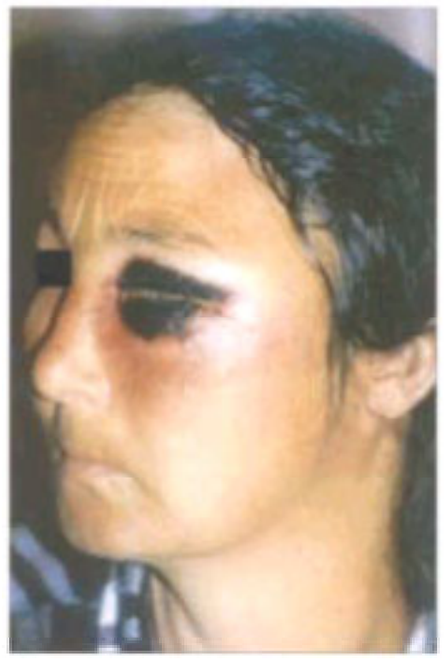


Figure 185-4 An anthrax lesion of the eyelids surrounded by erythema and massive edema extending from the left eye to the right and down to and beyond the neck. Such extensive edema is characteristic of anthrax. This lesion healed with therapy and left a deep scar.

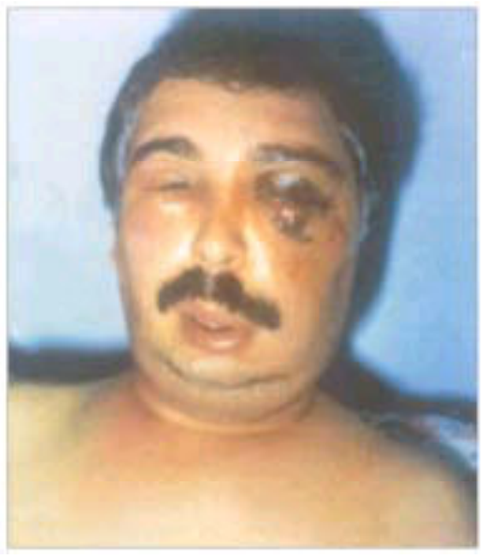


Figure 186.b-1 Lake Malawi.

Figure 186.b-2 Giant urticaria associated with Katayama syndrome after swimming in Lake Malawi. *Courtesy of Dr ME Jones, Edinburgh.*

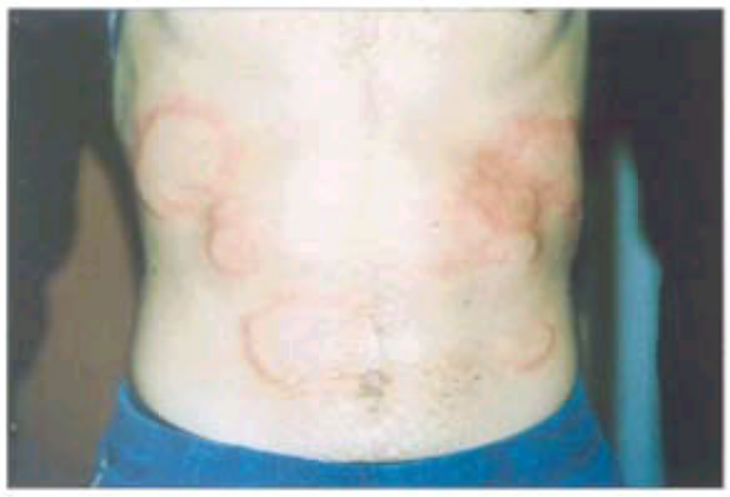


Figure 186.b-3 Empty ovum case and hatched miracidium of *Schistosoma haematobium* in semen. Note oligospermia, which usually resolves after treatment.

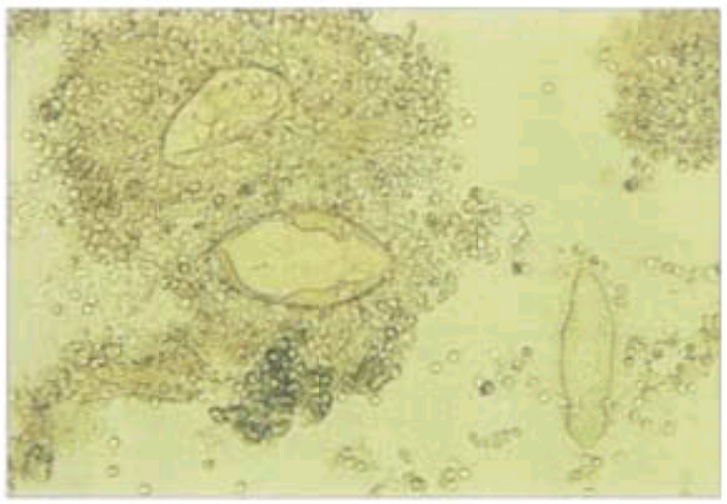


Figure 186.d-1 Heavy placental sequestration of malaria parasites is a common feature of malaria in pregnancy. (a) Placenta showing villous trees and maternal circulation in the intervillous spaces (broad arrows). (b) Photomicrograph showing trophozoites in maternal red blood cells in the intervillous spaces.

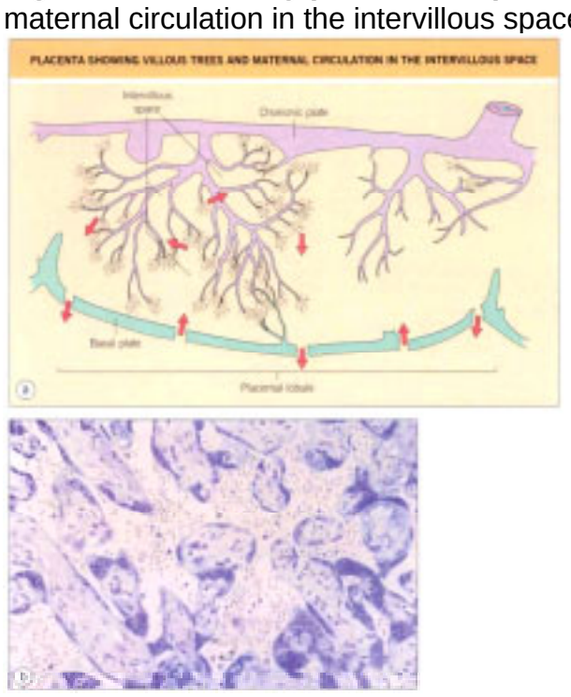


Figure 187-1a A disc diffusion sensitivity plate showing a fully sensitive coliform tested against a typical range of first-line antibiotics. *Courtesy of Dr M Cubbon, Brighton, UK.*

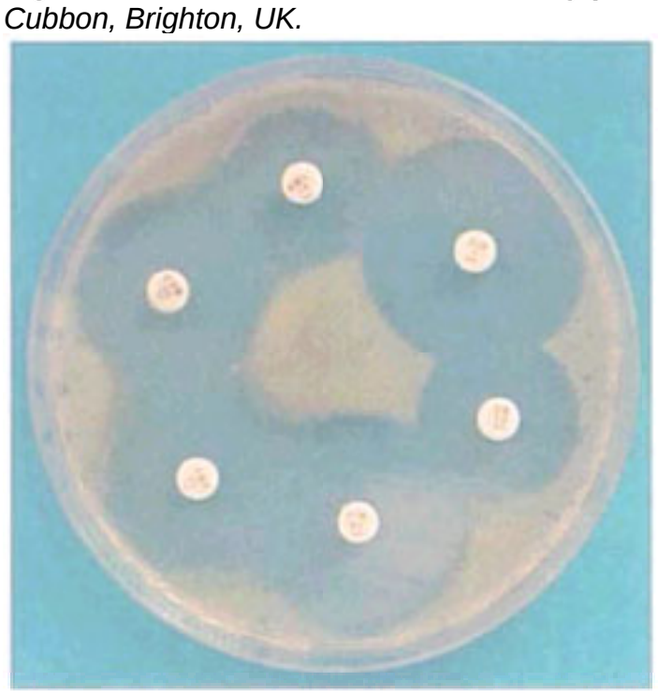


Figure 187-1b An E-test showing a methicillin-sensitive S. *aureus*. The MIC of oxacillin for this strain is 0.25 mg/L, and is obtained by noting the point at which the zone of inhibition intersects with the test strip. *Courtesy of Dr M Cubbon, Brighton, UK.*

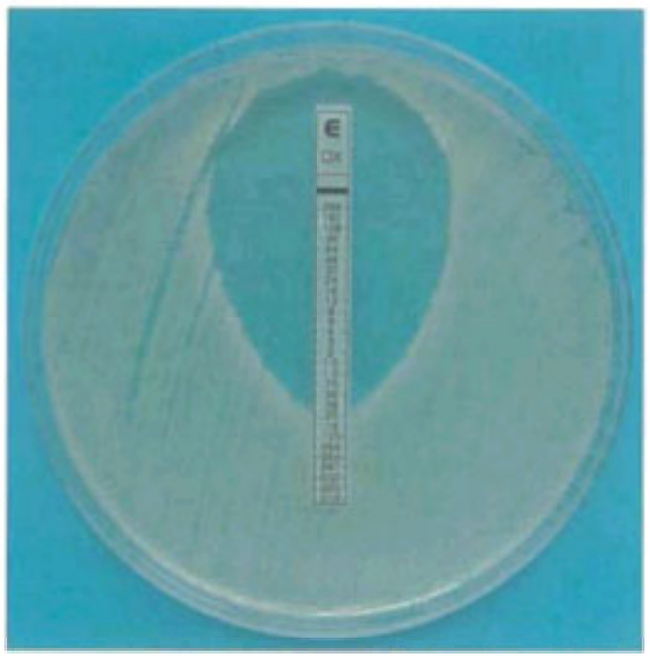


Figure 187-2 End-point determinations of the antimicrobial effect. See text for details. *Redrawn with permission of the American Society for Microbiology from Firsov et al.* [22]

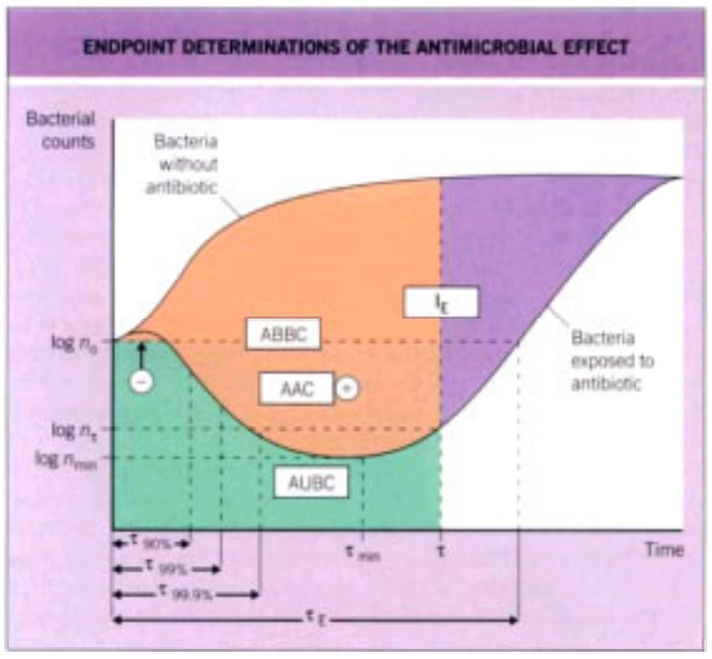


Figure 188-1 Diversity of ß-lactam antibiotics: main ring structures, names and representative antibiotics.

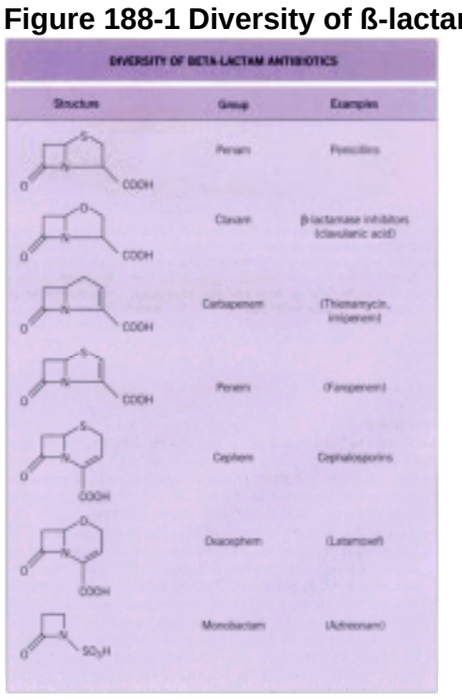


Figure 188-2 Site of action of antibiotics that perturb the synthesis of peptidoglycan. The peptidoglycan unit is formed in the cytosol of bacteria by the binding to uridine diphosphate (UDP)-*N*-acetylmuramic acid of a short peptide (the nature of which differs between bacteria). This precursor is then attached to a lipidic carrier and added to *N*-acetylglucosamine before crossing the bacterial membrane. At the cell surface peptidoglycan units are reticulated by the action of transglycosylases (catalyzing the polymerization between sugars) and of transpeptidases (catalyzing the polymerization between peptidic chains). The antibiotics act as follows: fosfomycin is an analog of phosphoenolpyruvate, the substrate of the *N*-acetylglucosamine-3-*o*-enolpyruvyl transferase synthesizing *N*-acetylmuramic acid from *N*-acetylglucosamine and phosphoenolpyruvate; cycloserine is an analog of D-Ala and blocks the action of D-Ala racemase and D-Ala:D-Ala ligase; bacitracin inhibits the transmembrane transport of the precursor; vancomycin binds to D-Ala-D-Ala termini and thus inhibits the action of transglycosylases and transpeptidases; and ß-lactams are analogs of D-Ala-D-Ala and suicide substrates for transpeptidases.

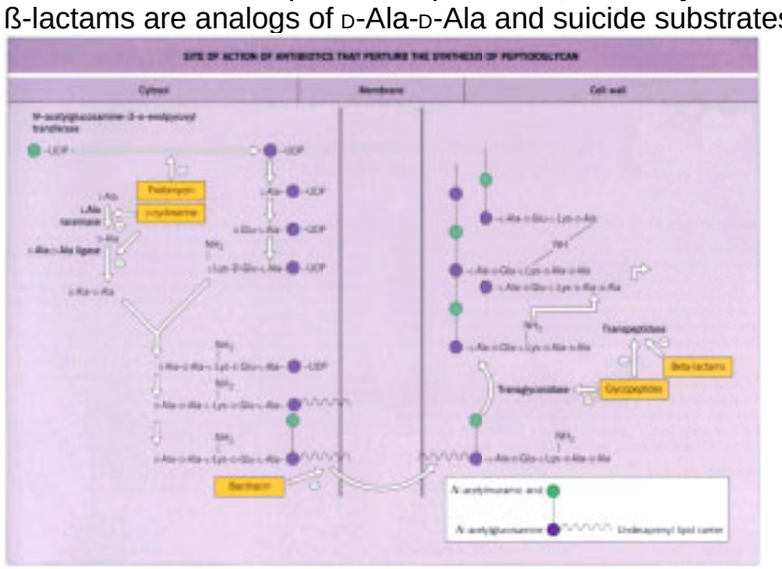


Figure 188-3 ß-Lactam antibiotics as substrates for transpeptidases and ß-lactamases. The left part of the illustration shows how a ß-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one ß-lactam per hour), making the enzyme inactive. The right part of the illustration shows that the same reaction occurs in the case of a ß-lactamase. Hydrolysis of the acylated enzyme is, however, very rapid (1000 ß-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

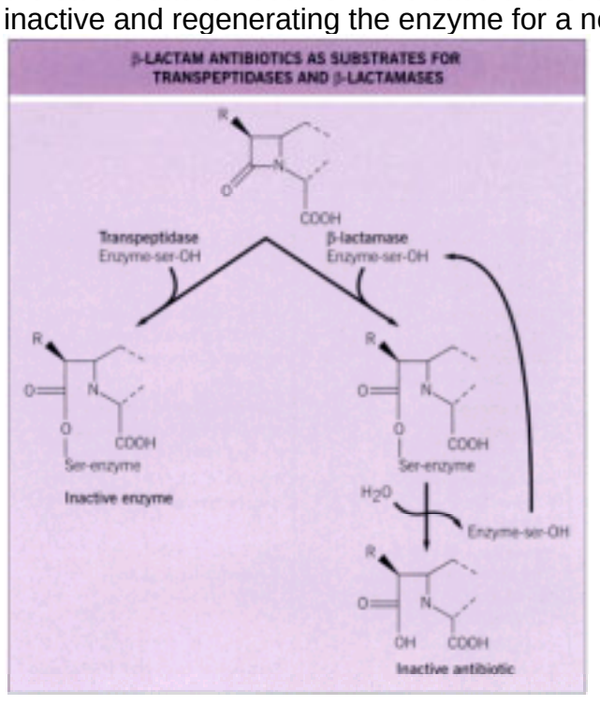


Figure 188-4 Structural modifications of ß-lactam antibiotics that overcome ß-lactamase degradation. A first strategy, applied in penicillins, cephalosporins, oxacephems and monobactams consists of the introduction of a large side chain on the nucleus, possibly containing a substituted imine or alkene. A second strategy, applied in oxacephems and cefoxitin consists of the introduction of a methoxy group on the ß-lactam ring.

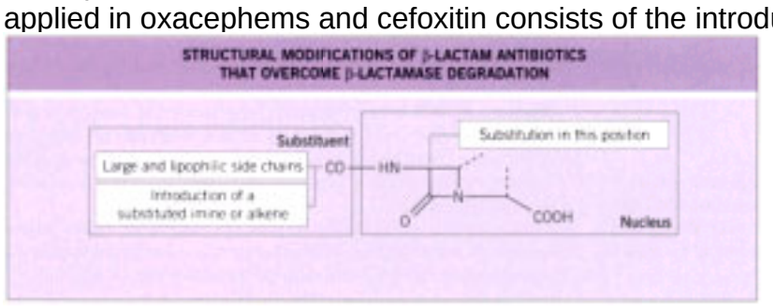


Figure 188-5 Analogy of structure between antibiotics acting on cell wall synthesis and the physiologic substrate. The two antibiotics act as analogs of the corresponding substrate.

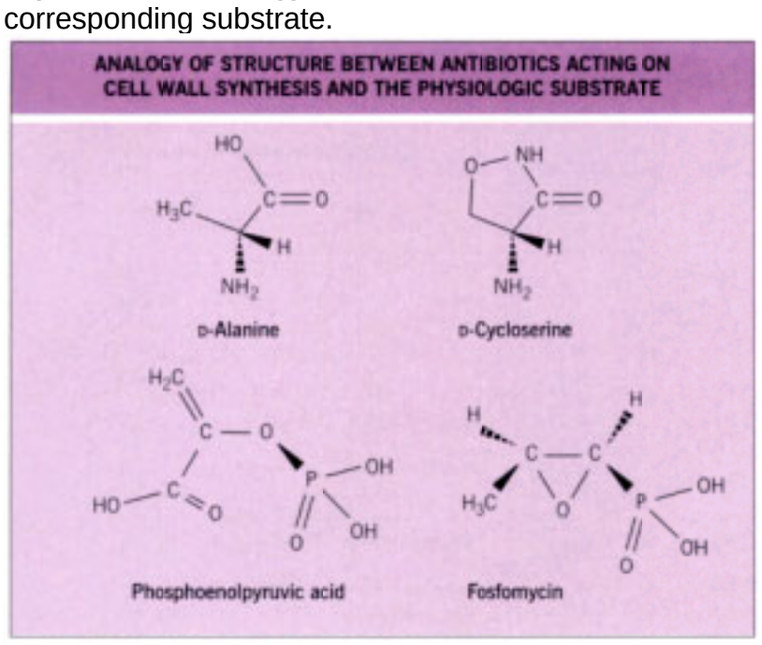


Figure 188-6 Structural formulae of the 2-deoxystreptamine-containing aminoglycosides. The numbering of the atoms shown here follows the recommendations from Nagabushe *et al.*[16] with the primed numbers (') being ascribed to the sugar attached to C4 of the 2-deoxystreptamine (as this C is of the R configuration) and the doubly primed numbers ('') being ascribed to the sugar attached to either the C6 (S configuration) for the 4,6-disubstituted 2-deoxystreptamine or the C5 (R configuration) for the 4,5-disubstituted 2-deoxystreptamine. Molecules indicated in bold denote the aminoglycosides in widespread clinical use.

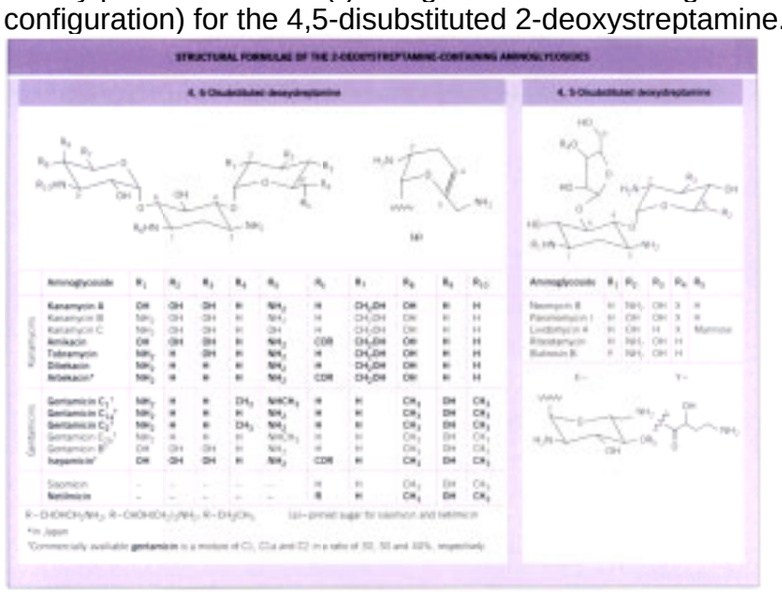


Figure 188-7 Major aminoglycoside-modifying enzymes that act on kanamycin C. This aminoglycoside is susceptible to the largest number of enzymes. The *N*-acetyltransferases (AACs) affect amino functions and the *o*-nucleotidyltransferases affect hydroxyl functions. Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (Roman numerals) with different substrate specificities (phenotypic classification). At least one enzyme is bifunctional and affects both positions 2" (*o*-phosphorylation) and 6' (*N*-acetylation). The main aminoglycosides used clinically on which these enzymes act are amikacin (A), dibekacin (DbK), commercial gentamicin (G), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T). The drug abbreviations that appear in parentheses are those for which resistance was detectable *in vitro* although clinical resistance was not conferred. *Data from Shaw et al.*[19]

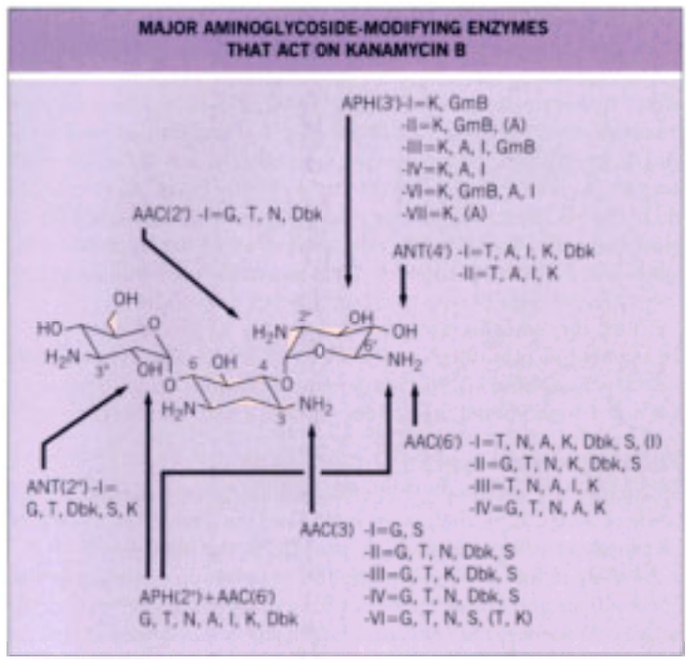


Figure 188-8 Accumulation, intrabacterial activity and efflux of tetracyclines. Tetracyclines diffuse freely through the extracellular membrane of Gram-negative bacteria. Penetration inside bacteria is an energy-dependent process depending on the pH and Mg^{2+} gradient between the extracellular medium of Gram-positive bacteria or the periplasmic medium of Gram-negative bacteria and the intracellular medium. Only the protonated form is highly diffusible, so accumulation is favored by lowering of the extracellular pH. Once inside the cytosol the tetracycline molecule forms a nondiffusible complex with Mg^{2+} . This type of complex with a bivalent cation is also the substrate of the efflux pumps present in the membrane of resistant bacteria and acting as H^+ antiports (pink circle). The antibacterial action of the teracyclines (T) is due to the binding to the 30S subunit of the ribosomes. In the pretranslocational state, tetracyclines inhibit the binding of aminoacyl tRNA (arrow 1) to the A-site (yellow part of the ribosome). In the post-translocational state, tetracyclines protrude in the P-site (white part of the ribosome) and inhibit the binding of the peptidyl tRNA (arrow 2). *Data from Geigenmüller and Nierhaus*[21] *and Yamaguchi et al.*[23]

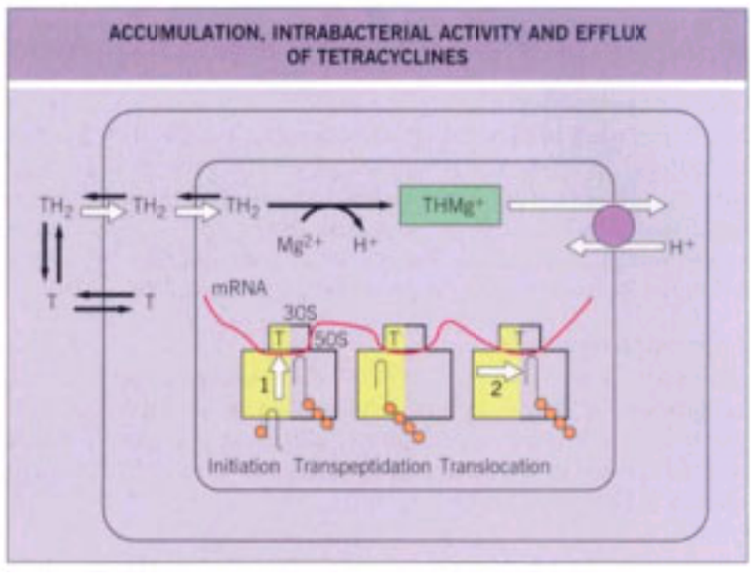


Figure 188-9 Chemical structure of the macrolides. The upper panel shows the degradation of erythromycin in the gastric milieu (substituents responsible for the instability of the miolecule are shown in gray). 16-membered macrolides and ketolides are intrinsically stable. The structural modifications conferring stability in acidic milieu to 14- and 15-membered macrolides are highlighted in gray in the middle panel. The lower panel compares the binding of macrolides and ketolides to the petidyl transferase site of the 50S subunit of ribosomes. Macrolides are characterized by a single anchoring point and ketolides by a double anchoring point, which increases the affinity of ketolides for wild type and methylated ribosomes.

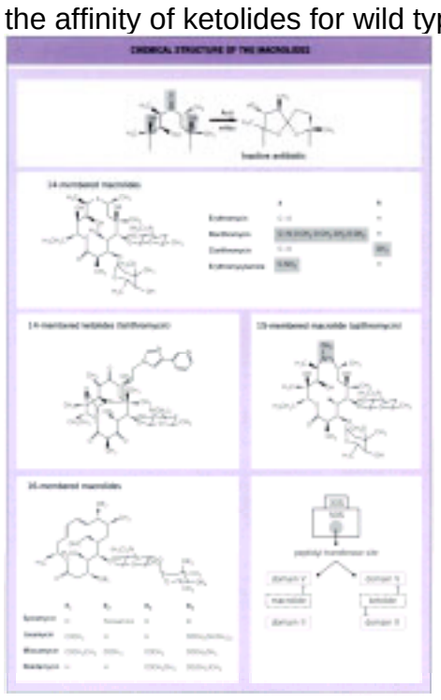


Figure 188-10 Structural activity relationship for linezolid, the first oxazolidinone, and mode of action. The drug prevents the formation of the ternary complex between mRNA, ribosome subunits and $tRNA^{met}$ necessary for protein synthesis.

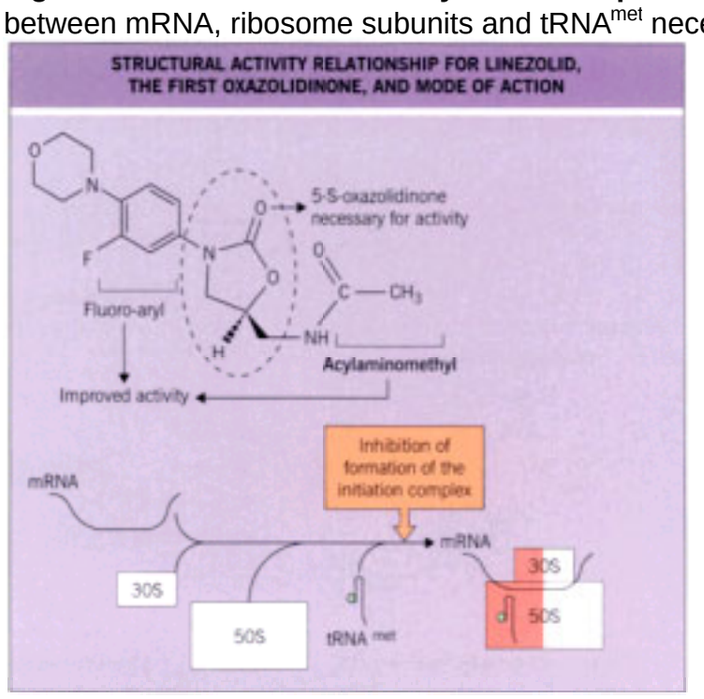


Figure 188-11 Structure-activity, structure-pharmacokinetics and structure-toxicity relationships of the fluoroquinolones. These considerations form the basis of the rational development of the new molecules of this class, which have a very extended spectrum (including Gram-positive bacteria and anaerobes), a long half-life and minimal phototoxicity and metabolic interactions.

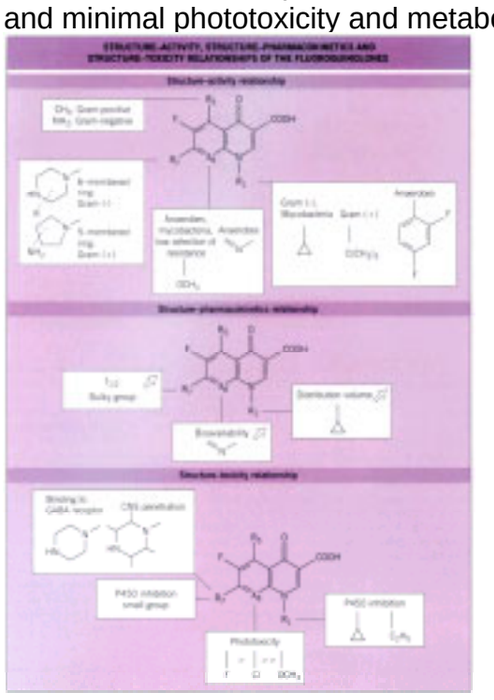


Figure 188-12 Ternary complex formed between DNA, DNA-gyrase or -topoisomerase IV and stacked fluoroquinolones. Subunits A form covalent bonds via Tyr122 with the 5' end of the DNA chain. The binding site for fluoroquinolones is located in the bubble formed during the local opening of the DNA molecule. The right panel shows the parts of the antibiotic molecules interacting with DNA, with the enzyme or favoring the stacking of the fluoroquinolone molecules. *Adapted from Shen et al.*[45]

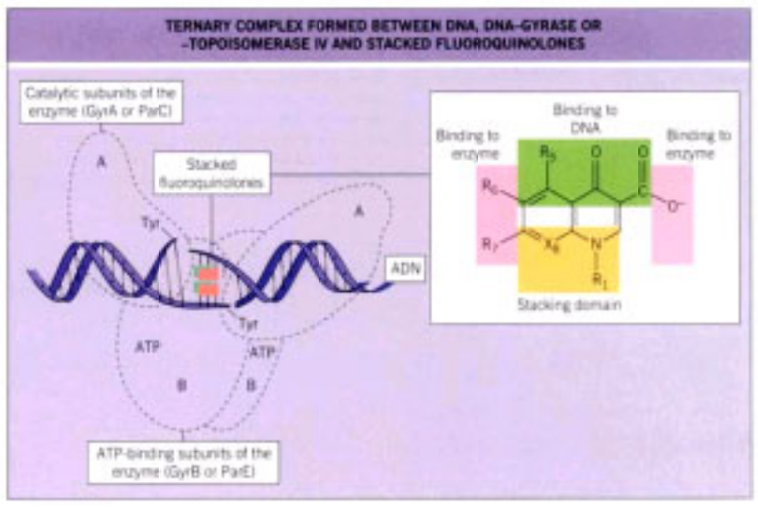


Figure 188-13 Modes of action of nitrofurans and nitroimidazoles. The modes of action include passage through the cell membrane, reduction to highly reactive products, interaction with intracellular targets and release of inactive end products.

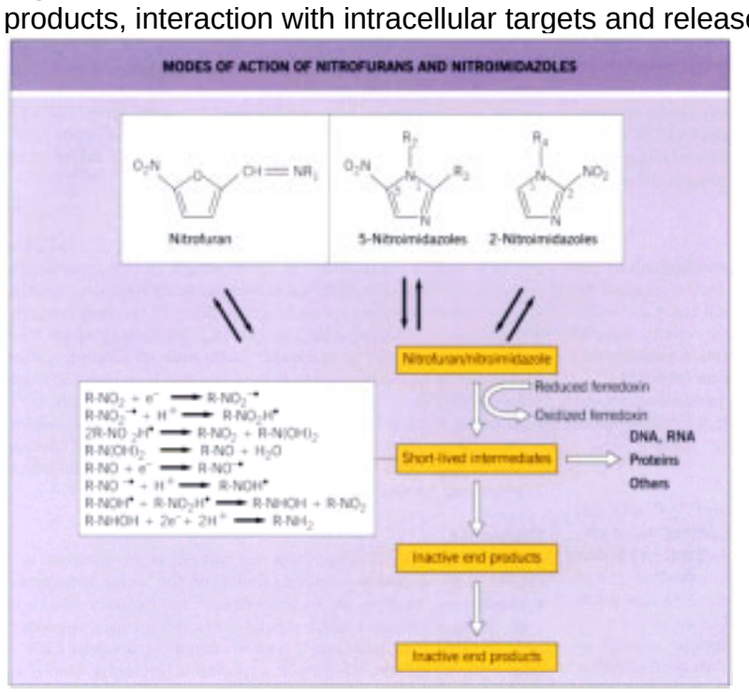


Figure 188-14 Mode of action of rifamycins. Synthesis of mRNA by RNA polymerase is shown in the upper panel and inhibition by rifamycins (R in the green squares) is shown in the lower panel. The RNA polymerase core is made up of four subunits, of which the ß' subunit binds to the DNA template and the ß subunit binds the ribonucleotide diphosphate (NDP; triangle). The s factor only participates to the initiation step by allowing for the recognition by the enzyme core of promoter sequences on the DNA template. Rifamycins bind to the ß subunit. They do not interfere with the binding of the nucleotide diphosphate, but rather inhibit the transcription initiation either by impairing the formation of the first phosphodiester bond or the translocation reaction of the newly synthesized dinucleotide.

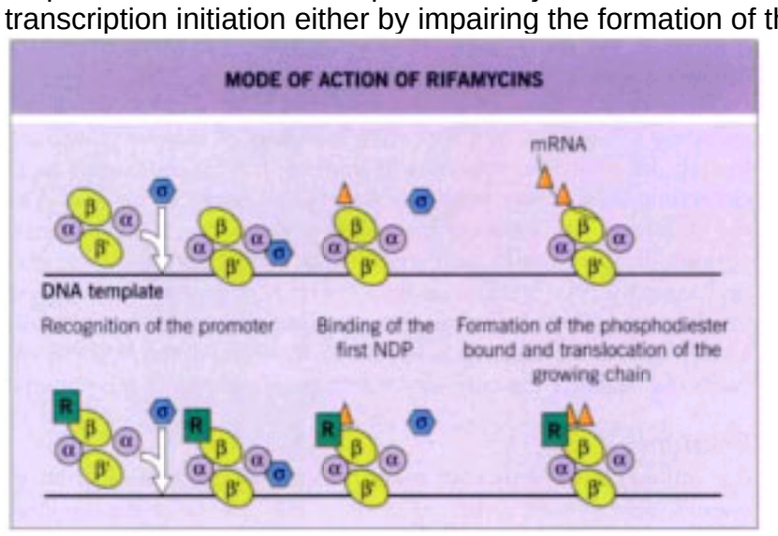


Figure 189-1 Mode of action and resistance of ß-lactam antibiotics in Gram-negative bacteria.

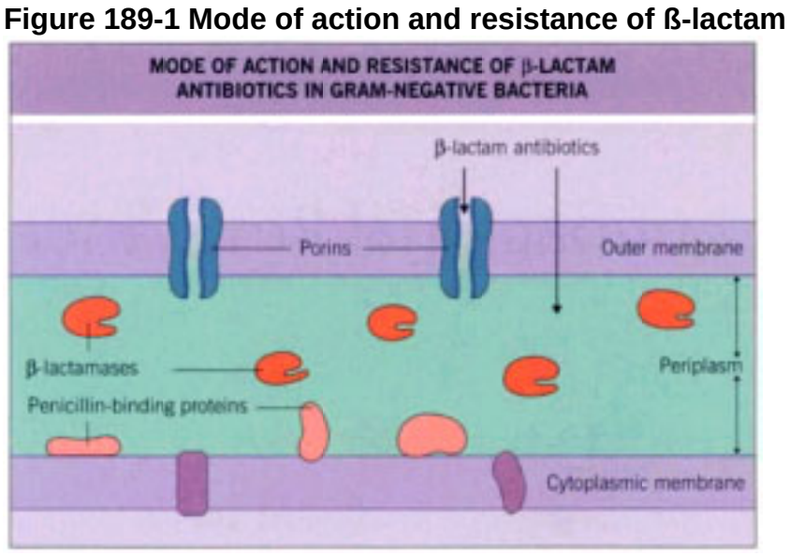


Figure 189-2 *mecA* region of different staphylococcal strains. IS, insertin sequence; mecA, methicillin-resistant gene; R1, regulatory element; Tn, transposon; vertical lines, position on restriction enzyme cleavage sites.

Figure 189-3 Peptidoglycan biosynthesis. ATP, adenosine triphosphate, Lac, lactate; UDP, uridine diphosphate. *Adapted from Shlaes and Rice.*[35]

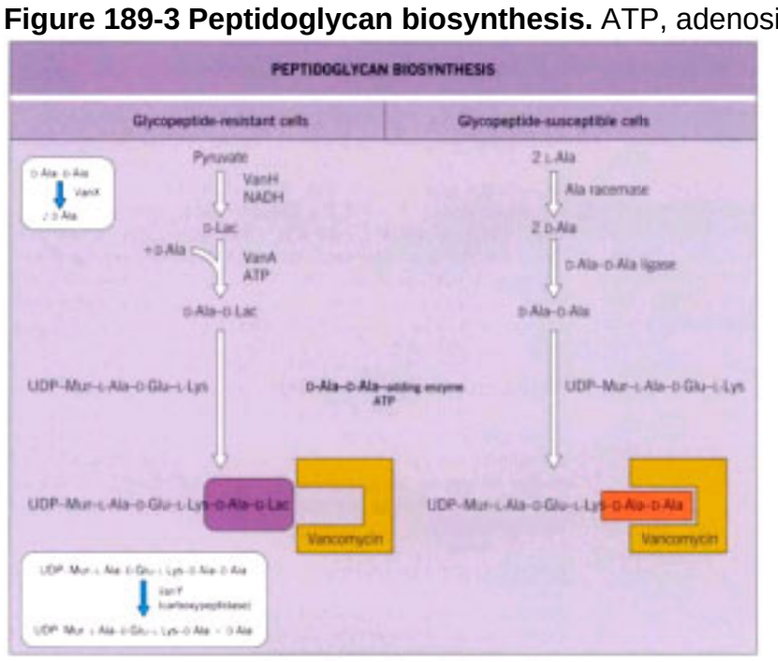


Figure 189-4 The MAR system and its regulation.

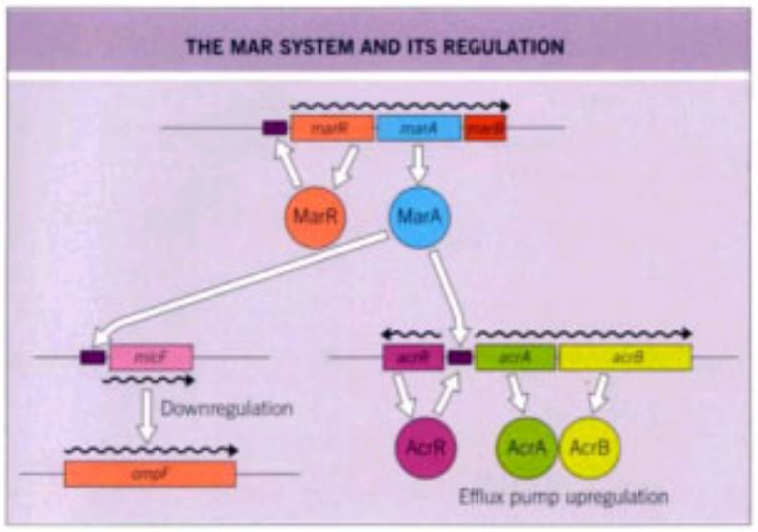


Figure 189-5 Class I integrons. Open arrows point in the direction of transcription from each promotor site. orf, open reading frame.

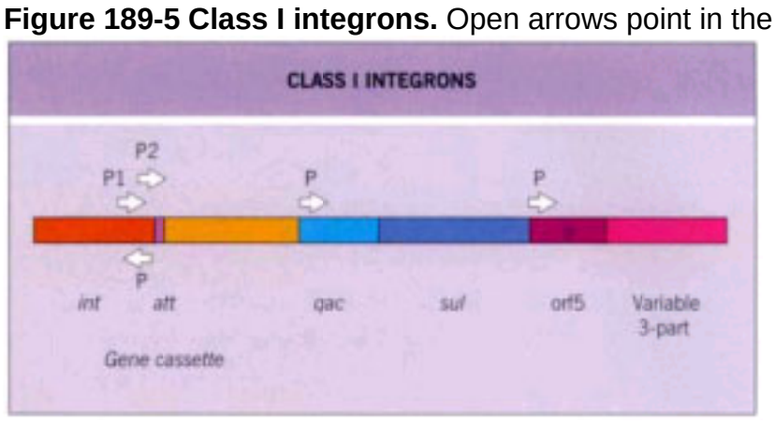


Figure 191-1 Clinical pathway for selection of patients for outpatient parenteral antimicrobial treatment of infections.

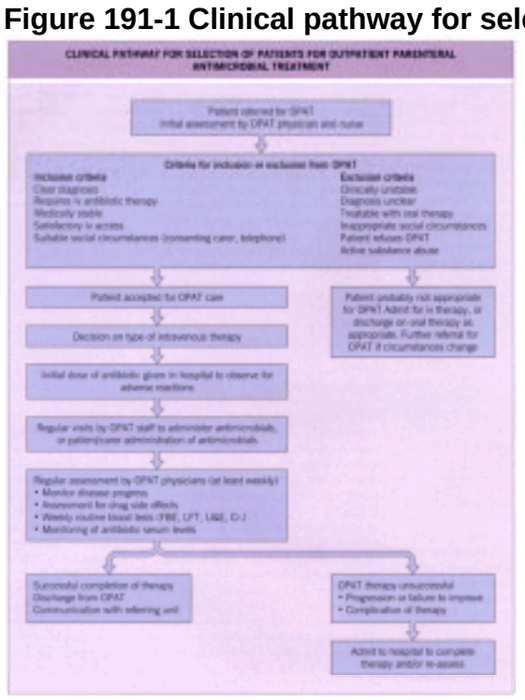


Figure 193-1 Concentrations of ß-lactam antibiotics in different tissues.

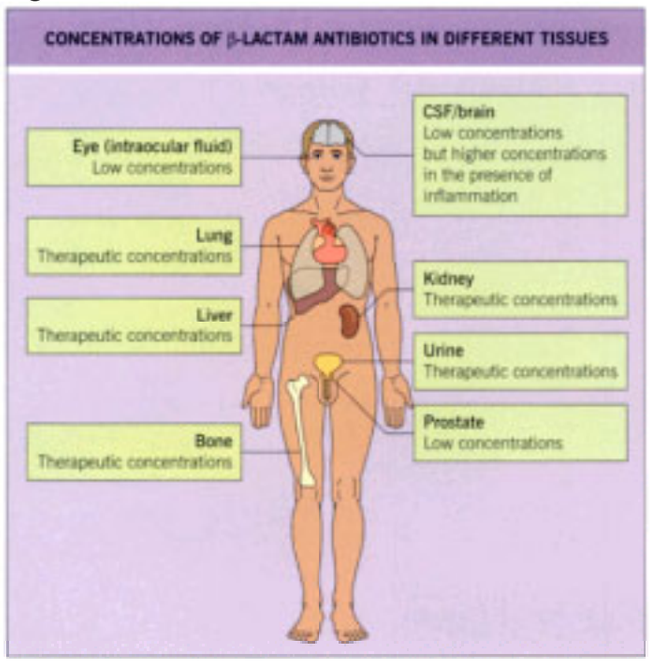


Figure 194-1 Classification of macrolides. *Adapted from Bryskier et al.*[1]

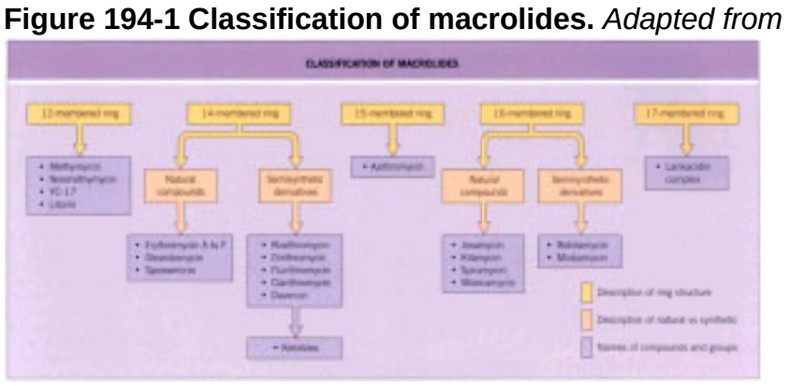


Figure 194-2 Possible molecular action of streptogramins. In the absence of streptogramins, the exit channel for peptide chains is free. In the presence of streptogramins, type A may induce a conformational change of L10 and L11, leading to an increase in the association of type B streptogramins for L24 and to a constriction of the exit channel. L10, L11 and L24 are the main proteins of the exit channel of peptide chains. *Adapted from Pechère.*[5]

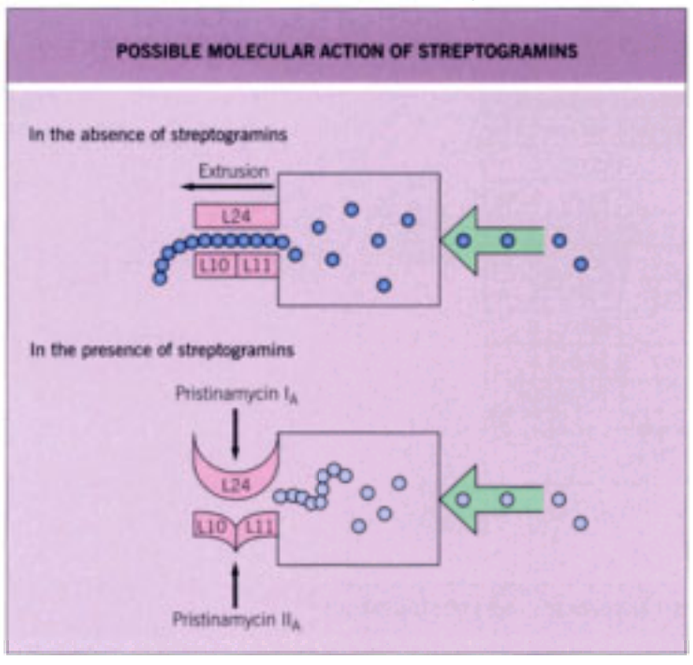


Figure 196-1 Chemical structures of the aminoglycosides. All aminoglycosides include an aminocyclitol (a central six-membered ring containing amino groups), which is linked to two or more amino- or non-amino-containing sugars by glycosidic bonds. For streptomycin, the aminocyclitol ring is a streptidine, whereas for the remainder of the clinically available aminoglycosides it is 2-deoxystreptamine. Neomycin contains approximately equal amounts of neomycin B (R_1 =H; R_2 =CH_2 NH_2) and neomycin C (R_1 =CH_2 NH_2 ; R_2 =H). Kanamycin is principally kanamycin A, as shown. Gentamicin is gentamicin C complex with roughly equal amounts of C_1 (R_1 =R_2 =CH_3), C_{1a} (R_1 =R_2 =H) and C_2 (R_1 =CH_3 ; R_2 =H).

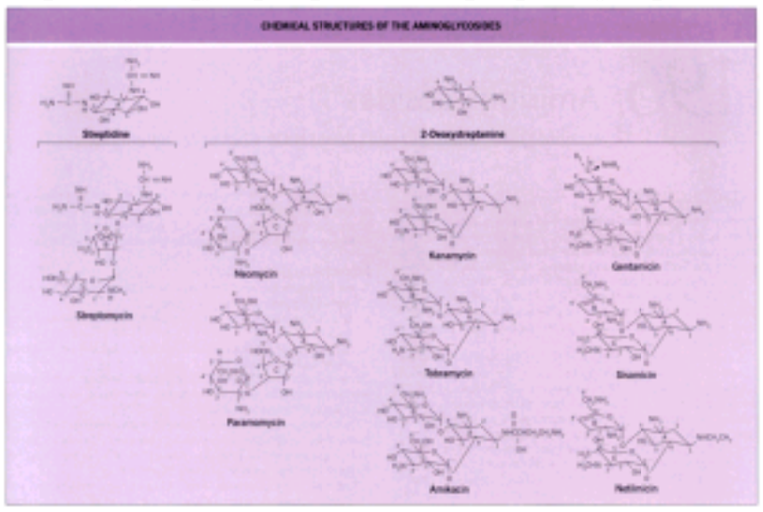


Figure 196-2 Simulated concentration versus time profiles for once-daily 7mg/kg and 5mg/kg gentamicin regimens in patients with varying degrees of creatinine clearance (Clcr).

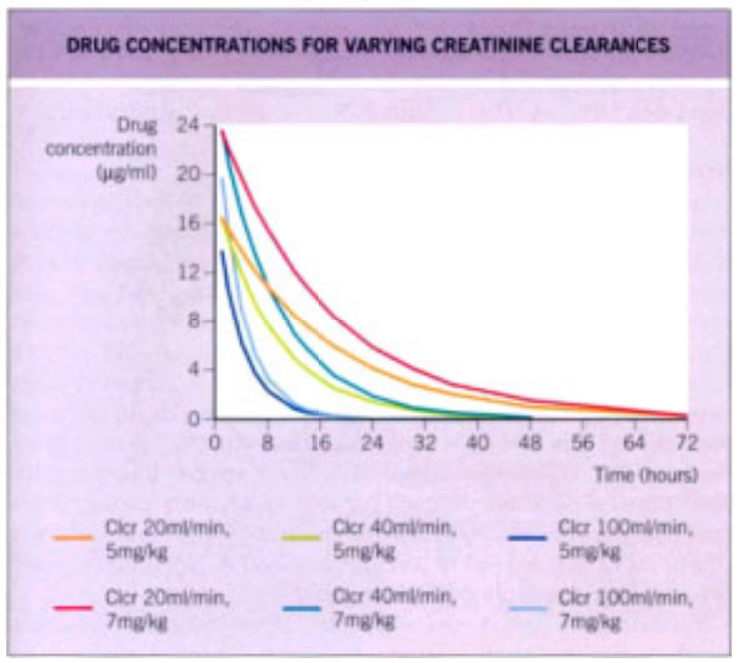


Figure 196-3 Simulated concentration versus time profiles for once-daily (7mg/kg q24h) and conventional (1.5mg/kg q8h) gentamicin regimens in patients with normal renal function.

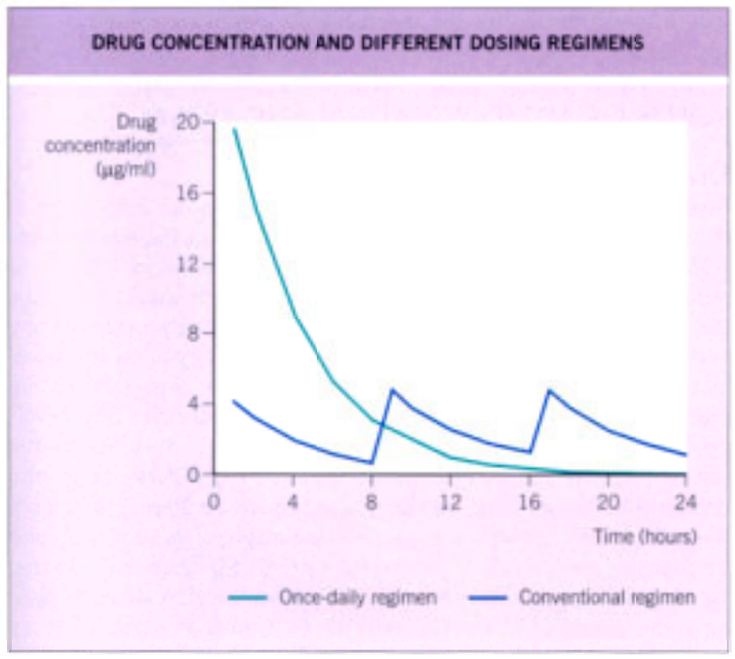


Figure 196-4 Hartford Hospital once-daily aminoglycoside nomogram for gentamicin and tobramycin using the 7mg/kg dose.

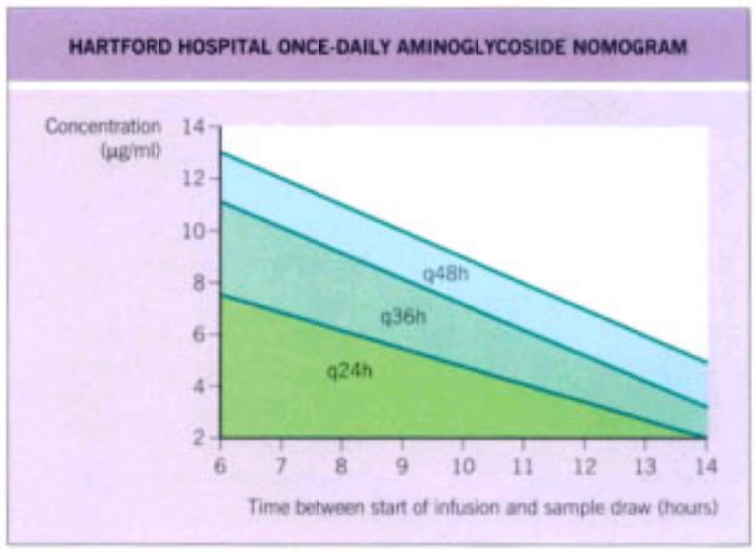


Figure 197-1 Mode of action of folate inhibitors.

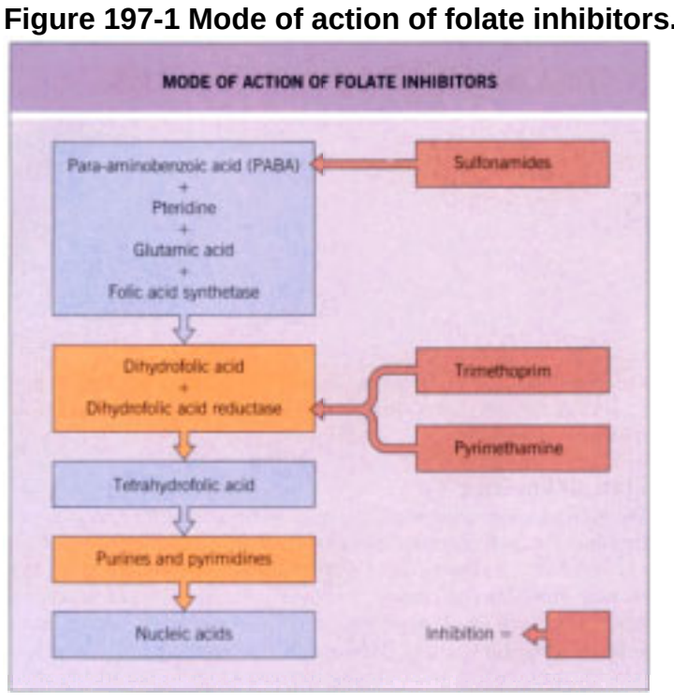


Figure 198-1 The evolution of quinolones.

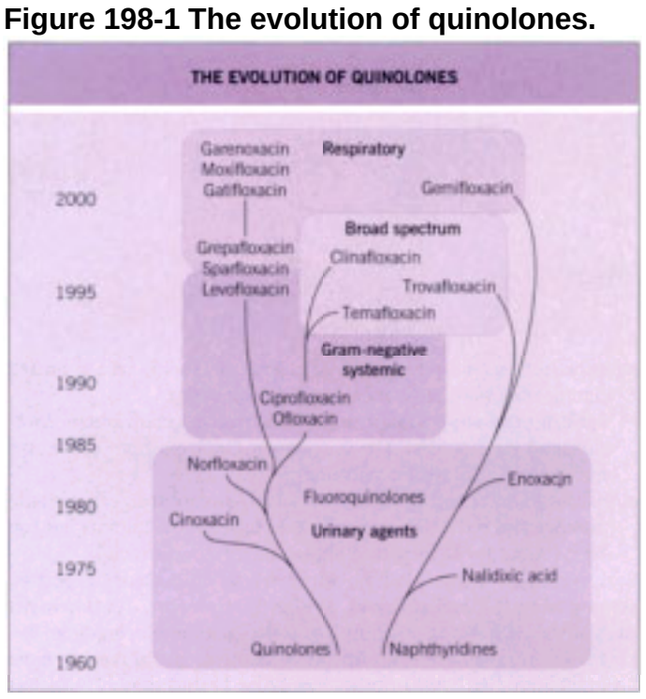


Figure 198-2 Structure of quinolones.

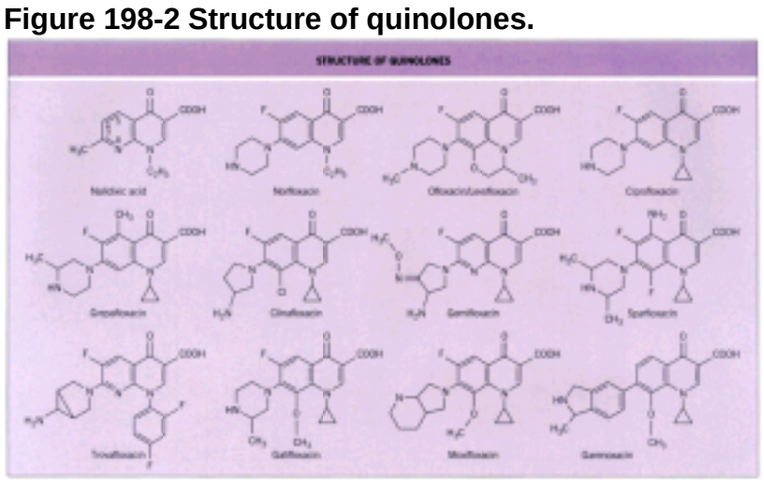


Figure 199-1 Elimination of vancomycin by anephric patients and by patients who have normal renal function. *Adapted with permission from Cunha et al.*[11]

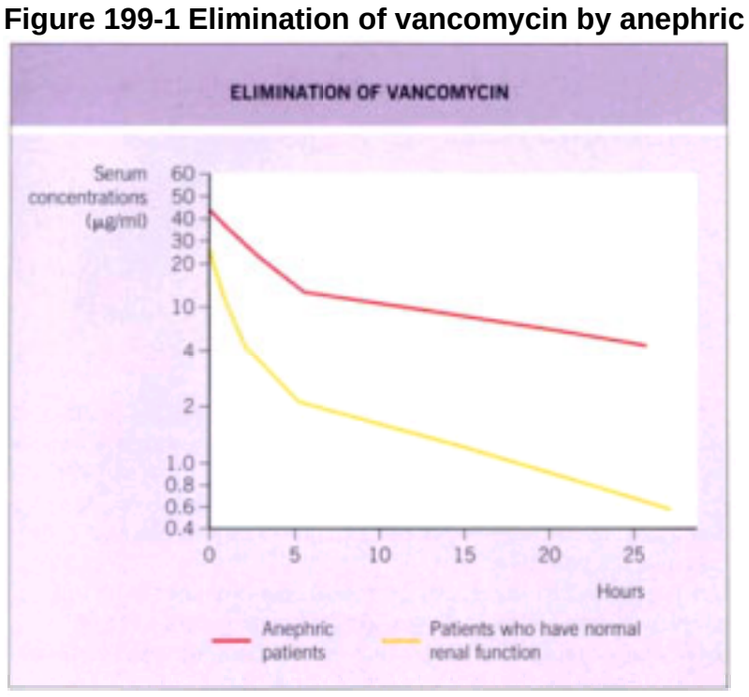
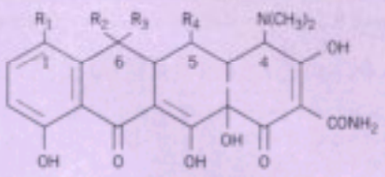


Figure 200-1 Molecular structure of some tetracyclines.

MOLECULAR STRUCTURE OF SOME TETRACYCLINES



	R_1	R_2		R_3	R_4
Chlortetracycline	Cl	CH_3		OH	H
Oxytetracycline	H	CH_3		OH	OH
Tetracycline	H	CH_3		OH	H
Demeclocycline	Cl	OH		H	H
Methacycline	H		$-CH_2-$		OH
Doxycycline	H	CH_3		H	OH
Minocycline	$N(CH_3)_2$	H		H	H

Figure 202-1 Targets of the antituberculosis agents.

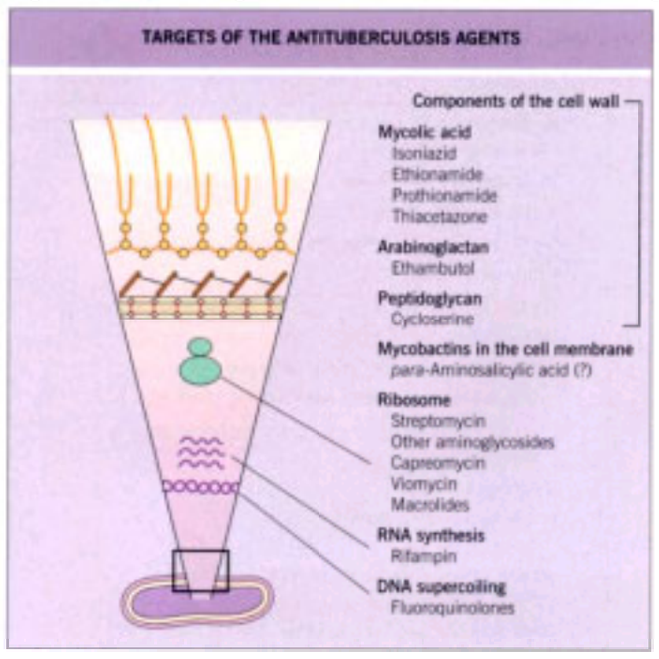


Figure 202-2 Severe dermal reaction to isoniazid. *Courtesy of Dr P Mwaba, Zambia.*

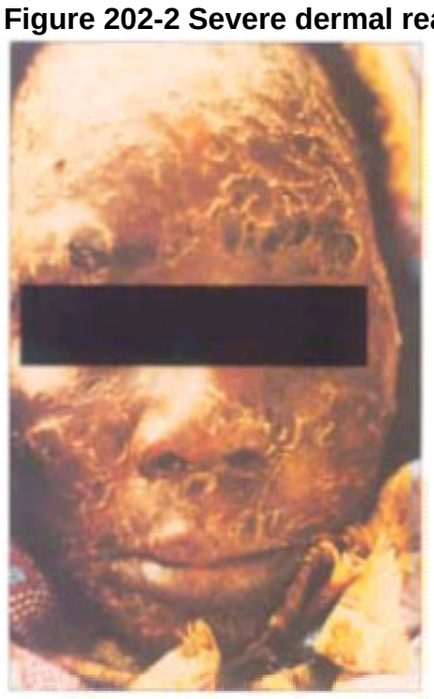


Figure 202-3 Erythema multiforme reaction to rifampin. *Courtesy of Dr P Mwaba, Zambia.*

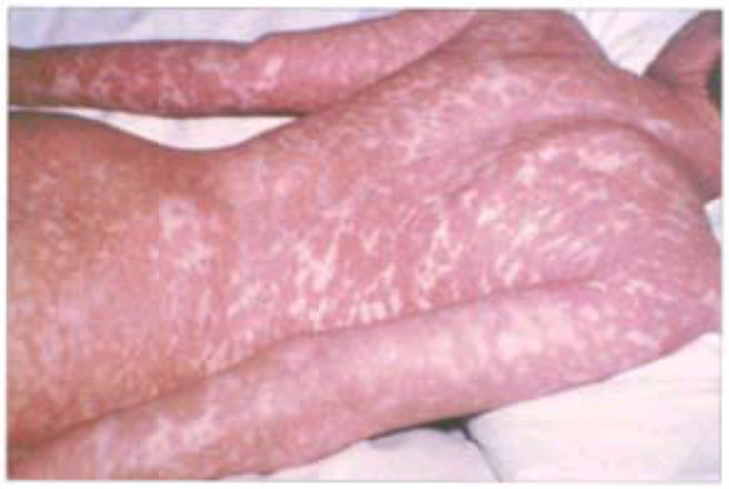


Figure 202-4 Stevens-Johnson syndrome induced by thiacetazone. *Courtesy of Dr P Mwaba, Zambia.*

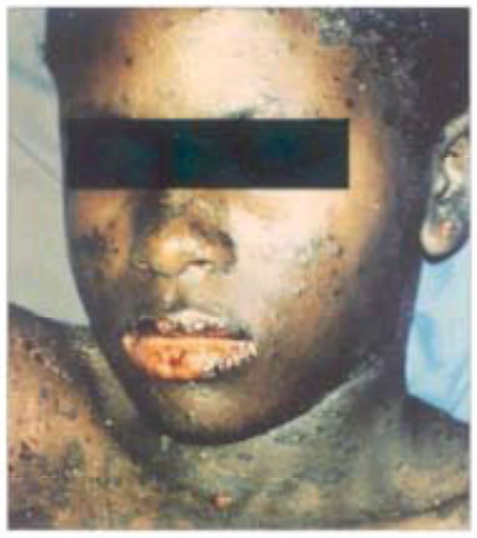


Figure 202-5 Global distribution of resistance to antituberculosis drugs. The resistance is given as a percentage of all isolates. *Data from the World Health Organization.*[32]

Figure 204-1 Life cycle of HIV-1 and major targets of antiretroviral agents.

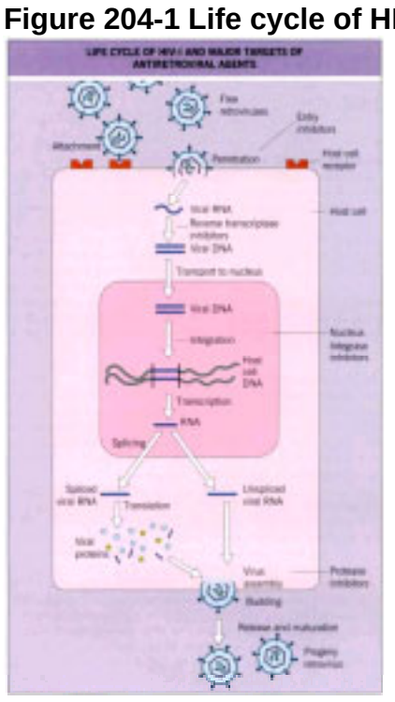


Figure 204-2 Chemical structures of approved nucleoside analog reverse transcriptase inhibitors.

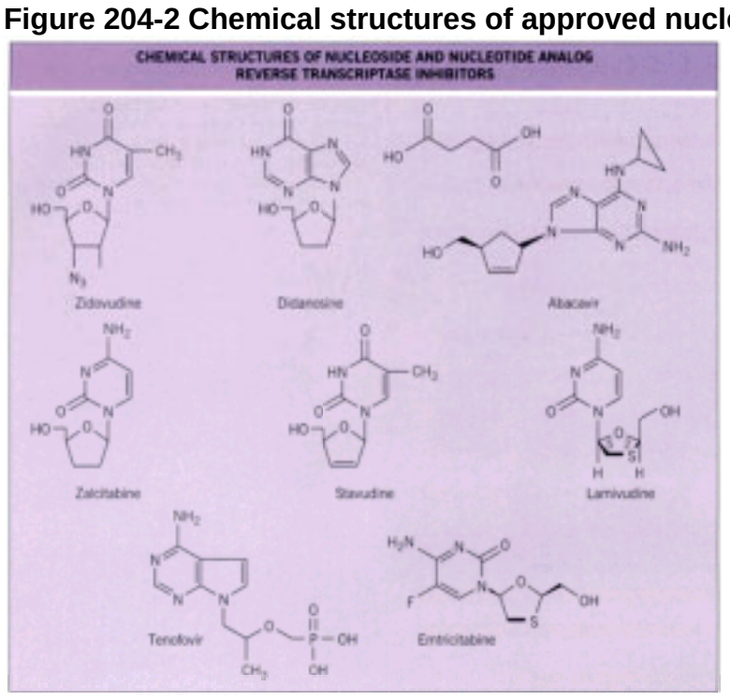


Figure 204-3 HIV drug resistance mutations. For each amino acid residue the letter above the bar indicates the amino acid associated with wild-type virus and the letter(s) below indicate the substitution(s) that confer viral resistance. The number shows the position of the mutation in the protein. HR1, first heptad repeat. *Courtesy of International AIDS Society — USA (for full details and footnotes see www.iasusa.org).*

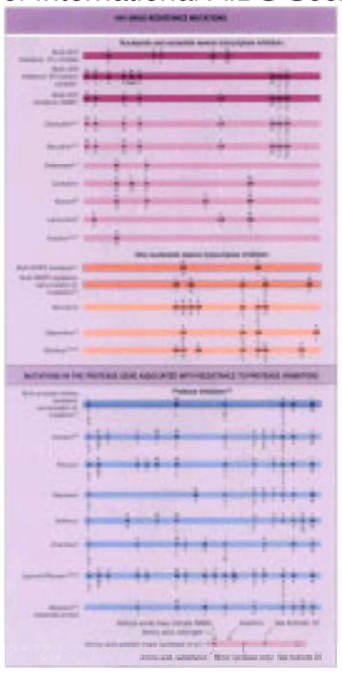


Figure 204-4 Chemical structures of approved non-nucleoside reverse transcriptase inhibitors.

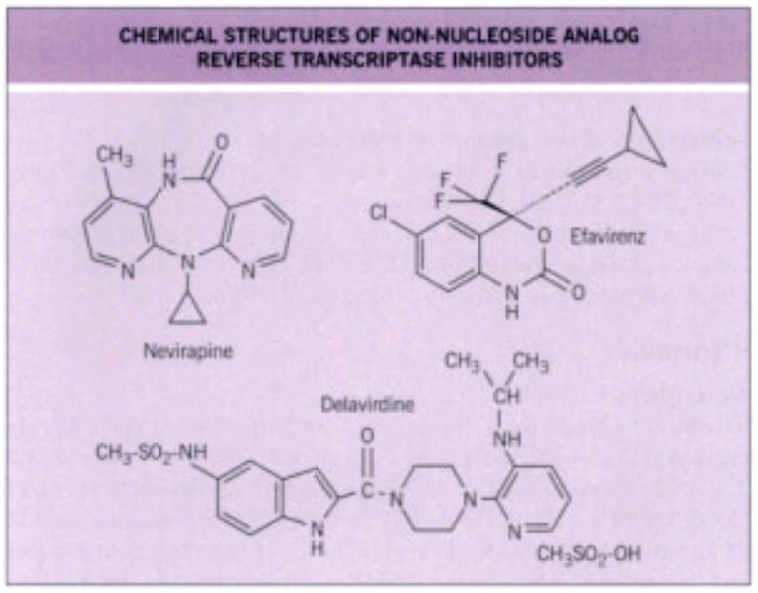


Figure 204-5 Chemical structures of approved protease inhibitors.

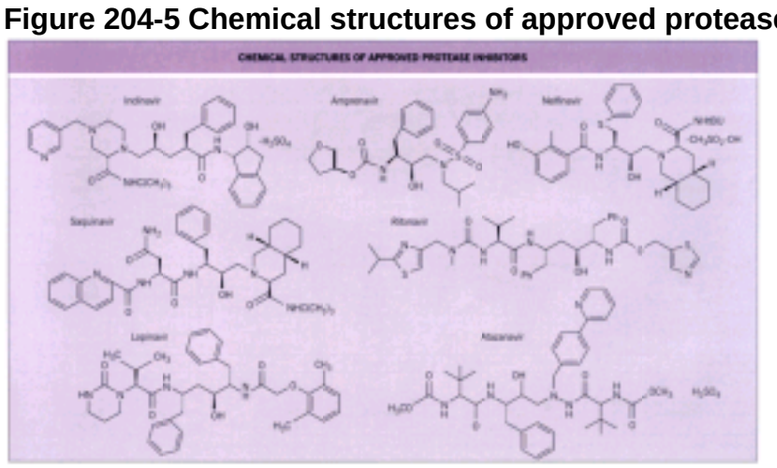


Figure 204-6 Mechanism of HIV fusion with host cell membrane and its inhibition by enfuvirtide (ENF, T-20).

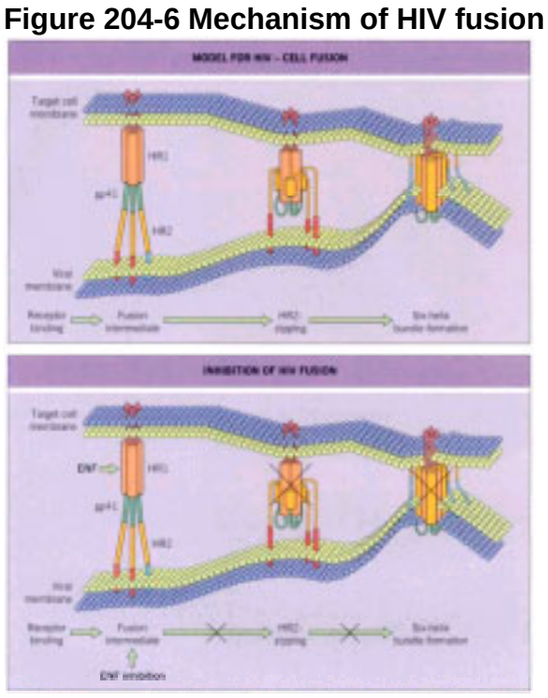


Figure 205-1 Activation of aciclovir is dependent on monophosphorylation via viral thymidine kinase (TK). Aciclovir triphosphate inhibits the activity of viral DNA polymerase, thus blocking viral replication. Penciclovir and ganciclovir are activated by similar mechanisms.

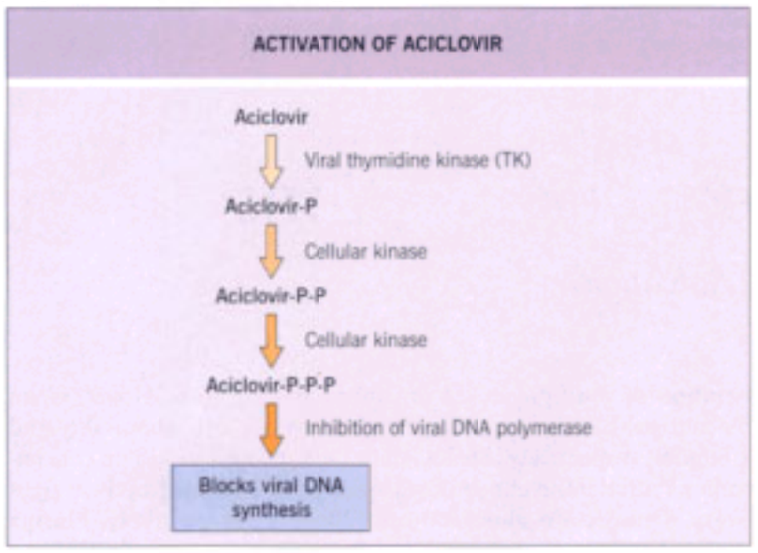


Figure 207-1 Adverse reactions to interferons.

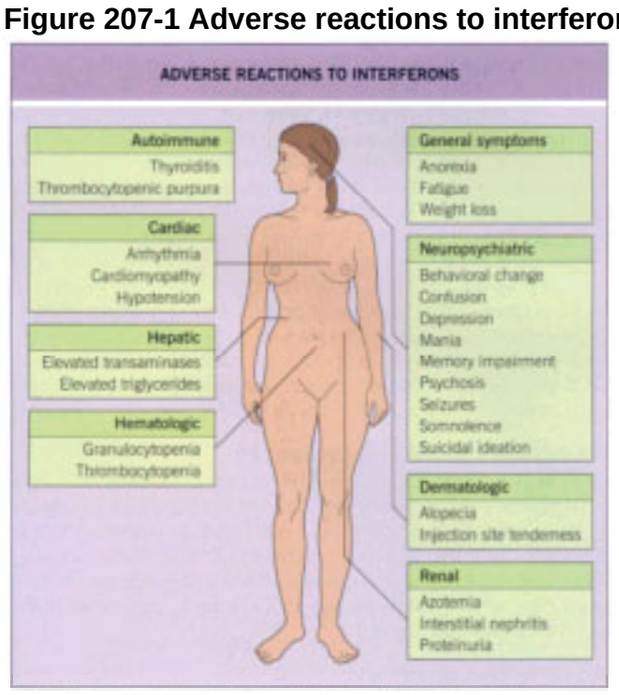


Figure 207-2 Hepatitis C virus life cycle and its inhibition.

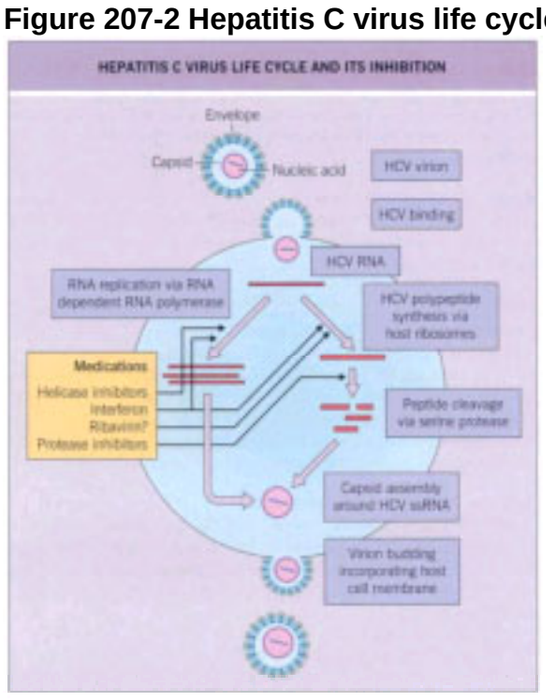


Figure 208-1 Chemical structures of amphotericin B and nystatin A1.

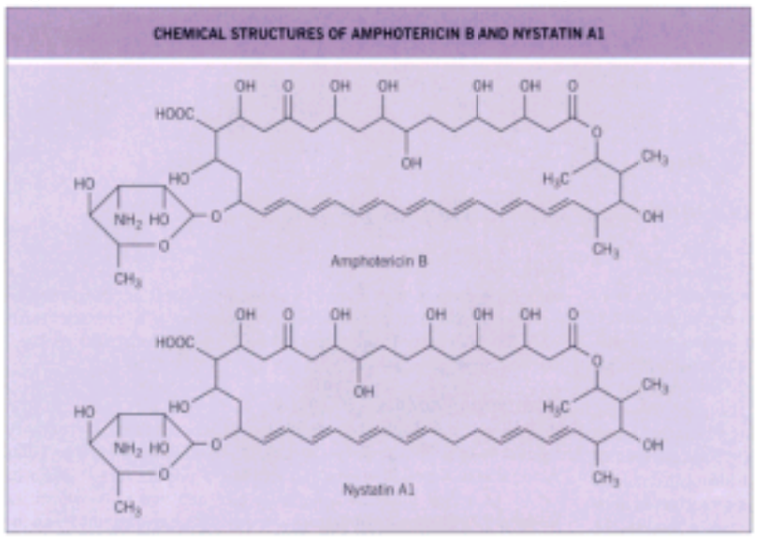


Figure 208-2 Biosynthetic pathway of ergosterol and points of activity by antifungal agents.

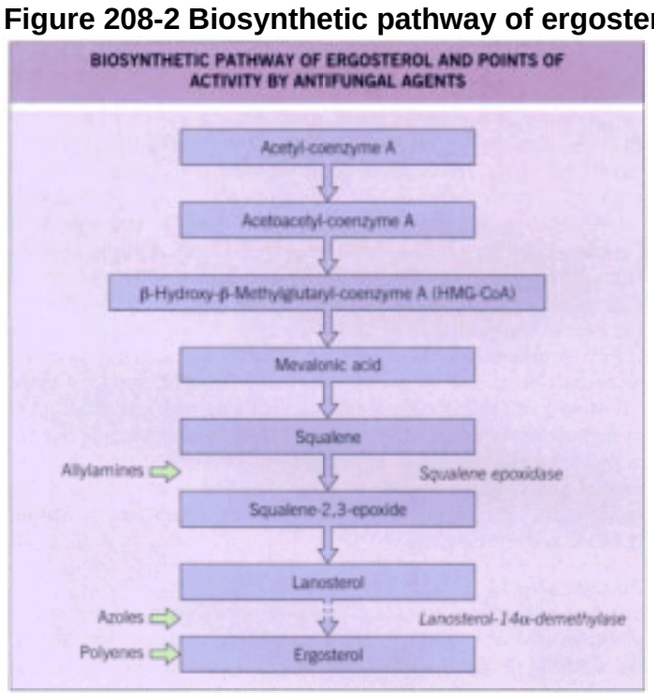


Figure 208-3 Comparison of the chemical structures of cytosine, flucytosine and fluorouracil.

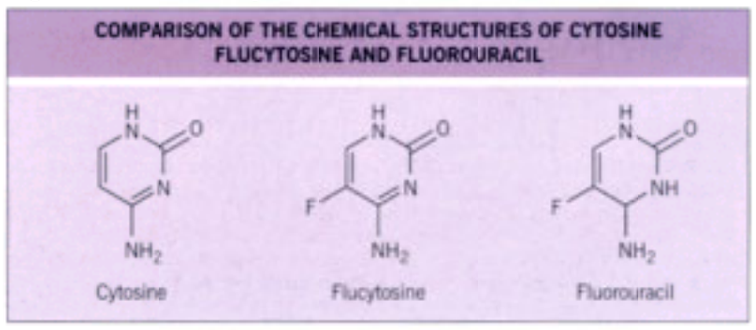


Figure 208-4 Chemical structures of fluconazole, itraconazole, voriconazole, posaconazole and ravuconazole.

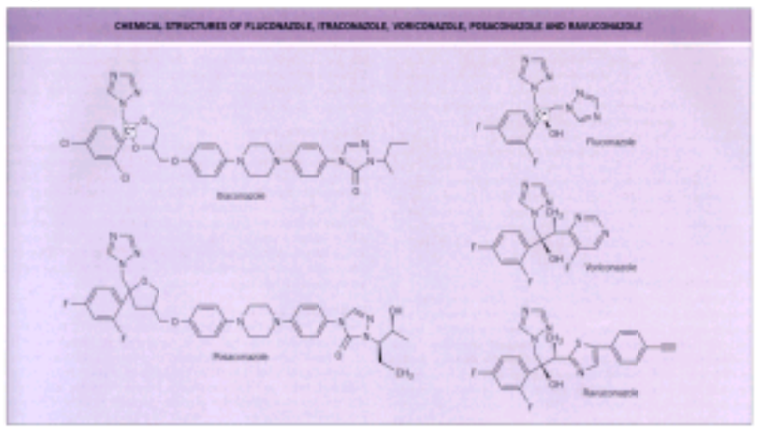


Figure 208-5 Mechanism of action of the echinocandins.

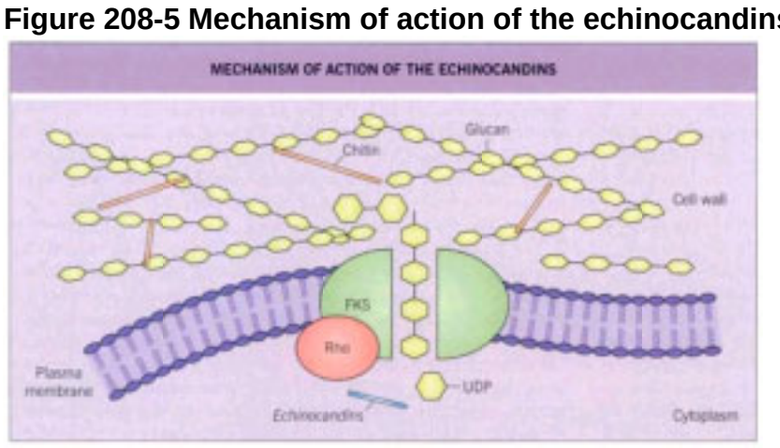


Figure 208-6 Structures of echinocandins currently in clinical practice or in clinical trials.

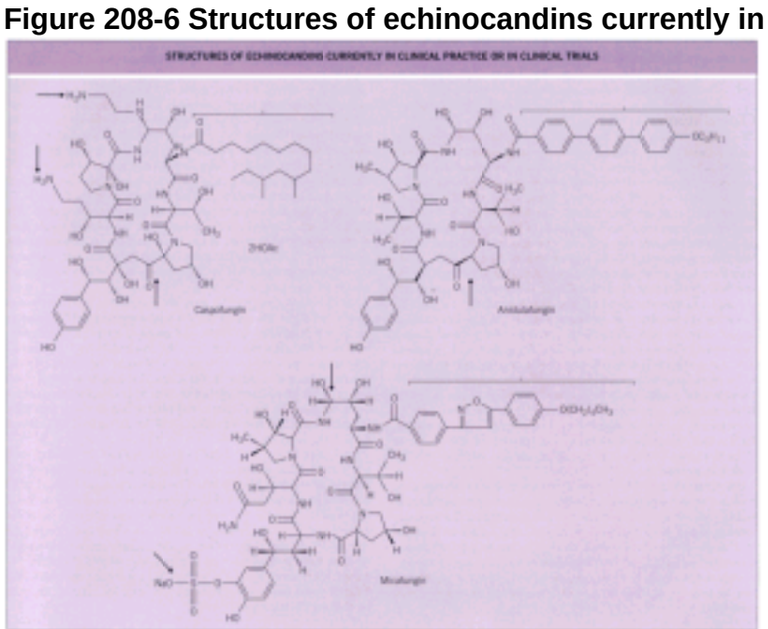


Figure 209-1 Distribution of chloroquine-resistant and chloroquine-sensitive *P. falciparum* malaria. *Adapted with permission from Lobel and Kozarsky.*[32]

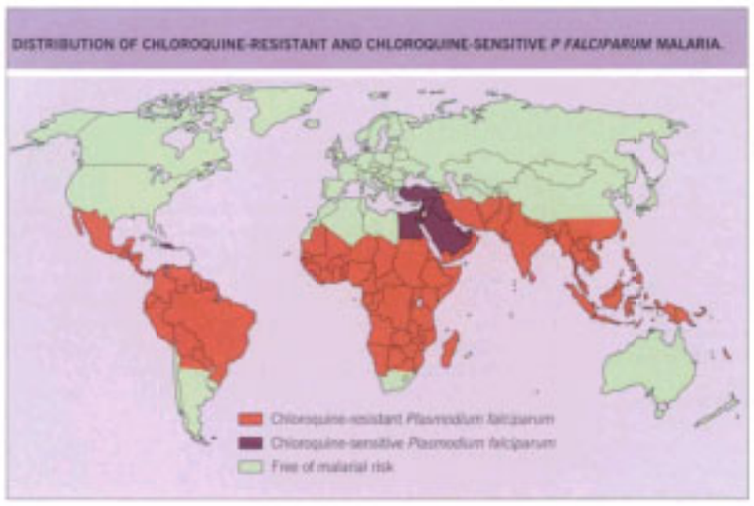


Figure 210-1 Simplified scheme of the steps in the inflammatory cascade as occur in (bacterial) infection. The block arrows indicate sites of intervention.

Figure 210-2 Incidence of infection associated with glucocorticosteroid use. In this meta-analysis of 71 placebo-controlled trials of prednisone, all trials in which there was a higher incidence of infection in the treated group (compared with the controls) were located above the isodose line of 700mg. This indicates that, independent of the regimen used in the trial, patients who had a cumulative dose of less than 700mg did not have an increased risk of infectious complications. *(With permission from Stuck et al.[75])*

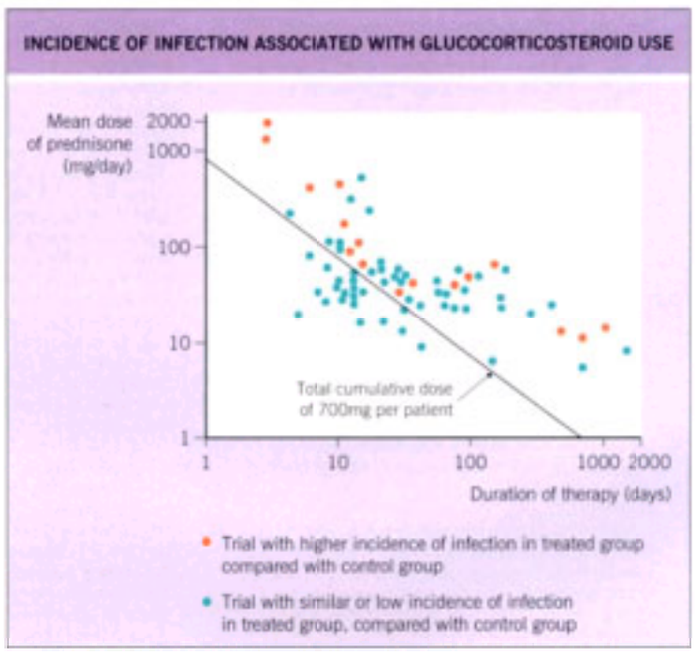


Figure 211-1 Rotavirus. Electron micrograph. *Courtesy of S Spangenberger.*

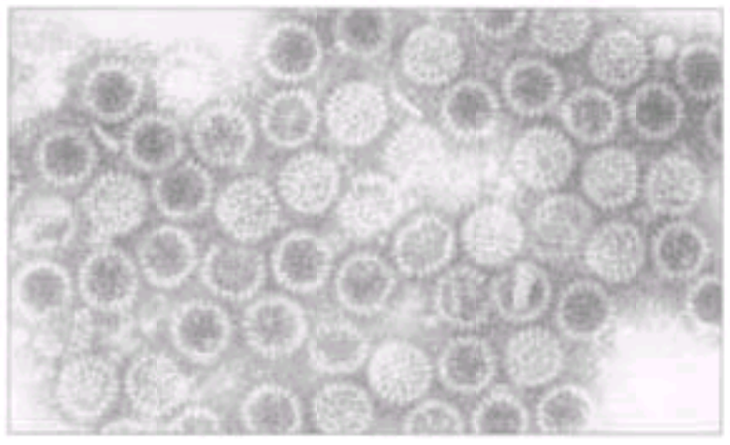


Figure 211-2 Rotavirus structure. Inner core is made of VP1, VP2 and VP3; inner capsid is made of VP6; outer capsid is made of VP7 and VP4. *Modified from Kapikian 2001.*[9]

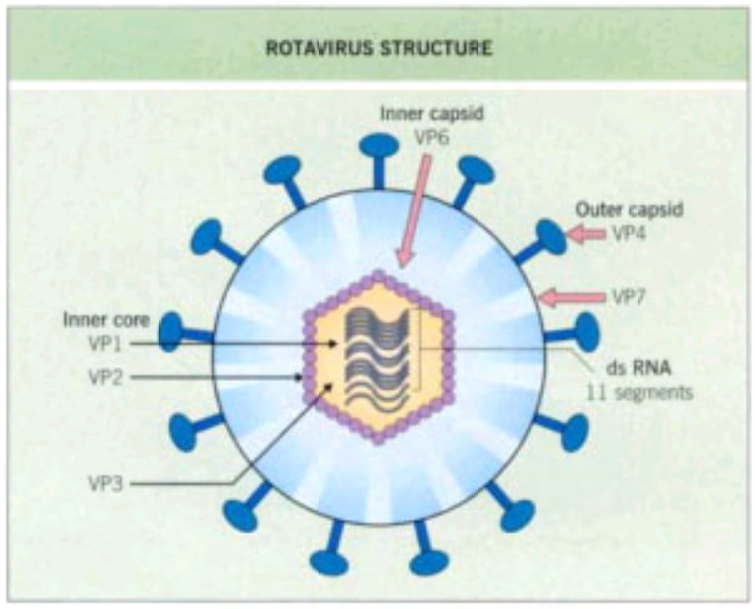


Figure 211-3 Norovirus. Electron micrograph. *Courtesy of C Humphrey, (CDC).*

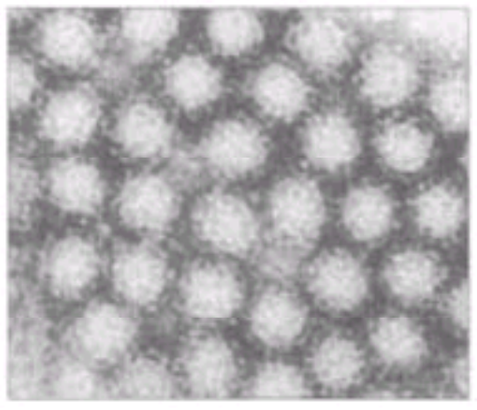


Figure 211-4 Sapovirus. Electron micrograph. *Courtesy of C Humphrey, (CDC).*

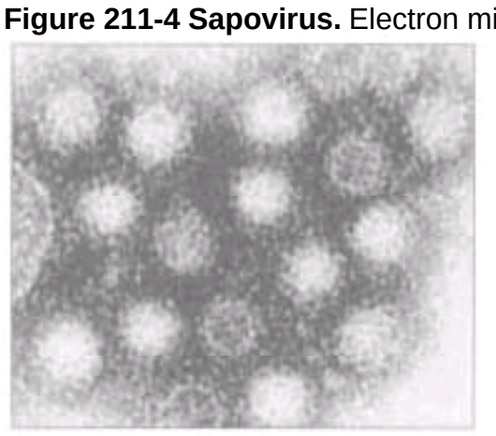


Figure 211-5 Enteric adenovirus. Electron micrograph. *Courtesy of S Spangenberger.*

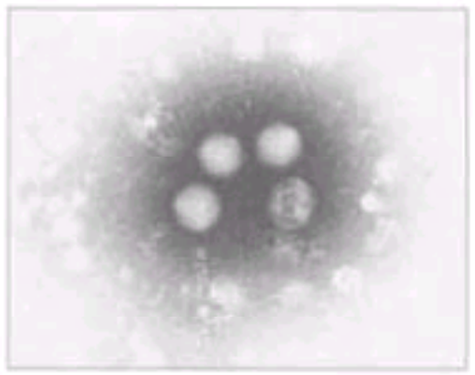


Figure 211-6 Astrovirus. Electron micrograph. *Courtesy of S Spangenberger.*

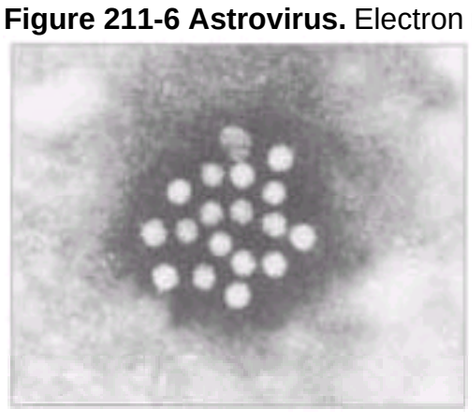


Figure 212-1 Annual measles notifications and subacute sclerosing panencephalitis (SSPE) cases in England and Wales between 1960 and 2000. The number of cases declined from the late 1960s, and there was a further decline after the introduction of MMR vaccine in 1988. *Data from the Office for National Statistics and CDSC.*

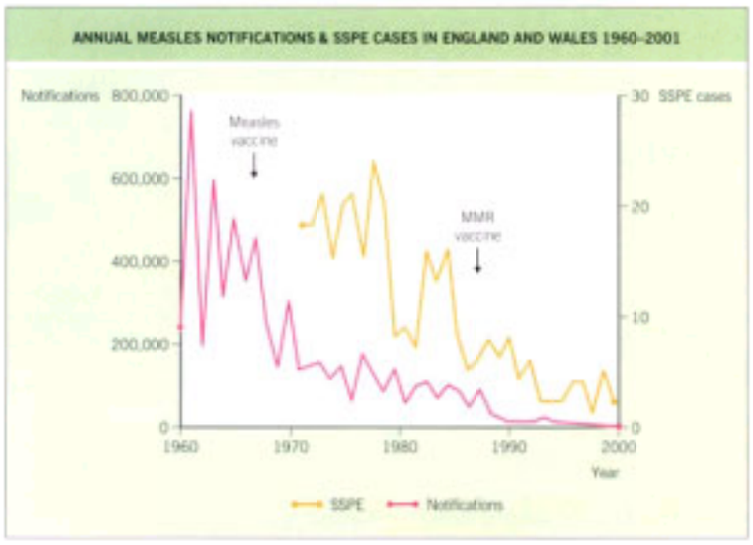


Figure 212-2 Measles. A disseminated erythematous rash can be seen over the trunk and arms.

Figure 212-3 Cancrum oris. Necrosis of the upper lip.

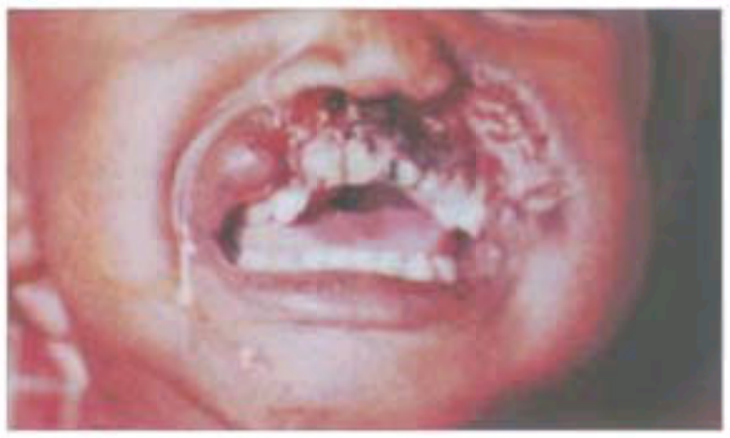


Figure 212-4 Cases of congenital rubella (CR) syndrome and infection in the UK. The number of confirmed cases was substantially reduced by the introduction of MMR vaccine in 1988. *Data from National Congenital Rubella Surveillance Programme and the PHLS Communicable Disease Surveillance Centre.*

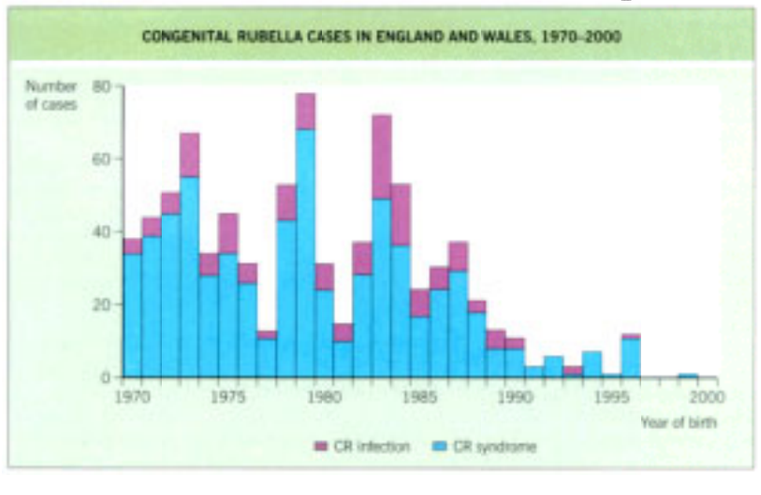


Figure 212-5 Serologic response in primary rubella. The development of specific IgG and IgM and the increasing avidity of specific IgG are illustrated.

Figure 212-6 Rubella. A pink macular rash can be seen on the forearm.

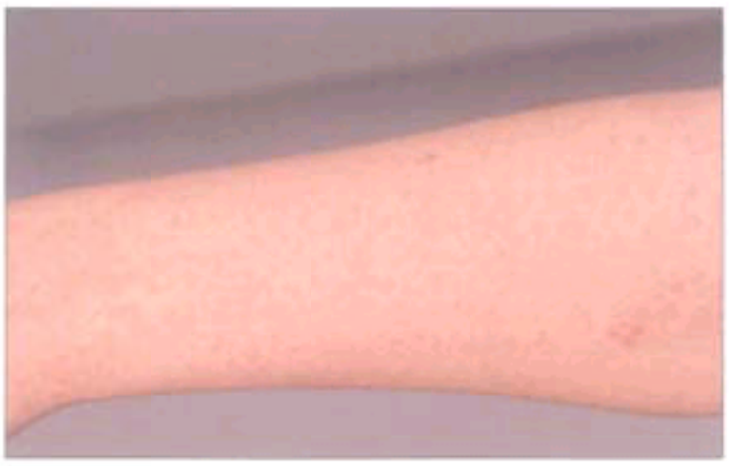


Figure 212-7 Electron micrograph of mumps virus. *Courtesy of Dr A Curry.*

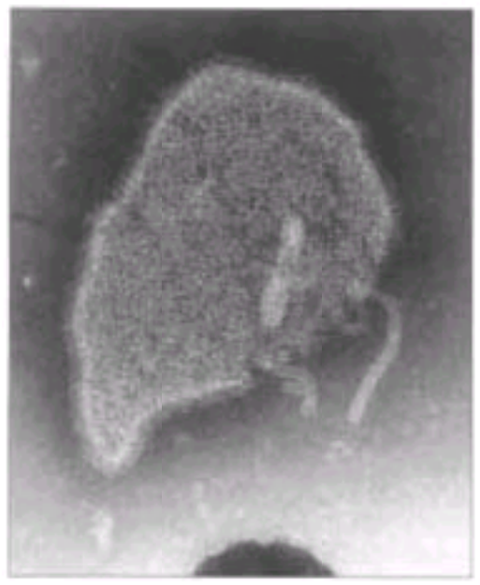


Figure 212-8 Mumps incidence reported to primary care physicians and number of laboratory diagnosed cases in England and Wales between 1962 and 2000. The number of cases declined in response to the introduction of MMR vaccine in 1988. *Data from the PHLS Communicable Disease Surveillance Centre.*

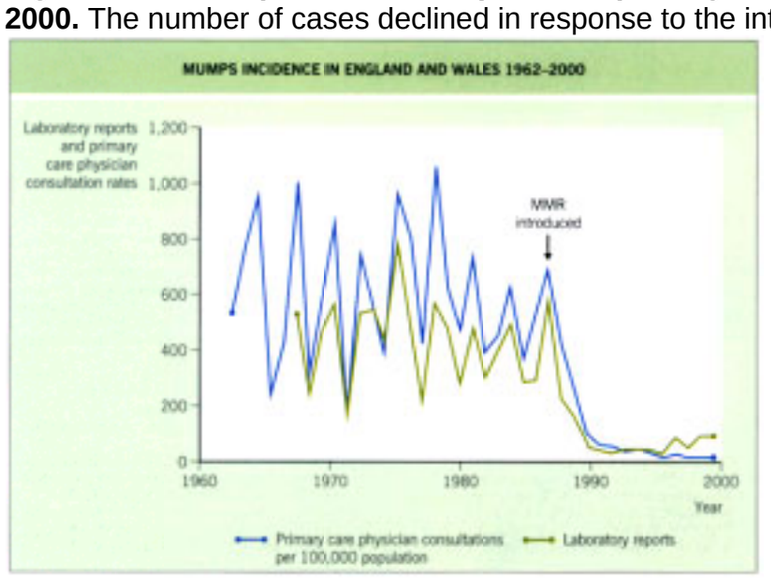


Figure 213-1 Poliovirus type 1. The particles in this electron micrograph are negatively stained with 0.5% uranyl acetate.

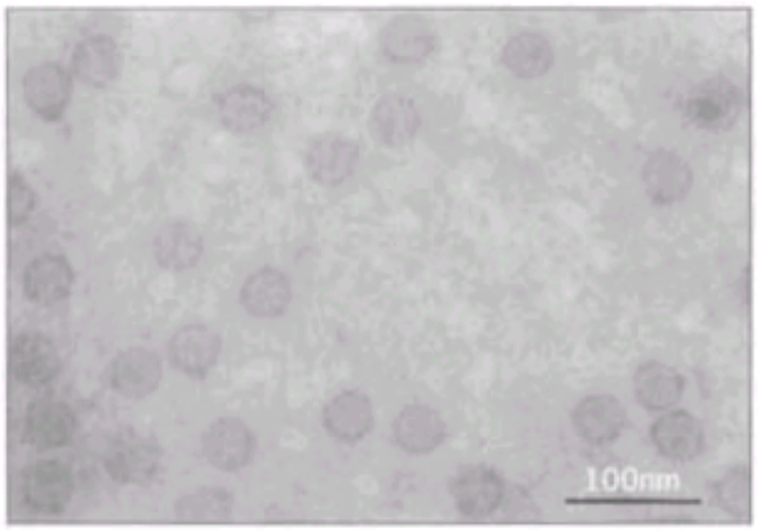


Figure 213-2 Organization of the poliovirus genome. The single-stranded genomic RNA of poliovirus (2.4×10^6 Da, approximately 7500 nucleotides) has positive-stranded sense and codes in a single open reading frame for capsid and functional proteins (modified according to Racaniello[3]). The boxes represent the coding region; the lines represent the nontranslated regions (NTR) at the 5'- and 3'-termini (5'- and 3'-NTRs). A small hydrophobic protein is covalently linked to the terminal uracil of the 5'-NTR and called 'virus protein genome linked' (VPg). The 5'-NTR has a significant secondary structure and contains the initiation site for translation at nucleotide position 741 (the internal ribosome entry site). The 3'-NTR (72 nucleotides) is polyadenylated (62 nucleotides on average). The coding region of the genome is translated into a large precursor polyprotein. Region P1 codes for the capsid proteins VP0 (precursor of VP4 and VP2), VP1 and VP3. Regions P2 and P3 code for functional proteins (e.g. 2A codes for a protease, 3B codes for VPg, 3C and 3CD code for proteases, and 3D codes for the RNA polymerase). Three proteases mediate processing of the precursor proteins: protease 2A releases the P1 capsid precursor from the nascent polyprotein, and the proteases 3C and 3CD mediate most of the other cleavages before and during virus assembly. Virus assembly is completed when a single RNA molecule is surrounded by its capsid with 60 protomers, in which the precursor VP0 is then cleaved into mature VP4 and VP2. It is postulated that the viral RNA is involved in this final cleavage.

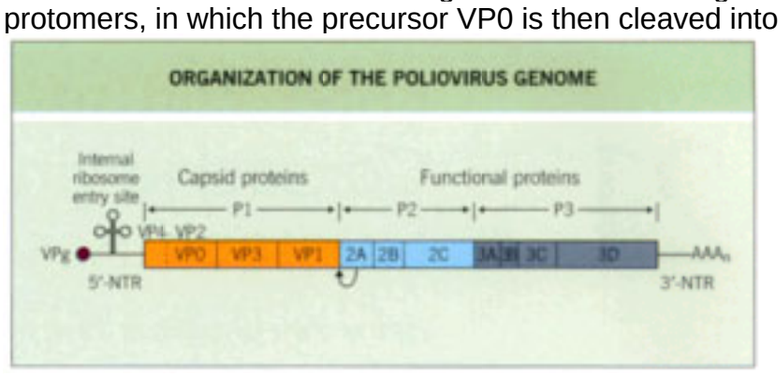


Figure 213-3 Epidemiologic survey of reported enterovirus infections. This epidemiologic survey by the German Association for Prevention of Virus Diseases (DVV) for the years 1984 and 1985 demonstrates that most infections with coxsackieviruses groups A and B and echoviruses in Germany occur during summer and autumn. *Data from Habermehl and Knocke.*

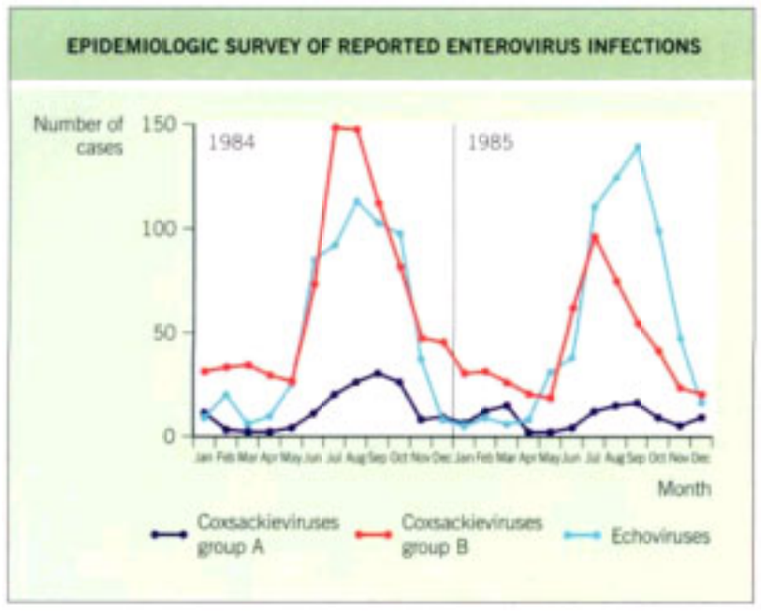


Figure 213-4 Receptor-mediated entry of poliovirus into host cells. The entry of poliovirus into HEp-2 cells is followed by transmission electron microscopy of ultrathin sections of synchronously infected cells.[31] [32] (a) Poliovirus (v) adsorbs at the cell surface immediately after infection (0 minutes after infection). (b) Beginning 1 minute after infection, poliovirus is located at areas of the cell surface that have clathrin-coated pits (cp), at which the surface membrane starts to invaginate. (c) Five minutes after infection, poliovirus is taken up by clathrin-coated vesicles (cv) into the cytoplasm. (d) Between 15 and 20 minutes after infection, poliovirus is within intracellular clathrin-free vesicles or endosomes (e), which are suggested as the sites of viral uncoating.

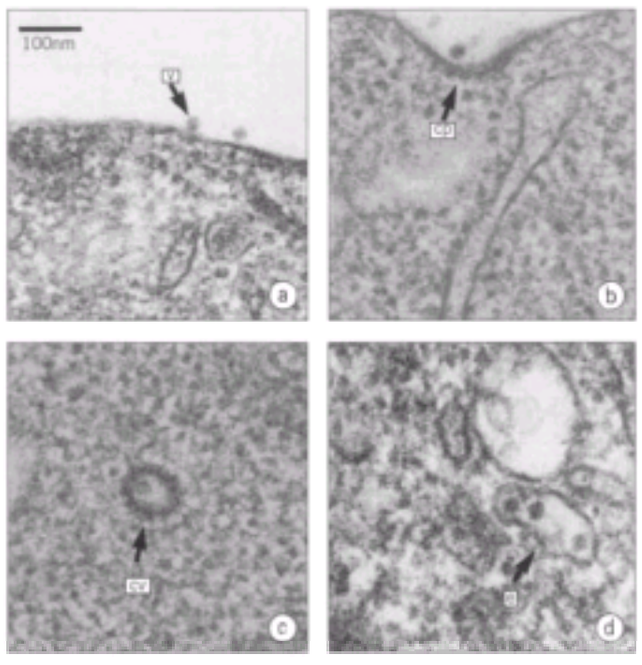


Figure 213-5 The reproduction cycle of poliovirus. Receptor-mediated entry of poliovirus is completed by the release of the viral RNA from the virus capsid (uncoating). The syntheses of viral protein and RNA are the next reproduction steps. The viral precursor polyprotein is autocatalytically cleaved by viral proteases, resulting in the viral RNA polymerase and, via several precursor proteins, in the virus capsid proteins (see Fig. 213.2). The viral RNA polymerase mediates viral transcription (i.e. de-novo synthesis of positive-stranded RNA via negative-stranded RNA templates). Maturation of the virus is completed by encapsidation of one molecule of positive-stranded RNA into a capsid with the complete set of proteins VP1, VP2, VP3 and VP4. RI, replicative intermediate.

Figure 213-6 The poliovirus-induced cytopathic effect. The cytopathic effect of poliovirus type 1 in monolayers of HEp-2 cells (a, c, e) is demonstrated in comparison to noninfected control cells (b, d, f) by light microscopy, scanning electron microscopy and transmission electron microscopy. [35] (a) Light microscopy of infected cells stained with hemalum-eosin shows rounded cells with pyknotic nuclei and condensed chromatin (8 hours after infection). (b) Light microscopy of control cells. (c) Scanning electron microscopy demonstrates severe rounding of the infected cells (12 hours after infection). The infected cells are characterized by elongated filopodia and microvilli at the cell surface; these are collapsed or even lost. (d) Scanning electron microscopy of control cells. (e) Transmission electron microscopy of ultrathin sections reveals infected pyknotic cell condensation of chromatin arranged in patches in a lobed nucleus and clusters of vesicles in the cytoplasm (8 hours after infection). (f) Transmission electron microscopy of control cells.

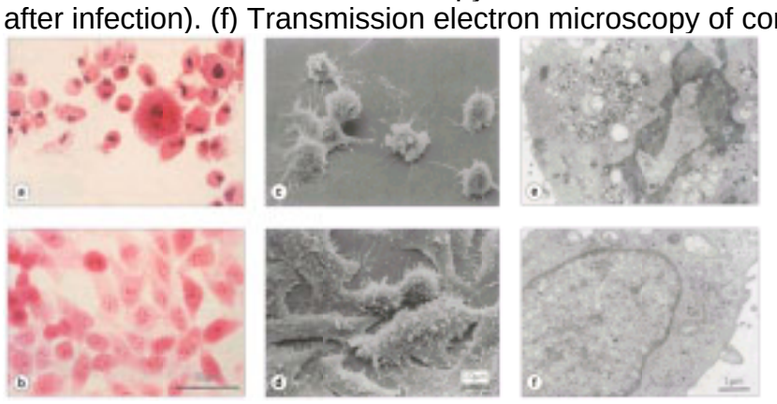


Figure 213-7 The course of infection with poliovirus.

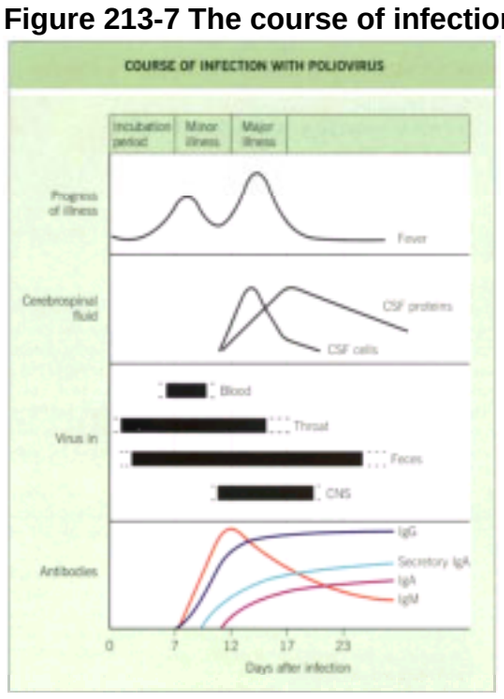


Figure 213-8 Fulminant enterovirus-induced myocarditis. In-situ hybridization of a ^{35}S-labeled enterovirus group-specific cDNA probe to the paraffinembedded autopsy heart tissue of an infant who died of acute enterovirus infection.[53] (a) Autoradiographic silver grains can be clearly localized to distinct infected myocytes, thereby providing the possibility of an unequivocal diagnosis of myocardial enterovirus infection. (b) Hybridization to myocardial cells was not observed when myocardial tissues were hybridized with the ^{35}S-labeled plasmid vector control probe, demonstrating the specificity of in-situ hybridization. Stained with hematoxylin and eosin. *Courtesy of R Kandolf, Tübingen.*

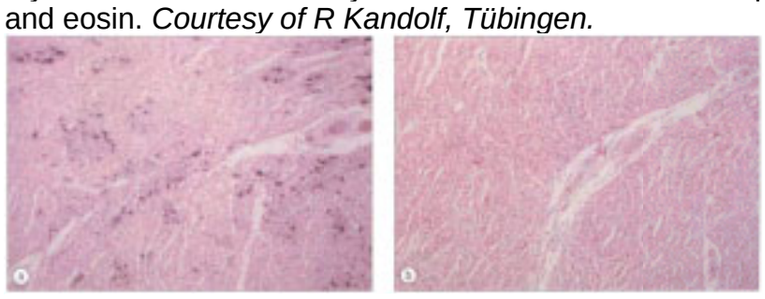


Figure 214-1 Hepatitis A virus. Note the vast number of virus particles present in a fecal extract.

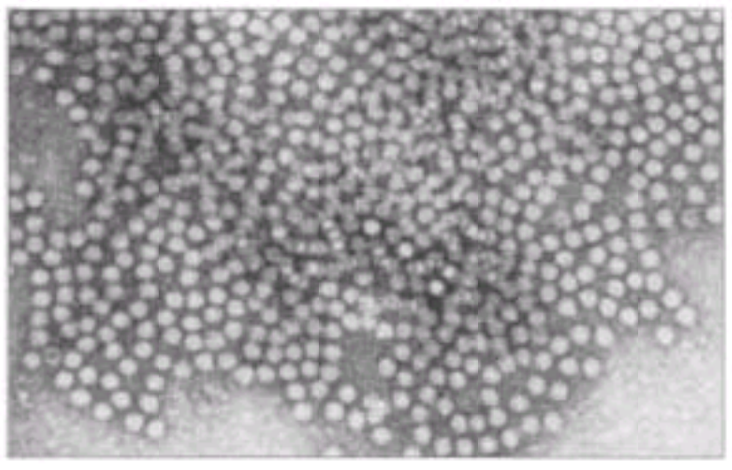


Figure 214-2 Course of acute hepatitis A.

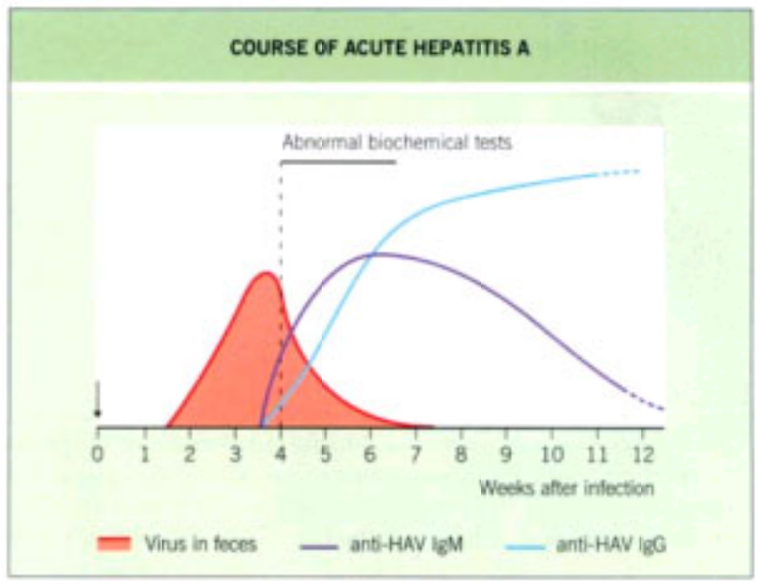


Figure 214-3 Histologic changes in the liver of a patient with acute hepatitis A.

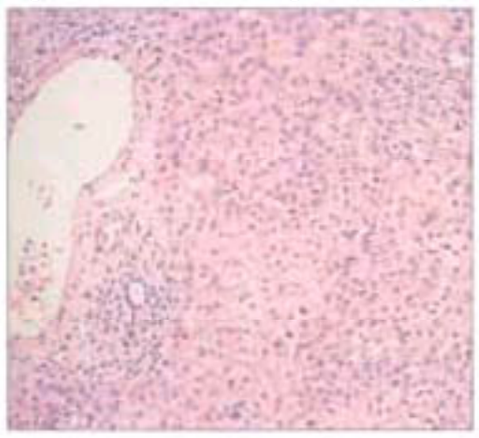


Figure 214-4 Serum from a patient with hepatitis B. The double-shelled particle is the complete virion. Tubular structures and 22nm HBsAg particles are present in small numbers.

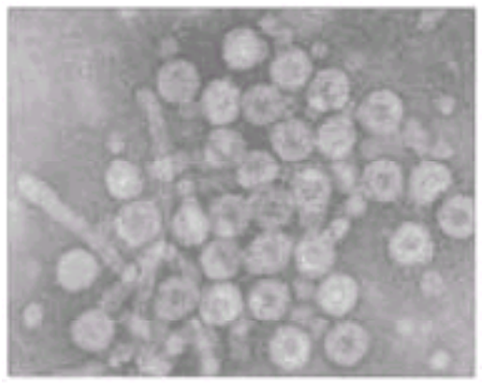


Figure 214-5 Hepatitis B viral genome.

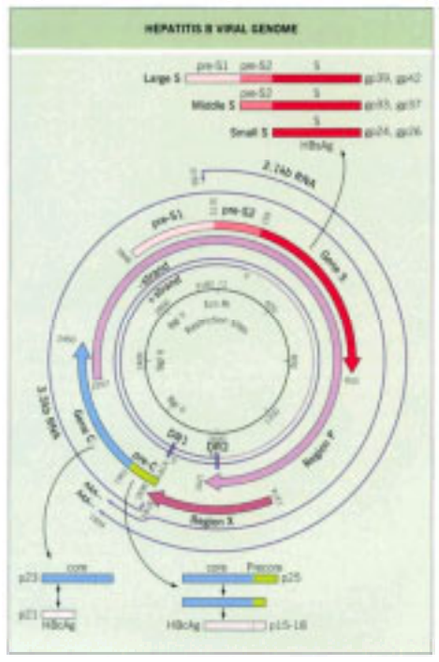


Figure 214-6 Possible consequences of hepatitis B virus infection in an adult.

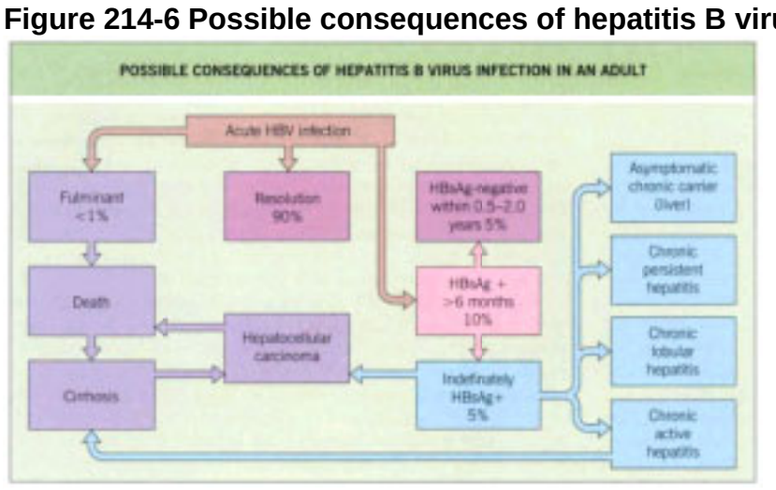


Figure 214-7 Hepatocellular carcinoma.

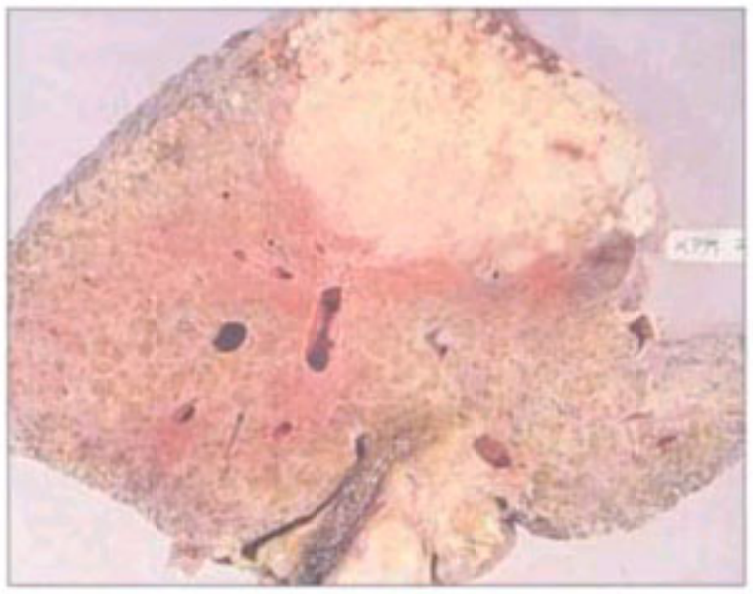


Figure 214-8 Hepatitis C viral genome.

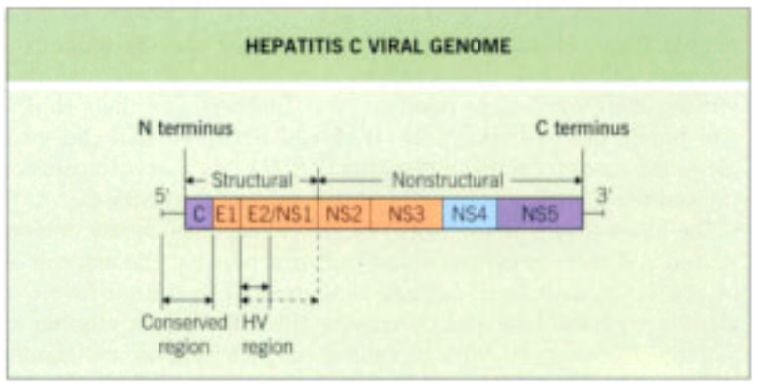


Figure 214-9 Hepatitis C virus active cirrhosis.

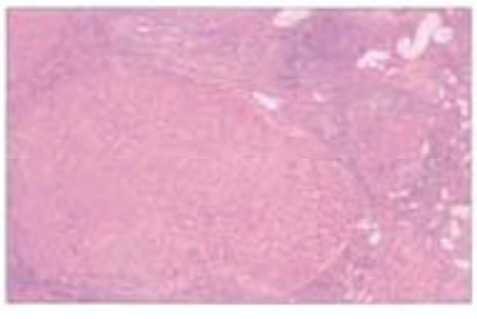


Figure 214-10 Acute hepatitis C.

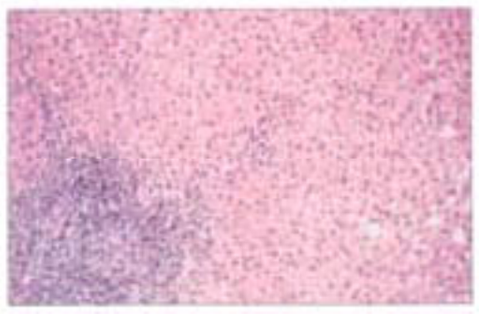


Figure 215-1 Organization of herpesvirus genomes. Inverted repeat sequences in VZV, HSV and CMV allow the genome to recombine in 2,4, and 4 isomers, respectively. Both HSV and CMV have a UL (long unique base sequence) and a US (short unique base sequence) each terminated by two sets of inverted repeated sequences. The repeated sequences allow the UL and US to invert relative to one another, so yielding four isometric forms of DNA. As there is only one set of inverted repeats in VZV, only two isomers of DNA can be produced. Both EBV and HHV-8 have only one isomeric form with several unique regions surrounded by direct repeated sequences.

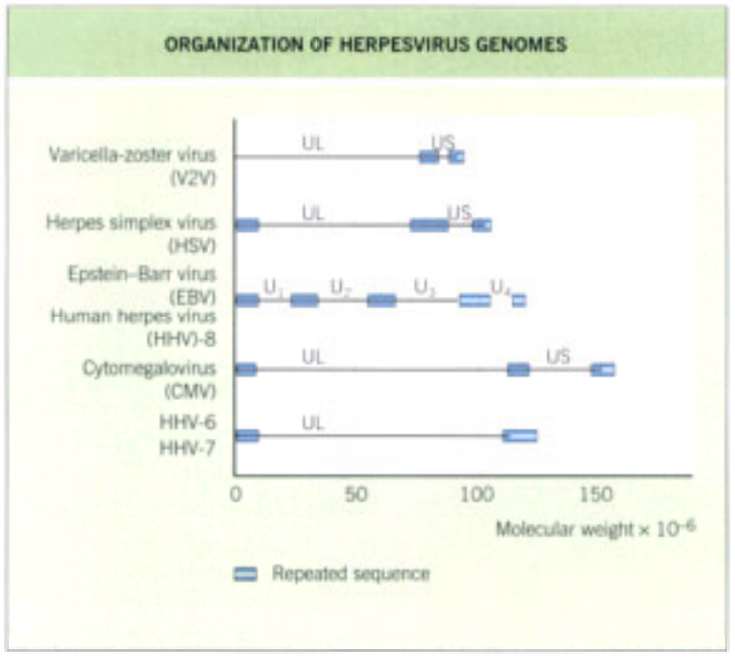


Figure 215-2 Enveloped virus particle. (a) Thin section. (b) Negative staining. These electron microscopic views (×140,000) show HSV. The DNA is surrounded by a nucleocapsid comprised of 162 individual protein subunits (150 hexavalent capsomers and 12 pentavalent capsomers) arranged in the form of an icosahedron. The nucleocapsid is in turn enclosed by the tegument and virus envelope bearing glycoprotein spikes. *Courtesy of Hans Gelderblom*.

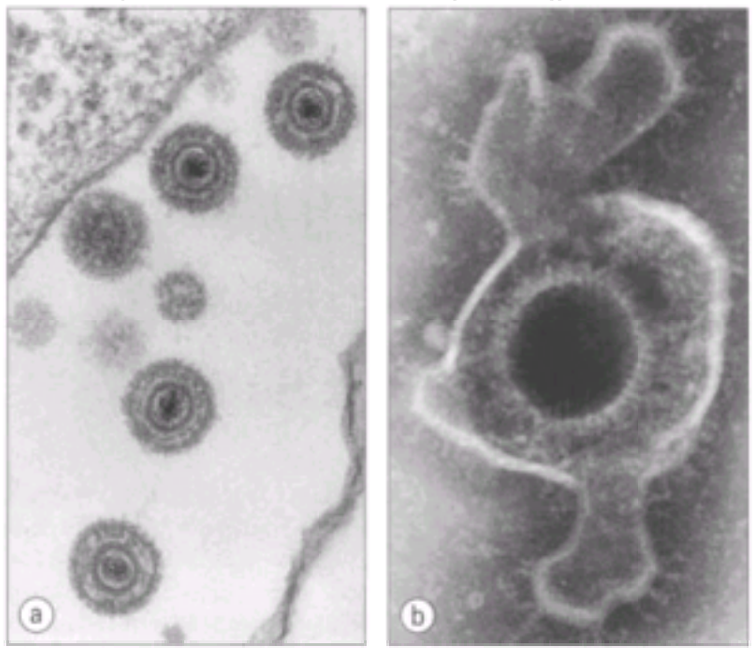


Figure 215-3 Phylogenetic relationship between the human herpesviruses and herpes B virus. The tree was created by neighbor-joining analysis of the glycoprotein B gene sequences. HVS, herpesvirus simiae (herpes B virus). *Adapted from Schultz, 2000.*[5]

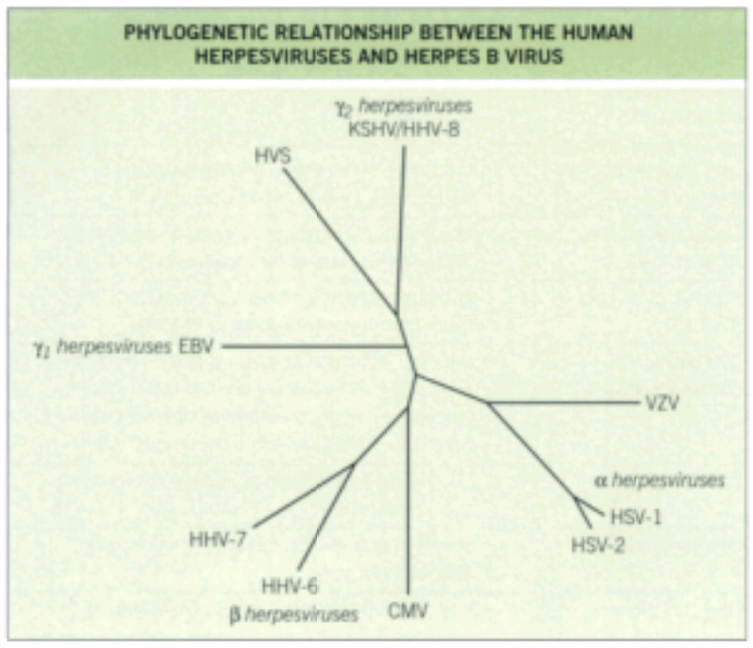


Figure 215-4 Herpesvirus replication. The tegument proteins effect the shut-down of host cell metabolism. On entry to the nucleus the DNA circularizes and binds a tegument protein and cellular factors to initiate transcription. Transcription and translation occur in three phases: immediate-early, early and late. Capsid proteins migrate into the nucleus and the viral DNA is encapsidated. The viral glycoproteins are extensively modified post-translationally by transit through the Golgi apparatus. The glycoproteins diffuse to the nuclear envelope. The nucleocapsids bud through the modified nuclear membrane and exit the cell via the endoplasmic reticulum or are released on cell lysis.

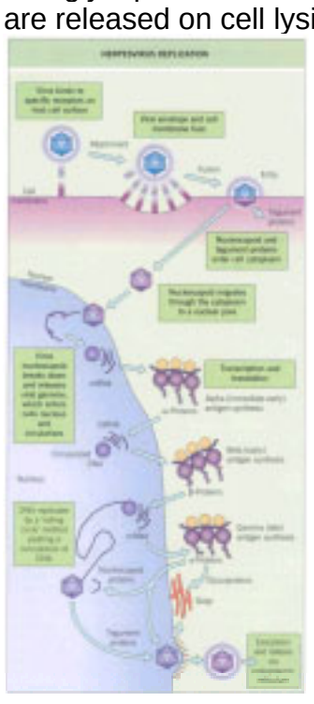


Figure 215-5 Pathogenesis of varicella-zoster virus infection.

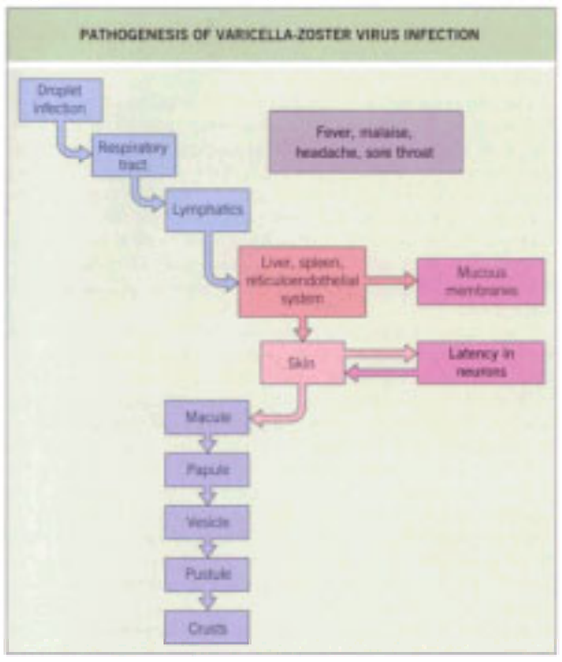


Figure 215-6 Pathogenesis of Epstein-Barr virus infections. Infection may result in lytic infection of the cell or cell immortalization, which can be distinguished by the production of virus and the expression of different viral proteins and antigens. T cells limit the outgrowth of EBV-infected cells. LMP, latent membrane protein; LP, Epstein-Barr nuclear antigen leader protein. *Adapted from Strauss et al., 1993.*[22]

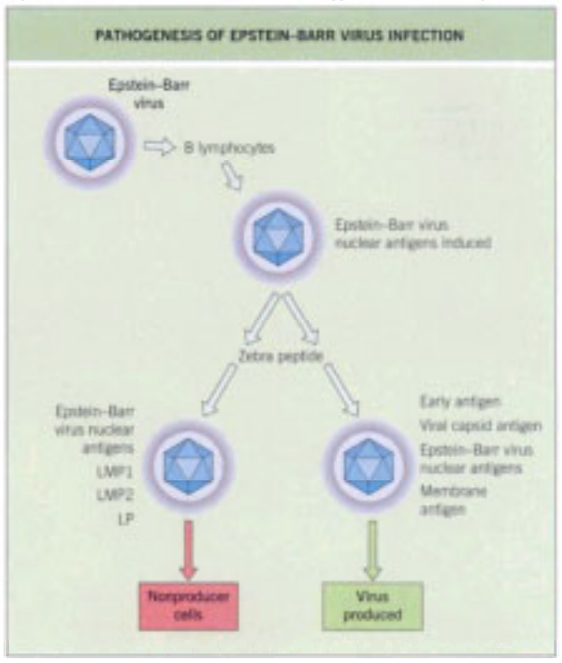


Figure 215-7 Diseases caused by herpes simplex virus-1 and herpes simplex virus-2.

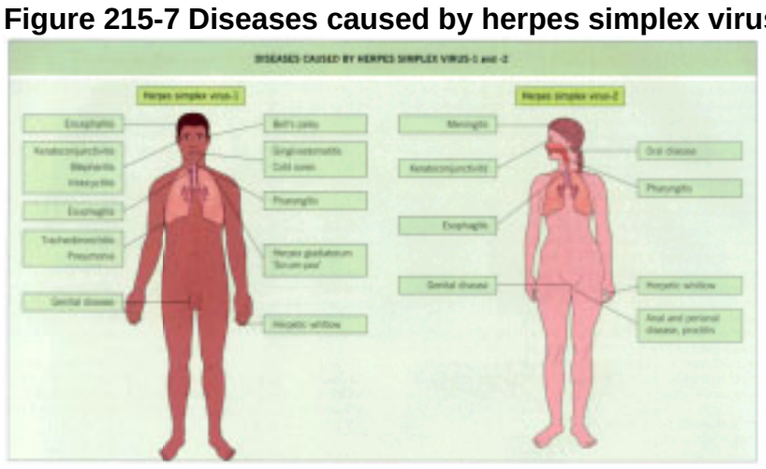


Figure 215-8 Mode of action of aciclovir. Phosphorylation is effected by virus-specified thymidine kinase. The aciclovir monophosphate is then converted to the triphosphate form by cellular kinases. Aciclovir triphosphate binds with high affinity to the virus-specified (but not host derived) DNA polymerase, leading to inactivation of the enzyme's activity. In addition, incorporation of aciclovir in the DNA blocks further chain elongation.

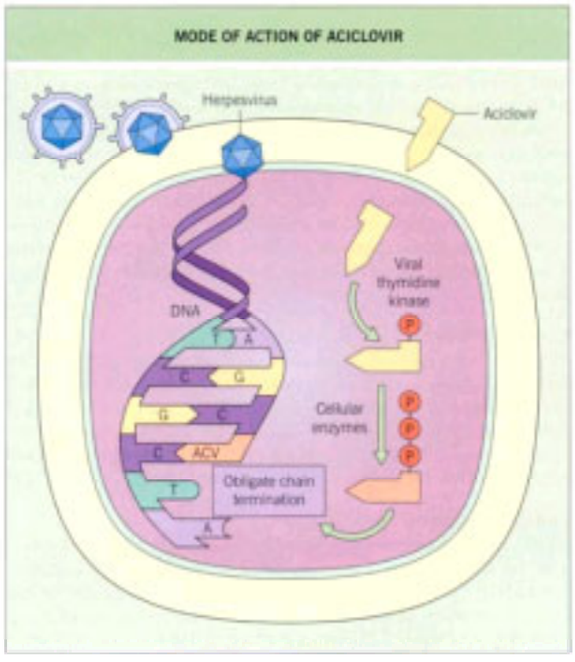


Figure 216-1 Human papillomavirus particles. The particles are nonenveloped, have icosahedral capsids and are 55nm in diameter. *Courtesy of Dr M Reissig.*

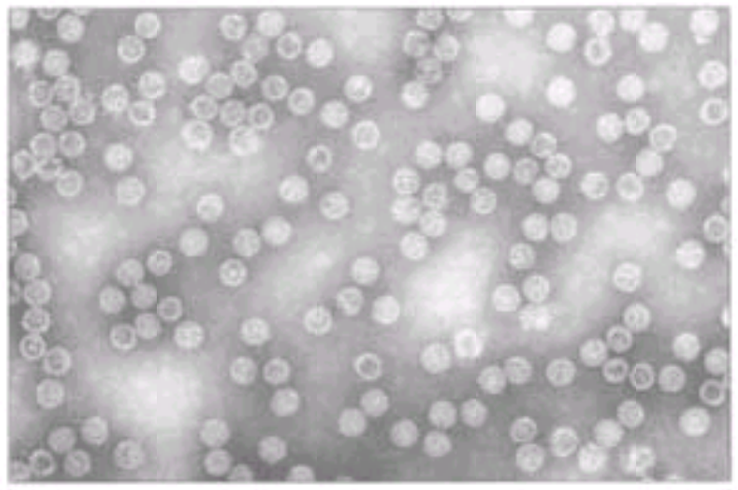


Figure 216-2 Worldwide distribution of HPV type in invasive cervical carcinoma. *The data are based on tests of over 900 cancers from different countries.*[7]

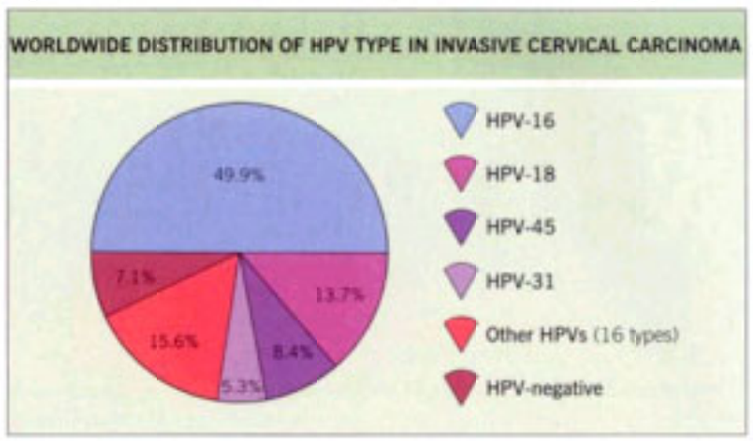


Figure 216-3 Effect of high-risk HPV E6 on the cell cycle. In normal cells (left), DNA damage results in increased p53 production, which leads to arrest of cell cycle in G1 phase, allowing the cell time to repair DNA damage. In cells infected with high-risk HPVs (right), the HPV E6 mediates degradation of p53, so there is no accumulation of p53, no cell-cycle arrest, and continued cell multiplication. This leads to genetic instability and accumulation of cellular mutations. *Courtesy of Dr TD Kessis.*

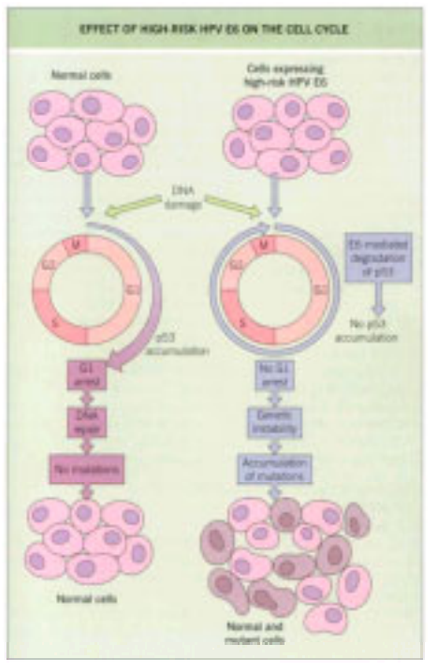


Figure 216-4 Gain of chromosome 3q in early cervical carcinoma. The figure displays a ratio image after comparative genome hybridization, in which normal reference metaphase chromosomes are hybridized with a mixture of differentially labeled tumor DNA (green label) and normal DNA (red label). The chromosomes are ordered in a karyogram-like fashion. Chromosome 3q is gained (over-representation of green label) and chromosomal band 13q21 is lost (over-representation of red label) in this carcinoma. *With permission from Heselmeyer et al.;*[10] *copyright (1996) National Academy of Sciences, USA.*

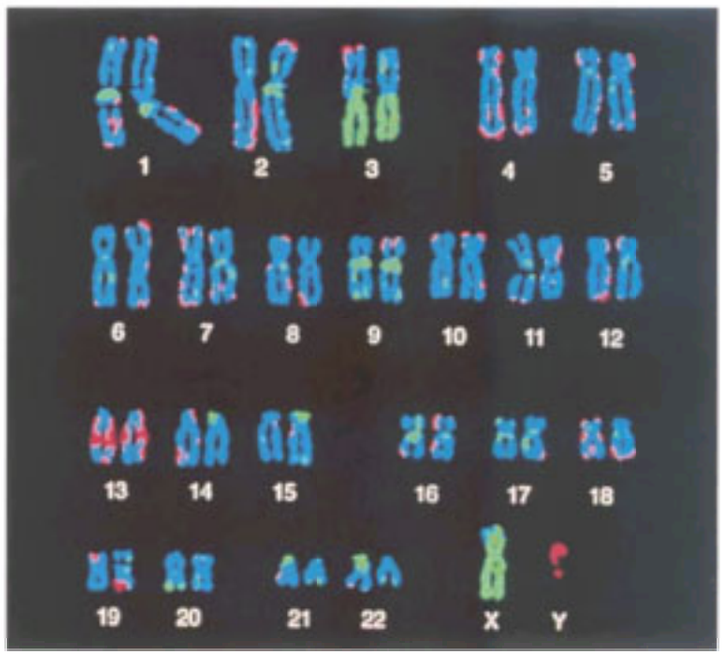


Figure 216-5 RRP of juvenile onset. (a) A respiratory papilloma on the vocal cord of a child. (b) Papilloma obstructing the respiratory tract. *Courtesy of Dr H Kashima.*

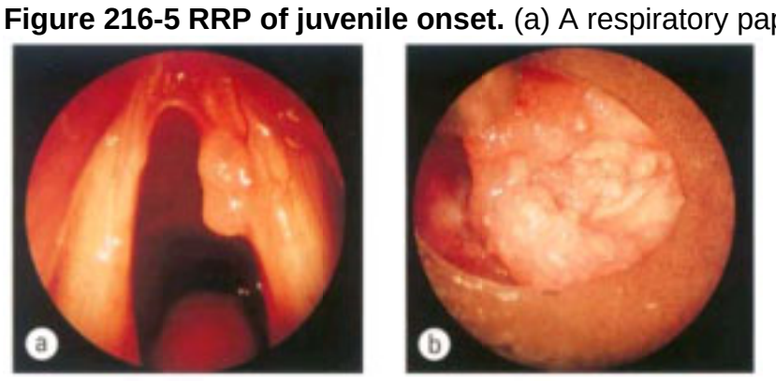


Figure 216-6 Foci of demyelination in PML. The foci in the superior frontal gyrus are the result of PML (Luxol fast blue stain). *With permission from Harrison and McArthur.*[26]

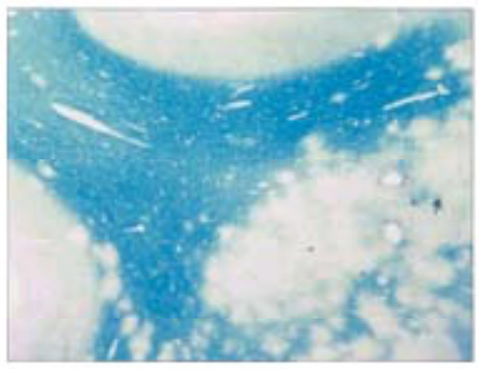


Figure 216-7 Enlarged oligodendrocytes in PML (stained with antiviral serum). *With permission from Harrison and McArthur.*[26]

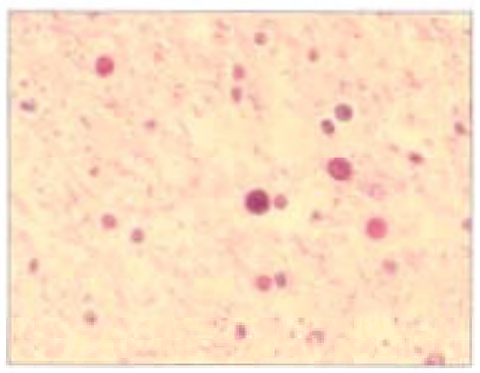


Figure 217-1 Electron micrograph of parvovirus B19 particles showing both full particles (short arrow) and empty capsids (long arrow).

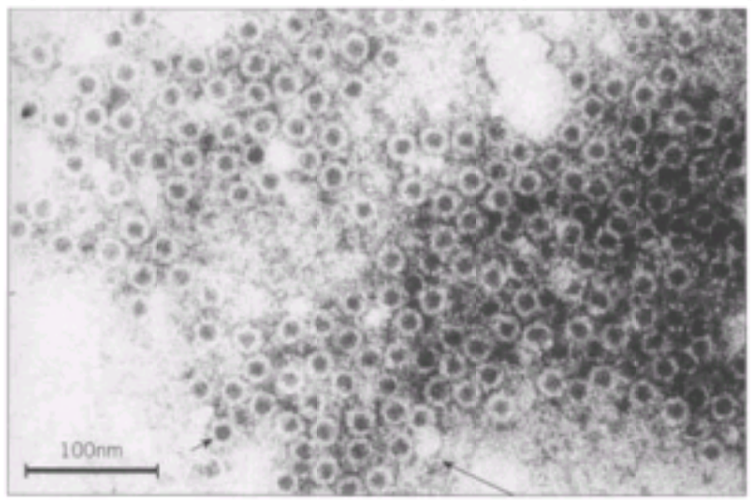


Figure 217-2 Course of parvovirus B19 infection. Volunteers were inoculated nasally with viremic serum and virologic, hematologic and clinical events observed.

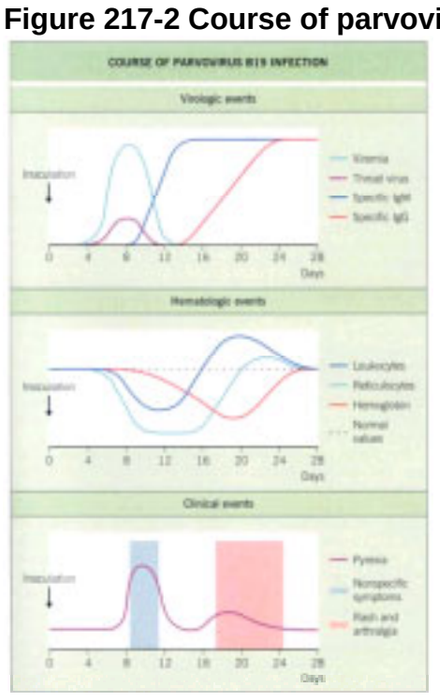


Figure 217-3 Classic 'slapped cheeks' of a child with erythema infectiosum, or fifth disease, caused by parvovirus B19. A lacy macular erythematous eruption is also present on the trunk but not shown. *Courtesy of Dr K Motton.*

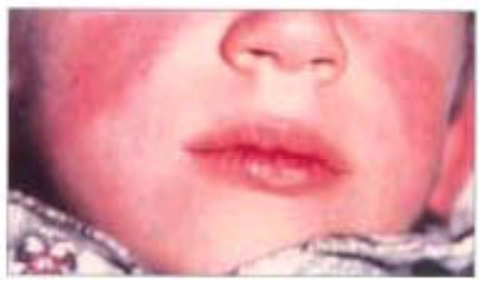


Figure 218-1 A negative-stained M form of molluscum contagiosum virus. Molluscum contagiosum virus from lesion material.

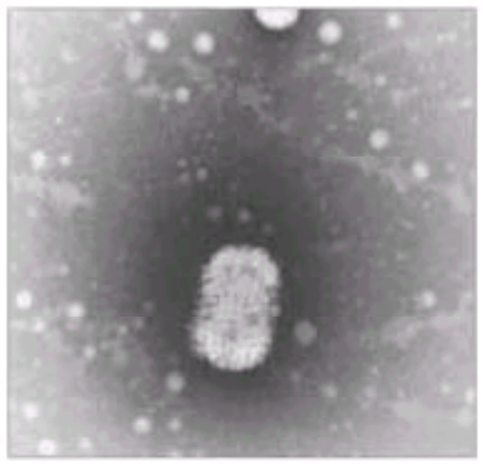


Figure 218-2 The cytoplasmic poxvirus replication cycle. Poxvirus virions containing early RNA transcription machinery attach to, and fuse with, the plasma membrane (uncoating I). Early genes are expressed that code for a variety of functions that modify the host cell for optimal virus replication, attentuate the host response to infection and mediate virus synthetic processes. After further uncoating (II), the virus genome is replicated via concatamers, late transcription factors are expressed from intermediate genes and late gene RNA is synthesized. Late genes encode the early transcription system, enzymes and structural proteins necessary for virion assembly, which commences with the formation of membrane structures in the intermediate compartment and the packaging of resolved unit length genomic DNA. The intracellular mature virion has two membranes derived from the intermediate compartment. It may remain in the cytoplasm or (in certain virus species) become occluded in an A-type inclusion body or become wrapped by a further two membranes in the Golgi and exported from the cell with the loss of one membrane (extracellular enveloped virions). The extracellular enveloped virions are thought to be most important in cell-to-cell spread and systemic disease. This replication scheme is based on the study of the prototypic poxvirus vaccinia.[1] Other poxvirus species probably vary from this model mainly in the types of growth factors and host response modifiers encoded by the virus and the amounts of extracellular enveloped virions produced. *Adapted from Moss.*[1]

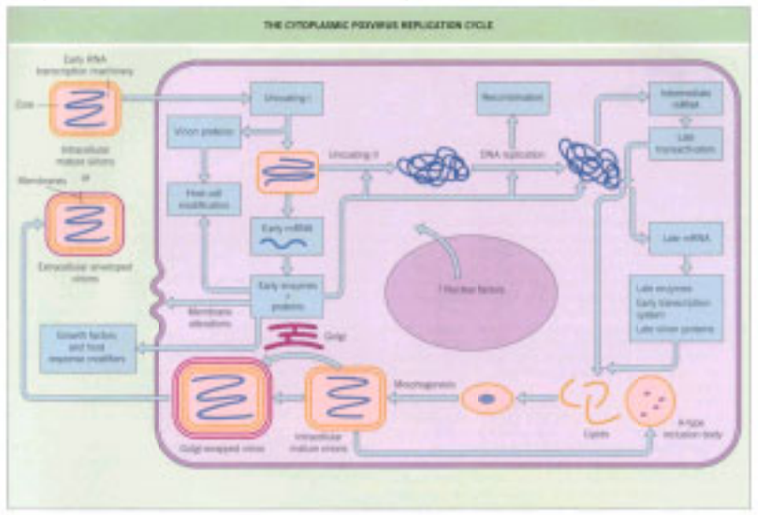


Figure 218-3 Molluscum contagiosum lesion. In this lesion a major and minor umbilicus has formed as a result of the hypertrophy of infected cells and hyperplasia of the basal cells, which caused a severe invagination of the epidermis but no loss of integrity of the basement membrane. The molluscum bodies stain as pink to purple acidophilic hyaline masses up to 37 × 27µm in size. Small arrows: molluscum bodies. Large arrows: epidermis-dermis boundary. (H & E.)

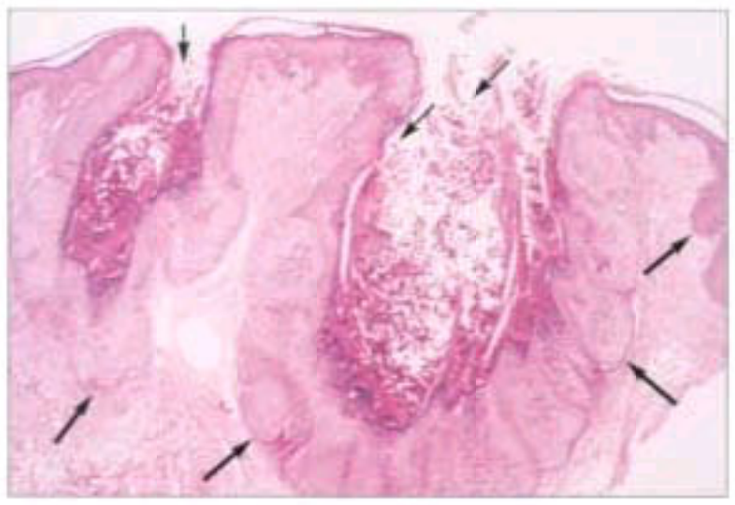


Figure 218-4 Molluscum contagiosum lesions. These are the more typical, but still large, lesions of molluscum contagiosum. *Courtesy of J Burnett.*

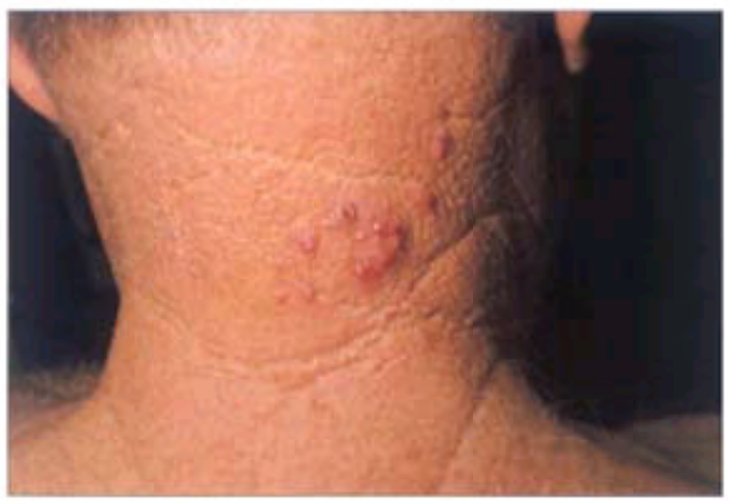


Figure 218-5 A typical orf lesion at the target stage of development. *Courtesy of Andrew Mercer.*

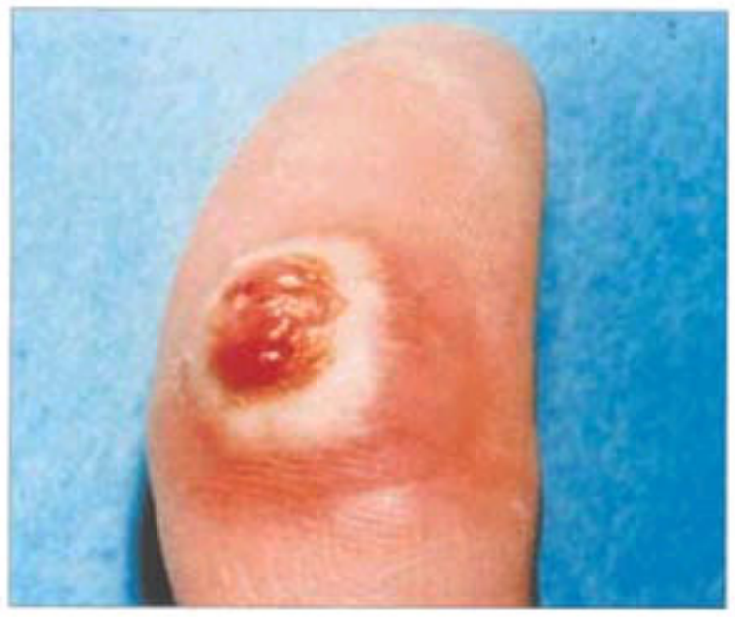


Figure 218-6 Monkeypox rash. A 7-year-old Zairian girl, 2 days after the onset of the rash. *Courtesy of M Szczeniowski.*

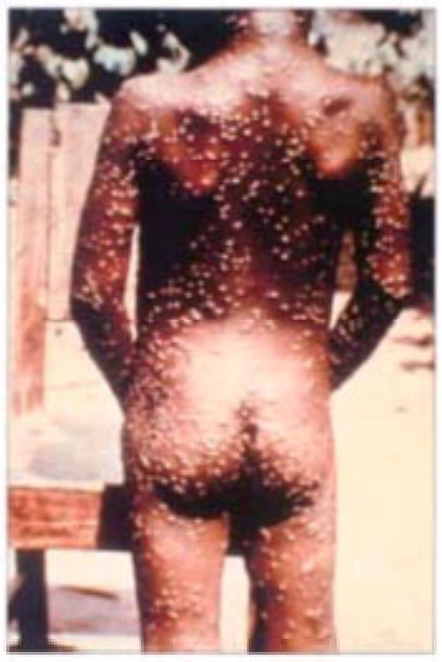


Figure 218-7 Primary and secondary lesions of cowpox. The primary lesion is at the early eschar stage (probably 2–3 weeks after infection), whereas the secondary lesion (below) is at the early vesicular stage. *With permission from Baxby et al.* [24]

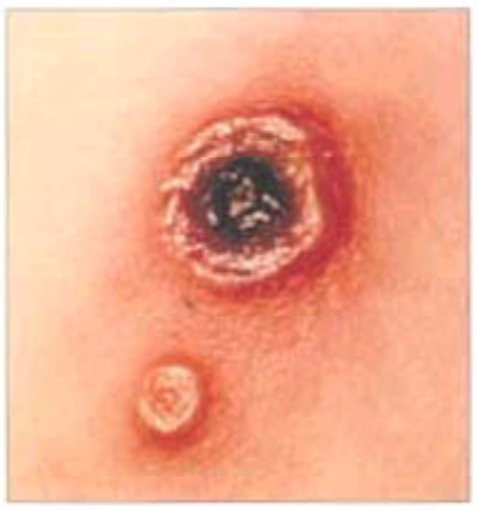


Figure 219-1 World distribution of rabies.

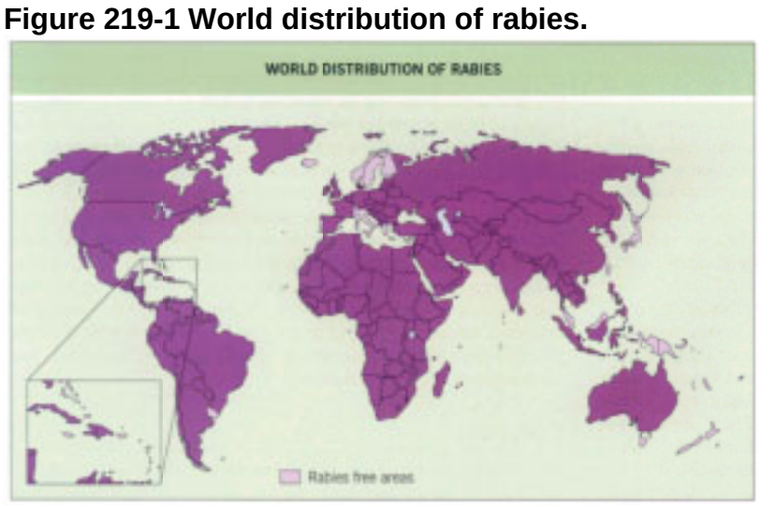


Figure 219-2 Negri bodies in cerebellar Purkinje cells in a human victim of rabies encephalitis. The intracytoplasmic dark-staining Negri bodies are marked with arrows. *Courtesy of the Armed Forces Institute, Bethesda, USA.*

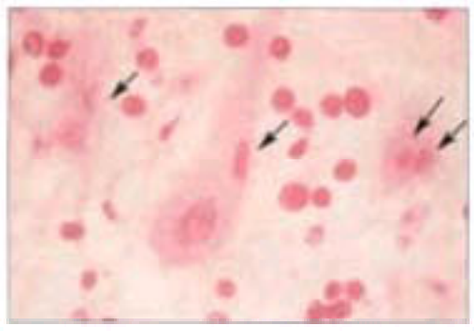


Figure 219-3 Pathogenesis of rabies.

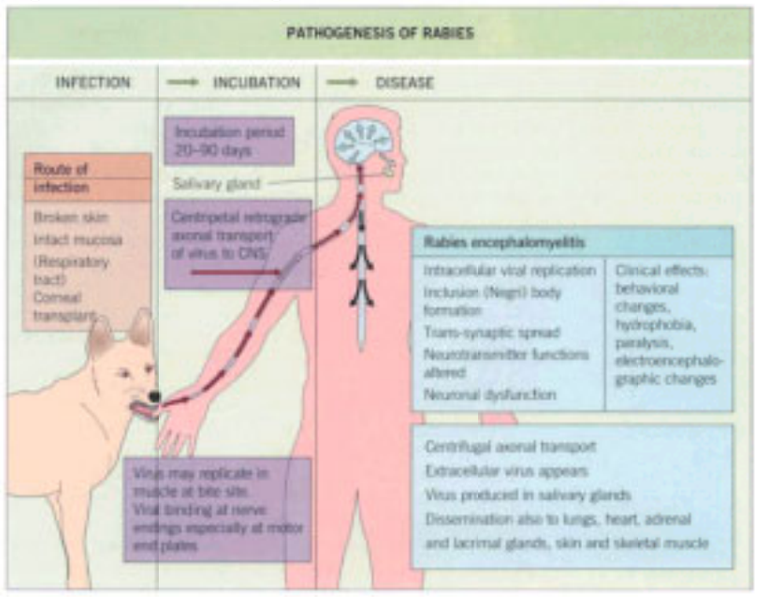


Figure 220-1 Cytopathic effect caused by adenovirus on Hep-2 cell line culture. (a) Uninoculated cell line. (b) Enlarged, refractile, rounded cells forming grape-like clusters.

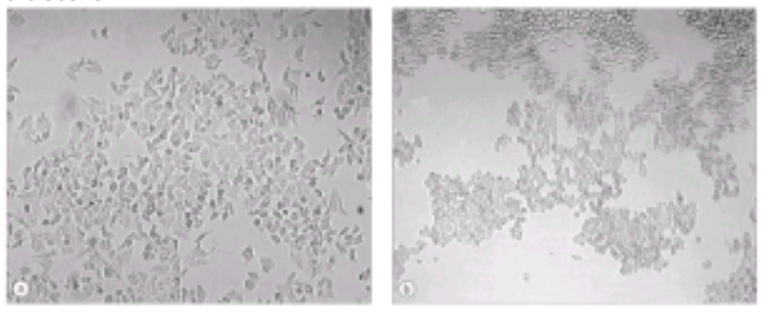


Figure 220-2 Cytopathic effect caused by rhinovirus on human foreskin fibroblasts (HFF) cell line culture. (a) Uninoculated cell line. (b) Formation of small teardrop- to oval-shaped highly refractile cells.

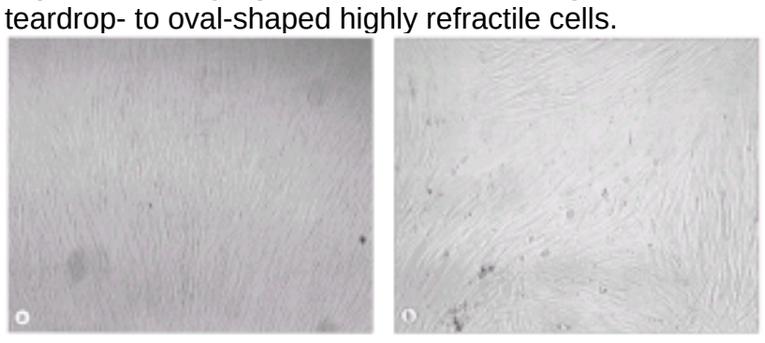


Figure 220-3 Cytopathic effect of RSV on Hep-2 cell line culture and identification of RSV antigen by means of IFA. (a) Uninoculated cell line. (b) Syncytia formation in cell line culture. (c) Positive cells coloring green under IF microscope.

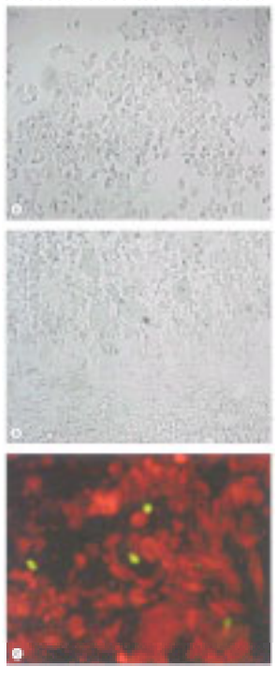


Figure 220-4 Identification of hemadsorbing viruses. (a) Uninoculated primary rhesus monkey kidney (PRMK) cell line. (b) Non-specific rounding or clumping of PRMK cells. (c) Positive hemadsorption of guinea-pig red blood cells.

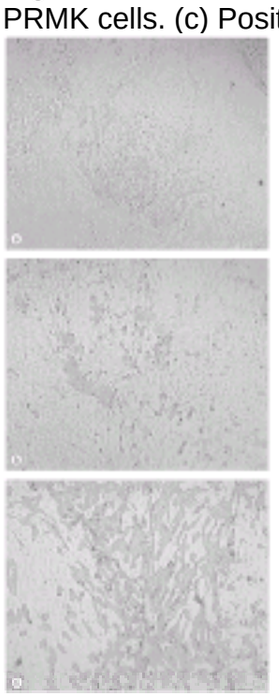


Figure 220-5 Differentiation of hemadsorbing viruses by means of IFA. (a) Negative control. (b) Influenza B positive. (c) Parainfluenza-3 positive, showing a comparatively more finely granular staining.

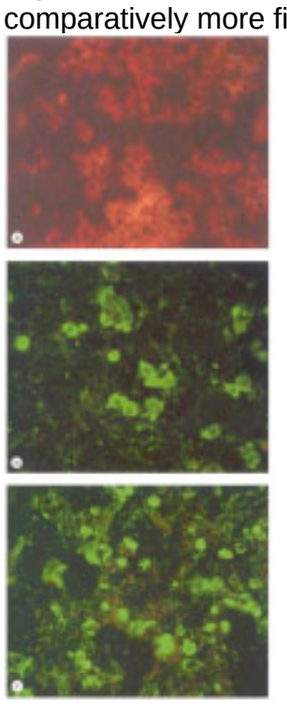


Figure 221-1 Typical electron microscopic view of retroviruses (HIV) showing an electron-dense core. *Courtesy of Dr Piet Joling.*

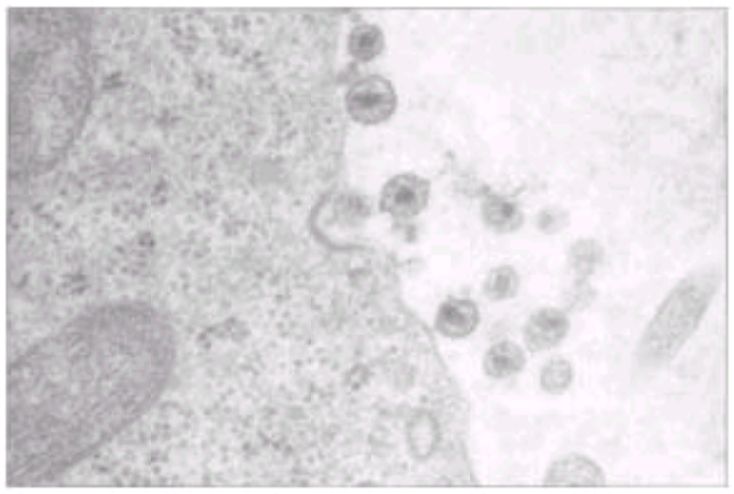


Figure 221-2 Structure of a retrovirus.

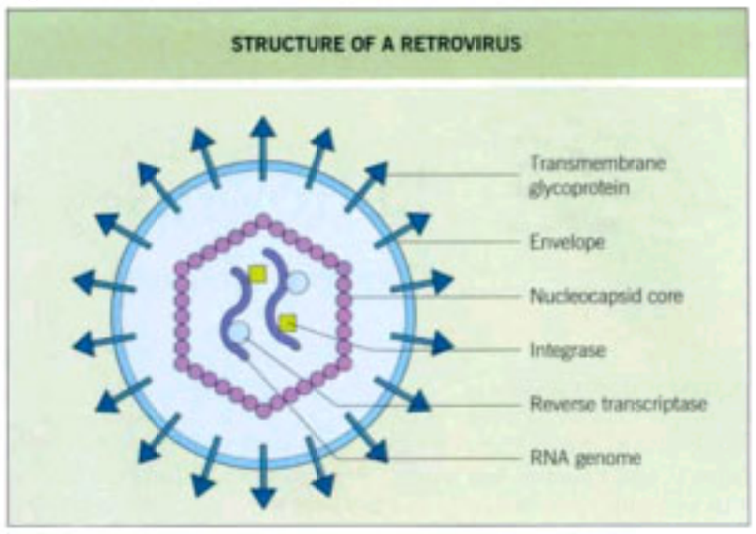


Figure 221-3 Genomic organization of three complex retroviruses. The diagram shows HIV-1, HIV-2 and HTLV. The components of the long terminal repeats are shaded. The open reading frames that encode viral proteins are depicted by unshaded boxes. *Adapted from Galasso et al.*[1]

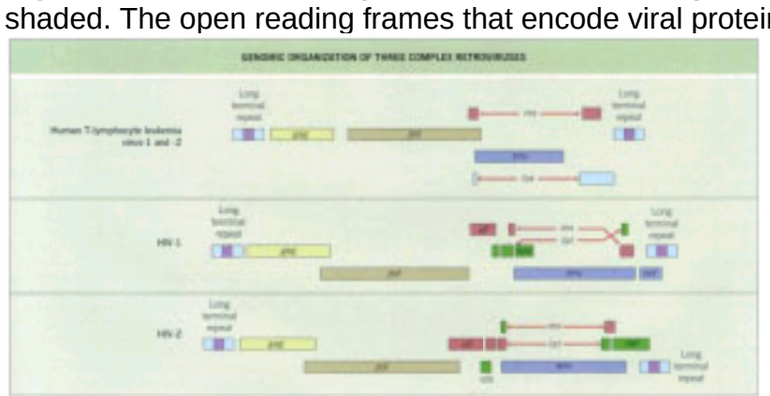


Figure 221-4 Replication cycle of retroviruses.

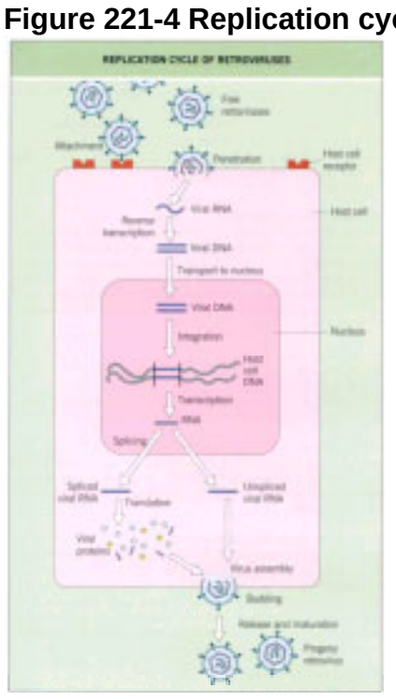


Figure 221-5 Typical 'clover leaf' appearance of nuclei of HTLV-1 induced adult T cell leukemic cells. *Courtesy of Steven M Opal.*

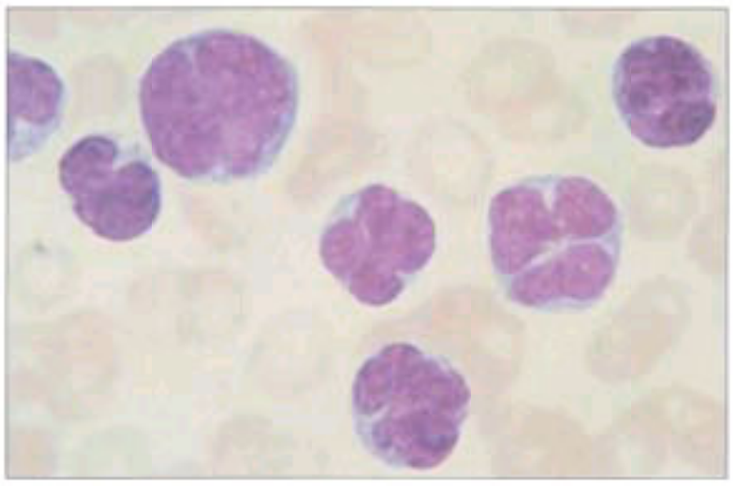


Figure 221-6 HIV-1 virion.

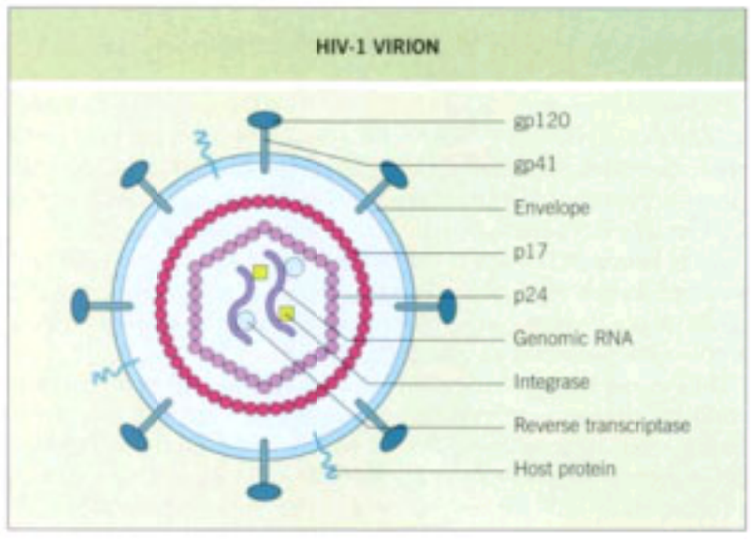


Figure 221-7 Dynamics of HIV-1 infection in vivo. Shown in the center is the cell-free virion population that is sampled when the viral load in plasma is measured. *Adapted from Perelson et al.*[54]

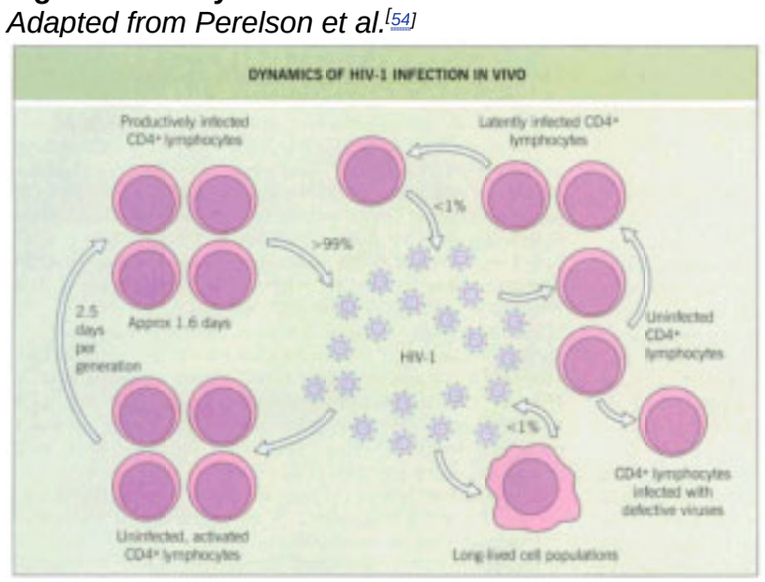


Figure 222-1 Maintenance of arbovirus cycle involving humans as the primary vertebrate host (e.g. urban dengue and urban yellow fever).

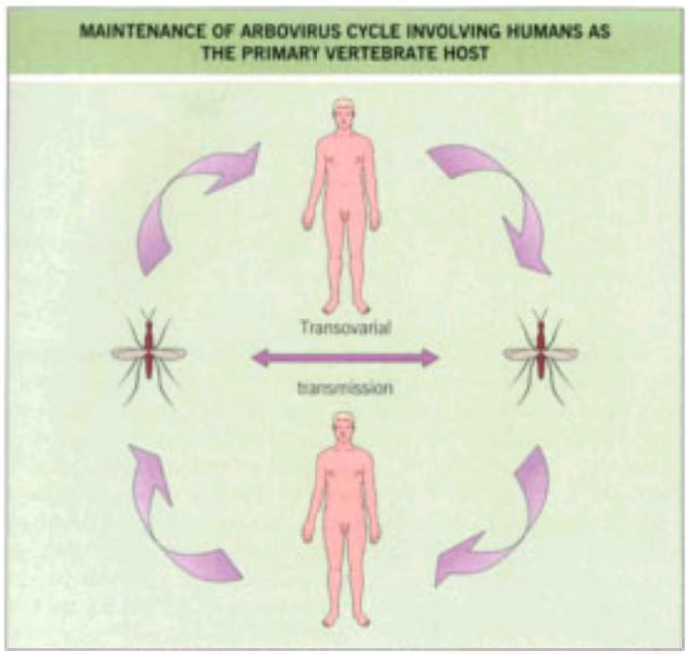


Figure 222-2 Sylvatic cycle of Western equine encephalitis (WEE) and St Louis encephalitis (SLE).

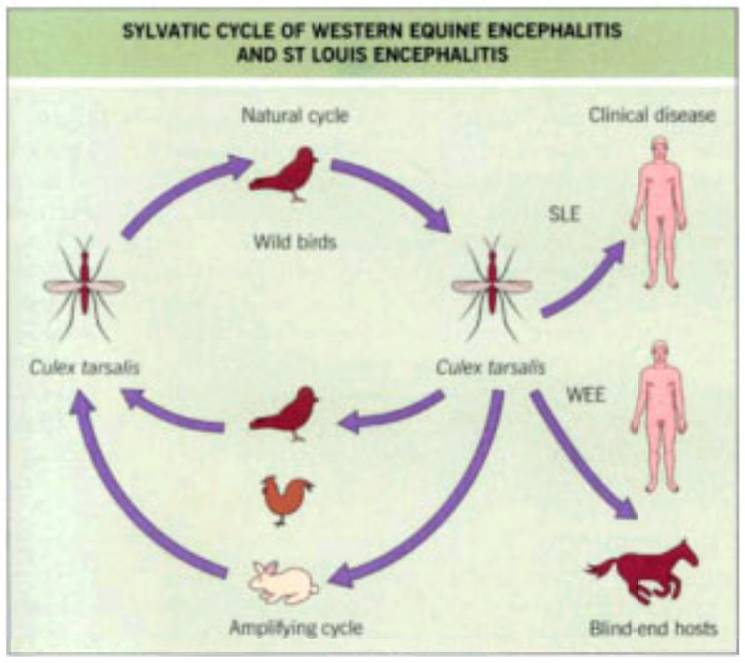


Figure 222-3 Arthropod sustained viral infection linked to tick transovarial transmission.

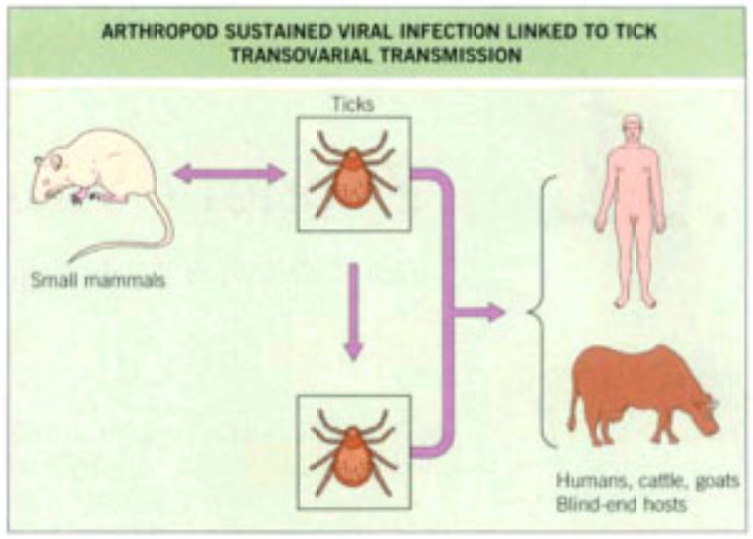


Figure 222-4 Distribution of reported cases of yellow fever, 1 January 1999. Dark purple areas show the worldwide distribution of reported cases.

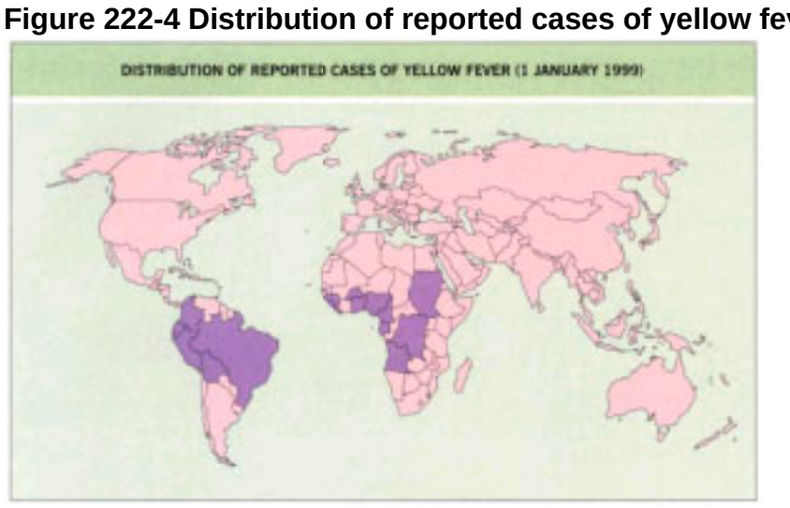


Figure 222-5 Histopathology of yellow fever in the liver. Yellow fever virus causes mid-zone necrosis of the liver tissue with sparing of hepatocytes around the central vain and the portal triads. *From Binford CH, Connor DH. Pathology of tropical and extraordinary diseases. Volume 1. Washington DC: Armed Forces Institute of Pathology; 1976.*

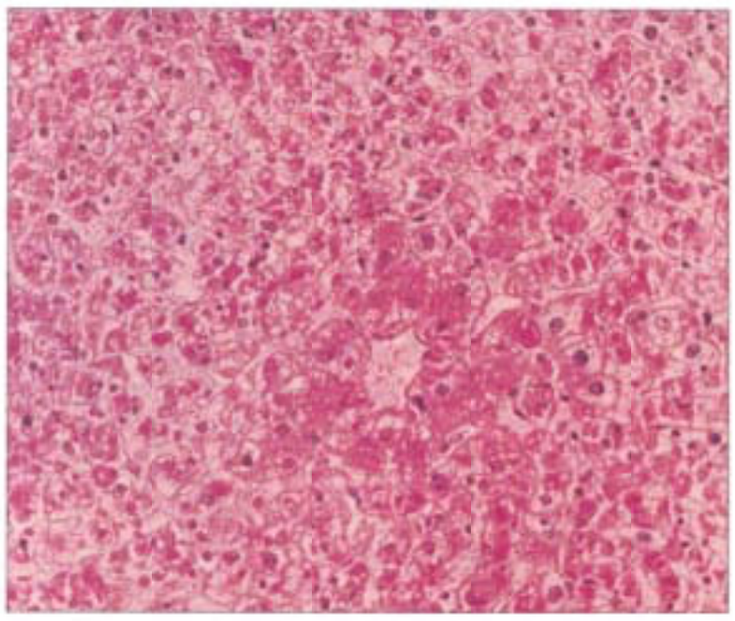


Figure 222-6 Histology of liver tissue in a fatal case of yellow fever. Note the Councilman bodies (arrowed), which are visible within degenerating hepatocytes.
From Binford CH, Connor DH. Pathology of tropical and extraordinary diseases. Volume 1. Washington DC: Armed Forces Institute of Pathology; 1976.

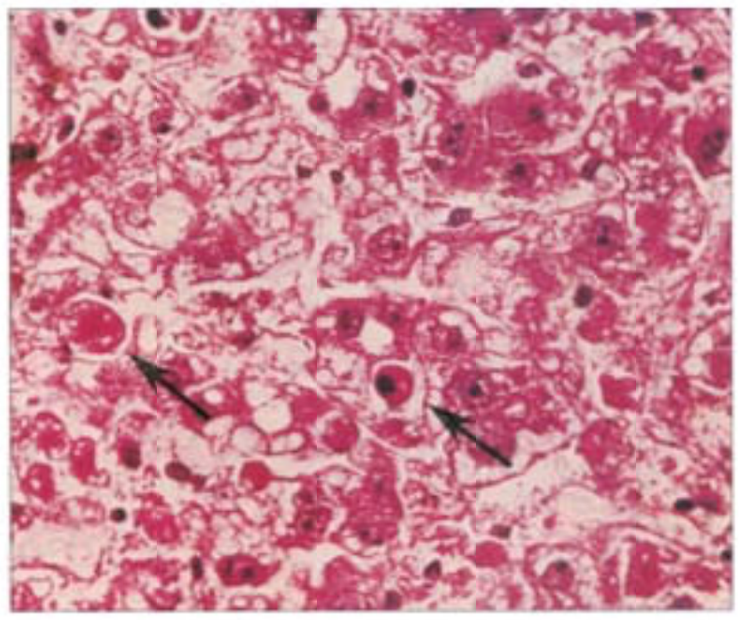


Figure 222-7 Arenaviruses. (a) Negative contrast electron micrograph of Lassa virus. (b) Arenavirus particle showing coiled nucleocapsid ribosomes and glycoprotein spikes. (c) Thin sections of infected Vero cells, showing extracellular virus, budding particles and intracellular inclusions. *Courtesy of G Lloyd, B Dowsett and ASR Featherstone.*

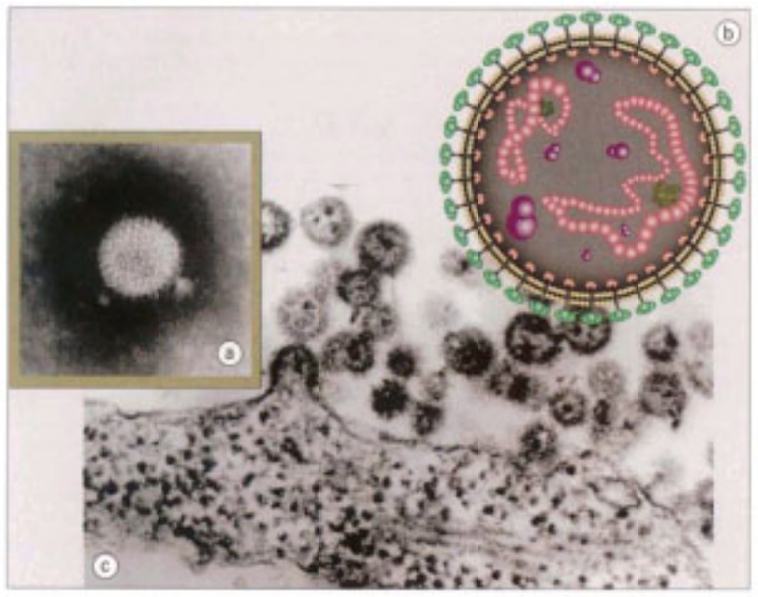


Figure 222-8 Distribution of pathogenic human arenavirus infection in South America and Africa, and year of first isolation of each virus.

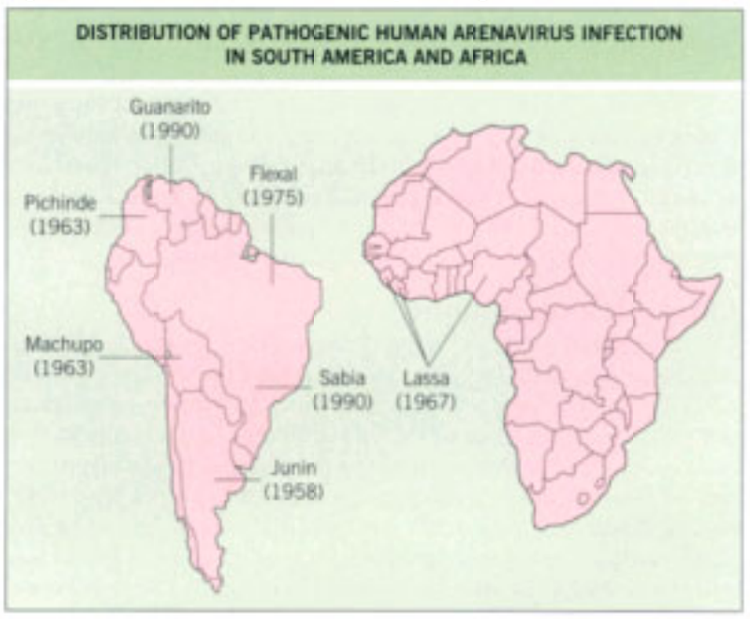


Figure 222-9 Comparison of effectiveness of diagnosis of Lassa fever in hospitalized patients by reverse transcriptase polymerase chain reaction (RT-PCR) and antibody detection by immunofluorescent antibody (IFA). (a) Samples from all patients were stratified with respect to the time since the onset of disease. The number of serum specimens in each group is indicated. (b) Samples were analyzed on a patient-by-patient basis with respect to time since admission to the hospital. *Adapted from Demby et al.* [26]

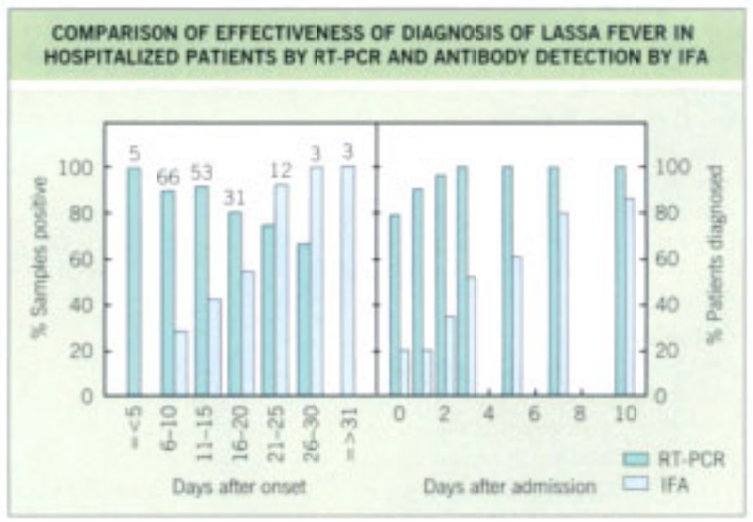


Figure 222-10 Lassa fever viral load in sequential samples from a case measured by real-time polymerase chain reaction.

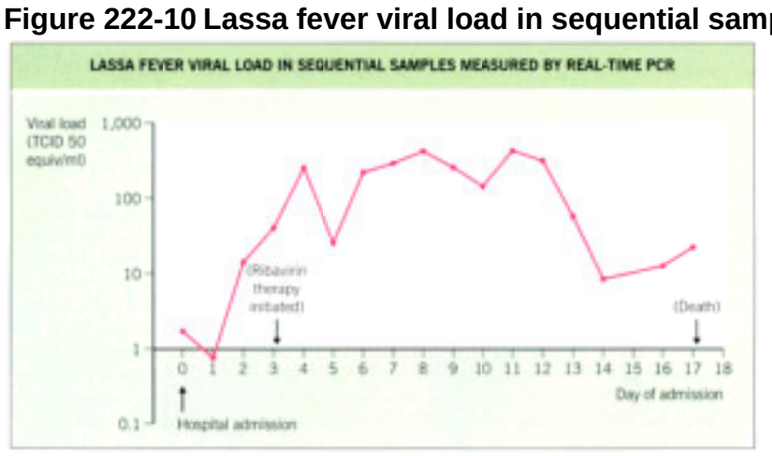


Figure 222-11 Filoviruses. (a) Filovirus particle in cross-section (not to scale) showing glycoprotein surface projections, nucleocapsid core inside the envelope. (b) Negative contrast micrograph of Ebola (Reston). (c) Intracellular filamentous particles showing nucleocapsid core. (d) Sections of Ebola (Republic of Congo, formerly Zaire) infected Vero cells, showing extracellular virus, budding particles. *Courtesy of G Lloyd, B Dowsett and ASR Featherstone.*

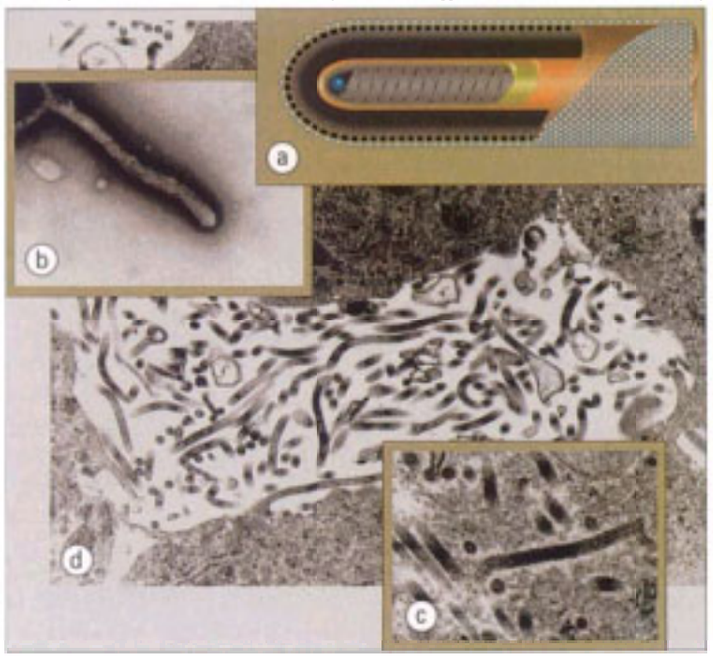


Figure 222-12 Distribution and dates of filovirus outbreaks in Africa.

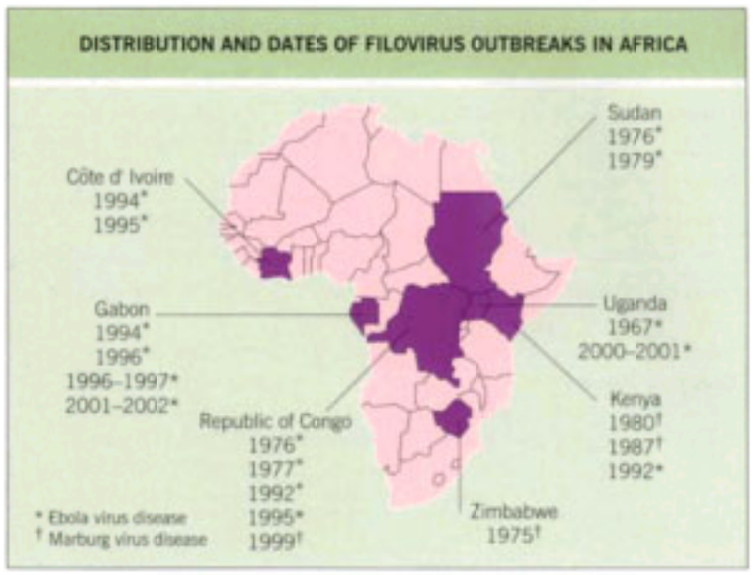


Figure 223-1 Immunodetection of PrP by Western blot in brain homogenates.

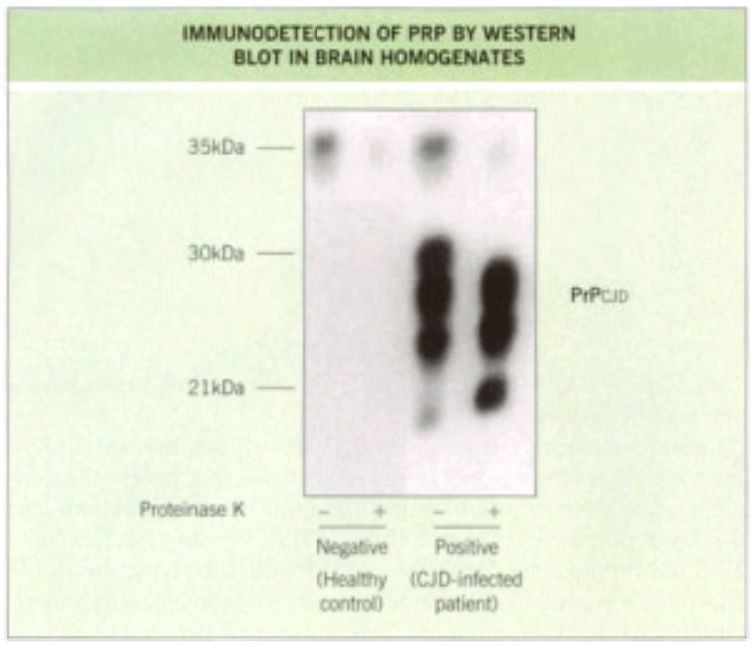


Figure 223-2 Human PrP. Representation of the 253 amino acid primary sequence of the human prion protein. Stop tranfert effector (STE) is a sequence that facilitates integration of the protein into the cell membrane.

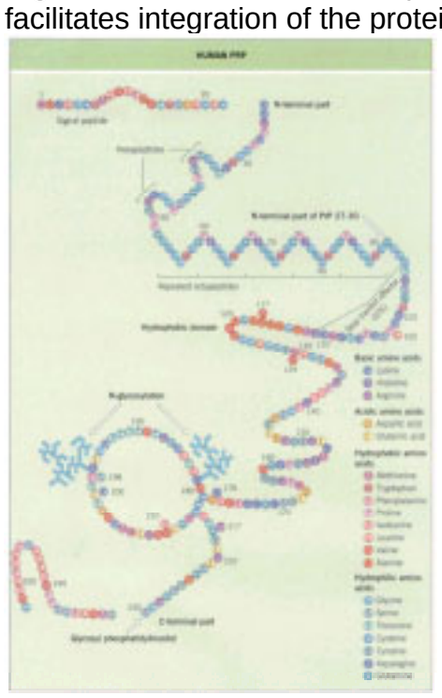


Figure 223-3 a-**Helices and** ß-**sheets in the murine PrP-c 121–231 fragment.** *Adapted from Riek et al.*[11]

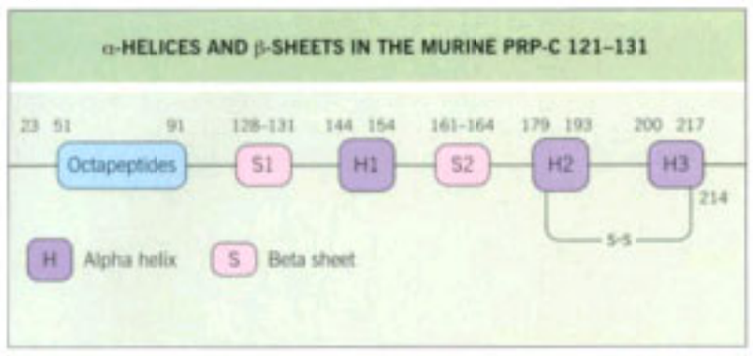


Figure 223-4 Gene structure of the PrP.

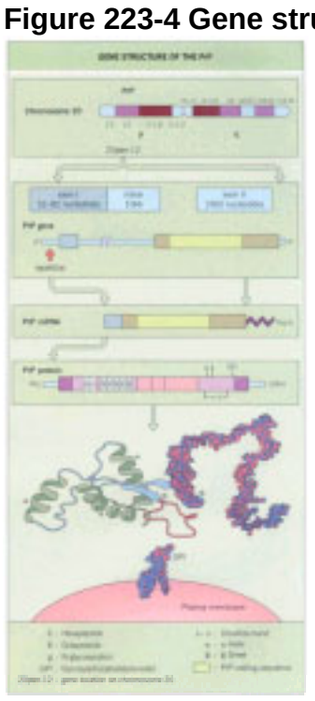


Figure 223-5 Prion hypothesis. *With permission from SB Prusiner, 1996.*

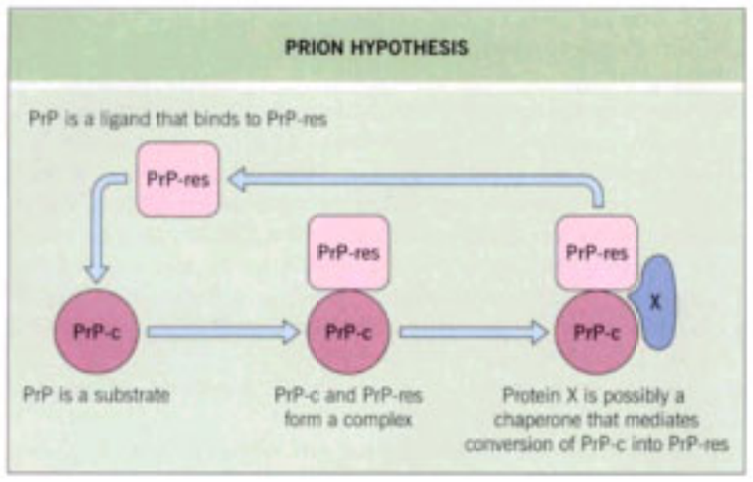


Figure 223-6 Molecular and cellular pathogenesis.

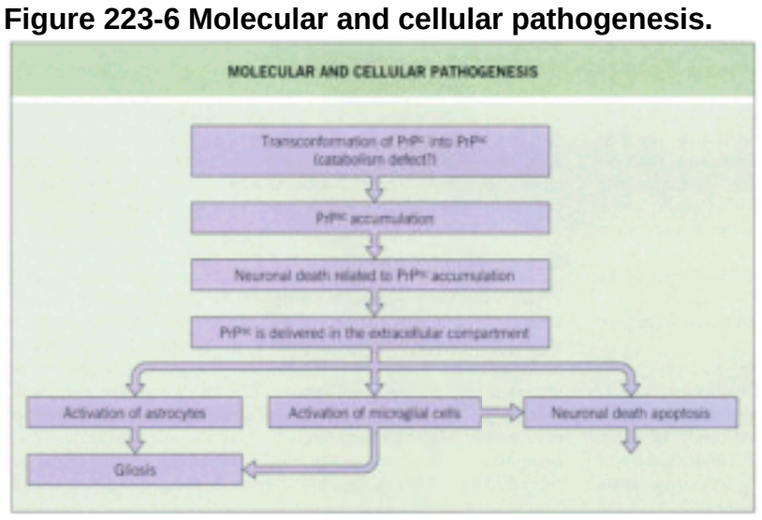


Figure 224-1 *Staphylococcus aureus* in a Gram stain of pus.

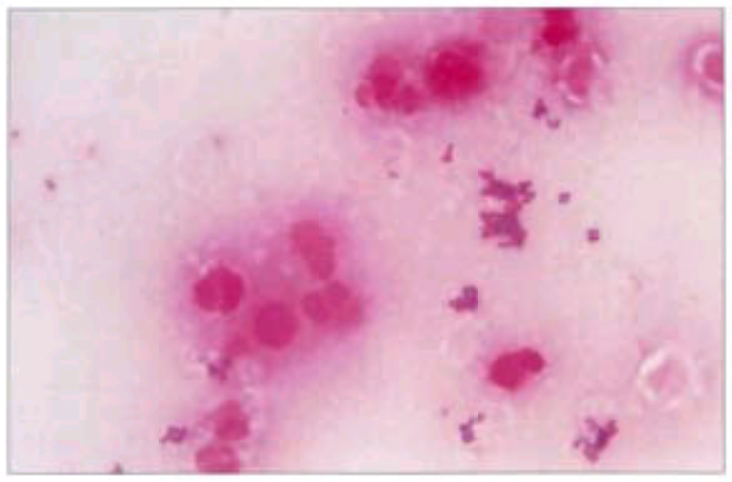


Figure 224-2 Relationship of *Staphylococcus* species. Dendrogram of the DNA relationships of *Staphylococcus* spp. and subspecies based on the relative percentage of DNA-DNA hybridization (reassociation) at optimal conditions. *Adapted with permission from Crossley and Archer.* [5]

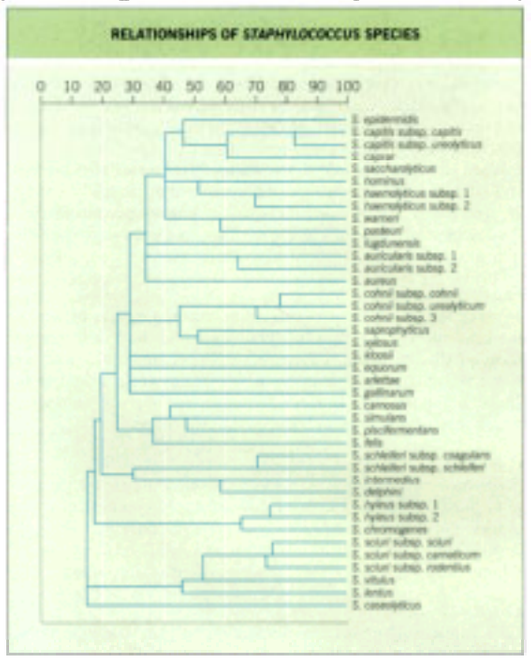


Figure 224-3 Phylogeny of *Staphylococcus* genus based on 16S rRNA sequences. *Adapted with permission from Crossley and Archer.*[5]

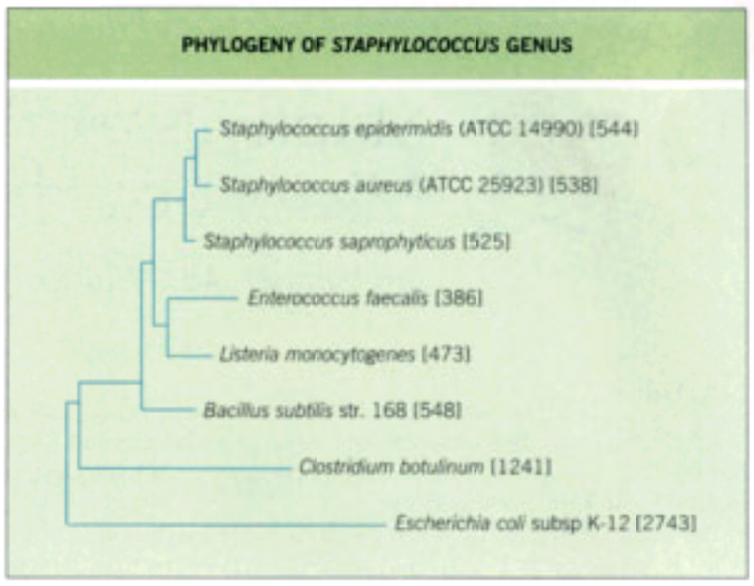


Figure 224-4 Structure of the peptidoglycan layer. The peptidoglycan layer consists of three integral parts. The glycan chains are built with 10–12 alternating *N*-acetylglucosamine (Glu) and *N*-acetylmuramic acid (Mur) subunits jointed with ß-1,4 glycosidic bonds. Vertical pentapeptide side chains are linked to the muramic acids subunits, and the side chains are in turn cross-linked with diagonal intrapeptide bridges. For example, the glycan chains in *Staphylococcus aureus* are cross-linked with pentaglycine bridges attached to L-glycine in one pentapeptide chain and D-alanine in an adjacent chain.

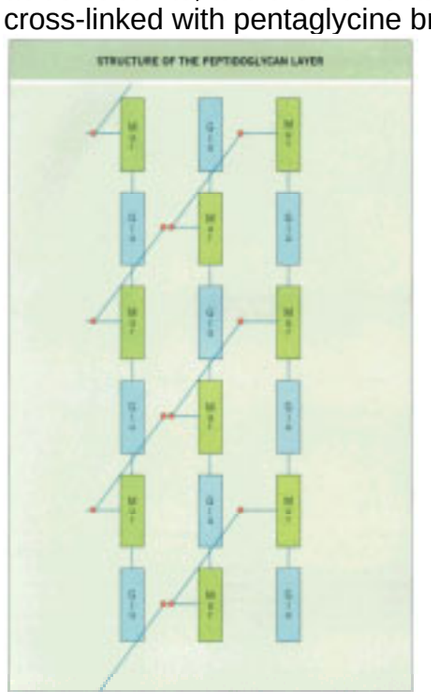


Figure 224-5 Structure and sorting of protein A. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus* and other Gram-positive bacteria. (a) Cell wall sorting of surface proteins consists of four distinct steps, which lead to the proteolytic cleavage of the polypeptide chain between threonine (T) and glycine (G). The carboxyl of threonine is subsequently amide-linked to the free amino group in the pentaglycine cross-bridge of the staphylococcal cell wall. The cell wall linkage of surface proteins in *S. aureus* (b) is compared with that proposed for other Gram-positive bacteria such as *Streptococcus pyogenes* (c) and *Listeria monocytogenes* (d). NacGlu, *N*-acetylglucosamine; NacMur, *N*-acetylmuramic acid. *Adapted with permission from Crossley and Archer.*[5]

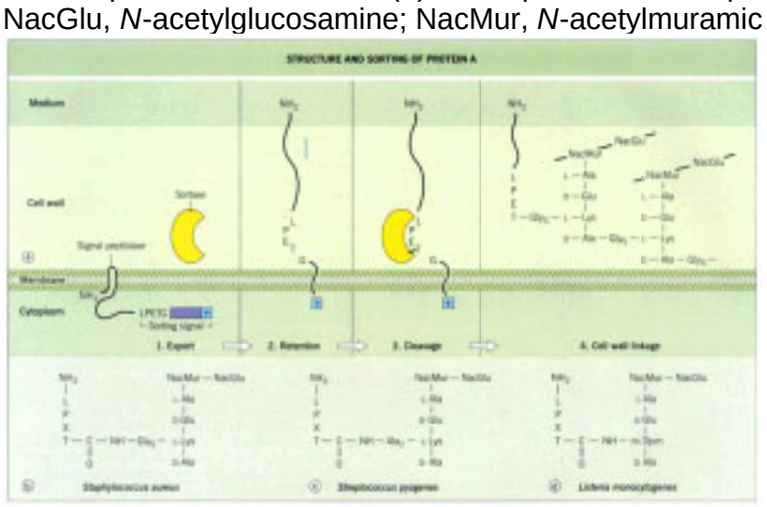


Figure 224-6 Structure of teichoic acid and linkage unit attaching teichoic acid to peptidoglycan in *Staphylococcus aureus*. The C2 and C4 positions of the ribitol residues are substituted by D-alanyl and *N*-acetylglucosamine residues. *Adapted with permission from Crossley and Archer.*[5]

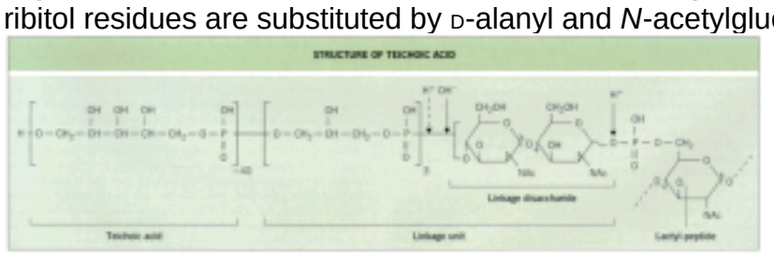


Figure 224-7 Phage type III of *Staphylococcus aureus.*

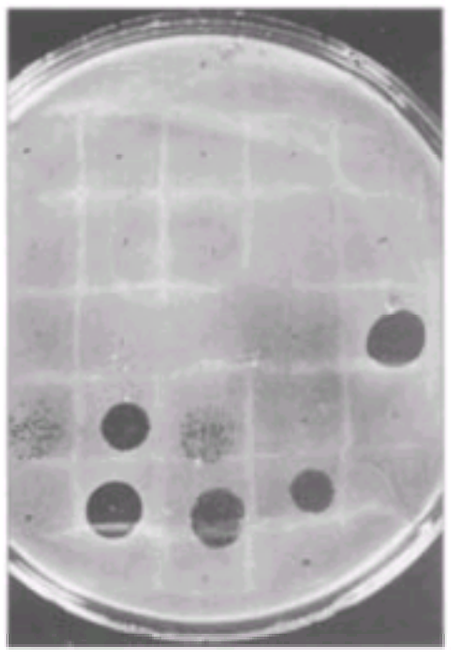


Figure 224-8 Bacterial adherence. The figure illustrates the events associated with bacterial (B) adherence to a biomaterial in relation to time and the molecular sequence in bacterial attachment, adhesion, aggregation and dispersion at substratum surface. A number of possible interactions may occur depending on the specificities of the bacteria or substratum system, the distance from the biomaterial and the stage of adherence. The attachment stage is mediated by non-specific forces. Adhesion is driven by specific adhesin-receptor interactions. The final aggregative step results in a bacterial macrocolony on the biomaterial surface in which the bacteria are firmly adherent to the biomaterial and each other. Bacterial exopolysaccharide blankets the macrocolony and may serve to improve the nutritional microenvironment and protect the bacteria from host defenses. In the dispersion phase, bacteria disaggregate, break loose from the macrocolony and drift free into the bloodstream. *Adapted with permission from Gristina AG. Biomaterial centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588. © 1987 American Association for the Advancement of Science.*

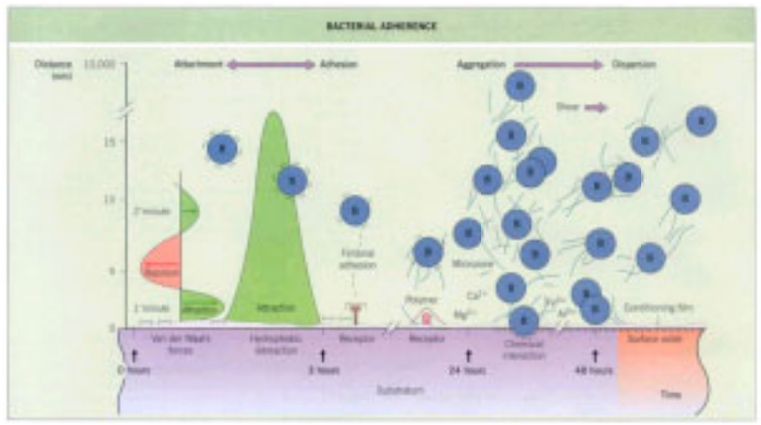


Figure 224-9 Opsonization of unencapsulated staphylococci. Opsonization through complement activation is primarily a function of C3b and iC3b. When antibody (ab) molecules bind to antigen, the antigen-antibody complex activates the first complement component, C1. C1 is then converted into an esterase, initiating the classical pathway. Additionally, some cell-wall components can activate the alternative pathway.

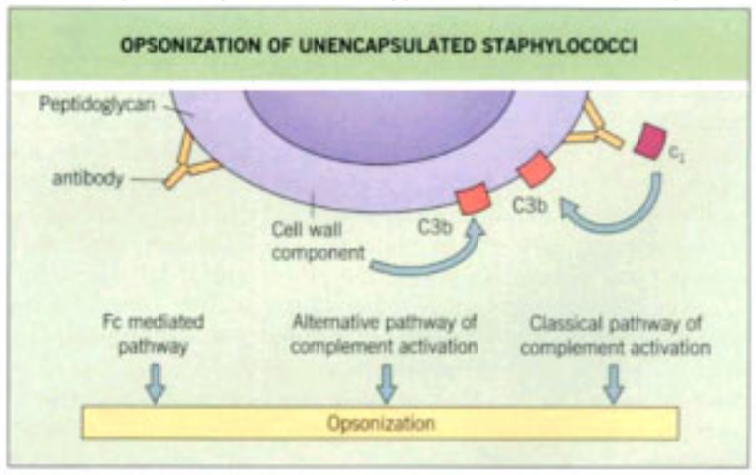


Figure 224-10 Prevention of opsonization in encapsulated staphylococci. (Left) The capsule of *Staphylococcus aureus* prevents binding of antibodies to peptidoglycan: no opsonization. (Right) The capsule prevents binding of opsonins on the cell wall of *Staphylococcus aureus* to complement and Fc receptors on PMNLs.

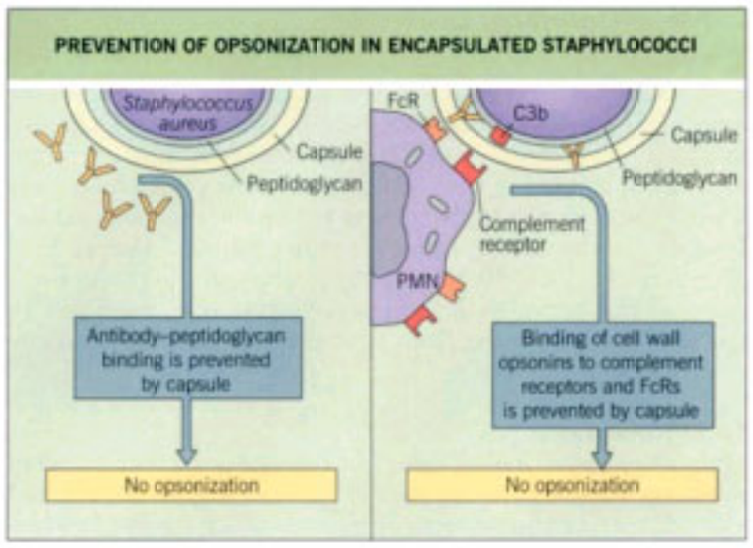


Figure 224-11 Differences between antigen and superantigen. Staphylococcal enterotoxin and TSST-1 act as superantigens, binding directly to MHC class II and the Vß chains of the T cell receptor (TCR) without the need for normal antigen processing.

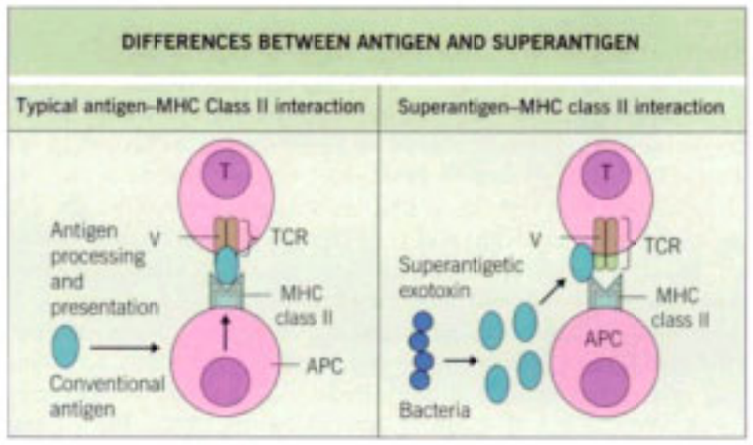


Figure 224-12 Potential sources for bacterial contamination of intravascular catheters. Bacteria gain access to the catheter by the following routes: contamination of the catheter hub, contamination of the infusate, transcutaneous migration and hematogenous seeding.

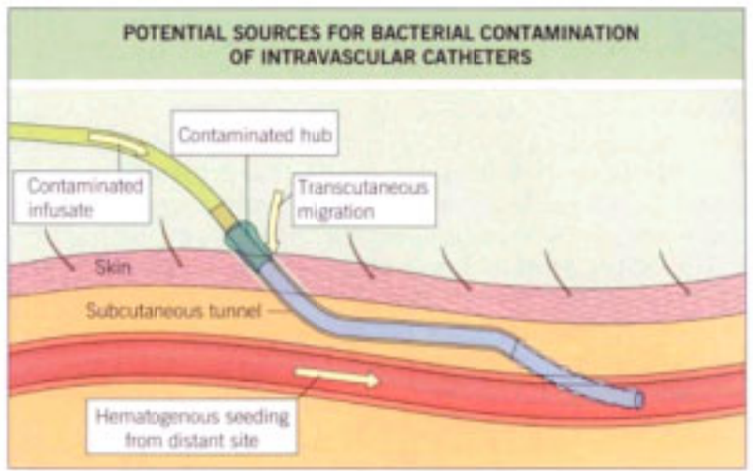


Figure 225-1 ß-**Hemolytic streptococci group A on a blood agar plate.** Note the clear ß-hemolytic zone.

Figure 225-2 a-**Hemolytic streptococci on a blood agar plate.** Note the small greenish zone surrounding the colonies, which is a characteristic of viridans streptococci.

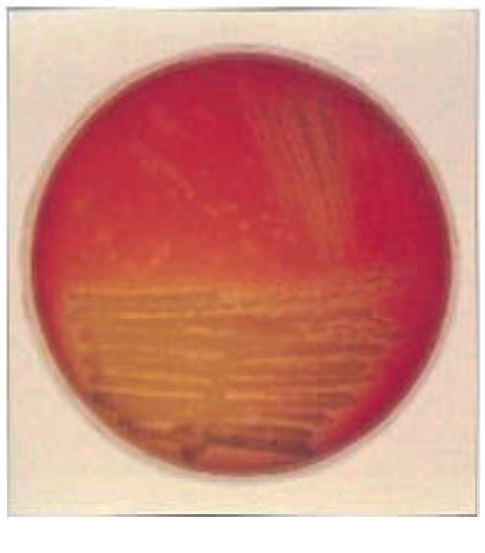


Figure 225-3 ?-**Streptococci on a blood agar plate.** Note the absence of hemolysis.

Figure 225-4 Bacterial interference. Inhibition of growth of ß-hemolytic streptococci by an a-hemolytic *Streptococcus* sp. No such inhibition is seen with other streptococci.

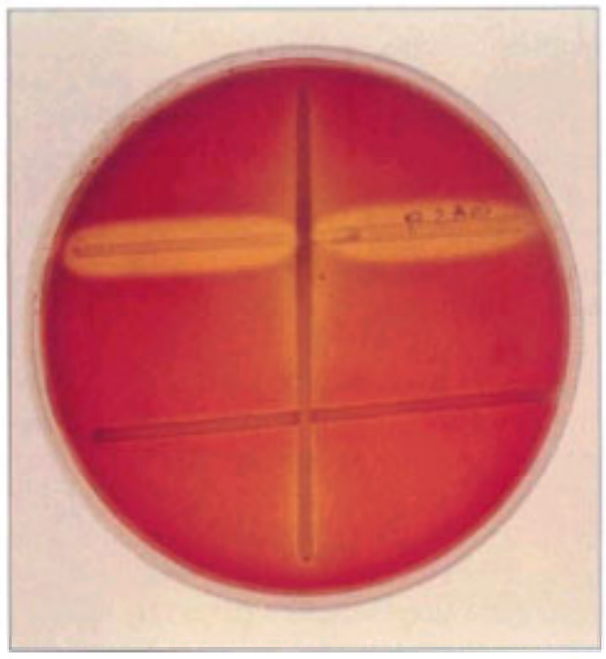


Figure 225-5 Gram-stain of ß-hemolytic streptococci group A.

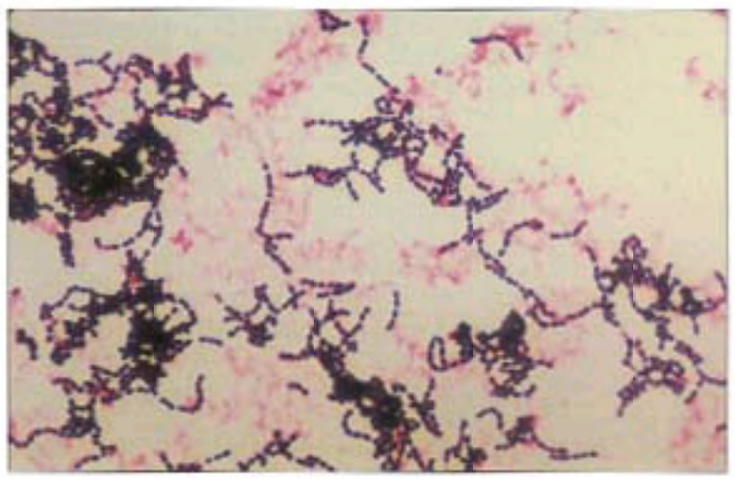


Figure 225-6 Gram stain of a sputum sample infected with *Streptococcus pneumoniae.*

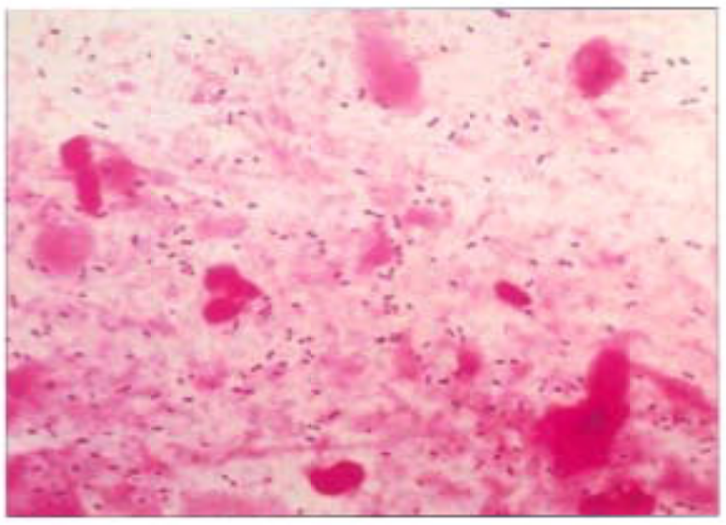


Figure 225-7 API-20 Strep tests for the identification of streptococci. (a) *Streptococcus equisimilis* ATCC 35666. (b) *Enterococcus faecium* ATCC 35667. (c) *Streptococcus mutans* ATCC 35668. (d) Interpretation scheme.

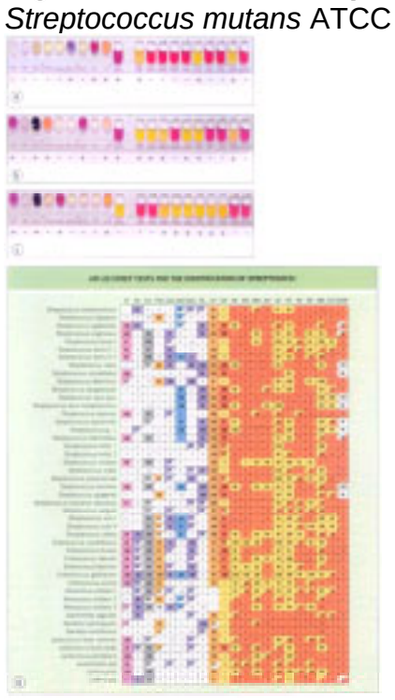


Figure 225-8 Latex agglutination for the identification of ß-hemolytic streptococci group A. (a) Positive agglutination. (b) Negative agglutination.

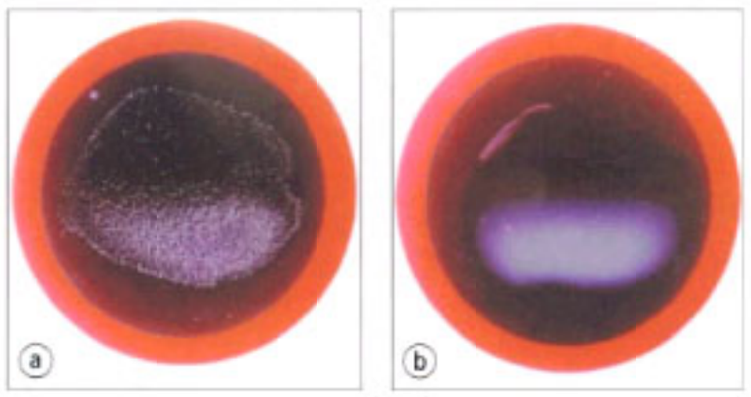


Figure 225-9 The CAMP test. Induced hemolysis of a group B streptococcus culture in the vicinity of a streak of *Staphylococcus* spp.

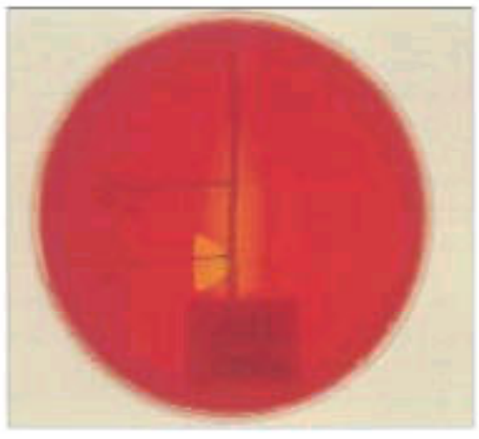


Figure 225-10 ***Enterococcus faecalis*** **on a blood agar plate.**

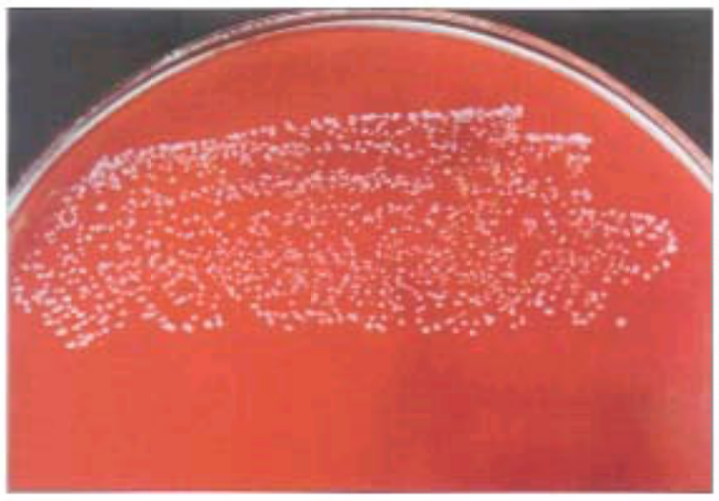


Figure 225-11 Mucoid *Streptococcus pneumoniae* on a blood agar plate.

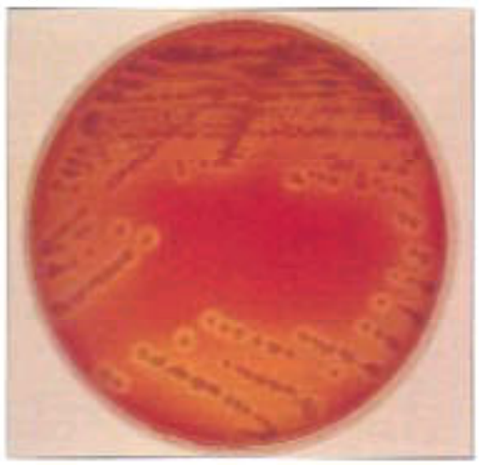


Figure 225-12 *Leuconostoc* on a blood agar plate.

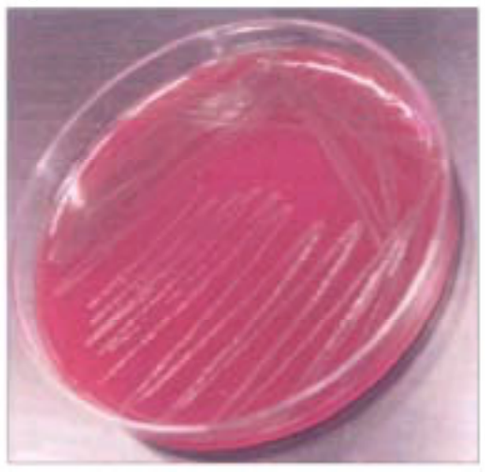


Figure 225-13 Virulence factors of group A streptococci. Spe, streptococcal pyrogenic exotoxins; SIC, streptococcal inhibitor of complement-mediated lysis; Ssa, streptococcal superantigens.

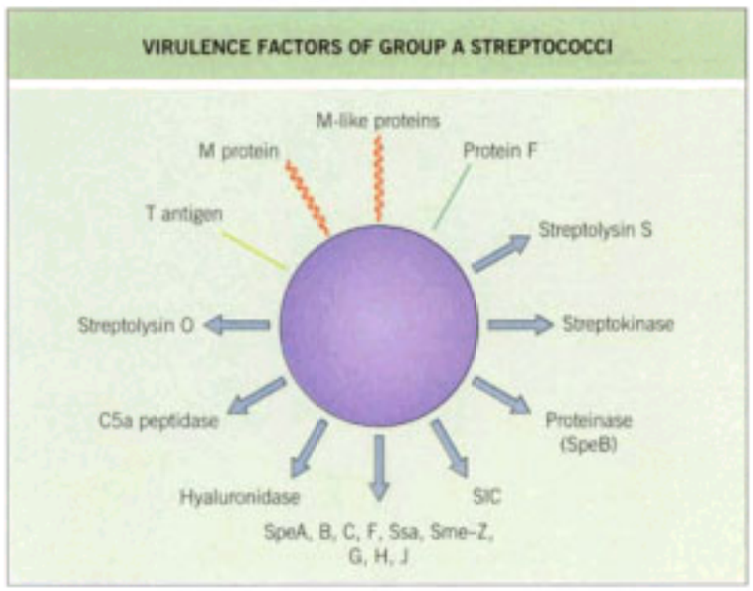


Figure 225-14 Electron microscopy of group A streptococcus. The fuzzy M protein layer can be seen protruding from the cell wall.

Figure 225-15 Group A streptococcal surface protein M. A, B and C represent various regions of the M protein characterized by different degrees of variability of the protein structure. H, S, A and Fib denote human serum albumin and fibrinogen binding structures, respectively.

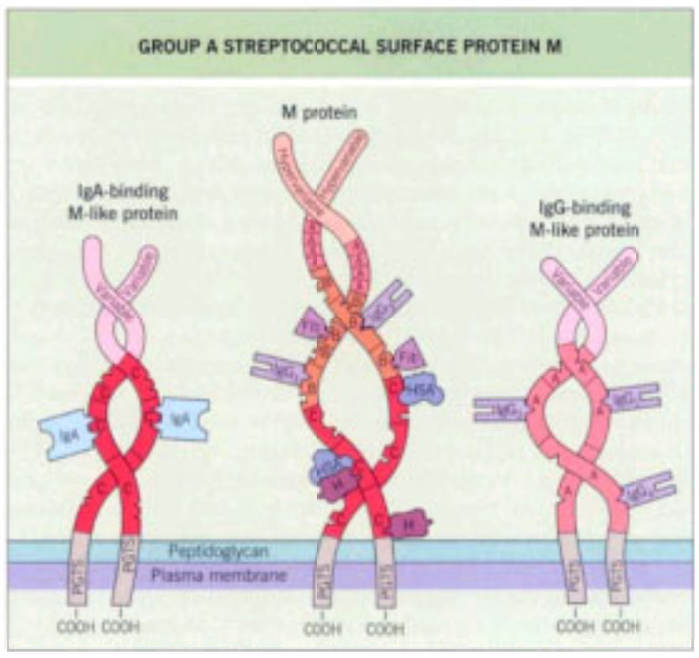


Figure 225-16 The role of streptokinase in acute poststreptococcal glomerulonephritis. Theoretic model of the pathogenesis of acute post-streptococcal glomerulonephritis as induced by streptokinase. The affinity of the streptokinase isotypes (Ska) is probably dependent on differences in the amino acid composition. This model explains the early deposition of C3b in the glomeruli that is typical of the early phase of acute post-streptococcal glomerulonephritis. Ska, streptokinase from Group A streptococci.

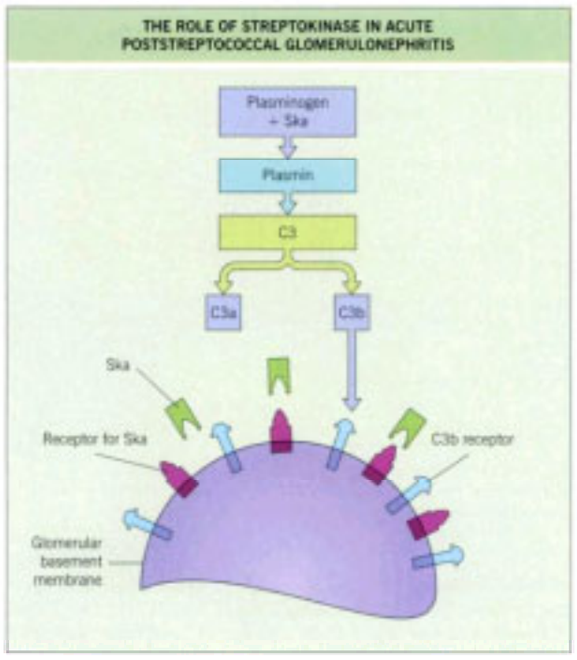


Figure 225-17 Pathogenesis of streptococcal toxic shock syndrome. In this model, patients who have opsonizing antibodies against the M antigen are protected against infection. In the absence of these antibodies, they may develop a serious infection if they are also lacking neutralizing antibodies against the streptococcal pyrogenic exotoxins which, as superantigens, may induce a cytokine cascade. This process will be hindered by the presence of toxin-neutralizing antibodies. The affinity of the toxins to the class II major histocompatibility complex of antigen presenting cells (APC) and T-cell receptors of specific Vß (variable region of ß chain of the T cell receptor) bearing T cells determines the outcome in each individual patient.

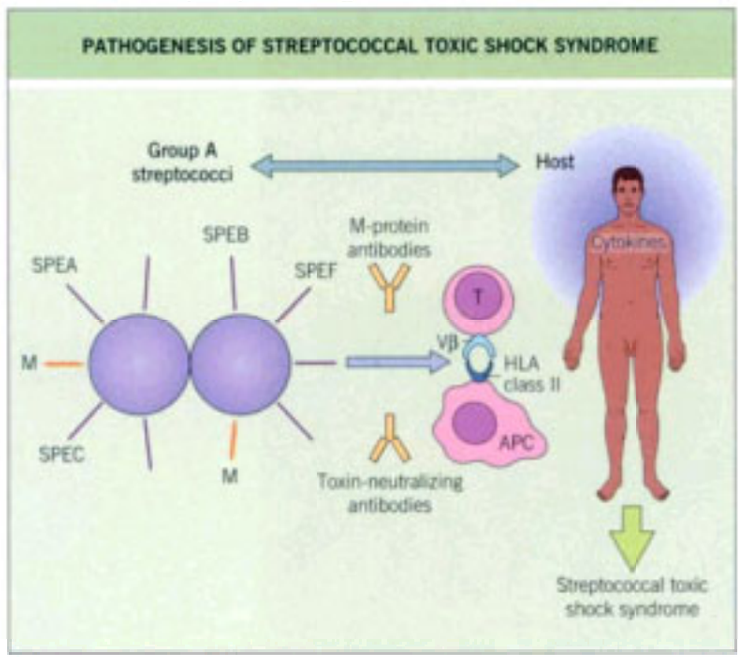


Figure 225-18 Impetigo in a child.

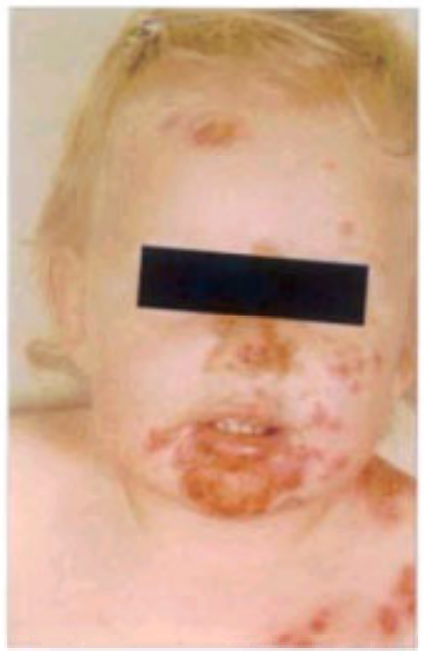


Figure 225-19 Erysipelas. Note the sharp demarcation of the affected skin.

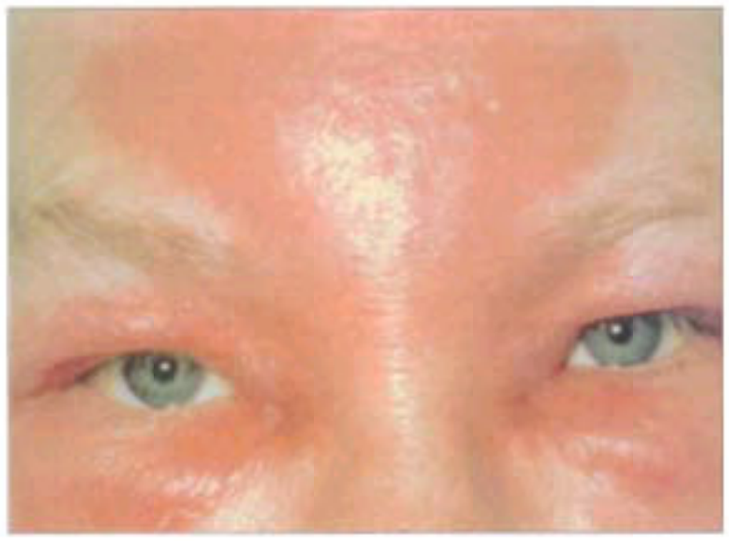


Figure 225-20 Necrotizing fasciitis caused by group A streptococci. There is only moderate erythema but at surgery there was extensive soft tissue damage.

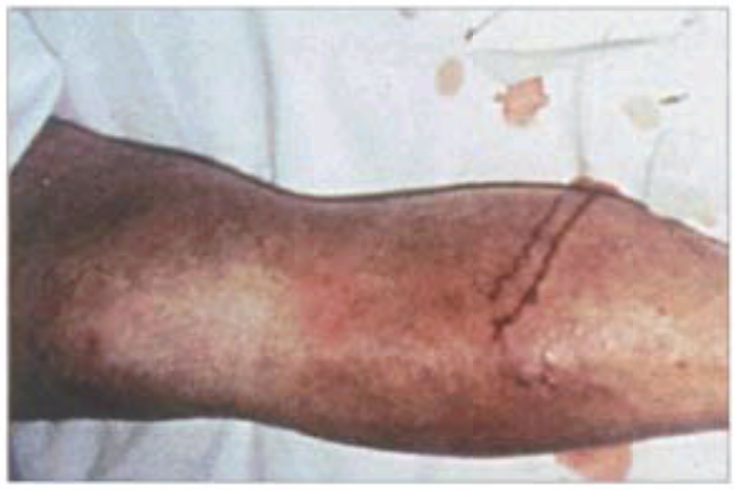


Figure 225-21 Worldwide frequency of isolation of penicillin-resistant pneumococci. Strains with MIC 0.1–2.0µg/ml are said to be intermediate resistant and high-level penicillin-resistant strains are defined as MIC > 2.0µg/ml.

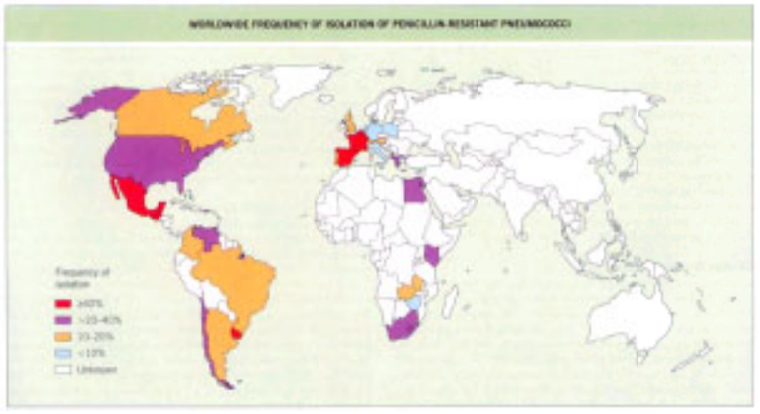


Figure 226-1 Tests for the identification of *Listeria monocytogenes.* (a) CAMP test. Enhanced hemolysis patterns for *L. monocytogenes* (left) and *Streptococcus agalactiae* (right) colonies are shown; these are growing adjacent to a streak of *Staphylococcus aureus* colonies in the center. (b) Demonstration of motility of *L. monocytogenes* grown in semisolid agar at room temperature. Note that the migration of the organism from the central stab is more pronounced at the surface of the soft agar, forming the typical umbrella-shaped pattern in both tubes.

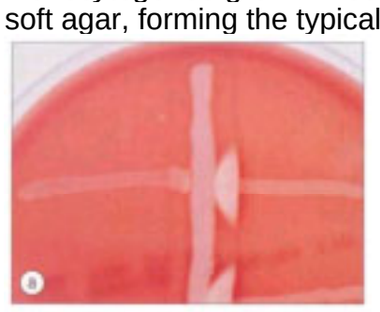


Figure 226-2 *Listeria monocytogenes* can invade both 'professional' phagocytic cells — polymorphonuclear leukocytes and monocytes — and nonphagocytic cells. Attachment of the bacteria to the surface of nonphagocytic cells may be determined by heparin sulfate recognition proteins on the surface of the organism. Once internalized, the bacterial product listeriolysin O will lyse the phagosome, liberating the bacteria into the cytoplasm, where they proliferate. After a few hours, actin filaments polarize at one end of the bacterium, propelling it to the internal membrane surface. The organism will break through the membrane to enter the exterior of the cell or an adjacent cell.

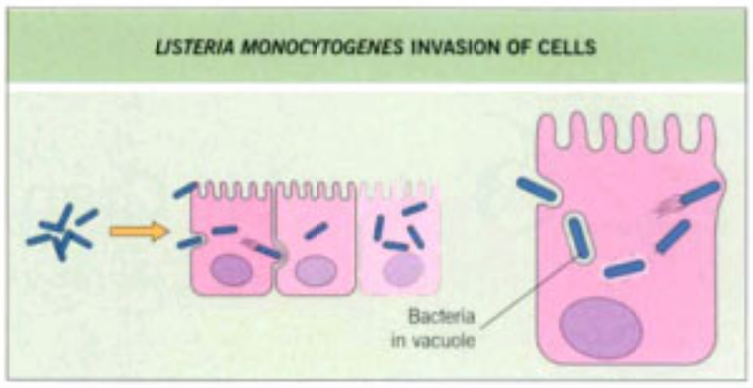


Figure 226-3 Phagocytosis of *Listeria monocytogenes.* Opsonized *L. monocytogenes* is phagocytosed by professional phagocytic cells through a complement-receptor-mediated process. Once ingested into a phagosome of a monocyte or macrophage, most bacteria are quickly killed by oxidative radicals (O_2^-), lysozyme, nitric oxide (NO) and other products of the cell. Tissue macrophages produce a variety of protein products essential for the activation of natural killer (NK) cells (interleukin(IL)-12), T cells (IL-1) and polymorphonuclear (PMN) leukocytes and other monocyte/macrophages (tumor necrosis factor (TNF)-a). These cells in turn produce cytokines, interferons (IFN), interleukins that cause cell proliferation (IL-2; macrophage colony stimulating factor (M-CSF)) or further activation of cell populations involved in elimination of the organism and of infected nonprofessional cells.

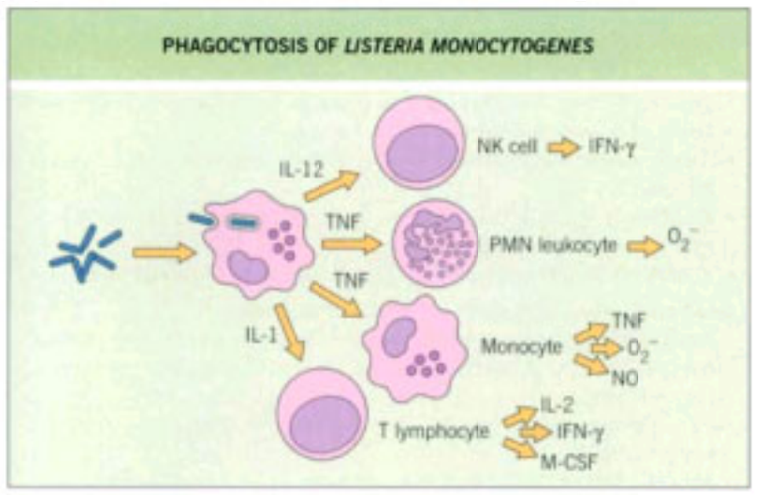


Figure 226-4 Recognition and destruction of *Listeria*-infected cells. Cytotoxic T lymphocytes and NK lymphocytes migrate to the site of infection and recognize and destroy *Listeria*-infected cells by the presence of listerial peptides on the surface of such cells. Intracellular bacteria are then exposed to the extracellular environment. Organisms in the extracellular space are efficiently phagocytosed and killed by polymorphonuclear leukocytes (PMN) and monocytes that have been primed or activated by cytokines (TNF-a, M-CSF, IL-8) and IFN-?.

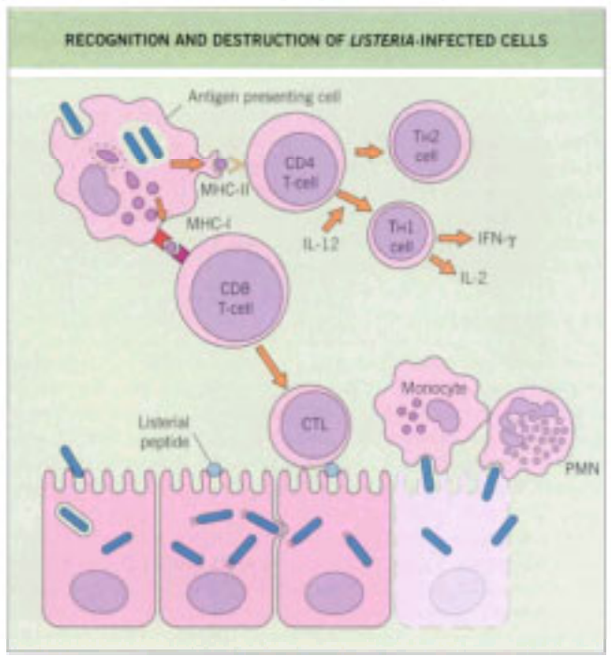


Figure 226-5 Gram stain of clinical specimen showing intra- and extracellular Gram-positive bacilli.

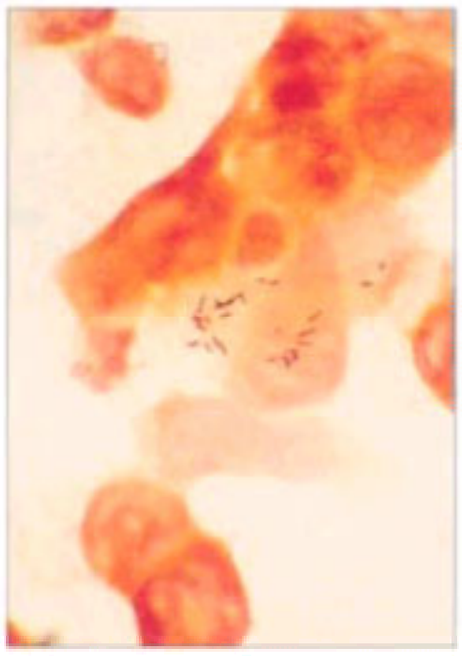


Figure 226-6 Skin rash on premature infant who has sepsis due to *Listeria monocytogenes.* This form of infection, known as granulomatosis infantisepticum, is characterized by disseminated microabscesses on the skin, spleen and liver. The elevated pale patches (1–2mm in diameter) on the skin are clearly seen in contrast to the bright erythema of surrounding skin.

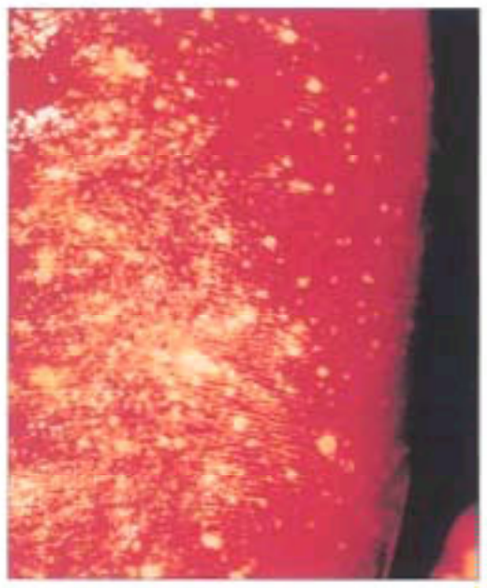


Figure 226-7 Phagocytosis of anthrax bacilli and ingestion of spores. Vegetative bacilli are relatively resistant to phagocytosis by polymorphonuclear leukocytes and monocytes, whereas spores are readily ingested. Once ingested by polymorphonuclear leukocytes or macrophage cells, however, the spores germinate into vegetative forms, which are able to reduce the normal production of bactericidal oxidative radicals by the cell. *Bacillus anthracis* produces lethal toxin (LT) and edema toxin (ET), which are cidal to cells and inhibit TNF-a production and oxidative radical production by phagocytic cells.

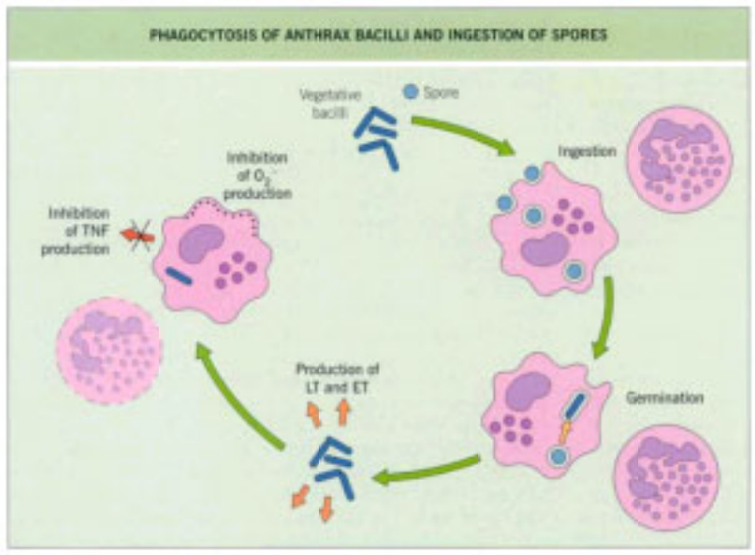


Figure 226-8 Cutaneous anthrax, demonstrating marked erythema, edema and vesicle rupture.

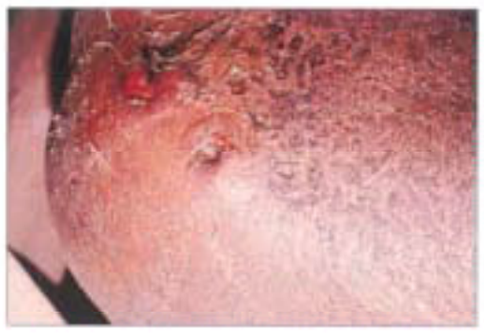


Figure 226-9 Chinese letter and picket-fence appearance of *Corynebacterium* spp. on Gram-stained smears.

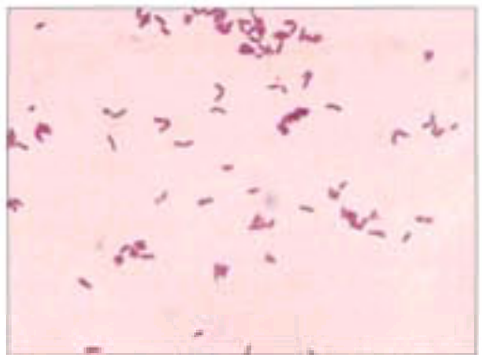


Figure 226-10 Molecular action of *Corynebacterium diphtheriae.* The B subunit of diphtheria toxin (DT) binds to host cell receptor. Proteolytic cleavage of the B subunit from the A subunit (the active DT) in cell endosome. The DT-A subunit escapes from the endosome into the cytoplasm, where it inhibits the transfer of triple code from mRNA to growing polypeptide at the ribosomes on the endoplasmic reticulum. It does this by modifying the enzyme elongation factor (EF)-2 that catalyzes this mRNA transfer.

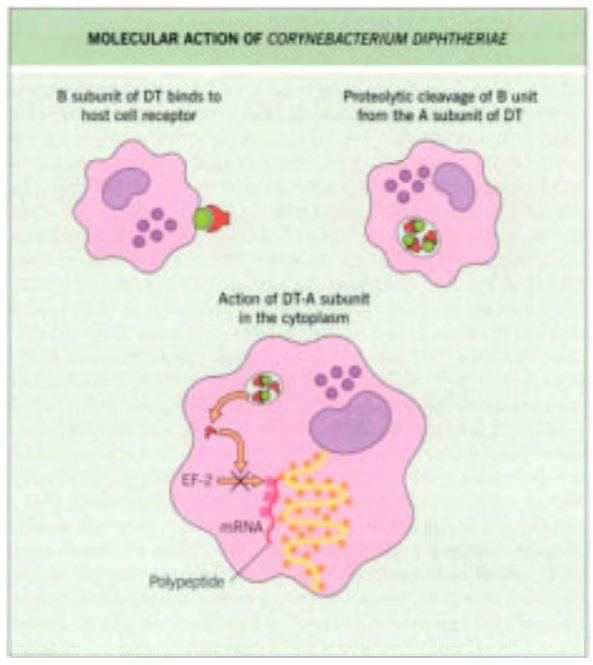


Figure 226-11 Detection of diphtheria toxin production. The Elek test detects DT production in a bacterial clinical isolate. A paper strip soaked in diphtheria antitoxin is placed perpendicular to a horizontal streak of bacterial growth. If it produces toxin, an arrow-shaped precipitin line develops in the agarose plate.

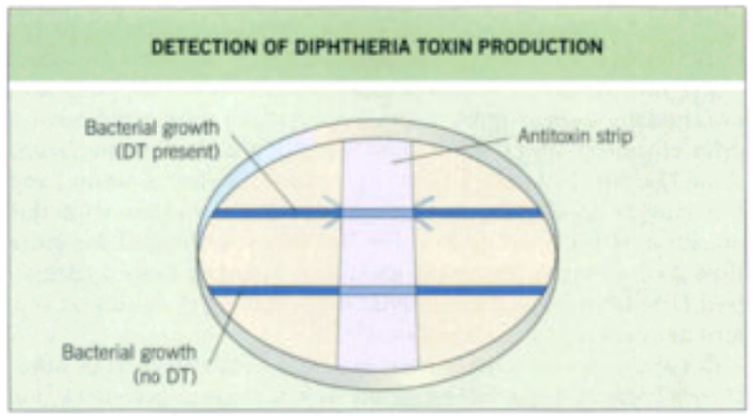


Figure 226-12 Management of a clinical diphtheria case. *Adapted from Farizo.*[61]

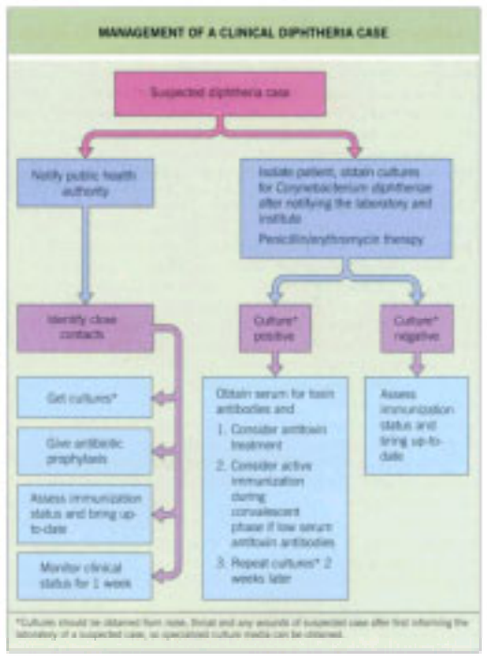


Figure 226-13 Colonies of *Nocardia asteroides,* showing a smooth, chalky-white appearance.

Figure 226-14 ***Nocardia asteroides*** **on Gram stain, showing extensively branched vegetative hyphae that break into short rods.**

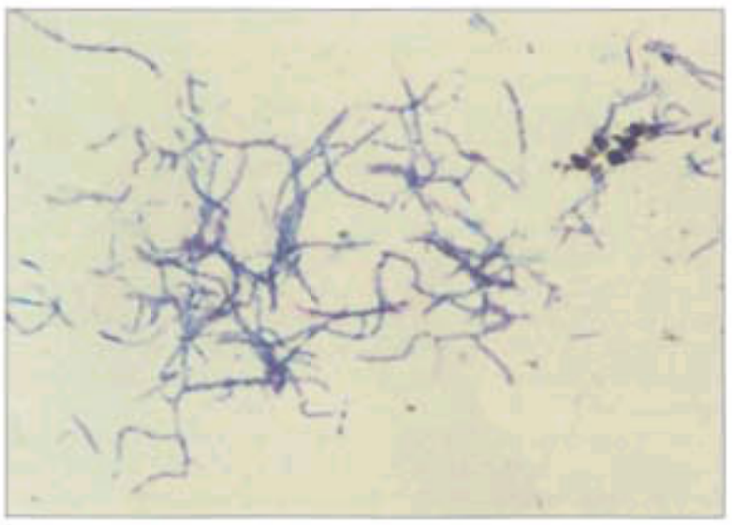


Figure 226-15 Primary cutaneous nocardial infection is characteristically painless, localized and slowly progressive. (a) There is marked swelling and erythema in this child's finger. (b) However, because the finger was painless the child was not brought to medical attention until the infection had progressed to involve the entire finger.

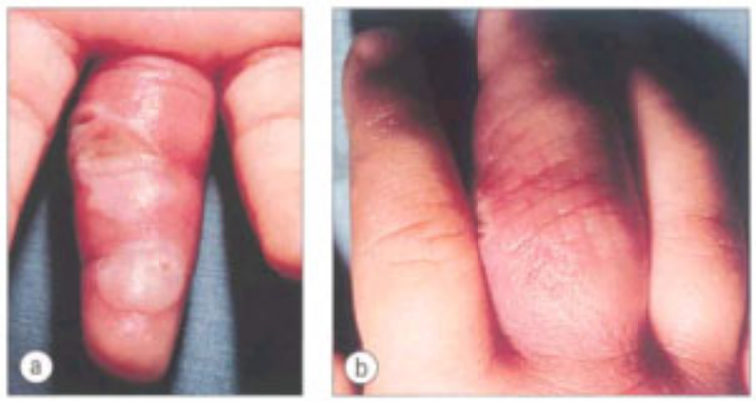


Figure 227-1 Neisseriaceae family. $T_{m(e)}$ is the melting temperature at which 50% of a hybrid is denatured. *(Adapted from Rossau et al.[5])*

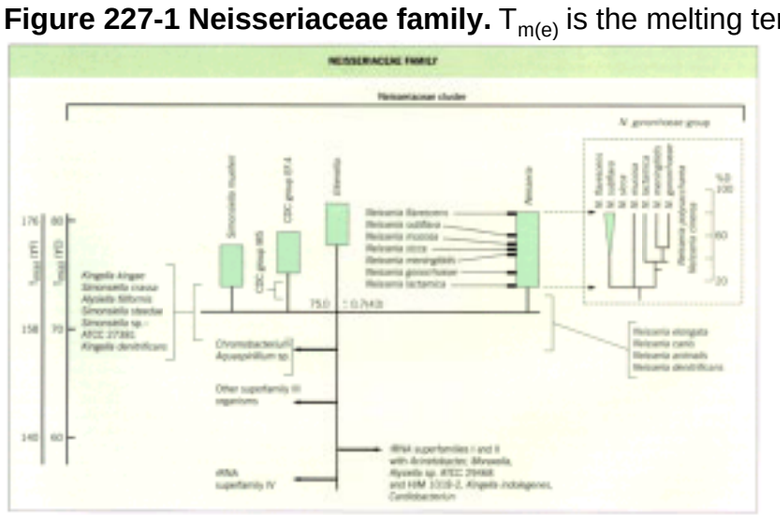


Figure 227-2 Structure of *N. gonorrhoeae.* *(Adapted from Mims et al. [9])*

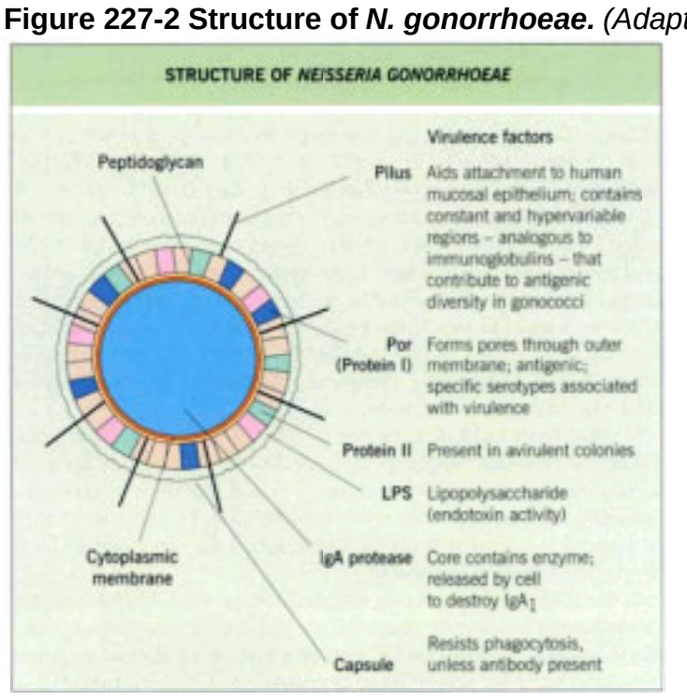


Figure 227-3 Epidemiology of meningococcal infection. *(Adapted from Jones.[36])*

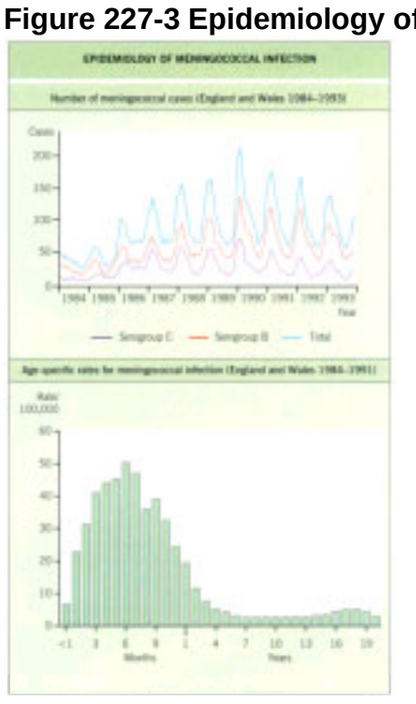


Figure 227-4 Steps in the pathogenesis of *N. gonorrhoeae* infection. *(Reproduced with permission from Weel.[40])*

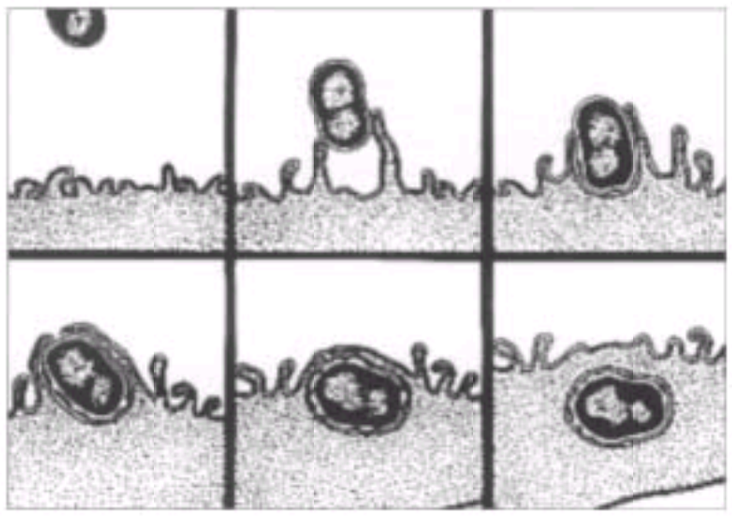


Figure 227-5 Pathogenesis of meningococcal infection. *(Adapted from Virji.[43])*

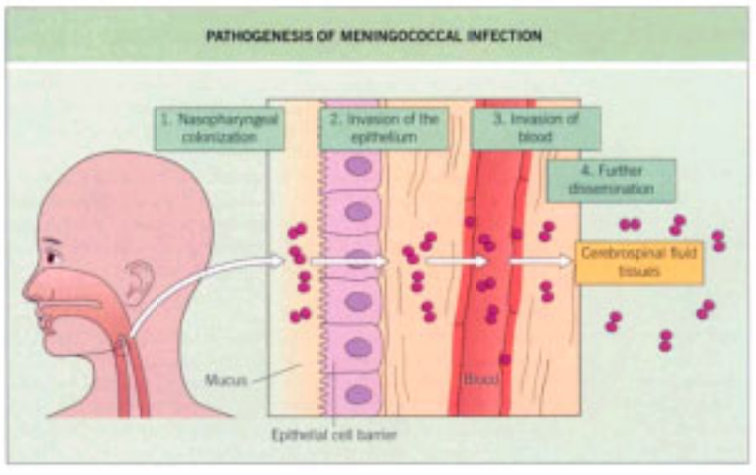


Figure 227-6 Bacterial meningitis. Exudate of acute inflammatory cells in the subarachnoid space. *Courtesy of P Garen. (Reproduced with permission from Mims et al. [9])*

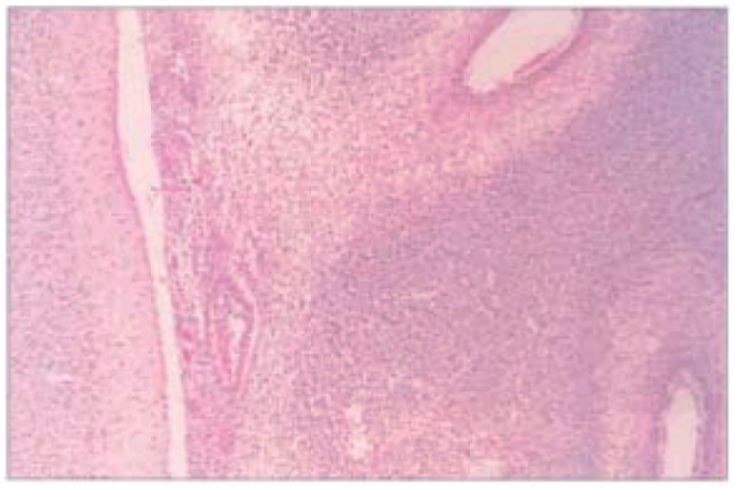


Figure 227-7 Gonococcal urethritis. *Courtesy of J Clay. (Reproduced with permission from Mims et al.[9])*

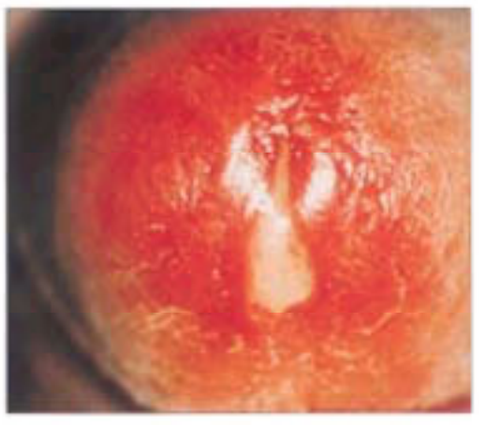


Figure 227-8 Disseminated gonococcal infection. (a) Skin lesions. *Courtesy of JS Bingham.* (b) Arthritis. *Courtesy of TF Sellers Jr. (Reproduced with permission from Mims et al.[9])*

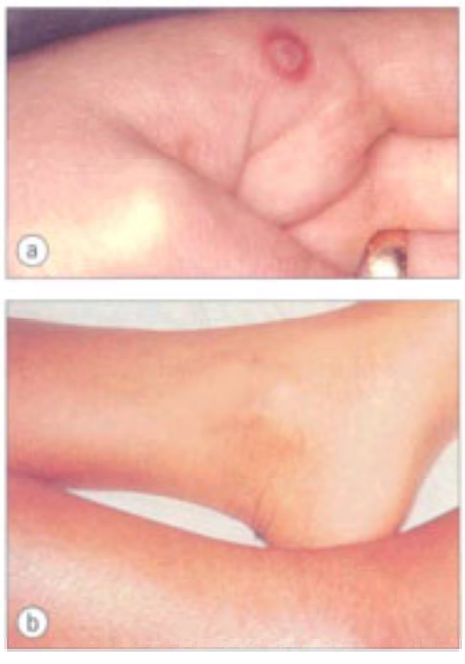


Figure 227-9 Typical rash of meningococcal sepsis. Fine erythematous macules and petechiae are present in some areas.

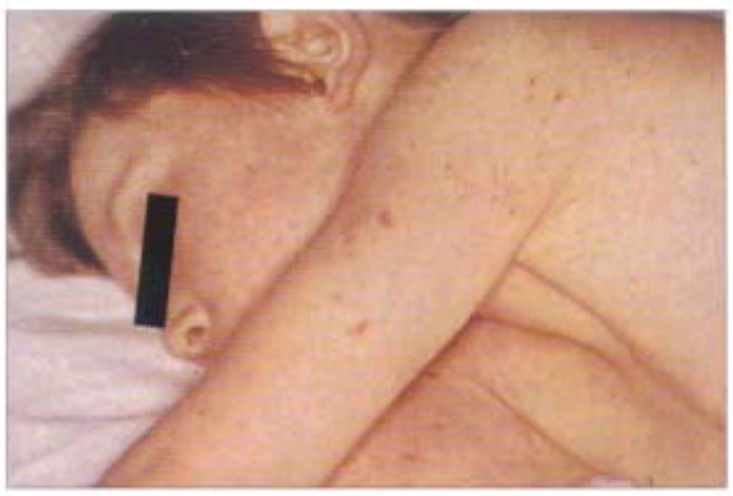


Figure 227-10 Gram stain of a urethral discharge from a male who has gonorrhea. Note the intracellular Gram-negative diplococci with neutrophils.

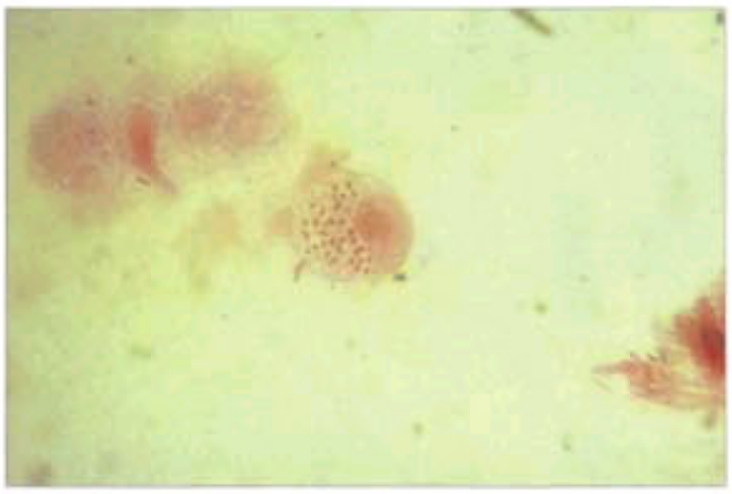


Figure 228-1 Major structural cell wall compounds of Enterobacteriaceae. The figure also illustrates the molecular organization of the outer membrane, and the most likely positions of outer membrane constituents are indicated. Lipopolysaccharide and phospholipid molecules are the major constituents of the asymmetric bilayer. Divalent cations (not indicated) are believed to play important roles in interactions of LPS: Only three types of protein are shown: the pore proteins (LamB protein not shown), OmpA protein and lipoprotein (their interactions with peptidoglycan and lipoprotein are not shown — that such interactions occur cannot be excluded). Several O-antigen chains are much longer than shown here. Enterobacterial core antigen has been omitted for simplicity. The P pili and fimbriae are discussed and illustrated in more detail in Chapter 1 .

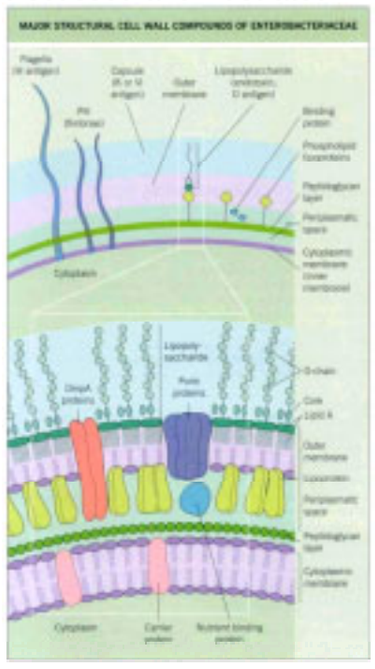


Figure 228-2 Longitudinal section of *Escherichia coli.* The bacterial cell is surrounded by a visible cell wall (outer membrane, cytoplasmic membrane and — in between — periplasm).

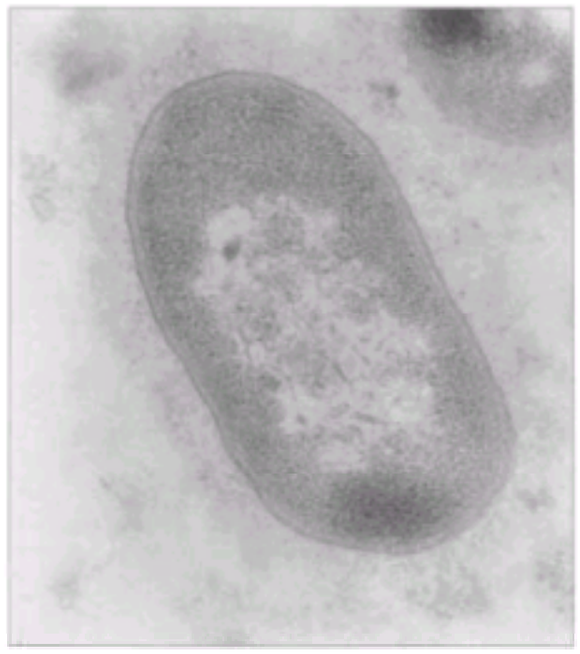


Figure 228-3 Mixed culture of two morphotypes of Enterobacteriaceae (*Escherichia coli* and *Salmonella* spp.) on blood agar plate.

Figure 228-4 Mixed culture of lactosefermenting colonies (red) and non-lactose-fermenting colonies (pale) on MacConkey agar plate.

Figure 228-5 *Salmonella-Shigella* agar plate showing growth of *Salmonella typhimurium* (pale colonies with black pigmentation indicating hydrogen sulfide production).

Figure 228-6 API-20E strip after 24 hours incubation at 95°F (35°C).

Figure 229-1 Key to identification of nonfermentative aerobic Gram-negative bacilli. Note that *Burkholderia mallei* has no flagella and is nonmotile; *Pseudomonas aeruginosa* is monotrichous; oxidase-negative organisms use carbohydrates (activity of a-glucosidase, ß-glucosidase, ß-galactosidase, ß-xylosidase); *Alcaligenes* spp. have degenerated peritrichous flagella, which are functional; *Chryseobacterium* spp. produce variably pigmented colonies due to yellowish-orange pigment; and *Stenotrophomonas* spp. (except nonpigmented mutants) produce yellow pigment.

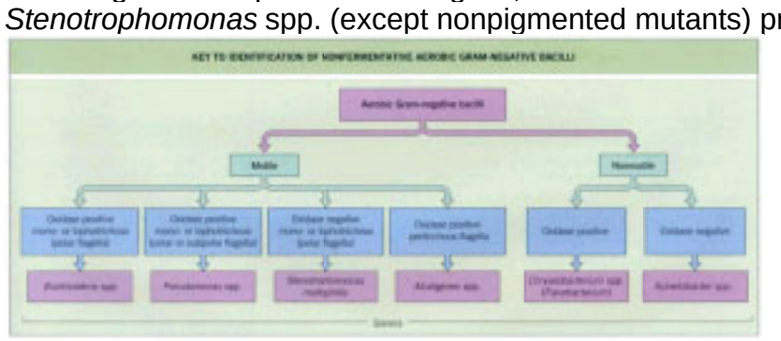


Figure 229-2 *Pseudomonas aeruginosa* monotrichous polar flagellum seen on electron microscopy. *Courtesy of Professor A Marty.*

Figure 229-3 *Pseudomonas aeruginosa* colonies on agar medium. *Courtesy of Professor E Bingen.*

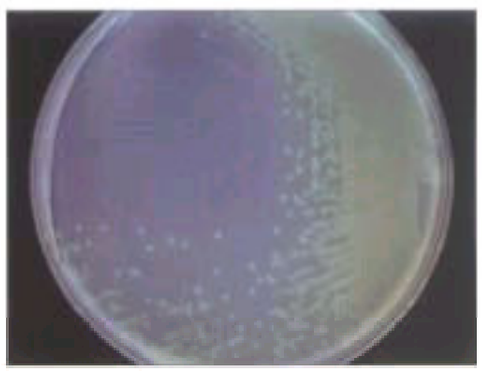


Figure 229-4 Anatomic pathology of *Pseudomonas aeruginosa* pneumonia showing acute inflammatory exudate, necrosis of alveolar membranes and fibrinous thrombosis in a venula. Hematoxylin-eosin stain. *Courtesy of Professor Groussard.*

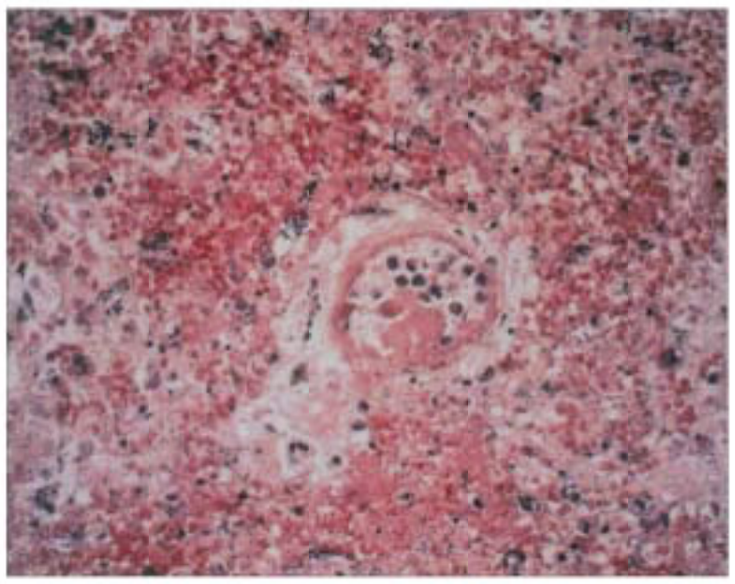


Figure 229-5 Mucoid colonies of a strain of *Pseudomonas aeruginosa* isolated from a patient who has cystic fibrosis. *Courtesy of Professor E Bingen.*

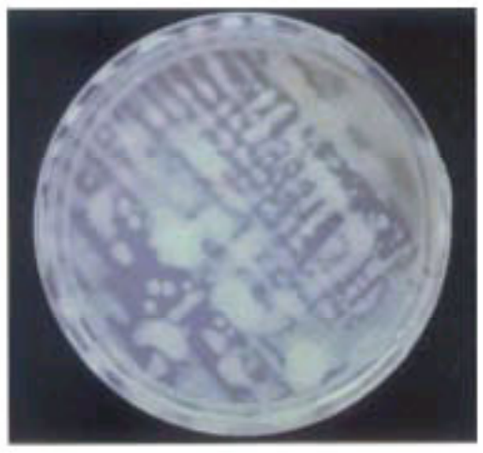


Figure 229-6 Burned leg that has been superinfected with *Pseudomonas aeruginosa.* *Courtesy of Professor H Carsin.*

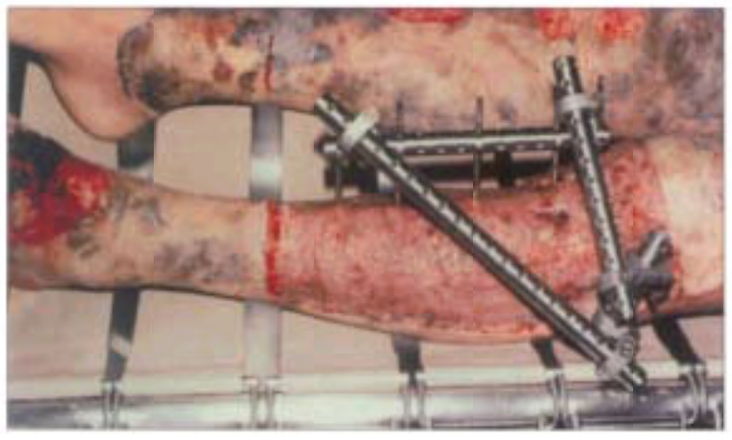


Figure 229-7 Burned abdominal wall that has been superinfected with *Pseudomonas aeruginosa.* *Courtesy of Professor H Carsin.*

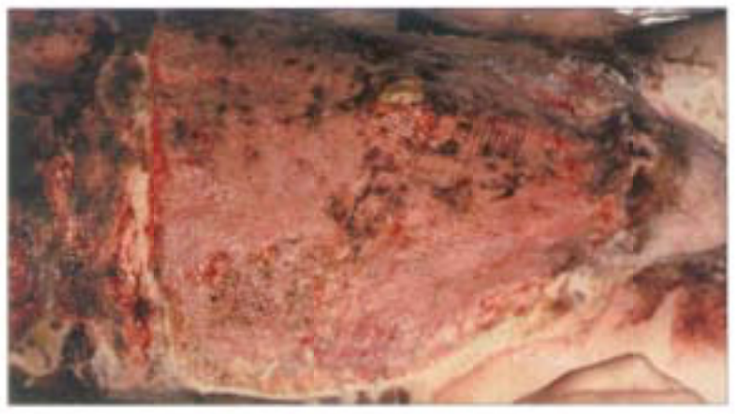


Figure 229-8 Morphology of *Acinetobacter baumannii* on Gram stain. Preparation from a lung infection in mice. *Courtesy of Dr ML Joly-Guillou.*

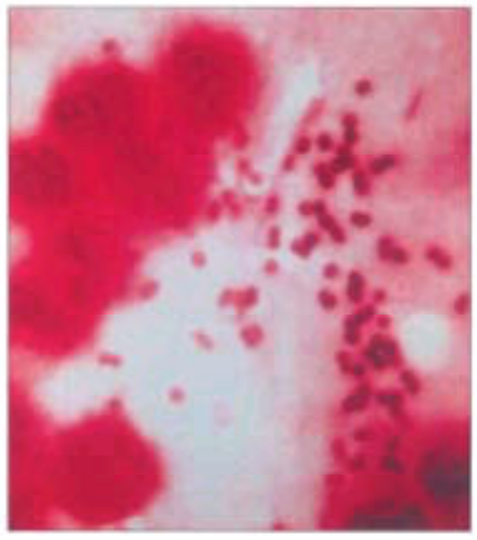


Figure 229-9 Colonies of *Flavobacterium-Chryseobacterium* group grown on Mueller-Hinton agar. *Courtesy of Professor H Monteil.*

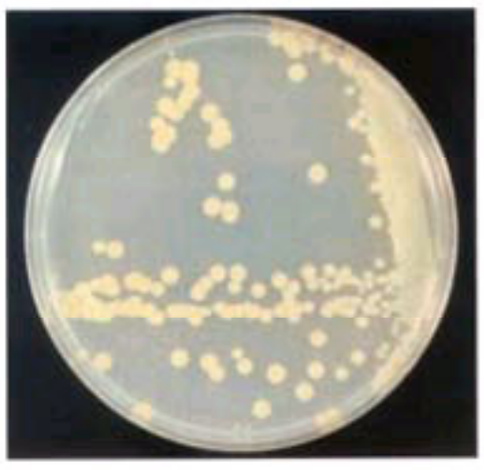


Figure 230-1 Cultured *H. pylori* in coccoid and bacilli forms, bound to immunomagnetic beads. *Adapted from Murray et al.* [106]

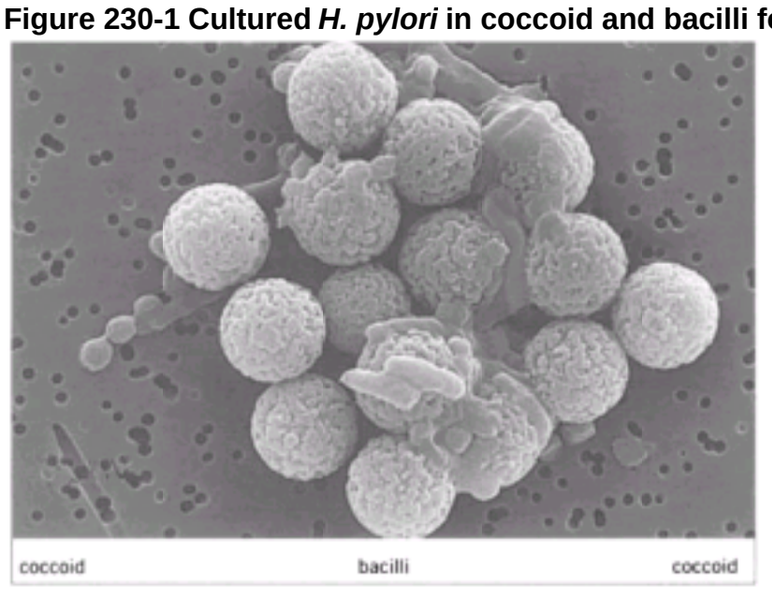


Figure 230-2 Spread of the *Vibrio cholerae* O1 El Tor pandemic in Central and South America, 1991–94. *(Adapted from Tauxe et al. [35])*

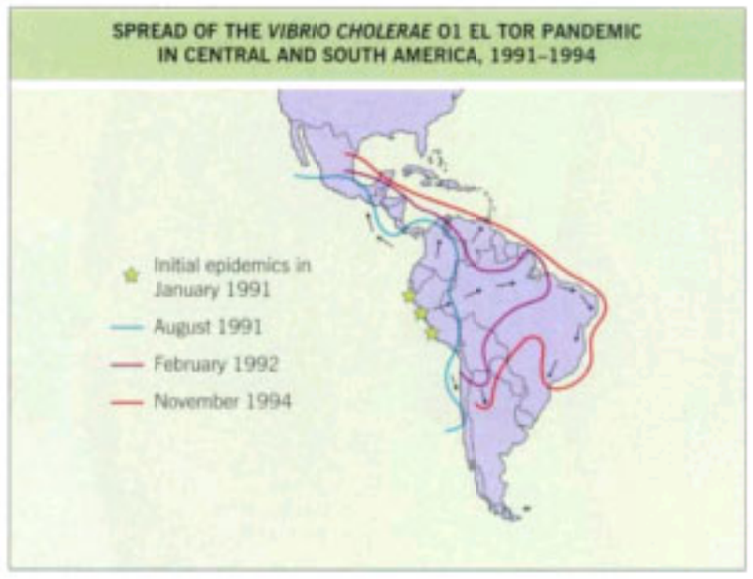


Figure 230-3 Mechanism of action of *Vibrio cholerae* toxin. The toxin binds to the GM1 ganglioside receptor on the intestinal mucosal cell membrane via the binding (B) subunits (a). The active portion of the A subunit enters the cell and activates adenyl cyclase (b), which results in the accumulation of cyclic adenosine monophosphate (cAMP), derived from adenosine triphosphate (ATP), along the cell membrane (c). The cAMP causes active secretion of sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-) and water (H_2O) out of the cell into the lumen of the intestine (d). *Adapted from Murray et al.*[106]

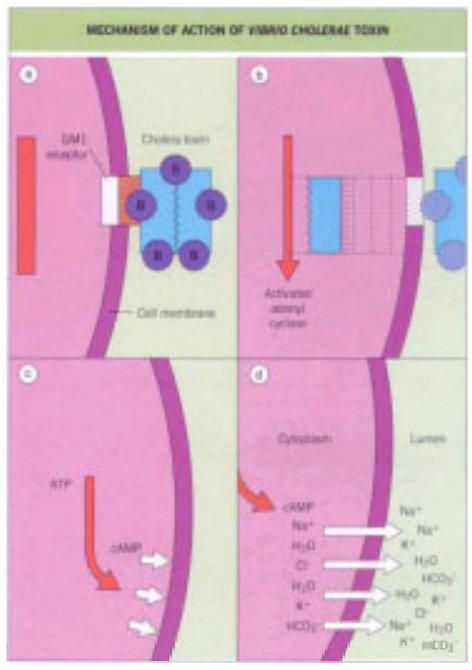


Figure 230-4 Helical structure of *T. pallidum* with the periplasmic flagella. *From Binford and Connor.*[107]

Figure 230-5 Secondary syphilis with typical skin rash. *(Reproduced with permission from Habif.*[58] *)*

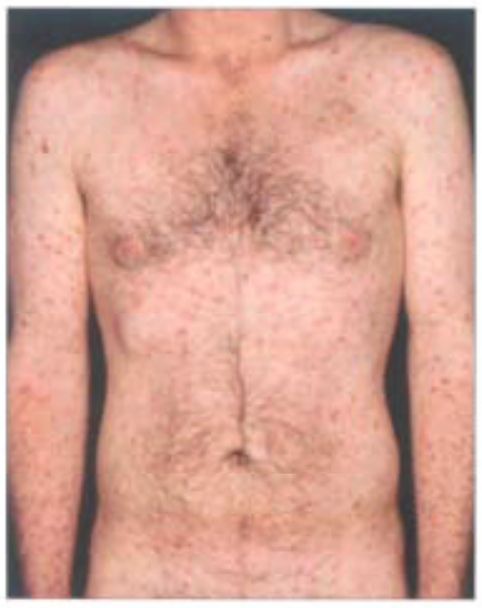


Figure 230-6 The papillomatous skin lesions of yaws. *(Reproduced with permission from Peters & Giles.[60])*

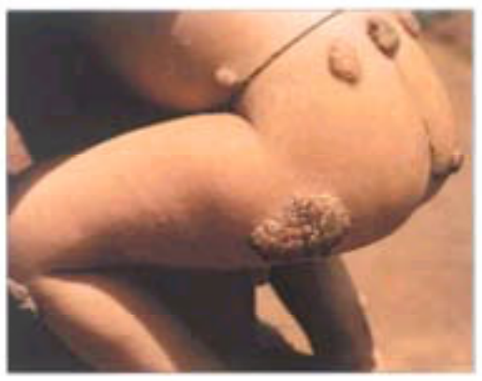


Figure 230-7 Giemsa stain of blood with *B. burgdorferi.* *Adapted from Murray et al.*[106]

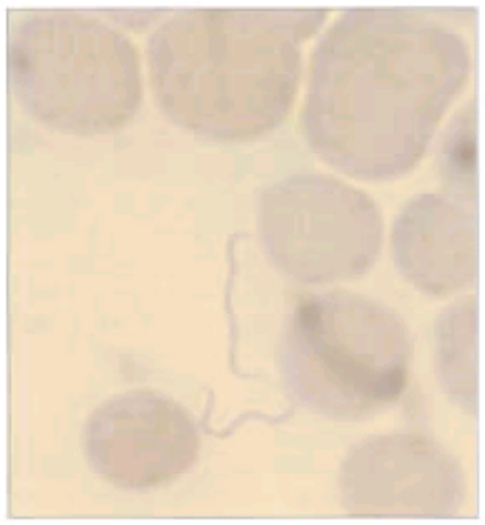


Figure 230-8 Typical erythema migrans rash. *Adapted from Murray et al.*[106]

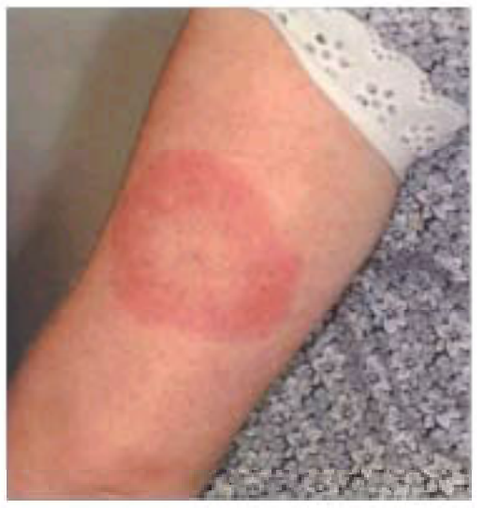


Figure 231-1 Whooping cough notifications — cases and deaths. Figures are for England and Wales 1940–2000. *Data supplied by the Public Health Laboratory Service Communicable Disease Surveillance Centre.*

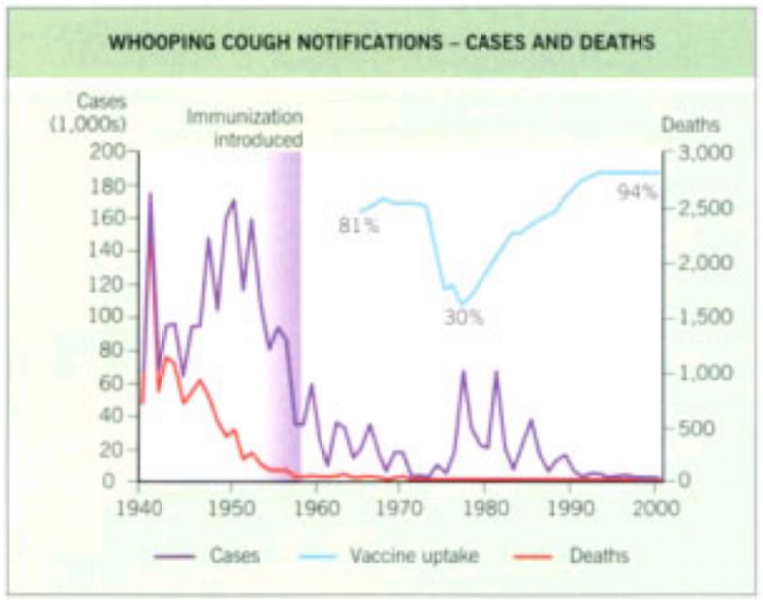


Figure 231-2 Colonies of *Bordetella pertussis* on charcoal blood agar.

Figure 231-3 Clinical manifestations of brucellosis.

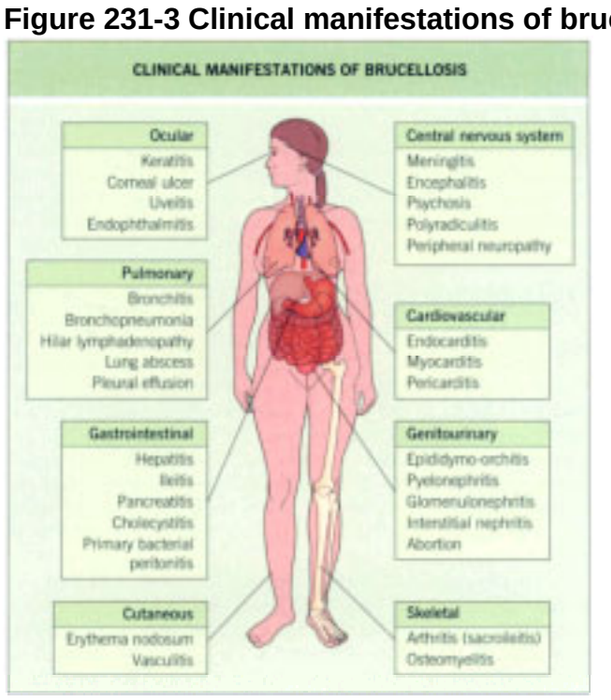


Figure 231-4 Clinical types of tularemia.

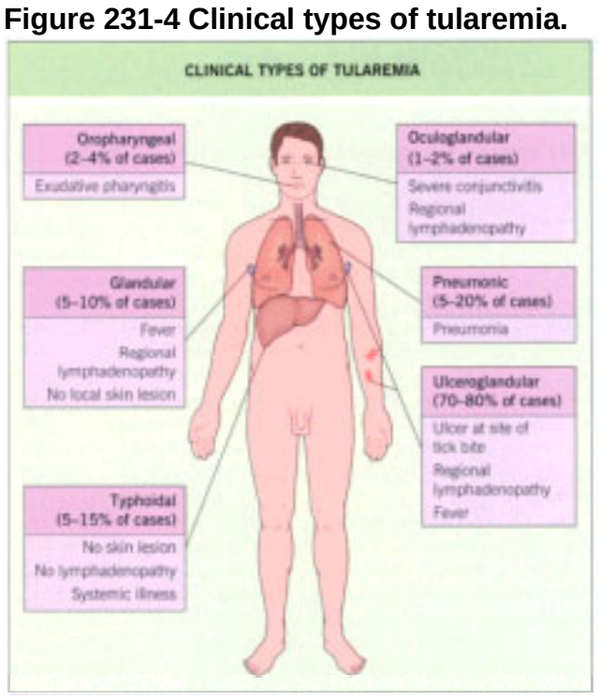


Figure 231-5 Incidence of invasive *Haemophilus influenzae* type b and noncapsulated *H. influenzae* disease. Figures are for the period from October 1990 to June 1997 in England.

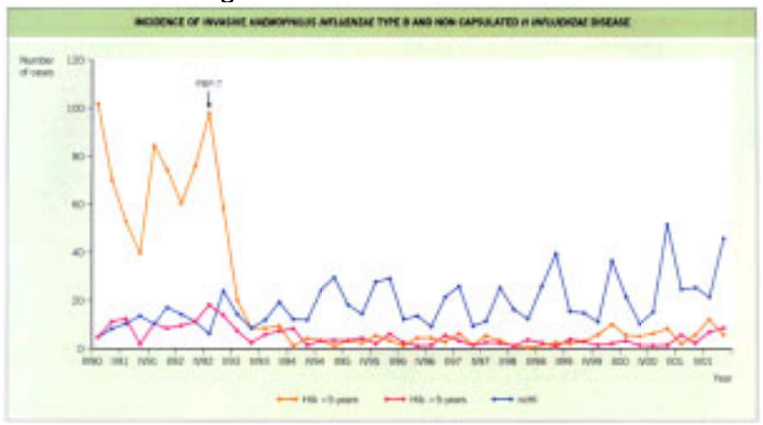


Figure 231-6 Growth factor requirement of *Haemophilus influenzae.* Strain of *H. influenzae* sown on Columbia agar plate. Filter paper disks containing X factor, V factor, and both X and V factors have been placed on the surface of inoculated plate, but colonies of *H. influenzae* grow only around the disk with both X and V factors.

Figure 231-7 Colonies of *Legionella pneumophila* on BCYE agar, showing the typical ground-glass appearance. *With permission from Harrison TG, Taylor AG (eds). A laboratory manual for Legionella. Chichester: Wiley; 1998.*

Figure 231-8 Gram-stained smear of sputum containing *Moraxella catarrhalis*. Sample from a patient suffering an acute exacerbation of chronic bronchitis showing Gram-negative diplococci and leukocytes (*Moraxella catarrhalis*).

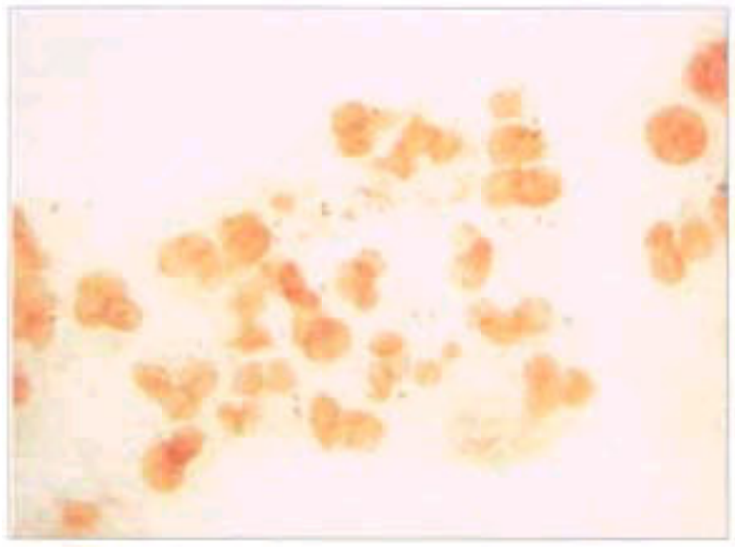


Figure 231-9 Clinical manifestations of *Pasteurella* infection in humans.

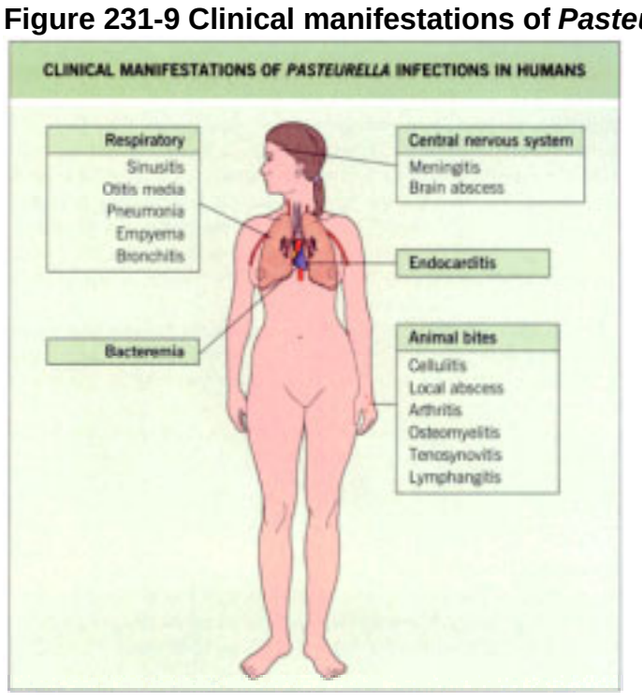


Figure 231-10 Transmission of plague.

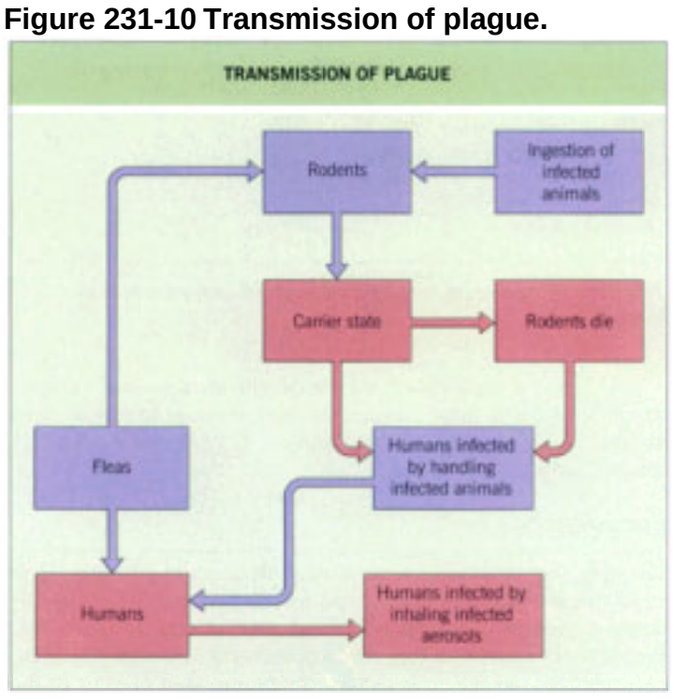


Figure 232-1 Gram stain of a perirectal abscess caused by polymicrobial aerobic and anaerobic flora.

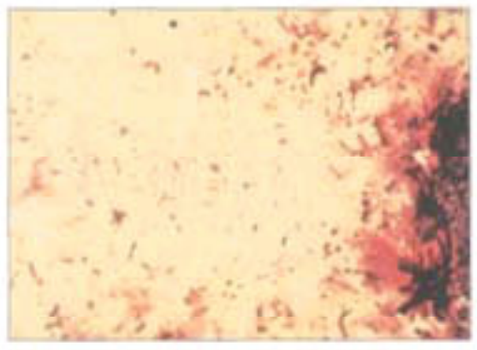


Figure 232-2 Gram stain of *Clostridium perfringens*.

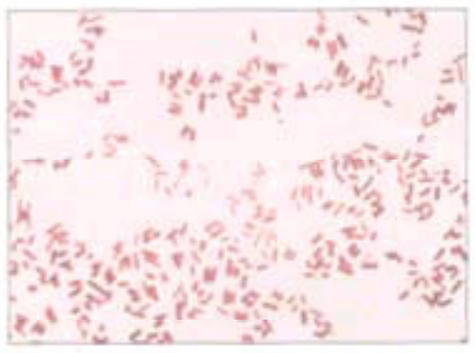


Figure 232-3 Gram stain of *Bacteroides fragilis.* *Courtesy of Mike Cox.*

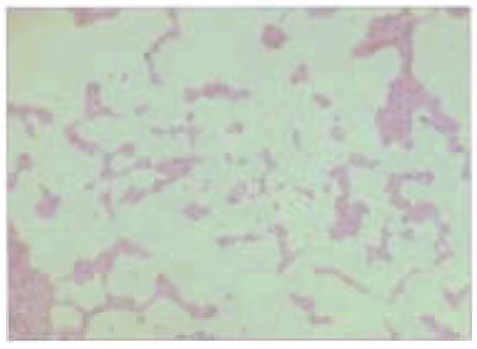


Figure 232-4 Gram stain of *Fusobacterium nucleatum.* *Courtesy of Mike Cox.*

Figure 232-5 Recovery of anaerobic bacteria in different infectious sites.

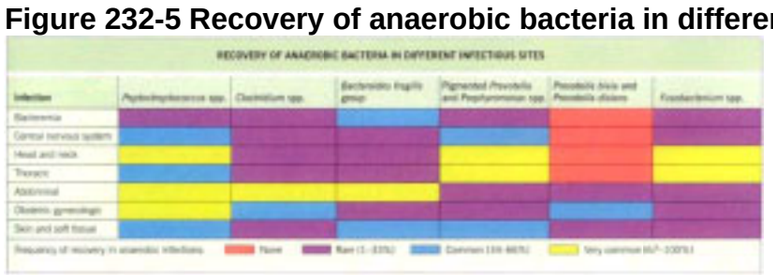


Figure 232-6 Anaerobic glove-box used in the microbiology laboratory for processing of specimens and identifying anaerobic bacteria. *Courtesy of Mike Cox.*

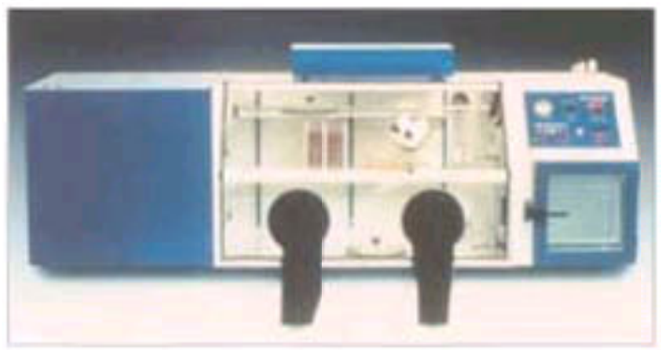


Figure 232-7 Infected diabetic ulcer.

Figure 232-8 Human bite wound.

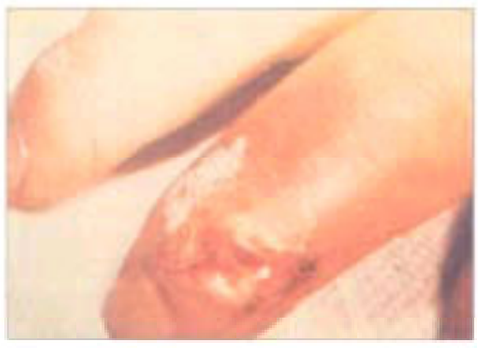


Figure 232-9 Polymicrobial necrotizing cellulitis. The initial lesion is a reddish-brown bled and is accompanied by local tenderness.

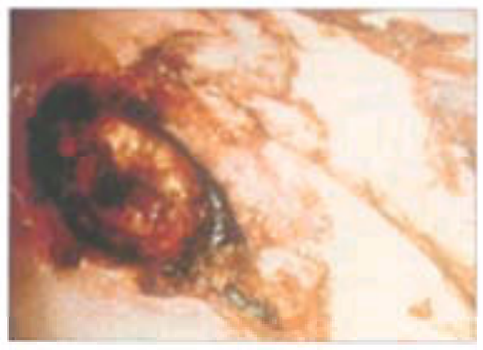


Figure 232-10 Necrotizing fasciitis caused by multiple aerobic and anaerobic bacteria. The fascia inspected through a surgical incision is swollen and dull in appearance, with areas of necrosis. A thin, brownish discharge exudes from the wound, with no pus.

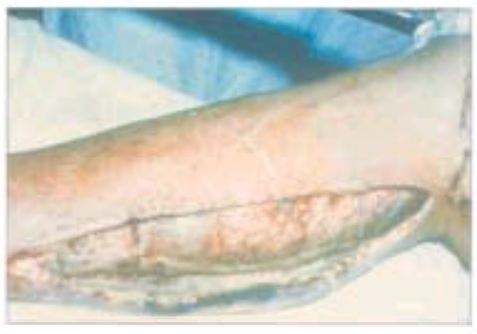


Figure 232-11 Anaerobic streptococcal myositis involving muscle and fascial planes.

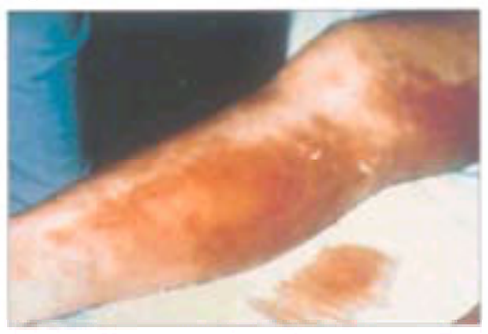


Figure 232-12 Distribution of organisms in subcutaneous abscesses, wounds, burns and decubitus ulcers.

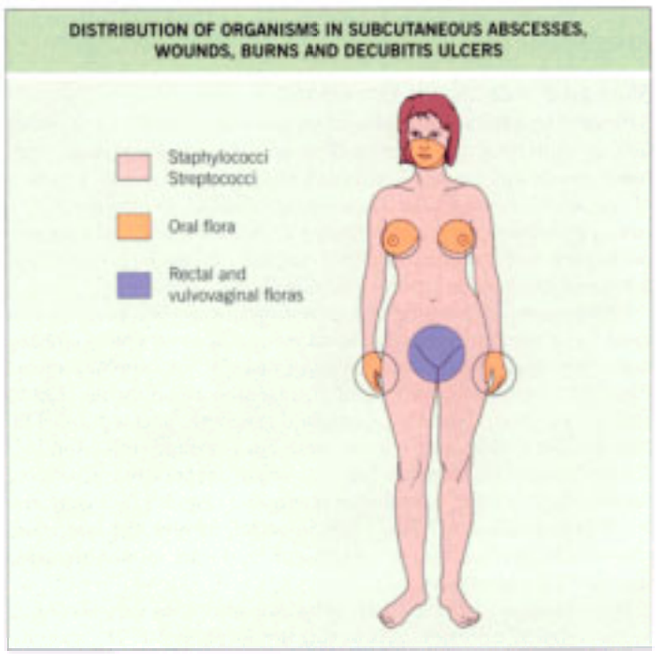


Figure 232-13 Susceptibility of anaerobic bacteria to antimicrobial agents.

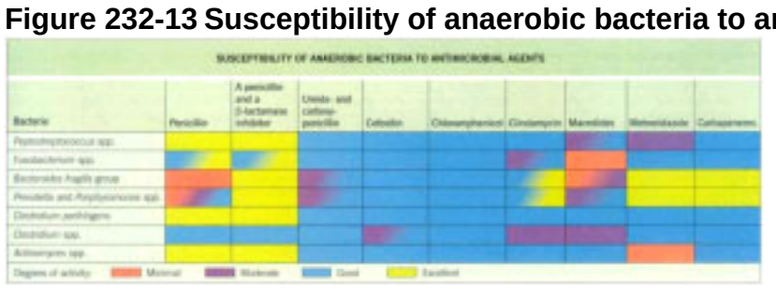


Figure 233-1 Microscopic clusters of three different species of mycobacteria. (a) Serpentine cording of *Mycobacterium tuberculosis*. (b) Cross-banding of *Mycobacterium kansasii*. (c) Loose clusters of *Mycobacterium avium* complex. *Photomicrographs taken from Attorri et al.* [5]

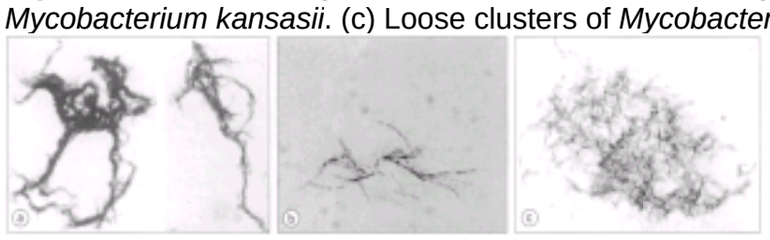


Figure 233-2 Mycobacterial cell envelope. This model displays the asymmetric array of the structural elements extending from the plasma membrane that surrounds the cytoplasm of the mycobacterial cell. The arabinogalactan is covalently linked to the peptidoglycan, which along with the lipoarabinomannan and phosphatidylinositol mannosides (PIM) are associated with the plasma membrane. The cell wall lipids are shown in a possible arrangement with the mycolates linked to the arabinogalactan. Two classes of polar lipids with medium and short chain fatty acids complement the varying hydrocarbon chains of the mycolates to create an even cell envelope. There is evidence for a small number of porins within the hydrophobic bilayer. *Adapted from Brennan and Draper.*[3]

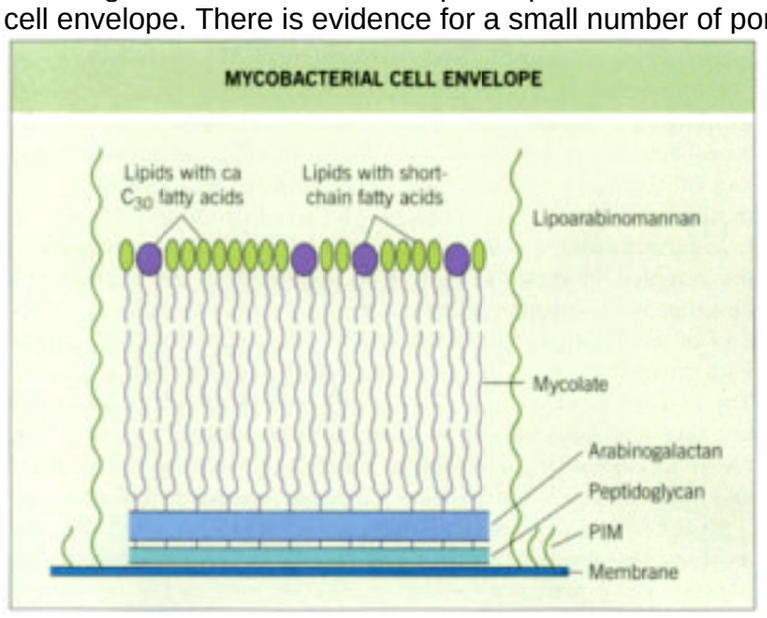


Figure 233-3 *Mycobacterium kansasii,* a photochromogenic mycobacterium, grown on Löwenstein-Jensen medium in light. *Courtesy of S Froman and A Gaytan.*

Figure 233-4 Phylogenetic tree of selected mycobacteria, based on 165 rRNA sequence information, including species whose genome sequence is completed (red) or is in progress (green). *Figure adapted from Brosch et al.*[6]

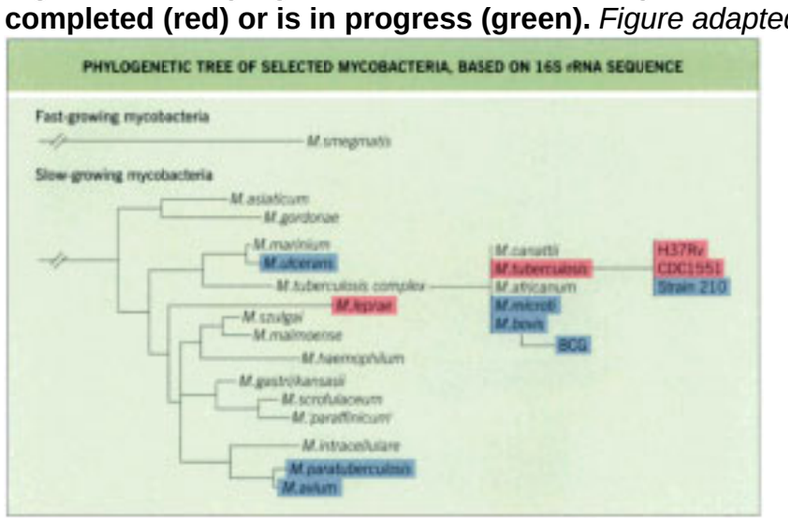


Figure 233-5 Estimated tuberculosis incidence rates, 2000. *Data and map adapted from World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO Report 2002, Switzerland, WHO/CDS/TB/2002.295.*[11]

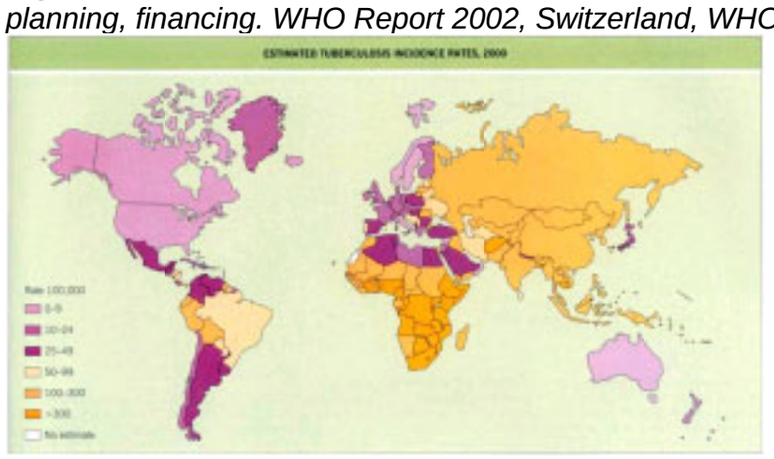


Figure 233-6 Macrophage phagocytosis and evasion of tubercle bacilli. The tubercle bacilli bind via lipoarabinomannan (LAM) (1) or complement receptors (2); phagocytosis occurs (3) with the activation of an oxidative burst (4); glycolipids (GL), sulfatides (ST) and LAM can downregulate the oxidative burst (5); reactive nitrogen intermediates may play a role in antimycobacterial activity (6), as does the acidic pH of the phagolysosome (7). Finally, the production of ammonia by tubercle bacilli may diminish the effect of reactive nitrogen intermediates (8) and contribute to the failure to form a phagolysosome fusion (9). Tubercule bacilli may evade the antimycobacterial activities of the phagolysosome by producing a hemolysin that releases the bacilli into the cytoplasm (10). NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; SOD, superoxide dismutase. *Adapted from Chan and Kaufmann.* [16]

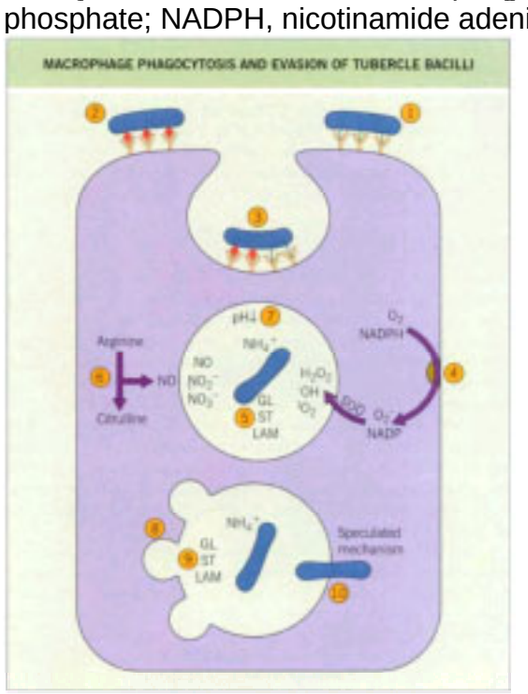


Figure 233-7 Primary isolate of *M. tuberculosis* grown from sputum on Löwenstein-Jensen medium displaying characteristic beige, rough and dry-appearing growth. *Courtesy of S Froman and A Gaytan.*

Figure 233-8 Primary isolate of *Mycobacterium tuberculosis* grown from sputum on Löwenstein-Jensen medium displaying 'cauliflower' or verrucose colonies. These are also characteristic of other mycobacteria including MAC and rapid growers, especially as a culture ages. *Courtesy of S Froman and A Gaytan.*

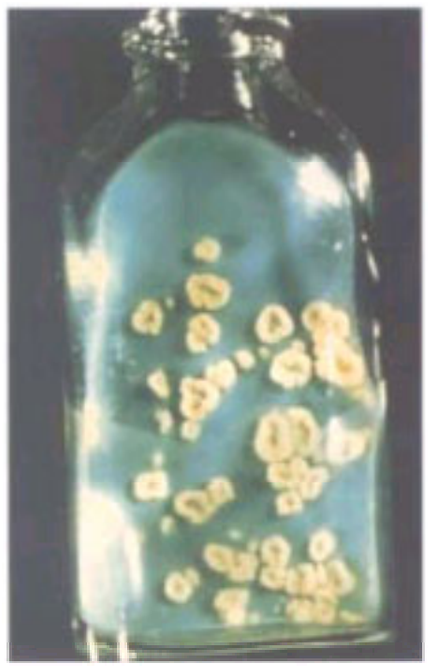


Figure 233-9 Immunoperoxidase stain of *Mycobacterium avium* complex in the bone marrow of a patient who has HIV infection and disseminated disease. The presence of mycobacteria in the bone marrow and blood of a patient is the microbiologic hallmark of disseminated MAC disease. The presence of mycobacteria in bone marrow sections using either an immunoperoxidase stain or a conventional acid-fast stain requires the presence of approximately 1.5×10^4 bacilli/g of bone marrow.

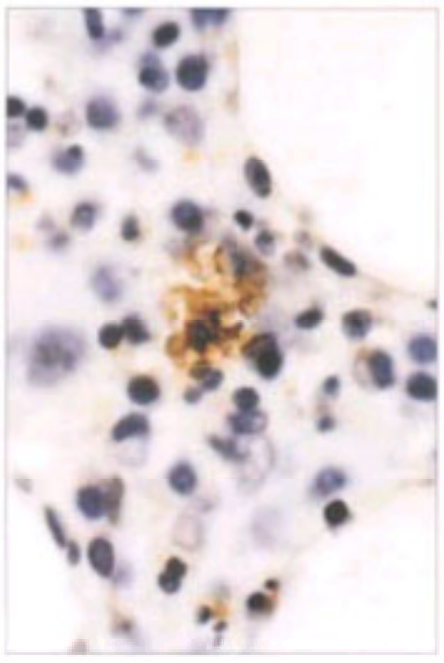


Figure 233-10 Ziehl-Neelsen acid-fast stain of sputum containing 4+ tubercle bacilli. *Courtesy of S Froman and A Gaytan.*

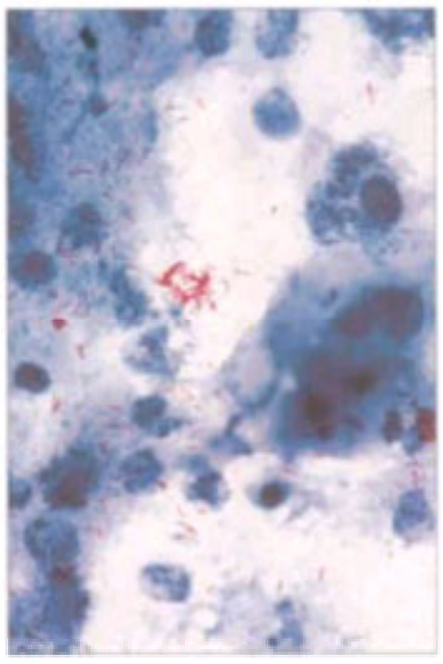


Figure 233-11 Sites of action or presumed sites of action of antimycobacterial agents. DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; PABA, *p*-aminobenzoic acid; PAS, *p*-aminosalicylic acid. *Figure adapted from Parsons et al.*[105] *and Young.*[106]

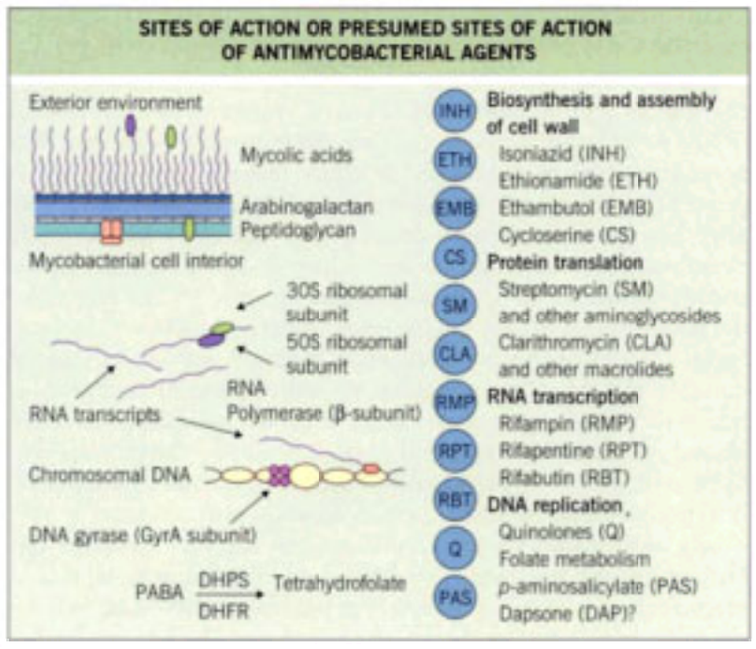


Figure 233-12 Acid-fast stain of a section of small intestine from a patient who has HIV infection and disseminated *Mycobacterium avium* disease. The photomicrograph shows many acid-fast bacilli within a villus tip of the intestinal tract biopsy. The cuboidal cells at the periphery of the tip are in disarray and appear abnormal with cell nuclei not evenly distributed at the base of the cells. There is no evidence of granuloma, but the overwhelming number of mycobacteria may be partially obscuring the host's cellular response. The histopathology is consistent with the symptoms of patients with MAC gastrointestinal tract infections, including abdominal pain, diarrhea and wasting. *Courtesy of LS Young.*

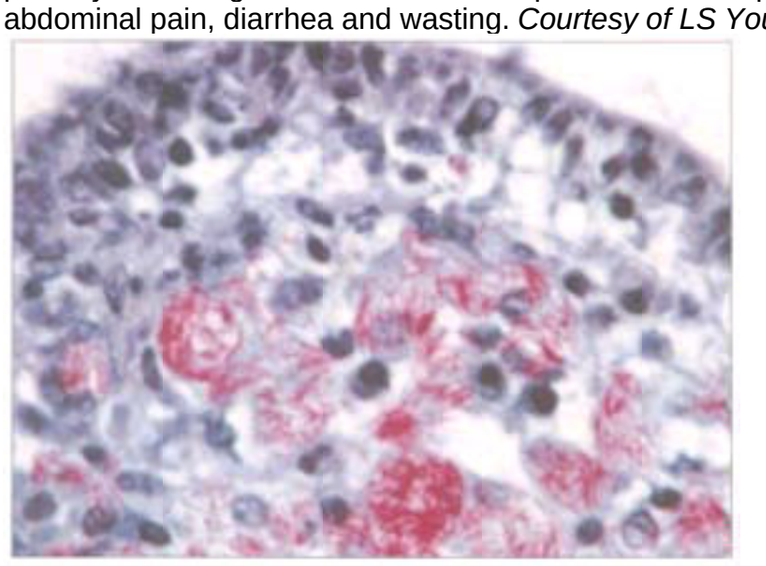


Figure 234-1 Electron micrograph of a mycoplasma organism. It has a diameter of approximately 300nm and lacks a rigid cell wall containing peptidoglycan.

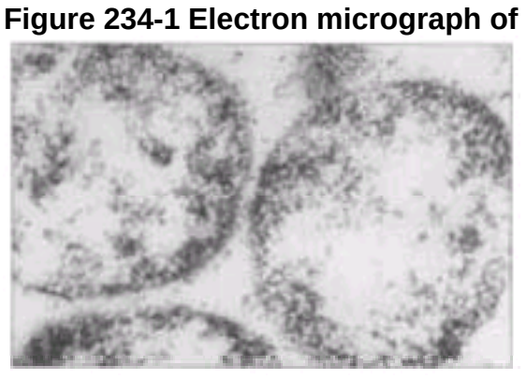


Figure 234-2 Electron micrograph of a mycoplasma organism producing filamentous structures in a broth culture. This gives the organism the appearance of a fungal mycelium. ('Myco' in mycoplasma refers to this feature.)

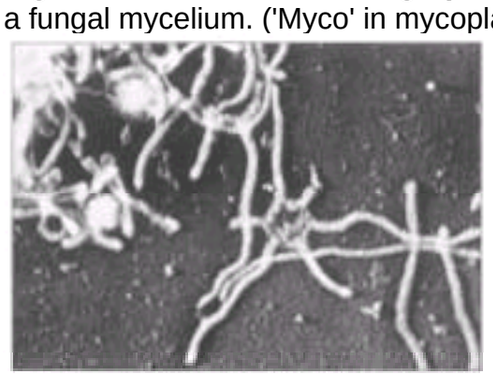


Figure 234-3 Modes of spread of *Mycoplasma hominis* to the upper genital tract. The canalicular spread to the tubes is indicated in the lower panel of the figure and the lymphatic spread to the parametria in the upper one.

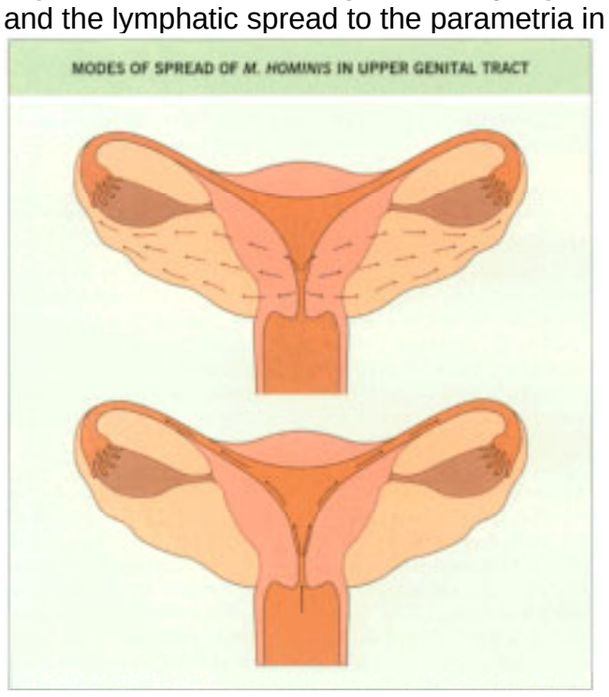


Figure 234-4 Swelling ('ballooning') of cilia in tissue cell cultures experimentally infected by *Mycoplasma hominis.*

Figure 234-5 Colonies of *Mycoplasma hominis* on PPLO agar with 'fried egg' appearance.

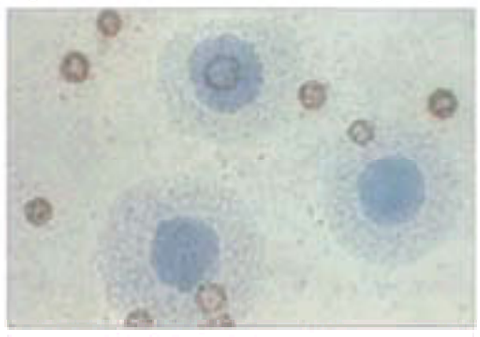


Figure 234-6 Colonies of *Mycoplasma pneumoniae* on PPLO agar with 'golf ball' appearance.

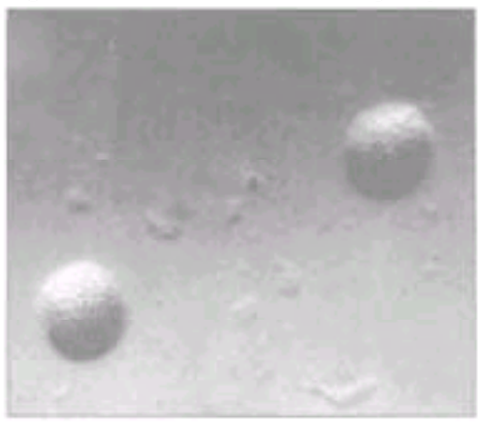


Figure 234-7 Kinetics of appearance of cold agglutinins and specific antibodies to *Mycoplasma pneumoniae* related to time after being taken ill with *M. pneumoniae* pneumonia.

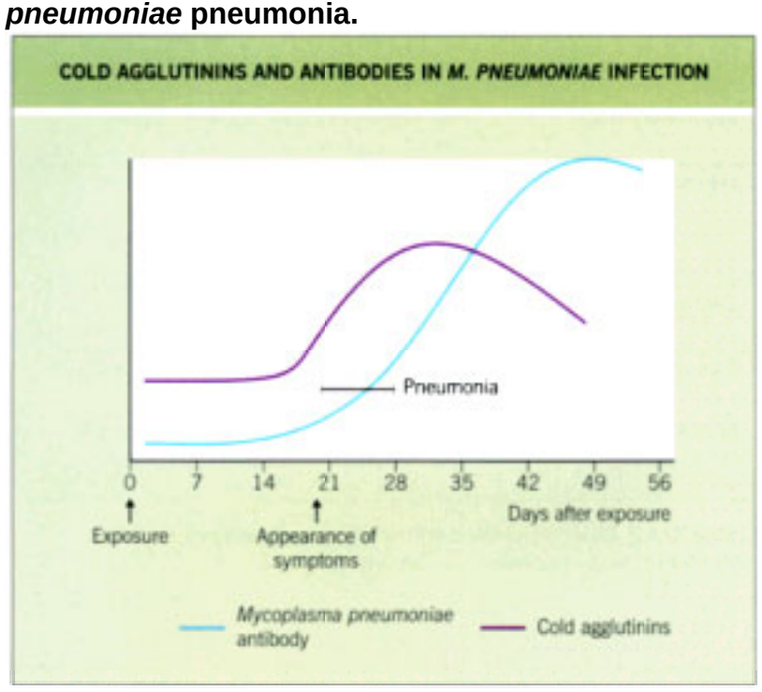


Figure 234-8 Section of parametrium from which *Mycoplasma hominis* was recovered. The patient had parametritis and developed an antibody response to the organism.

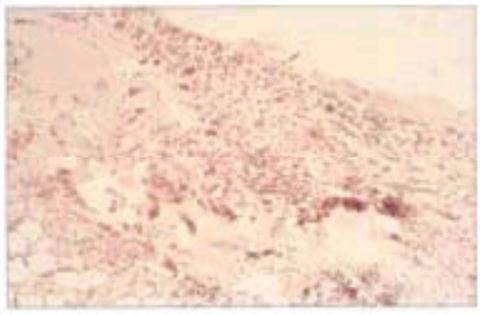


Figure 234-9 *Mycoplasma pneumoniae* pneumonia. There were few signs on auscultation.

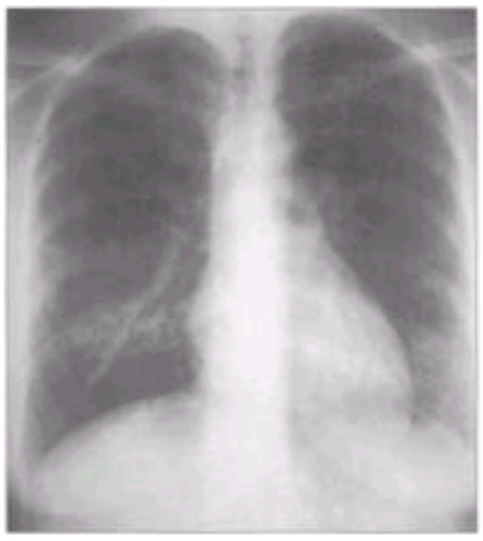


Figure 235-1 Phylogenetic relationships between rickettsias based on 16S rRNA gene sequences.

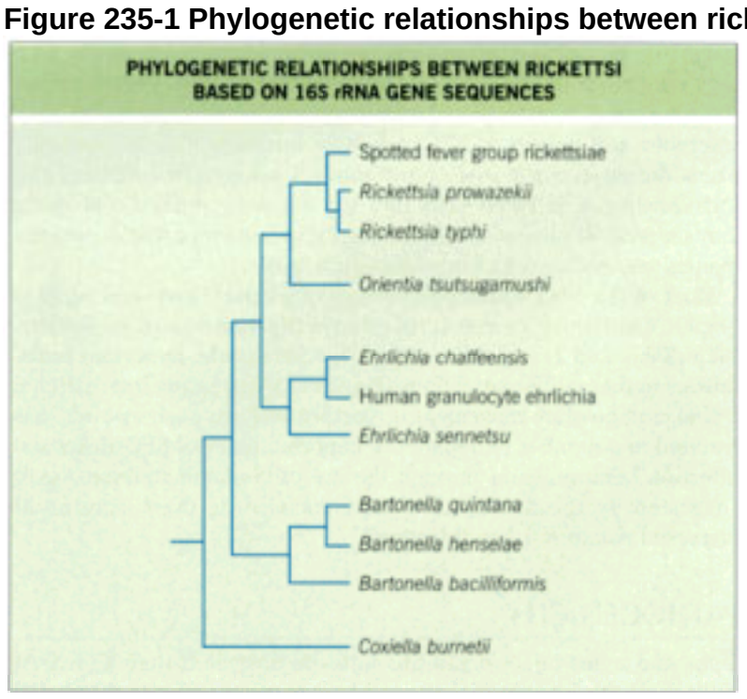


Figure 235-2 *Amblyomma hebraeum*, a typical tick vector of spotted fever group rickettsial infection.

Figure 235-3 Gimenez stain of tissue culture cells infected with spotted fever group rickettsias.

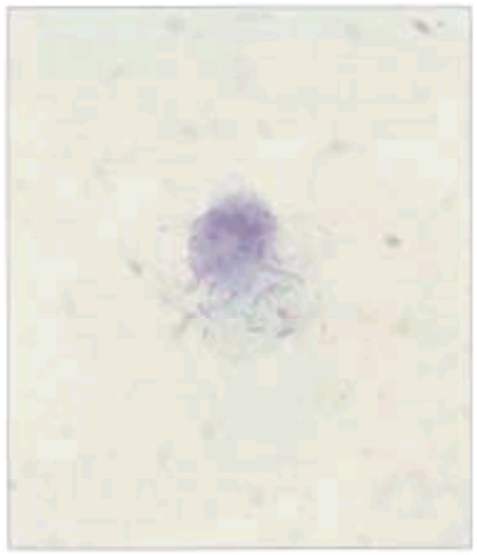


Figure 235-4 Western blot technique in spotted fever group infection. Western blot of pooled mouse antisera to *Rickettsia africae* — human isolate (lane 1), *Rickettsia africae* — tick isolate (lanes 2–4), *Rickettsia conorii* — Kenyan strain (lane 5), *Rickettsia conorii* — Moroccan strain (lane 6) and Israeli SFG rickettsia (lane 7). Molecular masses (in thousands) are shown.

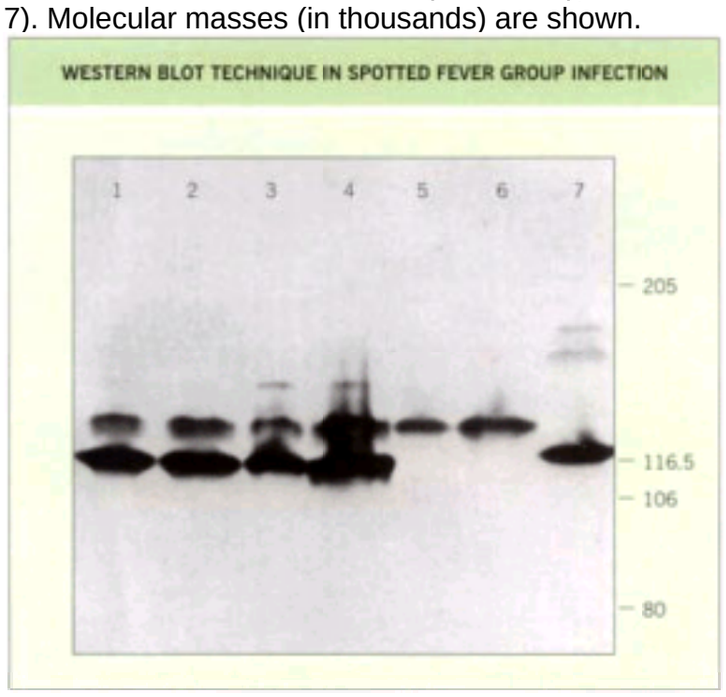


Figure 235-5 Multiple morulas of *Ehrlichia canis* in culture DH82 cells.

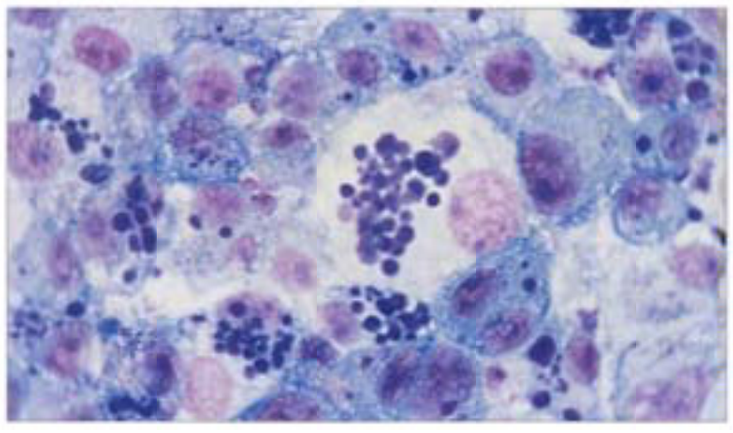


Figure 235-6 Colonies of *Bartonella henselae* on blood agar. *Courtesy of R. Birtles.*

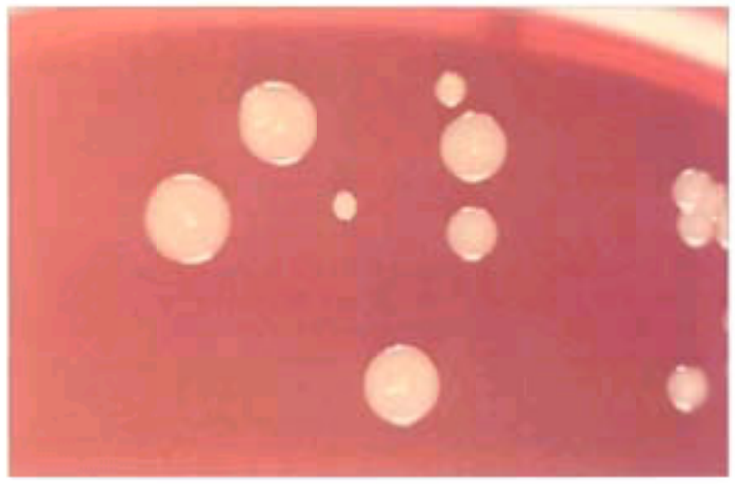


Figure 235-7 Skin lesions of bacillary angiomatosis. *Courtesy of P. Kelly.*

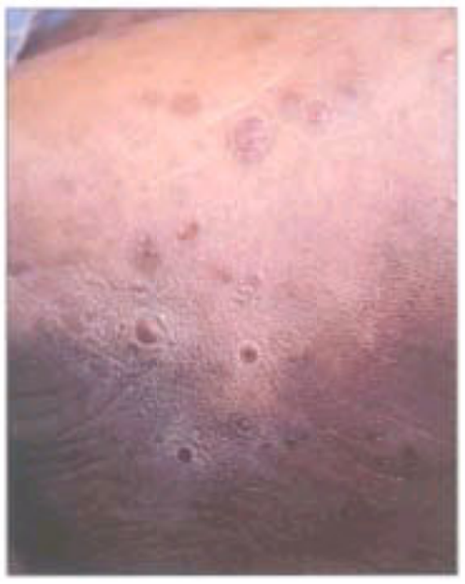


Figure 235-8 Kinetics of antibody responses to phase variants of *Coxiella burnetii*.

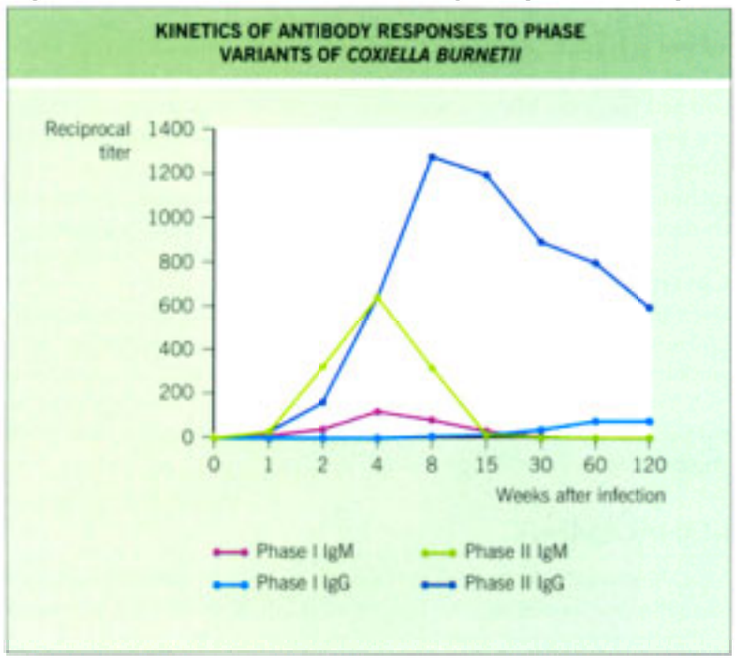


Figure 236-1 Life cycle of *Chlamydia* spp.

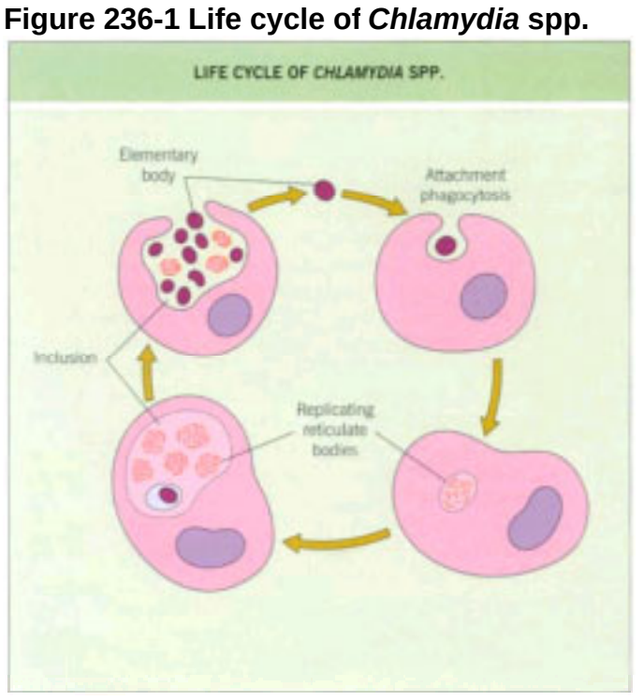


Figure 236-2 Structure of *Chlamydia* spp. *Courtesy of Dr A Matsumoto.*

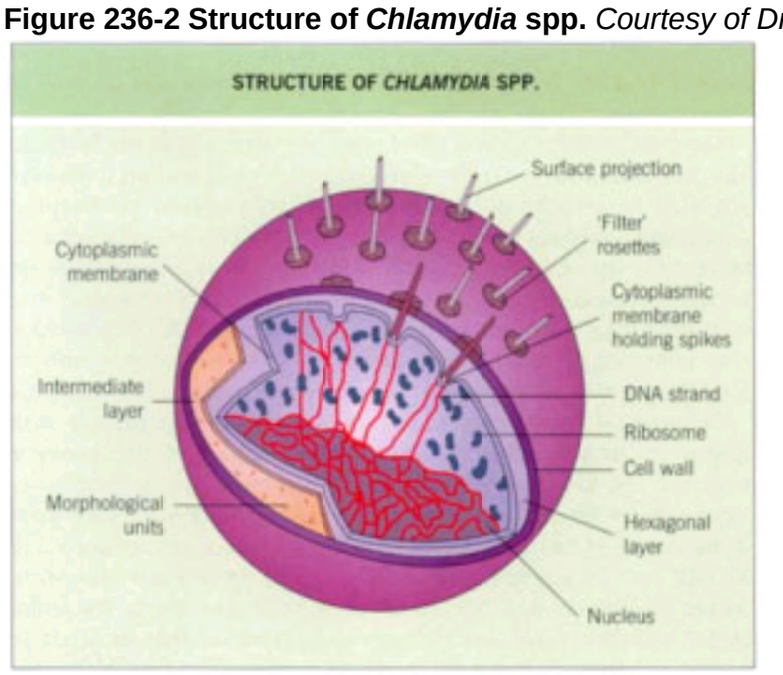


Figure 236-3 Chlamydial inclusion in confocal stereomicroscopy. *Chlamydia pneumoniae* cultured in human line (HL) cells are shown. Fluorescein isothiocyanate (FITC)-labeled anti-LPS monoclonal antibody strain. *Courtesy of Dr A Laurila.*

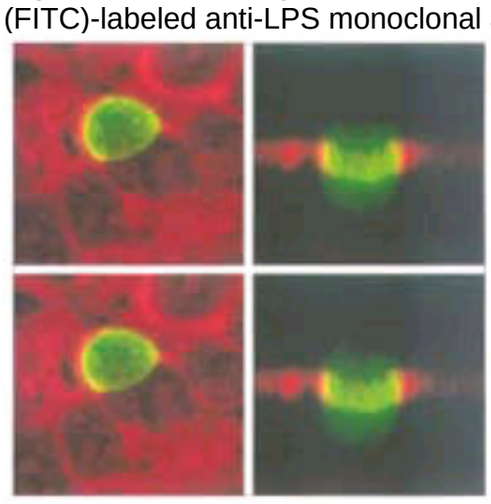


Figure 236-4 Electron microscopy of chlamydial inclusion. Reticulate bodies and transition stages to dense elementary bodies are shown. *Chlamydia pneumoniae* cultured in HL cells. *Courtesy of Dr CH von Bonsdorff.*

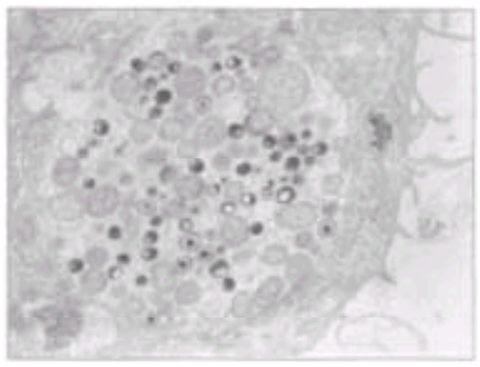


Figure 236-5 Chronic chlamydial infection in mouse lung. Note the infiltration of mononuclear cells. Hematoxylin and eosin stain. *Courtesy of Dr A Laurila.*

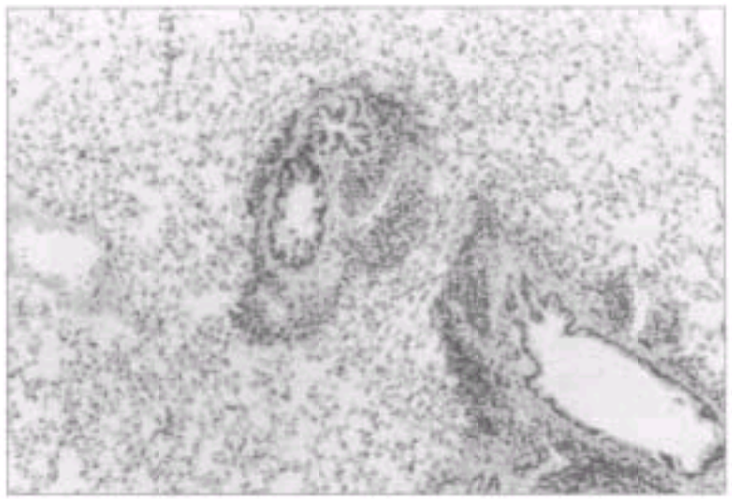


Figure 236-6 Mucopurulent cervicitis caused by *Chlamydia trachomatis*. *Courtesy of Dr J Paavonen.*

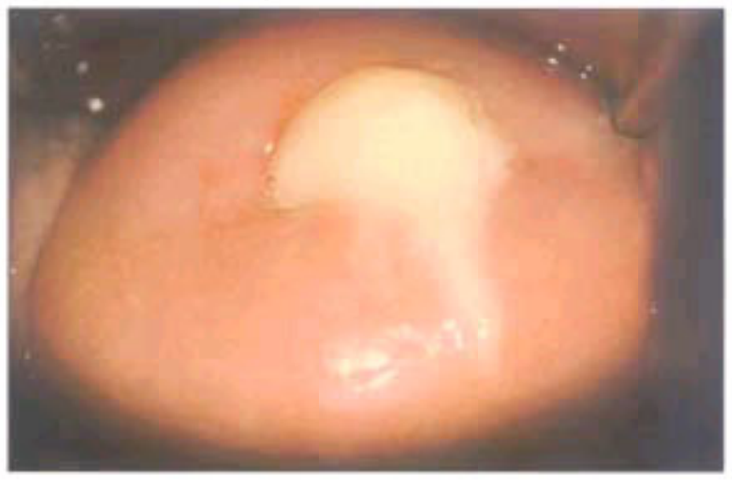


Figure 236-7 Ophthalmia neonatorum. *Courtesy of Dr M Puolakkainen.*

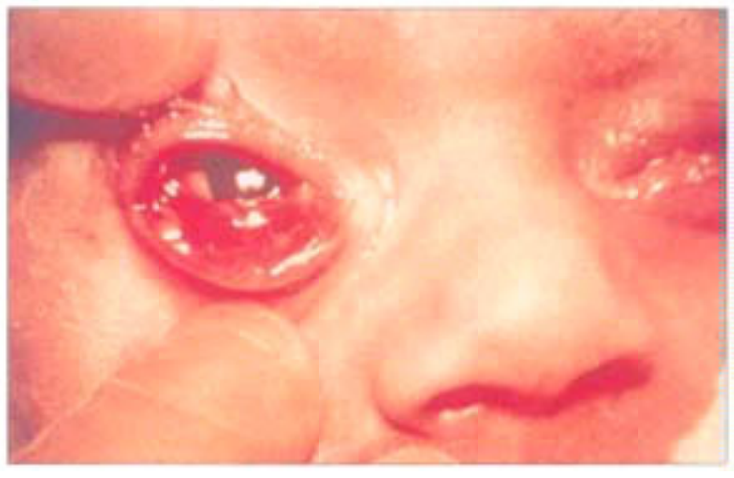


Figure 236-8 *Chlamydia pneumoniae* reinfection pneumonia in a 63-year-old male.

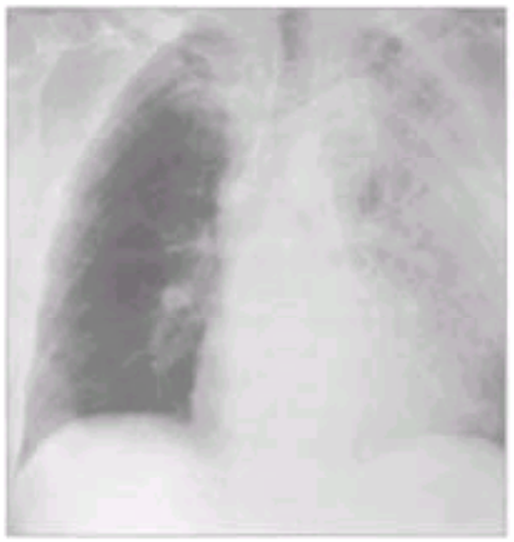


Figure 237-1 Colonies of *Candida albicans* on culture plate.

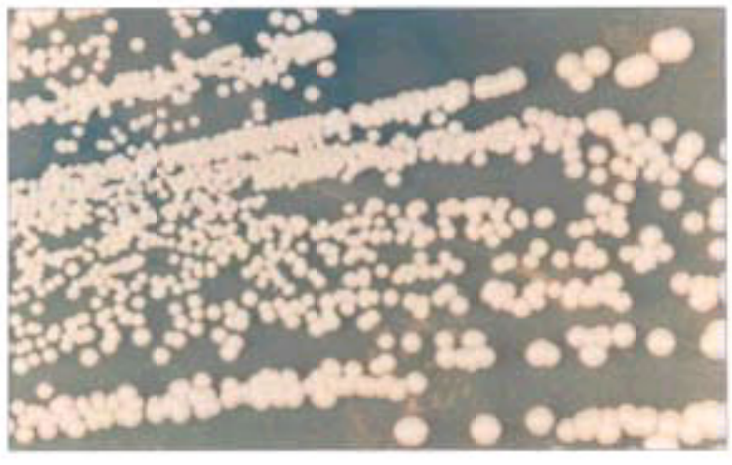


Figure 237-2 Candidal esophagitis. (a) Double-contrast radiography in candidal esophagitis. (b) Endoscopy of same patient.

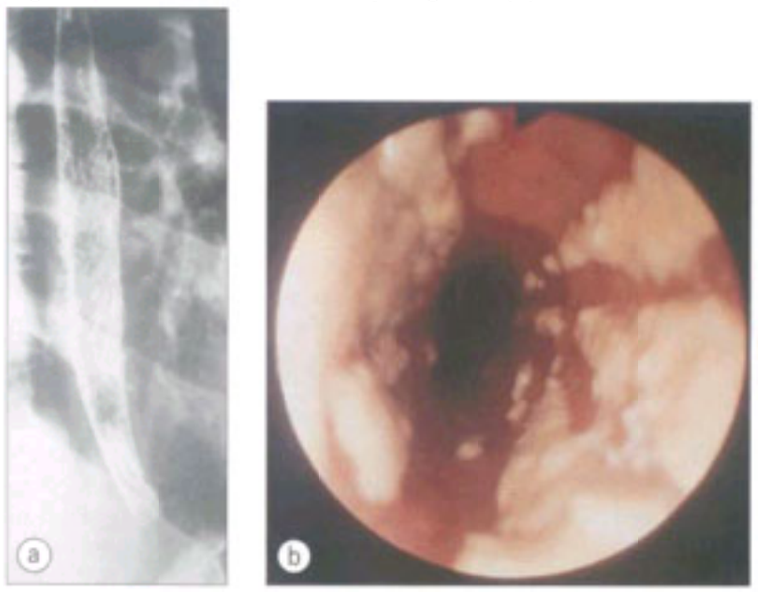


Figure 237-3 Retina of a patient who has lesions (arrows) due to disseminated candidiasis.

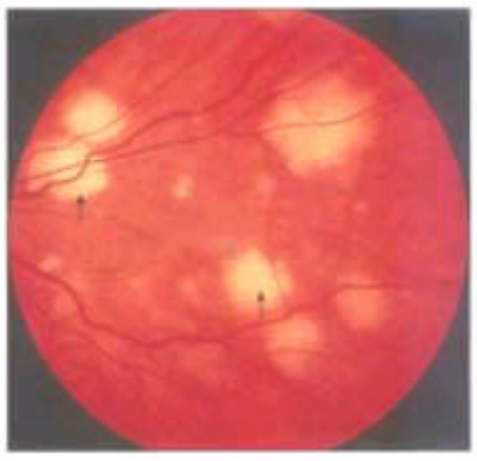


Figure 237-4 Hepatosplenic candidiasis. Multiple abscesses can be seen.

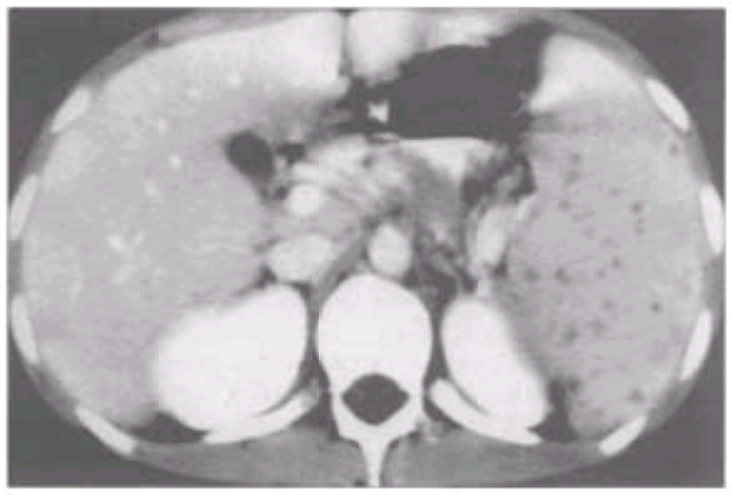


Figure 237-5 CT scan showing enlarged right hilar lymph node (top arrow) and a posterior infiltrate (bottom arrow) due to *Candida albicans.*

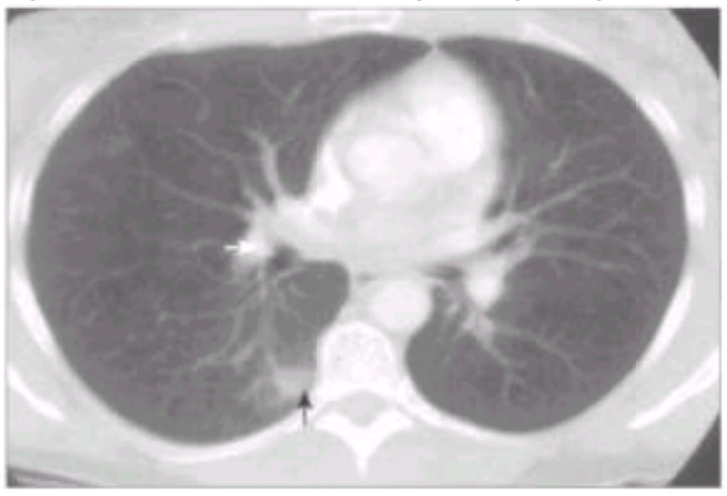


Figure 237-6 Targets and mechanisms of action of antifungal drugs.

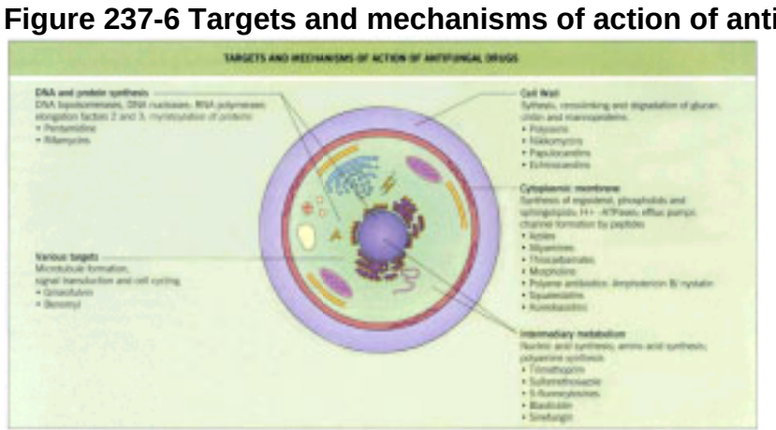


Figure 237-7 *Aspergillus* hyphae.

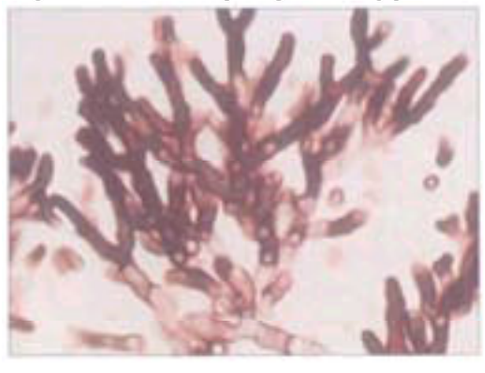


Figure 237-8 *Aspergillus* pneumonia.

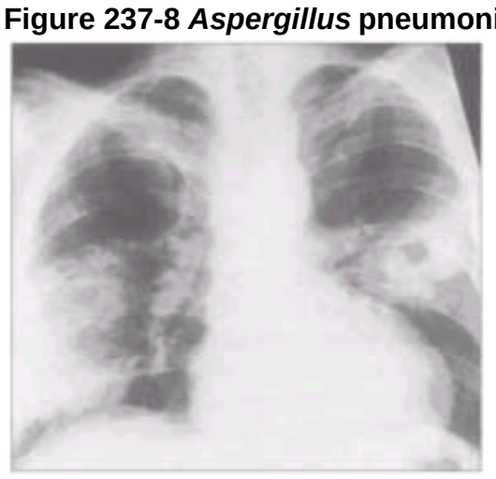


Figure 237-9 *Aspergillus* sinusitis. Involvement of right maxillary sinus with extension to adjacent structures and the brain (arrows). Coronal section.

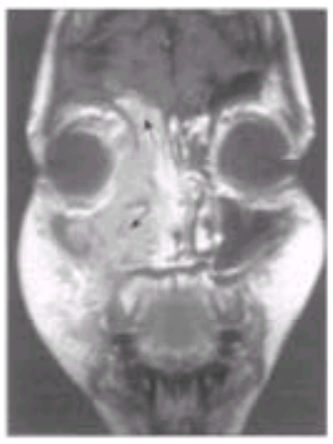


Figure 237-10 ***Cryptococcus neoformans.*** (a) Thinly encapsuiated. (b) With a thick capsule.

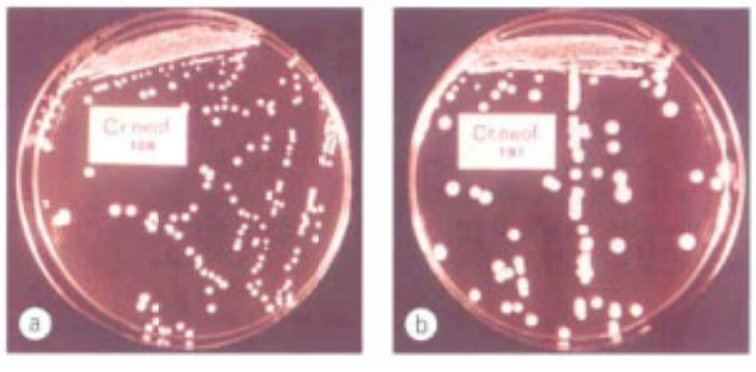


Figure 237-11 ***Cryptococcus neoformans.*** (a) Cytologic preparation of CSF, (b) India ink preparation, (c) Mayer's mucicarmine stain. *With permission from Jaster and Malecha. Copyright 1996 Massachusetts Medical Society. All rights reserved.*

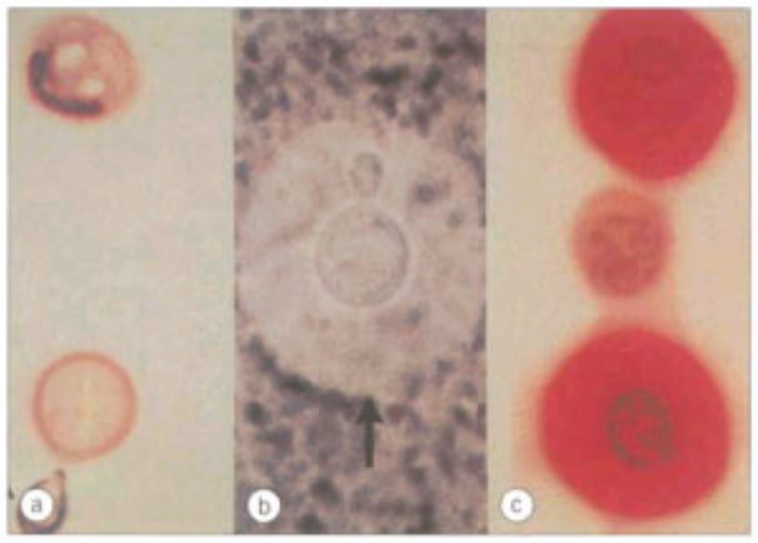


Figure 237-12 Histology of pulmonary cryptococcosis.

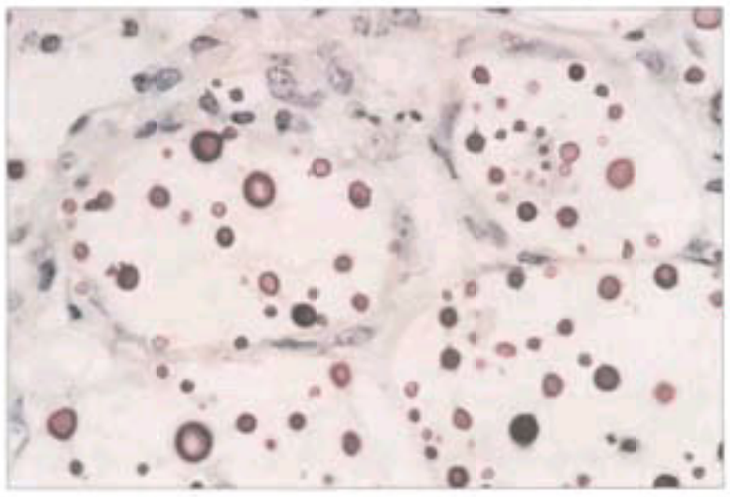


Figure 237-13 Lactophenol cotton blue preparation of *Absidia corymbifera.*

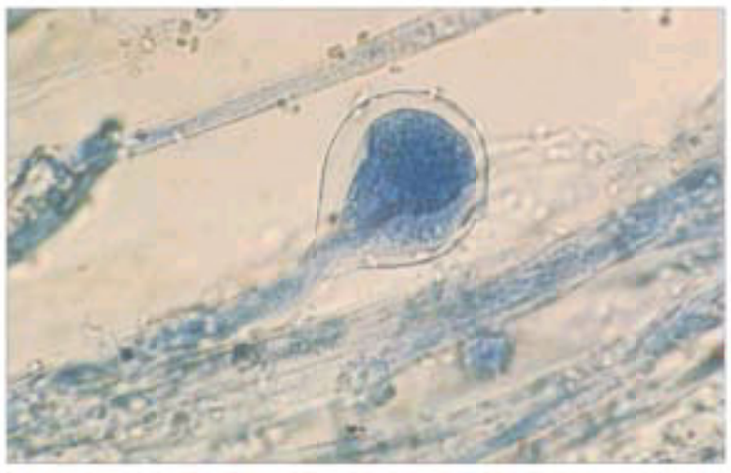


Figure 237-14 Patient with mucormycosis. Ocular invasion by Mucor in a patient with diabetes mellitus and ketoacidosis.

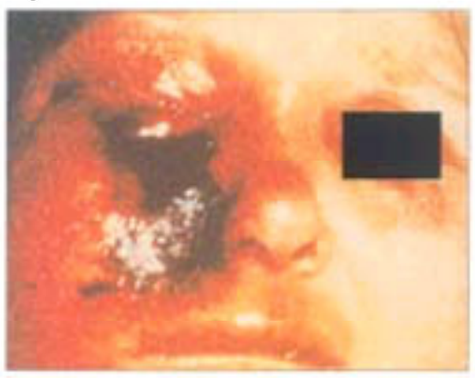


Figure 237-15 Lactophenol cotton blue preparation of *Penicillium brevi compactum.*

Figure 237-16 HIV-infected patient with *Penicillium marneffei* infection with molluscum contagiosum-like lesions.

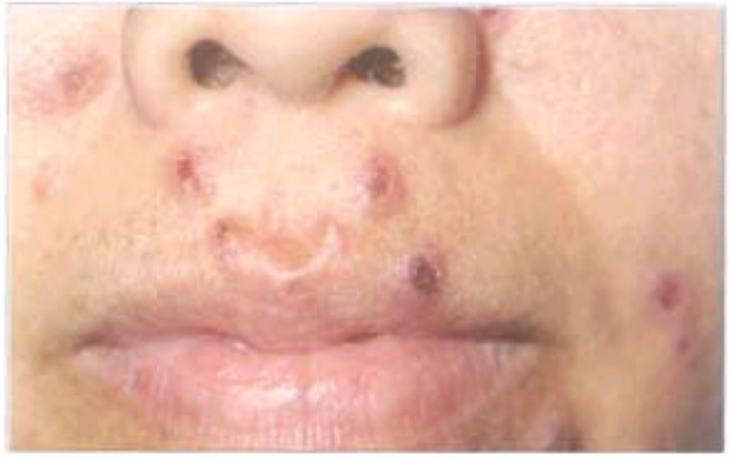


Figure 238-1 *Blastomyces dermatitidis,* mycelial form in culture at 86°F. This shows hyaline, septate, branching hyphae and short conidiophores bearing solitary oval-to-piriform conidia.

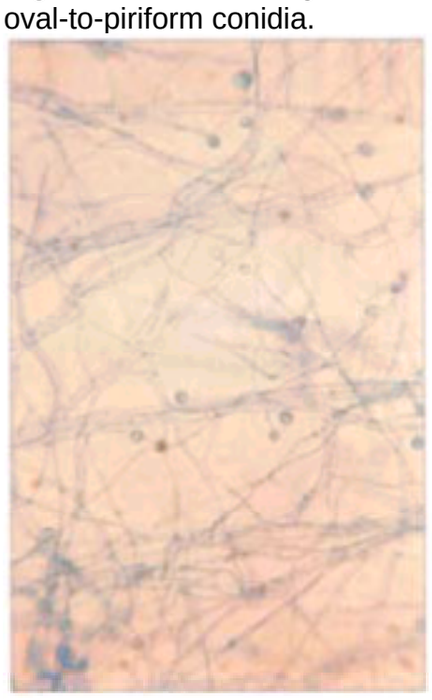


Figure 238-2 *Blastomyces dermatitidis,* yeast form in hematoxylin and eosin-stained section of microabscess from a cutaneous lesion. This shows large spherical yeasts characterized by thick, highly refractile cell walls, single budding and a broad attachment between the parent yeast cell and bud.

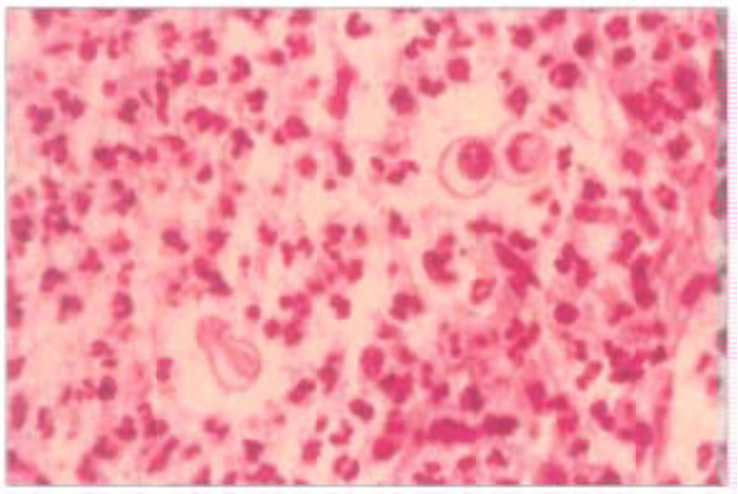


Figure 238-3 Initial cutaneous lesion of blastomycosis.

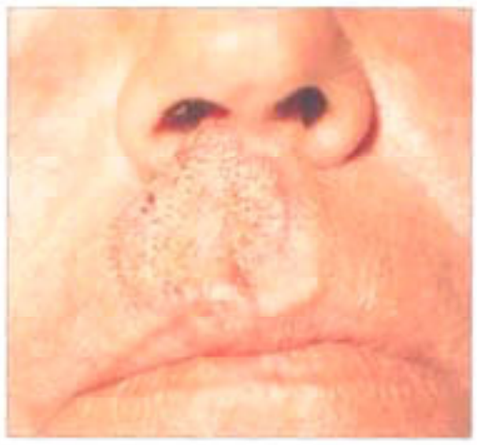


Figure 238-4 *Coccidioides immitis,* mycelial form in culture at 86°F. This shows hyaline, septate, branching hyphae and chains of arthroconidia, often in alternating cells.

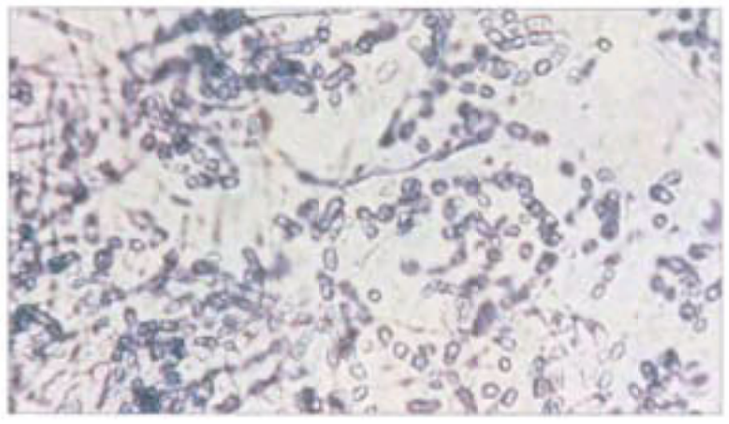


Figure 238-5 *Coccidioides immitis,* spherule in hematoxylin- and eosin-stained section of lung lesion. This shows refractile cell wall and internal endospores.

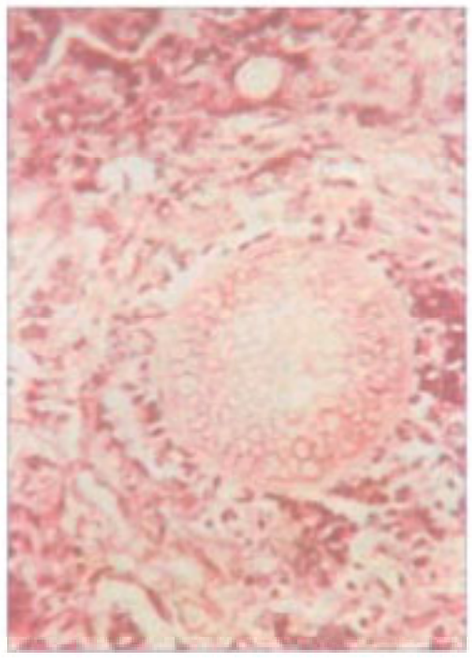


Figure 238-6 Coccidioidomycosis, showing hilar lymphadenopathy and a cavity in the left lung.

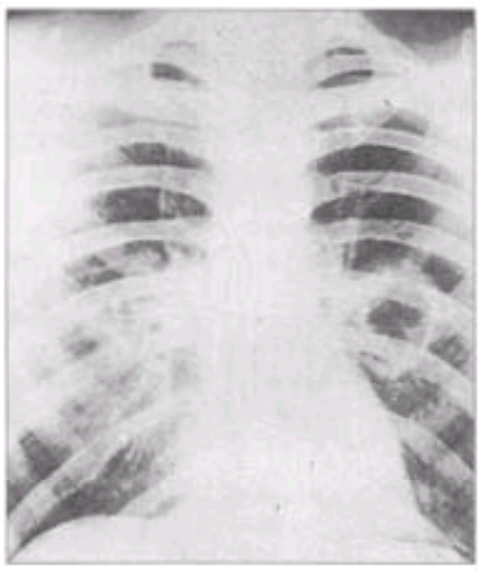


Figure 238-7 Allergic manifestations of infection with *Coccidioides immitis.* Erythema nodosum on the lower legs.

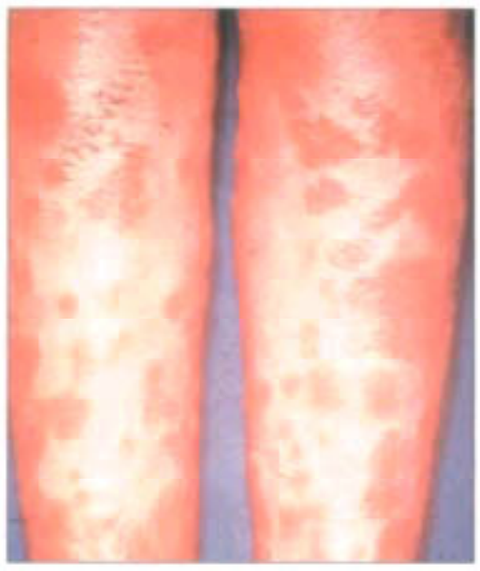


Figure 238-8 *Histoplasma capsulatum,* mycelial form in culture at 86°F. This shows hyaline septate branching hyphae, microconidia and large spherical macroconidia, with projections from the cell walls.

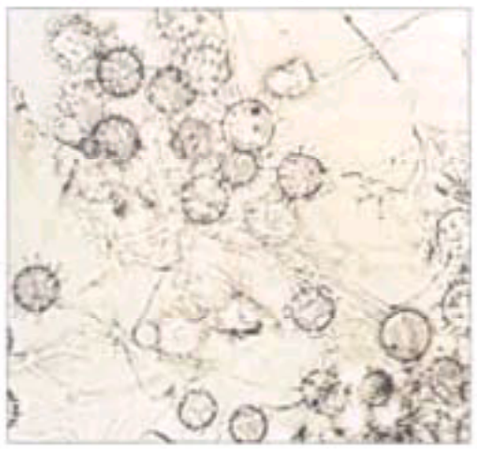


Figure 238-9 ***Histoplasma capsulatum.*** Small yeast cells packed inside macrophages in a Giemsa-stained smear of bone marrow aspirate.

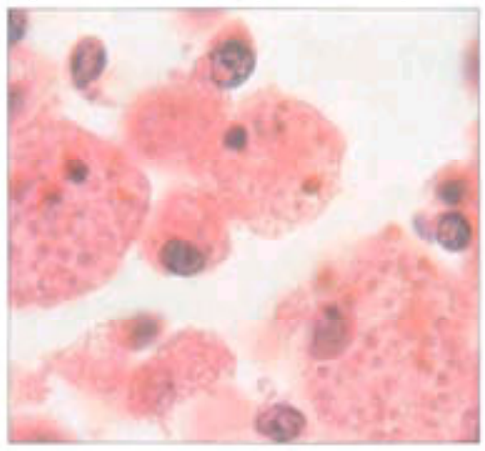


Figure 238-10 Histoplasmosis. (a) Acute pulmonary histoplasmosis showing hilar lymphadenopathy and diffuse infiltrates. (b) Hilar lymphadenopathy and miliary calcifications throughout both lungs.

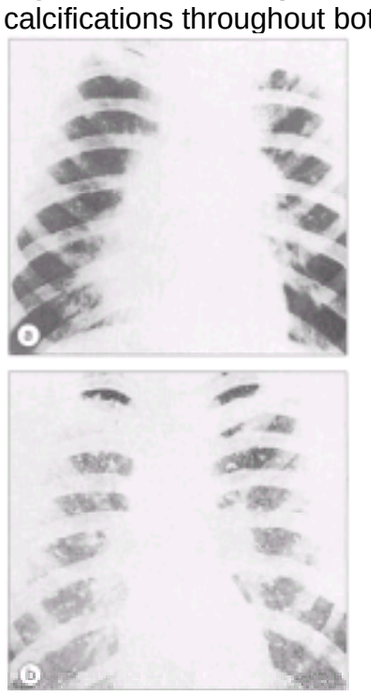


Figure 238-11 ***Paracoccidioides brasiliensis.*** Large multiply budding yeast cells in a potassium hydroxide preparation of a scraping of cutaneous paracoccidioidomycosis.

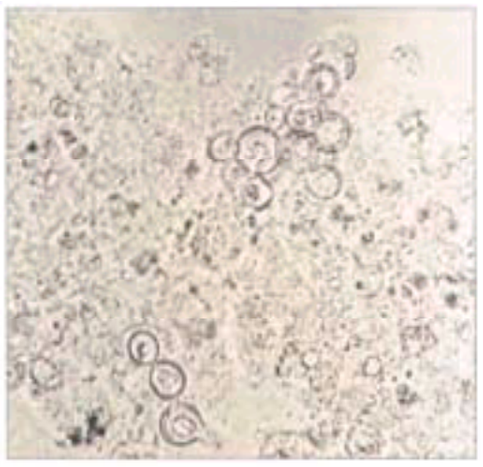


Figure 238-12 Cutaneous and mucocutaneous paracoccidiodomycosis.

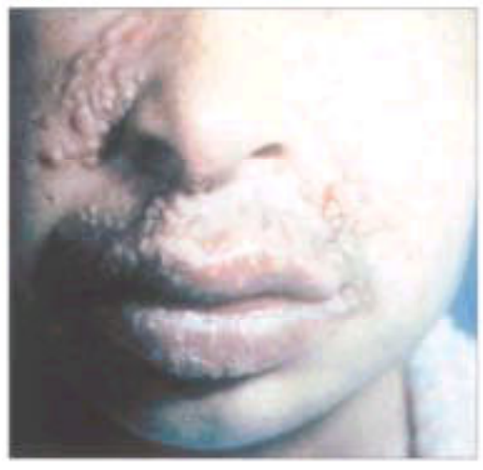


Figure 239-1 Chromoblastomycosis. Thick-walled, septate, dematiaceous muriform cells. *With permission from Richardson MD, et al. Slide atlas of fungal infection: subcutaneous and unusual fungal infections, Oxford: Blackwell, 1995.*

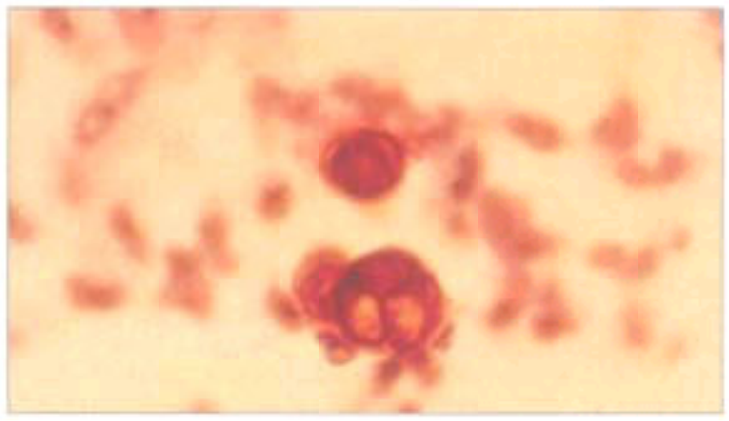


Figure 239-2 Lobomycosis. Yeast cells are attached to each other in short chains. Nonbudding and single-budding cells are also present.

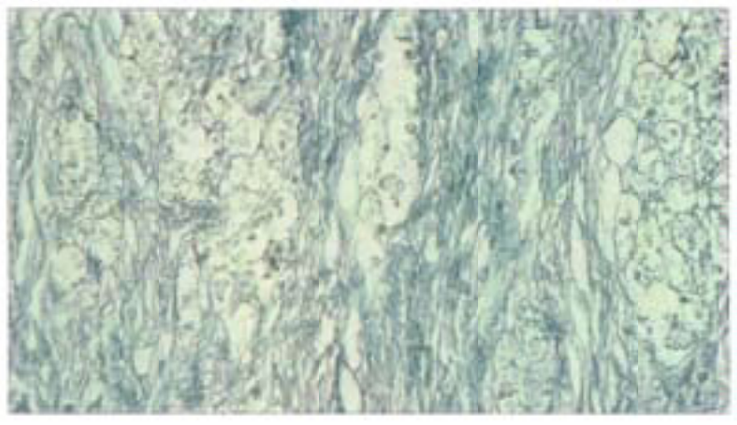


Figure 239-3 Granule of *Madurella mycetomatis.* The granules have a deeply pigmented periphery of compact hyphae. Randomly oriented, poorly pigmented fungal elements in the interior of the granule are less compact.

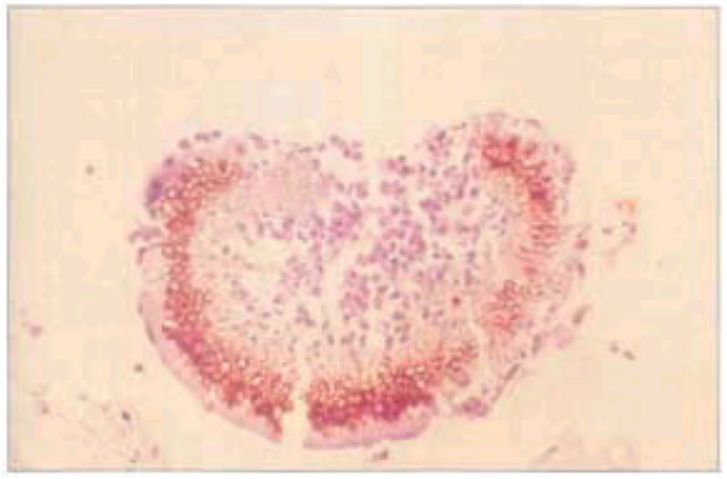


Figure 239-4 Subcutaneous phaeohyphomycosis caused by *Bipolaris spicifera.* The fungal elements are stained with Fontana-Masson, which accentuates and confirms the presence of melanin or melanin-like pigment in the fungal cell walls.

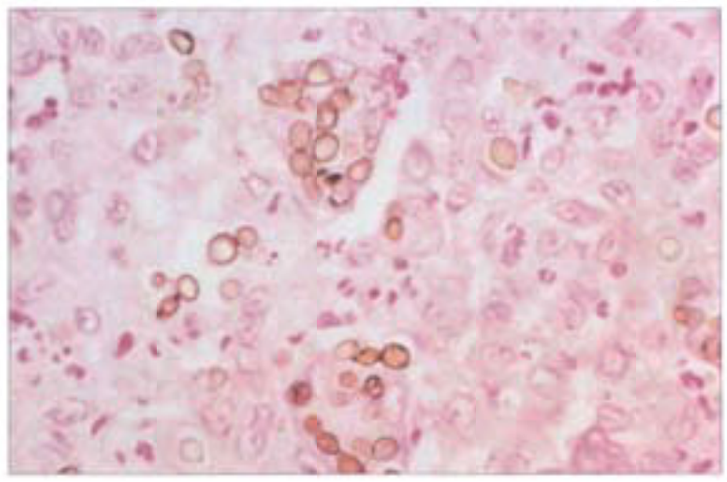


Figure 239-5 Rhinosporidiosis.

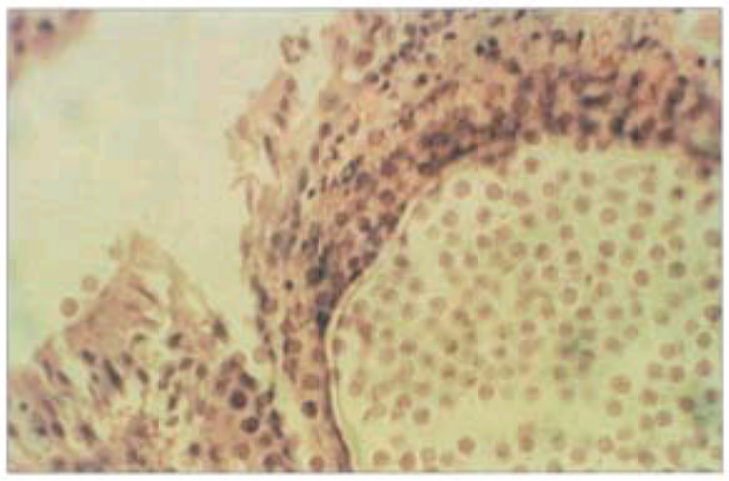


Figure 239-6 Asteroid body in cutaneous sporotrichosis. A yeast-like cell of *Sporothrix schenckii* with faintly basophilic, retracted cytoplasm is intimately surrounded by elongated spicules of Splendore-Hoeppli material.

Figure 239-7 Chromoblastomycosis lesions on the foot and ankle. The typical raised, crusted, verrucous lesions seen on the foot and ankle.

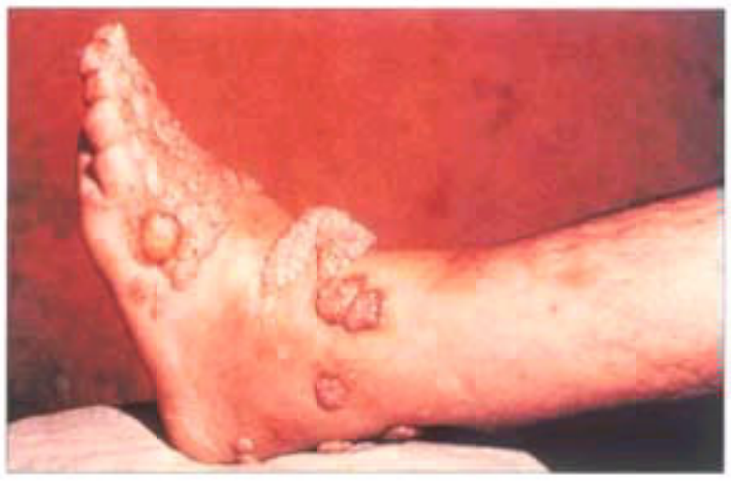


Figure 239-8 Chromoblastomycosis lesions on the ear. A crusty lesion on the lower part of the external ear.

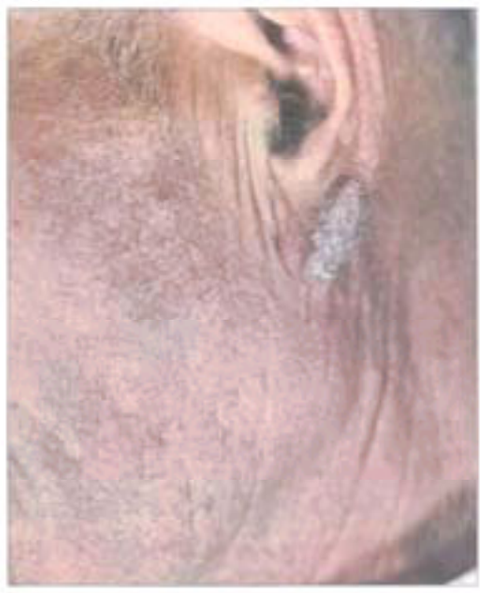


Figure 239-9 Rhinofacial conidiobolomycosis. *With permission from Richardson MD, et al. Slide atlas of fungal infection: subcutaneous and unusual fungal infections, Oxford: Blackwell, 1995.*

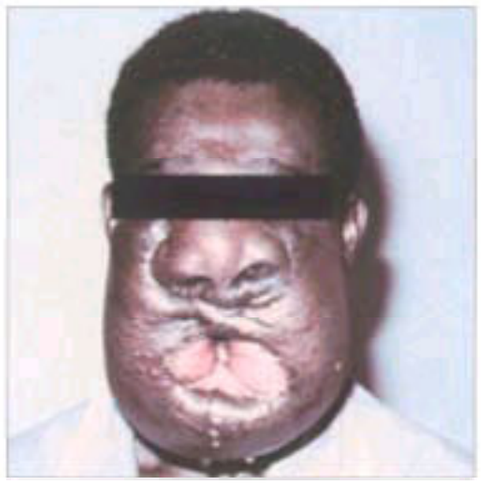


Figure 239-10 Mycetoma of the foot.

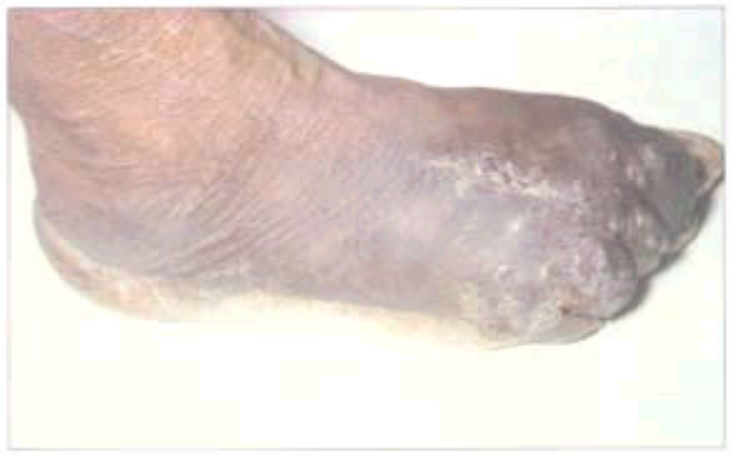


Figure 239-11 Localized lesion in sporotrichosis. The most common clinical presentation is a localized cutaneous or subcutaneous lesion, which develops at the site of implantation of the etiologic agent, *Sporothrix schenckii*.

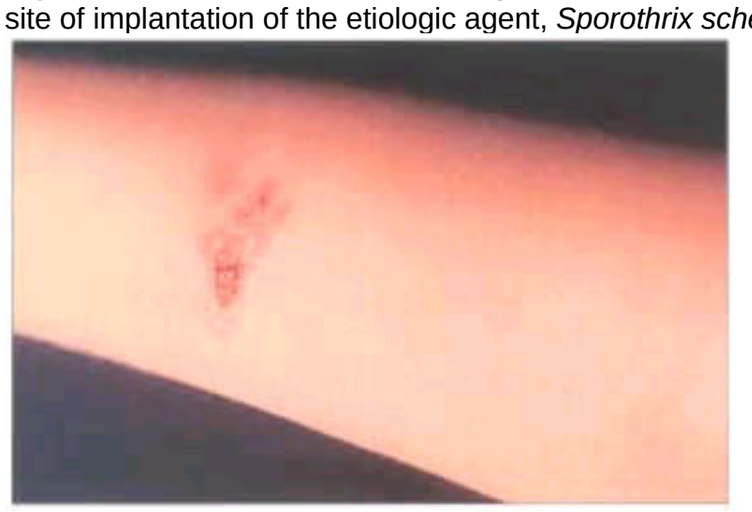


Figure 239-12 Lymphagitic spread of sporotrichosis. The spread is from a primary digital lesion up the dorsal surface of the forearm.

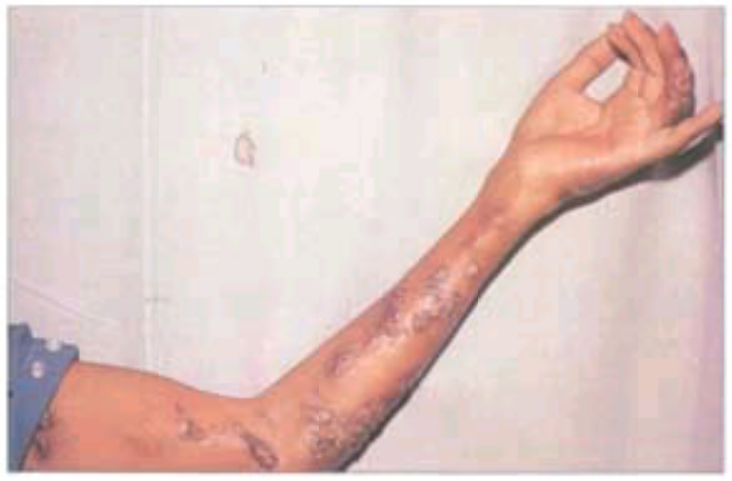


Figure 239-13 Multiple lesions in sporotrichosis. These skin lesions are a result of hematogenous spread.

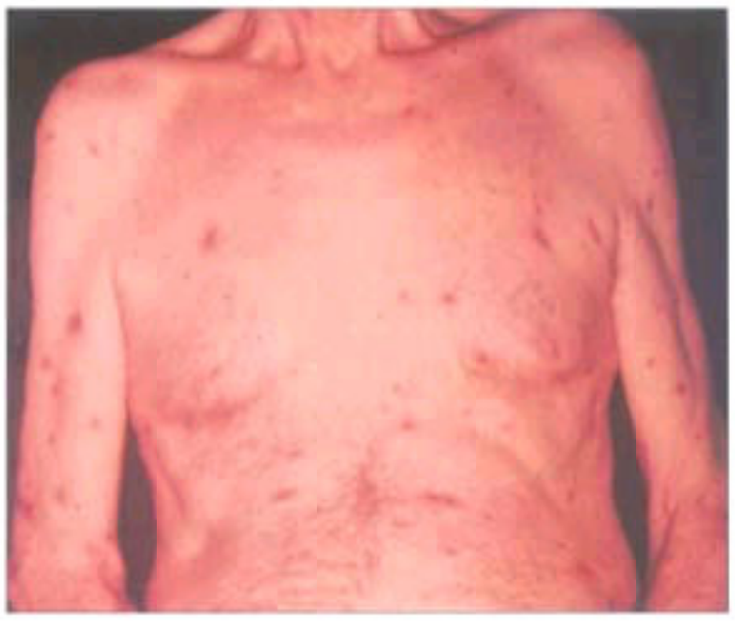


Figure 240-1 *Trichophyton rubrum* (heavily conidial Afro-Asiatic variant). Typical micromorphology of a dermatophyte in pure culture, showing micro- and macroaleurioconidia.

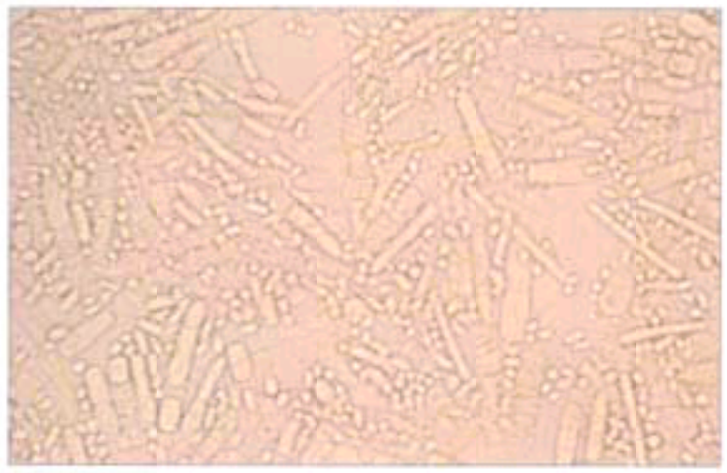


Figure 240-2 *Trichophyton mentagrophytes* complex: a zoophilic (animal-associated) isolate showing the 'granular' colonial morphology typical of such isolates. The strain depicted has been shown through mating to belong to the phylogenetic species *Trichophyton interdigitale* (sexual state called *Arthroderma vanbreuseghemii*), one of several genetic sibling species now known to make up the *T. mentagrophytes* complex.

Figure 240-3 Direct microscopy (NaOH slide) of palm skin showing distinctive dark fungal filaments of *Hortaea werneckii*, causal agent of tinea nigra.

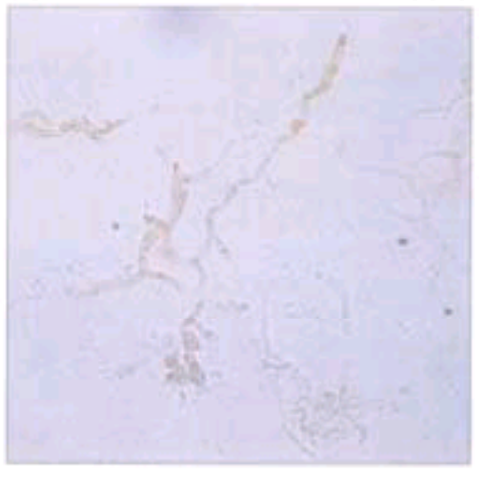


Figure 240-4 Tinea capitis, noninflammatory type. *Courtesy of Dr Natalie Roholt.*

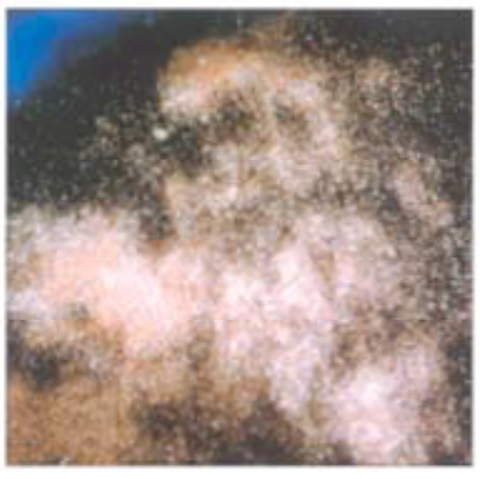


Figure 240-5 Tinea pedis, vesiculobullous variety. *Courtesy of Dr Natalie Roholt.*

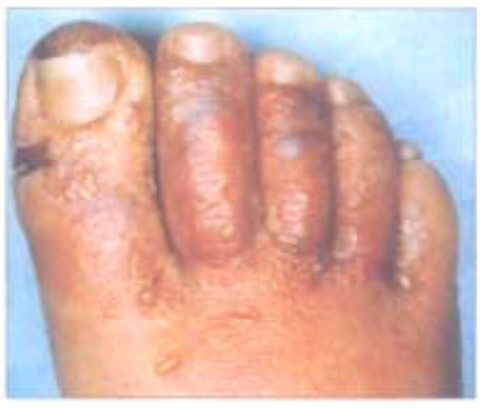


Figure 240-6 Tinea versicolor. *Courtesy of Dr Natalie Roholt.*

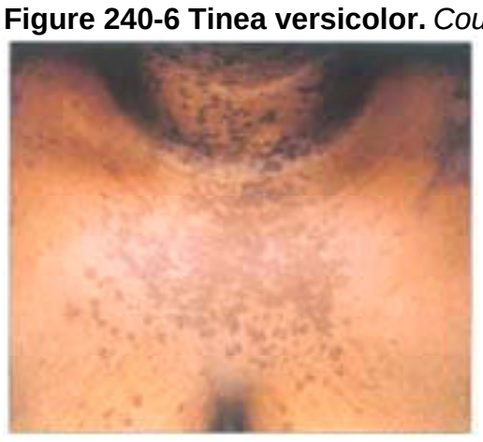


Figure 240-7 Onychomycosis (tinea unguium), distal and lateral subungual type. *Courtesy of Dr Natalie Roholt.*

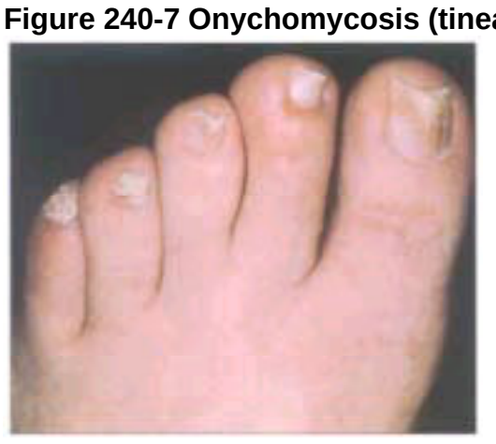


Figure 241-1 Pathogenesis of *Pneumocystis carinii* pneumonia. IFN, interferon; NO, nitric oxide; PMN, polymorph nuclear cell; SpA, surfactant protein A.

Figure 241-2 Hematoxylin and eosin stained section of lung tissue. Characteristic intra-alveolar foamy exudates can be seen in this patient, who has *P. carinii* pneumonia. *Courtesy of Michael Koss.*

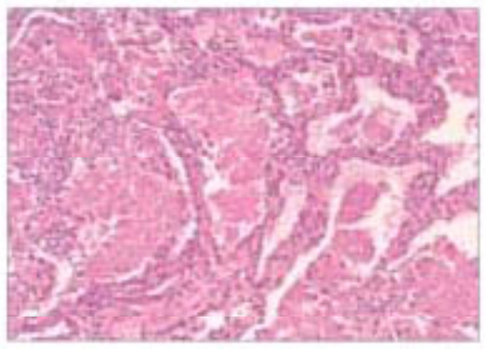


Figure 241-3 Monoclonal immunofluorescent stain. Monoclonal immunofluorescent stain of a BAL specimen (Merifluor stain) demonstrating *P. carinii* cysts that stain with a characteristic apple-green fluorescence. *With permission from Meridian Diagnostics.*

Figure 241-4 Gomori's methenamine silver stain of lung tissue. Gomori-stained section of lung tissue showing characteristic *P. carinii* cysts.

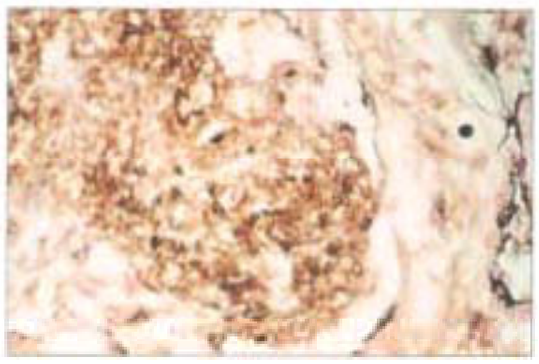


Figure 241-5 Wright-Giemsa stain of sputum. Wright-Giemsa stained specimen of an induced sputum specimen showing clusters of *P. carinii* organisms. *Courtesy of Maria Appleman.*

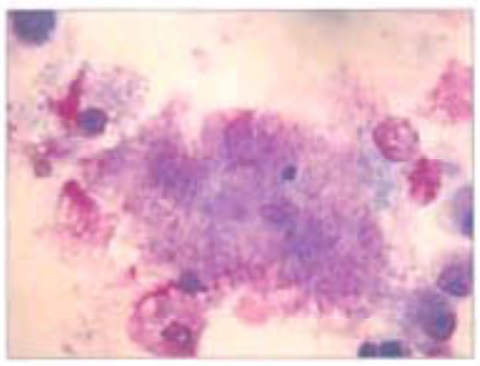


Figure 242-1 *Entamoeba histolytica.* (a) Trophozoite containing ingested red blood cells (the presence of red blood cells confirms the organism is the true pathogen, *E. histolytica*). (b) ***Entamoeba histolytica/E. dispar***, cyst containing four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: from the cyst morphology, it is not possible to differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*.

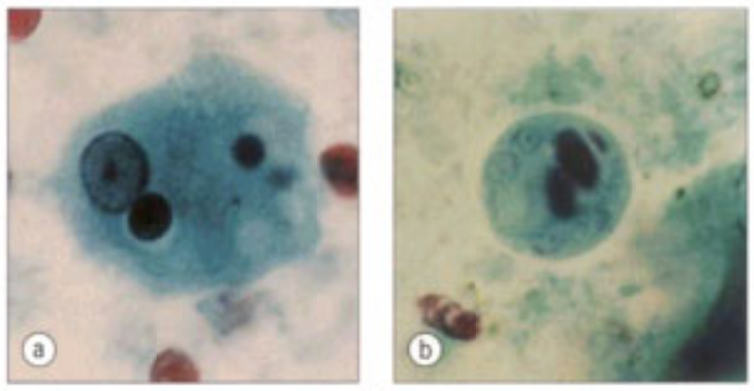


Figure 242-2 *Entamoeba hartmanni.* (a) Trophozoite. (b) Cyst containing up to four nuclei and chromatoidal bars with smooth, rounded edges (trichrome stain). Note: *E. hartmanni* measures less than *E. histolytica/E. dispar*, the trophozoite is <12µm and the cyst is <10µm.

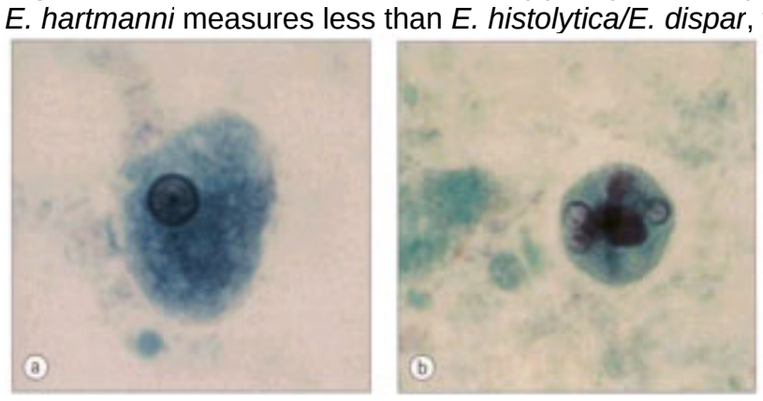


Figure 242-3 *Entamoeba coli.* (a) Trophozoite containing a single nucleus in which the karyosome is eccentric (tends to be centrally located in *E. histolytica/E. dispar*). (b) Cyst contains more than five nuclei (d'Antoni's iodine).

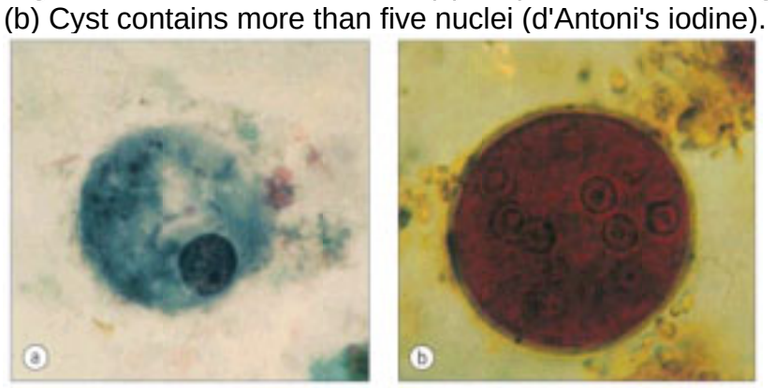


Figure 242-4 *Endolimax nana.* (a) Trophozoite containing a single nucleus with no peripheral chromatin (large karyosome only) and vacuolated cytoplasm. (b) Cyst containing four nuclei (three easily visible) (trichrome stain).

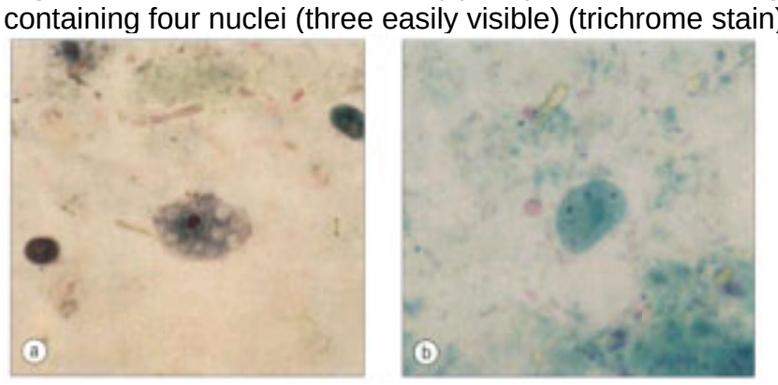


Figure 242-5 *Iodamoeba bütschlii.* (a) Trophozoite containing a single nucleus with a large karyosome and cyst containing a single nucleus and a large glycogen vacuole. (b) Cyst containing a single nucleus and a large glycogen vacuole — note the size of the karyosome (trichome stain). There is also a *Blastocystis hominis* central body form present at the lower left of the image.

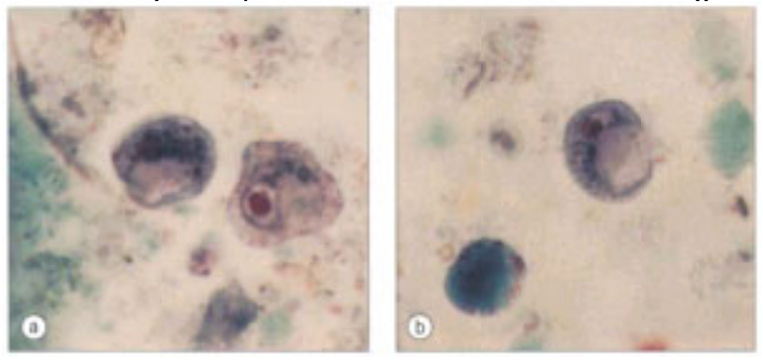


Figure 242-6 *Blastocystis hominis.* (a) Central body form with large 'empty' area (appears like a vacuole) with multiple nuclei around the edges (d'Antoni's iodine). (b) Three central body forms with the large empty area surrounded by nuclei (trichrome stain).

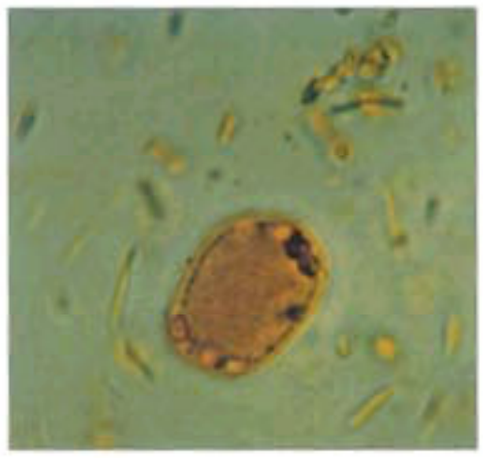


Figure 242-7 *Giardia lamblia.* (a) Trophozoites in mucus — note the sucking disk area, linear axonemes, curved median bodies and two nuclei (trichrome stain). (b) Cysts containing multiple nuclei, linear axonemes and curved median bodies (iron-hematoxylin stain).

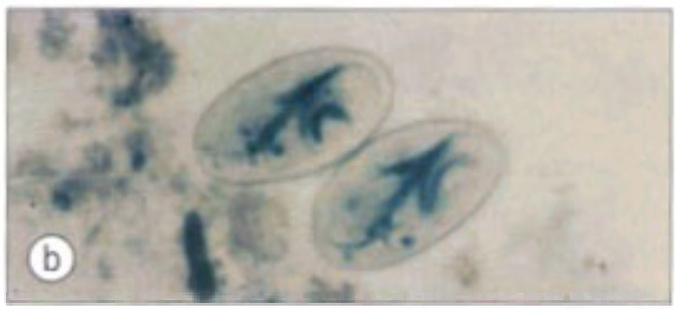


Figure 242-8 *Chilomastix mesnili.* (a) Trophozoite with single nucleus and clear feeding groove/cytostome. (b) Cyst containing single nucleus and curved fibril called the 'shepherd's crook' (trichrome stain).

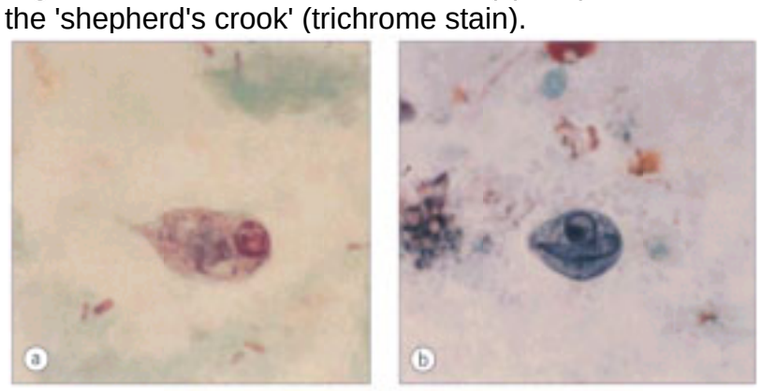


Figure 242-9 *Dientamoeba fragilis.* (a) Trophozoite with single nucleus fragmented into a 'tetrad' configuration. (b) Trophozoite containing two nuclei, each showing fragmented chromatin (trichrome stain). There is no known cyst form for this organism.

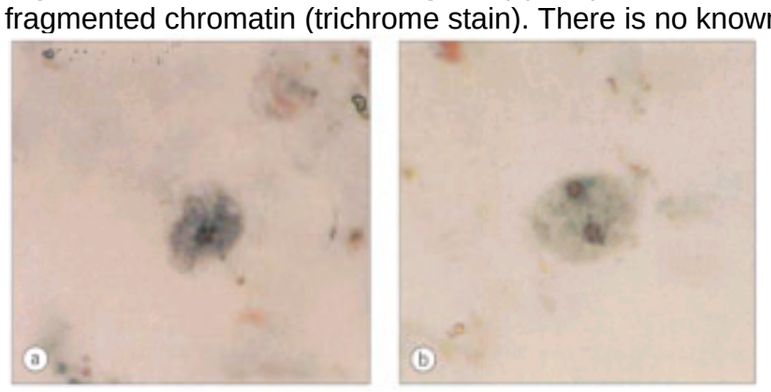


Figure 242-10 ***Trichomonas vaginalis.*** Trophozoites showing axostyle, flagella and part of the undulating membrane (smaller organism) (Giemsa stain). There is no known cyst form for this organism.

Figure 242-11 ***Balantidium coli.*** (a) Trophozoite — note the cilia around the edges ('fuzzy football'). (b) Cyst — note the cilia are difficult to see within the cyst wall (d'Antoni's iodine — light preparation, pale color).

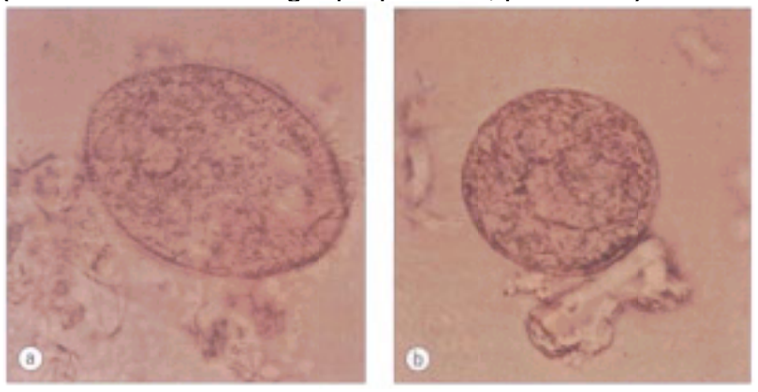


Figure 243-1 Intestinal cryptosporidial infection. Transmission electron micrograph of duodenal tissue of a patient with HIV infection showing two different developmental stages of *Cryptosporidium* spp. on the brush border of the mucosal surface: mature schizont with merozoites (right), undifferentiated zygote (left). *Cryptosporidium* spp. develop intracellularly just under the plasma membrane of the host cell. *Courtesy of MA Spycher.*

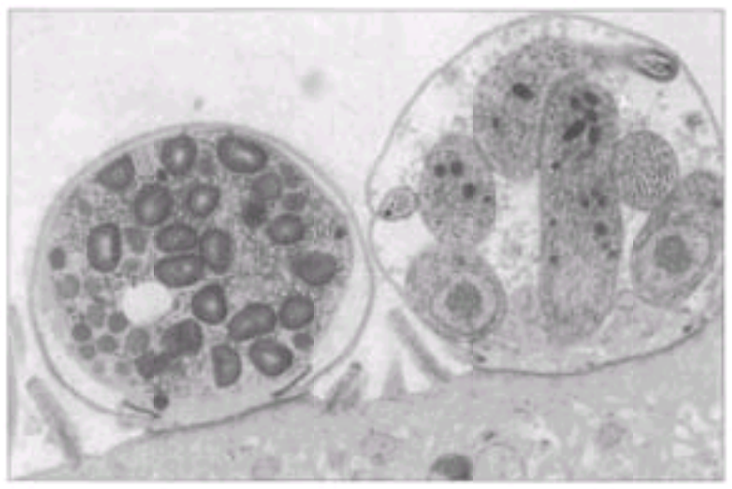


Figure 243-2 Intestinal *Enterocytozoon bieneusi* infection. Transmission electron micrograph showing duodenal epithelium of a patient with HIV infection who has *Enterocytozoon bieneusi* infection. The different developmental stages between the enterocyte nuclei (N) and the microvillous border include: (1) a proliferative plasmodium; (2) late sporogonial plasmodia; and (3) mature spores. *Courtesy of MA Spycher.*

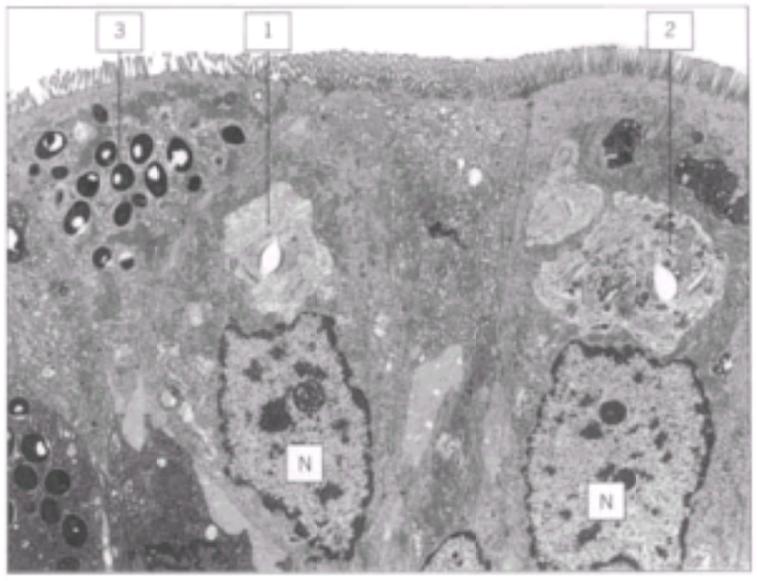


Figure 243-3 Acid-fast stained smears of fecal specimens showing intestinal coccidia. (a) *Cryptosporidium* spp., round, 4–6µm diameter. (b) *Cyclospora* spp., round, 8–10µm diameter. *Courtesy of EG Long.* (c) *Isospora belli*, elliptical, 23–33µm long and 10–19µm wide. Modified Kinyoun stain.

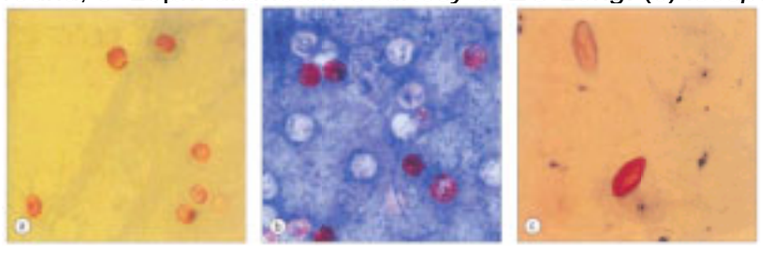


Figure 243-4 Intestinal *Cyclospora* infection. Transmission electron micrograph of duodenal epithelium obtained from a patient with HIV infection who has *Cyclospora cayetanensis* infection. A mature schizont filled with numerous merozoites is shown. *Courtesy of AM Deloul and FP Chatelet.*

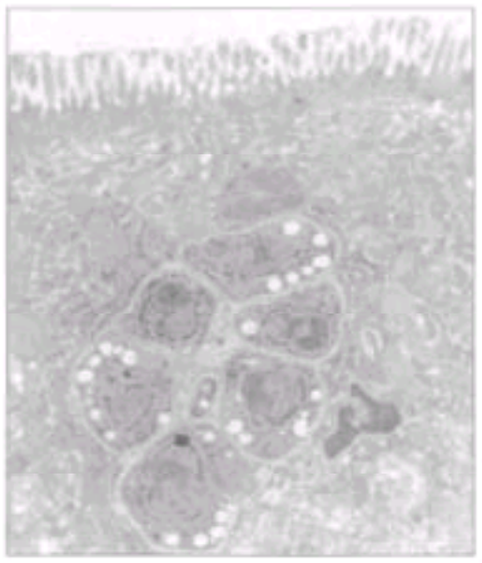


Figure 243-5 Detection of microsporidia in stool samples. Smear of a formalin-fixed, unconcentrated stool specimen of a patient who has AIDS and chronic diarrhea, showing pinkish-red-stained spores of *Enterocytozoon bieneusi*. Chromotrope staining (oil immersion).

Figure 243-6 Intestinal *Enterocytozoon bieneusi* infection. Terminal ileal tissue obtained by ileocolonoscopy in a patient who has AIDS and chronic diarrhea caused by *Enterocytozoon bieneusi* infection. Gram-positive or Gram-labile microsporidial spores (arrow) are found in supranuclear location within small intestinal enterocytes. Brown-Brenn stain.

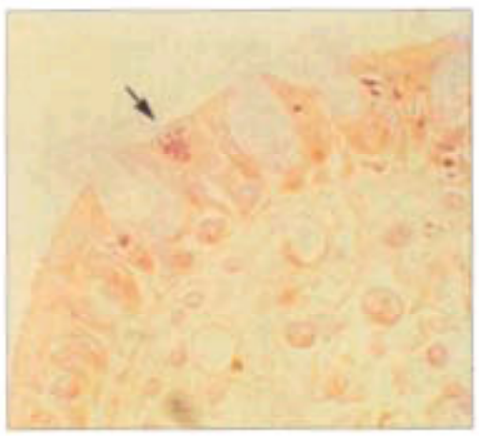


Figure 243-7 Detection of microsporidia in urine sediment. Urine sediment from a patient who has AIDS and disseminated *Encephalitozoon hellem* infection, showing Gram-labile intracellular and extracellular microsporidial spores. Gram stain (oil immersion).

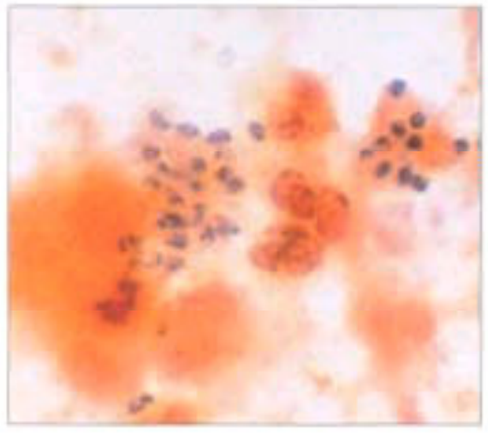


Figure 243-8 *Encephalitozoon intestinalis* (formerly *Septata intestinalis*): developing spores within enterocytes separated by a fibrillar matrix.
Encephalitozoon intestinalis develop within parasitophorous vacuoles, unlike *E. bieneusi*, which develops in direct contact with enterocyte cytoplasm. *Courtesy of MA Spycher.*

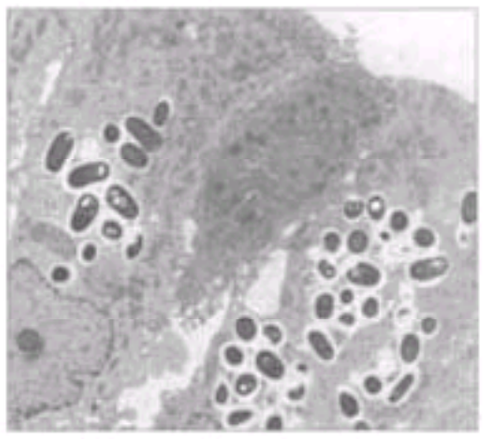


Figure 243-9 Keratopathy caused by *Encephalitozoon hellem.* Slit-lamp demonstration of punctate epithelial keratopathy in a patient who has AIDS and keratoconjunctivitis caused by *Encephalitozoon hellem*. Ocular microsporidiosis can often be diagnosed by examination under the light microscope of a smear obtained by a nontraumatic conjunctival swab. *Courtesy of M Diesenhouse and DA Schwartz.*

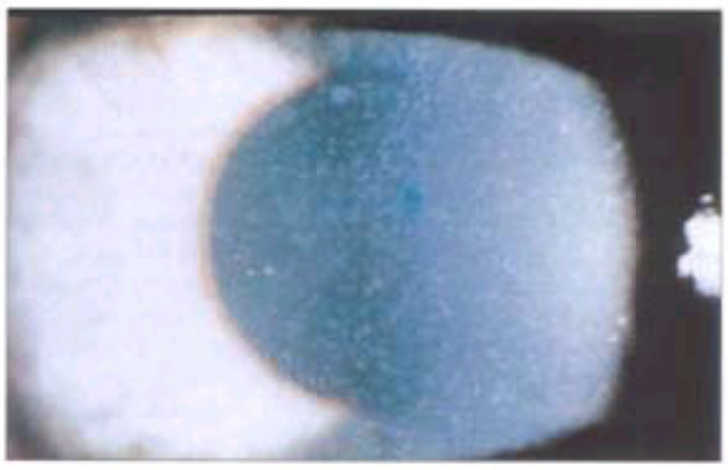


Figure 243-10 Cerebral microsporidiosis caused by *Encephalitozoon cuniculi* in a patient with HIV infection. The MRI shows multiple small contrast-enhancing, mostly ring-like, partly micronodular, lesions in hippocampal, mesencephal and intracortical regions (arrows), partly accompanied by slight edema, and congestion of the right ethmoid sinus. *Encephalitozoon cuniculi* was isolated from CSF. *From Weber et al.,*[36] *with permission from Massachusetts Medical Society.*

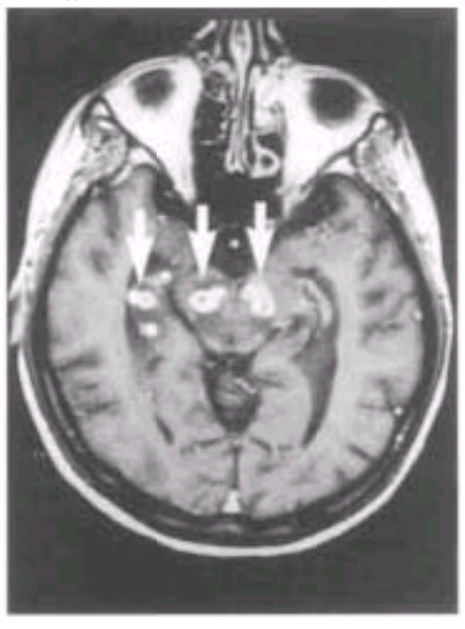


Figure 244-1 ***Naegleria fowleri.*** The trophozoite can be differentiated from the cyst by its characteristic lobopodial locomotion; both are taken from culture. Differential interference contrast.

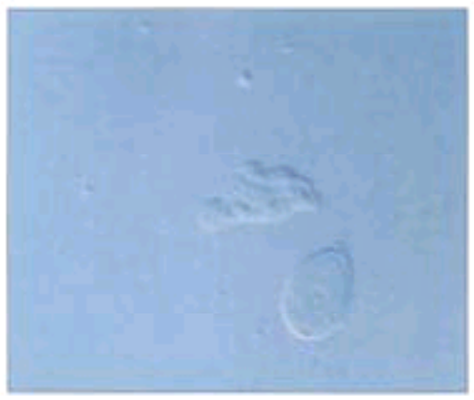


Figure 244-2 ***Acanthamoeba castellanii.*** The trophozoite has spiny acanthopodia whereas the cyst has an outer wrinkled ectocyst and a stellate endocyst; both are taken from culture. Differential interference contrast.

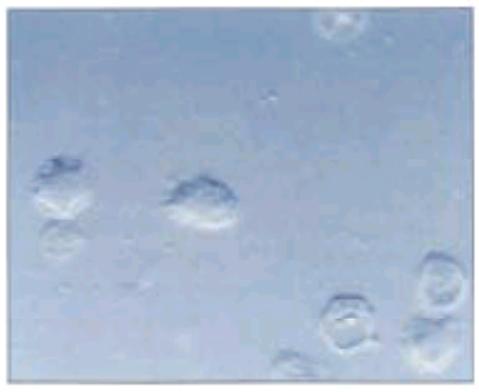


Figure 244-3 ***Balamuthia mandrillaris.*** The trophozoite is irregularly shaped whereas the cyst is spherical; both are taken from culture. Differential interference contrast.

Figure 244-4 CNS section demonstrating numerous trophozoites of *Naegleria fowleri.* Note the absence of cysts. (H & E.)

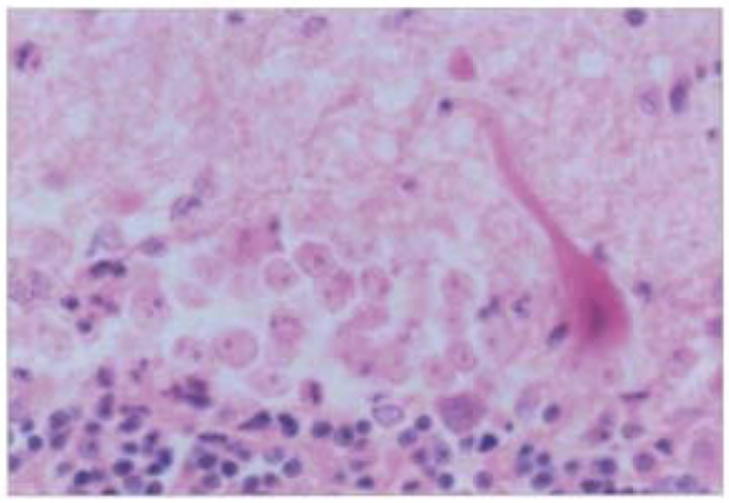


Figure 244-5 Brain section showing numerous trophozoites and a cyst (arrow) with typical features of *Acanthamoeba culbertsoni.* (Masson's trichrome.)

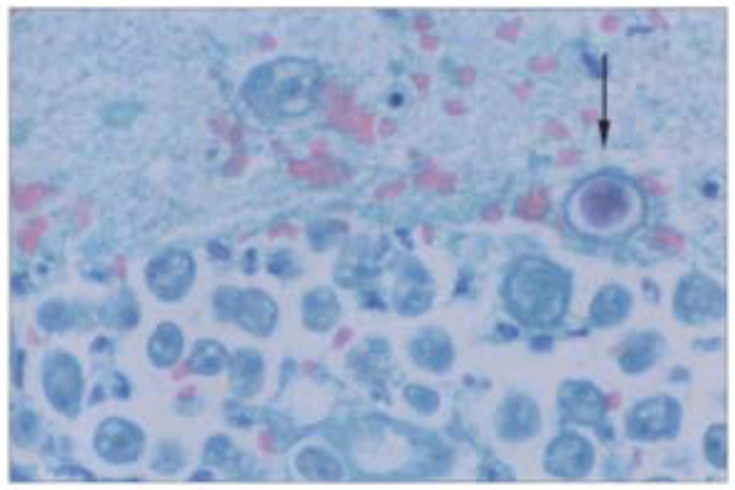


Figure 244-6 Trophozoites and cysts of *Acanthamoeba* sp. surrounding a blood vessel in a skin biopsy of a patient who has AIDS. A diffuse but modest inflammatory reaction is seen.

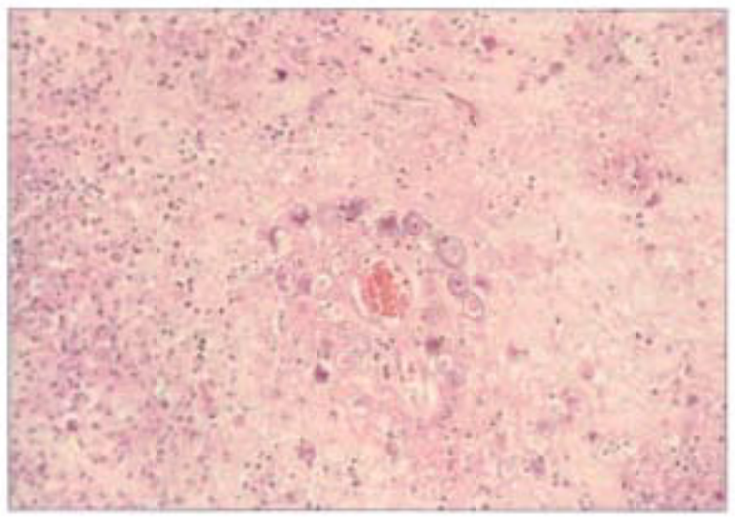


Figure 244-7 CNS section showing many trophozoites of *Balamuthia mandrillaris.* More than one nucleoli can be seen within the nuclei of the trophozoite.

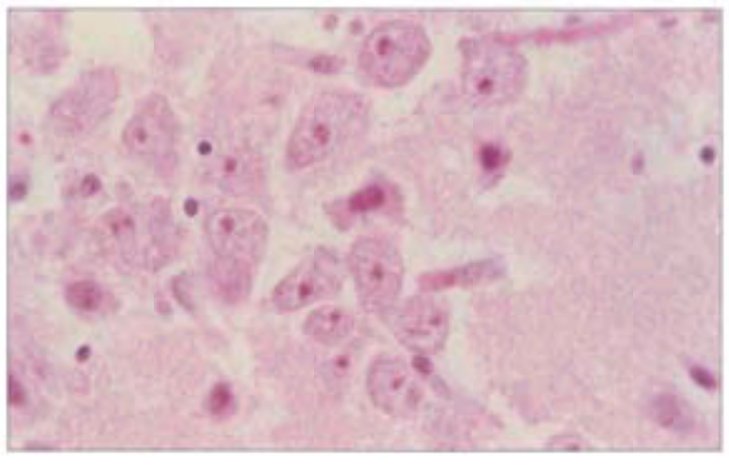


Figure 244-8 *Acanthamoeba* keratitis. Note the typical central or paracentral ring infiltrate. *Courtesy of Dr Theodore.*

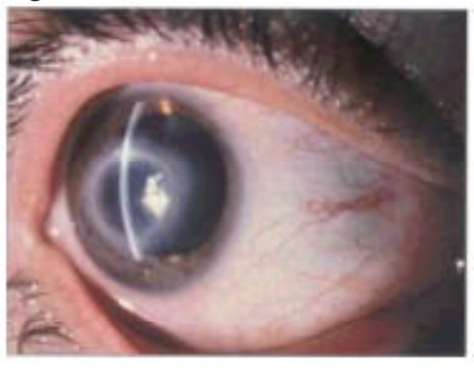


Figure 244-9 Characteristic star-shaped morphology of the cyst of *Acanthamoeba* sp. demonstrated by a corneal scraping stained with Hemacolor. *Courtesy of Dr Theodore.*

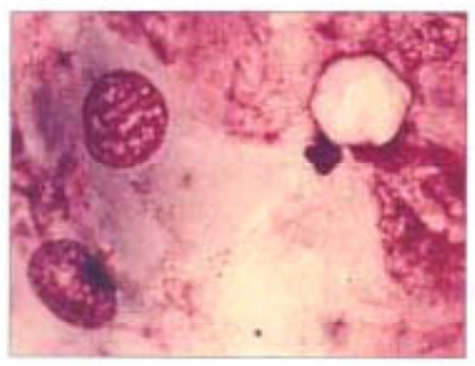


Figure 245-1 *Plasmodium vivax* life cycle. The life cycle starts when an infected mosquito feeds, inoculating the sporozoite form of the parasite, which infects the hepatocytes. The parasite cells then multiply (liver schizonts) and are liberated into the circulation to invade red cells where they grow (trophozoites) and divide (schizonts). Some trophozoites differentiate into gametocytes infective for mosquitoes.

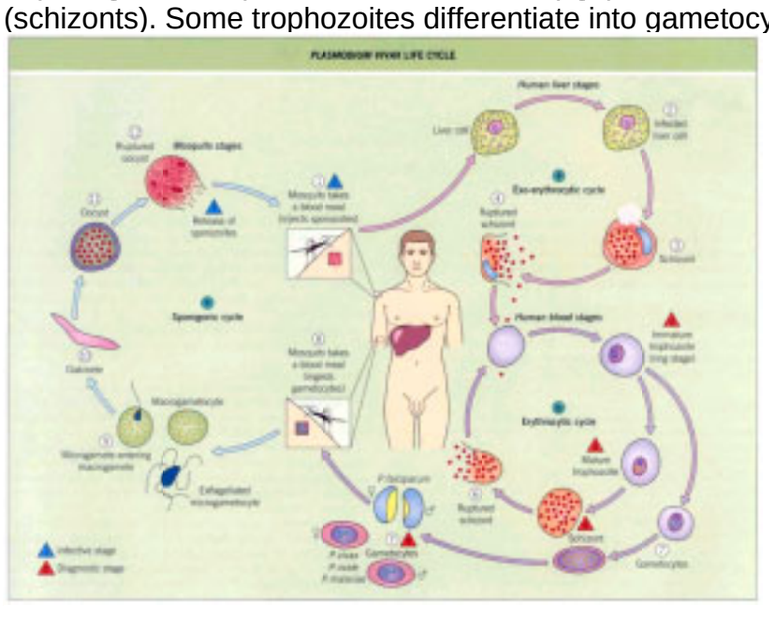


Figure 245-2 World distribution of malaria and areas of drug resistance, 2001.

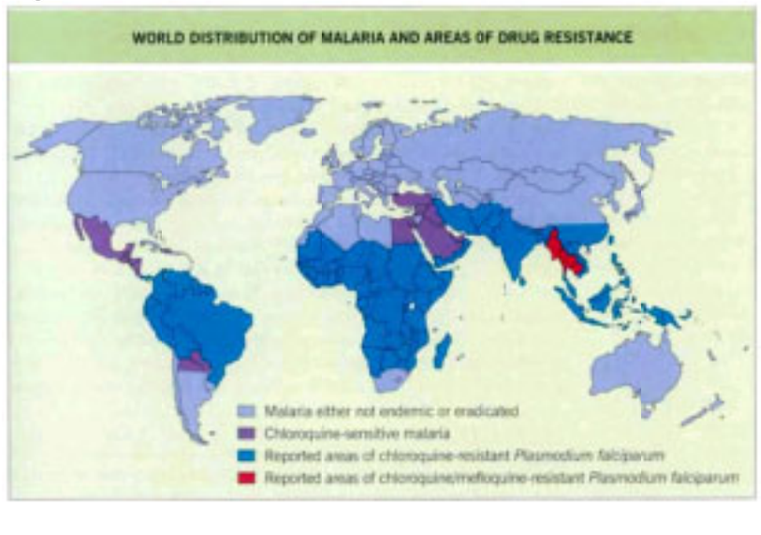


Figure 245-3 *Babesia* spp. Single and multiple intraerythrocytic parasites can be seen. The arrow marks a typical Maltese cross.

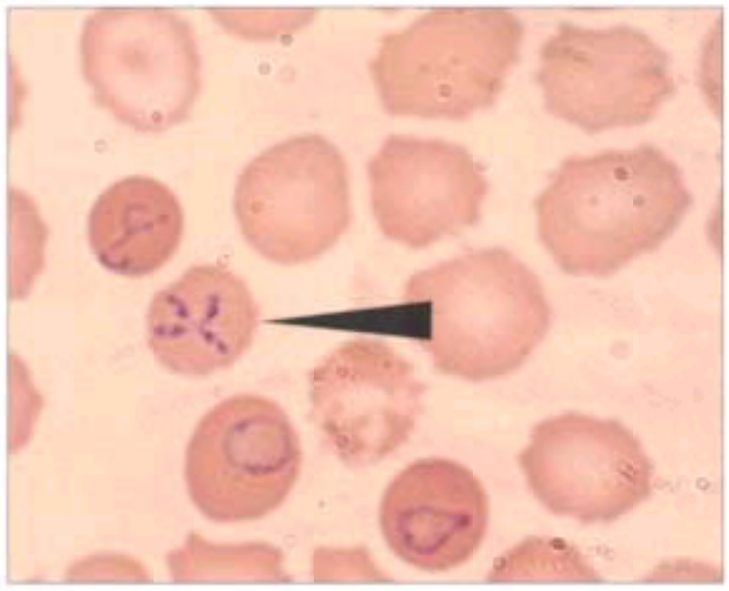


Figure 245-4 Toxoplasmic encephalitis in person who has AIDS. A cranial CT scan shows bilateral contrast-enhanced ring lesions with peripheral edema and mass effect.

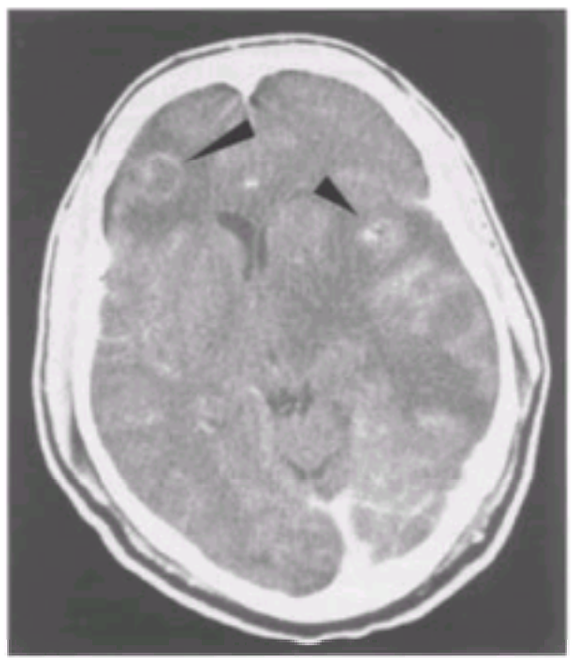


Figure 245-5 Fundoscopic image of toxoplasmic chorioretinitis. Most cases presenting in adults represent a late manifestation of a congenitally acquired toxoplasmosis.

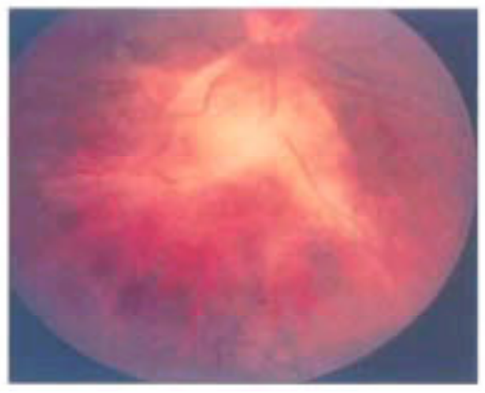


Figure 245-6 Toxoplasmosis risk management in pregnancy.

Figure 245-7 Geographic distribution of leishmaniasis. Visceral leishmaniasis (VL): 90% of cases occur in India, Bangladesh, Sudan and Brazil. Mucocutaneous leishmaniasis (MCL): 90% of cases occur in Bolivia, Brazil and Peru. Cutaneous leishmaniasis (CL): 90% of cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria.

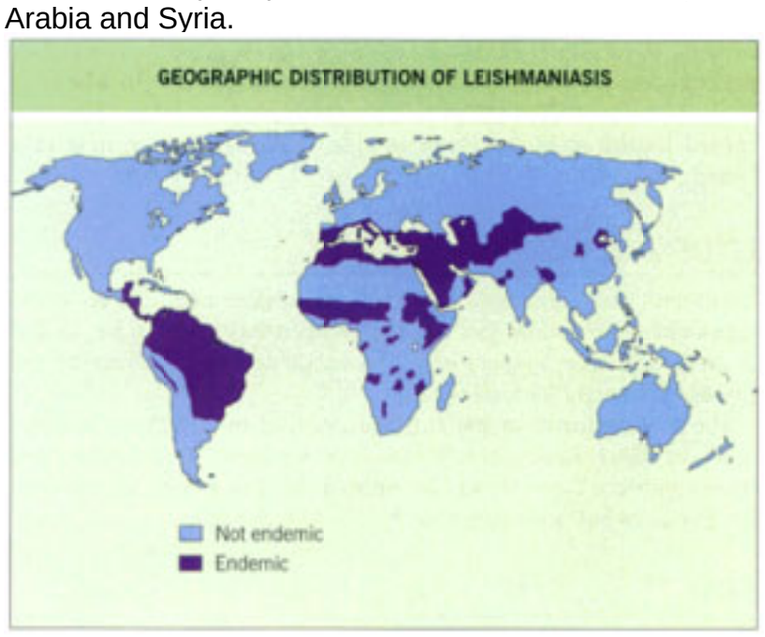


Figure 245-8 Cutaneous manifestation of kala-azar in AIDS.

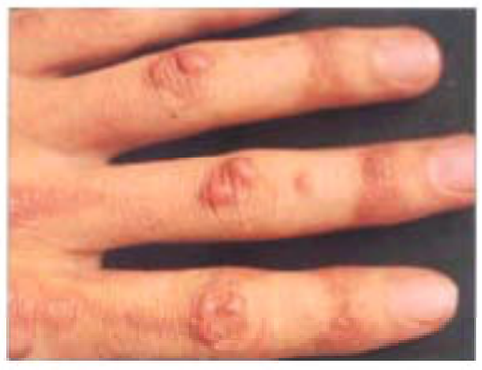


Figure 245-9 New World cutaneous leishmaniasis.

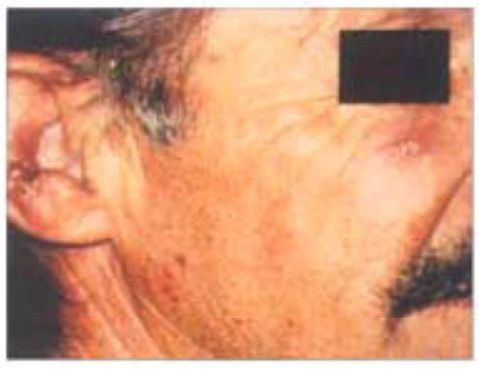


Figure 245-10 ***Trypanosoma brucei.*** Note the central nucleon, an undulating membrane and a clump of mitochondrial DNA called kinetoplast.

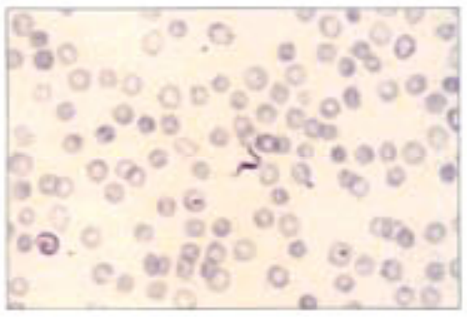


Figure 245-11 A dilated heart from a fatal case of chagasic cardiomyopathy. Chronic heart disease is the commonest cause of death in Chagas' disease. *Courtesy of Dr J Cohen, Cordoba, Argentina.*

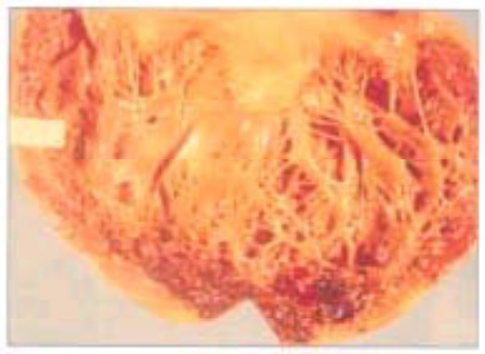


Figure 245-12 Chagasic megacolon at autopsy. Grossly enlarged colon as shown here or other duct dilatation is characteristic of chronic Chagas' disease. *Courtesy of Dr J Cohen, Cordoba, Argentina.*

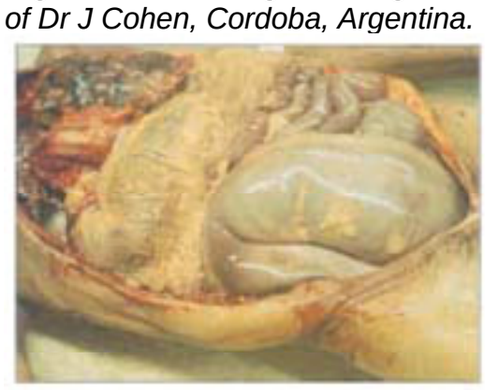


Figure 246-1 Life cycles of important human roundworms: adults living in the intestines.

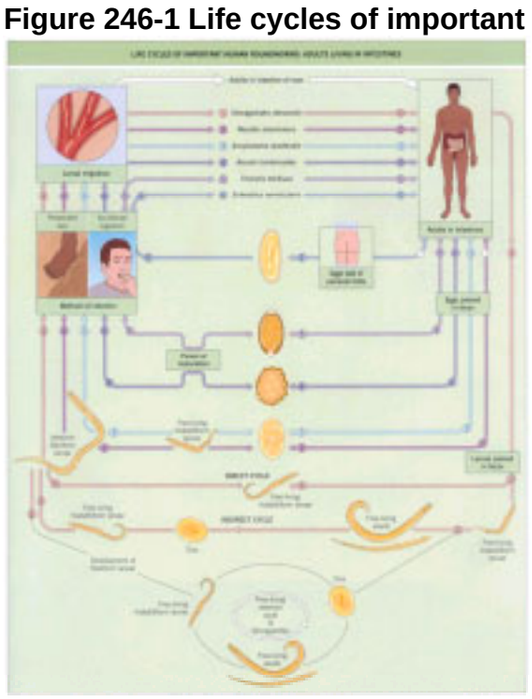


Figure 246-2 Life cycles of important human roundworms: adults living in tissues.

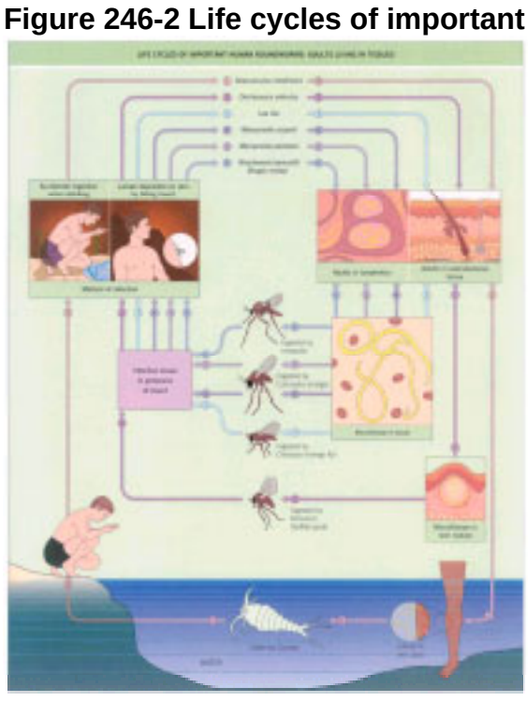


Figure 246-3 Life cycles of important human flukes: adults living in the liver, lungs, intestines and blood.

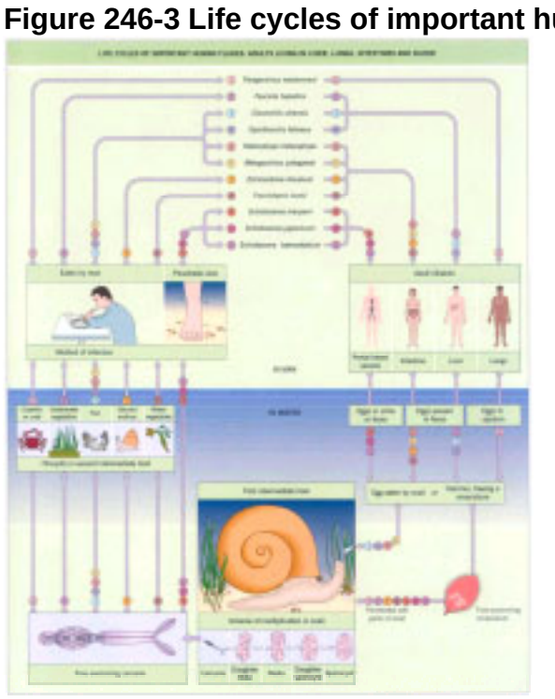


Figure 246-4 Life cycles of important human tapeworms: adults living in the intestines.

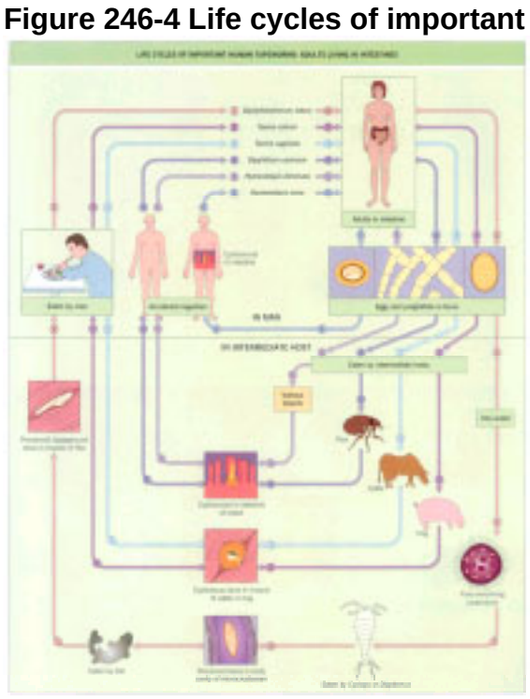


Figure 246-5 Life cycles of important human tapeworms; adults living in tissues and intestines and blood: humans are accidental hosts.

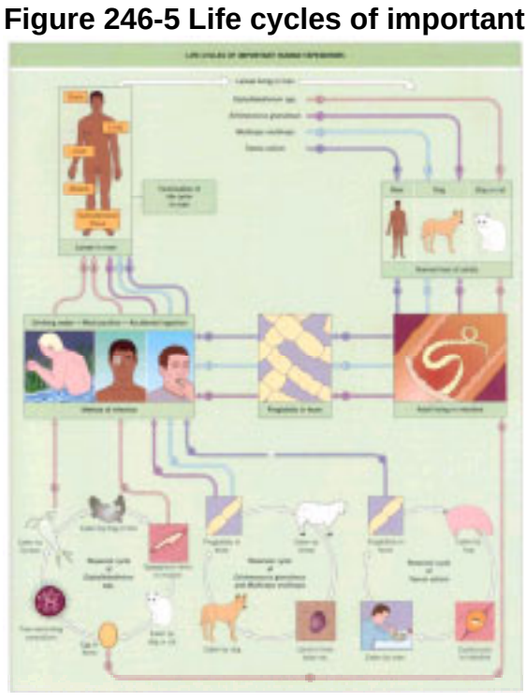


Figure 246-6 Relative size of helminth eggs. HHS Publication No. (CDC 89-8116)

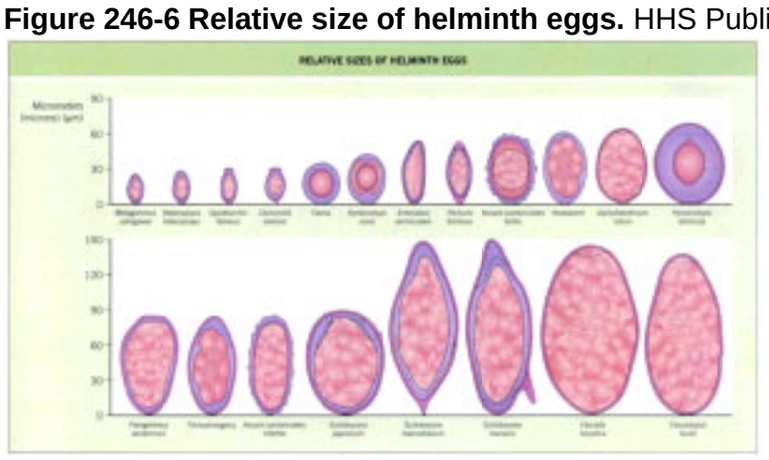


Figure 246-7 Hookworm and *Strongyloides* larvae. HHS Publication No. (CDC 89-8116)

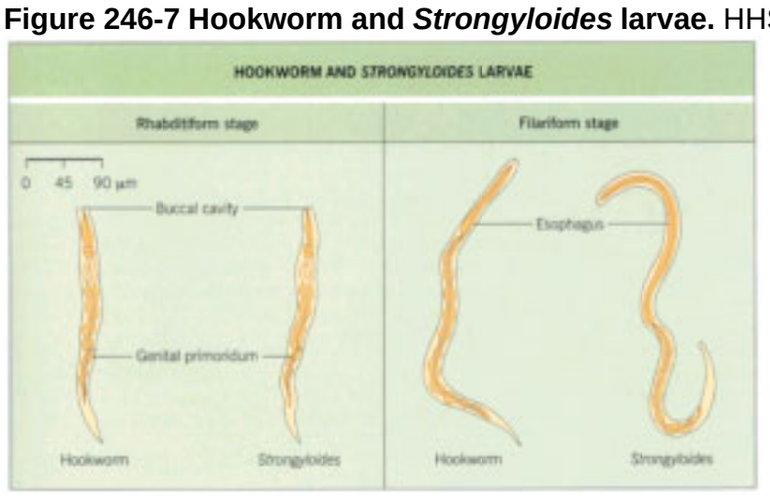


Figure 247-1 Adult *Pediculus capitis*, the human head louse. Note the prominent claws for grasping hair.

Figure 247-2 Egg or nit of human head louse attached to a hair shaft. Note that the operculum is missing indicating previous emergence of the larval louse.

Figure 247-3 Adult *Phthirus pubis*, the human crab louse. The bodies of these lice are rounder and more crab-like than those of head or body lice. *With permission from ASM Press.*

Figure 247-4 Adult *Ctenocephalides canis*, the dog flea. Note the strong muscular hind legs.

Figure 247-5 Adult bedbug *Cimex lectularius.* *With permission from ASM Press.*

Figure 247-6 Larva of *Dermatobia hominis*, the human botfly, following recovery from a furuncular skin lesion. *With permission from J Brad Thomas, David L Bergeron and James J Plorde.*

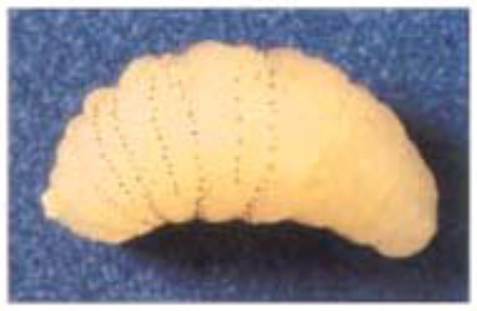


Figure 247-7 Female *Lactrodectus mactans*, the black widow spider. Note the characteristic red hourglass marking on the underside of the abdomen. *With permission from New England Journal of Medicine 1994;331:777.*

Figure 247-8 Nonengorged adult female *Dermacentor variabilis*, the common dog tick, of the family Ixodidae (hard ticks). *With permission from Northwest Infectious Disease Consultants.*

Figure 247-9 Nonengorged adult *Ornithodoros hermsi*, of the family Argasidae (soft ticks). *With permission from Northwest Infectious Disease Consultants.*

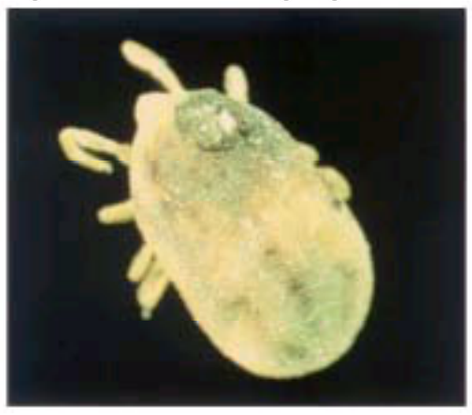


Figure 247-10 Nonengorged adult female *Ixodes scapularis*, the blacklegged tick or deer tick, of the family Ixodidae (hard ticks). *With permission from Northwest Infectious Disease Consultants.*

Figure 247-11 Crusted Norwegian scabies in a patient who has AIDS. *With permission from New England Journal of Medicine 1994;331:777.*

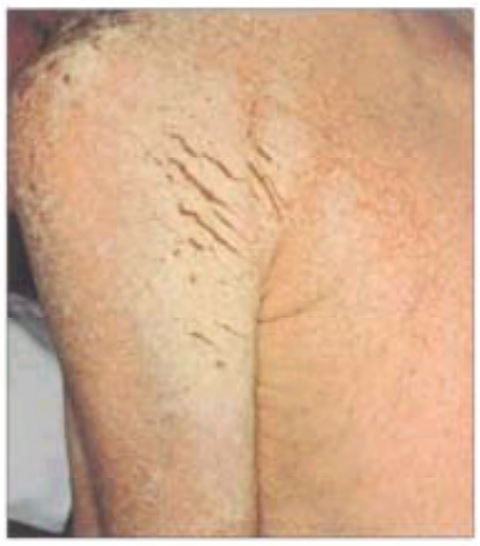


Figure 247-12 Adult *Sarcoptes scabiei*, the human itch or mange mite, seen in skin scrapings. *With permission from New England Journal of Medicine 1994;331:777.*